vanishing times that conforms to Monte Carlo simulations based on a fracture and end-depolymerization model. Our new model, extending previous work (Turner et al, Biophys. J. 91:1008-1013, 2006), characterizes the distribution of fragment lengths during depolymerization as a decreasing exponential that becomes steeper with time. Finally, we propose extensions of the model to characterize dissolution of fiber bundles and gels. The large number of fibers in a gel results in longer vanishing times. Applied to HbS, our model characterizes a pathological process. It is potentially applicable to other linear polymers that depolymerize by fracture and end-depolymerization and in which normal function depends on cyclic polymerization and depolymerization.

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A Repulsive Electrostatic Mechanism For Protein Translocation Through Type III Secretion System: Insights From Pulling Simulations Of MxiH Across The Needle Apparatus Of Shigella Flexneri

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Many gram-negative bacteria inject effector proteins to the host cell through the type III secretion injectosome that comprises of a basal body, a needle, and a tip. In the context of understanding the protein translocation mechanism, steered molecular dynamics (SMD) simulations have been performed to translocate an MxiH needle protein through the needle pore of *Shigella flexneri*. The needle apparatus is modeled implicitly to increase the computational efficiency. The energetics deduced from the SMD trajectories indicates that the translocation of MxiH is not favorable, which in fact, mimics the transportation of a chloride ion across the apparatus whose pore is highly electronegative in nature. Detailed analyses of structurally known proteins that pass through the injectosome reveal considerable electronegative patches on their surface. Further, the basal region has an electronegative pore. Intriguingly, similar feature is found in the flagellar filament of propelling flagellar secretion apparatus. Based on these observations, we propose a repulsive electrostatic mechanism by which the effectors/substrates pass through the type III injectosome and flagellar apparatus. This mechanism gains support from the fact that the transportation of a protein across these nanomachinery requires ATPase that provides the energy to overcome the initial electrorepulsive barrier.

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Solid-State NMR Studies of Gas Vesicle Structure

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Gas vesicles are gas-filled organelles that allow algae, bacteria and archaea to adjust their position in the water column for optimal illumination and aeration. The spindle-shaped vesicles are typically ~500 nm long and ~75 nm wide, with ~1.9 nm thick walls and their shells consist exclusively of protein, primarily the highly hydrophobic GvpA monomer (70 residues), with a permeability such that the vesicle is filled with gas of atmospheric composition. Electron microscopy has shown that the GvpA monomers are arranged in a low-pitched helix; infrared spectroscopy shows considerable beta-sheet content, in agreement with results from X-ray scattering; and atomic force microscopy shows beta-strands tilted at an angle relative to the vesicle axis that is consistent with X-ray scattering measurements on partially aligned vesicles. To gain further insight into the molecular structure and interactions that grant gas vesicles their remarkable physical properties, atomic resolution data is required. However, insolubility prevents the use of solution NMR or crystallography, and multiple scattering frustrates high-resolution electron microscopy. Here, we present the results of solid-state NMR experiments aimed at characterizing the structure of GvpA in intact, deflated gas vesicles from Anabaena flos-aquae. Fairly complete dipolar correlation spectra, indicating a largely rigid and well-ordered system, have allowed resonance assignments for ~80% of the protein sequence. These chemical shifts provide evidence for the presence of both beta-strand and alpha-helix elements in the GvpA backbone. Furthermore, certain regions of the sequence present duplicated resonances, which suggest that the basic structural subunit of gas vesicles is an asymmetric GvpA dimer. Finally, molecular mobility and preliminary tertiary structural characteristics are also discussed.

Protein Folding & Stability I

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Sequence-dependent Stability Of The Beta-helical Fold

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The left-handed beta helix is an intriguing structural motif in several known proteins. We attempt to elucidate the factors that contribute to its stability with a theoretical-computational approach. Combining a novel form of coarse-grained molecular dynamics with parallel tempering affords access to the regime of equilibration in short peptides. For example, this method has been used to reproduce important features of the helix-coil transition in polyalanine.
Three-layered beta-helical fragments are formed from various sequences that are superposed upon two backbone templates taken from sections of ideal type-I and type-II beta helices. Native, native-like, and various homogeneous sequences are simulated and their stability analyzed relative to the initial structure.
An all-atom potential energy and associated parameters drawn from the Assisted Model-Building and Energy Refinement package with slight modifications. The effects of aqueous solvent are treated with the generalized Born model and a recently-proposed hydrophobic potential of mean force. Larger conformational changes can be explored by treating non-bonded forces according to Brownian dynamics while simultaneously maintaining molecular geometry with a separate algorithm.

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Investigating the Origins of Fractional ψ-values in Protein Folding Transition States

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ψ-analysis has been used to characterize the inter-residue contacts that define the structure of the transition state ensemble (TSE) for three protein systems. ψ-values identify the degree to which an engineered bi-Histidine metal ion binding site is formed in the TSE. Values of zero or one indicate that the site is fully unfolded-like or native-like, respectively, while fractional values reflect a partial recovery of the binding-induced stabilization in the TSE. This method has been applied to three proteins, protein A (Baxa et al., 2008), Ubiquitin (Ub) (Krantz et al., 2004), and acyl-phosphotase (Pandit et al., 2006). In all three cases, the TSE captures ~70% of the respective native-state topology, as quantified by the relative contact order (RCO) metric. In light of the proposed ''70% rule'', a re-evaluation of the TSE of many small proteins, especially those characterized as polarized by mutational f-analysis, must be considered. While this ''70% rule'' is believed to be a general feature of most small proteins, the potential origin of fractional ψ-values remains to be investigated. All-atom Langevin dynamics (LD) simulations of TS models of Ub are performed with distance constraints on residue pairs having experimentally-determined ψ-values of unity. An analysis of the trajectories indicates that the fractional ψ-values of sites adjacent to unity sites tend to reflect distorted site geometries, while the residues for more distal, fractional values indicate that the sites are able to sample configurations where they are unfolded-like. Nevertheless, the simulations indicate that the unity ψ-values alone are sufficient to generate a TSE with a highly native-like topology. Furthermore, the calculation of f-values based on side-chain-sidechain contacts made in the TSE indicate that experimental f-values can dramatically under-report the amount of structure present even for highly buried residues.

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Electrostatic Interaction In The Unfolded States Of Proteins

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With recent recognition that the unfolded states of proteins play important and diverse roles in protein functions, some advances have been made in developing experimental techniques to help decipher residue-specific interactions. Here we present a molecular dynamics simulation based method that allows direct prediction of electrostatic interactions in the unfolded proteins under native conditions. The theoretical prediction is confirmed by measurements of pH-dependent folding free energies of a small model protein HP36.

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Non-Native Structure in the Unfolded Ensemble of a Prototypical β-Hairpin

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Two of the main challenges of modern molecular biology are the determination of the biologically active conformation of a protein from the information encoded in its amino acid sequence and the understanding of the series of events that brings this sequence to the native state. However, for a complete comprehension of the folding process, it's of fundamental importance not only to characterize the final *folded state* and the pathways that lead a protein there, but also to fully understand the nature of the ''starting point'', *i.e.,* the *unfolded ensemble*.