C98S) in a cardiac TnI core structure (McTnI-ND_{29}-Cys) did not affect the COOH-terminal conformation of TnI and preserved binding to TnT and TnC. McTnI-ND_{29}-Cys purified from bacterial culture was fluorescently labeled with the Alexa Fluor 532 dye and used to reconstitute troponin complex. After verifying the ratio of fluorophore to protein conjugation by spectrophotometer and SDS-PAGE, Ca^{2+}-titrations were performed for fluorescence intensity and polarization changes. The results demonstrated Ca^{2+} regulated conformational/environmental changes as well as flexibility change in the COOH terminus of TnI. Further experiments are performed to measure the Ca^{2+}-induced structural changes in reconstituted myofilaments to understand the function of TnI COOH terminal domain in calcium-regulation of muscle contraction.

1936-Plat
Cardiomyopathy Causing Mutations Stabilize an Intermediate State of Thin Filaments
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Congenital cardiomyopathies are initiated by changes in ATP hydrolysis and result in hypertrophy, fibrosis, and myofibrillar disarray. We studied the mechanism by which mutations in troponin and tropomyosin change ATPase rates and have linked several mutants to inappropriate switching between the inactive and active states of the actin thin filament. We have shown that troponin I mutants mimicking protein kinase C phosphorylation stabilize the inactive state of actin filaments whereas the Δ14 TnT mutant stabilizes the active state. We have now shown that two mutations on troponin I, R146G and R146W, which cause cardiomyopathy produce complex effects on ATPase activity. These TnI mutations produced increased ATPase rates in the absence of calcium and decreased rates in the presence of calcium compared to wild type. These differences were maintained at high actin concentrations. Saturating concentrations of the activator NEM-S1 equalized the rates of both the mutants and wild type. The NEM-S1 data rule out alterations in rate constants of transitions (i.e. product release) along the active pathway. The results from the R146G and R146W mutants have implications for the function of the 3 structural states of regulated actin that have been observed. That is, the results can be explained most readily if the mutants stabilize an intermediate state in both calcium and EGTA with an activity between that of the inactive and active states. In the past we have assumed that the intermediate state had properties identical to the inactive state. Our current data show that while the intermediate more closely resembles the inactive state it has unique properties. Our present results, as well as previous results, indicate that inappropriate stabilization of any state of regulated actin can result in cardiac dysfunction.

Platform AI: Protein Dynamics I

1937-Plat
Probing Conformational Motion of Serpin by Time-Resolved and Single Molecule Fluorescence
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Serpin (serine protease inhibitor) is a structural prototype for the study of the molecular mechanism of many diseases due to the conformational instability which leads to protein aggregation. The inhibitory function of serpin relys on a flexible loop undertaking a striking conformational transition, but this property also leaves serpin at risk of polymerization. We have investigated the conformational dynamics of the reaction center loop (RCL) of the plasminogen activator inhibitor-1 (PAI-1) by time resolved fluorescence spectroscopy. The RCL becomes more solvent exposed and exhibits faster rotation when PAI-1 interacts with an octapeptide which blocks the loop insertion pathway, indicating that the RCL is well displaced from the protein surface. A heterogeneous population model with three rotational correlation times has been developed to account for the ''dip and rise'' observed in fluorescence anisotropy decays. We have also employed single molecule FRET to probe the conformational change of serpin under different environment and the early stage of its ploymerization process. Preliminary results will be presented.

1938-Plat
Evolution of enzyme fold: Linking protein dynamics and catalysis
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Enzymes are dynamic molecules. In the past, enzymes have been viewed as static entities and their high catalytic power has been explained on the basis of direct structural interactions between the enzyme and the substrate. Recent evidence has linked protein dynamics to catalytic efficiency of enzymes. Further, motions in hydration-shell/bulk solvent have been shown to impact protein motions, therefore, function.
Theoretical and computational studies of protein dynamics linked to enzyme catalysis will be discussed. Investigations of cyclophilin A and dihydrofolate reductase have lead to the discovery of networks of protein vibrations promoting catalysis. Results indicate that the reaction promoting dynamics in these enzymes is conserved across several species. Moreover, we have characterized the protein dynamics of a diverse super-family of dinucleotide binding enzymes. These enzymes share very low sequence similarity and have different structural features. The results show that the reaction promoting dynamics is remarkably similar in this enzyme super-family.

1939-Plat
Function And Activity Of Von Willebrand Factor Is Regulated By A Hierarchy Of Mechanical Forces
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The von Willebrand factor (VWF) is a shear-flow sensitive multimeric protein. Under normal flow conditions VWF is in a globular state, it unfolds at high shear rates and is activated for adhesion at the blood vessel wall [Schneider et al. 2007 PNAS p7899]. The elongation of multimeric VWF results in a force pulling along the VWF length axis. Based on a model of the VWF A domain organization, we performed force probe molecular dynamics simulations. We reveal the basis of two force-sensing VWF functions, and test the results by experiments. Our results indicate a competition between VWF A2 domain and glycoprotein Ib (GPIb) for the same binding site of the VWF A1 domain. When the stretching force along VWF reaches a critical point, the A1 A2 interaction is lost. The domains remain connected by a linker that gives space for GPIb to bind to the A1 domain. We thus suggest a force-dependent platelet binding to VWF as mediated by GPIb, which is experimentally testable and represents an alternative mechanism to recently published studies [Chen et al. 2008 Biophys J p1303; Lou et al. 2008 PNAS p13847].
We show how proteolysis of the VWF is activated under shear conditions. The specific proteolytic site is buried in the VWF A2 domain [Sutherland et al. 2004 J Mol Model p259]. At extreme forces as present in high molecular weight VWF multimers, the A2 domain C terminus unfolds until the ADAMTS13 cleavage site is uncovered. Introducing a disulfide bond by mutagensis prevents VWF cleavage. This explains the size regulation of VWF by ADAMTS13: larger multimers involve higher pulling forces and therefore higher unfolding rates under shear flow. Larger VWF is cleaved faster, preventing blood clots and thrombosis [in preparation].

1940-Plat
Experimental Confirmation of an NtrC Transition Pathway Predicted by Targeted MD
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The infinitely short lifetime of transition states makes characterization extremely difficult. We have used a combination of molecular dynamics and experimental approaches to determine two important rate-limiting interactions involved in the allosteric transition of a signaling protein. Targeted MD simulations of the receiver domain of NtrC (Nitrogen Regulatory Protein C) were used to predict interactions that are important in stabilizing the transition state between the known inactive and active structures of the protein. Mutations were made to test these predictions and the rate of exchange between the two substates were measured by 15N-CPMG relaxation dispersion experiments. The results verify the importance of these key interactions in the transition pathway of NtrC. This work shows that targeted molecular dynamics together with experimental validation can be an invaluable tool at elucidating the structure and rate-limiting interactions of conformational transitions.

1941-Plat
Real-time 3D Tracking of Structural Transitions in Adenylate Kinase by Thermal Noise Imaging
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Proteins have to be flexible enough to support turn-over rates up to hundreds per second, yet stable enough to maintain their three-dimensional structure over hours and days. As result of thermal excitation they fluctuate between structural conformations. We measured thermally excited structural fluctuations in the Adenylate Kinase using a site-specifically attached nanoparticle