Form Approved Through 05/2004 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 56 characters, including spaces and punctuation.)*
National Center of Excellence: The Tissue Simulation Toolkit

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: RM 04-003 Title: National Centers for Biomedical Computing

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — New Investigator ☒ No ☐ Yes

3a. NAME *(Last, first, middle)*: Glazier, James Alexander

3b. DEGREE(S): Ph.D.

3c. POSITION TITLE: Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*:
Swain Hall West 159
727 East 3rd Street
Bloomington, IN 47405-7105

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Department of Physics

3f. MAJOR SUBDIVISION: Arts and Sciences

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: (812) 855-3735 FAX: (812) 855-5533

E-MAIL ADDRESS: glazier@indiana.edu

4. HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes
4a. Research Exempt ☐ No ☐ Yes — If "Yes," Exemption No.
4b. Human Subjects Assurance No.
4c. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☒ Yes
5a. If "Yes," IACUC approval Date: 6/12/2003, pending
5b. Animal welfare assurance no.: A4091-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
09/15/04	09/14/09	$3,272,686	$4,483,998	$16,381,749	$22,275,348

9. APPLICANT ORGANIZATION
Name: Indiana University
Address: P.O. Box 1847
Bloomington, IN 47402-1847

Institutional Profile File Number (if known): 577805

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1-356001673-A1
DUNS NO. 00-604-6700
Congressional District: 8

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Steven A. Martin
Title: Assistant Vice President for Research
Address: P.O. Box 1847
Bloomington, IN 47402-1847
Tel: (812) 855-3963 FAX: (812) 855-9943
E-Mail: spon2@iupui.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Michael A. McRobbie
Title: Vice President for Research
Address: P.O. Box 1847
Bloomington, IN 47402-1847
Tel: (812) 855-0516 FAX: (812) 855-9943
E-Mail: rugs@indiana.edu

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)* — DATE: 12/2/04

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* — DATE: 12/2/2004

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The National Center for Tissue Modeling (CTM) links four major Indiana universities: Bloomington, IUPUI, Notre Dame, and Purdue. By developing simple and adaptable open source tools and toolkits for cell-level simulations of tissue and organ development, the CTM will realize the twenty-year vision of modern systems biology – development of reliable multiscale simulations reaching from genome to organism. CTM tools will link seamlessly with both larger scale continuum models and microscopic models. The CTM includes with this proposal signed letters of agreement with Physiome, Virtual Cell, SciRun and BioSpice to support co-development, model integration and platform sharing. CTM simulations will be a hierarchical, organized set of biologically motivated levels where parameters and properties pass from coarser to finer scale models. The CTM will develop a simulation environment via the Tissue Simulation Toolkit (TST), enabling simulations that cover the mesoscale from single structured cells to aggregates of millions of cells, a scale neglected by other major Computational Biology Centers. Its core Cellular Potts Model (CPM) tool combines multiscale algorithms with scalable parallelism from PCs to supercomputers. This tool will be presented as a Grid service with a portal interface. Visualization tools will be based on SCIRun and the simulation framework on the CCA to facilitate model interconnection. The CTM is comprised of three major scientific components; computer science, model development and experiment, organized from a unified, interdisciplinary point of view. Experimental activity is devoted to three tractable problems in development and structural disease: vascular development, heart development, and limb regeneration, delivering parameters and test data for simulations and motivate modeling. The computer science activity delivers tools for modeling in conjunction with experiments. CTM member institutions will develop a set of shared interdisciplinary curricula, expanding successful outreach and training programs to educate and train a new generation of computational biologists.

PERFORMANCE SITE(S) *(organization, city, state)*

Indiana University, Bloomington, IN
Indiana University, Indiana University – Purdue University, Indianapolis (IUPUI), IN
University of Notre Dame, South Bend, IN
Purdue University, West Laffayette, IN
University of Kansas Medical Center, Kansas City, KS

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Glazier, James Alexander	Indiana University, Bloomington	Principal Investigator
Alber, Mark	University of Notre Dame	Coordinator, Notre Dame
Arenson, Andrew	Indiana University, IUPUI	Technical Specialist Software Support
Baker, Pauline	Indiana University, IUPUI	Participant, Visualization
Barabási, Albert-László	University of Notre Dame	Participant, Subcellular Modeling
Beleky-Adams, Teri	Indiana University, IUPUI	Participant, Regeneration Experiments
Borner, Katy	Indiana University, Bloomington	Participant, Subcellular Modeling
Bosron, William	Indiana University, IUPUI	Education and Outreach Coordinator
Chaturvedi, Rajiv	University of Notre Dame	Software Manager, Notre Dame
Chernoff, Ellen	Indiana University, IUPUI	Coordinator, Regeneration Experim.
Conway, Simon	Indiana University, IUPUI	Participant, Heart Experiments

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☐ Yes ☐ No

Name	Organization	Role on Project
Czirok, Andras	University of Kansas Medical Center	Participant, Heart Experiments
Dan, Debasis	Indiana University, Bloomington	Postdoctoral Researcher, CPM
Davisson, Vincent Jo	Pudue University	Exerimental Coordinator, Purdue
Field, Loren	Indiana University, IUPUI	Coordinator, Heart Experiments
Firulli, Anthony	Indiana University, IUPUI	Participant, Heart Experiments
Fox, Geoffrey Charles	Indiana University, Bloomington	Coordinator, Tool Development
Gannon, Dennis	Indiana University, Bloomington	Participant, Grid Software
Goodson, Holly	University of Notre Dame	Coordinator, Notre Dame Education
Grow, Matthew	Indiana University, IUPUI	Coordinator, Experimental Support
Hart, David C.	Indiana University, Bloomington	Coordinator, CPM Parallelization, Grid
Heiland, Randy	Indiana University, IUPUI	Participant, Computer Visualization
Jacobson, Stephen	Indiana University, Bloomington	Participant, Experimental Support
Little, Charles	University of Kansas Medical Center	Participant, Vascular Experiments
Lumsdaine, Andrew	Indiana University, Bloomington	Participant, CPM Development
March, Keith	Indiana University, IUPUI	Coordinator, Vascular Experiments
Marrs, James	Indiana University, IUPUI	Participant, Regeneration Experiments
Merks, Roeland	Indiana University, Bloomington	Postdoctoral Researcher, Vascular
Moffett, David	Purdue University	Participant, Software Support
Niebur, Glen	University of Notre Dame	Participant, Finite Element Simulation
Papakhian, Mary	Indiana University, Bloomington	Coordinator, Software Maintenance
Pidaparti, Ramana	Indiana University, IUPUI	Participant, Finite Element Simulation
Ramkrishna, Doraiswami	Purdue University	Participant, Subcellular Simulation
Repasky, Richard	Indiana University, Bloomington	Participant, Model Evaluation
Rhodes, Simon	Indiana University, IUPUI	Participant, Regeneration Experiments
Robinson, J. Paul	Purdue University	Participant, Experimental Support
Rongish, Brenda	University of Kansas Medical Center	Participant, Vascular Experiments
Schnell, Santiago	Indiana University, Bloomington	Coordinator, Subcellular Simulation
Setayeshgar, Sima	Indiana University, Bloomington	Coordinator, Finite Element Simulation
Shankar, Anurag	Indiana University, Bloomington	Coordinator, Data Repository
Sheppard, Raymond W	Indiana University, IUPUI	Participant, CPM Development
Shou, Weinian	Indiana University, IUPUI	Participant, Heart Experiments
Simms, Stephen	Indiana University, Bloomington	Participant, Grid Support
Stewart, Craig	Indiana University, Bloomington	Coordinator, Software Support
Stocum, David	Indiana University, IUPUI	Coordinator, Experimental Core
Wernert, Eric	Indiana University, Bloomington	Coordinator, Visualization
Wuchty, Stefan	University of Notre Dame	Postdoctoral Researcher, Subcellular
Yoder, Mervin	Indiana University, IUPUI	Participant, Vascular Experiments

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

Appendix *(Five collated sets. No page numbering necessary for Appendix.)*

Check if Appendix is Included

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited. ☒

Number of publications and manuscripts accepted for publication *(not to exceed 10)*

Other items (list):

Appendix I – Letters of Support and Participation

Appendix II – Relevant Publications (43 reprints)

CUMULATIVE BUDGET

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 09/15/2004 THROUGH: 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Core 1	Research				402,837	119,001	521,838
Core 2	Research				326,620	65,594	392,214
Core 3	Research				401,084	117,177	518,261
Core 4	Computer Support				135,881	43,849	179,730
Core 5	Education Training				45,000	14,522	59,522
Core 6	Outreach				55,000	17,749	72,749
Core 7	Administ-ration				90,000	29,043	119,043
	SUBTOTALS →				1,456,422	406,935	1,863,357

CONSULTANT COSTS

EQUIPMENT *(Itemize)*
Core 5 - $20,000 — 20,000

SUPPLIES *(Itemize by category)*
Core 2 - $4,500; Core 3 - $164,726; Core 5 - $5,000; Core 7 - $5,000 — 179,226

TRAVEL
See individual cores for details — 82,500

PATIENT CARE COSTS
INPATIENT
OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
See individual cores for details — 268,685

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 2,413,768
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	576,577
	FACILITIES AND ADMINISTRATIVE COSTS	282,341
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 3,272,686

SBIR/STTR Only: FEE REQUESTED

CUMULATIVE BUDGET

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		1,863,357	1,095,735	1,914,969	2,076,474	2,114,839
CONSULTANT COSTS						
EQUIPMENT		20,000	20,000	0	0	0
SUPPLIES		179,226	168,852	159,028	14,500	13,500
TRAVEL		82,500	87620	91880	88500	88500
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		268,685	230,251	233,408	247,381	228,327
SUBTOTAL DIRECT COSTS		2,413,768	2,412,458	2,399,285	2,426,855	2,445,166
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	576,577	584,850	593,439	548,569	554,631
	F&A	282,341	286,322	297,423	278,456	281,609
TOTAL DIRECT COSTS*		3,272,686	3,283,630	3,290,147	3,253,880	3,281,406

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 16,381,749***

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

* Total amounts requested without indirect costs on subcontractual agreements are:

Year 1	Year 2	Year 3	Year 4	Year 5	**Total**
$2,990,345	$2,997,308	$2,992,724	$2,975,424	$2,999,797	**$14,955,598**

Please see justification for each core for detailed information.

BUDGET NOTES
Cumulative Budget

COST SHARE TABLE

Organization	Financial	In-Kind
Purdue University		½ Postdoctoral Researcher
IUPUI School of Science		15 percent effort for Dr. Chernoff 10 percent effort for Dr. Rhodes
IUPUI School of Medicine	$25,000/year	
Notre Dame	$50,000/year $70,000/once $100,000/year (existing for Center)	5 percent effort by Dr. Barabasi 5 percent effort by Dr. Niebur 5 percent effort by Dr. Goodson
IUB Research and the University Graduate School	$100,000/year	
IUB College of Arts and Sciences	$30,000/year $250,000/year (existing for Center)	1 Full Time Faculty Position Startup Funds for 1 Faculty 25 percent effort by PI Glazier 10 percent effort by Dr. Setayeshgar Out-of-state fee remissions for graduate students

Core 1: Computational Tools

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Geoffrey Fox	Co-PI	12	10	214,200	21,420	6,912	28,332
Craig Stewart	Co-PI	12	5	95,621	4,781	1,543	6,324
Andrew Lumsdaine	Investigator	10	12	99,680	11,962	3,860	15,822
Randy Heiland	Visual. Lead	12	50	92,000	46,000	14,844	60,844
Charles Moad	Visual. Development	12	80	53,000	42,400	13,682	56,082
Mary Papakhian	Programming Manager	12	5	75,480	3,774	1,218	4,992
Subtotals from next page	See next page				272,500	76,942	349,442
SUBTOTALS →					402,837	119,001	521,838

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

TRAVEL
Domestic - $14,000; International - $3,000 — 17,000

PATIENT CARE COSTS
INPATIENT
OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
Graduate Student Fee Remission - $11,678
Non-capital equipment - $10,500 — 22,178

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD — **$** 561,016

CONSORTIUM/CONTRACTUAL COSTS
DIRECT COSTS — 100,000
FACILITIES AND ADMINISTRATIVE COSTS — 50,000

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* → **$** 711,016

SBIR/STTR Only: FEE REQUESTED

Core 1: Computational Tools (additional personnel)

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Additional personnel	Principal Investigator						
Sr. Programming Analyst	Programming	12	50	75,000	37,500	12,101	49,601
Sr. Visualization Analyst	Visualization	12	100	75,000	75,000	24,203	99,203
Postdoctoral Fellows (2)	Res. Assoc.	12	100	60,000	120,000	38,724	158,724
Graduate Students (2)	Res. Assist.	12	100	20,000	40,000	1,914	41,914
SUBTOTALS →					272,500	76,942	349,442

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ -
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		$ -

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		521,838	537,493	553,618	638,930	658,097
CONSULTANT COSTS						
EQUIPMENT			20,000			
SUPPLIES						
TRAVEL		17,000	17,000	17,000	17,000	17,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		22,178	12,028	12,389	42,401	32,858
SUBTOTAL DIRECT COSTS		561,016	586,521	583,007	698,331	707,955
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	100,000	100,000	100,000	100,000	100,000
	F&A	50,000	50,000	50,000	50,000	50,000
TOTAL DIRECT COSTS		711,016	736,521	733,007	848,331	857,955

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 3,886,830***

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $3,636,830.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 1: Computational Tools

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Geoffrey Fox	**Co-PI**	**10**	**Calendar**

Geoffrey Fox will perform overall supervision of the Core I activities. He is an internationally recognized expert, in distributed computing and parallel computing. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Craig Stewart	**Co-PI**	**5**	**Calendar**

Craig Stewart will perform overall supervision of the Core I activities. He is an internationally recognized expert biological computing. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Andrew Lumsdaine	**Investigator**	**12**	**Academic**

Andrew Lumsdaine will also perform overall supervision of the Core I activities. His expertise lies in parallel computing. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Randy Heiland	**Visualization Lead**	**50**	**Calendar**

Randy Heiland will act as lead on visualization research and development. He will oversee the management of the visualization research aspects of the project. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Charles Moad	**Visualization Development**	**80**	**Calendar**

Charles Moad will be a key visualization research developer for this project. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Mary Papakhian	**Programming Manager**	**5**	**Calendar**

Mary Papakhian will perform overall supervision of the Core I software, hardware and database infrastructure. Her primary responsibilities will be in systems administration. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN	**Senior Programmer Analyst**	**50**	**Calendar**

Core I of this proposal calls for completely rebuilding the code base of the Cellular Potts Model (CPM), to optimize performance and parallelize the implementation. This person would complement the existing HPC Support Team with specific expertise in stochastic dynamics and reaction-diffusion equations. The proposed salary is based on current rates necessary to attract such expertise. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN **Senior Visualization Analyst** **100** **Calendar**

An additional senior visualization analyst will be hired to work on software installation and maintenance and SCIRun development. The proposed salary is based on current rates necessary to attract such expertise. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN - 2 **Postdoctoral Fellows** **100** **Calendar**

These fellows will develop fully OGSI and CCA compliant interfaces to the Tissue Simulation Toolkit. The proposed salaries are based on current rates at IUB. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN – 2 **Graduate Research Assistants** **100** **Calendar**

Fox and Lumsdaine will also supervise one graduate student throughout the grant period, and an additional graduate student during years four and five. Graduate students will receive training in computational biology, expected to make them attractive for both academic and industry scientific programming positions. The proposed salaries are based on current rates at IUB (20 hours per week constitutes full-time employment for graduate students). Fringe benefits for each graduate student include annual fee for health insurance, currently $957.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
We request $20,000 for purchase of visualization equipment (1 Jon-E-Box, a 3D-Stereo Display Unit), which will take place in year 2.

SUPPLIES:
None.

TRAVEL:
We request $17,000 to finance travel between sites as well as support for travel to appropriate workshops and conferences.

OTHER EXPENSES:
We request $11,678 in year 1 to cover in-state fee remissions for two graduate students that will perform research associated with this portion of the project ($5,839 per student). Subsequent years are increased by 3 percent to adjust for inflation and cost-of-living increases. In addition, we plan to hire three additional graduate students in years 4 and 5. The difference between out-of-state ($19,622 per student) and in-state ($5,839 per student) tuition will be paid by the College of Arts and Sciences of Indiana University (total cost share in year 1 $27,566; total cost share for all years $238,073). Additionally, we request $10,500 in years 1 and 4 for purchase of non-capital equipment.

CONSORTIUM/CONTRACTUAL COSTS:
The University of Notre Dame subcontractor will receive $72,000 for support of two postdoctoral fellows and $20,000 for support of one graduate student annually. Additional $8,000 per year will be used to finance travel between sites and attendance of relevant symposia. Indirect costs are calculated at 50 percent of total modified direct costs. Please see Notre Dame subcontract justifications for detailed information.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
James A. Glazeir	Principal Investigator	3	100	128,400	35,310	7,090	42,400
Santiago Schnell	Investigator	3	73	72,000	14,400	2,892	17,292
Ramana Pidaprti	Investigator	3	64	76,622	13,409	2,692	16,101
Sima Setayeshgar	Investigator	3	36	63,000	6,300	1,265	7,565
Programmer (TBN)	Programmer	12	100	50,000	50,000	16,135	66,135
Postdoctoral Fel. (3 TBN)	Res. Assoc.	12	100	30,000	90,000	29,043	119,043
Grad. Students (7 TBN)	Res. Assist.	12	100	See justif.	117,201	6,477	123,678
SUBTOTALS →					326,620	65,594	392,214

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	4,500
TRAVEL Domestic - $12,500; International - $4,000	16,500
PATIENT CARE COSTS — INPATIENT	
PATIENT CARE COSTS — OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Graduate Student Fee Remission - $70,100 Non-capital equipment - $14,000 Publication costs - $5,000	89,100
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	**$** 502,314
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	183,442
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	91,990
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →	**$** 777,746

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		392,214	363,108	374,001	405,936	417,493
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		4,500	4,500	4,500	4,500	4,000
TRAVEL		16,500	16,500	16,500	16,500	16,500
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		89,100	77,203	79,369	87,980	90,470
SUBTOTAL DIRECT COSTS		502,314	461,311	474,370	514,916	528,463
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	183,442	183,963	184,510	185,087	185,599
	F&A	91,990	92,261	99,511	92,845	93,111
TOTAL DIRECT COSTS		777,746	737,535	758,391	792,848	807,173

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 3,873,693***

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $3,403,975.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 2: Modeling and Application

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
James A. Glazier	**Principal Investigator**	**100**	**Summer**

Prof. Glazier is PI on this project and is also director of the Biocomplexity Institute at IU which is coordinating the entire effort. Dr. Glazier will coordinate all model development and implementation and is responsible for directing faculty hiring and seeking outside funding to support this project. His summer salary will allow him to devote a substantial fraction of his time to the scientific issues associated with model development and implementation as well as to organizational issues. We request salary to reimburse 11 weeks of Dr. Glazier's effort. Fringe benefits for this position are calculated based on Indiana University standards at 20.08 percent.

Santiago Schnell	**Investigator**	**73**	**Summer**

Dr. Schnell will be leading the modeling efforts on regeneration and will be responsible for development of sub-cellular modeling tools and integration of the CPM with our own and external sub-cellular modeling tools. We request salary to reimburse 8 weeks of Dr. Schnell's effort. Fringe benefits for this position are calculated based on Indiana University standards at 20.08 percent.

Ramana Pidaparti	**Investigator**	**64**	**Summer**

Dr. Pidaparti will be assisting in model development for the regeneration BMP in Core 3 and will assist with development and integration of our own and external finite element modeling tools. We request salary to reimburse 7 weeks of Dr. Pidaparti's effort. Fringe benefits for this position are calculated based on Indiana University standards at 20.08 percent.

Sima Setayeshgar	**Investigator**	**36**	**Summer**

Dr. Setayeshgar will lead the modeling effort on heart development and coordinate with BMP projects on heart development (BMP2) and will lead modeling and integration efforts on our own and external finite element and continuum modeling tools. She will also be leading the educational component of this project. We request salary to reimburse 4 weeks of Dr. Setayeshgar's effort. Fringe benefits for this position are calculated based on Indiana University standards at 20.08 percent.

TBN	**Programmer**	**100**	**Calendar**

The Programmer, TBN, will work entirely on software implementation, support and upgrading of the CPM code and will also assist with specific model development to correspond with BMP priorities. The programmer will work closely with faculty investigators, other staff, postdoctoral researchers and graduate. Qualifications for the programmer will include C++ and scientific programming experience, user interface design experience and entry level management experience. Note compared to the Core 1 staff, the Core 2 programming will often be experimental and explore new modeling techniques. The requested Core 1 staffing will take the base Core 2 software and build into deployable tools.

We expect an M.A. or Ph.D. in Computer Science, Informatics or a Computational Quantitative Science. The programmer will work and receive training in a wide variety of computational biology techniques. We expect that his experience in this position will make him or her very attractive for both industry and academic scientific programming positions. In addition we expect that we will be able to support this position beyond the

termination of this grant using Biocomplexity Institute funds from other sources.

The salary of $50,000 is based on standard rates current in the Department of Computer Science and the School of Informatics. Fringe benefits are calculated based on the Indiana University rate for support staff of 32.27%.

TBN – 3 Postdoctoral Fellows 100 Calendar

The salary rate is based on current salary levels in the Departments of Physics and Biology at IUB and may be increased with partial funding from the Biocomplexity Institute. The extra postdoctoral fellow funded the first year will work to bootstrap full development and deployment of the CPM code. The salary for that postdoc will come from Biocomplexity Institute funds in year two. For the remaining period, one postdoctoral fellow will have responsibility for additional algorithm design for the CPM, the other will work primarily on developing specific biological models and their implementations for the BMP related simulations. We expect that each postdoctoral fellow will serve a two or three year term and receive both scientific and technical training suitable for either an academic or industrial careers. They will be expected to write and publish their research and present it at scientific conferences. They may also participate in grant writing for their projects. We expect a Ph.D. in Computer Science, Informatics, a Computational Quantitative Science or Quantitative or Computational Biology.

Three postdoctoral fellows ($30,000 annual compensation for each) will be involved in this project in year 1 to jumpstart the efforts. In subsequent years we will reduce the number of postdocs to two. Fringe benefits for these positions are calculated according to Indiana University standards at 32.27 percent of base salary.

TBN – 7 Graduate Research Assistants 100 Calendar

The graduate students working with Dr. Glazier will include two graduate students focusing on algorithm and CPM development and one on the development of detailed limb regeneration simulations. The additional students in years four and five will work on initial development of the detailed biological simulations of the BMPs to be instituted in year four. They will be supported by Biocomplexity Institute funds after the termination of this grant. The expected time to Ph.D. completion is five years for each graduate student. Graduate students may enroll through any department in the college of science.

The students in the first two years are expected to enroll through either the existing disciplinary programs or the new IUB Biophysics syllabus. Students enrolling in year three or after may also enroll through the planned Scientific Computing Ph.D. program. All students will learn fundamentals of Mathematical and Computational Biology, Experimental Biology, Informatics and Computer Science and will conduct rotations in experimental laboratories. Thus their training will be an example of the interdisciplinary approach we present in Core 5. Students will also write and publish articles on their research and present at USA and international scientific meetings and attend USA and international interdisciplinary biology training courses. Our goal is to provide the trained biomedical workforce outlined in the NIH roadmap.

Seven graduate students will be involved in this project in years 1-3. In years 4 and 5 we plan to increase the number of students to nine. Annual base salaries for graduate students vary depending on their affiliation – two graduate students will be hired through Indiana University School of Informatics and will be paid $13,000; one student will work for Indiana University Engineering School and will be paid $19,200; remaining students will be hired through College of Arts and Sciences (COAS) and their compensation is budgeted at $18,000. Fringe benefits for graduate students include annual health fee (currently $957 for students hired in Bloomington, and $735 for students in Indianapolis).

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
None.

SUPPLIES:
We request 4,500 in year 1 to cover other costs, which include photocopying, office supplies, small computer equipment, publication and page charge expenses and other standard computational laboratory expenses.

TRAVEL:
We request $16,500 annually to finance travel between sites as well as support for travel to appropriate workshops and conferneces for students, postdocs and staff.

OTHER EXPENSES:
We request $70,100 in year 1 to cover fee remissions for seven graduate students that will perform research associated with this portion of the project. Amout of fee remissions varies depending on student's affiliation. Two students hired through Indiana University School of Informatics require full fee remission at $19,622 each annually; fee remission for School of Engineering are $7,500 annually; fees for College of Arts and Sciences students are $5,839. Years 2-5 are increased by 3 percent to adjust for inflation and cost-of-living increases. In addition, we plan to hire two additional graduate students in years 4 and 5. Difference between out-of-state ($19,622 per student) and in-state ($5,839 per student) tuition for four COAS students will be paid by the College of Arts and Sciences (total cost share in year 1 $55,132; total cost share for all years $353,851). Additionally, we request $14,000 in year 1 for purchase of non-capital equipment and $5,000 annually to cover publication expenses.

CONSORTIUM/CONTRACTUAL COSTS:
The University of Notre Dame subcontractor will receive $108,000 for support of three postdoctoral fellows and $60,000 for support of three graduate student annually. Additional $2,000 per year will be used to finance travel between sites and attendance of relevant simposia. Indirect costs are calculated at 50 percent of total modified direct costs. Please see Notre Dame subcontract justifications for detailed information.

Purdue University subcontractor will receive $13,442 in year 1 to cover Dr. Ramkrishna's effort. Indirect costs are calculated at 52 percent of total modified direct costs. Please see Purdue University subcontract justifications for detailed information.

Core 3: Biologically Motivating Projects - total

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*		TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
BMP1-see detailed budg.	Research				139,246	44,936	184,182
BMP2-see detailed budg.	Research				60,513	18,917	79,430
BMP3-see detailed budg.	Research				151,146	43,515	194,661
Mathew Grow	Investigator	12	10	68,289	6,829	2,204	9,033
Programmer (TBN)	Programmer	12	50	41,200	20,600	6,648	27,248
Graduate Student (TBN)	Res. Assist.	12	100	22,750	22,750	957	23,707
SUBTOTALS →					401,084	117,177	518,261

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)* BMP1 - $58,995 BMP2 - $70,192 BMP3 - $25,339 Other - $10,200		164,726
TRAVEL BMP1-$4,000		4,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* Graduate Studetn Fee Remission - $5,839 BMP2 - $14,068 Other - $500		20,407
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 707,394
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	116,841
	FACILITIES AND ADMINISTRATIVE COSTS	50,077
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 874,312

SBIR/STTR Only: FEE REQUESTED

Core 3: Biologically Motivating Projects - total

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		518,261	527,968	537,967	528,201	543,070
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		164,726	154,352	144,528	0	0
TRAVEL		4,000	4,120	3,380	0	0
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		20,407	21,019	21,650	0	0
SUBTOTAL DIRECT COSTS		707,394	707,459	707,525	528,201	543,070
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	116,841	120,255	123,782	73,635	75,328
	F&A	50,077	51,532	53,036	38,290	39,171
TOTAL DIRECT COSTS		874,312	879,246	884,343	640,126	657,569

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 3,935,595***

SBIR/STTR Only Fee Requested				

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $3,703,490.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 3: Biologically Motivating Projects

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment

BMP 1 **Research**

Please see detailed justification for BMP1.

BMP 2 **Research**

Please see detailed justification for BMP2.

BMP 2 **Research**

Please see detailed justification for BMP3.

Future BMPs **Research**

Salaries and fringe benefits for Biologically Motivating Projects in years 4 and 5 are estimated based on year 3 averages from BMP1, BMP2 and BMP3. Detailed budgets are not provided per RFA instructions.

Mathew Grow **Investigator** **10** **Calendar**

Dr. Matthew Grow, assistant director of the Center for Medical Genomics (CMG) will serve as an advisor for interpreting transcriptional data, and as a "gene expression liaison" both internally between project participants and externally between the Biocomplexity Institute and other centers in the integration of gene expression tools. We request salary reimbursement for 10 percent effort by Dr. Grow. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN **Programmer** **100** **Calendar**

An analyst with programming experience (including Java) and a background in biology will provide assistance both in gene expression data analysis and the integration of outside gene expression tools with this proposal's modeling tools This person will be trained on CMG software as a user and developer, will assist with expression analysis, and will provide assistance to the Center for Tissue Simulation in the form of user training, advice on the user interface, and guidance on software integration. Plus, working at the CMG, they can assist Center members with expression data interpretation (if and when that is needed). Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN **Graduate Research Assistant** **100** **Calendar**

A graduate research assistant will conduct the experiments described in the gradient cell assay section. Graduate student support is requested for three years. We request support for one graduate student (for years 1-3). Base salary is $22,750; fringe benefits include annual health insurance fee, currently $957.

All salaries and fringe benefits are adjusted annually by 3 percent (with exception of BMP3 – no increase required).

EQUIPMENT:
None.

SUPPLIES:
Please see justifications for each BMP for detailed information about supplies necessary for the projects. In addition, funds are requested each year for materials and supplies to support the proposed gradient cell assay experiments. Fabrication projects as proposed tend to be intensive with regards to materials and supplies costs. Microfabrication substrates, photomasks, photoresists, developers, and etchants are requested in addition to chemical and biochemical reagents related to the gradient cell assays. A computer controlled flow control system will be developed to operate the microfluidic chamber, and components to build this system are included in the supplies. Optical supplies such as filters, objectives, and positioners are also included to modify existing fluorescence microscopes to conduct the requisite assays. Supplies are requested for three years.

TRAVEL:
Please see justifications for BMP1 for detailed information.

OTHER EXPENSES:
We request $5,839 in year 1 to cover fee remissions for one graduate student that will perform research associated with this portion of the project. Difference between out-of-state ($19,622 per student) and in-state ($5,839 per student) tuition will be paid by the College of Arts and Sciences (total cost share in year 1 $13,783; total cost share for all years $42,601). Additionally, we request $14,068 in year 1 for Wells Center Research Core Use Fee (see BMP2 for details) and $500 for miscelaneous expenses (such as telecommunications costs, and personal computer expenses, e.g., basic software and internet connection fees). These other expenses are requested for three years.

CONSORTIUM/CONTRACTUAL COSTS:
Purdue University subcontractor will receive $67,622 in year 1 to reimburse efforts by Dr. Robinson and one postdoctoral fellow. Indirect costs are calculated at 52 percent of total modified direct costs. Please see Purdue University subcontract justifications for detailed information.

University of Kansas Medical Center subcontractor will receive $31,732 in year 1 to reimburse efforts by Dr. Little, Dr. Czirok and Dr. Rongish. Indirect costs are calculated at 47 percent of total modified direct costs. Please see University of Kansas Medical Center subcontract justifications for detailed information (also listed under BMP1).

University Medical Diagnostics Associates (UMDA) will receive $5,495 in year 1 to reimburse efforts by Dr. March. Please see UMDA subcontract justifications for detailed information (also listed under BMP2).

University Pediatrics Associates (UPA) will receive $11,992 in year 1 to reimburse efforts by Dr. Yoder, Dr. Conway and Dr. Firulli. Please see UPA subcontract justifications for detailed information (also listed under BMP1 and BMP2).

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Loren Field	Principal Investigator	12	5	141,110	7,056	2,277	9,333
Anthony Firulli	Investigator	12	5	61,800	3,090	997	4,087
Simon Conway	Investigator	12	5	60,000	3,000	968	3,968
Weinian Shou	Investigator	12	5	72,000	3,600	1,162	4,762
Postdoctoral Fellows (3.5)	Postdoctoral	12	100	35,000	122,500	39,532	162,032
SUBTOTALS →					139,246	44,936	184,182

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		70,192
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* Wells Center Research Core Use Fee - $14,068		14,068
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 268,442
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	6,558
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 275,000

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		184,182	189,707	195,398	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		70,192	64,048	57,719	0	0
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		14,068	14,490	14,925	0	0
SUBTOTAL DIRECT COSTS		268,442	268,245	268,042	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	6,558	6,755	6,958	0	0
	F&A	0	0	0	0	0
TOTAL DIRECT COSTS		275,000	275,000	275,000	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 825,000**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 3: Biologically Motivating Projects – BMP1

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Loren Field	**Principal Investigator – BMP1**	**5**	**Calendar**

Dr. Field will devote 5 percent effort to this project. He will be involved in the direct supervision of the other personnel in the project, and is responsible for the design of the experimental approaches for studies examining clonal cardiomyocyte proliferation in the ventricular wall. He is also responsible for the identification and screening of the transgenic animal models that will be generated in this application. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Anthony Firulli	**Investigator**	**5**	**Calendar**

Dr. Firulli will devote 5 percent effort to this project. He will be responsible for the design of the experimental approaches and involved in the direct supervision of studies aimed at deducing additive vs. synergistic effects of cardiac transcription factors on heart development. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Simon Conway	**Investigator**	**5**	**Calendar**

Dr. Conway will devote 5 percent effort to this project. He will be responsible for the design of the experimental approaches and involved in the direct supervision of studies focused on modeling the development and stem-cell based repair of cardiac valves. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Weinian Shou	**Investigator**	**5**	**Calendar**

Dr. Shou will devote 5 percent effort to this project. He will be responsible for the design of the experimental approaches and involved in the direct supervision of studies aimed at modeling the molecular and cellular regulation of cardiac ventricular trabeculation and compaction. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN – 3.5	**Postdoctoral Fellows**	**100**	**Calendar**

Mark Soonpaa, Ph.D. (Postdoctoral Fellow). We request funds for 100% salary for year 01. Dr. Soonpaa will be responsible for the embryo culture and imaging experiments for the studies examining clonal cardiomyocyte proliferation in the ventricular wall (Aim 1). Dr Soonpaa is well experienced in these techniques and his participation is required for successful completion of the study.

TBN Postdoctoral Fellow. We request funds for 100 percent salary for a postdoctoral fellow to be appointed for year 01. This individual (Ph.D., M.D. or M.D./ Ph.D.) will be responsible for the embryo culture, in situ hybridizations and imaging experiments for the studies deducing additive vs. synergistic effects of cardiac transcription factors on heart development proposed in Aim 2. Hiring a qualified postdoctoral fellow is absolutely required for successful completion of the study.

Manabu Maeda, M.D. (Postdoctoral Fellow). We request funds for 50% salary for year 01. Dr. Maeda will be responsible for the analyzing cell cycle and apoptosis rates in neonatal and adult cardiac valves, in the

absence and presence of exogenous stem cells (Aim 3). Dr Maeda is highly experienced in the requisite histologic techniques and his participation is needed for the successful completion of the study.

Hanying Chen, M.D. (Postdoctoral Fellow). We request funds for 100 percent salary for year 01. Dr. Chen will be responsible for the embryo culture and imaging experiments for the studies aimed at modeling the molecular and cellular regulation of cardiac ventricular trabeculation and compaction (Aim 4). Dr Chen is well experience in these techniques and his participation is required for successful completion of the study.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
None.

SUPPLIES:
We request $70,192 for supplies in year 1 as indicated below.

Animal per diems ($36,405): The proposed experiments all utilize existing transgenice mouse resources. Based on current operations, we anticipate the need to maintain a steady state census of 300 mice to perform the proposed experiments (consisting of MHC-EGFP, MHC-cycD1 and p57 -/- mice for aim 1, Nkx2.5 -/- and HAND2 -/- mice for aim 2, ACT-EGFP and control non-transgenic mice for aim 3, and Ncx-RFP, Tie2-GFP, BMP-10 -/-, FKBP12 -/-, Tie2 -/- and endoglin -/- mice for aim 4, which will be used at various stages of the project). These funds will enable us to generate the requisite embryonic, neonatal and adult hearts for the proposed experiments. The Indiana University School of Medicine Animal Facility in Indianapolis per diem charges are $0.37/mouse/day. The yearly costs for maintaining a colony of 300 mice is $0.37 x 365 days x 300 mice or $47,268 per year. Please note that the funds needed to maintain breeding stocks are covered by the respective primary grants for these models.

Cell culture reagents ($9,000): Fetal calf serum and other media components (pipettes, antibiotics, base media, plastic and glassware) are required for the organ culture experiments proposed in Aims 1, 2 and 4.

Histologic Reagents ($10,000): Reagents for monitoring cell cycle progression (tritiated thymidine, BrdU, PCNA antibodies, etc) and apoptosis (ISEL detection kits, anti-activated caspase 3 antibodies, BAX antibodies, etc) are required. 35S-rNTPs and didgoxin-labelign reagents are required for gene expression analyses. Photographic emulsion is required to develop autoradiographic based assays.

PCR reagents ($4,787): Taq polymerase, nucleotides, oligonucleotide primers, and reverse transcriptase are required for genotyping experimental animals and for RT-PCR based gene expression quantiation.

Instrument time for confocal and two-photon molecular excitation analyses ($10,000). The proposed experiments will use a good deal of microscopy time at the Indiana Center for Biological Microscopy. All of the investigators in this applications are members if the Indiana University Cancer Center, and as such are eligible for the discount user flat rate of $20/hr microscopy time. We anticipate an average usage of approximately 10 hours per week, or 500 hours per year.

Indiana University School of Medicine has committed an additional $25,000 per year in support for this project (please see letter of commitment from Dr. Ora Hirsch Pescovitz, Executive Associate Dean for Research Affairs).

TRAVEL:

Given the close proximity between the laboratories in Indianapolis and Bloomington, no funds are required to maintain an integrated working relationship.

OTHER EXPENSES:

Wells Center Research Core Use Fee: The Wells Center for Pediatric Research maintains shared core facilities for the use of all member investigators. Centralization of these facilities permits superior management control and operating efficiency, and enables certain economies of scale to be achieved in the purchase of supporting supplies and services. A portion of the costs related to the use and upkeep of these facilities is paid by institutional funds; however, the remainder is charged to member investigators' grants through an approved cost system. Since tracking the actual usage of these shared facilities by project is not possible, the costs are allocated to each project based on the number of FTE's working on the project. The per FTE cost for the current fiscal year is $3,517.00. The rate is adjusted annually using actual costs from the prior year as a basis. The requested $14,068 for Facility Maintenance covers the 4 laboratory FTE's budgeted for this grant.

CONSORTIUM/CONTRACTUAL COSTS:

University Pediatrics Associates (UPA) will receive $6,558 in year 1 to reimburse efforts by Dr. Firulli and Dr. Conway. Dr. Conway and Dr. Firulli receive compensation from two sources as faculty members with the Pediatrics Department of Indiana University School of Medicine. The first source is from the Indiana University Pediatrics Department, as represented in the budget under Personnel. The second source is from a third-party, non-profit corporation, University Pediatric Associates, whose mission is to support the instruction, research, and public service missions of Indiana University. This portion of salary compensation is represented in the budget under Consortium/Contractual Costs as a subcontract with UPA. It is certified that the two sources of income comprise a single compensation package for these faculty members. Please see UPA subcontract justifications for detailed information.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Keith L. March	Principal Investigator	12	20	49,690	9,938	3,207	13,145
Mervin C. Yoder	Investigator	12	5	72,460	3,623	1,169	4,792
Brian H. Johnstone		12	20	62,115	12,423	4,009	16,432
Jingling Li		12	40	41,030	16,412	5,296	21,708
Postdoc. Fellow (TBN)		12	50	36,234	18,117	5,236	23,353
SUBTOTALS →					60,513	18,917	79,430

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)* Animal Purchases - $10,000 LARC - $10,000 Lab supplies - $18,995 Cell Culture supplies - $20,000		58,995
TRAVEL		4,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 142,425
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	42,661
	FACILITIES AND ADMINISTRATIVE COSTS	14,914
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 200,000

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		79,430	81,813	84,267	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		58,995	54,765	51,270	0	0
TRAVEL		4,000	4,120	3,380	0	0
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		142,425	140,698	138,917	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	42,661	43,941	45,260	0	0
	F&A	14,914	15,361	15,823	0	0
TOTAL DIRECT COSTS		200,000	200,000	200,000	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 600,000***

SBIR/STTR Only Fee Requested				

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $553,902.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 3: Biologically Motivating Projects – BMP2

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Keith L. March	**Principal Investigator – BMP2**	**20**	**Calendar**

Dr. Keith L. March will oversee all portions of the Core 3 project with respect to the design, execution, and interpretation of experiments, as well as interactions with collaborating investigators and manuscript preparation involving the synthesis of molecular, structural, and biophysical data.

Dr. March is applying as part of a 5/8ths joint appointment specified by a formal Memorandum of Understanding between Indiana University and the Richard Roudebush VA Medical Center. Note that the "Effort on Project" listed on the budget page is actually the percentage of appointment, per the NIH guide Vol. 18, No. 27. It is certified that there is no possibility of dual compensation for the same work, or of an actual or apparent conflict of interest regarding such work. Details of the joint appointment follow:

	IU Title: Assoc Prof of Medicine		*VA Title: Staff Physician*
	University %	VA %	Total %
Clinical	0.00	15.38	15.38
Admin	1.54	1.54	3.08
Teaching	6.15	3.08	9.23
Research	53.85	18.46	72.31
TOTAL	61.54	38.46	100.00

Mervin C. Yoder	**Co-PI – BMP2**	**5**	**Calendar**

Dr. Yoder will provide expertise in isolation of endothelial progenitor cell subtypes from murine yolk sac, and evaluating their stage of differentiation prior to injection and after transplant. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Brian H. Johnstone	**20**	**Calendar**

Dr. Johnstone will provide expertise in isolation and characterization of adipose stromal cells, and evaluation of their differentiation status before injection and after transplant harvest. He will also assist in the overall data collection and management, statistical analysis, data interpretation and manuscript writing. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Jingling Li	**40**	**Calendar**

Jingling Li will be responsible for performing the isolation and flow cytometry characterization of the ASCs. She will also work closely with Drs. March and Little in evaluating the fates of cells injected into the chimeric models, using molecular and histology techniques. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN **50** **Calendar**

The TBN Research Technician will work closely with Dr. Yoder and be responsible for cell isolation, immunostaining, sorting, analysis and transplantation of endothelial progenitor cells derived from murine yolk sac. This technician will also work in concert with Drs. Little and Rongish on the murine allantoids injections and evaluations. Fringe benefits for this position are calculated based on Indiana University standards at 28.90 percent.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
None.

SUPPLIES:
The following amoutns have been requested for supplies: animal Purchases - $10,000; LARC - $10,000; lab supplies - $18,995; and cell culture supplies - $20,000.

TRAVEL:
We request $4,000 in year 1 to finance travel between sites as well as support for travel to appropriate workshops and conferneces.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
University of Kansas Medical Center as a subcontractor will receive $31,732 in year 1 to reimburse efforts by Dr. Little, Dr. Czirok and Dr. Rongish. Indirect costs are calculated at 47 percent of total modified direct costs. Please see University of Kansas Medical Center subcontract justifications for detailed information.

University Medical Diagnostics Associates (UMDA) will receive $5,495 in year 1 to reimburse efforts by Dr. March. As a faculty member of the Department of Medicine, Dr. March receives compensation from two non-VA sources, Indiana University and University Medical Diagnostics Associates (UMDA). An equal percentage of each source is requested in this proposal, with the UMDA salary paid as a subcontract. This combined salary mechanism has been reviewed and approved by the Office of Secretary, Department of Health and Human Services. Please see UMDA subcontract justifications for detailed information (also listed under BMP1).

University Pediatrics Associates (UPA) will receive $5,434 in year 1 to reimburse efforts by Dr. Yoder. As a faculty member of the Department of Pediatrics, Dr. Yoder receives compensation from two non-VA sources, Indiana University and University Pediatrics Associates (UPA). An equal percentage of each source is requested in this proposal, with the UMDA salary paid as a subcontract. This combined salary mechanism has been reviewed and approved by the Office of the Secretary, Department of Health and Human Services. Please see UPA subcontract justifications for detailed information.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
David L. Stocum	Principal Investigator	3	100	132,758	43,146	8,664	51,810
Ellen A.G. Chernoff	Investigator	10	15		0	0	0
Simon Rhodes	Investigator	10	10		0	0	0
Postdoctoral Fellows (3)	Postdoctoral	12	100	36,000	108,000	34,851	142,851
SUBTOTALS →					151,146	43,515	194,661

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)* Animal Care - $12,000 Animals - $3,500 Antibodies - $5,000 Molecular biology supplies - $4,839		25,339
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 220,000
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 220,000

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		194,661	194,661	194,661	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		25,339	25,339	25,339	0	0
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		220,000	220,000	220,000	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		220,000	220,000	220,000	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 660,000**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 3: Biologically Motivating Projects – BMP3

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
David L. Stocum	**Principal Investigator – BMP3**	**100**	**Summer**

Dr. David L. Stocum is the lead investigator for the limb/fin regeneration project. This will be his primary area of effort. Summer salary is requested at 32.25% of base salary (13 weeks). Fringe benefits are requested at the standard amounts for the Indiana University system - 20.08 percent for summer salary.

Ellen A.G. Chernoff	**Investigator**	**15**	**Academic**

Dr. Chernoff will devote 15 percent of her effort towards this project. No salary reimbursement is requested.

Simon Rhodes	**Investigator**	**10**	**Academic**

Dr. Rhodes will devote 10 percent of her effort towards this project. No salary reimbursement is requested.

TBN – 3	**Postdoctoral Fellows**	**100**	**Calendar**

Three post-doctoral fellows are requested. One to work under the supervision of each of the following: Dr. Stocum, Dr. Chernoff and Dr. Marrs (Dr. Marrs will be involved in this project as a consultant). Salaries reflect NIH levels. Raises will be provided from other sources. Fringe benefits are requested at the standard amounts for the Indiana University system - 32.27 percent.

EQUIPMENT:
None.

SUPPLIES:
Fundign for supplies is requested for the following: animal care - $12,000, animals - $3,500, antibodies - $5,000 and molecular biology supplies - $4,839.

TRAVEL:
None.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

Core 4: Computer Support

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Craig Stewart	Co-PI	12	5	95,621	4,781	1,543	6,324
Daniel Lauer	Computer Syst. Admin.	12	100	56,100	56,100	18,103	74,203
Sr. Programmer	Programmer	12	100	75,000	75,000	24,203	99,203
SUBTOTALS →					135,881	43,849	179,730

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		10,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
Non-capital equipment - $7,000		7,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 196,730
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	97,766
	FACILITIES AND ADMINISTRATIVE COSTS	50,838
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 345,334

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		179,730	185,122	190,675	236,938	244,046
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL		10,000	10,000	10,000	10,000	10,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		7,000			7,000	
SUBTOTAL DIRECT COSTS		196,730	195,122	200,675	253,938	254,046
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	97,766	101,676	105,743	109,972	113,439
	F&A	50,838	52,872	54,986	57,185	58,988
TOTAL DIRECT COSTS		345,334	349,669	361,405	421,095	426,473

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 1,903,976***

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $1,629,107.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 4: Computer Support

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Craig Stewart	**Co-PI**	**5**	**Calendar**

Craig Stewart will perform overall supervision of the Core IV activities. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

Daniel Lauer	**Computer System Administrator**	**100**	**Calendar**

Mr. Lauer will be responsible for the installation, maintenance and debugging of software installed on Indiana University's supercomputer systems for use by the projects of the Tissue Modeling Center, and will work closely with computing staff at Purdue University to coordinate the installation and maintenance of software on Purdue's systems for this same purpose. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN	**Programmer**	**100**	**Calendar**

This person will install, maintain and debug new software created for this project. An additional programmer will be hired at 50 percent in years 4 and 5 to aid in final release of the software. Salary rate for this person is estimated from current rates for a similar position, adjusted for an annual 3 percent increase. Note that these positions cover support and the core 1 positions development of the software tools. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
We request $10,000 annually to support travel between sites as well as support for travel to appropriate workshops and conferneces.

OTHER EXPENSES:
We request $7,000 in years 1 and 4 for purchase of non-capital equipment (updates for workstations and software).

CONSORTIUM/CONTRACTUAL COSTS:
Purdue University subcontractor will receive $97,766 in year 1 to cover Mr. Moffett's effort. Indirect costs are calculated at 52 percent of total modified direct costs. Please see Purdue University subcontract justifications for detailed information.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 09/15/2004 THROUGH: 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Sima Setayeshgar	Lead Coordinator	10	10	63,000	0	0	0
Course Developer (1.5)	Course Development	12	100	30,000	45,000	14,522	59,522
SUBTOTALS →					45,000	14,522	59,522

CONSULTANT COSTS	
EQUIPMENT *(Itemize)* Videoconferencing equipment (one-time purchase) - $20,000	20,000
SUPPLIES *(Itemize by category)*	5,000
TRAVEL	10,000
PATIENT CARE COSTS — INPATIENT	
PATIENT CARE COSTS — OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Advertising and public relations - $20,000	20,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	**$** 114,522
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →	**$** 114,522

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		59,522	94,499	55,236	56,893	36,270
CONSULTANT COSTS						
EQUIPMENT		20,000	0	0	0	0
SUPPLIES		5,000	5,000	5,000	5,000	5,000
TRAVEL		10,000	15,000	20,000	20,000	20,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		20,000	10,000	10,000	0	0
SUBTOTAL DIRECT COSTS		114,522	124,499	90,236	81,893	61,270
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		114,522	124,499	90,236	81,893	61,270

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 472,420**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 5: Education and Training

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Sima Setayeshgar	**Lead Coordinator**	**10**	**Academic**

Dr. Setayeshgar will devote 10 percent of her time to the educational component of this project. No salary reimbursement is requested (the effort will be supported by the College or Arts and Sciences in the amount of $46,405 over 5 years).

TBN	**Course Developers**	**100**	**Calendar**

1.5 FTE will be devoted to course development in years 1 and 2. 1 FTE will continue through years 3 and 4; by year 5 all courses will be functional. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

The Course development assistants may be full time or part time. They will assist faculty in development of new courses, including transfer of course notes to PowerPoint, preparation of programs needed for courses, creation of course related web sites, preparation of all course related materials and texts and coordination and preparation of courses for sharing between institutions including adoption of courses developed elsewhere by IUB and transfer of IUB courses to other institutions.

These assistants will require both scientific and web experience and will be recruited either at the M.A. or Ph.D. level in a scientific discipline. Part time senior Ph.D. students in appropriate departments may also be suitable. Because course development will be most intense at the beginning of the project, when we will be developing and deploying a large number of courses, the number of FTE equivalents decreases gradually from 1 1/2 in years one and two to 1/2 in years three and four. We do not anticipate needing to deploy additional courses in year five.

TBN	**Administrative Coordinator**	**100**	**Calendar**

The Administrative coordinator will handle day to day secretarial duties associated with student admissions and teaching faculty support and will coordinate course development. Duties may also include support of textbook ordering, T.A. hiring and student interactions. The expected training level is a B.A. in business, or an appropriate humanities or social science area. This position will begin in year 2. Fringe benefits are calculated based on Indiana University standards at 32.27 percent.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:

We request $20,000 in year 1 for a one-time purchase of videoconferencing equipment. This equipment will be installed in classrooms at IUB and IUPUI to allow course sharing/distance learning between locations. It may also be used for coordination of meetings in this and other cores.

SUPPLIES:
We request $5,000 annually for miscellaneous supplies, such as software updates, teleconferencing fees and production of various training manuals and software documentation.

TRAVEL:
We request $10,000 in year 1 to support travel, primarily long-term graduate student exchange for training between institutions. This amount is increased in years 2 and 3 to reflect the growing number of senior graduate students within the project.

OTHER EXPENSES:
We request $20,000 in years 1 for advertising expenses. Advertising will include preparation and distribution of promotional materials for student recruiting and minority outreach recruiting and for education related publication costs. It will also include coordination and distribution of relevant course materials.

CONSORTIUM/CONTRACTUAL COSTS:
None.

COST SHARE:
$20,000 per year of the funds dedicated to the CTM by the Indiana University Office of the Vice President for Research will be allocated to this core to support inter-institutional travel and exchange costs and other educational expenses supporting collaborative research and education.

Core 6: Outreach

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*		TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Geoffrey Fox	Coordinator	12	0	214,200	0	0	0
Outreach Coordinator	Outreach	12	100	30,000	30,000	9,681	39,681
Graphic Web Designer	Web Designer	12	50	50,000	25,000	8,068	33,068
SUBTOTALS →					55,000	17,749	72,749

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* Advertising and public relations - $10,000 Annual workshop - $40,000 Training courses - $35,000 Minority outreach - $15,000		100,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 172,749
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	24,500
	FACILITIES AND ADMINISTRATIVE COSTS	12,250
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 209,499

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		72,749	74,931	77,179	79,494	81,879
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		100,000	100,000	100,000	100,000	100,000
SUBTOTAL DIRECT COSTS		172,749	174,931	177,179	179,494	181,879
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	24,500	24,500	24,500	24,500	24,500
	F&A	12,250	12,250	12,250	12,250	12,250
TOTAL DIRECT COSTS		209,499	211,681	213,929	216,244	218,629

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 1,069,982***

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $1,008,732.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 6: Outreach

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Geoffrey Fox	**Coordinator**	**0**	**Academic**

The outreach efforts described as Core VI of this proposal will be supervised by Geoffrey Fox. Dr. Fox is manager of the Community Grids Laboratory at Indiana University. His efforts will be reimbursed from his Core 1 salary.

TBN	**Outreach Coordinator**	**100**	**Calendar**

The Outreach Coordinator will supervise programs for students from minority-serving institutions, and will coordinate faculty efforts to reach the full range of the diversity of the future workforce. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN	**Graphic Web Designer**	**50**	**Calendar**

The Graphic Web Designer (TBN) will construct the web presence of this program, and should be experienced in user-centered, visually appealing design and expert in dynamic web page programming (should for example be familiar with Perl, Cold Fusion, Dreamweaver, and Photoshop). Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

The proposed salaries are based on current rates necessary to attract such expertise. All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

OTHER EXPENSES:
Advertising and public relations - $10,000; Annual workshop - $40,000; Training courses - $35,000; Minority outreach - $15,000.

CONSORTIUM/CONTRACTUAL COSTS:
The University of Notre Dame subcontractor will receive $24,500 to support one annual workshop, seminar series and short-term visitors. Indirect costs are calculated at 50 percent of total modified direct costs. Please see Notre Dame subcontract justifications for detailed information.

COST SHARE:

$40,000 of the funds allocated to the CTM by the Indiana University Office of the Vice President for Research will be devoted to collaborative outreach efforts for activities such as publicity, recruiting, seminars, and visitor support.

Core 7: Administration

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
James A. Glazier	Principal Investigator	10	25	128,400	0	0	0
Sr. Admin. Manager	Administ-ration	12	100	40,000	40,000	12,908	52,908
Sci. Project Manager.	Project Manager	12	100	50,000	50,000	16,135	66,135
SUBTOTALS →					90,000	29,043	119,043

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		5,000
TRAVEL		25,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* Advertising and public relations - $10,000		10,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 159,043
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	54,028
	FACILITIES AND ADMINISTRATIVE COSTS	27,185
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 240,256

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		119,043	122,614	126,293	130,082	133,984
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		5,000	5,000	5,000	5,000	4,500
TRAVEL		25,000	25,000	25,000	25,000	25,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		10,000	10,000	10,000	10,000	5,000
SUBTOTAL DIRECT COSTS		159,043	162,614	166,293	170,082	168,484
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	54,028	54,456	54,904	55,375	55,765
	F&A	27,185	27,408	27,640	27,885	28,088
TOTAL DIRECT COSTS		240,256	244,478	248,837	253,342	252,337

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 1,239,250**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

***** Total amount requested without indirect costs on subcontractual agreements is $1,101,044.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Core 7: Administration

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
James A. Glazier	**PI**	**25**	**Academic**

Prof. Glazier is PI on this project and is also director of the Biocomplexity Institute at IU which is coordinating the entire effort. Dr. Glazier will coordinate all model development and implementation and is responsible for directing faculty hiring and seeking outside funding to support this project. His academic year salary will allow him to devote a substantial fraction of his time to organizational issues associated with this project.

Dr. Glazier will devote 25 percent of his time towards management and coordinator of the center. No salary reimbursement is requested (the effort will be supported by the College of Arts and Sciences in the amount of $236,445 over 5 years).

TBN	**Senior Administrative Manager**	**100**	**Calendar**

The Senior Administrative Manager will oversee coordination and administration of the entire project including hiring, funding and grant compliance issues. The incumbent will also coordinate workshops and training courses in conjunction with the Outreach Coordinator.

The expected training level is a B.A. or M.A. in business, or appropriate humanities or social science area with extensive work experience in organizational management. We expect that experience in this position will make the incumbent very attractive for both industry and academic scientific management positions. In addition, we expect to support this position beyond termination of grant funding via the Biocomplexity Institute and funds from other sources. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

TBN	**Scientific Project Manager**	**100**	**Calendar**

The Scientific Project Manager will oversee coordination and administration of all scientific aspects of the entire project including funding and grant compliance issues. The incumbent will also coordinate educational and research issues between institutions.

The expected training level is an M.A. or Ph.D. in a scientific discipline with extensive work experience in scientific management. We expect experience in this position will be very attractive for both industry and academic scientific management. In addition, we expect to support this position beyond termination of grant funding via the Biocomplexity Institute and funds from other sources. Fringe benefits for this position are calculated based on Indiana University standards at 32.27 percent.

All salaries and fringe benefits are adjusted annually by 3 percent.

EQUIPMENT:

None.

SUPPLIES:

We request $5,000 for miscellaneous supplies. Supplies include general office and computer supplies and miscellaneous expenses related to day-to-day administration.

TRAVEL:

We request $25,000 annually to support travel. Travel includes support for meetings of the Executive and External Advisory Committees and for long and short term scientific visits. It will also support travel by the Senior Administrative Manager and Scientific Project Manager to attend workshops on grant and scientific management. Foreign travel represents our expectation that many visitors and External Advisory Committee members may be foreign nationals. We also expect that Dr. Glazier will attend a number of international conferences each year in conjunction with his duties as project director.

OTHER EXPENSES:

We request $10,000 for advertising and public relations expenses. The advertising and public relations budget will partially cover web costs for the Center, preparation, printing, and distribution of promotional materials designed to increase awareness of Center activities and uptake and use of Center programs.

CONSORTIUM/CONTRACTUAL COSTS:

The University of Notre Dame subcontractor will receive $45,480 as compensation to professional project manager. Indirect costs are calculated at 50 percent of total modified direct costs. Please see Notre Dame subcontract justifications for detailed information.

Purdue University subcontractor will receive $8,548 to reimburse effort by Dr. Davisson. Indirect costs are calculated at 52 percent of total modified direct costs. Please see Purdue University subcontract justifications for detailed information.

COST SHARE:

The Indiana University Bloomington College of Arts and Sciences (COAS) will provide the following support for the CTM: funding for one full-time faculty member plus associated startup costs, and $30,000 per year in discretionary funds. In addition, COAS allocates $250,000 per year to the Indiana Biocomplexity Institute, whose programs will be closely associated with the CTM. The Indiana University Commitment to Excellence Program also will contribute two million dollars in equipment and five faculty lines to the Biocomplexity Institute.

Notre Dame Subcontract – Cumulative Budget

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Core 1	Research				80,000	12,000	92,000
Core 2	Research				150,000	18,000	168,000
Core 6	Outreach				0	0	0
Core 7	Administ-ration				37,900	7,580	45,480
SUBTOTALS →					267,900	37,580	305,480

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	0
SUPPLIES *(Itemize by category)*	0
TRAVEL Core 1 - $8,000; Core 2 - $2,000	10,000
PATIENT CARE COSTS – INPATIENT	0
PATIENT CARE COSTS – OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)* Core 6: Workshop - $11,500 Seminar Series - $8,000 Support for short-term visitors - $5,000	24,500
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	**$** 339,980
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →	**$** 339,980

SBIR/STTR Only: FEE REQUESTED

Notre Dame Subcontract – Cumulative Budget

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		305,480	305,480	305,480	305,480	305,480
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL		10,000	10,000	10,000	10,000	10,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		24,500	24,500	24,500	24,500	24,500
SUBTOTAL DIRECT COSTS		339,980	339,980	339,980	339,980	339,980
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A**	169,990	169,990	169,990	169,990	169,990
TOTAL DIRECT COSTS		509,970	509,970	509,970	509,970	509,970

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 2,549,850**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

** F&A costs for Notre Dame subcontract (at 50% of direct costs). These values are included in the calculation of the total direct costs for the entire project but do not count towards the $3 million limit. Total amount requested for Notre Dame subcontract without indirect costs is $1,699,900.

Please see justifications for each core for detailed information.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Core 1	Principal Investigator						
Mark Alber	Coordinator	9	0		0	0	0
Albert Barabasi	Coordinator	9	0		0	0	0
Postdoctoral Fel. (2 TBN)	Res. Assoc.	12	100	30,000	60,000	12,000	72,000
Graduate Student (TBN)	Res. Assist.	12	100	20,000	20,000	0	20,000
SUBTOTALS →					80,000	12,000	92,000

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		8,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 100,000
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 100,000

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		92,000	92,000	92,000	92,000	92,000
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL		8,000	8,000	8,000	8,000	8,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		100,000	100,000	100,000	100,000	100,000
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		100,000	100,000	100,000	100,000	100,000

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 500,000**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Notre Dame Subcontract – Core 1: Computational Tools

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Mark Alber	**Coordinator**	**0**	**Academic**

Dr. Alber is a coordinator on this project on Notre Dame campus and is also a director of the Notre Dame Interdisciplinary Center for the Study Biocomplexity. Dr. Alber will coordinate development at Notre Dame of tools that will go into modeling specific submodels, in particular tissue modeling tools. He will be 1) leading efforts on extending CPM framework to include polarity; 2) application of the developed tools to apply them to limb bud development and limb regeneration; 3) building connections to other modules, specifically RK modules for the tools under coordination.

Albert-Laszlo Barabasi	**Investigator**	**0**	**Academic**

Dr. Barabasi will be leading the modeling efforts on theoretical aspects of Networks and their application to experimental biological subprojects. Under supervision of Dr. Alber and others, this will lead to developing API's for tissue modeling tools so as to allow interaction with network models in general.

TBN – 2	**Postdoctoral Fellows**	**100**	**Calendar**

Two postdoctoral fellows ($30,000 annual compensation for each) will be involved in this part of the project all through the project, one each with Dr. Alber and Dr. Barabasi. Fringe benefits for these positions are calculated according to University of Notre Dame standards at $6,000 for each fellowship. The salary rate is based on current salary levels in the Departments of Physics. In the first year they will work to bootstrap the various projects. They will have responsibility for implementing the basic tools for tissue modeling and their API with network models. In later years, they will carry out additional algorithm design and implementation of integrated framework. This will be followed by work required on developing specific biological models and their implementations for the BMP related simulations. We expect that each postdoctoral fellow will serve a two or three year term and receive both scientific and technical training suitable for either an academic or industrial careers. They will be expected to write and publish their research and present it at scientific conferences. They may also participate in grant writing for their projects. We expect a Ph.D. in Computer Science, Biophysics, Informatics, a Computational Quantitative Science or Quantitative or Computational Biology. We expect that each postdoctoral fellow will serve a two or three year term and receive both scientific and technical training suitable for either an academic or industrial careers. They will be expected to write and publish their research and present it at scientific conferences. They may also participate in grant writing for their projects. We expect a Ph.D. in Computer Science, Informatics, a Computational Quantitative Science or Quantitative or Computational Biology.

TBN	**Graduate Research Assistant**	**100**	**Calendar**

One graduate student will be involved in this project in years 1-5 with Dr. Alber. Annual base salary for one graduate student will be $20,000. The student is expected to enroll through the existing disciplinary programs with concentrations in mathematical biology, biophysics or bioengineering. Student will be appointed as a fellow of the ICSB and will receive a Certificate at the time of graduation. The student will learn fundamentals of Mathematical and Computational Biology, Experimental Biology, Informatics and Computer. Thus this training

will be an example of the interdisciplinary approach we present in Core 5. Student will also write and publish articles on his/her research and present at USA and international scientific meetings and attend USA and international interdisciplinary biology training courses. Our goal is to provide the trained biomedical workforce outlined in the NIH roadmap.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
We request $8,000 annually for 5 years to finance travel between sites as well as support for travel to appropriate workshops and conferneces.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Core 2	Principal Investigator						
Mark Alber	Coordinator	9	0		0	0	0
Albert Barabasi	Coordinator	9	5		0	0	0
Glen Niebur	Coordinator	9	5		0	0	0
Postdoctoral Fel. (3 TBN)	Res. Assoc.	12	100	30,000	90,000	18,000	108,000
Grad. Students (3 TBN)	Res. Assist.	12	100	20,000	60,000	0	60,000
SUBTOTALS →					150,000	18,000	168,000

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		2,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 170,000
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 170,000

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		168,000	168,000	168,000	168,000	168,000
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL		2,000	2,000	2,000	2,000	2,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		170,000	170,000	170,000	170,000	170,000
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		170,000	170,000	170,000	170,000	170,000

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 850,000**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Notre Dame Subcontract – Core 2: Modeling and Application

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Mark Alber	**Coordinator**	**10**	**Academic**

Prof. Alber is a coordinator on this project on Notre Dame campus and is also a director of the Notre Dame Interdisciplinary Center for the Study Biocomplexity (ICSB). Dr. Alber will coordinate all model development and implementation at the University of Notre Dame. He will be also leading efforts on extending of CPM simulation framework to include polarity; 2) extending of limb bud development model to limb regeneration; 3) developing appropriate tissue modeling tools as a part of the above effort; 4) building connections to RK modules in the CPM parts under coordination. While working on modeling efforts on regeneration he will be involved in development of sub-cellular modeling tools and integration of the CPM with our own and external sub-cellular modeling tools.

Laszlo Barabassi	**Investigator**	**5**	**Academic**

Dr. Barabasi's will be leading the modeling efforts on theoretical aspects of biological networks. In particular, he will focus on the development of methods which allow the calculation of fluxes and determination of switches in metabolic networks.. In addition, he will lead the development of algorithms which allow an assessment of reaction rates and substrates concentrations which will be used to establish connections to RK models.

Glen Niebur	**Investigator**	**5**	**Academic**

Dr. Niebur will be leading the modeling efforts on FEM implementation and Constitutive model development for Cancellous bone tissue including theoretical aspects and its implementation in codes, and their proposed interactions with other modules. He will also conduct experiments the data for which will be used in validating various tissue level models.

TBN – 3	**Postdoctoral Fellows**	**100**	**Calendar**

Three postdoctoral fellows ($30,000 annual compensation for each) will be involved in this project all through the project. Fringe benefits for these positions are $6,000 for each position calculated according to University of Notre Dame standards.

There will be one postdoctoral fellow each working for Alber, Niebur, and Barabassi.

The salary rate is based on current salary levels in the Departments of Physics, Maths and Engineering at. In the first year they will work to bootstrap the various projects. They will have responsibility for implementing the basics required for the execution of the models. In later years, they will carry out additional algorithm design for the CPM, Constitutive models for the bones and Networks respectively. This will be followed by work required on developing specific biological models and their implementations for the BMP related simulations. We expect that each postdoctoral fellow will serve a two or three year term and receive both scientific and technical training suitable for either an academic or industrial careers. They will be expected to write and publish their research and present it at scientific conferences. They may also participate in grant writing for their projects. We expect a Ph.D. in Computer Science, Informatics, a Computational Quantitative Science or Quantitative or Computational Biology.

TBN – 5 Graduate Research Assistants 100 Calendar

Five graduate students will be involved in this project in years 1-5. Annual base salary for each of three graduate students will be $20,000. Notre Dame as a part of its commitment will provide support in the amount of $50,000 for two additional students working on Core 2 annually.

The students will be involved with Barabasi (2) (Physics), Alber (2) (Mathematics and Physics) and Niebur (1) Aero Spave and Mechanical Engineering.

The graduate students working with Dr. Alber will focus on algorithm and code development for cell polarity and detailed limb regeneration simulations. He will work on initial development of the detailed biological simulations of the BMPs. He will be supported by ICSB funds after the termination of this grant.

The graduate student working with Dr. Niebur will focus on algorithm and FEM code development for Cancellous bone tissue constitutive models and the validation simulations. He will work on getting experimental data on tissue behavior. He will develop connection between CPM modeling approaches and FEM based continuum methods.

The graduate students working with Dr. Barabasi will focus on algorithm and code development for scale free networks and application to proposed biological problems. He will be supported by ICSB funds after the termination of this grant.

The expected time to Ph.D. completion is five years for each graduate student. The students are expected to enroll through the existing disciplinary programs with concentrations in mathematical biology, biophysics or bioengineering. Students will be appointed as fellows of the ICSB and will receive a Certificate at the time of graduation. All students will learn fundamentals of Mathematical and Computational Biology, Biophysics, Experimental Biology, Informatics and Computer Science and will conduct rotations in experimental laboratories. Thus their training will be an example of the interdisciplinary approach we present in Core 5. Students will also write and publish articles on their research and present at USA and international scientific meetings and attend USA and international interdisciplinary biology training courses. Our goal is to provide the trained biomedical workforce outlined in the NIH roadmap.

EQUIPMENT:

University of Notre Dame commits $70,000 first year in capital eqipment category. This funds will be used for setting up computational biology cluster which will consit of 64 nodes Beowulf cluster and will provide the much neede high end computing platform specifically devoted to Computational Biology projects on Notre Dame campus. These nodes will be added to the existing BoB cluster at Notre Dame, and the administration will be embedded in the Science Computing Facility structures, providing the experience and personnel already in place to make the proposed cluster functional quickly. This will also facilitate the training required for parallelizing the codes, and their efficient execution on the cluster. 25% of the cluster time will be reserved for outreach activities.

SUPPLIES:

None.

TRAVEL:

We request $20,000 for 5 years to finance travel between sites as well as support for travel to appropriate workshops and conferneces.

Notre Dame Subcontract – Core 6: Outreach

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Core 6	Principal Investigator						
Mark Alber	Coordinator	9	0		0	0	0
Holly Goodson	Coordinator	9	5		0	0	0
SUBTOTALS →					0	0	0

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* One workshop - $11,500 Seminar Series - $8,000 Support for short-term visitors - $5,000		24,500
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 24,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 24,500

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*						
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		24,500	24,500	24,500	24,500	24,500
SUBTOTAL DIRECT COSTS		24,500	24,500	24,500	24,500	24,500
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		24,500	24,500	24,500	24,500	24,500

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 122,500**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Notre Dame Subcontract – Core 6: Outreach

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Mark Alber	**Coordinator**	**0**	**Academic**

Prof. Alber is a coordinator on this project on Notre Dame campus and is also a director of the Notre Dame Interdisciplinary Center for the Study Biocomplexity. Dr. Alber will coordinate organization of a workshop On Notre Dame campus. 5 such workshop have been already held. He will be also coordinating Computational Biology Seminar and short-term visitors program

Holly Goodson	**Investigator**	**5**	**Academic**

Dr. Goodsoni is an associated director of the Notre Dame Interdisciplinary Center for the Study Biocomplexity. She will be assisting Dr. Alber in organizing all outreach activities of the Center on Notre Dame campus

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

OTHER EXPENSES:

One Workshop	$11,500
Seminar Series	$ 8,000
Support for short-term visitors	$ 5,000

CONSORTIUM/CONTRACTUAL COSTS:
None.

Notre Dame Subcontract – Core 7: Administration

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 09/15/2004	THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*		TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Core 7	Principal Investigator						
Mark Alber	Coordinator	9	0		0	0	0
Project Manager	Administr.	12	100	37,900	37,900	7,580	45,480
SUBTOTALS →					37,900	7,580	45,480

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 45,480
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 45,480

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		45,480	45,480	45,480	45,480	45,480
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		45,480	45,480	45,480	45,480	45,480
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		45,480	45,480	45,480	45,480	45,480

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 227,400**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Notre Dame Subcontract – Core 7: Administration

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Mark Alber	**Coordinator**	**0**	**Academic**

Prof. Alber will initiate a search for a Project Manager and will supervise his/her activities.

Holly Goodson	**Co-coordinator**	**0**	**Academic**

Dr. Goodson will assist Dr. Alber.

TBN	**Project Manager**	**100**	**Calendar**

The Project Manager, TBN, will manage the entire development of software implementation over its life cycle. This will include requirements gathering, design, and development activities, task assignment and supervision for Tissue Modeling Tools and support and upgrading of the existing CPM code. He will also assist with specific model development to correspond with BMP priorities. He will work closely with faculty investigators, other staff, postdoctoral researchers and graduate students. He will present results at relevant conferences. He may also participate in grant writing for appropriate projects. The salary of $37,900 with fringe benefits of $7,580 is based on a compromise between standard rates current in the Department of Computer Science and industry standards. Qualifications for the programmer will include C++ and scientific programming experience, user interface design experience.

We expect a Ph.D. in Engineering, Science, Computer Science, Informatics or a Computational Quantitative Science with experience in handling large scale software projects over geographically disbursed locations. He must have preferably worked on computational biology software, algorithms development, and must have a background in formal software development techniques as well as technical training in scientific/ engineering area. We expect that his/her experience in this position will make him/her very attractive for both industry and academic positions.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

COST SHARE:
Notre Dame has committed $50,000 per year toward funding for the CTM in addition to $70,000 startup costs. Current institutional funding for the Interdisciplinary Center for the Study of Biocomplexity, which is expected to partially support programs for the CTM due to the close association of their missions, is $100,000 per year.

Purdue Subcontract – Cumulative Budget

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Core 2	Research				7,775	2,667	10,442
Core 3	Research				41,866	16,756	58,622
Core 4	Computer Support				69,092	28,674	97,766
Core 7	Administ-ration				6,365	2,183	8,548
SUBTOTALS →					125,098	50,280	175,378

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*
Core 2 - $2,000
Core 3 - $8,000

10,000

TRAVEL
Core 2 - $1,000; Core 3 - $1,000 — 2,000

PATIENT CARE COSTS — INPATIENT

OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD — **$** 187,378

CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS

FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* → **$** 187,378

SBIR/STTR Only: FEE REQUESTED

Purdue Subcontract – Cumulative Budget

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		175,378	182,174	189,241	196,589	202,651
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		10,000	10,000	10,000	10,000	10,000
TRAVEL		2,000	2,000	2,000	2,000	2,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		187,378	194,174	201,241	208,589	214,651
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A**	97,437	100,970	104,645	108,466	111,619
TOTAL DIRECT COSTS		284,815	295,144	305,886	317,055	326,270

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 1,529,170**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

** F&A costs for Purdue subcontract (at 52% of direct costs). These values are included in the calculation of the total direct costs for the entire project but do not count towards the $3 million limit. Total amount requested for Purdue subcontract without indirect costs is $1,006,033.

Please see justifications for each core for detailed information.

Purdue Subcontract – Core 2: Modeling and Application

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Doraiswami Ramkrishna	Co-PI	12	5		7,775	2,667	10,442
SUBTOTALS →					7,775	2,667	10,442

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		2,000
TRAVEL		1,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 13,442
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 13,442

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		10,442	10,963	11,510	12,087	12,599
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		2,000	2,000	2,000	2,000	2,000
TRAVEL		1,000	1,000	1,000	1,000	1,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		13,442	13,963	14,510	15,087	15,599
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		13,442	13,963	14,510	15,087	15,599

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 72,601**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Purdue Subcontract – Core 2: Modeling and Application

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Doraiswami Ramkrishna	**Investigator**	**5**	**Calendar**

Dr. Ramkrishna has extensive expertise in reaction kinetics modeling of metabolic and regulatory networks. He will participate in the sub-cellular networks modeling projects in Cores 2 and 3 and will assist with evaluation of outside reaction kinetics modeling tools as well as in development of our own reaction kinetics models.

Dr. Ramkrishna and his postdoctoral associate will be responsible for modeling of sub-cellular processes and relate to the experimental group in Core 2. The 5 percent release time is for the PI to manage the project by interacting with (i) the post-doctoral fellow on a regular basis on a supervisory role, (ii) other PI's in the project, and (iii) to generate technical publications and presentations.

The University will cost share a post-doctoral associate, trained in reaction kinetics and applied mathematics, will be concerned with the following: (i) interact with the PI, (ii) interact with the reaction kinetics group to construct metabolic pathway in terms of elementary units, (iii) interact with the experimental group generating data on intra and extracellular variables and formulate pathway models, (iv) formulate identification problem (determination of model parameters) and simulate model, (v) interact (along with PI) with the extracellular modeling group for negotiating model simplification and link of intracellular variables to the extracellular ones.

Fringe benefits for this position are calculated based on University of Purdue standards at 34.30 percent. Subsequent years are increased by 4 percent.

EQUIPMENT:
None.

SUPPLIES:
We request $2,000 annually for laboratory supplies (S & E costs are mainly computational in nature).

TRAVEL:
We request $1,000 anually to enable the Dr. Ramkrishna and/or the postdoctoral associate to travel to meetings for presentation of the work.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

COST SHARE:
The value of the half-time postdoctoral assistant is $49,718 in the first year (including fringe benefits) plus 4% per year in subsequent years.

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
Joseph Paul Robinson	Co-PI	12	5		6,630	2,274	8,904
Postdoctoral Fel. (TBN)	Res. Assoc.	12	50		35,236	14,482	49,718
SUBTOTALS →					41,866	16,756	58,622

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		8,000
TRAVEL		1,000
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 67,622
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 67,622

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		58,622	60,559	62,564	64,635	66,328
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		8,000	8,000	8,000	8,000	8,000
TRAVEL		1,000	1,000	1,000	1,000	1,000
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		67,622	69,559	71,564	73,635	75,328
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		67,622	69,559	71,564	73,635	75,328

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 357,708**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Purdue Subcontract – Core 3: Biologically Motivating Projects, Support Technology

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Joseph P. Robinson		**5**	**Calendar**

Dr. Paul Robinson is the Deputy Director for Cytomics and Imaging Technology Core Facilities in the Bindley Bioscience Center at Purdue University. He will direct the work on the vascular development team involving the growth and analysis of progenitor endothelial cells in the extracellular matrix. His laboratory is also where the flow cytometry will be executed f. His laboratory will also help provide the informatics capabilities to serve this project in the context of developing a data set for computational modeling. His direction will be critical in assessing if the experimental content is appropriate and consistent with the overall computational modeling effort.

Fringe benefits for this position are calculated based on University of Purdue standards at 34.30 percent. Subsequent years are increased by 4 percent.

TBN	**Postdoctoral Fellow**	**50**	**Calendar**

A Postodoctoral Associate in the Cytomics and Imaging Technology Core Facilities in the Bindley Bioscience Center at Purdue University will be responsible for developing the growth conditions in the extracellular matrix to model capillary tube formation (vascular development team). This person will also be implementing the measurement systems for analyzing the cellular markers (flow cytometry) and the protein profiling efforts associated with the cellular signaling modeling efforts.

Fringe benefits for these positions are calculated based on University of Purdue standards at 40.50 percent. Subsequent years are increased by 4 percent.

EQUIPMENT:
None.

SUPPLIES:
We request $8,000 for costs associated with the experimental work in the extracellular matrix studies and the imaging data systems needed to support the computational modeling efforts in the proposed Center.

TRAVEL:
$1,000 annually is requested to support travel between collaborating institutions.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

Purdue Subcontract – Core 4: Computer Support

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator						
David P. Moffett	Coordinator	12	10		11,422	4,740	16,162
Computer Programmer	Programmer	12	100		57,670	23,934	81,604
SUBTOTALS →					69,092	28,674	97,766

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 97,766
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 97,766

SBIR/STTR Only: FEE REQUESTED

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		97,766	101,676	105,743	109,972	113,439
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		97,766	101,676	105,743	109,972	113,439
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		97,766	101,676	105,743	109,972	113,439

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 528,596**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Purdue Subcontract – Core 4: Computer Support

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
David Moffett	**Coordinator**	**10**	**Calendar**

Purdue University will provide computational support for the Center of Excellent in Tissue Modeling. In order to do this, in collaboration with Indiana University, we will provide network connectivity, PUNCH (an environment that web-enables tools that otherwise are not functional via the web), and a significant number of CPU hrs of the appropriate types to run the applications users start from PUNCH. Further, we will port & maintain applications in the PUNCH environment as need be over the course of the Center's existence.

David Moffett will supervise the programmer and assure that the availability of computer resources. The programmer will do the application porting and maintenance into the PUNCH environment.

TBN	**Programmer**	**100**	**Calendar**

The programmer will do the application porting and maintenance into the PUNCH environment.

Fringe benefits for these positions are calculated based on University of Purdue standards at 40.50 percent. Subsequent years are increased by 4 percent.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

Purdue Subcontract – Core 7: Administration

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Vincent Jo Davisson	Principal Investigator	12	5		6,365	2,183	8,548
SUBTOTALS →					6,365	2,183	8,548

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 8,548
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 8,548

SBIR/STTR Only: FEE REQUESTED

Purdue Subcontract – Core 7: Administration

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		8,548	8,976	9,424	9,895	10,285
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		8,548	8,976	9,424	9,895	10,285
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		8,548	8,976	9,424	9,895	10,285

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 47,128**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
Purdue Subcontract – Core 7: Administration

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
V. J. Davisson	**PI – Purdue Subcontract**	**5**	**Calendar**

Dr. V. Jo Davisson is the co-Director of the Bindley Bioscience Center and is responsible for the overall coordination and administration of the research activities at Purdue University that are associated with this grant. He will serve on the Executive Committee for the proposed Center and provide scientific direction on the Purdue campus to stimulate computational research activities. His effort will also be focused on developing additional experimental systems that will be suitable and important for the further development of the cellular signaling and metabolism reaction pathways modeling. As a Director in Discovery Park he is ideally suited for matching interested investigators with expertise at the interface of experiment and computational at Purdue with the further development of the proposed Center.

Fringe benefits for this position are calculated based on University of Purdue standards at 34.30 percent. Subsequent years are increased by 4 percent.

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

University of Kansas Medical Center Subcontract

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Charles Little	Principal Investigator	12	5	171,900	8,595	2,407	11,002
Andras Czirok	Investigator	12	10	69,568	6,757	1,892	8,649
Brenda Rongish	Investigator	12	10	94,375	9,438	2,643	12,081
SUBTOTALS →					24,790	6,942	31,732

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 31,732
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 31,732

SBIR/STTR Only: FEE REQUESTED

University of Kansas Medical Center Subcontract

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		31,732	32,684	33,665	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		31,732	32,684	33,665	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A**	14,914	15,361	15,823	0	0
TOTAL DIRECT COSTS		46,646	48,045	49,488	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 144,179**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

** F&A costs for University of Kansas Medical Center subcontract (at 47% of direct costs). These values are included in the calculation of the total direct costs for the entire project but do not count towards the $3 million limit. Total amount requested for University of Kansas Medical Center subcontract without indirect costs is $98,081.

Please see next page for detailed justification.

BUDGET JUSTIFICATION
University of Kansas Medical Center Subcontract (Core 3)

PERSONNEL:

Name	Role on Project	Percent Effort	Type of Appointment
Charles D. Little	Principal Investigator – Kansas Sub.	5	Calendar

Dr. Little is a Consortium Director and will provide expertise in embryology and vascular cell behavior. He will also be involved in the computational analysis of cellular behaviors observed using time-lapse imaging. Dr. Little will also oversee the work of Dr. Rongish on cell behavior in murine allantoides.

Andras Czirok	Investigator	10	Calendar

Dr. Czirok (Research Assistant Professor) will devote 10 percent of his effort to this project. He will design algorithms and implement software for the time-lapse imaging system; he will also work with Drs. Little and Rongish to perform computational analyses on cell motion observed in the dynamic imaging system.

Brenda J. Rongish	Investigator	10	Calendar

Dr. Rongish (Assistant Professor) will devote 10 percent of her effort to this project. She will perform embryo culture and microinjection of marker antibodies and cells into the avian embryo. She will also work with Drs. Czirok and Little to track cells over the recording period and compute their velocities. In addition, she will be responsible for preparing murine tissue explants (allantoides) for culture and time lapse recording to monitor cell behavior under the supervision of Dr. Little.

Fringe benefits for these positions are calculated at 19.647 percent of salary requested plus medical insurance ($4,147 times the percent effort charged to the grant).

EQUIPMENT:
None.

SUPPLIES:
None.

TRAVEL:
None.

OTHER EXPENSES:
None.

CONSORTIUM/CONTRACTUAL COSTS:
None.

University Medical Diagnostics Associates Subcontract

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: 09/15/2004 THROUGH: 09/14/2005

PERSONNEL *(Applicant organization only)*					DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Keith L. March	Investigator	12	20	27,080	5,416	79	5,495
SUBTOTALS →					5,416	79	5,495

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 5,495
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 5,495

SBIR/STTR Only: FEE REQUESTED

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		5,495	5,660	5,830	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		5,495	5,660	5,830	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		5,495	5,660	5,830	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 16,985**

SBIR/STTR Only Fee Requested

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Dr. Keith March receives compensation from two sources as faculty members with the Department of Medicine of Indiana University School of Medicine. The first source is from the Indiana University Department of Medicine, as represented in the budget under Personnel. The second source is from a third-party, non-profit corporation, University Medical Diagnostics Associates (UMDA). This portion of salary compensation is represented in the budget under Consortium/Contractual Costs as a subcontract with UMDA. It is certified that the two sources of income comprise a single compensation package for these faculty members. Fringe benefits associated with Dr. March's compensation from UMDA are calculated at 1.45 percent.

University Pediatrics Associates Subcontract

Principal Investigator/Program Director (Last, First, Middle): Glazier, James Alexander

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 09/15/2004 THROUGH 09/14/2005

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Mervin C. Yoder	Investigator	12	5	99,440	4,972	462	5,434
Anthony Firulli	Co-Investigator	12	8.3	27,000	2,250	209	2,459
Simon Conway	Co-Investigator	12	10	37,500	3,750	349	4,099
SUBTOTALS →					10,972	1,020	11,992

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 11,992
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		**$** 11,992

SBIR/STTR Only: FEE REQUESTED

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		11,992	12,352	12,723	0	0
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		11,992	12,352	12,723	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		11,992	12,352	12,723	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$ 37,067**

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Dr. Yoder, Dr. Conway and Dr. Firulli receive compensation from two sources as faculty members with the Pediatrics Department of Indiana University School of Medicine. The first source is from the Indiana University Pediatrics Department, as represented in the budget under Personnel. The second source is from a third-party, non-profit corporation, University Pediatric Associates (UPA), whose mission is to support the instruction, research, and public service missions of Indiana University. This portion of salary compensation is represented in the budget under Consortium/Contractual Costs as a subcontract with UPA. It is certified that the two sources of income comprise a single compensation package for these faculty members. Fringe benefits associated with compensation from UMDA are calculated at 9.30 percent.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
James Alexander Glazier	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Harvard College, Cambridge, MA (magna cum laude)	B.A.	1984	Physics and Mathematics
University of Chicago, Chicago, IL	M.A.	1987	Physics
University of Chicago, Chicago, IL	Ph.D.	1989	Soft Condensed Matter
Les Houches NATO Summer School, France		1986	Complexity Theory
AT&T Bell Laboratories, Murray Hill, NJ	Postdoctoral	1989-1991	Neuroscience
Research Institute of Electrical Communication, Tohoku University, Sendai, Japan	Postdoctoral	1991-1993	Neurobiology
Marine Biology Laboratory, Woods Hole, MA		1991	Neurobiology
National Institute of Genetics, Mishima, Japan		1992	Biology

A. Positions and Honors

1988 – 1989 Grainger Graduate Fellowship , Department of Physics, University of Chicago, Advisor, Prof. Albert Libchaber
1989 Visiting Fellow, Department of Physics, University of Western Australia, Perth, Australia
1989 – 1991 Postdoctoral researcher AT&T Bell Laboratories, Murray Hill, NJ, Advisor, Dr. David Tank
1989 – 1994 Consultant, Department of Genetic Epidemiology, University of Utah, Salt Lake City, UT
1991 Visitor, Department of Physics, Trinity College, Dublin, Ireland
1991 – 1993 JSPS/NSF Postdoctoral Fellowship, Research Institute of Electrical Communication, Tohoku University, Sendai, Japan
1992 – 1997 Assistant Professor, Department of Physics, University of Notre Dame, Notre Dame, IN
1997 – 2002 Associate Professor, Department of Physics, University of Notre Dame, Notre Dame, IN
1999 May-Nov. Visiting Full Professor, Research Institute of Electrical Communication, Tohoku University, Sendai, Japan
2000 Visiting Scientist, Laboratory of Physical Spectrometry, University of Grenoble, Grenoble, France
2001 – 2002 Director, Interdisciplinary Center for the Study of Biocomplexity, University of Notre Dame, Notre Dame, IN
2002 – present Professor, Department of Physics, Adjunct Professor, Department of Biology and School of Informatics, Indiana University, Bloomington, IN

B. Selected peer-reviewed publications

1. Belmonte, A. L., Vinson, M. J., Glazier, J. A., Gunaratne, G. H. and Kenny, B., "Trajectory scaling functions at the onset of chaos: experimental results," *Physical Review Letters* **61**, 539 (1988).
2. Stavans, J. and Glazier, J. A., "Soap froth revisited: dynamical scaling in the two-dimensional froth," *Physical Review Letters* **62**, 1318 (1989).
3. Glazier, J. A. and Libchaber, A., "Quasiperiodicity and dynamical systems: an experimentalists' view," *IEEE Transactions on Circuits and Systems* **35**, 790 (1988). Reprinted in Hao Bai-Lin, *Chaos II*, (World Scientific, 1990), 299.
4. Glazier, J. A., Anderson, M. P. and Grest, G. S., "Coarsening in the two-dimensional soap froth and the large-q potts model: a detailed comparison," *Philosophical Magazine B* **62**, 615 (1990).

5. Glazier, J. A. and Weaire, D., "The Kinetics of cellular patterns," *Journal of Physics: Condensed Matter* **4**, 1867 (1992).
6. Berthelsen, C., Glazier, J. A. and Skolnick, M. H., "Global fractal dimension of human dna sequences treated as pseudorandom walks," *Physical Review A* **45**, 8902-8913 (1992).
7. Graner, F. and Glazier, J. A., "Simulation of biological cell sorting using a two-dimensional extended potts model," *Physical Review Letters* **69**, 2013-2016 (1992).
8. Glazier, J. A. and Graner, F., "Simulation of the differential adhesion driven rearrangement of biological cells," *Physical Review E* **47**, 2128-2154 (1993).
9. Glazier, J. A., "Grain growth in three dimensions depends on grain topology," *Physical Review Letters* **70**, 2170-2173 (1993).
10. Gonatas, C. P., Yodh, A. G., Leigh, J. S., Glazier, J. A. and Prause, B., "Magnetic resonance images of coarsening inside a foam," *Physical Review Letters* **75**, 573-576 (1995).
11. Glazier, J. A., Raghavachari, S., Berthelsen. C. L. and Skolnick, M. H., "Reconstructing phylogeny from the multifractal spectrum of mitochondrial dna sequences," *Physical Review E* **51**, 2665-2668 (1995).
12. Raghavachari, S. and Glazier, J. A., "Spatially coherent states in fractally coupled map lattices," *Physical Review Letters* **74**, 3297-3300 (1995).
13. Mombach, J. C. M., Glazier, J. A., Raphael, R. C. and Zajac, M., "Quantitative comparison between differential adhesion models and cell sorting in the presence and absence of fluctuations," *Physical Review Letters* **75**, 2244-2247 (1995).
14. Prause, B., Glazier, J. A., Gravina, S. and Montemagno, C., "Magnetic resonance imaging of a three dimensional foam," *Journal of Physics: Condensed Matter* **7**, L511-L516 (1995).
15. Takeshita, T., Segawa, T., Glazier, J. A. and Sano, M., "Thermal turbulence in mercury," *Physical Review Letters* **76**, 1465-1468 (1996).
16. Mombach, J. C. M. and Glazier, J. A., "Single cell motion in aggregates of embryonic cells," *Physical Review Letters* **76**, 3032-3035 (1996).
17. Jiang, Y., Levine, H. and Glazier, J. A., "Possible cooperation of differential adhesion and chemotaxis in mound formation of dictyostelium," *Biophysical Journal* **75**, 2615-2625 (1998).
18. Jiang, Y., Swart, P. J., Saxena, A., Asipauskas, M. and Glazier, J. A., "Hysteresis and avalanches in two-dimensional foam rheology simulations," *Physical Review E* **59**, 5819-5832 (1999).
19. Raghavachari, S. and Glazier, J. A., "Waves in diffusively coupled bursting cells," *Physical Review Letters* **82**, 2991-2994 (1999).
20. Stott, E., Britton, N. F., Glazier, J. A. and Zajac, M., "Stochastic simulation of benign avascular tumor growth using the Potts Model," *Mathematical and Computer Modeling* **30**, 183-198 (1999).
21. Glazier, J. A., Segawa, T., Naert, A. and Sano, M., "Evidence against 'ultrahard' thermal turbulence at very high rayleigh numbers," *Nature* **398**, 307-310 (1999).
22. Rieu, J. P., Upadhyaya, A., Glazier, J. A., Ouchi, N. B. and Sawada, Y., "Diffusion and deformations of single hydra cells in cellular aggregates," *Biophysical Journal* **79**, 1903-1914 (2000).
23. Beysens, D., Forgacs, G. and Glazier, J. A., "Embryonic tissues are viscoelastic materials," *Canadian Journal of Physics* **78**, 243-251 (2000).
24. Beysens, D. A., Forgacs, G. and Glazier, J. A., "Cell sorting is analogous to phase ordering in fluids," *Proceedings of the National Academy of Sciences (USA)* **97**, 9467-9471 (2000).
25. Zajac, M., Jones, G. L. and Glazier, J. A., "Model of convergent extension in animal morphogenesis," *Physical Review Letters* **85**, 2022-2025 (2000).
26. Jiang, Y., Asipauskas, M., Glazier, J. A., Aubouy, M. and Graner, F., "*Ab Initio* derivation of stress and strain in fluid foams," in *Foams, Emulsions and their Applications*, P. Zitha, J. Banhart and G. Verbist editors (Verlag MIT Publishing, Bremen, Germany, 2000), 297-304.
27. Mombach, J. C. M., de Almeida, R. M. C., Thomas, G. L., Upadhyaya, A. and Glazier, J. A., "Bursts and cavity formation in hydra cell aggregates: experiments and simulations," *Physica A* **297**, 495-508 (2001).
28. Upadhyaya, A., Rieu, J. P., Glazier, J. A. and Sawada, Y., "Anomalous diffusion in two-dimensional hydra cell aggregates," *Physica A* **293** , 549-558 (2001).
29. Oiwa, N. and Glazier, J. A., "The Fractal structure of the mitochondrial genomes," *Physica A* **311**, 221-230 (2002).

C. Research Support

Current

NAG3-2366 (Glazier) 9/1/01-11/30/04
NASA Glenn Research Center/Microgravity Research Division
"Diffusive Coarsening of Liquid Foams in Microgravity"
The major goal of this project is to develop three-dimensional Magnetic Resonance Imaging techniques to conduct studies of the evolution of three-dimensional liquid foams in the absence of gravity.
Role: PI

IBN-0083653 (Glazier) 9/1/01-8/31/05
NSF/Division of Integrative Biology and Neuroscience
"Biocomplexity – Multiscale Simulation of Avian Limb Development"
The major goal of this project is to develop a comprehensive model of avian limb structural development based on physical processes including, cell adhesion, chemotaxis and haptotaxis models of cell migration, mitosis, and reaction-diffusion equation models of cell differentiation.
Role: PI

NRA 01-OBPR-06 (Forgacs) 3/01/03-2/28/06
NASA Fundamental Space Biology Program
"Morphogenesis in Microgravity"
The major goal of this project is to study the effects of simulated microgravity on embryonic development, which is particularly sensitive to changes in cell adhesion and migration.
Rode: Co-PI

Completed

DMR 00-89162 (Veretennikov) 09/01/02-12/31/03 (no cost extension to 11/04)
National Science Foundation (subcontract from University of Notre Dame)
"Effects of Local Interfacial and Flow Dynamics on Foam Drainage and Coarsening"
The major goal of this project is to study the effects of foam wetness, shape and size distribution and of liquid properties on foam flow through constrictions and past obstacles in a two-dimensional visualization cell.
Role: Co-PI

0089162 (Glazier) 01/01/01-12/31/02
NSF/Division of Materials Research
"Effects of Local Interfacial and Flow Dynamics on Foam Drainage and Coarsening"
The major goal of this project is to develop a theory for the rheology of foams under gravity, particularly a theory of the flow of liquid in thin surfactant stabilized films and to conduct experiments to validate this theory.
Role: PI

DE-FGO299ER45785 (Glazier) 10/1/99-9/30/02
Department of Energy
"Fingering Instabilities, Collapse, Avalanches and Self-Organized Criticality in Liquid Foams"
The major goal of this project is to study the rearrangements that occur during flows of two-dimensional liquid foams.
Role: PI

INT-98-02417-OC (Glazier) 5/01/98-4/30/01
National Science Foundation/CNPq
"U.S.-Brazil Cooperative Research: Cellular Patterns"
The major goal of this project was to conduct international collaborative research on biophysics, including experiments on chick embryo and hydra cell aggregates and to develop computer simulations of cell aggregation and bursting during hydra regeneration.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mark S. Alber	Professor of Mathematics, Concurrent Professor of Physics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Moscow Institute of Technology, Moscow, Russia	M.S. (Honors)	1983	Applied Mathematics
University of Pennsylvania, Philadelphia, PA	Ph.D.	1990	Mathematics

A. Positions and Honors

1990	Instructor, University of Pennsylvania, Philadelphia, PA
1990 – 1996	Assistant Professor, Department of Mathematics, University of Notre Dame, Notre Dame, IN
1993	Visiting Fellow, The Fields Institute for Research in Mathematical Sciences, Canada
1993 – 1994	Sabbatical at UC Berkeley, CA, Institute for Advanced Study, Princeton, NJ, and Mathematical Sciences Research Institute (MSRI), Berkeley, CA
1994	Visiting Scientist, CNLS, Los Alamos Natl. Lab., NM
1995, 1996	Visiting Fellow, Basic Research Institute in the Mathematical Sciences (BRIMS), Hewlett-Packard Research Lab., Bristol, UK
1996 – 2001	Tenured Associate Professor, Department of Mathematics, University of Notre Dame, Notre Dame, IN
1997 – 2000	Member of the Executive Committee of the Center for Applied Mathematics, University of Notre Dame, Notre Dame, IN
2000	Visiting Member, Institute for Mathematics and its Applications (IMA), University of Minnesota, Minneapolis, MN
2000 – 2001	Sabbatical at Stanford University, Palo Alto, CA
2001	Member of the NSF Panel
2001	Senior Fellow, Institute for Pure and Applied Mathematics (IPAM), UCLA, CA
2001 – present	Professor, Department of Mathematics, University of Notre Dame
2001 – 2002	Associate Director, Center for the Study of Biocomplexity, University of Notre Dame, Notre Dame, IN
2002 – present	Director, Center for the Study of Biocomplexity, University of Notre Dame, Notre Dame, IN

B. Selected peer-reviewed publications

Book:

Alber, M.S., Hu, B. and Rosenthal, J., editors, *Current and future directions in applied mathematics.* Papers from the international symposium held at the University of Notre Dame, Notre Dame, IN, April 1996, (BirkhŠuser Boston, Inc., Boston, MA, 1997) pp.261.

Special Issue of a Journal: Co-Editor-in-Chief, A Special Issue on Multiscale Modeling in Biology of a SIAM Journal of Multiscale Modeling and Simulation (to appear in 2004).

Papers:

1. Alber, M.S. and Alber, S.J., “Hamiltonian formalism for finite-zone solutions of integrable equations,” *C. R. Acad. Sci. Paris Math.* **301**, 777-781 (1985).
2. Alber, M.S. and Alber, S.J., “Hamiltonian formalism for nonlinear Schrodinger equations and sine-Gordon equations,” *Journal of the London Mathematical Society* **36**(2), 176-192 (1987).
3. Alber, M.S., “On integrable systems and semiclassical solutions of the stationary Schrodinger equations,” *Inverse Problems* **5**, 131-148 (1989).
4. Alber, M.S., “Hyperbolic Geometric Asymptotics,” *Asymptotic Analysis* **5**, 161-172 (1991).

5. Alber, M.S. and Marsden, J.E., "On Geometric Phases for Soliton Equations," *Communications in Mathematical Physics* **149**, 217-240 (1992).
6. Alber, M.S. and Marsden, J.E., "Resonant Geometric Phases for Soliton Equations," *Fields Institute Communications* **3**, 1-26 (1994).
7. Alber, M.S., Camassa, R., Holm, D.D. and Marsden, J.E., "The geometry of peaked solitons and billiard solutions of a class of integrable pde's," *Letters in Mathematical Physics* **32**, 137-151 (1994).
8. Alber, M.S., Camassa, R., Holm, D.D. and Marsden, J.E., "On Umbilic Geodesics and Soliton Solutions of Nonlinear PDE's," *Proceedings of the Royal Society London Ser. A* **450**, 677-692 (1995).
9. Alber, M.S., Luther, G.G. and Marsden, J.E., "Complex Billiard Hamiltonian Systems and Nonlinear Waves, Algebraic aspects of integrable systems," *Progr. Nonlinear Differential Equations and Applications* **26**, Birkhauser, Boston, MA., 1-16 (1997).
10. Alber, M.S., Luther, G.G. and Marsden, J.E., "Energy Dependent Schrodinger Operators and Complex Hamiltonian Systems on Riemann Surfaces," *Nonlinearity* **10**, 223-242 (1997).
11. Alber, M.S., Luther, G.G., Marsden, J.E. and Robbins, J.M., "Geometric phases, reduction and Lie-Poisson structure for the resonant three-wave interaction," *Physica D* **123**, 271-290 (1999).
12. Alber, M.S., Luther, G.G., Marsden, J.E. and Robbins, J.W., "Geometry and control of three-wave interactions," *Fields Institute Communications* **24**, 55-80 (1999).
13. Alber, M.S., Camassa, R., Fedorov, Y., Holm, D.D. and Marsden, J.E., "On billiard solutions of nonlinear PDE's," *Physics Letters A* **264**, 171-178 (2000).
14. Alber, M.S., "N-Component integrable systems and geometric asymptotics," in *Integrability: the Seiberg-Witten and Whitham equations*, H.W. Braden and I.M. Krichever editors (Gordon and Breach Science Publishers, Amsterdam,) 1-10 (2000).
15. Alber, M.S., Lfuther, G.G. and Miller, C., "On Soliton-type solutions of the equations associated with N-component systems," *Journal of Mathematical Physics* **41**, 284 -316 (2000).
16. Alber, M.S., Camassa, R. and Gekhtman, M., "On billiard weak solutions of nonlinear PDE's and Toda flows," *CRM Proceedings & Lecture Notes* **25**, 1-11 (2000).
17. Alber, M.S., Luther, G.G., Marsden, J.E. and Robbins, J.W., "Geometry and control of χ^2 processes and the generalized Poincare sphere," *Journal of the Optical Society of America B* **17**, 932-941(2000).
18. Alber, M.S. and Fedorov, Y., "Wave solutions of evolution equations and hamiltonian flows on nonlinear subvarieties of generalized jacobians," *Journal of Physics A: Mathematical and General* **33**, 8409-8425 (2000).
19. Alber, M.S., and Miller, C., "On Peakon solutions of the shallow water equation," *Applied Mathematics Letters* **14**, 93-98 (2001).
20. Alber, M.S., and Fedorov, Y., "Algebraic geometrical solutions for certain evolution equations and hamiltonian flows on nonlinear subvarieties of generalized jacobians," *Inverse Problems* **17**, 1017-1042 (2001).
21. Alber, M.S., Camassa, R., Fedorov, Y., Holm, D.D. and Marsden, J.E., "The complex geometry of weak piecewise smooth solutions of integrable nonlinear pde's of shallow water and dym type," *Communications in Mathematical Physics* **221**, 197-227 (2001).
22. Alber, M.S. and Kiskowski, A., "On Aggregation in CA Models in Biology," *Journal of Physics A: Mathematical and General* **34**, **48**, 10707-10714 (2001).
23. Chaturvedi, R., Izaguirre, J. A., Huang, C., Cickovski, T., Virtue, P., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A., and Glazier, J. A., "Multi-model simulations of chicken limb morphogenesis," *Lecture Notes in Computer Science*, Volume **2659**, Springer-Verlag, New York, 39-49 (2003).
24. Alber, M.S., Kiskowski, M.A., Glazier, J.A., and Jiang, Y. "On cellular automaton approaches to modeling biological cells, mathematical systems theory in biology, communication and finance," *IMA* Volume **134**, Springer-Verlag, New York, (2003).
25. Izaguirre, J. A., Chaturvedi, R., Huang, C., Cickovski, T., Coffland, J., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S.A., and Glazier, J.A., "CompuCell, a multi-model framework for simulation of morphogenesis," *Bioinformatics* (accepted for publication).
26. Alber, M.S., Kiskowski, M.A., Glazier, J.A., and Jiang, Y., "Lattice gas cellular automata model for rippling and aggregation in myxobacteria," *Physica D* (accepted for publication).

C. Research Support

Current

NSF IBN-0083653 9/1/00-8/31/05
National Science Foundation/Division of Integrative Biology and Neuroscience
"Biocomplexity – Multiscale Simulation of Avian Limb Development"
The major goal of this project is to develop a comprehensive model of avian limb structural development based on physical processes including cell adhesion, chemotaxis and haptotaxis models of cell migration, mitosis, and reaction-diffusion equation models of cell differentiation.
Role: Co-PI

Completed

Burroughs Wellcome Fund
Support grant for "Biocomplexity Workshop V: Multiscale Modeling in Biology" held at the University of Notre Dame from August 14-17, 2003.
Role: Organizer

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrew D. Arenson	Principal INGEN Data Specialist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Rice University, Houston, TX	B.A.	1992	Computer Science

A. Positions and Honors

1992-1994 Systems Analyst, Environmental Science & Engineering, Rice University, Houston, TX
1994-1999 Computer Programmer III, Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX
1999-2001 Senior Consultant, Solutions Delivery, Proxicom, San Francisco, CA
2002- Member, Steering Committee, Central Indiana Information Technology Professionals
2002- Principal INGEN Data Specialist, University Information Technology Services, Indiana University, Indianapolis, IN

B. Selected peer-revied publications

1. Hoskins, et al. “A BAC-based physical map of the major autosomes of Drosophila melanogaster,” *Science* **287**(5461):2271-4 (2000).
2. Lander, E., et al. “Initial sequencing and analysis of the human genome,” *Nature* **409**, 860 - 921 (2001).

C. Research Support

Current

NIH/NIAA 9/03 – 9/06
Stewart, C. A., Shankar, A., et al. **“**Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders”
The purpose of this grant is to unify the research being conducted on disorders resulting in children from alcohol consumption by pregnant women. The Informatics Core is providing a standardized data repository and help with statistical analysis.
Role: Lead Developer

Completed

Lilly Endowment 2000-08
Brater, D.C., Pescovitz, O.H., et al. “INGEN – the Indiana Genomics Initiative”
The purpose of this grant is to develop a world-class genomic-based medical research program based on the existing excellence of the IU School of Medicine. Stewart is a named investigator and director of the Information Technology Core. The purpose of the Information Technology core is to provide the advanced supercomputing, visualization, and high performance storage facilities required for INGEN researchers to perform cutting-edge genomic and biomedical research.
Role: Data Specialist

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
M. Pauline Baker	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Cornell University, NY	B.A.	1974	Psychology
Syracuse University, NY	M.S.	1977	Education
Western Illinois University, Macomb, IL	M.S.	1981	Computer Science
University of Illinois, Champaign, IL	Ph.D.	1990	Computer Science

A. Positions and Honors

1981-1985	Assistant Professor, Computer Science Department, Western Illinois University
1990 -1997	Research Scientist, Visualization, National Center for Supercomputing Applications, UIUC
1997 -1999	Associate Director: Visualization & Virtual Environments, National Center for Supercomputing Applications, UIUC
1996-2001	Senior Research Scientist, Beckman Institute for Advanced Technology, University of Illinois
1999-2001	Division Director: Data Mining and Visualization, National Center for Supercomputing Applications, University of Illinois
2002-present	Senior Research Scientist, Visualization and Virtual Reality, National Center for Supercomputing Applications, University of Illinois
2002-present	Distinguished Scientist and Director, Pervasive Technology Labs at Indiana University Indiana University – Purdue University Indianapolis
2002-present	Associate Professor, School of Informatics and Computer Science Department Indiana University – Purdue University Indianapolis

B. Selected peer-reviewed publications

1. Baker, M. P., and Bushnell, C., “After the Storm: Considerations for Information Visualization”, *IEEE Computer Graphics and Applications*, (May 1995).
2. Baker, M. P. and Wickens, C. D., “Human Factors in Virtual Environments for the Visual Analysis of Scientific Data”, *NCSA-TR032 and Institute of Aviation report ARL-95-8/PNL-95-2*, (August 1995).
3. Crutcher, R, Baker, M. P., G. Baxter, et al., “Radio Synthesis Imaging – A Grand Challenge HPCC Project”, *International Journal of Supercomputing Applications*, (1996).
4. Peters, E. L. and Baker, M. P., "Comparing Middleware Support Systems for Collaborative Visualization,” *U.S. Army Corps of Engineers Waterways Experiment Station Technical Report*, Vicksburg MS, (Fall 1997).
5. Baker, M. P. and Stein, R., "BattleView: Touring the Virtual Battlefield", *Proceedings of 2nd Annual Symposium, ARL Federated Lab for Advanced and Interactive Displays*, (Feb. 1998).
6. Baker, M. P., Bock, D., Heiland, R. and Stephens, M., "Visualization of Damaged Structures", *Army Corps of Engineers Waterways Experiment Station Technical Report*, Vicksburg MS, (1998).
7. Stein, R., Shih, A. and Baker, M. P., “Visualization of Water Quality in the Chesapeake Bay”, *Proceedings of IEEE Visualization 2000*, Salt Lake City, UT, (Oct. 8-13, 2000).
8. Hearn, D.D., and Baker, M. P., *Computer Graphics with OpenGL*, (C Edition), Prentice-Hall, (2004).

C. Research Support
Current

DOE SciDAC
Simulating core collapse supernovae
Developing advanced visualization techniques appropriate for the team.
Role: Investigator

NSF
Inverse Ocean Modeling.
Developing remote visualization methods and techniques for visualizing large covariance matrices.
Role: Co-PI

NSF
Project REVITALISE
Joint project with East Carolina University.
To enhance the professional experience of teachers in rural communities by exploring the use of visualization in the classroom and using Web-based collaboration tools to build a support community.
Role: Co-PI

Completed
NSF
Project ChemVizII
Works with local teachers and their students to explore the use of advanced 3-dimensional visualization techniques to teach chemistry concepts.
Role: Co-PI

Army Research Lab
Federated Lab for Advanced and Interactive Displays
Use of advanced computer graphics for situation awareness and battlefield visualization.
Role: Invenstigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Albert-László Barabási	Emil T. Hofman Professor of Physics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Bucharest, Romania	--	1989	Physics & Engineering
Eotvos Lorand University, Budapest, Hungary	M.S.	1991	Physics
Boston University, MA	Ph.D.	1994	Physics

A. Positions and Honors

Professional Experience:

1989-1991 Research Institute for Technical Physics, Hungarian Academy of Sciences, Budapest, Research Assistant
1991-1992 Boston University, Teaching Assistant
1992-1994 Boston University, Research Assistant
1994-1995 IBM, T.J. Watson Research Center, Postdoctoral Associate; advisor Dr. G. Grinstein
1995-1999 University of Notre Dame, Assistant Professor
1999-2000 University of Notre Dame, Associate Professor
2000-present University of Notre Dame, Hofman Professor of Physics

Honors and Other Professional Activities:

1990-1991 Republican Fellowship of the Republic of Hungary
1990-1991 Fellowship of Cel Foundation, Budapest, Hungary
1991-present TEMPUS Fellowship, European Community, University of Koln
1997 NSF CAREER Award
1998 ONR Young Investigator Award
1999 Distinguished Scholar Lecturer, College of Science, University of Notre Dame
2000 Keynote Speaker, Colocation Summit, Washington D.C.
2001 Nivo Prize for the best physics article Fizikai Szemle (Hungary)
2002 Presidential Award, University of Notre Dame
2002 Editorial Board, *ComPlexUs* and *Fractals*
2002 ISI: Fast Breaking Paper in Physics (*Reviews of Modern Physics* **76**, 69 (2002))
2002 ISI: Highly Cited (*Nature* **407**, 651 (2000))
2002 Keynote Speaker, Biotechnology Ventures, San Francisco
2003 Keynote Speaker, 4th Georgia Tech International Conference in Bioinformatics, Atlanta, GA
2003 Editorial Board of *Internet Mathematics*
2003 Elected Fellow of the American Physical Society

B. Selected peer-reviewed publications

Publications (related to the present project):

1. A.-L. Barabasi and R. Albert, “Emergence of scaling in random networks,” *Science* **286**, 509-512 (1999).
2. R. Albert, H. Jeong, and A.-L. Barabasi, “Attack and error tolerance of complex networks,” *Nature* **406**, 378-381 (2000).
3. H. Jeong, B. Tombor, R. Albert, Z.N. Oltavi, and A.-L. Barabasi, “The large-scale organization of metabolic networks,” *Nature* **407**, 651-654 (2000).

4. H. Jeong, S. P. Mason, A.-L. Barabasi, and Z.N. Oltvai, "Lethality and centrality in protein networks," *Nature*, **411**, 41-12 (2001).
5. J. Podani, Z.N. Oltvai, H. Jeong, B. Tombor, A.-L. Barabasi, and E. Szathmary, "Comparable system-level organization of Archaea and Eukaryotes," *Nature Genetics*, **29**, 54-56 (2001).
6. E. Ravasz, A.L. Somera, D. A. Mongru, Z. N. Oltvai and A.-L. Barabasi, "Hierarchical Organization of Modularity in Metabolic Networks," *Science*, **297**, 1551-1555 (2002).
7. S. Wuchty, Z. N. Oltvai and A.-L. Barabási, "Evolutionary conservation of motif constituents in the yeast protein interaction network," *Nature Genetics* **35**, 176-179 (2003).

Other Publications (Books):
1. A.-L. Barabasi and H. E. Stanley, *Fractal Concepts in Surface Growth*, Cambridge University Press, Cambridge (1995).
2. A.-L. Barabasi, M. Krishnamurthy, F. Liu, and T. Pearsall (eds.), *Epitaxial Growth – Principles and Applications*, Materials Research Society, Werrendale, PA (1999).
3. A.-L. Barabasi, *Linked: The New Science of Networks*, Perseus, Boston (2002).

Book Chapters:
1. K. Furdyna, S. Lee, A.-L. Barabasi, and J. L. Merz, "Self-Organized Low-Dimensional II-VI Nanostructures," in *II-VI Semiconductor Materials and Their Applications*, edited by M. C. Tamargo (Gordon and Breach Science Publishers, 1999).

Journal Articles (Selected from over 80 referred papers):
1. B. Suki, A.-L. Barabasi, and K. Lutchen, "Lung tissue viscoelasticity: A mathematical framework and its molecular basis," *Journal of Applied Physiology* **76**, 2749-2759 (1994).
2. B. Suki, A.-L. Barabasi, Z. Hantos, F. Petak, and H. E. Stanley, "Avalanches and power law behavior in lung inflation," *Nature* **368**, 615-618 (1994).
3. I. Daruka and A.-L. Barabasi, "Dislocation free island formation in heteroepitaxial growth: a study at equilibrium," *Physical Review Letters* **79**, 3708-3711 (1997).
4. U. Frey, M. Silverman, A.-L. Barabasi, and B. Suki, "Irregularities and power law distributions in the breathing pattern in preterm and term infants," *Journal of Applied Physiology* **85**, 789 (1998).
5. C.-S. Lee, B. Janko, I. Derenyi, and A.-L. Barabasi, "Reducing vortex density in superconductors using the 'rachet effect', *Nature* **400**, 337-340 (1999).
6. A. Czirok, A.-L. Barabasi, and T. Vicsek, "Collective motion of self-propelled particles: kinetic phase transition in one dimension," *Physical Review Letters* **82**, 209 (1999).
7. Z. Neda, E. Ravasz, Y. Brechet, T. Vicsek, A.-L. Barabasi, "Self-organization in the Concert Hall: the Dynamics of Rhythm Applause," *Nature* **403**, 849-850 (2000).

C. Research Support

Current

National Institute of Health 12/01/2001-11/30/2006
"Topologic Properties of Metabolic Networks"
An integrated experimental-theoretical project to predict and test the consequences of gene deletion.
Role: Principal Investigator

Completed

Department of Energy 09/01/2001-08/31/2002
"The Organization of Complex Metabolic Networks"
The major goals of this project are to develop theoretical and computational tools to offer integrated computer models for E. coli metabolism.
Role: Principal Investigator

National Science Foundation 08/01/2000-07/31/2002
“Dynamics of complex networks”
The major goal of this project is to investigate the statistical mechanisms of complex, non-biological networks.
Role: Principal Investigator

National Science Foundation 06/01/1997-05/31/2001
“Driven Interfaces in Porous Media”
The major goals of this project are to characterize the morphology and statistical properties of interface moving in porous media, with application to fluid flow in soil and rocks.
Role: Principal Investigator

Office of Naval Research 06/01/1998-05/31/2001
“Spatial Ordering of Self-Assembled Quantum Dots”
The major goals of this project are to study statistically the formation of self-assembled quantum dots using the tools of statistical mechanics.
Role: Principal Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Teri Louise Belecky-Adams	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wyoming, Laramie, WY	B.S.	1985	Psychology
University of Cincinnati College of Medicine, Cincinnati, OH	Ph.D.	1994	Cell Biology, Neurobiology and Anatomy

A. Positions and Honors

1985-1989	Research Assistant II, Department of Otolaryngology and Maxillofacial Surgery, University of Cincinnati School of Medicine
198-1994	Graduate Research Assistant, Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati School of Medicine
1994-1996	Post Doctoral Fellow, Wilmer Eye Research Institute, Johns Hopkins School of Medicine
1996-2001	Research Associate, Wilmer Eye Research Institute, Johns Hopkins School of Medicine
2001-present	Assistant Professor, Department of Biology, Indiana University-Purdue University at Indianapolis

B. Selected peer-reviewed publications

1. Belecky, T.L. and Smith, D.V. “Postnatal development of palatal and laryngeal taste buds in the hamster,” *J. Comp. Neurol*. **293**: 646-654 (1990).
2. Brining, S.K., Belecky, T.L. and Smith, D.V. “Taste reactivity in the hamster,” *Physiology and Behavior* **49**(6): 1265-72 (1991).
3. Belecky-Adams, T., Wight, D.C., Kopchick, J.J., and Parysek, L.M. “Intragenic sequences are required for cell type-specific and injury-induced expression of the rat peripherin gene,” *J. Neurosci*. **13**: 5056-5065 (1994).
4. Belecky-Adams, T.L., Cook, B.E., and Adler, R.A. “Correlations between terminal mitosis and differential fate of retinal precursor cells in vivo and in vitro: Analysis with the ‘window-labeling’ technique,” *Dev. Biol*. **178**:304-315 (1996).
5. Belecky-Adams, T.L., Tomarev, S., Li, H.-S., Ploder, L., McInnes, R.R., Sundin, O., Adler, R. Prox 1, Pax-6, and Chx10 homeobox gene expression correlate with phenotypic fate of retinal precursor cells,” *Invest. Ophthalmol. Vis. Sci*. **38**: 1293-1303 (1997).
6. Weng, J, Belecky-Adams, T.L., Adler, R., Travis, G.H. “Identification of two rds/peripherin homologs in the chick retina,” *Invest. Ophthalmol. Vis. Sci*. **39**: 440-443 (1997).
7. Belecky-Adams, T.L., Scheurer, D., and Adler, R. “Activin family members in the developing chick retina: expression patterns, protein distribution, and in vitro effects,” *Dev. Biol*. **210**: 107-23 (1999).
8. Adler, R., Belecky-Adams, T. “Cell fate determination in the chick embryo retina,” *Cell Fate and Lineage Determination*. Ed. Sally Moody, Academic Press, San Diego, 463-474 (1999).
9. Adler, R., Tamres, A.N., Bradford, R.B. and Belecky-Adams T.L. “Microenvironmental regulation of visual pigment expression in the chick retina,” *Developmental Biology* **236**: 454-464 (2001).
10. Belecky-Adams, T.L. and Adler, R. “Expression of bone morphogenetic proteins, receptors, and binding proteins during development of the chick retina,” *J. Comp. Neurol*. **430**: 562-572 (2001).
11. Belecky-Adams, T., Adler, R. and Beebe, D. “Bone morphogenetic protein signaling and the initiation of lens fiber cell differentiation,” *Development* **129**, 3795-802 (2002).
12. Adler, R. and Belecky-Adams, T. L. “The role of bone morphogenetic proteins in the differentiation of the ventral optic cup,” *Development* **129**, 3161-71 (2002).

13. Belecky-Adams**,** T.L. Michael Holmes, Yuqing Shan, C. Susan Tedesco, Carla Mascari, Ajay Kaul, David C. Wight, Randal E. Morris, Mark Sussman, Jack Diamond, Linda M. Parysek. "An intact intermediate filament network is required for collateral sprouting of small diameter nerve fibers," *J. Neurosci.* **23***,* 9312-9319 (2003).

C. Research Support

Current

Agency: March of Dimes Birth Defects Foundation 2/1/2003 - 1/31/2005
Basil O'Connor Starter Scholar Award (5-FY2003-5)
"The regulation and potential role of PAX2 in the development of the eye"
The goal of this proposal is to 1) test the hypothesis that PAX2 is necessary for mitosis of ventral retinal precursors in the closure of optic cup choroid fissure, and 2) determine if bone morphogenetic proteins directly regulate PAX2 cis-acting elements.
Role: PI

Completed

Agency: Purdue University 6/2002 - 8/2002
Type: Summer Research Salary Support
"BMPs in the Differentiating retina"
The goal of this proposal was to obtain preliminary data regarding the function of BMPs in the ventral retina in order to obtain extramural funding.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Katy Börner	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Technology Leipzig, Germany	Master	1991	Engineering in Electronics
University of Kaiserslautern, Germany	Ph.D.	1997	Computer Science

A. Positions and Honors

1991 - 1994	Research Assistant in BMBF funded projects GOSLER and FABEL, University of Technology Leipzig, Department of Mathematics and Computer Science
1992 - 1995.	Ph.D. Fellowship Studienstiftung des Deutschen Volkes e.V., Germany
1992 - 1998	Professional Services (Technical Development)
1993	Development Engineer, Horiba LTD, Automotive Instruments Development Department, Kyoto, Japan
1994 - 1996	Research Assistant in the BMBF funded project FABEL, University of Freiburg, Center for Cognitive Science
1994	Ph.D. Studies, Boston University, Department of Computer Science
1996 - 1998	Research Associate and Instructor, University of Bielefeld, Faculty of Technology
1998 - 1999	Visiting Assistant Professor, Computer Science Department, Indiana University, Bloomington
1999 - present	Faculty Member, Indiana University Advanced Visualization Laboratory
1999	Visiting Assistant Professor, School of Library and Information Science, Indiana University, Bloomington,
2000 - present	Assistant Professor School of Library and Information Science and Core Cognitive Science Faculty, Indiana University, Bloomington,.
2001 - present	Adjunct Assistant Professor School of Informatics, Indiana University
2001 - present	Fellow, Center for Research on Learning and Technology, Indiana University
2001 - present	Member of the VLearn3D Advisory Committee
2001 - present	Fellow, Center for Social Informatics, Indiana University
2003 - 2004	Pervasive Technology Labs Fellowship, Indiana University
2003 - 2004	SBC Fellow (formerly Ameritech Fellow), Round 4
2003 - 2008	NSF CAREER Award
2003	Trustees Teaching Award ,School of Library and Info. Science, Indiana University,.
2003	Outstanding Junior Faculty Award. Indiana University, Bloomington

B. Selected peer-reviewed publications

Journal Articles

1. Börner,K., “Efficient case-based structure generation for design support,” Artificial Intelligence Review **16**, (2), Kluwer Academic Publishers, 87-118 (Oct. 2001).
2. Mane, K. and Börner, K., “Mapping Topics and Topic Bursts in PNAS”, Mapping Knowledge Domains, Arthur M. Sackler Colloquium (2003).
3. Börner, K., Maru, J. and Goldstone R.,” The Simultaneous Evolution of Author and Paper Networks,” Mapping Knowledge Domains, Arthur M. Sackler Colloquium (2003).
4. Börner, K. and Penumarthy, S., “Social Diffusion Patterns in Three-Dimensional Virtual Worlds,” *Information Visualization Journal*, **2** (3), 182-198 (2003).

5. Boyack, K. W. and Börner, K,," Indicator-Assisted Evaluation and Funding of Research: Visualizing the Influence of Grants on the Number and Citation Counts of Research Papers, " Journal of the American Society of Information Science and Technology, Special Topic Issue on Visualizing Scientific Paradigms **54** (5), 447-461 (2003).
6. Börner, K., Chen, C. and Boyack, K.," Visualizing Knowledge Domains", Annual Review of Information Science & Technology,**37**, Cronin, B. Ed., Information Today, Inc./American Society for Information Science and Technology, Medford, NJ, 179-255 (2003).

Books and Book Chapters

1. Börner, K.,"Konzeptbildende Analogie: Integration von Conceptual Clustering und Analogem Schließen zur effizienten Unterstützung von Entwurfsaufgaben," Dissertation, INFIX Verlag (1997).
2. Börner, K.,"Twin Worlds: Augmenting, Evaluating, and Studying Three-Dimensional Digital Cities and Their Evolving Communities, *Makoto Tanabe*, Van den Besselaar, P. and Ishida, T. (Eds), *Digital Cities II: Computational and Sociological Approaches*, Springer Verlag, LNCS 2362, 256-269 (2002).
3. Börner, K., "Visual Interfaces for Semantic Information Retrieval and Browsing," Groimenko V. and Chen, C. (Eds.), *Visualizing the Semantic Web: XML-based Internet and Information Visualization*, Springer Verlag, 99-115 (2002).
4. Börner, K. and Chen C. (Eds), "Visual Interfaces to Digital Libraries," Springer Verlag, LNCS 2539 (2002).
5. Börner, K. and Penumarthy, S.,"Maps of Virtual Worlds," *Information Design Source Book*. Institute for Information Design Japan, Graphic-Sha, Japan (Oct. 2003).
6. Börner, K., Penumarthy, S., DeVarco, B. J. and Kerney, C., "Visualizing Social Patterns in Virtual Environments on a Local and Global Scale," *Digital Cities 3: Local information and communication infrastructures: Experiences and challenges,* Springer, Amsterdam, (September 19-21, 2003).
7. Börner, K., "Computers and Knowledge Sharing," *Encyclopedia of Community: From the Village to the Virtual World*, Christensen, K. and Levinson, D. (Eds.), Sage, Thousand Oaks, CA, 314-316 (2003).

Refereed Conference and Workshop Publications

1. Börner K. and Lin, Y.-C., "Visualizing Chat Log Data Collected in 3-D Virtual Worlds," *Information Visualisation Conference*, London, 141-146 (July 25-27, 2001).
2. Börner, K. and Zhou, Y., "A Software Repository for Education and Research in Information Visualization," *Information Visualisation Conference*, London, 257-262 (July 25-27, 2001).
3. Börner, K., "iScape: A collaborative memory palace for digital library search results*," Proceedings of the International Conference on Human-Computer Interaction*, Smith, M. J., Salvendy, G., Harris, D. and Koubek R. J. (Eds), New Orleans, LA, *Usability Evaluation and Interface Design* **1**, Lawrence Erlbaum Associates, London, 1160-1164 (August 5-10, 2001).
4. Feng, Y. and Börner, K., "Using Semantic Treemaps to Categorize and Visualize Bookmark Files," *Visualization and Data Analysis 2002*, *Proceedings of SPIE* **4665,** Erbacher, R. F., Chen, P. C., Grohn, M., Roberts, J. C. and Wittenbrink, C. M. (Eds), San Jose, CA, 218-227 (January 20-25, 2002).
5. Börner, K., Hazlewood, R. and Lin, S.-M., "Visualizing the Spatial and Temporal Distribution of User Interaction Data Collected in Three-Dimensional Virtual Worlds," *Sixth International Conference on Information Visualization*, IEEE Press, London, 25-31 (July 10-12, 2002).
6. Börner, K., Feng, Y. and McMahon, T., "Collaborative Visual Interfaces to Digital Libraries," Second *ACM+IEEE Joint Conference on Digital Libraries*, Marchionini, G. & Hersh, W. (Eds), ACM Press, Portland, OR, 279-280 (July 14-18, 2002).

C. Research Support

Current

Indiana University 2004
"Outstanding Junior Faculty Award"
Awarded for research on the validation and deployment of *Knowledge Domain Visualization* (KDV) techniques.
Role: PI

Pervasive Technology Labs Fellowship 09/2003 – 08/2004
"Data-Code-Computing Infrastructure for Data Mining, Modeling, and Visualization Research and Education"
Awarded for the design of an infrastructure for data mining, modeling and information visualization.
Role: PI

NSF IIS-0238261 award 09/2003-08/2008
"CAREER: Visualizing Knowledge Domains"
Funds the systematic development, validation, and deployment of KDV techniques.
Role: PI

NSF DUE-0333623 award 09/ 2003-08/2005
"Project ENABLE: Learning through Associations in a Grid based Bioinformatics Digital Library"
Aims to create and extensible networked association-based bioinformatics learning environment.
Role: Investigator

SBC (formerly Ameritech) Fellow Grant 05/2003-06/2004
"Information Visualization Learning Modules"
Funds the design, deployment, and evaluation of information visualization learning modules.
Role: PI

National Academy of Sciences The colloquium took place May 9-11, 2003.
Arthur M. Sackler Colloquium on Mapping Knowledge Domains.
Role: Associate Organizer

NSF SES Major Research Instrumentation Grant 08/2002-07/2007
"Development of a Spatial-Experimental Laboratory for Research and Policy Analysis Related to Complex Systems"
The lab will support cutting edge experimental research in the behavioral sciences and will benefit multiple departments at IU.
Role: Investigator

Completed

SRI 08/2002-03/2003
Center for Innovative Learning Technologies (CILT) grant no. EIA-0124012 from the National Science Foundation to SRI International under subcontract to Indiana University
"Building Blocks for Virtual Worlds: Design Principles for a Starter Kit for Educational Virtual Worlds"
A large scale online study was conducted to identify major obstacles in the usage of virtual worlds for educational purposes. Based on the results of this study, a toolkit was developed that applies data analysis and visualization techniques to support the design, usage, evaluation, and study of educational virtual worlds.
Role: PI

NIH Demo Fund 08/2001-12/2002
"Mapping Aging Research"
This research analyzed and visualize the impact of governmental funding on the amount and quality of research publications. For the first time, grant and publication data appear interlinked in one map. Resulting visualizations show an information landscape of aging-related data – providing NIH with a tool to discover general patterns and trends.
Role: PI

Indiana University's High Performance 2000-2001
Network Applications Program
"iUniverse - Creating a Collaborative Information Universe for IU"

The project established one of the most sophisticated interface technologies for desktop computers at IU. The technology, a "3D Virtual Reality Chat & Design Tool" by Activeworlds.com, Inc. allows building compelling, multi-modal, multi-user, navigable, collaborative virtual environments in 3D that are inhabited by avatars (acting as placeholders for human users) and provide means for interacting with the objects in the environment, with embedded information sources and services or with other users and visitors of the environment. The technology is available to faculty, staff and graduate students at IU.
Role: PI

Academic Equipment Grant 04/2001
EDUD-7824-010346-US by SUN microsystems
“Creating a 3-D Collaborative Information Universe”
This grant provides the hardware for the i-Universe (see project above).
Role: PI

Indiana University's High Performance 1999-2000
Network Applications Program
“LVis - Digital Library Visualizer”
The project developed and evaluated new techniques to support the navigation through complex information spaces. It created and texted diverse multi-modal, virtual reality interface that map data stored in digital libraries onto "information landscapes" that can then be explored by human users in a natural manner. The 2-D and 3-D prototype interfaces visualize search results from the Dido Image Bank, Department of the History of Art, IU and ISI’s web of science.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
William F. Bosron	Professor of Biochemistry and Molecular Biology, & Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bowling Green State University Bowling Green, Ohio	B.S.	1967	Chemistry and Biology
University of Cincinnati, Cincinnati, Ohio	Ph.D.	1973	Biological Chemistry
Harvard Medical School, Boston, Massachusetts	Postdoctoral	1975	Biological Chemistry

A. Positions and Honors

1972-1975 Postdoctoral Research Fellow, Biological Chemistry, Harvard Medical School and Peter Bent Brigham Hospital (Bert L. Vallee, advisor)
1975-1979 Assistant Professor of Medicine and Biochemistry, Indiana University School of Medicine
1979-1986 Associate Professor of Medicine and Biochemistry, Indiana University School of Medicine
1979-Present Graduate Faculty, Indiana University Graduate School
1986-Present Professor of Biochemistry and Molecular Biology, and of Medicine, Indiana University School of Medicine
1990 Visiting Professor, Institute of Biotechnology, University of Cambridge, England
1994-Present Assistant Dean for Graduate Studies, Indiana University School of Medicine
1997-1998 Director, Center for Structural Biology, Indiana University School of Medicine
1999-Present Director, INGEN Biotechnology Training Program, Indiana University School of Medicine
2000-2003 Director, INGEN Scholars Program, Indiana University School of Medicine

Awards and other professional activities:

1986-1990 Editorial Board of *Alcoholism: Clinical and Experimental Research*
1992-1996 NIAAA ALCB-1 Biochemistry, Physiology and Medicine Study Section (became ALTX-1 Alcohol and Toxicology Study Section in 1996)
1992-2002 NIAAA MERIT (R37) award, Structure and function of liver alcohol dehydrogenases
1996-2001 Editorial Board of *Journal of Biological Chemistry*
1999-2002 Editorial Board of *Alcohol Health and Research World*.

B. Selected peer-reviewed publications

Representative publications since 1996 (out of 106):

1. Davis, G.J., Bosron, W.F., Stone, C.L., Owusu-Dekyi, K, and Hurley, T.D., "X-ray structure of the $\beta_3\beta_3$ alcohol dehydrogenase. The contribution of ionic interactions to coenzyme binding," *J. Biol Chem.* **271**:17057-17061 (1996).
2. Zhang, J., Dean, R.A., Brzezinski, M.R. and Bosron, W.F. **"**Gender-specific differences in activity and protein content of cocaine carboxylesterase in rat tissues," *Life Sci.* **59**:1175-1184 (1996).
3. Kamendulis, L.M., Brzezinski, M.R., Pindel, E.V., Bosron, W.F. and Dean, R.A. "Metabolism of cocaine and heroin is regulated by the same human liver carboxylesterases," *J. Pharmacol. Exp. Therapeutics.* **279**:713-717 (1996).
4. Yang, Z.-N., Bosron, W.F. and Hurley,T.D., "Structure of human χχ alcohol dehydrogenase: A glutathione-dependent formaldehyde dehydrogenase," *J. Mol. Biol.* **265**:330-343 (1997).
5. Kedishvili, N.Y., Gough, W.H., Chernoff, E.A.G., Hurley, T.D., Stone, C.L., Bowman, K.D., Popov, K.M., Bosron, W.F. and Li, T.-K., "cDNA sequence and catalytic properties of an alcohol dehydrogenase in the chick embryo ADH-F that oxidizes retinol and 3β, 5α-hydroxysteroids," *J. Biol. Chem.* **272***:*7494-7500 (1997).

6. Pindel, E.V., Kedishvili, N.Y., Abraham, T.L., Brzezinski, M.R., Zhang, J., Dean, R.A. and Bosron, W.F., "Purification and cloning of a broad substrate specificity human liver carboxylesterase that catalyzes the hydrolysis of cocaine to ecgonine methyl ester and benzoic acid," *J. Biol. Chem.* **272**:14769-14775 (1997).
7. Xie, P., Bosron, W.F. and Hurley, T.D., "X-ray structure of human Class IV $\sigma\sigma$ alcohol dehydrogenase: structural basis for substrate specificity," *J. Biol. Chem* **272**:18558-18563 (1997).
8. Brzezinski, M.R., Spink, B.J., Dean, R.A., Berkman, C.E., Cashman, J.R., and Bosron, W.F., "Human liver carboxylesterase hCE-1: Binding specificity for cocaine, heroin and their metabolites and analogs," *Drug Metab. Dispos.* **25**:1089-1096 (1997).
9. Edenberg, H.J. and Bosron, W.F., "Alcohol dehydrogenases," in *Comprehensive Toxicology*, Vol. 3, Biotransformation, ed. F.P. Guengerich, Pergamon Press, New York (1997), 119-131.
10. Kedishvili, N.Y., Gough, W.H., Davis, W.I., Parsons, S.M., Li, T.-K., Bosron, W.F., **"**Effect of cellular retinol-binding protein on retinol oxidation by human class IV retinol/alcohol dehydrogenase and inhibition by ethanol," *Biochem. Biophys. Res. Commun.* **249**:191-196 (1998).
11. Zhang, J., Burnell, J.C., Dumaual, N. and Bosron, W.F., "Binding and hydrolysis of meperidine by human liver carboxylesterase hCE-1," *J. Pharmacol. Exp. Therapeutics* **290**:314-318 (1999).
12. Stone, C.L., Jipping, M.B., Owusu-Dekyi, K., Hurley, T.D., Li, T.-K., and Bosron, W.F., "The pH-dependent binding of NADH and subsequent enzyme isomerization of human liver $\beta_3\beta_3$ alcohol dehydrogenase," *Biochemistry* **38**:5829-5835 (1999).
13. Humerickhouse, R., Lohrbach, K., Li, L., Bosron, W.F. and Dolan, M.E., "Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2," *Cancer Research* **60**: 1189-1192 (2000).
14. Sanghani, P.C., Stone, C.L., Ray, B.D., Pindel, E.V., Hurley, T.D. and Bosron,W.F., "Kinetic mechanism of human glutathione-dependent formaldehyde Dehydrogenase," *Biochemistry* **39**:10720-10729 (2000).
15. Bosron, W.F. and Ramchandani, V. A., "Alcohol Metabolism," in: *Report of a Subcommittee of the National Advisory Council on Alcohol Abuse and Alcoholism on the Review of the Extramural Research Portfolio for Biomedical Research*. http://www.niaaa.nih.gov/extramural/biomedrpt.htm#experts (2000).
16. Crabb, D. W., Pinairs, J., Fang, H.M., Leo, M.A., Lieber, C.S., Tsukamoto, H., Motomura, K., Miyahara, T., Ohata, M., Bosron, W., Sanghani, S., Kedishvili, N., Shiraishi, H., Yokoyama, H., Miyagi, M., Ishii, H., Bergheim, I., Menzl, I., Parlesak, A. and Bode, C., "Alcohol and Retinoids," *Alcoholism: Clinical and Experimental Research* **25** *(5):*207S-217S (2001).
17. Ramchandani, V.A., Bosron, W.F. and Li, T.-K., "Research advances in ethanol metabolism," *Pathologie Biologie* **44***:* 676-682 (2001).
18. Bosron, W.F. and Hurley, T.D., "Lessons from a bacterial cocaine esterase," *Nature Structural Biology* **9***:*4-5 (2002).
19. Bosron, W.F. and Sanghani, S.P., "Identification and substrate specificity of human carboxylesterases hCE-1 and hCE-2," *Drug Metabolism Disposition* **30l***:*488-492 (2002).
20. Sanghani, P.C., Robinson, H., Bosron, W.F. and Hurley, T.D., "Human glutathione-dependent formaldehyde dehydrogenase. Structures of apo, binary and inhibitory ternary complexes," *Biochemistry* **41***:*10778-86 (2002).
21. Sanghani, S.P., Davis, W.I., Dumaual, N.G., Mahrenholz, A. and Bosron, W.F., "Identification of microsomal rat liver carboxylesterases and their activity with retinyl palmitate," *Eur J Biochem* **269**: 4387-4398 (2002).
22. Sanghani, P.C., Bosron, W.F. and Hurley, T.D., "Human glutathione-dependent formaldehyde dehydrogenase. Structural changes associated with ternary complex formation," *Biochemistry* **41***(51):*15189-94 (2002).
23. Sanghani, P.C., Robinson, H., Bennet-Lovsey, R., Hurley, T.D. and Bosron, W.F., "Structure-function relationships in Human Class III alcohol dehydrogenase (Formaldehyde dehydrogenase)," *Chemico-Biological Interactions* **143-144***:*195-200 (2003).
24. Sanghani, S.P., Quinney, S.K., Fredenburg, T.B., Sun, Z., Davis, W.I., Murry, D.J., Cummings, O.W., Seitz, D.E. and Bosron, W.F.**,** "Carboxylesterases expressed in human colon tumor tissue and their role in CPT-11 hydrolysis," *Clin. Cancer Res.* **9***:*4983-4991 (2003).

C. Research Support

Current

R21 CA93833-01 2/1/02-1/31/04
NIH
"CPT-11 activation by carboxylesterases in colon cancer."
Examination of the hydrolysis of CPT-11 and APC by human carboxylesterases and correlation of tumor activity with response to CPT-11 therapy and associated toxicity.
Role: PI

R01 DK063141-A1 07/01/03-06/30/06
NIH
"Retinoid Metabolism in Hepatic Stellate Cells"
Examination of the expression and kinetic properties of retinoid metabolizing enzymes in rat and human hepatic stellate cells during activation to myofibroblast-like cells.
Role: PI

Completed

R37 AA07117-14 02/01/92-01/31/02
NIH
"Structure and Function of Liver Alcohol Dehydrogenases"
Determination of the catalytic properties and three-dimensional structure of human alcohol dehydrogenase isoenzymes.
Role: PI

R21 AA12413-01 8/1/00-6/30/02
NIH
"Retinoid Metabolism in Stellate Cells"
Examination of the expression and kinetic properties of retinoid metabolizing enzymes in rat and human hepatic stellate cells during activation to myofibroblast-like cells.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Rajiv Chaturvedi	Post Doc RA

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Indian Inst. Of Technology, Kanpur, India	B. Tech.	1988	Mech. Eng.
Indian Inst. Of Technology, Kanpur, India	M. Tech.	1991	Mech. Eng. (TFE)
Indian Inst. Of Technology, Bombay, India	Ph.D.	2000	Mech. Eng. (CFD)

A. Positions and Honors

1986-1988 GOI scholarship during bachelor studies
1988-1989 Design Engineer, Kinetics Technologies India
1989 – 1991 Institute scholarship
1991-1992 Engineer, Gas Authority of India Ltd.
1992-1996 Institute scholarship
1996-2001 (concurrent with Ph. D. work): Industrial positions in managing, consulting, business development, engineering positions with technical software development companies (namely, ComputerVision, Parametric Technology Corporation, Tata Consultancy Services, Concio technologies)
2001-Present Post Doc RA at University of Notre Dame, joint appointment with Departments of Maths and Computer Science. Responsibilities included teaching a graduate course in Scientific Computation.

B. Selected peer-reviewed publications

1. Chaturvedi, R. and Kant, K., “Heat Transfer Augmentation via Turbulence Promotion,” *Journal of Energy, Heat and Mass Transfer* (1991).
2. Chaturvedi, R. and Roy, D. P., “Non Reflecting Boundary Conditions and Their Use in Estimating the Effects of Coolant Channel Failure in a PHWR,” *Nuclear Engineering and Design*, (1999).
3. Chaturvedi, Rajiv, Izaguirre, Jesus A., Huang, ChengBang, Cickovski, Trevor, Virtue, Patrick, Thomas, Gilberto, Forgacs, Gabor, Alber, Mark, Hentschel, George, Newman, Stuart A., and Glazier, James A., “Multi-model simulations of chicken limb morphogenesis," *Computational Science - ICCS 2003*: International Conference, Melbourne, Australia and St. Petersburg, Russia, *Proceedings, Part III*, **2659** Sloot. P. M. A., Abramson, D., Bogdanov, A. V., Dongarra, J. J., Zomaya, A. Y. and Gorbachev. Y. E. (Eds), Springer-Verlag, New York, 39-49 (June 2-4, 2003).
4. Izaguirre, J. A., Chaturvedi, R., Huang, C., Cickovski, T., Coffland, J., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A. and Glazier, J. A., "CompuCell, a multi-model framework for simulation of morphogenesis" *Bioinformatics* (accepted for publication) - Manuscript ID: BIOINF-2003-0175-03/162 (2004).

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ellen A. G. Chernoff	Associate Professor of Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The University of Chicago, IL	B.A.	1969 – 1973	Biology (Honors)
The University of Chicago, IL	Ph.D.	1973 – 1978	Biology, Developmental
University of Pennsylvania/Med, Philadelphia, PA	Postdoctoral	1978-1979	Anatomy, Developmental
Temple University, Philadelphia, PA	Visit. Scientist	1979 – 1980	Biology, Cell and Develop.
Case Western Reserve University, Cleveland, OH	Sr. Res. Assoc.	1980 – 1986	Biology, Cell and Develop.

A. Positions and Honors

Positions

1986-1992 Assistant Professor of Biology, Indiana University-Purdue University Indianapolis
1992-present Associate Professor of Biology, Indiana University-Purdue University Indianapolis
2001-present Director, IU Center for Regenerative Biology and Medicine (IUPUI)

Appointments

1988 Visiting Scientist, Eli Lilly Corp., CNS Research Group
1992-present Adjunct Professor, Medical Neuroscience Program, Indiana University School of Medicine
1995-1996 Sabbatical Visiting Scientist, Elanco/ Eli Lilly Research Laboratories, Developmental Biology Group

Honors

1972-1973 Francis Bundick Scholarship for Biochemistry/Biology, Undergraduate
1973-1974 University Pre-doctoral fellowship University of Chicago
1974-1978 NIH Cell Biology Training Grant (Pre- doctoral) Dept of Biology, University of Chicago
1978-1979 NIH Analysis of Development Training Grant (Post-doctoral) Dept of Anatomy, Univ. of Pennsylvania
1982-1984 American Heart Association, Northeast Ohio Affiliate, Inc. Post-doctoral Fellowship
1997 Marcus Singer Medal for furthering research in regeneration and developmental biology

Professional Activities

1985-present Journal article reviews for: Scanning Electron Microscopy; International Journal of Developmental Biology; Development, Growth, and Differentiation; Roux's Archives of Developmental Biology; Developmental Biology; Developmental Dynamics, Moseby Publishers' Biology textbooks; Journal of Vacuum Science and Technology A; Canadian Journal of Zoology, Developmental Genetics and Evolution, PNAS, Proc. Indiana Nat'l Acad. Sci-Reviews, Current Neurovascular Research
1986-present Ad hoc reviews of grant proposals for the NSF, USDA, the Indiana University Alcohol Center, Spinal Cord Research Foundation
1991 NIH site visit panel member, Program Project proposal on spinal cord injury research, for the Scientific Review Branch of the National Institute of Neurological Disorders and Stroke
1993 Co-Organizer, International Meeting on the Cellular and Molecular Biology of Urodeles, Indianapolis and Bloomington
1997, 1998 Co-organizer and Chair, Midwest Developmental Biology Meeting
1999 Co-Organizer International Marcus Singer Symposium, Univ. of Illinois
2002 NSF Partnerships for Innovation review panel

B. Selected peer-reviewed publications

1. Chernoff, E.A.G. and Overton, J. "Organization of the migrating chick epiblast edge; attachment sites, cytoskeleton and early developmental changes," *Develop. Biol*. **72**, 291-307 (1979).
2. Chernoff, E.A.G. and Lash, J.W. "Cell movement in somite devlopment in the chick. Inhibition of segmentation," *Develop. Biol.* **87**, 212-219 (1981).
3. Chernoff, E.A.G. and Hilfer, S.R. "Calcium dependence and contraction in somite formation," *Tissue and Cell* **14**, 435-449 (1982).
4. Chernoff, E.A.G., Maresh, G.A. and Culp, L.A. "Localization and characterization of a large, neurite-associated glycoconjugate," *J. Cell Biol.* **96**, 661-668 (1983).
5. Chernoff, E.A.G., Munck, C.M., Mendelsohn, L.G., and Egar, M.W., "Primary culture of axolotl spinal cord ependymal cells," *Tissue and Cell* **22**, 601-613 (1990).
6. O'Hara, C.M., M.W. Egar, and Chernoff, E.A.G., "Reorganization of the ependyma during axolotl spinal cord regeneration: Changes in intermediate filament and fibronectin expression," *Developmental Dynamics,* **193**, 103-115 (1992).
7. Chernoff, E.A.G. and Chernoff, D.A., "AFM images of collagen fibers," *Journal of Vacuum Science and Technology A*, **10**, 596-599 (1992).
8. O'Hara, C.M. and Chernoff, E.A.G., "Growth factor modulation of injury-reactive ependymal cell proliferation and migration," *Tissue and Cell,* **26**, 599-611 (1994).
9. Chernoff, E.A.G., Stocum D.L., "Developmental aspects of spinal cord and limb regeneration," *Development*, *Growth and Differentiation*. **37**, 133-147 (1995).
10. Chernoff, E.A.G., "Spinal cord regeneration: a phenomenon unique to urodeles?" *International J. Develop. Biol.* **40**, 823-831 (1996).
11. Kedishvili, N.Y., Gough, W.H., Chernoff, E.A.G., Hurley, T.D., Stone, C.L., Bowman, K.D., Popov, K.M., Bosron, W.F. and Li, T.-K., "cDNA sequence and catalytic properties of a chick embryo alcohol dehydrogenase (ADH-F) that oxidizes retinol and 3β,5α-hydroxysteroids," *J. Biol. Chem.* **272**, 7494-7500 (1997).
12. Chernoff, E.A.G., Henry, L.C. and Spotts, T., "An ependymal cell culture system for the study of spinal cord regeneration," *Wound Rep. Reg.* **6**, 435-444 (1998).
13. Chernoff, E.A.G., O'Hara, C.M., Bauerle, D. and Bowling, M., "Matrix metalloproteinase production in regenerating axolotl spinal cord," *Wound Rep. Reg* **8**, 282-291 (2000).
14. Chernoff, E.A.G., Clarke, D.O., Wallace-Evers, J.L., Hungate-Muegge, L.P. Smith, R.C. "The effects of collagen synthesis inhibitory drugs on somitogenesis and myogenin expression in cultured chick and mouse embryos," *Tissue and Cell,* **33**, 97-110 (2001).
15. Chernoff, E.A.G., Sato, K., Corn, A. and Karcavich, R.E. "Spinal cord regeneration, intrinsic properties and emerging mechanisms," *Sem Cell Develop Biol* **13**, 361-368 (2002).
16. Nye, H.L., Cameron, J., Chernoff, E.A.G. and Stocum, D.L. "Regeneration of the urodele limb: a review," *Develop. Dyn.* **226**, 280-294 (2003).
17. Chernoff, E.A.G., Stocum, D.L., Nye, H.L.D. and Cameron, J.A., "Urodele spinal cord regeneration and related processes," *Develop. Dyn.* **226**, 295-307 (2003).
18. Nye, H.L.D., Cameron, J., Chernoff, E.A.G. and Stocum, D.L., "Extending the table of stages of normal development of the axolotl: limb development," *Develop. Dyn.* **226**, 555-560 (Cover picture) (2003).
19. Showalter, A.D., Yaden, B.C., Chernoff, E.A.G. and Rhodes, S.J., "Cloning and analysis of axolotl ISL2 and LHX2 LIM-homeodomain transcription factors," *Genesis* (Developmental Genetics) In press (2004).

C. Research Support

Current

SB021-006 (Stocum) 10/1/03-11/30/04
Phase 2 SBIR DARPA
"Use of low-level electromagnetic fields to advance tissue regeneration"
Subcontract through The Technical Basis, LLC, Loma Linda CA.
Role: (Co-PI)

Eli Lilly and Company (Chernoff) 8/1/01-5/14/04
"Regenerative Biology and Medicine: A Search for Regeneration Stimulating Molecules in *Xenopus laevis* Spinal Cord"
Role: Principal Investigator

0093092 (Chernoff) 10/1/00–9/30/03 (no-cost extension to 3/31/04)
NSF
“Partnerships for innovation: a center of excellence in regenerative biology”
The major goal is to develop a research Center for Regenerative Biology and Medicine that will investigate limb and spinal cord regeneration in amphibians.
Role: Principal Investigator

Completed
031400-0063 (Stocum) 8/26/00–8/26/02 (no-cost extension to 12/31/03))
State of Indiana 21st Century Research and Technology Fund
“Novel approaches for tissue and organ regeneration”
The major goal was to develop a research Center for Regenerative Biology and Medicine that will investigate limb and spinal cord regeneration in amphibians.
Role: Co-investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME OF SPONSOR (CO-SPONSOR)	POSITION TITLE
Simon J. Conway	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Essex, England	B.S. (Hons)	1986 – 1989	Cell Biology
University College London (MRC), England	Ph.D.	1989 – 1993	Genetics & Biometry
University College London (ICH), England	Postdoctoral	1993 – 1996	Molecular Embryology

A. Positions and Honors

Current and Previous Positions:

Apr-June 1995	Postdoctoral Research Traveling Fellowship, Heart Development Group, Medical College of Georgia, Augusta, USA. Sponsor: Regents Prof. Margaret Kirby, Ph.D.
Apr 1996-Jun 2000	Assistant Professor, Institute of Molecular Medicine & Genetics/CBA, MCG, Augusta.
July 2000-April 2003	Tenured Associate Professor, IMMAG/CBA, MCG, Augusta, USA.
Feb-March 2001	Visiting Scientist, The Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Belgium. Sponsor: Prof. Peter Carmeliet, MD.
April 2003 – present	Adjunct Faculty, Graduate Studies, Medical College of Georgia, Augusta, USA
April 2003 – present	Tenured Associate Professor, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, IUPUI.
Oct 2003 – present	Member, Regulation of Cell Growth Program, Indiana University Cancer Center.

Honors and Awards:

1989-1993	Medical Research Council (UK) Post-graduate Studentship.
1990	European Molecular Biology Organization (EMBO) Post-graduate Training Award.
1998-2003	British Heart Foundation (BHF) Post-doctoral Fellowship.
1998-2000	Basil O'Connor Starter Scholar Award, March of Dimes Birth Defects Foundation.
1998-2003	NIH National Heart, Lung & Blood Institute R29 & RO1 grants.
1999-2003	American Heart Association Southern/Ohio Valley Research Consortium, Committee 1.
2000	NIH NHLBI – Special Emphasis Panel (PO1).
2000	Outstanding Young Faculty Award, School of Medicine, Medical College of Georgia.
2000	Winner of Young Investigators Award, (American Section) International Society for Heart Research.
2001	Distinguished Research Award, Graduate School, MCG.
2001	H. Victor Moore Research Grant, Children's Heart Program, MCG.
2001	NIH Special Study Section, ZRG1.
2003-present	NIH NHLBI CVA Study Section Ad Hoc reviewer & National Science Foundation Grant Reviewer.
2003-2007	Renewal of NHLBI RO1 HL60714 grant.

B. Selected peer-reviewed publications

1. Conway, S.J., "*In Situ* hybridization of cell and tissue sections." In: *Methods in Molecular Medicine, Molecular Diagnosis of Cancer*. Edited by: F.E. Cotter, Humana Press, Totowa, NJ, USA. **15:**193-206 (1996).
2. [1st] Conway, S.J., [1st] Henderson, D.J. and Copp, A.J., "*Pax3* is required for cardiac neural crest migration in the mouse: evidence from the (Sp^{2H}) mutant," *Development*, **124:** 505-514 (1997).
3. Conway, S.J., Henderson, D.J., Kirby, M.L., Anderson, R.H. and Copp, A. J., "Development of a lethal

congenital heart defect in the *splotch* (*Pax3*) mutant mouse," *Cardiovascular Res*., **36:** 163-173 (1997).
4. Conway, S.J., Godt, R.E., Hatcher, C., Leatherbury, L., Zolotouchnikov, V.V., Brotto, M.A.P., Copp, A.J., Kirby, M.L. and Creazzo, T.L. Neural crest is involved in development of abnormal myocardial function," *J. Molecular and Cellular Cardiology*, **29:** 2675-2685 (1997).
5. # Munn, D., Zhou, M., Attwood, J., Bondarev, I., Conway, S.J., Marshall, B., Brown, C. and Mellor, A.L., "Prevention of allogeneic fetal rejection by tryptophan catabolism," *Science,* **281:** 1191-1193 (1998).
6. Conway, S.J., "Novel expression of the *goosecoid* transcription factor in the embryonic mouse heart," *Mechanisms of Development,* **81:** 207-211 (1999).
7. Clark, T.G., Conway, S.J., Scott, I.C., Labosky, P., Winnier, G., Bundy, J., Hogan, B.L. and Greenspan, D.S., "*mTolloid-like 1* is necessary for normal septation/positioning of heart," *Development,* **126:** 2631-2642 (1999).
8. Winnier, G., Kume, T., Deng, K., Rogers, R., Bundy, J., Raines, C., Hogan, B.L.M. and Conway, S.J., **"**Roles for the winged helix transcription factors MF1 and MFH1 in cardiovascular development revealed by non-allelic non-complementation of null alleles," *Dev. Biology,* **216:** 16-27 (1999).
9. Koushik, S.V., Bundy, J and Conway, S.J., "Sodium-calcium exchanger is initially expressed in a cardiac-restricted pattern within the early mouse embryo," *Mechanisms of Development,* **88:** 119-122 (1999).
10. *# Lee, Y., Song, A.J., Baker, R.K., Micales, B.K**.,** Conway, S.J. and Lyons, G.E., "*Jumonji,* a nuclear protein that is necessary for normal heart development," *Circulation Research,* **86:** 932-938 (2000).
11. # Conway, S.J., Bundy, J., Chen, J., Dickman, E., Rogers, R. and Will, B.M., "Abnormal neural crest stem cell expansion is responsible for the conotruncal heart defects within the *Splotch (Sp^{2H})* mouse mutant," *Cardiovascular Research,* **47:** 314-328 (2000).
12. *Koushik, S.V., Wang, J., Rogers, R., Moskofidis, D., Lambert, L., Creazzo, T. and Conway, S.J., "Targeted inactivation of the sodium-calcium exchanger (*Ncx1*) results in the lack of a heartbeat and abnormal myofibrillar organization," *FASEB J.* (March 12) 10.1096/fj.00-069fje & (Summary), **15:** 1-4 (2001).
13. Kruzynska-Frejtag, A., Machnicki, M., Rogers, R., Markwald, R.R. and Conway, S.J., **"***Periostin* (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation," *Mechanisms of Development*, **103:**183-188 (2001).
14. [1st] Henderson, D.J., [1st] Conway, S.J., [1st] Greene, N., Gerrelli, D., Murdoch, J.N., Anderson, R.H. and Copp, A.J., "Cardiovascular defects associated with abnormalities in midline development in the *loop-tail* mouse mutant," *Circulation Research,* **89:** 6-12 (2001).
15. *# Müller, J.G., Isomatsu, Y., Koushik, S.V., O'Quinn, M., Zile, M., Conway, S.J. and Menick, D.R., "Cardiac Specific Expression and Hypertrophic Upregulation of the Feline Na^+/Ca^{2+} Exchanger gene H1-promoter in a transgenic mouse model," *Circulation Research*, **90:**158-164 (2002).
16. Koushik, S.V., Chen, H., Wang, J. and Conway, S.J., "Generation of a conditional *loxP* allele of the *Pax3* transcription factor that enables selective deletion of the homeodomain," *Genesis,* **32:**114-117 (2002).
17. Lagutina, I., Conway, S.J., Sublett, J. and Grosveld, G., "PAX3-FKHR knock-in mice show developmental aberrations but do not develop tumors," *Mol.Cell Biol.,* **22:**7204-16 (2002).
18. Conway, S.J., Kruzynska-Frejtag, A., Kneer, P.L., Machnicki, M. and Koushik, S.V., "What cardiovascular defect does my prenatal mouse mutant have, and why?" *Genesis,* **34:** 1-21 (2002).
19. Yamagishi, H., Maeda, J., Hu, T., McAnally, J., Conway, S.J., Kume, T., Meyers, E.N., Yamagishi, C. and Srivastava, D., "*Tbx1* is regulated by tissue-specific forkhead proteins through a common *Sonic hedgehog* responsive enhancer," *Genes & Development,* **203:** 269-281 (2003).
20. Stalmans, I., Lambrechts, D., Desmet, F., Jansen, S., Wang, J., Maity, S., Kneer, P., von der Ohe, M., Swillen, A., Maes, C., Gewillig, M., Molin, D., Hellings, P., Boetel, T., Haardt, M., Compernolle, V., Dewerchin, M., Emanuel, B., Gittenberger-de Groot, A.C., Esguerra, C., Scambler, P., Morrow, B., Driscol, D., Moons, L., Carmeliet, G., Behn-Krappa, A., Devriendt, K., Collen, D., Conway, S.J.* and Carmeliet, P.*, "VEGF: a modifier of the *del22q11* (DiGeorge) syndrome?" *Nature Medicine,* **9:** 173-182 (2003).
21. Hill W.D., Hess D.C., Martin-Studdard A., Carothers J.J., Zheng J., Hale D., Maeda M., Fagan S.C., Carroll J.E., and Conway S.J., "*Sdf-1 (cxcl12*) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury," *J. Neuropath & Experimental Neurology* (in press).
22. Kruzynska-Frejtag, A., Wang, J., Maeda, M., Rogers, R., Krug, E., Hoffman, S., Markwald, R. and Conway, S.J. "*Periostin* is expressed within the developing teeth at the sites of epithelial-mesenchymal interaction," *Developmental Dynamics* (in press).

Denotes cover article; # denotes accompanying editorial; [1st] denotes joint authorship.

C. Research Support

Current

RO1 HL60714 (renewal) 4/03-3/07
National Heart, Lung and Blood Institute
"Molecular Mechanisms of Neural Crest-Related Heart Defects"
The major goal of this grant is to determine whether the Splotch mouse mutant neural crest cells are inherently defective - in an attempt to identify the mechanism/s responsible for the resultant conotruncal heart defects.
Role: PI

RO1 HL33756-17 7/02-6/07
National Heart, Lung and Blood Institute
"Mucoploysaccharide metabolism in cardiac anomalies"
The major goal of this grant is to determine the role mucoploysaccharids play in valve formation. PI: R. Markwald
Role: Co-I

PO1 HL52813 1/99-12/04
National Heart, Lung and Blood Institute
"Cardiac Valvoseptal Morphogenesis"
Goal of this PPG is to investigate the multiple cell interactions and remodeling events that gives rise to the four-chambered heart. Prof. Roger Markwald (Medical University of South Carolina) is the PI.
Role: Consortium Leader

PO1 HL48788-10 8/03-7/08
National Heart, Lung and Blood Institute
"Load-Induced Cardiac Hypertrophy in the Adult Mammal"
Goal of this PPG is to investigate causes/consequences of cardiac hypertrophy. (PI: Prof. George IV Cooper, MUSC).
Role: Co-I, Project 3

Grant-In-Aid 1/04-12/05
American Heart Association -Midwest Affiliate
"Characterization of vascular defects in the sodium-calcium exchanger (Ncx1) knockout mouse"
Goal of this grant is to investigate the mechanism of vascular degeneration in mutant embryos that lack a heartbeat.
Role: PI

Completed

Post-doctoral Fellowship 7/00-6/02
AHA/Southeast Affiliate
"Molecular Study of the failing embryonic Splotch heart"
Role: Sponsor

RO1 HL60104 5/98-4/03
NHLBI
"Calcium Handling in the Splotch Mouse Mutant"
Role: PI

Grant-in-Aid 7/02-6/04 (2nd Yr returned)
AHA-SE Affiliate
"Embryonic Heartbeat"
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andras Czirok	Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Eotvos University, Budapest, Hungary		1991-1994	Physics
Eotvos University, Budapest, Hungary	M.Sc.	1994-1996	Statistical Physics
Eotvos University, Budapest, Hungary	Ph.D.	1996-2000	Biological Physics
Univ. of Kansas Medical Center, Kansas City, KS	Postdoc.	2000-	Developmental Biology

A. Positions and Honors

Positions

1996 – 1999 Graduate Student, Department of Biological Physics, Eotvos University, Budapest, Hungary
1999 – 2000 Junior Fellow, Instit. of Advanced Study, Collegium Budapest, Budapest, Hungary
2000 - Postdoctoral Fellowship in Anatomy & Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS, Advisor: Charles D. Little, Ph.D.
2001 - Assistant Professor, Dept. of Biological Physics, Eotvos University, Budapest, Hungary

Honors

1991 XXII. International Physics Olimpiade, gold medal
1992, 1993 Ortvay National Contest in Physics: I. prize
1993, 1995 Award of the Faculty of Natural Sciences of Eotvos University
1992 – 1996 Scholarship of the Hungarian Republic
1993, 1995, 1997 National Conference for Student Scientist (OTDK): I. prize
1997 Pro Scientia Prize of Hungary
1999 Junior Fellow, Inst. of Advanced Study, Collegium Budapest, Hungary
2000 – 2001 NSF-NATO Postdoct. Fellowship in Developmental Biology, Univ. of Kansas Medical Center
2001 KUMC Training Program in Biomedical Research
2002 Award of the Hungarian Physical Society

Research experience at foreign institutions

1993 - 1996	Tel Aviv University	Host: E. Ben-Jacob	(6 months)
1995 - 1997	University of Notre Dame	Host: A.-L. Barabasi	(2 months)
1994 - 1997	Boston University	Host: H.E. Stanley	(6 months)
1997 - 2000	Medical University of South Carolina	Host: Charles D. Little	(5 months)

B. Selected peer-reviewed publications

On bacterial colony formation

1. Ben-Jacob, E., Shochet, O., Tenenbaum, A., Cohen, I., Czirok, A. and Vicsek ,T., “Generic modelling of cooperative growth patterns in bacterial colonies,” *Nature* **368**, 46 (1994).
2. Ben-Jacob, E., Cohen, I., Shochet, O., Czirok, A. and Vicsek, T., “Cooperative formation of chiral patterns during growth of bacterial colonies,” *Phys*. Rev. Lett. **75**, 2899 (1995).
3. Czirok, A., Janosi, I. M. and Kessler, J. O., “Oxytactic Bioconvection Patterns in Suspensions of Bacillus subtilis,” *J. Exp. Biol* **203**, 3345-3354 (2000).

4. Czirok, A., Matsushita, M. and Vicsek, T., "Theory of periodic swarming of bacteria: application to Proteus mirabilis," *Phys. Rev. E*. **63**, 31915-31926 (2001).
5. Janosi, I.M., Czirok, A., Silhavy, D. and Holczinger, A., "Is bioconvection enhancing bacterial growth in quiscent environments?" *Envir. Microb*. **4**: 525-31 (2002).

On theoretical analysis of collective migration

1. Vicsek, T., Czirok, A., Ben-Jacob, E., Shochet, O. and Cohen, I., "Novel type of phase transition in a system of self-driven particles." Phys. Rev. Lett. **75**, 1226 (1995).
2. Czirok, A., Stanley, H. E. and Vicsek, T., "Spontaneously ordered motion of self-propelled particles," *J. Phys* A **30**, 1375 (1997).
3. Czirok, A., Barabasi, A.-L. and Vicsek, T., "Collective motion of self-propelled particles: kinetic phase transition in one dimension," *Phys. Rev*. Lett. **82**, 209 (1998).

On in vitro cell motility

1. Czirok, A., Schlett, K., Madarasz, E. and Vicsek, T., "Exponential Distribution of Locomotion Activity in Cell Cultures," *Phys. Rev*. Lett. **81**, 3038 (1998).
2. Schlett, K., Czirok, A., Tarnok, K., Vicsek, T. and Madarasz, E., "Dynamics of cell aggregation during in vitro neurogenesis by immortalized neuroectodermal progenitors," *J. Neurosc. Res.* **60**, 184-194 (2000).
3. Hegedus, B., Czirok, A., Fazekas, I., Babel, T., Madarasz, E. and Vicsek, T., "Locomotion and proliferation of glioblastoma cells in vitro: statistical evaluation of videomicroscopic observations," *J. Neurosurgery* **92**, 428-434 (2000).
4. Kornyei, Zs., Czirok, A., Vicsek, T. and Madarasz, E., "Proliferative and migratory responses of astrocytes to in vitro injury," *J. Neurosc. Res.* **61**, 421-429 (2000).
5. Twal. W. O., Czirok, A., Hegedus, B., Knaak, C., Chintalapudi, M. R., Okagawa, H., Sugi, Y. and Argraves, W. S., "Fibulin-1 suppression of adhesion and motility," *J. Cell Sci.* **114**, 4587-4598 (2001).
6. Mehes, E., Czirok, A., Hegedus, B., Vicsek, T. and Jancsik, V., "Laminin-1 increases motility, path-searching and process dynamism of rat and mouse Muller glial cells in vitro," *Cell Motil. Cytoskel.* **53**: 203-213 (2002).
7. Hegedus, B., Zach, J., Czirok, A., Lovey, J. and Vicsek, T., "Irradiation and Taxol treatment result in non-monotonous, dose-dependent changes in the motility of glioblastoma cells," J. *Neuro-Onclogy* (in press, 2004).

C. Research Support

Current

Mathers Charitable Foundation (Little, C., PI) 2/1/02-1/31/05
G. Harold and Leila Y.
Vascular pattern analysis: A systems approach
The major goals of this project are to analyze vascular patterning in terms of primordial endothelial cell VEGF signaling, to examine the role integrin □v□3 plays in vascular motility and cell protrusive activity, and to establish a computational bioassay designed to quantify endothelial dynamics in vivo.
Role: Research Assistant Professor

NIH/NIHLB (Little, C., PI) 8/1/02- 7/31/07
1R01HL68855-01
Computational Biology of Vascular Cell Behavior.
The aims of this project are to use time-lapse microscopy and computational bioassays to examine the role of VEGF and integrins in formation of the primary vascular bed using the whole avian embryo as the experimental system.
Role: Research Assistant Professor

NIH/NIHLB (Rongish, B., PI) 09/30/02-09/29/05
1R01HL73700-01
Computational study of fibrillins in cardiovascular development.

The goals of this project entail the use of 4D optical scanning microscopy and computational analyses to examine and quantify fibrillin fibril motion and assembly in the cardiovascular system of normal and experimentally perturbed avian embryos.
Role: Research Assistant Professor

Completed

NSF-NATO (Czirok, A., PI) 10/01/00-9/31/01
Postdoctoral Fellowship
Biological Matrix During Embryonic Development
The major goals of this project are to elicit and study defective microfibril assembly in tissue culture and in the embryo, and to compare normal and disrupted assembly of microfibrils by fluorescent time-lapse microscopy.
Role: PI (Postdoctoral Fellow)

KUMC (Czirok, A., PI) 7/1/01-6/30/02
Training Program inBiomedical Research
Biological Matrix During Embryonic Development
The major goals of this project are to elicit and study defective microfibril assembly in tissue culture and in the embryo, and to compare normal and disrupted assembly of microfibrils by fluorescent time-lapse microscopy.
Role: PI (Postdoctoral Fellow)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Debasis Dan	Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
R. K. Mission College, India	B.S. (Hons)	1995	Physics
University of Poona, India	M.S.	1997	Physics
Institute of Physics, India	M.Phil.	1998	Physics
Institute of Physics, India	Ph.D.	2003	Statistical Physics
Department of Physics, Indiana University, US.	Postdoctoral	Current	Biophysics

A. Positions and Honors

1995	Topped UGC, India fellowship
1997-1999	Junior Research Fellow, Department of Atomic Energy,India.
1999	Fellow of CSIR, India.
1999-2003	Senior Research Fellow, Department of Atomic Energy, India.
2003-present	Post Doctoral Fellow, Indiana University, Bloomington.
2001-Present	In the board of referees for Physical Review and Physica

B. Selected peer-reviewed publications

1. A Biologically Inspired Ratchet Model of Two Coupled Brownian Motors, *Physica A* **318**, 40-47 (2003).
2. Giant diffusion and coherent transport in tilted periodic inhomogeneous systems, *Phys. Rev. E* **66**, 041106 (2002).
3. Bona fide Stochastic Resonance: A view point from stochastic energetics, accepted in *Physica A.*

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Vincent Jo Davisson	Professor Medicinal Chemistry & Molecular Pharmacology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wittenberg University, Springfield, OH	B.A.	1978	Chemistry
Indiana Univ., School of Med., Indianapolis, IN	M.S.	1983	Biochemistry
University of Utah, Salt Lake City, UT	Ph.D.	1988	Organic Chemistry
University of California, San Francisco, CA	Postdoctoral	1987-89	Biochemistry

A. Positions and Honors

Positions:

1978-1979 Research Technician, Dept of Biochemistry, Indiana University School of Medicine
1979-1981 Research Organic Chemist, Eli Lilly Laboratories, Indianapolis, IN
1987-1989 Postdoctoral Fellow, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, with Professor D.V. Santi
1989-1993 Assistant Professor, Department of Medicinal Chemistry and Pharmacology, Purdue University, West Lafayette, IN
1994-1999 Associate Professor, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN
1998-present Associate Head, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN
1999-present Professor, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN
2001-present Co-Director Purdue Discovery Park, Purdue University, West Lafayette, IN

Honors, Awards and Other Professional Activities

1978 American Institute of Chemists Undergraduate Award
1986 University of Utah Graduate Student Research Prize
1988 NIH Postdoctoral Trainee
1989 Damon Runyon-Walter Winchell Fellowship
1997 American Chemical Society, Division of Biological Chemistry Nomination Committee
1997-2001 National Institutes of Health Review Panel Member, Bioorganic and Natural Products Chemistry
1999-present Purdue University Scholar

B. Selected peer-reviewed publications

1. Firestine, S. M., Poon, S.-W., Mueller, E. J., Stubbe, J. and Davisson, V. J., "Reactions catalyzed by AIR carboxylases from *Escherichia coli* and *Gallus gallus*: a case for divergent catalytic mechanisms?" *Biochemistry* **33**, 11927-11934 (1994).
2. Chen, X.-Y., Chen, Y., Heinstein, P. and Davisson, V. J., "Cloning, expression, and characterization of the δ-(+)-cadinene synthase: a catalyst for cotton phytoalexin biosynthesis," *Archives of Biochemistry and Biophysics* **324**, 255-266 (1995).
3. Parker, A. R., Moore, J. A., Schwab, J. M. and Davisson, V. J., "*Escherichia coli* imidazoleglycerol phosphate dehydratase: spectroscopic characterization of the enzymatic product and the steric course of the reaction." *Journal of the American Chemical Society* **117**, 10605-10613, (1995).

