polymers (i.e., polymer crystal structure not maintained during simulation) and extensive class-II force fields (CFF, PCFF, COMPASS, etc.) have not been parameterized for use in proteins. We have extended the CHARMM code so as to use a dedicated class-I force field for the protein, a class-II force field (image bond extended CFF, or newly implemented PCFF) for the polymer surfaces, and tuned electrostatic and van der Waals parameters for the interphase interaction. Results will be presented on the insufficiency of class-I force field for polymers and the suitability of the use of dual (one class-I and one class-II) force fields for solid-liquid interphase interactions relevant for protein adsorption on PLA polymers.

2088-Pos Board B58

Improving Molecular Mechanics Force Fields By Comparison Of Microsecond Simulations With Nmr Experiments

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Molecular dynamics simulations and NMR spectroscopy provide complementary approaches to the study of protein structure and dynamics. We have carried out several molecular dynamics simulations of globular proteins and compared the results to a range of NMR experiments that probe the structure and dynamics of these proteins. In particular, simulations on the microsecond timescale allow full sampling of the rotamer distribution of most of the protein side chains. Comparisons with NMR data suggest that, for some residues, this distribution may be incorrectly reproduced by common force fields. We quantified these discrepancies by performing simulations of small helical peptides and comparing the side-chain rotamer distributions with those found in the Protein Data Bank. The potentially problematic residues identified with this procedure were corrected by suitable modification of the force field terms. The performance of the modified force field was evaluated against NMR spectroscopy data.

2089-Pos Board B59

Extracting The Causality Of Correlated Motions From Molecular Dynamics Simulations

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We present a new method to extract the causality of correlated motions from molecular dynamics simulations. Applications of the method to the folded DNA-bound Ets-1 transcription factor show that helix H4 responds to the motion of helix H1, and that helix HI-1 responds to the motion of helix H4. Our calculations reveal how the presence of DNA is transmitted through the protein, ultimately leading to the unfolding of HI-1 upon DNA binding.

2090-Pos Board B60

Numerical Techniques to Optimize Free Energy Estimation Using Thermodynamic Integration

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Free energy estimation using thermodynamic integration (TI) involves numerically approximating an integral based on a limited set of discrete data points. These discrete data points represent the free energy slope as a function of the switching variable lambda for TI simulations. We present several numerical techniques for generating optimal free energy estimates utilizing polynomials, instead of the often-used quadrature, to fit the data and thus reduce the bias and uncertainty of the resulting estimates. The specific techniques utilized in our current study are Lagrange and Newton interpolation, cubic spline, and polynomial regression. To further improve the overall accuracy of free energy estimates using these techniques, we also investigated the use of non-equidistant lambda values (based on Chebyshev nodes) for thermodynamic integration simulations. Our results demonstrate that the use of non-equidistant lambda values and high degrees of polynomials gives the more accurate and precise free energy estimates compared to that of trapezoidal quadrature. Regression, in particular, offers the greatest flexibility that permits the degree of polynomial to vary for any desired accuracy without imposing any limitations on the number of lambda values.

2091-Pos Board B61

Free Energy Landscape of Biomolecules from Multiple Non-Equilibrium Molecular Simulations

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Computer simulations of biomolecules, e.g. molecular dynamics (MD), have gained widespread popularity in analyzing their behavior. One of the useful applications to reveal functional mechanisms of biomolecules is free energy calculation. Most of the current free energy calculation methods, however, rely heavily on the assumption that each trajectory approximates a quasi equilibrium ensemble of a target molecule. Since its violation may cause artifacts, practical use of short independent parallel simulations performed on massive parallel computer is still limited in the case of the system with slow equilibration time such as biomolecules. Hence it is highly demanded to develop the methods without this assumptions.

We propose "Multiple Markov transition Matrix Method", an algorithm by which a stationary probability distribution is estimated from non-equilibrium multiple MD trajectories independently generated with distinct Hamiltonians. Based on the Markovity assumption, we reconstructed a Markov transition matrix from the trajectories. Combining umbrella sampling technique and maximum likelihood estimation, we developed an optimization procedure to calculate the potential of mean force (PMF). The details will be described in the presentation.

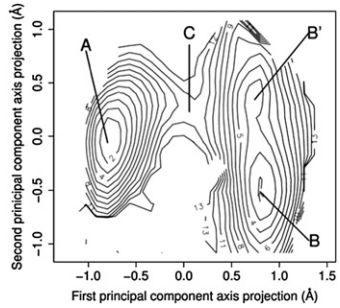

Figure 1:. Free energy landscape of Met-enkephalin calculated from non-equilibrium simulations using this method.

