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Hamin Jeon 16 April 2013

Physical Characteristics of DAP DNA

by

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Physical Characteristics of DAP DNA

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An abstract of a thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment of the requirements of the degree of Bachelor of Sciences with Honors

Department of Physics

2013

Abstract

Physical Characteristics of DAP DNA

by Hamin Jeon

The flexibility and torsional stiffness of DNA can influence important biological processes including nucleosome formation and DNA wrapping. To study the significance of these DNA properties, we characterized the effects of tension and torsion on 2,6 diaminopurine substituted DNA (DAP DNA) by using magnetic tweezers (MTs). By obtaining plots of DNA extension as a function of torsion applied to the DNA under different tensions, the helical repeat of DAP DNA in left-handed form and the relative extension of DAP DNA in left-handed form to right-handed form were computed. Force vs. extension curves of DAP DNA under different amounts of torsion were also plotted, and used to calculate the torsional constant, 62.0 $\pm$ 21.6 nm, and critical torque, 3.73×10^{-21} N×m of DAP DNA. The computed critical torque value was different from the value reported in the literature[3] for normal DNA.

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Chapter 1: Introduction

1.1 Motivation

In numerous biological functions, a mechanism that involves DNA bending may be essential. For instance, nucleosomes, which consist of 147 base pairs of DNA wrapped around a histone octamer,[2] require bending of DNA for their formation. During nucleosome formation, DNA needs to wrap around the histone octamer and certain DNA flexibility can better accommodate large curvatures around the octamer[2]. Topoisomerase enzymes, which can regulate overwinding and underwinding of DNA, also depend on the flexibility of DNA. Indeed, topoisomerases wrap DNA around one of their domains and, thus, the elasticity of DNA may influence the enzyme function. In DAP DNA, all base pairs are held together by three hydrogen bonds. This characteristic makes DAP DNA to be stiffer than normal DNA[1] without substantially altering the DNA sequence. Thus, DAP DNA can be used as a tool to investigate the effect of DNA stiffness on different biological systems. In poly (GC) DNA, base pairs are also connected by three hydrogen bonds. However, its sequence is significantly altered and it is hard to prepare since several ligation steps are necessary to synthesize a long fragment of poly (GC) DNA. Thus, my goal is to characterize DAP DNA in order to use it in studies where DNA torsional and bending elasticity may be important.

1.2 DNA Supercoiling

The DNA helix, in its crystallographic, relaxed B form, has a turn every 10.4 base pairs (bp). DNA may form supercoils when the long axis of the helix crosses itself. Therefore, the topology of DNA can be characterized by a term, the linking number, Lk=Tw+Wr, in which Tw (twist) represents the helical winding of the two DNA strands around one another, and Wr (writhe) represents DNA's coiling about its own axis[1]. When DNA writhes (or supercoils, or buckles), its extension decreases.

Some proteins and enzymes can either (un)twist DNA, or introduce supercoils[1]. DNA is normally a right-handed helix (B-DNA), but when it is unwound, it can transform into a left-handed structure, or L-DNA. L-DNA can have different elastic and helical properties and, thus, in this work, I studied the physical properties of unwound DAP DNA.

1.3 Magnetic Tweezers

Magnetic tweezers (MTs) is an instrument that can manipulate a single DNA molecule by applying tension and torsion. A photograph of the instrument that I used to perform this research can be found in Dr. Qing Shao's published literature[1] as Figure 2.1.

A chamber containing immobilized DNA molecules tethering a super-paramagnetic bead is put on the microscope stage. Above the stage, there is a pair of permanent magnets, which can either be rotated, or moved vertically. When rotated, the magnets can apply twist and, thus, torsion to the DNA. When moved vertically, the magnets' position relative to the sample can be changed which results in different tensions applied to the DNA. The effect of the magnets is shown in more detail in the Figure 1 below.

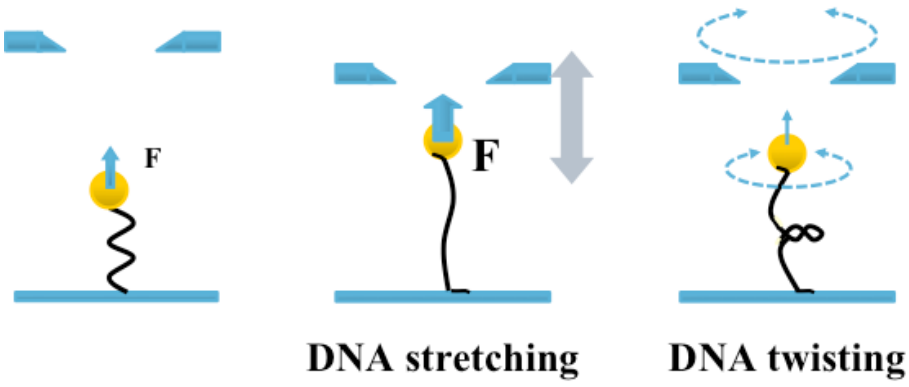

Figure 1: Illustration of magnetic tweezers' magnets applying tension and torsion to DNA (provided by Dr. Mónica Fernández-Sierra).

In the second panel in Figure 1, the magnets are closer to the DNA tether than those in the first panel and, thus, they apply a stronger stretching force to the DNA. This results in the stretching of the DNA molecule and an increase in its extension. In the third panel in Figure 1, the magnets are rotated, which results in twisting of the DNA. The objective in the

microscope focuses the light from a light-emitting diode (LED) onto the FireWire camera. The camera then captures the image of the field of view that contains the DNA-bead system, via a MATLAB based program interface.

When the light from the LED illuminates the paramagnetic beads, some of it is scattered. The interference between the scattered and unscattered light produces a diffraction pattern made of concentric rings around the bead, which depend on the distance between the bead and the focal point of the objective lens. During the calibration process, the diffraction patterns of a bead stuck to the surface of the coverslip is used as a reference and compared to that of the DNA-tethered bead. The extension of the DNA tether can be then calculated.

1.4 DAP DNA

2,6 Diaminopurine (DAP) substituted DNA is a molecule in which adenine bases are substituted by DAP. DAP and thymine form three hydrogen bonds, whereas only two hydrogen bonds are formed between adenine and thymine (AT) base pairs. Therefore, DAP DNA has a different elasticity from normal DNA. Figure 2 below illustrates the hydrogen bonding in different base pairs.

