GRAPHENE SUPPORTED HAFNIUM OXIDE NANOPORES FOR DNA SENSING

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THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Bioengineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT

The completion of the Human Genome Project in 2001\textsuperscript{1} has served as both an inspiration and a challenge for researchers in the past decade. Understanding the genetic makeup of organisms is crucial for early disease detection, one of the driving forces behind personalized medicine. In this work, state-of-the-art sequencing technologies are reviewed and compared with next-generation sequencing technologies. In particular, solid-state nanopores are investigated and recent developments in the field are discussed. The interdisciplinary effort from researchers to establish solid-state nanopores as a viable sequencing platform is thriving on multiple fronts including surface charge engineering for DNA capture\textsuperscript{2} and conductance modulation\textsuperscript{3} in nanopores, nanowire transistors for localized detection\textsuperscript{4}, ultra-thin membrane fabrication using graphene\textsuperscript{5}, and the exploration of alternative nanopore materials for biosensing applications\textsuperscript{6}. In this work, the development of a new solid-state nanopore sensor consisting of hafnium oxide suspended by functionalized graphene is reported along with DNA transport properties and dielectric characterization of the film in solution.
ACKNOWLEDGEMENTS

First I would like to thank my adviser, Prof. Rashid Bashir, for his guidance and support during this project. I am thankful for the opportunity to be a part of his group, for his encouragement, patience, and for his friendship. I am especially thankful for the help, support, wonderful hours of work together, and encouragement of Dr. Jiwook Shim. I would also like to express sincere gratitude to Dr. David Estrada for the many helpful discussions and encouragement throughout this process. In addition I would like to thank Shouvik Banerjee for his insightful discussions and support. I am also thankful for Dr. Glennys Mensing, who gave me great suggestions on difficult topics related to fabrication.

I would also like to thank the MNTL staff for their hard work and effort. I would especially like to thank Edmund Chow, Yaguang Lian, and Michael Hansen for their invaluable advice and training in the cleanroom. I am also thankful for supporting funds from the National Science Foundation graduate fellowships program and the National Institutes of Health. Most importantly, I would like to thank my lovely wife Lorna for her tremendous support and my wonderful parents.
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CHAPTER 1: INTRODUCTION

James Watson and Francis Crick discovered the molecular structure of DNA in 1953, a double helix of complementary base pairs that contains the genetic instructions for human life. The molecule consists of a sugar phosphate backbone held together by hydrogen bonds between the nucleotides adenine, thymine, cytosine, and guanine. DNA has a diameter ranging from approximately 2-2.4nm and a persistence length of 50nm, or 150 base pairs. Since the completion of the Human Genome Project in 2001, researchers have been trying to significantly decrease the cost of genome sequencing. The so-called $1000 dollar genome, a catchphrase coined by the National Human Genome Research Institute, has never been closer to becoming a reality with the scientists and engineers of various research groups and industry labs entering what they call a ‘heated race’ for personalized medicine. Remarkably, the cost of DNA sequencing has been decreasing over time in similar fashion to Moore’s law as state of the art sensors become integrated with solid state technology (as shown in Figure 2).

The ultimate goal of high throughput and fast DNA sequencing is to usher in an era of personalized medicine where the medical community will be able to detect diseases earlier and treat them more effectively. This is a challenging feat since the human genome contains 3.1 billion chemical nucleotide bases. The average gene consists of 3000 bases, but sizes vary greatly. In order to bring down the cost of full human genome sequencing, there needs to be a technology that is specific and high throughput, in addition to using a limited amount of reagent to save cost.

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The human genome project was accomplished using the shotgun sequencing method. In this method, chain terminating nucleotides are used to decipher an unknown strand of DNA. In order to get enough DNA to accomplish this, researchers amplify existing DNA by incorporating the sequence into a plasmid vector. A plasmid vector is a circular piece of DNA that is separate from a bacterial cell’s chromosomal DNA. DNA fragments can be amplified by mixing E. coli with recombinant vector DNA and allowing the bacteria to replicate. Once the DNA fragments were separated from their bacterial DNA plasmids and considered ready for sequencing, the strands were sequenced using the Sanger Sequencing method. This is similar to a PCR reaction, where DNA is denatured at a higher temperature (90C) and primers anneal to it. At lower temperatures (approximately 50C), the DNA polymerase attaches to the unwound strand and synthesizes a duplicate strand via nucleotide incorporation.
Figure 1: Schematic of sanger sequencing method\textsuperscript{10} showing DNA fragmentation, cloning using a plasmid vector, and sequencing using chain terminating nucleotides.

In the case of Sanger sequencing, chain-terminating nucleotides that have been fluorescently labeled are mixed in with the strands to be sequenced\textsuperscript{7}. These nucleotides consist of a dideoxyribonucleoside triphosphate (ddNTP) instead of a deoxyribonucleoside triphosphate (dNTP). In other words, they have a hydrogen atom instead of a hydroxyl group attached to the 3’ carbon atom, which makes formation of a phosphodiester bond with the next nucleotide triphosphate impossible. If there are chain-terminating nucleotides present in the solution, then the sequence will stop at fluorescently labeled set points that can be deciphered later by cross referencing with a computer (see Figure 1).
While shotgun sequencing is effective, there is a drive to cut down sequencing time by using less reagents and driving a faster reaction time. For this reason, both biological and solid-state nanopores have emerged as potential technologies for driving the cost of DNA sequencing down even further. The principle behind using nanopores is similar to the Coulter counter, whereby a thin membrane is sandwiched in between two fluidic reservoirs containing an electrolyte solution. It is possible to sense current traveling across the two chambers if there is a nanometer scale aperture in the thin membrane between both solutions. By measuring ionic conductance across the membrane, it is possible to detect single molecule translocation when the ionic current decreases, indicating an obstruction in the nanometer-sized aperture between both fluids.

By applying a voltage across the system, negatively charged DNA will be electrophoretically driven through the nanopore, away from the negatively charged side of the chamber. Nanopores provide a single molecule approach to DNA sensing and hold a potential
for great impact in the sequencing community since it is both label-free and amplification-free.

In addition to DNA sequencing, nanopores can also be used to detect proteins and DNA-protein interactions based on changes in conductance as single molecules traverse the pore. From a signal to noise ratio point of view it is most favorable to have the thinnest membrane possible for both sequencing and detection of DNA-protein complexes.

Ultra-thin graphene membranes are quite attractive due to their atomic-scale thickness and mechanical strength. However there are also challenges associated with the organic membrane, such as increased hydrophobic character in the nanopore and potential for DNA sticking onto the basal plane surface. In this work, we examine hafnium oxide solid-state nanopores for DNA sensing. Graphene is used as a supporting structure and locally induced crystallization is observed on the hafnium oxide membrane upon irradiation by a transmission electron microscope beam.
CHAPTER 2: LITERATURE REVIEW AND PREVIOUS WORK

2.1 Biological nanopores

As mentioned earlier, nanopores are a potential candidate for performing low cost, amplification free, and label-free DNA sequencing. Naturally occurring nanopores, or biological nanopores, offer distinct advantages for DNA sensing. Biological nanopores are reproduced heterogeneously in nature, they can be bioengineered to express specific properties in terms of shape and size, and they are produced by cells with high precision\textsuperscript{11}. Two biological nanopores have attracted considerable research interest for various reasons: alpha hemolysin and Mycobacterium smegmatis porin A (MspA).

