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Studying the role of cooperative hydration in stabilizing folded protein states

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ABSTRACT

Understanding and modelling protein folding remains a key scientific and engineering challenge. Two key questions in protein folding are (1) why many proteins adopt a folded state and (2) how these proteins transition from the random coil ensemble to a folded state. In this paper we employ molecular dynamics simulations to address the first of these questions. Computational methods are well-placed to address this issue due to their ability to analyze systems at atomic-level resolution. Traditionally, the stability of folded proteins has been ascribed to the balance of two types of intermolecular interactions: hydrogen-bonding interactions and hydrophobic contacts. In this study, we explore a third type of intermolecular interaction: cooperative hydration of protein surface residues. To achieve this, we consider multiple independent simulations of the villin headpiece domain to quantify the contributions of different interactions to the energy of the native and fully extended states. In addition, we consider whether these findings are robust with respect to the protein forcefield, the water model, and the presence of salt. In all cases, we identify many cooperatively hydrated interactions that are transient but energetically favor the native state. Whilst further work on additional protein structures, forcefields, and water models is necessary, these results suggest a role for cooperative hydration in protein folding that should be explored further. Rational design of cooperative hydration on the protein surface could be a viable strategy for increasing protein stability.

1. Introduction

Two important questions have been identified in protein folding (Creighton, 1985). First, why do many proteins adopt folded conformations rather than forming an ensemble of unfolded conformations? Second, how do folded proteins transition from an ensemble of unfolded conformations to the correct folded conformation? In this paper we address the first question of why proteins fold. More accurately, we attempt to identify why currently-used computational models predict that proteins fold. The two questions above have been studied extensively using experimental and computational techniques (Anfinsen and Scheraga, 1975; Baker, 2000; Ben-Naim, 1991; Best and Hummer, 2010; Bryngelson et al., 1995; Dill, 1990; Dobson et al., 1998; Eisenberg and McLachlan, 1985; Fersht et al., 1992; Hartl and Hayer-Hartl, 2009; Kauzmann, 1959; Levinthal, 1968; Levy and Onuchic, 2006; Martin et al., 1991; Miranker et al., 1993; Nicholls et al., 1991) (Berger and Leighton, 1998; Duan and Kollman, 1998; Freddolino et al., 2010; Levitt, 1976; Levitt and Warshel, 1975; Li and Scheraga, 1987; Pande et al., 2003; Piana et al., 2011; Shaw et al., 2010; Sippl, 1993; Snow et al., 2002; Sugita and Okamoto, 1999; Voelz et al., 2010; Wang and Wang, 1999). Studies on protein-folding kinetics have highlighted the importance of intermediate states (Ptitsyn et al., 1990), cooperativity (Dill et al., 1993), free energy barriers (Shastry and Roder, 1998), and folding funnels (Bryngelson et al., 1995). Studies on protein-folding thermodynamics have focused mainly on the balance between two (non-mutually exclusive) types of intermolecular interactions (Ben-Naim, 1991; Dill, 1990; Hendsch and Tidor, 1994; Lazaridis et al., 1995; Myers and Pace, 1996; Pace et al., 1996, 2011, 2014; Strickler et al., 2006):

1) Hydrogen-bonding/polar interactions – Folded states commonly exhibit numerous hydrogen-bonding interactions between protein residues (McDonald and Thornton, 1994). However, these must compete with hydrogen-bonding interactions between the protein and the surrounding water

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and thus the balance of terms must be considered (Ben-Naim, 1991; Fernández et al., 2002; Fersht et al., 1985; Levy and Onuchic, 2006).

2) Hydrophobic/non-polar interactions – Folded states also exhibit numerous non-polar interactions between the protein residues (Nicholls et al., 1991). Again, these must compete with interactions between the protein and the surrounding water.

In this work we consider a third mechanism in addition to those above: cooperative hydration of protein surface residues. The presence of water at the protein surface leads to a complex network of hydrogen bonds, with water molecules bridging interactions between protein residues. It has been suggested that this allows residues to be mutually solvated, forming strong cooperative interactions (Ben-Naim, 2011; Busch et al., 2013). Cooperative hydration effects have been identified as important in numerous other contexts (Guzmán et al., 2006; Okada and Tanaka, 2005; Pastorczak et al., 2013; Schellman, 1987). Thus, we are particularly interested in the prevalence of cooperative hydration and its effect on protein folding. Whilst analyses of polar and non-polar contributions to the energy and free energy of protein folding have been performed previously (Lazaridis et al., 1995; Makhatadze and Privalov, 1993; Privalov and Makhatadze, 1993; Robertson and Murphy, 1997), to our knowledge this is the first study that considers the difference between direct and water-mediated hydrogen-bonding interactions. We address this by considering the three mechanisms described above using average energies from long-timescale molecular dynamics (MD) simulations (Fenley et al., 2014; Roy et al., 2014). As a test case, we consider the villin headpiece, one of the mainstays of protein-folding studies (Duan et al., 1998; Fernández et al., 2003; Freddolino and Schulten, 2009; Jayachandran et al., 2006; Kubelka et al., 2003; Lee et al., 2000; McKnight et al., 1996; Mittal and Best, 2010; Wang et al., 2003). We explore the native state, a fully extended state, and three intermediate states (see Fig. 1).

