plasma membrane. In some exocytic events, we observed a 'triggering' mechanism where a collision of a small vesicle preceded the fusion event. Using quantum dot labeled IgG molecules (QD-IgG) we have imaged the entire 3D trafficking itinerary on the endocytic pathway at the single molecule level. We observed that QD-IgG molecules exhibit complex itineraries before and after endocytosis. Quantitative analysis of the data reveals that QDs can be tracked with high accuracy, which is difficult to achieve with other conventional imaging techniques. The present technique provides an invaluable tool to study the 3D trafficking pathways in cells and provides insight into the intracellular events that precede (follow) exocytosis (endocytosis).

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1800-Plat Identification Of Early Endocytic Structures After Stimulation Of Pancreatic Acinar Cells

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Our recent work challenges the classical model of granule fusion and collapse during secretion in epithelial cells (Palade 1975). We show that granules don't collapse but persist at the plasma membrane for many minutes (Thorn *et al.* 2004) with exocytosis being terminated by closure of the fusion pore (Larina *et al.* 2007). However, the next step, that of endocytic recovery of the granule membrane, still remains unclear. Here we used the HRP-DAB method to follow early steps of endocytosis using transmission electron microscopy. In pancreatic tissue fragments stimulated for 1 minute with Acetylcholine and left to recover for 10 minutes we observed unstained granules fused to the plasma membrane with open fusion pores; consistent with the protracted fusion events seen in live cells (Thorn *et al.* 2004). Electron-dense DAB reaction product was observed in large vesicles that were predominantly located towards the apical end of the cells. The measured diameter of these electron-dense vesicles was 450 +/− 17 nm (mean diameter +/− SEM, n=44, from 3 independent preparations). These labeled vesicles are statistically smaller in size compared to the total population of zymogen granules (748 +/− 11 nm, n=230, mean diameter +/−SEM). We conclude that the labeled vesicles are surprisingly large and, while this might suggest that whole granules are endocytically recaptured, it is still unclear why they are smaller than the total population of zymogen granules.

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Platform AN: Protein Folding & Stability

1801-Plat A Coarse Grained Model Of Protein Collapse With Explicit Solvent

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We study the collapse transition of a lattice based protein model including an explicit coarse-grained model of a solvent. This model accounts for explicit hydrophobic interactions, and it is studied by Monte Carlo simulation. The protein is modeled as self-avoiding random walk with nearest neighbor interactions on a two dimensional lattice. Without the solvent, universal quantities of the chain around the collapse transition temperature are well known. Hydrophobicity is then modeled through a lattice of solvent molecules in which each molecule can have Q states depending of an orientation variable. Only one state is energetically favored, when two neighboring solvent molecules are both in the same state of orientation. The monomers are placed in interstitial position of the solvent lattice, and are only allowed to occupy sites surrounded by solvent cells of the same orientation. The potential of mean force between two interstitial solute molecules is calculated, showing a solvent mediated attraction typical of hydrophobic interactions. We then show that this potential increases with the energy of hydrogen bond formation as it appears in the model, while its characteristic range decreases. More importantly, we show that the chain embedded in the solvent undergoes a collapse transition, with the temperature of the transition being shifted relative to that of the chain in isolation. We calculate several critical exponents near the collapse transition, and we observe that their values are not conserved in presence of the explicit solvent.

1802-Plat How Do Chemical Denaturants Affect the Mechanical Folding and Unfolding of Proteins?

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Single molecule atomic force microscopy (AFM) has evolved into a powerful tool to investigate the folding/unfolding dynamics of proteins. In single molecule AFM experiments, proteins are forced to undergo unfolding/folding reactions along a pre-defined reaction coordinate determined by the stretching force acting on the protein of interest. Here we combined single molecule AFM with chemical denaturation to investigate the effect of chemical denaturants guanidinium chloride (GdmCl) on the mechanical folding and unfolding kinetics of a small protein GB1. Upon increasing the concentration of GdmCl, we observed a systematic decreasing in the mechanical stability of GB1, indicating the softening effect of the chemical denaturants on the mechanical stability of proteins. This mechanical softening effect originates from the reduced free energy barrier between the folded and unfolding transition state, which decreases linearly as a function of the denaturant concentration. Chemical denaturants, however, do not shift the mechanical unfolding pathway or alter the distance between the folded and transition states. We also found that the folding rate constant of GB1 is slowed down by GdmCl in mechanical folding experiments. Combining the mechanical folding and unfolding kinetics of GB1 in GdmCl solution, we developed the "mechanical chevron plot" as a general tool to understand how the chemical denaturants influence the mechanical folding and unfolding kinetics and free energy diagram in a quantitative fashion. This study demonstrates the great potential in combining chemical denaturation with single molecule AFM techniques to reveal the invaluable information of the energy landscape underlying protein folding/unfolding reactions.

