

Protein Folding & Stability II

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Direct Characterization of Hydrophobic Hydration during Cold and Pressure Denaturation

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Many computational approaches for large biomolecular systems renormalize the role of the solvent into the definition of effective inter-residue interactions by coarse-grained models to access biologically relevant time-scales. However, the discrete nature of water and its effects on protein stability, dynamics and function cannot be explored in a predictive way with models that implicitly describe the solvent. We present here, a new off-lattice coarse-grained protein-like polymer model combined with a coarse-grained water model to correctly capture the solvent contribution in determining the stability of the collapsed state and molecular interactions. The water model includes tetrahedral interactions and correctly describes the phase diagram of water. The protein model exhibits pressure, cold and thermal denaturation. We will present the methodology and the results of how the length of a protein model and sequence of hydrophobic and hydrophilic monomers affects protein stability. We will show a mechanistic picture of how changes of hydrophobic hydration drive cold and pressure denaturation of proteins.

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Myoglobin. Addition of Chemical Details to the Coarse-Grained Model Enhances Resolution of Key-Features of RMS-Fluctuation in Monte-Carlo Simulated Data and Produces Universal Distribution of Amino-Acid Content into Four Transitional Groups

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Mechanism that is driving (un)folding transitions in a protein is one of the oldest biological devices ever built by evolution. Protein is a product of a DNA-RNA-Protein line. The mechanism of (un)folding must be universal. Protein reaches very high packing density during folding. These four conditions outline our side-chain backbone based Core-Shell model. The model develops the idea that the self-assembly takes place simultaneously at two - the peptide and side-chain, backbone levels. The Sliding Mechanism of (un)folding transitions developed in the Core-Shell model states, that under unfolding conditions, the rigid-peptide plane "softens" and the side-chain backbone performs transverse discrete moves across the peptide backbone. Sliding mechanism provides a residue's stereochemistry-specific score function that places the residue in one of four distinct Transitional groups.

Present work is a computational study of fluctuations of folded protein at near unfolding temperature. Our computational model of protein - a rigid-peptide plane model with Gō interactions, is simple enough to allow a step-by-step addition of details. We showed earlier, that there is a reasonable qualitative agreement between NMR, X-ray and the simplified model simulated data for small proteins. Here we show that a) though the simulation data is not accurate due to the lack of chemical details in the forcefield, the rms-fluctuation profile of simplified model Myoglobin detects better all key features of conformational fluctuations of native state protein; b) the addition of Ramachandran propensities improves resolution of computational key-features. The distribution of rms-fluctuation data into four Transitional Groups obtained for amino acid content of Myoglobin is strikingly similar to the distribution of 20 amino acids into Transitional groups - a proof on universality.

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Size, Shape and Motions of the SH3 Domain of the *Drosophila* Adapter Protein Drk

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The SH3 domain is a commonly found modular binding domain that functions to mediate protein-protein interactions and has been a frequent target of protein folding and other biophysical studies. The N-terminal SH3 domain (6.8 kDa) of the *Drosophila* adapter protein Drk is marginally stable under physiological conditions and exists in nearly 50/50 equilibrium between the folded and unfolded states, with a slow conversion rate of 2.2 s^{-1} (1). Due to the dynamic ensemble nature of this unfolded state under non-denaturing conditions it is difficult to characterize structurally. Experimental data have been used to define sets of heterogeneous conformations but ensemble averaging minimizes the information content (2). Importantly, little is known about inter-conversion rates within the unfolded ensemble.