Alpha hemolysin is a porin secreted by bacteria that causes the lysis of red blood cells \textsuperscript{12}. The alpha hemolysin pore consists of a 1.4nm vestibule and a 2.6nm wide beta barrel.

Researchers can incorporate this biological nanopore into a lipid bilayer for DNA sensing measurements\textsuperscript{11}. Kasianowicz et. al first reported current blockade and time distributions for single-stranded DNA through an alpha hemolysin pore \textsuperscript{13}. While it is possible to thread ssDNA through alpha hemolysin, it is virtually impossible to sequence DNA since the translocation velocities are so high (on the order of 1 million nucleotides per second).
The ability of naturally occurring alpha hemolysin may be limited in DNA sequencing, but Clarke et al reports an alternative approach that involves genetic modification of the alpha hemolysin pore to distinguish individual monophosphates that have been cleaved from a single strand of DNA. Conceptually the idea is simple: use an exonuclease to digest a strand of DNA whilst detecting the released nucleosides with a hemolysin pore that has been modified with a covalently attached adapter molecule. The permanent adapter allows for single monophosphate identification as shown in Figure 4, where each base produces a different blockade signature in terms of the ionic current.

**Figure 3:** Schematic of alpha hemolysin in a lipid bilayer membrane

**Figure 4:** Nucleotide event distributions with permanent adapter. (a) Single channel recording from mutated nanopore showing nucleotide discrimination. (b) Corresponding residual current histogram of nucleotide binding events.
DNA sequencing via genetically modified alpha hemolysin, with the aid of exonuclease digestion, is plausible in principle. An alternative is to use a different porin such as Mycobacterium smegmatis porin A (MspA), an octameric protein with a nanopore that is suitable for passage of single stranded DNA. In 2008, Butler et. al identified the porin as a suitable candidate for nucleic acid detection due to its distinct geometry; the nanopore has a 1nm long wide and 1nm long constriction with a wider region surrounding it. However, MspA is not without its drawbacks. Wild-type MspA appears to have a high density of negative charges that surround the nanopore area, making it very difficult for single stranded DNA translocation. Site-directed mutagenesis was necessary in order to observe ssDNA passage through the nanopore. Studies done on mutated MspA reveal that DNA translocation as well as sequencing is possible under the right conditions. Derrington et. al demonstrated proof of principle DNA sequencing with MspA by changing its structure via site directed mutagenesis. Figure 5 shows the crystal structure of MspA.
2.2 Solid state nanopores

While biological nanopores offer distinct advantages such as reproducibility in pore formation and low noise characteristics, solid state nanopores have emerged as a viable counterpart due to their increased lifetime and robustness. The principle behind using solid-state nanopores is the same: driving charged molecules through a nanopore using an applied voltage. The magnitude of the ionic current is dependent on the concentration of ions in the solution and the size of the nanopore. The conductance of the nanopore depends on the geometry of the pore as well as the thickness of the membrane as shown in Eqn. However, it has recently been shown that for thinner membranes the conductance value starts to scale linearly with the pore diameter as opposed to following a square dependence as shown in the conductance equations below\textsuperscript{18}. This is in experimental agreement with the results presented by Garaj et. al where graphene is studied as a transelectrode membrane and the conductance becomes linearly proportional to the pore diameter\textsuperscript{19}. This dependence arises from the atomic scale thickness of the graphene membrane.

\[
G = \sigma \frac{\pi d^2}{4l}
\]

\[
G = \sigma \left[ \frac{4l}{\pi d^2} + \frac{1}{d} \right]^{-1}
\]

In the equation above, \( G \) is for conductance, \( d \) is diameter, \( l \) is the membrane thickness, and \( \sigma \) is the conductivity of the media. In terms of DNA translocation through a solid state nanopore, the conductance depends on the change in the effective diameter of the nanopore with and without DNA present inside the pore. The equations below show how to calculate the change in conductance from a DNA molecule translocating through the nanopore\textsuperscript{18}. It can also be seen
from the equations above that the thickness of the membrane has an influence on the signal to noise ratio. For thinner membranes the conductance becomes greater, producing a larger signal output. Thus, it is always preferable to drive nanopore systems to thinner membranes.

\[
\Delta G = G_{\text{open pore}} - G_{\text{with DNA}} = G(d) - G(d_{\text{with DNA}})
\]

\[
d_{\text{with DNA}} = \sqrt{d^2 - d_{\text{with DNA}}^2}
\]

Silicon nitride nanopores were first sculpted by Li et al. by using a focused ion beam\(^2\). These membranes are highly favored in the nanopore community due to their low stress and chemical inertness, but process control over thickness is difficult. An alternative material that has recently been used for nanopore formation is aluminum oxide deposited via atomic layer deposition (ALD). ALD is a self-limiting process that allows for subnanometer control over deposition thickness. Venkatesan et al. demonstrated aluminum oxide nanopores that were more mechanically robust than their silicon dioxide counterparts and exhibited lower 1/f noise characteristics\(^2\). In addition, dose-dependent nanocrystallite formation was shown to reduce DNA translocation speeds by several orders of magnitude\(^1\).

**Noise in solid-state nanopores**

Both biological and solid state nanopores are subject to limitations based on temporal and spatial resolution. The latter refers to the resolution of the device, namely whether single base pairs can be distinguished with the technology. In contrast, temporal resolution refers to the speed at which the molecule translocations. There is also the issue of noise: current fluctuations in the nanopore conductance arise due to several factors and limit the viability of nanopores as a potential candidate for nucleic acid base identification. Flicker (or 1/f), thermal (Johnson), and
capacitive (dielectric) noise are all present in the system when conducting nanopore measurements. All of these are dominant at different frequencies as shown in Figure 6. In order to improve sensor performance, researchers have tailored nanopore fabrication toward noise reduction\textsuperscript{22}. It has been shown that increasing the capacitance of the system and reducing the hydrophobicity of the pore area can reduce noise\textsuperscript{23}.

\[
S(f) = \frac{A_0}{f} + a_1 + a_2f + a_3f^2
\]

**Figure 6:** Regions of noise in solid state nanopores divided into frequency regimes\textsuperscript{24}.

The power spectral density (PSD) of noise in solid state nanopore systems can be described by the second order polynomial shown in Equation where \( f \) is frequency and \( a_0, a_1, a_2, \) and \( a_3 \) are contributions of the different noise sources (flicker, thermal, dielectric, and capacitive)\textsuperscript{23}. The power spectral density plot shows that at lower frequencies the noise is dominated by a \( 1/f \) characteristic whereas at high frequency (greater than 10khz) the noise is dominated by dielectric noise. The dielectric noise is a consequence of the capacitance of the chip. As mentioned before, solid state nanopores are typically fabricated with silicon nitride or silicon dioxide. Smeets et al extracted the capacitive loss associated with silicon nitride and showed a
significant deviation from the behavior of an ideal insulator. Thus, there are losses in the nanopore system that give rise to fluctuations in the ionic current signature.

