DNA Characterization with Solid-State Nanopores and Combined Carbon Nanotube across Solid-State Nanopore Sensors

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Abstract

A DNA molecule passing through a nanopore in a liner and sequential fashion allows for unprecedented interrogation of the polymer. Adding transverse electrodes that are comparable in size and sensitive to the DNA molecule, can further the attempts to rapidly sequence DNA.

Carbon nanotubes are comparable in size and interact strongly with the DNA molecule. This makes them an excellent choice for integration with nanopores. Only the section of the carbon nanotube in immediate proximity to the nanopore should be sensitive to the DNA molecules. Atomic layer deposition of metal-oxides passivates the sections of the carbon nanotube that are not to interact with the DNA molecule. The coating also protects the thin film interconnects leading to the carbon nanotube. Hafnium oxide is superior to aluminum oxide in chemical resistance and electrical insulation but leads to a high failure rate of the carbon nanotube across nanopore devices. Aluminum oxide, combined with gold thin film interconnects to the carbon nanotube, produced the first functioning devices in electrolyte. These devices had concurrently functioning ionic (current across the nanopore) and transverse (current...
through the carbon nanotube) channels. No concurrent DNA translocation signal was recorded on the ionic and nanotube current traces. Analyzing the translocation events recorded on the ionic channel indicated that double-stranded DNA (dsDNA) passed through the carbon nanotube articulated nanopore an order of magnitude slower than it would have through a comparable unarticulated nanopore. The slower translocation observed is a necessary condition for sequencing.

Investigating dsDNA translocation under various experimental conditions led to the discovery of a new interaction between the molecule and small nanopores. A dsDNA molecule is trapped when the electric field near the nanopore attracts and immobilizes a non-end segment of the molecule at the nanopore orifice without inducing folded translocation. In this demonstration of the phenomenon, the ionic current through the nanopore decreases when the dsDNA molecule is trapped by the nanopore. By contrast, a translocating dsDNA molecule under the same conditions causes an ionic current increase. Finite element modeling results predict this behavior for the conditions of the experiment.
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Chapter 1: Introduction to Nanopores for DNA Sequencing

1.1 Nanopores

Nanopores are tiny holes in a biological or a solid-state membrane. Typically one nanopore connects two reservoirs filled with potassium chloride. For the purposes of charactering DNA, the molecules are introduced into one of the two reservoirs and electrophoretically driven through the nanopore into the other chamber. Translocation of DNA through alpha-hemolysin dates back to 1996 [1]. Kasianowicz and coworkers noticed that the drops in ionic current through a single voltage biased alpha-hemolysin pore can be attributed to the brief presence of the DNA molecule as it translocates and temporarily reduces the nanopore’s ionic current. The length of the blockades is approximately linearly dependent on the molecule’s length. Four years later, DNA homopolymer discrimination was demonstrated [2]. Length and base sensitivity suggest that nanopores can be used for rapid DNA sequencing. Single base discrimination was demonstrated only one year later [3]. Two key steps were taken towards sequencing recently in which alpha-hemolysin pores were genetically modified to both control rate of capture of DNA and translocation speed [4, 5]. A new protein pore, Mycobacterium smegmatis porin A (MspA), has recently been demonstrated to be ten times more specific than alpha-hemolysin at base recognition [6]. Controlling translocation rate in a modified alpha-hemolysin or an MspA pore
can yield a DNA sequencing device. Further information can be found in the following review articles [7, 8].

Biological pores are sensitive to temperature, pH, and solution composition. They have a rather limited lifetime and useful voltage range. While genetic engineering has allowed some modification, the structure of biological nanopores is mostly pre-determined and limits their applications. Integration of electrodes with biological nanopores to aid DNA sequencing or control translocation is at present impossible.

Solid-state nanopores on the other hand are significantly more stable and open the door for the fabrication of more complicated semiconducting devices. Typically these consist of a 2-20 nm diameter hole in a 30-500 nm thick silicon nitride (Si$_3$N$_4$) membrane. Golovchenko and coworkers demonstrated the first solid-state nanopore suitable for DNA detection in 2001 [9]. Two years later dsDNA translocation was demonstrated through solid-state nanopores [10]. These nanopores were fabricated through sputter closing larger pores in low stress Si$_3$N$_4$ with a 3 kV argon ion beam. Another fabrication technique involves conformal atomic layer deposition (ALD) of a metal-oxide to close down larger pores down to the dimensions needed for DNA detection [11, 12]. This approach also allows control of the nanopore surface charge and the DNA-nanopore interaction. Another common fabrication approach uses a focused electron beam to directly drill the nanopore into a Si$_3$N$_4$ membrane [13]. This work utilizes electron beam fabricated solid-state nanopores to probe capture of
dsDNA at the orifice of the nanopore and ALD closed solid-state nanopores for transverse electrode devices.

1.2 Transverse Electrodes and Nanopores

While unarticulated nanopores hold promise for DNA sequencing, they also have certain inherent limitations. Translocations of < 15 mer oligomers through alpha-hemolysin [14] and carbon nanotubes through solid state nanopores [15] suggest that the resulting blockade is convoluted signal from the bases occupying the entire length of the channel. One way around that limitation would be the use of ultra-thin membranes such as graphene [16, 17]. Those may offer single base resolution.

Regardless, direct translocation through nanopores is too quick to allow for an accurate current measurement at the rate of ~1 µs/nucleotide. Biological nanopores using additional molecules to control translocation speed have their own disadvantages.

A set of tunneling electrodes at the nanopore can distinguish bases and potentially control translocation rate. Theory suggests that electron tunneling can identify bases in single stranded DNA without functionalization [18, 19]. Both bare gold electrodes [20] and functionalized electrodes [21] allow experimental tunneling current distinction between the four DNA nucleotides. Incorporating electrodes into a nanopore remains difficult. Some groups report the fabrication of nanopores [22, 23]
with integrated transverse electrodes. In these cases the electrode gaps either were large or were not used for the detection of analyte. Ivanov and coworkers claim concurrent ionic and tunneling detection of DNA with platinum electrodes but the shape of both the ionic blockades (too long and deep) and the tunneling events suggests that these may not have been single DNA molecules [24]. Nanopores drilled through contacted nanowires have recently yielded a concurrent ionic and transverse electrode signal from translocating DNA [25]. Single-walled carbon nanotubes (SWCNTs) are more robust and electrochemically inert than thin film electrodes and nanowires at the relevant length scale for DNA sequencing making their integration with nanopores advantageous.
Chapter 2: Experimental Procedures

2.1 Freestanding Membrane Fabrication

Freestanding membranes are the platform used for all experiments presented in this work. A 4 inch p-doped, 1-20 $\Omega$*cm, 500 $\mu$m thick silicon substrate is used for all devices. Cornell’s Nanoscale Science and Technology Facility deposits 300 nm of low-pressure chemical vapor deposition (LPCVD) stoichiometric Si$_3$N$_4$ directly onto those wafers for devices that will be integrated with transverse electrodes. Devices that will not contain transverse electrodes, have 2 $\mu$m of thermal silicon dioxide (SiO$_2$) grown prior to the deposition of 100 nm of LPCVD low-stress, silicon-rich Si$_x$N$_4$ (the subscript x is greater than 3). The low-stress film has nominal tensile stress of 180 MPa, compared to 1200 MPa for stoichiometric films [26]. While the Si$_3$N$_4$ on SiO$_2$ chips provides low capacitance and a thinner stable membrane for an optimal signal to noise ratio, they are unsuitable for integration with SWCNTs. High stress Si$_3$N$_4$ provides the smoother surface necessary for SWCNT integration. Both stacks produce freestanding membranes with experiment specific optimized properties and go through the same initial processing steps.

Potassium hydroxide (KOH) doesn’t etch silicon nitride while it etches the (111) Si plane much slower than the (100) plane. This can be used to produce
freestanding membranes [27]. Optical lithography is used to define an array of open squares of size $w$ in photoresist spun onto the wafers used. Reactive ion etching with carbon tetrafluoride removes the exposed silicon nitride in those squares. The photoresist protects the silicon nitride everywhere else. After reactive ion etching the photoresist is removed with acetone. KOH etches the exposed Si squares to produce trapezoidal pits terminating at either the SiO$_2$ (if present) or the Si$_3$N$_4$ layer. If the Si is 500 µm thick and given that the angle between the (111) and the (100) plane is $\cos^{-1}(1/\sqrt{3})$, a freestanding membrane of size $w - \sqrt{2} \times 500 \mu m$ is produced. The processing steps in the high stress Si$_3$N$_4$ case is schematically illustrated in Figure 2.1. For further detail on freestanding membrane fabrication refer to Marc Gershow’s thesis [28]. KOH etching produces an array of membranes spaced 3 mm apart on the Si wafer and consisting of either 300 nm Si$_3$N$_4$ or 100 nm of Si$_x$N$_4$ on top of 2 µm of SiO$_2$. 
Figure 2.1. Schematic representation of the freestanding membrane fabrication process.
2.2 Electron Milled Nanopores

Electron beam drilled nanopores are fashioned in thin 80 nm thick Si₃N₄ membranes. These membranes provide a better signal to noise ratio in DNA translocation experiments but require removal of the SiO₂ immediately surrounding the nanopore before drilling. Anisotropically KOH etched Si₃N₄ on SiO₂ on Si wafers are diced into 3 by 3 mm chips. A batch of devices is then processed with a focused ion beam (FIB). A 2 µm square subsection of each Si₃N₄ on SiO₂ membrane is bombarded with 50 keV Ga⁺ ions until approximately 75% of the SiO₂ is sputtered away. Any residual organic contamination is removed from the chips with a 10 minute submersion in a 55 °C, 1:1:5 mixture of NH₄OH:H₂O₂:H₂O. Following a deionized water (DI) rinse, the chips are submerged in 40% NH₄F for 7 minutes. Ammonium fluoride etches away approximately 700 nm of SiO₂ isotropically. This is about an order of magnitude higher than what others have observed [29] which suggests that the thermal SiO₂ likely has some inherent defects in addition to the residual FIB damage. When the SiO₂ is isotropically removed, a 2.5 µm square freestanding section of pristine Ga⁺ and defect free Si₃N₄ is left (Figure 2.2a). The ammonium fluoride also removes 20 nm of the Si₃N₄ film bringing membrane thickness down to 80 nm.

The focused electron beam of a JEOL 2010F Transmission Electron Microscope (TEM) is used to drill the nanopores into the 2.5 µm square mini-membranes. After carefully calibrating the electron optics on a test sample, a device
is loaded and the mini-membrane located. At this point, the beam is turned off for 5 minutes to allow for stage drift stabilization. When I restart the beam I verify that thermal drift is less than 0.1 nm/s and bring the beam to crossover (into focus) in the middle of the mini-membrane. Drilling a pore with the largest condenser aperture and spot size (brightest configuration possible) takes 10-20 seconds. During that time, I shift the beam to correct for drift, similar to Dave Hoogerheide’s approach [30]. Nanopores with a predetermined diameter ranging from 2 nm to 10 nm are drilled. As soon as drilling is done an image is acquired (Figure 2.2b) and the beam is immediately turned off to minimize further undesired pore modification by the electron beam. Nanopore devices are stored in dry nitrogen boxes until used for translocation experiments.

Figure 2.2. Schematic representation of the FIB removal of SiO₂ followed by an SiO₂ etch and TEM milling of the nanopore (a). An image of a 3 nm diameter TEM milled nanopore (b). Nanopores smaller than 5 nm assume the triangular shape of the beam as defined by its field emission source.
2.3 Fabrication of Nanopore-Carbon Nanotube Devices

2.3.1 Overview

Integrating a contacted SWCNT across the diameter of a nanopore can circumvent some of the inherent nanopore limitations. Our earlier attempts relied on random alignment of a SWCNT during growth across one nanopore in an array of hundreds, followed by selective closure of the pores that were unused [31]. This presented a number of problems both with the fabrication and the integration of the devices into the flow cell environment for DNA translocation.

Figure 2.3 presents schematically the proposed SWCNT across a nanopore device and the current fabrication approach. Thin film interconnects contact three SWCNTs electrically. The SWCNT with the desired electrical properties is selected. I FIB drill a larger than desired for sequencing nanopore in the proximity of the selected SWCNT. Contact mode AFM is used to pull the nanotube across the nanopore. A 20-30 nm thick film of ALD metal-oxide is deposited. This film insulates the SWCNT and the thin film contacts from the electrolyte. It also closes down the nanopore to its target diameter without coating the suspended part of the SWCNT.
Figure 2.3. A complete SWCNT across a nanopore device is presented in (a). The fabrication involves contacting the SWCNT (b), milling a 70-90 nm FIB pore near the SWCNT (c), using contact mode AFM to align the SWCNT with the nanopore (d) and finally applying an ALD coating to close down the pore to ~10 nm and insulate the contacts.
2.3.2 Optical Lithography and Large Contact Patterning

Large thin-film metallic contacts are patterned onto wafers that will be used for SWCNT integrated devices. Lift-off resist LOR20B under a layer of Shipley 1818 along with standard optical lithography processing are used to define four contacts onto each 3 mm chip. Each contact consists of 20 nm of electron beam evaporated SiO$_2$, 5 nm of Cr and 20 nm of Mo. Following lift-off with Nanoremover PG, the wafer is diced (Figure 2.4a). The layer of SiO$_2$ helps minimize the chance that pinhole defects in the Si$_3$N$_4$ will allow the contact to short electrically to the underlying Si. Before further processing sample devices from each wafer are tested for electrolyte breakdown. Figure 2.4b shows the range of pinhole free Si$_3$N$_4$ breakdown voltages for 14 contacts. Prior to the measurements presented in Figure 2.4b, the thin-film electrodes and the Si$_3$N$_4$ film were exposed to simulated chemical vapor deposition (CVD) growth conditions. Exposure to 200 mL H$_2$ and 1.0 L CH$_4$ flow at 900 °C for 10 minutes mimics a later processing step. Contacts resting on top of pristine Si$_3$N$_4$ have negligible leakage currents of $< 500$ pA at 8 V to the substrate. A measured breakdown voltage of $\sim150$ V (Figure 2.4b) across a 300 nm Si$_3$N$_4$ film yields a dielectric breakdown field of 5 MV/cm, which is consistent with the literature [32]. If atomic force microscopy (AFM) images show no contamination and less than 10% of the contacts fail at $\sim 100$ V bias the wafer is considered of sufficient quality for future processing.
Figure 2.4. Large contact definition through optical lithography and physical vapor deposition in (a). Current from the large contacts to the Si substrate vs applied voltage bias in (b). The almost vertical spike in the current represents the breakdown voltage. Measurements from 14 contacts are plotted with an average breakdown voltage of ~150 V.
2.3.3 Carbon Nanotube Growth

Two electron beam lithography (EBL) steps precede SWCNT catalytic growth. The electron beam resist stack that provides the best balance between resolution, ease of lift-off and minimal residual contamination consists of PolyMethylMethacrylate (PMMA) on top of MethylMethacrylate (MMA). The resist is exposed in a Raith 150 30 kV electron beam writer and developed with standard methylisobutylketone (MIBK) and isopropanol solvent. Film evaporations are carried out either in thermal or electron beam physical vapor deposition chambers. The lift-off chemical of choice is acetone followed by an isopropanol rinse. Boiling acetone combined with the low molecular weight of the MMA resist leaves a minute amount of polymer contamination which is removed with 5-10 s of 100 W oxygen plasma at 500 mTorr. Chlorinated solvents, when used for lift-off leave residual chlorine on the surface, as observed by scanning electron energy-dispersive X-ray spectroscopy. Residual chlorine may interfere with SWCNT catalytic growth.

