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 GA**TA**TC sequence. It's own DNA is methylated in the first adenine, $GA_{CH3}TATC$, 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, GA**TA**TC, the cognate methylated sequence, G$\mathbf{A_{CH3}}$**TA**TC and the non-cognate sequence, GA**AT**TC, 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, G**A**TATC, 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 (αS) 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 αS 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 αS 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

simulations. The mutant models were obtained by mutating the NMR minimized average structure of αS (PDB ID = 1XQ8). Atomistic simulations for both wild-type and mutated αS in explicit water were conducted for at least 30 ns at four different temperatures (i.e., 310 K, 330 K, 350 K and 372 K) using the CHARMM22/CMAP force field. For wild-type αS, the percentage of α-helix changed by less than 5% from that of the NMR structure regardless of simulation temperature. For each mutant αS, the percentage of α-helix differed by 10% or less regardless of simulation temperature. In all simulations, the acidic tail of αS remained predominantly random coil. Moreover, at 372 K, beta sheet was temporarily observed within a small region (approximately 3%) for the wild-type and A30P mutant.

2300-Pos
Protein Trajectory Reconstruction Strategies from FRET-Derived Distance Constraints
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Critical to almost all biological processes, proteins are truly the cell's functional building blocks. The remarkable specificity and diversity of protein function are consequences of native conformational fluctuations, or protein dynamics, in the aqueous cytoplasmic environment. While crystallographic and NMR-based methods have dramatically improved our structural interpretation of proteins, extending that knowledge to predict and describe these dynamic processes remains a major open challenge. Merging Förster Resonance Energy Transfer (FRET) data from single molecule experiments with Elastic-Network protein Models (ENM), we reconstruct dynamic adenlyate kinase (ADK) trajectories over extended time scales and throughout its catalytic process.
With exceptional temporal resolution, single-molecule FRET experiments can produce inter-residue distance traces between modified residues within a single protein. Here, this distance is treated as a perturbation of the equilibrium distance from the PDB structure, reduced to an elastic network with only one alpha-carbon atom at each node. Normal Mode Analysis (NMA) reveals the natural directions of motion most responsible for the (inter-residue) distance perturbation, and these same eigen modes inform the geometry of all other (non-tagged) nodes. By reconstructing the entire protein network at each FRET time point, we have determined a maximum-likelihood estimation of the protein trajectory and can analyze the spatial dynamics of regions both proximal and distal to the tagged residues. Moreover, we suggest an automated solution for the non-trivial dye placement of fluorophore pairs that maximize information content of resultant distance traces. The combination of FRET data and ENM techniques access timescales beyond those of MD, but we show agreement between our method and atomistic ADK simulations from different starting structures in smaller temporal windows.

2301-Pos
Conserved Hydrophobic and Hydrophilic Bond Interaction Networks in ErbB Family Kinases
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The EGFR/ErbB/HER family of kinases contains four homologous receptor tyrosine kinases that are important regulatory elements in key signaling pathways. To elucidate the atomistic mechanisms of activation in the ErbB family, we perform molecular dynamics simulations on the three members of the ErbB family with known kinase activity, EGFR, HER2 (ErbB2) and ErbB4, in different molecular contexts: monomer vs. dimer, wildtype vs. mutant. Using bioinformatics and fluctuation analyses of the molecular dynamics trajectories, we relate sequence similarities to similarities in specific bond-interaction networks and similarities in collective dynamical modes. We find that in the active conformation of the ErbB kinases, key loop motions are coordinated through conserved hydrophilic interactions, an activating bond-network consisting of hydrogen bonds and salt bridges. While the inactive conformations show a less extensive inactivating bond network, they sequester key residues and disrupt the activating bond-network. Both conformational states are also stabilized through context-specific hydrophobic interactions. We show that the functional (activating) asymmetric kinase dimer interface forces a corresponding change in the hydrophobic and hydrophilic interactions characterizing the inactivating bond network, resulting in the motion of the αC-helix through allostery. Some of the clinically identified activating kinase mutations of EGFR also act in a similar fashion, disrupting the inactivating bond network. Our molecular dynamics study reveals a fundamental difference in the sequence of events in EGFR activation in comparison to the Src kinase Hck. This work is funded by NSF grants CBET-0730955 and CBET-0853539.

