Real-time visualization of DNA structural transitions under mechanical stress.

MULTI-COLOR DETECTION APPLICATION NOTE

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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 force increase—the DNA contour transition at around 65 pN. During it is well established that bare ssDNA, the overstretching transition length increases by 70%. Next force increase—the DNA contour transition at around 65 pN. During

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 nms⁻¹, 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-bond 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 nick distributed randomly along the DNA template.

As soon as RPA started binding concurrently with ssDNA 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 tweezers-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.

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