Ultra-Thin Solid-State Nanopores: Fabrication and Applications

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Ultra-Thin Solid-State Nanopores: Fabrication and Applications

A dissertation presented
by
Aaron Tzeyang Kuan
to
The School of Engineering of Applied Sciences
in partial fulfillment of the requirements
for the degree of
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Ultra-Thin Solid-State Nanopores: Fabrication and Applications

ABSTRACT

Solid-state nanopores are a nanofluidic platform with unique advantages for single-molecule analysis and filtration applications. However, significant improvements in device performance and scalable fabrication methods are needed to make nanopore devices competitive with existing technologies. This dissertation investigates the potential advantages of ultra-thin nanopores in which the thickness of the membrane is significantly smaller than the nanopore diameter. Novel, scalable fabrication methods were first developed and then utilized to examine device performance for water filtration and single molecule sensing applications.

Fabrication of nanometer-thin pores in silicon nitride membranes was achieved using a feedback-controlled ion beam method in which ion sputtering is arrested upon detection of the first few ions that drill through the membrane. Performing fabrication at liquid nitrogen temperatures prevents surface atom rearrangements that have previously complicated similar processes. A novel cross-sectional imaging method was also developed to allow careful examination of the full nanopore geometry.

Atomically-thin graphene nanopores were fabricated via an electrical pulse method in which sub-microsecond electrical pulses applied across a graphene membrane in electrolyte solution are used to create a defect in the membrane and
controllably enlarge it into a nanopore. This method dramatically increases the accuracy and reliability of graphene nanopore production, allowing consistent production of single nanopores down to subnanometer sizes.

In filtration applications in which nanopores are used to selectively restrict the passage of dissolved contaminants, ultra-thin nanopores minimize the flow resistance, increasing throughput and energy-efficiency. The ability of graphene nanopores to separate different ions was characterized via ionic conductance and reversal potential measurements. Graphene nanopores were observed to conduct cations preferentially over anions with selectivity ratios of 100 or higher for pores as large as 20 nm in diameter, suggesting that porous graphene membranes can be used to create highly effective cation exchange membranes for electrodialysis filtration. These surprisingly high selectivities cannot be explained by current models of ionic conduction in graphene nanopores, motivating the development of a new model in which elevated concentrations of mobile cations near the graphene surface generate additional ion selectivity.
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1 INTRODUCTION

1.1 BIOLOGICAL NANOPORES

Nanopores are very small holes in thin membranes. As the only connections between the two chambers on either side of the membrane, nanopores regulate which ions or molecules can pass from one chamber to the other. In this way, nanopores serve as a crucial link between the nanoscale environment inside the nanopore and the net flow of ions or molecules. As a result, nanopores serve a plethora of functions in nature, and are an essential part of the nanoscale machinery of the cell.

All bacterial, plant, and animal cells are enclosed by lipid bilayers, which prevent the passage of practically everything except small uncharged molecules. Indeed, many vital elements of cells like proteins, DNA, and ions are transported into and out of the cell by protein nanopores traversing the lipid membrane. In addition to regulating transport into and out of cells, nanopores are also a critical element of cognitive signaling. Ion channels, essentially ion selective nanopores, are instrumental for producing action potentials, which enable fast signal propagation between neurons and provide the basis for cognitive computation.

Biological nanopores are also a rich source of inspiration for engineered nanotechnologies. Protein nanopores can be employed ex vivo in bioengineered DNA-sequencing devices, in which DNA molecules are pulled through a single nanopore and concurrent modulations in the ionic current through the pore are analyzed to deduce the DNA sequence. As a result of directed research over the last 20 years, nanopore-
based DNA sequencing is now a rapidly growing technology offering fast read times and potentially unlimited read lengths. In this capacity, biological nanopores have also inspired and informed efforts towards sequencing with synthetic nanopores.

1.2 SOLID-STATE NANOPORES

In recent decades, the advent of nanotechnology has enabled the fabrication of synthetic nanopores, usually named solid-state nanopores in reference to the rigid membrane materials that were originally developed for solid-state electronics. For the same reasons that biological nanopores are so ubiquitous in nature, solid-state nanopores have promising potential for filtration and molecular sensing applications.

There are a number of reasons that future generations of nanopore devices may be based on solid-state materials, such as silicon nitride or graphene, instead of protein pores in lipid bilayers. Biological proteins, enzymes, and lipid bilayers are only stable in a limited range of environments, and can be easily destroyed by mechanical stresses, high electric fields, temperature extremes and harsh pHs. Solid-state nanopores, on the other hand, can be engineered to be much more robust, and can be easily modified and tuned by altering fabrication processes. Pore modifications in solid-state nanopores can also be extended to integration into electronic devices. For example, integrated transistors near the pore\textsuperscript{14-17} can be used to detect DNA translocations, potentially allowing parallel sensing from large arrays of nanopores in a single fluidic well.
Currently, the precise and reproducible fabrication of solid-state nanopores suited for DNA sequencing and filtration applications still remains a considerable challenge. Precise control of both the nanopore area and length of the nanopore constriction (i.e. the membrane thickness near the pore) is needed. Indeed, ultra-thin nanopores possess important application advantages, such as superior spatial resolution for sensing and low flow resistance for filtration. Over the last decade, interest in nanopore DNA sequencing has spurred the development of a number of fabrication strategies that have produced solid-state nanopore devices capable of detecting single molecules of DNA. However, these fabrication strategies generally produce pores too thick to resolve single DNA bases. Furthermore, existing fabrication methods tend to be labor-intensive, low-yield, and expensive, making a transition from academic research to industrial scale difficult.

1.3 Organization of the Thesis

This dissertation describes new methods for fabricating ultra-thin nanopores and presents experimental results characterizing these nanopores for filtration and molecular sensing applications.

Chapter 2 explores the fabrication of nanopores in silicon nitride, traditionally regarded as the material of choice for solid-state nanopores due to its structural properties and chemical stability. A novel fabrication method for precise and scalable fabrication of very thin silicon nitride nanopores is developed. Additionally, a new
technique for imaging nanopores in cross-section is introduced, which allows the direct measurement of nanopore geometry. The results presented in this chapter were published in an article in *Applied Physics Letters* entitled “Nanometer-Thin Solid-State Nanopores by Cold Ion Beam Sculpting”\(^\text{18}\).

Chapter 3 investigates the fabrication of atomically-thin graphene nanopores. A novel method for creating and enlarging graphene nanopores in solution using high-voltage electrical pulses is introduced. This new method allows reliable fabrication of single subnanometer graphene nanopores for the first time, and drastically improves the ease and feasibility of graphene nanopore experiments. The results presented in this chapter were published in an article in *Applied Physics Letters* entitled “Electrical Pulse Fabrication of Graphene Nanopores in Electrolyte Solution”\(^\text{19}\).

Chapter 4 characterizes the ability of graphene nanopores to separate different ionic species with filtration applications in mind. Measurements of ion selectivity as a function of pore size reveal that even rather large pores (20 nm in diameter) can effectively filter out chlorine anions, suggesting that porous graphene membranes can be very efficient membranes for electrodialysis filtration. A new model of conductance in graphene nanopores is presented to explain the mechanism behind the surprisingly high selectivities observed. The results presented in this chapter were published in an article in *Nature Communications* entitled “Ion Selectivity of Graphene Nanopores”\(^\text{20}\).

Chapter 5 is a discussion of the implications of the results contained within this dissertation. The remaining steps for achieving DNA sequencing and water
desalination with ultra-thin solid-state nanopores are outlined. Additionally, we discuss possible future investigations into the mechanisms of electrical pulse fabrication and ion conduction through ultra-thin nanopores.

Appendices A, B, and C present supporting material for Chapters 2, 3, and 4, respectively. Important concepts are explored in more detail, and supplemental data is present where necessary. Appendix D examines the prospects of DNA sequencing with graphene nanopores, which in principle should be the thinnest and most sensitive nanopore sensors. We find that while sequencing signals of graphene nanopores are very responsive, they are also hampered by high levels of low-frequency noise. A preliminary investigation of strategies for mitigating this low-frequency noise is presented.
1.4 REFERENCES


2 FABRICATION OF THIN SILICON NITRIDE NANOPORES

2.1 OVERVIEW

The first solid-state nanopores used to detect individual DNA molecules were created in silicon nitride (SiN\(_x\)) membranes by Jene Golovchenko and colleagues using a method called “ion beam sculpting” (IBS), a method that resulted from initially counterintuitive results\(^1\). The original idea was to mill a bowl-shaped cavity on one side of a SiN\(_x\) membrane and sputter away the membrane from the opposite side using a broad ion beam. By monitoring the transmitted ions in real time using a single ion detector, one could observe when the membrane surface intersected the cavity, forming a nanopore, and stop the sputtering when the pore reached the desired diameter (Fig 2.1a,b). However, this ion sculpting system\(^2\) did not work as predicted. No transmitted ions were detected until after the entire membrane had been sputtered away. Control experiments starting with through larger holes (~100 nm in diameter) instead of cavities revealed that the pores shrink rather than expand under the beam. This phenomenon was exploited to accurately close holes down to pores <10 nm, using the transmitted beam to monitor the pore size (Fig 2.1c).
Fig. 2.1 - Ion Beam Apparatus and Process
(a) Schematic of ion sculpting system. (b) Proposed process for creating a nanopore. (c) Pore closing process.
Atomic force microscopy (AFM) revealed that the pore-closing process involves the accumulation of a volcano-like mound of material in and around the pore\textsuperscript{3}. These nanometer sized pores were capable of detecting single molecules of dsDNA as they were electrophoretically pulled through the pore (known as a “translocation”), as well as distinguishing when DNA strands entered the pore in folded or unfolded configurations\textsuperscript{4}. While the IBS pore closing method affords precise control of the nanopore diameter (down to ~1 nm), the relatively thick volcano-shaped pores are not ideal for high resolution sensing. Furthermore, the exact material constituency of the material that defines IBS nanopores is still unknown, and recent measurements have even suggested that they could be composed of carbon contaminants rather than silicon nitride (see Appendix A.6).

Therefore, recent efforts have been directed towards achieving the original pore opening strategy (Fig 2.1a) by working at low temperatures. It has been observed that pore closing rates slow down as the temperature is lowered, and below about 4º C pores no longer close at all, presumably due to the “freezing out” of the surface diffusivity of adatoms\textsuperscript{1,5}. This led the investigators to propose a semi-quantitative adatom diffusion model in which the ion beam activates mobile surface atoms that accumulate near the pore as a result of the electric field caused by the accumulation of charge on the membrane\textsuperscript{1,3,5,6}. Alternatively, it has also been suggested that viscous flow of an irradiated surface layer causes the rearrangement of atoms near the pore\textsuperscript{7}. 
In Section 2.2, we demonstrate a cold ion sculpting technique that allows nanopore fabrication without pore closing effects. We found that this method not only produces thinner nanopores than the pore closing methods, but also is more easily reproducible because the final pore geometry is directly related to the shape of the original cavity.

In Section 2.3, we measure the thickness of the pore edges with high resolution, using a new protocol that allows cross-sectional TEM imaging of nanopores. Previously, the geometry of solid-state pores has been measured using electron tomography (ET)\textsuperscript{8,9}, energy-filtered TEM (EFTEM)\textsuperscript{9–11} and atomic force microscopy (AFM)\textsuperscript{11}, but the none of these methods have achieved subnanometer resolution maps of the pore interior. Therefore, we developed a new sample preparation technique that involves filling the pore with heavy metal, extracting it from the chip and depositing on a TEM grid so that it can be imaged in cross-section.

In Section 2.4, we demonstrate the feasibility of CIBS nanopores as single molecule sensors by performing ionic current noise analysis and DNA translocation experiments. These experiments suggest that ultra-thin CIBS nanopores have increased sensitivity to translocating molecules, but a precise determination of their ability to distinguish different DNA bases must await more complicated experiments.

Appendix A contains supporting material for this chapter, including a comparison of the CIBS method to alternative pore fabrication methods and detailed descriptions of relevant experimental methods. Also included is an investigation of the
material properties of the “volcano” material of ion beam sculpted pores and the role that carbonaceous contaminants may play in the pore closing process.

2.2 Cold Ion Beam Sculpting Method

A focused ion beam (FIB) machine (FEI Micron, 50 keV Ga) was used to mill a bowl-shaped (~100 nm diameter) cavity on one side of a ~250 nm thick free-standing silicon nitride (SiN) membrane. Then, in an ion sculpting apparatus described elsewhere\(^5\) (Fig. 2.1a), the membrane was cooled below 173 K using liquid nitrogen and sputtered from the side opposite the cavity using a ~1 mm diameter pulsed (\(\Delta T = 100\) ms) beam of 3 keV argon ions with flux \(F = 1.65 \pm 0.28\) ions/(nm\(^2\)·s). Ions transmitted through the membrane during sputtering were monitored using an electrostatic analyzer and a single ion detector (Channeltron). At first, no ions are observed because the membrane blocks the ion beam. As the membrane surface is sputtered away, it eventually breaks through the bottom of the bowl-shape cavity on the opposite surface, creating a nanopore with a thin constriction (Fig. 2.2a). After breakthrough, the ions are transmitted with a count rate proportional to the area of the nanopore. This allows one to monitor the pore size in real-time and halt the process when the pore reaches a desired diameter.

To demonstrate this control we created pores of different diameters using the same argon ion flux \(F\) but varying the count rate at which the sputtering process was
stopped. The diameter $D$ of the pore is related to the instantaneous ion count rate at the end of the ion sculpting process $c(t_f)$ by

$$c(t_f) = \left(\frac{F\pi}{4}\right) D(t_f)^2$$  \hspace{1cm} (2.1)

However, the number of ions counted during the $i$th pulse $C_i$ is the average of $c(t)$ over the pulse length $\Delta T$. Using a third order Taylor approximation for $c(t)$, we can approximate $c(t_f)$ using $C_i$ from the final three pulses, defining the adjusted final ion count $\tilde{C}_f$ as

$$\tilde{C}_f \equiv c(t_f) \Delta T \approx C_f + \frac{1}{2}(C_f - C_{f-1}) + \frac{1}{3}(C_f - 2C_{f-1} + C_{f-2})$$  \hspace{1cm} (2.2)

and obtain an estimate of the pore diameter

$$D(t_f) \approx \frac{2}{\sqrt{\pi}} \sqrt{\frac{\tilde{C}_f}{F\Delta T}}$$  \hspace{1cm} (2.3)

In Fig. 2.2b, the light blue band shows the expected relationship between adjusted ion counts and pore diameter given above; its width is determined by the systematic uncertainty in the ion flux. We also measured the diameters of the CIBS nanopores in a TEM (JEOL 2100) for each data point in Fig. 2.2b. The diameters and vertical error bars were determined from plan view TEM images (Fig. 2.2b, insets) using a radial averaging algorithm$^{12}$, and the horizontal error bars account for counting statistics. For pores $\geq 10$ nm, the prediction of diameter based on $\tilde{C}_f$ is very accurate, but pores $< 10$ nm appear larger than expected. This is most likely due to pore opening by electron sputtering during plan view TEM imaging. The accuracy of pore diameter
control ultimately depends on the sputter yield, which determines how many ions one counts for a given amount of material removed. For argon ions on silicon nitride, pores as small as 6 nm in diameter could be fabricated reproducibly. Sputtering using ions with lower sputtering yields should allow even smaller pores to be fabricated.
Fig 2.2 - Cold Ion Beam Sculpting Method

(a) Count rate trace of cold ion beam sculpting (CIBS) of a 50 nm diameter nanopore in a SiNₓ membrane. Data points indicate count rates for successive ion pulses. The line is merely a guide to the eye and is not a fit to the data. Inset: Plan-view TEM image of resulting nanopore. (b) Nanopore diameter as a function of adjusted final ion counts. The thick blue line indicates the expected pore diameters based on the ion flux; its width is due to uncertainty in the ion flux. Data markers represent diameters of CIBS nanopores fabricated in a SiN membrane as measured by TEM. Insets: TEM images of the nanopores from which the nanopore diameters were measured.
2.3 CROSS-SECTIONAL IMAGING OF NANOPORES

To characterize the profiles of the nanopore edges, we imaged cross-sections of the nanopores (along the plane of the membrane) using TEM. First, we coated the membrane on both sides with a 20 nm thick atomic layer deposition (ALD) of halfnium oxide (HfO$_2$)\textsuperscript{13} that fills the nanopore conformally, creating a nanopore mold. This mold was then protected by depositing metal layers on both sides (100 nm Ag and 1 µm Pt on cavity and flat sides, respectively). Using a dual beam FIB-SEM system (Zeiss), we excised from the membrane a lamella (~2 µm thick) containing the filled nanopore, transferred the lamella to a TEM grid using a micro-manipulator (Omniprobe), and thinned it until it was electron transparent (~100 nm, Fig 2.3a)\textsuperscript{14}. It is important to note that the pore is contained completely within the lamella – because the sample is imaged by transmission, the pore does not need to be cut in half by the FIB beam. Because of its higher atomic number, the HfO$_2$ gives strong scattering contrast compared to the SiN$_x$ membrane. As a result, a projected cross-sectional image of the nanopore is produced (Fig. 2.3b-e, top). The resolution of this cross-sectional image is limited by the lamella thickness and varies from sample to sample. We determined the resolution empirically\textsuperscript{15} by examining the edge between the HfO$_2$ layer and the SiN membrane away from the pore; for most samples the resolution was ~1 nm.
Fig 2.3 - Cross-Sectional Imaging of Ion Beam Sculpted Nanopores.

