

Development of a Solid-State Nanopore Platform Toward High-Throughput, Ultra-Sensitive, and Robust Single-Molecule Analysis of Nucleic Acids and Proteins

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Abstract

Solid-state nanopore sensors consist of a nanoscopic hole in a thin free-standing dielectric film (a membrane) bridging independent reservoirs of salt solution to interrogate the analytes present therein. Nanopores have demonstrated promising results in applications spanning DNA sequencing, biomarker detection, information storage and energy harvesting. Recently, researchers in the field have aimed to interrogate proteins with enough precision to extract their sequences. Despite their versatility, solid-state nanopores have advanced slowly experimentally due to obstacles and knowledge gaps that need to be explored.

A few challenges are addressed in this thesis, attempting to provide insight for future steps in this direction, namely: 1) extracting surface charge density is key to studying the effect of membrane material properties on nanopore transport, fabrication conditions and subsequent chemical functionalization, 2) unwanted adsorption between the biomolecules and the sensor's surface during the electrophoretic capture and translocation process hinders sensing performance and results in clog events, 3) small proteins or DNA nanostructures can be challenging to fully resolve, leading to missed or attenuated molecular event signatures, and 4) reducing membrane thickness to enhance the signal-to-noise ratio and the spatial resolution leads to faster sensor degradation over time for SiN.

For the first objective, I present an experimental characterization platform capable of measuring the surface charge density of silicon nitride as a membrane material. The platform allows high-pressure streaming current measurements in the picoampere range, providing enough resolution to extract the zeta potential and surface charge density of new materials.

Next, I explore an alternative anti-fouling protocol for the possibility of increasing sensing time while interrogating long and flexible single-stranded DNA (ssDNA). This analyte clogs the sensor in less than 15 seconds, while after surface modification, we collect relevant data for ~45–60 minutes before the first clog event. This improved protocol preserves noise performance comparable to pristine nanopores,

even after surface modification. Furthermore, I evaluate the surface properties of these modified nanopores to confirm the successful binding of the polymer on the surface through zeta potential measurements.

Moreover, I develop a trapping procedure that takes advantage of the surface characterization platform to reduce the translocation velocity of complex analytes, thus investigating pressure-voltage actuation for analytes of known velocity while determining the impact of this mode of translocation on dwell time. This actuation approach provides an up to 6× reduction in translocation velocity for DNA nanostructures and proteins respectively, facilitating the extraction of high-fidelity event signatures.

Finally, I perform an exploration of nanopore membrane microfabrication processes and materials to improve signal amplitude while reducing noise limitations. Here, I present an alternative deposition approach for SiN thin films with a lower thermal budget, namely ion beam deposition (IBD) compared to the traditional Low-Pressure Chemical Vapor Deposition (LPCVD). Independently, I explore the properties of a sub-5 nm annealed Hafnium oxide (HfO₂) membrane deposited by Atomic Layer Deposition (ALD), supported by an LPCVD SiN, in terms of film stability and sensing performance. Future work involves combining the lower thermal budget SiN deposition as a mechanical support for a sub-3 nm ALD HfO₂ membrane. This approach will offer an ultra-thin solid-state nanopore architecture with reduced degradation, enhanced signal-to-noise ratio, and bypass the annealing step due to the temperature mismatch between depositions.

Ultimately, my goal is to pave the way for more efficient and sensitive nanopore-based technologies, contributing to the fields of genomics, proteomics, and beyond.

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To my parents, I would have never made it this far without your constant encouragement and support. To my sisters, I wish you the best life has to offer.

Statement of Originality

The material presented in this thesis resulted from research performed over the course of my PhD degree under the supervision of Dr. Vincent Tabard-Cossa at the University of Ottawa.

Chapter 1 is a literature review of key concepts discussed in this thesis, introducing nanopore fundamentals, microfabrication principles and surface characterization in the nanopore context.

Chapter 2 (published as “Unraveling clogging of solid-state nanopores by selective polymer coating: surface potential measurements and sensing of long single stranded DNA” in *RSC Applied Interfaces*) contains a peer-reviewed article as first author in collaboration with Dr. Zachary Roelen, who assisted me in initial experimental validation and provided key edits to the final manuscript prior to publication. I wrote the first draft, collected and analyzed all the data presented in the article. This work was performed following a conference publication in IEEE SAS 2023 where I presented the developed platform and preliminary results, with the help of my co-authors Dmytro Lomovtsev who helped me in flow cell design, and Gengyang Mu who introduced me to membrane microfabrication.

Chapter 3 (submitted as “Pressure-Voltage modulation of passage dynamics for enhanced nanopore biosensing” but not yet published) contains a manuscript in collaboration with Dr. Martin Charron, who prepared the velocity profiling molecules, derived the final expression for the effective charge of DNA and provided key edits to the final manuscript. This work improved on the previously developed pressure-regulated platform to study the effect of this additional force on translocation dynamics of double-stranded DNA, nanostructured DNA and proteins.

Chapter 4 (published as “A method for fabricating CMOS back-end-of-line-compatible solid-state nanopore devices” in *Nanotechnology*) contains a first author paper in collaboration with Chunhui Dai with equal contributions. Specifically, Chunhui developed, collected and analyzed data pertaining to

material characterization while I developed, collected and analyzed data pertaining to nanopore microfabrication and characterization. All authors contributed to the writing of the final manuscript.

Chapter 5 contains results of ongoing research that would potentially lead to a journal article. Specifically, I worked with Michel Stephan, a research engineer in the group, to microfabricate the presented nanopore devices. The instrumentation presented in the high-throughput section was developed primarily by Dmytro Lomovtsev, who designed and manufactured multi-channel flow cells and PMDS micro-gaskets, and Michel Stephan who collaborated with him to implement hardware parallelization. Mathieu Gibeault and Michel Stephan developed the software interface to control the multi-channel fabrication unit. The data presented was collected and analyzed from my own experiments.

Nanopore data analysis was performed initially through Nanolyzer, software developed by Dr. Kyle Briggs, followed by Poriscope, successor to Nanolyzer developed by Kyle in collaboration with Carolina Gonzalez, an undergraduate student in the group.

List of Contributions

Publications

Bouhamidi, M. Y., Roelen, Z., Tabard-Cossa, V. (2025). Unraveling clogging of solid-state nanopores by selective polymer coating: surface potential measurements and sensing of long single stranded DNA. *RSC Applied Interfaces*, 2, 1661-1673.

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Conference presentations

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Bouhamidi, M. Y., Stephan, M., Nag, J., Bedau, D., Tabard-Cossa, V. (2026). Poster: Towards Ultra-Thin and Stable Solid-State Nanopores. 2026 Canadian Semiconductor Science and Technology Conference (CSSTC), Ottawa, ON.

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List of Acronyms

μ	Electrophoretic mobility [$\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$]
<i>AFM</i>	Atomic force microscopy
Al_2O_3	Aluminum oxide
<i>ALD</i>	Atomic layer deposition
<i>BEOL</i>	Back end of line
<i>bp</i>	Base pair [1 bp = 0.34 nm]
<i>c</i>	Concentration [mol/L]
<i>CBD</i>	Controlled breakdown
<i>CMOS</i>	Complementary metal-oxide-semiconductor
<i>D</i>	Diffusion coefficient [m^2/s]
<i>Da</i>	Daltons [1 Da = 1.66×10^{-27} kg]
d_{DNA}	Double-stranded DNA diameter [m]
<i>DI</i>	De-ionized water
d_{pore}	Nanopore diameter [m]
<i>dsDNA</i>	Double-stranded DNA
<i>e</i>	Elementary charge [C]
<i>E</i>	Electrical field [V/m]
<i>EDL</i>	Electrical double layer
E_y	Young's modulus [N/m^2]
<i>f</i>	Frequency [Hz]
<i>F</i>	Faraday constant [C/mol]
F_{EP}	Electrophoretic force [N]
<i>FIB</i>	Focused Ion Beam
<i>G</i>	Conductance [S]
HfO_2	Hafnium oxide
<i>I</i>	Current [A]
<i>IBD</i>	Ion beam deposition
<i>J</i>	Flux [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]
k_B	Boltzmann constant [J/K]
L_{pore}	Nanopore thickness or length [m]
<i>LPCVD</i>	Low-pressure chemical vapor deposition
<i>n</i>	Number density of ions [m^{-3}]
<i>pI</i>	Isoelectric point
<i>q</i>	Net charge [C]
<i>r</i>	Radius of a particle [m]
<i>R</i>	Universal gas constant [$\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$]
<i>RIE</i>	Reactive-ion etching
<i>RMS</i>	Root mean square
<i>sccm</i>	Standard cubic centimeters per minute

SiN	Silicon nitride
$ssDNA$	Single-stranded DNA
t	Time [s]
T	Temperature [K]
TEM	Transmission Electron Microscopy
v	Flow velocity [m/s]
V	Voltage [V]
x	Displacement in the x-direction [m]
y	Displacement in the y-direction [m]
z_i	Nonzero valence of species i
ΔT	Change in temperature [K]
ϵ	Permittivity of the medium [F/m]
η	Viscosity constant of the medium [Pa·s]
λ_D	Debye length [m]
σ	Surface charge density [C/m ²]
σ_s	Salt conductivity [S/m]
Φ, ζ	Surface/Zeta/Electrostatic potential [V]

1. Introduction

Nanopores are nanoscale channels that bridge two otherwise independent fluidic reservoirs.¹ Typically, these reservoirs contain electrolyte solutions, meaning salts dissolved in water, enabling an electrochemical readout via submerged electrodes such as Ag/AgCl. When a potential difference is applied across the nanopore, dissolved ions behave analogously to electrons in a conductor, establishing a continuous ionic current through this nanometric aperture. This ionic current provides a first-order approximation of the channel dimensions through current-voltage (I – V) measurements, as the magnitude of current flow scales with the cross-sectional area available for ion transport.²

Once the sensor's physical dimensions are characterized, the nanopore can be leveraged for molecular analysis. Charged biomolecules, including but not limited to DNA and proteins, can be introduced into the electrolyte on one side of the membrane and electrophoretically driven through the pore under an applied voltage bias. These biomolecules, which adopt an effective charge in solution through their protonation state, transiently obstruct the flow of ions during translocation. This migration is observed as a momentary reduction in ionic current, referred to as a current blockage event. By analyzing these events and extracting event amplitude (the depth of current reduction) and duration (the time required for complete translocation), biomolecular properties (shape, volume, and effective charge) can be readily inferred.^{3,4} This principle, first demonstrated by Coulter in the 1950s for micron-scale particles, has been progressively refined to achieve single-molecule sensitivity at the nanoscale.⁵

1.1 Nanopore Fundamentals

1.1.1 Principles of Operation

The fundamental principle behind solid-state nanopore sensors is based on the measurement of ionic current as biomolecules translocate through the nanopore. When a voltage is applied across the membrane, ions in the electrolyte solution generate an ionic current that flows through the nanopore. As a biomolecule passes through the pore, it partially blocks the flow of ions, causing a temporary change in the ionic current (Figure 1.1(a)). When a biomolecule such as DNA or protein translocates through a nanopore, it is akin to threading a needle at the nanoscale. This process is governed by multiple forces, including electrophoresis, electroosmosis, diffusion, and entropic barriers, each playing a crucial role in driving or impeding the molecule through the tiny pore.⁶

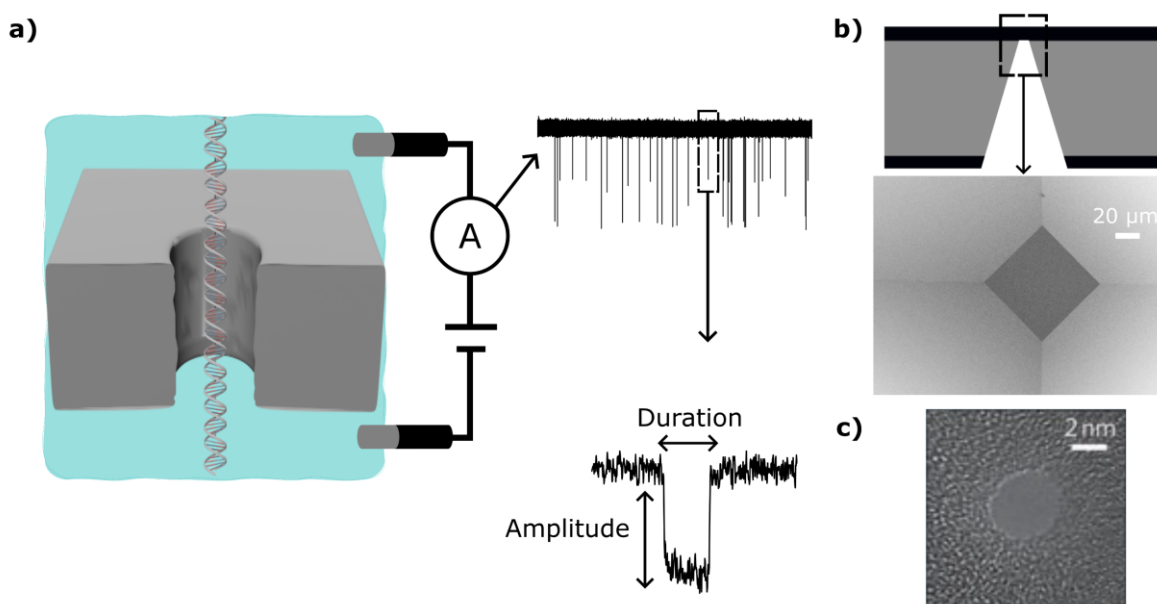


Figure 1.1 Nanopore sensing principle. a) Schematic of a nanopore sensor submerged in solution containing double-stranded DNA (left). A typical current trace shows event translocations following an applied bias voltage, with a zoom-in on a typical unfolded event (right). b) Cross section view of a SiN chip (top) with an SEM image of a SiN free-standing membrane taken from the backside of the chip (bottom). c) TEM image of a 3.5-nm SiN nanopore. Image adapted from Wanunu *et al.*⁷, with permission.

1.1.2 Ion Transport and the Nernst-Planck Equation

Ion transport governs nanopore science, where an electrical current is observed due to the flux of ions in liquid. The Nernst-Planck equation is a flux equation, combined with conservation of mass, that describes the motion of charged species under the combined influence of diffusion (concentration gradients) and an applied electrical field. As adapted by Albrecht *et al.* for nanopore applications, the Nernst-Planck equation under an applied field and no pressure gradient for a given ionic species i reduces to a one-dimensional form that captures the essential physics of ion movement through the pore:⁸

$$j_i = -D_i \cdot \left[\frac{dc_i}{dx} - c_i \cdot \frac{z_i \cdot F}{RT} \cdot E \right] \quad (1.1)$$

where D_i , z_i , F , R , T , and E are the diffusion constant and the nonzero valence of the species, the Faraday constant, the universal gas constant, the absolute temperature, and the electric field respectively.

The first term within brackets represents diffusion driven by concentration gradients, while the second term represents ion migration driven by the electric field. The relative magnitude of these terms determines whether transport is diffusion-limited or drift-dominated. This transport mechanism acts on positively and negatively charged species in solution, leading to a net ionic current through the nanopore, as observed when an electrical field is applied across the membrane.

1.1.3 Nanopore Conductance

The conductance of a nanopore can be modeled as a salt-dependent parameter that reflects the combined contributions of bulk electrolyte within the pore volume and surface charge effects at the pore walls. At low salt concentrations, typically below 100 mM, the ionic current is dominated by interactions between counterions and the charged nanopore walls, as the Debye screening length becomes comparable to or larger than the pore radius. In this regime, the conductance is highly sensitive to surface

charge density and pH. Conversely, at high salt concentrations of 1 M or above, the current is governed primarily by the electrophoretic mobility μ of charge carriers in solution, with surface contributions effectively screened over distances much smaller than the pore diameter.

For a cylindrical nanopore of diameter d_{pore} and length L_{pore} in KCl solution, which is the most common electrolyte system due to the similar mobilities of K^+ and Cl^- ions, Smeets *et al.* expressed the conductance G as a sum of bulk and surface contributions:²

$$G = \frac{\pi d_{pore}^2}{4 L_{pore}} \left((\mu_K + \mu_{Cl}) n_{KCl} e + \mu_K \frac{4\sigma}{d_{pore}} \right) \quad (1.2)$$

where n is the number density of potassium or chloride ions, e is the elementary charge, σ is the surface charge density term, accounting for the additional contribution of counterions screening the charged pore walls.

Under typical high-salt sensing conditions of 1 M or above, the contribution of surface charge to nanopore conductance is greatly reduced by charge screening effects, with the Debye length shrinking to sub-nanometer dimensions. In this regime, the conductance expression can be simplified to account primarily for the sensing region within the nanopore, namely the channel resistance. However, this simplified model neglects the contribution of converging electric field lines at the pore mouth, known as access resistance, which becomes significant in high aspect-ratio pores where $L_{pore} \leq d_{pore}$. Access resistance arises because ions must converge from a macroscopic reservoir into the nanoscale pore entrance, creating a region of heightened resistance immediately adjacent to the pore. The complete nanopore conductance is therefore best described as the inverse of three resistors in series: the channel resistance and two access resistances, one at each pore entrance:⁹

$$G = (R_{channel} + 2R_{access})^{-1} = \sigma_s \left(\frac{4L_{pore}}{\pi d_{pore}^2} + \frac{1}{d_{pore}} \right)^{-1} \quad (1.3)$$

where σ_s is the salt conductivity in solution.

In the extreme case of two-dimensional materials such as graphene, where membrane thickness can be as low as 0.3 nm or a single atomic layer, aspect ratios become exceptionally high, and the conductance simplifies further to $G = \sigma_s d_{pore}$.¹⁰ This indicates that access resistance completely dominates the transport behavior.

From equation 1.3, the nanopore diameter can be extracted by solving the quadratic form for a known channel length. This expression yields two roots, with the positive root corresponding to the physical diameter:

$$d_{pore} = \frac{G}{2\sigma_s} \left(1 + \sqrt{1 + \frac{16\sigma_s L_{pore}}{\pi G}} \right) \quad (1.4)$$

This approach provides a non-destructive method for pore size characterization.

1.1.4 Current Blockage During DNA Translocation

As a biomolecule translocates through the nanopore, it physically excludes volume that would otherwise be occupied by conducting ions, causing a temporary reduction in the ionic current. The magnitude and duration of the blockage event are influenced by the physical properties of the nanopore, including surface charge,¹¹ length,⁹ and diameter,¹² and the translocating molecule, including cross-sectional area,¹³ flexibility,¹⁴ and effective charge.¹⁵

This change in observed conductance, $G_{Open\ pore} - G_{DNA}$, was first modeled by the conductance of the unobstructed volume inside the nanopore, treating the molecule as a cylindrical insulator displacing

electrolyte.⁹ Adopting this linear approximation for simple unfolded blockage of dsDNA, the expected blockage current for a given nanopore thickness is:¹⁶

$$\Delta I = \sigma_s \frac{V\pi d_{DNA}^2}{4L_{pore}} \quad (1.5)$$

where V is the applied voltage and d_{DNA} is the effective diameter of DNA, approximately 2.2 nm including its hydration shell. This simple model predicts that blockage amplitude scales linearly with salt conductivity and applied voltage, and inversely with pore length.

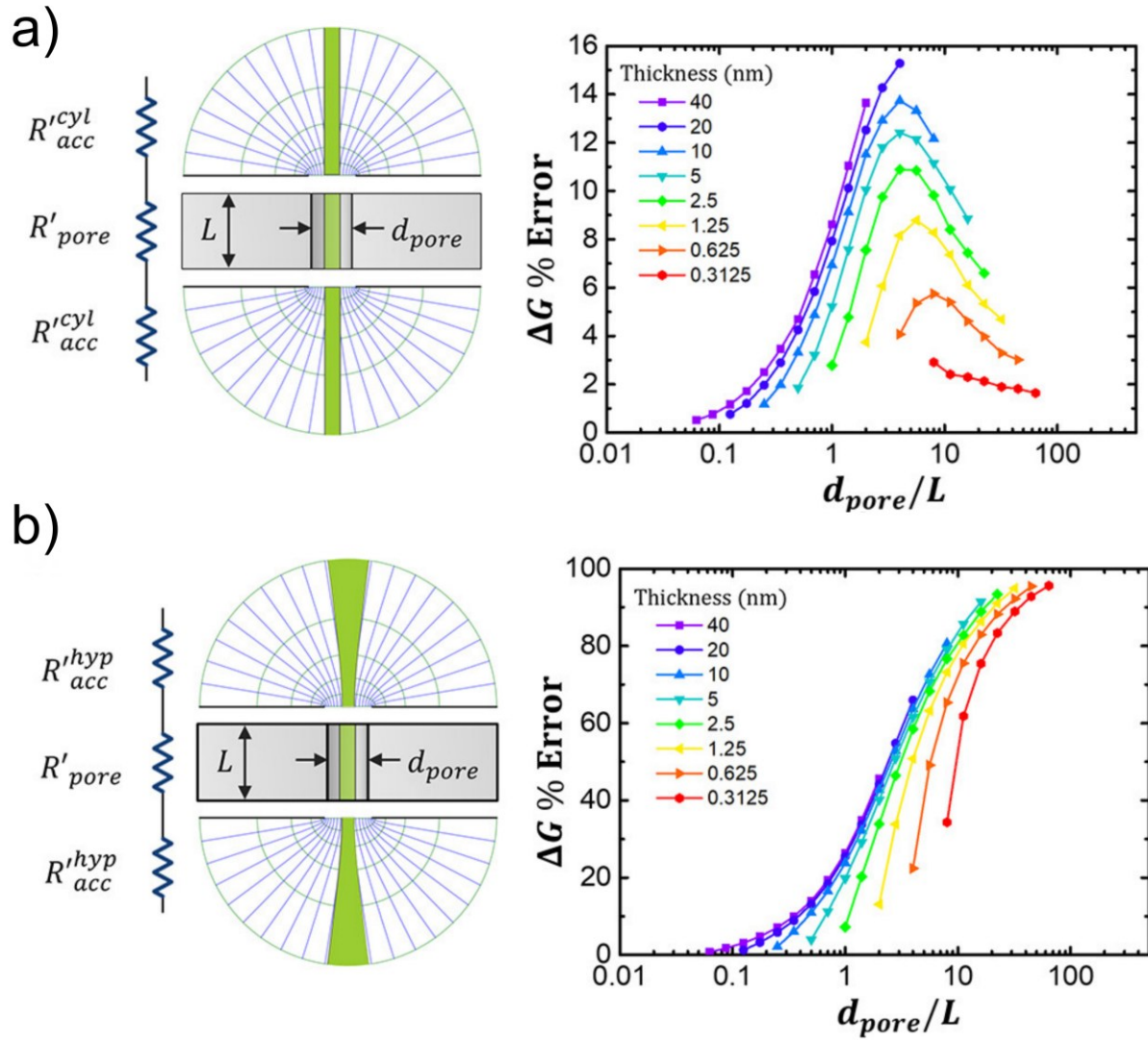


Figure 1.2 Nanopore conductance modeling for DNA translocation. Conductance modeling and error percentage calculated between simulation and model prediction including a) access resistance, b) no access resistance considerations. Image adapted from Charron *et al.*¹⁷, with permission.

