vanishing times that conforms to Monte Carlo simulations based on a fracture and end-depolymerization model. Our new model, extending previous work (Turner et al, Biophys. J. 91:1008-1013, 2006), characterizes the distribution of fragment lengths during depolymerization as a decreasing exponential that becomes steeper with time. Finally, we propose extensions of the model to characterize dissolution of fiber bundles and gels. The large number of fibers in a gel results in longer vanishing times. Applied to HbS, our model characterizes a pathological process. It is potentially applicable to other linear polymers that depolymerize by fracture and end-depolymerization and in which normal function depends on cyclic polymerization and depolymerization.

398-Pos Board B277

A Repulsive Electrostatic Mechanism For Protein Translocation Through Type III Secretion System: Insights From Pulling Simulations Of MxiH Across The Needle Apparatus Of Shigella Flexneri

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Many gram-negative bacteria inject effector proteins to the host cell through the type III secretion injectosome that comprises of a basal body, a needle, and a tip. In the context of understanding the protein translocation mechanism, steered molecular dynamics (SMD) simulations have been performed to translocate an MxiH needle protein through the needle pore of *Shigella flexneri*. The needle apparatus is modeled implicitly to increase the computational efficiency. The energetics deduced from the SMD trajectories indicates that the translocation of MxiH is not favorable, which in fact, mimics the transportation of a chloride ion across the apparatus whose pore is highly electronegative in nature. Detailed analyses of structurally known proteins that pass through the injectosome reveal considerable electronegative patches on their surface. Further, the basal region has an electronegative pore. Intriguingly, similar feature is found in the flagellar filament of propelling flagellar secretion apparatus. Based on these observations, we propose a repulsive electrostatic mechanism by which the effectors/substrates pass through the type III injectosome and flagellar apparatus. This mechanism gains support from the fact that the transportation of a protein across these nanomachinery requires ATPase that provides the energy to overcome the initial electrorepulsive barrier.

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Solid-State NMR Studies of Gas Vesicle Structure

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Gas vesicles are gas-filled organelles that allow algae, bacteria and archaea to adjust their position in the water column for optimal illumination and aeration. The spindle-shaped vesicles are typically ~500 nm long and ~75 nm wide, with ~1.9 nm thick walls and their shells consist exclusively of protein, primarily the highly hydrophobic GvpA monomer (70 residues), with a permeability such that the vesicle is filled with gas of atmospheric composition. Electron microscopy has shown that the GvpA monomers are arranged in a low-pitched helix; infrared spectroscopy shows considerable beta-sheet content, in agreement with results from X-ray scattering; and atomic force microscopy shows beta-strands tilted at an angle relative to the vesicle axis that is consistent with X-ray scattering measurements on partially aligned vesicles. To gain further insight into the molecular structure and interactions that grant gas vesicles their remarkable physical properties, atomic resolution data is required. However, insolubility prevents the use of solution NMR or crystallography, and multiple scattering frustrates high-resolution electron microscopy. Here, we present the results of solid-state NMR experiments aimed at characterizing the structure of GvpA in intact, deflated gas vesicles from Anabaena flos-aquae. Fairly complete dipolar correlation spectra, indicating a largely rigid and well-ordered system, have allowed resonance assignments for ~80% of the protein sequence. These chemical shifts provide evidence for the presence of both beta-strand and alpha-helix elements in the GvpA backbone. Furthermore, certain regions of the sequence present duplicated resonances, which suggest that the basic structural subunit of gas vesicles is an asymmetric GvpA dimer. Finally, molecular mobility and preliminary tertiary structural characteristics are also discussed.

Protein Folding & Stability I

400-Pos Board B279

Sequence-dependent Stability Of The Beta-helical Fold

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The left-handed beta helix is an intriguing structural motif in several known proteins. We attempt to elucidate the factors that contribute to its stability with a theoretical-computational approach. Combining a novel form of coarse-grained molecular dynamics with parallel tempering affords access to the regime of equilibration in short peptides. For example, this method has been used to reproduce important features of the helix-coil transition in polyalanine.
Three-layered beta-helical fragments are formed from various sequences that are superposed upon two backbone templates taken from sections of ideal type-I and type-II beta helices. Native, native-like, and various homogeneous sequences are simulated and their stability analyzed relative to the initial structure.
An all-atom potential energy and associated parameters drawn from the Assisted Model-Building and Energy Refinement package with slight modifications. The effects of aqueous solvent are treated with the generalized Born model and a recently-proposed hydrophobic potential of mean force. Larger conformational changes can be explored by treating non-bonded forces according to Brownian dynamics while simultaneously maintaining molecular geometry with a separate algorithm.