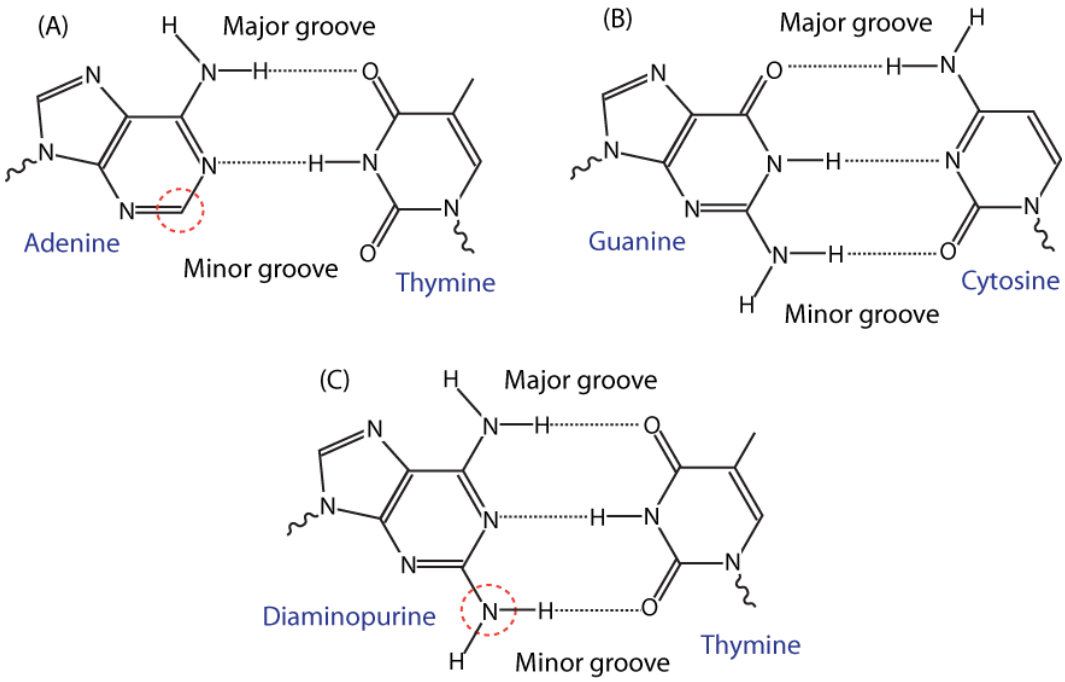

Figure 2: Illustration of adenine and thymine, guanine and cytosine, and DAP and thymine base pairs.

DAP DNA molecules were labeled with digoxigenin molecules at one end and attached to a glass microscope coverslip, which was coated with antidigoxigenin[2]. The DAP DNA was also biotin-labeled at the opposite end, which was linked to a paramagnetic bead coated with streptavidin [2]. A chamber made of two coverslips was then mounted on the microscope stage and torsion and tension were applied to the DNA tethers by either rotating the magnetic tweezers or varying the distance between the magnets and the chamber.

By observing the change in DNA extension as a function of torsion at different tensions, multiple plots of the tether extension as a function of number of turns were obtained at different levels of tension (Figure 5). The point on each plot at which the complete transition from right-handed DNA to left-handed DNA form occurs was recorded, and was used to compute the helical repeat of L-DNA. By studying the change in extension of the tether based on different amount of torsion applied, the transition behavior was also observed.

To compute both the torsional constant and the critical torque of DAP DNA (the amount of torque required for the transition from B-DNA to L-DNA form to occur), multiple plots of tension as a function of DNA tether extension were obtained. For each plot, a different number of turns was introduced to the tether, either in the direction of winding (positive), or unwinding (negative). Then, a polynomial equation was fitted through each plot that involved positive twist or negative twist and the area between both fit curves were calculated. After a certain amount of twist is applied, the DNA tether will start to coil about itself and its extension will decrease. The point at which the decrease in extension occurs is called the buckling point and the supercoils may also be called plectonemes. When plotting tension as a function of DNA tether extension, the tension is varied from a low to high number, to stretch the DNA and pull out the plectoneme structure. Thus, the area between fit

curves represents the difference between the amount of work done to stretch an overwound DNA molecule and to pull an underwound DNA. Then, based on the work by Strick et al.[3], by calculating the slope from a plot of $\sqrt{\frac{(l*\Delta)}{k\mathrm{B}*\mathrm{T}*2\pi^2}}$ as a function of number of turns applied to the tether, the torsional modulus of DAP DNA can be calculated. In $\sqrt{\frac{(l*\Delta)}{k\mathrm{B}*\mathrm{T}*2\pi^2}}$, l represents the contour length of the DNA, Δ represents the difference in the amount of work done to stretch an overwound and pull an underwound DNA, k_B represents the Boltzmann constant, and T represents the temperature at which the experiment was done. The critical torque can be also computed as $2\pi \times n_c \times C/l$, according to the literature[3] and in the equation, n_c equals the critical number of turns at which B-DNA starts to transition into L-DNA and C represents the torsional modulus.

Chapter 2: Methods

2.1 Preparation of DAP DNA

The DAP DNA introduced into the chamber consisted of a 3050 bp-long main fragment ligated to a 1000 bp-long biotin-labeled tail at one end and a 1000 bp-long digoxigenin-labeled tail at the other. The ligation protocol is shown in the Table 1 below.

ligation reaction volume (ul) =	30						
average MW/bp (g/mol/bp) =	650						
termini molar ratio =	3						
assumed ligation efficiency =	30						
desired amount of ligation (ng) =	150						
	length (bp)	MW	concentration (ng/ul)	concentration (nM)	picomoles to mix	ng to mix	volume (ul)
main fragment (pUC18_nuB104 frag.)	3050	1982500	114	0.058	0.152	302.0	2.6
biotin tail fragment	1000	650000	45	0.069	0.457	297.0	6.6
dig tail fragment	1000	650000	81	0.125	0.457	297.0	3.7
reaction mix							
main DNA	2.6	1.3					
bio tail DNA	6.6	3.3					
dig tail DNA	3.7	1.8					
10X ligase buffer	3.0	1.5					
ligase (10 U/ul)	1.0	0.5					
H2O	13.1	6.5					
total	30.0	15.0					

Table 1: Protocol for the ligation of DAP DNA main fragment, biotin-labeled tail, and digoxigenin-labeled tail

The DAP DNA main fragment used in this research was prepared by using Polymerase Chain Reaction (PCR). During the PCR process, a desired region was amplified by using the plasmid DNA, PuC18-nuB104, a sense primer, S/pUC18-nuB104/2623-NgoMIV, and an anti-sense primer, A/pUC18-nuB104/2043-ApaI. The protocol for the PCR process is shown in the Table 2 below.

label				1	2	3	4	5	6	7	8		
PCR volume (ul)	30												0.04
			bp									master mix	extra
H2O				22.02	22.02	22.02	22.02	22.02	22.02	22.02	22.02	176.16	183.21
ThermoPol polymerase bfr (NEB)	10	X		3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	24.00	24.96
pUC18-nuB104 (diluted 10X)	125	ng/ul		0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.64	0.67
Taq Polymerase (NEB)	2.5	U/ul		0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	4.00	4.16
DAP (Trilink)	10	mM		0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	4.80	4.99
dGTP (Fermentas)	10	mM		0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	4.80	4.99
dCTP (Fermentas)	10	mM		0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	4.80	4.99
dTTP (Fermentas)	10	mM		0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	4.80	4.99
A/pUC18-nuB104/2043-ApaI (Tm = 56.3)	10	uM		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	8.00	8.32
S/pUC18-nuB104/2623-NgoMIV (Tm = 54.1)	10	uM	2000	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	8.00	8.32
total (ul)				30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	240.00	249.60
	volume from master mix			30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	residue	9.60
initial denaturation (min)		1:00		95	°C								
denaturation time (min)		0:10		94	°C								
annealing time (min)		0:15		52.6	°C								
elongation time (min)		3:05		68	°C								
number of cycles		30											
finishing elongation (min)		5:00		68	°C								
re-annealing (min)		5:00		55	°C								
re-annealing (min)		3:00		37	°C								
		forever		4	°C								

Table 2: PCR protocol used to produce a DAP DNA main fragment

The PCR product was then purified using QIA quick Gel Extraction kit (QIAGEN) to remove any excess primers, nucleotides, and protein. The resulting product was then digested with NgoMIV and ApaI to make the main DNA fragment. The digested sample was purified again with QIAGEN to produce a final main fragment of 3050 bp in length.