Recently, Charron *et al.* proposed an improved model that takes into account the access resistance of the pore in determining nanopore conductance when DNA translocations are performed (Figure 1.2). The change in nanopore conductance due to dsDNA translocations becomes:¹⁷

$$\Delta G = \left(\frac{4L}{\sigma_s \pi (d_{pore}^2 - d_{DNA}^2)} + \frac{(1 + \frac{1}{4} (\frac{d_{DNA}}{d_{pore}})^2 + \frac{9}{64} (\frac{d_{DNA}}{d_{pore}})^4)}{\sigma_s \pi d_{pore}} \right)^{-1} \quad (1.6)$$

This improved model yields lower percentage differences in conductance variation compared to the Kowalczyk *et al.* model,⁹ specifically for long narrow 3D channels ($d_{pore} \ll L_{pore}$) and 2D membranes ($d_{pore} \gg L_{pore}$). The access resistance contribution becomes particularly important for high aspect-ratio pores and when the translocating molecule partially occludes the pore entrance region, modifying the convergence of electric field lines.

1.1.5 Forces Governing Translocation

To understand the mechanisms behind these biomolecular translocations, the forces acting on the analytes are presented in this section.

Brownian motion

Starting from simple Brownian motion, a particle suspended in liquid undergoes random fluctuations under thermal forces.¹⁸ The mean square displacement in N dimensions is described by the Einstein relation:

$$\overline{x^2} = 2NDt \quad (1.7)$$

where D is the diffusion coefficient, x the displacement and t the time. The diffusion coefficient itself is given by the Stokes-Einstein equation:¹⁹

$$D = \frac{k_B T}{6\pi\eta r} \quad (1.8)$$

where η is solution viscosity and r the particle hydrodynamic radius.

This Brownian motion is a rapid phenomenon on the nanometer scale, with D generally on the scale of $10^{-10} - 10^{-11} \text{ m}^2/\text{s}$ for biomolecules.²⁰ It is present even under an electrical field and leads to spatial and temporal fluctuations in the measured current. For example, DNA passing through the pore can experience thermal fluctuations, observed as forward and backward motion within the nanopore signal. This behavior is particularly evident when the driving force is weak or the molecule experiences temporary sticking. Brownian motion is also responsible for molecular diffusion, the primary driver for bringing molecules from the bulk solution close enough to the pore to be captured by the electric field. This process becomes rate-limiting in dilute samples.²¹

Electrophoresis

DNA and proteins, by their charged nature in aqueous solution, tend to migrate based on their size and charge under the influence of an applied electrical field. Electrophoresis is the phenomenon where charged particles move in a medium toward the electrode with the opposite charge. The fundamental equation describing this movement is the electrophoretic force:²²

$$F_{EP} = qE \quad (1.9)$$

where q is the net charge of the particle, and E is the electric field.

For nanopore systems, the electric field is highly non-uniform, concentrated within and immediately adjacent to the pore. This creates a capture region within which electrophoretic forces dominate over diffusion. For DNA, which is a long polyelectrolyte, the effective linear charge density is only about 20-30% of the bare phosphate charge due to counterion condensation described by Manning's theory.²³ This is a phenomenon where a fraction of counterions remain closely associated with the DNA backbone, effectively neutralizing a portion of its charge.

Electroosmosis

The movement of biomolecules through the nanopore is also influenced by electroosmotic flow, meaning the motion of fluid in a channel with a charged surface under an applied electrical field. When a charged surface such as silicon nitride, which carries negative silanol groups at physiological pH, contacts an electrolyte, it attracts a layer of counterions that can be mobilized by an electric field. These mobilized counterions drag the surrounding fluid through viscous coupling. Based on the Navier-Stokes equation for incompressible flow, simplifications yield a relationship between the electrostatic potential and fluid velocity:²⁴

$$\varepsilon E \frac{d^2 \phi}{dy^2} = \eta \frac{d^2 v}{dy^2} \quad (1.10)$$

where ε is the permittivity of the medium, ϕ is the electrostatic potential, η is the viscosity, and v is the fluid velocity.

In the case of nanopores, where the electrolyte solution is electrically neutral and the electrostatic potential away from the walls is negligible beyond the Debye length, the fluid velocity at the center of the channel is extracted by integrating Equation 1.10 twice and applying boundary conditions:²⁵

$$v_{EOF} = - \frac{\varepsilon \cdot E \cdot \phi_0}{\eta} \quad (1.11)$$

where ϕ_0 is the electrostatic potential at the channel wall, known as the zeta potential. The negative sign indicates that for negatively charged walls, meaning negative ϕ_0 , electroosmotic flow is directed opposite to the electric field direction, toward the negative electrode.

1.2 Nanopore Device Fabrication

Now that we have established the forces governing capture and translocation of biomolecules, we shift our attention to fabrication of nanopore sensors. The creation of solid-state nanopore devices relies on established microfabrication techniques adapted from the semiconductor industry. Understanding these processes is essential for interpreting the material choices and device architectures explored in subsequent chapters. This section provides a broad exploration of nanopore device fabrication, covering membrane materials and deposition methods, membrane patterning and release, nanopore formation techniques, and characterization approaches.

1.2.1 Membrane Materials and Deposition Methods

Nanopores exist in nature, most commonly as transmembrane protein channels embedded in cellular membranes. These proteins facilitate the selective passage of ions and molecules essential for cellular function. Biological nanopores offer atomically precise structures, identical from pore to pore, and have been successfully employed for DNA sequencing and single-molecule analysis.^{26,27} However, their lipid bilayer supports are mechanically fragile and sensitive to environmental conditions, limiting their stability and integration with complex microfluidic systems.²⁸

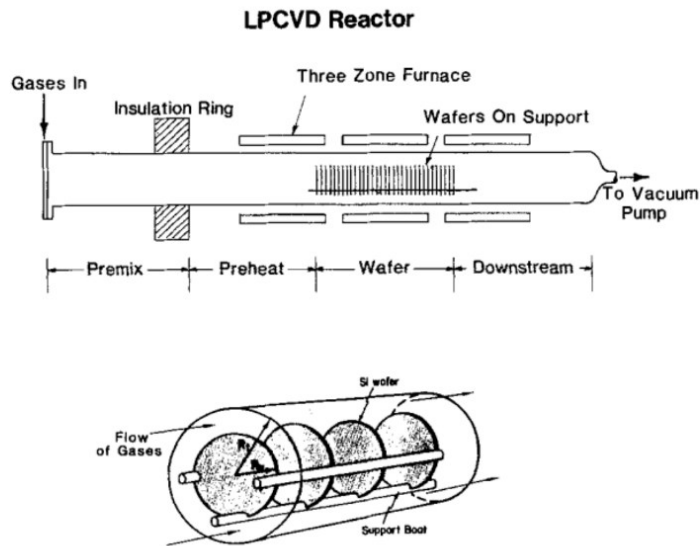
Synthetically, silicon-based technologies have sought to create analogues of these naturally occurring channels with enhanced durability and tunability. Silicon nitride (SiN) and hafnium oxide (HfO₂) have emerged as ideal dielectric materials for nanopore sensing due to their mechanical strength, chemical inertness, low electrical noise, and compatibility with standard microfabrication processes.^{11,29} These thin films are deposited directly on bare or oxide-insulated silicon substrates, with thicknesses from 10–50 nm for SiN and 2–10 nm for high- κ dielectrics like HfO₂.

Three primary deposition techniques are relevant to nanopore fabrication:

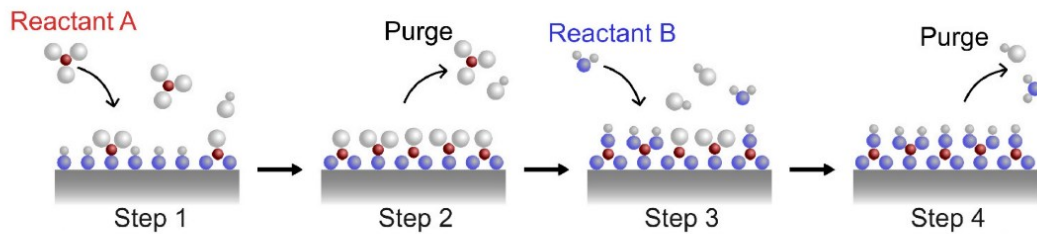
Low Pressure Chemical Vapor Deposition (LPCVD) remains the gold standard for silicon nitride membranes (Figure 1.3(a)). This technique involves the chemical reaction of gaseous precursors at low pressures, typically in the 0.1–0.3 Torr range, and elevated temperatures above 800 °C to form a uniform film on a substrate.³⁰ For silicon nitride deposition, typical precursors are dichlorosilane (SiH_2Cl_2) and ammonia (NH_3), which react to form stoichiometric SiN with excellent dielectric properties and etch resistance. LPCVD SiN films exhibit low stress, high density, and pinhole-free morphology, which are ideal properties for nanopore membranes.

Atomic Layer Deposition (ALD) has emerged as a critical technique, with a highly precise film deposition that allows for the creation of ultra-thin films with excellent uniformity and control over thickness at the atomic level (Figure 1.3(b)). The process involves sequential, self-limiting exposure of the substrate to different gaseous precursors, which react on the surface in a saturating manner.³¹ This ensures that only a single atomic layer is deposited per cycle. Between precursor pulses, inert gas purges remove excess reactants and byproducts. This cyclic process enables digital thickness control simply by counting cycles. For nanopore applications, ALD is particularly useful for depositing high- κ dielectrics that offer enhanced chemical stability, tunable surface charge, and reduced low-frequency noise compared to conventional SiN.³²

a)



b)



c)

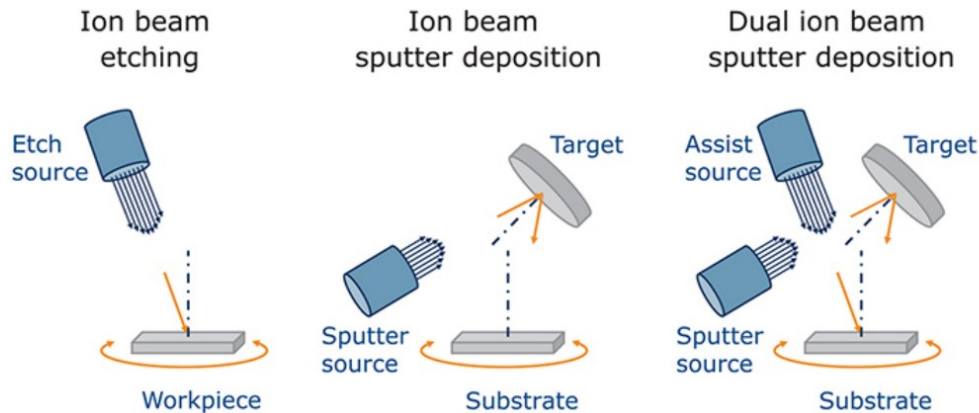


Figure 1.3 Thin film deposition techniques. a) Schematic of an LPCVD reactor, presenting the compartments required to premix, preheat, deposit and release precursor gases. Image adapted from Roenigk *et al.*³³, with permission. b) Schematic of ALD, where reactants A and B are sequentially introduced and purged to control the film thickness, one layer of atoms at a time. Based on a figure from Piechulla *et al.*³¹, licensed under CC BY-NC-ND 4.0. c) Schematic of IBD, where a material sputtered from a target is deposited on the substrate. Image adapted from Bundesmann *et al.*³⁴, with permission.

Ion Beam Deposition (IBD) is a physical vapor deposition technique that uses an ion beam, typically argon ions, to sputter material from a target onto the substrate (Figure 1.3(c)). Operating at room temperature and pressures below 0.1 Torr, IBD offers excellent control over film composition, density, and thickness through precise tuning of ion energy and incidence angle.³⁵ Unlike chemical vapor methods, IBD is a Physical Vapor Deposition (PVD) approach capable of sputtering virtually any material, including metals, dielectrics, and compounds, without requiring volatile precursors.

The choice of deposition method and material directly impacts nanopore performance. For instance, residual stress from thermal expansion mismatch between film and substrate can cause membrane buckling or cracking.^{36,37} In principle, if the film contracts more than the substrate during cooling, it develops tensile stress. On the contrary, if it contracts less, compressive stress results. Stress management through material selection, deposition parameter optimization, and annealing strategies is critical for reliable nanopore fabrication.

1.2.2 Membrane Definition and Release

Once deposited, the membrane must be defined and released from the substrate through etching processes. These processes create free-standing thin films suspended over a cavity, providing the necessary geometry for nanopore formation and fluidic access.

Wet etching involves the use of liquid chemical solutions to remove unwanted material from a substrate through chemical dissolution reactions. Wet etching can be classified into two main types. Isotropic etching results in uniform material removal in all directions, producing curved profiles. Anisotropic etching results in direction-dependent removal, producing crystallographically defined geometries.³⁸ The choice between isotropic and anisotropic etchants depends on the desired feature geometry and the crystallographic nature of the material being etched.

Potassium hydroxide (KOH) etching is an anisotropic process targeting single-crystal silicon, exploiting the different etch rates of crystallographic planes (Figure 1.4(a)). KOH etching is typically performed at 80–85 °C using 30 wt.% KOH solution, with a potential isopropyl alcohol additive to improve surface quality.³⁹ This achieves etch rates of approximately 1 µm/min at 80 °C for the <100> plane. The process preferentially etches silicon in the <100> plane approximately 400 times faster than the <111> plane, producing V-shaped grooves with sidewalls at a precise 54.7° angle to the surface.⁴⁰ This anisotropy enables the creation of well-defined membrane windows with sloping sidewalls that provide mechanical support. Silicon nitride serves as an effective etch stop with very low etch rates below 1 nm/min, making it ideal for masking KOH etching.⁴¹

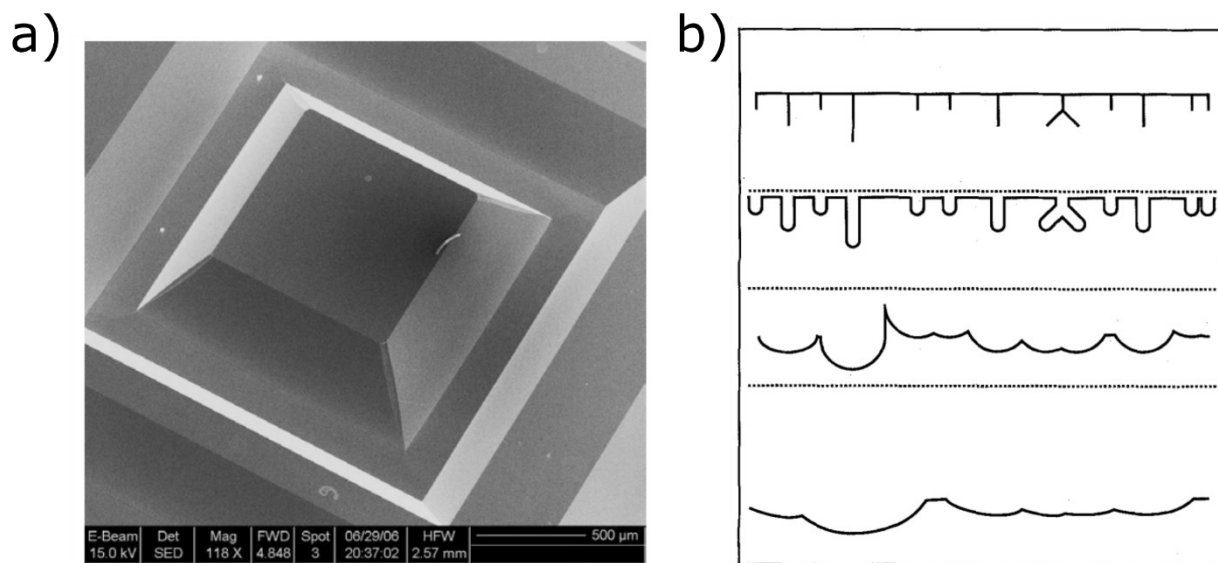


Figure 1.4 Wet etching directionality. a) SEM image of a mesa structure defined after KOH etching of a Si substrate with a SiN etch mask, demonstrating the V-groove pattern and anisotropic etching process. Image adapted from Fan *et al.*⁴⁰, with permission. b) Schematic of a glass substrate etched by HF over time (top to bottom), where the cracks on the surface are etched isotropically to form a cup-like surface. Image adapted from Spierings⁴², with permission.

Hydrofluoric acid (HF) etching is an isotropic process primarily targeting silicon dioxide (SiO₂) through the reaction $\text{SiO}_2 + 6\text{HF} \rightarrow \text{H}_2\text{SiF}_6 + 2\text{H}_2\text{O}$ (Figure 1.4(b)). HF etching is typically performed at room

temperature, with HF solutions varying in concentration from dilute 5% for controlled, slow etching to concentrated 49% for rapid removal.⁴¹ The etch rate also depends on oxide density, with thermal wet oxide etching slower than deposited dry oxide. For silicon nitride, Xia *et al.* showed that chemical etching in HF leads to pinhole formation on the membrane surface after extended exposure.⁴³ This effect facilitates pore formation through these uncontrolled defects. While this can complicate reproducible fabrication, with careful control it can be exploited for pore formation. Annealed ALD HfO₂ has demonstrated excellent stability during HF wet etching, with negligible etch rates and no pinhole formation.⁴⁴ This makes it suitable as a protective layer or membrane material in HF-based processes.

Dry etching uses ionized gases to remove material through a combination of chemical reactions and physical sputtering, offering greater precision and anisotropy than wet etching. Specifically, Reactive Ion Etching (RIE) involves placing a substrate inside a vacuum chamber and introducing reactive gases such as SF₆ or CF₄. The generated plasma dissociates the feed gases into reactive radicals, ions, and electrons. Ions are accelerated toward the substrate by the bias potential, etching the wafer primarily in the vertical direction. Simultaneously, the reactive radicals chemically etch material isotropically. The synergy between ion bombardment and radical chemistry enables highly anisotropic profiles with minimal undercut.^{45,46} This process is performed at room temperature with careful control of gas chemistry, pressure, and power to balance etch rate, selectivity, and profile control.⁴⁷ Specific to nanopore formation, RIE is employed to create defined regions for the subsequent KOH wet etching and formation of free-standing dielectric membranes with high precision.⁴⁸

1.2.3 Film Characterization

Accurate characterization of film properties ensures reproducible device performance and enables validation of microfabrication steps involving material removal for example. Two commonly used techniques provide complementary information about film thickness and topography.

Ellipsometry is a non-destructive optical technique measuring thickness and optical properties of thin films by analyzing changes in the polarization state of light upon reflection from the film surface. The polarization change is characterized by two parameters: the amplitude ratio (ψ) and phase difference (Δ) between incident and reflected components.⁴⁹ By fitting these measured parameters to an optical model of the film stack, thickness can be determined. Ellipsometry is particularly valuable for monitoring deposition processes and verifying film thickness uniformity across wafers.

Atomic Force Microscopy (AFM) is a high-resolution imaging technique using a tip on a flexible cantilever to scan the film surface and measure topography through tip-sample interactions. As the tip scans the sample's surface, a feedback loop tracks a laser's deflection on the cantilever's tip as measured by a photodiode, and translates the recorded signal into a topographical map.⁵⁰ This method provides true three-dimensional surface profiles with lateral resolution down to 1 nm and vertical resolution below 0.1 nm, enabling visualization of etch profiles, and surface roughness following etching steps.

1.2.4 Nanopore Formation Techniques

Since the 1990s, various techniques have been developed to form nanoscale pores in suspended membranes, each offering distinct advantages in terms of control, cost, and accessibility.⁵¹

Initially, nanopores were formed by Transmission Electron Microscopy (TEM) or Focused Ion Beam (FIB) milling of suspended thin films under vacuum (Figure 1.5(a)).⁵² This process requires specialized cleanroom equipment such as transmission electron microscopes or focused ion beam systems to gradually remove material until a nanometric channel is formed.⁵³ While this method offers excellent control over pore geometry and position, it is time-consuming, expensive, and requires access to sophisticated instrumentation.

