The Use of Entropic Cages For Trapping DNA and Controlling its Configurations in Nanopore Studies

by

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Nanopores can be used as sensors to probe the structure of single polymers. In a typical experiment, a thin insulating membrane containing a nanometer-scale pore separates two reservoirs of electrolyte solution. The nanopore acts as an ionic channel, permitting a current of ions to flow between the reservoirs when a voltage bias is applied to them. The presence of a single polymer inside the nanopore causes a change in the current, which is the basis of the sensing mechanism. Previous studies have quantified the dynamics of DNA molecules passing through the nanopore by electrophoresis, and examined the time course of the current to infer the physical structure of translocating polymers. In this thesis, we enhanced the ability of nanopores to study single molecules and to realize biosensing applications by juxtaposing a solid-state nanopore with a micrometer-sized cavity with a sub-micrometer opening in it. We used this newly created structure to investigate the origin of the viscous drag on DNA during translocations, and to characterize the same DNA molecule before and after it underwent a chemical reaction while entropically trapped inside the cavity.

First, we describe studies of electrophoretic DNA translocations of asymmetric nanopore-cavity structures whose dimensions were designed to control the initial configurations of the DNA molecules. The structures comprise a thin SiN membrane with a nanopore that leads into a 400 nm-high cavity, which is in turn covered by a 400 nm thick SiN membrane with a circular opening whose diameter was about 200 nm. This structure maintained a gap between the nanopore and a DNA coil translocating from the cavity side, but not one translocating from the nanopore side. The additional viscous drag on the DNA segment extending from the coil to the nanopore slowed translocations from the cavity side to less than half the average
speed of translocations from the nanopore side. The speeds converged as the opening enlarged from 200 nm to micrometer diameters. That result can be explained by the DNA coil, whose radius of gyration is $R_g \approx 0.73 \mu m$, squeezing further into the opening. Our experimental results compare favorably with a quantitative model of DNA translocation speeds, similar to models by Grosberg and by Ikonen, which account for the initial configuration of the DNA coil.

Next, we used the nanopore-cavity structures to enable analyses of the same DNA molecule before and after chemical interactions modified its structure. We used 1.5 $\mu m$ high cavities as cages which entropically trap single molecules next to a nanopore, despite the presence of a few-hundred-nanometer-wide opening. We probed the dynamics of DNA molecules inside the cage by electrically driving them in through the nanopore, removing the driving force for preset pause times, and then driving them back out through the nanopore. The saturating recapture time and high recapture probability after long pauses, their agreement with a convection-diffusion model, and the observation of trapped DNA under fluorescence microscopy all confirmed that the cage stably confines DNA. At the same time, entropic cages remained permeable to small molecules. To demonstrate these capabilities, we used a restriction endonuclease to sequence-specifically cut trapped DNA molecules into fragments whose number and sizes were analyzed upon exiting through the nanopore. The work presented in this thesis illustrates new and useful ways in which entropic cages can control a single DNA molecule before or after it translocates a nanopore.
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Chapter One

Introduction
1.1 DNA studies using nanopores

DNA is an important bio-polymer because it encodes genetic information [1]. Though the diameter of the double-stranded DNA molecule is only 2 nm [2], a nanometre-scale pore in a thin membrane can be made to detect a single molecule and sense its physical structure while in solution as it passes through the pore [3,4]. Kasianowicz et al. [3] first used a biological membrane channel, α-hemolysin, to study individual polynucleotide RNA molecules. The basic experimental set-up for nanopore sensing experiments is shown in Fig.1.1a. The α-hemolysin protein channel is a heptamer with a 2.6 nm diameter aperture leading into a slightly wider vestibule that abruptly narrows to a transmembrane domain with a 1.5 nm constriction [5, 6]. The channel connects two reservoirs of ionic solution, called cis and trans. A voltage applied between the reservoirs results in the flow of ions through the channel, as shown in Fig.1.1a. Kasianowicz et al. [3] detected transient current blockages after they added poly[U] to the negatively-charged cis side. Fig.1.1b presents the distribution of blockages according to the lifetime of the blockage, which shows three well-defined peaks. The lifetime of events in the first peak did not change when the experiment was repeated with different poly[U] lengths. Based on this observation, the authors argued that the first peak was due to collisions of RNA molecules with the mouth of the pore, and that those molecules did not translocate the nanopore. The lifetimes of the events in the second and third peaks, on the other hand, increased with poly[U] length. In addition, the lifetime of those events was inversely proportional to the applied voltage. Based on that dependence of lifetime on polymer length and applied voltage, the authors argued that those current blockages were due to the polymer translocating the nanopore by electrophoresis. They proposed that the existence of two peaks in the lifetime distribution reflected the polymer transiting the nanopore in either the 3’ to 5’ or the 5’ to 3’ orientation. The asymmetric structure of a DNA
Figure 1.1: Using a biological nanopore to study biopolymers. (a) Sketch of a nanopore translocation experiment. (b) The distribution of lifetimes of channel blockages caused by poly[U] translocating a α-hemolysin ion channel. (Adapted from [3]) (c) (Left) A current trace caused by the capture and translocation of a 6-bp DNA hairpin through the α-hemolysin nanopore. (Right) A molecular model of these events. (adapted from [6])

strand is what gives rise to those two distinct possibilities. In hindsight, this very first use of nanopores as biopolymer sensors already made it evident that the time course of the ionic current is rich in information about the structure and dynamics of single molecules.

Under the appropriate conditions, nanopores can even probe detailed aspects of the structure of nucleic acids. Vercoutere et al. [6], for example, used the α-hemolysin ion channel to discriminate individual DNA hairpin molecules, as shown in Fig. 1.1c (left). They detected three current levels, A, B, and C, corresponding to three
distinct molecular configurations shown in Fig. 1.1c (right). Level A is the open pore current, indicating no molecule in the ion channel. Level B is the DNA molecule stuck in the ion channel. This occurs because only linear single-stranded DNA can traverse the 1.5 nm limiting aperture, therefore the hairpin loop (yellow) must span the pore entrance, with the 6 bp of the stem (white and red) extending into the vestibule. Level C indicates molecules passing through the nanopore, which is possible when a hairpin loop breaks and the molecule opens into a single strand. The corresponding current trace shows a blockage, initially caused by a hairpin, but ending with a sharp downward spike due to the translocation of the linearized molecule. The authors also demonstrated that the duration of level B is proportional to the free energy of the hybridized loop, *i.e.* the more stable of the loop, the longer the time required for it to break up, confirming that level B was indeed caused by a hairpin loop stuck in the channel. This study illustrated how obtaining detailed information about the DNA configuration requires the ionic current trace to be interpreted with a good microscopic understanding of the nanopore and the target molecule.
In the quest for a versatile biosensor, nanopores in solid-state materials present advantages over biological nanopores. The advantages include very high stability, control of the diameter and length of the channel, adjustable surface properties, and the potential for integrating nanopores into arrays, with other devices, or types of sensors [8]. Fabricating nanopores with diameters measuring less than 10 nm, however, is not possible using conventional silicon processing techniques. Li et al. [4] first developed a method called “ion beam sculpting” to make solid-state nanopores. The technique is illustrated in Fig.1.2a. A focused ion beam (FIB) machine first milled a ∼100 nm hole in a free-standing membrane of silicon nitride. That membrane was then placed in a custom high-vacuum chamber, into the path of a beam of 3 keV ions directed at the surface. The ions caused the diameter of the hole to slowly shrink. The changing size of the hole could be monitored in real-time by detecting the rate at which ions in this case flying through vacuum passed through the hole. That rate provided a signal to indicate when to stop the process. Interestingly, the authors observed that, depending on the ion rate and temperature, pores could enlarge or shrink. This, and the real-time knowledge of the diameter of the hole, allows for fine-tuning of pores in the nanometer range.

Storm et al. [7] developed an alternative way to make solid-state nanopores using a transmission electron microscope (TEM), illustrated in Fig.1.2b. They observed that a highly focused electron beam would drill a pore, while a less focused electron beam would make the pore shrink. They could therefore tune the nanopore size by adjusting the beam intensity within the TEM. That technique has become the preferred method for making solid-state nanopores in the field.

Nanopore sensors have enabled fundamental studies on nucleic acid molecules [3,4,6,9–16] and show promise for use in a variety of biosensing applications [17–23].
In this case, we will make use of the versatility of solid-state fabrication techniques to create nanopore devices whose physical structure enables new fundamental studies and new biosensing applications.

### 1.2 The forces acting on DNA during translocation

The speed of DNA translocations and its uniformity are important for biosensing applications. Much effort has therefore been devoted to studying the driving forces and the viscous drag that determine the speed of DNA translocations.

The driving force has been measured directly by Keyser et al. \[13\] and Van-Dorp et al. \[15\] using optical tweezers. Fig.1.3a shows their experimental setup. They attached DNA polymers to a polystyrene bead, which was held by optical tweezers. The bead was brought close to a voltage-biased solid-state nanopore, so that the end of a DNA molecule would eventually be drawn through the nanopore. The electric force stretched the DNA and pulled the bead a distance $\Delta Z$ from the center of the optical trap, as shown in Fig.1.3b. By measuring the bead’s displacement, the authors were able to quantify the force applied to the DNA molecule in the nanopore. They measured the dependence of the force on the applied voltages and found they to be linearly related with a ratio of $0.24 \pm 0.02 \text{ pN-mV}^{-1}$ \[13\]. The mechanism of the driving force is understood to be a combination of the electrophoretic force on the charged DNA and the viscous shear force from fluid dragged along the side of the DNA by counterions in the electric double layer.

The driving force is balanced by the friction force on a molecule moving through
Figure 1.3: Direct force measurements on DNA in a solid-state nanopore [13]. (a) Experimental setup. DNA was bound to a bead by streptavidin-biotin. The electric field drove the DNA through the nanopore, pulling the bead a distance, \( Z \). (b) The measured bead position and current blockage, showing a current drop when a DNA was driven into the nanopore. The position of the bead jumped at the same time, indicating there was a force pulling the bead.

the nanopore. Different models have been developed to explain the drag force during translocation. The first dynamical model was by Lubensky and Nelson [24], who focused on single-stranded DNA passing through the \( \alpha \)-hemolysin channel, and argued that the friction was between the DNA molecule and the narrow channel of the protein pore. Fig.1.4a sketches the interaction of the DNA with the nanopore. Fig.1.4b shows the energy landscape, \( U(x) \), of the DNA as it translocates the nanopore. The authors treated the DNA molecule as a particle moving in the free energy field, \( U(x) \). It was proposed that each base needed to overcome a free energy barrier to travel through the nanopore. The probability distribution of the particle \( P(x,t) \) obeys the Smoluchowski equation

\[
\frac{\partial P}{\partial t} = D_0 \frac{\partial}{\partial x} \left[ \frac{\partial P}{\partial x} + \frac{U''(x) - F}{k_B T} P \right],
\]

where \( D_0 \) is the diffusion coefficient, \( U''(x) \) is the gradient of the free energy, and \( F \) is the constant driving force. The first term in the right side of equation (1.1) is the
diffusion term and the second term is the drift term.

Lubensky and Nelson’s theory explained the dynamics of single-stranded DNA and RNA translocations of the α-hemolysin ion channel, where the interaction between DNA the pore is very strong and the viscous drag force on the molecule outside the nanopore can be neglected. Their model predicts that the translocation time should be linearly proportional to the length of the molecule, $L$.

The model of Lubensky and Nelson is not applicable to the translocation of dsDNA through a solid-state nanopore, however, because solid-state nanopores are typically too wide to interact strongly with each base of a DNA. Storm et al. [11] observed a power-law scaling relationship between the translocation time and the length of dsDNA, i.e. $t \sim L^{1.27}$, as shown in fig[1.5]. Storm’s experiment indicated that the coil outside the nanopore contributes significantly to the drag on the moving polymer. To explain the super-linear dependence of translocation time on molecule length, Storm et al. proposed a model of the drag force on a moving sphere whose radius is $R_g$, the radius of gyration of the DNA coil outside the nanopore. The sphere shrinks as more DNA segments translocates the nanopore according to the assumption of the model. The authors argued the Stokes drag on the surface was
\[ F = 6\pi \eta R v_{blob} = 6\pi \eta R_g \frac{dR_g}{dt}, \]

where \( \eta \) is the viscosity, \( R_g \) is the radius of gyration of the DNA coil on the cis side, and \( dR_g/dt \) is the speed of the center of mass of that coil. This model assumes that the DNA coil maintains a quasi-equilibrium configuration during translocation, as shown in fig. 1.5.

The assumption that \( R_g \) equals its equilibrium value at all times appears inconsistent with the fact that the Zimm time, which is the characteristic time for a polymer to relax to its equilibrium configuration, is much longer than the translocation time. The Zimm time is 700 ms for \( \lambda \) DNA, whereas the molecule translocates the nanopore in only 2 ms. This means that the translocation process in fact drives the DNA far out of equilibrium.

Figure 1.5: (a) Experimental dependence of dwell time on DNA length. (b) Schematic of Storm et al.’s theoretical model, which assumes that DNA translocates the nanopore while maintaining a quasi-equilibrium configuration. (Adapted from [11])

Grosberg et al. [26] recognized that DNA translocations are typically too fast for a long polymer to maintain quasi-equilibrium, so they proposed a model in which only part of the molecule is in motion toward the pore at a given time. They argued
that only this moving segment of the molecule contributes to the drag. Fig. 1.6a illustrates their model of the translocation process. The nanopore is treated as a sink that attracts the polymer. The polymer is divided into several segments, separated by folds with local minimum distances from the sink. When the translocation starts, the closest segment (blue) becomes stretched and moves towards the nanopore. During that time, only the first segment experiences the drag force, while the other segments perform a random walk and otherwise have no influence on the translocation. After the first segment has translocated the nanopore, the next segment (red) starts to stretch and translocate. This process repeats until all the DNA segments have unfolded and translocated the nanopore. According to Grosberg et al.’s model, the instantaneous translocation speed changes in a manner determined by the initial configuration of the coil. Ikonen et al. [27] proposed a similar model, framed in terms of the propagation of tension along the moving part of the molecule. Using similar assumptions as Grosberg et al., Lu et al. [28] performed computer simulations to explain the velocity fluctuations observed in translocation experiments. Their model is illustrated in Fig. 1.6b, which shows 3 molecules initially translocating from different initial configurations. Molecule 1 was compact and close to the nanopore when it started translocating. That configuration resulted in a short moving segment and a high average translocation velocity. By contrast, molecule 3 was extended far away from the nanopore when it started translocating, therefore its translocation proceeded more slowly.

