2288-Pos
Obtaining Functionally Relevant Protein Structural Transitions Using a Combined Physics/Structure-Based Coarse-Grained Model
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The function of proteins is intimately tied to their dynamics and their ability to undergo structural transitions. These transitions are rare events, difficult to model computationally. Recently, we have developed ELNEDIN, a modeling approach that combines an elastic network with a physics based coarse-grained force field. We have shown that ELNEDIN can reproduce reliably the equilibrium dynamics of proteins. Here evaluate the ability of ELNEDIN to identify directions of conformational change that are functionally relevant, and to produce fully reversible transitions during the course of a simulation. To this end we selected a set of 15 protein systems known to exist in at least two conformational states (open/closed). ELNEDIN models of the open and closed state were simulated via molecular dynamics. The directions of the low frequency motions extracted from the trajectories were compared to the direction of the experimentally known conformational change. The main findings of these computational experiments are that: (1) ELNEDIN models based on the open(unliganded/apo) state of a protein system are more likely to identify correctly the direction of conformational change induced by the binding of the ligand than models based on the holo (liganded) state; (2) the degree of collectivity of the functional transition is the single most important predictor of the ability to identify computationally the direction of conformational change; (3) this is best achieved when the functional transition is of the hinge type, followed by shear type and worst for the unclassified type; (4) finally, in some cases, e.g. HIV-1 protease, fully reversible structural transitions can be observed during the course of a single simulation. These findings suggest that ELNEDIN models could be used towards understanding of the mechanistic processes associated with ligand-binding by allowing real-time simulations of such events.

2289-Pos
Mechanical Response of the Coiled Coil
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First discovered by Crick, coiled coils are a prevalent ropelike protein motif formed by two or more α-helices. Coiled coils are found in many functionally diverse protein complexes, some of which are involved in gene regulation, muscle contraction and cell signaling. Since they undergo functional bending, twisting, buckling and stretching motions, understanding the mechanical response of coiled coils is crucial for describing the conformational states of these proteins. The energetic of a coiled coil involves a competition between elastic deformation and hydrophobic interaction of residues of each helix. In this work, we present an energetic and geometric investigation of coiled coils using a coarse-grained elastic model. In this model, we treat α-helices as elastic rods where each rod interacts with another exclusively through beads representing the hydrophobic residues. One interesting result is that our model estimates the persistence length of a coiled coil dimer as 165 nm which is less than twice the persistence length of a single α-helix. We have validated our results using steered molecular dynamics simulations and we discuss our results and possible applications of the model to higher level complexes.

2290-Pos
Solution Dynamics of Monoclonal Antibodies: Experimental and Computational Approach
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Recombinant humanized monoclonal IgG1 antibodies are a major class of protein therapeutics. The efficacy of these antibodies may be attributed to solution dynamics of these proteins. Antibodies have three major domains with two Fab domains and an Fc domain. The Fc domain is connected to the Fab domain via a single peptide linker (hinge). It has been demonstrated previously that hinge region provides certain degree of flexibility to the antibody molecule. We are using time-resolved fluorescence anisotropy in conjunction with molecular dynamics simulations to map the different motions around the hinge region. In the experimental part of this study we conjugate the engineered cysteines on the antibody molecule to the fluorescence probes to extract two complementary types of information: a. rotational correlation times of different parts of the molecule (Fab and Fc regions) using time-correlated fluorescence anisotropy; b. distance distribution between Fab arms of the antibody by measuring FRET between the donor and acceptor fluorescence probes on the Fab domains of the same antibody molecule. In the computational part of this study we employ both coarse-grained dynamics modeling and all-atom molecular dynamics simulations to characterize the molecular motions of the antibody, including the motions around the hinge region. In addition, we apply mutual information analysis to the results of the simulations to characterize the correlation of motions between different antibody domains.
These tools will allow us to characterize the changes in solution dynamics of the antibodies as a result of storage conditions, different concentrations and interactions with excipients and other proteins.

2291-Pos
Low Frequency Motions of a Carboxylesterase and their Relation to Substrate Selectivity and Catalytic Activity
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Carboxylesterase (CEs) are ubiquitous enzymes responsible for the detoxification of xenobiotics. CEs can metabolize and hydrolyze a variety of esterified drugs, including the anticancer agent CPT-11. The specificity of CEs for a particular substrate or inhibitor depends on the enzyme's molecular structure and the dynamics of conformational substructures when a substrate is bound. We have used computational techniques to understand differences in substrate selectivity of CEs. First, we used 10ns molecular dynamics simulations (MD) to identify the loop region of high fluctuation in a CE from *B. subtilis* - pnbCE. Then we used normal mode analysis to find the lowest frequency mode which represented the largest global motion of pnbCE. Both computational methods were able to identify these two flexible loop regions. Our hypothesis is that the molecular dynamics of this loop region is correlated with substrate conversion efficiency for selected CEs. These experiments provide the first data toward testing this hypothesis.

2292-Pos
Solvent Molecule Bondability and Effect on Mechanical Proteins Transition State and Stability - A Smd Study
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The solvent environment plays an integral role in physiological processes in the living cell. Changes in solvent environment or properties are a chemical signal that may induce changes in the mechanical response in a protein system. In this steered molecular dynamics study, using simulations we stretch to unfold the mechanical protein titin in aqueous and non-aqueous environment and reveal the atomic details and mechanism of interactions between solvent and proteins when subjected to steering force. Titin is a mechanically stable protein which is able to resist force due to a force bearing topology element - antiparallel beta sheet, stabilized by 6 native hydrogen bond contacts. In our study we observe individual solvent molecules bridging the stabilizing native hydrogen bonds in the force bearing patch. Solvent molecules also modulate the distance to the transition state. We investigate the distance to transition state of the unfolding reaction as related to solvent molecule size and we also introduce the concept of solvent molecule bondability - the capability of a solvent molecule to bridge the native hydrogen bond contact in more than one way, as determined by solvent molecule polarity and topology. Features of the simulations were also matched with previously reported experimental results.
Since The distance to transition state determines the mechanical stability of a protein, changing the solvent composition is a novel way to fine tune mechanical properties. Our investigation provides insights of the properties of the unfolding reaction pathway and possible mechanisms of mechanical protection when such proteins are subject to mechanical stress.

2293-Pos
The Biological Channelling of a Reactive Intermediate
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The channelling of intermediates through buried molecular channels in multienzyme subunits is a topic of considerable interest as it is thought to occur in many different enzymes. This process effectively shields reactive or poisonous intermediates from the rest of the organism and ensures direct, rapid transport from one active site to the other. Many of these molecular channels have been identified using techniques such as X-ray crystallography, but little is known about the internal mechanisms they use to transport their intermediates. In this study, various computational methods were applied to investigate the proposed channelling activity of the bifunctional enzyme 4-hydroxy-2-ketovalerate aldolase-aldehyde dehydrogenase (acylating) (DmpFG). This enzyme breaks down its substrate (4-hydroxy-2-ketovalerate) into pyruvate and acetyl-CoA in two steps that occur at two different locations, or active sites, within the protein. The intermediate acetaldehyde formed in the first active site of DmpFG is toxic to the bacteria and release into the bulk media would not be advantageous to the organism. Instead, it has been hypothesised