2.2 Preparation of a Flow Chamber

To prepare a chamber, two glass microscope coverslips of 24 by 50 mm dimension were used. They were cleaned with ethanol and two double-sided adhesive tape strips were then attached along the sides of one coverslip (Figure 3). Vacuum grease was used as spacer and was put on the coverslip inside the tape strips. The other cover slip was then placed on top of the coverslip with tape strips, which secured them together. Pictures of a typical flow chamber are shown in Figure 3 and 4 below.

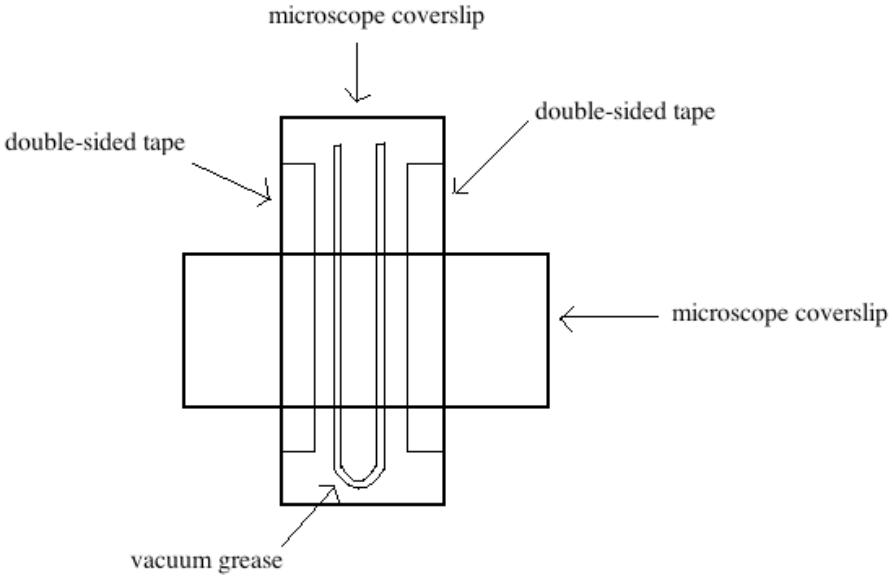

Figure 3: Flow chamber design.

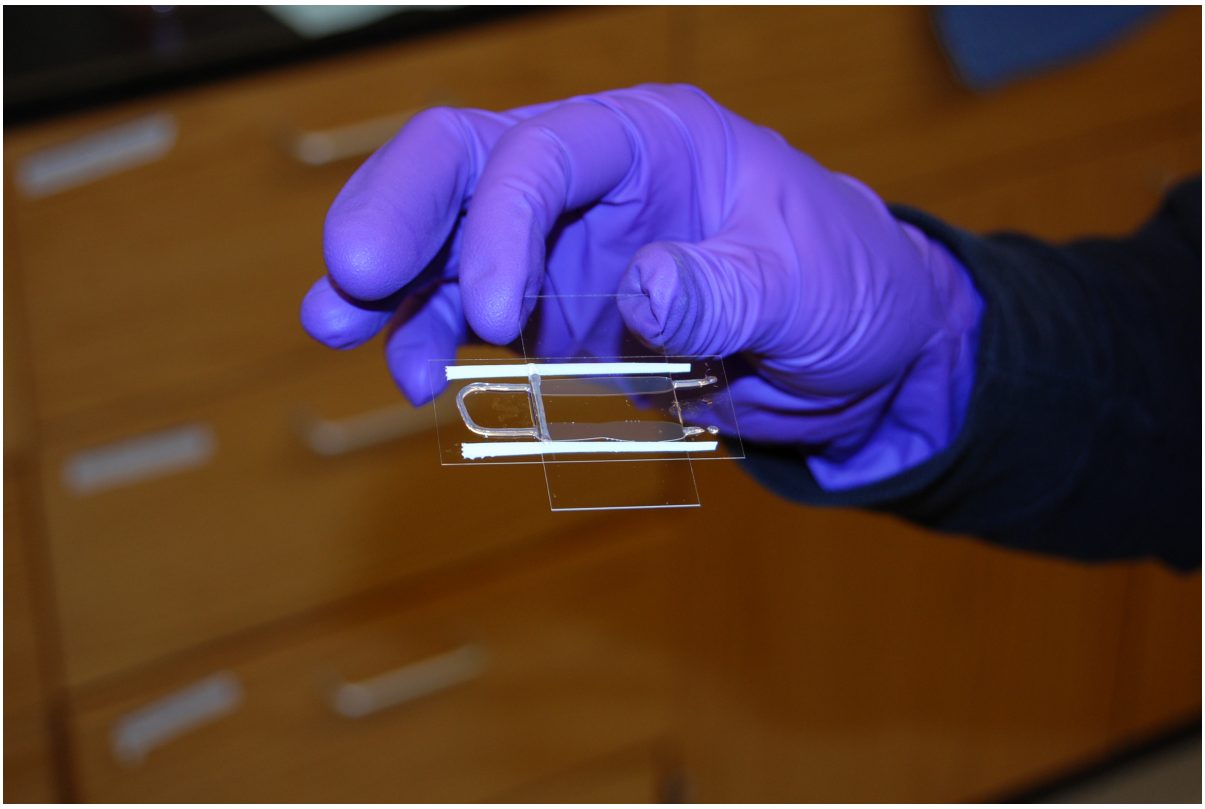

Figure 4: A sample picture of a flow chamber.

After preparing the flow chamber, 0.1 µl of 10 mg/ml paramagnetic beads (1-µm diameter, MyOne beads coated with streptavidin) was diluted in 50 µl of phosphate buffered saline (PBS) and was put into the flow chamber. After ten minutes of incubation, 200 µl of PBS was

applied to wash away any beads floating in the chamber. Then, 60 µl of 20 µg/ml anti-digoxigenin was put into the chamber, which was incubated at 4 °C overnight. The chamber was washed again with 200 µl of PBS four times and 80 µl of 100 µg/ml bovine serum albumin (BSA) was applied. The chamber was incubated for an hour and was washed with 200 µl of lambda buffer (10 mM Tris-HCL (pH 7.4), 200 mM KCl, 5% dimethyl sulfoxide (DMSO), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2 mM dithiothreitol) four times. At the same time, 0.3 µl of the MyOne bead was washed twice with 200 µl of PBS and then diluted in 10 µl of PBS. To the diluted beads, 0.7 µl of DAP DNA was added and the mixture was diluted again in 150 µl of lambda buffer. The bead and DAP DNA mixture was inserted in the flow chamber, which was then incubated for an hour. Finally, the chamber was washed twice with 150 µl of lambda buffer to remove unbound DNA tethers or beads, and then it was used for an experiment.

2.3 Magnetic Tweezers Experiment

The flow chamber was put on the stage of the magnetic tweezers microscope and the position of the oil-immersion objective was adjusted so that the oil made contact with the bottom of the chamber. The custom MATLAB program used to control our magnetic tweezers setup was then started. A real time video of the tethered beads was shown on the program screen. The position of the magnets relative to the chamber was then adjusted so that the force was around 1 pN. The field of view on the screen covers a portion of the area in the chamber and, thus, the field has to be changed to look for DNA tethers. The focus is usually adjusted to a plane above the DNA-tethered beads and, hence, the DNA-tethered beads are closer to focus than the stuck beads, which serve as depth position reference. This causes the DNA-tethered beads to have a smaller number of diffraction rings and look smaller than the reference beads.

