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Faculty positions in Systems Biology

Dear Prof. Dr. Yves Brun,

Prof. Dr. James A. Glazier cordially invited me to apply for a faculty position in Systems Biology at Indiana University, Bloomington and I was excited to hear about the current expansion in this area.

Setting out as a biologist I soon turned to theoretical work on computational modeling of coordinated cell movement in *Dictyostelium* morphogenesis. During the years I stepped down to the molecular level and currently work on spatio-temporal pattern formation of the actin-cytoskeleton. A major component of my work is the development of novel quantitative imaging methods to integrate experimental and model data.

I am looking for a good research environment which offers opportunities for close collaboration with experimental groups and would welcome to expand my future teaching activities.

I am looking forward to hearing from you.

Yours sincerely,

Till Bretschneider

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CV, list of publications, research statement and teaching philosophy, selected reprints (Bretschneider et al., 1999, 2004; Diez et al., 2005, Dormann et al., 2002), references

Till Bretschneider – Research

Overview

My main research interest are the principles of self-organization involved in the motion of animal cells. Before a cell can start to move it has to polarize and establish a cell front which is functionally distinct from the cell rear. Thus an extracellular signal, a gradient of a chemical attractant for example, is translated into an intracellular gradient pattern of regulatory proteins which defines the direction of motion. At the onset of motion cells elongate and develop a thin membrane protrusion at the leading edge. The driving force that pushes the cell membrane forward is polymerization of actin into filaments which form a dense branched network. Self-organization of the front-rear axis is based on short range autocatalytic activation of the front and long range inhibition of the rear. The positive-feedback at the front is based on phospholipids (PIP_3) , integral components of the cell membrane, and GTPases, regulatory signal proteins which signal to the actin cytoskeleton. The players of the negative feedback loop are phosphatases for PIP_3 and factors which inhibit the corresponding GTPases.

Other examples of self-organized spatio-temporal patterns which control cell motion are the co-ordinated movement patterns of cells during tissue formation in embryogenesis or wound healing, and the assembly of the actin cytoskeleton on the molecular level. Although these phenomena of self-organization occur on different length and time scales many generic aspects of pattern formation are understood very well on theoretical grounds and can be formulated by mathematical models. However even for simple patterns like the establishment of a cell front, where we know most of the involved molecular players, we do not have enough quantitative experimental data to validate any of the postulated mathematical models.

Following a systems-biology approach my goal is to bridge this gap between theory and experiment.

On the experimental side I am developing novel software tools for quantitative imaging and apply them to analyze data obtained from advanced fluorescence video-microscopy. In particular I am studying the spatio-temporal dynamics of protein redistribution patterns in live cells at high resolution and correlate it with cell motion. Wild-type and mutant cells defective in particular pathways leading to cytoskeleton reorganization help to identify the role of regulatory proteins.

My main incentive to use computational models is to integrate the experimental data and test competing model assumptions in order to validate or rule out conclusions drawn from the experiment. I have been applying stochastic many particle models to problems of structure formation, e.g. the formation of multicellular aggregates or supramolecular actin associated complexes. Using hybrid approaches I have combined such discrete models with continuous reaction-diffusion type models which are more suitable to describe distribution patterns of abundant signalling molecules like phospholipids in the cell membrane.

Previous and Current Work

Models for *Dictyostelium*-Morphogenesis

Upon starvation the eukaryotic micro-organism *Dictyostelium* enters a 24h developmental cycle which ends with the formation of a fruting body consisting of dormant spores supported by a stalk. The collective aggregation and formation of multicellular stages of about 10-100 thousand cells is organized by chemotaxis to periodic signals of the chemoattractant cAMP[1] emitted by the cells.

Using mathematical models based on individual cells I could show that chemotaxis toward cAMP and mechanical cell-cell-interaction in the form of adhesion and pressure can not only account for the three dimensional shape of cell aggregates at different stages of *Dictyostelium* development, but also for the complex motion of individual cells within these multicellular stages (Bretschneider et al., 1995, 1997, 1999)[2].

Models for Co-operative Motion of Cells on Elastic Substrates

A generalized version of the previous model was used to show that co-operative motion of cells can also be mediated by mechanical long-range interactions (tensiotaxis, Alt et al., 2003, Bretschneider 2004). In this model cells move on top of a two-dimensional visco-elastic substrate on which they pull at discrete sites of adhesion. Aggregation of cells can be explained by the assumption that the force exerted by a cell depends on the stress/rigidity of the underlying matrix, which has been shown in experiments by other labs.