The models of DNA translocation dynamics in which only the moving segment of a molecule contributes to the viscous drag are favoured within the theoretical literature. However, a direct test of this model was not possible previously because there was no way to control the configuration of a DNA molecule at the start of a translocation. We addressed this problem by designing a device with a micrometer-
Figure 1.6: (a) Grosberg et al.’s model \cite{26} of polymer translocation dynamics in which the segment moving to the nanopore contributes the viscous drag. (b) Initial polymer configurations (top) and the distribution of translocation times (bottom), simulated by Lu et al. \cite{28}.

sized cage on top of a solid-state nanopore that could control the initial configuration of the molecule by introducing a well-defined gap between the nanopore and the polymer coil. In chapter 3 we performed translocation experiments using this device to test the theoretical models, and thereby confirm that the dominant drag force is due to the moving segment of the molecule approaching the nanopore from outside.

1.3 Molecular probe enhanced bio-sensing of nanopore

Nanopores are particularly well suited for probing chemical interactions between DNA and other molecules \cite{29,31} because those interactions often substantially modify the structure of a DNA molecule. For example, oligonucleotide hybridization probes \cite{32} bind to specific DNA target sequences and create bulges that are easy to detect when they pass through the nanopore. Applying this principle, Singer et al. \cite{21} mapped the locations of target sequences on long, double-stranded DNA
molecules, as shown in Fig. 1.7a. They targeted the DNA with oligomers of γPNA, which bound to the complementary sequence along the DNA and caused additional current blockage when those hybridized regions passed through the nanopore. Similarly, Wanunu et al. [20] identified different microRNAs using sequence-specific hybridization probes and a solid-state nanopore sensor, as shown in Fig. 1.7b.

To correctly infer that a chemical change to the structure of a DNA molecule has occurred, it is crucial to know the structure of that molecule before the chemical interaction. This presents a serious epistemic challenge, as nanopore biosensors should ideally discover the characteristics of a molecule without prior knowledge. In chapter 4, we report our solution to this problem. We can use a nanopore to characterize the same DNA molecule before and after it interacts with another molecule. For that, we devised what amount to single-molecule test tubes, in which DNA can react without diffusing away.

We applied the principle of entropic trapping to confine single DNA molecules in the cavity of our nanopore device structures. That principle is based on the tendency of polymers to form a coil in free solution to maximize their configurational entropy. Han et al. [33] demonstrated how configurational entropy can be used to trap a DNA coil in a synthetic porous structure. Fig. 1.8 illustrates the nanofluidic device they used. DNA tends to stay in the micrometer-deep channel because there is an entropic cost to squeezing a large DNA coil through the surrounding nanochannel in a distorted configuration. That entropic cost prevents the molecule from spontaneously escaping when its radius of gyration is larger than the height of the nanochannel [34,35].

In Chapter 4 we describe experiments in which an entropic barrier was erected to trap DNA in a cage next to a nanopore. The 200 nm-diameter opening of the
Figure 1.7: Studies of DNA-probe interactions using a nanopore. (a) The experiment of Singer et al. [21], demonstrating the detection of the sequence-specific binding oligonucleotide of probes along DNA. (b) The experiment of Wanunu et al. [20], demonstrating the detection and identification of miRNA (blue) hybridized with sequence-specific oligonucleotide probes.
Figure 1.8: (a) Schematic of the nanofluidic device developed by Han et al. for entropic trapping of DNA molecules [33]. The sketch shows DNA relaxed in the micro channel, whose depth was about 1 µm, and where the DNA had lower free energy. The micro channels are bordered by 90 nm-high nanoslits. (b) Free energy landscape for DNA in the channel.

cage is small enough to stably confine a long DNA molecule, but big enough for us to introduce molecular probes to study the DNA. The same DNA molecule can therefore be interrogated by the nanopore before and after it interacts with probes while trapped in the cage. We demonstrate these capabilities by using a restriction endonuclease to cut a trapped DNA molecule into fragments, whose number and size are measured by the nanopore upon leaving the cage.
CHAPTER TWO

Methods
The work in this thesis is based on a new type of nanopore device, which combines a solid-state nanopore with a micrometer-scale cavity that we call an “entropic cage”. These devices enabled us to control the configurations of DNA before or after a molecule translocates the nanopore, and to trap single molecules. In this chapter, we describe how we fabricate of nanopore/cage structures, how we use them in DNA sensing experiments, and how we image single DNA molecules trapped inside them.

2.1 Device Fabrication

2.1.1 Overview of the device design.

The basic structure of the nanopore/cage is illustrated in Fig. 2.1. The cavity and nanopore are made in a $50 \mu m \times 50 \mu m$, 3-layer freestanding membrane. Fig. 2.1a shows the three key parts of the structure. There is a cylindrical opening through the top SiN layer (cyan), a disk-shaped cavity in the middle layer, made of SiO$_2$ (magenta), and a nanopore through the bottom, 20 nm-thin layer, made of SiN. The cylindrical opening was typically $\sim 200$ nm in diameter, however that dimension was varied for studies reported in Chapter 3. The cavity was typically $\sim 2 \mu m$ in diameter, and between 200 nm and 1.5 $\mu m$ in height. The typical diameter of the nanopore was $\sim 10$ nm. The membrane in Fig. 2.1a was suspended over a 5 mm $\times$ 5 mm Si chip as shown in Fig. 2.1b.

Fig. 2.1c shows a transmission electron microscopy (TEM, JEOL 2100F) image of the device. The wide grey disk is the cavity. The bright circle in the center is the 200 nm-diameter opening. The inset shows a magnification of the nanopore. Fig. 2.1d shows an optical microscopy image of the device, showing the square base of
Figure 2.1: (a) 3-D rendering of the nanopore/cage structure. (b) The structure of the chip. (c) TEM image of the cavity and nanopore in the membrane. (d) Image of the device taken by an optical microscope at 10× magnification. (e) Image of the whole chip.
the mesa in the silicon chip, whose side length is 610 µm, which leads to the square, 3-layer membrane. Fig. 2.1 is an image of the entire chip.

2.1.2 The deposition of multiple thin films on silicon substrate

The nanopore/cage devices were fabricated in multi-layer free-standing membranes on silicon substrate. In this subsection, we describe the preparation of those coated silicon wafers. Fig. 2.2 illustrates the sequence deposition steps. All deposition procedures were performed at the Center for Nanoscale Systems at Harvard University.

We started with a 4 inch-diameter, 400 µm-thick, (100) orientation, phosphorus-doped N type Si wafer. We first cleaned the wafer by following a standard procedure called the RCA clean. We put the wafer into a 7:2:2 solution of deionized water (DI):ammonium hydroxide (NH₄OH):hydrogen peroxide (H₂O₂), commonly known as RCA-1, at 70°C for 10 mins. This step removed the ionic and heavy metal atomic contaminants. We then put the wafer in a 7:2:2 solution of DI:hydrochloric acid (HCl):hydrogen peroxide (H₂O₂), commonly known as RCA-2, at 70°C for 10 mins. This step cleaned the insoluble organic contaminants. This step also oxidizes the Si surface slightly, producing a ~5 nm SiO₂ layer. We next dipped the wafer in 100× diluted HF aqueous solution for 30 s to etch away the ~5 nm thin SiO₂ layer. This step leaves the silicon surface hydrophobic. We rinsed the wafer with DI water after each step to rinse away the cleaning chemicals.

We deposited a 400 nm thick layer of low-stress SiN on both sides of the wafer by low-pressure (< 200 MPa) chemical vapor deposition (LPCVD), as shown in Fig. 2.2b. We used a Tystar Mini Tytan 4600 to deposit the SiN at 825°C by the
Figure 2.2: Deposition process of multi-layer film on Si wafer. (a) The raw Si wafer. (b) 400 nm SiN layer was deposited on both sides of the wafer by LPCVD. (c) SiO$_2$ layer was deposited on one side by PECVD. (d) Another 20 nm-thick SiN layer was deposited.
The pressure in the chamber was 200 mTorr [36]. This low pressure tends to reduce unwanted gas-phase reactions and improve film uniformity across the wafer. The intended film thickness was 400 nm. Since the deposition rate tended to drift over time, we needed to adjust the deposition time accordingly. We found it useful to refer to the logged deposition rates from previous users of the instrument to achieve the most accurate deposition results. The average growth rate was 5.32 ± nm/min. The uniformity of the thickness was ~1.5% across a 4 inch-diameter wafer. X-ray photoelectron spectroscopy (XPS) showed the elemental composition of the layer to be Si$_3$N$_2$.4.

The middle SiO$_2$ layer was next deposited on one side of the wafer by plasma-enhanced chemical vapor deposition (PECVD), as shown in Fig. 2.2c. We used a Surface Technology Systems Chemical Vapor Deposition (STS PECVD) [36] tool to perform the SiO$_2$ deposition by the reaction

$$3 \text{SiH}_4 + 6 \text{N}_2\text{O} \rightarrow 3 \text{SiO}_2 + 4 \text{NH}_3 + 4 \text{N}_2.$$ 

The precursor gasses flowed into the chamber were silane (SiH$_4$) at 12 sccm, nitrous oxide (N$_2$O) at 1420 sccm, and nitrogen (N$_2$) at 392 sccm. The pressure in the chamber was kept at 550 mTorr. The RF power was 60 W and delivered at low frequency (380 kHz). The temperature was 300°C at the plate that holds the wafer and 250°C at the lid above the wafer. The deposition rate was 79 nm/min. We adjusted the deposition time to make devices with 200 nm, 400 nm, 800 nm, and 1.5 µm layers for different experiments.
We annealed the SiO$_2$ layer before depositing the outer 20 nm-thick layer of SiN. A Mini Tytan 4600 furnace was used to heat the wafer to 800°C overnight. This step was designed to remove residual H$_2$ and make the SiO$_2$ layer denser, while avoiding cracking of the outer SiN layer. After the annealing process, we followed the same SiN deposition process as before to deposit 20 nm of SiN, shown in Fig. 2.21.

We measured the thickness of each layer after each deposition step using an ellipsometer (WVASE32, Woollam Spectroscopic Ellipsometer) [36]. The ellipsometer directs a laser beam, which may be decomposed into an $s$ and a $p$ component, on the wafer. The $s$ component oscillates perpendicular to the plane of incidence and parallel to the sample surface, while the $p$ component oscillates parallel to the plane of incidence. The amplitudes of the $s$ and $p$ components, after reflection and normalized to their initial value, are denoted by $r_s$ and $r_p$, respectively. The complex reflectance ratio, $\rho$, is calculated by

$$\rho = \frac{r_p}{r_s} = \tan(\Psi)e^{i\Delta},$$

(2.1)

where $\tan(\Psi)$ is the amplitude ratio upon reflection, and $\Delta$ is the phase shift. $\Psi$ and $\Delta$ are related to the refractive index and thickness of the individual layers of the sample and to their sequence. The instrument’s software calculated the thickness of the film from the Fresnel equations [37].

After completing the deposition process, we coated both surfaces with photoresist to avoid contaminating them while transporting the wafer to other labs for subsequent work. We cut the wafer into 30 mm $\times$ 30 mm squares using an automatic dicing saw (Disco, DAD-321).
2.1.3 Creating nanopore/cage devices

Fig. 2.3 illustrates the fabrication procedures we followed to make the nanopore/cage devices, starting with the 30 mm × 30 mm chips.

We first put a chip in acetone, then 2-propanol, and then DI water for 3 min each while sonicating to remove the protective photoresist. We next covered the surface with S1818, a positive photoresist, by spinning (with The Cee Model 100) at 3000 rpm for 30 s (Fig. 2.3b). We patterned the chip using a chromium mask and a Karl Suss MJB-3 Mask Aligner, which exposed the chip to UV light with a wavelength of 402 nm. The UV light breaks down the long chain molecules of the photoresist under the transparent parts of the mask. We developed the chip in 1:1 solution of MF312:DI water for 1 min, which dissolved away the broken-down areas of photoresist and left the surface coating with a profile like the one illustrated in Fig. 2.3c. We then etched away the exposed SiN layer in a reactive plasma of CF$_4$ gas [38] using a plasmatherm machine (Model 790 RIE-PECVD). Fig. 2.3d shows how the photoresist protected parts of the SiN layer from etching. After the reactive ion etch, we cleaned the chip again with acetone, 2-propanol, and DI water, in sequence.

We placed the chip in 40% KOH at 70°C for ~12 hours to etch the exposed Si along the (111) crystal planes [39], which resulted in a pyramid shape, as shown in Fig. 2.3e. We confirmed that the etch had terminated at the 3-layer membrane on the opposite surface by looking through the membrane. The 3-layer membrane is transparent to visible light. Fig. 2.3f shows the chip after KOH etching. We also checked the membrane under an optical microscope to make sure the membrane was clean and uniform (Fig. 2.1d).

We used a focused ion beam (FIB, FIE Helios) to mill a circular opening through
Figure 2.3: (a)-(h) Procedure for fabricating the opening-cavity-nanopore structure. (i) The picture of the chip after KOH etching. (j) A pattern of FIB drilling holes during the calibration process of the FIB drilling rate. (k) The image of the cavity taken by an optical microscope at 100× magnification. (l) The TEM image of the nanopore.
the 400 nm SiN layer and partially into the SiO$_2$ layer, as shown in Fig. 2.3f. The diameter of that opening was typically 200 nm, but could be easily enlarged. To ensure the FIB would stop milling in the SiO$_2$ layer, we calibrated the milling rate as shown in Fig. 2.3j. We milled a series of holes using an arithmetically increasing dose of ions. After milling that test array, we checked the chip using TEM. A comparison of the TEM and FIB images showed that a bright disk observed with the FIB indicated that we had completely milled through the membrane. We calculated the FIB milling rate using the threshold dose for milling through the full 3-layer membrane, and the total membrane thickness, and assuming that the milling rate for SiO$_2$ and SiN were the same.

We created a cylindrical cavity in the SiO$_2$ layer by immersing the device in buffered HF, as shown in Fig. 2.3g. The buffered HF selectively etched SiO$_2$ at the rate of ~100 nm/min. The etching time was selected to achieve a cavity of the desired diameter. We stopped the etch by rinsing the device in DI water. We then checked the device under a microscope to make sure the FIB drilling and the buffered HF etching had been successful, as shown in Fig. 2.3k. The pink circle in the center of that image indicates a successfully etched cavity. We occasionally observed no pink disk in the centre because the FIB had not drilled through the SiN layer, which left the SiO$_2$ layer protected by SiN from buffered HF etching. The buffered HF etched SiN layer very slowly, at a rate of only ~1 nm/min.

We drilled a nanopore at the bottom SiN layer by TEM, as shown in Fig. 2.3h. We condensed the electron beam down to a spot in a few nanometers in diameter. The electron beam imports enough energy to remove the material from the membrane, and prolonged irradiation eventually leads to the formation of a hole in the 20 nm SiN film. Fig. 2.3 shows the TEM image of the nanopore.
2.2 DNA Translocation Experiments

In this section, we briefly describe the procedures we followed to prepare our nanopore/cage structures for sensing DNA in translocation experiments.

