# CONFINING INDIVIDUAL DNA MOLECULES IN A NANOSCALE CONE

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## CONFINING INDIVIDUAL DNA MOLECULES IN A NANOSCALE CONE

By

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### ROBERT DAVIDSON PETERS, B.Sc.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science

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## Abstract

This thesis details our progress toward developing an experimental method that will study conical confinement effects on deoxyribonucleic acid (DNA). Using micropipettes with tip diameters on the order of  $\sim 1 \ \mu m$ , we study T4 bacteriophage DNA in the small radii of the micropipette tips to build upon our understanding of the entropic force due to confinement. Using two separate methods, evaporative flow and applied electric field, we are able to force the DNA molecules into confinement at the micropipette tip. Labeling the DNA chains with a YOYO-1<sup>®</sup> fluorescent dye, we image the motion of the chains after the applied force is removed. We observe that the DNA molecules move away from the tip of the micropipettes and the dynamics are well parametrized by our theoretical model for a polymer in conical confinement. However, when our experimental protocol is performed using 1  $\mu$ m polystyrene beads instead of DNA, we still see motion of the beads away from the tip after stopping the applied force. Studying polystyrene beads in various solvent conditions, we determine that due to the strict boundary conditions of our current experimental setups, ions in the DNA solvent cause the majority of particle motion seen in our DNA experiments, not polymer entropic effects. Using evaporative flow as our confining force, these dynamics are caused by a diffusion of concentrated ions at the tip. When the applied electric field was used to induce confinement, the excess dynamics occur due to a polarization of ions in the micropipette solution. Regardless of cause, these solvent ion dynamics mask the polymer entropic confinement effects in our current micropipette experiments. Using the knowledge we have gained through this study, we propose modifications which build upon our current experimental procedure, eliminating the effects due to evaporation or polarization of solvent ions. We hope that these proposed changes will allow us to successfully measure the entropic force in axisymmetric, continuously changing confinement.

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## Chapter 1

## Introduction

As polymer molecules are confined, the number of available states for the molecule decreases, reducing the entropy. Studying the entropic cost of confining polymers, and more specifically DNA, is not only of interest to fundamental polymer physics, but is essential for understanding biological systems including the ultra close packing of DNA in the nucleus or supercoiling of DNA through enzymatic processes. In the case of close DNA packing in the nucleus, the chain winds tightly around histone molecules, organizing into a structure which drastically reduces the size of the polymer to almost 1/40000<sup>th</sup> the natural DNA size [1]. Entropic costs due to such significant conformation restrictions are poorly understood, and require fundamental study of the entropic force induced by confinement.

Though the polymer entropic cost inside many confinement structures has been studied, the ultimate goal of our research is to develop an experimental procedure which will confine a DNA molecule in a cone. By monitoring the dynamics in such a symmetric system, we hope to measure the entropic cost of confining DNA molecules in a continuous gradient of confinement.

A review of the polymer physics associated with confined and unconfined polymers is presented, followed by a brief description of some experimental complications associated with using electric fields in microfluidics. We then introduce previous studies which look at the entropic cost and investigate the technological applications associated with confining polymers and DNA. A detailed description is then given for the most recent experimental procedure we have developed to confine DNA molecules in a cone.

We will then present results of the dynamics of DNA near the tip of a cone followed by a control test using polystyrene beads. Using the polystyrene beads, we learn that our current experimental procedure induces fluid dynamics due to the ionic nature of the DNA solvent. Having learned about the excessive dynamics caused by the solvent, we present a set of future experiments which should remove these effects and could potentially study the entropic force due to confinement in a cone.

### 1.1 Review

Polymer physics written by Rubinstein and Colby is an excellent treatment of polymer theory [2]. In what is to follow, we recap some of the important results.

### **1.1.1** Polymer Conformations

Polymers are chains composed of repeating molecular units commonly referred to as monomers. On a monomer length-scale (< nm), the molecular units are connected by stiff covalent bonds; however, given a sufficient number of monomers, the chain may be seen as flexible. Flexibility of the chain may arise due to a variety of mechanisms depending on the polymer. In double-helix DNA, each bond is flexible, whereas in polystyrene, the flexibility occurs due to the three possible angles in the covalent bonds between successive monomers.

Regardless of mechanism, the flexibility of a polymer is important, allowing the chain to take on new conformations rather than a straight, stiff line of monomers. On the length scale of the entire polymer, thermal fluctuations are influential as they cause the chain to "wiggle" around, exploring many different conformations. The exploration of various conformations is of fundamental interest for our study as we build upon our understanding of polymer entropy, S.

The entropy of a polymer is directly related to the number of possible conformations,  $\Omega$ , of a chain by the traditional equation for entropy

$$S = k \ln \Omega, \tag{1.1}$$

where k is the Boltzmann constant. As in any physical system, the free energy is always minimized when the entropy is maximized. Thus, to maximize entropy, the polymer molecule prefers to explore all conformations available with equal probability.

#### **Ideal Polymers**

The ideal chain is a polymer in which there are no physical interactions between monomers separated by a large distance along the chain [3]. One example of ideal chains in practice is polymers in a melt. In a polymer melt, there exist only polymers with no outside solvent. Therefore, for monomers separated by a large distance on the same chain, the interaction is identical to an interaction with a monomer on a nearby different chain [3]. A simple model of an ideal polymer is the freely-jointed chain (FJC) model. In the FJC model, the distance between monomers that experience no physical interaction with each other is often referred to as the Kuhn length, b. The entire freely-jointed chain consists of N randomly oriented segments of length b. For the discussion of ideal polymers, all calculations will be performed using the FJC model. Although the FJC is a simple model, the statistics are identical to those of all other ideal chain models.

#### **Polymer Size**

One method to describe the size of the polymer is the contour length,  $l_c$ , simply defined as

$$l_c = Na, \tag{1.2}$$

where N is the number of monomers in the polymer chain and a is the length of each individual monomer. For flexible polymer chains the contour length is a poor descriptor of the actual size of the molecule as  $l_c$  only represents the largest conformation of the chain, where all monomers line up straight.

There are two variables commonly used to describe the characteristic size of a polymer molecule: the end-to-end distance,  $\overrightarrow{R}_{ee}$ , and the radius of gyration,  $R_g$ . The end-to-end distance is simply the measurement of distance from the beginning to the



Figure 1.1: Diagram for the definition of the end-to-end distance.

end of the polymer chain (see Figure 1.1).  $\overrightarrow{R}_{ee}$  is mathematically represented as

$$\vec{R}_{ee} = \sum_{i}^{N} \vec{r'}_{i}, \qquad (1.3)$$

where  $\overrightarrow{r}_i$  is the vector representing the  $i^{th}$  Kuhn segment. Since the polymer conformation is constantly fluctuating, a polymer may not be characterized by a single end-to-end distance, but the average over all possible values (ensemble average). Taking the ensemble average of all conformations is trivial for the freely-jointed chain model if we recall that the chain can be thought of as uncorrelated segments of length b. We Follow the derivation by Rubinstein and Colby where the freely-jointed chain is treated analogous to a random walk with steps of size b [2].

In a random walk of N steps, the ensemble average is always zero. Therefore, the mean-square end-to-end distance,  $\langle \vec{R}_{ee}^2 \rangle$ , is a more accurate measure of polymer size.  $\langle \vec{R}_{ee}^2 \rangle$  can be described as

$$\left\langle \overrightarrow{R}_{ee}^{2} \right\rangle = \sum_{i=1}^{N} \sum_{j=1}^{N} \left\langle \overrightarrow{r}_{i} \cdot \overrightarrow{r}_{j} \right\rangle.$$
(1.4)

Using the fundamental definition of the dot product, we get  $\overrightarrow{r}_i \cdot \overrightarrow{r}_j = b^2 \cos \theta_{ij}$ , where  $\theta_{ij}$  is simply the angle between the *i*-th and *j*-th segment. Substituting into Equation 1.4, we find

$$\left\langle \overrightarrow{R}_{ee}^2 \right\rangle = b^2 \sum_{i=1}^N \sum_{j=1}^N \left\langle \cos \theta_{ij} \right\rangle.$$
 (1.5)



Figure 1.2: Various polymer architectures. Due to the varying number of ends for each architecture,  $R_g$  is a more commonly used measure of polymer size.

For all segments  $i \neq j$ ,  $\langle \cos \theta_{ij} \rangle = 0$  as the steps are uncorrelated, while for i = j,  $\langle \cos \theta_{ij} \rangle = 1$ . When summing over all *i* and *j*, the result is *N*. Taking the square root of  $\langle \vec{R}_{ee}^2 \rangle$ , we achieve a measurement of polymer size

$$\sqrt{\left\langle \vec{R}_{ee}^2 \right\rangle} = \overline{R}_{ee} = \sqrt{N}b, \qquad (1.6)$$

where we will refer to  $\overline{R}_{ee}$  as end-to-end distance hereafter. Equation 1.6 shows us that the size of an ideal polymer scales as  $\sqrt{N}$ , identical to a random walk with step size of length b.

