3031-Pos Board B78

Measuring Intramolecular Diffusion During Protein Folding Using an Ultrarapid Microfluidic Mixer

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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.

3032-Pos Board B79

Exploring the Folding Landscape of Lambda Repressor with Microfluidic Mixing

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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

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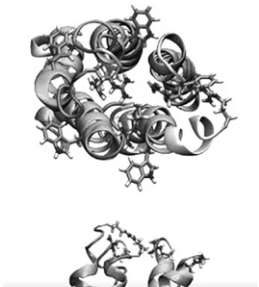
The success of ab-initio folding, adsorption and insertion of membrane proteins using implicit membrane models has been demonstrated. Of particular interest is the application of such novel methods to small channel-forming and antimicrobial peptides, as well as larger and more challenging membrane proteins. 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

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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

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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

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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

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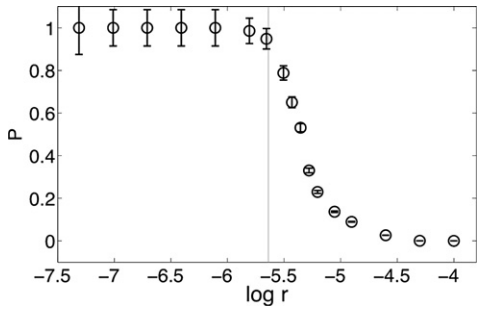
A growing number of experiments and simulations detect long-lived metastable and intermediate states in proteins, which suggests that protein folding does not

always occur on funneled energy landscapes. We introduce a theoretical framework to understand folding in the presence of metastable and intermediate states by considering the statistics of protein conformational dynamics on rugged energy landscapes. Our analysis reveals that, even for the most frustrated proteins, reliable folding can occur on rugged energy landscapes and is sensitive to the rate that external parameters are adjusted to induce folding. When folding is reliable, there is always a well-defined reaction path leading to the native state.

We test the predictions of our statistical analysis using simulations of a model protein. In the accompanying figure we plot the probability P to inhabit the native state after inducing folding by reducing the temperature at rate r. Reliable folding only occurs below a limiting rate that is correctly predicted by theory (red line).



3038-Pos Board B85

2*D*-string Theory of Biomolecular Bundle Space

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The *Biomolecular Bundle Space* is presented as a topological space with a *finite group* structure of size 36, DO_{GU}. The helical patterns of 2*D*-strings replace vibrational patterns of 1*D*-strings. Possible configurations of these helical patterns form 9 distinct *polyhedra*, from tetrahedron to icosahedron, and the *fundamental element* is a pair of equilateral triangles forming the *diamond simplex*. These polyhedra are distributed over the 3*D*-branes reflecting the DO_{GU} group structure. The two-sheet *3D-branes* are a *torus* for the sheet having the *symmetric* diamond simplex, and a *Klein bottle* for that having the *asymmetric* diamond simplex. The torus brane represents the backbone structure of the *nucleic acids, DNA, RNAs* and the Klein bottle brane that of *proteins*. We present the *fundamental elements* of the bundle space *B* and its *projection p* to the corresponding *base space X*. The base space has, as a translational symmetry, congruence (*mod* 6). The *transcription code* (Genetic Tableau) is directed by the *Transcription Shuttle* tetrahedron, the *translation code* (*tRNA+rRNA*) by the *Translation Key* truncated tetrahedron, and the *René Thom Cobordism code* (Protein Space) by the entire set of 9 distinct polyhedra of the biomolecular bundle space. The relative rotational energies of the polyhedral elements of the DO_{GU} group are calculated by the organizing centers of the *René Thom's catastrophes*. This classification provides a unified approach to analyze the relationships within the bundle space.

3039-Pos Board B86

Microsecond Explicit Solvent Molecular Dynamics Simulations of Protein Folding

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Explicit solvent molecular dynamics (MD) simulations of protein folding offer information on the protein folding process with tremendous temporal and spatial resolution. Long timescale protein folding trajectories can aid in interpretation of experimentally observed protein folding kinetics, and in the design of new fast-folding mutants. At the same time, protein folding simulations offer a demanding test for MD force fields to assess their accuracy in describing long-timescale conformational transitions in proteins. Until recently, however, little overlap existed between the timescales accessible to simulations and the time required for small proteins to fold. Recent experimental advances have lead to the discovery and characterization of a variety of proteins which fold on the 1-10 microsecond timescale; at the same time, increased computational power has made multiple microsecond timescales accessible for explicit solvent MD simulations.

