UNIVERSITY OF
PENNSYLVANIA
MEDICAL CENTER

University of Pennsylvania School of Medicine
Hospital of the University of Pennsylvania

Martin Muschol, Ph.D.
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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Dear Prof. de Ruyter van Steveninck,

I am applying for a faculty position associated with the Biocomplexity Institute at Indiana University.

I received my Ph.D. in Physics from the City University in New York in the laboratory of Prof. Herman Cummins. My graduate work focused on experiments to validate theories of pattern formation in non-equilibrium systems.

During my time as research associate at the University of Alabama in Huntsville I expanded into biological systems, investigating protein-protein interactions in aqueous solution and their relation to the difficulties of protein crystallization and the problem of aggregate formation.

I am currently a research assistant professor in the Neuroscience Department of the University of Pennsylvania. I use advanced optical techniques to probe the cellular mechanisms underlying short-term plasticity. Neuronal plasticity is a basic property of neuronal networks required for adaptation, learning and memory. To support this project I have secured funding through an NIH research training award (Apr. 2000 – Mar. 2004/ 442 K direct cost).

My broad interests in complex systems, experimental and computational neurobiology, and advanced optical techniques of cellular and molecular biology clearly overlap with several research initiatives at your Institute for Biocomplexity. Hence, I anticipate many opportunities for successful collaboration.

I look forward to the opportunity to discuss this position with you in person.

Sincerely,

M. Muschol

attachments: cv, research statement, teaching statement

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RESEARCH STATEMENT

Synapses are the pivotal relay stations for transmission and processing of information between neurons. Understanding synaptic transmission is essential for elucidating the process of learning and memory formation - and for unraveling the causes of important neurological disorders. One basic characteristic of synaptic transmission is plasticity, i.e. the ability of synapses to adjust the strength of their connection in response to previous activity. But how do synapses regulate their efficacy of release following stimulation? My research over the past several years has suggested a novel mechanism for generating synaptic plasticity via calcium-mediated, localized feedback on presynaptic excitability (Muschol et al, 2003). The first project, ***"Presynaptic Mechanisms of Short-Term Plasticity"***, (1) tests my hypothesis of *localized excitability changes* in presynaptic terminals and investigates the underlying mechanisms, and (2) determines the *detailed spatio-temporal pattern of calcium changes* within individual terminals and how this pattern regulates release.

Determining the 3-D structure of biological macromolecules is basic for understanding their biological activity, for revealing the causes of pathological dysfunctions, and for identifying promising sites for targeted drug development. Difficulties of crystallizing proteins, however, are often a critical bottleneck preventing x-ray structure determinations. The second project, ***Protein Phase Diagrams and Simplified Models of Macromolecular Interactions***, targets this problem by using protein interaction parameters to predict phase diagrams of aqueous protein solutions. This model of protein interactions and phase separation is equally relevant for understanding protein aggregation common to many neurodegenerative diseases.

Presynaptic Mechanisms of Short-Term Plasticity

Modulation of Local Excitability in Nerve Terminals and its Role in Short-Term Plasticity

Could nerve terminals be local "switches" regulating their own excitability depending on the pattern of stimulation? Optical measurements of action-potential modulation in the intact neurohypophysis suggest this possibility (Muschol et al., 2003). I plan to determine the origin of the dramatic changes in tissue excitability and test my model of "stuttering conduction". Using confocal imaging and fast voltage-sensitive dyes, I will directly visualize stimulation-induced changes in excitability along an individually stained axonal arbor. To ascertain the role of the Ca-activated K-current as source of the local failures, I will apply electrophysiology, pharmacological means or knock-out mutants (which have recently become available). Selective localization of the relevant K_{Ca} channel within nerve terminals/ swellings, but not axonal segments, will be established with immuno-gold labeling.

Spatio-Temporal Dynamics of Near-Membrane Calcium and Vesicle Release

Calcium is the primary trigger that activates many different biochemical and biophysical cascades which regulate release. Knowing the precise spatio-temporal pattern of calcium elevation is essential for understanding how different patterns of activity result in facilitation or depression of release and, hence, synaptic transmission. I have previously shown that the efficacy of release in the neurohypophysis is tightly coupled to a frequency-dependent modulation of calcium transients (Muschol and Salzberg, 2000). How the distinct modulations of Ca^{2+} influx and residual Ca^{2+} collude to alter release at the level of individual nerve terminal remains unclear. More specifically, what is the precise spatio-temporal pattern of near-membrane Ca^{2+} elevation that drives release? And how does it correlate to the observed dependencies of Ca^{2+} influx and residual Ca^{2+}? Building

on my experience with optical techniques, I have put together a set-up for total internal reflection fluorescence microscopy (TIRFM). This technique permits the visualization of fluorescence changes in the immediate vicinity (~50-200nm) of the plasma membrane. Measurements will be performed on isolated nerve terminals, loaded either with low-affinity Ca^{2+} indicators or with acidotrophic dyes as markers of secretory granules. Using this approach I will be able to determine near-membrane calcium changes within terminals and compare them with the amplitude and time-course of the volume-averaged signals seen in standard epi-fluorescence. Furthermore, I plan to determine the precise spatial and temporal correlation between near-membrane calcium elevation and vesicle fusion in this peptidergic system.