4. Schiffer, C. A., Clifton, I. J., Davisson, V. J., Santi, D. V. and Stroud, R. M., "Crystal structure of human thymidylate synthase: a structural mechanism for guiding substrates into the active site." *Biochemistry* **34**, 16279-16287 (1995).
5. Tesmer, J. J. G., Klem, T. J., Deras, M. L., Davisson, V. J., Smith, J. L., "The crystal structure of GMP synthetase: a paradigm for 2 enzyme families and novel catalytic triad." *Nature Struct'l Bio.***3**, 74-86 (1996).
6. Chen, X.-Y., Wang, M., Chen, Y., Davisson, V. J. and Heinstein, P., "Cloning / heterologous expression of a second (+)-δ-cadinene synthase from *Gossypium arboreum," J. of Natural Products* **59**, 944-951 (1996).
7. Schmuke, J. J., Davisson, V. J., Bonar, S. L., Geehsling-Mullis, K. and Dotson, S. B., "Sequence analysis of the *Candida albicans ADE2* gene and physical separation of the two functionally distinct domains of the phosphoribosylaminoimidazole carboxylase," *Yeast* **13**, 769-776 (1997).
8. Hoops, G. C., Zhang, P., Johnson, W. T., Paul, N., Bergstrom, D. E. and Davisson, V. J., "Template directed incorporation of nucleotide mixtures using azole-nucleobase analogs," *Nucleic Acids Research* **25**. 4866-4871 (1997).
9. Firestine, S. M., Misialek, S., Toffaletti, D., Klem, T. J., Perfect, J. and Davisson, V. J., "Biochemical role of the *Cryptococcus neoformans* ADE2 protein in fungal *de novo* purine biosynthesis," *Archives of Biochemistry and Biophysics* **351**, 123-134 (1998).
10. Zhang, P., Johnson, W. T., Klewer, D., Paul, N., Hoops, G., Davisson, V. J. and Bergstrom, D. E., "Explor'y studies on azole carboxamides as nucleobase analogs: thermal denaturation studies on oligodeoxyribonucleotide duplexes containing pyrrole-3-carboxamide," *Nucleic Acids Research* **26**, 2208-2215 (1998).
11. Deras, M. L., Chittur, S. V. and Davisson, V. J., "N^2-Hydroxyguanosine 5'-monophosphate is a time dependent inhibitor of *Escherichia coli* guanosine monophosphate synthetase," *Biochem* **38**, 303-310 (1999)
12. D'Ordine, R. L., Klem, T. J. and Davisson, V. J., "N^1-(5'-phosphoribosyl)adenosine-5'-monophosphate cyclohydrolase: purification & characterization of a unique metalloenzyme," *Biochem.***38**, 1537-1546 (1999).
13. Klewer, D. A., Hoskins, A., Zhang, Peiming, Davisson, V. J., Bergstrom, Donald E., and LiWang, Andy C. (2000) "NMR Structure of a DNA duplex containing nucleoside analog 1-(2'-deoxy-δ-D-ribofuranosyl)-3-nitropyrrole and the structure of the unmodified control." *Nucleic Acids Research*, **28**, 4514-4522.
14. Chittur, S. V., Chen, Y. and Davisson, V. J. (2000) "Expression and purification of imidazole glycerol phosphate synthase from *Saccharomyces cerevisiae*." *Protein Expression and Purification* **18**, 366-377.
15. Klewer, Douglas A., Zhang, Peiming, Bergstrom, Donald E., Davisson, V. J., and LiWang, Andy C. (2001) conformations of nucleoside analogue (1-(2'-deoxy-□-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide in different DNA sequence contexts." *Biochemistry* **40**(6), 1518-1527.
16. Klem, T. K., Chen, Y. and Davisson, V. J., "Subunit interactions and glutamine utilization by *Escherichia coli* imadazole glycerol phosphate synthase," *Journal of Bacteriology* **182**, 989-996 (2001).
17. Chittur, S. V., Klem, T. J., Shafer, C. M. and Davisson, V. J., "Mechanism for acivicin inactivation of triad glutamine amidotransferases," *Biochemistry* **40**, 876-887 (2001).
18. Donovan, M. Schmuke, J. J., Fonzi, W. A., Bonar, S. L., Gheesling-Mullis, K., Jacob, G. S., Davisson, V. J. and Dotson, S. B., "Virulence of an ADE2 deficient *Candida albicans* strain in an immune-suppressed murine model of systemic candidiasis," *Infection and Immunity* **69**(4), 2542-2548 (2001).
19. Chaudhuri, Barnali N., Lange, Stephanie C., Myers, Rebecca S., Chittur, Sridar V., Davisson, V. Jo and Smith, Janet L., "Crystal structure of imidazole glycerol phosphate synthase: A tunnel through a (β/α)8 barrel joins two active sites," *Structure* **9**, 987-997 (2001).
20. Wu, W., Bergstrom, D. E. and Davisson, V. J,, "A Combination Chemical and Enzymatic Approach for the Preparation of Azolecarboxamide Nucleoside Triphosphate," *J. Org. Chem* **68**, 3860-3865 (2003).
21. Myers, R. S., Jensen, J. R., Deras, I., Smith, J. L. and Davisson, V. J., "Substrate-Induced Changes in the Ammonia Channel for Imidazole Glycerol Phoshpate Synthase," *Biochemistry* **42**, 7013-7022 (2003).
22. Chaudhuri, B. N., Lange, S. C., Myers, R. S., Davisson, V. J. and Smith, J. L., "Towards Understanding the Mechanism of the Complex Cyclization Reaction Catalyzed by Imidazole Glycerolphosphate Synthase: Crystal Structures of a Ternary Complex and the Free Enzyme" *Biochemistry* **42**, 7003-7012 (2003).
23. Zhang, D., Xie, Y., Mrozek, M.F., Ortiz, C., Davisson, V.J., Ben-Amotz, D., "Raman Detection of Proteomic Analytes," *Anal. Chemistry* 10.1021/ac0345087 (published online Sept. 20, 2003).
24. Paul, N., Nashine, V. C., Hoops, G., Zhang, P., Zhou, J., Bergstrom, D. E., Davisson, V. J., "DNA Polymerase Template Interactions Probed by Degenerate Isosteric Nucleobase Analogs," *Chemistry and Biology 2003* **10**: 815-825 (2003).
25. Zheng, O., Takats, Z., Blake, T.A., Gologan, B., Guymon ,A. J., Wiseman, J. M., Oliver, J. C., Davisson, V. J. and Cooks, R. G., "Preparing protein Microarrays by soft landing of mass-selected ions" *Science* **301**, 1351-1354 (2003).

C. Research Support
Current

RO1 GM 53155 (Bergstrom) 7/1/99 - 6/30/02
National Institutes of Health
"Modified Nucleosides as Tools for Molecular Biology"
Specific aims of the project are the design, synthesis, and physicochemical properties of nucleoside analogues derived from five-membered azole rings will be studied.
Role: Co-PI

1 R21 CA91116-01 (Davisson) 4/1/01-3/31/03
National Institutes of Health
"Enzyme Targets, Apoptosis and Purine Metabolism"
Specific Aims: To assess inhibition of central steps in de novo purine biosynthesis as an approach toward induction of programmed death of tumor cells (apoptosis). This initial phase of the project aims to validate ADE2 as a molecular target for the experimental ansamycin class of antitumor agents and to assess the relative importance of ADE2 as a target for cancer chemotherapy.
Role: PI

Completed
RO1 GM45756-09 (Davisson) 7/1/97 - 6/30/01 (no cost extension)
National Institutes of Health
"Enzymes of the Histidine Biosynthetic Pathway"
Specific Aims: The major goals of this project are to characterize several of the mechanistic details concerning enzymes in the histidine biosynthetic pathway.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Loren J. Field	Professor of Medicine and Pediatrics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
State University of New York at Oswego	B.A.	1978	Biology and Chemistry
State University of New York at Buffalo	M.S.	1980	Molecular Biology
State University of New York at Buffalo	Ph.D.	1982	Molecular Biology
State University of New York at Buffalo	Postdoctoral	1983	Cell & Molecular Biology
Roswell Park Memorial Institute, Buffalo, NY (NIH Fellow in 1985)	Postdoctoral	1983 – 1985	Molecular Biology

A. Positions and Honors

1986-1990 Staff Investigator, Senior Staff Investigator, Cold Spring Harbor Laboratory, NY
1990 Associate Professor, Indiana University School of Medicine, Krannert Institute of Cardiology
1995 Professor of Medicne, IU School of Medicine, Krannert Institute of Cardiology
1998 Professor of Pediatrics and Medicine, Indiana School of Medicine, Wells Center

B. Selected peer-reviewed publications

1. Field, L.J., Bobek, L.A., Brennan, V.E., Reilly, J.D. and Bruenn, J.A., "There are at least two yeast viral dsRNAs of the same size: An explanation for viral exclusion," *Cell* **31**:193-200 (1982).
2. Field, L.J., "Atrial natriuretic factor-SV40 T antigen transgenes produce tumors and cardiac arrhythmias in mice," *Science* **239**:1029-1033 (1988).
3. Field, L.J., "Cardiovascular research in transgenic animals," *Trends in Cardiovascular Medicine* **1**: 141-146 (1991).
4. Katz, E., Steinhelper, M.E., Daud, A. Delcarpio, J.B., Claycomb, W.C. and Field, L.J., "Cardiomyocyte proliferation in mice expressing a-cardiac myosin heavy chain-SV40 T antigen transgenes," *American Journal of Physiology* 262 (Heart and Circulatory Physiology **31**):H1867-1876 (1992).
5. Daud, A.I., Lanson, N.A. Jr., Claycomb, W.C and Field, L.J., "Identification of SV40 large T-Antigen associated proteins in cardiomyocytes from transgenic mice," *Am. Journal of Physiology* 264 (Heart and Circulatory Physiology **33**):H1693-H1700 (1993).
6. Jones, L.R. and Field, L.J., "Residues 2-25 of phospholamban are insufficient to inhibit the Ca++ transport ATPase of cardiac sarcoplasmic reticulum," *Journal of Biological Chemistry* **268**:11486-11488 (1993).
7. Soonpaa, M.H., Koh, G.Y., Klug, M.G. and Field, L.J., "Formation of nascent intercalated discs between grafted fetal cardiomyocytes and host myocardium," *Science* **264**:98-101 (1994).
8. Kim, K.K., Soonpaa, M.H., Daud, A.I., Kim, J.S., Koh, G.Y. and Field, L.J., "Tumor suppressor gene expression during normal and pathologic myocardial growth," *Journal of Biological Chemistry* **269**:22607-22613 (1994).
9. Koh, G.Y., Kim, S.J., Klug, M.G., Park, K., Soonpaa, M.H. and Field, L.J., "Targeted expression of TGF-ß1 and neovascularization in hearts bearing intracardiac grafts," *Journal of Clinical Investigation* **95**:114-121 (1995).
10. Koh, G.Y., Soonpaa, M. H., Klug, M.G., Pride, H.P., Zipes, D.P., Cooper, B.J. and Field, L.J., "Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs," *Journal of Clinical Invsetigation* **96**:2034-2042 (1995).
11. Klug, M.G., Soonpaa, M.H. and Field, L.J., "DNA synthesis and multinucleation in embryonic stem cell-derived cardiomyocytes," *American Journal of Physiology* **269** (Heart and Circulatory Physiology 38):H1913-1931 (1995).
12. Klug, M.G., Soonpaa, M.H., Koh, G.Y. and Field, L.J., "Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts," *Journal of Clinical Investigation* **98**:216-

224 (1996).
13. Kim, K.K., Daud, A.I., Wong, S.C., Pajak, L., Tsai, S.-C., Wang, H., Henzel, W.J. and Field, L.J., "Mouse RAD50 has limited epitopic homology to p53 and is expressed in the adult myocardium," *Journal of Biological Chemistry* **271**:29255-29264 (1996).
14. Sutton, J., Costa, R., Klug, M., Field, L.J., Xu, D., Largaespada, D.A., Fletcher, C.F., Jenkins, N.A., Copeland, N.G., Klemsz, M. and Hromas, R., "Genesis, a winged helix transcriptional repressor with expression restricted to embryonic stem cells," *Journal of Biological Chemistry* **271**:23126-23133 (1996).
15. Soonpaa, M.H., Kim. K.K., Franklin, M., Pajak, L. and Field, L.J., "Cardiomyocyte DNA synthesis and multinucleation during murine development," *American Journal of Physiology* **271**:H2183-2189 (1996).
16. Soonpaa, M.H., Koh, G.Y., Pajak, L., Jing, S., Wang, H., Franklin, M., Kim, K.K. and Field, L.J., "Cyclin D1 over-expression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice," *Journal of Clinical Investigation* **99**:2644-2654 (1997).
17. Pajak, L., Jin, F., Xiao, G.H., Soonpaa, M.H., Field, L.J and Yeung, R.S., "Sustained cardiomyocyte DNA synthesis in whole embryo cultures lacking the TSC2 gene product," *American Journal of Physiology* **273**:H1619-1627 (1997).
18. Soonpaa, M.H. and Field, L.J., "A survey of studies examining mammalian cardiomyocyte DNA synthesis," *Circulation Research* **83**:15-26 (1998).
19. Neumann, J., Boknik, P., DePaoili-Roach, A., Field, L.J., Rockman, H.A., Kobayashi, Y. and Jones, L.R., "Targeted over-expression of phospholamban to mouse atrium depresses Ca2+ transport and contractility," *Journal of Molecular and Cellular Cardiology* **30**:1991-2002 (1999).
20. Nakajima, H., Nakajima, H.O., Soonpaa, M.H., Jing, S. and Field, L.J., "Heritable lympho-epithelial thymoma resulting from a transgene insertional mutation," *Oncogene* **19**:32-38 (2000).
21. Tsai, S.-C., Pasumarti, K., Pajak, L., Franklin, M., Patton, B., Henzel W.J., and Field, L.J., "SV40 Large T Antigen binds a novel BH3 containing pro-apoptosis protein in the cytoplasm," *Journal of Biological Chemistry* **275**:3239-3246 (2000).
22. Nakajima, H., Nakajima, H.O., Salcher, O., Dittiè, A.S., Dembowsky, K., Jing, S. and Field, L.J., "Atrial, but not ventricular, fibrosis in mice expressing a mutant TGF-ß1 transgene in the heart," *Circulation Research* **86**:571-579 (2000).
23. Pasumarthi, K.B.S., Nakajima, H., Nakajima, H.O., Jing, S. and Field, L.J., "Enhanced cardiomyocyte DNA synthesis during myocardial hypertrophy in mice expressing a modified TSC2 transgene," *Circulation Research* **86**: 1069 – 1077 (2000).
24. Reinlib, L. and Field. L.J., "Transplantation: Future Therapy for Cardiovascular Disease? An NHLBI Workshop," *Circulation* **101**:e182-e187 (2000).
25. Huh, N.E., Pasumarthi, K.B.S., Soonpaa, M.H., Jing, S., Patton, B. and Field, L.J., "Functional abrogation of p53 is required for T-Ag induced proliferation in cardiomyocytes," *Journal of Molecular and Cellular Cardiology* **33**:1405-1419 (2001).
26. Pasumarthi, K.B.S., Tsai, S.-C., and Field, L.J., "Co-expression of mutant p53 and p193 render embryonic stem cell-derived cardiomyocytes responsive to the growth-promoting activities of adenoviral E1A," *Circulation Research* **88**:1004-1011 (2001).
27. Zandstra, P.W., Bauwens, C., Yin, T., Liu, Q., Schiller, H., Zweigerdt, R., Pasumarthi K.B.S., and Field, L.J., "Scalable production of embryonic stem cell–derived cardiomyocytes," *Tissue Engineering* **9**:767-98 (2003).
28. Rubart, M., Wang, E., Dunn, K.W. and Field, L.J., "Two photon molecular excitation imaging of Ca^{2+} transients in Langendorff-perfused mouse hearts," *American Journal of Physiology* **284**:C1645-C1688 (2003).
29. Rubart, M., Pasumarthi, K.B.S., Nakajima, H., Soonpaa, M., Nakajima, H.O. and Field, L.J., "Physiologic coupling of donor and host cardiomyocytes following cellular transplantation," *Circulation Research* **92**:1217-1224 (2003).

Selected review articles/book chapters

1. Pasumarthi, K.B.S. and Field, L.J., "Cardiomyocyte cell cycle regulation. *CircRes* **90**:1044-1054 (2002).
2. Pasumarthi, K.B.S., Daud, A.I. and Field, L.J., "Regulation of cardiomyocyte proliferation and apoptosis", in *Reactivation of the Cell Cycle in Terminally Differentiated Cells*, Marco Crescenzi, Ed., from the Molecular Biology Intellegence Unit 17, Landes Biosciences, Inc., 11-27 (2002).
3. Dowell, J.D., Rubart, M., Pasumarthi, K.B.S., Soonpaa, M.H. and Field, L.J. "Myocyte and myogenic stem cell transplantation in the heart," *Cardiovascular Research* **58**: 336-350 (2003).
4. Dowell, J.D. Field, L.J. and Pasumarthi, K.B.S., "Cell cycle regulation to repair the infracted myocardium," *Heart Failure Reviews* **8**:293-303 (2003).

C. Research Support

Current

1 RO1 HL45453 7/90-3/07
NIH, National Heart Lung and Blood Institute
"Cloning genes that regulate myocardiocyte proliferation"
This grant is concerned with identifying genes that regulate cardiomyocyte cell cycle using an SV40 transgenic mouse model for gene discovery
Role: PI

1 RO1 HL69119 9/01-9/05
NIH, National Heart Lung and Blood Institute
"Bone marrow stem cells and cardiac repair"
This grant is concerned with comparing the cardiomyogenic potential of a number of different bone marrow stem cells to embryonic stem cells following in vitro differentiation and following transplantation into the heart.
Role: PI

1 R01 HL75609 12/03-11/07
NIH
"Cyclin D2 and cardiac regeneration"
This grant is concerned with examining the degree to which forced expression of cyclin D2 can effect regenerative growth post MI.
Role: PI

1 P30 CA82709 9/99-6/04
NIH
"Cancer Center Support Grant"
This grant provides funds to defray the costs of generating transgenic and knock-out mice for members of the Indiana University Cancer Center.
Role: PI on Transgenic and Knock-out Mouse Core

1 RO1 HL66362 9/30/00-8/31/04
NIH, National Heart Lung and Blood Institute
"Electrophysiologic consequences and prevention of remodeling due to hypertrophy and fibrosis"
This grant is concerned with the electrophysiologic effects and arrhythmia vulnerability in mouse models of hypertrophy and fibrosis and to determine whether genetic alterations to prevent hypertrophy and fibrosis prevent electrophysiologic abnormalities.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME OF SPONSOR (CO-SPONSOR)	POSITION TITLE
Anthony B. Firulli	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Roger Williams College, Bristol, RI	B.S.	1983-1987	Chemistry & Biology
Roswell Park Cancer Institute, Buffalo, NY	Ph.D.	1987-1993	Molecular Biology

A. Positions and Honors

Research and Professional Experience:

1993 – 1995 Postdoctoral Fellowship, Dept. of Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, Houston, TX

1995 – 1997 Postdoctoral Fellowship, Hamon Center for Basic Cancer Research, U.T. Southwestern Medical Center, Dallas, TX

1997 – 2003 Assistant Professor, Department of Physiology, University of Texas Health Science Center at San Antonio, TX.

2003 – present Associate Professor Department of Pediatrics Wells center for Pediatric Research, Riley Hospital for Children Indiana University Medical School Indianapolis IN.

Awards and Honors:

1986 – 1987 Who's Who Among Students in America Universities and Colleges

1986 – 1987 Member Alpha Chi National Honor Society, Chapter President in 1987

1987 Alpha Chi Edward L. Davis Award

1987 American Chemical Society, High Achievement in Chemistry Award

1993 – 1996 Muscular Dystrophy Association, William C. Gibson Neuromuscular Disease Research Fellowship

B. Peer-reviewed publications

1. Davis, T.L., Firulli, A.B., and Kinniburgh, A.J. "Ribonucleoprotein and Protein Factors Bind to an H-DNA c-myc DNA Element: Possible Regulators of the c-myc Gene," *Proc. Natl. Acad. Sci. USA.* **86**, 9682-86 (1989).
2. Firulli, A.B., Maibenco, D.C., and Kinniburgh, A.J. "The Identification of a Tandem H-DNA Structure in the c-myc Nuclease Sensitive Promoter Element," *Biochem. Biophys. Res. Comm.* **185**, 264-270 (1992).
3. Firulli, A.B., Maibenco, D.C., and Kinniburgh, A.J. "Triplex Forming Ability of a c-myc Promoter Element Predicts Promoter Strength," *Arch. Biophys. Biochem.* **301**, 1236-1242 (1994).
4. Kinniburgh, A.J., Firulli, A.B., and Kolluri, R. "DNA Triplexes and the Regulation of the c-myc Gene." *Gene*. **149**, 93-100 (1994).
5. Lilly, B., Galewsky, S., Firulli, A.B., Schulz, R., and Olson, E.N. "D-MEF2: A MADS Box Transcription Factor Expressed in Differentiating Mesoderm and Muscle Cell Lineages During Drosophila Embryogenesis." *Proc. Natl. Acad. Sci. USA* **91**, 5662-5666 (1994).
6. Miano, J.M., Firulli, A.B., Olson, E.N., Hara, P., Giachelli, C.M., and Schwartz, S.M. "Restricted Expression of Homeobox Genes Distinguishes Fetal from Adult Human Smooth Muscle Cells." *Proc. Natl. Acad. Sci. USA.* **93**, 900-905 (1996).
7. Firulli, A.B., Miano, J.M., Weizhen, B.I., Johnson, D., Casscells, W., Olson, E.N., and Schwarz, J.J. Myocyte Enhancer-Binding Factor-2 Expression and Activity in Vascular Smooth Muscle Cells: Association with the Activated Phenotype." *Circ. Res.* **78**, 196-204 (1996).

8. Molkentin, J. D., Firulli, A.B., Black, B.L., Martin, J.F., Hustad, C.M., Copland, N., Jenkins, N., Lyons, G., and Olson, E.N. "MEF2B is a Potent Transactivator Expressed in Early Myogenic Lineages." *Mol. Cell. Bio.* **16**, 3814-3824 (1996).
9. Firulli, A.B., and Olson, E.N. "Modular Regulation of Muscle Gene Transcription: A Mechanism for Muscle Cell Diversity." *Trends in Genet.* **13**, 364-369 (1997).
10. Firulli, A.B., McFadden, D.G., Lin, Q., Srivastava, D., and Olson, E.N. "Heart and Extraembryonic Mesodermal Defects in Mouse Embryos Lacking the bHLH Transcription Factor Hand1." *Nature Genetics* **18**, 266-270 (1998).
11. Firulli, A.B., Hann, D., Kelly-Roloff, L., Schwartz, S.M., Olson, E.N., and Miano, J.M. "A Comparative Molecular Analysis of Four Rat Smooth Muscle Cell Lines." *In vitro Cellular and Developmental Biology-Animal.* **34**, 217-226 (1998).
12. Firulli, B. A., Hadzic, D. B., McDaid, J. R., and Firulli, A. B. "The bHLH Transcription factors dHAND and eHAND Exhibit Dimerization Characteristics that Suggest Complex Regulation of Function." *J. Biol. Chem.* **275** (43) 33567-33573 (2000).
13. McFadden D.G., Charite J., Richardson, J.A., Srivastava, D., Firulli, A.B. and Olson E.N. "A GATA-dependent right ventricle enhancer controls dHAND transcription in the developing heart." *Development*. **127**, 5331-5341 (2000).
14. Firulli A.B, and Thattaliyath B.D. "Transcription factors in cardiogenesis: The combinations that unlock the mysteries of the heart." *Inter. Rev. Cytology: A Survey of Cell. Biology* **214**, 1-62 (2002).
15. Thattaliyath B.D. Firulli, B.A. and Firulli, A.B. "The basic-helix-loop-helix transcription factor hand2 directly regulates transcription of the atrial naturetic peptide gene." J*. Molec. Cell Cardiol.* **34**, 10 1293-1296 (2002).
16. Thattaliyath B.D., Livi C.B, Steinhelper, M.E., Toney G.M. and Firulli, A.B. "HAND1 and HAND2 are expressed in the adult rodent heart and are modulated during cardiac hypertrophy." *Biochem. Biophys. Res. Comm.* **297**, 4870-4875 (2002).
17. Firulli, A.B. "Another hat for myocardin." *J. Molec. Cell Cardiol.* **34**, 10 1293-1296 (2002).
18. Firulli, A.B. "A HANDful of questions: the molecular biology of the hand-class of basic helix-loop-helix transcription factors." *Gene* **312**, 27-40 (2003).
19. Xu, H. Firulli, A.B. Wu, X., Zhang, X., and Howard, M.J. "Phox2a is a downstream target of hand2 in the tissue-specific expression of noradrenergic characteristics of sympathetic ganglion neurons: role of camp and pka signaling in hand2 regulated differentiation and dbh promoter activity." *J. Dev. Biol.* **262**, 183-193 (2003).
20. Firulli, B.A., Howard, M.J., McDaid, J.R., McIlreavey L, Dionne K.M., Centonze V.E., Cserjesi P., Virshup, D. and Firulli, A.B. "Phosphorylation of HAND1 and HAND2 is regulated by the protein phosphatase 2a containing the regulatory subunit b56□ and the protein kinases pka and pkc: implications for the control of tissue specific gene expression." *Mol. Cell.* **12**, 1225-1237 (2003).

Books

Genetic Control of Heart Development. (1997). Eds. E.N. Olson, R.P. Harvey, R.A. Shultz, J.S. Altman. Coeds. C. Biben, B. Black, R. Cripps, A.B. Firulli. HFSP, Strasbourg.

Selected Abstracts

1. Firulli, B. A., Hadzic, D. B., McDaid, J. R., and Firulli, A. B. The bHLH transcription factors dHAND and eHAND can form efficient homo and heterodimers suggesting E protein independent transcriptional regulation. The Southwest Regional Meeting of the Society for Developmental Biology. Houston Texas March 10-12, 2000.
2. Firulli B.A., McDaid J.M., McIlreavey L., Chang J. Cserjesi P., Virshup, D. and Firulli A.B. (2002). PKC, PKA and PP2A modulate dimerization of HAND class bHLH factors: A mechanism for tissue specific gene expression Weinstein Cardiovasular Development Conference Salt lake City Utah May 16-19
3. Firulli B.A., McDaid J.M., McIlreavey L., Chang J. Cserjesi P., Virshup, D. and Firulli A.B. (2002). PKC, PKA and PP2A modulate dimerization of HAND class bHLH factors: A mechanism for tissue specific gene expression 2ndinternational symposium basic helix-loop-helix genes regulators of normal development and indicators of malignant development. Amsterdam, the Netherlands October 20-22.
4. Reyes M., Steinhelper M.E., Escobedo, D. Jaramillo L.L., Firulli A.B., Freeman, G.L., and Feldman M.D. (2002). Augmented myocardial fibrosis caused by aortic banding in mice genetically deficent in the agntioxidant MnSOD. American Heart Association Scientific Sessions. 2002 November 17-19.

5. Xu, H., Firulli, A.B, Zhou, X. and M.J. Howard. (2002) Neuron subtype-specific gene expreession: identification of regulatory elements in HAND2. Neuro Science 2002 Orlando November 2-7, 2002.

C. Research Support

Current

#1-FY02-276 (Firulli, PI) 06/01/02-05/31/05
March of Dimes
Understanding the molecular control of HAND proteins in congenital heart disease & extraembryonic mesodermal development
The major goal of this proposal is to look at the role of phosphorylation/dephosphorylation of HAND1 in extraembryonic development. The specific focus is on identification of the critical residues within HAND1 and how addition of phosphates affects biological function. Using an in vitro trophoblast system RCHOI cells to determine HAND1 biological activity in trophoblasts.

2 RO1HL61677-05 (Firulli, PI) 9/31/03-10/01/07
National Institutes of Health
Transcription Factors Involved in Heart Development
The major goal is to continue to increase the understanding of how the molecular pathways control cardiogenesis through study of the functional mechanisms of bHLH factors play in the cardiogenic process. Protein-protein interactions between transcription factors convey tissue specific gene expression and dissecting out the proteins present in these cardiac-specific complexes and deducing the nature of their interactions is paramount to understanding how tissue specific gene programs are implemented.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Geoffrey C. Fox	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Cambridge University, Cambridge	B.A.	1961 – 1964	Mathematics
Cambridge University, Cambridge	M.A.	1968	Theoretical Physics
Cambridge University, Cambridge	Ph.D.	1964 – 1967	Theoretical Physics
Institute for Advanced Study, Princeton	Postdoc	1967 – 1968	Theoretical Physics
Lawrence Berkeley Lab., Berkeley	Postdoc	1968 – 1969	Theoretical Physics
Cavendish Laboratory, Cambridge	Postdoc	1969 – 1970	Theoretical Physics
Brookhaven National Laboratory, Long Island	Postdoc	1970	Theoretical Physics
Caltech, Pasadena	Postdoc	1970 – 1971	Theoretical Physics

A. Positions and Honors

1964	Senior Wrangler Part III Mathematics Cambridge
1967 – 1968	Member of School of Natural Science, Inst. for Advanced Study, Princeton, New Jersey
1971 – 1974	Assistant Professor of Physics, California Inst. of Tech.
1973 – 1975	Alfred P. Sloan Foundation Fellowship
1974 – 1988	Associate Professor of Physics, Executive Officer of Physics (1981-83), Dean for Educational Computing (1983-1985). Associate Provost for Computing (1986-1988), Professor of Physics (1979-1990), California Inst. of Tech.
1989 – present	Editor Concurrency: Practice and Experience, John Wiley, Inc.
1990 – present	Fellow of the American Physical Society
1990 – present	Adjunct Professor, Computer Science, Rice University
1990 – 2000	Director of Northeast Parallel Architectures Center, Syracuse University
1990 – 2002	Professor of Physics and Computer Science, Syracuse University
1997 – 1999	President WebWisdom.com Corporation
2000 – present	Chief Technologist Anabas Corporation
2000 – 2001	Professor of Computer Science, Associate Director of School of Computational Science and Information Technology and Director of the Computational and Information Science Laboratory, Chief Technologist of Office of Distributed and Distance Learning, Florida State University
2001 – present	Professor of Computer Science, Informatics, Physics. Indiana University
2001 – present	Director of Community Grid Laboratory; Pervasive Technology Laboratories at Indiana University

B. Selected peer-revied publications

1. Fox, G. C. and Holmes, K. C. “An Alternative Method of Solving the Layer Scaling Equations of Hamilton, Rollett, and Sparks,” *Acta Cryst.* **20**, 886 (1966).
2. Fox, G. C. “Methods for Constructing Invariant Amplitudes Free from Kinematic Singularities and Zeros,” P*hys. Rev.* **157**, 1493 (1967).
3. Fox, G. C. and Hey, A. J. G., “Non-diffractive Production of Meson Resonances,“*Nuclear Physics* **B56**, 386 (1973).
4. Fox, G. C., (with experimental groups at Caltech, UCLA, Chicago Circle, Fermilab and Indiana University), “Observation of the Production of Jets of Particles at High Transverse Momentum and Comparison with

Single Particle Reactions," *Phys. Rev. Letters* **38**, 1447 (1977).
5. Fox, G. C., Feynman, R. P., and Field, R. D., "Correlations among Particles and Jets with Large Transverse Momenta," *Nuclear Physics* **B128**, 1 (1977).
6. Fox. G. C., "Phenomenology of Asymptotically Free Theories in Deep Inelastic Scattering, I: Electron and Muon Scattering," *Nuclear Physics* **B131**, 107 (1977).
7. Fox, G. C. and Wolfram, S., "Observables for the Analysis of Event Shapes in e+e- Annihilation and Other Processes," *Phys. Rev. Letters* **41**, 1581 (1978).
8. Fox. G. C., Bhalla, U., Furmanski, W., Nelson, M. Wilson, M. and Bower, J. "Structural Simulations of Neural Networks Using a General-Purpose Neural Network Simulator and a Hypercube Concurrent Computer," in *Proceedings of the Third Conference on Hypercube Concurrent Computers and Applications*, Volume 2, edited by G. C. Fox, ACM Press, 977-999 (1988).
9. Fox, G., Johnson, M., Lyzenga, G., Otto, S., Salmon, J. and Walker, D., *Solving Problems in Concurrent Processors-Volume 1*, Book published by Prentice Hall, March 1988.
10. Rose, K., Gurewitz, E., and Fox, G. C. "Statistical mechanics and phase transitions in clustering," *Physical Review Letters* **65**(8), 945-948 (1990).
11. Fox, G., Angus, I., Kim, J. and Walker, D., *Solving Problems in Concurrent Processors-Volume 2*, Book published by Prentice Hall, 1990.
12. Rose, K. Gurewitz, E. and Fox, G., "Vector Quantization by Deterministic Annealing," *IEEE Trans. Inf. Theory* **38**, 1249 (1992).
13. Fox, G. C., Messina, P. and Williams, R., "Parallel Computing Works!," Morgan Kaufmann, San Mateo Ca, 1994.
14. Fox, G., "Internetics: Technologies, Applications and Academic Fields" Invited Chapter in Book *:Feynman and Computation,* edited by A.J.G. Hey, Perseus Books (1999).
15. Fox, G. C., "From Computational Science to Internetics: Integration of Science with Computer Science," *Mathematics and Computers in Simulation, Elsevier,* **54** 295-306 (2000). http://www.npac.syr.edu/users/gcf/internetics2/
16. Fox, G.C. and Coddington, P.D. "Parallel Computers and Complex Systems," in *Complex Systems* edited by Terry R.J. Bossomaier and David G. Green, Cambridge University Press, 2000.
17. Fox, G.C., Hurst, K., Donnellan, A. and J. Parker, "Introducing a New Paradigm for Computational Earth Science – A web-object-based approach to Earthquake Simulations", a *chapter in AGU monograph on Physics of Earthquakes*, edited by John Rundle and published by AGU in 2000.
18. Lee, H.-K., Carpenter, B., Fox, G., Lim, S. B. on *Benchmarking HPJava: Prospects for Performance*; Proceedings of Sixth Workshop on "Languages, Compilers, and Run-time Systems for Scalable Computers" (LCR 02) http://grids.ucs.indiana.edu/ptliupages/publications/HPJavafeb02.pdf
19. Fox, G. and 10 co-authors, "The Online Knowledge Center: Building a Component Based Portal," Proceedings of the International Conference on Information and Knowledge Engineering, Las Vegas, June 2002. http://grids.ucs.indiana.edu/ptliupages/publications/ike02okc.pdf
20. Fox, G. and 14 co-authors, "An Architecture for e-Science and its Implications" in *Proceedings of the 2002 International Symposium on Performance Evaluation of Computer and Telecommunications Systems*, edited by Mohammed S.Obaidat, Franco Davoli, Ibrahim Onyuksel and Raffaele Bolla, Society for Modeling and Simulation International, 14-24 (2002). http://grids.ucs.indiana.edu/ptliupages/publications/spectsescience.pdf
21. Pierce, M., Youn, C., Balsoy, O., Fox, G., Mock, S. and Mueller, K., *Interoperable Web Services for Computational Portals,* Proceedings of SC02 November 2002.
22. Shrideep Pallickara and Geoffrey Fox *NaradaBrokering: A Distributed Middleware Framework and Architecture for Enabling Durable Peer-to-Peer Grids* in Proceedings of ACM/IFIP/USENIX International Middleware Conference Middleware-2003, Rio Janeiro, Brazil June 2003 http://grids.ucs.indiana.edu/ptliupages/publications/NB-Framework.pdf
23. Geoffrey Fox and 10 co-authors, *Collaborative Web Services and Peer-to-Peer Grids* proceedings of 2003 Collaborative Technologies Symposium (CTS'03) Orlando January 2003. http://grids.ucs.indiana.edu/ptliupages/publications/foxwmc03keynote.pdf
24. Berman, F., Fox, G. and Hey, T. "Grid Computing: Making the Global Infrastructure a Reality," book published 2003 by John Wiley. http://www.grid2002.org
25. Mehmet A. Nacar, Marlon Pierce and Geoffrey C. Fox *Developing a Secure Grid Computing Environment Shell Engine* to be published in special issue on Grid computing in Journal of Neural Parallel and Scientific Computations (NPSC) http://grids.ucs.indiana.edu/ptliupages/publications/npsc_gceshell.pdf

C. Research Support

Current

Lilly Foundation: Community Grids Laboratory 7/01/01-12/31/04
"Core Community Grids Lab Support"
Develop collaboration as a Web service and pervasive access to Grids using PDA's.
Role:Lead Design of new collaborative tools in a Grid architecture

User Environments 9/1/03-8/31/06
"Middlewre for the Development of Grid Computing"
National Science Foundation
Role: Lead OGCE Collaboration in developing portlets for computational Grids

Earthquake Science HPCC 4/1/02-3/31/05
National Aeronautics and Space Administration: JPL
Develop software engineering approach for large scale parallel computing based on web services. Customize computing portal to support Earth science applications.
Role: Architect of Grid and Software Engineering approach for SERVOGrid

High Perf. Web-Grid Service 4/1/02-3/31/04
National Science Foundation: NCSA
Identify opportunities for use of Grid Services in NASA aeronautical applications. Demonstrate with prototypes.
Role: Investigate role of Semantic Grid in Earth Science Applications

Indiana CS Research Infrastructure 7/1/02-6/30/07
National Science Foundation
Computer Infrastructure to support Indiana University Computer Science Department Research.
Role: Grid Server farm applications supporting new CS department hardware

Portal Web Services 4/1/02-3/31/05
Department of Energy SciDAC
Develop architecture of Grid Computing Environments and apply to DoE Fusion applications.
Role: Develop Grid Portals for DoE Science Grid

Dist. Knowledge Mgmt System 4/1/02-3/31/05
CRANE NSWC
Research and prototype middleware for a Fleet maintenance system for use by US Navy.
Role: Develop Messaging System and Portal for CRANE NSWC

Completed

PET Core and OKC Project 10/1/01-9/30/02
Department of Defense: Mississippi U.
Develop and support deployment of the Online Knowledge Centre for the Department of Defense High Performance Computing Modernization Office. Develop distributed authoring, XML-based messaging and other tools.
Role: Architect and Prototype Online Knowledge Center for DoD HPCMO

PACI Partner ET and EOT 10/1/01-9/30/02
National Science Foundation via NCSA
Develop outreach to minority serving institutions including collaborative portals for education and computing. Support large scale simulations on NSF supercomputers using Grid compatible portals.
Role: Develop Collaboratory for Indian Tribal Colleges. Develop CORBA based Computational Portal

Programming Fortran to Java 7/6/01-11/30/02
National Science Foundation
Research novel parallel computing paradigms exploiting advanced features of Java but consistent with other languages like Fortran. Develop MPI Java binding and HPJava compiler.
Role: Develop Parallel Compiler for Java Language

PACI Partner ET and EOT 10/1/01-9/30/02
National Science Foundation via NCSA
Design Collaboratory for Indian Nation Tribal Colleges. Work with Grid Forum to categorize and integrate different approaches to Grid Computing.
Role: Design Collaboratory for Indian Tribal Colleges. Develop initial architecture of Grid-based computational portals

Collaboration Technology
Department of Defence:HPTi 10/1/01-9/30/02
Design and prototype audio/video conferencing as a Web Service.
Role: Design Web service based Collaboration system

PACI Partner ET and EOT 10/1/02-9/30/02
National Science Foundation via NCSA
Continue outreach and develop messaging and other services for computing portals. Research special features of a collaborative portal for education.
Role: how how Jetspeed can integrate research and education portals

PET Gateway Portal 10/1/01-9/30/02
Department of Defense: Mississippi University
Demonstrate interoperable portals for DoD HPCMO applications.

PET Core and OKC Core 10/1/02-9/30/03
Department of Defense: Mississippi University
Continue to research technologies for Online Knowledge Center (OKC) working with US Army Corps of Engineers Center at Vicksburg Miss. Develop workflow mechanism to support approval of new material in OKC.
Role: Refine DoD HPCMO Online Knowledge Center

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Dennis Gannon	Professor and Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Davis, CA	B.S.	1965–1969	Mathematics
University of California, Davis, CA	M.S.	1969–1971	Mathematics
University of California, Davis, CA	Ph.D.	1971–1974	Mathematics
University of Illinois, Champaign, IL	Ph.D.	1976–1980	Computer Science

A. Positions and Honors

1975 – 1976	Lecturer in Mathematics, California State College, San Bernadino
1980 – 1985	Assistant Professor, Dept. of Computer Science, Purdue University, West Lafayette, IN
1982	Best Paper, International Conference on Parallel Processing
1983 – 1985	IBM Faculty Research Fellow
1985	Associate Professor, Dept. of Computer Science, Purdue University, West Lafayette, IN
1985 – 1995	Associate Professor, Department of Computer Science, Indiana University, Bloomington, IN
1989 – 1995	Research Directory, Center for Innovative Computer Applications, Indiana University, Bloomington, IN
1995 – present	Professor of Computer Science, Indiana University, Bloomington, IN
1997 – present	Chair, Department of Computer Science, Indiana University, Bloomington, IN
1998 – 2000	Director of Enabling Technologies, NASA Information Power Grid Project
2000 – present	Science Director, Pervasive Technology Laboratories, Indiana University, Bloomington, IN

B. Selected peer-revied publications

1. Norman, M., Beckman, P., Bryan, G., Dubinski, J., Gannon, D., Hernquist, L., Keahey, K., Ostriker, J.P., Shalf, J., Welling, J. and Yang, S., “Galaxies Collide on the I-WAY: An Example of Heterogeneous Wide-Area Collaborative Supercomputing," *Journal of Supercomputer Applications,* **10**(3), (1996).
2. Keahey, K. and Gannon, D., “PARDIS: A Parallel Approach to CORBA”, Proceedings 6th IEEE International Symposium on High Performance Distributed Computation, August 1997, Portland. (Selected for Best Paper Award)
3. Diwan, S. and Gannon, D., “A Capabilities Based Communication Model for High-Performance Distributed Applications: The Open HPC++ Approach,” Proceedings of the Second Merged Symposium IPPS/SPDP, April, 1999.
4. Armstrong, R., Gannon, D., Geist, A., Keahey, K., Kohn, S., McInnes, L., Parker, S. and Smolinski, B., “Toward a Common Component Architecture for High-Performance Scientific Computing,” Proceedings High Performance Distributed Computing Conference 1999.
5. Johnston, W., Gannon, D. and Nitzberg, B., “Grids as Production Computing Environments: The Engineering Aspects of NASAs Information Power Grid,” Proceedings High Performance Distributed Computing Conference 1999.
6. Bramley, R., Chiu, K., Diwan, S. and Gannon, D., Govindaraju M., Mukhi N., Temko B., Yechuri M., “A Component Based Services Architecture for Building Distributed Applications”, Proceedings High Performance Distributed Computing Conference 2000.
7. Johnston, W., Gannon, D., Nitzberg, B., Woo, A., Thigpen, B. and Tanner, L. A., “Computing and Data Grids for Science and Engineering,” proceeding *IEEE SC2000*.

8. Slominski, A., Govindaraju, M., Gannon, D. and Bramley, R., "Design of an XML based Interoperable RMI System: SoapRMI C++/Java 1.1," International Conference on Parallel and Distributed Processing Techniques and Applications, Las Vegas, Pages 1661-1667, June 25-28 2001.
9. Krishnan, S., Bramley, R., Govindaraju, M., Indurkar, R., Slominski, A., Gannon, D., Alameda, J. and Alkaire, D., "The XCAT Science Portal," *Proceedings SC2001*, Nov. 2001, Denver.
10. Gannon, D., Bramley, R., Fox, G., Smallen, S., Rossi, A., Ananthakrishnan, R., Bertrand, F., Chiu, K., Farrellee, M., Govindaraju, M., Krishnan, S., Ramakrishnan, L., Simmhan, Y., Slominski, A., Ma, Y., Olariu, C. and Rey-Cenvaz, N., "Programming the Grid: Distributed Software Components, P2P and Grid Web Services for Scientific Applications," *Journal of Cluster Computing* **5**(3), 325-336 (2002).
11. Ramakrishnan, L., Rehn, H., Alameda, J., Ananthakrishnan, R., Govindaraju, M., Slominski, A., Connelly, K., Welch, V., Gannon, D., Bramley, R. and Hampton, S., "An Authorization Framework for a Grid Based Component Architecture," Grid 2002 Workshop at SC2002, Baltimore Oct. 2002.
12. Chiu, K., Govindaraju, M. and Gannon, D., "The Proteus Multiprotocol Library," in *Proceedings of the 2002 Conference on Supercomputing*, November 2002.
13. Gannon, D. and Reynders, J., "Parallel Object Oriented Libraries," chapter 14 in *CRPC Parallel Computing Handbook*, J. Dongarra, I. Foster, G. Fox, K. Kennedy, L. Torczon, and A. White, editors, Morgan-Kaufmann Publishers, 2002.
14. Gannon, D., Ananthakrishnan, R., Krishnan, S., Govindaraju, M., Ramakrishnan, L. and Slominski, A., "Grid Web Services and Application Factories," to appear in *State of the Grid 2002,*" Fox, Berman, Hey, editors.

C. Research Support

Current

Department of Energy 8/1/01 - 7/31/04
"Middleware Technology to Support Science Portals: a Gateway to the Grid" with R. Bramley

Department of Energy 8/15/01 - 8/14/06
"Center for Component Technology for Terascale Simulation Software" with R. Bramley
Part of a distributed center involving Lawrence Livermore National Labs, Los Alamos National Labs, Sandia Laboratories, the University of Utah, Oak Ridge National Laboratory and Argonne National Labs.

National Science Foundation – MRI 9/1/01 - 8/31/04
"Creation of the AVIDD Data Facility" with R. Bramley, et. al.
Funding for a large data and compute cluster for interdisciplinary science.

Completed

National Science Foundation 10/99-10/02
"GrADS: Grid Advanced Development Systems"
Subcontract through Rice University. A study of compilation and scheduling strategies for grid computing.

Department of Energy 10/97-9/00
"A High Performance Software Component Architecture for SciTL"
Research on software component systems for distributed and parallel applications.

National Science Foundation 1997-2002
"National Computational Science Alliance", subcontract from NCSA
Partner in the NCSA Alliance working on software for scientific applications on parallel and distributed systems.

Univ. of Illinois DARPA subcontract. 6/13/97-6/12/00
"An Integrated, Language-Directed Performance Prediction, Measurement and Analysis Environment"
Analysis tools for distributed applications

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Holly V. Goodson	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Princeton University, Princeton, NJ	B.A.	1984-1988	
Stanford University, Stanford, CA	Ph.D.	1988-1995	
University of Geneva, Switzerland		1995-1999	

A. Positions and Honors

Professional positions and experience

1995 -1999 Post Doctoral Fellow, Department of Cell Biology, University of Geneva
2000 - present Assistant Professor, Dept. of Chemistry and Biochemistry, University of Notre Dame

Honors

1988 -1993 National Science Foundation Predoctoral Fellowship
1991 Society of General Physiologists' Award, MBL Physiology course
1995 -1996 EMBO Long Term Fellowship
1996 -1999 Helen Hay Whitney Foundation Fellowship

Local and national service

2000 -2001 Biochemistry admissions coordinator
2001 Organizer of ASCB Special Interest Subgroup Meeting “Microtubule Plus-end Tracking Proteins” (with Dr. Kevin Vaughan of Notre Dame)
2001 - 2002 Founding Associate Director of ND Center for the Study of Biocomplexity
2001 - 2004 Member American Heart Association Study Section (GA 4b, Molec. Signalling 2)
2001 - present Founding coorganizer of “The Chicago Cytoskeleton” meetings
2001 - present Member of Executive Committee, ND Center for the Study of Biocomplexity
2002 - present Biochemistry representative to Graduate Studies Committee
2003 Session Chair for Gordon Conference: “Contractile and Motile Systems”

B. Selected peer-reviewed publications

1. Diamantopoulos, G. S., Perez, F., Goodson, H. V., Batelier, G., Melki, R., Kreis, T. E. and Rickard, J. E., "Dynamic localization of CLIP-170 to microtubule plus-ends is coupled to microtubule assembly," *Journal of Cell Biology* **144,** 99-112 (1999)
2. Goodson, H. V., Warrick, H. M. and Spudich, J. A., "Specialized conservation of surface loops of myosin: evidence that loops are involved in determining functional characteristics," *Journal of Molecular Biology* **287**, 173-85 (1999).
3. Eckley, D. M., Gill, S. R., Melkonian, K. A., Bingham, J. B., Goodson, H. V., Heuser, J. E. and Schroer, T. A., ”Analysis of dynactin subcomplexes reveals a novel actin-related protein associated with the arp1 minifilament pointed end,” *J Cell Biol*. **147**, 307-20 (1999).
4. Perez, F., Pernet-Gallay, K., Nizak, C., Goodson, H. V., Kreis, T. E. and Goud, B., “CLIPR-59, a new trans-Golgi/TGN cytoplasmic linker protein belonging to the CLIP-170 family,” *Journal of Cell Biology* **156**(4), 631-42 (2002).
5. Lebrand, C., Corti, M., Goodson, H., Cosson, P., Cavalli, V., Mayran, N., Faure, J. and Gruenberg, J., “Late endosome motility depends on lipids via the small GTPase Rab7,” *EMBO J.* **21**(6),1289-300 (2002).

6. Goodson, H. V. and Hawse, W. F., "Molecular Evolution of the Actin Family," *Journal of Cell Science* **115**(Pt 13), 2619-22 (2002).
7. Askham, J. M., Vaughan, K. T., Goodson, H. V. and Morrison, E. E., "Sructure/function analysis of EB1: EB1 forms mutually exclusive complexes with APC and the $p150^{Glued}$ subunit of dynactin," *Molecular Biology of the Cell* **13**(10), 3627-3645 (2002).
8. Goodson, H. V., Barr, S. A., Stalder, R., Valetti, C., Kreis, T. E. and Schroer, T. A., "CLIP-170 interacts with dynactin complex and the APC-binding protein EB1 by different mechanisms," *Cell Motility and the Cytoskeleton* **55**(3), 156-73 (2003).
9. Gregoretti, I., Lee, Y.-M. and Goodson, H. V., "Molecular Evolution of the Histone Deacetylase Family: Functional Implications of Phylogenetic Analysis," *Journal of Molecular Biology*, (in press).
10. Pfluger, S. L., Goodson, H. V., Moran, J. M., Ruggiero, C. J., Ye, X., Emmons, K. M. and Hager, K. M., "A Receptor for Retrograde Transport in the Apicomplexan Parasite, *Toxoplasma gondii*." (Submitted*).*

C. Research Support

Current

American Heart Association 1/1/2001 – 12/31/2004
Scientist Development Grant
"Identification and Characterization of CLIP-170-Related Microtubule-Binding Proteins (the "ClipR" family)
Role: PI

National Institutes of Health (RO1) 4/1/2003 – 3/31/2008
"Interactions between CLIP-170 and Microtubules"
Role: PI

Completed

United States Department of Agriculture 9/1/01 – 8/31/03
RX2210-810-ND
Principal Investigator: Elmendorf (Georgetown Univerisity)
"Flagellar Function and the Role of Motility in Virulence of Giardia lamblia"
Role: Subcontract to perform biochemical analysis of tubulin binding

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Matthew Wayne Grow	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Purdue University, West Lafayette, IN	B.S.	1985–1993	Neuro & Animal Physics
University of Texas at Austin, Austin, TX	Ph.D.	1994–1999	Molecular Biology

A. Positions and Honors

1993 Independent Researcher, Southwestern Research Station, Portal, AZ
1993 Teaching Assistant, Purdue University, W. Lafayette, IN
1993 Student Researcher, Laboratory of Dr. John Anderson, Purdue University, W. Lafayette, IN
1994 – 1995 Instructional Coordinator, Purdue University, W. Lafayette, IN
1994 – 1996 Program in Molecular Biology Fellowship, University of Texas
1995 Teaching Assistant, University of Texas at Austin, Austin, TX
1996 Teaching Assistant, University of Texas at Austin, Austin, TX
1996 – 1998 David Bruton Fellowship, University of Texas at Austin
1997 – 1998 University of Texas Tuition Scholarship, University of Texas at Austin
1998 Teaching Assistant, University of Texas at Austin, Austin, TX
1998 Dorthea Bennet Memorial Fellowship, University of Texas at Austin
1994 – 1999 Graduate Research Assistant, Lab of Paul Kreig, University of Texas at Austin, Austin, TX
2000 – 2001 Postdoctoral Fellow, Laboratory of Dr. Mark Fishman, Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA
2001-present Assistant Professor, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

B. Selected peer-revied publications

1. Zorn, A.M., Grow, M.W., Patterson, K.D., Ebersole, T.A., Chen, Q., Artzt, K., and Krieg, P.A., “Remarkable sequence conservation of transcripts encoding amphibian and mammalian homologues of quaking, a KH-domain RNA-binding protein,” *Gene* **188**(2), 199-206 (1997).
2. Newman, C.S., Grow, M.W., Cleaver, O.B., Chia, F. and Krieg, P.A., “Xbap, a vertebrate bagpipe-related gene is expressed in developing craniofacial structures and in the anterior gut primordium,” *Developmental Biology* **181**, 223-233 (1997).
3. Patterson, K.D., Cleaver, O.B., Gerber, W.V., Newman, C., Grow, M., and Krieg, P.A., “Homeobox genes in cardiovascular development,” *Current Topics in Developmental Biology* **40**, 1-44 (1998).
4. Grow, M.W., and Krieg, P.A., “Tinman function is essential for vertebrate heart development: Elimination of cardiac differentiation by dominant inhibitory mutants of the tinman-related genes, XNkx2-3 and XNkx2-5,” *Developmental Biology* **204**, 187-196 (1998).
5. Newman, C. S., Reecy, J., Grow M. W., Ni, K., Boettger, T., Kessel, M., Schwartz, R. J., and Krieg, P. A., “Transient cardiac expression of the tinman-family homeobox gene, XNkx2-10,” *Mechanisms of Development* **91**, 369-373 (2000).
6. Breese, M. R., Stephens, M. J., McClintick, J. N., Grow, M. W. and Edenberg, H. J. “Labrat LIMS: an extensible framework for developing laboratory information management, analysis, and bioinformatics solutions for microarrays,” *SAC 2003* (2003).

C. Research Support

N/A

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
David Charles Hart	Manager, High Performance Computing Support

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Purdue University, West Lafayette, IN	B.S.	1972	Mathematics
University of California, Berkeley, CA	M.S.	1974	Mathematics
University of California, Berkeley, CA	Ph.D.	1980	Mathematics

A. Positions and Honors

1980-1986 Assistant Professor, Department of Mathematics, University of Florida
1986-1992 Assistant Professor, Department of Mathematics, University of Cincinnati
1992-2000 Visiting Assistant Professor, Department of Mathematics, Indiana University
1992-1998 Mathematical Computing Specialist, Center for Statistical and Mathematical Computing, Indiana University
1998-present Manager, High Performance Computing Support Team, University Information Technology Services, Indiana University
2000 Organizer, Symposium on Scientific Computing on Commodity Clusters, First SIAM Conference on Computational Science and Engineering

B. Selected peer-reviewed publications (Publications selected from 14 peer-reviewed publications)

1. Stewart, C.A., D. Hart, D. K. Berry, G. J. Olsen, E. Wernert, W. Fischer. “Parallel implementation and performance of fastDNAml - a program for maximum likelihood phylogenetic inference,” Proceedings of SC2001, Denver, CO. (2001). http://www.sc2001.org/papers/pap.pap191.pdf
2. Hart, D., D. Grover, M. Liggett, R. Repasky, C. Shields, S. Simms, A. Sweeny, P. Wang. “Distributed Parallel Computing Using Windows Desktop Systems,” Proceedings of the International Workshop on Challenges of Large Applications in Distributed Environments (2003). http://www.indiana.edu/~rac/papers/CLADE2003.doc

C. Research Support

Current

NSF Prop #0338618 (grant # not yet available, announced 9/29/03)
“The Indiana/Purdue Grid” (IP-grid)
The major goal of this project is to enhance the capabilities of NSF’s Extensible Terascale Facility and put unique resources online for the nation’s research and education community.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Randy Wayne Heiland	Associate Director, Scientific Data Analysis Lab, Indiana University Pervasive Technology Labs

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Eastern Illinois University, Charleston, IL	B.S.	1976-1979	Computational Math
University of Utah, Salt Lake City, UT	M.S.	1982-1985	Computer Science
Arizona State University, Tempe, AZ	M.A.	1989-1992	Mathematics

A. Positions and Honors

1979-1982 Computer Programmer, Caterpillar Tractor Co., Peoria, IL.
Software development for a graphics library

1982-1985 Teaching/Research Assistant, University of Utah, Salt Lake City, UT.
Computer Graphics/Modeling Group, Computer Science Dept. TA for Fortran programming course. RA/thesis on user interface for the Alpha-1 modeling (B-splines) package.

1985-1987 Computer Scientist, Center for Industrial Research, Oslo, Norway.
Computer-Aided Geometric Design Group. Writing software for commercial CAGD software package. Computer graphics.

1988-1989 Teaching/Research Assistant, Arizona State University, Tempe, AZ.
CAGD Group, Computer Science Dept. TA for introductory computer graphics course. RA: writing software for medical data visualization and computer-aided geometric design applications.

1989-1992 Research Assistant, Arizona State University, Tempe, AZ.
Dynamical Systems Group, Mathematics Dept. Visualizing/analyzing data from CFD/turbulence researchers. Thesis involved an open source package, KLTool, for analyzing spatiotemporal data.

1992 Graduate Research Assistant, Advanced Computing Lab, Los Alamos National Lab, Los Alamos, NM. Parallel visualization applications for ocean/climate simulation data.

1993-1997 Computer Scientist, Environmental Molecular Sciences Lab, Pacific Northwest National Lab, Richland, WA. Computational chemistry visualization. Medical (ultrasound) data visualization. Parallel computing applications.

1997-2002 Visualization Scientist, NCSA: A variety of visualization/VR-related projects for NCSA/Alliance and DoD scientists. Extensive use of open source software. Parallel and distributed computing applications. High-resolution tiled display.

2002 Instructor, Fall Semester, Parkland Community College, Champaign, IL
Taught computer graphics programming using OpenGL

1999-2003 Project lead for VisBench, NCSA: Project involving remote visualization and analysis of large data. Based on a distributed component architecture involving servers and clients communicating via CORBA or Web Services. Used VTK, MATLAB, Java, Python.

2002-2003 Senior Research Scientist, NCSA visualization lead for the NSF TeraGrid Project.

2003-present Associate Director, Scientific Data Analysis Lab, Pervasive Technology Labs at Indiana University

B. Selected peer-reviewed publications

1. Armbruster, D., Heiland, R., Kostelich, E., and Nicolaenko, B., “Phase-space analysis of bursting behavior in Kolmogorov flow,” *Physica D* **58** (1992).
2. Armbruster, D., Heiland, R., and Kostelich, E.J., “KLTool: A tool to analyze spatiotemporal complexity,” *Chaos* **4** (2) (1994).
3. Stone, E., Armbruster, D., and Heiland, R., “Towards analyzing the dynamics of flames,” Fields Institute Communications, *Pattern Formation: Symmetry Methods and Applications*, J.Chadam, M.Golubitsky, W.Langford, B.Wetton (Eds.), American Mathematical Society (1996).
4. Littlefield, R.J., Heiland, R.W. and Macedonia, C.R. , “Virtual reality volumetric display techniques for three-dimensional medical ultrasound”, *Ultrasound in Health Care in the Information Age*, H. Sieburg, S. Weghorst, and K. Morgan (Eds.), IOS Press and Ohmsha (1996).
5. Heiland, R., Baker, M.P. and Tafti, D., “VisBench: A framework for remote data visualization and analysis,” *Springer Verlag Lecture Notes in Computer Science (LNCS)*, Proceedings of the 2001 International Conference on Computational Science, San Francisco (2001).

C. Research Support

N/A

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Stephen C. Jacobson	Associate Professor, Indiana University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Georgetown University, Washington, DC	B.S.	1988	Mathematics
University of Tennessee, Knoxville, TN	Ph.D.	1992	Chemistry
Oak Ridge National Laboratory, Oak Ridge, TN	Postdoctoral	1992-95	Chemistry

A. Positions and Honors

Employment

1992 – 1995 Alexander Hollaender Distinguished Postdoctoral Fellow, Oak Ridge National Laboratory
1995 – 2003 Research Staff, Oak Ridge National Laboratory
2000 – 2003 Adjunct Assistant Professor, University of Tennessee, Knoxville
2003 – present Associate Professor, Indiana University, Bloomington

Other Experience and Professional Memberships

2000-2003 Scientific Committee, Lab Automation Annual Conference and Exhibition
2000-2003 Scientific Committee, SmallTalk: Microfluidics, Microarrays, and BioMEMs Conf
2001 Program Committee, Fifth International Conference on Miniaturized Chemical and Biochemical Analysis Systems, uTAS
2001-2002 Secretary, East Tennessee Section of the American Chemical Society
2001-present Editorial Advisory Board, *Journal of Separation Science*,.
2002-2003 Oak Ridge National Laboratory Seed Money Proposal Review Committee
2002-present International Editorial Advisory Board, *Lab on a Chip*, Royal Society of Chemistry
2003-present Board of Directors, Association of Laboratory Automation

Honors

1992 John A. Dean Award for the Top Analytical Chemistry Student, The University of Tennessee
1996 Technical Achievement Award, Lockheed Martin Energy Research
1996 Significant Event Award, Lockheed Martin Energy Research
1996 R&D 100 Award, R&D Magazine
2001 R&D 100 Top 40, R&D Magazine

B. Selected peer-reviewed publications

1. Khandurina, J., Jacobson, S. C., Waters, L. C., Foote, R. S. and Ramsey, J. M., "Microfabricated Porous Membrane Structure for Sample Concentration and Electrophoretic Analysis," *Analytical Chemistry* **71**, 1815-1819 (1999).
2. Jacobson, S. C., Ermakov, S. V. and Ramsey, J. M., "Minimizing the Number of Voltage Sources and Fluid Reservoirs for Electrokinetic Valving in Microfluidic Devices," *Analytical Chemistry* **71**, 3273-3276 (1999).
3. Schrum, D. P., Culbertson, C. T., Jacobson, S. C. and Ramsey, J. M., "Microchip Flow Cytometry Using Electrokinetic Focusing," *Analytical Chemistry* **71**, 4173-4177 (1999).
4. Jacobson, S. C., McKnight, T. E. and Ramsey, J. M., "Microfluidic Devices for Electrokinetically Driven Parallel and Serial Mixing," *Analytical Chemistry* **71**, 4455-4459 (1999).
5. Fister, J. C., Jacobson, S. C. and Ramsey, J. M., "Ultrasensitive Cross-Correlation Electrophoresis on Microchip Devices," *Analytical Chemistry* **71**, 4460-4464 (1999).
6. Hadd, A. G., Jacobson, S. C. and Ramsey, J. M., "Microfluidic Assays of Acetylcholinesterase Inhibitors," *Analytical Chemistry* **71**, 5206-5212 (1999).
7. Kutter, J. P., Jacobson, S. C. and Ramsey, J. M., "Solid Phase Extraction on Microfluidic Devices," *Journal of Microcolumn Separations* **12**, 93-97 (2000).

8. Alarie, J. P., Jacobson, S. C., Culbertson, C. T. and Ramsey, J. M., "Effects of the Electric Field Distribution on Microchip Valving Performance," *Electrophoresis* **21**, 100-106 (2000).
9. Dunn, W. C., Jacobson, S. C., Waters, L. C., Kroutchinina, N., Khandurina, J., Foote, R. S., Justice, M. J., Stubbs, L. J. and Ramsey, J. M., "PCR Amplification and Analysis of Simple Sequence Length Polymorphisms in Mouse DNA Using a Single Microchip Device," *Analytical Biochemistry* **277**, 157-160 (2000).
10. Soughayer, J. S., Krasieva, T., Jacobson, S. C., Ramsey, J. M., Tromberg, B. J. and Allbritton, N. L., "Characterization of Cellular Optoporation with Distance," *Analytical Chemistry* **72**, 1342-1347 (2000).
11. Ng, K. C., Ford, J. V., Jacobson, S. C., Ramsey, J. M. and Barnes, M. D., "Polymer Microparticle Arrays from Electrodynamically Focused Microdroplet Streams," *Review of Scientific Instruments* **71**, 2497-2499 (2000).
12. Ermakov, S. V., Jacobson, S. C. and Ramsey, J. M., "Computer Simulations of Electrokinetic Injection Techniques in Microfluidic Devices," *Analytical Chemistry* **72**, 3512-3517 (2000).
13. Khandurina, J., McKnight, T. E., Jacobson, S. C., Waters, L. C., Foote, R. S. and Ramsey, J. M., "Integrated System for Rapid PCR-Based DNA Analysis in Microfluidic Devices," *Analytical Chemistry* **72**, 2995-3000 (2000).
14. Gottschlich, N., Culbertson, C. T., McKnight, T. E., Jacobson, S. C. and Ramsey, J. M., "Integrated Microchip-Device for the Digestion, Separation and Postcolumn Labeling of Proteins and Peptides," *Journal of Chromatography B* **745**, 243-249 (2000).
15. Lazar, I. M., Ramsey, R. S., Jacobson, S. C., Foote, R. S. and Ramsey, J. M., "Novel Microfabricated Device for Electrokinetically Induced Pressure Flow and Electrospray Ionization Mass Spectrometry," *Journal of Chromatography A* **892**, 195-201 (2000).
16. Liu, Y., Foote, R. S., Culbertson, C. T., Jacobson, S. C., Ramsey, R. S. and Ramsey, J. M., "Electrophoretic Separation of Proteins on Microchip," *Journal of Microcolumn Separations* **12**, 407-411 (2000).
17. Liu, Y., Foote, R. S., Jacobson, S. C., Ramsey, R. S. and Ramsey, J. M., "Electrophoretic Separation of Proteins on a Microchip with Noncovalent, Postcolumn Labeling," *Analytical Chemistry* **72**, 4608-4613 (2000).
18. Culbertson, C. T., Jacobson, S. C. and Ramsey, J. M., "Microchip Devices for High Efficiency Separations," *Analytical Chemistry* **72**, 5814-5819 (2000).
19. Alarie, J. P., Jacobson, S. C. and Ramsey, J. M., "Electrophoretic Injection Bias in Microchip Valving," *Electrophoresis* **22**, 312-317 (2001).
20. Gottschlich, N., Jacobson, S. C., Culbertson, C. T. and Ramsey, J. M., "Two-Dimensional Electrochromatography/Capillary Electrophoresis Microchip Device," *Analytical Chemistry* **73**, 2669-2674 (2001).
21. McKnight, T. E., Culbertson, C. T., Jacobson, S. C. and Ramsey, J. M., "Electroosmotically Induced Hydraulic Pumping with Integrated Electrodes on Microfluidic Devices," *Analytical Chemistry* 73, 4045-4049 (2001).
22. McClain, M. A., Culbertson, C. T., Jacobson, S. C. and Ramsey, J. M., "Flow Cytometry of *Esherichia coli* on Microfluidic Devices" *Analytical Chemistry* **73**, 5334-5338 (2001).
23. Culbertson, C. T., Jacobson, S. C. and Ramsey, J. M., "Diffusion Coefficient Measurements Using Microfluidic Devices," *Talanta* **56**, 365-373 (2002).
24. Broyles, B. S., Jacobson, S. C. and Ramsey, J. M., "Sample Filtration, Concentration and Separation Integrated on Microfluidic Devices," *Analytical Chemistry* **75**, 2761-2767 (2003).
25. Tsouris, B., Culbertson, C. T., DePaoli, D. W., Jacobson, S. C., de Almeida, V. F. and Ramsey, J. M., "Electrohydrodynamic Mixing in Microchannels," *AIChE Journal* **49**, 2181-2186 (2003).
26. Ramsey, J. D., Jacobson, S. C., Culbertson, C. T. and Ramsey, J. M., "High Efficiency, Two-Dimensional Separations of Protein Digests on Microfluidic Devices," *Analytical Chemistry* **75**, 3758-3764 (2003).
27. McKnight, T. E., Melechko, A. V., Guillorn M. A., Merkulov, V. I., Doktycz, M., Culbertson, C. T., Jacobson, S. C., Lowndes, D. H. and Simpson, M. L., "Effects of Microfabrication Processing on the Electrochemistry of Carbon Nanofiber Electrodes," *Journal of Physical Chemistry* **107**, 10722-10728 (2003).
28. McClain, M. A., Culbertson, C. T., Jacobson, S. C., Allbritton, N. L., Sims, C. E. and Ramsey, J. M., "Microfluidic Devices for the High Throughput Chemical Analysis of Cells," *Analytical Chemistry* **75**, 5646-5655 (2003).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Charles D. Little	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
California State Polytechnic University, Pomona, CA	B.S.	1973	Biology
University of Pittsburgh, Pittsburgh, PA	Ph.D.	1977	Cell Biology
Harvard Medical School, Boston, MA	Postdoctoral	1979	Developmental Biology
University of California, San Diego, CA	Postdoctoral	1981	Cell Biology

A. Positions and Honors

1973-1977 Pre-doctoral Fellowship in Ophthalmology Research Laboratory, Eye and Ear Hospital, Pittsburgh, PA. Advisor: Robert L. Church, Ph.D.
1977-1979 Research Fellow in Medicine, Developmental Biology Lab, Massachusetts General Hospital and Harvard Med. School, Boston, MA. Mentor: Thomas F. Linsenmayer, PhD
1979-1981 Postdoctoral Fellow, Department of Biology, Univ. of California, San Diego, La Jolla, CA
1979-1982 Mentor: S.J. Singer, Ph.D.
1981-1987 Assistant Professor of Anatomy and Cell Biology, Univ. of Virginia, Charlottesville, VA
1987-1993 Associate Professor of Anatomy and Cell Biology (with tenure), Univ. of Virginia, Charlottesville, VA
1993-2000 Professor of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC
1993-2000 Co-Director, Cardiovascular Developmental Biology Center, MUSC, Charleston, SC
1995-1996 Visiting Director, Program in Developmental Mechanisms, National Science Foundation
2000-present Professor of Anatomy and Cell Biology, Univ. of Kansas Med. Center, Kansas City, KS

Honors

1978-1979 Fellow of Damon Runyon - Walter Winchell Cancer Fund
1979-1981 Senior Postdoctoral Fellow, American Heart Assoc.
1982-1984 Basil O'Conner Starter Grant, March of Dimes Birth Defects Foundation
1992 Site visit teams for NIHLB PO4 applications, U. of Washington, Seattle; U. of Iowa (1993)
1988 Member NIH Special Study Section "Basic Biology of Cardiac Development"
1986 Member NIH Special Study Section "Devel. and Different. of Airway Epithelium"
1985-1989 Advisory Panel, Program in Developmental Biology, NS
1989-1992 Member of the Board of Trustees, Society for Developmental Biology
1992 Advisory Panel, Graduate Research Training, National Science Foundation
1990-1993 Director, Graduate Program in Cell and Molecular Biology, Univ. of Virginia
1990-1994 Member Pathobiochemistry Study Section, NIH
1995-1996 Director, Program in Animal Developmental Mechanisms, NSF
11/01-8/02 Interim Senior Associate Dean for Research, Univ. of Kansas Med Cntre
2003-present Editorial Board: Developmental Dynamics and Embryo Today
2003-present Chair, Cardiovascular Development, American Heart Assoc.

B. Selected peer-reviewed publications

1. Little, C.D., Church, R.L., Miller, R.A., and Ruddle, F.H., “Evidence for a different type of collagen produced by a Teratocarcinoma-derived cell line, TSD4: evidence for a new molecular form of collagen,” *Cell* **10**:287-295 (1977).

2. Linsenmayer, T.F. and Little, C.D. “Embryonic neural retina collagen: in vitro synthesis of high molecular weight forms of type ii plus a new genetic type,” *Proc. Natl. Acad. Sci*. U.S.A. **75**: 3235-3239 (1978).
3. Linsenmayer, T.F., Hendrix, M.J.C. and Little, C.D., “Production and characterization of a monoclonal antibody to chicken type I collagen,” *Proc. Natl. Acad. Sci. U.S.A*. **76**:3703-3707 (1979).
4. Davis, L.A., Ogle, R.O., and Little, C.D., “Embryonic heart mesenchymal cell migration in laminin,” *Dev Biol* **133**: 37-43 (1989).
5. Little, C.D., Piquet, D.M., Davis, L.A. and Drake, C.J., “The distribution of laminin, collagen type IV, collagen type I and fibronectin in chicken cardiac jelly/basement membrane,” *Anat. Rec*. **224**: 417-425 (1989).
6. Drake, C.J., Davis, L.A., Hungerford, J.E. and Little, C.D., “Perturbation of ß1 integrin-mediated adhesions results in altered somite cell shape and behavior,” *Dev. Biol*. **149**:327-338 (1991).
7. Gallagher, B.C.; Sakai, L.Y. and Little, C.D., “Fibrillin delineates the primary axis of the early embryo,” *Develop. Dynamics*, **196**: 70-78 (1993).
8. Drake, C.J., Cheresh, D.A., Little, C.D., “An antagonist of integrin □ v □ 3 prevents maturation of blood vessels during embryonic neovascularization,” *J. Cell Sci*. **108**:2655 (1995).
9. Drake, C.J., Little, C.D., “Exogenous VEGF induces malformed and hyperfused vessels during embryonic neovascularization,” *Proc. Natl. Acad. Sci*, USA. **92**:7657-7661 (1995).
10. Bouchey, D., Drake, C.J., Little, C.D., “Distribution of connective tissue proteins during development and neovascularization of the epicardium,” *Cardiovasc. Res*., **31**:E104-E115 (1996).
11. Bouchey, D., Argraves, W.S, and Little, C.D., “Fibulin -1, vitronectin and fibronectin in valvuloseptal development,” *Anat. Rec.* **244**:540-551 (1996).
12. Hungerford, J.E., Owens, G.K., Argraves, W.S., Little, C.D., “Development of the vessel wall as defined by vascular smooth muscle and ECM markers,” *Dev. Biol*., **178**:375-392 (1996).
13. Hungerford, J.E., Hoeffler, J.P., Bowers, C.W., Dahm, L.M., Falchetto, R., Shabanowitz, J., Hunt, D.F., Little, C.D., “Identification of a novel marker for primordial smooth muscle and its differential expression pattern in contractile versus noncontractile cells*,” J. Cell Biol*., **137**: 925-937 (1997).
14. Drake, C.J., Brandt. S.J., Trusk, T.C., and Little, C.D., “TAL1/SCL is expressed in endothelial progenitor cells/angioblasts and defines a dorsal-to-ventral gradient of vasculogenesis,” *Dev. Biol.* **192**:17-30 (1997).
15. Rongish, B. J., Drake, C.J., Argraves, W.S. and Little, C.D., “Identification of the developmentally expressed JB3 antigen as avian fibrillin-2,” *Dev. Dynamics*, **212**: 461-471 (1998).
16. Drake, C.J. and Little, C.D., “VEGF and vascular fusion: implications for normal and pathological vessels,” *J. Histochem. & Cytochem*. **47**:1351-1355 (1999).
17. Drake, C.J., LaRue, A., Ferrara, N., and Little, C.D., “VEGF regulates cell behavior during vasculogenesis,” *Dev. Biol.* **224**:178-188 (2000).
18. Rupp, P.A. and Little, C.D, “Integrins in vascular development,” *Circ.Res.* **89**: 566-572 (2001).
19. Czirok, A., Rupp, P.A., Rongish, B.J., Little, C.D., “Multi-field 3D scanning light microscopy of early embryogenesis. *J. of Microscopy* **206**:209-217 (2002).
20. Visconti, R.P., Barth, J.L., Argraves, Keeley, F. and Little, C.D., “Fibrillins, tropoelastin and fibulin-1 are expressed during early morphogenesis of the vertebrate body plan,” *Matrix Biology*, **22**:109-11 (2003).
21. Rupp, P.A., Rongish, B.J., Czirok, A.C., and Little, C.D., “Culturing of avian embryos for time-lapse imaging,” *Biotechniques* **34**:274-278 (2003).
22. Rupp, P.A., Czirok, A., and Little, C.D., “Novel approaches for studying blood vessel morphogenesis, in vivo,” *Trends in Cardiovascular Medicine*, **13**:283-288 (2003).
23. Czirok, A., Rongish, B.J. Little, C.D., “Extracellular matrix dynamics during vertebrate axis formation.” In press, *Develop. Biol.* (2003).
24. Rupp, P.A., Czirok, A. and Little, C.D., “Endothelial cell dynamics during vasculogenesis.” In revision after initial review, *Development* (2003).

C. Research Support

Current

G. Harold and Leila Y. (Little, PI) 12/01/01-11/30/04
Mathers Charitable Foundation
"Vascular pattern analysis: A systems approach"
The major goals of this project are to implement a systems-approach for the study of embryonic endothelial cell motility and pattern formation of the primary vascular bed. The efforts include software and instrumentation development for computational analyses of morphogenesis *in situ* and to generate virtual models of embryonic endothelial cell behavior.
Role: PI

1R01HL73700-01 (Rongish, PI) 09/30/02-09/29/05
NIH/NIHLB
"Computational study of fibrillins in cardiovascular development"
The goals of this project entail the use of 4D optical scanning microscopy and computational analyses to examine and quantify fibrillin fibril motion and assembly in the cardiovascular system of normal and experimentally perturbed avian embryos.
Role: Co-PI

1R01HL68855-01 (Little, PI) 8/1/02- 7/31/07
NIH/NIHLB
"Computational Biology of Vascular Cell Behavior"
The aims of this project are to use time-lapse microscopy and computational bioassays to examine the role of VEGF and integrins in formation of the primary vascular bed using the whole avian embryo as the experimental system.
Role: PI

Completed

1RO1-HL-57645-05 (Little, PI) 9/30/96-8/31/02
NIH/NHLBI
"Assembly of the vessel wall in health and disease"
The major goals of this project are: 1) test whether the vessel wall is a composite of smooth muscle from two sources- endothelium and mesenchyme; 2) evaluate the function of specific integrins during smooth muscle investment of aortic endothelium, and 3) characterize proteins with unique expression patterns during early vascular morphogenesis.
Role: PI

1 F32 HL68457-01 (Rupp, PI) 08/01/01-07/31/03
NIH/NHLBI
"Vascular Fusion: interplay between VEGF and integrins"
The major goal of this project is to quantitatively analyze VEGF-mediated cell behavior during de novo blood vessel formation. Computational time lapse imaging will be used to describe the behavior of embryonic endothelial cells.
Role: Sponsor

PO1-HL-52813-06 (Markwald, PI) 10/1/00-12/31/04*
NIH/NHLBI
C.D. Little, Director, Consortium III
"Cell behavior during endocardial morphogenesis"
The major goal of this project is to assess endocardial cell behavior during formation of the endocardial cushions.
Role: Consortium Director
*As of 12/03, Dr. Little's project no longer receives any funding from this award.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrew Lumsdaine	Associate Professor of Computer Science

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge, MA	B.S.	1984	Electrical Engineering and Computer Science
Massachusetts Institute of Technology, Cambridge, MA	M.S.	1986	Electrical Engineering
Massachusetts Institute of Technology, Cambridge, MA	Ph.D.	1992	Electrical Engineering and Computer Science

A. Positions and Honors

1992	Visiting Fellow, Army High Performance Computing Research Center, Minneapolis, MN
1992 – 1997	Assistant Professor, Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN
1997 – 2001	Associate Professor, Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN
1999 – 2000	Scientist (visiting faculty), Lawrence Berkeley National Lab (on one year sabbatical from Notre Dame)
2001 – present	Associate Professor, Computer Science Department, Indiana University, Bloomington, IN
2001 – present	Director, Open Systems Laboratory, Pervasive Technology Laboratories, Indiana University, Bloomington, IN

Affiliations
The Association for Computing Machinery
The Institute of Electrical and Electronic Engineers
The Society for Industrial and Applied Mathematics

Honors

1984 – 1985	General Motors Graduate Fellowship
1987 – 1990	American Electronics Association/Dynatech Faculty Development Fellowship
1995	NSF CAREER Award
1996	CSE Department Undergraduate Teaching Award

B. Principal publications

1. William Gropp, Steven Huss-Lederman, Andrew Lumsdaine, Ewing Lusk, Bill Nitzberg, William Saphir, and Marc Snir, *MPI – The Complete Reference: Volume 2, The MPI-2 Extensions, MIT Press,* 1998.
2. Jeffrey M. Squyres, Andrew Lumsdaine, William L. George, John G. Hagedorn, Judith E. Devaney, "The Interoperable Message Passing Interface (IMPI) Extension to LAM/MPI," Proceedings 2000 MPI Developers Conference.
3. Jeremy Siek and Andrew Lumsdaine, "Concept Checking: Binding Parametric Polymorphism in C++", First Workshop on C++ Template Programming, Erfurt, Germany, 2000.
4. L.R. Petzold, U. Ascher, H.T. Banks, J. Crowley, W. Gander, L. Greengard, M. Heath, A. Lumsdaine, C. Moler, T. Oden, R, Schnabel, K. Steward, A. Trefethen, "Graduate Education in Computational Science and Engineering," *SIAM Review,* 43(1): 163-177, Mar 2001.

5. Jeremy Siek, Lie-Quan Lee, and Andrew Lumsdaine, *The Boost Graph Library—User Guided and Reference Manual,* Addison-Wesley, 2002.
6. L.S. Blackford, J. Demmel, J. Dongarra, I. Duff, S. Hammarling, G. Henry, M. Heroux, L. Kaufman, A. Lumsdaine, A. Petitet, R. Pozo, K. Remington, R.C. Whaley, "An Updated Set of Basic Linear Algebra Subprograms (BLAS)," *ACM Transactions on Mathematical Software* 28(2): 135-151 Jun 2002.
7. Thomas Naughton, Stephen L. Scott, Brian Barrett, Jeff Squyres, Andrew Lumsdaine, and Yung-Chin Fang, "the Penguin in the Pail – OSCAR Cluster Installation Tool", "The 6th World Multi Conference on Systemics, Cybernetics and Informatics" (SCI2002), 2002, Invited Session of SCI'02, Commodity, High Performance Cluster Computing Technologies and Applications, Orlando, FL, USA.
8. Sibylle Schupp, D.P. Gregor, B. Osman, David R. Musser, Jeremy G. Siek, Lie-Quan Lee, Andrew Lumsdaine: Concept Based Component Libraries and Optimizing Compilers. IPDPS 2002.
9. Todd Veldhuizen and Andrew Lumsdaine, "Guaranteed Optimization: Proving Nullspace Properties of Compliers," 2002 Static Analysis Symposium (SAS '02) Madrid, Spain, Sept, 17-20, 2002.
10. Lie-Quan Lee and Andrew Lumsdaine, Generic Programming for High Performance Scientific Applications, ACM Java Grande -- ISCOPE 2002 Conference, November, 2002.
11. J. Jarvi, G. Powell and A. Lumsdaine: The Lambda Library: Unnamed functions in C++. Software Practice and Experience, 33(3), 2003.
12. A. Lumsdaine and D. Wu, "Krylov-Subspace Acceleration of Waveform Relaxation," *SIAM Journal on Numerical Analysis,* 41(1): 90-111, 2003.
13. Brian Barrett, Jeff Squyres, and Andrew Lumsdaine. Integration of the LAM/MPI environment and the PBS scheduling system. In Proceedings, 17th Annual International Symposium on High Performance Computing Systems and Applications, Quebec, Canada, May 2003
14. Lie-Quan Lee and Andrew Lumsdaine, The Generic Message Passing Framework, IPDPS'03, April 2003.
15. John Mugler, Thomas Naughton, Stephen L. Scott, Brian Barrett, Andrew Lumsdaine, Jeffrey M. Squyres, Benoit des Ligneris, Francis Giraldeau, and Chokchai Leangsuksun. OSCAR Clusters. In Proceedings of the Ottawa Linux Symposium (OLS'03), Ottawa, Canada, July 23-26, 2003.
16. Jaakko Jarvi, Jeremiah Willcock, and Andrew Lumsdaine. Concept Controlled Polymorphism. In Frank Pfennig and Yannis Smaragdakis, editors, Generative Programming and Component Engineering, volume 2830 of LNCS, pages 228-244, September 2003.
17. Jaakko Jarvi, Andrew Lumsdaine, Jeremy Siek, and Jeremiah Willcock. An Analysis of Constrained Polymorphism for Generic Programming. In Kei Davis and Joerg Striegnitz, editors, Multiparadigm Programming in Object Oriented Languages Workshop (MPOOL) as OPPSLA, Anaheim, CA, October 2003.
18. Jeffrey M. Squyres and Andrew Lumsdaine. A Component Architecture for LAM/MPI. In Proceedings, Euro PVM/MPI, October 2003.
19. Sriram Sankaran, Jeffrey M. Squyres, Brain Barrett, Andrew Lumsdaine, Jason Dell, Paul Hargrove, and Eric Roman. The LAM/MPI Checkpoint/Restart Framework: System Initiated Checkpointing. In LACSI Symposium, October, 2003
20. Ronald Garcia, Jaakko Jarvi, Andrew Lumsdaine, Jeremy Siek, Jeremiah Willcock., "A Comparative Study of Language Support for Generic Programming, " Proceedings, OOPSLA '03, 2003
21. Lie-Quan Lee and Andrew Lumsdaine, "Generic Programming for High Performance Scientific Applications," Accepted for publication *Concurrency Practice and Experience.*
22. Jeremiah Willcock and Andrew Lumsdaine, "MPI for .NET," Accepted for publication *Concurrency Practice and Experience.*
23. Jiangtao Cheng, J.P. Morris, John Tran, Andrew Lumsdaine, N.J. Giordano, L.J. Pyrak-Nolte, "Single Phase Flow in a Fracture: Micro-Model Experiments & Network Flow Simulation," to appear in *International Journal of Rock Mechanics.*

C. Research Support

Current

Department of Defense 9/20/03-3/29/05
"Parallel Graph Toolkit"

A study of the use of the generic programming paradigm for the implementation of parallel graph algorithms and data types.
Role: PI

ANI-0330620 8/15/03-7/31/06
National Science Foundation
"Scalable Fault Tolerance for MPI"
A study of fault tolerance mechanisms for distributed and parallel computing middleware and development of an infrastructure to support those mechanisms.
Role: PI

ACI-0219884 9/1/02–8/31/05
National Science Foundation
"ITR: A Paradigm of Parallel Programming for Morton-Ordered Matrices," (with David Wise, PI).
A study of parallel and high-performance programming for linear algebra using recursively defined data structures.
Role: Co-PI

EIA-0202048 2002–2007
National Science Foundation
"CISE Research Infrastructure: A Research Infrastructure for Collaborative, High-Performance Grid Applications," (with David Wise, PI, co-PIs Beth Plale, Geoffrey Fox, Randall Bramley, Kay Connelly, David Leake, Dennis Gannon).
Infrastructure grant to support projects in Grid computing, parallel and distributed middleware, data-intensive computing, and security.
Role: Co-PI

EIA-0131354 9/15/01–9/14/04
National Science Foundation
"NGS: Open Compilation for Self-Optimizing Generic Components"
Study of the use of the abstraction mechanisms underlying generic software components in the context of optimizing compilers.
Role: PI

Completed

Lawrence Berkeley National Lab 4/1/03-12/31/03
"Checkpoint Restart Support for LAM/MPI"
Development of kernel-level checkpoint-restart mechanisms for parallel and distributed computing using LAM/MPI
Role: PI

ACI-9982205 4/1/00–3/31/03
National Science Foundation
"High Performance Software Components for Scientific Computing"
Application of the generic programming paradigm for high-performance scientific computing.
Role: PI

Sandia National Laboratory 6/15/01–9/30/01
"Fault Tolerance Enhancements to LAM/MPI"
Study of fault tolerance approaches for MPI and preliminary design for their implementation in LAM/MPI.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Keith L. March, MD	Professor of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
St. Francis College, Fort Wayne, IN	B.S.	1979	Biology/Chemistry
Indiana University, Bloomington, IN	Ph.D.	1983	Biochemistry
Indiana University School of Medicine	M.D.	1985	Medicine
Indiana University School of Medicine		1988	Internal Medicine Residency
Indiana University School of Medicine		1990	Cardiology Clinical Fellowship

A. Positions and Honors

1985 - 1988 Internship and Residency, Indiana University School of Medicine
1988 - 1990 Cardiology Fellowship, Indiana University Medical Center
1990 - 1995 Assistant Professor of Medicine, Indiana University Medical Center
1990 - present Research Associate, Krannert Institute of Cardiology
1995 - 2003 Associate Professor of Medicine, Indiana University Medical Center
1999 - 2003 Associate Professor of Cellular & Integrative Physiology, Indiana University Medical Center
1999 - 2/2002 Director, Indiana University MD/PhD Combined Degree Program
1999 - present Director, Indiana Center for Vascular Biology & Medicine
2002 Adjunct Associate Professor of Biomedical Engineering, Purdue University
2003 Professor, Indiana University

Honors

1979 - 1985 Insurance Medical Scientist Scholarship
1981 Robert Chernin Award for Outstanding Graduate Research
1983 Alpha Omega Alpha Medical Honor Society
1983 Robert W. Bullard Outstanding Medical Science Student Award
1984 John H. Edwards Fellowship for Highest Distinction as Graduate Student
1989 USCI Cardiology Fellowship Award

B. Selected peer-reviewed publications

1. Hathaway D.R., March K.L., "Vascular biology," in: Kelley, W.N., ed. *Textbook of Internal Medicine*, 3rd Ed., Philadelphia: Lippincott-Raven. 66-72 (1997).
2. Baldwin, A.L., Wilson, L.M., Gradus-Pizlo, I., Wilensky, R., March, K.L., "Effect of atherosclerosis on transmural convection and arterial ultrastructure: implications for local intravascular drug delivery," *Arter Throm Vasc Bio.* **17**(12):3365-3375 (1997).
3. Pyles, J.M., March, K.L., Franklin, M., Mehdi, K., Wilensky, R.L., Adam, L.P., "Activation of MAP kinase *in vivo* follows balloon overstretch injury of porcine coronary & carotid arteries," *Circ Res.* **81**(6):904-910 (1997).
4. Sindermann, J.R., March, K.L., "Heparin responsiveness in vitro as a prognostic tool for vascular graft stenosis: a tale of two cell types?" *Circulation* **97**(25):2486-2490 (1998).
5. Stoll, H.P., Carlson, K., Keefer, L.K., Hrabie, J.A., March, K.L., "Pharmacokinetics and consistency of pericardial delivery directed to coronary arteries: direct comparison with endoluminal delivery," *Clin Cardiol.* **22**(1 Suppl 1):I10-I16 (1999).
6. March, K.L., Woody, M., Mehdi, K., Zipes, D.P., Brantly, M., Trapnell, B.C., "Efficient in vivo catheter-based pericardial gene transfer mediated by adenoviral vectors," *Clin Cardiol.* **22**(1 Suppl 1):I23-I29 (1999).
7. Dickson, T.J., Gurudutt, V., Nguyen, A.Q., Kumfer, K., Maxted, W., Brown, J., Mahomed, Y., Sharp, T., Aufiero, T.X., Fineberg, N., March, K.L., "Establishment of a clinically correlated human pericardial fluid bank: evaluation of intrapericardial diagnostic potential," *Clin Cardiol.* **22**(1 Suppl 1):I40-I42 (1999).

8. Kraemer, R., Nguyen, H., March, K.L., Hempstead, B., "NGF activates similar intracellular signaling pathways in vascular smooth muscle cells as pdgf-bb but elicits different biological responses," *Arter Thromb Vasc Biol.* **19**(4):1041-1050 (1999).
9. Wilensky, R.L., Mehdi, K., Sowinski, K.M., Baek, S.H., March, K.L.. "Increased intramural retention after local delivery of molecules with increased binding properties: implications for regional delivery of pharmacologic agents," *J Cardiovasc Pharmacol Ther.* **4**(2):103-112 (1999).
10. March, K.L., Sandusky, G., Fan, L., "Hyperplasia in multiple smooth muscle tissues in transgenic mice expressing a temperature-sensitive sv40 t-antigen under the control of smooth muscle alpha-actin regulatory sequences," *Oncogene.* **18**(25):3773-3782 (1999).
11. Stoll, H.P., March, K.L., "Intracoronary brachytherapy to prevent restenosis following coronary intervention: is it ready for clinical use?" *Biomedical Research* (2000).
12. Sindermann, J.R., Fan, L., Weigel, K.A., Troyer, D., Muller, J.G., Schmidt, A., March, K.L., Breithardt, G., "Differences in the effects of hmg-coa reductase inhibitors on proliferation and viability of smooth muscle cells in culture," *Atherosclerosis.* **150**(2):331-341 (2000).
13. Hou, D., Rogers, P.I., Toleikis, P.M., Hunter, W., March, K.L., "Intrapericardial Paclitaxel delivery inhibits neointimal proliferation and promotes arterial enlargement after porcine coronary overstretch," *Circulation.* **102**(13):1575-1581 (2000).
14. Wang, S., Bray, P., McCaffrey, T., March, K.L., Hempstead, B.L., Kraemer, R., "p75NTR mediates neurotrophin-induced apoptosis of vascular smooth muscle cells," *Am J Pathol.* **157**: 1247-1258 (2000).
15. Sindermann J., Adam, L., March, K.L., "Molecular and cellular physiology of differentiated vascular smooth muscle. In: Willerson, J.T., Cohn, J.N., eds. Cardiovascular Medicine, 2nd Ed. Churchill Livingstone. 1275-1285 (2000).
16. Stoll, H.P., Hutchins, G.D., Winkle, W.L., Nguyen, A.T., Hou, D., Appledorn, C.R., Romeike, B., March, K.L., "Liquid-filled balloon brachytherapy using (68)ga is effective and safe because of the short 68-minute half-life: results of a feasibility study in the porcine coronary overstretch model," *Circulation*. **103**(13):1793-1798 (2001).
17. Stoll, H.P., Hutchins, G.D., Winkle, W.L., Nguyen, A.T., Appledorn, C.R., Janzen, I., Seifert, H., Rube, C., Schieffer, H., March, K.L., "Advantages of short-lived positron-emitting radioisotopes for intracoronary radiation therapy with liquid-filled balloons to prevent restenosis," *J Nucl Med.* **42**(9):1375-1383 (2001).
18. Baek, S.H., Hrabie, J.A., Keefer, L.K., Hou, D.M., Fineberg, N., Rhoades, R., March, K.L., "Augmentation of intrapericardial nitric oxide (no) level by a prolonged-release no donor reduces luminal narrowing after porcine coronary angioplasty," *Circulation* **105**(23):2779-2784 (2002).
19. Sindermann, J.R., Smith, J., Kobbert, C., Plenz, G., Skaletz-Rorowski, A., Solomon, J.L., Fan, L., March, K.L., "Direct evidence for the importance of p130 in injury response and arterial remodeling following carotid artery ligation," *Cardiovasc Res.* **54**(3):676-683 (2002).
20. Boekstegers, P., Raake, P., Al Ghobainy, R., Horstkotte, J., Hinkel, R., Sandner, T., Wichels, R., Meisner, F., Thein, E., March, K.L., Boehm, D., Reichenspurner, H., "Stent-based approach for ventricle-to-coronary artery bypass," *Circulation* **106**(8):1000-1006 (2002).
21. Sindermann, J.R., Babij, P., Klink, J.C., Kobbert, C., Plenz, G., Ebbing, J., Fan, L., March, K.L., "Smooth muscle-specific expression of sv40 large tag induces smc proliferation causing adaptive arterial remodeling," *Amer J Phys Heart.* **283**(6):H2714-H2724 (2002).
22. Hou, D.M., March, K.L., "A novel percutaneous technique for accessing the normal pericardium: a single-center successful experience of 53 porcine procedures," *J. Invas Cardiol.* **15**(1):13-17 (2003).
23. Hou, D.M., Maclaughlin, F., Thiesse, M., Panchal, V.R., Bekkers, S.C.A.M., Wilson, E.A., Rogers, P.I., Coleman, M.C., March, K.L., "Widespread regional myocardial transfection by plasmid encoding del-1 following retrograde coronary venous delivery," *Cath and Card Interv.* **58**(2):207-211 (2003).
24. Rehman, J., Li, J., Orschell, C.M., March, K.L., "Peripheral blood 'endothelial progenitor cells' are derived from monocyte/macrophages and secrete angiogenic growth factors," *Circulation.* **107**:1164-1169 (2003).
25. Rehman, J., Considine, R.V., Bovenkerk, J.E., Williams, C.A., Li, J., Jones, R.M., March, K.L., "Obesity is associated with increased levels of circulating hepatocyte growth factor," *J Amer Col Card.* **41**(8):1408-1413 (2003).
26. Sindermann, J.R., Kobbert, C., Bauer, F., Skaletz-Rorowski, A., Hohage, H., Plenz, G., Breithardt, G., March, K.L., "Vascular ligation response is independent of p107: stressing the role of the related p130," *Amer J Phys Heart.* **285**(2):H915-918 (2003).

Book – March, K.L., ed. Gene Transfer in the Cardiovascular System: Experimental Approaches and Therapeutic Implications. Boston: Kluwer Academic Publishers (1997).

C. Research Support

Current

Veteran's Administration Merit Review Award 2000 – 2005
"Molecular Mechanisms Directing Artery Remodeling"
This grant evaluates mechanisms of large artery remodeling.
Role: Principal Investigator.

Pfizer, Inc., 2002 - 2003
"Effects of Statins on Circulating Endothelial Progenitor Cells"
This grant supports studies of endothelial progenitor cells.
Role: Principal Investigator

National Institutes of Health (T32) 2002-2007
"Research Training Program in Pediatrics"
This is a fellowship/training grant.
Role: Co-Investigator (Principal Investigator, Mary Dinauer)

U.S. Civilian Research & Development Foundation 2003-2005
"Angiogenesis Control by the Urokinase System: Biochemical Mechanisms and Gene Therapy Approaches to Ischemia"
This grant involves the molecular mechanisms of urokinase effects on angiogenesis.
Role: Principal Investigator

Edwards Lifesciences, LLC. 2003-2004
"Optimization of Gene Delivery in Rabbit Hind Limb and Porcine Ischemia Models"
Role: Principal Investigator

Eli Lilly & Company, Inc. 2003-2005
"Utilizing the Multipotency of Human Adipose Stromal Cells"
Role: Principal Investigator

Completed

National Institutes of Health (R01) 1997-2002
"Smooth Muscle Diversity and Cell Cycle Control"
This grant evaluates smooth muscle cell cycling in transgenic animal models.
Role: Principal Investigator

Veteran's Administration Merit Review Award 1997-2000
"Vascular Smooth Muscle Immortalization and Cell Cycle Control"
This grant supported transgenic mouse modeling of vascular disease.
Role: Principal Investigator

American Heart Association 2001
Student Scholar Research Grant,
"Intramyocardial Cell Engraftment via Retrograde Coronary Venous Delivery"
This grant supported intramyocardial cell delivery studies.
Role: Sponsor

National Institutes of Health (R21) 1998-2002
"Non-Invasive Myocardial Revascularization Using Targeted Proton Beam Irradiation for Induction of Angiogenesis"
Role: Principal Investigator

National Institutes of Health (SBIR) 2000 – 2002

"Ultrasound Assisted Transmural Drug Delivery"
This grant evaluates the effects of ultrasound on the artery wall.
Role: Principal Academic Investigator

Valentis, Inc. 2000 – 2002
"Retrograde Coronary Delivery of Plasmids for Myocardial Angiogenesis"
This grant evaluates the expression and efficacy of a del-1 plasmid administered into the heart.
Role: Principal Investigator

National Institutes of Health 1995-2000
"Nonimmune Defense against Tuberculosis in the Lung"
The co-investigator assisted with confocal microscopy in this grant.
Role: Co-Investigator (Principal Investigator, Dr. William Martin)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
James A. Marrs	Associate Professor of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Illinois at Urbana-Champaign, IL	B.S.	1984	Biology
University of Illinois at Chicago, IL	Ph.D.	1990	Biology
Stanford University, CA	Postdoctoral	1991 – 1994	Epithelial Cell Biology

A. Positions and Honors

Positions and Employment

1991-1994 Postdoctoral Fellow, Department of Molecular and Cellular Physiology, Stanford University. Sponsor: W. James Nelson.
1995-2001 Assistant Professor of Medicine, Physiology and Biophysics, Indiana University Medical Center.
2001-present Associate Professor of Medicine, Physiology and Biophysics, Indiana University Medical Center (with tenure).

Other Experience and Professional Memberships

1997 *Ad hoc* reviewer, National Science Foundation.
1998-2000 *Ad hoc* reviewer, VISC study section, National Institutes of Health.
1999-2000 *Ad hoc* reviewer, NICHD Program project grant, National Institutes of Health.
Dec. 2000 *Ad hoc* reviewer, NIDDK Program project grant, National Institutes of Health.
2002- present *Editorial Board Member,* American Journal of Physiology: Cell Physiology
Dec. 2003 *Ad hoc* reviewer, NIDDK Program project grant, National Institutes of Health.

Honors

1989-1990 Laboratory for Molecular Biology, University of Illinois at Chicago, predoctoral fellowship.
1991-1994 National Institutes of Health, postdoctoral fellowship.
1994-1995 American Heart Association, postdoctoral fellowship: *declined because of concurrent ACS award*.
1994-1995 American Cancer Society, postdoctoral fellowship.
1994 *Invited speaker*, FASEB 1994 Annual Meeting, Anaheim CA.
1994 National Eye Institute Fellowship to attend *Fundamental Issues in Vision Research* course at Woods Hole Marine Biology Laboratory.
1997 *Organizer and Moderator*, Symposium on Cell-Cell Adhesion Junctions, Annual Meeting of the American Society of Nephrologists.
2001 *Invited Speaker*, Eighth International Workshop on Developmental Nephrology, International Pediatric Nephrology Association, Victoria, BC.
2002 *Minisymposium platform presentation*. American Society for Cell Biology 2002 Annual Meeting, San Francisco, CA.

B. Selected peer-reviewed publications

1. Marrs, J. A., E. W. Napolitano, C. Murphy-Erdosh, R. W. Mays, L. F. Reichardt and W. J. Nelson. "Distinguishing roles of the membrane-cytoskeleton and cadherin mediated cell-cell adhesion in generating different Na^+, K^+-ATPase distributions in different polarized epithelia," *J. Cell Biol.* **123**: 149-164 (1993).

2. Marrs, J. A., C. Anderson-Fisone, M. C. Jeong, I. R. Nabi, C. Zurzolo, E. Rodriguez-Boulan, and W. J. Nelson, "Plasticity in epithelial cell phenotype: modulation by expression of different cadherin cell adhesion molecules," *J. Cell Biol.* **129**: 507-519 (1995).
3. Jou, T.-S., D. B. Stewart, J. Stappart, W. J. Nelson, and J. A. Marrs, "Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex," *Proc. Natl. Acad. Sci. USA* **92**: 5067-5071 (1995).
4. Mays, R. W., W. J. Nelson, and J. A. Marrs, "Generation of epithelial cell polarity: roles for protein trafficking, membrane-cytoskeleton, and E-cadherin-mediated cell adhesion," *Cold Spring Harbor Symp. Quant. Biol.* **60**: 763-773 (1996).
5. Marrs, J. A., and W. J. Nelson, "Cadherin cell adhesion molecules in differentiation and embryogenesis," *Int. Rev. Cytol.* **165**: 159-205 (1996).
6. Gopalakrishnan, S., N. Raman, S. J. Atkinson and J. A. Marrs, "Rho GTPase signaling regulates normal tight junction assembly and protects tight junctions from disassembly during cellular injury," *Am. J. Physiol. **275** (Cell Physiol.**44**)*: C798-C809 (1998).
7. Molitoris, B. A., and J. A. Marrs, "The role of cell adhesion molecules in ischemic acute renal failure," *American Journal of Medicine*. **106**: 583-592 (1999).
8. Troxell, M.T., Chen, Y.-T., Cobb, N., Nelson, W.J., Marrs, J.A., "Cadherin function in junctional complex rearrangement and posttranslational control of cadherin expression," *Am. J. Physiol*. **276** (*Cell Physiol*.**45**): C404-C418 (1999).
9. Liu, Q., Marrs, J.A., Raymond, P.A., "Spatial correspondence between R-cadherin expression domains and retinal ganglion cell axons in developing zebrafish," *J. Comp. Neurol.* **410**: 290-302 (1999).
10. Liu, Q., Sanborn, K.L., Cobb, N., Raymond, P.A., Marrs, J.A., "R-cadherin expression in the developing and adult zebrafish visual system," *J. Comp. Neurol.* **410**: 303-319 (1999).
11. Troxell, M.T., Gopalakrishnan, S., McCormack, J., Poteat, B.A., Pennington, J., Garringer, S.M., Schneeberger, E.E., Nelson, W.J., Marrs, J.A., "Inhibiting cadherin function by dominant mutant E-cadherin expression increases the extent of tight junction assembly," *J. Cell Sci*. **113**: 985-996 (2000).
12. Troxell, M.L., Loftus, D.J., Nelson, W.J., and Marrs, J.A., "Mutant cadherin effects on epithelial tubulogenesis, invasion, and transformation," *J. Cell Sci*. **114**: 1237-1246 (2001).
13. Jin, Y., Atkinson, S.J., Marrs, J.A., Gallagher, P.J., "Myosin II light chain phosphorylation regulates membrane localization and apoptotic signaling of tumor necrosis factor receptor-1," *J Biol Chem.* **276**: 30342-30349 (2001).
14. Babb, S.G., Barnett, J., Doedens, A.L., Cobb, N., Liu, Q., Sorkin, B.C., Yelick, P.C., Raymond, P.A., and Marrs, J.A., "Zebrafish E-cadherin: expression during early embryogenesis and regulation during brain development," *Developmental Dynamics* **221**: 231-237 (2001).
15. Liu Q., Marrs, J.A., Chuang, J.C., and Raymond, P.A., "Cadherin-4 expression in the zebrafish central nervous system and regulation by ventral midline signaling," *Brain Res Dev Brain Res.* **131**: 17-29 (2001).
16. Liu, Q., Babb, S. G., Novince, Z. M., Doedens, A. L., Marrs, J. A., and Raymond, P. A., "Differential expression of cadherin-2 and cadherin-4 in the developing and adult zebrafish visual system," *Vis. Neurosci.* **18**: 923-933 (2001).
17. Gopalakrishnan, S., K. W. Dunn, and J. A. Marrs, "Rac signaling, but not Rho signaling, protects epithelial adherens junction assembly during ATP-depletion," *Am. J. Physiol. Cell Physiol.* **283**: C261-C272 (2002).
18. Liu, Q., Londraville, R.L., Azodi, E., Babb, S.G., Chiappini-Williamson, C., Marrs, J., Raymond, P.A., "Up-regulation of cadherin-2 and cadherin-4 in regenerating visual structures of adult zebrafish," *Exp. Neurol.* **177**: 396-406 (2002).
19. Gopalakrishnan, S., Dunn, K. W. and J. A. Marrs, "Testing effects of signal transduction pathways on cadherin junctional complex assembly using quantitative image analysis," *Methods* **30**: 218-227 (2003).
20. Gopalakrishnan, S., M. A. Hallett, S. J. Atkinson and J. A. Marrs, "Differential regulation of junctional complex assembly in renal epithelial cell lines," *Am. J. Physiol. Cell Physiol.* **285**: C102-C111 (2003).
21. Novince, Z. M., E. Azodi, J. A. Marrs, P. A. Raymond, Q. Liu, "Cadherin expression in the inner ear of developing zebrafish," *Gene Expr Patterns.* **3**: 337-339 (2003).
22. Liu, Q., Kerstetter, A. E., Azodi, E., and J. A. Marrs, "Cadherin-1, -2, and -11 expression and cadherin-2 function in the pectoral limb bud and fin of the developing zebrafish," *Developmental Dynamics* **228**: 734-739 (2003).

C. Research Support

Current

NIH/NIDDK, 1 P01 DK53465 12/01/99-11/30/04
Principal Investigator, Bruce Molitoris; Subproject Director, James A. Marrs
"Actin Dysregulation in Ischemia: Mechanisms and Effects" (Subproject: Junctional complex dysregulation during ischemic injury.)
The objective of this project is to examine effects of ischemic injury on epithelial cell junctional complexes and to determine whether dysfunction of junctional complexes is regulated by Rho-GTPase signaling or cadherin cell adhesion.

NIH/NEI, 2 R01 EY11365-06 02/1/02 – 01/31/07
Principal Investigator: James A. Marrs (Co-PI, Qin Liu)
"Cell Adhesion Function in the Visual System"
These experiments will test the function of cadherin cell adhesion molecules in the developing zebrafish visual system. Cloning and characterization of cDNA clones encoding cadherin molecules will be completed. Developmental expression patterns in the visual system and relevant visual centers in the brain will be studied. Function blocking experiments will be performed to determine the role of these cell adhesion molecules in visual system development.

NIH/NIDDK, 1P50 DK61594 04/01/02-03/30/04
Principal Investigator, Bruce Molitoris; Pilot Project Director, James A. Marrs
"Zebrafish as a model to study kidney development"
The objective of this project is to determine whether morpholino oligonucleotide inhibition of zebrafish genes that regulate cystic kidney formation, glomerulus formation and cadherin cell adhesion can be used as a model of metanephric kidney development. Although we will examine cadherin cell adhesion molecules, among other genes, different methods will be used to examine visual system, rather than pronephros formation and function development.

Pending
NIH/NIDCD, R01 DC006436 07/01/04-06/30/09
Principal Investigator, James A. Marrs (Co-PIs, Kate Barald, Univ. of Michigan and Qin Liu, Univ. of Akron)
"Cadherins in the Developing Zebrafish Inner Ear"
The proposed experiments will examine the function of cadherin cell adhesion molecules in the developing zebrafish inner ear. Developmental expression patterns of cadherins will be determined during critical stages of inner ear morphogenesis and neurogenesis. Function blocking experiments will be performed to determine the role of these cell adhesion molecules in inner ear development.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Roeland M. H. Merks	Visiting scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Utrecht University, The Netherlands	Drs. (=Msc)	1997	Theor. & Dev'mtl Biology
Tokyo University, Japan	-	1998	Theoretical Biology / Complex Systems Non-linear Dynamics
Santa Fe Institute, Santa Fe, NM	-	2001	Complex Systems
University of Amsterdam, The Netherlands	PhD	2003	Computational Biology

A. Positions and Honors

Nov 1997 - Feb 1998	Guest Researcher, Utrecht University, The Netherlands
Apr 1998 - Dec 1998	Guest Researcher, Tokyo University, Japan (Theoretical Biology/ Non-linear dynamics). Funded by Nuffic (Netherlands organization for international cooperation in higher education) grant: "Talentenprogramma".
Feb 1999 - Apr 2003	Ph.D. Student, University of Amsterdam
May 2003 - Jun 2003	Guest Researcher, Utrecht University, The Netherlands (Theoretical Biology)
Nov 2003 - Nov 2004	Visiting Scientist, Indiana University, Bloomington, IN (Biophysics)

B. Selected peer-reviewed publications

1. Merks, R. M. H., Hoekstra, A. G. and Sloot, P. M. A., "The moment propagation method for advection-diffusion in the lattice Boltzmann method: validation and Péclet number limits," *J. Comput. Phys* **183**, 563-576 (2002).
2. Merks, Roeland M. H., Hoekstra, Alfons G., Kaandorp, Jaap A. and Sloot, Peter M. A., "Models of coral growth: Spontaneous branching, compactification and the Laplacian growth assumption," *Journal of Theoretical Biology* **224**, 153-166 (2003).
3. Merks, Roeland, Hoekstra, Alfons, Kaandorp, Jaap and Sloot, Peter, "Spontaneous branching in a polyp oriented model of stony coral growth," *International Conference on Computational Science (ICCS)* **2329** Springer-Verlag, Berlin, of *Lecture Notes in Computer Science* Sloot, P. M. A., Kenneth-Tan, C. J., Dongarra, Jack J. and Hoekstra, Alfons G. (Eds), Amsterdam, the Netherlands, 88-96 (2002).
4. Merks, R. Hoekstra, A., Kaandorp, J. and Sloot, P., "A problem solving environment for modelling stony coral morphogenesis," In *Computational Science - ICCS 2003* **2657**, Melbourne, Australia, of *Lecture Notes in Computer Science,* St. Petersburg, Russia, Sloot, Peter M. A., Abramson, David, Bogdanov, Alexander V., Dongarra, Jack J., Zomaya, Albert Y. and Gorbachev, Yuriy E. (Eds), 639-648 (2003).
5. Merks, R. M. H., Hoekstra, A. G., Kaandorp, J. A. and Sloot, P. M. A., "Diffusion limited growth in laminar flows," *International Journal of Modern Physics* **C**, (in press).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
David P Moffett	Associate VP for Research Computing

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Purdue University, West Lafayette, IN	B.S.C.E.	1988	Civil Eng. w/Comp. Sci.
Purdue University, West Lafayette, IN	M.S.C.E.	1990	Civil Eng.
Purdue University, West Lafayette, IN	Ph.D.	2004 pend.	Civil Engineering / Heuristic Optimization

A. Positions and Honors

Positions and Employment

1977-1980 Service Manager, Home Computer Center, Indianapolis, IN
1980-1981 Project Engineer – Energy Management, Energy Micro-Systems, Inc, Indianapolis, IN
1981-1983 Production Manager, Energy Micro-Systems, Inc, Indianapolis, IN
1983-1987 Manager Information Systems, Ritron Inc, Carmel, IN
1990- Consultant in Computer Science & Engineering, (self), Lafayette, IN
1995-1998 Transportation Engineer, Bernardin, Lochmueller & Associates, Evansville, IN
2002- Associate VP for Research Computing, ITaP, Purdue University, W. Lafayette, IN

Other Experiences and Professional Memberships

1974 Audio Engineering Society
1988 American Society of Civil Engineers
2000 IEEE Computer Society
2000 Registered Professional Engineer (PE) in Indiana
2001 http://acme.ecn.purdue.edu. a compute cluster

B. Selected peer-reviewed publications

1. Moffett, David P., "Weaving Section Analysis Using Video License Plate Surveys", *7th Conference on the Applications of Transportation Planning Methods*, U.S. Department of Transportation, Transportation Research Board, etal., Boston, MA, (March 1999).
2. Moffett, David P., "Improvements In Transportation Planning Network Modeling Mechanics", *7th Conference on the Applications of Transportation Planning Methods*, U.S. Department of Transportation, Transportation Research Board, etal., Boston, MA (March 1999).
3. Fricker, Jon D. and Moffett, David P., "Urban Transportation Network Calibration", *7th Conference on the Applications of Transportation Planning Methods*, U.S. Department of Transportation, Transportation Research Board, etal., Boston, MA, (March 1999).
4. Moffett, David P., Jeong, Garrett D, “Clustering with FreeBSD”, *BSDCon 2000*, Monterey CA (Oct 2000).
5. Moffett, David P, “License Plate Matching & Open Source Software*”, 8th Conference on the Applications of Transportation Planning Methods*, U.S. Department of Transportation, Transportation Research Board, et al, Corpus Christi, TX (April 2001).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Glen L. Niebur	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The University of Minnesota, Minneapolis, MN	B.M.E.	1986	Mechanical Engineering
The University of Minnesota, Minneapolis, MS	M.S.M.E.	1995	Mechanical Engineering
The University of California, Berkeley, CA	Ph.D.	2000	Mechanical Engineering

A. Positions and Honors

1982 -1986 National Merit Scholar,
1986 -1990 Control Data Corporation, Senior Programmer Analyst, CIM Research and Development
1990 -1996 Mayo Medical Center, Research Engineer, Orthopedic Biomechanics Laboratory
1996 - 2000 The University of California, Berkeley, Graduate Research Assistant, Department of Mechanical Engineering
2001 - The University of Notre Dame, Assistant Professor, Department of Aerospace and Mechanical Engineering
1982 University of Minnesota, Institute of Technology Merit Scholar
2002 Orthopaedic Research Society, NIRA Recipient – ABJS award for best basic science study

B. Selected peer-reviewed publications

1. King, G. J. W., Itoi, E., Risung, F, Niebur, G.L., Morrey, B. F. and An, K. N., “Kinematics and stability of the Norway elbow,” *Acta Orthopedica Scandinavica* **64** (6), 657-663 (1993).
2. King, G. J. W., Itoi, E., Niebur, G. L., Morrey, B. M. and An, K. N., "Motion and laxity of the capitellocondylar total elbow prosthesis," *Journal of Bone and Joint Surgery-American Volume* **76**, 1000-8 (1994).
3. Itoi, E., King, G. J. W., Niebur, G. L., Morrey, B. F. and An, K. N, "Malrotation of the humeral component of the capitellocondylar total elbow replacement is not the sole cause of dislocation," *Journal of Orthopedic Research* **12**, 665-671 (1994).
4. Imaeda, T., Niebur, G., An, K. N. and Cooney, W. P., "Kinematics of the trapeziometacarpal joint after sectioning of ligaments," *Journal of Orthopedic Research* **12**, 205-10 (1994).
5. Imaeda, T., Niebur, G., Cooney, W. P. III, Linscheid, R. L., and An, K. N., "Kinematics of the normal trapeziometacarpal joint," *Journal of Orthopedic Research* **12**, 197-204 (1994).
6. Luo, Z., Niebur, G. L. and An, K. N., "Determination of the proximity tolerance for measurment of surface contact areas using a magnetic tracking device," *Journal of Biomechanics* **29** (3), 367-373 (1996).
7. Imaeda T., Cooney W. P., Niebur, G.. L., Linscheid R. L. and An, K. N., "Kinematics of the trapeziometacarpal joint - a biomechanical analysis comparing tendon interposition arthroplasty and total-joint arthroplasty," *Journal of Hand Surgery-American Volume* **21** (4), 544-553 (1996).
8. Liu, J., Hughes, R. E., Smutz, W. P., Niebur, G. and An, K. N., "Roles of deltoid and rotator cuff muscles in shoulder elevation," *Clinical Biomechanics* **12** (1), 32-38 (1997).
9. Imaeda, T., Niebur, G., Cooney, W. P., Linscheid, R. L. and An, K. N., "Ligament length during circumduction of the trapeziometacarpal joint." *Journal of Orthopedic Science* **2**, 319-327 (1997).
10. Ishikawa, J., Niebur, G. L., Uchiyama, S., Linscheid, R. L., Minami, A., Kaneda, K. and An, K. N., "Feasibility of using a magnetic tracking device for measuring carpal kinematics," *J Biomech* **30** (11-12), 1183-6 (1997).
11. Smutz, W. P., Kongsayreepong, A., Hughes, R. E., Niebur, G., Cooney, W. P. and An, K. N., "Mechanical advantage of the thumb muscles." *Journal of Biomechanics* **31**, 565-570 (1998).

12. Hughes, R. E., Niebur, G., Liu, J. and An, K. N., "Comparison of two methods for computing abduction moment arms of the rotator cuff," *J Biomech* **31** (2) 157-60 (1998).
13. Ishikawa, J., Cooney, W. P., Niebur, G., An, K. N., Minami, A. and Kaneda, K., "The effects of wrist distraction on carpal kinematics." *Journal of Hand Surgery-American* **24**, 113-120 (1999).
14. Imaeda, T., Niebur, G., Cooney, W. P., Linscheid, R. L. and An, K. N., "Ligament length during circumduction of the trapeziometacarpal joint after ligament sectioning." *Journal of Musculoskeletal Research* **3** (3), 183-194 (1999).
15. Uchiyama, S., Cooney, W. P., Niebur, G., An ,K. N. and Linscheid, R. L.., "Biomechanical analysis of the trapeziometacarpal joint after surface replacement arthroplasty," *Journal of Hand Surgery-American* **24** (3) 483-90 (1999).
16. Niebur, G. L., Yuen, J. C.; Hsia, A. C., and Keaveny, T. M., "Convergence behavior of high-resolution finite element models of trabecular bone," *Journal of Biomechanical Engineering* **121** (6), 629-35 (1999).
17. Kuechle, D. K., Newman, S. R., Itoi, E, Niebur, G. L., Morrey, B. F.and An, K. N., "The relevance of the moment arm of shoulder muscles with respect to axial rotation of the glenohumeral joint in four positions," *Clinical Biomechanics* **15** (5), 322-29 (2000).
18. Uchiyama S., Cooney, W. P. III, Linscheid, R. L., Niebur, G. and An, K. N., "Kinematics of the proximal interphalangeal joint of the finger after surface replacement," *Journal of Hand Surgery-American* 25 (2), 305-12 (2000).
19. Niebur, G. L., Feldstein, M. J., Yuen, J. C., Chen, T. J.and Keaveny, T. M., "High resolution finite element models with tissue strength asymmetry accurately predict failure of trabecular bone," *Journal of Biomechanics* **33** (12), 1575-1583 (2000).
20. Niebur, G. L., Yuen, J. C., Burghardt, A. J. and Keaveny, T. M., "Sensitivity of damage predictions to tissue level yield properties and apparent loading conditions," *Journal of Biomechanics* **34** (5) 699-706 (2001).
21. Jaasma, M. J., Bayraktar, H. H., Niebur, G. L. and Keaveny, T. M., "Biomechanical Effects of Intraspecimen Variations in Tissue Modulus for Trabecular Bone," *Journal of Biomechanics* **35**, 237-46 (2002)
22. Niebur, G. L., Feldstein, M. J. and Keaveny, T. M., "Biaxial compressive failure properties of trabecular bone," *Journal of Biomechanical Engineering* **124** (6), 699-705 (2002).
23. Bayraktar, H. H., Morgan, E. F., Morris, G. E., Niebur, G. L. and Keaveny, T. M., "Similarity of trabecular tissue and cortical bone mechanical properties," *Journal of Biomechanics,* In Press (2003)
24. Liu, X., Wang, X. and Niebur, G. L., "Effects of damage on the orthotropic material symmetry of bovine tibial trabecular bone," *Journal of Biomechanics* **36** (12), 1753-1759 (2003).
25. Wang, X., Liu, X. and Niebur, G. L., "Preparation of on-axis cylindrical trabecular bone specimens using micro-CT imaging", *Journal of Biomechanical Engineering*, In Press (2004).

C. Research Support

Current

Medtronic/Sofamor Danek (Niebur, G.L.) 11/01/02-4/30/04
The role of fusion mass size, location, and stiffness in the strength of lumbar interbody fusion
The goal of this work is to investigate how fusion mass size and placement affect the the strength of interbody fusion in order to assist in pre-surgical planning.
Role: PI

National Institutes of Health (Ryan K. Roeder, PI) 10/01/02-9/30/05
AR49598
Staining techniques for Micro-CT imaging of microdamage
The goal of this work is to develop stains composed of heavy metals that will preferentially stain damaged regions of bone, allowing the damage to be visualized in X-ray and CT.

DePuy Orthopaedics (Niebur, G.L.) 8/31/03-8/30/05
New Generation Bioscaffolds for Musculoskeletal Reconstruction
The goal of this work is to develop a porous ingrowth scaffold for fibrous soft tissue in order to optimize the load transfer from a tendon or ligament to an implanted device.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mary Papakhian	Manager, Research and Technical Services, Indiana University Information Technology Services

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Western Michigan University, Kalamazoo, MI	B. A.	1971	English
Western Michigan University, Kalamazoo, MI	M. A.	1973	English

A. Positions and Honors

1984 – 1986	Systems Programmer/Analyst, Data Solutions, Inc., Bloomington, Indiana
1996 – 1999	Research Systems Team Leader, Indiana University
1986 – 1989	Systems Programmer/Analyst, Indiana University
1989 – 1996	Senior Systems Programmer/Analyst, Indiana University
1999 – present	Manager, Research and Technical Services, Indiana University

B. Selected peer-reviewed publications

Papakhian, M., "Comparing Job-Management Systems: The User's Perspective," *Computational Science and Engineering* **5**(2), IEEE Computer Society (April-June, 1998).

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ramana M. Pidaparti	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Andhara University, India	B.S.	1980	Civil Engineering
India Institute of Science, India	M.S.	1982	Aeronautical Engineering
University of Maryland, College Park, MD	M.S.	1985	Aerospace Engineering
Purdue University, West Lafayette, IN	Ph.D.	1989	Aeronautics & Astronautics

A. Positions and Honors

1989 - 1993 Assistant Professor, Mechanical Engineering, Indiana University Purdue University Indianapolis
1993 - 1999 Associate Professor, Mechanical Engineering, Indiana University Purdue University Indianapolis
1999 - present Professor, Mechanical Engineering, Indiana University Purdue University Indianapolis

Honors

1992 The Whitaker Foundation Research Award
1999 Abe Max Distinguished Professor Award, IUPUI
2000 TERA (Teaching Excellence Recognition Award), IUPUI
Member, Technical Committee on "Intelligent Systems", AIAA
2002 ASME Fellow
2003 Frank E. Burley Distinguished Professor Award, IUPUI

B. Selected peer-reviewed publications

1. Pidaparti, R. M., Y. Liu and R. A. Meiss, "A viscoelastic material model to represent smooth muscle shortening," *Journal of Bio-Medical Materials and Engineering* **7**, 171-177 (1997).
2. Pidaparti. R. M. and Y. Liu, "Bone stiffness changes due to microdamage under different loadings," *Journal of Bio-Medical Materials and Engineering.* **7**, 193-203 (1997).
3. Pidaparti, R. M. V., B.A. Merril and N. A. Downton, "Fracture and material degradation properties of cortical bone under accelerated stress," *Journal of Biomedical Materials and Research* **37**, 161-165 (1997).
4. Chen, J., U. Akyuz, L. Xu and R. M. V. Pidaparti, "Stress analysis of the human temporomandibular joint," *Medical Engineering and Physics Journal.* **20**, 565-572 (1998).
5. Burr, D.B., C. H. Turner, M. R. Forwood, and R. M. Pidaparti, "Quantification of bone damage and crack morphology and its effect on skeletal fragility: response to Zioupos," *Journal of Biomechanics* **32**, 213-215 (1999).
6. Pidaparti, R. M., Q. Y. Wang and D.B. Burr, "Modeling fatigue damage evolution in bone," *Journal of Bio-Medical Materials and Engineering* **11**, 69-78 (2001).
7. Pidaparti, R. M., and A. Vogt, "Experimental investigation of poisson's ratio as a damage parameter for bone fatigue," *Journal of Bio-Medical Materials Research* **59**(2), 282-287 (2002).
8. Sarma, P. A., R. M. Pidaparti, and R. A. Meiss "Anisotropic properties of a tracheal smooth muscle tissue," *Journal of Bio-Medical Materials Research* **65A**, 1-8 (2003).
9. Sarma, P. A., R. M. Pidaparti, P. N. Moulik, and R. A. Meiss "Non-linear materials models for tracheal smooth muscle tissue," *Journal of Bio-Medical Materials Engineering*, **13**(3) 235-246 (2003).
10. Meiss, R. A. and R. M. Pidaparti, "Mechanical determinants of the length-tension relationship in airway smooth muscle," *Journal of Applied Physiology*, (in press) (2004).

C. Research Support

Current

National Science Foundation 06/01/01-05/31/04
"Developing durable composite system for industrial and infrastructure applications"
Role: PI

National Science Foundation 09/15/01-09/01/04
"Three-dimensional surface corrosion growth model for materials design"
Role: PI

National Science Foundation 09/15/01-09/01/04
"Mechanical effects of induced changes in the extracellular matrix of visceral smooth muscle tissues"
Role: co-PI

Indiana 21st Century Fund 09/01/03-08/31/05
"Multi-Scale Methodology for the Design of Active Materials"
Role: Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Doraiswami Ramkrishna	Harry Creighton Peffer Distinguished Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bombay University, Bombay, India	B.(Chem) Eng.	1960	Chemical Engineering
University of Minnesota, Minneapolis, MN.	Ph.D.	1965	Chemical Engineering

A. Positions and Honors

1964-1965	Instructor, University of Minnesota
1965-1967	Assistant Professor, University of Minnesota
1967-1972	Assistant Professor, Indian Institute of Technology, Kanpur
1972-1975	Associate Professor, Indian Institute of Technology, Kanpur
1975-1977	Professor, Indian Institute of Technology, Kanpur (on leave)
1974-1975	Visiting Associate Professor, University of Wisconsin
1975-1976	Visiting Professor, University of Minnesota
1976-1994	Professor, Purdue University
1982-1983	University Grants Commission Visiting Professor, Indian Institute of Science, Bangalore
1983	Dr. G.P. Kane Professor, Bombay University
1987	AIChE Alpha Chi Sigma Award in Chemical Engineering
1988	George T. Piercy Distinguished Visiting Professor, University of Minnesota
1994-present	H.C. Peffer Distinguished Professor, Purdue University
1994	'UDCT DIAMOND' Award, Department of Chemical Technology, Bombay University
1994	Melchor Visiting Professor, University of Notre Dame
1996	Fellow of the American Institute for Medical and Biological Engineering
1998	Distinguished Lecturer, The State University of New Jersey, Rutgers, Piscataway, NJ
1998	AIChE R. H. Wilhelm Award in Chemical Reaction Engineering
1999	Dow-Professor Sharma Distinguished Fellow in Chemical Engineering at Bombay University
2001	Senior Humboldt Award to visit the Max Planck Institute, Magdeburg, Germany
2001	Fulbright Award to visit Germany (declined).
2001	Honorary Fellow of the Indian Institute of Chemical Engineers
2001	Kvaerner Powergas, N. R. Kamath Memorial Lecturer at Chemcon 2001, Chennai, India
2004	Honorary Doctor of Science, University of Minnesota

B. Selected peer-reviewed publications

1. Kumar, S. and Ramkrishna, D., "On the Solution of Population Balance Equations by Discretization-III. Nucleation, Growth and Aggregation of Particles," *Chem. Eng. Sci.*, **24**, 4659-4679 (1997).
2. Varner, J. and Ramkrishna, D., "Application of Cybernetic Models to Metabolic Engineering. Investigation of Storage Pathways," *Biotech. & Bioeng.*, **58**, 282-291 (1998).
3. Varner, J. and Ramkrishna, D., "Mathematical Models of Metabolic Pathways," *Current Opinion in Biotechnology*, **10**, 146-150 (1999).
4. Varner, J., D., Ramkrishna, D. and Bailey, J. E., "A Self-Optimizing Adaptive Model of Glucose Catabolism in Escherichia coli. Prediction of Network Response to Amplification of Key Glycolytic Enzymes," *Biotechnology Progress* (1999).
5. Ramkrishna, D. and Schell, J., "On Self-Similar Growth," *J. Biotechnology*, **71**, 255-258, (1999).

6. Cote, A., Delgass, W. N. and Ramkrishna, D., "Investigation of Spatially Patterned Catalytic Reactors," *Chem. Eng. Sci.* (ISCRE issue), **54**, 2627-2635 (1999).
7. Ramkrishna, D. and Aris, R., "The Beauty of Self-Adjoint Symmetry," *Ind. Eng. Chem. Research* (Special Issue in honor of Roy Jackson), **38**, 845-850 (1999).
8. Tobin, T. and Ramkrishna, D., "Modeling the Effect of Drop Charge on Coalescence in Turbulent Liquid-Liquid Dispersions," *Can. J. Chem. Eng.*, **77**, 1090-1104 (1999).
9. Varner, J. and Ramkrishna, D., "Nonlinear Analysis of Cybernetic Models-I. Guidelines for Model Formulation," *J. Biotechnol.*, **71**, 67-103 (1999).
10. Varner, J. and Ramkrishna, D., Metabolic Engineering From A Cybernetic Perspective: Aspartate Family of Amino Acids," *Metabolic Eng.*, **1**, 88-116 (1999).
11. Varner, J. and Ramkrishna, D., "Metabolic Engineering From A Cybernetic Perspective II. Qualitative Investigation of Nodal Architectures and Their Response to Genetic Perturbation," *Biotechnology Progress*, **15**, 426-438 (1999).
12. Varner, J. and Ramkrishna, D., "Metabolic Engineering from a Cybernetic Perspective-I. Theoretical Preliminaries," *Biotechnology Progress*, **15**, 407-425 (1999).
13. Varner, J. and Ramkrishna, D., "Mathematical Models of Metabolic Pathways," *Current Opinion in Biotechnology*, **10**, 146-150 (1999).
14. Guardia, M. J., Gambhir, A., Europa A., Ramkrishna, D. and Wei-Shou, H., "Cybernetic Modeling and Regulation of Metabolic Pathways in Multiple Steady States of Hybridoma Cells, *Biotechnology Progress*, **16**, 847-853 (2000).
15. Kumar, S., Pirog, T. and Ramkrishna, D., "A New Method for Estimating Creaming/Settling Velocity of Particles in Poly-Dispersed Systems," *Chem. Eng. Sci.*, **55**, 1893-1904 (2000).
16. Cote, A., Delgass, W. N. and Ramkrishna, D., "Spatially Patterned Catalytic Reactors. Feasibility Issues," *Chem. Eng. Sci.* Jubilee Volume: ISCRE 16; Chemical Reaction Engineering Beyond 2000, Ed. Burghardt, A., Pohorecki, R., and Cybulski, A., (Part B) **56**, 1011-1019 (2001).
17. Namjoshi, A. and Ramkrishna, D., "Bifurcation Analysis of Cybernetic Models for Continuous Bioreactors with Mixed Substrate Feeds," *Chem. Eng. Sci*., (Mashelkar Festschrift issue), **56**, 5593-5607 (2001).
18. Mahoney, A. and Ramkrishna, D., "Efficient Solution of Population Balance Equations with Discontinuities by Finite Elements," *Chem.Eng.Sci.*, **57**, 1107-1119 (2002).
19. Ramkrishna, D., "On Aggregating Populations," *Industrial & Engineering Chemistry Research* (Shinnar special issue) in press.
20. Mahoney, A., F. Doyle and Ramkrishna, D., "Inverse Problems in Population Balances: Growth and Nucleation from Dynamic Data," *A.I.Ch.E. Jl.*, **48**, 981-990 (2002).
21. Ramkrishna, D. and Mahoney, A., "Population Balance Modeling. Promise for the Future," *Chem. Eng. Sci.,* (Invited Flagship paper), **57**, 595-606 (2002).
22. Ramkrishna, D., "On Bioreactor Models for Control," *Journal of Process Control*, **13**, 581-589 (2003).
23. Hamilton, R. A., Curtis, J. S. and Ramkrishna, D., "Beyond Log-Normal Distributions; A Hermite Spectral Method for Solving Population Balance Equations," *A.I.Ch.E. Jl.,* **81**, 2328-2343 (2003).
24. Namjoshi, A., Wei-Shou, H. and Ramkrishna, D., "Unveiling Steady State Multiplicity in Hybridoma Cultures: The Cybernetic Approach," *Biotech. & Bioeng*., **81**, 80-91 (2003).
25. Song, H.-S., Ramkrishna, D., Trinh, S., Espinoza, R. L. and Wright, H., "Multiplicity and Sensitivity Analysis of Fischer-Tropsch Bubble Column Slurry Reactors: Plug-Flow Gas and Well-Mixed Slurry Model," *Chem. Eng. Sci.*, **58,** 2759-2766 (2003).
26. Ramkrishna, D. and Amundson, N. R., "Mathematics in Chemical Engineering. A Fifty Year Introspection," *A.I.Ch.E. Jl.*, **82**, 1-16 (2004).

C. Research Support

Current

NSF 08/15/01-07/31/04

"Investigation of Bubble Size Distribution"

Investigates the formation of gas bubbles in a column using Laser Doppler Anemometry with a view to developing statisical population balance models for the prediction of their sizes.

Role: Investigator

NSF 10/01/00 – 03/31/04
"Metabolic Engineering, Optimization & Control of Ethanol Production in *Escherichia Coli*"
Investigates metabolic engineering strategies by developing metabolic models, adapting them to experimental measurement of numerous metabolites, and developing optimal designs of genetic changes for promotion of ethanol production in engineered strains.
Role: Investigator

Conoco, Inc. 07/01/99 – 06/30/04
"Reactor Engineering Modeling and Analysis"
This is an investigation of Fischer-Tropsch synthesis in collaboration with Conoco Chemicals for developing efficient reactor configurations for high quality hydrocarbon fuels from synthesis gas with safe operating limits.
Role: Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Richard R. Repasky	Bioinformatics consultant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
North Carolina State University, Raleigh, NC	B.S	1978	Wildlife Biology
University of British Columbia, Vancouver, BC	M.S.	1984	Zoology
Indiana University, Bloomington, IN	Ph.D.	1993	Zoology
University of Toronto, Toronto, ON	Postdoctoral	1993-1995	Evolutionary Biology
Indiana University, Bloomington, IN	Postdoctoral	1996	Evolutionary Biology
University of California at Santa Cruz, CA	Postdoctoral	1998	Evolutionary Biology

A. Positions and Honors

1999 Visiting Instructor, Indiana University, Bloomington, Indiana, USA
1999 - 2001 Unix Consultant, Indiana University, Bloomington, Indiana, USA
2001 - Bioinformatics Consultant, Indiana University, Bloomington, Indiana, USA

B. Selected peer-reviewed publications

1. Repasky, R. R., “Temperature and the northern distributions of wintering birds,” *Ecology* **72**, 2274-2285 (1991).
2. Repasky, R. R. and Schluter, D., “Habitat distributions of wintering sparrows along an elevational gradient: tests of alternate hypotheses,” *Journal of Animal Ecology* **63**, 569-582 (1994).
3. Repasky, R. R. and Schluter, D., “Habitat distributions of wintering sparrows: foraging success in a transplant experiment,” *Ecology* **77**, 452-460 (1996).
4. Repasky, R. R., “Using vigilance behavior to test whether predation promotes habitat partitioning,” *Ecology* **77**, 1880-1997 (1996).
5. Rowe, L., Repasky, R. R. and Palmer, A. R., “Condition-dependent symmetry in sexually selected traits: fluctuating asymmetry or antisymmetry?,” *Evolution* **51**, 1401-1408 (1997).

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Simon James Rhodes	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Sheffield, England, UK	B.S. (Hons)	1981 – 1984	Biochemistry
Purdue University, West Lafayette, IN	Ph.D.	1984 – 1991	Biochemistry/Molec. Biol.
University of California, San Diego, CA	Postdoctoral	1991 – 1995	Molecular Medicine

A. Positions and Honors

1989-1990	National Cancer Institute Center Student Fellowship
1991-1993	American Cancer Society Postdoctoral Fellowship
1992-present	Reviewed manuscripts for Proc. Natl. Acad. Sci. USA, Mol. Cell. Biol., Dev. Biol., Development, J. Biol. Chem., Mol. Endocrinol., Genome Res., Genes Dev., Nature, Am. J. Physiol., Mamm. Genome, Trends Endocrinol. Metab., Endocrinology, Comp. Gen. Endo.
1994-1997	Special Fellowship of the Leukemia Society of America
1995-2001	Assistant Professor of Biology, Indiana University-Purdue University Indianapolis
1995-present	Indiana University Cancer Center Member
1996-present	Adjunct Professor of Biochemistry & Molecular Biology, Indiana Univ. School Medicine
1997	Indiana University Teaching Excellence Recognition Award
1997 and 1998	Co-organizer and Chair, Midwest Developmental Biology Meeting
1998	Outstanding Faculty Award, Indiana University-Purdue University at Indianapolis
2000-present	Endocrine Society Minority Affairs Mentor
2000 and 2001	Endocrine Society Minority Affairs Shortcourse Visiting Professor
2001-present	Associate Professor of Biology, Indiana University-Purdue University Indianapolis
2001	Indiana University Trustees Teaching Excellence Award
2001	Reviewed grants for Wellcome Trust, U.K.
2002	*Ad hoc* review of grants for NSF, USDA, and NIH/NIDDK
2002	NIH Biochemical Endocrinology (BCE) Study Section
2003	*Ad hoc* grant reviews for NSF
2003	*Ad hoc* grant reviews for Canadian Institutes of Health Research

B. Selected peer-reviewed publications

1. Sloop, K. W., Meier, B.C., Bridwell, J. L., Parker, G. E., McCutchan, S. A., and Rhodes, S. J., "Differential activation of pituitary hormone genes by human Lhx3 isoforms with distinct DNA binding properties," *Mol. Endocrinol* **13**, 2212-2225 (1999).
2. Feister, H., Torrungruang, K., Thunyakitpisal, P., Parker, G. E., Rhodes. S. J., and Bidwell, J. P., "The NP/NMP4 transcription factors have distinct osteoblast nuclear matrix subdomains," *J. Cell. Biochem*. **79**, 506-517 (2000).
3. Sloop, K., Walvoord, E., Showalter, A., Pescovitz, O. H. and Rhodes, S. J., "Molecular analysis of LHX3 and PROP1 in pituitary hormone deficiency patients with posterior pituitary ectopia,". *J. Clin. Endo. Metab*. **85**, 2701 (2000).
4. Sloop, K., Showalter, A., Von Kap-Herr, C., Pettenati, M. and Rhodes, S., "Analysis of the human LHX3 neuroendocrine transcription factor gene: mapping to the subtelomeric region of chr 9," *Gene* **245**, 237-243 (2000).
5. Smith, R. and Rhodes, S. J., "Applications of developmental biology to medicine and animal agriculture," *Prog. Drug Res.* **54**, 155-198 (2000).
6. Parker, G. E., Sandoval, R. M., Feister, H. A., Bidwell, J. P. and Rhodes, S. J., "The homeodomain coordinates nuclear entry of the Lhx3 neuroendocrine transcription factor and association with the nuclear matrix," *J. Biol. Chem.* **275**, 23891-23898 (2000).

7. Thunyakitpisal, P., Alvarez, M., Tokunaga, T., Onyia, J. E., Hock, J., Rhodes, S. J. and Bidwell, J. P., "Cloning and functional analysis of a novel family of nuclear matrix transcription factors (NP/NMP4) that regulate type I collagen expression,"*. J. Bone Min. Res*. **16**, 10-23 (2001).
8. Sloop, K. W., Parker, G. E., Hanna, K., Wright, H. and Rhodes, S. J., "LHX3 transcription factor mutations associated with combined pituitary hormone deficiency impair the activation of pituitary target genes," *Gene* **265**, 61-69 (2001).
9. Rhodes, S. J. and Banner, L. R., "'Are your cells pregnant?' - relating biology laboratory exercises to everyday life," *The American Biology Teacher.* **63**, 514-517 (2001).
10. Parker, G. E., Sloop, K. W. and Rhodes, S. J., "Transcriptional control of the development and function of the hypothalamic-pituitary axis," *Dev. Endocrinol: from Research to Clinical Practice*. 3-42 (2001).
11. Blazer-Yost, B. L., Butterworth, M., Hartman, A., Parker, G. E., Faletti, C., Els, W. J. and Rhodes, S. J. "Characterization and imaging of A6 epithelial cell clones expressing fluorescently-labeled ENaC subunits," *Am. J. Physiol. Cell Physiol.* **281**, C624-C632 (2001).
12. Sloop, K. W., Parker, G. E. and Rhodes, S. J., "Transcriptional regulation in mammalian pituitary development and disease," *Curr. Genom*. **2**, 379-398 (2001).
13. Bridwell, J. L., Price, J. R., Parker, G. E., McCutchan, S. A., Sloop, K. W. and Rhodes, S. J., "Role of the LIM domains in DNA recognition by the Lhx3 neuroendocrine transcription factor," *Gene* **277**, 239-250 (2001).
14. Bidwell, J. P., Torrungruang, K., Alvarez, M., Rhodes, S. J., Shah, R, and Watt, A. J., "Involvement of the nuclear matrix in the control of skeletal genes: the NMP1 (YY1) NMP2 (Cbfa1), and NMP4 (Nmp4/CIZ) transcription factors," *Crit. Rev. Euk. Gene Exp.* **11**, 1-20 (2001).
15. Alvarez, M. B., Thunyakitpisal, P., Rhodes, S. J., Everett, E. T. and Bidwell, J. P., "Assignment of Nmp4 to mouse chromosome 6 band F1 flanked by D6Mit134 and D6Mit255 using radiation hybrid mapping and fluorescence in situ hybridization," *Cytogenet. Cell Genet.* **94**, 244-245 (2001).
16. Smith, T. P. L., Showalter, A. D., Sloop, K. W., Rohrer, G. A., Fahrenkrug, S. C., Meier, B. C, and Rhodes, S. J., "Identification of porcine Lhx3 and SF1 as candidate genes for QTL affecting growth and reproduction traits in swine," *Anim. Genet.* **32**, 344-350 (2001).
17. Sloop, K. W., Dwyer, C. and Rhodes, S. J., ''An isoform-specific inhibitory domain regulates the LHX3 LIM homeodomain factor holoprotein and the production of a functional alternate translation form," *J. Biol. Chem.* **276**, 36311-36319 (2001).
18. Cushman, L. J., Showalter, A. D. and Rhodes, S. J., "Genetic defects in the development and function of the anterior pituitary gland," *Ann. Med.* **34**, 179-191 (2002).
19. Showalter, A. D., Smith, T. P. L., Bennett, G. L., Sloop, K. W., Whitsett, J. A. and Rhodes, S. J., "Differential conservation of transcriptional domains of mammalian Prophet of Pit-1 proteins revealed by structural studies of the bovine gene and comparative functional analysis of the protein," *Gene* **291**, 211-221 (2002).
20. Torrungruang, K., Shah, R., Alvarez, M., Bowen, D. K., Pavalko, F., Elmendorf, E., Hock, J., Rhodes, S. J. and Bidwell, J. P., "Osteoblast intracellular localization of Nmp4 proteins," *Bone*. **30**, 931-936 (2002).
21. Torrungruang, K., Alvarez, M., Shah, R., Rhodes, S, J., Onyia, J. E. and Bidwell, J. P.,"DNA binding and gene activation properties of the Nmp4 nuclear matrix transcription factors" *J. Biol. Chem*., **277**, 16153-16159 (2002).
22. Freking, B. A., Murphy, S., Wylie, A., Jirtle, R., Rhodes, S. J., Keele, J., Leymaster, K. and Smith, T. P. L., "Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals," *Genome Res.* **12**, 1496-1506 (2002).
23. Palakal, M., Stephens, M., Mukhopadhyay, S., Raje, R. and Rhodes. S. J., "Identification of biological relationships from text documents using efficient computational methods," *J. Bioinf. Comput. Biol.* **1**, 1-34 (2003).
24. Alvarez, M., Rhodes, S. J. and Bidwell, J. P., "Context-dependent transcription: all politics is local," *Gene* In press, (2003).
25. Walvoord, E. C., Sloop, K. W., Dwyer, C. J., Rhodes, S. J. and Pescovitz, O. H., "Severe short stature and endogenous growth hormone resistance in twin brothers without growth hormone gene mutations," *Endocrine* **21**, 289-295 (2003).
26. Savage, J. J., Yaden, B. C., Kiratipranon, P. and Rhodes, S. J., "Transcriptional control during mammalian anterior pituitary development," *Gene* **319**, 1-19 (2003).
27. Tran, P., Savage, J. J., Ingraham, H. A. and Rhodes, S. J., "Molecular genetics of hypothalamic-pituitary axis development," *Pediatric Endocrinology: Mechanisms, Manifestations and Management*, In press (2003).
28. Showalter, A. D., Yaden, B. C., Chernoff, E. A. G. and Rhodes. S. J., "Cloning and analysis of axolotl ISL2 and LHX2 LIM-homeodomain transcription factors," In press (2003).

C. Research Support

Current

R01 HD42024-01 (Rhodes) 4/01/02–3/31/07
NIH/NICHD
"Role of LHX3 protein isoforms in pituitary development"
The major goals of this project are to investigate the role of human LHX3 proteins.
Role: PI

0131702 (Rhodes) 5/1/02-4/30/05
NSF
"Regulation of LIM homeodomain neuroendocrine transcription factor function"
The major goals are to study phosphorylation of the Lhx3 protein and to determine the biochemical properties of the Lhx4 protein.
Role: PI

0093092 (Chernoff) 10/1/00–9/30/03 (currently on no-cost extension)
NSF
"Partnerships for innovation: a center of excellence in regenerative biology"
The major goal is to develop a research Center for Regenerative Biology and Medicine that will investigate limb and spinal cord regeneration in amphibians.
Role: Co-investigator

Completed

031400-0063 (Stocum) 8/26/00–8/26/02 (no-cost extension to 8/03)
State of Indiana 21st Century Research and Technology Fund
"Novel approaches for tissue and organ regeneration"
The major goal was to develop a research Center for Regenerative Biology and Medicine that will investigate limb and spinal cord regeneration in amphibians.
Role: Co-investigator

0081944 (Mukhopadhyay) 9/1/00-8/31/03
NSF
"ITR/IM: an active, personalized, adaptive, multiformat biological information delivery system"
The goal of this project was to develop novel bioinformatics data retrieval computer tools. Dr. Rhodes serves to provide biological examples for bioinformatic analysis.
Role: Co-investigator

99-35205-8248 (Rhodes) 9/30/99–9/30/01 (no-cost extension to 9/03)
USDA
"Coordinated control of pituitary development and function by homeodomain proteins"
The major goals of this project were to map the Prop1 and Lhx3 genes in swine.
Role: PI

9729669 (Rhodes) 2/28/98-2/28/01 (no-cost extension to 2/02)
NSF
"Activity of the P-Lim homeoprotein in pituitary organogenesis"
The major goal was to biochemically characterize the mouse P-Lim transcription factor.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
J. Paul Robinson	Professor of Immunopharmacology Professor of Biomedical Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of NSW, Sydney, Australia	B.Sc.(Hons)	1975	Microbiology
University of NSW, Sydney, Australia	M.Sc	1980	Micro/Immuno
University of NSW, Sydney, Australia	Ph.D.	1983	Immuno/Path
St. Vincent's Hospital, Sydney, Australia	Postdoctoral	1983-1984	Immunology
University of Michigan, Ann Arbor, MI	Postdoctoral	1984-1986	Pathology

A. Positions and Honors

Positions

1985 – 1988	Research Coordinator, Cytometry Laboratories, Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA
1986 –1988	Research Investigator, Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA,
1988 –1993	Associate Professor, Department of Physiology and Pharmacology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA, July
1993 -	Professor of Immunopharmacology, School of Veterinary Medicine, Purdue University
1988 - present	Director, Purdue University Cytometry Laboratories, West Lafayette, Indiana, USA
1999 -	Professor of Biomedical Engineering, Department of Biomedical Engineering, Schools of Engineering, Purdue University

Awards

1979 – 1980	Anthony Stuart Immunology Scholarship, Medical School, University of New South Wales
1981	Commonwealth Postgraduate Scholarship
1981 – 1983	National Health and Medical Research Council (of Australia) Biomedical Postgraduate Fellowship
1997	Honorary Life Member, Iberian Society for Cytometry
2002	Gamma Sigma Delta Award of Merit, Research

B. Selected peer-reviewed publications

Books Published

1. *Handbook of Flow Cytometry Methods*; 2nd Edition, J. Paul Robinson, Z. Darzynkiewicz, P. Dean, L. Dressler, H. Tanke, L. Wheeless, Eds, Wiley-Liss. New York, ISBN 0-471-59634-5 (1993)
2. *Methods in Cell Biology, Cytometry, Vol. 63*, 3rd Edition, Z. Darzynkiewicz, J. Paul Robinson, and H. Crissman, Eds, Academic Press, New York (2000).
3. *Emerging Tools for Single-Cell Analysis*, G. Durack and J. P. Robinson, Eds, Wiley-Liss, New York (2000).

Publications

1. Voytik-Harbin, S. L., Brightman, A. O., Waisner, B. Z., Robinson, J. P. and Lamar, C. H., "Small intestinal submucosa: a tissue-derived extracellular matrix that promotes tissue-specific growth and differentiation of cells in vitro," *Tissue Eng*. **4**,157-174 (1998)
2. Al-Majali, A. M., Asem, E. K., Lamar, C, Robinson, J. P., Freeman, J. and Saeed, A. M., "Insulin modulates intestinal response of suckling mice to the Escherichia coli heat-stable enterotoxin," *Adv. Exp. Med. Biol.* **473**, 113-23 (1999).

3. Blanton, J. R. Jr., Grant, A. L., McFarland, D. C., Robinson, J. P. and Bidwell, C. A., "Isolation of two populations of myoblasts from porcine skeletal muscle," *Muscle Nerve* **22**, 43-50 (1999).
4. Brightman, A. O., Rajwa, B. P., Sturgis, J. E., McCallister, M. E., Robinson, J. P., and Voytik-Harbin, S. L., "Time-lapse confocal reflection microscopy of collagen fibrillogenesis and extracellular matrix assembly in vitro," *Biopolymers* **54**, 222-234 (2000).
5. Asem, E. K., Feng, S., Stingley-Salazar, S. R., Turek, J. J., Peter, A. T. and Robinson, J. P., "Basal lamina of avian ovarian follicle: influence on morphology of granulosa cells in-vitro," *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.***125**, 189-201 (2000).
6. Roeder, B. A., Kokini, K., Sturgis, J. E., Robinson, J. P. and Voytik-Harbin, S. L., "Micromechanics of extracellular matrix: three-dimensional microstructure under load," *ASME International Mechanical Engineering Congress and Exposition, Advances in Bioengineering*, (conference paper), New York, New York (2001).
7. Roeder, B. A., Kokini, K., Sturgis, J. E., Robinson, J. P. and Voytik-Harbin, S. L., "Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure," *J. Biomech Eng.* **124**, 214-222 (2002).
8. Luo, J., Li, N., Robinson, J. Paul and Shi, R., "Detection of reactive oxygen species by flow cytometry after spinal chord injury," *Journal of Neuroscience Methods* **120**, 105-112 (2002).
9. Voytik-Harbin, S. L., Roeder, B. A., Sturgis, J. E., Kokini, K. and Robinson, J. P., "Simultaneous mechanical loading and confocal reflection microscopy for 3D micro-biomechanical analysis of biomaterials and tissue constructs," *Microscopy and Microanalysis* **9** (1), 74-85 (2003).
10. Bassoe, C.-F., Li, Nianyu, Ragheb, Kathy, Lawler, Gretchen, Sturgis, Jennie and Robinson, J. Paul, "Cytometric Investigations of Phagosomes, Mitochondria and Acidic Granules in Human neutrophils," *Cytometry* **51B** (1), 21-9 (Jan 2003).
11. Wu, Jun, Rajwa, Bartomiej, Filmer, David L., Hoffmann, Christoph M., Yuan, Bo, Chiang, Ching-Shoei, Sturgis, Jennie, Voytik-Harbin, Sherry and Robinson, J. Paul, "Automated quantification and reconstruction of collagen matrix from 3D confocal datasets," *Journal of Microscopy* **210** (2),158-165 (2003).
12. Wu, Jun, Rajwa, Bartomiej, Filmer, David L., Hoffmann, Christoph M., Yuan, Bo, Chiang, Ching-Shoei, Sturgis, Jennie, and Robinson, J. Paul, "Analysis of orientations of collagen fibers by novel fiber-tracking software," *Microscopy & Microanalysis* **9**, 574-580 (2003).
13. Koulov, A. V., Stucker, K. A., Lakshmi, C., Robinson, J. P. and Smith, B. D., "Detection of apoptotic cells using a synthetic fluorescent sensor for membrane surfaces that contain phosphatidylserine," *Cell Death and Differentiation* **10**, 1357-9 (2003).
14. Voytik-Harbin, Sherry L., Roeder, Blayne A., Sturgis, Jennifer E., Kokini, Klod and Robinson, J. Paul, "Simultaneous Mechanical Loading and Confocal Reflection Microscopy for Three-Dimensional Microbiomechanical Analysis of Biomaterials and Tissue Constructs," *Microscopy & Microanalysis* **9**, 74-85 (2003).

Selected Book Chapters (of 18)

1. Robinson, J. P. and Turek, J. J., "Microscope image processing and analysis," *Wiley Encyclopedia of Electrical and Electronics Engineering* **13**. Webster, J.G. (ed.). New York, Wiley-Interscience, 1999.
2. Robinson, J. P., Overview of flow cytometry and microbiology. In: Current Protocols in Cytometry. Robinson, J. P., Darzynkiewicz, Z., Dean, P.N., Orfao, A., Rabinovitch, P.S., Stewart, C.C., Tanke, H.J., and Wheeless, L.L. (eds.). New York, John Wiley, 1999.
3. Voytik-Harbin, S. L., Rajwa, B.P., and Robinson, J. P., Three-dimensional imaging of extracellular matrix and extracellular matrix-cell interactions. Methods in Cell Biology. 63, 583-597, 2001.
4. Robinson, J. P., Principles of Confocal Microscopy. In: Methods in Cell Biology, Cytometry 3rd Edition, Part A. , Darzynkiewicz, Z., Crissman, H.A., and Robinson, J. P., New York, Academic Press, 2000.
5. Robinson, J. Paul, Ben Gravely White Light Scanning Digital Microscopy In: G. Durack and J. P. Robinson (Eds.), Emerging Tools for Single Cell Analysis: Advanced in Optical Measurement Technologies. (pp. 291-306. Wiley-Liss, New York, NY, 2000

Electronic Publications

1. Cytometry CD-ROM Vol. 7, Cytomics II, Producer and Publisher: J. Paul Robinson, 5000 distribution, ISBN 0-9717498-8-4 (May 2003).

C. Research Support

Current

Purdue University-Internal Grant 1/1/96- 12/31/04
VP's Reinvestment Program, Center for Computational Image Analysis and Visualization
Aim: This project has been funded to provide high quality image processing and analysis. Complex imaging protocols and advanced instrumentation are made possible under this program.
PIs: Drs. Turek, Sacks, Allebach and Robinson

Indiana/Ohio Heart 01/01/99- 12/31/03
Modulation of Coronary Arterial Function by Estrogen: Potential Role in Protection against Oxidant Injury
Aim: to study the effects of estrogen on endothelial function. PI: David Bell,
Co-PI: J. Paul Robinson

NIH 04/01/01- 11/30/03
S10-RR15781
Aim: This is an instrumentation grant for the purchase of a militia-photon microscope for Purdue University.
PI: J. Paul Robinson

National Cancer Institute (NIH) 4/1/00-12/31/04
NCI-2P30CA23168-18
Cancer Center Cytometry Laboratory Core Facility
Aims: To provide core support to members of Purdue Cancer Center for flow cytometry and image cytometry.
PI: Borch
Co-PI: J. Paul Robinson

NIH 7/01/00- 6/30/03
R019M55266
Acute Repair of Spinal Injury with Fusigens
Aim: This program will study injury and repair mechanisms of spinal cord. PI: R. B. Borgans,
Co-PI: J. Paul Robinson

NSF (Awarded 3/1/99) 03/02/02- 05/31/04
IGERT: Program on Therapeutic & Diagnostic Devices.
Aims: this program funds graduate students in an NSF graduate program. This is a training program designed to bring students into a much better understanding of how therapeutic devices are constructed and operate.
PI: N. A. Peppas
Co-PI: J. P. Robinson

USDA (Awarded 04/01/00) 3/01/02- 02/28/04
#58-1935-9-010
Aim: This program involved the development of microchip devices for detection of food borne pathogens.
PI: W. R. Woodson
Co-PI: J. P. Robinson

NIH 04/01/02- 3/31/03
R01-9M55266-01A2
Synthesis of Triggerable Fusogens for Membrane Bilayers
Aim: This proposal has been studying the mechanisms of formation of vesicles for transport of molecules through membranes. PI: D. H. Thompson
Co-PIs: I. Szleifer and J. Paul Robinson

American Heart Association 12/02 - 12/04
0350612Z
Aging, Physical Activity and Toll-like Receptor 4
To determine whether a 12-week combined endurance and resistance exercise training program influences the cell-surface expression of toll-like receptor 4 in young and older sedentary subjects. PI: M. G. Flynn
Co-PI: J. P. Robinson

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Brenda J. Rongish	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Kansas State Univ., Manhattan, KS	B.S.	1988	Biol & Sec. Ed.
Univ. of Iowa, Iowa City, IA	Ph.D.	1994	Anatomy
MUSC, Charleston, SC	Postdoctoral	1994-1997	Cell Biol/Anat
KUMC, Kansas City, KS	Postdoctoral	1997-2000	Anat/Cell Biol

A. Positions and Honors

1988 - 1994 Graduate Student with Robert J. Tomanek, Department of Anatomy, University of Iowa, Iowa City, IA
1994 - 7/97 Post-doctoral Fellowship with Charles Little, Department of Cell Biology & Anatomy, Medical Univ. of South Carolina, Charleston, SC
8/97 - 7/00 Post-doctoral fellowship with William Kinsey, Department of Anatomy & Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS
8/00 - 12/02 Research Assistant Professor, Department of Anatomy & Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS
1/03 - Assistant Professor, Department of Anatomy & Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS

1991, 1993 Sigma Xi Grant-in-Aid of Research
7/94 - 5/95 MUSC Health Sciences Foundation Postdoctoral Fellowship
5/95 - 8/95 NIH National Research Service Award Postdoctoral Fellowship
9/95 - 6/97 American Heart Association (SC Affiliate) Postdoctoral Fellowship
11/99 - 7/00 Postdoctoral Trainee, KUMC Reproductive Biology Center NIH Training Grant
11/02 NIH SBIR/STTR Study section panelist
10/03 - NIH ZRG1 F05 (20)L Fellowships Study section

B. Selected peer-reviewed publications

1. Torry, R. J. and Rongish, B. J., “Angiogenesis in the uterus: potential regulation and relation to tumor angiogenesis,” *American Journal of Reproductive Immunology* **27**, 171-179 (1992).
2. Rongish, B. J., Torry, R. J., Tucker D. C.,and Tomanek, R. J., “Neovascularization of embryonic rat hearts cultured in oculo closely mimics in utero coronary vessel development,” *Journal of Vascular Research* **31** (4), 205-215 (1994).
3. Schraeger, J. A., Canby, C. A., Rongish, B. J., Kawai, M. and Tomanek, R. J., “Normal left ventricular diastolic compliance following regression of hypertrophy,” *Journal of Cardiovasc Pharmacology* **23**, 349-357 (1994).
4. Rongish, B. J., Torry, R. J. and Tomanek, R.J., “Coronary neovascularization of embryonic rat hearts cultured in oculo is independent of thyroid hormones,” *American Journal of Physiol* **268**:(Heart Circ. Physiol. 37) H811-H816 (1995).
5. Little, C. D., and Rongish B. J., “The extracellular matrix during heart development,” *Experientia* **51**, 873-882 (1995).

C. Research Support

Current

NIH/NHLBI (Rongish) 9/30/02-9/29/05
1RO1HL73700-01
Computational study of fibrillins in CV morphogenesis
The major goals of this project include the use of 4-D optical sectioning microscopy and computational analyses to examine and quantify fibrillin fibril motion and assembly in the cardiovascular systems of normal and experimentally perturbed avian embryos.
Role: PI

Completed

KUMC Research Institute (Rongish) 2/1/01-1/31/02
Lied Basic Science
Pilot Research Award
Fibrillins in cardiovascular Development: Dynamic Imaging of Microfibril Assembly
The major goals of this project are to document normal microfibril assembly in cardiovascular development and any malformation(s) associated with perturbing this microfibril assembly.
Role: PI

AHA Heartland Affiliate (Rongish) 07/01/01-06/30/03
#0160369Z
Beginning Grant-in-Aid
Fibrillins in Cardiovascular Development: Dynamic Imaging of Microfibril Assembly
The major goals of this project are to document normal microfibril assembly in cardiovascular development and any malformation(s) associated with perturbing this microfibril assembly.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Santiago Schnell	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Universidad Simón Bolívar, Caracas, Venezuela	Licence	1991-1996	Biology
Mathematical Institute, University of Oxford, UK	D. Phil.	1998-2002	Applied Mathematics
Dept. of Statistics, University of Oxford, UK		2002	Bioinformatics
Mathematical Institute, University of Oxford, UK	Postdoctoral	2003-	Mathematical Biology

A. Positions and Honors

1991 - 1994 IDEA Foundation Scholar, Instituto de Estudios Avanzados (IDEA), Valle de Sartenejas, Caracas, Venezuela
1996 Honourable Mention in Biology (for outstanding research thesis), Universidad Simón Bolívar, Valle de Sartenejas, Caracas, Venezuela
1996 - 1997 Founding Member, Bioethical Committee, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT), Caracas, Venezuela
1997 - 1998 Senior Scientist, Procter & Gamble, Latin American Product Division, Caracas, Venezuela
1998 - 2002 José Gregorio Hernández Research Fellow and Scholar, Academia Nacional de Medicina, Caracas, Venezuela and Pembroke College, University of Oxford, Oxford, UK
1998 - 2002 ORS Award, Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom, London, UK.
1998 - 2002 Senior Scholarship, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT), Caracas, Venezuela
1999 - 2002 Lord Miles Senior Scholar in Science, Pembroke College, University of Oxford, Oxford, UK
2000 - 2001 Retained Lecturer, Pembroke College, University of Oxford, Oxford, UK.
2001 College Lecturer, Brasenose College, University of Oxford, Oxford,UK.
2001 - 2004 Junior Research Fellow, Christ Church, University of Oxford, Oxford, UK.
2002 MRC Postdoctoral Research Fellow, Genome Analysis and Bioinformatics Group, Department of Statistics, University of Oxford, UK.
2003 - 2004 Ordinary Research Fellow of the Wellcome Trust, Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, UK
2004 – pres. Assistant Professor, School of Informatics, Indiana University, Bloomington, Indiana, USA

B. Selected peer-reviewed publications

1. Schnell, S. and Mendoza, C., “Enzymological considerations for a theoretical description of the Quantitative Competitive Polymerase Chain Reaction (QC-PCR),” *Journal of theoretical Biology* **184**, 433-440 (1997)
2. Schnell, S. and Mendoza, C., “A closed-form solution for time-dependent enzyme kinetic,” *Journal of theoretical Biology* **187**, 207-212 (1997)/
3. Schnell, S. and Mendoza, C., “Theoretical description for polymerase chain reaction. *Journal of theoretical Biology* **188**, 313-318 (1997).
4. Schnell, S. and Mendoza, C., “Time-dependent closed form solutions for fully competitive enzyme reactions,” *Bulletin of Mathematical Biology* **62**, 321-336 (2000).
5. Schnell, S., Maini, P. K., “Clock and induction model for somitogenesis,” *Developmental Dynamics* **217**, 415-420 (2000).
6. Schnell, S. and Maini, P. K., “Enzyme kinetics at high enzyme concentration,” *Bulletin of Mathematical Biology* **62**, 483-499 (2000).

7. Schnell, S. and Mendoza, C., "Enzyme kinetics of multiple alternative substrates," *Journal of Mathematical Chemistry* **27**, 155-170 (2000).
8. Collier, J. R., McInerney, D., Schnell, S., Maini, P. K., Gavaghan, D. J., Houston, P. and Stern, C. D., "A cell cycle model for somitogenesis: mathematical formulation and numerical simulation," *Journal of theoretical Biology* **207**, 305-316 (2000).
9. Schnell, S. and Mendoza, C., "A fast method to estimate kinetic constants for enzyme inhibitors," *Acta Biotheoretica* **49**, 109-113 (2001)
10. Schnell, S. and Maini, P. K., "Enzyme kinetics far from the Standard Quasi-Steady-State and Equilibrium Approximations," *Mathematical and Computer Modelling* **35**, 137-144 (2002).
11. Schnell, S., Maini, P. K., McInerney, D., Gavaghan, D. J. and Houston, P., "Models for pattern formation in somitogenesis: a marriage of cellular and molecular biology," *Comptes Rendus Biologies* **325**,179-189 (2002).
12. Schnell, S. and Maini, P. K., "A century of enzyme kinetics: Reliability of the K_M and v_{max} estimates," *Comments on Theoretical Biology* **8**, 169-187 (2003).
13. Baker, R. E., Schnell, S. and Maini, P. K., "Formation of vertebral precursors: Past Models and Future Predictions," *Journal of Theoretical Medicine* **5**, 23-35 (2003).
14. Schnell, S., and Mendoza, C., "The condition for pseudo-first-order kinetics in enzymatic reactions is independent of the initial enzyme concentration," *Biophysical Chemistry* **107**, 165-174 (2004).
15. Schnell, S. and Turner, T. E., "Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws," *Progress in Biophysics & Molecules Biology*, (in press).
16. McInerney, D., Schnell, S., Baker, R. E. and Maini, P. K., "A mathematical formulation for the cell cycle model in somitogenesis: analysis, parameter constraints and numerical solutions," *Mathematical Medicine and Biology - A Journal of the IMA*, (in press.

C. Research Support

Current

"Reliability of the kinetic parameter estimates on enzyme catalised reactions" Initiated approx. Oct 1995
Supported by:

- Jose Gregorio Hernandez Award (Academia Nacional deMedicina, Venezuela; Pembroke College, Oxford)
- ORS Award (Committee of Vice-Chancellors and Principals of the Universities the United Kingdom)
- Programa de Cofinanciamiento Institucional IVIC-CONICIT (CONICIT,Venezuela)
- Lord Miles Senior Scholarship in Science (Pembroke College)
- Christ Church (University of Oxford, Oxford)
- Wellcome Trust (London, UK).

In collaboration with Prof Philip K Maini (Centre for Mathematical Biology, University of Oxford) and Prof Claudio Mendoza (Physics Center, IVIC, Venezuela).

Summary: Many biochemists have devoted their attention to the art of accurately determining the.kinetic parameters of enzyme catalised reaction employing rate expressions derived with a number of approximations such as the quasi-steady-state assumption, the pseudo-first order approximation, and equilibrium approximation, among others. Unfortunately, biochemists have not studied the conditions under which most of these rate expressions can be employed. As a consequence, the rate expressions of catalised reactions have been used on a number of occasions outside of the conditions for which they are valid.

Overall goals and responsibilities: We have been deriving a number of approximate solutions for the time-course of several enzyme catalised reaction and have been determining in which regions of the parameter space the approximations are valid.
Role: Prinicipal Researcher

"Biochemical kinetics in intracellular environments with macromolecular crowding" (Schnell)
(Initiated Oct 2002)
Supported by:

- Christ Church (University of Oxford)
- Wellcome Trust (London, UK)

In collaboration with Thomas E Turner (MSc student supervised by the applicant).

Summary: Conventional kinetics based on the law of mass action, the power-law approximation and standard stochastic reaction models fail to describe reactions *in vivo* conditions, because they assume the rate coefficients to be constant. When minimal obstructions to diffusion are present, the rate constant approach is reasonable, but in the presence of significant obstruction to diffusion due to macromolecular crowding, logarithm of the rate coefficient decays linearly on the logarithmic timescale, and so the rate coefficient is time-dependent.

Overall goals and responsibilities: Advances in transition state theory and computer simulations are providing new insights into the sources of reaction kinetics and enzyme catalysis in *in vivo* conditions. We are developing a computer simulation framework for understanding the effects of macromolecular crowding in intracellular reactions. The overall goal is identify and quantify the effects of macromolecular crowding in intracellular reactions and propose a mechanism of how reactions work in *in vivo* conditions.
Role: Principal Researcher

Completed
"Models for Pattern Formation in Somitogenesis: Incorporating the Effects of Fibroblast Growth Factor-8, Cell Adhesion Molecules and Cell Motility, and Hox Genes."
(Oct 2001 – Jan 2004)
Supported by:
- Wellcome Trust (London, UK)
- Christ Church (University of Oxford)

In collaboration with Daragh McInerney and Ruth Baker, D Phil students, (University of Oxford.

Summary of the research project: Segmentation is a basic characteristic of many animal species including man, and usually corresponds to a repetition, along the anterior-posterior axis, of similar structures during early development. In humans, segmentation is most obvious at the level of the vertebral column and its associated muscles, and also in the peripheral nervous system. Functionally, segmentation is critical to ensure the movements of a rod-like structure, such as the vertebral column. The segmented distribution of the vertebrae derives from the earlier metameric pattern of embryonic structures known as somites. Recent evidence in a number of vertebrate embryos indicates that segmentation of the embryonic body relies on at least two molecular clocks for its proper functioning. In humans, mutations and environmental factors disturbing the molecular clocks result in abnormal segmentation of the vertebral column giving rise to several clinical disorders.
Over the past few years, our knowledge of the molecular mechanisms underlying vertebrate segmentation has increased dramatically. However, despite considerable experimental work, the mechanism of somite formation is still a major unresolved problem in developmental biology. In areas of biology, such as neurophysiology, mathematical modelling has led to fundamental insights and discoveries through a process of synthesis and integration of experimental observations. In such cases, mathematical modeling techniques are used as a research tool, comparable with any powerful laboratory technique, for hypotheses testing and for making experimentally verifiable predictions. The applicant employed mathematical modelling to investigate the mechanism of segmentation during somite formation. Studying the developmental mechanisms in vertebral patterning will aid in the identification of protective or potentially disruptive factors for normal somitogenesis and could potentially impact on treatments for the prevention of vertebral patterning disorders. Moreover, a study of somitogenesis has provided an ideal opportunity to investigate multiscale effects linking recent findings at the molecular level to those at the cellular level.

Aims accomplished: The overall goal of this project was to use mathematical modelling to link experimental results at the molecular level with those at the cell and tissue levels in somitogenesis to provide a deeper understanding of the mechanisms that drive spatiotemporal patterning during somite formation. The accomplished aims were: (i) To incorporate recent experimental findings on the effects of FGF-8 on somite size; (ii) To model cell motion and response to cell adhesion molecules during the aggregation process that leads to somite formation; and (iii) To investigate different hypotheses concerning the subsequent somite differentiation process that leads to unique anatomical features depending on position along the cranio-caudal axis.
Role: Principal Researcher, sponsored by Prof Philip K Maini (Centre for Mathematical Biology, University of Oxford)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAM	POSITION TITLE
Sima Setayeshgar	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge, MA	B.S.	1990	Physics & Mathematics
California Institute of Technology, Pasadena, CA	Ph. D.	1997	Physics

A. Positions and Honors

Aug 2003 -present	Assistant Professor of Physics, Indiana University, Bloomington
Oct 2000 - Aug 2003	Council on Science and Technology Postdoctoral Fellow, Princeton University
Sep 1998 -Oct 2000	Postdoctoral Fellow, Caltech
Jun 1998 - Sep 1998	Visiting Scholar, MIT
Oct 1997 - Jun 1998	Postdoctoral Research Associate, Northeastern University
1990	AMITA (Assoc. of MIT Alumnae) Senior Merit Award
1993	ASCIT (Assoc. of Students at Calif. Inst. of Tech.) Teaching Award
1995	ASCIT (Assoc. of Students at Calif.Inst. of Tech.) Teaching Award
2000 - 2003	Princeton Council of Science and Technology Postdoctoral Fellowship
2001	Newton Institute of Sciences (U. of Cambridge) Fellowship

B. B. Selected peer-reviewed publications

1. Setayeshgar, S. and Cross, M. C., "Turing Instability in a Boundary-Fed System," *Phys. Rev. E* **58**, 4485-4500 (1998).
2. Setayeshgar, S. and Cross, M. C., "Numerical Bifurcation Diagram for the Two-Dimensional Boundary-Fed CDIMA System," *Phys. Rev. E* **59**, 4258-4264 (1999).
3. Setayeshgar, S. and Bernoff, A. J., "Scroll Waves in the Presence of Slowly Varying Anisotropy with Application to the Heart," *Phys. Rev. Lett* **88**, 028101 (2002).