We first cleaned a nanopore device in Nanostrip (Cytantek corporation) at 75°C for two hours. We mounted the device in a home-made testing cell (Fig. 2.4a), where the chip separated two reservoirs of buffered ionic solution. We designate the reservoir leading to the nanopore as the ‘nanopore side’, and the reservoir leading to the opening of the cavity as the ‘opening side’. The only channel connecting the two reservoirs was through the nanopore, the cavity, and the opening. We injected solutions into the reservoirs using a syringe and plastic tubing. The reservoirs were separately contacted by Ag/AgCl electrodes, connected to an electrometer (Axon Axopatch 200B). Fig. 2.4b shows the signal path. The electrometer biased the opening side to a voltage \( V \) relative to the nanopore side, and measured the resulting current, \( I \). The current was digitally sampled by a digitizer (Digidata 1440A, Axon Instruments) at 250 kHz after passing through an analog, 10 kHz, low-pass Bessel filter (Stanford Research Systems). We used a field-programmable gate array (FPGA) card to enable real-time control over \( V \) in response to the ionic current signal or pre-programmed delays. The whole system was controlled by a desktop computer. Prior to a DNA sensing experiment, the conductance of each device was tested in the presence of 1 M KCl buffer to ensure that the standard deviation of the baseline current was no greater than 0.01 nA with \( V = 100 \text{ mV} \). We annealed the DNA at 65°C for 10 min and then quickly cooled to room temperature in order to separate the complementary overhanging ends of \( \lambda \) DNA and thereby avoid concatenated or circular molecules.
Figure 2.4: (a) Chip holder used to perform DNA translocation experiments. (b) Schematic of the measurement and control systems used for DNA sensing experiments. The arrow between different equipments indicates the signal transmission direction.
2.3 Fluorescence Imaging Setup

In this section, we describe our procedures for imaging single DNA molecules that we trapped inside the cage, as described in Chapter 4.

Fig. 2.5 illustrates our fluorescence microscopy setup for observing fluorescently labelled DNA molecules trapped inside a device. After completing a DNA trapping experiment, we removed the nanopore chip from its fluidic chuck, rinsed it in DI water to wash away any DNA stuck to the membrane, placed the chip on a microscope cover glass with the opening side facing up, added a small drop of YOYO-1 solution (Life technologies, Y3601, Excitation/Emission (nm):491/509 ) to the opening side, and then placed another cover glass on top to prevent evaporation. We mounted the chip, sandwiched between cover glasses, on an inverted microscope (Nikon, ECLIPSE TE2000-U) equipped with a 100×, oil-immersion objective lens (Nikon, CFI Apo TIRF), an ultraviolet light source (EXFO X-Cite 120), a fluorescence imaging filter set (C125492, Chroma Technology Corp), and an EMCCD camera (Andor, iXon). Fig. 2.5a is a schematic of the system which indicates the components. Fluorescence images were recorded every 10 s using a 50 ms exposure time and a mechanical shutter that minimized photobleaching. Fig. 2.5b shows the stage used for holding the nanopore chip above the objective. Fig. 2.5c shows the spectrum of the filter set, which matched the YOYO-1 excitation requirement and our light source.
Figure 2.5: (a) Schematic of the fluorescence microscopy setup. (b) An image, showing the copper sample holder with the chip in the centre, and the transparent cover glass covering the chip. (c) The spectrum of the fluorescence filter set.
Chapter Three

Probing the Influence of Coil Configuration on DNA Translocation Dynamics in Solid-State Nanopores
In Chapter 1, we described the importance of the translocation speed in nanopore experiments, and how its dependence on the configuration of DNA at the onset of translocation has yet to be directly verified in an experiment. In this Chapter, we report experiments that probe the leading theoretical models directly. Fig. 3.1 illustrates our approach for controlling the initial configuration of DNA during translocation and probing the resulting translocation speed. It shows the configuration of a DNA molecule as it begins to translocate the nanopore from the opening side of a nanopore/cage device. The bulk of the DNA coil remains outside the opening, away from the nanopore. The basic idea we seek to exploit is that the cavity-opening structure creates a well defined gap between the DNA coil and the nanopore. As a result, the length of the moving segment of a DNA molecule can be increased compared with translocations of a conventional nanopore in a single, flat membrane. We anticipate this will cause the average translocation speed, $v$, to decrease according to the theoretical picture put forward by Grosberg [26], Lu [28], and Ikonen [27].

Figure 3.1: The use of asymmetrical nanopore structures in DNA translocation experiments. (a) A cut-out 3D sketch shows the DNA coil on the opening side as it begins to translocate the nanopore. (b) TEM images of a device.
We used devices with a 400 nm thick SiO$_2$ layer for these experiments, which when added to the 400 nm inner layer of SiN, resulted in a gap of 800 nm between the nanopore and the plane of the cavity opening. We could further control the length of the moving segment by adjusting the diameter of the opening. Widening the opening allows the DNA coil to descend into the cavity, closer to the nanopore. Fig. 3.1b shows the TEM image of a typical device. The bright disk in the centre is the 200 nm diameter opening that was drilled by FIB. The 2.3 $\mu$m wide dark grey disk is the cavity. The zoomed-in inset shows the nanopore in the thin SiN membrane. The smallest opening diameter in our devices was $D = 200$ nm, which was more than 6 times smaller than the coil diameter, $2R_g = 1.4 \mu$m [40]. Since the area of the opening was $\sim 400$ times larger than the area of nanopore, the voltage drop across the opening and the cavity was negligible. The widest opening tested was $D = 2 \mu$m.

### 3.1 Experimental results

We performed translocation experiments using our asymmetrical nanopore/cage devices. We added 12.5 $\mu$g/ml $\lambda$DNA in each reservoir of ionic solution (1 M KCl, 10 mM Tris, 1mM EDTA, pH=8). We first applied a positive voltage, $V$, to drive the DNA through the nanopore and into the cavity. The presence of a DNA molecule inside the nanopore caused a measurable decrease in the ionic current [10]. We recorded such translocation events with positive $V$ for 10 minutes before flipping the sign of the voltage to drive DNA molecules through the device in the opposite direction. We recorded such translocations from the opening side for an additional 10 minutes while maintaining the negative $V$. Fig. 3.2 presents typical current traces from translocation events in either direction. That figure also illustrates possible configurations of the DNA at the onset of each translocation. Fig. 3.2a (left) shows
Figure 3.2: DNA translocating the asymmetric nanopore in opposite directions. (a) A sketch showing the configurations of a DNA molecule as it begins translocating the asymmetric nanopore device in opposite directions. The cage structure keeps the coil away from the nanopore during translocation. (b) Corresponding current traces from typical translocations.
a DNA configuration as it begins to translocate the nanopore from the nanopore side, with its coil docked next to the nanopore. Fig. 3.2a (right) shows a DNA configuration as it begins to translocate the nanopore from the opening side, with its coil kept away from the nanopore by the opening and the cavity. Fig. 3.2b shows that the DNA took a longer time to translocate the nanopore from the opening side than from the nanopore side. We also noticed that DNA blocked slightly more current when it translocated from the nanopore side than from the opening side.

We identified translocation events in the raw data using a custom Matlab program, which classified events with a current change of at least $\Delta I = 0.08 \text{nA}$ and lasting at least 300 ps as translocations. For each translocation, we studied the event charge deficit (ECD) \cite{12}, which is the integrated current blockage over time,

$$ECD = \int_{\text{event}} \Delta I(t) dt.$$  \hspace{1cm} (3.1)

ECD corresponds to the area under a translocation event, as shown in Fig. 3.2b (right).

Fig. 3.3a compares the normalized distribution of ECDs for $\lambda$DNA molecules translocating a device in either direction for devices with opening diameters of 200 nm (top) and 680 nm (bottom) and with $|V| = 100 \text{mV}$. We found two peaks in every ECD distribution. The smaller peak was always centered around $ECD=0.1 \text{pC}$. We attribute these events to translocations by DNA fragments \cite{16} and exclude them from further analysis. The larger peaks were always observed at an ECD value above 0.1 pC, however the precise location depended strongly on the size of the device opening and on the direction of translocation. We attribute the events in the larger peaks to translocations by intact $\lambda$DNA molecules. The systematic variation in the ECD values reflects real differences in the translocation dynamics.
Figure 3.3: (a) Normalized ECD distributions for devices with a 200 nm diameter opening (top) and a 680 nm diameter opening (bottom) with $V = 100 \text{mV}$. White bars indicate translocations from the nanopore side. Grey bars indicate translocations from the opening side. There were 327 translocations from nanopore side and 400 from opening side for the device with $D = 200 \text{nm}$. There were 2457 translocations from the nanopore side and 293 from the opening side for the device with $D = 680 \text{nm}$. The curves are Gaussian fits of the major peaks. The red curve is for translocations from nanopore side. The black curve is for translocations from the cavity side. (b) A sketch showing how devices with different opening diameters, $D$, extended the DNA moving length differently during translocation. (c) Normalized distributions of $\Delta I$ samples for the same experiments as in (b). (d) The dependence of $\Delta I_o/\Delta I_n$ (black, left axis), and $I_o/I_n$ (red, right axis), on the opening diameter. The error bars are similar the symbol size.
In the device with the 200 nm diameter opening, translocations from nanopore side and from the opening side resulted in peaks in the ECD distributions centered at ECD=0.28 pC and ECD=0.52 pC, respectively. The translocation experiments using the device with \( D = 680 \) nm revealed the effect of allowing the coil to descend significantly into the cavity, as illustrated in Fig. (3.3b). The major peak in the ECD distribution remained near ECD=0.24 pC for translocations from the nanopore side, where it was observed in the device with \( D = 200 \) nm. On the other hand, the peak shifted back significantly to around ECD=0.3 pC for translocations from the opening side. For each device and each translocation direction, we fitted a Gaussian to the major peak in the ECD distribution. We obtained \( ECD \), the most probable value of the ECD, from these Gaussian fits.

Fig. (3.3c) shows the normalized distributions of \( \Delta I \) samples for the same two devices for translocations in either direction. \( \Delta I = 0 \) corresponds to current flowing through an open pore. The peaks at higher values of \( \Delta I \) in each figure were caused by DNA molecules translocating the nanopore. The first such peak corresponds to a single DNA molecule threading the nanopore in a unfolded configuration. The peaks at higher \( \Delta I \) values were caused by DNA translocating the nanopore in folded configurations. We used a Gaussian function to fit the peaks in the \( \Delta I \) distributions. The value we obtained for the center of the first peak, which was the highest peak, corresponds to the most probable current blockage caused by an unfolded DNA translocation, \( \Delta I \). We further distinguish the \( \Delta I \) induced by translocations from opening side and from the nanopore side with \( \Delta I_o \) and \( \Delta I_n \), respectively.

Fig. (3.3d) plots the dependence of \( \Delta I_o/\Delta I_n \), the ratio of current changes induced by translocations from the opening side and from the nanopore side, on \( D \), for the 16 different devices tested. Surprisingly, a DNA molecule typically blocked more current when it translocated from the nanopore side than when it translocated from
the opening side, \( i.e. \Delta I_o < \Delta I_n \). That asymmetry in the current blockage was not due to an asymmetry in the conductance of the nanopore, as we verified by measuring the baseline current in either direction, \( I_o \) and \( I_n \). Fig. 3.3d plots the dependence of \( I_o/I_n \) on \( D \). No directional bias was observed.

Figure 3.4: Dependence of the average translocation velocity, \( \nu \), normalized by \( \nu \) for translocations from the nanopore side with \( V = 100 \text{mV} \), on the magnification of the applied voltage. Experimental data are presented for devices with opening diameters of \( D = 200 \text{nm} \) (diamonds) and \( D = 680 \text{nm} \) (circles). The lines are linear fits to the experimental data. The red line and data indicate translocations from the nanopore side. The black line and data indicate translocations from opening side.

We are interested in how the average translocation speed, \( \nu \), depends on the moving length of the molecule. To measure the average translocation speed, it is necessary to account for the fact that DNA molecules often translocate in folded
configurations, and consequently complete the translocation in a shorter time than if they had translocated linearly from end to end with the same average speed. We therefore applied a procedure to mathematically unfold the molecules and thereby infer the unfolded translocation time \( \tau \). We obtain

\[
\tau = \frac{ECD}{\Delta I},
\]

(3.2)

where the \( ECD \) is the most probable value of the ECD found by Gaussian fits of the larger peaks in Fig. 3.3a and \( \Delta I \) is the most probable value of the current blockage obtained by Gaussian fits to the first blockage peak in the distributions of current samples. We obtain the average translocation speed from

\[
v = \frac{L}{\tau},
\]

(3.3)

where \( L = 16.5 \mu m \) is the molecule’s contour length and \( \tau \) is obtained from equation 3.2. We denote \( v_n \) and \( v_o \) for translocations from the nanopore side and the opening side, respectively.

We estimated the error in \( v \) using a bootstrapping method \[42\]. We first divided each experimental dataset into 5 subsets. We used the method described above to find \( ECD \) and \( \Delta I \) for each subset. We then took the errors in the \( ECD \) and \( \Delta I \) to be the standard deviations for the 5 corresponding subsets of data. The error in \( v \) is obtained as propagating the errors from \( ECD \) and \( \Delta I \), assuming they are uncorrelated.

Fig. 3.4 shows the dependence of \( v \) on \( V \) for devices with \( D = 200 \) nm and \( D = 680 \) nm. We normalized \( v \) by its value at \( V = 100 \) mV when the DNA translocated from the nanopore side to account for the systematic variations between different
devices, such as the precise thickness of nanopore membrane \[43\]. \(v_o\) and \(v_n\) increased linearly with \(V\) for both devices. In all cases, translocations proceeded more slowly from the opening side than from the nanopore side, \(i.e. v_o < v_n\) for the same \(V\). The device with \(D = 200\) nm showed a larger difference between \(v_o\) and \(v_n\) than the device with \(D = 680\) nm.

**Figure 3.5:** Dependence of the DNA mobility ratio, \(v_o/v_n\), on the opening diameter for \(|V| = 100\) mV. The error in \(D\) arose from the blur in the TEM image and the asymmetry of the opening circle. The dashed curve is the prediction of the simple theoretical model described in the text. Each data points in Fig. 3.4 and this figure were calculated by at least 70 events and at most 2994 events, with 806 events on average.

