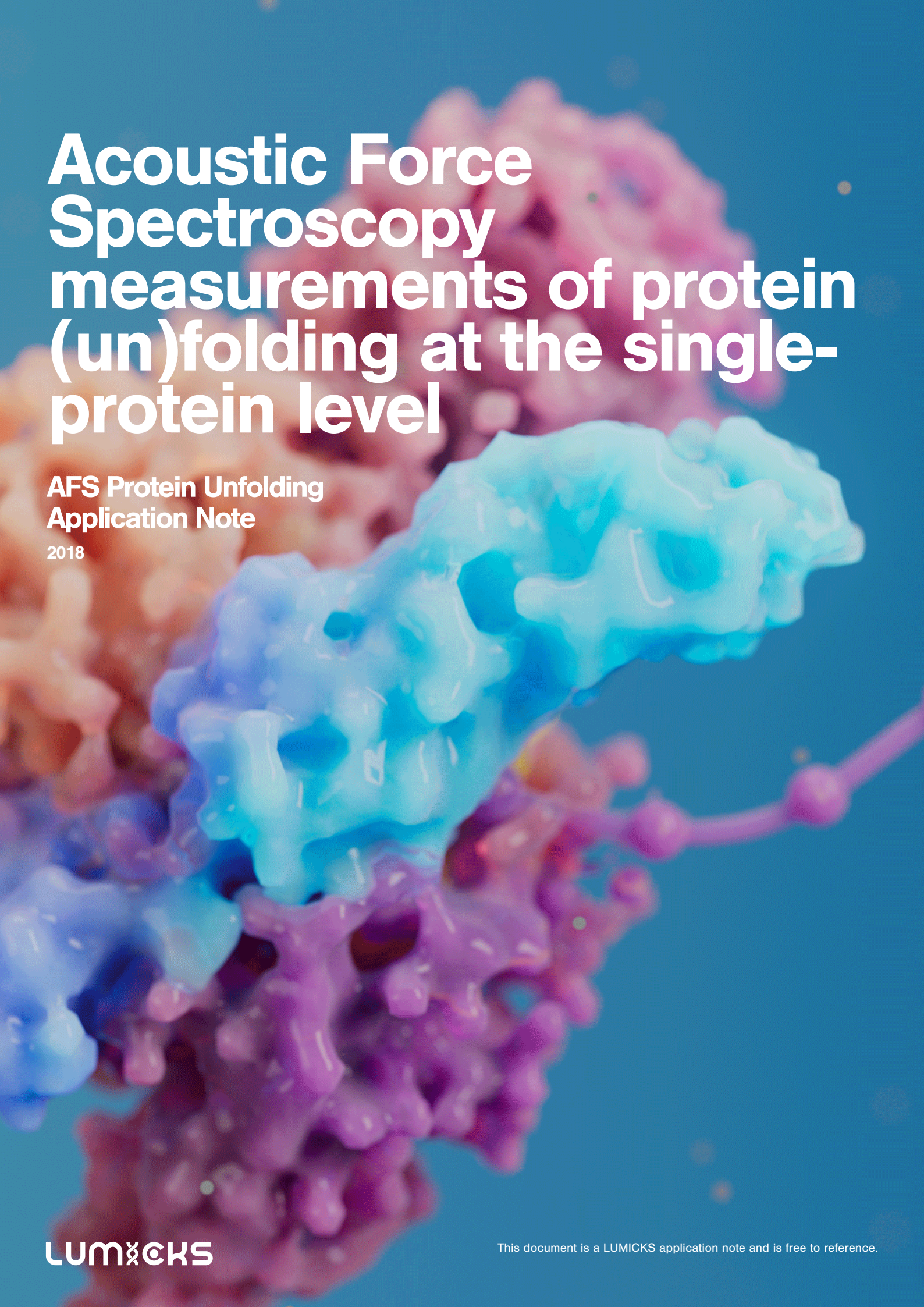


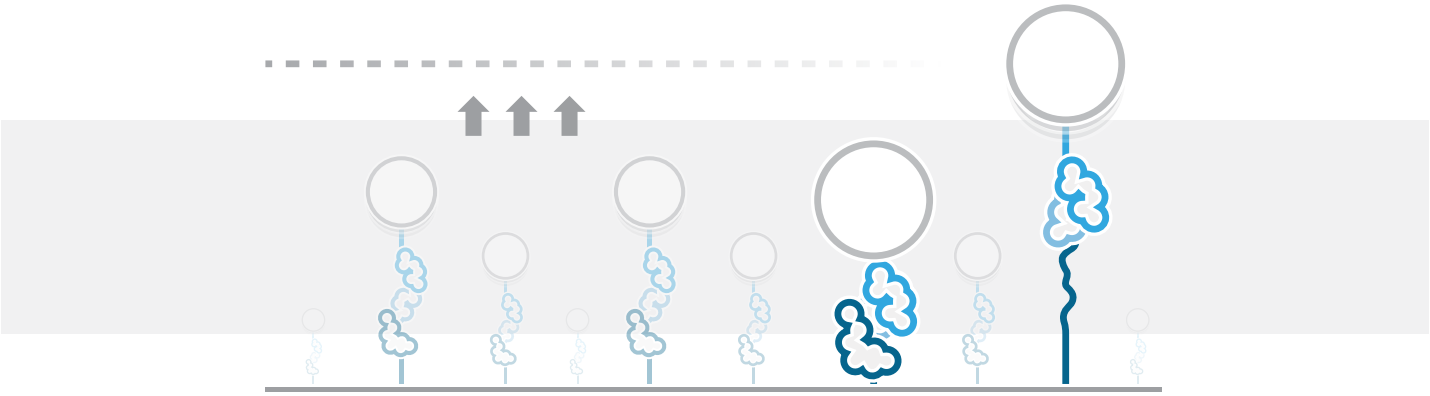
Acoustic Force Spectroscopy measurements of protein (un)folding at the single-protein level

AFS Protein Unfolding Application Note

2018

LUMICKS

AFS PROTEIN UNFOLDING
Application Note



1 A schematic representation of a protein unfolding experiment using AFS. Protein tethers are attached to the glass surface of the flow cell, while the opposed end of the protein tether is coupled to a polystyrene bead.

Study Protein Unfolding using Acoustics

Single-molecule force spectroscopy (SMFS) technologies have become prominent as powerful tools for the investigation of the biomechanics associated with protein structure and for the study of protein unfolding pathways. In turn, the information accessed by SMFS tools provides valuable contributions to research and drug development. In particular, SMFS can aid in revealing the molecular mechanisms underlying a wide range of human pathologies that are believed to stem from the formation and aggregation of misfolded proteins, such as Alzheimer's and Parkinson's disease.

However, current SMFS tools lack the ability to study multiple protein

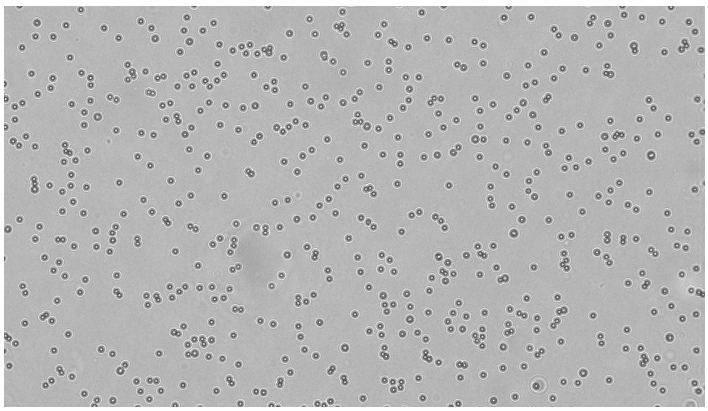