The radius of gyration is an alternative method of describing the size of a polymer molecule, and it more versatile and widely used than the average end-to-end distance. This is because for different polymer architectures, the number of ends of the polymer is not necessarily 2, making end-to-end distance a useless measurements. (See Figure 1.2).

Radius of gyration,  $R_g$  is a measurement of the distribution of mass in relation to

the centre of mass of the molecule,  $\overrightarrow{R}_{cm}$ .  $R_g$  is mathematically represented as

$$R_g^2 \equiv \frac{1}{N} \sum_{i=1}^{N} \left( \overrightarrow{R}_i - \overrightarrow{R}_{cm} \right)^2, \qquad (1.7)$$

where  $R_i$  is the directional vector pointing to a monomer or segment of the chain. If we once again consider that a polymer molecule does not exist in a single shape but explores many, then we must take the average size over all possible conformations. When derived by Rubinstein and Colby, a linear chain is found to have radius of gyration of

$$\sqrt{\langle R_g^2 \rangle} = R_g = b \sqrt{\frac{N}{6}} = \frac{\overline{R}_{ee}}{\sqrt{6}}.$$
(1.8)

From Equation 1.8 we see that the radius of gyration is related to the average endto-end distance for a linear polymer molecule, and also scales as  $N^{1/2}$ . Regardless of the chosen model, the ideal chain size will always obey these  $N^{1/2}$  statistics for size of the polymer.

#### **Real Polymers**

As stated earlier, polymer melts contain chains which are ideal, scaling in average size by the square root of the number of monomers, or Kuhn segments. Unfortunately, this scaling of polymer size does not always hold true in a polymer solution. A solution consists of two components: polymer chains and solvent. A polymer solution is considered dilute when the concentration of polymers is sufficiently small that the polymer chains do not overlap. When polymers overlap in a solution due to high concentration, the solution is described as being semi-dilute. Much work has gone into understanding the properties of DNA in semi-dilute solutions [4, 5, 6, 7]. Of particular interest to our study of polymer confinement is that due to the increased concentration of polymers in semi-dilute solution, the chains were shown to penetrate further into confinement than a dilute solution [6]. In general, confinement effects are shown to be reduced when dealing with entangled semi-dilute solutions [7], and thus we have chosen to perform our experiments in the dilute regime, maximizing the measurability of confinement effects.

Unlike in a polymer melt, in a dilute polymer solution chains interact with the



Figure 1.3: Depending on the polymer conditions, the size of the polymer will be vastly different for a chain of large number of Kuhn segments,  $N_b$ 

solvent instead of with other polymers. Interaction with the solvent governs the statistics of the polymer molecule size as outlined in. If the polymer has a repulsive interaction with the solvent, meaning the monomers prefer to interact with the other monomers, the chain will collapse, maximizing monomer contact. Solvents which cause polymer collapse are referred to as poor solvents. Alternatively, the polymer may experience an attractive interaction, causing the polymer to swell, exposing more monomers to the solvent. Solvents which cause swelling of polymer chains are referred to as good solvents. Under certain conditions, the polymer chain may interact with the solvent in a manner physically identical to how the chain interacts with itself, resulting in what is called a  $\theta$ -solvent. A  $\theta$ -solvent is the only type of solvent in which a polymer in dilute solution will act like an ideal chain (see all solvent conditions in Figure 1.3).

The statistical scaling of the polymer size with N varies depending on the solvent conditions. For a  $\theta$ -solvent, the polymer size will scale as  $N^{1/2}$ , as the polymer is ideal. In a poor solvent, the polymer can be modeled as collapsing into a globule. Thus by assuming dense packing, the polymer size will roughly scale as  $N^{1/3}$ . In contrast, for a polymer in a good solvent, the scaling of the polymer size is seen to be  $N^{\nu}$ , where  $\nu \approx 3/5$ . The calculation of this scaling  $\nu \approx 3/5$  for a polymer in a good solvent was first calculated by Flory, resulting in the term Flory chain. More accurate simulations have shown that  $\nu \approx 0.587597$  [8] for a real chain in a good solvent. Thus, for a polymer in solution, the radius of gyration is given as

$$R_g = b \left(\frac{N}{6}\right)^{\nu},\tag{1.9}$$

where  $\nu = 1/3$ , 1/2, or  $\approx 3/5$  depending on whether the polymer is in a poor,  $\theta$  or good solvent.

### **1.1.2** Electrophoresis and Microfluidics

Electrophoresis is the motion of charged particles relative to a stationary liquid, under the influence of an applied electric field [9]. In microfluidic devices, electrophoresis becomes much more complex as electrokinetic effects occur, causing the fluid to flow [10]. When a dielectric such as glass or any silica material is immersed in an electrolyte, such as aqueous solution, a surface charge appears. This surface charge originates from the Si-O-H terminal located at the surface of the glass, which becomes protonated, leaving a negative charge along the surface [10]. In a solution with ions, a layer of positive charges will form along the surface, creating what is called an electrical double layer [10]. These positive ions are mobile, whereas the surface charge is not. Therefore, when an electric field is applied, the entire layer of positive ions moves along the surface of the structure [10]. In microfluidic structures, the motion of the uniform ion layer pulls the solution at the surface through viscous drag, creating plug flow within the channel commonly referred to as the electro-osmotic flow (EOF) [10].

In order to reduce the effect of EOF in microfluidic experiments, a common treatment is to coat the surface with a substance that prevents the formation of a uniform electrical double layer. Polymers with medium adsorption strength are the most effective at preventing this electrical double layer [11], and polyvinylpyrrolidone (PVP) is one of the best commercially available [12].

### 1.1.3 de Gennes "Blob Theory"

The following is adapted from an argument made by de Gennes and supplemented with treatment by Rubinstein and Colby. [2, 3]

One of the simplest methods of describing forces and symmetric constraints on polymer chains is the blob model developed by deGennes. The basis for his scaling argument comes from the fact that whether confinement or a physical force is affecting the conformation of a chain, the local conformational freedom and polymer size statistics remain. For example, if a linear chain is stretched by an equal force pulling



Figure 1.4: For a linear polymer being pulled at both ends with a force of f, the molecule may be visualized as a sequence of blobs of size  $\xi$ , whose statistics are unperturbed by the external force. [3]

on both ends, the resulting conformation of the chain may be visualized as a string of connected blobs with size  $\xi$ . Any stretching observed is caused by the blobs lining up sequentially, not by restricting the local conformations of the chain. (See Figure 1.4)

The de Gennes scaling concept also applies when polymer molecules are compressed within confining structures. For example, in a cylindrical 2-dimensional confinement, if interaction with the walls is ignored, we can use the blob picture to explain the equilibrium conformation for real chains in either a good solvent (Flory chain) or  $\theta$ -solvent (ideal chain). The schematic description can be seen in Figure 1.5. For both ideal and Flory chains, portions of the polymer which are smaller than the diameter of the cylinder, D, will not be affected by the constraint, therefore  $\xi \approx D$ . If we define the number of monomers which are contained within a blob as g, then since blobs of size D are unaffected by the cylindrical constraint,

$$D \approx bg^{1/2}$$
 for ideal chains, and (1.10)

$$D \approx bg^{3/5}$$
 for Flory chains. (1.11)

Still using the blob model, we may consider the length of tube which the polymer occupies,  $R_{\parallel}$ . For an ideal chain, the physical interaction between pairs of blobs is identical to the interaction between the blobs and the surrounding medium. Therefore, in the following derivation performed by Rubinstein and Colby, we assume the blobs will perform a random walk, once again maximizing their entropy, which using



Figure 1.5: Difference between real and ideal polymer in cylindrical confinement.

Equation 1.10 yields,

$$R_{\parallel} \approx D\left(\frac{N}{g}\right)^{1/2} \approx bN^{1/2}.$$
 (1.12)

Equation 1.12 tells us that for an ideal chain, the equilibrium size along the axis of the cylinder is unperturbed. The polymer will be compressed in the radial direction, but since all interactions are neglected for an ideal chain, the blobs are allowed to overlap.

In contrast, for a Flory chain, the blobs will repel each other, as a higher concentration of monomers will increase the free energy. Thus, in order to allow more contact between the chain and the solvent, a line of connected blobs is formed inside the cylinder, yielding a  $R_{\parallel}$  of,

$$R_{\parallel} \approx D\left(\frac{N}{g}\right) \approx \left(\frac{b}{D}\right)^{2/3} Nb.$$
 (1.13)

Notice that the length of the chain along the axis of the cylinder is increased when the chain is confined within the tube, now scaling as N, compared to the  $N^{3/5}$  scaling of an unconfined Flory chain. Therefore, as the diameter of the cylinder decreases, the length of the polymer will increase, unlike the ideal chain where  $R_{\parallel}$  will remain the same.

