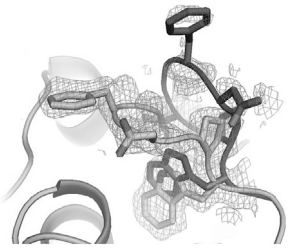
collectively responsive to perturbations. As a result, the fundamental interplay between conformational heterogeneity and biological functions such as catalysis and allostery remains poorly understood. To overcome these obstacles, we develop new algorithms to identify hidden alternative conformations and test whether they form cooperative interaction networks across a set of benchmark proteins. We also combine these computational tools with novel X-ray crystallography experiments to characterize the inherent conformational heterogeneity of the diabetes therapeutic target PTP1B and identify new susceptible allosteric sites. Our approach will open new avenues for the design of novel therapeutics and dynamically enabled protein functions.



3213-Plat
Determination of the Individual Roles of the Linker Residues in the Inter-Domain Motions of Calmodulin using NMR Chemical Shifts
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Many protein molecules are formed by two or more domains whose structures and dynamics are closely related to their biological functions. It is challenging, however, to determine the structural properties of these multidomain proteins because of their conformational heterogeneity. Here, we characterize the inter-domain motions in the calcium-bound state of calmodulin (Ca^{2+}-CaM) using NMR chemical shifts as replica-averaged structural restraints in molecular dynamics simulations. We find that the conformational fluctuations of the inter-domain linker, which are largely responsible for the interdomain motions of CaM, can be well described by exploiting the information provided by chemical shifts, as these parameters are particularly sensitive to the local geometry of polypeptide chains. We thus identify ten residues in the interdomain linker region that change their conformation upon substrate binding. Five of these residues (Met76, Lys 77, Thr79, Asp80 and Ser81) are highly flexible and cover the range of conformations observed in the substrate-bound state, while the remaining five (Arg74, Lys75, Asp78, Glu82 and Glu83) are much more rigid and do not populate conformations typical of the substrate-bound form. The ensemble of conformations representing the Ca^{2+}-CaM state obtained in this study is in agreement with residual dipolar coupling, paramagnetic resonance enhancement and small-angle X-ray scattering measurements, which were not used as restraints in the calculations. These results thus illustrate how CaM binds to its substrates by interdigitated residues in the interdomain linker that follow either the conformational selection or the induced fit mechanisms.

3214-Plat
The Role of Protein Dynamics in Calmodulin Target Recognition
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We aimed to elucidate the role of protein dynamics and stability in target recognition, a process critical in understanding how proteins "choose" the appropriate target. We chose to examine the ubiquitous protein calmodulin (CaM), an essential secondary messenger of calcium signaling with over 300 identified protein targets. CaM is highly flexible and dynamic and its conformational plasticity is essential for accommodating diverse targets. CaM exists as an ensemble of conformers fluctuating around a large flat energy minimum, where conformational sampling permits a continuum population of structures with similar energies. To investigate the role of conformational sampling we measured CaM properties under conditions of excluded volume, where non-reactive polymers were incorporated into experimental conditions. The polymers effectively limit the 3D space a protein can occupy and thus influence its conformational sampling by redistributing the probabilities of each conformational state and modulating the energy barriers between them. We observed that volume exclusion stabilized compact conformations of CaM which had minimal impact on steady state affinity for Ca^{2+} or for protein targets. We observed reduced association rates with targets and importantly, volume exclusion significantly decreased the rate of conformational transition from the initial encounter complex to the natively bound complex. This transition is dependent on polymer size, with smaller polymers correlated to larger decreases, as well as temperature, where lower temperatures which impede conformational dynamics decreased rates the most significantly. We conclude that stabilization of compact conformers and dampened protein dynamics induce conformational frustration during transition to the native complex, where sampling of productive transition state intermediates is encumbered by high energy barriers. Our data is in support of an induced fit binding mechanism where CaM and its target must undergo mutually adjusted step-wise conformational searches to find the natively bound complex.

3215-Plat
Insights into Allostery from the Local Elastic Constants of a Protein
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Allostery connects subtle changes in a protein's potential energy surface (induced by perturbations such as ligand-binding) to significant changes in its function. Understanding this phenomenon and predicting its occurrence are major goals of current research on biophysics and molecular biology. Here we introduce a novel approach for studying complex structural transformations such as those typical for allostery. We show that the calculation and analysis of atomic elastic constants of a known allosterically regulated protein, lac repressor, highlights regions that are particularly prone to suffer structural deformation and are experimentally linked to allosteric function. The calculations are based on a high resolution, all-atom description of the protein, but are computationally inexpensive when compared to currently used methods that employ the same resolution. We also show that, for the present system, lower resolution models yield qualitatively different results, indicating the importance of adequately describing the local environment surrounding the different parts of the protein.

3216-Plat
A Substrate Channel in Nitrogenase Revealed by a Molecular Dynamics Approach
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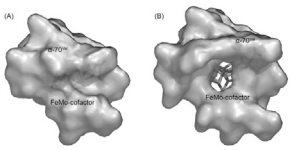
Mo-dependent nitrogenase catalyzes the biological reduction of N2 to 2NH3 at the FeMo-cofactor buried deep inside the MoFe protein. Access of substrates, such as N2, to the active site is likely restricted by the surrounding protein, requiring substrate channels that lead from the surface to the active site. Earlier studies on crystallographic structures of the MoFe protein have suggested three putative substrate channels. Here, we have utilized sub-microsecond atomistic molecular dynamics simulations to allow the nitrogenase MoFe protein to explore its conformational space in an aqueous solution at physiological ionic strength, revealing a putative substrate channel not previously reported. The viability of the proposed channel was tested by examining the free energy of passage of N2 from the surface through the channel to FeMo-cofactor, with discovery of a very low energy barrier. These studies point to a viable substrate channel in nitrogenase that appears during thermal motions of the protein in an aqueous environment that approaches a face of FeMo-cofactor earlier implicated in substrate binding.



Symposium: Structures of Membrane Fusion

3217-Symp
Solid-State NMR Structural Measurements and Models of the HIV and Influenza Fusion Proteins in Membranes
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Solid-state NMR (SSNMR) has been applied to probe the structures and membrane locations of domains of the HIV gp41 and the influenza virus HA2 membrane fusion proteins. Advantages of SSNMR include: (1) use of membrane bilayers without detergent and with lipid and cholesterol composition similar to that of host cells; and (2) preparation of samples under conditions very similar to those used for functional vesicle fusion assays. The *N*-terminal ~20-residue HA2 fusion peptide (IFP) domain has helix-turn-helix structure and in detergent there are reports of: (1) an open interhelical topology with a hydrophobic interhelical pocket and inverted V membrane insertion; and (2) a closed topology with tightly-packed antiparallel helices and membrane contact with a hydrophobic protein surface. SSNMR measurements in membranes lacking cholesterol show that the closed structure is predominant. Both the IFP and the corresponding *N*-terminal gp41 fusion peptide (HFP) have predominant oligomeric β sheet structure in membranes with 30 mole% cholesterol which is typical for viral and host cell membranes. SSNMR measurements for HFP show that there is antiparallel arrangement of adjacent stands with a broad distribution of antiparallel registries. The central regions of the antiparallel sheets