core near the helical centers and to stabilize the helical termini. Additional mutations both stabilized and destabilized the turn/loop sequence. The effect of the mutations on the overall thermodynamic stability was measured using CD and IR spectroscopies. Five isotopically labeled variants of a selected stabilized and destabilized mutant were synthesized to further study the influence of the mutations on the structural stability of the labeled segments. The effects of the mutations on the global and, especially, local unfolding provide important clues about the stabilization of the helix-turn-helix motif by specific interactions. Our results support and refine the proposed folding mechanism and point out the significance of tertiary interactions for the stability of alpha-t-alpha.

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In Vitro Interactions Between Model Proteins and Amyloid Inhibitors

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Protein folding is an essential requirement for most biological functions. Therefore, if folding of proteins can be specifically modulated, we have the power to tune/inhibit protein function, disease progression, and create novel analytical tools. This is an inter-disciplinary research project that aims to find small molecules that selectively modulate protein-folding reactions. The small molecules used in the study are bicyclic 2-pyridone derivatives . These compounds were originally designed to inhibit the assembly of bacterial pili, and are peptidomimetics that were directed to block the periplasmic chaperone PapD required for pilus biogenesis. A subgroup of these compounds are designed to inhibit the formation of curlin-based bacterial biofilms. Curlicides and pilicides thus are potential new antibiotics to fight bacterial infections. The curlicides bind the major curlin protein CsgA inhibiting its oligomerization into amyloid fibrils Moreover, some of these compounds have been found to inhibit β-amyloid peptide fibrillization. During the project we studied the interactions of some selected compounds with Pseudomas aureginosa azurin (beta-sheet model protein), Borrelia burgdorferi Vlse and human alpha-synuclein. We have found that two of the compounds we have studied, namely FNO75 and its fluorescently labelled analogue CB84 are interacting with VlsE directly, while binding to azurin occurs upon refolding of the protein after heat denaturation. Moreover, wéve tested the effect of the compounds on alpha synuclein fibrillization. Surprisingly, it was found that FNO75, which is known to inhibit curli and beta-fibrillization was speeding up the aggregation of alpha synuclein.

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Engineering of Acid Insensitive Proteins

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pH-driven conformational changes in proteins are essential for a variety of physiological processes (e.g. Bohr effect and root effect of hemoglobin, activation of influenza and other viruses, etc). To expand our understanding of some of the principles that govern pH-dependent conformational processes in proteins, we attempted to engineer an acid-insensitive form of staphylococcal nuclease. The acid-unfolding of this protein has been characterized previously; the highly cooperative acid unfolding transition of the wild type protein is coupled to preferential binding of multiple protons by the acid unfolded state. The pKa values of His, Asp and Glu residues in this protein have been measured with NMR spectroscopy previously. We attempted to increase resistance towards acid denaturation by removing carboxylic groups that titrate with perturbed pKa values in the native state. Substitutions aimed at modulating the pKa values indirectly were also tested, as were the effects of ionic strength and of different types of ions for modulating electrostatic effects. Efforts to eliminate the acid sensitivity of SNase that were focused on electrostatic effects succeeded to a degree, but the magnitudes of the shifts in the midpoint of acid unfolding of the protein were surprisingly modest. The most successful way of enhancing the acid resistance of this protein involved widening the gap between the native and the acid unfolded state by stabilization of the native state. This was achieved either through substitutions and loop deletions or through use of protective osmolytes such as TMAO, sucrose or with sulfate. Our results suggest that the engineering of proteins that switch with changes in pH, or acid resistant proteins, might need to focus more on deep understanding of the structural origins of stability than on the electrostatic contribution to stability.

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Elucidating the Effects of Mutation upon C-Type Lysozyme through Quantitative Stability/Flexibility Relationships

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The Distance Constraint Model (DCM) [1, 2] is a computational modeling scheme that integrates thermodynamic and mechanical descriptions to compute Quantitative Stability/Flexibility Relationships (QSFR) of protein structure. We have previously employed the mDCM to predict melting temperatures of c-type lysozyme mutants with a maximum error of 4.3% [3]. Going further, we now assess the differences in other QSFR quantities across the dataset. The model is parameterized by fitting to experimental heat capacity curves. Subsequently, a large number of mechanical descriptions of protein flexibility are calculated. Pronounced changes in rigidity and flexibility at sites remote from the mutation are common. Drastic changes occur in the backbone flexibility at the mutation site and neighboring residues within 6Å. Increases in rigidity and flexibility are nearly equal, and collectively occur more than sites without change. Interestingly, the β-domain becomes flexible in all cases, likely leading to domain unfolding related to aggregation [4]. We also present changes in pairwise residue-to-residue couplings that can affect functional collective motions.

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2165-Pos Board B151

Molecular Simulations of the Configurational Entropy of the Trp-Cage Miniprotein

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Understanding conformational entropy changes in globular protein folding is essential to both predictive models of protein structure and the development of high-affinity pharmaceuticals. The conformational entropy of the Trp-cage miniprotein is calculated using all-atom molecular dynamics simulations and the probability distributions of dihedral angles per residue. We use two force fields, Amber-99SB and Amber-94, in water and water-urea solvents in our calculations. We compute the configurational entropy using models that included correlations within individual amino acids and with nearest neighbors. We find that the entropy depends on sequence and position of the chain on the protein. Neighboring residues are shown to only have a less than 10% influence on individual residue entropies. AMBER-94 force field is shown to decrease the entropy of the alpha helical region in the unfolded state, suggesting anomalous residual helix formation in the unfolded state. Unexpectedly, the water-urea solution is shown to destabilize the alpha helical region in the folded state while stabilizing the alpha helical region in the folded state. The results of this project give new context with which to examine Flory's isolated-pair hypothesis as well as the Levinthal paradox. The results could be used to further examine the assumptions underlying the interpretation of protein NMR experiments that attempt to measure the configurational entropy.

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Measuring Sub-Microsceond Protein Folding Kinetics with Independent Probes

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Determining the rate of forming the truly folded conformation of ultrafast folding proteins has been an important issue for both experiment and simulation. The double-norleucine mutant of the 35-residue villin subdomain has been the focus of recent computer simulations with atomistic molecular dynamics because it is currently the fastest folding protein, with folding times less than 1 microsecond as measured by tryptophan fluorescence in laser temperature jump experiments. However, the molecular dynamics simulations reported folding times much longer than the times for fluorescence changes. To establish the folding time with greater certainty, we have therefore employed an independent method. In this experiment the decay of the tryptophan triplet state measured by triplet-triplet absorption spectroscopy monitors the change in the population of the unfolded state. The experimental rates from the two methods will be critically compared with each other and with the rates from simulations.

T.C. and M.B. made equal contributions to this work.