



# Race to the native state

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In contrast to other biopolymers such as RNA, most foldable protein sequences have a single, unique native structure, which is the most stable conformation. Understanding this remarkable property has been the long-held goal of protein folding research (1, 2). Once folded, proteins will undergo transitions between different conformational states within the native free-energy basin, often associated with their function; however, in most cases, these can be thought of as only local changes or deformations of structure that do not change the overall fold. On the other hand, a small number of proteins have been identified in which a more profound rearrangement of the fold can occur, with the most extreme examples being protein sequences that can switch to a completely different folded state as a function of environmental conditions or ligand binding (3). An example in which a subtle change of fold is used for functional regulation is found in the serine protease inhibitor family known as serpins. In this case, it appears that the initially formed “native” structure is not the thermodynamically most stable fold. In PNAS, Rao and Gosavi (4) investigate the origin of this kinetically controlled folding for a prototypical serpin,  $\alpha$ 1-antitrypsin.

Serpins are an abundant type of protease inhibitor, most commonly inhibiting chymotrypsin-like serine proteases (5) by a suicide mechanism: The protein initially folds to an active, inhibitory fold (Fig. 1, *Top Left*) in which the exposed reactive center loop (RCL) serves as a recognition motif for proteases. Binding of the protease results in cleavage of the RCL loop and covalent linkage of the serpin to the protease. At the same time, the remainder of the RCL loop inserts into a  $\beta$ -sheet in the serpin structure. Interestingly, a similar uncleaved “latent” fold with an inserted RCL sequence also exists (Fig. 1, *Bottom Left*) and, moreover, is more thermodynamically stable than the inhibitory fold. However, the inhibitory structure is formed first, presumably because it is under kinetic control. Some serpins, such as plasminogen activator inhibitor-1, can also spontaneously convert from the inhibitory form to the latent form

(dashed arrow in Fig. 1), providing yet another mode of regulation. The fascinating complexity of the serpin inhibition mechanism has been illuminated by the success of experimental structural biology in identifying many of the stable states populated by these proteins (including the examples shown in Fig. 1 and more) (5). In addition, equilibrium and kinetic folding studies have indicated the formation of a stable folding intermediate on the folding pathways of serpins, including  $\alpha$ 1-antitrypsin (6). Nonetheless, ensemble experiments can only probe stable or metastable states, and not the detailed mechanisms for their interconversion. Filling in these gaps is the natural domain of theory and simulation, although the problem is still challenging owing to the time scales involved. An excellent example is the above-mentioned spontaneous conversion of plasminogen activator inhibitor-1 from the inhibitory form to the latent form, a process that occurs on a time scale of hours. By using a recently developed path-sampling algorithm, the mechanism of this transition could be studied in recent all-atom simulations, with results very consistent with experimental changes of rate obtained for mutants (7).

Many mechanistic questions about serpin folding and conformational transitions remain. Foremost among these is why the active, inhibitory form wins the initial race to the native (inhibitory) state over the thermodynamically more stable latent form. Rao and Gosavi (4) now address this question using a structure-based, coarse-grained model in which the only residue pairs with attractive interactions are those formed in a given target structure. Such models are motivated by the energy landscape theory of protein folding, which posits that the folding free-energy landscape is funneled toward a single native state (8–11). While such a model may at first seem inconsistent with the folding scenario of serpins, it allows direct folding to the alternative latent structure to be studied, without the competing inhibitory fold. By computing separate folding free-energy landscapes for the inhibitory and latent structures, the authors found a free-energy barrier for folding to the latent form that was significantly

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