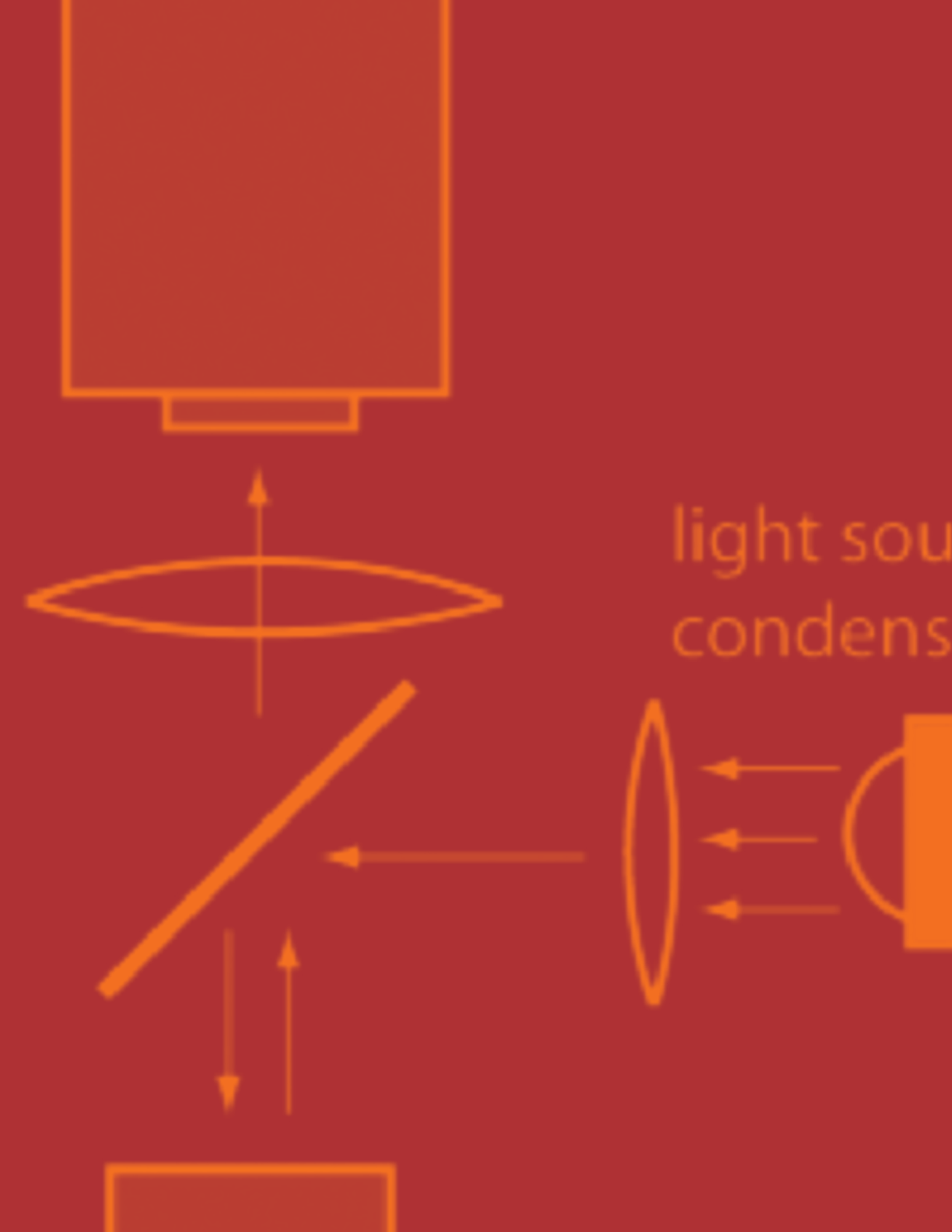
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Development of a Magnetic Tweezer Apparatus for the Manipulation of Single DNA Molecules

