Force Characterization of Protamine Condensed DNA

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Abstract

DNA has been known to react with different molecules to form configurations with entirely different material properties than generic "strand" DNA. In particular, when exposed to protamine, a small, arginine rich protein primarily involved in spermatogenesis, DNA is transformed from its generic strand state to an ultradense toroid. We seek to study the physical properties of this toroid to understand how much force is required to unwind the toroid with particular attention to how the DNA unravels as we apply more force to it. We accomplish this via optical trapping were upon we attach a bead to one end of a DNA strand, allow it to condense, and then pull on the bead via laser until the toroid unravels back into strand DNA. It is our hope that this will better allow us to characterize the interactions of protamine with DNA and the possible uses of this interaction in nanomaterial applications.
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Chapter 1

Introduction

1.1 DNA as a nanomaterial

Recently DNA has received an increased amount of attention in the fields of biology and material science. In the former, due to its role as the carrier and expresser of genetic material, and in the latter, due to the unique properties that DNA possess. In particular material scientists are interested in its nature as a nanomaterial as well as its ability to self-assemble into pre-determined structures. For example, recent work on DNA nanostructure self-assembly and applications by Tian et al. (2016)[1], demonstrate how DNA can be used form basic strand motifs that themselves can be manipulated to form more complex macro-structures. Work by Ong et al. (2016)[2] takes an alternative approach by treating DNA as bricks to be laid in a larger structure instead of strands.
that can be woven together to create macro-structures. Lu et al. (2016) detail various processes by which DNA nanostructure frames can be constructed with or incorporated into entirely inorganic nanostructure complexes which exhibit properties of both DNA and the inorganic core. These papers demonstrate novel lines of materials research that would allow DNA based materials to work outside of their traditional biological contexts. Even then, due to the unique position DNA is in, such lines of work might even lead to breakthroughs in a biological context as demonstrated by Wolfert et al. (1996) and Linko et al. (2015) where both show the viability of DNA based nanostructures as a vehicle for drug delivery.

We are interested in the properties of DNA folded by protamine molecules. This DNA has been shown to be significantly more rigid, condensed, and protected from UV rays than regular strands of DNA. In particular, due to
Figure 1.2: (a) A canonical representation of a nucleotide, or a DNA base segment. One of four bases are attached to a deoxyribose molecule. (b) The neutral form of the deoxyribose molecule. In order to form a nucleotide, the deoxyribose molecule must lose the $-OH$ at 1' in order to append a base and also lose the $-OH$ on the $CH_2OH$ at 5' in order to attach the phosphate group. Adapted from OpenStax Microbiology[8].

the increased rigidity and genetic fidelity associated with protamine-nucleated DNA, we hope that our characterization of DNA will allow the development of protamine-DNA based nanostructures.

### 1.2 DNA structure

Generic DNA is composed of two strands connected via hydrogen bonding, arranged in a double helix configuration. Each strand being composed of a number of deoxyribose molecules. Deoxyribose is the name of a specific form of monosaccharides, or simple sugar. Attached on the outer end of the DNA
is a phosphate group. This deoxyribose-phosphate structure is often called
the sugar phosphate backbone, or just phosphate backbone. The phosphate
backbone is bonded to sequence a of nucleobases, or bases. We present a vi-

sual representation of this configuration in Figure 1. The bases - adenine (A),
guanine (G), thymine (T), and cytosine (C) - are themselves subdivided into
two groups, the pyrimidines and purines, which we elaborate further on in
Figure 2. The particular nature of DNA lends itself to the establishment of
certain selection rules when pairing bases. In general, pyrimidines and purines
do not bond with others of the same group. In DNA specifically, a cytosine
base must bind with a guanine base and a thymine base must bond with an
adenine compliment. In this sense, we are able to talk about conjugate pairs
of bases. On a more macroscopic scale, if two DNA strands are bound, then
their nucleotide bases are conjugate to each other and we say the two strands
are complimentary. For example, if one strand has the sequence ACG, the
conjugate strand will have TGC. We present a macroscopic visualization in
Figure 3.

