Single-molecule DNA (over)stretching using optical tweezers

The advent of techniques to mechanically manipulate single (bio)molecules has sparked large efforts to precisely study the mechanical and elastic properties of proteins, protein fibers, DNA, RNA, etc. Two widely used techniques in this area are atomic force microscopy (AFM) and optical tweezers. Optical tweezers complement AFM at the lower end of the force regime: forces of typically a few hundred picoNewtons down to fractions of a picoNewton can be assessed using optical tweezers. This has allowed for, among other things, the precise measurement of forces and displacements exerted by individual motor proteins. In this report, we focus on the use of optical tweezers for force spectroscopy on single DNA molecules, and on the range of applications that this technique offers to learn not only about DNA itself, but also about the mechanics and thermodynamics of protein-DNA interaction.

DNA is a so-called *semi-flexible* polymer. This means that it has an intrinsic stiffness and therefore resists sharp bending. From the physics point of view, the elasticity of DNA therefore influences its dynamics in an interesting manner. Biologically speaking, the elasticity of DNA affects a wide variety of cellular processes, including protein-induced DNA bending, twisting or looping.

Since the first single-molecule stretching experiments were performed [1], many reports on the elasticity of double-stranded DNA (dsDNA) have appeared, including many model studies that now encompass most of the experimental data. The unique helical structure formed by two intertwined and base-paired strands (see Figure 1) determines its elastic behavior, including the intriguing phase transition called 'overstretching'. During this transition, DNA gains almost a factor two in length without the need to pull harder. Below, we will demonstrate these individual regimes experimentally.

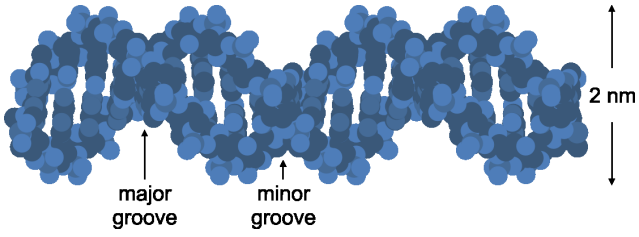

Fig. 1 Structure of double-stranded DNA. Two single strands, held together by hydrogen bonds between individual bases, are wound around each other, giving rise to major and minor grooves.

Experiment description

Optical tweezers platform

We have performed our measurements of DNA elasticity using JPK's NanoTracker™, an off-the-shelf optical-tweezers platform designed for high-resolution quantitative nanomanipulation (Figure 2). The NanoTracker™ is equipped with two optical traps, which can both be independently steered and used for tracking or force measurements. Force measurements are performed using back-focal-plane interferometry on InGaAs quadrant photodiodes, which can be calibrated online using the power spectrum analysis [2] feature of the software. Apart from this, the NanoTracker™ software allows full control of all instrument hardware, such as trap/sample positioning or trap stiffness adjustments, but also the microfluidic flow (see below). Moreover, a wide range of data acquisition options are built in.

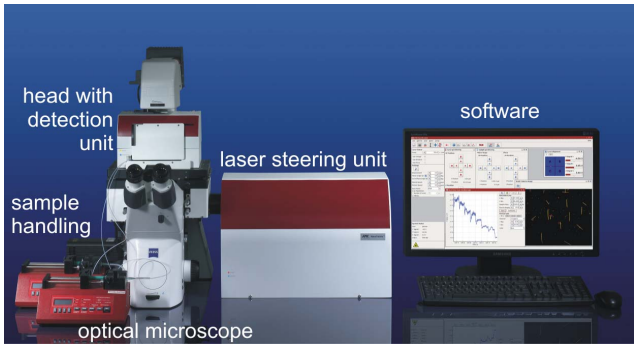

Fig. 2 JPK NanoTracker™, a dual-beam force-sensing optical tweezers platform, in the configuration used for the measurements described here.