To augment this analysis, we also consider the role of water using inhomogeneous fluid solvation theory (IFST), a statistical mechanical method that considers the contribution of individual hydration sites to the free-energy of the system. IFST has been used previously to study small-molecule hydration (Huggins, 2014b; Huggins and Payne, 2013; Lazaridis, 2000), protein-ligand binding (Haider and Huggins, 2013; Li and Lazaridis, 2005; Young et al., 2007), and artificial host-guest systems (Nguyen et al., 2012). We are also interested in how the choice of system treatment affects the predictions. Thus, we consider the effect of different water models, different force fields, and the presence or absence of salt.

2. Methods

2.1. System setup

We model five states of the of villin headpiece domain (McKnight et al., 1996): one native, one extended, and three intermediate states. The native state of the villin headpiece N68H mutant was taken from PDBID 1YRF (Chiu et al., 2005). An extended protein structure was generated from the protein sequence using Schrödinger's peptide builder script, setting the phi angles to −71.6° and the psi angles to 135° for proline residues and the phi angles to −135° and the psi angles to 135° for all other residues (Zagrovic et al., 2002). The use of a fully extended state allows us to compare the native structure to a structure in which protein-water interactions are maximized. This is useful because it allows us to assess the balance of protein-protein and water-water contacts in the native state with protein-water interactions in the extended state. However, it is important to note that this will likely to lead to an overestimate of the total energy difference between the folded and unfolded states. The structures of the three intermediate states were taken from the study of Freddolino and Schulten (2009) which was generated using the CHARMM22 forcefield (Mackerell et al., 2004) with 200 mM sodium and chloride ions and TIP3P water (Jorgensen et al., 1983). In addition to multiple states, we considered a number of different treatments of the system. A list of the simulations performed is shown in Table 1.

Each starting structure was first subjected to gradient minimization with the appropriate CHARMM energy function using NAMD for 100,000 steps. We used the CMAP correction for CHARMM22. The systems were then neutralized with two chloride ions. The sodium and chloride ions were assigned parameters from Lamoureux and Roux (Lamoureux and Roux, 2006). The next stage in preparation was to generate a large water shell around each system with the SOLVATE program version 1.0 from the Max Planck

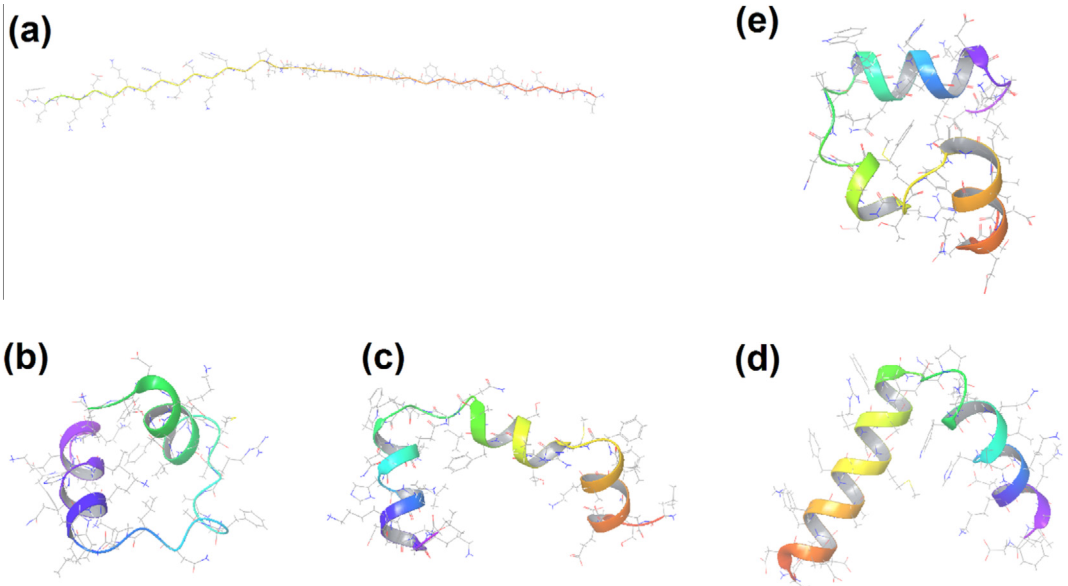

Fig. 1. (a) Extended, (b–d) intermediate, and (e) native states of the villin headpiece domain.

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