1/f noise is ubiquitous in both biological and physical systems. Interestingly it is present in heart beat rhythms and neural activity as well as semiconductors. The origin of this phenomenon has not been explained completely and is still the subject of much research. In solid state nanopores, 1/f noise has been attributed to a variety of physical factors including surface charge fluctuations at the nanopore surface as well as the mobility of charge carriers. Excessive 1/f noise has also been attributed to nanobubbles present in the nanopore and has been shown to be reduced by addition of a hydrophilic oxide layer. Hence, the hydrophilic properties of the nanopore are important in reducing 1/f noise. Minimizing noise at both low frequency and high frequency bandwidths is important in order to improve sensitivity and feasibility of nanopore sensors.

2.3 Nanopore drilling

Nanopores may be drilled using a focused convergent electron beam. The process consists of an electron source, typically a field emission gun, which releases a beam of electrons down the TEM column. The three condenser lens setup shown in Figure effectively demagnifies the gun crossover as much as possible in order to produce a focused, convergent beam on the membrane specimen. The beam causes direct atomic displacement which causes sputtering of the inorganic solid state material. The equation which relates the threshold energy \( E_t \) needed to displace an atom from the lattice of a material, which depends on both the atomic bond type \( E_d \) as well as the atomic weight of the atom \( A \), is shown below.
This equation confirms that the larger the atom, the larger the threshold energy. Figure 7 shows a cross section of the TEM column down to the specimen. There are three lenses shown, condenser lens 1, 2, and 3. Condenser lens 1 produces an image of the gun crossover, which comes from the source of electrons that the TEM emits. Condenser lens 2 is turned off and the rays are directed through a small aperture. The beam of electrons is then focused by the C3 lens. This type of setup ensures that the demagnification of the gun crossover is maximized; rendering a nanometer sized focused spot on the specimen.

\[
E_t = \frac{\left(\frac{100 + AE_d}{5}\right)^{1/2} - 10}{20}
\]

**Figure 7:** Condenser lens setup in order to produce a focused convergent beam using TEM\(^\text{27}\).

The diameter of a nanopore can be tuned by adjusting the beam current density of the electron gun. Kim et al has shown that nanopore contraction occurs when defocusing the beam.
and reducing intensity to $10^6$ e/nm$^2$. Typical beam current densities range on the order of $10^8$-$10^9$ e nm$^{-2}$. The electron beam induced material sputtering process may also lead to selective decomposition of weaker bound atoms in the material. For instance, it has been shown that Al$_2$O$_3$ nanopores fabricated via electron beam induced sputtering are oxygen deficient, thereby leaving an aluminum rich region in the nanopore area. Theoretical investigations also point to localized heating as a possible mechanism for the induced knock on damage of atoms upon electron beam exposure. Temperature rise during electron induced damage can be attributed to the sum of the elastic energy loss as well as the inelastic energy loss. The equation below shows the e-beam induced temperature increment, where $J$ is the current density of the beam, $k$ is the thermal conductivity, $R$ is the radius of the e-beam bombardment area, $D$ is the thermal diffusivity, $C_v$ is the specific heat, and $t$ is the e-beam exposure time.

$$\Delta T_e \approx \frac{3JQ}{8\varepsilon\kappa} R^2 \ln\left(1 + \frac{4Dt}{R^2}\right)$$

### 2.4 Alternative applications for nanopores

In addition to DNA detection, it has been recently shown by our group (Banerjee, Shim, Rivera, et al) that nanopores are a useful tool for studying the electrochemical exchange of ions at graphene edges without interference from defective sites in the basal plane. This was accomplished by seeding the graphene surface with a metal and depositing aluminum oxide dielectric on top via atomic layer deposition. The graphene edge embedded structure provides a unique capability to study the electrochemical exchange at an individual graphene edge where we found current densities as high as $1.2 \times 10^4$ A/cm$^2$. We also report ionic current modulation in the nanopore by biasing the embedded graphene terminal with respect to the electrodes in the
fluid. The high electrochemical specific current density for a graphene nanopore-based device can have many applications in sensitive chemical and biological sensing, and energy storage devices. The following section, including two figures (Figure 7 and Figure 8), was adapted with permission from Banerjee, S., Shim, J., Rivera, J., Jin, X., Estrada, D., Solovyeva, V., You, X., et al. (2012). Electrochemistry at Edge of Single Graphene Layer in a Nanopore. ACS nano, 7(1), 834–843. Copyright 2012 American Chemical Society. Graphene has attracted tremendous interest in the scientific world over the recent years due to its unique electronic, thermal, and optical properties. It has shown great promise in the field of electronics, biological and chemical sensing, and energy storage applications. Studies on graphene electrochemistry have suggested the ability of graphene-based electrodes to carry a large amount of current at electron transfer rates superior to graphite and carbon nanotube (CNT) electrodes. The relative abundance of carbon on earth combined with widespread knowledge of carbon-based chemistries and stability makes the study of graphene-based electrochemistry extremely exciting.

Graphene sheets have two types of electron transfer sites—edge and basal. Edge sites have already been demonstrated to possess enhanced electron transport rates and reactivity in studies of CNT ends. Graphene has a higher theoretical specific surface area (2630 m2/g) than graphite and CNTs (1315 m2/g) and provides motivation for study of heterogeneous electron transfer rates. In addition, graphene can carry significant current densities without degradation from electro-migration which typically causes significant damage in ultrathin metal films. Current densities as high as $2 \times 10^9$ A/cm$^2$ have been reported for nanoscale interconnects based on graphene grown by chemical vapor deposition (CVD). The graphene edge plane atoms have been reported to have significantly higher electron transfer rates compared to basal planes in
electrochemical studies on both highly ordered pyrolytic graphite as well as on multiple layers of graphene.\textsuperscript{42,43}

Graphene-modified glassy carbon electrodes have been reported to have much greater electrochemical response than unadulterated glassy carbon electrodes to molecules like paracetamol, hydrazine, glucose, and ethanol dopamine as well as heavy metals.\textsuperscript{37,39} Zhou et al.\textsuperscript{44} demonstrated the ability of chemically reduced graphene oxide electrodes to distinguish the electrochemical current signal from the four bases of DNA, which could not be distinguished with graphite and glassy carbon electrodes. Another important application of graphene electrochemistry is in energy storage devices. The specific capacitance of chemically modified graphene was found to be up to 1352 F/g, and extremely high energy densities up to 85.6 Wh/kg at room temperature have been reported.\textsuperscript{45} Furthermore, graphene and hybrid graphene-based electrodes have been used to increase specific capacities of Li+ ion based batteries, improving power density and cyclic performance, while maintaining mechanical integrity at high current densities.\textsuperscript{46}

Despite extensive studies on graphene sheets and graphene doped electrodes, the electrochemical properties of isolated graphene edges remain relatively unexplored. Here, we demonstrated a graphene edge embedded nanopore (GEEN) structure to isolate graphene edge electrochemical activity from basal plane activity. Transmission electron microscopy (TEM) based sculpting offers potential for control on graphene edge structures. Furthermore, we demonstrate the use of the embedded graphene edge to modulate the ionic flux in the nanopore. Along with a conductive graphene terminal of thickness equivalent to the distance between two adjacent base pairs in dsDNA (0.34 nm), this could provide a basis for single DNA molecule analysis with measurement methodologies like tunnelling or electrochemical redox reactions.
The fabrication of graphene nanopores using a TEM has been demonstrated previously and used to sense biomolecules like polynucleotides and DNA protein complexes\textsuperscript{47-5}. In this study, we fabricated GEENs in stacked graphene and dielectric layers using a focused electron beam in a TEM (200 keV), and measured the electrochemical current exchange at the graphene edge embedded within the nanopore. The top Al\textsubscript{2}O\textsubscript{3} dielectric layer isolates the basal plane electrochemical activity. We demonstrate the very high electrochemical current density as well as the first known study of electrochemical current exchange at the graphene (potentially as thin as single layer) edge in an ionic solution. The combination of nonlinear diffusion at nanoscale electrodes, an enhanced concentration gradient of ions in the vicinity of the nanopore\textsuperscript{48} and high electron transfer rates at damaged edges of graphene\textsuperscript{42} creates a unique system with high electrochemical current densities.