(a) Exploded view of sample lamella prepared for cross-sectional imaging. Conformal HfO$_2$ deposition provides a high-contrast mold for electron imaging. After capping with protective metal layers, the sample lamella is excised in a FIB-SEM and loaded onto a grid for cross-sectional TEM imaging. (b-e) Top: Cross-sectional TEM images of nanopore edges with cubic polynomial fits of edge profiles (black curves). Bottom left: Larger field of view cross-sectional TEM images. Bottom right: Plan view TEM images of nanopores. (b) 6 nm diameter CIBS nanopore from a deep cavity. (c) 33 nm diameter CIBS nanopore from a shallow cavity. (d) 20 nm diameter room-temperature ion sculpted nanopore. (e) 6 nm diameter electron drilled nanopore.
Since CIBS pores have rounded edges, the most meaningful and important measure of the nanopore profile is the edge radius of curvature. The edge profiles of the pores were determined from the cross-sectional TEM images by following a constant intensity contour at the intensity value corresponding to the edge of the membrane, which was determined by inspecting a line profile across the interface between the SiN membrane and the HfO$_2$ layer. The edge radius was extracted from this contour $(x_i, y_i)$ using a cubic least-squares fit $x_i - X_o = A(y_i - Y_o)^3 + B(y_i - Y_o)^2$, where the radius of curvature is $R = 1/2B$ (black curves in Fig. 2.3b-d, top). The uncertainty in $R$ was propagated from the standard errors in the parameter $B$ from the least-squares fit and the uncertainty in the intensity value defining the contour.

Fig. 2.3b shows a cross-sectional image of a CIBS nanopore with edge radius $R = 1.5 \pm 0.3$ nm. The incident argon ions sputtered the top side of the membrane where a $\sim 5$ nm thick layer implanted with argon is visible. This depth is consistent with simulations performed using the ion stopping simulation SRIM$^{16}$. The pore diameter $D = 6.6 \pm 0.3$ nm was measured from the plan view TEM image taken before the pore was filled with HfO$_2$ (Fig. 2.3b, bottom right). A larger field-of-view cross-sectional image (Fig. 2.3b, bottom left) reveals the shape of the cavity formed with the FIB on the bottom side of the membrane.

Very different pore profiles can be obtained from different cavity geometries, which depend on how deeply the cavities are milled into the SiN membrane. Fig. 2.3c shows a nanopore fabricated using a shallow cavity milled in an $\sim 80$ nm thick SiN
membrane. The bottom of this cavity (Fig. 2.3c, bottom left) is much flatter than the deep cavity shown in Fig. 2.3b. The cross-sectional TEM image (Fig. 2.3c, top) reveals a smaller edge radius ($R = 1.0 \pm 0.3 \text{ nm}$) than the deep cavity CIBS pore. Away from the cavity, the SiN membrane has been thinned to ~30 nm. The pore diameter measured from the plan view image (Fig. 2.3c, bottom right) $D = 33 \pm 3 \text{ nm}$ is consistent with the cross-sectional profile. Although the cavity geometry seems to have some effect on the edge radius, we hypothesize that the minimum edge radius for CIBS pores is ultimately limited by the ion penetration depth. This is because edges with radii much smaller than the ion range are quickly sputtered from the back and side surfaces. Therefore, even thinner edges may be obtained by using heavier ions or lower ion energies, both of which exhibit smaller penetration depths. The top surface of the CIBS pores shown in Fig. 2.3b and 2.3c are observed to deflect downwards close to the pore. We attribute this effect to biaxial compressive stresses induced in the membrane by the FIB during cavity formation; its effects have been observed in free-standing silicon nitride films by Kim et al\textsuperscript{17}.

For comparison, we also imaged a silicon nitride nanopore fabricated by room temperature ion sculpting\textsuperscript{1}. Fig. 2.3d demonstrates that it has a larger edge radius ($R = 5.6 \pm 0.2 \text{ nm}$) than the CIBS pores. The large field-of-view cross-sectional image of the room temperature ion sculpted pore reveals the volcano-like mound of accumulated adatom material (Fig. 2.3d, bottom left), which is significantly less dense than and
probably quite different from the original membrane material. The nature of this material is examined in more detail in Appendix A.6.

An electron beam drilled nanopore\textsuperscript{18} was also imaged (Fig. 2.3e, see also Appendix A.1), revealing that the pore has an asymmetric hourglass shape. Since the walls of the pore are almost straight, the thickness of the pore is approximately the thickness of the membrane (~80 nm). One can produce thinner pores with the electron beam drilling method by starting with thinner membranes: Wanunu et al. have demonstrated that SiN membranes as thin as 6 nm are robust enough to support electron beam drilled nanopore devices\textsuperscript{11}.

2.4 FLUIDIC PORE CHARACTERIZATION

To demonstrate the feasibility of CIBS nanopores as single molecule sensors, we measured the noise power spectral densities (PSD) (Fig. 2.4a), and translocations of 10 kbp dsDNA (New England Biolabs) (Fig. 2.4b) through an ~8 nm diameter nanopore similar to that shown in Fig. 2.3b. Nanopores were characterized in a 100 mM KCl/10 mM Tris/1 mM EDTA solution at pH 10 using a custom flowcell with Ag/AgCl electrodes and a patch clamp amplifier (Axopatch) filtered through a 20 kHz low pass filter (see Appendix A.2 for details). The pore exhibited a zero-bias conductance of $G = 27.9 \pm 0.5$ nS (see Appendix A.3 for details). At low frequencies (< 100 Hz), the power spectral density is dominated by $1/f$ noise, whereas at higher frequencies only
thermal (Fig. 2.4a, dashed line) and capacitively coupled preamplifier noise are observed\textsuperscript{19,20} (see Appendix A.4 for details). At 100 mM salt, DNA translocation can be detected by ionic current enhancements due to the counter ions around the DNA molecule acting as mobile charge carriers\textsuperscript{21} (see Appendix A.5 for details). Here we detected gel-purified 10 kbp fragments of \( \lambda \) dsDNA (New England Biolabs, \( \sim 12.5 \mu g/mL \)) with a 200 mV bias (Fig. 2.4b), observing predominantly unfolded events (white traces). The histogram of the current enhancement versus event duration shows clustering around events with median current enhancements of 57 pA and median duration of 132 \( \mu s \).

Fig 2.4 Nanofluidic Characterization of a Cold Ion Beam Sculpted Nanopore
(a) Noise power spectral density of current through an 8 nm diameter nanopore with no bias (cyan) and with 200 mV bias (red) in 100 mM KCl, pH 10. The dashed line indicates expected thermal noise. (b) Histogram of 108 DNA translocation events with a 200 mV bias (10 kbp dsDNA in 100 mM KCl solution), showing clustering around 57 pA, 132 \( \mu s \) events. White traces: 3 typical current-time traces of translocation events.
2.5 SUMMARY

We have shown that nanopores with edge radii as small as 1 nm and diameters as small as 6 nm can be reliably fabricated using cold ion beam sculpting. Practical upscaling of this process can be achieved using wafer-scale lithography methods (photolithography and RIE) to prepare the cavities and a broad ion beam to create the pores. Moreover, we believe that even smaller and thinner pores can be obtained with CIBS using lower energies and/or different sputter ions. We have presented cross-sectional images of nanopores that clearly demonstrate the advantages of CIBS nanopores and offer an unprecedented perspective on solid-state nanopore devices.

2.6 REFERENCES


3 Fabrication of Graphene Nanopores

3.1 Overview

Graphene membranes are the thinnest possible barrier to ions or molecules, and are mechanically robust enough to withstand fluidic or pressurized environments. Recently, intense interest has been focused on using graphene for nanopore applications, because its extreme thinness confers unique advantages, such as superior spatial resolution for nanopore DNA sequencing\textsuperscript{1,2} and low flow resistance for nanofiltration\textsuperscript{3,4} and gas separation\textsuperscript{5,6}. Graphene was first isolated by Novoselov, Geim, and colleagues in 2004\textsuperscript{7} by mechanically exfoliating single flakes from a block of graphite. Depositing these flakes on prepared scaffolds demonstrated that the 2D crystal is strong and stable enough to exist suspended at room temperature, despite previous notions that such structures were intrinsically unstable\textsuperscript{8}. Garaj, Golovchenko, and colleagues first demonstrated in 2008 that single layer graphene membranes can be used as a barrier in a fluidic environment. They exfoliated graphene flakes onto silicon nitride apertures, selected single layer regions, and used a TEM to drill nanopores that were able to detect double stranded DNA\textsuperscript{9}. Around the same time, graphene nanopores were also fabricated by in the Drndic and Dekker groups, although the membranes in these studies were likely multilayer graphene considerably thicker than monolayer graphene\textsuperscript{10,11}. 

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While exfoliated graphene was sufficient for proof-of-principle studies, it was too time-consuming even for research-scale pore fabrication. Since exfoliated graphene flakes are generally not larger than 10 μm, each membrane had to be transferred separately, and individually screened for single-layer areas. However, reliable growth of large area (cm scale) single-layer graphene is now achievable using chemical vapor deposition (CVD) on copper foils (see Appendix B.1 for details). These graphene films can be transferred to arrays of apertures all at once, allowing the preparation of suspended graphene membranes to be performed at scale (see Appendix B.2 for details). Therefore, the TEM drilling step (see Appendix B.3 for details) has become the major bottleneck for graphene nanopore fabrication. While TEM drilling allows one to unambiguously fabricate a single nanopore in a pristine membrane and immediately verify its size via electron imaging directly after drilling, it comes with the drawbacks of high equipment costs, low throughput, and a tendency to introduce contamination (see Appendix B.4 for details). On the other hand, bulk methods such as ion bombardment, plasma etching, or UV ozone treatment can scalably create pores but cannot precisely control their size or number.

Ideally, a nanopore fabrication method would be both scalable and precise. In this chapter, we demonstrate an electrical pulse fabrication method that requires only a simple fluidic cell and modest electronics, yet dramatically increases the accuracy and reliability of graphene nanopore production, allowing consistent production of single nanopores down to subnanometer sizes. This method is an improvement on electron
drilling in precision, reliability, convenience, cost, and scalability, with the only major drawback being that pores cannot be immediately imaged after fabrication.

3.2 Electrical Pulse Fabrication Method

A schematic of the experimental setup is shown in Fig. 3.1a. Suspended single layer graphene membranes (Fig 3.1b,c) are loaded into a fluidic cell and wet on both sides with 1 M KCl solution. Ag/AgCl electrodes are used to monitor transmembrane current (at 100 mV DC bias) or apply short, 250 ns voltage pulses across the membrane that create or enlarge nanopores in the membrane. Such short pulses were used to minimize the amount of membrane material removed during each pulse, which translates into accurate control over nanopore size. The pulse length of 250 ns was selected to be as short as possible while still being long compared to the RC charging time of the device (Fig 3.1e,f). Fig. 3.1d shows a representative experimental example of pore nucleation (creation of a very small nanopore) and progressive enlargement culminating in a 2.2 nm pore. At first, the membrane exhibits very little leakage current ($\ll 1$ nA), indicating that it is defect-free. 7 V nucleation pulses are repeatedly applied until a discernable jump in current is observed, indicating the nucleation of a nanopore. Since it usually takes several pulses before a pore is nucleated, it is unlikely that more than one pore will be created in the first successful nucleation pulse. After pore nucleation, lower voltage enlargement pulses of 5 V are successively applied to controllably increase the pore size.
Fig 3.1 - Electrical Pulse Fabrication

(a) Experimental setup, including suspended graphene membrane. (b) TEM micrograph of a suspended graphene membrane. (c) Electron diffraction of a suspended graphene membrane. (d) Experimental current data from the fabrication of a 2.2 nm pore. Electrical pulses are indicated by vertical bars (including 1 s measurement pauses). Pore nucleation is indicated by a sharp increase in current after a 7 V pulse. The pore is then enlarged with the repeated application of 5V enlarging pulses until the desired pore size (right-hand vertical axis) is achieved. (e) Approximate circuit diagram of nanopore setup. (f) A 5V, 250 ns input pulse (blue trace) was measured using a 10kΩ/50Ω voltage divider (exponential rise and fall presumably due to ~2 pF of parasitic capacitance). The response of a suspended graphene membrane device shows that the membrane is charged to the full voltage (\(V_{in} - V_{out} \approx 5 \text{ V}\)) for most of the duration of the pulse. (g) Change in diameter due enlargement pulses, \(\Delta D_E\), plotted as a function of the calculated pore diameter before the pulse, showing that the changes in diameter are approximately independent from the pore diameter. (h) IV curves of a graphene membrane before and after pore fabrication (5.5 nm pore). (i) Noise power spectrum of the same nanopore with 100 mV applied bias. The red line is a 1/f fit to the data.
The measured transmembrane current can be converted into a real-time measure of the pore diameter (right-hand vertical axis, Fig 3.1d) using the analytical approximation\(^\text{18,19}\)

\[
D = \frac{G}{2\sigma} \left( 1 + \sqrt{1 + \frac{16\sigma T}{\pi G}} \right) \tag{3.1}
\]

where \(D\) is the pore diameter (nm), \(G\) is the trans-membrane conductance (nS), \(\sigma\) is the solution conductivity (S/m), and \(T = 0.6\) nm\(^9\) is the effective thickness of the graphene. While 5 V pulses are usually ineffective for nucleating pores, their ability to enlarge pores once they have been nucleated is perhaps due to the increased reactivity at graphene edges\(^\text{20,21}\), weaker bonding of edge atoms compared to bulk\(^\text{16}\), or elevated temperatures in the pore due to Ohmic heating\(^\text{22}\). The change in diameter for each enlarging pulse is generally independent of the size of the pore before the pulse is applied (Fig. 3.1g). After the electrical pulsing process, nanopores show approximately linear I-V curves with stable conductance (Fig 3.1h). The as-fabricated graphene nanopores display considerable low frequency noise with a 1/f-like spectrum, the reduction of which remains an important technological challenge (Fig. 3.1i). However, the noise levels of electrically fabricated graphene nanopores are not significantly different from that of TEM drilled graphene nanopores in other studies\(^\text{2,15,23,24}\) and seems to be intrinsic to graphene nanopores, rather than a consequence of fabrication method.
To determine the accuracy of control over nanopore size, we gathered statistics on the electrical pulse fabrication process for many nanopores. The distributions of changes in nanopore diameter due to successful nucleation and enlarging pulses ($\Delta D_N$ and $\Delta D_E$, respectively) calculated from conductance measurements are plotted in Fig. 3.2a. These data suggest that pores as small as $\Delta D_N = 0.5 \pm 0.3$ nm (mean ± S.D.) can be nucleated and enlarged in steps of $\Delta D_E = 0.1\pm0.3$ nm. Note that $\Delta D_E$ was sometimes negative, which suggests that pulses may sometimes induce rearrangement or addition of atoms at the pore edge, decreasing the size of the pore. When attempting to fabricate a pore of a given size, there is always a small chance that a large $\Delta D_E$ can overshoot the target diameter. To understand these statistics, we simulated fabrication pulses by randomly choosing values of $\Delta D_N$ and $\Delta D_E$ from the distributions shown in Fig. 3.2a, applying successive pulses until a target diameter was reached or exceeded (Fig. 3.2b). The simulation shows that pores can be reliably fabricated within 0.2 nm (inset). Given that bond lengths in graphene are about 0.14 nm, these results suggest that enlargement pulses usually remove less than a single ring of atoms from the pore edge, allowing atomic-scale control of nanopore size.
Fabrication of single nanopores in silicon nitride (SiN$_x$) membranes via controlled dielectric breakdown has recently been demonstrated as a viable alternative to other fabrication techniques such as TEM drilling or ion beam sculpting. While it might be expected that exposure to high voltages would similarly induce damage in graphene membranes, it is not immediately obvious that the distinct physical and chemical properties of graphene would allow single pores to be fabricated with any degree of control. In particular, the dielectric breakdown mechanism responsible for pore fabrication in SiN$_x$ depends on the slow accumulation of structural defects throughout the membrane thickness over $10^1 - 10^5$ seconds, whereas the removal of any atoms from a graphene membrane immediately creates