The particular selection rules for DNA base pairings present us with an in-
teresting opportunity. Since the conjugate pairs are predefined, if we engineer
a strand of arbitrary bases, then we know with certainty the base sequence of
the complimentary strand. This property, with the advent of nanoscale engi-
neering, is currently having significant ramifications on how DNA is viewed
as a nanomaterial. As early as 1996, Mirkin et al.(1996)[9] and Alivisatos
et al.(1996)[10] manage to synthesize a previously theorized compound where
the compound demonstrates both the properties of an inorganic material as
well as that of DNA. They both successfully created DNA-gold nanoparticle complexes using DNA oligonucleotides, chains of nucleotides, to create a framework for the creation and aggregation of gold crystals. Since then, the techniques associated with DNA-based nanofabrication have improved dramatically and the resulting sophistication has in turn allowed for the development of DNA based nanomachines of surprising complexity, including those that operate via biological signals [11]. For example, Linko et al.(2015)[5] and Yildiz et al.(2011)[12] both detail the process by which specifically modified DNA nanoparticles could be designed to carry precise dosages of drugs to specific targets with relatively low risk. Seeman et al.(1999)[13] and Yurke et al.(2000)[14] both designed and synthesized DNA nanoparticle complexes that are able to act as mechanical "switches" - with the latter’s configuration even able to use its own DNA as fuel. Other work done by Yan et al.(2002)[15] pushes this application even further by demonstrating that functionalnanome-
Figure 1.4: (a) A three dimensional representation of DNA in double helix form. (b) A representation of conjugate base pairings. (c) Molecular arrangement of DNA. Adapted from OpenStax Microbiology [8].

Mechanical rotors can be constructed using DNA.

1.3 DNA-protamine interactions

Protamine is one of the primary agents in the late-stage spermatogenesis (sperm production) cycle of many animals. In humans, or more generally mammals, there are two types of protamine[16], henceforth referred to as P1 and P2 respectively. P1 protamines are generally 49 to 50 amino acids long and primarily responsible for the condensation of DNA in mammalian spermatozoon. Spectroscopy on free protamine solutions suggest that P1 protamines
Figure 1.5: (a) Diagram of the DNA-protamine condensation process adapted from Santos et al. (2004)[19]. (b) Real-time data showing a lambda-phage DNA strand being condensed in a salmine assay. The DNA (bright string) is attached to a microsphere (black point) that is 1 micron across. Adapted from Balhorn et al. (2000)[20].

Do not have a tendency to form macrostructures [17], however, upon making contact with DNA, P1 protamines begin to restructure the DNA by binding to the major groove of the DNA double helix, generally with one P1 protamine molecule per turn of the DNA double helix [18]. P2 protamines are mostly similar to P1 protamines (there is as much as 70% sequence identity between the two depending on the species [16]) and comprise a much different function in the production of spermatozoon. Structurally, P2 protamines are marginally larger than their P1 counter parts (P2 protamines in mice are usually 63 amino acids longs [16]) and are able to bind zinc atoms, incorporating them into the DNA complex [16]. While the exact purpose of the appended zinc atom is as of yet unknown, it is well understood that in humans, mice, and hamsters, P2
protamines bind zinc to DNA at approximately one atom of zinc per molecule of DNA [21]. This irregular addition is not insignificant, studies have shown that the presence of the P2 protamine attached zinc plays a critical role in the maturation of sperm chromatin in stallions [22]. In addition, the ratio of P1 to P2 protamines has been linked with fertility in male bull populations [23]. We will be using salmine in our experiment, a P1-like protamine found naturally in salmon that has similar structure and function to mammalian P1 protamines [16]. Henceforth, when we refer to protamine, we mean salmine.
1.4 Objective

Our goal is to measure the force it takes to unwind the protamine-nucleated DNA as a function of DNA extension. We will do this by attaching a nanoscale bead to one end of the protamine-DNA toroid and a glass plate to the other end. Afterwards, we will place the assay in optical microscope where two superimposed lasers will be focused in the field of view of the microscope. Using these two lasers, we will pull on the bead end of the DNA in what is called an optical tweezers configuration. Our hope is to obtain a force vs extension curve of the DNA which will allow us to categorically rule out different models of DNA looping.
Chapter 2

Methods

2.1 Sample Chamber Preparation

In order to image DNA under the microscope, we require a suitable vessel in which to store and carry the DNA solution. We use sample chambers produced in-house composed of a glass cover slip attached via tape to a microscope slide. We prepared the sample chamber by first taking a glass cover slip (Fisherbrand 12-544-B, 40 mm by 22 mm by 0.16-0.19mm) and scrubbing it with a 3:1 DI-soap solution (Dawn Platinum) for one minute using a toothbrush. Next, we rinsed the cover slip in DI for roughly ten seconds. In order to ensure that no organic residues remain, the cover slip must be dried quickly, so the cover slip is then put through multiple thirty second rounds of drying in a 1000 W microwave. We need to open the microwave for a minute every one to two
cycles in order to allow the air in the microwave to dehumidify otherwise the microwave overheats.