Subsequently, glass capillary technology was adapted to produce nanopipettes.^{54,55} These quartz-based nanopores are fabricated by laser-assisted pulling of heated glass capillaries until the desired tip diameter is achieved. Nanopipettes offer advantages including low cost, ease of fabrication, and conical geometries that concentrate electric fields. However, their tip diameters are typically larger than membrane-based pores and their geometry is less precisely controlled.

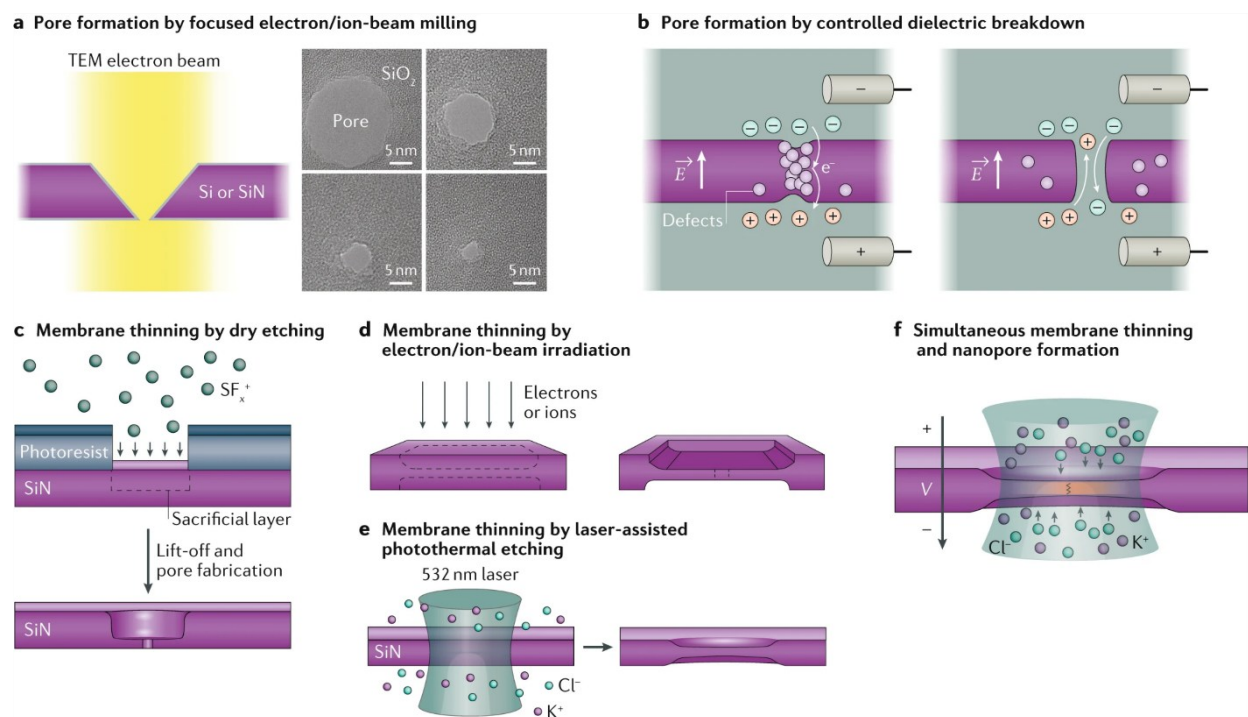


Figure 1.5 Nanopore formation techniques. a) TEM/FIB drilling of Si or SiN free-standing membranes. b) Controlled breakdown fabrication of solid-state nanopores. Pore formation post membrane thinning by either c) dry etching, d) electron/ion-beam etching, or e) laser-assisted etching. f) Simultaneous membrane thinning by photothermal etching and pore formation by controlled breakdown. Image adapted from Xue *et al.*³, with permission.

More recently, the principle of dielectric breakdown, commonly observed as damage in silicon chips subjected to excessive voltages, was adapted for nanopore fabrication.^{56,57} Figure 1.6 depicts the controlled breakdown (CBD) method. A sustained voltage is applied across a submerged membrane while leakage current is monitored. The applied electric field, typically above 0.5 V/nm, generates defects

through stress-induced bond breaking, eventually creating a conductive path. When a sharp increase in current is detected, indicating formation of a complete fluidic channel, the voltage is immediately halted to prevent uncontrolled pore growth (Figure 1.6(a)). The resulting nanopore, with a diameter measured by an I-V sweep, is often smaller than the analytes of interest. By applying brief 1-5 second voltage pulses below the original breakdown value, subsequent I-V measurements show increased pore diameters through field-induced etching of the pore walls (Figure 1.6(b)). A feedback algorithm can be employed to achieve the desired pore size with sub-nanometer precision (Figure 1.6(c)). This technique enables in-situ nanopore formation with minimal expertise and cost compared to earlier methods, democratizing access to nanopore technology. Recent variations on CBD fabrication include chemical tuning to modify electrolyte composition for controlling breakdown kinetics,⁵⁸ laser-assisted localization using focused light to heat and weaken specific membrane regions,⁵⁹ and membrane thinning to reduce fabrication time or improve spatial resolution.⁶⁰

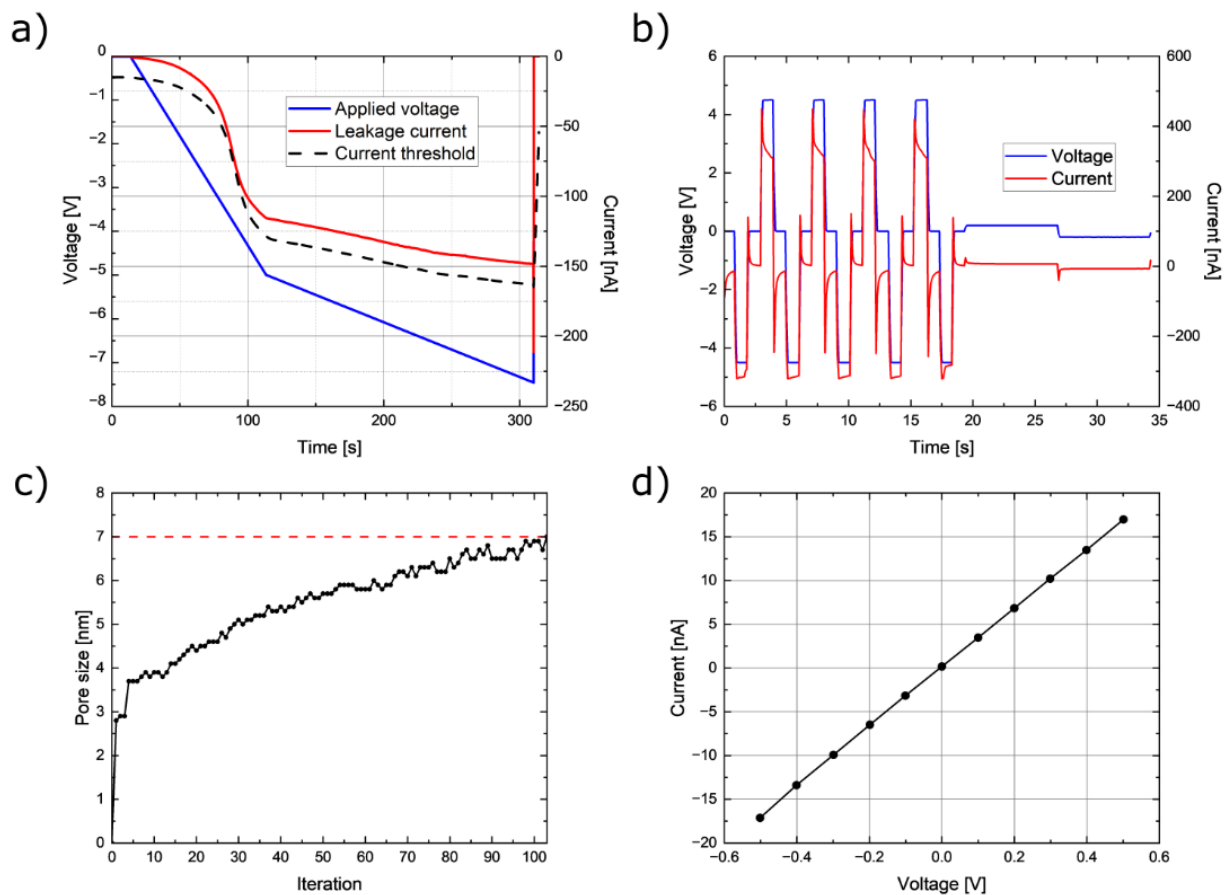


Figure 1.6 Controlled Breakdown protocol. a) Pore formation following a breakdown event as depicted by a sharp increase in current magnitude greater than the set current threshold. b) Pore growth post-fabrication by 1 s square 4.5 V voltage pulses. c) Feedback algorithm progress to track growth until target size is reached. d) Post-conditioning I-V curve to estimate pore size from conductance measurements.

Several research efforts have demonstrated specific applications of these techniques. For example, ALD deposition of Al_2O_3 on FIB-drilled pores in SiN membranes enabled modified surface charge density and reduced $1/f$ noise while maintaining angstrom-scale control over pore diameter through sequential deposition cycles.³² Similarly, ALD deposition of zinc oxide (ZnO) on quartz substrates yielded significantly increased translocation durations for negatively charged dsDNA compared to SiN pores, attributed to the positively charged ZnO surface at neutral pH which enhances electrostatic interactions with DNA.⁶¹ This

demonstrates how material choice can be used to tune translocation dynamics without modifying pore diameter.

ALD deposition of HfO_2 on etched SiN windows enabled sub-2 nm pore formation via TEM-drilling with 160-minute durability during continuous voltage application.¹¹ Translocations of dsDNA through HfO_2 pores were comparable to SiN pores, while ssDNA exhibited stronger pore-wall interactions evidenced by longer dwell times. This suggests that HfO_2 may offer advantages for applications requiring enhanced interactions with single-stranded nucleic acids. Furthermore, a similar approach was adopted to extend the lifetime of nanopore sensors.⁶²

Figure 1.7 illustrates recent approaches to form solid-state nanopores. Metal-assisted chemical etching of Si nanopores, using 10-40 nm Au particles as etch catalysts, forms ~3 nm pores in 12 nm thick Si films (Figure 1.7(a)).⁶³ This approach was validated by 2 kbp dsDNA translocations showing predominantly unfolded events. This indicates pore diameters only slightly larger than the 2.2 nm DNA cross-section and offers a fabrication route without requiring beam-based equipment. More recently, ~20 nm SiN nanopores were formed on a wafer-scale level by extreme ultraviolet (EUV) technology, paving the way for highly multiplexed nanopore sensing pending fluidic technology development and size tuning capabilities (Figure 1.7(b)).⁶⁴

Efforts to reduce membrane thickness have included local thinning by helium ion microscopy (HIM) or HF etching to achieve sub-10 nm membranes.^{60,65} This reduction in membrane thickness is critical for improving spatial resolution in applications such as DNA sequencing, where single-base sensitivity requires ultrathin membranes. Thus, achieving thicknesses comparable to biological lipid bilayers while maintaining the robustness of solid-state materials.

1.3 Noise in Nanopore Measurements

Enabling these nanoscale experiments are patch-clamp amplifiers. Standard instrumentation such as the Axopatch 200B achieves this through a resistive feedback headstage that converts current to voltage with high gain and low noise.⁶⁶ Advanced amplifiers employ capacitive feedback or integrated architectures to achieve even higher bandwidths up to 10 MHz by reducing the effective input capacitance and enabling faster sampling rates, though often at the cost of increased complexity or reduced dynamic range.⁶⁷

Inherent to ionic current recordings are unwanted signal fluctuations that contribute to noise and limit the minimum detectable signal. A detailed analysis of noise sources across frequency ranges enables systematic identification and mitigation of dominant noise contributions.

The total current noise power spectral density $S_I(f)$ can be expressed as a sum of frequency-dependent terms.⁶⁸

$$S_I(f) = \frac{A}{f} + B + Cf + Df^2 \quad (1.12)$$

where A/f represents flicker noise, B represents white noise sources, and Cf and Df^2 represent dielectric and capacitive noise respectively.

White noise sources present at all frequencies include thermal noise, also known as Johnson noise, from the thermal fluctuation of charge carriers in the nanopore resistance, given by $4k_B T/R_{pore}$. They also include shot noise from the discrete, random nature of ion transport. Additionally, protonation noise arises from the dissociation of surface groups, which depends on salt concentration and pH.⁶⁹

Low-frequency noise, also known as $1/f$ noise or flicker noise, is observed as fluctuations in the recorded baseline current that scale inversely with frequency. This noise contribution has been attributed to various physical phenomena.^{70,71} However, no single mechanism has been universally accepted as the dominant source. Empirically, $1/f$ noise magnitude correlates with pore size and surface properties.^{72,73}

High-frequency noise sources include dielectric noise, which is dominated by the chip capacitance and the dielectric loss of the membrane material. Dielectric noise scales linearly with frequency and becomes significant above a few kilohertz. Capacitive noise arises from the total capacitance at the input of the amplifier, including the device capacitance from the chip, membrane, and the amplifier's own input capacitance. This noise scales quadratically with frequency and ultimately limits the achievable bandwidth.

1.3.1 Noise Reduction Strategies

Strategies to reduce nanopore high-frequency or root mean square (RMS) noise target the last two contributions through device design and material selection (Figure 1.8). High-frequency noise has been reduced by isolating the membrane with a low-capacitance silicon oxide layer for silicon-based substrates.⁷⁴ This effectively separates the highly conductive silicon from the membrane. Another approach reduces the exposed area to liquid by covering the chip surface with a thick, low-loss dielectric layer such as PDMS or laser-machined glass, minimizing the capacitive coupling between electrolyte and substrate (Figure 1.8(a)).^{73,75} Transferring SiN thin films to quartz substrates through wafer bonding and selective silicon removal has also been shown to reduce device capacitance by eliminating the conductive silicon layer entirely (Figure 1.8(b)). This approach enables discrimination of ssDNA homopolymers, such as poly-A versus poly-T, based on their subtle differences in blockage amplitude.⁷⁶ Adopting insulating substrates such as fused silica, instead of traditional silicon, yields a four-fold reduction in RMS noise at 50 kHz due to the low capacitance of fused silica (Figure 1.8(c)).⁷⁷ Designing custom CMOS amplifiers with on-chip capacitance cancellation enables resolution of ssDNA features in the 100 ns range with an SNR of 10 by actively compensating for input capacitance (Figure 1.8(d)).⁶⁷

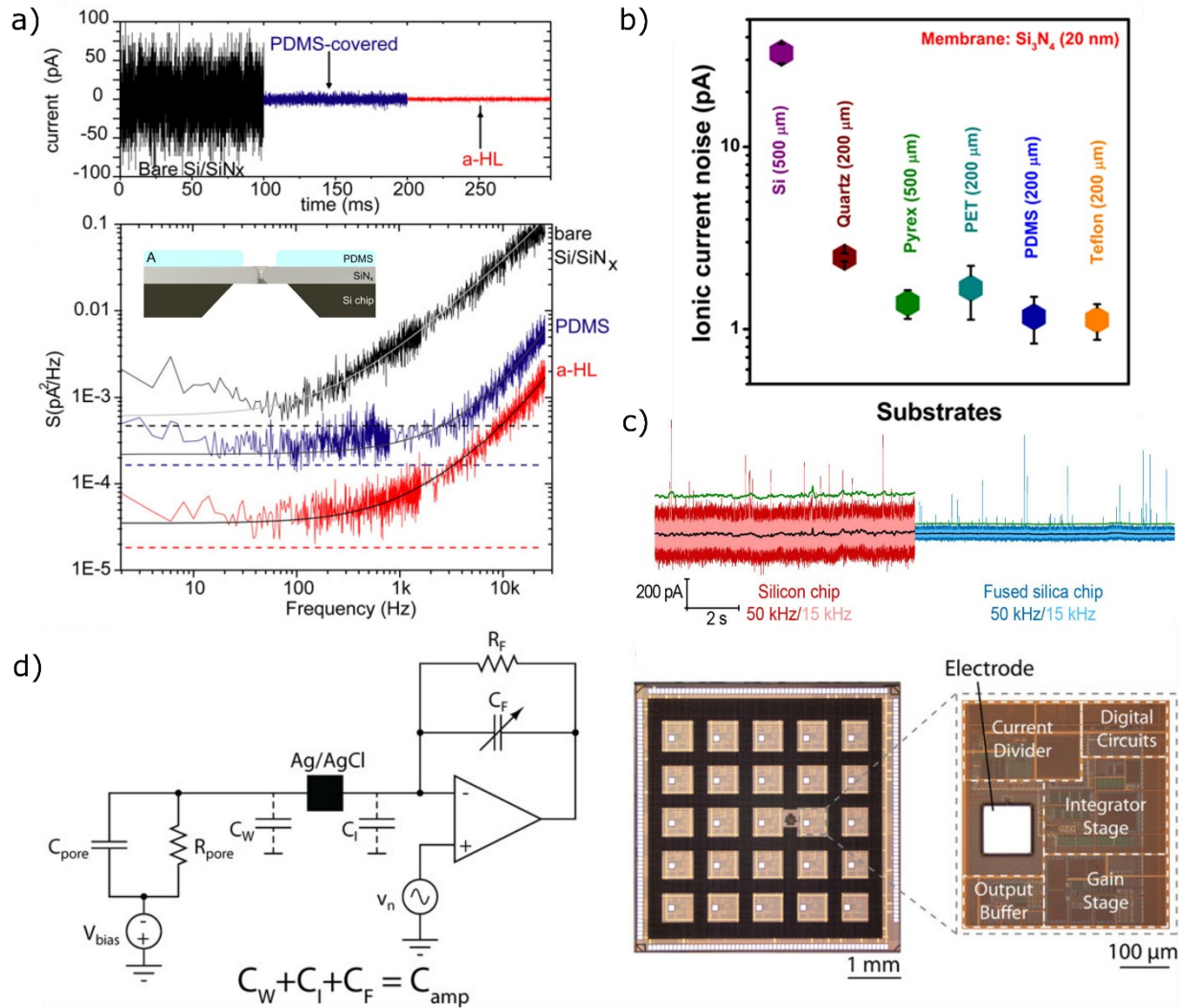


Figure 1.8 Potential strategies to reduce high-frequency noise contributions. a) PDMS-coated SiN chips lead to an order of magnitude reduction in RMS noise. Image adapted from Tabard-Cossa *et al.*⁷³, with permission. b) By selecting an insulating substrate compared to the standard Si, the ionic current noise is drastically lowered. Image adapted from Lee *et al.*⁷⁶, adapted under license CC BY 4.0. c) A one-to-one comparison of RMS noise recorded at 15 and 50 kHz between silicon and fused silica substrates. Image adapted from De Vreede *et al.*⁷⁷, with permission. d) Optimizing the amplifier circuit by reducing the input capacitance of the amplifier leads to a reduction in RMS noise and increase in recording bandwidth. Image adapted from Shekar *et al.*⁶⁷, with permission.

1.4 Signal Enhancement Strategies

While noise reduction improves sensor performance by lowering the detection threshold, maximizing signal amplitude is equally important for achieving high signal-to-noise ratios. The signal in nanopore sensing arises from transient current blockages by translocating analytes. The larger and longer the blockage, the easier it is to detect and characterize. Several complementary approaches have been developed to enhance either event amplitude (Figure 1.9) or duration (Figure 1.10).

1.4.1 Enhancing Event Amplitude

Event amplitude, meaning the depth of current reduction during translocation, scales with the volume of electrolyte displaced by the analyte and the salt conductivity of the solution. Increasing LiCl concentration from 1 M to 4 M improves signal amplitude by a factor of 2 (Figure 1.9(a)).¹⁵ Conversely, increasing nanopore diameter from 5 nm to 65 nm decreases current blockage amplitude by a factor of 2 for the same analyte (Figure 1.9(b)).⁹ This occurs because the fractional volume occupied by the molecule decreases relative to the pore volume. This creates a fundamental trade-off: larger pores accommodate larger analytes but produce smaller signals, while smaller pores yield larger signals but exclude larger analytes. Similarly, decreasing nanopore thickness from 20 nm to 5 nm during layer deposition increases current blockage amplitude by a factor 4 for single-stranded DNA (ssDNA).⁷⁶ Other efforts include two-step etching processes to reduce membrane thickness and improve signal amplitude as confirmed by DNA translocation experiments.^{65,78}

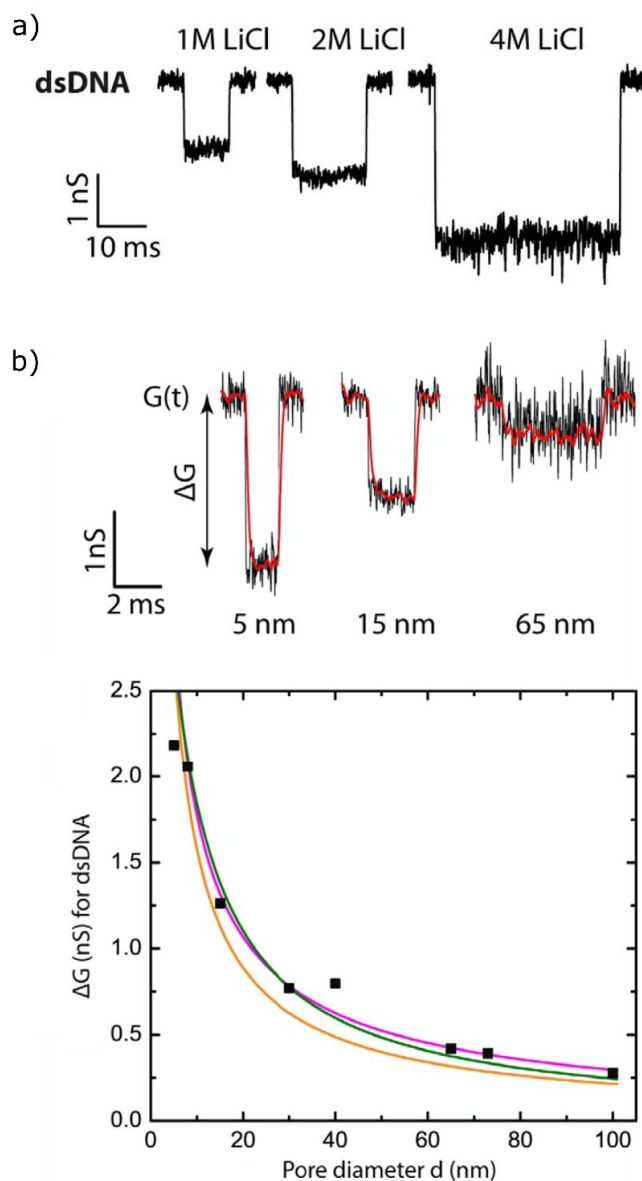


Figure 1.9 Signal enhancement strategies. a) Increasing the ionic strength from 1 M to 4 M LiCl leads to a deeper current blockage for lambda DNA for the same nanopore. Image adapted from Kowalczyk *et al.*¹⁵, with permission. b) Decreasing the nanopore diameter leads to a smaller sensing volume and an increase in current blockage for lambda DNA. Image adapted from Kowalczyk *et al.*⁹, with permission.

