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

Mikolai Fajer[1], Susan Taylor[1,2], J. Andrew McCammon[1,2].
[1]University of California, San Diego, La Jolla, CA, USA, [2]Howard Hughes Medical Institute, La Jolla, CA, USA.

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

Vikas P. Jain, Raymond S. Tu, Charles Maldarelli.
City College of City University of New York, New York, NY, USA.

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

Mai Zahran[1], Isabella Daidone[1,2], Petra Imhof[1], Jeremy C. Smith[1,3].
[1]IWR, University of Heidelberg, Heidelberg, Germany, [2]Dipartimento di Chimica Ingegneria Chimica e Materiali, University of L'Aquila, Coppito (AQ), Italy, [3]Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

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

Sam W. Fan, Richard A. George, Naomi L. Haworth, Lina L. Feng, Jason Y. Liu, **Merridee A. Wouters**.
Victor Chang Cardiac Research Institute, Sydney, NSW, Australia.

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

Dmitry V. Matyushov.
Arizona State University, Tempe, AZ, USA.

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

Hongyi Yang, James C. Patterson.
UAB, Birmingham, AL, USA.

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