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Abstract

Magnetic tweezers are a simple yet powerful tool for the micromanipulation of single molecules. In this study, a vertical magnetic tweezer apparatus was constructed for research on the micromechanics of DNA, DNA supercoiling, and DNA-protein interactions. By joining DNA molecules to magnetic beads and attaching these tethers to the wall of a flow cell, discrete forces were applied to extend the molecules by adjusting the vertical position of the magnets, and a torque was applied by rotating the magnets. The mechanical response of the molecule was then observed in real time by measuring the position of the bead along the stretching axis. To achieve a viable system, standard models of DNA extension and supercoiling were tested using the apparatus with bare DNA tethers. In addition to the intrinsic mechanical properties of single DNA molecules, DNA-protein interactions were characterized by injecting a protein solution into the flow cell and measuring the mechanical response of the tethered DNA. Investigations of the enzyme Topoisomerase V and other biomolecules are proposed.

Introduction

Recent advances have enabled scientists to explore biophysics at the level of single molecules. Many researchers involved in single-molecule research have directed their attention to deoxyribonucleic acid (DNA). DNA contains an organism's genetic code in the form of a long double-helical molecule several orders of magnitude longer than the cell in which it is contained. Consequently, the DNA must be compacted to fit inside a cell. This condensation is achieved by various proteins binding to and bending the DNA until it is folded into a form that fits conveniently in the cell. In order to transcribe the genetic code during cell division, the compacted DNA structure must be disassembled. With all of this condensation and decondensation, it is clear that understanding the intrinsic mechanical properties of DNA, and how they are affected by DNA-binding proteins, is crucial to understanding how DNA functions in living cells.

One important molecular conformation that assists in DNA condensation is the supercoil, a twisted and compacted state that occurs inside bacterial cells. Supercoiling can be simply demonstrated by firmly holding a telephone cord at both ends and twisting. If the ends are held at constant force as the cord is twisted, it will contract and relieve stress by forming intertwining braids called *plectonemes.*[1]

Supercoiling can occur when a DNA molecule is torsionally constrained in the same way as a telephone cord when both ends are held. DNA is naturally constrained as a circular plasmid in prokaryotes such as bacteria, and it is thought that regions of the molecule are constrained and topologically independent from one another when anchored to the nuclear scaffolding in eukaryotic chromatin.[1] Supercoiling plays an important role in DNA compaction, transcription, and repair, and a group of enzymes called topoisomerases mediates the unwinding and unknotting of the molecule required for these functions.[2]

Precise knowledge of the micromechanics and structural changes of supercoiled DNA could greatly assist fields as diverse as genetics, cell biology, and nanotechnology, all of which employ DNA for a variety of purposes. In this research, a magnetic tweezer apparatus was constructed, tested, and demonstrated to be an effective tool for this investigation. While experiments here used bare DNA molecules, this study opens up several avenues for research, most notably the study of the interactions between DNA molecules and topoisomerases.

Background

Various tools have been developed over the past 15 years to manipulate single DNA molecules and investigate their mechanical properties. Typically, one end of a DNA molecule is chemically attached to a glass surface and the other to a mechanical force sensor or a magnetic or nonmagnetic bead. Forces are applied by the mechanical sensor magnets, laser tweezers, or hydrodynamic flow, respectively. The resulting measurements of force and position provide information about the mechanical response of the molecule.[3] The first of these investigations was that of Smith et al., which used a combination of magnets and hydrodynamic flow to study the elasticity of DNA.[4]

