Frontiers in Biophysics Jan. 23rd, 2009
Title and Abstract List for Poster Session

1. **Borowski, P.**
   Title: Influence of noise and bifurcation structure on subthreshold oscillations in mixed mode oscillations

   Abstract: Mixed mode oscillations are observed in different classes of neurons. Various mathematical models have been proposed to describe this dynamical behavior, in particular a detailed conductance-based model and a simpler FitzHugh-Nagumo-type model. We compare these different models, focusing on the fundamentally different underlying bifurcation structures and the effect noise has on the dynamics. The goal is to present measures for closer comparison of the models with experimental data that help identifying the underlying mechanism.

2. **Cheng, J. T. J.**
   Title: Studying the effect of membrane composition on the antimicrobial peptides aurein 2.2 and 2.3, using CD and NMR spectroscopy

   Abstract: Our previous research of α-helical cationic antimicrobial aurein peptides has shown that amidated aurein peptides effectively perturb the DMPC/DMPG bilayers (model bacterial membranes), while displaying minor effects on the DMPC bilayers (model mammalian membranes) [1]. Our recent solution-state calcein release assays, solid-state oriented CD and ^{31}P NMR studies on the POPC/POPG membranes have suggested that the bilayer thickness and PG content have significant impacts on the behaviour of short-chain aurein peptides in different membrane environments [2]. We have found that differences in peptide-membrane interactions are concentration-dependent and peptide-specific in various model membranes examined.
   [1] Pan, Y.-L. *et al.* 2007. *Biophys. J.* 92: 2854-2864.
   [2] Cheng, J.T.J. *et al*. 2009. *Biophys. J.* (in press).

3. **Dahabieh, M.**
   Title: Sequence-dependence of RNA intermediates in the folding of purine riboswitches

   Abstract: Riboswitch regulation of gene expression requires ligand-mediated RNA folding. From the fluorescence lifetimes of bound 2-aminopurine we resolve three RNA conformers (Co, Ci, Cc) of G-/A-sensing riboswitches from Bacillus subtilis: The ligand and magnesium-binding affinities, alongside mutagenesis results, suggest Co and Ci are partially unfolded species compromised in loop-loop interactions present in the fully-folded Cc. The G-sensing riboswitch is more preorganized, and its ligand-mediated folding is more effective, less magnesium-dependent, and less debilitated by mutation, than the A-sensing riboswitch. We propose that sequence-dependent RNA dynamics tune the degree of induced-fit and kinetic discrimination in ligand binding, and gene regulation efficacy.

4. **Das, R.**
   Title: Analysis of single particle tracks to quantify binding kinetics of cell surface receptors.

   Abstract: Dynamics of cell surface molecules are influenced by their interactions with intracellular proteins, such as mediators of cytoskeletal attachment, or signaling proteins

downstream from a membrane receptor. We have developed a novel analysis of single particle tracks based on a hidden Markov model that identifies the kinetics of these binding interactions. We applied this technique to analyze single particle tracks of the cell surface integrin receptor LFA-1 in T cells and quantify its interaction with the actin cytoskeleton. Our analysis reveals 2D diffusion coefficients of LFA-1 in its free and bound forms and the rates of transition between the two states.

5. **Downing, B.**
   Title: Probing the mechanical response of elastin with optical tweezers

   Abstract: Elastin is a structural protein which is vital to the proper functioning of many human tissues, such as skin, lung and blood vessels. Our goal is to quantitatively characterize its mechanical properties at the single molecule level using optical tweezers. The task is complicated by the fact that elastin is one of the shortest molecules to be characterized in this manner. One way to address the challenges this raises is to use a DNA handle to extend the molecule. We will evaluate the effectiveness of this technique, including possible artifacts it may introduce into our measurements.

6. **Dwyer, R. J.**
   Title: Artificial nanopores for biophysical investigations and bioanalytical applications.

   Abstract: A nanopore is a powerful single molecule detector that consists of a nanometre-diameter hole in an ultrathin insulating membrane. We will outline a hybridization-based DNA assay using nanopore force spectroscopy to provide single base pair discrimination between two analyte oligomers. The high sensitivity and straightforward electrical operation can allow for DNA recognition in a simple nanopore-based device without the need for costly and time-consuming DNA amplification, fluorescent labelling, or optical readout. We will additionally outline our efforts to develop artificial nanopores suitable for *clinic-ready* devices. Finally, we will discuss nanopore force spectroscopy as a biophysical tool for probing intermolecular interactions.