Fabrication of our graphene embedded edge nanopore (GEEN) begins with a suspended hydrophilic supporting membrane of stacked layers of 50 nm Al\textsubscript{2}O\textsubscript{3}, 200 nm Si\textsubscript{3}N\textsubscript{4}, and 50 nm Al\textsubscript{2}O\textsubscript{3} is fabricated using deep reactive ion etching (DRIE). Subsequently, a hole of 300 ± 40 nm is formed in the supporting membrane using a focused ion beam (FIB). The graphene−Al\textsubscript{2}O\textsubscript{3} stack is then formed on the supporting membrane with the FIB hole by transferring graphene films grown by CVD.

We note that the hydrophilic nature of the supporting membrane helps spread the water more evenly during the graphene transfer steps and improves the smoothness of the transferred graphene/PMMA stack. The Raman spectroscopy maps of the graphene 2D to G peak intensity ratios (I\textsubscript{2D}/I\textsubscript{G}) were reported as well as the the full-width at half-maximum of the 2D peak show our growth process results in a mix of monolayer and bilayer graphene, similar to our previous work. The first graphene layer (G1) in our stack spans the FIB hole and acts as a mechanical
support for deposition of the subsequent graphene and dielectric layers of our architecture. We note that subsequent to the graphene transfers, the membranes are annealed in an Ar/H2 atmosphere at 400 °C to remove PMMA residue remnant from the transfer process.49

To ensure uniform nucleation of the subsequent Al₂O₃ deposition (D1) onto the chemically inert graphene basal planes, a metallic seed layer of Al (2 nm thick) is evaporated onto the graphene.⁵⁰ Al₂O₃ is a suitable choice as the dielectric because of its excellent mechanical stability⁶ and reduction in 1/f noise compared to Si₃N₄ and SiO₂ membranes.¹² ALD is chosen as it offers subnanometer control over dielectric thickness in addition to being a conformal deposition technique and a low temperature process, making it compatible with the previously transferred graphene layers.

![Figure 8: Leakage test on various thicknesses of Al₂O₃.](image)

(a) Leakage current density measured for Al₂O₃ on conductive silicon. Al₂O₃ thickness less than 10 nm showed leakage current greater than 1 nA/mm² at 500 mV, but thicker Al₂O₃ (>10 nm) showed much greater insulation over the voltage range of −500 to +500 mV. (b) Leakage current density for Al₂O₃ deposited on graphene. Leakage current is observed to be fairly high up to 20 nm-thick Al₂O₃. Also the leakage is significantly higher for positive voltage at Ag/AgCl electrode. Al₂O₃ at 24 nm thickness displays decent insulation from leakage.³¹
The thickness of the dielectric deposited is 24 nm, a value established through extensive leakage testing in fluidic environments (Figure 8). Similar thicknesses of dielectric have been reported to provide effective isolation in ionic fluid environments in transistor-based devices.\textsuperscript{51} A second graphene layer (G2) is transferred onto D1 and annealed in an Ar/H\textsubscript{2} atmosphere. This layer is contacted using Ti/Au contacts and insulated by depositing another 24 nm of Al\textsubscript{2}O\textsubscript{3} (D2) as described above.

**Figure 9:** Electrochemical measurements for embedded graphene nanoelectrode. (a) Schematic diagram of measurement setup. For the drain–source measurement (gray), the source is connected to ground and voltage applied at the drain. For drain–gate (red) and drain–source (blue) measurements, the gate is connected to ground and voltage is applied to the other terminal. (b) Current–voltage curve of nanopore ionic current and electrochemical behavior of graphene edge through 5 nm nanopore. Identical currents through the drain–gate and source–gate pathways indicate electrochemical exchange at the exposed graphene edge. (c) Conductance dependence on pore diameter. Drain–source conductance shows a square dependence on pore diameter, while gate current exchange shows a fairly linear dependence on pore diameter consistent with electrochemical exchange at cylindrical nanopore wall. The slight variation from linear dependence may be attributed to varying graphene sheet thickness on various regions of the membrane; 5, 9, 14, and 20 nm diameter nanopores were used in this study. All experiments are performed in 1 M KCl, 10 mM Tris, 1 mM EDTA at pH 7.6.\textsuperscript{31}

In summary, we present the investigation of electrochemical current exchange at CVD-grown graphene edges within a nanopore. We demonstrate the ability of our graphene embedded nanopore structures to study electrochemistry at graphene edges isolated from electrochemical contributions of the basal plane. We observed electrochemical current densities on the order of
104 A/cm², 3 orders of magnitude higher than those reported for carbon nanotubes and much higher than those reported for graphene surface electrochemical studies. The high currents are attributed to a combination of the nanopore edge structures produced by electron beam sculpting along with the convergent diffusion mechanisms due to nanosized electrodes, which have been reported to enhance ionic flux of reactive species. We also demonstrated the modulation of ionic current by the use of the embedded conductive graphene terminal. Numerical simulations were performed to confirm the transistor like characteristics of the device. Extremely high electrochemical current densities have exciting applications for both chemical and biological sensing as well as energy storage. The scaling of these structures by producing arrays of nanopores could enable multiple applications.

Nanopore/nanofluidic transistors

In the past decade or so, solid state nanopores have emerged as suitable candidates for single molecule studies. These have been used for detection of DNA, proteins, lambda DNA, as well as DNA methylation. In addition, there have been numerous studies done on nanopores as nanoscale fluidic channels. Researchers have demonstrated the possibility for manipulating ionic transport through surface charge modulations in the nanopore. In effect, nanopores are turned into ionic field effect transistors by embedded an electrode in the nanopore membrane and passivating the nanopore sidewall with a dielectric of choice. Electrostatic control of charges through the nanopore has been realized in a number of configurations. Jiang et. al demonstrated charge regulation in nanopore IFETs by looking at the source-drain current in a nanopore with varying applied potentials at the gate sandwiched in between two insulators. Their results are in well agreement with their electrofluidic gating model. Control of DNA capture by nanopore transistors was recently achieved by Paik et. al. They used electrically...
gated pores that were larger in size, on the order of 200nm, to regulate DNA transport. In the paper, they showed that by applying a positive gate voltage they were able to reduce sodium ion concentration on the pore wall, thereby allowing DNA passage through the nanopore. In contrast, low gate voltage attracts sodium ions to the pore wall and results in DNA rejection from the nanopore.
CHAPTER 3: MATERIALS AND METHODS

