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

Daniel W. Farrell[1], Tatyana Mamonova[2], Maria Kurnikova[2], Michael F. Thorpe[1].
[1]Arizona State University, Tempe, AZ, USA, [2]Carnegie Mellon University, Pittsburg, PA, USA.

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

Hiroshi Fujisaki, Akinori Kidera.
RIKEN, Wako, Japan.

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

Juan R. Perilla, Anu Nagarajan, Oliver Beckstein, Thomas B. Woolf.
Johns Hopkins University, Baltimore, MD, USA.

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

Artem B. Mamonov, Daniel M. Zuckerman.
University of Pittsburgh Medical School, Pittsburgh, PA, USA.

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

Xin Q. Zhang, Daniel Mark Zuckerman.
University of Pittsburgh, Pittsburgh, PA, USA.

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

Bin W. Zhang, Daniel M. Zuckerman, David Jasnow.
Univ. of Pittsburgh, Pittsburgh, PA, USA.

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

Reza Sharifi Sedeh, Mark Bathe, Klaus-Jürgen Bathe.
Massachusetts Institute of Technology, Cambridge, MA, USA.

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

proteins is sought, corresponding to the large-scale collective motions of atoms. Here, the subspace iteration method is applied to all-atom representations of proteins to demonstrate its suitability to protein normal mode analysis. Important properties are that computational cost increases linearly with the required number of lowest eigenpairs and the method is robust computationally. Additionally, the procedure is particularly well suited to cases where numerous analyses are performed for nearby conformational substates, such as in conformational pathway analysis. Finally, the method is amenable to parallel implementation.

Biomolecular NMR Spectroscopy

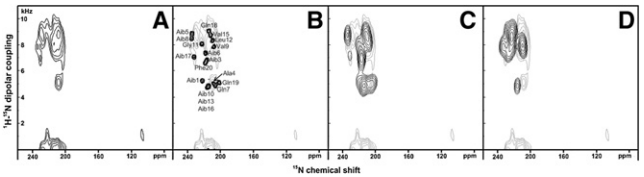
2101-Pos Board B71

Structure And Alignment Of Membrane-associated Peptaibols By Oriented 15N And 31P Solid-state NMR Spectroscopy

Evgeniy Salnikov[1,2], Herdis Friedrich[3], Xing Li[4], Philippe Bertani[1], Siegmund Reissman[3], Christian Hertweck[3], Joe O'Neil[4], Tatiana Ovchinnikova[5], Sergei Dzuba[2], Jan Rapp[6], **Burkhard Bechinger[1]**.
[1]University Louis Pasteur, Strasbourg, France, [2]Russian Academy of Sciences, Novosibirsk, Russian Federation, [3]University of Jena, Jena, Germany, [4]University of Manitoba, Winnipeg, MB, Canada, [5]Russian Academy of Sciences, Moscow, Russian Federation, [6]University of Leiden, Leiden, Netherlands.

Peptaibol antimicrobial peptides are produced by fungi and are characterized by a high content of hydrophobic amino acids, and in particular alpha-isobutyric acid Aib. Here several peptides from this family were uniformly labeled with ^{15}N, purified and reconstituted into oriented phophatidylcholine lipid bilayers and investigated by ^{15}N and ^{31}P solid-state NMR spectroscopy. Whereas alamethicin (20 residues) adopts transmembrane alignments in POPC or DMPC the much shorter ampullosporin A (15) and zervamicin (16) exhibit comparable configurations only in 'thin' membranes. In contrast the latter compounds are oriented parallel to the surface in 'thick' bilayers indicating that hydrophobic mismatch has a decisive effect. Two-dimensional ^{15}N chemical shift - ^{1}H-^{15}N dipolar coupling solid-state NMR suggests that in their transmembrane configuration ampulosporin and alamethicin adopt mixed alpha-/3_{10}-helical structures due to the restraints imposed by the membranes and the bulky Aib residues. The ^{15}N solid-state NMR spectra also provide information on the helical tilt angles, the details of this analysis depend on the appropriate choice of the ^{15}N chemcical shift tensor.

Figure: PISEMA spectra of alamethicin (A) and simulations of spectra resulting from 3_{10} (B,C) and mixed 3_{10}/α-helical conformations (D).



2102-Pos Board B72

Functional and Shunt States of Bacteriorhodopsin Identified and Characterized by Multidimensional DNP-Enhanced Solid State NMR

Melody L. Mak-Jurkauskas[1], Vikram S. Bajaj[1], Marina Belenky[2], Judith Herzfeld[2], Robert G. Griffin[1].
[1]Massachusetts Inst Technology, Cambridge, MA, USA, [2]Brandeis University, Waltham, MA, USA.

