Response to Comment on “Force-Clamp Spectroscopy Monitors the Folding Trajectory of a Single Protein”

It is encouraging to see that the protein-folding trajectories observed after a force quench (1) have raised interest within the scientific community (2). The comment of Best and Hummer accurately points out that the mechanical coupling between the folding domains could mask stepwise folding (3). However, their simulations fail to predict our experimental observation that all the domains fold cooperatively at the end of the measured folding trajectories.

In a typical force-quench experiment, a ubiquitin chain is fully unfolded and extended before quenching the pulling force to a low value to trigger folding. The resulting folding trajectories are marked by four distinct phases (1, 4). The first phase is a rapid drop in the length of the unfolded protein associated with elastic recoil (5). This phase is followed by a prolonged plateau (phase 2) that implies a search in the conformational energy landscape (6), which ends in a faster final contraction in length that can sometimes be resolved into two processes (phases 3 and 4). The folded state of all the domains is reached at the end of phase 4. Fig. 1 shows several of these folding trajectories where the length is scaled by the number of ubiquitin repeats in the chain (from 1 to 7). They are similar in both the shape and amplitude of the changes in length. In particular, the fast contraction in length corresponding to the final transition between the ends of phases 2 and 4 is measured to be 14.6 ± 1.5 nm per protein domain, independent of the stretching force or the number of domains in the chain. It is striking that the folding trajectory of a single ubiquitin (gray trace in Fig. 1) superimposes well with the scaled trajectories of ubiquitin chains containing up to seven repeats. This result can only be explained by a highly cooperative folding between the domains in a chain at the end of the trajectory.

In addition, as we previously reported [figure S4, Supporting Online Material in (1)], folding, as determined by the recovery of mechanical stability, was never observed during the long plateau phase 2. Folding was observed in only 7% of the cases during phase 3 and in over 93% after reaching the end of phase 4. This further reinforces the conclusion that the folding of multiple domains is cooperative between the individual domains, with the final chain collapse accompanying the formation of native contacts and folding. By contrast, the trajectories generated by Best and Hummer predict that folding is equally likely anywhere along the pathway, a prediction that fails to reproduce our experimental observations.

Although the shape of the folding trajectories for ubiquitin chains is qualitatively different between our experiments and their simulations, both approaches reveal a complex folding path for individual ubiquitin proteins (7). This is highlighted by the well-resolved intermediates that are observed in the single ubiquitin folding trajectories measured with force-clamp spectroscopy (1) as well as the single module folding trajectories of Best and Hummer.

The concept of a “two-state” folding reaction has been a useful simplification that allowed generations of biochemists to interpret their observations of folding/unfolding reactions measured from bulk quantities of proteins (8). Inevitably, observing folding trajectories at the single-molecule level can no longer be described so simply and therefore necessitates statistical mechanics and a more detailed description of the folding energy landscape (9, 10). In particular, the mechanism by which a protein recovers its folded length after a force quench is still unknown. It is inescapable that the physics of polypeptide collapse under a stretching force will play a crucial role in explaining our trajectories. Unfortunately, a theoretical understanding of these phenomena is lacking. The cooperativity observed at the end of all our folding trajectories is puzzling but clear and cannot be explained by uncorrelated, Markovian models. Explaining this cooperativity will help understand the physical mechanisms underlying protein folding.

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References
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