heating pulse unfolds the protein and the fast cooling transition allows it to refold. As a demonstration of our system's capabilities, we have studied the folding behavior of BBL, a small, fast-folding protein, under various denaturant conditions.

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Structure and Dynamics of Molten Globular Intermediates Encountered during the Unfolding of Barstar

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The modelling of the folding and unfolding reactions of small proteins as two-state processes has provided us wealth of information on the kinetics and thermodynamics of these processes and the stability and pathway of folding. Hidden in this misleading simplicity are various intermediate states including the molten globular (MG) forms. The nature of solvation of MG forms has acquired considerable importance in the context of identifying the forces that drive protein folding and unfolding reactions.

In this work we monitored the solvent accessibility during the unfolding of barstar, a small single domain protein, by following the bimolecular quenching constant (k_q) associated with dynamic fluorescence quenching of the single and buried tryptophan (Trp-53) by either acrylamide or potassium iodide. A pico-second time-domain fluorimeter capable of acquiring data every 200 ms of unfolding was used. Analysis of the time-dependence of k_q showed the presence of an intermediate with solvent accessibility very similar to that of the unfolded (U) state. However, structural indicators such as steady-state fluorescence intensity and time-resolved anisotropy of Trp-53 and near UV-CD indicated the presence of another intermediate within ~ 10 ms, the zero time associated with the initiation of unfolding. Taken together, our data is consistent with the following model for the process of unfolding. N à DMG à WMG à U, where DMG and WMG refer to 'dry' and 'wet' MG forms, respectively. The demonstration of the presence of a DMG has strong implications in dissecting the overall process of folding and unfolding into individual steps, apart from revealing the forces that lead to protein folding.

2282-Pos Board B52

Conformational Flexibility and the Mechanism of Allosteric Transitions in Calmodulin

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Conformational dynamics and flexibility is often essential to protein's function. Calmodulin (CaM) is a well-characterized calcium binding protein that takes functional advantage of its considerable intrinsic conformational flexibility. We investigate the allosteric open/close transition of the domains of CaM through simulations of a multiple basin, topology-based model. Our primary focus is to clarify how main chain flexibility influences the mechanism for allosteric transitions of flexible proteins. In particular, we compare the simulated transition mechanisms of the domains of CaM, which are topologically similar, but differ significantly in their conformational flexibility and dynamics. The simulated transition mechanisms are described at the residue level in terms of local structural order parameters that can be compared with predictions from a coarse grained variational model of allostery (Tripathi and Portman, J. Chem. Phys. 135 075104 (2011)).

2283-Pos Board B53

GNEIMO Constrained Dynamics Method: A Tool for Protein Structure Refinement and Conformational Changes

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Constrained molecular dynamics methods, wherein the high frequency degrees of freedom are placed as hard holonomic constraints in the dynamic model of the protein have been developed several decades ago, but these methods are not used widely. There are several bottlenecks in using these methods, the most formidable being the computational time taken for solving the coupled equations of motion. Spatial operator algebra(SOA) techniques reduces the computational time for solving the equations of motion by two orders of magnitude. We have developed a computational framework called GNEIMO, that uses the SOA techniques combined with all-atom force field and appropriate integrators to solve the constrained equations of motion. The generalized constrained molecular dynamics method GNEIMO, allows the user to ''freeze and thaw'' torsional degrees of freedom as fit for the problem studied.

We will demonstrate the use of GNEIMO method in protein structure refinement of low resolution homology models. Starting from low resolution homology models we observed that the all-torsion GNEIMO method leads to a 2Å improvement in RMSD to the crystal structure, while the all-atom molecular dynamics method disrupts the starting model further. The GNEIMO method also showed enrichment in the population density of native-like conformations. We have also tested out the GNEIMO method for studying conformational transitions between two well characterized (crystal structures) conformations of a protein. Long time scale dynamics with GNEIMO on calmodulin and fasciculin shows that the transitions from one conformation to another happen with more facility than with all-atom MD simulations.

2284-Pos Board B54

Models with Energy Penalty on Inter-Residual Rotation Address Insufficiency of Conventional Elastic Network Models

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In this study, I present to our knowledge a new elastic network model, which addresses insufficiencies of two conventional models_ the Gaussian network model (GNM) [1] and the anisotropic network model (ANM)[2]. It has been shown previously that the GNM is not rotation-invariant due to its energy, which penalizes rigid-body rotation (external rotation). As a result, GNM models are found contaminated with rigid-body rotation, especially in the most collective ones. A new model (EPIRM) is proposed to remove such external component in modes[3]. The extracted internal motions result from a potential that penalizes interresidue stretching and rotation in a protein. The new model is shown to pertinently describe crystallographic temperature factors (B-factors) and protein open↔closed transitions. Also, the capability of separating internal and external motions in GNM slow modes permits reexamining important mechanochemical properties in enzyme active sites. The results suggest that catalytic residues stay closer to rigid-body rotation axes than their immediate backbone neighbors. I show that the cumulative density of states for EPIRM and ANM follow different power laws as functions of low-mode frequencies. When using a cutoff distance of 7.5 A, The cumulative density of states of EPIRM scales faster than that of all-atom normal mode analysis and slower than that of simple lattices.

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2285-Pos Board B55

The Dynasome as the Missing Link Between Protein Structure and Function

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We investigated the relationship between protein structure, dynamics, and function using recently developed metrics quantifying their dynamical and structural similarity. The metrics are based on a comprehensive set of 34 dynamics and 24 structure descriptors, which map proteins into corresponding vector spaces. The distances in these spaces provide a straightforward similarity measure and, further, allow to quantify to which extent protein structure relates to dynamics. We analyzed this relation for a representative set of 112 proteins, the dynamics of which were obtained from atomistic simulations. The structure-versus-dynamics distance distribution of all 6216 protein pairs (Figure) showed that for many pairs structure and dynamics are correlated (red). There are notable exceptions (blue), however, indicating disjoint (similar structure/different dynamics) and adjoint (different structure/similar dynamics) behavior. Apparently, these dynamics contain additional information beyond structural information. For example, the snake toxin (3ebx) is structurally similar to other (human) proteins, while its disjoint dynamics differ markedly. This behavior is rationalized considering its function which requires flexibility to block a wide range of receptors, but sufficient rigidity to escape proteolytic degradation. This approach thus holds the promise of improved protein function predictions.