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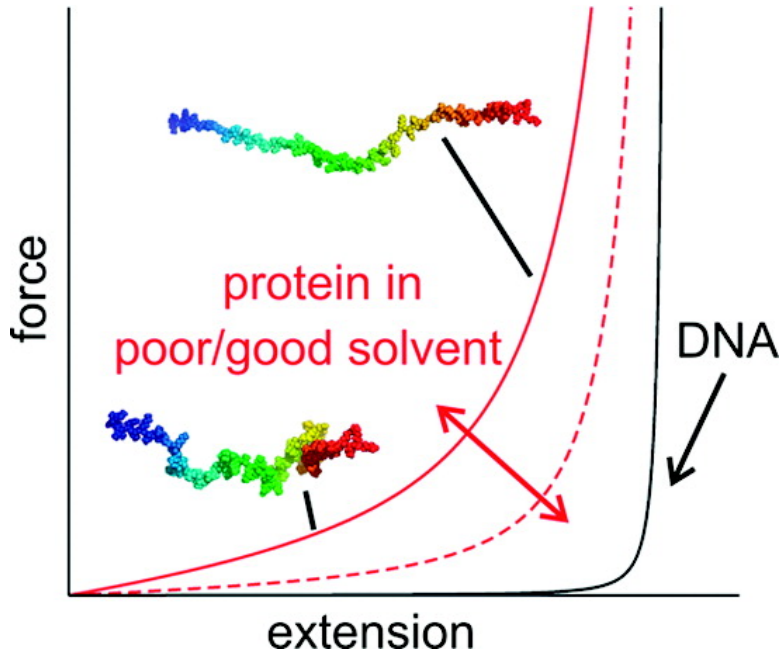

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Dissecting Entropic Coiling and Poor Solvent Effects in Protein Collapse

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Protein folding to specific functional structures is preceded by a largely unspecific protein collapse into a molten globule state which reflects protein elasticity. Contributions to protein elasticity, however, are not yet well understood. Experimental measurements on single protein chains using force spectroscopy reveal a force−extension behavior in reasonable agreement with polymer random coil models such as the worm-like chain model (WLC).[1] WLC fits to such data yield an effective persistence length in the range $p_{eff} = 0.3-0.6$ nm (Figure 1a)[2,3] as a measure of protein elasticity, in agreement with end-to-end distances of loops in protein structures.[4] Surprisingly, recent measurements at low force again do not reveal any departure from purely entropic chain behavior.[5] Consequently, a major effort has been focused on the development of theories for protein elasticity solely based on chain entropy,[6,7] with departures from entropic coiling behavior only considered at high force in the form of enthalpic stretching.[8,9] The apparent random coil behavior, however, is not in agreement with the common notion of protein hydrophobic collapse. Protein unfolding involves exposure of hydrophobic side chains buried in the protein core of the folded state, and hence, water is a poor solvent for unfolded or disordered proteins.[10] Recent FRET,[11] AFM,[2] or NMR experiments[12] reveal signatures for hydrophobic collapse in primary refolding events. Poor solvent conditions drastically change the equilibrium and dynamical properties of polymers[13,14] and also entail pronounced deviations from random coil stretching behavior, as predicted[15,16] and recently shown by AFM studies of polystyrene.[17]

We aim to resolve the contradiction between the hydrophobic nature of unfolded proteins and their apparent worm-like chain behavior. To assess the entropic chain contribution to protein elasticity, which corresponds to the coiling behavior of a protein in a good solvent (gs), we employ an all-atom random coil model of the protein ubiquitin[18] using a modified force field, in which only bonded interactions and the repulsive part of the Lennard−Jones interactions (C12) were kept. This entropic chain incorporates the correct volume exclusion, backbone geometry, and conformational freedom of the protein and ignores nonlocal interactions and solvent-induced effects. It therefore corresponds to an unfolded protein chain in an optimal solvent. The force−extension curve of this model, determined from MD simulations, closely follows WLC behavior, with a persistence length of $p_{gs} = 1.2$ nm, a finding remarkably sequence-independent (Suppl. Figure 1). In this particular context, we refer to this chain as a "stiff chain" because it has a persistence length roughly three times larger than the commonly assumed values (Figure 1a). Given the rigidity of the π-conjugated amide bond and the restriction of the two remaining dihedrals to a specific region on the Ramachandran plot, the obtained persistence length of approximately three amino acids in length is very reasonable.[13,19] It is also in quantitative agreement with recent findings for unfolded proteins[11,20] and with the high population of extended conformations expected in the absence of nonlocal interactions and secondary structure.[21]

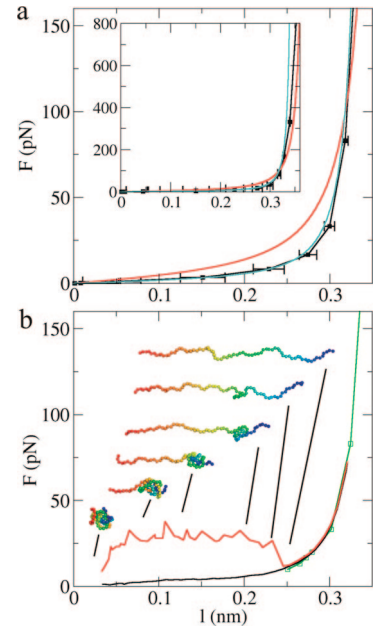

Figure 1. Force−extension behavior in good and poor solvents. (a) For a protein in a good solvent obtained from all-atom simulations of ubiquitin as an entropic chain (black). A WLC fit gives a persistence length of $p_{gs} = 1.2$ nm (cyan), remarkably different to a WLC with $p_{eff} = 0.4$ nm experimentally found (red). (b) For a protein in a poor solvent obtained for a coarse-grained (bead−spring model) hydrophobic chain (red), in comparison to the dwell length of the same model under force−clamp (green, compare Suppl. Figure 3) and to good solvent conditions (black). l is the average length per residue.