The first EBL step defines a set of alignment marks consisting of 5 nm Cr and 15 nm Pt at the corners of a 200 µm square field of view. The second EBL step references the alignment marks and defines a 10 by 1 µm carbon nanotube growth catalyst pad. The pad consists of 1 nm of Fe on top 10 nm of Al₂O₃. SWCNTs will grow from the catalyst pad which is centered, with better than 50 nm accuracy, with respect to the reference alignment marks (Figure 2.5).
SWCNTs are grown in a custom made (CVD) chamber. Argon and hydrogen gases are flown continuously through the chamber at rates of 300 sccm and 160 sccm respectively. The temperature in the chamber is raised to 900 °C and 1600 sccm of CH₄ is flown for 10 minutes before allowing the chamber to cool back down to room temperature. Methane serves as the carbon source for the iron catalyzed SWCNT growth [33]. A film comprised of 5 nm Cr, 30 nm Au and 20 nm SiO₂ (backgate) is deposited on the back of the chip. This produces a batch of devices with randomly aligned SWCNTs originating from the iron catalyst pads.
2.3.4 Contacting Carbon Nanotubes and FIB Nanopore Drilling

Establishing a reference system on the back of the chip is necessary. FIB is used to mill a set of alignment marks on the back of the membrane. The 200 nm square alignment marks only penetrate about 70-80% of the way through the membrane. Due to the high residual stress of the Si$_3$N$_4$ film, these alignment marks produce AFM observable topographical features on the top surface of the membrane. AFM imaging of the top of the chips reveals concurrently the positions of the catalyst pad, the SWCNTs that grew from it and the FIB alignment marks. AFM is performed in tapping mode which is gentle enough not to damage the SWCNTs. Using the AFM mapping, SWCNTs can be addressed with better than 50 nm precision through EBL. Interconnects between selected SWCNTs and the large contacts are defined through EBL. Up to three SWCNTs are contacted using the optically defined large contact pads on each chip. Interconnects between the SWCNTs and the large contacts consist of 1 nm Cr, 20 nm Pd (or Au) and 20 nm SiO$_2$.

Oxygen plasma will destroy the SWCNTs if used at this stage to remove the thin layer of residual MMA resist. This presents a significant problem to precisely moving the SWCNTs using contact mode AFM, as the SWCNTs are fixed to the surface by the contamination. Using the lower molecular weight MMA resist (Copolymer MMA 8.5) and aggressive lift-off with boiling acetone helps reduces but doesn’t eliminate polymer contamination. Placing the devices back into the CVD
chamber and chemically reducing the organic contamination away in an Ar/H₂ environment at 375 °C restores the pristine surface of the membrane without damaging the SWCNTs. All components of the device except for the backgate and the interconnects have already experienced these conditions.

Electrically characterizing the contacted SWCNTs allows for selection of the best performing SWCNT on each chip. Depending on the intended application either a semi-conducting or a metallic SWCNT is selected. In both cases I look for low contact resistance. Using the AFM mapping which connects the top and back reference systems a profiled 70-90 nm diameter pore 150 ± 25 nm away from the SWCNT chosen is FIB milled (Figure 2.3c). Using identical processing steps and milling conditions calibration pores are drilled into membranes that do not contain SWCNTs. TEM images of those calibration chips determine the exact diameter of the nanopores on the SWCNT devices. The starting nanopore diameter determines the number of ALD cycles necessary for the target final nanopore size.

The bowing FIB alignment marks are clearly visible on the AFM image containing the map of the SWCNTs. An error of 180 nm in aligning the FIB marks, which are 18 µm apart, would result in positional error of the nanopore of only 0.01 nm. Adding the SWCNT positional uncertainty due to AFM tip convolution and drift of 5-10 nm and another 5-10 nm error from FIB stage drift results in positional accuracy of ± 25 nm. This positional accuracy combined with the fact that Ga⁺ penetration depth at 50 kV into Si₃N₄ is only 20-30 nm, allows drilling of nanopores in close proximity to the SWCNTs without damaging them.
2.3.5 Carbon Nanotube-Nanopore Alignment and Pore Closing

Stoichiometric Si$_3$N$_4$ has surface roughness that is lower than that of low stress films. This allows for easier AFM manipulation of the SWCNTs. Moving a SWCNT the required distance to bring it in alignment with the nanopore can take as many as 100 careful steps of moving the AFM tip in contact mode across the surface in paths effecting the desired repositioning (Figure 2.6).
Figure 2.6. An aligned contacted SWCNT across a nanopore in (a). (b)-(d) show incremental steps in the AFM alignment process. The interconnects and the SWCNTs appear white in these images.

After each step the SWCNT and the nanopore are reimaged in tapping mode AFM and new contact mode paths are designed and executed. This iterative feedback
process allows alignment of the nanotube to within $3.7 \pm 2.3$ nm from the center of the nanopore, as measured from the TEM images of 9 different devices. Two of those 9 chips are aligned to better than 1 nm (Figure 2.7). Special care is taken to minimize damage to the SWCNT while working in contact mode. The AFM tip never touches the suspended section of the SWCNT. Damaging the suspended section of the SWCNT would compromise the subsequent ALD processing.

Figure 2.7. Presented in (a) is a TEM image of a SWCNT aligned with the nanopore. ALD closes down the nanopore conformally while the suspended section remains uncoated (b).

ALD produces a precise conformal coating on both the nanopore and the electrodes of the device [34]. The defect free SWCNT section across the nanopore remains uncoated [35]. TEM as well as lower energy SEM electron beams introduce sufficient damage and contamination on the suspended section of the SWCNT for ALD growth intitation [36]. Due to the imaging constraint, pores milled under
identical conditions are used to determine the number of cycles necessary to achieve the target final device nanopore size. Figure 2.7a shows a device that was TEM imaged before ALD closing of the nanopore. Uncertainty in the initial nanopore size combined with SWCNT-nanopore alignment limitation places the lower limit for the final nanopore diameter at about 6 nm. ALD nucleates and grows on the MoO₃ surface of the large contacts as well as the SiO₂ evaporate on top of the Pd (or Au) interconnects and the Au backgate. This leaves only the section of nanotube over the nanopore exposed (Figure 2.7b).

After ALD, SWCNTs are electrically characterized one last time. Moving the SWCNTs with the AFM introduces some defects, lowering the maximum conductivity of the device (Figure 2.8). Neither FIB milling nor ALD coatings seem to affect the conductivity significantly. ALD can shift curves somewhat due to a shift of the potential level of the SWCNT with no detrimental effect to the overall device operation. Selecting the right ALD metal-oxide for these devices was challenging and will be discussed in detail in the next chapter.
Figure 2.8. Conductance of a SWCNT as a function of a backgate potential through the key processing steps. AFM alignment introduces some defects which decrease conductivity.

2.4 Flow Cell Device Integration

A flow cell made of Polyether Ether Ketone (PEEK) is used to introduce electrolyte and DNA to the nanopores. The flow cell allows efficient replacement of the electrolyte on either side of the device as well as the capability to introduce and flush out DNA. Polydimethylsiloxane (PDMS) gaskets seal to the surface of the devices forming the two reservoirs on the opposing sides of the nanopore. Ag/AgCl electrodes set the voltage across the nanopore and measure the resulting ionic current between the chambers. For more details on the flow cell design, consult Marc Gershow’s thesis [28]. In this work I’ve modified the flow cell to allow contact to the SWCNTs but the original design is otherwise unchanged.
All flow cell parts are rigorously cleaned between experiments to eliminate contamination. All components are rinsed with DI H₂O and then submerged in 1 N HCl for 10 minutes to help eliminate any residual DNA. HCl is rinsed with DI H₂O. The flow cell parts are then ultrasonicated for 10 minutes in DI H₂O, followed by a 10 minute ultrasonication in 190 proof ethanol (EtOH) and a final DI H₂O rinse. Everything is dried with N₂.

In order to contact the SWCNT in the flow cell a thin wire is run in-between the chip’s top surface and the top PDMS gasket. A 10:1 ratio of polymer to curing agent produces soft PDMS gaskets that seal well around the wire. Only the 200-400 µm circular opening in the center of the gasket has access to the electrolyte when the flow cell and chip are assembled. The 1 mm central opening diameter of the backside gasket is slightly larger than the membrane pit opening which allows for easy fluid exchange. Gaskets are soaked in EtOH to remove short non cross-linked polymers on the surface, rinsed with DI H₂O, N₂ dried and oxygen plasma cleaned before being fitted to the flow cell.

A drop of silver paint applied to the surface of the large contacts, prior to ALD, allows an unimpeded electrical connection between the thin film contacts and the wires leading to them. A coating of diluted Corona Dope covering the chip’s edges prevents the wire from shorting to the Si substrate. The chip is placed on the bottom PDMS gasket which is mounted on the bottom half of the flow cell. The thin copper wires are optically aligned with the silver paint on the contacts leading to the
SWCNT. Additional silver paint can be carefully applied to the wire and the thin film contacts as long as it doesn’t overflow from the contact surface. Optical alignment is used to center the top PDMS gasket’s opening over the membrane. The top PDMS gasket attached to the corresponding half of the flow cell is brought into contact with the chip. The top PDMS gasket seals around the thin wires and the chip’s surface. These thin wires lead to larger metal connectors that are attached to the perimeter of the flow cell and connect to a pre-amplifier.

Devices that lack SWCNTs are processed in a similar way. Firmer 5:1 gaskets can be used since they don’t have to seal around any wires. After the device is loaded different electrolytes can be introduced in order to wet the surfaces and the pore prior to DNA introduction. An Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA) sets the ionic bias on the two sides of the nanopore and measures the resulting current through it. A second Axopatch 200B is used to monitor and voltage-bias the SWCNT. The ionic current signal is filtered with an eight-pole low-pass Bessel filter and then digitized at a 250 kilosamples/s rate. For one channel measurements of the ionic current the low-pass cutoff frequency is set between 20 and 90 kHz depending on noise levels and the expected event durations. In dual channel recording mode the low pass cutoff is usually set to 10 kHz for both channels. Recorded current traces contain the DNA translocation events.

Following data collection, events are located in the current traces and fitted. A non-linear median filter allows for easy detection of the event’s leading edge even in low signal to noise situations. The located events were then least-squares fitted,
taking into consideration the low pass filter effect on the event shape. Both the search
and the fitting algorithms were implemented in MATLAB code (MATLAB software;
The MathWorks, Natick, MA).
Chapter 3: Atomic Layer Deposition on Nanotube-Nanopore Devices

An ideal ALD film has to controllably reduce the diameter of a large nanopore, resist etching and leave only the suspended section of the SWCNT device exposed to interact with the DNA molecules.

3.1 Basics

ALD is a self limiting step-by-step form of CVD, which produces conformal coatings with very precise thickness. In ALD alternating gases are flown over a substrate, reacting with each other only on the substrate’s surface. A reagent is introduced in the chamber that contain the device. Once all the surface sites have undergone the desired chemical reaction, residual excess reagent is purged. Volatile undesired byproducts of this half of the chemical reaction are purged as well. The complimentary reagent is introduced and allowed to react. Excess reagent, along with the volatile byproducts of this half of the chemical reaction is purged. This cyclical self-limiting process allows for precise film thickness deposition (Figure 3.1).
Figure 3.1. Schematic representation of the ALD process. The first precursor is introduced (white triangles), saturates the surface and then excess is purged. Then the second precursor (green triangles) is introduced until all the surface sites react (form blue rectangles) and excess is purged. The cycle repeats.

In order to limit the chemical reaction to the surface, the substrate is maintained at a higher temperature than its surroundings. If the temperature of the substrate is too low, the chemical reaction simply won’t proceed or will be reaction rate limited. Physical absorption of un-reacted precursors can occur at lower than ideal substrate temperatures. If on the other hand the temperature is too high, the precursor may decompose or desorb from the substrate before it has a chance to react (Figure 3.2). Temperature also determines whether the film is amorphous or polycrystalline. Only a narrow precursor and product dependent temperature range will result in an ALD film with the desired properties.
Another important consideration that works in tandem with substrate temperature is the purge time between reagent introductions. If too short, the volatile byproducts of the reaction and the precursor itself may still be present in the chamber when the complimentary reagent is introduced. In that case the two chemicals may react, if temperature is high enough, in the gaseous phase and lead to CVD. This results in an uncontrolled, higher than expected, deposition rate. Excessively long purge times on the other hand lead to precursor desorption and decomposition on the surface and produce a lower than expected per cycle rate (Figure 3.2).

Figure 3.2. Schematic depicting the different processes resulting from various temperature and purge time parameters. In the optimal temperature and purge time range for the selected precursors a predictable and stable per cycle ALD rate is produced.
There are several important considerations in ALD precursor selection. Precursor chemicals must have a vapor pressure high enough to enter the reaction chamber. Precursors must have high surface reactivity and generate reaction sites for their complimentary reagents. Reaction byproducts must not react with the film and the precursors must not be self-reactive. Precursors must react with at least some of the chemical surface groups on the uncoated surface to initiate the ALD process.

