range of members of the myosin family. In recent work, we have combined this approach with the use of small molecule effectors of myosin motor activity. We identified pentabromopseudilin (PBP) and related halogenated alkaloids as potent inhibitors of myosin-dependent processes such as isometric tension development, unloaded shortening velocity, and in vitro motility. Coupling between the actin and nucleotide binding sites is reduced in the presence of these inhibitors. PBP-induced changes in rate constants for ATP-binding, ATP-hydrolysis and ADP dissociation extend the time required per actin-activated myosin ATPase cycle. Additionally, the ratio of time spent per ATPase cycle in strong and weak binding states is shifted by PBP and related compounds in favor of non-force generating states. To elucidate the binding mode of these compounds, we crystallized their complexes formed with the Dictyostelium myosin-2 motor domain. In every case, the electron density for the small molecule inhibitor is unambiguous. All compounds bind to a novel allosteric site near actin-binding residues at the tip of the 50-kDa domain. The residues involved in the binding of this new class of inhibitors are only moderately conserved between the members of the different myosin classes. This is consistent with the observed differences in IC50 values. The results of molecular modeling studies show that these isoform-specific variations in the extent of inhibition can be predicted at least in trend for each of the new compounds.

2849-Symp
The collective mechanics of myosin in muscle
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2850-Symp
An Integrative Analysis of the Muscle Myosin Motor Using Genetic and Transgenic Tools
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We use *Drosophila melanogaster* to investigate the mechanisms by which myosin heavy chain isoforms contribute to muscle-specific cyto-architectures and contractile properties. We expressed various myosin isoforms in transgenic indirect flight muscles. Along with our collaborators, we examined 1) ATPase, *in vitro* actin sliding, step size and structure of the isolated myosin, 2) ultrastructure and mechanical properties of muscle fibers, and 3) locomotory abilities of the transgenic organisms. We found that isoform-specific differences in myosin cause relatively small structural variations in muscle assembly, but are critical to myofibril stability and function. ATPase, *in vitro* motility and fiber mechanical assays show that embryonic and indirect flight muscle isoforms represent slower and faster myosins, respectively. We have used a series of chimeric transgenes to study the function of the alternative domains that vary among the *Drosophila* myosins. We found that the converter is a key determinant of isoform-specific properties, and that some mechanisms of fine tuning myosin function differ from those of vertebrates.
Drosophila point mutants also help define the function of structural domains of myosin. We analyzed point mutations in the transducer domain near the nucleotide-binding pocket. While increased myosin ATPase and actin sliding ability of one transducer mutation induce dramatic degeneration of the indirect flight muscles, another mutation that reduces these properties does not affect myofibril stability. The effects of these mutations on the *Drosophila* heart are disparate as well, with increased function yielding restrictive cardiomyopathy and decreased function leading to dilated cardiomyopathy. These phenotypes parallel those arising from myosin mutations in humans. *Drosophila* may thus serve as a useful model for studying the molecular basis of cardiomyopathy, as well as mechanisms of its suppression.

Platform AX: Protein Dynamics II

2851-Plat
Slow Correlated Movement of Structural Elements in Hemoglobin and Myoglobin
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Structural fluctuations of proteins in solution are often reflections of functional movements and appear to follow low energy pathways that have evolved to accommodate conformational changes triggered, for instance, by binding to ligands or catalysis of reactions. The study of these fluctuations can provide substantial insight into protein function. The spatial extent and dynamics of intramolecular movements in proteins are highly dependent on the environment of the protein, being particularly sensitive to the concentration of proteins and other constituents in the solution. Consequently, studies as a function of protein concentration may provide further insight into the nature of these fluctuations than is possible when experiments are carried out at a single protein concentration. Wide-angle x-ray solution scattering (WAXS) and neutron spin echo spectroscopy (NSE) provide substantial information about the spatial extent and time scale, respectively, of structural fluctuations in solution. They are particularly well suited to the study of slow, correlated movements that are central to many protein functions, providing information complementary to that obtainable by x-ray crystallography and NMR. Using these approaches, we have characterized the spatial and temporal properties of hemoglobin and myoglobin across a wide range of environments. For these proteins, the spatial extent and dynamics of correlated movements both increase rapidly as protein concentration decreases below about 50 mg/ml. A combined analysis of WAXS and NSE data indicates that the slow correlated fluctuations in these molecules are dominated by movement of relatively rigid alpha helices within the subunits. These fluctuations are highly dependent on the ligation state of the molecules and are altered by mutations that impact the function of the molecules.