C. Research Support

Indiana University, Bloomington, College of Arts and Sciences Start-up Funds

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Anurag Shankar	Manager, Distributed Storage Services

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Lucknow University, India	B.S.	1979	Physics, Mathematics, Astronomy
University of Roorkee, India	M.S.	1981	Physics
West Virginia University, Morgantown, WV	M.S.	1983	Physics
University of Illinois, Urbana, IL	Ph.D.	1990	Astronomy

A. Positions and Honors

1981-1984 Teaching and Research Assistant, Dept. of Physics, West Virginia University, Morgantown, WV
1983 Sigma Pi Sigma, Physics Honor Society
1984-1990 Teaching and Research Assistant, Dept. of Astronomy, University of Illinois, Urbana, IL
1993-1994 Research Associate, Dept. of Astronomy, University of Arizona, Tucson, AZ
1993-1995 Adjunct Research Associate, Dept. of Astronomy, Indiana University, Bloomington, IN
1995-1997 Senior Systems Programmer, Computing and Information Services, Brown University, Providence, RI
1997-1999 Manager, Unix Workstation Support Group, University Information Technology Services, Indiana University, IN
1997-present Adjunct Assistant Professor, Dept. of Astronomy, Indiana University, Bloomington, IN
1999-present Manager, Distributed Storage Services Group, University Information Technology Services, Indiana University, IN

B. Selected peer-reviewed publications

1. Shankar, A. and Bernbom, G., "Building a Massive, Distributed Storage Infrastructure at Indiana University," *Proceedings of the 10th NASA Goddard Conference on Mass Storage Systems and Technologies, College Park, MD*, 285-289 (Apr. 2002).
2. Shankar, A., Meglicki, G., Russ, J., Yang, H. and Garrison, E. C., "Building a Massive Data Infrastructure for the Masses," *Proceedings of the Supercomputing 2002 Conference, Baltimore, MD* (Nov. 2002).
3. Shankar, A., Meglicki, G., Russ, J., Yang, H. and Garrison, E. C., "Building and Supporting a Massive Data Infrastructure for the Masses," *Proceedings of the SIGUCCS 2002 Conference*, Providence, RI, 134-138 (Nov 2002).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Raymond William Sheppard	Principal Analyst, High Performance Computing Support

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Mississippi State University, Mississippi State, MS	B.S.	1997	Physics & Spanish
Mississippi State University, Mississippi State, MS	B.S.	1997	Computer Science

A. Positions and Honors

1979-1986 Naval Tactical Data Systems/Combat Systems Technician (Senior) U.S. Navy
1987-1989 Asst. Electrical Engineer, Ingalls Shipbuilding, Pascagoula, MS
1990-1997 Physics Instructor; A/V Technician, Mississippi State University
1998-2002 Senior Program Engineer, Northrop-Grumman
2002-pres Principal Analyst, Indiana University

B. Selected peer-reviewed publications

1. Peggion, G., Wallcraft, A. and Sheppard, R. W., “The Power of Autotasking,” *Mississippi Academy of Sciences,* 46 (1), (January 2001).
2. Cruise, R. B., Sheppard, R. W. and Moskvin, V., "Parallelization of the Penelope Monte Carlo Particle Transport Simulation Package," *Nuclear Mathematical and Computational Sciences: A Century in Review, A Century Anew*, Gatlinburg, TN, CD-ROM, American Nuclear Society, LaGrange Park, IL (April 6-11, 2003).
3. Stewart, C. A., Hart, D., Sheppard, R. W., Li, H., Cruise, R., Moskvin, V. and Papiez, L., “Parallel computing in biomedical research and the search for peta-scale biomedical applications,” *ParCo*, (2003).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Weinian Shou	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wuhan University, China	B.S.	1984	Cell Biology
Shanghai Institute of Cell Biology, China	Ph.D.	1991	Developmental Biology
M. D. Anderson Cancer Center, Houston, TX	Postdoctoral	1991–1994	Developmental Biology
Baylor College of Medicine, Houston, TX	Postdoctoral	1994–1997	Mouse Development

A. Positions and Honors

1997 – 1999 Assistant Professor, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX

1999 – present Assistant Professor, Department of Pediatrics, Department of Biochemistry and Molecular Biology Indiana University School of Medicine, Indianapolis, IN

B. Selected peer-reviewed publications

1. Shou, W. and Chuang, H., "The competency differences between superficial and inner cell layer of presumptive ectoderm of Xenopus embryos," *Chinese J. Cell Biol.* **11**, 52-62, (1989).
2. Shou, W., "Peptide growth factors and mesoderm induction," *Chinese J. Cell Biol*. **12**, 118-130 (1990).
3. Shou, W., "Mesoderm induction and the related gene expression," *Chinese J. Cell Biol.* **13**, 88-96 (1991).
4. Shou, W. and Chuang, H., "The effects of injection of anti-TGFβ-antibodies into developing Xenopus embryos," *Acta Biol. Exper. Sinica* **25**, 122-137 (1992).
5. Shou, W. and Chuang, H., "The immunohistological studies of TGFβ-like proteins in Xenopus early embryos," *Acta Biol. Exper. Sinica* **25**, 113-121 (1992).
6. Shou, W., Song, Q., Chen, Y. and Qiang, R., "The transcription of TGFβ-like gene in Xenopus early embryos," *Acta Biol. Exper. Sinica* **25**, 105-112 (1992).
7. Li, X., Shou, W., Kloc, M., Reddy, B. and Etkin, L.D., "Xenopus nuclear factor 7 (Xnf7) associated with mitotic spindle," *Exp. Cell Res.* **231**, 473-481 (1994).
8. Li, X., Shou, W., Kloc, M., Reddy, B. and Etkin, L.D., "Cytoplasmic retention of Xenopus nuclear factor 7 (Xnf7) prior to the mid blastula transition utilizes a unique anchoring mechanism involving a retention domain and several phosphorylation sites," *J. Cell Biol.* **124**, 7-17 (1994).
9. Shou, W., Li, X., Wu, C., Cao, T., Kuang, J., Che, S. and Etkin, L. D., "Finely tuned regulation of cytoplasmic retention of Xenopus nuclear factor 7 by phosphorylation of individual threonine residues," *Mol. & Cell Biol.* **16**, 990-997 (1996).
10. Matzuk, M. M., Kumar, T. R., Shou, W., Coerver, K. A., Lau, A., Behringer, R. and Finegold, M. J., "Transgenic models to study the roles of inhibins and activins in reproduction, oncogenesis and development," *Recent Progress in Hormone Research*. **52**, 124-157 (1996).
11. Shou, W., Gou, Q., Dong, J. and Matzuk, M. M., "Transgenic models for studying adrenal, ovarian and testicular cancer," *Adrenal glands, vasuclar system, and hypertension,* ed. Vinson, G. P., *J. Endocrinology, Inc.,* Bristol, 281-292 (1997).
12. El-Hodiri, H. M., Shou, W. and Etkin, L. D., "Xnf7 function in dorsal ventral patterning of the Xenopus embryo," *Dev. Biol.* **190**, 1-17 (1997).
13. Etkin, L. D., El-Hodiri, H. M., Nakamura, H., Wu, C. F., Shou, W. and Gong, S. G., "Characterization and function of Xnf7 during early development of Xenopus," *J. Cellular Physiology*. **173**, 144-146 (1997).

14. Lau, A., Shou, W., Guo, Q., Matzuk, M. M., "Transgenic approaches to study the functions of the transforming growth factor β superfamily members, *Inhibin, activin, and follistatin: Recent advances and future views,* Ed. T. Aono, H. Sugiro, and W. W. Vale, Springer-Verlag, New York, 20-243 (1997).
15. Shou, W., Woodruff, T. K. and Matzuk, M. M., "Role of androgens in testicular tumor development in inhibin- deficient mice," *Endocrinology*. **38**, 5000-5005 (1997).
16. Shou, W., Aghdasi, B., Armstrong, D. L., Guo, Q., Bao, S., Charng, M., Mathews, L. S., Schneider, M. D., Hamilton, S. L. and Matzuk, M. M., "Cardiac defects and altered ryanodine receptor function in FKBP12-deficient mice," *Nature* **391**, 489-492 (1998).
17. Bassing, C. H., Shou, W., Heitman, J., Matzuk, M. M. and Wang, X.-F. "FKBP12 is not required for the modulation of transforming growth factor beta receptor I signaling activity in embryonic fibroblasts and thymocytes," *Cell Growth and Differentiation* **9**, 223-228 (1998).
18. Gold, B. G., Densmore, V., Shou, W., Matzuk, M. M. and Gordon, H. S., "The immunophilin FKBP52 (not FKBP12) mediates the neurotrophic action of FK506," *J. Pharmaco Exp. Ther.* **289**(3), 1202-1210 (1999).
19. Xu, X. H., Su, B. Barndt, R., Chen, H., Xin, H., Yan, G., Zhuang, Y., Fleischer, S. and Shou, W., "FKBP12, but not FKBP12.6, is the only FK506 Binding Protein involved in immunosuppressant-induced T cell Inactivation," *Transplantation* **73**, 1835-1838 (2002).
20. Dubois, S., Shou, W., Haneline, L., Fleischer, S., Waldmann, T. and Müller, J. R., "Distinct Regulation of Interleukin-2 and 15-Induced Proliferation in T Lymphocytes by FK506-binding Proteins 12 and 12.6," *PNAS* 100(24): 14169-14174 (2003).
21. Chen, H., Shi, S., Acosta, L., Li, W., Lu, J., Bao, S., Chen, Z., Yang, Z., Schneider, M. D., Chien, K. R., Conway, S. M., Yoder, M. C., Haneline, L. S., Franco, D. and Shou, W., "BMP-10 is essential for maintaining cardiac growth during murine cardiogenesis," *Development* (in press).
22. Zhong, L., Li, W., Yang, Z., Chen, L., Li, Y., Weigel-Kelly, K. A., Yoder, M. C., Shou, W. and Srivastava, A., "Improved transduction of primary murine hepatocytes by recombinant adeno-associated virus 2 vectors in vivo," *Gene therapy* (in press).

C. Research Support

Current

R01 HL70259 04/2002-

National Institutes of Health

"Role of BMP signaling in cardiac development"

The major goal of this project is to analyze the role of BMP in regulating cardiac development at early stage.

Role: PI

Showalter Research Foundation 07/2003-

"The Role of TGF beta and BMP in Caridac function at midgestation"

The major goal of this project is to create a genetic mutated BMP-deficient mice and study the role of BMP and BMP/TGFbeta mediated signaling in cardiac function.

Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Stephen C. Simms	Senior Technical Advisor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wabash College, Crawfordsville, IN	B.A.	1988	Mathematics and Music
Roosevelt University, Chicago, IL	M.M	1991	Music Composition

A. Positions and Honors

1991 - 1996	Associate Instructor, Department of Music, Indiana University
1992 - 1998	Computer Consultant, University Computing Services, Indiana University
1997	Visiting Lecturer in Music, Indiana University Purdue University Columbus
1997-2000	Visiting Lecturer in Music, Marian College
1998	Unix/Macintosh Support Coordinator, Support Center, University Information Technology Services, Indiana University
1999	Unix Systems Specialist, Unix Workstation Support Group, University Information Technology Services, Indiana University
1999 - 2000	Unix Systems Administrator, Research and Technical Services, University Information Technology Services, Indiana University
2000 - 2002	Manager, Unix Workstation Support Group, University Information Technology Services, Indiana University
2002 -	Senior Technical Advisor for Research and Technical Services, University Information Technology Services, Indiana University

Synergistic Activities

1. Participation in setup and staging for iGrid2000 in Yokohama.
2. Worked with ATLAS researchers to get AtlasGrid running on Indiana's AVIDD cluster.
3. Helped with cycle-harvesting project using Condor.
4. Helped with development of Simple Message Brokering Library for use with Condor.
5. Developing algorithmic video software for use in live performance.
6. Assisted with Craig Stewart's Bioinformatics tutorial at SC2003.
7. Assisted with Indiana's winning HPC Challenge project at SC2003

B. Selected peer-reviewed publications

1. Stewart, C. A., Peebles, C. S., Papakhian, M., Samuel, J., Hart, D., and Simms, Stephen, "High Performance Computing: Delivering Valuable and Valued Services at Colleges and Universities," *Proceedings of SIGUCCS*, Portland, OR, (October 2001).

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Craig A. Stewart	Director, Research and Academic Computing

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wittenberg University, Springfield, OH	B.A.	1981	Biology, Mathematics
Indiana University, Bloomington, IN	Ph.D.	1988	Biology

A. Positions and Honors

1981-1984 Associate Instructor, Department of Biology, Indiana University
1984-1985 Computer Consultant, Bloomington Academic Computing Services, Indiana University
1985-1986 Applications Programmer, Bloomington Academic Computing Services, Indiana University
1986-1991 Manager, Business Computing Facilities, School of Business, Indiana University
1991-1995 Director, Center for Statistical and Mathematical Computing, UCS, Indiana University
1995-1996 Manager, Support Center, University Computing Services, Indiana University
1996-1997 Assistant Director for Research Computing Services, University Computing Services, Indiana University
1997-present Director, Research and Academic Computing
2000-present Director, Indiana Genomics Initiative Information Technology Core
2003-present Special Assistant for the Life Sciences, Office of the Vice President for Research
2003-present Adjunct Associate Professor, Department of Medical Genetics
Spring 2003 Visiting Faculty Member, Faculty of Computer Science, University of Stuttgart, Stuttgart, Germany

Honors

2003 High Performance Computing Challenge Award, at Supercomputing 2003 conference (www.supercomp.org), for the project “Global analysis of arthropod evolution.” This project used maximum likelihood analysis of DNA data consuming thousands of hours of computer time to analyze the phylogenetic relationships of arthropods. Stewart lead an international team that created a global grid of supercomputers spanning every continent except Antarctica to perform this analysis.

B. Selected peer- reviewed publications

1. Stewart, C.A. Diurnal variation in minimal thermal conductance of the white-footed mouse (Peromyscus leucopus), the golden hamster (Mesocricetus auratus), and the eastern woodrat (Neotoma floridana). Ph.D. dissertation, Indiana University (1988).
2. Stewart, C.A., Tan T.W., M. Buckhorn, D. Hart, D. K. Berry, L. Zhang, E. Wernert, M. Sakharkar, W. Fischer, D.F. McMullen. Evolutionary biology and high performance computing. In: R.F. Enenkel. Software Tools for Computational Biology (2000).
3. http://www.cas.ibm.com/archives/1999/workshop_report/bio.html
4. Zorn, M., et al. Computational Biology and High Performance Computing. Tutorial presented at SC2000, Dallas, Nov. 2000. Also printed as a special report of Lawrence Berkeley National Laboratory (2000). http://cbcg.lbl.gov
5. Stewart, C.A., C.S. Peebles, M. Papakhian, J. Samuel, D. Hart, S. Simms. High Performance Computing: Delivering Valuable and Valued Services at Colleges and Universities. Proceedings of SIGUCCS, Portland, OR, October 2001. http://www.indiana.edu/~rac/staff_papers.html.

6. Stewart, C.A., D. Hart, D. K. Berry, G. J. Olsen, E. Wernert, W. Fischer. Parallel implementation and performance of fastDNAml - a program for maximum likelihood phylogenetic inference. Proceedings of SC2001, Denver, CO, November 2001. http://www.sc2001.org/papers/pap.pap191.pdf
7. Peebles, C.S., C.A. Stewart, B.D. Voss, S.B. Workman. Measuring quality, cost, and value of IT services in higher education. Proceedings of Educause, Indianapolis, IN, October 2001.
8. http://www.indiana.edu/~rac/staff_papers.html.
9. Peebles, C.S., C.A. Stewart, B.D. Voss, S.B. Workman. Measuring quality, cost, and value of IT services in higher education. Proceedings of the American Quality Congress, Charlotte, NC, May 2001.
10. Samuel. J.V., Wilhite, K.J., Stewart, C.A. Getting More for Less: A Software Distribution Model. Paper presented at the Educause Conference, October 1-4, 2002, Atlanta, GA. Available from http://www.indiana.edu/~rac/staff_papers.html.
11. Stewart, C.A., et al. Opportunities for Biomedical Research and the NIH through High Performance Computing and Data Management (2003). Published by the Coalition for Advanced Scientific Computing (http://www.casc.org/paper.html).
12. Stewart, C.A., D. Hart, R. Repasky, M. Papakhian, A. Shankar, E. Wernert, A.D. Arenson, G. Bernbom. Information technology support for post-genomic biomedical research. Proceedings of SIGUCCS 2003 Conference, Sante Fe, NM, October 2003.
13. Stewart, C.A., D. Hart, R.W. Sheppard, H. Li, R. Cruise, V. Moskvin, L. Papiez. Parallel computing in biomedical research and the search for peta-scale biomedical applications. Proceedings of ParCo2003 Conference, Dresden, Germany, September 2003 (in press).

C. Research Support

Current

Lilly Endowment (private charitable trust) 2000-2008

"INGEN – the Indiana Genomics Initiative"

The purpose of this grant is to develop a world-class genomic-based medical research program based on the existing excellence of the IU School of Medicine. The purpose of the Information Technology core is to provide the advanced supercomputing, visualization, and high performance storage facilities required for INGEN researchers to perform cutting-edge genomic and biomedical research.

Role: Investigator and Director of the Information Technology Core.

NIH/NIAAA 2003-2006

"Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders"

The purpose of this grant is to unify the research being conducted on disorders resulting in children from alcohol consumption by pregnant woman. The Informatics Core is providing a standardized data repository and help with statistical analysis.

Role: Principal Investigator

NSF 2003-2008

"IP-Grid - Indiana Purdue Grid"

Grant from the National Science Foundation. The purpose of this grant is to join the information technology resources of Indiana University and Purdue University to the NSF-funded Teragrid. The Teragrid is a national system of supercomputers, storage facilities, and specialized instrumentation designed to enable next-generation scientific research. Indiana University's contributions to the Teragrid will focus on our supercomputing resources, storage facilities, and especially on the array of life sciences data sets we manage and make available for use by scientific researchers.

Role: Investigator

IBM 2002-2004

Joint Development Contract with IBM. Inc.

Development of the Protein Family Annotator. The purpose of this project is to create a distributed database of annotated data for families of proteins.

Role: Co-PI

Completed

NSF Major Research Infrastructure Grant 2001-2003
"Creation of the AVIDD Data Facility: A distributed facility for managing, Analyzing and Visualizing Instrument-Driven Data"
The purpose of this grant was to create a distributed facility of massive Linux clusters, storage systems, and visualization facilities to enable real time analysis of streams of data from advanced digital instruments. This grant resulted in the creation of a distributed suite of Linux clusters that currently are listed as the fastest system of distributed clusters in the world on the list of the world's 500 fastest supercomputers (www.top500.org). In addition, this grant resulted in the creation of a 3-D visualization system suitable for in-lab work. This system, which is a 50" cube, has been successfully commercialized. The AVIDD system has enabled many scientific breakthroughs, including several in the life sciences.
Role: Investigator

IBM Inc. Shared University Research grant award 2001-2002
"Information Technology Applications for the Life Sciences"
This grant enabled the creation of several open-source software applications for the life sciences.
Role: Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
David L. Stocum	Professor of Biology and Dean, School of Science

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Susquehanna University, Selinsgrove, PA	B.A.	1961	Biology and Psychology
University of Illinois, Champaign, IL	M.S.	1963	Zoology
University of Pennsylvania, Philadelphia, PA	Ph.D.	1968	Cell and Developmental Biology

A. Positions and Honors

1968 – 1990	Assistant Professor through Professor, University of Illinois, School of Life Sciences, Department of Cell and Structural Biology, Champaign, IL
1974 – present	AAAS Fellow, Section G (Biological Sciences).
1974 – 1976	Director, Honors Biology Program, University of Illinois, School of Life Sciences, Department of Cell and Structural Biology, Champaign, IL
1984 – 1986	Acting Head, Department of Anatomical Sciences, School of Life Sciences, University of Illinois, Champaign, IL
1984 – 1990	Professor, College of Medicine, University of Illinois
1986 – 1987	Associate in the Center for Advanced Study, University of Illinois, Champaign, IL
1988	Singer Medallion for Regeneration Research
1989 – present	Professor of Biology and Dean, School of Science, Indiana University-Purdue University Indianapolis, Indianapolis, IN

B. Selected peer-reviewed publications (of 92 total)

1. Stocum, D. L., "Regeneration of symmetrical hindlimbs in larval salamanders," *Science* **200**, 790-793 (1978).
2. Nardi, J. B. and Stocum, D. L., "Surface properties of regenerating limb cells: evidence for gradation along the proximodistal axis," *Differentiation* **25**, 27-31 (1983).
3. Crawford, K. and Stocum, D. L., "Retinoic acid coordinately proximalizes regenerate pattern and blastema differential affinity in axolotl limbs," *Development* **102**, 687-698 (1988).
4. Stocum, D. L., "Wound Repair, Regeneration and Artificial Tissues," *Austin: RG Landes Co*, 221 (1995).
5. Stocum, D. L. "Stem cells in regenerative biology and medicine," *Wound Rep Reg* **9**: 429-442 (2001).
6. Nye, H. L. D., Cameron, J. A., Chernoff, E. A. G. and Stocum, D. L., "Regeneration of the urodele limb: a review," *Dev Dynam* **226**, 280-294 (2003).
7. Stocum, D. L., "Amphibian regeneration and stem cells." *Regeneration: Stem Cells and Beyond*, Heber-Katz E, ed. Heidelberg: Springer, 1-70 (2004).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Eric Andrew Wernert	Senior Scientist & Manager, UITS Advanced Visualization Lab

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bellarmine College, Louisville, KY	B.A.	1985	Mathematics, Computer Science, Secondary Education
Indiana University, Bloomington, IN	M.S.	1991	Computer Science
Indiana University, Bloomington, IN	Ph.D.	2000	Computer Science (Cognitive Science minor)

A. Positions and Honors

Positions and Employment

1985-1989 Mathematics and Computer Science Teacher, St. Xavier High School, Louisville, KY
1989-1992 Associate Instructor, Computer Science Department, Indiana University, Bloomington, IN
1992-1996 Undegraduate Course Coordinator & Instructor, Computer Science Department, Indiana University, Bloomington, IN
1992-2000 Lecturer for Computer Graphics and Virtual Reality (part time), Computer Science Department, Indiana University, Bloomington, IN
1996-1997 Graphics Software Specialist, UITS Center for Innovative Computer Applications, Indiana University, Bloomington, IN
1997-1999 Senior Research Programmer & CAVE Team Leader, UITS Virtual Reality / Virtual Environments Lab, Indiana University, Bloomington, IN
1999-2000 Acting Manager and Senior Research Programmer, UITS Advanced Visualization Lab, Indiana University – Bloomington and Indiana University – Purdue University, Indianapolis
2000- Senior Scientist and Manager, UITS Advanced Visualization Lab, Indiana University – Bloomington and Indiana University – Purdue University, Indianapolis
2002- Visiting Assistant Professor, Computer Science Dept., Indiana University, Bloomington, IN

Honors

1981-1985 Presidential Scholarship, Bellarmine College, Louisville, KY
1985 Faculty Merit Award in Education, Award for Excellence in Math and Science, & Archbishop's Medal for Highest Academic Achievement - Bellarmine College, Louisville, KY
1988 Selected Participant, NASA Educational Workshop for Math and Science Teachers – NASA Langley Research Center, Langley, VA
1989-1990 College of Arts and Sciences Fellowship - Indiana University, Bloomington, IN

B. Selected peer-reviewed publications

1. Wernert, E. A.,"A Unified Environment for Presenting, Developing, and Analyzing Graphics Algorithms," *Computer Graphics* (August 1997).
2. Hanson, A. J. and Wernert, E. A., "Constrained 3D Navigation with 2D Controllers," *Proceedings of IEEEVisualization '97*,175-182 (1997).
3. Hanson, A. J. and Wernert, E. A., "Image-Based Rendering with Occlusions via Cubist Images," *Proceedingsof IEEE Visualization '98*, 327-334 (1998).

4. Hanson, A. J., Wernert, E. A. and Hughes, S. B., “Constrained navigation environments,” *Scientific Visualization: Dagstuhl '97 Proceedings,* Hagen, Hans, Nielson. Gregory M. and Post, Frits, eds, IEEE Computer Society Press, 95-104 (1999).
5. Wernert, E. A. and Hanson, A. J., “A framework for assisted exploration with collaboration,” *Proceedings of IEEE Visualization '99*, IEEE Computer Society Press, 241-248 (1999).
6. Stewart, C. A., Tan, T. W., Buckhorn, M., Hart, D., Berry, D. K., Zhang, L., Wernert, E. A., Sakharkar, M., Fischer, W. and McMullen, D. F., “Evolutionary biology and high performance computing,” *Software Tools for Computational Biology*, Enenkel, R. F., ed. (2000).
7. Wernert, E. A. and Hanson, A. J., “Tethering and reattachment in collaborative virtual environments,” *Proceedings of IEEE Virtual Reality 2000*, IEEE Computer Society Press, 292 (2000).
8. Hanson, A. J., Fu, Chi-Wing and Wernert, E. A., “Very large scale visualization methods for astrophysical data,”*Data Visualization 2000*,. Proceedings of the Joint Eurographics and IEEE TVCG Symposium on Visualization, May 29-31, 2000, de Leeuw, W. and van Liere, R., eds., Amsterdam, the Netherlands, Springer-Verlag, 115-124 (2000).
9. Stewart, C.A., Hart, D., Berry, D. K., Olsen, G. J., Wernert, E. A. and Fischer, W., “Parallel implementation and performance of fastDNAml – a program for maximum likelihood phylogenetic inference,” *Proceedings of SC2001*, Denver, CO, (November 2001).
10. Hanson, A. J., Fu, Chi-Wing and Wernert, E. A., “Visualizing cosmological time,” *Proceedings of Dagstuhl 2000*, 21-26 May 2000, Dagstuhl, DE, (2002).
11. Wernert, E. A., Berry, D. K., Huffman, J. N. and Stewart. C. A., “Tree3D - A System for Temporal and Comparative Analysis of Phylogenetic Trees, “*Proceedings of IEEE Information Visualization 2003 – Interactive Poster Session*, Seattle, WA, (October 2003).

C. Research Support

Current

NSF (McRobbie) 09/01/01 – 08/31/03
0116050
NSF/ EIA-Major Research Instrumentation
“Creation of the AVIDD Data Facility: A Distributed Facility for Managing, Analyzing and Visualizing Instrument-Driven Data”
This grant enables the acquisition of computation, storage and visualization resources to manage and analyze very large data from projects including crystallography, medical imaging, high-energy physics, and geology.
Role: Planning and implementation of visualization components

HIN/HIAAA, (Foroud) 09/29/03-09/28/06
U01 AA014809-01
“A Cross-Cultural Longitudinal Assessment of FASD (U24 Core)”
The imaging core of this project will collect and analyze 3D facial images from a range of subjects to perform standard and novel anthropometric measurements for more effective diagnosis of FAS and FASD.
Role: Co-Investigator for 3D Imaging Core

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Stefan Wuchty	Postdoctoral Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Vienna, Vienna, Austria	B.S.	1997	Biochemistry
University of Vienna, Vienna, Austria	M.S.	1998	Theoretical Biochemistry
University of Vienna, Vienna, Austria	Ph.D.	2002	Theoretical Biochemistry

A. Positions and Honors

1997 Effort award of the University of Vienna, Vienna, Austria
1998-1999 Research Associate, IPHT, Jena, Germany
1998-1999 Research Associate, MERCK KGaA, Darmstadt, Germany
1999-2002 Research Associate, European Media Laboratory Ges.m.b.H., Heidelberg, Germany
2002-present Postdoctoral Research Associate, Department of Physics, University of Notre Dame

B. Selected peer-reviewed publications

1. Schober, A., Schlingloff, G., Tomandl, D., Köhler, J. M., Mayer, G., Gross, A., Wuchty, S., Diefenbach, B and Wurziger, H., "Chemical and Biochemical Synthesis and Screening in Silico," *Microsystems Technologies 98, 6th. Internat. Conf. on Micro-, Electro-, Opto- and Mechanical Systems and Components*, Reichl, H. and Obermayer, E. (eds.), Potsdam 451-456 (1998).
2. Wuchty, S., Fontana, W., Hofacker, I. L. and Schuster, P. K., "Complete Suboptimal Folding of RNA and the Stability of Secondary Structures," *Biopolymers* **49**, 145-165 (1999).
3. Wuchty. S., "Scale-free Behavior in Protein Domain Networks," *Mol. Biol. Evol* **18** (9), 1694-1702 (2001).
4. Wuchty, S., "Interaction and Domain Networks of Yeast," *Proteomics* **2**(12), 1715-1723 (2002).
5. Wuchty. S., Oltvai, Z. N. and Barabási, A.-L., "Evolutionary conservation of motif constituents in the yeast protein interaction network," *Nature Genetics* **35**, 176-179 (2003).
6. Wuchty, S., "Small-Worlds in RNA," *Nucl. Acids Res.,* **31**, 1108-1117 (2003).
7. Wuchty, S. and Stadler, P. F., "Centers of large networks," *J. theoret. Biol*., **223**, 45-53 (2003).
8. Wuchty, S., Oltvai, Z. N. and Barabasi, A.-L., "Evolutionary conservation of motif constituents within the yeast protein interaction network," *Nature Genetics*, **35** (2), 176-179 (2003).
9. Wuchty, S., Ravasz, E. and Barabasi, A.-L., "The Architecture of Biological Networks," in *Complex Systems Science in Biomedicine,* T.S. Deisboeck, J., Kresh, Y. and Kepler, T. B. (eds.), Kluwer Academic Publishing, New York (2003).
10. Barabasi, A.-L. Oltvai, Z. N. and Wuchty, S., "Characteristics of Biological Networks," in *Complex Systems,* Ben-Naim, E. Frauenfelder, H. and Toroczkai, Z. (eds.), Springer Lecture Notes in Physics, New York, (2003).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mervin C. Yoder	Professor of Pediatrics and of Biochemistry and Molecular Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Malone College, Canton, OH	B.A.	1975	Biology
Indiana State University, Terre Haute, IN	M.A.	1976	Physiology
Indiana University School of Medicine, Indianapolis, IN	M.D.	1980	Medicine

A. Positions and Honors

1975	Magna Cum Laude, Malone College
1979	Alpha Omega Alpha, Indiana University School of Medicine, Indianapolis, IN
1980 – 1983	Resident Physician in Pediatrics, The Children's Hospital of Philadelphia, PA
1980	M.D. with Highest Distinction, Indiana University School of Medicine, Indianapolis, IN
1983 – 1985	Fellow in Neonatology, The Children's Hospital of Philadelphia, PA
1983	Senior Pediatric Resident Teacher Award, University of Pennsylvania
1985 – 1991	Assistant Professor of Pediatrics, Indiana University School of Medicine, Indianapolis, IN
1990 – 1991	Assistant Professor of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN
1991 – 2000	Associate Professor of Pediatrics and of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN
1998	Prenatal Research Society
2000 – present	Professor of Pediatrics and of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN
2001	American Pediatric Society

B. Selected peer-reviewed publications (from > 120).

1. Yoder, M. C., Du, X.-X. and Williams, D. A., "High proliferative potential colony-forming cell heterogeneity identified using counterflow centrifugal elutriation," *Blood* **82** (2), 385-391 (1993).
2. Yoder, M. C., Papaioannou, V. E., Breitfeld, P. P. and Williams, D. A., "Murine yolk sac endoderm- and mesoderm-derived cell lines support in vitro growth and differentiation of hematopoietic cells," *Blood* **83** (9), 2436-2443 (1994).
3. Clapp, D. W., Freie, B., Srour, E., Yoder, M. C., Fortney K. and Gerson, S. L., "Myeloproliferative sarcoma virus directed expression of □-galactosidase following retroviral transduction of murine hematopoietic cells," *Exp Hematol* **23**, 630-638 (1995).
4. Yoder, M. C., King, B., Hiatt, K, Mukherjee, P. and Williams, D. A., "Growth of bone marrow high proliferative potential colony forming cells during in vitro co-culture with murine embryonic yolk sac cell lines," *Blood* **86**,1322-1330 (1995).
5. Wang. X., Yoder, M. C., Zhou, S. Z. and Srivastava, A. "Parvovirus B19 promoter at map unit 6 confers replication competence and erythroid specificity to adeno-associated virus 2 in primary human hematopoietic progenitor cells," *Proc Natl Acad Sci* **92** (26), 12416-12420 (1995).
6. Traycoff, C. M., Cornetta, K., Yoder, M. C., Davidson, A. and Srour, E. F., "Ex vivo expansion of murine hematopoietic progenitor cells generates classes of expanded cells possessing varying levels of bone marrow repopulating potentials," *Exp Hematol* **24**, 299-306 (1996).
7. Yoder, M. C., Cumming, J., Hiatt. K., Mukherjee, P.and Williams, D. A., "A novel method of myeloablation to enhance engraftment of adult bone marrow cells in newborn mice," *Biol Blood Marrow Transplant* **2**, 59-67 (1996).
8. Leemhuis, T., Yoder, M. C., Grigsby, S., Eder, P. and Srour, E. F., "Isolation of primitive human bone marrow hematopoietic progenitor cells using Hoechst 33342 and rhodamine 123," *Exp Hematol* **24**:1215-

1224 (1996).
9. Dutt. P., Hanenberg, H., Vik, T., Williams, D. A. and Yoder, M. C., "A recombinant human fibronectin fragment facilitates retroviral mediated gene transfer into human hematopoietic progenitor cells," *Biochem Mol Biol Internat* **42** (5), 909-917 (1997).
10. Ponnazhagan, S., Yoder, M. C. and Srivastava, A., "Adeno-associated virus 2-mediated transduction of murine hematopoietic cells with long-term repopulating ability and sustained expression of a human globin gene in vivo," *J Virol* **71** (4), 3098-3104 (1997).
11. Yoder, M. C. and Hiatt, K., "Engraftment of embryonic hematopoietic cells in conditioned newborn recipients," *Blood* **89**, 2176-2183 (1997).
12. Yoder, M. C., Hiatt, K. and Mukherjee, P., "In vivo repopulating hematopoietic stem cells are present in the murine yolk sac at day 9.0 postcoitus," Proc *Natl Acad Sci USA* **94**, 6776-6780 (1997).
13. Yoder, M. C., Hiatt, K., Dutt, P., Mukherjee, P., Bodine, D. M. and Orlic, D., "Characterization of definitive lymphohematopoietic stem cells isolated from the day 9 murine yolk sac," *Immunity* **7**, 335-344 (1997).
14. Ponnazhagan. S., Wang. X.-S., Kurpad. C., Yoder, M. C., Srour, E. F. and Srivastava, A., "Adeno-associated virus 2-mediated transduction of primary human bone marrow-derived $CD34^+$ hematopoietic cells: Donor variation and correlation of expression with cellular differentiation," *J Virol* **71** (11), 8262-8267 (1997).
15. Orlic, D., Girard, L. J., Anderson, S. M., Pyle, L. C., Yoder, M. C., Broxmeyer, H. E. and Bodine, D. M., "Identification of human and mouse hematopoietic stem cell (HSC) populations expressing high levels of mRNA encoding retrovirus receptors," *Blood* **91** (9), 3247-3254 (1998).
16. Dagher, R., Hiatt, K., Traycoff, C., Srour, E. and Yoder, M. C., "c-Kit and CD38 are expressed by reconstituting hematopoietic cells present in the murine yolk sac," *Biol Blood Marrow Transplant* **4**:69-74 (1998).
17. Haneline, L. S., Gobbett, T. A., Ramani, R., Carreau, M., Buchwald, M., Yoder, M. C. and Clapp, D. W., "Loss of FancC function results in decreased hematopoietic stem cell repopulating ability," *Blood* **94** (1):1-9 (1999).
18. Orschell-Traycoff, C. M., Hiatt, K., Dagher, R. N., Rice, S., Yoder, M. C. and Srour, E. F., "Homing and engraftment potential of Sca-1+Lin- cells fractionated on the basis of adhesion molecule expression and position in cell cycle," *Blood* **96** (4),1380-1387 (2000).
19. Palis, J., Chan, R. J., Koniski, A., Patel, R., Starr, M. and Yoder, M. C., "Spatial and temporal emergence of high proliferative potential hematopoietic precursors during murine embryogenesis," *Proc Natl Acad Sci USA* **98** (8), 4528-4533 (2001).
20. Srour, E. F., Jetmore, A., Wolber, F. M., Plett, A., Abonour, R., Yoder, M. C. and Orschell-Traycoff, C. M. , "Homing, cell cycle kinetics and fate of transplanted hematopoietic stem cells," *Leukemia* **15** (11), 1681-1684 (2001).
21. Badylak, S. F., Park, K., Peppas, N., McCabe, G. and Yoder, M. C., "The composition of bioscaffolds affects in-vivo cell recruitment and alters the pattern of tissue repair," *Exp Hematol* **29** (11),1310-1318 (2001).
22. Wolber, F. M., Leonard, E., Michael, S., Orschell-Traycoff, C. M., Yoder, M. C. and Srour, E. F., "Roles of spleen and liver in development of the murine hematopoietic system," *Exp Hematol* **30**,1010-1019 (2002).
23. Xie, X., Chan, R. J., Starr, M., Johnson, S. A., Li, W., Morrison, P. and Yoder, M. C., "Thrombopoietin promotes mixed-lineage and megakaryocytic colony forming unit growth but inhibits primitive and definitive erythropoiesis in cells isolated from the early yolk sac," *Blood* **101** (4), 1329-1335 (2003).
24. Ferkowicz, M., Li, W., Starr, M., Xie, X., Morrison, P., Johnson, S., Shelley, C. and Yoder, M. C., "CD41 expression defines the onset of primitive and definitive hematopoiesis in the mouse embryo," *Development* **130**, 4393-4403 (2003).
25. Li, W., Johnson, S. A., Shelley, W. C., Ferkowicz, M., Morrison, P., Li, Y. and Yoder, M. C., "Primary endothelial cells isolated from the yolk sac and paraaortic splanchnopleure (P-Sp) support the expansion of adult marrow stem cells in vitro," *Blood* **102** (13), 4345-4353 (2003).
26. Ingram, D. A., Huddleston, H., Tan, B., Yang, F.C., Wenning, M. J., Orazi, A., Yoder, M. C. and Kapur, R., "A functional *p85* gene is required for normal murine fetal erythropoiesis," *Blood* (in press).
27. Guo, Y., Chan, R., Ramsey, H., Li, W., Xie, X., Shelley, W. C., Martinez-Barbera, J. P., Bort, B., Zaret, K., Yoder, M. C. and Hromas, R., "The homeoprotein Hex is required for hemangioblast differentiation," *Blood* (in press).
28. Chan, R. J., Johnson. S. A., Li, Y., Yoder, M. C. and Feng, G. S., "A definitive role of Shp-2 tyrosine phosphatase in mediating embryonic stem cell differentiation and hematopoiesis," *Blood* (in press).

C. Research Support

Current

R01 HL58881 (Srivastava, P.I.) 9/1/97 – 6/30/05
NIH
"Parvovirus vectors for human gene therapy"
The major goal of this application is to analyze the ability of parvovirus vectors to transduce human and mouse stem cells and to observe erythroid restricted expression of the transferred genes.
Role: Co-Investigator

R01HL63169 (Yoder, P.I.) 7/1/99 – 6/30/04
NIH
"Endothelial cell role in yolk sac stem cell development"
The major goal of this application is to study endothelial and stem cell development in the murine yolk sac.
Role: PI

P60 HL 53586 (Dinauer, P.I.) 3/15/00-2/28/05
NIH
"Gene Replacement Therapy in Hematopoietic Stem Cells"
The major goal of Project 3 is to evaluate whether introduction of the murine homologue of Fanconi Anemia Type C will restore normal hematopoiesis in *FancC -/-* mice.
Role: Co-P.I. on Proj. 3

R01 HL55716 (Srour, P.I.) 4/1/00 – 3/31/04
NIH
"Cell cycle progression and hematopoietic potential"
The major goal of this application is to examine the utility of hematopoietic stem cells from different tissues to serve as hematopoietic repopulating cells following transplantation.
Role: Co-investigator

R01 HL 63219 (Clapp, P.I.) 9/1/00 – 8/31/05
NIH
"Hematopoiesis and Leukemic Transformation in *Fac* Mice"
The major goal of this application is to develop aplastic and leukemogenic models of Fanconi anemia using *Fancc -/-* mice.
Role: Co-PI

R01 HL/DK65570 (Srivastave, P.I.) 6/1/01-5/31/06
NIH
"Hematopoietic stem cell transduction by AAV2 vectors"
The major goal of this application is to examine the efficiency and long-term expression of genes in hematopoietic cells transduced with AAV2 vectors.
Role: Co-Investigator

R01 HL69156 (Srour, P.I.) 9/30/01 – 8/31/05
NIH
"Common muscle, hematopoietic, and neural stem cells"
The major goal of this application is to determine whether a stem cell with a particular cell surface phenotype resides in multiple tissues and exhibits the capacity to give rise to many different tissues in vivo upon transplantation.
Role: Co-Investigator

P50 DK61594 (Molitoris, P.I.) 6/1/02 – 5/37/07
NIH
"Center for advance renal microscopic analysis (Project 3)"
The major goal of this project is to develop transgenic animals expressing endothelial specific proteins.
Role: Co-Investigator, Project 3

R44 HL65864 (Pollok, P.I.) 2/1/03 – 1/31/05
NIH
“A novel frozen storage system for cord blood cells”
The major goal of this project is to analyze progenitor cell content of blood after prolonged cryopreservation.
Role: Co-Investigator

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: Substantial PI laboratories are available at all Institutions involved in the project. The Center for Medical Genomics at Indiana University School of Medicine occupies 4,000 square feet of state-of the-art laboratory space in the new Biotechnology Resource and Training Center (BRTC) building. The researchers in the Biologically Motivating projects in Core Three occupy either brand new research space in the Indiana University Cancer Center or newly renovated laboratory space in the Reilly Children's Hospital at the Indiana University School of Medicine. Indiana University, Bloomington, personnel are mostly located in the newly renovated Lindley, Chemistry and Informatics buildings and in renovated space in Swain West Halls. We also have a substantial commitment of state-of-the-art laboratories in the Multidisciplinary Sciences Buildings I (80,000 sq.ft.) and II (140,000 sq.ft.), to begin construction in Spring 2004 and Spring 2005 and the planned 250,000 sq.ft. Informatics Building planned for 2006. All laboratories associated with the Notre Dame Center for the Study of Biocomplexity are appropriately equipped for the planned projects. Notre Dame is currently constructing a new Science teaching Building (150,000 sq.ft.) which will free a significant amount of additional office and research laboratory space in existing departmental Buildings and is planning a large building to house interdisciplinary research centers. Purdue faculty associated with the Bindley Biosciences Center will occupy a flexible research building (26,000 sq.ft.) to be completed by Spring of 2005. Please see attached for individual laboratory information.

Clinical: Not relevant

Animal: The Science Animal Resource Center at Indiana University-Purdue University, Indianapolis, provides the 2,400 square foot multiple room physical facilities, a three member staff, and routine animal care/ health monitoring and maintenance of the physical facility. Indiana University Medical Center houses various animals in the Laboratory Animal Resource Center (LARC), where the animals are housed in an AALAC-approved facility with oversight of all animals provided by four veterinarians. Please see attached for detailed information about each facility.

Computer: See attached

Office: Office facilities will be provided by the Department of Physics, University Information Technology Services, the School of Informatics, the pervasive technologies Laboratories and the Departments of Computer Science and Chemistry at Indiana University, Bloomington. Indiana University School of Medicine and IUPUI School of Science also supply all investigators with office space in their home departments, as does the University Information Technology Services location at IUPUI. All Biocomplexity Center faculty members at the University of Notre Dame have individual offices in their home departments. Bindley Biosciences Center members at Purdue have offices through the Center. Most Project Directors have their own Administrative Assistants. Secretarial support is available to individual faculty through their department offices. Please see attached for individual office information.

Other: The Institute will purchase videoconferencing equipment for all locations to facilitate timely and efficient inter-site communication.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

See attached

COMPUTER RESOURCES

Indiana University Computing Systems

FACILITIES: INFORMATION TECHNOLOGY AND COMPUTING SYSTEMS

Indiana University has a long history of providing computing, visualization and data resources in support of computational biology, bioinformatics and medical research. The Indiana Genomics Initiative funding, awarded by the Lilly Endowment in December, 2000, resulted in a major expansion of these facilities. As a result, biologists and biomedical researchers have since utilized more than 1.6 million hours of CPU time on Indiana University's supercomputer systems. Purdue University also has a long history of providing the cyberinfrastructure required for research in bioengineering. Recently, Indiana University and Purdue University have collaborated to combine these significant resources. In October, 2003, Indiana University and Purdue University were awarded funding from the National Science Foundation to become part of the Extensible TeraScale Facility. This funding has allowed IU and Purdue to create a grid of supercomputing, visualization, and data facilities within the State of Indiana's three largest research campuses (Indiana University Purdue University Indianapolis, Purdue University West Lafayette, and Indiana University Bloomington). This IP-grid (Indiana Purdue grid) is interconnected via the State of Indiana's I-Light high-speed optical fiber network. The aggregate rate of growth of Indiana and Purdue systems since 1996 has been aggressive, outstripping a Moore's Law rate, with peak theoretical capacity doubling every 15 months. Indiana and Purdue expect growth to continue in these facilities. These facilities would be available to the projects of the seven cores of the proposed National Center of Excellence in Tissue Modeling. Table 1 shows the computational resources which will be available to this proposed center.

Computational Resources

IU and Purdue offer diverse hardware architecture platforms. The Sun V1280s and several of the IBM SP nodes offer significant SMP capacity. All are well balanced in disk and memory. The AVIDD Linux cluster includes 10 TB of disk storage space and an Itanium-based component. The Purdue Linux clusters provide an additional aggregate compute capacity of over 1 TeraFLOPS.

Both institutions have extensive distributed computing implementations. At Purdue this involves Condor and United Devices (UD) software. Purdue's Condor instance will include over 1,000 hosts by the end of Summer 2003 for Condor and Condor/G's use. The United Devices deployment includes more than 2,300 Windows/XP hosts that are part of the instructional labs. These resources can be accessed via the familiar PBS job management system. In an experiment with IU's Condor flock of 1,010 Windows-based PCs, researchers harvested 60,000 CPU hours of compute time during a week of wall clock time.

IBM SP Systems at IU and Purdue

The IU IBM SP System comprises two geographically distributed IBM RS/6000 SP systems connected to form one logical system with a total theoretical peak compute capacity of 1.005 TeraFLOPS (1.005 trillion FLoating OPerations per Second), a total memory capacity of 452 gigabytes (452 billion bytes), and a total disk capacity of 5.3 terabytes (5.3 trillion bytes). The nodes within each SP system are interconnected via a low-latency, high-speed (150 megabytes/second) network using crossbar switch technology, referred to as the SP Switch. The IU IBM SP includes a total of 632 processors.

The Purdue SP system, like the IU SP system, is comprised of multiple WinterhawkII, NighthawkII and Power4 SP nodes, with an aggregate peak compute capacity of 0.56 TeraFLOPS. Please see

IU's AVIDD (Analysis and Visualization of Instrument-Driven Data) Facility

AVIDD includes a suite of four distributed Linux clusters. Two identical clusters, each with 208 2.4 Ghz Prestonia processors, form the core of this suite of clusters. These clusters are located 53 miles apart, in Indianapolis and the other in Bloomington. Each local cluster includes a Myrinet interconnect. In addition, the

two clusters are connected via Gbit Ethernet via a private network \x{2013} using two dedicated fibers of the I-Light network (http://www.i-light.org/) and two Force10 E600\x{2122} switches, each configured with 120 ports of 1000Base-TX (Gigabit Ethernet over copper). There is in addition a 36-processor cluster based in the newest Intel Itanium processors located in Indianapolis, and a smaller PentiumIII-based cluster located in IU-Northwest in Gary, Indiana. The peak theoretical capacity of the AVIDD Linux clusters is 2.16 TFLOPS.

The supercomputers operated by Indiana and Purdue Universities are connected by a unique, university owned- and operated- network called I-Light. I-Light (http://www.i-light.org/) is a dedicated, high-bandwidth network that connects supercomputer systems located in West Lafayette, Indianapolis, and Bloomington. The geographic distribution of these supercomputer assets provides a unique level of reliability and resilience in the face of disaster or malicious attack on the buildings that house these supercomputers. The geographic distribution of these facilities assures that computational and simulation systems will remain operational (albeit at reduced capacity) even in the event that two of the three machine rooms (West Lafayette, Indianapolis, and Bloomington) becomes inoperable. This level of resilience has clear and profound benefits in offering a reliable infrastructure for the proposed simulation center.

IU's Research Database Complex

This serves research database needs, providing a large-memory SMP environment, Oracle software, and large disk storage. The RDC comprises two Sun V1280 systems, each with 12 CPUs and 96GB memory and 6TB shared disk, dedicated research database use, and a Sun V880 system with 4 CPUs and 8GB memory serving as a WWW front-end to the databases. It is used by IU's leading database researchers and serves several large biomedical and GIS datasets.

Table 1: Computational Resources

Supercomputers	TFLOPS Capacity	RAM system total	Disk system total
Indiana University	***3.21***	***1.21 TB***	***27.77 TB***
Sun V1280s (2)	0.05	0.20 TB	6.5 TB
IBM SP WinterhawkII, NighthawkII, Regatta nodes; Power3+ & Power4	1.00	0.45 TB	8.31 TB
AVIDD Linux Clusters (Pentium4; IBM IA64)	2.16	0.56 TB	15.58TB
Purdue University	***1.65***	***0.84 TB***	***19.62 TB***
IBM SP WinterhawkII, NighthawkII, Regatta nodes; Power3+ & Power4	0.56	0.42 TB	4.60TB
Intel Cluster (Pentium3 & Pentium2)	0.61	0.32 TB	14.02 TB
AMD Cluster (Dual 1.6Ghz)	0.48	0.10 TB	1.00 TB
Grid resources			
IU Condor Pool	1.67		
Purdue Condor and UD Pool	1.69		

STORAGE

Table 2 shows the storage resources that IP-grid would bring to the National Center for Biomedical Computing. The growth rate of storage facilities within the IP-grid has been dramatic, doubling every 14 months. Our universities' commitment to advanced storage technologies predicts that storage resources contributed to the National Center for Biomedical Computing will also gain in significance over time.

IU's Massive Data Storage System

IU's massive data storage system (MDSS) utilizes the High Performance Storage System (HPSS) software to make available to IU researchers a total storage capacity of roughly 1.6 petabytes. A hierarchical storage management (HSM) system by design, HPSS uses a hierarchy of storage media transparently to provide massive, near-line data storage capacity. Users can access data over the network from central research hosts (IBM SP, Steel, AVIDD) and from personal workstations, using either parallel FTP or hsi (Hierarchical Storage Interface) clients for high performance data transfer, or the convenient Distributed Computing Environment Distributed File System (DCE DFS). Data stored in the MDSS are mirrored between IU Bloomington and IUPUI over Indiana's high performance I-Light network to protect against disasters. Individual researchers are allowed to store up to 500 gigabytes of mirrored data without charge. Usage beyond 500GB is available on a cost-share basis.

Since 1999 Indiana University has employed the HPSS as the basis for its massive data storage system, with a fast front-end disk cache and tape storage in STK 9310 robotic tape silos. IU was the first HPSS installation to implement distributed data movers, and now has redundant, distributed tape storage in Bloomington and Indianapolis connected via the I-Light network. Data written to IU's HPSS system is copied simultaneously to STK tape robots located at both campuses, providing highly reliable disaster protection. This is critical for life sciences data, which is irreplaceable once it's lost.

Purdue's DXUL Storage System

Purdue's DXUL deployment supports the university with 50 TB of tape archival service. Efforts are underway to implement a Data Grid to distribute the large quantities of data fully integrated into the computational grid. As pieces of the Open Grid Services Architecture become production quality through local testbed projects, these resources will be moved onto the Grid, allowing data mining in parallel using local clusters.

Table 2: Storage Resources

Storage Facility Type	Total Capacity
IU -- Disk Storage	24.0 TB
IU -- Tape Storage	520.0 TB
Purdue -- Disk Storage	51.8 TB
Purdue -- Tape Storage	50.0 TB
TOTAL	**645.8 TB**

Visualization

Following are the units that focus on visualization at IUB, IUPUI, and Purdue:

- *Advanced Visualization Lab (AVL).* Indiana University's Advanced Visualization Lab (AVL) has important development programs in VR technology, particularly in constrained navigation, visualization in the life sciences, and the arts. The Lab provides expert consulting, research support, educational opportunities, and hardware and software resources for scientific visualization, virtual reality, high-end computer graphics, and visual tele-collaboration. Resources include high-end graphics workstations and clusters; projection-based virtual reality devices; very high-resolution displays; special-purpose, volume-rendering hardware; haptic display devices; and tools for tele-conferencing and tele-collaboration. The AVL welcomes partnerships with researchers and educators from all IU departments and campuses. For

additional information on AVL hardware and software resources, see http://www.avl.iu.edu/technology.

- *The Purdue Envision Center.* This interdisciplinary effort integrates large-scale visualization, graphics, and haptic devices. Recent and ongoing research supported by Center staff includes projects in the life sciences, audiology, engineering, communications, theater, computer science, geology, and management. The Center's first project, focused on the 9/11 terrorist attack, included a simulation of the plane crashing into the Pentagon, and data animation to determine the cause of the building's structural failure.
- *Visualization and Interactive Spaces Lab.* This Lab at IUPUI, directed by M. Pauline Baker, develops unique interactive spaces combining physical devices, projectors, cameras and sensors, hand-held tablets, PDAs, and game controls, to build the infrastructure for "smart spaces" that enable display of and interaction with data. A popular display at SC2002 was the interactive map providing geographical data ranging from views of the earth down to restaurant advice for the Baltimore area.

Table 3: Advanced Visualization Facilities

Facility Type	Number	Location	Special notes
CAVE™	1	IU	Three 8' walls
Immersadesk™	1	IU	
John-E-Box™	4 installed; 4 more by 7/1/2004	IU	IU design; consistent with NCSA Display-Wall-in-a-Box
Display Walls	2 installed;10 more by 7/1/2004	IU	Consistent with NCSA Display-Wall-in-a-Box
Fakespace Flex reconfigurable Wall & CAVE™	1	Purdue	Wall 8'x30', 4-sided CAVE™
Tiled Display Wall	1	Purdue	7'x14' screen with 12 tiles 4096x2304 resolution
Access Grid Node	6	(2) @ Purdue; (4) @ IU	Video conferencing and meetings
Freespace	1	Purdue	3 wall 8'x8'x8' cave-like full immersion

Specialized HPC Facilities

Indiana University was an early adopter of GRAPE (GRAvity PipE) systems. Applications that use GRAPE boards are repeat finalists for and winners of the annual Gordon Bell prize at the IEEE/ACM SC conference series. Indiana University currently has two systems useful for biomedical computing called MD-GRAPEs (Molecular Dynamics GRAvity PipE) The MD-GRAPE is designed to calculate forces, such as gravity, Coulomb, Ewald and Leonard-Jones forces, on large numbers of particles/bodies, as well as determining nearest neighbors Each MD-GRAPE has a peak compute capacity of 64 GigaFLOPS. These systems will be available to the National Center of Excellence in Tissue Modeling.

DATASETS AND DATA SERVICES

Life Sciences Datasets

Indiana University is host to a number of important datasets of special significance to researchers in the life sciences. These are described below.

- *Life Science Data Repository.* Indiana University has created a "Central Life Sciences Data" (CLSD) facility that provides a centralized means of access to disparate public life sciences data sources. Data access is centralized; the data sets are organized in a federated fashion using Information Integrator and DB2 from IBM. They include NCBI's Genbank and many other NCBI databases as well as databases from SwissProt and Kegg. The current volume of these data sets is in aggregate 1.2 TB. Half are accessible from within CLSD. A more detailed description of CLSD data services is included below.
- *Flybase* and *euGenes.* Indiana University is the primary source for Flybase, the authoritative resource for Drosophila (fruit fly) genome information. euGenes presents eukaryotic genome information organized using the Gene Ontology controlled vocabulary.
- *Regenstreif Institute.* The Regenstreif Institute manages one of the oldest continually operating Medical Informatics systems in the world, in operation since 1972 and containing more than 300-million patient data records. The Regenstreif Institute is currently working to make de-identified patient data available for research purposes; these data will be available (with Institutional Review Board approval) via the National Center for Biomedical Computing Ethosource. Ethosource, a new international center for biomedical computing initiative, will create a systematic, comprehensive, and massive repository of animal behavior data organized under a controlled vocabulary.

Centralized Life Sciences Data

The Centralized Life Sciences Data (CLSD) service provides a single, SQL-based interface to a selection of widely used biomedical datasets, including BIND, ENZYME LIGAND, LocusLink, UniGene, dbSNP, SGD, KEGG PATHWAY and a variety of NCBI BLAST databases. CLSD runs on Indiana University's IBM SP supercomputer, using IBM DB2 database software and IBM's Information Integrator software for retrieving data from multiple, heterogeneous data sources.

CLSD is already used by three research applications, including a Web interface designed to allow intuitive queries by biomedical researchers, a Web-based analysis platform for biomedical researchers who are customers of a DNA microarray facility, and as part of the biomedical data reporting section of a laboratory information management system.

Besides these production systems, there are multiple biomedical research applications in development that plan to make use of CLSD.

CLSD uses software programs called "parsers" to transform datasets from their native format into relational databases. Data files are copied across the Internet from their original sources to supercomputers at Indiana University. These files are converted to relational database format using a parser that is specific to each particular data source.

The resulting relational database files are then imported into IBM's DB2 relational database management system. IBM's DiscoveryLink software and the CLSD software allow biomedical researchers to transparently query any or all of these diverse data sets without necessarily knowing where any particular piece of data originally resided. This allows, for example, the joining of data from different databases into a single record of information presented to the researcher. CLSD also incorporates BLAST datasets, permitting a researcher to run a BLAST job as if executing a database query.

Clinical and research labs can also make their data available to other IU investigators via CLSD. CLSD and its underlying database products enable clinicians and researchers to provide data securely and with control over who has access to the data being provided. Data may be contributed via a wide variety of relational and non-

relational formats, including DB2, Oracle, Sybase, SQL Server, Informix, flat files, Excel, and XML. This system allows a quick, simple, and secure means for sharing data while assuring that all researchers with rights to access the data are making use of the most current versions of those data. The CLSD system, user interface, and several of the underlying '"parser" programs were written by Indiana University's University Information Technology Services staff.

PLANNED GROWTH

Physical Facilities

Informatics and Communications Technology Complex, IUPUI

A strong physical center for information technology on the Indianapolis campus is an important element in the University's plans for leveraging its IT resources and investments, and key to ensuring that the University community has access to the full range of IT resources and services. In Indianapolis, the new Informatics and Communications Technology Complex (ICTC) is slated to open in Summer 2004 on the southwest corner of Michigan and West Streets. The ICTC will house UITS staff, the Office of the Vice President for IT, the machine room, a 24-hour Student Technology Center, a Support Center, and training facilities. It will be the University's center of telecommunications; an anchor of the Indianapolis-Bloomington technology corridor; and a hub of I-Light, the Indiana optical fiber infrastructure linking IUB, IUPUI, and Purdue University. IU's statewide data, voice, and video networks will converge and be managed from the ICTC, and IU's network will connect to global and national centers for biomedical computing networks. One of the most advanced sites for pioneering work in a broad range of information technology fields, the ICTC will house the IUPUI-based Pervasive Technology Labs and the School of Informatics with its New Media Program, the School of Journalism, and Program in Music. IUPUI, IUB, and Purdue are all active participants in building the life sciences corridor in Central Indiana and are contributors to the BioCrossroads initiative that is encouraging growth in a range of life-sciences-related fields in Indiana.

The complex will be an intellectual and symbolic bridge between the University and the Indianapolis community. Consolidating and relocating IUPUI's considerable IT resources at a high-profile crossroads of campus and community will make them highly visible and readily accessible for partnerships beneficial to local economic development and will provide a tangible symbol of IU's commitment to building the information economy in Indiana.

Computer Information Building, IUB

Construction of the Computation and Information Building (CIB) on the Bloomington campus will provide faculty, staff, and students with much-needed access to IUB's heavily used IT resources, staff, and expertise. Consolidating these in a central campus location, the CIB will also be home to supercomputing, massive data storage, and enterprise information systems. The CIB will provide important security and structural protection for the University's costly, mission-critical IT equipment: the central computational and storage facilities that support research and the University's enterprise information systems. The CIB will also house the Pervasive Technology Labs based at IU Bloomington. The design for the CIB has been approved.

Supercomputer Resources Expansion Plans

Currently Indiana University and Purdue University are discussing a plan to jointly acquire a 20-30 TFLOPS IBM BlueGene system (~10,000 processors) within the next two years. (A 65,536-processor, 180 TFLOPS BlueGene prototype will be installed at Lawrence Livermore National Lab in December, 2004). This specialized, massively parallel system would be used for protein-folding and other biomedical applications to produce simulations of real-time evolution with temporal and optical resolutions not currently possible.

University of Notre Dame Computing Systems

University wide computing facilities at Notre Dame:

Institute wide scientific computing facilities at Notre Dame are embedded in the administrative structures of the University's Science Computation Facilities (SCF) (http://www.science.nd.edu/scf/). The use of established communication channels and the involvement of the experienced staff of the SCF allows efficient startup and operation of the new updates to computing instrumentation that is regularly undertaken, resulting in minimization of auxiliary costs through the use of floating and site licenses for commercial software that is already available in the SCF. SCF maintains a twenty-four workstations graphics cluster (SGI O2) located in the Department of Chemistry and Biochemistry. Students taking courses predominantly use these workstation clusters. The Office of Information Technology (OIT) (http://www.nd.edu/~ndoit)at the University of Notre Dame currently maintains a 32 CPU (300 MHz R12000) SGI Origin2000 as a resource for the entire university. A PBS queuing system (see http://www.nd.edu/~sgi) is run on it to share the overall CPU time between various researcheres.

The BoB cluster (http://bob.nd.edu) at Notre Dame is listed among the top 500 supercomputers at http://www.top500.org/list/2002/11/. This configuration reaches a peak performance of 707 Gflops and was benchmarked at 197.2 Gflops over all processors. In the list (11/02) of the fastest supercomputers in the world (http://www.top500.org), this results in position 461, only 0.1 Gflops behind the significantly more expensive Sun Fire 15K/144, which occupies ranks 379-460. BoB is one of only 15 self-made clusters in this list. The design philosophy for BoB is a large number of jobs over a smaller number of processors, allowing a significant percentage of the theoretical peak performance to be reached.

There are several SUN workstation clusters distributed over the campus, which are maintained by OIT. In addition, a group of researchers in the College of Engineering maintains the HYDRA cluster of workstations, which currently consists of 32 dual-processor SUN UltraSparc servers. This experimental cluster is mostly used for the development of new MPI technology and is not generally available for large production runs. The Computer Science and Engineering department has recently installed the ISS Cluster. The system is a Linux cluster consisting of 44 dual-Xeon nodes with a Myrinet interconnect and a 1 Terabyte RAID storage subsystem. This cluster will be used to support their research in multi-agent systems, molecular dynamics, and parallel computer architectures.

Disk space is available through the network file system, AFS.

Local Computing Facilities for Computational Biology Researchers:

Individual researchers have available to them SUN workstations comprising of Sparc Ultra-6 and similar processors running 64 bit Solaris 8. In addition we have single and dual processors Intel based machines running on Windows and Linux, which are mainly used for developmental purposes. Production runs are carried out on the machines on the clusters listed above. For backups, we use a local external hard disk of 80 GB capacity.

Purdue University

The Bindley Bioscience Center

The Bindley Bioscience Center (BBC), one of five centers in Discovery Park at Purdue University, initiates and facilitates multi-investigator, multidisciplinary research that blends life sciences and engineering. Discovery Park was established to bring together researchers from a wide range of disciplines to foster a synergy of faculty, students, business and industry for the exploration of new ideas, technologies and products. Other Centers within the Park include the Birck Nanotechnology Center, the Burton D. Morgan Center for Entrepreneurship, the e-Enterprise Center, and the Discovery Learning Center.

Research Themes targeted by the BBC include 1) chemical and structural biology, 2) virus research and technology, 3) innovative biological instrumentation, and 4) integrated tissue engineering systems.

The BBC has developed four Research Cores in the following areas: 1) Computational Life Sciences and Informatics, 2) Genomics Research and Technology, 3) Analytical and Functional Proteomics, and 4) Cytomics and Imaging Technology.

The BBC will play a critical role in the development of infrastructure that will link many research centers including the Purdue Cancer Center. A highly flexible research building with 26,000 sq ft of assignable space will be completed by Spring of 2005. The space will house core infrastructure for interdisciplinary Life Sciences and Bioengineering research on campus. It will also house those Centers, Program projects, Institutes, and Consortia that have alignments with a Sponsored Program.

The BBC also fosters and develops plans for support of the interdisciplinary Research Center and Institutes. In addition, there are initiatives that connect the interdisciplinary Life Sciences and Engineering efforts to other campuses, institutions, or companies. These program areas and initiatives focus the investments for strengthening the infrastructure and expand the research capacity to implement visionary interdisciplinary research initiatives and promote engagement activities in Indiana to advance economic development.

The leadership structure of the BBC is composed of an Executive Team including the co-Directors, four Deputy Directors for Research Cores, Managing Director, Administrative Coordinator. The co-Directors V. Jo Davisson (Medicinal Chemistry and Molecular Pharmacology) and George Wodicka (Biomedical Engineering)of the BBC report directly to the Executive Director of Discovery Park/Vice Provost for Research at Purdue University. An External Advisory Board, and a Scientific Advisory Panel (internal) are also used to help guide the directions and operations of the BBC.

Purdue Discovery Park

Purdue University has established an on-campus research initiative called Discovery Park that was publicly announced on October 2001. Located in a 40-acre site on the west campus, Discovery Park is composed of a cluster of research Centers that will be a focus of interdisciplinary research activities. A major component of the Discovery Park was made possible by a $26 million grant from the Lilly Endowment. In addition, there are major gifts associated with the planned buildings that are being leveraged with state and University support to create a $100 million enterprise. While the activities of the Centers are distributed around the West Lafayette campus at this point, it is anticipated that the four buildings and their associated programs will be in residence in the Discovery Park by early 2005.

Purdue has recently focused its mission through the creation of a University Strategic Plan. There are clear priorities and associated metrics by which the University will measure its future success. Such a focus is recognized as necessary to move to the highest level of academic excellence in preparing students and the State of Indiana for a new economy in the 21st Century. Purdue Discovery Park will serve the University in achieving its Strategic Plan by offering the capacity to harness the best of what higher education can offer. It is doing so by creating an environment and the resources to:

- Increase the research capacity of the University in targeted areas to build upon existing strengths and to promote excellence at the international level. The three primary areas of study are nanoscience-engineering, bioscience-engineering, and e-enterprises.
- Integrate management, research, and education with excellence in science, engineering, technology, agriculture, pharmacy and veterinary medicine.
- Improve the research capacity of Purdue faculty through the creation of new facilities and program-based research opportunities.
- Gain greater impact by leveraging investments to attract additional resources and national visibility to the University and the State of Indiana.

The Centers and their corresponding facilities in the Discovery Park are not designed around existing academic disciplines. They are designed as models for interdisciplinary science and technology integrated with the many innovative components of the University. The physical proximity of these four Centers is a key

consideration in the conceptual plan. Each of these four Centers will be linked to the Center for Entrepreneurship where space and a synergistic environment is provided for chemists, engineers, life scientists, economists, computer scientists, management professionals and others to meet and exchange ideas.

Central Indiana Life Sciences

The Central Indiana Life Sciences Initiative is an example of the leadership and vision from the business and University communities to engage in partnerships for the development of advanced technologies that promote economic development. Purdue is expected to be a player in the education of highly trained individuals that will drive the innovation for the future economy in the State of Indiana. The Central Indiana Corporate Partnership, the City of Indianapolis, Indiana University, Purdue University and the Indiana Health Industry Forum lead the Central Indiana Life Sciences Initiative, which seeks to increase the number of jobs, businesses and research opportunities in the Life Sciences industry in Central Indiana. The primary focus for the life sciences-based industries include pharmaceuticals, medical devices and instruments, hospitals and laboratories, food and nutrition, organic and agricultural chemicals, and research and testing. Purdue Discovery Park in cooperation with its partner institutions (Notre Dame University and Indiana University) will enable the academic research efforts to foster the growth of its students and faculty while working toward the future economy.

Cytomics and Imaging Research Core in the Bindley Bioscience Center at Purdue University

Cell analysis & cell separation: The Core is capable of supporting a variety of cell analysis research needs. Cell analysis using flow cytometry allows the isolation and subsequent analysis of individual cells using multivariate analysis. Advanced multi-laser analysis instruments notably the Epics Altra high speed cell sorter with 4 lasers, including a Coherent Enterprise II UV laser and a Coherent Krypton multiline laser in addition to the air cooled Argon and HeNe laser are present in the core. The instrument can sort cells into sterile environments. In addition to this, the core has an Epics Elite cell sorter which is used for development projects, particularly related to instrument modifications, enhancements or any alterations necessary to perform experiments that cannot be performed in more routine instruments. For analysis, the core has two FC500 6 color dual laser analyzers both with automated 32 tube carousels. Each instrument has multivariate analysis software. An Epics XL 4 color single laser Cytometer is also available for basic routine flow cytometry analysis. For specialized microbial operations, the core has a Bio-Rad Bryte which has a lamp based excitation source and is particularly sensitive to very small particles. A significant analytical software suite is available in the core for multivariate analysis.

Laser Capture Cell microdissection: Cell types undergoing molecular changes, such as those thought to be most definitive of the disease progression, may constitute less than 5% of the volume of the tissue biopsy sample. Therefore, microdissection is essential to apply molecular analysis methods to study evolving disease lesions in actual tissue where morphologic characterization is required. The core has a Leica micro-dissection platform, combining automated upright microscope architecture, three-dimensional optical control of the dissecting laser beam and the dissected area, non-contact tissue sampling and motorized post-dissection handling.

Biological Imaging: There are multiple imaging technologies available in the cytomics & Imaging core. Basic imaging using standards high quality microscopes allows high resolution imaging in brightfield or fluorescence excitation. To achieve this the core has a Nikon 1000R upright fully automated fluorescence microscope with a SPOT high sensitivity, high resolution digital camera is also present. A Nikon Labophot upright fluorescence microscope and a Nikon inverted 300 fluorescence microscope with a DVC 250 line-scanner confocal microscope is also available. More advanced capabilities exist in other confocal microscopes which can create 3D images using optical slicing. Systems are available to do UV through near IR imaging. A Bio-Rad MP 1024 UV/vis confocal microscope is mounted on Nikon inverted microscope. This unit is ideal for UV live cell imaging. Also available is a Bio-Rad Cellmap 2 color confocal microscope mounted on a Nikon E800 upright

microscope. The unit has a 405 Violet laser and a 488 blue diode laser for excitation. The most advanced capability in fluorescence imaging is a Bio-Rad MP 2100 Multiphoton microscope that has the capability of advanced live cell imaging, including live embryos, deep tissue imaging and even functional brain imaging. This instrument also have a Becker & Hinkl life-time module linked to two high speed PMTs that allow for simultaneous Multiphoton imaging and lifetime imaging.

Analytical Software: The core has a large range of software packages for advanced and basic image processing.

High resolution Imaging

Atomic Force Microscopy specifically for biological samples provides the highest possible resolution for live cell systems. Instruments specifically designed to study biological systems operate under very different conditions to similar technologies operating the area of materials sciences. AFM instruments are available in the core to provide high resolution analysis specifically for biological samples.

Multispectral Imaging: The latest technology under development in the center involves multispectral imaging. We currently have several instruments available for use that are testbeds for new technology development. For example we have an AOTF (Acousto Optical Tunable Filter) on an upright brightfield/fluorescence microscope. This unit can collect up to 400 images at 1.5 nm increments from 370 to nearly 800 nm. This allows the analysis of multispectral data sets for the potential isolation of autofluorescence or specific target oriented molecules of interest.

Advanced Image Processing: The core provides multidimensional image processing – 1D, 2D, 3D and 4D image analysis using sophisticated software programs. These packages are directly linked to the detections systems and can extract complex structures and reproduce them in multidimensional space. Such analytical tools are both qualitative and quantitative. We have several packages that are high level image analysis packages such as Image ProPlus, Metamorph and VoxelView.

High Content-High Throughput Systems

The core is developing capabilities in high content and high throughput analysis typical of systems where thousands of samples must be analyzed daily. These systems require advanced computational support to provide analysis. Current technologies include microtiter based fluorescence imaging instruments which use sensitive cameras and advanced image based computational analysis.

Protein Profiling

The Core has a unique resource in a state-of-the-art automated, two-dimensional fractionation system expressly designed for high-resolution analysis of complex protein mixtures. The ProteomeLab PF 2D provides an integrated solution that effectively resolves hundreds to thousands of proteins present in cell lysates. The ProteomeLab PF 2D system can generate data maps from two dimensions of separation for easy comparison using the ProteomeLab Software Suite supplied with the system. Liquid fractions can be collected and stored for future analysis or the eluent can be connected directly to ESI-mass spectrometry. Liquid fractions can be stored or transferred to a MALDI plate spotter, or directly connected to an electrospray source. The PF 2D's unique combination of chromatofocusing followed by non-porous reverse phase chromatography provides high resolution of proteins, while delivering information that can be related to 2D gel data. The ProteomeLab PF 2D's second dimension is capable of resolving hundreds of proteins in great detail from each first-dimension fraction. Fraction collection from the first dimension is quality-controlled with an in-line pH monitor, then automatically injected into the second dimension. As a result, PF 2D requires much less time and attention than traditional labor-intensive techniques. The entire 2 dimensional separation process can be performed in less than 20 hours.

The Protein Sequence and Analysis Laboratory also offers a range of protein chemistry analyses and training for researchers in their application. Included are 2D gel analyses in a variety of formats, in-gel proteolytic digestions with excision of the digest for mass spectrometric analysis, electrophoretic transfer of gel spots to PVDF membranes, and database searching algorithms for interpretation of the data. The gel manipulations can be done either manually or robotically. More traditional protein analyses include Edman sequencing,

amino acid composition analyses, and proteolytic digestions followed by peptide separations on microbore HPLCs.

Protein Identification

The Analytical and Functional Proteomics Core in the Bindley Bioscience Center has a complementary set of mass spectrometry based facilities as well as liquid chromatography as well as two-dimensional gel electrophoresis laboratories. The various instruments provide analytical capabilities include a Bruker Reflex III MALDI-TOF, Esquire LC/MS/MS, ABI QSTAR, and ABI Mariner ES-TOF. These instruments are dedicated to the identification and quantification of proteins and peptides. Two of these instruments are directly interfaced to capillary HPLC units. Each of these instruments can be accessed through either direct use or on a fee for service basis. This Core also works in cooperation with the Campus-wide Mass Spectrometry facility, which also houses 8 mass spectrometers with a full range of ionization sources capable in several locations around campus capable of identifying a wide mass range of molecules. There are also additional two full range of ionization options is provided gas or liquid chromatographs is available. Advice on sample preparation, selection of the proper type of MS equipment for the problem, and training in interpreting the data is available from the operators and the campus-wide coordinator of the facility.

Consulting and Data Processing: The Core provides excellent expertise for the design, preparation and analysis of experimental profiles. Consultants are available to advise researchers in the correct techniques and training opportunities are available on a weekly basis.

OTHER EQUIPMENT AVAILABLE FOR USE BY THE BIOCOMPLEXITY INSTITUTE

The Indiana Center for Biological Microscopy (IUPUI School of Medicine)

The recent confluence of technical developments in optical microscopy, and the digital technologies of image deconvolution and three-dimensional image representation have made high resolution three-dimensional imaging possible. Funding from Indiana University, Indiana University Medical School, the Division of Nephrology, the NIH and the Lilly Endowment has given Indiana University a world-class center for biological microscopy, equipped with a comprehensive set of the best examples of these technologies. The Indiana Center for Biological Microscopy is equipped with two combined confocal and 2-photon microscopes (Zeiss 510-NLO, Bio-Rad MRC-1024MP), an ultraviolet-visible confocal microscope (Zeiss LSM-510), a high-speed confocal microscope (Perkin-Elmer Ultraview), a high resolution cooled CCD imaging and image deconvolution microscope (Applied Precision Deltavision System) and dedicated microinjection and micromanipulation system equipped for high resolution DIC and video microscopy. The Center is also equipped with numerous Pentium and Silicon Graphics workstations running advanced image processing software for analysis of both two-dimensional and three-dimensional images.

It is the objective of the imaging facility to apply, develop, and combine these imaging technologies to provide researchers with a rational, integrated approach to microscopic imaging. The unique strength of the imaging facility is that it not only provides researchers with hands-on access to state-of-the-art imaging equipment, but also with the benefit of close interaction with the facility staff. Providing consultation, training and experimental assistance, the facility staff provides researchers with the opportunity to optimally apply the imaging technology most appropriate to each particular research question. The Center is also actively involved in research into biological imaging, resulting in the development and dissemination of new methods of microscopy and digital image analysis software. The products of these activities are disseminated through a program of education, including seminars, courses and individual training. Details of the Center may be found at: http://134.68.94.222/Imaging/index.htm.

The facility represents a strong institutional commitment to optical imaging technology development. The imaging suite is part of a laboratory renovation costing Indiana University a total of 2.2 million dollars. An additional sum of nearly $360,000 was appropriated by the University for imaging equipment through the Research Infrastructure Fund. In 1998 an additional $390,000 was allotted to Dr. Dunn through the Indiana University Strategic Directions Initiative program to develop image processing software and to upgrade the confocal microscope to 2-photon capability. In 2001, the Lilly foundation awarded a sum of $2.9 million to the imaging facility to expand its capacity and capabilities, as part of the Indiana Genomics initiative. In 2003 the Nephrology Division and the Imaging Facility will move into 18,000 sq. ft. of new laboratory space.

Currently, the Center is a core resource for a $6.6 million Cancer Center Grant, a $5.6 million NIH Program Project grant in Nephrology, a $1.2 million NIH Molecular Imaging center planning grant and a $5.6 million NIH Pediatric Hematology program project grant. In July of 2002, Center investigators received $3.5 million from the NIH as the center was designated a National O'Brien Center for Renal Microscopy.

Center Personnel

The imaging equipment, and the facility personnel were selected in response to the need for high resolution, three-dimensional optical imaging to support a modern research effort into renal cell biology. The equipment assembled represents a logical, comprehensive and powerful combination of technologies that have been productive for both the Nephrology section and for other Indiana University investigators.

Kenneth Dunn, Ph.D., Director For the past six years, Dr. Dunn has overseen the development of the Nephrology Division imaging facility and is currently the director of the facility. Dr. Dunn has 14 years of experience in quantitative fluorescence microscopy and 12 years of experience with quantitative confocal microscopy including three years of running an interdepartmental imaging facility at Columbia University (see

Biosketch). In the seven years since joining the Nephrology group, Dr. Dunn has focused his research on applying quantitative confocal microscopy to characterize endocytic sorting and acidification in polarized MDCK cells. Dr. Dunn has developed numerous techniques of quantitative image analysis and has spent the past six years developing the image processing software to quantify cellular parameters for his own research as well as that of various members of the Nephrology group. Dr. Dunn oversees the scientific direction of the imaging facility, advises principal investigators on appropriate experimental approaches to imaging studies and represents the imaging facility in seminars and courses.

Exing Wang, Ph.D., Microscopy Specialist. Dr. Wang is currently the co-manager of the Nephrology Division's imaging facility. He has a Ph.D. in Biophysics from Ohio State University. He has worked with Dr. Dunn for the past four years and has extensive training and expertise in light microscopy, image processing and image analysis. Dr. Wang trains researchers, schedules use of facility equipment, and maintains facility instruments.

Connie Temm-Grove, Ph.D., Microscopy Specialist. Dr. Temm-Grove is currently the co-manager of the Nephrology Division's imaging facility. She received her Ph.D. from the University of Bielefeld, Germany, and managed an optical microscopy facility at the University of Arizona. Her research in microvascular cell biology has given her significant experience in multi-color immunofluorescence microscopy. She trains researchers, schedules use of facility equipment, and coordinates educational Center activities.

Jeffrey Clendenon, Image Analysis Specialist. Mr. Clendenon joined the facility in Spring of 2000. Jeff has a bachelor's degree in electrical engineering and over 20 years in scientific computing and digital image analysis. In the brief period Jeff has been with the group he has been solely responsible for the development of the VOXX 3D rendering software, as well as the Corr3D program. Jeff continues to develop the VOXX software, is developing methods to optimize 3D image collection and analysis, and works closely with biomedical researchers, writing programs specifically to their needs.

Jason Byars, Image Analysis Apecialist. Jason Byars works with Jeff Clendenon to write image analysis software. He also develops and troubleshoots hardware control of microscope systems, and manages the facility computer systems and computer network.

Specific Descriptions of Major Microscope Systems

Core biotechnology facilities within the Department of Biochemistry are provided as a campus resource. This core facility is utilized for peptide synthesis and automated fluorescent DNA sequencing. (See http://www.bbf.iu.edu/ for more information about services).

Laser scanning confocal, and 2 photon microscope system
- Biorad MRC-1024 confocal mounted on a Nikon Eclipse-200 inverted microscope
- Krypton-Argon and Argon lasers - fluorescence excitations at 488, 514, 568 and 647 nm
- Titanium-Sapphire laser pumped with 5 W Argon laser, tunable from 700 to 1000 nm
- 3 internal epi-imaging detectors and 3 trans-imaging detectors
- 3 external detectors for 2 photon imaging
- 800 Mhz Pentium Computer with a 21 inch color monitor
- TMC air flotation isolation table

Laser scanning confocal, and 2 photon microscope system
- Zeiss 510 NLO confocal/2photon microscope system
- Argon and He-Ne lasers - excitations at 351, 364, 458, 488, 514, 543 and 633 nm
- 2 internal epi-imaging detectors and META spectral detector
- Titanium-Sapphire laser pumped with 10 W Argon laser, tunable from 700 to 1000 nm

UV laser scanning confocal microscope system
- Zeiss LSM-510 UV confocal microscope
- Argon and He-Ne lasers - excitations at 351, 364, 458, 488, 514, 543 and 633 nm
- 4 internal epi-imaging detectors and 1 trans-imaging detector (equipped for DIC)
- Zeiss Axiovert 100M inverted microscope and Zeiss Axioscope upright microscope

Perkin-Elmer Ultraview confocal microscope
- Mounted on Nikon Eclipse 200 inverted microcope
- Equipped with Argon, Krypton-Argon and HeCd lasers – excitations at 442, 488, 514, 568, 647 nm
- High speed Hamamatsu chilled CCD detector
- Piezoelectric stage control, automated XYZ stage control

Applied Precision Deltavision imaging system
- Nikon Eclipse 300 inverted microscope
- Cooled CCD detector with Kodak KAF1401EG1 chip, 500 KHz transfer rate
- 2 meter fiber optic illumination scrambler for either trans- or epi-illumination
- High resolution DIC optics
- High precision API XYZ stage controller
- Silicon Graphics O2 200 MHz computer, with 320 Mb of RAM and a 13 Gb hard drive
- TMC air flotation isolation table

Nikon Diaphot inverted epifluorescence microscope
- DIC and phase optics
- Diagnostics Instruments SPOT RT color CCD detector
- Photometrics Star-1 cooled CCD imaging detector
- Dage MTI CCD-72 video CCD camera with a Dage 104722 image intensifier
- Metamorph microscope control and image acquisition software
- Eppendorf 5170 micromanipulator and Eppendorf 5242 microinjector
- Kinetics air flotation isolation table

Nikon Optiphot upright epifluorescence microscope
- Diagnostics Instruments SPOT RT color CCD detector

Image processing and presentation systems
- Silicon Graphics Octane 270 MHz workstation, with 10Gb hard drive and 128 Mb RAM
- Applied Precision Deltavision deconvolution and rendering software
- 2 1.4 GHz Pentium computers, with Hewlett/Packard CD recorders and 21 inch color monitors
- Metamorph, Adobe Photoshop, Deltagraph, Microsoft Excel and Powerpoint software
- Image processing software written in house, including VOXX, 3DSPOTS, CORR3D
- Kodak XLS 8650PS dye sublimation printer

The Indiana Center for Biological Microscopy is equipped with the following major equipment: 1) LSM510-Meta laser scanning microscope system equipped for either confocal or multiphoton imaging from Carl Zeiss; The system is facilitated with Ar (458, 488, 514 nm) and He-Ne lasers (543, 633 nm) for confocal imaging and a Femtosecond Titanium-Sapphire laser, tunable between 680 nm and 1000 nm and pumped by a 10W CW green laser, for two-photon imaging. Four external detectors are available for multiphoton imaging. 2) Perkin-Elmer UltraVIEW spinning disk confocal system equipped with an ORCA ER cooled CCD camera (Hamamatsu), and laser lines at 442 nm, 488nm, 514 nm and 568 nm. Fast frame rate (up to 80 frames per second); two-color,four-dimensional imaging (3D over time) can be done with the fast Piezo Z-controller. 3) A Bio-Rad MRC1024 laser scanning confocal microscope equipped with Krypton-Argon (488, 568, 647 nm) and Argon visible lasers, and for 2-photon operation, a tunable Titanium-Sapphire laser, using a 5W Millennia diode solid state pump laser. The system is equipped with 3 imaging photometers in both the epi-illumination and transillumination paths, as well as three channel external detectors for 2-photon imaging. 4) A Zeiss LSM-510 confocal microscope equipped with a UV Argon Laser (351 nm, 364 nm), Argon laser and two He-Ne lasers, a visible Argon laser (458, 488, 514nm) and two Helium-Neon Lasers (543 nm and 633 nm). The microscope is equipped with four epifluorescence or reflection PMT detectors and one transillumination PMT detector.

LSM510-Meta laser scanning microscope system equipped for either confocal or multiphoton imaging from Carl Zeiss: With the newly developed "Meta detector" system, which captures and deconvolves the total emission spectrum of each pixel, this system is capable of distinguishing a large number overlapping fluorophores in a sample. The system is facilitated with Ar(458,488, 514 nm) and He-Ne lasers (543, 633 nm) for confocal imaging and a Femtosecond Titanium-Sapphire laser, tunable between 680 nm and 1000 nm and

pumped by a 10W CW green laser, for two photon imaging. Four external detectors are available for multiphoton imaging.

Perkin-Elmer UltraVIEW spinning disk confocal system: This system is equipped with an ORCA ER cooled CCD camera (Hamamatsu), and laser lines at 442 nm, 488nm, 514 nm and 568 nm. Fast speed (up to 80 frames per second!) and low photobleaching together make this system a very powerful tool for live cell imaging. Two-color, four-dimensional imaging (3D over time) can be done with the fast Piezo Z-controller. This system should be ready soon.

A Bio-Rad MRC1024 laser scanning confocal microscope is equipped with Krypton-Argon (488, 568, 647 nm) and Argon visible lasers, and for 2-photon operation, a tunable Titanium-Sapphire laser, using a 5W Millennia diode solid state pump laser. The system is equipped with 3 imaging photometers in both the epi-illumination and transillumination paths, as well as three channel external detectors for 2-photon imaging. The imaging system is suspended on a TMC air flotation isolation table.

A Zeiss LSM-510 confocal microscope is equipped with a UV Argon Laser (351 nm, 364 nm), Argon laser and two He-Ne lasers, a visible Argon laser (458, 488, 514nm) and two Helium-Neon Lasers (543 nm and 633 nm). The microscope is equipped with four epifluorescence or reflection PMT detectors and one transillumination PMT detector.

A Deltavision wide-field imaging system from Applied Precision for epifluorescence and phase contrast imaging is equipped with high-speed fluorescence excitation and emission filter wheels, high precision API XYZ motorized stage controller, and an illumination lightpath scrambled by means of a 2 meter fiber optic scrambler. Images are collected by means of a cooled CCD detector, equipped with a Kodak KAF1401EG1 chip, 1317 by 1035 pixels at 12 bits per pixel). The system is equipped for excitation ratio measurements of BCECF and FURA-2

A Nikon Diaphot 200 inverted epifluorescence microscope is equipped with DIC and may also be equipped with a SPOT color camera or a cooled CCD camera or an intensified video camera. An Eppendorf micromanipulator and microinjector is mounted onto the stage of the microscope.

Image Processing Capabilities

The Center is equipped with several Pentium workstations devoted to image analysis. We have developed several image processing programs that run on Pentium systems. Voxel-based Rendering Software (VOXX) is a three-dimensional image rendering software developed in the Nephrology Imaging Facility by Jeff Clendenon. Corr3D is a program that compares two image volumes and calculates the Pearson's correlation coefficient for the intensity distributions of the two images, using a user-specified, or calculated region of interest. 3DSpots is a program that both segments and quantifies the fluorescence of three-dimensional objects. This program segments objects according to their individual intensity properties.

We have also acquired commercial software as necessary. The Applied Precision Deltavision image deconvolution software runs on a Silicon Graphics Octane SE. The Deltavision software, developed in collaboration with David Agard and John Sedat of U.C.S.F. (Hiraoka et al., 1991) supports several levels of image deconvolution, including the fast nearest-neighbor algorithm, as well as the more rigorous and complete constrained iterative process. Neurolucida image analysis software, along with the Confocal Module (Microbrightfield, Inc. Colchester, VT) permits user-specified segmentation and analysis of complex 3-dimensional images. The PC-based software provides quantification of object fluorescence as well as various morphometric parameters that have been primarily used in neuroscience studies. This software is valuable for analysis of objects whose edges are not easily detected by automated image processing procedures.

All microscopy systems and computers are connected to a 100Base-TX Ethernet switch. Microscope image data may be written directly to the hard drive of the associated computer, for later storage on recordable or rewritable CDs. Data may be also be archived via a 4 Gb 4 DAT mm tape drive.

The Center for Regenerative Medicine (Indiana University – Purdue University Indianapolis)

The research team occupies 10 laboratories and accompanying office space totaling nearly 10,000 square feet on the campuses of Indiana University (Bloomington and Indianapolis), Indiana State University, and the University of Illinois. Equipment available in individual labs includes bright-field, phase, Nomarski, and fluorescent optical microscopes; laminar flow hoods; thermal cyclers; minicentrifuges; gel electrophoresis apparatus; power supplies; refrigerators; tissue culture incubators; and computers. In addition, there is large-scale sharing of common equipment at each campus, including low-temperature freezers; ultracentrifuges; scintillation counters; fluorescence-activated cell sorters; gel dryers; gel visualization and photography equipment; confocal microscope; autoclaves; digital camera systems; tissue culture facilities; and histology/cytology support facilities, including bone histomorphometry.

AAALAC (American Association for Accreditation of Laboratory Animal Care) approved, full staffed animal quarters exist on each campus, supported by animal per deim charges. The facilities and programs meet the requirements of Federal Law (89-544, 91-579) and NIH regulations. Protocol forms are all approved and on file for this project. IUPUI has core centers for genomics and proteomics.

Departmental offices and Dean's offices at each campus provide grant accounting and clerical support. The Center for Regenerative Biology and Medicine at IUPUI is administered by the Director, Ellen Chernoff, and a part-time administrative assistant.

Center Personnel

David L. Stocum, Ph.D. (Cell and Developmental Biology), Principal Investigator

Background: David Stocum was an NIH Fellow at the University of Pennsylvania from 1965-1968. He joined the faculty of Life Sciences at the University of Illinois Urbana-Champaign in 1968, where he was promoted through the ranks to Full Professor of Cell and Structural Biology. He also held administrative positions at Illinois as Director of the Honors Biology Program (1974-1976) and Acting Head of the Department of Anatomical Sciences (1984-1986). He has served on a wide variety of NSF, NIH, and Veterans Administration scientific panels. In 1989, he moved to the Indianapolis campus of Indiana University as Professor of Biology and Dean of the School of Science. Stocum's research interests are in the areas of cell and developmental biology, regenerative biology, and regenerative medicine. His work has been funded by the National Science Foundation, the National Institutes of Health, and, more recently, by a grant from the State of Indiana 21st Century Research and Technology Fund. He has published over 85 papers in the area of limb regeneration and regenerative biology and medicine, as well as one book titled "Wound Repair, Regeneration, and Artificial Tissues". He has given numerous invited presentations around the world. Stocum is the founder of the Indiana University Center for Regenerative Biology and Medicine.

Current Appointments: Professor of Biology and Dean, School of Science, Indiana University-Purdue University Indianapolis; Investigator, Center for Regenerative Biology and Medicine.

Professional Societies and Awards: Society for Developmental Biology, Society for Wound Repair, Regenerative Medicine and Stem Cell Biology Society (charter member), American Association for the Advancement of Science, Federation of American Scientists for Experimental Biology, and American Association of Anatomists. He was selected as an Associate in the Center for Advanced Study, University of Illinois (1986). He was elected a Fellow, Section G, Biology, of the AAAS in 1988, and was the 1988 awardee of the Singer Medal for Regeneration Research.

Research expertise: Amphibian limb and neural development and regeneration; regenerative medicine

Recent publications: Stocum DL 2001 Regenerative biology and medicine: a new era in bioengineering. Cardiac and Vasc Reg 1:157-169. Stocum DL 2001 Rx for tissue restoration: regenerative biology and medicine. Korean J Biol Sci 5:91-99.

Ellen A.G. Chernoff, Ph.D. (Cell and Developmental Biology)

Current Appointments: Associate Professor, School of Science, Indiana University-Purdue University Indianapolis; Director, Center for Regenerative Biology and Medicine.

Professional Societies and Awards: Singer Medal for Regeneration Research.

Research Expertise: Neural development and regeneration.

Recent Publications: Chernoff EAG, O'Hara CM, Bauerle D, Bowling M 2000 Matrix metalloproteinase production in regenerating axolotl spinal cord. Wound Rep Reg 8:282-291. Chernoff EAG, Clarke DO, Wallace-Evers JL, Hungate-Muegge LP, Smith RC. 2001 The effects of collagen synthesis inhibitory drugs on somitogenesis and myogenin expression in cultured chick and mouse embryos. Tissue Cell 33:97-110.

Simon J. Rhodes, Ph.D. (Biochemistry)

Current Appointments: Associate Professor of Biology and Associate Professor of Biochemistry and Molecular Biology, Indiana University-Purdue University Indianapolis; Member, Indiana University Cancer Center; Investigator, Center for Regenerative Biology and Medicine.

Professional Societies and Awards: Endocrine Society, Endocrine Society Visiting Professor; Outstanding Young Professor, IUPUI; Trustees Teaching Award, IUPUI.

Research Expertise: Neuroendocrine development and regeneration.

Recent publications: Sloop KW, Dwyer C, and Rhodes SJ. 2001. J Biol Chem 276: 36311-36319. Sloop KW, Parker GE, and Rhodes SJ. 2001. Curr Genomics 2: 379-398.

Teri Belecky-Adams, Ph.D. (Neurobiology, Cell Biology, and Anatomy)

Current Appointments: Assistant Professor, Department of Biology, School of Science, Indiana University-Purdue University Indianapolis; Investigator in the Center for Regenerative Biology and Medicine.

Professional Societies and Awards: Association for Research in Vision and Ophthalmology, American Society for Cell Biology, Society for Neurosciences; Knights Templar Eye Foundation Award, Purdue Research Faculty Award (2001)

Research expertise: retinal development and regeneration; diseases of the retina.

Recent publications: Belecky-Adams TL, Adler R 2001 Expression of bone morphogenetic proteins, receptors, and binding proteins during development of the chick retina. J Comp Neurol 430:562-572. Adler R, Tamres AN, Bradford RB, Belecky-Adams T. 2001 Microenvironmental regulation of visual pigment expression in the chick retina. Dev Biol 236:454-464.

Michael King, Ph.D. (Biochemistry)

Current Appointments: Associate Professor of Biochemistry and Molecular Biology, Indiana University School of Medicine, Terre Haute Center for Medical Education, Indiana State University; Investigator, Center for Regenerative Biology and Medicine.

Professional Societies and Awards: Regenerative Medicine and Stem Cell Society (charter member).

Research Expertise: molecular biology of *Xenopus* development and regeneration.

Recent Publications: King MW 2001 Rapid and non-radioactive screening recombinant libraries by PCR. In: Methods in Molecular Biology Vol 192, 2nd ed. "PCR Cloning Protocols". Humana Press, Inc, Totowa, NJ, pp. 377-384. Krishnan P, King MW, Neff AW, Sandusky GE, Bierman KL, Grinnell B, Smith RC 2001 Human truncated Smad6 (Smad 6s) inhibits the bone morphogenetic pathway in *Xenopus laevis*. Dev Growth Diff 43:115-132.

Anton Neff, Ph.D. (Biological Structure)

Current Appointments: Associate Professor of Anatomy and Cell Biology, Indiana University School of Medicine, Indiana University Bloomington; Senior Fellow, Indiana University Molecular Biology Institute; Investigator, Center for Regenerative Biology and Medicine.

Professional Societies and Awards: American Association of Anatomists.

Research Expertise: Amphibian embryonic development and regeneration.

Recent Publications: Krishnan P, King MW, Neff AW, Sandusky GE, Bierman KL, Grinnell B, Smith RC. 2001 Human truncated Smad6 (Smad 6s) inhibits the bone morphogenetic pathway in *Xenopus laevis.* Dev Growth Diff 43:115-132. Neff AW, Dent AE, Armstrong JB 1996 Heart development and regeneration in urodeles. Int J Dev Biol 40:719-725.

Anthony Mescher, Ph.D. (Developmental Biology)

Current Appointments: Professor of Anatomy and Cell Biology, Indiana University School of Medicine, Indiana University Bloomington; Investigator, Center for Regenerative Biology and Medicine. Research expertise: amphibian limb regeneration.

Recent publications: Mescher, A.L., G.W. White and J.J. Brokaw (2000) Apoptosis in regenerating and denervated, nonregenerating urodele forelimbs. Wound Repair and Regeneration 8: 110-116.

Matthew W. Grow, Ph.D. (Biochemistry)

Current Appointments: Assistant Professor of Biology and Associate Professor of Biochemistry and Molecular Biology, Indiana University-Purdue University Indianapolis; Assistant Director of Center for Medical Genomics; Member, Center for Regenerative Biology and Medicine; Member, Indiana Biocomplexity Institute; Member, Indiana Center for Vascular Biology and Medicine

Professional Societies and Awards: Society for Developmental Biology; Society for Applied Computing.

Research Expertise: Cardiovascular development and microarray technologies.

Recent publications: Breese, M. R., Stephens, M. J., McClintick, J. N., Grow, M. W., and Edenberg, H. J. (2003). Labrat LIMS: an extensible framework for developing laboratory information management, analysis, and bioinformatics solutions for microarrays. *SAC 2003*.

The Center for Medical Genomics (Indiana University School of Medicine)

Microarrays offer researchers a powerful tool, allowing the expression levels of thousands of individual genes to be monitored at once. Indeed, many of the projects described in this proposal will use microarray data as one of the base elements for creating theoretical metabolic and gene expression network models. After these initial theoretical models have been constructed, however, continued testing and *in vivo* validation of these models will be required. Through the use of microarrays, researchers will be able to design initial network models, test their theoretical models at the level of transcriptional regulation, and further refine their models. The Center for Medical Genomics (CMG) at the Indiana University School of Medicine offers a wide range of

services and expertise in microarray technology. The following is a description of the benefits that the CMG will provide to participants in this proposal.

The Center for Medical Genomics was established and is supported by the Indiana Genomics Initiative and Indiana 21st Century Research and Technology Fund to provide high-throughput gene expression and genotyping services to academic researchers across the state of Indiana. The CMG offers consultation, infrastructure, and expertise in the use of both Affymetrix Genechips® and custom spotted microarrays. In addition to handling all physical aspects of microarray work following RNA submission, the CMG also assists investigators with data interpretation. For this bioinformatics support, the CMG has designed the Labrat LIMS and Microarray Data Portal, which together form a robust system for storing and analyzing microarray data. Overall, the Center for Medical Genomics offers researchers a one-stop solution for researchers' gene expression needs.

Affymetrix Genechips®

There are currently Affymetrix Genechip® microarrays available for a wide range of organisms, including human, mouse, rat, *Drosophila*, *C. elegans*, and yeast (for a complete listing, see www.affymetrix.com). The CMG provides all of the resources and services necessary to take advantage of Genechip® technology. Resources for Affymetrix chip processing include an Agilent 2100 Bioanalyzer, two Affymetrix fluidic wash stations and a Genechip® scanner. After consultation with CMG staff on experimental design and on RNA preparation, researchers may submit total RNA samples for processing. The CMG provides full in-house handling and quality control of all samples and will notify the researcher when results are ready (turn-around time is approximately two weeks – special conditions may apply).

For Genechip® data analysis, the CMG utilizes the Affymetrix Suite 5 (MAS 5) software in conjunction with the Microarray Data Portal (MDP). Through the web-based MDP, researchers can access raw expression data from their experiments, annotate their samples (compliant with MGED standards, http://www.mged.org) and perform a large number of analyses. Basic options include the sorting and filtering of the data based on a standard two sample t-test, fold change, log fold change, nonparametric statistics (Wilcoxon), and the fraction of Present calls in each sample set determined by Affymetrix™ MAS5 software. Researchers may also perform k-means, Estimation Maximization (EM), and hierarchical clustering of their data using the Microarray Data Portal. Additional bioinformatics support may be arranged on a collaborative basis.

Spotted Microarrays

As an alternative to Genechip® microarrays, the CMG offers a spotted microarray service. This alternative may be considered by researchers using organisms for which there are no available Genechips® or when a custom microarray is a cost-effective option for a particular project. Researchers may choose to fabricate a custom spotted microarray, purchase commercially-available spotted arrays, or obtain spotted microarrays from collaborators. Following consultation, researchers may submit total RNA to the CMG for hybridization and data analysis.

For custom microarray fabrication, the CMG can print oligonucleotides, amplified cDNA, and genomic DNA probes. Unless noted otherwise, these probes will be spotted onto GAPS II aminosilane-coated slides and passed through a series of tests to assure quality and uniformity. Each barcoded microarray will be recorded into the CMG's Labrat LIMS database, along with all available probe information.

Additional Services

In addition to the microarray services described above, the Center for Medical Genomics also provides the following services (costs must be agreed upon beforehand)

- 96 and 384 well PCR
- 96 well agarose gel electrophoresis
- Automated PCR setup
- Automated PCR product purification (384 well format)

- High-throughput SNP Genotyping
- Colony/Phage picking from libraries
- Filter array gridding
- 94 and 384 well library handling (plate replication/merging)
- “Cherry Picking” individual clones from plated sets

Resources and Equipment

The CMG is located at the Biotechnology Research and Technology Building (BRTC) on the IUPUI campus in Indianapolis. The CMG occupies 2500 sq ft of laboratory space, in addition to 500 sq ft of space for Dr. Matthew Grow’s laboratory. Addtionally, there is a 300 sq ft computer lab, 150 sq ft of freezer space, and 6 offices. The CMG also has access to the BRTC shared resources, including 2 conference rooms and a state-of-the-art multimedia training center. The training center has networked computers at each of 28 stations and built-in teleconferencing capabilities, making it ideal for local and long distance software training.

The following is a listing of the major instruments in use at the CMG.

- Agilent 2100 Bioanalyzer
- Affymetrix 417 arrayer
- Affymetrix 428 scanner
- 2 Affymetrix Genechip® fluidic wash stations
- Affymetrix Genechip® scanner
- 10 dual block 384-well thermocyclers
- 5 96-well thermocyclers
- Hamilton MPH-96, 96 channel liquid handling robot
- Sequenom MassArray System
- Virtek Chipwriter Pro microarray printer
- Virtek Chipwriter colony picking module
- Virtek Chipwriter liquid “cherry picking” module
- A dual-1Ghz Linux-based server with 670GB RAID, for database storage and performance of the computationally complex calculations necessary for array analysis.

Summary

For the participants in Biocomplexity Institute, the microarray support offered by the Center for Medical Genomics greatly expands the capability to successfully execute and interpret microarray experiments. The CMG will assist the participants of this proposal in utilizing Center’s resources to the fullest extent. To encourage Genechip® use, the CMG will provide a total of 40 Affymetrix Genechips® to participants of this proposal at a subsidized cost of $600/chip. This amount includes the cost of the Genechip®, RNA processing and hybridizations, and basic data analysis (as described above). Distribution and use of these Genechips® will be controlled by the executive committee of this proposal. Additional Genechips® may be purchased and used in accordance with standard CMG pricing and policy.

Also included in the budget is a portion of the material costs associated with spotted microarray fabrication and use. In the project proposed by Dr. Matthew Grow, a 15,360-feature *Xenopus* embryonic heart microarray is planned. The budget reflects a portion of the material costs associated with the construction and use of 136 of these *Xenopus* heart microarrays.

In order to offset a portion of operational costs, the CMG is requesting coverage be provided for 20 per cent of the yearly service contract costs for Affymetrix microarray equipment and the Virtek Chipwriter microarray printer. Additionally, the CMG is requesting an amount to cover 10 per cent of the salary and benefits for an RS07 level technician and 10 per cent of the salary and benefits for a postdoctoral research fellow. These personnel would devote a portion of their time toward assisting participants in the Biocomplexity Institute with gene expression analysis and network model building.

As mentioned earlier, the CMG has developed a robust LIMS database and front-end interface. The Center would welcome the opportunity to explore the further development of its system with participants in this

proposal. Some possible avenues worth investigating include: data warehousing for the Biocomplexity Institute, automated theoretical network modeling based on gene expression data, and the development of a collaborative electronic notebook.

The Science Animal Resource Center (IUPUI School of Science)

The IUPUI School of Science animal care facility, the Science Animal Resource Center (SARC), is maintained to support the research and teaching missions of the School of Science faculty and students. The mission of the University is undergraduate and graduate education, and, to that end, the University recognizes the link between research and a quality educational experience. The Department of Biology investigator labs use their equipment to study animal tissue regeneration and development, cancer, and immunity. The Department of Psychology investigator labs use their equipment in experimental rat studies on drugs of abuse; including pharmacological, neurological and neurochemical experiments on drug reinforcement and the effects of acute and chronic drug exposure.

The Science Animal Resource Center aids the teaching and research missions of the School of Science. The SARC provides the 2,400 square foot multiple-room physical facilities, a three-member staff, and routine animal care/ health monitoring and maintenance of the physical facility. The staff consists of a director of operations and two technicians. Currently, eight rooms are dedicated for housing (900 square feet), two rooms are dedicated for aseptic surgery (230 square feet), one room is dedicated for general procedures (64 square feet), and nine rooms provide support functions. All animal rooms have 24-hour temperature control monitoring and are kept within a five degrees range of 72^{o} F. Sanitization requirements are met by using two 130-square-foot mechanical washers in combination with sanitizing agents or equipment assisted hand washing. Animal housing consists mostly of 1,500 solid bottom polypropylene species and size appropriate caging and stainless steel lids placed on 35 vertical racks. One thousand five hundred glass bottles and tops provide access to tap water for each species, except for aquatic species. Aquatic species are provided specialized cages and treated water mixtures. Each surgery area provides direct ventilated hoods and anesthetic gas vaporizers. The Indiana University School of Medicine Laboratory Animal Resource Center provides contract veterinary services for all species housed at the SARC.

The Institutional Animal Care and Use Committee (IACUC) and SARC staff provide a mechanism for insuring the humane use of animals and provide investigators with direct access to advice and assistance. The SARC staff or Attending Veterinarian provide training in proper animal care and use to investigators conducting additional procedures. The SARC is committed to following the recommendations found in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), the standards of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), the regulations of the United Stated Department of Agriculture (USDA), and the policy of the Public Health Service (PHS); administrated and coordinated by the Office of Laboratory Animal Welfare (OLAW) at the National Institute of Health (NIH).

Laboratory Animal Resource Center (LARC) – Indiana University Medical Center

Indiana University Medical Center houses various animals in the Laboratory Animal Resource Center (LARC), where the animals are housed in an AALAC approved facility with oversight of all animals provided by four veterinarians. All procedures outlined in the Guide for the care and use of laboratory animals (National Research Council, National Academy Press, Washington, 1996) are followed. All food, bedding, and cages are autoclaved. Each animal cage is topped with a filtered lid. Specific temperatures, humidity, and light/dark cycles are maintained. Surrogate animals are kept with the inbred strains to sample monthly for respiratory, gastrointestinal, or other pathogens. Access to LARC is limited to specific personnel via a card key system.

Animal Resources Center, Indiana University, Bloomington

Indiana University, Bloomington Campus, maintains and manages approved animal facilities by way of the USDA Animal Welfare Act annual certification and the PHS/NIH Animal Welfare Assurance.

Complete veterinary diagnostic and clinical services are provided by the Bloomington campus veterinarian. Several exceptional facilities for reknown breeding and distribution of distinctive species are maintained. Routine animal care in the various facilities is performed by certified animal technicians with extensive experience in handling animals. The Bloomington campus IACUC oversees and maintains diligent purview over all facets of the animal care and use program.

MAJOR EQUIPMENT IN THE LABORATORIES OF PROJECT LEADERS AND SELECTED COLLABORATORS

- **Mark Alber (Department of Mathematics, University of Notre Dame)**

Computer: Dr. Alber has a personal computer in his office. A Computer Lab is available for graduate students. Full-time computer support staff is available.

Office: Dr. Alber has a private office in the Mathematics Department with telephone services.

Other: The University maintains a science library, easily accessible to the Mathematics Department, with current periodicals and journals, support staff, and computer databases.

- **Andrew Arenson (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **M. Pauline Baker (Pervasive Technology Labs, Indiana University)**

Laboratory: Dr. Baker has a Visualization and Interactive Spaces Lab, a component of the Pervasive Technology Labs at Indiana University. This lab includes high-end visualization equipment, including systems for stereoscopic viewing, as well as novel configurations for table-top display and wire-free user interaction. The lab space is located near the medical school of Indiana University in Indianapolis. Development of high-end visualization capabilities for this project will be conducted in this lab space.

The Advanced Visualization Lab of Indiana University also includes a number of high-end graphics and visualization capabilities that can be made available for projects. In Bloomington, the AVL program supports a CAVE virtual reality theater. In Indianapolis, the AVL program provides an ImmersaDesk for stereoscopic viewing, a tiled high-resolution display wall, and a Reach-In stereo viewing and haptic interaction station.

- **Albert-László Barabási (Department of Physics, University of Notre Dame)**

Computer: The Notre Dame group currently has two sun, one IBM RS/6000 workstation, and four PC's (two Pentium II 350 MHz, two Pentium III 450 MHz) that will be used for data analysis purpose. In additional, we have access to the University of Notre Dame's computer facilities, 161 Sun Ultra 30 workstations and 17 Silicon Graphics workstations. Our university has Internet-2 connection for better access to public data on the web.

Office: The PI's office is located on the second floor of the NSH building (room 203) and is approximately 1,000 square feet. Rooms for postdocs and students are also on the same floor, with 500 square feet each. All offices are equipped with Internet-2 connection to ND computer facilities.

Major Equipment: These offices are equipped with high-speed Ethernet access (10 Mbps), and there are plans to install 100 Mbps lines. The Barabási research group currently has two Sun, one IBM RS/6000 workstations, and four PC’s (two Pentium II 350 MHz, two Pentium III 450 MHz) that will be used for data analysis purposes. There is a storage capability of over Pb, that could be used to store some of the large databases on network topology. The Barabási group has access to the UND’s computer facilities that are described above.

- **Teri Belecky-Adams (Department of Biology, College of Science, Indiana University – Purdue University Indianapolis)**

Laboratory: Dr. Belecky-Adams' laboratory is found at Indiana University Purdue University Indianapolis, which is the largest campus in Indiana, houses the state of Indiana's only four- year medical school, and is the most highly federally funded campus in Indiana. The Department of Biology has 24 faculty members whose expertise range from intracellular signaling and transcription factors to physiology and regeneration. The department grants both M.S. and Ph.D. degrees. Dr. Belecky-Adams' individual lab space occupies 800 square feet in the Department of Biology. This lab space is immediately adjacent to approximately 3,000 square feet of core equipment space for the department.

Animal: Animal care facilities and programs meet the requirements of Federal Law (89-544, 91-579) and NIH regulations. The facilities are also accredited by the American Association for Accreditation of Laboratory Animal Care (AALAC).

Computer: A Macintosh G4 with 60 GB hard drive, zip and jazz drives, and CD writer/drive; a Dell Optiplex GX240 Pentium 4 with 80GB hard drive, CD/DVD writer/drive, and zip drive; a Macintosh 7100 with CD and three-quarter inch floppy drive are available. All computers are connected via the internet to several departmental laser printers and color laser printer.

Office: Office space is approximately 160 square feet adjacent to the laboratory space and is equipped with a desk, a computer desk, computer filing cabinets, and bookshelves.

Major Equipment: Belecky-Adams' lab contains two CO_2 tissue culture incubators; a six-foot laminar flow hood; a 600-egg variable humidity incubator; a micropipette puller; a PLI-100 picoinjector; a micropipette beveler; two Zeiss stereomicroscopes, and two regular Wild stereomicroscopes; a liquid nitrogen storage tank; two eppendorf microcentrifuges; an -80° freezer and a -20° freezer; a refrigerator; protein and DNA electrophoresis equipment; a Cytospin 4 cytocentrifuge; a Leitz upright fluorescence/brightfield microscope; a Zeiss Axiovert 25 inverted microscope; an Axiocam cooled B&W digital camera; an Axiocam color camera; and axiovision software.

The core facilities contain two autoclaves;, two scinitilation counters; a gamma radiation counter; two table-top centrifuges; two high speed centrifuges; two ultracentrifuges; a Storm phosphorimager; a Coulter counter; three computers; a Milli-Qultrapure water system; two walk-in cold rooms; two 6-foot lamina flow hoods; a Nikon Eclipse upright; a Nikon Eclipse inverted; a Spot digital camera system; a spectrophotometer; a histology core with paraffin embedding capabilities; a microtome; an ultramicrotome; a cryostat; four PCR machines; and molecular core with UV light box and camera set up.

Other: The School of Science and School of Medicine offer facilities that are available on a time share or recharge basis, including a computer cluster; a cryostat; three confocal microscopes; electron microscopes; a Zeiss upright microscope with MetaMorph image analysis capabilities; core molecular biology services; and proteomics core. The School of Medicine also houses the Medical Sciences Library.

- **Katy Borner (School of Library Science and Informatics, Indiana University Bloomington)**

Laboratory: Borner's Information Visualization Laboratory will be used for software development and testing, project meetings, and informal usability studies.

Computer: The laboratory is equipped with a DELL PowerEdge 1300 PC and two Sun Ray 100 Enterprise Appliance CRTs connected to a Sun Enterprise 250 Server with two 400MHz CPUs, each with 2-MB cache, 2GB memory, and four 36GB 10,000 RPM drives.

Major Equipment: Formal usability studies will be conducted in the SLIS Usability Laboratory, a state of the art facility (http://www.slis.indiana.edu/hcilab/new/labdes.html) that provides an interruption-free, controlled environment with sophisticated facilities for capturing, editing, and analyzing live user performance data in a variety of formats. The lab design follows the classic usability testing lab design, incorporating separate testing

and observation rooms. Several observation monitors supplement a one-way mirror allowing clients to observe sessions.

- **William Bosron (School of Medicine, Indiana University)**

Laboratory: Approximately 500 square feet of newly constructed laboratory space in L3-339 in the Biotechnology Research and Training Center in the Department of Biochemistry and Molecular Biology is dedicated to research projects. In addition approximately 1250 square feet of space in the biotechnology training space is available for research and includes cell culture facilities and shared instruments.

Clinical: Clinical specimens for immunohistochemical analysis will be collected from liver transplant donors and recipients. Patients will be seen by Dr. Lawrence Lumeng (division head) and the physician staff of the Division of Gasteroenterology of Indiana University School of Medicine at Clarian Health Services Hospitals. Dr. Lumeng is an Investigator of the NIAAA Indiana Alcohol Research Center.

Animal: Approved facilities for storage of laboratory animals and surgical space is available on the first floor of the Biotechnology Research and Training Center

Computer: There are many IBM-style laboratory computers for data collection and analysis in the laboratories. Access to supercomputing facilities is available at Indiana University.

Office: Drs. Bosron, Sanghani and Sanghani have individual offices in the Biotechnology Research and Training Center. The office of the Department of Biochemistry and Molecular Biology provides overall support.

Major Equipment: In the general biochemical laboratory there are refrigerated chromatography cabinets; UV column monitors; Waters model 600 chromatograph for protein purification; and several Pharmacia protein purification systems; -20° and -70° freezers; Beckman preparative and table top centrifuges; electrophoresis units; PCR cyclers; three Class II A/B3 type Biosafety cabinets; constant temperature cell culture incubators; an incubator for culture; a Nikon cell culture microscope; and a Zeiss Axiovert 200 manual Fluorescence microscope.

The analytical laboratory (L3 336) contains an HP 1100 HPLC system with fluorescence and UV detectors; a Hewlett-Packard GC-Mass Spectrometer with autosampler and analysis software; Cary 300, Cary 50 and Perkin-Elmer Lambda 6 double spectrophotometers; a SPEX Floromax-2 fluorescence spectrometer; an Applied Photophysics SX.18MV-R Stopped-Flow reaction analyzer; Beckman capillary electrophoresis; and a Spectromax UV/VIS plate reader.

In the adjacent Department of Biochemistry are the following shared facilities: ABI 377XL DNA sequencing facilities; a Finnigan MAT LCQ ESI/MS attached to a capillary HPLC; Micromass Q-TOF and MALDI mass spectrometers; a Phosphorimager; scintillation counters; constant temperature warm and cold rooms; ABI 7700 sequence detection system of real-time PCR; a New Brunswick Bioflow 3000 Fermentor with 10L capacity for insect cell preparation; a Thermo Spectronic French Pressure Cell Press; and Pharmacia FPLC and Bio-Rad Biologic LP protein purification systems.

- **Rajiv Chaturvedi (Department of Computer Science, University of Notre Dame)**

See University of Notre Dame Computing Systems.

- **Ellen Chernoff (Department of Biology, College of Science, Indiana University – Purdue University Indianapolis)**

Laboratory: Dr. Chernoff has a 600 square foot laboratory well equipped for microscopy with new upright and fluorescence microscopes (one DIC, one phase contrast) with digital imaging systems and a fluorescence stereomicroscope. The laboratory is equipped with laminar flow hood and incubators for animal surgery and

cell culture. The lab has all of the equipment used in for PCR, cloning, Northern blot and Western blot analysis. Laboratory personnel all have computers.

- **Simon Conway (School of Medicine, Indiana University – Purdue University Indianapolis)**

Laboratory: Dr. Conway's laboratory occupies1,000 square feet (two 500 square feet modules) in the HB Wells Center for Pediatric Research located on the Indiana University Medical Center campus. The laboratory is fully equipped with a fume hood, post-doc/student/research assistant desks, and ample space for storage of supply stocks and small equipment. In addition, there is full access to all Wells Core space including histology; darkroom with automated autorad processors; digital imaging for gel capture and phosphoimager; cold rooms; tissue culture core facilities (incubators, hoods, microscopes); and Common Use areas that contain most of the large equipment for the labs such as –80^{o}C freezers and refrigerated table-top & low, middle, high speed ultracentrifuges. Confocal Microscopy is available to the project through the Imaging Core Facility & Media prep, and glassware washing facilities are provided in the Wells Center. The Wells Center occupies a total of 27,550 square feet on this campus, with 13,550 square feet in the newly constructed Research Institute, and another 14,000 square feet in Riley Hospital. The Hospital and the Institute are directly connected via an enclosed sky-bridge. A macromolecular facility, including peptide synthesis, DNA sequencing, protein analysis, three crystallographers, an Area Detector, and oligonucleotide synthesizer is also available in the Department of Biochemistry and Molecular Biology across the street in the Medical Sciences Building.

Animal: A newly renovated mouse facility is located on the third floor of Riley Hospital for Children, one floor above the West section of the Wells Center. The facility is maintained by the Laboratory Animal Resource Center at Indiana University, and includes eight dedicated rooms for housing mice and a ^{137}CS-irradiator located in contiguous space. A new transgenic facility is located in the Research Institute, and it includes five additional rooms and two microinjection facilities.

Computer: All of the data acquisition instruments (gel scanner, Affymetrix/Incyte GEMTools and digital camera etc.) are fully networked to the PI's office computer system.

Office: Dr. Conway occupies a 150 square foot office adjacent to the laboratories. The Wells Center houses two conference rooms, each with adequate seating for 35 individuals. These rooms are equipped with slide and overhead projectors and house the journal collection, which includes issues up to one year old of 25 journals. The Medical School Library is located directly across the street for older issues. Administrative support is available to all Wells Center investigators.

Major Equipment: Major equipment available includes Zeiss SV11 & 6 dissecting scopes (with Spot digital cameras); two Eppendorf PCR gradient blocks; Molecular Biology Workstations; hybridization incubators and protein/DNA/RNA electrophoresis equipment; Zeiss Axioscope-2 with light-/dark-field; and a DIC-phase/fluorescence (with digital camera). Additionally, Dr. Yoder (adjacent lab) has Mettler AG104 Analytical Balance; Beckman L Preparative Ultracentrifuge; Beckman J6M/E centrifuge, J2-21 and GP centrifuge; Forma CO_2 and Hereus auto-zero tissue culture incubators; Coulter counter and channelizer; Coulter 753 Cell sorter; Hoeffer fluorometer; Nikon L-Ke microscope with MicroflexPPM 35mm photomicrographic attachment; Zeiss light microscope; Nikon TMS phase contrast microscope; Gilford spectrophotometer; Revco -70°C freezer; and an icemaker.

Other: The HB Wells Center for Pediatric Research currently has 25 faculty members and has recently established a Cardiovascular Development Group to complement its well established Hematopoietic Development and Stem Cell Group. Two additional groups are currently being set-up: the Pulmonary and the Comparitive Medicine Groups. The Cardiovascular Development Group is highly interactive and is housed in contigous laboratory space and shares facilities and major equipment. The Group is led by Loren Field, Ph.D. (cardiomyocyte cell cycle and terminal differentiation) and includes Weinian Shou, Ph.D (FKBP-mediated signaling), Joseph Ruiz, Ph.D. (novel *msk* kinase pathway), and two newly recruited cardiovascular developmental biologists: Anthony Firulli, Ph.D. (dHAND & eHAND) and Simon J. Conway, Ph.D. (congenital

heart defects and mouse mutants). A further recruit of a clinician-scientist for the Cardiovascular Development Group is currently underway.

- **Andras Czirok (Kansas University Medical Center)**

See description for Charles Little.

- **Debasis Dan (Biocomplexity Institute, College of Arts & Sciences, Indiana University Bloomington)**

See description for James A. Glazier.

- **Jo Davisson (Bindley Bioscience Center, Purdue University)**

Laboratory: RHPH: The current facilities include ,700 square feet of space with half the space dedicated to synthetic chemistry; the remainder is equipped for standard biochemical analyses. Cold room space is shared within the lab. A Hi-Tech SF-61DX2 stopped flow spectrophotometer with emission monochromator for fluorescence detection; a Hi-Tech RFQ-63 quench flow apparatus; Cary 3 and Cary 4 UV-VIS spectrophotometers; and Beckman HPLC with photodiode array are available in the laboratory for these studies. Two NMR spectrophotometers (Brüker ARX300 and DRX500 MHz) and a Packard robotic liquid handling system are also available.

HANS: Laboratories facilities for the co-PI include an 1,800 square foot laboratory dedicating 98 linear feet to synthesis chemistry with an additional 40 linear feet for instrumentation and equipment. A 350 square foot room is dedicated to PCR, DNA sequencing and gel purification.

Computer: A laboratory for computational Biochemistry is available and maintains sequencing analysis software package and the GCG programs. In the department there is access to Vector NTI, Origin Data analysis software and Sybyl structural analysis software. Computer facilities are available within the department for data analysis, searching of nucleotide sequences and molecular modeling. The P.I. and co-P.I. both maintain multi-platform computer facilities to Departmental Servers and the campus wide network.

Office: Dr. Davisson has an office with adequate space within the Department of Medicinal Chemistry and Molecular Pharmacology. The co-P.I.'s office is located within the Hansen laboratory.

Major Equipment: Shared Equipment equipment includes full broad-banded 250 MHz, 300 MHz and 500 MHz spectrometers, which are housed within the department and are available for research purposes by trained users. There is also a 4-channel 600 MHz system with pulse field gradients in the Department of Chemistry that is available for use by professional staff and students with advanced training. A campus-wide mass spectrometry facility has capabilities for MALDI, FAB EI, CI experiments with GC and HPLC interfaces along with a high resolution instrument. A Fluoromax 2 spectrofluorimeter is available for use, as is a Tecan UV/fluorescence plate reader. Analytical ultracentrifugation is available for user-based data collection. The relevant department equipment includes liquid scintillation counters; low speed, high speed, and ultra centrifuges; thermocyclers; a film processor; a phosphorimager; a scanning densitometer; a polarimeter; a 10L fermentor; incubators; tissue culture facilities; floor shaker-incubators; upright shaker incubators; computer graphics; NIH Level 3 biohazard safety cabinets; a -80º C freezer; and autoclaves. In HANSEN Chromatron, Gilson FC-80M, FC0100, and FC-203 fraction collectors are available, as are five gel apparatus with power supplies; Semimicro balances and an electronic balance; a DNA synthesizer; Milligen/Biosearch 8700 and an inert atmosphere system. In addition, other scientific equipment is located here including a freezer dryer; rotary evaporators; an FT-IR system; a PCR thermocycler by Perkin Elmer; an ion analyzer; and an assortment of scientific centrifuges including high speed, micro, and evaporator. Available electrophoresis apparatus includes a Transilluminator system; photochemical apparatus; a pump; a drying oven; and a Molecular Dynamics Phosphorimager.

Other: Machine, electronic and glassblowing shops are housed in other departments on campus and are available for service on a charge-back basis. Secretarial support (20 per cent) is provided by the department. The Purdue Structural Biology group in the Department of Biological Sciences has world-class research facilities and active training programs. The research environment at Purdue in fields related to those proposed are active and as a member of the Purdue University Program in Biochemistry and Molecular Biology, there exists a network of scientific colleagues for consultation. The Purdue Genomics Center maintains a state-of-the art DNA sequencing facility that is well equipped to handle the demand of the proposed mutagenesis experiments.

- **Loren Field (School of Medicine, Indiana University Bloomington – Purdue University)**

Laboratory: The molecular analyses will be performed in the Herman B. Wells Center for Pediatric Research, Indiana University Medical School, Indianapolis. Dr. Field's laboratory encompasses approximately 1,000 square feet on the third floor of Riley Hospital for Children. Within the Institute, there are multiple cold rooms; bacteriological incubators; CO_2 incubators; darkrooms (with photographic and x-ray equipment); biohazard hoods and autoclaves, all of which are available for the general use of the staff.

Animal: The Indiana University School of Medicine currently operates the Lab Animal Resource Facility in IB Building which is accredited by the American Association for the Accreditation of Laboratory Animal Care and is currently under review. The facility is staffed by full-time animal technicians and veterinarians who are available for both periodic and emergency consultation.

Computer: The Herman B. Wells Center for Pediatric Research houses a computer facility with many services, including the Intelligenetics DNA and protein manipulation and analysis programs, data bank searches and word processing. Canon laser printers and word processing stations for the secretaries are also available.

Office: Requisite office space and secretarial services are available.

Major Equipment: Wells Center core equipment includes Scintillation counters (beta, gamma); and Beckman low speed and ultracentrifuges. The field laboratory contains two Leitz embryo microinjection stations (including Leitz microscopes with Nomarski optics; micromanipulators; Defronbrune microforges; Kopf needle pullers; CO_2 incubators, etc.; a Leitz microscope with darkfield, phase contrast and fluorescence optics; a Cryostat; a complete cell culture lab with three CO_2 incubators; a Laminar flow hood; a Flexcell strain unit; electroporator; Physiology lab including physiograph with A-D interface, three complete direct blood pressure stations, ECG electrodes and couplers; a molecular biology lab including three Cetus PCR thermocyclers; assorted bacterial incubators and environmental chambers, assorted water baths, Western transfer stations, electroelution equipment; equipment for DNA cloning, analytical and preparative analyses, and sequencing; three Beckman microfuges; a Sorval centrifuge; a UV spectrophotometer; and miscellaneous molecular equipment.

Wells Center core equipment includes scintillation counters (beta, gamma), and Beckman low-speed and ultracentrifuges with assorted rotors.

- **Anthony Firulli (School of Medicine, Indiana University – Purdue University Indianapolis)**

Laboratory: The molecular analyses will be performed in the Herman B. Wells Center for Pediatric Research, Indiana University Medical School, Indianapolis. Dr. Field's laboratory encompasses approximately 1,000 square feet In the Wells Center on the third floor of the R4 Research Building. Within the Institute there are multiple cold rooms; bacteriological incubators; CO_2 incubators; darkrooms (with photographic and x-ray equipment); and biohazard hoods and autoclaves all of which are available for the general use of the staff.

Animal: The Indiana University School of Medicine currently operates the Lab Animal Resource Facility in IB Building, which is accredited by the American Association for the Accreditation of Laboratory Animal Care and is currently under review. The facility is staffed by full-time animal technicians and veterinarians who are available for both periodic and emergency consultation.

Computer: The Herman B. Wells Center for Pediatric Research houses a computer facility with many services, including the Intelligenetics DNA and protein manipulation and analysis programs, data bank searches and word processing. Canon laser printers and word processing stations for the secretaries are also available.

Office: Requisite office space and secretarial services are available.

- **Geoffrey Charles Fox (Departments of Computer Science, Informatics, and Physics, Indiana University)**

Laboratory: Geoffrey Fox is the Director of the Community Grids Laboratory. This Laboratory is a research organization funded partially by the Lilly Foundation focused on technologies to support distributed scientific research and education. It develops Grid systems with personal digital assistant interfaces and collaborative portals. The laboratory has six scientific researchers (four junior, two senior) and about 25 students. It is supported by the administrative staff of the Pervasive Technology Laboratories and occupies about 4,000 square feet in the Indiana University Research Park.

Computer: This laboratory uses the major resources of UITS and the Indiana Computer Science Department described separately. All staff students have modern workstations and the server technology of the laboratory is hosted on 20 two-processor rack mounted Linux systems with two 8-processor Sun Fire V880 systems. The former are arranged as a Grid server farm. The latter have 16 gigabytes of memory each to support the complex Java applications developed in the laboratory. Two 2-processor Ultra-60 Sun Servers serve as laboratory general purpose systems. Many software packages are used including Oracle 9i, other application servers, Java Message Service, XML databases and many Apache products including Axis, Jetspeed and Tomcat. The laboratory has excellent networking with a gigabit/second link to the campus computer center.

Other: The laboratory has installed high-end audio video conferencing systems using Access Grid technology for communicating both with outside collaborators and internally with the Computer Science department, which is about one mile from the laboratory. There are several smaller Polycom systems, which run a shared-application collaborative server.

- **Dennis Gannon (Chair, Department of Computer Science, Indiana University Bloomington)**

Laboratory: The Computer Science Department operates a facility consisting of several hundred workstations and servers (Unix and NT) and has a staff of seven full-time professionals to manage it. For this project the department has access to a new high performance computing cluster (32 Intel Itanium nodes) and a large (2 Terabyte) data server. This system is designed to support software research for interdisciplinary applications such as those proposed by the biocomplexity projects described here. This compute cluster is also designed to be compatible with the NSF NCSA distributed teragrid architecture, which is the hub of the new cyber-infrastructure for scientific computation. The department will soon have a 2 Gbps connection to the Abilene research backbone. The Department works closely with the University Information Technology Service which operates our large production Itanium Cluster Grid and the 1-Tflop SP-2 system.

Computer: This laboratory uses the major resources of UITS and the Indiana Computer Science Department described separately. All staff students have modern workstations and the server technology of the laboratory is hosted on eight two-processor rack mounted Linux systems with two four-processor Sun Fire V480 systems to be added soon. The latter have 16 gigabytes of memory to support the complex Java applications developed in the laboratory. Two two-processor Ultra-60 Sun Servers currently serve as laboratory general purpose systems. We use many software packages including Oracle 9i, other application servers, Java Message Service, XML databases and many Apache products including Axis, Jetspeed and Tomcat. The laboratory has excellent networking with a gigabit/second link to the campus computer center.

Other: The laboratory has installed two high-end Audio Video conferencing systems using Access Grid technology which it uses for communicating both with outside collaborators and internally with the Computer

Science department, which is about one mile from the laboratory. We have several smaller Polycom systems and run shared application collaborative servers.

- **James A. Glazier (Department of Physics, Indiana University Bloomington)**

Laboratory: Dr. Glazier's laboratory is currently under renovation. When completed it will consist of 1,300 square feet of laboratory space including Dr. Glazier's 150-square-foot office and a 300-square-foot student office. The laboratory will be equipped with two rooms for microscopy and a cell culture room, all with laminar flow biohazard hoods and integral UV sterilization.

Computer: There are eight workstations of various types, and numerous high-end PCs and Macintosh Computers.

Major Equipment: Dr. Glazier has, or currently has on order, two fluorescence microscopes equipped for video microscopy; a spinning disk confocal microscope; a low temperature freezer; CO^2 incubators; centrifuges; millipore water filtration; an autoclave; and miscellaneous small laboratory equipment. In addition he has primary access to a wet AFM.

- **Matthew Grow (School of Medicine, Indiana University)**

Laboratory: The Center for Medical Genomics (CMG) currently devotes approximately 1000 square feet of laboratory space to microarray operations, plus an additional 300 square foot computer lab and office space. Dr. Grow has 500 square feet of laboratory space in the Biotechnology Research and Training Center (BRTC) and shares an additional 2,500 square feet of space with the CMG core facilities.

Computer: The CMG has built a dual-1Ghz Linux-based server with 670GB RAID for database storage and performance of the computationally complex calculations necessary for array analysis. A wireless network and custom database with barcode capability that will enable sample tracking throughout the microarray fabrication, QC, and hybridization processes has been designed. For personal workstations and robot control, the CMG and Dr. Grow's lab have 12 up-to-date PCs and six Apple computers with full technical support from the Department of Biochemistry and Molecular Biology. Finally, a separate server supports the production version of the Microarray Data Portal (MDP).

Office: Dr. Grow maintains office on the second floor of the BRTC.

- **David C. Hart (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Randy Heiland (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Stephen Jacobson (Department of Chemistry, College of Arts & Sciences, Indiana University Bloomington)**

Laboratory: Approximately 1,250 square feet of laboratory space is available for micromachining, microchip diagnostics and experiments. This space includes 100 square feet of class 10000 cleanroom and 300 square feet of class 1000 cleanroom with class 100 fume hoods for micromachining and assembly operations.

Computer: Eight computers are available for instrument control, data acquisition, and analysis. Most of these computers are equipped with data acquisition cards and LabView software.

Major Equipment: For microchip fabrication, equipment includes a spin coater; two class 100 fume hoods; mask aligner/exposure system; two etch baths; a nanopure water system; a programmable furnace; a stylus profiler; a scanning electron microscope; a thermal evaporator; and a magnetron sputtering system. Equipment for microchip diagnostics and fluorescence detection includes three optical tables; two inverted fluorescence microscopes; two observation microscopes; two frame transfer CCD cameras; two video cameras; five computer controlled high voltage sources; an oscilloscope; two picoammeters; argon ion and helium-neon lasers; four photomultiplier tubes; and four current amplifiers. In addition, a reactive ion etch system and confocal microscope are in the process of being purchased.

Other: State-of-the-art machining and electronics design and fabrication facilities are available in the Chemistry Department at IU. The machine shop will help with drilling and dicing the microfabricated devices, and the electronics shop will assist in developing control circuits for fluid transport. A full service glass shop is also available.

- **Charles Little (Kansas University Medical Center)**

Laboratory: Dr. Little's laboratory consists of 1,000 square feet of standard lab bench space within a larger 2,000-square-foot lab suite in Wahl Hall West. Within this suite are fully equipped tissue culture and microscopy- microinjection rooms.

Animal: The lab contains CO_2 cell culture incubators for cell and avian embryo culture. Avian embryos are currently not regulated by NIH guidelines and are stored and handled within the existing lab space. To utilize avian eggs there is a 15C egg-holding refrigerator and bench top 37°C humidifying incubators for egg incubation. Timed pregnant mice will be used as a source of embryonic allantoides. Mice will not be housed, but will arrive at the institution on the day of use. All animal protocols meet institutional laboratory animal resources and NIH standards of care.

Computer: Dr. Little has a Power MacIntosh G4 and a Powerbook G4 computer and access to fourteen PCs, which run on both Windows and Linux and are loaded with word processing and graphics software. The lab has two HP Laserjet 2100TN and two HP Deskjet 970 Cxi printers and a flatbed scanner. The lab has seven high speed internet connections to handle the transfer of large volumes of data. An Apple Xserve dual 1.33 Mhz 1 gig RAM 60Gb HD server with an Xserve RAID 2.25 terabyte, 512+512 Cache and service contract is also available.

Major Equipment: Dr. Little also has access to electrophoresis equipment; a gel dryer; standard and -80°C freezers; CO_2 incubators; a thermal cycler; hybridization ovens; bench top centrifuges; Mettler balances; and orbital shakers. The lab has two fume hoods and two tissue culture hoods. Microscopy equipment includes three (3) automated time-lapse work stations; a 2002 fully automated Leica upright microscope; a 2002 fully automated Leica inverted microscope; a 1998 upgraded Leitz photo-microscope with epifluorescence illumination; a photomultiplier detector for photography; and Leitz fluotar objectives for epifluorescence and differential interference contrast. In addition, for preparative purposes, a Leitz high resolution epifluorescence stereomicrosope is available. A Leitz Diavert microscope equipped with Hoffman contrast objectives as well as phase contrast objectives is available as are four research grade stereomicroscopes with darkfield, brightfield, and variable angle illuminated bases, three of which are equipped with cameras. The lab has a Medical Systems PL1-100 pico-injector with a Narishige four-channel hydraulic micromanipulator workstation. Time lapse digital imaging is performed with a Photometrics Quantix or Q imaging digital camera and computer-controlled, motor driven, X,Y,Z stage positioning software.

Other: Departmental investigators have immediate access to high speed centrifuges, an ultracentrifuge, a UV spectrophotometer and a scintillation counter. The imaging center has scanning and transmission EM equipment, a Zeiss LSM 510 (confocal) microscope, and high resolution color printers. A phosphoimager, chemiluminescence imager and an x-ray film developer are also available. A Biotechnology core in WHW performs peptide synthesis and sequencing.

- **Andrew Lumsdaine (Department of Computer Science, Indiana University Bloomington)**

Laboratory: The Open Systems Lab conducts research on software platforms for pervasive technologies. Particular areas of investigation include scientific and engineering applications, scalable computing, and peer-to-peer distributed computing. With the goal of enabling more productive and efficient software development in practice, the lab also develops tools and processes for collaborative, decentralized software development. The lab provides a stable environment where these and other tools are deployed and where developers worldwide collaborate on software development in an open environment.

Computer: This laboratory uses the major resources of UITS and the Indiana Computer Science Department described separately. All staff and students have modern desktop workstations; the server and computational needs of the laboratory are supplied by four two-processor rack mounted Unix systems as well as an 8-node Linux cluster. The laboratory has excellent networking with a gigabit/second link to the campus computer center.

- **Keith March (School of Medicine, Indiana University – Purdue University Indianapolis)**

Laboratory: The principal facilities available to the project include five laboratories: a molecular biology lab; a cell culture lab; a physics lab; a small animal proceduaral lab; and a microscopy/imaging and histology lab. The total space of these facilities is 3,750 square feet. The labs are equipped with equipment appropriate to the proposed research. The flow cytometry and sorting equipment is available through the use of co-investigator Edward Srour's laboratory.

Clinical: Clinical laboratory space will be provided through the use of the General Clinical Research Center (GCRC) where co-investigator Robert Considine has a clinic and ongoing research.

Animal: The Laboratory Animal Research Center will render housing, care, and appropriate husbandry to the animals.

Computer: Ten PCs and five Macs, all with Internet, Medline, and Office Suite capabilities, a scanner and a color laser printer are available. In the cell culture, molecular, transgenic, and microscopy labs, all computers have digital imaging systems.

Office: Three hundred square feet of office space is occupied. Secretarial and administrative support is available through the Center, along with facsimile and copy machines.

Major Equipment: The flow cytometry core laboratory has BD FACS caliber analysis and sorting equipment.

There are shared facilities, which include two walk-in cold rooms; tissue homogenizers; ultracentrifuges (Beckman L5-50, J2-21 and TL-100); an Eppendorf centrifuge (5415C); a cool down centrifuge (Labnet Company); and a computer-assisted analysis ELISA system. A Programmable Thermal Controller (MJ research, Inc) PCR machine is also available.

Other: The Indiana Center for Vascular Biology & Medicine and The Krannert Institute of Cardiology provide a favorable environment for training and actively facilitate interdisciplinary study. Over twenty physicians and scientists work in basic and applied cardiovasucalar research and are available for consultation. Collaboration is common and is encouraged. The facilities are well suited for conducting the proposed experiments.

Departmental investigators have immediate access to high speed centrifuges, an ultracentrifuge, a UV spectrophotometer, and a scintillation counter. The imaging center has scanning and transmission EM equipment, a Zeiss LSM 510 (confocal) microscope, and high resolution color printers. A phosphoimager, chemiluminescence imager and an x-ray film developer are also available. A Biotechnology core in WHW performs peptide synthesis and sequencing.

- **James Marrs (School of Medicine, Indiana University, Indiana University – Purdue University Indianapolis)**

Laboratory: Dr. Marrs has 1,500 square feet of new laboratory space (Research Building 2, 950 W. Walnut) that includes tissue culture facilities, general equipment space, storage and a cold room. The laboratory is well equipped for molecular biology, cell biology, histology and biochemistry studies. In addition, there is full access to the imaging facility that is housed on the same floor as the laboratory and houses the microscope equipment.

Animal: Zebrafish are housed in the Laboratory Animal Resource Center in an adjacent research building. Oversight of these facilities comes from the Laboratory Animal Resource Center, which provides inspection, compliance and veterinary support. Antibodies are produced using commercial services (BAbCO/Covance, Richmond CA).

Computer: A Pentium 4 PC is available for general purposes, including data analysis and image processing (Photoshop). There are four Pentium 4 PCs available for general laboratory purposes, including sequence analysis (Vector NTI Software) and image processing (MetaMorph and Photoshop). Within the Division and the Imaging Facility, there are considerable computing resources and computing support.

Office: Dr. Marrs has a 100 square foot office. The fellows have a shared office.

Major Equipment: The laboratory has a Leica CM3050 cryostat to prepare sections for *in situ* hybridization and immunohistochemistry experiments. There are two Leica dissecting microscopes with a Narishige micromanipulator microinjection set-up that can be equipped with the imaging facility's SPOT CCD detector for image acquisition.

The division's core equipment is extensive. A partial list includes super speed centrifuge; floor model ultraspeed centrifuge; table top ultracentrifuge; assorted rotors for centrifuges; darkroom with x-ray film developer; BioRad Fluor-S MultiImager (gel documentation system and phosphoimager) with Pentium III PC to drive instrument and run Quantity One software; tissue culture facilities (laminar flow hoods, inverted microscopes, CO_2 incubators); MilliQ UF water purification system; thermal cyclers; gel dryer and speedvac system; spectrophotometer; fluorometer; UV/Vis/Fluorescent microtiter plate reader; BioRad EconoSystem column chromatography system; console and bench top incubator shakers; and scintillation counter.

In addition to the imaging facility equipment listed above, the research division employs a full-time electron microscopy technician. There is access to a Philips CM 120 Transmission Electron Microscope housed in the Department of Pathology at the Veteran's Administration Hospital on campus. Although electron microscopy experiments are not proposed in this application, such capabilities may become useful, and the personnel and facilities have access to the laboratory at no cost.

- **Roeland Merks (Biocomplexity Institute, Indiana University Bloomington)**

See description for James A. Glazier.

- **David Moffett (University Information Technology Services, Indiana University)**

See Cytomics and Imaging Research Core in the Bindley Bioscience Center at Purdue University.

- **Glen Niebur (Department of Aerospace Engineering, School of Engineering, University of Notre Dame)**

Laboratory: The University of Notre Dame Tissue Mechanics Laboratory and Biomaterials Laboratory are located in 1,200 square feet of space.

Major equipment includes a canco μCT-80 micro-computed-tomography scanner and computer system with 80 GB of hard disk space, and a DLT backup drive that is capable of imaging bone specimens up to 80 mm in diameter. The maximum spatial resolution is 10 μm per pixel. Complete three-dimensional morphology software allows analysis of both cortical and trabecular bone architectural parameters. A Nikon Eclipse ME 600 equipped with epifluorescence capability, with filters tuned for viewing of alizarin, xylenol, and calcein, is used for microscopic analysis.

Furnaces and ovens, including a vacuum oven, are present in the laboratory for drying and ashing of specimens. Analytical balances are available. Glassware and appropriate chemical measurement and handling supplies are present.

Specimen preparation equipment includes two diamond wafering saws, a Beuhler two wheel grinding and polishing station, band saw for gross cutting, a drill press with diamond coring bits for cutting of cylindrical specimens. The laboratory has fine dissection tools such as scalpels, forceps etc.. Appropriate safety equipment including eyewash and shower, fume hoods, and chemical storage cabinets are present. Two 20-cubic-foot chest freezers and a combination refrigerator freezer are used for specimen storage.

The Material Testing Facility at Notre Dame is a shared facility to which Dr. Niebur has unlimited access. The laboratory has an Instron 8821 servo-hydraulic biaxial load frame with Instron model 8800 controller, two MTS servo-hydraulic load frames, and three screw-driven testing machines. Computerized data acquisition is available for all systems. Two full-time technicians provide support for experimental setup, data aquisition and maintenance. A 1000 lb./500 in-lb. axial torsion load cell and an Epsilon Technologies biaxial extensometer are dedicated to Dr. Niebur's research. Additional load cells and measurement equipment are available in the laboratory.

Through his participation in the Center for Molecularly Engineered Materials, Dr. Niebur has access to a centralized, shared materials characterization facility, which includes a Quantachrome Autosorb-1 nitrogen adsorption isotherm analyzer;a Scintag XDS 2000 x-ray diffractometer; a TA instruments Model SDT 2960 simultaneous DTA - TGA thermal gravimetric analyzer; scanning electron microsocopes; a transmission electron microscope; and other major equipment.

Computer: The Tissue Mechanics Laboratory computer facilities include an SGI Octane workstation with two GB of RAM and an AMD Opteron 2 processor workstation with three GB of RAM. A total of 330 GB of shared disk space is available on the two systems. A DLT backup drive is connected to the SGI workstation for backup of both systems. High performance computing systems include a 20 processor SGI Origin and a 64 processor Linux cluster, which are maintained by the University High Performance Computing Complex. Dr. Niebur has access to these machines on a shared basis. Software includes ABAQUS and MARC finite element analysis applications, TrueGrid and Hypermesh for finite element mesh generation, IDL and Matlab for image processing and general mathematics. A campuswide SAS license is used for statistical analysis.

Personal computers are available in the laboratory and throughout the campus network for documentation, graphics, and statistical analysis. All staff and students have access to Unix workstation accounts and PC accounts with shared data space via AFS maintained by the campus Office of Information Technology.

Office: Sufficient office space is provided for all personnel. Dr. Niebur has a private office. Graduate students share office space in proximity to the laboratory. All offices include computer, printing, and internet access.

Other: The Department of Aerospace and Mechanical Engineering maintains a full machine shop including a CNC mill and rapid prototyping equipment. Full time machinists and technicians staff the shop and are available to produce test fixtures or other mechanical parts.

- **Mary Papakhian (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Ramana Pidaparti (Department of Mechanical Engineering, School of Engineering and Technology, Indiana University Bloomington – Purdue University Indianapolis)**

Laboratory: Dr. Pidaparti's Experimental Mechanics and Advanced Materials Lab in the Department of Mechanical Engineering is equipped with the devices and instruments necessary to conduct mechanical and ultrasound testing of materials. A wet lab in Biomedical Engineering Program is approximately 500 square feet. Routine laboratory equipment include a pH meter; a conductivity meter; digital balances; plate stirrers; a mini-centrifuge; a water bath; an incubator; a gel electrophoresis apparatus for DNA and proteins; a UV illuminator; PCR thermo-cycler (Perkin Elmer Gene Amp 2400); and a PCR/UV workstation. AFM is housed in an approximately 150-square-foot. room attached to the wet lab. IU Bloomington has a newly renovated space consisting of a 1,200-square-foot laboratory with sterile cell culture facilities, atomic force microscopy, scanning confocal microscopy and normal epifluorescence microscopy. A fluorescence cell sorter for genetic modification/ tranfection experiments; a "Tracker" for measuring dynamic surface tenion in liquids; a liquid nitrogen refrigerator; and centrifuges, autoclaves and all the usual bio-lab equipment are also available.

Computer: All three investigators on this project have modern desktop personal computers (Macintosh Power PC's or Pentium II's). Silicon Graphics workstations with extensive software capabilities are available in the Computational Molecular Science Facility in the School of Science, and Sun workstations are available in the offices and computational mechanics and Fluid Dynamics laboratories as well as in a cluster supported by the Purdue School of Engineering. General-purpose finite element software such as ANSYS, ABAQUS, PRO/E, and PATRAN are available in the Computational Mechanics Laboratory in the Department of Mechanical Engineering. Imaging tools such as atomic force microscopy and fluorescence microscopy are connected to the dedicated PC. The Department of Electrical and Computer Engineering at IUPUI has licenses for Mentor Graphics tools, Synopsys/Avant! Tools, and TannerEDA tools for ASIC, FPGA and MEMS design and fabrication. IUB has enormous central computing facilities generally shared with IUPUI.

Office: Office space exists for all investigators in the respective departments. Accommodations for undergraduate students and graduate students will be made available in their chosen departments.

Major Equipment: The IUPUI campus has a full spectrum of computer tools, device inspection equipment and chemistry laboratory facilities to insure the ability to investigate fabricated MEMS devices. Integrated circuit fabrication facilities also exist and are available at Purdue University since the School of Engineering and Technology at IUPUI is a Purdue University extension.

A Digital Instruments Nanoscope III Scanning Probe Microscopy System composed of a contact mode Atomic Force Microscope (AFM) and an Electrochemical Scanning Tunneling Microscope, is available for the proposed project.

A scanning electron microscope (Amray 1000A, KLA-Tencor, MA) is available in Electron Microscopy Facility in the Department of Anatomy and Cell Biology at IUPUI. The sample will be dried using a critical dryer (Samdri-780, Tousimis Research Co., MD), and the sample surface will be coated with gold palladium using a sputter (Hummer V, Anatech Ltd., VA).

A Nikon E600 fluorescence microscope with a Metamorph high-resolution digital imaging workstation is housed in the bioengineering lab. The CCD camera (Photometrics series 200) with 1317 X 1035 pixel resolution is attached to the microscope. A Nikon Diaphot inverted fluorescence microscope is also available for the proposed project. This microscope is equipped with two-channel fluorescence filters (FITC and Texas Red) and a CCD camera.

Other: Machine and electronic shop services are available through the Departments of Mechanical Engineering at IUPUI.

- **Doraiswami Ramkrishna (Department of Chemical Engineering, Schools of Engineering, Purdue University)**

Computers: Chemical Engineering (ChE) has a variety of computing resources at its disposal. These resources are made up of Windows NT workstations, Windows NT servers, Windows TS servers, Ultrasparc workstations, Ultrasparc servers, and Alpha servers.

The Windows NT workstations are comprised of 46 Windows NT 4.0 workstations in two PC labs. The Windows NT Servers consist of one Windows NT Domain controller; four Windows NT Backup Domain controllers; and two Windows NT Wins servers. The Windows Terminal Servers contain two Windows Terminal Servers (quad processor configurations.) The Ultrasparc workstations contain 13 Ultra 5 Sun Workstations (Unix lab.) The Ultrasparc Servers contain three Ultra Enterprise E450 Sun servers (quad processor configurations) and one Ultra Enterprise E6500 Sun Server (16 processor configuration.) The Alpha Servers consist of one DS40 Compaq Alpha server (quad processor configuration) and one ES20 Compaq Alpha server (dual processor configuration.)

There is more than 650GB of backed up storage space available within the ChE central network. In addition the ChE servers have over +20GB of RAM available for computational use.

- **Richard Repasky (University Information Technology Services, Indiana Universit)**

See Indiana University Computing Systems.

- **Simon Rhodes (Department of Biology, College of Science, Indiana University – Purdue University Indianapolis)**

Laboratory: Dr. Rhodes has a 1,000-square-foot main laboratory in a seven-year-old building and also shares a nearby two-room (300 square foot) tissue culture laboratory with one other investigator. The main laboratory is well equipped for molecular biology and biochemistry.

Animal: The nearby 2,000-square-foot animal facility is AAALAC certified and includes seven housing rooms, two cage washing rooms, three storage rooms, three surgery/procedure rooms, and an office. An animal attending veterinarian is available for routine inspection and emergency service. The School of Science IACUC oversees all animal care and has independent USDA Registration/OPRR Assurance.

Computer: The laboratory/office is equipped with four MacIntosh and two Dell computers with network access to the Indiana University Medical Library, the IUPUI Library, the Internet, Medline, e-mail, and laser printers/scanners. In addition, the School of Science maintains a cluster of networked computers that are located within the building and are fully available to the investigator and students.

Office: Dr. Rhodes has a 150-square-foot office located adjacent to the laboratory. In addition, the Department office is close by and contains copying, typing, mai, and facsimile facilities.

Major Equipment: Dr. Rhodes' main laboratory contains electrophoresis equipment (for agarose gels, SDS-PAGe, etc.); two luminometers; two thermal cyclers; high and low voltage power supplies; protein blotting apparatus; a vacuum oven; a hybridization oven; water baths; circulating water baths; a pH meter; balances; vortexers; pipetmen; a shaking incubator; general-use incubators; stir/heat plates; microcentrifuges; freezers; gel dryers; a microwave oven; a Geiger counter; a chemical hood; shaking platforms, etc.

The tissue culture room contains a Baker laminar flow hood, a Forma double CO_2 incubator, a Nikon diaphot inverted microscope, a water-bath and a refrigerator/freezer.

Department facilities are located in rooms adjacent to the laboratory and include two dark rooms with photographic equipment/x-ray film developer and computereized gel documentation system; two cold rooms; a

30°C room; a 37°C room; autoclaves; and an ice machines. An equipment room with ultracentrifuges; high-speed centrifuges; clinical centrifuges; a scintillation counter; a speed-vac; ultra-low freezers; a spectrophotometer; a phosphorimager; a Coulter cell counter; a Leica cryomicrotome; a Leica Histokinette tissue processor; a vacuum infiltrator/paraffin dispenser; a microtome; a microtome knife sharpener., etc. are also available.

- **J. Paul Robinson (School of Veterinary Medicine, Department of Basic Medical Sciences, Purdue University)**

Laboratory: The facilities available to Dr. Robinson include a total of 4500 square feet of space consisting of 5 laboratories of which one is for cell culture and protein chemistry, one for imaging, one for flow cytometry , one for microbiology and the other for general biology. The laboratory has a cold room, ice machine, and water purification system.

Computer: Dr. Robinson's laboratory has a reasonably large computer base – including 7 high speed rack mounted servers servicing over 50 PC computers. The computer system is Novel networked within the PIs laboratory and the system operates completely independently of the campus backbone, although we are connected to the campus backbone and utilize that system. The computer system has over 1 terabyte of in house storage. The laboratory has over 350 licensed software packages in our database of which several are high level image analysis packages such as Image ProPlus, Metamorph and VoxelView.

Office: The P.I. has an office within the Hansen Hall laboratory space.

Major Equipment: Shared Equipment avaialbe: Flow Cyometry: Epics Elite cell sorter with 4 lasers, Epics Altra high speed sorter with 4 lasers, 3 FC500 analysers both with dual lasers and automated carousels. An automated 4 color XL Cytometer is also available. For microbial cytometry we have a Biorad Bryte lamp based Cytometer. All instruments have multivariate analysis software. For image Analysis we have a Bio-Rad MP 2100 multiphoton microscope mounted on a Nikon inverted microscope with a Mai Tai 10 Watt laser. The instrument has a Becker 7 Hinkl life time module connected to 2 high speed PMTs. A Bio-Rad MP 1024 UV/vis confocal microscope is mounted on Nikon inverted microscope. This unit is ideal for UV live cell imaging. Also available is a Bio-Rad Cellmap 2 color confocal microscope mounted on a Nikon E800 upright microscope. The unit has a 405 Violet laser and a 488 blue diode laser for excitation. A Nikon 1000R upright fully automated fluorescence microscope with a SPOT high sensitivity, high resolution digital camera is also present. A Nikon upright fluorescence microscope and a Nikon inverted 300 fluorescence microscope with a DVC 250 line-scanner confocal microscope is also mounted. A Perkin Elmer LS50B spectrofluorometer is available for fluorescence analysis. For protein analysis we also have a Beckman-Coulter Proteome LabTM fully automated protein profiling system.

- **Brenda Rongish (Kansas University Medical Center)**

See description for Charles Little.

- **Ann Rundell (Department of Biomedical Engineering, School of Engineering, Purdue University)**

Laboratory: Associated Experimental work will be conducted in the Dr. Rundell's 1,000-square-foot laboratory that also houses graduate student office space, a cell culture facility, and standard laboratory equipment.

Computer: The laboratory and office are equipped with seven PC-based computers, a digital scanner and laser and ink jet printers with software for computational biology, word processing, structure drawing, graphing, internet access and electronic mail. The Engineering Computer Network System at Purdue University provides and supports multiple computing environments including C++ and MATLAB. For information exchange, all computers are networked and connected to the Internet via Ethernet.

Office: Dr Rundell's 120-square-foot office is located in the Potter Engineering Building within a suite of Biomedical Engineering Faculty offices that also houses the departmental-supported secretarial support. Dr. Rundell's graduate students have desk space within the laboratory in the Potter Engineering Building.

Major Equipment: Major equipment housed within the Rundell laboratory includes dual and single-chamber, water-jacketed CO_2 incubators for mammalian cell culture; a laminar flow safety cabinet; a Leica DM IRB inverted microscope; an autoclave; centrifuges; equipment for protein electrophoresis; Ohaus Explorer Balance; a ventilation hood; a vacuum oven; and an -80°C freezer. The Bindley Bioscience Center Protein Separation and Analysis facility provides state-of-the-art proteomic analysis including protein sequencing, 2D gel separation systems and MALDI-TOF MS analysis. The facility includes an Odyssey infrared imager (LI-COR) for quantitative western analysis. The Transgenic Mouse Core Facility provides services for the production of transgenic and gene-targeted mice for Purdue investigators.

Other: The Department of Biomedical Engineering is headed by Dr. George Wodicka, who also serves as co-director of the Bindley Biosciences Center, which forges interdisciplinary research initiatives between the life sciences and engineering departments. Research is supported by state-of-the-art departmental and university-wide instrumentation including five superconducting NMR spectrometers; a mass spectrometry facility with low, medium and high resolution instruments and a computer graphics facility; an NMR facility; a Macromolecular Crystallography facility; an Analytical Cytology facility; a DNA Sequencing Facility; and a Transgenic Mouse facility. Electronics shop personnel are available within the Departments of Biomedical Engineering and Electrical and Computer Engineering. Computer support personnel are available within the Department of Biomedical Engineering.

- **Sima Setayeshgar (Department of Physics, College of Arts & Sciences, Indiana University Bloomington)**

Computer: Dr. Setayeshgar has a desktop computer (IMac) and two Dell workstations (Pentium 4, 3.0 GHz, 1 GB Memory, 240 GB HD each).

Office: Dr. Setayeshgar's office is located in the Department of Physics, Indiana University Bloomington and is furnished with all necessary equipment.

- **Santiago Schnell (School of Informatics, Indiana University Bloomington)**

The applicant has been provided with $125,000.00 of research start-up funds within a four-year time frame. In addition, the School of Informatics has provided the applicant with an office outfitted with standard furniture, a personal computer and a phone

- **Anurag Shankar (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Raymond W. Sheppard (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Weinian Shou (School of Medicine, Indiana University Bloomington–Purdue University Indianapolis*)***

Laboratory: Dr. Shou's laboratory includes 650 square feet of the HB Wells Center for Pediatric Research located on the Indiana University Medical Center campus. The Wells Center occupies a total of 27,550 square feet on this campus, with 13,550 square feet in the newly constructed Cancer Research Institute, and another 14,000 square feet in Riley Hospital. The Hospital and the Institute are directly connected by a sky-bridge. The laboratory is in the West section. The Wells Center facility includes tissue culture core facilities (incubators, hoods, microscopes) dedicated to ES cell work, and major equipment facility (including low, middle, high speed, and ultracentrifuges). The laboratory is equipped with two hybridization ovens; a PTC-100 thermal cycler; a SmartSpec 3000; a Leica MZ12.5; a Leica DMLRB; two Forma CO_2 incubators; analytic balance; and electrophoresis set-ups, etc.

Animal: A newly renovated mouse facility is located on the third floor of Riley Hospital for Children, one floor above the west section of the Wells Center. The facility is maintained by the Laboratory Animal Resource Center at Indiana University, and includes eight dedicated rooms for housing mice and a ^{137}CS-irradiator located in contiguous space. A new transgenic facility is located in the Cancer Research Institute, and it includes five additional rooms and two microinjection facilities.

Computer: Dr. Shou's office houses a Pentium computer with an attached HP Laserjet 5L printer. These computers, as well as those in their laboratories and the Wells Center administrative staff offices, are connected to the Wells Center Novell network, which has various word processing, spreadsheet, graphics, database, e-mail, and other shared software. These computers can also access the Internet, and through it, the VAX mainframe computer in Bloomington, Indiana, which contains the GCG DNA sequence analysis software.

Office: Dr. Shou's office is adjacent to his laboratory. The Wells Center houses two conference rooms each with adequate seating for 35 individuals. These rooms are equipped with slide and overhead projectors and house the journal collection, which includes issues up to one year old of 25 journals. The Medical School library is located directly across the street for older issues. Administrative support is available to all Wells Center investigators.

Major Equipment: Common equipment areas are conveniently located in the laboratory and contain all necessary equipment for molecular and cell biology research including ultracentrifuges; a scintillation counter; a gamma counter; gel dryers; a sonicator; bacterial incubators; a FACScan flow analysis machine; -80^{o} freezers; a phosphoimager; and multiple low and high speed centrifuges. Tissue culture core areas contain biological hoods, incubators, tissue culture microscopes, refrigerators and centrifuges. Darkrooms contain cameras and UV light boxes for gel photography and a Kodak X-Omat for x-ray film processing. One dark room is set up for fluorescence microscopy and contains a Nikon Axiophot scope and camera. One dark room is set up for *in situ* hybridization studies. Cold rooms contain centrifuges, FPLC apparatus, and media storage areas.

Other: Oligonucleotide synthesis and glassware washing facilities are provided in the Wells Center. A macromolecular facility, including peptide synthesis, DNA sequencing, protein analysis, three crystallographers, anArea Detector, and oligonucleotide synthesizer is also available in the Department of Biochemistry and Molecular Biology.

- **Stephen Simms (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Craig Stewart (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **David Stocum (Department of Biology, School of Science, Indiana University, Purdue University)**

See section on the Center for Regenerative Biology.

- **Charles Turner (School of Medicine, Indiana University – Purdue University Indianapolis)**

Laboratory: The Orthopaedic Laboratories at Indiana University include 5,000 square feet of renovated lab space in Fesler Hall, 600 square feet of lab space in the new Biotechnology Research and Training Center, and 1,000 square feet of lab space in the Medical Research Building. Orthopaedic Research also has two cell culture facilities, a large equipment room, a dark room, a cold room, a calcium imaging center, and a patch clamp facility.

Animal: The animal quarters in the Indiana University School of Medicine were recently constructed as part of the new Biotechnology Research and Training Center (BRTC), which is an AAALAC accredited facility employing a full-time veterinarian to supervise animal care. Adequate space for new transgenic mouse colonies is available. The BRTC also houses the Skeletal Genetics Lab (under Dr. Turner), which houses the x-ray densitometry and microimaging equipment as well as a small biomechanics testing system for mouse bones.

Computer: Office computers are available to all faculty and staff. Laboratories are equipped with at least one personal computer. All computers are connected to the University network and have access to massive data storage facilities.

Office: Orthopaedic Research has twelve offices and six cubicles.

Major Equipment: The following major equipment is available: Scanco uCT 20 microCT; MTS Alliance mechanical testing system; a PIXImus mouse bone densitometer; a Stratec SA+ pQCT imaging system; an Enduratec ELF 3200 low force mechanical testing system; three electromechanical and piezoelectric mechanical loading systems for mouse bone; a Reichert-Jung 2050 Supercut microtome; two Jung 820 Histocut microtomes; Reichert-Jung Polycut microtomes; two Buehler Isomet Low Speed sectioning saws; three DDK diamond wire sectioning saws; a Shandon automatic processor (PathCenter, Shandon); an Embedding Center (Tissue-tek, Sakura); and an automatic stainer. The following microscopes are available: two Nikon Optiphot research microscopes for brightfield; a Nikon Optiphot linked to Bioquant IV image analysis system with CCD camera attachment; a Leitz DMRXE linked via SPOT RT digitizing camera (Diagnostics Instruments, Sterling Heights, MI) and SPOT RT3.0 morphometry software to Dell computer.

- **Eric Wernert (University Information Technology Services, Indiana University)**

See Indiana University Computing Systems.

- **Stefan Wuchty (Department of Physics, University of Notre Dame)**

See description for Albert-László Bárabisi.

- **Mervin Yoder (School of Medicine, Indiana – Purdue University Indianapolis)**

Laboratory: Dr. Yoder has a 900 square foot laboratory within the 20,000-square-foot Herman B. Wells Research Center. There are adjacent warm and cold rooms, three tissue culture facilities, and common equipment rooms within the Center. Computers (three Power Macs and one PC) are located in the laboratory

Animal: Mice will be housed in the Indiana University School of Medicine Laboratory Animal Resource Center. This Center is approved by the American Association for Accreditation of Laboratory Animal Care. Contiguous to this space is a Gamma cell 40 Cesium irradiator for total body irradiation of small animals.

Computer: Computer facilities include: three Power Macs, three PCs, one Laser Jet printer, one AppleScanner, and one Slidewriter. All computers are connected to the Wells Center computer network (Novell based) and through this network to Indiana University VAX computers and the Internet.

Office: The principal investigator has a 160-square-foot office adjacent to his laboratory that contains a Power MAC computer and color printer. Secretaries are supported by departmental funds. Macintosh computers and a variety of software are available in the office area. There is an imaging room on the floor with an up-to-date slide writer, scanner, and color printer.

Major Equipment: Available major equipment includes: a Mettler AG104 Analytical Balance; a Mettler PB 1501; a Digital Analytical Balance;a Beckman L Preparative ultracentrifuge; a Beckman J6M/E centrifuge J2-21, and GP centrifuge; a Forma CO_2 tissue culture incubator; a Hereus auto-zero tissue culture incubator; a Baker laminar flow hood; a Coulter counter and channelizer; a Coulter 753 cell sorter; a Hoeffer fluorometer; Nikon L-Ke microscope with Microflex PPM 35 mm photomicrographic attachment; Zeiss light microscope; Nikon TMS phase contrast microscope; a Leica Wild M8 dissecting microscope; an Intralux 5000-1 light source; a Dubnoff shaking water bath; a Corning 125 pH meter; a Gilford spectrophotometer; a Bio-Rad electrophoresis power supply; an Ephortec horizontal Gel apparatus; a Helena cellulose acetate gel apparatus; a Kenmore refrigerator/freezer, a Revco -70°C freezer; and an icemaker.

Other: Electronics and machine shops are also present at the Indiana University Medical Center and are available on a fee-for-service basis.

CORE I: TISSUE SIMULATION TOOLKIT DEVELOPMENT

I.1 COORDINATOR:

Geoffrey Fox (IUB)

1.2 PARTICIPANTS:

Craig Stewart (IUB), Andrew Lumsdaine (IUB), David Hart (IUB), Randy Heiland (IUB), Dennis Gannon (IUB), Marlon Pierce (IUB), Debasis Dan (IUB), Jo Davisson (Purdue), David Moffett (Purdue), Mark Alber (ND)

I.3 INTRODUCTION AND OVERVIEW:

The goal of the Center for Tissue Modeling (CTM) is to develop the ability to model complete organisms with a hierarchical suite of models ranging from genomic information and subcellular properties to tissues, organs, and organ systems. We plan to accomplish this within the next two decades. Such a hierarchical approach to modeling matches the hierarchical structure of biological structures, which all work together to ensure the survival and reproduction of the organism of which the structures are a part.

The center is comprised of three major components: computer science, modeling, and experiment, addressed from a unified, interdisciplinary point of view. The CTM's long-term goal is to develop the computational architecture and technologies to support comprehensive multi-scale models of cells and cell communities, and to test their applicability to a wide-range of biological phenomena. Initially we will focus on developmental biology, but we intend to pursue other important areas amenable to our techniques in additional proposals. The models will address scales from subcellular genetic control networks and protein networks. At the cellular level, we will emphasize cell polarity, cell migration and cell-cell interactions (e.g., adhesion) as different components in the unified computational model. At the supercellular level, the center studies will include the aggregation of cells into tissues and tissues into organs, as well as later detailed modeling of Biofilms.

It is essential to understand that the CTM is not operating alone. Rather it must operate within the context defined by best practice in both the computer science and biological communities. Furthermore, tools and implementation strategies must be chosen not just by technical requirements but also by consideration of technical life cycles and support issues. Interactions with outside communities, such as other NIH centers funded under this program, as well as with the international community, are also essential. To succeed, an effort such as this must be built on a consistent set of biologically-motivated levels wherein the parameters and properties of each level are based on the results of lower levels. Consistency is critical in two directions: hierarchically within a given modeling environment and between different modeling efforts through use of agreed-upon standards.

The Center for Tissue Modeling will start where Indiana University's expertise is greatest, due to our success in implementing the Cellular Potts Model. We will then adopt, adapt and build at levels above and below. The biomedical research community needs, ultimately, the ability to choose among tools to select the most appropriate for a particular task. To maximize potential, it will be the CTM's practice to interface with existing modeling resources to the greatest extent possible.

Figure 1.1 sketches our multiscale view of systems biology. At scales above the Potts model, we will average into continuum models of organs. We will approach this goal using an integrated experimental and theoretical/computational approach. From computer science we require grid-based CyberInfrastructure, new parallelization algorithms, new ways of connecting multi-scale processes and seamless merging of levels of abstraction.

A mix of phenomenology, experimentation and computer science is essential for large-scale systems biology simulations. Biological simulations differ essentially in their scientific methodology and information technology challenges from those familiar in fields that can base models directly on "fundamental equations," e.g.,

molecular dynamics, computational fluid dynamics (CFD) and quantum chromodynamics (QCD). In complexity

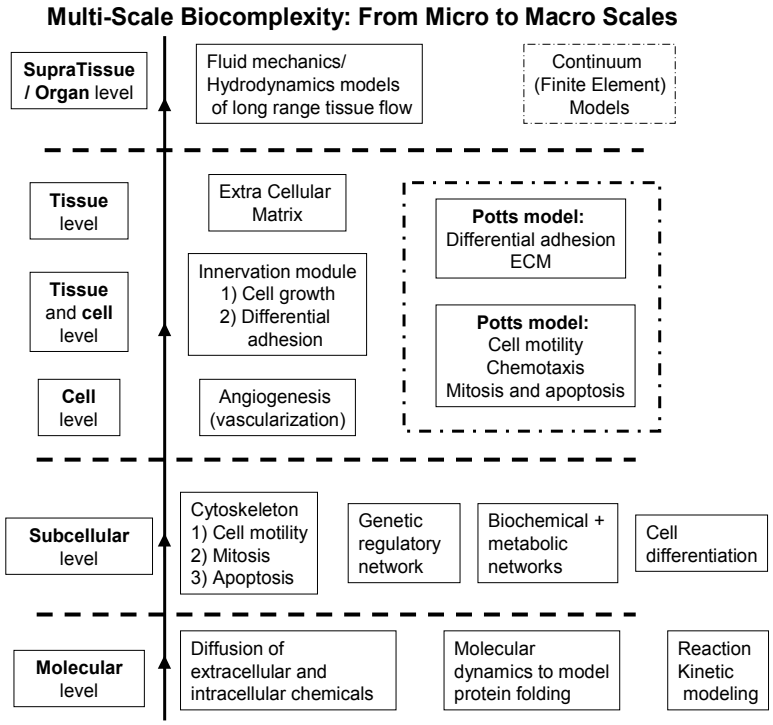


Figure 1.1: Scales of integrated biological modeling.

the equations simulated are not "fundamental"—they arise through a complex interplay between modelers, the simulations themselves and experimentalists using intuition to develop appropriate abstractions and approximations. Fields like CFD and QCD have great computational challenges, and the simulations involve difficult numerical procedures applied, however, to relatively fundamental equations derived by the classic interplay between theory and experiment. Other areas lack such accessible ways to systematically develop the abstractions to describe their complex behavior. In all areas multi-scale and linked models have proven a daunting challenge; most examples involve heroic efforts and limited success.

Systems biology has a natural set of abstractions—levels of hierarchy and components within levels—corresponding to biological structure. Furthermore, biological structures within one organism have arisen under the influence of natural selection and the need for components to act together assuring the survival and reproduction of the biological entity. Thus there is an overarching force (evolution) that forces all levels of the hierarchy within one organism to function as an integrated whole, suggesting that the functional interconnections between levels of hierarchy will prove tractable with hierarchical computer modeling approaches.

