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
Michael W. Clarkson[1], James Fraser[2], Thomas Alber[2], Dorothee Kern[1].
[1]Brandeis University, Waltham, MA, USA, [2]University of California - Berkeley, Berkeley, CA, USA.
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
Shehu Ibrahim, Georg Blaser, Angel Orte, Sophie Jackson, David Klenerman.
Cambridge University, Cambridge, United Kingdom.
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
Julius T. Su.
Caltech, Pasadena, CA, USA.
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.

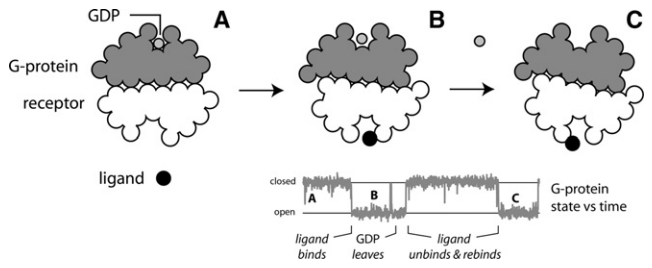

Figure 1. Ligand binding causes the receptor to switch (**A**), which induces the G-protein to adopt an open conformation (**B**), which causes GDP to be released (**C**). Then (not shown) GTP binds, reversing the conformational shift and causing the G-protein and receptor to separate from each other.

2857-Plat
Investigation of the Regulatory Mechanism of the ZAP-70 Immunological Signaling Enzyme
Peter J. Bond, Josè D. Faraldo-Gómez.
Max Planck Institute of Biophysics, Frankfurt am Main, Germany.
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
Paul C. Whitford[1], Alexander Schug[1], Jeffrey K. Noel[1], Shachi Gosavi[1], Scott P. Hennelly[2], Kevin Y. Sanbonmatsu[2], Jose' N. Onuchic[1].
[1]UC San Diego, San Diego, CA, USA, [2]Los Alamos National Laboratories, Los Alamos, NM, USA.
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
Ruchuan Liu[1,2], Sergi Garcia-Manyes[2], Atom Sarkar[3], Carmen L. Badilla[2], Julio M. Fernandez[2].
[1]Natiaonal University of Singapore, Singapore, Singapore, [2]Columbia University, New York, NY, USA, [3]Ohio State University, Columbus, OH, USA.