2852-Plat
Method to Characterize the Global Dynamics of Proteins by Temperature Dependent Phosphorescence Lifetime Measurements. Application for the Allosteric Effect in Hemoglobin
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At the glass transition temperature of proteins, large scale, anharmonic motions of the conformation become activated. It has been shown that in this temperature range the phosphorescence lifetime of Trp residues that is long (several s) at cryogenic temperatures become efficiently quenched. We report the elaboration of a systematic method based on this observation with the purpose to use this effect for characterizing the global conformational dynamics of proteins. In this method, special conditions were determined under which the onset temperature of phosphorescence quenching can be clearly separated from the slaving effect of fluctuations activated in the solvent, and the relevance of this temperature for the global dynamics of the protein has been verified by parallel experiments on human alpha-oxyFe-betaZn-Hemoglobin, w.t. horse heart Myoglobin and Trp mutant forms of a two-domain protein, yeast phosphoglycerate kinase. The method was used to characterize the role of global dynamics in the allosteric response of human hemoglobin to the binding of effectors as Cl, IHP, DPG, BZF. The quenching effect showed a two state behavior with a characteristic onset temperature sensitive to the presence and quality of allosteric effectors. The data points were fit by a simple two state model and the thermodynamic parameters - enthalpy and entropy changes characteristic for the quenching effect were discussed in terms of oxygen hopping models. The study on hemoglobin verifies the concept that in allosteric regulation, the global dynamics of the protein tetramer plays important role.
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2853-Plat
Computational Methods for Predicting Sites of Functionally Important Dynamics
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Understanding and controlling biological function of proteins at the atomic level is of great importance; allosteric mechanisms provide such an interface. Experimental and computational methods have been developed to search for residue mutations that produce changes in function by altering sites of correlated motion. These methods are often observational in that altered motions are achieved by random sampling without revealing the underlying mechanism(s). We present two deterministic methods founded on structure-function relationships that predict dynamic control sites (i.e. locations that experience correlated motions as a result of altered dynamics).
The first method (''static'') is based on a single structure conformation (e.g. the wild type (WT)) and utilizes a graph description of atomic connectivity. The local atomic interactions are used to compute the propagation of contact paths. This description of structure connectivity reveals flexible locations that are susceptible to altered dynamics.
The second method (''dynamic'') is a comparative analysis between the normal modes of a WT structure and a mutant structure. A mapping function is defined that quantifies the significance of the motions in one structure projected onto the motions of the other. Each mode is considered up- or down-regulated according to its change in relative significance. This description of altered dynamics is the basis for a motion correlation analysis, from which the dynamic control sites are readily identified.

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:
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[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.

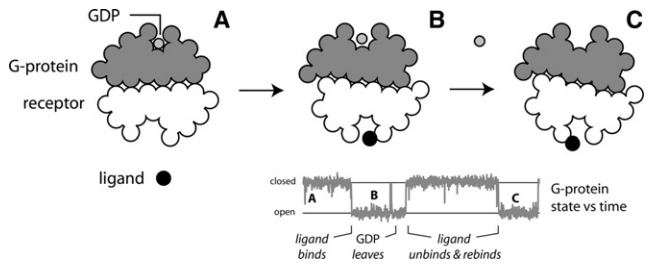

Figure 1. Ligand binding causes the receptor to switch (**A**), which induces the G-protein to adopt an open conformation (**B**), which causes GDP to be released (**C**). Then (not shown) GTP binds, reversing the conformational shift and causing the G-protein and receptor to separate from each other.

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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