We next took a microscope slide (Corning, plain, pre-cleaned, 75 mm by 25 mm by 0.96-1.06 mm glass) and set it on a specially constructed mold. Before we place the cover slip on the microscope slide, we first applied double-sided tape vertically on the microscope slide as to make a corridor through which we can flow solution. Afterwards, we placed the cover slip into the mold and lightly pressed the regions connected to the tape to ensure connectivity. To ensure that the connection is airtight, the microscope slide is then lifted from the mold and we applied pressure on the cover slip again on the tape-connected region. Afterward, we applied epoxy to the sides of the cover slip in order to seal the interior of the flow chamber. Refer to Figure 2.1 for a visual representation.

2.2 Assay Production

2.2.1 Stuck Bead Assay

We used 4% carboxylate modified (CML) beads produced by Invitrogen\textsuperscript{W}. First, we diluted with deionized water to one part in a thousand. We then vortex the solution to mix it. Afterwards, we pipetted 15 \(\mu\)L of solution onto a sample chamber. Then, we added 20 \(\mu\)L of 1 M magnesium acetate solution
Figure 2.1: (a) Mold with microscope base. (b) Microscope base with double sided tape. (c) Set the cover slip on vertically on the base. (d) Notice the air bubbles in the tape. (e) Pressed out air bubbles. (f) Apply epoxy to seal the chamber.
and allow it to sit for two minutes. The positively charge magnesium ions act as a screen towards the negatively charged beads and glass allowing the bead to stick to the cover slip via Van der Waals forces. Then, we pipetted another 200 µL of DI, suctioning any solution that is pushed out of the sample chamber as we add DI, in order to sweep away any excess magnesium.

### 2.2.2 Free Bead Assay

We used beads 4% carboxylate modified (CML) produced by Invitrogen™. First, we diluted with deionized water to one part in a thousand. Next, we proceed to vortex the bead solution to mix it. Then we proceed to sonicate the beads (Qsonica Sonicator Model Q500, 500W 2kHz along with the Qsonica Oasis 180 Chiller keeping the system at 4 °C) at two second intervals (2s on, 2s off) for a total time of one hour. Sonication acts to break up clusters of beads to ensure that the solution is monodisperse. Afterwards, we pipette 15 µL into a sample chamber.

### 2.2.3 TPM DNA Assay

We first took Spherotech Streptavidin (SA) coated Polystyrene 1% w/v bead stock and extracted 60 µL of stock per two sample chambers. Next, the stock is mixed with 200 µL of 0.4% Phos-Tween solution. The solution is then
centrifuged for two minutes at 10,000 rpm. Then we remove the supernatant. We repeat this process three times in order to separate the beads from solution. Afterwards, the beads are resuspended in 30 μL of wash buffer (See Appendix A). The new solution is then sonicated using two seconds cycles (2s on, 2s off). Next, we begin preparing the DNA to mix into solution. First we make 30 μL of 200 μM DNA per two sample chambers. We then prepare a solution of 20 μg/ml anti-dig (See Appendix A). Anti-dig is an antibody that binds to a digoxigenin antigen on the DNA, tethering the DNA to the surface. We then flow 50 μL of the anti-dig solution onto the sample chambers. The sample chambers are left to rest for an hour in a humidity chamber. After the hour wait, we mix the bead solution with the DNA solution. Afterwards, we wash each slide by flowing wash buffer through twice. After ten minutes, we repeat the wash. Then, we flow 30 μL of the bead-DNA solution onto the slide. Lastly, we allow it to rest for an hour at room temperature.

2.3 The Optical Trap

2.3.1 The Microscope

We use the Nikon™ Eclips Ti-E inverted microscope to look at the sample. We direct the laser through the back of the microscope via a large opening which redirects the beam with a dichroic mirror to the objective (Figure
2.2). In order to adjust the position of the sample with precision, we place a PI® P-733.3CD piezo nanopositioning stage above the objective. The stage is equipped with a holder designed to fit our sample chambers. We then are able to control the position of the sample chamber externally via the PI® E-712 Digital Piezo Controller which moves the stage based on commands we can send via LabView™.

2.3.2 The Trap

We use two lasers in our configuration. The first laser is the Coherent Matrix 1064-10-CW, which has a Gaussian profile. We are able to control the
power of the laser in real time using the accompanying Matrix laser proprietary software. This is the laser we use to trap beads. We generally leave the power of this laser in the 1.0-2.0 W range. In addition, we also filter this beam through a Thorlabs™ P5-1064PM-FC-2 single-mode fiber optic cable as to eliminate unwanted higher order modes.

We have a second detector laser with a wavelength of around 945 nm and variable power which we control with an external controller. This laser is primarily responsible for tracking the movement of the bead as we pull on the DNA. We detect the beads using the second laser by measuring the backscattered light via a Quadrant Photodiode (QPD) which we will elaborate further on in section 2.4.