We performed multiple-microsecond folding simulations of two well-characterized fast-folding proteins, namely the villin headpiece subdomain and Pin1 WW domain. The villin headpiece folds to a native state after ~6 microseconds of molecular dynamics simulation. Furthermore, a common folding mechanism is observed in multiple simulations from different starting conditions, where all secondary structure elements form over 1-2 microseconds after an initial hydrophobic collapse, but the native structure is only obtained after a complete dissociation and rearrangement of the secondary structure elements relative to each other. In the case of the WW domain, the protein misfolds in all simulated folding attempts. Conformational free energy calculations indicate that the WW domain's native state, a three-stranded beta sheet, is significantly higher (~ 9 kcal/mol) in free energy for the force field (CHARMM22/CMAP) used than several misfolded helical structures obtained from folding simulations. Our results agree with several other recent studies in suggesting a bias toward helical secondary structure in modern MD force fields.

3040-Pos Board B87

Ab Initio Determination of Tryptophan Fluorescence Quenching by Histidine Cation in HP35-N27H, Barnase, and T4Lysozyme

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The fast folding, 35-residue villin headpiece, HP35, has been at the center of numerous protein folding rate simulations. Eaton et al. have experimentally followed the folding with the N27H mutant, plausibly because the protonated His27 quenches the fluorescence from Trp23 in the folded form by electron transfer, but not when unfolded. Because of this, at least in some simulations of the folding, a major criterion for the folded form is close proximity of His27 to Trp 23. Protonated His is indeed a potent quencher of Trp fluorescence in solution and in some, but not all, proteins. For quenching to occur the energy of the Trp-to-His charge transfer(CT) state must be low enough to be in resonance with the excited state of the Trp. This resonance is dictated by the electric potential difference between the Trp and His due to local protein environment. In some proteins the electric field enables quenching, and in others it does not. We have carried out QM-MM simulations of quenching by His cation for Trp23 in in villin, Trp94 in barnase and Trp138 in Q105H T4 lysozyme using ab initio electronic coupling [Callis et al J. Phys. Chem. B; 2007; 111(35); 10335-10339] Preliminary results indicate that for villin and T4 lysozyme the lowest CT state is that of the amide backbone of Trp. In contrast, the lowest CT state for barnase has His cation as the electron acceptor. We have also computed electronic coupling matrix elements between 3-methylindole and imidazole cation for all three proteins. The average coupling is 58, 329, and 2 cm-1 for the T4 lysozyme, barnase, and villin cases, respectively, suggesting that the change of Trp fluorescence upon folding in villin is not because of quenching by His27.

3041-Pos Board B88

Cu Involvement In Prion Oligopeptide Stability: Experiments And Numerical Simulations

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The enormous sociological impact of neurodegenerative diseases (like Alzheimer disease, Transmissible Spongiform Encephalopathies, Parkinson disease, etc.) has pushed the attention of researchers towards the study of the rôle played by metals in the misfolding process, as they are regarded as a possible concurrent cause of protein aggregation and plaque formation.

Metals are, in fact, essential players in many of the fundamental activities of cells. Storing, metabolism and trafficking of metals through the cellular membrane and within the cytoplasm is mediated by many proteins via well tuned mechanisms because of the toxicity of free ions.

With a combination of X-ray Absorption Spectroscopy (XAS) technique and numerical ab initio simulations we have investigated the physico-chemical basis of the aggregation phenomenon, which is suspected to be at the basis of the development of the amyloidosis.

In this presentation we will summarize the results of the our experimental and numerical investigations aimed at understanding the possible rôle of Cu in stabilizing the Prion protein

structure and in the formation of protein polymers.

3042-Pos Board B89

Folding Pathway And Free Energy Landscape Of Villin Headpiece Subdomain HP35 Studied by String Method

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The string method with swarm of trajectories is applied here to find the most probable folding pathways of the 35-residue villin headpiece subdomain (HP35). The converged pathway, represented by 61 discrete images, fully