The Smith approach was advanced by Strick et al. in part by eliminating hydrodynamic flow, thereby improving the signal-to-noise ratio.[1,2] In the Strick apparatus, a DNA molecule is tethered to a glass slide on one end and a magnetic bead on the other by oligonucleotides, one labeled with biotin and the other with digoxigenin. These labels provide specific binding affinities, allowing easy attachment of the molecule to surfaces functionalized with complimentary ligands. Magnetic tweezers are used to hold the bead at constant force, torsionally constraining the DNA molecule. The force can be adjusted by translating the magnets along the optical axis, and torque can be applied by rotating the magnet assembly. This approach has the advantage of simply determining the force (F) by calculating the molecule's rigidity (k) from the bead's thermal fluctuations by invoking the equipartition theorem,

$$\frac{F}{l} = k_x = \frac{K_B T}{\langle \delta x^2 \rangle},$$

where l is the molecular extension, k is the Boltzmann constant, T is the absolute temperature and $\langle \delta x^2 \rangle$ is the mean-square fluctuation transverse to the stretching axis.[2]

Using a magnetic field to torsionally constrain the DNA molecules allows experimenters to investigate the structure and topology of supercoiled DNA, which, as discussed above, plays a crucial role in many of the molecule's functions. DNA supercoiling can be characterized by three quantities.[1] The first, twist (*Tw*), is the number of times the two strands of DNA twist around each other in the helical structure. Relaxed DNA has one twist per 10.4 base pairs (bp). The second quantity, writhe (*Wr*), can be thought of as "the number of crossings of the molecule axis over itself, when the molecule shape is projected onto a plane."[5] In extended DNA, *Wr* is 0, although it can be increased or decreased through the formation of plectonemes.

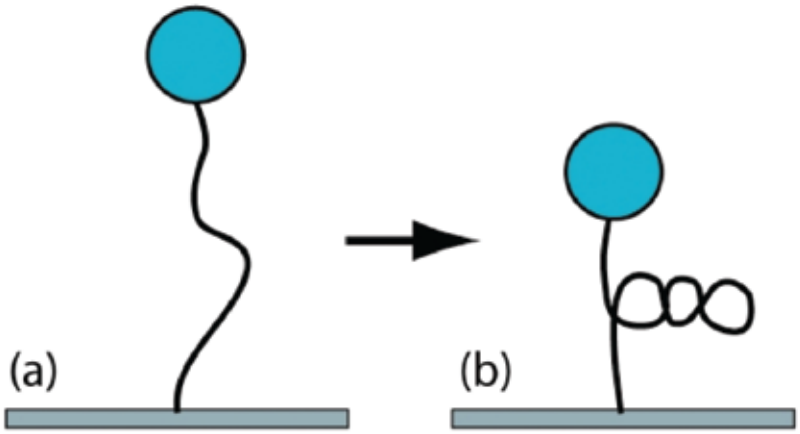

Figure 1. Transition of a DNA tether from (a) extended to (b) plectonemic states as it is supercoiled by twisting. In many models (and this schematic), DNA is treated as a uniform, semiflexible polymer.

In a constrained DNA molecule, twist and writhe are connected to a third, topologically invariant quantity, called the linking number (*Lk*) by a mathematical theorem:

$$Lk = Tw + Wr.$$

Although Lk is a topological constant, a DNA molecule's values of Tw and Wr vary under different conditions, as seen in the difference between the extended and plectonemic states (Figure 1). In the Strick set-up, the value of *Lk* can be increased or decreased ("overwinding" or "underwinding") by an integral number. A useful measure of this change from the relaxed linking number, Lk_0, is the degree of supercoiling,

$$\sigma = \frac{Lk - Lk_0}{Lk_0} = \frac{\Delta Lk}{Lk_0}.$$

Plasmid DNA within bacteria typically have a nonzero degree of supercoiling ($\sigma \approx -0.05$). While plectonemes can be introduced artificially by rotating the magnets of magnetic tweezers, cells use enzymes — proteins that bind and alter the structure of their ligands — to manipulate DNA conformations.

Adding a solution of enzymes to a flow cell containing a DNA molecule constrained by a magnetic field and continuously measuring the molecule's extension allows real-time observation of the effect of the enzyme on the molecule.