The experiments presented in this work use two ALD metal-oxides; \( \text{Al}_2\text{O}_3 \) (alumina) and \( \text{HfO}_2 \) (hafnia). In the case of alumina ALD the precursors are trimethyl-aluminum (TMA) and water. Silicon nitride surfaces contain silanol groups which react with the TMA to initiate the ALD process. When TMA comes in contact with the silanol groups, aluminum bonds covalently to the oxygen replacing the hydrogen atom. The other two methyl groups remain bound to the aluminum atom and are available for the next step. When water is introduced the methyl groups are replaced by hydroxyl groups which provide the reaction sites for the next TMA cycle. The per-cycle rate is stable and reproducible for a substrate temperature of 250 °C, 10-30 s TMA and 30-90 s water purge times. Reactor pressure is 250 mTorr with 10 sccm of purging nitrogen gas flow introduced close to the precursor flow control valves. Amorphous alumina is produced for the entire temperature range allowed by the precursors.

Hafnia deposition uses tetrakis-dimethylamido-hafnium (TDMAH) and water as precursors. Due to its very high dielectric constant hafnia has attracted significant attention in field effect transistor fabrication [37]. The chemical process is very
similar to that of TMA and water. The dimethylamine surface groups react with water leaving hydroxyl groups available for the next TDMAH introduction cycle. Working with tetrakis-diethylamido-hafnium (TDEAH) proved difficult due to its low vapor pressure making TDMAH held at 80 °C the preferred precursor (Figure 3.3). TEM images confirm that, for temperatures under 150 °C mostly amorphous hafnia forms while at 250 °C the film is polycrystalline, consistent with the observations of others [38]. Hafnia ALD requires longer 30-90 s TDMAH and water purge times. Both hafnia and alumina have advantages and shortcoming when used as passivation layers in SWCNT-nanopore devices.

![Figure 3.3. Vapor pressure of TDMAH as a function of temperature (reproduced from [39]). Sufficient vapor pressure (over 1 Torr) is necessary to introduce the gas into the ALD reactor.](image)

Figure 3.3. Vapor pressure of TDMAH as a function of temperature (reproduced from [39]). Sufficient vapor pressure (over 1 Torr) is necessary to introduce the gas into the ALD reactor.
3.2 Deposition Rates and Film Structure

Chamber geometry, purge times and temperature all affect the per-cycle ALD rate. There are several ways film thickness can be measured. Ellipsometry can be quite accurate but requires an adjustment for the native oxide layer that forms on the silicon surface. Otherwise the measured deposited film thickness would appear higher than it is. While ALD is conformal when the amount of precursor used is sufficient to saturate the surfaces, both initiation and the per-cycle rate can vary in the non-planar nanopore geometry. Removal of the volatile reaction byproducts as well as the precursor itself from nanopores that are only several nanometers in diameter is more difficult than it is on a flat surface. For those reasons we choose to primarily use TEM images of ALD closed nanopores to measure deposition rate (Figure 3.4). Alumina deposition at 250 °C results in a rate of 1.1 Å/cycle. It took approximately 29 cycles to initiate growth due to the surface properties of the Si₅N₄ (Figure 3.5c).
Figure 3.4. ALD alumina deposition on a nanopore. From the initial (a) and final pore sizes (b) we can determine the per-cycle rate.

When the pore diameter becomes very small film growth rate increases (Figure 3.5b). Difficulty removing the excess physically adsorbed precursor from the nanopore when it’s only several nm in diameter results in higher than expected per-cycle rate. Higher temperature and purge times help but can degrade the precursor or lead to desorption in other areas of the device. We can see the more electron transparent overgrowth forming if we stop the process at the right time (Figure 3.5a). This puts a lower bound of 5 nm on the final nanopore diameter under optimal temperature and purging conditions with the current reactor and precursors. Alumina did not grow on the suspended sections of defect free SWCNTs (Figure 3.6d).
Hafnia comes in two flavors. Amorphous hafnia grows at a rate of 1.015 ± 0.002 Å/cycle, from native silicon oxide calibrated ellipsometry measurements, at a reactor temperature of 150 °C. While superior in many ways these films are unsuitable for SWCNT-nanopore devices due to physical adsorption of the precursor onto the suspended section of the SWCNTs (Figure 3.6a). If we raise the reactor
temperature to 250 °C we obtain polycrystalline hafnia (Figure 3.6c). Long purge times of 90 s per TDMAH introduction and 90 s per water introduction and the higher temperature lead to some precursor desorption and degradation resulting in a rate of 0.86 ± 0.05 Å/cycle. Polycrystalline hafnia ALD limits final pore diameter to about 10 nm due to the uneven circumference resulting from the crystal growth (Figure 3.6c). The suspended sections of the SWCNTs remain fully uncoated with polycrystalline hafnia (Figure 3.6b).
Figure 3.6. TEM images of ALD films. Undesired amorphous hafnia growing along a suspended carbon nanotube (a). Polycrystalline hafnia leaving the tube pristine (b) TEM image of the hafnia crystal formation at 250 °C (c). Alumina closed nanopore with a SWCNT across (d).

Working with mixed alumina/hafnia films yielded some encouraging preliminary results. It is unclear whether most of the physical absorption of TDMAH leading to the overgrowth of hafnia along the SWCNTs happens in the initiation
cycles of the ALD deposition or towards the end. To test that I deposited 50 cycles of alumina followed immediately by 200 cycles hafnia. The addition of alumina decreased the hafnia overgrowth along the SWCNTs somewhat at 140-150 °C (compared to amorphous hafnia) and to a lesser degree at 240-250 °C (compared to polycrystalline hafnia). While encouraging the benefit was not sufficient to merit adoption of the laminate films.

### 3.3 Film Properties in Electrolyte

The nature of the ALD film surface is important to the successful fabrication of a SWCNT-nanopore device. Hydrophobic ALD surfaces can make it very difficult to wet the nanopores especially considering that the surrounding PDMS gaskets and PEEK are all hydrophobic (Figure 3.7a). A WCNT across the nanopore would further hinder the wetting process. I can distinguish between the surface of the membrane being dry and the nanopore being dry by the increased amplitude of the high frequency components of the nanopore current power spectrum (capacitive noise). If the capacitive noise is present but there is no conductance through the pore the surface is wet but the pore isn’t.

In the order to measure how well different solutions wet the surfaces of different ALD coatings I looked at the equilibrium area of a 3 µL droplet on the film. Results from the experiment are consistent with expectations based on work done with the flow cell. Alumina wets easier than hafnia. Ethanol wets both alumina and
hafnia better than HCl which wets the surfaces better than 1 M KCl at pH 8 (Figure 3.7b). Depositing an ~1.1 nm thin layer of alumina on top of a hafnia film results in easy wetting of the surface, equivalent to that of an alumina only film.

Figure 3.7. Schematic representation of a wet membrane surface (bottom) with a dry nanopore resulting from the same contact angle $\theta$. Area of a 3 µL droplet being on an ALD surface (b). Larger droplets mean better wetting of the surface. Optical image in (c) demonstrating how the area of the droplet is measured (software post processed and analyzed to extract the area).
Using ethanol to wet the nanopore carries a higher risk of SWCNT failure. Ethanol has to be replaced with water rapidly because mixing releases dissolved gasses which sometimes de-wet the nanopore. Degassing all the solutions immediately prior to use helped. DI water is then replaced with the desired electrolyte for the experiment. Hydrochloric acid offers a good compromise, because it’s easier to replace with water or even directly with a large amount of buffered electrolyte. Ethanol wetting is the most reliable method when SWCNTs passivated with hafnia are involved. With alumina direct KCl solution introduction was usually sufficient to wet the nanopore.

Aside from wetting the metal-oxide surface we also need to consider the film’s resilience in the electrolyte. High pH etches ALD Al₂O₃ films while HfO₂ films are stable in extremely basic environments for extended periods of time. ALD Al₂O₃ films remain relatively un-affected at pH values bellow 6, compared to 5.0 for anodic alumina [40], while HfO₂ films are stable even at pH 14. That feature of the HfO₂ films allows dsDNA denaturing with high pH leading to single stranded DNA translocation.

Chemical resistance of the film affects its durability which determines the time we have to carry out the experiment. After the film is degraded, significant electrochemistry occurs at the thin-film metal interconnects leading to the SWCNTs. A good measure of the film’s ability to protect the interconnects can be obtained from the value of the ionic current between the thin film metal electrode and the Ag/AgCl electrode through the electrolyte. Low currents mean that the ionic and SWCNT
channels are not strongly coupled and equally importantly mean that the thin film electrodes are not being degraded. Once the leakage current becomes large enough, the electrodes are irreversibly eroded. Current leakage through the ALD films was measured with the test chips show in (Figure 3.8a). A cross consisting of 3 µm wide thin-film interconnects is coated with the ALD film of interest. Only the central 300 µm of the cross is exposed to the electrolyte. The rest of the surface is covered with a PDMS gasket. A 20 nm film of HfO₂ insulates about 1 order of magnitude better than the same thickness Al₂O₃ (before it etches) in 1 M KCl pH 7 solutions (Figure 3.8b). In both cases ALD was performed on Pd contacts covered with SiO₂ to aid ALD nucleation.

Using Cr/Au/SiO₂ instead of Cr/Pd/ SiO₂ interconnects improves the durability of the contacts. Gold is more resistant to electrochemical reactions and fares better even with a less then perfect ALD passivation layer. A Cr/Au/SiO₂ thin-film electrode without an ALD film has a leakage current comparable to that of the more electrochemically active Cr/Pd/SiO₂ electrode with Al₂O₃. Combining gold and hafnia would seem like the best choice. In reality due to the nanopore wetting issue and a high failure rate of hafnia coated SWCNTs devices, Cr/Au/SiO₂/Al₂O₃ interconnects in pH 6 or lower solutions performed better.
Figure 3.8. Optical image (a) of a silicon nitride membrane (the lighter colored rectangle) with thin-film test electrodes (the crossing lines). The electrodes are biased with respect to the electrolyte that is covering the central area of the device and the leakage current is recorded. The leakage current through hafnia is a lot lower than that through alumina (b).

Mixed hafnia under alumina films provide the insulation properties of the hafnia and the wetting properties of alumina. Sacrificing the alumina layer at high pH aids wetting. While electrically these films were very stable in electrolyte (as good as hafnia alone) and they wet easily, the SWCNTs failed much in the same way as they did with pure hafnia film coatings. The issue of SWCNTs coated with an ALD film failing will be further discussed in the next chapter.
Chapter 4: Carbon Nanotube Devices in the Flow Cell and DNA Interactions

4.1 Single Wall Carbon Nanotube Basics

Single wall carbon nanotubes are cylindrical shells with a diameter of several nanometers, consisting of a single rolled up graphene sheet. Examples of nanotubes consisting of several sheets of graphene were first observed by Ijima in 1991 [41]. Two years later SWCNTs were discovered [42]. Due to their unique mechanical, electrical and optical properties, SWCNTs have attracted a lot of attention and are uniquely well suited for probing biomolecules.

Each SWCNT can be unambiguously defined by the chiral vector along the surface of the graphene sheet that becomes the SWCNT’s circumference (Figure 4.1). The chiral vector \( \vec{C}_h \) is defined as follows,

\[
\vec{C}_h = n\vec{a}_1 + m\vec{a}_2
\]

where \( \vec{a}_1 \) and \( \vec{a}_2 \) are the graphene lattice vectors and \( m \) and \( n \) are integers. The chiral index \((n,m)\) uniquely identifies each SWCNT and defines its properties.
The diameter $d$ of an $(n,m)$ SWCNT is from geometric considerations,

$$d = \frac{|C_k|}{\pi} = \frac{\sqrt{3}a_0(m^2 + mn + n^2)^{1/2}}{\pi}$$  \hspace{1cm} (4.2)

where $a_0$ is the nearest neighbor carbon-carbon separation ($1.421$ Å in graphite) [43].

Figure 4.1. Different chiral vectors along the graphene sheet. The rolled up chiral vector becomes the SWCNT's circumference.
The energy dispersion relationship of a SWCNT determines its electrical properties. To obtain the dispersion relationship, we start with the graphene dispersion relationship and quantize it along the circumference of the SWCNT. In 1947 P.R. Wallace did the first tight binding approximation calculation for graphite ignoring interactions between the planes [44]. Ignoring overlap of electrons on adjacent sites the dispersion relationship for a wave vector \( \vec{k} \) is:

\[
E(\vec{k}) = E_r \pm \gamma_0 \left( 1 + 4 \cos \frac{\sqrt{3} k_x a}{2} \cos \frac{k_y a}{2} + 4 \cos^2 \frac{k_z a}{2} \right)^{1/2} \tag{4.3}
\]

where \( \gamma_0 \) is the nearest-neighbor transfer integral and \( a = \sqrt{3} a_0 (2.46 \text{ Å}) \) is the in-plane lattice constant (Figure 4.2).
Figure 4.2. On the left, a graphene sheet and its dispersion relationship. When rolled up into a SWCNT, a slice of the dispersion relationship is selected producing a semi-conducting or a metallic SWCNT.

Taylor expanding Equation 4.3 for small \( |\vec{k}| = k \) near the K-points where the valence and conductance bands overlap gives us a linear dispersion relationship for metallic (no gap) SWCNTs,

\[
E(\vec{k}) = \pm \gamma_0 \frac{\sqrt{3}ka}{2} \quad (4.4)
\]
Depending on chirality, SWCNTs can be semi-conducting with a gap of varying size or metallic with the simplified dispersion relationship in Equation 4.4.

Electrons in SWCNTs, are in the absence of magnetic fields, 2 fold degenerate. This sets the maximum value of the conductance of a metallic SWCNT with two channels (overlap points between the valence and conductance bands),

\[ G_{\text{max}} = \frac{4e^2}{h} = 4G_Q \]  

(4.5)

where \( e \) is the electron charge, \( h \) is Plank’s constant and \( G_Q \) is defined as the conductance quantum [45]. I’ll sometimes express SWCNT conductance in units of \( G_Q \).

Ballistic transport resulting in conductance close to 4 times the conductance quantum has been demonstrated in SWCNTs [46]. Most SWCNTs have lower values of peak conductance due to contact resistance, Schottky barriers and reduced carrier availability and mobility. Less than perfect charge carrier transmission from the contact to the SWCNT can be characterized with a contact resistance \( R_c \) that is independent of shifts in the SWCNT’s Fermi level. In addition to the less than unitary transmission coefficient, depending on the work function of the metal interconnects leading to the SWCNTs and the gap of semi-conducting SWCNT a Schottky barrier may be formed (Figure 4.3). When a Schottky barrier is present, electrons or holes have to tunnel through a depleted charge layer that forms at the interface of the SWCNT and the contact. The tunneling distance can be reduced or increased through
capacitively shifting (gating) the Fermi level. A change in the SWCNT Fermi level leads to conductance modulation and results in Schottky barrier based SWCNT field effect transistors (FETs) [47].

