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Thomas Connor Bishop

Joint Faculty Appoints Program

November 12, 2003

Prof. Rob deRuyter, Head Biocomplexity Search
Department of Physics
Swain West 165
727 East Third Street
Indiana University
Bloomington, IN 47405-7105

Dear Prof. deRuyter,

Please accept the following as my application for Assistant Professor of Theoretical and Computational Biocomplexity and Biophysics in the Department of Physics at Indiana University Bloomington.

My expertise is biophysics and math biology. I have been formally trained in Physics (B.S.), Applied Math (M.S.), and Chemical Physics (Ph.D.). My primary research interests are two molecular events affecting gene regulation through the hormone response mechanism. This gene regulatory mechanism plays a critical role in numerous physiologic functions for human and wildlife species. Thus, my motivation is decidedly biological while my goal is to investigate the molecular basis of significant human and environmental health issues.

My approach is theoretical and computational and combines chemistry, computer science, mathematics and physics. My efforts to develop and analyze new methods have addressed basic scientific problems (e.g. multiple time stepping schemes for numerical integration, fast multipole algorithms, distributed/shared memory parallel computing, mathematical and numerical analysis of the PDEs describing elastic rod dynamics). I have modeled protein-DNA and ligand-receptor systems, immobilized artificial membranes, organic molecules, DNA and antibodies. I have utilized *ab initio* and semi-empirical quantum mechanics, molecular dynamics, simulated annealing, homology modeling, peptide design and free energy calculation techniques. I have maintained high performance computer networks for these purposes. My work is interdisciplinary.

Why I am I looking for a new position now?
I currently hold simultaneous tenure track positions at Tulane and Xavier Universities through the NSF Joint Faculty Appointments Program (JFAP). This program's goal is to build bridges and infrastructure between historically black colleges and universities and majority white universities. As an EPSCoR program the emphasis is on research and should include undergraduates (Tulane and Xavier) as well as graduates (Tulane). As a founding member of the New Orleans Protein Folding Intergroup and the Center for Computational Sciences at Tulane and Xavier Universities, I achieved the goals of building bridges and infrastructure. However, I have not been able to effectively achieve my academic goals within the confines of my joint appointments.

What type of position do I seek?
I wish to join an existing or emerging interdisciplinary collaborative team that desires to incorporate my research expertise into its agenda. Such a position enables me to advance my research goals rapidly and provides a strong basis for me to build a vigorous independently funded computational structural biology research program. The ideal position allows me to utilize my training in physics, applied math, and chemistry to teach graduates and undergraduates because I truly enjoy teaching

Thank you for taking the time to review my application.

Sincerely,

Thomas Connor Bishop, PhD.

Enclosures: *curriculum vitae*, research plan, statement of teaching philosophy & interests, and three letters evaluating my teaching from students.

Research Plan **Thomas C. Bishop**

The Hormone Response Mechanism is the unifying theme of my research. The methods of choice are molecular dynamics simulation and mathematical modeling. The points of interest are specific molecular events in this mechanism.

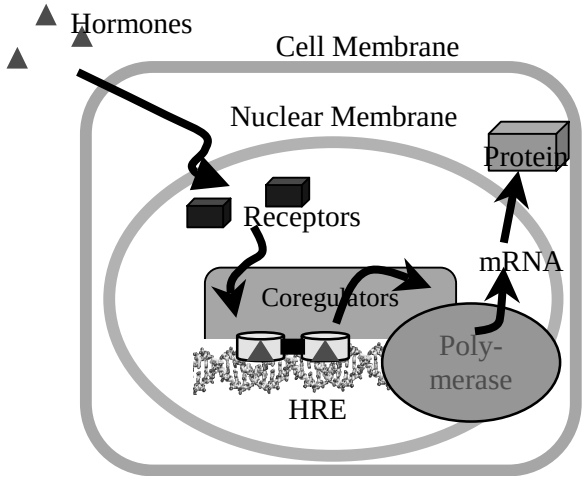
Background

The endocrine system regulates numerous physiologic functions including homeostasis, growth and development, the expression of secondary sexual characteristics, and cell differentiation. The hormone response mechanism is the common signaling pathway for all of these physiologic functions. The mechanism utilizes a receptor (steroid, thyroid, or retinoic acid) to affect ligand dependent gene regulation. Human and wildlife species including mammals, birds and invertebrates possess these receptors and the associated signaling pathways.