1.4.2 Enhancing Event Duration

Event duration, meaning the time required for complete translocation, determines the temporal resolution required for detection and the information that can be extracted from event substructure. Longer events enable higher-resolution sampling of molecular features but reduce throughput. Event duration can be increased through multiple mechanisms (Figure 1.10).

Ion size significantly affects translocation kinetics (Figure 1.10(a)). Smaller ions such as Li^+ exhibit stronger binding to the DNA backbone, enhancing charge screening and reducing effective linear charge density. This yields translocation times up to 10 times longer than with K^+ , although with a reduced capture rate.¹⁵

Temperature modulation similarly affects translocation through its influence on viscosity and diffusion coefficients (Figure 1.10(b)). Lower temperatures increase solution viscosity and reduce thermal energy, decreasing translocation velocity for 48.5 kbp dsDNA. However, low temperatures also reduce signal amplitude as reported through conductance blockage variations.⁷⁹

Solution viscosity affects translocation velocity through the Stokes drag on the moving molecule (Figure 1.10(c)). Increasing viscosity through water-organic mixtures, such as adding glycerol, shows a linear dependence of event duration on viscosity. However, this comes at the cost of reduced current blockages due to lower ionic mobility, which can compromise signal detection if viscosity becomes too high.^{80,81}

Gel interfaces dramatically alter translocation dynamics by introducing steric interactions beyond the pore itself (Figure 1.10(d)). Interfacing the nanopore with agarose or polyacrylamide gels slows translocation from 35 μs to 2900 μs for 5 kbp dsDNA. This occurs because the DNA must navigate through the gel matrix while partially extruded from the pore. The effect scales with gel concentration and crosslinking. Polyacrylamide's smaller pore size yields an order-of-magnitude increase in dwell time even for short DNA such as 100 bp fragments.⁸²

Pore diameter strongly influences polymer-pore interactions. Reducing pore diameter from 6 nm to 2.9 nm increases steric and electrostatic interactions between DNA and the pore walls (Figure 1.10(e)). This yields a dramatic increase in event duration for 500 bp DNA as the molecule experiences increased friction during passage. For pores only slightly larger than the DNA diameter, translocation enters a regime where wall interactions dominate.^{12,83}

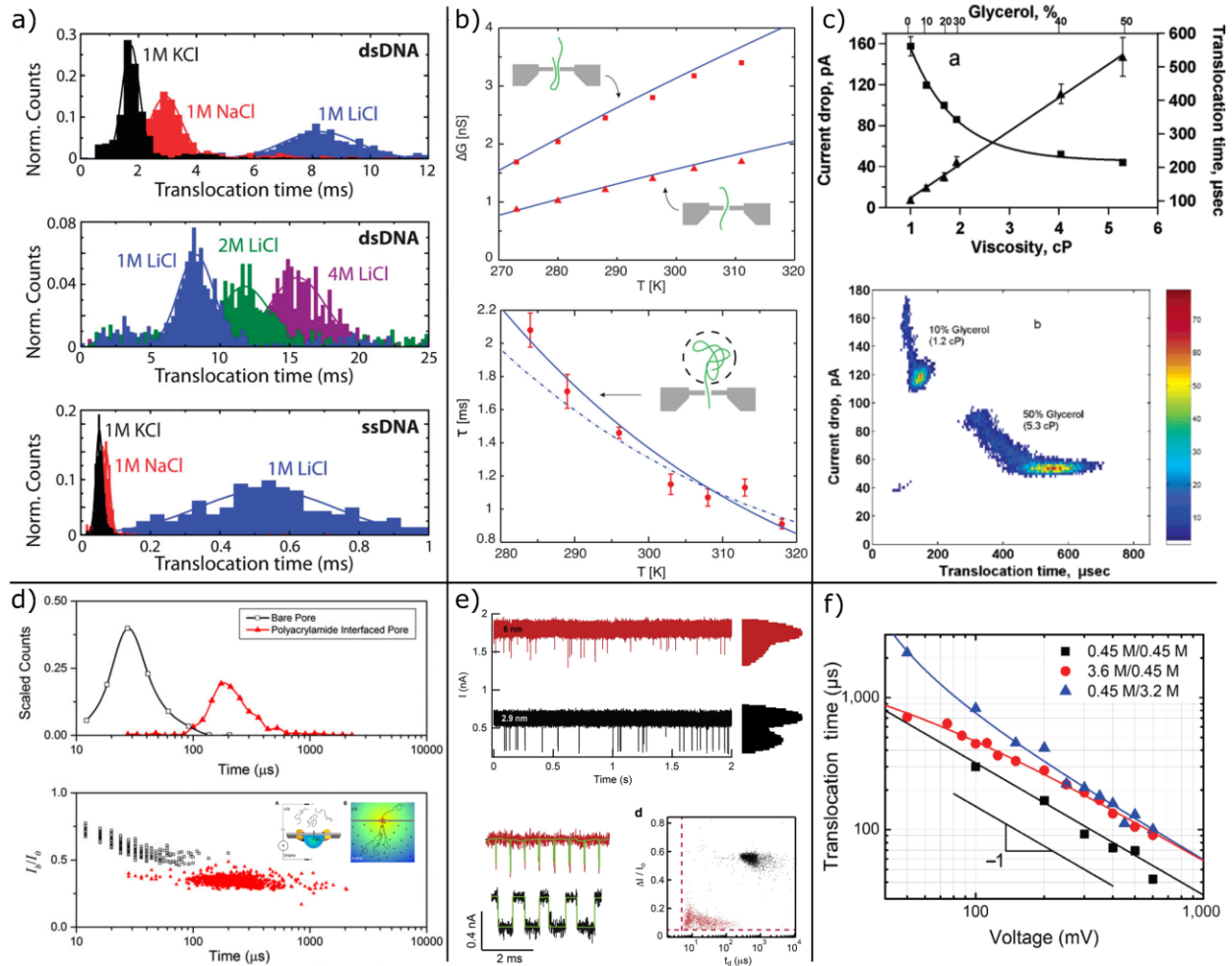


Figure 1.10 Translocation time modulation approaches, including a) Ion species and salt concentration. Image adapted from Kowalczyk *et al.*¹⁵, with permission. b) Temperature. Image adapted from Verschueren *et al.*⁷⁹, with permission. c) Viscosity. Image adapted from Fologea *et al.*⁸⁰, with permission. d) Gel interfaces. Image adapted from Waugh *et al.*⁸², with permission. e) Pore diameter. Image adapted from Carson *et al.*¹², with permission. f) Salt gradients. Image adapted from Charron *et al.*⁸⁴, with permission.

Salt gradients across the nanopore, established by maintaining different electrolyte concentrations on each side, modify the electric field distribution (Figure 1.10(f)). With high concentration on the trans side and low concentration on the cis side, the electric field extends further into the cis reservoir, increasing capture rate.⁷ Meanwhile, translocation time is reduced by a factor of ~1.5 compared to symmetric conditions due to the asymmetric ion distribution.⁸⁴

1.4.3 Trapping Approach

Beyond passive modulation of translocation parameters, active trapping mechanisms can confine analytes within the sensing region for extended periods, enabling detailed characterization of molecular structure and dynamics. The nanopore electroosmotic trap, or NEOTrap, achieves this by docking a DNA origami sphere on a lipid bilayer-coated nanopore, creating a nanocavity that captures proteins with hour-long residence times.⁸⁵ The lipid bilayer provides a fluid, low-friction surface that minimizes nonspecific interactions, while the DNA sphere acts as a size-selective filter. This enables label-free discrimination of proteins by size and shape based on their relative blockage.

1.5 Pressure Controlled Platform

Pressure-controlled platforms offer an additional degree of freedom for controlling analyte transport, independent of electrophoretic forces (Figure 1.11). By applying a pressure gradient across the membrane, a pressure-driven flow can be established that either assists or opposes electrophoretic translocation, enabling precise tuning of net velocity.

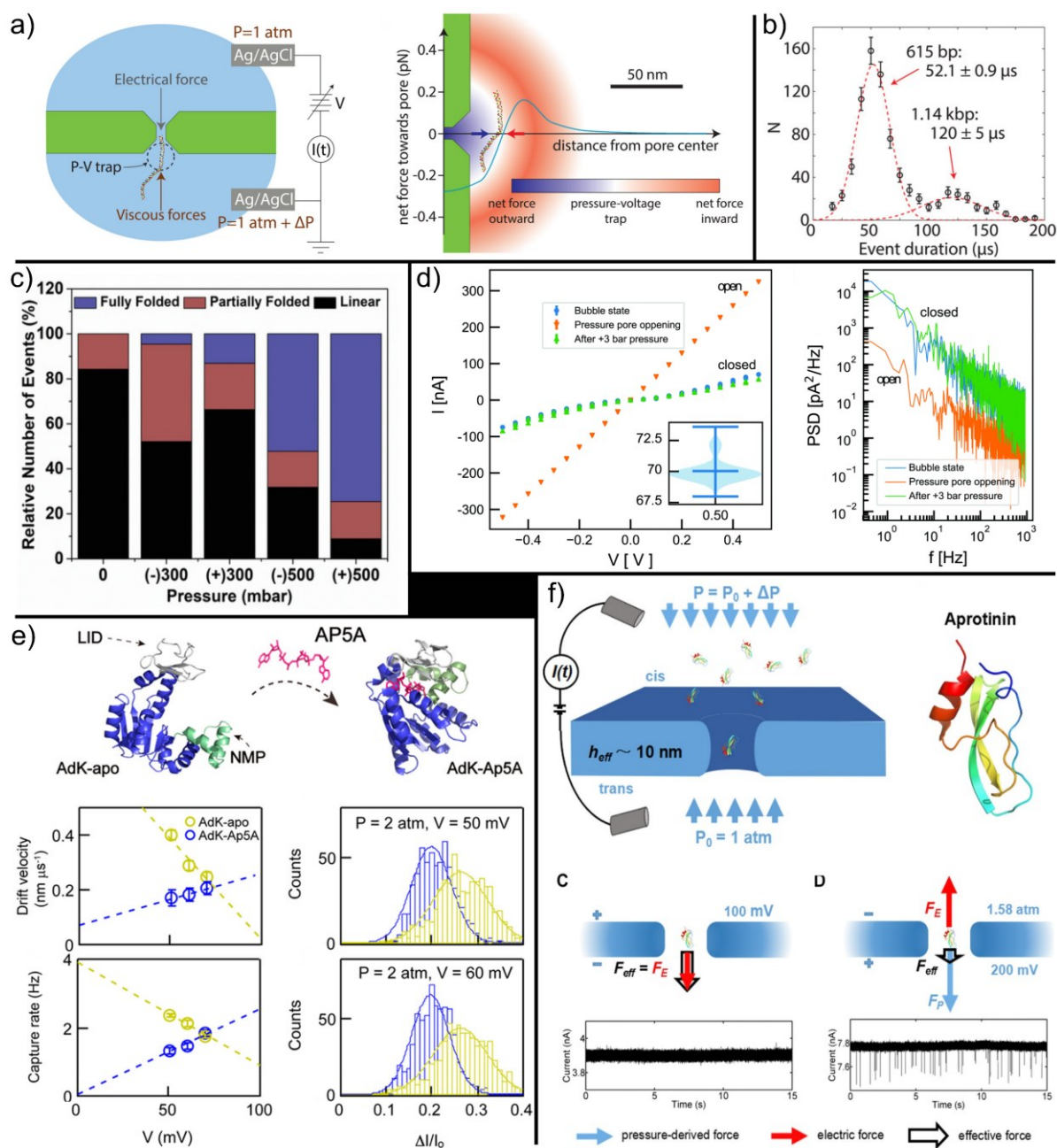


Figure 1.11 Pressure-voltage platforms. a) Formation of a pressure-voltage trap at the nanopore entrance. Image adapted from Hoogerheide *et al.*⁸⁶, reproduced under an ACS AuthorChoice open-access license. b) DNA length discrimination demonstrated by 0.6 and 1.14 kbp dsDNA. Image adapted from Lu *et al.*⁸⁷, with permission. c) Modulated folding of 48.5 kbp dsDNA in nanopipettes. Image adapted from Sharma *et al.*⁸⁸, with permission. d) Improved wetting capabilities and noise reduction facilitated by pressure enhanced flow. Image adapted from Marion *et al.*⁸⁹, with permission. e) Enzyme flexibility and conformation interrogated by a pressure-assisted sensing regime. Image adapted from Hu *et al.*⁹⁰, with permission. f) Detection of Aprotinin (6.5 kDa), a small protein elusive during voltage-only sensing. Image adapted from Li *et al.*⁹¹, with permission.

Golovchenko's group pioneered this approach (Figure 1.11(a)), demonstrating a 2-3 $\times$ reduction in translocation velocity for 3.27 kbp dsDNA under driving pressure and opposing voltage compared to voltage-only bias.^{86,87} By balancing electrophoretic and pressure-driven forces, molecules could be temporarily trapped at the pore entrance. This enabled discrimination between 0.6 and 1.14 kbp fragments based on their specific response to the applied forces (Figure 1.11(b)), and even detection of neutral PEG molecules that are invisible to voltage-driven sensing.

Xiao's group used pressure control to study the repeated entry and exit of anchored DNA molecules.⁹² They observed multiple translocation attempts from single molecules as they fluctuated in and out of the pore under near-equilibrium conditions.

Freedman's group examined translocation dynamics in glass nanopipettes under pressure-voltage control.⁸⁸ They reported systematic effects on current blockage amplitude, folding dynamics, and gating behavior for 48.5 kbp dsDNA (Figure 1.11(c)).

Radenovic's group investigated pressure effects on nanobubble transport and pore wetting.⁸⁹ They showed that applied pressure could displace trapped bubbles that cause rectification and excess $1/f$ noise, restoring linear I-V characteristics and reducing low-frequency noise (Figure 1.11(d)).

Wanunu and Zhao differentiated proteins based on the width of their current blockage distributions, a measure of conformational flexibility.⁹⁰ They studied how the enzyme adenylate kinase (AdK) changes its conformational dynamics upon binding the inhibitor Ap5A as captured by a driving pressure flow and retarding voltage (Figure 1.11(e)).

Zhao's group demonstrated detection of a 6.5 kDa protein, aprotinin, which was completely undetectable under voltage-only conditions due to its fast translocation (Figure 1.11(f)).⁹¹ Under optimized pressure-voltage balance, capture rate increased five-fold and events became detectable. However, this delicate

balance proved sensitive to protein size. For larger proteins such as BSA at 66.5 kDa, the optimal conditions differed substantially, indicating the need for analyte-specific optimization.

1.6 Protein Sensing

Building on this understanding of DNA translocation, the field has shown interest in proteomics. Specifically, the field has slowly evolved from simply detecting proteins to characterizing them in sophisticated ways and developing methods for specific identification (Figure 1.12).

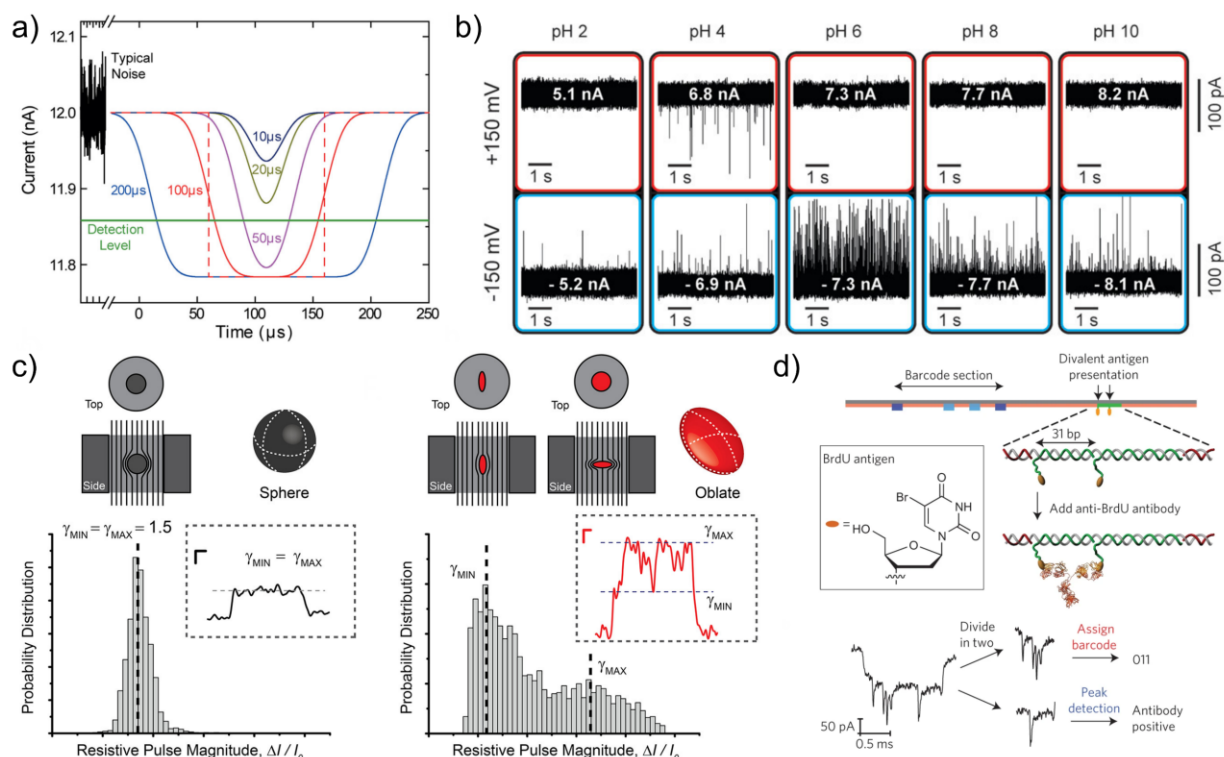


Figure 1.12 Solid-state nanopore sensing of proteins. a) The translocation duration of proteins requires high-bandwidth instrumentation. Image adapted from Plesa *et al.*⁹³, with permission. b) pH modulation allows the understanding of driving forces during protein translocation. Image adapted from Firnkes *et al.*⁹⁴, with permission. c) Estimation of a protein's shape and volume based on event signature. Image adapted from Houghtaling *et al.*⁹⁵, with permission. d) Multiplexed protein sensing facilitated by DNA nanostructures. Image adapted from Bell *et al.*⁹⁶, with permission.

The focus was initially on understanding the forces at play during protein translocation in solid-state nanopores. Specifically, researchers reported that electroosmotic flow is critical during protein translocation.⁹⁴ Due to the negatively charged nanopore walls and the low salt concentration adopted, the electroosmotic force drives protein capture and dominates the electrophoretic force, which is usually assumed to govern capture (Figure 1.12(b)). Next, by tuning the solution's pH close to a protein's isoelectric point (pI), its net charge is reduced, dramatically slowing its translocation and enabling the detection of a small protein (ubiquitin, 8.5 kDa) that would otherwise be too fast.⁹⁷

While protein translocation is of interest, fundamental challenges remain in terms of temporal resolution, non-specific adsorption and clogging, and lack of intrinsic specificity. Proteins, being small and globular, translocate through pores in microseconds, which is faster than the bandwidth of most amplifiers (Figure 1.12(a)), leading to 99.9% of events being missed for sub-30 kDa proteins.⁹³ Next, the high surface-to-volume ratio of a nanopore makes it prone to protein sticking, leading to clogged pores, noisy signals, and terminated experiments.⁹⁸ Finally, bare nanopores sense volume exclusion, providing information on the protein size but lacking specificity on the protein's identity.

Attempts at overcoming these challenges include various studies where:

Larkin *et al.* showed that using custom high-bandwidth amplifiers (e.g., 250 kHz filtering) in conjunction with thin, small-diameter pores (where $d_{pore}/d_{protein} < 1.5$) is essential.⁹⁹ This increases confinement and the signal-to-noise ratio, allowing for the detection of a much larger fraction of the fastest events and enabling the extraction of meaningful diffusion and volume parameters.