(un)folding events simultaneously, making the investigation of protein folding an excessively time-consuming process. Employing acoustics to manipulate and apply force on hundreds of biomolecules in parallel represents an ideal approach for obtaining high data throughput. In this application note we present Acoustic Force Spectroscopy (AFS™) as a new technological route to perform single-molecule manipulation experiments for the study of protein unfolding.

In this technique, a piezo element resonantly excites planar acoustic waves over a microfluidic chip. These resonant acoustic waves are capable of applying forces on microspheres with different density than the surrounding medium. Thus, by applying acoustic forces, multiple biomolecules individually tethered between a surface and a microsphere can be stretched synchronously (**Figure 1**).

In a typical field of view, hundreds of microspheres can be tracked in real-time and manipulated in parallel, resulting to the collection of a large data set that provides insights into the stochastic and heterogeneous behavior of individual biomolecules[1] (**Figure 2**).

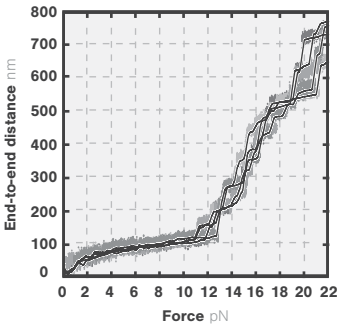
In this pilot study that was conducted at the lab of Prof. Yan Jie (National University of Singapore), we demonstrate for the first time how AFS can be used to manipulate and apply tension on individual proteins. By exerting a controlled level of tensions on a Talin protein we induce (un)folding transitions in a regulated manner, while simultaneously recording protein extension with high accuracy.

