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**
**John S. Schreck**, Jian-Min Yuan.
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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

a multiproperty reaction coordinate and conformational clustering. In this way, structures along the pathways are assigned native, intermediate and denatured states and the properties of these states are calculated and compared. The unfolding of the CTPRs is initiated by the loss of contacts between two repeat motifs which leads to the destabilization and subsequent unfolding of those repeat domains as intra-helix contacts are lost. The unfolding of individual repeats leads to partially unfolded species in agreement with experiment.

**3310-Pos**

**An Fft-Based Method for Modeling Crowding Effects when Both Test Proteins and Crowders are Represented at the Atomic Level**

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Macromolecular crowding affects protein folding, binding, and aggregation, and such effects have been studied by computer simulations. In direct simulations of test proteins mixed with crowders, the proteins have been represented at a coarse-grained level and the crowders modeled as spheres; protein-crowder interactions are assumed to be repulsive. Our recently developed postprocessing approach has allowed test proteins to be represented at the atomic level [1]. In this approach, the motions of a test protein and those of the crowders are followed in two separate simulations. The effects of crowding are then modeled by calculating $\Delta\mu$, the crowding-induced change in the chemical potential of the test protein. For a repulsive type of protein-crowder interactions, $\Delta\mu$ is related to the fraction, $f$, of allowed placements of the test protein into a box of crowders. An algorithm has been developed to calculate $f$ for spherical crowders. Here we present a new algorithm that enables the calculation of $f$ for atomistic crowders. We express $f$ as the correlation function of two spatial functions, one defined for the crowders and one for the test protein. The correlation function was calculated by fast Fourier transform. As the first application, we studied the effects of ellipsoidal crowders on the folding and binding free energies of atomistic proteins, and found that the nonspherical shapes of the crowders lead to greater stabilization effects than spherical crowders of the same volume. This finding has significant physiological implications since the macromolecules inside cells have many different shapes. Additional applications to proteins as crowders and other *in vitro* crowding agents are underway, marking a major step toward realistic modeling of intracellular environments.

[1] S. Qin, and H.-X. Zhou, Biophys J **97**, 12 (2009).

**3311-Pos**

**Negative and Positive Design in Protein Folding and Thermodynamic Stability: Insights from Computational Mutagenesis and Simulations**

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Negative and positive components of protein design are crucial for stability and uniqueness of native proteins. The main goal of this work is to investigate mutual work of positive and negative components of design via the effects of non-specific single and multiple mutations on protein thermodynamic stability and folding dynamics. Proteins representing all four major fold types are under consideration. All mutations are done according to single-nucleotide polymorphism, and coarse-grained protein models with Cα representation are constructed based on native-centric approach and are used in Molecular Dynamic (MD) simulations with Langevin dynamics. Inclusion of non-native interactions to the protein dynamics increases the folding/unfolding transition temperatures compared to the model without non-native interactions regardless of protein type. Depending on mutation types and where they are located, changes in thermodynamic stability consistent with experiments are observed. Mutations can also affect the population of transition-state conformations and folding/unfolding dynamics. Positive and negative components are indispensible parts of protein design, and they should be considered in all experimental and computational studies of protein structure and folding. In particular, specific roles of non-native repulsive interactions illuminated in this work calls for in-depth exploration of the role of unfolded conformations in thermodynamic stability and kinetics of protein folding.

**3312-Pos**

**Towards Comprehensive Analysis of Protein Family Quantitative Stability/flexibility Relationships**

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The Distance Constraint Model (DCM) is a computational modeling scheme that uniquely integrates thermodynamic and mechanical descriptions of protein structure. As such, quantitative stability/flexibility relationships (QSFR) can be computed. Using comparative QSFR analyses, we have previously investigated the give-and-take between thermodynamics and mechanics across a small number of protein orthologs, ranging from 2 to 9 [1-3]. However, a comprehensive protein family analysis requires consideration of hundreds of proteins. Consequently, homology models are necessary to fill in the structural gaps. As a first step towards such comprehensive analyses, herein we assess the differences within QSFR quantities calculated from the human c-type lysozyme x-ray crystal structure and homology models constructed from various orthologs. We parameterize our current minimal DCM (mDCM) by fitting to experimental $C_p$ curves. All models are able to reproduce the experimental $C_p$ curve. Interestingly, the least squares fitting error is not correlated to homology model accuracy. We present quantitative differences within various QSFR metrics between the x-ray and model structures, and establish thresholds on model accuracy based on their ability to reproduce the QSFR metrics of the x-ray structure.

[1] Livesay and Jacobs (2006). *Proteins*, 62: 130-143.
[2] Livesay et al. (2008). *Chem Central J*, 2:17.
[3] Mottonen et al. (2009). *Proteins*, 75:610-627.

**3313-Pos**

**Huntingtin: Stability and Interaction with Molecular Partner from Computational Biophysics Studies**

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Huntington disease is a neurodegenerative disorder producing motor, cognitive and psychiatric symptoms. It is caused by a trinucleotide CAG repeat gene mutations, encoding an expanded polyglutamine (polyQ) tract in the respective protein. Proteolytic processing of mut-Htt lead to the formation of short N-terminal polyQ-containing fragments that have the propensity to aggregate and cause neurodegeneration. These fragments form insoluble β-sheet aggregates that are the hallmark of the disease. Here we shall present a simulation study aimed at pinpointing key factors for the structural stability of polyQ aggregates based on classical molecular dynamics simulations and first-principles calculations. Such study is complemented by a structural prediction of a complex between F-actin and the N-terminal part of mut-Htt, which it is proposed to bind F-actin and to trigger cell apoptosis. This may play an important role in determining the aggregation potential of mut-Htt in cells.

**3314-Pos**

**Analysis of Site-Specific Folding of Helix-Turn-Helix Proteins with Statistical-Mechanical Models**

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Isotopically-edited IR spectroscopy can provide detailed site-specific information about the protein folding mechanism. Our equilibrium unfolding studies of two simple helix-turn-helix (*hth*) proteins revealed complex, heterogeneous processes, which involve structurally diverse ensembles of partially folded intermediates. In order to obtain a consistent picture of the folding mechanism, and insights into its physical origins, it is necessary to connect the sets of site-specific experimental data within a framework of a model, which can explain the observations in terms of the structural and energetic properties of the protein. We have analyzed the experimental data, circular dichroism (CD) and infrared (IR) which included spectra of multiple $^{13}C$ isotopically labeled variants, for both model *hth* proteins using Ising-like statistical-mechanical models. We implemented the Muñoz-Eaton (ME) model, which can be enumerated exactly using efficient transfer matrix methods, and Galzitskaya-Finkelstein (GF) model in double- and triple-sequence approximation. Model parameters were optimized by simultaneously fitting the complete set of data for each protein. With a single parameter for the contact energy, neither variant was capable of simultaneously fitting all the experimental data. However, with Miyazawa-Jernigan residue-specific potentials the GF models closely reproduced the site-specific unfolding, as well as the CD. The ME model, on the other hand, did not improve. For both model proteins, the results are consistent with the proposed folding mechanism and demonstrate that simple, Ising-like statistical mechanical model for protein folding is capable of correctly reproducing multiple site-specific sets of folding experimental data.

**3315-Pos**

**A Physics-Based Approach for Understanding Foldability**

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Statistical coupling analysis (SCA) indicates that in addition to the conservation of amino acid composition at individual site, the coupling information between sites is necessary and sufficient to specify a protein fold.[1] To