401-Pos Board B280

Investigating the Origins of Fractional ψ-values in Protein Folding Transition States

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ψ-analysis has been used to characterize the inter-residue contacts that define the structure of the transition state ensemble (TSE) for three protein systems. ψ-values identify the degree to which an engineered bi-Histidine metal ion binding site is formed in the TSE. Values of zero or one indicate that the site is fully unfolded-like or native-like, respectively, while fractional values reflect a partial recovery of the binding-induced stabilization in the TSE. This method has been applied to three proteins, protein A (Baxa et al., 2008), Ubiquitin (Ub) (Krantz et al., 2004), and acyl-phosphotase (Pandit et al., 2006). In all three cases, the TSE captures ~70% of the respective native-state topology, as quantified by the relative contact order (RCO) metric. In light of the proposed ''70% rule'', a re-evaluation of the TSE of many small proteins, especially those characterized as polarized by mutational f-analysis, must be considered. While this ''70% rule'' is believed to be a general feature of most small proteins, the potential origin of fractional ψ-values remains to be investigated. All-atom Langevin dynamics (LD) simulations of TS models of Ub are performed with distance constraints on residue pairs having experimentally-determined ψ-values of unity. An analysis of the trajectories indicates that the fractional ψ-values of sites adjacent to unity sites tend to reflect distorted site geometries, while the residues for more distal, fractional values indicate that the sites are able to sample configurations where they are unfolded-like. Nevertheless, the simulations indicate that the unity ψ-values alone are sufficient to generate a TSE with a highly native-like topology. Furthermore, the calculation of f-values based on side-chain-sidechain contacts made in the TSE indicate that experimental f-values can dramatically under-report the amount of structure present even for highly buried residues.

402-Pos Board B281

Electrostatic Interaction In The Unfolded States Of Proteins

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With recent recognition that the unfolded states of proteins play important and diverse roles in protein functions, some advances have been made in developing experimental techniques to help decipher residue-specific interactions. Here we present a molecular dynamics simulation based method that allows direct prediction of electrostatic interactions in the unfolded proteins under native conditions. The theoretical prediction is confirmed by measurements of pH-dependent folding free energies of a small model protein HP36.

403-Pos Board B282

Non-Native Structure in the Unfolded Ensemble of a Prototypical β-Hairpin

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Two of the main challenges of modern molecular biology are the determination of the biologically active conformation of a protein from the information encoded in its amino acid sequence and the understanding of the series of events that brings this sequence to the native state. However, for a complete comprehension of the folding process, it's of fundamental importance not only to characterize the final *folded state* and the pathways that lead a protein there, but also to fully understand the nature of the ''starting point'', *i.e.,* the *unfolded ensemble*.

In this presentation we illustrate in detail the folding mechanism of a prototypical beta-hairpin, namely the C-terminal fragment of protein GB1, by means of all-atom molecular dynamics (MD) simulations in explicit solvent, using metadynamics to accelerate the sampling of standard MD and reconstruct the free energy of the process.
Our results show clearly that the unfolded ensemble of this protein does not comply with the classical view of a collection of disordered coil conformations. Indeed we found out that a fully stretched configuration is unstable towards the formation of a turn in the central region of the peptide. This loop can assume two different conformations: a native-like one, which eventually leads to the 2:4 native structure, and a non-native turn which characterizes the ensemble of the unfolded states among which an ordered 3:5 misfolded structure is particularly stable.
Our results, corroborated by several experiments, support the growing idea that the unfolded ensemble of proteins and even of small polypeptides can be characterized by some form of non-native structure.

404-Pos Board B283
Beta-barrel Proteins that Reside in the E. coli Outer Membrane In Vivo Demonstrate Varied Folding Behavior In Vitro
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Little is known about the dynamic process of membrane protein folding, and few models exist to explore it. We have doubled the number of *Escherichia coli* outer membrane proteins (OMPs) for which folding into lipid bilayers has been systematically investigated. We cloned, expressed, and folded nine OMPs: outer membrane protein X (OmpX), OmpW, OmpA, the *crcA* gene product (PagP), OmpT, outer membrane phospholipase A (OmpLa), the *fadl* gene product (FadL), the *yaet* gene product (Omp85), and OmpF. These proteins share a transmembrane β-barrel motif, but vary in barrel size and primary sequence. We quantified their ability to fold into a matrix of bilayer environments by SDS-PAGE. Several trends emerged from these experiments: higher pH values, thinner bilayers, and increased bilayer curvature promote folding of all OMPs. Increasing the incubation temperature promoted folding of several OMPs but inhibited folding of others. We discovered that OMPs do not have the same ability to fold into any single bilayer environment. We show that while environmental factors are influential, OMP folding must also be modulated by intrinsic protein properties. To rationalize the differences in folding results between OMPs, we explored their kinetic profile and determined their resistance to thermal denaturation. Although these proteins share a common structural motif and have evolved to reside in the same bilayer environment *in vivo*, we found that these OMPs vary in their folding behavior.