Fig. 3.2 - Electrical Pulsing Statistics
(a) Histograms of changes in pore diameter due to successful 7 V nucleation ($\Delta D_N$) and 5 V enlargement pulses ($\Delta D_E$). Dotted lines indicate averages, which suggest that pores as small as $\Delta D_N = 0.5 \pm 0.3$ nm (mean ± S.D.) can be nucleated and enlarged in steps of $\Delta D_E = 0.1\pm0.3$ nm. (b) Simulation of pore fabrication generated by randomly choosing values $\Delta D_N$ and $\Delta D_E$ from the distributions shown in (a) until the target diameter is reached or exceeded. Inset: median error in diameter is 0.2 nm for pores larger than 0.5 nm.
pores within $10^{-6}$ s. It is actually quite surprising that single pores can be reliably formed and enlarged in graphene, as the formation of multiple pores or catastrophic failure of the membrane might initially seem more likely. Because experimental studies of nanopores in graphene (as well as other 2D materials such as boron nitride$^{34}$ and molybdenum disulfide$^{35}$) are currently dependent on TEM drilling, which is particularly challenging and contamination-prone for atomically-thin membranes, we anticipate the electrical pulse fabrication method shown here to be of considerable practical usefulness, as well as motivate further research on the nanoscale chemistry and mechanics of graphene.

3.3 DNA TRANSLOCATION EXPERIMENTS

DNA translocation experiments were performed after electrical pulse fabrication to confirm that only a single pore of the appropriate size is created and to demonstrate the suitability of these nanopores for single molecule measurements. To prevent DNA from adhering to the graphene surfaces, the membranes containing a nanopore were first coated on both sides with a self-assembled coating consisting of aminopyrene “foot” molecules that adhere to the graphene surface and tethered polyethylene glycol 4-mer (PEG4) “brushes” that render the surface hydrophilic and discourage DNA sticking (developed by Schneider et al.,$^{14}$ Fig. 3.3a). The coating reduces the conductivity of the pore, which can be approximated by$^{14,18,19}$
\[ G' = \sigma \left[ \frac{4T'}{\pi (D')^2} + \frac{1}{D'} \right]^{-1} = \sigma \left[ \frac{4(T + 2L)}{\pi (D - 2\Delta R)^2} + \frac{1}{(D - 2\Delta R)} \right]^{-1} \]  

(3.2)

where \( L \) is the effective length of the polymer, \( \Delta R \) is a measure of how much the coating obscures the pore opening, \( T = 0.6 \) nm is the effective thickness of the graphene\(^9\), and \( D \) is the diameter of the pore before coating given by eq. 3.1 (Fig. 3.3b). A least squares fit of eq. (2) to measured pore conductances (Fig. 3.3c) gives \( L = 1.7 \pm 0.5 \) nm (mean ± S.D.) and \( \Delta R = 0.1 \pm 0.2 \) nm, both physically reasonable values given the length of the PEG\(_4\) brush (1.56 nm) and the pyrene-graphene spacing (0.35 nm\(^3\)). It is worth noting that these conductance measurements can already distinguish a single nanopore from multiple smaller ones, since smaller pores are affected more substantially by the coating. Indeed, generalizations of eq. 3.2 to include multiple pores produce predictions inconsistent with the data (Fig. 3.3c, dashed lines), indicating that the fabrication method reliably produces a single pore.
Fig 3.3 – Pore Coating Experiment
(a) Self-assembled hydrophilic coating used to prevent DNA from sticking to graphene membranes (developed by Schneider et al.\textsuperscript{14}). Two step pore coating chemistry combines aminopyrene “foot” and polyethylene glycol 4-mer (PEG\textsubscript{4}) “brush” molecules. (b) Diagram of coated pore, illustrating increased thickness L and reduced diameter ΔR. (c) Measured pore conductances before and after coating, shown with predictions from an analytical model (eq. 3.2) with best fit coating parameters L = 1.7±0.5 nm and ΔR = 0.1±0.2 nm. Dashed lines show predictions for multiple equally sized pores, confirming that the electrical pulse method consistently produces a single pore.
After pore coating, 10 kbp double stranded DNA (dsDNA) fragments were injected on the grounded side of the membrane and electrophoretically driven through the pore with a 200 mV DC voltage bias in 3 M KCl. The threading of a DNA molecule as it passes through the pore causes a transient reduction in the current through the pore, known as a translocation event. The magnitude of the current blockage is associated with the cross-sectional diameter of dsDNA (~2.3 nm), whereas the duration depends on the length of the DNA fragment (~6 µm). A scatter plot of more than 200 translocation events through a representative nanopore (Fig. 3.4a) shows that most events have similar current blockages and durations corresponding to the monodisperse sample of injected DNA fragments. The tail in the distribution of translocation durations suggests that dsDNA sometimes temporarily sticks to the pore or membrane despite the polymer coating.
Fig 3.4 - DNA Translocations

(a) Scatter plot of current blockages and translocation durations for dsDNA translocations through an 8.0 nm coated graphene nanopore (10 kbp fragments, 3M KCl, 200 mV bias, n = 202). (b) DNA translocation blockages as a function of open pore current for 8 pores of different diameters. Calculated pore diameters are shown on the upper horizontal axis. Individual translocation events are plotted as small dots; large markers indicate pore averages, with error bars indicating the median 50% of events. The solid line indicates computational predictions from a finite element model of a pore threaded by dsDNA. This is not a fit to the data, as all free parameters have already been determined from Fig. 3c. Agreement between data and model confirms that calculated pore diameters are accurate. (c) Axisymmetric finite element model of the pore used for the computational predictions of open pore currents and DNA translocation blockages for various pore diameters. The color map in the images shows the magnitude of the current density (5 nm diameter, 3 M KCl, 200 mV bias).
To confirm that the nanopores match the sizes computed from their conductances, we performed DNA translocation experiments with several pores of different diameters and compared the current blockages to computational predictions from a finite element model of appropriately sized pores (Fig. 3.4b,c). This model included electrostatics and fluid dynamics (coupled Poisson-Boltzmann and Navier-Stokes equations) and was solved using the COMSOL Multiphysics software (COMSOL, Inc.). Uncoated graphene membranes were modeled as an insulating membrane of thickness $T = 0.6$ nm. Coated graphene membranes used $T = 4.1$ nm ($0.6$ nm + $2L$), and the DNA was modelled as an insulating, charged rod 2.3 nm in diameter. For such thin nanopores, the current blockage is quite sensitive to the diameter of the pore, allowing this measurement to serve as a consistency check on the nanopore diameters. It is important to note that the predicted curve in Fig. 3.4b is not a fit, as all model parameters have been determined via eq. 3.2. Although the model is only a simple approximation of the physical system, the data lies close to the predicted curve, verifying that the model accurately encapsulates the geometry of the fabricated pores. The slight deviations from the model predictions may be due to interactions between the DNA and the PEG brushes or other complications not included in the model. Transmission electron microscope (TEM) imaging was also attempted to directly verify the pore sizes, but turned out not to be a precise measure of pore size due to hydrocarbon contamination and/or damage from the electron beam (see Appendix B.4 for details).
3.4 MECHANISM OF PORE FORMATION

Determining the mechanisms responsible for electrical pulse nanopore formation and enlargement is a difficult problem. Redox reactions between graphene and water are likely responsible for removing carbon atoms from the lattice\textsuperscript{39}, but in this context oxidation and reduction half-reactions could act from opposite sides of the membrane, supporting an electrochemical current. Since the membrane is atomically thin, it is even possible that the half-reactions are coupled through the graphene and occur simultaneously. The fact that the method produces predominantly single nanopores suggests that the process is initiated via a fluctuation that randomly selects a site for a nanopore nucleation and then is limited to that site during enlargement. Given the extremely small amount of material being removed from the membrane (<100 atoms per pulse), it is unlikely that direct studies of reaction products will be feasible to study the mechanisms of nanopore formation. In this work we have already exploited the acute sensitivity of transmembrane current measurements to the removal of such small numbers of atoms from the membrane. Perhaps careful electrical measurements during the pulsing process could provide additional information. Nevertheless, a full mechanistic understanding of electrical pulse fabrication probably must await further fundamental research on graphene chemistry under high electric fields.
3.5 **SUMMARY**

We have shown that nanometer-sized single holes in graphene membranes can be fabricated via either TEM drilling (Appendix B.3) or Electrical Pulse Fabrication. While TEM drilling allows precise imaging of the nanopore immediately fabrication, it has many practical drawbacks that motivated the development of the pulse technique. By dramatically increasing the accuracy, reliability, and ease of graphene nanopore production, this electrical pulse method will help unlock the technological and scientific potential of graphene nanopores.

3.6 **REFERENCES**


4  **ION SELECTIVITY IN GRAPHENE NANOPORES**

4.1  **OVERVIEW**

Water scarcity is one of the most difficult global challenges we face today. Currently, more than one third of the world’s population live in water-stressed areas\(^1\). As population growth continues to outpace development of water infrastructure in many countries, desalination (the removal of salts from seawater) at high energy efficiency will likely become a vital source of fresh water. Currently, about half of desalinated water comes via distillation, technology that purifies seawater by evaporating it and recondensing the pure water. Distillation is gradually being replaced by newer technologies because it is energy inefficient\(^2\) (the extra heat of vaporization is not necessary to separate salt from water). The other half of desalinated water is mostly produced via reverse osmosis (RO), in which seawater is pressurized against a membrane that allows water to pass but retains salt ions (Fig 4.1a). Reverse osmosis is currently the most energy efficient technique, with state-of-the-art systems consuming only about twice the thermodynamic minimum energy required to desalinate water\(^3\) (the free energy of mixing). However, pre and post-treatment of the water, transportation, and construction of the plants and surrounding infrastructure all increase the total energy input required to produce desalinated water. Despite being a mature technology, RO membranes still have a lot of potential for improvement via new membrane materials. In particular, current RO membranes suffer from a large flow resistances and membrane fouling (clogging of the membrane by contaminants)\(^2\).
Fig 4.1 - Desalination Techniques
(a) Reverse Osmosis: saltwater feed is pressurized against a semipermeable membrane that allows the passage of water but not dissolved salts. (b) Electrodialysis: seawater feed is fed along parallel anion-permeable and cation-permeable exchange membranes. Under the electric field, dissolved salt ions are removed through one of the exchange membranes depending on their charge.

It has long been known that biological ion channels can selectively filter particular ions\(^4\), but similar filtration applications for solid-state nanopores have not been explored until recent advances have allowed fabrication of subnanometer sized pores. Solid-state nanopores are attractive for filtration applications because their properties can be easily tuned to optimize their effectiveness. Broadly speaking, porous membranes can be characterized by their filtration selectivity and filtration resistance. The selectivity indicates what percent of ions or impurities present in the original solution (feed) are excluded from the filtered solution (diluate), and the resistance (hydrodynamic or electrical) indicates how much energy (through the pressure or electric field) needs to be consumed to filter at a target rate. While nanopores are unlikely to improve on the already excellent selectivities achievable with current
membrane technologies, there is a significant potential for reduction of filtration resistance. The current state-of-the-art reverse osmosis membranes are generally composed of many layers of polymeric material, and have a trade-off between selectivity and resistance (both increase with membrane thickness). Ultra-thin nanopores, on the other hand, could potentially achieve high selectivities with much lower resistance.

As the thinnest possible membrane material, graphene is an attractive material choice for desalination. Recent investigations have suggested that porous graphene membranes can attain orders of magnitude higher flow rates than commercial reverse osmosis (RO) membranes while still providing excellent salt rejection. Unfortunately, RO salt rejection depends on a very tight distribution of subnanometer pores. A few large pores in a membrane can contribute large unselective water fluxes, impairing salt rejection. Thus, viable RO membranes depend on the complete elimination of pores larger than a nanometer or so, which remains a difficult fabrication challenge (see Appendix C.1 for more details).

However, graphene membranes with larger holes may be highly efficient membranes for electrodialysis (ED), an alternative desalination technique in which an applied electric field is used to remove unwanted ions through pores in an ion selective membrane. For seawater desalination, a stack of alternating cation-exchange (pass cations but block anions) and anion-exchange membranes are used to produce freshwater and concentrated waste streams (Fig 4.1b). The direction of the electric field
can be periodically reversed (called electrodialysis reversal, or EDR), which causes the product and concentrate streams to swap\(^\text{17}\). This does not undo the desalination because the product is collected continuously as a stream. The advantage of EDR is that it automatically defouls the membranes, drastically increasing their lifetime. Although currently electrodialysis techniques are not generally economically competitive with distillation and RO, they are often used in combination with other techniques, such as pretreatment for RO of highly concentrated feeds. Furthermore, with the introduction of new membrane materials such as graphene, electrodialysis may become an increasingly attractive option for desalination.

Recent theoretical predictions suggest that graphene nanopores that are too large for RO may still be suitable for electrodialysis (ED) if they are electrostatically charged, allowing them to separate anions from cations\(^\text{18-20}\). It is likely that the edges of graphene nanopores have chemical groups containing deprotonatable oxygen atoms, such as carboxyl or hydroxyl groups (similar to chemical groups found at edges of graphene sheets\(^\text{21}\)). Therefore, at neutral or basic pH, the graphene pore edges should be negatively charged. These charges will repel anions in solution over the length scale of the Debye screening length (beyond this distance, the charge is screened out and not “seen” by ions in solution). Therefore, it is expected that pores smaller than the Debye length in radius (~1 nm in 100 mM salt) should be strongly cation selective. This notion of ion selectivity involves comparing ionic fluxes of one type of ion versus another, and involves no explicit measurement of or comparison to the water flux. Ion selectivity has
been methodically explored for biological ion channels, but is much less well-explored in solid-state pores. While a few recent studies have made some measurements of ion selectivity in graphene nanopores\textsuperscript{14,15}, the topic has yet to be examined in detail. In particular, previous experimental investigations of ion selectivity have been limited to subnanometer pores, and do not examine the relationship between pore size and selectivity.

In order to determine the suitability of graphene pores for ED, we measured selectivity in graphene nanopores over a wide range of pore sizes. Our results indicate that graphene nanopores preferentially permit the passage of K\textsuperscript{+} cations over Cl\textsuperscript{-} anions with selectivity ratios of over 100 and conduct monovalent cations up to 5 times more rapidly than divalent cations. Surprisingly, the observed K\textsuperscript{+} /Cl\textsuperscript{-} selectivity persists in pores even as large as 20 nm in diameter, suggesting that high throughput, highly selective graphene ED membranes can be fabricated without the need for subnanometer control over pore size. We hypothesize that these high K\textsuperscript{+} /Cl\textsuperscript{-} selectivities might be caused by increased cation density along the graphene surface, allowing the entire graphene membrane to act as a cation collector for the pore. Numerical simulations were performed that show that such a model can produce results similar to our experiments\textsuperscript{22}. 
4.2 CATION / ANION SELECTIVITY

Here we examine ion selectivity (cations vs. anions, as well as amongst different cations) of single graphene nanopores with an emphasis on the relationship between ion selectivity and pore size. Studying single nanopores in small-area suspended membranes eliminates the problem of intrinsic defects and allows a fundamental examination of the nanopore properties. Inherent in this kind of approach is the assumption that a porous membrane containing many pores of similar size would have similar ion selectivity to a single pore.