Though we can visually study the conformations of real and ideal chains in confine-

ment, the free energy or entropic cost of confinement is of great physical importance as well. If we recall that lengths of chain which are  $\leq g$  do not experience any change in their conformation statistics, then we can estimate that for every blob, there is a cost of  $\approx kT$  in energy. Essentially, the chain loses a degree of freedom for every blob, since chain segments longer than g are not allowed to explore outside the confinement, preventing a large number of possible conformations. With Equations 1.10 and 1.11, we find the free energy costs of confinement,  $F_{conf}$  to be

$$F_{conf} \approx kT \frac{N}{g} \approx kTN \left(\frac{b}{D}\right)^2$$
 for ideal chains and (1.14)

$$F_{conf} \approx kT \frac{N}{g} \approx kT N \left(\frac{b}{D}\right)^{5/3}$$
 for Flory chains. (1.15)

Thus, as the diameter decreases, the free energy cost of confinement increases for both cases. Though the motion of the polymer is restricted from moving radially out of the confinement, the chain can still navigate back and forth through the tube in a motion called reptation. Reptation is simply a random walk in one dimension as the chain moves back and forth within a tube.

#### **1.1.4** Entropic Force due to Confinement

In the case of a constant confinement, such as the cylindrical one described above, though a free energy cost exists, the polymer reptates back and forth randomly as the chain is not in contact with areas of lower free energy. If the degree of confinement were to vary, the gradient in the entropy, and more generally the free energy for the polymer molecule, would force the polymer molecule to move toward the less confined region. This form of the entropic force is known as the confinement entropic force [13].

As an example, consider a cylindrically confining structure which has two regions with different radii (See Figure 1.6). Using Equation 1.15, a real polymer in good solvent which sits at the barrier between these two regions will experience a free energy of

$$F_{conf} = kT \left( N_1 \left( \frac{b}{D_1} \right)^{5/3} + N_2 \left( \frac{b}{D_2} \right)^{5/3} \right),$$
(1.16)



Figure 1.6: A polymer which exists between the two regions with different radii will experience a force toward the wider cylinder due to the increase in entropy.

where  $N_1$  and  $N_2$  are simply the number of Kuhn segments in the cylinders of width  $D_1$  and  $D_2$  respectively.

Using Equation 1.16, every segment which moves into the wider cylinder of width  $D_2$ , decreases the free energy cost of confinement for the entire polymer. Minimization of free energy drives the confinement entropic force, moving polymers into regions with less confinement. The confinement entropic force will not arise in spatially constant confinement structures, as the change in confinement is what drives the motion of the polymer [13], much like a metal chain sitting on a frictionless table. If an entire metal chain is resting on the table (polymer is in a constant confined region), there is no motion, and the chain will remain on the table. However, if part of the chain is left hanging off the table (polymer is between  $D_1$  region and  $D_2$  region), the chain will fall to the floor, minimizing the chain's potential energy (free energy).

#### 1.1.5 Previous Experiments/Simulations

The dynamics of polymers, especially DNA, in various confinement structures has been of great interest in recent years. The ability to image individual molecules has provided the means for studying properties of single chains rather than extracting information from measurements performed in the bulk. Single molecule imaging has accelerated our understanding of polymer dynamics, and allowed us to probe the unique and complex dynamics of polymers in different confinement geometries.



Figure 1.7: In the presence of a nanohole, the polymer dynamics have been studied in both the cases where the polymer is driven and not driven through a nanopore.

#### Nanopore Confinement

One of the most prevalent confinement structures of interest is the nanopore (Figure 1.7), in part due to the potential of advanced technologies for sequencing long DNA molecules. The Sanger method of decoding DNA chains developed in 1977 provided a method for decoding a DNA sequence using gel electrophoresis [14]. In this method the chains are terminated at specific points resulting in strands of various lengths. After gel electrophoresis, the lengths of the strands are determined, and using multiple chain terminators yields a complete description of the sequence for the DNA.

Though the Sanger method has proven useful, even the most effective variations are only accurate for sequence lengths on the order of ~ 1000 base pairs, much less than a typical genome length [15]. For example, the Escheria coli genome contains  $4.6 \times 10^6$  base pairs [16]. Due to the amount of time and DNA required to use variations of the Sanger method in decoding genomes, alternate methods of decoding are desirable to reduce cost [17].

Nanopores have been shown to provide the possibility of quick (1bp/10ns) and cost-effective sequencing of DNA chains [17, 18]. By applying an electric field, the DNA molecule is forced to travel through the nanopore one base at a time. As the DNA chain translocates through a nanopore, the ionic current that is measured

will depend on which base pair is passing through the nanopore [17]. The nanopore technique is advantageous for the length of DNA chain it could potentially sequence in one experiment, as the method uses base-by-base decoding. Base-by-base decoding means the measurement at any one instance will be independent of the history of the experiment, imposing no limit to the potential length of DNA which could be sequenced [18].

Beyond the practical application of DNA sequencing, interest has been placed on understanding the physics of DNA and polymer molecules in nanopore confinement. In addition to the case of a polymer forced through a nanopore like in the sequencing experiments [19], theoretical and simulative studies have been performed on the dynamics of a non-driven polymer trapped in a nanopore [20, 21, 22]. Though the dynamics of moving through a hole may seem simple, much controversy was created over the scaling exponent,  $\nu$ , associated with the escape time,  $\tau$ , for a polymer exiting a nanopore. Though  $\tau \sim N^{\nu}$  was a widely accepted model for escape time, various simulative and theoretical approaches achieved different scaling exponents  $\nu$ , confusing the scientific community. The theoretical escape time exponents that have been found range from  $\nu = 3$  assuming a purely diffusive process over an energy barrier [22] to  $\nu = 2$  where the process is assumed to be dominated by dynamics and friction inside the pore [21]. Exponents of  $\nu = 2.2, 2.4, 2.52, 2.588$  have also been found, though usually through using varying lengths of polymers, hole sizes, hole shapes and whether the polymer is assumed at equilibrium [20].

The discrepancy in values for  $\nu$  has only recently been explained in an article by Hendrick de Haan and Gary Slater [20]. Performing Langevin dynamics (LD) simulations, they varied the size of the circular pore through which the polymer was translocating. By simply varying the radius of the hole, they were able to achieve nearly all scaling exponents,  $\nu$ , which had been found in previous studies.

The nanopore system shows that even if the geometry of confinement is extremely simple, the dynamics can be very complex and vary significantly based on simple parameters such as the width of confinement, hydrostatic interactions and length of the polymer [20].

#### Confinement Geometries for Sorting DNA

Another confinement geometry for DNA which has been studied extensively through simulation and experiment is the entropic trap [23]. The entropic trap geometry consists of a wide channel through which DNA can travel under the influence of an electric field. Along the direction of travel, the channel height varies in a periodic, step function from a depth that is similar to the  $R_g$  of the DNA (~ 1  $\mu$ m), to a height in which the DNA is confined significantly (~ 90 nm). Applying high electric fields, the DNA molecules were found to move through both the thin and thick regions quickly. Applying low electric fields, the molecules would never traverse the 90 nm thin regions due to the high entropic barrier. However, in intermediate electric fields, a 'trapping' was witnessed at the boundary between the thick and thin regions that was dependent on the DNA length, thus the naming of the geometry as an entropic trap [23].

The entropic trap was experimentally studied using fluorescently labelled DNA so that individual molecules could be tracked under the influence of an electric field [23, 24]. In the thicker regions, the DNA molecules formed approximately spherical blobs until they reached the boundary between the thin and thick regions, becoming 'trapped'. At the boundary, DNA molecules were found to eventually stretch a small portion of the chain into the thin region after a characteristic time,  $\tau$  [23]. Once the initial stretch formed, the rest of the molecule followed sequentially, quickly crossing the thin region. The trapping process is repeated at each boundary between a thick and thin region. Upon analyzing the statistics of the varying trapping times, the characteristic trapping time was found to be shorter for longer DNA molecules. The reason was not due to a variance in size of the entropic barrier that was to be overcome, but due to the higher surface area of long polymer in contact with the thin region where the molecule was trapped [23]. The larger amount of polymer in contact with the thin region increased the probability of forming the initial stretch into the thin region, allowing for quicker passage through a channel with multiple 'traps' [23]. These experimental results were verified by simulations carried out using a Monte Carlo simulation [25].

It was noticed by Han et al. that the entropic trap geometry was ideal for separating molecules of different sizes, as the escape times for T2 and T7 DNA, 164 kbp and 37.9 kbp respectively, were found to have a trapping lifetime ratios,  $\frac{\tau_{TT}}{\tau_{T2}}$ , as high as 1.9 (for 4  $\mu$ m period between successive traps) [23]. In a study more concentrated on DNA separation, the structure was shown to be one to two orders of magnitude faster for separation than conventional gel electrophoresis, while providing similar resolution [24].