405-Pos Board B284
Bistable Entropy Landscape of Sequences and Folds of Proteins
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The evolutionary capacity of a protein fold is defined as the number of sequences that match a particular structure. The logarithm of the evolutionary capacity is proportional to the sequence entropy. We computed the sequence entropies for a representative set of protein structures from the Protein Data Bank (1590 folds). For each of the structures we sample about ten million sequences acceptable to the target fold, and estimate the evolutionary capacity for a range of energies with telescoping ratios. The calculations are conducted with three empirical energy functions that were designed for different tasks in computational biology. The probability of observing a protein with a capacity normalized with respect to length is doubly peaked. The proteins at the peak of lower capacity are most likely to belong to the SCOP classes of all-alpha and the alpha/beta classes. The other peak of capacity includes structures from all-beta and the alpha+beta classes similarly to the overall distributions. Experimentally determined mutants of each protein in the set were collected using BLAST with E-value cutoff of 10^{-10}. The evolutionary capacity and experimentally determined number of mutants are positively correlated for proteins at the peaks. Among all the proteins within the two peaks, a HIV reverse transcriptase at the higher peak has the maximum number of mutants. HIV virus is known for its high evolution rate, which corresponds to high evolutionary capacity in our research. We also examine the network in which sequences flip between alternative folds. The network density and dynamics will be reported.

406-Pos Board B285
Amyloid β Proteins, Modified by a Lipid Oxidation Product, Are Nucleation Sites for Fibril Formation on Lipid Membranes
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Oxidatively damaged lipid membranes are known to promote the aggregation of amyloid β (Aβ) proteins and fibril formation. When lipid membranes contain ω-6 polyunsaturated fatty acyl chains, 4-hydroxy-2-nonenal (HNE) is produced during oxidative stress. We previously demonstrated that HNE modifies the three His residues in Aβ proteins by Michael addition, which increases the hydrophobicity and affinity of Aβ proteins for the membrane surface, and promotes the aggregation of unmodified Aβ proteins into fibrils. There are two different mechanisms by which the promotion of fibril formation may occur. HNE-modified Aβ proteins may act as catalytic templates that stabilize monomers in a fibrillar conformation, but do not ultimately become a part of the fibril (template mechanism). Alternatively, they may act as seeds that reduce a kinetic barrier to adopt a fibrillar conformation by becoming a part of the fibril (seed mechanism). In this report, the concentrations of HNE-modified Aβ proteins in fibrils, in solution, and in lipid vesicle membranes were monitored in order to distinguish between these two mechanisms.
Results indicate that HNE-modified Aβ proteins associate primarily with lipid membranes, suggesting that HNE promotes fibril formation by the template mechanism, and that relatively little HNE-modified Aβ is incorporated into fibrils. This result is pathophysiologically significant because such templates are not consumed by the process of fibril formation and may be long-lived. The formation of isolated templates may also account for the patchy distribution of amyloid fibril plaques in brain tissue afflicted with Alzheimer's disease.

407-Pos Board B286
Rescuing Functional Protein from Amyloid-Like Structure
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Formation of amyloid deposits is the molecular background of several diseases. Protein oligomers and aggregates formed in the process are connected to the observed pathogenesis. Amyloids represent the end stage of a multi-step aggregation cascade. Although some recovery of the enzyme activity from amyloid deposits has been reported in the case of the lysozyme (Booth II et al., Nature 385 (1997) 787-93), the recovery of the active structure from amyloid or amyloid-like deposits has not been studied. Here we show that phosphoglycerate kinase can be refolded into the biologically active structure from amyloid-like fibrils. First, amyloid-like fibrils were grown from phosphoglycerate kinase. The conversion of the protein structure was confirmed by electron microscopy, enzyme activity assays, as well as by Congo red and Thioflavin T binding measurements. Next, the protein was refolded into its native structure. Biological equivalence of the reference and recovered enzyme was confirmed by enzyme activity and differential scanning calorimetry measurements. We found that stabilizing the native fold is not enough for the efficient recovery of the native enzyme. The aggregates have to be destabilized before the formation of the native structure is initiated.

408-Pos Board B287
Dissecting the N-terminal Helical Domain of Apolipoprotein B
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High levels of low density lipoprotein (LDL, the bad cholesterol) are associated with cardiovascular disease, the leading cause of death in western countries. Very low density lipoprotein (VLDL, the precursor to LDL) secretion requires proper folding of the N-terminal domains of apolipoprotein B (ApoB). The N-terminal domain of ApoB is homologous to lipovitellin, whose structure is known. It is composed of a β-barrel, followed by a helical domain and two β-sheet domains. There is little direct structural information on any part of ApoB. Structural studies of the N-terminal domain of ApoB are complicated by aggregation in the absence of lipids. Our initial goal was to isolate an independent folding domain suitable for study by NMR. Two constructs encoding ApoB6.4-8 and ApoB6.4-9 were found to be cooperatively folded and show helical CD spectra. Single point mutations (e.g. L343V) within this region of ApoB have been shown to cause Familial Hypobetalipoproteinemia (FHBL). FHBL is characterized by low levels of plasma ApoB-containing lipoproteins. These mutations result in retention and degradation of ApoB in the ER. Despite the conservative mutation, our hypothesis is that the mutations cause a structural defect in this helical domain which disrupts efficient secretion of VLDL.

409-Pos Board B288
Peptide Structure Stabilization: A Study Of Aromatic-aromatic Interaction And pH Effect On A β-hairpin Stability
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Aromatic side chains in proteins are often involved in aromatic pairs, most of which form interacting networks of three or more aromatic side chains. (Petsko