3031-Pos Board B78

Measuring Intramolecular Diffusion During Protein Folding Using an Ultrarapid Microfluidic Mixer

Steven A. Waldauer[1], Avinash Kane[2], Olgica Bakajin[2], Lisa Lapidus[1].
[1]Michigan State University, East Lansing, MI, USA, [2]Lawrence Livermore National Laboratory, Livermore, CA, USA.

Cysteine - Tryptophan contact quenching has been a valuable tool to measure intramolecular diffusion between specific amino acids. Until now however, this measurement has only been possible at equilibrium conditions. In this presentation, we present a novel technique combining contact quenching measurements with an ultrarapid microfluidic mixer. The mixer uses a serpentine mixing region to obtain mixing times within 50 μs and is deep etched in a fused silica substrate to allow UV absorbance measurements. With this instrument, we are able to measure quenching rates from 100 μs to milliseconds following mixing. This new experiment should prove a valuable new probe in measuring protein folding kinetics by observing the loss of conformational disorder.

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Exploring the Folding Landscape of Lambda Repressor with Microfluidic Mixing

Stephen DeCamp, Steven A. Waldauer, Lisa J. Lapidus.
Michigan State University, East Lansing, MI, USA.

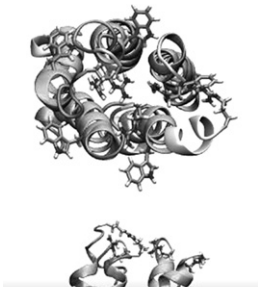
The folding kinetics of the λ-repressor protein have been explored with an ultrafast microfluidic mixer with a mixing time of 4 microseconds. Mutants with high stability (T_m=73°C), medium stability (T_m=54°C), and low stability (T_m=47°C) were tested and found to have an Arrhenius rate dependence around the cold denaturation temperature. Additionally, a surprising increase in molecular rate as the temperature approaches the heat denaturation temperature was also observed. Finally, the medium and low stability mutants exhibit a fast (molecular) rate which is not detected by T-jump experiments. These results suggest that chemical denaturation samples different pathways in the folding landscape than heat denaturation.

3033-Pos Board B80

Simulating The Folding And Assembly Of Viral Ion Channels

Jakob P. Ulmschneider.
University of Heidelberg, Heidelberg, Germany.

The success of ab-initio folding, adsorption and insertion of membrane proteins using implicit membrane models has been demonstrated. Of particular interest is the application of such novel methods to small channel-forming and antimicrobial peptides, as well as larger and more challenging membrane proteins. Are folding and insertion pathways comparable to experiment, to explicit bilayer simulations, or to the popular coarse-grain approaches? Simulations in the microsecond range performed by us reveal striking differences in folding and insertion pathways, as well as the predicted structure and thermodynamic behavior of small membrane bound peptides and motifs. Can the underlying models and parameters be tuned to overcome these discrepancies? We will present a newly developed semi-implicit Generalized Born membrane model, and the latest results of folding and oligomerization studies of viral channel formers.



3034-Pos Board B81

Insights On Protein Structure And Dynamics From Multiple Biased Molecular Dynamics Simulations

Fabrizio Marinelli[1], Stefano Piana[2], Fabio Pietrucci[1], Nevena Torodova[3], Irene Yarovsky[3], Alessandro Laio[1].
[1]International School of Advanced Studies (SISSA/ISAS), Trieste, Italy, [2]Curtin University of Technology, Perth, Australia, [3]RMIT University, Melbourne, Australia.

Exhaustive sampling of protein conformations is necessary to understand the molecular basis of protein structure and function. To achieve a sufficient exploration an enhanced sampling method is often required. Bias exchange metadynamics (J. Phys. Chem. B 2007, 111, 4553-4559) is a recently developed technique based on coupling several metadynamics simulations by a replica exchange scheme. Using this technique it was possible to reversibly fold the Trp cage miniprotein in explicit solvent using only 40 ns of simulation on 8 replicas. We show that using the same technique it is possible to fold, also in explicit solvent, even larger proteins. In particular, it is possible to predict the effect of a point mutation on Villin and Advillin Headpieces (J. Mol. Bio. 2008, 375, 460-470) and to obtain biologically relevant thermodynamic and kinetic informations on insulin chain B.