We employ two telescopes each with a pair of two lens (Figure 2.2) in our configuration. The purpose of these devices is two fold. The first is to change the width, of the laser beam which is important for maximizing the signal. If the width of the beam is larger than an opening it goes into, the beam is clipped and we effectively lose power on the beam. The second is to collimate the beams, focusing it in the place of the objective. The first of the telescopes is in front of the Matrix laser. This one serves to change the width of the trap beam so that it has the right diameter at the lens just before being sent into the fiber cable (FC in Figure 2.2). The second of the telescopes changes the width of both beams so that it matches the opening in the back of the objective.

We had initially aligned the two mirrors such that the beam in the sample plane was in the conjugate plane of the back focal point. However, the laser
drifts due to forces outside our control, so we employ Gimbal mirrors (Figure 2.2). We can independently change the position of each laser by adjusting the position of the corresponding Gimbal mirror located before the second telescope. Each mirror has two rotating knobs which control the in the x or y translation of the relevant laser. This allows for a relatively quick way of superimposing each laser on top on each other should they drift apart.

We place a 4 ND filter in front of the cameras to prevent them from being burned from the lasers. We also place a half wave plate mounted on a Thor-Labs™ K10CR1 Rotating Stepper Mount as well as a polarizing beam splitter in front of the Matrix beam after it is filtered by the fiber optic cable. This allows us to control the intensity of the trap by simply changing the orientation of the incoming light. In order to get a detection signal, we place a half wave plate and then a quarter wave plate in front of the detector beam in order to induce an orientation. This induces a phase on the detector beam such that it is out of phased with the trap beam. The detector beam is then passed then filtered through an dichroic mirror and superimposed on the Matrix beam. Since the two beams are are different phases relative to each other, we are able to use a polarizing beam splitter (PBS in Figure 2.2) which selectively reflects or transmit light to redirect the backscattered detector beam into the QPD, which gives us a signal.

We operate two cameras attached to the trapping microscope, the CoolSnap EZ and the Digital Color CCP IV-CCAMZ Camera. The CoolSnap EZ is able to record videos directly from the field of view using parameters such as the total number of frames, frame rate, and exposure time. In addition, Coolsnap
Figure 2.3: Both cameras imaging the same region. (a) Coolsnap EZ (b) Digital Color CCP

EZ is able to detect both laser beams. The Digital Color CCP Camera filters out the lasers so we can easily image the beads. A benefit of this is that we are able to see beads get trapped directly.

We use a ThorLabs™ SHB1T shutter with an accompanying ThorLabs™ controller to block the trap beam as needed. The shutter is controlled directly via LabView™.

2.4 Bead Detection and Data Acquisitions

2.4.1 Signal Acquisitions

We utilize a tethered particle motion (TPM) assay in our experiment where one side of the strand of DNA is attached to the cover slip and a microbead is
Figure 2.4: (a) The general sweeping procedure (b) Data from another experiment. Black is the QPD signal, red is the fit. Both adapted from Devenica et al. (2017) [26]

attached to the other free end of the DNA strand. We shine a detector beam on the bead which scatters the light. We measure the amount of backscattered light using a Quadrant Photodiode (QPD) which returns a voltage value depending on when the scattered light is located on the QPD. This value in of itself is meaningless. We need to be able to convert the voltage value of the QPD into physical units. To this value, we hold the bead in the center of the trap beam and then scan over with the detector laser, moving the detector through the bead. The resulting QPD signal (Figure 2.4a) should only occur over the diameter of the bead with an approximately linear region near the center of the bead. We then fit this region and use the resulting slope of the voltage on the QPD to measure the movement of the mirror in nm (Figure 2.4b).
Figure 2.5: Force diagram of the bead system representing the $x$ and $z$ direction. Adapted from Devenica et al. (2017)[26].

2.4.2 Force and Extension Calibration

Determining the force on the trap is a complicated process related to the traps interaction with the microbead on the DNA which we devote the entirety of chapter 3 to. In short, we use the potential of the bead interaction with the trap laser to determine a force constant which we used to measure the force based on the extension. We use three different methods which involve measuring different pieces of information in order to independently determine the value of the force constant.

As for the extension of the DNA, we can solve for it base on the geometry of the system (Figure 2.5). We know from direct measurements the value of $z_{\text{bead}}$ and $x_{\text{stage}}$. To solve for the extension of the DNA, we adopt the model from Wang et al. (1997)[27]:

20
\[ x = \frac{z_{\text{trap}} - z_{\text{bead}}}{\sin[\tan^{-1}(\frac{z_{\text{trap}} - z_{\text{bead}}}{x_{\text{polym}} - x_{\text{bead}}})]} - r, \]  

(2.1)

where \( r \) is the radius of the bead, \( z_{\text{bead}} \) is the height of the bead, and \( x_{\text{polym}} \) is the value of the displacement due molecular complex linking the DNA to the glass surface. In practice, this is a measured value.

