force-extension curves qualitatively match those from recent AFM pulling experiments on CTPR by Chiba, et al. We will extend these studies to include larger numbers of repeats and measure ensembles of pulling trajectories to capture the statistics of the unfolding events.

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Dodging The Crisis Of Folding Proteins With Knots

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We prove that proteins with nontrivial topology, containing knots and slipknots, have the ability to fold to their native states without any additional external forces invoked. Our studies are based on a simple coarse-grained model with interactions modeled only by the native contacts. We demonstrate that folding of knotted proteins YibK and YbeA proceeds through an intermediate configuration with a slipknot. Analysis of topological barriers and backtracking associated to these trajectories reveals to which extent various native contacts are responsible for a folding process. From this we conclude how to modify their strength to get more realistic model, with a higher ratio of properly folded structures. We also discuss how folding properties of knotted proteins are affected when additional chains are attached to one or both termini, and when protein does not have knot topology.

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Continuous Dissolution Of Structure During The Unfolding Of A Small Protein

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An unresolved question in the field of protein folding is whether a protein unfolds in a two-state (N↔U) cooperative manner with only two species being populated during the entire unfolding reaction or in a non-cooperative fashion with a continuum of intermediate forms being populated. To make a definitive distinction between the two has been a difficult challenge, because of the difficulty in identifying and quantifying populations of different species present together during the unfolding reaction.

Time-resolved fluorescence resonance energy transfer (TR-FRET) method can differentiate and measure selectively the populations of N, U and I forms, if present together. In this method, energy transfer efficiency is estimated by collecting the decays of fluorescence intensity of the donor fluorophore in the presence or absence of an acceptor. When such fluorescence intensity decays are analyzed by the maximum entropy method (MEM), distributions of fluorescence lifetimes are obtained, which can be used to generate a distribution of distances between the donor and acceptor.

In this study, a multi-site, TR-FRET methodology coupled to MEM analysis has been used, for the first time, to study the time evolution of the probability distributions of four intra-molecular distances in the small plant protein monellin, as it unfolds starting from the native state. Surprisingly, one distance is seen to increase completely in a gradual manner, while the increase in the other three distances appears to have both discrete and gradual components. Hence, the protein is found to sample many intermediate conformations, characterized by different intra-molecular distances, before unfolding completely. The observed data can be explained by a simple physical model based upon swelling of a homopolymer chain undergoing diffusive dynamics according to the Rouse model.

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Experimental Studies on Protein Folding in the Presence of the Hsp70 Chaperone System

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The Hsp70 chaperone system (including DnaK, DnaJ and GrpE in bacteria) plays a vital role in preventing aggregation and assisting protein folding. While a lot of the chaperone biochemistry has already been worked out, very little is know about the interaction of DnaK and DnaJ with substrates. So far, such interaction had only been probed with small peptide or protein substrates incapable of independent/efficient refolding. This paucity of information has precluded studies on the competition between inter-molecular binding and intra-molecular folding. Such competition is important for cell viability because the accessibility of DnaK to substrate binding is modulated by the intrinsic rates of substrate folding/unfolding and by the substrate's thermodynamic stability. We developed a computational kinetic model to predict the interaction of protein substrates exhibiting two-state folding behavior with DnaK, DnaJ and GrpE. We found that, under physiological conditions, only proteins that fold slowly and/or have moderate thermodynamic stability are predicted to bind chaperones during their folding cycle.

Experimental protein folding kinetics followed by stopped-flow in the presence and absence of the Hsp70 chaperone system shows good agreement with the predictions by the computational model. Furthermore, gel filtration and reverse phase chromatography data further support the stopped-flow results by providing evidence for DnaK-DnaJ-substrate interactions at equilibrium. This result is in agreement with the predictions of the computational model. In summary, the combination of experiments and computational predictions developed in this work is a powerful tool to help unveiling the relations between protein folding and chaperone binding.

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Folding Mechanism Of The Z Mutant Of Human Antitrypsin Studied By H/D Exchange

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The serpins are a unique family of serine protease inhibitors which possess an inherent ability to fold to their active metastable state and avoid thermodynamically more stable conformation. This metastability is crucial for their biological function as they undergo large conformational changes to the stable relaxed (R) state during protease inhibition. Though dramatic conformational mobility of serpins is required for their activity, this also renders them vulnerable to misfolding and polymerization. Misfolding and polymerization are observed in some genetic variants of plasma serpins such as antitrypsin, antichymotrysin and antithrombin III leading to a diverse range of diseases including cirrhosis, dementia, thrombosis, angioadema and emphysema. Studies on the Z variant of human α1 antitrypsin (α1-AT) suggested that extremely retarded folding traps the molecule in an intermediate conformation which has a high tendency to form polymeric aggregates. However, there is no specific report on the nature of the folding intermediate. In the present study we have tried to follow the refolding pathway of the Z variant of α1-AT by hydrogen/deuterium exchange mass spectrometry. Hydrogen/ deuterium exchange mass spectrometry is a powerful method in analyzing the folding/ unfolding of a protein in a region specific manner. We observed different refolding kinetics for different parts of the protein. For most of the regions only 25-30% refolding was observed even after 20 hours. Maximum refolding of 60% was observed for residues 120-142 containing helix E. These studies will shed new light on the nature of the polymerization prone folding intermediate of the Z variant.

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Urea H-bonds to the peptide group, but Gdm does not

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Great attention has been devoted to studying protein conformational stability for about one century. Denaturants have played a prominent role in these investigations for decades, but it is still unclear what the exact mechanism of denaturant action on proteins is. On a more general level, denaturants have been found to preferentially interact with proteins, particularly with the peptide backbone. A prominent model proposes that urea accumulates at the backbone through H-bonding. Guanidine has been proposed to additionally engage in planar stacking interactions.

We test these models using hydrogen-deuterium exchange (HX). When denaturant H-bonds to a peptide group, HX is blocked. Thus, denaturant-dependent slowing of HX is a direct measure of peptide-denaturant H-bonding. We find that urea strongly H-bonds to the peptide group, evidenced by a conspicuous drop in HX rate. Guanidine, in contrast, barely reduces the HX rate. Thus guanidine is likely to denature proteins by an entirely different mechanism than urea. While the urea H-bonding is sufficient to account for its experimental preferential interaction with peptides, guanidine probably accumulates at the peptide group through stacking interactions.

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The Effect of High Concentration Salt on the Structure, Stability, and Aggregation of RecA

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The *Escherichia coli* protein, RecA, is critical for maintaining genetic integrity. RecA catalyzes DNA pairing and strand exchange reactions that are utilized in