

Mitochondria are the energy producing organelles of eukaryotic cells. Owing to their endosymbiotic evolutionary history, they contain their own genome (mtDNA) that encodes for thirteen proteins essential for ATP production. In mammalian cells, multiple mtDNAs are compacted into protein-DNA complexes called "nucleoids". A major component of these nucleoids is the mitochondrial transcription factor A (TFAM), a member of the high mobility group (HMG) family of proteins. This abundant protein binds DNA with little sequence specificity, and is able to coat the entire mtDNA molecule. It not only serves a role in mtDNA packaging, but is also required for mitochondrial transcription. At this point, dynamics of the TFAM-DNA interaction remain unclear.

Experiments on single DNA molecules offer a very direct way to study TFAM dynamics. Tethered Particle Motion (TPM) experiments show that the system quickly equilibrates with the buffer, and that the end-to-end distance of the DNA decreases upon TFAM binding. Manipulations of single DNA molecules with two optical traps offer an explanation: TFAM decreases the DNA's stiffness (persistence length). A possible molecular mechanism for this decrease is that TFAM introduces bends in the DNA.

Adding single-molecule fluorescence to the dual optical trap illuminates TFAM's binding behavior. Literally seeing TFAM on the DNA, we can derive on- and off-rates, and determine that TFAM does not bind cooperatively. Interestingly and seemingly unrelated to its role in DNA organization, we also observe that TFAM can rapidly bind single-stranded DNA (ssDNA), but not when the ssDNA is under tension. The physiological function of TFAM binding to ssDNA could be related to its regulation of transcription.

Symposium 9: Biophysics of the Failing Heart

1153-Symp

What is the Effect of A Familial Hypertrophic Cardiomyopathy Mutation on Cardiac Myosin Function?

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Familial hypertrophic cardiomyopathy (FHC) is a clinically and genetically heterogeneous disease which is a major cause of heart failure. The landmark discovery that a point mutation at residue 403 (R403Q) in the β -myosin heavy chain (MHC) can cause a lethal form of FHC was made in 1990, but the effect of this mutation on the functional properties of human cardiac myosin remains poorly understood. One problem has been that the prevalent mouse model for FHC expresses predominantly α -MHC. The β -MHC, however, is the predominant isoform in the ventricles of all larger mammals. Even though the α - and β -MHC share > 90 % sequence identity, they differ ~ 2-fold in enzymatic and mechanical properties, raising the possibility that the effect of a disease mutation may depend on the isoform backbone. To address this question we used a transgenic mouse model in which the endogenous α -MHC was replaced with transgenically encoded β -MHC. A His-tag was cloned at the N-terminus of α - and β -MHC, along with the R403Q mutation, to facilitate isolation of myosin or its head subfragment-1 (S1). We find that the steady-state ATPase activity and *in vitro* motility of mouse α -MHC is enhanced by the R403Q mutation, as reported previously, but the R403Q mutation in a β -MHC background shows a slight reduction in activity. A more in-depth analysis of the R403Q phenotype is being undertaken by stopped-flow kinetics to measure the nucleotide turnover in these mutant S1 isoforms. In order to determine the extent of species-dependent differences, we are comparing the functional properties of β -cardiac myosin in the mouse with those in the rabbit, a model system which more closely resembles humans in protein composition and disease phenotypes.

1154-Symp

Reduced Responsiveness to β -Adrenergic Agonists in Murine cMyBP-C Cardiomyopathy

