showed ~95% of the dihedral angles in the allowable regions of the Ramachandran plot. Our model reveals that the cavity walls of TRiC exhibit an overall positively charged surface property, the opposite of GroEL. The interior surface chemical properties likely play an important role for TRiC's unique substrate specificity.

1158-MiniSymp
Counting Hydrolyzed ATP On Single Tric Nanomachines in Solution
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Single biomolecules can be localized in aqueous solution using an Anti-Brownian Electrokinetic (ABEL) trap, which uses fluorescence imaging combined with actively applied electrokinetic forces in a microfluidic geometry. With this device, we have explored the ATP-induced cooperative transitions in the important multi-subunit eukaryotic chaperonin TRiC, a model for other multisubunit enzymes. ATP is labeled with Cy3 and incubated with Atto647 labeled TRiC in the presence of AlFx. The ATPs bind to up to 16 subunits in TRiC and are locked in their binding sites by AlFx after hydrolysis. A rotating 532nm confocal excitation beam is used for both trapping and measuring, and each TRiC complex is trapped until complete photobleaching of the Cy3 occurs. The number of photobleaching steps in the fluorescent intensity trace yields the number of hydrolyzed ATPs. In a separate measurement the total number of TRiCs and the number of TRiCs with one or more ATPs are determined. From these measurements, we obtain the distribution of the number of ATPs on each TRiC at different incubation ATP concentrations. As the ATP concentration increases from 25uM, the position of the major peak remains at 7-8 ATP/chaperonin while the height of the peak increases. For ATP concentrations above 200uM, all the TRiCs are found to have 7-8 ATP bound with a smaller probability of 6 or 9 ATP bound, and no other peak appears up to an ATP concentration of 1.5mM, suggesting each ring hydrolyzes at most 4 ATP. Only when the ATP concentration is lower than 25uM does the peak position move to smaller numbers. While the averages of our distributions can be fit with MWC model, the distributions themselves depart from the model. This new method may be applied to study the cooperativity behavior of other multi-subunit enzymes.

1159-MiniSymp
Reconstituting EphA2-Ephrin Signaling with Supported Membranes
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The Eph family of receptor tyrosine kinases mediates cell patterning and tissue organization via their ability to govern intercellular interactions. Eph activation occurs when cell surface Eph receptors encounter their ligands, the ephrins, presented on the membrane of an adjacent cell. The resultant intracellular signaling strongly influences whether the eventual outcome of the cell-cell contact event will be adhesion or repulsion. Here, we examine the complex interplay between spatial, mechanical and biochemical regulation mechanisms in EphA2 signaling.
We recapitulate the native intercellular signaling geometry using a hybrid junction between live human breast cancer cells expressing EphA2 and a supported membrane functionalized with ephrin-A1. After cells contact the membrane for 1 hour, we observe spatial reorganization of EphA2-ephrin-A1 complexes on multiple lengthscales as well as pronounced changes in cell shape and signaling activity.
To further probe the reorganization phenomenon, we have investigated a panel of membrane-associated effector proteins with functions associated with motility and cytoskeletal remodeling to determine how they are affected by EphA2 reorganization. These observations provide new insight regarding the cellular transduction of spatio-mechanical feedback into biochemical signaling.

1160-MiniSymp
Engineering Vesicle Membranes for Cellular Reconstitutions
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Cells routinely sample their local environment, process this information through internal signaling, and respond accordingly. However, the sheer complexity that underlies cellular behavior makes cells notoriously difficult to understand and control. To address these issues, traditional cell biological approaches are often complemented with in vitro reconstitutions aimed at building 'cell-like' systems from individual components. Such reconstitutions can provide functional insights into biological processes and have great potential for improving drug delivery. However, these studies are still limited by the technical challenge of assembling giant lipid vesicles with embedded transmembrane (TM) proteins and complex cytosolic components.

Here we present a method for incorporating TM proteins into giant unilamellar vesicles (GUVs) with simultaneous control over the encapsulated components. This method is an extension of a previously developed microfluidic jetting technique for creating GUVs from planar bilayers, analogous to blowing bubbles from a soap film. The technique can now be used to incorporate TM proteins with controlled orientation and create an asymmetric lipid bilayer composition in order to assemble increasingly sophisticated 'cell-like' systems.
With this approach, we demonstrate encapsulation of small unilamellar vesicles (SUVs) carrying the vesicular fusion machinery (vSNAREs) into GUVs carrying the target fusion machinery (tSNAREs), closely mimicking the organization of synaptic vesicles in cells. Using fluorescence microscopy we track the location of SUVs in the lumen of GUVs and study the role of vesicle docking in the process of SNARE-mediated membrane fusion.

