show that rodlet assembly is possible without additional beta strand formation in the flexible loop regions.
Hydrophobins are small amphipathic proteins characterized by their unique bond pattern of eight Cysteine amino acids and a resulting similar tertiary structure, although only marginal sequence conservation exists within the family. In this work we elucidate the structure of the assembled rodlets in Class-I hydrophobins. The initial model of the EAS mutant is created using homology modeling of the solution structure of EAS allowing for a rigid structure omitting most of the flexible loop regions. A large population of possible docking structures is further generated using a generic protein-protein docking approach and filtered for candidates with distinct amphipathicity to account for the presence of an air-water interface.
The resulting structures are further relaxed in the all-atom-free energy force-field PFF02[1] using the POEM (Protein Optimization using Energy Methods) program package in parallel relaxation runs. Relaxation of the whole population was possible using the distributed volunteer computing platform POEM@HOME (http://boinc.fzk.de/).
[1] A.Verma, W.Wenzel, A Free-Energy Approach for All-Atom Protein Simulation, Biophysical Journal, Volume 96, Issue 9, 2009, P. 3483

3299-Pos
Modeling Protein Stability
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We will present our recent model of protein thermodynamics to better understand the role of different forces in determining the origin of protein stability. A long standing question in the field of protein biochemistry has been how does a protein achieve enhanced stability. This has lead to careful thermodynamic studies of several thermophilic proteins to understand the origin of high stability in these proteins. However, conclusions have often been contradictory. Our recent model elucidates thermodynamics of these proteins and comparison of thermophilic and mesophilic protein thermodynamics indicates a possible general strategy that proteins may employ to gain high stability. Our analysis is based on several thermophilic and mesophilic proteins with different fold and sequence. We compare relative roles of enthalpy, entropy and specific heat in stability determination. Our model qualitatively explains experimental data and also provides an explanation for apparently conflicting findings from different experimental studies. Furthermore, we predict stability based on our model and demonstrate quantitative agreement with experimental data.

3300-Pos
A Toy Model for Calculating the Rate and Position of Amyloid Fibril Dissociation
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In a previous paper we used a statistical model to calculate the form of the dependence of the amyloid association rate constant on the size of an unfolded polypeptide chain (Hall, D and Hirota, N., (2009) *Biophys. Chem.* **140***, 122-128*). In the current work we use a Langevin dynamics based simulation to examine the breakage/dissociation rate of an amyloid fibril in a solution environment. We treat the protein monomers in the amyloid fibril as point particles enclosed by a monomer shell. These encapsulated monomers are joined together by virtual bonds which break when extended beyond a certain limit. The solvent environment is treated as a viscous fluid capable of producing random fluctuating forces that are sensitive to the relative position, but not the velocity of the monomer units making up the amyloid fibril. The simulation results suggest how the rate of fibril breakage /dissociation will alter in response to changes in certain characteristic properties of the amyloid, namely the bonding arrangement, the constituent polypeptide size, the strength of the fibril bond and the existence of bond defects in the fibril. Additionally we use the simulation results to make comment on the likelihood of any position bias with regards to the event of fibril fragmentation - a finding which has important consequences for the 'infectivity'of amyloid fibres *in vivo*.

3301-Pos
Cooperative Folding Kinetics of BBL Protein and Peripheral Subunit-Binding Domain Homologues
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Recent experiments claiming that Naf-BBL protein follows a global downhill folding raised an important controversy as to the folding mechanism of fast-folding proteins. Under the global downhill folding scenario, not only do proteins undergo a gradual folding, but folding events along the continuous folding pathway also could be mapped out from the equilibrium denaturation experiment. Based on the exact calculation using a free energy landscape, relaxation eigenmodes from a master equation, and Monte-Carlo simulation of an extended Munoz-Eaton model that incorporates multiscale-heterogeneous pairwise interactions between amino acids, here we show (1) that the very nature of a two-state cooperative transition such as a bimodal distribution from an exact free energy landscape and biphasic relaxation kinetics manifest in the thermodynamics and folding-unfolding kinetics of BBL and peripheral subunit-binding domain homologues. Our results provide an unequivocal resolution to the fundamental controversy related to the global downhill folding scheme, whose applicability to other proteins should be critically reexamined.
(1) Proc. Nat'l. Acad. Sci. (USA), Vol.105, 2397-2402 (2008).