Figure 4.3. Band diagram of the SWCNT in contact with a metal before charge flows (a). Electrons flow to the metal which has a lower Fermi level (b). This results in extra holes at the interface and their unimpeded flow across the interface. If the Fermi level of the metal falls above that of the SWCNT holes are depleted at the interface and a Schottky barrier is formed (c-d). Choosing a metal with high work-function can produce ohmic (barrier free) contacts for the p-channel (hole dominated).
An ohmic contact doesn’t exhibit a Schottky barrier. Unaltered semi-conducting SWCNTs are p-doped as a result of atmospheric oxygen exposure [48]. Using metals with a large enough work functions to place the contact’s Fermi level bellow that of the SWCNT results in ohmic contacts, for hole dominated transport (Figure 4.3b). In this case, electrons migrate to the contact increasing the p-type charge carriers in the SWCNT at the interface. If the metal contact’s Fermi level is higher than that of the SWCNT, electrons will flow into the SWCNT creating a hole-depleted layer at the interface. This layer presents a Schottky barrier to the p-conductance channel (Figure 4.3d). Gold with its 5.10-5.47 eV work function has been shown to contact SWCNTs ohmically [49]. Palladium works even better with its 5.22-5.6 eV work function [50]. When DNA interacts with the SWCNTs it can affect the Schottky barrier at the contact. Asymmetric conductance modulation of the hole and electron channels is observed, if the contacts are not passivated and the Schottky barrier is modified.

Let’s consider a simple semi-quantitative model for the conductance change of the p-channel of a SWCNT when its Fermi level is modified through a gate. Capacitively coupling to a SWCNT modifies the electron’s energy and thus the Fermi level. This can be achieved through a backgate electrode that addresses the contacts as well, a top gate electrode that addresses the SWCNT alone or a wetgate electrode that uses electrolyte to couple to the SWCNT [51]. For zero or positive potential on the gate, the resistance of a hole dominated semi-conducting SWCNT is high due to the lack of charge carriers. This is the “off” state of the p-channel and the energy
difference between the valance band and the Fermi level $E_b$ is much larger than $kT$ resulting in low conductance,

$$G \sim e^{-E_b/kT} \quad (4.6)$$

where $G$ is the conductance of the SWCNT. As the gate potential becomes negative the barrier is lowered and conductivity increases (Figure 4.4),

$$\delta E_b = e\alpha \delta V_g \quad (4.7)$$

where $\alpha$ is a coupling coefficient that in a perfect FET would have the value of 1. Equations 4.6 and 4.7 describe the thermally activated exponential increase in the SWCNT conductance as its Fermi level approaches the valence band. This is the sub-threshold swing and represents the most gate sensitive part of the FET’s conductance curve. For the perfect $\alpha = 1$ case we can obtain a 10 fold change in the SWCNT’s conductivity for a gate potential change of 60 mV. Sensitivity close to 60 mV / decade has been demonstrated [33]. This is sufficient to detect the charge of the Debye screened dsDNA backbone a few nanometers away from the SWCNT.

Conductivity for more negative gate voltages, past the thermal activation region, is determined by charge carrier density and is linearly coupled to the gate potential. In a long SWCNT with scattering sites (defects) we observe non-ballistic transport. We can add a Drude-like resistance model to the quantum resistance and
the contact resistance to arrive at the final p-channel resistance of an ohmically contacted SWCNT,

\[ R = \frac{h}{4e^2} + R_c + \frac{L}{\mu C_g |V_g - V_{g0}|} \]  

(4.8)

where \( \mu \) is the mobility of the charge carriers, \( V_g \) is the gate voltage, \( V_{g0} \) is the threshold voltage at which the SWCNT begins conducting and \( C_g \) is the capacitive coupling to the gate per unit length. Once the number of carriers becomes high enough that the contact and quantum resistances dominate, the gate modulation of the SWCNT conductance levels off (Figure 4.4).
Figure 4.4. Conductance $G$ of the p-channel of a SWCNT as a function of gating voltage. The conductance $G$ depends on different parameters depending on the operating regime.

The capacitive coupling between the gate and the SWCNT can be determined from geometric considerations. In a backgate geometry a 3 nm diameter nanotube on top of 300 nm of Si$_x$N$_4$ with relative permittivity of 7.0 has capacitance per unit length of

$$C_{bg} = \frac{2\pi \varepsilon \varepsilon_0}{\ln(4h/d)} \sim 6 \times 10^{-11} \, F/m$$  \hspace{1cm} (4.9)
This is two orders of magnitude lower than the capacitance of an electrolyte gate,

\[
C_{wg} = \frac{2\pi \varepsilon \varepsilon_0}{\ln(1 + 2 \lambda_D / d)} \sim 9 \times 10^{-9} F / m
\]  

(4.10)

where \( \lambda_D \sim 1 \text{nm} \) for 0.1 M KCl electrolyte is the Debye screening length and \( d \) is the diameter of the SWCNT \( \sim 3 \text{ nm} \). The dielectric constant of water is 80.1.

Quantum capacitance dominates the total capacitance of the liquid gate, while negligible in the case of the backgate geometry. It originates from the energy dependent change in the number of electron states of the SWCNT,

\[
\frac{dN}{dE} = D(E)
\]  

(4.11)

where \( D(E) \) is the density of states of the SWCNT, \( N \) is the number of states available and \( E \) is the energy of the electron. Then the quantum capacitance \( C_q \) is (assuming full occupancy of the states),

\[
C_q L = \frac{dQ}{dV} = \frac{edN}{dE / e} = D(E)e^2
\]  

(4.12)
The density of state of a SWCNT at the Fermi level [45] is,

\[ D(E_f) = \frac{8L}{h\nu_f} \]  \hspace{1cm} (4.13)

where \( L \) is the length of the SWCNT, \( h \) is Planck’s constant and \( \nu_f \) is the Fermi velocity. A Fermi velocity of \( 8\times10^5 \) m/s results in \( C_q \sim 4\times10^{-10} \) F/m.

Capacitance of the SWCNT with respect to the gate is added in series to the quantum capacitance,

\[ 1/C = 1/C_q + 1/C_{\text{geometric}} \]  \hspace{1cm} (4.14)

which is dominated by the smallest capacitance of the system. We can disregard the geometric capacitance, which is 2 orders of magnitude larger than its quantum counterpart, in the case of the liquid gate. In the case of the backgate we can disregard the quantum capacitance. Based on the capacitance, the quantum dominated liquid gate is expected to couple approximately 10 times better than the backgate to the SWCNT (Table 4.1).
<table>
<thead>
<tr>
<th>Gate</th>
<th>Quantum Capacitance</th>
<th>Geometric Capacitance</th>
<th>Total Capacitance</th>
</tr>
</thead>
<tbody>
<tr>
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<td>~400 pF/m</td>
<td>~60 pF/m</td>
<td>~60 pF/m</td>
</tr>
<tr>
<td>Liquid Gate</td>
<td>~400 pF/m</td>
<td>~9000 pF/m</td>
<td>~400 pF/m</td>
</tr>
</tbody>
</table>

Table 4.1. Relevant capacitance for the gate coupling in the backgate and liquid gate geometries.

A molecule can modify the conductance of a SWCNT in four distinct ways. A voltage shift in the conductance vs gate voltage curve will occur if a charged molecule wraps itself around the SWCNT. A negatively charged molecule will shift the curve to the right. If there is a Schotky barrier, negatively charged molecules adsorbing onto the electrodes will increase their work function and improve p-channel while reducing n-channel conductance. Molecules adsorbing onto the SWCNT could also change the capacitive coupling of the liquid gate and the mobility of the charge carriers. This will modify the slope of the linear region of the conductance curve in accordance with Equation 4.8.

In the case of very small valence to conductance band gaps and metallic tubes, the n-channel contributes significantly to the total conductance (with a different threshold voltage). Adding electron and hole conductance together produces a curve with a well defined minimum conductivity but no true off state. Changes in the conductance curve of the SWCNT as it interacts with DNA can be explained through the model presented above.
4.2 Hafnia Coated Carbon Nanotubes in the Flow Cell

Excellent chemical resistance and electrical insulating properties make hafnia the ideal passivation film. Unfortunately all attempts to use hafnia coated SWCNT across nanopore devices failed in the flow cell. Either the nanopore didn’t wet (no ionic conductance) or else the SWCNT conductivity was lost.

SWCNT failure over the nanopore due to static build up during the introduction of the solution was suspected initially. A number of unsuccessful experiments were carried out in an attempt to prevent SWCNT failure due to electrostatic discharge. Special attention was paid to maintaining everything grounded to minimize static build up. Certain components of the system were in some experiments left floating. For example the ionic electrodes would be grounded but the SWCNT left floating to prevent rapid discharge. High humidity (60% RH) environment was maintained. Loading a device and submersing the assembled flow cell into a grounded ethanol bath instead of pushing solution in also led to SWCNT failure.

To confirm that the ALD film was the culprit we carried out experiments with uncoated carbon nanotubes on the surface of a membrane (no nanopores). SWCNTs remained conductive when electrolyte was introduced. The uncoated contacts were not electrochemically stable but at low source-drain SWCNT biases they remained intact through the experiment. SWCNT devices lacking nanopores and ALD, that went through the burn-off process (used to remove residual MMA) also functioned in
the flow cell. This left either the ALD film or the suspended part of the SWCNT as the failure point.

Quantifying the static build up can suggest procedure modifications to minimize it. We kept track of the potential of the Ag/AgCl electrode in the flow cell and a thin film Cr/Mo electrode exposed to the solution. Static build-up was significant and strongly correlated with the speed at which the electrolyte was introduced (Figure 4.5). The conclusions from these experiments were that slow introduction of the solution is key, HCl produces less static build up than EtOH, neutralizing charge with a ZeroStat gun has negligible effect and that glass syringes generate less static than plastic ones. We attempted introducing EtOH into the flow cell with glass syringes and only relying on gravity to push the solution slowly into the flow cell (with the syringes vertical and above the level of the cell). Unfortunately that setup (see Figure 4.6) didn’t result in a working hafnia-SWCNT device either.
Figure 4.5. Rapid introduction of ethanol into the flow cell (a). Top trace is the Ag/AgCl potential and the bottom curve is the potential of a thin film surface electrode. The static build up decreased when ethanol is introduced very slowly (b). Green vertical line indicates the time of ethanol introduction.

Figure 4.6. Optical image of the two channel setup with gravity fed solution from glass syringes in an attempt to minimize static build up.
Unloading the devices from the flow cell without compromising them is complicated. This makes examination of failed devices difficult. We have to disassemble the flow cell in a DI H₂O bath in order to prevent the device from drying, then rinse thoroughly before transitioning to EtOH and critical point drying (CPD). Any residual contamination will be concentrated into the nanotube-nanopore area if we have to contend with a receding meniscus. Even with the CPD additional contamination and damage are introduced to the device.

Three unloaded hafnia coated devices gave us some hints about the failure mode. All had ionic conductance in the flow cell but lost conductivity through the intact SWCNT. Figure 4.7d shows a transmission electron micrograph of the SWCNT across the nanopore suggesting that failure occurred away from the nanopore. An AFM image before ALD deposition overlaid with an SEM image after unloading the failed device (Figure 4.7b) confirms that residual stress has not in any way displaced the SWCNT during electrolyte introduction. Figure 4.7c shows an AFM line section along the tube right after AFM alignment that points to two possible buckling points of the tube (height spikes). It is possible that the SWCNT buckled at those points during AFM alignment and failed completely at them due to the electrostatic discharge or the mechanical stress accompanying the nanopore wetting. The SWCNT didn’t appear to fail over the nanopore, nor shift during electrolyte introduction.

Grounding one of the contacts leading to the SWCNT on two different failed devices and imaging them in a scanning electron microscope (SEM) revealed the point of electrical failure (Figure 4.8). Change in SEM contrast along the SWCNTs
suggests that they failed away from the nanopore. The grounded segments of the SWCNTs appear brighter in those images representing enhanced secondary electron emission. The points at which the SWCNTs stopped conducting (failed) lead to the change in contrast. SEM, TEM and AFM images suggest that the failure occurs away from the nanopore. AFM manipulation of the SWCNT could be buckling the tube making it more susceptible to failure. This prompted us to FIB drill the nanopores 100-150 nm away from the SWCNTs compared to 250 nm previously. The reduced distance required less AFM manipulation, lowering the chance of buckling the SWCNT. This resulted in several functioning alumina coated devices.
Figure 4.7 Possible buckle points in (c) are circled in red on the height section along the SWCNT. The path of the line section is shown in (a). If the tube buckles it would appear higher on the image. Composite image of the same SWCNT after failing in the flow cell (b). A grayscale SEM image of the failed device is overlaid on top of the original AFM image before ALD. In this TEM image of the same SWCNT across the nanopore, the SWCNT appears to be mechanically intact (d).
Figure 4.8. SEM images of failed SWCNTs across nanopores coated with hafnia. These devices were carefully unloaded and critical point dried after failing in the flow cell and have one contact grounded (the bright part of the SWCNT). The change in secondary electron emission along the SWCNT suggests failure away from the nanopore.

4.3 Single Stranded DNA Interaction with Carbon-Nanotubes

Short single stranded DNA molecules bind to and can be used to disperse hydrophobic SWCNTs [52]. The binding is both base and length dependent. Conductivity changes of the SWCNTs with the introduction of 30 base cytosine (C) homopolymers was investigated. To characterize this interaction 200 by 300 nm slits in a Si₃N₄ membrane were bridged with contacted SWCNTs (Figure 4.9a). Using a micro-pipette, 2 µL of 1 M KCl at pH 8.6, TE buffer (10 mM TRIS, 1 mM EDTA) was introduced on top of the hafnia passivated device. Micromanipulators bring a
small Ag/AgCl electrode into contact with the electrolyte and the response of the SWCNT to the Ag/AgCl electrode is measured. 1 µL of 30 C ssDNA (500 µg/mL) is introduced and the changes in the SWCNT conductance are recorded (Figure 4.9b). Initially the dominant change was an increase in the p-channel conductance suggesting higher mobility, lower contact to SWCNT resistance or an increase in the p-carrier density. If the effect originated from Schottky barrier modification we’d expect a drop in the n-channel conductance which increased as well. After waiting another 5 minutes an electrostatic shift in the conductance and further increase in the p and n-channel conductances were observed. This can result from a concurrent increase in the capacitive coupling to the gate and a drop in the contact resistance to the SWCNT or more likely due to an increase in carrier mobility.

