

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**Ekaterine Kortkhonjia¹, Relly Brandman², Ilya Chorny², Julian Kissman¹, Tom Patapoff¹, Bruce Kabakoff¹, Ken Dill², Trevor Swartz¹.¹Genentech, South San Francisco, CA, USA, ²Simpota, San Francisco, CA, USA.

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**Xiaozhen Yu¹, Monika Wierdl², Philip Potter², Randy Wadkins¹.¹University of Mississippi, University, MS, USA, ²St. Jude Children's Research Hospital, Memphis, TN, USA.

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 multi-enzyme 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-ketovaleate aldolase-aldehyde dehydrogenase (acylating) (DmpFG). This enzyme breaks down its substrate (4-hydroxy-2-ketovaleate) 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

that acetaldehyde moves directly from one active site to the other using a 29 Å channel identified in previous crystallographic studies. The aim of this study is to determine the energetic feasibility of the channelling event and to verify the roles of the proposed checkpoints at the entry (Tyr-291) and exit (three hydrophobic residues called the 'hydrophobic triad') of the channel which were also identified previously. Here we show our progress towards a complete mapping of the free energy surface for the passage of acetaldehyde through the channel. The data to date suggest that it is energetically feasible, and in fact highly probable, that acetaldehyde moves through the protein in this manner; however, further simulations are required to verify this.

2294-Pos

Dynamics of the PKA C-Subunit Major Conformational States Using REXAMD

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Molecular dynamics simulations can yield insight into the role of protein dynamics in allostery and binding cooperativity. Recent work by Masterson et al. (reference 1) on protein kinase A (PKA) has shown that the binding of ATP and the substrate-like inhibitor PKI to the catalytic sub-unit is highly cooperative. Various biochemical techniques identified specific residues important to the allosteric network. We present replica exchange accelerated molecular dynamics (REXAMD) simulations of the different states of the PKA catalytic sub-unit, linking the protein dynamics with the known cooperativity. References:

1) Masterson, L. R.; Mascioni, A.; Traaseth, N. J.; Taylor, S. S.; Veglia, G. Proceedings of the National Academy of Sciences 2008, 105, 506-511.

2295-Pos

Dynamic Peptide Folding and Assembly for DNA Separations

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The phenomenon of counterion-mediated DNA-condensation is fundamental to most DNA related activity in the cell, from chromosome packaging to control over translational mechanisms. Developing synthetic systems to manipulate DNA-condensation is essential for the development of biotechnologies for gene encapsulation and DNA-separation. We investigate the dynamics of the DNA-condensation by using our model peptide where the interaction between DNA and peptide is non-specific. We have designed a peptide that shows switchable surface activity, where the folded form of the peptide is amphiphilic and the unfolded form is not amphiphilic. The peptide is α -helical, containing 23 amino acids with variation in the number and distribution of hydrophobic and charged amino acids. The designs incorporate hydrophobic residues on one side (leucines and alanines) and hydrophilic residues on the opposite side so that helix is surface active. The secondary structure has been characterized by using circular dichroism spectropolarimetry, and we show that the peptide has a transient secondary structure as a function of monovalent salt concentration. The behavior of the peptide at air-water interface is characterized by pendant drop/bubble method and modeled accordingly. Our hypothesis is that the unfolded peptide is in equilibrium with the folded peptide in the bulk solution but in presence of DNA, the unfolded peptide folds and then binds to DNA. Critical Aggregate concentration of the peptide for DNA condensation is determined by using multi angle light scattering, which is also used to calculate the radius of gyration and molecular weight of these condensates. We investigate the kinetics of the condensation process by using Circular dichroism in Stop-flow mode and also by isothermal titration calorimetry.