Once a DNA tether and a nearby stuck bead were found, several tests were performed on the tether. The length of the tether was first determined by estimating how much the focus needs to be changed for the images of the DNA-tethered and reference beads to be similar. The length determined from this estimation must be similar to the contour length of about 1 μm. The DNA tether was also tested to see whether it was nicked. If the tether was nicked, it did not form plectonemes when twisted. Only unnicked DNA tethers were chosen. A test was performed to also see whether the tether bead had multiple DNA molecules bound. The bead can have multiple biotin binding sites and, thus, more than one DNA molecule could bind to the bead. For the experiment, a single DNA-tethered bead is needed, which forms plectonemes only if it is supercoiled in the direction of wrapping of the DNA helix at a force larger than 1 pN. However, a bead with multiple DNA tethers can form plectonemes when twisted in either direction.

After these tests, the magnet position was changed to apply low tension to the tether. The tether was twisted in both negative and positive directions by applying different number of turns and a plot of DNA extension as a function of number of turns was obtained. This plot, also called a chapeau curve, was used to test the symmetry in plectoneme formation in both positive and negative supercoiling regions. After testing the symmetry from the chapeau curve, the extension data of the DAP DNA tether was gathered as a function of the force applied to the tether. The plot of extension vs. force, which is also called a force-extension curve, was obtained by varying the distance between the magnet and the tether and was fitted to a worm-like-chain model. Before further examining the tether, the force-extension curve's close fit to the worm-like-chain was confirmed.

To calculate the torsional constant of DAP DNA, several more force-extension curves were obtained. After applying the same amount of twist in both positive and negative

directions to the DNA tether, force-extension curves were gathered by varying the magnet position. This process was repeated by applying different amount of twist each time.

By applying different tensions, more chapeau curves were also obtained. At different magnet positions, which apply different tension, chapeau curves were collected until the extension of the DNA almost reached zero when negative twist was introduced. From this data, the transition and the elastic behaviors of DAP DNA and its transition to L-DNA under different tension and torsion were studied.

Chapter 3: Results and Discussion

By applying different amounts of tension (represented by force) and torsion (represented by number of turns), the changes in DAP DNA's extension were observed, as shown in Figure 5. Such data was also collected by Dr. Qing Shao (Figures 6 and 7) and Dr. Mónica Fernández-Sierra (data not shown).

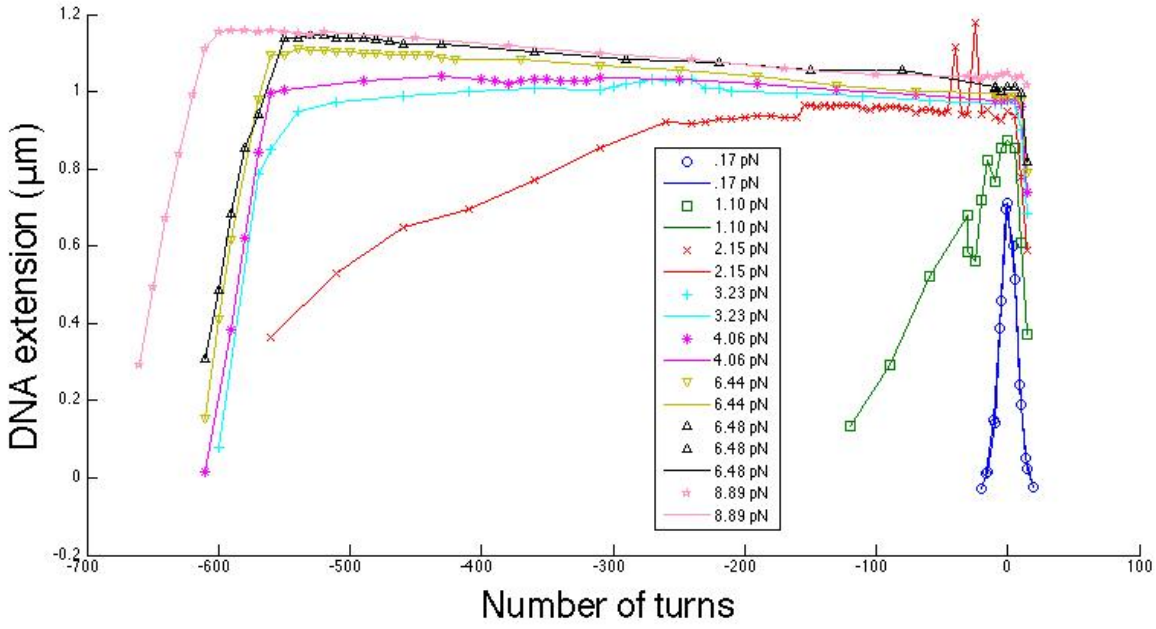

Figure 5: Plot of the extension of DAP DNA as a function of the number of turns.

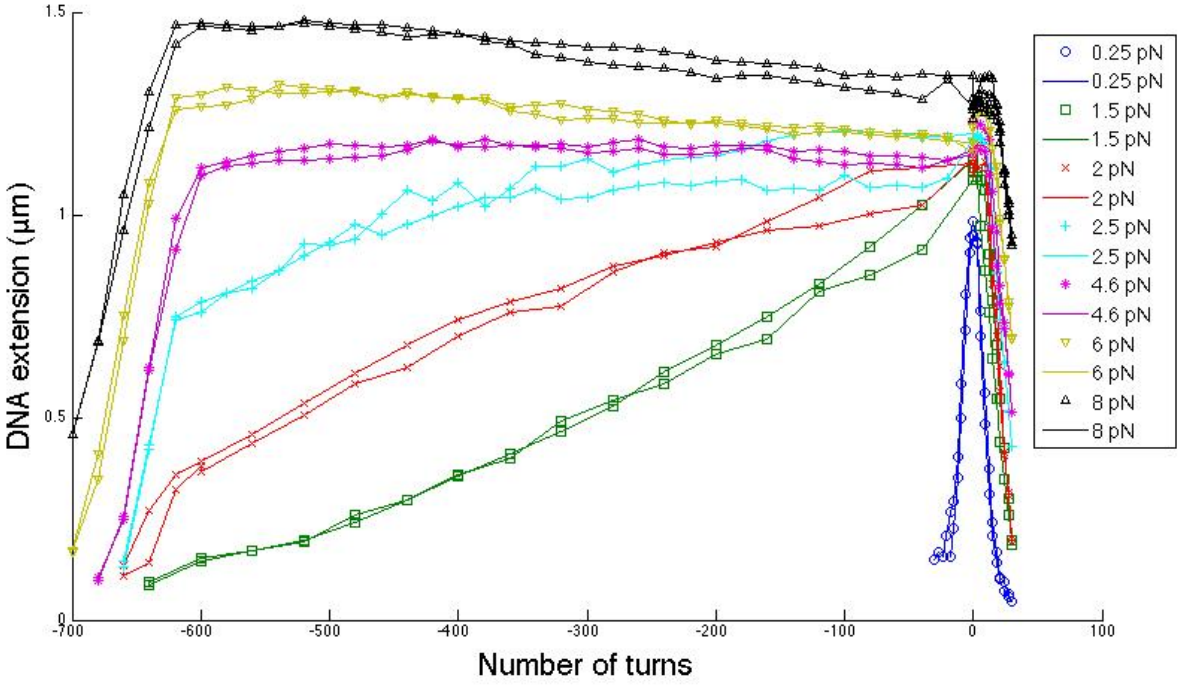

Figure 6: Plot of the extension of DAP DNA as a function of the number of turns (collected by Dr. Qing Shao).