3.1 Membrane fabrication

Membranes consisting of stacked layers of Al₂O₃ and Si₃N₄ were fabricated on 300 (+/- 2 μm thick double-side polished) silicon wafers purchased from SiliconQuest International. Wafers are piranha cleaned (1:1 H₂SO₄/H₂O₂) for 15 min before depositing Al₂O₃ via ALD (Cambridge Nanotech). Al₂O₃ (50 nm) was deposited at a platen temperature of 250°C using tetramethyl-aluminum (TMA) and water vapor precursors. Subsequently, 200 nm of low-stress Si₃N₄ is deposited (STS Mesc PECVD system) using a mixed-frequency recipe (high frequency, 6 s at 13.56MHz, platen power of 20W; and low frequency, 2s at 380 kHz, platen power of 60W) with precursors SiH₄ and NH₃ at flowrates of 40 and 55 sccm, respectively, at a platen temperature of 300°C. Another 50 nm of Al₂O₃ (ALD) is deposited with the same parameters as described before.

Optical lithography is used to define 80μm square windows on the back of the wafer with the aid of plasma resistant Megaposit SPR-220 photoresist and an ABM Flood Exposure (model 60) tool. Before starting the lithography process, the wafer was O₂ descummed for 1 minute at 100W using a Jupiter RIE tool. SPR-220 photoresist is spun at 3000rpm for 30 seconds and soft-baked (2 minutes at 60C and 1 minute at 110C). In order to protect the front side of the wafer, 5 um of KMPR 1010 photoresist was spun on the front side of the wafer at 3000rpm for 30 seconds for protection. The wafer is soft-baked for 5 minutes followed by flood exposure for 90 sec and later hardbaked for two minutes.

The wafer is then placed inside an STS Pegasus ICP DRIE for 20 minutes and 80um square membranes are suspended using a Bosch etching process. The Bosch etch consists of
alternating between passivation (C₄F₈) and etching (SF₆) gasses in order to create a vertical sidewall. The process leaves a suspended membrane that is approximately 300nm thick with the aluminum oxide layer serving as a stop layer. Careful treatment of the membranes is required as they are quite fragile. After fabricating the membranes, the structures are ready to be milled using an FEI Focused Ion Beam tool. In order to make sure the aluminum oxide would serve as a stop layer in practice, we deposited 48nm of Al₂O₃ on a bare silicon wafer and tested the thickness using an ellipsometer. Three spots were covered with tape on the wafer and it was placed inside the STS Pegasus ICP RIE tool where we ran a Bosch Etch process to detect the etch rate. The aluminum oxide was etched at a rate of about 1nm/min according to this process after verifying with ellipsometry.

**Figure 10:** Advanced Bosch etching process showing cross sectional view passivation, depassivation, and etching steps.⁵⁶
**Figure 11:** SEM image of nanopore chip sidewall after Bosch etching process in STS Pegasus ICP RIE

**Figure 12:** Cross sectional membrane process flow (left) and 300nm FIB hole (right)
In order to mill structures in the 300nm membranes, samples are loaded into the FEI FIB DB235 and pumped down to an internal pressure of 8.6e-6 Torr. In contrast to electron microscopy where electrons are generated and guided to the sample surface, the focused ion beam consists of ions generated by a gallium source. Gallium ions are favorable since gallium is metallic with a low melting temperature and its surface potential allows for both ionization and field emission. Ions are heavier than electrons and thus gain a higher momentum when colliding with the sample substrate. Since heavy ions can transfer their momentum to atoms in the sample lattice, decompositional sputtering is possible as sample atoms are dislocated at similar speed and energy as the incident ions. The removal of atoms from their lattice is also known as milling. The FIB consists of two pole pieces that aim approximately 54 degrees away from each other. For general use, the FIB may be used as an SEM since it also has an electron gun.

After the sample has been loaded and pumped down, the beams in the emission gun are turned on. The electron gun allows for less invasive imaging of the sample and is set at a rate of 15kV while the ion beam is set at 30pA/30kV. The first step in milling a sample is to set the sample stage at the eucentric height (the point at which the electron and ion beams converge onto a tilted axis). First, a single particle near the membrane surface is used to focus the image optically. The free working distance is then set to 5.2. The sample is then tilted 5 degrees followed by 52 degrees. The z-height is adjusted such that the gun crossover is at the same particle you started with. At this point the sample is at the eucentric height and the ion column is aligned with the electron column. At this point the sample is ready to be milled by the ion beam. 300nm nanopores were drilled in this configuration with a drill time of 30 seconds, dwell time of 10us, overlap of 50%, and magnification of 500kx.
Figure 13: FIB ion column and electron column setup

### 3.2 Graphene growth and transfer process

Large-area synthesis of graphene on copper was first reported by Li et. al. Graphene was grown directly on copper using a chemical vapor deposition process and transferred onto our aluminum oxide substrate. The process of chemical vapor deposition consisted of placing a piece of copper foil into a furnace (Atomate CNT Furnace). First, the copper is annealed at 1000°C to increase the grain size of the substrate, thereby providing a higher yield of graphene. Methane, argon, and hydrogen were introduced into the quartz tube where the copper foil was placed under low vacuum. Chemical adsorption of carbon atoms takes place on the copper substrate which serves as a catalyst. The catalytic ability of transition metals such as nickel and copper for graphitic carbon formation arise from empty states in their d-orbitals and the ability to provide low energy pathways. The low solubility of carbon along with the ability to stabilize carbon on its surface makes copper a suitable catalyst for graphene growth. Table shows a detailed description of the graphene growth process.
Figure 14: Chemical vapor deposition of graphene showing the deposition of carbon atoms onto a copper substrate⁶⁰

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<th>Step</th>
<th>Duration</th>
<th>Heat, C</th>
<th>Methane, sccm</th>
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<th>Hydrogen, sccm</th>
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Table 1: CVD graphene recipe

The difference in thermal coefficients between the copper substrate and the graphene film results in so-called graphene wrinkles. However, in contrast to mechanical exfoliation (Ref), the CVD process yields a large sheet of graphene (as large as the substrate) to work with. PMMA is spun onto the copper substrate after chemical vapor deposition. The PMMA is spun at 3000rpm for 30 seconds and baked at 200C. An oxygen plasma treatment is used to remove residual graphene from the back of the copper foil, thereby enabling backside etching of the copper. The process summary has been tabulated below in detail. The copper is then placed in copper etchant
overnight to get rid of the copper foil and leave a suspended sheet of graphene. The process steps were as follows:

1. Spin coat desired piece of Copper foil with PMMA 495 A2 for 3000 RPM for 30 secs
2. Bake at 200C for 120sec
3. Spin coat with PMMA 950 A4 3000 RPM for 30 secs
4. Bake at 200C for 120sec
5. Etch backside graphene in O$_2$ plasma, 100 mTorr, 20 sccm O$_2$, Power 100 Watts (20%), 30-40 secs.
6. Place foil in 1M FeCl$_3$/or oxone solution with PMMA side up (foil should float on top of etchant)
7. After complete etching of copper foil (overnight) transfer PMMA/graphene film to DI water, rinse for about 5 mins
8. Transfer to 10% HCL solution and etch residual copper for 5 mins/or change this step to water
9. Transfer to DI water, rinse for about 5 mins
10. Transfer to substrate
11. Let dry for good adhesion for 2-3hrs, or put on hot plate @40C for 30 min (start heating the plate when out the sample there)
12. Spin coat with 950K PMMA at 3000 rpm for 30secs JEOL film to protect membrane.
13. Bake at 200 C for 120 secs
14. Remove PMMA in 1:1 Methanol:Methylene Chloride (DHM) solution
15. Clean the chip on IPA bath and dry slowly
16. Anneal with the following parameters:
<table>
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<th>Argon, sccm</th>
<th>Hydrogen, sccm</th>
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<td>20 min</td>
<td>1000</td>
<td>200</td>
<td>50</td>
<td>760</td>
</tr>
</tbody>
</table>

Table 2: CVD graphene anneal recipe

Figure 15: Schematic representation of graphene transfer process

After the overnight etch is complete, glass slides were piranha cleaned in 1:1 \( \text{H}_2\text{SO}_4: \text{H}_2\text{O}_2 \) for 15 minutes. The graphene is transferred from the copper etchant to DI water using a glass slide. It is subsequently transferred from the DI water to 10% HCl to DI water once again (the graphene sheets are allowed to sit in the aforementioned solutions for at least 5 minutes in between transfers). This process ensures a clean graphene film that is ready to be
transferred onto our membranes. The graphene is later annealed at 400C for 1.5 hours after being transferred to the aluminum oxide substrate (see table above). This facilitates the removal of PMMA residues from the graphene film.

**Metal seed layers**

Atomic layer deposition is a self-limiting process that is based on pulsing gas precursors sequentially into an enclosed chamber. The main difference between this technique and chemical vapor deposition is that in ALD processes, what would be a CVD reaction is broken down into two half reactions. By controlling the rate of these two reactions you are able to achieve atomic scale control over film thickness. In order for the process to work, the surface on which the film is growing must be hydrogenated freely when exposed to water. In air, water vapor is adsorbed on silicon surfaces that form a hydroxyl group. In the atomic layer deposition process of Al₂O₃ over a silicon surface, water vapor is first dispensed to hydroxylate the substrate, followed by a pulse of tri-methyl aluminum. The tri-methyl aluminum is a molecule that consists of an aluminum center and is flanked by three methyl groups. Chemical adsorption leads to a reaction between the TMA and adsorbed hydroxyl groups, producing methane as the reaction product.
Figure 16: Atomic layer deposition of aluminum oxide\textsuperscript{61}
Methane byproduct is purged from the chamber using nitrogen. After pulsing water into the chamber again, the trimethyl aluminum precursor on the surface reacts with water to make Al₂O₃ and the process starts all over again. Unfortunately, graphene does not have dangling bonds that can react with ALD precursors and hydroxyl groups do not adsorb on its inert surface. It has been found that deposition of high k dielectrics via ALD only takes place at defect sites on the graphene due to the presence of dangling bonds at those sites. Since the sp² hybridized graphene sheet has no out of plane covalent bonds sticking out of its inert basal plane, several methods have been incorporated in order to functionalize the surface of graphene to make it more suitable for atomic layer deposition.

NO₂/ozone, fluorination, low-k polymer seeding, and e-beam evaporation on graphene have all been explored. Using an ozone based approach, it is possible to create gate capacitance structures as thin as 9.5nm. Interestingly, the exposure of graphene to XeF₂ has allowed researchers to cover graphene in fluorine adatoms that can react as nucleation sites for initial ALD precursors to react with. Fluorination of graphene results in high thermal and chemical stability, as well as favorable mechanical properties. So-called perfluorographene has also been investigated, involving the fluorination of graphene using XeF₂ at room temperature; however, these structures leaves more nonfluorinated sites in the graphene sheet. Also, the deposition of thin metal films via electron beam evaporation is becoming an increasingly popular method for graphene functionalization. In this work, titanium was chosen as the seed layer due to its lower surface diffusion when compared to other seed layers such as aluminum oxide.
Figure 17: SEM images of graphene surface before and after TiO$_2$ seed layer deposition followed by ALD of HfO$_2$

Chemical functionalization of graphene via nitrogen dioxide, polymer coating such as addition of monolayer PMMA, and application of organic self-assembled monolayers (SAMs) have offered a potential solution to the problem of high k dielectric deposition on graphene surfaces\textsuperscript{69} by making a more reactive surface. In this work, we evaporated 2nm of titanium onto our graphene substrate, followed by oxidation of the titanium film into titanium dioxide. Titanium seed layers have been shown to be superior to their aluminum counterparts due to a lower surface roughness\textsuperscript{68}.
3.3 Hafnium oxide as a nanopore material

Solid-state nanopores are typically formed via focused ion beam or electron beam methods in thin silicon nitride or silicon dioxide suspended membranes. While commercially available membranes provide valuable platforms for nanopore formation, there is an increasing drive for membranes that are not only thin but robust. Recently it has been shown that it is possible to fabricate ultra-thin graphene nanopores for DNA detection\textsuperscript{5}. However, defects in the basal plane of CVD graphene such as pinholes can result in leakage currents through the membrane, resulting in limited lifetime and durability of the nanopore platform.

In an effort to mitigate the trade-off between thickness and durability, we have combined an atomic layer deposition approach with the structural support of graphene in order to fabricate a hafnium oxide solid-state membrane. Hafnium oxide has recently been incorporated as a gate dielectric in the semiconductor industry for the fabrication of state-of-the-art CMOS transistors\textsuperscript{70} but it has also emerged as a promising candidate for biosensing applications due to its chemical inertness and stability in aqueous environments\textsuperscript{71}. Moreover the isoelectric point of hafnium oxide is approximately 7.0\textsuperscript{72,73}, making it intrinsically uncharged in biological solutions and thus an ideal candidate for nanopore applications. In addition, hafnium oxide is a high-k dielectric ($\varepsilon=21.0$)\textsuperscript{74} with superior permittivity compared to SiO$_2$ and Al$_2$O$_3$. Thus, hafnium oxide also has the potential to advance the field of nanopore transistors with greater gate capacitance and possibly reduced leakage effects.

The crystallization of hafnium oxide has also been studied extensively in gate dielectric applications. Annealing hafnium oxide has been shown to increase the capacitance of the dielectric for both bio-sensing and pH sensing applications. Annealing hafnium oxide thin films
deposited via atomic layer deposition has several effects on the material properties. Tapily et. al has shown through nanoindentation studies that the as-deposited amorphous state of hafnium oxide is harder (18 GPa) than the polycrystalline (15 GPa) form obtained by annealing at higher temperatures such as 600C.

In this work, hafnium oxide was annealed at 500C for 20 minutes in Ar/H\textsubscript{2} mixture. Locally induced crystallization was observed upon electron beam exposure during nanopore drilling, a phenomena that is likely the result of localized heating. Similar to TiO\textsubscript{2} and WO\textsubscript{3} systems\textsuperscript{77}, hydrophilicity appears to be induced in HfO\textsubscript{2} systems with increased temperature as well. Thus, the crystallized area may exhibit increased hydrophilicity and thus facilitate nanopore wetting (see results in Chapter 4).