Protein Phase Diagrams and Simplified Models of Macromolecular Interactions

Crystallization of proteins from solution still relies on time- and material consuming random tests of "promising" solution conditions. I have suggested that globular proteins exhibit a "universal" phase diagram (Muschol and Rosenberger, 1997) that could be determined from straight-forward measurements of interaction parameters in solution (Muschol and Rosenberger, 1995). This phase diagram has peculiar features. In particular, the presence of a metastable liquid-liquid phase appears essential for identifying suitable crystal nucleation and growth conditions in phase space. This hypothesis has since been supported by simplified models of colloidal and macromolecular interactions (Auer and Frenkel, 2001; ten Wolde and Frenkel, 1999).

Using two or three "model proteins" with known crystallization conditions (e.g. lysozyme, BSA and concanavalin), I will compare the actual phase diagrams with model predictions based on interaction parameters derived from light scattering measurements. Similarly, the role of the metastable liquid-liquid phase separation in inducing crystal nucleation will be determined. This model will then be applied to predict suitable crystallization conditions for novel proteins awaiting structure determination.

Long-Term Goals and Interests

The long-term goal of my work on neuronal plasticity is to derive general principles governing short-term plasticity, as well as to discern system-specific adaptations. Therefore, I am planning to study short-term plasticity in various areas of the central nervous system. The investigation of protein interactions and their phase diagrams will be expanded to the problem of protein aggregation in neurodegenerative diseases, an area of pressing need for advancement. The use of optical techniques will provide the common framework tying the work on these different projects together in my laboratory.

References:

Auer, S. and Frenkel, D (2001). *Prediction of absolute crystal-nucleation rate in hard-sphere colloids.* Nature **409**:1020-1023.

Muschol, M., P. Kosterin, M. Ichikawa, and B.M. Salzberg (2003). *Activity-Dependent Depression of Excitability and Calcium Transients in the Neurohypophysis Suggests a Model of "Stuttering Conduction".* J. Neurosci., in press.

Muschol, M. and B.M. Salzberg (2000). *Dependence of Transient and Residual Calcium Dynamics on Action-Potential Patterning during Neuropeptide Secretion.* J. Neurosci. **20**:6773-6780.

Muschol, M. and F. Rosenberger (1997). *Liquid-liquid Phase Separation, Precipitate Formation and Crystallization in Supersaturated Lysozyme Solutions.* J. Chem. Phys. **107**: 1953

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TEACHING EXPERIENCE AND PHILOSOPHY

Throughout my academic career I have sought out teaching opportunities. Originally I intended to become a high school teacher, enrolling in a master's program at the University of Regensburg (Germany). During my Ph.D. training at City College I had the opportunity to teach students of widely disparate interests (liberal arts, life science, engineering and physics majors). My teaching experience ranges from lecture-hall courses with over 100 students, to recitation sections and experimental laboratory courses, to the personal interactions with undergraduates, graduate students and postdoctoral trainees in several research laboratories.

My teaching philosophy is based on a few fundamental principles from which a more concrete set of techniques and guidelines is derived. First and foremost, teaching has to serve the requirements of a modern, technology-oriented and democratic society. Therefore, it must provide even non-science students with an understanding of the "scientific method" and its ever-increasing importance for the future. For science-oriented students, it must provide fundamental skills with for analyzing scientific problems, and the ability to transfer those skills to their respective fields of specialization.

While the standard frontal lectures in science courses are an effective tool to convey a large body of knowledge in a short period of time, they often leave the students disengaged. I attempt to enhance active participation of students in the learning process in several ways. First of all, I try to introduce specific topics (e.g. action potentials in neuronal systems) with problems encountered in every-day life (how do our nerve cells transmit signals over long distances). I illustrate its relevance in many different circumstances (fundamental output signal of neurons throughout the nervous system), and show how specific physical principles provide a solution (linear vs. non-linear cable equation). These fundamental principles, then, need to be consolidated in the minds of the students on as many different cognitive levels as possible. This requires hands-on experimental laboratories integrated into the course material, targeted homework that applies the new principles to specific problems, and the use of computer simulations and audiovisual materials to further reinforce new concepts.

I also promote work in small groups. For example, I assign students to perform library and text-book research on a given topic and report their findings back to the rest of the class as part of their course work. Students are encouraged to work on class assignments together and to discuss their solutions. I find that this approach helps less advanced students catch up to their peers while requiring advanced students to clearly communicate their ideas and skills. These skills become critically important for their later careers where group work, ability to interact, and communication skills are critical for success.

My overall inclination is to limit the amount of material presented in a course in favor of fostering student interest and student participation. I hope that this approach provides the students with more of the skills they require when they attempt to solve real-life scientific problems.