Graphene nanopores were fabricated using a recently reported electrical pulse method that enables rapid fabrication of very small single nanopores, as well as controllable, in situ enlargement of the nanopore\textsuperscript{23} (see Chapter 3 for details). This method allows measurements to be performed for multiple pore sizes with a single sample, which would be extremely difficult and time consuming using electron beam drilling fabrication methods\textsuperscript{24–28}. In order to create a pore, freestanding graphene membranes were placed in a flow-cell between two fluid reservoirs filled with 1M KCl as schematically depicted in Fig. 4.2a. Ultra-short, high voltage pulses were applied across the membrane to nucleate and enlarge single nanopores. A transmission electron microscope (TEM) image of a graphene membrane after electrical pulse fabrication is shown in Fig. 4.2b. The outline of the pore can be clearly seen in a close-up of the image shown in Fig 4.2c. Although TEM imaging can be used to measure pore sizes as was done here, preparing and imaging samples after solution-based experiments is labor-
intensive and low-yield. Therefore, for the bulk of our experiments, we estimated the pore size based on the measured conductance of the nanopore in 1 M KCl solution using an analytical model of pore conductance\textsuperscript{23,24,29,30}, given by

\[
G = \sigma \left( \frac{4t}{\pi D^2} + \frac{1}{D} \right)^{-1}
\]  

(4.1)

where \( G \) is the pore conductance, \( \sigma \) is the solution conductivity (105 mS/cm), \( t \) is the effective thickness of the graphene membrane (0.6 nm\textsuperscript{24}), and \( D \) is the pore diameter. Fig. 4.2d shows the outline of the pore obtained via the TEM imaging (grey) compared to the estimated size of the pore based on the conductance of the pore in 1 M KCl at pH 2 (black circle). The close agreement suggests that Eq. 4.1 does an adequate job of estimating the pore size, precluding the need to image every sample with TEM. While the exact mechanism that produces electrically pulsed nanopores is not yet fully understood, it likely involves the oxidation of carbon at the pore edge\textsuperscript{23}, which results in carboxyl or other protonatable edge groups\textsuperscript{31} that bestow a negative charge on the edge of the pore at neutral and higher pH (Figure 4.2a, inset). Previous research on comparatively thick, insulating solid-state nanopores has demonstrated that negative charge at the periphery of the pore repels anions and attracts cations, which conduct the bulk of the ionic current\textsuperscript{32–34}. However, such electrostatically controlled ion selectivity was thought to be negligible for pores in which the diameter is significantly larger than the membrane thickness\textsuperscript{35}. Indeed, recent measurements have shown that sub-2 nm intrinsic defects in chemical vapor deposition (CVD) graphene membranes can
distinguish between mono and divalent cations, but comparable measurements for larger pores have not been performed\textsuperscript{15}. 

Figure 4.2. - Experimental Setup  
\textbf{(a)} Cross-sectional diagram of a suspended graphene nanopore sample immersed in electrolyte solution. Ag/AgCl electrodes that contact the solution via agarose salt bridges are used to measure ionic current through the nanopore or enlarge the nanopore using electrical pulses. \textbf{Inset:} Illustration of anion and divalent cation rejection in a negatively charged nanopore. \textbf{(b)} TEM image of suspended graphene membrane after a pore has been created via electrical pulse fabrication. White box indicates the location of the nanopore \textbf{(c)} Close-up of area containing a nanopore. The sides of the image are 30 nm in length. \textbf{(d)} Comparison of the size of the pore with estimation of the pore size calculated from the pore conductance via eq. 4.1. The grey outline is traced from the TEM image and the black circle is calculated from the conductance $G$. 

\[ G = 82 \text{ nS} \]
To measure cation/anion selectivity, current-voltage (I-V) curves were performed with a variety of KCl concentration gradients across the pore (Figure 4.3a). The voltage bias was applied via Ag/AgCl electrodes contacting the fluidic cell via agarose salt bridges used to eliminate the potential generated from redox reactions on electrodes in different salt concentrations. K\(^+\) and Cl\(^-\) ions were selected as a representative cation/anion pair because they have very similar bulk mobilities (see Appendix C.2), and therefore exhibit negligible liquid junction potentials, about 1 mV for our measurements (as calculated by the Henderson equation\(^36\)). Therefore, within experimental error (~5 mV), the measured voltages were equal to the voltage drop across the graphene membrane. An example set of I-V curves for a 3 nm pore is shown in Figure 4.3b. When there is no applied voltage (\(V_{app} = 0\)) both K\(^+\) and Cl\(^-\) ions diffuse from high to low concentration, and a net current (short-circuit current) is produced only if one ion diffuses at a higher rate than the other through the pore (Figure 4.3b, upper inset). The direction of this short-circuit current is consistent with the net flow of positive charges from high to low concentration, immediately indicating that the pore is cation selective. While the short-circuit current can identify selectivity, it is not a direct quantitative measure of selectivity because it also depends strongly on the conductance of the nanopore. A better choice is the reversal potential \(V_{rev}\), the applied potential at which the net current is zero (Figure 4.3b, lower inset), which in the well-known Goldman-Hodgkin-Katz (GHK) model does not explicitly depend on pore size\(^4,37\). By assuming that each ion species contributes a current given by the Nernst-Planck equation parametrized by an effective diffusion constant \(D_i^*\) that is different for each ion.
species $i$, the GHK model presents a quantitative measure of selectivity that is useful for comparing selectivity amongst different pores\textsuperscript{4,32,34}. In this context, the selectivity ratio $S_{GHK}$ is defined as $D_{K^+}^*/D_{Cl^-}^*$, which is equal to the ratio of drift currents from each ion when there is no concentration gradient (but see Appendix C.3). The reversal potential is related to the selectivity of the pore via the GHK voltage equation

$$V_{\text{rev}} = \frac{k_b T}{e} \ln \left( \frac{S_{GHK} c_{\text{high}} + c_{\text{low}}}{S_{GHK} c_{\text{low}} + c_{\text{high}}} \right),$$

(4.2)

where $S$ is the selectivity ratio, $c_{\text{high}}$ and $c_{\text{low}}$ are the solution concentrations in the fluid reservoirs, $e$ is the electron charge, $k_b$ is the Boltzmann constant, and $T$ is the solution temperature.

The K$^+$/Cl$^-$ selectivity ratio $S_{GHK}$ was calculated for each pore by fitting the reversal potentials to the GHK voltage equation (Eq. 4.2, Figure 4.3b, lower inset). Figure 4.3c shows the selectivity ratio $S_{GHK}$ plotted as a function of pore size for four samples at pH 8. The lower horizontal axis is the measured conductance in 1 M KCl, while the upper axis shows the estimated pore diameter based on eq. 4.1. The K$^+$/Cl$^-$ selectivity ratios at pH 8 were generally above 100, values comparable to biological ion channels and polymer membranes many microns thick\textsuperscript{38}. Surprisingly, the selectivity ratios were not significantly reduced until the pores were larger than about 20 nm. These high selectivities contrast with previous measurements by O’Hern et al.\textsuperscript{14} on graphene membranes with many subnanometer pores, which reached a maximum selectivity ratio of $S_{GHK} = 1.3$. It is possible that the high K$^+$/Cl$^-$ selectivities measured
here are unique to electrically pulsed pores, or that a small number of large pores or tears in O’Hern et al.’s centimeter-scale membranes drastically reduced selectivity by introducing parallel paths of non-selective ion flow. Indeed, pores larger than 100 nm (where the graphene pore is almost as large as the supporting silicon nitride aperture) showed minimal selectivity ($S_{GHK} < 5$, Figure 4.3c). A control aperture with no graphene yielded $S_{GHK} \approx 2$ (Figure 4.3c, open circle), indicating that the aperture itself contributes non-zero but comparatively minimal selectivity. $S_{GHK}$ was also measured at different solution pHs to examine whether or not K$^+$/Cl$^-$ selectivity is influenced by protonation/deprotonation of chemical groups (Figure 4.3d). The measured selectivity for a 3 nm pore drops significantly between pH 6 and 4, and is negligible by pH 2. This pH dependence has a similar progression to deprotonation of edge groups expected at graphene edges (such as carboxyls)$^{21}$, suggesting that deprotonation of chemical edge groups is necessary for cation/anion selectivity.

While the presence of negatively charged groups on the pore edge can explain strong K$^+$/Cl$^-$ selectivity in subnanometer sized pores, it cannot account for larger pores (radius $\gg$ Debye length $\approx 1$ nm in 100 mM KCl), where the edge charge is screened out by mobile counterions$^{33,35}$. Moreover, in ultra-thin nanopores larger than a few nanometers in diameter, the pore conduction is mostly determined by access resistance extending out into the fluid$^{24,29,30}$, which is largely unaffected by the charge at the edge of the pore. Therefore, a different mechanism is needed to explain the high selectivities for large nanopores.
Figure 4.3 - K⁺/Cl⁻ Selectivity
(a) Schematic of experimental setup: a concentration gradient and electric potential are simultaneously imposed across the nanopore and the net ionic current is measured. (b) Measured I-V curves for several concentration ratios. Upper inset: zero bias current indicates that K⁺ ions pass more easily than Cl⁻. Solid line is a linear fit. Lower inset: reversal voltage as a function of concentration ratio, along with fit to the GHK voltage equation (Eq. 4.2), which is used to calculate selectivity. (c) K⁺/Cl⁻ selectivity ratio as a function of pore size for several nanopores; different markers indicate different samples. The conductance in 1M KCl (lower x-axis) is used to calculate the pore diameter (upper x-axis). The open circle indicates a control aperture with no suspended graphene. (d) K⁺/Cl⁻ selectivity ratio as a function of pH for a 3 nm pore (black diamonds), showing that selectivity increases with pH. In contrast, a sample with most of the graphene removed (gray circles) shows little selectivity at any pH.
4.3 MECHANISM FOR HIGH SELECTIVITY IN LARGE PORES

The persistence of strong K⁺/Cl⁻ selectivities in pores as large as 20 nm is surprising and suggests that previously uncharacterized mechanisms may be responsible. We propose that the graphene surface (not just the pore edge) carries a pH-dependent surface charge due to deprotonatable oxygen-containing chemical groups on the graphene surface (oxidized graphene) or attached to hydrocarbon contaminants on the graphene surface. This surface charge, which is negative at neutral and positive pH values, attracts a screening cloud of positive counterions, while also repelling anions in solution. Because these mobile cations near the surface are so concentrated, they contribute a large cation-selective ion current, causing the total ionic current to be cation-selective.

To test the plausibility of this hypothesis, we measured pore conductance with various KCl concentrations at pH 2 and 8 (Fig. 4.4a). These measurements were taken on an 8.5 nm diameter nanopore, the same pore for which TEM imaging was shown in Fig. 4.2b,c. The presence of surface charge on the entire graphene surface causes the conductance at pH 8 to be significantly higher than at pH 2, even at salt concentrations high enough that the surface charge is screened out (Debye length << pore diameter). The conductance data shown in Fig 4.4a clearly shows this effect, with conductance at pH 8 considerably higher than at pH 2 for all concentrations below 3 M.

To quantitatively test the surface charge hypothesis, we numerically solved the Poisson-Nernst-Planck (PNP) equations for a 2D axisymmetric pore geometry with a
variable surface charge on the suspended graphene surface (see Appendix C.4 for details). The conductance data agrees with a model of an 8.5 nm pore with surface charge $\sigma = -0.6 \text{ C}/\text{m}^2$ (Fig 4.4a). Initially, this surface charge density seems surprising high, considering that pristine graphene should not have any deprotonatable surface groups. However, it is possible that the high voltage pulses used to fabricate the nanopore oxidize the graphene surface, as well as hydrocarbon contaminants on the graphene surface$^{39,40}$. As a comparison, graphene lightly treated with oxygen plasma was measured to have a surface charge density of $-0.24 \text{ C}/\text{m}^2$ at pH 7$^{41}$. In order to illustrate how this surface charge density causes high $\text{K}^+/\text{Cl}^-$ selectivity in this system, we examined two example paths in the numerical simulation (Fig 4.4b): along the center of the pore (path 1, dotted line) and along the surface of the graphene (path 2, solid line). A plot of ion concentrations along the paths (Figure 4.4c) shows increased $\text{K}^+$ concentration and decreased $\text{Cl}^-$ concentration along the entirety of the surface path (path 2). As a result, the $\text{K}^+$ current density is elevated and the $\text{Cl}^-$ current density reduced over the surface path (Figure 4.4d). $\text{K}^+/\text{Cl}^-$ selectivity is therefore a result of both increased $\text{K}^+$ current and reduced $\text{Cl}^-$ current. Since these highly selective surface current paths are also highly conductive due to the elevated cation concentrations, they can cause even large pores to be very selective.
Figure 4.4. - Surface Charge Model of Ion Selectivity
(a) Conductance measurements as a function of KCl concentration for an 8.5 nm pore (the same pore shown in Fig 4.2b,c,d). Blue and red dots show experimental data at pH 2 and pH 8, respectively. Blue and red dotted lines show numerical predictions for a numerical Poisson-Nernst-Planck (PNP) model (see Appendix C.4 for details) with surface charge density of 0 C/m² and -0.6 C/m², respectively. (b) Diagram of the numerical PNP system with negative surface charge (σ = -0.6 C/m²). The K⁺ concentration is plotted in a 31 mM KCl environment with 100 mV applied across the pore. (c) K⁺ and Cl⁻ concentrations plotted along the two illustrative paths shown in (b). The gray bar indicates the thickness of the nanopore. Path 2 along the surface of the graphene has elevated K⁺ and decreased Cl⁻ concentration. (d) K⁺ and Cl⁻ current densities plotted along the two illustrative paths shown in (b). Path 2 shows much greater K⁺ current than Cl⁻ current, which results in K⁺/Cl⁻ selectivity. The overall pore selectivity results from these highly selective, highly conductive current paths. (e) Comparison of measured reversal potentials and predictions from the model for a 10:100 mM concentration gradient. Experimental data are shown as black dots. Blue and red lines indicate numerical predictions with and without surface charge, respectively.
In order to quantitatively evaluate whether this model can account for large measured selectivities, we simulated reversal potential measurements for a 10:100 mM concentration gradient and directly compared them to the experimental results shown in Figure 4.3. Figure 4.4e shows the experimentally measured and numerically simulated reversal potentials as a function of pore size. Without the surface charge on the graphene membrane, the reversal potential and selectivity drop rapidly for pores larger than 1 nm, but the simulation, including the surface charge, models the measured data much better, predicting K⁺/Cl⁻ selectivity ratios above 100 for pores as large as 5 nm. The spread in the experimental data most likely indicates that the surface charge and pore shape vary from sample to sample.

With this surface charge mechanism for ion selectivity in mind, we must be careful in the interpretation of reversal potential data using the GHK equation (Eq. 4.2). In the GHK model, the ion selectivity does not depend on salt concentration. However, the surface charge model implies that selectivity would decrease with increasing salt concentration, because the surface charge is screened out more strongly in high salt solutions. Indeed, the reversal potential (and therefore selectivity) is lower at higher salts, although there is significant sample-to-sample variability on how much the selectivity drops off at salt concentrations of 1M or higher (see Appendix C.5 for details). Therefore, while the GHK model is useful for estimating selectivities at a given salt concentration, it cannot be interpreted as a full physical model because it does not encapsulate the vital electrostatic effects of surface charge. In comparison, the numerical
PNP model is a more complete model but does not offer the convenience of analytical solutions.