One set of enzymes studied using this technique is topoisomerases, which, as previously mentioned, mediate unwinding and unknotting of cellular DNA. Topoisomerases achieve this by changing a constrained molecule's linking number; they are divided into two types based on their mechanism. Type I topoisomerases cut a single strand of DNA, allowing the other to rotate. Depending on the exact mechanism, this changes the value of *Lk* by either ±1 (type IA), or by a value with a magnitude greater than one (type IB). Type II topoisomerases cut both strands and allow another double-stranded DNA (dsDNA) to pass through before resealing the molecule.

Approach

Experiments were performed by vertically extending DNA molecules along the optical axis of a high-numerical aperture microscope objective using magnetic tweezers ("vertical MT") specifically built for this research. In comparison with magnetic tweezers that extend DNA in the focal plane, vertical MT have lower extension resolution but are superior at making measurements of shorter molecules.[6] Additionally, vertical MT are easier to set up and use, but lack the ability to easily observe fluorescent markers along a DNA molecule. The apparatus used in this research is similar to the Strick apparatus previously discussed, but instead uses a normal objective and places the magnet assembly underneath the flow cell (Figure 2). Vertical MT systems based on this newer model have previously been used in the investigation of topoisomerases and chromatin formation.[6–8]

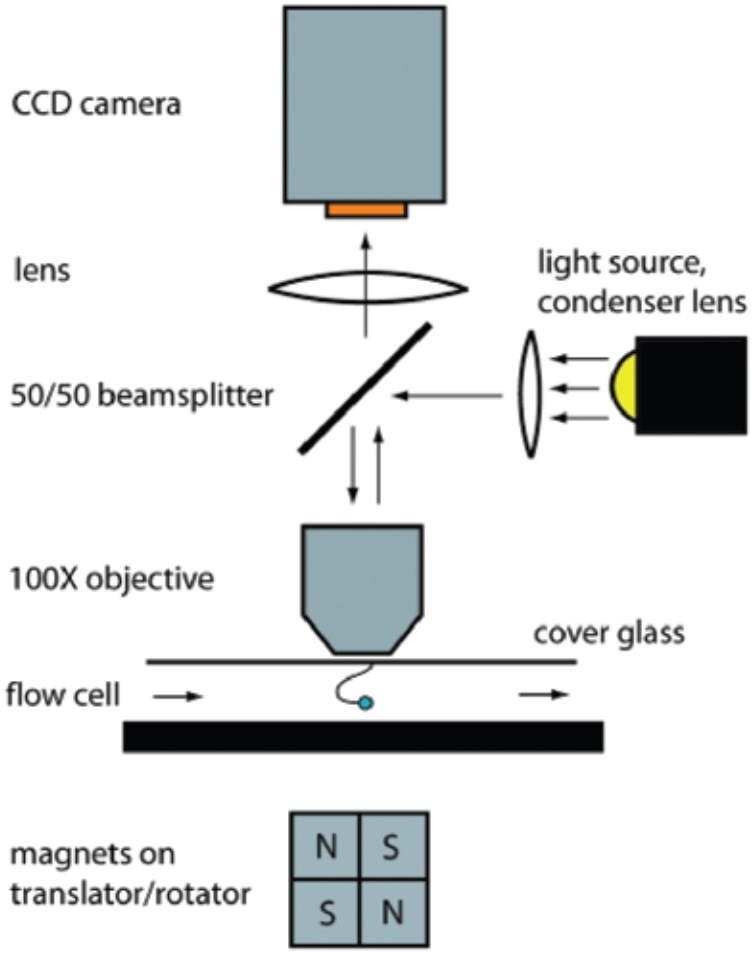

Figure 2. Schematic representation of the vertical magnetic tweezer apparatus.