Fig. 3.5 shows the dependence of mobility ratio, \(v_o/v_n\), on \(D\) for all 16 devices tested. We performed the bi-directional translocation experiments with \(V = 100\) mV using devices with \(D\) ranging from 200 nm to 2000 nm. We observed the smallest
value of \( \frac{v_o}{v_n} \approx 0.4 \), corresponding to the largest asymmetry in the translocation speed, in a device with \( D = 200 \text{ nm} \), the smallest opening tested. The ratio \( \frac{v_o}{v_n} \) rose as \( D \) increased. The rise was rapid at first, and then it gradually asymptoted as \( D \) grew beyond about 1 \( \mu \text{m} \) in diameter. We found the highest value of \( \frac{v_o}{v_n} = 0.83 \), corresponding to the lowest asymmetry in the translocation speed, when \( D = 2 \mu \text{m} \).

### 3.2 Modelling the viscous drag during translocation

In this section, we develop a physical model to explain the slowing of the DNA translocation speed caused by the presence of a cavity with a narrow opening. The slowing occurs when DNA translocates from the opening side.

**Figure 3.6:** Modelling the viscous drag during translocation. (a) The driving force, \( \vec{F}_{\text{drive}} \), is balanced by the drag inside the nanopore, \( \vec{F}_{\text{in}} \), and the drag outside the nanopore, \( \vec{F}_{\text{out}} \). (b) The geometry of the DNA descending into the cavity by a hard sphere model.

Fig. 3.6a illustrates driving force and the drag forces acting on a DNA molecule.
during translocation. The driving force, $\vec{F}_{\text{drive}}$, is the electric force inside the nanopore \cite{13}, which is linearly proportional to the voltage drop across the nanopore, i.e. $\vec{F}_{\text{drive}} = \alpha V$, where $\alpha$ is a constant. We divided the viscous drag force into two parts, the force acting on DNA inside nanopore, $\vec{F}_{\text{in}}$, and the force on DNA outside the nanopore, $\vec{F}_{\text{out}}$. We modelled $\vec{F}_{\text{in}}$ as the Stokes drag on a cylinder moving through a viscous fluid inside another cylinder \cite{44}. We modelled $\vec{F}_{\text{out}}$ as a cylinder moving in the axial direction in an unbounded viscous fluid \cite{45}.

Happel’s method \cite{46} was used to derive the viscous drag on a cylinder moving in a larger cylinder, which obtained the following expression for the Stokes drag per unit length, $F'_{\text{in}}$ \cite{44},

$$
\frac{F'_{\text{in}}}{2\pi \mu v} = \frac{\sigma^2 - 3 + 4 \frac{\ln(\sigma)}{\sigma^2 - 1}}{(\sigma^2 + 1)\ln(\sigma) - (\sigma^2 - 1)},
$$

where $\mu$ is the mobility of DNA in free solution, $v$ is the translocation speed of DNA molecule, the dimensionless parameter $\sigma = \frac{R}{r}$ is the ratio of the outer cylinder’s radius, $R$, and the smaller cylinder’s radius, $r$. The expression we derived in Eq. 3.4 differs from Roger’s result \cite{44} by the last term in denominator. In Roger’s paper, this term is 1 instead of $\sigma^2 - 1$. Roger’s result is clearly erroneous because the denominator becomes zero when $\sigma$ reaches some value between 1 and 2, i.e. $(\sigma^2 + 1)\ln(\sigma) - 1 = 0$, which is not physical.

In our problem, $r = 1$ nm is the radius of the DNA strand and $R = 10$ nm is the nanopore radius. With these values, the right side of Eq. 3.4 becomes 0.73, which we treated as a constant for all devices. The logarithm of $\sigma = \frac{R}{r}$ changes very slowly as the size of the nanopore varies, so this approximation is reasonable.

We followed Burger’s derivation \cite{45} to calculate the viscous drag on a cylinder
moving in the axial direction through a viscous fluid without boundary. We obtained
Stokes drag per unit length, $F'_{out}$,

$$
\frac{F'_{out}}{2\pi \mu v} = \frac{1}{\ln \left( \frac{l}{r} \right) - 0.72},
$$

(3.5)

where $l$ is the length of the cylinder. We set $l = R_g = 0.73 \mu m$ and $r = 1 \text{ nm}$ for our
problem. With these values, the right side of Eq. 3.5 becomes 0.17, which we take
to be a constant for the same reason as before.

Inserting these calculated constants into Eqs. 3.4 and 3.5 yields the following
expression for the total viscous drag force, $F_{drag}$,

$$
F_{drag} = 2\pi \mu v (0.73 l_{in} + 0.17 l_{out}).
$$

(3.6)

Balancing the driving force with the total drag force, we obtain the expression
for the translocation velocity,

$$
v = \frac{1}{2\pi \mu} \cdot \alpha \cdot \frac{V}{0.73 l_{in} + 0.17 l_{out}},
$$

(3.7)

where $l_{in} = 20 \text{ nm}$ is the length of DNA inside the nanopore and $l_{out}$ is the moving
length of DNA outside the nanopore.

Eq. 3.7 correctly predicts a linear relationship between $v$ and $V$, as shown in
Fig. 3.4 In addition, Eq. 3.7 gives the translocation velocity as a function of the
length of the moving segment of DNA outside the nanopore, which we could control
in experiments with the asymmetric nanopore/cage devices. We modelled the DNA
coil as a sphere with radius $R_g$ which did not shrink during the translocation, as
Fig. 3.6b shows. We justify this by considering the Zimm time [47], $\tau_{zimm} = 170 \text{ ms}$
for λDNA in water at room temperature, which is the time for the molecule to fully relax to its equilibrium conformation. Translocations from the nanopore side, which typically last ~2 ms, are too fast to allow the coil to relax and shrink in size. For translocations from the opening side, we estimated the time for the end of the DNA entering the opening to arrive at the nanopore, \( t_e \), by calculating the transit time for a particle with the same mobility as DNA to drift in the electric fields from outside the opening to the nanopore. We obtained \( t_e \sim 25 \) ms, which is ~7 times smaller than \( \tau_{zimm} \). Furthermore, as soon as the end of the molecule arrives at the nanopore, the molecule translocates within 4 ms. These short times indicate that the coil should not shrink significantly before completion of the translocation.

According to Grosberg’s theoretical model [26], the segment of the DNA molecule that is closest to the nanopore is driven towards the nanopore while the other segments of the molecule remain dormant and consequently experience little drag. The characteristic length of the moving segment is \( R_g \). After the first extended DNA segment has translocated, the second segment starts to move towards the nanopore, and the process repeats until all the segments have translocated. In each case, the characteristic moving length is expected to be \( R_g \) for translocations from the nanopore side. We expect the characteristic moving length for translocations from the opening side to be longer by an amount equal to the distance that the coil was kept away by the cavity.

The sketch in Fig. 3.2a indicates the moving segment in red. For translocations from the nanopore side, \( l_{\text{out}}^n = 0.73 \) µm. For translocation from the opening side, we treated the DNA coil as a hard sphere that descended into the cavity as far as it was allowed by the size of the circular opening, as shown in Fig. 3.6b. According to the picture, the moving length increases to \( l_{\text{out}}^o = 800 \) nm + \( \sqrt{R_g^2 - (D/2)^2} \), where 800 nm was the distance between the nanopore and the plane of the opening.
The asymmetry in the translocation speed, $v_o/v_n$, predicted by our model, is

$$\frac{v_o}{v_n} = \frac{0.73 l_m + 0.17 l_{out}^o}{0.73 l_m + 0.17 l_{out}^n} \quad (3.8)$$

The dashed line in Fig. 3.5 plots the asymmetry predicted by Eq. (3.8). In the limit of small opening diameter, $v_o$ approaches about half $v_n$, reflecting the fact that the moving length is approximately double. As the opening widens, $v_o$ rises toward $v_n$, and the two are predicted to be equal when $D = 2R_g$ and the coil can fit all the way into the opening. Our hard sphere model did not perfectly follow the experimental results, however, since the coil was in dynamic equilibrium rather than being a hard sphere. The experimental results of $v_o/v_n$ never increased to 1, possibly because the molecule must elongate before translocation due to the cage structure, which increased the moving length.

We performed experiments on devices with the thicker SiO$_2$ layers. The thickness tested were 800 nm and 1.5 µm, which we hoped would control the moving length. The results we obtained could not be explained by our current model. In some experiments, the ECD distribution for translocations from the opening side showed a broad spread between 0 pC and $\sim 10$ pC, without forming a distinct peak. Other experiments resulted in events that all had ECD $< 0.1$ pC for translocations from the opening side.

We calculated the electrophoretic drift time for a DNA molecule to enter the opening and arrive at the nanopore to be $t_e = 90$ ms and 330 ms for devices with SiO$_2$ thicknesses of 800 nm and 1.5 µm, respectively. These times are comparable to $\tau_{zimm}$, which means our assumption of fast translocation processes does not apply. This long $t_e$ allows the DNA molecules explore more configurations as they make their way to and through the nanopore. For example, some molecules might remain largely
outside the opening when one end finds the nanopore and begins to translocation, which would result in a long moving length and correspondingly large ECD. Other molecules might find their way into the cavity and adopt a compact configuration before starting to translocate, which would result in a low ECD. It would also be possible to find configurations that are intermediate between the extremes, which would explain the wide distribution in ECD. DNA translocations through these thick devices are therefore different from the picture we proposed in Fig. 3.1. Since thick devices could not reliably maintain a gap between the DNA coil and the nanopore at the onset of translocation, we could not use these experiments to test Grosberg’s dynamical theory.

3.3 Summary

The asymmetric translocation dynamics by long DNA polymers across nanopore/cage devices reveal that the DNA molecule’s configuration outside of the nanopore plays an important role in determining $v$. We tested the model of Grosberg [26], which assumes that the moving segment of the molecule is the dominant contribution to the drag during translocation, by showing the $v$ decreased due to the cage. We also showed that the asymmetry in $v$ due to the direction of translocation diminishes as the diameter of the opening increases, reflecting the ability of the DNA molecule to approach closer to the nanopore. These experiments provide a new way to physically control the speed of translocation.
An Entropic Cage for Trapping DNA Near a Nanopore
Nanopores are nanometre-sized holes in thin insulating membranes that serve as sensors for the analysis of single DNA molecules \[3,4,6,8,16,18,22,48,50\]. In a typical experiment, the membrane separates two reservoirs of ionic solution and a voltage bias \(V\) is applied between the reservoirs. The resulting ionic current through the nanopore \(I\) is the basis of the sensing mechanism. The voltage bias also drives DNA molecules through the nanopore by electrophoresis \[13,15\], as shown in Fig. 1.3, and the presence of a molecule inside the nanopore causes a measurable change in \(I\). The goal of the analysis is always to infer the structure of a molecule from the time course of \(I\) \[6,49,50\]. While solid-state nanopores have yet to show sensitivity to the different DNA bases, like their biological counterparts, they can be used in combination with chemical probes that induce detectable modifications of the structure of a molecule based on its sequence. For example, oligonucleotide hybridization probes \[32\] and certain proteins \[51,52\] will bind to a specific DNA target sequence and create bulges that block additional ionic current when they pass through the nanopore. Using this principle, Singer et al. \[21\] mapped binding locations of peptide nucleic acid oligomers along double-stranded DNA, as shown in Fig. 1.7a, and Wanunu et al. \[20\] identified microRNAs by detecting their binding to oligomeric sequence probes, as shown in Fig. 1.7b.

Many other biochemical strategies can easily be envisioned. In each case, however, it is crucial for the correct interpretation of the results to know the structure of the DNA molecule before its interaction with the chemical probe. Nanopores should ideally reveal molecular characteristics without prior knowledge. The solution is to use the nanopore to characterize the same DNA molecule before and after it interacts with the chemical probe, and for that purpose, nanopores need to be coupled with single-molecule test tubes in which DNA molecules can react without diffusing away.

Here we report the use of our newly-designed devices that juxtapose a solid-state
nanopore with a cage for trapping a single DNA molecule [53, 55]. The cage traps DNA entropically [33], as shown in Fig.4.1a: there is an entropic cost to squeezing a large DNA coil through the opening in a distorted configuration which prevents the molecule from escaping when its radius of gyration is larger than the opening [34, 35]. Small molecules like hybridization probes and restriction enzymes can nevertheless diffuse through the opening without difficulty. We present experimental evidence that single DNA molecules can be stably trapped in a cage and recovered with nearly perfect efficiency. Finally, we used a restriction endonuclease to cut DNA [56] held in the cage and detected the resulting fragments to demonstrate that a nanopore can analyse DNA before and after a chemical reaction.

4.1 Nanopore-cage structures and experiments.

Fig. 4.1a illustrates the nanopore-cage structure and Fig.4.1b shows transmission electron microscope (TEM) images of a typical one. The detailed nano-fabrication procedure was described in Chapter 2. For these experiments, we used devices with a 1.5 \( \mu \)m-thick \( \text{SiO}_2 \) layer. The cavity was large enough to accommodate a \( \lambda \) DNA molecule, which is 16.5 \( \mu \)m long [13] and has a radius of gyration that is \( R_g = 730 \) nm [40] in equilibrium.

In our DNA sensing experiments, the chip separated two reservoirs of buffered ionic solution (1 M KCl, 10 mM Tris, 1 mM EDTA, at pH=8), one contacting the opening of the cavity (the ‘opening side’), and the other contacting the nanopore and containing 1.25 \( \mu \)g/mL \( \lambda \)DNA (the ‘nanopore side’). The filtered output of the current amplifier was simultaneously monitored by a digitizer (Digidata) and by a FPGA (NI PCIe-6251) running a custom LabView program. The FPGA actively
controlled $V$ by means of an analog voltage command line to the electrometer.

### 4.2 Dynamics of DNA in an entropic cage.

We investigated the dynamics of $\lambda$ DNA molecules in the entropic cage with ‘ping-pause-pong’ experiments, illustrated in Fig. 4.2a. A current trace from a typical experiment is shown in Fig. 4.2b. The application of $V = 100 \text{ mV}$ resulted in an open-pore current of 9.73 nA. When a DNA molecule translocated the nanopore into the cage, it caused a transient decrease in $I$ \cite{4}. We call this first translocation signal the ‘ping’. We maintained the positive voltage bias for 2 ms following the ping. The voltage bias was then removed for a pause time, $t_p$, during which the molecule could relax and diffuse within the cavity. We varied $t_p$ in experiments between 2 ms and 50 s. Following the pause, we flipped the voltage to $V = -100 \text{ mV}$ for 5 s. We usually observed another transient conductance drop, which we call the ‘pong’ and attribute to the same DNA molecule returning through the nanopore. The interval of time
between the voltage reversal and the pong is \( t_r \), the recapture time.