Instead of relying on a single metric (like dwell time), the field is moving toward using the full richness of the signal. The distribution of current blockages gives information on flexibility.^{90,100} The shape of the

pulse within an event gives information on orientation and dipole moment (Figure 1.12(c)).⁹⁵ The interplay of multiple levels within an event reveals unfolding pathways.¹⁰¹

To achieve specificity during protein translocation, Bell and Keyser presented a novel solution using DNA nanotechnology.⁹⁶ A large, engineered DNA nanostructure acts as a carrier. It contains a unique barcode (pattern of DNA hairpins) for identification and a specific binding site (e.g., an antigen) to capture a target antibody. This transforms the non-specific nanopore into a highly multiplexable sensor, as demonstrated by a 3-bit 8-member library and the approach could be extended with different capture agents for a wide range of targets (Figure 1.12(d)).

Yusko *et al.* enhanced protein detection while extending nanopore lifetime by engineering a lipid bilayer.¹⁰² It provides a fluid, non-adsorptive surface that virtually eliminates clogging and suppresses electroosmosis, allowing for the study of native protein dynamics. Hence, the study of surface characteristics enhances sensor performance while surface modifications improve robustness during protein sensing.

1.7 Surface Chemistry and Modifications

1.7.1 Electrostatic Interactions

Electrostatic interactions play a crucial role in the behavior of ions and biomolecules within the nanopore, influencing everything from baseline conductance to translocation dynamics and analyte capture rates. The electric double layer, meaning the region adjacent to a charged surface where counterions accumulate and co-ions are depleted, forms spontaneously when any charged surface contacts an electrolyte. This double layer, with thickness characterized by the Debye length, affects the ionic current by modifying the local ion concentration and creates electroosmotic flow when an electric field is applied.²⁴

In solution, surface groups can ionize depending on pH and ionic strength. Silicon nitride surfaces present both silanol (Si-OH) and amine (Si-NH₂) groups, with an isoelectric point around pH 4-6.^{103,104} Below this pH, the surface carries net positive charge from protonated amines. Above this pH, the surface carries net negative charge from deprotonated silanols. DNA carries negatively charged phosphate groups, while proteins expose amine and carboxyl groups on their surfaces, leading to pH-dependent net charge that determines their electrophoretic behavior.

These charged species attract counterions present in solution to form the electric double layer. The Debye length λ_D characterizes the thickness of this layer and determines the distance over which electrostatic interactions are screened.¹⁰⁵

$$\lambda_D = \sqrt{\frac{\epsilon k_B T}{2 N_A e^2 c}} \quad (1.13)$$

where c is the ionic strength or salt concentration and N_A is the Avogadro constant.

For monovalent electrolytes at room temperature, equation 1.13 simplifies to $\lambda_D \approx \frac{0.3}{\sqrt{c}} [nm]$. This means that at 1 M salt, the double layer is only approximately 0.3 nm thick. At 1 mM salt, the double layer extends to approximately 10 nm, potentially exceeding the pore radius. This strong dependence of screening length on salt concentration underlies many of the salt-dependent phenomena in nanopore sensing.

1.7.2 Electrostatic Potential and Surface Charge Density

Due to their charged nature, surfaces in contact with electrolyte develop an electrostatic potential that decays with distance as the double layer forms. The Poisson-Boltzmann equation relates this potential to the underlying charge distribution, providing a theoretical framework for understanding surface effects.

For planar surfaces with monovalent symmetric electrolyte, it takes the form:¹⁰⁶

$$\frac{d^2\phi}{dx^2} = \frac{ce}{\varepsilon} \cdot \left(e^{\frac{e\phi(x)}{k_B T}} - e^{-\frac{e\phi(x)}{k_B T}} \right) \quad (1.14)$$

where ε is the permittivity of the medium and $\phi(x)$ is the electrostatic potential at a distance x from the surface.

This nonlinear equation describes how the potential evolves from its surface value to the bulk, with the exponential terms accounting for the Boltzmann distribution of ions.

For low potentials where $|\phi| \ll k_B T/e$, approximately below 25 mV at room temperature, the solution to the Poisson-Boltzmann equation yields the Debye-Hückel approximation for surface potentials:¹⁰⁵

$$\phi(x) = \phi_0 \cdot e^{-x/\lambda_D} \quad (1.15)$$

This simple exponential decay shows that the potential is confined to within a few Debye lengths of the surface. Higher salt concentrations, meaning shorter λ_D , compress the double layer closer to the surface.

The Grahame equation relates the surface charge density σ to the surface potential ϕ_0 based on the Gouy-Chapman theory for a planar surface:¹⁹

$$\sigma = \sqrt{8c\varepsilon k_B T} \cdot \sinh\left(\frac{e\phi_0}{2k_B T}\right) \quad (1.16)$$

For small potentials ($e|\phi| \ll k_B T$), this expression further linearizes to:

$$\sigma = \frac{\varepsilon\phi_0}{\lambda_D} \quad (1.17)$$

These relationships are critical for engineering analyte-pore interactions.

1.7.3 Surface Preparation

Materials tend to either accept or repel water molecules, a property quantified by measuring the contact angle formed at the solid-liquid-vapor interface. Hydrophilic surfaces with contact angles below 90° are wetted by water, with water spreading to minimize interfacial energy. Hydrophobic surfaces with contact angles above 90° resist wetting, with water beading up. For nanopore applications, hydrophilic surfaces are highly desired because they facilitate capillary filling of narrow channels and reduce bubble entrapment.^{70,89}

Several surface treatments have been developed to render silicon nitride hydrophilic. Piranha solution, consisting of 3:1 concentrated H₂SO₄ to 30% H₂O₂ at 90 °C for 1 hour,⁷³ aggressively oxidizes organic contaminants and enhances hydrophilicity. Similarly, RCA1 (SC-1) clean, consisting of 5:1:1 DI H₂O, 29% NH₄OH, and 30% H₂O₂ at 80 °C for 15 minutes,¹⁰⁷ provides gentler oxidation and cleaning. A thorough DI water rinse follows each protocol to remove residual chemicals. These treatments promote formation of a thin oxide layer, approximately 1-2 nm thick, on the nitride surface. This increases silanol group density and thereby enhances hydrophilicity while providing reactive sites for subsequent functionalization.

1.7.4 Surface Modification

Beyond simple cleaning and activation, deliberate surface modification enables tuning of nanopore properties for specific applications.¹⁰⁸ Under continuous voltage bias, gradual accumulation of adsorbed molecules can lead to irreversible clogging. This is observed as a permanent, stepwise reduction in nanopore conductance that does not recover with voltage reversal or flushing. Such clogging terminates experiments prematurely and remains a major obstacle to long-duration sensing applications.

Polymer brushes, meaning dense layers of polymer chains tethered to the surface, can be created by either grafting pre-formed polymers to reactive surface sites, known as grafting to, or by synthesizing polymers directly from surface-immobilized initiators, known as grafting from.²⁴

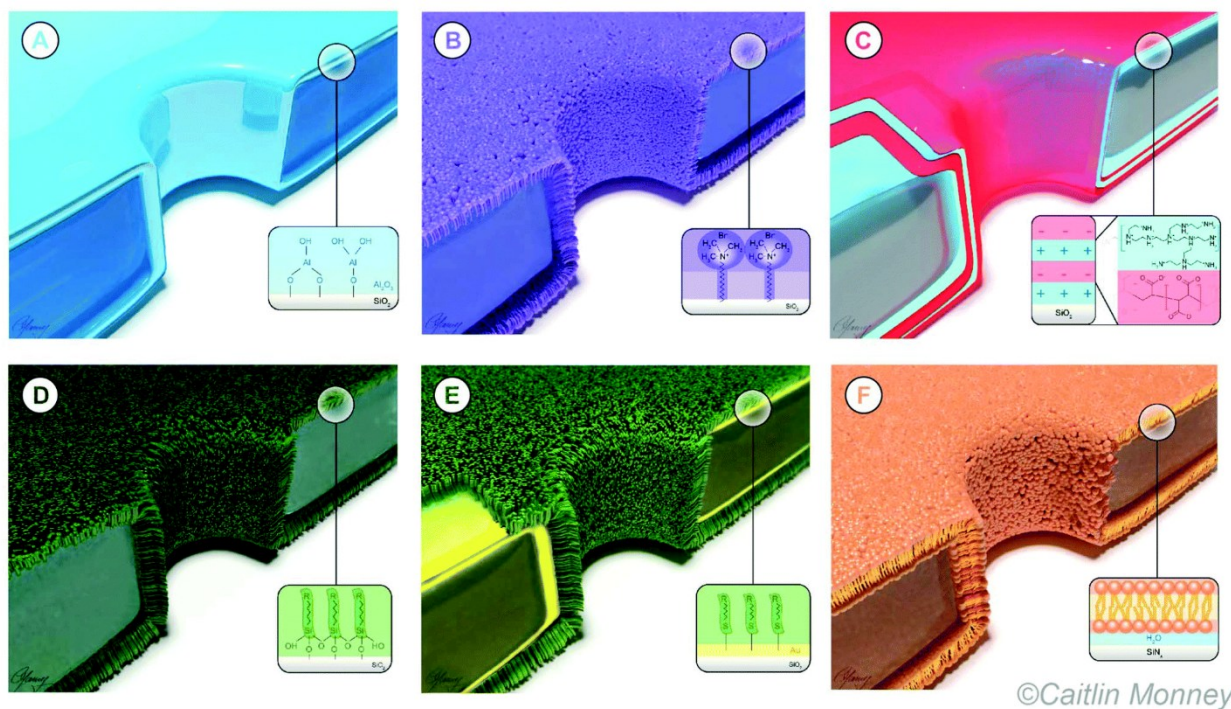


Figure 1.13 Surface coating approaches to improve solid-state nanopores' robustness. Image adapted from Eggenberger *et al.*¹⁰⁸, with permission under license CC BY 3.0.

For solid-state nanopores, end groups are engineered to covalently bond with the oxidized surface, typically through silane chemistry, while the polymer extends into solution (Figure 1.13). The devices are typically submerged in polymer coating solutions with controlled incubation times from minutes to hours to achieve a dense protective layer.⁹⁸ These extended chains can serve multiple functions. For example, neutrally charged polymers like polyethylene glycol (PEG) passivate surfaces and resist analyte adsorption.^{109,110} Alternative surface modification techniques include light-activated physisorption, where UV irradiation induces crosslinking of adsorbed polymer layers.¹¹¹ Another approach involves formation of fluid lipid bilayers, biomimetic coatings that dramatically reduce nonspecific adsorption.¹⁰²

However, most surface modification strategies improve sensor robustness to the detriment of noise performance, where $1/f$ noise is typically increased by the presence of these extended polymers at the nanopore walls.

1.8 Thesis Objectives

This thesis addresses the limitations in current solid-state nanopore sensing by systematically investigating surface properties, modification strategies, translocation control, and device architecture.

These investigations are organized around four core objectives.

First, developing a robust characterization platform is essential for studying surface properties, particularly surface charge density of the nanopore walls, which can modulate analyte-pore interactions and the dynamics of transport. Current literature has primarily focused on SiN using TEM-drilled pores within narrow salt ranges, leaving a significant gap in understanding for novel materials like HfO_2 or Al_2O_3 , or pores fabricated with alternative techniques such as CBD. To bridge this gap, Chapter 2 establishes a high-pressure streaming current platform with picoampere sensitivity. This platform enables systematic investigation across varied pH, salt concentration, and pressure ranges, yielding the first comprehensive surface charge dataset for CBD-fabricated pores.

Building upon this foundational characterization, the second objective addresses the critical issue of sensor longevity. Unwanted analyte adsorption and surface interactions severely limit sensor performance. Therefore, Chapter 2 also proposes a strategy for coating a nanopore membrane with an anti-fouling polymer that covalently binds to oxidized silicon nitride without detrimentally altering the low-frequency noise properties of the nanopore sensor. By systematically comparing membrane-only versus fully coated nanopores, Chapter 2 elucidates the dominant mechanisms and location of pore clogging, thereby enabling the rational design of minimal, highly effective coatings necessary for long-duration sensing involving challenging analytes like flexible ssDNA.

The third objective focuses on the precise control of analyte translocation velocity, and is addressed in Chapter 3. When molecular events are too short even for the highest-bandwidth recordings (~MHz) or when molecular substructure must be precisely resolved to extract encoded information, enhanced velocity control is important. In Chapter 3, I employ a voltage-pressure regime to precisely control translocation duration, effectively creating a tunable trap at the pore entrance. By balancing electrophoretic and pressure-driven forces, this method aims to extend event durations for nanostructured DNA and protein passages, allowing for detailed characterization of molecular features currently beyond the temporal resolution of state-of-the-art voltage-driven sensing. This approach is validated with well-characterized DNA origami structures of known geometry and low molecular weight proteins such as GFP and Cytochrome c.

The last objective is addressed in Chapters 4 and 5, which tackle the limitations inherent in current materials for nanopore membranes. Silicon nitride, although widely adopted, faces limitations including gradual chemical dissolution under typical biosensing experimental conditions, manifested as pore expansion and increased noise. Furthermore, the trade-off between membrane thickness and spatial resolution mandates architectural alternatives that are more robust. Therefore, we first investigated ion beam deposition (IBD) as an alternative, CMOS-back-end-of-line (BEOL)-compatible method for producing high-quality SiN membranes. Building on this foundation, we extended our research scope to high- κ dielectric materials, particularly hafnium oxide (HfO_2) deposited by atomic layer deposition (ALD). HfO_2 offers superior chemical stability and tunable thickness deposition,⁴⁴ but a fabrication workflow for ultra-thin membranes remains a challenge. Chapter 4 presents the fabrication and analysis of IBD SiN membranes, while Chapter 5 details the fabrication and characterization of HfO_2 membranes.

Finally, Chapter 6 synthesizes these diverse findings to discuss implications for future nanopore applications in proteomics, diagnostics, and single-molecule biophysics.

2. Unraveling clogging of solid-state nanopores by selective polymer coating: surface potential measurements and sensing of long single stranded DNA

This chapter is published in *RSC Applied Interfaces* as “Unraveling clogging of solid-state nanopores by selective polymer coating: surface potential measurements and sensing of long single stranded DNA” (2025) by Mohamed Yassine Bouhamidi, Zachary Roelen, and Vincent Tabard-Cossa.

2.1 Abstract

Solid-state nanopores have emerged as powerful tools for single-molecule analysis, with applications ranging from genomics to diagnostics. However, challenges such as surface interactions and clogging have limited their applicability, and a better characterization of the nanopore wall surfaces and control of the surface coating would provide valuable information to tackle these problems. Here, employing a platform capable of high-pressure streaming current measurements in the picoampere range, we characterize the surface potential of solid-state nanopores and perform selective surface coating to elucidate the mechanism for molecular clogging. By examining different operating conditions, such as pore diameter, salt concentration, and pH, we discuss optimizations for precise streaming current measurements. We validate the performance of the setup by studying both bare SiN and polymer-coated nanopores fabricated by the controlled breakdown method. To address the issue of nanopore clogging, we investigate localized functionalization approaches either prior to or after pore formation. Our results allow us to establish that outer membrane coating is sufficient to provide antifouling properties during translocation of long single-stranded DNA, and provide insight into the leading causes of nanopore clogging.

2.2 Introduction

Nanopores are small holes in thin insulating membranes used to analyze single molecules such as DNA, RNA, and proteins.^{4,26,112} When immersed in a salt solution, these molecular-sized channels produce an ionic current in response to a voltage gradient. The passage of charged analytes by electrophoresis through the pore, one at a time, causes variations in this electrical signal. The resulting current blockages can provide critical information about the molecule's structure and composition.³

Nanopores, made in solid-state materials, such as SiN, offer a versatile platform for different biosensing applications by allowing for the tuning of the pore size to match the analyte. Recent advances in the controlled breakdown (CBD) method of nanopore fabrication have democratized access to solid-state nanopores of the desired size from 2 to 20 nm and expanded their field of use.^{56,57} Yet, despite this technological progress, typical surface properties (such as roughness, charge density, precise pore shape and surface coating) of solid-state nanopores present significant challenges.

A major factor limiting the sensing performance of solid-state nanopores is the level of $1/f$ noise^{68,71,73,113} in the ionic current signal (dominating up to 1 kHz), which has been linked to surface charges, as well as to the roughness and hydrophobicity of the nanopore membrane, factors which can promote nanobubble formation and trapping.^{70,72,114} Furthermore, the levels of electrical interference arising from the protonation of the surface are greatly impacted by the working conditions of salt concentration and pH, and also lead to an increase in white noise (present at all frequencies).⁶⁹ Typically, surface charge density is determined experimentally using the streaming potential method¹¹⁵ or is estimated theoretically through the expected degree of dissociation of silanol and amine groups.¹⁰⁴ Here, the streaming potential technique is favored for direct surface potential measurements, since the pressure actuation across the nanopore directly probes the motion of ions near the nanopore wall, neglecting the contribution from the bulk.

Surface interactions between the molecule and the pore also directly affect the sensing duration of an experiment, which usually terminates due to an irreversible clog where the nanopore's conductance is irrevocably reduced despite repeated voltage reversal. Mitigating clogging issues has been the subject of on-going studies of solid-state pores.^{108,116–119} Techniques to promote nanopore anti-fouling properties have included location-agnostic functionalization of the SiN surface *via* silanization of carboxylic acid and polyethylene glycol (PEG),^{109,110} grafted PEG brushes,¹²⁰ photohydrosilylation of acidic, basic and nonionizable end groups,¹¹¹ or coating with a fluid lipid bilayer.^{102,121} While localized functionalization has been studied in anodic aluminum oxide nanoporous membranes, where inner and outer wall chemical modification enables a wide range of properties such as anti-interference, signal enhancement and ion gating,¹²² a localized approach to chemical modification has yet to be studied in SiN and at the single-nanopore level. Addressing these challenges related to surface properties requires careful characterization of the surface charge density of bare and functionalized surfaces to control the quality of the surface coating and relate it to observations of improved sensing performance.

To shine some light into the clogging issue, optical observation of molecules to sense molecules translocating nanopores has been performed over the years to provide additional information beyond the traditional electrical channel. Dual optical-electrical platforms were developed to add parallelization and specificity to the nanopore measurements.^{123–125} Of interest to this work are fluorescence studies that tracked the capture and passage of molecules through nanopores to gain a better understanding of the transport process.¹²⁶ Indeed, an optical microscope-nanopore platform can provide relevant spatial information about the molecule leading up to the translocation through the pore, whereas the electrical signal is most sensitive when the molecule is inside the pore. By labeling DNA strands with fluorescent dyes, a spatial resolution of $\sim 1\ \mu\text{m}$ has been achieved by confocal laser scanning microscopy.^{116–118} Meanwhile, through stimulated emission depletion (STED) super-resolution microscopy, the observation of the capture region around a nanopore with a $\sim 100\ \text{nm}$ resolution was achieved.¹²⁷ However, while

these optical measurements offer advantages in terms of spatial resolution outside the pore, the temporal resolution is limited by the frame rate of the camera, offering snapshots of the molecule in the millisecond range, much slower than the translocation timescale through the pore, typically in the microsecond range. Details of the transport process and mechanisms of clogging are thus not well resolved optically.

In this work, we performed streaming current measurements using a modified version of our previously developed platform,¹²⁸ to investigate the effect of parameters such as salt concentration, pH, and pore diameter on the surface potential of SiN nanopores. Unlike traditional nanopore measurements in which a voltage is applied across the membrane,⁸ streaming current measurements are driven by applying a transmembrane pressure gradient. Similarly to electroosmosis, the streaming current is a result of the motion of excess ions in the electric double layer (EDL) shielding the charges on the nanopore wall. We show that the isoelectric point of SiN pores from the batch of chips used and fabricated by CBD is approximately 4.4, agreeing with the values previously observed ranging from ~4-5.^{103,129} Finally, we propose a protocol for the selective coating (interior-exterior) of CBD-fabricated nanopores and compare the zeta potential of functionalized nanopores to that of bare SiN surfaces. In particular, by studying the translocation of 7 kilo-nucleotide (knt) single-stranded DNA (ssDNA), we show that coating only the surrounding membrane surface with an antifouling polymer, while leaving the nanopore walls unfunctionalized, is enough to impart significant improvements in sensing times, and in the number of translocated molecules during nanopore experiments.

2.3 Results and discussion

2.3.1 Platform for nanopore fabrication, characterization, and functionalization

Solid-state nanopores made in 20 nm thick SiN by the controlled breakdown method⁵⁷ were used in this study. To enable high-precision zeta potential measurements of the pore surface, we designed a pressure-driven system housed in a Faraday cage (Figure 2.1(a)). Following system calibration, we applied

pressure sweeps up to 0.3 MPa (in steps of 0.05 MPa), systematically recording the resulting streaming current for a duration of 30-120 seconds. The *trans* side of the nanopore was kept at atmospheric pressure as a reference, while the *cis* side was biased using a mechanical pressure regulator. These measurements formed the basis for extracting zeta potential and associated surface charge density values of nanopores under different operating conditions and surface treatments, which we explore in subsequent sections. To eliminate issues related to electrode potential drift when switching between solutions of different salt concentration and/or pH, a 40% PEGDA salt bridge^{130,131} was used to decouple the Ag/AgCl electrodes from the sensing medium (*i.e.*, keeping them consistently immersed in reservoirs of 3 M KCl, pH 8). This improvement significantly reduced current drift in the 1-10 picoampere range that the streaming signals occupied, allowing for accurate measurements while also extending the electrode lifetime.