Manipulating the ligand activation step by administering Selective Estrogen Receptor Modulators (SERMs) is a key strategy in the treatment of hormone responsive cancers, fertility regulation and hormone replacement therapies. Ligand activation may also be inadvertently activated or suppressed by exposure to diverse chemicals of natural or synthetic origin. Such chemicals are known as Endocrine Disrupting Chemicals (EDCs) and have been associated with adverse health effects in human, livestock and wildlife species. Many EDCs and SERMs interact directly with the estrogen receptor to achieve their effect.

Hormone Response Mechanism

The hormone response mechanism is typically described as a 7 step process. My research addresses Step 3: Ligand Activation and Step: 5 DNA Binding.



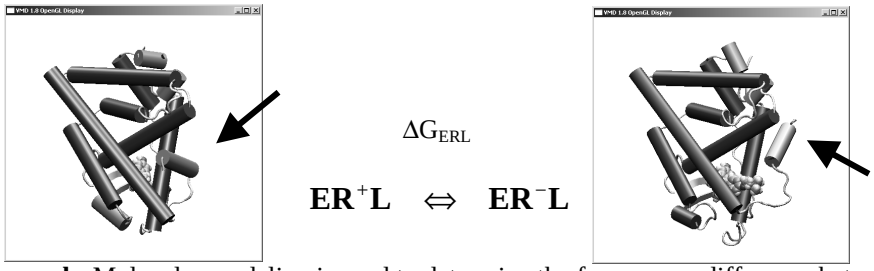
A simplified diagram of the hormone response mechanism adapted from Hormones 2nd Ed. by Norman and Litwack.

Step 1) Hormones (triangles) are secreted into the blood stream and dissociate from plasma transport proteins, not explicitly shown. **Step 2)** The hormones diffuse into the nucleus. (Only nuclear activation is shown). **Step 3 and 4)** Hormones interact with receptors in the nucleus converting the inactive form (blocks) to an active form (cylinders) and causing heat shock protein (HSP90) to dissociate from the receptor. **Step 5)** Activated receptor binds as a dimer to the Hormone Response Element (a specific sequence of DNA in the promoter region) and recruits various transcription coregulators(large box). This may either induce or repress gene expression. **Step 6)** Transcription yields a protein product. **Step 7)** The increase or decrease of protein product generates the final biologic response.

Step 3: LIGAND ACTIVATION

The estrogen receptor has a tripartite pharmacology determined by 1) ligand structure, 2) receptor subtype [ERα or ERβ] and conformation of the ligand-receptor complex, and 3) interaction of the ligand-receptor complex with coregulators.

Our hypothesis is that the conformation of the ligand-receptor complex is an equilibrium between agonist ($\mathbf{ER^{+}L}$) and antagonist ($\mathbf{ER^{-}L}$) conformations regardless of whether the ligand itself is an agonist or antagonist. The following reaction summarizes this idea.



Summary of Approach: Molecular modeling is used to determine the free energy difference between agonist(left) and antagonist(right) conformations of the receptor. The primary difference in conformation is the orientation of "helix 12" indicated by an arrow. We have developed methods to induce this conformational transition using simulated annealing, thus we can follow a folding pathway from agonist to antagonist with ligand bound.

If ΔG_{ERL} is large compared to the free energy associated with coregulator binding, then the receptor conformation with the lowest free energy dominates and is the primary determinant of the gene regulatory effect. If ΔG_{ERL} is small compared to the free energy associated with coregulator binding, then coregulators may determine the gene regulatory effect.