For each translocation, we computed the event charge deficit (ECD), which is the induced current change integrated over the duration of the event. Previous studies showed that ECD is primarily related to the length of a DNA molecule and not the folding configuration in which it translocates \[10, 12\]. Fig.4.3a shows a scatter plot indicating the ECD of each ping and \( t_r \) of the corresponding pong for \( t_p = 30 \text{ ms} \). Most of the data clustered in a major group centered around \( \text{ECD} = 0.4 \text{ pC} \) and \( t_r = 20 \text{ ms} \). A small number of molecules with low ping ECD was widely distributed in \( t_r \). Fig.4.3B shows the distribution of ECD values, which has a major peak near 0.4 pC and a separate minor grouping around 0.1 pC. We attribute the pings in the major group to translocations of intact \( \lambda \) DNA molecules, and those in the minor group to \( \lambda \) DNA fragments \[16\]. We used the threshold \( \text{ECD} = 0.2 \text{ pC} \) to distinguish between intact molecules and fragments. For the analysis which follows we only considered the intact molecules.

Fig.4.4a shows the dependence of the mean return time \( \langle t_r \rangle \) on \( t_p \). \( \langle t_r \rangle \) increased with \( t_p \) until \( t_p \approx 700 \text{ ms} \), where it saturated at \( \langle t_r \rangle \approx 250 \text{ ms} \) (blue data). We determined that the saturation in \( \langle t_r \rangle \) was due to the presence of the cage by performing the following control experiment. We applied the same ping-pause-pong procedure, only this time we used a device whose cavity and opening diameters were so much larger than the equilibrium coil size of \( \lambda \) DNA that the cavity could not possibly impede the molecule’s motion. Because of this, these devices behave as if there were no cage at all. The results of these control experiments are shown in Fig.4.4b (red data). \( \langle t_r \rangle \) increased with \( t_p \) over the full range of pause times tested. The longest pause, \( t_p = 50 \text{ s} \), gave \( t_r = 2 \text{ s} \), which was nearly an order of magnitude longer than the \( t_r \) plateau value observed in experiments with a cage.
Figure 4.2: Ping-pause-pong experiments. (a) Illustrations of the ping, pause, and pong steps of our experiment. (b) A typical current trace from a ping-pause-pong experiment, showing the ping and pong translocation signatures, and illustrating $t_d$, $t_p$ and $t_r$. The current transients near .02s and 5.39s were caused by sudden changes in $V$. Slow drift of the current baseline caused the jump in the current at the break during pause.
Figure 4.3: ECD of ping and \( t_r \) of the corresponding pong. (a) Scatter plot showing ECD and \( t_r \) of the pings. (b) Histogram of ECD values (right) when \( t_p = 30 \text{ ms} \). Data attributed to fragments are shown in grey; these were excluded from subsequent analyses.

The cage also significantly enhanced the probability of recapturing a molecule, defined as the fraction of ping-pause-pong cycles where the ping was followed by a pong. Fig. 4.4b plots the dependence of the recapture probability on \( t_p \) for translocations into a cage and for translocations across a device with no cage. In the absence of a cage, the recapture probability decreased precipitously with \( t_p \) for \( t_p > 200 \text{ ms} \). The longest pause in the cage-free experiments was 5 s because beyond that, pongs were very rarely detected. By contrast, the recapture probability remained high in devices with a cage, even for \( t_p = 50 \text{ s} \) (100% for two devices and > 80% for a third device).

We ensured that our measurements of \( t_r \) and the recapture probability were not confounded by molecules that were left over on the opening side of the device from an earlier ping-pause-pong cycle, where, for example, a captured molecule failed to trigger a ping or was not recaptured within the 5 s allotted. Between sets of experiment with different \( t_p \), we checked that the opening side was clean by applying \( V = -100 \text{ mV} \) for 1 minute. We usually detected no translocations during this
Figure 4.4: Dynamics of DNA in an entropic cage. (a) Dependence of $\langle t_r \rangle$ on $t_p$ for devices with an entropic cage (blue) and without (red). Different symbols indicate different devices. The error bars show a standard deviation of $t_r$. 70 ping-pause-pong cycles on average were recorded for each $t_p$. The solid lines are predictions of the theoretical model described in the text and detailed in the Supplementary Information. (b) Dependence of the recapture probability on $t_p$. The symbols and lines have the same meanings as in (a). The precise dimensions of the devices used are listed in the end of the chapter.
time. Only in a few experiments was a single event observed during 1 minute, which indicated that molecules left over from previous ping-pause-pong cycles had negligible influence on subsequent experiments.

4.3 Dynamical modelling.

To guide our interpretation of the experiments, we modelled the dynamics of DNA in a ping-pause-pong cycle theoretically, and compared the predictions with our experimental results. Our approach was similar to the one used by Gershow and Golovchenko [14] to model the capture and recapture of DNA without a pause step or a cage. We treated λ DNA as a charged particle drifting due to the electric fields outside the nanopore and diffusing at the same time. When a voltage was applied, it induced hemispherically symmetric electric fields that decayed as the inverse square of the distance from the nanopore, as one would expect from a point source of current injected into fluid with a uniform conductivity. We calculated the evolution of the particle’s probability density distribution $c(r, t)$ as a function of time $t$ and radial distance from the nanopore $r$ for the ping and pause phases by numerically solving the convection-diffusion equation,

$$\frac{\partial c(r, t)}{\partial t} = D \nabla^2 c(r, t) - \mathbf{v} \cdot \nabla c(r, t), \quad (4.1)$$

where $\mathbf{v}$ is the electrophoretic drift velocity and $D = 0.5 \mu m^2/s$ is the diffusion constant of λ DNA in bulk solution [40].

$\mathbf{v}$ is related to the electric field outside nanopore, $E$, and the electrophoretic mobility of DNA, $\mu$, by $\mathbf{v} = \mu E$. We took the value of mobility to be $\mu = 2.2 \times$
10^{-4}\,\text{cm}^2\text{V}^{-1}\text{s}^{-1} in 1\text{M KCl} based on the theoretical estimate by Grosberg \[57\], which is consistent with mobility measurements by Stellwagen and Stellwagen \[58\] if those measurements are extrapolated to a salinity of 1\text{M}. The electric field $\mathbf{E}$ is related to the current density $j$ and the conductivity of 1\text{M} KCl at room temperature $\sigma = 10\,\Omega^{-1}\text{m}^{-1}$ by $\mathbf{E} = \frac{j}{\sigma}$. We modelled the nanopore as a point source of current injected into a uniform ohmic fluid, therefore $j$ is spherically symmetric and decays with radial distance as $1/r^2$. We find $j = \frac{I}{2\pi r^2}$, where $I = 10\,\text{nA}$ is the open-pore ionic current. The radial drift velocity is therefore

$$v = \frac{|\mu I|}{2\pi \sigma r^2}. \tag{4.2}$$

Using eqs. 4.2 and 4.1 and rewriting eq. 4.1 in spherical coordinates, we obtain

\begin{align*}
\text{Ping phase : } \frac{\partial c(r,t)}{\partial t} &= \frac{1}{r^2} \frac{\partial}{\partial r} r^2 \left( - \frac{|\mu I|}{2\pi \sigma r^2} c(r,t) + D \frac{\partial c(r,t)}{\partial r} \right), \\
\text{Pause phase : } \frac{\partial c(r,t)}{\partial t} &= \frac{1}{r^2} \frac{\partial}{\partial r} r^2 \left( D \frac{\partial c(r,t)}{\partial r} \right). \tag{4.3}
\end{align*}

The diffusion term is common to the ping and pause phases, while the ping phase has an additional term that describes the electrophoretic convection of particles away from the nanopore.

The initial condition is

$$c(r,0) = \frac{\delta(r-r_0)}{2\pi r_0^2}, \tag{4.4}$$

which corresponds to the particle being located at $r = r_0 = 100\,\text{nm}$ immediately after translocating the nanopore. We imposed a no-flux boundary condition at $r = r_c = 50\,\text{nm}$ to model the experiment without a cage. We imposed two no-flux boundary conditions to model the experiment with a cage, one at $r = r_c$ and the other at
$r = 1.5 \mu m$. These boundary conditions are expressed as

\begin{align*}
\text{No cage} & \quad \begin{cases} 
\frac{\partial c}{\partial r}(r_c, 0) = 0 \\
\frac{\partial c}{\partial r}(r \to \infty, 0) = 0
\end{cases} \\
\text{Cage} & \quad \begin{cases} 
\frac{\partial c}{\partial r}(r_c, t) = 0 \\
\frac{\partial c}{\partial r}(r = 1.5 \mu m, t) = 0
\end{cases} \quad (4.5)
\end{align*}

We note that these boundary conditions differ from the one imposed by Gershow and Golovchenko, who instead imposed a sink for particles at $r = r_c$, i.e. $c(r_c, t) = 0$, during the ping phase. A sink does not conserve probability, so a fraction of the particles could be lost. That fraction should be negligible as long as the electric fields drive particles away from the sink, as was the case in the ping phase of the calculations of Gershow and Golovchenko. The problem would have become significant in our calculations, however, because the particles are free to diffuse back to the nanopore during the pause phase.

To implement our numerical calculations, we made eq. (4.3) dimensionless using a rescaled radial distance, $x \equiv r/L$, and a rescaled time, $s \equiv t/\tau$, where $L = \frac{\mu I}{2 \pi \sigma D} \approx 7 \mu m$ and $\tau = L^2/D \approx 78 s$. ($L$ is the extent of the drifting domain, beyond which DNA diffuses nearly freely, as if it was in the bulk solution \[57\]. $\tau$ is the time scale for DNA to diffuse a distance $L$ away.) In these rescaled units, eqs. (4.3) become

\begin{align*}
\text{Ping phase} : \quad & \frac{\partial c(x, s)}{\partial s} = \frac{1}{x^2} \frac{\partial}{\partial x} \left( -c(x, s) + x^2 \frac{\partial c(x, s)}{\partial x} \right) \quad (4.6) \\
\text{Pause phase} : \quad & \frac{\partial c(x, s)}{\partial s} = \frac{1}{x^2} \frac{\partial}{\partial x} \left( x^2 \frac{\partial c(x, s)}{\partial x} \right) \quad (4.7)
\end{align*}

We used Matlab and its “pdepe” solver to numerically solve for the evolution of $c(r, t)$. We further simplified calculations by imposing a no-flux boundary condition at $r = 20 \mu m$ rather than at infinity for the case where there was no cage. This sped
Figure 4.5: Theoretical modelling of ping-pause-pong experiments. (a) $u(r, t)$ in the cage immediately after the ping (red) and after maintaining the positive $V$ for 2 ms (black). (b) $u(r, t)$ in the cage after pause times of $t_p = 2$ ms, 20 ms, 200 ms, and 5 s, as indicated. (c) (top) Dependence of $t_r$ on $r$ at the outset of the recapture phase. The red dashed line indicates $r = 3.8 \mu$m, the maximum distance from which a particle was recaptured. (Bottom) $u(r, t)$ at the outset of the recapture phase for an experiment with no cage. The shaded region indicates the domain from which molecules were not recaptured.

up calculations without affecting their accuracy because the probability of a particle diffusing 20 $\mu$m was negligible. Finally, we mimicked the delta function in the initial condition, eq. 4.4, with a narrow Gaussian distribution, $c(x, 0) = \frac{1}{N} \cdot \exp \left( -\frac{(x-x_0)^2}{2\pi\sigma^2} \right)$, where we chose $\sigma = \frac{0.1 \text{nm}}{L}$ and where the distribution is normalized by setting $N = \int_{x_{\text{c}}}^{\infty} \exp \left( -\frac{(x-x_0)^2}{2\pi\sigma^2} \right) dx$. 

Fig. 4.5a plots the radial probability density, $u(r, t) = 2\pi r^2 c(r, t)$, of a particle in a cage at the beginning and at the end of the ping step. (The radial probability
density gives the probability of finding the particle between \( r \) and \( r + dr \), whatever the angular coordinates.) The initial condition is a delta function at \( r = 100 \) nm (red line). The distribution shifts outward and broadens from applying the positive \( V \) for an additional 2 ms. The solutions for the case where there was no cage were nearly identical. This is sensible since the particle did not interact with the boundary of the cage after 2 ms; the radial probability density is centered at \( r \approx 0.4 \mu \text{m} \), less than one third of the distance to the boundary of the cage (1.5 \( \mu \text{m} \)), and the width of the distribution is only \( \sim 0.1 \mu \text{m} \). These results confirm that the molecule remains inside the cavity during the ping phase.

The probability distribution at the end of the ping phase was taken to be the initial condition for the pause phase and eq. 4.7 was solved numerically. Fig. 4.5b shows \( u(r, t) \) for a particle in a cage after different \( t_p \). The distribution broadens due to diffusion, eventually reaching the nanopore and then the edge of the cavity as \( t_p \) increases. After a 5 s pause, diffusion smoothed the probability density nearly completely inside the cavity. (Note that when \( c \) is uniform, \( u \) is parabolic.) Fig. 4.5c (bottom) shows \( u(r, t) \) for different \( t_p \) for the case where there is no cage. In this case, the distributions broaden well beyond \( r = 1.5 \mu \text{m} \) with increasing \( t_p \).

Ideally, we should calculate the distribution of recapture times by solving the convection-diffusion equation with an inward driving force, making the nanopore a sink for particles and using as the initial condition the probability distribution at the end of the pause phase. This approach unfortunately encounters by singularities in the electrophoretic force, in the gradient of the probability density, and consequently in the capture rate. We chose to avoid these problems by ignoring the effects of diffusion in the recapture (pong) phase. Instead, we analytically calculated the trajectory of a particle drifting due to the electrical field according to eq. 4.2. The
equation of motion of the particle is

$$\frac{dr}{dt} = \frac{|\mu I|}{2\pi \sigma r^2}.$$  \hspace{1cm} (4.8)

The motion can be integrated to find the relationship between the initial \( r \) and \( t_r \)

$$t_r = \frac{2\pi \sigma r^3}{3\mu I}.$$  \hspace{1cm} (4.9)

Fig. 4.5c (top) illustrates the dependence of \( t_r \) on \( r \) at the outset of the recapture phase. During that phase, we maintained the negative \( V \) for 5 s. Any particles that were not recaptured within 5 s were considered lost. The red dashed line in Fig. 4.5c indicates \( r_{\text{max}} = 3.8 \mu \text{m} \), the far edge of the domain from which molecules were recaptured. The domain from which molecules are lost is indicated in Fig. 4.5c (bottom).

We calculated the mean recapture time, \( \langle t_r \rangle \), from the probability distribution at the end of the pause phase and the \( r \)-dependent recapture time given by eq. 4.9.

$$\langle t_r \rangle = \frac{\int_0^{r_{\text{max}}} t_r(r) \cdot u(r, t_p) \, dr}{\int_0^{r_{\text{max}}} u(r, t_p) \, dr}.$$  \hspace{1cm} (4.10)

5 s was the maximum recapture time that could contribute to the mean. The resulting relationships between \( \langle t_r \rangle \) and \( t_p \) for ping-pause-pong experiments with and without a cage are plotted in Fig. 4.4.