1803-Plat Energetics of Infinite Homopolypeptide Chains: A New Look at Commonly Used Force Fields

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Empirical force fields for proteins come in a variety of flavors. Despite their ubiquitous use in biomolecular simulations, the differences and similarities between them remain poorly understood. In this work, we present a novel method for comparing the long-range part of force fields in the presence of a maximally cooperative network of hydrogen bonds. The method is based on mapping the potential energy surface of an infinite polypeptide chain in the gas phase using cylindrical coordinates (the twist and pitch) as geometry descriptors. We apply our method to an infinite poly-alanine chain and consider the AMBER99, AMBER99SB, CHARMM27, OPLS-AA/L fixed partial-charge force fields and the protein-specific version of the AMOEBA polarizable force field. Results from our analysis are compared to those obtained from high-level density-functional theory (DFT) calculations.

We find that all force fields produce stronger stabilization of the helical conformations as compared to DFT. Only AMBER99/AMBER99SB can satisfactorily reproduce all three helical conformations (pi, alpha, and 3-10) seen in DFT calculations. Qualitative differences between CHARMM27 and the other force fields considered in our study, as well as the implications for the force fields' abilities to capture the cooperative nature of hydrogen-bond networks, are discussed.

1804-Plat Models of hexameric amyloid-beta(1–42) peptides in aqueous solutions and stability tests with Discrete Molecular Dynamics simulations

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Amyloid-β peptides (Aβ) have been linked to neurotoxicity in Alzheimer's disease. Among the alloforms of Aβ, the 42-residue long Aβ42 is the most toxic, aggregates faster, and forms larger oligomers (up to hexamers) *in vitro* during early aggregation in aqueous solution. Here we present models of hexameric Aβ42 in aqueous solution, and for the first time, analyze their stabilities using Discrete Molecular Dynamics (DMD) simulations. Various simulation parameters, such as overall time steps and temperature, were obtained by analyzing the stability of proteins of known structure. The results were temperature-dependent: the proteins were almost frozen at the lowest temperature, were relatively stable at intermediate temperatures, and were destabilized at high temperatures. At low temperatures, α-helices were more stable than β-strands. To choose the implicit solvent models, we applied Kyte-Doolittle and Miyazawa-Jernigan scales, and found no substantial differences. The hydrophobic core region of the hexameric Aβ42 was modeled as a six-stranded antiparallel β-barrel formed by residues 29–42, while the outer region was modeled to have either α-helices or β-strands. DMD stability tests for these two hexameric Aβ42 models showed that the model with outer β-strands was more stable. In both hexameric Aβ42 models, the central β-barrel region remained stable.

1805-Plat Trpzip-Based Beta-Hairpin Equilibrium and Temperature Jump IR Studies Enhanced by Site-Specific Isotope Labeling

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Beta-hairpins may be the smallest folding units in a protein, and two antiparallel beta-strands connected by a turn make the simplest model system for analysis of the interactions and dynamics of beta-sheets. We have studied site-specific conformational dynamics by use of equilibrium and temperature-jump kinetic IR-spectroscopy with site-specific enhancement via isotopic labelling of the amide with 13C=O in isotopically labeled variants of a modification of Cochran's 12-residue tryptophan zipper peptide, TrpZip2. Equilibrium measurements reflect decreased stability of the hairpin cross-strand H-bonds at the turn and the termini. Spectral analysis of single and doubly labeled species is used to determine specific coupling levels. 13C=O groups introduced at different amide positions lead to distinguishable cross-strand coupling of the labelled residues which is lost on unfolding. These labels have distinct frequency patterns and different thermal behaviors depending on their position in the hairpin and reflect the local structural variation along the strands. Relaxation kinetics upon laser-induced T-jumps of ~10 C have time constants of a few microsec that decrease with increase of the initial temperature of the peptide before the temperature jump. Analysis of the data supports a multistate folding process, consistent with the hydrophobic collapse hypothesis for hairpin folding, but it is not possible to clearly define a folding and unfolding rate.