We can determine \( z_{\text{bead}} \) based off \( z_{\text{trap}} \) and \( x_{\text{bead}} \) by the relation:

\[ z_{\text{bead}} = \frac{z_{\text{trap}}}{(K_x/K_z)(\frac{x_{\text{polym}} - x_{\text{bead}}}{x_{\text{bead}}}) + 1}, \]  

(2.2)

where \( K_x \) and \( K_z \) are the trap stiffness (see Chapter 3) of the the trap in the \( x \) and \( z \) directions respectively. For our particular configuration, we can take the ratio of \( K_z \) to \( K_x \) to be approximately 5.

We record the value of \( x_{\text{stage}} \) with \( a \) in real time. This is accomplished by first initializing position of the stage before we pull the DNA and then determining the displacement from that initial location as we pull the DNA. To determine \( z_{\text{trap}} \), we move the stage in \( z \) until it hits the surface and move back a certain distance. Again, we initialize the stage to a predetermine location and then move the stage up until the stage bumps into the bead. Then we move down a set distance and use the displacement from the initial location as \( z_{\text{trap}} \). For more information, see section 2.4.3.

2.4.3 Procedure for Measuring DNA Length

We can reduce the process of taking the force-extension curve into roughly four steps (Figure 2.6). The first step, the bead alignment, requires manually
trapping a bead using a live view of one of the cameras mentioned in section 2.3.3. The next step is the beam alignment which is the process outlined in section 2.4.1 where the QPD voltage scaling is determined by sweeping the detector bead. The third step is the height calibration where we determine the value of $z_{\text{trap}}$ or the height of the trap above the cover slip. This is done by physically moving the stage in the $z$, producing the curve in Figure 2.6c. The curve shows the surface a bump. Using that bump as a reference, we move to a location a set distance from the surface of the assay. Lastly, we use the movement of the bead to measure the force and extension as outlined in section 2.4.2. We plot both force and extension and use a linear fit to derive an expression for force as a function of extension near the center.
Chapter 3

Trap Stiffness Calibration

3.1 Trap Stiffness

The trap stiffness, $k$, is a measure of how tightly the trap holds onto something. In more physical terms, this is a measure of the steepness of the laser’s potential, $U$. To understand the relationship between the laser’s potential and trap stiffness, we must first assume that the size of the bead is significantly smaller than the wavelength of light. In this case, the potential energy of the bead, $U$, is the negative dot product of $p$, its induced dipole moment, and the local electric field, $E$:

$$U = -p \cdot E. \quad (3.1)$$
In addition, we can relate the bead’s induced dipole moment to \( \alpha \), its polarizability and \( E \), the local electric field:

\[
p = \alpha E.
\]  

(3.2)

From Eq 3.2 and Eq. 3.3, it follows that

\[
U = -p \cdot E = -\alpha E^2 = -\alpha I.
\]

(3.3)

which shows a simple proportionality between the intensity and potential.

Since our laser profile is Gaussian, we know that near the center of the beam the behavior of the potential should be approximately parabolic and thus there should be a region where the gradient of the potential is approximately linear. We model this center region using Hooke’s Law,

\[
F = k x_{bd}.
\]

(3.4)

Then in the relevant regime, the force increases linearly either as a function of \( x_{bd} \), the distance of the bead from the center of the trap, or \( k \), the stiffness of the trap. In order to determine the force as a function of \( x_{bd} \), the value of \( k \) must first be determined.

In principle, \( k \) is controllable by either the shape of the laser or the intensity of the laser. However, since the shape of our laser’s intensity profile is set by an optical fiber cable which is unable to be modified conveniently, we must resort to a method where we can control the stiffness based on intensity alone. We
Figure 3.1: The equipartition and power spectrum methods both determine the value of $k$ by measuring the statistical properties of the bead under Brownian motion.

do this by sending the laser through a half-wave plate to select a particular polarization and then using polarizing beam splitter to filter out all light of a certain polarization. We can then directly change the intensity of the beam by changing the orientation of the half-wave plate. Using this configuration, we then use three different methods in order to calibrate the value of $k$: the drag method, equipartition method, and power spectrum method.

3.2 Equipartition

From statistical mechanics, the equipartition theorem states that the potential energy, $U$, per degree of freedom is equal to half the thermal energy,
Figure 3.2: Raw position data as a function of time.