1161-MiniSymp
A Light-Gated, Potassium-Selective Glutamate Receptor for the Optical Inhibition of Neuronal Firing
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Genetically-targeted, light-activated ion channels and pumps have recently made it possible to manipulate activity in specific neurons and thereby probe their role in neuronal circuits, information processing and behavior. Here, we describe the development of a K+-selective ionotropic glutamate receptor that inhibits nerve activity in response to light. The receptor is a chimera of the pore region of a K+-selective bacterial glutamate receptor and the ligand binding domain of the light-gated mammalian kainate receptor LiGluR (iGluR6/GluK2). This new hyperpolarizing light-gated channel is turned ON and OFF by brief light pulses even at moderate light intensities. After optical activation the silencing of neuronal activity persist in the dark for extended periods, a feature that will prove advantageous for the dissection of neural circuitry in behaving animals.

1162-MiniSymp
Computational Nanomedicine: Simulating Protein Misfolding Disease
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Protein misfolding diseases such as Alzheimer's Disease (AD) and Huntington's Disease (HD) are challenging to study experimentally. Thus, computational methods, if they are able to be sufficiently accurate and reach sufficiently long timescales, can naturall contribute in this challenging area. I will discuss our recent results using novel methods within the Folding@home distributed computing project on progress towards a molecular understanding of protein aggregation involved in AD and HD. Specifically, I will detail our extensions to simulation Markov State Model methodology as well as specific predictions that arose from these simulations and experimental validation of these predictions. These results lead to a novel hypothesis for the structural basis of Abeta aggregation as well as the ability to explain existing experimental data.

Platform X: Membrane Protein Functions

1163-Plat
Using Giant Uni-Lamellar Vesicles to Study Ion Channel Activity and Interactions
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Unraveling the complicated dynamics of ion channel activity and interactions requires experimental systems in which ion channel activity and distribution can be measured while controlling ion channel concentration and membrane composition, tension and voltage. Many of the constraints imposed by existing techniques, such as planar Black Lipid Membranes (BLM), could potentially be circumvented by using Giant Uni-Lamellar Vesicles (GUVs). To explore this possibility, a method was developed to produce GUVs containing KvAP, a bacterial voltage-gated potassium channel [1]. Protein was first purified, fluorescently labeled and reconstituted into Small Unilamellar Vesicles (SUVs) which were then used to grow GUVs via an electro-formation procedure [2]. Incorporation of the fluorescently-labeled channels was confirmed via confocal microscopy while channel activity was studied with the patch-clamp technique. In parallel, attempts were made to adapt the "whole-cell" patch-clamp geometry for GUVs. While the absence of a cytoskeleton made the "whole-GUV" configuration quite difficult, this geometry is possible and its further development is important since it permits simultaneous control of the voltage inside the GUV and measurement of the entire membrane current. These results confirm the potential of GUVs for ion channel studies, and experiments measuring the effect

of membrane tension, composition and curvature on KvAP activity and distribution are currently underway.

[1] Ruta et. al., Nature, Volume 422, p180-185 (2003)
[2] Montes et. al., Biophysical Journal, Volume 93, p3548-3554 (2007).

1164-Plat
Lipid Membrane Composition has A Dramatic Effect on the Dynamics of the GlpG Rhomboid Protease from Escherichia Coli

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Intramembrane proteases cleave transmembrane substrates to liberate physiologically important molecules. The proteolytic activity of these enzymes can be significantly influenced by the composition of the lipid membrane. Here, we find that the composition of the lipid membrane has a dramatic effect on the motions of the GlpG rhomboid serine protease from Escherichia coli.

In a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) lipid bilayer, conformational changes of two critical structural elements of the protease, the cap loop close to the active site and the regulatory loop L1, occur within 40ns of unconstrained molecular dynamics simulations. In contrast, in a 1-palmytoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (POPE) lipid bilayer, a conformational transition of the cap loop is observed only after ~80ns, ~20ns after that of loop L1. This sensitivity of the enzyme motions on the lipid membrane composition is explained by differences in how POPC and POPE lipid headgroups hydrogen bond among themselves, and with protein amino acids. Tight interactions between a lipid headgroup and the active site restrict the dynamics of the cap loop.

An atomistic description of the structure and dynamics of the protease:substrate complex is a critical first step towards understanding how the protease works. Molecular dynamics simulations of GlpG together with the Spitz model substrate reveal that docking of the substrate to the enzyme involves a complex interplay of changes in the structure and dynamics of the substrate, the protease, and the surrounding lipid molecules.