2092-Pos Board B62

The Extrapolated Motion Protocol For Molecular Dynamics Simulations: Predicting Large-scale Conformational Transitions In Mechanosensitive Channels

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Gating of ion channels involves large structural rearrangements and timescales posing challenges for conventional MD simulations. Forces imposed in steered MD protocols may lead to unnatural distortions, whereas near-physiological gradients produce small motions capturing just the local direction. We developed a new computationally efficient protocol allowing to 'continue' the observed small-scale motion and drive the protein along a self-chosen pathway. It was tested on bacterial channels MscL and MscS for which the initial outward motion of helices is pre-defined by membrane tension. The motion was initiated with a small (0.1-0.5 A) radial displacement of all atoms of the barrel away from the axis (step 1), followed by energy minimization (2), 1 ps relaxing MD simulation (3), and symmetry-restrained energy minimization (4). The conformational change resulting from this first cycle was linearly extrapolated with a small amplification coefficient and the three structure-relaxing steps (2-4) were repeated completing the next cycle. A sequence of 50-100 extrapolation/relaxation cycles produces a smooth pseudo-continuous trajectory revealing substantial conformational changes while preserving most of the secondary structure. The character of motion was sensitive to the amplification coefficient with 1.00 producing local oscillations, 1.05 - consistent moderate-scale motions, and 1.10 - larger transitions reaching instability. When applied to MscL, the method reproduced the characteristic iris-like gating supported by experiments. Extrapolations of the compact MscS model with reconstructed N-termini predicted barrel expansion with tilting and straightening of the kinked pore-lining helices. Extrapolations started with random thermal fluctuations produced trajectories similar to those started with a pre-conceived displacement. Open conformations of MscS reproducibly closed in extrapolations. Resting and open models of MscS based on families of extrapolated trajectories were refined in all-atom MD simulations, tested for conductance and received support by experiments.

2093-Pos Board B63

Collective variable-based calculations in NAMD

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The computational power of parallel clusters and supercomputers, and of the macromolecular simulation softwares typically used has been rapidly increasing in the recent years. One of the consequences is the higher demand for methods to analyze the dynamics and conformational space of biomolecular complexes. Several free energy calculation and enhanced sampling techniques have been developed in the past years, but only rarely they have been implemented altogether within a consistent "toolkit". Here, we introduce a new generalized interface for all those methods which rely on the definition of a set of collective variables. The code, implemented as a collective variables C++ module for NAMD (version 2.7), allows researchers in this field to choose

quickly the optimal setup for their calculations, and (if necessary) extend its functionality with an extremely small effort, unprecedented in the codes currently available. Also, the module is highly autonomous from the other NAMD source files, and can be easily adapted to other simulation programs as well. The set of features and their options will be introduced. Applications using the methods implemented so far (umbrella sampling, steered MD, adaptive biasing force and metadynamics) and make specific use of their combined advantages, will also be presented.

2094-Pos Board B64

Generating Pathways for Free Energy Calculations in Proteins Using Constraint-Based Conformational Sampling

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Constraint-based sampling [1] is a computational method for quickly exploring the allowed motions of a protein. Sampling of protein conformations is guided by a set of geometric constraints instead of a molecular mechanics force field. The geometric constraints preserve covalent bonding geometry, maintain favorable non-bonded contacts, and prevent steric overlap. We have tuned the constraints so that sampled conformations are low in energy according to a molecular mechanics force field (Amber). In this work, we apply the constraint-based sampling method in a targeted fashion to generate a pathway between two conformational end states in the protein dihydrofolate reductase (DHFR). The pathway we generate bridges the so-called ''closed'' and ''occluded'' states of DHFR, a transition that involves loop rearrangement near the binding site and relative rotations of subdomains. We then use this pathway as a starting point for free energy calculations. By performing molecular dynamics umbrella sampling [2] along the pathway, we obtain the free energy difference between the end states. Although the generated pathway is not necessarily the actual transition pathway, accurate calculation of the free energy difference between end states only requires that the pathway be low in free energy in the umbrella sampling method.

[1] Wells S, Menor S, Hespenheide B M, and Thorpe M F. Constrained geometric simulation of the diffusive motions in proteins. *Phys Bio* **2** S127-S136 (2005).
[2] Mamonova T and Kurnikova M. Structure and energetics of channel-forming protein-polysaccharide complexes inferred via computational statistical thermodynamics. *J Phys Chem* **110**(49) 25091-25100 (2006).