1806-Plat Folding-unfolding dynamics of Titin explored with Force-clamp Optical Tweezers

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Titin is a giant filamentous muscle protein that spans the half sarcomere and functions as a molecular spring, a sarcomeric template, and possibly as a mechanosensor. Early single-molecule manipulation experiments revealed force-driven domain unfolding and refolding in titin, the kinetics of which varied with the instantaneous force determined by the rate of stretch and the complex stiffness of the nanomechanical system. To understand the kinetic detail in mechanically-driven unfolding and refolding, constant-force measurements are desired. Here we explored the unfolding and refolding dynamics of skeletal-muscle titin by using a custom-built force-feedback optical tweezers apparatus. The fast (up to 500 Hz) feedback allowed us to monitor rapid changes in molecular extension at forces clamped at values as high as 150 pN and as low as 1 pN. It was possible to record tens of consecutive high-and-low force-clamp cycles on the same single titin molecule. At high forces titin extended in discrete steps of ~30 pN that correspond to single-domain unfolding events. Domain unfolding followed an overall single-exponential function. At low forces, the extension of titin changed in three relatively distinct phases. A rapid (~10 ms) shortening phase was followed by one characterized by large length fluctuations that lasted up to several seconds. Finally, titin contracted in an apparently cooperative, rapid (~100 ms) process. The details of time-dependent length changes during refolding were different in subsequent force-clamp cycles, suggesting that the molecule maintains no memory of an exact folding path. Probe stretches at various stages during refolding suggest that the molecule may acquire intermediate states transiently stabilized in shortened state.

1807-Plat Passive Microfluidic Mixers for Studies of Protein Folding

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The purpose of this study is to design, fabricate and characterize microfluidic mixers to investigate the kinetics of protein secondary structure formation with Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy. SRCD allows us to use wavelengths below 220 nm where differences between the CD spectra of random coil and the various secondary structure types are most pronounced. Microfluidic mixing allows a fast initiation of the protein folding reaction. By combining this with SRCD, we can clarify an intense debate in the protein folding community as to when, in the process of folding, the secondary structure content forms.

We designed, fabricated and characterized mixing in a serpentine-shaped microfluidic mixer. Folding is initiated through the rapid dilution of denaturant with buffer. In our mixer, a protein solution with high denaturant concentration meets with a buffer solution right before the serpentine-shaped region. In this region, the two solution get mixed in the laminar flow regime by virtue of diffusion and chaotic advection. Spectroscopic measurements are performed downstream in an "observation channel". Kinetic measurements of SRCD have been completed on the Cold Shock Protein (CSP). Using a mixer with 10 serpentine turns, we measured the formation of 32% secondary structure content within 1.3 ms after mixing was completed.

I will present further improvements and characterization of the "serpentine mixer" using a parameter that we call a mixing metric, M. This parameter is based on the standard deviation of the fluorescence intensity. M=0 represents a completely unmixed state, and M=1 represents a completely mixed state. Fluorescein dye and buffer are mixed in the ratio 2:1. Intensity scans taken at various flow rates indicate that a mixer design with five serpentine turns is capable of achieving a mixing metric M = 0.934 within a mixing time of 100 μs.

1808-Plat Mapping The Mechanical Response Of The Enzyme Guanylate Kinase With The Allosteric Spring Probe

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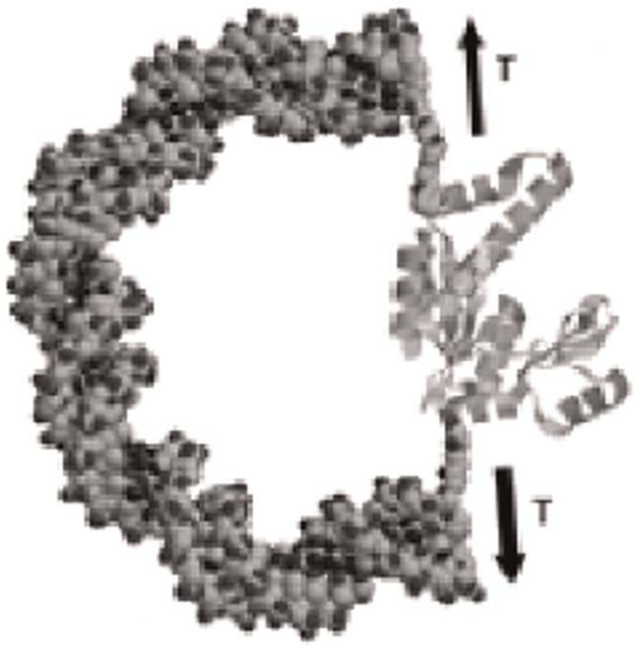
Protein molecules are semi-rigid objects with organized but fluctuating conformation. For guanylate kinase which catalyzes phosphoryl transfer between ATP and GMP, a large conformational change upon substrate binding (~ 1nm in amplitude) occurs which is essential for enzymatic activity. By mechanically stretching the molecule, different conformational states could be probed.