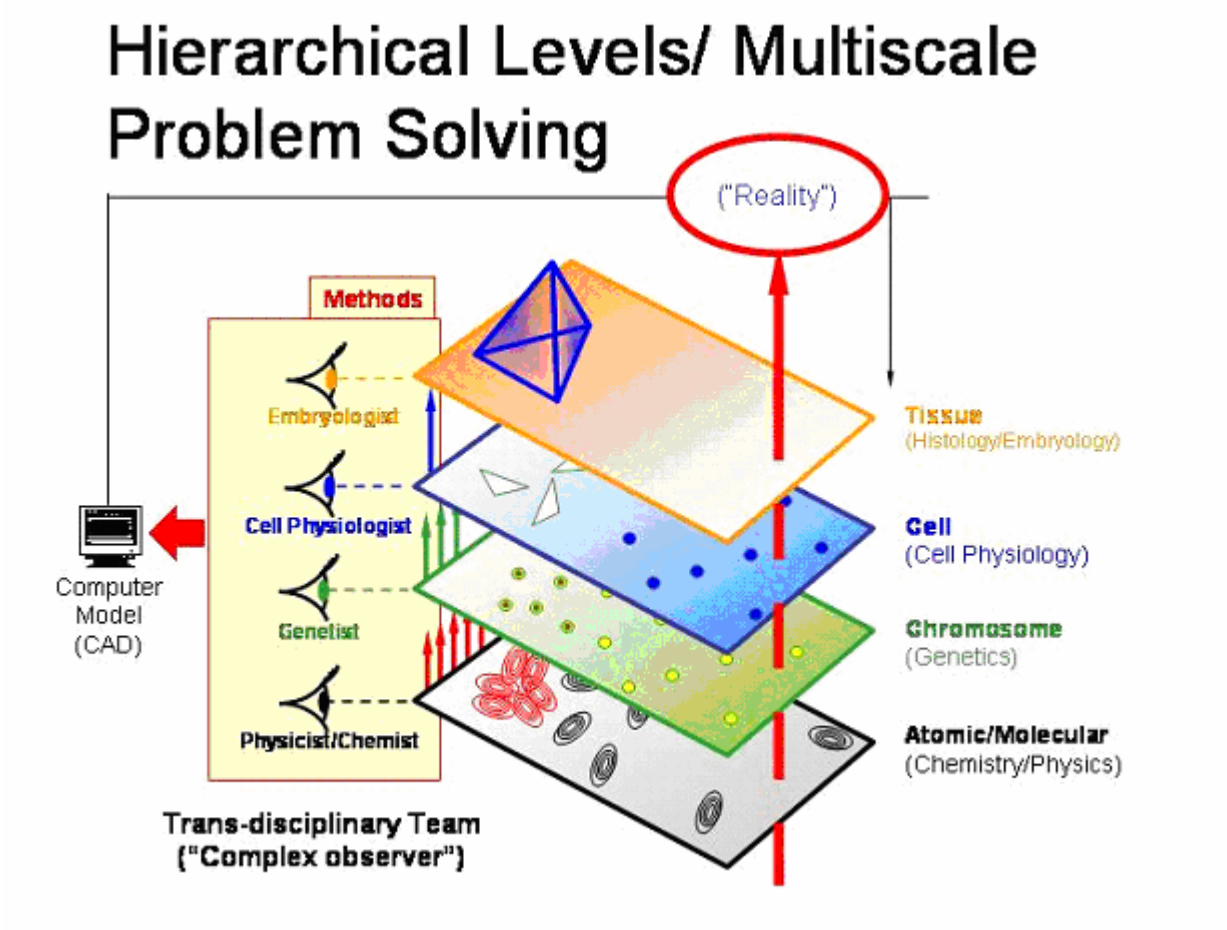


Figure 1.2 Hierarchical models and Multiscale problem solving diagrammed across multiple dimensions (size scale and methods)

Dr. James Glazier and collaborators have constructed a computational cellular automaton model to study biological pattern formation in morphogenesis. Based on this intuitive feature and their successes in simulations, we expect that systems biology simulations can be precise enough to lead to new insights and to

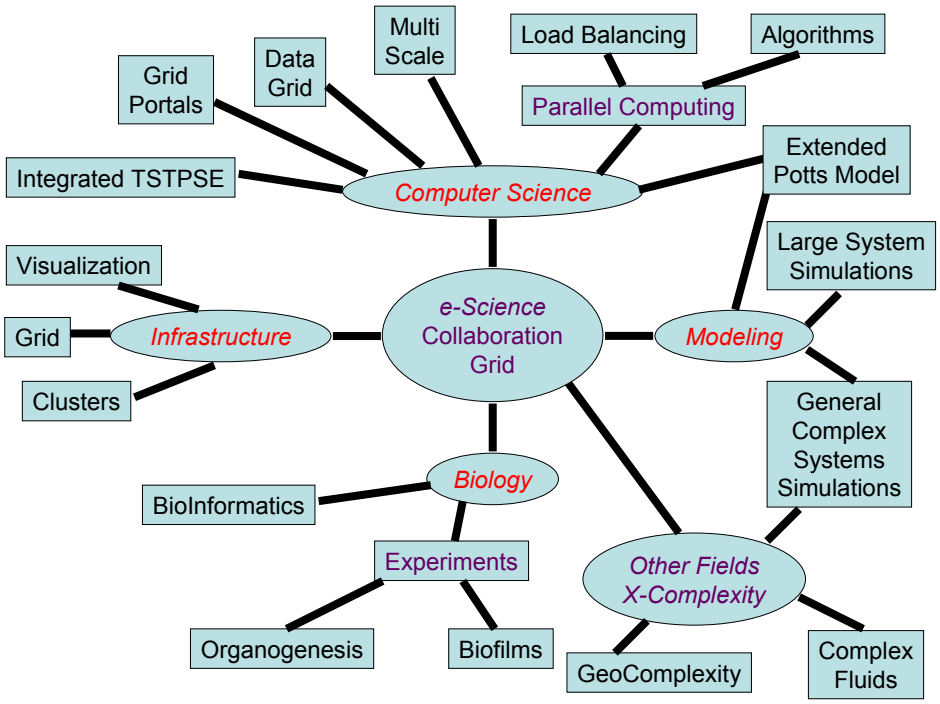


Fig. 1.3: Integration of computer science, modeling and experiment for complexity.

suggest new experiments. The CTM will develop approaches to systems biology as modeled in Figure 1.3. We will produce new ways to express and simulate multiple levels of abstraction and techniques to integrate the rich sources of data from different experiments and to cope efficiently with large-scale parallel decomposition of such heterogeneous dynamic problems.

In our algorithm work in core 1, for example, we will develop multi-scale algorithms building on traditional multigrid but able to describe scale-relevant physical effects like cell boundaries. Further, we will support Monte Carlo methods of the Potts model simulations that exemplify algorithms and computational approaches of general applicability to complex system simulation. We will package the tools from core 1—the Tissue Simulation Toolkit, or TST—as a scalable computational environment.

By “scalable” we mean scalable in both function and in requirements placed on the computing environment. A computer model will be of use to biomedical researchers only if it can become part of everyday research practice. Thus our intent is to provide tools that can be run on a modern laptop computer. Of course, more challenging problems may require a larger computer server or even a supercomputer. The largest, most thorough simulations will require the use of grids.

The methods used by a practicing biomedical researcher to access these tools may range from an open source add-on to a commonly used commercial mathematical software package, to a standards-compliant portal that allows access to one large server or a grid of supercomputers. By leveraging the relevant computer science standards for such access, we will assure that our work is of the greatest possible utility in the short run, and of the greatest possible viability and maintainability in the long run.

As we develop the Tissue Simulation Toolkit we will incorporate the advice of usability experts at Indiana University and focus on implementing suggestions and guidance provided by the biomedical researchers involved in the project. Since our goal is to create a tool that is widely usable by traditional biomedical researchers, core 1 will collaborate carefully with core 4 (which delivers training and support). We will pay particular attention to the suggestions and feedback of biomedical researchers who do not have extensive backgrounds in advanced computing technologies.

I.4 INCORPORATION OF EXISTING STANDARDS AND TOOLS:

Our efforts will be based on a philosophy of “evaluate, adopt, adapt and expand, and develop only where needed”, and can be thought of in terms of levels of function and perception operating within computing environments in a fashion similar to biological function and perception in the natural environment. A fundamental principle for the Center for Tissue Modeling will be to build new tools only where necessary. Standards are not useful for their own sake. Table 1 presents a listing of several markup languages currently used in biology and biomedical research. Table 2 shows a listing of existing modeling efforts used in biology.

SBML (Hucka *et al*., 2003) is the most widely used markup language for systems biology, and it focuses on biochemical pathways. It is itself based on XML (Bray *et al*., 1998) and created in compliance with UML (http://www.omg.org/uml). SBML Level 1 (Hucka *et al*., 2003) addresses non-spatial biochemical processes. Cell models of which we are aware are more likely to be consistent with SBML than any other biology markup language (cf. Table 2). Importantly, the developers of SBML are working with other key efforts, such as the CellML effort being developed by the Physiome Project (Hunter and Borg, 2003; http://www.physiome.org/). The importance of standardization with SBML is indicated by the creation of a tool for translating from KEGG pathway data to SBML-compliant files (http://www.sbml.org/kegg2sbml.html).

Table 1. Biomedically-oriented markup languages

Name	Web reference	Focus (quoted from each effort’s web site)	Version	Current status
SBML	www.sbml.org/	"models of biochemical reaction networks. SBML is applicable to metabolic networks, cell-signaling pathways, genomic regulatory networks, and many other areas in systems biology"	Level 2	Active
BioPAX	www.biopax.org/	"The goal of the BioPAX group is to develop a common exchange format for biological pathways data"	Level 1 0.5.1	Roadmap forward completed Dec 2003
CellML	www.cellml.org/	"The purpose of CellML is to store and exchange computer-based biological models"	1.0 - 1.1	Active
AnatML	www.physiome.org.nz/anatml/pages/	"AnatML … [is a] language for storing geometric information and documentation …"		No updates since 2000
FieldML	www.physiome.org.nz/fieldml/pages/	"FieldML is an XML-based language for describing time-varying and spatially-varying fields"	No full version yet	active but early
Proteomics Standards Initiative	http://psidev.sourceforge.net/	"The Proteomics Standards Initiative (PSI) aims to define community standards for data representation in proteomics to facilitate data	1.0	Active; publication of first full version in press

Name	Web reference	Focus (quoted from each effort's web site)	Version	Current status
		comparison, exchange and verification"		

Table 2. A summary of key biological modeling efforts throughout the world.

url	Focus & Function	Open Source?	Web accessible?	SBML support?
http://eminch.gmxhome.de/pathscout11/	Pathways	free; license terms not clear	no	Y
http://www.integrativebioinformatics.com/processdb.html	search bio databases	no	yes	developing
http://www.sbw-sbml.org/oldindex.html	Systems Biology Workbench (SBW) is a modular, broker-based, message-passing framework for simplified application intercommunications.	LGPL	no	Y
http://www.ucl.ac.uk/oncology/MicroCore/microcore.htm	pluggable suite of programs specifically designed for the analysis of microarray data.	free; license terms not clear	no	Y
http://icb.med.cornell.edu/crt/SigPath/index.xml	Information system designed to support quantitative studies on the signaling pathways and networks of the cell	no comment	yes	Y
http://info.anat.cam.ac.uk/groups/comp-cell/StochSim.html	general biochemical simulator in which each molecule or complex is represented	GPL	no	Y
http://www.sysbio.pl/stocks/	Stochastic simulations of biochemical	GPL	no	developing

url	Focus & Function	Open Source?	Web accessible?	SBML support?
	reaction networks			
http://teranode.com/products.htm	Teranode technology enables closed-loop discovery, development and production for life sciences.	Commercial	no	Y
http://sourceforge.net/projects/trelis	graphical Monte Carlo simulation tool for modeling the time evolution of chemical reaction systems involving small numbers of reactant molecules	GPL	no	Y
http://www.nrcam.uchc.edu/vcell_development/vcell_dev.html	models of reactions within spatial compartments	no comment	yes	Y
http://www.cds.caltech.edu/~hsauro/	Related to but simpler than Jarnac or Jdesigner	no comment	no	Y

Hunter and Borg (2003) lay out the multiple levels of biological hierarchy in a clear fashion, as is also done in Figure 1.4 (downloaded from www.physiome.org). The Physiome Project identifies a number of levels of hierarchy and the associated markup languages (MLs) that go with these levels of hierarchy within the plans for the Physiome Project: CellML (cell processes), TissueML (a markup language for describing tissues), AnatML (a markup language designed for describing organs – so far most advanced as regards heart models), and PhysioML (a markup language for describing physiological processes). The leaders of the Physiome project CellML development and the leaders of SBML have begun coordinating their work (Hucka *et al*., 2003).

The focus of our core simulation development lies in the range between two cells interacting and an entire tissue (i.e., 2 to ~10^6 cells).

An explicit part of our strategy in developing the tissue simulation toolkit is to focus on levels of biological organization not already well addressed in existing models at the intracellular to single-cell level (e.g., Virtual cell [http://www.nrcam.uchc.edu/index.html] or BioSpice [https://community.biospice.org/] and organ level [e.g., cardiac models associated with the Physiome project http://www.physiome.org/]).

Clearly it would be futile to create a markup language specifically for the range of biological phenomena on which our efforts will focus. (The creation of something called ABunchOfCellsML would not be a step forward). Rather, it is more important that we extend the capabilities of SBML and/or CellML so that biological phenomena at the level of 2 to 10^6 cells can be modeled effectively, and models at this scale can interact effectively with models at smaller and larger scales.

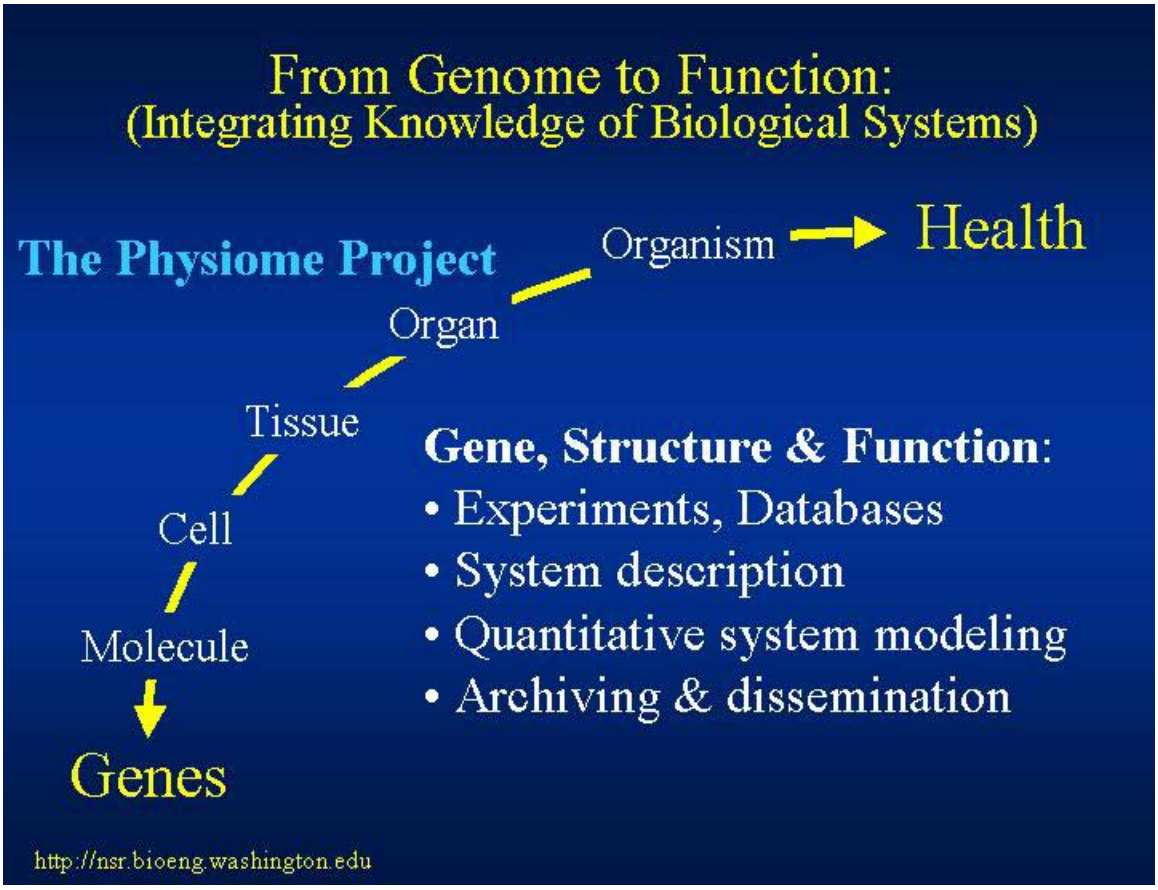


Figure 1.4 "From Genome to function." Focus of the Tissue Simulation Toolkit developed by the CTM will be from 2 to ~10^6 cells – the range from just above one cell to the tissue in the figure above.

The extension of existing standards such as SBML to handle the Cellular Potts Model requires development in two critical areas: the metadata and markup language itself, and solvers that will actually turn the model definition into an executable program.

Leveraging SBML as a standard systems biology markup language and leveraging other relevant standards in computer science will be beneficial in creating a tool suite that may be used on scales of computing from a laptop computer to a national grid of supercomputers such as the TeraGrid (www.teragrid.org) or BIRN. The next section introduces the Cellular Potts Model in some detail.

I. 5 THE CELLULAR POTTS MODEL:

Our overall goal is to develop an improved understanding of the multiscale phenomena of tissue morphogenesis and organogenesis using an integrated experimental and theoretical/computational approach. In this section we will describe the basic features of the Cellular Potts Model (CPM). After that, we will discuss the computer implementation of the CPM in more detail in core 1. Additional information on details of the CPM and its use with biologically motivating projects will be discussed in cores 2 and 3.

Movement of cells and tissues are of pre-eminent importance in understanding tissue formation during development. They are often described by the term morphogenesis. There are several types of morphogenetic movements that occur in many different circumstances during development. Examples include:

- Cellular aggregation to form clumps, e.g., in cartilage formation.

- Formation and maintenance of boundaries by differential adhesion, e.g., for different germ layers.
- Mesenchymal to epithelial transition to form shell sheets, e.g., formation of coelomic epithelium.
- Transformation between cell sheets, tubes and vesicles.
- Chemokinesis or chemotaxis of individual cells, e.g., in wound healing.

We think we know the molecular identity of most of the key components in the cytoskeleton, and have a qualitative view of growth and decay of microfilaments and operation of motor protein. But a proper understanding of morphogenetic movements obviously involves explanation at level of physical forces and their effects on cells and tissues. This is a difficult area as it involves both elasticity and viscosity. Mathematical modeling is the only means of providing a quantifiable framework for integrating such numerous processes across many temporal and spatial scales. It is clear that no single model can encompass a factor of 10^9 in spatial scale or temporal scale and a reasonable approach is therefore to develop models for a limited range and to link the parameters of this hierarchy of models. A key product of our investigation will be a flexible, general purpose simulation program based on the widely used and well-validated Cellular Potts Model (CPM), which can both reproduce quantitatively observed developmental phenomena and predict the results of genetic or biochemical intervention during development and adult morphogenetic processes.

I.5.i WHAT IS THE CPM?

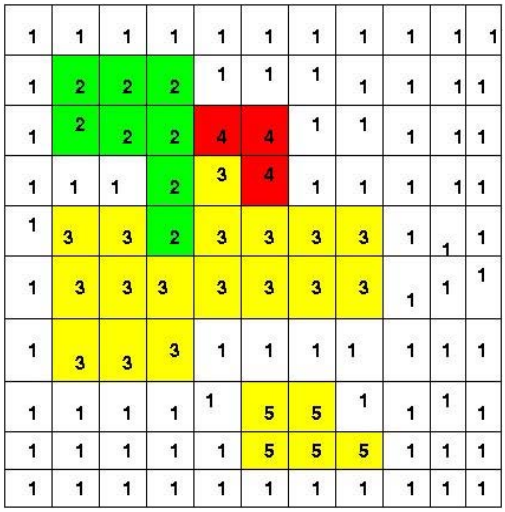


Figure 1.5 The figure shows a typical configuration of Potts model in 2D. The numerals indicate spin values. The colors indicate cell type. A cell is collection of simply connected lattice points with same spin value. The number of lattice points in a cell is its volume and number of lattice points on its boundary (interface with other spin value) is its surface area. By convention ECM is a cell with spin 1.

The Cellular Potts Model (CPM) is a classic example of stochastic cellular automata (CA) model. It is a generalized Ising model where each lattice site can be in one of the q different states (q > 2). A group of simply connected lattice points in the same state in it is referred as a cell. Hence each cell has definite volume and surface area (which can be mapped to real biological cells after simple normalization). CPM is an energy-based model wherein cellular interactions are phenomenologically modeled through cell-cell interaction as well as self energies like volume energy, surface energy, etc. An effective energy E and "fields," e.g., the local concentrations of diffusants, conveniently describe their interactions, motion, differentiation and division. The effective energy mixes true energies, like cell-cell adhesion, and terms that mimic energies, e.g., the response of a cell to a chemotactic gradient. Given an effective energy we can calculate the resulting cell motion, since differences in energy produce forces, F. Since cells in tissues move in an extremely viscous environment, the velocity of the center of mass of the cell, v, not acceleration, is proportional to force, with an effective cell mobility, m. Thus

$$\boldsymbol{v} = \boldsymbol{m} \nabla \boldsymbol{E} \qquad (1.1)$$

Equation (1.1) implies that cells move to minimize their total effective energy. In what follows we discuss briefly, the important energy contributions and fields we incorporate in our simulations (these are described in detail in core 2).

Cell Adhesion: We can describe the net interaction between two cell membranes by an effective cell-type dependent binding energy per unit area, $J_{\tau,\tau'}$, where τ is the type of the cell on either side of the link, which incorporates both specific (e.g., integrins, cadherins) and nonspecific interactions (e.g., elastic effects due to cell deformations. In the CPM the effective cell-cell interaction energy is:

$$\boldsymbol{E}_{\boldsymbol{adhesion}} = \sum_{(i,j)(k,l)\,neighbors} \boldsymbol{J}_{(\tau(\sigma(i,j)),\tau(\sigma(k,l)))}\left(1-\delta_{\sigma(\boldsymbol{i},\boldsymbol{j}),\sigma(\boldsymbol{k},\boldsymbol{l})}\right) \qquad (1.2)$$

where the Kroneker r δ, ($\delta(\sigma,\sigma')=0$ if $\sigma \neq \sigma'$ and $\delta(\sigma,\sigma')=1$ if $\sigma=\sigma'$), ensures that only surface sites between neighboring cells contribute to the cell adhesion energy.

I.5.ii CELL VOLUME, MITOSIS AND APOPTOSIS:

At any time *t*, a cell, of type, τ, has a volume $v(\sigma,t)$ and surface area $s(\sigma,t)$. Since cell volume can fluctuate, e.g., due to changes in osmotic pressure, we describe the cell volume in terms of an effective volume elasticity, $\lambda_{\boldsymbol{V}}$, and target volume $\boldsymbol{v}_{\boldsymbol{target}}(\sigma,t)$. We define a membrane elasticity $\lambda_{\boldsymbol{s}}$, and a target surface area $\boldsymbol{s}_{\boldsymbol{target}}(\sigma,t)$

$$\boldsymbol{E}_{\boldsymbol{vol}} = \lambda_{\boldsymbol{v}}\left(\boldsymbol{v}(\sigma,t) - \boldsymbol{v}_{\boldsymbol{target}}(\sigma,t)\right)^2 \qquad (1.3a)$$

$$E_{area} = \lambda_s\left(s(\sigma,t) - s_{target}(\sigma,t)\right)^2 \qquad (1.3b)$$

When a cell grows, both $v(\sigma,t)$ and $s(\sigma,t)$ increase, otherwise $\boldsymbol{v}_{\boldsymbol{target}}(\sigma,t)$ and $\boldsymbol{s}_{\boldsymbol{target}}(\sigma,t)$ are constant.

I.5.iii MEMBRANE FLUCTUATIONS:

How do cells move? In mixtures of liquid droplets, the thermal fluctuations of the droplet surfaces cause diffusion (Brownian motion) leading to energy minimization. Cytoskeletally driven cell membrane ruffling of a few micrometers (μm) has no need to be thermal and cell and tissue dynamics might depend sensitively on details of the fluctuations spectrum. The simplest assumption is that an effective temperature, T, drives cell membrane fluctuations. We can describe these fluctuations statistically using Monte-Carlo Boltzmann dynamics (Metropolis *et al.*, 1953), where T defines the size of the typical fluctuation. If a proposed change in configuration produces a change in effective energy, ΔE, we accept it with probability:

$$\boldsymbol{P}(\Delta E) = \begin{matrix} 1, \Delta E \leq 0 \\ e^{-\Delta E/kT}, \Delta E > 0 \end{matrix} \qquad (1.4)$$

where k is a constant converting T into units of energy.

I.5.iv CHEMOTAXIS:

Chemotaxis requires additional fields to describe the local concentrations of the molecules diffusing in extracellular space. Let the molecule's diffusion constant be *d*, its decay rate □, and let it be secreted or absorbed at the surface of cells in a history dependent way , s_c , x,t . To model a specific form for s_c requires experimental measurement of the concentrations of diffusing morphogens in the tissue. The equation for the field then is

$$\frac{\partial C(x)}{\partial t} = d\nabla^2 C(\vec{x}) - \Gamma C(\vec{x}) + \sum_{\sigma} s_C(\sigma, \vec{x}, t). \qquad (1.5)$$

The cell executes a biased random walk, which averages to directed motion in the direction of the gradient. Instead of solving the chemotactic diffusion equation, we approximate the Laplacian by an averaging process and the diffusion is achieved self consistently within the framework of the Potts model. Besides diffusion we also achieve advection using this framework (discussed in detail in core 2).

I.5.v WHY CPM?

CPM removes the drawbacks of molecular dynamics simulation on the one hand, where molecular level information has to be provided thereby limiting the size of the simulation, and on the other hand it captures local quantities/fluctuations, which are absent in the mean field models. The contribution of each energy term can be systematically studied and experimentally verified. Furthermore, unlike continuum models where *ad hoc* cell-cell interactions are difficult to justify, CPM compensates for the lack of detail knowledge by a reasonable set of logic-based rules, instead of cooking up some effective force for dynamical equations. Moreover, because of the high speed of simulations of CPM, a wide range of possibilities can be explored which would be impossible with more traditional methods based on differential equations

I.5.vi A NEW CELLULAR POTTS MODEL (CPM) CODE:

I.5.vi(1) Potts Model Simulation Environment

The current Potts model simulation environment (Figure 1.6) consists of a modular C++ software package and a visualization system. The software implements a Metropolis-based Monte Carlo algorithm and supports modeling biological cells on an *nxnxn* lattice and simulating the chemical concentration gradients necessary for chemotaxis. The visualization system consists of OpenGL (http://www.opengl.org/) and VTK-based (http://public.kitware.com/VTK/) applications that generate 3D representations of the lattice and provide basic animation capabilities.

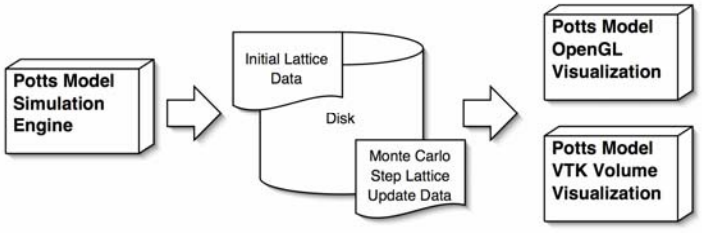


Figure 1.6: The Potts Model Simulation Environment

I.5.vi. (2) POTTS MODEL SIMULATION ENGINE:

The Potts model simulation is implemented as a modular system, with main controlling engine that calls out to different modules. Figure 1.6 shows the modular layout of the code.

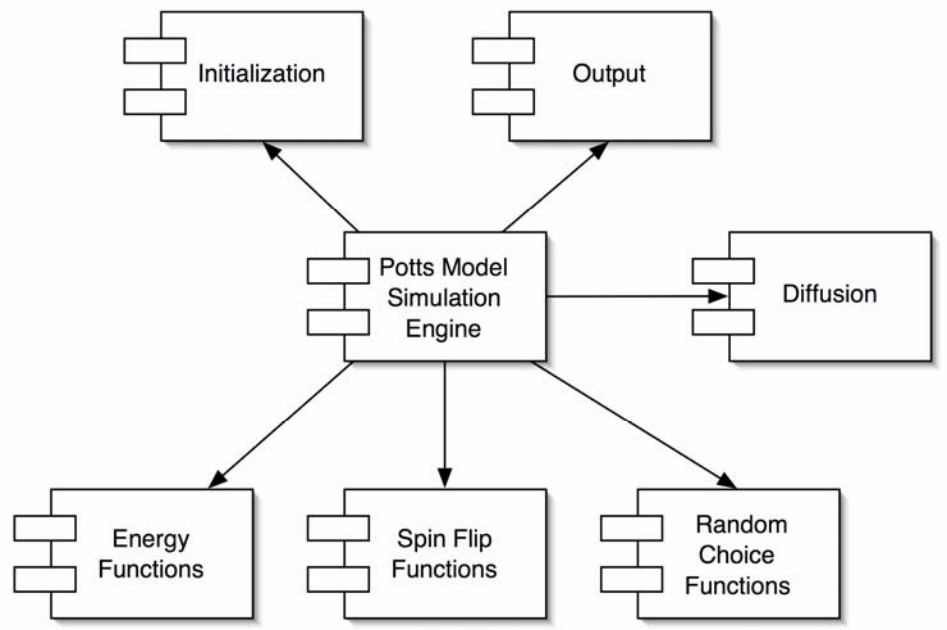


Figure 1.7: Potts Model Simulation Modules (UML Component Diagram)

Each module is implemented as one or more C++ files. The choice of modules is a compile-time decision controlled in the makefile. Selecting the functions at compile-time rather using a run-time linking system allows the code to maintain a higher level of performance while still enabling modularity. Because the code is currently small and used by individual researchers, this burden of managing modules at compile-time is low. As the environment expands and the simulation includes a parallel version, other approaches need to be considered to maintain performance and modularity without impacting usability.

The simulation engine contains the main Monte Carlo loop and calls out the different modules to perform the actual operations on the matrix. The Pseudo-code for the simulation engine is in Listing 1, below.

```
1:  # Initialize the simulation
2:  RandomSeed()
3:  InitializeLattice()
4:  OutputLattice()
5:
6:  # Monte Carlo Loop
7:  For n Monte Carlo steps:
8:    CalculateDiffusion()
9:
10:   # Select a point and compute its energy change
11:   point = ChooseRandomPoint()
12:   delta_E = ComputeEnergyChange(point)
13:
```

```
14:  # Metropolis Step (determine prob(flip))
15:  if delta_E <= 0.0:
16:    flipProb = 1.0
17:  else:
18:    flipProb = exp(-beta * delta_E)
19:
20:  FlipPoint(point, flipProb)
21:
22:  OutputUpdate(point)
23:
24: # End of Monte Carlo Loop
```

Listing 1: Potts Model Simulation Pseudo-code

The engine calls out to the *Initialization* module to setup the lattice (line 3). The Initialization module creates the biological cells and initializes the chemical concentrations in the extra-cellular material (ECM). Different Initialization modules can be used depending on the experiment being run. For instance, one module may simply create a random distribution of cells whereas another may setup the lattice to precisely emulate the initial conditions from *in-vivo* experimental data.

Before entering the main loop, the engine uses the *Output* module to output a complete copy of the lattice (line 4). The simulation results are saved in this initial full lattice file and another file that saves the results of each lattice update (line 22). This makes it possible to view the lattice at every Monte Carlo step without using an excessive amount of storage space.

The Monte Carlo loop (lines 6-24) drives the execution of the simulation using functions defined in the *Energy*, *Spin Flip*, and *Random Choice* modules. First, a random lattice point is selected as a candidate for spin flipping (line 11). The Random Choice module performs the actual point selection. Different implementations of this module can impose extra restrictions on the selection of the lattice point. For instance, it can ignore all points that are internal to a cell or the ECM or only allow updates on cells of a certain type.

Next, the Energy module computes the change in energy that would occur if this point were to flip its spin (line 12). The Hamiltonians are implemented in this module. Different modules here allow for experimentation with different energy functions and different combinations of the coefficients for these functions. For instance, one version may exclude the contribution of surface area and place a stronger emphasis on volume.

Based on the results of the energy functions, the Metropolis algorithm is applied to determine the probability of a spin flip occurring at the selected point (lines 14-18). No calls to external modules are made here.

With the probability computed, the Spin Flip module is called to perform the actual flip (line 20). This module computes a random number and performs the flip if probability is less than this random number. Different Spin Flip modules perform different types of updates on the lattice. A simple module will simply change the spin value for the point. More complex ones also enable tracking of parameters for the cells such as area and volume.

Finally, the results of the update are saved to the update file (line 22).

I.5.vii Basic CPM Visualization System:

The Basic CPM consists of two main 3D visualization tools: a fast OpenGL based viewer for quick analysis and VTK-based volume visualization tool for more detailed analysis.

I.5.vii(1) OpenGL Visualization:

The OpenGL visualization tool (Figure 1.8) reads in the lattice data for the biological cells and the update information. Using this, it renders an animation of the biological cells as the simulation evolves. Because ECM and chemical concentration data is not displayed, only a small amount of processing is needed and results can be viewed quickly.

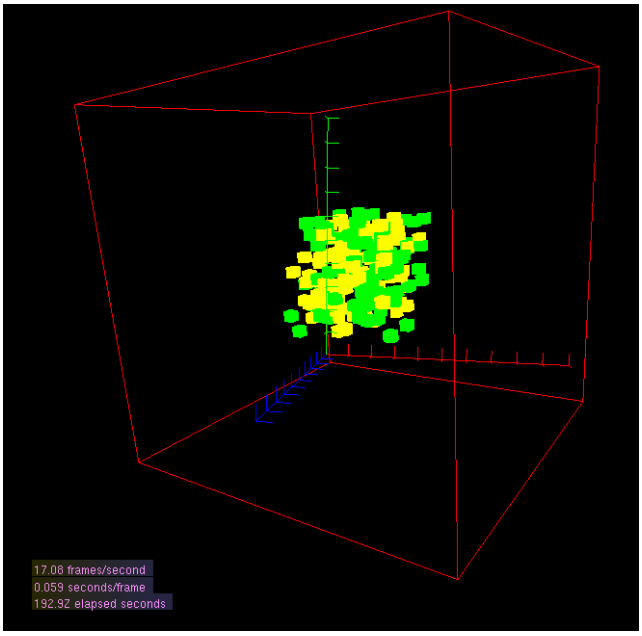


Figure 1.8: Potts OpenGL Visualization Tool

I.5.vii(2) VTK Volume Visualization:

The Potts VTK volume visualization tool allows for more detailed visual analysis of the simulation data but requires more processing to generate images. Like the OpenGL tool, the VTK tool displays the biological cells. Additionally, it uses volume visualization and iso-contouring techniques (Figures 1.9 and 1.10) to enable exploration of the ECM and chemical concentration gradients.

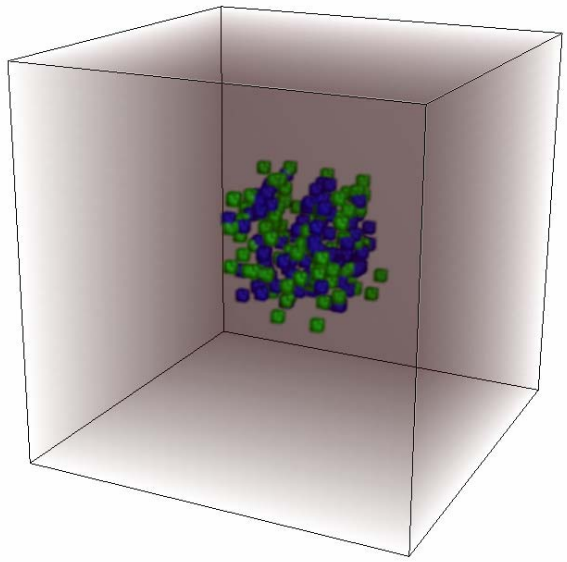


Figure1.9: Potts Volume Visualization with chemical concentration displayed as a density cloud

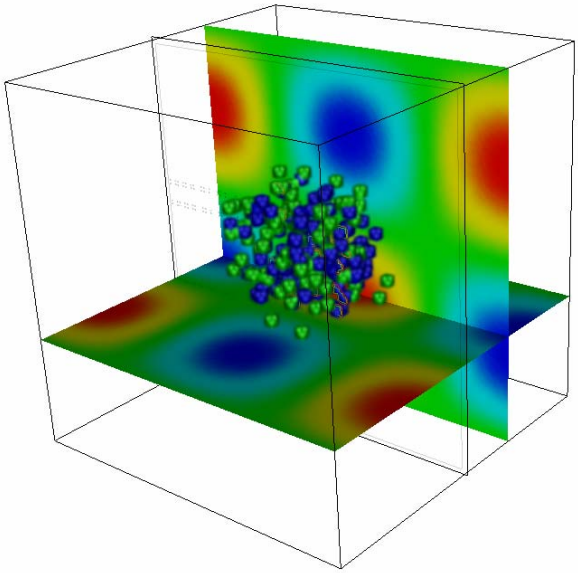


Figure 1.10: Potts Volume Visualization with chemical concentrations sampled as cutting planes and iso-contours

I.5.viii DESKTOP VERSION OF NEW CPM CODE

Our intent is to create a Tissue Simulation Toolkit, with the CPM code described above as its core that is scalable in several ways. That the model must be scale across orders of magnitude and levels of hierarchy of biology goes without saying. To be a successful tool for transforming biomedical research, however, it is also necessary that the Tissue Simulation Toolkit scale across several orders of magnitude of power of computing systems. This scalability should range from a laptop computer being used by a biomedical researcher in a lab or during idle time on a plane to a massive grid of the US's largest supercomputers. Furthermore, the laptop or desktop version of the software must seem manageable to the traditional biomedical researcher, and thus should leverage the robustness and graphical capabilities of widely available mathematical software.

Mathematica(TM) (http://www.wolfram.com/) is a widely used mathematical software package, and our initial efforts to create a desktop environment will focus on building a Tissue Simulation Toolkit that may be used in conjunction with Mathematica(TM).

There already exists a SBML module for Mathematica(TM), called MathSBML (http://www.sbml.org/mathsbml.html). Mathematica(TM) is itself a commercial software package, but it is one that is widely used, well supported, and as likely to be persistent into the future as any commercial mathematical software package. It is sufficiently inexpensive that it does not seem especially burdensome to presume that researchers modeling biological phenomena on a desktop or laptop computer should buy a copy of Mathematica(TM) in addition to the computer itself, particularly given the high quality of the underlying mathematical routines and graphical facilities available. MathSBML is open source, and the applications programming interface (API) for Mathematica(TM) is available.

Our work in creating a desktop version of the Tissue Simulation Toolkit will focus then on two areas: creating a way to express input to the CPM code as an ABML model (which necessitates extending SBML, as already discussed), and linking the C code described above to Mathematica(TM) through the Applications Program Interface for Mathematica(TM) .

This means that other researchers will be free, if they wish, to extend anything we create to interoperate with other commercial or open source mathematical packages depending on needs and preferences. This approach also leverages the graphical capabilities and robustness of Mathematica(TM) while assuring that the future of our development work is not tied inextricably to the fortunes of Wolfram Research Inc., the makers of Mathematica.(TM)

I.5.ix SCALING UP – PARALLELIZATION OF THE NEW CPM CODE

The Potts model simulation environment as currently implemented is a single-threaded application that readily handles 100-cube (1000,000 point) lattices. This has proven effective for developing the Hamiltonians for basic cellular interactions and exploring techniques for implementing chemotaxis directly on the Potts lattice. However, as the simulations grow to include large-scale cellular systems, the lattice size will also grow, reaching the point where a single processor is no longer sufficient. Thus, it will be necessary to extend the current model to effectively scale beyond one processor. Our goal is to allow scientists to routinely use 10,000-cube (10^{12} point) lattices for the tissue and organ simulations.

In addition to handling larger lattice sizes, another important motivation for the parallel implementation of the Potts model is simply the increased power desktop users have available. It is not uncommon for a desktop system to have two processors and an individual researcher to have immediate access to a small cluster. As hardware becomes even cheaper, it is expected that this will eventually be the rule rather than the exception. It is essential that the tools scientists are using take full advantage of their desktop environment.

The main challenge in parallelizing traditional Monte Carlo systems is maintaining statistical randomness across all nodes in the parallel implementation. For shared memory systems, this can be solved using a global random number generator. However, with the higher dependency on cluster-based supercomputers and, more recently, grid-based ones, the problem becomes more difficult. A common approach for small lattices has every node run the same random number generator but only update its lattice when the random point falls in their range. As the lattice grows, the point selection becomes the bottleneck as most random points fall outside the current node's range. This makes it difficult to scale a single simulation across many nodes. To take advantage of large clusters, many independent small lattice simulations are run in parallel, with the results averaged. This does not adequately address the need to run on larger lattices in a reasonable time frame. A component of the parallelization research will focus on this.

Conventional domain decomposition is the natural basis for parallel implementations of most complex systems simulations, including the ones used in this project. We must solve three problems – load balancing; ensuring good synchronization between nodes of the MPP; and minimizing communication costs. Synchronization is difficult in dynamic Monte Carlo Metropolis algorithms like the Cellular Potts Model. We cannot update in

parallel spins that interact directly with each other. However, as the spins have essentially local interactions, we have in other cases produced efficient algorithms as in the irregular melting problem studied in sec. 14.2 of Fox *et al.,* 1994. Efficient legal update orders then lead to the third problem – arranging for blocked communication to minimize effects of latency on fine-grain message traffic. Here tools like CHAOS [Chaos] have pioneered efficient approaches and we will adopt this or a similar approach for our parallel runtime tool.

The Potts model implementation will be a main component of the multi-scale framework of the Tissue Simulation Toolkit. While traditional, self-contained multi-scale techniques such as multigrid (http://www.multigrid.com/) can be parallelized in a reasonably straightforward manner, the multi-scale techniques that will eventually drive the toolkit will ultimately integrate disparate applications running at different speeds on different systems. Maintaining a high level of performance and parallelism across this environment will be a challenge that will require a significant amount of effort. An adaptive model for program design, similar to that employed by Dongerra and coworkers in optimizing the mathematical library LAPACK (Chen *et al.*, 2004) is clearly necessary. That is, we must build a tool within the software that will interrogate its operating environment and determine what sort of facilities are available for computation. The software must then be sufficiently sophisticated to employ the appropriate versions of the components that make up the software to deliver an optimal mix of accuracy and performance for the software user (and at the same time report clearly to the user exactly what is being done).

Creating an implementation of the Cellular Potts Model that will scale from a laptop to a single supercomputer involves the capability to change the scale and detail used in the models, and a certain suite of software engineering challenges.

However, scaling code up to run on many processors on one large supercomputer is not the end of the scalability issue. First, no matter how large one supercomputer is, many supercomputers linked together through a computational grid can run larger suites of calculations than one computer. Second, there is a natural similarity between a linked hierarchical system of computational models of biological processes and computational grids. The architecturally natural solution to the challenge presented by unprecedented and massive computations in the form of hierarchical linked models is to solve each model on one (or more) computing systems, each linked together by a high-speed network. Such assemblages of massive computing systems linked together through high speed networks are referred to as computational grids. Grids will play an important role as one of the platforms the system will execute on and draw components from. grid computing and the Tissue Simulation Toolkit are discussed in detail in the following section.

I.6 OVERVIEW OF THE GRID APPROACH:

Grids, e-Science and CyberInfrastructure are linked concepts driving hundreds of projects in many continents and countries. Furthermore, they are creating major commercial interest from all the leading vendors, including HP, IBM, Microsoft, Oracle and Sun. As with many emerging ideas, there is no clear agreement as to what is a grid, and even less agreement as to its appropriate technology.

A reasonable working definition is that a grid is a suite of computers – typically large computers – linked together by high-speed networks to create an integrated system. Foster and Kesselman (1999) described the idea of a computing grid as follows:

“The *grid* is an emerging infrastructure that will fundamentally change the way we think about – and use – computing. The grid will connect multiple regional and national *computational grids* to create a universal source of computing power. The word ‘grid’ is chosen by analogy with the electric power grid, which provides pervasive access to power and, like the computer and a small number of other advances, has had a dramatic impact on human capabilities and society. We believe that by providing pervasive, dependable, consistent, and inexpensive access to advanced computational capabilities, databases, sensors, and people, computational grids will have a similar transforming effect, allowing new classes of applications to emerge.”

The prediction made by Foster and Kesselman has certainly been well borne out by computer science research thus far. The grid has proven a lightning rod around which a considerable amount of computer

science research has focused. The idea of computational grids has also been expanded to incorporate concepts of data grids, collaborative grids, and most recently Cyberinfrastructure. “Cyberinfrastructure” is a term developed by the National Science Foundation to refer to a grid of supercomputers, massive data storage systems, and advanced digital instruments linked together by high speed networks. The two premier examples of the cyberinfrastructure concept within the US are the NIH-funded BIRN project (http://www.nbirn.net) and the NSF-funded TeraGrid (http://www.teragrid.org/). Indiana and Purdue Universities are already partners in the TeraGrid initiative, and Indiana University manages the Abilene Network, a key component of the network linking the TeraGrid together. The TeraGrid is comprised of computing centers that are part of the National Computational Science Alliance (NCSA) (http://www.ncsa.uiuc.edu/) and the National Partnership for Advanced Computing Infrastructure (NPACI) (http://www.npaci.edu/). The current structure of the TeraGrid is depicted in Figure 1.11

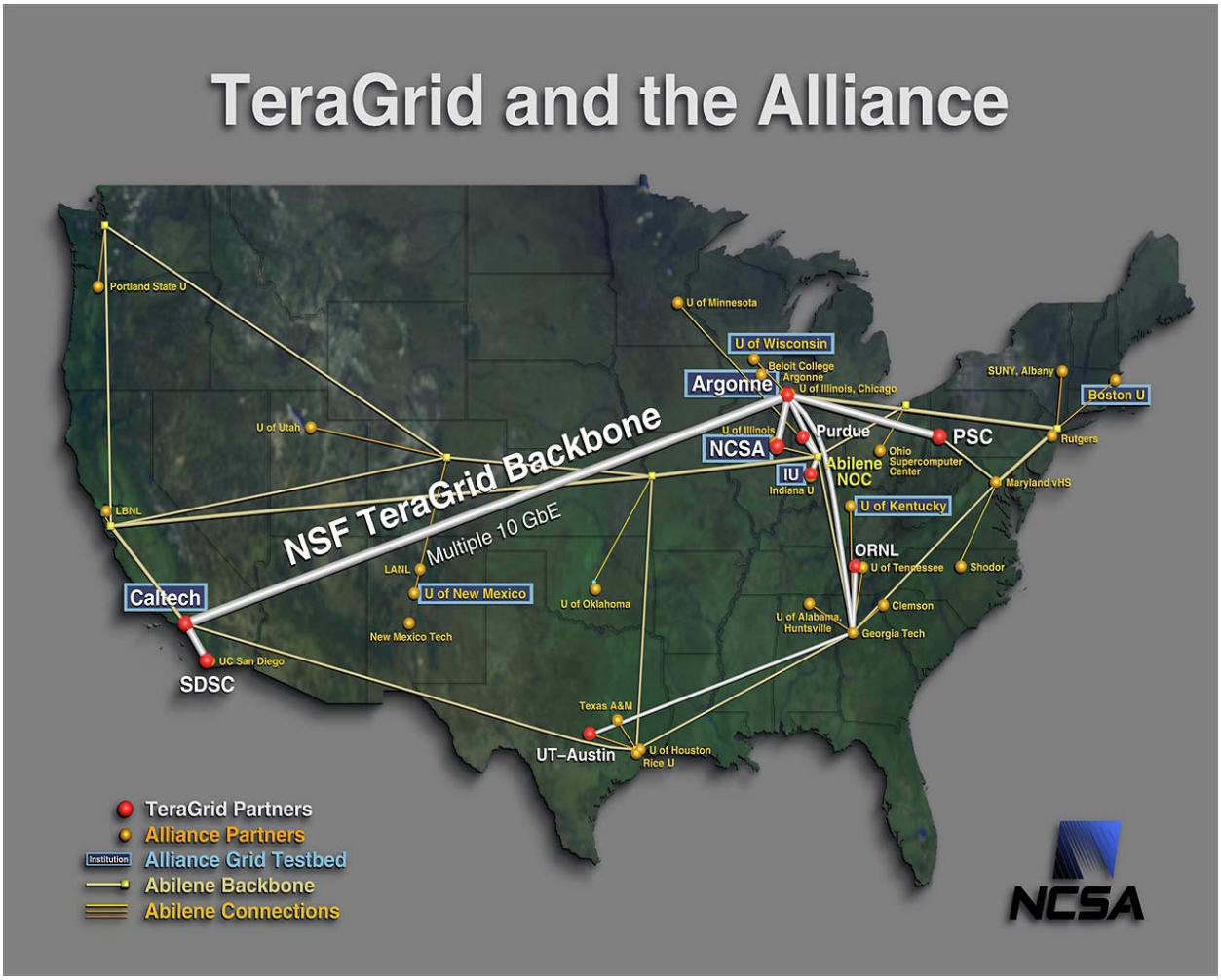


Figure 1.11. The TeraGrid – one example of a national Cyberinfrastructure in the US (from http://www.teragrid.org/img/tg_map_1280.jpg)

Lastly, e-Science refers “… to the large scale science that will increasingly be carried out through distributed global collaborations enabled by the Internet. Typically, a feature of such collaborative scientific enterprises is that they will require access to very large data collections, very large scale computing resources and high performance visualization back to the individual user scientists” (http://www.nesc.ac.uk/nesc/define.html).

Pioneering biology Grids include BIRN (http://www.nbirn.net) in the USA and MyGrid and DiscoveryNet in the UK (http://mygrid.man.ac.uk/, http://www.gridoutreach.org.uk/docs/pilots/discovery.htm). Indiana University leads an award-winning demonstration tying together a global grid of computers including hundreds of

processors spanning six continents (Keller *et al.*, 2003). These probe different areas including data sharing, meta-data (Semantic Grid) and workflow. Although we will use all these capabilities, our tissue modeling problem emphasizes rather different features because of the need to support scalable high performance simulations with a larger "compute/data access" ratio than the best known biology Grids. That is, there will be a greater emphasis on advanced computations relative to the amount of raw data handled than is typical of the major ongoing biologically-oriented grid projects.

Grids can be characterized in many ways, one of which is to view them as instantiations of loose virtual organizations or collaborations. In this light grids support the international virtual research organization in e-Science or, in our case, its specialization to biology. Grids commonly link the different components of the international biology research community – people, data, computers and networks. For example, iVDGL (international Virtual Data Grid) is a grid project specific to the high-energy physics community (http://www.ivdgl.org/). BIRN is likewise a grid focused on biological imaging. We will create an extensible and open grid focused on Tissue Simulation – TSTGrid (Tissue Simulation Toolkit Grid).

I.6.i OVERVIEW OF GRID STANDARDS:

The success of this proposal does not depend on the details of grid technology. We have three groups participating, led by Fox, Lumsdaine, and Stewart, who are leaders in the grid community and its research. We will develop all tools to be grid-aware and deliver them in a fashion compatible with best practice at the time. We plan to structure our architecture so that we can easily adjust to and incorporate changes due to the vagaries of the development of grid architectures and preferred systems.

A critical strategy in being able to abstract and hide the details of grid technology will be compliance with the relevant existing and commonly used grid standards. There are three critical aspects to the relevant standards for grid computing: the OGSI model, the Web services model, and the Common Component Architecture model. These are described in some detail below:

Open Grid Services Infrastructure (OGSI) and Open Grid Services Architecture (OGSA). These standards define the basic communications, security, and interoperability standards supported by the Global Grid Forum (http://www.gridforum.org/). The Global Grid Forum is recognized internationally as the key standards setting body for basic grid infrastructure. Within the US the Globus Project (http://www.globus.org) is the key effort to create the basic components required to create and operate a grid. Indiana University has long been involved in use of Globus technology, and will provide the core infrastructure needed by the Center for Tissue Modeling.

Web Services describe an "…XML format for describing network services as a set of endpoints operating on messages containing either document-oriented or procedure-oriented information" (http://www.w3.org/TR/wsdl). Web Services are a standard managed by the W3C (World Wide Web Consortium), and this standard can be thought of as operating at a higher level in the hierarchy of computing grids than the OGSI/OGSA standards. Web Services make it easy for disparate computing systems to work together in loosely coupled ways, based on the exchange of information via XML. The natural affinities of this style of computing with the coupled hierarchical models discussed throughout this proposal are clear. Equally clear is the leverage created by an XML-based scheme for grid computing (Web Services) and XML-based schemes for describing biological models (e.g., SBML and CellML).

Common Component Architecture (CCA). The Common Component Architecture Form (http://www.cca-forum.org/) defines a set of standards that identify in considerable detail the way different program modules interoperate. The CCA is important for several reasons. First, compliance with the CCA standards turns programs into program modules that can be tied together in ways that suite the particular needs of different researchers. Furthermore use of the CCA standard permits incremental improvements to code. Suppose a computer scientist develops a new stochastic simulation routine that is better than previous efforts. Incorporating such a module into an application built on the CCA standard is straightforward. Furthermore, CCA is the key to interoperability with other important biomedical simulation codes, specifically SCIRun (Johnson *et al.*, 2002)

I.6.ii TSTGRID:

TSTGrid will be a specific implementation of existing standards mentioned above in a fashion that will permit the operation of the Tissue Simulation Toolkit on a grid, and its integration within a grid framework with other biological simulation codes. By using grid standards, both for biology metadata and more general standards like OGSA-DAI (http://www.ogsadai.org.uk/) for databases, we will maintain compatibility between these outside Grids and our TSTGrid. The general architecture of TSTGrid is shown in fig. 1.12 with system (computer, databases) and application services.

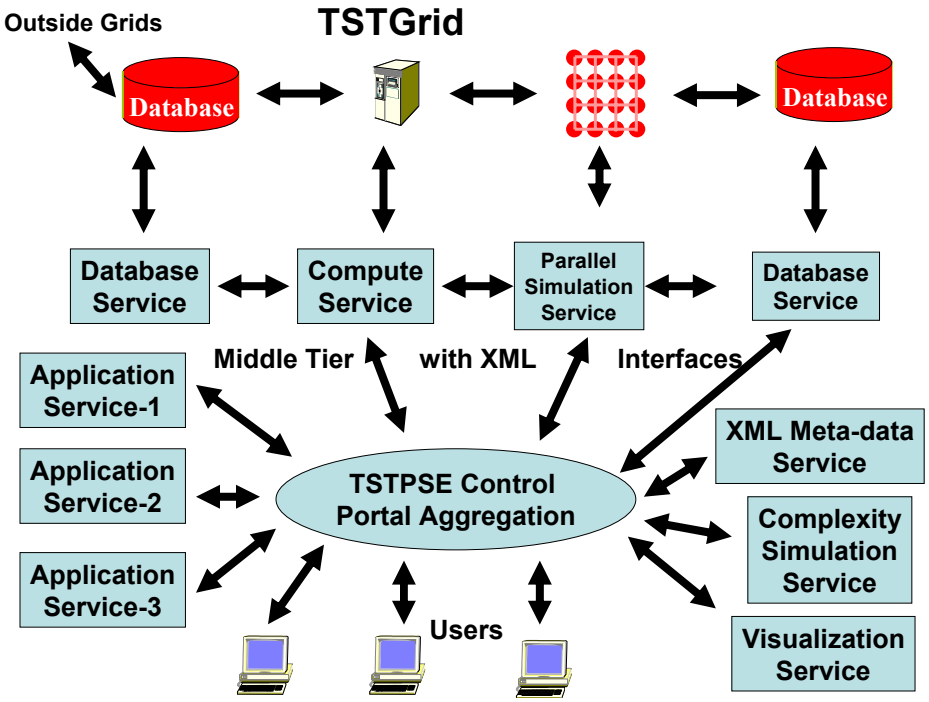


Fig. 1.12: Data and Compute resources, middleware and portal for the TSTGrid. Typical Application services are shown The Meta-data and Complexity Simulation Service are detailed in fig. 1.4

I.6.iii TSTGRID AND GRID RESOURCES:

We will adopt the model of the NSF TeraGrid (http://www.teragrid.org/) where grid technology links multiple parallel machines to data sources and researchers. In fact, the participation of Indiana University and Purdue University in the TeraGrid will create the supercomputing and data storage infrastructure on which to pilot test and operate TSTGrid. Indiana and Purdue University resources will be available through allocations processes operated by the TeraGrid and by the Center for Tissue Modeling itself.

The computational resources of the TSTGrid itself will include more than 3 TeraFLOPS of computational capability. (A TeraFLOPS is one trillion mathematical operations per second). One of the resources to be included in TSTGrid is the 1 TeraFLOPS IBM SP supercomputer operated by Indiana University. Purdue operates a one-half TeraFLOPS SP as well. Indiana recently installed a 2.2 Teraflop 408 node Linux cluster called AVIDD (Analysis and Visualization Instrument Driven Data). Resources like the NSF TeraGrid and those of other NIH centers will be accessed through TSTGrid by using standard grid service views of these resources. This is already available for many resources through our collaborations with the NCSA alliance, NASA JPL, NSF TeraGrid and Middleware Initiative NMI and DOE Science Grids (http://www.ncsa.uiuc.edu/, http://www.jpl.nasa.gov/, http://www.teragrid.org, http://doesciencegrid.org/). Internationally this can be

extended as needed through our numerous collaborations, especially the OMII (Open Middleware Infrastructure Institute) of the UK e-Science program (http://www.rcuk.ac.uk/escience/).

The TSTGrid will deploy complex middleware software that we will implement in Java with each module running on Linux or Solaris servers. We have a mix of PC Linux boxes with various configurations and new 8-processor large memory Sunfire servers to support this middleware. We will upgrade this infrastructure as needed.

I.6.iv GRID SOFTWARE INFRASTRUCTURE:

Grid technology is rapidly evolving (Berman *et al.*, 2003). Efforts like the VDT (Virtual Data Toolkit) from the Globus/Condor team (http://www.lsc-group.phys.uwm.edu/vdt/) have produced relatively robust base software. VDT needs significant enhancement to support the Web service OGSA (Open Grid Service Architecture), but we are confident that we will be able to deploy OGSA compliant software, with prototypes today and improving robustness and functionality by 2004-2005. We note that Globus Toolkit 3 (http://www-unix.globus.org/toolkit/) has been adopted successfully by some groups, but it is not easily used for "deployment" due to the software immaturity and expected changes in the underlying OGSI architecture. We collaborate with the UK e-Science program (http://www.escience-grid.org.uk/), which has similar grid requirements and several biology applications (http://www.bbsrc.ac.uk/escience/Welcome.html). This program has produced the OGSA-DAI Grid database interface [OGSA-DAI] and will commit major resources to ensure robust Data Grid software.

Many aspects of TSTGrid require active research for the foreseeable future, and our project will benefit from this research. However, a suitable approach is emerging for building the interfaces to grid environments – web based access mechanisms called grid portals (GCE, 2003; Fox *et al.*, 2002). DOE CHEF (http://www.chefproject.org/) and NSF NCSA PACI teams that include Indiana are adopting portlets and Apache Jetspeed technology [http://jakarta.apache.org/jetspeed/site/; Krishnan *et al.*, 2001; Balsoy *et al.*, 2002]. Like everything else, there is a standard for "portlets" (the individual components of a portal). We will adopt this software model and this standard for the TSTGrid portal and structure our user interface in the component fashion the portlet standard enables. Portals will provide seamless access to the different simulation back-ends used in our collaboration as well as allowing the insertion of software modules we develop. Our team leads the recently funded NSF NMI program to harden and deploy portlet-based grid services (http://www.nsf-middleware.org/). This NMI project involves the universities at Indiana, Michigan and Texas as well as NCSA and Argonne. The TSTPSE (Problem Solving Environment) corresponds to the portlet-based portal interfacing with a suite of grid services. We intend to encourage NIH to adopt this architecture for all their centers so external users can pick and choose the tools they want from both NIH and other sources and build portals customized for particular biological tasks.

We will develop the TSTGrid environment assuming that all remote data resources are OGSA–DAI compliant. We will develop new repositories to this standard and if necessary produce proxy wrappers to needed external repositories. Our chosen applications are rich in types of data but do not require high bandwidth access, so such wrapping should not produce unacceptable overheads. We describe our proposed research in interfacing simulation engines to the grid below.

1.6.v TSTGRID DATA GRID:

We will need to support data repositories for information both specific to the center and specific to the tools produced by the center. The center data includes the results of our experiments and data associated with our tools and simulations. The tool-associated data is exemplified by both defining meta-data and visualization data sets produced by the visualization tool. The simulation data is illustrated by the provenance meta-data defining the origin and history of simulation associated results. In addition to CTM data, we need to provide access to a richer and more voluminous set of data generated by other NIH centers and the worldwide community. We adopt a conventional two level meta-data plus file approach storing the experiment and project

meta-data in an XML-based data repository driving our entire environment. It will hold user, software, tool, biological parameter and “complexity scripts” as Fig. 1.4 depicts. We expect to implement this as a federation of distributed OGSA-DAI compliant Xindice XML databases (http://xml.apache.org/xindice/). This meta-data, using technology like Apache Slide (Goland *et al.*, 1999), will allow files for large volume data to be specified in the meta-data. The TSTGrid architecture may need to support a rich data assimilation model in out years of the project. The “data deluge” (http://eu-datagrid.web.cern.ch/eu-datagrid/) is not initially a major focus of this proposal, as we do not expect to need more than a few hundred data values to specify the initial Potts model simulations.

However, this will change in two ways. Increasing the sophistication of the CPM will require more parameters. Greatly many more parameters will be involved in simulations as soon as we begin to integrate simulations across multiple scales. We will design our data grid tools so they can be extended in this fashion, to adopt and be compatible with the bioinformatics data grid resources of other projects. We will need to have this capability from the start in order to access such databases. We will support it by using the OGSA-DAI standard and developing a tool that allows us to build proxies that map between native and OGSA-DAI formats. This will provide both the initial access-only mode and later creation of large databases.

I.7 TST PROBLEM SOLVING ENVIRONMENT:

The TST Problem Solving Environment is the basic computational core on which TSTGrid operates. This simulation tool will provide the usual job submittal service, but in addition we will allow it to control and specify further capabilities, such as load balancing for our parallel simulations. Our approach is that running high performance computing (HPC) applications and tools will use the TSTGrid middleware to manage their meta-data and control parameters leading to the simulation meta-data service implementation shown in Fig. 1.13. Internally to running simulations, we will provide a tool that implements one of the many load-balancing algorithms now available (see recent review in chapter 18 of Dongarra *et al.*, 2002). We will give this tool a portal interface allowing experimentation with different approaches controlled by parameters managed by the TSTGrid Meta-data service. As the project progresses, we will build or adopt existing appropriate run-time algorithms and the needed middleware support to support the parallel and multi-scale algorithms developed in our project. All parallel simulations will assume that parallel programs operate using the Message Passing Interface (MPI, http://www-unix.mcs.anl.gov/mpi/), the predominate standard for parallel programming.

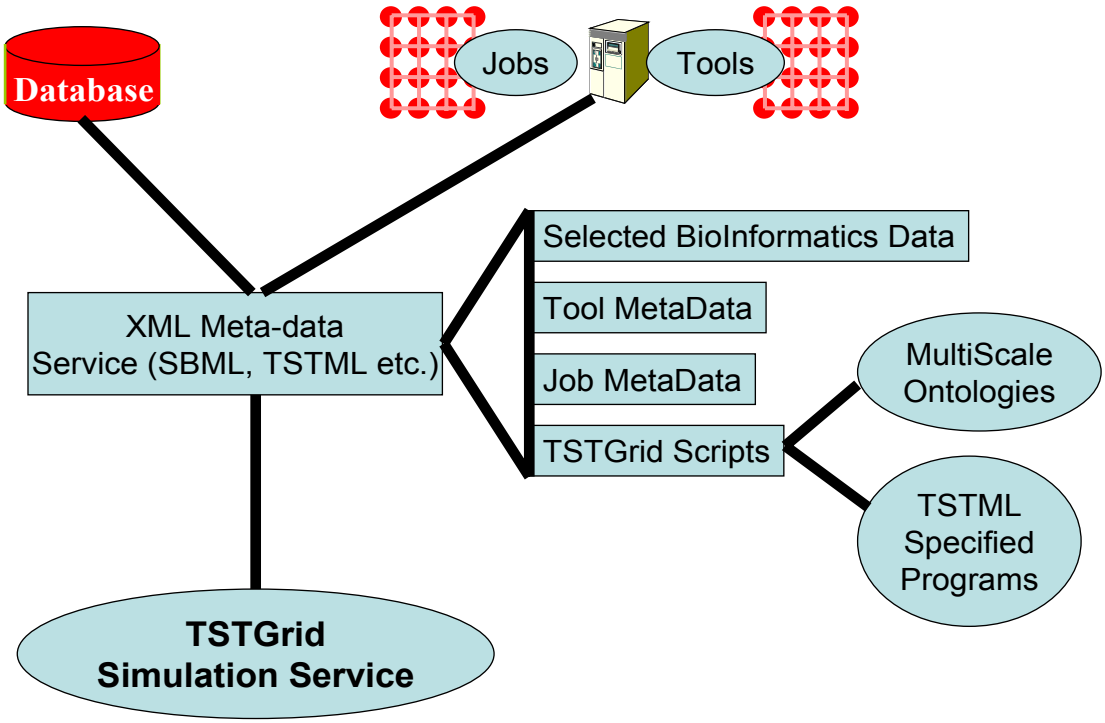


Fig. 1.13: Some details of the Complexity Simulation and XML Meta-data Services shown in Fig.1.12.

Conventional domain decomposition is the natural basis for parallel implementations of most complex systems simulations, including the ones used in this project. We must solve three problems – the load balancing discussed above; ensuring good synchronization between nodes of the MPP; and minimizing communication costs. Synchronization is difficult in dynamic Monte Carlo Metropolis algorithms like the Cellular Potts Model. We cannot update in parallel spins that interact directly with each other. However, as the spins have essentially local interactions, we have in other cases produced efficient algorithms as in the irregular melting problem studied in Fox *et al.* (1994). Efficient updates then lead to the third problem – arranging for blocked communication to minimize effects of latency on fine-grain message traffic. Here tools like CHAOS (http://www.cs.umd.edu/projects/hpsl/chaos/) have pioneered efficient approaches, and we will adopt this or a similar approach for our parallel runtime tool.

I.8 APPLICATION WEB SERVICES:

The bottom left of Fig. 1.12 indicates that we will implement all applications as Web Services. These services capture the meta-data of simulations in the same way that the parallel simulation service described above captures the load-balancing (and other parallel computing) meta-data. Our gateway portal has developed effective general approaches and our “wizards” (http://www.xmlnuggets.org/indexpage.html) will provide a general way of rapidly implementing simulation codes as Web services. This involves specifying needed metadata to define applications precisely. The schemas and ontologies for this will be taken where possible from those produced by Web, grid and biological communities. As mentioned above, we currently expect to adopt SBML as our base biology standard markup language, but we will also attempt to establish interoperability with CellML.

I.9 MULTI-SCALE MULTI-MODEL MULTI-ABSTRACTION TOOL FOR TSTPROBLEM SOLVING ENVIRONMENT:

Multiple levels of abstraction are present in all complex systems. We typically model them as heterogeneous hierarchical structures. As we move up the hierarchy, we introduce new abstractions and a process for deriving the parameters at the higher scale from those at the lower. We derive multi-scale models by various methods, which mix theory, experiment and phenomenology. In some physical systems, the renormalization group is a powerful, elegant approach; in chemistry, moving from shorter to longer length scales, the description changes from quantum physics to classical molecular dynamics. In many cases, collective modes abstract phenomena described at a lower level by particle based descriptions. In several fields, e.g., climate and earthquake science, we diagonalize largely observational correlations to find the dominant patterns or higher-level abstractions. Mathematical descriptions of differential equation and particle systems have derived hierarchical descriptions (multigrid or “fast multipole”), which are “just clever” averaging of the parameters at one level to derive automatically those at a higher level. These ideas have led to the fastest numerical solvers for partial differential equations and particle dynamics. Multigrid and fast multipole “automate” the choosing of the simulation scale. With some other methods, the modeler must choose the scale or abstraction “by hand.”

Biology and earth science are intrinsically more heterogeneous and complicated than most cases with which we have high performance computing experience. Nevertheless we expect similar strategies to be useful. Indeed the current successes in biomedical modeling come from studying the dynamics of coarse grain abstractions generated by the insight gained from experiment and theoretical studies. Earthquake science has had recent successes with pattern dynamics (Tiampo *et al.*, 2002). Sometimes the cross-scale linkage is not easy, as the difficulty of understanding protein folding from molecular dynamics and in linking particle dynamics simulations to friction models for earthquake fault slippage illustrates.

One critical idea for multi-scale support in the TST Problem Solving Environment is that the integration framework must have two key interfaces. These allow the modeler to specify both the linkage of the descriptions across scales as well the criteria to decide the level of representation. These mimic interfaces familiar with the multigrid method. Generalizing most of the cross-scale linkages discussed above should be straightforward. The TST Problem Solving Environment should support general linkages like multigrid, fast multipole, pattern dynamics and user defined. In each case the TST Problem Solving Environment must support the two cross-scale interfaces (relating parameters and scale/abstraction choice criteria).

The second key idea in a multi-scale approach is to base it on grid workflow technologies designed to support the coarse scale integration we need. We will use Web Services to support multi-scale abstractions, because the generality and power of this approach outweighs the performance loss: setting up transitions between scales divides into control logic, which is not computationally demanding and transforming (interpolating) simulation variables between scales which must run on a parallel machine. We discuss the fine scale support below. Correspondingly, we will invoke transformations and the actual simulations on the parallel processors from control logic running as a web service in the middle tier. This logic is the complexity simulation service in Figs. 1.11 and 1.12. The multi-scale support Web service tool is a novel idea that we will evaluate different workflow technologies for specifying and implementing the multi-scale abstractions and their relationships. As discussed in a recent workshop in Edinburgh (http://www.nesc.ac.uk/esi/events/303/) there are many approaches to workflow, or rather dataflow, as is often needed in scientific Grids. Three popular approaches to workflow are scripting (see e.g., Indiana’s HPSearch effort http://www.hpsearch.org/); XML-based workflow specifications, as in BPEL4WS (Andrews *et al.*, 2003) and WSCL (Banjeri *et al.*, 2002); and the fascinating agent/semantic web initiative (http://www.w3.org/2001/sw/). Initially we expect to follow the semantic web and use ontologies (http://www.w3.org/2001/sw/) to define each abstraction in frameworks like RDF (http://www.w3.org/RDF/) and OWL (http://www.w3.org/2001/sw/WebOnt/). Expressive abstractions and efficient implementation will require much research and experience. Fox is co-chair of the Grid Forum Semantic Grid research group and we will take advantage of the best tools in this area.

I.10 SBML AND TST:

We have defined the scope of the center to be simulations and models covering the cellular through macro-cellular realms (the latter also called the continuum region). We cover roughly the regime from two cells interacting to aggregations of a million cells. Thus core 1 explicitly includes work on tools interfacing with outside computational biology activities covering both continuum models and the property of cells in chemotaxis, reaction kinetics and genetic regulatory network areas. These are the functional interfaces we must support with outside groups. There are also architectural interfaces needed, for we must use Grids, parallel computing, multiscale, metadata and visualization for our multi-cellular simulations. However in all these areas we must choose approaches that are both best practice and compatible with tools using these features but for other computational purposes. Our TSTGrid must interoperate with that of other biology grids, like BIRN; our parallel simulations must allow coupling with other parallel simulations at lower and higher scales; we must not invent unnecessary new metadata but work with standards like CellML and SBML. At present we believe that our first effort will be to extend SBML to include the entities and interactions required to express Cellular Potts Models.

I.11 COMPATIBILITY WITH THE COMMON COMPONENT ARCHITECTURE:

A primary goal for the software component is compatibility with other development efforts, so that the cell, tissue and organ models might be readily combined. To this end, compliance with community-developed open software standards is essential. Of particular importance is the Common Component Architecture (http://www.cca-forum.org/), depicted in schematic form in Figure 1.14. The basic concept behind the Common Component Architecture (CCA) is that large application suites can be built by tying together program modules, as long as the modules accept each other's output as input, perhaps after some processing. More formally, CCA is a systematic way of encapsulating special functionality and behaviors of a piece of software into reusable units: a framework of standard rules of behavior for objects. CCA was recognized as one of the "Top Ten Science Achievements in 2002" by the DOE Office of Science.

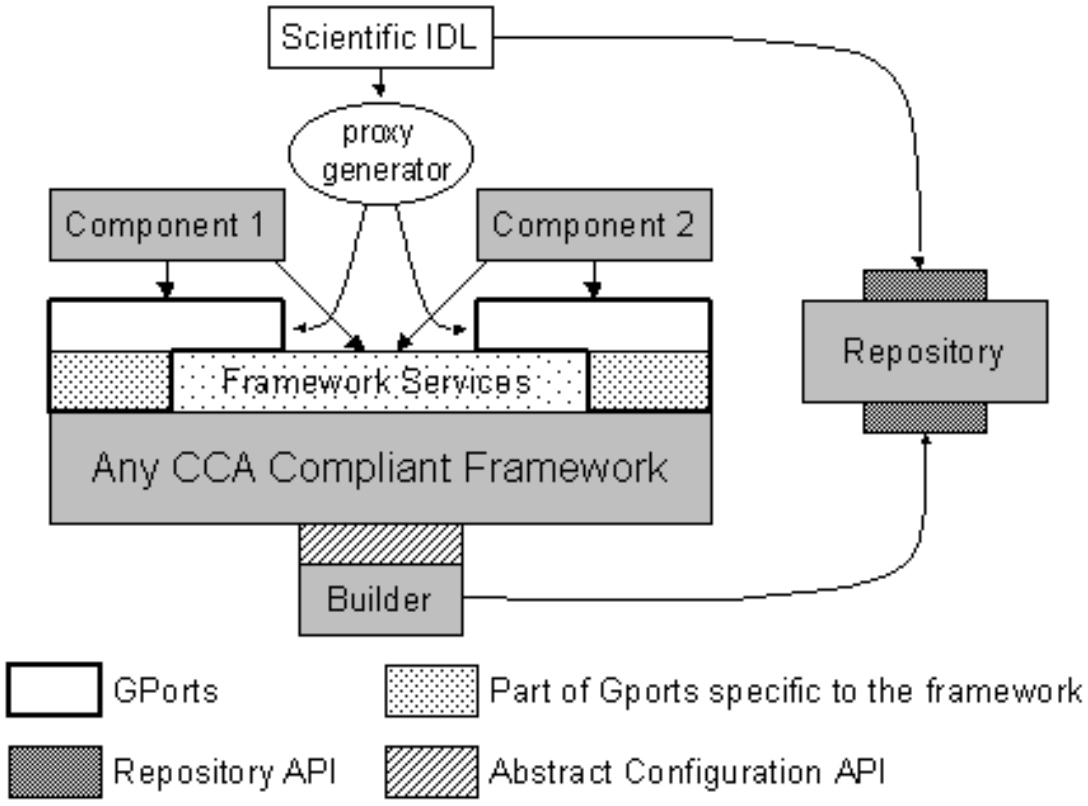


Figure 1.14. Basic Structure of the Common Component Architecture [From "Toward a Common Component Architecture for High-Performance Scientific Computing," by Rob Armstrong, Dennis Gannon, Al Geist, Katarzyna Keahey, Scott Kohn, Lois McInnes, Steve Parker, and Brent Smolinski, Proceedings of the 1999 Conference on High Performance Distributed Computing]

The problem of interoperating software components was faced in the desktop computing world when business users needed to connect word-processing, database, spreadsheet, and mailing software to create complete business applications. The highly successful solution was the use of component architectures: software is encapsulated into reusable components communicating via well-defined interfaces. Components differ from standard libraries or subroutines. They typically run in their own thread of control or process, composition of them consists of connecting binaries without a recompilation and linking phase, and they interact on a peer-to-peer basis, with no component designated as a master. Examples of component architectures include Microsoft's ActiveX/DCOM and Sun's JavaBeans and JavaStudio. They allow users to tie together disparate and sophisticated resources, running on networked machines, as easily as dragging and dropping boxes in a computer window.

Computational biology presents extraordinary challenges to the component approach, and the component architectures for desktop applications cannot be directly used for large-scale CS&E computing. Typical desktop components exchange data on the order of a few hundred bytes, while computational biology components need to exchange data on the order of hundreds of megabytes. A more fundamental problem is the nature of the data objects. The desktop world has a relatively small and homogeneous set of data objects to exchange. In contrast, the data objects in computational biology are of greatly differing types, including symbolic mathematical expressions, terabyte database queries and results, large geometry files, massive discretized field data, and data distributed across parallel supercomputers. The set of computational biology data objects is rapidly evolving and growing, requiring dynamic methods for adding components and interfaces. In spite of

the challenges, extending component architectures to the full range of computational biology endeavors will greatly facilitate the exchange of computer programs, data, and collaborative research.

Indiana University has a taken leadership role in designing component architectures. IU research currently targets component interface standards and mechanisms, including the division of tasks between components, their interfaces, framework managers most suitable for computational science, and hierarchical information systems which can present results from a component system at multiple levels of abstraction. The DOE ASCI project (http://www.itrd.gov/pubs/lue01/asci.html) in particular has prompted recognition of the necessity for CS&E component systems, and IU is active in the development of standards for the Common Component Architecture (CCA) Forum. Thus, compliance with CCA standards will be easy for the Center for Tissue Modeling to achieve.

Furthermore, IU has already produced successful problem solving environments in other areas, in particular the LSA (Linear System Analyzer), a problem-solving environment for large sparse systems of equations (http://www.extreme.indiana.edu/pseware/LSA/LSAhome.html). The LSA encapsulates several existing methods for manipulating and solving sparse systems, and allows users to rapidly compose these methods as components running on a distributed network of computers, to explore and compare solution strategies. A GUI written in Java provides both a visual interface and the control mechanism for the overall grid-based system. Thus, creating the Tissue Simulation Toolkit in a CCA-compliant form will be a straightforward exercise in adhering to standards that IU has helped establish and worked with effectively.

Use of the CCA standard is a critical component of our plans for compatibility with other efforts, such as SCIRun (http://www.sci.utah.edu/). SCIRun is a problem-solving environment (PSE) that allows the interactive construction, debugging, and steering of large-scale scientific computations.

I.12 SCALING FUNCTION FROM THE GENETIC REGULATORY NETWORK TO THE ORGANISM:

1.12.i COMPARISON OF CELL MODELS:

We will provide access to and use of computer models of cell function in a fashion consistent with the modus operandi of this proposal: evaluate, adopt, adapt and extend, develop. In this phase of work we will focus on the range from evaluation to adaptation and extension of models at biological scales above and below the range of tools that we will develop within the Center for Tissue Modeling.

Evaluate. At present there are few facilities available where one can run some simple models of biological processes via the web. There is no facility where a researcher can find and execute all – or even two – of the various models of biological processes within the same computational environment for purposes of comparison. A list of current biomedical computer models is provided in Table 1. Our first step in terms of execution of our proposal and in developing services for the community will be to download all available open source models in the domains of biological science we will consider (genetic regulatory networks to the organism), and install them on IU's 2 TFLOPS Linux cluster. This will permit our team to evaluate the performance of these applications in a single consistent environment. This will allow our research and development endeavors to be built upon best-of-breed models in all categories. We will also provide some sort of interface, via a Web portal whenever possible, so biomedical researchers can run these models in a comparative fashion using their own data. We plan to provide access to computational resources without limit to researchers involved in the proposed Center for Tissue Modeling, and provide resources on a “best effort” basis to the biomedical research community at large.

Adopt. Based on our evaluations, we will adopt one suite of tools – no more than one per level of biological organization or structure – and adopt them as the basis of our modeling efforts. Our initial explorations suggest that we will likely choose to link with Virtual Cell (http://www.nrcam.uchc.edu/index.html) at levels of detail below the Potts model, and SCIRun (http://www.sci.utah.edu/) and other tools being developed in conjunction with the SCIRun group (http://www.sci.utah.edu/) at scales larger than those handled by the Potts model. A

critical part of the adoption process will be creating a curated repository of model parameters and model output. This repository will be reliably stored in IU's massive data storage system. Input parameters and results will be stored using consistent nomenclature. GO (Gene Ontology Consortium, 2000) will be used to describe cell structures, genes, and biochemical pathways. A consistent method will be developed for describing the input parameters and the results.

Adapt and extend. Once we have identified best-of-breed models at scales other than those that have been identified, considerable effort will be invested in making tools interoperable. We will develop interfaces for existing models that are compliant with relevant standards. Table 2 is a list of biologically and biomedically-oriented markup languages. A scan of Table 1 suggests that SBML is the standard and most widely used markup language. Thus, we will aim for consistency with SBML first and foremost as the markup language standard. We will also strive for compatibility with CellML whenever possible.

In summary, the efforts to create a repository of cell models at scales other than those developed by the Center for Tissue Modeling, and our efforts to adopt, adapt and extend these models, will be tremendously valuable in the development of tools for computational biology. This effort will address a key need in the simulation of biological systems. Furthermore, this effort will permit the Potts model and related tools developed within the proposed Center for Tissue Modeling to link with the best existing models at smaller and larger scales of biological function, thus ensuring that the proposed Tissue Simulation Toolkit will be as effective, useful, and accurate as possible.

I.12.ii KEY EXISTING SYSTEMS:

The next four subsections detail projects that we view as critical other efforts, with which our efforts must be interoperable. Among the letters of support are included letters from the leaders of each of the following four modeling initiatives; we have similarly sent letters of support to the leaders of each of these four initiatives. The content of the letters is very simple: we have committed to interoperability between the Tissue Simulation Toolkit and each of the following for modeling systems. Each of the following four initiatives has likewise indicated that they will use the Tissue Simulation Toolkit for modeling at the scale of biological organization that we have mapped out as our primary area of development focus, from 2 to ~10^6 cells.

I.12.ii.(a) THE PHYSIOME PROJECT:

Website: http://www.bioeng.auckland.ac.nz/physiome/physiome_project.php

The following overview is from the Physiome Project Website:

“The Physiome Project is a worldwide public domain effort to provide a computational framework for understanding human and other eukaryotic physiology. It aims to develop integrative models at all levels of biological organization, from genes to the whole organism, via gene regulatory networks, protein pathways, integrative cell function, and tissue and whole organ structure/function relations. Current projects include the development of:

- ontologies to organize biological knowledge and access to databases
- markup languages to encode models of biological structure and function in a standard format for sharing between different application programs and for re-use as components of more comprehensive models
- databases of structure at the cell, tissue and organ levels
- software to render computational models of cell function such as ion channel electrophysiology, cell signaling and metabolic pathways, transport, motility, the cell cycle, etc. in 2 & 3D graphical form

- software for displaying and interacting with the organ models which will allow the user to move across all spatial scales

An important goal of the project is to develop applications for teaching physiology."

Interoperability between applications and tools that are part of the Physiome project is accomplished using XML-based languages. The central markup language is CellML, which we have already stated is a target for our efforts in terms of interoperability.

I.12.ii.(b) VIRTUAL CELL:

Website: http://www.nrcam.uchc.edu/index.html

Introduction from: J. Schaff, L.M. Loew, (1999)

"A general computational framework for modeling cell biological processes, the 'Virtual Cell', is being developed at the University of Connecticut Health Center. The Virtual Cell is intended to be a tool for experimentalists. Models are constructed from biochemical and electrophysiological data mapped to appropriate subcellular locations in images obtained from a microscope. Chemical kinetics, membrane fluxes, and diffusion are thus coupled and the resultant equations are solved numerically. The results are again mapped to experimental images so that the cell biologist can fully utilize the familiar arsenal of image processing tools to analyze the simulations."

The philosophy driving the "Virtual Cell" project requires a clear operational definition of the term "model". The idea is best understood as a restatement of the scientific method. A model, in this language, is simply a collection of hypotheses and facts that are brought together in an attempt to understand a phenomenon. The choice of which hypotheses and facts to collect and the manner in which they are assembled themselves constitute additional hypotheses. A prediction based on the model is, in one sense, most useful if it doesn't match the experimental details of the process--it then unequivocally tells us that the elements of the model are inaccurate or incomplete. Although such negative results are not always publishable, they are a tremendous aid in refining our understanding. If the prediction does match the experiment, it can never guarantee the truth of the model, but should suggest other experiments that can test the validity of critical elements; ideally, it should also provide new predictions that can, in turn, be verified experimentally. The Virtual Cell is itself not a model. It is intended as a computational framework and tool for cell biologists to create models and to generate predictions from models via simulations. To assure the reliability of such a tool, all the underlying math, physics, and numerics must be thoroughly validated. To assure the utility and accessibility of such a tool to cell biologists, the results of such simulations must be presented in a format that may be analyzed using procedures comparable to those used to analyze the results of experiments."

Virtual Cell is accessed through Java/RMI and a Web portal, and both our effort and the SCIRun group (personal communication) anticipate creating a CCA-compliant mechanism for interacting with Virtual Cell.

I.12.ii.(c) BIOSPICE:

Website: https://community.biospice.org/:

"Our goal is to create an open source framework and toolset for modeling dynamic cellular network functions and around this to develop a user community committed to using, extending and exploiting these tools to further our knowledge of biologic processes.

In collaboration with other BioSPICE Community members, we will develop, license, distribute, and maintain a comprehensive environment that integrates a suite of analytical, simulation, and visualization services to aid biological researchers engaged in building computable descriptions of cellular functions. Starting from primary data and progressing to experimentally validated mathematical models of cell systems, our environment will offer a comprehensive substrate for efficient research, collaboration and publication."

BioSpice bears mention in some detail because of its architectural sophistication. Because of this, it will be both important and straightforward to establish multiple functional linkages between the Tissue Simulation Toolkit and BioSpice.

Multiuser operation. A single BioSpice installation serves multiple users, and this is reflected in the system's emphasis on collaboration and security. Authorized users of the system log in to obtain secure access to BioSpice's services, and data they store and retrieve are protected by ownership and permissions. In this way, data sharing and collaboration are supported while security and integrity of their data is maintained. Furthermore, users can attach levels of authority and review to the information they share, allowing hypothetical and preliminary data to be distinguished from accepted and established data.

Extensibility. The default build and installation provides a skeleton implementation of the BioSpice architecture. Developers can easily use the open specifications of the system's interfaces, object types, and database schemas to extend BioSpice's functionality. Particularly accessible is BioSpice's simulation framework, which allows developers to wrap existing simulation code in components that can be accessed systemwide. All elements of BioSpice are open source, allowing developers to further customize any aspect of a BioSpice installation.

Platform independence. Many pieces of the BioSpice software are written in Java, allowing users to run the system on a wide array of platforms. And because the system's components communicate through CORBA as a default, BioSpice can be scaled across several platforms that are not necessarily of the same type. BioSpice's database requirements are met by a number of relational databases, including several freely available open source packages such as MySQL and PostgreSQL, running on a wide selection of operating systems and hardware.

Interoperability. By using a database system which uniquely identifies objects, separate BioSpice installations can share and exchange user data. Moreover, BioSpice's strategy of using database schema corresponding to widely accepted bioinformatics data formats allows import and exchange with existing repositories of information, such as NCBI, GenBank, and others.

Components of BioSpice. The individual components of BioSpice form a layered architecture with a database and essential services at its core. Web applications and graphical interfaces allow users to access the data and services provided by the system. Software projects contributing to BioSpice include the following:

- *PathwayBuilder* provides users a visual environment for creating and editing reaction pathways, allowing expression of transcription, molecular interactions, catalysis, and other biomolecular phenomena. Pathways are displayed with symbols commonly used in metabolic diagrams and familiar to those in the molecular biology community. The PathwayBuilder can communicate with the rest of the BioSpice system for access to biological data and simulation kernels.
- *BioDB* is a set of databases, data translators, and object servers that store and provide access to biological data, using widely-used data types in an object-relational system. Clients can employ the BioDB interface layer, via CORBA, to manipulate the data as native-language objects. BioDB fully maintains object ownership, data authority levels, and editing permissions, much like files on a file server; users can maintain private data objects, or choose to share them with peers on the system. The databases used by the basic installation of BioSpice use data representations adapted from several widely-used sources, such as the National Center for Biotechnology Information (NCBI) and GenBank.
- *XMLdbmsAPI* is a custom adaptation and extension of **XMDBMS**, allowing translation between XML data conforming to standard bioinformatics DTD's and data objects within BioDB. As XML-formatted data gains popularity in the bioinformatics community, the XMLdbmsAPI will provide BioSpice compatibility with a broad range of third-party tools and systems.
- *Schemonster*, an ASN.1-to-SQL compiler, was written primarily as a tool to convert the **NCBI** and **BIND** data types into database definitions compatible with BioDB's object-relational scheme. It also generates a mapping specific to the schema for use by XMLdbmsAPI. Users can employ

Schemonster to generate new databases within BioDB quickly and easily from other ASN.1 data type definitions.

The *BioSpice Server* is a set of CORBA components allowing clients authenticated, secure access to BioDB data channels, simulation monitors and components, graphical display data, and related services. The BioSpice project provides precompiled client code for C, C++, Java, and Perl for rapid development of new software that can communicate with the server components and BioDB.

I.12.ii.(d) SCIRUN:

Website: http://www.sci.utah.edu/research/pse_fields.html

Description from Website:

"SCIRun is a Problem Solving Environment (PSE), and a computational steering software system. SCIRun allows a scientist or engineer to interactively steer a computation, changing parameters, recomputing, and then revisualizing--all within the same programming environment. The tightly integrated modular environment provided by SCIRun allows computational steering to be applied to the broad range of advanced scientific computations that are addressed by the SCI Institute.

Implementation of a computational steering environment requires a successful integration of many aspects of scientific computing. Specific examples include performance analysis, geometric modeling, numerical analysis, and scientific visualization, all of which need to be effectively coordinated within an efficient computing environment. SCIRun is both a tool for the applications scientist or engineer as well as a test for new approaches in integrated large-scale scientific computing."

Integration with SCIRun will be achieved via compliance with the CCA standard. Currently, only portions of SCIRun are CCA-compliant; however, the developers have identified full CCA compliance as a top priority (Johnson, 2002). SCIRun plays a central role in our visualization plans.

I.13.iii INTEROPERABILITY OF THE TISSUE SIMULATION TOOLKIT AND OTHER INFORMATION TECHNOLOGY TOOLS DEVELOPED AT INDIANA UNIVERSITY – GENE EXPRESSION:

One of the long-term goals of this proposal is to integrate our Tissue Simulation Tools with other computational tools so that biological processes can be better modeled and analyzed at the organ, tissue, cellular, and molecular levels. Linking generated tissue models to gene expression data is an important element in accomplishing this goal. To this end, we will establish a "gene expression support group," led by Dr. Matthew Grow at the Indiana University Center for Medical Genomics (CMG). Its goals and services are described below:

I.12.iii(a) Maintain current lists of tools for gene expression analysis and transcriptional network modeling, distribute this information to center members, and assist in the interpretation of relevant gene expression data.

The gene expression support group will work to identify and evaluate both basic gene expression analysis tools and transcriptional network modeling software. The support group will also provide advice on microarray experimental design, and will help Center for Tissue Modeling members on the interpretation of both their own microarray data, and that in publications or public databases. There is already software capable of generating putative functional relationships between genes and their products based on genomic, proteomic, and bibliometric data (such as PathwayAssist from Stratagene). Along with the experimental groups in this

proposal, the gene expression support group will utilize public and private (when available) networking tools to generate networks and relationships specifically relevant to each experimental group. The support group will also serve as a conduit between experimental groups, attempting to identify areas of overlapping interest (such as vascular endothelial cell differentiation and endocardial differentiation/trabeculae formation). Whenever possible, common genomic elements between experimental groups will be highlighted and relevant results discussed.

I.12.iii(b) Plan future connectivity of Tissue Simulation Tools and Gene Expression/Transcriptional Networking Tools developed at other NIH Centers for Biomedical Computing and elsewhere

We anticipate that one or more of the NIH Centers for Biomedical Computing will focus on developing toolsets for generating theoretical transcriptional networks based on gene expression (i.e., microarray) data. In attempt to capitalize on the synergistic potential of cooperativity between centers, we will actively pursue opening communications with other Biomedical Computing Centers working in this area within the first year of operation. The goal of this communication will be to develop long-term strategies for future connectivity and data exchange between the various proposed tools.

The gene expression support group will also work closely with other center collaborators, and will investigate and advise the center on integrating or linking the Tissue Simulation Tools to outside gene expression tools or databases.

In order to establish good communication between each of the experimental groups, and to provide knowledgeable assistance in the area of gene expression data interpretation, it is desirable for the gene expression support group to have knowledge and experience in those areas of research. Dr. Grow has extensive experience in the area of cardiovascular development, having worked on the role of the *tinman*-related genes (including *XNkx2-5*) in heart development (Grow and Krieg, 1998), and continuing research into the importance of the homeobox gene *Hex* for vasculogenesis. Additionally, Dr. Grow is an active member of the Center for Regenerative Biology and Medicine, and is currently overseeing the fabrication and use of a regenerating *Xenopus* limb bud spotted microarray. The gene expression support group will be located within the Center for Medical Genomics (CMG). Established by Dr. Howard Edenberg with support from the Indiana 21st Century Fund and the Indiana Genomics Initiative (INGEN), the CMG is a gene expression and genotyping core facility, specializing in high-throughput SNP genotyping, Affymetrix GeneChips® and custom-spotted microarrays. Additionally, the CMG has established a bioinformatics group in order to fully support these services (see next section). To date, the CMG has processed over 1000 Affymetrix GeneChips®, and has accumulated a considerable amount of experience in the analysis of microarray data. Additionally, Dr. Grow oversees the CMG spotted microarray facility, a full service facility for printing, hybridizing, and interpreting custom spotted arrays.

I.12.iii(c) Continue software development for management of bioinformatics data – Labrat and MDP:

We have developed an informatics tool, the Microarray Data Portal (MDP), a Java servelet that provides users with web-based access to a wide variety of algorithms and tools useful for the analyses of gene expression data. The interface for this tool is shown in Figure 1.15. Using MDP, two way comparisons of data may be performed using a two sample t-test, fold change, log fold change, and nonparametric statistics (Wilcoxon). Sorting and filtering are allowed on both the p value from a t-test and the average ratio and fold change of the data. Series analyses may be performed using k-means analysis, Estimation Maximization (EM), and hierarchical clustering. Additionally, singular value decomposition (SVD) views of the data are possible (Alter *et al.*, 2000).

Tracking and filtering of data may be performed based on the information from a variety of external bioinformatics sources. This includes KEGG (Kanehisa *et al.*, 2002) pathway information from LIGAND (Goto *et al.*, 2002), Gene Ontology (GO) terms (Ashburner *et al.*, 2000), and chromosomal location. When possible, gene information is linked to common bioinformatics databases such as LocusLink (Pruitt *et al.*, 2000), GenBank (Benson *et al.*, 2004), and Unigene (Schuler, 1997). Users may download their generated data in a tab delimited form, format compatible with the GenMap program, or the MAGE-ML format (Spellman *et al.*, 2002).

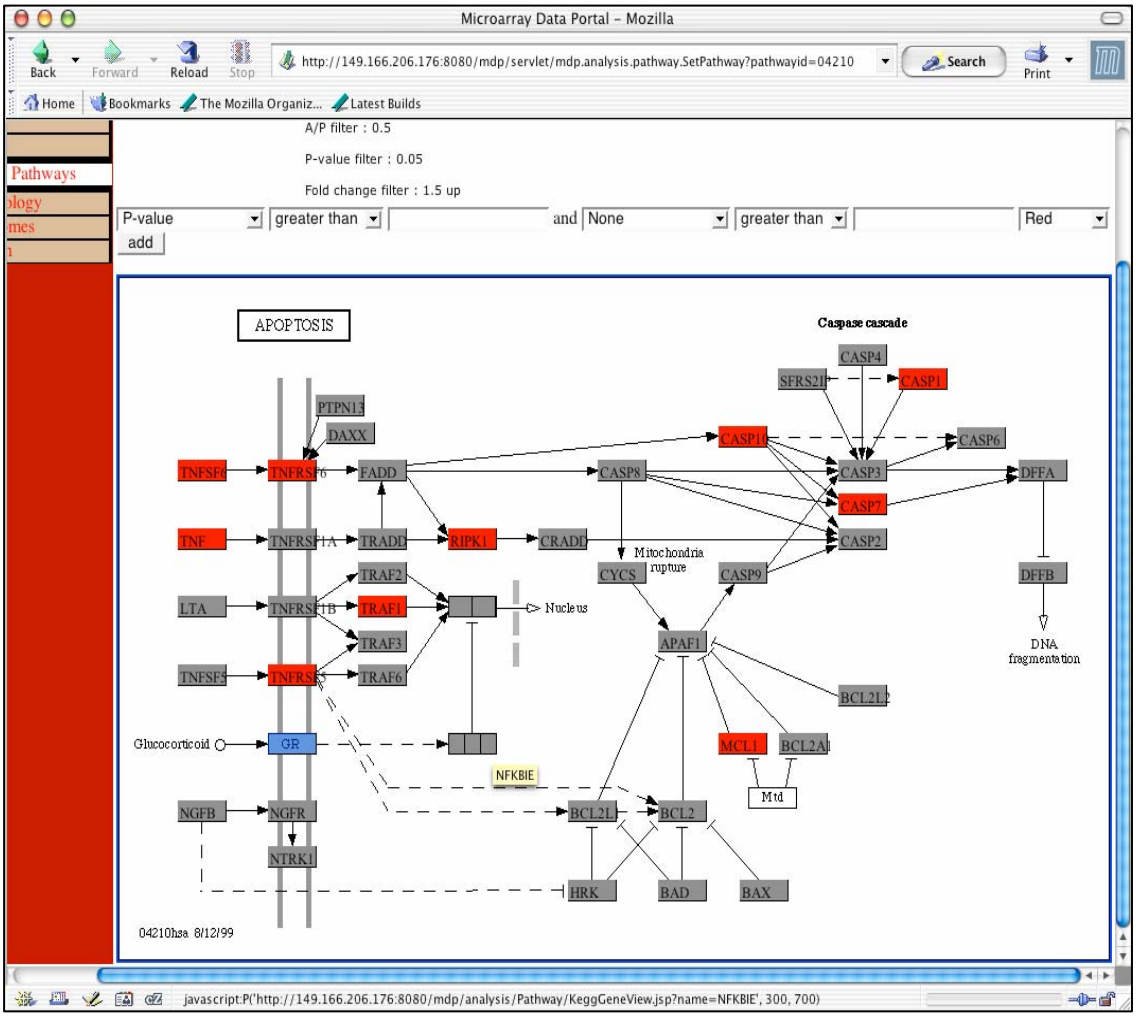


Figure 1.15 Screen shot of MDP displaying a KEGG pathway linked to gene expression results.

Sample information and center workflow is stored and managed using the Labrat LIMS system. This LIMS is linked to the MDP and was developed at the CMG for managing microarray data. The flexibility of this system and the easy-to-use interface is attractive to many diverse labs, however, and commercialization of this product is now in progress.

I.12.iii(d) Transcriptional Data and Application to Experimental Projects:

Initial efforts of the Center for Tissue Modeling will be focused on the generation of computational models to support each of the experimental projects. These projects, however, will also benefit greatly from the anticipated accumulation of public gene expression data, from transcriptional profiling conducted by members

within our center, and from expected advances in transcriptional analysis tools. The following are some examples of how gene expression data may be used to enhance specific experimental platforms.

I.12.iii(e) Grid Service Architectures and Problem Solving Environments:

Grid and Web service architectures are evolving toward a common service-oriented architecture (Booth *et al.*, 2003). Grids represent collections of resources, often managed by different groups and aggregated into a single "virtual organization." All of the resources, including data, science applications such as the Cellular Potts Model code described earlier, and computing resources are accessed via Grid Web services. The collection of these resource services and their associated pervasive information, security, and collection/aggregation/orchestration services collectively define a service-oriented grid.

Service-oriented Grids such as we propose have two important characteristics: XML-based service descriptions (WSDL and extensions http://www.w3.org/TR/wsdl) and XML-based messages (such as SOAP http://www.w3.org/2000/xp/Group/). This architecture and these programming language-independent service and message abstractions provide an important decoupling of service instances (that will be distributed over remote resources) and the user interface environments that one builds to access these services. In our architecture, service and message definitions are enough to allow us to define a wide range of user environments: downloadable, sophisticated graphical user interfaces (GUIs) built with TCL/TK, Java, Python and so on; "shell"-like environments that allow command-line access to remote resources and support user scripting, and Web browser-based computing portals. The latter is the subject of the rest of this section. However, we wish to emphasize that the service architecture does not preclude any number of user interface strategies. This is an important consideration, since users will have widely varying requirements, which may change over time and location—a power-user of the biology grid who normally relies on a "grid shell" system for scripting may greatly appreciate a portal interface when traveling.

I.12.iii(f) Component-Based Portals:

A computing portal is a Web browser-based interface to remote collections of grid resources. Computing portals are not passive interfaces: they typically allow users to securely access remote computing and data resources for launching applications on supercomputers and clusters, manipulating remote files, and monitoring jobs. Computing portals also allow the leveraging of more typical Web portal tools such as news feeds, calendar/address book services, member-only areas for sharing documents, and so on.

Computing portals to Grids are common development efforts. A recent special issue of Concurrency and Computation: Practice and Experience (Fox and Hey, 2002) identified over 25 such projects, and the field has been represented by the grid computing environments research group of the Global Grid Forum. The field has reached an important maturity level: computing portal efforts have recently undergone a major shift toward supporting interoperability through the adoption of a component-based "portlet" approach. Portlets are portal components that manage the user interface to a particular portal service: the HTML for a calendar service or an SBML-based search engine would be managed by different portlet instances. The HTML nuggets of these instances can be aggregated into a single, customizable browser interface. In the service oriented architecture, portlet instances also act as clients to manage remote service invocations. A portlet thus has two interfaces: one for generating displays and another for handing the remote Web service invocations to a particular grid resource.

The key feature of the portlet approach is reusability. Portlets are aggregated into computing portals in well-defined ways. Thus, when one has developed a particular portlet to a useful service, this component can be reused in other projects. One should, for example, not put effort into building a calendar service for a portal if one can instead plug in a portlet that has the desired functionality.

Portlet-based portals are becoming commonplace. The Jetspeed project (http://jakarta.apache.org/jetspeed/site/) from Jakarta represents an early, open source implementation of the

portlet concept. More recently, commercial companies such as IBM, Sun, Oracle, and others have developed portlets standards (JSR 168, http://jcp.org/aboutJava/communityprocess/final/jsr168/index.html).

Portlet approaches are being adopted by the computing portals community for the reusability they offer. This approach has also proven to be a useful one in distributed development efforts: different teams can be responsible for building individual capabilities that can be integrated into a common portal rather quickly.

I.12.iv TST PROBLEM SOLVING ENVIRONMENT PORTAL COMPONENTS:

Indiana University team members are participants in three portlet-based projects that can be leveraged to build the initial TST PSE system: the Alliance Portal, the DOE SciDAC Portal Services collaboratory, and the NSF National Middleware Initiative's Open Grid Computing Environments (OGCE Collaboratory) project (http://www.alliances.bearingpoint.com/, http://www.scidac.org/, and http://www.nsf-middleware.org/). Collectively, these projects possess a host of available capabilities that we may draw upon. These are summarized in the table. Categories include:

- Community Portlets: calendars, newsgroups, news feeds, document sharing areas, and so on.
- Basic Grid Portlets: Interfaces to common computing grid services such as user proxy certificate managers (for authentication), remote command execution, and file management.
- Science Application Portlets: these are portlet interfaces to domain-specific portal capabilities, such as HTML "wizard"-style form assistance for creating input files for running applications such as the Cellular Potts Model, and portlets accessing visualization, analysis, and post processing services.

Table 3 includes a partial list of current and soon-to-be available portlets from the OGCE (http://www.ogce.org/). These can be used to quickly build an initial biology portal system. By taking advantage of the existing general portlet capabilities, we may concentrate efforts on the specific requirements of the TST Problem Solving Environment, such as access to biology portal codes and data.

One area of particular importance for development is a portlet-building wizard for the TSTGrid. As new applications come online, we must provide a simple template wizard that will allow these applications to be deployed automatically. This template wizard will allow the Center for Tissue Modeling to distribute support for maintaining applications and more easily support third-party participation.

Figure 1.16 and 1.17 are screen shots illustrate two portlet-based interfaces: one for earthquake simulation and the other for accessing fusion grid resources. The Web launch form, visualization, and logbook each represent a different portlet.

Portal Capabilities	Provider	Description	Status	Next Steps
Grid login	TACC	Get Grid proxy cert when logging into Jetspeed.	Available	Integrate with IU portlet-based MyProxy
Grid Proxy Certificate Manager	IU	Get MyProxy certs after logging in.	Available	Integrate with TACC authentication.
GPIR Portlets	TACC	View, interact with portal information data, such as machine and job status.	Available	Point to FG resources
MDS/LDAP Browsers	IU (ANL)	Basic Globus MDS browsing and navigating	Available	Complete.
Document Share	UM	A group accessible repository for documents, presentations, etc. Standard WebDAV	Avaialble	Develop Web service module.
Calendar/Meeting planner	UM	Provide an interactive, sharable calendar.	Available	Convert into a modular Web Service.
Newsgroups, citation managers, discussion groups	IU, UM	Provide discussion forums, feature requests, etc. Uses NaradaBroker, JMS/RSS/Semantic Grid services	Available	More closely integrate with portal container (Jetspeed).
File Transfer	IU	Support access to GridFTP	Available	Integrate with FG.
Audio/Video Collaboration Portlets	IU	Display and manage Access Grid clients (Vic) Integrates with XGSP H323/SIP Web Service	Demonstration	Integrate with improved client libraries and Autonomic messaging.
OGSA-DAI Portlets	IU	User interface to OGSA/DAI services	Demo/Prototype Available	Consider bridges to SRB/MDSplus

Table 3. A selection of portlets available from the OGCE collaboratory.

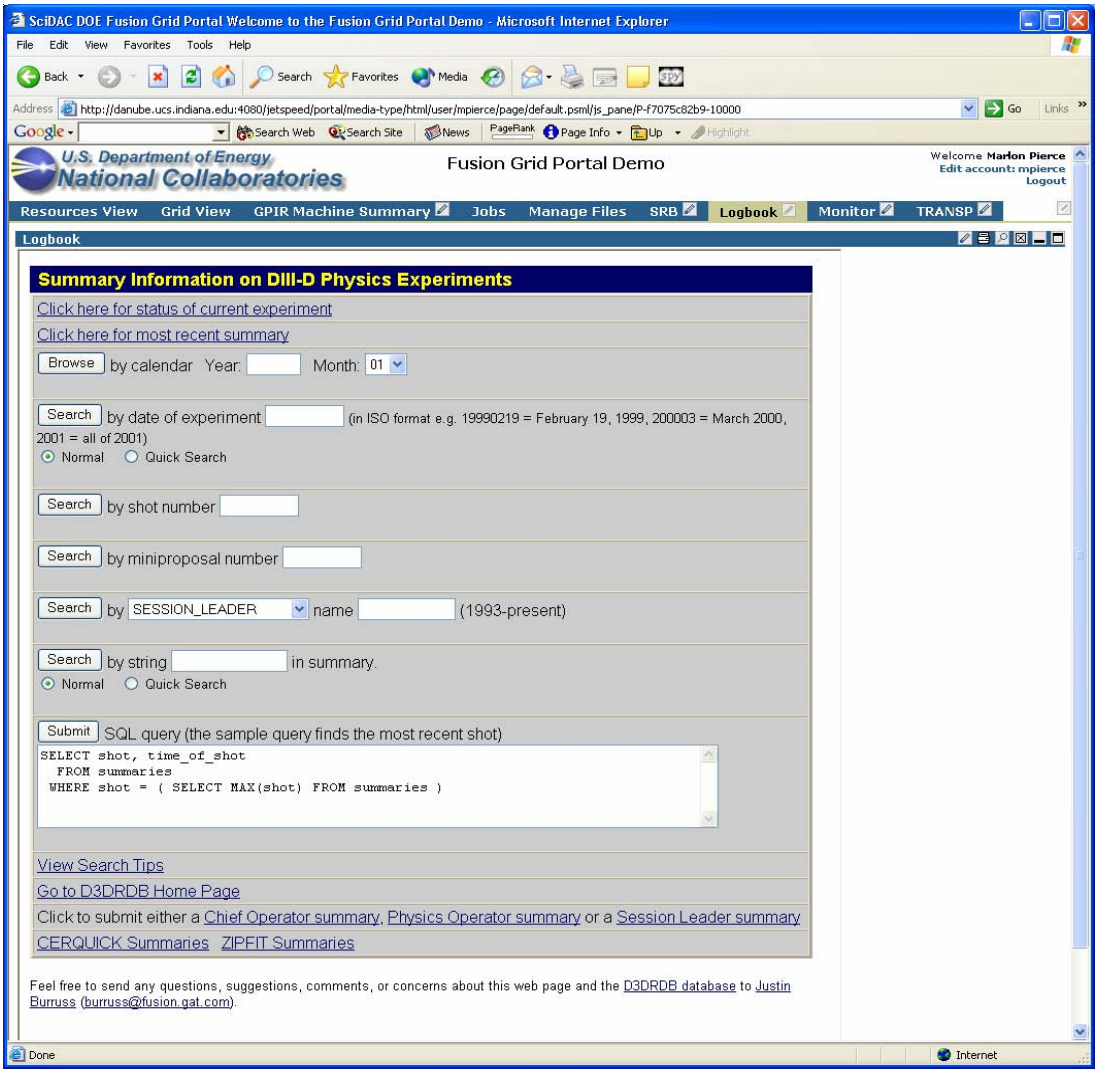


Figure 1.16: The screen shot shows one portlet display, a log book form page for viewing fusion shot results. The top menu bar shows several other available portlet components ("Resource View", "Grid View", "GPIR Machine Summary", etc.)

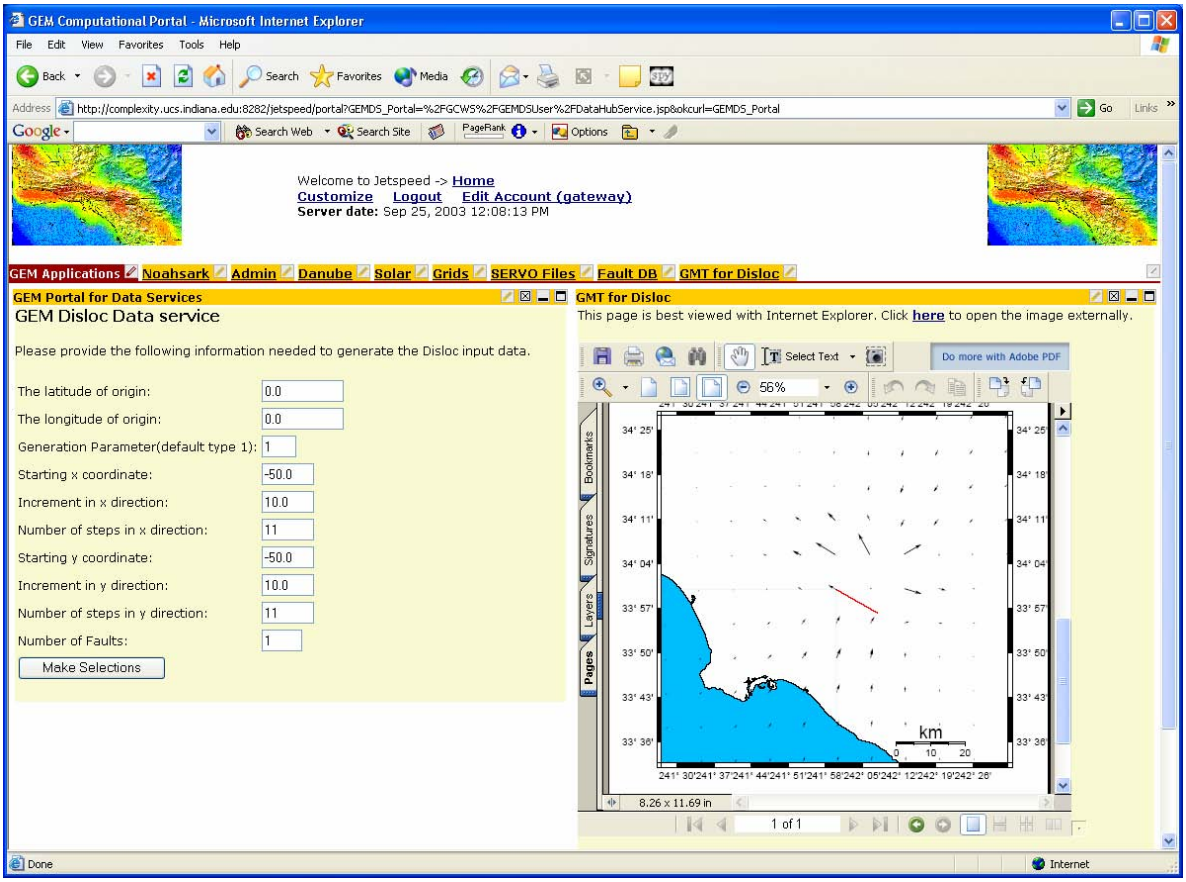


Figure 1.17 Screen shot of a computing portal for earthquake simulation built from portlet components. The left portlet display shows form parameter fields, the right shows a simple visualization of model results (calculated surface stress vectors over Southern California). The horizontal menu bar lists several other component interfaces.

I.13 ADVANCED VISUALIZATION:

The data that will be used and generated by the Center for Tissue Modeling will require appropriate visualization and analysis tools. The question becomes -- what is appropriate? We offer our views on that in this section. One thing is certain, scientific visualization, since being proposed as an independent discipline nearly 20 years ago (McCormick, 1987), has played a crucial role in establishing computational science as a pillar for modern day scientific research. In this section, we summarize our experience with scientific visualization and outline a plan for incorporating it into the proposed work.

Three cooperating groups at Indiana University will carry out the visualization components of this core. These groups complement each other in areas of resources, expertise, and missions, and their collaboration will ensure a high level of integration, accessibility, and innovation for the visualization tools in support of the simulation science.

The Scientific Data Analysis (SDA) Lab (sda.iu.edu) is part of the Pervasive Technology Labs at Indiana University that seeks to advance science by collaborating with researchers who have challenging problems in scientific data management, analysis, and visualization. The SDA Lab has an ongoing project with Molecular Kinetics, Inc. (located in Indianapolis) for the analysis of disordered proteins. Additionally, the Lab has a project with the Air Force Research Lab to provide immersive visualization capabilities for their new VR lab.

The Open Systems Lab (OSL, osl.iu.edu) is another lab within the Pervasive Technology Labs that conducts research on software platforms for pervasive technologies. Particular areas of investigation include scientific and engineering applications, scalable computing, and peer-to-peer distributed computing. Members of the OSL have been instrumental in the design of the new CPM system and code, and have developed the OpenGL and VTK visualization prototypes.

The Advanced Visualization Lab (AVL, avl.iu.edu) is a unit of the Research and Academic Computing division of University Information Technology Services (UITS) at Indiana University. The AVL provides expert consulting, research support, educational opportunities, and hardware and software resources for scientific visualization, virtual reality, high-end computer graphics, and visual tele-collaboration. The AVL has a number of ongoing visualization projects with the Indiana Genomics Initiative (INGEN) involving biomolecular systems, biomedical imaging, and genomics-related data.

I.13.i VISUALIZATION REQUIREMENTS:

As outlined above, the primary goals of this consortium are to develop computer models that simulate organogenesis at varying length scales, and to compare and verify the validity of these models against experimental results. Our simulations will, to a large degree, be based on our extensive experience with the Cellular Potts Model (CPM). The results produced by the model dictate certain requirements for an accompanying visualization and analysis tool. (For brevity, we henceforth refer to a visualization tool, realizing that visualization and analysis are often synonymous, especially with the tools that exist today.)

The first requirement for our visualization system is that it be capable of handling both 2-D and 3-D spatial data, as well as their time-dependent extensions. An example output from a CPM on a 2-D spatial domain is shown in Figure 1.18.

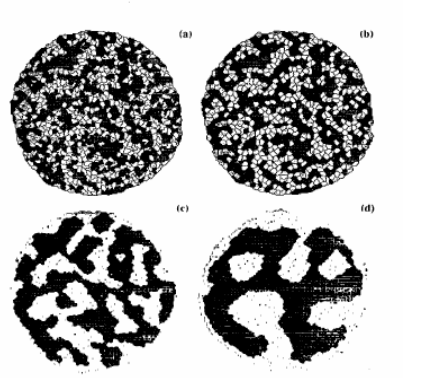


Figure 1.18 2-D CPM showing cellular aggregation.

Adding one spatial dimension, lifting us into 3-D (Figure 1.19), offers a challenge to all visualization systems, both in terms of the underlying visualization algorithms and the rendered results. The flat screen of a computer monitor is still the ubiquitous display medium. Fortunately, modern graphics hardware is capable of displaying very large polygonalized models at interactive rates, allowing for dynamic inspection (rotating, zooming, panning, slicing, etc.) of datasets and providing valuable 3-D cues to human vision. In addition, the most popular graphics PC-based cards now come with graphics library extensions that provide very nice visual special effects which have proven to be useful not only in Hollywood, but in scientific visualization as well.

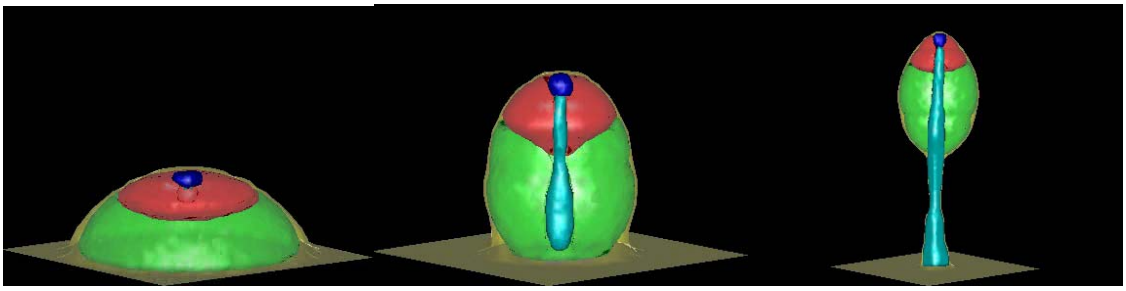


Figure 1.19. 3-D CPM depicting the evolution of slime mold.

As the CPM simulation advances in time, one would see local dynamics of both the cell locations and cell boundaries, as well as the global shape dynamics of the aggregate. A useful property of a visualization system would be to quantify these dynamics and display any coherent structures, or patterns, that may exist. We have explored one such technique for decomposing spatiotemporal data (Armbruster, 1992; Armbruster, 1994; Stone, 1999) and could readily adopt it to CPM. Sample results using this algorithm for CFD data (air-flow over a 3-D turbine blade to dissipate heat) is shown in Figure 1.20 (Heiland, 2001). However, pattern detection and tracking is a rather specialized algorithm for spatiotemporal data. One would not expect to find it in a general-purpose visualization system. But this raises an important point – we believe that a visualization system needs to be extensible. That is to say, it should provide an easy mechanism for adding new, or modified, capabilities. And this point brings us to the issue of open source software and the NIH software dissemination goals for this RFA.

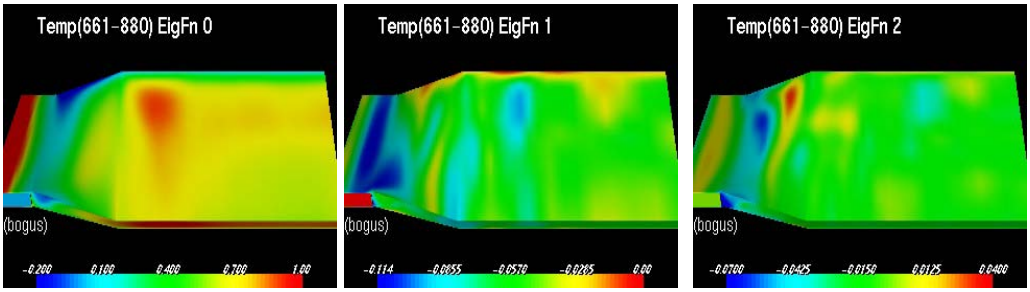


Figure 1.20 Coherent structures from spatiotemporal CFD data

I.13.ii VISUALIZATION SOFTWARE:

We are strong proponents of open source software and have used and contributed to various packages falling under this label for several years. In this section, we present our experience with some of these packages.

I.13.ii(a) THE VISUALIZATION TOOLKIT (VTK):

The Visualization Toolkit (Schroeder, 1996; Schroeder, 2003) grew out of an effort started at General Electric Corporate R&D Center in the mid-1990s. This led to the spin-off formation of Kitware, Inc., in 1998, a company devoted to the continued development and support of the open source VTK software and related projects. Except for a very small "patented algorithms" subset, all of VTK is open source. Members of the SDA have used (and contributed to) the VTK software for about eight years and are therefore quite familiar with this package and with the very large community of users and developers behind it. In addition, members of the AVL are currently utilizing VTK for projects in FEA visualization and parallel rendering. We believe VTK offers a well-designed foundation of extensible visualization software on which one can build tools for the proposed work. Unfortunately, VTK by itself is not the end solution. While VTK provides a very broad base of visualization software (about 700 C++ classes), including some algorithms for computational geometry, it does

not provide an end-user tool. That is to say, there is no graphical user interface that comes with VTK – it is simply a suite of libraries.

I.13.ii(b) INSIGHT TOOLKIT (ITK):

In 1999, the NIH National Library of Medicine funded the development of what became the Insight Toolkit (ITK) (Ibanez, 2003). ITK is, in short, an open source software package for image segmentation and registration. It was initially designed to support the Visible Human Project. ITK does not, however, explicitly provide any visualization capabilities. Since ITK, like VTK, is an open source project, there is a large community of developers who contribute to it. Some of the key institutions supporting it are Kitware, Inc., Insightful Corp., GE Corporate R&D, and the University of Utah. ITK is currently being used for research on volumetric segmentation and filtering within the IU School of Medicine. An all-day ITK workshop held at the annual IEEE Visualization conference in October 2003 provided us with even more motivation to find additional interesting ITK-related applications. We believe its various algorithms for 2-D and 3-D segmentation would be quite useful for the CPM results, as well as in vitro experimental results. While at the workshop, we learned more about the connection between ITK and SCIRun, a visualization package from the University of Utah.

I.13.ii(c) SCIRUN:

SCIRun is a problem-solving environment (PSE) that allows the interactive construction, debugging, and steering of large-scale scientific computations. Using a component-based visual programming and dataflow model, SCIRun provides a “computational workbench” that allows scientists to interactively design, modify, and visualize running simulations (Johnson 2002). The effectiveness of SCIRun’s extensible design and standards-based implementation has been demonstrated through the BioPSE and Uintah problem solving environments, both of which build upon the SCIRun core. SCIRun has also been successfully integrated with Matlab, MySQL, and the Genesis multicellular model (SCI, 2002). SCIRun is being actively developed and supported by the Scientific Computing and Imaging (SCI) Institute at the University of Utah.

Like other visualization systems that predate SCIRun (e.g., AVS, IRIS Explorer, and OpenDX), the network operates in a data-flow fashion – data typically originates at a reader module (input from a file) and then flows through the network until it reaches some terminating node, typically a render module that displays an image (Figure 1.21).

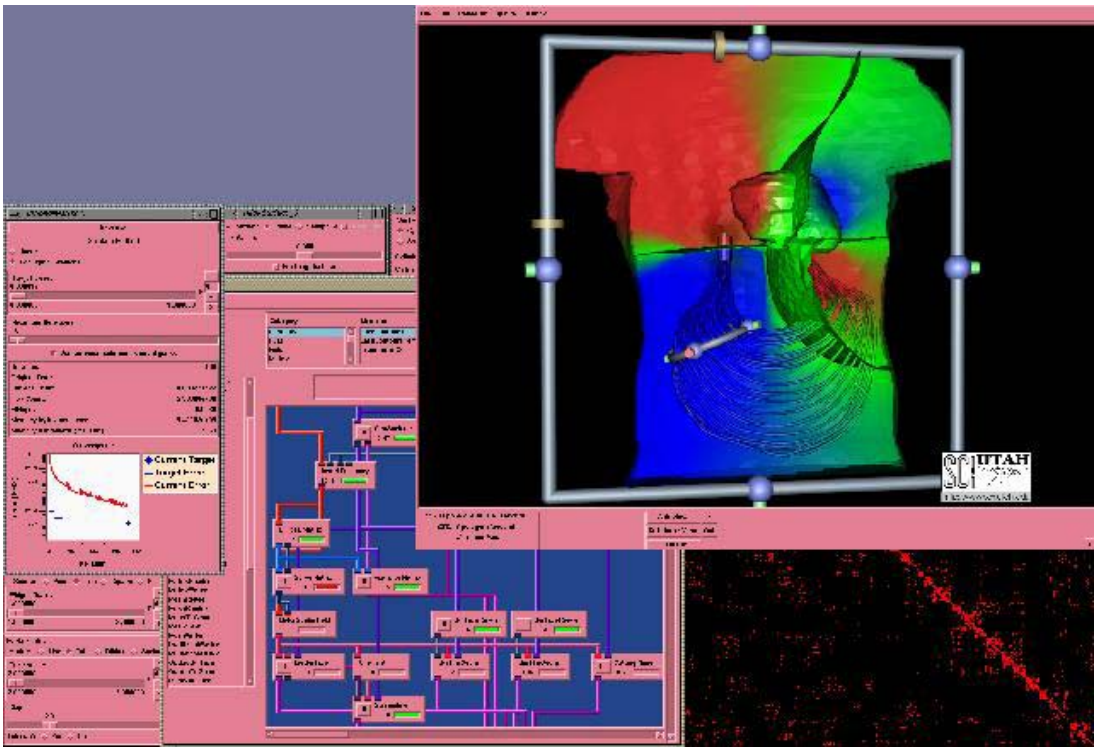


Figure 1.21 A sample SCIRun session with a render window (Johnson 2002)

SCIRun was originally targeted for applications in computational medicine and promoted the notion of computational steering. A user can steer a computation (embedded in the network) by interactively changing parameters associated with a running simulation. The ability to steer a large-scale simulation places the scientist “in the loop”, making cause-effect relationships more evident and allowing the scientist to develop better intuition about the model, algorithm and parameters. This can be particularly important within this core as the evaluation of multiple existing cellular models and the refinement of tissue models are key objectives.

Members of the SDA have followed the development of SCIRun over the years and included it in a report (Heiland, 1998) on co-processing (i.e., computational steering) tools for visualization. SCIRun certainly falls under the category of extensible visualization systems. In fact, picking up from the previous section, the SCIRun developers have provided an XML-wrapping mechanism to automatically incorporate ITK modules into their system. And the latest information we have received from the SCIRun developers is that they now provide a similar mechanism for wrapping VTK modules. (One should also realize that, to some degree, there is duplication of functionality in SCIRun and VTK due to their history of independent development.)

While a few other computational steering frameworks exist, none of the others have had the extensive testing, refinement, and application to biomedical problems that SCIRun has. Thus, we plan to rapidly evaluate the compatibility of SCIRun with the Tissue Simulation Toolkit so as to concentrate on issues of integration, extension, and support. In the unlikely event that SCIRun cannot meet our criteria for a visualization system, then we would either seek an open source alternative or, as a last resort, build our own graphical user interface on top of VTK, ITK, and other open source packages.

I.13.ii(d) PARAVIEW:

One viable alternative to SCIRun might be the ParaView system, also available from Kitware, Inc. ParaView, depicted in Figure 1.22, is built on top of VTK and consists of a graphical user interface and a mechanism for client-server based (remote) visualization. Also, ParaView is designed to handle very large datasets by

allowing for a parallel visualization pipeline. We have experience using ParaView for large data visualization using MPI on a Linux cluster. The potential downside of ParaView is that it is currently going through rapid design changes. On the other hand, these design changes will no doubt make this a better and more extensible system.

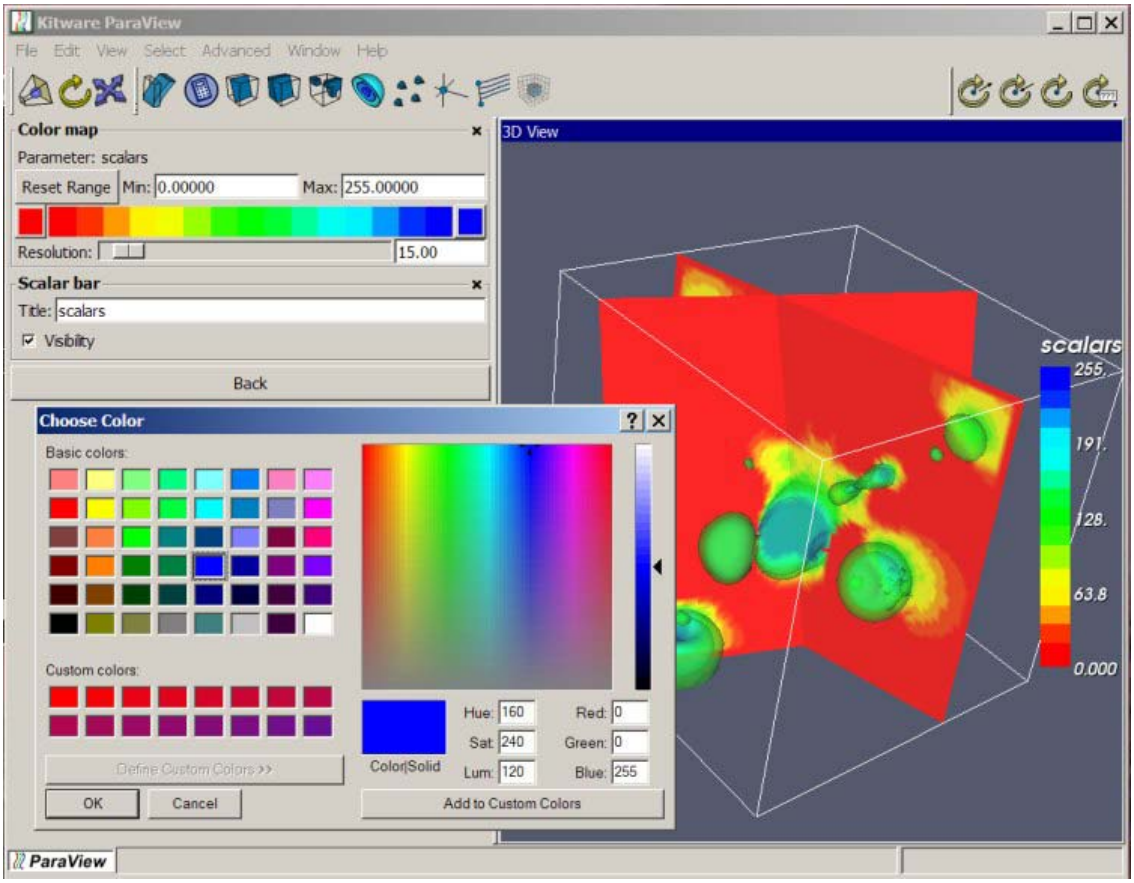


Figure 1.22. A ParaView session. (www.paraview.org)

I.13.iii VISUALIZATION SOFTWARE FOR THE LIFE SCIENCES:

The groups comprising the visualization team have considerable experience in developing, extending, and applying visualization tools to the areas of bioinformatics, computational chemistry, and computational fluid dynamics. The SDA has an ongoing project for the analysis of disordered proteins that involves the use of online protein databases, neural networks, computational geometry, and interactive 3-D visualization. As part of this project, members of the SDA Lab have been actively experimenting with the protein/molecular visualization packages DeepView and Protein Explorer as well as custom tools. The SDA has also been involved in various medical imaging projects (Littlefield, 1996). The AVL has ongoing visualization projects with the Indiana Genomics Initiative (INGEN), the IU School of Medicine, and the Departments of Chemistry and Biology involving biomedical imaging, biomolecular systems and genomics-related data (Figure 1.19).

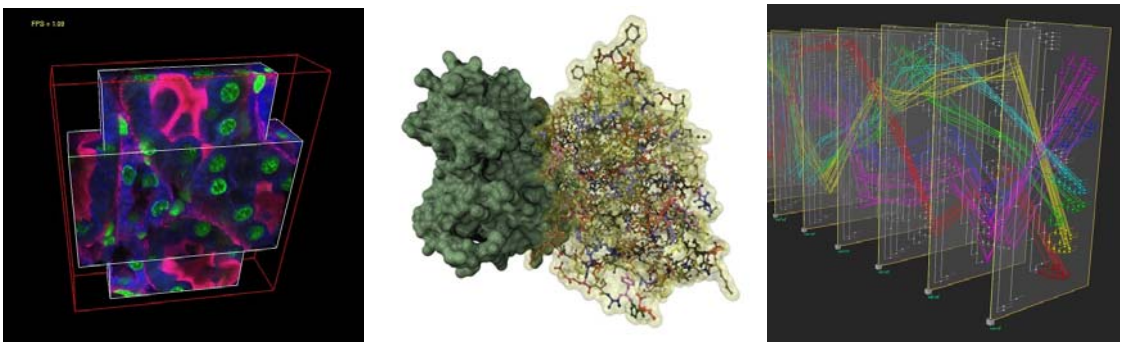


Figure 1.23. Examples of custom life sciences applications developed by the Advanced Visualization Lab. Left: kidney microtubules visualized with the 3DIVE volume render; Middle: a protein structure visualized using the XMView/CMView package; Right: a sequence of phylogenetic trees visualized in the Tree3D program. (www.avl.iu.edu/ingenviz)

I.13.iv VISUALIZATION SOFTWARE SUPPORT PLAN:

While SCIRun is a powerful and mature system, it is only recently that its developers have begun to address the issues of running it in a heterogeneous and distributed grid-based environment. In addition, many of the components of the SCIRun-based PSEs are not CCA-based. An important component and contribution of the planned local support is to further the refinement and development of SCIRun in several areas identified by the SCI group, including support for remote visualization, cluster-based visualization, computation on distributed memory systems and clusters, and full conversion to the CCA model (Johnson 2002).

Staff in the AVL will support the installation of SCIRun at CTM sites, and will work with members of the SDA and OSL to integrate the Tissue Simulation Toolkit with SCIRun. The SDA and AVL will collaborate on extensions to the SCIRun framework, particularly those needed to utilize the tool in a grid environment. The groups will also collaborate to identify and implement novel visualization techniques within the chosen framework to provide the most effective presentation of the simulation data, and to enable these visualizations for advanced displays.

I.13.v GRID-BASED VISUALIZATION:

We bring significant grid-based visualization experience to this project. The VisBench project (Heiland, 2001) was an effort to provide various data visualization and analysis services that could run on central servers with high-speed access to large data stores. When coupled with grid middleware for moving data, it became a prototype grid visualization tool for the NSF TeraGrid project. We provided a very lightweight Java client (Figure 1.20), a graphical user interface designed for general-purpose VTK and application-specific MATLAB backend services. Initially we used CORBA as our middleware, but later replaced it with a much simpler XML-RPC protocol.

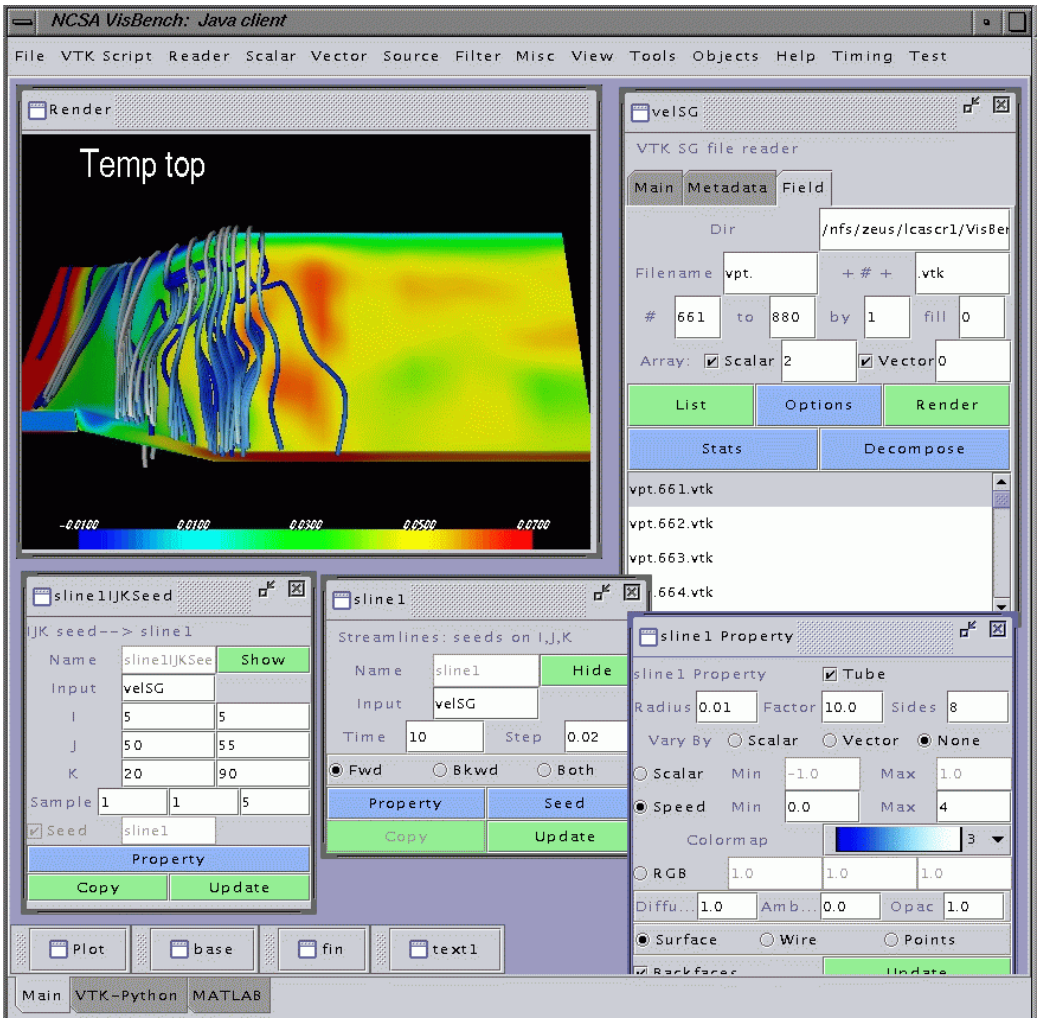


Figure 1.24. The VisBench Java client (Heiland, 2001)

I.13.vi COLLABORATIVE VISUALIZATION:

Projects of this magnitude, spanning several institutions, can greatly benefit from some sort of collaborative visualization tools. Using such tools, researchers can share results in real-time with remote colleagues. While there are various commercial tools that address these needs in one fashion or another, we prefer the open source collaborative environment known as the Access Grid™ (AG) (www.accessgrid.org) (Figure 1.21), developed at Argonne National Lab. We have several Access Grid nodes (virtual meeting rooms) within our respective labs, as well as people who develop code for the AG Toolkit. It is worth pointing out that the latest AG Toolkit now uses the Globus Grid middleware. More specifically, it uses Python Globus (pyGlobus). And Python, an Open Source Initiative certified scripting language, has been our scripting language of choice for several years. There is a wealth of open source packages that use Python-packages for numerical computing, for graphing and visualization, and for user interface development. The AG Toolkit, for example, uses wxPython, an open source package for graphical user interfaces. We advocate the use of wxPython for GUI-development as it provides rapid prototyping, cross-platform support, and a large choice of widgets.

Figure 1.25 An Access Grid™ node

I.13.vii DISPLAY HARDWARE:

We realize that most of the time researchers sit in front of their office workstations to visualize results from their simulations or experiments. However, there are occasions for researchers to use other display environments. Quite often this involves the use of larger displays that allow small groups to view and interact with the data being presented. Typically larger displays are equipped with immersive capabilities, including stereo projections and motion tracking of the viewer, as well as six degree-of-freedom input devices. The extreme example of this is the CAVE™, as well as large, multi-titled display walls. More recently however, IU has been actively involved in more affordable, scaled down, PC-based alternatives.

A particularly exciting innovation at Indiana University is the development and commercialization of a passive 3D stereo device called the John-e-Box (Figure 1.22), developed at the AVL. The John-e-Box has a screen large enough to produce a very impressive feeling of three dimensions, yet the entire device is small enough to be very practical as a piece of “lab scale” computing equipment (it is roughly a 50” cube overall). IU has commercialized the John-e-Box through a company called CAE-Net, Inc. (www.cae-net.com), located in Indianapolis, Indiana.[1] Indiana University has recently completed the deployment of eleven John-e-Boxes on the IU Bloomington, IUPUI, and IU Northwest campuses. John-e-Boxes will provide a practical tool for 3D visualization among partner institutions and users of the TST.

[1] John-E-Boxes are available for purchase at an educational price of $25,675, making them quite reasonable for use in many biomedical research laboratories.

Figure 1.26 The commercial version of the John-e-Box

In addition to the John-e-Box, CTM researchers will be able to utilize other advanced displays on IU's Bloomington and Indianapolis campuses, including the UITS-sponsored CAVE and tiled display systems, Access Grid Nodes, a larger format passive stereo display (the VisBox), and the Perceptable interactive tabletop display (developed at IUPUI's Visualization and Interactive Spaces Lab.)

I.14 CORE 1 INTEGRATION, EVALUATION AND SUPPORT ACTIVITIES:

Integration and evaluation are ongoing tasks to ensure that tools are properly prepared and deployed. One important issue is the software engineering strategy used in the writing the code for each tool. One could define this formally using an agile software engineering strategy that has had some success in distributed academic environments. However, we intend to exploit the software engineering advantages of our Web (Grid) service architecture and require the following process be followed in CTM software development:

- All groups will develop modules using WSDL (Web Service Definition Language) specification for all interfaces;
- The WSDL specifications will be discussed and agreed on before major software development is started;
- All software modules will be described by provenance metadata describing their pedigree and annotations to enrich the bare WSDL describing the interfaces. This will build on current Semantic Grid activities like MyGrid and ICENI developing (RDF/OWL) metadata enriched Web services;
- There will be a CMT metadata resource (hosted in a Xindice XML database) storing all the above metadata and supporting versioning as interfaces, their schemas evolve as we gain experience;
- All software will be maintained in a CVS repository.

The integration task will be staffed to monitor this process and to enable the architectural discussions needed to define the WSDL interfaces. This task ensures that critical metadata is entered in a consistent fashion. The strategy is natural for distributed components, and, as argued by Fox, can be used directly in any case where a delay of about a millisecond can be tolerated, corresponding to the case where explicit Web Service-style messaging can be used at the input and output of each module. High performance modules communicating by low latency method calls, or using parallel computing MPI messaging, have interface overheads measured in microseconds and cannot directly use the WSDL interface. Here we can use the CCA (Common Component Architecture) approach and develop several tools to map WSDL specification into low latency interactions. We will require all major interfaces, whether internal methods or external Web services, be specified by the metadata enhanced WSDL.

Tools will be developed according to an agreed schedule with intermediate deliverables and a well-defined internal test and evaluation process. Testing will involve both users in the core 2 and 3 teams and professional QA (quality assurance) covering respectively the functional and technical sides of the tools. Testing will be an iterative process and tools may of course need major revisions and perhaps occasionally need to be abandoned. Adequate documentation will be a requirement. After the tools have passed this internal process, they will be made available through the CTM web site described in core 6. This final step will also review the licensing issues to verify compliance with the NIH open software requirement. This involves the universities for locally developed software, but one must also verify the use and redistribution policy of outside software used in the tools. Anybody downloading tools will be asked to register an email address so we can offer better support.

Careful life-cycle support enters a new phase when a tool is released. Core 4 will provide support for new products as they are released, and will provide phone, email, and online consulting help for users of production versions of the software produced by the Center for Tissue Modeling.

I.15 INITIAL DELIVERABLES AND TIME-LINE FOR CORE 1:

As described earlier, the tools listed above we have identified as possibilities to be evaluated during the first few years of the center. In the first year we will build tools as projects with a one to two-year time frame. We will phase in new tools as other projects end in a fashion to best support the work in cores 2 and 3, as well as with input from NIH and outside users. The decision on initial and follow-up tools to be developed will be made by the management team in consultation with NIH.

Our anticipated timeline is shown. Note that duration listed below is that of the major development – ongoing refinement and bug fixes will occur after the initial focused development phase.

Deliverable	**Start Time**	**Duration**
Internal grid infrastructure with basic GT3 level capabilities with service registries and meta-data catalog	Year 1	1 year
Gather detailed information from researchers using CPM concerning its data structure and possibilities for interactive visualization.	Year 1	1 year
Install and develop a working knowledge of Univ of Utah's SCIRun visualization package. Maintain core visualization software, i.e., keep up with latest versions. Ensure developed code continues to work. Regression testing.	Year 1	4 years
Tools supporting WSDL-based software engineering	Year 1	1 year

Deliverable	Start Time	Duration
Portlet-based biology portal based on existing NSF NMI and alliance portals	Year 1	1 year
Redesign, rewrite code base for serial Potts model	Year 1	2 years
SCIRun-based Potts model visualization	Year 1	1 year
Sequential Potts model with increasing capabilities	Year 1	3 years
Application web services for Potts and sub-cell and continuum models	Year 2	2 years
Scalable parallel Potts model	Year 2	3 years
Tools supporting outside dissemination of center tools	Year 2	1 year
OGSA-DAI database for internal data	Year 2	6 months
Explore “large” display (JohnEBox,etc) environments for CPM-related visualization.	Year 2	2 years
Wizard for construction of new portals with portlets for major outside resources and full support of SBML and other metadata	Year 2	2 years
TSTPSE–Problem Solving Environment	Year 2	2 years
Experiment with algorithms used for (quantifiable) pattern detection and tracking in the CPM data. Show results to/work closely with end users, i.e., CPM experts.	Year 2	1 year
Maintain project’s publicly-available visualization/analysis tools. Create a help-desk.	Year 2	2 years
Grid workflow support of multi-model simulations	Year 2	1 year
Meta-data tools to support integration of models	Year 2	2 years
Multi-scale Potts model	Year 3	2 years
Run-time to support parallel Potts model with load balancing	Year 3	1 year
Grid meta-data resource managing dynamic data and simulation requirements	Year 3	2 years
Distribution of production version of code to SourceForge and other open source archives	Year 3	Ongoing
TSTGrid Problem Solving Environment	Year 3	2 years

Deliverable	Start Time	Duration
Provide CPM-based visualization tools that are ready for "public consumption", i.e., ready for download and use by the broader (tissue simulation) community, including documentation.	Year 3	1 year
Explore "small" displays (handhelds) for CPM-related visualization.	Year 3	2 years
Explore visualization within a grid environment.	Year 3	3 years
Integrated multi-scale model from sub-cellular through Potts to continuum models	Year 4	2 years
Focus on the final versions of publicly-available visualization/analysis tools, finalize all Users' Guides.	Year 5	1 year

I.16 EXIT STRATEGY:

Our exit strategy is implicit in every aspect of the plans for core 1. For the foreseeable future, the universities involved in the Center for Tissue Modeling will play majors roles nationally and internationally in advanced information technology and biomedical research. Maintenance of the software technology we develop as a result of this project will be maintained by ongoing university base budgets. This is already done with several software packages previously developed and released as open source by Indiana University.

Should a time come when further development, management, and maintenance of the software should shift away from the CTM, our strategies in software development will make transfer straightforward. We are developing new software only where necessary and maintaining interoperability with other key projects. Any of these "partner" projects could become a logical new "home" for the Tissue Simulation Toolkit if necessary. Furthermore, no later than in year 3 of this project will we move production versions of all codes to SourceForge.Net, a public repository of open source software. We will also escrow code with the NIH and NLM. Therefore, the code base created in this project will be available to the public at large on an ongoing basis. The fact that we will use accessible software engineering practices, documenting the code internally with comments, means that the code base will be intellectually as well as physically available. The combination of ongoing commitment by the universities that comprise the Center for Tissue Modeling, and the commitment to open source practices, ensures that NIH investment in the Tissue Simulation Toolkit will reap benefits for years and decades to come.

CORE II: MODELING AND MODEL INTEGRATION

II. 1 PROJECT COORDINATOR: James A. Glazier (IUB), Sima Setayeshgar (IUB), Santiago Schnell (IUB), Mark Alber (Notre Dame)

II.2 PROJECT PARTICIPANTS: Albert-Laszlo Barabasi (Notre Dame), Katy Börner (IUB), Rajiv Chaturvedi (Notre Dame), Andras Czirok (University of Kansas Medical Center), Dan Debasis (IUB), Andrew Lumsdaine (IUB), Roeland Merks (IUB), Glen Neibur (ND), Ramana Pidaparti (IUPUI), Doraiswami Ramkrishna (Purdue), Stefan Wuchty (Notre Dame)

II.3 GENERAL INTRODUCTION:

While core 1 focused on tool development and computer science isuses, this core addresses the biological connections of the mathematical and computational models used to simulate biological processes at various length scales. It discusses these at the philosophical, mathematical, and computational levels of multiscale modeling. It reviews existing simulations and their relevance to the overall goal of a comprehensive multiscale model of tissues and provides technical details about the underlying biology and implementation and integration of these models. It then discusses the applications of these methods to the three Biologically Motivating Projects.

We first discuss subcellular and reaction kinetics models (which can simulate one or a few cells), next the CPM and cell level models (which can simulate up to ~10^6-10^8 cells), then supercellular and continuum models (which can simulate whole organs and organogenesis). We then discuss how we can integrate the different scales of models and how these techiques apply to heart and vascular development and to limb regeneration. In each case we introduce the modeling concepts, review existing models of this type and discuss the details of the modeling method.

The difference between a simulation tool and a simulation or computer model is important. The tool relates to the simulation as a program such as MSWord relates to a novel. We must also distinguish the whole hierarchy of abstractions from a qualitative biological model of a process (e.g., a signal transduction pathway diagram), to a mathematical model (e.g., the set of differential equations which describe the pathway), to a computational model (*e.g.*, a specific implementation of the mathematical model within a tool framework) to a simulation, which includes all of the parameters and data as well as the details necessary to quantiatively determine the predictions of the computational model. These last two live inside (or are written in) the simulation tool which is a language and environment for modeling and simulation.

II.3.i BACKGROUND — DEVELOPMENTAL EXPERIMENTS AND MODELS:

The methods of study of biological development have changed completely in the past twenty years. Classical developmental studies from the 1800s to the late 1970s focused on physiology and morphology. Classic measurements included studies of overall changes in morphology of tissues and organs, rates of cell mitosis and death, cell tracking experiments and fate mapping. This period saw the enunciation of the two main hypotheses for developmental patterning, self-organization and positional coding. In the 1980s, dramatic advances in genetic and molecular biology allowed detailed descriptions of genetic and biochememical events in embryological development, eclipsing classic cell and tissue level methodologies. Initially these biomolecular techniques provided single pieces of information, *e.g.*, about gene expression (*e.g.*, *in situ* hybrydization would reveal which genes were active at given locations in a tissue). More recent techniques, including microarrays and proteomics and the unprecedented ability to control gene expression (*e.g.*, by knockouts, morpholino, *etc*....) provide information on molecular pathways controlling metabolism, cell signalling and gene regulation. Lee Hood and others are now proposing microfluidic lab-on-a-chip techniques which will be able to make hundreds of proteomic and genomic measurements in parallel

(Heath *et al.,* 2003). Similarly, the development of *in vivo* multispectral (GFP/RFP...) labeling and two-photon microscopy has revitalized cell level work by allowing fate-mapping experiments in much greater quantitative detail and *in vivo*.

In parallel with experimental techniques, modeling and computational tools have developed for many scales: the molecular and subcellular, as epitomized by molecular dynamics models of individual molecules, and Reaction Kinetics (RK) models of metabolic, signalling and regulatory pathways; at the level of the cell, the Cellular Potts Model (CPM) and a variety of other methods; and at the level of tissues and organs, continuum, finite element models such as Physiome and SCIRun. Each of these levels has specific advantages and goals arising from the fundamentals of the biology and the biological heirarchy of molecule, macromolecular cluster, cell, tissue, organ and organism sets the mathematical approach of the model. Each level provides certain types of insight into the rules which govern biological function at that scale.

In many ways, the dream of being able to “compute an organism” from genome to animal seems tantalizingly close. The unprecedented flood of molecular level experimental data may allow us to understand how genes and proteins work collectively in cells, but to develop an organism we still need to understand how genetic and molecular signals lead to the physical processes that give an organism its structure. To understand the mechanisms underlying development requires bridging the gaps between experimental observations at the biochemical and genetic level, the cellular level and the level of whole tissues and organs. Metaphorically, we can think of a living organism as an automobile: classic developmental research involved driving the automobile, photographing it, measuring its fuel consumption, *etc.* The early genomics approach provided a pile of disconnected parts with a list of their names. Pathway mapping shows which parts connect to which. Now we need to understand how a motor works, how the subsystems of the car function, the physics of combustion and movement, the interaction of car and driver...

A back-of-the envelope calculation shows that we will never be able to build a computer to build a computer able to model every atom in a living organism from first principles. Even if we could, we would merely reproduce the organism, not understand it. Understanding inevitably requires some degree of abstraction and simplification. Instead, to achieve intelligent multiscale integration, we need insight into the organizing principles at each scale, then to link the scales into a comprehensive model. We must be able to abstract the results of microscopic models and use them to develop phenomenological descriptions at coarser scales, to provide the parameters for coarser scales or to directly control larger scale simulations. Otherwise parameters must come from experiments. Going from large to small, we need more macroscopic results to validate microscopic models in conjunction with experiment.In all cases, the only criterion for a valid model is its ability to predict experimental biological behaviours quantitatively.

In other areas of biology, such as neurophysiology, mathematical and computational modelling has led to fundamental insights and discoveries through synthesis and integration of experimental observations. Such models function as research tools, comparable to powerful experimental methods, for hypothesis testing and for making experimentally verifiable predictions. Model development requires a constant feedback between experiment and simulation. Experiment provides the structures and parameters as well as the initial mechanistic insight for simulation. Simulation then attempts first to postdict experimental results, then to predict new experiments and finally to understand the laws which govern aspects of biological behavior. Comparison between simulation and experiment leads to new experimental questions and to mode refinement. This cycle between model and experiment repeats until we arrive at an acceptable, predictive understanding of biological processes.

Because the core of the Tissue Simulation Toolkit (TST) is the Cellular Potts Model (CPM), we concentrate on links to models one level above and one level below it, *i.e.* Reaction Kinetics (RK) and single cell models and continuum tissue and organ models. We do not focus on models further away in scale, such as molecular dynamics models of individual molecules or population dynamics models of interacting individuals in an environment. Because our initial Biologically Motivating Projects (BMPs) focus on development, we also do not initially address very important issues like the modeling of the nervous system (and Toolkits like GENESIS and NEURON) or specific models of cardiac electrophysiology like Cardiome. Our eventual goal is to integrate these areas as well, but our initial five-year project is already ambitious.

II.3.2 HYPOTHESIS-DRIVEN SYSTEMS BIOLOGY:

For a long time, developmental biologists relied on conceptual models to understand the mechanisms of development. Such a model describes a hypothetical mechanism using text and figures, and suggests further experimentation that could support or reject its hypotheses. With the advent of high throughput experimental procedures, such as DNA sequencing, microarray data, and combinatorial chemistry, the number of known interacting players significant to development (genome, proteins, cell types, extracellular matrix computers, *etc*.) becomes so large that relying exclusively on conceptual models is no longer feasible. Moreover, parallel developments in the physics of complex systems have shown that groups of many interacting particles often exhibit collective behavior that we cannot intuitively understand from the behavior of the individual particles. Thus, paradoxically, additional *information* may make the most central classical tool generating new *knowledge* and *understanding* of development — the conceptual model — no longer helpful.

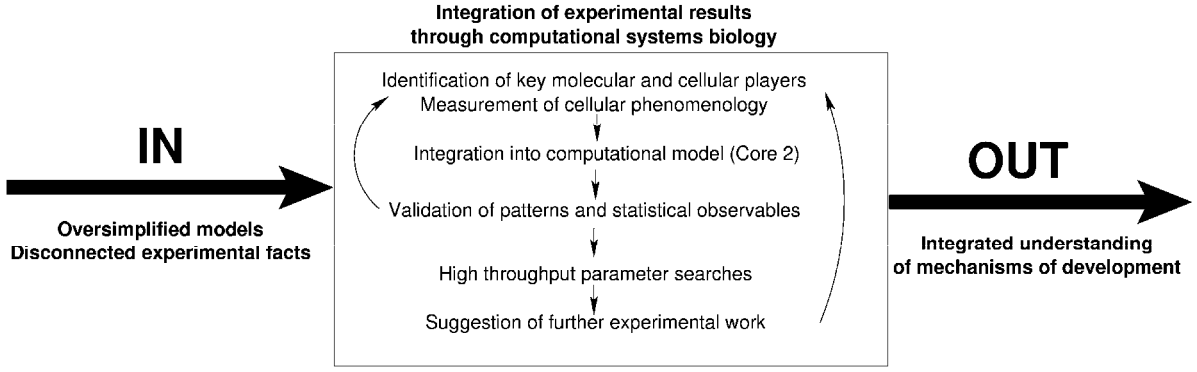


Figure 1. Schematic description of the systems biology approach.

A true understanding of developmental mechanisms requires deployment computational tools that can help organise and model the many interations between the interacting elements of development. We might contrast this *systems biology approach* (Kitano 2002) and Fig. 1). to a caricature of the traditional approach, which blindly lists all the parts of a biological system. However, a systems biology does not essentially differ from more traditional scientific approaches, except that it exchanges *computational models* for traditional *conceptual models* to generate new hypotheses.

II.3.iii BIOLOGICALLY MOTIVATING PROJECTS:

We have selected our initial Biologically Motivating Projects (heart development, vascular development and limb regeneration) for a number of reasons. They address fundamental issues of developmental biology, employ similar experimental techniques which allow synergy between experiments (*e.g*., all use cell tracking, stem cell methods and fate mapping) and demand similar tools for modeling. All are of great clinical importance and have first rate experimental teams within the Indiana research community. In addition, they serve as a model process for studying pattern formation and a number of different aspects of embryogenesis, such as the role of biological clocks during development, gene expression and cell differentiation, cell-cell signaling and signalling cascades, cell migration and adhesion. They also involve a number of related morphological changes including epithelim and tube formation, branching, apoptotic pruning, differential growth, mesenchymal condensation and invasive migration. As such, they serve as important paradigmatic models for investigating multiscale effects.

II.4 SPECIFIC AIMS:

- To develop the Cellular Potts Model (CPM), which treats individual cells, their collective interactions, interactions with extra cellular matrix and chemical dynamics, in a self-consistent manner to describe the many biological mechanisms which contribute to development
- To develop specific models within the CPM framework for our three BMPs

- To establish the parameters (and their experimental valves – see Core 3) needed for the CPM models for our three BMPs
- To validate these models against the BMP experiments
- Develop homogenization techniques and software links to connect the CPM with smaller scale models—initially Virtual Cell and BioSpice and to larger scale models—initially Physiome and SCIRun
- To use the small scale models to calculate parameters for the CPM and to generate phenomenological models for use with the CPM
- To use the CPM reults to validate the microscopic models
- To use the CPM to calculate parameters for Physiome and SCIRun and to generate phenomenological models for use with these models
- To use the large scale model results to validate the CPM
- The diagram below shows the specific scales that we plan to address with our simulation tools

Level (by color)	Biophenomenon	Model
Supra Tissue/ Organ Level	Integrated multiphysics models	
Tissue level Tissue and cell level Cell level	Cells -Division -Differential adhesion -Growth Extra Cellular Matrix	Cellular Potts model: Differential adhesion ECM Cell motility
Subcellular level	Cytoskeleton: Cell motility, Mitosis, Apoptosis Genetic regulatory network Cell differentiation	Cell Type change map ("state" diagram)
Molecular Level	Diffusion of extracellular and intracellular chemicals Production of morphogens	Reaction Diffusion mechanisms (e.g., TFG-β) Cell level ODE's (e.g., SAM

Table 1. Subcellular Models

II.5 SUBCELLULAR MODELS:

II.5.i INTRODUCTION:

Multicellular organisms are not born fully formed. Rather, they arise by a relatively slow process of progressive change known as development. In nearly all cases, the development of a multicellular organism begins with a single cell (zygote) which divides mitotically to produce all the cells of the body.

One of the major functions of development is the production and organization of all the diverse types of cells in the body. The zygote gives rise to muscles cells, skin cells, neurons, blood cells and all the other cells types. The generation of cellular diversity is *differentiation*; the process that organizes the different cells into tissues and organs is *morphogenesis*. Cells interact with one another and rearrange themselves to produce organs, in *organogenesis*.

The differentiation of cells reflects one of the major phenomena of embryogenesis: the cell selects or commits to a particular developmental pathway. The genomes of the differentiated cells remain the same;

the pattern of gene expression, that is, the *phenotype*, changes. Some differences between cells in the early embryo originate from the unequal distribution of cytoplasmic determinants localized in the egg before cleavage, but most arise later from local differences in the environment of the cells. Therefore, cells often must choose one among several possible developmental fates in response to a chemical signal such a hormone, growth factor or specific substrate.

Undifferentiated cells sense and process stimuli via signal-transduction cascades. These molecular circuits detect, amplify, and integrate diverse external signals to generate responses such as changes in the metabolism, or gene expression. Metabolic signals or signal-transduction cascades regulate genetic circuits which control gene expression. Metabolism is the highly integrated network of chemical reactions controlling the cells' energy transduction. Since enzymes catalyse almost all cells' metabolic reactions, strictly regulating their activity according to the needs of the cell or organism is essential. Characteristically, gene-expression and translation-level control mechanisms regulate enzyme synthesis, while rapid regulatory mechanisms act directly on enzymes. These complex interactions lead to developmental differentiation and pattern formation.

Subcellular models address a number of these issues: how cells absorb and use materials from their environment (metabolism), how they respond to and create signals in their environment (signal transduction) and how they change their gene expression (genetic regulation).

Classical pathway diagrams describe the successive interactions of enzymes, substrates, and other chemicals as directed graphs bring a *network*. To move from this qualitative description to a quantitative, we must make simplifying assumptions about the nature and kinetics of these interactions. The most common framework for descriptions is *Reaction Kinetics* (RK) , which describes each interaction as an ODE and the network as a system of many coupled ODEs.

II.5.ii SPECIFIC AIMS:

Our overall goal is to use reaction kinetic models predicatively to support larger scale models either by providing calculated coarse-grained parameters, to generate tractable phenomenological models. A major limitation of existing modelling tools is that they employ one of the three different kinetics regimes to describe complex biochemical pathways: discrete and stochastic, continuous and stochastic and continuous and deterministic. Most tools model reactions in the continuous and deterministic regimes using the law of mass action and quasi-steady-state kinetics or to control the large-scale model directly.. Mass action and quasi-steady-state kinetics are valid only under certain conditions, which do not always apply in *in vivo*. We must determine the most valid regime and model for the reactions we consider.

Within this context, we will address the following specific items:

1. Evaluate existing RK models
2. Adopt the best RK model
3. Improve the RK model if necessary
4. Devlop hooks to extracellular modeling frameworks (CPM and Continum models)
5. Collect input data for RK models from literature
6. Generate experimental input data for RK models from our specific experimental systems
7. Determine the appropriate regime and model for each BMP
8. Use the output of RK model to generate CPM or other larger scale model parameters for our specific model systems
9. Develop our own phenomenological models to run the CPM without RK
10. Run the RK models inside each cell in the CPM to directly control the CPM
11. Validate the CPM/RK combination experimentally
12. Investigate the Continuum/RK combination experimentally
13. Develop additional RK related material that is not included in the adopted RK model
14. Extend compatibility to other RK models

II.5.iii BACKGROUND:

The development of a multicellular organism with differentiated cells requires three main processes:

1. Gene regulation. All cells in the tissues of an organism have the same genetic information. Differentiation arises because different genes are active.
2. Cell reproduction and survival. Differentiation states are heritable through cell division. Differentiated cells must fulfil their required functions in tissues and survive. (Usually they must also produce.)
3. Spatial patterns. Differentiated cells form a specific and repeatable spatial pattern, either through 'self-organization' and/or 'external specification'.

All these processes work through and require controlled sequences of complex biochemical reactions and other physico-chemical processes such as transport and diffusion. Most biochemical reactions continually taking pace in living organisms involve proteins called enzymes, which act as remarkably efficient catalysis.

The complexity of biological and biochemical processes often requires the development of mathematical and computer models to help understand the phenomena under consideration. Numerous modelling toolkits deal with such biocomplexity. From such models and modelling toolkits we must select biochemically and biologically pausible RK models. In the next section we discuss some kinetic models and their corresponding mathematical representations, which cover a large range of real reactions, and some general types of complex reaction phenomena (such as biochemical pathways and metabolism.) Evaluating RK models is essential to construct modeling toolkits, such as the TST which reflect the specific properties of a biochemical phenomenon.

II.5.iii.1 PARAMETERS:

Subcellular models required a number of parameters depending on the biochemical reactions or physico-chemical processes being simulated. They also depend on the timescales at which the simulated reactions occur. Key parameters are the rate of equilibration, equilibrium constants, reaction rates, rate constants, and enzyme kinetic parameters such as the apparent maximum velocities, the Michealis-Menten constants, the Hill coefficients and other allosteric constants. Other important constants include the steady-state concentration of reactants, their basal synthesis and degradation rates, diffusion coefficients and transport rates. Dilution coefficients due to cell division, cell volumes and masses, cell growth rates, cell cycle times are necessary for models involving cell growth and division. To account for genetic regulation requires parameters such as transcriptional and translation delays. Most importantly of all, the retro pathology needs to be mapped in detail, since missing stages or interactions can completely change its qualitative behavior.

II.5.iii.2 DATA SOURCES:

While the supply of data needed for RK subcellular models is increasing rapidly, we are still ignorant of many crucial pathways and parameter values. The current experimental focus is uneven, with much more effort devoted to pathway mapping than to determining kinetic constants. Nevertheless, we fell it is appropriate to start building the simulation tools and models now so that they are ready as the necessary data becomes available.

Most of the modelling toolkits we have discussed obtain their data from reviews of the published literature on the problems under consideration. We discuss those references in the specific subsection for each BMP below. We can also obtain parameters by least square fitting of related experimental data or by extrapolating or guessing from benchmark valves.In certain cases we must look carefully at our simulation's sensitivity to changes in model parameters. In all cases we must worry about the reliability and accuracy published values, especially whether they were obtained under comparable conditions. A cell in a tissue and the same cells *in vitro* often have different physiologies, patterns of gene expression, and concentrations of key molecules. Adequate information to judge data reliability is often not .

Other sources include:

1. The Center for Medical Genomics (CMG), which, as a participant in this proposal (see Core 3), is committed to providing gene expression data using their MicroArray Data Portal, which is key input information for our TST. The MicroArray experiments that the CMG carries out is support at the BMPs.
2. Parameters obtained from simulations in other modeling toolkits such as V-CELL, BioSpice and SCIRun.
3. Monograph and internet database literature, as sources of kinetic and biochemical pathway data. The book *Biochemical Pathways: An Atlas of Biochemistry and Molecules Biology*, edited by Gerhard Michal, provides a general survey of numerous pathways related to our BMPs. Various internet databases provide more detailed and current information and help locate original papers and data. The following table gives a selection of these data (local copies of many of these databases are maintained at IU):

Name	**Address**	**Species**	**Data available**
KEGG	http://www.genome.ad.jp/kegg/	General	Pathways structure, Enzyme characteristics, Protein sequence, DNA sequence
LIGAND	http://www.genome.ad.jp/dbget/	General	Enzyme characteristics
BIOCHEMICAL PATHWAYS	http://www.expasy.org/cgi-bin/search-biochem-index	General	Pathways structure, Enzyme characteristics
WIT	http://wit.mcs.anl.gov/	General	Pathways structure
ENTREZ	http://www.ncbi.nlm.nih.gov/Entrez/	General	Pathways structure, Enzyme characteristics
ENZYME	http://ca.expasy.org/enzyme/	General	Enzyme characteristics
PDB	http://www.rcsb.org/pdb/	General	Protein structure
CATH	http://www.biochem.ucl.ac.uk/bsm/cath/	General	Protein structure
SCOP	http://scop.mrc-lmb.cam.ac.uk/scop/	General	Protein structure
SWISS-PROT	http://ca.expasy.org/sprot/	General	Protein sequence
TRANSFAC	http://www.gene-regulation.com/	Eukaryotes	Transcription factors, their genomic binding sites and DNA-binding profiles
PATHO	http://www.gene-regulation.com/	Eukaryotes and humans	Pathologically relevant mutated forms of transcription factors and transcription factor binding sites
S/MARt DB	http://www.gene-regulation.com/	General	Scaffold/matrix attached regions and the nuclear matrix proteins that are involved in the interaction with the nuclear matrix
CYTOMER	http://www.gene-regulation.com/	Human and Mouse	Gene expression sources which list physiological systems, organs and cell types
TRANSCompel	http://www.gene-regulation.com/	Eukaryotes	Composite regulatory elements affecting gene transcription

Name	Address	Species	Data available
TRANSPATH	http://www.gene-regulation.com/	Eukaryotes	Signal transduction pathways, in particular those that aim at transcription regulatory components
PEDANT	http://pedant.gsf.de/	General	Protein sequence
PDD	http://www-lecb.ncifcrf.gov/PDD/	Human	Enzyme characteristics, protein sequence and DNA sequence of proteins involved in disease
GENBANK	http://www.ncbi.nlm.nih.gov/	General	DNA sequence
dbEST	http://www.ncbi.nlm.nih.gov/dbEST/	General	DNA sequence
GDB	http://www.gdb.org/	Human	Enzyme characteristic, protein sequence, DNA sequence
OMIM	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM	Human	Enzyme characteristic, protein structure, protein sequence and DNA sequence of genes and genetic disorders

Table 2: Internet database literature

Below we tabulate a number of approaches researchers have taken to model cellular and subcellular phenomena. The list provides an overview of various approaches, with typical examples wherever applicable. In the table below, serial numbers 1 to 5 consider models devoted to cell dynamics and the complex outcomes like tissue formation and patterning that results from it. The approaches can be broadly categorized as follows. *Continuum based models of cell behavior* (Serial nos. 1, 2, 3), are more interested in gross behaviors and properties like cell membrane phenomena, cell densities. However, continuum approaches have considered scales of cellular phenomena too, for example, FEM based models for cells in serial no. 1. *Discrete models* (Serial nos. 4, 5), based on a CA approach, consider individual cells when trying to describe dynamics and motions of such cells and the resulting social behavior. The third category deals with *subcellular scales* where there are a multitude of phenomena of biological interest- reaction pathways, genetic processes, protein folding, etc., Serial nos. 6, 7, and 8 are examples of such efforts.

Often these modeling approaches are complementary to each other; hence, an interaction between such systems is a way forward to consider a biological system as a whole.

S. No.	Title	Description	Author/Institution	Reference
1	FEM based (continuum) models of individual cell phenomena	Here, though the phenomena under consideration are at the scale of individual cells and subcellular (for example, membrane permeability) a continuum approach is taken. Numerics is usually FEM based.	Numerous, for example Laboratory of Biocybernetics, Univ. Ljubljana / http://lbk.fe.uni-lj.si	Pavlin M., Pavselj, N., Miklavcic, D., 2002, Dependence of Induced Transmembrane Potential on Cell Density, Arrangement, and Cell Position Inside a Cell System, (IEEE Trans. Biomed. Engg., Vol 49, No. 6, June 2002)
2	Constitutive equations of tissue mechanics, continuum approach	Gross properties of bone tissue, FEM solutions of continuum equations, experimental measurements, does not consider individual cells	Glen Niebur, Tissue Mechanics Lab, University of Notre Dame / http://cauchy.ame.nd.edu/	Niebur, G L, Yeh, O C, and Keaveny, T M. "Damage evolution in trabecular bone is anisotropic". in 48th Annual meeting of the ORS. 2002. Dallas (2002).

S. No.	Title	Description	Author/Institution	Reference
3	Reactor diffusion continuum models	Cell density used as continuum variable, does not consider individual cells	Hentschel, Newman, http://www.mathcs.emory.edu/~tglimm/Index.html	Abstract at: http://www.eps.org/aps/meet/MAR02/baps/abs/S4700008.html
4	CPM based discrete models with phenomen-ological energy formalisms	Models individual cells dynamics to generate tissue scale phenomena, mesoscale model. It has been integrated into continuum models for morphogen diffusion and into Regulatory Networks describing cell differentiation. Hence, CPM is evolving as a *framework* to allow for integration of various modeling approaches, as this proposal stresses.	Glazier, http://www.biocomplexity.indiana.edu/ Alber, http://www.nd.edu/~icsb/	Chaturvedi, R., Izaguirre, J. A., Huang, C., Cickovski, T.,Virtue, P., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A., and Glazier, J. A., "Multi-model simulations of chicken limb morphogenesis", LNCS, Volume 2659, Springer-Verlag, New York, 39-49 (2003)
5	LGCA and other rule based discrete CA models	Models cells and cell colonies using rule based CA	Alber, http://www.nd.edu/~icsb/	Alber, M.S., Kiskowski, M.A., Glazier, J.A., and Jiang, Y. "On Cellular Automaton Approaches to Modeling Biological Cells", Mathematical Systems Theory in Biology, Communication, and Finance, IMA Volume 134, Springer-Verlag, New York, (2003)
6	E-cell: Hybrid model using continuum as well as discrete approachces	Modeling subcellular biochemical and genetic processes, utilizes an hybrid and integrative approach at subcellular scales	http://www.e-cell.org/	Kinetic simulation of signal transduction system in hippocampal long-term potentiation with dynamic modeling of protein phosphatase 2A by Kikuchi,S., Fujimoto,K., Kitagawa,N., Fuchikawa,T., Abe, M., Oka, K., Takei,K., and Tomita,M., published in the November issue of *Neural Networks*
7	M-Cell Monte Carlo simulations of subcellular molecules	Behavior and variability of real systems comprising finite numbers of molecules interacting in spatially complex environments inside cells. Upward compatibility with CPM possible.	Bartol, Computational Neurobiology Lab., The Salk Instt. Stiles, Biomedical Applications, Pittsburg Supercomputing Center. http://www.mcell.cnl.salk.edu/	Franks, K. M., Bartol, T. J., and Sejnowski, T. J., An MCell model of calcium dynamics and frequency-dependence of calmodulin activation in dendritic spines, *Neurocomputing* 38-40, 9-16, (2001).
8	Virtual Cell: Simulations of reaction pathways	Models biochemical and electrophysiological mechanism describing individual reactions. Experimental microscopic image data describe their subcellular locations.	National Resource for Cell Analysis and Modeling http://www.nrcam.uchc.edu/	J. Schaff, C. Fink, B. Slepchenko, J. Carson, L. Loew, (1997) "A General Computational Framework for Modeling Cellular Structure and Function", Biophys. J. 73:1135-1146.

Table 3. Example list of cellular and subcellular models

II.5.iv PRIOR WORK AND ACCOMPLISHMENTS:

II.5.iv.1 IUB GROUP:

Santiago Schnell studied the metabolome (metabolomics), which the analysis of multiple interactions of gene products in biological networks. Schnell's interests in this area are 1. understanding the dynamics of complex biochemical reactions to determine this kinetic parameters; 2. studying methods for simplifing the reprersentation of complex biochemical reactions and for determining the conditions of their validity; 3. describing the physical-chemistry of biochemical reactions *in vivo*; and 4. the developing methods for deducing the mechanisms of complex biochemical reactions.