2095-Pos Board B65

Conformational Transition Path Sampling For Proteins

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Defining a reaction path (or reaction coordinate) is an essential step to understand chemical reactions, conformational change, or ligand-binding processes in proteins, and it is also important to consider protein-protein associations, for example, in immunology. However, conventional molecular dynamics simulation methods often fail to find appropriate reaction paths even with very huge computing facilities. This is because such reactions occur much more slowly than the computationally feasible time, and the sampling efficiency of such reaction paths can be very low especially for large proteins. Recent advance in transition path sampling techniques helps us to circumvent this annoying situation, but the application of such methods to large proteins has been rarely done. Using such transition path sampling methods, we examine the conformational change of a protein, adenylate kinase, after ligand binding. In this work, we propose a novel coarse-grained model for the protein to describe the ligand-binding processes in a realistic way. The purpose of this study is to clarify the conformational transition pathways in the protein, that is, there are two moving domains in the protein, and we try to understand which domain moves first to make a transition from the open to closed structures at finite temperatures. To quantify the result, we calculate the free energy surface along such a reaction path. We compare a zero-temperature path (intrinsic reaction path) and finite-temperature paths, and discuss the difference in terms of conformational entropy and other quantities. We furthermore carry out the transition-path study of the protein using the corresponding all-atom model, and discuss the difference between the coarse-grained and all-atom models.

2096-Pos Board B66

Computing Transitions in Macromolecular Systems: Dynamic Importance Sampling

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Understanding and predicting conformational change in macromolecules is central to linking structure and function. Performing straight-forward all-atom molecular dynamics would, in principle, enable sampling of conformational changes. However, the time-scale for functionally important transitions, exceeds the usual molecular dynamics timescales by several orders of magnitude. For example, with large amounts of computer time all these transitions could be observed with good statistics and the results collected simply by waiting long enough. Thus to sample on longer time-scales requires the development of biased molecular dynamics methods, where the bias can be applied and corrected for at the end. In our approach, called 'Dynamic Importance Sampling' we generate a series of independent trajectories that are conditioned on starting and ending in defined conformations. Trajectories are generated using two different algorithms: one uses a soft-racheting scheme based on stochastic trajectories and the other uses information from the set of normal modes. The algorithms, which require no initial pathway, are capable of rapidly determining multiple pathways between known states. The associated probablity scores, determined by correcting for the bias, allows us to rank order the most likely pathways. We will present examples from three-helix bundles and other systems for both analysis and possible experimental work.

2097-Pos Board B67

Improving The Computational Efficiency Of Non-Dynamical Approaches For Equilibrium Sampling Of All-Atom Protein Models

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We have been pursuing non-dynamical sampling methods which employ precalculation and storage of partial results, but limited energy calls during ''production'' sampling. Specifically, we have been using polymer-growth strategies to sample implicitly solvated all-atom polypeptides. Our original implementation was not efficient compared to standard Langevin dynamics (LD) simulations. We now describe a variety of technical advances - mostly in implementation, rather than in the algorithm - which have led to unprecedented efficiency compared to LD for several polypeptides. The efficiency comparison was performed using a novel statistical tool developed in our group.

2098-Pos Board B68

Evaluating The Effective Sample Size Of Equilibrium Molecular Simulations Using Automatically Approximated Physical States

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In order to assess ''convergence'' in molecular simulations and to quantify the efficiency of competing algorithms, we need a reasonable and universally applicable estimate of the ''effective sample size,'' N_eff. For equilibrium sampling, we suggest the most undamental definition of N_eff to be that number governing the variance in populations of physical states measured from multiple independent simulations. We demonstrate a simple automated procedure for approximating physical states and show that the resulting estimates for N_eff agree well with intuitive transition counts. A wide variety of biomolecular systems are successfully analyzed. Our approach can be applied to systems with unknown physical states and to modern non-dynamical algorithms, such as those based on the ''exchange'' mechanism.The necessary software for estimating N_eff will be freely available on our website.

2099-Pos Board B69

The "Weighted Ensemble" Path Sampling Method Can Find Target States Blindly And Automatically

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The computational sampling of rare transition events is a well appreciated challenge in chemical and biomolecular systems. Previously, we employed the ''weighted ensemble'' (WE) approach to path sampling and found it efficient in a simple protein model (PNAS, 104:18043, 2007). However, one drawback of the original WE formulation, and of other path sampling methods, is the requirement for a previously known target state and/or approximate reaction coordinate. We show that an improved, fully ''blind'' WE method does not require choosing any coordinates in advance. We demonstrate the correctness of the new approach, and quantify its efficiency, using a previously studied united-residue model of calmodulin. In addition, we have performed WE simulations using the CHARMM package to study alanine dipeptide. We find multiple structurally distinct pathways, highlighting the strength of WE in sampling multiple barrier-separated pathways.

2100-Pos Board B70

Accelerated Subspace Iteration Method for Protein Normal Mode Analysis

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Normal mode analysis is commonly employed to elucidate the conformational dynamics of proteins and related biological function. In typical applications, only a small subset of the complete set of frequencies and normal modes of