fibrosis, cancer and severe burns. AP consists of two domains: a C-terminal calcium-binding domain containing glycine- and aspartate nonapeptide repeats, characteristic of the Repeats in ToXin (RTX) proteins, and an N-terminal proteolytic (passenger) domain. The exoprotease is secreted through a type I secretion system (TSS) composed of a tripartite complex formed by an ABC-transporter, a membrane fusion protein, and a TolC-like outer membrane protein. TISS substrates vary widely in size and function and include toxins, lipases, and proteases. The conserved feature of the substrate proteins is the presence of one or more RTX motifs. Previously, we have demonstrated that calcium regulates multiple conformations of AP. Calcium-induced RTX domain folding serves to chaperone the folding of the protease domain. Here we show that disruption of the calcium-binding sites alters both the affinity and cooperativity of calcium-induced folding, measured in the RTX domain and in the full-length protease, and that the binding sites are not iso-energetic. We have also evaluated the role of calcium in the secretion of the protease, as previous studies have suggested that calcium may facilitate protease secretion. Protein secretion was efficient when the passenger domain was maintained in an unfolded conformation and secreted into medium with high calcium concentrations. Secretion efficiencies decreased with mutations in the RTX domain and with passenger domains that were stable and folded in the bacterial cytoplasm. From these results we conclude calcium regulates protease conformation and may contribute to secretion efficiencies by maintaining specific protein conformations during translocation. This results provide a basis for understanding the calcium-associated secretion RTX proteins from multiple bacterial pathogens.

2036-Pos Board B55
Assessing the Contribution of Cavity Density to Protein Partial Molar Expansion by High-Pressure NMR

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Staphylococcal nuclease (SNase) is a model protein folding system. We recently reported that the magnitude of internal void volumes primarily determines proteins sensitivity to pressure (1) and that the tridimensional position of cavities can modulate the folding free-energy landscape (2). Here we characterize the effect of temperature on pressure denaturation of these previously studied cavity containing variants of SNase (L125A and 192A) to address the thermodynamic parameter will provide unique insight into protein behavior in the pressure-temperature plane.

References:

2037-Pos Board B56
Internal Cavities and their Role as Determinants of Pressure Unfolding of Proteins

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The mechanisms of chemical and heat denaturation of proteins are fairly well established. In contrast, the determinants of pressure unfolding are not well understood. Volume is the conjugate variable of pressure and therefore the key parameter that describes protein sensitivity to pressure. The volume change upon unfolding (\(\Delta V\)) is thought to originate from empty cavities present in the native state and absent in the unfolded state. Staphylococcal nuclease was used to examine this issue systematically. It has a small cavity in its hydrophobic core, of volume comparable to a water molecule. Enlargement of the cavity with site-directed mutagenesis increased the \(\Delta V\) measured by pressure denaturation. The enlarged cavities are large enough to hold several water molecules, but no internal water molecules were observed inside the cavities in any of the seventeen crystal structures determined thus far. Intriguingly, the location of the cavity affected the relationship between cavity size and measured \(\Delta V\). To further examine the role of cavities in the pressure unfolding of nuclease, systematic studies were performed with variants of one of the following characteristics: (1) greatly enlarged cavities, (2) naturally occurring cavity volume eliminated, (3) polar residues lining artificial cavities so as to hydrate the cavities. For all the variants, crystal structures and thermodynamic stabilities were obtained and \(\Delta V\) of unfolding were measured with both Trp fluorescence and NMR spectroscopy. The crystal structures are useful to characterize the cavities and as starting structures for structure-based calculations. The highly detailed description of the mechanism of pressure unfolding emerging from these studies with high pressure Trp fluorescence and NMR spectroscopy can elucidate aspects of the conformational energy landscapes of proteins not readily accessible with other experimental approaches.

2038-Pos Board B57
Langevin Dynamics Simulations to Predict Sidechain Dihedral Angle Distributions

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Despite recent improvements in computational methods for protein design, we still lack a predictive understanding of protein structure and interactions. We perform Langevin dynamics simulations of protein models that include only hard-sphere and geometric constraints to investigate the distribution of sidechain dihedral angles. We first study dipeptides of nonpolar amino acids as a function of the backbone dihedral angles and cooling rate, and compare the sidechain dihedral angle distributions to those of protein crystal structures in the protein data bank. In addition, we predict the sidechain dihedral angle propensities in the core region of the protein ROP and several mutants. The studies serve as a first step in developing the ability to quantitatively rank the energies of designed protein constructs.