### 3.4 Experimental setup

#### Contact angle measurements

In order to determine the wettability of our substrate, contact angle measurements were performed using a goniometer (model). The setup was first calibrated using a 4mm sphere. The sample was placed on the stage and a small water droplet was dropped onto the substrate. Contact angle measurements are useful in determining hydrophilicity of a surface, surface energy, and adhesion. The Young equation is used to determine the shape of the liquid interface and consists of three terms that describe thermodynamic equilibrium between three phases, namely the liquid phase, the solid phase, and the gas/vapor phase.

A schematic of how the contact angle is mathematically determined is shown below. YLG, YSL, and YSG are parameters in the Young equation that describe the liquid phase, solid
phase, and gas/vapor phase, respectively. The equilibrium contact angle can be determined by the Young’s Equation shown below. For hydrophilic surfaces, the contact angle will be less than 90 degrees; while for hydrophobic surfaces, the contact angle will be 90 degrees or greater.

\[ 0 = Y_{SG} - Y_{SL} - Y_{LG} \cos(\theta_c) \]

**Figure 18:** Schematic of contact angle calculation vectors

Contact angle measurements were performed using a CAM 100 Optical Tensiometer Lite. Optical tensiometers are used to characterize material surface properties as well as contact angles. In this work, we performed contact angle measurements using this technology. The optical goniometer (also known as tensiometer) analyzed the drop shapes and the captured image was analyzed using a profile fitting method based on Young’s equation. The sessile drop was used for this purpose, a reproducible optical method that is widely accepted in the literature as contact angle measurement. In order to make the measurements, the camera was calibrated according to CAM100 Theta specifications and images were captured.

**Figure 19:** Contact angles for super-hydrophobic, hydrophilic, and hydrophobic surfaces.
Data acquisition system

Nanopores were O$_2$ plasma treated at 100W for 30 seconds prior to introducing the chips to our fluidic chamber setup. The chamber is composed of two fluidic reservoirs and two O-ring seals to prevent leaks and keep the nanopore chip compressed in between the two cells. The setup ensures that the only electrical connection in the cell is at the membrane region of the chip exposed to fluid, thereby allowing for nanopore measurements. Ag/AgCl electrodes were prepared by soldering silver wire together to a gold plated contact pin and later exposing the silver end to a sodium hypochlorite solution. The reversible silver/silver chloride electrodes ensure a uniform potential with minimal noise contribution to the ionic current. Nanopore experiments were performed with an Axopatch 200B patch clamp amplifier (10kHz low pass filter) and sampled with Digidata 1440A at 100khz.

![Fluidic cell used in nanopore measurements](image)

Figure 20: Fluidic cell used in nanopore measurements

Nanopore chips were stored in the cool, dry environment of a nitrogen box. In order to mount the chips, the following procedure was used:

1. Wash nanopore chamber with copious amounts of DI water in order to get rid of impurities and traces of DNA inside the chamber.
2. Place chip on glass slide suspended in between two pieces of tape. O2 plasma clean for 30 seconds at 50% power (approximately 100W).
3. Wash o-rings with copious amounts of DI water to provide a smooth surface and place o-rings on both sides of the nanopore chamber.

4. Carefully mount the chip on top of one of the o-rings in the chamber. Careful placement of the chip so that it is centered on the electrochemical cell will reduce possible breaking when screwing the two ends together.

5. Place the second half of the chip, with o-ring in place, on top of the nanopore chip and slide the two screws through both half cells. Seal the two chambers together (adjusting until finger tight).

6. Once the nanopore chip is secured between the two half cells, first introduce 200mL of DI water into both fluid reservoirs.

7. Prepare 1M KCl solution buffered at pH of 7.5.

8. Flush DI water subsequently removing water from the outlet side of the chamber after adding it through the inlet side.

9. Add 200uL of 1M KCl through the inlet side, flushing the chamber continuously at least three times as before.

10. Place the chamber into the faraday cage, adding the silver/silver chloride electrodes on both ends and measure the ionic current using pCLAMP software.
CHAPTER 4: RESULTS AND DISCUSSION

4.1 Locally induced crystallization of hafnium oxide under TEM exposure

Solid-state nanopores continue to uphold considerable promise as both a bio-sensing and sequencing technology. The interdisciplinary effort from researchers to establish solid-state nanopores as a viable sequencing platform is thriving on multiple fronts including surface charge engineering for DNA capture\(^2\) and conductance modulation\(^3\) in nanopores, nanowire transistors for localized detection\(^4\), ultra-thin membrane fabrication using graphene\(^5\), and the exploration of alternative nanopore materials for biosensing applications\(^6\). High-k materials are being widely adopted by the semiconductor industry for the fabrication of state-of-the-art CMOS transistors due to their superior gate oxide capacitance values when compared to traditional materials like SiO\(_2\). Robust, high-k oxides that are capable of being incorporated in aqueous environments are of interest for biosensing applications where a large gate capacitance is required. In particular, HfO\(_2\) has attracted widespread interest by the biosensor community due to its chemical stability, pH sensitivity, and high dielectric constant. HfO\(_2\) also has an isoelectric point of 7.0\(^7\), making its surface uncharged at physiological pH and a stable candidate for DNA-nanopore experiments.

Annealing dielectric films results in reduced oxygen vacancies, passivation of interface traps, and overall improvement in dielectric constant\(^8\). However, different films can also exhibit structural degradation at higher temperatures due to the polycrystallization of the film resulting in grain boundaries that promote leakage pathways across the dielectric. In this work, we report nanopore formation on as-deposited and annealed HfO\(_2\) films deposited on functionalized graphene as a first step toward high-k, hafnium oxide nanopore transistor platforms. Graphene is
a single layered hexagonal sheet of sp\textsuperscript{2} hybridized carbon atoms with remarkable mechanical characteristics and electrical properties. It is used here as an electron transparent mechanical support for our HfO\textsubscript{2} structures.

Here, we demonstrate locally induced crystallization of hafnium oxide films on graphene with the use of transmission electron microscopy. As-deposited and annealed HfO\textsubscript{2} films were characterized in an electrolyte-oxide-silicon configuration to ascertain dielectric quality in 1M KCl solution. In order to assess the impact of crystallization on nanopore functionality, we measured the contact angle for amorphous and crystallized HfO\textsubscript{2} films and found that hydrophilicity increases with post-deposition annealing. Hence, increased hydrophilicity and improved wettability is expected in the pore region due to localized heating from electron beam irradiation. We also analyzed noise frequency characteristics in the nanopore for annealed and as-deposited films to verify pore wettability. Finally, we show DNA translocation through HfO\textsubscript{2} nanopores.