Critical point drying the device and characterizing it through the backgate revealed a capacitive coupling that was approximately 10 times weaker than the liquid gate. The n-channel was ~35 % more conductive out of the electrolyte. This was likely the result of a decreased n-channel Schottky barrier as the p-channel remained unaffected to slightly constricted. Removing the ssDNA with a 15 minute 60 °C phenol-chloroform bath and critical point drying again restored the conductance roughly to its original value (Figure 4.9b step 4). The n-channel conductance was higher after ssDNA removal and drying compared to the initial dry characterization.

Two undesired effects may contribute to the changes observed. The absorption of ssDNA on the hafnia surface and surface charge changes in response to
local pH can both shift the conductance curve of the SWCNT. Ideally the device should only be sensitive to the interaction of the ssDNA with the SWCNT. Accounting for all the possible effects allows for a certain degree of decoupling. The changes in the SWCNT conductance originated from the interaction of the ssDNA with the suspended section of the SWCNT proving the feasibility of the detector operation.

Figure 4.9. AFM image of a carbon nanotube across 200 by 300 nm slits in a silicon nitride membrane (a). Presented in (b) is the conductance of a SWCNT when wet (solid lines) and dry (broken lines). The Ag/AgCl electrode sets the liquid gate potential while the backgate potential is set by a metallic coating on the back of the membrane. We introduced 30 C ssDNA in step 1. After additional time has elapsed the conductance is further modified (step 2). In step 3 we CPD the device, and in step 4 we striped the ssDNA with phenol and chloroform. The backgate potentials (broken lines) are 10 times the “Liquid Gate Potential” values indicated on the x-axis.

Short homopolymer-SWCNT interactions were also observed in the flow cell.

In this case the SWCNT was centered across a nanopore and the ionic conductance
was recorded. This device had a 20 nm alumina passivation layer. Liquid gate conductance response shifted approximately +150 mV after the addition of 1.5 µg of 30 C homo-polymers to the 1 M KCl TE at pH 4.66 electrolyte (Figure 4.10a). I attempted to absorb and desorb ssDNA molecules on and away from the SWCNT by varying the potential of the SWCNT and the voltage bias across the nanopore. Clearly observable steps in the ionic conductance occurred when the nanotube was maintained at a -100 mV bias (no current through it), while electrolyte was ground on the side of the SWCNT and at -100 mV at the other side of the membrane. In this situation we’d expect negatively charged ssDNA to be removed from the SWCNT. Steps in the ionic current suggest reversible adsorption of the ssDNA molecules to the SWCNT (Figure 4.10b). This is consistent with the ssDNA-SWCNT mediated SWCNT conductance modulation previously observed.
4.10. SWCNT across a nanopore device in the flow cell. (a) Change in SWCNT conductance response to the wet-gate after the introduction of 30 C ssDNA. (b) Observed steps in the ionic channel conductance attributed to the adsorption and desorption of the homopolymers.
4.4 Double Stranded DNA and Carbon-Nanotube Nanopore Devices

Experiments dsDNA and SWCNT-nanopore devices inside the flow cell produced some interesting data. The first device that had concurrently functioning SWCNT and an ionic channels was short lived. This device was fabricated according to the procedures described in Chapter 2 with Cr/Pd/SiO₂ interconnects and then coated with 200 cycles (22 nm) of alumina. Relative humidity was maintained at 50% and special attention was paid to minimizing electrostatic discharge through all the loading steps. A 2 M KCl pH 3.0 TE degassed solution was pushed into the flow cell.

The SWCNT remained conductive after the introduction of the electrolyte. The conductance of the SWCNT decreased as the low pH electrolyte protonated the alumina surface making it more positive and capacitively coupling to the SWCNT, reducing the number of holes available (Figure 4.11a). Ionic conductance was not observed at this point and more electrolyte had to be pushed through the flow cell to force the wetting of the nanopore. Pushing additional 3 cc of the same electrolyte wet the nanopore, resulting in ionic current. Sweeping the nanotube’s source drain potential with the ionic reservoirs grounded through the Ag/AgCl electrodes led to the almost immediate failure of the nanotube at a bias of about 6 mV (Figure 4.11a). The resulting increase in the ionic current (Figure 4.11b) suggests that the entire ALD film inside the nanopore along with the suspended SWCNT section were removed.
Figure 4.11. (a) Current through the SWCNT vs applied source-drain bias. Grey, before introduction of electrolyte, broken green line after introduction of electrolyte. The blue line is the first sweep of the SWCNT bias while the electrolyte was grounded and led to irreversible failure. Current sweep after failure in red (leakage through the electrolyte over 4 mV). In (b), ionic current before (blue) and after SWCNT failure (green).

Since the both hafnia and alumina ALD films caused a number of issues, a Cr/Au contacted SWCNT device without ALD was used next. This tunneling device (Figure 4.12) was exposed to 1 M KCl pH 4.66 TE buffer in the flow cell. Unfortunately the noise level due to the SWCNT and ionic channels coupling through the electrolyte overwhelmed any potential dsDNA translocation signals (Figure 4.13). Leaving the SWCNT floating reduced the ionic channel noise level and dsDNA translocation current blockade events were recorded (Figure 4.14). The most probable translocation duration was 1275 ± 134 µs (95 % confidence) from 106 events at 200 mV across the nanopore, with the SWCNT floating (Figure 4.14b). Translocation time is equivalent to a speed of 0.267 ± 0.028 cm/s. This is about one order of magnitude slower than what we have observed with regular nanopores. Interaction of
the dsDNA with the SWCNT slows down the translocation process, which is critically important to sequencing.

Figure 4.12. Tunneling SWCNT across a nanopore device without ALD coating.

Figure 4.13. (a) Ionic channel noise with Cr/Au electrodes (dark blue) and Cr/Au/Alumina electrodes (light blue). Note the extra noise in the 1 – 10 kHz range. (b) Noise in SWCNT current. Dark red is the case of Cr/Au electrodes and red includes an alumina passivation layer. Excess noise without the alumina is observed.
The next device presented had Cr/Au/SiO$_2$ interconnects to a continuous SWCNT across a nanopore. It was passivated with 300 cycles (~33 nm) of alumina ALD. The electrolyte used in the flow cell was again 1 M KCl TE buffer at pH 4.66. Noise was significantly lower in this case compared to the bare electrode case (Figure 4.13). Better insulation between the Ag/AgCl electrodes and the SWCNT interconnects allowed sweeping of the ionic and SWCNT voltage biases concurrently without failure of the device. After running a ssDNA experiment (Figure 4.10), the device was flushed and 10 kb dsDNA was introduced. Ionic current blockade events were observed as a result of dsDNA translocation. Source-drain bias on the SWCNT of 10 mV was maintained. Ionic channel events here were again about an order of magnitude longer than what is expected without a SWCNT (this time across the nanopore). Unfortunately no clearly observable concurrent events were recorded on the SWCNT and the ionic current channels. Further experiments will be needed to conclusively confirm or rule out the possibility of non-tunneling and tunneling SWCNT detection of dsDNA as it translocates through a nanopore.
Figure 4.14. (a) Probability scatter plot of 10 kb dsDNA translocation events with an unbiased SWCNT across a nanopore. 106 total events. (b) The time distribution of the events recorded in (a). Average duration is $1275 \pm 134 \mu s$. 
4.5 Conclusions and Future Directions

Fabricating a fully functional SWCNT-nanopore device and integrating it with the flow cell is challenging. ssDNA interacts with the SWCNT and does so in a base specific way. The effect of ssDNA on SWCNT conductance was measured and reversed. Base contrast was not explored. Removing the adsorbed ssDNA molecules with a phenol-chloroform solvent was effective and would allow for the reuse of the same device with other molecules. Adsorption and desorption of the ssDNA molecules can be observed on the ionic current channel if the active part of the SWCNT is across a nanopore.

Using the right ALD film with SWCNT devices is crucial. Hafnia seemed very promising due to its excellent electric insulation and passivation characteristics but I was unable to pinpoint the SWCNT failure mechanism to which it led. Alumina on the other hand etches quickly at pH above 7.0 but can be successfully used in combination with gold electrodes at lower pH. Leakage is negligible and the noise coupling between the two channels allows ionic current dsDNA translocation detection. Translocation speed through SWCNT articulated nanopores was approximately one order of magnitude slower than normal. Slower translocation is desirable for a sequencing device. At the time of writing this thesis the most promising approach to realizing a SWCNT-nanopore device involves gold electrodes and an alumina ALD film.
Fabricating tunneling devices with the process described above is trivial. The device presented in Figure 4.12 was the result of direct FIB drilling through the SWCNT. It would be easier to cut the tube across the membrane using the membrane itself to shield all but the suspended part of the SWCNT from directional oxygen plasma. Only the exposed part over the nanopore will be attacked. Various moderate energy ion bombardments from the back of the chip combined with a feedback mechanism on the SWCNT conductivity would also work. Simply running a very high current through the SWCNT is likely to lead to the thermal failure of the suspended section.
Chapter 5: DNA Trapping at the Orifice of a Nanopore

Working with SWCNT articulated nanopores led to an unexpected discovery. In an attempt to prolong the useful life of SWCNT-nanopore devices lower molarity electrolyte was used. Under these conditions a decrease in the ionic current preceding some of the dsDNA translocation events was observed.

This new phenomenon is best explored with small 3-5 nm diameter Si₃N₄ unarticulated nanopores. The observed decrease in the current is in contrast to the enhancement during translocation. We posit that the decrease in current is the result of the electric field near the nanopore immobilizing the dsDNA molecule at the orifice of the nanopore.

Simpler electron beam fabricated nanopore without SWCNTs were used to explore the newly observed phenomenon (Figure 5.1). Current traces containing the new feature were analyzed to extract the current levels during trapping for a range of applied ionic voltages. Finite-element current simulations of the open-pore, translocation and trapped geometries confirm that the proposed trapping model is consistent with the experimental data.

Two necessary simulation parameters are calculated analytically. The ionic mobility of the electrolyte solutions decreases with increasing KCl concentration. An effective mobility for each species is calculated based on the electrolyte solution’s measured conductivity. The other parameter calculated is the charge of the Si₃N₄
surface. The electrolyte’s pH determines the average number of protons bound to the 
$\text{Si}_3\text{N}_4$ surface chemical groups and the membrane’s surface charge.

Simulation data is used to estimate the probability of dsDNA trapping. The 
change in free energy of a dsDNA molecule entering the trapped state determines the 
likelihood of trapping. The entropic, electrostatic and fluid drag forces on a trapped 
dsDNA are examined. Experimental and simulation trapping data allow for a rough 
estimate of the dsDNA molecule’s persistence length.
Figure 5.1. Schematic of the dsDNA translocation experiment. Ag/AgCl electrodes set the potential of the electrolyte on the two sides of the nanopore. This potential difference drives the negatively charged dsDNA molecules through the nanopore or traps it at the orifice. Inset shows a TEM image of a typical nanopore.
5.1 Effective Ionic Mobility and Surface Charge Calculations

In order to predict the nanopore currents an analytical model of the effective mobility of the ions in the solution and the surface charge of the Si₃N₄ pore is necessary. Ion mobility decreases with increasing ionic concentration due to interactions. Surface charge of the nanopore is dependent both on pH and ionic strength.

5.1.1 Electrolyte Conductivity and Effective Ionic Mobility

Electrical conductivity is linked to resistivity by the simple relationship \( \kappa = 1/\rho \) and is measured in Siemens per meter. In the case of electrolyte solutions it’s convenient to divide the conductivity by the molarity \( c \) of the solution to obtain the molar conductivity \( \Lambda_m \),

\[
\Lambda_m = \kappa / c \quad (5.1)
\]

For an idealized strong electrolyte, \( \Lambda_m \) is constant and the conductivity of the solution is linear with the electrolyte concentration. In real symmetrical electrolyte we need to consider the frictional force of the solvent on the ions through Stokes’ Law, the electrophoretic effect and the relaxation retarding effect. The relaxation effect
refers to the solution and counter-ion atmosphere relaxing around the moving ion. The time-scales for that relaxation are on the order of nanoseconds. The Debye-Hückel-Onsager [53] model takes into account the factors enumerated above to predict the electrolyte conductivity at low molar concentrations,

\[ \Lambda_m = \Lambda_0 - S \sqrt{c_{\text{actual}}} \]  
\[ S = a + b \Lambda^0 \]  

Where \( \Lambda^0 \) is the molar conductivity in the zero concentration limit. The constant \( a \) depends on temperature, relative permittivity and viscosity and accounts for electrophoresis while \( b \) depends on temperature and relative permittivity only and accounts for relaxation. This equation is very similar to the empirically derived,

\[ \Lambda_m = \Lambda_0 - K \sqrt{c_{\text{stoich}}} \]  

in which \( K \) is a fitting constant. While Equation 5.2 captures the basic structure of the empirically derived law in Equation 5.3 and expands significantly on the Debye-Hückel model, it only predicts conductivity accurately for concentrations on the order of 1 mM. Fuoss and Onsager improved the model extending its usability up to about 40 mM with a concise abbreviated derivation of about 100 pages which included a number of perturbations to the Debye-Hückel-Onsager theory [54]. The theoretical
models provide us with insight into the relevant physics. The high molar concentrations used in this work necessitate experimental measurement of the ionic mobility through solution conductivity.

The conductivity of KCl solutions has been standardized and we can refer to ASTM Standard D1125-77 for very precise values at 25C (Table 5.1).

<table>
<thead>
<tr>
<th>Molarity</th>
<th>µS/cm @ 25 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>146.93</td>
</tr>
<tr>
<td>0.01</td>
<td>1408.8</td>
</tr>
<tr>
<td>0.1</td>
<td>12856</td>
</tr>
<tr>
<td>1.0</td>
<td>111342</td>
</tr>
<tr>
<td>0.1 KCl TE pH 9.1</td>
<td>13220 ± 60</td>
</tr>
<tr>
<td>1.0 KCl TE pH 7.0</td>
<td>106630 ± 510</td>
</tr>
</tbody>
</table>

Table 5.1. Conductivity of standard reference KCl solutions and measured conductivity of two solutions used in trapping experiments.