Bacteriorhodopsin (bR) is a 26 kDa archaeal membrane protein that harvests light energy to create an ion gradient across the cell membrane. Photoisomierzation of the retinilydene chromophore is coupled to ion translocation via a sequence of photocycle intermediates. Here we apply selective multidimensional solid-state NMR to uniformly ^{13}C,^{15}N-labeled bR in its native membrane to obtain chemical shifts in the chromophore of cryogenically trapped bR photointermediates. This is made feasible by using 250 GHz radiation to stimulate dynamic nuclear polarization (DNP), whereby the large spin polarization of unpaired electrons in exogenous free biradicals is transferred to nuclei. Subsequent N-C-C transfers in the NMR experiment allow us to distinguish four discrete substates of the L intermediate. Three of these are shunts that revert to the resting state of the protein upon thermal relaxation, while one L substate, labeled as 'persistent L' in our earlier 1D experiments, relaxes to the M state and is therefore deemed functional. Functional L has the strongest counterion, as indicated by its Schiff base (SB) nitrogen chemical shift. It also has a fully planarized 13-*cis* C13=C14 bond, as indicated by the gamma effect on the C12 chemical shift. These results are consistent with indications from time-resolved optical spectrometry and QM/MM studies of multiple barriers on the way to SB deprotonation. On the other hand, they are inconsistent with models in which the C13=C14 bond is twisted until Schiff base deprotonation. The experiments also demonstrate the use of DNP-enhancement at cryogenic temperatures to investigate mixed states of a membrane protein by multi-dimensional NMR. The results presented here would have been impossible without the availability of DNP to enhance spin polarization that is spread over multiple atoms in multiple protein states.

2103-Pos Board B73

C15=N Torsion Measured by DNP-Enhanced Solid State NMR in Bacteriorhodopsin Intermediates

Alexander B. Barnes[1], Melody L. Mak-Jurkauskas[1], Yoh Matsuki[2], Johan Lugtenburg[3], Judith Herzfeld[2], Robert G. Griffin[1].
[1]Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Brandeis University, Waltham, MA, USA, [3]Rijksuniversiteit te Leiden, Leiden, Netherlands.

Bacteriorhodopsin is a 26 kDa light-driven ion pump that establishes an ion gradient across the membrane of *Halobacterium salinarium.* Although it has been characterized extensively by a wide range of techniques, structural details pertinent to its mechanism are still under scrutiny. Of particular interest is chromophore torsion that would orient the protonated Schiff base favorably toward the proton acceptor until proton transfer occurs. Thus, a measurement of the C15=N torsion in L, the intermediate directly preceeding proton transfer, would yield evidence supporting one of the models proposed for the proton transfer. By performing dipolar recoupling between ^{13}C labels at retinal-C14 and Lys-Cϵ, we determined the distance between the labeled sites, and thus the torsion angle around C15=N.

Utilizing the sensitivity available with DNP (Dynamic Nuclear Polarization), only 7.4 hours is needed to record a 2D spectrum from 15 mg of protein, even when the intensities of interest are divided in a roughly 60:40 ratio, corresponding to two different intermediates. This demonstrates the utility of DNP-SSNMR in obtaining precise quantitative measurements in membrane proteins, even in mixed states.

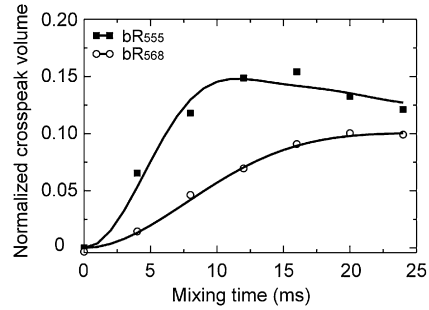


Figure 1. SSNMR recoupling build-up curve of retinal-14C, K216-Cϵ distance in bacteriorhodopsin. Data were fit to yield 3.11 +/− 0.02Å between 14C and Cϵ in bR_{555}, and 3.90 +/−0.08 Å in bR_{568}.

2104-Pos Board B74

Influence of Dynamics on The Analysis of Solid-State NMR Data From Membrane-bound Peptides

Erik Strandberg[1], Santi Esteban-Martin[2], Jesús Salgado[2], Anne S. Ulrich[1,3].
[1]Forschungszentrum Karlsruhe, Eggenstein-Leopoldshafen, Germany, [2]Universitat de Valencia, Valencia, Spain, [3]University of Karlsruhe, Karlsruhe, Germany.

By isotope labeling of membrane-bound peptides, typically with ^{2}H, ^{19}F, or ^{15}N, solid-state NMR experiments can yield data from which the orientation of peptides in a native membrane environment can be determined. Such an orientation is defined by a tilt angle and an azimuthal rotation angle.

Here we show that to obtain correct values of the orientation angles, it is important to include dynamics in the analysis of the NMR data. Nevertheless the effects of dynamics are different depending on the type of isotope labeling and NMR experiment considered.

To analyze the influence of dynamics in detail, we generated virtual NMR observables using a model peptide undergoing explicit Gaussian fluctuations of the orientation angles. For simulated ^{2}H- or ^{19}F-NMR data, even moderate motions were found to have a large influence, as calculated tilt values are consistently much too small, unless dynamics is properly considered. A simple