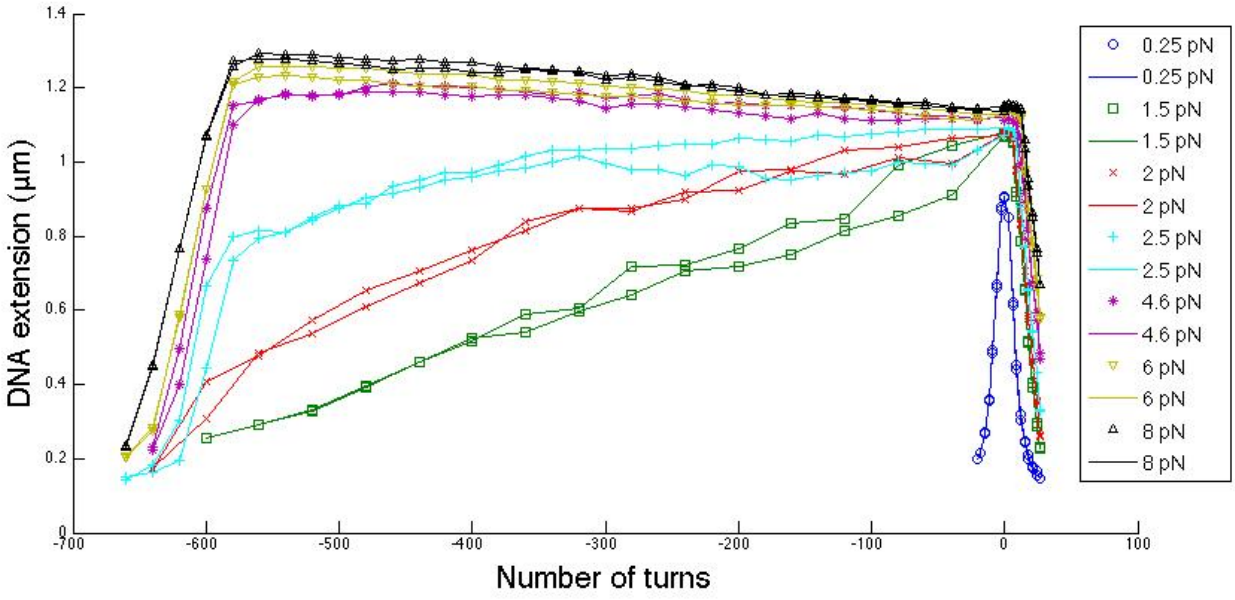

Figure 7: Plot of the extension of DAP DNA as a function of the number of turns (another data set collected by Dr. Qing Shao).

By combining the data that Dr. Qing Shao, Dr. Mónica Fernández-Sierra, and I collected, Figures 8 and 9 were obtained. To compute the L-DNA helical repeat, the total length of the DAP DNA in base pairs was divided by the number of turns at which the complete transition into L-DNA form happened after subtracting the natural twist amount of

DAP DNA. The point at which the DAP DNA was completely transitioned into L-DNA from B-DNA form was measured from Figures 5,6 and 7, and from other chapeau curves that are not shown here. To calculate the relative extension of L-DNA to B-DNA (Figure 9), the extension of the DAP DNA completely in L-DNA form was divided by the extension of DAP DNA in B-DNA form.

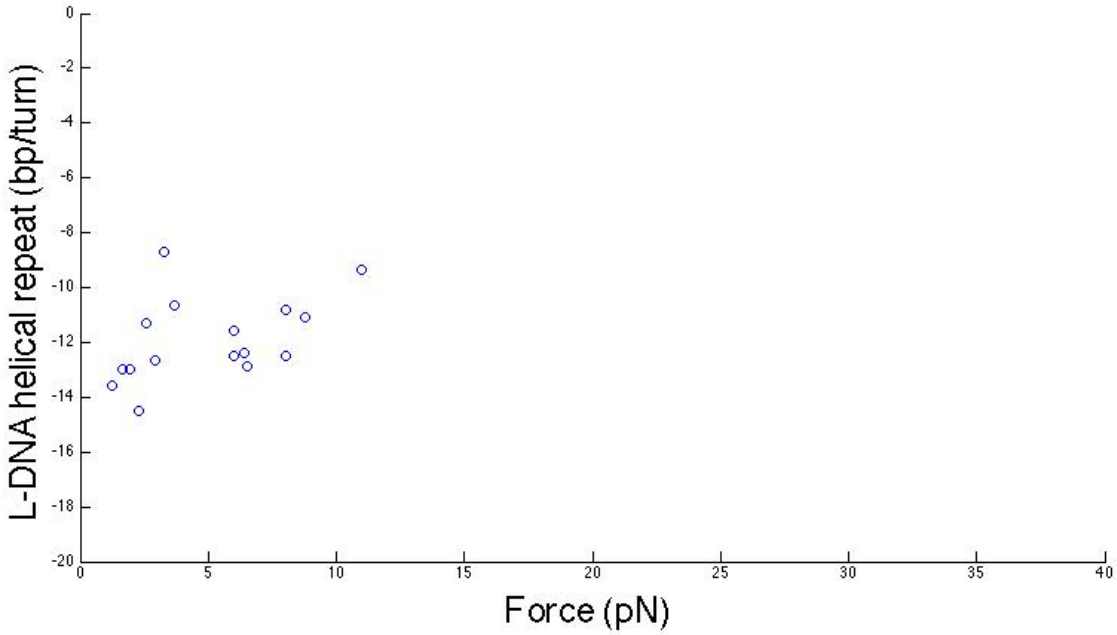

Figure 8: Plot of L-DNA helical repeat data as a function of force.

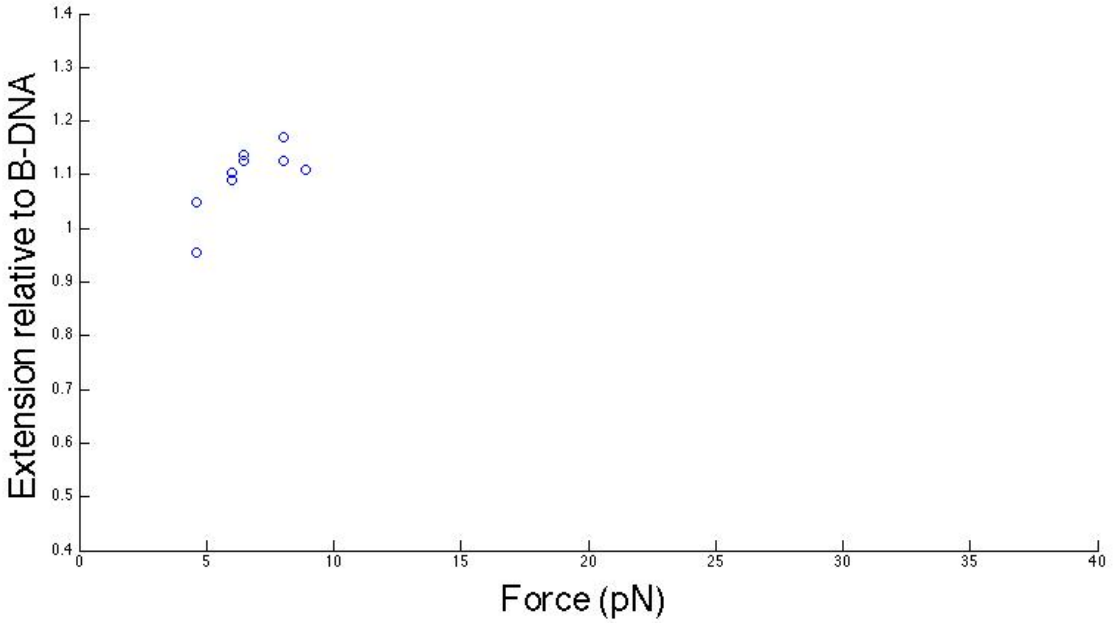

Figure 9: Plot of extension of L-DNA relative to B-DNA.

