et al., Science, 1985, 229, 23-28) Analysis of neighboring aromatic groups can lead to improved understanding of protein folding mechanisms and stability. We have used the β-hairpin forming peptide Trpzip2 (Cochran et al., PNAS, 2001, 98, 5578-5583) as a template to study the effect of aromatic-aromatic interaction on peptide stability. Optical spectra (ECD, FTIR) and NMR of the original TZ2 peptide and its Tyr and Val-substituted mutants were measured to characterize their conformation and thermal stability. Compared to the strongly interacting Trp-Trp edge-to-face geometry, Tyr-Tyr shows much weaker aromatic-aromatic interaction. These aromatic-aromatic interactions were also compared to simple hydrophobic interaction by substituting Val for two interacting Trp residues in this peptide. Aromatic interaction showed a stronger effect than hydrophobic interaction for stabilization of this peptide. The thermal unfolding process of three Val mutants was studied under both neutral and acidic conditions by IR and ECD. We see more random coil content at acidic pH than those at neutral pH from analysis of the IR amide I' band, which reflects peptide secondary structure, and the transition temperatures obtained using both IR and CD are lower at acidic pH than at neutral pH, which suggests that this peptide is less stable at acidic pH.

410-Pos Board B289

Molecular Partition Functions For Amino Acids And Beyond

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The Distance Constraint Model (DCM) provides a novel paradigm for describing protein thermodynamics [1] based on a Free-Energy Decomposition (FED), which assigns free energy contributions to specific molecular interactions. Unique to the DCM, non-additivity in entropy components is explicitly calculated using constraint theory as the total free energy of a system is reconstituted from its molecular parts. In prior work, a minimal DCM (mDCM) involving three empirical fitting parameters has been used to successfully predict protein flexibility and stability in proteins [1-4]. Although the mDCM captures essential physics, it is based on an oversimplified FED involving effective energy and entropy parameters universally applied to all residues. Employing, in part, principal component analysis, we constructed a residue-specific energy and entropy spectrum that depends on secondary backbone conformation states. The resulting molecular partition function predicts surprisingly rich temperature-dependent energy and entropy contributions. This method is general, and works for any type of chemical group and other types of molecular interactions, such as hydrogen bonds. Our new FED is self-consistent, complete and computationally simple. It defines the first all-atom DCM parameterization that is analogous to a molecular mechanics force field. This work is supported by NIH R01 GM073082, and we gratefully acknowledge partial support for H. Wang from the Charlotte Research Institute through a Duke Postdoctoral Fellowship.

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411-Pos Board B290

Lin-12/Notch Repeat B: The Effects Of Disulfide Bonding And Hydrophobic Residues On Its Autonomous Folding

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Notch proteins are transmembrane proteins involved in controlling cell differentiation, cell growth, and cell death. An extracellular negative regulatory region (NRR) contains three contiguous LIN-12/Notch Repeats (LNRs) - LNRA, LNRB, and LNRC. Previous work from our lab showed that the total number of residues N-terminal to the first cysteine residue from human Notch1 LNRB (hN1LNRB) was critical for correct disulfide bond formation. Because of its more central location in relation to the rest of the protein, hN1LNRB participates in extensive interactions via its hydrophobic residues in the context of the full Notch protein. When expressed and folded in isolation, some of these residues are expected to be exposed to solvent and possibly contribute to the requirement of the additional N-terminal residues for autonomous *in vitro* folding with formation of the correct disulfide bonds. To test this hypothesis we mutated W52 to A52 and compared its folding pattern to that of the wild-type hN1LNRB. The effect of the total number of disulfide bonds on the autonomous folding of hN1LNRB was also investigated. In this study, the first of three pairs of disulfide bonds in hN1LNRB was eliminated by mutating C45 and C69 to A. Two mutant forms with two pairs of disulfide bonds were folded *in vitro* under the same conditions as the wild-type and the folding patterns were compared. The data and comparative analysis we present in this work demonstrate the importance of specific hydrophobic interactions and the total number of disulfide bonds as key determinants for the correct folding of an LNR module in addition to the total number of amino acids and Ca^{2+} ion coordination within the module.

412-Pos Board B291

Lattice Model Studies of Designability and Alpha-helix to Beta-sheet Transitions of Short Peptide Chains

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The folding kinetics of lattice peptide chains are studied using a directed Markov-chain model. The small-world characteristics of the conformation space has been noted in recent publications, encouraging further study in the move set topology. We extend this idea to study the entropic effects that result from different move sets and energy functions. Markov-chain models allow for an efficient exploration of the steady-state probabilities, parametrized by temperature. This is an improvement over Monte-Carlo simulations, where an efficient exploration of the entire state-space requires some form of adaptive temperatures scheme. Interesting results are shown at intermediate temperatures, where higher energy states are favored over their lower counter-parts due to their connectivity in the move set. We also provide evidence for rudimentary alpha-helix to beta-sheet transitions for specific peptide configurations and the designability of such structures. Given a transition we can elucidate the kinetics of the pathways between them using first passage times.

413-Pos Board B292

Atomistic Modeling of Macromolecular Crowding Predicts Modest Increases in Protein Folding and Binding Stability

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Theoretical models predict that macromolecular crowding can increase protein folding stability, but depending on details of the models (e.g., how the unfolded state is represented), the level of stabilization predicted can be very different [1, 2]. In this study we represented the folded and unfolded states realistically, with conformations sampled from explicit-solvent molecular dynamics simulations at room temperature and high temperature, respectively. We then designed an efficient algorithm to calculate the allowed fraction, f, when the protein molecule is placed inside a box of crowders. The allowed fraction corresponds to an increase of $\mu = -k_B T \ln f$ in chemical potential. The difference in μ between the folded and unfolded states predicts the effect of crowding on the folding free energy. Even when the crowders occupied 35% of volume, the stabilization reached only 1.5 kcal/mol for cytochrome b_{562}, a 106-residue four-helix bundle protein. This prediction of modest stabilization is consistent with experimental data [3, 4]. Interestingly, we found that a mixture of different sized crowders exerts a greater effect than the sum of the individual species of crowders, in agreement with an earlier model [5]. The stabilization of crowding on the binding stability of barnase and barstar, based on atomistic modeling of the proteins, is similarly modest. The atomistically detailed calculation results can be fitted to a fundamental measure theory, affording the theory predictive power.

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414-Pos Board B293

Forced Unfolding of CTPR proteins

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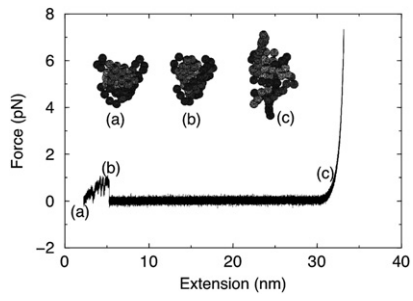
We perform Brownian dynamics simulations of coarse-grained models for CTPR proteins to study the dynamics of unfolding while the proteins are subjected to stretching forces. We use a variant of an HP model with cooperative attractive interactions that give rise to a cascade of bond breaking events during unfolding. In the attached figure, we show a force versus extension curve for CTPR2 in which a single cooperative unfolding event occurs near an extension of 5 nm and the 'worm-like-chain' behavior for pulling out thermal fluctuations of the unfolded chain occurs near 30 nm. The inset shows the structure of CTPR2 during the unfolding process; note that initially the superhelices must twist so that CTPR2 can stretch. Our



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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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.

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 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.

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, 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.

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