Table 5.1 confirms what we expected from Equation 5.2. At 1 mM KCl concentration, the measured conductivity is very close to the uncorrected value of 149.84 µS/cm [50] while at 1 M it deviates significantly from the uncorrected value of 149.84 mS/cm. If we were to substitute the appropriate values in Equation 5.2 for 1 M KCl we’d actually get a correction larger than $A^0$ which is not surprising given the low concentration range in which the equation applies. Conductivity values of the 0.1 M KCl at pH 9.1 and 1.0 M at pH 7.0 (10 mM TRIS and 1 mM EDTA buffer for both) are presented in Table 5.1. These measured conductivities serve as the basis for the effective mobilities and diffusion constants used to model the system.
At pH 9.1 we are dealing with OH\(^-\) ions that are 4 orders of magnitude less in concentration than the 100 mM KCl ions. Even though OH\(^-\) ions are 2.61 times as mobile as Cl\(^-\) their contribution to the conductivity can be included as a perturbation in the mobility of the Cl\(^-\) ions. Likewise the contributions of the TRIS and EDTA ions can be treated as a perturbation due to their low mobilities and concentrations.

According to Kohlrausch’s law we can separately treat the conductivity of potassium and chlorine ions and add their contributions at the end [55]. The ionic mobility is the constant of proportionality between the drift speed of an ion and the applied electric field,

\[ v_d = \mu_{ionic} E \]  \hspace{1cm} (5.4)

We can relate the current flux \( J \) to the drift speed,

\[ J = v_d ne = \mu_{ionic} neE \]  \hspace{1cm} (5.5)

where \( n \) is the number of ions per cubic meter and \( e \) is the electron charge.
Considering that $J = \kappa E$ we conclude from Equation 5.5 that,

$$\kappa = \mu_{ionic} n e = \mu_{ionic} c N_a e$$  \hspace{1cm} (5.6)

Where in Equation 5.6 we replaced the density $n$ by the chemical concentration $c$ multiplied by Avogadro’s number $N_a$. Solving for $\mu_{ionic}$ from Equations 5.6 and 5.1,

$$\Lambda_m = \kappa / c = \mu_{ionic} N_a e$$  \hspace{1cm} (5.7)

$$\mu_{ionic} = \frac{\Lambda_m}{N_a e}$$

The diffusion constant for the same ion is by the Einstein relation,

$$D = \frac{k_b T}{e} \mu_{ionic} = \frac{k_b T \Lambda_m}{N_a e^2}$$  \hspace{1cm} (5.8)
The mobility per ion and per unit charge $\mu_v$ is a useful number used in our simulations,

$$\mu_v = \frac{\mu_{\text{ionic}}}{N_a e} = \frac{\Lambda_m}{(N_a e)^2} \quad (5.9)$$

We need to decouple the conductivity contributions of the Cl$^-$ and the K$^+$ ions from the total measured value for the solution. The infinite dilution molar conductivities of the potassium and chlorine ions are 73.5 and 76.34 $\text{S} \cdot \text{cm}^2/\text{mol}$ respectively [50].

Let’s define $r_0$ to be the ratio of the infinite dilution molar conductivities of K$^+$ and Cl$^-$,

$$r_0 = \frac{\Lambda^0_{\text{K}^+}}{\Lambda^0_{\text{Cl}^-}} = \frac{73.5}{76.34} = 0.963 \quad (5.10)$$

Then for a measured total molar conductivity $\Lambda = \Lambda_{\text{K}^+} + \Lambda_{\text{Cl}^-}$ of a KCl solution we have an approximate effective ionic mobility $\mu_{\text{ionic}}^\text{CT}$,

$$\mu_{\text{ionic}}^\text{CT} = \frac{\Lambda_{\text{Cl}^-}}{N_a e} \approx \frac{\Lambda}{1 + r_0 \frac{1}{N_a e}} \quad (5.11)$$

where we assume that the ratio of the mobilities of the potassium and chloride ions, remains approximately the same with concentration.
Similarly, 

\[
\mu_{\text{ionic}}^+ = \frac{\Lambda^+}{N_a e} \approx \frac{\Lambda}{1 + 1/r_0 N_a e}
\]  

(5.12)

Using Equations 5.11 and 5.12 and the measured conductivities presented in Table 5.1 we can calculate effective ionic mobilities. In a 1 M KCl solution an effective molarity of 1.01 M results in \(\mu_{K^+} = 5.37 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}\) and \(\mu_{Cl^-} = 5.58 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}\).

In a 100 mM KCl solution the effective mobilities are \(\mu_{K^+} = 6.11 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}\) and \(\mu_{Cl^-} = 6.35 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}\) for an effective molarity of 110 mM. Ionic mobility in the 1 M KCl solution is reduced by 12% compared to the 100 mM KCl solution.

5.1.2 Surface Charge

Chemistry at the interface between the electrolyte solution and the Si\(_3\)N\(_4\) surface determines the charge density of the nanopore. Electrolyte pH dependent addition and removal of H\(^+\) ions to the nanopore surface dominates its surface charge. Si\(_3\)N\(_4\) has 3 surface groups that interact with the solution (Figure 5.2). Amine groups play a small role in determining the Si\(_3\)N\(_4\) surface charge. Interaction with the atmosphere and the solution produces an interface that has twice as many oxygen
atoms as it does nitrogen [56]. The two oxide groups involved are siloxane and silanol. Silanol groups are the only ones that are involved in a considerable charge transfer through pH dependent protonation and deprotonation [57].

At high pH nearly all the surface H⁺ ions would rather go into solution and we have SiO⁻ groups dominating the Si₃N₄ surface and making it negative. At low pH the abundance of H⁺ ions in the solution leads to protonation of the silanol groups. This makes the Si₃N₄ surface positive through an increased SiOH₂⁺ group density. There’s an in between pH value defined as the isoelectric point at which the Si₃N₄ surface has an equal number of SiO⁻ and SiOH₂⁺ groups along with neutral SiOH groups. At the isoelectric point the Si₃N₄ surface is charge neutral.

Figure 5.2. The surface groups on a Si₃N₄ membrane. Amine groups (left most), deprotonated silanol groups (SiO⁻), neutral silanol and doubly protonated silanol along with siloxane (right most) groups depict the relevant species. Surface charge is negative and dominated by SiO⁻ groups in the pH 6-14 range.
When the electrolyte conditions deviate from the isoelectric point pH the ratio of the negative SiO$^-$ to the neutral SiO$_2$ groups changes. We’ll consider the Behrens-Grier model [57] and neglect the contributions of the amine, the siloxane and the SiOH$_2^+$ groups. The H$^+$ concentrations are over 8 orders of magnitude too low at pH 7 for any significant number of SiOH$_2^+$ groups to exist [56]. The chemical reaction responsible for the bulk of the Si$_x$N$_4$ surface charge is,

$$SiO^- + H^+ \xrightleftharpoons{K} SiOH$$

with equilibrium constant $K$. The rate constant $K$ determines the ratio of surface groups for a given H$^+$ concentration,

$$K = \frac{\Gamma_{SiO^-} [H^+]_0}{\Gamma_{SiOH}}$$

(5.14)

where $\Gamma_{SiO^-}$ is the surface density of SiO$^-$ and $\Gamma_{SiOH}$ of SiOH groups. These surface densities add up to the total density of surface groups $\Gamma$. The surface charge is,

$$\sigma = -e\Gamma_{SiO^-}$$

(5.15)
We can rewrite Equation 5.14 in terms of the logarithmic rate constant $pK$,

$$pK = -\log_{10}(K),$$

Combining Equations 5.15 and 5.16 results in,

$$10^{pH-pK} \frac{\Gamma_{SO^-}}{\Gamma_{SiOH}} = \frac{\Gamma_{SiOH}}{\Gamma_{SiOH}}$$

We need to modify Equation 5.17 to account for the reduced/enhanced presence of $H^+$ ions near the charged surface compared to the bulk. A Boltzmann factor relates the surface $H^+$ concentration to the bulk concentration,

$$[H^+]_0 = [H^+]_{bulk} e^{-\beta \psi_0}$$

where $\psi_0$ is the surface potential, $e$ is the elementary charge magnitude, $\beta^{-1} = kT$. 

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Including the difference in the surface and bulk \( \text{H}^+ \) concentrations into equation (5.17) results in,

\[
10^{p\text{H}^- - pK} (\Gamma e + \sigma) + \sigma e^{-\beta \psi_0} = 0
\] (5.19)

Given the logarithmic rate constant \( pK \), the total number of surface sites \( \Gamma \) and another relationship between the surface potential and surface charge we can calculate the surface charge for any pH. Poisson-Boltzmann formalism, applied to a flat surface, results in the Grahame equation [58] which relates the surface charge and the diffusive potential,

\[
\sigma = \frac{2\varepsilon\varepsilon_0 \lambda_d}{\beta e} \sinh \left( \frac{\beta e \psi_d}{2} \right)
\] (5.20)

where \( \lambda_d \) is the Debye screening length. Stern theory [59] connects the surface potential \( \psi_0 \) to the diffusive potential \( \psi_d \) through the layer’s phenomenological capacity \( C \),

\[
C = \frac{\sigma}{\psi_0 - \psi_d}
\] (5.21)
Equations 5.19-5.21 can be solved self consistently for the surface charge. Figure 5.3 shows the surface charge dependence on pH in the high pH range for 1.01 M and 110 mM (effective molarities) KCl solutions. The Si₃N₄ surface charge for the 1 M KCl pH 7.0 and the 100 mM KCl pH 9.1 solutions are 69.8 mC/m² and 140.2 mC/m² respectively. The parameter values used are listed in Table 5.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value Used</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Site Density ( \Gamma ) (nm⁻²)</td>
<td>2.33</td>
<td>3.0 [56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.0 [57]</td>
</tr>
<tr>
<td>Stern Capacity ( C ) (F/m²)</td>
<td>2.9</td>
<td>2.9 [57]</td>
</tr>
<tr>
<td>( pK )</td>
<td>6.75</td>
<td>6.2 [56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5 [57]</td>
</tr>
</tbody>
</table>

Table 5.2. Values used in the numerical solution for surface charge presented in Figure 5.3.

Figure 5.3. Surface charge in mC/m² vs pH value. Numerical solution of Equations 5.19-5.21 with the parameters in Table 5.2. Red curve is for 0.11 M KCl and the blue curve is for 1.01M KCl solutions.
5.2 Nanopore Conductivity Model

5.2.1 Geometry and Meshing

Using the calculated ionic mobility and surface charge values from above, we constructed a model for the nanopore. Finite-element simulations on the open pore, trapped dsDNA and translocating dsDNA geometries were carried out. Computational limitations made a two-dimensional axis-symmetric model necessary. Figure 5.4 shows sections of the open pore, dsDNA translocating and dsDNA at the orifice of the nanopore geometries used to calculate the resulting currents. The 80 nm thick membrane separates the top and bottom reservoirs of electrolyte. The nanopore is formed by rounding off the vertices that define the membrane. The vertex at the most narrow point of the nanopore is 2.2 nm away from the center line and 10 nm bellow the mid-point of the membrane. The other two points that form the pore cone are at a radius of 6 nm from the center line and at the surface of the membrane, 30 nm below and 50 nm above the apex of the nanopore. The 3 vertices of the membrane that define the pore geometry are rounded off with a 15 nm fillet radius. The cone has fairly steep walls. This differs from the typical geometry of an electron beam closed nanopore. It is however consistent with tilted transmission electron micrographs of the electron beam opened nanopores we have produced. The reservoirs on both sides of the membrane are spherical in shape with a radius of 500 nm. At the 500 nm boundary bulk values for all the parameters are assumed.
Figure 5.4. Sections of the model geometry of the open pore (a), dsDNA translocation (b) and dsDNA trapped at the opening of the nanopore (c). Left panels represent the entire model (500 nm radius). Middle panels zoom in on the nanopore and right most panels show the dsDNA in red along with the meshing detail. The membrane is white in color in all images. Electrolyte is teal colored in non-meshed images.
Translocating dsDNA is depicted as a 150 nm long 2.2 nm diameter cylinder coaxial with the nanopore. The edges are rounded off with a 1 nm fillet radius. dsDNA obstructing the pore opening is modeled as a torus due to cylindrical symmetry restrictions of the model. The torus has a 4 nm radius from the center of the pore. It is 30 nm or -32 nm away from the mid-point of the nanopore depending on the side from which dsDNA is originating. The diameter of the dsDNA obstructing the pore is modeled at 2.2 nm and 2.8 nm. A Stern layer of immobile charge results in the 2.8 nm diameter model.

Meshing details are crucial. The bulk of the electrolyte has a maximum triangular element size of 50 nm with a maximum element growth rate of 1.1. dsDNA surfaces and the apex of the pore are restricted to a maximum element size of 0.08 nm while the cone of the pore has an upper element size limit of 0.16 nm. The membrane surface outside the pore is restricted to a 5 nm maximum element size. Up to 12 rectangular boundary layer elements are defined along the surfaces where most of the physics occurs. These elements grow in thickness at a rate of 1.2 and further improve the interfacial resolution by a factor of about 10 (Figure 5.4). As a first approximation the simulation was carried out on a mesh that was about 5 times less detailed than the one described above. The results from the two meshes differed by less than one percent on average.
5.2.2 Theory Behind the Simulations

The ionic current through the nanopore is calculated by solving the coupled electrostatics, fluid-dynamics and drift-diffusion equations. The first component of the nanopore model accounts for electrostatic interactions. We’ll first consider the equations governing the electric fields inside the electrolyte and then the appropriate boundary conditions. The bulk of the electrolyte is water which is treated as a dielectric medium. Free charge $\rho_v$ in the electrolyte originates from the difference in potassium and chlorine ion concentrations as calculated from the Nernst-Plank equations,

$$\rho_v = N_a e (c_{K^+} - c_{Cl^-})$$  \hspace{1cm} (5.22)

The resulting displacement field from Gauss’s law,

$$\nabla \cdot \bar{D} = \rho_v$$  \hspace{1cm} (5.23)

\vspace{1cm}

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We can obtain the electric field and the electric potential on the mesh,

$$\vec{E} = \frac{\vec{D}}{\varepsilon_r \varepsilon_0}$$  \hspace{1cm} (5.24)$$

$$\Delta V = -\int_{C} \vec{E} \cdot d\vec{l}$$

where $\varepsilon_0$ is the permittivity of free space and $\varepsilon_r = 80.2$ is the relative permittivity of water.