Based on Figure 8, which represents the data obtained for DAP DNA, L-DNA helical repeat value approximately ranges from -11 to -14. This value is comparable to the data obtained by Sheinin et al. for normal DNA, for which the L-DNA helical repeat is approximately -13[4]. According to Figure 9 and the data collected by Sheinin et al[4], the relative extension of L-DNA to B-DNA of DAP DNA follows a similar pattern to that of normal DNA as the relative extension changes in the force range of about 4.5 to 9 pN. In both data set, the relative extensions for both DAP and normal DNA are also measured above 1, which indicate that the L-DNA extension is greater than that of B-DNA for both DAP and normal DNA. Also, in Figures 5-7, there exist positions where DAP DNA transitions from right-handed form to left-handed form and to further study such areas, the following equations can be used. By using these equations, the fraction of DAP DNA in L-DNA and B-DNA form can be calculated.

$$d_{measured}(F) = \nu * d_L(F) + (1-\nu) * d_B(F) \quad \text{-Eq. (1)}^5$$

$$\nu = (d_{measured}(F) - d_B(F)) / (d_L(F) - d_B(F)) \quad \text{-Eq. (2)}^5$$

In Eq. 1 and 2, $d_{measured}$ represents the DNA extension at a position of interest, ν represents the fraction of left-handed DNA, d_L represents the DNA extension at which the DAP DNA is completely transformed into left-handed from, and d_B represents the DNA extension when no external torsion is applied to DNA, or where DAP DNA is only in right-handed form.

Next, the torsional constant and the critical torque of DAP DNA were computed by using force extension curves, which were obtained after applying a certain number of turns to the tether in both positive and negative directions.

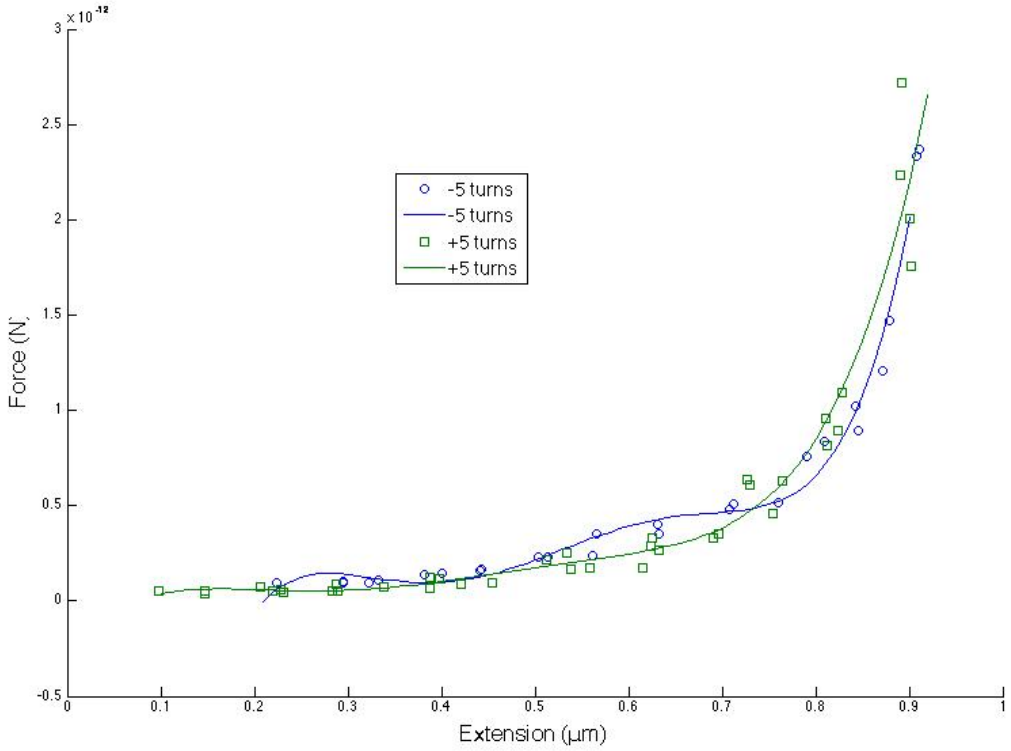

Figure 10: Force extension plots when ±5 turns were applied.

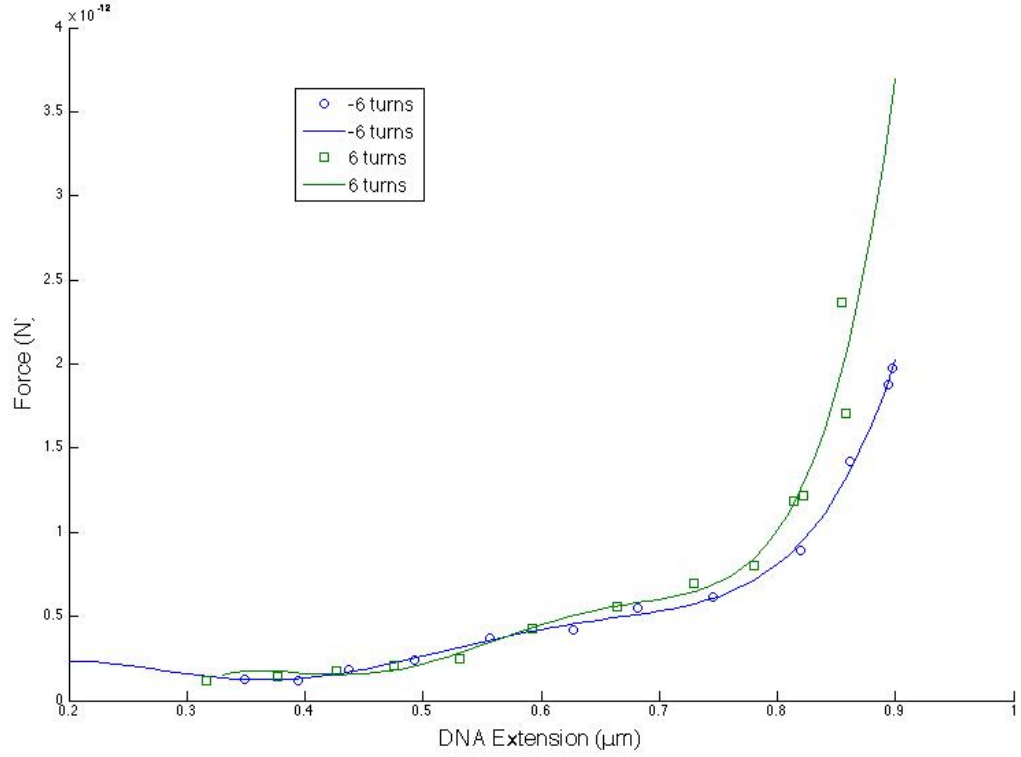

Figure 11: Force extension plots when ±6 turns were applied.

