Probing Protein Folding Kinetics with High-resolution, Stabilized Optical Tweezers

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Single-molecule techniques provide a powerful means of exploring molecular transitions such as the unfolding and refolding of a protein. However, the quantification of bi-directional transitions and near-equilibrium phenomena poses unique challenges, and is often limited by the detection resolution and long-term stability of the instrument. We have developed unique optical tweezers methods that address these problems, including an interference-based method for high-resolution 3D bead tracking (\(\sim 1\) nm laterally, \(\sim 0.3\) nm vertically, at \(> 100\) Hz), and a continuous autofocus system that stabilizes the trap height to within 1-2 nm longterm [1,2]. We have used our instruments to quantify the force-dependent unfolding and refolding kinetics of single protein domains (e.g. spectrin in collaboration with E. Evans). These single-molecule studies are presented, together with the accompanying probabilistic analysis that we have developed. References: 1. W.P. Wong, V. Heinrich, E. Evans, Mat. Res. Soc. Symp. Proc., 790, P5.1-P5.10 (2004). 2. V. Heinrich, W.P. Wong, K. Halvorsen, E. Evans, Langmuir, 24, 1194-1203 (2008).