Protein folding

Protein Folding and Conformational Changes Application Note - C-Trap Dymo®

2017

LUMICKS

PROTEIN UNFOLDING
Application Note

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.

Study Multi-domain Protein Unfolding Steps

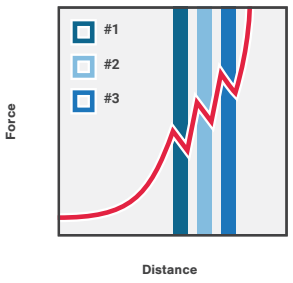
The biological function of many macromolecules such as proteins and RNA are tightly coupled to their conformation and their conformational dynamic. For example, the correct folding of a protein into its native state is essential for its biological function. Misfolded or unfolded proteins are known to 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 unfolding processes as they happen in real-time.

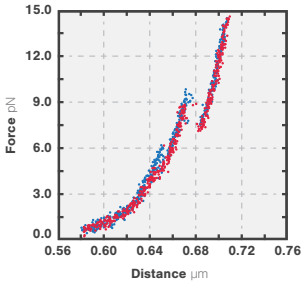
Owing to their outstanding sensitivity and their ability to apply mechanical forces it is possible to directly manipulate single proteins and observe how they change conformation by measuring their length change down to the sub-nm level. In this application note we perform a demonstrative experiment investigating the folding pathway of the protein Calmodulin (CaM), the primary Calcium-binding protein in the human body, using the high resolution optical tweezers developed by LUMICKS.

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.

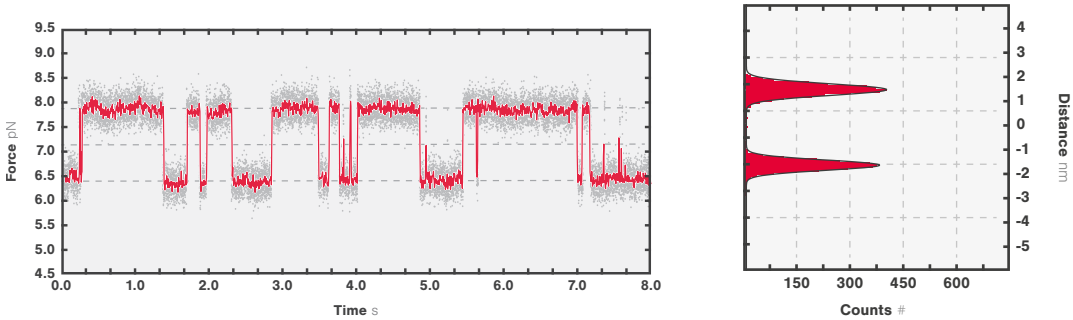
Using laminar flow microfluidics and our automated *in situ* assembly procedure, single CaM proteins can be specifically suspended between two optically-trapped microspheres using DNA linkers connected to the N- and C- terminal domains (**Figure 1**).



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



3 Force-extension (blue) and force retraction (red) cycle of full length CaM at 10 mM Ca2+. Pulling and retraction speed is 100 nm/s. Bead diameter is 1.0 µm and trap stiffness is 0.284 pN/nm. In the Figure the 200 Hz data is shown.



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 Hz (red line). The two most populated states can be clearly distinguished also from the histogram in the inset (right panel) showing two peaks at 6.5 ± 0.1 pN and 7.8 ± 0.09 pN (mean ± standard deviation).

In a first experiment, we recorded the mechanical stretching and retracting behavior of full-length CaM at the calcium concentration of 10 mM Ca^{2+}. 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-loop-helix domains. Hence, we conclude that unfolding events can be resolved within the force-extension curves by using force spectroscopy because of the high force and distance resolution (<0.1 pN and <0.3 nm at 100 Hz) of the C-Trap instrument. This type of measurement is well suited for comparative studies between the folding profiles of wild-type proteins versus those of altered or damaged proteins.

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 on a single CaM molecule and equilibrium fluctuations between three different states were 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 experiment 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.

Studying the folding and unfolding dynamics as well as conformational changes of single proteins represent a valuable method to produce groundbreaking discovery in the field of biology and biophysics.

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 further characterization of 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.

Ultra-stable measurements with absence of drift

For folding/unfolding investigations that require monitoring small and transient conformational changes in real-time with high stability, the absence of signal drift over time is crucial. To demonstrate the ultra-stable capability of LUMICKS' optical tweezers over time, we performed a second experiment.

Here, we ran a passive distance clamp experiment, during which we monitored DNA conformational transitions for over 500 seconds (**Figure 5a**).

When looking at a 10 s inset of the complete trace, fast transitions between multiple states are clearly visible (**Figure 5b**). In fact, when we performed histogram analysis for both the complete 520 s trace and two 50 s sections distributed along the main trace (**Figure 5c**, **5d** and **5e** respectively), up to 4 states displaying DNA breathing corresponding to up to 60 base pairs were resolved.

1 Stigler, J., Ziegler, F., Gieseke, A., Gebhardt, J. C. M. & Rief, M. The Complex Folding Network of Single Calmodulin Molecules. Science (80-.). **334**, 512–516 (2011).

2 Stigler, J. & Rief, M. Calcium-dependent folding of single calmodulin molecules. Proc. Natl. Acad. Sci. **109**, 17814–17819 (2012).

Sample obtained with courtesy of UC Berkeley, Bustamante Lab.

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