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Identifying Two-State Transitions by Microcanonical Analysis: Coarse-Grained Simulations of Helical Peptides

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Two-state folding is an important feature in protein thermodynamics. It describes the transition between a native and a denatured state without intermediates that are populated at equilibrium. A widely used test for a two-state transition is a calorimetric criterion which probes features in the canonical specific heat curve. However, this criterion does not suffice to identify a two-state transition [Zhou et al., Protein Science, 8 (1999)]. Nevertheless, a microcanonical analysis, where the density of states is directly measured, can provide unambiguous information about the nature of the transition.

In this work, we use generalized-ensemble simulations to calculate the density of states of a simplified implicit solvent, four bead per amino acid coarse-grained model which is not biased to a protein's native state. The thermodynamics of different helical model peptides are studied. Our results show strong correlations between the energetics at the transition temperature and structural rearrangements of the native state: as chain length increases, the helix breaks into bundles and tertiary contacts become important. Statistical models have suggested that cooperativity in helical bundles arises from the interplay between secondary and tertiary interactions (e. g. [Ghosh and Dill, JACS, 131, 2306 (2009)]). Our results corroborate this picture: only an amino acid sequence that can stabilize a well-defined hydrophobic core in a helical bundle will exhibit a first-order like transition. We illustrate this by performing a microcanonical analysis of a 73-residue long de novo three-helix bundle.

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Tryptophan Fluorescence Quantum Yield Predictions in the Study of Protein Folding. Ac-Trp-Ala-Ala-Ala-His-NH2 and Villin Headpiece Subdomain

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In the study of protein folding dynamics followed by tryptophan (Trp) fluorescence, a common motif has emerged. This motif has the following amino acid sequence, Trp-X-X-X-His+. Two examples are the villin headpiece subdomain (HP35) (PNAS 2005 102, 7517) and a synthetic peptide Ac-Trp-(Ala)3-His+-NH2 (Angew. Chem. Int. Ed. 2009, 48, 5628). The common assumption is that the strongly quenching His+ contacts the Trp in the helical folded form, leading to a lower fluorescence intensity. For HP35, however, both experiment and our 12 ns QM-MM simulation show that the quenching is mostly by the Trp backbone amide. The electronic coupling between the Trp ring and amide is constantly large and the energy gap between the fluorescent state and the amide charge transfer state is small. In contrast, quenching by electron transfer to His+ in the folded form of HP35 is often diminished due to large Trp-His distances when the His+ is salt bridged with Glu72 or Trp is in a cation-pi stacking with Lys65. Preliminary MD simulations on Ac-Trp-(Ala)3-His+-NH2, on the other hand, show that in the helical form, Trp and His+ are virtually always in close contact. We are currently investigating the ratio of amide vs His+ quenching in the folded and unfolded forms.

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Exploring the Kinetics and Folding Pathways of Trp-cage using a Kinetic Network Model

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Protein folding is a slow process occurring via a cooperative transition of many degrees of freedom. It is therefore difficult to obtain meaningful information on the kinetics and mechanism of such a process from all-atom simulations. Advanced sampling methods such as replica exchange molecular dynamics (REMD) can significantly enhance the ability to obtain accurate canonical populations in complex systems. However, the kinetic information is lost due to temperature swaps. We take advantage of the power of REMD, collecting the samples from the REMD simulation and organizing them into a kinetic network. We show that kinetic information such as folding pathways, Pfold values and folding rate constants can be obtained from this network either by direct simulation using the Gillespie algorithm or by linear algebra and graph theory. Clustering techniques can also be applied to the network to vary the size and complexity of the network to required resolution. The technique is illustrated for the Trp-cage mini-protein. The results from the kinetic network are analyzed and projected on a small number of appropriate reaction coordinates for a clear interpretation of folding pathways and the folding mechanism.

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Insights from Molecular Simulations into the Temperature-Induced Collapse of Unfolded Proteins

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Single molecule Förster resonance energy transfer (FRET) and dynamic light scattering experiments on the unfolded state of a small cold shock protein have shown a compaction with increasing temperature, in contrast to expectations for a simple polymer (1). A comparable collapse of the intrinsically disordered protein prothymosin a suggests that the temperature-dependence of the hydrophobic effect is not the sole reason for the collapse. We have used all-atom replica exchange molecular dynamics simulations with explicit solvent to investigate the origin of the collapse. We find that the results of the simulation are dependent on the protein force field, and particularly on the solvent model employed. Use of the TIP4P-Ew water model together with the Amber ff03* protein force field (2) produces qualitatively similar results to experiment, however with TIP3P water, the protein is found to expand with temperature; this probably reflects the more accurate temperature dependent properties of TIP4P-Ew. The simulations suggest that collapse is correlated with the formation of additional intramolecular hydrogen bonds and loss of hydrogen bonds to water, as well as the formation of more turn and beta-structure, consistent with CD measurements. Finally, we have also used simulations of simple model compounds to investigate the molecular origin of the temperature dependence of the intrachain interactions.

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An Alternately Charged Residue Cluster at the N-terminal End Forms a Ring System and Dynamically Regulates Calsequestrin Polymerization

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Calsequestrin (CASQ2) mediated calcium buffering and release is the key for muscular contraction and relaxation. Upon Ca^{2+} binding CASQ2 undergoes polymerization in a linear fashion by front-to-front dimerization and back-to-back packing to form the wire-shaped structures as observed by electron microscopy. Being enriched in negatively charged residues, the C-terminal half of the molecule has been considered to be important for Ca^{2+} binding capacity and function of CASQ. However, the recent finding of R33Q mutation leading to lethal **Catecholaminergic Polymorphic Ventricular Tachycardia** (CPVT) in human indicates importance of N-terminal end. By protein sequence analysis we have found a cluster (DGKDR) of alternating positively and negatively charged residues in the N-terminal end, that include residue R33, conserved from *C. elegans* to both CASQ isoforms in human. Systematic deletion and charge neutralization mutagenesis was coupled to circular dichroism, limited proteolysis, turbidimetric assays and computational molecular dynamics to illustrate that the cluster works as a molecular switch. Molecular dynamics studies illustrate the dipolar arrangement in the cluster brings about a critical flip of D32 residue essential for stabilization of dimer by formation of hydrogen bond network and arrange the cluster into a ring system. Results show that Ca^{2+}-induced CASQ2 aggregation is reversible, non-linear and can be resolubilized to native conformation by Ca^{2+}-chelation with EGTA. However CASQ2 mutation with alteration in the charge pattern in the cluster, including the R33Q mutation, disrupt the ring system and reduce the backbone flexibility, thus impairing the response to Ca^{2+}-induced aggregation and Ca^{2+}-chelation by EGTA leading to loss of reversibility of polymerization. We propose that under increased physiological demands the R33Q mutant fails to undergo dynamic conformational interconversions necessary to cope with increased Ca^{2+} handling and thereby lead to CPVT.

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Modeling and Simulation of Rodlet Assembly of Hydrophobin EAS in an All-Atom Free-Energy Forcefield

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In this investigation we simulate the rodlet assembly of an Class-I hydrophobin mutant EAS D15D8 in all-atom free-energy Monte-Carlo simulations. Results