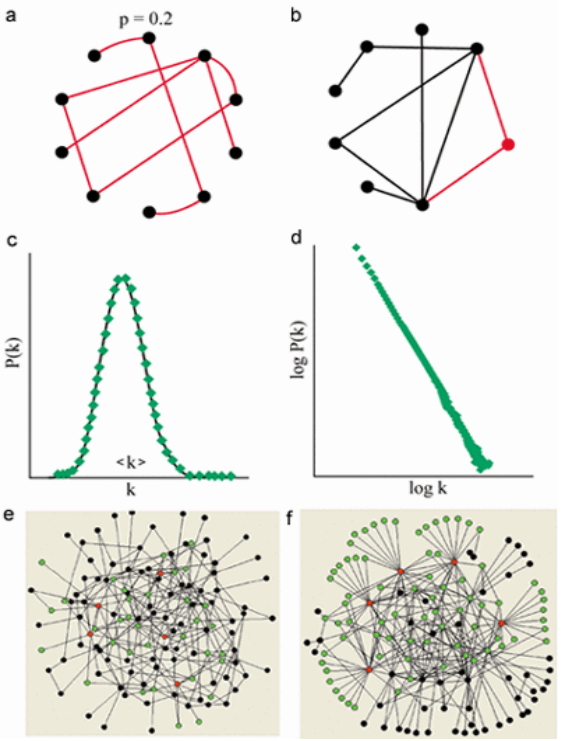


Figure 2: Illustration of an (a, e) exponential and (b, f) scale-free network. Connectivity distribution P(k) of (c) exponential and (d) scale-free network, giving the probability that a given node is connected with k other nodes.

His major accomplishment, developing new procedures for calculating kinetics parameters from progress curves involved the creation of a new mathematical description for numerous enzyme-catalyzed reactions formulated in the same terms as the experimental measurements. These new procedures reduced the number of experimental assays necessary for the determination of kinetic parameters. Furthermore, he has also determined the conditions under which velocity expressions derived by various assumptions (*e.g.,* quasi-steady-state approximations, lumping, and pseudo-first order approximations) are valid (Schnell and Maini, 2003)).

He has also found that conventional methods based on rate constants fail to describe reactions in *in vivo* conditions. Under minimal obstruction to diffusion, the rate constant approach is valid but in the presence of significant obstruction due to macromolecular crowding, the logarithm of the rate coefficient decays linearly on a logarithmic timescale, so the rate coefficient is time-dependent (*i.e.,* not constant*)* (Schnell and Turner, 2004).

Finally, Schnell and his collaborators have succesfully combined the interactions between molecular clocks and cell coordination to model segmentation formation (*somitogenesis*) during early development (Schnell *et al.,* 2002).

Sima Setayeshgar brings expertise on modeling tools and computational algorithms for studying chemical and biological systems. She has worked on numerical simulations of stationary Turing pattern formation in chemical reaction-diffusion systems, using realistic models of the chemical kinetics and experimental geometry (Setayeshgar, 1998, 1999). She has also worked on nonlinear spatiotemporal patterns in excitable media, such as the heart; in particular, her work has shown analytically for the first time the crucial role of the anisotropy inherent in cardiac tissue on the stability of nonlinear waves, with important implications for the onset of arrhythmias (Setayeshgar, 2002).

Setayeshgar also brings experience on biophysical modeling of intracellular processes: She has worked on understanding the role of diffusive noise on the accuracy of biochemical signaling, important in gene expression as well as external signal transduction (Setayeshgar, 2003a). She has also worked on modeling and simulating signal transduction and motor response leading to chemotactic behavior in a population of bacteria. In this work, a Monte Carlo description of these intracellular processes for each cell was combined with a macroscopic time-stepping scheme for the evolution of the population. This hybrid computational approach has built on and extended the new multiscale method of Path Dynamics (see section on multiscale modeling), and will serve a prototype for the development of similar multiscale methods within TST. Setayeshgar will participate primarily in the modeling and CPM-based simulations of the cardiac development experimental platforms (See Core 3). She will also work with the rest of the computational core in building averaging and lifting algorithms (see below) to interface CPM with (i) subcellular models of reaction kinetics in one limit, as well as (ii) continuum models and methods at the other limit.

II.5.iv.2 NOTRE DAME GROUP:

Albert-László Barabási's Group at Notre Dame has focused on the large-scale topological organization and function of cellular networks. They examined the topological properties of the core metabolic networks of 43 different organisms based on data deposited in the WIT (now ERGO) database (Overbeek *et al.,* 2000). In the metabolic network, nodes are the substrates that connect to each other through the metabolic reactions. Their results indicate that in *E. coli* and 42 other organisms representing all three domains of life the probability that a given substrate participates in *k* reactions follows a power-law distribution, *i.e*., metabolic networks are scale-free (Jeong *et al*., 2000). They also found that the diameter of these network is constant for all organisms. The metabolic networks of the 43 organisms consist of many small, highly connected topological modules which ultimately combine in a hierarchical manner into larger, less cohesive units, with their number and degree of clustering following a power law (Ravasz *et al*., 2002; see Figure 2). Recent experimental evidence shows that this organization has a hierarchical modularity which closely overlaps with known metabolic functions, and global transposon mutagenesis indicates that enzymes within such modules possess preferentially essential or non-essential phenotypes (Gerdes *et al.*, 2003). Further flux balance analysis (Edwards, 2002) for *E. coli* metabolism shows that a connected high-flux reaction backbone dominates metabolic activity. The overall flux distribution is has a distinct power law. This analysis also revealed that *E. coli* responds to changes in growth conditions by reorganizing this high flux backbone in a discrete fashion while leaving small fluxes largely unaltered (Almaas *et al*., 2004).

II.5.iv.3 PURDUE GROUP:

The Purdue group has experience in stochastic simulations of particulate systems in general including biological populations. Ramkrishna (Shah *et al*., 1976, 1977) published a series of stochasticmethods in the chemical engineering literature independently of SSA and at the same time. Since then stochastic simulation has been a routine feature of Purdue research (see Ramkrishna curriculum vitae for list of complete works).

Ramkrishna's has developed metabolic framework that successfully models diverse uptake patterns in bacterial cultures in mixed substrate environments reproducing both the sequential uptake patterns of mixed carbon substrates in the famous *diauxie* experiments of Monod, and the simultaneous uptake patterns of organic acids. Their modelling approach also describes conflicting trends in biomass concentration in chemostat cultures under dual limiting conditions of carbon and nitrogen substrates, and

the steady state multistable behavior of chemostat mammalian cell cultures. The model correctly predicted two start-up strategies to obtain high cell density steady states. The same model also describes the behavior of the wild-type organism as well as two additional engineered genetic strains.

II.5.v KINETIC MODELLING OF BIOCHEMICAL REACTION NETWORKS:

Mechanistic studies of reactions are important in biochemistry for several reasons: 1. Improved understanding of the functional role of different molecules requires knowing the mechanism of specific reactions and the nature of key intermediates; 2. Understanding the control (or regulation) of pathways requires some hypothesis about reaction mechanisms; and 3. Kinetic modelling, the basis of reaction kinetics, requires comprehensive information about reaction mechanisms. Kinetic models allow simulation of complicated pathways, even whole-cell simulations, which are proving to be an increasingly important predictive tool in the post-genomic era (Noble, 2002). Kinetic modelling typically requires time series data on biochemical response to different conditions and stimuli. Deducing reaction mechanisms from time series data remains something of an art, with few attempts to derive general approaches until recently (Crampin *et al.,* 2004).

A full reaction mechanism is the set of elementary steps that specifies how a chemical reaction takes place. Elementary reaction steps are those which we cannot be decompose to reveal reaction intermediates which we might indentify as separate chemical entities. A comprehensive description of the reaction therefore requires the number of chemical species and processes, the sequence of their interactions and the rate laws governing the elementary reaction velocities. A description composed solely of elementary reaction steps aims to chart the progress of actual molecular events.

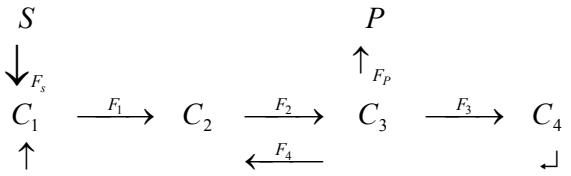
The goal of a mechanistic study is to clarify the nature of reaction intermediates and their interrelations (how they react with, or transform into, each other) and to determine the rate of these transformations. We can identify into two aspects: a mechanism has a connectivity (or wiring diagram), and the individual interactions need appropriate kinetic properties or rate laws. Therefore, the mathematical models describing complex biochemical reaction mechanisms torn in two groups according to their structure: stoichiometric and kinetic models. The former use the time invariant characteristics of the reactions and the latter both the stoichiometry and reaction rates.

II.5.v.1 STOICHIOMETRIC MODELS:

The topology reflects the relationships between intermediates, the stoichiometry of the reaction steps and of the overall mechanisms. We can think of a reaction mechanism as a network that connects the various reactants, intermediates, and products. Stoichiometric models concern the proportional changes in the concentrations of the reacting species. These proportions result from the topological structure of the reaction mechanism, which indicates what species are link to each other by reactions. This point of view can lead to important general results about the properties of reactions. In contrast to the kinetic models, which deal with velocity changes and vary in time, the stoichiometric models are structurally invariant. The stoichiometric properties do not depend on the mathematical description of the rate laws and are often better known than the kinetic parameters of the reactions. However, stoichiometric models have a major drawback: their lack of regulatory information, which only kinetic models include, limits their predictive power.

II.5.v.2 KINETICS MODELS:

Kinetic models deal with biochemistry. Their formulation requires both the reaction stoichiometry and detailed information about the kinetics. Kinetic models combine kinetics with known reaction pathway stoichiometry. Consider the following stoichiometric pathway scheme:



. (II. 1)

In this reaction scheme, we consider a biochemical pathway in which a substrate S_1 synthesizes a product P a through the intermediates C_i. The pathway has negative feedback in C_1 triggered by the end intermediate C_4.

Now, based on the stoichiometry we can write the mass balance equation for the rate of change of intermediate C_3 as:

$$\frac{d[C_3]}{dt} = 2F_2 - (F_3 + F_p), \qquad \text{(II. 2)}$$

where the F_i are the reaction rates. Here we assume no mass flow due to convection or diffusion. The algebraic expression for the reaction rates will depend on the kinetics under consideration. Many biochemists employ experimentally derived phenomenological rate laws. However, the literature often reports more accurate kinetics. Mathematical approaches to representing the kinetics of a complex biochemical pathway include (Burrage *et al.,* 2004):

1. Directed graphs in which the chemical species are vertices ad the interactions between the species are the edges.
2. Bayesian networks in which the vertices correspond to random variables that describe a reaction while the network defines a joint probability density function
3. Boolean networks in which a chemical species is either on or off
4. Ordinary Differential Equations (ODEs) in which rate equations represent the concentrations of chemical species. The most common rate laws are mass action, Michaelis-Menten and Allosteric kinetics, and the Hill power-law approximation.
5. Delay Differential Equations (DDEs) which introduce time delays into the ODEs to describe the time delay between the initiation of transcription and the initiation of translation of genes or between the initiation and finalization of protein translation
6. Partial Differential Equations (PDEs) which take into account spatial structure of cells and tissues.
7. Stochastic Differential Equations (SDEs) and Stochastic Simulation Algorithms (SSA) for which we have to decide whether to work with chemical concentrations or with individual molecules

The most common methods and modeling tools in the literature are: ODEs using mass action, Michaelis-Menten, Allosteric and Hill kinetics, Stochastic Differential Equations (SDEs) and Stochastic Simulation Algorithms (SSAs).

II.5.v.3 ODES AND THE LAW OF MASS ACTION:

ODEs obtained from a mechanism by the law of mass action determine the behaviour of a homogenous chemical system. The rate of any given elementary reaction is proportional to the product of the concentrations of the reactants in the elementary process. This law was postulated more than a century ago to describe empirical observations about the rate of chemical reactions though it is consistent with results of non-equilibrium thermodynamics (see for example, Keizer, 1987). The proportionality constant, known as the rate constant, depends on the reaction conditions (temperature, solvent, pH, *etc*). Experimentally, biochemists generally try to hold the reaction conditions constant to avoid dealing with higher-order effects due to variation of the rate constants.

Let us consider an example. Applying the law of mass action, the governing equations of the reaction:

$$S_1 + S_1 \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} S_2 , \qquad \text{(II. 3)}$$

are

$$\begin{aligned} \frac{d[S_1]}{dt} &= -2k_1[S_1]^2 + 2k_{-1}[S_2] \\ \frac{d[S_2]}{dt} &= k_1[S_1]^2 - k_{-1}[S_2] \end{aligned} . \qquad \text{(II. 4)}$$

In this example, the stoichiometric coefficients and exponents are integers, but for reactions in nonideal solutions, they can be noninteger (Othmer, 1981). This is part of the basis of the power-law formalism, which it will be discussed below.

II.5.v.4 ODES AND ENZYME KINETICS:

Enzyme catalysed reaction can be described in detail by incorporating all the elementary steps of the enzyme–substrate association–dissociation, isomeration of intermediates and formation of products. The major problem of this approach is that produces systems of highly-nonlinear differential equations with a many parameters. These systems are stiff, have multiple timescales and are computational demanding to solve numerically.

The kinetic modelling of enzymatic reaction can be simplified considerably if the overall reaction is studied with the aid of the quasi-steady-state or equilibrium approximations. Consider the simplest enzymic reaction, in which there is a reversible association between an enzyme E and a substrate S yields an intermediate enzyme--substrate complex C that irreversibly breaks down to form a product P:

$$S + E \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} C \overset{k_2}{\rightarrow} P + E . \qquad \text{(II. 5)}$$

Under the quasi-steady-state conditions, the substrate and product concentrations in reaction scheme (MMreac) are mathematically described by the Michaelis–Menten (MM) equation (Boyde 1980)

$$v = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = \frac{v_{\max}[S]}{K_M + [S]} , \qquad \text{(II. 6)}$$

where v_{max} is the maximum velocity, and $K_M=(k_{-1}+k_2)/k_1$ is the Michaelis–Menten constant. This equation is arguably among the most important in biochemistry.

Clearly the advantage of employing the quasi-steady-state or equilibrium approximations is that reduce the order of the governing differential equation system and the number of parameters. The disadvantaged of reduction is that its are only valid under certain conditions, which limit the applicability of the reduce model to a small region of the parameter domain (Schnell and Maini, 2003).

An important aspect of enzyme kinetics in complex biochemical pathways is the effect of inhibitors and activators, or the intervention in reactions of allosteric enzyme. In all these cases, the derivation of the quasi-steady-state rates laws generally involved solving a system of linear algebraic equations for the enzyme-containing species. The number of concentration variables by considering that the total enzyme concentration is conserved. This is one of the most common assumptions made in the modelling of complex biochemical pathways. It is important to note that the overall rates laws for the enzymatic reaction exhibit a number of hallmark behaviours, which are unique to enzymatic reaction.

II.5.v.5 ODES AND THE POWER-LAW FORMALISMS (S-SYSTEMS):

Savageua (1969, 1976) proposed the power-law approximation as an alternative approach for modelling reactions following non-ideal kinetics, like those occurring under molecular crowding within cells

(Savageau, 1992). The power-law approximation is a phenomenological extension to the law of mass action where the enzyme kinetics rate expressions are linearised in terms of the concentrations (Heinrich and Rapopport 1974; Palsson *et al.*, 1985) or in terms of the reaction parameters (Kenden and Caplan, 1965; Rottenberg, 1973). A number of essential properties of the rate laws can be modeled with this approximation, but it fails to describe many important biochemical effects such as saturation and sigmoidicity (Heinrich and Schuster, 1996).

An alternative modelling formulation based less on physical principles and more on mathematical amenity. In contrast to the law of mass action and Michaelis-Menten equations the ``synergistic-system''(S-system) assumes a general formulation where the rate of change of a state variable is equal to the difference of two products of variables raised to (non-integer) powers:

$$\frac{dx_i}{dt} = \alpha_i \prod_{j=1}^{n} x_j^{g_{i,j}} - \beta_i \prod_{j=1}^{n} x_j^{h_{i,j}} \text{ for } i = 1,...,n. \qquad \text{(II. 7)}$$

In the law of mass action, the kinetic orders are given by the stoichiometric coefficients, while in the power-law approximation they are phenomenological parameters which may or may no be integer, and can be either positive or negative.

A negative stoichiometric coefficient does not immediately appear to be a very physically meaningful, but they are an important aspect in the power law approximation to describe the effects of inhibitors. Unfortunately, such negative exponential can lead to singularities in the rate laws rendering useless from the mathematical point of view.

Forty years ago, it was acceptable to employ the power-law formalism when we did not count with the present computer power to handle complex fractional mathematical expression such as the Michaelis–Menten equation. However, the application of the power-law approximation seems inappropriate unless our aim is to model complex biochemical reaction where the detailed kinetics description is not available.

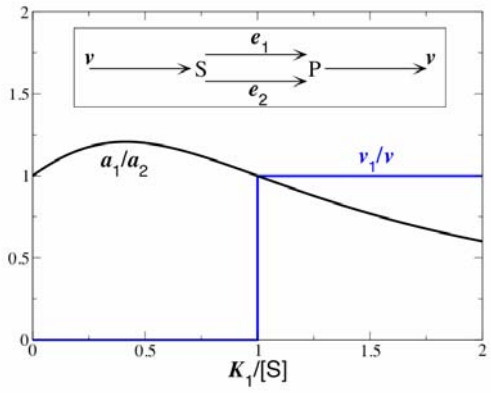
II.5.v.6 OTHER MODELING FORMALISMS:

There is, in any modeling effort, some arbitrariness about the choice of mathematical formulation. However, different mathematical formulations are better able to capture one or other features of the data, and represent the underlying hypotheses of a theory more or less faithfully. There is currently an active debate in the biochemical modelling literature as to the appropriate modelling formulation for biochemical network kinetics (Schnell and Turner, 2004). The traditional approach, which encompasses the law of mass action and power-law approximation, described above, has been the basis for kinetic modelling for over a century, in particular in modelling enzyme kinetics. This approach naturally extends to include partial differential equation models for spatially distributed processes, such as reaction-diffusion models, which are derived from conservation equations, or coupled high dimensional systems of ODEs for compartmental spatial modelling, and to stochastic differential equations (Langevin equations) to include noise-driven processes, including situations in which variability for example due to low concentrations (small populations of molecules) plays an important role (for example in regulation of gene expression, where small numbers of regulatory proteins interact with DNA binding sites in the gene's promoter region (McAdams and Arkin, 1999)). Several other approaches have proven to be useful in some situations, these have been recently review by Schnell and Turner (2004).

Simulations of stochastic gene expression (McAdams and Arkin, 1997) based on studies of biochemical reaction networks by Arkin and Ross (1994) suggest that an activated promoter produces variable numbers of proteins in short bursts at random time intervals. As a result, the time between successive events in regulatory cascades can differ greatly across a cell population.

Many molecules that control genetic regulatory circuits act at extremely low intracellular concentrations in inhomogeneous environments. Resultant fluctuations (noise) in the reaction rates can cause large random variation in rates of development, morphology and the instantaneous concentration of each molecular species in each cell. For regulatory reliability cells use redundancy in genes as well as redundancy and extensive feedback in regulatory pathways. We shall model such noisy processes in inhomogeneous environments using Monte Carlo techniques (Krauss *et al.*, 1996).

II.5.vi PREDICTING THE LOCATION AND BEHAVIOR OF METABOLIC SWITCHES:



This simple example illustrates how the constrained optimization method allow us to determine the existence of potential metabolic switches. Consider two isoenzymes (1 and 2) catalyzing the conversion of the substrate S into the product P. In this case, the total enzyme concentration (1) is given by $[e]=a_1v_1+a_2v_2$. The optimal reaction rates (v_1,v_2) are those minimizing [*e*] subject to the mass conservation constraint $v_1+v_2=v$.

In general isoenzymes have different kinetic properties that justify their differential use depending un the current physiological conditions, therefore we use two different kinetic models for each iosenzyme. One enzyme will be described by an irreversible Michaelis-Menten rate equation model and the other by a Hill type rate equation model with exponent 2, *i.e.* $v_1=[e_1]/a_1$ and $v_2=[e_2]/a_2$ with

$$a_1=\frac{1}{k_1}\left(1+\frac{K_1}{[S]}\right) \qquad a_2=\frac{1}{k_2}\left(1+\left(\frac{K_2}{[S]}\right)^2\right)$$

where K_1 and K_2 are the saturation constants for the substrate S corresponding to isoenzymes 1 and 2, respectively. For simplicity we set $K_1=K_2$. The plot at the left show that there is a certain range of [S] where $a_1>a_2$ ($a_1<a_2$) and all the flux go over the second (first) pathway. If we wouldn't know the rate equation models we could assign random values to a_1 and a_2 discovering that in some cases the pathway 1 (or 2) is active while the other is inactive, indicating the existence of a potential metabolic switch. The method can be extended to pathways of arbitrary topology to identify lest obvious novel metabolic switches.

Much of the emphasis of the research done in kinetics models has been on the application of techniques in system dynamics, non-linear systems and control theory for understand the system under consideration. However, little attention has been paid to efficient reduction methods which would simplify the complexity of the kinetic model for further analysis and simulations. The Barabasi Group at Notre Dame has developed a constrained optimization method that provides a systematic approach to identify the pathways whose overall function can be optimized by their selective usage, predicting the potential existence of metabolic switches (pathways that get activated or inactivated, due to changes in the physiological conditions). The strength of this approach comes from the fact that the kinetic parameters of the rate equation models for each reaction, which are currently unknown for most metabolic reactions, are not necessary for predicting the existence or the location of a metabolic switch. In fact, the location of potential metabolic switches is solely determined by the network topology and the stochiometry of the underlying reactions. Indeed, these parameters are required only if we wish to determine the precise physiological condition at which the switch is activated. The very existence of a metabolic switch is independent of these parameters, being encoded into the topological and stoichiometric properties of the metabolic network. Therefore, potential metabolic switches are uncovered from a mere knowledge of the metabolic wiring diagram.

Preliminary calculations for the E. coli central metabolism indicate that our method predict the existence of some known metabolic switches. The competition of the glucose and lactose uptake pathways offers the first example of an experimentally well-characterized metabolic switch whose existence is predicted by our principle.

The optimal reaction rates are those that minimize fluxes subject to the mass conservation constraint for each metabolite (the sum of the reaction rates consuming each metabolite must equal the sum of the reaction rates producing that metabolite, multiplied by the respective stoichiometric coefficients). Mathematically, if $S_{\alpha i}$ is the stochiometric matrix, reflecting the extent to which metabolite α is consumed ($S_{\alpha i}<0$) or produced ($S_{\alpha i}>0$) in reaction i. Thus, the mass conservation constraints are given by

$$\sum_{i=1}^{N} S_{\alpha i} v_i = 0 \quad . \qquad \text{(II. 8)}$$

One of the reactions, i = N (for simplicity), represents the biomass production rate, where its stochiometric coefficient reflects the experimentally determined biomass composition.

The strength of the method is that a prior knowledge of the $\{a_i\}$ parameters is not necessary to locate potential switches (see box above). Therefore, we generate random sets of parameters $\{a_i\}$ by assigning random values to a_i. For a given $\{a_i\}$ parameter set some reactions will be active ($v_i>0$) and other inactive ($v_i=0$). Changing $\{a_i\}$, which mimics changes in metabolite concentrations, we can obtain different subsets of active and inactive reactions. Based on this description, we can divide the reactions into two classes, those that remain active (or inactive) for any $\{a_i\}$ parameter set, and those that switch from an active to an inactive, or vice versa, when the $\{a_i\}$ parameter set is altered. Reactions or pathways belonging to the latter group can be identified as potential metabolic switches since their state, active or inactive, depends on the particular conditions of the cell, modeled by the $\{a_i\}$ parameter set.

II.5.vii REGULATION AND CONTROL OF BIOCHEMICAL PATHWAYS/METABOLIC REACTIONS:

It will also be of interest to investigate metabolic pathways using the framework developed at Purdue that began with analysis of bioreactors and has recently been expanded to encompass detailed treatment of large biological pathways. The distinctive nature of this reaction kinetic approach is the modeling of metabolic regulation through the control of the syntheses and activities of the different enzymes catalyzing the different metabolic reactions. This approach has enjoyed considerable success in describing experimentally observed diverse uptake patterns of nutrients in bacterial and mammalian cell cultures and correlate steady state multiplicities in the latter. In contrast with other approaches based on metabolic flux balancing, the calculation of internal fluxes are not dependent on *specification* of external ones from experiments. Instead both external and internal fluxes are *mutually* coupled in the theory and are calculated together based on optimality principles requiring efficient utilization of resources for enzyme syntheses

rather than interpolating between specified limits as in linear programming approaches. Thus the framework provides for the coupling of intracellular processes to extracellular ones. In other words, it is designed to be a fully predictive framework.

The application of these models to batch (Kompala *et al.*, 1986), fed-batch and continuous reactor systems, in which bacteria have been subjected to multiple carbon substrates (Baloo and Ramkrishna, 1991a,b), complementary carbon and nitrogen substrates (Straight and Ramkrishna, 1994b) have been very successful in describing the diverse variety of uptake patterns observed (Ramakrishna *et al.*, 1996). In applying the models to mammalian cell cultures, the models have been not only able to predict the experimentally observed multiple steady states for Hybridoma cultures (Namjoshi *et al.*, 2001; Namjoshi *et al.*, 2003) but also explain start-up strategies for the more desirable steady states.

II.6 CELLULAR POTTS MODEL:

II.6.i INTRODUCTION:

In many ways, the level of the cell is most natural scale at which to model development. While the intenal RK networks of the cell are extremely complex, with interactions among tens of thousands of different proteins, enzymes and other biomolecules and with macromolecular machines and complex spatial organization, the number of phenomenological behaviors of a cell is large but limited. Cells can reproduce, die, move spontaneously or in response to extracellular cues, absorb or secrete chemicals, apply mechanical forces and change shape and polarize. Certain specialized cells like neurons and myofibers also have complex electrical behaviors. Thus a phenomenoloical description of these processes allows an enormous simplification compared to RK and other microscopic models, which permits us to simulate many millions of cells at once. The Cellular Potts Model (CPM) provides a convenient modeling framework to address this level of abstraction.

II.6.ii SPECIFIC AIMS:

Our primary aim is to develop and refine the CPM, to develop specific simulations of our BMP processes, and to collect experimental parameter values to allow predictive modeling with the CPM at the BMPs and thus validate our models:

1. To develop the CPM into a reliable modeling tool for widespread use
2. To add modules to the CPM to reproduce a broad range of cellular phenomena
3. To validate the CPM against experiments
4. To build sample models within the CPM to make its use easier
5. To build an API to enable modular integration with models at other scales
6. To build a set of validating numerical experiments
7. To use the CPM in conjunction with other models to simulate the BMPs

II.6.iii BACKGROUND:

The formation of intricate patterns and topologically diverse structures from simple cells has long fascinated mathematical and computational biologists. The prime examples of patterning are excitable media and Turing patterns. We know a number of genetic network and developmental mechanisms, which can generate such pattern via simple rules. Most of these mechanisms adopt a classical view of development where pattern formation depends on the assumption that cells respond to an internal developmental programme by differentiating, migrating or changing their shape in response to external and internal cues.

We can also describe such phenomena with cellular automata (CA) models. Here pattern and structures arise self-consistently due to mechano-chemical coupling among “cells.” CA assumes that Equilibrium/Steady state configurations are minimum energy configurations. The Cellular Potts Model

(CPM) is a classic stochastic CA model, a generalized Ising model where each lattice site can be in one of *Q* different states (*Q* > 2). A group of simply-connected lattice points in the same state forms a *cell*. Hence, each cell has definite volume and surface area (which we can map onto real biological cells after simple normalization). The CPM is an energy-based model that models cell interactions phenomenologically through cell-cell interaction as well as self energies like volume energy, surface energy, *etc*. It avoids the drawbacks of molecular dynamics simulations, which require molecular level information, limiting the size of the simulation, yet captures local quantities/fluctuations which are absent in mean field/continuum models. The contribution of each biological mechanism translates into an energy term which we can systematically study and verify experimentally.

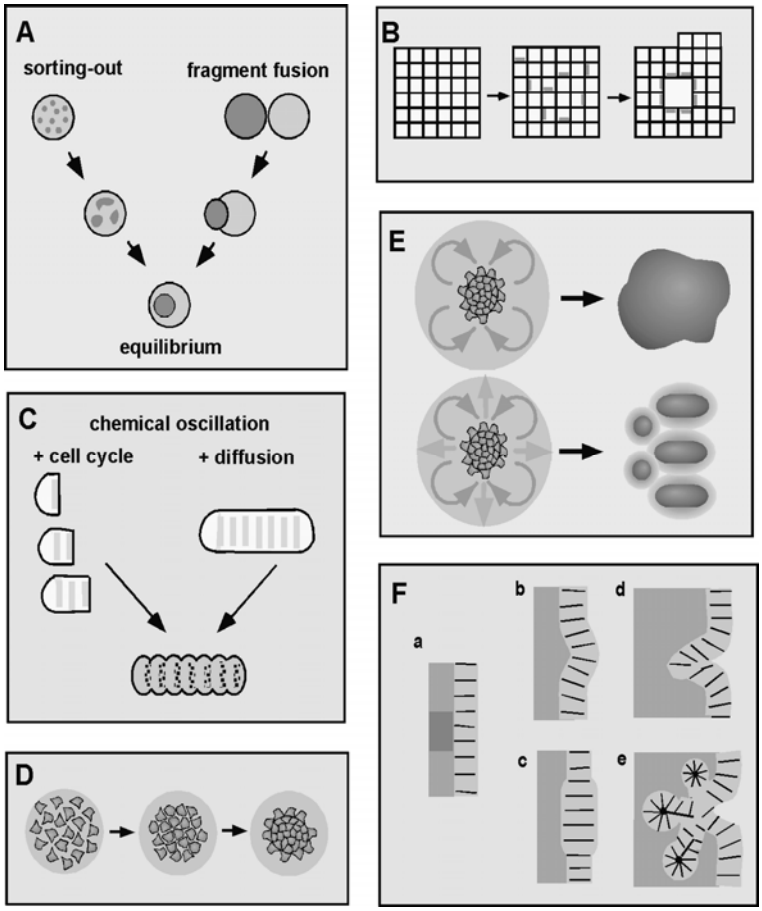


Figure 4 (A) Schematic representation of the behavior of intermixed cells and corresponding tissue fragments for two differentially adhesive cell populations (Steinberg, 1998). (B) Schematic view of the formation of a lumen or internal cavity by differential adhesion in an epithelioid tissue consisting of polarized cells (Newman, 1998). (C) Schematic representation of two modes of tissue segmentation that can arise when the tissue's cells contain a biochemical circuit that generates a chemical oscillation or "molecular clock," and the oscillating species directly or indirectly regulates the strength or specificity of cell adhesivity (Newman, 1993). (D) Schematic representation of mesenchymal condensation, which occurs during skeletal morphogenesis and many other developmental processes. (E) Morphogenesis of connective tissue, such as cartilage rods and nodules, occurs by the interplay of diffusible activators and inhibitors of mesenchymal condensation (Newman, 1996; Newman and Tomasek, 1996). (F) Types of epithelial morphogenesis (Newman and Müller, 2000).

II.6.iv BIOPHYSICAL BASIS FOR THE CELLULAR POTTS MODEL:

In 1962 Steinberg proposed that differences in cell-cell adhesion energies (differential adhesion) could explain many patterns observed *in vitro* and during development (Steinberg, 1963). This fundamental observation inspired a number of energy-based models of tissues, including the extended large-*Q* Potts model. When multiple types of cells from a primitive animal or an embryo are dissociated, randomly

intermingled and then re-aggregated, they re-establish coherent homotypic domains, the less cohesive types forming layers surrounding the more cohesive types. This *cell sorting* offers insight into the mechanisms governing morphogenesis. Sorting has long been studied using organisms including *hydra* (Armstrong, 1989). Numerical simulations of cell sorting using the extended large-Q Potts model started almost a decade ago (Graner *et al*., 1992) (Glazier *et al*., 1992). They showed that random cell fluctuations and differential adhesion between different cell types suffice to simulate various biological phenomena, including cell sorting, tissue engulfment and tissue rounding. Potts model simulations can also describe non-biological phenomena like grain growth (Glazier *et al*., 1992) and foam rheology (Jiang *et al*., 1998; Hogeweg, 2000, VanOss *et al*., 1996). Potts model plus reaction diffusion equations can describe the entire life cycle of the slime mould *Dictyostelium discoideum* (Maree, 2000), the first successful example of computing an organism. Because of its flexibility and simplicity of implementation, Potts model methods are increasingly common in complex biological simulations of organogenesis (Levine *et al*., 1996; Jiang *et al*., 1998; Hogeweg, 2000; VanOss *et al*., 1996).

Morphogenesis is the shaping and molding of living tissues in three-dimensional space. In addition to its role in development, morphogenesis is central to regeneration, wound healing, and various pathologies. During morphogenesis, tissue masses may disperse, form internal foci of cell condensation, lengthen or shorten, or acquire lumens. They can also form sheets which may invaginate or evaginate, or develop one or more internal boundaries which restrict or prohibit cell mixing. Such compartmentalized tissues can either physically separate, or remain attached and engulf, or be engulfed by, one another. These processes produce the various body plans and organ forms characteristic of multicellular organisms, as well as tumors, abnormal polyps, and fibrotic lesions.

Differentiation is the biochemical diversification of cell types according to well-ordered lines of descent, or lineages. Differentiation is dynamic and involves networks of transcription factors controlling one another's expression and the expression of other target genes. *Pattern formation* is the establishment of specific spatial arrangements of differentiated cell types.

Figure 4 summarizes how the "generic" properties of differential cell-cell adhesion, cell excitability, and cell polarity can generate recognizable properties of embryos and developing organs. In many cases these generic mechanisms may be just a starting point both developmentally and evolutionarily, with fine-tuned genetic mechanisms combining to produce the complex developmental mechanisms of modern organisms (Newman and Müller, 2000).

Recently a fine-tuned genetic mechanism has been reported to play a vital role in vertebrate segmentation [Figure VI. 1 (C)]. Segmentation is a basic characteristic of many animal species including man, and usually corresponds to a repetition, along the anterior-posterior axis, of similar structures during early development. In humans, segmentation is most obvious at the level of the vertebral column and its associated muscles, and also in the peripheral nervous system. The segmented distribution of the vertebrae derives from the earlier metameric pattern of embryonic structures known as somites (Schnell *et al.,* 2002). In a number of vertebrate embryos indicates that segmentation of the embryonic body relies on at least two molecular clocks for its proper functioning (Collier *et al.,* 2000). Segmentation seems to be controlled by counting mechanism for cells in the PSM produced by a stationary phase gradient of a product of a molecular clock. Cell maturation is the result of motion along this gradient. The formation of a new intersomite boundary seems be triggered by a cell-signaling pathway when a certain threshold is reached; the timing of this response will correspond to a certain number of completed cycles controlled by the molecular clock (Schnell and Maini, 2000).

II.6.v CPM IN BIOLOGY:

Groups throughout the world use the CPM to simulate cell aggregation, pattern formation, organization of slime moulds, angiogenesis and tumors. The simulation results have been validated against experimental evidence. The Cellular Potts Model is one of the few modeling tools that are highly scalable, realistically encompassing from cellular level to tissue level to organ level and in principle to the whole organism. It also makes modeling different experimental conditions and connecting to models at other scales relatively easy.

II.6.vi PARAMETERS FOR THE CPM:

Like all biological models, the CPM requires a large number of parameters, although the number is much smaller than that required for a large-scale reaction kinetics model. Unlike the RK model which requires a limited number of controls for each reactor and where the complexity arises from the number of reactions (and often the needed data is qualitative or stoichiometric rather than quantitative). The CPM requires more different parameters per cell but has relatively few cell types. Generally these parameters are much less well identified than for RK models. The CPM stands to one side of the data deluge.

The types of parameters differ as well: The CPM requires measurement of classical histological and physiological type as well as more molecular information: Fate mapping, cell tracking and measurements of cell adhesion and chemo- and hapto -tactic response are all crucial. In some cases, the technology to make such measures is poorly developed (we propose some technological development in Core 3 Section).In other cases the technology exists but the measurements have not been made, either because such 'classic' measurements are unfashionable or because they repeat quantitatively, qualitative experiments of decades ago. In any case, the availability of data is much less than for RK models, and we designed our BMPs both to allow and provide these measurements and to allow us to validate the CPM simulations in a scientifically and clinically significant context in which we must integrate classical and modern molecular biological techniques.

The input parameters required in a biological model within the CPM framework correspond to the number of biological mechanisms included in the model, which we must choose on the basis of the phenomena to be modeled.

Key parameters of the CPM include:

1. The size and shape of the tissue/organ domain, which will vary as the tissue develops
2. The size and density of packing of the cells
3. The extent of membrane fluctuation or filiopodial or lamellipodial protrusion or ruffling activity which corresponds to the amplitude of fluctuations, the model temperature
4. Mean Cell velocities and correlation times
5. Type dependent binding energies per unit area which include both specific (*e.g.*, integrins, cadherins) and nonspecific interactions (*e.g.*, elastic effects due to cell deformation)
6. The distribution of adhesion sites on the cell membrane
7. Parameters to control cell polarity and shape, which might require assigning additional attributes to lattice sites describing a cell
8. Parameters to control cell differentiation, specifically, a state parameter that determines if the cell is differentiated and a list of types the cell can differentiate into if it is not yet differentiated
9. Parameters to control cellular oscillations. These will describe the cycles the cell undergoes and what other parameters are affected at each stage in the cycle.
10. Cell volumes and the parameters which control it. Since cell vollume canfluctuate, *e.g.*, due to osmotic pressure, we describe cell volume in terms of an effective volume elasticity and target volume.
11. Similarly membrane elasticity and target surface area
12. Chemotaxis parameters: response of cells to chemical signals including the threshold level of chemical concentration required for the cell to respond to the relative strength of this interaction, and dose-response, and adaption curves
13. Parameters governing the refractory period for a cell's response to chemical cue
14. Specific responses to chemical cues. Description of differentiation rules.

15. Parameters for cell division including size at division, cleavage plane orientation, size of daughter cells
16. Parameters for apoptosis: Rate of cell death, decision parameters for which cells die.

These parameters can be controlled by a model of genetic regulation, an example of which is given by Hogeweg (2000). She included in each cell of the CPM an abstraction of genetic regulation, the Boolean or Kaufman network (Kaufmann, 1969). A Boolean network contains a number of “genes” each of which can be either turned “ON” or “OFF”. The state is controlled by two other “genes” and a Boolean function, such as “AND”, “OR” or “XOR”, which acts as a model of the regulatory sequences of the gene. For example, when two of the input genes are turned ON and the Boolean function is AND, the next state of the gene will be ON. The multiple stable states that occur in such Boolean networks are considered 'cell types', while cell-cell adhesion and cell-ECM adhesion is controlled by a number of “cell adhesion genes” in the boolean network. In the TST, we will include the option to incorporate realistic biochemical kinetics models to control some or each of the CPM parameters described above. Thus we will be able to include cell differentiation as the biochemical network is driven to an alternative stable state. Also, we will include biochemical kinetics models describing the expression of cellular adhesion molecules and integrins, or models that describing processes the cell cycle, the expression of cell adhesion molecules and integrins, or the excretion chemical signals. Such models are straightforwardly translated into the CPM parameters described above.

II.6.vi.1 PARAMETERS FOR MECHANICAL PROPERTIES OF NON-CELLULAR MATERIALS:

Some non-cellular materials are modeled directly in the CPM. For instance, the fluid medium in which chemical diffusion occurs can be modeled directly on the CPM grid. To accurately model the behavior of the non-cellular materials, certain mechanical properties are necessary. These include:

1. Parameters for controlling the liquid medium such as viscosity, density, compressibility
2. Rigidity parameters for the cytoskeleton
3. Motility parameters that determine how the cells are allowed to move through the extra-cellular material

II.6.vi.2 PARAMETERS FOR DIFFUSING CHEMICALS:

Chemicals that are small enough can diffuse through the cellular and extracellular space. Some chemicals may actively be transferred across cell membranes. Many of these are morphogens, produced by the cell. Some diffuse and/or decay while others do not. Hence the two major mechanisms at play here are diffusion and reaction. The parameters of importance, thus are:

1. Morphogens kinetic parameters such as rate of production, absorption, and decay of morphogens
2. Diffusion coefficient of the morphogens
3. Parameters specific to the numerical implementation- geometry of domain, grid, parameters associated with specific numerical scheme used (various FDM/ FEM other discretizations)

II.6.vi.3 PARAMETERS FOR RK AND NETWORK MODELS:

These models provide control inputs into the CPM by controlling various cell responses like differentiation, cell adaption, etc. They in turn are controlled by parameters like:

1. Chemical mechanism in gene circuits
2. Signal transduction and biochemical pathways
3. Kinetics parameters for the chemical species involved in these reactions.

4. Parameters in the ODE models governing the regulatory networks.
5. Integration of RK/network models with cell descriptions can use the abstraction of a Cell State change map.

II.6.VII DATA SOURCES:

Most of our experiments in the BMPs and our support for technological development aim to determine CPM parameters. One advantage of the CPM is that surface adhesion molecules and extracellular matrix in morphogenesis are well characterized and the other parameters are also experimentally measurable in principle. The cell size sets the spatial scale, while measuring the diffusion constant establishes the time scale. In the continuum Turing case (Turing, 1952), besides the unidentified activator and inhibitor molecules, the parameters relate very indirectly to experiments. Atomic Force Microscopy can directly measure the cell adhesiveness and cell-ECM binding strength.

II.6.VIII PRIOR WORK AND ACCOMPLISHMENTS:

II.6.viii.1 IUB AND NOTRE DAME:

Dr. Glazier was the original developer of the CPM for biological applications.

Using a single model which includes only differential surface energies an area constraint with isotropic cells and without including detailed membrane or cytoskeletal properties, (Glazier *et al.*, 1992, 1993) reproduced various observed biological phenomena of cell sorting between two cell types simply by varying the surface energies between the cells and the medium. The simulations explored the cell behaviours, both qualitatively by characterized simulated patterns and quantitatively by measuring the statistics of the pattern. The simulation suggested that energy minimization was important for cell sorting. They could distinguish between spontaneous or neutral processes having power-law time dependence from activated processes which have logarithmic time dependence. Technau and Holstein observed such logarithmic time dependence in cell sorting in *hydra* aggregates (Technau *et al.*,1992).

Dr. Glazier's and Dr. Alber's laboratories have designed and developed the CompuCell program which handles the CPM, reaction-diffusion and rule based cellular automata in an integrated fashion. The program allows flexible specification of parameters and visualization.

Simulations using CompuCell have reproduced patterns of precursor morphogens (TGF-β and fibronectin) and mesenchymal condensation resembling experiments in cartilaginous tissue (see below). Binaries and simulation results are available from the link: http://www.nd.edu/~lcls.

In the development of the animal embryo changes of form are especially practical during gastrulation when extensive cell rearrangement forms. During these rearrangements groups of cells move coherently over distances very large compared to cell dimensions. Extensive experiments, particularly on embryos of the frog, show a characteristic and widespread type of rearrangement, "convergent extension." For example, in the development of axial structure such as precursors to the vertebrate spinal column, known as somites (Baker *et al.,* 2003). Recently, it has been demonstrated that chick somite formation occurs in a six-step process (Kulesa and Fraser, 2003), which includes cell de-adhesion, complex cell movements and tissue shaping. Preliminary analyses show that the somite pulls apart from the presomitic mesoderm, rather than cleanly slicing off. Cells that exchange across the forming inter-somitic boundary rapidly alter gene expression to match new neighbors. In the laboratory, we are now examining whether: 1) perturbations to certain key genes affect particular steps of the somite sculpting process; 2) cells, inhibited from moving during somite boundary formation, continue to alter their gene expression and; 3) mouse somite shaping takes place in a similar multi-step process as in chick. Here an active group of cells, originally roughly isodiametric, elongate, while on a somewhat slower time scale, the cells intercalate between each other. The intercalation is in the direction of alignment so that the number of cells in that direction decreases while the number of cells in directions perpendicular to the alignment increases. The elongation increases the overall length of the group of cells in the direction of alignment and decreases the length in orthogonal directions (since the volume stays roughly constant). Glazier *et al.* used the CPM to show that important aspects of convergent extension result from the tendency of the active cells to minimize their total energy, provided that they interact with a non-uniform surface (adhesive) energy satisfying certain conditions. This

single simple property suffices to cause cell extension, alignment, and intercalation in the direction of alignment, the characteristics of convergent extension. The final aspect ratio is independent of the initial configuration and on the anisotropy.

Mesenchymal condensation is a critical step in much early morphogenesis. In developing connective tissues, mesenchymal cells are initially dispersed in a hydrated ECM. In a crucial, common step in the formation of structures like skeletal elements, feather germs, blood vessels, or epithelial kidney tubules, these cells transiently reorganize into compact clusters through mesenchymal condensation**.** The vertebrate limb develops from masses of mesodermally-derived mesenchymal tissue that emerge from the body wall. Experiments support mechanisms involving generation of local adhesive differentials based on deposition of new extracellular matrix and production of cell-surface adhesion molecules (Hall, 1995; Newman, 1996; Miur, 2000).

The CPM reproduces much of the density dependent phenomenology of *in vitro* chick limb chondrogenic mesenchymal condensation. Unlike the Turing mechanism where chemical diffusion drives patterning, in the CPM the pattern results from adhesion biasing cell diffusion. This mechanism also differs from those like chemotaxis which require long-range cell signaling and movement.`

II.6.ix ENERGY MINIMIZATION FORMALISM:

The central element of the CPM is an energy minimization formalism to simulate the movement and properties of spatially extended cells undergoing cytoskeletally driven fluctuations. Extensive quantitative experiments have shown that it successfully reproduces the behavior of simple cell aggregates (Beysens *et al.*, 1998, 2000; Mombach and Glazier, 1996; Mombach *et al.*, 1995; Rieu *et al.*, 2000; Upadhyaya *et al.*, 2001).

The fundamental entities in our model are individual cells. An effective energy *E* and "fields," *e.g.*, the local concentrations of diffusants, conveniently describe their interactions, motion, differentiation and division. The effective energy mixes true energies, like cell-cell adhesion, and terms that mimic energies, e.g., the response of a cell to a chemotactic gradient. Given an effective energy we can calculate the resulting cell motion, since differences in energy produce forces, ***F.*** Since cells in tissues move in an extremely viscous environment, the velocity of the center of mass of the cell, *v*, not acceleration is proportional to force, with an effective cell mobility, *m*. Thus $\vec{v} = m\vec{F}$; *i.e.*,

$$\vec{v} = m\vec{\nabla}E \,. \qquad \text{(II.9)}$$

The above equation implies that cells move to minimize their total effective energy. In what follows we discuss the various energy contributions and fields we incorporate in our simulations.

We can implement this equation in a simulation in a number of ways, using center, vertex, boundary or finite element methods (Graner, 1993; Graner and Sawada, 1993). Up to the accuracy of our approximations all methods yield the same results. We have found that the CPM optimizes trade offs between efficiency, complexity of code and accuracy. (Graner and Glazier, 1992; Glazier and Graner, 1993; Jiang *et al.*, 1998; Marée, 2000).

II.6.X DESCRIPTION OF THE CELLULAR POTTS MODEL:

We now give a brief description of the CPM and the relation between the underlying biology and the physical terms in the Hamiltonian. The CPM model is a Cellular Automaton model on a lattice with each lattice site having a unique spin value σ Its simplest form, the Ising model, has two spin values. The spins interact with each other by obeying some standard rules of physics for the net energy called a Hamiltonian, e.g., the interaction energies of these spins. The number of distinct spin values determines the degrees of freedom of the lattice, its phase structure and the critical exponents of the model. The underlying lattice can in general be in one or more dimension. The equilibrium/steady state configuration of such a multidimensional array of spins corresponds to the global minimum of this Hamiltonian. The evolution of the configuration aims to find this minimum, given the Hamiltonian, constraints and boundary conditions. Analytical results are possible only for specific geometries of the lattice like the Bethe lattice. In general we

have to resort to various numerical techniques to reach this equilibrum. Monte-Carlo techniques are convenient.

The cellular Potts model (CPM) is biological in context. A cell in CPM is defined as a collection of lattice sites with same spin value. Each cell has an unique spin value but can have a non-unique type, as depicted in the Figure below, where the numerals indicate cell spin and the color cell type. The difference between spins define the cell boundary. Hence each cell has a volume and a surface area. Each cell in the CPM can represent an actual biological cell (under suitable normalization of scales), the number of spins and their distribution being determined by the needs of the specific system/experiment being modeled. The interaction of neighboring different spins models the surface tension and the adhesivity of the cell membrane. Influences of external field like chemical concentration gradient and various constraints like volume constraint, surface area constraint, *etc*. can also be imposed to model different situations.

II.6.X.1 CELL ADHESION:

Cell adhesion is necessary for multicellularity. Its alteration is also central to many, morphogenetic changes. Many types of cell-surface proteins are adhesive under various conditions and modern organisms use several different adhesion mechanisms. Experimentally, a mixture of cell types with different quantities or types of adhesion molecules on their surfaces will sort out into islands of more cohesive cells within lakes of their less cohesive neighbors. Eventually, through random cell movement, the islands coalesce to establish an interface across which cells do not intermix (Steinberg,1998; Wei *et al.*, 2003). Figures 2 and 3 in section III.1.i show computational results of how this happens in some of our simulations. Two or more differentially adhesive cell populations present within the same tissue mass can self-assemble into multilayered structures, comprising distinct non-mixing compartments. The phenomenon resembles what happens when we pour two immiscible liquids, such as oil and water, into the same container. The following figure (Wei *et al.*, 2003) shows how this happens in experiments with precartilage and mesenchymal cells *in vitro*.

We can describe the net interaction between two cell membranes by an effective cell-type dependent binding energy per unit area, $J_{\tau,\tau'}$, where τ is the type of the cell on either side of the link. This incorporates both specific (e.g., integrins, cadherins) and nonspecific interactions (*e.g.*, elastic effects due to cell deformations, (Drasdo and Forgacs, 2000) as measured with laser tweezers (Sato-Maeda *et al.,* 1994), compression apparatus (Foty *et al.*, 1996), or micropipette aspiration techniques (Merkel *et al.*, 1999). In the CPM the effective cell-cell interaction energy is:

$$E_{adhesion} = \sum_{(i,j)(k,l)neighbors} J(\tau(\sigma(i,j),\tau(\sigma(k,l)))(1-\delta_{\sigma(i,j),\sigma(k,l)}), \qquad \text{(II.9)}$$

where the Kroneker δ, ($\delta(\sigma,\sigma')$=0 if $\sigma\neq\sigma'$ and $\delta(\sigma,\sigma')$=1 if $\sigma=\sigma'$), ensures that only surface sites between neighboring cells contribute to the cell adhesion energy.

Based on energy minimization under differential adhesion (DAH) Steinberg (1963) predicted that in an aggregate of two cell types, if the heterotypic energy is greater than the homotypic, the lowest energy configuration has the less cohesive cell type completely surrounding a sphere of the more cohesive cell type. Many assays have observed this classic cell sorting (Steinberg and Takeichi, 1994). Complications arise because the cell adhesion molecules may change both in quantity and identity (Bozzaro and Ponte, 1995). We model all such changes as variations in cell-specific adhesivity. Cell-cell interactions are adhesive, thus the coupling energy is negative (Upadhyaya, 2001), $\boldsymbol{J_{\tau,\tau'} < 0}$. For the surface energy term, the membrane breaks up to try to maximize its surface areas (and hence minimize its energy).

II.6.x.2 CELL VOLUME, MITOSIS AND APOPTOSIS:

At any time *t*, a cell, of type, τ, has a volume $v(\sigma,t)$ and surface area $s(\sigma,t)$. Since cell volume can fluctuate, e.g.,, due to changes in osmotic pressure, we describe the cell volume in terms of an effective volume elasticity, λ, and target volume $v_{target}(\sigma,t)$. We define a membrane elasticity, λ', and a target surface area $s_{target}(\sigma,t)$:

$$E_{vol} = \lambda_v (v(\sigma,t) - v_{target}(\sigma,t))^2 \qquad \text{(II.10)}$$
$$E_{area} = \lambda_s (s(\sigma,t) - s_{target}(\sigma,t))^2$$

When a cell grows, both $v(\sigma,t)$ and $s(\sigma,t)$ increase, otherwise $v_{target}(t)$ and $s_{target}(t)$ are constant. Mitosis occurs either when the cell reaches a fixed, type-dependent volume, or when the ratio between cell surface area and cell volume reaches a critical value (Drasdo, 1996). For a specific form see Drasdo and Forgacs (2000). We create two daughters with their plane of separation along the parent cell's longest axis and new target areas $v_{target}/2$, based on experiments that show well-defined orientation of cleavage planes with mitotic spindles aligned according to the positions of neighboring cells(White and Borisy, 1983). We can simulate apoptosis simply by setting the cell's target volume to zero.

This simple model reproduces the observed cell arrangements in a variety of plant tissues (Mombach *et al.*, 1993) and avascular tumors (Stott *et al.*, 1999; Drasdo *et al.,* 1995).

II.6.x.3 RIGID BODY CONSTRAINTS:

A cell can undergo deformation from its equilibrium shape due to the shearing force of fluid motion or neighboring cells. To account for this strain energy, we subdivide a cell into N parts. Let the center of mass of the i-th part of the cell be denoted by R_i. Then the distance between the neighboring center of mass is $R_i - R_j$, where *j* is the nearest neighbor of *i*. If the equilibrium distance between these two points is $|R0_{ij}|$, then the strain energy can be written as

$$E_{strain}=\lambda_{strain}(\Sigma_{i>j}|R_i - R_j| - |R0_{ij}|)^2, \qquad \text{(II.11)}$$

where *j* is the nearest neighbors of ith subcell and $i > j$. Hence under equilibrium condition the strain energy is zero.

The coefficient λ_{strain} is called Lame coefficient relates to the bulk modulus and modulus of rigidity. Here we have simplified by ignoring the cell's inhomogenity and anisotropy.

II.6.x.4 EXTRACELLULAR MATRIX:

In contrast to epithelioid and epithelial tissues, in which cells directly adhere to one another over a substantial portion of their surfaces, connective tissues consist of cells suspended in an extracellular matrix (ECM). Additional morphogenetic mechanisms depend on changes in the distance between cells, the effects of cells on the organization of the ECM, and the effects of the ECM on the shape and cytoskeletal organization of cells that typically occur in connective, but not epithelioid, tissues.

As in epithelioid tissues, immiscible boundaries can develop in connective tissues, such as the interface between the flank of a developing vertebrate embryo and an emerging limb bud (Heintzelman *et al.*, 1978).

The formation of elaborate cell-ECM adhesive structures in developmentally mature connective tissues permits physical forces originating within cells to contribute to tissue morphogenesis (Newman and Tomasek, 1996). In particular, fibers can transmit the intracellular forces necessary for cell shape changes

and migration to the surrounding cells and ECM, mechanically stressing the whole tissue (Beloussov *et al.*, 1975; Grinnell, 1994; Ingber *et al.*, 1994). In such cases the tissue no longer exhibits liquid-like behavior (since the cells are not independently mobile), but rather behaves as an elastic solid.

We model unconfined ECM, liquid medium and solid substrates, as generalized cells with the property that their target area always tracks their current area. ECM is similar to a secreted signaling molecule except that the secreted material does not diffuse. If a cell secretes a unit of ECM, the ECM's target area increases by one unit. Similarly if a cell absorbs a unit of ECM, the ECM target area decreases by one. If the ECM is solid, then local concentrations of ECM cannot fluctuate or move, if liquid, it behaves exactly like a normal cell. We must define the interaction energy between each cell type and the ECM.

II.6.x.5 DIFFUSION AND HYDRODYNAMICS:

We subdive the ECM or fluid into small fluid particle cells having properties including differential adhesivity, surface constraints, volume constraints, *etc*. The elasticity coefficients are very small compared to those of the large biological cells. Hence, the fluid particle cells can move relative to each other if local pressure gradient develops due to the movement of biological cells generating advection. The fluid cells carry chemical concentration (morphogens secreted from the membrane of the biological cells) which diffuse among neighboring cells by local averaging rules. Since the Laplacian in a diffusion equation averages the neighboring densities (Morse and Feshbach, 1953) we approximate it as

$$\Delta c(x) \approx \frac{(< c(x,t) > - c(x,t))}{R^2}, \qquad \text{(II.12)}$$

where $< c(x,t) >$ ⟨ $c(\boldsymbol{x},t)$ ⟩ is the average density in a sphere of radius R ($R \rightarrow 0$) about $\boldsymbol{x}$, i.e.,

$$< c(x,t) >= \frac{3}{4\pi R^3} \int_V c(x+r)dr \,. \qquad \text{(II.13)}$$

In our simulation we take the average over the neighboring cells. We are thus able to obtain both diffusion and advection within the frame work of CPM. The simulation correctly duplicates the flow behavior at low Reynolds number flow.

II.6.x.6 MODELING CELL SHAPES AND POLARITY:

Cells, as in myxobacteria colonies, can be elongated. In addition, they can have a phenomenological "head" and "tail", with the cells attaching to each other at unlike "poles". We can model such polarity extending the Potts grid, where in addition to the "spin," a pixel may have other attributes. To maintain an elongated "elliptical" or "tubular" cell shape, we assign to certain pixels in the cells special "cytoskeletal" properties to form the "foci" of the ellipse, or the "axis" or "backbone" of the tubular shape. The other pixels comprising the cells tend to remain in shape due to an energy penalty associated with the distance from the "foci" or "axis" pixels.

We align the pixels at the two ends of the "axis" "head" or "tail" polarity. An energy term corresponding to the interaction of two dipoles causes the head of the "following" cell will align with the tail of the "leader" cell.

II.6.x.7 CELL POLARITY VIA HAMILTONIAN TERM:

Cells are capable of generating protrusive movements by internal mechanisms which allow them to move through their surrounding environment. Although a cell's intrinsic polarity may cause it to move in a preferred direction relative to its own symmetry, its direction of movement relative to other cells in the embryo will be random unless it experiences external signals or forces. Diffusible chemical signals in the

form of polypeptide growth factors can cause cells to migrate preferentially in a given direction, typically up the gradient of the factor, in *chemotaxis*. Alternatively, bound molecules, either on the surfaces of adjacent cells or in the extracellular matrix, can provide adhesive gradients that guide cell movements in a preferred direction, in *haptotaxis*.

We describe one approach to handling cell polarity below, which relies on the assignment of an energy term associated with a polarity vector. The previous section describes another approach that attempts to add more information at a finer scale to the CPM.

Hogeweg *et al. (to be published) h*ave recently introduced another model of cell polarity, which uses local membrane fluctuations. In their model, the dissipation energy (see below) that is needed to extend a phylopodium is a local property of each membrane site of the CPM, and it differs for each of the copying direction. In this way the membrane is “stiff” in some directions and locations, while it is more flexible elsewhere. These stiffnesses develop over the course of the simulation and are determined by previous copying steps. In this way cells may align and intercalate, a process that may explain the “convergence-extension” (Keller, 1985) that drives the elongation that occurs at the neurulation stage in many vertebrate embryos.

The distribution of cell adhesion molecules is typically nonuniform along the cell membrane, due to cell polarity. We simulate polarity by defining an orientation vector for each cell and allowing *J* to vary as a function of the position on the cell surface with respect to the orientation vector. Extremely polar cells characterized by their apical and basal surfaces (Bozzaro and Ponte, 1995; Kuspa *et al.*, 1992; Roberson *et al.*, 1980), contact their neighbors along the lateral part of their membranes to form epithelial sheets. The sheet has a local spontaneous curvature, $c_{\square\square}$ at site $\square$(Lipowsky, 1991) determined exclusively by the preferred shape of the individual cell at $\square$. The curvature of a surface at point $\square$ is $1/r_{\square}$, where *r* is the local radius of curvature. Bending the sheet distorts the me

$$E_{\text{Polarity}} = \kappa \sum_{\text{cell membranes}} \left(\frac{1}{r_\sigma} - c_\sigma \right)^2 , \qquad \text{(II.14)}$$

II.6.x.8 MEMBRANE FLUCTUATIONS:

How do cells move? In mixtures of liquid droplets, the thermal fluctuations of the droplet surfaces cause diffusion (Brownian motion) leading to energy minimization. Cytoskeletally driven cell membrane ruffling of a few micrometers ($\square$m) has no need to be thermal and cell and tissue dynamics might depend sensitively on details of the fluctuations spectrum. The simplest assumption is that an effective temperature, *T*, drives cell membrane fluctuations. We can describe these fluctuations statistically using Monte-Carlo Boltzmann dynamics (Metropolis, 1953), where *T* defines the size of the typical fluctuation. If a proposed change in configuration produces a change given by

$$P(\Delta E) = \begin{cases} 1 : \Delta E \le 0 \\ \mathrm{e}^{-\Delta E / kT} : \Delta E > 0 \end{cases} , \qquad \text{(II.15)}$$

where *k* is a constant converting *T* into units of energy.

The formation and breakage of CAM bonds is dissipative. Therefore, Hogeweg *et al.* corrected the classical Boltzmann index evolution dynamics to

$$P(\Delta E) = \begin{cases} 1 : \Delta E \le H_{Diss} \\ \mathrm{e}^{-\Delta E / kT} : \Delta E > H_{Diss} \end{cases} , \qquad \text{(II.16)}$$

where, H_{diss}, represents the dissipation costs of deforming a boundary.

For realistic values of the simulation parameters measured for *Hydra* cell aggregates, the simulated diffusion constants have the correct value (Rieu *et al.*, 2000). In chick retina, the spectrum of velocities of a

single pigmented cell in a neural cell aggregate is identical to that of a simulated cell (Mombach and Glazier, 1996).

II.6.x.9 Correlated Cell Motion:

Once a cell begins to move in a given directions, it tends to continue because forming or destroying a leading edge takes time. While persistence times vary greatly, for limb mesenchymal cells a reasonable time is one minute (Upadhyaya *et al.*, 2000). We can include a constraint on the cell velocity of our usual

$$\boldsymbol{E_{velocity}} = \sum_{all\,cells} \lambda_c''(\vec{V}(\sigma,t) - \vec{V_{target}}(\sigma,t))^2 \quad , \qquad \text{(II.17)}$$

$$E_{Velocity} = \sum_{all\_cells} \lambda''_c$$

where $\vec{V}$ is the instantaneous velocity of a cell due to an index change, V_{target} is the velocity of the cell averaged over a time interval (or at the previous time step) and λ'' is the correlation strength. If λ''=0 the cells execute Brownian random motion and if λ'' is very large they move continuously in one direction at fixed velocity like keratocytes.

II.6.x.10 CHEMOTAXIS:

Chemotaxis requires additional fields to describe the local concentrations of the molecules diffusing in extracellular space (or, in the presence of gap junctions, through cells as well). Let the molecule's diffusion constant be *d*, its decay rate Γ, and let it be secreted or absorbed at the surface of cells in a history dependent way, $s_c(\sigma,x,y,z,t)$. To model a specific form for s_c requires experimental measurement of the concentrations of diffusing morphogens in the tissue. The equation for the field th

$$\frac{\partial C(\vec{x})}{\partial t} = d\nabla^2 C(\vec{x}) - \Gamma C(\vec{x}) + \sum_{\sigma} s_C(\sigma,\vec{x},t) \quad , \qquad \text{(II.18)}$$

We describe the cell's response to the chemotactic field by an effective chemical potential, $\mu\sigma$, which may be time dependent, e.g., in a refractory period $\mu\sigma$ will be nearly 0 or the cell may adapt, in which case the value of $\mu\sigma$ depends on the cell's previous exposure to *C* described by a set of ODEs. $\mu\sigma$>0 yields repulsion, and $\mu\sigma$ <0 attraction. The effective chemi

$$E_{Chemical} = \mu\sigma C(\vec{x}) \quad , \qquad \text{(II.19)}$$

We can measure $\mu(\sigma$ through dynamic flow experiments as described in Core 3. The cell executes a biased random walk, which averages to di

$$\vec{\boldsymbol{v}}(\sigma) = -\mu(\sigma)\vec{\nabla} C(\vec{x}) \quad , \qquad \text{(II.20)}$$

which has the same form as equation (VI.1), justifying our energy treatment.

II.6.x.11 PATTERN FORMATION BY REACTION-DIFFUSION:

Besides treating the motion of individual cells we must simulate the diffusion of morphogens, chemoattractants and chemorepellants. Turing (1952) introduced the classic reaction-diffusion equation model of differentiation to treat pairs of continuous fields rather than individual cells. His formalism carries over to the CPM. If *A* and *B* are diffusing morphogens, which evolve like the diffusants in chemotaxis (Turing, 1952):

$$\begin{aligned} \partial A(\vec{x})/\partial t &= d_A \nabla^2 A(\vec{x}) - \Gamma_A A(\vec{x}) + f(A(\vec{x}), B(\vec{x})), \\ \partial B(\vec{x})/\partial t &= d_B \nabla^2 B(\vec{x}) - \Gamma_B B(\vec{x}) + g(A(\vec{x}), B(\vec{x})), \end{aligned} \quad \text{(II.21)}$$

If *A* is excitatory and *B* inhibitory and *B* diffuses faster than *A*, *dA*<*dB*, then an initial uniform distribution of *A* and *B* is unstable and the concentration field will evolve into domains (Turing, 1952). Cells, instead of moving, can change their parameters, e.g., their membrane adhesivities and elasticities, in response to *A* and *B,* as a function, h, of the surface concentrations of the chemoattractants, where h is defined by a model for the internal state

$$\frac{\partial \lambda_\sigma}{\partial t} = \mathrm{h}\left(\oint_{\text{surface of } \sigma} A \, \mathrm{d}^2 s, \quad \oint_{\text{surface of } \sigma} B \, \mathrm{d}^2 s, \ldots \right). \quad \text{(II.22)}$$

II.6.x.12 MODELING THE INTERNAL STATE OF THE CELL:

The closest link between experimental molecular biology and computation is at the level of the modeling of the internal state of the cell. Modeling the internal state of a cell is necessary to understand how cells differentiate. We must model the regulatory mechanisms of gene expression, signal transduction cascades, and cellular metabolism to determine the internal cell state.

If cells differentiate primarily as a result of their history and not due to positional signals, then the cell carries a set of internal variables describing its state, which evolve according to a set of internally defined ODEs. These states are general logic gates formed by biochemical reaction networks (Arkin and Ross, 1994). The necessary signal to switch on a gene depends on a biochemical reaction network (BRN).

On the multicellular level, a gradient of a diffusible morphogen based on purely reaction-diffusion mechanisms persists only in small tissues ~ 1 mm, otherwise the time required for diffusive exchange of molecules is too long. In larger organisms and tissues, particular genes have to be activated in response to signals, setting off a gene cascade, which requires autocatalytic activation of genes (Meinhardt, 1978). More complex interactions allow the space-dependent activation of several genes under the influence of a single gradient (Meinhardt, 1978).

To model spatial variations of chemical concentration arising from the BRN inside the cell we need to incorporate the relevant boundary conditions at the cell membrane and nucleus (Hentschel and Fine, 1994, 1996). We can find these heterogeneous concentrations by solving numerically the relevant reaction-diffusion equation subject to biologically plausible conditions for the fluxes at the evolving free boundary of the tissue. Experiments must determine their functional form and magnitude.

II.7 CONTINUUM MODELING:

To model organs and organisms at the level of tissue, engineers have used classical solid and fluid mechanics approaches developed for complex materials. This approach is complementary to CPM modeling of tissue and cell-cluster scales since it focuses not on individual cells, but the gross stress strain properties of the biomaterial.

II.7.i FINITE ELEMENT MODELING OF BONE:

II.7.i(a) SPECIFIC AIMS:

To develop our links to the finite element models of SCIRun and Physiome, Dr. Glen Neibur, our finite element expert will first develop two well-understood finite element solid tissue models as testcases for the CPM continuum model. These cases also apply directly to the bone regeneration component of Core 3 BMP-Limb Regeneration.

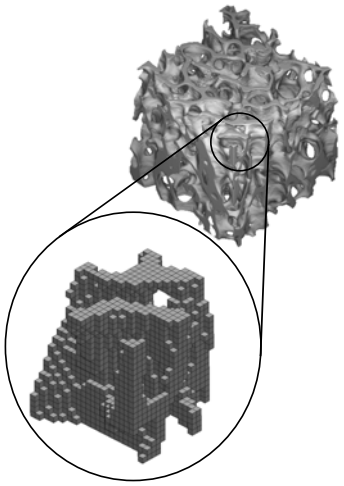


Figure 4: A high-resolution finite element model of cancellous bone, containing over 120,000 elements. The inset shows a magnified view of 1/16 of the total model.

The aims are:

1. to develop well-validated finite element testcases to transfer to Physiome and SCIRun
2. To use these testcases of the basis for integration of these packages with the CPM and TST

The specific testcases will be:

1. Microscale Modeling of Cancellous Bone Damage
2. Damage Mechanics Constitutive Model for Cancellous Bone

II.7.i(b) BACKGROUND:

Glen Niebur has developed and extended continuum solid mechanics constituitive models of tissues to address various aspects of bone deformation and fracture. As a further extension to his work, we will incorporate a damage-plasticity constitutive model for trabecular tissue (the material making up individual struts is cancellous bone) into a high-resolution finite element model of cancellous bone. We will calibrate the material parameters for the constitutive model to agree with the macroscopic experimentally measured behavior.

II.7.i(c) EXISTING MODELS:

Microscale Modeling of Cancellous Bone Damage(Aim 1)

High-resolution finite element models will provide geometrically accurate, specimen-specific models of experimental specimens (Fig. 4). Dr. Neibur will modify his existing finite element code to incorporate damage at the trabecular tissue (microstructural) level. The code currently incorporates a bilinear constitutive model that does not allow for permanent deformation or unloading at either the tissue or macroscopic level. We will incorporate these features along with the capability to analyze geometrically nonlinear behavior (large displacements). The code takes advantage of the characteristics of voxel-based meshes to improve solution efficiency. Because some of these characteristics may be lost treating a complex nonlinear material model, he will also port his model to SCIRun and Physiome.

The constitutive model for the trabecular tissue extend that of Fondrk and Davy for cortical bone. Cortical bone behavior is a reasonable starting point for modeling trabecular tissue behavior, and accurately simulates macroscopic cancellous bone yield behavior after calibration of the yield parameter. This model is isotropic and one-dimensional. An isotropic formulation for trabecular tissue has been successful in capturing the experimental behavior of cancellous bone for both elastic and yield properties. The one-dimensional formulation will apply to the principal stress and strain of the trabecular tissue, assuming that these are primarily oriented along the length of trabeculae. Multiaxial damage models are also commonly applied only to principal strains. We will not incorporate Viscoelastic effects or Trabecular fractures which play a minor role in macroscopic modulus reduction.

High-resolution (20 μm) images of each specimen will be obtained by micro-computed tomography prior to mechanical testing and converted to finite element models using a voxel-conversion technique [40]. The models will first apply to boundary conditions simulating axial compression and torsion to calculate the undamaged elastic properties. The models will then be apply the same damaging loads applied to the specimens experimentally, resulting in damage and permanent deformations. We will calculate the axial and torsional stiffnesses and the corresponding modulus reductions following damage and compare them to the experimental data.

A parametric study on a subset of five specimens for each damage loading mode (Compression, Tension, and Torsion—fifteen total specimens) will determine the dependence of the permanent deformation and modulus reductions on the parameters of the tissue level constitutive model. Excluding viscous effects, the damage model proposed by Fondrk requires four independent parameters to describe the damage-plasticity behavior. Using the experimental data from the three loading modes, we will adjust the four parameters from the initial values to agree with the experimental data.

Finite Element Model Validation:

We will use the adjusted parameters based on 15 specimens to simulate the behavior of 15 additional specimens (five from each group), which will validate the model predictions by comparison to experimental measurements. We will also compare the predicted tissue level damage from the models to histological measurements for ten specimens. If we cannot validate the models, we will seek an alternative formulation for damage at the tissue level.

Damage Mechanics Constitutive Model for Cancellous Bone:

We will use the previous results to develop a damage mechanics constitutive model for cancellous bone. In the long term, this model will apply to limb regeneration, to the design and analysis of orthopaedic implants, and to the study of damaged induced remodeling in cancellous bone.

Our theoretical development will begin by determining values for a damage tensor that relates the measured stress to the effective stress, based on the work of Kracjcinovic, Lemaitre, and Murakami:

$$\begin{aligned}\mathbf{S} &= (\mathbf{I}-\mathbf{D})\tilde{\mathbf{S}} \\ \mathbf{S} &= \left\{(\mathbf{I}-\mathbf{D})^{1/2}\tilde{\mathbf{S}}_{dev}(\mathbf{I}-\mathbf{D})^{1/2}\right\}_{dev} + \eta(1-D_H)\tilde{S}_H\mathbf{I}\end{aligned} \qquad \text{(II.23)}$$

where **S** is the measured stress and $\tilde{\mathbf{S}}$ is the effective stress for undamaged bone for the same strain, and **I** is the fourth-order identity tensor. The subscript *dev* indicates the deviatoric part and the subscript *H* the hydrostatic part of a tensor. **I** is the second-order identity. The fourth-order tensor **D** of $(4)_1$ reduces to the second-order tensor, **D**, in $(4)_2$ assuming orthotropic damage. We will then evaluate the damage tensors for a series of loads to find a law of evolution relating **D** to the loading history.

Based on the formulation in $(4)_2$, the ratio of the undamaged elastic modulus to the damaged elastic modulus along any orthotropic direction is [61]:

$$\frac{E_1}{\tilde{E}_1} = \frac{1}{9}\left[\frac{4+2\frac{\nu_{12}E_1}{E_2}+2\frac{\nu_{13}E_1}{E_3}}{1-D_1} + \frac{1+2\frac{\nu_{12}E_1}{E_2}-\frac{\nu_{13}E_1}{E_3}}{1-D_2} + \frac{1+2\frac{\nu_{13}E_1}{E_3}-\frac{\nu_{12}E_1}{E_2}}{1-D_3}\right] + \frac{1-\frac{\nu_{12}E_1}{E_2}-\frac{\nu_{13}E_1}{E_3}}{3(1-\eta D_H)} \qquad \text{(II.24)}$$

Here, E_1, E_2, and E_3 are the undamaged elastic moduli along the three orthotropic directions; $\tilde{E}_1$, $\tilde{E}_2$, and $\tilde{E}_3$ are the corresponding moduli after damage; D_1, D_2, and D_3 are eigenvalues of **D**; D_H is the trace of **D**, and η is a parameter to allow appropriate variations in the Poisson's ratios. Analogous equations apply to the

moduli along directions 2 and 3, and the three independent Poisson's ratios of an orthotropic material. Hence, modulus measurements along two directions provide six equations for the four unknowns, D_1, D_2, D_3, and η in (5). Using our calibrated and validated computational models, we will calculate all three undamaged and damaged moduli and Poisson's ratios by the representative volume technique [102], yielding **D**. We will calculate the damage tensor for ten specimens damaged in uniaxial on-axis compression at five strain levels ranging from 1.2% to 2%, and for the *same* specimens damaged in transverse compression, and pure shear. This repeated damage controls the effects of interspecimen heterogeneity. If the second-order tensor model fails to describe the damage behavior, we will calculate the full fourth-order tensor instead.

Given measured values of the damage parameter, we will calculate the applied damaging strains, and resulting accumulated (plastic) strain and evolution laws for the damage parameter as a function of applied loads and architecture.

$$\dot{\mathbf{D}} = \left(\frac{Y}{S}\right)^s \left|\dot{\mathbf{e}}^p\right|; Y = \int \widetilde{\mathbf{S}} d\mathbf{e}^{eff} \qquad \text{(II.25)}$$

with Y the effective strain energy, equivalent to the strain energy density release rate, $\mathbf{e}^p$ the plastic strain, $\mathbf{e}^{eff}$ the effective strain, and *S* and *s* are the material parameters to be identified. We also need a strain limit, $\mathbf{e}_D$ below which damage does not occur. [47, 53, 74, 80] Thus, the parameters *S*, and *s* depend on trabecular architecture. Scalar parameters should depend only on the relative density of the specimens, as Y and $\mathbf{e}^p$ incorporate the dependence on architecture.

Constitutive Model Evaluation:

We will test the model's capability to describe cancellous bone damage mechanics by comparing to experimental data.

II.7.iii IMMERSED BOUNDARY METHOD:

Biofluid dynamics is characterized by the interaction of elastic incompressible tissue with viscous incompressible fluid. In some cases the elastic tissue is active, like muscle or actin filaments, which means that it can exert active forces on the surrounding medium. The Immersed Boundary Method (Peskin and McQueen, 1989) is both a mathematical formulation and a computational method for biofluid dynamic problems. In the immersed boundary formulation, the equations of fluid dynamics are used in an unconventional way, to describe not only the fluid but also the immersed elastic tissue with which it interacts. In the computational scheme that is motivated by this formulation, the fluid equations are solved on a fixed (Eulerian) cubic lattice, and elastic forces are computed from a Lagrangian representation of the immersed elastic tissue, the material points of which move freely through the cubic lattice of the fluid computation. The two components of this Eulerian/Lagrangian scheme are linked by a smoothed version of the Dirac delta function, which is used to apply elastic forces to the fluid, and to interpolate the fluid velocity at the representative material points of the elastic tissue.

This methodology has been applied to the heart and its valves; the inner ear; the swimming of fish, sperm, and micro-organisms; the deformation and locomotion of cells; the clotting of blood; the collapse of thin-walled veins; and the regulatory contractions of muscular arterioles. Adaptive, parallel versions of the Immersed Boundary Method have been developed for simulating fluid mechanics of the heart (Roma *et al.*, 1999).

In conjunction with CPM, we propose to adopt the Immersed Boundary Method for modeling and simulating several of the motivating experimental projects, in particular the cardiac experiments (see Core 3). Such a Cartesian mesh method with embedded geometry can easily and robustly produce a mesh in a very complicated domain. In exchange, these methods push the difficulty of dealing with the cut cells at the boundary, which finite difference and finite element methods are faced with, onto the "flow solver." There is an accuracy issue since the mesh is extremely irregular at the boundary; and there is a stability issue for explicit schemes, since the cell volumes can be orders of magnitude smaller than the regular cell volumes. The TST computational core will work on such algorithmic issues, extending the applicability of the Immersed Boundary Method from a fluids-basedAdopting this continuum scheme should corroborate results obtained from CPM; used as an intermediate-scale (multicellular and small tissue-level)

computational method, in some cases, it is likely to interface more easily than CPM with larger scale and whole organ computational frameworks, such as SCIRun.

II.8 MULTISCALE INTEGRATION:

II.8.i BACKGROUND: MULTISCALE MODELING:

The existence of multiple space and/or time scales is a recurring bottleneck in computational modeling of physical processes. Important examples that have received decades of attention include coarsening in magnetic spin systems, patterns in fluids, defect dynamics in solids, and molecular dynamics of macromolecules. In the last case, which is the most developed area of multiscale modeling in biology, the evolution of the physical configuration of a macromolecule from the initial to the final state typically occurs on the time scale of milliseconds, while the interactions between constituent components must be resolved on the picosecond time scale.

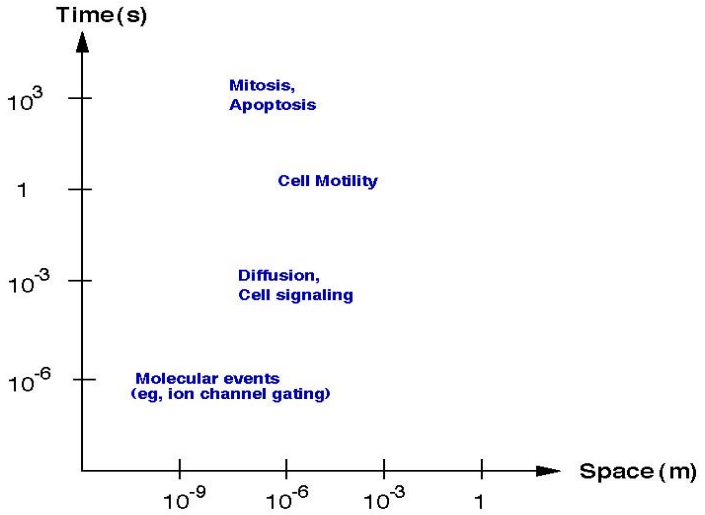


Figure 5. Time and space scales common in biological systems.

Spatially all biological tissues are organized in a hierarchy with features ranging in size from sub-micron to meters. In bone tissue, for example, osteocytes exist at a scale of 10 microns in lacunar spaces, osteons can be seen at a scale of 100 microns with many lacunar spaces, and finally, at a scale of 1000 microns or 1mm, we can see the individual plates of bone that may contain osteons or trabecular packets. It is important to understand how the hierarchy works together to create the overall physical behavior – stiffness in the case of bone and conductivity in the case of cardiac tissue, for example.

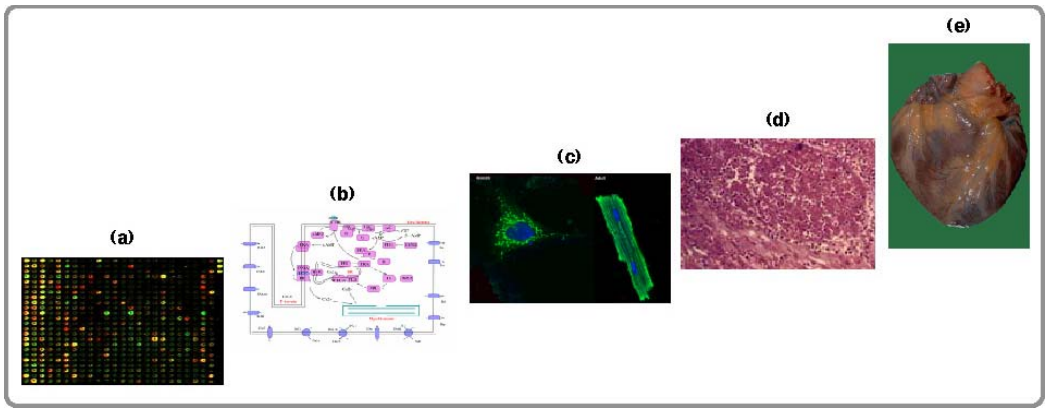


Figure 6. Examples of different scales in biology. a) Microarray data (small molecules, proteins), b) Pathway diagram (intracellular interactions), c) Single cells, d) Tissue, e) Organ.

Traditional multiscale techniques, such as the Multigrid Method (Briggs *et al.*, 2000), Domain Decomposition (Lions, 1988, 1989, 1990), Adaptive Mesh Refinement (Berger and Oliger, 1984; Berger and Colella, 1989), the Fast Multipole Method (Barnes and Hut, 1986) and the Conjugate Gradient Method (Meijirink and Vandervorst, 1977) have focused on efficiently resolving the fine scale. In contrast, modern multiscale methods aim at reducing the computational complexity by utilizing the scale separation and adopting *different* computational approaches on *different* scales. Indeed, this approach is not surprising, as different laws of physics are required on different space and time scales: For example, on the macroscale (>millimeters), fluids are accurately described by density, temperature and velocity fields which obey continuum Navier-Stokes equations. However, on the scale of the mean-free path of the fluid particles, it is necessary to use kinetic theory (Boltzmann's equations) to get a more detailed description. Averaging, where the leading order behavior of a slow time-varying variable is replaced by its time-average value, and Homogenization, where approximate equations are obtained to leading order in the ratio of fine and coarse spatial scales are examples of powerful analytical techniques (Bensoussan *et al.*, 1978; Torquato, 2001). The Quasi-continuum Method (Tadmor *et al.*, 1996), Patch Dynamics (Theodoropoulos *et al.*, 2000; Kevrekidis *et al.*, 2002; Gear *et al.*, 2002) and the Heterogeneous Multiscale Method (E and Engquist, 2003a,b; Abdulle and E, 2003; E *et al.*, 2003; Vanden-Eijnden, 2003) are examples of recent numerical approaches.

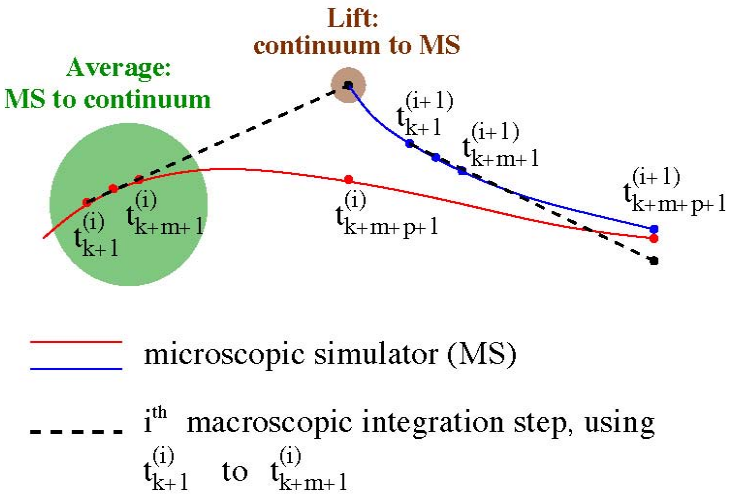


Figure 7. Cartoon sketch of multiscale computational scheme that alternates between the microscopic simulator (e.g., CPM) and the macroscopic time-stepper (e.g., finite difference or finite element schemes).

The multiscale Patch Dynamics method, for example, combines processing on fine space/time scales according to governing microscopic descriptions with macroscopic changes on a coarse computational grid.

The “coarse," or macroscopic simulator, generates a map from the coarse variables at time *t* to those at time *t+T*, where *T* is typically much larger than characteristic microscopic time scales in the system. This map is not obtained directly from the macroscopic evolution equations, which do not exist for complex biological systems, but rather through short time evolution intervals of appropriately initialized microscopic simulations. The initial macroscopic variables are *lifted* to microscopic variables to initialize the microscopic simulation. At the completion of a burst of microscopic simulation, the microscopic variables are *restricted* back to macroscopic variables, providing an approximation to the macroscopic time step. This provides a chord of the macroscopic solution at each spatial point, which is an approximation to the time derivative of the macroscopic solution. This value can then be used in any conventional continuum numerical method for the macroscopic equations (see Figure 4). This approach, including coarse-graining in space, has been applied in several physical and biological microscopic contexts, and the results appear to be promising (Makeev *et al.*, 2002; Hummer and Kevrekidis, 2003). It offers a general framework for the computer-assisted analysis of systems whose dynamics are given at a microscopic/stochastic “fine" level, but for which we are also interested in averaged, macroscopic information. Such a hybrid scheme allows efficient simulation of the macroscopic behavior, and may provide insight into macroscopic model equations. As an example, we have studied the chemotactic response of a population of bacterial cells, arrived at from Monte Carlo simulation of the intracellular signal transduction and motor response, using such an approach (Setayeshgar, 2003b). We plan to evaluate, adapt and adopt existing multiscale algorithms for simulating tissue level behavior, using CPM at the cellular and subcellular scales (microscopic simulator) and continuum methods, such as finite difference and finite element methods at the tissue scale.

Furthermore, given the small volumes and often small numbers of key constituent substrates involved in intra- and intercellular processes, fluctuations in the numbers of molecules are significant. Hence, simulation of intra- and extracellular biochemical processes must include both deterministic and stochastic models and computational schemes. The accepted method for simulating the stochastic time evolution of chemically reacting systems is Gillespie’s Stochastic Simulation Algorithm (SSA) (Gillespie, 1976). The Gillespie procedure is exact in the sense that it yields a stochastic realization of the Chemical Master Equation. In principle, the SSA could be used to simulate all of the chemical species and reactions of interest, but the algorithm inherently captures every detail, which is in general not required for determining the correct output of various biochemical networks. Although methods have been proposed (Gibson and Bruck, 2000) to speed up the SSA, by itself it remains too slow for practical simulation of realistic biological systems. Hence, building on recent work (Gillespie and Petzold, 2003), we will use the Gillespie stochastic simulation algorithm as merely a starting point for our development of new multiscale tools for simulating subcellular biochemical reactions.

Homogenization and averaging are the mathematical/computational process by which local properties are obtained on a coarse space/time grid from the variables on fine scales. The literature on analytic studies of homogenization problems in random and periodic heterogeneous materials is extensive (Bensoussan *et al.*, 1978; Torquato, 2001). As an example, homogenization of cardiac tissue continues to be an active area of theoretical research: Recent work has shown that the standard method to homogenize the inhomogeneous resistivity of cardiac tissue due to the discreteness of gap junctions can fail to give correct predictions about propagation speed and propagation failure (Keener, 2000). This shortcoming can be overcome with a more careful look at the homogenization procedure. At both interfaces, TST must devise accurate ***1) averaging tools***, for going from CPM to continuum variables or from subcellular/cellular to CPM variables, and ***2) lifting tools***, for reinitializing variables in a higher dimensional space from those in a lower dimensional space (continuum to CPM or CPM to subcellular/cellular space) (Setayeshgar, 2003b).

II.8.II EXISTING TOOLS AND MODELS:

The number of relevant biological simulation tools is very large; a complete list and description of their benefits and drawbacks would occupy this entire grant application. In this proposal we therefore concentrate on widely distributed and adopted models and simulations, which can interface directly with the CPM and TST. We are aware of the many beautiful specialized simulations that address important biological mechanisms and also of a number of broad and useful toolkits. Our omission of particular models does not reflect a judgment of their value or ignorance of their existance. We have discussed toolkits at length in Core 1. Here we focus on specific simulation tools. Further, we concentrate on the relevant

aspects of the simulation tools with which we have signed letters of cooperation. (See Appendix 1 – Virtual Cell, Biospice, Physiome and SCIRun.)

In addition we will not discuss proprietary or commercial software whose use the current RFA excludes. Thus we will not discuss Entelos' Corporation's PhysioLab (http://www.entelos.com), one of the most sophisticated and complete models of cell metabolism.

The list below provides a good overview of various approaches researchers have taken to model cellular and sub cellular phenomena. Serial numbers 1 to 5 consider models devoted to cell dynamics and the complex outcomes like tissue formation and patterning that results from it. The approaches can be broadly categorized as follows. Continuum-based models of cell behavior (Serial nos. 1, 2, 3), are more interested in gross behaviors and propoerties like cell membrane phenomena, and cell densities. However, continuum approaches have considered scales of cellular phenomena too, for example, FEM based models for cells in serial no. 1. Discrete models (Serial nos. 4, 5), based on a CA approach, consider individual cells when trying to describe dynamics and motions of such cells and the resulting social behavior. The third category deals with subcellular scales where there are a multitude of phenomena of biological interest-reaction pathways, genetic processes, protein folding, *etc.*; items 6, 7, and 8 are examples of such efforts.

Often these modeling approaches are complementary to each other, hence an interaction between such systems is a way forward to consider a biological system as a whole.

Table 4: Example list of cellular and subcellular models:

Item	Title	Description	Author/Institution	Reference
	KINSIM	KINSIM is a computer simulation software for simulating reaction mechanisms with up to 100 species and 100 reaction steps and include other improvements such as allowing global fitting following multiple species.	Carl Frieden (Washington University School of Medicine, USA) Website: http://biochem.wustl.edu/cflab/message.html	B. A. Barshop, R. F. Wrenn and C. Frieden, (1983) Analysis of numerical methods for computer simulation of kinetic processes: development of KINSIM--a flexible, portable system. Anal Biochem 130, 134-145
	GEPASI	Gepasi is a software package for modeling biochemical systems. It simulates the kinetics of systems of biochemical reactions and provides a number of tools to fit models to data, optimize any function of the model, perform metabolic control analysis and linear	Pedro Mendes (Virginia Bioinformatics Institute, USA) Website: http://www.gepasi.org	Mendes, P. (1993) GEPASI: A software package for modelling the dynamics, steady states and control of biochemical and other systems. *Comput. Applic. Biosci.* **9**, 563-571.

Item	Title	Description	Author/Institution	Reference
		stability analysis. MEG (Model Extender for Gepasi) is a module program for the modelling of complex, heterogeneous, cellular systems.		
	DBSolve	DBsolve is integrated environment software for creating, modeling, analysis and fitting to the experimental data models of metabolic pathways and other biochemical models.	Igor Goryanin (GlaxoSmithKline, UK) Website: http://biosim.genebee.msu.su/dbsdownload_en.html	Goryanin, I. Hodgman, T. C. and Selkov, E. (1999). Mathematical simulation and anlysis of cellular metabolism and regulation. Bioinformatics, 15, 749-758
	SCAMP	SCAMP is a general purpose simulator of metabolic and chemical networks. It is based around a simple metabolic language.	Herbert Sauro (Keck Graduate Institute) Website: http://www.fssc.demon.co.uk/Scamp/scamp.htm	Sauro, H. M (1993) SCAMP: a general-purpose simulator and metabolic control analysis program. CABIOS, Vol 9, no. 4, Pages 441-450
1	FEM based (continuum) models of individual cell phenomena	Here, though the phenomena under consideration are at the scale of individual cells and subcellular (for example, membrane permeability) a continuum approach is taken. Numerics is usually FEM based.	Numerous, for example Laboratory of Biocybernetics, Univ. Ljubljana / http://lbk.fe.uni-lj.si	Pavlin M., Pavselj, N., Miklavcic, D., 2002, Dependence of Induced Transmembrane Potential on Cell Density, Arrangement, and Cell Position Inside a Cell System, (IEEE Trans. Biomed. Engg., Vol 49, No. 6, June 2002)
2	Constitutive equations of tissue mechanics, continuum approach	Gross properties of bone tissue, FEM solutions of continuum equations, experimental measurements, does not consider individual cells	Glen Niebur, Tissue Mechanics Lab, University of Notre Dame / http://cauchy.ame.nd.edu/	Niebur, G L, Yeh, O C, and Keaveny, T M. "Damage evolution in trabecular bone is anisotropic". in 48th Annual meeting of the ORS. 2002. Dallas (2002).

Item	Title	Description	Author/Institution	Reference
3	Reactor diffusion continuum models	Cell density used as continuum variable, does not consider individual cells	Hentschel, Newman, http://www.mathcs.emory.edu/~tglimm/Index.html	Abstract at: http:// www.eps.org /aps/meet/MAR02 /baps/abs/ S4700008.html
4	CPM based discrete models with phenomen-ological energy formalisms	Models individual cells dynamics to generate tissue scale phenomena, mesoscale model. It has been integrated into continuum models for morphogen diffusion and into Regulatory Networks describing cell differentiation. Hence, CPM is evolving as a *framework* to allow for integration of various modeling approaches, as this proposal stresses.	Glazier, http://www.biocomplexity.indiana.edu/ Alber, http://www.nd.edu/~icsb/	Chaturvedi, R., Izaguirre, J. A., Huang, C., Cickovski, T.,Virtue, P., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A., and Glazier, J. A., "Multi-model simulations of chicken limb morphogenesis", LNCS, Volume 2659, Springer-Verlag, New York, 39-49 (2003)
5	LGCA and other rule based discrete CA models	Models cells and cell colonies using rule based CA	Alber, http://www.nd.edu/~icsb/	Alber, M.S., Kiskowski, M.A., Glazier, J.A., and Jiang, Y. "On Cellular Automaton Approaches to Modeling Biological Cells", Mathematical Systems Theory in Biology, Communication, and Finance, IMA Volume 134, Springer-Verlag, New York, (2003)
6	E-cell: Hybrid model using continuum as well as discrete approachc es	Modeling subcellular biochemical and genetic processes, utilizes an hybrid and integrative approach at subcellular scales	http://www.e-cell.org/	Kinetic simulation of signal transduction system in hippocampal long-term potentiation with dynamic modeling of protein phosphatase 2A by Kikuchi,S., Fujimoto,K., Kitagawa,N., Fuchikawa,T., Abe, M., Oka, K., Takei,K., and Tomita,M., published in the November issue of *Neural Networks*
7	M-Cell Monte Carlo simulations	Behavior and variability of real systems comprising finite numbers of	Bartol, Computational Neurobiology Lab., The Salk Instt.	Franks, K. M., Bartol, T. J., and Sejnowski, T. J., An MCell model of calcium dynamics and frequency-dependence of

Item	Title	Description	Author/Institution	Reference
	of subcellular molecules	molecules interacting in spatially complex environments inside cells. Upward compatibility with CPM possible.	Stiles, Biomedical Applications, Pittsburg Supercomputing Center. http://www.mcell.cnl.salk.edu/	calmodulin activation in dendritic spines, *Neurocomputing* 38-40, 9-16, (2001).
8	Virtual Cell: Simulations of reaction pathways	Models biochemical and electrophysiological mechanism describing individual reactions. Experimental microscopic image data describe their subcellular locations.	National Resource for Cell Analysis and Modeling http://www.nrcam.uchc.edu/	J. Schaff, C. Fink, B. Slepchenko, J. Carson, L. Loew, (1997) "A General Computational Framework for Modeling Cellular Structure and Function", Biophys. J. 73:1135-1146.

II.8.III DETAILS ON SELECTED MODELS:

As an example of some of the many other important simulation tools currently available, we must discuss four examples: StochSim, Stocks, E-Cell and M-Cell, all of which may be targeted for integration with the TST in the future.

The next few sections detail existing systems that are available for subcelluar modeling:

II.8.iii.1 STOCHSIM:

Carl Firth developed one of the first stochastic approaches based on the SSA of Gillespie (1977) (1998) as part of a study of bacterial chemotaxis. He sought a realistic way of representing the stochastic features of signaling pathways and to handle the large number of individual reactions (Firth and Bray, 2000). Individual software objects represented molecules and their complexes. Reactions between molecules occur stochastically, according to probabilities derived from known rate constants.

StochSim quantizes time into discrete, independent time intervals where the mot rapid reaction in the system determines the timestep. At the start of the simulation, the user fixes the maximum number of molecules. In each time interval, the code selects a molecule and another object (either a molecule or a pseudo molecule) at random. For two molecules, any reaction is bimolecular whether for one molecule or pseudo-molecule, it is unimolecular. Whether another random number exceeds a threshold determines if a reaction will occur.

StochSim is slower than the SSA for a small set of simple biochemical reactions, especially when the number of molecules is large. However, if the molecules can exist in multiple states, StochSim may be faster and also more reliable. An extended StochSim incorporating an explicit 20-lattice spatial representation of nearest-neighbour interactions of molecules (such as clustered receptors on a membrane) has predicted the structural arrangement of the chemotaxis receptor complex (Shimizu *et al.*, 2000).

II.8.iii.2 STOCKS:

Kierzek's STOCKS (2002) is a sophisticated implementation of the SSA. STOCKS simulates both cell growth and division. A cell doubles its volume in a single generation by letting its volume grow as a function of generation time. At each simulation time step, the rates of all the second order reactions are divided by the current volume. When the system reaches the generation time, all of the reactants that model DNA elements double (implemented by an additional set of reactions and the numbers of all other molecules reduced by a factor of two, the volume of the cell resets and the next generation time simulates the behaviour of a new cell.

II.8.iii.3 E-CELL:

E-Cell is a modeling and simulation environment for biochemical and genetic processes. It aims to serve as a universal simulation platform that can integrate any sets of different simulation algorithms, including differential-equation based models, diffusion-reaction, Gillespie's algorithm, cellular automata and GMA/S-System. It is designed to conduct efficient simulations on a cell model consisting of subsystems with different time scales and spacial resolutions. This modelling tool only deals with sub-cellular phenomena.

II.8.iii.4 M-CELL:

M-Cell is a general Monte Carlo simulator of cellular microphysiology. It is focussed on modelling biological signal transduction pathways. It has been mainly employed to deal with the microphysiology of synaptic transmission, but its application to other phenomena is possible, such as statistical chemistry, diffusion theory, single channel simulation and data analysis, noise analysis, and Markov processes. M-Cell simulations can provide unique insights into the behavior and variability of real systems comprising finite numbers of molecules interacting in spatially complex environments.

M-Cell is a microscopic modeling tool and hence large scale simulation of tissues and organs are not feasible with this system. However, M-Cell can provide valuable input to our Potts model like diffusion coefficient of morphogens, presence of inhomogeneity, distribution of receptors on the cell membrane.

II.8.iii.5 VIRTUAL CELL:

Virtual Cell (developed by Leslie Lowe and Cowolkers at The National Resource for Cell Analysis and Modeling located at the University of Connecticut Health Centers (http://nrcam.uchc.edu) is a general framework for the spatial modeling and simulation of cellular physiology. Its modelling tool can deal with compartmental models expressed mathematically as Ordinary Differential Equations (ODEs) and spatial models expressed mathematically as Partial Differential Equations (PDEs).

In Virtual Cell, a *model* object represents the physiological model of the cell or subsystem under study. Each model consists of a collection of *species* (e.g., calcium, ATP, *etc*.), *reactions* (*e.g*., enzyme kinetics, receptor binding, membrane fluxes), and *structures* (*e.g*., cytosol). The *structure* objects define mutually exclusive compartments within the cells and the membranes that isolate them. Using this definition, the plasma separates the extracellular region from the cytosol (and hence the ER, nucleus, *etc*.). These structures contain *species* and a collection of *reactions* that describe the biochemical behavior of each compartment. Average geometric information, such as surface to volume ratios for the appropriate features completes the necessary information for a single- point compartmental simulation. The *species* are objects that identify molecular species, and are either *membrane* or *volume species*. A surface density for each membrane describes the *membrane species*. A concentration and a diffusion constant describe the *volume species*. The *reactions* are objects that completely specify the reaction kinetics. *Reactions* are collections of related *reaction steps* (*e.g*., membrane receptor binding, calcium buffering), and *membrane fluxes* (*e.g*., flux through an ion channel). Arbitrary algebraic expressions represent the fluxes and reaction rates. These expression objects are capable of basic algebraic simplification, partial differentiation, binding to the appropriate Parameters and Species, and numeric evaluation. Finally, the *geometry* objects represent the

cellular geometry (based on segmented images) and can map directly to the corresponding cellular features. The specified geometry can currently be 2-D or 3-D segmented images with the appropriate scaling information to properly define a simulation domain.

II.8.iii.6 CELLO:

The number of models adddressing cell level phenomena is much smaller than that addressing subcellular and whole organ phenomena. None of the major models we mentioned in Section YYY above addresses the scale of modeling. However, a few somewhat comparable models do exist.

Cello (http://mbi.dkfz-heidelberg.de/mbi/research/cellsim/cello/index.html), developed independently at Deutsche Krebsforschungszentrum's (DKFZ, the German Cancer Research Center), Medical and Biological Informatics division. In some respects, modeling the CPM like the CPM Cello is generic and extensibile, using Object Oriented software design. However, the CPM integrates a mechanism for signaling by morphogen fields, which is not available in Cello. The CPMs's level of multi-model integration is conceptually superior to Cellos'.

II.8.IV INTEGRATION:

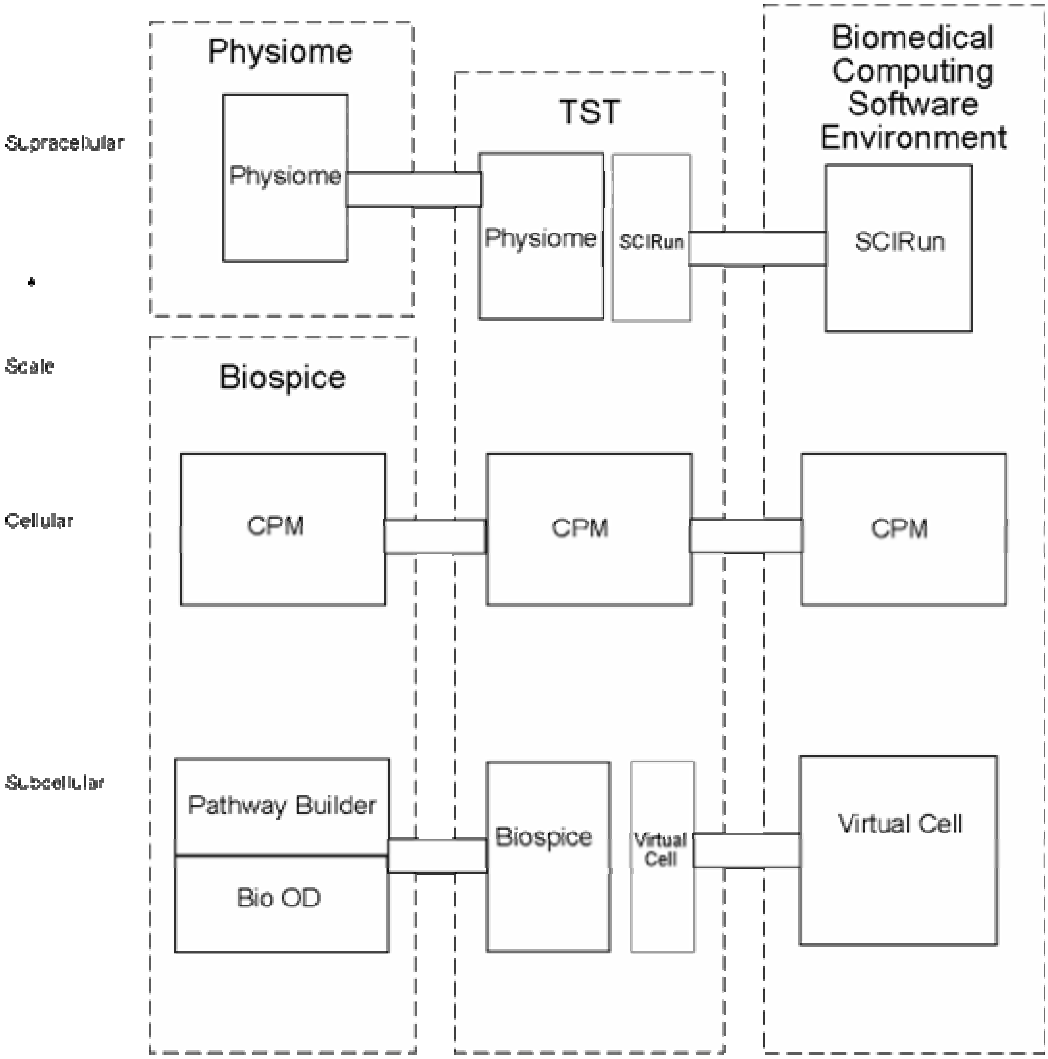


Figure 8: TST proposes to use CPM as its computational core, interfacing with state-of-the-art existing and proposed efforts at the subcellular and continuum ends.

Because tools are written in many different languages for many different platforms, we must have a standard system for interoperability. Some tools already have such support in the form of well-defined methods for extension and embedding. For example, SCIRun defines a complete extension system that uses C libraries and XML definitions for modules.

However, not all potential applications use a standard for interoperability and even when they do, it may not be general enough. To increase interoperability, all software we develop under this grant will use the Common Component Architecture (CCA). The CCA is a methodology and framework researchers from national laboratories and academic institutions developed to define common protocols for interoperation in high-performance applications. All new development under this grant will meet CCA standards. Because many applications already have different interoperation mechanisms, when possible, we will budget time and staff to help make pre-existing tools CCA compliant as well.

Virtual Cell is an excellent tool for validating models and generating microscopic properties of systems in which diffusion of molecules, cell membrane and volume, surface area and cell polarity are important factors Many of these parameters are also essential inputs to our Cellular Potts Model.

Both Virtual Cell and SCIRun include additional tools (*e.g.*, for visualization) that make them attractive and wide ranging toolkits for biological simulations. However, they do not currently include a cell-level simulation tool. Integration with Virtual Cell uses Java/RMI and Web portals.

For these reasons we have actively sought the cooperation of Dr. Loew and the NRCAM to integrate our CPM and TST with Virtual Cell and to coordinate this proposal with Dr. Loew and Dr. Johnson's (University of Utah) proposal to this NIH RFA to create a center for Integrated Biomedical Software Systems (CIBSS). (See attached letters of support.) This coordination includes Virtual Cell and SCIRun in our tissue simulation toolkit and making our tissue simulation tools available through the CIBSS's proposed Biomedical Computing Software Environment (BCSE) to further this integration we will build our tissue simulation tools using the BCSE's CCA software architecture. We have agreed with Drs. Loew and Johnson (see attached letter) to work together to ensure seamless integration between our respective tools. This integration is particularly important because the CPM represents the intermediate cell-level simulation currently lacking from the BCSE.

As we noted in Core 1, BioSpice is a large-scale open source toolkit for subcellular modeling. Of the many BioSpice tools, the most relevant to our proposal are PathwayBuilder and GeneChip DB, which provide experimental data and parameters for the tools for simulating the operation of Reaction Kinetics networks. BioSpice's current focus is exclusively subcellular. The data and simulation tools in BioSpice can directly generate many of the input parameters for the CPM. Similarly the experiments proposed in our BMPs, especially those at the genomic/molecular level, will generate data that can enhance the GeneChip DB.These experiments may also benefit from BioSpice simulations directly and help to validate BioSpice models. Thus BioSpice is complementary rather than competitive with our CPM and TST. As with Virtual Cell and SCIRs, we have signed a letter of agreement with Dr. Arkin (see Appendix) to work together to integrate TST tools, especially the CPM, with BioSpice and to make these mutually available as part of each other's toolkits. Again our goal is to achieve seamless parameter passing between BioSpice and the CPM, as discussed in the section on Multiscale Modeling, to use BioSpice to generate simple phenomenological models of genetic regulations and signal transduction networks related to our BMPs and to allow multiple BioSpice simulations to directly control individual cells in the CPM.

II.8.iv.3 CPM INTERFACE TO MICROSCOPIC MODELING:

The subcellular models can just as well be adapted to discrete approaches like the CPM based on association from experimental observations of one or more intracellular variables with extracellular state variables in the CPM. Thus we can envisage a Monte Carlo approach in which random numbers for "waiting times" can be generated during which single cells can be updated for their internal states using any of the subcellular models in this proposal. Such an approach has the flexibility of numerical solution for very complex models given adequate computational facilities.

Of interest is the following description of the linkage between the CPM framework and Gene Regulatory Networks, that shows the way the various subprojects in this core will be linked. In our model of chicken limb development (Chaturvedi *et al.*, 2003), for example, we model a rudimentary Gene Regulatory Network (GRN) sufficient for chondrogenesis. We do this by allowing cells of different types and define the conditions under which the type may change. Hence, cells differentiate from initial stem cells into the specialized types in the developed organism. Though every cell is unique and modeled as such under the CPM framework, we identify cells with broadly similar structure and behavior as a "differentiation state" or type. In the chicken limb we consider, cell types of interest are the initial undifferentiated mesenchymal cells, which can mitose (divide), and produce various morphogens depending on the cues they receive from their external environment. We model differentiation using a "state-change" map. Each "state" in this map corresponds to a limb chondrogenesis cell type. The behavior of a cell depends on its type and its parameters within the state. Change of a cell from one type to another corresponds to cell differentiation. The "state change map" sets down the rules governing type change, they take into account several chemical fields at the intra- and inter-cellular level.

Thus, for the chicken limb, we assume all cells in the active zone of developing limb are of mitosing (*i.e.*, dividing) type. A mitosing cell in the active zone upon sensing a threshold local concentration of TGF-β changes to the fibronectin producing type. Such a cell is also capable of upregulating its cell-cell adhesion (the parameter $J_{\tau,\,\tau'}$ ($J_{\text{cell,cell}}$) in the CPM decreases). Cells that have not experienced local threshold levels of activator are of the mitosing and condensing type, responding to but not producing fibronectin.

Models of biological systems that involve more complex regulatory and signaling networks as described in the various subprojects will benefit greatly by integration of existing software packages like M-Cell with our tissue modeling tools. The abstract basis for networks provided by the Barabasi group will help in gaining important insights into specific implementations and also in the general behaviors arising out of the complex interactions between these two modules. It will lead to a holistic viewpoint combining "genetic" and "generic" factors that together shape Biocomplexity of living systems.

We have included Reaction-Diffusion as part of our CPM tool. The CPM tool uses various mechanisms beyond the CPM framework, including a classic PDE based approach to describe the morphogen fields. We can also include diffusion in the CPM on the same grid as the CPM lattice.

In general, CPM and field models complement each other since they usually encompass two different scales. The CPM uses a lattice to describe discrete processes at a cellular scale, field models usually arise from a continuum viewpoint, usually of diffusible entities such as small morphogen molecules. Continuum models introduced a grid while solving the PDEs numerically, and complexity arises from the complex geometry of the spatial domain and its movement. Hence, integration between the two modeling techniques requires matching of the possibly heterogeneous grids. Thus, in the chicken limb simulations, the point of interaction between the morphogen field that affects the cells and the cell itself is the spatial coordinate where they interact. To complicate matters, the morphogen molecule may exist only inside a cell, only outside it, or may permeate the whole of tissue space. If the molecule is not small and does not "dissolve" (*e.g.*, Fibronectin in the Chicken limb), the implementation must know that the two entities cannot coexist at the same spatial coordinate.

In the last decade, many different tools have addressed different aspects of computational systems biology. These tools provide varying degrees of support for different types of simulation, from microscopic Reaction Kinetics to macroscopic finite element simulations. Additionally, many form part of larger framework efforts that attempt to group together similar tools in complete toolkit packages. Because of the framework needed

to handle multi-scale simulation, we intend to leverage the existing applications as simulation parts of our TST.

II.8.iv.4 CPM INTERFACE TO MACROSCOPIC MODELING:

II.8.iv.4.a INTEGRATION WITH PHYSIOME:

We will achieve Integration Interoperability between TST applications and tools that are part of the Physiome project using XML-based languages. The three main languages are CellML, AnatML, FieldML. Additionally, many public databases are available under this Physiome project that can be downloaded directly or accessed via the Internet.

II.8.iv.4.b INTEGRATION WITH THE SCIRUN PROBLEM SOLVING ENVIRONMENT:

We propose to use the SCIRun Problem Solving Environment (PSE) and computational steering software system developed by the Scientific Computing and Imaging (SCI) Institute at the University of Utah as the primary continuum computational and visualization interface of the TST. NIH partially funds the SCI Institute as a NIH Center for Bioelectric Field Modeling, Simulation, and Visualization, headed by Professor Christopher Johnson who has provided a letter of support (Appendix I). SCIRun allows a user to interactively steer a computation, changing parameters, recomputing, and then revisualizing — all within the same programming environment. The tightly integrated modular environment provided by SCIRun allows computational steering to apply to the broad range of advanced scientific computations that the SCI Institute currently addresses. These include the study of bioelectric signals from the heart and the brain, medical imaging, surgical planning, reservoir modeling in geoscience, diffusion of airborne pollutants in environmental science, and combustion in chemical and fuels engineering.

II.8.iv.4.c GENESIS AND SCIRUN: A SUCCESSFUL PROTOTYPE:

Recently, Butson *et al.* developed a suite of tools that provide simultaneous simulation, visualization, and analysis of networks of neurons within the SCIRun: They used the Genesis program (Wilson and Bower, see **http://www.sci.utah.edu/pubs**) to numerically solve a multicompartmental, multicellular model of piriform cortex and to send the membrane potentials, intracellular currents, and spike times to a MySQL database using a customized SQL interface. An associated model in SCIRun retrieves the numerical data and passes them to other SCIRun modules for visualization of the membrane potentials in each compartment of each cell in the piriform cortex. SCIRun uses the intracellular current of each cellular compartment as initial conditions to solve the forward field problem, and then flexibly visualizes these results within a realistic head model. This successful effort increases our confidence that we will be able to integrate the CPM and TST tools in the SCIRun framework.

II.9 APPLYING OUR MODEL TO SPECIFIC BMPS:

To generate, refine and validate quantitative computational models of key embryonic development processes and their dependence on selected molecular cues. These models will refine our understanding of the mechanisms of embryonic development, and enable high throughput parameter searches, that will suggest specific experimental tests. Such computationally aided hypothesis construction will enable experimental biologists to more quickly focus in on the physicochemical parameters of the cells that are predicted to be significant determinants of pattern formation.

To build a Cellular Potts model of Matrigel endothelial cell cultures, in which the cellular parameters can be easily changed by experimental biologists. Such a model will provide a powerful tool to understand the

initial stages of vascular development, and to understand the effects of experimental treatments that alter the cells' biophysical properties.

To build computational model of *in vivo* vascular development, more specifically the incorporation of adipous stromal cells (ASCs) in the early quail and in the mouse chorion (see core 3), which will aid the experimental researcher in understanding which properties of the cell behavior are determinant for migration and incorporation of ASCs and hemangioblasts, targeting their experiments to pre-treatments that will specifically alter these cell behaviors.The motivating experimental BMPs of this proposal – regeneration and cardiovascular development – are crucial areas of research in biomedicine. Although our development of computational tools will focus on these areas, the Tissue Simulation Toolkit will not be just another suite of tools under SCIRun. Rather, we intend to use this state-of-art product as the framework for interfacing the CPM level description of tissues with continuum-level descriptions using the proposed experimental platforms as a testbed. The tools for interfacing the microscopic and macroscopic descriptions of these experimental models—cardiac and vascular tissue, or amphibian and zebrafish limb regeneration—are a central and novel feature of this proposal.

II.9.i VASCULAR DEVELOPMENT:

Blood vessels develop can develop *de novo*, through the recruitment and subsequent aggregation of endothelial cells (EC) and pericytes. This process, called *vasculogenesis*, occurs primarily in early embryogenesis. Later during embryonic development new vessels are primarily develop from existing vessels. New vessels originate from pre-existing vessels by sprouting, a process called *sprouting angiogenesis* or by means of division of blood vessels through *intussusceptive angiogenesis* Djonov *et al.*(2003). In adult mammals, physiological neovascularization is thought to occur primarily in the female reproductive system and during wound healing, and in many pathological processes, tumour growth (Carmeliet and Jain, 2000), diabetic retinopathy, psoriasis and rheumatoid arthritis.

Sprouting angiogenesis roughly involves the following steps. Stimulated by growth factors, including proteins of the vascular endothelial growth factor family (VEGF), endothelial cells excrete proteolytic enzymes, locally dissolving the basal membrane. Endothelial cells and pericytes proliferate, and migrate into the surrounding tissue, chemotactically attracted by growth factors. The endothelial cells aggregate into a string, and they form a lumen. A basal membrane is formed, possibly excreted by the pericytes, and a new blood vessel has been formed. The role of the pericytes is controversial. The traditional view holds they stabilize a primary vascular network formed by the endothelial cells. However, recent work suggests that in many organ systems pericytes and endothelial cells migrate at the same moment or that they may even be the “pioneers” of sprouting angiogenesis Gerhardt and Betsholtz (2003).

As more and more of the molecular key players in blood vessel formation are elucidated, the precise role of each of these and their interaction becomes ever more difficult to understand. The regulatory pathways of the many growth factors, extra-cellular matrix proteins, and receptors are accurately known. Through knock-out experiments, biologists have elucidated the role of each of the key players and identified many of these as potential drug targets. But it is still largely unknown how each of these players interact, when and where they become active, and how their effect on the endothelial cells and pericytes affects the angiogenetic process. Moreover, there is evidence that cellular traction forces on the extracellular matrix and the consequent stresses and strains play an essential role in the development of blood vessels. We are only at the beginning to understand how the biophysical processes of cell movements and the resultant strains and stresses on the extra-cellular matrix interact with cell regulation to conduct blood vessel growth (see *e.g.,* Manoussaki, 2003 and Namy *et al.*, submitted).

To understand the mechanisms of development, for a long time developmental biologists could rely on conceptual models. In such a model, a hypothetical mechanism is described using text and figures, and further experimentation is suggested that could support or reject the hypothesis. But with the advent of high throughput experimental procedures, such as DNA sequencing, microarray data, and the number of known interacting players (genome, proteins, cell types, extracellular matrix, etc.) in most developmental processes is growing so rapidly that it is no longer feasible to rely exclusively on conceptual models. Moreover, parallel developments in the physics of complex systems have shown that systems consisting of many interacting particles often exhibit collective behavior that cannot be intuitively understood from the

behavior of the individual particles. Thus with the gain of more *information*, the most central tool in generating new *knowledge* and *understanding* of development—the conceptual model—may no longer be usable.

Hence, to come to a true understanding of developmental mechanisms, it is essential to deploy computational tools that can help in organising and modeling the many interactions between the interacting elements of development. Such an approach is often called *systems biology* (Kitano (2002) and Fig. 1). Such an approach is often contrasted to a caricature of the traditional approach, that would blindly list all the parts of a biological system. But in fact a systems biology approach is not essentially different from the more traditional scientific approach, except for the fact that to generate new hypotheses, the traditional *conceptual models* have been exchanged for *computational models*.

Indeed, systems biology has already shown to be succesful in understanding processes in vascular development. Many researchers have used continuum models to study angiogenesis, in particular its effect on tumour growth (see for example Breward *et al.,* 2003). However, such models mostly only consider the density of blood vessels because they are interested in how the blood flow affects tumor growth. Our experimental collaborators are interested in the fates of individual cells, and for them it thus essential to understand vasculogenesis at the level of the individual cells, and to understand how the microstructure of the vessels develops. Other, spatial continuum approaches are able to model pattern formation, but although these approaches are very powerful, also in these models the morphogenesis cannot be understood at cell level. Using time-lapse video, Gamba *et al.*(2003) and Serini *et al.*(2003) demonstrated that endothelial cells taking part in vasculogenesis tend to migrate towards sites of higher cell densities, possibly attracted by a chemotactic signal. They applied this experimental observation in a partial differential equation (PDE) model that describes the endothelial cells as a viscous fluid, attracted by a diffusing chemical excreted by the endothelial cells. With low initial cell density, the cells aggregate to several disconnected clusters. When a threshold density is crossed however, the pattern switches to a single connected, percolating structure. The patterns formed in this model closely reproduce the experimental observations. These papers beautifully demonstrate how physical (percolation) theory can be applied to understand experimental observations on capillary network formation.

Although these chemotaxis based computer simulations closely resemble the observations in the *in vitro* experimental system, later theoretical and experimental work suggests that chemotaxis may not be the primary driving force in vascular network formation. According to this work (Manoussaki (2003) and Namy *et al.*, submitted), the extracellular matrix is mechanically remodelled through cell traction, by which the endothelial cells are haptotactically attracted. The role of strains and stressses in the extracellular matrix is confirmed through experiments.

These computational approaches impressively establish the use of systems biology in vascular biology. But until now, they have only generated hypotheses about cell cultures, where only endothelial cells are considered. We believe that the time has now come to take the next step, and apply the systems biology approach to vasculogenesis *in vivo*.

II.9.i.1 MODELING STAGES:

II.9.i.1.a STAGE I: Modelling of *in vitro* Capillary Network Formation with the Tissue Simulation Tool:

In order to capture *in vivo* blood vessel formation in a simulation model, several simplifications have to be made both in the experimental system and in the computational model. Rather than attempting to simulate a full *in vivo* experimental system right from the start, initially we will focus on simulating capillary network formation in Matrigel cell cultures (see Fig. 7 of Core 3). Although such cell cultures have altready been modeled by other groups using a more traditional continuum (partial diffential equation) approach, building such a model using the tissue simulation tools offers several advantages. Firstly, the data obtained from the experimental work (core 3) can be more directly translated into TST model parameters than into continuum parameters, because these measurements provide data on individual cell behavior. Measurements obtained from cell cultures are: (see Core 2)

1. Velocity
2. Trajectory
3. Persistence time of motility; *i.e.*, extent of random vs. directed motile activity
4. Cell shape and orientation (major and minor axial lengths and apparent vector of the major in the observed (x,y) plane
5. Rates and amplitudes of axial length ratio variability (protrusiveness)
6. Cell replication
7. Velocity-field maps
8. Deformation-rate plots (tissue deformations versus vascular pattern formation)
9. Degree of positive or negative interactions with other cells
10. Topological changes in pattern (degree of connectivity)
11. Correlations between whole embryo or tissue motion and vascular cell motion (DIC optics+fluorescence optics)

We will simulate aggregates of hundreds to thousands of cells, focusing on the tissue scale, where intercellular adhesive and inductive interactions, and interactions with the extra-cellular matrix are taken into account. The effects of cell adhesion and the interaction with the extracellular matrix are most naturally captured when the cells are modelled individually. The cellular Potts model (CPM), a lattice-based Monte-Carlo method originally developed to study the structure of soap froths (Holm *et al.,*1991)) and later extended to simulate differential adhesion driven cell rearrangement Glazier and Graner (1993), is very well suited for this purpose. It focusses on morphogenesis due to cell rearrangement in cell-aggregates caused by differential cell-cell adhesion through cellular adhesion molecules (CAMs), and quantitatively reproduces cell sorting experiments Glazier and Graner (1993). In the CPM, biological cells are represented on the lattice by a patch of sites in the same state, where the state identifies a cell. Cell-cell contacts are represented as bonds between sites of unlike state, where the bond strength is determined by the types of "cellular adhesion molecules" (CAM) of the cells to which both sites belong. During a Monte-Carlo step, the cells attempt to make new bonds to neighboring cells, trying to copy the state of a site into a neighbouring site where a Hamiltonian favours stronger bonds over weaker bonds. An example is given in Fig. 3.

Some of the experimental measurements can be directly used as modell inputs; techniques are available to implement them in the TST. Cell replication, cell velocity, persistence of motitilty, and cell shape and orientation can be directly set as model parameters (see description of the CPM above). Measurements do not apply to individual cell behavior, and they cannot be used as model inputs. They provide data about the collective cell behavior that emerges through the interplay between the cells and the extracellular environment. Such observables include the velocity fields, the degree of positive or negative interaction with other cells, deformation-rate plots and correlations between whole embryo or tissue motion and vascular cell motions. These measurements thus provide important handles to validate the behavior of the computational model.

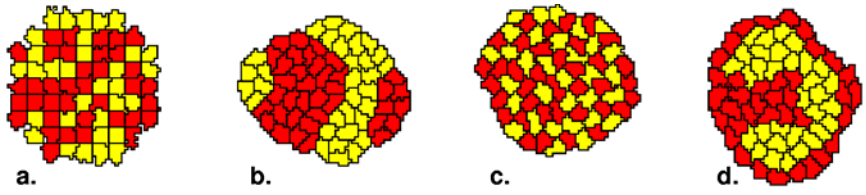


Figure 9: Simple set-up of the cellular Potts model (CPM) Glazier and Graner (1993). (a) Initial condition, (b-d) results for various bond-strength settings between dark cells, light cells and the surrounding medium.

The CPM is becoming a standard computational tool in the study of biological morphogenesis, and has been used to simulate the full development of the cellular slime mould *Dyctostelium discoideum* (Glazier and Graner, 1993) convergent extension in early vertebrate embryos Zajac *et al.*(2000), tumour invasion Turner and Sheratt (2002) and skeletal formation in the vertebrate limb (S.A. Newman and J.A. Glazier, in preparation).

We will use a hybrid CPM and partial differential equation (PDE) model (Glazier and Graner, 1993). The endothelial cells and the extracellular matrix will be modelled using the CPM, while the diffusion of small molecules (VEGF, PAI-1, proteolytic enzymes, etc.) will be modelled using PDEs. The cells will exhibit experimentally confirmed behaviour and excrete VEGF (after hypoxic stress), chemotactically migrate upstream VEGF-gradients, which will be hindered by the ECM's stiffness. Furthermore the cells will excrete fibrinolytic enzymes, attach to the ECM through integrins located at the cell surface, whose expression is Induced by PAI-1. Some authors stress furthermore stress the importance of viscoelastic mechanical feedback of the endothelial cells with the extracellular matrix.

These processes are all elegantly modelled using recent extensions to the hybrid CPM/PDE that we described in Section VII.2. The model parameters will be estimated using a dual-method approach. Firstly, the biophysical and chemical properties of the ECM in the quail will be measured in the lab. The excretion rate of growth factors by the pericytes and endothelial cells will be measured. The cellular response to chemotactic and haptotactic gradients will estimated using the microfluid chambers developed at IUB (see core III). Using the statistical measurements obtained from imaging experiments (Rupp *et al.* 2003) , we will be able to obtain a number of important modelling parameters. Cell motility will be characterised by calculating the mean square displacement of the cells, which defines a diffusion coefficient and the mean free path, or *peristance length* of the cells. The peristance length is an important parameter in the CPM, which can be set by introducing velocity correlations, as described above. Moreover imaging experiments can measure the persistence time, allowing us to fine tune the simulation parameters and better match developmental experiments. Thus, we can determine which parameters are irrelevant, and which parameters significantly affect the vasculogenesis process. This will greatly streamline our research efforts, since it will facilitate a focus on the key players in vasculogenesis, while it may prevent extensive research efforts on mapping out the role of factors that appear not to strongly affect vasculogogenesis. Finally, high-throughput, systematic parameter screenings of the simulation models may help elucidating the origin and mechanisms of pathologic vascular development. Mapping the vascular patterns obtained in the simulations against databases of known anomalous vascular patterns may help finding which parameters have to attain very specific values to obtain a particular pattern, and which parameters may vary (and are thus less relevant). Again, this will focus experimental research to the factors are most likely affected in a pathologic situation.

II.9.i.1.b STAGE II: Modelling the Delivery of Adipous Stromal Cells in *in vivo* Cultures:

In the second stage of the project we will attempt to model the *in vivo* experimental system that is proposed in Core III. This experimental system consists of essentially two-dimensional, chimeric avian and mammalian model systems.: *ex ovo* cultures of the quail embryo (Rupp *et al.* 2003), and the mouse allantois isolated an cultured *ex utero,* both of which can undergo extensive vascular development and assembly under conditions favorable to microscopic study (see core 3). These cultures are essentially flat and relatively thin, which creates excellent opportunities for imaging and computational modeling, because to first approximation we can work with two-dimensional simulation models.

For this step the succesful conclusion of stage I is an essential precondition. Once that stage is completed, we have captured and understood the essential features of endothelial cell behavior in a computational model, and only then we can start adding the behavior of the other cells in the quail embryo cultures, such as pericytes.

To the model described in section 2.1, we will add the following elements: 1) Interaction with the overlaying and underlaying mesodermal cell layers, 2) the possibility to systematically vary the adhesion to the extracellular matrix, which will aid the experimental researcher in understanding how the cell surface molecules determine the delivery of ASCs and hemangioblasts.

This will provide an important tool to the experimental biologists in Core III. These models will refine their understanding of the mechanisms of embryonic development, and enable high throughput parameter searches, that will suggest specific experimental tests. Such computationally aided hypothesis construction will enable the experimental project s to more quickly focus in on the physicochemical parameters of the cells that are predicted to be significant determinants of pattern formation.

II.9.i.1.c STAGE III: Tube Formation:

Initially, our focus is to understand vasculogenesis in the early quail, and to understand the formation of vascular networks in cell cultures of endothelial cells. In these experimental systems, vasculogenesis takes place in an two-dimensional space, and to first approximation the proposed two-dimensional model will be sufficient. In these two-dimensional simulations the developing vessels are represented by strings of endothelial cells. To obtain more realistic simulations, we will extend our work to three-dimensions and allow for the formations of tubes. Although we do now routinely carry out three-dimensional CPM simulations, the formation of actual blood vessels with a lumen calls for an extension of the CPM model. In a parallel track, Dr. Debasis Dan in the group of Prof. Glazier at Indiana University, Bloomington, is now developing such an extension, which involves the introduction of cell polarity. To obtain a ring like structure from a cell aggregate the differential adhesivity will be position dependent. Suppose an oblong cell which has a very high adhesivity to its own type at its short sides, while its long sides are non-adhesive. Such cells may form long strips of cells. To form tubes, the strips have to bend over. The bending energy of these cells may be provided by chemo-attractants (for example VEGF). This scheme can be extrapolated further by assuming that the upper and lower part of the rings have different adhesivity than the anterior and posterior, which will result in self-assembly (stacking) to form long tubes.

With the CPM alone we can currently not model cell *layers* in which the constituent cells are coupled through thight junctions, such as in epithelia. Recently, several methods have been developed to include cell polarity in the CPM, and these methods may allow us to model tightly integrated epithelia in the future. Another, perhaps more efficient possiblility may be to model epithelial cell layers as a whole with finite element models. Finite element models are routinely interfaced with lattice based models (such as the CPM) by iterative discretization of the finite elements (see *e.g.*, Merks *et al.*, 2003) for which fast algorithms are available. The movements of the finite element model, and the surface tension of the finite element with the CPM will be expressed using a Hamiltonian, allowing for fast and straightforward intergration with the CPM.

Initially, this track will focus on developing the techniques to model non-uniform distributions of adhesion strength on the cells, and it will involve an investigation of putative mechanisms to build vascular tubes through self-assembly. After the initial phase has completed, more experimental input is needed. We will need measurements of the distributions of cell adhesion molecules, or of the distribution of the adhesive strength.

II.9.i.1.d STAGE IV: Effects of Fluid Flow:

An important process in the formation of vascular networks is "pruning". After an initial network has formed, and blood starts flowing through it, blood vessels that receive more blood will be selected and reinforced, while the other vessels will die. To model this process, in a separate track we are developing methods to model fluid flow using the CPM. This will enable straight-forward interaction of the cells and the surrounding fluid. To do so, we break up the ECM into small hypothetical fluid cells (much smaller than the actual biological cells). These fluid cells have most of the properties of biological cells, like adhesivity, volume constraint and surface constraint. The chemical concentration is assumed to be uniform within this cell and diffusion occurs by averaging the neighbouring concentrations (Morse and Feshbach, 1953). With this implementation we are able to achieve both diffusion and advection within the CPM framework.

II.9.i.2 CONCLUSIONS:

We propose to build computational models of *in vitro* and *in vivo* experimental systems of vascular development. These models will provide important insights in the mechanisms of vasculogenesis, because it wil help us to understand how the behaviour of individual cells, their interaction with other cells and the extracellular matrix, and their gene expression patterns drive vascular development. Moreover, the screening of large ranges of parameters values, will enable us to find alternative results of the pattern

formation process. Such results may a) suggest experimental manipulations which will validate the model, and b) bear similarity to know pathological vasculogenesis, for which the model may provide new hypotheses that can be tested by experiments. Thus, this tool will increase the understanding of vascular pattern formation and focus and speed up the experimental cycle.

II.9.ii HEART DEVELOPMENT:

See Computational Platform, under Core 3.

II.9.iii LIMB REGENERATION:

Our work on chicken limb has demonstrated the use of CPM as a framework for modeling regeneration at the cellular and tissue scale. Our chick work also demonstrates the linkages between different scales that we have implemented in our simulations (Izaguirre *et al.*, 2003; Chaturvedi *et al.*, 2003). For additional details see Core 3.

Figure 9 shows experimental chondrogenesis, and Figures 10 and 11 show our simulation results.

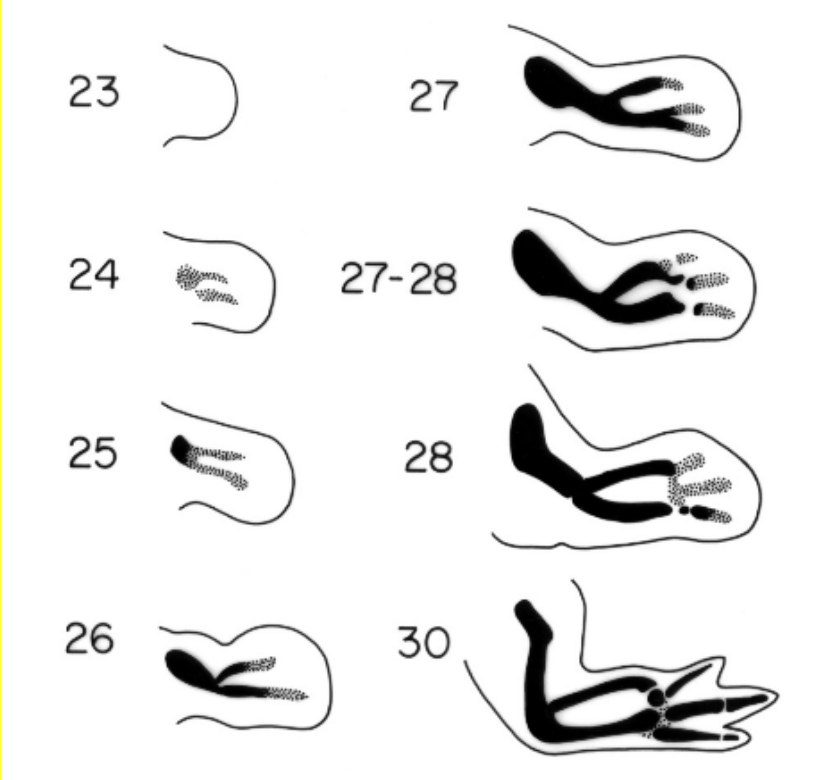


Figure 9: Skeletal Pattern formation: stages in a chicken limb bud.

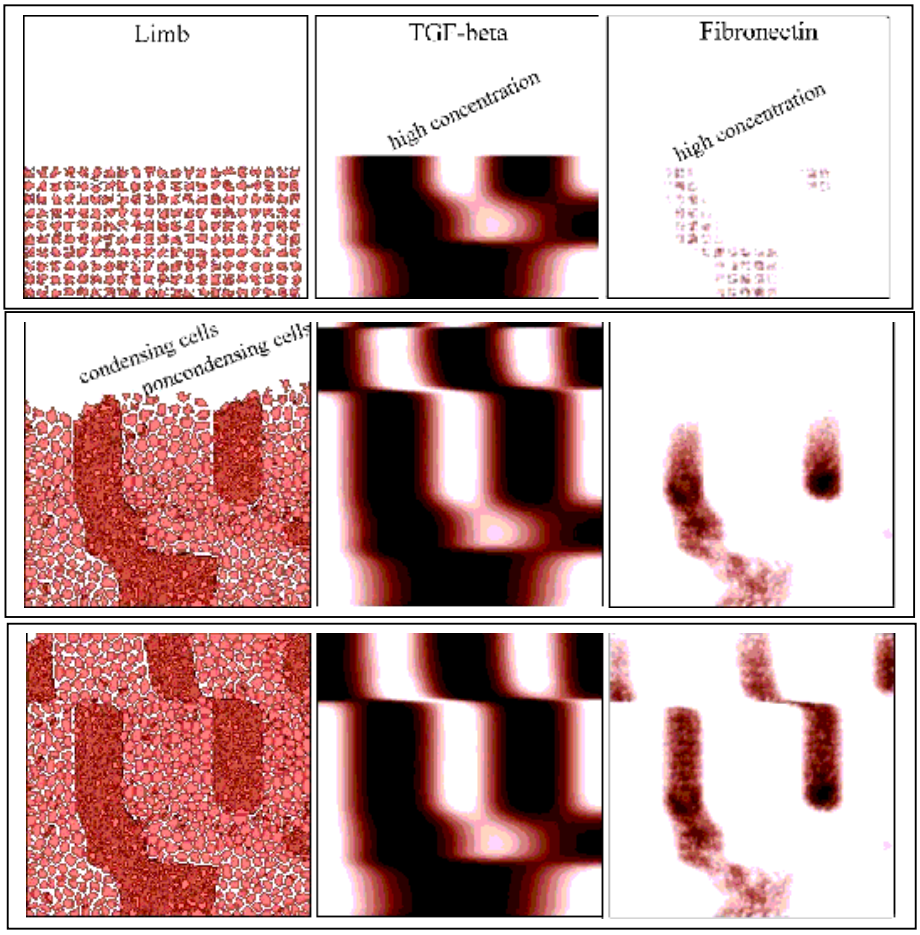


Figure 10: Condensation of cells into chondrogenic patterns- 2D simulations at different times in a simplified rectangular domain (see Chaturvedi *et al.*, 2003l Izaguirre *et al.*, 2003).

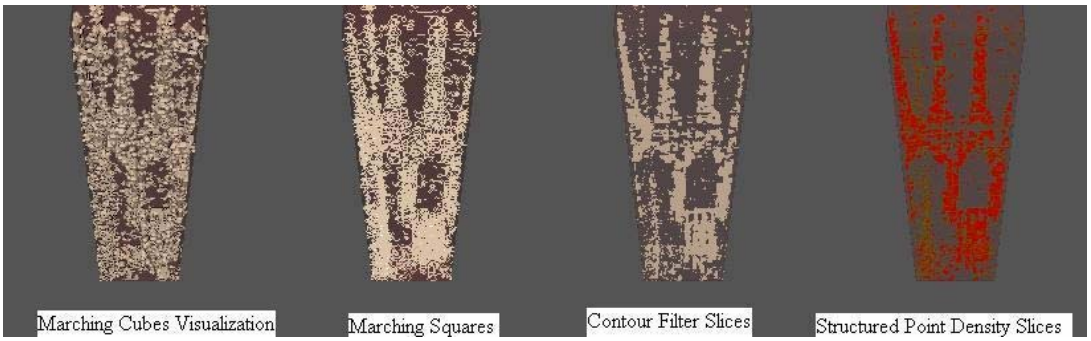


Figure 11: Condensation of cells into chondrogenic patterns. Various visualization mechanisms bring out different features of the cell distribution after 1340 Monte Carlo Steps (MCS). These simulations use the complete three-dimensional Schnakenberg system of prepatterning equations.

We will develop a parametric model of blastema formation into a solid conic structure. Using data obtained by time-lapse video for longitudinal- and cross-sections of different stages of limb regeneration. First, we will develop a geometric representation will be developed using tools such as MIMICS, ProE or Matlab. The software will provide x, y and z surface coordinates. Once we know the morphology of the blastema formation we will begin to establish a tissue model using the information obtained through a finite/boundary element analysis.

Our hypothesis is that limb regeneration involves tensile and compressive strains mediated through cells, cell connections and the extracellular environment. This hypothesis is in concert with recent theory (Henderson and Carter, 2002). Limb regeneration involves tissue differentiation, direction, growth and anisotropic growth rates leading to a specific morphology. The various factors affecting the regeneration process are mediated through the mechanical environment involving growth related strains. The mechanical environment involving growth related strains mediates the various factors affecting regeneration.

We will model the various regeneration phases obtained through time-lapse video. We will digitize the coordinate data of the regenerating surface at fixed time intervals, initially 2hrs. From a morphology movie, we will estimate the strains that would be responsible for regeneration growth if the limb were a visco-elastic solid, using both a solid model and a finite element mesh. The finite element model will use ANSYS general-purpose software and the stress analysis will use the developed CPM. Experiments will attempt to measure the limb's material properties during regeneration, starting with linear elastic material properties.

Loading of the finite element model will produce strains in the digitized models. A series of finite element models will be built corresponding to various morphology of limb regeneration and we will calculate strain distributions at various locations to compare to experimental data. We will refine the simulation models by changing loading and geometry until the calculated strains agree with the experiment.

Limb regeneration growth depends on cell mitosis rate, cell hypertrophy, and ECM production. Mechanically, growth relates to strains. We will extend our model to explore this connection by combining the CPM with FEM models. One fundamental problem in blastema formation is identifying cell-mediated factors determining limb regeneration size and shape.

II.10 EXIT STRATEGY:

It is expected that funding through the Indiana Genomics Initiative will provide resources needed for continued support and development of TST through 2008. In the third year of the project, we will also apply for R01 and NSF funding to continue algorithm development and application of TST to other biologically motivating projects, such as bone regeneration, which also has strong representation at the IU Medical School. The strong history of the IU School of Medicine, and the aggressive building and growth projects currently underway (a $70M building is being started, after the completion of two other new buildings in the last 3 years) suggests that the overall funding stream for this research will be strong and ongoing. In addition, the State of Indiana's Biocrossroads consortium is initiating several public-private partnerships to promote technology transfer and commercialization. Many of the BMPs have direct or indirect links to projects targeted for commercial investment. Thus, there is a strong mix of funding from private charitable endowments (The Lilly Foundation, Inc. support for the Indiana Genomics Initiative), public grants (NIH,

NSF, and others), and opportunities for private commercial development. The funding base then seems diverse, reliable, and strong.

II.11 EDUCATIONAL SIGNIFICANCE:

Training and teaching are integral parts of the research program at IUB, IUPUI, Notre Dame and Purdue. Graduate and undergraduate students perform a significant proportion of the research in our theoretical and computational groups. The development and support of the Tissue Simulation Toolkit will provide varied opportunities for participation from graduate and undergraduate students interested in the computational and life sciences. With growing quantitative biology initiatives at the various campuses (for example, as part of the newly formed School of Informatics and expanding Biophysics Program at IUB), we expect the computational and modeling activities of this proposal to synergistically benefit from and contribute to the educational core of our institutions.

II.12 TIMELINE:

Quarter	Year 1	Year 2	Year 3	Year 4	Year 5
One	Interact with Core 2 members on choice of cell line, join other subcellular modeling groups in collecting information on metabolic information. Collate reaction sets, and secure detailed pathway of cell line. Interact with extracellular modeling group to assess relevant extracellular factors for tissue scale modeling	Interact with experimental group on collection of subcellular process data. Develop methodology for model identification.	Collect data for different cell types, and metabolic data. Begin a new phase of investigation incorporating gene regulatory networks into metabolic models. Include cyclic AMP balances and couple with protein synthesis. Interact vigorously with Stochastic modeling group	Interact with Stochastic modeling group to report findings and discuss variations found between their approach and that spearheaded at Purdue. Interact with experimental group in Core 3 and examine cytometric measurements of protein distributions with model trends	Application of methodology developed with comprehensive model for different cell types encountered in cardiac development.
Two	Formulate metabolic models and scope literature for any available data.	Implement methodology for model identification.	Begin formulation of single cell (comprehensive) model including both metabolic and genetic regulation.	Investigate various links to extracellular variables through metabolic model enhanced with gene regulatory features.	Continue investigation of different cell types for cardiac development.
Three	Simulate models for preliminary results.	Simulate models for comparison with data	Begin simulation of complete model for the first cell system identified.	Interact with Tissue modeling group to investigate the impact of comprehensive model	Continue investigation of different cell types for cardiac development
Four	Cross check with other subcellular modeling efforts and compare results.	Interact with Core 3 members to identify additional single cell systems for modeling.	Compare model simulations with those made without gene regulation.	Begin scoping different cell types for analysis with global model	Interact with all modeling groups to evaluate impact of intracellular modeling approach

CORE III: EXPERIMENTAL EFFORTS

III.1 COORDINATORS:

Keith L. March (IU School of Medicine, IUPUI), Loren J. Field (IU School of Medicine, IUPUI), David L. Stocum (Department of Biology, IUPUI)

III.2 PARTICIPANTS:

Anthony Firulli (IU School of Medicine, IUPUI), Simon Conway (IU School of Medicine, IUPUI), Weinian Shou (IU School of Medicine, IUPUI), Charles Little (Kansas University Medical Center), Merv Yoder (IU School of Medicine, IUPUI), Brian Johnstone (IU School of Medicine, IUPUI), Jingling Li (IU School of Medicine, IUPUI), Ellen Chernoff Department of Biology, IUPUI), Simon Rhodes (Department of Biology, IUPUI)

III.3 GENERAL INTRODUCTION:

We have chosen three focused, interrelated Biologically Motivating Projects (BMPs), which focus on the morphogenesis of heart development, vascular development, and limb regeneration. These projects combine quantitative experiments and computer simulations, build upon the mutually complementary strengths of the researchers at the center, and illustrate a broad variety of fundamental developmental mechanisms. These three projects provide a unified and logical program for research and exploit intersecting projects in areas of outreach, bioinformatics and education. This core contains two parts—detailed descriptions of the three BMPs and a brief summary of common experimental techniques relevant to these experiments and available as core resources for current and future BMPs

III.3.i Goals:

The goals of Core 3 are to:

- Generate the biological data necessary to develop computational simulations of the systems studied.
- Use these computational simulations to predict the effects of particular experimental treatments or perturbations.
- Validate simulations through follow-up experimentation and analysis.
- Refine simulations as tools for continuing research.

These projects address a number of key goals of the whole program, which are to:

- Understand how cellular decisions are made at the molecular level.
- Elucidate the structure and properties of molecular and control networks.
- Determine the mechanisms that give rise to large-scale cell migration and the patterning of differentiation.
- Understand how cell and extracellular matrix (ECM) properties interact with tissue geometry to give rise to specific function.

III.3.ii Introduction to Morphogenesis:

III.3.ii.a General Background/Definitions:

Organogenesis is the establishment of functionally coordinated arrangements of tissues during embryonic development. It consists of three main processes, *morphogenesis*, *differentiation*, and *pattern formation*, which we can separate conceptually, although they typically occur in coordination with one another in space and time.

Morphogenesis is the shaping and molding of living tissues in three-dimensional space. In addition to its role in development, morphogenesis is central to regeneration, wound healing, and various pathologies. During morphogenesis, tissue masses may disperse, form internal foci of cell condensation, lengthen or shorten, or acquire lumens. They can also form sheets that may invaginate or evaginate, or develop one or more internal boundaries that restrict or prohibit cell mixing. Such compartmentalized tissues can either physically separate, or remain attached and engulf, or be engulfed by, one another. These processes produce the various body plans and organ forms characteristic of multicellular organisms, as well as tumors, abnormal polyps, and fibrotic lesions.

Differentiation is the biochemical diversification of cell types according to well-ordered lines of descent, or lineages. Because differentiation is a dynamical process that involves networks of transcription factors controlling one another's expression and the expression of other target genes, we consider it primarily as part of the suncellular section of this application (Core 2, part 1).

Pattern formation is the establishment of specific spatial arrangements of differentiated cell types.

III.3.ii.b Morphogenesis:

Multicellular organisms exhibit dramatic modifications in shape and form as they develop, modifications associated with successive changes in the spatial organization of specialized (differentiated) cell types, such as chondrocytes, neurons, blood cells, and vascular epithelia. We call this process morphogenesis. How functional and spatiotemporal specialization takes place remains an open question for cell and developmental biologists. Cells interact with other cells, with extracellular matrix, and with diffusible molecules. Differentiation and cell migration may occur simultaneously or sequentially. These processes, which generate the body plan and the various organs, depend on regulated gene expression (and provide the basis for new gene expression), coordinated cell movement, and tissue rearrangement, creating an enormously complex network. However, we can discover dynamical and organizational rules at various levels.

Morphogenesis is one of the major outstanding problems in the biological sciences. It concerns the fundamental question of how biological form and structure are generated. Morphogenesis encompasses a broad scope of biological processes. It concerns adult as well as embryonic tissues, and includes an understanding of the maintenance, degeneration, and regeneration of tissues and organs as well as their formation. Morphogenesis also addresses the problem of biological form at many levels, from the structure of individual cells, through the formation of multicellular arrays and tissues, to the higher order assembly of tissues into organs and whole organisms. While related to the field of developmental biology with its traditional emphasis on the control of gene expression and the acquisition of cell fates, morphogenesis investigates how this regulation of cell fates contributes to the form and structure of the organism and its component parts.

III.3.iii Rationale and Benefits of Computational Simulation:

III.3.iii.a Introduction:

Computer simulations that allow the separate representation and control of individual mechanisms are essential for disentangling the complex interacting phenomena of embryonic pattern formation (the spatiotemporal control of cell differentiation) and morphogenesis (the generation of the appropriate three-dimensional relationships among tissues). Such simulation allows integration of these data with other experimental results. Ultimately the simulations should be multiscale and quantitatively predict phenomenology.

III.3.iii.b Interplay of Physical and Genetic Mechanisms in Organogenesis:

Embryos and tissues develop forms that express highly complex, genetic programs. Nevertheless, embryos, organs, and healing and regenerating tissues assume many forms resembling those that physics produces in nonliving materials. Often genes determine morphogenesis by mobilizing physical forces and properties. Core 2 describes a range of generic mechanisms underlying or contributing to tissue morphogenesis, which living and nonliving materials shared and which thus allow modeling that uses variations of the methods of computational physics.

Single cells, as independent signal-processing units, interact with their environment (including diffusible chemicals and extra cellular matrix, ECM) and communicate with each other through their membranes. Findings in material science suggest that physical properties such as the adhesiveness of the cell surface, the viscoelasticity of the intracellular milieu and the extracellular matrix, and the cell's biosynthetic response ("excitability"), all of which are genetically regulated, can alter in response to external stimuli and can determine macroscopic tissue properties and behavior.

Physical mechanisms depending on the material properties of cells and tissues profoundly affect morphogenesis. These material properties depend on the gene products expressed by the cells and tissues, as well as on other components, such as water and ions, which gene products typically control. Thus looking at tissue morphogenesis as a physical phenomenon and looking at it as a genetic phenomenon are compatible. Changes in gene expression mobilize most physical processes we consider, although many immediate signals and biochemical modifications at the cellular level may not require direct genetic instructions. Chemical reaction, diffusion, and ECM deposition generate extracellular signals; cells respond to these signals and undergo patterned changes in functional or adhesive state; and sequential changes of cells' surface properties, in turn, modify the chemical environment and ECM configuration, thus driving morphogenetic behavior.

Modeling morphogenetic processes such as the development of the circulatory system and limb regeneration illustrate a broad variety of fundamental developmental mechanisms. Using experimental data, computational modeling of these processes should allow for the integration and understanding of both the physical and genetic mechanisms underlying morphogenesis. Morphogenesis serves as a model process for studying pattern formation and a number of different aspects of embryogenesis, such as the role of biological clocks during development, gene expression and cell differentiation, cell-cell signaling and signaling cascades, cell migration and adhesion. Moreover, the computational studies of these processes provide an ideal opportunity to investigate multiscale effects linking findings at the subcellular level to those at the cellular and supracellular level.

III.3.iv Technical and Scientific Commonalities between Projects:

Our approach to research is interdisciplinary, combining embryology and molecular biology with bioimaging and computational modeling. Participants in this core will study limb regeneration and vascular and cardiac development. In each of these processes, many of the same proteins play key roles; TGF-β, FGFs, BMPs, and fibronectin are universally significant. Additionally, these diverse tissues may also exhibit similar physical movements. In view of this universality, all our Subprojects consist of the same three parts:

- A set of experiments focusing on the known morphogenetic mechanisms and the way that they give rise to the physical phenomena observed (cell migration, patterning, *etc*.).
- The use of experimentation and modeling to study the cell-cell adhesive interactions and cell-growth factor interactions, and genetic control systems involved in development.
- Genetic perturbation and analysis to identify important regulatory and other genes that play roles in controlling and implementing the developmental phenomenon.

Technically, many of these experiments will utilize the same methods, such as transfection, retroviral infection, transgenic and knockout approaches, and electroporation with DNA promoter-reporter and endogenous protein-GFP constructs – which allow direct quantitative visualization of gene and protein expression during cell migration, aggregation, and spatiotemporal patterning *in vitro* and *in vivo*. In addition, fluorescently labeled antibodies will determine *in vivo* the subcellular localization and structural organization of important proteins.

The targeted perturbation of particular genes of interest through misexpression and/or inhibition provides further insights into genetic mechanisms.

III.3.v Medical and Basic Science Motivations:

Studying the developmental mechanism in these patterning processes will aid the identification of protective or potentially disruptive factors for normal circulatory system and limb development and could potentially impact on treatments for the prevention of numerous disorders. Moreover, a study of these developmental processes provides an ideal opportunity to investigate multiscale effects linking experimental findings at the molecular level to those at the cellular level.

Cardiovascular disease is one of the leading health threats facing the public today. Further understanding of the underlying regulatory mechanisms of cardiovascular development will greatly advance potential treatments of a wide range of diseases. As discussed above, limb regeneration shares common biological ground with cardiovascular development. Not only does limb regeneration research offer future treatments for wound recovery, but understanding the process of dedifferentiation and pattern formation can have a tremendous impact in treating damaged hearts. Given the fact that damaged heart tissue has a limited capacity for self-repair, a knowledge of regenerative processes may unlock previously unknown methods for treating heart disease.

III.4 HEART DEVELOPMENT:

III.4.i Introduction:

The cardiovascular system forms the vertebrate embryo's first functional organ. At present, however, the heart, while well studied, remains little understood. Much of what we know about early heart development was discovered through observation and embryonic manipulation. Only the advent of molecular biology has made possible the study of the genetic pathways involved.

III.4.ii Experimental Platforms:

Although the morphogenetic transformation of the primitive heart into a four-chambered structure is fairly well characterized from an anatomical standpoint, issues pertaining to the origin of the heart forming cells, regulation of cardiomyogenic induction and proliferation, and the molecular regulation of morphogenesis during early embryonic life remain largely unanswered. Through the use of a variety of genetic technologies in a number of different species, molecules responsible for regulating several key steps of cardiac development have been identified. In the mouse, both traditional transgenic and gene targeting experiments have identified a number of genes that play a key role in cardiomyocyte differentiation and proliferation, as well as organ morphogenesis and/or chamber specification (Firulli *et al.,* 2002; Pasumarthi *et al.,* 2002; Solloway *et al.,* 2003). Although these experiments have provided great insight in to the molecular regulation of cardiac differentiation, proliferation and organogenesis, most studies have been largely two-dimensional in nature and none have embraced the notion of generating predictive computer simulations of cardiac development. To begin to accumulate baseline data and computational tools to generate three-dimensional predictive models, we propose to study four key steps in cardiac development. These four "experimental platforms" will complement ongoing, NIH-funded projects in four independent laboratories within the Herman B. Wells Center for Pediatric Research. They are:

III.4.ii.a Experimental Platform 1: Modeling the effects of cardiomyocyte proliferation on the developing ventricular wall:

Retroviral tagging experiments in chicks suggest that proliferation of individual progenitor cells within the tubular heart gives rise to transmural, cone-shaped growth units which ultimately assemble with one another, resulting in the formation of three-dimensional ovoid structures, which contribute to the formation of the ventricular wall. Studies in this experimental platform will provide baseline-imaging data to model the growth of individual transmural growth units within the normal embryonic heart. Data will include spatio-temporal information of cardiomyocyte position, number, and division rates. We will use this information to generate a computer simulation of this stage of cardiac organogenesis. We will then initiate *in silico* experiments wherein

the division rate of individual progenitor cell is altered. We will, in parallel, initiate imaging studies wherein the division rate of individual progenitor cells are similarly altered via genetic manipulation. Real-time imaging data from the genetically altered progenitor cells will be compared to those generated *in silico* to directly test the predictive capacity of the simulation.

III.4.ii.b Experimental Platform 2: Deducing additive vs. synergistic effects of cardiac transcription factors on heart development:

To date, a large number of transcription factors have been shown to be essential for the morphological formation of the developing heart. For example, mice lacking the homeobox transcription factor *Nkx2.5* exhibit incorrect cardiac looping and the apparent lack of a left ventricle. Conversely, mice lacking the bHLH transcription factor *HAND2* exhibit morphological defects in the developing right ventricle. Studies in this experimental platform will analyze spatio-temporal images of cardiac development in normal hearts as well as hearts from mice lacking either the *Nkx2.5* or the *HAND2* gene. Data will include cataloging the overall morphology of the hearts, as well as the anatomical position, relative size, and cell content of ventricle and atrial chambers. We will use this information to generate a simulation model the three-dimensional spatio-temporal pattern of cardiac structure and gene expression. We will then initiate *In silico* experiments to predict the spatio-temporal pattern of cardiac structure and gene expression in *Nkx2.5*, *HAND2* double mutant mice. In parallel, we will initiate imaging studies of cardiac development in the in *Nkx2.5*, *HAND2* double mutant mice. We will compare this imaging data to those generated *in silico* to directly test the predictive capacity of the simulation.

III.4.ii.c Experimental Platform 3: Modeling the development and stem cell-based repair of cardiac valves:

Morphogenesis of the cardiac valves is critical for appropriate development of the four-chambered heart. Studies in this experimental platform will determine if cell transplantation can repair the cardiac valves *in situ*. Initial studies will establish the spatial and temporal patterns of cell apoptosis and proliferation in valves from neonatal and adult mouse hearts. We will use these data to model cell proliferation and/or apoptotic rates during valve development, and to predict *in silico* how rates of endogenous cell proliferation and/or apoptosis must alter to maintain normal valve size and morphology following the addition of exogenous cells. In parallel, additional experiments will image adult valves following colonization with exogenous EGFP-expressing marrow-derived stem cells. In addition, we will image valves from non-colonized and colonized hearts after mechanical injury. We will then compare the results of these manipulations will then be compared to the computer models to test their predictive value for simulating cell maintenance and turnover within normal and injured adult valves.

III.4.ii.d Experimental Platform 4: Modeling the molecular and cellular regulation of cardiac ventricular trabeculation and compaction:

Interactions between the endothelial cells of the endocardium and the proliferating cardiomyocytes play a key role in driving the formation and compaction of the ventricular trabeculae. Data from knockout mice indicate that BMP-10 is a key regulator of this process. Studies in this experimental platform will use transgenic reporter mice to simultaneously image the spatio-temporal interactions between endothelial cells and cardiomyocytes in three-dimensions during critical stages of trabeculation in normal and BMP-10 knockout hearts. Data will include morphologic quantitation of the anatomical attributes of the “endocardial out-pockets” (which reflects the ability of endocardium to recruit myocytes), as well as those of the nascent trabecula *(i.e.,* cell number, division rates, and relative position to the endothelial out-pockets). We will use these data to generate a dynamic simulation of ventricular trabeculation, and also to predict *in silico* the effects of relative BMP-10 expression vs. the extent of ventricular trabeculation. In parallel, we will perform imaging experiments to generate a BMP-10 dose-response curve using BMP-10 deficient hearts cultured *in vitro* in the presence of varying concentrations of exogenous cytokine. We will compare the resulting imaging data to those generated *in silico* to directly test the predictive capacity of the simulation.

Collectively, these studies will attempt to model four key aspects of cardiac development, namely, the relationship between cardiomyocyte cell cycle activity and ventricular wall formation (Platform 1), the combinatorial effects of lineage determining transcription factors on cardiac morphogenesis and gene

expression (Platform 2), the role of cell proliferation *vs*. apoptosis in valve morphogenesis and homeostasis (Platform 3), and the relationship between cytokine signaling and trabecular out-growth (Platform 4). The experiments capitalize on the principal investigators' established and funded expertise. We have made particular effort to incorporate additional experiments that use established genetic resources to test the validity of the computer simulations. Thus, the proposed experiments will provide important baseline data for modeling key aspects of cardiac development. Additionally, once we can establish the predictive value of the *in silico* simulations, we can apply these models directly to experiments aimed at modeling regenerative growth of diseased hearts.

III.4.iii Experimental Platform 1: Modeling the effects of cardiomyocyte proliferation on the developing ventricular wall (Loren Field, coordinator):

Although the morphogenetic transformation of the primitive heart into a four-chambered structure is fairly well characterized from an anatomical standpoint, issues pertaining to the origin of the heart forming cells, regulation of cardiomyogenic induction and proliferation, and the molecular regulation of morphogenesis during early embryonic life remain largely unanswered.

III.4.iii.a Specific Aims

Proper development of the ventricular musculature depends in part upon well-coordinated regulation of cardiomyocyte cell cycle activity and migration in the embryonic heart. Retroviral tagging experiments in chicks using β-galactosidase-expressing viruses provided initial insight into this complex process. These studies revealed that upon myogenic differentiation, proliferation of a single infected myocyte in the tubular heart gives rise to transmural, cone-shaped growth units (Mikawa *et al.*, 1992a; Mikawa *et al.*, 1992b; Fischman and Mikawa, 1997). Mikawa *et al.* hypothesized that the assembly of multiple transmural growth units contributes to the three-dimensional ovoid structure of the ventricular walls. More recent studies with neonatal and adult transgenic mice carrying chimerically expressed reporter genes further supported this conclusion (Meihac *et al.*, 2003). Although these studies provided important insight into the relationship between the progenitor cells in the primitive heart and the subsequent three-dimensional structure of the ventricular wall, the analyses were static and largely two-dimensional in nature. Consequently the extent to which cell cycle activity contributes to the formation of a three-dimensional structure has not been quantitatively determined. The studies proposed in this experimental platform will generate baseline data to facilitate the development of a computer simulation of the formation of transmural growth units. In addition, we will perform experiments *in silico* and *in situ* to model and experimentally determine, respectively, the consequences of altered cardiomyocyte cell cycle activity on the morphogenesis of the transmural growth units. We will pursue the following:

1. Specific Aim 1: Image analysis in cultured explanted hearts of transmural growth units derived from cardiomyocyte progenitors with normal cell cycle activity.

2. Specific Aim 2: Image analysis in cultured explanted hearts of transmural growth units derived from cardiomyocyte progenitors with genetic modifications resulting in enhanced cell cycle activity.

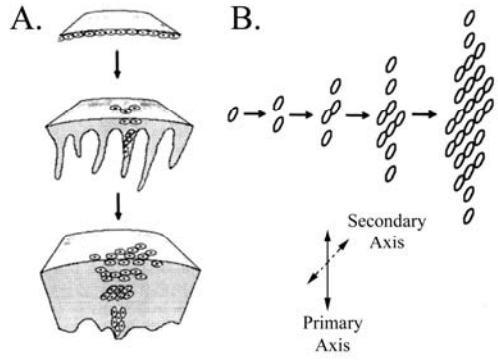
3. Specific Aim 3: Computer simulation of transmural growth units derived from cardiomyocyte progenitors with normal or genetically enhanced cell cycle activity.

The proposed experiments will provide information describing the real-time formation of the cardiac growth units in terms of cell number, division rates and anatomical position. These data will allow us to generate *in silico* models providing three-dimensional morphogenetic information or normal cardiac development, and additionally can apply directly to therapies which rely on enhancing cardiomyocyte proliferation to effect myocardial repair in diseased hearts.

III.4.iii.b Background and Significance:

Traditional tritiated thymidine incorporation studies show that Cell cycle activity is an intrinsic component of cardiac differentiation and morphogenesis. An exceedingly high level of DNA synthesis (labeling indices approaching 70%) occurs in the precardic mesoderm of the myoepicardial plate at embryonic day 8 (E8) of mouse development (Rumyantsev, 1991; Erokhnia, 1968a and 19668b). The onset of cardiomyogenic differentiation (*i.e.*, the first appearance of a cardiomyocyte phenotype) accompanies transient reduction in the tritiated thymidine-labeling index that recovers to approximately 45% by E11 during mouse ontology (Rumyantsev, 1991; Erokhnia, 1968a and 19668b). A similar modulation of cell cycle activity occurs during early cardiac development in the chick (Rumyantsev, 1991). The high rate of cell cycle activity during the early stages of cardiomyogenic differentiation undoubtedly contributes to the "ballooning" of ventricular cardiomyocytes from the tubular heart as the morphologic studies of Moorman and colleagues describe in (Christoffels *et al.*, 2000). As morphogenesis of the ventricle progresses, the tritiated thymidine-labeling index in cardiomyocytes of the compact layer is twice that of those in the inner trabeculae (Rumyantsev, 1991; Erokhnia, 1968a and 19668b). The overall rate of cardiomyocyte proliferation gradually declines during later stages of embryogenesis, and cardiomyocyte cell cycle activity ceases shortly after birth (Rumyantsev, 1991; Soonpaa *et al.*, 1996; Clubb and Bishop, 1984; Li *et al.*, 1996). Subsequent increases in heart size in neonatal and adult life arise predominantly from hypertrophic growth of terminally differentiated cardiomyocytes.

Figure 1.



Retroviral tagging experiments in chicks using □-galactosidase-expressing viruses have provided insight into the interplay between cardiac cell cycle and morphogenic formation of the heart (Mikawa *et al.*, 1992a; Mikawa *et al.*, 1992a; Mikawa *et al.*, 1992b; Fischman and Mikawa, 1997). These studies indicate that upon myogenic differentiation, proliferation of a single infected cardiomyocyte in the tubular heart gives rise to transmural, cone-shaped growth units. The growth units exhibited a primary axis, which corresponded to the elongation of the cluster. In addition, a secondary axis of rows of cells formed. The angle between the primary and secondary axes varied, suggesting that independent events impact growth orientation (see Figure 1, adapted from Mikawa *et al.*, 1992b). As the ventricular wall matured, growth units became transmural and contained rows of cells oriented at progressive angles to one another. Mikawa hypothesized that assembly of these cone-shaped growth units contributed to the three-dimensional ovoid structure of the ventricular walls. The gradient in the rate of cardiomyocyte proliferation observed between the compact layer and the inner trabeculae (Rumyantsev, 1991; Erokhnia, 1968a; Erokhnia, 1968b) would account for the formation of the cone-shaped growth units. They demonstrated that cell death (*i.e.*, apoptosis) did not contribute to this process. More recent studies examining neonatal and adult transgenic mice carrying chimerically expressed reporter genes (Meilhac *et al.*, 2003) further refined the pattern of developmental cardiomyocyte growth in morphogenetic terms. An initial phase of growth observed early in development was characterized by dispersion of progenitor cells along the venous-arterial axis of the heart tube. By embryonic stage 9.5, a more coherent pattern of cell growth began to emerge where progeny cells formed growth units (similar to those in the chick model).

Recent analysis of the proliferative capacity of individual embryonic cardiomyocytes cultured at clonal density supports the general concept that coordinated cell cycle regulation contributes to the formation of cone-shaped growth units (Burton *et al.*, 1999). These studies revealed that the absolute proliferative capacity of individual cardiomyocytes isolated from E15 rats varied greatly. We would anticipate such variation of growth potential between individual cells given the intrinsic differences in cell cycle activity between cells from the compact zone and the trabeculae at any given time during cardiac development. These studies also suggested that daughter cells from a given progenitor tended to divide a similar number of times prior to terminal differentiation. The symmetrical structure of the cone-shaped growth units would depend on similar growth potential in daughter cells.

Knowledge of the interplay between cell proliferation and morphogenic development of the transmural growth units is important if we wish to understand how a relatively simple contractile tube can develop into a complex four-chambered pump. Moreover, understanding the interplay between cell cycle regulation and morphology is a key element in developing regenerative medicine approaches of heart repair. Although the retrospective chick and mouse histochemical experiments described above provide important insight, they are static, providing no kinetic information. To generate data suitable for modeling the dynamic morphogenesis of the transmural growth units, we propose to use chimeric fetal mice that express an EGFP reporter transgene (Rubart *et al.*, 2003) in a limited number of cardiomyocytes in the tubular heart. We will harvest the embryos and allow the hearts allowed to develop *in vitro* using established organ culture techniques. Repeatedly, we will optically image the individual hearts, and the progressive growth and assess morphogenesis of discrete clusters of EGFP-positive cardiomyocytes (originating from individual progenitor cardiomyocytes) in real time. We will monitor cell doubling to determine mitosis rates, and use imaging software to accumulate cell position and/or migration data in three-dimensions. Indeed, we can only address the potential contribution of cardiomyocyte migration to the formation of the ventricular wall via experiments. We will use the resulting information to generate mathematical models for the real-time development of the transmural growth units, to model *in silico* the consequences of increased cardiomyocyte mitosis rates on the morphogenic development of the transmural growth units. In parallel to these computational studies, we will generate additional chimeric animals with EGFP-tagged cardiomyocytes genetically programmed to have increased mitosis rates (using established genetic models). We will harvest and image embryos as described above. The resulting data will test the fidelity of the modesl and refine them to enhance their predictive capabilities.

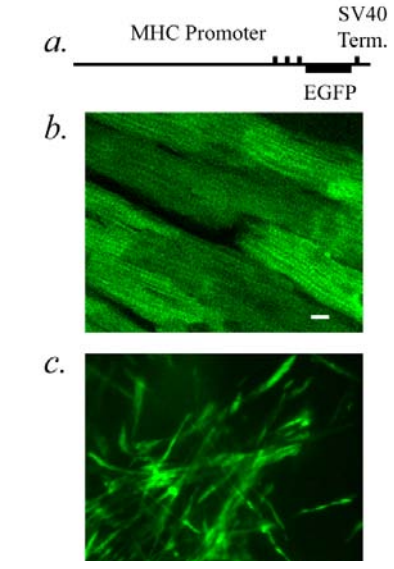
The proposed experiments will build on the existing published data for the formation of cardiac growth units in the developing heart (Mikawa *et al.*, 1992a; 1992b; Fischman and Mikawa, 1997; Meilhac *et al.*, 2003), and should provide definitive information needed to establish the relationship between cardiomyocyte cell cycle activity and ventricular wall formation. The Field laboratory has a longstanding interest in developing therapeutic strategies based on increasing the number of functional cardiomyocytes in diseased hearts. The approaches include strategies based on the reactivation of cell cycle activity in surviving cardiomyocytes (HL75609, HL45453), as well as on strategies employing the transplantation of cardiomyocytes or cardiomyogenic stem cells (HL69119). A major component of these projects has been the generation of transgenic models with lineage restricted reporter genes, the generation of transgenic models with altered cardiomyocyte cell cycle activity, and the development of techniques suitable for *in vitro* fluorescent assessment of cardiac shape and function. The data generated in this proposal will directly complement and enhance the approaches of these actively funded studies.

III.4.iii.c Preliminary Results:

III.4.iii.c.1 Generation of reporter mice with chimeric transgene expression:

The experiments in Aims 1 and 2 require a fluorescence-based reporter transgene to monitor the morphogenesis of transmural growth units in the developing ventricle. We have recently developed a transgenic reporter mouse (designated MHC-EGFP [Rubart *et al.*, 2003]) that uses the alpha-cardiac myosin heavy chain (MHC) promoter to target expression of enhanced green fluorescent protein (EGFP) to cardiomyocytes (Figure 2*a*). We have generated nine transgenic lineages that show two patterns of transgene expression. Several lines exhibited very high penetrance of transgene expression, with virtually all of the cardiomyocytes exhibiting EGFP positivity (Figure 2*b*). Other lines exhibited a mosaic pattern of transgene expression, with anywhere from <1 to ca. 20% of the cardiomyocytes exhibiting EGFP fluorescence in the adult heart (Figure 2*c*). This mosaic pattern of transgene expression resembles that the Buckingham group saw using a β-galactosidase reporter in the retrospective analyses of cardiac growth unit formation (Meilhac *et al.*, 2003). Interestingly, the genetic background of the mice influenced the level of mosaic transgene expression. The transgenic lineages were originally

Figure 2.

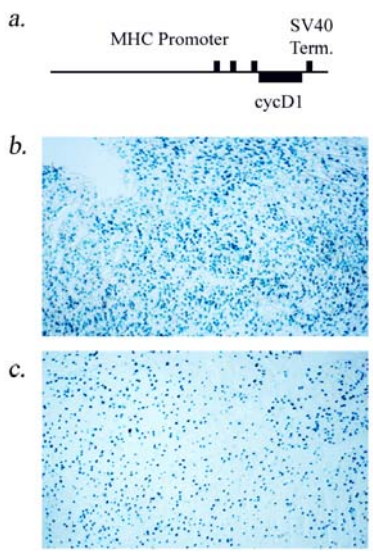


generated in C3HeB/FeJ inbred mice; after crossing the mosaic-expressing mice into a DBA/2J background, 100% of the cardiomyocytes expressed EGFP. Crossing these animals back into the C3HeB/FeJ background once again resulted in highly mosaic transgene expression. The MHC-EGFP mice show ventricular transgene expression during development with ventricular EGFP fluorescence throughout cardiac development, despite the low levels of endogenous MHC gene expression in development (Gulick *et al.*, 1991).