4.4 CATION / CATION SELECTIVITY

To measure selectivity amongst different cations, including divalent cations, we directly compared pore conductance in different cation-chloride solutions without a concentration gradient (Figure 4.5a). This direct comparison can be made because the current due to $Cl^-$ anions at pH 8 is negligible for small pores (as shown in the previous section). It is important to note, however, that different cations have significantly different electrophoretic mobilities, which result in differences in conductivity in bulk solution (see Appendix C.2). To account for these differences we introduce a normalized conductance $g_i$ for each cation

$$g_i = \frac{G_i}{\mu_i/\mu_{K^+}}$$ (4.3)

where $G_i$ is the measured nanopore conductance in cation-chloride solution, $\mu_i$ is the bulk electrophoretic mobility of the cation, and $\mu_{K^+}$ is the mobility of $K^+$ ions. Any observed differences in normalized conductance indicate inter-cation selectivity of the pore. Figure 4.5b shows normalized conductances for a variety of mono and divalent cations for several small nanopores 2-4 nm in diameter. It is immediately evident that divalent cations show much lower normalized conductance than monovalent cations, which agrees with recent results on sub-2 nm defects in CVD graphene membranes$^{15}$. Even amongst monovalent cations, the normalized conductances appear to follow the
general trend $K^+ > Na^+ > Cs^+ > Li^+$. These results indicate that graphene nanopores can distinguish strongly between mono and divalent cations, and weakly amongst monovalent cations.

To examine how inter-cation selectivity depends on pore size, we measured normalized cation conductances for a pore during sequential stages of electrical pulse enlargement (Figure 4.5c). To characterize the relative selectivities for different cations, we define the inter-cation selectivity ratio (relative to $K^+$) as

$$S_i = \frac{g_i}{g_{K^+}}, \quad \text{(Eq. 4.4)}$$

This definition of selectivity gives us $S_i = 1$ for a nanopore that does not distinguish between a cation $i$ and $K^+$. As the pore is enlarged, the same ordering $K^+ > Na^+ > Cs^+ > Li^+ \gg Ca^{2+} > Mg^{2+}$ is preserved, but the selectivity of all cations is reduced as the pore size increases. For pores larger than 20 nm, no significant inter-cation selectivity remains. The deviations of $g_i$ from 1 for such large pores are likely due to chlorine flux, which begin to contribute to current at >20 nm pore diameter.

While a mean-field PNP model incorporating surface charge could largely explain the large $K^+/Cl^-$ selectivities, it cannot explain the smaller inter-cation selectivities measured here. As far as a PNP model is concerned, two monovalent ions are exactly the same, and the differences in valence between mono and di-valent salts do not result in significantly different ion selectivity. It is likely that the inter-cation selectivity is sensitive to molecular-scale details such as ion hydration and ion-ion interactions. Such molecular mechanisms of ion selectivity are quite complex and have been deciphered in detail only in biological ion channels, with the support of X-ray
crystal structures and targeted mutagenesis\textsuperscript{42}. Therefore, we will not speculate here as to the mechanisms of inter-cation selectivity. However, our observations that graphene nanopores can also noticeably select amongst different cations should strongly encourage further theoretical and experimental investigations.

Figure 4.5. - Inter-cation Selectivity

(a) Schematic of experimental setup: pore conductance is measured in a variety of 100 mM cation-chloride solutions. (b) Normalized conductance for 4 different nanopores 2-4 nm in diameter; different markers indicate different samples. Monovalent cations pass more easily than divalent cations. (c) Inter-cation selectivity ratio (relative to K\textsuperscript{+}) of a graphene nanopore as a function of pore size. The conductance in 1 M KCl (lower x-axis) is used to estimate pore size using Eq. 4.1. Inter-cation selectivity decreases as pore size increases and is no longer significant above 20 nm.
4.5 Summary

We have shown that graphene nanopores up to about 20 nm in diameter show $K^+/Cl^-$ selectivity ratios over 100 and monovalent/divalent cation selectivities up to 5. The $K^+/Cl^-$ selectivities can be explained by elevated concentrations of mobile cations near the graphene. Future work studying the source of these increased surface concentrations (which could include mechanisms other the fixed surface charge examined here) is still needed to complete our understanding of the mechanism responsible for ion selectivity. Altering the surface charge by modifying the surface or using different thin materials may also allow nanopores to select for anions instead of cations. Although we have limited this study to single pores, we expect that large-area porous membranes containing many nanopores 20 nm or smaller will retain high selectivity while supporting orders of magnitude larger ionic currents. Previous investigations of the desalination potential of graphene have focused on subnanometer diameter pores for reverse osmosis, but the results shown here suggest that such strict fabrication limitations are not necessary. The loosening of the pore size upper bound from <1 nm to 20 nm means that existing techniques for creating porous graphene membranes can likely be used to create highly effective cation exchange membranes for electrodialysis. Furthermore, these surprising observations indicate that atomically thin nanopores can behave quite differently than their thicker counterparts, and should continue to be a rich platform for studying nanoscale mechanisms of ion transport.
4.6 REFERENCES


29. Kowalczyk, S. W., Grosberg, A. Y., Rabin, Y. & Dekker, C. Modeling the conductance and DNA blockade of solid-state nanopores. Nanotechnology 22,


5 DISCUSSION

In this dissertation, I have demonstrated methods for scalable fabrication of ultra-thin solid-state nanopores in silicon nitride and graphene membranes. These new methods should make industrial scale production of ultra-thin nanopores feasible for DNA sequencing, desalination, or other applications. I have also shown that graphene nanopores have several desirable properties, including increased sensitivity to translocating molecules, reduced resistance to ionic flow, and strong cation selectivity. However, there still remain several challenges for realizing sequencing and desalination applications with solid-state nanopores. Additionally, this work has also raised some interesting fundamental questions about ionic flows in ultra-thin nanopores. This chapter identifies these challenges and opportunities and offers some suggestions of how future work might address them.

5.1 DNA SEQUENCING

DNA sequencing with solid-state nanopore is still awaiting a crucial result – a demonstration of a solid-state nanopore with sufficient signal-to-noise ratio (SNR) to detect differences in individual DNA bases. While the use of ultra-thin nanopores increases the sensitivity of the signal, it also increases the noise. In solid-state nanopores, this increased noise usually has a $1/f$-like spectrum, which implies that it overlaps with the sequencing signal and cannot be filtered out. Initial measurements
with graphene nanopores suggest that the ultra-thin nanopores do not improve the SNR enough to allow sequencing (see Appendix D.4, also Heerema et al.).

In order to take advantage of the increased sensitivity of ultra-thin nanopores, the $1/f$ noise must be suppressed. Therefore, the origin of this noise needs to first be identified. Measurements on nanopores in other 2D materials such as boron nitride (BN)\(^2\) and molybdenum disulphide (MoS\(_2\))\(^3\) have shown similar noise characteristics to graphene. Therefore, it seems that the increased $1/f$ noise is intrinsic to ultra-thin nanopores. Initial investigations suggest that this noise may be due to mechanical fluctuations of the atomically thin membrane (see Appendix D.4, also Heerema et al.).

Careful mechanical measurements (perhaps using environmental AFM) and modeling are needed to test this hypothesis. Feng et al.\(^4\) have recently reported identification of isolated DNA nucleotide molecules in atomically-thin molybdenum disulphide (MoS\(_2\)) nanopores, but convincing physical explanations of how the $1/f$ noise was overcome and why such small molecules reside in the pore long enough to be identified were lacking.

A further complication is the sticking and clogging of DNA molecules in and around the pore. Bare graphene is the most troublesome, while coated graphene, other 2D materials, and silicon nitride fare better. However, all solid-state nanopores are much more susceptible to sticking and clogging than biological nanopores, which almost never irreversibly clog. A more fundamental understanding of the interaction of DNA molecules with solid-state surfaces is long overdue. Perhaps single-molecule force
studies (using optical tweezers, for example) and fluorescence studies could be combined to approach this understanding.

Reliable methods of controlling DNA motion during sequencing are also needed. Since enzymatic motors have already been successfully used with biological pores, they are the most obvious method. Other methods, such as employing two pores to pull on the DNA simultaneously in opposite directions, or creating a strong electrostatic trap that can be used as a ratchet, may ultimately be more desirable but still require a lot of development.

Although we have eliminated the most time-consuming and expensive TEM drilling step by introducing the new cold ion sculpting and electrical pulse fabrication methods, industrial scaling of nanopore devices for DNA sequencing still depends on further engineering. It is desirable to use arrays of nanopores instead of a single pore, allowing parallel measurements that produce larger data sets that ultimately lead to more accurate sequencing results. However, in a nanopore array, each nanopore needs its own current amplifier to avoid cross-talk between different pores. The integration of current amplifiers has been demonstrated with single nanopores\textsuperscript{5}, but not arrays of pores. The cold ion beam sculpting method seems quite amenable to adding current amplifiers, as nanofabrication of the amplifier circuits could be performed in concert with the milling of the bowl shapes. Creating arrays of graphene nanopores is probably more difficult. With many suspended graphene membranes, it is difficult to avoid rips
and tears. In order to tolerate a few broken membranes, each membrane will probably have to be electrically isolated, which complicates the fabrication process.

5.2 **Desalination**

In Chapter 4, we showed that graphene pores as large as 20 nm can be highly cation-selective. In order to actually perform desalination, however, we need a porous membrane containing many pores 20 nm or smaller. Several methods, such as plasma treatment, ozone treatment, ion bombardment, and chemical etching, have already successfully produced such porous membranes. It is likely that the electrical pulse fabrication method can be combined with ion bombardment to produce porous membranes. An experiment demonstrating this and determining the level of control over the size distribution of pores would be quite useful. Furthermore, it remains to be shown that a porous membrane retains the same selectivity as a single pore.

For a complete electrolysis (ED) desalination system to benefit from ultra-thin membranes, anion-selective pores are also necessary. It is possible that chemical modification of the graphene surface or use of other ultra-thin or 2D materials may achieve this. A study demonstrating control over the type of ion selectivity will also go a long way towards a complete understanding of the mechanism behind ion selectivity.

It is also likely that the fabrication difficulties in producing uniformly subnanometer pores will be eventually overcome, allowing reverse osmosis (RO) to be achieved. At this point, a comparison of the efficiencies of RO and ED with ultra-thin
pores will be interesting. While RO has traditionally been more efficient, the large ion fluxes allowed via 20 nm ion-selective pores may change this calculus. In any case, it seems likely that ultra-thin membranes will become an important material for desalination and filtration systems.

5.3 MECHANISM OF ELECTRICAL PULSE FABRICATION

The mechanism of how electrical pulses create and enlarge nanopores in graphene is still unknown. Given the miniscule amount of material that is removed by each pulse (< 100 atoms), it is difficult to design experiments that can probe this process. Because the ionic current through even a very small nanopore is measurable, it might make sense to measure the current flowing during the pulsing. In our fabrication experiments in Chapter 3, we do not measure the current passing through the membrane during the pulse, mostly to protect the patch-clamp amplifier from large input voltages. However, even if one could safely measure the current during the pulse, it would be difficult to distinguish the current through the membrane from the capacitive current from charging the membrane. A clever measurement circuit may circumvent this issue. Such experiments may also be combined with scanning tunneling microscopy (STM) to probe the atomic structure of the pore as it is enlarged. Sculpting a material on the atomic scale with feedback control is a new and exciting idea that is interesting on a fundamental level as well as for its practical applications.
5.4 **ION CONDUCTION IN ULTRA-THIN PORES**

Initial experiments on ultra-thin nanopore gave generally emphasized practical applications, but it is becoming more and more clear that the basic model of such pores is incomplete. We showed in Chapter 4 that a naïve model of ion conduction in graphene nanopores fails to predict the large cation selectivities observed for pore as large as 20 nm. Our surface charge hypothesis begins to improve the model, but measurements of inter-cation selectivity already reveal that a Poisson-Nernst-Planck model is also insufficient to explain everything that is going on. Careful studies of ion selectivity across the parameter space of electrolyte species, salt concentration, solution pH, pore size, pore thickness, applied voltage, surface properties, etc. are warranted, along with analytical and numerical modeling. In the task of understanding ionic current, we have the vast literature of ion channels to guide and inspire us.

5.5 **CONCLUSION**

By removing experimental bottlenecks for producing ultra-thin solid-state nanopores, we have facilitated experiments that reveal both promising and disheartening facts about DNA sequencing and filtration applications. Regardless of the ultimate practical usefulness of these nanopores, these results have revealed several interesting and puzzling phenomena that encourage further investigation and understanding of ionic current through ultra-thin nanopores. In addition to making new measurements possible, these new fabrication methods also allow quicker and more reliable pore fabrication, which translates into experiments that can be replicated
and verified more thoroughly. Hopefully these methods and experimental observations will contribute to a more complete understanding of the fascinating phenomena that occur on the nanoscale.

5.6 REFERENCES


A. SUPPORTING INFORMATION FOR CHAPTER 2

A.1 ALTERNATIVE NANOPORE FABRICATION METHODS

Since the first experiments with ion sculpted nanopores, there has been an incentive for other laboratories to develop alternative pore fabrication methods that did not require building custom vacuum systems. Indeed, setting up the ion beam sculpting system involves a considerable time investment both in building the system and fine-tuning the fabrication protocol.

A method for creating solid-state nanopores using electron beams in a transmission electron microscope (TEM) was developed by Cees Dekker and colleagues in 2003\(^1\). They showed that a focused electron beam can drill through thin (~100 nm) silicon oxide (SiO\(_2\)) or SiN\(_x\) membranes, forming nanopores as small as a few nanometers in diameter. Pores could also be shrunk under broad-beam exposure to 300 keV in the same TEM. They hypothesized that the driving force for this pore closing was surface tension of a fluidized layer of viscous SiO or SiN, similar to the viscous flow model of IBS. This electron drilling method allows immediate and accurate measurement of the pore size via TEM imaging. The thickness of nanopore is determined by the membrane thickness, and the lower limit is depends on the stability of the membrane. Devices with SiN membranes as thin as 6 nm have been successfully used to detect molecules\(^2\). Because electron microscopes are a standard piece of equipment in many laboratories and shared-use facilities, they tend to be more
accessible to researchers than an ion beam sculpting. However, for industrial applications, ion beam sculpting is actually a more scalable and cost-efficient technique. This is mostly because it does not require advanced electron optics, instead using a broad ion beam with minimal ion optics. Therefore, as solid-state nanopores transition from proof-of-principle experiments in academic research labs to mass production for commercial products, it is likely that ion beam fabrication methods will be preferred over electron drilling.

A third method of solid-state nanopore fabrication, called dielectric breakdown, was developed by Vincent Tabard-Cossa and colleagues in 2014 to address the issue of scalability\textsuperscript{3-5}. Thin SiN\textsubscript{x} membranes without pores are immersed in electrolyte solution (typically 1M KCl) and a large voltage bias is applied across the membrane. After prolonged exposure to the field (~10 sec – 10 hrs), a defect suddenly opens up in the membrane, causing ionic current to flow across the membrane. Using the current as a measure of pore size, the voltage source is turned off when the pore is the desired size, allowing precise control of the nanopore size down to about 1 nm. This dielectric breakdown method was discovered independently of the electrical pulse method we developed for graphene pores, but is a very similar method. Both methods are performed in the same fluidic cell used for experiments, making pore fabrication more convenient and less expensive. These methods make nanopore fabrication possible for researchers without access to advanced nanotechnology equipment, and will therefore expand and accelerate nanopore research.
A.2 Nanofluidic Setup

In this thesis, most experimental measurements are made in a setup in which the nanopore is immersed in an aqueous electrolyte solution (Fig A.1a), typically Potassium Chloride (KCl). The nanopore is the only fluid connection between the two fluid reservoirs on opposite sides of the membrane. Silver / Silver Chloride electrodes are used to apply a voltage bias to the two reservoirs, setting up an electric field across the nanopore. Charged ions in solution move under the electric field, setting up an ionic current. A low-noise patch-clamp amplifier (Axopatch 200B) is used both to apply the voltage bias and to measure the ionic current. Because the nanopore is the limiting constriction for flow of ions, most of the voltage drop occurs in or near the pore. Typical ionic currents for solid-state nanopores are 100 pA – 50 nA, depending on the size and thickness of the pore. The current is generally low-passed filtered at 20 kHz to remove high-frequency noise.