We obtained the recapture probability, \( P \), by calculating the fraction of particles, initially distributed according to \( c(r, t) \) at the end of the pause phase, that would
return to the nanopore within 5 s, according to eq. 4.9

\[ P = \int_0^{r_{\text{max}}} u(r, t_p) dr \] (4.11)

The resulting relationships between \( P \) and \( t_p \) are plotted in Fig. 4.4b for experiments with and without a cage. The probability of recapturing a molecule from the cage is unity no matter how long \( t_p \) is because the edge of the cage is within \( r_{\text{max}} \). Without a cage, the probability drops with \( t_p \) once diffusion begins to broaden the particle’s probability distribution beyond \( r_{\text{max}} \).

We calculated the return rate, \( R(t_r) \), defined as the fraction of returning molecules per unit recapture time, \( t_r \), from the distribution of molecules after the pause (Fig. 4.5), and the relationship between \( r \) and \( t_r \) (Fig. 4.5c), calculated from eq. 4.9

\[ r = \left( \frac{3\mu I}{2\pi \sigma} \cdot t_r \right)^{\frac{1}{3}}. \] (4.12)

The distribution of \( R(t) \) is related to the distribution of \( u(r, t_p) \) by

\[ u(r, t_p) dr = R(t) dt \]

\[ \Rightarrow R(t) = u(r, t_p) \frac{dr}{dt} \]

\[ = u(r, t_p) \cdot \frac{1}{3} \cdot \left( \frac{3\mu I}{2\pi \sigma} \right)^{\frac{1}{3}} \cdot t_r^{-\frac{2}{3}} \] (4.13)

The black line in Fig. 4.6 plots the dependence of \( R(t) \) on \( t_r \) predicted for different \( t_p \) without any fitting parameters. The grey bars in Fig. 4.6 plot the normalized experimental return rate distribution. Note that the theoretical line ends at \( t_r = 3.21 \text{ ms} \) for \( t_p > 200 \text{ ms} \), which corresponds to the return time of a molecule from 1.5 \( \mu \text{m} \) away from the nanopore. According to our deterministic model, all molecules within 1.5 \( \mu \text{m} \) away from the nanopore are recaptured.
Figure 4.6: The dependence of return rate, $R(t)$, on return time, $t_r$, for cage experiments. Figures are layered out according to their pause time, $t_p$. The black line is the theoretical prediction, which is defined in eq. 4.13. The grey bar is the experimental results.
Figure 4.7: The dependence of return rate, $R(t)$, on return time, $t_r$, for nocage experiments. Figures are layered out according to their pause time, $t_p$. The black line is the theoretical prediction, which is defined in eq. 4.13, with normalized $u_n(r, t_p)$ defined in eq. ?? . The grey bar is the experimental results.
We performed the same calculation of DNA dynamics in device with no cages. Fig.4.6 and Fig.4.7 demonstrates that the theoretical prediction quantitatively agreed with our experimental results.

4.4 Attempts to Trap DNA in a Thin Device

We tried but failed to stably trap λDNA in thin cavities. Fig.4.8a shows the sketch of devices with different cavity heights for DNA trapping experiments. The top image shows the thick device (1.5 µm-thick SiO₂), and the bottom figure shows the thin device. Fig.4.8b plots the recapture probability as a function of \( t_p \) for devices with cavities that were 400 nm and 800 nm high. The probability decreased as \( t_p \) increased. Fig.4.8c plots the \( \langle t_r \rangle \) as a function of \( t_p \). \( \langle t_r \rangle \) increased but did not show a clear plateau as the 1.5 µm-thick devices did. In addition, the longest \( t_r \) was 1 s, which was \( \sim 4 \) times longer than the plateau value (\( \sim 250 \text{ ms} \)) measured in the thick device experiment.

Simulations \[59\] and experiments \[60\] have shown that the DNA molecule forms a tight coil when it emerges from the nanopore. The coil size can grow with the time during the pause because the molecule is able to relax toward equilibrium. The coil ultimately reaches to the edge of the cavity since the cavity height is smaller than the diameter of the \( \lambda \)DNA coil \((2R_g = 1.46 \mu m)\). The free energy of \( \lambda \)DNA is higher in its squeezed configuration than in its equilibrium configuration. So the escape of a molecule from the small cavity is entropically favoured. The recapture probability started to decrease with time after \( t_p \approx 500 \text{ ms} \), which was in the same order as the longest relaxation time of the polymer \[61\].
4.5 Visualizing an entropically trapped DNA molecule.

We visually confirmed that the entropic cage stably trapped λ DNA in a separate series of experiments. After a DNA trapping experiment, we removed the nanopore chip from its fluidic chuck, rinsed it in DI water to wash away any DNA stuck to the membrane, placed the chip on a microscope cover glass with the opening side facing up, added a small drop of YOYO-1 solution (5 μM solution of YOYO-1 dye (Invitrogen) in 20 mM Tris-HCl, 1 mM EDTA, at pH 8.0) to the opening side, and then placed another cover glass on top to prevent evaporation. We mounted the chip sandwiched between cover glasses on an inverted microscope (Nikon, ECLIPSE TE2000-U) equipped with a 100×, oil-immersion objective lens (Nikon, CFI Apo TIRF), an ultraviolet light source (EXFO X-Cite 120), a fluorescence imaging filter set (C125492, Chroma Technology Corp.), and an EMCCD camera (Andor, iXon). Fig 4.9a illustrated our viewing setup. The YOYO-1 could diffuse through the opening of the cage and stain the DNA molecule inside while we observed the process optically.

We located the cavity in white light mode (Fig 4.9b) and then monitored that
Figure 4.9: Imaging a trapped DNA molecule. (a) Schematic showing one drop of solution containing YOYO-1 fluorescent dye added to the opening side of a chip on an optical microscope. (b) An image of a device with a trapped DNA molecule illuminated by white light. The bright square is the 3-layer membrane. The dark disk indicated by a white dashed circle is the cavity. (c) A fluorescence image of the same device as in (b). (d) A fluorescence image of a cage that was emptied of DNA in a ping-pong-pong experiment. The brightness and contrast settings of the images in (c) and (d) were identical. The exposure time was 50 ms. The spot in (c) remained bright for all 154 frames. Both (c) and (d) are the 50th frames of their respective experiments so the incubation times in YOYO-1 were the same. We tried three devices with trapping DNA and two devices with no DNA. They showed the same result as in (c) and (d).
region in fluorescence mode for about 25 minutes. Fluorescence images were recorded every 10 s using a 50 ms exposure time and a mechanical shutter that minimized photobleaching. A bright spot appeared in the location of the cavity (Fig. 4.9c), showing that a DNA molecule was trapped inside. Fig. 4.9d shows the results of a control experiment where we used full ping-pause-pong procedure to pull the DNA molecule into the cavity and then remove it before adding the drop of YOYO-1 solution. As expected, no fluorescence was visible since no molecule was in the cage. We repeated the imaging experiment three times with DNA trapped in. And all of them showed a shining spot in the center.

4.6 Cutting trapped DNA molecules with a restriction endonuclease.

We used an entropic cage to subject trapped DNA molecules to a sequence-specific biochemical reaction, as illustrated in Fig. 4.10a. Buffer (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 1 mM DTT, at pH=7.9) containing the SmaI (400 units/mL SmaI, New England Biolab, R0141S) restriction endonuclease was added to the reservoir on the opening side, while the reservoir on the nanopore side was filled with a similar buffer that contained 1.25 µg/mL λ DNA instead of the enzyme. We chose this buffer since it is active at room temperature (24°C). We used the ping-pause-pong technique to draw a DNA molecule into the cage, where it could interact with SmaI during the pause, and then reverse the voltage so that the nanopore would interrogate the products of the reaction. SmaI cuts double-stranded DNA at a particular recognition sequence found at three sites along λ DNA. We therefore expected a λ DNA molecule entering the cage to be cut into
as many as 4 pieces, with the number of pieces increasing as the reaction progresses to completion.

Fig. 4.10b shows a typical current trace obtained with $t_p = 5\text{ s}$. Applying $V = 200\text{ mV}$ drew a DNA molecule into the cage through the nanopore, in this case causing an increase in $I$ because the DNA is highly charged, and entrains enough counterions into the nanopore to increase its conductance in low salinity enzyme buffer [62]. After the pause, the voltage was flipped to $V = -200\text{ mV}$ and maintained for 5 s. From the single ping, we obtained two pong signals in this case, indicating that a molecule was trapped inside the cavity, cut, and pulled back through in pieces. We varied $t_p$ in our experiments between $t_p = 1\text{ s}, 5\text{ s},$ and 20 s to probe the restriction reaction after different incubation times. At each $t_p$, we repeated the experiment at least 60 times. We also performed control experiments with no SmaI present and $t_p = 5\text{ s}$. Fig. 4.11a shows the distribution of the number of pongs observed per ping-pause-pong cycle ($N_{pong}$) for the different incubation times and for the experiment with no enzyme. Between 1 and 4 molecules were recaptured per cycle. Without SmaI, 98.6% of the experimental cycles returned only one pong. With SmaI present, the probability of detecting multiple pongs in a cycle increased with $t_p$, from 17.3% at $t_p = 1\text{ s}$ to 50.0% at $t_p = 20\text{ s}$.

To confirm that multiple pong signals were caused by fragments of the original molecule, we verified that the fragments were smaller than the original molecule and compared the total size of the recaptured fragments in a cycle with the size of the original molecule, using ECD as a measure of size. Fig. 4.11b shows the distributions of $\frac{\text{ECD}_{pong}}{\text{ECD}_{ping}}$, the ECD values of the pongs relative to the corresponding ping, grouped by $N_{pong}$ (white bars). That figure also plots the distributions of $\frac{\Sigma \text{ECD}_{pong}}{\text{ECD}_{ping}}$, the total ECD of all the pongs in a cycle relative to the corresponding ping (grey bars). When $N_{pong} = 1$, the distribution of $\frac{\text{ECD}_{pong}}{\text{ECD}_{ping}}$ was centered around 1, indicating that
Figure 4.10: Enzymatic cutting of trapped DNA. (a) Illustration of the experiment. (b) A typical current trace showing a single ping followed by two pongs after a pause of $t_p = 5 \text{s}$. The ECD of the ping is 0.10 pC and those of the pongs are 0.05 pC and 0.04 pC, sequentially. The current transients near 0.01 s and 5.4 s were caused by sudden changes in $V$. (c) Distributions of pongs per ping-pause-pong cycle, $N_{pong}$, for three different $t_p$ with SmaI present, and for $t_p = 5 \text{s}$ without SmaI. (d) Distributions of $\frac{ECD_{pong}}{ECD_{ping}}$ (white bars) and $\frac{\Sigma ECD_{pong}}{ECD_{ping}}$ (grey bars). Both distributions are normalized by the total number of counts, respectively.
Figure 4.11: Enzymatic cutting of trapped DNA. (a) Distributions of pongs per ping-pause-pong cycle, $N_{pong}$, for three different $t_p$ with SmaI present, and for $t_p = 5\text{s}$ without SmaI. The sample sizes are, 76 cycles for no enzyme experiment, 62 cycles for $t_p = 1\text{s}$ experiment, 93 cycles for $t_p = 5\text{s}$ experiment, 82 events for $t_p = 20\text{s}$ experiment. (b) Distributions of $\overline{ECD}_{pong}$ (white bars) and $\Sigma ECD_{pong} ECD_{ping}$ (grey bars). Both distributions are normalized by the total number of counts, respectively.

the molecule was not cut because its size did not change. As more pongs were detected per cycle, the distribution of $\frac{ECD_{pong}}{ECD_{ping}}$ shifted to lower values, indicating that the recaptured molecules were becoming smaller than the original one. The peak of the $\frac{\Sigma ECD_{pong}}{ECD_{ping}}$ distribution remained near 1 for all $N_{pong}$, however, indicating that the total size of the recaptured molecules was the same as the original.

We confirmed that the multiple pongs observed in the enzymatic DNA cutting experiments were not caused by enzyme molecules translocating the nanopore. We
applied $V = -200 \text{ mV}$ for 1 minute between sets of experiments using a different $t_p$. No translocation signals were detected, which indicated the SmaI molecule is too large to translocate the nanopore or its translocation signal is too short to be detected.

### 4.7 Discussion

We have entropically trapped single DNA molecules in an entropic cage and performed bio-chemical reactions on the trapped single molecules. We demonstrated trapping the DNA stably in the cage by driving the molecule into the cage from the nanopore, letting the DNA relax in the cage for a preset time, and recapturing the DNA at almost 100% efficiency and saturated recapture time. The recapture time and recapture probability both agree with our model, which treated the molecule as a particle drifting by the electric field and diffusing at the same time. We confirmed the trapping by observing the trapped fluorescence DNA in a microscope. To demonstrate the device’s ability to perform bio-chemical reactions on single molecule and probe the reaction results, we performed the experiment which used the enzyme to cut the DNA at sequence-specific sites.

### 4.8 The Dimension of The Devices in The Chapter

Table 4.1 lists the diameters of the nanopore, the opening to the entropic cage, and the disc-shaped cavity for each device used in the experiments described in the main
text. The dimensions were measured by TEM.

<table>
<thead>
<tr>
<th>Device</th>
<th>Nanopore diameter (nm)</th>
<th>Opening diameter (nm)</th>
<th>Cavity diameter (µm)</th>
<th>Location of data in the main article</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.9</td>
<td>230</td>
<td>2.25</td>
<td>Fig.4.4, blue circles</td>
</tr>
<tr>
<td>2</td>
<td>8.5</td>
<td>230</td>
<td>2.28</td>
<td>Fig.4.4, blue squares</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
<td>240</td>
<td>2.30</td>
<td>Fig.4.4, blue diamonds</td>
</tr>
<tr>
<td>4</td>
<td>10.2</td>
<td>1560</td>
<td>6.12</td>
<td>Fig.4.4, red circles (no cage)</td>
</tr>
<tr>
<td>5</td>
<td>9.1</td>
<td>5320</td>
<td>7.32</td>
<td>Fig.4.4, red squares (no cage)</td>
</tr>
<tr>
<td>6</td>
<td>9.4</td>
<td>230</td>
<td>3.30</td>
<td>Fig.4.9d</td>
</tr>
<tr>
<td>7</td>
<td>14.0</td>
<td>290</td>
<td>2.72</td>
<td>Fig.4.9b and c</td>
</tr>
<tr>
<td>8</td>
<td>8.7</td>
<td>240</td>
<td>2.46</td>
<td>Fig.4.11</td>
</tr>
</tbody>
</table>

Table 4.1: Measured dimensions of nanopore devices and the locations of the corresponding results in the main article.
CHAPTER FIVE

Summary and outlook
In this thesis, we used a micrometer-sized entropic cage next to a solid-state nanopore to manipulate and study single DNA molecules in new ways. We first used our device to address a question about the fundamental physics of voltage-driven DNA translocations, specifically, “What is the nature of the viscous drag on the part of a DNA molecule outside the nanopore as it is pulled through?” We used the entropic cage of our device to create a gap between the DNA coil and the nanopore at the onset of translocation, which extended the moving segment during translocation. Enlarging the opening of the cage allowed the coil to come closer to the nanopore in a tunable fashion. In these ways, the entropic cage enabled experiments where, for the first time, the configuration of a DNA molecule at the onset of translocation was controlled systematically. The result of increasing the moving length was a decrease in the average translocation speed, which we quantitatively compared with the theoretical models. Our measurements thereby provided direct experimental evidence for the theoretical model that attributes the major contribution to the viscous drag during translocation to the moving segment of a coil that is otherwise dormant.