$k_B T$:

$$U = \frac{k_B T}{2},$$

(3.5)

where $k_B$ is the Boltzmann constant and $T$ is the temperature of the system. Since the intensity profile of the beam is Gaussian, the trap is harmonic. Thus, the energy of the system can also be expressed as,

$$U = \frac{k \sigma^2}{2},$$

(3.6)

where $k$ is the trap stiffness and $\sigma$ is the standard deviation of the bead’s displacement. Substituting and replacing Eq. 3.6 into Eq. 3.7 yields,

$$k = \frac{k_B T}{\sigma^2}.$$

(3.7)
Thus, by measuring the standard deviation of the bead's motion, we are able to determine the trap stiffness.

To take data, we measure the voltage vs time values of the bead in the trap off the QPD (Figure 3.2). Afterwards, we convert the voltage data to position data using the method described in 2.4.1 and calculate the standard deviation of the position over the whole time period (Figure 3.3). Assuming a \( k_0T \) value of 4.1 pN-nm, we are then able to use Eq. 3.8 to calculate the trap stiffness. We then repeat this process at various intensities in order to determine \( k \) as a function of \( I \).
Figure 3.4: Frequency data obtained by taking the power spectral density on data like Figure 3.3.

3.3 Power Spectrum

If we place the bead in the center of the trap as in Figure 3.1, the bead will oscillate about the point like a simple harmonic oscillator with energy determined by the shape of the beam profile. While the mean displacement of the bead should be zero, the instantaneous displacement should non-zero. The range of instantaneous displacements is itself determined by the frequency of the bead’s oscillations. If we were to take position data as a function of time and transform it into frequency space (Figure 3.5), we would be able to extract the corner frequency, $f_c$, which we are able to relate to the trap stiffness through a series of constants.

Mathematically, the movement of the bead should be random which we
can express as the following Langevin equation [28]:

$$m\ddot{x} = -k_x x_{bd} - \gamma \dot{x} + F_L, \quad (3.8)$$

where $k_x$ is the stiffness of the bead, $x_{bd}$ is the displacement on the bead, $\gamma$ is the drag coefficient, and $F_L$ is term accounting for Brownian motion. In our case, since the bead is in equilibrium, $m\ddot{x}$ is equal to zero. If we perform a Fourier transform on Eq. 3.8, we would yield the following one dimensional Lorentz-like power spectrum, denoted as $S_x$:

$$S_x(f) = \frac{k_BT}{\gamma \pi^2 (f_c^2 + f^2)}, \quad (3.9)$$

where $k_B$ is the Boltzmann constant, $T$ is the temperature, $\gamma$ is the drag coefficient, $f$ is the frequency and $f_c$ is the corner frequency. The relationship between $k$ and $f_c$ is:

$$k_x = 2\pi \gamma f_c. \quad (3.10)$$

We repeat this measurement for $k_x$ and $k_y$ as a function of $I$ (Figure 3.4). The trap stiffnesses follow the following relations:

$$k_{x,\text{EQP}} = 0.003(3)W + 100(100), \quad (3.11)$$
$$k_{y,\text{EQP}} = 0.008(1)W + 200(40), \quad (3.12)$$
$$k_{x,\text{PG}} = 0.003(2)W + 900(100), \quad (3.13)$$
$$k_{y,\text{PG}} = 0.003(1)W + 240(22). \quad (3.14)$$

30
Figure 3.5: $k_x$ and $k_y$ as a function of $I$ for both equipartition and power spectrum methods. Dotted lines correspond to EQP measurements, solid lines correspond to PS measurements.

The values that we obtain from the EQP and PS methods are thus in agreement.

### 3.4 Drag

The drag method (Figure 3.6) involves moving the stage and recording $x_{bd}$ as well as force on the bead due to the acceleration from the stage. First, the stage is moved in order to create a flow that forces the bead out of the trap. Next, we measure the force of the flow and $x_{bd}$ as it moves at velocity $v$. Based on the drag-force equation,

$$F = -\gamma v,$$

(3.15)

should we know $\gamma$, we should be able determine the force induced on the bead. We know from the Stoke’s drag equation, that we can calculate $\gamma$ based on
Figure 3.6: The drag method involves inducing a force on the entire system and watching the restorative force of the laser.

\[ \gamma = 6\pi \eta r_{\text{bead}}, \]  

(3.16)

where \( \eta \) is the viscosity of the solution and \( r_{\text{bead}} \) is the radius of the bead. Since the bead system is in equilibrium, we also know that from Eq. 3.8

\[ F_{\text{trap}} = -F_{\text{drag}} + F_L. \]  

(3.17)

However, we know that \( F_L \) also average to zero, so we must have

\[ F_{\text{trap}} = -F_{\text{drag}}. \]  

(3.18)