Application of this ideal equilibrium expression is complicated by the fact that the conformation of the ligand-receptor complex may change significantly from known x-ray structures when different ligands are docked or may be intermediate between agonist ($ER^{+}L$) and antagonist ($ER^{-}L$) conformations, thus the need for simulated annealing. Our approach allows us to explicitly address the tripartite receptor pharmacology, conformational dependencies, and the well-known tissue specific response of the nuclear receptors: some ligands function as agonists in one tissue and antagonists in another.

The long term goal of our ligand-activation studies is to computationally determine the free energy of binding of numerous compounds to ERα and ERβ as a means of quantitatively analyzing the role of the ligand-receptor conformation in agonism and antagonism. The compounds to be studied include endogenous ligands, pharmaceuticals, industrial chemicals, and natural compounds to which human and wildlife species are either deliberately or inadvertently exposed.

Our approach can be readily expanded to analyze other aspects of the 7 step mechanism such as a) coregulator interactions, b) ligand interaction with other receptors (e.g. progesterone or androgen), c) affects of mutants and species specific isoforms of a given receptor, d) ligand interaction with transport proteins or enzymes. Specific Aim #2 provides the tools to efficiently accomplish such computations and relate the results to the entire biologic mechanism, effectively allowing for a molecular mechanics based systems biology analysis.

Ligand Activation Specific Aim #1: Develop a computational protocol for determining the free energy of binding (FEB) of diverse ligands to agonists and antagonist conformations of the α and β isoforms of the estrogen receptor (ER) ligand binding domain and access the validity (accuracy and precision) of these methods.

To develop our computational protocol, 14 test compounds have been chosen because they are well characterized experimentally and represent a broad range of chemical diversity. Two different FEB methods will be tested and compared for this purpose:

1) Molecular Mechanics/Poisson Boltzmann Solvent Accessible Surface Area (MM/PBSA)
2) Molecular Mechanics/Generalized-Born-Solvent Accessible Surface Area (MM/GBSA)

Ligand Activation Specific Aim #2: Design and implement a Molecular dynamics Database Management Tool (MDbMT).

MDbMT will be the interface to a database of molecular simulations that also manages simulation setup, control, and analysis (computational protocols), data sharing (results queries), dissemination (web interface) and archiving. MDbMT is a general molecular modeling tool for managing simulations and its relation to arbitrary text-based molecular modeling tasks and thus useful for any molecular modeling study.

Ligand Activation Specific Aim #3: Apply the MDbMT and optimized free energy of binding protocol to determine the physicochemical basis of estrogen receptor agonism and antagonism for approximately 100 endogenous, pharmaceutical, industrial and natural chemicals.

By relating the free energy of binding to agonist and antagonist conformations we bridge a gap in data between binding affinity and gene induction assays and provide atomic information about ligand-receptor interactions not revealed by solution assays.

Step 5: DNA BINDING

Single molecule experiments readily demonstrate that i) forces and torques are transmitted through DNA, ii) are interrelated with DNA conformation and sequence, and iii) are produced by proteins that act on DNA in an ATP dependent manner. This mechanical view of biological molecules has gained wide acceptance with the advent of nanoscale technologies that enable direct measurement and application of forces and torques on single molecules (e.g. Atomic Force Microscopy). For DNA its mechanical properties as a nanofiber, including its charge, are fundamental to any number of biologic mechanisms.

Using molecular dynamics simulations we have demonstrated that the estrogen receptor and the glucocorticoid receptor bend DNA upon binding to their respective hormone response elements. The observed bending is supported by experimental studies. The ability of proteins to deform DNA is not unique to hormone receptors; many proteins and drugs induce specific deformations in DNA. The question remains what effect do these conformational changes have on the dynamics of DNA and the transmission of forces and torques through DNA.