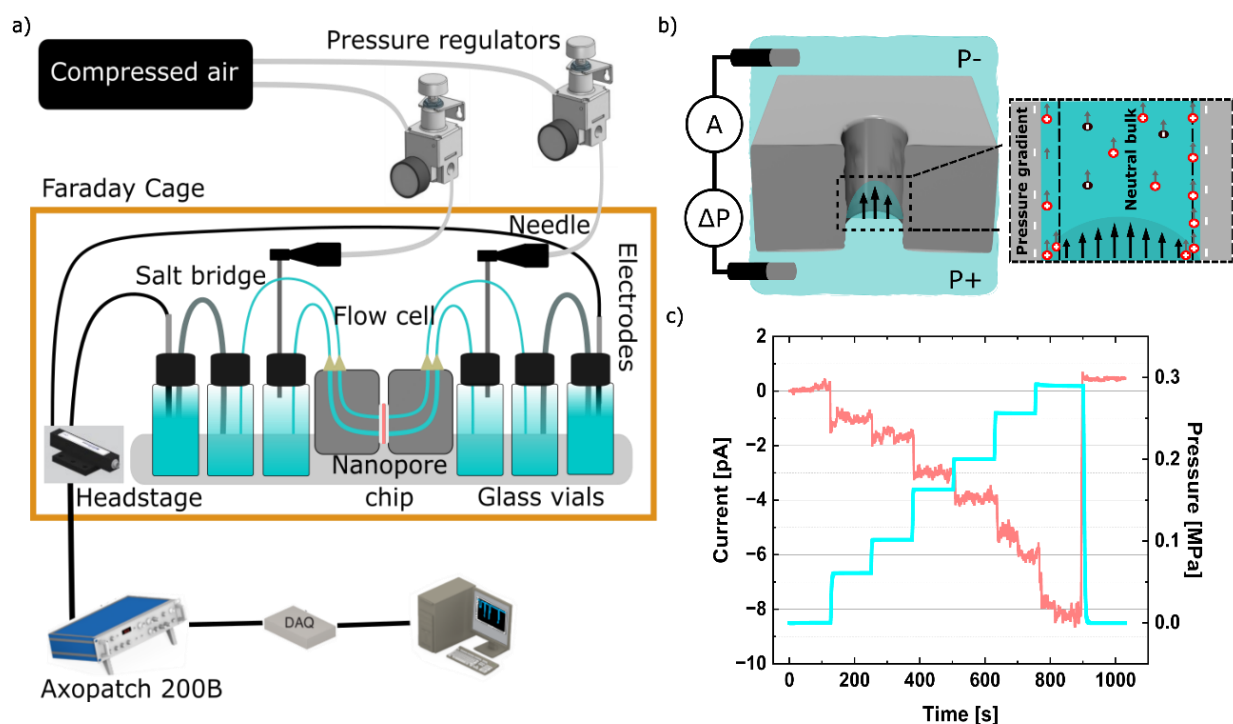


Figure 2.1 Platform for fabrication, functionalization, and characterization of solid-state nanopores. a) System diagram depicting the different components of the voltage and pressure-controlled platform used to perform low-noise current measurements. **b)** Schematic highlighting the working principle of the streaming current method inside a nanopore. **c)** Typical I_{stream} (red) and ΔP (blue) curves vs. time for a streaming current measurement (10 nm SiN nanopore in 0.1 M KCl pH 8).

Figure 2.1(b) depicts the principle of the streaming current that arises upon the application of a pressure difference across a nanopore due to the presence of surface charges on the pore wall. Figure 2.1(c) shows a typical time trace of the streaming current, I_{stream} , generated under changes of transmembrane pressure gradient ΔP . These pressure gradient sweeps allow for the extraction of the pore interior zeta potential ζ . This metric characterizes the amount of net charge on the nanopore wall, as observed by an electrokinetic measurement such as the recording of streaming current. Under conditions of low or weak surface potentials in the Debye-Hückel regime, the zeta potential can be expressed as:¹⁹

$$\zeta = \frac{\eta L \Delta I_{stream}}{\varepsilon_r A \Delta P} \quad (2.1)$$

in terms of the viscosity η and dielectric constant ε_r of the surrounding fluid (assumed uniform and in a laminar flow regime), the length L and cross-sectional area A of the nanopore, the pressure gradient applied across the nanopore ΔP (approximating that the pressure drop across the nanopore dominates over that of the access region, valid when $\sqrt{A} \lesssim L$), and measured streaming current I_{stream} .

By recording the streaming current under a sweep of pressure gradients, the zeta potential of the nanopore surface can be calculated from the slope of I_{stream} vs. ΔP using Eqn (2.1), when $\lambda_D < d$ (where λ_D and d are the Debye length and nanopore diameter respectively). This approximation assumes the fact that the Hagen-Poiseuille law (laminar flow in a uniform circular channel) is respected for the nanopore with aspect ratios $d/L \sim 1$, used here.¹³² The surface charge density, a property of the material interface with the solution, can also be estimated from the measured zeta potential using the Grahame equation for cylindrical nanopores:¹⁰⁴

$$\sigma = \frac{2\varepsilon_r k_B T}{\lambda z e} \left[\sinh\left(\frac{ze\zeta}{2k_B T}\right) - \frac{2\lambda}{d} \tanh\left(\frac{ze\zeta}{4k_B T}\right) \right] \quad (2.2)$$

where z , e , k_B and T are the valence of ions, the elementary charge, the Boltzmann constant and the absolute temperature, respectively.

Here, we adopt the Debye length equation for monovalent salts at 25 °C as:

$$\lambda [nm] = \frac{0.304}{\sqrt{C}} \quad (2.3)$$

where C is the salt concentration in units of $mol L^{-1}$. From Eqn (2.1)–(2.3), we see how key experimental parameters—such as pore shape, salt concentration, and pH—can thus affect the surface charge density of a nanopore, through the Debye length and the streaming current observed.

Equipped with a setup for performing streaming current measurements, we next examined the effects of different operating parameters (pore size, salt concentration, and pH) on surface potential in order to optimize the measurement conditions. Figure 2.2(a) plots the streaming current under a

transmembrane pressure for three nanopores of varying diameters (10 to 25 nm) and identical nominal membrane thickness in 100 mM KCl pH 8, and shows a significant increase in streaming current magnitude with pore diameter. For example, at a fixed pressure gradient of 0.3 MPa, values of ~ 10 pA are observed for the 10 and 12 nm pores, compared to ~ 35 pA for the 25 nm diameter pore. We note that although the nanopore cross-sectional area A increases in the sequence of pore diameters tested, zeta potential calculations using Eqn (2.1) for these nanopores give relatively consistent values of -7.3 ± 1.4 , -7.0 ± 0.5 and -7.0 ± 0.3 mV for $d = 10, 12$ and 25 nm, respectively. The slight differences in extracted zeta potentials, being within the calculated standard deviations, lead us to conclude that the zeta potential stays constant for these nanopore dimensions (even for the case when $d > L$). On the other hand, the 4-fold increase in observed streaming current for the 25 nm pore *versus* the ~ 10 nm ones is a significant factor that should be considered when performing these measurements, for which the noise and drift are often in the 1-10 pA range, and thus translates into a similar ~ 4 -fold increase in the signal-to-noise ratio, facilitating the measurement.

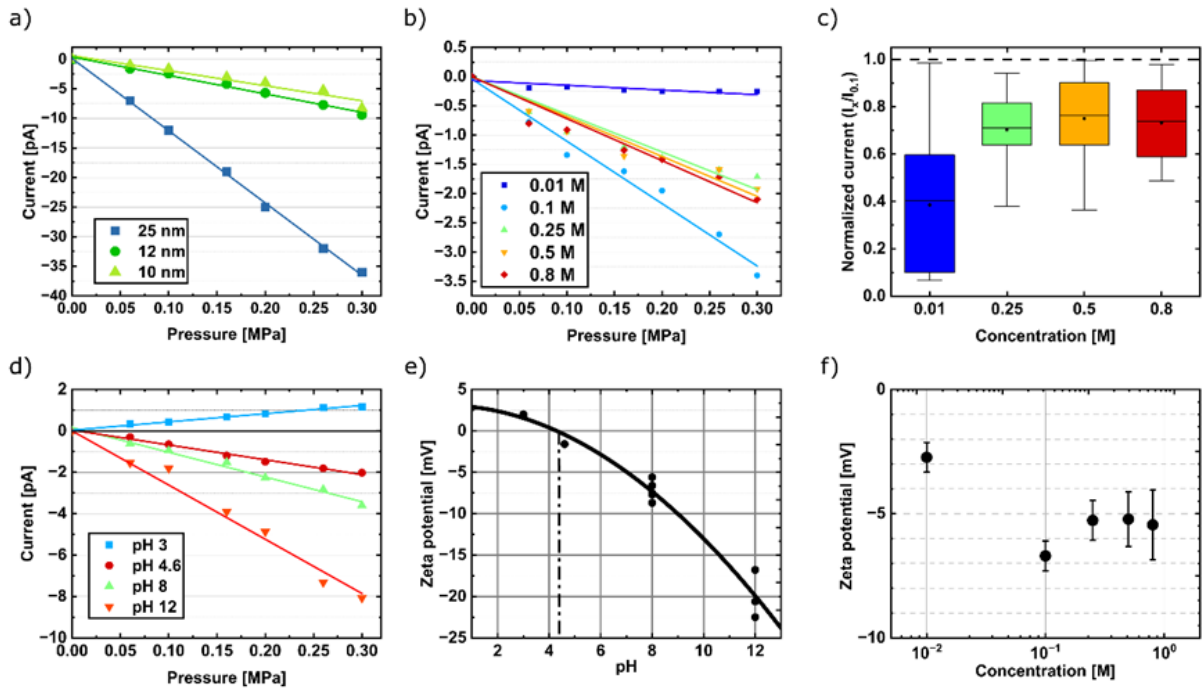


Figure 2.2 Effect of SiN pore diameter, salt solution concentration and pH on zeta potential inside the nanopore. a) Streaming current measurements *versus* applied transmembrane pressure in 0.1 M KCl pH 8 for different nanopore diameters. b) Streaming current *versus* applied pressure gradient for a 9 nm nanopore in KCl solutions of different salt concentrations, pH 8. c) Normalized streaming current for different KCl concentrations, pH 8, for three nanopores ranging from 9 to 13 nm in diameter, and relative to their values at 0.1 M KCl. The dashed line represents the normalized current value reached at 0.1 M KCl. Six data points (at different transmembrane pressures) are collected during a pressure sweep, with 2-3 sweeps performed for each device tested, and the error bars indicate the 25% and 75% data quartiles respectively. The line and dot in the 50% quartile box represent the median and mean value respectively. d) Streaming current *versus* applied pressure gradient for a 9 nm nanopore in 0.1 M KCl at varying solution pH. e) Measured nanopore zeta potential in 0.1 M KCl at varying solution pH for ten nanopores ranging from 8 to 25 nm in diameter. Dashed line is a guide to the eye for the isoelectric point at $\zeta = 0$ mV. f) Measured nanopore zeta potential for different KCl concentrations at pH 8 for three nanopores ranging from 9 to 13 nm in diameter. Error bars indicate the pooled standard deviation for these samples.

The salt concentration of the surrounding fluid also influences the magnitude of the streaming current by governing the ion screening effects of the pore wall surface charge. Indeed, the extent of the screening effect (as characterized by the Debye length) is inversely proportional to the square root of the solution's salt concentration C as observed in Eqn (2.3); a lower C thus leads to a longer λ_D and increases the extent of electrostatic interactions. Figure 2.2(b) and (c) highlight how this property of the salt

concentration affects the magnitude of the streaming current signal in a SiN nanopore. Figure 2.2(b) plots the streaming current *versus* applied pressure gradient for varying KCl concentrations at pH 8 for a 9 nm diameter nanopore. At either end of the salt concentration sweep ranging from 0.01 M to 0.8 M, the magnitude of the streaming current is reduced (*e.g.* < 1 pA for pressure gradients above 0.2 MPa in 0.01 M KCl) which we attribute to either the sparse number of ions available in the Stern layer at low salt concentration or by a greater screening of the surface at high salt concentrations ($\lambda_D = 0.34$ nm for 0.8 M KCl). Furthermore, when operating at >1 M KCl pH 8 ($\lambda_D = 0.30$ nm), these measurements become challenging to reproduce and interpret due to the finite sizes of the ions, both freely diffusing and in the adsorbed layer of counterions on the surface as described by the Stern model, when compared to the Debye lengths in the single angstrom range. Figure 2.2(c) plots the streaming current measurements of multiple pores (diameters 9-13 nm) over a salt concentration sweep, normalized by their values at 0.1 M KCl pH 8. It shows that, on average, the maximum signal-to-noise ratio is reached near this intermediate value ~0.1 M, with an asymptotic ~25% decrease in magnitude for ≥ 0.25 M KCl with these 20 nm thick nanopores. A similar, non-monotonic trend has been observed previously in silica nanochannels and was found to agree with chemical equilibrium models.¹¹⁵

Figure 2.2(f) presents the measured variation in zeta potential over a sweep of bulk salt concentration. The zeta potential is measured from 0.01 M to 0.8 M KCl, pH 8, and exhibits an analogous trend to the streaming current, with the highest surface potential recorded at 0.1 M KCl (-6.70 mV compared to -2.73 mV at 0.01 M and -5.45 mV at 0.8 M). Firnkes *et al.*⁹⁴ reported a monotonically decreasing surface potential magnitude in from 0.1 to 0.4 M KCl, which is consistent with our results in that range. Meanwhile, calculated values of surface charge density ranged from -1.2 mC m^{-2} to -12.0 mC m^{-2} for bulk KCl concentrations between 0.01 M and 0.8 M at pH 8 (see Figure 2.8 of the SI). Note from Eqn (2.2) that the surface charge density is, to first order, proportional to the zeta potential, while being inversely proportional to the Debye length or the square root of the bulk concentration. These

calculated surface charge density values, which follow a power law (with saturation at lower concentrations due to the challenge in extracting the streaming current explained earlier), qualitatively illustrate the differing rates of change between the zeta potential and the Debye length in varying KCl concentration.

We note, however, that these calculated surface charge densities, based on ζ values extracted under the streaming current method, should be considered as approximations, as they are known to deviate greatly from those of more direct (*e.g.* charge titration) measurements.¹⁹ Furthermore, the assumptions that underline the simplified streaming current model (Eqn (2.1)), from which our surface charge density estimates are derived, will not strictly hold as the zeta potential tends towards or exceeds the Debye-Hückel limit of ~25 mV. The surface charge density estimates can also be compared to values extracted from the conductance model of the nanopore:²

$$G = \frac{\pi d^2}{4L} \left[(\mu_K + \mu_{Cl}) n_{KCl} e + \mu_K \frac{4\sigma}{d} \right] \quad (2.4)$$

where μ_K , μ_{Cl} , n_{KCl} are the electrophoretic mobility of potassium and chloride ions, and the number density of potassium chloride ions, respectively (using values of $\mu_K = 7.616 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $\mu_{Cl} = 7.909 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$). Sections 2.7.3 and 2.7.4 of the SI present a detailed comparison of surface charge densities extracted from both measurement techniques and hypothesize that the observed differences are related to the initial assumptions made for the streaming current method and the distance away from the wall at which the measurement is performed.

Waduge *et al.*¹⁰⁰ reported that at 0.4 M KCl in pH 7.8, TEM drilled pores have a zeta potential of -7.9 ± 1.6 mV as measured with the streaming potential technique. In previous streaming current measurements, we reported a zeta potential value of -7.4 ± 1 mV at 100 mM KCl in pH 8,¹²⁸ and our present experimental data reveals a value of -5.22 ± 1.2 mV for 0.5 M KCl in pH 8 (see Figure 2.2(f)). Although our experimental data falls within the measurement error reported, potential explanations for the lower mean value observed could include differences in the silicon nitride stoichiometry, wafer to

wafer variations, and surface cleanliness. We also note that pores were fabricated by CBD directly in KCl aqueous solution in the present work and thus streaming current measurements here probe a freshly exposed surface inside the nanopore. Conversely, with nanopores fabricated by TEM drilling, an additional step of piranha cleaning is required to facilitate wetting.⁵¹ This step further modifies the surface by promoting the formation of an oxide layer on top of the native silicon nitride and so is expected to result in surfaces featuring higher degrees of silanol dissociation exposed to solution, and thus higher measured streaming currents and zeta potentials. The solution's pH also strongly influences the surface charge density of the SiN membrane, again through silanol group dissociation.²⁴ These are well-established equilibrium equations for SiN in basic and acidic solutions:



Depending on the pH, *i.e.*, the concentration of hydrogen ions $[\text{H}^+]$ in the bulk solution, the magnitude of the streaming current signal is impacted at a fixed salt concentration. To quantify this effect, Figure 2.2(d) plots the streaming current *versus* an applied pressure gradient for a 9 nm pore at 100 mM KCl with varying pH and shows that, starting from pH 8 ($[\text{H}^+] = 10^{-8}$ M), a decrease in concentration of hydrogen ions to pH 12 ($[\text{H}^+] = 10^{-12}$ M) leads to an increase in the slope of the streaming current response with applied pressure, resulting for example in current magnitudes of -3.6 pA and -8 pA at 0.3 MPa applied pressure for pH 8 and 12, respectively. Similarly, an increase in hydrogen ions concentration from 10^{-8} M to $10^{-4.6}$ M (pH 4.6) in the bulk solution leads to a decrease in streaming current magnitude and slope. The sign of the slope is also reversed when crossing the isoelectric point of the SiN membrane, turning the surface from being negatively charged at pH 4.6 to being positively charged at pH 3, as exemplified by values measured at 0.3 MPa of +1.2 pA at pH 3 compared to -2.1 pA at pH 4.6. The change in polarity of the current directly corresponds to a change in charge polarity of the ions shielding the surface.

Figure 2.2(e) presents measured ζ values over a pH sweep for different nanopores ($N = 10$) of diameters ranging from 8 nm to 25 nm at a fixed salt concentration of 100 mM KCl (zeta potential is observed to be independent of pore dimensions in this range, as discussed above). The zeta potential is measured at -7.5 ± 0.9 mV at pH 8 and increases in magnitude (becomes more negative) as the bulk solution pH is increased to reach -20.5 ± 2 mV at pH 12. Similarly, by decreasing the bulk solution pH, the zeta potential becomes more positive and reaches a value of $+1.7 \pm 0.3$ mV at 100 mM pH 3 after crossing the isoelectric point. This trend can also be translated to an estimated surface charge density after factoring in the solution's Debye length *via* Eqn (2.2). Indeed, in this 100 mM KCl solution, the surface charge density is calculated to be $+1.2 \text{ mC m}^{-2}$, -5.0 mC m^{-2} , and -14.2 mC m^{-2} at pH 3, pH 8 and pH 12 respectively (see Section 2.7.1 of the supporting information).

By fitting the collected pH data to a power law, the isoelectric point of our CBD-fabricated SiN nanopores can be determined by the intercept where the ζ - or σ -value is zero. As reported by Lin *et al.*,¹⁰³ the isoelectric point of SiN is expected to be in the pH range of 4-5, for pores made by CBD and TEM drilling. Meanwhile, Sheetz *et al.*¹²⁹ report a value of 4.3 for CBD fabricated pores, as determined by nanopore conductance measurements (compared to the streaming current method adopted here). By interpolating within our fitted data, we obtain a SiN isoelectric point of 4.4 ± 0.1 for CBD fabricated pores, which is in good agreement with the above values, irrespective of the choice of fabrication method. We note here that the pH value was assumed to be the one measured in the bulk, whereas previous studies with silica nanopores showed that the recorded pH near the nanopore wall can differ by up to one unit from this bulk value when inspected by fluorescent dyes on the nanopore wall.¹³³

Together, these measurements serve as a reference for the surface characteristics of SiN nanopores in the reported conditions of bulk salt concentration and pH, as well as validate the capabilities of our developed system to characterize the surface of nanopores.

2.3.2 Characterization of anti-fouling polymer coated nanopores through pore conductance, noise and zeta potential

Having optimized the conditions for surface potential characterization on bare SiN nanopores, we next explored providing an antifouling property to the nanopore surface or the surrounding membrane in order to improve sensing performance and investigate the mechanisms behind nanopore clogging. Functionalizing the nanopore surface can minimize surface interactions between the translocating analyte and the pore and thus reduce clogging artifacts, which are major limitations for long-duration biosensing, especially for samples with complex background mixtures¹³⁴ or certain biomolecules particularly prone to pore fouling like amyloid-beta.¹⁰² Here, we propose a protocol based on silane chemistry to preferentially functionalize the membrane surface and/or the pore interior. This protocol utilizes PAcrAm-g-PMOXA (SuSoS AG), as previously described by Awasthi *et al.*⁹⁸ for coating SiN nanopores. While this polymer has demonstrated potential in extending sensing time for protein detection through helium-ion-milling (HIM)-drilled pores, its application to nanopores fabricated by CBD has not been previously reported.

In particular, the CBD fabrication method facilitates the selective functionalization of the membrane and/or of the nanopore interior since the pore is formed *in situ*, *i.e.* directly in solution, with this technique. The membrane coating can thus be applied prior to pore formation and avoid being exposed to the potentially damaging vacuum and irradiation conditions encountered in energetic beam drilling fabrication methods.⁵¹ CBD fabrication therefore readily facilitates three distinct coating scenarios: (1) coating the membrane surface before nanopore fabrication, resulting in a pristine, unmodified pore interior and functionalized membrane; (2) simultaneous functionalization of the membrane and nanopore interior post-fabrication; or (3) initial chemical modification of the membrane followed by incubation with a different coating post-nanopore fabrication, leading to distinct coatings inside and outside the pore.