Fluorescence correlation spectroscopy (FCS) experiments performed on the wild-type and on a mutant that stabilizes the folded state (T22G) provided the hydrodynamic radii of the folded ($R_{h,f}$), unfolded ($R_{h,u}$) and denatured ($R_{h,d}$) states at 20°C. Quite surprisingly, the results indicate that the unfolded state under non-denaturing conditions is less compact than the chemically-denatured state ($R_{h,u}/R_{h,f}=1.31 \pm 0.05$ vs. $R_{h,d}/R_{h,f}=1.21 \pm 0.03$). However, this can be explained by introducing adequate shape factors in previously reported NMR/SAXS data. In addition the FCS measured compactness of unfolded state is in an excellent agreement with PFG-NMR value of $R_{h,u}/R_{h,f}=1.30 \pm 0.01$ (2). Furthermore, FCS analysis reveals a fast 200 ns process within the unfolded ensemble. To resolve slow (milli)second conformational dynamics, dual cysteine mutant (E2C-60C) was labelled with the Bodipy FI/Alexa 647 FRET pair and single proteins were encapsulated in surface-tethered liposomes for long observations. This study provides further insights into the unfolded state heterogeneity and its importance in protein folding in general.

1.M Tollinger et al., J. Am. Chem. Soc.;123(46):11341-11352 (2001).

2.WY Choy et al., J. Mol. Biol.;316(1):101-112 (2002).

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Four-State Folding of a SH3 Domain: Salt-Induced Modulation of the Stabilities of the Intermediates and Native State

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Intermediates detected in the folding pathways of small proteins often serve as 'stepping stones', since they possess significant structural information. For many of the apparent two-state folders, it is merely a question of using the right folding conditions, or of using the right experimental probe, before a folding intermediate is revealed. The refolding of the PI3 kinase (PI3K) SH3 domain (previously assumed two-state, $U \leftrightarrow N$), had been shown to commence with the formation of a non-specific structure-less collapsed globule, U_C^1 . Kinetic folding studies were performed on this protein with the addition of a stabilizing co-solute to investigate the interplay of collapse and structure formation in the earliest steps of folding. A molten globule-like, on-pathway intermediate, L, stabilized by 500 mM Na_2SO_4 , was detected within 6 ms of the initiation of folding. Both in the absence and presence of 500 mM Na_2SO_4 , unfolding experiments also revealed an on-pathway native-like intermediate, M. The folding mechanism was therefore modelled using a minimal linear four-state mechanism (given below) which includes intermediates detected both before (U_C and L) and after (M) the rate-limiting step of the folding reaction. $U \leftrightarrow (U_C \leftrightarrow L) \leftrightarrow M \leftrightarrow N$ Ongoing experiments aimed at elucidating the structural properties of L using tyrosine fluorescence, ANS fluorescence and multi-site FRET coupled to rapid mixing techniques indicate that L is a specific structural component of the collapsed ensemble in equilibrium with U_C . Similar experiments are also being performed to characterize the structural properties of the unfolding intermediate, M.

Reference:

1. Dasgupta, A. & Udgaonkar, J. B. (2010). Evidence for initial non-specific polypeptide chain collapse during the refolding of the SH3 domain of PI3 kinase. *J Mol Biol* **403**, 430-45.

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Hexafluoroisopropanol Induced Helix Sheet Transition of Stem Bromelain Correlation to Function

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Stembromelain is a proteolytic phytoprotein with a variety of therapeutic effects. Understanding its structural properties could provide insight into the mechanisms underlying its clinical utility. Stem bromelain was evaluated for its conformational and folding properties at the pH conditions it encounters when administered orally. It exists as a partially folded intermediate at pH 2.0. The conformational changes to this intermediate state evaluated using fluorinated alcohols known to induce changes similar to those seen in vivo. Studies using circular dichroism, fluorescence emission spectroscopy, binding of the hydrophobic dye 1-anilino-8-naphthalene sulfonic acid and mass spectrometry indicate that treatment with 10-30% hexafluoroisopropanol induces the partially folded intermediate to adopt much of the native protein's secondary structure, but only a rudimentary tertiary structure, characteristic of the molten globule state. Addition of slightly higher concentrations of hexafluoroisopropanol caused transformation from an α -helix to a β -sheet and induced formation of a compact nonnative structure. This nonnative form was more inhibitory of cell survival than either the native or the partially folded intermediate forms, as measured by enhanced suppression of proliferative cues and initiation of apoptotic events. The nonnative form also showed better antitumorigenic properties, as evaluated using an induced two-stage mouse skin