III.4.iii.c.2 Generation of transgenic mice with enhanced cell cycle activity:

The experiments proposed in Aim 2 require transgenic mice with enhanced cardiomyocyte cell cycle activity in the developing ventricles. Our laboratory has generated a number of transgenic mice with altered cardiomyocyte cell cycle activity in the developing ventricle (reviewed in Pasumarthi and Field, 2000). We will initially test two different transgenic mouse models that exhibit either mild or marked enhanced cardiomyocyte cell cycle activity during development. MHC-cycD1 transgenic mice use the mouse MHC promoter to target expression of a cyclin D1 cDNA (Figure 3*a*) (Soonpaa *et al.*, 1997). At birth MHC-cycD1 mice have approximately 3-fold more cardiomyocytes than their non-transgenic littermates. Figure 3*b vs. c* shows the increased cardiomyocyte content. The sections shown were from MHC-cycD1 / MHC-nLAC double transgenic heart (panel *b*) or MHC-nLAC single transgenic mice (panel *c*). The MHC-nLAC transgene results in nuclear localized □-galactosidase activity, and the cardiomyocyte nuclei were identified by increased blue content in X-GAL stained sections indicates increased cell content (Soonpaa *et al.*, 1997). We will also use MHC-TAG transgenic mice 9 (Katz *et al.*, 1992). In these mice, the rat MHC promoter targets expression of the SV40 early region, which encodes the Large T Antigen oncoproteins (Figure 4*a*). Transgene expression results in a marked proliferative response in the developing ventricle; Figure 4*b* shows an E12 embryonic heart from an MHC-TAG mouse stained for T Antigen (black signal); note the wide-spread transgene expression in the atria and ventricles. Figure 4*c* shows a survey photomicrograph of a neonatal MHC-TAG heart stained with H and E; Hematoxylinphyllic staining shows hyperplasia of transmural growth units. In Aim 2, the MHC-cycD1 mice will be used to induce mildly enhanced cardiomyocyte cell cycle activity, whereas the MHC-TAG mice will be used to induce markedly enhanced cardiomyocyte cell cycle activity.

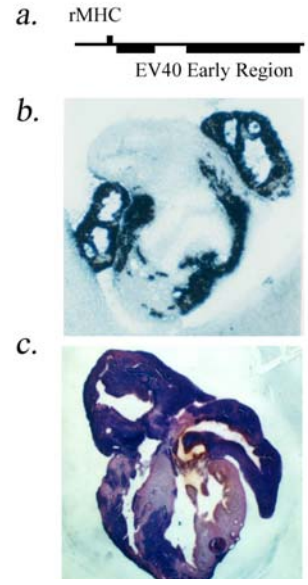
Figure 3.



III.4.iii.d Research Plan:

Our general experimental approach is to generate data suitable for modeling the dynamic growth of transmural growth units comprised of cardiomyocytes with normal (Aim 1) or enhanced (Aim 2) cell cycle activity. We will then use the experimental data to generate computer simulations of transmural growth unit morphogenesis (Aim 3).

Figure 4.



III.4.iii.d.1 Specific Aim 1: Image analysis in cultured explanted hearts of transmural growth units derived from cardiomyocyte progenitors with normal cell cycle activity:

We will use expression of the MHC-EGFP transgene to mark cardiomyocytes in the developing ventricle for image analysis. Our initial approach simply uses MHC-EGFP lines with intrinsically mosaic transgene expression as described in the Preliminary Results section (*i.e.*, MHC-EGFP mice maintained in the C3HeB/FeJ background). We will harvest embryos from timed pregnancies (determined via the presence of vaginal plugs) at E9.5, and dissect the hearts in DMEM containing 10% FBS, and washed twice in PBS and cultured in DMEM containing 1% FBS. (Conway *et al.*, 1997; Rentschler *et al.*, 2002). Studies from the Conway laboratory (PI of Experimental Platform #3) (Conway *et al.*, 1997) have shown that hearts from

this early stage of development undergo fairly normal morphogenesis for as long as 72 hrs in culture. We will image the individual hearts repeatedly using either single-photon confocal laser scanning microscopy (BioRad 1024 laser scanning confocal system fitted to a Nikon Eclipse inverted microscope with a X60 water immersion, NA 1.2 objective) or two-photon microscopy (Zeiss LSM510-Meta confocal microscope system equipped with a tunable Titanium-Sapphire laser and fitted to a Zeiss Axioscope with water dipping objectives). The latter imaging approach has increased tissue penetration depth and reduced photodamaging of the tissue due to less out-of-focus photobleaching. We will process vertical series of two-dimensional images will be processed using a voxel-based three-dimensional imaging program (Voxx) capable of near real-time rendering that was recently developed in the Center for Biological Microscopy at Indiana University Medical School (Clendenon *et al.*, 2002). This volume visualization software enables interactive inspection and manipulation of complex three-dimensional images.

To simultaneously establish the orientation of the EGFP-tagged cardiomyocytes with other cardiomyocytes in adjacent and/or intermingling growth units, we will stain the mosaic-expressing hearts with di-4-anepps (a voltage-sensitive dye that stains sarcolemmal membranes) prior to imaging. We have used this fluorophore to image t-tubules in *in situ* adult cardiomyocytes (Rubart *et al.*, 2003). Importantly, di-4-anepps and EGFP emission spectra are readily separated (500-550 vs. 560-650 nm, respectively), thus enabling us to ascertain the position/relationship of the tagged cardiomyocytes to the remainder of the myocardium. This approach allows us to assess the progressive growth and morphogenesis of discrete clusters of EGFP-positive cardiomyocytes (originating from individual progenitor cardiomyocytes). We will monitor cell doubling to determine mitosis rates, and use Voxx software to accumulate cell position/migration in three-dimensional. In two-photon imaging mode, both fluorophores are conveniently excited at a common wavelength of 810 nm, thereby eliminating the need to repeatedly readjust excitation wavelengths.

III.4.iii.d.1.A Anticipated results, potential problems, and potential solutions:

We anticipate that imaging MHC-EGFP expressing cardiomyocytes in the cultured hearts with mosaic transgene expression will be straightforward. If using naturally occurring mosaic mice (*i.e.*, the mice maintained in a C3HeB/FeJ background) is problematic, we will use traditional embryologic approaches to produce true chimeras comprised in part of cells from non-transgenic DBA/2J mice and in part from MHC-EGFP mice maintained in a DBA/2J background (Robertson, 1987), by generating aggregation chimeras where we control the relative content of the MHC-EGFP expressing cells by varying either the donor cell number or the age of the donor MHC-EGFP embryo. Alternatively, if necessary we will introduce the MHC-EGFP transgene into embryonic stem (ES) cells, and generate chimeric hearts by injecting the ES cells into donor blastocysts. In this case, simply varying the number of stem cells injected into the blastocyst will control the relative content of MHC-EGFP expressing cells in the embryonic heart. In the unlikely event that our primary and back-up approaches fail, we will use the approach of Buckingham and colleagues (Meilhac *et al.*, 2003), with intragenic recombination activating an EGFP reporter, rather than an β -galactosidase reporter.

Imaging the hearts at 3-hour intervals should suffice to monitor cardiomyocyte cell division, based on the estimated rates of cell cycle activity from E9.5 through E12.5 of development (Rumyantsev, 1991; Erokhnia, 1968a; Erokhnia, 1968b). For such experiments, we can transfer the hearts to a perfusion chamber on the microscope stage for imaging (ca. 10 minutes will be required to image an entire transmural growth unit). If a more frequent scan rate is required, or if difficulties are encountered in re-accessing a given growth unit, we will generate a perfusion chamber with tempered and gassed media and perform the culture entirely on the microscope stage.

III.4.iii.d.2 Specific Aim 2: Image analysis in cultured explanted hearts of transmural growth units derived from cardiomyocyte progenitors with genetic modifications resulting in enhanced cell cycle activity:

After we collect data for cell cycle activity and morphogenesis of normal transmural growth units, we will study growth units with altered cardiomyocyte mitosis rates. We will initially analyze growth units expressing the MHC-TAG transgene, as this model has a marked increase in cardiomyocyte cell cycle activity. We will intercross MHC-EGFP mice with 100% transgene penetrance (*i.e.*, mice maintained in the DBA/2J background, see Preliminary Results) with MHC-TAG mice (also in a DBA/2J background), and intercross the resulting double transgenic F_1 animals to generate mice homozygous for both the MHC-EGFP and MHC-TAG

transgenes. Once the resulting breeding stocks are in hand, we will intercross the double transgenic mice with a non-transgenic animal; all embryos from such crosses will carry both the MHC-EGFP and MHC-TAG transgene. We will harvest four-cell embryos, remove the *zona pellucida* via hyaluronidase digestion, and use the cells to generate aggregation chimeras with cells prepared from non-transgenic embryos using standard approaches (Robertson, 1987). We will re-implant the aggregation chimeras into pseudopregnant foster mothers and allow them to develop to the equivalent of E9.5. We will then harvest the resulting embryos, dissect and culture the hearts and individual transmural growth units as described above. We will use the resulting imaging data to generate three-dimensional reconstructions of transmural growth unit morphogenesis over time. If necessary, we can regulate the relative content of the MHC-EGFP / MHC-TAG cells in the resulting hearts by varying the transgenic donor cell number and age. We will use a similar approach to generate and image hearts from chimeric mice carrying both the MHC-EGFP and MHC-cycD1 transgenes.

III.4.iii.d.2.A Anticipated results, potential problems, and potential solutions:

We anticipate that increasing the rate of cardiomyocyte mitosis will alter morphology of the resulting growth units, affecting the neighboring and intermingling growth units. We do not anticipate any difficulties in the imaging or culturing experiments. The same issues and solutions regarding the generation of chimeric animals addressed in Aim 1 also apply to the Aim 2 experiments. Since we will be imaging the morphogenesis of individual transmural growth units over time, the presence of multiple EGFP-expressing growth units in an individual heart is not problematic (in contrast to the retrospective histochemical studies, where this multiplicity constrained the experimental design). Such re-implantation of aggregation chimeras will delay developmental. We will directly compare developmental timing in normal embryos to aggregation chimera embryos, if necessary harvesting the experimental aggregation chimeras at a later developmental date. Since aggregation chimeras develop normally, and since the kinetics of development do not alter after implantation, harvesting the aggregation embryos at a slightly later embryonic age will not impact the parameters under study.

III.4.iii.d.3 Specific Aim 3: Computational analysis of transmural growth units derived from cardiomyocyte progenitors with normal or genetically enhanced cell cycle activity:

Please see Computational Platform.

III.4.iv EXPERIMENTAL PLATFORM 2: Deducing additive vs. synergistic effects of cardiac transcription factors on heart development (Anthony Firulli):

III.4.iv.a Specific Aims:

Heart formation involves the specification, differentiation, and morphological patterning of mesodermal- and neural crest-derived precursor cells within the developing embryo. The breakdown of the molecular networks humans (occurring in 1 out of 1000 live births). As cardiac malformations represent such a large human health issue, a fundamental understanding of the genetic programs that control heart development is essential for developing better treatments and cures. Although cardiogenesis has been well studied in various developmental systems, we understand surprisingly little about the molecular mechanisms that direct heart formation. Current research in cardiogenesis is gaining a better understanding on the transcriptional control of cell specification, differentiation, and morphogenesis of the cells that contribute to heart formation. Our specific focus centers on the HAND sub-class of basic helix-loop-helix (bHLH) transcription factors and the NK-class homeodomain protein Nkx2.5. Targeted gene deletion via homologous recombination (*i.e.,* knockout or null mice) allows us to study the effects of transcription factors on cardiogensis. *HAND2* knockout mice exhibit cardiac abnormalities that result in embryonic lethality at E9.5 as a result of a reduction in cells composing the right ventricle. In contrast, *Nkx2.5* knockout mice exhibit embryonic lethality at E9.5 as a result of a cardiac looping defect, resulting in the reduction of cells composing the left ventricle. Although *HAND2, Nkx2.5* compound knockout mice (Yamagishi *et al.*, 2001), have a marked reduction in total ventricular content, whether the phenotype reflects a simple additive effect of the individual mutation or if the two transcription factors act synergistically. (*i.e.*, the combined transcriptional activity is greater than the additive contributions of each transcription factor separately) is not clear. Our hypothesis is that the resultant phenotype observed in the *HAND2, Nkx2.5* compound null mice is not simply an additive composite of the individual knockout

phenotypes, but reflects a synergy between these two transcription factors. Our recent studies indicate that HAND2 and Nkx2.5 synergistically regulate the *atrial naturetic peptide* (*ANP*) gene. To directly address our hypothesis, we plan to simulate the cardiac morphogenic abnormalities observed in both the *HAND2* and *Nkx2.5* knockout mice using *in vivo* data collected from null mice via confocal reconstruction microscopy. We will also incorporate the known effects on cardiac gene expression into the developmental simulation. Direct comparison of a simulated intercross between *HAND2* and *Nkx2.5* with actual data generated *in vivo*, can determine if additive contributions of these two cardiac phenotypes recapitulates the double null phenotype or if interactions between HAND2 and Nkx2.5 collaboratively orchestrate a common subset of the cardiac gene program. We propose the following studies:

III.4.iv.a.1 Specific Aim 1:

a) Collect wild-type mice between E7.5 and E11.5 and generate three-dimensional reconstructions of the developing heart using laser-scanning confocal image analysis.

b) Collect *HAND2* and *Nkx2.5* null mice between E7.5 and E10.5 and generate three-dimensional reconstructions of the developing heart using laser-scanning confocal image analysis.

c) Collect *HAND2*, *Nkx2.5* compound knockout mice between E7.5 and E10.5 and generate three-dimensional reconstructions of the developing heart using confocal imaging analysis.

III.4.iv.a.2 Specific Aim 2: Computational analysis of wild type*, HAND2*, *Nkx2.5*, and *HAND2-Nkx2.5* cardiogenesis:

This study will contribute to the characterization of the molecular mechanisms involved in cardiac gene transcription and development, leading to a greater understanding of cardiogenesis. This information will help fine-tune the current molecular framework controlling heart development, and will provide a more useful understanding of molecular pathways that contribute to congenital heart disease.

III.4.iv.b Background and Significance:

Transcriptional control of cell type specification and differentiation is central to the formation of various organs within the developing embryo, as exemplified by the heart. Cardiomyocytes concurrently undergo specification and morphologic patterning, which formulates the linear heart tube (Firulli and Thattaliyath, 2002). This structure later loops asymmetrically, ultimately forming the four cardiac chambers. Many transcription factors are essential for cardiogenesis. In most instances deletion of the transcription factor(2) does not affect cardiac cell specification, but compromises the morphological formation of the developing heart. Examples of cardiac morphological defects include mice lacking the homeobox transcription factor *Nkx2.5* (*i.e.*, *Nkx2.5* null mice) (Lyons and Parsons *et al.*, 1995; Kasahara and Lee *et al.*, 2000), which have incorrect cardiac looping and fewer cells in the left ventricle. Mice null for the bHLH transcription factor *HAND2* exhibit morphological defects in the developing right ventricle (Firulli and Thattaliyath, 2002; Srivastava *et al.*, 1997). A recent report indicated that both right and left ventricle structures reduce to a small number of cells that fail to expand and pattern in mice with compound *Nkx2.5, HAND2* mutation (Yamagishi *et al.*, 2001), but did not study development in a comprehensive manner in either the single or the compound mutant mice, and so could not discriminate between a simple additive effect resulting from the loss of the transcription factors vs. a synergistic effect. Additional phenotypes could arise due to the potential synergistic actions of these two transcription factors on target genes. Indeed, preliminary results clearly indicate that these two factors synergistically regulate the ANP gene.

We propose to generate a computer simulation of heart tube formation and asymmetric looping. We will generate this simulation using data obtained from normal embryonic hearts, as well as hearts from *Nkx2.5* null embryos and from *HAND2* null embryos. We will perform spatio-temporal laser-scanning confocal imaging of wholemount *in situ* hybridizations between embryonic day 7.5 and embryonic day 11.5. We will determine expression of *HAND1, HAND2, Nkx2.5*, and several other several key marker genes. We will process this data using a voxel-based imaging program to generate a simulation to recapitulate the three-dimensional spatio-

temporal pattern of gene expression and cardiac structure (*i.e.*, overall shape of the hearts as well as the anatomical position, relative size, and cell content of ventricle and atrial chambers). Once generated, the simulation will predict the spatio-temporal gene expression pattern and gross cardiac structure in *Nkx2.5*, *HAND2* double mutant mice based on the assumption that the effects of the two mutations will be additive. In parallel, *Nkx2.5*, *HAND2* compound knockout mice will be generated and subjected to structural and spatio-temporal gene expression analyses as described above. Data generated via computer simulation will be directly compared with the actual data obtained from *Nkx2.5,HAND2* double-knockout mice. If the compound phenotype is purely additive in nature, the simulation of the resultant morphological defects should mirror the *in vivo* experiments. If synergistic interactions between the factors come into play, the simulation and whole animal experiments should not correlate. Although the published data provides some clues as to the anticipated phenotype in the *Nkx2.5*, *HAND2* compound knockout mice (Yamagishi *et al.*, 2001), our analyses will provide a much more comprehensive temporal assessment of the mutant phenotypes, which is critical if we wish to distinguish between additive vs. synergistic effects in the compound mutation.

The experiments, if successful, would help to discriminate between additive and synergistic effects in animals with multiple genetic mutations. These data will complement our actively funded studies on the molecular mechanism of HAND2 in cardiac development (HL61677). In particular, the simulation will allow us to better characterize the interplay between HAND2 and other cardiac-expressed factors and help reveal the factors with which HAND2 combinatorially interacts to drive the cardiac transcriptional program.

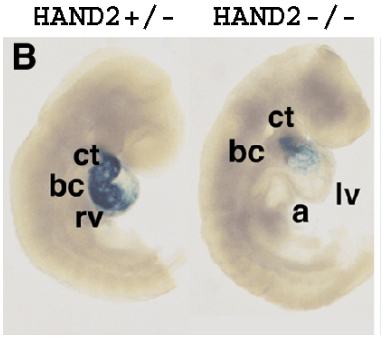


Figure 5: Wholemount analysis of HAND2 promoter expression in E9.5 wild type (left) and HAND2 -/- (right) mice. Note the strong LACZ staining in the right ventricle (rv) of wild-type embryo. In HAND2-/- the right ventricle is absent allowing visualization of the common atria (a) from the right side. HAND2 LACZ expressing cells are reduced in

III.4.iv.c Preliminary Results:

Our laboratory has both *HAND2* and *Nkx2.5* knockout mice lines. Published reports and data in Figure 5 show that HAND2 mice have fewer cells that make up the future left ventricle. As seen in Figure 5, β-galactosidase staining of cells expressing *LACZ* from a 5.5kb proximal region of the *HAND2* promoter (HAND2LACZ mice) (Kasahara and

Lee *et al.*, 2000) shows the right ventricle-restricted expression of *HAND2* (blue) within an E9.5 embryonic heart. Intercross of this transgenic line with the *HAND2* knockout mice shows cells that should be expressing HAND2 (assuming no significant auto-regulation of the HAND2 promoter). A reduced number of blue cells which corresponds to the regions of the developing heat tube that are fated to form the *cono truncus*, *bulbus cordus*, and right ventricle is apparent (Figure 5).

Nkx2.5 null mice also exhibit a lethal cardiac phenotype and die between E9.0 and E10 as a result of a heart looping defect and reduction of cells contributing to left ventricle formation (Lyons *et al.,* 1995; Kasahara and Lee *et al.*, 2000). Figure 6 shows that *Nkx2.5* -/- mice exhibit pericardial edema reflective of heart failure. Intercross of the HAND2LACZ and *Nkx2.5* mutant mice shows that the outflow tract and right ventricle are present but not cardiac looping. (Figure 6).

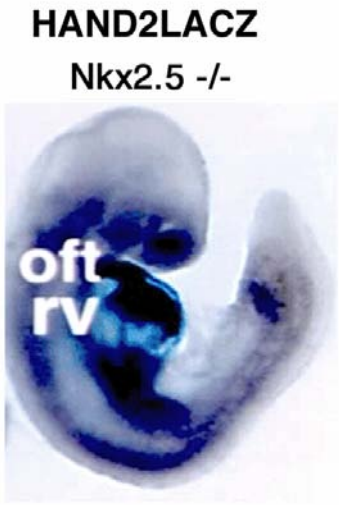


Figure 6: Wholemount analysis of *HAND2* reporter gene expression in an E9.5 *Nkx2.5* null mice. *HAND2* expression in the right ventricle (rv), which is positioned

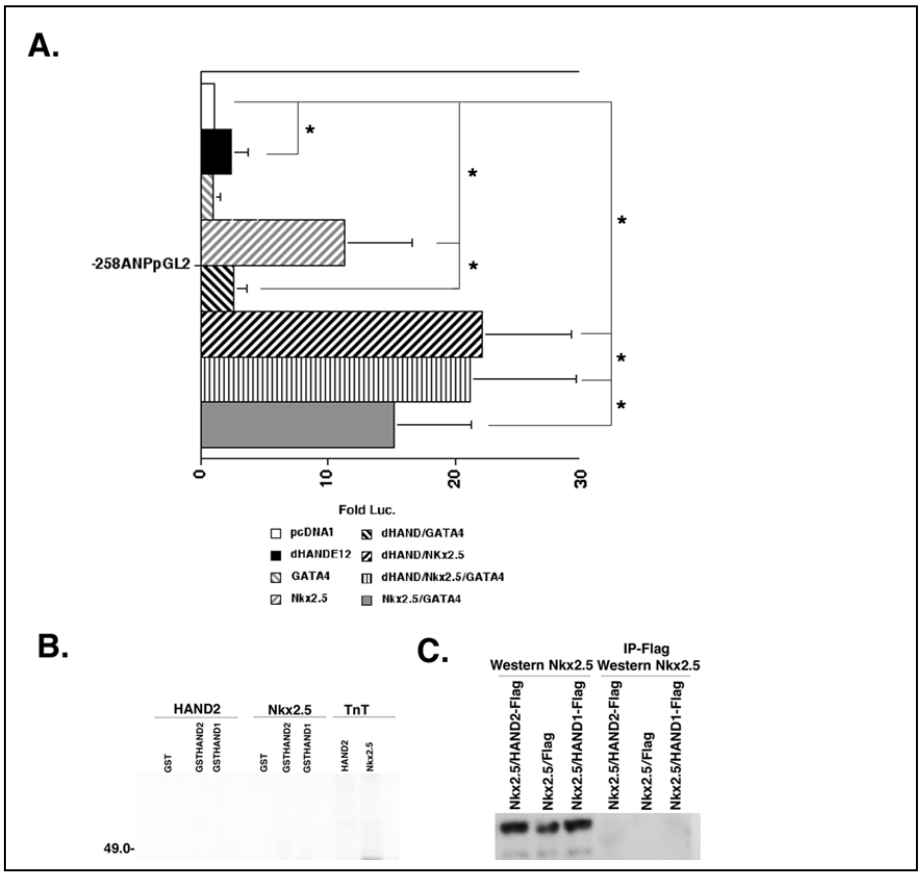


Figure 7: A) HAND2 exhibits transcriptional synergy with Nkx2.5 in the regulation of *ANP*. Nkx2.5 augments *ANP* transcription by 10-fold. HAND2 upregulation is 3-4 fold. Nkx2.5/HAND2 increases activity of *ANP* by more than 20-fold over control. **B**) Pull-down analysis shows direct interaction of Nkx2.5 with HAND proteins. We incubated. ^{35}S labeled Nkx2.5 and HAND2 w for 2hrs with GST protein alone or GST-HAND1 or HAND2 proteins, washed, them through an SDS-PAGE, dried, and exposed. **C**)We cotransfected IP-westerns showing HAND-Nkx2.5 interaction *in vivo* Nkx2.5 +/- HAND1 or –2 epitope tagged with flag in 293 cells. We performed IP with flag conjugated agarose beads. We then western blotted protein lysates and IP-retained protein with Nkx2.5-specific polyclonal antibody. Both flag-tagged HAND1 and –2 IP Nkx2.5 indicating interaction *in vivo*. *-statistical significance at level of confidence P<0.05. Error bars standard error.

Our recent studies showed that *HAND2* and *Nkx2.5* can synergistically activate transcription of the *ANP* promoter (Thattaliyath *et al.*, 2002). Figure 7 shows that coexpression of *HAND2* with a 3.9kb proximal region of the *ANP* promoter driving *lucfierase* gene expression increases 3 to 4-fold activation of *ANP* transcription (Figure 7A). Coexpression of *Nkx2.5* with *ANP luciferase* increases 9-fold transcription while coexpression of *HAND2* and *Nkx2.5* results in 20-fold activation, indicating transcriptional synergy (Figure 7A) (Thattaliyath *et al.*, 2002). To deduce the nature of the observed transcriptional synergy, we monitored protein-protein interaction between HAND2 and Nkx2.5 (Figure 7B). Biochemical pull-down assays using (Mulholland and Gotlieb, 1996) S labeled HAND2 and Nkx2.5 and GST-HAND2 fusion protein show that HAND2 can form a homodimer and heterodimer with HAND1 (Figure 7B). Furthermore, results show that labeled Nkx2.5 protein also associates with HAND1 and HAND2, perhaps explaining the synergy (Figure 7B). To determine if protein-protein interactions between HAND factors and Nkx2.5 could occur in living cells, we performed immunoprecipitation-western (IP-westerns) with flag epitope tagged HAND1 and 2 and Nkx2.5 in HEK293 cells (Figure 7C). IPs of HAND1 and HAND2 detect significant Nkx2.5 protein whereas IP of a construct containing only the flag epitope shows no significant interaction with Nkx2.5 (Figure 7C). Together, these results show that *HAND2* and *Nkx2.5* regulate shared transcriptional targets within the cardiac transcriptional program, in part mediated by protein-protein interactions between these two factors.

III.4.iv.d Research Plan:

The phenotypes of *HAND2* and *Nkx2.5* knockout mice show compromised formation of the right ventricle (*HAND2*) and left ventricle (*Nkx2.5*). Intercross of these two knockout mice reduces both ventricles, with only a few cells within the heart expressing ventricle specific genes (Yamagishi *et al.*, 2001). At the molecular level HAND2 and Nkx2.5 cooperate in the regulation of cardiac-expressed genes, and can physically interact at the protein level (Thattaliyath *et al.*, 2002). Is the phenotype Yamagishi *et al.,* described simply an additive consequence of the *HAND2* and *Nkx2.5* phenotypes or does the ability of these two transcription factors to co-regulate genes within the cardiac program make the double null phenotype more severe than the sum of the *HAND2* and *Nkx2.5* single phenotypes?

To address this question directly, we will determine he morphogenic patterning and spatio-temporal gene expression of cardiac markers in wild type, *HAND2* null, and *Nkx2.5* null mice. We will render these data into a three-dimensional simulation of cardiac development. We will use the TST to predict the phenotype of *HAND2, Nkx2.5* compound null mice based on the assumption that the combined phenotype will be additive. In parallel, we will generate and analyze *HAND2, Nkx2.5* compound null mice, comparing these *in silico* hearts with data obtained by *in vivo* analysis, and will directly determine the nature of the compound phenotype.

III.4.iv.d.1 Specific Aim 1a: To collect wild-type mice between E7.5 and E11.5 and generate three-dimensional reconstructions of the developing heart using confocal image analysis:

We will mate ACT-EGFP mice and isolate litters from timed pregnancies ranging from E7.5 to E11.5. ACT-EGFP mice express constitutively an EGFP fluorescent reporter gene in all cell types (Jackson Laboratories, Bar Harbor Maine). We will dissect embryos within each litter in ice-cold PBS and fix overnight in 4% paraformaldehyde. After fixation, embryos will be washed in PBS and determine more specific staging by counting somites. As embryonic hearts show some variation, 5 representative embryos from each 1/2-day time point (*i.e.*, E7.5, E8.0, E8.5, etc.) will be imaged. We will image the individual hearts repeatedly as in Specific Aim 1. For this portion of the data collection, we will image 45 embryos, providing a representation of wild-type heart variation during development.

We will use embryos from the ranged embryonic stages for qualitative wholemount *in situ* hybridization with a number of cardiac marker genes, to deduce the regional expression throughout the heart (*i.e.*, expansion or reduction from wild type expression). The marker genes included are *Nkx2.5*, *ANP*, *myosin light chain 2 atria* (*MLC2A*), *MLC2 ventricle* (*MLC2V*), *HAND1*, *HAND2* and *myosin heavy chain* (*MHC*). These marker genes are chosen as follows: *MLC2A* and *2V* mark atria and ventricle cardiomyocytes during cardiogenesis (Firulli and Thattaliyath, 2002; Harvey *et al.,* 1999). *HAND1* and *HAND2* mark the left and right ventricle respectively (Firulli and Thattaliyath, 2002). *ANP* is expressed in atria and ventricle chambers, and we have shown it to be a synergistic transcriptional target of HAND2 and Nkx2.5. (Firulli and Thattaliyath, 2002; Harvey *et al.*, 1999) *MHC* is an embryonic cardiac myosin expressed throughout the entire heart, marking all cardiomyocytes. After RNA hybridization of non-radioactive labeled probes immunodetection of the probe with a conjugated antibody allows visualization of expression by TRITC fluorescence. As a counter stain to the red, we will use the MHC-EGFP highexpressing line described by Dr. Field (Figure 2), to observe the entire heart via GFP signal and the regional expression of the specific marker gene in red.

III.4.iv.d.2 Specific Aim 1b: To collect *HAND2* and *Nkx2.5* null mice between E7.5 and E10.5 and generate three-dimensional reconstructions of the developing heart using confocal image analysis:

Our plan for completing Specific Aim 1b is identical to that employed in Aim 1a with the exception that we will be isolating embryos from *HAND2* or *Nkx2.5* knockout mice. Since these animals will be generated via mating of heterozygous mutant animals, only 1 out of 4 embryos will be homozygous mutant for the *HAND2* or *Nkx2.5* null alleles, respectively. We will confirm proper identification of embryos by polymerase chain reaction (PCR) genotyping of yolk sac tissue. At early time points (E7.5-E8.5), we will take great care to carefully remove all maternally derived tissue that can confound genotyping analysis. Older embryos (E10-E10.5) may be necrotic and already being reabsorbed. Should animals of these later time points not be suitable for *in situ* analysis, we will exclude them. As we have successfully used animals as old as E9.5 without observing confounding

necrosis, we foresee little difficulty obtaining the key data hallmarking the phenotypic defects for the computational simulation.

III.4.iv.d.3 Specific Aim 1c: To collect *HAND2,Nkx2.5* compound knockout mice between E7.5 and E10.5 and generate 3-D reconstructions of the developing heart using confocal image analysis:

The strategy is identical to that in Specific Aims 1a and 1b. These experiments will intercross compound heterozygous *HAND2-Nkx2.5* mice and isolate embryos at desired embryonic time points. We will carry out the identification of the double null embryos by PCR genotyping. This breeding strategy will result in 1 out of 16 embryos yielding the desired genotype and thus a large number of compound heterozygous females will be required to generate the required embryos for the *in situ* analysis. As we have all reagents and have experience with employing the required technologies, we expect little difficulty obtaining this data.

Limits, Alternative approaches

As we have a good deal of experience working with these lines of knockout mice, embryo dissections, and employing wholemount *in situs* successfully, we foresee little difficulty collecting this data. Should use of the MHC-EGFP mouse line not provide 100% expression in the cardiomyocytes, we can employ immunodetection using the MF20 cardiac specific antibody or use βMHC using a FITC conjugated secondary antibody. We expect variation in the shapes and sizes of individual hearts from wild type and mutants, which we will collects by sample size.

III.4.iv.d.4 Specific Aim 2. Computational analysis of *wild-type HAND2*, *Nkx2.5*, and *HAND2-Nkx2.5* cardiogenesis:

See Computational Platform.

III.4.v EXPERIMENTAL PLATFORM 3: MODELING THE DEVELOPMENT AND STEM CELL-BASED REPAIR OF CARDIAC VALVES (SIMON J. CONWAY):

III.4.v.a SPECIFIC AIMS:

Morphogenetic remodeling of the single-channel pulsatile early tubular heart into a dual-channel, synchronously beating four-chambered heart with one-way valves is critical for *in utero* survival (Conway *et al.*, 2003). During development, cell proliferation and cell death respond to a wide variety of intra- and extra-cellular cues so that the developing valves retain precisely the type and number of cells needed to proceed into maturity. In contrast, terminally differentiated adult collagenous valvular tissue seems to have limited regenerative capacity so any significant damage can result in heart failure. To date little attention has been paid to cell replacement and/or augmentation of terminally differentiated valvular tissues using stem cell based approaches.

Studies in this experimental platform will determine if cell transplantation approaches can engender repair of the cardiac valves *in situ*. Initial studies will establish the spatial and temporal patterns of cell apoptosis and proliferation in valves from neonatal and adult mouse hearts. These data will help generate computer simulations of cell proliferation and/or apoptotic rates during valve development. Additionally, experiments *in silico* will determine how rates of endogenous cell proliferation and/or apoptosis must change to maintain normal valve size and morphology following the addition of exogenous cells. In parallel, *in situ* experiments will image adult valves following colonization with exogenous EGFP-expressing bone marrow-derived stem cells. After mechanical injury, we will image valves from non-colonized and colonized hearts. We will then compare the results of these manipulations to the computer models to test their predictive value for simulating cell maintenance and turnover within normal and injured adult valves. These studies will complement and enhance our currently funded NIH studies (HL52813) cardiac valvo-septal morphogenesis.

Specific Aim 1: Image analysis of the spatial and temporal patterns of cell apoptosis and proliferation in valves from fetal, neonatal and adult normal mouse hearts.

Specific Aim 2: Image analysis of adult valves following colonization with exogenous EGFP-expressing bone marrow-derived stem cells.

Specific Aim 3: Image analysis of adult valves from non-colonized and bone marrow-derived EGFP-colonized hearts following mechanical injury.

Specific Aim 4: Computational analysis of cell proliferation and apoptosis within both normal and mechanically injured hearts following either non-EGFP or EGFP stem cell augmentation.

III.4.v.b Background and Significance:

Diseased valves that do not open or close properly can cause secondary damage to the heart. If pharmacological intervention fails to rectify valve function, the diseased valves are routinely replaced with valves from human cadavers, pigs or cows, or with mechanical valves. However, surgically transplanted pig, cow and/or mechanical valves have a limited functional halflife, necessitating multiple procedures in younger patients. Additionally, human cadaver valves can present other health hazards by eliciting an immune response. To bypass these drawbacks, investigators are trying to use tissue-engineered valves made from a patient's own cells using either cadaver collagen/elastin scaffolds with living cells removed or by seeding the diseased valves directly with stem cells. We do not know how these sparsely cellularized relatively inert valves will respond to 'extra' grafted or colonizing cells, or their effect on valve morphology, cell proliferation and death.

Even though valvular morphogenesis is critical for *in utero* survival (Conway *et al.*, 2003), many aspects of development, maturation and remodeling required for formation of adult cardiac valves remain unclear. For instance, we know much about the anatomical, cellular and molecular events of endocardial cushion formation and specification, but little about how these primitive swellings are remodeled to give rise to fully functioning sculpted valves attached to chordae tendineae and specific papillary muscles. Indeed, we know even less about the maintenance and turnover of all the various cell types in postnatal valves. Although we often think of the cardiac valves as relative inert collagenous tissue, they are in fact composed of multiple cell types that have different embryonic origins: these include cardiomyocytes, neuronal, endocardial, smooth muscle and the heterogenous valvular interstitial (fibroblasts) cells (Conway *et al.*, 2003; Bartram *et al.*, 2001). The valvular interstitial cells are contractile, have some characteristic features of smooth muscle cells, communicate with each other via gap junctions, secrete valvular matrix, and can regulate repair processes following injury (Mulholland and Gotlieb, 1996). Surprisingly, the cell lineage and origin of the valvular interstitial cells are presently unknown. Although we have described cell death (van den Hoff *et al.,* 2000) and proliferation (Sedmera *et al.*, 2003) during embryonic cardiac development, we have few details regarding cell death and proliferation within the neonatal/adult valvular tissues. This gap in our required knowledge of cardiovascular morphogenesis is surprising, particularly given recent rapid progress in many areas of molecular cardiology and treatment of cardiovascular disease. Particularly encouraging are the initial results from the tissue engineering, cardiomyocyte cell cycle manipulation, cell transplantation and mobilization of endogenous stem cell fields (Pasumarthi and Field, 2002; Sedmera *et al.*, 2003; Kehat and Gepstein, 2003; Oh *et al.*, 2003). However, we have paid little attention to cell replacement and/or augmentation of terminally differentiated valvular tissues using these novel approaches.

The embryonic heart develops from the precardiac mesoderm and is populated by several extra-cardiac cell types. Following lineage allocation and colonization of the heart anlagen, inductive tissue interactions and intercellular signaling that regulates cell proliferation and the acquisition of lineage-specific morphological and molecular characteristics controls differentiation of the lineage precursors. Differential proliferation plays a major role in growth and remolding of the heart tube into the mature four-chambered structure (Sedmera *et al.*, 2003; Jeter and Cameron, 1971). Recently, we have identified zones of low myocyte proliferation at both the venous and arterial poles of the mouse embryo heart, as well as in the atrioventricular region (Sedmera *et al.*, 2003). The labeling index was distinctly higher in non-myocardial populations (endocardium, epicardium, and cardiac cushions), and ventricular trabeculae showed lower proliferative activity than the ventricular compact

layer after their appearance at E9.5. We have observed low labeling in the pectinate muscles of the atria from E11.5, and the His bundle, bundle branches, and Purkinje fiber network likewise lacked labeling (Sedmera *et al.*, 2003). Between the formation of the tubular heart and birth, cell number increases several-fold increase. While the embryonic heart undergoes rapid proliferation during both normal development to increase cardiac mass and in response to injury, the post-natal heart has more constraints that curtail cell proliferation. Indeed, during neonatal life growth (particularly in the cardiomytocytes) changes from hyperplastic to hypertrophic (Soonpaa and Field, 1997). Conversely, low-level proliferation of non-myocytes continues throughout life, and the development of the coronary vasculature also continues well after birth (Sedmera *et al.*, 2003). Although a number of studies have examined the adult proliferative capacity of cardiomyocytes (Soonpaa and Field, 1997) and cardiac fibroblasts (Agocha *et al.*, 1997) and the heart during development (Sedmera *et al.*, 2003; Jeter and Cameron, 1971)**,** the proliferative capacity of the post-natal valves is unknown.

Cell death is also a common integral feature of the development of many mammalian tissues/organs. Two well-known examples of programmed cell death (apoptosis) are the cell deaths associated with fusion of the neural folds and removal of interdigital mesenchymal cells during digit formation. Currently, we know much about the mechanisms of cell death, as a cadre of proteins controlling apoptosis in mammals have been identified (*i.e.*, receptors/ligands, caspases, cytochrome c, Apaf-1, Bcl-2 family and inhibitory apoptosis proteins). We have identified two major pathways of apoptosis, the receptor-mediated and the mitochondrial apoptotic pathways (Mirkes, 2002). Surprisingly, several groups have reported that different tissues within the early post implantation mammalian embryo are differentially sensitive to the cell death-inducing potential of teratogens, from exquisite sensitivity of cells in the developing central nervous system to complete resistance of cells in the developing heart (Mirkes, 2002; Poelmann *et al.,* 2000). Significantly, the resistance of heart cells relates directly to the failure to activate the mitochondrial apoptotic pathway (Mirkes, 2002). However, distinct spatial and temporal distributions of apoptosis are present in the embryonic heart that parallel the morphologic changes and tissue differentiation during heart development, suggesting that apoptosis is crucial to the transformation of the heart from a simple tube to a complex multichambered pump (Cheng *et al.*, 2002). Apoptosis mainly occurs in the non-myocardial compartment of the embryonic heart, and the first distinctive zone of apoptosis arises in the outflow tract (Watanabe *et al.,* 1998) (site of future aorta and pulmonary trunks and aortic and pulmonary valves). Subsequently, a remarkable level of apoptosis appears in the atrioventricular cushions (site of future mitral and triscupid valves) mid-gestation, and drops significantly thereafter (Cheng *et al.*, 2002). Furthermore, an apoptotic population surrounds the developing conduction system, which may trigger subsequent signal cascades (involving the activation of latent transforming growth factor beta) and play a role in differentiation (Poelmann *et al.,* 2000). Finally although aberrant apoptosis often accompanies cardiac anomalies (Bartram *et al.*, 2001; Poelmann *et al.*, 2000) apoptosis probably does not occur in the adult valves.

III.4.v.c Preliminary Results:

In broad agreement with previously published patterns of cell proliferation (Sedmera *et al.*, 2003; Jeter and Cameron, 1971) we found that the E14 fetal hearts had high levels of DNA-synthesizing cells in the myocardium and all four valves, and that rates of proliferation differed between the fetal compact and trabeculated zones (Fig. 8) following a ^{3}H-thymidine injection in pregnant female mice (200μCi i.p. at 28 Ci/mM). We harvested the fetuses after 4 hours. We dipped serial 10 □m cryostat sections in nuclear emulsion and exposed for 12 days as previously described (Pasumarthi and Field, 2002; Soonpaa and Field, 1997). The rates of proliferation were similar between all four valves (in spite of their different stages of maturation and colonization by different extra-cardiac cell types; data not shown) and comparable to the mitotically active compact zone. The labeling index of E14 compact zone is ~32% (^{3}H-labeled cells/total cells) and the valves is ~26% (^{3}H-labeled cells/total cells). However, when we examined serially sectioned adult hearts, the levels of DNA synthesis were drastically lower (Fig. 8; these mice express a nuclear localized □-galactosidase reporter only in cardiomyocyte nuclei, as described in experimental platform #1) (Pasumarthi and Field, 2002; Soonpaa and Field, 1997). Adult cardiomyocytes have a labeling index of ~0.0005%[42], and by assessing all the ^{3}H-labeled non-nLAC stained cells/total number of non-nLAC stained cells our preliminary data suggests that the adult valves have a labeling index of ~0.6% (*i.e.*, more than 1000-fold higher).

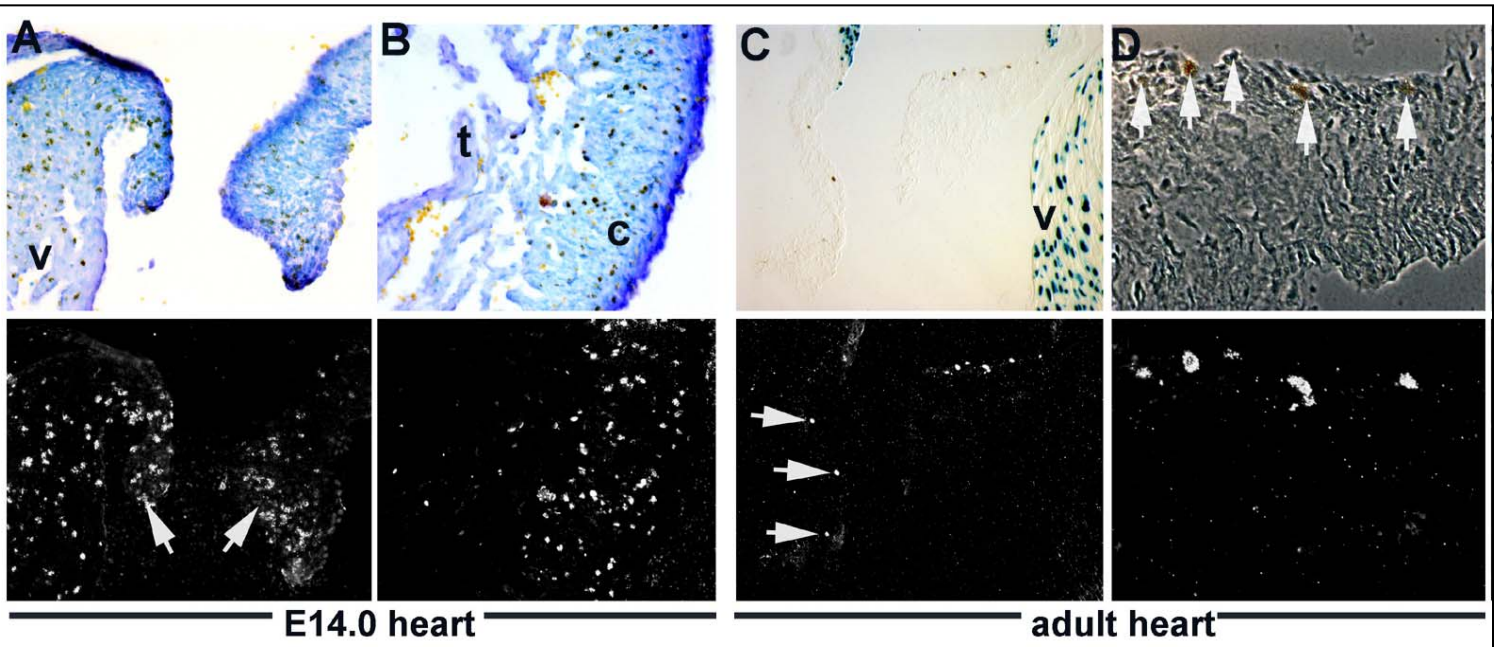


Figure 8. Fetal and adult hearts following assessment of DNA synthesis. Note, top panels are light field and bottom panels are dark field. A) 10μm cryostat section of E14.0 four-chambered view of tricuspid valve of heart following toludine blue counter-staining of ^{3}H-thymidine stained proliferating nuclei. Note that the labeling is similar between the ventricle (v) and valve leaflets (indicated by arrows). B) However, the E14.0 compact zone (c) has significantly more labeled nuclei than the less proliferative trabecular zone (t). C) 10μm cryostat section of adult of tricuspid valve of MHC-nLAC heart following ^{3}H-thymidine staining of proliferating nuclei. Note that there is limited DNA synthesis in the valve leaflets (3 arrows) and none within β-galactosidase marked cardiomyocytes. D) High power view of C. Note that there are 5 ^{3}H-thymidine stained nuclei on the ventricular aspect of the valve (indicated by 5 arrows). Magnification: A, B, & C = x100; D = x200.

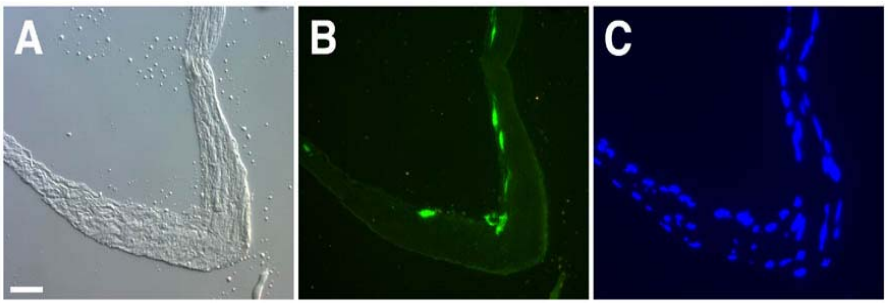


Figure 9. Bone marrow EGFP-derived cells more densely populate the ventricular aspect of the valves. A) Differential interference contrast and B) epifluorescence images of 3μm sections of a heart valve from a EGFP-transplanted mouse highly enriched for EGFP^{+} cells on its ventricular leaflet. C) Hoechst nuclear staining reveals the relatively cellular nature of the valve. Bar = 25μm.

In order to study the potential impact of bone marrow-derived hematopoietic stem cells (HSCs) on heart development/homeostasis, we entered into a collaboration with Dr. Chris Drake (MUSC) to provide us with hearts from mice transplanted with HSCs from enhanced green fluorescent protein (EGFP) mice that express cytoplasmic EGFP in all cells under the ubiquitous actin promoter. Dr. Drake and his colleagues have developed an efficient technique to produce mice with clonal hematopoietic engraftment by combining FACS cell sorting and short-term suspension culture of bone marrow-derived cells (Masuya *et al.*, 2003; Ishikawa *et al.*, 2003). In order to define the temporal and spatial contribution of HSCs to valve homeostasis, he determined the distribution and/or number of $EGFP^+$ engrafted cells in transplanted mice at 1 & 3 months following transplantation. The analyses revealed no gross changes in the total number or distribution of cells within each valve, despite the presence of a large number of $EGFP^+$ cells (data not shown). Thus, the presence of $EGFP^+$ cells in the valve tissue after three months indicates long-term reconstitution of the bone marrow by the transplanted $EGFP^+$ HSCs. Careful analysis of the sites of engraftment of $EGFP^+$ cells into the valves suggests that the ventricular aspect of the valve contains more $EGFP^+$ cells than the atrial aspect (Fig. 9). We are currently investigating whether this asymmetry is specific to engrafted $EGFP^+$ cells or if it represents an asymmetrical distribution of ECM under normal physiological conditions. Future studies will reveal if the number of $EGFP^+$ HSCs remains constant and if these cells proliferate and/or die.

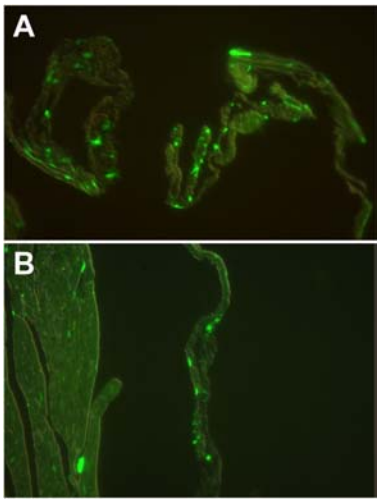


Figure 10. Cells derived from a single EPFP-colony also engraft into the cardiac valves. A and B depict $EGFP^+$ cells engrafted into the leaflets of the mitral valve and the aortic valve, respectively. Magnification = 630x.

Cells derived from a single EPFP-colony can also engraft into the cardiac valves one month after transplantation (Fig. 10). Additionally, in order to biochemically profile the stem cell-derived populations in the valves, we subjected the transplanted hearts to various RNA probes and antibodies to detect mRNA or proteins that would indicate of the cell type. We hypothesize that the HSC-derived $EGFP^+$ cells are fibroblastic. Analysis indicates that HSC-derived, $EGFP^+$ cells express mRNAs for pro-collagen (data not shown), strongly suggesting that the engrafted $EGFP^+$ cells share characteristics with fibroblasts (*i.e.,* the expression of collagen [van der Kamp and Nauta, 1979]). To further evaluate the fibroblastic nature of the engrafted $EGFP^+$ cells, we evaluated the expression of the collagen receptor, discoidin domain receptor 2 (DDR2). DDR2 is a receptor tyrosine kinase (RTK) with the unusual property of being activated by an extracellular matrix protein triple-helical fibrillar collagen (collagens types I–III and V). DDR2 is mainly expressed in mesenchymal cells and regulates chondrocyte, hepatic stellar cell, and fibroblast proliferation (Vogel *et al.*, 2000). In the context of the valves, we reasoned that DDR2 expression is likely associated with the fibroblast. Preliminary data indicates that cells expressing EGFP also expressed DDR2 (Fig. 11). This observation is consistent with our finding of pro-collagen expression in $EGFP^+$ cells and further supporting the fibroblastic nature of the engrafted cells.

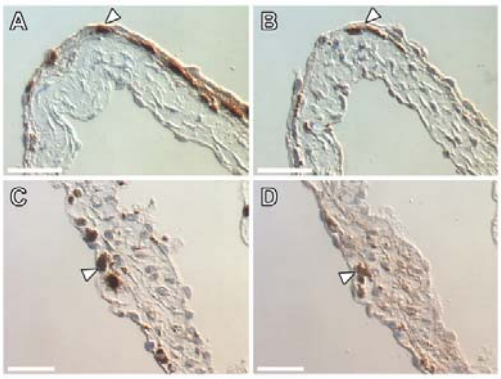


Figure 11. Immunohistochemical analysis of Discoidin Domain Receptor 2 (DDR2) expression by $EGFP^+$ (HSC-derived) cells in serially-sectioned (3μm) adult valve. A and C) DIC images of peroxidase-labeled $DDR2^+$ cells, B and D) DIC images of

III.4.v.d Research Plan:

III.4.v.d.1 Specific Aim 1: Image analysis of the spatial and temporal patterns of cell apoptosis and proliferation in valves from fetal, neonatal and adult normal mouse hearts:

Since the geometry of the valves is crucial for normal cardiac function and survival, understanding the dynamic interplay of cell proliferation and death within adult valves is proportional. We propose to comprehensively determine the spatial and temporal patterns of apoptosis and proliferation in timed neonatal (1, 7 & 14 day) and adult (2, 4 & 6 months) murine hearts using TUNEL *in situ* end-labeling technique (Vector Labs) for detecting fragmented DNA, and ^{3}H-thymidine labeling (Amersham) as a marker for DNA synthesis/compared to Hoechst-stained total cell nuclei counts using established methods (Pasumarthi and Field, 2002; Soonpaa and Field, 1997; Cheng *et al.*, 2002; Conway *et al.*, 2000; Koushik *et al.*, 2001). We will use at least five serially sectioned hearts at each specific for each assay and count at least ten random grids (each containing ~300-400 nuclei) per heart. Also, we will access cardiac expression of cell proliferation (PCNA) and apoptosis-related proteins (proapoptotic Bax and Fas as well as antiapoptotic Bcl-2) by immunofluorescence to verify initial apoptosis and proliferation analysis. We will compile digital images from sequential histologic sections into a three-dimensional rendering using the Voxx software. We will use these data to determine the baseline changes in apoptosis and proliferation during postnatal cardiac remodeling and within the normal adult heart, prior to assessment of apoptosis and proliferation in altered valves. These data will provide the baseline for simulation and predicting the likely outcomes of polymeric grafting and stem cell addition and colonization on the largely cellular valve leaflets and concomitant effects upon cell proliferation and apoptosis. These data will predict the degree of cell proliferation and/or apoptosis required to maintain normal valve morphology and what defects are likely to occur when we alter either cell proliferation or apoptosis.

III.4.v.d.2 Specific Aim 2: Image analysis of adult valves following colonization with exogenous EGFP-expressing bone marrow-derived stem cells:

A second series of experiments will focus on augmenting the number of cells in the adult valves, with the ultimate aim of determining if stem cell delivery can effect reparative growth. These studies are based on initial observations (collaboration with Dr. C.J. Drake, MUSC) that efficient colonization of the valves follows bone marrow reconstitution using EGFP-expressing marrow-derived stem cells. Dr. Drake will provide us with at least three hearts at several time points post transplantation. We will transplant the lethally irradiated C57BL/6-Ly-5.1 host mice with HSCs isolated via FACS cell sorting of bone marrow cells from transgenic EGFP mice (C57BL/6-Ly-5.2 background [HSCs sorted based on the presence or absence of the following lineage markers: Lin(-), Sca-1(+), c-kit(+), CD34(-)]). Intriguingly, preliminary histochemical analyses have revealed that the number of EGFP donor cells in the valves remains constant for as long as three months post transplantation and that the EGFP-expressing cells express several valve fibroblast markers (procollagen, DDR2). We will similarly serially analyze hearts at 6 and 12 months post transplantation. Since the valves are of normal size and function normally, we predict that endogenous cell proliferation will have decreased and/or apoptosis will have increased to maintain homeostasis and normal valve morphology. We will study morphology, cell cycle and apoptoses in animals with stem cell transplants as described above, and use the resulting data to simulate the dynamic changes that occur in valves from the transplanted animals. Once again, we will compile digital images from sequential histologic sections into a three-dimensional rendering using Voxx. Additionally, we will perform *in silico* experiments to simulate the consequences of altered donor cell proliferation and/or apoptotic rates on valve seeding and remodeling.

III.4.v.d.3 Specific Aim 3: Image analysis of adult valves from non-colonized and bone marrow-derived EGFP-colonized hearts following mechanical injury:

In parallel, additional experiments on EGFP-seeded hearts that have been mechanically injured following catheter insertion will determine the potential reparative consequences on both the host and donor stem cells. We will specifically determine if both cell types, only the donor and not the host cells or *vice versa* exhibit altered cell cycles and/or apoptosis following injury repair, as described above. We will then compare the results of these manipulations to the computer simulations, to enable us to develop further hypotheses to address the role of cell maintenance and turnover within neonatal and adult valves. The ability to promote

valvular repair using autologous donor cells as described above, if successful, could have important clinical implications.

III.4.v.d.4 Specific Aim 4: Computational analysis of cell proliferation and apoptosis within both normal and mechanically injured hearts following either non-EGFP or EGFP stem cell augmentation:

See Computational Platform.

III.4.vi EXPERIMENTAL PLATFORM 4: THE MOLECULAR AND CELLULAR REGULATION OF CARDIAC VENTRICULAR TRABECULATION AND COMPACTION (WEINIAN SHOU):

III.4.vi.a Specific Aims:

Cardiac ventricular trabeculation and compaction are important morphogenetic processes during cardiogenesis. Ventricular trabeculae contribute to the maturation of the developing ventricular chambers, which include the formation of the ventricular wall and septum, papillary muscles, and cardiac conduction cells. Abnormal trabeculation and/or compaction can result in numerous congenital heart defects. In this study, we will apply the complementary techniques of molecular biology, genetics, optical imaging and computer simulation to investigate and simulate the genetic and cellular programs that regulate the dynamic process of cardiac ventricular trabeculation and compaction. To do so, we propose following aims:

Specific Aim 1: Generate transgenic reporter mice to differentially label the myocardium and endocardium.

Specific Aim 2: Image analysis of the dynamics of ventricular trabeculation and compaction in normal and abnormal developing hearts.

Specific Aim 3: Image analysis of a BMP-10 dose-response study.

Specific Aim 4: Computational analysis of cardiac trabeculation and compaction in normal and mutant embryos.

III.4.vi.b Background and Significance:

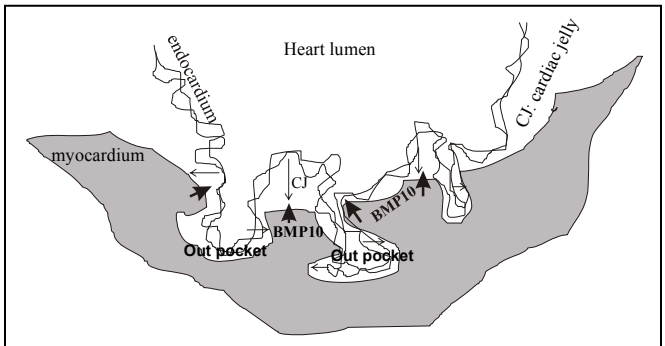


Figure 12: Hypothetical model for ventricular trabeculation.

During midgestation of mammalian embryonic development, the newly formed embryonic heart grows rapidly in cell number and size and undergoes a series of morphological and functional changes in response to the increasing hemodynamic load (Srivastava and Olson, 2000). At embryonic day 9.0 (E9.0), the interaction between the endothelial cells of the endocardium and the proliferating cardiomyocytes leads to the formation of a loose interwoven meshwork of myocardial fibers (the so-called ventricular trabeculae). By E14.5, these trabecular structures become more compact toward the epicardial surface, and the intertrabecular recesses reduce to capillaries. This unique morphogenetic process is *ventricular trabeculation and compaction* (Icardo, 1984; Berne, 1979; Rumyantsev, 1991), and is critical to normal spatio-temporal control of cardiac growth and chamber maturation. The alteration of cardiac trabeculation and compaction in patients leads to the congenital cardiac disease, noncompaction of myocardium (Ichida *et al.*, 2001). The initial stages of trabeculation (at E9.0 - E9.5) triggers the penetrated endocardium through the cardiac jelly and envaginating along discrete points on the myocardium to form “out pockets” directed toward the myocardium (Figure 12). Further expansion of primitive trabecular myocardium between E9.5 to E13.5, via either myocyte recruitment and/or proliferation, is important for maturation trabecula. Later in development

(E13.5–E14.5), these trabecular structures rapidly undergo “compaction” and become part of wall, papillary muscles, interventricular septum, and conductive cells, respectively. One scientific challenge is to determine the molecular mechanism that regulates the dynamics of cardiac trabeculation and compaction.

We will simulate the dynamic process of ventricular trabeculation and compaction in normal developing hearts. Subsequent studies will examine the impact of several candidate genes that have been implicated in regulating the process. BMP-10 (Bone Morphogenetic Protein 10) and its associated pathways play critical regulatory roles. Our ultimate goal is to put the genetic programs in the context of the dynamics of ventrticular trabeculation and compaction. Our experimental approach will use transgenic reporter mice to differentially label the myocardium and endocardium with different fluorescent markers. Expression of red fluorescent protein (RFP) will target the embryonic cardiomyocytes using the cardiac specific H-Ncx (Sodium-calcium exchanger) promoter. We will breed these animals, designated H-Ncx-RFP, to existing Tie2-GFP transgenic mice. In the resulting double transgenic animals, expression of RFP driven by the H-Ncx promoter will produce red fluorescence signals in the developing myocardium, while GFP driven by the Tie2 promoter will produce green fluorescence in the endocardium. We will isolate whole embryonic hearts (H-Ncx-RFP/Tie2-GFP positive) at different stages, and put them in our established *in vitro* culture system. We will use two-photon laser scanning microscopy to image the cultured hearts and create a series of three-dimensional renderings with emphasis on monitoring the interaction between endocardium and myocardium (initiation of trabeculation), recruitment of cardiomyocytes into primitive trabeculae (re-organization), and the outgrowth of trabecular structure (proliferation). We will perform similar experiments in mice lacking the BMP-10 gene. We will use these data to generate a dynamic simulation of the process of ventricular trabeculation in the presence and absence of BMP-10. We will perform additional *in silico* experiments to predict the effects of varying BMP-10 content on trabecular formation. We will perform *In vitro* BMP-10 dose-response experiments in parallel to test the predictive capability of the simulation.

The successful execution of these aims would generate a vast amount of data to support computer simulation of ventricular trabeculation and compaction. This approach may help in the eventual clinical diagnosis of noncompaction defects in patients, providing valuable functional-genomic tools for us to evaluate new genes and mutants and complement and enhance our current NIH-funded studies (HL70259) on the role of BMP signaling in chamber maturation.

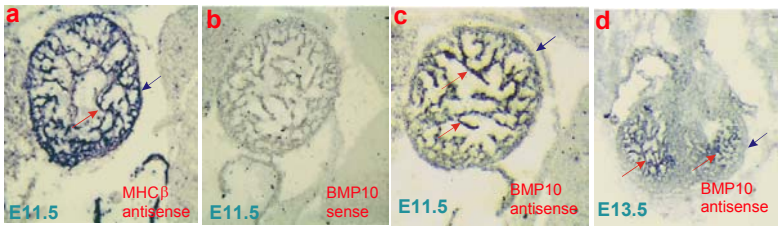
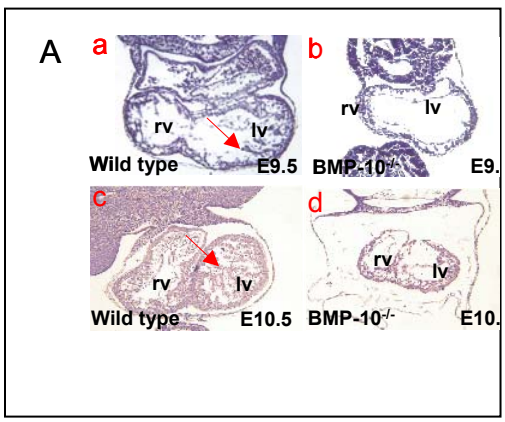


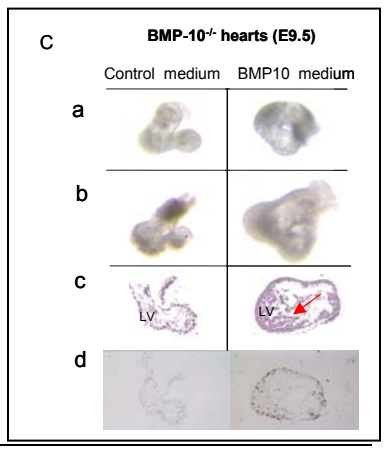
Figure 13: BMP-10 expression is associated with ventricular trabeculae. Red arrows indicate the ventricular trabeculae, while blue arrows indicate the compact wall. We used MHCβ probe as a positive control, BMP-10 sense probe is used negative control.

III.4.vi.c Preliminary Results:

III.4.vi.c.1 Cardiac-specific cytokine BMP-10 expression associates with cardiac trabeculation:

Figure 14: Functional analysis of BMP-10 in ventricular trabeculation. (A) BMP-10 deficient hearts fail to develop ventricular trabeculae. (B) Primitive trabeculae were formed in BMP-10 deficient heart, but fail to grow further. (C) BMP-10 conditioned medium is able to rescue BMP10 mutant hearts. (a) Before the culture, (b) after the culture, (c) histological analysis of cultured hearts, and (d) ^{3}H-thymidine labeling to assess the proliferative activity. Red arrows indicate the trabeculae, white arrows indicate the endocardium. v: ventricle; a: atrium; lv: left ventricle; rv: right





BMP-10 is a peptide growth factor that belongs to the TGF-β superfamily and was initially identified as being up-regulated in a hyper-trabeculated heart from mice deficient in FK506 Binding Protein 12 (FKBP12) (Shou *et al.*, 1998). FKBP12 is a negative regulator of BMPs/TGFβ/activins. FKBP12-deficient hearts developed myocardial hyperplasia and excessive ventricular trabeculae. BMP-10 is the only BMP that has cardiac-restricted expression. We have carefully analyzed the expression pattern of BMP-10 in the developing mouse heart using wholemount and section *in situ* hybridization analysis. As in Figure 13 shows, BMP-10 expresses transiently in the ventricular trabecular myocardium from E9.0 to E13.5, making BMP-10 a strong candidate molecule for regulating cardiac trabeculation and compaction. That BMP-10 is up-regulated in FKBP12-deficient hearts further suggests that BMP-10 is a positive regulator of myocardial growth and trabeculation.

III.4.vi.c.2 Functional analysis of BMP-10 in cardiac development:

We have used gene targeting to generate BMP-10 deficient mice. BMP-10 homozygous mutants die *in utero* at E10.5 due to severe defects in cardiac development, which include a hypoplastic ventricular wall and a lack of ventricular trabeculation (Figure 14). To better understand if the lack of trabeculation in the BMP-10-deficient heart was due to a failure of endocardial outpocketing (*i.e.*, initiation of trabeculation) or a failure of a myocardial response (*i.e.*, further expansion of trabecular myocardium through either myocyte recruitment or growth), we used confocal microscopy to analyze the structural relationship between the endocardium and the myocardium in BMP-10 deficient hearts at E9.25. As Figure 14B shows, endothelial receptors for vascular endothelial growth factor (Flk1; green signal) normally express in the BMP-10 deficient endocardium like wild type and littermate controls. We also maintained MF20 expression (anti-myosin heavy chain; blue signal) in the BMP-10 deficient myocardium despite severe hypoplasia. The developing endocardium was in normal proximity to the ventricular wall and the primitive ventricular trabeculae were present in the BMP-10 mutants. These findings strongly suggest that BMP-10 was not essential for the initiation of cardiac trabeculation or myocyte recruitment, but was essential for the outgrowth of the trabeculae. Furthermore, BMP-10 conditioned medium rescued the growth-deficient phenotype in BMP-10 deficient hearts (Figure 14). The ^{3}H-thymidine labeling index in the rescued mutant hearts was significantly higher than that using only control medium (12.1%±1.3, mutant heart in BMP-10 conditioned medium versus 5.3%±1.1, mutant hearts in control medium, $p<0.001$).

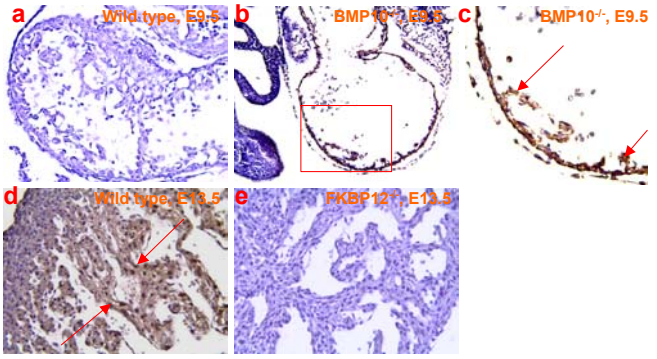


Figure 15: The p57^{kip2} expression is up-regulated in BMP-10-deficient heart and down-regulated in FKBP12-deficient hearts in which BMP-10 is up-regulated.

Induction of p57^{kip2} expression (a negative cell cycle regulator) associates closely with maturation of the ventricular trabeculae (Kochilas *et al.*, 1998). Thus, p57^{kip2} provides a good surrogate marker for cardiac cell cycle exit within the ventricular trabeculae during chamber maturation. Immunohistochemistry staining revealed that p57^{kip2} was up-regulated and ectopically expressed throughout the ventricular wall in BMP-10 deficient hearts at E9.5 as compared to littermate controls (Figure 15). Furthermore, FKBP12 deficient hearts (E13.5) exhibit significantly lower p57^{kip2} expression within the trabecular myocardium compared to littermate controls (Figure 15). Given that FKBP12 deficient hearts have elevated BMP-10 levels and an over-production of trabeculae, this further supports the notion that BMP-10 provides a potent positive growth signal during the regulation of ventricular trabeculae outgrowth.

III.4.vi.c.3 Signals from endocardium regulate BMP-10 expression:

As discussed above, the endocardium, which provides the between developing myocardium triggers ventricular trabeculation. induction of BMP-10 expression signals from the endocardium BMP-10 expression in Tie2-endoglin-deficient mice. Tie2 and receptors expressed by the vascular endothelial cells that and TGFβ (Arthur *et al.*, 2000), deficient in Tie2 or endoglin have that result in abnormal outpocketing of initial interaction and endocardium, To determine if the dependents on itself, we analyzed deficient and endoglin are endocardium and bind angiopoietin respectively. Mice endothelial defects development of the vasculature system and the heart, leading to embryonic lethality at E10.5. Significantly, BMP-10 is down-regulated in Tie2 null and endoglin-null hearts as wholemount *in situ* hybridization demonstrates. (Figure 16). This finding suggests that endocardially-derived signals regulate BMP-10 expression. Importantly, Tie2 null and endoglin-null hearts also have a hypoplastic trabeculae phenotype.

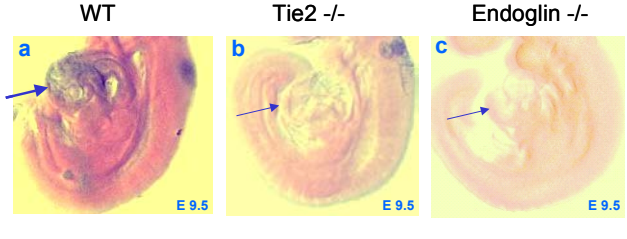


Figure 16: BMP-10 expression is down-regulated in Tie2-deficient and endoglin-deficient hearts. Arrows indicate hearts.

The presence of BMP-10 in the developing ventricle at E9.0 is critical for the outgrowth of ventricular trabeculae, and further that signals from endocardium regulate expression of BMP-10. Thus, the level of BMP-10 present in myocardium appears to determine the degree of trabeculation, as multiple independent knockout mouse models demonstrate.

III.4.vi.d Research Plan:

Our overall goal is to characterize the dynamics process of trabeculae formation using mouse molecular genetic tools, imaging techniques, and computer simulation. Initial experiments will generate the transgenic models needed to image endothelial cell and myocardial cell interactions in normal hearts and in hearts lacking BMP-10. These data will generate computer simulations of cardiac trabeculation, and to predict the effects of varying BMP-10 content during development. In parallel, additional *in vitro* experiments will directly determine the impact of varying BMP-10 content during development in order to establish the predictive capabilities of the simulations. To achieve these goals, we will implement the following research plan:

III.4.vi.d.1 Specific Aim 1: Generate transgenic reporter mice to differentially label the myocardium and endocardium:

Transgenic Tie2-GFP mice, which express GFP in endothelial cells (including the endocardium), are freely available to us (Motoike, 2000). Therefore, our immediate goal will be to generate H-Ncx-RFP transgenic mice. The 1.8 kb cardiomyocyte-specific promoter of Ncx1 gene (H-Ncx) has been well characterized and previously used to drive luciferase reporter gene expression within the developing myocardium as early as E8.0 (Muller *et al.*, 2002) (Figure 17). This promoter targets strong and reproducible reporter gene expression to cardiomyocytes throughout development and into adulthood. We will use this H-Ncx promoter (provided by Dr. S.J. Conway, Experimental Platform #3) to drive

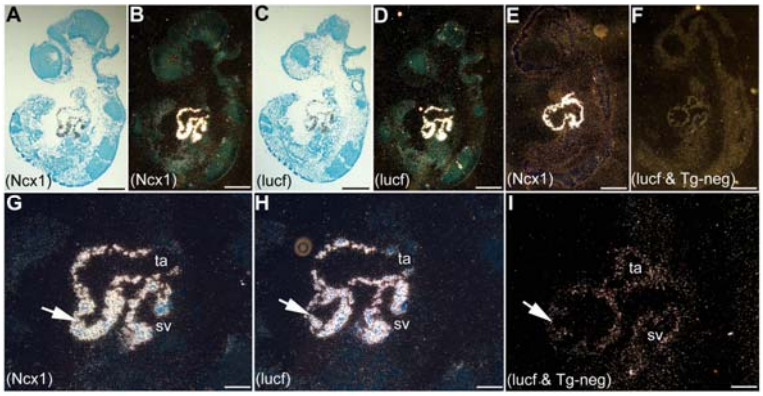


Figure 17: *In situ* analysis of H-Ncx promoter activity. A, B, and G, H-Ncx expression in early embryonic heart. C, D, and H, cardiac-specific expression of luciferase driven by H-Ncx promoter. E, F, and I, transgenic negative control.

expression of the fluorescence marker RFP to label the developing myocardium. We have extensive expertise generating and analyzing transgenic mice and do not anticipate any difficulties. We will breed H-Ncx-RFP transgenic to Tie2-GFP mice to create a compound transgenic strain that possesses both transgenes, H-Ncx-RFP and Tie2-GFP, which will allow us to simultaneously yet differentially label and examine the myocardium and endocardium using our heart - organ culture *in vitro.*

III.4.vi.d.2 Specific Aim 2: Image analysis of the dynamics of ventricular trabeculation and compaction in normal and abnormally developing hearts:

We have already established and used heart explant *in vitro* organ culture to analyze our BMP-10 null heart phenotypes (see Preliminary Results). Using this system we will be able to carefully document and analyze dynamic real-time cardiac development. For our initial experiments, we will isolate fluorescence labeled embryonic hearts carrying both the H-Ncx-RFP and Tie2-GFP reporter genes at different stages of development (E9.0-E13.5), and we will put them into our *in vitro* culture. Since each reporter gene segregates in a Mendelian fashion, ¼ of the embryos from a given intercross will have the desired genotype. Two-photon laser scanning microscopy will image the cultured hearts and create a series of three-dimensional to monitor the dynamic, transient and/or permanent interactions between the endocardium and myocardium. The stages we will analyze will be initiation of trabeculation (E9.0-E9.5); recruitment of cardiomyocytes into primitive trabeculae (E9.0-E10.5); outgrowth of trabecular structure (E9.5-E12.5); and the compaction of trabecular myocardium (E12.5-E13.5). First, we will document the number, size, and structure of the endocardial “out pockets” in E9.0 and E9.5, which will reflect the ability of the endocardium to recruit cardiomyocytes. Second, we will determine the number and size of primitive trabeculae, and the distance between myocardial trabecular structure adjacent to endocardial “out-pockets” in E9.0 and E9.5 hearts. Third, we will measure the outgrowth rate of ventricular trabeculae in 6-hour and 12-hour periods in E9.0-E9.5, E10.0-E10.5 and E12.0-E12.5 hearts. We will acquire Image data on a two-photon microscope (Zeiss LSM510-Meta confocal microscope) and we will process a vertical series of two-dimensional images using the Voxx software as described in Experimental Platform 1. We will perform additional experiments using embryonic hearts carrying both the H-Ncx-RFP and Tie2-GFP reporter genes in a BMP-10 null genetic background. Since the reporter genes and each BMP-10 mutant allele segregate in a Mendelian fashion, only 1/16th of the embryos from a given intercross will have the desired genotype. We will confirm BMP-10 genotype via PCR analysis. We will explan, cuture, and image hearts as described above. Using the resulting imaging data, we will generate 3-D reconstructions of cardiac trabeculation in the presence and absence of BMP-10.

III.4.vi.d.3 Specific Aim 3: Image analysis of a BMP-10 dose-response study:

Data presented in the Preliminary Results Section indicate that exogenous BMP-10 can rescue the hypoplastic trabecular outgrowth/noncompaction phenotype in cultured BMP-10 deficient hearts. BMP-10 dose-response experiments should be able to determine the precise relationship between BMP-10 signaling (content and timing of exposure) and ventricular development. We will generate embryos carrying the H-Ncx-RFP and Tie2-GFP reporter genes in a BMP-10 null genetic background as described above. We will harvest embryos, and we will dissect and culture the hearts in the presence of different BMP-10 concentrations. We will image the hearts and will perform a three-dimensional rendering as described above. Additional experiments will determine if sustained BMP-10 signaling is required to rescue the hypoplastic phenotype, or if transient exposure is sufficient. The resulting imaging data will be used to generate three-dimensional reconstructions of cardiac trabeculation in the presence of varying concentrations of BMP-10.

III.4.vi.d.4 Specific Aim 4: Computational analysis of cardiac trabeculation and compaction from normal and genetic manipulated mutant hearts:

III.4.vi.d.4.A Modeling of Cardiac Trabeculation:

While the Shou laboratory has been involved primarily in experimental approaches, we have begun discussions with other Consortium members on the modeling of cardiac trabeculation using the CPM. Our identification of the spatiotemporal expression of growth and differentiation factors such as BMP-10 under conditions of normal cardiogenesis and impaired cardiogenesis in the FKBP12 mutant mouse, in conjunction with earlier studies on expression of BMP-2/BMP-4 (Nakajima *et al.*, 2000), BMP-6 (Solloway *et al.*, 1998), and BMP-5 and BMP-7 (Solloway and Robertson, 1999) provide a basis for modeling the relevant morphogen fields, which in the case of myocardial development regulate cell proliferation. Moreover, as with limb morphogenesis (see Section VI.9.ii, above), the extracellular matrix protein fibronectin increases at the onset of trabeculation (Icardo and Manasek, 1983), providing a potential haptotactic component that the CPM is ideally suited to handle. Finally, an existing biomechanical model of cardiac trabecualtion (Taber and Zahalak, 2001) lacks specific reference to growth-regulating morphogen and haptotactic fields, although it provides a plausible account of mechanical aspects. We plan to devise a more comprehensive model taking both aspects into account using the CPM.

III.4.vii COMPUTATIONAL PLATFORM: COMPUTATIONAL MODELING OF CARDIAC DEVELOPMENT:

Experimental Platforms 1–4 address four key aspects of cardiac development: 1) the relationship between the cardiomyocyte cell cycle activity and ventricular wall formation; 2) the combined effects of specific transcription factors on cardiac morphogenesis; 3) the role of cell proliferation and apoptosis in valve morphogenesis and maintenance; and 4) the interaction between the endothelial cells of the endocardium and proliferating cardiomyocytes in driving trabeculation. These four platforms are concerned fundamentally with factors regulating the morphology of the developing heart and the application of these studies to regenerative growth of diseased hearts. The Cellular Potts Model (CPM) is one of few available computational frameworks for simulating growing, differentiating and moving cellular domains comprised of unit cells. As such, it is ideally suited as the computational workbench to complement these experimental studies.

III.4.vii.a Platform 1:

Platform 1 will use CPM to model proliferation of genetically altered progenitor cells in the ventricular wall in three dimensions, as a function of the rate of mitosis, which is an easily adjustable parameter in CPM calculations. We expect to simulate the formation of transmural growth units and their assembly into three-dimensional ovoid structures, as a function of the division rate of the progenitor cells as well as other Potts parameters.

III.4.vii.b Platform 2:

Platform 2 will motivate the formulation a minimal model of the gene regulatory motif involving transcription factors Nkx2.5 and HAND2, shown to be essential for morphological formation of the developing heart. Such a model is likely to be stochastic in nature, given the fact that transcription factors are present in the cell often in small copy numbers. We expect fluctuations in these numbers to be significant in the combined expression of the corresponding genes. The CPM interface to this experimental platform provides an example of one the unique computational capabilities of TST: An intracellular process, such as a gene regulatory motif, stipulates effective parameters to the Potts model, in turn leading to different rates of proliferation, differentiation and adhesion, determining overall morphology of the tissue. We expect CPM modeling to be particularly relevant to this experimental platform during early stages of heart development, when the morphology is as yet not too intricate.

III.4.vii.c Platform 3:

Platform 3 will use CPM to investigate spatiotemporal patterns of cell apoptosis and proliferation during valve development as well as repair of damaged valves. These rates, as well as the complex valve geometry, are straightforwardly prescribed within the CPM framework.

III.4.vii.d Platform 4:

Platform 4 will use CPM to study the role of cytokine signaling on cardiomyocyte proliferation. Using RFP- and GFP-tagged genes expressed in the myocardium and endocardium, respectively, two-photon microscopy will be used to dynamically follow the interaction between the endocardium and myocardium *in vitro* and its role on trabeculation. The role of BMP-10, known to be a positive regulator of trabeculation, will be incorporated into CPM by formulating a minimal mechanistic model of its regulation by endocardially-derived signals. CPM simulations, analogous to the imaging of *in vitro* development of ventricular trabecular outgowth, will be performed and compared with experiments.

In the above, the emphasis will be (a) on the predictive role of simulations for the various experimental platforms, (b) in suggesting new experimental parameters to be explored, as well as (c) to complement these experiments in parameter regimes that may not be experimentally accessible. We expect that in addition to basic investigation of fundamental mechanisms of cardiac development suggested by these four experimental platforms, TST will be in a good position to interface with the experiments underway by the Alliance for Cell Signaling (AfCS) on the cardiomyocyte model cell as they become available (Sambrano *et al.*, 2002).

III.5 VASCULAR DEVELOPMENT (KEITH L. MARCH, CHARLES D. LITTLE, AND MERV YODER):

III.5.i INTRODUCTION:

The formation of the blood vessels (vasculogenesis) is a challenging problem. There are three major constraints on the constructions of the blood vessels. The first constraint is physiological: embryonic cells must obtain nourishment before there is an intestine, use oxygen before they have lungs, and excrete wasted before they have kidneys. The fore the developing embryo has needs that are different from those of the adult organism, and its circulatory system reflects those differences. The second constrain is evolutionary. For example, the mammalian embryo will extend blood vessels to the yolk sack even though there is no yolk therein. Thus, even our physiology does not require such structure; our embryonic condition reflects our evolutionary history. Finally, the third constrain is physical. According to the laws of fluid movement, the most effective transport of fluids is performed by large tubes. As the ratios of the blood vessel gets smaller, resistance to flow increases as r^{-4} (Poiseulle's law). A blood vessel that is half as wide as another has a resistance to flow 16 times greater. However, diffusion of nutrients can take place only when blood flows slowly and has access to the membrane. There is a paradox in vasculogenesis: the constraints of diffusion mandate that the vessels be small, while the laws of hydraulics mandate that the vessels be large. Living organisms have solved this paradox by evolving circulatory system with hierarchy of vessel sizes. This hierarchy if formed very early in development. By having large vessels specialized for diffusion and small vessels specialized for diffusion, nutrients and oxygen can reach the individual cells of the developing organism. But there is another physical problem. If fluid under constant pressure moves directly from a large-diameter pipe into a small-diameter pipe, the fluid velocity increases. The evolutionary solution for this problem was the emergence of smaller vessels branching out from a larger one, so that collective cross-sectional area of all the smaller vessels is greater than that of the larger vessel. This relationship is known as Murray's law: the cube of the radius of the parent vessel approximates the sum of the cubes of the radii of the smaller vessels. The construction of any circulatory system must negotiate among these physical, physiological and evolutionary constraints. This makes the development of the circulatory system a complex biological challenge.

III.5.ii SPECIFIC AIMS:

Recent descriptions of pluripotent cells derived from a number of adult as well as embryonic sources, able to incorporate into and influence the development of nascent vasculature, have led to the concept of employing such cells as therapeutic tools for manipulation of angiogenesis and arterial remodeling. Such cells have been characterized in terms of their ability to facilitate tissue function by the acceleration of the endogenous processes of angiogenesis and arteriogenesis that in turn contribute to the restitution of tissue perfusion in areas subject to pathologic reductions of blood flow. Examples of cell types that have been shown to possess such activity include endothelial precursor cells obtained from yolk sac or embryonic sources, or found in the bone marrow of adult organisms, as well as most recently adult endothelial precursors (ADEPs) located among pluripotent adipose stromal cells (ASCs) derived from adult organisms. While it is clear that such cells indeed can augment the formation of capillaries, the mechanisms by which this occurs are not well-defined, and likely include both providing instructive signals as well as supplementing the number of component cells available to participate in local vessel formation. In addition, little study has been directed to characterizing the dynamic behavior of such cells as they assemble into vessels in either embryonic or in adult systems.

Understanding the self-assembly of a vascular system from component cells is an important problem from both therapeutic and developmental perspectives. The direct imaging of individual cells undergoing temporal changes, during development and vascular assembly, provides unique insights into the mechanisms by which cells develop a functional vascular network. We have recently established methodology to permit such dynamic imaging of vascular development at cellular resolution levels over organism-scale spatial fields, by taking advantage of two complementary embryonic systems which are essentially flat and relatively thin, thus providing excellent properties for imaging. One such system is the quail embryo isolated ex ovo, and another is the mouse allantois isolated and cultured ex utero, both of which can undergo extensive vascular development and assembly under conditions favorable to microscopic study.

The availability of these models for studying vascular pattern formation, coupled with the interest in understanding mechanisms governing the participation of both and embryonic- and adult-derived cells such as endothelial precursors and pluripotent adipose stromal cells in the formation of nascent vasculature, suggested study of the behavior of these cells, both readily available from murine sources, upon introduction into the quail embryo or mouse allantois respectively. Recent data has demonstrated that murine endothelial cells as well as endothelial progenitor cells can indeed migrate and pattern in response to embryonic patterning signals in quail hosts, supporting the feasibility of their study in such chimeric embryo systems.

The hypotheses of this project accordingly are that selected embryonic-derived endothelial precursor cell populations of defined potential, as well as adult-derived endothelial precursor cells derived from adipose tissue will exhibit cell- and environment-specific behaviors when implanted into developing embryonic systems, which will be based on the endogenous molecular programs of the cells and environment as well as modulable by exogenous factors; and that the detailed real-time recording of behavioral parameters for individual cells and cell populations will provide data for generating, refining, and validating quantitative computational models of key embryonic development processes such as vasculogenesis, and their dependence on selected molecular cues. These models will refine our understanding of the mechanisms of embryonic development, and enable high throughput searches for cell parameters that play the pre-eminent roles in determining their behaviors; these will in turn suggest specific experimental tests. Such computationally-aided hypothesis construction will enable ongoing and future experimental projects to be more quickly focused on those physicochemical parameters of the cells that are predicted to be significant determinants of pattern formation (see also Core 2).

III.5.ii.a Specific Aim 1:

Definition of the migration, lineage fate, and incorporation of murine embryonic- or adult-derived endothelial precursor cells implanted into a developing heterologous quail embryo or mouse allantois system as a *function of their individual surface markers and degree of pre-specification.*

The degree of differentiation and related commitment of both adult adipose stromal-derived progenitor cells as well as yolk-sac-derived hemangioblast progenitor cells is expected to play a critical role in determining their capability of proceeding along specified differentiation pathways. Defined endothelial precursor cell

populations, derived from yolk sac (embryonic) or adipose (adult) sources, characterized by their surface marker phenotypes and fluorescently labeled, will be evaluated with respect to their dynamic behavior monitored following injection into both the quail embryo and the mouse allantois, with particular attention to the characteristics of incorporation into the various components of the nascent vasculature. The movements and ultimate incorporation and differentiation of these cells will be studied as a function of their initial differentiation status as reflected by their marker profiles. Computational studies will include searches through different combinations cell adhesion strengths between the precursor cells and extracellular environments, increasing understanding of the biophysical determinants of cell behavior that will direct further experimentation. Information from these analyses will then be used to direct choices of cell selection for delivery in further experiments designed to help in understanding the effects of the extracellular environment in governing the behavior of these cells.

III.5.ii.b Specific Aim 2:

Evaluation of the dependence of the migration and incorporation of embryonic- or adult-derived endothelial precursor cells implanted as above, upon *the cues received from the embryonic microenvironment*, as determined by the time and site of injection as well as the genomic information resident in the host environment.

The growth factor and matrix environment surrounding pluripotential cells such as both the embryonic- and adult- derived endothelial precursors play a critical role in instructing the cells to proceed along specified differentiation pathways. The dynamic behavior and ultimate loci and extent of incorporation of defined labeled yolk sac (embryonic) or adipose stromal (adult) progenitor cell populations will be evaluated following injection into both the quail embryo and the mouse allantois, as a function of the state of development of the recipient embryo or allantois, the site of injection in the quail embryo, and the growth factor status of the allantois. The quail embryo system provides an ideal setting for the assessment of spatiotemporal cues for vascular patterning, given the availability of the entire formative vascular tree to the “reporter” cells; while the mouse allantois system, while limited in anatomic extent, provides an excellent setting to evaluate mechanisms even for genetic alterations lethal to the development of entire embryos. Experiments will be performed to evaluate the particular hypothesis that the genetic overexpression or partial deletion of VEGF in the allantois system will lead to alterations in the survival, mobility, differentiation, and integration of progenitor cells of both sources. The data obtained from these experiments will uncover key mechanisms governing the behavior of these progenitor cells, as well as providing a target for simulation by the cellular behavior models being developed and tested elsewhere in this proposal.

III.5.ii.c Specific Aim 3:

Characterization of the manner in which the migration and incorporation of embryonic- or adult-derived endothelial precursor cells introduced at a particular locus will be programmed or modulated by pre-treatment of the cells or their environment with *exogenously provided factors which signal differentiation* along particular pathways.

The extent to which exposure of embryonic- and adult- derived endothelial precursors to particular growth factors individually or in combination can modulate their degree of differentiation and lineage commitment, thus affecting their behavior upon introduction into the area of a developing vasculature, will be studied by exposing these defined murine progenitor cell populations to factors promoting endothelial differentiation either prior to implantation into the quail embryo and the mouse allantois systems, or by co-injecting the progenitor cells along with these factors at supra-physiologic levels, and comparing the dynamic behavior and ultimate fate of these cells with their behavior and fate in the absence of the super-imposed environmental manipulation. Again, the data obtained from these experiments will provide information concerning the inputs determining the specification of these progenitor cells, as well as extending the data derived from Aims 1 and 2 so as to provide opportunities for further refinement of the cellular behavior models being developed and tested elsewhere in this program. The computational model will aid us in understanding that cell properties are key determinants of migration and incorporation of ADEPs and EDEPs, and will target future experiments to pre-treatments that will specifically alter these cell behaviors.

III.5.iii BACKGROUND AND SIGNIFICANCE:

III.5.iii.a Adipose tissue derived pluripotent cells:

The discovery of pluripotent cells in the adipose tissue (Zuk *et al.*, 2001) has revealed a novel source of cells that may be used for autologous cell therapy to regenerate tissue. The pluripotent cells reside in the "stromal" or "non-adipocyte" fraction of the adipose tissue; they were previously considered to be pre-adipocytes, *i.e.* adipocyte progenitor cells, however recent data suggest a much wider differentiation potential. Zuk *et al.,* (2001) were able to establish differentiation of such subcutaneous human ASCs *in vitro* into adipocytes, chondrocytes and myocytes (Zuk *et al.*, 2001). These findings were extended in a study by Erickson *et al.*, which showed that human ASCs could differentiate *in vivo* into chondrocytes (Erickson *et al.*, 2002) following transplantation into immune-deficient mice. More recently, it was demonstrated that human ASCs were able to differentiate into neuronal cells (Safford *et al.*, 2002), osteoblasts (Dragoo *et al.*, 2003), and cells expressing endothelial phenotypes (Preliminary Data below). Such adipose stromal cells (ASCs) can be obtained in large quantities, in the range of 10^8 to 10^9 cells, following routine liposuction of subcutaneous adipose tissue. This ready accessibility in turn has suggested the notion that they might provide for a particularly feasible and attractive form of autologous cell therapy requiring either no ex vivo expansion or relatively limited expansion. However, relatively little is known about the biological characteristics of ASCs, the factors governing their fate and function, and their specific ability to differentiate along hemangioblast pathways. It is clear that such studies will provide critical foundations for evaluating the potential of these cells. The definition of the identity of these cells as well as investigations probing their ability to differentiate into cells that give rise to components of the vasculature may suggest practical strategies for modulating physiological processes such as collateral vessel formation. The recent identification of a specific population of pluripotential cells of non-hematopoietic origin, resident in other tissues such as muscle, brain, and marrow (Howell *et al.*, 2002; Howell *et al.*, 2003), has suggested the existence of a common murine stem cell characterized by a CD45-/Sca-1+/c-kit- cell-surface phenotype. Our data suggests that this phenotype is shared by a substantial fraction of murine ASCs, and that the analogous CD45-/CD34+/c-kit- phenotype is characteristic for a major fraction of human ASCs.

III.5.iii.b Expression of endothelial precursor markers on adipose stromal cells:

As we will show in our preliminary data section, we have found that a large proportion (~70%) of freshly isolated human subcutaneous ASCs express the cell surface marker CD34. ASCs also contain a small population of cells that are positive for CD34 as well as for the endothelial-specific marker VE-Cadherin. However, the majority of the ASCs are CD34(+)/VE-Cadherin(-), thus suggesting that they are not mature endothelial cells, but are instead similar to primitive cells of the hemangioblast lineage, thus representing adult-derived endothelial precursors (**ADEP**s). Our data also show that murine ASCs express Sca-1 (Stem Cell Antigen-1), which is a marker of both murine pluripotent stem cells (Uchida and Weissman, 1992) and murine endothelial progenitor cells (Dimmeler *et al.*, 2001).

III.5.iii.c Yolk sac-derived endothelial precursor cells:

We (Merv Yoder), have recently identified surface marker characteristics that reliably distinguish cells from the murine embryonic yolk sac that are 1.) cells that are endothelial progenitors initially devoid of hematopoietic activity but capable of re-acquiring hematopoietic activity after exposure to stromal cells; 2.) cells that are irreversibly committed endothelial progenitors without evidence of inducible hematopoietic activity; and 3.) cells that are irreversibly committed hematopoietic progenitors (Li *et al.,* in press). Cells obtained from an 8.25 day post-coitus timepoint which are Tie2+/Flk1dim/CD41- manifest both endothelial and hematopoietic potential; while cells which are Tie2+/Flk1bright/CD41- are committed endothelial progenitors; and cells which are Flk1dim/CD41+ exhibit solely hematopoietic potential. These studies establish the feasibility of isolating murine embryonic-derived endothelial precursor **(EDEP)** cells with either uncommitted or committed phenotype, for study of their respective behaviors when placed in environments permitting dynamic assessment of vascular pattern formation, as below.

III.5.iii.d Origin of endothelial cells in the embryo:

The first step of blood vessel formation in the gastrulating murine embryo is the differentiation of endothelial cells from mesodermal precursors (Risau and Flamme, 1995) Mesoderm-derived angioblasts are the

endothelial precursors that arise in the yolk sac mesoderm. Differentiation, expansion, and coalescence of angioblasts into the initial vascular network are called vasculogenesis. Angioblasts first appear in the yolk sac with hematopoietic precursors in aggregates of cells that will ultimately form blood islands. The second step of vasculogenesis is comprised of endothelial cell formation into a "honeycomb-like" capillary plexus that later remodels into an arterial and venous system with an intervening capillary bed. A number of endothelial growth factors, extracellular matrix molecules, cell adhesion molecules, and transcription factors are required for the successful morphogenesis of the vascular system (Hanahan, 1997).

The essential role of endothelial growth factors and their receptors in vasculogenesis and angiogenesis has been demonstrated by disruption of the genes encoding murine vascular endothelial growth factor (VEGF), Flk-1 and Flt-1 (receptors for VEGF) via gene targeting in embryonic stem cells and the generation of mutant mice. Flk-1 appears essential for the development of endothelial cells from angioblast precursors as knock-out mice lacking Flk-1 expression fail to form endothelial cells and capillaries in the yolk sac and the embryos die early in gestation (Shalaby *et al.*, 1995). VEGF, the primary known ligand for Flk-1, is also essential for vasculogenesis. Surprisingly, animals that were heterozygous for VEGF expression ($VEGF^{+/-}$) as well as animals totally deficient in VEGF expression ($VEGF^{-/-}$), were noted to have abnormal capillary development, angiogenic capillary sprouting, and large vessel development and to die early in development. (Campbell *et al.*, 2001). Of interest, modest increases in VEGF expression (2-fold) are also embryonic lethal as embryos display excessive angiogenesis and abnormal cardiovascular and vascular development (Miquerol *et al.*, 2000). Accordingly, VEGF has been chosen as a key target for studies in this proposal, as these will provide an important opportunity for testing models and parameters that relate local levels of this factor to cellular behavior.

III.5.iii.e Dynamic imaging of vascular development in the Quail embryo cultured *ex ovo*:

The avian embryo is well suited for the study of vertebrate blood vessel morphogenesis. Like mammals, birds are warm-blooded and require a high performance cardiovascular system for survival. Furthermore, it has been determined that primary vasculogenesis in mice and birds progresses in a virtually identical manner (Drake and Fleming, 2000).

The major advantage of using avian, as opposed to mouse, embryos is that both experimental treatment and direct observation at the earliest developmental stages are readily accomplished. The experimental equivalent of a conditional gene knockout can be accomplished in avians, and the effects on vascular morphogenesis examined at precisely timed intervals. This is accomplished by introducing an experimental reagent directly into the vascular primordia, *in vivo*. Earlier studies by others determined that the precise delivery of reagents to the splanchnopleure, the site of initial intraembryonic vasculogenesis, was not feasible using embryos on the yolk; thus the *ex ovo* culture method was devised (Little and Drake, 2000). Our approach permits accurate and precise delivery of experimental reagents into the splanchnopleural ECM, situated between the endoderm and splanchnic mesoderm, at stages when vasculogenesis is occurring (stages 6-10). Injected embryos are then cultured on yolk/agar beds (Drake *et al.*, 1992) to continue their development.

Another advantage of using quail embryos is that analysis of vascular development is readily accomplished for several reasons: First, initial vessel formation occurs, for the most part, in a single optical plane; Second, the embryo progresses from being avascular at 2 somites to having an intact circulatory network only 9 hours later; and third, vessel morphogenesis occurs in the absence of blood flow and peri-endothelial cells (Drake *et al.*, 1998). The availability of robust markers of quail endothelial cells and their precursors further aid analysis, especially for monitoring vascular assembly. The mouse monoclonal antibody, QH1, recognizes an epitope present on the surface of quail primordial endothelial cells (Pardanaud *et al.*, 1987), and we have used this marker successfully in our microscopic analysis of avian vasculogenesis (Drake *et al.*, 1995; Drake and Little, 1999; Drake *et al.*, 2000), as shown below on Preliminary Data, figures 12-13. We have also determined that avian endothelial cells, like their murine counterparts (Kallianpur *et al.*, 1994), express the transcription factor, TAL1/SCL, and that this expression precedes QH1 expression. TAL1 expression defines a subpopulation of cells (angioblasts) within the splanchnic mesoderm (Drake *et al.*, 1997), which are committed to an endothelial fate, but are not yet QH1 positive. Both QH1 and TAL1/SCL markers are detected once cells enter the splanchnopleural ECM, where lumen and vascular network formation occurs.

In regards to this application, use of time-lapse imaging of specific markers has already allowed us to establish that vascular networks in the bird and mouse embryo are expanded by both the ongoing recruitment of angioblasts and by endothelial cell protrusive activity (Drake *et al.*, 1997; Drake and Fleming, 2000). Analyzing the time-lapse data of QH1-labeled cells, in reverse, allows the fate and behavior of endothelial cells to be followed back in time to a period even before formation of mesoderm (gastrulation). While this approach does not label every endothelial cell it has the advantage that high-resolution optics can be used to critically analyze the dynamic behavior of individual cells in situ throughout vasculogenesis. Further the digital image files can be mined as a rich source of quantitative data. For example, endothelial cell velocity, persistence of motility, trajectories, velocity field plots and other computational parameters; moreover, all these data can be compared and correlated to the global motions of the intact embryo. Thus, for example changes in formation of the dorsal aorta or the sinus venosus can be correlated with vertebral axis extension and formation of the foregut, key morphogenic deformations that profoundly influence vascular pattern formation (Rupp *et al.*, 2004).

A third advantage of the quail embryo culture is that it enables two-dimensional modeling with the Tissue Simulation Toolkit. Although with the tools that we currently have available, and with the tools that will be developed in core I, three-dimensional simulation studies are routinely carried out, the initial use of two-dimensional model approximations is a powerful and fruitful research strategy. A two-dimensional domain helps to more easily observe and analyze the behavior of the model, and greatly simplifies statistical analysis and comparison with the experimental systems. Endothelial cell velocity, persistence of motility, trajectories, and velocity field plots are readily obtained from experiment and simulation alike, using identical methods (see *e.g.* Ouchi *et al.*, 2003). This enables one-to-one quantitative comparison of model and experiment. Moreover, the computational requirements are small relative to three-dimensional models, which makes it possible to carry out high-throughput parameter studies, directing and facilitating the construction of new hypotheses for the experimental research. Once the two-dimensional experimental system and the model is well understood, we will be able to proceed to three-dimensional systems.

III.5.iii.f Allantois and culture of isolated allantois tissue:

The mouse allantois is the future umbilical component of the chorioallantoic placenta and ultimately differentiates into the major artery and vein and surrounding connective tissue joining mother and embryo. Unlike the human allantois (consisting of both endoderm and mesoderm), the mouse allantois is composed entirely of mesoderm, which is relevant since endothelial cells are derived solely from the mesoderm. This makes the allantois a good model system in which to study vasculogenesis. The initial formation of the allantoic bud has been reported to begin between E7.0 and E7.25 (Downs and Davies, 1993; Drake and Fleming, 2000). While it is not known how the allantois initially forms, it grows by addition of mesoderm from the posterior streak, and ends by the time the third somitic pair has formed (Tam and Beddington, 1987). Fusion with the chorion occurs at approximately E8.25, which is the equivalent of a 6-7 somite mouse embryo (Figure 2). At this same time vessel formation begins by vasculogenesis (Drake and Fleming, 2000) but this does not depend on chorionic fusion (Downs and Gardner, 1995). We have used our computational imaging approach as described above to examine the dynamics of vascular pattern formation in explanted wild type mouse allantoides. This data will be used for comparison to a two-dimensional model constructed with the TST, which will provide computationally-aided hypothesis generation.

III.5.iii.g Growth and Visualization of Cells in extracellular matrix:

Cell-cell communication within the 3-D molecular framework of the extracellular matrix (ECM), collectively gives rise to differentiated form and function within tissues and organs. The realization that the ECM not only provides a mechanical framework for tissue architecture but also plays an active role in regulating the signaling process altered our understanding of how this complex scaffold network regulates cell behavior. Both biochemical and biophysical signals from the ECM modulate fundamental cellular activities, including adhesion, migration, proliferation, differential gene expression, and programmed cell death (Adams and Watt, 1993; Lelievre *et al.*, 1996). Similarly, the cell can modify its ECM environment by modulating synthesis, degradation, and organization of specific matrix components. We have investigated an intact interstitial extracellular matrix known as intestinal submucosa for use as a cell culture substrate (Voytik-Harbin *et al.*, 1998) as well as synthesized matrices (Brightman *et al.*, 2000). Collagen represents the predominant

component of the extracellular matrix, so it is not surprising that 3-D substrates prepared from this macromolecule have been extensively applied to study cell behavior ***in vitro*** **(Ehrmann and Gey, 1956; Elsdale and Bard, 1972)**. Type I collagen is most widely used because of its relative abundance and ease of purification. This collagen type is prepared from virtually any tissue or organ, with the most convenient sources being tendon, dermis, and bone. A purified collagen preparation from bovine dermis (Vitrogen) is available from Collagen Aesthetics (Palo Alto, CA).

Likewise, we (Jo Davisson and Paul Robinson) and others have applied this technique for surface and volume visualization of intact ECM biomaterials as well as 3-D reconstituted matrices consisting of individual (*e.g.*, collagen) or mixtures of ECM components (Brightman *et al.*, 2000; Friedl *et al.*, 1997; Gunzer *et al.*, 1997). Reflected light from collagen fibers has been collected simultaneously by fluorescence from cells stained with vital fluorochromes to monitor the dynamic process of cell migration through a 3-D collagen matrix (Friedl *et al.*, 1997). More recently, we provided the first account of confocal reflection microscopy (CRM) in a time-lapse mode for studying collagen fiber formation (fibrillogenesis) and ECM assembly ***in vitro*** **(Brightman *et al.*, 2000; Robinson *et al.*, 1999; Voytik-Harbin *et al.*, 1998)**. With time-lapse CRM, both kinetic and 3-D structural information can be collected simultaneously as ECM components polymerize from a soluble to a gel phase. Taken together, CRM offers a useful technique for investigating biological processes such as vasculogenesis in living systems involving the ECM and ECM-cell interactions that occur in multiple dimensions.

III.5.iii.h Vascular development of heterologous endothelial progenitors in chimeric embryo systems:

Recent studies by Ambler et al (2001, 2003) have demonstrated the feasibility of studying molecular mechanisms underlying murine embryonic cell migration and fate following the implantation of these cells into developing quail embryos. This chimeric approach was used to demonstrate the ability of murine angioblasts derived from somatic mesoderm, as well as stem-cell derived endothelial cell progenitors to migrate extensively and colonize the appropriate vascular beds, forming mosaic vessels with avian endothelial cells. The murine progenitors were found able to respond to global patterning cues, and to require both the VEGF-A gene and its flk-1 receptor to pattern properly. These data form a key basis for the use of the chimeric embryo approach in this proposal.

To summarize, this proposal will harness the capability of embryo-scale, high-resolution dynamic imaging-based data acquisition coupled with static morphometric and molecular analyses of cell fate, to permit the comparative study of defined embryonic vs. adult-derived vascular precursor cells with respect to their behavior in developing vascular systems, and to provide key data for the development and testing of initial computational models for vascular development.

III.5.iii.i Significance of Research:

The ready availability of large numbers of autologous cells with the capabilities of facilitating blood vessel growth and repair or of bone marrow repopulation, would be very significant clinically for patients with coronary or peripheral tissue ischemic, restenosis or atherosclerosis. One long-term goal of this research accordingly is to define the parameters that govern the vasculogenic activity of embryonic-derived endothelial precursor cell populations available from extraembryonic sources (yolk-sac); and to specifically compare this with the vasculogenic activity of the adult-derived endothelial precursors resident within the readily-available source of adult adipose tissue, in the context of development of a nascent vasculature. Ultimately, clinical therapies which might be implemented for diseases involving inadequate vascular supply could involve a minimally-invasive procedure such as liposuction to harvest adipose tissue, followed by any processing required to obtain an appropriately-defined and functionally consistent population of cells with the desired characteristics. Because of the large numbers of cells available from such harvests, this processing might simply include isolation / separation steps in the absence of any need for expansion; or conversely might include culturing in the presence of either specific media, growth factors, or even genetic material for the purpose of directing subsequent cellular behavior. The experiments described in this proposal will help clarify the biological responses of these cells to various environments, thus laying the foundation for future studies of therapeutic utility for angiogenesis and vessel repair.

III.5.iii.j Study of Complex Systems and computationally-aided hypothesis construction:

Until recently, developmental biologists had limited ways of gathering data over a long-term experiment. They relied on data gained from various points throughout the experiment and then speculated on what happened to get from point A to point B. Now, technology is available that allows the measurement and quantification of behaviors, both at the cellular and tissue levels. This technology, dynamic computational microscopy, combines biological questions with computer modeling to describe the behavior of a system. The long-term goal of this technology is to understand complex biological processes that involve the simultaneous interaction of multiple tissues during morphogenic events. This systems approach requires the detailed collection and analysis of large quantities of data about a developmental system in a normal environment. Once those data are obtained, the experimental system can be analyzed against the control model system in a manner that is quantifiable and testable. This systems approach also permits data acquisition that is typically obscured during a normal time-course experiment and will allow researchers to gather information throughout the entire length of the experiment, collecting data every few minutes instead of hours. The use of computational models helps the researcher to test how a particular cell behavior will affect the formed pattern, and will help him/her to quickly search through a large number of such cell behaviors. Such computationally aided hypothesis construction will efficiently direct the experimental efforts to the relevant experimental tests, and increase understanding of the morphogenetic mechanisms that drive biological development.

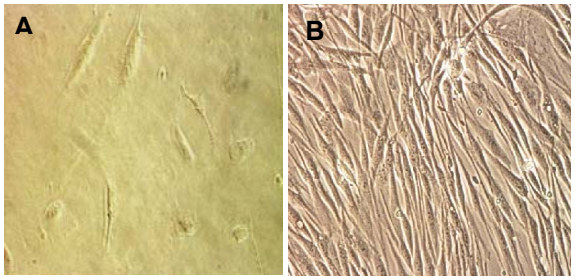


Fig 18: Phase contrast micrographs of a sub-confluent (left)

III.5.iv PRELIMINARY STUDIES / PREVIOUS WORK:

Isolation and expansion of murine adipose stromal cells (ASCs):

To isolate ASCs, subcutaneous adipose tissue (1-2 grams) is obtained from adult mice (*e.g.*, 4 months of age) and processed as we have described for human cells (Rehman, in press, 2004). ASCs are plated at 1000-10,000 cells/ cm^2. The majority of ASCs attach to the flask and when cultured in EGM2MV media, for example, can be expanded significantly (Figures 18, 19) with their growth rate decreasing when they reach confluency. These experiments demonstrate that ASCs can be readily isolated and rapidly expanded ex vivo from relatively small amounts of adipose tissue, thus laying the groundwork for using autologous ASCs in research and clinical settings. Human cardiovascular cell therapy studies suggest that 10^8 to 10^9 autologous cells may be required for clinical

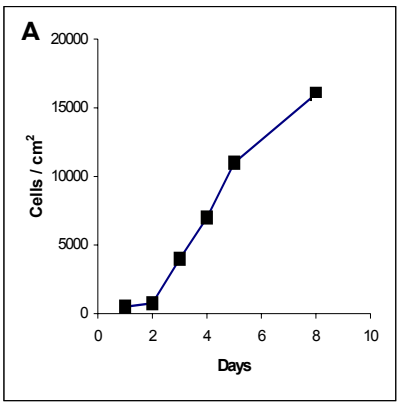
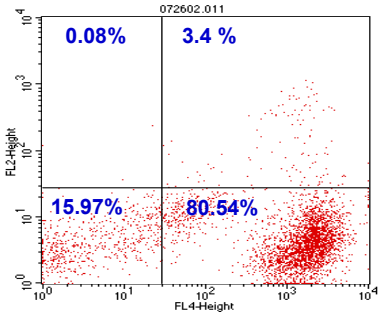


Fig 19: *In vitro* expansion of (A) human and (B) mouse ASCs.



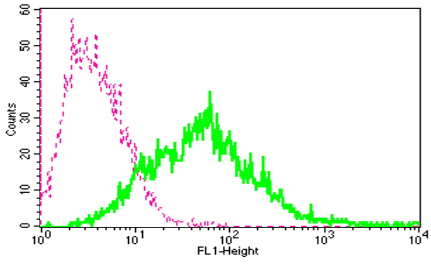


Fig 21: Flow cytometric analysis of Sca-1 expression (green) and the corresponding isotype control (pink).

applications. Considering the fact that liposuction can yield up to 3 liters of subcutaneous fat tissue in a single outpatient procedure, such cell numbers can be easily obtained from patients with little or no expansion.

III.5.iv.a Expression of stem cell markers on ASCs:

To evaluate the expression of the stem cell marker CD34, ASCs were freshly isolated from human adipose tissue and labeled with fluorescent antibodies against CD34 (BD Biosciences). As CD 34 is also expressed on mature endothelial cells and endothelial progenitor cells, the isolated cells were co-labeled with an antibody directed against human VE-cadherin (BD Biosciences), a highly specific marker of endothelial cells (Martin-Rendon and Watt, 2003; Rafii, 2000). The results indicate that the majority of cells are CD34+/VE-cadherin-, thus suggesting that most human adipose tissue derived stromal cells are **non-endothelial CD34-positive stem or progenitor cells**. The human adipose stromal cell fraction also contains a small but distinct population of CD34+/VE-cadherin+ cells, which may represent either mature endothelial cells or endothelial progenitor cells.

The expression of stem cell markers by murine ASCs was also examined. Murine ASCs were cultured, expanded in EGM2MV media with 20% fetal bovine serum and passaged when confluent. Cells at passages 0 - 3 were subsequently labeled with an antibody directed against the specific murine stem cell marker Sca-1 (Stem Cell Antigen-1). As shown in data from a representative experiment in Figure 21, > 70% of cultured murine ASCs express the stem cell marker Sca-1, as do other skeletal muscle-derived pluripotent cells described by our collaborators, Drs Srour and Yoder. The high level of expression of CD34 in human ASCs and of Sca-1 on murine ASCs complements the data on ASC differentiation potential (Erickson *et al.*, 2002; Safford *et al.*, 2002; Zuk *et al.*, 2001)*,* and lends support to the concept that ASCs have stem cell characteristics.

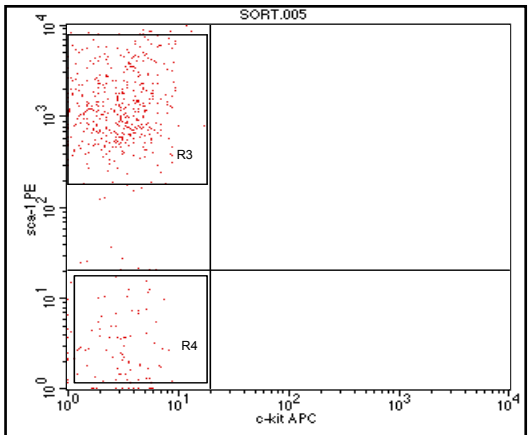
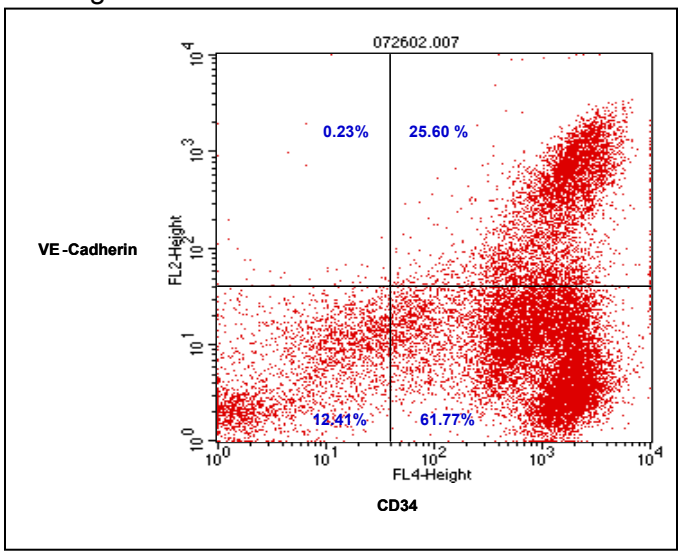


Fig 22: Flow cytometric analysis of murine ASCs gated on CD45(-) cells. R3 represents a Sca-1+/CD45-/c-kit- population, while the R4 cells are Sca-1(-)CD45(-)c-kit(-)

III.5.iv.b Flow cytometric evaluation of murine ASCs:

Our previous data have shown the expression of Sca-1 on murine ASCs and therefore suggested similarity between the subcutaneous ASC population and muscle derived Sca-1+/CD45-/c-kit- cells previously described as pluripotential by our collaborator Dr Mervin Yoder. We further defined the phenotype of murine ASCs by co-staining for the markers CD45 and c-kit. As was shown in Figure 5, murine ASCs contain a significant Sca-1+/CD45-/c-kit- cell population. These findings support the similarity of murine ASCs and the muscle derived stem cells. This further suggests that ASCs may have a hematopoietic potential similar to what has been shown for the muscle-derived stem cells (Howell *et al.*, 2002; Royer *et al.*, 2002), and also represent adult- derived endothelial precursors.



III.5.iv.c ASCs represent adult-derived endothelial precursors (EDEP):

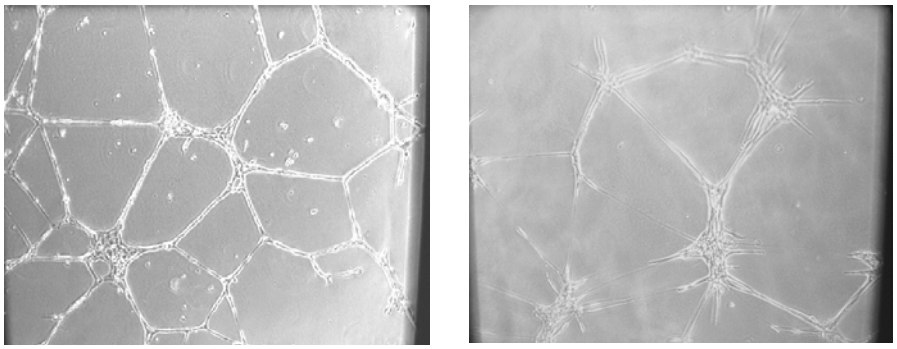


Fig 24: Human aortic endothelial cells (left) and human adipose stromal cells (right) were plated overnight on Matrigel.

A major pathway by which ASCs could enhance angiogenesis is by differentiation of ASCs into a vascular cell phenotype. Since ASCs are already known to differentiate into a number of cell types like muscle, bone and neural cells, we evaluated whether ASCs can develop phenotypes that correspond to either vascular endothelial cells or vascular smooth muscle cells. Adherent human ASCs plated on plastic were evaluated after several days by flow cytometry for the expression of the stem cell marker CD34 and the endothelial marker VE-Cadherin, (Figure 23). While the majority of cells are still CD34+/VE-Cadherin-, there has emerged a substantial proportion of cells that are CD34+/VE-Cadherin+; this finding has been replicated with samples from several donors. This is in contrast to freshly isolated ASCs, which express very low levels of VE-Cadherin prior to plating (Figure 20). The appearance of a CD34+/VE-Cadherin+ cell population suggests that differentiation towards an endothelial phenotype may have occurred. This experiment also demonstrates that the adherent ASC population continues to express high levels of the stem cell marker CD34 both in conjunction with the expression of VE-cadherin, as well as in a VE-cadherin-negative population. In parallel experiments (data not shown), it was found the human ASC population began to express an additional endothelial marker, the VEGF receptor-2, (KDR or flk-1) following passage, whereas they did not express this marker at detectable levels when freshly isolated; this finding supports the interpretation of direction towards an endothelial lineage.

To examine whether ASCs can also manifest typical endothelial behavior *in vitro*, their phenotype on Matrigel was determined. Mature endothelial cells when plated overnight on Matrigel (BD Biosciences) form tube- and cord-like structures, reminiscent of a capillary network. We plated human subcutaneous ASCs that had been cultured in EGM2MV media on Matrigel overnight and discarded non-adherent cells on the following day. Human aortic endothelial cells were also plated as a positive control in a separate Matrigel well (Figure 24). The endothelial cells formed the expected tube and cord-like structures. Interestingly, ASCs also formed similar structures, further supporting the description of ASCs as adult- derived endothelial precursor (ADEP) cells.

Together, these data suggest that ASCs can develop phenotypes of specific vascular cells, in a fashion that is directly responsive to their growth factor and matrix environments, suggesting a pliability of phenotype in these cells. These data also support the notion that ASCs might be able to contribute to angiogenesis or arteriogenesis directly by providing differentiated cells for incorporation into nascent vascular structures.

III.5.iv.d Systemic delivery of ADEPs accelerates reperfusion of ischemic skeletal muscle:

The hindlimbs of nude mice were unilaterally ligated and treated systemically with either control media or $5x10^5$ human ADEPs. Representative images of control-treated and ADEP treated hindlimbs are shown in Fig.25A and Fig.25B, suggesting marked improvement of perfusion in ADEP treated mice. Quantitative analysis performed by Laser Doppler imaging confirmed that freshly plated ADEPs (Fig. 25C) and cultured passage 1 ADEPs (Fig. 25D) were both able to markedly enhance the perfusion in the ischemic hindlimb, when compared to control-injected mice ($p<0.05$). The significant improvement was already observed on day 5 after treatment and was sustained until day 10, when the experiment was terminated due to the developing severe hindlimb necrosis in the control-treated animals. These experiments demonstrate the angiogenic potential of ADEPs in the context of a nascent adult vasculature. The exact nature of this effect (whether by direct engraftment into nascent vasculature or through providing enhanced growth factors at the site of injury, or by a combination of both mechanisms) requires further experimentation.

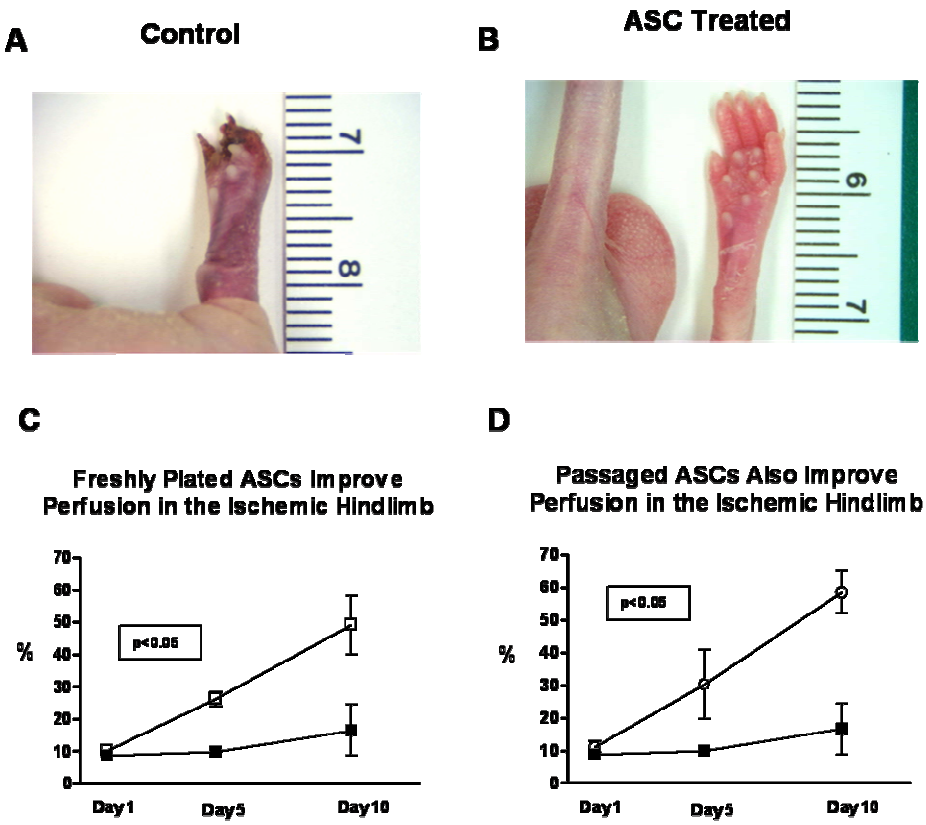


Fig 25. Treatment of hindlimb ischemia in nude mice with either control medium, $5X10^5$ ASCs plated for 1-2 days or $5x10^5$ cultured and passaged ASCs. Representative photos of control-treated (A) or ASC-treated (B) ischemic hindlimbs on day 5 are shown. Laser Doppler imaging, presented as the percentage of perfusion in the ischemic leg compared to the non-ischemic leg, at days 1, 5, and 10. Injection with either ASCs plated for 1-2 days (C) or long-term cultured ASCs was compared to medium-injection.

III.5.iv.e Transfection of ADEPs with green-fluorescent protein (GFP) and in vivo localization

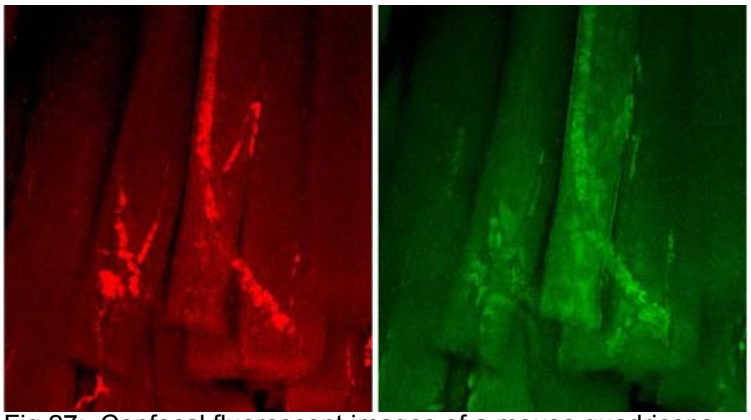


Fig 27: Confocal fluorescent images of a mouse quadriceps muscle bundle showing CD31 staining (red) and GFP expression (green).

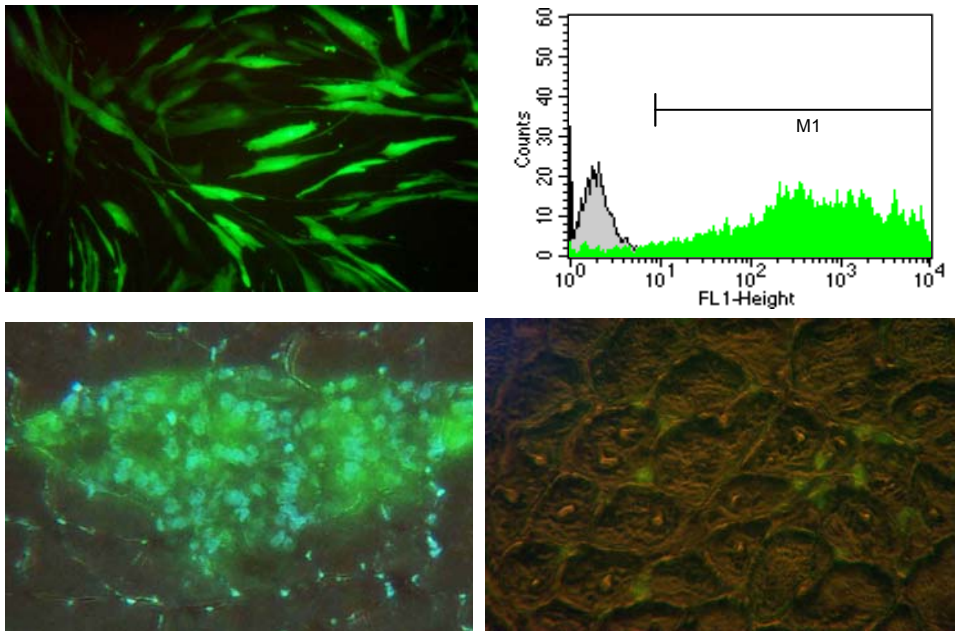


Fig 26. Transduction of human ASCs with the GFP-encoding lentivirus vector. (A) GFP-expressing cultured ASCs at 1 day following transduction (10x magnification). (B) Flow cytometric analysis of GFP-expressing ASCs confirms the high transduction efficiency seen in panel A. (C) GFP-expressing ASCs were injected into mouse tibialis anterior (TA) muscle and visualized microscopically (40x magnification) the next day after staining nuclei with DAPI (blue fluorescing nuclei). (D) Engrafted GFP-expressing ASCs in TA visualized at 1 wk following (40x magnification).

We examined whether ADEPs could be transduced and effectively labeled by lentiviral vectors as well as mammalian expression plasmids. The use of integrating lentiviral vectors will allow effective identification and ultimately clonal analysis (by integration site determination) of cells derived from ADEPs following their introduction in vivo; as well as provide a basis for the use of ADEPs as cellular “vectors” for gene therapy and supplement effects of the endogenously secreted growth factors. Human ADEPs were isolated and cultured as above. Confluent ADEP cultures at passages 0 through 4 were exposed to a lentiviral vector (VSV-G-pseudotyped HIV-1-based virus, provided by our collaborator Dr. Ken Cornetta) expressing green fluorescent protein (GFP) under control of the CMV promoter (Sastry *et al.*, 2002). GFP expression during *in vitro* expansion was assessed by flow cytometry on days 1, 3 and 7 post transfection. Transduction efficiency, assessed by manual counting of GFP+ cells and flow cytometric analysis, confirmed that at each passage,

greater than 90% of the cells were positive for GFP, a level which was maintained over the time in culture (Figure 26 A and B). One day after injection, cells had not spread outside the injection point (Figure 26C); however, after one week, ADEPs had dispersed among resident cells throughout into the muscle (Figure 26D). Approximately 23% of the injected cells were detected microscopically after one week. These data support the genetic marking of the ADEP populations with lentiviral vectors, and the tracking of GFP-labeled cells in vivo; as well as the feasibility of transduction of ADEP with genes encoding active factors in future experiments not specifically included in this proposal.

III.5.iv.f *In vivo* assessment of spatial deployment of GFP-labeled cells incorporated into nascent vasculature by confocal microscopy and three-dimensional reconstruction:

We have established the ability to evaluate the spatial features of GFP+ cells homing and incorporating into vasculature in the murine hindlimb ischemia model, by the use of confocal microscopy and reconstruction from images acquired at micron-scale increments of depth. As an example, a mouse which underwent marrow ablation with subsequent transplantation with bone marrow obtained from mice containing a transgene expressing GFP under control of the Tie2 (endothelial-selective) promoter (Motoike *et al.*, 2000), underwent ligation and excision of the femoral artery to produce the hindlimb ischemia model. Seven days after the creation of ischemia, the tissue was fixed and stained en bloc for the CD31 antigen with a red chromophore and for desmin with a infrared chromophore (not represented here). Figure 27 depicts paired images of the quadriceps femoris muscle detecting CD31 in red, while the green fluorescence represents GFP. These images provide evidence for the incorporation of bone marrow-derived cells with Tie-2 driven GFP expression that in many cases co-localizes with the CD31 stain, confirming their endothelial-like nature. Such incorporation of GFP was not evident in contralateral non-ischemic limbs. These data form the groundwork for imaging that will be employed to determine incorporation patterns of EDEP and ADEP cells into tissues in the proposed Aims.

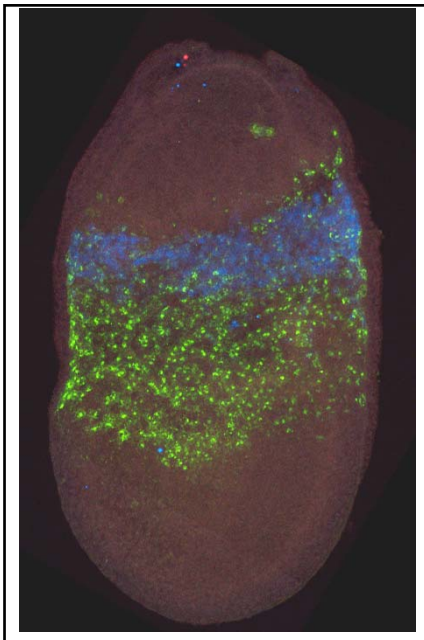


Fig. 28. Antibody staining of primitive EDEPs (Flk1+; green) and HSCs (CD41+; blue) in a mouse embryo (E7.5).

viii.) Isolation and expansion of yolk-sac derived hemangioblasts

We have recently reported on the derivation of methods (as described in Background) using surface markers to specifically identify the angioblasts and early endothelial cells as they emerge from the extraembryonic mesoderm. (Ferkowicz, Development,

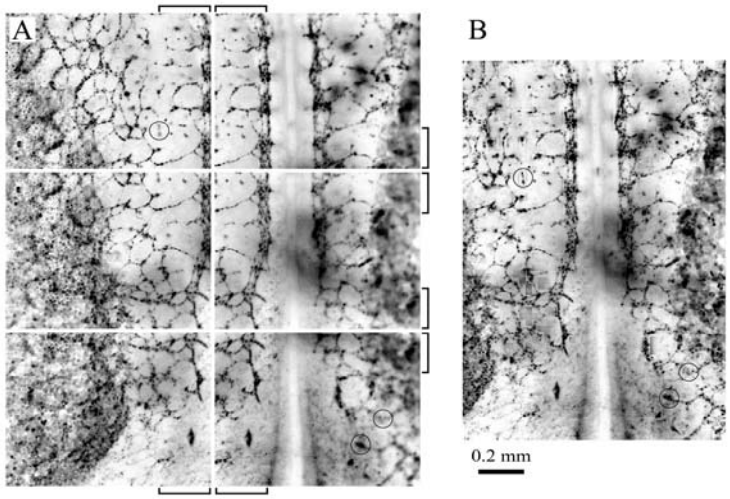


Fig. 29. High resolution visualization of vasculogenesis. (**A**) Montage of overlapping epifluorescent images taken at one time into a high resolution. (**B**) Final merged image once the individual fields have been normalized, merged and collapsed.

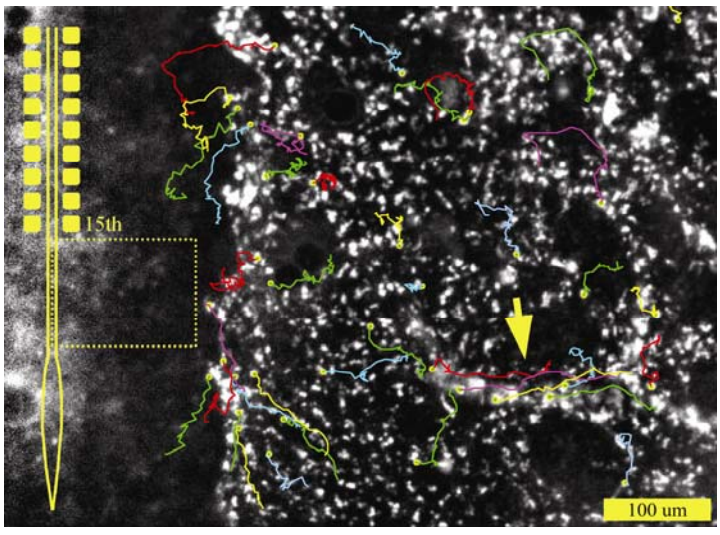


Fig. 30. Trajectory of endothelial cells labeled with fluorescently tagged-QH1 antibody during an 11 hr time period. Colored lines represent the path of cells and arrow indicates an individual cell migrating along an existing vessel. The area of the embryo analyzed is at the left.

2003). As noted in the appended article, the mesoderm cells are brachyury^{+}Flk1^{+} (not shown), while the endothelial precursors (EDEP) lose brachyury expression and only express Flk1 (green below). The Flk1+ endothelial precursors further differentiate into multipotent cells (Tie2+Flk1dimCD41-) that possess the potential to become both endothelial cells or hematopoietic cells, or just endothelial cells (Tie2+Flk1BrightCD41-). In data not shown, the Flk1+ cells isolated that co-express Tie2 but not CD41 represent the multipotent endothelial precursors as described in detail in the appended manuscript (Ferkowicz, Development 130:4393, 2003). We will focus on the *in vitro* and in vivo differentiation of the multipotent endothelial precursor cells (Tie2+Flk1dimCD41-) in the present experiments and examine the role of the microenvironment in promoting endothelial cell differentiation

Figure 11. Day 7.5 mouse embryo stained with monoclonal antibodies and imaged with confocal microscopy. The green cells are Flk1+ endothelial precursors and blue cells are the CD41+primitive hematopoietic progenitor cells. Not shown are the brachyury expressing mesoderm cells.

III.5.iv.g Dynamic imaging of avian vasculogenesis:

We have designed a system to study endothelial cell behavior at cellular resolution over an extended spatial and temporal field in the quail embryo. The embryo is cultured under temperature, humidity, and pH / nutrient conditions appropriate to maintain health and ongoing development over several days; and rested on a support that allows natural tissue deformation, permits development of the appropriate tension fields required for morphogenesis, and is compatible with high-resolution microscopy. Vasculogenesis occurs over most of the intraembryonic region, which is imaged at cellular resolution by novel image processing software interfaced with automated microscopes as developed by the Little laboratory. Typically, eight slightly overlapping fields of view (x,y plane) are recorded in multiple focal planes (z-axis) in both DIC and epifluorescence optical modes at intervals of several minutes over periods of 8-24 hours. The individual time-lapse images are processed into registered, focal-drift-corrected and positional-drift corrected montages in which adjacent (x,y,z) image planes are woven together (Figure 29) (Czirok, 2002); the coalescence of cellular components into vascular structures can thus be readily observed in a movie format.

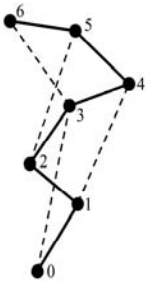


Fig. 30. Hypothetical schematic of an average displacement of an endothelial cell over a 6 hour time interval (solid line) and over 3 hours (dashed line).

III.5.iv.h Tracking of streaming and motion of individual endothelial cells:

Following labeling with a pulse of micro-injected fluorescent probes, we have demonstrated the feasibility of identification and tracking of individual cells through successive images. The motion of labeled cells is established relative to an anatomic frame of reference, such as the intersomitic clefts. The resulting trajectories clearly reveal the migratory behaviors of the tracked cells, and have shown the coordinated streaming behavior of endothelial cells following their labeling by a fluorescent endothelial-specific marking antibody, QH1 (Figure 30). This motility analysis has clearly confirmed contribution of a large number of endogenous endothelial progenitor cells to the formation of the vasculature, particularly the dorsal aorta and the omphalomesenteric vessels.

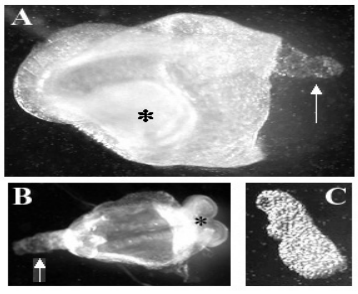


Fig. 31. E8.0 mouse embryo and allantois. (A) E8.0 mouse embryo after removal of Reichert's membrane and the ectoplacental cone (B) The same embryo as shown in A. The amnion has been cut away and the embryo has been flattened. (C) An allantoic explant after removal from the posterior end of the embryo.

III.5.iv.i Quantification of endothelial cell motility data

Statistical analyses of the endothelial cell trajectories acquired as above yields substantial quantitative information. For example, the average displacements of endothelial cells over a sequence of time intervals may be determine (Figure 30). For short time intervals, the measured velocity of cells determines the average displacement; while for longer intervals, the average displacement depends progressively more on the degree to which cells change direction (persistence of motion). Such information may be used in evaluating the effects of particular biological inputs; Figure 16(=7 in TCM) reveals the increase in endothelial cell motility produced by the

introduction of exogenous vascular endothelial growth factor by injection into quail embryos. These experiments also revealed quantifiable increases in endothelial cell protrusive activity, differentiation of greater numbers of precursor cells into identifiable endothelial phenotypes, proliferation, and fusion behavior (data not shown), with larger vessels formed, and vessels formed in regions that should have been avascular (Drake *et al.*, 1995; Drake *et al.*, 2000) .

III.5.iv.j Visualization of dynamic endothelial cell behavior in mouse allantoides:

Drake and Fleming (2000) and Downs *et al.* (2001) have shown that primary vasculogenesis in mice and avians occurs in virtually the same manner. Mouse allantoides are entirely mesodermal tissue, making them desirable for the study of vasculogenesis (Figure 32). Another advantage to examining allantoides is that, unlike in the avian model, there are no large-scale anatomical movements to account for in the digital imaging. Therefore, we have extended the above studies into the mouse embryo by examining the allantois, supported in culture and optically evaluated as described above for the quail embryo; this has allowed comparable investigation of vasculogenesis while taking advantage of the genetic and molecular approaches available in the mouse, not currently available in the avian system. The murine system also affords a greater range of reagents (*e.g.* inhibitors, activators, antibodies) that may be utilized. It has been shown that allantoic explants, when cultured in the presence of an antibody against CD34, a transmembrane cell surface glycoprotein that is expressed on vascular endothelial cells, display no perturbation of *in vitro* development (Drake, personal communication). Preliminary work in our laboratory has demonstrated that a directly conjugated CD34 antibody (CD34-Cy3) is detectable by our microscopy system and allows us to follow labeled endothelial cells over a 24 to 48 hour period without noticeable photobleaching effects or loss of the antibody signal from bleaching or normal protein turnover. Fluorescence exposure time is approximately 100 msec per acquired image. Figures 33 and 34 are representative images of the development of the allantoic explant over a 42-hour incubation period using DIC and fluorescence optics.

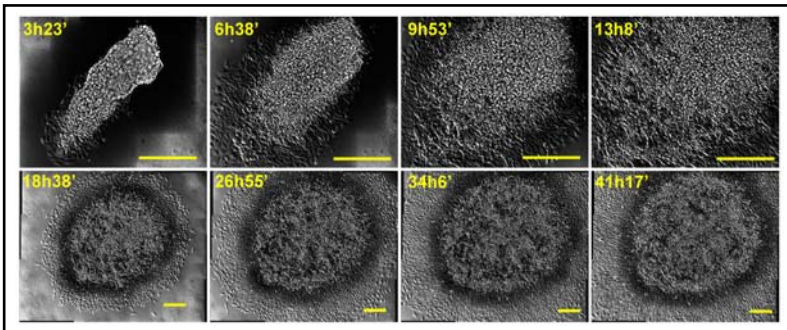


Figure 33. Development of a CD-1 allantois over a period of 42 hours using DIC optics.

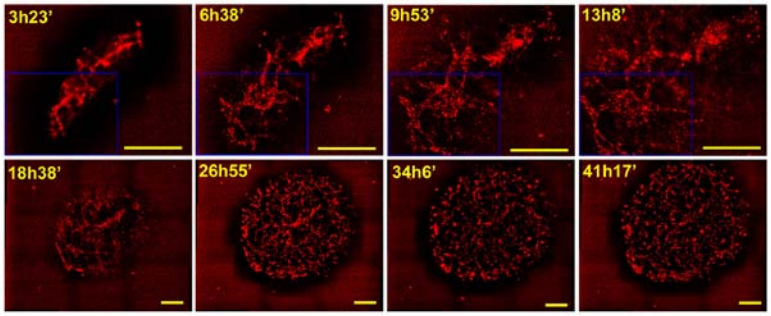


Figure 34. Development of a CD-1 allantois over a period of 42 hours using epifluorescence optics. This allantois is labeled with CD34-Cy3 and is the same allantois as shown in Figure 3.

Using this system, it has been shown (Dr. Chris Drake and Mr. Paul Fleming (2000), and we have confirmed, that exogenous VEGF added to allantois cultures leads to a hyperfusion of vessels, creating more and thicker blood vessels than seen in control allantoides. This is the same behavior that was exhibited in the avian embryo as above.

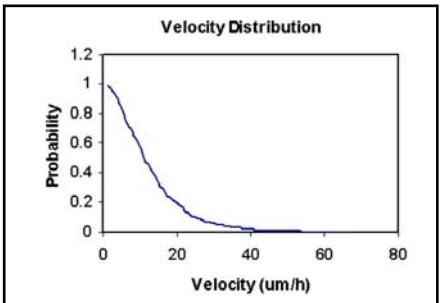


Fig. 35. Percentage of cells moving at shown velocities.

This indicates a conservation of VEGF activity across species, and supports the complementary use of the whole-quail and murine allantois systems.

Quantitation of dynamic computational imaging in the mouse allantois

The same techniques of computer-controlled microscopy and image processing algorithms described above {Czirok, 200; Rupp, in preparation) have been successfully applied to the mouse allantoides shown here, with the exception that a Bioptechs

chamber was used to generate the mouse allantoic data. A high-resolution digital CCD camera was used to gather the individual images, taking successive images approximately every 15 minutes. Figure 35 is an example of one type of data we can extract from the dynamic data sets.

In conclusion, these preliminary data support the key hypotheses of our proposal that well-characterized populations of endothelial progenitor cells can be evaluated for cell- as well as environment-specific behaviors when implanted into two complementary developing embryonic systems; that these behaviors will be based on the endogenous molecular programs of the cells and environment as well as modulable by exogenous factors; and that the detailed real-time recording of behavioral parameters for individual cells and cell populations will provide data for generating and refining quantitative models of key embryonic development processes and their dependence on selected molecular cues.

III.5.v EXPERIMENTAL DESIGN AND METHODS:

The overall experimental design will evaluate the behavior of defined populations of vascular progenitor cells isolated from adult ASC (ADEP) or embryonic yolk sac sources (EDEP), and implanted into embryonic systems undergoing development and patterning of a nascent vasculature. The experiments will define the cell-intrinsic as well as environmental parameters that determine the capability of each of these cell types to participate in this development, by both incorporation into as well as modulation of induction of the nascent vascular structures. The detailed quantitation of the dynamic, structural, and molecular behaviors and features of these cells in association with their participation in vascular development will provide a wealth of information for construction and refinement of both the tissue / cellular-scale and intracellular models relevant to the vascular system.

III.5.v.a Specific Aim 1. Definition of the migration, lineage fate, and incorporation of embryonic- or adult-derived endothelial precursor cells implanted into a developing heterologous embryo or allantois system as a function of their individual surface markers and degree of pre-specification:

Specific Aim 1 will examine for differences of behavior among two marker-defined populations each of EDEP or ADEP cells. **Aim 2** will investigate the effects of spatial, temporal, and genetic differences of the embryo context on the vascular patterning of implanted cells. The final **Aim 3** will extend the first two Aims by determining how EDEP or ADEP cell modulation by *in vitro* cues as well as cues from the embryonic context instruct the cells with respect to vasculogenesis. Together, these studies will help delineate the comparative behaviors of adult- vs. embryonically-derived progenitor populations with respect to the formation of nascent vasculature, and accordingly may provide information about the mechanisms by which vascular progenitors may influence the progression of vascular remodeling in the context of cardiovascular disease.

III.5.v.a.1 Rationale:

The degree of differentiation and related commitment of both adult adipose stromal-derived progenitor cells as well as yolk-sac-derived hemangioblast progenitor cells is expected to play a critical role in determining their capability of proceeding along specified differentiation pathways. Defined adipose stromal (adult) or yolk sac (embryonic) progenitor cell populations, characterized by their surface marker phenotypes and fluorescently labeled, will be evaluated with respect to their dynamic behavior monitored following injection into both the quail embryo and the mouse allantois, with particular attention to the characteristics of incorporation into the various components of the nascent vasculature. The movements and ultimate incorporation and differentiation of these cells will be studied as a function of their initial differentiation status as reflected by their marker profiles. Information from these analyses will then be used to direct choices of cell selection for delivery in further experiments designed to help in understanding the effects of the extracellular environment in governing the behavior of these cells.

III.5.v.a.2 Donor Cells and Recipient Systems Overview- Aim 1:

Recipient Systems / Donor Cells	Quail Embryo, ex ovo, injected into the splanchnic mesoderm	Mouse allantois, injected into splanchnic mesoderm
ADEP: Sca-1+/c-kit-/CD45-/Flk1dim Sca-1+/c-kit-/CD45-/Flk1bright	Studies: -dynamic properties; -fate of incorporation; -molecular profile of incorporated cells	SAME
EDEP: Tie2+/Flk1dim/CD41- Tie2+/Flk1bright/CD41-	SAME	SAME

III.5.v.a.3 Isolation and selection of donor mouse ADEP populations for injection into developing quail embryo *ex ovo* and developing mouse allantois:

In order to facilitate tracking and identification of the implanted donor cells as well as their derivative cells, without respect for their ultimate fate, we will universally utilize donor cells derived from mice which possess a ubiquitously-expressed endogenously fluorescent protein throughout their body, i.e., mice expressing EGFP protein under the control of the beta-actin promoter. Populations of these cells will be obtained from subcutaneous adipose tissue of mice ~4 months of age, as has been routine (Rehman, 2003; Rehman, in press). Typically the subcutaneous tissue from each animal yields ~250,000 adipose-derived stromal cells. These cells will then undergo analytical followed by sorting flow cytometry, to obtain purified populations with the marker characteristics above. The cells will be available for injection into the chimeric models, and comparative analysis with regard to dynamic behavior, fate, and gene expression.

III.5.v.a.4 Isolation and selection of donor mouse EDEP populations for injection into developing quail embryo *ex ovo* and developing mouse allantois:

Again, the experiments will employ fluorescently labeled donor cells, serived from mice expressing EGFP as above. Matings will be arranged, and pregnancies timed from the detection of vaginal plugs. Embryos will then be removed for cytometric preparation of the appropriate EDEP populations from the yolk sac (Ferkowicz, 2003) for subsequent injections.

III.5.v.a.5 Implantation of donor cells into developing quail embryo:

At embryonic stage 6, partially or fully characterized mouse donor stem cells isolated by flow-sorting according to the markers noted in the above table, from each of the populations of interest, will be drawn into a polished glass micropipet and an introduced into the splanchnic mesoderm space in the recipient quail embryo host. To minimize, damage the cells will be drawn and injected using the low ballast pressure of the Medical Instruments Picoinjector. This will insure that the cells are not subjected to high shear forces and will remain healthy. Approximately 100 cells will be introduced in a 5nl bolus of Hank's embryo buffer. In most cases the Hank's buffer will also contain 1ng/nl of fluorescently-conjugated QH1, the quail endothelial cell marker. We have also successfully used this method with good cell survival using dispersed mouse allantois cells (data not

shown). We have been injecting antibodies and experimental reagents into quail embryos undergoing vasculogenesis for over a decade. This procedure is very robust and well established.

Since the mouse cells will be genetically tagged with GFP, we will use QH1 IgG conjugated with a far red visual marker, most likely Alexa 647, to permit double-epifluorescence imaging together with the DIC mode. Thus, three optical states will be monitored in time lapse. The instrumentation for this approach is fully operational in Dr. Little's lab. Based on the success of static imaging studies by Ambler *et al.* (Ambler *et al.*, 2001; Ambler *et al.*, 2003) we expect to follow the vascular fate of the various cell types in the context of the vasculogenic stage avian. All of our computational tools for examining and quantifying vessel assembly and vascular pattern formation will accordingly be brought to bear. From a data acquisition standpoint this mouse-quail chimera system is no different from previous time-lapse studies in the Little lab (Czirok *et al.*, 2002; Rupp *et al.*, 2003; Rupp *et al.*, 2004).

III.5.v.a.6 XXMouse allantois culture and implantation of donor cells:

Mouse BL6.beta-actin-EGFP embryos of embryonic day (E) 7.5-8.5 will be microdissected free of the uterine muscle and decidual tissue and placed in cold embryonic phosphate buffered saline (ePBS). Reichert's membrane and the ectoplacental cone will be removed, the amnionic sac will be torn away and the embryo flattened. The allantois will then be extracted, washed in cold embryonic phosphate buffered saline (ePBS) and pipetted into fibronectin-coated Delta T culture dishes (Bioptechs, Butler, PA) containing Dulbecco's modified Eagles' medium (DMEM), 10% fetal bovine serum (FBS), 1% penicillin streptomycin, and 1% L-glutamine. Explants may readily be cultured at 37˚C in a 5% CO_2-containing atmosphere for 12-24 hours.

Allantoides will be initially cultured for 2 hours, after which 100 cells of each of the above populations, isolated by flow-sorting as noted in the above table, will be incubated briefly in a hanging drop culture to form a small aggregate, which will be physically positioned in contact with the recipient allantois. Alternatively we can also gently triturate the allantoic cells and mix them with the isolated embryonal or adult progenitor cells, reaggregate in a hanging drop and place into our custom-made time-lapse microscopy culture chamber (Rupp *et al.*, 2003). Then, DIC (differential interference contrast) time-lapse digital imaging will be performed throughout a 24 hour incubation period to observe the assembly of the vascular network, in conjunction with fluorescence time lapse digital imaging of the GFP signal, marking the donor cells, and a CD34 fluorescent conjugate, used to follow endothelial-specific movements

III.5.v.a.7 Computational analysis of stem cell dynamics and patterning in vivo and explant culture:

As presented above, we have (Czirok *et al.*, 2002) developed the technology and required software for acquiring dynamic data sets. Using computer-controlled microscopy and image processing algorithms, Czirok *et al.* (2002) were able to follow development of the quail embryo at very early stages (stages 6-9) when vasculogenesis is occurring {Czirok, 2002; Rupp, in preparation). These same techniques for image gathering and image assembly were used to generate the preliminary data using the mouse allantoides shown in Preliminary Data, with the exception that the Bioptechs chamber was used to generate the mouse allantoic data presented in this proposal. A high-resolution digital CCD camera was used to gather the individual images, taking successive images approximately every 15 minutes. Dr. Czirok and Erica Perryn are currently (February 2004) devising computational algorithms specifically for assessing the behavior of allantois-derived vascular precursor cells. Basically there are features of the culture systems that can be controlled-for in the computational realm. For example, the allantois cells rapidly spread in an attempt to "cover" their substratum, consequently the vascular precursor cells are "chasing" the mesothelial cells spreading across the substratum. Czirok and Perryn are devising algorithms that "subtract" or account for the culture-based "spreading behavior."

III.5.v.a.8 Examples of parameters to be collected and quantified in detail include the following:

-Velocity

-Trajectory

-Persistence time of motility; *i.e.*, extent of random vs. directed motile activity

-Cell shape and orientation (major and minor axial lengths and apparent vector of the major in the observed (x,y) plane

-Rates and amplitudes of axial length ratio variability (protrusiveness)

-Cell replication

-Velocity-field maps

-Deformation-rate plots (tissue deformations versus vascular pattern formation)

-Degree of positive or negative interactions with other cells

-Topological changes in pattern (degree of connectivity)

-Correlations between whole embryo or tissue motion and vascular cell motion (DIC optics+fluorescence optics)

-Computational algorithms yet to be defined

These computational analyses will be conducted on both the donor and host primordial vascular/stems cells using double fluorescence (see text above). Either transgenic-tags (GFP) or directly-conjugated CD34 Ab will be used to label the mouse donor cells, while the endogenous quail vascular precursor cells are tagged with the QH1 antibodies. All parameters will be obtained as functions of time and intra-embryonic position for each of at least 100 individual donor and 200 host cells per embryo preparation, provided that this number can be optically identified and tracked clearly throughout the time of the experiment. The parameters will be correlated with one another, as well as compared with the matched parameters from each of several (n=10) replicates for each condition tested, in order to establish intra- and inter-embryonic variability and to provide a statistical foundation for comparisons among the 4 groups of cell types planned for evaluation in each of the model embryonic systems.

III.5.v.a.9 Computational modeling of stem cell dynamics and patterning:

The parameters measured above will be used as an input to the Tissue Simulation Tool developed elsewhere in this project. Each of these parameters can be easily set in the computational model (see core 2). Recently it has also become possible to set the persistence of motion in the Cellular Potts model. The algorithms defined for the analysis of the patterns and dynamics in the *in vitro* experiments will be used to analyze and validate the results of the computational model. Once the computational model has been validated, the computational parameters will be varied in a systematic manner, thus constructing a so-called “parameter-space” in which the alternative behaviors of the system are mapped out. These alternative behaviors may suggest new experiments, or provide mechanistic explanations for previously observations in mutated or experimentally altered systems. Core 2 of this project will develop and analyze these models, and will also provide working tools to researchers of in core 3 who can use these tools to direct their experimental efforts to new behaviors of the system as predicted by the computational models.

III.5.v.a.10 Determination of fates of donor cells in chimeric embryo culture:

At 12, 24, and 48 hours after implantation of the donor cells into the chimeric embryos, the embryos will be removed from culture and fixed using standard techniques (Little and Drake, 2000) which will permit unambiguous identification of the location and fate of the cells derived from the donors by morphological criteria supplemented by fluorescent immunostaining for appropriate vascular markers (*e.g.*, CD34, CD31, Tie-2, Flk-1, eNOS, and VE-cadherin for endothelial cells) when necessary.

III.5.v.a.11 Identification of molecular expression phenotype of donor cells incorporated into chimeric embryo as a function of the site of incorporation:

At 12, 24, and 48 hours following implantation, another parallel set of embryos will be removed from culture, and cells bearing the donor fluorescent mark will be retrieved from the embryos by a combination of laser-microdissection when necessary and enzymatic dissocation followed by fluorescent automated cell sorting to achieve as homogenous a population (or set of populations) of fluorescently-labeled cells as feasible. If the donor-derived cells have incorporated into multiple organs or have adopted widely variable characteristics by microscopic visualization, then the microdissection will be employed prior to the FACS isolation of cells from each region or of each apparent type. This approach will yield cells that will be molecularly phenotyped both in a targeted and comprehensive fashion, by 1.)multicolor flow cytometry to determine the co-expression patterns of several known endothelial markers (CD34, CD31, Tie-2, Flk-1, eNOS, and VE-cadherin, as above); and 2.) expression array evaluations to be conducted by the relevant core. The results of this analysis will be correlated with the input cell types, the dynamic behavior of the cells, and their time-dependent fates, as appropriate for the analysis intended to integrate data from the multiple scales ranging from the subcellular level to cell motility and pattern assembly.

III.5.v.a.12 Potential limitations and alternative approaches:

A potential pitfall is the possibility that the injected cells of murine origin might be restricted in their ability to respond to molecular cues in the quail embryo. This seems to be an issue of limited significance given the data (Ambler *et al.*, 2001; Ambler *et al.*, 2003) which demonstrated the ability of murine endothelial precursor cells to migrate appropriately and incorporate into developing quail vasculature, and indeed to incorporate into the vessels.

III.5.v.b Specific Aim 2.) Evaluation of the dependence of the migration and incorporation of embryonic- or adult-derived endothelial precursor cells implanted as above, upon *the cues received from the embryonic microenvironment*, as determined by the time and site of injection as well as the genomic information resident in the host environment:

III.5.v.b.1 Rationale:

The growth factor and matrix environment surrounding pluripotential cells such as ADEPs or EDEPs play a critical role in instructing the cells to proceed along specified differentiation pathways. The dynamic behavior and ultimate loci and extent of incorporation of defined labeled adipose stromal (adult) or yolk sac (embryonic) progenitor cell populations will be evaluated following injection into both the quail embryo and the mouse allantois, as a function of the state of development of the recipient embryo or allantois, the site of injection in the quail embryo, and the growth factor status of the allantois. The quail embryo system provides an ideal setting for the assessment of spatiotemporal cues for vascular patterning, given the availability of the entire formative vascular tree to the “reporter” cells; while the mouse allantois system, while limited in anatomic extent, provides an excellent setting to evaluate mechanisms even for genetic alterations lethal to the development of entire embryos. Experiments will be performed to evaluate the particular hypothesis that the genetic partial deletion or overexpression of VEGF in the allantois system will lead to alterations in the survival, mobility, differentiation, and integration of progenitor cells of both sources. The data obtained from these experiments will uncover key mechanisms governing the behavior of these progenitor cells, as well as providing a target for simulation by the cellular behavior models being developed and tested elsewhere in this proposal.

III.5.v.b.2 Donor Cells and Recipient Systems Overview- Aim 2:

Donor Cells \ Recipient Systems	Quail Embryo, ex ovo, injected into : - **either the splanchnic mesoderm or heart field** at: -**Timepoints:** 2-, 4-, and 6- somite embryos	Mouse allantoides with genotypes of either: -**VEGF overexpression; normal (+/+); or +/-**
ADEP: Sca-1+/c-kit-/CD45-/Flk1 phenotype as determined to exhibit best vascular pattern incorporation in Aim 1	Studies: -dynamic properties; -fate of incorporation; -molecular profile of incorporated cells	SAME
EDEP: Tie2+/ CD41-/ Flk1 phenotype as determined to exhibit best vascular pattern incorporation in Aim 1	SAME	SAME

III.5.v.b.3 Isolation and selection of donor mouse adult ADEP populations and extra-embryonic EDEP populations for injection into developing quail embryo ex ovo and developing mouse allantois:

As in Aim 1, tracking and identification of the implanted donor cells as well as their derivative cells will be facilitated by a ubiquitously-expressed endogenously fluorescent protein EGFP. This aim will employ only 2 cell types, one each from the ADEP and EDEP populations, which will be chosen as the type from each of these categories that exhibited the most robust vascular incorporation in the experiments of Aim 1. The cells will be obtained as described in Aim 1.

III.5.v.b.4 Implantation of donor cells into developing quail embryo at variable timepoints and locations:

At embryonic stages with 2, 4, or 6 somites respectively, 100 cells of each of the above populations, isolated in pure populations by flow-sorting according to the markers noted in the above table, will be implanted by micro-injection as described above, into either the splanchnic mesoderm, or into the region of the heart field. This study will evaluate the comparative behavior of the injected cells placed into these two distinct regions of active vasculogenesis and vascular patterning.

III.5.v.b.5 Implantation of donor cells into mouse allantois in culture:

Since the VEGF system is critical in its quantitative regulation, as is clear from the dose-dependence of VEGF alleles described in the background, we have chosen this molecule for testing using the allantois system. Probing this system is of particular interest, given the early lethality in hemizygous embryos, making impossible the evaluation of vasculogenesis in such whole mouse embryos. Allantoides obtained by microdissection as in Aim 1, at 8dpc, will be cultured for two hours and then exposed to aggregates of about 100 cells of each of the above populations, obtained from embryonic (EDEP) or adult (ADEP) mice, with a normal complement of VEGF alleles.

III.5.v.b.6 Experimental outcomes:

Dynamic behaviors of donor and recipient cells in vivo in chimeric embryo culture; fates of donor cells; and molecular expression phenotype of donor cells incorporated into chimeric embryo as a function of implantation site and genetic background of the recipient milieu

Parameters will be collected and evaluated in detail, including the physical data for both donor cells and their derivative cells, as well as host endothelial cells, exactly as in the experiments of Aim 1. The fates and molecular phenotype of the donor cells will likewise be determined in parallel with the approaches utilized in Aim 1.

III.5.v.b.7 Potential Problems:

While we recognize that the early lethality of the VEGF -/- embryos may render impractical studies using such allantois, we have worked with the VEGF-/+ embryos and expect based on our observations that we will indeed be able to isolate an appropriate amount of allantois tissue from this genotype. If however the mass of VEGF-/+ tissue should prove a restrictive factor, then we would anticipate the pooling of cell aggregates from more than one allantois as required. ADEPs may show markedly delayed or incomplete engraftment. The laboratory of Charlie Little is well-skilled in these techniques and we accordingly we do not anticipate difficulty in conducting these studies.

III.5.v.c Specific Aim 3. Characterization of the manner in which the migration and incorporation of embryonic- or adult-derived endothelial precursor cells introduced at a particular locus are programmed and modulated by exposure of the cells to *exogenously provided factors* or to instructions from the embryonic environment which signal differentiation along particular pathways:

III.5.v.c.1 Rationale:

Aim 1 addresses the dynamic behavior and incorporation of **various marker-defined populations of adult-derived endothelial precursors** (ADEPs) or embryonic-derived **endothelial precursors** (EDEPs) provided in the context of heterologous model systems undergoing vascular development and pattern formation; while Aim 2 addresses the dynamic behavior and incorporation of these cells as dependent on **spatial as well as genetic cues in the embryonic environment.** In Aim 3, we will evaluate the manner in which the properties of these progenitor cells are influenced by exogenous endothelial differentiation factors either prior to or concomitantly with implantation, and characterize key instructive signals to which these cells respond by endothelial differentiation and participation in vasculogenesis.

The extent to which particular growth factors individually or in combination are responsible for the differentiation and lineage commitment of EDEPs or ADEPs, determining their behavior upon introduction into the area of a developing vasculature, will be studied using two approaches, as follows:

-First, **in Aim 3a**, these defined progenitor cell populations will be exposed to factors promoting endothelial differentiation prior to implantation into the quail embryo and the mouse allantois systems; and the dynamic behavior of these cells both in vivo and within *in vitro* vasculogenesis assays will be compared with their behavior and fate in the absence of stimulation by specific factors.

-Second, **in Aim 3b**, complementary experiments will be conducted comparing the *in vitro* vasculogenesis behavior of EDEP and ADEP cells upon their initial isolation; following re-isolation from the chimeric embryonic

environments, having been instructively programmed for vasculogenesis in that context; and finally after stimulation by particular factors *in vitro* in an effort to artificially recreate key instructive inputs of the embryonic environment. The data obtained from these experiments will provide information concerning the inputs determining the specification and differentiation of these progenitor cells, as well as extending the data derived from Aims 1 and 2 so as to provide opportunities for further refinement of the cellular behavior models being developed and tested elsewhere in this program.

III.5.v.c.2 Donor Cells and Recipient Systems Overview- Aim 3:

Donor Cells \ Recipient System	Quail Embryo, ex ovo, injected into splanchic mesoderm	Mouse allantoides, prepared as disperse chimeras or as co-cultured aggregates	*In vitro*, Extracellular Matrix (ECM) assay for vascular tube formation
ADEP: Sca-1+/c-kit-/CD45- phenotype as determined to exhibit best vascular pattern in Aim 1, **+/- incubation** in VEGF alone or endothelial growth medium cocktail, **+/- hypoxia**	Studies **(3a)** : -dynamic properties;	**3a**, SAME	**3b**, ability to conduct vasculogenesis *in vitro*
EDEP: Tie2+/ CD41- phenotype as determined to exhibit best vascular pattern in Aim 1, **+/- incubation** in VEGF alone or endothelial growth medium cocktail **+/- hypoxia**	**3a**, SAME	**3a**, SAME	**3b**, ability to conduct vasculogenesis *in vitro*
ADEP or EDEP which have been **re-isolated following incubation** in either the quail or allantois models	----------	----------	**3b**, ability to conduct vasculogenesis *in vitro*

III.5.v.c.3 Aim 3a. To define growth and environmental factors (individually or in combination) that modulate the behavior of EDEPs or ADEPs in their participation in vasculogenesis *in vivo*:

III.5.v.c.3.i Modification of donor mouse ADEP and EDEP populations prior to injection into developing quail embryo *ex ovo* and developing mouse allantois:

As in Aim 1, tracking and identification of the implanted donor cells as well as their derivative cells will be facilitated by a ubiquitously-expressed endogenously fluorescent protein EGFP. This aim will employ only 2 cell types, one each from the ADEP and EDEP populations, which will be selected as in Aim 2, as the type from each of these categories which exhibited the most robust vascular incorporation in the experiments of Aim

1. The cells will be obtained as described in Aim 1. Rather than direct injection without prior culture as in Aims 1 and 2, they will then be treated in the following ways before injection:

- control media conditions (endothelial basal media, EBM, with minimal added growth factors) ; or
- addition of VEGF-A over a range of doses (0.1-20 nM), with culture for 48 hours; or
- addition of a pre-defined endothelial growth factor cocktail, as has been optimized for microvascular endothelial cell culture conditions (Endothelial Growth Media, EGM-2MV) (Rehman, 2003), with culture for 48 hours; or
- hypoxia to 1 or 5%, for 48 hours

Following exposure to these culture conditions, the extent to which the EDEP and ADEP cells (so, 2 cell types x 4 conditions) have been modulated by these environments will be characterized by 2 major types of experiments: Implantation into the vasculogenic embryos as in Aims 1 and 2 (this sub-Aim, 3a); and placement into *in vitro* extracellular matrices which are known to support vasculogenesis and capillary tube formation (sub-Aim 3b, detailed below) (Davis *et al.*, 2002). In the latter experiments, the behavior of these cells that have been treated in vitro will be compared with the same cell types that have been re-isolated after implantation and residence in the chimeric embryonic environment.

III.5.v.c.3.ii Implantation of donor cells into developing quail embryo and mouse allantois in culture:

At embryonic stage 6, 100 cells of each of the above populations, isolated in pure populations by flow-sorting according to the markers noted in the above table, will be implanted into the quail embryo as described in Aims 1 and 2, for subsequent time-lapse imaging using the same techniques. This approach will permit direct comparison of the behavior of the cell populations cultured under the four conditions outlined above, with the behavior of the freshly isolated cells that were the focus of the earlier studies,

Similarly, mouse allantoides will be obtained by microdissection at 8dpc and cultured for two hours, after which 100 cells of each of the above populations, isolated in pure populations by flow-sorting according to their markers defined in Aims 1 and 2, will be implanted as above into the cultured mouse allantois, derived from mice with a normal complement of VEGF alleles.

III.5.v.c.3.iii Dynamic behaviors of factor-exposed donor and recipient cells *in vivo* in chimeric embryo culture:

Physical parameters will be collected and evaluated in detail for both donor cells and their “derivative cells” which have undergone the treatments outlined above, as well as host endothelial cells, using methods paralleling those used in the experiments of Aim 1.

III.5.v.c.4 Aim 3b. To define the growth and environmental factors (individually or in combination) that program the EDEPs or ADEPs to differentiate and commit to lineage in the formation of capillary tubes in a three dimensional extracellular matrix (ECM). This *in vitro* model will enable the identification of the key factors required for differentiation and the associated subcellular signaling processes:

III.5.v.c.4.i Rationale:

In Aim 3b, we will evaluate the manner in which the properties of these progenitor cells are influenced by exogenous endothelial differentiation factors either prior to or concomitantly with implantation in an extracellular matrix model. The selection of specific stimuli (growth factors or stress factors, *e.g.*, hypoxia) will be enabled in part by the information reflected by the cellular phenotyping in Aim 1 and the results from Aim 3a. In turn, each of these principal factors will be correlated with the dynamic behavior and progenitor cell differentiation-incorporation in the heterologous model systems undergoing vascular development and pattern formation (Aims 1 and 2). As a new model system, the extracellular matrix (ECM) will be used in building a subcellular pathway modeling effort in core 2 that will provide a unique experimental and computational basis for linking with the tissue modeling approaches.

The requirements for growth factors and environmental stimuli will provide a basis for establishing the biochemical changes (and therefore mechanistic information) related to differentiation in the vascularization models. Also, the classification of cell types discussed in Aim 1 will enable a molecular basis for sorting the cells derived from within the newly formed tissue in the heterologous model systems. Aim 2 addresses the dynamic behavior and incorporation of these cells as dependent on genetic cues in the embryonic environment. Therefore, a link between the tissue level modeling efforts derived from the imaging information from Aims 1 and 2 will be made to the subcellular modeling effort in core 2 by defining and validating the biochemical changes related to cell specific signaling pathways, of which VEGF-mediated signaling is the exemplary case addressed by Aim 2. The primary surface receptors (markers) identified in Aims 1-3 will also allow for the hypothesis-based approach to assessing the presence of specific cellular signaling pathways. A protein profiling strategy will be used to characterize the status of signaling markers in the specific cell types using the *in vitro* extracellular matrix model for capillary tube formation (Davis *et al.*, 2002).

III.5.v.c.4.ii Phenotype of donor cells prior to and after "instruction" either *in vitro*, following incorporation into vasculogenic embryonic environment, or adjacent to vasculogenic tissue:

Three alternative approaches will be considered to define the growth and differentiation factors required for capillary tube formation in the 3-D extracellular matrix.

1) Pre-treatment of the endothelial precursors (ADEPs) or embryonic-derived endothelial precursors (EDEPs) with growth factors or environmental stress (alone or in combination) before introducing to a defined ECM. The selection of growth factors will be guided by the knowledge of the surface receptors in Aim 1 and the success of promoting different phenotypes in the *ex vivo* models (Aim 3a). In addition, the ECM can be varied as especially with respect to addition of factors like VEGF to modulate the balance between capillary tube formation and regression (Davis and Bayless, 2003).

2) Isolate differentiated cells from the allantois and/or embryonic models the differentiated and classified endothelial cells by flow cytometry and expose to the ECM. The experiment will certainly offer a platform for analyzing the fate of these cells in the ECM as a function of the developmental stage in the ex vivo model. In this case, the work in Aim 1 will define the complexity of cellular subtypes and offer a rationale for selection of the cells to be sorted and transferred to the ECM. As above, the variation of ECM substrates can be assessed based upon the resident spatial location of these endothelial cells in the ex vivo models.

3) Combine progenitor cells with tissue explant from the embryo or allantois in the ECM to observe capillary tube formation. This would be a novel approach to stimulate the proliferation and differentiation of the progenitor cells in the ECM. It would enable the experimental assessment of how the physical environment in combination with the biochemical factors that reside in the embryo or the allantois may promote or inhibit the capillary tube formation in the ECM.

The general work-flow for the analysis of the cells growing in culture will be performed to link the phenotypes with the *ex vivo* models. Imaging methods for cells and tissue in the ECM are well established in the Cytomics and Imaging Core at Purdue University (see Experimental Techniques). In addition, new biochemical information to link the biochemical characteristics of the cellular signaling processes to the structural/functional alterations in the scaffold environment. The increase in cell number in this ECM will enable the cells to be sorted by phenotypes (Aim 1) and further analyzed by cellular fractionation methods. Protein profiles including antibody pull-downs to assess surface receptor content and to analyze status of specific signaling pathways will be performed on systems with adequate cells to enable us to link the signal transduction events with the subsequent alterations in 3-D structure.

III.5.vi CAPILLARY FORMATION IN 3-D EXTRACELLULAR MATRIX (ECM):

One of the advantages of using the ECM 3-D model is the ability to study the formation of microvessels, an area that has attracted a significant amount of attention recently (Bayless & Davis, 2002; Davis *et al.,* 2000; Davis *et al.,* 2001; Davis *et al.,* 2002). It is known that the formation of tubes in a 3-D matrix is absolutely dependent upon the interaction between the EC and the matrix itself via integrins and a variety of signaling events although the molecular mechanisms of the processes are not known. This sub-Aim will focus on monitoring the cellular interactions and molecular expression of cell surface glycoproteins and adhesion molecules. Many of these molecules are available for mouse systems including the following adhesion molecules and selectins (CD106, CD11b, CD11a, CD11c, CD18, CD31, CD34, CD44, CD44v6, CD49b, CD49d, CD49f, cd54, CD62L, JAM-2, MadCam-1) or growth factors (VEGF, EGF, GM-CSF, FGF, G-CSF, IGF-1, NGF-2.5S) all from Serotec. Using a combination of confocal or multiphoton imaging (via the Cytomics & Imaging Core) we will be able to monitor the expression of these molecules on cultured cells, as their three-dimensional pattern is changing. Secondly we will focus on expression of a variety of growth factors that will assist us in indicating the signal transduction pathways during this vessel formation process. All of the imaging will be performed under CO_2 and temperature controlled conditions using time-lapse 3-D imaging, which is known as 4-D imaging. One of the unique advantages of the proposed system is the ability to break down the matrix at specific points and extract all of the cells from the cultures so that they can be re-sorted using flow cytometry for more detailed phenotypic analysis. Tube formation can me monitored in a manner similar to that proposed for monitoring the reorganization of the collagen matrix, except that the resolution and data sets for tube formation will be much smaller and will utilize standard image processing approaches available in Image Pro Plus.