Microfluidic buffer control

The two optical traps provided by the NanoTracker™ can be used to suspend a single DNA molecule between two

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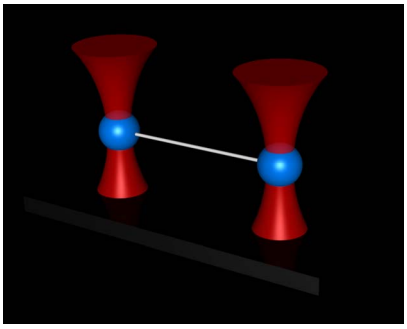

Fig. 3 Schematic illustration of a DNA molecule captured and stretched between two traps. By moving one or both traps, the tension in the suspended DNA can be tuned and the molecule can be stretched.

streptavidin-coated polystyrene beads, as depicted in Figure 3. To obtain a specific connection to the beads, the DNA of bacteriophage lambda is modified to have several biotin groups at its termini. The construction of such trapped DNA benefits greatly from microfluidic buffer flow, which allows for the controlled step-by-step assembly of the bead-DNA-bead construct, as explained in Figure 4.

Performing a DNA stretching experiment

By controllably changing the distance between the two traps at the DNA's extremities, we can apply tension to the DNA. Since the NanoTracker™ was designed as a force-sensing optical-tweezers instrument, one can accurately measure the forces applied. In an alternative configuration, one of the DNA's extremities could have been attached to the sample surface, allowing manipulation either by moving the trapped particle on the other end or by moving the sample using a piezo-actuated XYZ stage. We can thus measure the 'force versus extension' characteristic of a single DNA molecule, i.e., its elastic response to forces.

Figure 5 shows a typical measurement of the highly non-linear elasticity of double-stranded DNA. Even when the DNA is slack, i.e., when the DNA ends are much closer together than the length of the DNA polymer, a finite force has to be exerted in order to fix its ends. This finite force is of *entropic* nature: due to the continuous bombardment by solvent molecules, the DNA actually 'wants to' attain as many conformations as possible. Keeping the DNA at a fixed end-to-end distance, one precludes a tremendous

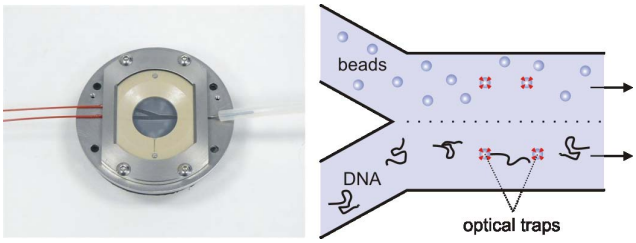

Fig. 4 Picture and schematic illustration of the multi-channel microfluidic flow cell used for the capturing of DNA between two traps. A separate channel is available for the controlled supply of beads and DNA. After capturing two beads in the optical traps, the sample is moved into the DNA channel. DNA binding to the bead held upstream is stretched out, allowing to 'fish' for the other end by moving the second trap. Stretching experiments are then performed without applying flow.

number of conformations, lowering the entropy of the DNA. The further one pulls, the more conformational states are precluded, and hence the higher this entropic force is. When the DNA is (almost) pulled taut, one actually starts to increase the length of the DNA contour by stretching the DNA backbone. This stretching occurs linearly with extension, as for a 'Hookean' spring. This regime is called the *enthalpic* regime. This regime tells us the intrinsic spring stiffness of a DNA molecule.

The entropic and enthalpic regimes can be mathematically described by the so-called (extensible) *worm-like chain* model (WLC) [3,4]:

$$F = \frac{k_B T}{L_p}\left[\frac{1}{4}\left(1-\frac{L}{L_0}+\frac{F}{S}\right)^{-2}-\frac{1}{4}+\frac{L}{L_0}-\frac{F}{S}\right]$$

with F the force on the DNA, L the end-to-end distance (i.e., the extension) of the DNA with contour length L_0. The parameter L_p is the DNA's *persistence length*, a temperature-dependent measure for the bending rigidity (~50 nm); S represents the DNA *stretch modulus* (1000–1500 pN [4]). The Boltzmann constant k_B times the absolute temperature T represent the thermal energy. Figure 5b shows a fit of the elasticity data to the WLC model (red line).