Elastic rod theory provides a mathematically rigorous framework for addressing this question. By mapping genomic information to sequence specific mechanical properties of DNA the effects of such variability on conformation and dynamics can be analyzed. The effects of DNA binding proteins and drugs can also be readily incorporated into such a model as for example a change in linear density, stiffness or conformation.

a) $\dot{\vec{p}} + \vec{\omega} \times \vec{p} = \vec{P}' + \vec{\Omega} \times \vec{P}$

b) $\dot{\vec{m}} + \vec{\omega} \times \vec{m} = \vec{M}' + \vec{\Omega} \times \vec{M} + \vec{\Gamma} \times \vec{P}$

c) $\dot{\vec{\Gamma}} + \vec{\omega} \times \vec{\Gamma} = \vec{\gamma}' + \vec{\Omega} \times \vec{\gamma}$

d) $\dot{\vec{\Omega}} + \vec{\omega} \times \vec{\Omega} = \vec{\omega}'$

Summary of approach: Comparison of mathematical analysis, numeric analysis, and molecular dynamics simulations. Left: The equations of motion of an elastic rod in an internal coordinate reference frame a) balance of linear momentum, **p** and force, **P**, b) balance of angular momentum, **m** and torque, **M**, c) and d) continuity relations: rotation, **Ω**, and translation, **Γ**, of cross-sections along rod centerline; rotation**, ω**, and translation, **γ**, of cross-sections as function of time. **Middle:** Snapshots of bend-shear dynamics as predicted by continuum rod theory. **Right:** Similar motion excited in an all atom molecular dynamics simulation. The snapshots represent the same time and length scales, ~150bp DNA and 300ps. The continuum and discrete models yield very similar results and good agreement with asymptotic approximations.

Our hypothesis is that changing the conformation of DNA and/or its mechanical properties changes the dynamic behavior of the DNA. These effects are an integral part of gene regulation.

To test this hypothesis we are using a continuous medium elastic rod theory to predict the relevant time, energy and length scales and to identify the most significant parameters controlling these observables (e.g. mass, charge, viscosity etc.). Since the continuum theory is a first order approximation that neglects many important details, the predictions are tested against all atom molecular dynamics simulations as a means of validating and improving the model.

The long term goal is to determine how forces and torques are transmitted through DNA and relate to biologic outcomes, namely gene regulation. Our model will be employed to predict and analyze the effects of DNA binding proteins, adducts and cancer causing agents which bind to DNA or intercalate between basepairs. Hormone receptors are the primary DNA binding proteins of interest.

To develop the model, I will initially focus on linear DNA as a means of avoiding self-contact and conformational dependencies. The mathematical analysis is scalable and therefore applies to linear segments of condensed chromatin, entire chromosomes or any nanofiber for which suitable material properties can be determined.

Research Plan **Thomas C. Bishop**

DNA Binding Specific Aim #1: Mathematical Analysis: Develop the mathematical analysis of elastic rods appropriate for DNA and flexible nanofibers in general.

A first order approximation of DNA as an elastic rod should respect the fact that DNA is chiral and should allow for all types of deformation, namely bend, shear, stretch, twist, and extension. Many elastic rod models are more restricted, e.g. unshearable or inextensible. Thus, the first task is to analyze an elastic rod model that does not have such restrictions.

Recently we obtained solutions describing the planar bend-shear motion of an untwisted rod and the twist-extension dynamics of a chiral rod. Twist-extension is the most biologically relevant as such motion is required during transcription.

Planned mathematical developments are:

a) Construct a twist-extension phase diagram and compare to experimentally determined phase diagrams.
b) Develop a perturbation scheme to investigate the stability of twist-extension and bend-shear motions.
c) Investigate the effects of chirality in our bend-shear analysis. Chirality requires twist-extension coupling and bend-shear requires extension, thus all modes are coupled in bend-shear motion of a chiral rod.

DNA Binding Specific Aim #2: Develop Compute Engine: Develop a compute engine capable of solving the equations of motion for an elastic rod that allows for arbitrarily complex internal and external forces and torques.

We have developed Mathematica notebooks and a compute engine in C++ to study the equations of motion. Our compute engine utilizes a 4th order Runge-Kutta method to solve the ODE obtained from discretizing the equations of motion by the method of lines.