7. **Emberly, E.**
   Title: Insulator sequences regulate chromatin structure via epigentic marks

8. **Gauthier, M.**
   Title: Control of DNA replication by anomalous reaction-diffusion kinetics

   Abstract: We propose a simple model for the control of DNA replication in which the rate of initiation of replication origins is controlled by protein-DNA interactions. Analyzing recent data from *Xenopus* frog embryos, we find that the initiation rate is reaction limited until nearly the end of replication, when it becomes diffusion limited. Initiation of origins is suppressed when the diffusion-limited search time dominates. To fit the experimental data, we find that the interaction between DNA and the rate-limiting protein must be subdiffusive.

9. **Hamidi, M.**
   Title: Optimizing channel capacity of a genetic cascade in a variable 2-state environment

   Abstract: Cells respond to their extracellular environment through interaction with molecules of interest in the cell's exterior. This initiates a cascade of reactions resulting in the expression

of a gene of interest. The state of the extracellular space can be modeled as the input and the expression levels of the gene at the end of the cascade as the output of a noisy information channel. The capacity of this channel to transmit information about the extracellular space can be calculated as a function of biochemical constants that define the cascade. Capacity of channels of different constants are calculated and compared with those in natural systems.

10. Holloway, D.
Title: Pattern selection in plants: coupling chemical dynamics to surface growth in 3D

Abstract: I study the interplay between the pattern formation of growth catalysts on a plant surface and the expansion of the surface to generate organismal shape. I use the Brusselator reaction-diffusion model, on an initially hemispherical shape. Chemical pattern catalyzes local area increase, which is accommodated by outward movement of surface elements, producing shape change. This mechanism successfully generates some of the fundamental shapes in plant development: tip growth; tip flattening; dichotomous and higher-order branching (making whorled structures); and control of branching plane in successive dichotomous branching. Experimental work aims to further quantify the fit between pattern and shape change.

11. Horst, A. v. d.
Title: Calibration of holographic optical tweezers for force measurements on biomaterials

Abstract: Holographic optical tweezers (HOTs) offer interactive control over multiple traps in 3D, which suggest their suitability for mechanical probing of complex systems such as cells or elastomeric protein networks. Thus far, however, HOTs are not commonly used in quantitative biophysical experiments. To perform quantitative force measurements, parameters such as trap stiffness and its position dependence, range of trap steering, and minimum step size are of key importance.

In our HOT setup we were able to control and detect ~1nm steps. In addition, we found that the stiffness was independent of the precise trap configuration, demonstrating that HOTs can be used in quantitative force measurements on soft biomaterials.

12. Huang, H.
Title: Determining the inter-domain contact sites in CTP:phosphocholine cytidylyltransferase

Abstract: CTP: phosphocholine cytidylyltransferase (CCT) catalyzes the rate-limiting step in the synthesis of phosphatidylcholine. Our current working hypothesis for CCT activation is that in the soluble form, the lipid-binding domain (M), interacts with the other domains in CCT and exerts inhibition on CCT activity. Upon membrane binding, the inhibition is released due to the departure of domain M from CCT, and subsequent attachment and insertion of domain M to the membrane lipid bilayer. To validate this model, single cysteine mutants throughout domain M were made through site-directed mutagenesis. Interdomain contact sites were examined via oxidation, photo-cross-linking and mass spectrometry.

13. Jollymore, A.
Title: Nanomechanical properties of tenascin-X revealed by single-molecule force spectroscopy

Abstract: The manner in which nature tunes the mechanical stability of proteins is a poorly understood mechanism, yet these nanomechanical properties determine much of the

functionality exhibited in vivo, especially in proteins commonly exposed to force. Tenascin-X (TN-X) is an extracellular matrix (ECM) protein that plays an important role in regulating the structure and mechanical properties of the ECM, an environment subject to continual mechanical force. Investigating the mechanical properties of TN-X via single molecule atomic force spectroscopy (AFM) reveals that the elastic behaviour of TN-X when mechanically unfolded originates in its constituent fibronectin type III (FnIII) domains. An investigation of these domains within full length as well as an FnIII fragment reveals that the mechanical stability, contour length, and unfolding kinetics are analogous among the thirty different FnIII domains despite large divergences in sequence between them. Differing domains exhibit apparently dissimilar refolding behaviour. An investigation of an individual domain, contained within a polyprotein chimera containing GB1, reinforces the notion that the mechanical behaviour of full length TN-X originates within individual FnIII domains. These results allow for the exploration of how the nanomechanical properties exhibited by the full-length protein are specifically engineered within constituent, seemingly structurally deviating domains. Discerning how nanomechanical properties arise could also be elucidated in the comparison between TN-X and previous single molecule AFM studies done on tenascin-C and FNIII-containing fibronectin to establish how subtle differences in structure are translated into differing mechanical properties.