Fluidic measurements were all performed using a custom Polyether Ether Ketone (PEEK) fluidic cell designed by Marc Gershow (Fig. A.1b). This two piece fluidic cell is designed to form water-tight seals to both sides of a nanopore chip via Polydimethylsiloxane (PDMS) gaskets. The electrolyte solutions in each reservoir can be easily switched using the inlet and outlet ports of the cell. DNA molecules or other molecules of interest can be injected near the nanopore using the injection ports.
The 3 mm silicon chips that house the membranes and nanopores are prepared using standard nanofabrication techniques. Briefly, (1) a silicon nitride thin film is grown on a silicon wafer, (2) optical lithography is used to remove ~700 µm square of SiN<sub>x</sub> in a 3 mm grid pattern, (3) a wet KOH etch is used to etch through the silicon and release a suspended SiN<sub>x</sub> membrane, (4) a wafer dicer is used to cut the wafer into 3 mm chips, each containing a suspended membrane.

Because nanopores are so small, it is not always easy to get the electrolyte solution to wet the inside of the pore. Nanoscale air bubbles can get trapped in the pore and stubbornly prevent wetting. A pore that is not wet does not conduct at all, and looks like a membrane without a pore. Wetting is especially problematic if the pore is hydrophobic because air bubbles are attracted to the membrane and pore. Silicon nitride nanopores are generally treated with an oxygen plasma before wetting. This aggressively oxidizes the nitride surface, making it hydrophilic, as well as burning off any surface contaminants. The fluidic cell is then filled with ethanol, which has a much less polar than water and has a lower surface tension. Once the pore is wet with ethanol, the ethanol is directly replaced with electrolyte solution.

Graphene membranes cannot be oxygen plasma cleaned because the membrane also reacts with oxygen plasma. This limitation combined with the fact that graphene is intrinsically hydrophobic initially makes wetting graphene nanopores quite challenging<sup>6,7</sup>. We developed a new gas-assisted wetting protocol that enables complete and reliable wetting of graphene nanopores (see Appendix D.2 for details).
Fig A.1 - Nanofluidic Setup for Nanopore Experiments
(a) Schematic for nanofluidic experiments. The nanopore is immersed in electrolyte solution, a voltage bias is applied across the nanopore membrane, and the ionic current through the pore is measured. (b) Design of fluidic cell (Marc Gershow), showing how the nanopore chip is mated to various inlet and outlet ports.
A.3 Nanopore Conductance

The most basic and fundamental characterization of a nanopore is a measurement of its conductance (inverse of resistance), obtained by measuring the slope of a current-voltage (IV) curve. For small voltage biases (<500 mV), measured IV curves tend to be linear. Some exceptions exist, such as subnanometer diameter pores, highly charged pores, and pores with highly asymmetric geometry. Barring these exceptions, a nanopore filled with electrolyte solution behaves similarly to a resistor. Ions in solution drift in response to the electric field, leading to current density given by equation A.1.

\[
\vec{J} = -\frac{F^2 D c}{RT} \vec{E}
\]  

(A.1)

Where F is Faraday’s constant, D is the diffusion constant of the ion, c is the concentration of the ion, R is the ideal gas constant, and T is the temperature. Equation A.1 applies to each ion present in the solution, so the total current density is given by Ohm’s Law

\[
\vec{J} = -\sigma \vec{E}
\]  

(A.2)

Where the conductivity \(\sigma = \frac{F^2}{RT} \sum D_i c_i\) is determined by concentration and properties of the various ions in solution. If the solution is treated like a uniform conductive medium, then the pore conductance can be straightforwardly be calculated from its geometry. For a cylindrical pore, we have
\[ R_p = \frac{1}{\sigma} \left( \frac{4L}{\pi d^2} \right) \]  \hspace{1cm} (A.3)

where \( L \) is the length of the pore and \( d \) is the diameter. This calculation, however, neglects any additional voltage drop from the pore mouth to the electrode far away from the pore. For example, an infinitely thin membrane (\( L = 0 \)) does not have zero resistance as Eq. A.3 suggests, but exhibits an access resistance given by

\[ R_a = \frac{1}{\sigma d} \]  \hspace{1cm} (A.4)

For long and narrow pores (\( d \ll L \)), Eq. A.3 is more appropriate, but for very thin pores like graphene (\( d \gg L \)), Eq. A.4 is better\(^9\). Eq. A.5 treats the access and pore resistances in series and is a decent approximation for the all pore thicknesses\(^10\).

\[ R_{tot} = \frac{1}{\sigma} \left( \frac{4L}{\pi d^2} + \frac{1}{d} \right) \]  \hspace{1cm} (A.5)

The actual pore geometry is usually not perfectly cylindrical. Electron beam drilled nanopores tend to have hourglass-like shapes due to the focusing of the electron beam. In this case, the diameter and thickness parameters \( d \) and \( L \) can still be used to characterize pores, but with the understanding that these are “effective” length scales.

In treating \( \sigma \) as a constant we have assumed that the concentration of ions is constant everywhere. However, if the pore walls have a surface charge, they will attract an excess of the oppositely charged ions in solution (the Debye screening cloud), leading to an increase in effective conductance due to the excess mobile charge carriers\(^11,12\). In these cases where there the net charge in solution can be nonzero, the motion of ions under the electric field can create solvent flows, called electroosmotic
flow (EOF). However, electroosmotic flow tends to be small effect in nanopore systems, and is usually neglected.

A.4 Ionic Current Noise

For DNA sequencing and other nanopore sensing applications, the ionic current noise limits how sensitively the pore can detect differences in current blockade signals. Noise can be caused by many difference sources, including thermal (Johnson) noise, the measurement apparatus, and the nanopore itself. Noise is often characterized by the root-mean-square (RMS) signal given by

\[ \Delta I_{RMS} = \sqrt{\int_0^T \left( \frac{I(t) - I_{avg}}{T} \right)^2 dt} \]  

(A.6)

Where \( \Delta I_{RMS} \) is an approximation of the average magnitude of the current noise, \( T \) is the length of the signal, \( I(t) \) is the current measured time \( t \), and \( I_{avg} \) is the average current. Although the RMS current immediately gives an estimate of what sized signals can be distinguished over the noise, it does not capture the frequency distribution of the noise, which determines how much of the noise can be filtered out to improved signal to noise ratio (SNR).

To measure the frequency distribution of the noise, one calculates the power spectral density (PSD), given by

\[ S_f(f) = \frac{2}{T} |\bar{I}(f, T)|^2 \]  

(A.7)
where $S_i(f)$ is a function of frequency with units nA^2/Hz, and $\tilde{I}(f, T)$ is the Fourier transform of the current $I(t)$ taken over the time window $T$. Some noise sources, such as amplifier noise coupled through the capacitance of the membrane, are significant only at high frequencies (>10 kHZ). If the DNA relevant sequencing signal happens on the timescale of ms to s, then the entire signal can be low-pass filtered to improve SNR. All solid-state nanopores exhibit noise in the low frequency range with a spectrum approximately proportional to 1/frequency. This 1/f noise is much more problematic, as it overlaps with the relevant signal frequencies. The origin 1/f is still largely unknown, and is analogous to mysterious 1/f noise in many other systems, such as solid-state transistors and radio broadcasts. By selecting appropriate filters, once can isolate the bandwidth of interest for the signal and reduce to RMS noise to

$$\Delta I_{RMS}(f_{min}, f_{max}) = \sqrt{\int_{f_{min}}^{f_{max}} S_i(f) \, df}$$  \hspace{1cm} (A.8)

which can be considerably less than the full bandwidth noise given in Eq. A.6.
A.5 DNA Translocations

DNA translocations are typically measured by injecting DNA fragments into one of the fluid reservoirs and applying a DC voltage in the direction that pulls DNA through the pore. The passage of DNA molecules are observed as transient reductions in ionic current (Fig A.2). Translocations of free DNA molecules (as opposed to molecule attached to molecular motors) depend on the length of the DNA molecules and the magnitude of the voltage bias, but are typically quite fast (~1 μs to 1 ms in duration). Single (ssDNA) or double stranded DNA (dsDNA) can be translocated, but the blockage levels are about twice as deep for dsDNA due to the larger cross-section of the molecule. For pores significantly larger than the cross-sectional diameter of the DNA (~1.4 nm for ssDNA, ~3 nm for dsDNA), the DNA molecules can translocate through the pores in folded conformations, leading to multi-level current blockades.

The magnitude of the current blockade due to a translocating is difficult to predict because it depends on many physical parameters, including the salt concentration, voltage bias, pore size, pore geometry, pH, DNA conformation, and the effective charge of the DNA. For example, a DNA molecule within a pore reduces the ionic current by sterically blocking ions, but also increases the current by providing excess ions in the pore in the form of its Debye cloud. Thus, if the salt concentration in the solution is low enough (< 400 mM), then the DNA actually increases the current, leading to current enhancements instead of blockages. For certain conditions, simple analytical and numerical approximations have been used to model experimental
data, but no accurate general model has not been demonstrated. Indeed, Oxford Nanotechnology’s MinION system performs base-calling by essentially comparing ionic current data to exhaustive empirical reference tables, rather than a first-principles physical model.

Fig A.2 - DNA Translocations
Illustration of a DNA translocation event. The ionic current through the nanopore is continuously measured. When the DNA molecule is pulled into the pore, the current is reduced, remaining at lower level until the molecule completely translocates through the pore.
A.6 Material Analysis of Ion Beam Closed Nanopores

Cross-sectional TEM imaging revealed that the material that accumulates during ion beam sculpting to close down a pore is much less dense than the silicon nitride membrane (see Chapter 2, Fig. 2.3d). In light of this striking observation, it is possible that the “volcano” is primarily made up of carbonaceous material that is present on the sample as a contaminant. If this is the case, then the ion sculpting process primarily describes the movement of surface contaminants, rather than Si or Ni adatoms dislodged by the ion beam. Indeed, carbon contamination is always a concern because it can be accumulated during lithographic processing or during sample handling in air.

We investigated the material constituency of this “volcano” material using energy-dispersive X-ray spectroscopy in the TEM (EDS). In this measurement, an X-ray detector counts and sorts X-rays that are emitted as a result of electron bombardment of the sample. The X-ray energy is equivalent to the energy lost by the electrons as they travel through the sample. The possible X-ray energies are generally constrained by atomic transitions in the sample material, which allows the EDS signal to be used to identify the material makeup of the sample.

A ~50 nm nanopore in a SiN<sub>x</sub> membrane was created using a focused ion beam, then closed completely using ion beam sculpting and room temperature. Fig A.3a shows a plan-view TEM image of the pore after closing. The outline of the original pore is clearly visible, but the interior of the pore is completely filled with thin amorphous
material. A single-point EDS measurement of the ion beam closed material (Fig. A.3a,c, red) reveals that this material has a much higher carbon content than the SiNₓ membrane (Fig. A.3a,c, blue). To verify that the closed material is largely carbonaceous, we then subjected the entire sample to an oxygen plasma treatment (100W, 5min) and imaged again. After the plasma clean, the ion beam closed material was completely absent, suggesting that it was burned off by the oxygen plasma. This suggests that the “volcano” is almost entirely made up of carbonaceous material susceptible to oxygen plasma removal.

Although unlikely, it is possible that the carbonaceous material in the interior of the pore was accumulated by carbon contamination during electron imaging rather than the ion sculpting process. To rule out this interpretation, we prepared a cross-sectional lamella of an ion beam closed nanopore and analyze it with using scanning TEM (STEM) EDS. The deposition of protective metal layers before the lamella excision (see Fig. 2.3a) encapsulates any carbonaceous contaminants deposited during ion sculpting, allowing us to distinguish it from other contamination sources. The large image is an annular dark field STEM image, which has reverse contrast (darker means lower density). The “volcano-like” material is noticeably less dense than the rest of the membrane (see also Fig 2.3d). A conventional TEM image of the same area (inset, top right) confirms this observation. EDS maps of areas near the pore (left) and far away from the pore (right) reveal a clear carbon-rich layer (red channel) present only near the
pore in the “volcano”. Together with the point-EDS measurements, this result clearly shows that the ion sculpted material is largely carbonaceous.

The possibility that carbon contamination is heavily involved in ion beam sculpting motivated us to do a careful examination of the ion beam closing protocol. Adding vigorous cleaning steps immediately before ion closing such as oxygen plasma (100W, 5min) or piranha etch (sulfuric acid and hydrogen peroxide, 5:1 ratio, 85° C for 15 min) caused pores not to close under ion beam exposure. A controlled deposition of carbonaceous contaminants (800 mM Methyl Methacrylate allowed to dry on chip) caused previously cleaned sample to close.

These experiments, taken together, demonstrate that in our preparation carbon contaminants play a central role in ion beam sculpting closing. In Chapter 2, we avoided these complications by working at low temperatures. However, it seems that vigorous sample cleaning procedures may allow the process to be performed at room temperature. While our experiments do not rule out the possibility that material rearranged by ion beam closing could be silicon nitride, as has been generally accepted in the literature17–21, we have established that carbon contamination can close pores under ion beam irradiation, and must be carefully controlled in ion beam sculpting experiments.
Fig. A.3 - Material Analysis of Ion Beam Closed Nanopores

(a) Plan-view TEM image of 50 nm pore completely closed by ion sculpting. The outline of the original ~50 nm hole can be seen, but the interior is completely filled with material. (b) Plan-view image of ion sculpted pore after oxygen plasma etch. The material on the interior of the pore has been completely removed. (c) Single-point EDS measurements of the SiNₓ membrane (red) and the ion sculpted material on the interior of the pore (blue). The ion sculpted material has a large carbon peak that is absent in the membrane material. (d) Cross-sectional STEM-EDS map of an ion beam closed nanopore. Large image: angular dark field STEM image of the pore. Top-right inset: conventional TEM image of the pore pore. Colored maps: EDS maps of relevant X-ray energies corresponding to prominent transitions for various elements taken near the pore (left) and far away from the pore (right). The material near the pore contains a carbon-rich layer that is absent far away from the pore.
A.7 References


B. SUPPORTING INFORMATION FOR CHAPTER 3

B.1 GRAPHENE GROWTH

Controllable growth of large area, single crystal graphene on has been an important goal ever since the discovery of the 2D material. For nanopore applications, CVD growth\(^1,2\) has been the preferred method because the graphene can be transferred onto any substrate because the metal foils can be selectively etched away. CVD graphene growth was first achieved on polycrystalline nickel foils\(^3\) or thin films\(^4,5\). The Ni surface is heated to ~1000° C in the presence of gaseous hydrocarbons, typically methane (CH\(_4\)). Carbon from the vapor source dissolves into the nickel surface, creating a reservoir of carbon atoms. As the Ni surface is rapidly cooled, the carbon segregates out onto the surface and crystallizes into graphitic layers. If the Ni is cooled fast enough, the growth of graphene can be limited to a few layers (and single layers in some areas)\(^3\). Graphene has also been grown on Ni using ion implanted carbon instead of a vapor source. This method allows some control over the average film thickness as the total amount of carbon is limited by the implantation dose\(^6\). However, with graphene growth on Ni it remains difficult to grow large areas uniform thickness graphene, in particular uniformly single layer graphene. Ruoff and colleagues discovered that CVD growth on copper (Cu) results in predominantly single layer graphene, with less than 5% multilayer graphene by area\(^2\). It is thought that the very low solubility of carbon in Cu limits the dissolution of carbon into the metal, forcing crystal growth to happen at the surface and arresting the growth at a single layer.
Growth experiments using different carbon radioisotopes have confirmed that graphene grown on Cu occurs by surface adsorption while growth on Ni is a precipitation process.7

In addition to thickness homogeneity, graphene grain size can be important for nanopore and other applications. The presence of grain boundaries can hinder electronic transport and reduce the mechanical strength of the membrane8,9. Therefore, it is desirable to have grains as large as possible. Graphene grains begin to grow from nucleation sites on the Cu surface, but the exactly what causes nucleation is still not fully understood. Polishing the copper surface10 or using a molten surface11 has been shown to reduce the number but not eliminate grain nucleation. Limiting the partial pressure of the carbon source (usually methane) also leads to fewer and less dense nucleation12,13. In this regime, single grains as large as mm scale can be produced, but the growth process is necessarily very slow. At this point, CVD graphene growth on Cu is still a bit of an art, as small changes in processing can lead to significant differences in grain size or membrane quality. However, for most of the studies in this thesis, only very small areas of graphene are suspended, generally 200 nm on a side or less, so that relatively fast growth recipes that result in ~1-10 µm grains were sufficient.