Another confinement geometry studied using DNA is a 2-dimensional pillar array [13, 26]. Using  $\mu$ m sized separation between pillars, DNA chains in an electric field became hooked around the pillars for certain periods of time. Though the frequency and length of time taken to unhook a chain was characteristic of the geometry, the hooking process did not seem to be length dependent, so the authors concluded this structure was not a viable option for DNA separation [26]. Conversely, nanopillars with a separation of ~ 125 nm were found to provide a possible option for DNA separation [13]. With various DNA at a boundary between a non-pillared region and a nanopillar region, an electric field was used to force the chain to extend into the area between the pillars.

If the electric field was turned off before the DNA molecule had fully entered the pillared region, a confinement entropic force would cause the DNA chain to recoil back into the non-pillared region where the entropy was much higher for the chain. However, if the DNA completely entered the pillared region, when the electric field was released, there was no entropic force acting on the DNA, allowing the chain to remain confined within the pillared region [13]. Using intermittent pulses of electric field, the recoil served as a mechanism for separating various DNA lengths, as by forcing the shorter DNA to fully extend into the pillared region, when we turned off the electric field, longer chains would recoil back from confinement. The small chains would remain confined in the nanopillar region, thus separated from the longer chains [13]. The entropic trap stresses the importance of gradient in confinement to induce a confinement entropic force, as the recoil only occurred at the boundary between the pillared and non-pillared regions. The authors found that the force caused by confinement was constant at the boundary, causing the DNA recoil to speed up as there was less extension into the pillared region [13].

As will be outlined in the next subsection, entropic stretching forces decay as a function of the portion of DNA which is stretched [27], whereas confinement entropic

force will remain constant, depending mainly on the geometry.

The study of dynamics in the entropic trap and nanopillar confinements has focused on the practical applications of studying DNA in confinement. Though the physics is extremely interesting and complex, these two confinement geometries provide only discrete changes in confinement, minimizing the amount we can learn about the confinement entropic force.

### Entropic Relaxation Experiments and Conformational Analysis in Confinement

In the previously described geometries, the DNA and other polymers were studied in a system where they had access to both a confined and unconfined state. The variation in confinement initiated an entropic force which caused the molecule to move into the unconfined region. There is however another entropic force which was introduced in section 1.1.3 which arises solely due to the stretching or compression of a chain.

Many experimental methods have been attempted to study the force associated with elongating a DNA chain. Using hydrodynamic flow to elongate a DNA molecule, the relaxation of the chain could be observed [28], or using optical tweezing or magnetic bead techniques, the stretching force could be studied directly [29, 30].

The entropic relaxation of chains inside confinement to their equilibrium conformation has also been studied. Experiments have been performed in nanotubes (symmetrical 2-D confinement) [27, 31] as well as nanoslits (1D confinement) [32] to study the relaxation of DNA chains after an applied stress. In nanotubes, compressing or stretching of a DNA chain resulted in a relaxation to the blob conformation outlined in section 1.1.3, provided the width of the tube was larger than the Kuhn length of the chain [27, 31]. By monitoring the fluorescence intensity of a DNA molecule stretched within a nanotube, relaxation was found to occur in a method predicted by the direct experiments on the stretching force for a DNA chain [30], where the entropic force decreased as the chain approached equilibrium, thus slowing down its relaxation [27, 31].

Relaxation in nanoslits was also studied, a structure best described as having a thin channel height with infinite size in the x-y plane. When chains were elongated in nanoslits, the DNA relaxed with two separate exponential decay times [32]. The authors determined the two separate relaxation mechanisms were associated with a quick relaxation of the DNA chain into a linear link of blobs, as predicted by the de Gennes model, followed by a slower relaxation where the blobs would orient themselves to be more random inside the x-y plane of the slit.

Relaxation experiments provide information on the equilibrium conformations of polymer molecules in confinement as well as the dynamics associated with perturbing confined chains from equilibrium. Unfortunately the entropic force associated with relaxation is fundamentally different from the entropic force due to confinement. For relaxing polymers, the force diminishes as the polymer approaches equilibrium conformation. In the case of the confinement entropic force, the magnitude is determined by the gradient in polymer confinement, and thus does not have to decrease as the polymer approaches its equilibrium state.

### 1.2 Goals

Though many studies have been performed that look at entropic forces inside confinement, the dynamics and statics of DNA relaxing to equilibrium while in confinement studies only the force associated with elongating a DNA chain. Similarly, the studies performed on polymers in a gradient of confinement, although concerned with the confinement entropic force (hereafter referred to as simply the entropic force), are mostly focused on the technological advances using geometries with discretely changing confinements.

Our goal is to develop an experimental technique that will provide a better fundamental understanding of the entropic force. By studying the dynamics of an individual DNA molecule in a cone, a highly symmetrical, continuously changing confinement, we will be able to measure the entropic force for a single chain in a range of confinements. (See Figure 1.8)

Conical confinement hosts all the properties we desire in a confinement structure for these experiments. The geometry is axisymmetric with a continuous change in width near the cone apex. The uniqueness of using conical structures for confinement is that we can describe the width of the structure at all points using only 2 variables, the tapering angle  $\theta$  and the distance from the tip of the cone, x. By monitoring



Figure 1.8: A conical confinement provides a highly symmetrical confinement geometry to study the entropic force.

the position of the polymer under the influence of the entropic force in a cone, we can measure the magnitude of the entropic force for a wide range of confinements in a single experiment. Monitoring these dynamics will provide us with much more information on the magnitude of the entropic force in a spatially varying confinement than previous studies in discretely changing geometries.

Our experiment will not only provide a unique method for studying the entropy of an individual linear polymer, but our goal is to use our technique to understand the entropic cost of many more complex systems such as ultra compacted DNA in the nucleus, supercoiled DNA or various common polymer architectures (star polymers, dendrimers, circular polymers, etc.).

For our ideal experimental setup, we require a force to move DNA molecules into confinement at the tip of a conical structure. Upon release of this external force, we will monitor the position of the molecule over time. Our procedure should eliminate all external forces acting on the DNA in order to study the effects due to only one effect, the entropic force. Using a theory described in the next chapter, we aim to uncover the dependence of the entropic force, and more fundamentally the entropy of a polymer molecule, on the tapering angle for the cone,  $\theta$  and the size of the DNA chain. M.Sc. Thesis - R.D. Peters

McMaster University - Physics and Astronomy

## Chapter 2

# Theory for Entropic Force in Conical Confinement

The highly symmetric, continuous change in width of a cone is very conducive to theoretical treatment. As opposed to the sudden changes in confinement experienced in previous studies, in a cone, a polymer is able to continuously explore a range of confinements. A proper theoretical analysis of a polymer in conical confinement will thus yield a more fundamental understanding of the strength and dependencies of the entropic force.

The theory of a polymer in a conical confinement was derived in 1977 by Brochard and de Gennes [33]. In this article, the authors used the fact that a cone is the same as a cylindrical confinement with a continually changing radius. Recognizing that the diameter of the cone was equal to

$$D = x \tan \theta, \tag{2.1}$$

Brochard and de Gennes made a simple substitution into the free energy given in Equation (1.15). Assuming that the tapering angle,  $\theta$ , is very small, the authors calculated the free energy cost of confinement to be

$$F_{cone} \approx kTN \left(\frac{b}{x\theta}\right)^{5/3} \approx kT \left(\frac{R_F}{x\theta}\right)^{5/3},$$
 (2.2)

where  $R_F = bN^{3/5}$  is the size of a polymer in good solvent and T is temperature. To

determine the entropic force on a polymer as a function of distance from the cone tip x, differentiation of the energy gave

$$\frac{dF_{cone}}{dx} = f_{ent} \approx \frac{T}{x} \left(\frac{R_F}{x\theta}\right)^{5/3},\tag{2.3}$$

where  $f_{ent}$  is the entropic force for a polymer confined in a cone [33].

Noting that a polymer molecule in a solvent is not in an inertial system, but a dissipative one, we can state that  $f_{ent} \propto v$ , where v is velocity of the molecule [10]. Unfortunately, measuring the velocity of a molecule can be quite difficult, but the position of a molecule is easy to monitor. Thus, we used the force in Equation 2.3 to derive the position of the molecule as a function of time. By simply integrating the velocity,  $v = \frac{dx}{dt}$ , through separation of variables, we find the position, x, of a polymer in a cone behaves as

$$\int dx x^{8/3} \approx \frac{T R_F^{5/3}}{\theta^{5/3}} \int dt \tag{2.4}$$

$$x = A(t+t_0)^{3/11} - x_0, (2.5)$$

where  $A(\theta, R_F, T, \eta) \sim T\left(\frac{R_F}{\theta}\right)^{5/11}$ ,  $\eta$  is the solvent viscosity,  $x_0$  and  $t_0$  are experimental constants and x and t are the measured position and time. Since we can not experimentally confine a polymer molecule to the apex of a cone where x = 0, we require the experimental offsets  $x_0$  and  $t_0$ . The relevance and meaning of the variables in Equation 2.5 will be further discussed in the next chapter.