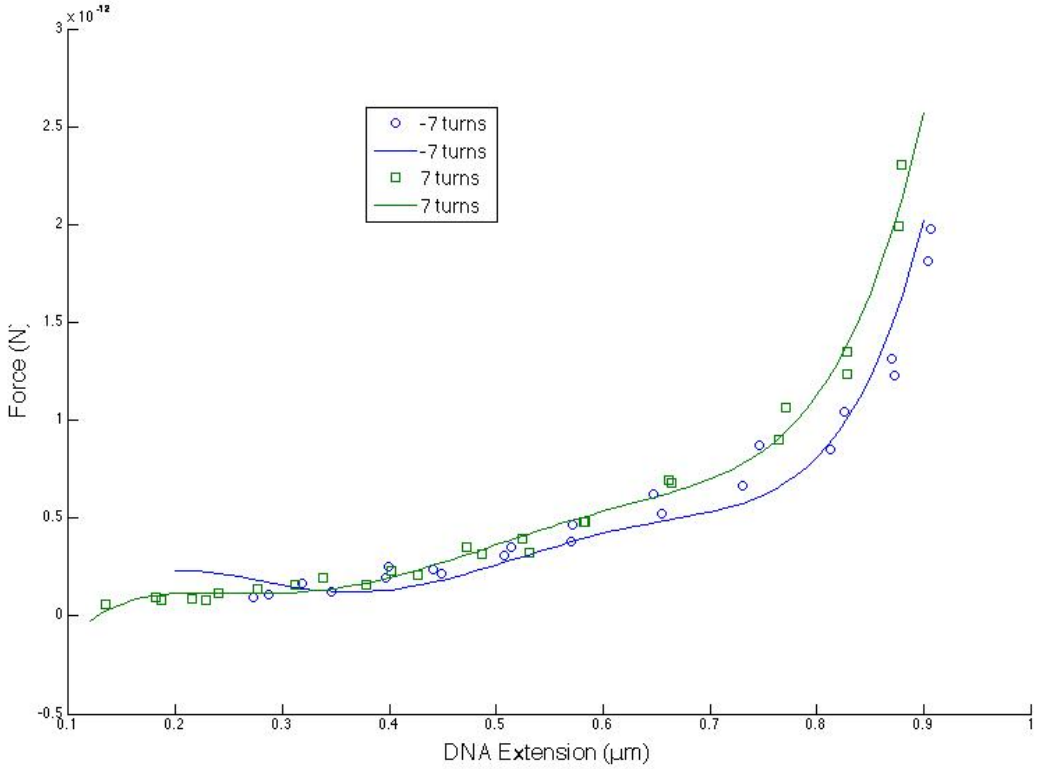

Figure 12: Force extension plots when ±7 turns were applied.

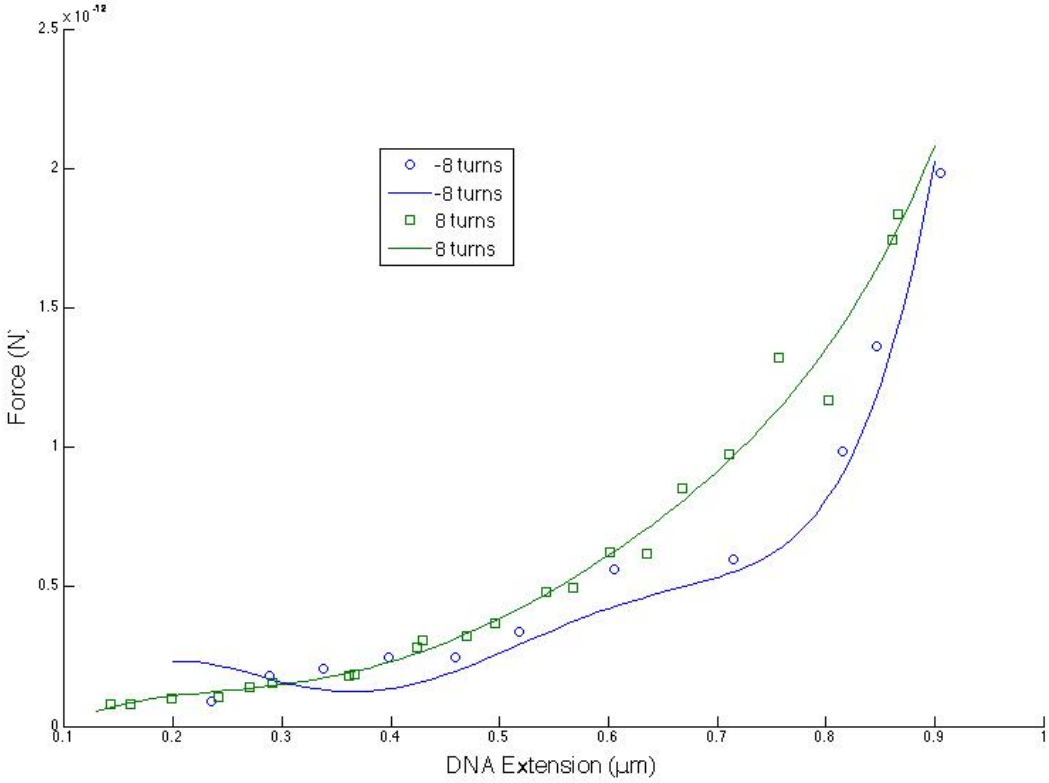

Figure 13: Force extension plots when ±8 turns were applied.

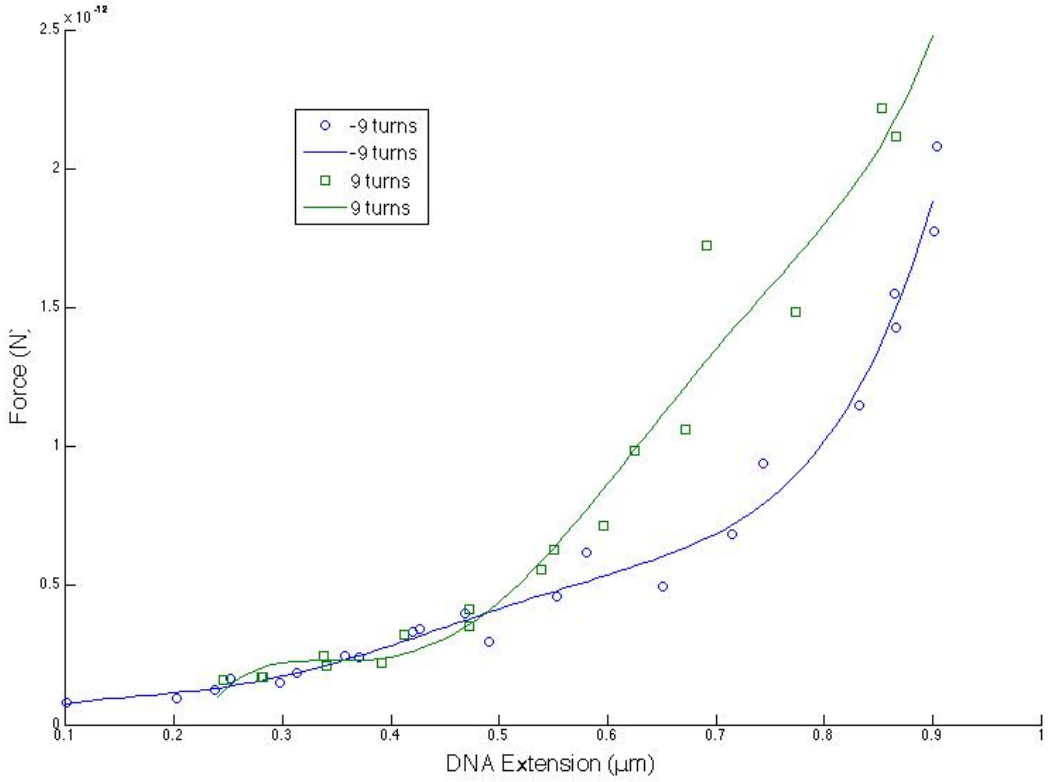

Figure 14: Force extension plots when ±9 turns were applied.

