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
Yan Jiang, Nick Douglas, Nick Conley, Judith Frydman, W.E. Moerner.
Stanford University, Stanford, CA, USA.
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
Jay T. Groves, **Rebecca Petit**.
UC Berkeley, Berkeley, CA, USA.
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
David L. Richmond, Eva M. Schmid, Sascha Martens,
Jeanne C. Stachowiak, Nicole Liska, Daniel A. Fletcher.
UC Berkeley, Berkeley, CA, USA.
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
Harald Janovjak[1], Dirk Trauner[2], Ehud Y. Isacoff[1].
[1]UC Berkeley, Berkeley, CA, USA, [2]University of Munich, Munich, Germany.
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
Vijay Pande.
Stanford Univ, Stanford, CA, USA.
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
Sophie Aimon, **Gilman ES Toombes**, Patricia Bassereau.
Institut Curie, Paris, France.
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