A key feature of our compute engine is that a description of the elastic rod in internal coordinates and in Cartesian coordinates is available at each time step of a simulation. Thus internal and external forces and torques can be readily incorporated into the model. Internal forces and torques are a convenient method of representing a bound drug or DNA-binding protein. External forces and torques are necessary to represent interaction with media, electrostatic interactions or self-contact.

Planned developments of the compute engine are:

a) Complete the object-oriented implementation in C++ including GUI and scripting capabilities.
b) Incorporate mapping between elastic rod and all atom models of DNA. 3DNA(available from Rutgers) will be used to convert between DNA helical parameters and atomic coordinates. This creates a powerful tool for multi-scale simulations using continuum and atomic based molecular mechanics models.
c) Implement internal forces and moments that properly account for chirality.
d) Implement external forces and moments arising from viscosity, self-contact, and electrostatics.

DNA Binding Specific Aim #3: All atom molecular dynamics simulations: Develop molecular dynamics simulation techniques that allow all atom simulations to be directly compared to the elastic rod model.

We have demonstrated that an all atom molecular dynamics simulation of 158bp of DNA in the absence of solvent supports a bend-shear type wave motion described by the elastic rod model. The predicted time and length scales, as determined by the wave velocity from each model, are in very good agreement. To date, all atom molecular dynamics simulations attempting to demonstrate a twist only or extension only motion as predicted by elastic rod theory have failed. The DNA exhibited writhe during the molecular dynamics simulation suggesting twist and extension cannot be excited independently.

Planned molecular dynamics simulations of DNA include investigations of:

a) The propagation of coupled twist-extension waves through DNA as predicted for chiral rods in the absence of solvent.
b) The effects of electrostatic cut-off on wave propagation in the absence of solvent.
c) The dissipation of wave motion into DNA thermal motion in the absence of solvent.
d) The dissipation of wave motion into thermal motion of the surrounding media.

Statement of Teaching Philosophy & Interests **Thomas C. Bishop**

Teaching Philosophy

A teacher is an instructor, advisor, and mentor. My goal is to continually develop my understanding of each of these roles and my effectiveness in serving others through teaching.

An instructor is a guide through subject matter, providing motivation and encouragement as needed. This requires careful attention to the dynamics of a class as a whole and to the abilities and background of each student. I am an enthusiastic teacher who naturally stimulates learning.

An advisor is responsible for evaluating each student and directing his or her academic choices. In the sciences, evaluations are based on understanding and application, not only knowledge or skill. I try to craft course assignments, discussions and exams to reflect each evaluation criteria and properly weigh them for the degree level. An effective advisor also teaches students to assess their abilities as part of their career development process. I believe my background gives me a strong basis for advising students with diverse interests and career goals.

Finally, a mentor realizes that learning requires the integration of disparate experiences inside and outside the classroom and serves as an example of how to achieve this integration as a learning philosophy. I strive to achieve such integration in the classroom, in my academic pursuits, and in my personal life.

The enclosed letters from students indicate my success as a teacher.

Teaching Interests

I seek an appointment that allows me to develop and teach basic and advanced courses compatible with my training in physics, math and chemistry and/or my research interests. I am also interested in collaborating with other teachers to incorporate molecular visualization, modeling and analysis into their courses.

Courses Taught

Biophysical Chemistry, University of California at Berkeley
Fate and Transport of Toxic Substances in the Environment, Tulane University
Student Seminar Presentations, Env. Health Sci. Master's Requirement, Tulane University
Quantum Mechanics, Xavier University
Physical Chemistry Lab, Xavier University
Molecular Modeling, Xavier University

Course Lectures

Terrorism: A Public Health Challenge (2 Lectures), Tulane University
Structure and Function of Biomolecules (2 Lectures and 2 modeling labs), Tulane University

Independent Studies and Lab Rotations

I have supervised nearly 20 undergraduate and graduate students in independent research projects and laboratory rotations that included sequence analysis, homology modeling, molecular dynamics simulations, database design, algorithm development, and numerical analysis.