Then if we apply a known force to the bead, we can by Eq. 3.3 determine the resulting velocity of the bead. If we record \( x_{\text{bd}} \) as well, we can determine \( k \) by
directly by,

\[ k = \frac{dF_{\text{drag}}}{dx_{bd}} \approx \frac{\gamma \Delta v}{\Delta x_{bd}} \approx 6\pi \eta r_{bd} \frac{\Delta v}{\Delta x_{bd}}. \quad (3.19) \]

If our PS or EQP methods had disagreed, then this method would have given us insight into the validity the disagreement. Since measuring the drag force is a direct measurement that makes no assumptions beforehand about the physics of the system other than it being a bead being pulled by a force, we can categorically rule out the other methods depending if the data provided by this method is linear or not. However, since the \( k_w \) of the other methods agree and we believe the divergence in the \( k_y \) values is due to a sampling error, we did not have to resort to this method.
Chapter 4

Tether Particle Motion (TPM)

4.1 Theory of DNA Folding

4.1.1 Worm-like chain (WLC) model

In order to understand the models of DNA folding, one first has to understand how naked DNA behaves. The model that is commonly used to describe naked DNA is called the worm-like chain model. In this model, one can imagine DNA, or in general any polymer chain, as a series of segments that together form a long chain. Each of these individual segments interacts with the next segment in the chain depending on the thermodynamic and chemical properties of the molecule in question. We characterize how the chain behaves by the quantity called the persistence length, $L_p$, which is the average length
of a "straight" segment. If the persistence length of a material is, say 50 nm, then one might think of it as straight at the scale of 50 nm. Similarly if a material has a low persistence length - say 2 nm, it will appear balled up, and be flexible at the 50-nm scale.

We adopt the modified Marko-Siggia description of the WLC as presented in Wang et al.(1997)[27]:

\[ F = \left( \frac{k_b T}{L_p} \right) \left[ \frac{1}{4 (1 - \frac{x}{L_0} + \frac{F}{K_0})^2} - \frac{1}{4} \frac{x}{L_0} - \frac{F}{K_0} \right], \]  

(4.1)

where \( F \) is the force, \( K_0 \) is the elastic modulus or a measure of how resistant the material is to elastic deformity, \( x \) is the extension, \( L_p \) is the persistence length, \( L_0 \) is the contour length or the full extension length, \( k_b \) is the Boltzmann constant, and \( T \) is the absolute temperature in kelvin. Based on this model, we can fit the force on the WLC based on the two parameters \( L_p \) and \( L_0 \). Notice the term in round brackets contains the energy term, \( k_b T \), divided by the persistence length. If we keep the energy constant and change \( L_p \), we can change the scaling of the force without changing the energy. Physically, this means that we are changing the entropy of the system, thus \( L_p \) is the variable we will use to measure the entropy of the system. \( L_0 \) is simpler to understand, for if we change it then we are quite explicitly changing the length of the DNA molecule, which is an enthalpic change.
4.1.2 Entropic vs Enthalpic processes

In the context of the worm-like chain model, the behavior of how DNA is folded as it transforms into a toroid from a naked DNA strand can be determined by looking at how entropic or enthalpic the DNA looping is. Imagine a string of some sort, let’s say a shoelace. If we pick up the shoelace and drop it, we would expect it to fall in such a way that the resulting geometry of the shoelace is not a perfectly straight line. If we were to grab and pull the ends of the shoelace, it would be progressively turned back into a straight string. We can say that this process drains the entropy out of the system. In the context of DNA folding, this would be extending the DNA to its $L_o$ length. Now consider pulling on the shoelace even more. So much so, that the inner fabric of the shoelace breaks and the shoelace extends. In this case, the
structure of the shoelace is changed. We would call the analogous process in DNA to be an enthalpic process. In the context of DNA folding, this would correspond to pulling on the bonds of the DNA molecule. In the context of the DNA toroid, this would correspond to unlooping the DNA since we are breaking the bonds and changing the structure of the itself. In Figure 4.1a, we can see that if the scaling of the force increases at constant $L_0$, then Eq. 4.1 would suggest that the entropic term increases, which at constant $k_B T$ means that $L_p$ is decreasing. Similarly in Figure 4.2b, if we look at Eq. 4.1 for the enthalpic term, to the first order, we can imagine changing $L_0$ as stretching the original F-x curve.

4.2 Models of DNA Folding

While it is understood that the final result of DNA condensation by protamines is a toroid, the pathway by which the folding occurs is not well understood. There are currently three models that could describe the folding. They are Bind & Bend, $L_p$ Change, and Partial Loop (Figure 4.2).