To clarify the role of poor-solvent effects, a simplified coarse-grained model of a protein-like entropic chain was employed consisting of 76 spheres at the positions of C-α atoms of ubiquitin (0.5 nm in diameter) connected by springs, with angle restraints chosen to give the backbone stiffness of ubiquitin. The force−extension behavior of this chain in a poor solvent was calculated by adding an effective pairwise interaction potential to mimic the hydrophobic effect, estimated from the attraction between small hydrophobic spheres in water (Figure 1b). At low to intermediate end-to-end distances of the chain, some of the spheres are involved in forming a globule via nonlocal contacts, and extension requires ∼20 pN to enforce a continuous globule-to-coil transition. For extensions larger than l ∼0.23 nm, the hydrophobic chain shows the same force−extension behavior as the purely entropic chain, since the penalty for chain bending then exceeds the benefit from contact formation. An analytical derivation of the stretching response of

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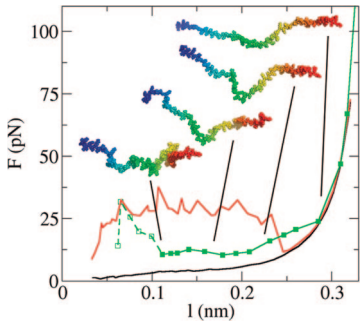

Figure 2. Force extension behavior for a protein chain in poor solvent, for which the hydrophobic attractions between residues are assigned to the side chains (green). This model shows moderate deviation from the entropic chain (dashed black) for $l > 0.1$ nm.

worm-like chains in poor solvents (Supporting Information (SI) and Suppl. Figure 2) confirmed this finding in that no significant contraction of the poorly solvated chain over the noninteracting chain was observed in this regime. We expect the hump-like profile to be a characteristic of a polymer in a poor solvent, when the length scale of the attractive forces (~1 nm) is similar or smaller than the persistence length (1.2 nm), i.e. for relatively stiff chains. As expected, the drop in force at medium extension and the resulting hump disappear for a softer polymer ($p_{gs} = 0.4$ nm) in a poor solvent, modeled here by omitting angle constraints in the coarse-grain model (Suppl. Figure 4). The force plateau directly passes into the entropic chain behavior, since nonlocal contact formation is not hampered by bending rigidity even for large chain extensions. A plateau[15,16] for flexible chains and a hump-like shape for comparably stiff chains have been predicted theoretically[22] and have been found in force spectroscopy measurements of single hydrophobic polymer chains in water.[17]

Proteins are stiff polymers ($p_{gs} = 1.2$ nm) in poor solvents, yet their experimental force−extension behavior does not exhibit a hump but instead approximately follows an apparent worm-like chain behavior. As an important feature of proteins and a possible explanation for this discrepancy, hydrophobic forces are expected to be strongest between the protein's hydrophobic side chains. To investigate the effect of side chains, we determined the force−extension profile of an all-atom protein chain in which pairwise attractions are exclusively assigned to the side chains (Figure 2, green curve; see SI for details). As expected, interside chain interactions endure up to significantly larger extensions, as reflected by an offset in force from the respective behavior in good solvent up to $l = 0.30$ nm. The attractive side chain interactions thus allow proteins in poor vs good solvents appear softer, instead of causing the pronounced hump of other stiff polymers. Our simple model with hydrophobic interactions also quantitatively captures the major features of protein collapse in water after force quench as observed by force spectroscopy,[23,18] namely the average dwell length (0.8 of the 100 pN length at 10 pN) and the high cooperativity of the coil-to-globule transition (Figure 1b and Suppl. Figure 3).

Our study suggests that the persistence length commonly used to analyze AFM force−extension data reflects an effective protein elasticity incorporating entropic as well as attractive interactions, most likely of hydrophobic nature, which lower the persistence from $p_{gs} = 1.2$ nm to p_{eff} ~0.6 nm measured in water.[11,5] The low force regime ($F < 20$ pN) is dominated by nonlocal, supposedly hydrophobic interactions, while in the high force regime stiff entropic chain behavior and enthalpic stretching are dominant. According to our model, the strong hydrophobic effect in water compacts an unfolded protein against its bending rigidity. The high chain stiffness combined with solvent mediated attraction between side chains consequently results in a force−extension behavior pronouncedly different from the plateau (Suppl. Figure 4) found for the relatively flexible polystyrene chains in water[17] or the hump-like behavior (Figure 1) found for simple stiff chains.[22] We thus find that the incorporation of a correct entropic chain persistence into coarse-grain models frequently used for collapse and folding simulations is crucial,[24,25] while the details of a chosen elasticity theory to describe protein collapse (worm-like chain versus freely rotating chain) is of minor importance, because the dominant contributions are not due to chain entropy. The recent advances in force spectroscopy studies in conjunction with solvent dependency of protein collapse will help test our predictions.

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Supporting Information Available: Supplementary force−extension curves, collapse trajectories, derivation of a modified worm-like chain formulation, and simulation methods.This material is available free of charge via the Internet at http://pubs.acs.org.

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