The equations above can be solved for the electric field given ionic concentrations and a set of boundary conditions. We assume no significant field penetration into the Si₃N₄ and apply a surface charge boundary condition,

$$-\hat{n} \cdot \vec{D} = \rho_s$$  \hspace{1cm} (5.25)$$

where $\rho_s$ is the surface charge as derived from the Behrens-Grier model. The same boundary condition is applied at the surface of the dsDNA molecule. We assume the $-2e$ charge per base to be uniformly spread out on the surface of the 2.2 nm diameter cylinder representing dsDNA which results in a surface charge density of $-136.2$ mC/m². A spherically symmetric grounded surface sets the potential 500 nm from the nanopore to zero. Similarly, 500 nm away on the other side of the nanopore the potential on a spherical surface is set to the applied voltage value. Approximating bulk conditions 500 nm from the nanopore is valid. Expected axial potential is within
10 % of the electrode value 44 nm from the pore’s 4.4 nm diameter. Simulations show an even faster convergence to 3.48 %. We use quadratic discretization on the mesh and use the applied potential as the initial condition for the electrolyte.

The electric field leads to flow of the electrolyte. Fluid flow is described by the Navier–Stokes equations. We assume incompressible laminar flow and neglect the inertial term due to the low Reynolds number of the system (from simulation < 10⁻⁵). Under these conditions viscous flow dominates and we drop the momentum transfer and the convective force terms resulting in,

\[ \nu \nabla^2 \ddot{u} + \tilde{f} = \nabla p \quad (5.26) \]
\[ \nabla \cdot \ddot{u} = 0 \quad (5.27) \]

where \( \ddot{u} \) is the velocity field of the fluid, \( p \) is the pressure, \( \tilde{f} \) is the external volume force and \( \nu \) is the dynamic viscosity for water (0.001 Pa*s). Equation 5.27 is the mathematical equivalent to incompressible flow.
The external force $\vec{f}$ is obtained from the electric field and the free ionic charge,

$$\vec{f} = \vec{E} \rho_v$$  \hspace{1cm} (5.28)

Where we assume that the electrostatic force on the ions is directly transmitted to the fluid, given the $\sim$ns relaxation time of the system. The external force leads to electroosmotic flow of the solution.

A no slip, zero velocity, boundary condition is assumed along the entire Si₃N₄ surface and the surface of the trapped dsDNA molecule. When the molecule is translocating through the nanopore, the interfacial fluid velocity matches that of the molecule (no slip). The translocation velocity is assumed to be linearly proportional to the applied bias. Experimental data at 600 mV in a 100 mM KCl buffered solution resulted in a translocation speed of $6.0 \pm 1.8 \text{ cm/s}$. At a distance of 500 nm from the nanopore on both sides of the membrane a spherically symmetric zero pressure, zero viscous stress boundary condition is applied,

$$\rho = 0$$  \hspace{1cm} (5.29)
$$\nu \nabla^2 \vec{u} = 0$$

We use second order discretization of the velocity field and first order of the pressure on the mesh with zero velocity and pressure initial conditions.
Diffusion due to electrostatically established ionic concentration gradients and

drift in the electric fields are described by the Nernst-Planck equations. Coupled to

the electrostatic potential and velocity fields calculated from Maxwell’s and Navier–

Stokes’ equations the drift diffusion relation to the concentration is,

\[ \nabla \cdot (D \nabla c - \bar{u}c - \mu z e \vec{E}) = \frac{\partial c}{\partial t} \quad (5.30) \]

where \( D \) is the diffusion constant, \( \mu \) is the mobility, \( z \) is the charge of the ion and \( \vec{E} \) is

the electric field. The convection term has to be treated conservatively in the

calculations, \( \nabla \cdot (\bar{u}c) \neq c \nabla \cdot \bar{u} \) due to the high gradient in the concentration. Equation

5.30 determines the concentration distribution in the steady state.

The boundary conditions in this case are simple. Bulk ionic concentration on a

sphere 500 nm away from the pore on both sides of the membrane clamps the

concentration of both species on those boundaries. The Si₃N₄ surface and the dsDNA

surfaces are assumed to be impenetrable to ions.

Within the Nernst-Planck equations convective (electroosmotic) ion flow

originates from the fluid flow velocity field calculated with the Navier–Stokes

formalism. Electrophoretic flow is governed by the electric field determined by

Maxwell’s equations. There’s a third final contribution to the ionic current from

concentration driven diffusion. The diffusion constant for the ions used is connected

to their mobility by the Einstein relationship (Equation 5.8). Effective ion mobilities
Equilibrium electrolyte concentration is the initial condition applied on the quadratically discretized mesh outside the membrane. Solving the equations above predicts the current through the pore in the three geometries considered.

5.3 Experimental Results, Calculations and Discussion

5.3.1 Experimental Results

Figure 5.5 shows a segment of the ionic current trace through a nanopore, during a time interval that includes the new kind of molecular 10 kb dsDNA event that this paper presents. The electrolyte was 100 mM pH 9.1 KCl and the nanopore diameter was 5 nm. The applied voltage bias was 350 mV and resulted in an open pore current of 2.36 nA, as seen in regions A and E of Figure 5.5. The full event consists of an ~900 µs long ~180 pA decrease in the ionic current (region B of Figure 5.5), followed by a brief ~100 µs return to the open pore current (region C of Figure 5.5) and then a transient current enhancement in region D of Figure 5.5. The enhancement is consistent with a single unfolded molecule of dsDNA translocating through the nanopore [60].
Figure 5.5. Current trace of a trapping event followed by translocation. The current in region B decreases from the open pore level in regions A and E. Briefly the current returns to the open pore level, in region C, before the translocating dsDNA molecule increases the current, transiently, in region D.

The decrease in current shown in region B of Figure 5.5 contrasts with the enhancement expected from a dsDNA translocation and has not been previously reported in the literature. Only nanopores 5 nm and smaller in diameter displayed the new feature in region B, typically at a voltage bias above 300 mV.

Figure 5.6a shows the results of a similar experiment conducted at a 600 mV bias, where multiple events were recorded and displayed as a scatter plot. Each point
in the scatter plot represents a single event, indicating the observed average current increase and its duration. Average duration was $57.0 \pm 17.5$ µs equivalent to a mean dsDNA translocation speed of $6.0 \pm 1.8$ cm/s. Out of the 86 translocation events plotted in Figure 5.6a, 17 exhibited a decrease in the nanopore current similar to that seen in region B of Figure 5.5. The duration of this new feature ranged from 70 µs to 2550 µs. The latter is more than an order of magnitude longer than dsDNA translocation times at the same bias. The average duration of new feature was 914.1 µs which is comparable to the Zimm conformation relaxation time of a 1 kb dsDNA segment [61]. In most cases the return to the open pore current displayed in region C of Figure 5.5 was shorter than 8 µs and remained unresolved.

Figure 5.6b shows that when the electrolyte molarity is raised to 1 M KCl, typical dsDNA translocation events that now decrease the nanopore current are observed. This control experiment didn’t reveal depressed currents preceding the translocations like those in region B of Figure 5.5. Consistent with other reports, several types of translocation events were recorded (Figure 5.6b) [10, 62]. These were unfolded events in which one end of the dsDNA molecule enters the nanopore and several types of folded events in which two strands of the same dsDNA enter the pore simultaneously. During folding, translocation events displayed a current blockage that is approximately twice that of unfolded events. Figure 5.6b presents a scatter plot of 285 blockade events at 500 mV bias. Out of the 285 events 237 were unfolded, with average translocation duration of $166 \pm 61$ µs.
Figure 5.6. Scatter plots of dsDNA events in 100 mM (a) and 1 M (b) KCl solutions along with selected fitted event traces. In (a) 86 10 kb dsDNA translocation events at 600 mV in 100 mM KCl are plotted. Only unfolded events enhancing the current are observed. 17 of the 86 events show a decrease in the current before translocation due to trapping. Plotted in (b) are 285 10 kb dsDNA translocation events at 500 mV in 1 M KCl. Bottom right presents an unfolded event (current trace in red, fitted curve in blue). Middle right shows a dsDNA molecule folding close to one of its end as it enters the pore while top right shows a dsDNA molecule folding near its midpoint as it enters the nanopore. Arrows point to the areas of the scatter plot containing the fitted current traces.
5.3.2 Simulated Currents

Simulations of the current flow through the nanopore result in opposite sign current modulation during translocation and trapping. The model accurately predicts open nanopore and regular dsDNA translocation currents (supplementary materials contain Comsol model files). Figure 5.7a presents the $z$ component of the ionic current through a section at the apex of the nanopore. An excess number of potassium counterions accumulates along the nanopore surface due to its negative surface charge. This excess positive charge causes electroosmotic flow of the liquid due to the electric field. The no slip boundary condition limits the contribution of the electroosmotic flow to the total ionic current (Figure 5.7a). The high local ionic concentration near the surface leads to a high electrophoretic current, which accounts for most of the total current (Figure 5.7a). The potential drops rapidly near the apex of the nanopore producing a strong electric field (~15 mV/nm) that helps drive the electrophoretic ion flow (Figure 5.7b). The lack of a significant gradient in the ionic concentrations with respect to the $z$ coordinate is consistent with the predicted negligible diffusive current.
The current profile through the nanopore looks different when a dsDNA molecule translocates. At the low 0.1 M KCl concentration the negative charge of the nanopore wall and the dsDNA molecule both enhance the K⁺ concentration and the electrophoretic and electroosmotic currents (Figure 5.8a). The diffusive contribution to the current is negligible. A no-slip condition on the dsDNA surface results in a small negative electroosmotic current at that boundary. Positive counterions are dragged along with the dsDNA molecule against the field very near its surface. As the molecule translocates it blocks part of the nanopore’s ion flow. That decrease in current and the slightly negative electroosmotic current at the dsDNA surface are overwhelmed by the enhanced potassium ion concentrations and electrophoretic
current flow. The result is a net increase of the nanopore’s current during dsDNA translocation.

Figure 5.8. dsDNA translocation simulation at 600 mV. Plotted in (a) is the \( z \) component of the current through the apex of the pore. The electrophoretic ion flow (red line) dominates the total current through the pore (dark blue line). Electroosmotic flow (green line) contributes to the total current everywhere except right at the surface of the dsDNA molecule. Diffusive flow (aqua line) has negligible contribution. Presented in (b) is a 2D section of the total current around the dsDNA molecule and inside the nanopore (in nA/nm\(^2\)). The white box in the middle is the excluded volume due to the dsDNA molecule while the white sections on the left and right are the Si\(_3\)N\(_4\) membrane.

When dsDNA is trapped near the nanopore orifice the current enhancement resulting from the presence of the charged polymer is not sufficient to offset the current blocked. As expected the total current is enhanced near the dsDNA molecule (Figure 5.8b). Combined electrophoretic and diffusive current is enhanced as is the electroosmotic current. The increase in net ionic current near the dsDNA molecule comes up short of making up for the current blocked by the dsDNA molecule. This
results in a net decrease of the combined electrophoretic and diffusive current and the total current.

Figure 5.9. Total $z$ component current sections near the surface of the open pore (a) and near the dsDNA along the diameter of the pore (b) at 600 mV. In (b) we see an enhancement in the total current (dark blue) in close proximity to the dsDNA. This is offset by the zero current flowing through the dsDNA molecule itself and results in a decreased total current when the molecule is trapped. The dsDNA molecule begins at the rightmost edge of the graph in (b).

The model described above predicts the open pore and dsDNA translocation current levels accurately. Figure 5.10a compares experimental data with the calculated ionic currents for a range of applied voltage values in an open pore and during translocation. Positioning the apex of the nanopore 9.5 nm lower than the midpoint of the membrane in the simulations accurately captures the slight rectifying effect that the experimental data shows (lower conductivity at negative bias). Using the full unshielded charge of 2 electrons per base pair of dsDNA molecule produces current enhancements that are consistent with experimental measurements.
The same model is used to simulate the dsDNA trapping current levels. Calculations for 2.2 nm and 2.8 nm diameter dsDNA are presented. The 2.8 nm model fits the experimental data better and may be the result of a Stern layer that can’t be captured accurately by simulations. The predicted and experimental absolute values of the trapping current level are plotted in Figure 5.10b for a 2.8nm diameter dsDNA model. The inset of Figure 5.10b presents the experimental and predicted trapped current levels relative to the open pore current for both the 2.2 nm and 2.8 nm diameter dsDNA models. Predicted values are consistent with experimental data.

Translocation and trapping can produce opposite sign ionic current modulations according to both experiment and simulation. The total trapping current level is lower than that of the open pore when the obstructing torus is at the surface of the membrane but provides poor quantitative agreement with the experimental data. Full three dimensional models agree qualitatively with the results presented here but sufficient meshing detail is hard to achieve.

The nanopore modeled, and providing the data in Figure 5.6, was too large to prevent folded events in 1 M KCl. If dsDNA was trapped across the diameter of the nanopore and couldn’t translocate the simulation model predicts a dip in the conductivity of 85.8 pA at 500 mV. At 500 mV in 1 M KCl, measured peak to peak noise was 98.9 pA resulting in a S/N ratio of 0.8. In the 100 mM case the S/N ratio was 2.4 making it much easier to see the trapping phenomenon. The contrast between the translocation and trapping current modulations along with the better predicted S/N ratio at 100 mM KCl concentrations made detection of the new phenomenon possible.
Figure 5.10. Experimental (points) and simulated (solid lines) currents through the pore plotted for different ionic voltages across the nanopore (100 mM KCl, 10 kb dsDNA). In (a), dark blue solid line represents the simulated current value through the open pore and lighter blue squares are the experimental values. Dark red solid line represents the simulated current through the pore when dsDNA translocates and lighter red circles are the experimental values. (b) Dark orange line represents the simulated current value for a trapped dsDNA molecule, while the squares stand for the experimental data. Inset shows the same decrease in current plotted in (b) but relative to the open current value. Solid black line in the inset stands for 2.2 nm diameter dsDNA and predicts the lower voltage data better while a Stern layer (2.8 nm diameter dsDNA) fits better the higher voltage experimental data (orange line).
Figure 5.10 (Continued)
5.3.3 Probability of dsDNA Trapping near a Nanopore

Simulation results and analytical models allow the estimation of the probability of trapping a dsDNA molecule near a nanopore. The trapping well occurs due to the externally applied voltage bias and the resulting electric field. Simulations of the nanopore provide the value of the attractive potential at the opening of the nanopore which combined with the dsDNA charge results in an attractive force. Loss of entropy due to immobilizing the dsDNA molecule near the pore works against the trapping potential. When conditions are such that the trapped dsDNA molecule’s free energy decreases more than a $kT$ trapping is likely to occur,

$$\Delta F = \Delta F_{\text{entropy}} + \Delta F_{\text{field}} < -kT$$  (5.31)

where $\Delta F$ is the total change in free energy and the sum of the entropic and electrostatic components. We’ll calculate the entropic contribution and use simulation data along with a screened charge density on the dsDNA molecule to estimate the trapping probability.

