Optical tweezers measurements of protein folding/unfolding & conformational dynamics at the single-protein level.
PROTEIN UNFOLDING
Application Note

The biological function of many macromolecules such as proteins and RNA is tightly coupled to their conformation and their conformational dynamics. For example, the correct folding of a protein into its native state is essential for its biological function. Misfolded or unfolded proteins are known to be either (partially) inactive or to even display toxic functionality as aggregation of misfolded proteins is associated with multiple neurodegenerative diseases.

Studying how proteins fold correctly and undergo conformational changes to accomplish their biological function is therefore crucial to understand the underlying biological mechanism and how diseases arise. Single-molecule Force Spectroscopy (SMFS) represents an ideal tool to study these molecular phenomena because of their unique capability to isolate individual biomolecules and observe conformational transitions and refolding processes as they happen in real-time.

In a first experiment, we recorded the mechanical stretching and retracting behaviour of full-length CaM at the calcium concentration of 10 mM Ca²⁺. We recorded single CaM force-extension profiles at the pulling and retracting velocity of 100 nm/s (Figure 3). While the force is increased, two unfolding steps can be observed as a sudden drop of the force signal, corresponding to the unfolding of two helix-lobe-helix domains. Hence, we conclude that unfolding events can be resolved within the force-extension curve by using force spectroscopy due to the high-force and distance resolution (<0.2 pN and ~0.5 nm at 100 Hz) of the C-Trap instrument. This type of measurement is well suited for comparative studies between the unfolding profiles of wild-type proteins versus those of altered or damaged proteins.

Using laminar flow microfluidics and our automated in situ assembly procedure, single CaM proteins can be specifically suspended between two optically-trapped microspheres via DNA linkers and retracted behaviour of full-length CaM at 10 mM Ca²⁺ showing equilibrium dynamics between multiple states. Data is recorded at 50 kHz (grey line) and averaged at 200 ns (red line). The two most populated states can be clearly distinguished after the histogram in the inset shows two peaks at 6.5 ± 0.1 pN and 7.8 ± 0.09 pN (mean ± standard deviation).

We further characterize the properties of the folding/unfolding equilibrium dynamics of CaM by holding the optical traps at a fixed distance (hence imposing a force across the CaM protein), while protein length fluctuations are measured and the transition between short-living structural intermediate states are resolved (Figure 4). A force of 7.5 pN is applied across a single CaM molecule and equilibrium fluctuations between three different states are observed in real-time, reflecting folding and unfolding of CaM loop-helix-loop subdomains. A noticeably complex multi-state folding/unfolding pattern appears (Figure 4), as described previously in literature.1,2 This capability of measuring folding and unfolding transition of single proteins at equilibrium over long periods of time is a highly informative approach and is enabled by the low drift and excellent data quality obtained by the instrument. Using this approach, it is possible to quantify the dwell time and transition kinetics of different states. This information in turn can provide insights into the folding/unfolding pathway of the specific protein of interest.

In this application note we perform a demonstrative experiment and is enabled by the high-resolution optical tweezer-fluorescence microscope developed by LUMICKS: The C-Trap.10

Throughout the past decade, a series of groundbreaking experiments using single-molecule methods have been performed, laying down a novel description of how conformational changes are related to the generation of force and mechanical work along specific mechanical reaction coordinates as a result of the energy released because binding interactions or bond hydrolysis.

Studying the folding and unfolding dynamics as well as conformational changes of single proteins represents a valuable method to produce groundbreaking discovery in the field of biology and biophysics. Using the C-Trap optical tweezer-fluorescence technology, both unfolding and refolding can be observed, as well as highly detailed equilibrium dynamics. This, in turn, allows scientists to study intermediate states in the unfolding process, providing valuable information of the structure-function of the protein. Further studies in the field of protein folding and conformation will likely exploit the high-sensitivity of the C-Trap, together with the multicolor fluorescence single-molecule FRET capability, enabling to further characterize protein conformation by detecting changes in the FRET efficiency signal and force fluctuations simultaneously. This allows correlating global mechanical properties of the protein with local structural properties.

3. Sample obtained with courtesy of UC Berkeley, Dinstein Lab.

1 A schematic representation of a multi-domain protein unfolding experiment. A protein with three domains (1, 2, and 3) is attached to optically trapped microspheres via DNA linkers.

2 By changing the trap-trap distance we can apply a force across the protein and detect rupture events.

3 Force-extension (blue) and force retraction (red) curves of full-length CaM at 10 mM Ca²⁺. Pulling and retracting speed is 100 nm/s. Bead diameter is 1.5 µm and trap stiffness is 0.284 pN/nm. In the figure the 200 Hz data is above.

4 Full-length CaM protein at 10 mM Ca²⁺ showing equilibrium dynamics between multiple states. Data is recorded at 50 kHz (grey line) and averaged at 200 ns (red line). The two most populated states can be clearly distinguished after the histogram in the inset shows two peaks at 6.5 ± 0.1 pN and 7.8 ± 0.09 pN (mean ± standard deviation).