2296-Pos

Mechanism of DNA Recognition by EcoRV

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The restriction enzyme EcoRV destroys invading foreign DNA by inducing a sharp kink of 50 degrees and cleaving it at the center step of a GATATC sequence. Its own DNA is methylated in the first adenine, GA_{CH}3TATC, and is not cleaved by EcoRV. We report here on molecular dynamics simulations of the interaction of EcoRV with three DNA sequences: the cognate sequence, GATATC, the cognate methylated sequence, GA_{CH}3TATC and the non-cognate sequence, GAATTC, not cleaved by EcoRV. Simulations of the three DNA sequences unbound and bound to EcoRV and of unbound EcoRV are performed, to understand the recognition-cleavage process. The results suggest a three-step recognition mechanism: first, EcoRV is in an open state, ready

to bind to the DNA. When bound, EcoRV makes loose contacts with any DNA sequence. Then in a third step, taking place only for the cognate sequence, the DNA is kinked and bound deep enough in the protein to allow cleavage. This step is determined by an intrinsically higher flexibility of the cognate sequence and the formation of stronger hydrogen-bond interactions between DNA and protein than for the other two non-cleaved sequences. A crucial role of Asn185, forming hydrogen bonds with the first adenine of the recognition sequence, GATATC, could be determined from our simulations. In the EcoRV-methyl-DNA complex, as well as in the complex of a N185A mutant with the cognate sequence (TA), the formation of a hydrogen bond between Asn185 and the adenine is prohibited. The formation of a tight EcoRV-DNA complex is thus impossible and the energy gained upon complex formation becomes insufficient to kink the DNA despite its intrinsic flexibility. These findings elucidate in atomic detail the interplay between specific binding interactions in the complex and intrinsic properties of the DNA in the recognition process.

2297-Pos

Conformational Transitions Associated with Different Redox States of Di-Thiol Pairs

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Protein redox regulation is of growing interest because of its relevance to neurodegenerative diseases, cancer, diabetes and heart disease. Redox-active disulfides are best known for their catalytic functions but are increasingly being recognized for their roles in regulation of protein function.

Redox-active disulfides are, by their very nature, more susceptible to reduction than structural disulfides; and conversely, the Cys pairs that form them are more susceptible to oxidation. In this study, we searched for potentially redox-active Cys Pairs by mining structures of proteins in alternate redox states from the Protein Data Bank. Over 1,134 unique redox pairs of proteins were found, many of which exhibit conformational differences between alternate redox states. Our study is the first to systematically study these conformational changes. Several classes of structural changes were observed, proteins that exhibit: disulfide oxidation following expulsion of metals such as Zn; order/disorder transitions; changes in quaternary structure and major reorganisation of the polypeptide backbone in association with disulfide redox-activity. This latter group, also known as "morphing" proteins, challenge Anfinsen's thesis of a one-to-one mapping of sequence to structure, also known as the thermodynamic hypothesis. Our study shows the conformational state of morphing proteins can be influenced by redox conditions.

2298-Pos

Electrostatics of the Protein-Water Interface

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Electrostatic fluctuations within proteins are critical to their biological activity as carriers in electron transport chains typically requiring a significant number of single-electron hops. This mechanistic requirement poses the question of how a sufficient energetic efficiency is achieved. We present the results of numerical simulations of the statistics of electrostatic fluctuations at the protein/water interface. The statistics of the electrostatic potential fluctuations inside the protein is strongly non-Gaussian at high temperatures, but becomes consistent with the linear/Gaussian response below the temperature of the dynamical transition in proteins. At high temperatures the large, non-Gaussian electrostatic noise allows higher efficiency of electron transport chains which can be magnified by an order of magnitude compared to the predictions of Gaussian models. The appearance of non-Gaussian statistics is traced back to a significant polarization of the protein-water interface which slows its relaxation at lower temperatures and becomes kinetically frozen below the temperature of dynamical transition.

2299-Pos

Molecular Dynamics Simulations of Alpha-Synuclein at Various Temperatures

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Alpha-synuclein (α S) is a natively unfolded protein with a C-terminus that is enriched with acidic residues. Three independent mutants (i.e., A30P, A53T and E46K) were identified in the genetic study of familial Parkinson's disease. Wild-type α S has been shown to possess a consistent secondary structure composition (Thomas D. Kim et al. 2000) at various temperatures. Other studies indicate that the acidic tail of α S plays an important role in preventing the protein's aggregation (Sang Myun Park et al. 2002). Here we show that both the wild-type and mutated proteins have a similar response to heat in our MD