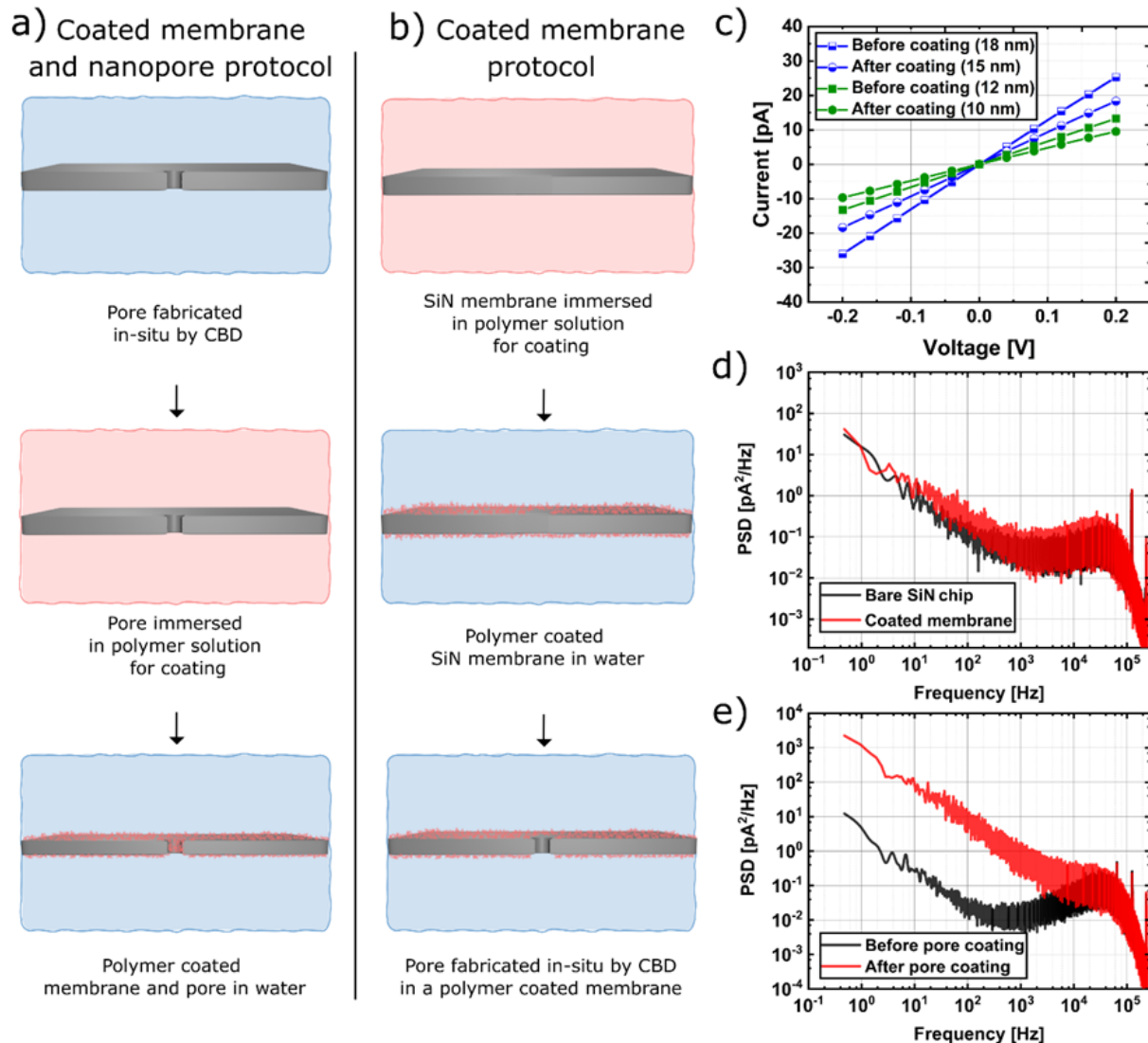


Figure 2.3 Schematic of functionalization protocol for preferential in-situ coating of SiN membranes and/or the pore interior and characterization through conductance and noise measurements. **a)** Protocol for coated membrane and pore interior: initial nanopore fabrication, incubation in 0.1 mg/mL PAcrAm-g-PMOXA solution for 45 minutes, and DI water rinse before injecting the sensing solution with the sample of interest. **b)** Protocol for coated membrane: incubation in 0.1 mg/mL PAcrAm-g-PMOXA solution for 45 minutes, DI water rinse before CBD nanopore fabrication, then injection of sensing solution with the sample of interest. **c)** I-V curves for two nanopores before and after interior surface coating. Estimated size is extracted from the slope of the data using a conductance model that assumes a cylindrical pore geometry.⁹ **d)** Current power spectral density for a bare 11 nm SiN membrane device compared to an 11 nm nanopore device with its outer membrane surface coated (Axopatch 200B, 100 kHz hardware low-pass filter). **e)** Current power spectral density for an initially 18 nm bare SiN device before and after coating of its inner pore surface (Axopatch 200B, 100 kHz hardware low-pass filter).

Figure 2.3(a) and (b) showcase two such coating strategies explored in this work using the PAcrAm-g-PMOXA polymer that targets the membrane surface and/or the pore interior. A cleaned SiN chip is mounted inside a flow cell, allowing for a sequence of solutions to be flushed onto the membrane surface to achieve functionalization, as well as providing electrical access to the membrane for a selectively placed step of nanopore fabrication by CBD in the protocol.

When the pore interior is coated, covalent bonding between the native silicon oxide sites on the SiN surface and the polymer's hydroxy (-OH) groups results in a neutrally charged interface of polymer extension (acrylamide) groups, as seen by the analyte in solution.

One metric to characterize the functionalization of the pore interior is by measuring the open pore conductance, which should be predictably reduced after narrowing the pore diameter by the thickness of the polymer coating. The pore's DC conductance was determined by performing a current-voltage (I-V) measurement from -200 mV to +200 mV in 3.6 M LiCl at pH 8. Figure 2.3(c) plots such I-V traces for two separate pores both before and after interior coating (Figure 2.3(a)), leading to estimates of their pore diameters through a conductance model for cylindrical pore geometries.⁹ As the polymer layer is expected to be approximately 1.5 nm thick,¹³⁵ the observed ca. ≈ 3 nm decrease in nanopore size after carrying out the coating protocol is consistent with having the pore interior fully coated.

Another means of characterizing the effect of antifouling coatings is by using power spectral density (PSD) measurements of the ionic current signal through the pore.⁹⁸ Figure 2.3(d) compares the PSDs of a membrane-only coated chip and a bare SiN chip (3.6 M LiCl pH 8, Axopatch 200B, 100 kHz hardware filter), and both are observed to display similar $1/f$ noise performance at ± 200 mV (both nanopores were ~ 11 nm in diameter). Compared to previous nanopore functionalization strategies which involve coating the pore interior,^{98,121} coating the membrane prior to pore fabrication eliminates the challenge of maintaining acceptable low-frequency noise performance after functionalization, which has been a factor limiting the widespread use of surface coatings to reduce clogs. Figure 2.3(e) highlights this

phenomenon by plotting PSDs of a single pore (18 nm) both before and after interior coating with the antifouling polymer, with the post-coating PSD observed to feature a noticeable $1/f$ noise increase. Such an increase in low-frequency noise when a pore is coated after fabrication ($\sim 100\times$ at 1 Hz) corresponds to pores with less stable open pore baselines, leading to molecular signals with reduced SNR and making molecular sensing challenging.

Finally, we investigated the zeta potential, as measured by streaming current, as a key metric for probing the nanopore interior after surface modification.¹³⁶ Figure 2.4(a) shows how the zeta potential across a pH range of 3 to 12 changes for the different types of devices tested, namely bare SiN membranes, coated membranes, and coated pore interiors, all immersed in a salt concentration of 100 mM KCl. As expected, the membrane coated devices, for which the pore interior remains a pristine SiN surface, exhibited similar zeta potential values to the bare SiN membrane devices across all tested pHs. Meanwhile, starting from pH 3, we observed a zeta potential of 0.9 ± 0.1 mV for fully coated nanopore devices compared to 2.0 ± 0.5 mV and 1.8 ± 0.4 mV for membrane coated and bare SiN pores respectively, representing a 50% decrease in the measured zeta potential between the bare SiN and fully coated nanopores. A similar behavior is exhibited between bare SiN and fully coated pores at pH 8, with a 56% decrease from -7.5 ± 1 mV to -3.3 ± 0.2 mV, and at pH 12, with a 72% decrease from -22.5 ± 3.8 mV to -6.3 ± 0.9 mV. Unlike polymer coatings with engineered end groups that react to solution pH,¹¹¹ the graft copolymer tested in this work is observed to passivate the surface with an associated reduction in magnitude of the measured zeta potential, avoiding a complete polarity inversion of the surface charge density at a given pH.

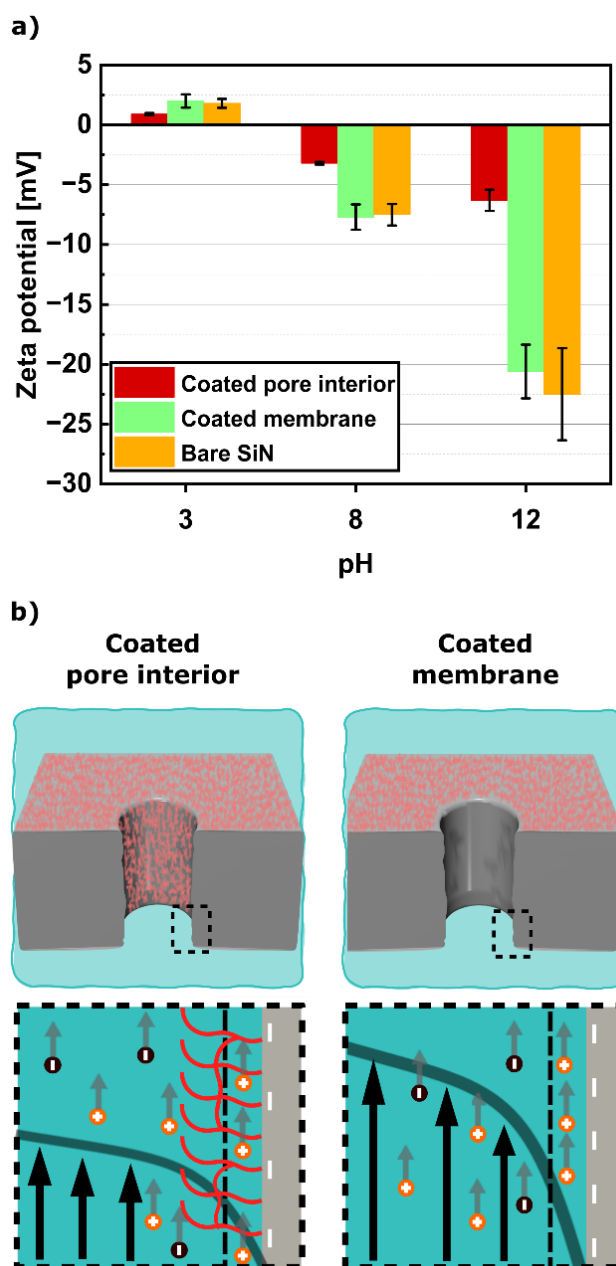


Figure 2.4 Surface characteristics of functionalized nanopores. a) Measured zeta potential as a function of pH for the different devices tested (bare SiN membrane N=10, coated membrane N=2, and coated pore devices N=2). Six data points (at different transmembrane pressures) are collected during a pressure sweep, leading to six zeta potential data points per sweep, with 2-3 sweeps performed for each device tested. Data points are then averaged with a calculated standard deviation for each device at each pH value and the error bars indicate the pooled standard deviation from all devices sharing a common pH and coating type. b) Schematic of the nanopore streaming current for devices under both coating protocols, illustrating the impact of the charge interface at the pore wall on the bulk solution flow.

The observed reduction in zeta potential is attributed to the covalent bonding of the polymer on the surface, which decreases liquid flow inside the channel due to the rigidity and density of these extended structures (Figure 2.4(b)). Another possible explanation could be derived from the surface dissociation reactions (Eqn 2.4 and 2.5). As polymer adhesion to the surface increases, there is a reduction in free dissociated silanol groups, leading to fewer reaction sites at the interface between ions in the Debye layer and the surface. Consequently, this would result in decreased accumulation of counterions, and ultimately a reduced streaming current (and thus zeta potential or calculated surface charge density) being measured for polymer-coated nanopores.

2.3.3 Impact of nanopore functionalization on ssDNA translocation

Finally, in addition to surface characterization measurements, we investigated the changes in translocation characteristics associated with the surface functionalization. Specifically, we explored the anti-clogging properties of these two different strategies by recording the translocation of a linearized 7 knt long ssDNA genome from the M13 phage. This analyte is commonly used as the scaffold for DNA origami nanostructure assemblies and has previously shown a high propensity for clogging bare SiN solid-state nanopores.¹¹⁰ Figure 2.5 summarizes nanopore sensing experiments of M13 ssDNA in both bare and polymer-coated SiN membranes. In a bare 11-nm SiN nanopore, running a 5 nM M13 sample led to a clog event in less than 15 seconds, following 7 translocations (Figure 2.5(a)). In contrast, nanopores featuring an outer membrane coating, with their inner pore surfaces either also coated or left bare, exhibited a significant increase in event counts and sensing durations. Figure 2.5(b) shows the current trace at the start and end of an M13 ssDNA translocation experiment through an 11-nm pore coated only on its outer membrane surface. This pore recorded 3560 translocations of 7 knt long ssDNA M13 over the course of 45 minutes before permanently clogging. Section 2.7.5 of the SI additionally shows data for a 14-nm pore coated on the outer membrane only, with 13 813 events in 3120 seconds of recording.

Similarly, two coated-interior pores (10 and 15 nm) were observed to register 2795 and 10 169 translocations of 7 knt ssDNA over durations of 2680 and 3720 seconds, respectively, before clogging (see Section 2.7.5 of the SI). These results suggest that nanopore clogging behaviors are primarily a membrane surface interaction issue, with the analyte sticking point occurring somewhere outside of the pore interior. This general picture is consistent with the fact that the electric field strength is expected to be much weaker outside the pore than within its inner volume, and so proportionately smaller electrophoretic forces will be available in these regions when attempting to successfully dislodge a molecular clog. Overall, these translocation experiments highlight the effectiveness of surface coatings in extending sensing durations from seconds to ≥ 1 hour (from <10 to ~ 1000 - $10\,000$ events, statistically sufficient for most studies) and shine light on the mechanism of nanopore clogging, *i.e.* of an analyte pinned outside the pore on the membrane and not necessarily stuck on the pore wall itself as proposed by others,^{108,116–118,127} a phenomenon which currently widely impacts the field. Previous studies of nanopore clogging involved fluorescence microscopy to track polymers and conditions of pore clogging, yet the spatiotemporal resolution (~ 0.1 - $1\,\mu\text{m}$, $\sim 1\,\text{ms}$) of these measurements makes it difficult to elucidate where the polymer is pinned relative to the nanometer dimensions of the pore. Furthermore, fluorescent dye labeling of DNA is known to affect its physical properties,¹³⁷ potentially increasing the likelihood of clogging, contrary to our present, label-free investigation of the clogging mechanism.

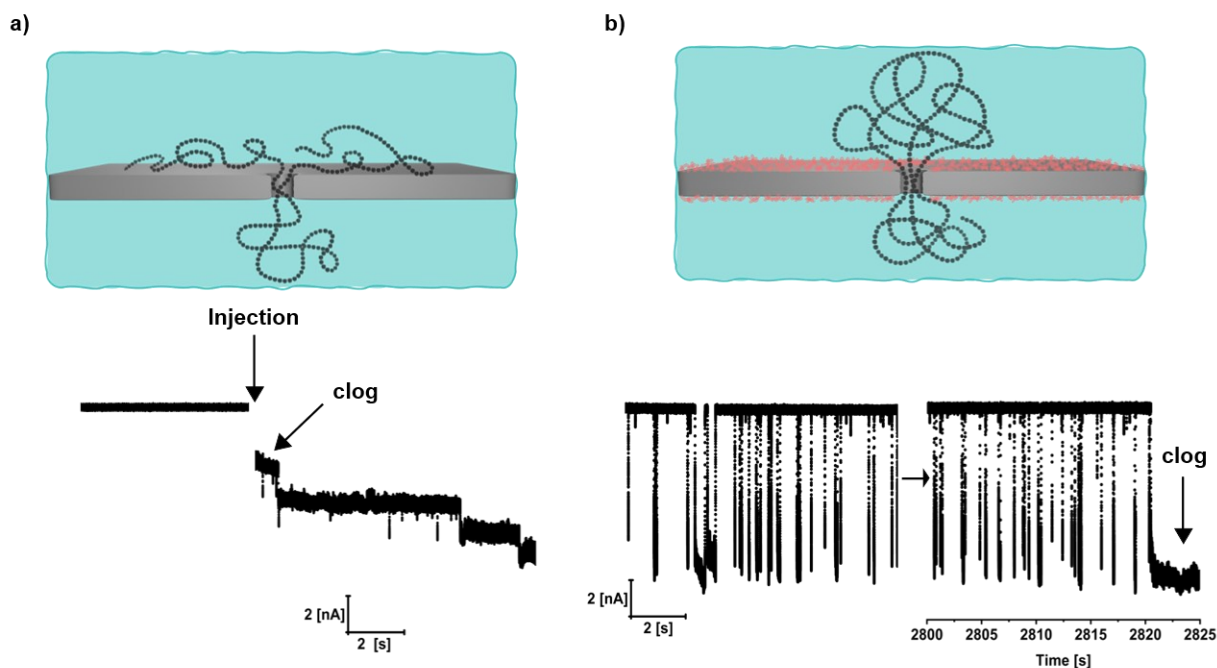


Figure 2.5 Characterization of functionalized SiN nanopores for enhanced sensing duration of ssDNA (M13 scaffold, 7 knt). a) Schematic of ssDNA translocating a bare SiN nanopore device (top) and ionic current traces (200 mV, 3.6 M LiCl) of a bare nanopore (11 nm) before and after ssDNA sample injection (bottom). b) Schematic of ssDNA translocating through a coated-membrane SiN nanopore device, demonstrating its anti-fouling capabilities (top), and ionic current traces (200 mV, 3.6 M LiCl) of a coated-membrane nanopore for the initial 15 seconds post ssDNA sample injection and, later, directly preceding an irreversible clogging event, demonstrating a ~45-minute increase in sensing time after functionalization (bottom).

Building on these observations, Figure 2.6(a) and (b) present scatter plots of maximum current blockage *versus* dwell time for the translocation events of two of the aforementioned coated pores, together representing both types of coating strategy. The smaller diameter nanopore (11 nm), with only its outer membrane coated, exhibited notably longer and more widely varying dwell times when passing M13 ssDNA (with a most probable duration, τ_{peak} , of ~10 000 μ s, see Figure 2.6(a)) compared to a 2-kbp dsDNA control molecule ($\tau_{peak} \approx 100 \mu$ s). In contrast, the larger nanopore (15 nm), with its coated inner walls, exhibited shorter, narrowly distributed M13 ssDNA dwell times ($\tau_{peak} \approx 126 \mu$ s, seen in Figure 2.6(b)). This reduction in dwell time could possibly result from the presence of the polymer coating, which should act to shield the interior walls of the nanopore from interacting with the translocating sample, as well as

to reduce the strength of electroosmotic flow opposing the electrophoretic transport of negative analytes, due to the lower effective surface charge (as characterized by zeta potential in the previous section). To further investigate this behavior, Figure 2.11 presents a scatter plot of max current blockage *versus* dwell time for a 10 nm pore with coated inner walls and 14 nm pore with outer membrane coating. The observed dwell time increase with pore narrowing was found to still hold independently of the coating type, leading us to conclude that this particular dwell time variation is independent of the coating location, but is rather related to the pore diameter. It is interesting to note that the observed blockages for this 7 knt ssDNA are much deeper than the blockage of dsDNA, with both pores showing a comparable mean blockage of $\Delta I_{peak} \approx 8-9$ nA. This value is *ca.* 14-fold deeper than the measured single-file passage of dsDNA which resulted in blockages of $\Delta I_{peak} = 0.6$ nA. This agrees with the previous observations of Kowalczyk *et al.*¹³⁸ that revealed that ssDNA and dsDNA are easily discriminated by their current blockages, with a reported blockage level at 2 nS for 7-kb dsDNA compared to ~6-15 nS for 3.7-knt ssDNA in 1 M KCl pH 8. This implies that the ssDNA translocates in a stretched blob configuration (many segments in the pore at the same time) and not a linearized chain, as, given the highly flexible nature of the ssDNA, the tension front does not fully propagate along the contour length of the polymer.¹⁴

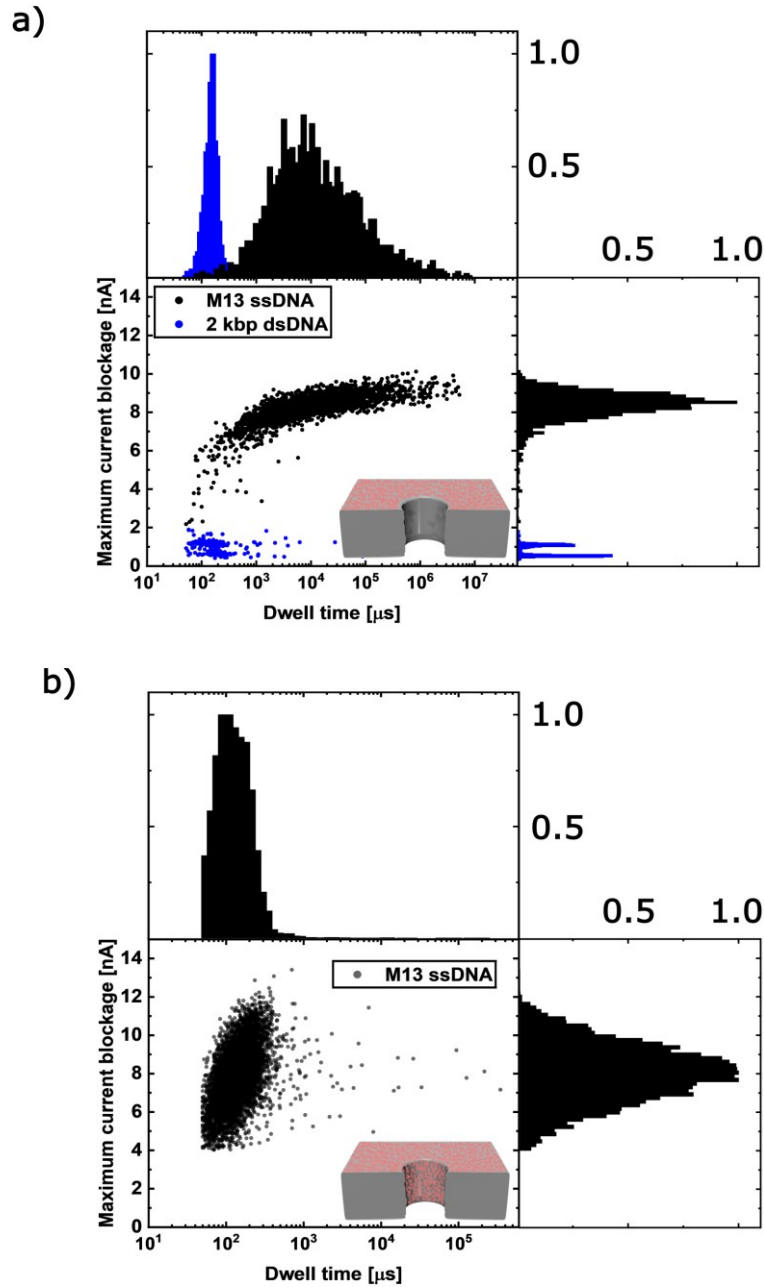


Figure 2.6 DNA translocation characteristics in functionalized nanopores in 3.6 M LiCl pH 8, 200 mV, sampled at 500 kHz and digitally low-pass filtered (8-pole Bessel) at 100 kHz. a) Scatter plot of maximum ionic blockage *versus* passage time for M13 7 knt ssDNA and 2 kbp dsDNA translocation events ($N = 3560$) for an 11 nm nanopore with its outer membrane coated with an antifouling polymer. b) Scatter plot of M13 7 knt ssDNA translocation events ($N = 10\,169$) for a 15 nm nanopore with its supporting membrane and interior wall coated.