4.2.1 Bind & Bend

In this model, protamine molecules bind to the DNA which forces the DNA to bend. The process of binding to the DNA causes a change in the bond angle of the system which would imply that this model is an enthalpy
dominated process. If this model is correct, we would first expect an entropic extension of DNA followed by a "popping" event where the DNA tears. Once this happens, the DNA extends and the force on the DNA decreases, much akin to what happens if you were the pull out a loop of string wrapped around a spool. If we were to measure the force vs extension curve of this behavior (Figure 4.3), we would get a series of popping events where the the DNA is pulled to the max length, unloops, and then is pulled to the max length again. When the DNA unloops, there is an abrupt decrease in force followed by an increase in extension. We call this a "pop". For the Bind & Bend model, we would expect a series of small pops where the extension gained from each pop is only a fraction of the total length of a loop.
4.2.2 \( L_p \) Change

As opposed to the Bind & Bend model, this model suggests that instead of bending the DNA, protamine binding causes a decrease in the persistence length of the DNA. Since \( L_p \) is the length of each "chain" in the DNA molecule, we can think of a decrease in the \( L_p \) as an increase in the total possible number of states the DNA can take and therefore an increase in the entropy of the DNA. You can imagine this as an ordered strand of DNA folding into a less ordered one. The force-extension curve predicted by this model would be of similar nature to that shown in Figure 4.1a, however with the addition of one popping event at the end. If we imagine the folding process going forward, the \( L_p \) decreases and then a loop forming event happens. This last popping corresponds to the unwinding of that the bond responsible for the loop formation.
4.2.3 Partial Loop

In the Partial Loop model, the protamine acts in a similar way as the Bind & Bend model in the sense that protamine binds with the DNA and serves to shape the DNA into a loop. However, this model assumes that protamine cooperatively binds with DNA such that a half loop might form all at once. The force-extension curve of this model shares the popping feature of the Bind & Bend model, however instead of multiple small pops, this model predicts smaller number of pops with a longer extension. For example, one pop could correspond to the first half of the DNA loop (Figure 4.4), and another pop could correspond to the other half.
4.3 Optical Trapping Data

Since we are interested in the effects of DNA-protamine interactions, we must first measure the F-x curve for naked DNA. Currently, we have measured the unfolding on naked DNA with the measured value of $L_p$ to be 50 nm ± 2 nm, which is consistent with the value of 50 nm found in the literature\cite{30,31}. The value is $L_o$ is merely the length of the DNA we choose to use. In this case, we used 1023 nm DNA. We as of yet have not measured the values of $L_p$ of protamine condensed DNA.
Chapter 5

Conclusions

Based on the values of the stiffness we measured using the EQP and PS methods (Eq.3.11-3.14), we conclude that behavior of the stiffness is approximately linear in our regime of interest. However, the fact that the data for x and y for both EQP and PS diverge from each other suggests that the beam is not entering the microscope in a perfectly symmetric manner. That is to say, it looks more like an ellipse than a circle when we view it via the cameras on the microscope.

We have in essence completed all the preparation in order to perform the experiment. Moving forward, we would like to actually do the experiment and gather the force-extension curve via the method described in section 2.4.3 and determine the pathways with which protamine folds DNA. Beyond that, we would like to study the order in which protamine folds with DNA. Along with the insight into mechanical interactions protamine has with DNA, this
line of work could possibly lead to the use of protamine-DNA interactions in
the construction of nanomaterials. For example, if we could learn what occurs
at each stage of the folding process along with the external conditions they
occur or no longer occur, we could create a sequence of conditions that would
drive the DNA into a specific shape. Such studies would not only shed more
light onto the biological nature of DNA, but also demonstrate the incredible
versatility DNA possesses as a nanomaterial.
Appendix A

Assay Components

A.1 Wash Buffer

To make 10 ml of Wash Buffer, add:
8.45 ml dd$H_2O$
250 µL 1M Triss-HCL (ph=7.5)
10 µL 1M $Mg(OAC)_2$
10 µL 1M $NaCl$
400 µL 10% Tween-20 0.4%
10 µL 1M Dithiothreitol
870 µL 35 mg/mL filtered BSA
into a suitable container and vortex until homogeneous. Keep refrigerated.
Lasts one week.
A.2 Bovine Serum Albumin (BSA)

To make 10 mL 35 mg/mL BSA, add:

9.4 mL ddH20

200 µL 1 M Tris-HCl

400 µL 10% Tween-20

350 mg BSA into a suitable container and vortex until homogeneous. Keep refrigerated. Lasts one week. To filter, put BSA into another container via syringe and filter.

A.3 Anti-dig

To make 100 µL of 20 µg/µL anti-dig solution, add:

90 µL 100 mM Na-phos (pH=7.5)

10 µL of 200 µg/mL anti-dig

into a suitable container and vortex. Keep refrigerated. Lasts one week.
Bibliography


[8] OpenStaxMicrobiology, Microbiology (OpenStax CNX, 2017), URL http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.9.