The dependence of the power law derived in Equation 2.5,  $x \sim t^{3/11}$ , is a byproduct of the statistics for the random walk of a polymer chain in a good solvent. However, it can be shown that for an ideal chain, the power law changes only slightly to become  $x \sim t^{1/4}$ . The prefactor A is of more interest for this study due to its dependence on the size of the polymer molecule, the tapering angle of the cone as well as temperature and viscosity. By studying the dependence of the prefactor on varying  $\theta$  and  $R_F$ , we will develop a better understanding of the entropic force in conical confinement.

## Chapter 3

## Experimental

### 3.1 Sample Preparation

### 3.1.1 Confinement Environment

Creating an axisymmetric continuous confinement gradient on the micro-scale is not an easily accomplished task using the traditional laser lithography and etching techniques utilized in previously mentioned experiments. In order to achieve our ideal experimental confinement geometry, we construct glass micropipettes. Although the micropipette tip structure is not a perfect cone along the entire structure, for experiments which take place at the small diameters near the tip, the tapering angle is locally constant and small (See Figure 1), allowing us to use Equation 2.5 for analysis of our results.

Our micropipettes are created from boro-silicate glass capillaries with an outer diameter (OD) of 1.0 mm and an inner diameter (ID) of 0.5 mm. Using a horizontal micropipette puller (Narishige model PN-30), the capillaries are heated at their centre



Figure 3.1: An example of a typical micropipette tip. The tapering angle is extremely small and constant over the field of view in the image.

and pulled at both ends to attain micropipettes with a tip OD of ~ 1  $\mu$ m. These micropipettes are pulled in such a way that the tips remain open, allowing us to fill them with a dilute solution of DNA.

### 3.1.2 DNA preparation

The DNA we use in our experiments is a T4 bacteriophage E.Coli DNA (Wako Scientific). The primary reason for selecting this particular DNA to study was its length of 169,000 base pairs (169 kbp). Using equation 1.9, given that the length of a DNA base pair is 0.34 nm and the Kuhn length is ~ 100 nm [34] the radius of gyration for our DNA molecule is  $R_g \approx 1.5 \ \mu$ m. This DNA size is large enough that we can confine the chains within our previously described glass micropipettes.

During and between experiments, the DNA is stored in a 0.5x Tris-Borate EDTA (TBE) buffer solution (5x TBE buffer, Sigma-Aldrich, diluted 10x in de-ionized H<sub>2</sub>O). This buffer solution is a good solvent for DNA and is necessary to prevent denaturation of the chain. Minimal imaging contrast between the DNA and the surrounding buffer solution necessitates that for effective particle tracking, the chain must be labelled with a fluorescent dye. We use YOYO<sup>®</sup>-1 (Molecular Probes, Invitrogen) dye to stain our DNA chains. YOYO<sup>®</sup>-1 is an intercalating dye, meaning the fluorescent molecule inserts itself between the backbone units of the DNA chain. This dye is more effective than traditional fluorescent dye molecules as the dye does not hang off the side of the DNA chain, altering the polymer physics and dynamics in experiment. YOYO<sup>®</sup>-1 also has the unique property that when attached to the DNA backbone, the dye experiences an enhancement in fluorescence intensity compared to when in free solution. Increases in fluorescence intensity of  $460 \times [35]$  and  $3200 \times [36]$  have been measured, the discrepancy is due to the difficulty in measuring the minimal fluorescence of YOYO<sup>®</sup>-1 in free solution. The increase in fluorescence is extremely valuable, as it results in negligible background fluorescence from excess dye remaining in the solution during experiment.

In order to stain the DNA with our YOYO<sup>®</sup>-1 fluorescent dye, we incubate a  $\sim 1.0 \text{ ng}/\mu\text{L}$  solution of T4 DNA in a 0.1  $\mu\text{M}$  solution of dye for one hour at room temperature. This protocol successfully labels the chains at a 5:1 bp:dye ratio, the highest concentration of dye molecules that may be intercalated with the DNA chain

before denaturing of the chain may occur [37]. For experiments in micropipettes, our resultant solution is diluted at a 9:1 ratio with 0.5x TBE to yield a DNA concentration of 0.1 ng/ $\mu$ L. As outlined in section 1.1.2, in order to suppress electro-osmotic flow, 2% (w/w) polyvinylpyrrolidone is added to the DNA solution before we fill the micropipettes.

### 3.1.3 Filling Micropipettes

Originally, we attempted to fill the micropipettes with DNA solution using capillary flow through the  $\mu$ m sized tip. Unfortunately, capillary flow was an extremely slow method of filling micropipettes, as we were attempting to fill sections as wide as 0.5 mm through a hole of only ~ 1  $\mu$ m in diameter.

Attempting to speed up the process, we used a suction pressure to pull the solution in faster, however the filling procedure still required hours for a sufficient amount of solution to flow through the micron sized tip into the micropipette. By using a 34 gauge MicroFil<sup>®</sup> (World Precision Instruments) luer lock syringe tip, we are able to inject the DNA solution directly into the back end (wide end) of the micropipette. The benefit of the MicroFil<sup>®</sup> is that the device is composed of plastic and fused silica, allowing for easy bending of the tip without breaking when inserted into the micropipette. Due to its long length (67 mm) and small OD (164  $\mu$ m) we can inject the DNA solution near the tip of the micropipette. For the smaller widths of the micropipette that the MicroFil<sup>®</sup> syringe tip is incapable of reaching, the capillary force described in Appendix A is strong enough to pull the solution to smaller radii, filling the remainder of the tip.

Unfortunately, when injecting solution, we introduce the major issue of air bubbles. Air bubbles near the tip of a micropipette have been shown to create a clogging pressure which is so great, no pressure supplied will expel the air from the micropipette [38]. As well, when an air bubble is created, the capillary force will be unable to fill the tip of the pipette with solution. In order to avoid air bubbles, we mount the syringe with the MicroFil<sup>®</sup> tip attached, onto a 3-D micromanipulator. We start continuous injection of DNA solution when the MicroFil<sup>®</sup> is in the wide section of the pipette so that by the time we reach the narrow sections of the micropipette tip, the air bubbles have been expelled from the syringe, and floated harmlessly away from the micro-scale tip. The entire process of filling micropipettes using the MicroFil<sup>®</sup> syringe tips takes mere minutes in comparison to the hours required for the previously attempted methods.

### 3.1.4 Polystyrene Beads

In addition to performing experiments in micropipettes with DNA, we also performed them with 1  $\mu$ m Polystyrene Polybeads<sup>®</sup> (Polyscience Inc.). The experiments with Polystyrene beads were a control, as the dynamics experienced by the beads would not be induced by the entropic cost of confinement. This allowed us to probe any dynamics caused by the solvent conditions or random motions of the particles in the micropipettes.

The Polybeads<sup>®</sup> are supplied in an aqueous environment with a concentration of  $4.55 \times 10^{10}$  particles/mL. This stock solution was diluted  $10000 \times$  so that the final concentration was  $4.55 \times 10^{6}$  particles/mL for experiments.

Measurements were made with the Polybeads<sup>®</sup> diluted in de-ionized water as well as  $0.5 \times$  TBE buffer solution and  $0.5 \times$  TBE buffer solution with 2% polyvinylpyrrolidone.

### 3.2 Evolution of Experimental Setup

### 3.2.1 Imaging and Electrophoresis

All fluorescence imaging is performed on an Olympus BX51 microscope equipped with a fluorescence attachment (Olympus Canada, model BX-URA2) and 2x magnification changer (Olympus Canada, model BX-2). The 2x magnification changer enables us to achieve the same resultant enlargement of our images with a lower magnification objective. This extra magnification is important, as the higher the magnification of the objective, the closer the lens must be to the object. Thus, with the magnification changer installed, we have more maneuverability in our experimental setup while maintaining the enlargement of a superior lens. Images were taken using an ultra low noise, cooled CCD camera (Roper Scientific). For measurements of the polymer



Figure 3.2: Though all DNA are moving upwards due to an electric field, we can monitor a single DNA (outlined with white circle). The expanded image displays the elongated nature of the DNA, ensuring we are not measuring the fluorescence of a simple spherical particle.

dynamics near the micropipette tip, we take sequences of images to monitor the position of the molecules as a function of time.

Initially, we ensured that imaging DNA under the influence of electrophoresis was achievable inside a geometry which would not confine the chains. We created a rectangular glass chamber by melting two parafilm strips between a glass slide and cover slip. Using an undiluted solution of fluorescently labelled DNA, we filled the chamber and placed wires in contact with each open end. Applying an electric potential across the chamber ( $\sim 20 \text{ V}$ ) we monitored the motion of the DNA. In Figure 3.2, we can see that tracking DNA particles in an unconfined environment under the influence of an electrophoretic force is experimentally possible using fluorescence imaging.

#### **3.2.2** Electrical Contact

Once we ensured that imaging and electrophoresis of DNA was possible in an unconfined environment, we needed to develop a method to apply electrodes to the two ends of a micropipette for the application of an electrophoretic force. The wide end is brought into electrical contact by simply inserting a wire into the back end of the micropipette. Maintaining electrical contact with the tip however is no trivial task. The complication of creating electrical contact occurs due to the fragility and bendability of the thin glass at the micropipette tip, the extremely small ( $\sim 1 \ \mu m^2$ ) area which requires electrical contact, and the necessity of minimal scattering or background brightness induced by the contact to enable high contrast fluorescence imaging.

