The methods are theoretically derived and applied using the canonical system dihydrofolate reductase. Both methods demonstrate a very high predictive value ($p < 0.005$) in identifying known dynamic control sites. These tools are suitable for allosteric investigations and may greatly enhance the speed and effectiveness of other computational and experimental methods.

2854-Plat
Cyclophilin Dynamics and Catalysis are Mechanistically Linked
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Millisecond motions in the cyclophilin active site correlate with the overall enzymatic turnover, but the structural nature of the conformational change has not yet been defined. Stringent analysis of ultra-high resolution crystal structures of the free enzyme, together with NMR chemical shift information, suggested that a single side-chain may be responsible for the observed dynamics. A mutation designed to restrict this motion indeed severely reduces the rate of the active-site motions and, strikingly, catalysis by the same amount as measured by NMR relaxation dispersion experiments. The reduction in catalytic power by restricting active site motions is on the same order as mutating the active site Arg responsible for the chemical step. These results illustrate on an atomic level how both dynamics and chemistry contribute to catalytic efficiency.

2855-Plat
Single Molecule Studies Of Ubiquitin Unfolding
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Our group has recently reported the use of a specially designed single molecule nanomixer[1] to study the unfolding kinetics of a yellow fluorescent protein (YFP)[2] based on the measurements of single-pair fluorescence resonance energy transfer (spFRET), between the intrinsic chromophore and a covalently attached dye, and single-molecule fluorescence two-colour coincidence detection (TCCD). In this work we extend the application of the technology to the small and fast folding ubiquitin doubly labelled for spFRET and TCCD measurements. We firstly explored a variety of labelling strategies and fluorophores for optimal double labelling of ubiquitin, a key issue in application of single molecule fluorescence methods to protein folding/unfolding. We also added a second micropipette to the nanomixer for double-jump experiments to monitor single-molecule refolding. Using this dual labelled sample we have then studied the unfolding and refolding of ubiquitin at the single molecule level.
References:
[1] S. S. White, S. Balasubramanian, D. Klenerman, L. Ying Angew. Chem. Int. Ed. 2006, 45, 7540-7543.
[2] A. Orte, T. D. Craggs, S. S. White, S. E. Jackson, D. Klenerman. J. Am. Chem. Soc., 130 (25), 7898-7907, 2008.

2856-Plat
Simulation Of Signal Transduction In Model Multiprotein Systems
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To simulate the dynamics of multiprotein machines, I have developed a method called *multiconformer Brownian dynamics (mcBD)*. In this method, proteins rotate and translate via Brownian motion while their conformations are varied among a prestored set of structures on a simplified energy landscape, taking into account inter-protein interactions. As an example, I build a simple model of a G-protein coupled receptor/G-protein complex, and show that ligand binding causes conformational shifts, which induce GDP to leave, GTP to bind, and the complex to dissociate (Figure 1). The two proteins couple their fast fluctuations together into large-scale coordinated functional motions, resulting in signal transduction. I vary the shapes, electrostatics, and energy landscapes of the proteins independently and examine the impact this has on the system's function. In one result, increasing the binding between proteins improves the fidelity of communication, but at the expense of overall switching frequency.

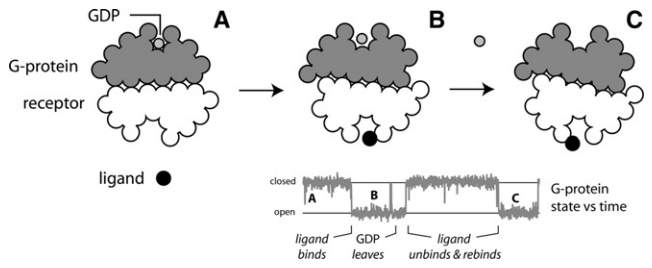

Figure 1. Ligand binding causes the receptor to switch (**A**), which induces the G-protein to adopt an open conformation (**B**), which causes GDP to be released (**C**). Then (not shown) GTP binds, reversing the conformational shift and causing the G-protein and receptor to separate from each other.