![Figure 5.1](image)

Figure 5.1: DNA translocation speed at different solution conditions. (a) Dependence of current drop and translocation time on viscosity. (b) Dependence of current drop and translocation time on ionic concentration of solution. (c) Distribution of translocation time at different applied and different temperature. (Adapted from [12])

An interesting implication of our results is that a nanopore/cage device offers a physical way to slow down DNA translocations, which is seen as a key step toward rapid single molecule identification [23]. Researchers have devoted considerable
effort to slowing down the translocation speed. For example, Fologea et al. [12] explored different solution conditions, including the ionic concentration, the viscosity, and the temperature, to see which would have a strong influence on the translocation speed, as shown in Fig. 5.1. Increasing the viscosity of the solution by adding glycerol increased the translocation time, as expected (Fig. 5.1a, triangles), but this came at the expense of the ionic signal intensity, which decreases with viscosity by the same amount (Fig. 5.1a, squares) because the conductivity of the solution, $\sigma$, is inversely proportional to the viscosity. Increasing the concentration of salt in solution also decreases the translocation speed (Fig. 5.1b) because the additional ions screen the DNA charge more effectively, resulting in a weaker driving force. At the same time, the increased salt concentration enhances the DNA detection signal. The slowing effect is modest, however, and the translocation time in fact plateaus for ionic strengths larger than 1 M. Finally, Fologea et al. found that lowering the temperature reduced the translocation speed (Fig. 5.1c) because the viscosity increased with decreasing temperature.

![Figure 5.2:](a) Ionic current traces from DNA translocations in different solutions. (b) Distribution of translocation times in different ionic solutions. (Adapted from [63])

In a different study, Kowalczyk et al. [63] examined the effect of different counterions on the translocation speed. They performed DNA translocation experiments with solutions of the monovalent salts, KCl, NaCl, and LiCl at the same concentration. Fig. 5.2a shows three events from those experiments while Fig. 5.2b shows the distributions of translocation times. The translocation speed was significantly
slower in the presence of LiCl than in the other solutions. The explanation was that
the smaller the size of the counterions, the stronger they bound to the DNA and
resulted a greater force transmitted from the ion to the DNA.

The methods of Fologea et al. \cite{12} and Kowalczyk et al. \cite{63} for reducing the
translocation speed of DNA required adjusting the contents of the solution, which
may not be permissible in many biosensing applications. Furthermore, the slowing
of DNA often came at the expense of detection signal. Our entropic cage devices,
on the other hand, can control the translocation speed by altering the initial config-
uration of the DNA molecule, which has no influence on the ionic current signal or
the chemical environment. Entropic cages or other nanofluidic structures that add
to the viscous force on a DNA (without altering the viscosity of the fluid) could be
broadly applied for nanopore sensing in different chemical environments.

![Figure 5.3](image)

**Figure 5.3**: DNA docked outside of the nanopore, causing the current first decreases and then
increases (Adapted from \cite{64} and \cite{65})

The ability of an entropic cage to create a gap between the DNA coil and the
nanopore creates new opportunities to investigate basic questions about the capture
and translocation of polymers. For instance, little is currently known about how
the end of a DNA molecule finds a small nanopore (<6 nm diameter) and threads
it. Kowalczyk et al. [64] and Vlassarev et al. [65] both observed what were called composite events under low salt conditions (100 mM KCl) and intermediate voltages (between 200 mV and 600 mV), where within each single event the current first decreased and then increased, as shown in Fig. 5.3. Kowalczyk et al. proposed a model which assumed that the DNA coil outside the nanopore caused the current decrease and the DNA translocation caused the current increase. They argued that the DNA coil first docked in the region of access resistance outside the nanopore, which caused the current decrease. The translocation caused current increase because the negatively charged DNA increased the conductance of nanopore [62]. Fig. 5.3a shows the model of Kowalczyk et al. [64]. Vlassarev et al., on the other hand, concluded that the decrease in current was caused by a small segment of DNA becoming jammed in the nanopore oriented parallel to the membrane. Fig. 5.3b shows the model of Vlassarev et al. [65]. It should be possible to use our nanopore/cage device to test their models. If the composite events indeed represent docking or jamming of DNA in the nanopore, these events should be observable for translocations from the nanopore side, but less frequently from the opening size. Furthermore, the ability to keep the DNA coil up to \( \sim 800 \text{ nm} \) away from the nanopore might enable us to choose between the two models, because only one of them relies on the DNA coil being in the region of access resistance.

In chapter 4 we demonstrated that entropic cages can also be used to capture single DNA molecules next to a nanopore. The ability to capture and constrain a single molecule allows us to study the physics of a molecule’s translocation of a nanopore in general since it affords us some control over the state of the molecule before reverse translocation [13, 14, 66–69]. Entropic traps also enhance solid-state nanopore technology by serving as single molecule test tubes in which a molecule, interrogated by the nanopore on the way in, can react and then be probed again
using the reverse translocation. As an example, we demonstrated the cutting of a trapped DNA molecule in the cavity, which can provide a way to simulate the restriction modification systems of bacteriophage resistance mechanisms \[70\] in vitro. In addition, compared to the widely-used genomic analysis method of optical mapping \[71, 73\], which uses a microscope to observe the results of cutting long, single DNA molecules with restriction enzymes, our device provided a fast and label-free way to study the enzymatic cutting of DNA in real time. Given careful choice of enzyme and more thorough study of the reaction conditions, one could use the device to study restriction fragment length polymorphism \[74\] and variation analysis \[75\].

![Figure 5.4](image)

**Figure 5.4:** (a) A signal recognition algorithm to decide whether the ping-pause-pong procedure should be applied to a particular molecule or not. (b) A device with the nanopore sandwiched by two cages. This device enables us to trap the molecule at cis side as well as trans side.

The ability to trap a single DNA molecule in a cage opens up a large number of possibilities for biosensing and molecular analyses. Fig 5.4 illustrates two possibilities. First, it is possible for a nanopore sensor in combination with a suitable signal recognition algorithm to decide whether the ping-pause-pong procedure should be applied to a particular molecule, or whether the system should keep scanning for a molecule with more desirable characteristics. This idea is illustrated in Fig. 5.4a. The significant advantage over this approach is that it avoids the need to purify
a DNA sample or amplify the interesting part of it by polymerase chain reaction (PCR) before an analysis. This approach could detect rare genomic variations or small subpopulations, which are increasingly recognized as important for assessing disease states and progression \cite{71,76}.

Second, it should be straightforward to extend our ability to trap a single DNA molecule in a cage, to trapping and shuttling a molecule back and forth between two adjacent cages. Currently, we can only trap the molecule at trans side, as molecules tend to diffuse away on the cis side. We can achieve trapping the molecule in both sides by fabricating a device illustrated in Fig. 5.4b, which features a nanopore sensor sandwiched between two cages. That device would enable us to perform another “ping-pause-pong” cycle on the same molecule on the cis side and interrogate it multiple times.

More significantly, in a device with an array of such back-to-back cage structures, molecules could be shuttled back and forth between the cages while different chemical probes are introduced into the trans side. The chemical probes would only interrogate the molecules trapped on that side. The opposing cages would therefore serve as “on” and “off” states, with the chemical probe serving as an operation that only acts on molecules in the “on” state. This idea could lead to massive parallelization of chemical analyses performed on single DNA molecules.
Appendix One

DNA as a polymer
In this section, I described the most important concept of polymer in my thesis, including the persistence length, $l_p$, the radius of gyration, $R_g$, the free energy of an ideal chain in confinement, $F$. Most of the work in this section followed deGennes’s theory in polymer physics.

Fig. A.1 demonstrates the concept of the persistence length, $l_p$. In polymer physics, we neglected the details of chemical bond. We treated the polymer as a chain with different persistent length, $l_p$. The polymer had many energy minima due to different configurations. $\Delta \epsilon$ is the energy difference between two minima of consideration. If $\Delta \epsilon < T$, the polymer configuration is easy to jump from one minimum to the other, i.e. it is extremely flexible, where $T$ is the thermal energy. The persistence length, $l_p$ was defined

$$l_p = l_0 \exp \left( \frac{\Delta \epsilon}{T} \right)$$

(A.1)

where $l_0$ is on the order of a few Angstroms. Equation (A.1) indicates that we treat the polymer as a stiff rod if its length is much smaller than $l_p$. Beyond $l_p$, the direction that the polymer points at has little correlation with the original direction.

Based on the assumption that the polymer is a stiff rod within some finite length, we treated the configuration of the whole polymer using a random walk model, as shown in Fig. A.1b. The end-to-end vector $\mathbf{R}$ is the sum of N “jump vectors”.

$$\mathbf{R} = \mathbf{r}_1 + \mathbf{r}_2 + \mathbf{r}_3 + \cdots + \mathbf{r}_N = \sum_n \mathbf{r}_n$$

(A.2)

where each of the $\mathbf{r}$ terms is a vector of length $l_k$. $l_k = 2l_p$ is the Kuhn length. Different $\mathbf{r}$ vectors have completely independent orientations. We got
(a) the average square end-to-end distance is linear in \( N \),

\[
\langle R^2 \rangle = \sum_{nm} \langle r_n \cdot r_m \rangle = \sum_n \langle r_n^2 \rangle = N l_k^2 \quad (= R_0^2),
\]  

(A.3)
since \( \sum_{n \neq m} \langle r_n \cdot r_m \rangle = 0 \). Qualitatively, the flexible polymer has a size \( R_0 \sim N^{1/2} l_k \). This length is defined as the radius of gyration of DNA.

(b) the distribution function for \( R \), defined by

\[
p(R) = \Xi_N(R) / \left( \sum_R \Xi_N(R) \right),
\]  

(A.4)
has a Gaussian shape when \( N \gg 1 \). In three dimensions

\[
p(R) \cong N^{-3/2} \exp \left( -\frac{3R^2}{2Nl_k^2} \right)
\]  

(A.5)

The entropy of the chain is defined

\[
S(R) = \ln \left[ \Xi_N(R) \right] = S(0) - \frac{3R^2}{2R_0^2}
\]  

(A.6)
And the free energy is

\[
F(R) = E - TS = F(0) + \frac{3TR^2}{2R_0}
\]  

(A.7)

Scaling analysis is very powerful for studying polymers. We demonstrated an example as the polymer is confined in a tube with diameter, \( D \), smaller than \( R_0 \), as shown in Fig. [A.1]. First, the confinement is only in one dimension- the radial direction. So the free energy should be a function of diameter, \( F(D) \). Second, we assumed the entropy is the only significant factor (no long-range van der Walls force
Figure A.1: Basic concept of polymer physics. (a) The definition of persistent length, $l_p$, which we assume the polymer as a stiff rod for the scale smaller than $l_p$. (b) The random walk model showing the ideal chain in a dilute solvent. (c) The polymer is confined in a cylinder with diameter $D$, resulting in increasing free energy due to reduced configurations.

in the cylinder.) We try to estimate the reduction in entropy $\Delta S$ due to confinement:

(a) The leading term in $\Delta S$ will be a linear function $N$. If we double the chain size, then $\Delta S$ doubles. (b) $\Delta S$ is dimensionless and depends only on the length ratio $R_0/D$. This leads to $\Delta S = -(R_0/D)^y \sim N^{y/2}$, and from (a) we must have $y = 2$. So the corresponding free energy is

$$F \simeq T \frac{R_0^2}{D^2} \quad (A.8)$$

We assume the polymer is a ideal chain in a dilute solvent. In reality, the polymer strand can not overlap with each other. deGennes modified Fig. A.3 as,

$$R_F \simeq l_k N^\nu, \quad (A.9)$$

where $d$ is the dimensionality, $\nu = \frac{3}{d+2}$ is the flory exponent, which successfully explains the scale of the polymer in a solution.

The concept of persistence length, $l_p$, radius of gyration, which we estimated
roughly \( R_0 \) and free energy of DNA are highly relevant concepts to the research of nanopore translocation.
APPENDIX TWO

The Operations of FIB Milling
Comparing to other standard fabrication procedure, the FIB milling step was newly developed and critical to the device performance. Here we describe its detailed operation to make the nanopore/cage devices. We first clicked the Start UI tab, input the username and password to open the software environment, as shown in Fig. B.1a. Our operation uses three tabs, which are beam control, navigation, and patterning. After loading the sample into the holder, we first click the beam control and pumped down the vacuum. This step takes about 10 minutes until the vacuum indicator turns green. Then we wake up the beam source. We use three sub-windows in the main monitor, which are electron beam, ion beam, and camera. We first activate the electron beam sub-window to observe the sample in low resolution, set by $63 \times$, 30.0 KeV, 21 pA, and 3 $\mu$s scanning cycle to view the sample. We click the navigation tab, position the sample to (0,0). After finding the sample, we use Tools→Auto contrast brightness to get a better view. In $800 \times$, we used stage→XT Align feature to align the sample. We then adjust the focus until we get a sharp view of the sample, 2) link Z to FWD, and 3) set the Z height to be 4 mm. Steps 1), 2), and 3) are repeated until the focus is stable. Then we tilt the sample at $15^\circ \rightarrow 30^\circ \rightarrow 45^\circ \rightarrow 52^\circ$ sequentially.