Figures 10-14 show the combined force-extension curves obtained when a certain number of turns was added in positive and negative directions. Polynomial fits were applied to each data set. Then, the difference in the amount of work done to stretch the overwound and to pull the underwound DAP DNA was calculated for each data set by weighing the area between each polynomial curve[6]. From this calculation, the following work difference values were obtained for each plot with 5, 6, 7, 8, and 9 turns introduced, respectively: 3.79×10^{-20}J, 8.47×10^{-20}J, 8.41×10^{-20}J, 1.33×10^{-19}J, 2.62×10^{-19}J. Based on the literature[3], the difference in work, D, can be represented in the following equation.

$$\Delta \equiv \frac{2\pi^2 C(n-n_c)^2}{l} \qquad \text{-Eq. (3)}^3$$

In the equation, C represents torsional modulus, n represents the number of turns applied to the tether, n_c represents the critical number of turns required for the DNA to transition into L-DNA form, and l represents the contour length of the DNA. In order to compute the torsional

constant value, which equals $C/(k_BT)$, the Eq. 3 can be rearranged to obtain the following plot, the squared slope of which equals the torsional constant value.

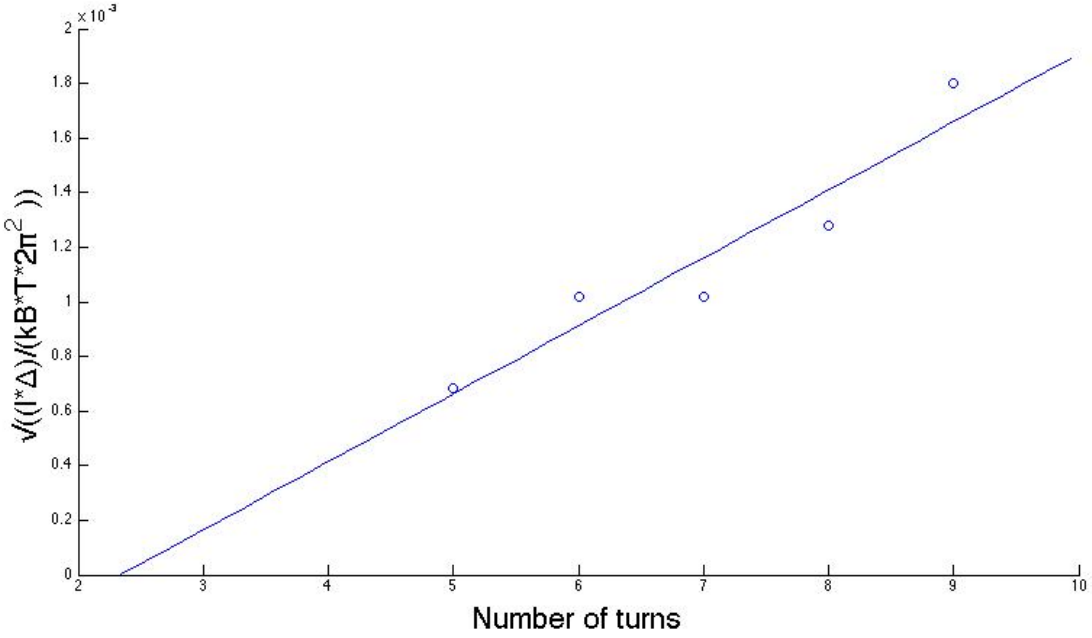

Figure 15: The plot of square root of amount of work done to stretch DNA as a function of the number of turns applied.

In the figure, for the y-axis value of $((l\times\Delta)/(k_B\times T\times 2\pi^2))^{0.5}$, l represents the contour length of the DNA, Δ represents the difference in the amount of work done to stretch an overwound and pull an underwound DNA, k_B represents the Boltzmann constant, and T represents the temperature at which the experiment was done. The slope of Figure 15 equals $(C/(k_BT))^{0.5}$, and from the linear fit applied to the data, the slope is approximated as 0.000249. By squaring the slope value, the torsional constant can be calculated as 62.0 ± 21.6 nm by using 296K as T value. From Figure 15, by noting the y-intercept value, n_c value can be also computed as approximately 2.3 turns. By using this value, previously calculated C value (from the torsional constant), the contour length value of 10^{-6}m, and the torque equation of $2\pi\times n_c\times C/l$, the critical torque at of DAP DNA can be computed as 3.73×10^{-21} N×m.

Chapter 4: Conclusion

The effects of torsion and tension on DAP DNA were studied in this work. By observing the change in DAP DNA extension as a function of torsion under different tensions, the transition of the DNA from right-handed to left-handed form was investigated. From the chapeau curves and Eq. 1 and 2, the fraction of DNA in B-DNA and L-DNA forms was computed, which can provide details of the transition of DNA. From the chapeau curves, the helical repeat of L-DNA and extension of L-DNA relative to B-DNA as functions of tension were also calculated, which can be used to characterize the DAP DNA. The results collected were comparable to the literature values[4] mentioned previously, which may indicate that the DAP DNA in L-DNA form may have similar characteristics when compared to the normal DNA. By gathering force-extension curves under different torsion, the torsional constant and critical torque value of DAP DNA were calculated as well. The calculated torsional constant and critical torque values were 62.0± 21.6 nm and 3.73×10^{-21} N×m, respectively. The torsional constant of DAP DNA was comparable to that of normal DNA, which equals 86 ± 10 nm^3. However, the critical torque of DAP DNA differed from that of normal DNA, which equals 9 $pN\times nm^3$. This difference may indicate DAP DNA and normal DNA's different torsional characteristics. The result also agrees with previous work by Dr. Qing Shao[1], which showed that DAP DNA tends to undergo transition change more readily than normal DNA does, which is congruent with the smaller critical torque value of DAP DNA compared to that of normal DNA.

In the future, more experiments could be performed to characterize DAP DNA further. Especially, the details of DAP DNA in L-DNA form are still to be investigated. For this purpose, more chapeau curves of DAP DNA can be gathered, from which the supercoiling level at which the complete transition from B-DNA to L-DNA occurs can be measured. Then, at that supercoiling level, a force-extension plot can be obtained, to which a worm-like-chain

curve can be fitted. From the fit, other parameters such as persistence length and the stretching modulus of DAP DNA in L-DNA form could be computed. DAP DNA is also known to exist in nature, specifically in Cyanophage S-2L[7]. Thus, DAP DNA may actually assume a biological role, the details of which can be investigated in the future.

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