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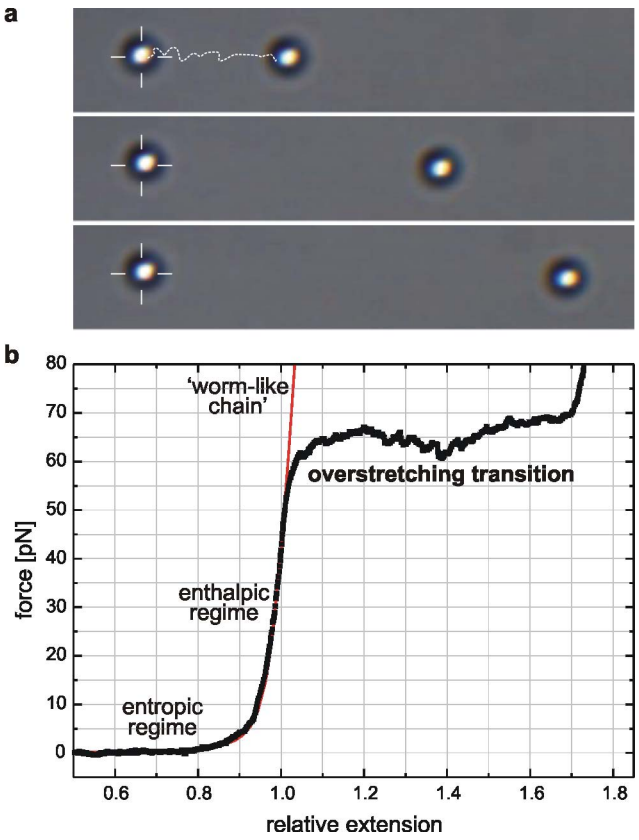

Fig. 5 DNA elasticity measurement using JPK's NanoTracker™. [a] Microscopy images of the pulling experiment (DNA is not visible). The lower two images show how the left bead is pulled slightly out of its trap at high force. [b] Force-extension graph of the experiment in panel a. As the DNA is pulled to larger extensions, it undergoes several phases of distinct elastic behavior. The red line is a fit to the so-called worm-like chain model [1,3,4]. The overstretching transition, typical for double-stranded DNA, is readily seen.

A remarkable feature is observed in Figure 5b at a force of around 65 pN: without the requirement of additional force, the DNA lengthens by almost a factor two. This characteristic phase transition, first reported during the late nineties by two groups in the same issue of Science [5,6], is called the *overstretching transition*. This transition reproducibly occurs at the same pulling force and is reversible. When the DNA is extended beyond ~170% of its contour length, the transition is complete and a steep increase in force is observed. Much debate exists in the literature about the structural nature of the transition. More and more evidence exists, however, for the transition being force-induced *melting*, i.e., the gradual breakdown of the interaction between the two strands.

Hysteresis upon DNA overstretching

The overstretching transition is in principle a fully reversible reaction. Nonetheless, upon relaxation of the molecule, significant hysteresis can be observed. The occurrence of hysteresis depends mainly on buffer conditions such as ionic strength [5], which may hamper the back-conversion to intact double-stranded DNA. Figure 6 shows an example of such hysteresis between stretching (forward) and relaxing (backward) of a DNA molecule. Even in the presence of hysteresis, the overstretching transition itself is typically fully reversible. In some cases though, the molecule can irreversibly change its elastic behavior, as seen in Figure 7. In this case, the DNA apparently lost part of one of its two strands, since the resulting elastic behavior seems to correspond to that of a hybrid single-stranded/double-stranded DNA molecule. It is likely that such irreversible changes occur on molecules that contain