3035-Pos Board B82

Reordering Hydrogen Bonds In The Protein Backbone In Hamiltonian Exchange MD Enhances Sampling Of Conformational Changes

Jocelyne Vreede[1], Maarten G. Wolf[2], Simon W. de Leeuw[3], **Peter G. Bolhuis**[1].
[1]University of Amsterdam, Amsterdam, Netherlands, [2]University of Technology Delft, Delft, Netherlands, [3]University of Leiden, Leiden, Netherlands.

Molecular simulation enables detailed insight into the mechanisms underlying protein conformational changes and is complementary to experiments. Studying a protein folding reaction at atomistic resolution with conventional Molecular Dynamics (MD) is unpractical due to the long time scales involved. These long time scales originate from the presence of local free energy minima from which it is not trivial to escape.

The replica exchange MD (REMD) method allows for the escape of such local minima by letting multiple replicas diffuse through temperature space while maintaining the canonical distribution at the temperature of interest. Instead of switching temperatures, the replicas can also exchange (part of) their Hamiltonians. A smooth interpolation between the regular Hamiltonian and one that enables swift conformational changes of the protein (e.g. by lowering barriers) enhances sampling of the conformational space.

As hydrogen bonds play an important role in conformational changes of proteins, including folding, it seems natural to bias these bonds specifically. We have developed biasing potentials that allow the breaking and formation of bonds between arbitrary hydrogen bond donors and acceptors in the protein backbone. Employing these potentials in a Hamiltonian REMD scheme we aim to reorder hydrogen bonds in the protein backbone, and thus speed up the conformational sampling.

For a simple beta-heptapeptide test system, we show that our hydrogen bond switching scheme is four times more efficient in sampling the conformational space of the peptide as conventional temperature REMD, while it also has the advantage of not having to know the stable states in advance.

Finally, we discuss the slow convergence for larger systems, which is often observed in REMD or Hamiltonian exchange. This is caused by replicas having a preference for specific biasing potentials.

3036-Pos Board B83

Force Propagation in Proteins From Molecular Dynamics Simulations

Wolfram Stacklies, Frauke Graeter.
CAS-MPG Partner Institute for Computational Biology (PICB), Shanghai, China.

Mechanical force is routinely applied to proteins in force probing experiments and simulations to observe a protein's response to external stress. A yet unanswered question is how force propagates through proteins. How do perturbations like an external force flow through protein scaffolds and how is this related to protein stability and function?

We here present a new method based on molecular dynamics simulations that allows visualizing stress propagation in proteins, resembling finite element analysis for macroscopic structures. Using this method we elucidate force distribution in I27, an immunoglobulin domain from human muscle titin and one of the most stable proteins known. Hereto we monitor alterations in forces between pairs of atoms in the folded state upon pulling the protein with a constant force. We find forces to be a more direct measure for internal strain than the only minor changes in atomic coordinates. We observe that the externally applied force is anisotropically distributed throughout the protein scaffold highlighting three prominent regions that contribute most of the protein's mechanical resistance. The functional relevance of the force distribution network is highlighted by unfolding simulations of in-silico mutants and, interestingly, by comparison with a network of coevolved residues found in the titin immunoglobulin family. Both networks show a remarkable overlap thereby suggesting that the force distribution pattern reflects evolutionary constraints used to render I27 a mechanically robust protein. We also show that the method can easily be extended to other types of perturbation including point mutations and allosteric signals, such as ligand binding or phosphorylation.

3037-Pos Board B84

The Free Energy Reaction Path Theory of Reliable Protein Folding

Gregg Lois, Jerzy Blawzdziewicz, Corey O'Hern.
Yale University, New Haven, CT, USA.

A growing number of experiments and simulations detect long-lived metastable and intermediate states in proteins, which suggests that protein folding does not