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2902-Pos Board B7

Unbiased Simulation of the Back and Forth Apo to Holo Conformations for the Calmodulin N-Terminal Nodule: A Sequence of Specific Events

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The 76-residues N-terminal nodule of the Calmodulin protein undergoes strong structural rearrangement as two calcium atoms bind or unbind from their EF-hand sites. Calmodulin is widely used in all kind of cells for a variety of tasks activated by the calcium ion. Calcium arrival allows 2 Helix-loop-Helix motives (EF-hands) to open cooperatively their hydrophobic core, allowing binding to protein targets. Numerous simulations have been made, with biased potential, to understand the folding pathway from either the apo to holo structures or vice versa. We present the results of an unbiased simulation using a detailed atomistic model extending the coarse-grained OPEP potential coupled with a multiscale saddle-point method for sampling configurations, identifying the two folding pathways and crucial interactions.

2903-Pos Board B8

Molecular Recognition Mechanism of Calmodulin Examined by Perturbation-Response Scanning

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Calmodulin (CaM) has a pivotal role as an intracellular Ca2+ receptor that is involved in calcium signaling pathways in eukaryotic cells [1,2]. Binding of Ca2+ and proteins or small organic molecules to CaM induces large conformational changes that are distinct to each interacting partner [1,3,4]. To design drugs that inhibit Ca2+-CaM formation, the molecular binding mechanism must be thoroughly understood.

In this study, we use a new tool called perturbation-response scanning that is based on systematically exerting directed forces on the residues of the protein [5] and recording the changes in fluctuation profiles as the response. Different conformations of CaM are investigated to locate domains or key residues controlling ligand binding and release. We perform 200 ns long MD simulations on the apo form of CaM. The simulation is divided into chunks which resemble different conformations of the apo form. Using the cross-correlation matrices obtained from these chunks as the kernel in linear response, we determine the residues whose perturbation yields the experimentally determined displacement profiles of the apo and holo forms. We find that it is possible to induce the different conformational changes relevant to the binding of five different ligands, by perturbing varying ligand binding residues, and/or residues on the distant helices in the single apo-form. The findings thus give information on how the flexible linker region acts as a transducer of binding information to distant parts of the protein. This new tool enables us to reveal the essence of ligand recognition mechanisms by which CaM controls a wide variety of Ca2+ signaling processes.

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2904-Pos Board B9

Modeling the Unbinding Mechanism of the Neutral and Anionic Semi-Quinone from the QA Site of Bacterial Reaction Centers Using Steered Molecular Dynamics Simulations

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Bacterial photosynthetic reactions centers are large integral membrane proteins that carry out a series of light initiated electron transfer reactions between bound co-factors. In the QA binding site ubiquinone is singly reduced to the anionic semi-quinone. Previous experimental studies using hydroxyl quinones at a pH above the pKa, showed that neutral and anionic quinone species have similar affinity for the QA site (Madeo et. al Biochemistry, 2005, 44 10994-11004). Despite this, anionic quinones dissociate from the QA site 10⊥3 times more slowly than neutral quinones. This suggests that there are large kinetic barriers created by the protein for the dissociation of anionic quinones that are not present in the neutral state. The present study further investigates these barriers by applying constant-velocity steered molecular dynamics (SMD) to compare the unbinding of the neutral native ubiquinone and its anionic semiquinone. The same starting structure (1aij.pdb) is used in ubiquinone and semiquinone simulations, with partial charges adjusted to represent the equilibrated residue charge states. All simulations were performed with the GROMACS package with the OPLS-AA force field. A structural analysis of the trajectories generated during the unbinding of quinones identifies residues of the protein making stronger interactions with semiquinone than with ubiquinone and thus possibly contributing to the kinetic barriers. The magnitude of the energetic barrier is compared to the measured dissociation rate of anionic hydroxyl quinones.

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2905-Pos Board B10

Hierarchical Elastic Network Modeling of cryo-EM Data

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Cryo-EM images of molecules are increasing in resolution, extending their uses from rigid-body docking to generation of atomistic density maps independent of prior structural information. Single molecule cryo-EM provides images of a molecule from many viewpoints, which are averaged and compiled to form a 3D reconstruction. This reconstruction determines the smallest possible hull enclosing the molecule, but sacrifices information about dynamic properties of the molecule intrinsically contained in the varied image set.

We suggest projecting the images into 3D space, without averaging out inconsistent densities, to form a probability map for the reconstruction. This map contains flexibility information neglected by earlier analyses of dynamics from density maps. To highlight different levels of molecular flexibility found in this map, we use a hierarchical elastic network model (hENM). The hENM uses the Markov transition probabilities between the voxels in the density map to segment the map into sets of multi-resolution nodes. Each voxel is initially taken as a node in the network, after-which the network is iteratively reduced to increasingly coarser sets of important nodes, forming a hierarchy. This method provides a unifying framework in allowing the derivation of dynamics concurrently with the study of information propagation through the network, with segmentation of the protein into structural sub-regions as an additional benefit. As these nodes highlight the core structural regions of the protein, they can also be used as anchor points for fitting high-resolution structures into EM maps. Preliminary results show excellent overlap between the dynamics computed using a Gaussian Network Model of the C-alpha atoms of proteins and those computed from our hENM nodes, validating the role of hENM in predicting intrinsic protein motions. With the greater goal of inferring function from dynamics, we work here to predict dynamics directly from cryo-EM images.

2906-Pos Board B11

Vectorial Network Model (VNM) for Protein Dynamics: An Analytical Tool for Protein Fluctuations

Yun Xu, Larisa Adamian, Jie Liang.

Dynamics play important roles in how proteins function. Coarse grained network model such as Gaussian Network Model (GNM) and Anisotropic Network Model (ANM) are widely used for studying dynamic changes of proteins. It provides an analytical treatment of different eigen modes of protein fluctuations based on the Gaussian assumption. However, GNM and ANM have limitations. For example, detailed mode analysis needed in these models often requires additional ad hoc knowledge for model selection. ANM can only describe mode specific relative movement between residues. It is difficult to combine different modes with proper weighting to describe the overall dynamic properties of a protein. Here we describe a new theoretical model that overcome these limitations. With a model where both potential energy and kinetic energy are accounted for, we can compute the magnitude and direction of fluctuation of each residue as well as their vectorial correlation analytically, without the aid of eigen mode analysis and with all modes accounted for properly. We describe how this approach can be used to identify the hinge region and model the hinge bending motion of calmodulin originally detected in NMR study.

2907-Pos Board B12

Towards Finding a Better Reaction Coordinate: NtrC and DIMS

Juan R. Perilla, Thomas B. Woolf.

All atom molecular dynamics is appealing due to the detailed insights towards protein function. But, all-atom molecular dynamics is limited due to the small amount of time spent in the transitions between stable states. Dynamic importance sampling (DIMS) enhances this sampling problem by biasing, with correction, to encourage sampling away from the stable states and to thus improve estimates of barrier crossing times. Nevertheless, DIMS has limitations based on the choice of order parameters that define intermediate states. To improve the representation, in DIMS, of order parameters, we have performed simulations of NtrC and have analyzed the transition populations. Our analysis, using techniques of nonlinear time series analysis and nonlinear dimensionality reduction, has enabled us to determine a new numerical method for finding reduced parameter descriptions of transitions in complex biomolecular systems. The results should enable an iterative approach to finding the best order parameter and at the same time improve convergence for estimates of reaction pathways, kinetics and relative free energy differences.