2857-Plat
Investigation of the Regulatory Mechanism of the ZAP-70 Immunological Signaling Enzyme
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ZAP-70 is a critical Syk-family protein tyrosine kinase (PTK) that functions in the initial step of T-cell receptor (TCR) signaling. Its importance is highlighted by mutations in ZAP-70 that cause severe combined immunodeficiency (SCID). Moreover, elevated levels of ZAP-70 are associated with T-cell proliferative diseases and chronic lymphocytic leukemia (CLL). Under normal conditions, T-cell activation by foreign antigen results in co-localization with ZAP-70, due to binding of its associated tandem SH2 domains (tSH2) to phosphorylated ITAM (Immunoreceptor Tyrosine Activation Motif) sequences present on the TCR ζ subunits. This leads to up-regulation of kinase activity and downstream initiation of an immune response. Experimental structures reveal that upon ITAM binding, the tSH2 must undergo a large conformational change. Of interest, therefore, is how ITAM-binding controls the tSH2 conformational equilibrium, and the subsequent proposed disassembly of the kinase domain. To this end, we have carried out 1.5 μs of molecular dynamics (MD) simulations of various ZAP-70 systems, composed of 50,000 to 70,000 atoms. These include the isolated tSH2, and variants of the ITAM-bound state. Collectively, our results suggest that one phosphotyrosine site regulates the tSH2 conformational equilibrium, whilst the other controls localization of the tSH2 to membrane-proximal ITAM targets. Using these results, along with those from targeted MD simulations, we have identified a set of order parameters that describe the tSH2 conformational transition. We have subsequently performed multidimensional potential of mean force (PMF) calculations, via umbrella-sampling, to describe the transition; a single such PMF for the tSH2 "switch" constitutes over 3 μs of simulation time. Thus, we have achieved an atomic level characterization of the free-energy landscape of the tSH2 in the presence and absence of the catalytic domain, with important consequences for the mechanism of signaling and autoinhibition in Syk-family kinases.

2858-Plat
All-atom Models For RNA And Proteins: Simulating Folding And Function
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The energy landscapes of RNA and protein folding are inextricably linked to biological function. Using All-atom structure-based models, we seek to determine the physical properties that govern folding and function of biopolymers. With these models we have studied folding, conformational rearrangements and ligand binding for a variety of protein and RNA systems. In one application of the model, we have characterized the complete folding/unfolding landscape of the SAM-1 riboswitch. Riboswitches are structured RNA fragments that alter their conformation in response to elevated concentrations of specific metabolites. To better understand the role and mechanism of ligand binding, we simulated the RNA in a bath of ligands. Key findings include the identification of the rate limiting step in SAM-1 folding and that ligand binding specifically targets this step. These results illustrate the close link between folding and function in this riboswitch. For protein function, results from Adenylate Kinase will be presented. Adenylate Kinase is an enzyme that undergoes large scale conformational rearrangements during catalysis. Using our All-atom models we have been able to quantify the free energy barriers associated with these rearrangements and identify key structural components that regulate these motions.

Platform AY: Emerging Single Molecule Techniques II

2859-Plat
Mechanical characterization of Protein L in the low-force regime by electromagnetic tweezers/evanescent nanometry
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Mechanical manipulation at the single molecule level of proteins exhibiting mechanical stability poses a technical challenge that has been almost exclusively approached by atomic force microscopy (AFM) techniques. However, due to mechanical drift limitations, AFM techniques are restricted to experimental recordings that last less than a minute in the high-force regime. Here we demonstrate a novel combination of electromagnetic tweezers and evanescent nanometry that readily captures the forced unfolding trajectories of protein L at pulling forces as low as 10~15 pN. Using this approach, we monitor unfolding and refolding cycles of the same polyprotein for a period of time longer than 30 minutes. From such long lasting recordings, we obtain ensemble averages of unfolding step sizes and rates that are consistent with single molecule AFM data obtained at higher stretching forces. The unfolding kinetics of protein L at low stretching forces confirms and extends the observations that the mechanical unfolding rate is exponentially dependent on the pulling force within a wide range of stretching forces spanning from 13 pN up to 120 pN, thereby excluding the presence of curvature in the rate-versus-force plot. Our experiments demonstrate a novel approach for the mechanical manipulation of single proteins for extended periods of time in the low-force regime, providing an ideal complement to force-clamp AFM, expanding the accessible regions of the unfolding energy landscape of a mechanically stable protein.

2860-Plat
Magnetic Tweezers Measurement of Single Molecule Torque
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Helical duplex DNA continually experiences torque from translocating macromolecular complexes and helix unwinding proteins. We have developed a magnetic tweezers methodology using a cylindrical magnet and magnetic nanorods to directly measure torsional stress, or resistive torque, as twists are introduced at low pulling forces. We demonstrate the utility of this method by measuring the resistive torque of single DNA molecules and, for the first time, single chromatin fibers.
Figure: New and conventional magnetic tweezers configurations. (a) In conventional magnetic tweezers, the field orients the induced dipole of the superparamagnetic bead horizontally, producing a strong horizontal angular trap that prevents angular fluctuations of the probe. (b) In the new configuration consisting of a vertical magnetic field and nanorod-bead construct, the magnetic field and the probe dipole align vertically, thus horizontal angular movements are not constrained. A weak horizontal force generates a weak horizontal angular trap allowing us to measure the torque applied to the molecule. (c) Scanning electron micrograph of magnetic Ni-Pt nanorods (bar = 1μm). (d) Bright-field image of nanorod-bead probe. Nanorod and bead self-assemble by magnetic attraction.

