fluorescence accounts for the popularity of characterizing proteins via this spectroscopic technique. However, molecular level detail that accounts for emission shifts is lacking. Here we address the problem of emission shifts caused by amino acid conformational changes in aqueous solution by examining a variety of tryptophan dipeptides in the zwitterionic and anionic states. In addition to fluorescence emission, we employ UV resonance Raman (UVRR) spectroscopy, molecular dynamics simulation, and ab initio calculations. In our previous study of TrpGly and GlyTrp dipeptide species, a correlation between fluorescence emission shifts and UVRR W10 band shifts and W7 band intensity ratios were discovered. Nanosecond molecular dynamics simulation coupled with energy minimization showed that the dipeptide species adopt two basic conformations: one with a 'stretched' backbone or one with a 'curled' backbone. For TrpGly zwitterions in the stretched conformation, the terminal amine cation is 2.44 Å from the pyrrole ring C3. The Stark effect predicts that a positive charge near the pyrrole ring blue shifts the emission maximum, which is observed. Remarkably, rotamers do not show a correlation with the dihedral angle, chi 2. Rather, the dihedral angle, chi 1, takes on three discrete values that account for the 'stretched' and 'curled' backbone conformations. We have continued in this manner to study Trp dipeptides with Glu, Lys, Tyr, His and Trp, showing that both the pattern of backbone interaction with the indole and the spectroscopic pattern of changes with dipeptide species persists in spite of residue variation in charge, aromaticity and size.

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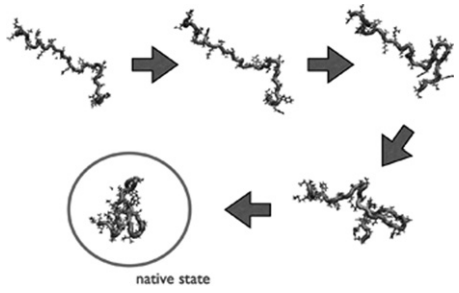
Atomistic Simulations of Dominant Pathways in Protein Folding: From a Simple WW Domain to a Complex a Knotted Protein

Pietro Faccioli, Silvio a Beccara, Tatjana Skrbic, Roberto Covino.
Trento University, Trento, Italy.

MD simulations of protein folding using parallel or distributed computing can at most assess small chains with folding times in the μs range. The Dominant Reaction Pathways (DRP) approach now allows to simulate the folding of medium-sized proteins on just few tens of CPUs. We discuss the results of the DRP simulations of the folding of two proteins with different size: the 35 aminoacid WW domain Fip35 and the 83 aminoacid knotted protein YibK. Our results for Fip35 agree with those obtained by Shaw and co-workers, indicating a dominant path in which the first hairpin folds before the second. However, at temperatures below the folding temperature a sub-dominant pathway emerges, in which the order of formation of the hairpins is reversed. This finding can explain the results of the phi-value analysis of Jäger and co-workers. Next, we present our results of the folding YibK, a much more complex protein with a self-entangled native structure and we discuss how the dominant folding pathway suggests a precise mechanism for knot formation.



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The Geometrical Origins of a Protein Folding Mechanism

Heiko Lammert, Jeffrey K. Noel, Jose N. Onuchic.
University of California San Diego, La Jolla, CA, USA.

The principle of minimal frustration, drawn from energy landscape theory, is realized in structure-based protein models. Folding mechanisms for many proteins are reproduced by these models exclusively on the basis of geometrical constraints inherent to the structure of the native state. We demonstrate how excluded volume interactions are instrumental in making these geometrical constraints effective, and we identify the interactions that determine the structurally polarized transition state of an SH3 domain.

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Entropic Insights from Millisecond Protein Simulations

Andrew T. Fenley, Michael K. Gilson.
U.C. San Diego, San Diego, CA, USA.