4-D imaging will be analyzed using 4-D analysis programs via Image Pro Plus (Media Cybernetics) and size, volume and fluorescence expression will be monitored (Wu *et al.,* 2003a, 2003b). Expression profiles of surface receptors (see above) will be related to number and 3-D relationship to vessel formation. Tube length as well as the number of intersection points will be monitored in 3-D space. By classifying the structural with the functional expression, molecular expression pathways will become more apparent.

III.5.vi.a Computational analysis:

Based on the evidence (Background) that biological functions of extracellular matrix (ECM) are highly correlated to not only its composition but also its structure, we propose to use the modeling of the structure to study its function in the context of vasculogenesis. We have previously shown how it is possible to integrate confocal microscopy imaging and image-processing techniques to analyze the microstructural properties of ECM providing a significant data set for future modeling. We have already published a report describing a two- and three-dimensional fiber middle-line tracing algorithm that may be used to quantify collagen fibril organization. We utilized computer simulation and statistical analysis to validate the developed algorithm. These algorithms were applied to confocal images of collagen gels made with reconstituted bovine collagen type I, to demonstrate the computation of orientations of individual fibers (Wu *et al.,* 2003).

To quantify collagen fibre organization quickly and accurately, we have developed novel middle-line tracing software based on our original algorithm, which was specifically tuned towards CLSM-BSL images of fibrous collagen. The software was able to compute fibre orientation, diameter and length on the basis of 3-D confocal datasets. The extracted quantitative data allowed us to build 3-D surface-rendered models. By culturing cells in the 3-D matrix and monitoring in 4-D space (3-D with time) we will collect data sets that will demonstrate the remodeling by the EDEP and ADEP cells following their exposure to the various conditions outlined above.

III.5.vi.b Potential limitations:

We recognize that the number of cells available for ECM culture following recovery from the experimental protocols outlined above will not be large. However, the ECM preparations are designed so that useful information regarding matrix remodeling and the extent of tube formation will be obtained by imaging of the regions of matrix surrounding groups of small numbers of cells, and the volume of matrix employed can be reduced as necessary to permit productive interactions among the cells.

III.5.vii FUTURE DIRECTIONS:

If, as we expect, morphological data in the quail embryo suggests engraftment and differentiation of EDEP or ADEP progeny along not only vascular but also other lineages which are not vascular (*i.e.*, not primary foci of this proposal), we plan to test the potential of *individual donor clones* to give rise to multiple lineages in the various in vivo models. Donor populations will be transduced by GFP-expressing lentiviruses. Following isolation of donor progeny by tissue digestion and GFP-based sorting as described in Aim 1 above, cells of distinct differentiated phenotypes will be evaluated by LM-PCR and sequence analysis (Dr. Ken Cornetta will conduct these experiments with us) to "fingerprint" their sites of proviral integration. Identity of integration sites across various cell types is evidence of clonal pluripotentiality.

In another key future direction, having defined the properties of murine ADEPs and EDEPs to either participate or modulate developmental vasculogenesis, then we will plan future studies (beyond the scope of this proposal, but in close parallel) extending this work to characterize the effects of native and growth factor-transduced **adult human** ADEPs as well as **extraembryonic human umbilical-cord** derived EDEPs on angiogenesis in these models of vascular development.

III.6 LIMB REGENERATION:

III.6.i INTRODUCTION:

For two centuries, the regeneration of the amphibian limb has been not only part of the most awesome instances of regulation, but also a model for vertebrate limb development. When a salamander limb is amputated, the remaining cells are able to reconstruct a complete limb with all its differentiated cells ranged in the proper order. It is remarkable that not only has the limb regenerated, but the remaining cells maintain the information specifying their position and the position of the cells that have been removed. In other words, the new cells construct only the missing structures and no more.

Upon amputation, epidermal cells from the remaining stump migrate to cover the wound surface. This single layer of cells then proliferates to form the apical ectodermal cap. The cells beneath this cap undergo dramatic dedifferentiation: bone cells, cartilage cells, fibroblast, muocytes, and neural cell lose their differentiated characteristic and become detached from each other. The well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the regeneration bastema, and these cells will continue to proliferate and differentiate to form the new structures of the limb.

So we are faced with a situation wherein the adult cells of an organism can return to an "embryonic" condition and begin the formation of the limb anew. Just as in embryonic development, the bastema forms successively more distal structures. Thus, the blastema must contain some positional information that directs a blastema on a stump containing a humerus neither to make another humerus nor to start immediately producing digits. Not only the blastema regenerate those structures beginning at the appropriate proximal-distal level in the limb, but the polarity of the anterior-posterior and dorsal-ventral axes also correspond to those of the stump.

Regenerative medicine is an exciting emerging branch of the biomedical sciences in which cell and tissue based therapies are applied to the treatments of disease. It encompasses the fields of tissue engineering, biomaterials, stem cell applications, and the study of associated human diseases. Knowledge from scientific inquiry about morphogenesis and its translation into the study of regenerative medicine will help us prevent birth defects, control abnormal tissue growth, slow the deterioration and aging of tissues, facilitate the repair, regeneration and replacement of injured tissues, and eventually even allow us to produce replacement tissues and organs *in vitro*.

The results of our research and modeling toolkits to be developed will aid national centers in regenerative medicine focused on developing and delivering therapies that reestablish tissue and organ function impaired by disease, trauma or congenital abnormalities. We hope that our work will foster the generation of scientific knowledge in regenerative medicine and to share that knowledge with researchers, clinicians and the public through educational activities, training and publications.

We are aware that this experimental section is less mature than the advanced heart and vascular development sections. The challenge and design of this proposal, however, is to develop tools not only for experimental groups already in advanced stages of morphogenic research, but also for those less advanced groups. Through tool integration, we expect the regeneration group to benefit from cardiovascular observations and simulations, and further expect the models generated by the regeneration studies to be of great use to the center as a whole.

III.6.ii BACKGROUND AND PRELIMINARY DATA:

Mitotically-competent cells residing within tissues accomplish tissue regeneration in multicellular organisms. In some cases, such as liver regeneration, these cells mitose while remaining fully differentiated. The tissues of most multicellular organisms, however, regenerate from *undifferentiated stem cells*. In the majority of cases, these cells are *reserve* stem cells, arrested in an early state of differentiation as cells around them continue to differentiate during embryonic development. Reserve stem cells have long been recognized as the source of renewal and regeneration for vertebrate tissues such as blood, immune cells, epithelia, bone, muscle, and olfactory bulb and nerve. Alternatively, regeneration in some higher animals can occur by the production of stem cells via de-differentiation (Gardiner *et al.*, 2002; Walder *et al.*, 2003; Nye *et al.*, 2003a; Stocum, 2004). The amphibian limb is a good example of a complex structure that can regenerate by dedifferentiation, and is a system where the growth, tissue patterning, and morphogenesis can be studied experimentally and simulated computationally. Understanding the mechanisms of amphibian limb regeneration will provide a toolkit for pharmacological and molecular manipulation to achieve regeneration of mammalian tissues and organs.

III.6.ii.a Events of Limb Regeneration:

An amputated amphibian limb goes through a typical series of morphological and histological stages (Figures 36, 37). Basal epidermal cells migrating from the cut edges of the skin closed the wound. Undifferentiated cells, generated by proteolysis of tissue extracellular matrix (ECM) and loss of phenotypic specializations, accumulate under the wound epidermis to form a regeneration blastema, while at the same time the apical wound epidermis thickens to form the apical epidermal cap (AEC). The AEC is the homologue of the apical ectodermal ridge (AER) of the embryonic limb bud in anuran and amniote embryos. Both the AER and the AEC are essential for the outgrowth of the limb bud or blastema. Urodele limb buds do not have a morphologically recognizable AER, but the distal ectoderm functions like one in promoting the outgrowth of the limb bud mesenchyme. The outer layers of the AEC are protective, whereas its basal layers appear to be anatomically and functionally equivalent to the AER of amniote embryonic limb buds. The cut ends of the nerves innervating the limb begin sprouting into the tip of the amputated limb within a few days after amputation. The sensory fibers innervate the wound epidermis, while the motor fibers ramify throughout the blastema cells, where they will eventually re-innervate the new muscles.

Once a blastema has formed, regeneration seems to largely recapitulate the events of limb ontogeny (Figures 36, 37). The AEC and the nerves provide growth and trophic factors essential for blastema cell survival and proliferation. To be clearly a survival factor or mitogen, a molecule must meet several criteria: (1) the AEC or nerves must secrete it into the blastema, (2) removal of the tissue should result in loss of the molecule, (3) neutralization of the molecule reduces blastema cell proliferation, and (4) the molecule can substitute for the AER or the nerves. Table 1 summarizes how well a number of candidate molecules meet these criteria. So far, only the iron-binding protein, transferrrin, meets all the criteria. None of the others have been as thoroughly tested, though their ability to substitute for the AEC or nerve strongly suggests that they are essential for survival and proliferation of blastema cells.

The undifferentiated cells divide by mitosis, enlarging the blastema into a conical bud. With further growth of the blastema, its cells withdraw from the cell cycle and differentiate to restore the histogenetic and morphogenetic patterns of the amputated parts. As muscles differentiate, by regenerating motor nerve fibers, they re-innervate. Differentiation and morphogenesis take place in a proximal to distal and anterior to posterior sequence, except that in the proximodistal (PD) axis, the digits appear to begin differentiation prior to the carpals or tarsals. In the anteroposterior (AP) axis, the radius differentiates faster than the ulna, and the anterior-most two digits appear first, followed by the remaining two (hand) or three (foot) digits. This anterior to posterior sequence of differentiation is unique to urodeles; the regenerates and embryonic limb buds of anuran

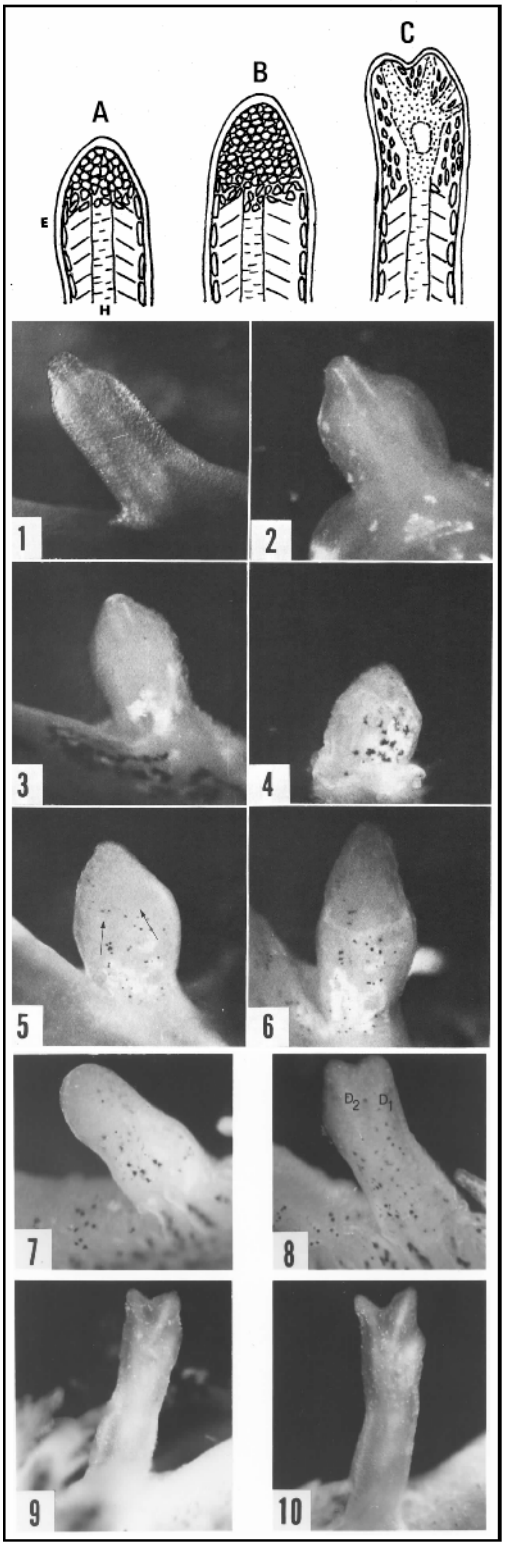


Figure 36. Forelimb regeneration in *Ambystoma maculatum*. **A.** Diagram of a 7-day upper arm regenerate at the medium bud stage. **B.** Diagram of an 8-day upper arm regenerate at the late bud stage. **C.** Diagram of a 10-day upper arm regenerate at the notch stage. **Panel 1.** External ventral view of a right upper arm regenerate, 2 days post amputation. Epidermal migration has resulted in the formation of a translucent cone of wound epidermis covering the protruding humerus. Mag. x114. **Panel 2.** Dorsal view of left upper arm regenerate, 4 days post amputation. **Panel 3.** Left upper arm regenerate, 5 days post amputation at the end of the stage of initial dedifferentiation. The humerus has a fuzzy outline due to its continuing dissolution and a small accumulation of blastema cells lies under the apical epidermis. **Panel 4.** Right upper arm regenerate, 6 days post amputation at the early bud stage. This stage has a characteristic small conical blastema at the limb tip. **Panel 5.** Left upper arm regenerate, 7 days post amputation at the medium bud stage. The medium bud blastema is still conical but longer than the earlier bud blastema. Arrows point to the junction of the wound epidermis and the old skin. **Panel 6.** Left upper arm regenerate, 8 days post amputation at the late bud stage. The blastema is still conical but flattened in the dorsal-ventral plane. **Panel 7.** Left upper arm regenerate, 9 days post amputation at the stage of early redifferentiation. **Panel 8.** Right upper arm regenerate, 10 days post amputation at the notch stage. D1, D2 = precartilage condensations of digits 1 and 2. **Panel 9.** Right upper arm regenerate, 12 days post amputation at the 2-fingerbud stage. **Panel 10.** Left upper arm regenerate, 14 days post amputation at the 3-fingerbud stage. Modified from Stocum (1979).

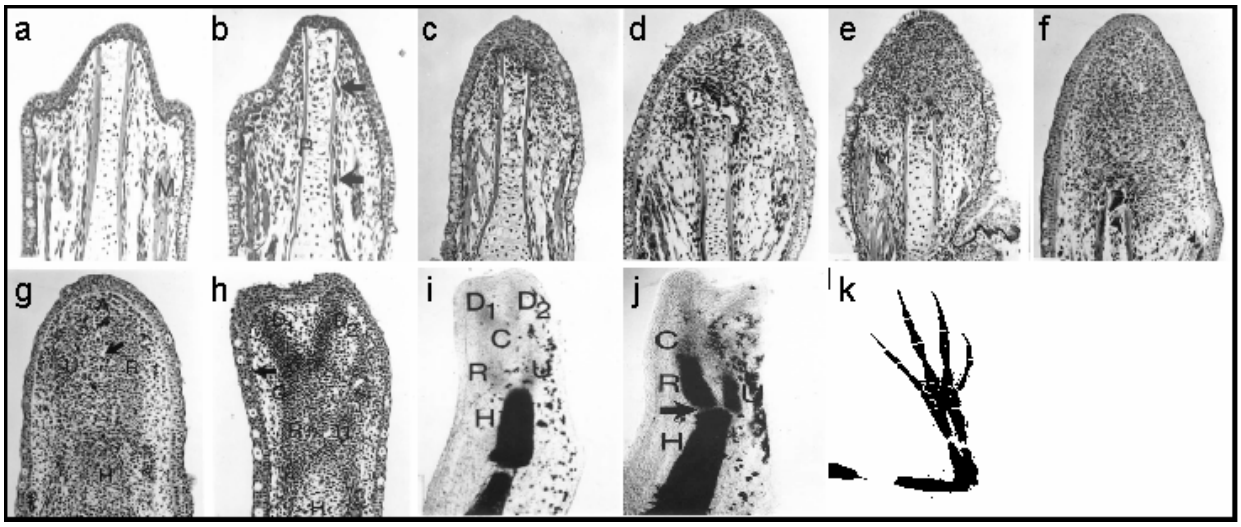


Figure 37. Stages of upper arm regenerates in *Ambystoma maculatum*. A-H, sections stained with Mayer's hematoxylin; I-K, whole mounts stained with Methylene Blue. Mag. 237.5. **a.** 2 days postamputation. The skin and muscle (M) have retracted for some distance proximal to the tip of the humerus. The basement membrane appears clearly as a dark line immediately beneath the epidermis. **b.** 4 days postamputation. Multinucleate osteoclasts (arrows) are present, eroding the periosteal bone cylinder (P). Cartilage matrix dissolves and chondrocytes dedifferentiate. Blastema cells derived from the muscle and connective tissue accumulate around the humerus distal to the cut muscle. **c.** 5 days postamputation. blastema cells accumulate under a thickened AEC. Dissolution of cartilage matrix has progressed to the level of the cut muscles, marking the end of the initial dedifferentiation stage. **d.** 6 days postamputation, early bud stage. The blastema enlarges by dedifferentiation and mitoses.. The AEC prominent. **e.** 7 days postamputation, medium bud stage, showing the large population of mitotically active blastema cells. The region of graded dedifferentiation from the tip of the humerus to the level of the cut muscle (M) will be a region of tissue repair, with new structures arising from the blastema distal to the humeral tip. **f.** 8-days postamputation, late bud stage. Osteoclasts are still present at the tip of the humerus. Overt signs of redifferentiation have not yet set in. **g.** 9 days postamputation, early redifferentiation. Chondrification has begun in the proximal portion of the regenerating humerus (H). The radius (R) and ulna (U) are faint condensations separated by a blood vessel (arrow). The apical mesenchyme (A) shows no overt signs of redifferentiation. **h.** 10-day notch stage regenerate. The precartilage condensations for the carpals (C) and digits 1 and 2 (D_1, D_2) are now prominent. Short condensations representing digits 3 and 4 are visible on the postaxial side of the hand. The radius (R) and ulna (U) are short and bowed. The humerus (H) is well chondrified. Proximal to distal and anterior to posterior (preaxial to postaxial) gradients of skeletal redifferentiation are evident. A thin basement membrane (arrow) has been formed everywhere in the regenerate except at its apex. The epidermis was thickened over digits 1 and 2 in this specimen, but not over the condensations of digits 3 and 4. **i.** 10-day notch stage regenerate. The proximal-distal gradient of skeletal redifferentiation is evident. H, humerus; R and U, radius and ulna; C, carpal mesenchyme; D_1 and D_2, digits 1 and 2. **j.** 12-day 2-fingerbud regenerate. The humerus (H) and radius-ulna (R, U) are well chondrified, and the carpal mesenchyme (C) is separating into three blocks. The elbow joint (arrow) is emerging. Proximal-distal and anterior-posterior gradients of chondrification are evident. X237.5. **k.** 25-day regenerate. X 114. Modified from Stocum, 1979.

Table 1: Partial restoration of regeneration in regeneration-deficient *Xenopus* limbs. Degree to which candidate molecules that the AEC or nerves express meet criteria for survival factor/mitogen status. Question mark indicates lack of test data. Fgf, fibroblast growth factor; Ggf, glial growth factor; Tf, transferrin; P, substance P (from Stocum 2004)

	Secreted into blastema	Removal reduces presence in blastema	Factor substitutes for tissue	Neurtalization abolishes effect
AEC				
Fgf1	**?**	**?**	**+**	**+**
Fgf2	**?**	**?**	**+**	**?**
Fgf8	**?**	**?**	**?**	**?**
Fgf10	**?**	**?**	**+***	**?**
NERVE				
Fgf2	**?**	**?**	**+**	**?**
Ggf2	**?**	**?**	**+**	**?**
P	**?**	**?**	**+**	**+**
Tf	+	+	+	+

amphibians and the limb buds of amniotes follow a posterior to anterior sequence. Differentiation along the dorsoventral (DV) axis appears to be simultaneous. The remainder of regeneration consists of growth to match the size of the unamputated limb.

III.6.ii.b Molecular Characterization of Blastema Cells:

In contrast to the reserve stem cells of many mammalian tissues, the dedifferentiated cells of the regeneration blastema have not been well-characterized in molecular terms. Aside from unusual presence of cytokeratins, molecules normally associated with epithelial cells, we know nothing of their regulatory pathways or surface antigenic features as associated with “stemness” in other animals. We are currently determining the molecular features of dedifferentiated cells of the blastema and comparing them to reserve stem cells such as satellite cells, hematopoietic stems cells and mesenchymal stem cells.

III.6.ii.c Do Reserve Stem Cells Contribute to the Blastema?

Another interesting question is whether or not reserve stem cells contribute to the regeneration blastema. Amphibian muscle and bone, as in other vertebrates, harbor satellite cells and mesenchymal stem cells used for repair of muscle injury and bone fracture, respectively, in a non-amputation context. A contribution of these cells to the blastema is possible but lacking direct evidence. Testing for a reserve stem cell contribution to the blastema is difficult, because it requires to selective labeling mesenchymal stem or satellite cells for tracking during limb regeneration. We might possibly use muscle explants to label satellite cells. ^{3}H-thymidine selectively labels the nuclei of satellite cells in newt muscle explants during the first six days of culture. We might pulse-label and track satellite cells selectively *in vivo* immediately after amputation to see if they enter the blastema, and if so, in what numbers. We might make salamanders with marked bone marrow cells by repopulating the marrow of irradiated animals with marked marrow cells (triploid, transgenic for LacZ or green fluorescent protein, opposite sex chromosomes, lipophilic tracker dyes), or by grafting marked hematopoietic regions of early embryos to unmarked hosts. We could then amputate the limbs of the marked animals and examine the blastema for the presence of marked cells.

III.6.ii.d Spatial Organization of Tissue Patterns in the Regenerating Limb:

Although individual blastema stem cells have histogenetic plasticity, the tissue organization of the blastema as a whole is self-determined from its inception. Grafting experiments on *Ambystoma* larvae show that the blastema self-organizes its tissue patterns, as opposed to being patterned by a set of inductive stimuli from the mature tissues adjacent to the blastema. Blastemas grafted to the dorsal fin, exchanged between forelimb and hindlimb, grafted to a different PD level, or rotated around the PD axis to disharmonize the AP and/or DV axes of blastema and adjacent tissues, always develop according to their origin with regard to limb type, PD level, and handedness. The fibroblasts and muscle of the limb carry the information for self-organization. The central importance of fibroblasts to patterning appears most clearly shown in experiments that grafted unirradiated skin to irradiated limbs in axolotls. The irradiated host muscle and cartilage tissues cannot contribute to the regeneration blastema that forms after amputation of these limbs. Nevertheless, the regenerate that forms, though lacking in muscle, has a normal skeletal pattern, indicating that the blastema cells derived from the dermal fibroblasts of the unirradiated skin were capable of organizing this pattern.

Retinoic acid (RA) can modify the positional information carried by fibroblast and muscle cells. RA simultaneously proximalizes, posteriorizes and ventralizes the position of individual blastema cells. Using RA in conjunction with *in vivo* assays has shown that the surface of the cell encodes the positional information carried by blastema cells (Crawford and Stocum, 1988a, b).

III.6.ii.e Transcription Factors and Signaling Molecules Involved in Specifying Positional Identity:

In amniote limb buds, distal, posterior, and dorsal signaling centers appear to provide the molecular signals that direct the outgrowth and three-dimensional patterning of the bud (Tickle, 2003, for review). The first of these is the AER, which controls growth and patterning along the PD axis. The second is the zone of polarizing activity (ZPA), located on the posterior margin of the limb bud that directs patterning in the AP axis. The third is the dorsal ectoderm, which directs patterning along the DV axis. These centers provide signals that activate transcription factors in cells that, in turn, specify their positional identity. We have only a rudimentary understanding of how tissue patterns form, but we know some of the signals and transcription factors involved and we know that the signaling centers interact in an interdependent way to generate the pattern. A current model for amniote limb buds is that FGF-8 induces the expression of FGF-10 in the prospective limb cells of the lateral plate mesoderm, which in turn induces FGF8 expression in the overlying ectoderm to establish the AER. FGF-8 from the AER maintains mesenchymal FGF-10 expression and induces SHH expression in the ZPA region. SHH induces FGF 4 expression in the posterior AER. A feedback loop between the ZPA and the posterior AER reciprocally maintains expression of SHH and of FGF-4. At this stage, the limb region has budded out and maintains proliferation of its mesechyme FGF-8 and FGF-4 from the AER. SHH serves mainly for AP specification of digit number and identity; how the more proximal elements of the AP pattern form is unknown. The Hox A and D genes also appear crucial to PD and AP patterning of the limb bud. Finally, the dorsal ectoderm expresses Wnt-7, which induces the transcription of *Lmx-1* in the dorsal mesoderm, which

specifies dorsality. The ventral ectoderm expresses En-1 and represses *Wnt-7* and thus *Lmx-1* expression, resulting in a ventral pattern. Further investigation must bridge the many gaps in this model, but it serve as a starting point to look at the specification of positional identity in the regenerating limb. Many of the same signaling molecules and transcription factors involved in patterning of the limb bud also express in the regenerating limb in similar (but not necessarily identical) temporal and spatial patterns.

Despite some differences in expression pattern, many of the same genes implicated in amniote limb development also express in the amphibian limb bud and regeneration blastema (Gardiner *et al.,* 2002). Two homeobox genes in newts associated with forelimb vs hind limb identity are *HoxC6*, expresses exclusively in forelimb blastemas, and *HoxC10*, expressed only in hindlimb blastemas. *Notophthalmus viridescens (Nv)Tbox 1*, a member of the T-box family of genes, expresses in the mesenchyme of adult newt forelimb regeneration blastemas exclusively, suggesting that it, too, helps specify limb identity. Homeobox genes that appear to play a role in patterning along the proximodistal axis of the regeneration blastema are *HoxD10* and *HoxA9* and *13*. The *HoxD/A* genes express in 3' to 5' order within each *Hox* cluster (lower numbers to higher numbers), in overlapping domains from proximal to distal in the mesenchyme of developing limb buds and regeneration blastemas. Thus *HoxD10* and *A9* expression associates with proximal structures and *HoxD13* and *A13* expression with distal structures. Expression of *NvTbox1* is two-fold higher in proximal than distal forelimb blastemas and retinoic acid increases expression of the gene two-fold in distal blastemas, suggesting that this gene helps specify PD positional identity as well.

Shh also expresses in early to medium bud stages of regenerating newt, axolotl, and *Xenopus* tadpole limbs, but not in amputated *Xenopus* hindlimbs as the tadpoles approach metamorphosis, coincident with the proximal to distal loss of regeneration. When transfected into the anterior cells of axolotl blastemas, *shh* evokes digit duplications and two patches of *Shh* expression form after reversal of the AP axis of the newt limb regeneration blastema with respect to the rest of the limb, which evokes supernumerary limb regeneration. The function of hedgehog proteins in patterning of the regeneration blastema is not yet clear, however. The relatively late expression of the *shh* gene (at medium bud) may mean that it is not necessary for AP patterning of most of the limb bud or regeneration blastema, but rather helps specify digit identity. Genetic analysis of patterning in the mouse limb bud indicates that the skeletal elements of the stylopodium and zeugopodium develop normally in the absence of *Shh.* Digits also develop in the absence of *Shh*, but they are iterative, so *shh* seems necessary for normal digit number and identity.

Investigations of gene expression associated with DV axial polarity during limb regeneration have been carried out in *Xenopus***.** The dorsal mesenchyme of *Xenopus* limb buds at stages 51-53 (regeneration-competent) and of regeneration blastemas formed after amputation through the zeugopodium at these stages expresses *Lmx-1*. Stage 55 blastemas, which formed only symmetrical cartilage spikes, do not express *Lmx-1*. We reversed the normal spatial relationship between the epidermis and mesodermal tissues by stripping the epidermis from the zeugopodial segment of the limb bud and reversing the DV axis of this segment with respect to the limb stump. Fresh epidermis of normal DV polarity grew over the reversed segment, which we then allowed to regenerate. When we performed this operation at stage 52, *Lmx-1* expression and structural pattern of the regenerate conformed to the epidermal polarity, but at stage 5, the original *Lmx-1* expression and structural polarity of the segment persisted in the regenerate. These results suggest that the effect of the wound epidermis on the expression of *Lmx-1* specifies the specific self-organization of DV polarity in the limb regeneration blastema up through stage 52 or 53; after that the polarity is fixed. The expression patterns of *Wnt-7a* and *En-1* in the regeneration blastema have not yet been reported.

III.6.iii EXPERIMENTAL PLATFORM 1: GENE EXPRESSION IN REGENERATING LIMB BUDS:

III.6.iii.a Background:

Mammals have latent regenerative capacity that is suppressed (Gage, 2000). In the limb, the gene *Msx1* is active only in undifferentiated mesenchyme of the early limb bud of all vertebrates and the limb regeneration blastema of amphibians (Robert *et al.*, 1991; Simon *et al.*, 1995). Forced expression of this gene in cultured mouse multinucleated muscle cells results in their cleavage and dedifferentiation into mononucleate myoblasts (Odelberg *et al.*, 2001). Furthermore, extract from regenerating newt limbs primes mouse myotubes to cleave, dedifferentiate, and proliferate (McGann *et al.*, 2001). These observations argue strongly for evolutionary conservation in mammals of a major part of the unique regenerative mechanisms deployed by amphibians and thus the generality of amphibian models to identify molecules that are up or down regulated to stimulate or inhibit regeneration.

Our research focuses on two amphibians: the frog, *Xenopus laevis*, and the axolotl, *Ambystoma mexicanum*. *Xenopus* is of particular value because many vital tissues regenerate strongly during tadpole stages, but lose this capacity as the animal undergoes metamorphosis. This difference allows a molecular comparison of the same tissues at regeneration-competent *vs*. incompetent stages. In addition, *Xenopus* has a large bioinformatics database. The axolotl regenerates throughout its lifetime and thus enables us to carry out similar molecular comparisons between mature, regeneration-incompetent *Xenopus* tissues and mature, regeneration-competent axolotl tissues. The limb is a complex structure of musculoskeletal tissue, skin, vascular, and peripheral nerves and is thus likely to be a rich source of molecules that promote regeneration.

III.6.iii.b Specific Aim 1. Examine the expression profiles of potential genes will in regeneration-competent and regeneration-deficient tissues by real-time quantitative PCR, gene arrays, and *in situ* hybridization:

We will consider genes expressed at two-fold levels above normal to be genes that have the potential to drive regeneration, and vice versa. We have elected to examine a substantial number of genes found by others to be developmentally regulated during embryogenesis of the tissues of interest (see Tasks). We have also identified and sequenced, by reciprocal subtraction of cDNA libraries, ~1700 clones of genes expressed at regeneration-competent or regeneration-incompetent early stages of hind limb tissues of Xenopus laevis (Nguyen *et al.*, 2001). Fifteen percent of these are redundant sequences. Of the remaining non-redundant sequences, nearly 80% are novel genes. We have gene-arrayed over 6000 clones from the cDNA library generated by subtraction of regeneration-incompetent cDNA from regeneration-competent cDNA and shown that 25% of these are upregulated by a factor of two or more, while the level of expression of other genes remains the same.

III.6.iii.c Specific Aim 2. Genes identified as regeneration-promoting or regeneration-inhibiting will be subjected to gain or loss of function assays:

We will carry out these assays by introducing sense or antisense genes driven by constitutive or tissue-specific promoters into cells *in vivo* or *in vitro*. Available methods for gene delivery include lipofection, electroporation, and the construction of transgenic animals. The CRBM already uses the first two approaches. We also have established transgenic mouse and amphibian technology

Lipofection of DNA carried in protein expression plasmids is a proven technique for transfecting genes into *Xenopus* cells. We will accomplish lipofection by injecting a lipid/DNA solution into cells (Brown, 1998). Alternatively, we can electroporate gene constructs into cells or tissues from regeneration-competent and incompetent stages maintained *in vitro*. A number of *Xenopus* cultured cell lines exist (Smith and Tata, 1991). We can obtain other cell lines and can easily culture tissue explants. We can then explant the transfected cells or tissue explants into regeneration-incompetent tissues, which we will injure or amputate to determine whether the transfected cells can dedifferentiate to become stem cells that transdifferentiate into other cell types.

Transgenesis allows the introduction of foreign genes regulated by tissue-specific or constitutive promoters into the genome of an egg. Constitutive promoters allow these genes to function later in tissues where they might normally be silenced. We will use the Amaya and Kroll (1996) method of restriction enzyme-mediated integration (REMI) to introduce regeneration-promoting genes under the control of a constitutive promoter into *Xenopus* sperm. In parallel, controls will receive reporter genes encoding GFP. We will then transplant the transfected sperm nuclei into the eggs. We will allow the transgenic eggs to develop to regeneration-incompetent stages, whereupon we will attempt regeneration of tissues. If the transfected gene(s) function to stimulate regeneration, regeneration competence should persist into normally regeneration-incompetent stages. Conversely, we can accomplish experiments designed to test loss of regenerative capacity by making animals transgenic for genes expressed at high levels at regeneration-incompetent stages. We would expect these constitutively active genes to inhibit regeneration at regeneration-competent stages.

III.6.iii.c.1 Metric Analysis:

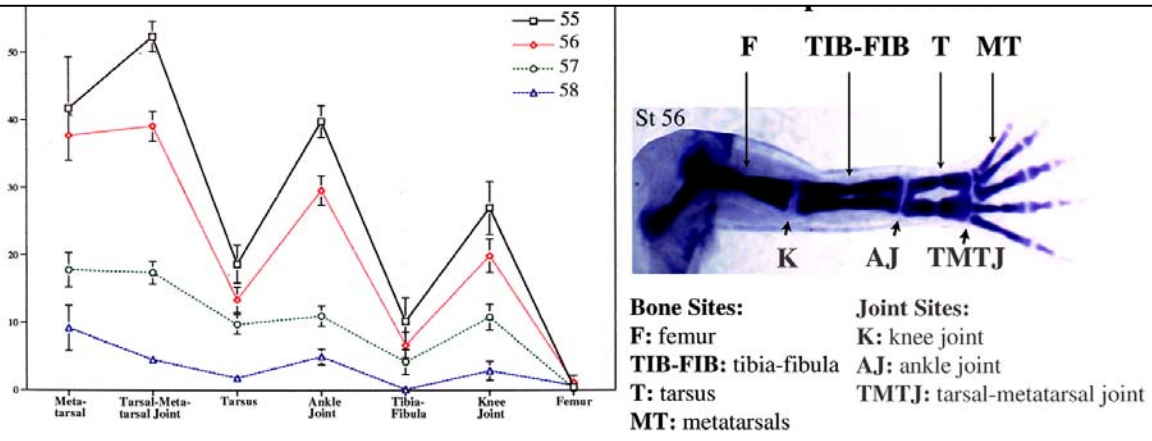
We will assemble quality of regeneration by examination of anatomical and histological structure. We have developed a quantitative scoring system for regeneration that is statistically rigorous, based on the anatomy and number of skeletal elements in the regenerated limb (Nye and Cameron, unpublished, Figure 39). We can evaluate limb function simply by comparing the movements of the regenerated limbs with those of limbs of control animals.

III.6.iv EXPERIMENTAL PLATFORM 2: AMPHIBIAN LIMB REGENERATION:

III.6.iv.a Background:

Intensive research on the development of the vertebrate limb skeleton, has identified many factors that affect its symmetries and polarities (reviewed in Ng *et al.*, 1999). However, we still do not understand the dynamics s by which the interactions of limb bud cells with various factors they produce result in a series of articulated, well-arranged rods and nodules of cartilage. Limb bud mesenchyme exhibits *self-organization*, so the cell surface-extracellular matrix (ECM) adhesive interactions that promote precartilage condensation *in vivo* also occur in a patterned fashion in isolated mesenchyme *in vitro*.

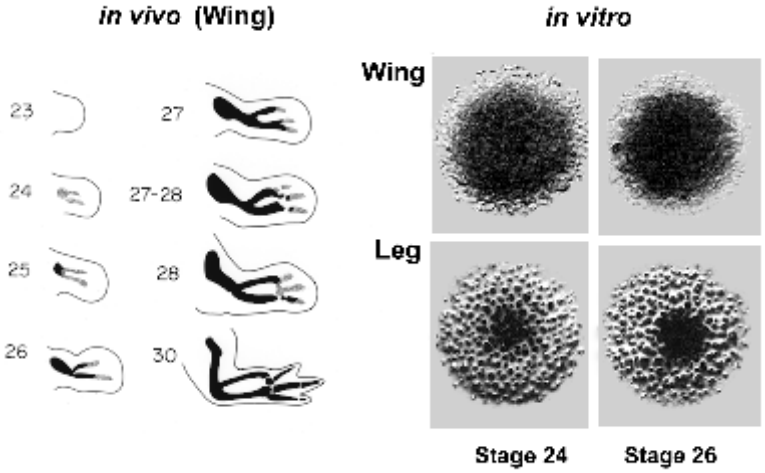
Figure 38. *Xenopus laevis* Regeneration Baseline. The graph on the left side of the figure displays the regeneration quality curves for seven amputation sites along the proximodistal axis of four increasingly older ages of tadpoles: stages 55 through 58. Shown on the right are the locations of those amputation sites on the right side of the figure; the skeletal element image is that of a Victoria Blue stained normally developing stage 56 hind limb. As the graph indicates, bony amputation sites' regeneration quality (the "valleys" in the curves) is significantly lower than that of cartilaginous amputation sites (the "peaks" in the curves) (Wolfe *et al.*, 2000; Nye and Cameron, unpubl.).



Precartilage mesenchymal cells first form tight aggregates or condensations (Hall and Miyake, 1992; 2000; Newman and Tomasek, 1996), then differentiate into cartilage, establishing the primordia for the bony skeleton. Chondrogenesis *in vivo* requires transient condensation (Hall and Miyake, 1992; 2000; Newman and Tomasek, 1996), and also *in vitro*, unless circumvented experimentally (Zanetti and Solursh, 1984). Precartilage condensation reflects changed cell-ECM and cell-cell interactions (Frenz *et al.*, 1989a, b; Mackie *et al.*, 1987; Oberlender and Tuan, 1994; Widelitz *et al.*, 1993), and leads to cytoskeletal-linked changes in cell shape (Zanetti and Solursh, 1984). Once condensations have formed, a cascade of signals occurs that ultimately results in the differentiation of the mesenchymal foci into cartilage (Smales and Biddulph, 1985; Gay and Kosher, 1985; Leonard and Newman, 1987; Zhang *et al.*, 1996).

Chicken precartilage mesenchyme in culture forms a continuous sheet or a set of isolated nodules of cartilage, depending upon whether it derives from the wing or leg bud (Downie and Newman, 1994; 1995) (Fig. 39). What determines where and when foci of condensation initiate?

Fig. 39. Left, Progress of chondrogenesis in the chick wing bud between days 4 and 7 of development. Solid black regions represent definitive cartilage; stippled areas represent precartilage condensation. Stages are those of Hamburger and Hamilton. Right, Morphology of representative Alcian blue-stained micromass cultures, prepared from mesenchyme isolated from the distal tips of stages 24 and 26 wing and leg buds and grown for six days in serum-free medium. Macroscopic images; culture diameters approximately 5 mm (Downie and Newman, 1994).



Earlier work suggested that the ECM protein fibronectin mediates condensation (Frenz *et al.*, 1989a, b; Downie and Newman, 1995; Gehris *et al.*, 1997). Recent work also strongly suggests that reaction-diffusion organizes limb skeletal patterning (Miura and Shiota, 2000a) in which the activator is one or more molecules of the TGF-ß family (Miura and Shiota, 2000b). These findings provide a basis for modeling limb skeletal pattern formation using the IST framework, in conjunction with information on the cohesivity and viscoelastic properties of limb bud precartilage mesenchyme.

III.6.iv.b Specific Aim 1. Measure and Model Changes in Regenerating Limb Shape:

III.6.iv.b.1 Rationale:

We must first put the measurable parameters of limb regeneration within the context of the overall shape changes of the limb regeneration blastema as it forms, grows and patterns into the different segments of the limb with their specific patterns of tissue differentiation.

The blastema forms as a solid conic structure with axes. We first need to know their relationships. The cone then flattens and undergoes more complex shape changes to produce the digits. These shape changes have never been measured or simulated.

We will do a time-lapse reconstruction of limb regeneration in axolotls using measurements of longitudinal and cross-sections prepared from different stages of limb regeneration. We can then use the measurements to validate our computer simulation. We will add increases in cell number due to generalized or localized cell division, loss of cells due to apoptosis and changes in cell density during differentiation to the simulation to produce a predictive algorithm. In future studies, we will use drug treatment and gene overexpression (via electroporation) to alter mitosis, apoptosis and cell density to test the predictive capacity of the simulation. We can then relate changes in the parameters outlined above during the different stages of regeneration to the simulation. We will then use the limbs described below to measure patterns of mitotic activity, apoptosis and cell density.

The baseline for simulation of limb regeneration will be the normal morphogenic changes during regeneration. Digital imaging will provide critical dimensions of the developing blastema and limb will show the morphological changes of the limb during regeneration.

III.6.iv.b.2 Experimental Procedure:

We will chill axolotl larvae with limb amputations to slow their movement and photograph them using a calibrated Nikon stereomicrocope with digital imaging. We will compare the regenerative changes with normal limb development in recent studies, extending the normal stages of axolotl limb development in a collaborative project involving the Cameron lab and the Stocum and Chernoff labs (Nye *et al.*, 2003b). Some details of the order in which limb structures differentiate vary between urodeles and other vertebrates, so we must compare regeneration to development in urodeles to determine features specific to regeneration (Nye *et al.*, 2003b)

We will develop a parametric description of the blastema as it grows as a conic structure that changes shape and a geometric model using data obtained from longitudinal and cross-sections of different stages of limb regeneration through time-lapse video using the tools such as MIMICS or ProE or Matlab software. The coordinates will be extracted from the software and will be used to relate parameters and geometric coordinates. We will then attempt to determine how growth and change in shape of the blastema depend on changes in the parameters. Once this model is established then a tissue model will be developed using the information obtained through a finite/boundary element analysis.

We will correlate the morphological changes with the appearance of cartilage condensations and differentiating muscle identified using histological analysis, molecular probes and marker proteins. We will use MyoD and formation of striated skeletal muscle to monitor myogenic commitment and differentiation. We will use chondroitin sulfate proteoglycan and cart1 to identify cartilage condensations and bone (Buckingham *et al.*, 2003; Stockdale *et al.*, 2000). In future studies these results will be correlated with expression of genes that suppress and stimulate differentiation of the limb core tissues.

III.6.iv.c Specific Aim 2. Measure Rates of Mitosis and Apoptosis as a Function of Location Within the Blastema and Stage of Development:

III.6.iv.c.1 Rationale:

Accumulation of cells through proliferation and reduction of cell numbers through apoptosis (programmed cell death) expand and control the shape of the regenerating limb.

III.6.iv.c.2 Experimental Procedure:

While visualizing patterns of mitotic and apoptotic activity in a living regenerating limb would be desirable, the technical difficulties are substantial. Measuring these parameters in sections of regenerating limbs fixed and processed at different stages of regeneration is more practical.

We will measure proliferation using anti-proliferating cell nuclear antigen (anti-PCNA, Sigma) (Zhang *et al.,* 2000) with a fluorescently labeled secondary antibody. We will label all nuclei with DAPI (a fluorescent DNA-intercalating dye). We will capture digital images using a calibrated microscope and measure dividing cells as the percentage of anti-PCNA and DAPI double labeled nuclei versus total DAPI labeled nuclei. We will count

all the labeled nuclei in every fourth cross-section from the tip of the blastema to its base. Using every fourth section prevents double counting of nuclei. To measure apoptosis at different stages of regeneration, we will use the TUNEL reaction. We will use DNA fragmentation as the marker of apoptosis, detected using the Roche TUNEL labeling kit.

III.6.iv.d Specific Aim 3: Measure the Pattern of Cell Density Change as the Blastema Grows and Changes Shape:

III.6.iv.d.1 Rationale:

As blastema cells develop into core tissues of the limb, they condense, changing the pattern of cell density. We can quantitate condensation and correlate it with the formation of muscles and skeletal elements.

III.6.iv.d.2 Experimental Procedure:

We will measure cell density per unit area in the same sections we use to determine mitotic activity. We will capture images with a calibrated microscope with digital imaging (Nikon) and measure cell profiles and area using Image Pro Plus software to express cell density as the number of nuclei/mm^2.

III.6.iv.e Specific Aims 4: Determine if Cell Motility Plays a Role in Blastema Formation and Morphogenesis

III.6.iv.e.1.i Rationale:

Cell motility may be important in both the formation of the blastema and in morphogenesis through the elongation and branching of emerging skeletal elements.

Dermal fibroblasts contribute nearly half of the cells of the limb regeneration blastema, with only two percent coming from cartilage and the rest from muscle (myogenic cells and connective tissue sheaths) (Muneoka *et al.,* 1986). Regeneration blastemas in small and large axolotls are about the same size. In a small axolotl the base of the blastema is equivalent in area to the cross-section of the limb stump. In a large animal, however, the blastema forms in the center of the cross-section and covers only a small area of it. What accounts for this restriction in size? Does the wound edge in a large animal contract toward the center so that the wound epidermis covers only a small area within which dedifferentiation occurs? Or, does diffusion restrict the size of the blastema so that dedifferentiated cells from the dermis of the skin migrate from the periphery toward the center to form the blastema?

As the blastema begins to redifferentiate, skeletal elements condense and elongate, including those of the digits at the tip of the blastema. Two major factors in elongation are continued cell division and the elaboration of extracellular matrix around chondrocytes. However, another factor neglected up to now with regard to skeletal elongation is the morphogenetic movement called convergent extension, in which cells in a fixed volume, intercalate with each other to retain the same volume, but elongate one axis at the expense of the others. Convergent extension is a major morphogenetic movement in gastrulation and neurulation (Keller, 2002; it may also play a role in the elongation and shaping of the regenerating limb.

III.6.iv.e.1.ii Experimental Procedures:

III.6.iv.e.1.ii.A Measurement of wound contraction:

We will amputate limbs of small (less than 100 mm length) and large (more than 200mm length) axolotls through the mid humerus. We will stain the edge of the cut skin with spots of Nile Blue sulphate around the circumference and follow the dye spots over the next several days. We will measure the area within the circumference of the skin to determine whether it stays the same (no contraction) or decreases (contraction).

In addition, we will examine the sprouting of blood vessels over the period of initial blastema formation in histological sections. What is the earliest time after amputation that blood vessels sprout? Must dedifferentiated cells be present for sprouting to occur and does sprouting occur only in areas of dedifferentiated cells capped by wound epidermis? Answers to these questions can lead to investigations of the factors involved in angiogenesis during limb regeneration.

III.6.iv.e.1.ii.B Detection of dermal fibroblast migration.:

We will label dermal fibroblasts in small and large axolotls with DiI and track the movements of the fibroblasts in sections over the period of initial blastema formation.

If initial diffusion constraints restrict the size of the blastema, we would expect to see that (1) angiogenesis does not begin until after an initial accumulation of dedifferentiated cells, (2) small axolotls will exhibit no dermal contraction, but large ones will, and (3) dermal fibroblasts will not migrate towards the center of the amputation surface in small axolotls, but will extensively in large axolotls.

III.6.iv.f Specific Aim 5 — Role of Fibronectin in Precartilage Condensations:

III.6.iv.f.1 Rationale:

In the developing vertebrate limb, discrete cellular condensations form before cartilage elements, to serve as primordia for the appendicular skeleton. The control of the size and spacing of these condensations is a key aspect of skeletal pattern formation.

III.6.iv.f.2 Experimental Procedure:

The 29 kDa N-terminal heparin-binding domain of fibronectin (FnNTD) is necessary for condensation in chicken limb mesenchyme (Frenz *et al.*, 1989a; 1989b; Downie and Newman, 1995 (Fig. 40).

Ectopic expression of chicken or human FnNTD or its mutated forms may enhance or inhibit the rate and extent of condensation in precartilage limb bud mesenchyme in high density micromass cultures (essentially two-dimensional disks), and whether the transfected tissue derives from fore or hind limbs. Endogenous fibronectin organizes differently *in vitro* and *in vivo* and plays different roles in mediating fore and hind limb cell condensations *in vitro* (Downie and Newman, 1995).

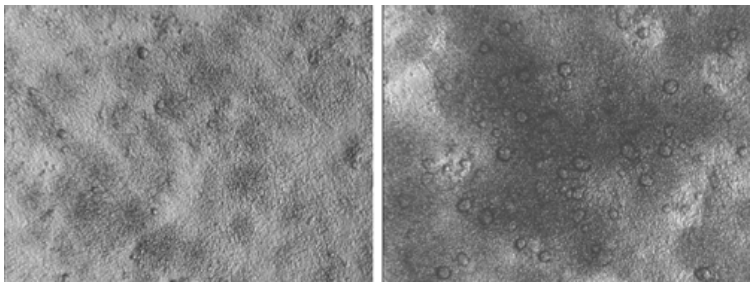


Fig. 40 Left, control leg cell culture, initial plating density 1.75×10^7 cells/ml, after 3 days. Right, leg cell culture, same plating density, transfected with capped mRNA encoding the initial 54 kDa of human fibronectin, preceded by natural secretion signal sequence. Control exhibits numerous discrete condensations (dark gray) separated by uncondensed cells; condensations in transfected culture are broad and confluent.

III.6.vEXPERIMENTAL PLATFORM 3: ZEBRAFISH FIN REGENERATION:

III.6.v.a Aim: Develop the Regenerating Zebrafish Fin as a Model Amenable to Simulation:

III.6.v.b Rationale:

Fish fin regeneration is homologous with limb bud regeneration, using basically the same set of mechanisms (termed epimorphic regeneration: wound healing, blastema formation, proliferation, morphogenetic gradient formation and differentiation) (Akimenko *et al.*, 2003). Indeed, where known, the molecules involved during specific steps of regeneration are homologous. Because the fish fin is a simpler structure with fewer cell types, the regeneration model simulation is significantly simpler to study, and thus more amenable to simulation. However, it is far less well characterized, and will require much preliminary work to establish it as a useful regeneration model.

III.6.v.c Background:

Fish fin regeneration has advantages as a model beyond simplicity. Zebrafish has emerged as an important model organism, mainly because of its use as a genetic model (Nusslein-Volhard, 1994; Haffter and Nusslein-Volhard, 1996). Consequently, some important molecules that regulate epimorphic regeneration in amphibians have been identified as the products of genes that were mutated and studied in zebrafish development (for example, retinoic acid biosynthetic enzymes). If genetic mutations are not available for genes that are of interest, antisense morpholino oligonucleotides can suppress the expression of any gene with known sequence (Ekker, 2000; Ekker and Larson, 2001). Furthermore, zebrafish grow rapidly, allows time lapse measurements of development, providing three-dimensional real-time imaging of epimorphic regeneration events. We can produce transgenic zebrafish that express GFP marker proteins under the control of gene promoter sequences relevant to regeneration. We can image these GFP marker proteins in time-lapse using two-photon microscopy and analyze them with Vaxx (http://www.nephrology.iupui.edu/Imaging/).

III.6.v.d Specific Aim 1: Map morphogenetic movements in fin regeneration:

We have previously labeled BrdU zebrafish to examine cell division during the growth of the fin regeneration blastema (Poleo *et al.*, 2001; Nechiporuk and Keating, 2002). These studies showed that all blastema cells are initially mitotically active. Subsequently, the proximal cells remain mitotically active, while distal cells show little or no mitotic activity. Using specific promoters that are regulated during mitosis (e g., cyclins), GFP reporters can serve in living zebrafish to examine mitotic activity using three-dimensional, time-lapse microscopy to provide a detailed four-dimensional map of cell divisions in the regenerating fin. We will use similar GFP reporter approaches to examine apoptosis, cell shape, cell migration and cell adhesion.

III.6.v.d.i Experiment Set 2:

Adhesion mediated by cadherins is an important regulator during embryogenesis. Marrs and colleagues have cloned several cadherin molecules in zebrafish (Liu *et al.*, 1999; Babb *et al.*, 2001; Liu *et al.*, 2001) (and unpublished results).

Cadherin-2 expresses specifically in cartilage structures in the developing zebrafish fin, and cadherin-2 is necessary for zebrafish fin development (Liu *et al.*, 2003) (see fig. 41). Many molecules required for fin development or limb development are also needed for regeneration. We propose to examine the role of cadherin-2 in zebrafish fin regeneration using zebrafish carrying mutations in the cadherin-2 gene (*parachute* and *glass onion*) (Lele *et al.*, 2002; Malicki *et al.*, 2003).

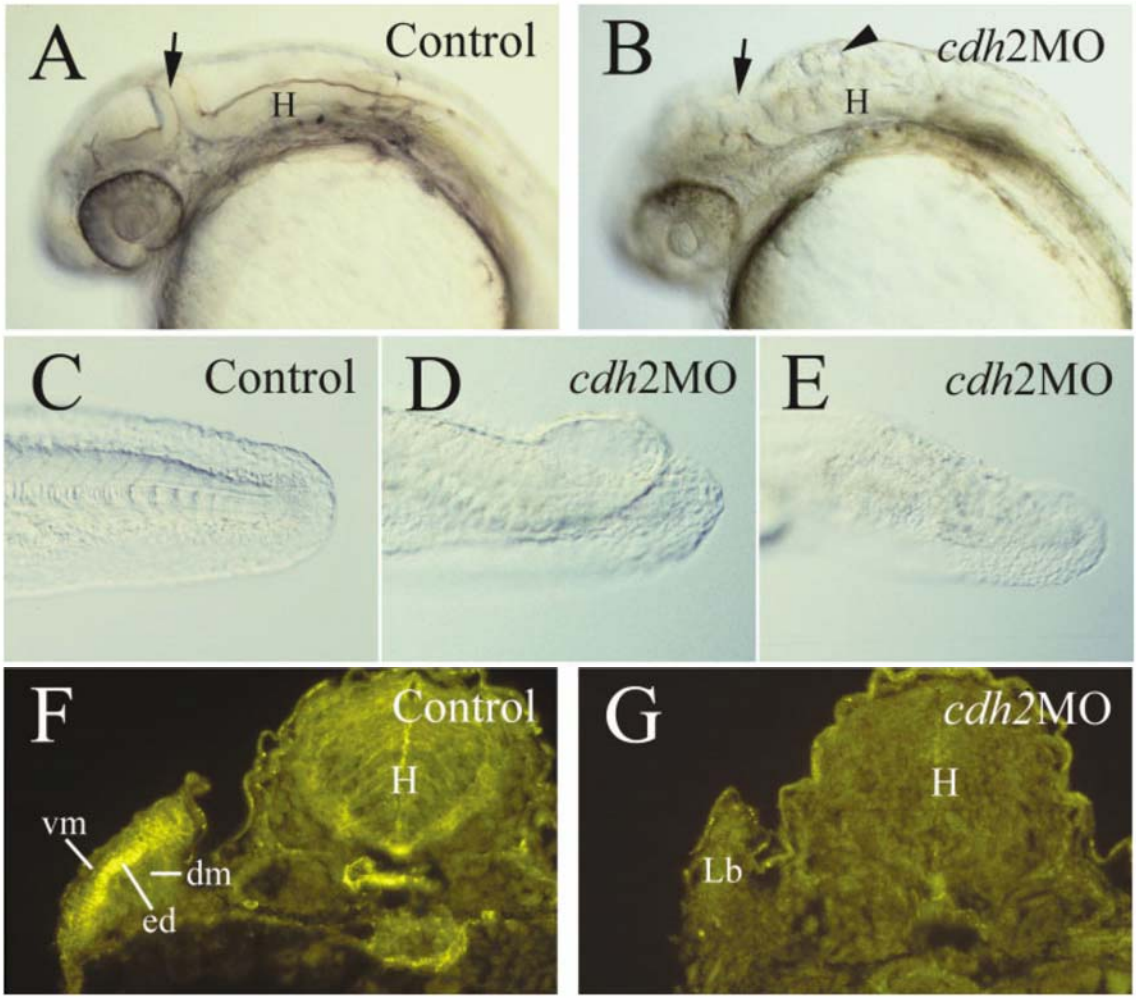


Figure 41. Embryos injected with *cdh2* morpholino antisense oligonucleotides (*cdh2*MO) phenocopy the *pac* and the *glass onion* mutants. A-E: Views from 24 hours postfertilization (hpf) embryos with anterior to the left and dorsal up. B illustrates defects in the midbrain and hindbrain (H). The arrows in A and B point to the midbrain-hindbrain boundary. The arrowhead in B points to loose cell aggregates in the fourth ventricle. Compared with the tip of the tail in control embryos (C), the *cdh2*MO embryos had split fins (D) or tail blisters (E). F,G: Cross-sections (dorsal is up) of Cdh2 immunostaining of control morpholino oligonucleotide (Control) -injected (F) and *cdh2*MO-injected embryos (G), both at 50 hpf. dm, dorsal musculature; ed, endoskeletal disc; Lb, limb bud; vm, ventral musculature.

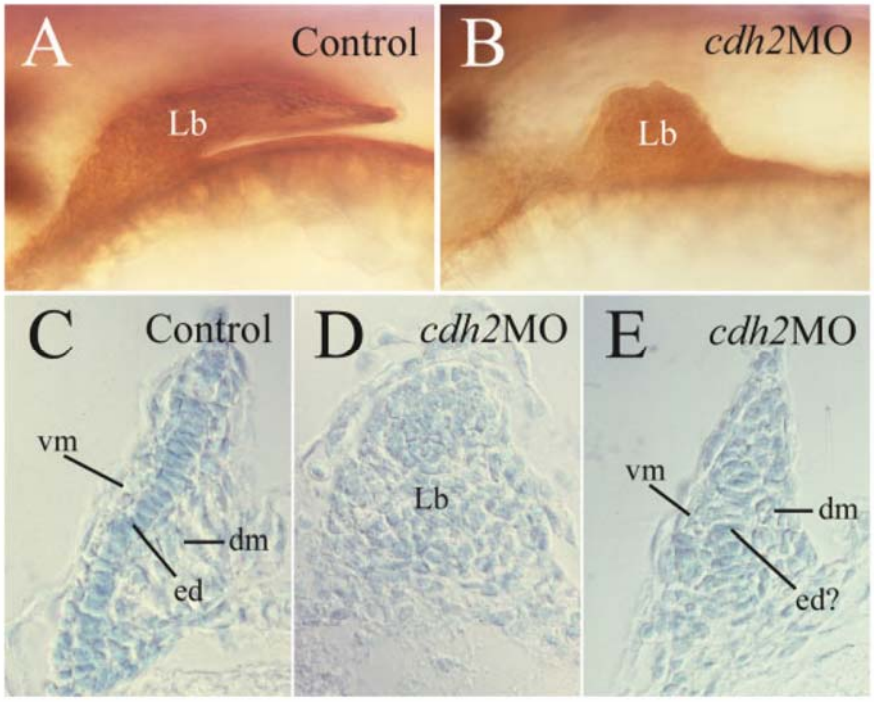


Figure 42 Effects of injection of *cdh2* morpholino antisense oligonucleotides (*cdh2*MO) on the development of the pectoral limb buds. A,B: Lateral views of the pectoral limb bud of whole-mount embryos stained with an anti-Hu antibody (Sigma). This antibody labels differentiating neurons. The staining in the limb buds, although nonspecific, makes their visualization easier. Dorsal is up and anterior is to the left. C-E: Cross-sections of limb buds (dorsal up) stained with Alcian blue showing anatomy of control (C) and *cdh2*MO-injected (D,E) embryos. ed?, possible endoskeleton; Lb, limb bud.

III.6.v.e Specific Aim 2: Tracking spatio-temporal expression of cadherin-2:

We propose to clone and characterize promoter sequences that drive cadherin-2 expression in the developing zebrafish embryo. Sanger Centre genome sequencing has already determined the zebrafish genomic sequences encoding the cadherin-2 (http://www.sanger.ac.uk/Projects/D_rerio/). In particular, we will examine promoter constructs to determine whether the developing fin recapitulates expression of cadherin-2. We will use these promoter constructs to drive GFP in transgenic zebrafish. Using this cadherin-2/GFP transgenic line, we can obtain four-dimensional imaging of n-cadherin expression during normal fin development and during fin regeneration. These findings will be extremely useful to simulate cell adhesion during regeneration, particularly in the forming dermal skeleton.

Together with our previous work, these important, modern tools applied to zebrafish fin regeneration may produce a superior experimental model for future simulation studies. We will develop these tools and obtain preliminary data during the initial years of the proposed studies.

III.6.vi COMPUTATIONAL MODELING:

Complete computer simulation of limb regeneration will require a great deal of data on the quantitative levels and spatial patterns of expression of the proteins involved in the growth and patterning of the regeneration blastema. Such data is not yet available in sufficient quantity to do computer simulation involving growth factor production and cell signaling in limb regeneration. However, while work on these aspects of regeneration is underway in our laboratories, we can address some basic quantifiable downstream phenomena and begin simulations: localization of mitotic cells, zones of apoptotic cells, and changes in cell density associated with remodeling and with differentiation. In future studies, we can correlate changes in these quantifiable factors with time and location of expression of genes associated with survival and proliferation of blastema cells, patterning of the limb and inhibition or potentiation of differentiation of limb core tissues. In addition, as we

outline below, we wish to develop the regenerating zebrafish fin as a valuable tool for obtaining data for use in computer simulation of growth, patterning and morphogenesis.

See Computational Methods.

III.6.vii TRANSLATION OF BASIC RESEARCH FINDINGS TO CLINICAL APPLICATIONS:

This proposal describes experiments that will provide the basic science data required to develop computer modeling techniques that predict limb development patterns and anomalies. In these and associated studies, we will continue to work with our other collaborators to pursue potential biomedical applications of molecular screens of regenerating amphibian limb and spinal cord. Similarly, the developing zebrafish fin will be used for studies of cell migration and appendage development. This proposal describes the basic science data collection required to model growth of these appendages.

III.6.viii TRAINING IN RESEARCH:

Training and teaching are integral parts of the research program in our laboratories. A significant proportion of all of the research in our laboratories is performed by graduate and undergraduate students. The P.I. also participates in the IUPUI Minority Research Scholars (MRS) program, with one MRS student currently involved in amphibian regeneration research. Research required to analyze the short-toe mutants is currently being performed by a post-doctoral fellow.

III.6.ix REGENERATION SUBPROJECT EXIT STRATEGY:

In the third year of the project we will apply for R01 or NSF funding for a project that includes continued application of computer modeling. The core of this project will be stem cell behavior in appendages. In the axolotl system we will examine the duplicative and toxic effects of retinoids on limbs and regeneration in a mutant strain that affects limb development. In the zebrafish system we will create transgenic animals with genes of interest in stem cell behavior. This work will build on studies currently underway in the Chernoff lab on stem cell behavior in axolotl spinal cord. We have cloned axolotl genes associated with stem cell behavior for the spinal cord project. The new project will be two-fold: a strong molecular biology component and measurements of the same parameters examined in the current proposal. This will allow us to determine if the limb duplicative and toxic effects of retinoids and the effects of genes associated with stem cells produce patterns of limb growth predictable by the computer model.

III.7 TECHNIQUES/RESOURCES:

III.7.i Gradient Cell Assays:

III.7.i.a Introduction:

One approach to studying complex bioanalytical problems is to develop microfabricated fluidic devices on which to conduct various chemical and biological assays (Auroux *et al.*, 2002; Reyes *et al.*, 2002). These devices are typically fabricated using micromachining techniques including photolithography, etching, thin film deposition, and cover plate bonding. Compared to state-of-the-art microelectronics, these devices are relatively simple in design and moderately easy to fabricate. However, when used for liquid phase analysis, these microfluidic devices have the potential to provide faster, more precise, and more sensitive strategies for solving complex problems. Experiments executed on microfluidic devices cannot only provide new information but also complement existing methodologies. Due to the small length scales, *e.g.*, 1-100 □m, fluids can be precisely manipulated in the nanoliter to femtoliter range by controlling the applied force, the geometry of the structure, and the properties of the materials. Until recently, chemists and biologists have not been able to manipulate molecules, fluids, and cells with the precision and accuracy these devices provide. Several simple yet elegant

examples have been demonstrated with these devices and have subsequently provided even greater opportunities for research using microfluidic systems.

While modern molecular biology has developed sophisticated biochemical techniques that span a number of areas, the quantitative control and measurement of biophysical properties, *e.g.*, cellular adhesion, chemotaxis, haptotaxis, are less satisfactory. The purpose of this portion of the project is to design, fabricate, and evaluate microfluidic systems capable of determining physical parameters, validating reaction kinetics, and providing parameters for Potts model simulations. The chemical gradients generated on the microfluidic devices will enable cell growth and death rates to be measured when cells are exposed to different concentrations of growth and inhibitory factors. Cell migration velocities, persistence times, polarities, and adhesion will also be assessed when the cells are subjected to various chemical gradients. For these assays, the chemical gradients can be varied both spatially and temporally.

III.7.i.b Specific Aims:

The specific aims of the gradient cell assays are:

1. Design, fabricate, and evaluate a static gradient cell chamber
2. Develop dynamic fluid control for the gradient cell chamber.
3. Conduct homogeneous, heterogeneous, and combination chemotaxis assays.

III.7.i.c Background:

Chemotaxis is oriented cell motion in response to an extracellular chemical concentration gradient that occurs during many biological processes including fertilization, development, inflammation, wound healing, and hematopoiesis (Devreotes and Zigmond, 1988). Several methods have been developed to assay chemotaxis including migration of cells under agarose layers (Cutler and Munoz, 1974), phagokinetic tract assays (Zetter, 1980), cell orientation assays (Zigmond, 1977), and Boyden chamber assays (Boyden, 1962). The most commonly used assays to measure chemotaxis are Zigmond or Boyden chamber assays, or modifications of these methods, *e.g.*, the Dunn chamber (Zicha *et al.*, 1991). The chemotactic response is analyzed by either measuring the distance traveled by the leading front of the cells (Zigmond) or by quantifying the number of cells on the lower surface of the filter (Boyden). Neither assay is quantitative, and both have serious limitations for investigating the mechanisms of chemotaxis or even for confirming the occurrence of chemotaxis (Zicha *et al.*, 1991). The concentration gradients are poorly defined and never reach a steady state, and temporal control of the gradients is not possible. In addition, these methods are laborious, subjective because counting is done by eye, and require a large number of cells. In order to overcome these deficiencies, precisely control the gradient, and track individual cells, microfluidic system are well suited for chemotaxis.

Over the past decade, a number of groups have been exploring both fundamental issues and applications of microfluidic devices for chemical and biochemical analysis, *i.e.*, lab-on-a-chip technologies (Auroux *et al.*, 2002; Reyes *et al.*, 2002). Interest in microfabricated instrumentation for chemical sensing and analysis has grown considerably primarily because these miniature instruments have the potential to provide information rapidly and reliably at low cost. Several groups including ours have taken the approach of developing functional elements including filters, valves, pumps, mixers, reactors, separators, cytometers, and detectors, and coupling these elements together to perform a complete assay under computer control. Examples of biochemical assays performed on microfluidic devices include immunoassays (Chiem and Harrison, 1998), restriction fragment analysis (Jacobson and Ramsey, 1996), PCR amplification and analysis (Waters *et al.*, 1998), a 96-capillary array for DNA separations (Shi *et al.,* 1999), and characterization of the *in vitro* propagation of viruses (Endler *et al.*, 2003). Microfluidic devices have been demonstrated for cell-based assays such as an electrokinetically driven microfabricated fluorescence activated cell sorter (FACS) (Fu *et al.*, 1999), a poly(dimethylsiloxane) (PDMS) flow cytometer using pressure-driven flow (Huh *et al.*, 2002), membrane potential measurements (Farinas *et al.*, 2001), delivering and removing reagents from cells with subcellular spatial selectivity (Takayama *et al.*, 2003), a cell culture system with isolation from the external environment by means of a permeable membrane (Inoue *et al.*, 2001), concentrating bacterial solutions using zone electrophoresis and isoelectric focusing in carrier-free solutions (Cabrera and Yager, 2001), and culturing mammalian cells in microchambers created by using patterned flows of etching solutions in PDMS microfluidic

devices (Takayama *et al.*, 2001). Among these many examples, the work most relevant to this project includes generating chemical gradients on a microfluidic device using a series of cross intersections (Jacobson *et al.*, 1999b) and a branched network of channels (Dertinger *et al.*, 2001). The latter channel design has been used to produce concentration gradients for neutrophil chemotaxis assays (Jeon *et al.*, 2001).

III.7.i.d Preliminary Studies:

Central to fluid manipulations on these microfluidic devices (microchips) is the dispensing and mixing of samples and reagents at the nanoliter to femtoliter scale. With electrokinetic and pressure driven material transport, dispensing and mixing are dictated by the applied potentials or pressures, the geometry of the channels, and the properties of the materials in those channels. Two basic valving elements that were developed at Oak Ridge National Laboratory (ORNL) are constant volume dispensing (Jacobson *et al.,* 1994a) and variable volume dispensing (Jacobson *et al.*, 1994b, 1999a). Also, we have designed and tested microfluidic structures to precisely proportion two or more fluids in different ratios by controlling both the applied electric potentials and channel geometry (Jacobson *et al.*, 1999b). Devices with tee intersections for mixing and cross intersections for dispensing that employ electrokinetic transport have been used for determining kinetics of enzymatic reactions (Hadd *et al.*, 1997) and inhibition of enzymatic activity (Hadd *et al.*, 1999).

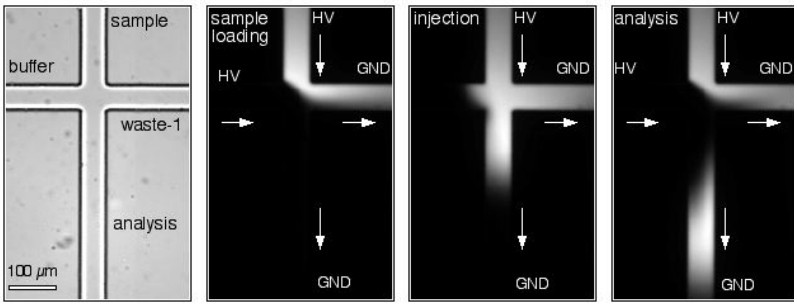


Figure 43. Image sequence for the gated injection valve.

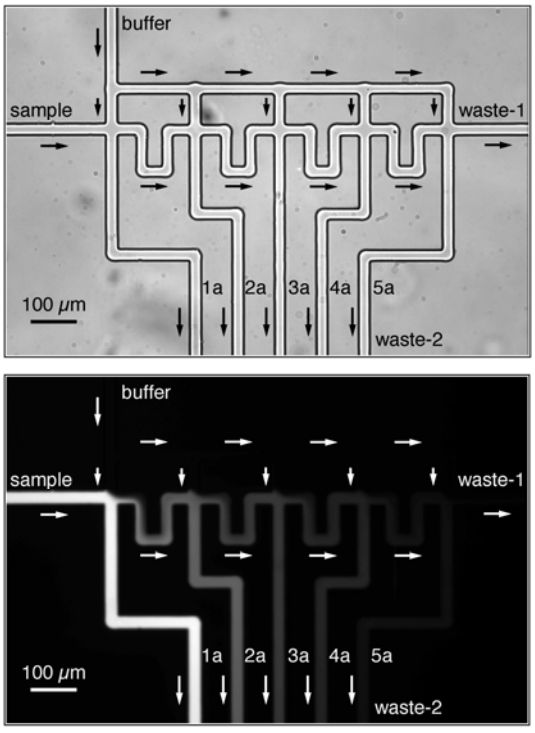


Figure 44. Images of a serial dilution microfluidic device (top) and serial dilution of sample with buffer (bottom).

In Figure 44 an electrokinetic version of the variable volume (gated) valve is shown. The sample (the bright regions) is transported through the injection cross toward the waste-1 reservoir. The potential applied to the buffer reservoir prevents sample transport into the analysis channel. To make an injection of the sample onto the analysis channel, the potential at the buffer reservoir is lowered or removed by opening a high voltage relay for a brief period of time (0.02 s or longer), and sample migrates into the analysis channel as in an electrokinetic injection. To break off the injection plug, the potential at the buffer reservoir is raised or reapplied. In this scenario, the electric field strength in the buffer channel is greater than the electric field strength in the analysis channel, and the electric field strength in the sample channel is less than the electric field strength in the waste-1 channel. The arrows indicate direction of flow for the sample and buffer streams. This valve has a precision of better than 0.5% relative standard deviation (rsd) for delivering 20 pL aliquots of sample. Also, this valve implementation can deliver operator selected volumes. The injection is biased by the relative electrophoretic mobilities of the sample ions as with conventional electrokinetic injections. The variable volume valve provides unidirectional fluid flow that can be advantageous for continuous flow conditions.

In addition, a microfluidic device for serial mixing of fluids has been demonstrated (Jacobson *et al.*, 1999b) at ORNL and is based on an array of cross intersections and sample shunting (see Figure 44). When the fluidic channels provide the appropriate voltage division, only a single fixed voltage or pressure source is required to transport and mix material. This minimizes the hardware necessary to operate the microfluidic device. In an effort to make the microchip architecture compact, multiple buffer, sample, or analysis channels terminate in single reservoirs. To test the serial mixing scheme, a sample was diluted with buffer in proportions dictated by the channel lengths, and sample fractions of 1.0, 0.36, 0.21, 0.12, and 0.06 were generated. The microchip in Figure 44 has five analysis channels (1a-5a), but the series of tee and cross intersections can continue for as many mixing intersections as needed. Figure 44 also shows a fluorescence image of the sample being mixed with buffer with 0.4 kV applied to the sample and buffer reservoirs with the waste-1 and -2 reservoirs grounded. The fluorescence signal decreases from channels 1a to 5a due to mixing of the sample with buffer.

At Indiana University, Hosek and Glazier have generated a chemical gradient on a microfluidic device made from poly(dimethylsiloxane) cast on an SU-8 mold (see Figure 45). The image was taken with a red dye transported through the flow chamber. The chamber is 2 mm wide and the channels entering at the top of the chamber are 200 □m wide. A subambient pressure was applied to the fluid reservoir at the bottom of the image. The serpentine channel pattern allows enough time for the dye and diluent to mix and a sufficient pressure drop to suppress flow rate through the chamber.

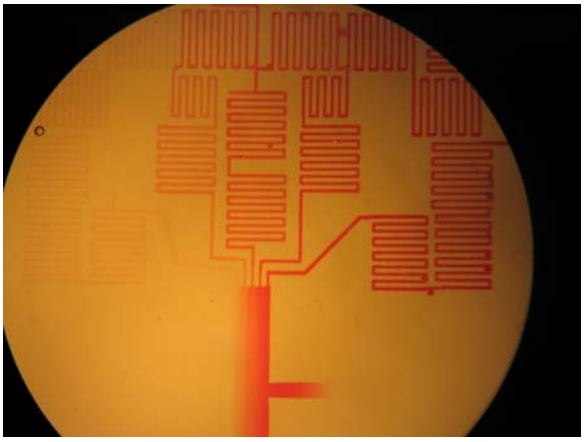


Figure 45. Image of gradient chamber made from SU-8

As seen in the experiments depicted in Figures 44 and 45, mixing occurs by diffusion alone. The Reynolds number, $Re = vl\rho/\mu$ where v is the velocity of the fluid (m/s), l is the cross-sectional dimension (m), ρ is the density of the fluid (for water, 1000 kg/m^3), and μ is the viscosity of the fluid (for water, 10^{-3} kg/(m·s)), describes the flow regime, laminar or turbulent. A Re < 2300 indicates laminar flow whereas a Re > 2300 is indicative of turbulence. In most microfluidic systems, the Reynolds number is typically < 1, and as a consequence, two or more steams will mix diffusively. Diffusion can be modeled in one dimension by the equation $\langle d^2 \rangle = 2Dt$ where d is the distance a particle moves in time t, and D is the diffusion coefficient. For a diffusion coefficient in the range of 10^{-6}–10^{-7} cm^2/s, mixing in a 10 μm wide channel takes about 0.5-5 s. Consequently, the channels need to be an appropriate length to allow sufficient time for components to mix.

III.7.i.e Research Design and Methods:

For the fabrication of the gradient cell chambers, two methods will be evaluated, and the assay performance on each type of device will be compared. Both fabrication techniques are in-house at IUB. The first method to be tested will use poly(dimethylsiloxane) (PDMS) as a substrate. The advantage of PDMS is the low cost of fabrication. However, tall features with small lateral dimensions, *e.g.*, < 10 □m, are not always easy to produce using this polymer. The fabrication technique will involve producing a negative mold out of SU-8 photoresist and casting the microfluidic structures in PDMS (McDonald *et al.*, 2001). PDMS has the advantages that the polymer is non-toxic, optically transparent down to 300 nm, and gas permeable. The gas permeability makes PDMS an attractive substrate for systems involving mammalian cell culture. PDMS is very hydrophobic (advancing contact angle of water, θ_a^{H20}~110°), but its surface can be converted for a period of time to a hydrophilic form (θ_a^{H20}~10°) by brief treatment with an oxidizing plasma (Chaudhury and Whitesides, 1991). Two pieces of PDMS or a piece of PDMS and glass can be irreversibly adhered to each other by oxidizing the surfaces to be contacted, presumably by a spontaneous dehydration of SiOH groups (≡SiOH + HOSi ≡ → ≡ SiOSi ≡ + H_2O) (Morra *et al.,* 1990).

The second fabrication method will use glass or fused silica substrates and cover plates. Glass and silica substrates offer the advantage that a wide variety of surface modification chemistries are available, and if surface modification is necessary to minimize or enhance interactions of the sample with the channel walls, the surfaces can be modified using standard silane chemistry. For fabrication on glass or fused silica substrates, the microchip substrates are covered with a thin chrome film (100 nm), an anti-reflective coating, and a positive photoresist. The microchip design is then transferred onto the substrate by UV flood exposure. The exposed photoresist is developed, the chrome film etched, and the substrate immersed in a dilute sulfuric acid solution as a cleaning step. Channels are etched into the substrate using a dilute, stirred buffered oxide etchant. Access holes are mechanically or ultrasonically drilled at the ends of the etched channels, and remaining photoresist and chrome film removed. The drilled substrate and an uncoated coverplate are then hydrolyzed, joined, ramped, and held at 550° C for glass and at 1050° C for fused silica for 10 hours to permanently anneal the substrate and coverplate. Microchip channels are treated with 1 N NaOH prior to use. This glass fabrication method can also be used to create the master for the PDMS molding instead of the SU-8 photoresist.

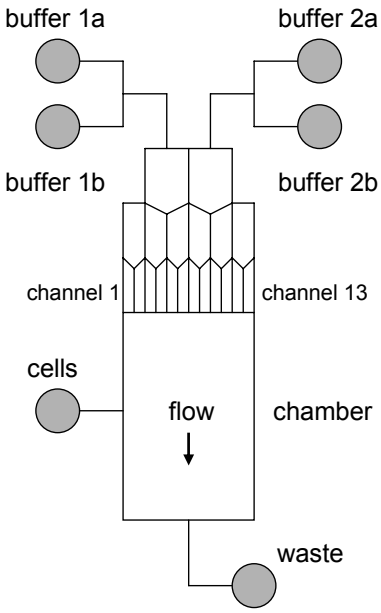


Figure 46. Schematic of gradient flow chamber.

In Figure 46, a schematic of the flow chamber for the chemotaxis experiments is depicted. The channel manifold is fabricated such that at each intersection the channels are bi- or tri-furcated to either pass the same concentration on to the next level or to mix its contents with an adjacent channel. The interior channels are trifurcated, and thus, the design requires fewer levels to generate a gradient than the binomial pattern (Dertinger *et al.*, 2001). The relative flows are controlled by keeping the channel cross-sections similar and adjusting the lengths of the channels appropriately. By simply varying the channel lengths and not the channel cross-section, a single photomask can be used for several fabrication processes. In the schematic, the cells are introduced into the gradient chamber at a point where diffusive mixing has smoothed the steps in the gradient.

Buffers 1 and 2 are mixed to generate a linear gradient of the buffer components at the top of the flow chamber. The number of channels entering the top of the chamber determines the number of discrete concentration levels. In Figure 46, channels 3 through 11 range from 0 to 100% of buffer 2, and to help minimize variations of the buffer concentrations near the edges of the chamber, channels 1 and 2 are 0% buffer 2 and channels 12 and 13 are 100% buffer 2.

As seen in Figure 47, the starting and stopping points of the gradient and the slope of the gradient can be controlled by varying the relative contributions of buffers 1a, 1b, 2a, and 2b entering the first bifurcation. Having active control of the mixing of the a and b portions of each buffer will enable a variety of chemotaxis gradients to be evaluated. This active control can be implemented in several ways including applying superambient pressures to the buffer 1 and 2 reservoirs, using a combination of super- and sub-ambient pressures at each of the reservoirs, or combining electro-osmotic and pressure driven flow. Also, different reagents can be placed in the buffer 1 and 2 reservoirs allowing chemical gradients of opposite slope and generating a chemotaxis minimum or saddle. Homogeneous, heterogeneous, or combination assays can be evaluated using this device. With homogeneous assays, the chemical gradient is contained in the buffer used for the assay. For the heterogeneous assays, the gradient will be chemically bound to the surface by running a gradient first and subsequently running the assay. A combination assay can be run where a heterogeneous gradient is bound to the surface and a homogeneous gradient will be contained in the assay

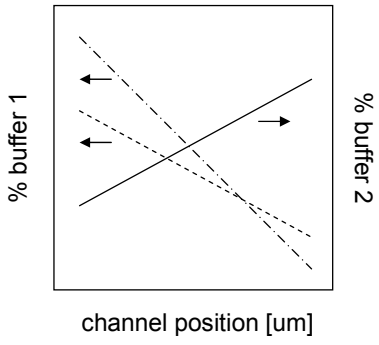


Figure 47. Variation of buffer 1 and 2 concentrations with channel position

buffer. In general, flexibility in the design of the gradient cell assay chamber will allow the operator to tune the cell assay to the cell line, reagent, and concentration range. White light and fluorescence imaging will be used to obtain the necessary parameters from these assays, and an inverted Nikon TE-2000 with a frame transfer CCD camera is available for these experiments.

III.7.i.f BIOLOGICAL IMAGING – TO SUPPORT EXPERIMENTAL AND SIMULATION TOOLS WITH IMAGING INSTRUMENTATION AND ANALYSIS TOOLS:

III.7.i.g Introduction:

Experimental study of tissue properties requires visualization of the tissues at high spatial resolution in three dimensions. Study of organogenesis and regeneration has similar requirements for spatial resolution and dimensionality, but also requires visualization of developing tissues through time. The Center for Medical Imaging at IUPUI already has and IUB plans to acquire under its Commitment to Excellence Program (budgeted at $1,000,000 for the instrument) multispectral two-photon confocal optical microscopes with the aim of following all or nearly all cells in a developing embryo simultaneously and in three dimensions. Our plans for using this instrument to track three-dimensional cell rearrangements during embryogenesis are described in section III.5.

These instruments will allow us to obtain data with the necessary spatial, temporal, and spectral resolution. However, acquiring suitable raw images is only part of the problem. After the two-dimensional digital data are acquired, we must analyze and interpret them to produce a useful three-dimensional picture. We will the ICBMS Vaxx software for this reconstruction.

III.7.i.h Two-Photon Laser Scanning Microscope (TPLSM) Imaging:

III.7.i.h.1 Goals:

1. Visualize in three dimensions time-dependent changes in cell structure, cell signaling, gene expression, and protein distribution.

2. Track in three dimensions the rearrangements of cells in cell aggregates and in developing embryos and tissues.

III.7.i.h.2 Introduction:

As shown in all three BMPs, fluorescent probes have become invaluable tools for the study of biological and non-biological samples. The combination of fluorescence with microscopy allows us to obtain detailed and selective information at the microscopic level. For instance, we can stain different structural and functional entities in biological cells selectively with (extrinsic) fluorescent probes so that they can be easily identified in images. Numerous fluorescent probe molecules are now available that enable selective imaging of the electric potential of membranes, DNA, spatial variations of free ion concentration and specific proteins within living cells.

A laser scanning microscope (LSM) illuminates fluorescent samples by focusing a laser to a diffraction-limited beam waist less than 1μm in diameter and raster-scanning it through the specimen. The combination of LSM with confocal microscopy provides excellent depth discrimination and improves spatial resolution within the plane of focus by forming the image through a pinhole placed in front of the detector, which acts as a spatial filter to select the emission from the plane of focus. Very good laser scanning micrographs result from the use of fluorescent markers that absorb and emit visible light. For stable fluorescent specimens, we can often improve image quality simply by increasing the exposure time or the illumination intensity.

However, a serious problem with standard confocal microscopy is photobleaching that results from the intense laser illumination. When photobleaching occurs, the number of photons available to form an image of the fluorescent specimen becomes fixed. Reducing the statistical noise by increasing the number of emitted photons is no longer possible. Biological specimens are particularly demanding in this regard. Photobleaching

of fluorophores whose concentration is often low to begin with, and in living cells the attendant destruction of vital cellular components, place strict limits on the amount of light available for imaging.

One very successful solution to this problem is “two-photon molecular excitation.” In two-photon confocal microscopy (TPLSM), very high local instantaneous intensity provided by the tight focusing in an LSM combined with the temporal concentration of a femtosecond pulsed laser causes excitation. With a colliding-pulse, mode-locked dye laser producing a stream of pulses with pulse duration about 100 fs at a repetition rate of about 80 MHz, the probability becomes appreciable for a dye molecule to absorb two long-wavelength photons simultaneously, thus combining their energy in order to reach its excited state. The microscope makes use of a tunable femtosecond titanium: sapphire laser enabling two-photon excitation of a broad range of fluorescent molecules, including UV probes. TPLSM is ideally suited for imaging cells *in vivo* due to its deeper tissue penetration and reduced phototoxicity.

We intend to optically record multiple cell types within a developing embryo simultaneously using fluorescent tags inserted in embryonic cells using GFP-expressing retroviruses. We will record cell and tissue movements in the developing embryos using multispectral, time-lapse fluorescent microscopy in three dimensions. Then we will analyze the recorded data using computers running sophisticated cell-tracking and color discrimination software capable of distinguishing the subtle movements that thousands of individual cells make and identifying a handful of genes that these cells express. We will integrate the gene expression and cell migration data collected using laser microscopes in order to understand the complex flows of information and interactions that occur during development within the spatial and temporal context of the maturing embryo.

The imaging of living cells and tissues using laser-scanning microscopy offers dramatic insights into the spatial and temporal control of biological processes. The availability of genetically encoded labels such as green fluorescent protein (GFP) offers unique opportunities to trace cell movements, cell signaling or gene expression dynamically in developing embryos. To unambiguously identify CFP, GFP, YFP, and RFP as well as conventional dyes, the multispectral TPLSM uses a liquid crystal tunable filter (LCTF) to collect

the emission spectrum of each pixel within the TPLSM image (Lansford *et al.*, 2001). The Zeiss 510 multispectral imaging system obtains the full emission spectrum from a single scan. Based on the fluorescent emission spectra, supervised classification and linear unmixing analysis algorithms identify the nature and relative amounts of the fluorescent proteins expressed in the cells. In optimal cases, this technique separates GFP from fluorescein. It can also distinguish up to four fluorophores that spatially overlap at single pixel resolution. This approach, as installed at the ICBM and planned at IUB offers the needed ability to concurrently image multiple colored, spectrally overlapping marker proteins within living cells.

III.7.i.h.3 Sample Application to Vascular Development BMP:

Soluble mixtures of ECM components are prepared from small intestinal submucosa, an intact interstitial ECM, as described previously (Voytik-Harbin *et al.,* 1998a). Unstained 3-D matrices of type I collagen or mixtures of ECM components are polymerized in Lab-Tek chambered coverglass (Nalge Nunc Int, Rochester, **NY)** and imaged using a Bio-Rad MRC1024 confocal microscope via a **60X,** 1.4 NA (numerical aperture) oil immersion lens. Optical settings are established and optimized on reconstituted matrices after polymerization is completed. An aliquot of soluble collagen or ECM preparations is then placed onto the heated (37°C) stage of the microscope and fibrillogenesis and fibril assembly imaged. Samples are illuminated with 488 nm laser light, and the reflected light is detected with a photomultiplier tube **(PMT)** using a blue reflection filter. For the Bio-Rad MRC1024 confocal microscope, instrument setup involved a beam splitter placed in position DI and a dichroic filter that reflects 488 nm light into PMT2 in position D2 as previously noted (Voytik-Harbin *et al.,* 1998a). Optical filters can be added in positions W1 and W3 for the simultaneous collection of fluorescence information in PMT1 and PMT3, respectively. A z-step of 0.2 pm is used to optically section the samples. Because the resolution of the z-plane is less than that of the x-y plane, the sampling along the z-axis may be different from that of the x-y plane. 2-D (*e.g.*, x-y) or 3-D (*e.g.*, x-y-z) sections demonstrating fibrillogenesis and assembly events are recorded at step intervals ranging from 1 to 5 sec for up 1 hr. Minimum time intervals required for image collection are dependent on the section size, resolution, and scanning mode (point versus line). For standardization of scanning depth, the scan head must be adjusted to a distance in the range of 20-50 pm from the upper surface of the coverglass.

Non-uniform background caused by interference and reflection from the optical pathway is removed from the images using a standard rank leveling procedure on each x-y section. Rank leveling consists of applying a multiple number of erosions followed by a Gaussian filter to each section to create a background approximation. This background approximation is then subtracted from the original section to enhance the signal to noise ratio. Three-dimensional images of reconstituted ECM biomaterials are either compiled into a single view projection using Laser Sharp image processing software (Bio-Rad, Hemel Hempstead, England) or compiled into a 3-D projection using Voxel-View reconstruction software (Vital Images, Minneapolis, MN) or Image Pro Plus (MediaCybernetics). Rank leveling can also be performed on 3-D images prior to reconstruction into 3-D views to improve image quality.

III.7.ii PREPARATION OF SAMPLES FROM SOLID TISSUE OR TISSUE MASSES:

III.7.ii.a Laser Microdissection (LMD):

Laser microdissection is a technique for the preparation of single cells, or small sections of tissue for the purposed of advanced analysis, PCR, or molecular phenotyping. The principle is the use of thermal energy to slice though tissue by using a thermal conductive layer beneath the tissue. A nitrogen laser with a small circular beam profile of less than 1 micron frequently supplies the thermal energy. Using high magnification objective to view the sample, it is possible to draw an area of interest using a drawing tool and this section will be sliced from the tissue and deposited into a small container for analysis. Depending upon the quality of the sample, and the resolution of the system, it is possible to isolate a single cell.

III.7.ii.b Cell Preparation:

Extraction of cells from the embryo/allantois for separation and further classification will be performed by standard flow cytometry techniques. Prior to analysis cells will be separated using the techniques already described above or for larger aggregated tissue, we will use the tissue desegregation procedures normally used for solid tumors. Briefly, small pieces of tissue (up to 10 mm^3 are placed into a Medicon unit and placed into a Medimachine (BD Biosciences, San Jose, Ca). The medicon unit spins at 80 rpm and contains a blade that disaggregates tissue into single cells. The internal part of the Medicons units contains a fixed, stainless steel screen with about 100 hexagonal holes around each of which 6 micro-blades are situated. The tissue is placed in the unit together with 0.5 to 1.5 mL of buffer and brought to the blades by an element rotating on the screen. Disaggregated tissue passes through the holes, and a micro-pump located under the screen supplies liquid through the holes to ascertain they are constantly cleaned. Tissue processing takes from 15 seconds to 1 minute per specimen and samples are immediately ready for staining or processing prior to flow cytometry.

III.7.ii.c Flow Cytometry:

Flow cytometry is a technique useful for the identification of population data. By evaluating a large number of particles or cells, it is possible to identify individual populations that are discriminated by one of several possible variables that are measured, or a significant number of additional parameters that can be extracted from the multivariate analysis. By using a laser to excite fluorochromes resident or attached to cells or particles, and then establishing a detection system that can separate the individual spectra and correlate all of these data, a complex multivariate analysis is possible. Because many of the spectra overlap, most flow cytometers have extensive technology involved in their detection systems to deal with the complex issue of spectral overlap. The compensation hardware and software is crucial to accurate detection of multi-colored particles. Particularly important, is the sorting or cell separation component of a flow Cytometer. This utilizes an electrostatic charge to separate particles based on charge. Using multivariate analysis, it is possible to physically separate cells in an aseptic operation for subsequent further analysis or culture. Several facilities exist within the components of this proposal to perform both advanced multivariate analysis and cell sorting.

III.7.iii PROTEIN PROFILING:

There are two traditional methods that involve arraying protein mixtures in two different dimensions based upon orthogonal physical properties will be used in these studies. The traditional methods employ two-dimensional gel electrophoresis involving separation by isoelectric focusing followed by mass. The visualization of proteins immobilized in the polyacrylamide matrix is offered by a variety of stains enabling a rendering of an array of spots or a map. Quantification of the protein content can be achieved with some

accuracy using differential image analysis. One distinct advantage of the gel-based system is that traditional antibody-based western blotting on appropriate membranes can be executed for the entire array allowing probing of a variety of possible proteins. Limitations to this approach are that the sensitivity of detection (the dynamic range of the measurement) and the rate of throughput.

A chromatographic arraying approach will offer a second strategy for the analysis of protein mixtures. In this case, the core facilities at Purdue have a state-of-the-art automated, two-dimensional fractionation system expressly designed for high-resolution chromatographic analysis of complex protein mixtures. The ProteomeLab PF 2-D provides an integrated solution that resolves hundreds to thousands of proteins present in cell lysates in approximately 20 hours of total operations. The system generates detailed protein maps for easy comparison using the ProteomeLab Software Suite supplied with the system data. Liquid fractions can be collected and stored for future analysis or the eluent can be connected directly to ESI-mass spectrometry. A variety of different first dimension separation modes can employ ion exchange, gel filtration, of chromatofocusing followed by non-porous reverse phase chromatography in the second dimension. Fraction collection from the first dimension is quality-controlled with an in-line pH monitor, then automatically injected into the second dimension. As a result, PF 2-D requires less time and attention than traditional labor-intensive nonautomated techniques. There is a gain in reproducibility when the system is appropriately standardized.

Both methods (gel and chromatographic) will be employed in this proposal in core 3 and require careful attention to achieve a reproducible profile. Each experimental system will require some optimization and standardization to achieve a useful comparative profiles. A minimum of 10^6 cells will be freshly collected from the extracellular matrix experiments and subcellular fractions will be created to isolate, soluble, or membrane bound fractions. It is anticipated that somewhere between 300-1000 proteins can be readily detected from this number of cells. Coupling with traditional western blotting experiments with specific antibodies for markers proteins indicated in the cell phenotyping and/growth factor combinations will enhance the sensitivity of this approach. In some cases, the affinity selection protocols (using immunoprecipitation to probe specific signaling pathway proteins) will be employed to assess specific protein complexes using the profiling methods. Initially both the gel and chromatography based profiling approaches will be used to arrive at complementary data. Most importantly, the protein profiles will be standardized by comparative analysis for each experimental system. The design of each experiment is then chosen with the idea of comparative analysis in mind.

III.7.iii.a PROTEIN IDENTIFICATION:

As needed, the fractions or spots from the 2-D gels will be further processed to identify the individual proteins and their states of post-translational modification. One advantage of the chromatography-based system is that liquid fractions can be stored or transferred to a MALDI plate spotter, or directly connected to an electrospray mass spectrometry source for protein identification. An adequate capacity to digest samples from 2-D gels and spotting them on MALDI plates before or after tryptic digestion are available for this project as well. The Analytical and Functional Proteomics Core in the Bindley Bioscience Center also has a complementary set MALDI-TOF and ESI based mass spectrometers that are dedicated to the identification and quantification of proteins and peptides. Collisional activation of the peptide ions can be used to induce further fragmentation resulting backbone bond cleavage to result in unit lose equivalent to the amino acid. This gas phase sequence information is critical in the verification of the proposed from the parent ion of the peptide sequence. This approach can also be highly informative with respect to the identification of the post-translational modifications present. Two of these instruments are directly interfaced to capillary HPLC units designed for peptide isolation and identification. Database searching is enabled through directly sequence matching or appropriate peptide mass matching from predicated peptide fragments based upon genomic data.

III.7.iii.b Extracellular Matrix Preparation and Imaging:

Soluble mixtures of ECM components are prepared from small intestinal submucosa, an intact interstitial ECM, as described previously (Voytik-Harbin *et al.,* 1998a). Unstained 3-D matrices of type I collagen or mixtures of ECM components are polymerized in Lab-Tek chambered coverglass (Nalge Nunc Int, Rochester, NY) and imaged using a Bio-Rad MRC1024 confocal microscope via a 60X, 1.4 NA (numerical aperture) oil immersion lens. Optical settings are established and optimized on reconstituted matrices after polymerization is completed. An aliquot of soluble collagen or ECM preparations is then placed onto the heated (37°C) stage of

the microscope and fibrillogenesis and fibril assembly imaged. Samples are illuminated with 488 nm laser light, and the reflected light is detected with a photomultiplier tube (PMT) using a blue reflection filter. For the Bio-Rad MRC1024 confocal microscope, instrument setup involved a beam splitter placed in position DI and a dichroic filter that reflects 488 nm light into PMT2 in position D2 as previously noted (Voytik-Harbin *et al.,* 1998a). Optical filters can be added in positions W1 and W3 for the simultaneous collection of fluorescence information in PMT1 and PMT3, respectively. A z-step of 0.2 pm is used to optically section the samples. Because the resolution of the z-plane is less than that of the x-y plane, the sampling along the z-axis may be different from that of the x-y plane. 2-D (*e.g.*, x-y) or 3-D (*e.g.*, x-y-z) sections demonstrating fibrillogenesis and assembly events are recorded at step intervals ranging from 1 to 5 sec for up 1 hr. Minimum time intervals required for image collection are dependent on the section size, resolution, and scanning mode (point versus line). For standardization of scanning depth, the scan head must be adjusted to a distance in the range of 20-50 pm from the upper surface of the coverglass.

Non-uniform background caused by interference and reflection from the optical pathway is removed from the images using a standard rank leveling procedure on each x-y section. Rank leveling consists of applying a multiple number of erosions followed by a Gaussian filter to each section to create a background approximation. This background approximation is then subtracted from the original section to enhance the signal to noise ratio. Three-dimensional images of reconstituted ECM biomaterials are either compiled into a single view projection using Laser Sharp image processing software (Bio-Rad, Hemel Hempstead, England) or compiled into a 3-D projection using Voxel-View reconstruction software (Vital Images, Minneapolis, MN) or Image Pro Plus (MediaCybernetics). Rank leveling can also be performed on 3-D images prior to reconstruction into 3-D views to improve image quality.

III.7.iii.c Gene Expression Support:

As described in core 1 and attached support materials, a gene expression support group will be formed for the following tasks:

1. Maintain current lists of tools for gene expression analysis and transcriptional network modeling, distribute this information to center members, and assist in the interpretation of relevant gene expression data.
2. Plan future connectivity of Tissue Simulation Tools and Gene Expression/Transcriptional Networking Tools developed at other NIH Centers for Biomedical Computing and elsewhere
3. Provide assistance in integrating gene expression tools and advise the Center for Tissue Simulation on usability and user interface issues.

Any proposed or future microarray experiments will be carried out at the Center for Medical Genomics (CMG), with assistance from the gene expression support group and Dr. Matthew Grow, Assistant director of the CMG.

III.8 TIMELINE:

Year	Subproject	Aim/ Platform	Progress
1	Heart	1	Spatiotemporal cardiomyocyte data collection
		2	Data collection – Wild-type, *Nkx2-5* and *HAND2* deficient mouse heart imaging
		3	Stem cell tracking – heart incorporation
		4	Trabeculation imaging for BMP-10 mutant and normal mouse hearts
	Vascular	1	Comparison of EDEPs vs. ADEPs Parameter quantitation
		2	

		3	
	Regeneration	1	Morphometric measurements differentiation marker identification cloning/localization of patterning genes
		2	Morphometric/mitotic measurements Cadherin studies Computational Tool Development
2	Heart	1	*In silico* model construction
		2	*In silico* model construction Double *Nkx2-5/HAND2* knockout generation
		3	*In silico* model construction
		4	*In silico* model construction
	Vascular	1	
		2	Spatiotemporal cue regulation Effects of VEGF measured
		3	Factor-rich vs. embryonic environmental studies
	Regeneration	1	Mitosis, apoptosis, and density studies cloning/localization of patterning genes
		2	Morphometric/mitotic measurements Cadherin studies Computational Tool Refinement
3	Heart	1	Simulation predictions compared to experimental results
		2	Predicted double knockout phenotype compared to simulation prediction
		3	Simulation compared to experimental data in normal vs. injured valves
		4	Simulation compared to experimental BMP-10 dose response
	Vascular	1	
		2	
		3	Factor-rich vs. embryonic environmental studies
	Regeneration	1	Mitosis, apoptosis, and density studies cloning/localization of patterning genes
		2	Cadherin studies Computational Tool Validation
4	Heart	1	Tool use continues on Independently-funded projects
		2	X
		3	X
		4	X

	Vascular	1	Tool use continues on Independently-funded projects
		2	X
		3	X
	Regeneration	1	Tool use continues on Independently-funded projects
		2	X
5	Heart	1	Tool use continues on Independently-funded projects
		2	X
		3	X
		4	X
	Vascular	1	Tool use continues on Independently-funded projects
		2	X
		3	X
	Regeneration	1	Tool use continues on Independently-funded projects
		2	X

III.9 EXIT STRATEGY:

By the conclusion of this project, the Tissue Simulation Tools should have links to, or integration with several externally-developed tools for gene expression analysis or transcriptional network modeling. Additionally, member of the Center for Tissue Simulation will have benefited from training on these systems and in the interpretation of increasing amounts transcriptional data (with assistance provided by the gene expression support group).

As the gene expression support group matures, there should be a natural progression to RO1 funded projects focused on the development of additional bioinformatic (genomic) tools, and additional collaboratory funding secured with research groups requiring advanced assistance with microarray-based experiments and data interpretation.

At the end of five years, the individual in the position of programmer/analyst in the support group will have acquired experience and skills that will make them extremely marketable. The combination of software development skills and gene expression data analysis experience will be sought after by employers in both the public and private sectors.

CORE IV: SUPPORT AND INFRASTRUCTURE

IV.1 COORDINATOR:

David Hart (Indiana University, Bloomington)

IV.2 PARTICIPANTS:

David Moffett (Purdue), Craig Stewart (IUPUI), Mary Papakhian (IUB), Anurag Shankar (IUB), Andrew Arenson (IUPUI), Steven Simms (IUB), Raymond Sheppard (IUPUI)

IV.3 INTRODUCTION:

One of the key goals of the Center for Tissue Modeling (CTM) is to create tools that a diverse community of biomedical researchers use widely. Equally as important in the long run is that computer scientists and computational scientists build onto and add to the CTM's tools, and that the Tissue Simulation Toolkit (TST) be interoperable with other computational biology tools. Both of these goals require good mechanisms for software dissemination, active participation in standards setting groups, and robust and nonbureaucratic mechanisms for code management and access.

A critical part of our support strategy is that all software involved in the Tissue Simulation Toolkit will be open source. Three groups will support this software: the CTM; our many partners who have indicated that they will use (and thus provide some support for) the TST; and the community of people who use this software themselves – since access to the source code implies the ability to make changes to fit community needs.

Indiana University is an established leader in computer support (Voss *et al*., 1995) and, in particular, has extensive expertise in delivery of online support services, including the IU Knowledge Base (http://kb.indiana.edu/), which has won several awards as an outstanding online support tool (http://kb.indiana.edu/data/aiwp.html?cust=658179.00635.131). Purdue University has successfully created simple-to-use computing tools accessible via the web. Both schools have significant expertise in managing software engineering.

Indiana University has a particularly strong track record supporting biomedical researchers (Stewart *et al*., 2001; Stewart *et al*., 2003; Stewart *et al.*, 2004). University Information Technology Services (UITS) conducts an annual (anonymous) survey of its users. Results are available online (http://about.uits.iu.edu/divisions/rac/usersurv.html), and referred publications discuss survey history details (Peebles *et al*., 2001). In last year's survey more than 97% of the researchers in the IU School of Medicine found support services from UITS to range from "satisfactory" to "excellent." UITS also has a strong track record delivering high performance computing tools that solve problems biomedical researchers actually want to solve (Stewart *et al*., 2004), as opposed to solving problems that we think biomedical researchers ought to want to solve. In the past three years, UITS has produced three new software packages to implement techniques requested by traditionally trained biomedical researchers (M.D.s or M.D./Ph.D.s), and enhanced another six software packages. This demonstrates something that in theory seems simple but in practice is rare: our computing centers are highly responsive to requests from the user communities they serve. This basic and simple philosophy will guide support by the CTM.

We will discuss the support and infrastructure components of the Center for Tissue Modeling in order starting from the components most obvious to the user (the user interface), moving to portions less visible to the ultimate end users (software engineering). In particular, we will focus on the following:

- User Interface.
- Typical usage patterns and user support for production software.

- CTM computer resources and software installation.
- User support for test software.
- Software engineering support.

Cores 5 and 7 extensively cover "Support" in the sense of training and outreach; to save space we will not repeat that discussion here, beyond noting that we recognize training as a key part of our overall software support strategy, and we will pay careful attention to this aspect of the support services required to create successful tools. In particular, we will work to provide training at a variety of locations, make training available online, and coordinate our training efforts with other initiatives such as DIMACS and NECSI (see attached letters of support). We would view it as a great success if the CTM and DIMACS offered training courses at each others' brick-and-mortar locations.

IV.4 USER INTERFACE:

Indiana University Purdue University Indianapolis, Purdue University, and Indiana University are uniquely well positioned to support this National Center of Excellence. Purdue's ground breaking web services system called PUNCH (http://punch.purdue.edu/), IU's Alliance Portal Project (http://www.extreme.indiana.edu/alliance/miledeliv/), and IU's knowledge-based help services (http://kb.indiana.edu/) are excellent models for providing high quality, nation-wide support.

We have proposed to make many existing cell and tissue simulation tools available for general purpose use via the web. We will build an application area web site that houses these tools. Tools with low computational impact will run locally on the web site host while tools with higher computational loads will be submitted to the normal job streams served by the systems we mention later in this core, based on the grid infrastructure Core 1 describes in detail.

Purdue has an extensive background in web-enabling pre-existing tools. PUNCH, the Purdue University Network Computing Hubs, already supports the National Science Foundation's Network for Computational Nanotechnology (http://nanohub.purdue.edu/), among other sites. Nanohub supports approximately 2500 users from all over the world, using over 50 tools. We expect the TST to operate similarly. Tools that migrate easily to the web are directly available while tools that do not easily port to the web use the PUNCH interface to interact with the user. PUNCH allows web enabling of command-line oriented tools without a large investment in code changes within the tool, simplifying upgrades to new tool versions with little effort or lost time.

While PUNCH provides highly useful experience in user interface design, helping to make interfaces for grid-aware portlets, IU's Alliance Portal Project goes further, providing direct experience with portlets and the service model, described in Core 1, we will use to create a Grid portal. The Portal Project delivers similar functionality to PUNCH, allowing more customization for application automation at the cost of more time-consuming application porting.

Operationally, tools that result in a large computational load on the nanohub have been enabled using either the Alliance Portal Project or PUNCH on a user-by-user basis. This case-by-case approach has proven to be a workable solution as long as the number of users does not grow too large. Computationally hard jobs can connect into the normal job streams at IU or Purdue depending on the computational and data resources needed and available. The university-owned I-light fiber facilitates job movement between universities. Both universities are part of IP-Grid (http://www.indiana.edu/~uits/cpo/teragrid092903/), which in turn is part of the NSF's Extended Teragrid facility (http://www.teragrid.org/).

IV.5 TYPICAL USAGE PATTERNS AND USER SUPPORT FOR PRODUCTION SOFTWARE:

A researcher who needs to use a tool will connect to a single web site, log in, navigate that site to a particular tool or chain of tools, fill in the appropriate web forms, and press submit. The site then will run the tool and return the result. Each user will have individual local storage on the Tissue Simulation Toolkit Grid (TSTGrid), as well as access to computational resources. As the researcher asks questions, context-sensitive help will be the front-line way of quickly providing answers. Where the tool actually runs isn't important to the researcher. Both the PUNCH and Alliance Portal Project environment manage the underlying tasks transparently and seamlessly, and this functionality will be provided for the Portlet (web portal) front end to the Tissue Simulation Toolkit. This Portlet approach should provide for simple and straightforward use of the Tissue Simulation Toolkit from a variety of workstations and from anywhere a biomedical researcher has access to the Internet.

Biomedical researchers who want to use the Tissue Simulation Toolkit will need two things: introductory documentation on how to get started, and assistance with problems or questions. Indiana University is known for the quality of its educational materials, including online documentation. We will develop an online course that covers both the biomedical modeling tools and use of the software itself. This courseware will be based on experience teaching to users in person (see Core 6 on outreach for more details on in-person course plans). Online educational materials have both advantages and disadvantages. One of key advantage is availability on demand, so that a researcher can begin learning about the software at any time, at their convenience. A key shortcoming is the loss of personal contact and spontaneity associated with the in-class education. Writing materials that address questions usually asked in a live class and using a question-and-answer format as part of the online presentation of materials can partially ameliorate this lack of warmth. We have successfully adopted this approach in material we have previously presented online.

A key demand, as the popularity of certain types of support books for computer software demonstrates, is quick and simple explanations that take a new user from starting up a software tool to actually achieving something with it. The first thing accomplished may be very simple, but it is essential to give new users a sense of accomplishment very quickly after they start the program. We will create documents that focus on providing a very simple learning curve from a user's first look at the software to "doing something." The Tissue Simulation Toolkit contains multiple individual tools. We will focus on providing pre-programmed examples of simple systems for each of the tools. For example, for the Cellular Potts Model (CPM) we plan to have simple examples of slime mold lifecycles and chick limb bud development. Even the most computer-phobic users will, with just a few mouse clicks and movement of slidebars in the interface, be able to watch simulations of developmental processes under "typical" parameters and then to vary these parameters to see their effect. We have a letter of support from Reify, Inc. (Cambridge, MA) indicating their interest in working with us to create easy 'commercial style' packaging that will appeal to and seem accessible even to quite naïve computer users from students to practicing clinicians. This strategy will help create a large user base – from hundreds of specialists in various subfields of developmental biology to thousands of medical students and tens of thousands of undergraduate and high school students. Of course, the TST will appeal to such a large user base if it has embedded in it a number of interesting and biologically realistic examples. We will use the Biologically Motivating Projects (BMPs) to collect the required data and prepare several example systems for simulation with the TST.

Once researchers have a basic familiarity with the Tissue Simulation Toolkit they will often have specific questions to which they want specific answers. Indiana University is a leader in the development of online support tools that answer common questions electronically: clearly, quickly, and 24 hours a day, 365 days per year (Hewitt and Stewart, 1996; http://kb.indiana.edu/).

Figure 4.1 shows the Knowledge Base main screen. Figure 4.2 shows the results of a search for IUBio – a biology web archive operated by Indiana University.

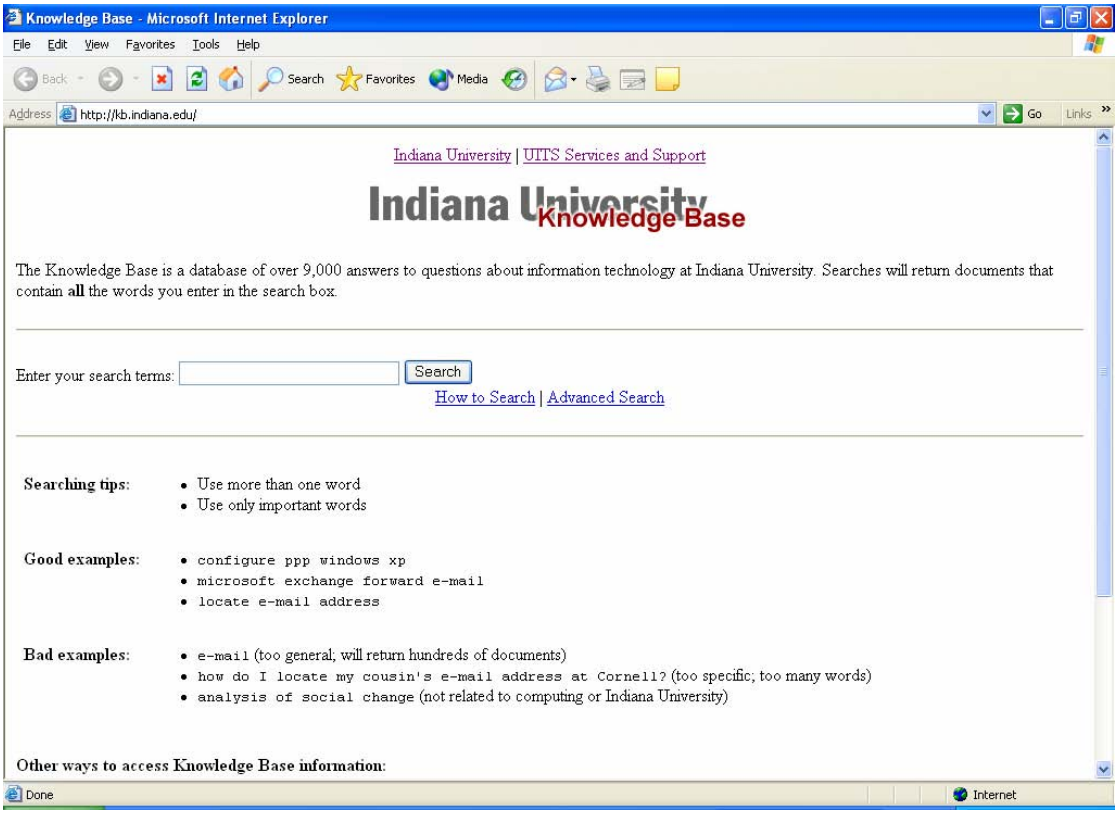


Figure 1. Main search screen of the Indiana University Knowledge Base (http://kb.indiana.edu/).

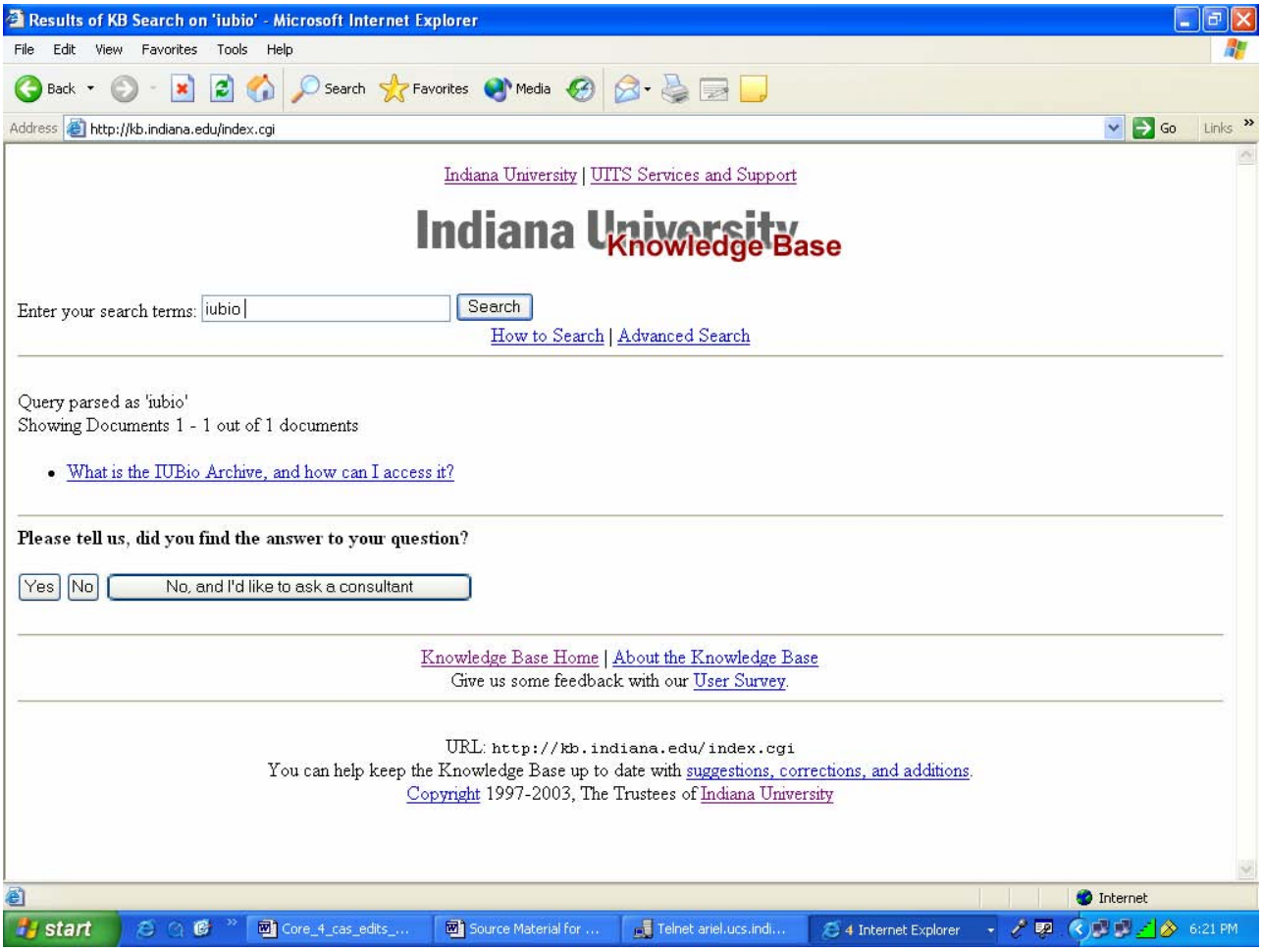


Figure 4.2 The results of a sample search of the IU Knowledge Base.

Figure 4.3 shows the key element in the success of the IU Knowledge Base – the IUBio KB entry.

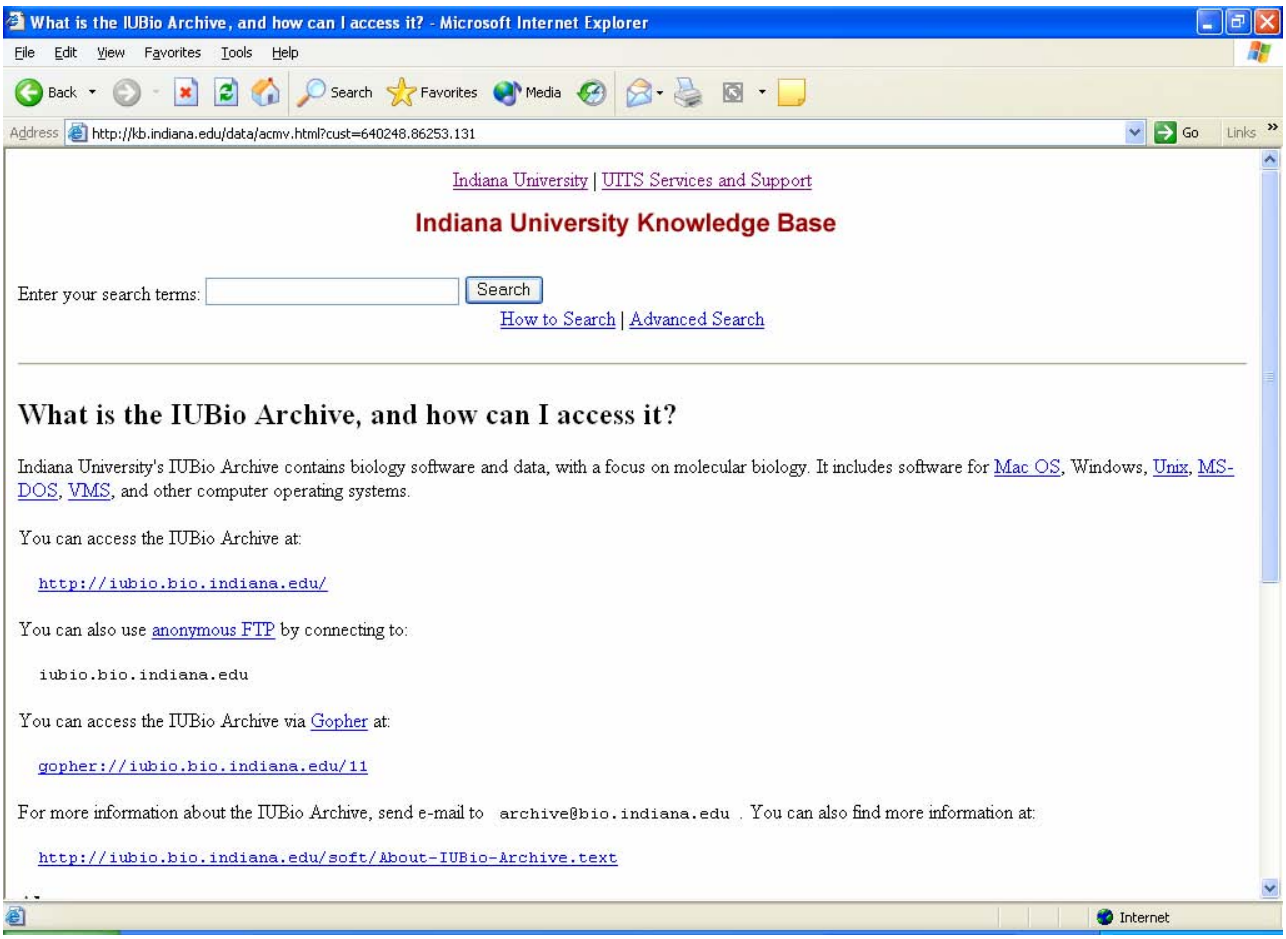


Figure 4.3. The IU Knowledge Base entry about the IUBio Archive (http://kb.indiana.edu/data/acmv.html).

The IU Knowledge Base has proved useful to computer users because it incorporates entries that are written in an easy-to-follow question and answer format, usually with pointers to further information. One of the important facilities of the IU Knowledge Base is that it supports "domains." A general query on the word "bio" results in dozens of potential KB entries as its result. Many of these results, however, have to do with the Basic Input and Output System of a microcomputer (its BIOS). Indiana University will create a domain of KB entries specifically for national and international users of the Tissue Simulation Toolkit. Users can search within this domain and find results that deal specifically with the TST.

Populating the Knowledge Base will come as a direct result of the delivery of more personal services – phone consulting (which we plan to provide 1-5 pm EST) and e-mail consulting. Such personalized consulting services serve two functions. First, when a question is asked that seems general in nature, the question generates the writing of a Knowledge Base entry— automatically populating the Knowledge Base in ways that respond to the actual experiences of users. Over time, as more and more entries accumulate in the Knowledge Base, users of the Tissue Simulation Toolkit will learn to start with the Knowledge Base and then call or send e-mail only if they do not find the answer to their problem in the Knowledge Base. (The Knowledge Base includes an option for users to indicate that they have not found the answer to their problem and submit a question via a web form, which is then answered by e-mail.)

The other reason for phone and email consulting is that sometimes problems require personal attention by a consultant for resolution. Personal contact is important, and we will provide it by having expert staff available to answer the phone half of each business day. We have used this half-day approach successfully in other specialized support situations.

A side effect of our commitment to establishing bilateral interoperability with other tools such as the Physiome Project, BioSpice, the San Diego Supercomputer Center, Virtual Cell and SCIRun creates an interesting challenge (We have written letters in support of projects involving these software tools, and they have likewise written letters supporting our project, included in the appendices). Interoperability implies a certain degree of shared responsibility, since we must provide support to people using any tool from any of these programs that we list as interoperable with the TST, and *vice versa*. We propose two principles to guide our activities in this area. One is that the key developer of each software tool be the party that provides detailed support for that product; and two, that the developer of each tool be responsible for assuring that its interoperability features work properly. In the long run, the IU Knowledge Base could be populated with help information on a wide variety of computational biology tools – the TST, and any and all tools that interoperate with it.

IV.6 ACCESSING AND USING THE SOFTWARE:

Users of the TST will have several options for using its software. One option will be to use the TST via a web interface, running on the advanced information technology infrastructure of the three universities participating in this grant. We also plan to install the TST at nationally shared Centers funded by the NIH and the NSF. Thus, users who wish to try the software, or users who want to perform “heroic” runs, will find that web-based availability of the software from these systems satisfies their needs.

“Heroically large” runs deserve special mention. Indiana and Purdue University systems are in part available for use through national allocations, and part through locally controlled allocations. The CTM will provide a minimum of 25% of the locally-managed allocations to the national user community in direct support of the TST. Major users of the TST will be assisted in applying for allocations on the nationally-funded supercomputer infrastructure provided by the NIH and the NSF.

The concept of scalability from the laptop to the TeraFLOPS (and beyond) requires that users of the TST be able easily to download the software, install it on their own computing system, and use it locally. We will make the source code for the TST easily accessible from web-based archives (including SourceForge.net). We will also provide precompiled binaries for Windows, Linux, and MacOSX (recognizing in the latter case that Macintosh are quite popular among many biomedical researchers). Indiana University runs archives for many types of software, and currently delivers tens of thousands of downloads of software packages per month. The additional load of providing access to the TST and its components will not burden the capacity of IU’s delivery systems for download services.

IV.7 CTM COMPUTER RESOURCES AND SOFTWARE INSTALLATION:

Core 4 expert staff will install software and add new tools to the TST. This staff will include both subject-area experts and information technology staff. They will initially evaluate the existing web capabilities of the tool and connect the tool to a traditional web site wherever possible. If, however, the tool is older or has a non-web user interface, they will then write a configuration script that describes the input and output from the tool. The Alliance Portal Project can bring to the web tools that need better web integration. We will add the application to the production environment after testing in the test environment. For typical tools, this entire process will take less than a person-week of time. In any case, as we bring the tool into the TST environment, we will need to create appropriate documentation for training and support and to connect it to the Knowledge Base.

We handle updates of already installed tools similarly. In the absence of major changes in user interfaces, typical updates will take less than a day.

The facilities section of this grant describes in detail the computer resources to be made available by the Center for Tissue Modeling to users of the Tissue Simulation Toolkit. A state-owned, high-speed fiber infrastructure called I-light interconnects Indiana University, Indiana University Purdue University Indianapolis, and Purdue University. This state-owned network yields effectively unlimited bandwidth between the campuses by simply improving the electronics on that fiber.

IP-grid, the state of Indiana's Globus-based computational grid, interconnects Purdue and Indiana to the TeraGrid, NSF's distributed infrastructure for open scientific research. Purdue and IU are both providing computational and storage facilities as part of their contribution to this leading-edge infrastructure. Indiana University has a long history of providing computing, visualization, and data resources in support of computational biology, bioinformatics and medical research. The Indiana Genomics Initiative funding, awarded by the Lilly Endowment in December 2000, resulted in a major expansion of these facilities. As a result, biologists and biomedical researchers have since used more than 1.6 million hours of CPU time on Indiana University's supercomputer systems. Purdue University also has a long provided the cyberinfrastructure required for research in bioengineering. Recently, Indiana University and Purdue University have collaborated to combine these significant resources. In October 2003, the National Science Foundation awarded Indiana University and Purdue University funding to become part of the Extensible TeraScale Facility. This funding has allowed IU and Purdue to create a grid of supercomputing, visualization, and data facilities within the State of Indiana's three largest research campuses (Indiana University Purdue University Indianapolis, Purdue University West Lafayette, and Indiana University Bloomington). This IP-grid (Indiana Purdue grid) is interconnected via the State of Indiana's I-Light high-speed optical fiber network. The aggregate rate of computational growth of the Indiana and Purdue systems since 1996 has been aggressive, outstripping Moore's Law with peak theoretical capacity doubling every 15 months. Both institutions expect growth to continue for these facilities. All of these facilities are available to the proposed National Center of Excellence for Tissue Modeling. Table 1 shows the local computational resources that will be available to the CTM.

Table 1. Computational resources available to support the TST

Supercomputers	**TFLOPS Capacity**	**RAM (system total)**	**Disk (system total)**
Indiana University	***3.21***	***1.21 TB***	***27.77 TB***
Sun V1280s (2)	0.05	0.20 TB	6.5 TB
IBM SP WinterhawkII, NighthawkII, Regatta nodes; Power3+ & Power4	1.00	0.45 TB	8.31 TB
AVIDD Linux Clusters (Pentium4; IBM IA64)	2.16	0.56 TB	15.58TB
Purdue University	***1.82***	***1.80 TB***	***22.1 TB***
Sun F6800s (5)	0.16	0.96 TB	2.5 TB
IBM SP WinterhawkII, NighthawkII, Regatta nodes; Power3+ & Power4	0.56	0.42 TB	4.60 TB
Intel Cluster (Pentium3 & Pentium2)	0.61	0.32 TB	14.02 TB
AMD Cluster (Dual 1.6Ghz)	0.48	0.10 TB	1.00 TB

Another critical facility will be convenient access to public databases that will inform parameter choices and modeling efforts by researchers using the Tissue Simulation Toolkit. The Centralized Life Sciences Data (CLSD) service provides a single, SQL-based interface to a selection of widely used biomedical datasets, including BIND, ENZYME, LIGAND, LocusLink, UniGene, dbSNP, SGD, KEGG PATHWAY and a variety of NCBI BLAST databases. CLSD runs on Indiana University's IBM SP supercomputer, using IBM DB2 database software and IBM's Information Integrator software to retrieve data from multiple, heterogeneous data sources.

A half dozen research applications already use the CLSD (http://www.indiana.edu/~rac/clsd/), including a Web interface designed to allow intuitive queries by biomedical researchers, a Web-based analysis platform for biomedical researchers who are customers of a DNA microarray facility, and as part of the biomedical data reporting section of a laboratory information management system. Besides these production systems, multiple biomedical research applications in development plan to use the CLSD.

CLSD uses software programs called “parsers” to transform datasets from their native format into relational databases. Data files are copied across the Internet from their original sources to supercomputers at Indiana University. A parser that is specific to each particular data source then converts these files to the relational database format.

IBM's DB2 relational database management system then imports the resulting relational database files. IBM's DiscoveryLink™ software and the CLSD software allow biomedical researchers to transparently query any or all of these diverse data sets without necessarily knowing where any particular piece of data originally resided. This transparency allows, for example, the joining of data from different databases into a single record of information presented to the researcher. CLSD also incorporates BLAST datasets, permitting a researcher to run a BLAST job as if executing a database query.

Clinical and research labs can also make their data available to other IU investigators via CLSD. CLSD and its underlying database products enable clinicians and researchers to provide data securely and with control over who has access to the data being provided. They may contribute data in a wide variety of relational and non-relational formats, including DB2, Oracle, Sybase, SQL Server, Informix, flat files, Excel, and XML. This system provides a quick, simple, and secure means for sharing data while ensuring that all researchers with rights to access the data make use of the most current versions of those data. Indiana University’s University Information Technology Services staff wrote the CLSD system and several of its underlying “parser” programs. They will develop Portlets for the CLSD to incorporate its data into the Grid portal strategy described in Core 1.

IV.7 USER SUPPORT FOR TEST SOFTWARE:

The nature of the software the Center for Tissue Modeling produces will require that, from time to time, users employ software that is made available via Grid services in test mode. All tools will come with test suites. This mode of operation implies that users will occasionally encounter problems that result from system malfunctions, not user errors. The discovery of actual bugs in a tool can trigger several possible reaction paths. First, some tools (“Bronze” support) are supported remotely and the bug should be referred back to the original organization that owns the tool. Other tools (“Silver” support”) — those that have come fully into the public domain— need at least to have their bugs documented. Finally (“Gold” support) — locally developed tools or tools that have been significantly modified locally — will need to be tracked and repaired.

To this end, we will use an open source problem tracking system called Bugzillia (http://www.bugzilla.org/). Bugzilla is a web-based problem tracking system with a wide variety of reporting, which the Bugzilla team actively supports. We have had good experiences with it on other projects. Genuine bugs here will serve as feedback to Core 1 as part of its software benchmarking process.

IV.8 SOFTWARE ENGINEERING SUPPORT:

The development of the software systems for this project presents numerous challenges, both in the development of individual tools and in their integration. Further challenges are presented by the presence of legacy code on numerous development platforms (FORTRAN, C, C++, Matlab), each with its own input and output data, visualization mechanisms, and control formats. To address these issues, we will develop a software architecture and development framework that promotes the integrated use (and reuse) of software artifacts (including legacy code). The framework will be driven by the interface specifications required for integration of software components into it. The specification of application-independent data formats will facilitate data interchange (and system integration). This integration will require:

- Software architecture and design that promotes reusability of software artifacts (including legacy code). Reuse has proved particularly difficult for parallel code that cannot use many standard commodity tools. However systematic use of parallel libraries has been successful.
- Developing interfaces for legacy and new code.
- Availability of data in application-independent formats.
- Toolboxes for users to develop their own applications from available functions.
- An appropriately designed and generic front end (GUI) that allows full exploitation of the power of the web. This GUI will use user-facing Web service ports as described earlier.
- A web-based environment for distributed collaborative software development.

Note that our use of Grid and Web Service technology has two complementary aspects. It provides the functional integration of software modules to drive sophisticated simulations and their linkage with data sources. Second, it provides a powerful software engineering framework suitable for distributed teams as those in the Center for Tissue Modeling.

Implementation: The development and integration challenges for this project occur on multiple scales. At the source code level, within individual programs, we seek to re-use source code components. At a larger scale, modules and libraries must inter-operate within applications. At a still larger scale, whole applications must be integrated to cooperate with each other. Different technologies will tend to be more effective (and more appropriate) at different levels of such an architecture. At the lowest level, object-oriented, generic, and generative programming techniques will provide source-code interfaces for re-use. At the application (highest) level, web services, grid services, and the common component architecture (CCA) will provide interfaces for integration. In between, at the library level, we may use techniques from both the source-code level and the application level.

Development: The use of a web-based software management system being developed as part of a separate research project at IUB (http://www.cvshome.org) will facilitate collaborative development between members of the project team. This web-based system will encourage and enable software best practices for development, including version control, unit and system testing, document management, and real-time collaborative tools, among others. We will also use the real-time collaboration technology to enhance cross-institution software development. Indiana University will maintain a CVS repository of software under development.

We plan to expend significant effort encouraging the open source philosophy to work effectively to support and advance the tools we create. We will create a thorough set of documentation for the TST itself and its interoperability tools – essentially creating an Applications Program Interface (API) standard for the TST. A working group will manage this API, under the direction of the organizational structure of the CTM detailed in Core 7.

To be effective, the open source philosophy has to be open to contributions from groups at major centers as well as from individual investigators. We will adopt the model of the Free Software Foundation, in that we will have a formal process for submitting code, we will invite the more technically-inclined range of the user

community to test contributed code, and then the CTM will certify approved production releases of the TST and component tools in the toolkit.

IV.9 MAINTENANCE AND END-OF-LIFE:

When large, collaborative, computer-intensive research projects in the US have been successful, one of the keys to their success has been that the project is designed in a fashion that ensures robustness of the project and availability of software and services, independent of the participation of individual institutions involved in the project. We will follow this approach.

As regards software availability, all software produced by the Center for Tissue Modeling will be Open Sourced (most likely under the terms of the Lesser GNU Public License, thus permitting subsequent commercialization of software we develop if the market deems it of value). At some point in the development process moving source code repositories from private CVS repositories managed within the CTM to a public repository such as SourceForge (http://www.sourceforge.org/) will be appropriate. We expect SourceForge to be a robust, ongoing repository for open source code. IU has an excellent ongoing history of supporting open source projects through its participation in providing publicly available mirrors of other source code archives (http://www.ussg.iu.edu/index.php?option=com_downloads, ftp://ftp.ussg.iu.edu). The Center for Tissue Modeling will also escrow copies of all source code directly with the NIH and NCRR.

Robustness of service delivery will come via the CTM's participation in the NSF-funded TeraGrid (http://www.teragrid.org/) and the NIH-funded NCRR.

The universities participating in the CTM are committed to its ongoing viability, and to the ongoing viability of the TST. The Indiana University Knowledge Base provides an example of the level of commitment and persistence of IU's involvement in computing support. The Knowledge Base has existed in one form or another since the days when a VAX 11/780 was a new and cutting-edge piece of high-performance computing – or in other words, since the early 1980s. The universities involved in the CTM are committed to its ongoing survival, and will seek funding from state sources, national sources, and private charitable trusts to ensure the viability and support of the TST in the long term. That base funding at Indiana University has permitted the persistence and development of a key component of the support mechanism for the TST is perhaps the best proof of the ongoing commitment to support and development of the software tools we create.

We plan to license the TST under open source terms that will permit commercialization of the code. If the code is successful, and we expect it to be, the market place should provide for its support.

A key part of our support strategy is the development and fostering of a community of expert users and programmers for the TST. All three participating institutions have a strong record of staff development, including formal career paths that provide for classification grade and salary advancement strictly on the basis of technical expertise. By providing mechanisms for the technical staff of the CTM to have a local career path, we will ensure that we retain institutional knowledge within the CTM. At the same time, we recognize that recruitment of staff away from the CTM will spread knowledge and expertise as well. This knowledge diffusion has already proved to be the case with other software projects managed by IU, such as our ongoing support of fastDNAml – a program for maximum likelihood inference of evolutionary relationships. At present three former student users of fastDNAml hold tenured or tenure-track positions at universities in the US, as well as staff that have been recruited away from Indiana University, carrying with them significant expertise with fastDNAml as they go, thus spreading understanding of this software and improving the research community's ability to support it through the open source process.

IV.10 INITIAL DELIVERABLES AND TIME-LINE FOR CORE 4: As described earlier, the activities listed above are those we have identified as possibilities to be evaluated during the first few years of the CTM. In the first year we will begin many projects with a one to two-year time frame. We will phase in new projects as other projects end, in a fashion to best support the work in other cores, with input from NIH and outside users.

Our anticipated timeline is below. Note that duration listed below is that of the major developments, we expect ongoing refinement and bug fixes after the initial focused development phase.

Deliverable	**Start Time**	**Duration**
Current TST running on PUNCH, Alliance Portal	Year 1	1 year
Tools supporting WSDL-based software engineering	Year 1	1 year
Portlet-based biology portal based on existing NSF NMI and alliance portals	Year 1	1 year
Create a help-desk.	Year 2	1 year
Maintain project's publicly-available visualization/analysis tools.	Year 2	2 years
Introductory documents in IU Knowledge Base	Year 2	1 year
Local CVS Repository and Bugzilla in place	Year 2	1 year
Wizard for construction of new portals with portlets for major outside resources and full support of SBML and other metadata	Year 2	2 years
Provide documentation for CPM and visualization tools	Year 2	2 years
Web portal provides grid access	Year 3	1 year
Tools developed at other NIH centers available online	Year 3	3 years
Code repository to SourceForge	Year 5	1 year

CORE V: EDUCATION AND TRAINING

V.1 COORDINATOR:

Geoffrey Fox

V.2 PARTICIPANTS:

James Glazier (IUB), Mark Alber (ND), Jo Davisson (Purdue), David Hart (IUB), Craig Stewart (IUB), Dennis Gannon (IUB), Andrew Lumsdaine (IUB)

V.3 INTRODUCTION:

In accordance with the priorities outlined in the NIH Roadmap, the educational goal of the Center for Tissue Modeling (CTM) is to educate scientists, clinicians and technologists to combine a deep knowledge of biology with the mathematical, computational, and physical sophistication needed to address the increasingly complex problems of post-Human-Genome-Project biology. The Center for Tissue Modeling builds on the foundation of the existing Indiana Biocomplexity Consortium (IBC). As such, the existing educational infrastructure of the IBC supports and integrates the educational goals of the CTM.

A fundamental feature of the IBC training programs, which we outline in more detail below, is that we require students to obtain cross-disciplinary training. In particular, those with a background in the biological sciences must obtain training in computational and physical science, and those with backgrounds in computational and physical science must obtain training in at least one aspect of the biological sciences in order to graduate from IBC training programs. Our goal is to produce researchers who are equally comfortable with the languages of developmental and cell biology, molecular biology, computer science, mathematics, and physics. The Institutions in the IBC are meeting these educational challenges through a range of coordinated approaches, including: creating new explicitly interdisciplinary graduate programs; developing new interdisciplinary courses to be made freely available to all consortium institutions; revising existing departmental graduate curricula to include a broader range of courses; offering cross enrollment between participating institutions; sponsoring long-term research visits by students, postdocs and faculty, seminars, journal clubs, and workshops with participants from diverse departments, disciplines, and institutions. We plan to take full advantage of electronic approaches to disseminating learning and information, including the placement of all IBC course materials in an online knowledge center. This archive will allow information sharing between Institutions and easy distance education via video-conferencing facilities and on-line enrollment.

V.4 ACCOMPLISHMENTS:

IUB's Department of Physics has in place a new interdisciplinary syllabus in biophysics and biocomplexity, which combines physics, biology and computer science courses with new specifically interdisciplinary courses. The new degree in biotechnology offered by the School of Medicine at IUPUI takes a similar interdisciplinary approach as does the Ph.D. program in scientific computation currently being developed jointly between the College of Arts and Sciences and the School of Informatics at IUB. The University of Notre Dame is in the process of instituting a certificate in the study of biocomplexity, and already has in place a series of new interdisciplinary courses, including a required semester of biology for all freshmen engineers and computer scientists. Purdue University is establishing a master's level computational life sciences program. This program, a seven-school, multi-discipline program covers bioinformatics, computational biology and systems biology from the modeling and theoretical point of view. These achievements demonstrate that interdisciplinary education is already thriving at CTM institutions and provide an ideal foundation for CTM education and training.

V.5 EDUCATIONAL PROGRAMS:

V.5.i INTRODUCTION AND OVERVIEW:

The current disciplinary divisions of science (e.g., biology, chemistry, physics, etc.) not only isolate specific research disciplines, but also partition the teaching of science as well. We must proceed beyond this state of affairs to address the educational needs of the scientists and technologists who will advance bioscience and medicine in the 21st century. Research in biomedical computing and systems biology requires students with a solid background not only in biology and computer science, but also physics and applied mathematics. The CTM and IBC will work together to provide training to graduate students, post-docs and faculty members, by developing programs with the following common features and goals:

- Development of explicitly interdisciplinary courses, especially at the interface between computational and biological sciences.
- Reduced specialization: long lists of required discipline-specific courses inhibit exploration of other fields and prevent acquisition of the expertise necessary for true interdisciplinary research.
- Cross-disciplinary training: in particular, the CTM programs require students with advanced training in biomedical and behavioral sciences to obtain training in computer science, and *vice versa*.
- Research co-supervision: students in these programs must have thesis co-advisors from different disciplines.
- Fellowships for long and short-term travel: the CTM will support research and training opportunities at institutions both inside the center and in the greater scientific community.
- Promotion of optimal use of technology for distance learning, including remote audio/visual interaction and web-based courses.
- Sponsorship and organization of graduate research seminars, colloquia, and workshops on interdisciplinary topics.
- Availability of all programs to interested participants at the post-doctoral and faculty levels.

In addition to these common features, each CTM training program has unique aspects. Program-specific attributes, explained in more detail below, allow participating researchers and institutions to experiment with new interdisciplinary courses, approaches, and perspectives. We will share the lessons learned from these different implementations at yearly workshops specifically dedicated to assessing the educational effectiveness of different CTM programs.

V.5.ii PHILOSOPHY OF EDUCATION AND TRAINING:

As an example of the philosophy guiding CTM educational efforts across participating institutions, we describe here the key aspects of the CTM programs presently in place on the IU Bloomington and Indiana University Purdue University Indianapolis campuses.

The ultimate goal of the CTM educational program is to graduate trained professionals ready to enter academia and industry with the skills necessary to contribute to science and medicine in the 21st century. Modern biomedical science is inherently interdisciplinary, and the key to creating interdisciplinary research opportunities in this field is not in *making* it happen, but in *letting* it happen. To accomplish this goal, we first remove the pressure on students to specialize. In a traditional mono-disciplinary structure, a student without

this pressure might feel lost or overwhelmed. To reduce this pressure we also fundamentally alter the structure of the student-department relationship. Most people feel more at home in one environment than another. Students interested in our programs pick a "home department" in which they feel most comfortable, or in which the nature of that discipline most suits or appeals to them. From there, students accepted into the CTM will be guided and supported both by their program and by a CTM faculty co-advisor in another department as they learn to reach through a variety of channels to gain the knowledge and skills necessary to combine and expand upon discipline-based approaches (see Core 7 for more information on student admissions and funding).

The CTM program includes active participation in research combining experience in theory, experiment, and computation. Students are required to have an advisor both in the home department and in the CTM, and are encouraged to establish strong relationships with members of multiple departments and institutions as well, for example, including members of their thesis committee. Thesis committee members will be from diverse and complementary fields. This body of faculty mentors plays an essential role in the CTM education, providing the breadth of the knowledge and experience necessary to support student research and training at the interface between traditional disciplines. Students are encouraged to participate in research at outside institutions and are able to do so without going through lengthy channels of approval. This outside experience may include long-term residences in remote laboratories for specialized training and research, or internships in industry. The Office of Research and the University Graduate School at IUB has committed $100,000/year to support multi-institutional educational and research efforts by the CTM, of which $40,000 will be used to provide long-term travel support.

We strongly encourage students to take advantage of existing outside opportunities for training, especially laboratory courses like those offered by the Marine Biological Laboratory at Woods Hole and Cold Spring Harbor, and theoretical and computational courses like those at the Santa Fe Institute and the University of Illinois, Urbana-Champaign. We will provide financial support to enable students to undertake such training. Students from center-participating institutions have an excellent track record of gaining admission to highly competitive courses such as those offered at DIMACS, NECSI and the University of Connecticut (see attached letters of support). Finally, whenever possible, we will arrange for students to spend at least one semester training in the laboratory of a member at another location. Again, we have already established a successful track record of such placements.

Students gain a number of distinct advantages from this type of open educational environment, and perhaps fluidity is foremost. During their participation in CTM programs, students are constantly collaborating with workers in different fields, reading papers from different fields, and participating in experiments that use techniques from different fields. They write papers with researchers in other fields. By the time they leave, no subject relating to biomedical science will be so foreign to them that they won't be able to incorporate some of what that field has to offer into their research and project some of their own expertise back into the pool. Students successfully completing a CTM program will ultimately obtain a degree or certificate, depending on the program chosen. Even more significant, they will have the practical knowledge necessary to conduct research at the interface between disparate disciplinary cultures.

We recognize that we cannot expect anyone to arrive an expert from the beginning in all necessary areas of biology, computer science and physical science. To make the coursework and cross-training the CTM programs require easier, we are developing a set of graduate-level bridge courses for sophisticated graduate students who are new to a particular area. Our first such course at IUB, "Genomics for Physicists and Mathematicians is being taught this year by Prof. Peter Cherbas, the director of the IUB's Center for Genomics and Bioinformatics. We are planning comparable courses in applied mathematics, computer science and physics for students who need this additional background. Since CTM programs may require more coursework than traditional disciplinary syllabi, we plan to make funding available to support students who wish to take additional courses or even semesters of courses. We feel that the extra course opportunities are much more important than haste in training, and the support supplied by the CTM can remove the impression on the part of departments that they are being asked to pay for 'unnecessary' courses.

Other participating institutions have already incorporated aspects of this model into their graduate programs and are examining other approaches for future implementation. The inter-institutional aspects of the CTM have

several important educational outcomes. First, we encourage departments to allow graduate students to conduct their Ph.D. research with any Center member regardless of home department or institution, provided that they also maintain a co-supervisor in the home department. In part to assist graduate students in finding appropriate co-supervisors, we are planning to organize a series of interdisciplinary and interdepartmental seminars with speakers to include all resident active members of the consortium. We also encourage students to choose Ph.D. committee members from other institutions. These meetings will also provide an opportunity for all CTM-supported graduate students and post-docs to present and communicate their work. Future plans include development of a summer school in mathematical biology for graduate students and postdocs.

V.5.iii OVERVIEW OF INDIVIDUAL GRADUATE TRAINING PROGRAMS:

V.5.iii.(a) Indiana University—IU Bloomington

Because physics education forms an ideal platform from which to explore issues in quantitative biology, we have extended the graduate physics program at IU Bloomington with a recognized Ph.D. degree in biophysics. Within the biophysics group we would like to create an intellectual climate where one can apply the precise language and unifying principles characteristic of physics to understand the intricate ways in which Life adapts matter to its needs. In keeping with this ideal we have introduced a curriculum for graduate students that guarantees an education with a solid core of physics courses, but one that also incorporates education in a number of topics in biology, selected because they form natural inroads for physicists into biology. The student we have in mind for this program is the traditional physics bachelor, who will have been exposed to the usual undergraduate courses in physics, but who will have little formal education in biology. We think it is very important, besides offering these students a core physics package, to expose them to biological topics early on. This means that the curriculum includes more general areas of physics (classical mechanics, electricity and magnetism, statistical physics and quantum physics), but substitutes biologically oriented subjects for topics in specialized fields, such as high energy, nuclear, and condensed matter physics. We have had several Ph.D. students participate under this program in the 2003-2004 academic year and anticipate annually growing enrollments for the foreseeable future.

IUB is in the middle of a substantial expansion in interdisciplinary biosciences/computational biology/biocomplexity. Between the School of Informatics and the Department of Physics, under the leadership of Dean Subbaswamy of the College of Arts and Sciences, Dean Dunn of the School of Informatics and Dr. Glazier, the faculty in this area has increased from two in 2002-2003 to five in 2003-2004 with four planned hires this year and a commitment for at least an additional 12 hires over the next three years, with a roughly equal balance between experiment and computation. One indication of the seriousness of this interdisciplinary effort is that several of the new faculty have neither M.A. nor Ph.D. degrees in the disciplinary of their home department.

Indiana University Bloomington is also in the process of establishing a Ph.D. program in scientific computation. An interdisciplinary committee of computational sciences faculty from disciplines including computational systems biology, molecular and biochemical modeling and simulations, theoretical biophysics and soft condensed matter theorists are developing the program with the goal of training students conceptually in the basics of scientific computation. They will then be allowed to choose mentors in any of the basic scientific disciplines. Scientists trained in this manner will be well equipped to function in an interdisciplinary environment. We expect the program to be approved and ready for implementation by the fall of 2005.

V.5.iii.(b) Indiana University—School of Medicine

The School of Medicine graduate faculty will propose an interdisciplinary Ph.D. minor in Tissue Modeling that would be associated with the Center for Tissue Modeling. There are currently ten separate basic biomedical science Ph.D. degrees affiliated with School of Medicine faculty that are described at http://www.medicine.iu.edu/~gradschl/Ph.D./index.html. These degrees are administered by the School of Medicine, located on the Indiana University – Purdue University at Indianapolis campus. There are approximately 200 Ph.D. or combined M.D./Ph.D. students in these programs. Two Ph.D. programs are interdisciplinary (medical neurobiology and medical biophysics). The medical biophysics has just been

reorganized to focus on biomolecular imaging and we will collaborate with the biophysics faculty in the Department of Physics at Indiana University Bloomington in this new degree focus.

The Indiana University Graduate School requires that all Ph.D. students complete a ~12 credit minor in a subject outside the core Ph.D. discipline. These minors can be in another discipline (either basic science or IUPUI school like Informatics, Computer Science, biomedical engineering) or in an interdisciplinary topic. We have successfully used the interdisciplinary minor in two ways: NIH Institute oriented minors and collaboration with disciplines outside the School. For example, we have established minors in Diabetes and Obesity, Cancer Biology and Aging. These minors have a defined teaching faculty with a director, special didactic courses and a seminar series. The goal is to write NIH T32 training grants in these areas, and we were successful in obtaining one from NIDDK last year. We have collaborated with the IU School of Informatics and Purdue Department of Biomedical Engineering on the new minors in bioinformatics and biomedical engineering, respectively. Students usually take 2-3 core courses from the academic unit and 1-2 elective courses from a focused list of courses. The minor representative serves on the student's advisory committee. Similarly, in our proposal for a Tissue Modeling minor, we will identify training faculty, select a minor director, identify core courses and outline a seminar series for the program. We believe that this is an outstanding way to expand interdisciplinary training in the Ph.D. programs at the School of Medicine and to foster research collaborations between School of Medicine faculty and the Center for Tissue Modeling.

In addition to the proposal of a Tissue Modeling minor, students on both the IUPUI and IUB campuses have the opportunity to participate in new courses being developed in biomedical imaging, biomedical engineering and biotechnology at the IUPUI campus.

V.5.iii.(c) Indiana University—School of Informatics

The School of Informatics at IU, initially operating at the Bloomington and IUPUI campuses, will eventually include all eight IU locations. It currently has over 1000 undergraduate and 175 graduate students. Of the latter 75 are in the Bioinformatics masters program. The CTM can benefit from their experience in setting up new educational initiatives both from a programmatic point of view and by the exchange of course offerings. We plan to develop biomedical computing courses that are cross-listed in Informatics. Further, several of the basic Informatics courses will be useful in preparing students in the different systems biology disciplines for cross disciplinary study; for example, Informatics has experience in preparing computer science-trained students in biology and vice-versa.

V.5.iii.(d) Purdue

Purdue University has established an interdisciplinary graduate specialization program in computational life sciences (CLS) at the master of science level. The program provides students with the opportunity to study a specific science or engineering discipline along with gaining skills in CLS. The objective is to produce students who are fluent with both computational tools and techniques in the life sciences. These skills, in turn, will help prepare them for discovery and implementation of algorithms that facilitate the understanding of biological processes. Computational life sciences combines interdisciplinary study in bioinformatics, Computational Biology, and Systems Biology. The program is expected to be offered in the fall of 2004.

V.5.iii.(e) Notre Dame

The Notre Dame Interdisciplinary Center for the Study of Biocomplexity (ICSB) supports education and research in the field of biocomplexity at both graduate and undergraduate levels. ICSB efforts include the sponsorship of interdisciplinary classes, a seminar series, distinguished lectures, and frequent workshops attracting researchers from across the nation and around the globe. As an institution, Notre Dame is greatly expanding opportunities for interdisciplinary education. The new Notre Dame Science Teaching Center, presently under construction, contains a laboratory dedicated to the teaching of interdisciplinary biological laboratory techniques. The Engineering Learning Center currently provides an excellent opportunity for students to study and work together on group projects. One major curricular change, supported and encouraged by the ICSB, has been the development of a biology class specifically for engineers and students

of the physical sciences. This course (first offered this spring) is required for all freshman engineers, and will be made available in a modified form to graduate students as well. In addition to curricular support and development, the ICSB provides financial support for education in the form of fellowships for the short and long-term visits of our students to other major institutions, as well as the short and long-exchange of faculty members between institutions. To further support interdisciplinary research and to recognize students with significant accomplishments in the field of biocomplexity, we are in the process of instituting a certificate in the study of biocomplexity. This program has a number of important aspects as described below, but in particular it creates an educational framework for students interested in problems at the boundaries between traditional disciplines and provides a measure of recognition and reward for those making significant achievements in these areas.

V.5.iv OVERVIEW OF OTHER TRAINING PROGRAMS:

V.5.iv.(a) POSTDOCTORAL PROGRAM:

At the postdoctoral level the CTM will require that all experimentalists also have a theoretical or computational supervisor and *vice versa*. Center workshops and seminars will provide a forum for postdocs to learn new methods. We will encourage postdocs to audit or enroll for credit in graduate course offerings outside their area of training.

At the Indiana University School of Medicine, there are approximately 260 postdoctoral fellows, including 11 institutional T32 training programs for pre- and postdoctoral fellows. The school is in the process of reorganizing the Graduate Division Office to charge a new associate dean with oversight of both pre- and postdoctoral training. The goal is to establish a postdoctoral database and specific training opportunities for postdoctoral fellows. This year we established a new one credit course, “Introduction to Research Ethics”, that is designed to meet needs for student and postdoc instruction in animal use, human subjects, conflict of interest, copyright and other topics important to research ethics. We also offer a voluntary Preparing Future Faculty program for students and fellows who desire discussions and opportunities in teaching in higher education.

V.5.iv.(b) TECHNICIAN TRAINING PROGRAM:

In response to the expanding frontier in biotechnology and the biomedical sciences, the Indiana University School of Medicine has created the Biotechnology Certificate Training Program. The program, which began in August 2002, offers a graduate certificate in biotechnology from the IU Graduate School. The program provides the latest instruction, hands-on laboratory courses and an interactive problem-based learning experience. The curriculum includes laboratory work in molecular biology/genomics, cell biology, and proteins and proteomics. It is designed for the continuing education of research technicians with baccalaureate degrees in academic and industrial laboratories as well as new college graduates who want to enhance their knowledge and skills in the latest biotechnology areas. The certificate program takes a minimum of one-and-a-half years to complete (three full-time semesters and one summer session); however, the program can be completed part-time in about two-and-a-half years.

The IU Biotechnology Training Program is part of the Indiana Genomics Initiative (INGEN), a landmark endeavor made possible with a $105 million grant from the Lilly Endowment to Indiana University. IU School of Medicine's top research faculty serve as program instructors. Students in the program have access to the most modern INGEN and School of Medicine core research facilities for cellular imaging, DNA sequencing, proteomics, gene expression, protein expression, flow cytometry and bioinformatics. A teaching laboratory in Indianapolis was newly completed for the 2002-03 academic year.

V.5.v. ADMINISTRATION AND INFRASTRUCTURE:

Through effective administration we will create new pathways for students to learn about and participate in research at the intersections of many fields in a way that is smooth and natural, so students will be able to cross departmental and institutional lines without feeling alien or having to risk straining loyalties.

Effective administration will mean managing the formal structure of interaction across departments and institutions. This will include people, policies, procedures, and also hardware such as video linkups, computing facilities, web based tools etc. A student will not need to go through laborious application procedures or tedious paperwork to benefit from resources at other departments or institutions. Clear procedures will be in place for this. Equipment can be shared easily with a minimum of bureaucratic hassle. Technology will be linked in a cooperative manner to make sure that all participating departments are using compatible hardware software and security protocols for easy communication. These details will be managed by support staff to free up students and researchers to do their academic work.

Global issues related to graduate education in the CTM, including cross evaluation of the various programs, will be addressed by a Graduate Studies Committee composed of one representative from each institution. These representatives will serve as liaisons between the CTM and participating departments and graduate programs. Other administrative issues relating to specific graduate programs are discussed in Core 7 or in the detailed program descriptions below.

V.5.vi DISTANCE LEARNING:

Indiana University and Purdue University own the I-Light network, an optical high-speed network that connects Purdue's West Lafayette campus, the Indiana University-Purdue University Indianapolis campus, and the IU Bloomington campus. State funding has recently been awarded the I-Light 2 project, which will extend this network to most college and university campuses in the State of Indiana. This network infrastructure will form the basis for a suite of high-speed, high-bandwidth, high quality tele-collaboration installations throughout the state. Tele-collaboration and distance learning will be enabled by use of Access Grid technology, discussed, in Core 1, which permits one-to-many instruction and many-to-many collaboration. An installed base of advanced visualization equipment (including John-E-Boxes, described in Core 1, installed at four of IU's eight campuses) will provide for high quality visualization experiences by students involved in distance learning and other collaborative activities.

This technology is already being used successfully in enabling two faculty members, one at IU, one at Purdue, to teach together a computer science class to a group of students taken from three campuses: Purdue West Lafayette, IUPUI (Indianapolis), and IU Bloomington. The technology is easily extensible and effective, and can be used not only for teaching and distance education throughout the state but throughout the world via the Access Grid. Courses will be offered for credit through IU's various distance education programs. The CTM plans to make full use of I-Light's capabilities in all facets of research, education, and outreach.

V.5.vii CONCLUSION:

Scientists and teachers emerging from our educational programs will be biologists who know how to take full advantage of computers for modeling and mathematical models for quantitative results; physicists who understand the fundamental questions needing to be addressed in medicine and biology such as the biomechanics of tumor growth, the fluid properties of blood, and the electrical impulses in neurons; computer scientists versed in the latest computational models being used in biology and medicine who understand the living systems being modeled. They will all have experience with the latest tools and technologies. With the power to draw from many sources and the ability to communicate fluidly across disciplines, graduates from CTM institutions will be ready to solve the problems waiting for them in the world at large.

To achieve this goal, we will study the successes of other institutions of integrative study, such as the Indiana Biocomplexity Institute at IUB, The Center for Complex Systems at Cornell University, and the Graduate Program for Cross Disciplinary Research Training in Mathematical Biology at the University of Utah. We will then expand our existing programs, initiatives and infrastructure at the participating institutions.

The CTM will be a vibrant educational atmosphere with as many doors, and as few walls as possible. Our past experiences with this type of cross-fertilization and networking is that research blossoms; researchers develop in a balanced way, both strong and flexible, that enables them to tackle the hardest problems without running out of tools, resources, or ideas. Since many of the necessary changes have already begun at the participating institutions, we can hope to achieve our goals quickly and in a cost effective manner.

V.5.viii GRADUATE PROGRAM DETAILS BY INSTITUTION:

V.5.viii.(a) IU Bloomington—Graduate Curriculum in Biophysics:

Over the last decades, biology has become noticeably more quantitative. Experimental techniques in molecular biology have evolved rapidly, leading to spectacular results. A host of technical developments in electronics, miniaturization, optics etc. has led to unprecedented levels of measurement accuracy in a wide variety of biological systems. In parallel, digital data recording has proliferated widely, and data storage and processing capabilities have increased dramatically. As a consequence of these developments, quantitative analysis has gained prominence within the biological community as a valuable addition to its intellectual arsenal. On the other hand, workers from traditionally quantitative fields, such as physics, find an ever wider variety of concrete interesting biological problems to work on. The result of all this has been a noticeable increase in movement of people and ideas across the disciplines. Tangible expressions of these trends on the Bloomington campus are the proposed construction of a multidisciplinary science building, the support for biocomplexity research through the commitment to Excellence (CTE) initiative, and the very recent recognition by IBM of Indiana University as a Life Sciences Institute of Innovation.

Initiatives such as these need to be matched by changes in educational opportunities, and these are indeed occurring at several physics departments throughout the country. In this spirit, recognizing that traditional undergraduate physics education forms an ideal platform from which to explore issues in quantitative biology, we are extending the graduate physics program at IU Bloomington with a recognized Ph.D. degree in biophysics starting in fall 2004. As to the formal requirements of the program, to qualify for the graduate program we propose to follow the model that was adopted earlier for the astrophysics program. In other words, the students should follow the core physics courses required for the first day of the qualifying exam (Classical Mechanics, Electricity and Magnetism I, and Statistical Physics). Biophysics students thus take the first half of the qualifying exam together with the “regular” physics students. The second day of the qualifying exam will be especially geared toward the biophysics component of the program, and may contain components in the overlap area between biophysics on the one hand and classical mechanics, statistical mechanics, and electrodynamics on the other. By having a requirement for two biophysics courses (Introduction to Biophysics, and Mathematical Biology) in the first year we should have enough material to assemble a written exam in biophysics. The biophysics portion of the exam will be prepared and administered by the biophysics group working with the curriculum and exams committee.

The program is flexible enough to allow students the choice to take the qualifying exam, or one of the two parts of the exam, directly in the first semester. Some students may have a more extensive background than just the bachelor’s degree, and we feel that it is in the student’s interest for this to be recognized by a formal exam. Of course, those students who wish to do this must be made aware of the risks and possible consequences of their choice.

V.5.viii.(a)(1) CURRICULUM FOR IU BLOOMINGTON BIOPHYSICS (TO BE IMPLEMENTED FALL 2004):

The proposed curriculum will contain the following courses:

- P575 (Introduction to Biophysics)
- P548 (Mathematical Biology)
- P581 (Modeling and Computation in Biophysics)
- P582 (Biological and Artificial Neural Networks)
- P583 (Signal Processing and Information Theory in Biology)
- P676 (Selected Topics in Biophysics)

With the exception of P548, all these courses are to be newly developed. Below are listed the short descriptions for these courses as they are proposed for the new syllabus:

Introduction to Biophysics: The aim of this course is to familiarize the students with a number of fundamental topics in Biophysics. Topics to be discussed are: Molecular level: biomolecules, chemical bonds, rates of reaction, biological membranes, receptors, ion channels, gating. Molecular assemblies: DNA and chromosomes, molecular motors and machines, cytoskeleton. Cellular level: sensory cells, nerve and muscle, cable properties. Multicellular organization: reaction-diffusion processes, pattern formation, morphogenesis. Sensory and motor systems: signal and noise, inference, neural computation. Behavior: psychophysics, animal behavior. Mathematical topics: Nernst equation, diffusion, Brownian motion, Hodgkin-Huxley equations, statistical inference, signal detection, ideal observer theory.

Mathematical Biology: Mathematical analysis of genetic and metabolic networks, molecular motors, actin and microtubule dynamics, oscillatory phenomena, chemotaxis and differential adhesion, coat patterning during embryonic development, wound healing, angiogenesis, neural development, limb development, tumor growth, population dynamics, flock models.

Modeling and Computation in Biophysics: Introduction to modeling and computational methods applied to phenomena in Biophysics. Topics: Population Dynamics. Reaction Kinetics. Biological Oscillators. Coupled Reaction Networks. Network Theory. Molecular Motors. Limit Cycles. Reaction Diffusion Models. The Heart. Turing Instability. Bacterial Patterns. Angiogenesis.

Biological and Artificial Neural Networks: Biological details of neurons relevant to computation. Artificial neural network theories and models, and relation to statistical physics. Living neural networks and critical evaluation of neural network theories. Students' final projects will consist of programming networks and applying them to current research topics.

Signal Processing and Information Theory in Biology: Probability and statistics. Filtering. Correlation functions and power spectra. Time invariant and time-varying systems. Shannon Information. Coding and decoding. Processing of sensory signals and other applications to neurobiology and psychophysics.

Selected Topics in Biophysics: The course will present current topics in biophysics research through lectures on recently published papers. The scope is widened by presenting those results in the context of earlier relevant work. In some cases, several papers may be treated that highlight a subject from different viewpoints, or even disagree in their conclusions. It is expected that students in this class participate actively by preparatory reading and participation in class discussions.

V.5.viii.(a)(2) DESCRIPTION AND MOTIVATION OF THE COURSE OF STUDY:

The following list of courses is not meant to be exhaustive, and students will also be able to choose approved classes from other departments and institutions.

Fall, Year 1:

All three proposed courses are mandatory: P521 and P506 are part of the standard physics requirements, and are topics for the first day of the graduate qualifying exam. The biophysics course for this semester is P575 (Introduction to Biophysics). The idea is to expose the students to a number of core subjects in biophysics, and to familiarize them with the work done within the biophysics group. Offering such a range of choices may pique their interest in specific topics, and may inform their choice of more biologically-oriented courses later on in the program.

Spring, Year 1:

In this semester we propose two required courses, namely a core statistical physics course (P556), and a course in Mathematical Biology (P548). In addition to this the students choose a course from a list of biological, psychological and neurophysiological topics. The fall and spring semester should have prepared the students to take the qualifying exam, modified as described above.

Fall, Year 2:

As in the spring semester we propose a core physics course (P511, Quantum Mechanics I). In addition we propose to require P581 (Modeling and Computation in Biophysics) because we feel that a student graduating from a biophysics program should be exposed to a high-level computational course.

Spring, Year 2:

This semester has one required course (P676 Selected Topics in Biophysics). For the two other courses the student has a broad range of choice, ranging over various disciplines. By this time the student should be associated with a research program, and we think that in this phase he/she should pick at least one or two courses that are relevant to the particular field of research.

V.5.viii.(b) Indiana University School of Medicine:

At the School of Medicine, students have the opportunity to enter through an individual Ph.D. program or through Open Admission where they have up to one year to find a laboratory mentor and program that best fits their educational needs. After identifying a mentor, an advisory committee is formed to monitor selection of graduate courses, including the tissue modeling minor option. The committee also serves to advise the student on research. After completing the qualifying exam and advancing to candidacy (usually after the first or second year), students form their research committee. With the mentor, the committee advises students on research directions. The mentor and this committee are the most important factors for success in research training. All students in the tissue modeling minor will take the Introduction to Research Ethics course by the end of their second year.

The curriculum for an interdisciplinary Ph.D. minor in Tissue Modeling will require development of a faculty proposal and approvals by the School of Medicine graduate advisors, IUPUI Graduate Affairs Committee and IU Graduate School. However, from previously approved proposals of interdisciplinary minors, a likely proposal would be: two core courses from the Bloomington biophysics curriculum such as P548 (Mathematical Biology) and P581 (Modeling and Computation in Biology) plus two elective courses from biophysics or relevant areas at IUPUI that are shown below.

Number	Department	Title
BME 601	Biomed Eng	Principles of Biomedical Engineering I
BME 602	Biomed Eng	Principles of Biomedical Engineering II

CSCI 548	Comp Sci	Introduction to Bioinformatics
D501	Anatomy	Functionally-Oriented Human Gross Anatomy
D502	Anatomy	Basic Histology
D527	Anatomy	Graduate Neuroanatomy
B500	Biochemistry	Introductory Biochemistry
B807	Biochemistry	Protein Structure and Function
B810	Biochemistry	Cellular Biochemistry and Regulation
F592	Physiol	Introduction to Biomolecular Imaging
F804	Pharmacology	Introduction to Pharmacology and Toxicology I
F814	Pharmacology	Introduction to Pharmacology and Toxicology II
G613	Physiol	Advanced Cellular Imaging
G614	Physiol	Advanced Molecular Imaging
G706	Physiol	Cell-Cell Communication
G818	Physiol	Integrative Cell Biol
G817	Graduate	Eukaryotic Cell Biology
G823	Graduate	Methods in Cell Biology
G841	Graduate	Methods in Proteomics
G865	Graduate	Fundamental Molecular Biology
G890	Graduate	Methods in Molecular Biology and Pathology
G910	Graduate	Advance Molecular Biology Methods
J805	Microbiology	Molecular Immunology
J828	Microbiology	Virology
K516	Biology	Molecular Biology of Cancer
I501	Informatics	Introduction to Informatics
I590	Informatics	Topics in Informatics
Q612	Genetics	Molecular and Biochemical Genetics

A very important issue for success in courses in the Tissue Modeling minor will be successful video conferencing of courses on the Bloomington, Indianapolis and West Lafayette (Purdue University) campuses.

The School of Medicine faculty have good experience with BME 601 and BME 602, which is a video conferencing course that has been in operation for three years between the Indianapolis and West Lafayette campuses. The success of the course is not only dependent on high quality equipment and support for conferencing but also the willingness of key faculty to meet periodically with students on the remote campus. Traveling of faculty to other campuses also enhances research collaborations among investigators.

V.6.viii.(c) ADMINISTRATION AND INFRASTRUCTURE - IU MEDICAL SCHOOL:

The Tissue Modeling minor at IUPUI will be administered by the associate dean for graduate studies in the School of Medicine in collaboration with the associate dean for the graduate school in Indianapolis. The formation of a core training faculty in Indianapolis will ensure that there is suitable participation of research faculty in the School of Medicine in the Tissue Modeling Center and the Tissue Modeling graduate minor. The resources for students to find good research mentors at the School of Medicine are excellent. There are 198 full, 123 associate and 6 affiliate members of graduate faculty in the School who can serve as mentors to the approximately 200 Ph.D. and M.D./Ph.D. students. The entire School of Medicine faculty generated $187 million in research funding on 1078 grants.

There are excellent core research resources at the School of Medicine http://www.medicine.iu.edu/research/cores/ largely due to the establishment of key core facilities through the INGEN project http://www.ingen.iu.edu/. As indicated above, laboratory training courses are being established by the Biotechnology Training Program faculty to assist students and postdocs in the use of core biotechnology resources. There is a state-of-the-art teaching facility in the new Biotechnology Research and Training Center with excellent video conferencing resources and computerized classroom and projection resources. These resources will be made available to students, postdocs and faculty affiliated with the Tissue Modeling Center.

V.5.ix PURDUE

V.5.ix.(a) COMPUTATIONAL LIFE SCIENCES:

Computational Life Sciences, positioned at the intersection of modern biology, quantitative modeling and high performance computing, is helping to provide a fundamental understanding of complex biological systems and offers the potential to significantly impact a wide variety of technologies, including drug discovery, novel therapies for human, animal and plant diseases, metabolic engineering and efficient production of traditional and high-value foodstuffs. Research in biology spans > 10 orders of magnitude in size, from the interactions within a population of animals that lead to coordinated behavior in ecosystems to the atomic interactions within and between populations of molecules in a cell. Traditional (reductionist) approaches in biology have studied individual components and rigorously defined their behavior. However, these components do not exist in isolation, and biology will only be completely understood by treating these phenomena as complex systems. We are experiencing a revolution in the increased technological capacities to rapidly generate large complex data sets that quantify the amount, spatial locations, and biochemical properties of molecules within individual cells or populations of cells. These data are available for the breadth of biological diversity, from humans to agronomically important crops to infectious bacteria or viruses. The chemical and mechanical properties can now be measured and simulated with high throughput methods, leading to an explosion in the rate of data generation; however, data generation must be linked to analysis and understanding. CLS is the field suited to turning this torrent of data into useful science and information. The program is expected to be available in the fall of 2004.

Computational Life Sciences can be divided into three major categories:

Bioinformatics: The research, development or application of computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those to acquire, store, organize, archive, analyze, or visualize such data.

Computational Biology: The development and application of data-analytical and theoretical methods, mathematical modeling and computational simulation techniques to the study of biological, behavioral, and social systems.

Systems Biology: The development of quantitative, mechanistic based models of the whole cell, collections of cells or large pieces of the cellular machinery, where the objective is an integrated picture that complements the reductionist viewpoint of molecular biology.

Purdue University has established an interdisciplinary graduate specialization program in computational life sciences (CLS) at the *MS level*. The program provides students with the opportunity to study a specific science or engineering discipline along with gaining skills in CLS. The aim of the program is to produce students who have learned about computational tools and techniques in the life sciences. These skills, in turn, will help prepare them for discovery and implementation of algorithms that facilitate the understanding of biological processes.

Six Charter Departments offer CLS specialization: Agronomy, Biological Sciences, Chemical Engineering, Computer Sciences, Statistics, Computer Technology, and Electrical and Computer Engineering. Currently, the CLS graduate committee consists of one representative from each Charter Department, as well as the director of the CLS program, the director of the CSE program, and the coordinator of the CRI educational programs.

Students who wish to join the CLS program are expected to have a strong interest in the various aspects of life sciences (see section 5). Their undergraduate training is expected to have provided them with a strong foundation in several areas of life science, engineering, statistics, and computing. Because some students may not have both the interdisciplinary breadth and depth needed for the CLS program, students are given the opportunity to round up their background with appropriate course work. Ten five-week modules are specifically designed to provide key concepts from upper level undergraduate courses in life sciences, engineering, statistics and computational methods. The purpose of these modules is to assist students in achieving a common preparation level for entry into higher level courses in the CLS program.

Counseling students in CLS is the primary responsibility of the major professor for research matters. Assistance to CLS students in routine educational matters is provided by the CLS representative of each participating department. Documents are available to guide the students in matters concerning specific requirements and procedures. The routine registrar and graduate school forms are signed by the CLS graduate representative, unless specifically directed otherwise.

