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.

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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.

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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

investigate the significance of coupling information, we simulate a repertoire of artificial WW domain sequences using a physics-based search method called ZAM (Zipping and Assembly method).[2] Our result shows that coupling information has a remarkable influence on the local contacts of N-terminal β-turn of WW domains. This turn would not form correctly if lack of such information. Interestingly, the formation of N-terminal β-turn has been determined as the nucleator and rate-limiting step experimentally.[3] We also identify specific crucial contacts at the beginning of folding process, and accomplish to predict the foldability of a WW sequence, based on its favor of these crucial contacts.

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3316-Pos

Prediction of H Exchange from Perfectly Funneled Structure Based Models

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Simulations based on perfectly funneled landscapes have been able to capture many of the fundamental aspects of protein folding. When frustration is low enough the topology becomes the main factor determining the folding process. In the most fundamental implementation of the minimal frustration principle only native interactions significantly contribute to the stabililization of the protein structure. Using these ideas and coarse grain models an extensive sampling of the energy landscape could be achieved. We explored the use of such models to interpret subtle dynamic motions near the native state and whether they are able to give a quantitative description of the native protein ensembles. For this aim we developed a method for the quantitative comparison of the local stability of proteins simulated using perfectly funneled structure based models, and detailed experimental measurements of single residue hydrogen/deuterium exchange of backbone amides (HDX) which depends on structural and dynamic properties. The method was applied to ubiquitin, cytochrome-C, HEWL, S6, and IkBalpha70-206. The predicted exchange patterns agree with the experimentally determined HDX protection factors under native conditions. A variety of simulation models with homogeneous, heterogeneous, additive as well as non additive contact potentials were evaluated for their agreement with experiment. We also compare the results obtained using different criteria for structurally defining the open and closed states based on the number of native contacts of each residue, the dynamics of hydrogen bonded residues or a combination of both criteria.

3317-Pos

Computational Prediction of Hotspots in Protein Misfolding for Rational Immunotherapy

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Physics-based algorithms can predict the misfolding mechanisms of proteins involved in aggregation-related diseases, including SOD1 whose misfolding template-directed conversion is involved in Amyotrophic lateral sclerosis and PrPc, wherein propagation of the misfolded protein is central to the the prion diseases. We have recently developed an algorithm capable of predicting thermodynamically likely regions for misfolding, by employing modeling which involves both atomistic interactions and surface-area based coarse-graining, along with a heterogeneous dielectric function inside the protein. Predictions based upon the algorithm are consistent with recent immunological assays that have uncovered disease-specific epitopes in SOD1 and prion protein, and point to diagnostic and therapeutic applications.
This research was performed in collaboration with Dr. Neil Cashman at the Brain Research Centre, University of British Columbia, and involved joint supervision of M.D./Ph.D. student Will Guest.

3318-Pos

Protonation/deprotonation Effects on the Stability of Trp-cage Miniprotein

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The effect on the folding/unfolding equilibrium of protonating the aspartic acid on the Trp-cage miniprotein is studied by explicit solvent moleclular dynamics simulations. Replica exchange molecular dynamics (REMD) simulations spanning the temperature range from 280K to 538K were carried out to the micro second scale using the AMBER99SB forcefield in explicit TIP3P water.
The root mean square distance from the backbone of the NMR structure shows two highly populated basins close to the native state with peaks at 0.6 A and 1.6 A which are consistent with previous simulations using the same forcefield. The fraction of folded replicas shows a drastic decrease because of the breaking of the salt bridge. However, significant populations of conformations with the arginine sidechain completely exposed to the solvent, but within the folded basin. This shows the possibility to reach the folded state without formation of the ion pair contrary to the expected.

3319-Pos

Force Field Dependence of Near-Equiilibrium Properties in a Beta Hairpin Peptide

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All-atom implicit solvent molecular dynamics simulations of the tryptophan zipper trpzip2 were carried out with a fast multiple time stepping integrator and a replica exchange method to improve sampling. Two modifications of the backbone dihedral angle potential energies in the AMBER ff99 parameter set were compared. Individual trajectories were run for over 375 ns, and aggregate simulation times were over 7.5 microseconds. Several measures of folding behavior in simulations begun from both folded and unfolded ensembles showed convergence to near-equilibrium values, allowing thermal phase behavior to be inferred and compared with experiment.

3320-Pos

The Mechanism of Geometrical Frustration in SH3

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Structure-based protein models build a minimally frustrated energy landscape to focus on the influence of geometrical factors on their dynamics, and they have demonstrated that the native structure is often sufficient to determine the folding mechanism. We customize structure-based models with a flexible interaction potential to investigate this geometrical control of the folding pathway. In the case of SH3 a polarized transition state results from the delayed formation of the N-terminal beta sheet. We isolate the contributions of the native contact map, of chain connectivity and of excluded volume interactions to identify their roles in the creation of this specific mechanism. While the native contacts are a direct expression of the native structure we find that the unspecific repulsion is essential to understand how geometrical frustration guides the folding process.

Heme Proteins

3321-Pos

A Biophysical-Biochemical Comparison of Hemoglobins from Mammoth, Asian Elephant, and Human

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This study is aimed at investigating the molecular basis of environmental adaptation of hemoglobin from tropical climate to low temperature in the Arctic region. We have carried out a biochemical-biophysical characterization of the structural and functional properties of hemoglobins from woolly mammoth (Hb WM) and Asian elephant (Hb AE) and compared those to human hemoglobins (Hb A and Hb A2) in 0.1 M phosphate buffer. Hb A consists of two α and two β subunits. Hb AE was found to contain two α subunits and two β/δ fusion subunits. Hb WM was expressed by inserting Asian elephant α-like and β/δ-like cDNA into our E. coli Hb plasmid (pHE2), and then introducing the mammoth-specific residue differences (αK5N, β/δT12A, β/δA86S, and β/δG101Q) into the Asian elephant plasmid. Since Hb AE and Hb WM contain β/δ fusion chains, we have also compared them to Hb A2, which contains δ chains instead of the β subunits present in Hb A. Oxygen affinity, Bohr effect, and cooperativity of oxygenation were measured at different temperatures and pH and 1H-NMR spectra were obtained for structural comparisons for each Hb. Our results show: (i) Hb AE has the higher O2 affinity as compared to Hb WM, Hb A2, and Hb A; (ii) the effect of an allosteric effector, inositol hexaphosphate (IHP), is the most prominent on Hb A2 as compared to Hb A, Hb AE, and Hb WM. 1H NMR results indicates that the α1β/δ1 and α1β/δ2 interfaces are perturbed in both Hb AE and Hb WM, whereas only the α1δ1 interface is perturbed in Hb A2 compared to Hb A. Hb AE and Hb WM have structural