3302-Pos
Fast Prediction of Protein Thermodynamics
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A computational method for a Flexibility And Stability Test (FAST) on three-dimensional protein structure is described. A four-dimensional free energy landscape defined by temperature and three order parameters is calculated in matter of minutes. The order parameters characterize structure and solvent properties. Specifically, they depict the number of native vs. disordered residues that are within a particular solvent state. Thermodynamic properties are derived from the free energy landscape, including stability curves and heat capacity over all experimentally accessible temperatures. Protein flexibility and correlated motions for a given thermodynamic and solvent condition are also calculated to highlight structural mechanisms. A Free Energy Decomposition (FED) is employed to account for essential enthalpy-entropy compensation mechanisms that include: hydrogen bonding, chemical diversity among residues, atomic packing, strain and vibration energy, pH effects, solvation effects such as clathrate water interacting with residues, hydrophobic effects due to water transfer from buried regions to bulk solvent, and network rigidity. Network rigidity is a long-range underlying mechanical interaction that accounts for conformational entropy nonadditivity during Free Energy Reconstitution (FER). In contrast to molecular simulation, FAST is based on a free energy functional that is solved using self-consistent mean field theory. Individual free energy components come from molecular partition functions that are parameterized from a combination of long all-atom molecular dynamics simulations in explicit solvent, and empirical fitting to experimental data. FAST is a unified model that accounts for several modes of protein denaturation driven by extreme: temperature, pH, and concentration of co-solute. Pressure dependence will also be incorporated in future work. Because of its computational efficiency, FAST can be used in high throughput applications (i.e., design) to assess the consequences of all FED components on the free energy. This work is supported by NIH grant R01-GM073082.

3303-Pos
Non-Covalent Interactions Involving Aromatic Residues in Protein Structures: Stability and Dynamics in Membrane and Globular Proteins using Molecular Dynamics Simulations
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Recent studies have revealed the importance of non-covalent interactions in proteins viz. conventional & non-conventional H-bonds. Importance of interactions involving π electron cloud of aromatic residues namely C-H $\cdots\pi$, D-H$\cdots\pi$, lone-pair$\cdots\pi$ and cation$\cdots\pi$ have recently been recognized. Most of the studies involved crystal structure analysis of proteins or ab-initio calculations. However, the dynamic properties such as stability and life time of these interactions through experimental studies have not been investigated due to difficulties in carrying out such experiments.
In this study, we carried out simulations on four globular and two membrane proteins with different secondary structural contents. The dynamic nature of six different non-covalent interactions was analyzed to identify their behavior over time within and across the different classes (all-α versus all-β) and different types (globular versus membrane) of proteins. Some of the properties analyzed were, fraction of each type of interaction that was maintained throughout the simulation, maximum residence time (MRT) and the life time of the interactions. Our preliminary investigation reveals that conventional H-bonds are dominant (~60%) interactions and is mostly due to main-chain functional groups. They are predominantly stable with a MRT of at least 10 ns, owing to their role in maintaining the secondary structure of proteins. Our analysis reveals that C-H$\cdots$O interactions involving the main-chain Cα and main-chain carbonyl oxygen atoms are the second most dominant interactions in the all-β-proteins. Large proportion of them is relatively more stable. Cation$\cdots\pi$ interactions are

less frequently observed (~2%), but surprisingly it is one of the most stable interactions. The longest MRT of many such interactions exceeds 20 ns. Such strong and stable interactions have implications in the biological activity of proteins, protein-ligand interactions and protein folding studies.

3304-Pos
Simplified Global Nonlinear Function for Fitness Landscape of Protein Design
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Computational protein design or inverse protein folding aims to generate amino acid sequences that fold into an a priori determined structural fold for engineering novel or enhanced biochemistry. For this task, a function describing the fitness landscape of sequences is critical for identifing correct ones that fold into the desired structure. An nonlinear kernel fitness function can be formulated by combining weighted Gaussian kernels centered around a set of native proteins and a set of non-protein decoys. This type of nonlinear fitness function has been shown to offer significant improvement over linear functions in computational blind test of global sequence design. However, this formulation is demanding both in storage and in computational time. We show that nonlinear fitness function for protein design can be significantly improved by using rectangle kernel and a finite Newton method. A blind test of a simplified version of sequence design is carried out to discriminate simultaneously 428 native sequences not homologous to any training proteins from 11 million challenging protein-like decoys. This simplified fitness function correctly classifies 408 native sequences (20 misclassifications, 95% correct rate), which outperforms other statistical linear scoring function and optimized linear function. The performance is also comparable with results obtained from a far more complex nonlinear fitness function with $>$ 5000 terms. Our results further suggest that for the task of global sequence design of 428 selected proteins, the search space of protein shape and sequence can be effectively parametrized with just about 3680 carefully chosen basis set of proteins and decoys, and we show in addition that the overall landscape is not overly sensitive to the specific choice of this set.

3305-Pos
Investigations into Alpha-Helix to Beta-Sheet Phase Transitions
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Statistical mechanical methods have been widely applied to the studies of problems in protein folding and protein aggregation. One of the best examples is the helix-coil transition, initiated 50 years ago by Zimm and Bragg, followed by Lifson, Roig and others. Their approach based on partition functions, transfer matrices, phase transitions, and other statistical methods has initiated a field, which is still active today. Due to close collaborations between theoretical and experimental researchers, the field of helix-coil transitions is considered to be one of the best developed ones. However, the same cannot be said about transitions involving sheet structures, such as sheet-coil or sheet-helix-coil transitions. The difficulties lie in the long-range nature of the residue interactions involved and the richness of sheet structures. To take steps toward solving these problems, we use a long-range multi-state model for the studies of conformation changes of proteins involving sheet, helix, and coil structures. The range of interactions is defined by the sequence distance of 2 residues in contacts and L is the longest of such distances. To date, we have investigated patterns of anti-parallel sheets with L=odd number, up to L=13 for which the partition function can be reduced into products of independent, nearest-neighbor chains. We show that the partition function can be put into an analytic, numerically exact form, based on which various thermodynamic quantities, such as the heat capacity, can be calculated.