Recently, epitaxial wafer-scale single crystal graphene growth has been reported using hydrogen-terminated Germanium (Ge) as the catalytic surface14. Apparently, graphene grains grow all aligned to the Ge surface and therefore multiple grains can
combine without grain boundaries. If this method proves reproducible, epitaxial growth of single crystal graphene will likely replace other growth strategies.

B.2 **Graphene Transfer**

Once graphene is grown, it needs to be transferred to the substrate. The transfer method is important because it can cause wrinkling and defects in the membrane, as well as introduce contaminants to the graphene surface. An ideal transfer method also minimizes manual handwork and is scalable to large areas of graphene. The most common method, often referred to as “wet transfer” is illustrated in Fig B.1. First, a support polymer, such as Poly methyl methacrylate (PMMA), is deposited on the graphene surface. Second, the growth substrate (usually Cu) is etching away by floating the metal foil graphene side up in a wet etchant. At the conclusion of the etch, the graphene-PMMA stack is left floating on the surface of the etchant due to surface tension. The etchant is now replaced with water, usually by using a dummy substrate to scooped out the PMMA-graphene stack and move it to a different bath. Then desired substrate is scooped under the graphene so that the exposed side of the graphene lies on the substrate. When the stack dries, the graphene adheres to the substrate. Lastly, the support polymer is etched away, leaving the graphene deposited on the substrate. Careful optimization of these transfer steps allows centimeter scale graphene membranes to be transferred by hand in a few hours. Automation and scaling of this process to larger areas and higher throughput is likely possible in an industrial setting.
Although the use of a polymer support layer is most convenient, it can leave behind carbonaceous residue on the graphene surface. Carbonaceous contaminants on graphene are ubiquitous and difficult to remove because most cleaning techniques that could remove the contaminants also destroy the graphene. Indeed, all graphene samples to date contain network of amorphous surface contaminants interspersed with atomically clean areas\textsuperscript{15}. For this reason, alternative support layers such as deposited gold\textsuperscript{16} have been used. Graphene can also be transferred directly to TEM without a support polymer if the grid provides a flexible, porous support surface\textsuperscript{17,18}. A similar idea is using a polymer support stamp that contacts the graphene in a grid pattern, leaving micron scale areas that do not ever contact the support stamp\textsuperscript{2,4,19}. These dry transfer techniques reduce the amount of surface contaminants, but do not completely eliminate them. The nature of these contaminants, which probably amount to less than a monolayer of material, is still not well understood. They may be deposited during the growth of the graphene or simply adsorb to the graphene from the air.

As with graphene growth, careful process development is the key to graphene transfer. In this thesis, most of the graphene is transferred using a wet transfer process with methyl methacrylate (MMA) as the support polymer. The use of several wash steps before scooping the graphene stack onto the substrate and a high-temperature cleaning anneal at the end of the process ensured that the graphene membranes were clean enough for experiments (approximately 50% atomically clean by area, as determined by TEM imaging).
**B.3 TEM DRILLING OF GRAPHENE NANOPORES**

Graphene nanopores can be drilled in a very similar manner to drilling of SiN$_x$\textsuperscript{20}. The electron beam is focused to a spot on the membrane surface, and the high intensity electron beam sputters a hole whose diameter is controlled by the size of the beam spot. In the JEOL 2010F electron microscope operating at 200 kV used for pore drilling, atomically clean areas basically have no contrast, and cannot be distinguished from vacuum. However, the networks of surface contamination on the surface give away the presence of the underlying graphene membrane. In this microscope, the resolution is limited by spherical aberrations in the objective lens, and is not high enough to resolve the individual atoms in the lattice. Nevertheless, the atoms in the lattice could be resolved using aberration-corrected microscopes\textsuperscript{18,21,22}. Once a pore is present, the contrast from the pore edge can be clearly imaged and the pore size can be measured. The sample is then ready to be used for fluidic experiments.
Unlike thicker solid-state membranes like SiNₓ, graphene drills very quickly. Under normal imaging conditions at 200 kV, pores can be drilled in about a second. The pore size is likely dependent on the drilling time, which is probably limited by how long it takes to manually condense and re-expand the beam. Therefore, pore fabrication is not very reproducible in terms of pore size. This is important because unlike other solid-state materials, graphene does not fluidize under the beam and allow controlled pore shrinkage. In principle, automatic control of the electron beam might improve pore size reproducibility but such strategies require non-trivial modification of the TEM beam-control hardware. Other technique improvements involving standard apertures and beam settings can already significantly increase control over pore size. Figure B.2 shows TEM images of drilled nanopores for different condenser apertures and spot sizes. The spot size settings adjust the two condenser lenses in concert to adjust the angle at which the beam arrives at the condenser aperture, which is a physical aperture with different diameters. By reducing the spot size and condenser aperture, we can reduce the beam intensity and the spatial size of the beam on the sample (the focal plane). In this way, the pore size is limited by the spot size and condenser settings, and can be controlled reproducibly. Pores as small as 1.2 nm can be reliably fabricated this way.

Although reproducibility of pore size can be achieved with careful technique development, the overall pore-drilling method is remains unreliable due to hydrocarbon contamination. Hydrocarbon contamination is a well-known problem in
both TEM and SEM imaging. The focused electron beam tends to deposit carbonaceous compounds on the illuminated area of the sample (Fig B.3). These contaminants are hydrocarbon residues from the microscope chamber or released from the sample that become fixed to the sample surface via electron-beam catalyzed reactions. Such contamination is generally mitigated by maintaining a clean vacuum in the microscope (using a cold finger and performing regular bake-outs). However, for suspended graphene, even a monolayer of contamination fundamentally changes the nature and thickness of the membrane. Indeed, contamination can be identified in the TEM image when the “tiger stripe” pattern of contaminants is replaced by a uniform pattern. Ironically, contaminated graphene can look “cleaner” than clean graphene. However, this stack of graphene and surface contaminants is more resistant to pore drilling and must be thicker than atomically clean graphene.

Graphene contamination under electron beams has been also been reduced using high temperature sample holders in the TEM\textsuperscript{24,25}. However, these techniques increase the complication and the process time of TEM drilling, further reducing fabrication throughput. Indeed, any scalable fabrication process cannot require the use of a TEM, because TEM samples are loaded and pumped one sample at a time. Furthermore, TEMs are very expensive to build and maintain, requiring sophisticated and stable lenses capable of focusing the beam down to nanometer sizes.
TEM drilling of graphene nanopores

TEM images of graphene nanopores fabricated by TEM drilling. The example images show the effect of various beam settings on the pore size. The fabricated pore size is also sensitive to the drilling time.

Contamination of suspended graphene membranes

(a) TEM image of a portion of a suspended graphene membrane, showing an atomically clean area. The image is 15 nm on a side. (b) The same area after about 1 minute of TEM imaging. Much of the formerly clean area is now covered by carbon contamination.
B.4 TEM Imaging of Electrical Pulse Fabricated Nanopores

Direct TEM imaging of pulse-fabricated graphene nanopores was very difficult and was not achieved reliably enough to serve as a precise verification of pore sizes. Contamination during drying of the sample, in air, and under the electron beam often obscured the pores. Locating small pores was difficult because the contrast of the pore edges is no stronger than the patterns of surface contamination on the graphene. Moreover, standard TEM electron energies (80-200kV) can create or enlarge pores in graphene during the search process\textsuperscript{18,24}. For larger pores, the pore can sometimes be successfully imaged, but the accuracy of pore size measurements is limited due to the complications just mentioned. Fig. B.4 shows a suspended membrane containing a single $\sim 10$ nm pore, which is near the middle of the aperture and approximately circular. Another successfully imaged pore is shown in Fig. 4.2bcd.
Fig. B.4 - TEM imaging of electrical pulsed nanopores
TEM image of a suspended graphene membrane after electrical pulse fabrication of a single ~10 nm pore. Bottom image is a closer zoom detailing the pore, which is near the middle of the aperture and approximately circular.
B.5 REFERENCES


C. SUPPORTING INFO FOR CHAPTER 4

C.1. GRAPHENE NANOPORES FOR REVERSE OSMOSIS

Atomically thin graphene membranes have generated considerable interest for use as filtration membranes because their atomic thickness presents minimal resistance to fluid or ion flow while retaining high structural integrity. Recent investigations have suggested that porous graphene membranes can attain orders of magnitude higher flow rates than commercial reverse osmosis (RO) membranes while still providing excellent salt rejection\(^1\)\(^{-7}\). Unfortunately, RO salt rejection depends on a very tight distribution of subnanometer pores. Since the water flux through such small pores is very small, a few large pores in the membrane can contribute large unselective water fluxes that impair salt rejection. Thus, the complete elimination of pores larger than a nanometer or so is paramount to viable RO membranes. Unfortunately, avoiding larger pores in single layer graphene membranes remains a difficult fabrication challenge. Work by Rohit Karnik and colleagues has shown that large-area CVD-grown single layer graphene membranes contain intrinsic defects 1-15 nm in diameter. These intrinsic defects can filter our large dyes such as tetramethylrhodamine dextran (70 kDa) but are not very selective to salts like KCl\(^8\). As a result, graphene membranes containing subnanometer pores (created via ion bombardment followed by edge-selective wet-etching) show only moderate selectivity due to leakage through these intrinsic defects\(^9\). In order to mitigate this problem, Karnik and colleagues have developed an atomic layer deposition defect-
sealing process that seals intrinsic defects before creating subnanometer pores. These membranes can filter multivalent salts (MgSO\(_4\)) and dyes (Allura Red, 496 Da), but do not successfully reject monovalent salts (NaCl). This continued difficulty is perhaps due to incomplete sealing of intrinsic defects or the presence of larger pores created after sealing. Indeed, almost any process of creating pores in graphene membranes will produce a distribution of pore sizes and pores on the large end of this distribution can significantly decrease selectivity in RO. Therefore, creating single layer graphene membranes suitable for RO remains a difficult fabrication challenge.

C.2. **Table of Ion Mobilities**

Following is a table of electrophoretic mobilities for ions studied in the paper. These values are from Hille et al. The mobility relative to K\(^+\) has also been tabulated to quantify the relative variation of mobilities amongst the different ions.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Electrophoretic Mobility (\mu_i [10^{-4} \text{ (cm/s) / (V/cm)}])</th>
<th>Relative Mobility (\mu_i/\mu_{K^+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>7.62</td>
<td>1.00</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>7.92</td>
<td>1.04</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>4.01</td>
<td>0.53</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>5.19</td>
<td>0.68</td>
</tr>
<tr>
<td>Cs(^+)</td>
<td>8.01</td>
<td>1.05</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>6.17</td>
<td>0.81</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>5.50</td>
<td>0.72</td>
</tr>
</tbody>
</table>
C.3. INTERPRETATION OF THE GHK EQUATION

Because the derivation of the GHK voltage equation contains several assumptions that may not apply in our graphene nanopore system\(^\text{10}\), we must be careful in applying it to calculate selectivity. While the selectivity defined as the ratio of effective diffusion constants \(S_{GHK} = \frac{D_{K^+}}{D_{Cl^-}}\) is well-defined in the context of the GHK model, this definition is not directly applicable to the more concretely-defined PNP system (see section C.4 for details), where the diffusion constants are given by bulk values (Table C.1). However, in the PNP model, we can directly define the selectivity as the ratio of \(K^+\) current to \(Cl^-\) current \(S_i = \frac{I_{K^+}}{I_{Cl^-}}\) measured in a symmetric salt situation \((c_{\text{high}} = c_{\text{low}})\). Unfortunately, this intuitive definition of selectivity cannot be used for the experimental data because \(K^+\) and \(Cl^-\) currents cannot be independently measured. Here we test whether or not the two definitions of selectivity are equivalent. Figure C.1 shows numerical calculations of selectivity \(S_i\) as a function of pore size. Because counter-ion screening of the surface charge depends on the solution concentration, selectivity \(S_i\) is also dependent on solution concentration (see section C.5 for details). The selectivities for lower concentrations (30 and 10 mM) show selectivities around 100 persisting for pores as large as 5 nm, which agrees with the reversal potential calculations in Figure 4.3.

In order to directly compare the two definitions of selectivity, we plotted \(S_{GHK}\) and \(S_i\) as a function of reversal potential. The black line shows \(S_{GHK}\) calculated using the GHK voltage equation with reversal potentials that were calculated from the PNP
model with a 10mM:100mM concentration gradient. The colored lines show $S_I$ calculated directly from the same PNP model without a concentration gradient. $S_I(c)$ is concentration dependent, whereas $S_{GHK}$ is not. However, for $c = 30$ mM (approximately the geometric mean of $c_{\text{high}}$ and $c_{\text{low}}$), $S_I$ is very similar to $S_{GHK}$. Therefore, we conclude that the GHK voltage equation is an acceptable model for calculating $K^+$/Cl$^-$ selectivity, with the understanding that $S_{GHK} \cong S_{PNP}(\sqrt{c_{\text{high}}c_{\text{low}}})$.

Figure C.1 - Comparison of GHK and PNP model selectivity
(a) Numerical calculations of selectivity $S_I$ as a function of pore size for different solution concentrations. (b) Comparison of two definitions of selectivity, $S_{GHK}$ and $S_I$. The colored lines show $S_I$ calculated directly from the PNP model without a concentration gradient. The black line shows $S_{GHK}$ calculated from the reversal potentials generated by the PNP model with a 10mM:100mM concentration gradient. $S_I(c)$ is concentration dependent, whereas $S_{GHK}$ is not.
C.4. Numerical Poisson-Nernst-Planck Model

Figure C.2a shows a diagram of the Poisson-Nernst-Planck system used to model a graphene nanopore, solved using COMSOL Multiphysics Software. The model solves for three scalar fields, the concentrations $c_{K^+}$ and $c_{Cl^-}$, and the electric potential $\psi$, given Poisson’s equation and the steady-state Nernst-Planck Equation for each ion.

\[
\nabla^2 \psi = \frac{eF}{\varepsilon}(c_{K^+} - c_{Cl^-}) \tag{C.1}
\]

\[
\nabla \cdot J_{K^+} = \nabla \cdot \left[ -FD_{K^+}\nabla c_{K^+} - \frac{F^2D_{K^+}}{RT}c_{K^+}\nabla \psi \right] = 0 \tag{C.2}
\]

\[
\nabla \cdot J_{Cl^-} = \nabla \cdot \left[ FD_{Cl^-}\nabla c_{Cl^-} - \frac{F^2D_{Cl^-}}{RT}c_{Cl^-}\nabla \psi \right] = 0 \tag{C.3}
\]

where $e$ is the electron charge, $F$ is Faraday’s constant, $\varepsilon$ is the relative permeability, $J_i$ is the current density for the ion $i$, $D_i$ is the diffusion constant for the ion $i$, $R$ is the universal gas constant, and $T$ is temperature. The relevant physical dimensions and boundary conditions are indicated on the diagram. Surface charge boundary conditions were imposed on the pore edge ($\sigma_p = -1 \text{ C/m}^2$) and on the graphene surface ($\sigma_{gr}$, variable) to model deprotonatable chemical groups on the pore edge and graphene surface, respectively.

Fig C.2b shows model predictions for the conductance of an 8.5 nm nanopore compared with the experimental data conductance data taken at pH 8 (the data shown in Fig. 4.4a). Fig. C.2c shows model predictions for the reversal potential in a 10:100 mM
concentration gradient compared to experimental data for many pores (the data shown in Fig. 4.4e). Several values of the surface charge density are plotted to demonstrate the effect of the surface charge. At high values of surface charge density ($< -0.4 \text{ C/nm}^2$), the predictions for conductance and reversal potential are not very sensitive to surface charge. The best fit value ($\sigma_{gr} = -0.6 \text{ C/m}^2$) should therefore be interpreted more as an order-of-magnitude estimate than a precise measurement of surface charge.