In the next step, we activate the ion beam to observe the sample, as shown in Fig [B.1b]. We set the ion beam at $149 \times$(smallest), 30.0 KeV, 9.7 pA, and 10 $\mu$s scanning cycle. We notice the aperture sized changed a lot due to intensive using. For example, we set the aperture at 9.7 pA, while the real ion beam current was 49.6 nA. So we carefully choose the aperture according to the size of pore we drill and the ageing of the aperture. We indicate the membrane in centre by red dashed square at Fig [B.1b]. Fig [B.1c] illustrates the zoom in of the membrane. We turn the nob to fine adjust the focus until the corner looked sharp. Then we drill a $\sim 200$ nm cycle through the membrane. Normally the hole looks elliptical, as shown in Fig [B.1d].
Figure B.1: The detailed recipe of milling a hole half-through the membrane. (a) The interface of the FIB controlling software. We listed the three tabs that we used in the process. (b) The zoomed out (146×) view of the chip. The dark square in the center is the thin membrane. (c) The zoom in (20k×) of the membrane. We adjusted the focus at the corner. (d) A drilled through hole for stigmation test. (e) A half-through hole.
We adjust the X and Y stigmation to make the hole more circular and shaper. We adjust the focus at the same. We repeat the viewing, adjusting stigmation and focus operation several times until the hole looked sharp and circular.

To drill a hole in certain depth, $h_{\text{aim}}$, we should take the ageing of aperture into consideration. Fig. B.1h shows the setting of ion beam was 9.7 nA while the real intensity was 48.6 nA. We made two assumptions. First, we achieved perfect focus and stigmation. Second, the FIB drill the hole in linear rate. We could input the drilling depth, $h_{\text{input}}$, by calculating

$$\frac{h_{\text{aim}}}{h_{\text{input}}} = \frac{48.6 \text{ nA}}{9.7 \text{ nA}}$$

$$\Rightarrow h_{\text{input}} = 0.2 \cdot h_{\text{aim}}$$  \hfill (B.1)

Or, we could calibrate the drilling rate as shown in Fig. 2.3h, if we find it hard to keep the focus perfect. After that, we click the navigation tab and move the stage by $\pm 5$ mm at X or Y direction, as needed, which was the chip size, to drill another hole.
Appendix Three

The Viscous Drag along a Cylinder
In chapter 3, we used the equation 3.4 and equation 3.5 to calculate the viscous drag per unit length, for the DNA inside and outside of the nanopore respectively. Here we show the detailed derivation for the two equations.

Fig. C.1a shows our model to calculate the viscous drag of DNA inside nanopore. We treated the nanopore as a larger cylinder with radius $R$, and the DNA as a smaller cylinder with radius $r$. We assumed this movement was cylindrical symmetric, i.e the DNA moved in the center of the nanopore and the velocity was only in $z$-axis. We followed Ui et al. \cite{44} and Happel et al. \cite{77} to derive equation 3.4. We started from the Navier-Stokes equation of Newtonian fluids,

$$\rho \left( \frac{\partial \vec{v}}{\partial t} + \vec{v} \cdot \nabla \vec{v} \right) = -\nabla P + \mu \nabla^2 \vec{v} + \vec{f},$$  \hspace{0.5cm} (C.1)

where $\rho$ is the density of the fluid, $\vec{v}$ is the velocity of the fluid, $P$ is the pressure on the fluid, $\mu$ is viscosity of the fluid, and $\vec{f}$ is the force acting on the fluid. Considering it is steady flow, $\frac{\partial \vec{v}}{\partial t} = 0$. The fluid is incompressible, so $\vec{v} \cdot \nabla \vec{v} = 0$. And there is no force acting on the fluid, $\vec{f} = 0$. We assumed the velocity is only in $z$-direction, $\vec{v} = w \hat{z}$. Therefore, the equation C.1 was simplified to

$$\mu \nabla^2 w = \nabla P$$  \hspace{0.5cm} (C.2)

We rewrote eq. C.2 at cylindrical coordinates.

$$\frac{1}{x} \frac{d}{dx} \left( x \frac{dw}{dx} \right) = \frac{1}{\mu} \frac{dP}{dz}$$  \hspace{0.5cm} (C.3)
The boundary conditions are

\[
\begin{aligned}
  w(x = r) &= -v \\
  w(x = R) &= 0 \\
  Q &= 2\pi \int_{r}^{R} w dx = 0
\end{aligned}
\] (C.4)

The top two boundary condition in equation \[\text{C.4}\] are the non-slippery at the surface of the DNA and nanopore. \(Q\) in the bottom of equation \[\text{C.4}\] is the net flow. We assumed there was no leak in our system, \(i.e\) the total amount of fluid in the \(\text{trans}\) and \(\text{cis}\) side were conserved. Integrate equation \[\text{C.3}\], we got the expression of \(w\)

\[
  w = C_3 x^2 + C_1 \ln x + C_2,
\] (C.5)

where \(C_1, C_2,\) and \(C_3\) are constants, we got their value by solving the three boundary conditions at equation \[\text{C.4}\].

The viscous drag per unit length acting on the inner cylinder is

\[
  F'_{in} = -2\pi r \mu \left( \frac{\partial w}{\partial x} \right) \bigg|_{x=r},
\] (C.6)

where \(2\pi r\) is the perimeter of the inner cylinder, \(\mu\) is viscosity, and \(\left( \frac{\partial w}{\partial x} \right) \bigg|_{x=r}\) is the shear rate at the inner cylinder surface. Using the expression of \(w\) in equation \[\text{C.5}\], we got

\[
  \frac{F'_{in}}{2\pi \mu w} = \frac{\sigma^2 - 3 + [4\ln \sigma / (\sigma^2 - 1)]}{[(\sigma^2 + 1) \ln \sigma] - (\sigma^2 - 1)},
\] (C.7)

where \(\sigma = \frac{R}{r}\) is a geometry constant. The equation \[\text{C.7}\] is the equation 3.4 in chapter 3. The equation \[\text{C.7}\] differs from \(\text{Ui et al.} \ [44]\) by one term in the denominator.
Figure C.1: The calculation of the viscous drag on a cylinder. (a) The model of the viscous drag of the molecule inside the nanopore: a smaller cylinder moving in another larger cylinder. (b) The results viscous drag of the smaller cylinder. The dashed line is Ui et al.’s [44] results. The solid line is the results of this work. (c) (top) The green function of a force applied at point A caused the fluid move at point B. (bottom) A cylinder moving in x-direction.

Ui et al. [44] got the following result

$$\frac{F_{in}'}{2\pi \mu v} = \frac{\sigma^2 - 3 + [4\ln\sigma / (\sigma^2 - 1)]}{[(\sigma^2 + 1) \ln\sigma] - 1},$$

where the last term in denominator is 1 instead of $(\sigma^2 - 1)$. Fig. C.1b shows the results of Ui et al. and the results derived in this work together. We noticed there is a singular when $\sigma$ reached some value between 1 and 2 for the results of Ui et al. Obviously, the first term in the denominator $\ln\sigma (\sigma^2 + 1) \to 0$ when $\sigma \to 1$, and $\ln\sigma (\sigma^2 + 1) \to \infty$ when $\sigma \to \infty$. So the denominator of Ui et al. could be 0 and cause blow up for the equation. On the other hand, our results make more sense. When $\sigma \to 1$, i.e. the inner cylinder and outer cylinder are infinitely close, $\frac{F_{in}'}{2\pi \mu v} \to \infty$. When $\sigma \to \infty$, i.e. inner cylinder is moving in a free solution, $\frac{F_{in}'}{2\pi \mu v} \to 0$.

Fig. C.1c shows our model to derive the viscous force on the DNA outside nanopore, which is equation 3.5 in chapter 3. We treated the DNA as a cylinder moving along its axis direction in a free fluid without boundary. The driving force caused the cylinder move, therefore generated a velocity field $\vec{v}(r)$ in the fluid. Fol-
ollowing Lisicki's derivation \cite{78}, we first studied the basic green function of a force, \( \vec{F} \), acting at point A, resulted in the fluid at point B moving at the speed of \( \vec{v} \), as shown in Fig. \[ C.1 \]: (top). The final speed of the fluid at point B is the integration of the green function in the whole space. The full system followed the navier-stocks equation

\[
\nabla p(\vec{r}) + \mu \nabla^2 \vec{v}(\vec{r}) = -\vec{F} \delta(\vec{r}) \tag{C.9}
\]

\[
\nabla \cdot \vec{v} = 0 \tag{C.10}
\]

where \( p(\vec{r}) \) is the pressure, \( \mu \) is viscosity, \( \vec{v}(\vec{r}) \) is the velocity, and \( -\vec{F} \delta(\vec{r}) \) is the force applied at \( \vec{r} = 0 \). For a incompressible fluid, \( \nabla \cdot \vec{v} = 0 \). We take the divergence of equation \[ C.10 \]

\[
\nabla^2 p(\vec{r}) = \nabla \left[ \vec{F} \delta(\vec{r}) \right] \tag{C.11}
\]

which is a Possion equation. We then applied Fourier transform to equation \[ C.11 \]

\[
k^2 \hat{p}(\vec{k}) = -i\vec{k} \cdot \vec{F} \Rightarrow \hat{p} = -i \frac{\vec{k} \cdot \vec{F}}{k^2} \tag{C.12}
\]

Then we applied Fourier transform to equation \[ C.10 \]

\[
i\vec{k} \left( \frac{\vec{k} \cdot \vec{F}}{k^2} \right) + \mu k^2 \hat{\vec{v}}(\vec{k}) = \vec{F} \tag{C.13}
\]

We find the expression for the velocity in \( \vec{k} \)-space

\[
\hat{\vec{v}}(\vec{k}) = \frac{1}{\mu k^2} \left( \vec{F} - \vec{k} \cdot \frac{\vec{k} \cdot \vec{F}}{k^2} \right) \tag{C.14}
\]

Using the definition of the inverse Fourier transform, we wrote down the expression
for the pressure and velocity.

\[
\bar{v}(\vec{r}) = \frac{1}{8\mu\pi^3} \int_{\mathbb{R}} dk \frac{e^{i\vec{k} \cdot \vec{r}}}{k^2} \left( \vec{F} - \frac{\vec{k}}{k^2} \cdot \vec{F} \right) \quad (C.15)
\]

\[
p(\vec{r}) = \frac{i}{8\pi^3} \int_{\mathbb{R}} dk \frac{e^{i\vec{k} \cdot \vec{r}}}{k^2} \vec{k} \cdot \vec{F} \quad (C.16)
\]

Then we got the expression, \(\bar{v}(\vec{r})\)

\[
\bar{v}(\vec{r}) = \frac{\vec{F}}{4\pi\mu r} - \frac{\vec{F}}{\mu} \cdot \nabla \left( \frac{r}{8\pi} \right) \quad (C.17)
\]

Using the fact that \(\nabla r = \vec{r}\) and \(\nabla \vec{r} = \mathbb{I}\), and applying the chain rule, we got

\[
\nabla \nabla r = \nabla \left( \frac{\vec{r}}{r} \right) = \frac{\nabla \vec{r}}{r} + \vec{r} \nabla \frac{1}{r} = \frac{\vec{r}\vec{r}}{r^3} \quad (C.18)
\]

So the equation \(C.17\) becomes

\[
\bar{v}(\vec{r}) = \frac{\vec{F}}{8\pi\mu r} \cdot \left( \mathbb{I} + \frac{\vec{r}\vec{r}}{r^2} \right) \quad (C.20)
\]

The component of \(\vec{v}\) can be written by

\[
v_i(\vec{r}) = \frac{1}{8\pi\mu r} \left( F_j \delta_{ij} + \frac{F_j r_i r_j}{r^2} \right) = \frac{1}{8\pi\mu r} \left[ F_i + \frac{(F_j r_j) r_i}{r^2} \right] \quad (C.21)
\]
We assign $\vec{F}$ in $x$ direction, $\vec{F} = (F_x, 0, 0)$. We get

$$
\begin{align*}
  u &= \frac{F_x}{8\pi \mu} \left( \frac{1}{r} + \frac{x^2}{r^2} \right) \\
  v &= \frac{F_x}{8\pi \mu} \frac{xy}{r^2} \\
  w &= \frac{F_x}{8\pi \mu} \frac{xz}{r^2}
\end{align*}
$$

which is the same results as Burgers al. [79].

Fig. C.1c (bottom) shows our model to calculate the viscous force on a cylinder with length, $L$, and radius, $r_0$. When at an element $d\xi$ of the axis a force $f(\xi)$ is introduced, the $u$ component of the velocity produced by this force at a point of the surface of the body, situated on the section made by the plane $x = constant$, is given by

$$
du = \frac{F_x}{8\pi \mu} f(\xi) d\xi \left[ \left( \frac{1}{(x - \xi)^2 + r^2} \right)^{1/2} + \frac{(x - \xi)^2}{((x - \xi)^2 + r^2)^{3/2}} \right]
$$

Non-slippery condition gives the velocity of the fluid on the surface is $-v$. So

$$
-v = u = \int_{-L/2}^{L/2} \frac{F_x}{8\pi \mu} f(\xi) d\xi \left[ \left( \frac{1}{(x - \xi)^2 + r^2} \right)^{1/2} + \frac{(x - \xi)^2}{((x - \xi)^2 + r^2)^{3/2}} \right]
$$

When the cylinder is symmetrical with respect to the plan $x = 0$, we used a polynomial function to approach $f(\xi)$.

$$
f(\xi) = -8\pi \mu v \left[ A_0 + A_1 \left( \frac{\xi}{L/2} \right)^2 + A_2 \left( \frac{\xi}{L/2} \right)^4 \right]
$$
Then we can integrate equation \[\text{C.25}\]. We got
\[
2 \left[ A_0 + A_1 \left( \frac{x}{L/2} \right)^2 + A_2 \left( \frac{x}{L/2} \right)^4 \right] \ln \frac{4 [(L/2)^2 - x^2]}{r^2} -
(2A_0 - 2A_1 + A_2) - (8A_1 - 2A_2) \left( \frac{x}{L/2} \right)^2
- \frac{31}{3} A_2 \left( \frac{x}{L/2} \right)^4 - 1 = 0
\]
(C.27)

We simplified the cylinder to an ellipsoidal body. In that case we have
\[
\left( \frac{x}{L/2} \right)^2 + \left( \frac{r}{r_0} \right)^2 = 1,
\]
(C.28)

where \(r_0\) is the equatorial radius of the ellipsoid. To satisfy equation \[\text{C.27}\], we chose the parameter
\[
A_0 = \frac{1}{2 \ln(L^2/r_0^2) - 2}, \quad A_1 = 0, \quad A_2 = 0
\]
(C.29)

The driving force is obtained from the integral:
\[
F = - \int_{-L/2}^{L/2} f(\xi) d\xi = 8\pi\mu L \left( A_0 + \frac{1}{3} A_1 + \frac{1}{5} A_2 \right)
= \frac{2\pi\mu Lv}{\ln(L/r_0) - 0.5}
\]
(C.30)
(C.31)

A more precise approach to the cylinder shape gives \[79\]
\[
F = \frac{2\pi\mu Lv}{\ln(L/r_0) - 0.72}
\]
(C.32)

which is the equation \[3.5\] in chapter \[3\].
Bibliography


[36] *Harvard CNS Tool Infomation*.