Flow cells with 40 µL chambers were constructed as previously discussed.[9] Samples used a 9,702 bp plasmid, pNG1175, provided in linear and end-labeled form by Nigel Grindley of Yale University. Labeling was achieved through ligation of 800 bp DNA fragments carrying biotin or digoxigenin (DIG) labels, making the overall length roughly 10.3 kilo base pairs (kb), and providing different specific labels on opposite ends of the molecule. The molecules were tethered to streptavidin-coated 2.8-µm-diameter superparamagnetic polystyrene beads, and the tethers were bound to the anti-DIG-functionalized glass of the flow cell in phosphate buffered saline (PBS). Forces and torques were applied to the beads by NdFeB permanent magnets and adjusted by translation using a stepper motor. When a magnetic force was applied to the tethered bead, the linkers on both ends constrained the DNA molecule. Rotating the magnet assembly reversibly increased or decreased the DNA molecule's linking number by integral numbers at constant force.

The beads in the flow cell were observed through a 100X 1.3 numerical aperture (NA) oil immersion objective using a charge-coupled device (CCD) camera; a piezoelectric system allowed translation of the objective in 10 nm intervals. Illumination was provided by a white LED using a condenser lens and a 50/50 beam splitter. Using bead-tracking software, forces were measured with the equipartition theorem as discussed above, and the molecule's extension was measured by a focusing algorithm that uses the piezoelectric system to compare the position of a tethered bead with that of a bead adsorbed to the glass.

After a sample was prepared, an adsorbed bead and a tethered bead in close proximity were located (Figure 3). Tethered beads exhibit distinct, constrained Brownian motion and are typically found in a different focal plane than adsorbed beads. Next, it was verified that the bead was tethered to a single molecule. DNA molecules are not directly observable in this approach, but the presence of a single molecule may be ensured by measuring the force-extension relationship. At higher forces (~10 piconewtons, pN), the molecule is expected to extend to roughly 3 µm, the full length of pNG1175; if two molecules are tethered, the value will be significantly smaller. As an additional check, single molecules should have a symmetric relationship between extension and the degree of supercoiling that develops an asymmetry at higher forces.[5] After verifying that a particular bead is tethered to a single molecule, the instrument was calibrated by measuring force as a function of magnet position. This calibration is required because individual beads have slightly different magnetizations.

Results and Discussion

The completed vertical MT apparatus (Figure 4) was first tested to verify that it could properly measure the force on and extension of a single DNA molecule, and to determine if the molecule could be extended and supercoiled. Once the apparatus was determined to be fully functional, the molecule was extended at various forces and twisted at a fixed force, and the resulting data were compared to standard models.

DNA extension was tested using a force (F) versus extension (z) plot by translating the magnet to vary the force between roughly 0.006 pN and 10 pN. Lower forces could not be achieved, as they marked the lower limit of magnet translation, and higher forces were not used because they stretched the DNA beyond its natural contour length. The resulting data (Figure 5) agree with previous single-DNA stretching experiments.[1–3]

The contour length (L) and persistence length (A) were calculated using data points for forces greater than 0.5 pN, and the asymptotic form of the semiflexible polymer model for bare, dsDNA,[5]

$$\frac{1}{\sqrt{F}} = -\frac{z}{L}\sqrt{\frac{4A}{k_B T}} + \sqrt{\frac{4A}{k_B T}},$$

where F is the force, z is the extension and $k_B T$ is the Boltzmann factor. The persistence length quantifies a polymer's stiffness and is roughly the length scale at which it coils randomly. The calculation of both quantities was achieved by a linear fit in a z versus $F^{-1/2}$ plot (r^2 = 0.9834, the coefficient of determination). The contour length was 3.32 µm, in strong agreement with the expected 3.43 ± 0.2 µm (assuming 0.3 nm/bp for dsDNA and uncertainty from the labels), and the persistence length, 40.8 nm, matched moderately well with typical estimates of 50 nm. The main sources of error were fluctuations in extension at low force and the use of a statistical quantity to calculate force.

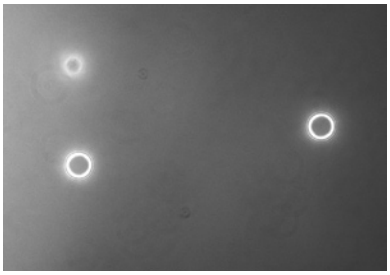

Figure 3. Three magnetic beads, as seen through the objective. The two in focus are adsorbed on the top of the flow cell, while the other is roughly 1 μm below them, attached to a DNA tether and moving by constrained Brownian motion.