2039-Pos Board B58
Probing Single-Molecule Protein Conformational Folding-Unfolding Dynamics: The Multiple-State and Multiple-Channel Energy Landscape

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The folding-unfolding dynamics of protein provides an important understanding of the protein conformational dynamics and functions. We have used single-molecule fluorescence resonance energy transfer combined with statistical data analysis to characterize enzyme and signaling protein fundamental conformational dynamics of Calmodulin (CaM) and kinase (6-Hydroxymethyl-7,8-dihydrotetrahydrophosphokinase, HPPK). The conformational dependence of FRET efficiency of GdmCl indicates the unfolding conformational transition of the proteins. At 2M of denaturant solvent, the majority of the HPPK and CaM protein molecules are under fluctuating folding-unfolding conformational changes, spending about half time in their native state and half time in their unfolded state. We obtained the fluctuation rates from the autocorrelation function analyses of the protein conformational fluctuation trajectories, and we have identified multiple intermediate states involving in bunched time dynamics and the related energy landscape. We had also analyzed the protein folding-unfolding pathways using detailed balance theoretical model analysis in order to understand the complex multiple-state and multiple-channel protein dynamics.

2040-Pos Board B59
Fast Protein Refolding Observed in Pressure-Jump Molecular Dynamics Simulation

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Pressure jump is known to induce fast protein folding. For a five-helix bundle \(\lambda\)-repressor fragment, a short refolding time of ~2 \(\mu\)s was reported in an earlier pressure-jump experiment. To investigate this pressure-jump induced fast folding behavior, all-atom molecular dynamics simulations of more than 33 \(\mu\)s in explicit solvent were carried out on the same \(\lambda\)-repressor construct. High-pressure denatured states, generated through a high-temperature unfolding and high-pressure equilibration simulation procedure, were found to contain a significant amount of helical structure. Upon pressure drop, the protein
refolded into the native state in 20 μs. The folding in the simulation is slower than the one measured in pressure-jump experiment, but faster than the folding time of 80 μs observed in temperature-jump experiment. A complete unfolding and refolding process was observed in the trajectory, which permitted the characterization of high-pressure denatured states and refolding pathway. The pressure jump simulations carried out for this study can be employed in the future to investigate slow-folding proteins through 10–100 μs molecular dynamics simulations by inducing a fast folding phase.

2041-Pos Board B60
Small Protein Folding using Weighted Ensemble Simulation: A Case Study of Trp-Cage
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The 20-residue Trp-Cage is a rapidly folding protein ideal for testing different strategies in folding simulations. The system was studied via the weighted ensemble (WE) approach where trajectories evolved according to Langevin dynamics in implicit solvent. In the WE method [Huber and Kim, Biophys. J., 1996], the configuration space is divided into arbitrary regions called bins. Trajectories arriving to new bins are replicated into identical daughter trajectories that inherit the history and a corresponding fraction of the parent’s weight. In general, WE is a parallel method that forces a system to explore timescales and regions of configuration space typically inaccessible in regular simulations. Unbiased estimates of rates and other properties are produced. Appropriate selection of bins remains a challenging aspect of WE, however, especially in non-trivial systems such as proteins. For Trp-cage, different binning strategies are explored using 1D and 2D binning coordinates with the goal of improving efficiency. Rate constants calculated in both the high and low friction regimes are compared with independent estimates.

2042-Pos Board B61
Quantifying the Drive-Response Relationships between Residues in Protein Folding
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In protein folding processes, mutual correlation and cooperativity are commonly used to describe the interactions between different residues in a protein. However, these metrics do not provide any information on the causality relationships between these residues. The drive-response relationships in protein folding processes are still poorly studied and, due to technical limitations, are difficult to measure experimentally. The information theory transfer entropy (TE) provides a direct measurement of causality between two times series. We have used this method to quantify the causality between residues in the folding processes of a number of molecular dynamics simulations for six small proteins, i.e., Trp-cage, BBA, Villin, WW domain, NTL9 and BBL (Lindorff-Larsen K. et al. 2011 Science. 334, 517-520). Instead of using one single TE value, we calculated the time-dependent TE using the residue-based Q-scores along the folding/unfolding processes. Preliminary analysis reveals that the residues in the hydrophobic cores are frequently involved in drive-response relationships, indicating the critical roles of these residues. The time-dependent TE also allows us to identify key events during the folding and evaluate the contributions from non-native contacts. We expect further studies may disclose more details on the causality relationships of the residues and the folding mechanisms of these proteins.