2861-Plat
A Novel Way To Combine Magnetic Tweezers and Fluorescence Microscopy For Single Molecule Studies
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Single-molecule experiments make it possible to look at and manipulate individual molecular systems in real time and do not suffer from averaging over a large number of unsynchronised events. In magnetic tweezers, one traps a single DNA molecule between a glass surface and a magnetic bead, and exerts controlled force and topological constraint on the double helix. However, visualization of events occurring along the molecule is made difficult by the geometry of the device, in which the trapped molecule is pulled perpendicular to the observation plane. We developed a new generation of magnetic tweezers that addresses this problem, while keeping the mechanical control of the trapped molecule. The DNA-bead system is injected into a microfluidic channel placed on a home-made epifluorescence microscope. The channel's top surface is coated with a reflective metallic layer and is tilted so that the whole length of the stretched tether's reflection will be located at the focus of the objective. We applied this instrument to the study of homologous recombination, a DNA double-strand break repair pathway playing an essential role in vivo in genome maintenance and replication. The functional form of the central protein in this process, RecA in bacteria and Rad51 in eukaryotes, is a nucleoprotein complex in which protein monomers assemble into a helical filament on single-stranded DNA. This filament promotes homologous pairing and strand exchange with duplex DNA. Despite the extensive literature on homologous recombination, several key aspects of the detailed molecular mechanism remain quite controversial, especially concerning how the nucleoprotein filament interacts with homologous DNA and exchanges strands. For the first time, our instrument allowed us to observe individual nucleoprotein filaments, labeled with quantum dots, interacting with a double-stranded DNA molecule held by the tweezers.

2862-Plat
First-principles Calculation Of DNA Looping In Tethered Particle Experiments
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We show how to calculate the probability of DNA loop formation mediated by regulatory proteins such as Lac repressor, using a mathematical model of DNA elasticity. Our approach has new features enabling us to compute quantities directly observable in Tethered Particle Motion (TPM) experiments; e.g. it accounts for all the entropic forces present in such experiments. Our model has no free parameters; it characterizes DNA elasticity using information obtained in other kinds of experiments. It can compute both the ''looping J factor'' (or equivalently, looping free energy) for various DNA construct geometries and repressor concentrations, as well as the detailed probability density function of bead excursions. We also show how to extract the same quantities from recent experimental data on tethered particle motion, and compare to our model's predictions. In particular, we present a new method to correct observed data for finite camera shutter time.
The model successfully reproduces the detailed distributions of bead excursion, including their surprising three-peak structure, without any fit parameters and without invoking any alternative conformation of the repressor tetramer. However, for short DNA loops (around 95 bp) the experiments show more looping than is predicted by the linear-elasticity model, echoing other recent experimental results. Because the experiments we study are done in vitro, this anomalously high looping cannot be rationalized as resulting from the presence of DNA-bending proteins or other cellular machinery. We also show that it is unlikely to be the result of a hypothetical ''open'' conformation of the repressor.
Ref: KB Towles et al, accepted for publication in Physical Biology.

2863-Plat
Single Molecule Investigations of HSP70 Proteins
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Heat Shock Proteins 70 (HSP70) are a class of proteins involved in protein folding and are highly upregulated when a cell is under stress. The protein consists of three functional domains: the N-terminal ATP binding domain where ATP and ADP can bind, the substrate binding domain which can bind small residues and the C-terminal domains which acts as a lid for the substrate binding domain. It is thought that the binding of ATP/ADP drives conformational changes in this protein.
Using burst analysis with pulsed interleaved excitation, we have performed single-pair Förster resonance energy transfer (FRET) experiments to investigated the distribution of conformations in HSP70 molecules under different conditions. The FRET efficiency is very sensitive to the distance between donor and acceptor on the scale of 2-10 nm and thus provides information over the conformation of the different HSP70 domains as they diffusion through the focus of our confocal microscope. As experiments are performed on single molecules, subpopulations can be directly observed. We have investigated the conformation of HSP70 by cloning a number of mutants that allowed specific labeling of the different domains. Experiments were performed with or without ATP, ADP, and nonhydrolysable ATP analogs as well as in the absence or presence of peptide substrates or cochaperons. We will present how the conformation and flexibility of HSP70 is influenced by these various interactions.

2864-Plat
Single-molecule Observations of Replisome Structure and Function
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DNA replication requires the coordinated activity of a large number of enzymes at the replication fork. Understanding the mechanisms controlling this organization requires a direct probing of the dynamics of fully functional replisomes during replication. Observations at the single-molecule level provide the most direct way to visualize the complex biochemistry of the replisome and to quantify the many transient intermediates essential to replication. We present a novel assay that combines the observation of individual fluorescently labeled proteins with the mechanical manipulation of DNA. Surface-tethered DNAs labeled