of the activation energy barrier, and Δx_U, the distance to the transition state. For all protecting osmolytes we measure ΔG_U increases, demonstrating that the I27 protein is stabilized. More striking is the measurement of Δx_U. Unfolding the I27 protein in water gives a $\Delta x_U = 2.5$ Å, a distance similar to the size of a water molecule. Water molecules have been identified as integral components of the unfolding transition state of the I27 protein, forming a solvent bridge between two β-strands. By varying osmolyte molecule size we rigorous test this solvent bridging hypothesis. We find that Δx_U correlates with osmolyte size for molecules ranging in size from 2.5 Å to 5.6 Å. However, for larger molecules (> 5.6 Å) Δx_U remains unchanged relative to the value measured in water, suggesting these osmolytes do not participate in solvent bridging in the transition state. These studies uniquely probe the length scales over which solvent molecules can modify the molecular architecture of the unfolding transition of a protein, an area which remains beyond the reach of other experimental techniques.

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Site-Specific Folding Dynamics of Isotopically Labeled Peptides Studied by Time-Resolved Infrared-Spectroscopy

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Peptides with well-defined secondary structure are ideal model systems for study of protein folding dynamics for specific, unique structures. IR techniques provide the necessary time resolution as well as have structural sensitivity, which arises from coupling of sequential residues, normally evidenced as a splitting or frequency shift of the amide centered transitions. The amide I region, mainly the C=O stretching vibrations of the polypeptide backbone, is the prime target band for secondary structure. Isotopic labeling of individual amide 13C=O groups can induce site-specific frequency shifts and provide insight into local structure. A nanosecond laser is used to excite the solvent and induce a fast temperature jump (~10 C), and relaxation dynamics are probed with a diode laser tuned to selected, structurally sensitive wavenumbers across the amide I absorption. Site-specific dynamics have been monitored for the thermal unfolding of an isotopically labeled beta-hairpin peptide, a 12-mer tryptophan zipper peptide, which has a hydrophobic core formed by four Trp residues, by use of cross-strand coupled 13C=O labeled variants [1]. Data for single labeled peptides provided a control. Mutants of this sequence with just two Trp residues were introduced to destabilize the hairpin selectively near the termini or near the turn. Differences in kinetic behavior have been found for the loss of beta-strand and the gain of disordered structure. The isotope-edited kinetics vary with labeling position along the hairpin backbone and the mutations show consistent patterns depending on position. Our data supports a multistate folding mechanism for this hairpin structure. Similarly obtained data for other model peptides provide useful basis for interpretation of the observations.

[1] Hauser, K., Krejtschi, C., Huang, R., Wu, L., Keiderling, T.A., J. Am. Chem. Soc. 130 (2008) 2984- 2992.

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Linear Response of Biomolecules To External Perturbations: Revisit Induce-fit

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Atoms in proteins, viewed as interlinked hubs, communicate with each other in complying with governed physical forces. The deviations of their positions from the mean, known as fluctuations, are essential in mediating functionally relevant biological processes maintaining one's daily life. The coupled fluctuations between pairs of atoms, the fluctuation covariance, can be determined analytically by Normal Mode Analysis, or numerically by MD simulations. Our study considers how this network of atoms reacts in response to external perturbations. Effects of such perturbations are exemplified by ligand- or (another) protein-induced conformational changes as well as the appreciated structural distortion of crystalline structures from its solution conformers. We assume the response of the system has linear departure from the mean under small perturbations on the Hamiltonian at equilibrium state. The formulated linear response theories, either time-dependent or -independent [1], says that the positional change of a given atom i is the accumulative sum of fluctuation covariance ij, at unperturbed state, multiplied by the force exerted on atom j. The time-dependent response function determined from MD simulation of carbonmonoxy myoglobin is used to track time-dependent conformational changes upon photo-dissociation of CO. The consequently obtained understanding of perturbation propagation is compared with experimental results. Also, the structural distortion of X-ray-characterized ubiquitin from its solution conformer can be well explained by the induce-fit theory, in a wider sense, while population-shift does not account for such a deviation.

[1] Ikeguchi M, Ueno J, Sato M, and Kidera A. (2005) Phys Rev Lett, 94, 078102.

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Dynamic Allostery In Proteins

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Allostery or signalling between two sites of a molecule is a widely present phenomenon in nature. Classically it is believed that the communication between distant sites proceeds via a series of conformational changes. Recently allosteric proteins without conformational change have been observed. This cannot be accounted for with the classical theory and therefore we have built a model that explains the long distance signalling in such cases. We believe that the signalling proceeds via a change in dynamic behaviour of the protein and we illustrate the feasibility of such an explanation on a model system, the Catabolite Activator Protein (CAP).

CAP displays negative cooperativity without conformational change upon binding two cAMP ligands. We have built a coarse grain model of multiple slow and fast modes and demonstrated how negative cooperativity can arise without conformational change. The slow, global modes are responsible for the allosteric behavior and result in a purely entropic contribution to the allosteric free energy (ΔΔG). Multiple slow modes need to be included to achieve the experimentally observed free energy values. In the real system compensating entropic and enthalpic terms are observed. Fast modes, despite being localized can couple to the slow modes and assist allostery. We show that they account for the split of ΔΔG into the entropic and enthalpic parts. The value of ΔΔG is proportional to the number of slow modes. The size of the compensating enthalpic and entropic terms increases with number of enslaved fast modes. We can therefore estimate how many slow and fast modes are taking part in the allosteric signaling.

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Impact of Hofmeister Salts on Structural Dynamics of Photoactive Yellow Protein

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Water is known as the lubricant of life. Without water, most proteins would lose their biological activities. Extensive studies have been carried out on how aqueous solutions with high concentration salts alter the stability and solubility of proteins. Such effects are thought to be mediated largely via salt-water interactions and water-protein interactions. This classic research field is known as the Hofmeister Series. We report the effects of Hofmeister salts on the structural dynamics of proteins. Photoactive yellow protein (PYP), a bacterial blue light photoreceptor protein, is employed as a model system in this study. Time-resolved FTIR spectroscopic techniques were used to probe the protein structural changes of PYP in response to blue light excitation. Our data demonstrate that high concentration salt solutions have profound effects on functionally important motions of PYP, including (1) the light triggered proton transfer pathway in the active site and (2) the large conformational changes associated with PYP receptor activation. We will discuss the significance of our study in relation with protein crystallization, and with other properties of the Hofmeister series.

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Linking Enzyme Conformational Dynamics To Catalytic Function With Single-molecule FRET

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Many enzymes endure sizable conformational remodeling on a timescale comparable to their catalytic cycle. In adenylate kinase (AK) from E. coli, this involves large-amplitude rearrangements of the enzyme's lid domain, which may be critical to the enzymes' catalytic function. We applied high-resolution single-molecule FRET developed in our laboratory to follow AK's domain movements on its catalytic timescale. This was achieved by recording and analyzing data photon by photon to rigorously account for counting noise, background, and cross talks. By utilizing a maximum entropy-based approach to remove photon-counting noise, the enzyme's entire conformational distribution was quantitatively recovered without a presumed model. Armed with precise single-molecule FRET dynamics measurements and comprehensive bulk kinetic