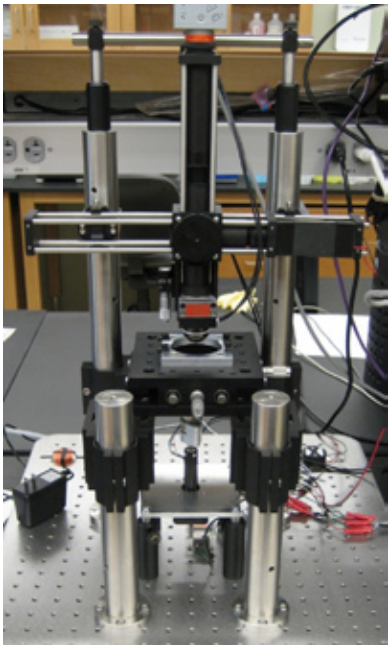

Figure 4. The completed magnetic tweezer setup. The apparatus is mounted on a platform that dampens external vibrations using compressed air. Both the translator/rotator system and the CCD camera are controlled by a computer.

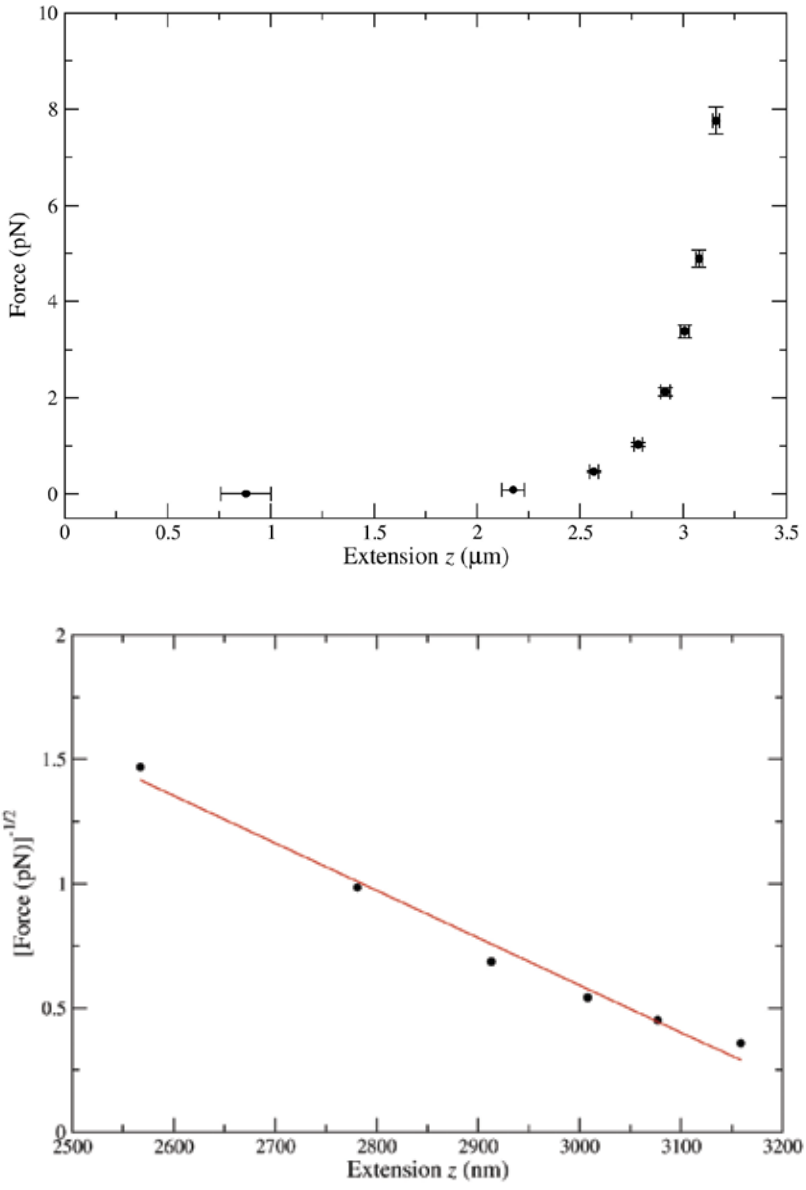

Figure 5. Extension-force data taken from a single tether of DNA and its fit (for $F > 0.5$ pN) to the asymptotic form of the semiflexible polymer model.

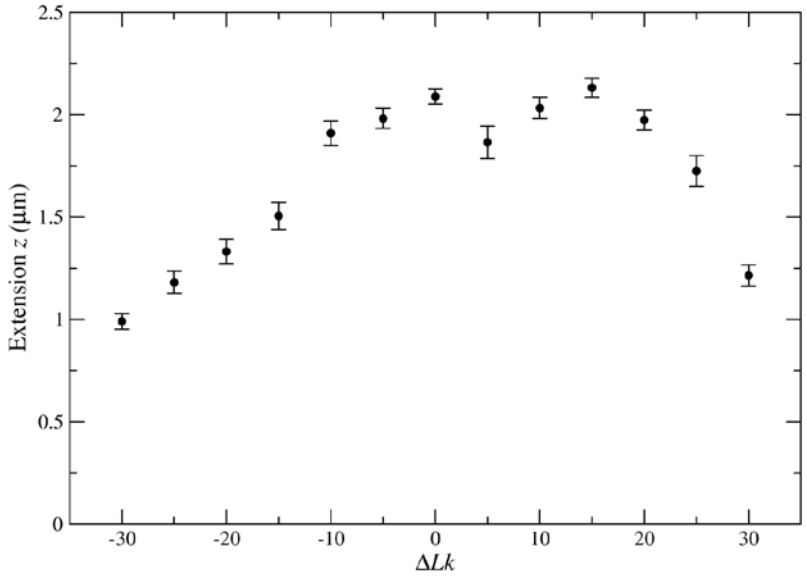

Figure 6. The response of a DNA molecule to changes in linking number by twisting at 0.5 pN. Points represent averages, and error bars the 95% confidence interval.

Additional extension data were taken for changes of linking number at a set force of 0.5 pN. Data was collected after every five rotations of the magnet, and one sweep was made for ΔLk varying from -30 to 30. A reverse sweep verified a lack of hysterisis. The resulting curve (Figure 6) suggests a decrease of extension caused by the formation of plectonemes as the DNA was twisted. As expected, the data are somewhere between the completely symmetric (for twisting at 0.2 pN) and completely asymmetric (1 pN) data that other groups have published. The symmetry breaking at high force is a result of the right-handed chirality of this form of DNA.

Conclusion

In observing both extension and twisting of single DNA molecules, the vertical MT apparatus adequately reproduces well-cited relationships between extension, force, and linking number, providing good estimates of contour length and persistence length and demonstrating the symmetry breaking between underwinding and overwinding of a DNA molecule. Significant errors exist in the data, especially at the low-force limit, but observations could be improved by increasing the acquisition time.

This project will continue with studies of DNA-protein interactions, particularly an investigation of Topoisomerase V (Topo V), a type I topoisomerase found in a hypothermophilic bacteria that lacks several key features of types IA and IB.[7] Vertical MT could further characterize the mechanism of this unusual high-temperature enzyme and determine if there is a difference in nonlinear friction between left-handed and right-handed DNA rotation.

Further research avenues are varied, and possible uses of this apparatus are not limited to DNA or DNA-protein studies. By finding or developing appropriate linkers, magnetic tweezers could probe the forces required to break an antigen-antibody bond or unfold proteins. Given their ease of use and success in manipulating single molecules, magnetic tweezers are an excellent tool for continued research on the nanometer scale.

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