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Transition States in Protein Folding

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Abstract. The folding dynamics of small single-domain proteins is a current focus of simulations and experiments. Many of these proteins are 'two-state folders', i.e. proteins that fold rather directly from the denatured state to the native state, without populating metastable intermediate states. A central question is how to characterize the instable, partially folded conformations of two-state proteins, in particular the rate-limiting transition-state conformations between the denatured and the native state. These partially folded conformations are short-lived and cannot be observed directly in experiments. However, experimental data from detailed mutational analyses of the folding dynamics provide indirect access to transition states. The interpretation of these data, in particular the reconstruction of transition-state conformations, requires simulation and modeling. The traditional interpretation of the mutational data aims to reconstruct the degree of structure formation of individual residues in the transition state, while a novel interpretation aims at degrees of structure formation of cooperative substructures such as α-helices and β-hairpins. By splitting up mutation-induced free energies into secondary and tertiary structural components, the novel interpretation resolves some of the inconsistencies of the traditional interpretation.

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1 Folding dynamics of small single-domain proteins

Proteins are biomolecules that participate in all cellular processes of living organisms. Some proteins have structural or mechanical function, such as the protein collagen, which provides the structural support of our connective tissues. Other proteins catalyze biochemical reactions, transport or store electrons, ions, and small molecules, perform mechanical work in our muscles, transmit information within or between cells, act as antibodies in immune responses, or control the expression of genes and, thus, the generation

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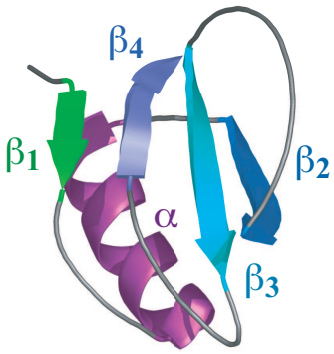

Figure 1: The structure of the protein CI2 consists of an α-helix packed against a four-stranded β-sheet [80]. CI2 is a two-state protein that folds from the denatured state to the native state without experimentally detectable intermediate states [12].

of other proteins [1]. Proteins achieve this functional versatility by folding into different, unique three-dimensional structures (see Fig. 1). The folding of proteins is a spontaneous process of structure formation and a prerequisite for their robust function. Misfolding can lead to protein aggregates that cause severe diseases, such as Alzheimer's, Parkinson's, or the variant Creutzfeldt-Jakob disease [2].

How precisely proteins fold into their native, three-dimensional structure remains an intriguing question [3,4]. Given the vast number of unfolded conformations of the flexible protein chain, Cyrus Levinthal argued in 1968 [5,6] that proteins are guided to their native structure by a sequence of folding intermediates. In the following decades, experimentalists focused on detecting and characterizing metastable folding intermediates of proteins [7]. The view that proteins have to fold in sequential pathways from intermediate to intermediate, now known as 'old view' [8,9], changed in the '90s when statistical-mechanical models demonstrated that fast and efficient folding can also be achieved on funnel energy landscapes that are smoothly biased towards the native state [10,11]. The stochastic folding process on these landscapes is highly parallel, and partially folded states along the parallel folding routes are instable rather than metastable. The paradigmatic proteins of this 'new view' are two-state proteins, first discovered in 1991 [12]. Two-state proteins fold from the denatured state to the native state without experimentally detectable intermediate states. Since then, the majority of small single-domain proteins with a length up to 100 or 120 amino acids has been shown to fold in apparent two-state kinetics, while larger multi-domain proteins often exhibit metastable folding intermediates [13–15].

The simplest model for a two-state process is classical transition-state theory. In transition-state theory, the folding rate of a two-state protein is assumed to have the form (see, e.g., [14])

$$k = k_o \exp[-G_{\text{T-D}}/RT], \tag{1.1}$$

where $G_{\text{T-D}}$ is the free-energy difference between the transition state T and the denatured