The configurational landscape of proteins and other macromolecules contains distinct and possibly long lived states that differ minimally in their total free energy. However, the similarity in the respective total free energy of these long lived states can mask drastic differences in their free energy profile, i.e. alternative balances of entropy and enthalpy. An accurate investigation of these differences requires an exhaustive search of the configurational space coupled with tools capable of elucidating the key interactions and correlations that govern the states and their associated transitions. Here we use the recently published Anton 1ms molecular dynamics (MD) trajectory of bovine pancreatic trypsin inhibitor (BPTI) coupled with the mutual information expansion (MIE) of the configurational entropy to develop an in depth analysis of a major conformational divide controlled by a single disulfide bridge. The disulfide bridge flips the system between two states that are similar in free energy but different in entropy and enthalpy. Due to the unprecedented level of configurational sampling from the 1ms MD trajectory, we are able to account for 3rd order correlation terms of the coordinates in the MIE formalism, which yield a non-negligible addition to the total configurational entropy. These novel calculations provide insight regarding the fundamental mechanisms of entropy-enthalpy compensation.

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Analysis of Native H/D Exchange Dynamics in EX1/EX2 Conditions using Structure Based Model Simulations

Patricio O. Craig[1], Ryan M.B. Hoffman[1], Joachim Lätzer[2], Patrick Weinkam[3], Elizabeth A. Komives[1], Peter G. Wolynes[4].
[1]UCSD, La Jolla, CA, USA, [2]Rutgers University, Piscataway, NJ, USA, [3]University of California at San Francisco, San Francisco, CA, USA, [4]Rice University, Houston, TX, USA.

The analysis of kinetic and thermodynamic data measured at single residue level, and the perturbation produced by mutagenesis or denaturant agents, may be useful for tuning simulation parameters, and provide a precise description of proteins structural dynamics. Phi value and hydrogen exchange are some of the experimental techniques that provide this type of information at different depths on the folding funnel. The development of methods to quantitatively compare these experimental parameters with simulation is required. We recently developed a method for the quantitative analysis of native HX protection factors in EX2 conditions using coarse grain structure based model simulations, which allow a fast and thorough analysis of both local and global unfolding transitions. This analysis applied to ubiquitin, chymotrypsin inhibitor, and Staphylococcal nuclease, allowed us to establish optimum structural definitions for the exchange competent and incompetent states. In this work, we extend the method to study the exchange dynamics of ubiquitin in the EX1 limit, which provides information about the rate of the opening reactions. A variety of simulation models with homogeneous, heterogeneous, additive as well as non additive contact potentials were evaluated for their agreement with experiment.

Acknowledgments: Financial support NIH.

2280-Pos Board B50

Inverse Temperature Jump System to Study Fast Protein Folding Kinetics

Mark E. Polinkovsky[1], Yann Gambin[2,3], Michael Erickstad[1], Ashok Deniz[2], Alex Groisman[1].
[1]University of California, San Diego, La Jolla, CA, USA, [2]The Scripps Research Institute, La Jolla, CA, USA, [3]University of Queensland, Brisbane, Australia.

A protein's conformation depends on the protein's chemical and physical environment, including the temperature, pH, and denaturant concentration. The changes in protein structure can be visualized by Förster resonance energy transfer (FRET) between donor and acceptor fluorescent dyes bound to separate residues on the protein. One method of studying protein folding kinetics is the temperature jump, where a protein sample is quickly heated by a laser pulse to trigger a change in the protein conformation. During and after the pulse, a fluorescence excitation laser illuminates the protein sample and the FRET signal is collected. Traditional temperature jump methods have very short heating times (~1 ns), facilitating the observation of protein folding events triggered by heating. However, due to the relatively large heated volume (~1 nL), the long cooling time (hundreds of μs) obscures cooling-driven protein folding events (tens of μs). In order to observe fast folding events, the protein sample is often placed in non-native conditions: its unfolded state is imposed by low temperatures, usually at a high denaturant concentration, so that the heating laser pulse will induce the protein to refold. We have designed a novel system, which we call the inverse temperature jump or iT-jump, that enables observations of fast folding transitions by cooling the protein sample on a timescale of <1 μs. The rapid cooling is made possible by placing the sample in a shallow microfluidic channel and tightly focusing the heating laser beam to a volume of ~1 fL. The proposed system inverts the existing T-jump paradigm, as the