Initially, we attempted to immerse the tip of the micropipette into buffer solution, creating electrical contact by placing the electrode in contact with the solution bath. Using this bath of buffer solution, the brightness of fluorescence was drastically reduced as much more scattering occurred in the extra layers that the fluorescence needed to travel through. In order to protect the lens from contact with the buffer solution, we placed a thin microscope slide cover between the objective and the buffer. The result was 4 different mediums the fluorescence was required to pass through to reach the objective: curved glass (the micropipette), buffer solution, flat glass (microscope cover slide) and air. The many layers and their varying indexes of refraction were an issue for scattering. As well, the objective could no longer be placed as close to the micropipette tip, restricting our use of highly magnifying lenses which have short focal lengths.

To avoid surrounding the micropipette tip with buffer solution as an electrical contact, we attempted to maneuver the micropipette into contact with a variety of conducting media. Using copper wire, copper wire covered in silver paint and agarose gel made with 0.5x TBE, we were unable to develop a satisfactory contact with the tip. Using the plain copper wire, the contact with the tip was minimal, and as soon as the tip came into contact with the wire it would begin to bend. We then painted the copper wire with silver conductive paint (Flash  $Dry^{\mathsf{M}}$  Silver Paint, SPI Supplies) to provide a softer electrical contact. Unfortunately, we found that the rough and highly reflective surface of the silver paint scattered and reflected light into the objective, making fluorescence imaging at the tip nearly impossible. The same difficulty was present when using the agarose gel as an electrical contact for the tip, as the gel was highly scattering, drastically reducing the fluorescence imaging contrast.

To provide a liquid electrical contact with the micron sized tip that did not scatter excessive background light, our current electrically conductive setup uses roomtemperature Mercury. We are able to suction a small amount of liquid mercury, using



Figure 3.3: A schematic of the electrically conductive setup.

a syringe, into either a glass capillary or a micropipette with a large tip diameter ( $\sim 200 \ \mu m$ ). We then induce a slight pressure using the syringe, forcing a small mercury droplet to be exposed at the end of the capillary with which the micropipette can come into contact. The schematic of our current setup is shown in Figure 3.3.

As seen in Figure 3.3, both the micropipette with DNA solution and the mercury pipette are attached to 3-dimensional micromanipulators (NewPort, models 423(mercury) and 462(micropipette)). These micromanipulators provide the ability to precisely move the micropipette into contact with the mercury droplet while ensuring that focal drift does not occur from movement of the microscope stage.

To perform experiments using this electrically conductive setup, we use the micromanipulators to achieve contact between the micropipette tip and the mercury droplet. Using a DC power supply, we apply an electric potential across the electrodes located in the mercury and the back of the micropipette to achieve electrophoresis of DNA, forcing them into the micropipette tip. After releasing the electric field, we monitor the motion of the DNA particles using fluorescence imaging.

Though our electrically conductive method is effective in creating an electrical contact with the micropipette, due to the high surface tension of mercury, the micropipette tip can be deflected during the measurement, exposing DNA solution to air and stopping electrical contact. Through exposing the micropipette to air during an electrically conductive experiment however, we noticed a flow of solution toward the tip. This flow allowed us to create an experimental setup without the need for electrical contact.

#### 3.2.3 Evaporative Flow Setup

When the micropipette tip is exposed to air, DNA molecules move toward the tip. As outlined in Appendix A, this is caused by evaporation near the tip of the micropipette, creating a flow of solution. In short, as solution evaporates from the tip, due to capillarity the remaining liquid prefers to flow toward the tip of the micropipette so that the surface at the smallest radii of the micropipette is still in contact with solution.

Our interest in creating flow of solution is for the purpose of replacing the electrophoretic force as the confining force. Using the naturally occurring evaporative flow, we can force DNA toward the micropipette tip, making our experimental procedure much more simple. Instead of electrical conductivity being a necessary requirement for the material we place the micropipette tip in contact with, the only requirement is that the material prevents evaporation, stopping the flow of solution toward the micropipette tip. Our concerns with the high surface tension of mercury are therefore avoided, preventing contamination of the solution from dirt deposited on the surface of the mercury and deflection of the micropipette tip.

As an evaporation stopper, we use Sylgard<sup>®</sup> 184 Silicone Elastomer (DOW Corning Corporation). The elastomer comes as a silicone encapsulant which can be cured to create a rubber. To create our evaporation stopper, we mix the curing agent and silicone encapsulant at room temperature. Using a 1.5 mm OD capillary tube with one end closed (Kimble Chase), we dip the closed end into the elastomer. We then heat the capillary in an oven at 150 degrees for 20 minutes, fully curing the mixture into a solid elastomer droplet at the end of the capillary (see Figure 3.4). The capillary is then attached to the 3-dimensional micromanipulator of our electrically conductive setup in place of the mercury droplet. By simply placing the tip of the micropipette into contact with the soft elastomer, we can stop evaporation and monitor the motion of DNA after evaporative flow has ceased.

We saw the evaporative flow experimental procedure to be ideal, as understanding the effects of an electric field applied in a microfluidic device can be difficult. Using evaporative flow prevents the occurrence of EOF or polarization of ions in the solution, which may induce forces on the DNA chain even after the electric field has been terminated.



Figure 3.4: An image of a micropipette coming into contact with a silicone elastomer droplet at the end of a capillary.

### 3.3 Image Analysis

Image analysis to determine the position of DNA molecules or polystyrene beads was performed using code written in MATLAB<sup>®</sup> (Mathworks). Since the only direction with a confinement gradient in our micropipette is along the axis of symmetry, before taking any measurements of position in the micropipette, the program has you create a line of reference along its axis. After creating the reference line, by simply identifying the position of the DNA molecule in each image using a cursor, the program will record the projection of that point onto the axis of symmetry, eliminating any measurement of radial movement. The result is a measurement of the DNA position along the axis of symmetry as a function of time, precisely what we have derived in Equation 2.5.

As described in section 2.1, the theoretical origin for our model exists where the apex of the cone would be, at x = 0. Unfortunately the cone apex does not exist in our field of view, or in practice at all, as our micropipette tips are open and not perfect cones. Thus, we take our measurements as the position of the DNA in relation to its location in the first image of the experiment. In Figure 3.5, we see that  $x_0$  simply represents the position of the DNA at the beginning of the experiment, and



Figure 3.5: In the cone, x = 0 is where the apex of the cone is located.  $x_0$  is simply the position where you start tracking the molecule and x refers to the measured position of the molecule throughout an experiment.

the measured position of the DNA through analysis is x. Upon fitting the results of a measurement of position vs. time using Equation 2.5, we will obtain fitted values for  $x_0$ ,  $t_0$  and A. As described in Chapter 2, we look to monitor the dependence of A on physical parameters, such as the size of the polymer molecule and the tapering angle of the micropipette in order to derive the proper form for entropy in a cone and the magnitude of the entropic force.

## Chapter 4

## Results

Experiments studying DNA dynamics were initially carried out using the electrically conductive setup. We then switched to performing experiments with the evaporative flow setup due to the difficulties of working with the high surface tension mercury. As a control for determining the contribution of solvent conditions and random particle diffusion in a cone to the dynamics we were measuring in our experiments with the DNA, we performed the same experimental procedures with polystyrene beads. Polystyrene beads, which exhibit no entropic effects due to confinement, exhibited similar dynamics to DNA, which we determined to be dependent on the ionic nature of the buffer solution. These solvent dynamics were found to mask the dynamics caused by entropic confinement effects of DNA in the micropipettes we used, making experimental modifications necessary for accurate study of the entropic force.

### 4.1 DNA

### 4.1.1 Electrically Conductive Setup

Figure 4.1 gives an example of the images which are obtained when performing an experiment with our current electrically conductive setup. The DNA molecule, indicated by the white circles in the image, is forced toward the tip (to the right) using an applied electric potential. At  $t \approx 400$  s, the electric field is turned off, causing the DNA molecule to move away from the tip (to the left) of the micropipette.

In Figure 4.2, we can see the position of the DNA molecule as a function of time



Figure 4.1: Dynamic response inside a micropipette, during and after applying an electric potential of V = 15 V between the back of the pipette and the mercury in contact with the tip.



Figure 4.2: The measured dynamics of the DNA shown in Figure 4.1 during and after electrophoresis. In Figure 4.1, position is measured in relation to the starting point before the electric field is applied, yielding a format where increasing value in position indicates approaching the tip (opposite to our theoretical model).

for this same experiment. Under the influence of the applied electric field, the DNA molecule initially moves rapidly towards the tip, slowing down as the chain reaches equilibrium with all the forces in the system (electrophoretic, entropic, EOF). Upon releasing the electric field at  $t \approx 400$  s, the DNA molecule immediately travels away from the tip. Since to our knowledge no other forces existed except surface interactions and viscous drag, of which neither is biased in any direction, we initially believed this motion was induced by the entropic force.