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Myosin binding protein C (MyBP-C) is a thick filament accessory protein that has both structural and regulatory roles in striated muscle contraction. We are studying the roles of the cardiac isoform of MyBP-C in mouse models in which the cMyBP-C gene has been disrupted, resulting in ablation of the protein, and in mice expressing mutant protein in which residues that are phosphorylated *in vivo* by PKA have been replaced with ala or asp. Ablation of cMyBP-C results in a cardiac phenotype similar to many inherited cardiomyopathies in humans, i.e., septal hypertrophy, increased arrhythmic activity, and systolic and diastolic dysfunction. Studies of isolated myocytes from wild-type and null mice suggest that cMyBP-C regulates the kinetics of

cross-bridge interaction with actin, a mechanism that is lost in the null mouse. Studies of myocytes from mouse lines expressing phosphorylation mutants of cMyBP-C indicate that PKA stimulation of contraction kinetics in myocardium is in large part due to phosphorylation of cMyBP-C, which appears to relieve a structural constraint on myosin and increases the likelihood of myosin binding to actin. Living myocardium expressing non-phosphorylatable cMyBP-C was found to exhibit depressed twitch force-frequency relationships and reductions in both frequency-dependent and β -agonist-induced acceleration of relaxation. These results can be explained by a model in which cMyBP-C phosphorylation accelerates cross-bridge interaction kinetics in wild-type myocardium, a regulatory mechanism that is lost in myocardium expressing the non-phosphorylatable mutant cMyBP-C. Supported by NIH R01 HL082900 and P01 HL094291.

1155-Symp

The Giant Elastic Protein Titin Role in Muscle Function and Disease

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No Abstract.

1156-Symp

Rescue of Familial Cardiomyopathies by Modifications at the Sarcomere Level and Ca^{2+} Fluxes

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Familial cardiomyopathies are commonly linked to missense mutations, deletions or truncations in sarcomeric, cytoskeletal, or intermediate filament proteins and give rise to hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) or restrictive cardiomyopathy. Although in the last two decades much information about the pathophysiology of genetically linked HCM and DCM has been provided by studies using transgenic animal models, there is still no therapy to prevent the development of the disease and increase survival in patients with HCM or DCM. Our emphasis here is on development of new therapies for treatment of HCM and DCM linked to mutations in thin filament proteins that are associated with increased and decreased myofilament sensitivity to Ca^{2+} respectively. We hypothesize that direct modifications of myofilament Ca^{2+} sensitivity and/or alteration in Ca^{2+} fluxes can serve as new therapeutic targets. Therefore if 1) HCM is associated with increased myofilament sensitivity to Ca^{2+} , interventions that desensitize the myofilament to Ca^{2+} may serve as potential new therapeutic targets and 2) DCM is associated with decreased myofilament sensitivity to Ca^{2+} , interventions that sensitize the myofilament to Ca^{2+} may serve as potential new therapeutic targets. There are several possible targets within myofilament proteins for altering myofilament Ca^{2+} sensitivity, in particular troponin I. Alterations in Ca^{2+} regulation by modification of sarcoplasmic reticulum Ca-ATPase (Serca2) or phospholamban levels are additional potential targets for HCM and DCM.

Minisymposium 2: Nanomedicine: Biophysical Approaches to Clinical Problems at the Nanoscale

1157-MiniSym

4.0 Å Cryo-EM Structure of the Mammalian Chaperonin: TRiC/CCT

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TRiC is a eukaryotic chaperonin essential for *de novo* folding of ~10% newly synthesized cytosolic proteins, many of which cannot be folded by other cellular chaperones. Unlike prokaryotic and archaeal chaperonins, each of its two rings consists of eight unique, but similar subunits. Using single particle cryo-EM, we determined the mammalian TRiC structure without any symmetry imposition at 4.7 Å resolution, which is the highest resolution asymmetric cryo-EM reconstruction to date. An analysis of this map allowed us to elucidate the relative orientation of the two rings and the two-fold symmetry axis location between them. A subsequent two-fold symmetrized map yielded a 4.0 Å structure, in which a large fraction of side chains and structural elements including loops and insertions appear as visible densities. These features permitted unambiguous identification of all eight individual subunits, despite their similarity. A $\text{C}\alpha$ backbone model of the entire TRiC complex was subsequently refined from initial homology models against the cryo-EM density based on our subunit identification. A refined all-atom model for a single subunit

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 naturally 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