Posters

Protein Structure and Conformation II

1050-Pos Board B1
Structural Rigidity Regulates Functional Interactions in the Hsp40-Hsp70 Molecular Machine
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Protein interactions commonly involve lock-and-key associations between interacting domains. Structural features of these binding partners regulate the nature and extent of their interactions. We report our investigations on specific physical characteristics required of the Hsp40 J-domain to stimulate ATP hydrolysis in the Hsp40-Hsp70 molecular chaperone machine. Characterization of functional and dysfunctional Hsp40-Hsp70 interactions using isothermal titration calorimetry (ITC), and nuclear magnetic resonance (NMR) spectroscopy reveals the importance of structural rigidity for Hsp40 function. Our results suggest that the functional J-domain acts like a semi-elliptical spring, whose resistance to bending in Hsp40-Hsp70 interactions modulates the ATPase domain conformational change and promotes ATP hydrolysis.

1051-Pos Board B2
Structural Studies of Soluble Guanylate Cyclase
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Soluble guanylate cyclase (sGC) is a ~150 kDa heterodimeric enzyme that plays a crucial role in the cardiovascular system by catalyzing the conversion of GTP into cGMP, a secondary messenger whose downstream effects include vasodilation and inhibiting platelet aggregation. Using a heme prosthetic group located at the β N-terminus, nitric oxide (NO) binds to sGC and induces a conformational change, increasing catalytic activity several hundred-fold. Inhibition of sGC activity through oxidative stress and NO-scavengers has been linked to cardiovascular disease. Due to a lack of structural data, how sGC transitions between basal and active states remains largely unknown. The ultimate goal in our lab is to provide a detailed structural mechanism for sGC activation to guide structure-based drug design of novel small molecule sGC activators to treat cardiovascular diseases.
We have recently solved the structure of the catalytic heterodimeric wild-type αβGC. Activity assay results showed that this construct only displays ~ 0.01% of full-length sGC basal activity, suggesting that additional sGC domains are required to align active site residues in a catalytically-competent position. Our efforts are now focused on strategies to overcome this limitation and obtain the structure of the active conformation of the heterodimeric catalytic domains.

1052-Pos Board B3
Gestation of a Glu Plasminogen Supra Fold via Molecular Dynamics Simulation
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Plasminogen (Pgn) is the precursor of plasmin, a proteinase that plays key roles in tissue remodeling, cell migration, fibrinolysis, *etc*. Pgn, M_r ~92 kD, is structured by seven in-tandem globular domains: an N-terminal preactivation peptide (PAN, 77 aa), five kringle (K) repeats (~ 80 aa each), and a trypsinogen-like serine protease zymogen (243 aa). Upon activation, Pgn undergoes a conformational change such that binding sites in kringles become accessible and able to interact with fatty acids and exposed lysyl side-chains. Via molecular dynamics (MD) simulations, we have investigated changes in inter-domain interactions that reflect supra-fold of Pgn. A model structure built by connecting sequential domains with fully extended flexible linkers was placed in a box of 282487 explicit water molecules and allowed to equilibrate for 420 ns via NAMD under CHARMM force field. During the MD, the protein folds as monitored by its radius of gyration (RG) that decreases from 157 Å to 34 Å, in good agreement with experimental observations (*Mangel WF, Lin BH, Ramakrishnan V. Science 1990;248:69-73*; *Ponting CP, Holland SK, Cederholm-Williams SA, et al. Biochim Biophys Acta 1992;1159:155-161*). The equilibrated structure is compact with a spiral spatial configurations of the in-tandem domains, as reported for the crystal (*Law RHP, Caradoc-Davies T, Cowieson N, et al. Cell Reports 2012;1:185-190*). By reversing the time course of the trajectory, dynamics insights are gained for the unfolding that accompanies the Pgn conformational change in the process of fibrinolysis. The PAN-K5 interaction was analyzed via free energy calculations as a function of relevant key inter-atomic distances. Two energy minima unveil configurations that exhibit strong and weak interactions between the PAN Lys 50 and the Asp516/Asp518 loci at the canonical K5 lysine binding site.

1053-Pos Board B4
Constrained Maximum Likelihood Estimation of the Abundances of Protein Conformation in a Heterogeneous Structural Ensemble from Small Angle X-ray Scattering Intensity Measurements
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A method is described for maximum likelihood estimation (MLE) of the relative abundances of different conformations of a protein in a heterogeneous mixture based on small angle X-ray scattering (SAXS) intensities.
This approach is of particular interest in situations where there are unknown, intermediate conformations, for instance, during catalytic cycling of a protein. First, an ensemble of structures is generated using molecular dynamics, crystallography or other technique. This ensemble is then clustered into sub-sets based on k-means clustering and the Cramer-Rao bound on the mixture coefficient estimation error. A sparse basis set that represents the space spanned by the measured SAXS intensities of the conformations of a protein is then generated from representative members of each cluster. Based on a statistical model for the intensity measurements, we show that the MLE approach can be expressed as a constrained convex optimization problem. Starting with a basis set generated from known conformations of the enzyme, adenylate kinase (ADK), we carried out Monte Carlo simulations to assess the performance of the proposed estimation scheme. We demonstrate the utility of the approach by identification of dominant conformations under different solution conditions and provide estimates of the abundance of minor species as a function of concentration of different ligands.

1054-Pos Board B5
Using Physics and Heuristics in Protein Structure Prediction
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Predicting the structure of proteins via computer simulations could be greatly accelerated by using our general knowledge of globular proteins. For example: globular proteins have hydrophobic cores. This kind of information provides many possible restraints, only a few of which are true. How can we leverage this information in simulations to accelerate folding at the same time that we use only the small part of the information that is correct?
We have recently developed a method called MELD (modeling employing limited data) that can deal with ambiguous and erroneous information in atomistic simulations allowing us to fold a set of 20 small proteins up to five orders of magnitude faster than in brute force atomistic simulations alone. We have recently competed in a blind test competition (CASP) which allowed to showcase this method for proteins over 100 residues long and under strict time constraints.

1055-Pos Board B6
Elucidating the Functional Significance of the C-Terminal Hypervariable Region (HVR) in K-Ras4A
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In humans, three ubiquitously expressed genes (HRAS, KRAS, NRAS) encode four different isoforms of the Ras protein. Ras is a member of the guanine nucleotide-binding protein superfamily, and acts as a "molecular switch" to effect diverse cellular activities, including proliferation, differentiation, and cell survival. Oncogenic mutations equally affect the highly-expressed K-Ras4B splice variant of KRAS, which is amongst the most frequently observed in human tumors, and the K-Ras4A splice variant, which exhibits low mRNA expression in cells. These isoforms share a nearly identical polypeptide