Effects of Integrin-dependent Substrate Attachment on Keratinocyte Lamellipodia Dynamics

This work comprises a detailed quantitative study of lamellipodia dynamics and cell polarity of human epidermal keratinocytes on substrates of varying adhesiveness (Libotte et al., 2001). It was shown that protrusion and retraction of the leading lamella are regulated by independent pathways. Using soluble cell-binding peptides which compete with substrate bound peptides we could further show that lamella dynamics depends on firm cell-substrate binding while the maintenance of cell polarity does not.

Quantitative Imaging of Spatio-Temporal Protein-Distribution Patterns

I have developed software (QuimP[3]) based on active contour methods to correlate cell motility with spatio-temporal patterns of regulatory proteins which upon activation translocate to

[1] cyclic 3',5'-Adenosinmonophosphate

[2] Corresponding movies on this and the following topics can be found on http://www.till-bretschneider.de .

[3] The image analysis software QuimP can be downloaded free for testing purposes from http://www.garching-innovation.de .

the cell-membrane. This method was applied to *Dictyostelium* phospholipid-signaling during chemotaxis (Dormann et al., 2002) to show that regulatory domain of PH-CRAC is required to remove it again from the membrane once it has bound. Quantitation of fluorescently labeled coronin binding to the cell cortex in photobleaching experiments (Bretschneider et al., 2002) revealed effective binding/unbinding rates for coronin to the actin cortex. QuimP was also used to analyze cortical DPAKa enrichment during cytokinesis (Müller-Taubenberger et al., 2002). Current work on imaging involves motility analysis of *Dictyostelium* formin mutants (Schirenbeck et al., 2005) and computation of dye diffusion in the cell membrane of keratocytes (Weisswange et al., 2005).

Analysis of Supramolecular Actin-Complexes

A major emphasis of my work has been the investigation of dynamic patterns of supramolecular actin-complexes associated with the Arp2/3 complex (Bretschneider et al., 2004; Gerisch et al., 2004; Diez et al., 2005). The analysis is based on data obtained by the combination of total internal reflection microscopy with double labeling of actin and its associated proteins. Using fluorescent markers specific for filamentous actin we find highly dynamic networks throughout the cell cortex polymerizing at rates of 3 μm/sec, whereby the dynamics is independent of leading edge activities. Inserted into this network are stationary, Arp2/3 containing foci with a well defined lifetime of around 15 seconds. Travelling waves of actin polymerization form fascinating patterns while propagating on top of the cortical actin network. Three dimensional analyses of such waves revealed that their architecture resembles very much the organization of actin at the leading edge or in phagocytic cups, respectively. These waves can be efficiently and persistently induced by moderate concentrations of the drug latrunculin A. Because of their longevity under these conditions, these actin waves are a perfect model system for future studies on the theoretical aspects of pattern formation and self-assembly of the actin system.

Research Plan

Future work will follow three lines:

- Quantitative imaging of the spatio-temporal dynamics of actin and its regulatory proteins. Functional context will be the reorientation of *Dictyostelium* cells in response to alternating chemotactic fields and mechanical stimulation by shear flow reversal. These data will be used to challenge existing models for gradient sensing and polarization.
- Software development of object-oriented image analysis programs for mapping of protein distribution patterns. The focus will be on regulatory protein networks at the cell membrane. The goal is to create spatio-temporal signal transduction maps from high throughput screening assays.
- Modelling different modes of actin organization. The role of formins and the Arp2/3 complex in the pattern formation of loose cortical networks and dense actin waves will be investigated by computational models.

Quantitative Imaging of Spatio-Temporal Actin Dynamics during Reorientation of Cells

An important biological question is: How does a cell retain its sensitivity to reorientate toward an extracellular stimulus once it is polarized? Plausible mechanisms for gradient sensing and polarization were recently povided by continous reaction-diffusion models based on phospholipid-signaling[4]. The underlying concepts of short-range activation and long-range inhibition go back to phenomenological models developed by Meinhardt[5]. We will validate current models based on the data obtained in reorientation experiments where cells are periodically stimulated first on one side, then on the opposite side. This approach has several advantages: Firstly, theoretical models can be challenged with quantitative time-course data of complex transient dynamics instead of simple steady state solutions. Secondly, we measure assembly and disassembly kinetics of the cytoskeleton repeatedly in the very same cell and can average over multiple responses. Thirdly, functional aspects can be investigated by changing the frequency of alternating stimulations to measure the minimum time interval required for establishing polarity or adaptation times.

