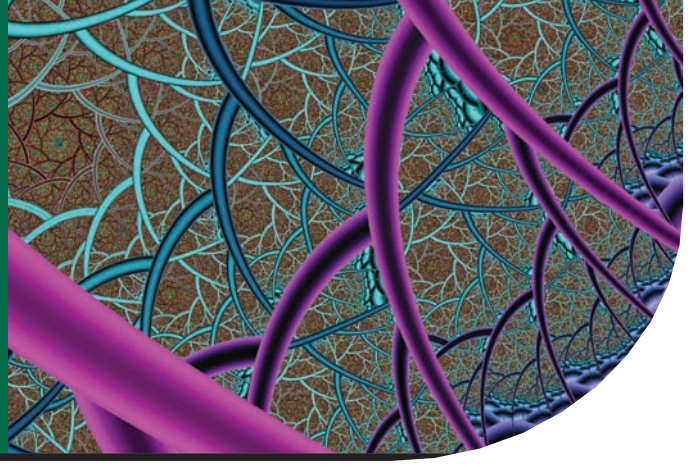

DNA as a Rubber Band: Single-Molecule DNA Stretching Using Optical Tweezers

The development of techniques to manipulate single molecules has led to large efforts to precisely study the mechanical and elastic properties of biomolecules such as proteins, protein fibers, DNA and RNA. Optical tweezers are a widely used technique in this area. They are sensitive in a biologically highly interesting force range: forces of typically a few hundred picoNewtons down to fractions of a picoNewton can be applied and measured using optical tweezers. This has allowed for, among other things, the precise measurement of forces and displacements exerted by individual 'motor proteins', enzymes responsible for the conversion of chemical into mechanical energy in biology. 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."***

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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 *(Figure 1a)* 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.

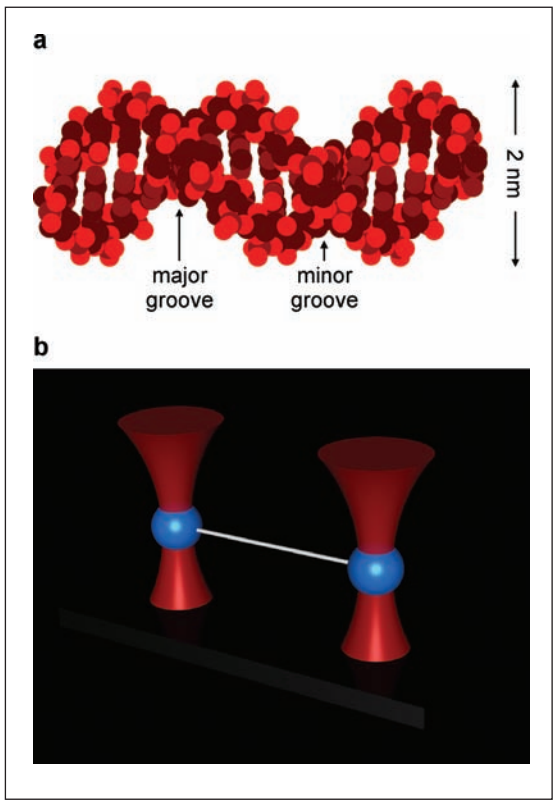

Figure 1a shows the unique helical structure formed by two intertwined and base-paired strands. Figure 1b shows that using two optical traps, a single DNA molecule can be suspended between two streptavidin-coated polystyrene beads.

EXPERIMENT DESCRIPTION

We have performed measurements of DNA elasticity using JPK's NanoTracker™, an off-the-shelf optical-tweezers platform designed for high-resolution quantitative nanomanipulation. Force measurements are performed using back-focal-plane interferometry on InGaAs quadrant photodiodes, calibrated using power spectrum analysis [2]. Using two optical traps, a single DNA molecule can be suspended between two streptavidin-coated polystyrene beads, as depicted in *Figure 1b*. To obtain a specific connection to the streptavidin-coated beads, the DNA (from the bacteriophage lambda) is modified to have several biotin groups at its termini.

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.

