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