Talin proteins belong to a class of mechanosensitive macromolecules that are involved in mediating cell attachment to the extracellular matrix Due to their key role in cellular processes and signaling pathways, in conjunction with their ability to undergo plastic conformational changes under force stimulation,

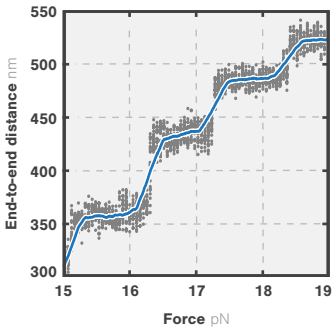


2 Typical field of view (FOV) displaying the simultaneously imaging of 4.5 µm polystyrene beads that are tethered to the surface with single protein-DNA molecules. The image is recorded with a 20x magnification objective.

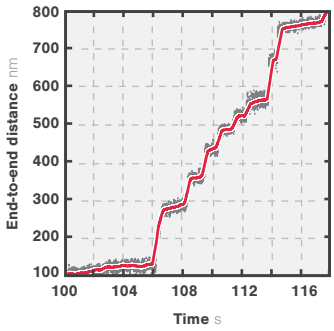
1 Sitters, G., Kamsma, D., Thalhammer, G., Ritsch-Marte, M., Peterman, E.J.G. & Wuite, G.J.L. Acoustic Force spectroscopy. Nature Methods **12**, 47-50 (2015).



3a Force-distance curves representing multiple stretching cycles of the same individual Talin protein.



3b Zoom- in of an individual force-distance curve corresponding to a single pulling cycle, covering a force ramp range of 4 pN.



3c Time-distance curve of the same molecule in figure 3b, displaying an 18 second range.

3 Unfolding traces of an individual Talin protein using AFS at a loading rate of 1 pN/s. Traces were obtained at 500 Hz (colored dots) and averaged to 50 Hz (using a moving box average, black lines).

Talin proteins have attracted active interest in the field of single-molecule biophysics

In the assay we used to demonstrate the possibility to study protein unfolding dynamics with AFS,

a Talin protein is tethered between a polystyrene bead and a glass surface using DNA linkers. By applying standing acoustic waves, a planar acoustic node is formed and the bead connected to the Talin molecule experiences a force directed towards this node. During the experiment, the force is modulated by changing the amplitude of the acoustic waves. Increasing the force on the tethered bead in a controlled manner, causes the different protein domains to unravel in a sequential fashion.

The experiment is performed by alternating stretching and relaxation cycles (loading rate was set at 1 pN/s) while simultaneously monitoring the end-to-end extension of each individual protein in real-time with nanometer-resolution (**Figure 3**).

In the force-extension curve shown in **Figure 3a**, we can see consecutive unfolding cycles of an individual Talin protein. By overlapping the unfolding traces of a single protein, individual domain unfolding events can be detected, enabling researchers to obtain valuable information to further understand protein structure and the protein's unfolding free-energy landscape.

The ultrasounds generated by the AFS instrument do not harm the structural integrity of the tethered biomolecules. Thus, proteins can consecutively unfold and refold for hours in a row, allowing to obtain multiple unfolding and refolding curves using a single protein substrate. This is highly valuable as the information collected from each protein is greatly increased as opposed to other SMFS methods which typically, after a limited amount of consecutive stretching and relaxation cycles, damage the protein system due to laser-induced phototoxicity or force-induced corruption, inevitably causing the termination of the measurements.

Figure 3b depicts an enlarged snippet of an individual force-distance curve corresponding to

