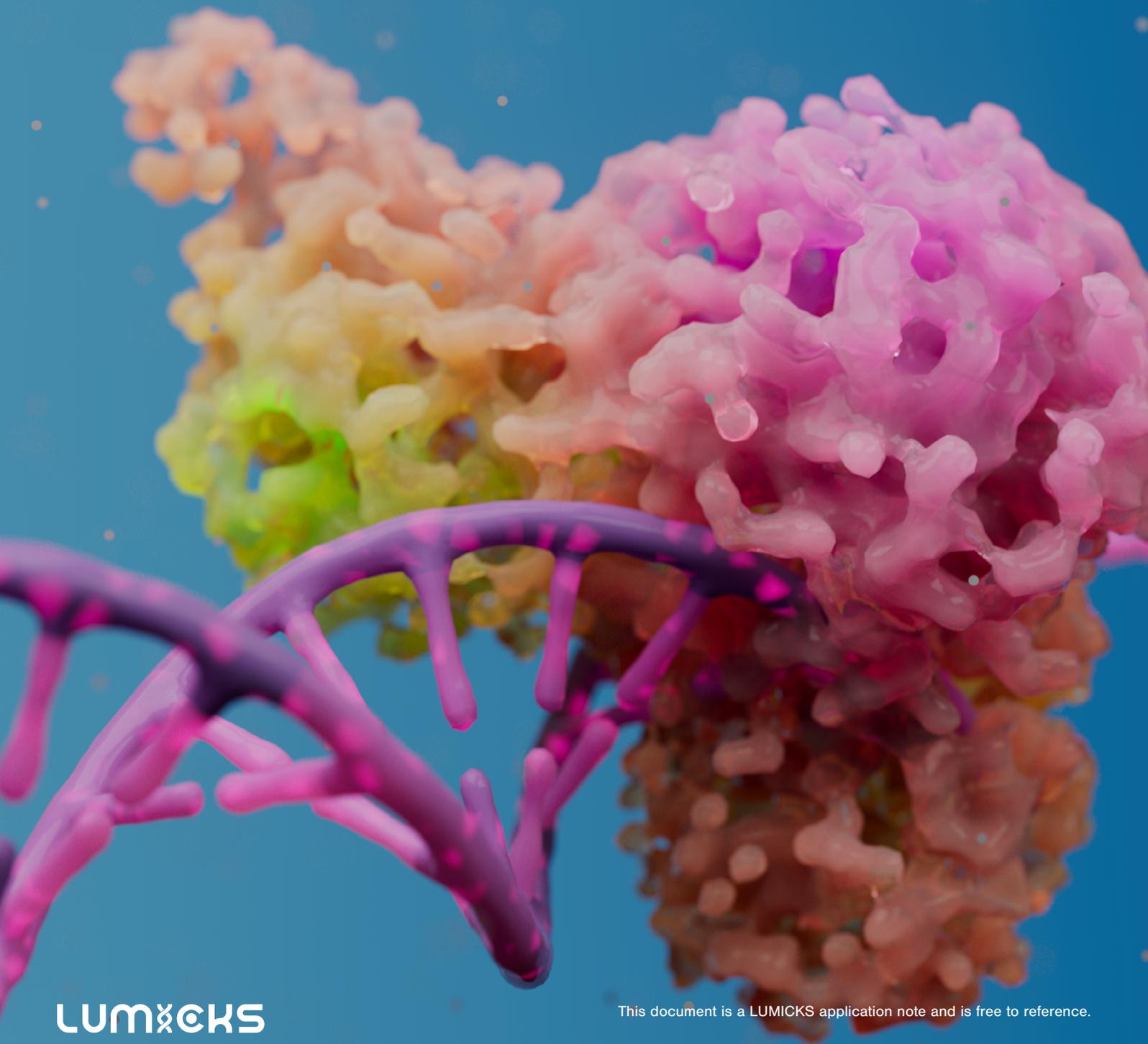


Real-time visualization of DNA structural transitions under mechanical stress

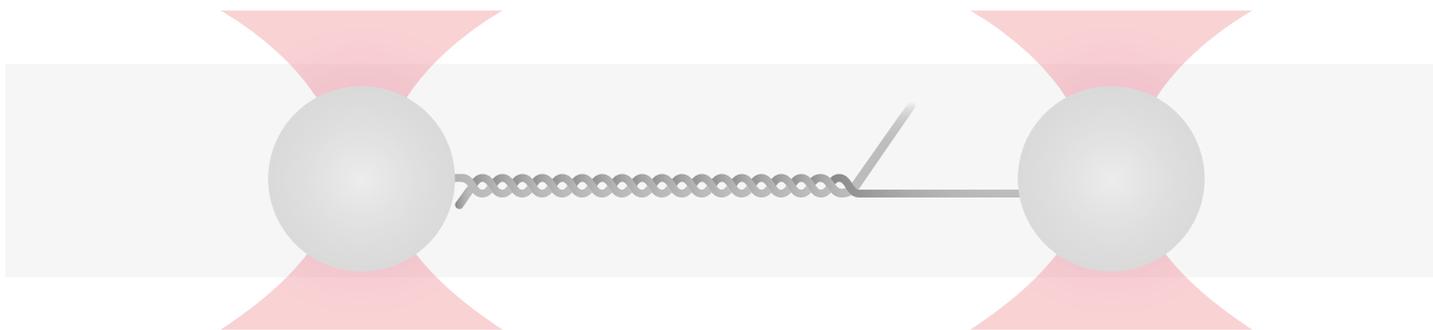
Multi-color Detection
Application Note

2017



MULTI-COLOR DETECTION

Application Note



1 A schematic representation of a DNA overstretching experiment. A double stranded DNA molecule is attached to optically trapped microspheres that are moved apart, thus inducing peeling from both ends.

Study Structural Transitions of DNA During Overstretching

Single-molecule force spectroscopy (SMFS) tools are widely used to study structural transitions of DNA during overstretching. However, as many of these tools only provide global information, the exact mechanisms that occur during these transitions remain unclear. Combining SMFS with visualization of local information could resolve this problem. In this application note we will discuss how LUMICKS' C-Trap™ technology combines high-resolution optical tweezers as a SMFS tool with confocal fluorescence microscopy to monitor the transition of double-stranded (ds) DNA to single-stranded (ss) DNA upon applying mechanical stress.

It is well established that bare dsDNA undergoes an overstretching transition at around 65 pN. During this transition—with virtually no force increase—the DNA contour length increases by 70%. Next to a conversion from B to S-form dsDNA, the overstretching transition consists of regions of melted DNA, caused either by unpeeling from

the termini or nicks, or by formation of melting bubbles. Because of the reproducibility of this behavior at around 65 pN for bare DNA, being able to measure changes on the force at which the overstretching plateau occurs in the presence of different binding partners or in different buffers, can reveal how those binding partner-DNA interactions can affect DNA structure and stability.