Figure C.2: Numerical PNP Model of Nanopore with Surface Charge
(a) Diagram of Poisson-Nernst-Planck system used to model a graphene nanopore, solved using COMSOL Multiphysics Software. (b) Model predictions for the conductance of an 8.5 nm nanopore compared with the experimental data conductance data taken at pH 8 (the data shown in Fig. 4.4a). (c) Model predictions for the reversal potential in a 10:100 mM concentration gradient compared to experimental data for many pores (the data shown in Fig. 4.4e). Several values of the surface charge density are plotted to demonstrate the effect of the surface charge.
C.5. Salting Out

In the GHK model, the reversal potential depends on the bulk concentration ratio \( \frac{c_{\text{high}}}{c_{\text{low}}} \) but not on the absolute value of the concentrations \( c_{\text{high}} \). In other words, selectivity does not depend on salt concentration. However, the surface charge model implies that selectivity decreases with increasing salt concentration because the surface charge is screened out more strongly in high salt solutions. The reduction of selectivity at high salt concentrations, an effect called “salting-out”, has previously been observed and modelled in biological porins\(^{12} \). To determine if the surface charge model accurately predicts this effect, we measured the reversal potential as a function of salt concentration for several nanopores with a 10:1 concentration ratio and compared the results to predictions from the PNP model (Fig. C.3). Indeed, the reversal potential (and therefore selectivity) is lower at higher salts, although there is significant sample-to-sample variability on how much the selectivity drops off at salt concentrations of 1M or higher. This trend agrees with predictions from the numerical PNP model (black line), which includes charge screening effects.
Figure C.3: Salting Out of Ion Selectivity
Reversal potential measured as a function of salt concentration for several nanopores (colored markers) with a 10:1 concentration ratio and compared the results to predictions from the PNP model (black line). The reversal potential (and therefore selectivity) is lower at higher salts, although there is significant sample-to-sample variability on how much the selectivity drops off at salt concentrations of 1M or higher.
C.6. REFERENCES


D. TOWARDS DNA SEQUENCING WITH GRAPHENE NANOPORES

D.1. OVERVIEW

DNA sequencing has played a central role in the development of both biological and solid-state nanopores. A large portion of funding for nanopore research in the U.S. has come from “$1,000 Genome Grants” offered by the National Institute of Health under the National Human Genome Research Initiative. The overarching goal of this initiative was developing genome technologies to the point where full genome sequencing is affordable and worthwhile for individuals and becomes a routine clinical diagnostic.

In 1996, Daniel Branton, Dave Deamer and colleagues demonstrated that single stranded DNA (ssDNA) could be electrophoretically driven through a *Staphylococcus aureus* α-hemolysin nanopore inserted into an artificial lipid bilayer\(^1\). The passing of the DNA polymers through the pore, often called “translocations”, were observed via drops in ionic current due to the DNA blocking part of the pore from ions while it is threaded through the pore. This work hypothesized that these current drops might be different for the four different DNA nucleotides adenine, cytosine, guanine, and thymine, raising the exciting prospect of long-read, label-free, and extremely fast DNA sequencing.

For nanopore DNA sequencing to be feasible, three conditions must be met\(^2,3\): (i) the nanopore diameter must be large enough to allow the passage of ssDNA (~1.4 nm) but not so large as to allow ssDNA to translocate in a folded state (~3 nm), (ii) the
constriction of the nanopore must be thin enough so that the ionic current signal is sensitive to only a single or a few nucleotides at a time (~0.3 nm per nucleotide), and (iii) each base must spend a long enough time in the nanopore constriction so that noise in the ionic current can be averaged out. Although α-hemolysin satisfies criterion (i), its constriction fits about 15 consecutive nucleotides at a time\(^4,5\), probably too many to reliably extract sequence information (criterion ii). Jens Gundlach, Michael Niederweis, and colleagues created biological pores with a thinner constrictions by mutating the porin MspA of *Mycobacterium segmatis*\(^6\). Meanwhile, Mark Akeson and colleagues addressed criterion (iii) by using DNA processing enzymes such as exonucleases\(^7\) or polymerases\(^8\) to slow down the translocation speed. In these experiments, the ssDNA threads the nanopore, but does not translocate through because the DNA-enzyme complex is too large to fit through the nanopore constriction. The enzyme then actively ratchets the DNA through the pore against the applied voltage according to its normal enzymatic activity, generally at speeds slow enough to integrate out high-frequency noise (>10 ms/nt)\(^9\). Enzymatic polymerase control was then combined with MspA nanopores to measure reproducible current traces that correspond to specific DNA sequences, establishing proof-of-principle demonstration of a complete nanopore sequencing system\(^10\). In 2015, Oxford Nanopore Technologies, a company spun out of Oxford University in 2005 by Hagan Bayley, Gordon Sanghera, and Spike Willcocks, began a community testing program for the MinION, a portable protein nanopore sequencing device. The technology behind the MinION has yet to be publically revealed, but scientists in the field believe that it is an MspA mutant using DNA
helicase enzymes for base ratcheting. The arrival of a commercial nanopore sequencer demonstrates that nanopore DNA sequencing is not only a topic of academic interest, but also a promising technology that will likely be competitive with other sequencing methods in the years to come.

Despite the recent successful applications of biological nanopores to DNA sequencing, there are a number of incentives to transition to devices based on solid-state nanopores. Biological proteins, enzymes, and lipid bilayers are only stable in a limited range of environments, and can be easily destroyed by mechanical stresses, high electric fields, temperature extremes and harsh pHs, while solid-state nanopores can be engineered to be much more robust. Additionally, solid-state nanopores can be modified and tuned as well as easily integrated into electronic devices. It is also anticipated that atomically thin graphene nanopores can achieve higher sensitivities than protein pores due to their extremely thin read head. Other atomically thin materials such as boron nitride\textsuperscript{11} and molybdenum disulfide\textsuperscript{12}, along with very thin materials such as thinned SiN$_x$,\textsuperscript{13} and halfnium oxide ALD\textsuperscript{14} have also been explored.

As a result of the vital importance of DNA sequencing applications, DNA translocation measurements are often used as a bench-mark characterization method for solid-state nanopores. Free translocations have been used to identify folding states of DNA\textsuperscript{15}, distinguish different nucleic acids (such as dsDNA, ssDNA, RNA, and tRNA)\textsuperscript{16}, and differentiate small DNA homopolymers\textsuperscript{17}. However, solid-state nanopore experiments in which the DNA is slowed down enough to allow discrimination of
different bases have been lacking. However, it may be the case that several of these experiments have been attempted but returned negative or inconclusive results due to issues like DNA sticking, low-frequency noise, and pore instability. Indeed, at this point, no conclusive demonstration of base-identification by a solid-state nanopore has been published.

In this appendix, we present results from experiments attempting to characterize the sensitivity of graphene nanopores to different DNA bases. These experiments have suffered from two major impediments: (1) DNA sticking and clogging and (2) low-frequency noise. We present some strategies that have been used to mitigate these problems, but a successful proof-of-principle demonstration of DNA sequencing will have to await future research.

D.2. FREE DNA TRANSLOCATIONS

Since the first experimental demonstrations of DNA translocations in graphene nanopores\textsuperscript{18–20} in 2010, a solid-state DNA sequencing experiment seemed imminent. However, several non-trivial obstacles soon became evident. Merchant et al. reported that less than 10% of their graphene nanopores exhibited translocations, with error modes including rips and tears, wetting problems, and unstable open-pore current\textsuperscript{19}. They found that coating the membrane with 5 nm of TiO\textsubscript{2} improved the pore characteristics, albeit at the cost of increasing the membrane thickness. The project here at Harvard\textsuperscript{18} was limited by similarly low yields, which is part of the reason that a
follow-up study took 3 years to complete\textsuperscript{21}. This work measured the magnitude of current blockages due to DNA translocations as a function of pore size, showing that signal gets larger as the pore gets smaller ("a molecule-hugging pore"). This data was difficult to collect because a substantial portion of the pores that showed DNA translocations quickly and irreversibly clogged, presumably due to interactions between the DNA bases and the hydrophobic graphene surface. Indeed, Schneider et al. reported that clogging issues made DNA experiments unfeasible with bare graphene nanopores, and introduced PEG coating that mitigate clogging\textsuperscript{22} (see Figure 3.3 for details).

We addressed the sample yield problem by developing the electrical pulse fabrication method described in Chapter 3, along with a gas-assisted wetting technique. The fluidic cell is first submerged in deionized water and ultrapure CO\textsubscript{2} gas (99.999\%) is flowed through both sides for 5 minutes to replace any air contacting the graphene membrane with CO\textsubscript{2}. Degassed 1M KCl solution (10 mM Tris buffer, 1 mM EDTA, titrated with KOH to pH 10) is then flowed through both sides of the cell with syringes (still underwater so that no air is introduced). The cell was then removed from the water, dried off with an N\textsubscript{2} gun, and connected to a current amplifier. Any residual CO\textsubscript{2} bubbles on the graphene surface are removed by hydroxyl ions in solution (CO\textsubscript{2} + 2OH\textsuperscript{-} \rightarrow CO\textsubscript{3}\textsuperscript{2-} + H\textsubscript{2}O), allowing the graphene to wet completely. We also adopted the PEG coating to reduce clogging. These pore fabrication and wetting strategies allowed us to gather a substantial number of free DNA translocations with \( \sim 50\% \) total yield (data shown in Fig. 3.4).
Fig. D.1 shows data from a translocation experiment with a ~5 nm graphene nanopore. The top panel is the raw data, showing significant variation of the open-pore current due to the low-frequency noise (see Fig. 3.1i). The free translocations (short downward spikes) can still be identified because they are so deep (~1.5 nA) due to the thin membrane\textsuperscript{18,21}. A 50 Hz highpass filter removes the slow variation of the open pore current and allows a much cleaner identification of the translocation events. Using this strategy, we were able to detect single-stranded (Fig. D.2a) and double-stranded DNA translocations (Fig. D.2b) in the same pore. The translocation blockages of the double-stranded DNA were clearly larger due to the increased cross-sectional area (Fig D.3c).
Fig D.1 - Raw DNA Translocation Data
**Top panel:** raw ionic current data for free DNA translocations through a ~5 nm graphene nanopore. The translocation events are seen as downward spikes.
**Bottom panel:** data filtered through 50 Hz highpass filter that removes low-frequency noise. Once the baseline current is stabilized like this, identification and characterization of DNA translocations is much more straightforward.

Fig D.2 - DNA translocations
(a) Concatenated ssDNA translocation events through a ~6 nm graphene nanopore.
(b) Concatenated dsDNA translocation events through the same nanopore. (c) Histogram of current blockages for ss and dsDNA, showing a clear different in blockage magnitude.
D.3. **IMMOBILIZED DNA BLOCKAGES**

Free translocations generally cannot be used for sequencing because the DNA does not spend enough time at each position to allow enough sufficient ionic current data to be recorded. In order to determine the sensitivity of coated graphene nanopores for DNA sequencing, we performed an immobilized DNA experiment. Poly A 30mers of single-stranded DNA were attached to neutravidin proteins, which are too large to fit through the nanopore (Fig D.3a). The molecules were prepared so that most neutravidin molecules have only one attached ssDNA fragment. When the DNA is pulled into the nanopore, it gets stuck when the neutravidin hits the pore, allowing a long-time measurement of the ionic current blockade. At moderate holding voltages (~50 mV or more), the DNA-neutravidin complex can be held in the pore indefinitely, until it is released by reversing the voltage bias (Fig D.3b). Performing this measurement with 30mers of each DNA base and comparing the ionic current blockages could in principle demonstrate base discrimination. However, in our experiment was clear that the low-frequency noise is so high that base discrimination would be impossible. Indeed, the ionic current differences between bases are expected to be a few percent of the total current blockage, but the open-pore current wanders by more than this amount. A low-pass filter cannot be used (as opposed to with free translocations) because the signals for immobilized or ratcheted DNA strands overlap with the low frequency noise. Clearly, DNA sequencing with graphene nanopores requires a strategy to significantly reduce the low frequency noise.
Fig D.3 Immobilized DNA blockages
(a) Schematic of Neutravidin DNA complex. A 30bp polyA fragment of ssDNA is attached to a neutravidin protein. The neutravidin is too large to fit through the nanopore and thus causes the molecule to be captured and immobilized by the pore.
(b) Raw ionic current data of capture events. Once captured, the molecule remains in the pore until it is released by reversing the voltage bias. While the capture events can be clearly distinguished, the low frequency noise makes detailed examination of the molecule impossible.

D.4. LOW-FREQUENCY NOISE IN GRAPHENE NANOPORES

Garaj et al.\textsuperscript{18} first noticed the high low-frequency noise in graphene nanopores and attributed it to conduction through pin-hole defects in the membrane. A control experiment using a pore in a very small area membrane (~20 nm) exhibited much lower noise. However, our recent experiments cast doubt on the pin-hole defect hypothesis. The reliability with which we can create single pores using electrical pulse fabrication (see Chapter 3 for details) suggest that defects large enough to conduct ions are absent from the membrane. Furthermore, we have observed that the noise is not significantly reduced after coating the membrane with PEG, which would presumably cover up pinhole defects.
Heerema et al.\textsuperscript{23} studied how low-frequency noise in graphene nanopores is affected by experimental conditions. They showed that the power spectral density of ionic current through graphene nanopores is dominated by a $1/f$-like spectrum (which agrees with our measurements, see Fig. 3.1h,i). They found that the magnitude of this $1/f$ noise varied considerably from sample to sample, but did not strongly depend on experimental factors like salt concentration, solution pH, and nanopore size. They did observe that pores in few-layer (10 or more) graphene membranes had significantly reduced noise, and hypothesized that the low-frequency noise is caused by mechanical vibrations of the membrane. Kumar et al.\textsuperscript{24} reported that replacing the silicon wafer with a quartz wafer significantly reduces low frequency nanopore noise, but did not present a physical argument for why the wafer material should be relevant to the nanopore noise properties.

With the goal of reducing low-frequency noise in mind, we fabricated graphene nanopore chips with a variety of aperture sizes (10 – 100 nm) and characterized the low-frequency noise of nanopores fabricated using electrical pulsing. Since control over apertures this small is difficult using a FIB, we measured the aperture area using TEM imaging after the noise measurements. Fig. D.4 shows the total RMS noise (1 Hz – 10 kHz, 100 mV applied voltage, normalized to 1 M KCl conditions) as a function of aperture size (the size of the suspended graphene membrane). In this frequency range the total RMS noise is dominated by $1/f$ noise. The trend of decreasing noise with smaller apertures, which was anecdotally noted by Garaj. et al.\textsuperscript{18}, is confirmed by our
data. As a comparison, measurements from other recent publications are also included\textsuperscript{23,25} in Fig. D.4. Although very small membranes (~10 nm) may achieve low enough noise levels for sequencing, they increase the difficulty of sample preparation. These results lend indirect support to the hypothesis that the low frequency noise is caused by mechanical vibrations, if we can safely assume that (1) larger membranes cause larger vibrations and (2) vibrations cause ionic current noise. While (1) is very likely to be true, a mechanism for (2) not obvious, and neither point has been directly measured. These questions are ripe for future studies.

![Graphene Nanopores RMS Noise](image)

**Fig D.4 Low Frequency Noise**

RMS noise in nA (1 Hz-10 kHz, 100 mV applied voltage, normalized to 1M KCl) for a variety of graphene nanopores, plotted as a function of the aperture diameter (the area of the suspended graphene). There is a clear trend of lower noise for smaller apertures. The typical noise levels of SiN\textsubscript{x} pores is included for reference. The grey line indicates the estimated magnitude of ssDNA blockages. Differences between bases are expected to be a few percent of the blockage magnitude.
D.5. **Summary**

We have addressed issues of device yield, wetting, and DNA-pore interactions, but low-frequency noise remains as a major impediment to DNA sequencing with graphene nanopores. While working with very small area graphene membranes can mitigate this problem, it increases the difficulty of sample preparation. Future studies are needed to develop a deeper understanding of the causes of this low-frequency noise. Such an understanding may reveal important secrets that nature has already applied in designing biological nanopores, which often do not exhibit any measurable 1/f noise.

D.6. **References**


