landscape of PGK reveals a multitude of barriers, pressure-jump experiments spanning timescales from microsecond to hours showed only one predominant several-minute-long kinetic phase. These observations may indicate that under pressure, the smaller kinetic barriers of PGK are smoothed out by the large positive activation volume of the transition state.

1318-Pos Board B48

Accelerated Monte-Carlo Simulations for All-Atom Protein Folding

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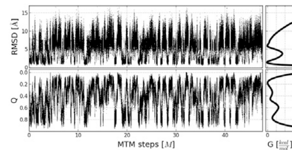
Recent advances in the computational description of proteins facilitate the atomic-level characterization of fast folding proteins using molecular-dynamics simulations[1]. However, due to the femtosecond timestep of an MD simulation, 10 ˆ 10 computationally expensive energy evaluations are required to reach timescales of the order of single folding events even for ultrafast-folding proteins. Stochastic simulation techniques can overcome this limiting factor by generating thermodynamic conformational ensembles without incorporating atomic vibrations, but most of these methods are inherently sequential. Here we investigate a parallel extension to the conventional Metropolis-Hastings algorithm, the Multiple-Try-Metropolis, enabling parallel simulation of reversible Markov-chains[2,3]. We successfully characterize the thermodynamic landscape of the Villin-headpiece-subdomain using a generalized MTM approach adapted to protein systems. We parameterized the polypeptide using the AMBER99SB-STAR-ILDN-forcefield with an implicit solvent model. In our simulations we could observe approximately two folding transitions per day on standard hardware, with a total of 109 transition events during 3*10ˆ9 energy evaluations. The MTM approach thus facilitates the efficient thermodynamic characterization of proteins on common computer architectures. The comparison with experimental resolved folding times of 8μs translates to a time equivalent of 16ps per MTM step.



[1] Shaw, Science-2010.
[2] Liu, JASA-2000.
[3] Pandolfi, JMLR-2010.

1319-Pos Board B49

Thermodynamic Characterization of Protein Folding using Monte Carlo Methods

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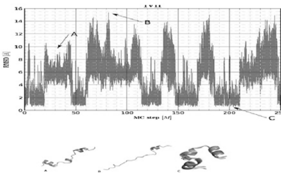
The study of protein folding has been a difficult challenge in molecular biology and simulation science.

Recent publications showed the reproducible folding of small protein using molecular dynamics simulations. This large conformational changes can be only achieved by using specialized supercomputers [1]. In contrast to molecular dynamics simulations, Monte Carlo based simulations are not constrained solving Newton's equation of motion and therefore may be used conventional computer architectures to sample the protein folding process. Here we show fast reproducible all-atom folding transitions of the villin headpiece (Figure 1) simulated using SIMONA[2], a Monte Carlo based simulation package for nanoscale simulations including a variant of the Amber99SB*-ILDN[3]. The results demonstrate the applicability of Monte Carlo simulation techniques to the investigation of large-scale conformational changes of proteins on conventional computer architectures. The computing time for observing folding/refolding events can be significantly reduced in comparison with molecular dynamics simulations. The thermodynamic characterization of simulated proteins can be compared with the experimental results.



[1] Shaw, D. E. et al. Science 330, 341-346 (2010).
[2] Wolf, M., Strunk, T. et al. J Comput Chem (2012).
[3] Lindorff-Larsen et al. Science 334, 517-520 (2011).

1320-Pos Board B50

Mechanistic Explanation of Different Unfolding Behaviors Observed for Transmembrane and Soluble β-Barrel Proteins

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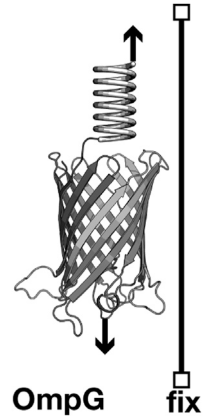
In response to mechanical stress, membrane proteins progress through sequences of major unfolding barriers, whereas soluble proteins usually must overcome only one major unfolding barrier. To gain insight into these markedly different unfolding behaviors, we applied force-probe molecular dynamics simulations and unfolded two β-barrel proteins, the transmembrane outer membrane protein G (OmpG) and the water-soluble green fluorescent protein (GFP). The simulations mimic with high precision the unfolding experiments and show that OmpG in the absence of a membrane and GFP circumvent high unfolding barriers by rotations and explore alternative unfolding pathways. Embedding OmpG in the lipid membrane restricts this search for pathways and forces the protein to cross high unfolding barriers. Likewise, restricting the rotation forces GFP to traverse high unfolding barriers in a similar manner to membrane-embedded OmpG. These results indicate that mechanically stressed proteins search alternative unfolding pathways by rotations and explain why membrane proteins generally show higher mechanical stability compared to water-soluble proteins.



1321-Pos Board B51

Deciphering Folding Pathways of Phage T4 Lysozyme: Influence of Multiple Conformations

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Understanding how a linear strand of amino acids folds into an active protein in the cell is still one of the challenges in biochemistry. Moreover, as quite a distinct amount of misfolded proteins are responsible for diseases, e. g. Alzheimer's. Thus, understanding the folding pathway, hence describing the energy landscape, of a protein is of critical importance. Here, we used the model enzyme lysozyme from the phage T4 (T4L), a simple two domain protein, which already exists in an equilibrium of at least three conformations in the folded state, a situation expected from most other proteins as well.

Thus, we created a set of double mutants of the cysteine-free pseudo-wild type by inserting an unnatural amino acid and a cysteine mutation, which builds up a network of distances spanning the enzyme. Later, we site-specifically labeled them using orthogonal chemistry with a Förster resonance energy transfer (FRET) dye pair. The unfolding process was monitored on the one hand using traditional ensemble methods like CD- spectroscopy and on the other hand with our fluorescence spectroscopic methods (ensemble steady-state fluorescence, ensemble time correlated single photon counting, single molecule fluorescence spectroscopy, filtered fluorescence correlation spectroscopy). Single molecule high precision FRET, with its high temporal and spatial resolution covering seven time decades, enables us to determine transition rates between conformational states in equilibrium and in denatured conditions. The combination of our fluorescence spectroscopic tool provides enough information to describe the energy landscape of the unfolding process of T4L and its paths to the unfolded conformations.

1322-Pos Board B52

Kinetic of Loop Formation in Polypeptides and Free Energy Landscapes

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The kinetics of contact formation between the two ends of a polypeptide has been the focus of extended interest in the recent and less recent past. One of the reasons is that contact formation is the elementary event underlying processes such as folding and binding. More importantly, it is experimentally measurable with increasing time and space resolution; it can be predicted theoretically for ideal polymers. Deviations from single exponential kinetics has been sometimes interpreted as a signature of a rugged, protein-like, free energy landscape. Here we present simulations of short peptides with different structural propensity and of a structured protein with different atomistic models. Results show a remarkable deviation from exponentiality