Analyses of reorientation experiments will be performed with QuimP-software (Dormann et al., 2002)) which can quantify the spatio-temporal distribution of fluorescently labeled proteins in the cell cortex and correlate it with cell motility. The software is used by several groups (G. Gerisch, Martinsried; P. van Haastert, Groningen; F. Bruckert, Grenoble) working on *Dictyostelium* to study redistribution of actin, myosin and associated proteins as well as phospho-

[4]see Postma M., Bosgraaf, L., Loovers, H.M. & Van Haastert, P.J.M. Chemotaxis: signalling modules join hands at front and tail. *EMBO reports* **5**:35-40 (2004) and references therein

[5]Meinhardt, H. Orientation of chemotactic cells and growth cones: models and mechanisms. *J. Cell Sci.* **112**:2867-2874 (1999).

lipids involved in signalling to the actin cytoskeleton. We will continue to collaborate with these labs to obtain data for a large number of regulatory proteins and mutants.

An experimental setup for studying cells under alternating shear flow has already been established by F. Bruckert's group[6]. Analysis with QuimP shows that actin disassembly at the old cell front is rapid compared to assembly at the new front. It is planned to study the dynamics of wild-type cells and different mutants (e.g. myosin-II-null, profilin-I/II-null) in order to decipher regulatory pathways of gradient sensing and polarization. In the group of J. Rädler (LMU München) a "chemotactic trap" is being constructed using microfluidic devices in order to study cells in well defined alternating chemotactic fields. We also plan to study cytoskeletal reorganisation of cells on flexible substrates which are periodically stretched from opposite sides.

A common feature of chemotaxing cells is splitting of the cell front into two pseudopodia, one of which is eventually retracted, while the other is reinforced. This phenomenon has never before been addressed theoretically. Since current models consider a fixed circular geometry for cells, we plan to develop more realistic models which also account for cell shape changes.

Cartography of Signal Transduction at the Cell Membrane

Quantifying the spatio-temporal dynamics of protein patterns is closely linked to problems in cartography. So far there is no standard representation of the "landscape" of cells in terms of a co-ordinate system that could be used to store data and make it accessible to statistical analysis in high-throughput assays. A fundamental problem which has to be solved is to account for shape changes and motility of cells. The focus will be at the cell membrane since this is the starting point for almost all signal transduction events in response to extracellular signals. A typical application will be high-throughut screening of motogen activity in pharmacological studies with neutrophils. A number of more general questions will be addressed: *How can these data be correlated with biological functions other than cell motility, for example gene expression? How can we chart this data independent of cell size and shape in order to perform high-throughput statistical analyses? Which data models facilitate the incorporation of experimental data into computational models?*

The starting point will be the software QuimP which is based on automatic segmentation of the cell cortex by active contours. In future further sophisticated segmentation procedures will be implemented to map the transport of proteins in and out of the nuclues. Optical flow methods, which have to be adapted to irregular moving grids, hold promise to measure fluxes of protein redistribution throughout the entire cell in 3D.

[6]Dalous J, Bretschneider T, Prassler J, Burghardt E, Ecke M, Müller-Taubenberger A, Gerisch G, Bruckert F. I. Mechanosensitivity and polarisation of *Dictyostelium* cells under shear stress. Cytoskeleton reorganisation and protein relocalisation after a flow reversal. *in preparation*, 2005

and fluorescent probes specific for filamentous actin we quantified actin network dynamics in the cortex of *Dictyostelium* cells with high temporal and spatial resolution (Bretschneider et al., 2004; Gerisch et al., 2004). Two types of actin networks can be distinguished: A loose network underneath the membrane forming the bulk actin cortex. Here polymerization occurs concomitantly with bundling of filaments at high rates of 3 μm/sec (Diez et al., 2005) and most likely is driven by formins[7]. Inserted into this loose network are dense stationary Arp2/3 containing patches. The Arp2/3 complex nucleates and branches actin filaments. Arp2/3 rich patches eventually initiate waves of actin polymerization which propagate on the planar subtrate attached cell membrane. Upon collision these waves can push the membrane forward and then resemble actin networks found at the leading edge.