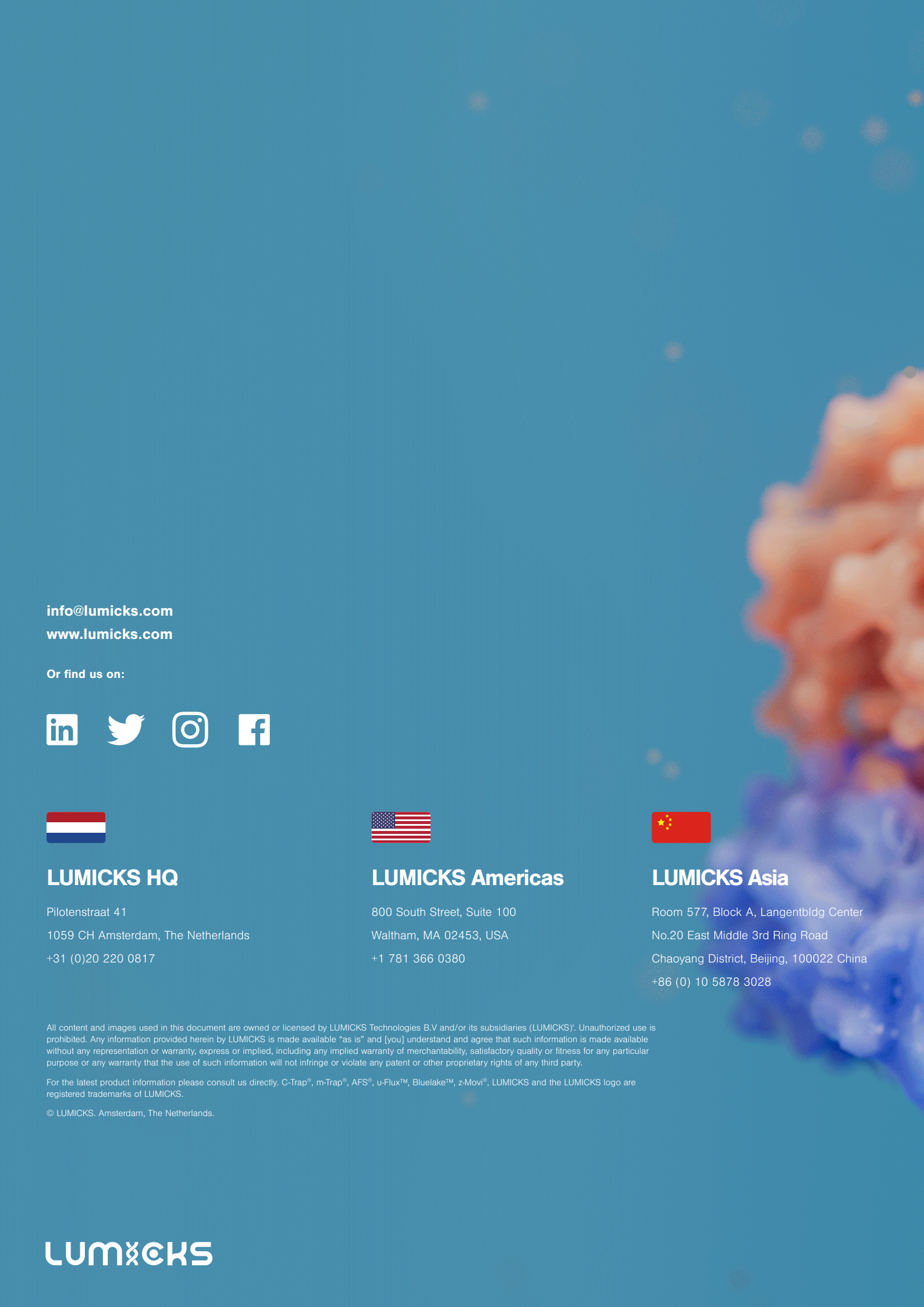
a single pulling cycle. Here, while ramping the force from 15 to 19 pN, we observe a series of four unfolding events – corresponding to four individual protein domains – ranging between 30 and 100 nm. The unfolding events can be clearly distinguished owing to the high-resolution distance measurement capability of the AFS technology. In particular, the AFS system features a position tracking accuracy of 2 nm along the x,y direction, 4 nm along the z direction (at the bandwidth of 25 Hz) empowering the user to investigate specific (un)folding steps in high detail.

In addition, the time-distance curve of the same molecule as in Figure 3b is given in **Figure 3c**, displaying an 18 second range. Generally, with the AFS it is possible to measure events ranging from milliseconds to >10 hours, allowing for the kinetic and thermodynamic characterization of the protein dynamics over a wide time regime.

Within this application note, by monitoring the unfolding steps of a single Talin protein and recording consecutive unfolding traces with high tracking resolution, we have demonstrated how AFS can be used to study protein unfolding. Investigating in detail the mechanism of the (un)folding pathways of single proteins represents a valuable method to produce groundbreaking discovery in the fields of biophysics and medicinal biology.

Further studies in the field of protein folding and protein-protein interactions will likely exploit the multiplexing capability of an AFS and allow the user to simultaneously monitor numerous proteins in parallel. This in turn will empower the user to boost significantly the experimental data throughput and perform statistical analysis of the mechanical properties of protein structures based on a single experiment, without compromising resolution.

Sample and data courtesy of NUS, Yan Jie group.



info@lumicks.com
www.lumicks.com

Or find us on:

LUMICKS HQ

Pilotenstraat 41
1059 CH Amsterdam, The Netherlands
+31 (0)20 220 0817

LUMICKS Americas

800 South Street, Suite 100
Waltham, MA 02453, USA
+1 781 366 0380

LUMICKS Asia

Room 577, Block A, Langentbldg Center
No.20 East Middle 3rd Ring Road
Chaoyang District, Beijing, 100022 China
+86 (0) 10 5878 3028