3293-Pos

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.

3296-Pos

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.

1. D. Nettels, S. Müller-Späth, F. Küstera, H. Hofmann, D. Haenni, S. Rüegger, L. Reymond, A. Hoffmann, J. Kubelka, B. Heinz, K. Gast, R. B. Best, B. Schuler. ''Single molecule spectroscopy of the temperature-induced collapse of unfolded proteins'', Proc. Natl. Acad. Sci., in press.
2. R. B. Best, G. Hummer. ''Optimized Molecular Dynamics Force Fields Applied to the Helix-Coil Transition of Polypeptides'', J. Phys. Chem. B, 113, 9004-9015 (2009).''

3297-Pos

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.

3298-Pos

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

show that rodlet assembly is possible without additional beta strand formation in the flexible loop regions.
Hydrophobins are small amphipathic proteins characterized by their unique bond pattern of eight Cysteine amino acids and a resulting similar tertiary structure, although only marginal sequence conservation exists within the family. In this work we elucidate the structure of the assembled rodlets in Class-I hydrophobins. The initial model of the EAS mutant is created using homology modeling of the solution structure of EAS allowing for a rigid structure omitting most of the flexible loop regions. A large population of possible docking structures is further generated using a generic protein-protein docking approach and filtered for candidates with distinct amphipathicity to account for the presence of an air-water interface.
The resulting structures are further relaxed in the all-atom-free energy force-field PFF02[1] using the POEM (Protein Optimization using Energy Methods) program package in parallel relaxation runs. Relaxation of the whole population was possible using the distributed volunteer computing platform POEM@HOME (http://boinc.fzk.de/).
[1] A.Verma, W.Wenzel, A Free-Energy Approach for All-Atom Protein Simulation, Biophysical Journal, Volume 96, Issue 9, 2009, P. 3483

3299-Pos
Modeling Protein Stability
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We will present our recent model of protein thermodynamics to better understand the role of different forces in determining the origin of protein stability. A long standing question in the field of protein biochemistry has been how does a protein achieve enhanced stability. This has lead to careful thermodynamic studies of several thermophilic proteins to understand the origin of high stability in these proteins. However, conclusions have often been contradictory. Our recent model elucidates thermodynamics of these proteins and comparison of thermophilic and mesophilic protein thermodynamics indicates a possible general strategy that proteins may employ to gain high stability. Our analysis is based on several thermophilic and mesophilic proteins with different fold and sequence. We compare relative roles of enthalpy, entropy and specific heat in stability determination. Our model qualitatively explains experimental data and also provides an explanation for apparently conflicting findings from different experimental studies. Furthermore, we predict stability based on our model and demonstrate quantitative agreement with experimental data.

3300-Pos
A Toy Model for Calculating the Rate and Position of Amyloid Fibril Dissociation
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In a previous paper we used a statistical model to calculate the form of the dependence of the amyloid association rate constant on the size of an unfolded polypeptide chain (Hall, D and Hirota, N., (2009) *Biophys. Chem.* **140***, 122-128*). In the current work we use a Langevin dynamics based simulation to examine the breakage/dissociation rate of an amyloid fibril in a solution environment. We treat the protein monomers in the amyloid fibril as point particles enclosed by a monomer shell. These encapsulated monomers are joined together by virtual bonds which break when extended beyond a certain limit. The solvent environment is treated as a viscous fluid capable of producing random fluctuating forces that are sensitive to the relative position, but not the velocity of the monomer units making up the amyloid fibril. The simulation results suggest how the rate of fibril breakage /dissociation will alter in response to changes in certain characteristic properties of the amyloid, namely the bonding arrangement, the constituent polypeptide size, the strength of the fibril bond and the existence of bond defects in the fibril. Additionally we use the simulation results to make comment on the likelihood of any position bias with regards to the event of fibril fragmentation - a finding which has important consequences for the 'infectivity'of amyloid fibres *in vivo*.