Here, we use high-resolution optical tweezers to both manipulate individual molecules, thereby inducing transitions in a controlled manner, but also to enable readings of force and distance values over time with sub-pN and sub-nm resolution. In order to distinguish melted (ssDNA) from double-stranded (dsDNA) regions, we use specific fluorescent markers, which are visualized using multicolor single-photon detection to determine their specific location along the DNA strand with high precision (<15 nm).

Using laminar flow microfluidics and our automated in situ assembly

procedure, an individual dsDNA molecule can be tethered between two optically-trapped microspheres (**Figure 1**). Then, the tethered construct can be transported into a channel containing both 2 nM Sytox Orange, a dsDNA fluorescent marker, and 3 nM fluorescently-labeled replication protein A (RPA), a ssDNA binding protein.

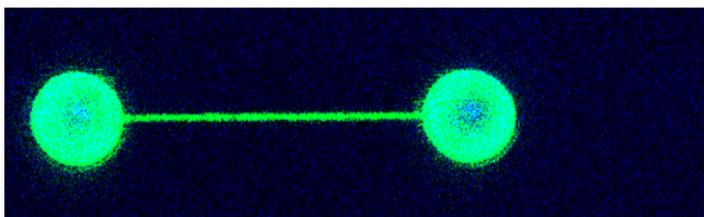
In a first experiment, we recorded fluorescent confocal images at different tensions to demonstrate the dual color detection capabilities of our system. First, we visualized dsDNA at a tension of 30 pN (**Figure 2a**). At this tension, the inter base-pair distance allows efficient binding of fluorescent Sytox molecules (green), thereby fully coating dsDNA.

In a second experiment, we apply an increasing level of tension until we reach the overstretching regime. At this level of tension the dsDNA started peeling from both ends. In this case, we observed the appearance of a structurally distinct region, corresponding to the binding of RPA to ssDNA (**Figure 2b, blue**).

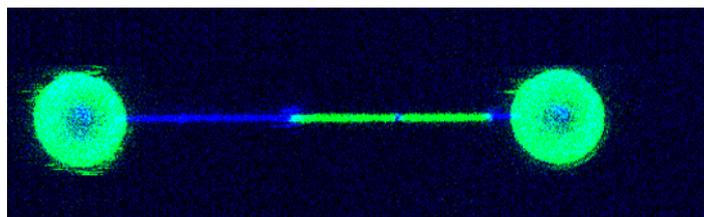
The presence of an RPA-stained DNA region other than at the two ends of the template indicates the occurrence of peeling initiating from DNA nicks.

