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.

415-Pos  Board B294  
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.

416-Pos  Board B295  
CONTINUOUS DISSOLUTION OF STRUCTURE DURING THE UNFOLDING OF A SMALL PROTEIN
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We have tried to follow the refolding pathway of the Z variant of 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.

417-Pos  Board B296  
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 known 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 develop a computational 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.

418-Pos  Board B297  
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, antichymotrypsin and antithrombin III leading to a diverse range of diseases including cirrhosis, dementia, thrombosis, angioedema 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.

419-Pos  Board B298  
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.

420-Pos  Board B299  
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
DNA recombination and DNA repair. Buffer and salt conditions influence the aggregation and activity of RecA. In low salt conditions, RecA is a DNA-dependent ATPase. However, prior research demonstrated that high salt concentrations allow RecA to hydrolyze ATP in the absence of DNA and at levels comparable to those obtained in the presence of DNA [Pugh, B. F. and Cox, M. M. (1988) Journal of Biological Chemistry 263, 76-83]. We have used circular dichroism (CD) and fluorescence spectroscopies to better understand the salt-induced effects on RecA structure and function. CD and fluorescence studies were performed in order to monitor the thermally induced unfolding of RecA in the presence of a variety of salts. We found that different salts had unique effects on RecA unfolding transitions and stability. Unfolding studies performed under salt conditions known to activate RecA’s ATPase activity showed unique, thermally stable RecA structures. A comparison of the influences of different ions on RecA unfolding will be presented. These studies may help to elucidate how different ions influence RecA activity, structure, aggregation, and stability.

421-Pos Board B300
Folding/Unfolding of Glycolipid Transfer Protein: Molten Globule-Like Intermediates?
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Glycolipid transfer proteins (GLTPs) are small, soluble, single-chain proteins (~24 kDa) that selectively accelerate the intermembrane transfer of glycolipids in vitro. The GLTP-fold is unique among lipid-binding proteins. However, little is known about GBF and GBP stability and folding/unfolding dynamics. Vice versa expressed GLTP, FPLC size exclusion chromatography showed peaks corresponding to monomer, multimer, and a third peak of intermediate elution volume. Unexpectedly, native gel electrophoresis showed that the intermediate protein peak migrated as monomer rather than dimer, raising the possibility of a molten globule-like state. Intrinsic GLTP tryptophan fluorescence showed a blue-shifted (~2nm) emission wavelength maximum (λmax), indicating an altered tryptophan environment compared to monomer. ANS binding resulted in a large blue shift (~20nm) in λmax and dramatically enhanced emission intensity (~120%). Far-UV-CD showed retention of ordered secondary structure (~95%), but substantially reduced cooperativity during thermally-induced melting. Near-UV-CD analysis of induced optical activity of GLTP Try/Tyr residues was insufficient to establish tertiary folding changes. To further evaluate GLTP unfolding intermediates, the effect of urea was studied. Trp emission changes suggested a two-step unfolding pathway involving intermediate formation at 4M urea and characterized by red-shifted Trp emission. Additional urea induced further unfolding marked by red-shifted Trp emission. Far-UV-CD analyses of the 4M urea-induced intermediate indicated ordered secondary structure and cooperative melting at lower temperature. However to native GLTP, but the near-UV-CD signal did not provide definitive insights into tertiary folding status. ANS binding showed 1nm blue shift and 60% increase in fluorescence intensity compared to untreated GLTP. Conditions are identified under which GLTP may exist in vivo with efficiency and fidelity. Among the most widely employed agents.

Osmolytes Control Peptide Folding and Aggregation
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Protein folding is a highly complex process that allows proteins to adopt their unique three-dimensional structures, which are essential for their function. Osmolytes, such as sugars and amino acids, are known to influence protein folding by altering the solvent properties and providing a protective environment. In this study, we investigate the role of osmolytes in controlling the folding and aggregation of a synthetic peptide, CALB-GasP, which is known to form amyloid-like aggregates. We used a combination of spectroscopic techniques, including circular dichroism (CD), fluorescence polarization (FP), and small-angle X-ray scattering (SAXS), to analyze the conformational changes and aggregation propensity of CALB-GasP in the presence of different osmolytes. Our results show that osmolytes can modulate the folding and aggregation of CALB-GasP by altering the solvent properties and providing a protective environment, thereby promoting the formation of stable and functional proteins. This research provides insights into the role of osmolytes in controlling protein folding and aggregation, which is critical for understanding the mechanisms of protein misfolding and aggregation in various diseases, including Alzheimer’s disease and amyloidosis. Our findings have implications for the design of therapeutic strategies to prevent and treat these diseases.