By site-directed mutagenesis, we prepared five different mutants of guanylate kinase from Mycobacterium Tuberculosis. Each mutant bears a pair of cycteines of specific sites where the allosteric spring probe is attached via heterobifunctional crosslinkers (Fig.1). To investigate the relation between conformation and enzymatic activity, we apply mechanical tension to perturb the structure, searching "hot spots" which respond with significant changes in enzymatic functions such as substrate binding, ATP hydrolysis, and phosphoryl transfer.

Figure 1. Schematic arrangement of the allosteric spring probe (shown as space-filled dsDNA) and guanylate kinase (ribbon). The mechanical tension T on the protein can be controlled by the degree of hybridization of the DNA spring.



Platform AO: Channel Regulation & Modulation

1809-Plat Regional Heterogeneity Of KATP Channel Structure And Function In The Heart: Atrial KATP Contains SUR1

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Sulfonylurea receptors (SURs) are obligate components of K_{ATP} channels, coassembling with Kir6.x subunits to form a tetradimeric protein complex. Different isoforms, SUR1 and SUR2, confer different physiological sensitivity to MgADP channel stimulation with important implications for the timing of K_{ATP} activation during metabolic stress. In the heart, both transcripts are expressed and studies of SUR2−/− animals clearly reveal that SUR2 is necessary for sarcolemmal K_{ATP} function in ventricular myocytes. The role of SUR1 in generating K_{ATP} in the heart is unknown, however, we recently demonstrated that both SUR1 and SUR2 can coassemble within the same octomeric channel, raising the possibility that both subunits are necessary. In the present study, we examine the role of SUR1 in generating sarcolemmal K_{ATP} channel activity in the mouse heart. Immunoblot analysis using a novel antibody raised against SUR1 confirms expression in the heart but, surprisingly, total K_{ATP} conductance induced by metabolic inhibition is not different between WT and SUR1−/− ventricular myocytes (3.00±0.47 vs. 3.14±0.59 nS/pF, respectively, n=4–6), indicating that SUR1 does not contribute to K_{ATP} in the ventricle and suggesting that there is regional variability in SUR1 expression. Indeed, SUR1 is readily detected in atrial, but not ventricular, proteins, suggesting that SUR1 is a component of the atrial K_{ATP} channel. Consistent with this notion, diazoxide-activated current was undetectable in whole cell experiments on SUR1−/− atrial myocytes (29.8±12.0 nS in WT vs. −2.9±3.7 nS in SUR1−/−, n=3–5) and little K_{ATP} current was detected in inside-out patch clamp experiments (71.15±17.74 pA in WT vs. 10.51±7.73 pA in SUR1−/−, n=4–6). Collectively, the data demonstrate a regional distribution of SUR1 in the heart and K_{ATP} heterogeneity that may have important implications for the response of the heart during metabolic stress.

1810-Plat Identification of a Critical Region Essential for Augmentation of KCNQ1 Potassium Channels by Both Chemical Opener and Auxiliary Subunit

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KCNQ1 (Kv7.1) encodes potassium channels that are predominantly expressed in cardiac and epithelial tissues. Assembly of KCNQ1 with KCNE1 (IsK, or minK) subunit forms the I_{Ks} current critical for membrane repolarization of cardiac action potential. Genetic mutations of the KCNQ1 gene cause reduction of I_{Ks} resulting in QT prolongation in humans. We recently reported that zinc pyrithione (ZnPy) potently activates neuronal KCNQ potassium channels (KCNQ2-5). However, the ZnPy effects on cardiac KCNQ1 potassium channel remain largely unknown. Here we show ZnPy potently augments KCNQ1 current (EC_{50} =3.5μM), exhibiting an increase in current amplitude, reduction of inactivation, and slowing of both activation and deactivation. However, coassembly of KCNQ1 with KCNE1 completely desensitizes the ZnPy-mediated augmentation. Consistent with the observation, native I_{Ks} current displays no sensitivity to ZnPy either. Site-directed mutagenesis reveals that residues in KCNQ1 critical for ZnPy effects and KCNE1 modulation are clustered together in the S6 region. Collectively, these results revealed that S6 segment harbors a region essential for augmentation of KCNQ1 potassium channels by both chemical opener and auxiliary subunit.

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1811-Plat Structural Determinants for KCNE4 Inhibition of KCNQ1

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KCNE proteins distinctly modulate voltage-gated potassium (Kv) channels. KCNE4 has a dramatic inhibitory effect on heterologously expressed KCNQ1 that differs substantially from the functional activation of this channel mediated by KCNE1 or KCNE3. Previous work first defined three amino acid residues within the transmem-