V.5.ix.(b) Curriculum for the CLS Specialization:

The CLS curriculum consists of a sequence of *CLS introductory modules* (five weeks each), *CLS core courses* and *CLS relevant courses* specified by the participating departments. No separate M.S. Degree examination is required for the CLS specialization. The plan of study, worked out by the student and the major Professor, must be approved by the graduate committees of both the participating department and the CLS program. Further details regarding the CLS M.S. requirements of each charter department are provided in section 7. Ph.D. students, in a CLS participating department, who wish to acquire the CLS specialization for the MS degree, will have access to the introductory modules, core courses, and relevant courses of the CLS program. (The requirements listed here may be superceded by the CLS requirements of participating departments.)

V.5.ix.(c) Courses in the CLS Curriculum : CLS Introductory Modules:

At the entry level, the Computational Life Sciences program offers, five-week modules (1 credit each) for CLS students having diverse backgrounds. The students and their major Professors may select from these

modules in order to best meet their individual needs. These five-week modules are offered in Life Sciences, Computational Methods, Engineering, Statistics and Computer Technology. The modules are designed to assist the CLS students in completing their background for studying the graduate core courses of the CLS specialization. It should be emphasized that a CLS introductory module in home department X is designed to educate those CLS graduate students not in department X. Therefore, students in department X would not receive credit for modules offered in department X. Additional conditions may be imposed by participation departments. All these modules will have relevance to concepts in Life Sciences. A brief summary of the proposed CLS introductory modules (five weeks each) are as follows.

V.6.ix(c)(1) Life Sciences:

Computational Methods for Life Sciences

Objective: These modules give students a working knowledge of a variety of Computer Science concepts and their applications in computational life sciences. Moreover, these modules will provide the students with the basic understanding of how to formulate computational life science problems and the basic tools for solving these problems. While the conceptual derivation and theories underlying the tools will be presented, primary emphasis will be on working with libraries and existing tools where possible. Throughout, connections to life science applications will be emphasized.

Engineering Models for Life Sciences

Objectives: Development of mechanistic models in a biological context (e.g. the kinetics would focus on reactions with inhibition that are common in enzyme catalyzed reactions, electrical conduction would be for neuron firing, solid mechanics would examine the bending of actin fibers, etc.). The models would be simple one-dimensional idealizations. Analytical solutions of models would be limited to algebra and linear first and second order ordinary differential equations the solutions of which would be taught in the course. Most solutions would be obtained using Matlab, where instruction in the use of Matlab would be provided both in class and via online tutorials.

Statistics for Life Sciences

Objectives: of the Statistics modules (Probability and Statistics) are to provide a foundation upon which later bioinformatics concepts will be laid. Fundamental concepts in probability, expectation, and Markov chains are necessary to understand much of the modeling performed in sequence alignment, data base searches, and mathematical processes. Concepts from Statistics will complement those topics covered in the probability module by providing the necessary tools for the analysis of experimental data and the associated biological interpretation. Many of these statistical tools are used for the analysis of data from quantitative trait locus (QTL) mapping, differential expression (gene and protein), evolution, and other biological applications.

Information Systems for Life Sciences

Objectives: These modules will provide students with the requisite foundation upon which subsequent specialized courses in computational life sciences systems analysis, design, and implementation will be based. The Information Systems Architecture Module (Module 1) will provide technical background concerning hardware, operating systems, CLS application software, networking, interfacing to life sciences instrumentation and the overall architectures and protocols required to build such systems. The Short course in Systems Analysis and Design (Module 2) will provide students with process-oriented experience in requirements gathering, system modeling, data modeling, and design of acceptance test and validation scenarios for CLS information systems.

V.5.ix.(c)(2) CLS Relevant Courses:

In addition to the above CLS core courses, the CLS Program maintains a list of CLS relevant courses offered by the participating departments. These courses have substantial bioinformatics or computational biology

relevance and are accessible to interested students from other departments. For more information, see the Program website.

V.5.x. UNIVERSITY OF NOTRE DAME

At Notre Dame, educational activities associated with the Center for Tissue Modeling will occur under the auspices of the Interdisciplinary Center for the Study of Biocomplexity (ICSB). At Notre Dame, the ICSB supports a vigorous program of biocomplexity seminars and colloquia. Speakers within the last year have included**.** A number of biocomplexity-related courses have already been implemented with the help of the ICSB, including a required biological science course for all freshmen engineers, and more are planned. At present, study by students interested in biocomplexity and related fields is accomplished within the confines of existing departments. However, we are in the process of instituting a "Certificate in the Study of Biocomplexity". This program has the following aspects:

To obtain a certificate in the study of biocomplexity, students will:

1. Be admitted to any department (the "home department") in the Schools of Science or Engineering

2. Complete all of the normal graduate requirements within their home department.

In addition, the student will:

1. Take at least two classes addressing the study of biocomplexity, at least one of which must be outside their "core" area*. Acceptance of these classes towards the certificate must be approved by the graduate studies committee. If acceptable to the home department, these classes may be counted towards the normal graduate requirements within the department, but if not acceptable, these classes will be in addition to the normal requirements.

2. Participate regularly in the biocomplexity seminar for at least two full years. This seminar has two components: a journal discussion for biocomplexity students and faculty, and seminars by outside speakers. Candidates for the certificate are expected to participate enthusiastically in both.

3. Choose as a member of their thesis committee a faculty member who is both outside their own department and outside their core area of study.*

4. Perform a thesis project that addresses at least one aspect of biocomplexity and is interdisciplinary in nature. The choice of topic and committee member must be approved by the biocomplexity graduate studies committee.

Students who have completed the necessary departmental and Certificate coursework and advanced to candidacy with an approved thesis topic will be eligible to compete for fellowships. Those receiving fellowships will be designated as "Fellows" of the ICSB for the remainder of their time at Notre Dame.

V.5.x.(a) ADMINISTRATION AND INFRASTRUCTURE – NOTRE DAME CERTIFICATE IN BIOCOMPLEXITY:

Because the students seeking this certificate will be normal members of their home departments, administrative loads associated with this program will be minimal. Students will normally apply to the program after their first year of graduate study, but as long as all requirements are met, the timing of admission is flexible. The certificate will be awarded upon graduation. Decisions on admissions and other issues associated with administration of the program will be made by members of the ICSB graduate studies committee (appointed by the ICSB executive board).

V.5.x.(b) INTERDISCIPLINARY COURSES PRESENTLY OFFERED BY NOTRE DAME FACULTY INCLUDE:

MATH 434/534: Mathematical and Computational Modeling in Biology and Physics

Introductory course on applied mathematics methods with emphasis on modeling of physical, mechanical and biological problems in terms of differential equations and stochastic dynamical systems. Students work in small groups on independent modeling projects and present them in class at the end of the course as well as at the spring “Biocomplexity Day” meeting. Syllabus includes: Linear and nonlinear systems of differential equations; nonlinear methods of empirical analysis; Markov processes in biology; applications of these methods to problems including population genetics, epidemiology, bioinformatics, and development.

Math 611: Nonlinear Dynamical Systems

Description: A dynamical system consists of an abstract phase space or state space, whose coordinates describe the dynamical state at any instant; and a dynamical rule which specifies the immediate future trend of all state variables, given only the present values of those same state variables. This self-contained course on nonlinear dynamical systems includes: review of the linear and nonlinear dynamical systems, bifurcation phenomena, and transition to chaos. The theory of nonlinear dynamical systems has applications to a wide variety of fields, from mathematics, physics, biology, and chemistry, to engineering, economics, and medicine, and examples are drawn from all of these fields.

Computer Science and Engineering 498J/598J: Simulation of Environmental Biocomplexity

This course will introduce and apply simulation theory and techniques to modeling Environmental Biocomplexity. Biocomplexity refers to the dynamic web of interrelationships between physical, biological, geochemical, hydrological, environmental, ecological, social, and economic systems. The study of biocomplexity includes systems that range from molecular to global in scale, and exhibit properties that depend not only on the individual actions of their components, but also the interactions among those components. Simulations will be built using the JAVA programming language, and packages such as SWARM, StarLogo and Mathematica. The course will focus on modeling the unique characteristics of biocomplexity using simulation and artificial life. Course requirements: students should have had a semester programming course (or equivalent).

CSE 598E. Computational Methods in Biomolecular Modeling

Study of algorithmic and computational issues in biomolecular modeling: multiple scale solvers for molecular dynamics, performance of several serial and parallel implementations, software engineering for scientific computing, and requirements for interactive modeling.

Chemistry 650, 651: Computational Chemistry I, II

An overview of the fundamental theory, methodology, and applications of computational chemistry. Topics include simulation techniques such as molecular dynamics and Monte Carlo as well as a wide range of quantum chemistry methods. Applications center on organic molecules and biological systems such as proteins and DNA. Hands-on computer experience is an integral part of these courses. (Fall and spring)

Chemistry 627 Molecular Biophysics

As science progresses towards atomic-level understanding of biological processes, it is often helpful to address biological questions in physical chemical terms. This merging of disciplines is referred to as molecular biophysics. Important biological processes that benefit from physical chemical thinking include protein folding, molecular recognition, and regulation of biological process by temperature, pH, and small molecules. Fields such as structure-based drug design, protein structure and prediction, structural studies of biological mechanisms, as well as advances in proteomics require biophysical thinking. Topics covered include a review

of classical thermodynamics, a primer in statistical mechanics, the hydrophobic effect, transition state theory, kinetics of macromolecular interactions, and influence of membranes on interaction thermodynamics. Course is discussion-based with a focus on problem solving; active student participation is required.

Chemistry 628: Bioinformatic Approaches to Protein Structure and Function

As biology moves into the post-genomic era, a significant challenge will be to utilize the enormous amount of sequence information present in our databases. A classic problem in biochemistry is the relationship between protein structure and function. This course will concentrate on using modern methods in bioinformatics and molecular evolution to extract information about protein structure and function from these still-expanding databases. Course will be hands-on and include a basic discussion of the theoretical underpinnings of the methods used. Topics include similarity searching, structure prediction algorithms, calculation and interpretation of phylogenetic trees, and three dimensional homology modeling. Graduate students and upper level undergraduates from any background in science or engineering are encouraged to participate.

V.6 TIMELINE:

Most of the activities are already in place, and many will be in place soon after the start of the center. Major academic initiatives such as the biocomplexity Ph.D. will take longer to introduce.

Year of Introduction	**DESCRIPTION**
1	Continue existing Interdisciplinary Classes and graduate programs Implement new classes and programs: IU Biophysics, ND Biocomplexity Certificate Continue existing seminars and workshops Review Curricula across institutions
2	Begin Computational Biology Ph.D. in IU Bloomington Review results of first year graduate programs recruiting and effectiveness Tune programs in response to this evaluation
3	Customize CTM website for outreach and training - Prepare CTM courses as Distance Education offerings - Offer all seminar proceedings online for dissemination to CTM members, students and the greater scientific community Institution of first annual meeting of all CTM faculty, students and postdocs Major evaluation of CTM education effectiveness
4	Continuation, with emphasis placed on developing additional new interdisciplinary courses.
5	Continuation, with major evaluation of the programs.

CORE VI: OUTREACH

VI.1 COORDINATORS:

James Glazier (IUB), Craig Stewart (IUB)

VI.2 PARTICIPANTS:

David Moffett (Purdue), Mary Papakhian (IUB), Andrew Arenson (IUB), Gilbert Rochon (Purdue)

VI. 3 INTRODUCTION

The Outreach Core will focus on outreach to several groups in several ways as follows:

- We will reach out to the community of traditionally-trained biomedical researchers (both NIH- and NSF-funded) to increase the adoption of computational biology tools by expert researchers and experimentalists whose research can be enhanced by incorporating computer modeling into their research program, thus enhancing biomedical experimental research and providing data that will improve the quality of the Tissue Simulation Toolkit.
- We will reach out to the community of traditionally-trained computer scientists, encouraging them to focus on adding new computational tools to the suite of back-end computational tools incorporated in the TST and encourage such scientists to partner with biomedical researchers in development of new models.
- We will reach out to current undergraduate and graduate students in areas spanning the life sciences and computer science, helping to create a new generation of scientists who, regardless of their core discipline, see a collaboration between computer and life sciences as a natural and profitable—and even essential—component of their research activities.
- We will reach out to individuals from traditionally underserved groups at all stages of their career to draw a broader spectrum of America's diversity into the field of computational biology.

The specific mechanisms by which we will accomplish these goals are as follows:

- Software repository and center web pages to allow the tools and results of the center to be accessed by the biology community.
- Short courses on the center tools offered in the center sites and in other NIH centers around the country. Theses courses will be given simultaneously by the Access Grid to allow easier remote participation.
- Center newsletter with printed and electronic versions.
- Center seminars with major presentations held in Access Grid-enhanced lecture halls, allowing full participation of outside participants.
- An annual center scientific workshop integrated into the highly successful semi-annual series already organized by IUB and Notre Dame
- Semi-annual "all hands" meetings bringing all center participants together for three-day meetings. The center advisory committee will meet concurrently.
- Visitor Program to bring experts to the participating institutions in the center.
- Industry outreach to allow exchange of information and to enhance use of our tools in the life science industry.

- Outreach to individuals from traditionally underserved groups to increase both the broad awareness of our tools and the number of qualified people entering the computational biology pipeline.

These items are detailed in the following sections of Core 6.

VI.4 SOFTWARE REPOSITORY AND CENTER WEB PORTAL

Easy access to facilities that can be used to run the Tissue Simulation Toolkit, and easy access to software for download, form critical elements of our outreach. Distributing access and source code easily will greatly facilitate adoption of the Tissue Simulation Toolkit across all of the categories of individuals outlined above.

We described our software dissemination and portal strategy in Core 1. Our portal will be built using the portlet architecture, and correspondingly all features in our portal will be delivered by services. We find this approach supports independent production of web page components. Furthermore, the same uniform approach supports conventional web pages, the interfaces to compute and data resources; it will be applied to both the TST Problem Solving Environment and the outreach web resources.

The outreach web portal must at once support the software dissemination and both specialist and generalist access to the center's knowledge base described in Core 4. We will use our existing portal research to offer through the portal the needed collaborative functions to participate in seminars and meetings. These portlets include Access Grid and Webcam support (using Java Applets), shared display for presentations and the full range of CHEF capabilities from Michigan. The latter has, for example, a calendar and text chat capability. Other portlets will access the center's technical reports, presentations and other documentation.

The software dissemination function of the center will include both documentation and software for download. The software will be provided with a clear open source license. A given tool will often use software packages from multiple sites and we will provide links to these and clear statements as to licenses they carry. We will always encourage use of software with simple open source licenses such as that used at Indiana University. The software in the repository will be divided into three categories: "Gold support" for tools produced by the center where we provide technical support and training. For the convenience of users of our portal, we will list software in "Silver" and "Bronze" categories. The "Bronze" category includes software considered useful by the center but for which only cursory checking has been performed. "Silver" support category corresponds to software that is not actively supported but for which we have performed some testing and checking to see that interfaces are compliant with data standards. Software "retired" from the "Gold" category—perhaps because it is no longer considered the best algorithm—would be placed in the "Silver" category. We expect to list outside software of importance to tissue modeling in either the Bronze or Silver categories. As discussed in Core 4, the Gold category software will be supported by:

- Bugzilla-based registry for tracking and reporting bugs
- Email access to technical staff for outside queries on the tools
- News groups for users of tools
- News items of changes and important issues with the tools

These capabilities will be supported in the center portal.

VI.5 SHORT COURSES ON CENTER TOOLS, TUTORIALS, AND APPEARANCES AT NATIONAL CONFERENCES

Groups supported in Core 1 to produce center tools will be required to provide both documentation and tutorials or short courses covering their tools and their interaction with other software. We would expect two short courses to be typical, although a popular tool with ongoing support could have many more. We will offer all short courses with audio-video conferencing and shared display collaboration support. The courses will be recorded for future access. We will support Access Grid, Polycom, RealNetworks streaming and VRVS

conferencing using the GlobalMMCS Web Service approach developed in the Community Grids Laboratory (http://www.globalmmcs.org). Any presentations or other electronic material used in the courses will be posted on the center portal.

These short courses will be complemented by new curricula described in Core 5 using the center tools and results. All courses will be assessed and the feedback used to improve later offerings. The web site will include a survey allowing us to decide which courses need to offered. Typically courses will be offered at Center locations with external users handled by the Access Grid support. However, we will work with other NIH centers to offer short courses in remote locations where they could have particular value.

The center will offer a minimum of four short courses on tissue modeling. The courses differ in content and intended audiences. Two courses will be aimed at biologists who wish to learn modeling techniques, one for graduate students and post-docs and one for faculty in mid-career. The other two courses will be aimed at computer scientists who wish to pursue modeling of biological systems. Again, one course will be for graduate students and postdocs, and one for mid-career faculty. Courses will emphasize modeling and make use the tissue-modeling toolkit. They will be taught on the campus of one of the participating institutions, and on-campus housing will be available to participants. Short courses will also be taught in compact form as tutorials at national conferences. Stewart is already well known for his tutorials on computational biology at the world's largest international conference, the annual SC conference (Stewart, 2003).

Also assuring rapid dissemination of early research results is our record of high-profile collaborative research displays at international conferences (http://www.research-indiana.org/, http://www.research-indiana.org/2002/, http://www.research-indiana.org/2001/). We recently hosted a statewide conference to disseminate information about the virtual campus grid and to foster collaborative research (http://www.iupui.edu/~ilight/workshop.html); this could easily include national TST users. Our researchers disseminate research experience via national and international standard-setting organizations and working groups. TMC PIs, Co-PIs, and investigators have leadership positions in the Global Grid Forum, the DOE-funded Common Component Architecture Forum, and NSF Network Middleware Initiative projects. The consortium member institutions also have leadership roles in functional areas (FA) with the DOD High Performance Computing Modernization Office Programming Environment and Training Program. IU's Dr. Geoffrey Fox is the FA lead for the Online Knowledge Center.

All of these efforts will have the effect of reaching out to all of the targeted individuals to create a large and diverse community of TST users.

VI.6 CENTER NEWSLETTER

We will feature an online newsletter where the items are both added by the center participants and by the newsletter editor. This will be supported by our portal where a secure service with a portlet interface will support users adding, deleting or editing newsletter contributions, technical documents and general portal material.

The portlet security model will support roles for users, including the ability to create new material, to edit all or some of the material and to suggest edits to material they are not authorized to change.

The online newsletter will be the basis of regular printed newsletters and center summaries that we currently expect to produce on a quarterly basis. These plans will of course be tailored to support any NIH center-wide policies that may be developed.

VI.7 CENTER SEMINARS

As in all such major academic activities, there will be many seminars of relevance to the center with themes varying from biological to computer science and their intersection. The online calendar in the portal will be used to announce these seminars. There will be seminar series organized by both the center in general and by the different core activities and held in a variety of locations among the participant universities and departments. We already have several locations enabled for audio-video conferencing and we will try to schedule as many

seminars as possible in rooms where we can use Access Grid or Polycom technology to allow participation by outside participants. This will of course help us bring the center participants together and further enhance our outreach to other NIH centers and the broader computational biology community.

VI.8 ANNUAL SCIENTIFIC WORKSHOP

The Center plans an annual workshop on tissue modeling. The goal of the workshops is to establish promising directions for research and to investigate new ideas. Workshops will be held in conjunction with national or international conferences on systems biology. Proceedings will be made available on the web and formally published as part of the conference proceedings or as special issues of journals. For example the best papers from 2nd IEEE International Workshop on High-Performance Computation Biology (HiCOMB 2003), will be published in a special issue of Concurrency and Computation: Practice and Experience edited by Geoffrey Fox who leads the Core 1 activities in our center.

The workshop will include the Cellular Potts Model as well as other approaches to tissue models and the areas of sub-cellular and continuum biology that interact with the tissue models. We will integrate this workshop with an existing highly successful series that holds semi-annual workshops that alternate between northern and southern Indiana locations.

The goal of the workshops is to establish promising directions for modeling and experiments and to investigate potential new areas of research. Workshops develop new inter- and intra-institutional collaborations, incorporating tutorials directed to students and researchers in disciplines outside the workshop focus. We have successfully obtained outside funding (NIH, NSF, Whitaker Foundation) in support of the workshops, with which we have subsidized student participation and increased each workshop's scope and size.

Members have extensive experience organizing international conferences. For example, members of the Notre Dame Applied Mathematics Group organized a 1996 International Conference on Current and Future Directions in Applied Mathematics. Dr. Mark Alber was a co-organizer of the Seventeenth International Symposium on Mathematical Theory of Networks and Systems at Notre Dame on August 12-16, 2002, where he is also co-organizer of a special mini-symposium on applications to biology. In addition, he has organized two invited mini-symposia on Applications of Nonlinear Dynamical Systems to Biology at the Fifth SIAM Conference on Control and its Applications, held jointly with the 2001 SIAM Annual Meeting in San Diego on July 11-14, 2001. (Notre Dame also participates in the Chicago Cytoskeleton workshop held monthly at Northwestern University.) Dr. James Glazier, besides organizing the Biocomplexity Consortium workshops, organized a mini-symposium on Organogenesis at the European Society of Mathematical and Theoretical Biology in Milan, Italy, in July 2002. The center will collaborate with other bio-centers in organizing large conferences.

VI.9 CENTER ALL HANDS MEETINGS

We will hold center-wide "all hands" meetings twice a year. These will alternate location between the participating institutions and feature tool and related research updates. All students associated with the center will be encouraged to present their work. These meetings will also allow planning meetings to steer the center work based on our and outside progress in the field. We also intend that the center advisory committee meet together at one or both of these meetings each year. These meetings will be the opportunity to demonstrate and get friendly feedback on early versions of tools and to practice course material being developed for outside users. We expect the meetings to last three days and we will locate them in rooms where we can use the Access Grid and Polycom technology. These "all hands" meetings will serve many functions in terms of outreach in the sense of building community. Such semi-informal meetings will be an ideal venue for the discussion of problems and development of collaborations between established biomedical researchers, established computer science researchers, and new students who will begin their careers viewing computational biology as a unique and important area of science.

VI.10 VISITORS PROGRAM

The visitors program provides an opportunity for faculty and graduate students from other institutions to take advantage of the center. The center provides facilities for research, a collegial atmosphere and covers some expenses. Visitors benefit from working in a group of people with similar interests. Visitors may stay for a few days up to a full year.

Visitors will be essential in ensuring that our work and the tools developed maintain both relevance to the broad community and embody the best available knowledge in the field. We will continue and enhance our existing practice to host visiting faculty and students for both short- and long-term visits, providing the opportunity to conduct experiments and analyses in the interdisciplinary environment of the center, and assist our members with conducting research elsewhere. Past faculty visitors include Dr. G. Thomas and Dr. R. de Almeida of Universidade Federal do Rio Grande do Sul, Brazil. Confirmed visitors to Indiana University Bloomington during the next year include Dr. Y. Hayakawa of Tohoku University, Japan, and Dr. Manuel Balaguera of the Universidad Javeriana, Columbia. This program is funded by a base component of the budget for the Center for Biocomplexity at Indiana University.

VI.11 INDUSTRY OUTREACH

Departments participating in the center as well as individual members have well-established collaborations with industry. Industry relations include both life science and computing-related companies, especially as the latter have identified computational biology as a key growth area. We already have strong contacts with H-P, IBM, Microsoft, and Sun on the computing side. Dr. Geoffrey Fox is the founder of a small company Anabas in the collaboration area. In life sciences our major relationships include Cardiogene AG, Genisphere Inc., Kraft Foods, Lilly (including many scientists at IUPUI and the Pervasive Technology Laboratories), and Morphogen Pharmaceuticals.

Industry collaborators benefit from the center in several ways: They can access the resources of the center and our experiences using computers and instruments. They can find a talented pool of students with the proper background for possible internships and future recruitment. The center coordinates enrollment of students in internship programs with industrial companies in biological research. Internship may result in students conducting research for their Ph.D. theses under guidance from an industry co-supervisor. Industry has an opportunity to participate in workshops and conferences on cutting-edge research. We will host short-term and long-term visits by industrial researchers to participate in specific scientific programs and lectures. Industry collaborators get rapid access to our results both in terms of science and supporting technology.

The benefits are, of course, two-way. Several of our students will take jobs with our industry collaborators. Computing companies can give us early access to new products and also to their architecture directions. For instance, our current research on grid technology is strongly guided by specific interactions with IBM as well as by general interactions with other vendors like HP, Microsoft and Sun at the Grid Forum. The life science companies can guide our research with their understanding of key practical issues. If a scientist spends time at institutions in our center, it is as much a benefit to us as it is the industrial collaborator.

As the project becomes established, we will review our industry partnerships and consider setting them up in a more formal fashion with a membership program offering different levels of involvement.

VI.12 OUTREACH TO INDIVIDUALS FROM TRADITIONALLY UNDERSERVED GROUPS

Center institutions are aware of the significant barriers to participation in interdisciplinary science. Each participating organization has resolved to effect disciplinary and cultural changes to reduce such barriers. We also recognize that traditionally underserved groups must play a major role in creating an interdisciplinary

cultural environment. Participating departments will actively recruit highly qualified students who reflect the diversity of the U.S. population as a whole.

The center will reach out to individuals from Traditionally Underserved Groups (TUGs) through its visitors program, its short courses and by assisting university efforts to recruit minority students. At all times the visitors program will reserve space for a faculty member visiting from a Minority Serving Institution (MSI). It will also reserve two slots in each short course for students from Minority Serving Institutions and pay all expenses of those students. The center will recruit for these positions by advertising at Minority Serving Institutions and other appropriate venues. Finally, the center will assist university efforts to recruit minority students in the following ways:

- advertise at historically black and Hispanic-serving institutions and within minority professional publications
- use search services with direct faculty follow-up contact to prospective students
- affiliate with consortia and professional societies that work to enhance minority representation in higher education
- fully fund campus visits for promising minority prospects

Purdue will be a host site for the NASA Research in Science and Engineering Program, which offers intensive summer research and training programs for students in historically black colleges and universities (HBCUs) and members of the Hispanic Association of Colleges and Universities (HACUs), and will offer training and use of the TST.

IU is teaching a new “Scientific Informatics” class at its Bloomington, Indianapolis, and Gary campuses. The Gary campus serves a higher percentage of students from underrepresented groups than any other Indiana college campus. We expect at least 20 African-American students per semester to take “Scientific Informatics,” which will include use of TST.

IU and Purdue computer and application sciences classes will reach hundreds of students using the IP-Grid, many from TUGs.

IU’s University Information Technology Services has a Minority Internship Program, providing paid internships (0.25% FTE) for women and minority students. Last year two interns worked in high performance computing. A minimum of two interns per year would have direct involvement in the ETF.

The Intel Foundation has provided funding to Purdue for a program to enhance diversity in information technology, science and engineering. Students from Purdue and minority-serving institutions receive funding to participate in relevant research and present their findings at major conferences and in peer-reviewed journals. Purdue will include use of IP-grid in this program.

The Community Grids Laboratory at Indiana University is working with a group of Minority Serving Institutions (MSIs) on the Community Grid of the Americas (CGA) aimed at bringing science to broader audiences, involving both information technology and discipline specific academic activities. It includes web service-based educational portals, a CGA Collaboratory and high-speed network links to participating MSIs. The project is centered in the AN-MSI collaboration between Educause (http://www.educause.org) and the EOT activity at the NSF supercomputer sites (http://www.eot.org). AN-MSI is an NSF-funded project involving 103 collaborating minority-serving institutions (MSIs)—historically black colleges and universities (HBCUs), Hispanic-serving institutions, and tribal colleges and universities. The goal of the AN-MSI project is to assist their member institutions in developing and upgrading their information infrastructure, and in developing educational and research applications utilizing information and communications technologies. CGA involves a variety of programs including faculty and student research (such as summer research experiences), workshops and collaborative classes. We will work with the CGA group to extend the current discipline focus of CGA from environmental science to include the biological sciences.

The University of Notre Dame (ND) is committed to recruiting into its graduate programs students from traditionally under-represented groups, including African-Americans, Asian-Americans, Hispanic-Americans, and Native Americans, as well as women in science and engineering where they are historically under-

represented. The graduate school at ND has an office dedicated to minority student recruitment, directed by Dr. Janice M. Poorman, associate dean for graduate recruitment and admissions. In close coordination with that office, our center will make a targeted effort to help participating departments recruit members of under-represented groups specifically into the TCM.

ND maintains active membership in ten different consortia and professional groups that promote graduate education for students from under-represented groups. These contacts will be used to promote graduate studies in biocomplexity at ND. For example, the national office of GEM (National Consortium for Graduate Degrees for Minorities in Science and Engineering) is located at Notre Dame. GEM will provide us with an annual list of minority candidates for graduate school, from which we will contact appropriate individuals and, together with various departments at ND, actively recruit them into the biocomplexity program.

VI.13 INITIAL DELIVERABLES AND TIME-LINE FOR CORE 6

As described earlier, the activities listed above are those we have identified as possibilities to be evaluated during the first few years of the center. In the first year we will begin many projects with a one- to two-year time frame. We will phase-in new projects as other projects end in a fashion to best support the work in other cores with input from NIH and outside users.

Our anticipated timeline is below. Note that duration listed is that of the major development—we expect ongoing refinement after the initial development phase.

Deliverable	**Start Time**	**Duration**
Newsletter	Year 1	6 months
Seminar series	Year 1	6 months
Portlet-based biology portal based on existing NSF NMI and alliance portals	Year 1	1 year
Scientific workshop	Year 1	4 years
All-hands meetings	Year 1	4 years
Create a help-desk	Year 2	1 year
Introductory documents in IU Knowledge Base	Year 2	1 year
Local CVS Repository and Bugzilla in place	Year 2	1 year
Short courses	Year 2	3 years
Visitor program	Year 2	3 years
Industrial partnerships	Year 5	1 year

CORE VII: ADMINISTRATION

VII.1 COORDINATORS:

James A. Glazier (Indiana University), Mark Alber (University of Notre Dame), Jo Davisson (Purdue University), David Stocum (IUPUI)

VII.2 PARTICIPANTS:

David Moffett (Purdue), Craig Stewart (IUPUI), Geoffrey Fox (IUB)

VII.3 SPECIFIC AIMS:

The goal of the administrative core is to fully support interdisciplinary research in systems biology and biomedical computing, focused initially on the projects described in the proposal. To achieve this goal, the Center must also support essential changes in scientific, academic, and educational culture necessary to further interdisciplinary inquiry. The organizational structure is designed to assist in:

- Integration of projects and support between institutions.
- Coordinated development of software tools.
- Continuous feedback between experiments and simulations.
- Deployment and validation issues for tools.
- Mechanisms for picking new biologically motivating projects.
- Setting up training programs for tool users.
- Outreach efforts.
- Reviewing new models of research and education for best practices.
- Disseminating information to the scientific community.

VII.4 CENTER STRENGTHS:

The proposed Center has many strengths. In recent years, departments at participating institutions have hired many faculty in Center-related areas who will contribute to this effort (see biographical sketches). Each institution brings particular areas of proficiency to the project: IUB in modeling of development, reaction kinetics networks and finite element models, tool development, grid and portal infrastructure, software support, parallel computing, massive data storage, computer science and microfluidics; IUPUI in vertebrate limb regeneration, cardiac development and disease, vascular development and disease, microarray technology and visualization; Notre Dame in computational modeling of development and networks and finite element models; Purdue in proteomics and reaction-kinetics modeling and software support. The Center helps coordinate hires in areas that will further this effort. Most important, institutions' research programs are complementary, covering all essential disciplines (computational, biological, and engineering-related).

- Indiana is an excellent location for the Center due to:
- Strong existing interdisciplinary systems biology and biomedical computing research community ties.
- Demonstrated institutional commitments to expanding systems biology and biomedical computing research and training.

- Historically strong and continually growing tradition of successful interdisciplinary centers.
- Vibrant graduate programs in all relevant departments.
- Highly leveraged investment.
- Strong state and industry support (*e.g*. PTL).
- Strong collaborations with outside research centers.

VII.4.i Established Interdisciplinary Research:

IUB and Notre Dame share one of the NSF-funded Biocomplexity initiatives on chick limb development, a project that brings together faculty from three departments with researchers at three outside institutions. This successful collaboration is one of the models for the CTM. IUB also is a part of a successful NSF Integrated Graduate Education and Teaching (IGERT) award with the University of Oregon, linking with IUPUI and the IU School of Medicine through the statewide INGEN Biotechnology program and the Center for Regenerative Biology and Medicine (CRBM), as well as through programs in medical sciences at IUB and Notre Dame which provide course training for students enrolled at IU School of Medicine.

VII.4.ii Institutional Commitment:

Individual participants have all been strongly committed to developing programs supporting systems biology and biomedical computing research well in advance of collaboration (see letters of support in Appendix I). Both IUB and ND are fully committed to interdisciplinary systems and computational biosciences and both are contributing substantial financial and material support ($458,000 per year in cash at IUB, plus cost shares, a new tenure track faculty hire and equipment matches and fee remissions; and approximately $170,000 per year at ND, plus cost shares, tuition and fee remission). The institutions currently participating in the Center have made a total of six computational biology-related hires in the past two years and have all made commitments for additional faculty hiring. IUB is committed to more than seven hires in the next two years (see the attached letters of support in Appendix I). In addition, construction will begin in March 2004 on the Multidisciplinary Sciences Building (MSB) I at IUB with 80,000 assignable square feet of space. The Center for Tissue Modeling will receive at least 6000sq.ft. of office and laboratory space in the MSB I. Space will also be available in the larger (and approved) MSB II (to start construction in 2005) and the planned Informatics Building (planned start date 2006). ND is currently planning a substantial Interdisciplinary Sciences Building to house the Center for Biocomplexity. In addition, the CTM benefits from the Biotechnology Resource and Training Center (BRTC), which houses the Center for Medical Genomics and the Biotechnology Training program at the IU School of Medicine, and will also benefit from the numerous faculty hires in related areas to which IU School of Medicine and IUPUI have committed.

VII.4.iii Tradition of Successful Centers and Indiana Collaborations:

All four participating institutions are home to a number of successful interdisciplinary initiatives, including the Center for Regenerative Biology and Medicine, the Center for Medical Genomics, the Center for Genomics and Bioinformatics, INGEN, the Pervasive Computing Laboratories, the Biocomplexity Institute and Consortium, and the Bindley Bioscience Center. Many of these include large scale collaborations of faculty from multiple departments, colleges and universities.

VII.4.iv Cooperation with External Centers:

The Center has a focus complementary to other major biosystems modeling centers. We therefore expect to work closely with existing and new NIH computational centers. Collaborating centers and projects include DIMACS, NECSI, BioSpice, SCIRun, Virtual Cell, the San Diego Supercomputing Center and Physiome and the Center for Mathematical Biology at Oxford. The Appendix includes letters of support in sharing and mutually supporting modeling tools and toolkits, education and training initiatives and in developing interoperability standards between software tools from these institutions. Cores 1 and 2 discuss in detail particular areas of focus in collaboration and cooperation.

VII.4.v Participation of the Center for Regenerative Biology and Medicine at IUPUI:

The Center for Regenerative Biology and Medicine at IUPUI (CRBM) was created in 2001 with $2.1 million in funding from the State of Indiana 21st Century Research and Technology Fund, the National Science Foundation, Eli Lilly & Co., and university matching funds. It is a joint effort among the School of Science of IUPUI, IU Medical, and the Medical Sciences Programs at IUB and Indiana State University. Dr. E. Chernoff (Dept. of Biology, IUPUI) directs the CRBM. CRBM associate director Dr. D. Stocum is a Center member. A nationally recognized scientific board and a commercial board from Indiana business and industry advise the CRBM. The center has three missions: research, academic training in regenerative biology and medicine, and technology transfer. We focus on the regeneration of neural, musculoskeletal, and cardiovascular tissues. A primary strategy is to discover differences between molecular pathways that define fibrosis (scarring) and those that regenerate the original tissue architecture. This will allow the design of a "molecular cocktail" of proteins or genes, which can be administered directly to a damaged tissue to induce regeneration by resident adult stem cells or by dedifferentiation of mature cells to create adult stem cells at the site of damage. This strategy bypasses all the immunological, logistical, ethical, and legal problems associated with obtaining and growing embryonic or adult stem cells. Compared to current practices and others under development, the CRBM approach would require less costly facilities, less retraining after recovery, and less expensive long-term healthcare.

VII.4.vi Participation of the Center for Medical Genomics at the IU School of Medicine:

The Center for Medical Genomics (CMG) was established and is supported by the Indiana Genomics Initiative and the Indiana 21st Century Research and Technology Fund to provide high-throughput gene expression and genotyping services to academic researchers across the state of Indiana. Dr. Howard Edenberg directs the CMG, with Dr. Matthew Grow overseeing spotted microarray operations. Dr. Grow is a Center member. For microarray needs, the CMG offers consultation, infrastructure, and expertise in the use of both Affymetrix Genechips® and custom spotted microarrays.

In addition to handling all physical aspects of microarray work following RNA submission, the CMG also assists investigators with data interpretation. For this bioinformatics support, the CMG has designed the Labrat LIMS and Microarray Data Portal (MDP), which together form a robust system for storing and analyzing microarray data. Through the web-based MDP, researchers can access raw expression data from their experiments, annotate their samples (compliant with MGED standards, http://www.mged.org) and perform a large number of analyses. Basic options include the sorting and filtering of the data, based on a standard two-sample t-test, fold change, log fold change, nonparametric statistics (Wilcoxon), and the fraction of Present calls in each sample set determined by Affymetrix™ MAS5 software. Researchers may also perform k-means, Estimation Maximization (EM), and hierarchical clustering of their data using the Microarray Data Portal.

The CMG's microarray support greatly expands Center members' capability to successfully execute and interpret microarray experiments. In order to assist and encourage members to use CMG resources to the fullest extent, the CMG will offer a variety of training workshops to students, faculty, and staff throughout the year. These workshops will be held in the state-of-the-art training facilities of the Biotechnology Research and Training Center (BRTC). When necessary, instruction will be teleconferenced via the web. The CMG also will work closely with Center researchers to train graduate students in transcriptional regulation and gene expression. Dr. Grow will suggest and develop microarray applications for the Center's BMPs.

VII.4.vii Participation of the Pervasive Technology Laboratories at IUB:

The Pervasive Technology Laboratories (PTL) at IUB, founded in 1999 with a $30 million grant from the Lilly Foundation, aim to move Indiana's economic focus from heavy manufacturing jobs to technology-based careers and industries. Individual labs, headed by distinguished scientists and supported by experienced technical staff and advanced IU students, work to improve information technology software in areas including grid computing, open systems, advanced communication networking, visualization, systems security, knowledge management, and other related focus areas in information technology, many of which are of central

importance to systems biology and biomedical computing.

To date the PTL have generated interest from several national companies, including technology heavyweights IBM, Sun Microsystems and Intel. The PTL are already leveraging existing IUB agreements with the Department of Defense, the Department of Energy, the National Center for Supercomputing Applications, the Naval Surface Warfare Center, Crane Division, and other organizations.

The Open Software, Visualization, and Community Grids Laboratory Directors are part of the Biocomplexity Consortium, as is the Science Director Dr. D. Gannon. The PTL also provide direct links to University Information Technology Services and the IUB Department of Computer Science. The Computer Science Departments of IUB and IUPUI have identified algorithms and software issues underlying biocomplexity as key focus areas.

The PTL will provide support and training for large-scale computation, parallel computing, image processing, visualization, and internet and database development for Center members. They will also establish software engineering principles and maintain a dedicated core of programmers to write commercial-quality code for projects. They will make major initial resources available for portals and simulation support for the CTM.

VII.4.vii Participation of the University Information Technology Services at IU:

The University Information Technology Services (UITS) at IU is home to a 1TeraFLOPS IBM SP supercomputer, a distributed Linux cluster (spanning IUB and IUPUI), significant data storage capacity (in excess of a Petabyte), high performance networking (such as Abilene, TransPAC, and I-Light) and support for mathematical and statistical software. The Research and Academic Computing (RAC) division of UITS has been actively involved in the past in supporting biomedical research and members of RAC currently act as the informatics core for INGEN and for the NIH-supported, Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Dr. Craig Stewart directs the activities of RAC and also acts as a Special Assistant for Life Sciences to the Office of the Vice President for Information Technology at IU. Dr. Stewart is a Center Coordinator.

VII.4.viii Cooperation with the Indiana Center for Biological Microscopy at IU School of Medicine:

The Indiana Center for Biological Microscopy (ICBM) has two combined confocal and 2-photon microscopes (Zeiss 510-NLO, Bio-Rad MRC-1024MP), an ultraviolet-visible confocal microscope (Zeiss LSM-510), a high-speed confocal microscope (Perkin-Elmer Ultraview), a high resolution cooled CCD imaging and image deconvolution microscope (Applied Precision Deltavision System) and dedicated microinjection and micromanipulation system equipped for high resolution DIC and video microscopy. The ICBM is also has numerous Pentium and Silicon Graphics workstations running advanced image processing software for analysis of both 2-dimensional and 3-dimensional images. The objective of the ICBM is to apply, develop and combine these imaging technologies to provide researchers with a rational, integrated approach to microscopic imaging. The unique strength of the ICBM is that it not only provides researchers with hands-on access to state-of-the-art imaging equipment, but also with the benefit of close interaction with the facility staff, who provide consultation, training and experimental assistance for researchers to optimally apply the imaging technology most appropriate to each particular research question. The ICBM is also actively involved in research into biological imaging, resulting in the development and dissemination of new methods of microscopy and digital image analysis software. The products of these activities are disseminated through a program of education, including seminars, courses and individual training. The facilities and skills of the ICBM will be available to consortium members on an at-cost basis.

For details on the ICBM see: http://www.nephrology.iupui.edu/Imaging/index.htm.

VII.5 GRADUATE PROGRAM ADMINISTRATION AND RECRUITING:

The Center will coordinate teaching efforts in systems biology and biomedical computing among institutions. We are in the process of reviewing program and departmental syllabi to determine best use of teaching and academic resources, especially in terms of bridging disciplinary boundaries. The Center will establish a formal curriculum review committee comprised of members of all departments to propose initial syllabus revisions.

Ongoing review will ensure currency. While Purdue's program in Computational Life Sciences is organized in five-week modules as opposed to the traditional sixteen-week terms used at the other institutions, course content across all curricula will be functionally synchronized and appropriate academic credit given for transfer students.

Center members will coordinate graduate and postdoctoral recruiting to improve the visibility of the program to applicants. Until we have completed the lengthy process of institutional and state approval for a Ph.D. program in biological computing, already begun at IUB, we will use departmental outreach as our base mechanism for recruitment. Currently individual departments receive many more applications from high quality students who would like to work on interdisciplinary problems than they can support from their existing stock of department-tied research and teaching assistantships. Departments will include Center informational materials in their promotional and admissions packets as soon as they are developed, a strategy that will allow the Center to focus on its specific priorities without having to address each institution's demographic particulars.

One of our first tasks will be to create promotional materials for graduate and postdoctoral recruitment. Initial packets will include:

1. A description of the CTM and its research and educational goals.
2. Faculty member profiles.
3. Center educational resources and program descriptions.
4. Descriptions of current and possible future projects.
5. Outside research and industry collaborations.
6. Funding availability.

We expect the interdisciplinary student salaries provided in this proposal to attract applications from students of exceptional quality.

An admissions committee comprised of a representative from each member department will teleconference frequently throughout the academic year to review scanned, electronically distributed graduate applications and recommend students to the Executive Committee for a decision on offers of funding. Departments are willing to admit all students the Center will support. The Center will adopt a policy of one-year terms on the admissions committee both to promote broad input into admissions decisions and to mitigate administrative demands on Center members.

VII.6 ORGANIZATIONAL STRUCTURE:

VII.6.i The Center for Tissue Modeling (CTM) overview:

To encourage the interdisciplinary collaboration and cooperation necessary to address systems biology and biomedical computing, we have created the Center for Tissue Modeling, which comprises the Biocomplexity Institute at Indiana University Bloomington (IUB**)**, the Interdisciplinary Center for the Study of Biocomplexity at Notre Dame (ND), and includes substantial participation by the Center for Regenerative Biology and Medicine at IUPUI, and the Center for Medical Genomics, the Department of Nephrology, and the Department of Pediatrics at the IU School of Medicine, as well as the Bindley Bioscience Center at Purdue. The CTM emphasizes multiscale modeling and quantitative experiments. The focus is on the physical manifestations that give rise to the complex structures of life. Unlike institutes spotlighting one level or system, the Center's approach is intrinsically multiscale, looking at determinants and mechanisms of development from molecular to organismal levels. We provide researchers from different fields with the opportunity to unite efforts and expertise to work on joint projects throughout the scalar spectrum of systems biology and biomedical computing.

VII.6.ii Management and Leadership Structure:

The overall organization structure is show in Figure 1. The basic structure is simple. Working groups will collaboratively pursue the best technical solutions to the various problems and challenges we propose to attack in this proposal. Lead scientists involved in the proposal will provide assistance and at times strategic direction decision-making. The project manager will ensure that deliverables are produced in a timely basis, and that results are disseminated broadly.

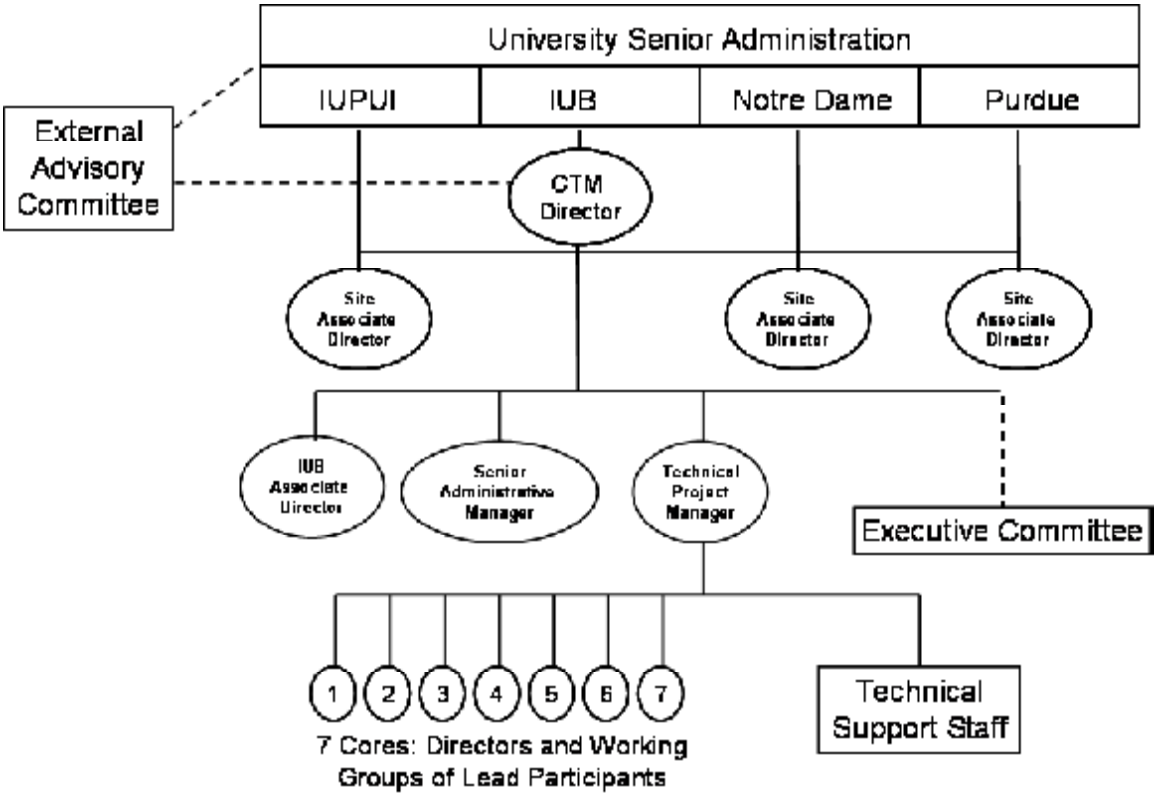


Figure 1. Organizational overview of the Tissue Modeling Center.

VII.6.iii Responsibility:

The CTM reports to the appropriate senior administrators at each institution. At IUB, the center reports to the dean of the College of Arts and Sciences (Dr. K. Subbaswamy), at IUPUI to the dean of Science (Dr. D. Stocum), at IU School of Medicine to the dean of the School of Medicine (D. C. Brater, MD), at Notre Dame to the vice president for research (Dr. J. Kantor), and at Purdue to Charles O. Rutledge (Executive Director, Purdue Discovery Park). The center as a whole reports to Dr. Subbaswamy via the executive committee, which is composed of members of all participating institutions.

VII.6.iv The Directors:

The responsible administrator at each institution (identified above) will appoint a director and an associate director to renewable four-year terms. The Center director at IU will be Dr. Glazier (Depts. of Physics and Biology and School of Informatics IU Bloomington). The associate directors will be Dr. Fox (Depts. of Computer Science and Physics and School of Informatics, IU Bloomington) and Dr. Sima Setayeshgar (Dept. of Physics, IU Bloomington). The director of the Notre Dame center will be Dr. Alber (Dept. of Mathematics, Notre Dame);

the associate director for ND is Dr. Holly Goodson (Dept. of Chemistry, University of Notre Dame). The director of the Purdue Center will be V. Jo Davisson (Dept. of Pharmacology); the associate director will be David Moffett (Research Computing Services). The senior Directors (Drs. Glazier, Fox, Stewart, Davisson, Stocum and Alber) all have extensive experience in large scale, interdisciplinary, multi institution project management.

The director will be responsible for:

1. Overseeing the functioning of the collaboration by providing scientific and administrative leadership.
2. Acting as a common thread between the administrative core, the executive committee, and the external advisory committee.
3. Overseeing the management of internal and external budgets, the submission of an annual report and other required reports to funding agencies.
4. Ensuring the dissemination of new results and tools (working in concert with the project manager).
5. Ensuring the compliance of the Center with university and NIH guidelines.
6. Holding an annual meeting seeking members' input on scientific and administrative issues.

VII.6.v Project Coordinators:

For each Biologically Motivating Project (BMP) project, the director will appoint a project coordinator who will be responsible for organizing collaborative research. The initial project coordinators will be Dr. Mathew Grow (IU School of Medicine, IUPUI), Dr Field (IU School of Medicine, IUPUI), Dr. March (IU School of Medicine, IUPUI) and Dr. Stocum (Department of Biology, IUPUI).

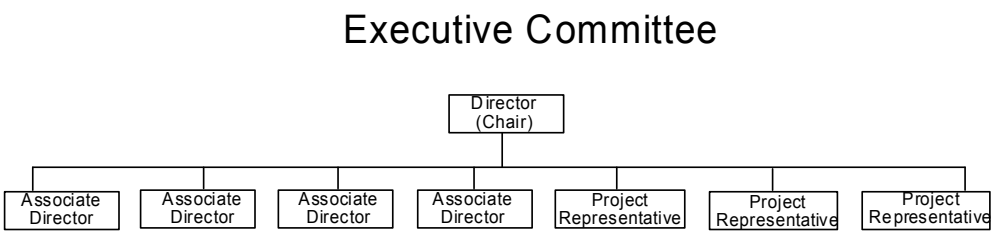
VII.6.vi Executive Committee:

The executive committee will consist of the director as chair, the associate directors and one representative from each of the three BMPs. The director will appoint these representatives for renewable three-year terms in consultation with the members in each research project. Representatives have primary responsibility for communicating Center priorities and policies to group members and for transmitting group member concerns to the Center leadership.

The executive committee will meet at least every four months to vote on graduate salaries and distribution of discretionary research support, including support for new BMPs. The committee will

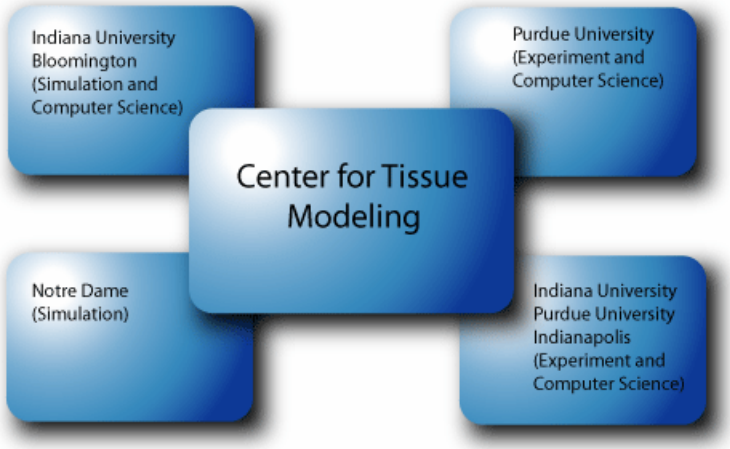
1. Advise the director on collaborative research strategies, priorities of research, and relations with other centers, departments, non-resident researchers, and other cooperating organizations.
2. Suggest speakers, short- and long-term visitors, and conference topics.
3. Assist in preparing the annual progress report on Center activities for submission to participating universities and NIH.
4. Meet regularly with university officers to discuss issues of hiring, space allocation and building plans, and to negotiate demands on staff and faculty time.

When a single member of the committee represents the committee as a whole in a meeting with university officers, a report on the discussion will be distributed to the entire committee within two weeks' time.



VII.6.vii Coordination of institutions:

The Executive Committee is responsible for institutional coordination. To assist in this and other Center activities, we have budgeted to install videoconferencing equipment at the four main participating Institutions. We expect to take full advantage of videoconferencing facilities, especially for ad hoc meetings. All members of the CTM have existing close collaborations with other members, either through the Indiana Biocomplexity Consortium, through various NSF sponsored supercomputing projects. All of the Coordinators have extensive experience managing large scale interdepartmental and inter institutional collaborative institutes and projects.



VII.6.viii Selection of Biologically Motivating Projects

The executive committee is responsible for selecting (with approval from the assigned NIH Science Officer) BMPs subsequent to those outlined in this proposal.

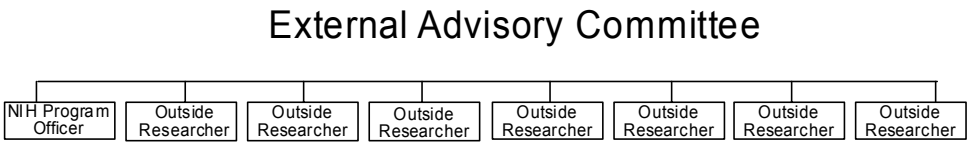
The choice of research projects balances the need for high quality research with the need to respond to a rapidly developing field. The Center initially will be tightly focused on the BMPs outlined in this proposal, but any member can propose new subprojects and reallocation of personnel in existing projects. All members are encouraged to submit written proposals for pilot projects to the Executive Committee at any time, which will be electronically distributed, reviewed, and discussed by the Committee as necessary. If approved, the Executive Committee will award small-scale funding which may be increased based on successful project review and availability of funds.

The CTM will assist BMP PI's in their search for outside funding to continue and expand their projects at the termination of their initial three year CTM support.

VII.5.ix External Advisory Committee:

Because of the size and complexity of the Center, expert scientific and organizational guidance is essential. In addition to the NIH appointed Program Officer, the external advisory committee will consist of eight recognized leaders in the scientific fields represented within the Center.

The advisory committee will visit the Center annually and prepare a report on its status with recommendations for improvements and changes in scientific direction and status with collaborators. The Center will bear all travel costs. The director will prepare a written response to this report and make it available to all members. In addition, the Center will invite the members of the advisory committee to participate in all workshops and conferences, and encourage advisory committee members to spend time at as short- term or long-term visitors.



VII.6.x Committee Vacancies and Other Considerations:

In the event of vacancies for any of the above positions, the executive committee will select a replacement for the unexpired term. Appointment requires permission of the member's department chair. The Center will limit the administrative responsibilities of untenured Committee members so as not to interfere with the member's professional progress.

VII.6.xi Membership:

Researchers at participating institutions and at other academic, research, and industrial institutions in the US and abroad may apply for membership in the Center. Each prospective member must closely associate with a Center project and must provide a detailed statement of research interests and relevance. The executive committee and director will review requests for membership. We expect all members to participate in workshops (subject to funding availability, the Center will bear travel costs for attendance) and to make their home research facilities available for research and training to participants and students. We will also encourage non-resident members to visit and send students to work closely with resident members. Exchange of students and faculty is essential to the proper functioning of an interdisciplinary environment.

Members must submit an annual, updated statement of research interests, a list of research accomplishments, publications, seminar and conference presentations, and a full list of visitors, students, and other members of their laboratory in NIH format and remain active in the work of the center.

VII.6.xii Technical Support Staff:

To maximize the use of research facilities, the Center will hire full-time programmers and system engineers to support computational resources, develop software, and assist with individual researchers' software development. Programmers must be skilled in both scientific (parallel) programming and the middleware needed to build portals and other grid components. The system engineer will also be responsible for project software engineering and customizing tools to support multi-institution collaborations, including creating the CTM intranet (grid). We seek creative technicians who will be able to take a proactive approach to a rapidly changing research and technological environment. A full time webmaster will be responsible for the Center website and distribution of information and documents such as the proceedings of workshops, posting of position openings and graduate and postdoctoral recruiting information. Critical data aspects of the project will be handled by the database and data curation staff hired by the Center for Genomics and Bioinformatics, who will also host this part of the project infrastructure. The Community Grids Laboratory will handle the grid, software engineering, and scientific computing staff and systems. Systems administration will be leveraged from existing staff at these two centers.

Audio-video conferencing and associated shared application servers will be supported by the Community Grids Laboratory, which already has several Polycom and Access Grid systems installed and has major research expertise in this field.

Growing career potential for technicians is assured. We anticipate the Center to be ongoing and expanding, therefore requiring additional technical support in the near future across all participating institutions. Successful technical staff will be promoted and provided the opportunity for additional training if necessary, which the Center will subsidize. Other interdisciplinary centers on each campus also are expanding technical support staff areas. In addition, all institutions have outstanding university-wide IT support organizations that offer a great variety of technical positions with excellent wages and growth potential.

VII.6.xiii Project Manager

Developing a new model of research fostering interdisciplinary inquiry necessitates developing a new model of research administration. Currently all institutions associated with this application are investing in discrete but contiguous interdisciplinary centers and academic curricula, devoting major capital and intellectual resources toward integrating disparate discipline-specific methodologies. The growing cohesiveness of and necessity for collaboration among these and national and international efforts has already begun to spark a new scientific culture that obscures both disciplinary and administrative boundaries. As a result, we envision that the project manager will play a crucial part in not only project development but also in advancing the Center itself.

Project manager positions for standard collaborative research have been traditionally undervalued. Interdisciplinary project management calls for a much greater, and different, administrative and scientific scope than discipline-specific efforts and must be nurtured and compensated according to the exceptional requirements of the role. We anticipate the new culture of interdisciplinary science will increasingly rely on the bridging skills of such managers, leading to their greater visibility and expanding responsibilities. We plan to actively cultivate innovative opportunities for scientific project managers in our evolving interdisciplinary research environment, starting with our collaborators as our research and administrative needs change. Currently accessible career paths for interdisciplinary project managers within academia include central research administration and academic program director positions as well as research development opportunities. We anticipate a growing and changing environment in the next decade during which new types of opportunities requiring diverse and blended administrative and research experience will evolve.

We seek a project manager with excellent technical management skills and a scientific background capable of promoting the needs of the Center in terms of ensuring projects remain on track technically and fiscally. Further the project manager must ensure integration of the different cores and the many projects within each core. The ability to communicate and negotiate with corporate, business, and government officials as well as research scientists and university administrators is essential. A person with a Ph.D. in a life or computational science area with significant research management experience would appear to be ideal. However, we believe that narrowing the focus of our search by requiring a science Ph.D. would be counterproductive to establishing a new culture for interdisciplinary inquiry. Those with a Ph.D. in a peripheral field, or an M.A. or M.S. with demonstrated involvement in advancing science and substantial research administration experience will be considered based on comprehensiveness of academic and business expertise balanced with the potential for adaptation and creativity. These attributes are essential in order to meet the rapidly changing demands of an interdisciplinary environment.

To assist both the Project Manager and Director, we also seek a Senior Administrative Manager who will be responsible for the operations of the Center, coordinating administrative operations including organizing meetings, inviting seminar speakers, coordinating job searches, and managing short-term projects in addition to secretarial assistance in the preparation of grant proposals, daily accounting and purchasing, travel coordination, managing Center personnel records and actions, and hiring and supervising temporary staff as necessary. The Senior Administrative Manager will be key not only to the fiscal administration of the Center but to the working order of all practical aspects of the collaborative process itself. The position with function as the primary liaison between the Center and departments, and will often be the first point of contact for those outside who are interested in the work of the Center. Qualifications include a strong business management background, master's degree preferred, with experience in a research-oriented environment, the ability to multitask and respond quickly to the changing needs of Center members. The ability to accurately represent the mission and goals of the Center is essential. Many career opportunities for administrative managers with

the diverse professional skills necessary for this position exist both within and outside academe. Central research administration at a middle senior level is one highly viable career path. Business and industry administration, especially for research-oriented organizations, is another excellent alternative, as well as senior administrative management positions in state and federal government.

VII.6.xiv Administration of Research and Salary Support

This grant will primarily support salaries for graduate students, postdocs, and visiting faculty. Our goal is to ensure that all support recipients conduct research that furthers the Center's projects. Members may apply to the executive committee for support for individuals or may apply for support for an open position to be filled by advertisement and interviews. In either case, the Center will strongly favor joint applications involving participation by at least two members in two different departments or institutions. In addition, applicants and research groups may request funding to cover the recipient's research expenses and equipment needs.

Salary and support will include:

- Twelve-month graduate student salaries renewable for up to three years.
- Graduate student salaries for summer research only.
- Resident postdoctoral salaries for one year, renewable for up to three years.
- Support for travel costs and tuition fees to enable resident students and members to work in remote laboratories.
- Support for students and members in remote laboratories to work at consortium institutions.
- Support for undergraduate student research during term time and summer terms.
- Salaries and expenses for visiting faculty for one or more semesters. We will encourage long-term visitors to spend part of their term at remote member locations.
- Support for short-term visitors.
- Limited research support to enhance members' consortium-related research, especially pilot projects and high-risk small-scale undertakings.

Nonresident members may apply for student and postdoctoral salaries. The consortium will request a waiver of normal overhead for these appointments. Supported non-residents, except for undergraduates, must attend consortium workshops and, whenever possible, conduct at least one month of research while resident at a consortium institution. We invite senior visitors to offer short-duration and for-credit courses as part of their residency. Support recipients should present seminars on their research to an appropriate forum. All support recipients must file a research report at the end of their term.

VII.6.xv Research Facilities

The center will include shared research facilities to further its research projects. The participating institutions are generous in their support for shared infrastructure, typically contributing a substantial cost share in the form of hard money salaries for staff or as funds for equipment purchase. Initial time on these facilities and experimental support for pilot projects will be free of charge for both members and non-members. After a successful pilot project, time and support on these instruments will also be available to any interested users on a cost basis. Users may pay their user costs from their own research funding or may apply to the Center for funding. Initial shared facilities include:

- Indiana University's major large-scale simulation and data storage facilities. These include a 1 TFLOP IBM SP Supercomputer, which is geographically distributed between IU's campuses in Indianapolis and Bloomington. Three TeraBytes (TB) of RAID 5 disk storage have been ordered for this system, specifically to provide storage space for biomedical data. IU also has a 64-processor Sun E10000

supercomputer, with a peak theoretical capacity of 52 GFLOPS. IU has recently installed a 2.2 TFLOPS Linux cluster, distributed (like the IBM SP) between Bloomington and Indianapolis. This system is configured specifically to deal with large data problems, with half a TB of RAM and 10 TB of spinning disk.

- The Community Grids Laboratory will make available to the center an 8 processor Sun Fire system with 0.8 terabytes of disk and 16 gigabytes of memory. This will host the infrastructure to support simulations, Grid, and portals needed by the center and is a contribution valued at $116,000.
- The project has budgeted a total of $300,000 (NIH and university match) to provide initial project central facilities for data, simulation, and grid integration services. In addition, we have funded modest system software and upgrade costs as well as upfront costs ($56,000) of audio-video conferencing systems to link center sites.

In addition, members agree to make major instrumentation in their own laboratories available for use by other members. Use of equipment in other collaborating centers will be available on a cost basis.

VII.6.xvi Faculty Development

Initially the Center's teaching faculty will consist of faculty in individual departments, faculty visiting the Center, and the Center's advanced postdoctoral fellows. To encourage faculty support for this effort, we will ask departments to provide teaching credit for teaching within the Center. We will encourage our postdoctoral fellows to teach specialized courses on systems biology and biomedical computing.

Creation of the Center will encourage concurrent appointments by departments. Such appointments are already standard for faculty hired as part of the Indiana Biocomplexity Consortium. As the Center develops, we will promote additional joint appointments between multiple departments. We have already made several joint faculty hires at the tenured faculty level. Shared appointments reduce the cost of appointment to individual departments and simplify the hiring of genuinely interdisciplinary researchers. We will also seek funding for soft-money faculty associated directly with the Center, outside a home department.

We expect faculty career development to be multifaceted. Each participating institution's central administration has pledged to support faculty promotion despite career barriers that hamper interdisciplinary researchers, which is to be expected in that all participating institutions currently support other established interdisciplinary centers. These organizations have begun to create a vigorous research environment primed to sustain recognition of scientific contributions that cross conventional boundaries. The Center for Tissue Modeling will create a bond between these separate efforts, magnifying their potential to generate a new academic culture which rewards research based on the advancement of knowledge without regard to disciplinary traditions. Faculty associated with the Center will be able to pursue career opportunities with departments at all Center institutions as well as the interdisciplinary organizations on each campus. In addition, national, international, business, industrial, and other non-academic research positions are increasing with the growing need for scientists able to merge and transcend disciplines.

The Center also plans to approach editorial boards of major journals regarding increasing the publication of interdisciplinary research articles.

VII.6.xvii Distance Education

Distance education is essential for the Center as it allows teachers with specialized knowledge in one area to deliver courses from one organization to students throughout the Center (and outside it). We have budgeted the infrastructure for distance programming that can share the collaboration technology aimed at research interactions. The Community Grids laboratory has substantial experience in this area. Their use of this technology has been to bring leading edge IT classes to minority serving institutions and government laboratories. For example, Dr. Fox taught Internet classes fall 1997-fall 2004 from Syracuse and Indiana to Jackson State University in Jackson, Mississippi.

We will use this technology to enhance our training and outreach programs and enhance the collaboration support infrastructure as need increases.

VII.7 LEVERAGING AND EXIT STRATEGY

The primary long-term impact of the Center on biomedical science will be the outcome of the research discussed in this proposal and in future projects. Impact will be heightened by the future contributions of students trained in this unique interdisciplinary environment. This grant will have immediate and decisive influence, increasing the amount and quality of interdisciplinary research and providing new foci for the education of graduate and post-doctoral students. It will begin to change the scientific community at large through workshops, training opportunities, software tools, and collaborations. The Center for Tissue Modeling will amplify the already substantial collaborative interactions among participating institutions and increase the likelihood of junior investigators obtaining independent support for their investigator-initiated projects. Existing efforts have already greatly improved success in student and faculty recruitment.

We expect future funding for the Center for Tissue Modeling to be based on individual project support from federal and non-federal sources as well as institutional support via indirect cost recovery tied to both research and education. We will also seek other long and short term private, federal and foundation support for Center expansion and continuation. While this is hardly a novel exit strategy, the economic benefit and social necessity that will undoubtedly continue to drive increasing investment in biosciences both nationally and internationally coupled with the exceptional strengths of the Center solidly ground our expectation.

All participating institutions have clearly recognized the importance of interdisciplinary education and research. Their support of interdisciplinary centers, as described in this proposal, will continue to grow. In addition, as the pool of qualified students (which we will help to groom) matriculates and begins to populate the ranks of business, industry, and academe over the next decade, a new culture of scientific inquiry will emerge, blending and obscuring disciplinary boundaries in such a way that the mission and goals of the CTM will become central to the missions and goals of funding agencies of all types.

Our ongoing quest for excellence in bioscience, education, collaboration, networking, and especially the value we place on creative potential in research and teaching will serve to keep the CTM vibrant. Focusing on best practices will be key to maintaining an energetic, responsive environment for interdisciplinary inquiry.

Our current base of institutions and individual members place us in a superb position to achieve both short- and long-term research and funding objectives, in addition to our outside supporting and collaborative network, which includes the University of Auckland, University of California Berkeley, the Center for Computational Biology and Bioinformatics, CIBSS, NECSI, Reify, and the University of California San Diego, a list that will grow in coming years.

VII.8 Timeline:

All the projects described here are currently operational except the selection of the external advisory committee, which will be chosen during the first months of year 1.

Year 1: Three or four planned faculty hires in Computational and Systems Biology

Year 2: MSB I Building online

Three of four planned faculty hires in Computational and Systems Biology

Year 3: MSB II Building online

Three or four planned faculty hires in Computational and Systems Biology

Initial selection of second round BMPs

Year 4: Implementation of second round BMPs

E. HUMAN SUBJECTS

None

F. VERTIBRATE ANIMALS

Core 3: Biologically Motivating Project 1 – Heart Development

Investigators: Loren Field, Anthony Firulli, Simon Conway and Weinian Shou.

1. Use of Animals

In general, the age of the mice will not exceed 12 months. Both male and female animals will be used. We anticipate that a steady-state census of ca 300 animals for both transgenesis and knock-out experiments.

2. Justification

The mouse is the best characterized laboratory animal used for biomedical research. In addition, mice are easy to maintain and store in reasonable numbers. Physiological experiments require that the control and experimental animals be as similar as possible; thus any changes in pathophysiology observed during the course of the experiment can be attributed directly to the specific manipulation or treatment performed. Mice by far provide the best genetically characterized experimental system; knowledge of the lineage map is extensive and the transgenic technology permits the introduction of new, defined genetic alterations. No other experimental animal system can afford these features. We estimate that 300 mice, at various ages, will be continuously maintained for the proposed experiments. All efforts will be made to minimize the number of mice being maintained.

3. Veterinary Care

The Indiana University Medical School Animal Resource Center is currently under consideration of accreditation by the American Association for Accreditation of Laboratory Animal Care. Animals are maintained in accordance with the applicable portions of the Animal Welfare act and the DHHS Guide for the Care and Use of Laboratory Animals. Veterinary care is under the direction of a consulting veterinarian boarded by the American College of Laboratory Animal Medicine. Additional veterinary staff and veterinary technicians provide a complete comprehensive program of diagnostics, preventive and clinical medicine at our facility. The consulting veterinary has authority to terminate all animal research which does not comply with current government regulations or Laboratory Policy.

4. Animal Concerns

Mice will be anesthetized during all surgical procedures. Moreover, any animals which are not recovering from surgical procedures will be immediately sacrificed. Animals in the colony will be continually monitored so as to identify any moribund animal. These animals will be immediately sacrificed.

Ketamine (100 mg/kg), xylazine (5 mg/kg) and morphine (2.5 mg/kg), administered intraperitonially, will be used to anesthetize mice for all surgical procedures. Anesthesia develops after 2-5 minutes, and surgical anesthesia lasts for ca. 60 minutes, which is more than enough to perform all of the necessary surgical procedures.

5. Euthanasia

Two methods of euthanasia will be used: cervical dislocation and carbon dioxide.

Cervical dislocation will be used to sacrifice mice to examine expression and/or functional consequences of intra-cardiac grafts. In addition, females used for generation of embryos for microinjection experiments will be sacrificed by the same method. This is a method of choice when the intention is to collect mouse tissues with minimal physiological alterations. Methods of euthanasia relying on chemical drugs were shown to alter pathological and histopathological observation, which is also the case for carbon dioxide.

Carbon dioxide will be used to euthanize nonexperimental mice (*i,e*., nontransgenic siblings) and rats. Carbon dioxide is a safe and humane way to kill large numbers of mice. Adult mice are unconscious within 10 seconds after placing them in the chamber filled with CO_2, and dead within 2-4 minutes.

Core 3: Biologically Motivating Project 2 – Vascular Development

Investigators: Dr. March, Dr. Yoder (School of Medicine, IUPUI) and Dr. Litte (Kansas University Medical Center)

All animal experimentation will be performed in accordance with Indiana School of Medicine and University of Kanas Medical School guidelines after obtaining IACUC and Biosafety approvals.

1. Use of Animals

Both quail and mice of both sexes will be used in this study. Embryos from both species or mature mice (approximately 4 months) will be used in these experiments. The approximate numbers of quail embryos will be 60 per year (180 total). Mouse strains to be used include C57BL/6J and C57BL/6J-EGFP. Approximately 60 mouse embryos (EDEP isolation) and 30 adult mice (AEDP isolation) will be used per year (270 total).

2. Justification

Vasculogenesis studies using mice and quail embryos are well founded in the literature. Both systems have distinct benefits for these studies: the quail due to ease of manipulating and observing embryos at the earliest stages of development (including the avascular stage) and the availability of imaging and computational tools; the mouse allantois due to its closer similarity to human physiology.

3. Veterinary Care

Animal care will be provided by either the Indiana University Medical School Laboratory Animal Resource Center of the Kansas University Medical College Laboratory Animal Resource (AAALAC accredited). Animals are maintained in accordance with the applicable portions of the Animal Welfare act and the DHHS Guide for the Care and Use of Laboratory Animals. Veterinary care is under the direction of consulting veterinarians boarded by the American College of Laboratory Animal Medicine. Additional veterinary staff and veterinary technicians provide a complete comprehensive program of diagnostics, preventive and clinical medicine at the facilities. The consulting veterinarians have authority to terminate all animal research which does not comply with current government regulations or Laboratory Policy.

4. Animal Concerns

a. Adult mice and quail will be anesthetized during all surgical procedures. Moreover, any animals which are not recovering from surgical procedures will be immediately sacrificed. Animals in the colony will be continually monitored so as to identify any moribund animal. These animals will be immediately sacrificed.

b. Ketamine (100 mg/kg), xylazine (5 mg/kg) and morphine (2.5 mg/kg), administered intraperitonially, will be used for anesthesia during all surgical procedures. Anesthesia develops after 2-5 minutes, and surgical anesthesia lasts for ca. 60 minutes, which is more than enough to perform all of the necessary surgical procedures.

5. Euthanasia

Carbon dioxide will be used to euthanize nonexperimental mice (ie nontransgenic siblings) and rats. Carbon dioxide is a safe and humane way to kill large numbers of mice. Adult mice are unconscious within 10 seconds after placing them in the chamber filled with CO2, and dead within 2-4 minutes.

CORE 3: Biologically Motivating Project 3: Limb Regeneration

Investigators: Stocum, Chernoff, Rhodes, Department of Biology, School of Science, IUPUI

The School of Science IACUC committee has provisionally approved all of the experiments described in this proposal. Full approval is pending. All experiments will be performed with Indiana University Institutional Biosafety Committee (IBC) approval.

1. Use of Animals

Species to be used: axolotl (*Ambystoma mexicanum*)

Age at sacrifice: three months post-hatching.

Sex: males and females

Numbers of animals to be used: 60 per year; total of 180

All of the axolotls used for this project will be bred by the Indiana University (Bloomington) Axolotl Colony, an NSF-funded facility that maintains normal and mutant axolotl lines. The primary short-toes mutant stock is in a white background, so white animals will be used for the normal limb regeneration studies as well.

Axolotls will be housed in the IUPUI School of Science animal facility. The animal facility is AAALAC certified, is 2,000 square feet, and includes seven housing rooms, two cage-washing rooms, three storage rooms, three surgery and procedure rooms, and an office. An animal technician and part-time animal caretakers provide all animal husbandry and maintenance. An attending veterinarian is available for routine inspections and emergency service, and the School of Science IACUC oversees all animal care and has independent USDA Registration/ OPRR Assurance Assurance Number = A4091-01). Housing conditions will be under normal food, water, and light, etc.

For limb regeneration studies, larval axolotl (Ambystoma mexicanum, larvae) forelimbs will be amputated following this procedure.

Species and specific considerations:

- Axolotls are purpose-bred, fully aquatic salamanders. They must be maintained in aquaria and kept cool.
- The only non-toxic anesthetic agents are transdermal.
- The only non-toxic analgesic agent is chilling.
- The sources of our procedures are instruction and publications of the Indiana University Axolotl Colony; Wright, KM and Whitaker BR (2001) Amphibian Medicine and Captive Husbandry. Krieger Publishing, Malabar, Florida. Chapter 9; advice of Indiana University SARC animal facility veterinarians.
- All instruments will be sterilized by steam autoclaving.
- Surgeon will wear mask, cap, clean lab coat, and sterile surgical gloves.
- Surgical area will include a sterile, horizontal laminar flow hood.
- Skin will be disinfected via soaking in Thimerosal 1:10,000 in the anesthetic solution.
- Anesthesia in aquatic amphibians is transdermal: sterile solution of tricaine methane sulfanate, 0.5g/liter artificial pondwater (20% Holtfreter's solution).
- Anesthetic monitoring: loss of righting reflex will be used to indicate onset and loss of twitch reflex from tail pinch will be used to indicate induction.
- Zeugopodial (wrist level) amputation performed using sterile iridectomy scissors. The animal is placed on a wet, sterile surgical cloth for the amputation. This procedure is very rapid.
- Recovery: animals will be kept cool (12°C) in refrigerated artificial pond water and the investigator will remain until all are well recovered as monitored by resumption of normal posture and activity. They will then be maintained at 12° until a wound epithelium has sealed the limb stump (2-4 hours observed hourly by stereo microscope). When healed the animals will be returned to the animal facility where they will be observed daily.
- Post-operative analgesia will be provided by chilling as described above.

Axolotls will be euthanized to obtain tissues for analysis. Animals will be deeply anesthetized for harvesting of regenerate tissue. Animal will then be killed by cervical dislocation with sharp scissors. Harvested tissue will be treated in the following ways:

- Analysis of mitotic activity
- Analysis of apoptosis
- Analysis of cell density
- Analysis of expression of muscle and cartilage differentiation markers
- Analysis of Hox gene expression

After analysis, axolotl carcasses and tissues will be disposed by freezing in bags followed by incineration. Morphometry of regenerating limbs will be performed on living animals.

2. Justification

The significance is that humans cannot regenerate limbs proximal to digit tips. Salamanders, such as the axolotl, are the only tetrapod vertebrates that can regenerate limbs at any stage of life. A better understanding

of this process in axolotls will provide insight that will assist in mediation of improved regeneration in mammals, including humans.

To better understand organ regeneration we must analyze the events that regulate these processes. Species such as microbes, insects, plants, and fungi, do not have limbs. *Axolotls* are a suitable species for this project because they are the most phylogenetically advanced animals that can regenerate limbs. Their limbs contain the same complement of bones (humerus, radius, ulna, etc) as human limbs. Previous studies by our laboratories and others have shown well conserved aspects as well as differences between amphibian limb regeneration and mammalian limb development. In other projects in our laboratories we are using primary cell culture methods to examine and manipulate the molecular biology of limb regeneration. However, in order to move forward in developing computer models for regeneration and morphogenesis, we must perform a number of more “old-fashioned” experiments using whole animals and limbs.

The numbers of axolotls to be used are calculated based upon several considerations. The numbers are sufficient to provide replicate sets of animals for studies of mitosis and cell density and differentiation markers in normal and mutant axolotls.

To ensure that our research is not unnecessarily duplicative, we perform weekly literature searches to ensure that the proposed experiments have not been performed elsewhere. We search the PubMed MEDLINE database using the following keywords: *alternatives, limb regeneration, limb development, amphibian limb, myogenesis, chondrogenesis, limb mutant*. We also search the MEDLINE database using the same keywords and the Indiana University Ruth Lilly Medical Library Ovid Database System. In this system, we search the *1997-to-present* database and the *1966 to 1996* database. These searches have not found publications describing experiments that are duplicative of the ones proposed in this application.

3. Veterinary Care

An attending veterinarian is available for routine inspections and emergency service. The School of Science IACUC oversees all animal care and has independent USDA Registration/ OPRR Assurance (Assurance Number = A4091-01). A full-time animal technician and part-time animal caretakers provide all animal husbandry and maintenance. All School of Science animal protocols are reviewed by the veterinarian before approval.

In all experiments, if an animal sustains more than a minor injury, appears quite ill (e.g. displays depressed activity, unnatural posture, loss of righting reflex, food avoidance, unexplained blood loss, inflammation or bloating), it will be euthanized. If the cause of the stress is unknown, the facility veterinarian will be contacted for advice (e.g. on whether she needs to perform a necropsy; potential risk to other animals, etc.). In such cases and at the conclusion of all experiments, euthanasia will be done by deep transdermal anesthesia followed by severing of the spinal cord at the base of the brain.

4. Animal Concerns

All individuals with animal contact must have completed the School of Science animal training course.

Normal and mutant axolotls will be purpose-bred animals obtained from the Indiana University Axolotl Colony, which is in full compliance with their Institutional animal welfare guidelines.

In all experiments, if an animal sustains more than a minor injury, appears ill (e.g. displays depressed activity, unnatural posture, loss of righting reflex, food avoidance, unexplained blood loss, inflammation or bloating, it will be euthanized. If the cause of the stress is unknown, the facility veterinarian will be contacted for advice (e.g. on whether she needs to perform an necropsy; potential risk to other animals, etc.). In such cases and at the conclusion of all experiments, euthanasia will be done by transdermal anesthesia$_2$ asphyxiation followed by cervical dislocation.

The only procedure that will be performed on live animals will be the limb amputation as described above. Care will be taken to provide a sterile environment and to perform the procedure rapidly (see above). Animals will be anesthetized and the level of anesthesia will be carefully monitored. Recovering animals will be kept cool and comfortable until they are well recovered as monitored by resumption of normal posture and activity. They will then be observed every 30 minutes for several hours. Post-operative analgesia will be provided by chilling as described above. Wound healing will be checked daily and general health as judged by food intake and behavior assessed. Records will be kept detailing health and status of all individuals. Limb amputation is

necessary in order to study limb regeneration. Axolotls vasoconstrict rapidly and blood loss is minimal. Drs. Stocum and Chernoff are experienced in this procedure. Sources consulted include:

- Wright, KM and Whitaker BR (2001) Amphibian Medicine and Captive Husbandry. Krieger Publishing, Malabar, Florida.
- http://www.indiana.edu/~axolotl/
- http://www.indiana.edu/~axolotl/newsletter/newsltr.html

5. Euthanasia

The method of euthanasia will be transdermal anesthesia followed by cervical dislocation. This method was selected because it is a humane method and because it does not adversely affect the tissues of the animals so that the experiments can be properly performed. This method is consistent with the recommendation of the Panel on Euthanasia of The American Veterinary Medical Association. Laboratory personnel will wear gloves, lab coats and will maintain complete and accurate records. Criteria for euthanasia:

- Poor health (failure to grow or symptoms as described above)
- Sacrifice to obtain experimental samples.

After analysis, axolotl carcasses and tissues will be disposed by freezing in bags followed by incineration.

CORE 3: Biologically Motivating Project 3 – Zebrafish Fin Regeneration

Investigator: J. Marrs (Division of Nephrology, Indiana University School of Medicine)

1. Use of Animals

Adult zebrafish are obtained from a local pet store and maintained under standard laboratory conditions in Aquatic Habitats tank systems at 27°C on a 14-hour light/10-hour dark cycle (Westerfield, 1995). Embryos are collected 30 minutes after first morning light, then incubated at 28.5°C for various times. Embryonic fish are sacrificed by overdose with anesthetic (0.1% 3-aminobenzoic acid ethyl ester) before procedures are performed. Adult fish are anesthetized, and then sacrificed by cervical dislocation before procedures are performed. Approximately 50 adult fish are used per year. Mostly, adults are maintained for breeding. After approximately one year, adults are replaced because breeding efficiency is reduced in older animals. Mutant embryos are obtained by breeding adult zebrafish that are heterozygous for mutant alleles. Mutant lines are obtained from the University of Oregon or Max-Planck-Institut für Entwicklungsbiologie Zebrafish Mutant Stock Center.

2. Justification

Zebrafish provide an extremely important opportunity to study developmental processes with reduced pain and suffering found in studies of mammalian species. Embryogenesis occurs outside the body, and large numbers of embryos can be collected without sacrificing the adult animals.

3. Veterinary Care

Laboratory procedures and animal facilities are overseen and regularly inspected by a staff veterinarian. Institutional laboratory animal resource committees approve methods and procedures and insure USDA and IACUC approval.

4. Animal Concerns

Anesthesia is applied prior to euthanasia. No survival surgery or invasive experimental procedures are proposed.

5. Euthanasia

Animals are placed in the anesthetic solution (0.1% 3-aminobenzoic acid ethyl ester) until their movement is severely slowed. Once full anesthesia is achieved, adult animals will be removed from the anesthetic solution and euthenized. Embryos are kept in the anesthetic solution for an additional 5-10 minutes, resulting in overdose. The anesthetic use has been described previously (Westerfield,1995) (alsopublishedontheweb: http://zfish.uoregon.edu/zf_info/zfbook/zfbk.html).

G. LITERATURE CITED

Abdulle, A. and Engquist, W. E (2003), "Finite difference heterogeneous multi-scale method for homogenization problems," *J. Comp. Phys.* **191**, 18-39.

Adams, J. C. and Watt, F. M. (1993) "Regulation of development and differentiation by the extracellular matrix," *Development* **117**, 1183-1198.

Agocha, A., Sigel, A. V. and Eghbali-Webb, M. (1997) "Characterization of adult human heart fibroblasts in culture: a comparative study of growth, proliferation and collagen production in human and rabbit cardiac fibroblasts and their response to transforming growth factor-beta1," *Cell Tissue Res*. **288**(1), 87-93.

Akimenko, M. A., Mari-Beffa, M., Becerra, J. and Geraudie, J. (2003) "Old questions, new tools, and some answers to the mystery of fin regeneration," *Dev. Dyn.* **226**, 190-201.

Alter, O., Brown, P. O. and Botstein, D. (2000) "Singular value decomposition for genome-wide expression data processing and modeling," *Proc. Natl. Acad. Sci. USA* **97**, 10101-10106

Amari, S.–I. and Nagaoka, H. (2002) *Methods of Information Geometry, Amer. Math. Soc.* (Oxford University Press)

Ambler, C. A., Nowicki, J. L., Burke, A. C. and Bautch, V. L. (2001) "Assembly of trunk and limb blood vessels involves extensive migration and vasculogenesis of somite-derived angioblasts," *Developmental Biology* **234**, 352-364.

Ambler, C. A., Schmunk, G. M. and Bautch, V. L. (2003) "Stem cell-derived endothelial cells/progenitors migrate and pattern in the embryo using the VEGF signaling pathway," *Developmental Biology* 257, 205-219.

Andrews, T., F. Curbera, H. Dholakia, Y. Goland, J. Klein, F. Leymann, K. Liu, D. Roller, D. Smith, Thatte, I. Trickovic, S. Weerawarana. 2003. "Specification: Business Process Execution Language for Web Services 1.1," Available from http://www-106.ibm.com/developerworks/webservices/library/ws-bpel/.

Armbruster, D., Heiland, R. and Kostelich, E. (1994) "KLTool: A tool to analyze spatiotemporal complexity," *Chaos* **4**, 421-424

Armbruster, D., Heiland, R., Kostelich, E. and Nicolaenko, B. (1992) "Phase-space analysis of bursting behavior in Kolmogorov flow," *Physica D* **58**, 392-401

Armstrong, P. (1989) "Cell sorting out: the self-assembly of tissues *in vitro*," *Crit. Rev. Biochem. Mol. Biol.* **24**, 119-149.

Arrayás, M., Ebert, U. and Hundsdorfer, W. (2002) "Spontaneous branching of anode-directed streamers between planar electrodes," *Phys. Rev. Lett*. **88**, 174502.

Arthur, H. M., Ure, J., Smith, A. J., Renforth, G., Wilson, D. I., Torsney, E., Charlton, R., Parums, D. V., Jowett, T., Marchuk, D. A., Burn, J. and Diamond, A. G. (2000) "Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development," *Developmental Biology* **217**, 42-53.

Asahara, T. and Isner, J. M. (2002) "Endothelial progenitor cells for vascular regeneration," *J. Hematother. Stem Cell Res.* **11**, 171-178.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M. and Sherlock, G. (2000) "Gene ontology: tool for the unification of biology," The Gene Ontology Consortium, *Nat. Genet.* **25**, 25-29.

Athanasius F. M. Marée and Hogeweg, Paulien (2002) "How amoeboids self-organize into a fruiting body: Multicellular coordination in Dictyostelium discoideum," *PNAS* **98**, 3879-3883.

Audoly, S., D'Angio, L., Saccomani, M. P. and Cobelli, C., (1998) "Global identifiability of linear compartmental models - a computer algebra algorithm,' *Biomed. Eng.* **45**, 36-47.

Auroux, P. A., Iossifidis, D., Reyes, D. R. and Manz, A. (2002) "Micro total analysis systems. 2. Analytical standard operations and applications," *Analytical Chemistry* **74**, 2637-2652.

Babb, S. G., Barnett, J., Doedens, A. L., Cobb, N., Liu, Q., Sorkin, B. C., Yelick, P. C., Raymond, P. A. and Marrs, J. A. (2001) "Zebrafish E-cadherin: expression during early embryogenesis and regulation during brain development," *Dev. Dyn.* **221**, 231-237.

Baek, S. H., Hrabie, J. A., Keefer, L. K., Hou, D., Fineberg, N., Rhoades, R. and March, K. L. (2002) "Augmentation of intrapericardial nitric oxide level by a prolonged-release nitric oxide donor reduces luminal narrowing after porcine coronary angioplasty," *Circulation* **105**, 2779-2784.

Bailey, A. S., Jiang, S., Afentoulis, M., Baumann, C. I., Schroeder, D. A., Olson, S. B., Wong, M. H. and Fleming, W. H. (2003) "Transplanted adult hematopoietic stem cells differentiate into functional endothelial cells," *Blood*, 13-19.

Baker, R. E., Schnell, and S., Maini, P. K. (2003) "Formation of vertebral precursors: Past Models and Future Predictions," *Journal of Theoretical Medicine* **5**, 23-35.

Baloo, S. and Ramkrishna, D. (1991a) "Metabolic Regulation in Continuous Cultures of Bacteria-I," *Biotechnology and Bioengineering* **38**, 1337-1352.

Baloo, S. and Ramkrishna, D. (1991b) "Metabolic Regulation in Continuous Cultures of Bacteria-II," *Biotechnology and Bioengineering* **38**, 1353-1363.

Balsoy, O., Aktas, M. S., Aydin, G., Aysan, M. N., Ikibas, C., Kaplan, A., Kim, J., Pierce, M., Topcu, A., Yildiz, B. and Fox, G. (2002) "The Online Knowledge Center: Building a Component Based Portal." *Proceedings of the International Conference on Information and Knowledge Engineering*.

Banerji, A., C. Bartolini, D. Beringer, V. Chopella, K. Govindarajan. A. Karp, H. Kuno, M. Lemon, G. Pogossiants, S. Sharma and S. Williams (2002) "Web Services Conversation Language (WSCL) 1.0," Available from http://www.w3.org/TR/2002/NOTE-wscl10-20020314/.

Barbash, I. M., Chouraqui, P., Baron, J., Feinberg, M. S., Etzion, S., Tessone, A., Miller, L., Guetta, E., Zipori, D., Kedes, L. H., Kloner, R. A. and Leor, J. (2003) "Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution," *Circulation* **108**, 863-868.

Barnes, J. and Nut, P. (1986), "A Hierarchical O(N-Log-N) force-calculation algorithm," *Nature* **324**, 446-449.

Bartram, U., Bartelings, M. M., Kramer, H. H., and Gittenberger-de Groot, A. C. (2001) "Congenital polyvalvular disease: a review," *Pediatr Cardiol.* **22**, 93-101.

Bellman, R. and Astrom, K. J. (1970) "On structural identifiability," *Math. Biosci*. **7**, 329-339.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. and Wheller, D. L. (2004) "GenBank: update," *Nucleic Acids Res*. **32**, D23-6.

Bensoussan, A., Lions, J. L. and Papanicolaou, G. (1978) "Asymptotic Analysis for Periodic Structures," (North Holland).

Berger, M. J. and Collela, P. (1989), "Local adaptive mesh refinement for shock hydrodynamics," *J. Comp. Phys.* **82**, 62-84.

Berger, M. J. and Oliger, J. (1984), "Adaptive mesh refinement for hyperbolic partial differential equations," *J. Comp. Phys.* **53**, 484-512.

Berman, F., Fox, G. and Hey, T. (2003) *Grid Computing: Making the Global Infrastructure a Reality* (John Wiley and Sons, Ltd, Chichester) (http://www.grid2002.org).

Berne, R. M. (1979) *"Handbook of physiology, section 2: The cardiovascular system," (Am. Physiol. Society)*. 30-41.

Bialek, W. and Setayeshgar, S. (2003a), "Physical limits to biochemical signaling," submitted to *Proc. Nat. Acad. Sci.,* (downloadable from Los Alamos preprint archive physics/0301001).

Booth, D. *et al* (2003) "Web Service Architecture." W3C Working Draft.

Boyde, T. R. C. (1980) *Foundation stones of biochemistry*, (Voile et. Aviron, Hong Kong).

Boyden, S. (1962) "The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes," *Journal of Experimental Medicine* **115**, 453-466.

Bray, T., Paoli, J. and Sperberg-McQueen, C. M. (1998) "Extensible markup language (XML) 1.0," (Available from http://www3.org/TR/1998/REC-xml-19980210).

Breese, M. R., Stephens, J. J., McClintick, J. N., Grow, M. W. and Edenberg, H. J. (2003) "Labrat LIMS: an extensible framework for developing laboratory information management, analysis, and bioinformatics solutions for microarrays," Proceedings of the 2003 ACM symposium on Applied Computing.

Breward, C. J. W., Byrne, H. M. and Lewis, C. E. (2003) "A multiphase model describing vascular tumour growth," *Bull. Math. Biol.* **65**, 609-640.

Briggs, W. L., Henson, V. E. and McCormick, S. F. (2000) *A Multgrid Tutorial*, (SIAM, Philadelphia, PA)

Brightman, A. O., Rajwa, B. P., Sturgis, J. E., McCallister, M. E., Robinson, J. P. and Voytik-Harbin, S. L. (2000) "Time-lapse confocal reflection microscopy of collagen fibrillogenesis and extracellular matrix assembly *in vitro*" *Biopolymers* **54**, 222-234.

Burrage, K., Tian, T., andBurrage, P. (2004) "A multi-scaled approach for simulating chemical reaction systems," *Prog. Biophys. Mol. Biol.,* (in press).

Burton, P. B. J., Raff, M. C., Kerr, P., Yacoub, M. H., and Barton, P. J. R. (1999) "An intrinsic timer that controls cell-cycle withdrawal in cultured cardiac myocytes," *Developmental Biology* **216**, 659-670.

Cabrera, C. R. and Yager, P. (2001) "Continuous concentration of bacteria in a microfluidic flow cell using electrokinetic techniques," *Electrophoresis* **22**, 355-362.

Campbell, A. I., Zhao, Y., Sandhu, R. and Stewart, D. J. (2001) "Cell-based gene transfer of vascular endothelial growth factor attenuates monocrotaline-induced pulmonary hypertension," *Circulation* **104**, 2242-2248.

Carmeliet, P. and Jain, R. K. (2000) "Angiogenesis in cancer and other diseases," *Nature* **407**, 249-257.

Chaturvedi, R., Izaguirre, J. A., Huang, C., Cickovski, T., Virtue, P., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A. and Glazier, J. A. (2003) "Multi-model simulations of chicken limb morphogenesis," in *Computational Science - ICCS 2003: International Conference,* June 2-4, 2003, Melbourne, Australia and St. Petersburg, Russia, *Proceedings, Part III*, Sloot, P. M. A., Abramson, D., Bogdanov, A. V., Dongarra, J. J., Zomaya, A. Y. and Gorbachev, Y. E., (eds) *Lecture Notes in Computer Science* **2659**, Springer-Verlag, New York, 2003), 39-49. (downloadable from Springer: http://link.springer.de/link/service/series/0558/tocs/t2659.htm).

Chaudhury, M. K. and Whitesides, G. M. (1991) "Direct measurement of interfacial interactions between semispherical lenses and flat sheets of poly(dimethylsiloxane) and their chemical derivatives," *Langmuir* **7**, 1013–1025.

Chen, J., Brodsky, S., Li, H., Hampel, D. J., Miyata, T., Weinstein, T., Gafter, U., Norman, J. T., Fine, L. G. and Goligorsky, M. S. (2001) "Delayed branching of endothelial capillary-like cords in glycated collagen I is mediated by early induction of PAI-1," *Am. J. Physiol.-Renal Physiol*. **281**, F71-F80.

Chen, Z., Dongarra, J., Luszczek, P. and Roche, K. (Jan 5-8, 2004) "LAPACK for Clusters Project: An Example of Self Adapting Numerical Softwaware," (Hawaii International Conference on System Sciences HICSS-37, Hilton Waikoloa Village, Big Island, Hawaii).

Cheng, G., Wessels, A., Gourdie, R. G., and Thompson, R. P. (2002) "Spatio-temporal and tissue specific distribution of apoptosis in the developing chick heart," *Dev. Dyn*. **223**, 119-33.

Chiem, N. H. and Harrison, D. J. (1998) "Microchip systems for immunoassay: an integrated immunoreactor with electrophoretic separation for serum theophylline determination," *Clinical Chemistry* **44**, 591-598.

Choi, K. (2002) "The hemangioblast: a common progenitor of hematopoietic and endothelial cells," *J. Hematother Stem Cell Res.* **11**, 91-101.

Christoffels, V. M., Habets, P. E. M. H., Franco, E., Campione, M., de Jong, F., Lamers, W. H., Bao, Z.-Z., Palmer, S., Biben, C., Harvey, R. P., and Moorman, A. F. M. (2000) "Chamber formation and morphogenesis in the developing mammalian heart," *Developmental Biology* **223**, 266-278.

Clendenon, J. L., Phillips, C. I., Sandoval, R. M., Fang, S., and Dunn, K. W. (2002) "Voxx: a PC-based, near real-time volume rendering system for biological microscopy," *Am. J. Physiol. Cell Physiol.* **282**, C213-C218.

Clubb, F. J. Jr. and S. P. Bishop (1984) "Formation of binucleated myocardial cells in the neonatal rat. An index for growth hypertrophy," *Lab. Invest.* **50**, 571-574.

Cogle, C. R., Wainman, D. A., Jorgensen, M. L., Guthrie, S. M., Mames, R. N. and Scott, E. W. (2003) "Adult human hematopoietic cells provide functional hemangioblast activity," *Blood*.

Collier J. R., McInerney. D., Schnell, S., Maini, P. K., Gavaghan, D. J., Houston, P. and Stern, C. D. (2000) "A cell cycle model for somitogenesis: mathematical formulation and numerical simulation," *Journal of theoretical Biology* **207**, 305-316.

Conway, S. J., Bundy, J., Chen, J., Dickman, E., and Rogers, R., (2000) "Decreased neural crest stem cell expansion is responsible for the conotruncal heart defects within the splotch (Sp(2H))/Pax3 mouse mutant," *Cardiovasc. Res*. **47**, 314-328.

Conway, S. J., Henderson, D. J., and Copp, A. J. (1997) "Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant," *Development* **124**, 505-514.

Conway, S. J., Kruzynska-Frejtag, A., Kneer, P. L., Machnicki, M., and Koushik, S. V. (2003) "What cardiovascular defect does my prenatal mouse mutant have, and why?" *Genesis*. **35**, 1-21.

Crampin, E. J., Schnell, S. and McSharry, P. E. (2004) "Mathematical and computational techniques to deduce complex biochemical reaction mechanisms," *Prog. Biophys. Mol. Biol.,* (in press).

Crawford, K. and Stocum, D. L. (1988a) "Retinoic acid coordinately proximalizes regenerate pattern and blastema differential affinity in axolotl limbs," *Development* **102**, 687-698.

Crawford, K. and Stocum, D. L. (1988b) "Retinoic acid proximalizes level-specific properties responsible for intercalary regeneration in axolotl limbs," *Development* **104**, 703-712.

Cutler, J. E. and Munoz, J. J. (1974) "A simple *in vitro* method for studies on chemotaxis," *Proceedings of the Society for Experimental Biology and Medicine* **147**, 471-474.

Czirok, A., Rupp, P. A., Rongish, B. J. and Little, C. D. (2002) "Multi-field 3D scanning light microscopy of early embryogenesis," *Journal of Microscopy* **206**, 209-217.

Davis, G. E. and Bayless, K. J. (2003) "An integrin and Rho GTPase-dependent pinocytic vacuole mechanism controls capillary lumen formation in collagen and fibrin matrices," *Microcirculation* **10**, 27-44.

Davis, G. E., Bayless, K. J. and Mavila, A. (2002) "Molecular basis of endothelial cell morphogenesis in three-dimensional extracellular matrices," *Anat. Rec*. **268**, 252-275.

de Haan, G., Nijhof, W. and Van Zant, G. (1997) "Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity," *Blood* **89**, 1543-1550.

Dertinger, S. K. W., Chiu, D. T., Jeon, N. L. and Whitesides, G. M. (2001) "Generation of gradients having complex shapes using microfluidic networks," *Analytical Chemistry* **73**, 1240-1246.

Devreotes, P. N. and Zigmond, S. H. (1988) "Chemotaxis in eukaryotic cells: a focus on leukocytes and dictyostelium," *Annual Review of Cell Biology* **4**, 649-686.

Dimmeler, S., Aicher, A., Vasa, M., Mildner-Rihm, C., Adler, K., Tiemann, M., Rutten, H., Fichtlscherer, S., Martin, H. and Zeiher, A. M. (2001) "HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway," *J. Clin. Invest*. **108**, 391-397.

Djonov, V., Baum, O. and Burri, P. H. (2003) "Vascular remodeling by intussusceptive angiogenesis," *Cell And Tissue Research* **314**,107-117.

Domen, J. and Weissman, I. L. (1999) "Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate," *Mol. Med. Today* **5**, 201-208.

Dongarra, J., Foster, I., Fox, G., Gropp. W., Kennedy, K., Torczon. L. and White, A. (2003) *Sourcebook of Parallel Computing*, (Morgan Kauffman)

Downie, S. A. and Newman, S.A. (1994) "Morphogenetic differences between fore and hind limb precartilage mesenchyme: relation to mechanisms of skeletal pattern formation," Dev. Biol. **162**, 195-208.

Downie S. A. and Newman, S.A. (1995) "Different roles for fibronectin in the generation of fore and hind limb precartilage condensations," Dev. Biol. **172**, 519-530.

Downs, K. M. and Davies, T. (1993) "Staging of gastrulating mouse embryos by morphological landmarks in the dissecting microscope," *Development* **118**, 1255-1266.

Downs, K. M. and Gardner, R. L. (1995) An investigation into early placental ontogeny: allantoic attachment to the chorion is selective and developmentally regulated," *Development* **121**, 407-416.

Downs, K. M., Temkin, R., Gifford, S. and McHugh, J. (2001) "Study of the murine allantois by allantoic explants," *Developmental Biology* **233**, 347-364.

Dragoo, J. L., Choi, J. Y., Lieberman, J. R., Huang, J., Zuk, P. A., Zhang, J., Hedrick, M. H. and Benhaim, P. (2003) "Bone induction by BMP-2 transduced stem cells derived from human fat," *J. Orthop. Res.* **21**, 622-629.

Drake, C. J, Hungerford, J. E. and Little, C. D. (1998) "Morphogenesis of the first blood vessels," *Ann. NY Acad. Sci.* **857**, 155-179.

Drake, C. J, LaRue, A., Ferrara, N. and Little, C. D. (2000) "VEGF regulates cell behavior during vasculogenesis," *Developmental Biology* **224**, 178-188.

Drake, C. J. and Fleming, P. A. (2000) "Vasculogenesis in the day 6.5 to 9.5 mouse embryo," *Blood* **95**, 1671-1679.

Drake, C. J. and Little, C. D. (1998) "The morphogenesis of primordial vascular networks *In vivo*," in Vascular Morphogenesis, *In Vivo, In Vitro, In Mente*. (Little, Sage and Mironov, eds., Birkhauser and Co)

Drake, C. J. and Little, C. D. (1999) "VEGF and vascular fusion: implications for normal and pathological vessels," *J. Histochem. and Cytochem.* **47**, 1351-1356.

Drake, C. J., Brandt. S. J., Trusk, T. C. and Little, C .D. (1997) "TAL1/SCL Is expressed in endothelial progenitor cells/angioblasts and defines a dorsal-to-ventral gradient of vasculogenesis," *Developmental Biology* **192**, 17-30.

Drake, C. J., Cheresh, D. A. and Little, C. D. (1995) "An antagonist of integrin alpha v beta 3 prevents maturation of blood vessels during embryonic neovascularization," *J. Cell Sci.* **108**, 2655-2661.

Drake, C. J., Davis, L. A., Hungerford, J. .E and Little, C. D. (1992) "Perturbation of beta 1 integrin-mediated adhesions results in altered somite cell shape and behavior," *Developmental Biology* **149**, 327-338.

Drake, C. J., LaRue, A., Ferrara, N. and Little, C. D. (2000) "VEGF regulates cell behavior during vasculogenesis," *Developmental Biology* **224**, 178-188.

Drake, C. J., Little, C. D. (1995) "Exogenous VEGF induces malformed and hyperfused vessels," *Proc. Natl. Acad. Sci., USA* **92**, 7657-766.

Duan, H. F., Wu, C. T., Wu, D. L., Lu, Y., Liu, H. J., Ha, X. Q., Zhang, Q. W., Wang, H., Jia, X. X. and Wang, L. S. (2003) "Treatment of myocardial ischemia with bone marrow-derived mesenchymal stem cells overexpressing hepatocyte growth factor," *Mol. Ther.* **8**, 467-474.

Dumont, D. J., Gradwohl, G., Fong, G. H., Puri, M. C., Gertsenstein, M., Auerbach, A. and Breitman, M. L. (1994) "Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo," *Genes Dev.* **8**, 1897-909.

Ehrmann, R. L. and Gey, G. O. (1956) "The growth of cells on a transparent gel of reconstituted rat-tail collagen," *J. Natl. Cancer Inst.* **16**, 1375-1403.

Eichmann, A., Pardanaud, L., Yuan, L. and Moyon, D. (2002) "Vasculogenesis and the search for the hemangioblast," *J. Hematother Stem Cell Res.* **11**, 207-214.

Ekker, S. C. (2000) "Morphants: a new systematic vertebrate functional genomics approach," *Yeast* **17**, 302-306.

Ekker, S. C. and Larson, J. D. (2001) "Morphant technology in model developmental systems," *Genesis* **30**, 89-93.

Elias F., Flament C., Glazier J. A, Graner F. and Jiang Y., (1999) "Foams Out of Stable Equilibrium: Cell Elongation and Side Swapping," *Phil. Mag. B* **79**, 729-751.

Elsdale, T. and Bard, J. (1972) "Collagen substrata for studies on cell behavior*," J. Cell Biol.* **54**, 626-637.

Ema, M. and Rossant, J. (2003) "Cell fate decisions in early blood vessel formation," *Trends Cardiovasc. Med.* **13**, 254-259.

Endler, E. E., Duca, K. A., Nealey, P. F., Whitesides G. M. and Yin J. (2003) "Propagation of viruses on micropatterned host cells," *Biotechnology and Bioengineering* **81**, 719-725.

Engquist, W. E. B. and Huang, Z. (2003), "Heterogeneous multi-scale method - a general methodology for multi-scale modeling," *Phys. Rev. B* **67**, 092101.

Engquist, W. E. B. and Huang Z. (2001), "Matching conditions in atomistic-continuum modeling of materials," *Phys. Rev. Lett*. **87**, 135501.

Engquist, W. E. B. (2003a), "The heterogeneous multi-scale method," *Comm. Math. Sci.* **1**, 87-133.

Engquist, W. E. B. (2003b), "Multiscale Modeling and Computation," *Notices of the AMS* **50**, 1062-1070.

Erickson, G. R., Gimble, J. M., Franklin, D. M., Rice, H. E., Awad, H. and Guilak, F. (2002) "Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo," *Biochem. Biophy.s Res. Commun.* **290**, 763-769.

Erokhnia, I. L. (1968a) "The proliferation and DNA synthesis during early stages of myocardial development," *Tsiotologiya* **10**, 162-172.

Erokhnia, I. L. (1968b) "Proliferation dynamics of cellular elements in the differentiating mouse myocardium," *Tsiotologiya* **10**, 1391-1409.

Farinas, J., Chow, A. W. and Wada, H. G. (2001) "A microfluidic device for measuring cellular membrane potential," *Analytical Biochemistry* **295**, 138-142.

Ferkowicz M. J., Starr M., Xie, X., Li, W., Johnson S. A., Shelley W. C., Morrison P. R. and Yoder, M. C. (2003) "CD41 expression defines the onset of primitive and definitive hematopoiesis in the murine embryo," Development **130**, 4393-4403.

Firulli, A. B. and Thattaliyath, B. D. (2002) "Transcription factors in cardiogenesis: The combinations that may unlock the mysteries of the heart," *Inter. Rev. Cytol*. **214**, 1-62.

Fischman, D. A. and Mikawa, T. (1997) "The use of replication-defective retroviruses for cell lineage studies of myogenic cells," *Methods Cell Biol.* **52**, 215-227.

Forrai, A. and Robb, L. (2003) "The hemangioblast - between blood and vessels," *Cell Cycle* **2**, 86-90.

Foster, I., Kesselman, C., Nick, J. M. and Tuecke, S. (June 22, 2002) "The Physiology of the Grid: An Open Grid Services Architecture for Distributed Systems Architecture," *Global Grid Forum* (Draft 2.9), http://www.ggf.org/ogsi-wg/drafts/ogsa_draft2.9_2002-06-22.pdf.

Fox, G. and Hey, A., eds. (2002) *Concurrency and Computation: Practice and Experience* **14**.

Fox, G., Gannon, D. and Thomas, M. (2002) "Editorial: A Summary of Grid Computing Environments." *Concurrency and Computation: Practice and Experience* **14**, 1035-1044.

Fox, G., Williams, R. and Messina, P. (1994) *Parallel Computing Works* (Morgan Kauffman).

Frenz D. A., Jaikaria, N. S., and Newman, S. A. (1989) "The mechanism of precartilage mesenchymal condensation: a major role for interaction of the cell surface with the amino-terminal heparin-binding domain of fibronectin," Dev. Biol. **136**, 97-103.

Frenz D. A., Akiyama S. K., Paulsen D. F. and Newman, S. A. (1989) "Latex beads as probes of cell surface-extracellular matrix interactions during chondrogenesis: evidence for a role for amino-terminal heparin-binding domain of fibronectin," Dev Biol. **136**, 87-96.

Friedl, P., Maaser, K., Klein, C. E, Niggemann, B., Krohne, G. and Zanker, K. S. (1997) "Migration of highly aggressive MV3 melanoma cells in 3-dimensional collagen lattices results in local matrix reorganization and shedding of alpha2 and beta1 integrins and CD44," *Cancer Res*. **57**, 2061-2070.

Fu, A. Y., Spence, C., Schere, A., Arnonld, F. H. and Quake, S. R. (1999) "A microfabricated fluorescence-activated cell sorter," *Nature Biotechnology* **17**, 1109-1111.

Gamba, A., Ambrosi, D., Coniglio, A., De Candia, A., Di Talia, S., Giraudo, E., Serini, G., Preziosi, L. and Bussolino, F. (2003) "Percolation morphogenesis and burgers dynamics in blood vessels formation," *Phys. Rev*. Lett., **90**, 118101.

Garcia, A. L., Bell, J. B., Crutchfield, W. Y. and Alder, B. J. (1999) "Adaptive Mesh and Algorithm Refinement using Direct Simulation Monte Carlo," *J. Comp. Phys.* **154**, 134-155.

Gardiner, D. M., Blumberg, B., Komine, Y. and Bryant, S. V. (1995) "Regulation of HoxA expression in developing and regenerating axolotl limbs," *Development* **121**, 1731-1741.

Gardiner, D. M., Endo, T. and Bryant, S.V. (2002) "The molecular basis of amphibian limb regeneration: integrating the old and the new," *Sem Cell Developmental Biology* **13**, 345-352.

Gay, S. W. and Kosher, R. A. (1985) "Prostaglandin synthesis during the course of limb cartilage differentiation *in vitro*," J. Embryol. Exp. Morphol. **89**, 367-382.

Gear, C. W., Kevrekidis, I. G. and Theodoropoulos, C. (2002) "Coarse Integration/Bifurcation Analysis via Microscopic Simulators: micro-Galerkin methods," *Comp. Chem. Eng*. **26**, 941-978.

Gehling, U. M., Ergun, S., Schumacher, U., Wagener, C., Pantel, K., Otte, M., Schuch, G., Schafhausen, P., Mende, T., Kilic, N., Kluge, K., Schafer, B., Hossfeld, D. K. and Fiedler, W. (2000) "*In vitro* differentiation of endothelial cells from AC133-positive progenitor cells." *Blood* **95**, 3106-3112.

Gene Ontology Consortium (2000) "Gene ontology: tool for the unification of biology," Nature Genetics **25**, 25-29.

Gerhardt and Betsholtz, C. (2000) "Endothelial-pericyte interactions in angiogenesis," *Cell and Tissue Research* **314**,15-23.

Gehris A.L., Stringa, E., Spina, J., Desmond, M. E., Tuan, R. S. and Bennett, V.D. (1997) "The region encoded by the alternatively spliced exon IIIA in mesenchymal fibronectin appears essential for chondrogenesis at the level of cellular condensation," Dev. Biol. **190**, 191-205.

Gillespie, D. T. (1977) "Exact stochastic simulation of coupled chemical reactions," *J. Phys. Chem* **81**, 2340-2361.

Glazier, J. A. and Graner, F. (1993) "Simulation of the differential adhesion driven rearrangement of biological cells," *Phys. Rev. E* **47**, 2128-2154.

Glazier, J. A. and Weaire, D. (1992) “Modelling Grain Growth and Soap Froth Coarsening: Past, Present and Future,” *Materials Science Forum* **27**, 94-96.

Godfrey, K. R. (1983) *Compartmental Models and their Application* (Academic Press, New York).

Godfrey, K. R. and DiStefano, J. J., III (1987) “Identifiability of model parameters,” *In Identifiability of Parametric Models* (E. Walter, Editor, Pergamon, Oxford).

Godfrey, K. R., Chapman, M. J. and Vajda, S. (1994) “Identifiability and indistinguishability of nonlinear pharmacokinetic models,” *J. Pharmacokinet. Biopharm*. **22**, 229-251.

Goland, Y., Whitehead, E., Faizi, A., Carter, S., and Jensen, D. (1999). “HTTP Extensions for Distributed Authoring-WEBDAV,” *Internet Engineering Task Force RFC 2518* (http://www.ietf.org/rfc/rfc2518.txt).

Goto, S., Okuno, Y., Hattori, M., Nishioka, T. and Kanehisa, M. (2002) “LIGAND: database of chemical compounds and reactions in biological pathways,” *Nucleic Acids Res.* **30**, 402-404.

Govindaraju, M., Krishnan, S., Chiu, K., Slominski, A., Gannon, D. and Bramley, R. (2003) “Merging the CCA Component Model with the OGSI Framework,” Proceedings of CCGrid2003, 3rd International Symposium on Cluster Computing and the Grid.

Graner, F. and Glazier, J. A. (1992) “Simulation of biological cell sorting using a 2-dimensional extended Potts model,” *Phys. Rev. Lett.* **69**, 2013-2016.

Grant, M. B., May, W. S., Caballero, S., Brown, G. A., Guthrie, S. M., Mames, R. N., Byrne, B. J., Vaught, T., Spoerri, P. E., Peck, A. B. and Scott, E. W. (2002) “Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization,” *Nat. Med.* **8**, 607-612.

Grow, M. W. and Kreig, P. A. (1998) “Tinman function is essential for vertebrate heart development elimination of cardiac differentiation by dominant inhibitory mutants of the tinman-related genes, XNkx2-3 and XNkx2-5,” *Developmental Biology* **204**, 187-96.

Gulick, J., Subramaniam, A., Neumann, J. and Robbins, J. (1991) “Isolation and characterization of the mouse cardiac myosin heavy chain genes,” *J. Biol. Chem.* **266**, 9180-9185.

Gunzer, M., Kampgen, E., Brocker, E. B., Zanker, K. S. and Friedl, P. (1997) “Migration of dendritic cells in 3D-collagen lattices. Visualisation of dynamic interactions with the substratum and the distribution of surface structures via a novel confocal reflection imaging technique,” *Adv. Exp. Med. Biol.* **417**, 97-103.

Hadd, A. G., Jacobson, S. C. and Ramsey, J. M. (1999) "Microfluidic biochemical detection and kinetics of acetylcholinesterase inhibitors," *Analytical Chemistry* **71**, 5206-5212.

Hadd, A. G., Raymond, D. E., Halliwell, J. W., Jacobson, S. C. and Ramsey, J. M. (1997) "Microchip device for performing enzyme assays," *Analytical Chemistry* **69**, 3407-3412.

Haffter, P. and Nusslein-Volhard, C. (1996) “Large scale genetics in a small vertebrate, the zebrafish,” *Int. J. Developmental Biology* **40**, 221-227.

Hall, B. K. and Miyake, T. (1995) “Divide, accumulate, differentiate: cell condensation in skeletal development revisited,” *Int. J. Dev. Biol.* **39**, 881-893.

Hanahan, D. (1997) “Signaling vascular morphogenesis and maintenance,” *Science* **277**, 48-50.

Hart, D., Lauer, D. and Stewart, C. A. (2000) “Performance of parallel programs on PC clusters: NT vs. Linux,” Presented at *SIAM Conference on Computational Science and Engineering*, http://www.siam.org/meetings/cse00/.

Harvey, R. P., Biben, C. Ellliot, D. A. (1999) *Heart development* (Harvey, R.P. and Rosenthal, N. *et al*., eds., Academic Press, London).

Heath, James R., Phelps, Michael E. and Hood, Leroy (September-October 2003) “NanoSystems biology,” *Molecular Imaging & Biology* **5**, 312-325.

Heiland, R., Baker, M. P. and Semeraro, B. D. (1999) "A survey of visualization tools for high performance computing," *Poster presentation at SIAM Parallel Processing for Scientific Computing.*

Heiland, R., Baker, M. P. and Tafti, D. (2001) “VisBench: a framework for remote data visualization and analysis,” *Proceedings of the 2001 International Conference on Computational Science*, Springer Verlag Lecture Notes in Computer Science (LNCS), San Francisco.

Heinrich, R. and Rapoport, T. A. (1974) “A linear steady-state treatment of enzymatic chains. Critique of the crossover theorem and a general procedure to identify interaction sites with an effector,” *Eur. J. Biochem.* **42**, 97-105.

Heinrich, R. and Schuster, S. (1996) *The regulation of cellular systems*, (Chapman and Hall, New York).

Henderson and Carter (Dec. 2002) "Mechanical Induction in Limb Morphogenesis: The Role of Growth-generated Strains and Pressures," *Bone*, **31** (6), 645-653.

Hewitt, D. and Stewart, C. A. (Dec. 1996) "Implementing online help services," Half-day tutorial given at *CAUSE96,* (San Francisco).

Hogeweg, P. (2000) "Evolving mechanisms of morphogenesis: On the interplay between differential adhesion and cell differentiation," *J. Theor. Biol.* **203**, 317-333.

Holm, E. A., Glazier, J. A., Srolovitz, D. J. and Grest, G. S. (1991) "Effects of lattice anisotropy and temperature on domain growth in the 2-dimensional Potts-model," *Phys. Rev. A* **43**, 2662-2668.

Hou, D. and March, K. L. (2003) "A novel percutaneous technique for accessing the normal pericardium: a single-center successful experience of 53 porcine procedures," *J. Invasive Cardiol.* **15**, 13-17.

Howell, J. C., Lee, W. H., Morrison, P., Zhong, J., Yoder, M. C. and Srour, E. F. (2003) "Pluripotent stem cells identified in multiple murine tissues," *Ann. NY Acad. Sci.* **996**, 158-173.

Howell, J. C., Yoder, M. C. and Srour, E. F. (2002) "Hematopoietic potential of murine skeletal muscle-derived CD45(-)Sca-1(+)c-kit(-) cells," *Exp. Hematol.* **30**, 915-924.

Huang, J. I., Beanes, S. R., Zhu, M., Lorenz, H. P., Hedrick, M. H. and Benhaim, P. (2002) "Rat extramedullary adipose tissue as a source of osteochondrogenic progenitor cells," *Plast. Reconstr. Surg*. **109**, 1033-1041; discussion 1042-1033.

Huang, W. E. and Z. (2001), "Matching conditions in atomistic-continuum modeling of materials," *Phys. Rev. Lett.* **87**, 135501.

Hucka, M., Finney, A., Sauro, H. M., Bolouri, H., Doyle, J. C., Kitano, H., Arkin, A. P., Bornstein, B. J., Bray, D., Cornish-Bowden, A., Cuellar, A. A., Dronov, S., Gilles, E. D., Ginkel, M., Gor, V., Goryanin, I. I., Hedley, W. J., Hodgman, T. C., Hofmeyr, J.-H., Hunter, P. J., Juty, N. S., Kasberger, J. L., Kremling, A., Kummer, U., Le Novere, N., Lowe, L. M., Lucio, D., Mendes, P., Minch, E., Mjolsness, E. D., Nakayama, Y., Nelson, M. R., Nielsen, P. F., Sakurada, T., Schaff, J. C., Shapiro, B. E., Shimizu, T. S., Spence, H. D., Stelling, J., Takahashi, K., Tomita, M., Wagner, J. and Wang, J. (2003) "The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models," *Bioinformatics* **19**, 525-531.

Huh, D., Tung, Y., Wei, H., Grotberg, J. B., Skerlos, S. J., Kurabayashi, K. and Takayama, S. (2002) "Use of air-liquid two-phase flow in hydrophobic microfluidic channels for disposable flow cytometers," *Biomedical Microdevices* **4**, 141-149.

Hummer, G. and Kevrekidis, I. G. (2003), "Coarse molecular dynamics of a peptide fragment: free energy, kinetics and long time dynamics computations*," J. Chem. Phys.* **118**, 10762.

Hungerford, J. E. and Little, C. D. (1999) "Developmental biology of the vascular smooth muscle cell," *J. Vasc. Res.* **36**, 2-27.

Hunter, P. J. and Borg, T. K. (2003) "Integration from proteins to organs: the Physiome Project," *Nature Reviews Molecular and Cell Biology* **4**, 237-243.

Ibanez, L., Schroeder, W., Ng, L. and Cates, J. (2003) *The ITK Software Guide*, (Kitware, Inc.).

Icardo, J. M., and Manasek, F. J. (1983) "Fibronectin distribution during early chick embryo heart development," Dev. Biol. **95,** 19-30.

Icardo, J. M. (1984) "The growing heart: An anatomical perspective," *Growth of the heart in health and disease* (Zak, R., ed., Raven Press) 41-79.

Ichida, F., Tsubata, S., Bowles, K. R., Haneda, N., Uese, K., Miyawaki, T., Dreyer, W. J., Messina, J., Li, H., Bowles, N. E. and Towbin, J. A. (2001) "Novel gene mutations in patients with left ventricular noncompaction or Bath Syndrome," *Circ*. **103**, 1256-1263.

Inoue, I., Wakamoto, Y., Moriguchi, H., Okano, K. and Yasuda, K. (2001) "On-chip culture system for observation of isolated individual cells," *Lab on a Chip* **1**, 50-55.

Ishikawa, F., Drake, C. J., Yang, S., Fleming, P., Minamiguchi, H., Visconti, R. P., Crosby, C. V., Argraves, W. S., Harada, M., Key, L. L. Jr., Livingston, A. G., Wingard, J. R. and Ogawa, M. (2003) "Transplanted human cord blood cells give rise to hepatocytes in engrafted mice," *Ann. NY Acad. Sci.* **996**, 174-185.

Isner, J. M. and Asahara, T. (1999) Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization," *J. Clin. Invest.* **103**, 1231-1236.

Itescu, S., Kocher, A. A. and Schuster, M. D (2003) “Myocardial neovascularization by adult bone marrow-derived angioblasts: strategies for improvement of cardiomyocyte function,” *Heart Fail. Rev.* **8**, 253-258.

Iwaguro, H., Yamaguchi, J., Kalka, C., Murasawa, S., Masuda, H., Hayashi, S., Silver, M., Li, T., Isner, J. M. and Asahara, T. (2002) “Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration,” *Circulation* **105**, 732-738.

Izaguirre, J. A., Chaturvedi, R., Huang, C., Cickovski, T., Coffland, J., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A., and Glazier, J. A., (accepted for publication) "CompuCell, a multi-model framework for simulation of morphogenesis" *Bioinformatics*, (Manuscript ID: BIOINF-2003-0175-03/162).

Jacobson, S. C. and Ramsey, J. M. (1996) “Integrated microdevice for DNA restriction fragment analysis,” *Analytical Chemistry* **68**, 720-723.

Jacobson, S. C., Ermakov, S. V. and Ramsey, J. M. (1999a) “Minimizing the number of voltage sources and fluid reservoirs for electrokinetic valving in microfluidic devices,” *Analytical Chemistry* **71**, 3273-3276.

Jacobson, S. C., Hergenröder, R., Koutny, L. B., Warmack, R. J. and Ramsey, J. M. (1994a) “Effects of injection schemes and column geometry on the performance of microchip electrophoresis devices,” *Analytical Chemistry* **66**, 1107-1113.

Jacobson, S. C., Hergenröder, R., Moore Jr., A. W. and Ramsey, J. M. (1994b) “Precolumn reactions with electrophoretic analysis integrated on a microchip,” *Analytical Chemistry* **66**, 4127-4132.

Jacobson, S. C., McKnight, T. E. and Ramsey, J. M. (1999b) "Microfluidic devices for electrokinetically driven parallel and serial mixing," *Analytical Chemistry* **71**, 4455-4459.

Jeon, N. L., Baskaran, H., Dertinger, S. K. W., Whitesides, G. M., Van De Water, L. and Toner, M. (2001) “Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device,” *Nature Biotechnology* **20**, 826-830.

Jeter, J. R. Jr. and Cameron, I. L. (1971) “Cell proliferation patterns during cytodifferentiation in embryonic chick tissues: liver, heart and erythrocytes,” *J. Embryol. Exp. Morphol.* **25**, 405-422.

Jiang, Y., Levine, H. and Glazier, J. A. (1998) “Possible cooperation of differential adhesion and chemotaxis in mound formation of *Dictyostelium*,” *Biophys. J.* **75**, 2615-2625.

Johnson, C. R., Parker, S., Weinstein, D. and Heffernan, S (2002) “Component-based problem solving environments for large-scale scientific computing,” *Journal on Concurrency and Computation: Practice and Experience* **14**, 1337-1349.

Kale, S., Karihaloo, A., Clark, P. R., Kashgarian, M., Krause, D. S. and Cantley, L. G. (2003) “Bone marrow stem cells contribute to repair of the ischemically injured renal tubule,” *J. Clin. Invest.* **112**, 42-49.

Kalka, C., Masuda, H., Takahashi, T., Kalka-Moll, W. M., Silver, M., Kearney, M., Li, T., Isner, J. M. and Asahara, T. (2000) “Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization,” *PNAS* 97, 3422-3427.

Kallianpur, A. R., Jordan, J. E. and Brandt, S. J. (1994) “The SCL/TAL-1 gene is expressed in progenitors of both the hematopoietic and vascular systems during embryogenesis,” *Blood* **83**, 1200-1208.

Kanehisa, M., Goto, S., Kawashima, S. and Nakaya, A. (2002) “The KEGG databases at GenomeNet,” *Nucleic Acids Res.* **30**, 42-6.

Kang, H. J., Kim, S. C., Kim, Y. J., Kim, C. W., Kim, J. G., Ahn, H. S., Park, S. I., Jung, M. H., Choi, B. C. and Kimm, K. (2001) “Short-term phytohaemagglutinin-activated mononuclear cells induce endothelial progenitor cells from cord blood CD34+ cells,” *Br. J. Haematol.* **113**, 962-969.

Kasahara, H., Lee, B. *et al.* (2000) “Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease,” *J. Clin. Invest.* **106**, 299-308.

Katz, E. B., Steinhelper, M. E., Delcarpio, J. B., Daud, A. I., Claycomb, W. C. and Field, L. J. (1992) “Cardiomyocyte proliferation in mice expressing alpha-cardiac myosin heavy chain-SV40 T-antigen transgenes,” *Am. J. Physiol.* **262**, H1867-H1876.

Kawamoto, A., Gwon, H. C., Iwaguro, H., Yamaguchi, J. I., Uchida, S., Masuda, H., Silver, M., Ma, H., Kearney, M., Isner, J. M. and Asahara, T. (2001) “Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia,” *Circulation* **103**, 634-637.

Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y. S., Milliken, C., Uchida, S., Masuo, O., Iwaguro, H., Ma, H., Hanley, A., Silver, M., Kearney, M., Losordo, D. W., Isner, J. M. and

Asahara, T. (2003) "Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia," *Circulation* **107**, 461-468.

Kedem, O. and Caplan, S. R. (1965) "Degree of coupling and its relation to efficiency of energy conversion," *Trans. Faraday Soc*. **61**,1897-1911.

Keener, J. P. (2000), "Homogenization and propagation in the bistable equations," *Physica D* **136**, 1-17.

Kehat, I. and Gepstein, L. (2003) "Human embryonic stem cells for myocardial regeneration," *Heart Fail Rev*. **8**, 229-36.

Keller, R. E., Danilchik, M., Gimmlich, R. and Shih, J. (1985) "Convergent extension by cell intercalation during gastrulation of *Xenopus laevis*," In *Molecular Determinants of Animal Form* (Edelman, G. M. ed., Alan R. Liss, New York) 111-141.

Keller, R., Stewart, C., Colbourne, J., Hess, M., Hart, D., Steinbachs, J., Woessner, U., Berry, D., Repasky, R., Mueller, M., Li, H., Stuart, G. W., Resch, M., Wernert, E., Buchhorn, M., Takemiya, H., Belhaj, R., Nagel, W. E., Sanielevici, S., Kofuji, S. T., Bannon, D., Nakajima, N., Badia, R., Miller, M. A., Park, H., Steens, R., Lin, F.-P., Brooke, J., Moffett, D., Wee, T. T., Newby, G., Poole, J. C. T., Hamza, R., Papakhian, M., Grundhoefer, L., Cherbas, P. and Trueman, J. (2003) "Global analysis of arthropod evolution," (Available online: http://www.sc-conference.org/sc2003/inter_cal_inter_cal_detail.php?eventid=10701#3).

Kevrekidis, I. G., Gear, C. W., Hyman, J. M., Kevrekidis, P. G., Runborg, O. and Theodoropoulos, K. (2002) "Equation-free multiscale computation: enabling microscopic simulators to perform system-level tasks," submitted *to Comm. Math. Science*, downloadable from Los Alamos Preprint Archive physics/0209043.

Kitano, H. (2002) "Systems Biology: A Brief Overview," *Science* **295** (5560),1662-1664.

Kocher, A. A. (2003) "Bone marrow-derived stem cells for ischemic hearts," *Wien Klin. Wochenschr.* **115**, 77-79.

Kochilas, L. K., Li, J., Buck, C. A. and Epstein, J. A. (1998) "$P57^{kip2}$ expression is enhanced during mid-cardiac murine development and is restricted to trabecular myocardium," *Pediatric Research* **45**, 635-642.

Kompala, D.S., Ramkrishna, D., Jansen, N. B. and Tsao, G. T. (1986) "Investigation of Bacterial Growth on Mixed Substrates. Experimental Evaluation of Cybernetic Models," *Biotechnology and Bioengineering* **28**, 1044-1056.

Koushik, S. V., Wang, J., Rogers, R., Moskophidis, D., Lambert, N. A., Creazzo, T. L. and Conway, S. J. (2001) "Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization," *FASEB J.* **15**, 1209-11.

Krishnan, S. et al (2001) "The XCAT Science Portal," *IEEE/ACM Supercomputing*.

Kulesa, P. M. and Fraser, S. E. (2002) "Cell dynamics during somite boundary formation revealed by time-lapse analysis," *Science* **298**, 991-995.

Lele, Z., Folchert, A., Concha, M., Rauch, G. J., Geisler, R., Rosa, F., Wilson, S. W., Hammerschmidt, M. and Bally-Cuif, L. (2002) "Parachute/n-cadherin is required for morphogenesis and maintained integrity of the zebrafish neural tube," *Development* **129**, 3281-3294.

Lelievre, S., Weaver, V. M. and Bissell, M. J. (1996) "Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: a model of gene regulation," *Recent Prog. Horm. Res.* **51**, 417-432.

Leonard, C. M. and Newman, S. A. (1987) "Nuclear events during early chondrogenesis: phosphorylation of the precartilage 35.5-kDa domain-specific chromatin protein and its regulation by cyclic AMP," Dev. Biol. **120**, 92-100.

Levine, H., Aranson, I., Tsimring, L. and Truong, T. V. (1996) "Positive genetic feedback governs cAMP spiral wave formation in *Dictyostelium*," *PNAS* **93**, 6382-6386.

Li, F., Wang, X., Capasso, J. M. and Gerdes, A. M. (1996) "Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development," *J. Mol. Cell Cardiol.* **28**, 1737-1746.

Lions, P. L. (1988) "On the Schwarz alternating method I," in *First International Symposium on Domain Decomposition Methods for Partial Differential Equations*, (Glowinski, R. *et al.,* eds., SIAM, Philadelphia) 2-42.

Lions, P. L. (1989) "On the Schwarz alternating method II," in *Domain Decomposition Methods*, (Chan, T. *et al*., eds., SIAM, Philadelphia) 47-70.

Lions, P. L. (1990), "On the Schwarz alternating method III," in *Third International Symposium on Domain Decomposition Methods for Partial Differential Equations*, (Chan, T. *et al*., eds., SIAM, Philadelphia) 202-223.

Little, C. D. and Drake, C. J. (2000) "Whole-mount immunolabeling of embryos by microinjection. Increased detection levels of extracellular and cell surface epitopes," *Methods Mol. Biol.* **135**, 183-189.

Littlefield, R. J., Heiland, R. W. and Macedonia, C. R. (1996) "Virtual reality volumetric display techniques for three-dimensional medical ultrasound," *Ultrasound in Health Care in the Information Age*, (Sieburg, H., Weghorst, S. and Morgan, K. eds., IOS Press and Ohmsha).

Liu, Q., Babb, S. G., Novince, Z. M., Doedens, A. L., Marrs, J. and Raymond, P. A. (2001) "Differential expression of cadherins-2 and cadherins-4 in the developing and adult zebrafish visual system," *Vis. Neurosci* **18**, 923-933.

Liu, Q., Kerstetter, A. E. Azodi, E. and Marrs, J. A. (2003) "Cadherin-1, -2, and –11 expression and cadherin-2 function in the pectoral limb bud and fin of the developing zebrafish," *Dev. Dyn.* **228**, 734-739.

Liu, Q., Sanborn, K. L., Cobb, N., Raymond, P. A. and Marrs, J. A. (1999) "R-cadherin expression in the developing and adult zebrafish visual system," *J. Comp. Neurol.* **210**, 303-319.

Ljung, L. and Glad, T. (1994) "On global identifiability for arbitrary model parametrizations," *Automatica* **30**, 265-276.

Lyons, I., Parsons, L. M., *et al.* (1995) "Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5," *Genes De*v. **9**, 1654-66.

Mackie K. P., Nairn A. C., Hampel, G., Lam, G. and Jaffe E.A. 1989. "Thrombin and histamine stimulate the phosphorylation of elongation factor 2 in human umbilical vein endothelial cells," J. Biol. Chem. **264**, 1748-1753.

Makeev, A. G., Maroudas, D. and Kevrekidis, I. G. (2002) "'Coarse' stability and bifurcation analysis using stochastic simulators: Kinetic Monte Carlo Examples," *J. Chem. Phys.* **116**, 10083.

Malicki, J., Jo, H. and Pujic, Z. (2003) "Zebrafish N-cadherin, encoded by the glass onion locus, plays an essential role in retinal patterning," *Developmental Biology* **259**, 95-108.

Manoussaki, D. (2003) "A mechanochemical model of angiogenesis and vasculogenesis," *ESAIM-Mathematical Modelling and Numerical Analysis* **37**, 581-599.

Marée, A. F. M. (2000) "From Pattern Formation to Morphogenesis, Multicellular Coordination in Dictyostelium discoideum," *Optima Grafische Communicatie*, Rotterdam.

Martin-Rendon, E. and Watt, S. M. (2003) "Stem cell plasticity," *Br. J. Haematol.* **122**, 877-891.

Masuda, H. and Asahara, T. (2003) "Post-natal endothelial progenitor cells for neovascularization in tissue regeneration," *Cardiovasc Res.* **58**, 390-398.

Masuya, M., Drake, C. J., Fleming, P. A., Reilly, C. M., Zeng, H., Hill, W. D., Martin-Studdard, A., Hess, D. C. and Ogawa, M. (2003) "Hematopoietic origin of glomerular mesangial cells," *Blood*. **15**, 2215-2218.

McAdams, H. H. and Arkin, A. (1999), "It's a noisy business! Genetic regulation at the nanomolar scale," *Trends Genet*. **15**, 65-69.

McCormick, B. H., DeFanti, T. A. and Brown, M. D. (1987) "Visualization in Scientific Computing," *Computer Graphics* **21**, 1-14.

McDonald, J. C., Metallo, S. J. and Whitesides, G. M. (2001) "Fabrication of a configurable, single-use microfluidic device," *Analytical Chemistry* **73**, 5645-5650.

McDougall, S. R., Anderson, A. R. A., Chaplain, M. A. J. and Sherratt, J. A. (2002) "Mathematical modelling of flow through vascular networks: implications for tumour-induced angiogenesis and chemotherapy strategies," *Bull. Math. Biol*. **64**, 673-702.

McFadden, D. G., Charite, J., *et al.* (2000) "A GATA-dependent right ventricular enhancer controls dHAND transcription in the developing heart," *Development* **127**, 5331-5341.

Meijerink, J. A. and Vandervorst, H. A. (1977) "Iterative solution method for linear systems of which coefficient matrix is a symmetric M-matrix," *Math. Comput.* **31**, 148-162.

Meilhac, S. M., Kelly, R. G., Rocancourt, D., Eloy-Trinquet, S., Nicolas, J. F. and Buckingham, M. E. (2003) "A retrospective clonal analysis of the myocardium reveals two phases of clonal growth in the developing mouse heart," *Development* **130**, 3877-3889.

Merks, R., Hoekstra, A., Kaandorp, J. and Sloot, P. (2003) "Models of coral growth: spontaneous branching, compactification and the Laplacian growth assumption," *Journal of Theoretical Biology* **224,** 153-166.

Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H. and Teller, E. (1953) "Equation of state calculations by fast computing machines," *J. Chem. Phys.* **21**, 1087-1092.

Mikawa, T., Borisov, A., Brown, A. M. C., and Fischman, D. A. (1992a) "Clonal analysis of cardiac morphogenesis in the chicken embryo using a replication-defective retrovirus: I. Formation of the ventricular myocardium," *Dev. Dyn.* **193**, 11-23.

Mikawa, T., Cohen-Gould, L. and Fischman, D. A. (1992b) "Clonal analysis of cardiac morphogenesis in the chicken embryos using a replication-defective retrovirus. III: Polyclonal origin of adjacent ventricular myocytes," *Dev. Dyn.* **195**, 133-141.

Miquerol, L., Langille, B. L. and Nagy, A. (2000) "Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression," *Development* **127**, 3941-3946.

Mirkes, P. E. (2002) "Warkany lecture: to die or not to die, the role of apoptosis in normal and abnormal mammalian development," *Teratology*. **65**, 228-39.

Miura, T., Komori, M. and Shiota, K. (2000) "A novel method for analysis of the periodicity of chondrogenic patterns in limb bud cell culture: correlation of *in vitro* pattern formation with theoretical models," *Anat. Embryol. (Berl.)* **201**, 419-428.

Mizuno, H., Zuk, P. A., Zhu, M., Lorenz, H. P., Benhaim, P. and Hedrick, M. H. (2002) "Myogenic differentiation by human processed lipoaspirate cells," *Plast. Reconstr. Surg.* **109**, 199-209; discussion 210-191.

Morra, M., Occhiello E., Marola, R., Garbassi, F., Humphrey, P. and Johnson D. (1990) "On the aging of oxygen plasma-treated polydimethylsiloxane surfaces," *Journal of Colloid and Interface Science* **137**, 11–24.

Motoike, T., Loughna, S., Perens, E., Roman, B. L., Liao, W., Chau, T. C., Richardson, C. D., Kawate, T., Kuno, J., Weinstein, B. M., Stainier, D. Y. and Sato, T. N. (2000) "Universal GFP reporter for the study of vascular development," *Genesis* **28**, 75-81.

Mulholland, D. L. and Gotlieb, A. I. (1996) "Cell biology of valvular interstitial cells," *Can. J. Cardiol*. **12**, 231-236.

Muller, J. G., Isomatsu, Y., Koushik, S. V., O'Quinn, M., Xu, L., Kappler, C. S., Hapke, E., Zile, M. R., Conway, S. J. and Menick, D. R. (2002) "Cardiac-specific expression and hypertrophic upregulation of the feline Na+-Ca2+ exchanger gene H1-promoter in a transgenic mouse model," *Circ. Res*. **90**, 158-164.

Murayama, T. and Asahara, T. (2002) "Bone marrow-derived endothelial progenitor cells for vascular regeneration," *Curr. Opin. Mol. Ther.* **4**, 395-402.

Namjoshi, A. and Ramkrishna, D. (2001) "Bifurcation Analysis of Cybernetic Models for Continuous Bioreactors with Mixed Substrate Feeds," *Chemical Engineering Science* **56,** 5593-5607.

Namjoshi, A., Hu, W.-S. and Ramkrishna, D. (2003) "Unveiling Steady State Multiplicity in Hybridoma Cultures: The Cybernetic Approach," *Biotechnology and Bioengineering* **81**, 80-91.

Nechiporuk, A. and Keating, M. T. (2002) "A proliferation gradient between proximal and msxb-expressing distal blastema directs zebrafish fin regeneration," *Development* **129**, 2607-2617.

Newman S. A. and Muller G. B. (2000) "Epigenetic mechanisms of character origination," *J. Exp. Zool.* **288**, 304-317.

Newman, S. (1998a) *Epithelial morphogenesis: a physico-evolutionary interpretation. In Molecular Basis of Epithelial Appendage Morphogenesis* (Chuong, C.-M. ed., R. G. Landes, Austin, TX) 341-358.

Newman, S. A. (1996) "Sticky fingers: Hox genes and cell adhesion in vertebrate limb development," *BioEssays* **18**, 171-174.

Nusslein-Volhard, C. (1994) "Of flies and fishes," *Science* **266**, 572-574.

Nye, H. L. D., Cameron, J. A., Chernoff, E. A.G. and Stocum, D. L. (2003a) "Regeneration of the urodele limb: a review," *Dev. Dyn.* **226**, 280-294.

Nye, H. L. D., Cameron, J., Chernoff, E. A. G. and Stocum, D. L. (2003b) "Extending the table of stages of normal development of the axolotl: limb development," *Dev. Dyn.* **226**, 555-560.

Oberlander, S. A. and Tuan, R. S. (1994) "Spatiotemporal profile of N-cadherin expression in the developing limb mesenchyme," Cell. Adhes. Commun. **2,** 521-537.

Odelberg S. J., Kollhoff, A. and Keating M. T. (2000) "Dedifferentiation of mammalian myotubes induced by msx1," Cell **103**, 1090-1099.

Oh, H., Bradfute, S. B., Gallardo, T. D., Nakamura, T., Gaussin, V., Mishina, Y., Pocius, J., Michael, L. H., Behringer, R. R., Garry D. J., Entman, M. L. and Schneider, M. D. (2003) "Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction," *PNAS* **100**, 12313-12318.

Orlic, D. (2002) "Adult BM stem cells regenerate mouse myocardium," *Cytotherapy* **4**, 521-525.

Orlic, D. (2003) "Adult bone marrow stem cells regenerate myocardium in ischemic heart disease," *Ann. NY Acad. Sci.* **996**, 152-157.

Ouchi, N. B., Glazier, J. A., Rieu, J.-P., Upadhyaha, A. and Sawada, Y. (2003) "Improving the realism of the cellular Potts model in simulations of biological cells," *Physica A* **329**, 451-458.

Palsson, B. O., Palsson, H. and Lightfoot, E. N. (1985) "Mathematical modelling of dynamics and control in metabolic networks. III. Linear reaction sequences," *J. Theor. Biol.* **113**, 231-259.

Pardanaud, L., Altmann, C., Kitos, P., Dieterlen-Lievre, F. and Buck, C. A. (1987) "Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells," *Development* **100**, 339-349.

Pasumarthi, K. B. S. and Field, L. J. (2000) "Strategies to identify cardiomyocyte cell cycle regulatory genes," *Molecular Approaches to Heart Failure Therapy* (G. Hassenfus and E. Marban, eds., Steinkopff Darmstadt Press) 333-352.

Pasumarthi, K. B. S. and Field, L. J. (2002) "Cardiomyocyte cell cycle regulation," *Circ. Res.* **90**, 1044-1054.

Peebles, C. S., Stewart, C. A., Voss, B. D. and Workman, S. B. (May 2001) "Measuring quality, cost, and value of IT services in higher education," *Proceedings of the American Quality Congress*, **(**Charlotte, NC).

Peebles, C. S., Stewart, C. A., Voss, B. D. and Workman, S. B. (Oct. 2001), "Measuring quality, cost, and value of IT services in higher education," *Proceedings of Educause*, (Indianapolis, IN), http://www.indiana.edu/~rac/staff_papers.html.

Peng, C. F., Wei, Y., Levsky, J. M., McDonald, T. V., Childs, G. and Kitsis, R. N. (2002) "Microarray analysis of global changes in gene expression during cardiac myocyte differentiation," *Physiol. Genomics* **9**, 145-155.

Peskin, C. S. and McQueen, D. M. (1989) "A three-dimensional computational method for blood flow in the heart. I. Immersed elastic fibers in a viscous incompressible fluid," *Journal of Computational Physics* **81**, 372-405.

Poelmann, R. E., Molin, D., Wisse, L. J. and Gittenberger-de Groot, A. C. (2000) "Apoptosis in cardiac development," *Cell Tissue Res*. **301**, 43-52.

Pohjanpalo, H. (1978) "System identifiability based on the power series expansion of the solution," *Math. Biosci*. **41**, 21-33.

Poleo, G., Brown, C. W., Laforest, L. and Akimenko, M. A. (2001) "Cell proliferation and movement during early fin regeneration in zebrafish," *Dev. Dyn.* **221**, 380-390.

Pruitt, K. D., Katz, K. S., Sicotte, H. and Maglott, D. R. (2000) "Introducing RefSeq and LocusLink curated human genome rexsources at the NCBI," *Trends Genet* **16**, 44-7.

Quirici, N., Soligo, D., Caneva, L., Servida, F., Bossolasco, P. and Deliliers, G. L. (2001) "Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells," *Br. J. Haematol* **115**, 186-194.

Rafii, S. (2000) "Circulating endothelial precursors: mystery, reality, and promise," *J. Clin. Invest.* **105**, 17-19.

Rafii, S., Heissig, B. and Hattori, K. (2002) "Efficient mobilization and recruitment of marrow-derived endothelial and hematopoietic stem cells by adenoviral vectors expressing angiogenic factors," *Gene Ther.* **9**, 631-641.

Ramakrishna, R., Ramkrishna, D. and Konopka, A. E. (1996) "Cybernetic Modeling of Growth in Mixed, Substitutable Substrate Environments. Preferential and Simultaneous Utilization," *Biotechnology and Bioengineering* **52**, 141-151.

Ramkrishna, D. (1982) "A Cybernetic Perspective of Microbial Growth," in *Foundations of Biochemical Engineering: Kinetics and Thermodynamics in Biological Systems*, (American Chemical Society Publication).

Ramkrishna, D. (2000) "Population Balances. Theory and Applications to Particulate Systems Engineering," Academic Press, San Diego.

Rathinam, M., Petzold, L. F., Cao, Y. and Gillespie D. T. (2003) "Stiffness in stochastic reacting systems: The implicit tau-leaping method," *J. Chem. Phys.* **119**, 12784-12794.

Rauscher, F. M., Goldschmidt-Clermont, P. J., Davis, B. H., Wang, T., Gregg, D., Ramaswami, P., Pippen, A. M., Annex, B. H., Dong, C. and Taylor, D. A. (2003) "Aging, progenitor cell exhaustion, and atherosclerosis," *Circulation* **108**, 457-463.

Relaix, F., Polimeni, M., Rocancourt, D., Ponzetto, C., Schafer, B. W. and Buckingham, M. (2003) "The transcriptional activator PAX3-FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Met signaling in vivo," Genes Dev. **17**, 2950-2965.

Rentschler, S., Zander, J., Meyers, K., France, D., Levine, R., Porter, G., Rivkees, S. A., Morley, G. E. and Fishman, G. I. (2002) "Neuregulin-1 promotes formation of the murine cardiac conduction system," *PNAS* **99**, 10464-10469.

Reyes, D. R., Iossifidis, D., Auroux, P.-A. and Manz, A. (2002) "Micro total analysis systems. 1. Introduction, theory, and technology," *Analytical Chemistry* **74**, 2623-2636.

Ribatti, D., Vacca, A., Roncali, L. and Dammacco, F. (2000) "Hematopoiesis and angiogenesis: a link between two apparently independent processes," *J. Hematother Stem Cell Res.* **9**, 13-19.

Risau, W. and Flamme, I. (1995) "Vasculogenesis," *Annu. Rev. Cell Dev. Biol.* **11**, 73-91.

Robertson, E. J. (1987) *Teratocarcinomas and embryonic stem cells: a practical approach* (ed., IRL Press, Oxford)

Robinson, J. P., Brightman, A. O., Kelley, M. R., Rajwa, B. P., Sturgis, J. E. and Voytik-Harbin, S. L. (1999) "Imaging technique for kinetic evaluation of collagen matrix assembly," *FASEB Journal* **13**, A344.

Roma, A. M., Peskin, C. S. and Berger, M. J. (1999) "An adaptive version of the immersed boundary method," *J. Comp. Phys.* **153**, 509-534.

Rottenberg, H., (1973) "The mechanism of energy-dependent ion transport in mitochondria*," J. Membr. Biol.* **11**, 117-137.

Royer, C. L., Howell, J. C., Morrison, P. R., Srour, E. F. and Yoder, M. C. (2002) "Muscle-Derived Cd45(-)Sca-1(+)C-Kit(-) Progenitor Cells Give Rise to Skeletal Muscle Myotubes *in Vitro*," *In Vitro Cell Dev. Biol. Anim.* **38**, 512-517.

Rubart, M., Pasumarthi, K. B. S., Nakajima, H., Soonpaa, M., Nakajima, H. O. and Field L. J. (2003) "Physiologic coupling of donor and host cardiomyocytes following cellular transplantation," *Circ. Res*. **92**, 1217-1224.

Rubart, M., Wang, X., Dunn, K. W. and Field, L. J. (2003) "Two-photon molecular excitation imaging of Ca2+ transients in Langendorff-perfused mouse hearts," *Circ. Res.* **92**, 1217-1224.

Rumyantsev, P. P. (1991) "Growth and hyperplasia of cardiac muscle cells," *Soviet Medical Reviews Supplement series* (Carlson, B.M., editor, Harwood Acad. Publishers, Chur, Switzerland)

Rupp, P. A. and Little, C. D. (2001) "Integrins in vascular development," *Circ. Res*. **89**, 566-572.

Rupp, P. A. Czirok, A. and Little, C. D. (2003) "Novel approaches for studying blood vessel morphogenesis, *in vivo*," *Trends in Cardiovascular Medicine* **13**, 283-288.

Rupp, P. A., Czirok, A. and Little, C. D. (2003) "Endothelial cell dynamics during vasculogenesis," Under revision after initial review, *Development*.

Rupp, P. A., Rongish, B. J., Czirok, A. and Little, C. D. (2003) "Culturing of avian embryos for time-lapse imaging," *Biotechniques* **34**, 274-278.

Safford, K. M., Hicok, K. C., Safford, S. D., Halvorsen, Y. D., Wilkison, W. O., Gimble, J. M. and Rice, H. E. (2002) "Neurogenic differentiation of murine and human adipose-derived stromal cells," *Biochem. Biophys. Res. Commun.* **294**, 371-379.

Sambrano, G. R., Fraser, I., Han, H., Ni, Y., O'Connell, T., Yan, Z. and Stull, J. T. (2002) "Navigating the signaling network in mouse cardiac myocytes," *Nature* **420**, 712-714.

Sastry, L., Johnson, T., Hobson, M. J., Smucker, B. and Cornetta, K. (2002) "Titering lentiviral vectors: comparison of DNA, RNA and marker expression methods," *Gene Ther.* **9**, 1155-1162.

Sata, M., Saiura, A., Kunisato, A., Tojo, A., Okada, S., Tokuhisa, T., Hirai, H., Makuuchi, M., Hirata, Y. and Nagai, R. (2002) "Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis," *Nat. Med.* **8**, 403-409.

Savageau, M. A. (1969) "Biochemical systems analysis. I. Some mathematical properties of the rate law for the component enzymatic reactions," *J. Theor. Biol*. **25**, 365--369.

Savageau, M. A. (1976) *Biochemical Systems Analysis: A study of function and design in molecular biology*, (Addison-Wesley, Reading, MA).

Savageau, M. A. (1992) "A critique of the enzymologist's test tube," in *Fundamentals of medical cell biology* **3A** (Bittar, E. ed., Academic Press, New York) 45-108.

Savageau, M. A. (1995) "Michaelis-Menten mechanism reconsidered: Implications of fractal kinetics," *J. Theor. Biol*. **176**, 115-124.

Savill, N. J. and Hogeweg, P. (1997) "Modelling morphogenesis: from single cells to crawling slugs," *J.Theor. Biol.* **184**, 229-235.

Schaff, J. C. and Lowe, L. M. (1999) "The virtual cell," *Pacific Symposium on Biocomputing* **4**, 228-239.

Schnell, S. and Maini, P. K. (2000) "Clock and induction model for somitogenesis," *Developmental Dynamics* **217**, 415-420.

Schnell, S. and Maini, P. K. (2003) "A century of enzyme kinetics: Reliability of the KM and v_{max} estimates," *Comments on Theoretical Biology* **8**, 169-187.

Schnell, S. and Turner, T. E. (2004) "Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws," *Prog. Biophys. Mol. Biol*., (in press).

Schnell, S., Maini, P. K., McInerney, D., Gavaghan, D. J. and Houston, P. (2002) "Models for pattern formation in somitogenesis: a marriage of cellular and molecular biology," *Comptes Rendus Biologies* **325**, 179-189.

Schuler, G. D. (1997) "Pieces of the puzzle, expressed sequence tags and the catalog of human genes," *J. Mol. Med.* **75**, 694-698.

Scientific Computing and Imaging Institute (2002), "SCIRun: A scientific computing problem solving environment," (http://software.sci.utah.edu/scirun).

Sedmera, D., Reckova, M., DeAlmeida, A., Coppen, S. R., Kubalak, S. W., Gourdie, R. G., and Thompson, R. P. (2003) "Spatio-temporal pattern of commitment to slowed proliferation in the embryonic mouse heart indicates progressive differentiation of the cardiac conduction system," *Anat. Rec*. **274A**, 773-777.

Serini, G., Ambrosi, D., Giraudo, E., Gamba, A., Preziosi, L. and Bussolino, F. (2003) "Modeling the early stages of vascular network assembly," *EMBO J*. **22**, 1771-1779.

Setayeshgar, S. and Bernoff, A. J. (2002) "Dynamics of scroll waves in anisotropic excitable media with application to the heart," *Phys. Rev. Lett.* **88**, 028101-028104.

Setayeshgar, S. and Cross, M. C. (1998) "Turing Instability in a Boundary-Fed System," *Phys. Rev. E* **58**, 4485-4500.

Setayeshgar, S. and Cross, M. C. (1999) "Numerical Bifurcation Diagram for the Two-Dimensional Boundary-Fed CDIMA System," *Phys. Rev. E* **59**, 4258-4264.

Setayeshgar, S., Gear, C. W., Othmer, H. G. and Kevrekidis, I. G. (2003b) "Application of coarse integration to bacterial chemotaxis," submitted to *SIAM Journal on Multiscale Modeling*, downloadable from Los Alamos preprint archive physics/0308040.

Setayeshgar, S., Keller, H. B. and Pearson, J. E. (2002) "Application of lifting to defective eigenvalue problems," submitted to *J. Comp. Phys.*, downloadable from Los Alamos preprint archive math-ph/0210063.

Shah, B. H., Borwanker, J. D. and Ramkrishna, D. (1976) "Monte Carlo Simulation of Microbial Population Growth," *Mathematical Biosciences* **31**, 1-23.

Shah, B. H., Ramkrishna, D. and Borwanker, J. D. (1977) "Monte Carlo Simulation of Particulate Systems," *American Institute of Chemical Engineers Journal* **23,** 897-904.

Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L. and Schuh, A. C. (1995) "Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice," *Nature* **376**, 62-66.

Shi, Y. N., Simpson, P. C., Scherer, J. R., Wexler, D., Skibola, C., Smith, M. T. and Mathies, R. A. (1999) "Radial capillary array electrophoresis microplate and scanner for high-performance nucleic acid analysis," *Analytical Chemistry* **71**, 5354-5361.

Shou, W., Aghdasi, B., Armstrong D. L., Guo, Q., Bao, S., Charng, M.-J., Mathews, L.S., Schneider, M. D., Hamilton, S. L. and Matzuk, M. M. (1998) "Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12," *Nature* **391**, 489-492.

Simper, D., Stalboerger, P. G., Panetta, C. J., Wang, S. and Caplice, N. M. (2002) "Smooth muscle progenitor cells in human blood," *Circulation* **106**, 1199-1204.

Skalak, T. C., Little, C. D., McIntire, L. V., Hirschi, K. K., Tranquillo, R. T., Post, M., and Ranieri, J. (2002) "Vascular assembly in engineered and natural tissues: breakout session summary," *Ann. NY Acad. Sci*. **961**, 255-257.

Smales, W. P., and Biddulph, D. M. (1985) "Limb development in chick embryos: cyclic AMP-dependent protein kinase activity, cyclic AMP, and prostaglandin concentrations during cytodifferentiation and morphogenesis," J. Cell. Physiol. **122**, 259-65.

Smith J. C., and Tata, J. R. (1991) "*Xenopus* cell lines," Methods Cell Biol. **36**, 635-654.

Solloway, M. J. and Harvey, R. P. (2003) "Molecular pathways in myocardial development: a stem cell perspective," *Cardiovasc. Res*. **58**, 264-277.

Solloway, M. J., Dudley, A. T., Bikoff, E. K., Lyons, K. M., Hogan, B. L. and Robertson, E. .J. (1998) "Mice lacking Bmp6 function," Dev Genet. **22**, 321-39.

Solloway, M. J. and E. J. Robertson (1999) "Early embryonic lethality in Bmp5;Bmp7 double mutant mice suggests functional redundancy within the 60A subgroup," Development **126,** 1753-1768.

Soonpaa, M. H. and Field, L. J. (1997) "Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts," *Am. J. Physiol*. **272**, H220-6.

Soonpaa, M. H., Kim, K. K., Pajak, L., Franklin, M. and Field, L.J. (1996) "Cardiomyocyte DNA synthesis and binucleation during murine development," *Am. J. Physiol.* **271**, H2183-2189.

Soonpaa, M. H., Koh, G. Y., Pajak, L., Jing, S., Wang, H., Franklin, M. T., Kim, K. K. and Field, L. J. (1997) "Cyclin D1 overexpression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice," *J. Clin. Invest.* **99**, 2644-2654.

Spellman, P. T., Miller, M., Stewart, J., Troup, C., Sarkans, U., Chervitz, S., Bernhart, D., Sherlock, G., Ball, C., Lepage, M., Swiatek, M., Marks, W. L., Goncalves, J., Markel, S., Iordan, D., Shojatalab, M., Pizarro, A., White, J., Hubley, R., Deutsch, E., Senger, M., Aronow, B. J., Robinson, A., Bassett, D., Stoeckert, C. J. Jr., and Brazman, A. (2002) "Design and implementation of microarray gene expression markup language (MAGE-ML)," *Genome Biol.* **3**, RESEARCH0046.

Srivastava, D. and Olson, E. N. (2000) "A genetic blueprint for cardiac development," *Nature* **407**, 221-226.

Srivastava, D., Thomas, T., *et al.* (1997) "Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, Dhand," *Nat. Genet.* **16**, 154-60.

Steinberg, M. S. (1963) "Reconstruction of tissues by dissociated cells," *Science* **141**, 401-408.

Steinberg, M. S. (1998) "Goal-directedness in embryonic development," *Integrative Biology* **1**, 49-59.

Stewart, C. A. (1998) "Maximum likelihood estimation of phylogenetic relationships with geographically distributed supercomputers," Presented at *CASCON*, (Toronto, Canada). .

Stewart, C. A., Vernon, D. and Grover, D. (1998) "Changing (almost) everything and keeping (almost) everyone happy," *Cause/Effect* **21**:39-46. (Available at www.educause.edu/ir/library/ html/cem9837.html).

Stewart, C. A., et al. (2003) "Information technology support for post-genomic biomedical research," (Presented at SIGUCCS 2003 Conference).

Stewart, C. A., *et al*. (2003) "Opportunities for Biomedical Research and the NIH through High Performance Computing and Data Management," (Published by the Coalition for Advanced Scientific Computing, http://www.casc.org/paper.html).

Stewart, C. A., Hart, D., Berry, D. K., Olsen, G. J., Wernert, E. and Fischer, W. (Nov. 2001) "Parallel implementation and performance of fastDNAml - a program for maximum likelihood phylogenetic inference," *Proceedings of SC2001*, (Denver, CO), http://www.sc2001.org/papers/pap.pap191.pdf.

Stewart, C. A., Peebles, C. S., Papakhian, M., Samuel, J., Hart, D. and Simms, S. (Oct. 2001) "High Performance Computing: Delivering Valuable and Valued Services at Colleges and Universities," *Proceedings of SIGUCCS*, (Portland, OR), http://www.indiana.edu/~rac/staff_papers.html.

Stewart, C. A., Tan T. W., Buckhorn, M., Hart, D., Berry, D. K., Zhang, L., Wernert, E., Sakharkar, M., Fischer, W. and McMullen, D. F. (2000) "Evolutionary biology and high performance computing," in *Software Tools for Computational Biology,* (Enenkel, R. F. ed.) http://www.cas.ibm.com/archives/1999/workshop_report/bio.html.

Stewart, C. A. (Nov. 2003) *Computational Biology, Bioinformatics, and High Performance Computing*. (Tutorial presented at SC2003, Phoenix).

Stockdale, F.E., Nikovits, W. Jr. and Christ, B. (2000) "Molecular and cellular biology of avian somite development," Dev. Dyn. **219**, 304-321.

Stocum, D. L. (2004) "Amphibian regeneration and stem cells," *Regeneration: Stem Cells and Beyond*, (E. Heber-Katz, ed., Springer, New York) 1-70.

Stone, E., Armbruster, D. and Heiland, R. (1996) "Towards analyzing the dynamics of flames," *Fields Institute Communications, Pattern Formation: Symmetry Methods and Applications*, (Chadam, J., Golubitsky, M., Langford, W. and Wetton, B. eds., American Mathematical Society,)

Straight, J. V. and Ramkrishna, D. (1994a) "Cybernetic Modeling and Regulation of Metabolic Pathways. Application to Growth on Complementary Substrates," *Biotechnology Progress* **10**, 574-587.

Straight, J. V. and Ramkrishna, D. (1994b) "Cybernetic Modeling and Regulation of Metabolic Pathways. Application to Growth under Multiply-Limiting Conditions," *Biotechnology Progress* **10**, 588-605.

Strauer, B. E., Brehm, M., Zeus, T., Gattermann, N., Hernandez, A., Sorg, R. V., Kogler, G. and Wernet, P. (2001) "Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction," *Dtsch. Med. Wochenschr.* **126**, 932-938.

Tadmor, E. B., Ortiz, M. and Phillips, R. (1996) "Quasicontinuum analysis of defects in crystals," *Phil. Mag. A* **73**, 1529-1563.

Takayama, S., Ostuni, E., LeDuc, P., Naruse, K., Ingber, D. E. and Whitesides G. M. (2003) "Selective chemical treatment of cellular microdomains using multiple laminar streams," *Chemistry & Biology* **10**, 123-130.

Tam, P. P. and Beddington, R. S. (1987) "The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis," *Development* **99**, 109-126.

Tateishi-Yuyama, E., Matsubara, H., Murohara, T., Ikeda, U., Shintani, S., Masaki, H., Amano, K., Kishimoto, Y., Yoshimoto, K., Akashi, H., Shimada, K., Iwasaka, T. and Imaizumi, T. (2002) "Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial," *Lancet* **360**, 427-435.

Technau, U. and Holstein, T. W. (1992) "Cell sorting during the regeneration of Hydra from reaggregated cells," *Developmental Biology* **151**, 117-27.

Thattaliyath, B. D., Firulli, B. A., *et al.* (2002) "The basic-helix-loop-helix transcription factor *HAND2* directly regulates transcription of the *Atrial Naturetic Peptide* gene," *J. Molec. Cell. Cardiol*. **34**, 1325-1344.

Theodoropoulos, K., Qian, Y. H. and Kevrekidis, I. G. (2000) "Coarse stability and bifurcation analysis using timesteppers: a reaction diffusion example," *PNAS.* **97**, 9840.

Tiampo, K. F, Rundle, J. B., McGinnis, S. and Klein, W. (2002) "Pattern Dynamics and Forecast Methods in Seismically Active Regions." *PAGEOPH*. **159**, 2429-2467.

Tickle C. (2003) "Patterning systems—from one end of the limb to the other," *Dev. Cell.* **4**, 449-458.

Tong, S. and Yuan, F. (2001) "Numerical simulations of angiogenesis in the cornea," *Microvasc. Res*. **61**,14-27.

Torquato, S. (2001) *Random Heterogeneous Materials: Microstructure and Macroscopic Properties,* (Springer-Verlag).

Turing, A. (1952) "The chemical basis of morphogenesis*," Phil. Trans. Roy. Soc. Lond. B* **237**, 37-72.

Turner, S. and Sherratt, J. A. (2002) "Intercellular adhesion and cancer invasion: a discrete simulation using the extended Potts model," *J.Theor. Biol*. **216** 85-100.

Uchida, N. and Weissman, I. L. (1992) "Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin-Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow," *J. Exp. Med.* **175**, 175-184.

van den Hoff, M. J., van den Eijnde. S. M., Viragh, S., Moorman, A. F. (2000) "Programmed cell death in the developing heart," *Cardiovasc. Res*. **45**, 603-20.

van der Kamp, A. W. and Nauta, J. (1979) "Fibroblast function and the maintenance of the aortic-valve matrix," *Cardiovasc. Res*. **13**, 167-72.

van Royen, N., Piek, J. J., Buschmann, I., Hoefer, I., Voskuil, M. and Schaper, W. (2001) "Stimulation of arteriogenesis; a new concept for the treatment of arterial occlusive disease," *Cardiovasc. Res.* **49**, 543-553.

Vanden-Eijnden, E. (2003), "Numerical techniques for multiscale dynamical systems with stochastic effects," *Comm. Math. Sci.* **1**, 385-391.

van Oss, C., Panfilov, A., Hogeweg, V. P., Siegert, F. and Weijer, C. J. (1996) “Spatial Pattern Formation During Aggregation of the Slime Mould *Dictyostelium discoideum*,” *J. Theor. Biol.* **181**, 203-213

Varner, J. and Ramkrishna, D. (1999a) "Metabolic Engineering from a Cybernetic Perspective-I. Theoretical Preliminaries," *Biotechnology Progress* **15**, 407-425.

Varner, J. and Ramkrishna, D. (1999b) "Metabolic Engineering from a Cybernetic Perspective II. Qualitative Investigation of Nodal Architectures and their Response to Genetic Perturbation," *Biotechnology Progress* **15**, 426-438.

Varner, J. and Ramkrishna, D. (1999c) "Mathematical Models of Metabolic Pathways," *Current Opinion in Biotechnology* **10**, 146-150.

Vaziri, H., Dragowska, W., Allsopp, R. C., Thomas, T. E., Harley, C. B. and Lansdorp, P. M. (1994) “Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age,” *PNAS* **91**, 9857-9860.

Vogel, W., Brakebusch, C., Fassler, R., Alves, F., Ruggiero, F. and Pawson, T. (2000) “Discoidin domain receptor 1 is activated independently of beta(1) integrin,” *J. Biol. Chem*. **25**, 5779-5784.

Voss, B., Stewart, C. A. and Jung-Gribble, D. (May 1996) “The leveraged support model,” Presented at *CAUSE95* and *CUMREC*.

Voytik-Harbin, S. L., Brightman, A. O., Waisner, B. Z., Robinson, L. P. and Lamar, C. H. (1998) “Small intestinal submucosa: A tissue-derived extra-cellular matrix that promotes tissue-specific growth and differentiation of cells *in vitro*,” *Tissue Eng.* **4**, 157-174.

Voytik-Harbin, S. L., Rajwa, B., Sturgis, J., McCallister, M. E, Brightman, A. O. and Robinson, J. P. (1998) “Time-lapse confocal reflection (TLR) imaging for the study of extracellular matrix assembly,” *Mol. Biol. Cell* **9**, 61a.

Walder, S., Zhang, F. and Ferretti, P. (2003) “Up-regulation of neural stem cell markers suggests the occurrence of dedifferentiation in regenerating spinal cord,” *Dev. Genes Evol.* **213**, 625-630.

Walter, E. (1982) *Identifiability of State Space Models*, (Springer, New York).

Walter, E. and Lecourtier, Y. (1982) “Global approaches to identifiability testing for linear and nonlinear state space models,” *Math. Comput. Simul*. **24**, 472-482.

Wang, D., Oparil, S., Feng, J. A., Li, P., Perry, G., Chen, L. B., Dai, M., John, S. W. and Chen, Y. F. (2003) “Effects of pressure overload on extracellular matrix expression in the heart of the atrial natriuretic peptide-null mouse,” *Hypertension* **42**, 88-95.

Watanabe, M., Choudhry, A., Berlan, M., Singal, A., Siwik, E., Mohr, S., Fisher, S. A. (1998) “Developmental remodeling and shortening of the cardiac outflow tract involves myocyte programmed cell death,” *Development*. **125**, 3809-20.

Waters, L. C., Jacobson, S. C., Kroutchinina, N., Khandurina, J., Foote, R. S. and Ramsey, J. M. (1998) “Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing,” *Analytical Chemistry* **70**, 158-162.

Witten, T. A. Jr. and Sander, L. M. (1981) “Diffusion-limited aggregation, a kinetic critical phenomenon,” *Phys. Rev. Lett*. **47**, 1400-1403.

Yamagishi, H., Yamsgishi, C., *et al.* (2001) “The combinatorial activities of Nkx2.5 and dHAND are essential for cardiac ventricle formation,” *Developmental Biology* **239**, 190-203.

Zajac, M., Jones, G. L. and Glazier, J. A. (2000) “Model of convergent extension in animal morphogenesis,” *Phys. Rev. Lett*. **85**, 2022-2025.

Zanetti, N.C. and Solursh, M. (1984) “Induction of chondrogenesis in limb mesenchymal cultures by disruption of the actin cytoskeleton,” J. *Cell. Biol.* **99**, 115-123.

Zetter, B. R. (1980) “Migration of capillary endothelial cells is stimulated by tumour-derived factors,” *Nature* **285**, 41-43.

Zhang, F., Clarke, J. D. W. and Ferretti, P. (2000) “FGF-2 up-regulation and proliferation of neural progenitors in the regenerating amphibian spinal cord in vivo” *Developmental Biology* **225**, 381-391.

Zicha, D., Dunn, G. A. and Brown, A. (1991) “A new direct-viewing chemotaxis chamber,” *Journal of Cell Science* **99**, 769-775.

Zigmond, S. H. (1977) “Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors,” *Journal of Cell Biology* **75**, 606-616.

Zorn, M., *et al*. (Nov. 2000) “Computational Biology and High Performance Computing,” *Tutorial presented at SC2000, Dallas*, (Also printed as a special report of Lawrence Berkeley National Laboratory), http://cbcg.lbl.gov.

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P., Lorenz, H. P. and Hedrick, M. H. (2001) “Multilineage cells from human adipose tissue: implications for cell-based therapies,” *Tissue Engineering* **7**, 211-228.

Principal Investigator/Program Director (last, First, Middle): Glazier, James Alexander

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ SBIR Phase I ☐ SBIR Phase II: SBIR Phase I Grant No. _____ ☐ SBIR Fast Track

☐ STTR Phase I ☐ STTR Phase II: STTR Phase I Grant No. _____ ☐ STTR Fast Track

☐ REVISION of application number: _____

(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

☐ COMPETING CONTINUATION of grant number: _____

(This application is to extend a funded grant beyond its current project period.)

INVENTIONS AND PATENTS *(Competing continuation appl. and Phase II only)*

☐ No ☐ Previously reported

☐ SUPPLEMENT to grant number: _____ ☐ Yes. If "Yes," ☐ Not previously reported

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

Name of former principal investigator/program director: _____

☐ FOREIGN application or significant foreign component.

1. PROGRAM INCOME ***(See instructions.)***

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/ certifications are provided in Section III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

•Human Subjects; •Research Using Human Embryonic Stem Cells• •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy• Vertebrate Animals• •Debarment and Suspension; •Drug- Free Workplace *(applicable to new [Type 1] or revised [Type 1] applications only)*; •Lobbying; •Non-Delinquency on Federal Debt; •Research Misconduct; •Civil Rights (Form HHS 441 or HHS 690); •Handicapped Individuals (Form HHS 641 or HHS 690); •Sex Discrimination (Form HHS 639-A or HHS 690); •Age Discrimination (Form HHS 680 or HHS 690); •Recombinant DNA and Human Gene Transfer Research; •Financial Conflict of Interest (except Phase I SBIR/STTR) •STTR ONLY: Certification of Research Institution Participation.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 6/17/2002 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	2,398,638	x Rate applied	50.50	% = F&A costs	$	1,211,312
b. 02 year	Amount of base $	2,320,224	x Rate applied	50.50	% = F&A costs	$	1,171,713
c. 03 year	Amount of base $	2,312,818	x Rate applied	50.50	% = F&A costs	$	1,167,973
d. 04 year	Amount of base $	2,311,974	x Rate applied	50.50	% = F&A costs	$	1,167,547
e. 05 year	Amount of base $	2,326,839	x Rate applied	50.50	% = F&A costs	$	1,175,054
					TOTAL F&A Costs	$	5,893,599

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

4. SMOKE-FREE WORKPLACE ☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*