14. Leung, S.

Title: Melting behavior of sphingolipids involved in biological signaling

Abstract: Sphingomyelin, a major component of mammalian cell plasma membranes, is converted to ceramide during apoptosis. To investigate the structural changes to the membrane that accompany this conversion, we have used deuterium nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) to study multilamellar vesicles composed of n-palmitoyl sphingomyelin (SPM) and n-palmitoyl sphingosine (CER). For the NMR experiments, either SPM or CER was deuterium-labelled on the palmitoyl chain. Both NMR and DSC show that CER markedly increases the onset temperature of SPM:CER membrane gel to liquid crystalline melting, as well as the temperature of the liquidus. Both techniques also reveal that for CER concentrations of approximately 30% or less, there is evidence of a three-phase line at 40 +/- 2oC. At high temperatures, a homogeneous liquid crystalline phase is formed. At physiological temperature, CER production in cell membranes is thus expected to increase the gel phase propensity of sphingolipid-rich regions of the plasma membrane.

15. Loosley, A.

Title: Response of biochemical networks to oscillating signals

Abstract: Recent experiments, motivated by techniques in electrical engineering, have shown that it is possible to reverse engineer a biological network by measuring the dynamical response of the network when driven by an oscillating source. We investigate the dynamical responses of three well studied biological networks and determine the ranges over which these dynamical responses are informative about the underlying network properties.

16. Morriss-Andrews, A.

Title: Coarse-grained molecular dynamics simulations of DNA using ellipsoids

Abstract: We are developing a coarse-grained computational model of DNA using ellipsoids, and simulating the DNA using molecular dynamics simulations. We represent the bases using

the RE^2 potential, as these molecules are better represented by ellipsoids than spheres. Our model gives greater resolution than traditional coarse-grained models but requires less computational power than all-atom simulations.

17. Pan, J.

Title: Effect of magnetic alignment on cellulose crystallinity

Abstract: Nanocrystalline cellulose samples, obtained by acid hydrolysis using different protocols, have been exposed to an external magnetic field in order to investigate the effect of the magnetic alignment on cellulose crystallinity. The crystallinity of cellulose films dried either in or out of the magnet, was characterized by ^{13}C CPMAS solid-state NMR. The increasing chiral nematic order through magnetic alignment was monitored by measuring the changes in induced circular dichroism.

18. Sabin, J.

Title: The impact of Poly(ethylenimine) on phospholipid bilayers as a function of ionic strength.

Abstract: The impact of PEI on phospholipids bilayer has important consequences for applications involving the transfection of cell membranes. Gene therapy, for example, uses PEI for the tranfection of target cells by plasmid DNA. The interaction between DMPC unillamelar vesicles and Poly-ethylenimine (PEI) was investigated under Differential Scan Calorimeter and NMR techniques at different concentrations of NaCl. The aggregation of the vesicles in presence of PEI was also studied by Light Scattering technique.

19. Samii, L.

Title: Simulation of movement of a molecular spider on 1D track

Abstract: Molecular spiders are synthetic bio-molecular systems which have “legs” made out of short single-stranded segments of DNA and two to six attached nucleic acid catalysts (deoxyribozymes) with phosphodiesterase activity.1 Spiders move on a surface covered with single-stranded DNA substrates. Spider motion proceeds as legs bind to the surface DNA, cleave this substrate, then dissociate and rebind elsewhere. Analysis of experimental results suggests that molecular spiders preferably move to areas covered with new substrates,1 i.e., they undergo biased diffusion. Since the spiders alter the surface, a proper description of the motion of a single spider requires knowledge of its entire trajectory. Towards this goal we have simulated a molecular spider moving on a 1D tack of ssDNA using the Monte Carlo method. The simulation is based on a simple scheme of kinetics of interactions between spider legs and substrate. We investigate the dependence of rate of diffusion, processivity, and directionality of motion on kinetic parameters of the spider mechanism and on the relative roles of specific and nonspecific binding interactions.

1. R. Pei, S. K. Taylor, D. Stefanovic, S. Rudchenko, T. E. Mitchell and M.N. Stojanovic. *J. Am. Chem. Soc.*, 2006, 128 (39), pp 12693–12699

20. Shaghaghi, M.

Title: A ^{2}H-NMR study of POPC/sterol membranes: some exciting anomalies

Abstract: In a recent study [1] it has been showed that the ^{2}H-NMR order parameter, M_1, of