In Figure 4.3, we concentrate on the DNA position as a function of time after the electric field has been removed. We can clearly see that DNA motion occurs only toward increasing x, away from the micropipette tip. There does seem to be a two-tier process though, where the motion of the DNA is halted for  $t \approx 50 - 150$  s. However, after  $\approx t = 150$  s, the molecule once again moves quickly away from the tip.

The period of immobility for the DNA made accurate analysis with our power law model for the entropic force impossible. The most likely explanation for the immobility of the DNA is that the chain adhered to the surface of the micropipette, halting its dynamics for a short period of time. Although the PVP in the solution is meant to adsorb to the surface of the pipette, it is unlikely that every part of the inside is coated with the PVP. While applying an electric field, the EOF may shear the PVP from the surface, leaving exposed glass to the DNA for some time after the electric field is released.

Though analysis was not possible due to the temporary immobility of the DNA on the glass surface, this experiment was successful in monitoring the dynamics for a polymer in conical confinement. Unfortunately, as described earlier, small perturbations including air fluctuations can cause the micropipette tip to deflect from the high surface tension mercury, exposing the micropipette tip to air and allowing evaporation. The high probability of perturbations make lengthy observations difficult with the electrically conductive method. As well, there was concern that by using an electric field in an ionic buffer solution with defined boundaries, we would polarize the solution in the micropipette. Polarization was an issue as it could affect the dynamics of the DNA even after we release the electric field.

For these reasons, we began experimenting with our evaporative flow setup which



Figure 4.3: Position vs. time for DNA motion following the application of an electric field. Micropipette drawing indicates position of tip, where increasing x indicates movement away from confinement.

provides a means for forcing the DNA toward the tip while allowing for a more consistent and effective removal of the force. We could thus perform experiments without polarizing the buffer solution or exposing the micropipette tip to air.

#### 4.1.2 Evaporative Flow Setup

Figure 4.4 gives an example of a measurement taken using the evaporative flow technique. Notice that the length of the time in which we can measure the dynamics of the DNA molecule is drastically increased using the new technique, measuring for  $\sim 700$  s in comparison to the  $\sim 200$  s achieved with the electrically conductive setup.

The data in Figure 4.4 is fit to our model for the dynamics of a polymer in a cone. In all fits of our model to the experimental data, we allow the prefactor A to vary along with the experimental constants  $x_0$  and  $t_0$ . Since the constants  $x_0$  and  $t_0$  are experimental constants which only depend on where the DNA molecule is in our theoretical cone when the experiment starts, the prefactor A is what yields information about the physics of our confinement geometry and the DNA being confined. If the dynamics of DNA under the influence of the entropic force are measured, we will build a fundamental understanding of the confinement effects by measuring the dependence  $A(\theta)$ . However, for the measurement shown in Figure 4.4, although the model seems to parametrize the results effectively, the motion of the DNA needed to be studied further in order to determine whether the entropic force was the cause of these dynamics.

In order to enhance the distance over which our measurements were taken, as we are limited to the field of view of our microscope objective we use an accumulation of many experiments. By taking multiple measurements in the same micropipette at different starting positions,  $x_0$ , we can obtain results for the motion of the DNA molecule over a wider range of the pipette. In Figure 4.5, we give an example of multiple experimental data sets taken in the same micropipette.

If we were in an inertial system, the speed of a molecule at a certain position in the pipette would be dependent on where the DNA started moving under the influence of the entropic force. An inertial system would necessitate that we analyze each curve separately. However, since we are in a dissipative system where  $v \propto f$ , for each position in our micropipette there is a specific velocity associated with it, i.e. there



Figure 4.4: The position of an individual polymer molecule after using evaporative flow to force the chain into the tip of the micropipette. The data is fit with the model for entropic force in Equation 2.5. Although the model agrees well with the data, we will show later that the dynamics are not induced by the entropic force through control experiments.



Figure 4.5: Position of DNA as a function of time after evaporative flow. The many curves represent different experiments run from different positions in the same micropipette.

are no inertial effects. Therefore, regardless of where our DNA starts moving, the molecule should move at the same speed for any specific position in the micropipette. This will results in the same trajectory for all DNA molecules if the dynamics are created by the entropic force.

By observing a constant in all images (such as a spot of dirt on the outside of the micropipette), we adjusted all measurements to the same frame of reference for x. Then by adjusting each measurements' time coordinate, we can create a master curve for position as a function of time in the micropipette. The resultant graph is shown in Figure 4.6 and displays the dynamics of the DNA molecule over a wider range of position in the same micropipette than could be provided by a single measurement.

Our concern with the results in Figure 4.6 is that the individual runs show a slowing in dynamics at different positions within the micropipette, contradicting theory. If the dynamics were caused by the entropic force, then theory predicts that all trajectories should be identical regardless of when the measurement is taken within the same micropipette.

In Figure 4.7 we see another set of data where the dynamics of multiple experiments with DNA molecules in the same micropipette were aligned to the same reference frame. It is even more evident in these results that the DNA molecules do not stabilize at the same point within the micropipette, seeming to have a clear difference in when the molecules stop moving away from the tip.

There are many possible explanations for the inconsistency between experiments including adsorption of the DNA to the glass surface or exposure of the tip to air causing slight evaporation which contradicts the entropic force. In order to determine the cause of the dynamics in our results, we performed experiments with Polystyrene beads where no entropic force would exist. This control experiment allowed us to determine the extent of dynamics caused by solvent conditions and the experimental setup without confinement entropic effects.

### 4.2 Polystyrene Beads

For our control experiments we used 1  $\mu$ m polystyrene beads which hosted a slight anionic charge, making them analogous to our DNA, but without polymeric entropy



Figure 4.6: Multiple experimental curves for position vs. time all aligned to the same reference frame, creating a Master Curve.



Figure 4.7: Multiple experiments run on the same pipette shifted to the same frame of reference. Although all in the same frame of reference, the DNA molecules seems to stop in some experiments at a different position within the pipette.



Figure 4.8: Polystyrene beads moving away from tip after stopping evaporative flow.

properties. Our expectation was to clearly observe the motion of the beads under the influence of the evaporative flow. Upon placing the silica elastomer in contact with the micropipette tip, if we were correct in assuming the dynamics we had observed for the DNA experiments were due to an entropic force, the beads would do one of two things. They would either stop moving toward the tip and begin random brownian motion or they would slow down drastically, moving toward the tip slowly if slight evaporation still occurred in the system.

As is evident in Figure 4.8, neither of these two possible outcomes occur. Instead, after stopping evaporative flow, the polystyrene beads immediately begin to move away from the tip in a similar fashion to what we observed with DNA. As the polystyrene beads have no polymer entropic properties, this provided clear evidence that the motion in our experiments is induced by either a charge interaction between the beads or the solvent and experimental method we are using.

In Figure 4.9, we graph the motion of a DNA molecule in comparison with that of polystyrene beads in both buffer solution and water after using the evaporative



Figure 4.9: DNA (triangles) and polystyrene beads in buffer solution (squares) and water (stars) in similar micropipettes.

flow method. Although these experiments were not performed within the same micropipette, all micropipettes were pulled with the same heating and pulling conditions, yielding what we believed to be extremely similar pipettes.

In Figure 4.9, we see that the DNA and polystyrene beads move in a similar fashion over the same length and time scale. Due to the minimal reaction of the polystyrene beads in de-ionized water after stopping evaporative flow, we determined that the dynamics in the buffer solution were a result of the ionic nature of our buffer solution and the evaporative flow technique used. These results indicate that if there were entropic confinement effects due to confinement occurring in the evaporative flow experiments, distinguishing between entropic confinement effects and the solvent dynamics in our current experimental procedure would be difficult.

### 4.3 Dependence of Dynamics on Solvent Contents

In order to understand what is creating the dynamics in our experimental procedure, we performed both evaporative flow and electrical conductivity measurements using polystyrene beads. Our results are summarized in Tables 4.1 and 4.2 using various solvents including de-ionized water, 0.5x TBE buffer solution and 0.5X TBE buffer with 2% PVP. A schematic is provided to demonstrate the mechanism we have associated with the dynamics of each response as well.

Solvent	Response	Response Schematic
TBE Buffer	Bead moves away from tip	
TBE Buffer without PVP	Bead moves away from tip	
De-Ionized Water	Negligible Response	

Table 4.1: Dynamic Response of Polystyrene Beads after Evaporation

The only solvent and experimental procedure which did not show the polystyrene beads retreating from the tip of the micropipette was when we performed the evaporative flow measurement in de-ionized water. Therefore, we conclude that the ions in the buffer solution are producing the force that pushes the DNA and polystyrene beads away from the tip.