2043-Pos Board B62
Deciphering the Protein Folding Code through Zipping Steps
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Earlier experiments suggest that the evolutionary information (conservation of amino acids and coevolution between amino acids) encoded in protein sequences is necessary and sufficient to specify the fold of a protein family. However, there is no computational work to quantify the effect of such evolutionary information on the folding process. Here we simulate a repertoire of native and artificial WW domain sequences using a physics-based protein structure search method called ZAM (Zipping and Assembly method), which samples conformational space effectively towards native-like conformations through zipping and assembly search mechanism. We explore the sequence-structure relationship for WW domains and find that the coevolution information has a remarkable influence on local contacts of N-terminal β-turn of WW domains. This turn would not form correctly in the absence of such information. Moreover, through maximum likelihood approach, we identify five local contacts that play a critical role in folding, using the contact probability of those five local contacts at the early stage of folding, we built a classification model. This enables us to predict the foldability of a WW sequence with 81% accuracy. Based on this classification model, we re-design the unfoldable WW domain sequences and make them foldable by introducing a few mutations that lead to stabilization of these critical contacts.

2044-Pos Board B63
Analyzing Protein Folding by High-throughput Simulations
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Molecular Dynamics allows investigating the dynamical properties of biomolecules. Protein folding simulations are computationally challenging when simulating all involved (solvent) atoms considering timescales of ms or slower. Native structure based models (SBM, Go-models) reduce computational complexity and have been proven to be a robust and efficient way for exploring the folding behavior of proteins [Schug and O’Brien 2010; Thirumalai, O’Brien et al. 2010]. They are based on energy landscape theory and the principle of minimal frustration. Using this framework, we simulate protein folding for a large set (~ 200) of non-homologous monomeric proteins sized from 50-150 amino acids in coarse-grained simulations, representing each amino acid by a single bead. A fully automated workflow implemented with the help of eSBM Tools (Lutz et al.) guides these simulations. From the simulations, we extract typical folding properties like phi-values, folding free energy landscape and transition state ensembles. We repeat the simulations for a variant SBM with flavored contact strengths pending on amino acids composition. The resulting database estimates the robustness of folding parameters, quantifies the folding behavior, compares the behavior to existing experimental data and can serve as a baseline for comparison to future experiments or simulations of protein folding. Schug, A. and J. N. O’Brian (2010). “From protein folding to protein function and biomolecular binding by energy landscape theory.” Curr Opin Pharmacol 10(6): 709-714.


2045-Pos Board B64
Simulations of the Thermodynamics and Structural Properties of the TC10b Trp-Cage Protein
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The Trp-cage is an extensively studied miniprotein. Most of the literature, however, studies the TC5b variant of this protein. Andersen et al., showed that by mutating the first four residues to a high alanine content, it is thought that the α-helix of the protein is further stabilized, resulting in a higher folding temperature for the TC10b variant than for the original, TC5b, sequence. In this study, we computationally investigate the higher folding temperature and its causes using Replica Exchange Molecular Dynamics (REMD). First, we show that indeed the TC10b variant has a higher folding temperature in our simulations than TC5b. We also find that the helical fraction is higher as well. Comparing the structural ensemble, we further found differences in the lack of a prevalent secondary substrate, as is the case for TC5b. We finally compute thermodynamics parameters that define the stability diagram, Delta G(P,T), and show that while many other parameters are identical, such as the compressibility, linear expansion coefficient etc., we find that the free energy of folding is more than twice that of TC5b. We conclude that our results support the contention that in most ways, the sequence yields an almost identical structure, with some slight differences, compared to TC5b, but is more stable. This work is supported by the NSF MCB-1050966 to AEG and CCNI at RPI.

2046-Pos Board B65
The Differences in Dynamics and Stability of the Wild Type Beta-Amyloid Aβ1-40, and AΔE22-Aβ1-39 (Japanese) Mutant, A Molecular Dynamics Study
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Alzheimer’s disease (AD) has been identified as a progressive, neurodegenerative disorder associated with protein misfolding due to the aggregation of monomeric β-amyloid proteins (Aβ) to form fibrillar plaques. Experimental attempts to purify and chemically analyze the structure of Aβ protofibrils and to elucidate the mechanism of fibril formation have yet to reveal much about the molecular etiology of AD, due to the low solubility and non-crystalline nature of Aβ. It has been shown experimentally that the AΔE22-Aβ1-39 (Japanese) mutation of the β-amyloid leads to production of typical