Entropy is lost as a result of the restricted volume available to the dsDNA molecule when it’s trapped. Each side of the flow cell contains about 1 µL of electrolyte which is equivalent to a volume of $10^9$ µm³. When the dsDNA molecule is free, its center of mass is translationally free to explore the entire volume. When immobilized near the nanopore the volume that is available to the molecule can be
estimated as the sum of the Gaussian gyration volumes [61] for each of the two segments,

\[ R_g = b \sqrt{\frac{N}{6}} \]  
\[ V_{\text{trapped}} = \frac{4\pi b^3}{6^{5/2}} \left( n^{3/2} + (N-n)^{3/2} \right) \]  

(5.32)

where \( b \) is the Kuhn length and is defined as twice the persistence length, \( N \) is the total number of Kuhn lengths in the molecule and \( n \) is the number Kuhn lengths in the shorter segment. The total gyration volume of the dsDNA molecule for \( n=1 \) is \( 1.6 \times 10^{-3} \) µm\(^3\) and \( 1.9 \times 10^{-3} \) µm\(^3\) for \( n=17 \), given a Kuhn length of 100 nm and 34 total segments in a 10 kb molecule. This results in an increase of the free energy

\[ \Delta F_{\text{translation}} = -kT \ln \left( \frac{V_{\text{trapped}}}{V_{\text{free}}} \right) \]

of 27.2 and 27.0 \( kT \) for \( n=1 \) and \( n=17 \) respectively.

When the dsDNA molecule is immobilized near a flat surface the number of conformational states available to the polymer is restricted due to steric interactions with the surface. The two segments of the molecule are restricted to the \( x > 0 \) domain if we consider the membrane to be a \( yz \) plane and the molecule pinned at \( \vec{r}_0 = (\varepsilon,0,0) \), \( n \) segments away from its shorter end.
The probability that the free end of the shorter segment of length \( n \) is at radius \( \tilde{r} \) is then by the mirror image method [63],

\[
G(\tilde{r}, \tilde{r}_0, n) = \left[ \frac{2\pi nb^2}{3} \right]^{-3/2} \frac{6x\varepsilon}{nb^2} \exp\left( -\frac{3r^2}{2nb^2} \right)
\] (5.33)

where \( \varepsilon \) is arbitrarily small. The steric constraint factor \( Z_s(n) \) is defined as,

\[
Z_s(n) = \int_{x>0} G(\tilde{r}, \tilde{r}_0, n) d\tilde{r} < 1
\] (5.34)

and represents the reduction in conformation states available for the shorter segment of the dsDNA molecule when trapped. If the molecule is free \( Z_s(n) = 1 \). From Equations 5.33 and 5.34 we can infer that \( Z_s(n) \) scales as \( n^{-1/2} \). The conformational increase in free energy when the dsDNA molecule is trapped due to both the shorter and longer segments is,

\[
\Delta F_{\text{steric}} = \frac{1}{2} kT \ln(n(N-n))
\] (5.35)
Combining the steric and translational increases in free energy when the molecule is immobilized $n$ Kuhn lengths from its shorter end we arrive at,

$$\Delta F_{\text{entropy}} = -kT \left( \ln \left( \frac{V_{\text{pinned}}}{V_{\text{free}}} \right) - \frac{1}{2} \ln(n(N-n)) \right)$$  \hspace{1cm} (5.36)

Figure 5.11 shows the loss in entropy (gain in free energy) in units of $kT$ as a function of $n$.

Figure 5.11. Total entropic increase in the free energy of the dsDNA molecule when it’s pinned $n$ Kuhn lengths from the shorter end.

The loss in entropy must be offset by a gain in electrostatic energy for the molecule to stay near the opening of the pore. The electrostatic potential near the pore
opening is obtained from simulation data. A single Kuhn length across the nanopore is considered. Due to the repulsion from the negative surface charge on the membrane surface, the 100 nm Kuhn length of dsDNA will experience a zero net force when its axis is 4.46 nm above the pore surface. In that configuration the central 36.85 nm will be pulled towards the nanopore by the applied external bias and the remaining 63.15 nm will be repelled. The Manning condensation charge of 0.48 $e$/ base results in 51.19 $kT$ of trapping energy at 600 mV. Figure 5.12 shows the pinning electrostatic energy (in $kT$ units) vs applied potential.

Figure 5.12. Electrostatic energy of dsDNA across the diameter of a nanopore as a function of applied voltage across the pore from simulation data. Entropic cost of trapping the molecule is indicated. Trapping occurs above 350 mV in this geometry.
The data in Figures 5.11 and 5.12 suggests that the electrostatic decrease in energy of the dsDNA molecule can offset the loss of entropy when the molecule is trapped. An external voltage bias of 100 mV can barely offset the loss in translational entropy. Only at an applied voltage bias of ~350 mV trapping is observed. The prediction of a threshold value and its magnitude are both consistent with the experimental results from a number of experiments.

Based on the model presented above we can also analyze the relevant forces when the dsDNA molecule is trapped. Adding drag on the dsDNA molecule resulting from the electroosmotic flow of the fluid shifts the trapped equilibrium position 0.05 nm away from the surface (to 4.51 nm). The maximum restoring force that has to be overcome to free the dsDNA molecule at a 600 mV applied voltage bias is 5.4 pN. This is significantly larger than the ~74 fN average conformational entropic force resulting from the reduced availability of conformation states with trapping. The force is the result of differentiating the free energy $F$ with respect to the $x$ coordinate (distance above the membrane surface). It ignores the volume restriction contribution to the free energy. The mirror image method results in a steric constraint factor of 0 when the molecule is at the surface. Using the slope obtained from the mirror image method (Equations 5.33 and 5.34) combined with a steric constraint factor value of $\frac{1}{2}$ at the surface results in a force of 74 fN when the dsDNA molecule is pinned near its end ($n=1$). This entropic force is sufficient to displace the molecule laterally 1 nm away from the axis of the nanopore eventually allowing capture of one of the molecule’s free ends. Once translocation begins the charge along the translocating
strands pushes the trapped segment an additional 3 nm away from the axis laterally and the 129.7 pN translocation force overwhelms any residual trapping potential. Trapping forces are roughly linear with applied bias.

5.3.4 Lower Bound on the dsDNA Persistence Length

Persistence length can be calculated from the force necessary to fold the dsDNA molecule into a nanopore of diameter $d$. Consider a beam with Young’s modulus $E$ and moment of inertia $I$ simply supported at two points distance $L$ apart (Figure 5.13). If $\theta$ is the angle between its two support points and a concentrated applied force in the middle of the beam, the system is described by the following differential equation,

$$EI \frac{d^2\theta}{dt^2} - F \cos(\theta) = 0$$

(5.37)

Figure 5.13. Schematic diagram of a simply supported beam.
If we multiply the above equation by \(d\theta/dl\) and integrate with proper boundary conditions we have

\[
\frac{1}{2} L = \sqrt{\frac{EI}{F}} \int_{\theta_0}^{\pi/2} \frac{\cos(\theta - \theta_0)}{\sqrt{\sin(\theta)}} \, d\theta
\]

(5.38)

Where \(\theta_0\) is the angle at which the beam collapses (folds). We can rearrange Equation 5.38,

\[
F = \frac{EI}{L^2} g(\theta_0)
\]

(5.39)

\[
g(\theta_0) = 4 \sin(\theta_0) \left( \int_{\theta_0}^{\pi/2} \frac{\cos(\theta - \theta_0)}{\sqrt{\sin(\theta)}} \, d\theta \right)^2
\]

The \(\theta\) dependent term in the equation above has a maximum value of 2.135 for an angle of 0.735 radians resulting in a critical concentrated force necessary to collapse the beam \(F_{\text{crit}}\),

\[
F_{\text{crit}} = \frac{EI}{L^2} \times 2.135
\]

(5.40)
A force density $K(l)$ along the beam modifies Equation 5.37 to,

$$E I \frac{d^2 \theta}{dl^2} - K(l) l \cos(\theta) = 0$$  \hspace{1cm} (5.41)

This is difficult to evaluate analytically. We can however for an arbitrary force density calculate an equivalent concentrated force. The nanopore is symmetric so it suffices to consider the force density from the axis to the zero-force (pivot) point (Figure 5.14).

Figure 5.14. Force density in pN/nm plotted for half a Kuhn length starting at the axis of the nanopore neglecting fluid drag on the molecule. When integrated the total force is zero. The pivot point is 18.428 nm from the axis. The repulsive force from 18.428 nm to 50 nm originates from the negative charge on the membrane surface. Simulation is carried out at 600 mV in 100 mM KCl. The force is based on the calculated electric field acting on the dsDNA Manning charge.
The force on dsDNA molecule is calculated using the electric field values from the nanopore model acting on an effective charge of -0.48 $e/\text{base charge}$. This is the same Manning condensation charge used in the previous section. The dsDNA molecule is not a part of the simulation in this case which is why the charge is adjusted analytically. The total force on the dsDNA between the axis of the pore and the pivot point at 18.428 nm is -2.23 pN. The force distribution can be approximated with an equivalent concentrated force of -2.23 pN at 6.85 nm from the axis of the nanopore,

$$F = \int_{0}^{18.428\text{nm}} K(r)dr = 2.2335\text{pN}$$

(5.42)

$$l = 18.428\text{nm} \times \frac{\int_{0}^{18.428\text{nm}} K(r)(18.428\text{nm} - r)*dr}{\int_{0}^{18.428\text{nm}} K(r)dr} = 6.8477\text{nm}$$

Axial symmetry dictates the presence of an equivalent force at -6.85 nm along the dsDNA molecule.

Equation 5.40 connects the Young’s modulus of the beam to a concentrated critical force at the middle of the beam. Figure 5.15 presents schematically the bending moment diagrams for a central concentrated force and a symmetrically applied force at two points along a beam. The maximum bending moment is responsible for the failure of the beam.
The equivalent focused central force producing the same maximum bending moment as the two point symmetric load is,

\[ F_{\text{crit}} = -2.2335 \, pN \frac{6.8477 \, nm}{18.428 \, nm} = -0.8300 \, pN \]  \hspace{1cm} (5.43)

Starting with the force distribution in Figure 5.14 the problem is reduced to a single concentrated force at the center of the dsDNA molecule.

Figure 5.15. Top, the shear force on a beam for the symmetric two point loading case (left) and the concentrated force problem (right). Bottom, corresponding bending moment diagrams for the same two problems.
Considering that there are no folded events at biases up to 600 mV in the 100 mM KCl but folded events are observed in 1 M KCl, a lower bound on the persistence length of dsDNA \( l_p = \frac{EI}{kT} \) (from [61]) can be calculated. Using Equation 5.40,

\[
l_p > \frac{F_{crt} (L/2)^2}{2.135kT}
\] (5.44)

The value of -0.830 pN with a pivot point \( L/2 = 18.428 \) nm yields a lower bound on the persistence length of 68.47 nm which given all the approximations is in good agreement with the accepted value of ~50 nm [64].

5.4 Conclusions

Under the right experimental conditions a negatively charged dsDNA molecule can become trapped at the orifice of a nanopore. The size of the nanopore and the strength of the electric field have to be within a narrow range so that the molecule becomes trapped but doesn’t buckle and translocate. The trapped molecule decreases the current through the nanopore in contrast to the current enhancement observed during translocation at low molarities. This phenomenon will affect capture
statistics in nanopores too small to allow folded events and may remain undetected at high molarities.

The possibility of trapping a charged molecule at the orifice of a solid-state nanopore suggests some interesting applications. If a transition from trapping a charged polymer to its folded translocation occurs at a certain applied voltage, the persistence length of the molecule can be calculated. Charged molecules that are too stiff to buckle for any reasonable applied voltage can be precisely positioned over the nanopore. For example, single-walled carbon nanotubes decorated with single-stranded DNA can in this fashion be electrophoretically aligned with a nanopore. Fabricating an array of nanopores can result in precise alignment and control surface mobility of a trapped molecule through the trapping force. Through controlling the surface mobility of a DNA molecule that is translocating through a larger nanopore one can control its translocation speed.
Chapter 6: Conclusions and Future Directions

Integrating SWCNTs with nanopores for dsDNA sensing remains a promising approach to rapid dsDNA sequencing. ssDNA interacts strongly with SWCNTs modifying their conductance. Finding the right passivation atomic layer deposition film is crucial to successful integration of the SWCNT-nanopore devices with an electrolyte and analyte containing flow cell. Different hafnia and alumina films have their advantages and shortcomings. The combination of gold interconnects to the SWCNTs with an alumina passivation film holds promise for concurrent ionic channel and SWCNT conductance channel dsDNA detection. Tenfold slower dsDNA translocation speeds are an important and unexpected advantage observed in SWCNT-nanopore devices. Transitioning to tunneling devices offers few fabrication challenges.

Trapping of dsDNA molecules at the orifice of a nanopore was observed for the first time during work at low molarities with SWCNT devices. The new phenomenon was explored with nanopore devices, without SWCNTs. When the applied voltage bias is high enough, the nanopore small enough and the molarity of the solution low dsDNA can become trapped at the orifice of a nanopore. It is crucial that the pore is small enough that the dsDNA molecule can not fold and translocate through it. Simulations confirm the opposite sign current modulation observed experimentally during translocation and trapping. Higher molarities should allow trapping as well but will result in a significantly worse signal to noise ratio and the
same sign change current modulation during trapping and translocation. Trapping of dsDNA at the orifice of a nanopore has implications for capture statistics, sequencing in solid state nanopores and can be used to probe persistence length of the molecule.
Bibliography