Solvent	Response	Response Schematic
TBE Buffer	Bead moves away from tip	
TBE Buffer without PVP	Bead moves away from tip	
De-Ionized Water	Bead moves away from tip	

 Table 4.2: Dynamic Response of Polystyrene Beads after Electrophoretic Force

For the evaporative flow method, as water evaporates from the tip, an excess amount of ions are deposited at the tip when using our buffer solution. A higher concentration of ions may be the cause of these dynamics, as when evaporation is stopped, the concentration gradient will create a mass diffusion of both positive and negative ions away from the tip. Since both the polystyrene beads and the DNA we use have an anionic charge, positive ions in the buffer solution will form an double electrical layer on the surface of the molecules [10]. Thus, the concentration gradient near the tip of the pipette will not only force the ions to all diffuse away from the tip, but the ions surrounding the DNA or polystyrene particle as well, dragging the larger molecules, similar to the way EOF drags the solution near the glass walls. Since the diffusion of particles in microfluidics is known to be slow, the time scale of ~ 1000 s is a reasonable result for the time period of dynamics for a diffusional process [10]. Clearly, in de-ionized water a concentration gradient mechanism will be minimal as there are essentially no ions in the solution. Thus, upon ceasing evaporation, there is no concentration gradient, creating no dynamics away from the tip.

For the electrically conductive method, regardless of the solvent used, we witnessed motion of the polystyrene beads away from the tip after releasing the electrophoretic force. The response of the ions after electrophoresis is slightly different than to evaporation, as instead of depositing an excessive amount of positive and negative ions near the tip of the pipette, the electric field polarizes the solution [10]. The polarization creates an excess amount of negative ions near the tip of the micropipette which need to diffuse away from the tip. One possible method for the dynamics we witness to occur is that as the ions are forced to diffuse slowly through the microfluidics, the excess of negative charges near the tip creates a slight electric field with the excess of positive ions away from the tip, forcing the polystyrene beads or DNA to move out of the tip. Once again, the diffusion can occur over a time-scale equivalent to the  $\sim 1000 \ s$  that we witness in our experiments [10].

## Chapter 5

## **Future Experiments**

It is clear that our current experimental methods are ineffective at measuring the entropic force accurately, as the dynamics created by our buffer solution overshadow those of DNA confinement. Though these results are unfortunate there is potential to build upon our current experimental setup and develop experiments which prevent buffer solution dynamics while managing to study polymer confinement in a micropipette.

One option for studying the physics of a linear polymer, if we are not concerned with using DNA, is to use an extremely long synthetic chain which has a good solvent that contains no ions. For example, polystyrene, which obeys good solvent statistics in toluene, is available in a molecular weight of 9100 kg/mol (Polymer Source Inc.), corresponding to a radius of gyration of ~ 150 nm. This polystyrene molecule will be possible to confine using an evaporative flow setup in commercially available pipettes which have a tip diameter of  $100 \pm 10$  nm (World Precision Instruments). Without any ions in the toluene, the extra dynamics observed in DNA experiments should be completely removed. As well, it has been shown that polystyrene may be labeled with fluorescent dyes, allowing our imaging process to remain the same [39].

Another possible experiment may be performed using our evaporative flow technique which does not study only a single linear polymer in confinement. We can graft long polymers, such as the 9100 g/mol polymer described above, onto nm sized beads. The model will no longer be simple, due to the combined effect of confining many polymer chains at once, however the experiment could still be performed in



Figure 5.1: Both the tip and the back end of the micropipette are immersed in a bath of buffer solution, eliminating evaporation and polarization of solution.

a non-ionic solvent, eliminating the extra dynamics. As well, experiments could be performed using star or branched polymers, which can yield  $R_g$ 's which are much larger than typical linear chains, but have much more complex theoretical models.

Preferably, we will still be able to study DNA inside the micropipette, as the ease of confining such a large molecule and the theory which has already been developed for a linear polymer make application and analysis much simpler. Thus, the experiment we will be performing next uses the electrically conductive method while immersing both the tip and the back end of the micropipette in a bath of buffer solution. Provided the bath of buffer solution is large enough, the effect of evaporation within the pipette will be negligible. As well, applying an electric field by placing electrodes in contact with the excess solution at each end of the micropipette is simple, and will allow the solute ions to travel out of the confinement structure, preventing polarization near the tip.

As described in the experimental section, a draw back of using buffer solution at the tip is that imaging the DNA through an extra layer of solution will reduce the fluorescence intensity measured. However, by immersing the objective into the buffer solution, we hope to still be able to detect enough fluorescence to monitor the DNA position, allowing accurate measurement of the entropic force after releasing the electric field. With no effects due to strict boundary conditions or the use of an ionic solvent, we hope this experimental procedure will provide the ideal setup for studying the entropic force. A schematic of the future experimental setup is shown in Figure 5.1.

## Chapter 6

## Conclusions

Confining DNA and other polymer molecules in a variety of geometries has yielded information on the forces associated with polymer confinement. The main goal for this study was to develop an experimental technique that would allow us to study the dynamics of DNA chains in a conical confinement. The continuous entropy gradient of the unique conical structure was extremely attractive for understanding the entropic force, which had previously been studied in only discretely changing geometries. We achieved this conical confinement using glass micropipettes and developed an experimental setup to monitor the DNA dynamics using fluorescence.

We were successful in forcing DNA molecules into the tip of the micropipettes using both evaporative flow and electrophoretic force. After forcing the DNA molecules to the micropipette tips, we released the applied force and monitored the dynamics. Though the results seemed to be in agreement with the theoretical model for entropic force of a polymer in a cone, by performing experiments with polystyrene beads we discovered that the dominant forces acting on our DNA molecules were not induced by reduction in entropy, but by solvent conditions and our experimental procedure. While we were unable to measure the entropic force accurately, our ability to image and manipulate the motion of DNA inside a conical microfluidic device was a great success.

Our current sample preparation and experimental procedures create excess dynamics, masking the effect of the entropic force, however our progress thus far is still advantageous. By understanding the limitations of our current technique, we have proposed a new procedure for measuring the entropic force of DNA in micropipettes. Having learnt from many caveats that have arisen through our studies, this new procedure should remove all excess dynamics associated with our current experimental setup, yielding dynamics under the influence of only the entropic force. The results from these experiments will be revolutionary, and the possibilities for studying much more complex systems, such as DNA wrapping around histones, supercoiled DNA and various other polymer architectures, will ensure many future studies using our technique.

## Appendix A

## **Capillarity and Evaporative Flow**

When a thin glass capillary is set into contact with a bath of water, the liquid will rise inside the capillary to a certain height. The liquids' tendency to rise up into the capillary, known as capillarity, is what drives the filling of our micropipette tips and the evaporative flow. Capillarity arises due to the energetic preference of the water to be in contact with the glass capillary as opposed to air, commonly referred to as wetting. An excellent theoretical treatment which was used as the source for the following is provided by de Gennes and Brochard-Wyart [40].

When filling a constant radius glass capillary with liquid, the solution will rise into a vertical capillary through capillarity to a height, H, of

$$H = \frac{2\gamma\cos\theta_E}{\rho g R},\tag{A.1}$$

where  $\gamma$  is the surface tension of the liquid in air,  $\theta_E$  is the contact angle the solution makes with the surface,  $\rho$  is the density of the liquid, g is the gravitational acceleration constant and R is the radius of the capillary.

Equation A.1 has been obtained by studying the surface energy gain due to maximizing contact with the glass surface balanced with the gravitational potential energy. The energy E of the liquid column in the capillary is represented as

$$E = -2\pi R H \gamma \cos \theta_E + \frac{1}{2}\pi R^2 H^2 \rho g.$$
 (A.2)

The first term in equation A.2 is the contribution of the surface energy gain through



Figure A.1: A schematic of the variables associated with the Laplace pressure argument in the capillary rise of solution in a pipette adapted from [40].

wetting, whereas the second term is the cost due to the gravitational potential energy. For the interest of our experiments, we can focus on the surface energy contribution to the system, as our glass micropipettes are placed horizontally, preventing any gravitational potential energy cost.

Referring to Figure A.1 and using a Laplace pressure argument, the pressure immediately underneath the surface of a meniscus in a capillary is found to be

$$P_A = P_0 - \frac{2\gamma\cos\theta_E}{R},\tag{A.3}$$

where  $P_A$  is the pressure at point A, just underneath the meniscus, and  $P_0$  is the atmospheric pressure. Equation A.3 shows that for a decrease in the radius of the capillary, R, the pressure which drives the motion further into the capillary will increase, hence why we see a higher column of liquid rise in thinner capillaries. The micropipettes used in our experiment have a radius which varies near the tip as a continuous gradient, monotonically decreasing in size. Therefore, using Equation A.3, the Laplace pressure associated with capillarity will force the solution into the smaller radii, thus filling the tip.

Similarly, when solution evaporates from the tip, the increase in the driving pressure of capillarity for smaller radii will cause solution to fill in the space that would be opened up due to the lost water. Thus, when evaporation from the micropipette tip occurs, the capillary force will create a flow of solution that will maintain wetting at the smallest radii of the tip.

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