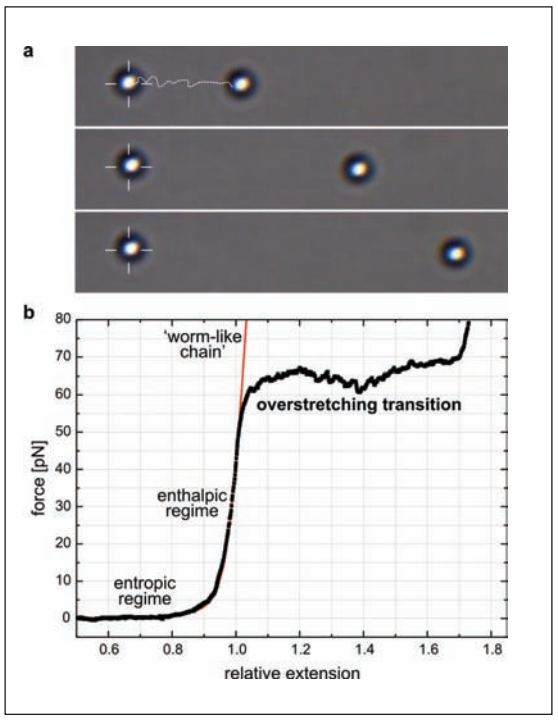

Figure 2a shows a typical measurement of the highly non-linear elasticity of double-stranded DNA. Figure 2b shows a fit of the elasticity data to the WLC model (red line).

Figure 2 shows a typical measurement of the highly non-linear elasticity of double-stranded DNA. Even when the DNA is slack, for example, 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 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 2b* shows a fit of the elasticity data to the WLC model (red line).

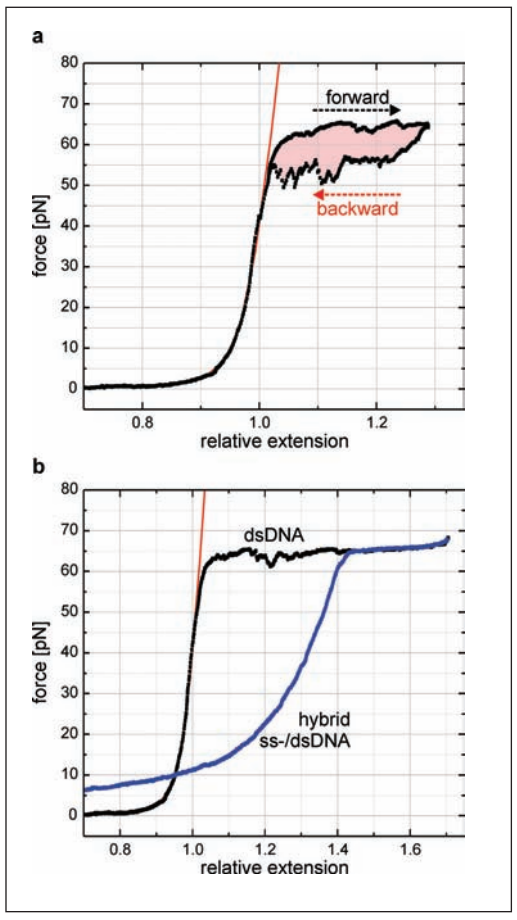

Figure 3a. An example of hysteresis between stretching (forward) and relaxing (backward) of a DNA molecule. Figure 3b. In some cases though, the molecule can irreversibly change its elastic behaviour.

A remarkable feature is observed in *Figure 2b* 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, for example, 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 3a* shows an example of such hysteresis between stretching (forward) and relaxing (backward) of a DNA molecule. Even in the presence of hysteresis,

Joost van Mameren with the NanoTracker

the overstretching transition itself is typically fully reversible. In some cases though, the molecule can irreversibly change its elastic behaviour, as seen in *Figure 3b*. In this case, the DNA apparently lost part of one of its two strands, since the resulting elastic behaviour 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 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 polymerisation 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 visualisation 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. It is to be expected that the emergence of commercial optical-tweezers platforms will help to spread this technology beyond the self-building enthusiasts to a broader group of life science researchers.