We further validate the presence of DNA melted regions by taking advantage of the C-Trap™ microfluidics system, and we apply a gentle buffer flow perpendicular to the DNA to elongate the complementary peeled ssDNA (**Figure 2c**).

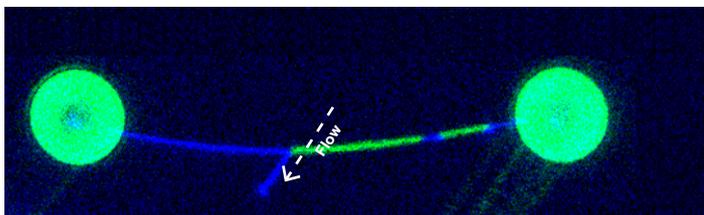
Finally, the full melting of dsDNA was induced by tension, resulting in a single molecule of ssDNA fully coated with bound RPA and tethered between optically trapped beads (**Figure 2d**).



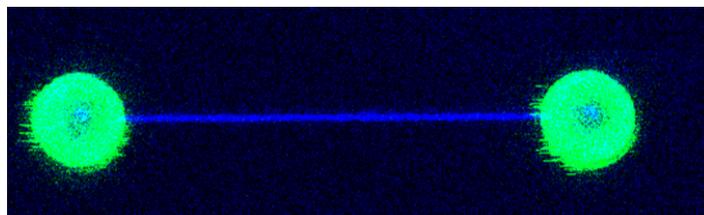
2a dsDNA at a force of 30 pN



2b Overstretching transition



2c Flow stretch



2d ssDNA at a force of 30pN

2 Dual color 2D confocal fluorescence images corresponding to different stages of the transition from dsDNA to ssDNA (from top to bottom). While green fluorescence indicates specific binding of Sytox Orange to dsDNA regions, blue signal corresponds to binding of RPA to ssDNA.

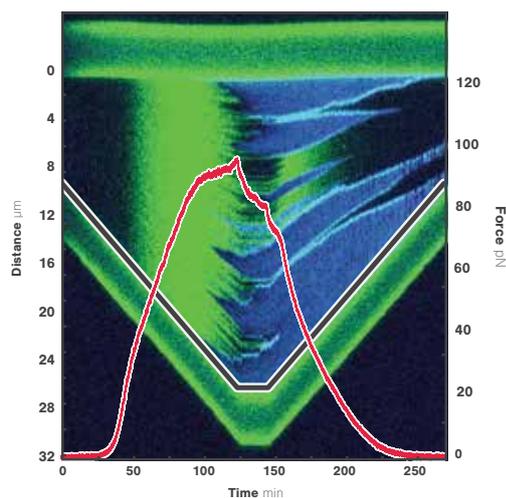
In order to correlate the visualization by fluorescence microscopy with quantitative force spectroscopy data, we performed an additional experiment in which we stretched a single dsDNA molecule with a constant velocity of 140 nm/s, while at the same time continuously recording force, distance and fluorescence (Figure 3).

The grey line indicates the end-to-end distance changes over time and the red curve indicates the time varying applied force on the DNA. By overlapping all data sets, we gained access to a force and an end-to-end value for each line scanned by confocal microscopy. Therefore, we can now directly attribute the initial binding of Sytox Orange to dsDNA at tensions of around 25 pN. Also, differently than for bare dsDNA, we did not observe an overstretching transition until reaching a tension of 90 pN. This phenomenon can be attributed to the fact that Sytox Orange stabilizes the dsDNA structure upon binding, making it more difficult to melt. Nevertheless, when reaching the overstretching transition, regions of blue fluorescence appeared, indicating a structural transition from dsDNA to ssDNA. Note that once again, peeling occurred not only from the DNA extremes but also from the nicks distributed randomly along the DNA template.

As soon as RPA started binding concurrently with dsDNA melting, a drop in the force signal was observed, indicating the stabilization of melted DNA, which being coated by RPA cannot be reannealed with its complementary strand to form dsDNA again. Finally, relaxing the molecule back under 25 pN, resulted in Sytox Orange dissociation while RPA remained bound.

Understanding how the structural properties of DNA change in the presence of small molecule ligands and how those interaction can be studied by means of mechanical manipulation at the molecular level can lead to ground-breaking discoveries in the field of biophysics and biology.

Using the C-Trap optical tweezer-fluorescence technology, structural transitions can be directly visualized and detected in real-time. In addition, having access to tension and extension of single molecules, provides scientists with valuable information on the structural properties of biomolecules and biopolymers.



3 Dual color fluorescence kymograph corresponding to the extension and retraction of a single molecule of dsDNA at a rate of 140 nm/s. End-to-end distance (grey) and force (red) data sets are overlapped to the fluorescent image for a true correlation of the data sets.

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