Hafnium oxide was deposited using atomic layer deposition on a functionalized graphene surface. The lack of dangling bonds on the basal plane of graphene makes atomic layer deposition difficult since there are no available sites for nucleation\textsuperscript{81}. For this reason, a thin metal seed layer was evaporated on graphene using an electron beam source. Titanium was chosen as the seed layer due to its high adsorption energy on graphene\textsuperscript{82} and low surface diffusion\textsuperscript{68}. The 2nm film of titanium was oxidized once exposed to air, resulting in a thin layer of TiO\textsubscript{2} on the graphene surface. HfO\textsubscript{2} was subsequently deposited on the functionalized graphene surface and the membrane was imaged using transmission electron microscopy. Figure 21 shows a schematic diagram of the nanopore fabrication procedure (left) in addition to TEM phase contrast images (right). Crystallization of the film was observed with prolonged exposure. Nanopores were
drilled in both as-deposited and annealed films as shown in Figure 21a. It can also be seen that the region surrounding the nanopore for both cases remains unaltered. Corresponding FFT images confirm amorphous to crystalline phase transitions in the case of as-deposited films and crystalline patterns for the annealed film.

**Figure 21**: TEM phase contrast images of as-deposited amorphous (i) and annealed (iv) HfO$_2$ films deposited on a graphene supported membrane. (ii) Nanopore drilled in amorphous HfO$_2$ film showing electron beam induced crystallization in the vicinity of the pore. (iii) HfO$_2$ bulk phase remains amorphous after nanopore formation. (v) Nanopore in annealed HfO$_2$ thin film. (vi) Annealed HfO$_2$ bulk phase remains crystallized. Figure 1b: FFTs of corresponding TEM image found in Figure 1a confirming amorphous (i',iii') and crystallized (ii',iv',v',vi') phases before and after nanopore formation.
Figure 22: TEM images of nanopores drilled in a 16nm silicon nitride film (left) and 16nm HfO$_2$ film (right). Corresponding FFTs indicate the absence of induced crystallization for silicon nitride films.

4.2 Hafnium oxide characterization in electrolyte solution

In order to verify the quality of as-deposited and annealed HfO$_2$ films in an aqueous environment, HfO$_2$ was deposited on polished, highly doped p-type silicon using atomic layer deposition. The electrolyte solution was dispensed onto a PDMS well on the HfO$_2$ surface and contacted using Ag/AgCl electrodes while the back of the silicon substrate was grounded. As shown in Figure 23, we first applied voltages in the range of +/- 500mV across the electrolyte-dielectric interface since that is the range for our nanopore measurements. Larger values led to dielectric breakdown for both films, albeit at different voltages. The increase in leakage current at a lower voltage for annealed films is attributed to the growth of grain boundaries in the dielectric after post-deposition annealing. In addition, the hydrophilicity of the surface was analyzed for HfO$_2$ deposited on both functionalized graphene and p-type silicon. Similar contact angle values confirm a uniform deposition over the functionalized graphene surface.
While contact angle values were similar for both substrates, there was an increase in hydrophilicity for both surfaces after post-deposition annealing. The influence of post-deposition annealing on the contact angle of dielectric films is known as thermo-induced hydrophilicity. This effect is attributed to the removal of surface contaminants, crystal phase transition, and changes in porosity during annealing. Figure 23 shows a contact angle difference of approximately 10 degrees for as-deposited versus annealed films. The contact angle for HfO₂ was also measured after annealing at 700°C, indicating a contact angle of 39 degrees. Thus, for increasing annealing temperatures, the hydrophilicity of the film continues to improve. Figure 24 also shows leakage current densities after annealing HfO₂ films at 700°C.

**Figure 23:** Characterization of ALD HfO₂ film in an aqueous environment. (a) Leakage current densities for as-deposited and annealed HfO₂ films in an electrolyte-oxide-silicon configuration. The dielectric breakdown of HfO₂ for higher voltages in 1M KCl is shown in (b), where the annealed film shows a higher leakage characteristic. (c) The contact angle for HfO₂ on silicon and for HfO₂ on metal-seeded graphene increases after annealing at 500°C, indicating thermo-induced hydrophilicity due to a crystal phase transition.
4.3 I-V characteristics and noise

In solid state nanopores, 1/f noise has been attributed to a variety of physical factors including surface charge fluctuations at the nanopore surface$^{25}$ as well as the mobility of charge carriers$^{26}$. Excessive 1/f noise has also been attributed to nanobubbles present in the nanopore$^{26}$ and has been shown to be reduced by addition of an oxide layer$^6$. In addition, reductions in 1/f noise have been reported after oxygen plasma and chemical treatment to make the pore more hydrophilic. Figure 25 shows 1/f noise values at voltages ranging from 100 to 300mV for annealed and as-deposited films, indicating pore wettability. Similar voltage values for annealed and as-deposited films suggest that the 1/f noise is dominated by local interactions at the pore itself. In addition, Figure 25b shows current vs voltage measurements for five different HfO$_2$ nanopore diameters.
Figure 25: Noise and I-V characteristics for nanopores drilled in HfO$_2$. (a) The magnitude of the 1/f noise scales with the applied voltage, indicating wettability of the pore. In comparison with annealed values, as-deposited values of 1/f noise are similar in magnitude, suggesting that the 1/f noise is dominated by ionic interactions at the crystallized nanopore as opposed to being influenced by the phase of the bulk membrane region. (b) Current vs. voltage characteristics for five nanopores of different sizes in 1M KCl solution.

4.4 DNA translocation

In order to verify our platform as a viable biosensing methodology, we performed DNA translocation experiments on HfO$_2$ nanopores. The electrolyte solution used was 1 M KCl, 10 mM Tris, 1 mM EDTA, pH 7.2 and the concentration of DNA was 5nM. DNA translocation events were detected from 200 to 500mV as blockades in the ionic conductance of the nanopore. The change in blockade conductance was plotted as a function of the applied voltage in order to confirm presence of DNA translocation. Also, translocation duration varied for DNA events as a function of applied voltage. Figure 26 shows representative data of DNA translocation events for four different voltages as well as the current blockade and dwell time distributions.
Figure 26: Double-stranded DNA translocation. (a) Sample time traces showing translocation of 1kbp dsDNA through a 4nm nanopore recorded at various voltages in 1M KCl solution. (b) Current blockade levels for DNA translocation events plotted as a function of voltage. (c) Duration of the translocation events corresponding to four different voltages.
CHAPTER 5: CONCLUSIONS

The aim of this work was to demonstrate DNA detection using HfO$_2$ based nanopores. Graphene, the single layered hexagonal sheet of sp$^2$ carbon atoms grown by chemical vapor deposition, was used as a structural support in the fabrication of hafnium oxide membranes. Transmission electron microscopy was used to drill nanometer sized holes in the films. As a result of this, locally induced crystallization of hafnium oxide was observed upon prolonged exposure to electron beam irradiation. The hafnium oxide films were deposited via atomic layer deposition on p type silicon and characterized in 1M KCl solution. Leakage currents were analyzed for annealed and as-deposited films, revealing higher current densities in crystallized films due to the nucleation of grain boundaries.

However, crystallization of the high k dielectric resulted in increased hydrophilicity, suggesting improved wettability in hafnium oxide nanopores. Power spectral density plots were acquired in order to verify 1/f noise trends that indicated wettability in hafnium oxide nanopores. Finally, the viability of hafnium oxide nanopores as a biosensing platform was verified by performing DNA translocation experiments. Hence, hafnium oxide is a suitable material for nanopore sensing applications due to its potential in high-k nanopore transistor applications, thermo-induced hydrophilicity, chemical inertness, and the ability to detect DNA transport.
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