3306-Pos
Importance of Protein Context on the Unfolding Pathways of β-hairpins
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Small β-hairpin peptides as well as three-stranded WW domains have been used as models for the folding of β-hairpins in larger proteins. Previous studies of the FBP28 WW domain proposed that side chain contacts between residues in the strands and not the precise order of backbone hydrogen bond formation guide β-hairpin folding. But how applicable is the folding of model systems, such as FBP28 WW domain, to the folding of β-structure in larger proteins with conventional hydrophobic cores? Here we present multiple unfolding molecular dynamics simulations of three proteins that share a double hairpin motif structurally similar to WW domains: cold shock protein A (CspA), cold shock protein B (CspB) and glucose permease IIA domain. The motif forms a sheet in both cold shock proteins while the double hairpin is part of a larger, 7-stranded β-sheet in the IIA domain. We characterized the unfolding pathways of each protein, all showing no consistent order to the loss of backbone hydrogen bonds, similar to the FPB28 WW domain. The smaller cold shock proteins both lose contacts between the β-hairpins and the hydrophobic core early in the unfolding simulations, while the larger, more complex IIA domain maintains contacts to the core and surrounding β-strands later in the simulations, resulting in a more varied unfolding pathway. We show that the larger protein context affects the details of the unfolding pathway of the double hairpin motif.

3307-Pos
Molecular Modeling of Folding in Lactam-Modified Conotoxins
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We report a method to model the conformational folding of α-conotoxins and the factors that affect the synthesis of specific regioisomers by using a combination of molecular dynamics methods to determine the geometric factors (S-S distances and C-N distances in lactam-modified α-conotoxins) and ab initio methods to determine the conformational energy and molecular orbital information. In the literature, the replacement of the Cys2-Cys7 disulfide bridge with a lactam bridge caused a complete loss of activity. However, exchanging the larger Cys3-Cys13 bridge led to analogues that exhibited considerable affinities for the receptor sites. In this work, we examine the effect of the exchange of the latter bridge by replacing Cys3 with an aspartate residue and the Cys13 with a basic amino acid. The results show that thermal fluctuations lead to configurations where a molecular orbital overlap between S-S atoms (Cys2-Cys7) can take place, leading to the proper regioisomer formation. Furthermore, ab initio methods predict adequate orbital overlap between the sulfur atoms. In addition, the amino acid proline appears to generate rigidity in its surrounding amino acids, specifically in at least the region controlling the relative orientation of the Cys2 and Cys7 residues. The length of the methylene chain of the basic amino acid at position 13 affects the probability of forming a lactam bridge between positions 7 and 13. With short chains (one methylene group between the backbone and the amino group), there never is any observed orbital overlap between the carbon and nitrogen atoms, possibly because of the rigidity of the backbone. The probability of robust overlap increases with longer chain size and it is expected to match the efficiency of the Cys2-Cys7 overlap when using lysine at position 13.

3308-Pos
Identification of Multiple Folding Pathways Shared by Three-Helix Bundle Proteins
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Protein domains can be clustered on the basis of shared structural similarity into folds, and a limited number of protein folds have been observed. The small number of proteins folds may imply a similarly constrained number of folding pathways. The degree to which dynamics vary within and between folds may provide broader insights to the protein folding problem. As an initial step, we are interested in the breadth of native dynamics and unfolding behavior within a single fold. Three members (EnHD, c-Myb, and hTRF1) of the three-helix bundle engrailed homeodomain family exhibit some outwardly different folding behavior linked by an shared underlying mechanism dependent on their relative helical propensity. However, further sampling is needed to extract consistent residue-level determinants of protein folding. Consequently, ten additional members of this three-helix bundle fold were selected for simulation based on their low sequence similarity. We have identified multiple unique initial unfolding events shared between three-helix bundles and are characterizing the source of the divergent folding events.

3309-Pos
Molecular Dynamics Simulations of Consensus Tetratricopeptide Repeat Proteins
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The Consensus Tetratricopeptide repeat (CTPR) is a designed 34 amino acid helix-turn-helix motif that occurs in tandem arrays. CTPR proteins provide the unique opportunity to study proteins lacking the long range interactions characteristic of globular proteins. Extensive experimental data from Forster resonance energy transfer (FRET), florescence correlation (FCS), circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies consistently show that the unfolding/folding of these repeats are not two-state folding pathway. Instead there are partially intermediate species along the pathway. To provide atomic detail to complement the experimental data we have performed molecular dynamics (MD) simulations in water of CTPR proteins with two and three repeats, totaling 1.2 μs of simulation time. Thermal unfolding simulations go from the native to the denatured state where all helical content is eliminated in agreement with CD experiments of CTPR proteins in chemical denaturant. We use a variety of methods to analyze the unfolding pathways including