Protein Folding & Stability II

2302-Pos
Rational Design of Acidic Human Fibroblast Growth Factor (hFGF-1) with Increased Stability and Mitogenic Activity
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Human acidic fibroblast growth factor (hFGF-1) belongs to a superfamily of 22 fibroblast growth factors (FGFs) that have a high affinity to heparin. It is a 16kD protein, which is involved in many cellular functions, such as, cell proliferation, cell differentiation, angiogenesis, tumor growth, and wound healing. In wound healing, the blood coagulation cascade is triggered to clog the wound. One of the proteases that participates in wound healing is thrombin, which converts fibrinogen to fibrin. Thrombin also cleaves wild type hFGF-1 and consequently decreases its wound healing potency. Thrombin is shown to specifically cleave FGF-1 at Arg136. In this context, in the present study we have designed several site-directed mutants at Arg136 to understand the role of this residue not only on the stability of FGF-1 but also on the FGF signaling process. The results of this study will be discussed in detail.

2303-Pos
Design of Novel Affinity Tags to Increase Recovery of Recombinant Proteins in Ther Soluble Forms
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One major challenge in the expression and purification of recombinant proteins is preventing proteins from entering inclusion bodies. While some proteins can be extracted and refolded from the insoluble inclusion bodies others cannot, such as those proteins containing a protein affinity tag. This study will examine those specific challenges and will explore potential solutions to those problems. We aim to exploit properties of osmolytes like betaine, sarcosine, arginine, and proline to design affinity tags that prevent the formation of inclusion bodies during over-expression of proteins in bacterial hosts. Results of the interactions of the designed affinity tag(s) with transition metals, like nickel and cobalt, will be presented. In addition, correlation between recombinant protein expression yields and the length of the affinity tag(s) will also be discussed.

2304-Pos
Direct Observation of Villin Folding in a Microfluidic Mixer
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Villin headpiece subdomain (HP35) is a 35 residues polypeptide that folds on the microsecond timescale. Therefore, it has been an attractive object of protein folding for both computational and experimental studies, with some disagreement between the two methods. In this work, we observed folding of this protein after dilution of high denaturant in an ultrarapid microfluidic mixer far below the thermal midpoint. Tryptophan fluorescence changes were observed at various times beyond mixing from 8 ~1500 μs at temperatures between 10 ~ 40 °C. It is similar with T-jump that fluorescence relaxes can be fit with two exponential decays. Most of the amplitude decays within the mixing time, in agreement with the T-jump observation. But a small population decays on the tens of microsecond timescale. This slow phase is in rough agreement with MD simulations by Ensign et al. (1) and suggests a simple two-state model is not adequate to describe the folding pathway.
1. Ensign DL, PM Kasson, VS Pande. 2007. Heterogeneity even at the speed limit of folding: Large-scale molecular dynamics study of a fast-folding variant of the villin headpiece. Journal of Molecular Biology 374(3):806-816.

2305-Pos
Molecular Basis for the Solvation and Reconstructive Denaturation of Proteins by Detergents
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Detergents are widely used for the biochemical and structural study of proteins. Non-ionic and zwitterionic detergents are used as membrane mimetics, where they solvate the hydrophobic regions of integral membrane proteins. In contrast, ionic detergents such as sodium dodecyl sulphate (SDS) (anionic) and lauryl-trimethylammonium chloride (LTAC) (cationic) are strong protein denaturants that unfold both soluble and membrane proteins. Not only does the SDS-unfolded state have high alpha-helix content, but SDS and other ionic