ACKNOWLEDGMENTS

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[1]. S. B. Smith, L. Finzi, and C. Bustamante. Direct mechanical measurements of the elasticity of single DNA molecules by using magnetic beads. Science, 258(5085):1122–1126, 1992.

[2]. K. Berg-Sorensen and H. Flyvbjerg. Power spectrum analysis for optical tweezers. Review of Scientific Instruments, 75(3):594–612, 2004.

[3]. C. Bustamante, J. F. Marko, E. D. Siggia, and S. Smith. Entropic elasticity of lambda-phage DNA. Science, 265(5178):1599–1600, 1994.

[4]. M. D. Wang, H. Yin, R. Landick, J. Gelles, and S. M. Block. Stretching DNA with optical tweezers. Biophysical Journal, 72(3):1335–1346, 1997.

[5]. S. B. Smith, Y. Cui, and C. Bustamante. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. Science, 271(5250):795–799, 1996.

[6]. P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J. L. Viovy, D. Chatenay, and F. Caron. DNA: An extensible molecule. Science, 271(5250):792–794, 1996.

[7]. I. Rouzina and V. A. Bloomfield. Force-induced melting of the DNA double helix. 1. Thermodynamic analysis. Biophysical Journal, 80(2):882–893, 2001.

[8]. I. Tinoco J., P. T. X. Li, and C. Bustamante. Determination of thermodynamics and kinetics of RNA reactions by force. Quarterly Reviews of Biophysics 39(4):325–360, 2006

[9]. P.Bechtluft, R.G.H. van Leeuwen, M. Tyreman, D. Tomkiewicz, N. Nouwen, H.L. Tepper, A.J.M. Driessen, S.J. Tans. Direct observation of chaperone-induced changes in a protein folding pathway. Science 318: 1458-1461 (2007).

[10]. K. Pant, R. L. Karpel, and M. C. Williams. Kinetic Regulation of Single DNA Molecule Denaturation by T4 Gene 32 Protein Structural Domains. Journal of Molecular Biology, 327(3):571–578, 2003.

[11]. I. D. Vladescu, M. J. McCauley, M. E. Nunez, I. Rouzina, and M. C. Williams. Quantifying force-dependent and zero-force DNA intercalation by single-molecule stretching. Nature Methods, 4(6):517–522, 2007.

[12]. G. J. L. Wuite, S. B. Smith, M. Young, D. Keller, and C. Bustamante. Single-molecule studies of the effect of template tension on T7 DNA polymerase activity. Nature, 404(6773):103–106, 2000.

[13]. J. van Mameren, M. Modesti, R. Kanaar, C. Wyman, G. J. L. Wuite, and E. J. G. Peterman. Counting RAD51 proteins disassembling from nucleoprotein filaments under tension. Nature, in press, 2008.

Perfect Focus System Ranked Second in Top Innovations of 2008

Nikon Instruments' exclusive Perfect Focus System (PFS) has been ranked second in the US life sciences journal, The Scientist's top innovations of 2008 – its first ranking of the best innovations to hit the life science market in the past year. The Scientist asked a panel of expert judges to sort through the year's offerings and pick the ones likely to have the biggest impact. The judges – David Piston, Simon Watkins, Klaus Hahn, and Steven Wiley are all known for pushing the technical boundaries and have collectively published more than 700 scholarly articles.

Nikon's PFS is the solution to focus drift – one of the biggest challenges in high resolution and live cell imaging, and is compatible with the innovative Ti inverted microscope series, launched at the end of 2007.

PFS is a hardware component that uses a half-moon shaped beam of infrared light to track optical offset and correct for it by sampling every five milliseconds. It holds focus both in time-lapse experiments and in short-term studies where acts such as perfusing a drug or moving a Z-stage might shift the sample. Similar systems exist, Ross says, but they don't work as well because they sample much less frequently.

Beaten only by a low-cost genome sequencing system, Nikon's PFS is, according to judge Simon Watkins, Vice Chairman and Head of the Department of Cell Biology and Physiology at the University of Pittsburgh School of Medicine: "Invaluable for any investigator performing microscope-based live cell analyses over an extended period of time. It maintains the specimens in focus regardless of temperature or vibration, such that you can conduct experimental manipulations during imaging, and continue the experiment for days."