3301-Pos
Cooperative Folding Kinetics of BBL Protein and Peripheral Subunit-Binding Domain Homologues
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Recent experiments claiming that Naf-BBL protein follows a global downhill folding raised an important controversy as to the folding mechanism of fast-folding proteins. Under the global downhill folding scenario, not only do proteins undergo a gradual folding, but folding events along the continuous folding pathway also could be mapped out from the equilibrium denaturation experiment. Based on the exact calculation using a free energy landscape, relaxation eigenmodes from a master equation, and Monte-Carlo simulation of an extended Munoz-Eaton model that incorporates multiscale-heterogeneous pairwise interactions between amino acids, here we show (1) that the very nature of a two-state cooperative transition such as a bimodal distribution from an exact free energy landscape and biphasic relaxation kinetics manifest in the thermodynamics and folding-unfolding kinetics of BBL and peripheral subunit-binding domain homologues. Our results provide an unequivocal resolution to the fundamental controversy related to the global downhill folding scheme, whose applicability to other proteins should be critically reexamined.
(1) Proc. Nat'l. Acad. Sci. (USA), Vol.105, 2397-2402 (2008).

3302-Pos
Fast Prediction of Protein Thermodynamics
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A computational method for a Flexibility And Stability Test (FAST) on three-dimensional protein structure is described. A four-dimensional free energy landscape defined by temperature and three order parameters is calculated in matter of minutes. The order parameters characterize structure and solvent properties. Specifically, they depict the number of native vs. disordered residues that are within a particular solvent state. Thermodynamic properties are derived from the free energy landscape, including stability curves and heat capacity over all experimentally accessible temperatures. Protein flexibility and correlated motions for a given thermodynamic and solvent condition are also calculated to highlight structural mechanisms. A Free Energy Decomposition (FED) is employed to account for essential enthalpy-entropy compensation mechanisms that include: hydrogen bonding, chemical diversity among residues, atomic packing, strain and vibration energy, pH effects, solvation effects such as clathrate water interacting with residues, hydrophobic effects due to water transfer from buried regions to bulk solvent, and network rigidity. Network rigidity is a long-range underlying mechanical interaction that accounts for conformational entropy nonadditivity during Free Energy Reconstitution (FER). In contrast to molecular simulation, FAST is based on a free energy functional that is solved using self-consistent mean field theory. Individual free energy components come from molecular partition functions that are parameterized from a combination of long all-atom molecular dynamics simulations in explicit solvent, and empirical fitting to experimental data. FAST is a unified model that accounts for several modes of protein denaturation driven by extreme: temperature, pH, and concentration of co-solute. Pressure dependence will also be incorporated in future work. Because of its computational efficiency, FAST can be used in high throughput applications (i.e., design) to assess the consequences of all FED components on the free energy. This work is supported by NIH grant R01-GM073082.

3303-Pos
Non-Covalent Interactions Involving Aromatic Residues in Protein Structures: Stability and Dynamics in Membrane and Globular Proteins using Molecular Dynamics Simulations
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Recent studies have revealed the importance of non-covalent interactions in proteins viz. conventional & non-conventional H-bonds. Importance of interactions involving π electron cloud of aromatic residues namely C-H $\cdots\pi$, D-H$\cdots\pi$, lone-pair$\cdots\pi$ and cation$\cdots\pi$ have recently been recognized. Most of the studies involved crystal structure analysis of proteins or ab-initio calculations. However, the dynamic properties such as stability and life time of these interactions through experimental studies have not been investigated due to difficulties in carrying out such experiments.
In this study, we carried out simulations on four globular and two membrane proteins with different secondary structural contents. The dynamic nature of six different non-covalent interactions was analyzed to identify their behavior over time within and across the different classes (all-α versus all-β) and different types (globular versus membrane) of proteins. Some of the properties analyzed were, fraction of each type of interaction that was maintained throughout the simulation, maximum residence time (MRT) and the life time of the interactions. Our preliminary investigation reveals that conventional H-bonds are dominant (~60%) interactions and is mostly due to main-chain functional groups. They are predominantly stable with a MRT of at least 10 ns, owing to their role in maintaining the secondary structure of proteins. Our analysis reveals that C-H$\cdots$O interactions involving the main-chain Cα and main-chain carbonyl oxygen atoms are the second most dominant interactions in the all-β-proteins. Large proportion of them is relatively more stable. Cation$\cdots\pi$ interactions are