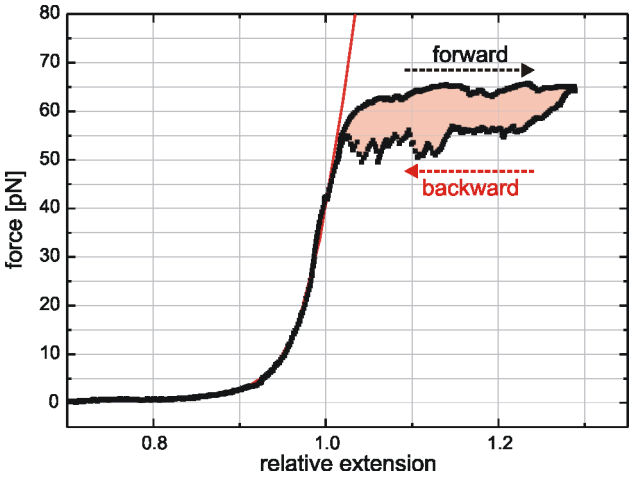

Fig. 6 Hysteresis upon DNA overstretching. When overstretching DNA, the elastic response may show hysteresis upon relaxation. The occurrence of this effect largely depends on buffer conditions, such as ionic strength. From the point on where the backwards curve meets the forward one, the DNA has returned to its native, double-helical state. Therefore even in the presence of hysteresis, the overstretching transition is reversible.

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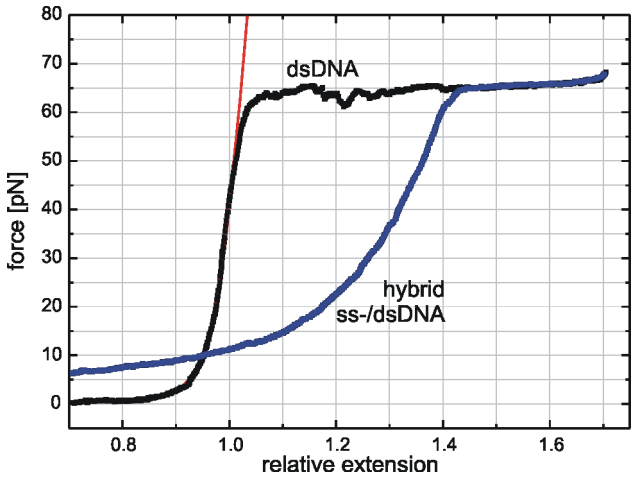

Fig. 7 Overstretching DNA with single-stranded breaks ('nicks') can yield to the irreversible (partial) conversion to a single-stranded DNA, as explained in the text. The black curve shows the first overstretching cycle. The blue curve shows the subsequent second stretching cycle, which has the signature of a hybrid double-stranded/single-stranded DNA molecule.

backbone interruptions in one of the strands ('nicks'). The occurrence of such changes supports the interpretation of the overstretching transition as a force-induced DNA melting transition [7].

What can we learn? Applications

Single-molecule force spectroscopy can teach us a lot about the intrinsic mechanical properties of (bio)molecules. The intricate elastic response of DNA, as described here, has been elucidated largely using optical-tweezers-based technology. Moreover, protein and RNA structure and dynamics can be unraveled with this technique [8,9]. Since the overstretching transition bears similarity to *thermal melting* of DNA, it can be used to study the kinetics and thermodynamics of DNA–protein or other DNA–ligand interactions [10,11].

Yet the mechanical control over a single molecule can also be exploited to study the functioning of biomolecules in a more biological context. For instance, DNA polymerase, the enzyme responsible for the duplication of the genome during cell division, can be forced to reverse its polymerization direction by controlling the tension in the DNA along which it moves [12]. This provides insight into how enzymes convert chemical energy ('fuel') into mechanical energy ('movement'). Similarly, DNA tension was recently used to reveal how recombinases, enzymes required for the repair of damaged DNA, function [13]. To this end, the visualization of DNA-bound proteins using sensitive fluorescence was performed simultaneously.

Obviously, optical-tweezers-based force spectroscopy is not limited to the study of DNA and related enzymes; the list of proteins successfully studied using optical tweezers would be too long to be included in this report.

Outlook

Notably, the NanoTracker™ is the first commercial optical-tweezers platform that offers a complete solution for such quantitative force spectroscopy measurements. To this end, it integrates versatile manipulation and sample control through microfluidics. On top of that, microscopic visualization can be flexibly enhanced using optical microscopy techniques such as DIC or fluorescence. JPK's vision has been to finally have optical-tweezers technology transcend the labs of self-building enthusiasts, by offering a complete solution for such demanding experiments.

Acknowledgments

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