1165-Plat
Energetics of Glycophorin A Dimerization in Mammalian Plasma Membranes

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Quantitative measurements of protein interaction strengths are crucial for describing signaling networks and predicting cellular responses to environmental stimuli. Of all interactions between biological macromolecules, interactions between membrane proteins are the least characterized, and their strength is often measured in model detergent and lipid systems that poorly mimic the complex biological membrane. Measurements in models systems, however, are not likely to yield accurate predictions, because interactions in the native cellular environment occur within the context of a crowded system.

Here we explore the utility of plasma membrane-derived vesicles as a model crowded environment for quantitative characterization of membrane protein interactions in mammalian membranes. In particular, we study the dimerization energetics of Glycophorin A (GpA), the primary sialoglycoprotein of human erythrocyte membranes, using the "quantitative imaging Föster resonance energy transfer (QI-FRET)" method. We determine the FRET efficiency, and the donor and the acceptor concentrations in single plasma membrane-derived vesicles loaded with GpA. These measurements yield, for the first time, the free energy of GpA dimerization in mammalian membranes. Supported by NSF MCB-0718841.

Li E, Placone J, Merzlyakov M, Hristova K (2008) Quantitative measurements of protein interactions in a crowded cellular environment. *Anal Chem* 80: 5976-5985

1166-Plat
Monitoring Proton Flux Quantitatively; Influenza Proton Channel A/M2

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An improved methodology for monitoring proton translocation across membranes is presented, along with results from the Influenza A virus proton channel A/M2.

We have constructed a liposome-production instrument which creates proteoliposomes from a lipid/detergent/protein mixture by gradually adding hydrophobic beads while continuously monitoring sample turbidity. The method is fast and reproducible, and facilitates enhanced control of key protein reconstitution parameters. It also enables synchronous measurements of protein-mediated ion flow and passive permeability across the bilayer. Two novel pH-sensors are presented: Glu3 and TCHP. These porphyrin-based probes are membrane-impermeable, do not interact with biological complexes, have physiologically appropriate pK, and display high extinction coefficients. Glu3 is also ratiometric in emission.

H+, K+ and Na+ permeabilities were determined in liposomes of different lipid and cholesterol composition. The effect of detergent/lipid and lipid/protein ratios on ion permeability was systematically investigated. The proton channel A/M2, key to Influenza A virus propagation and an antiviral drug target, was successfully reconstituted. The proton translocation rate was determined to 8.3 protons per second and A/M2 tetramer. We also found that the presence of protein in the bilayer enhanced the passive ion permeability. Lowering the protein/lipid ratio minimized this effect, and prolonged the measurement window of proton movements to several minutes, and thus resulted in the most reliable data.

Preliminary data from membrane-spanning subunits of respiratory Complex I are also presented.

1167-Plat
The Role of the Protein-Conducting Channel in the Membrane Insertion of Transmembrane Segments

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In all domains of life, the majority of membrane proteins are inserted into the membrane via a protein-conducting channel, also known as the SecY or Sec61 complex. In addition to a translocation pathway across the membrane, this channel features a unique lateral gate, which can open toward the membrane, permitting the sequential insertion of transmembrane segments (TMs). How this insertion occurs is still unclear, although a thermodynamic partioning between channel and membrane environments has been proposed. However, experiment- and simulation-based scales for the free-energy insertion cost of various amino acids differ, sometimes significantly as in the case of arginine (2-3 kcal/mol in experiment compared to 17 kcal/mol in simulation). Using free energy perturbation (FEP) simulations, we have calculated the insertion cost for an arginine located on a background poly-leucine helix, both in the center of a pure bilayer and in the center of a model of SecY featuring an open lateral gate. We find that the presence of SecY greatly reduces the membrane insertion cost for arginine, in agreement with prior simulations. We also consider the free energy cost for the insertion of the background helix from SecY to the membrane, which had been neglected previously.

1168-Plat
Nature as A Scaffold: The Rational Redesign of a Protein Pore

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Our major goal is to engineer a transmembrane protein pore that acts as a single-molecule nanopore probe for sensing double-stranded DNA (dsDNA), folded proteins and their complexes with interacting agents. The protein of choice is Ferric hydroxamate uptake protein component A (FhuA), a multifunctional outer membrane protein found in E. coli., which facilitates the uptake of Fe3+, along with phage binding and the translocation of small peptides. Using standard protein engineering, we modified the FhuA protein by removing the N-terminal 160 residue-long cork and by deleting several large extracellular loops to form an open protein pore with an elliptical cross section of ~ 49 x 36 Å. This engineered protein nanopore exhibits a stable open-channel activity for long periods of time, with a unitary conductance of ~5 nS. We show evidence that this engineered FhuA-based nanopore acts as a stochastic sensing element for detecting small folded proteins at single-molecule resolution.

1169-Plat
Modulation of the Lateral Mobility of Transmembrane Peptides with Hydrophobic Mismatch

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