POPC/ergosterol multi-bilayers at 25^{o}C increases linearly as a function of ergosterol concentration to 25 mol%, but did not increase further when more ergosterol was added. By contrast, M_1 for POPC/cholesterol bilayers increases linearly to at least 50% sterol. Now the structural difference between cholesterol and ergosterol is that ergosterol has an additional double bond in its fused ring (C7-8) and a trans double bond (C22-23) plus a methyl group (at C24) in its alkyl chain. The question then arises as to which of these structural changes is responsible for the observed saturation of the order parameter in POPC/ergosterol bilayers. In [1] it was shown that the M_1 of POPC/7-dehydrocholesterol (7-DHC) multilayers behaves similarly to that of POPC/cholesterol, increasing linearly with 7-DHC. Note that 7-DHC has an ergosterol fused ring structure but a cholesterol alkyl tail. To further explore this phenomenon, we determined the sterol concentration dependence of POPC containing brassicasterol, a plant sterol that has the same fused ring structure as cholesterol with the alkyl tail of ergosterol [2]. We found that POPC/brassicasterol bilayers exhibit the same saturation behavior in M_1 at 25^{o}C as POPC/ergosterol bilayers, but at a lower value of M_1. We also examined POPC/campesterol bilayers to evaluate the role of the C22-23 trans double bond in the saturation effect. The M_1 of POPC/campesterol multilayers behaves similarly to that of POPC/cholesterol, increasing linearly with campesterol concentration.We plan to investigate other sterols in order to understand the sensitivity of POPC/sterol membranes to the sterol's alkyl tail structure.
[1] Y-W Hsueh et al., (2007) *Biophys. J.* **92**:1606-1615.

[2] We are most grateful to Till Boecking for suggesting brassicasterol for this study.

21. Tait, A.

Title: Phosphorylation of U24 from Human Herpes Virus type 6 (HHV-6) and its potential role in mimicking myelin basic protein (MBP) in multiple sclerosis

Abstract: Myelin basic protein (MBP) from multiple sclerosis (MS) patients contains lower levels of phosphorylation at Thr97 than normal individuals. The significance of phosphorylation at this site is not fully understood, but it is proposed to play a role in the normal functioning of MBP. Human Herpesvirus Type 6 encodes the protein U24, which has tentatively been implicated in the pathology of MS. U24 shares a 7 amino acid stretch encompassing the Thr97 phosphorylation site of MBP: PRTPPPS. We demonstrate using a combination of mass spectrometry, thin layer chromatography and autoradiography, that U24 can be phosphorylated at the equivalent threonine. Phospho-U24 may confound signalling or other pathways in which phosphorylated MBP may participate, precipitating a pathological process.

22. Wang, E.

Title: Configuration entropy modulates the mechanical stability of protein GB1

Abstract: Loop elongation into a folded protein is expected to destabilize the mechanical stability and accelerate the mechanical unfolding kinetics of the protein. This expectation can be explained by the lost of configurational entropy with closure of an unstructured flexible loop using classical polymer theory, resulting in decreased in thermodynamic stability of the protein. Four mutated polyproteins of loop elongations of two, five, twenty-four, and forty-six amino acid residues into the second loop of GB1 are constructed, respectively and are to study the mechanical stability as well as the mechanical unfolding kinetics of the proteins by using single-molecule atomic force microscopy. Structures of the mutants are examined by far-UV

circular dichroism spectroscopy, in this the results show that all mutants fold properly and suggest that the insertion of residues on the second loop of GB1 is well tolerant without influencing the structure of the native GB1 protein. Single molecule AFM results give good agreement with our expectations and demonstrate that the significant role of the second loop region of GB1 in the mechanical unfolding stability. This study not only proves that the loop region plays key roles in the mechanical unfolding stability as well as mechanical unfolding kinetics of the protein due to the cost of the configurational entropy but also provides the information and direction towards the future work of nanomechanical and biomedical applications.

23. Wieczorek, A.

Title: Purification and mutation of collagen II to determine the effects of specific mutations on its mechanical properties

Abstract: Collagens are the most abundant proteins in the human body. They are triple-helical matrix proteins that make up connective tissues such as skin, cartilage and tendons, and are responsible for maintaining the structure of many tissues and organs. Mutations and improper folding result in a wide range of connective tissue diseases, including osteogenesis imperfecta (brittle-bone disease), various skeletal dysplasias, and Ehlers-Danlos syndrome. In order to gain insight into the effects of mutations, optical tweezers will be used to explore the mechanical properties of both native and mutated collagen molecules. Collagen will be produced in a mammalian cell line to ensure proper post-translational modification, and constructs will be made to introduce specific, disease-associated mutations into the collagen. Labels must also be introduced onto the ends of the collagen triple helix to enable attachment to beads for manipulation. This poster outlines the steps involved in the preparation and purification of the collagen for use in an optical trap.

24. Zhuang, S.

Title: Rational engineering a stronger extracellular matrix protein

Abstract: Rational tuning the mechanical stability of extracellular matrix proteins may open up the possibility to control their mechanical stability *in vivo*. By the integration of protein engineering, single molecule AFM and steered molecular dynamics simulation, we have rationally designed a mutant of third FnIII domain of extracellular matrix protein tenascin-C with enhanced mechanical stability. This is the first step toward engineering extracellular matrix proteins with defined mechanical properties and provides the possibility to investigate the role of mechanical stability of extracellular matrix proteins in mechanotransduction.