Until now theoretical work on the structure of actin networks has focussed on dense neshworks at the leading edge, and how they can generate force to push the membrane forward. Different approaches have been taken ranging from molecular dynamics simulations of actin polymerization[8] to continuous reaction-diffusion[9] or multi-phase flow models[10]. However, these models only consider networks in a steady state, but not their formation/break-down.

To study the differences in formin versus Arp2/3 based actin network assembly we will start by applying an evolutionary approach to phenomenological models of simulated networks. This strategy has been taken by Maly and Borisy[11] to study steady-state distributions of branching angles in lamellipodia. In contrast, we will focus on the dynamics of de novo formation and break-down and use stochastic many-particle models based on single F-actin filaments on the mesoscopic scale[12]. Different selection schemes will be tested which could account for different functions, such as selecting for maximum growth rates of a network or maximum force generation. Using such models will help to better understand the complex interplay of nucleation, branching, capping, bundling and severing involved in reorganisation of actin networks. Computational modeling will be used to analyse non-linear phenomena of self-organisation such as autocatalytic assembly of actin networks due to Arp2/3-induced branching and wave-like propagation of actin polymerization.

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[7]Higashida C., Miyoshi T., Fujita A., Oceguera-Yanez F., Monypenny J., Andou Y., Narumiya S. & Watanabe N. Actin polymerization driven molecular movement of mDia1 in living cells. *SCIENCE* **303**:2007-2010 (2004).

[8]Dickinson R.B. & Purich, D.L.. Clamped-filament elongation model for actin-based motors. *Biophys. J.* **82**:605-617 (2002).

[9]Mogilner A. & Edelstein-Keshet, L. Regulation of actin dynamics in rapidly moving cells: a quantitative analysis. *Biophys. J.* **83**:1237-1258 (2002).

[10]Alt W. & Dembo, M . Cytoplasm dynamics and cell motion: two-phase flow models. *Math. Biosci.* **156**:207-228 (1999).

[11]Maly, I.V. & Borisy, G.G. Self-organization of a propulsive actin network as an evolutionary process. *PNAS* **98**:11324-11329 (2001).

[12]Carlsson, A.E. Growth of branched actin networks against obstacles. *Biophys. J.* **81**:1907-1923 (2001).

Till Bretschneider – Teaching Philosophy

What makes a good systems biologist? Understanding complex biological regulatory systems requires integration of biology, mathematics, physics and computer sciences. The goal of teaching systems biology certainly cannot be to train students to become experts in all of these disciplines, but to give students of any discipline a solid background in non-linear dynamics. In practice I consider computational modelling to be the "nuts and bolts" of systems biology. Faced with mainly biology and biochemistry students, I found that nothing illustrates better problems of non-linear dynamics than solving a simple set of chemical rate equations on the computer. For many students it is a truly novel experience to see that the interaction of as few as three species can be so complex that it fools our intuition.

Currently I have students which come for six to eight weeks to do small research projects. After a short primer in computer programming they have to solve a given problem to evaluate what they have learned. In the project phase the students are free to pick a problem of their interest from the area of my own research. Before they start I emphasize that they write a short proposal about why they think the problem is interesting, the current state of the art, what they want to achieve and a project timeline. In my opinion the definition of small reachable milestones helps best to create the positive feedback required for successful learning. The advancement of the project and possible alternatives are discussed weekly to avoid stagnacy and keep up the motivation. In the end the work has to be summarized in the form of a small research article.

In short my major objective is to develop general problem-solving skills. Since systems biology is such a wide open subject, I believe that students thus will be prepared better for a rapidly evolving science, than by learning too many detailed facts. I hope to inspire students to share some of my enthusiasm for the beauty of complex biological systems.

I would welcome the oppurtunity to teach practical courses in mathematical methods for biology students or image processing. Since I'm visually impaired and cannot run an experimental lab or supervise laboratory students myself, I regret that I cannot give any practical courses in biology. However, I can very well imagine to give lectures in cell biology or developmental biology.