2.4 Conclusion

In this study, we presented an improved platform for performing low-noise streaming current measurements through nanopores in order to characterize the pore surface potential and associated surface charge density. Our developed voltage- and pressure-controlled setup enables high-pressure streaming current measurements in the 1-10 picoampere range with stable baselines. This allowed us to investigate the impact of pore size, salt concentration, and pH on the measured streaming current, and the trends of zeta potential and surface charge density with these parameters.

The streaming current platform was then used to characterize the surface of nanopores functionalized with an antifouling polymer coating. By adopting two different protocols tailored to preferentially target the outer membrane surface and/or the pore interior, we shed some light onto the mechanisms of nanopore clogging. To validate the effectiveness of the antifouling coating for extending the sensor lifetime, we translocated M13 DNA, a flexible 7 knt ssDNA genome that tends to otherwise instantly clog most bare SiN pores. Our findings point to one mechanism for “clog events” being associated with analytes irreversibly sticking to somewhere on the outer membrane surface near the pore (close enough for an end of the molecule to be captured), rather than within its interior volume as previously proposed.^{108,116–118} By implementing spatially selective functionalization of the device, we addressed a key issue that has so far limited the widespread use of surface coatings to minimize pore clogging, namely adding antifouling functionality to pores without increasing the low-frequency noise of their ionic current signals. This greatly increases the usefulness of such pores for biosensing, thus paving the way for longer duration applications, including nanostructured DNA molecules for biomarker detection^{134,139} and emerging protein sensing schemes.¹⁴⁰

2.5 Methods

Reagents: Potassium chloride (KCl), potassium hydroxide (KOH), lithium chloride (LiCl), lithium hydroxide (LiOH), hydrochloric acid (HCl), and HEPES (N-(2-Hydroxyethyl)-piperazine-N'-(2ethanesulfonic acid) buffer were purchased from Sigma-Aldrich. All solutions used in this study were prepared with ultrapure deionized water (18.2 M Ω -cm at 25 °C) from a Milli-Q system. Conductivities of all prepared solutions were measured using a conductivity meter (Thermo Fisher Scientific, 018020MD). Solutions of 0.01 M, 0.1 M, 0.25 M, 0.5 M, 0.8 M KCl were diluted from a stock solution of 1 M KCl buffered with 10 mM HEPES at pH 8. Solutions of 0.1 M KCl at pHs of 3, 4.6, 8, 12 were prepared by dropwise titration of HCl or KOH into a master solution of 0.1 M KCl, pH 8 while using a pH meter (SB80PC VWR, 89231-580).

Pressure actuation and sensing: Mechanical pressure regulators were adopted in this study for a pressure actuation range of 0–0.3 MPa (SMC, IR2010-N02-R). Pressure sensing was based on a flow-through pressure sensor (MPS3, Elveflow), providing a voltage reading of 5–10 V that directly translates to a pressure reading using a calibration curve (a 5 V offset with a linear conversion of 10 psi/V was taken from the sensor datasheet).

DNA samples: Linear dsDNA fragments of 2000 base pairs were purchased from Thermo Fisher (NoLimits, SM1701) as our calibration sample. Flexible ssDNA samples were purchased from Tilibit Nanosystems (M13mp18, type p7249). Both samples were mixed with 3.6 M LiCl buffered with 10 mM HEPES at pH 8 to reach a sample concentration of 5 nM in an 80 μ L volume.

Salt bridge: Photo initiator Irgacure or 2-Hydroxy-4'-(2-hydroxyethoxy)-2methylpropiophenone (Sigma-Aldrich, 410896) was first dissolved in 99% (wt/wt) Ethanol (VWR, 200004-488) at a concentration of 10% (w/v), then mixed with Pure PEGDA (Sigma-Aldrich, 437441) and 3 M KCl pH 8, reaching a final PEGDA concentration of 40% (v/v) with a 1% (w/v) photo initiator concentration. This mixture was introduced in ~20 mm long PTFE tubes with an external diameter of 1/32 in. (Cole Parmer) using a low vacuum setup, then exposed to UV light in the 450 nm range until cured. Finally, Ag/AgCl electrodes were

submerged in a vial filled with 3 M KCl electrolyte and connected to a separate sensing vial (containing an electrolyte of variable salt concentration and pH, see Figure 2.1) through the developed PEGDA-filled tubing. This provided an electrical connection between the two vials (≤ 10 k Ω access resistance) while decoupling the direct exposure of the electrode to the sensing solution, thus reducing electrode drift during sensing as well as degradation over time.

Surface coating: PAcrAm-g-PMOXA (poly(acrylamide)-g-(PMOXA₅₀, 1,6-hexanediamine, 3-aminopropyldimethylsilanol)) was obtained from SuSoS AG, Switzerland. The dried sample was dissolved to a concentration of 1 mg/mL in ultrapure water (18.2 M Ω -cm) containing 10 mM HEPES buffer (pH 7.4), then diluted 10 times to reach a polymer concentration of 0.1 mg/mL. Chips were piranha cleaned (3:1 H₂SO₄ and 30% H₂O₂) for 1 hour at 90 °C, followed by a DI water rinse before moving to an RCA1 clean (5:1:1 DI water, NH₄OH, and 30% H₂O₂) for 15 minutes at 80 °C, then dried using N₂. After mounting in a flow cell, one of two coating protocols is followed (see Figure 2.3). For outer membrane surface and pore interior coating, the CBD protocol is first performed in 1 M KCl solution to create a nanopore of the desired size (factoring in an assumed ~ 3 nm reduction in diameter after the later coating step). After a DI water flush, the polymer coating is introduced into the flow cell at a 0.1 mg/mL concentration and incubated for 45 minutes for optimal results. A second DI water flush is required before finally adding the sensing solution for streaming current or DNA translocation experiments. For coating just the outer membrane surface, the 0.1 mg/mL polymer solution is directly injected into the flow cell after mounting the chip containing an intact membrane and is again incubated for 45 minutes. DI water is then flushed to rinse out excess coating polymer and the subsequent CBD pore fabrication step is performed. This results in a nanopore of the desired size featuring bare inner walls and coated outer membrane.

Nanopore sensing: All nanopores used in this work were made on 5 mm square chips featuring 60 $\times$ 60 μ m free-standing membranes of 20 nm thin SiN, deposited at Addison Engineering, Inc (Low stress LPCVD Nitride, film tensile stress $< 250 \pm 50$ MPa) and manufactured in-house at the uOttawa NanoFab

facilities. Ionic current sensing was carried out on a single-channel patch-clamp amplifier (Axopatch 200B, Molecular Devices). An acquisition card (NI USB-6353, National Instruments) was used to interface between the amplifier, the pressure sensor, and the computer.

Signal processing: Streaming current data was processed from the acquisition card and software low-pass filtered (8-pole Bessel) at 10 Hz. This data processing was performed using a custom Python script. DNA translocation data was sampled at a rate of 500 kHz and software low-pass filtered (8-pole Bessel) at 100 kHz. An in-house implementation of the CUSUM+ algorithm is used to extract relevant information from recorded current traces (Nanolyzer, Northern Nanopore Instruments).¹⁴¹

2.6 Acknowledgment

The authors would like to acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC) [funding reference number RGPIN-2021-04304, I2IPJ 586238-23], and Oxford Nanopore Technologies. MYB would like to thank Dr. Shan Zou (Carleton University) for fruitful discussions. We also thank Dmytro Lomovtsev, Gengyang Mu, Matthew Waugh, and Martin Charron for many training sessions around flow cell design, device microfabrication and insightful discussions about streaming current concepts.

2.7 Supporting Information

2.7.1 Dependence of surface charge density on pH at fixed KCl concentration

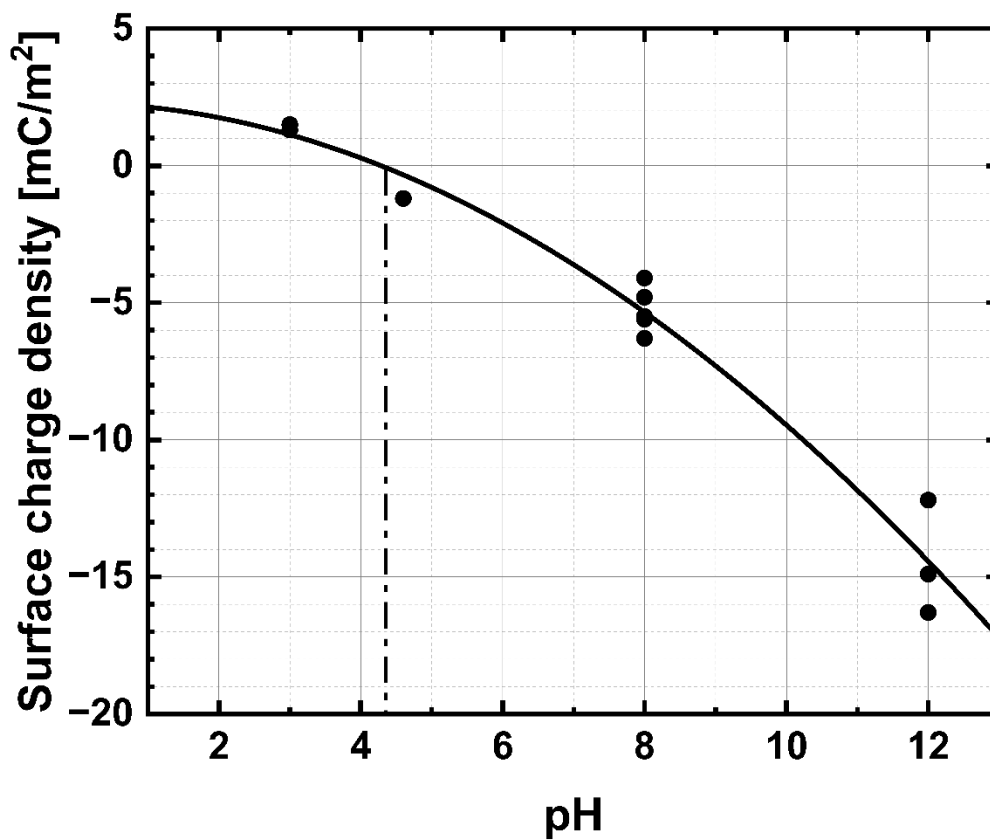


Figure 2.7 Effect of solution pH on surface charge density for SiN nanopores at 100 mM KCl (9 to 25 nm diameter pores in a 20 nm thick membrane, N = 10). The surface charge density is calculated from the zeta potential, based on the streaming current technique.

2.7.2 Dependence of surface charge density on KCl concentration at fixed pH

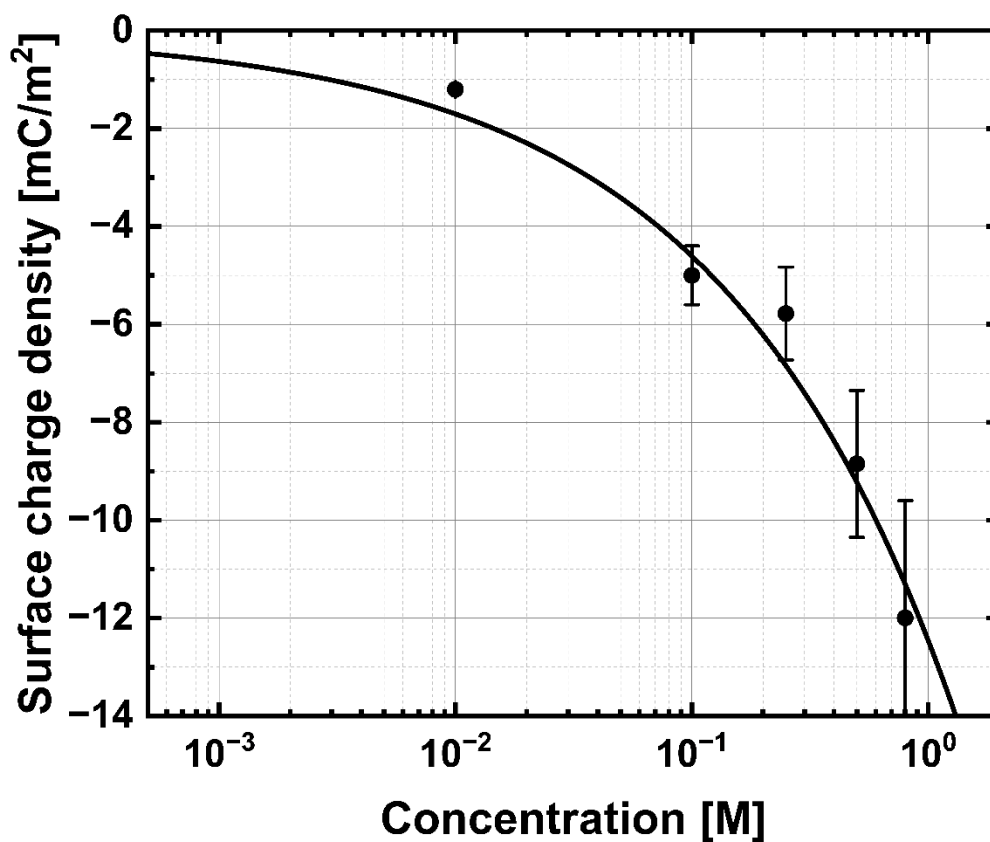


Figure 2.8 Effect of salt solution concentration on surface charge density for an 11 nm SiN nanopore at pH 8. The surface charge density is calculated from the zeta potential, based on the streaming current technique. Six data points (at different transmembrane pressures) are collected during a pressure sweep, leading to six surface charge density data points per sweep, with 2–3 sweeps performed. All the data points are then averaged for each concentration and error bars indicate the standard deviation of the mean.

2.7.3 Conductance extracted surface charge density characteristics

Figure 2.9 depicts the calculated surface charge density from the conductance model² for varying salt concentrations and pH for an 11 nm nanopore. Starting from panel (a), the measured conductance is plotted over a concentration sweep in the 0.01–0.8 M KCl range. Next, panel (b) shows conductance measurements over a pH sweep for the same 11 nm nanopore, with the lowest conductance value approximating the isoelectric point of SiN membranes. From these conductance measurements over a KCl concentration and pH sweep, we extract the surface charge density characteristics of this 11 nm nanopore in panels (c) and (d).

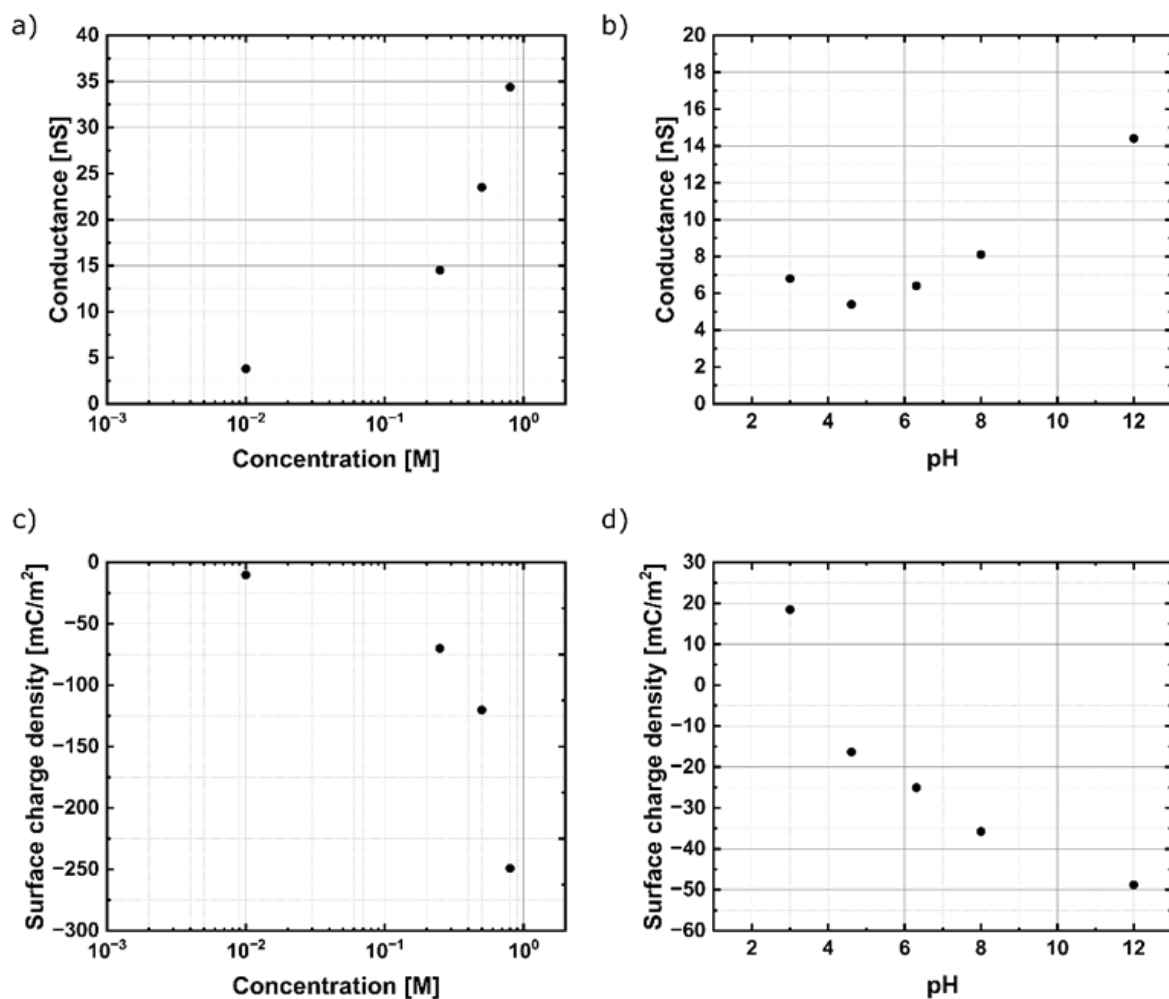


Figure 2.9 Conductance extracted surface charge density for an 11 nm SiN nanopore. a) Conductance measurements over a concentration sweep at pH 8. b) Conductance measurements over a pH sweep in 100 mM KCl. c) Calculated surface charge density from the conductance measurements over a concentration sweep at pH 8. d) Calculated surface charge density from the conductance measurements over a pH sweep in 100 mM KCl.

2.7.4 Dependence of the surface charge density approximation on the measurement distance from the nanopore surface

Streaming current extracted surface charge densities show an underestimation of the surface charge density when compared to the conductance model. Figure 2.10 clearly highlights this trend, where the streaming current extracted surface charge density normalized by the conductance extracted surface charge density is plotted over a KCl concentration sweep at fixed pH.

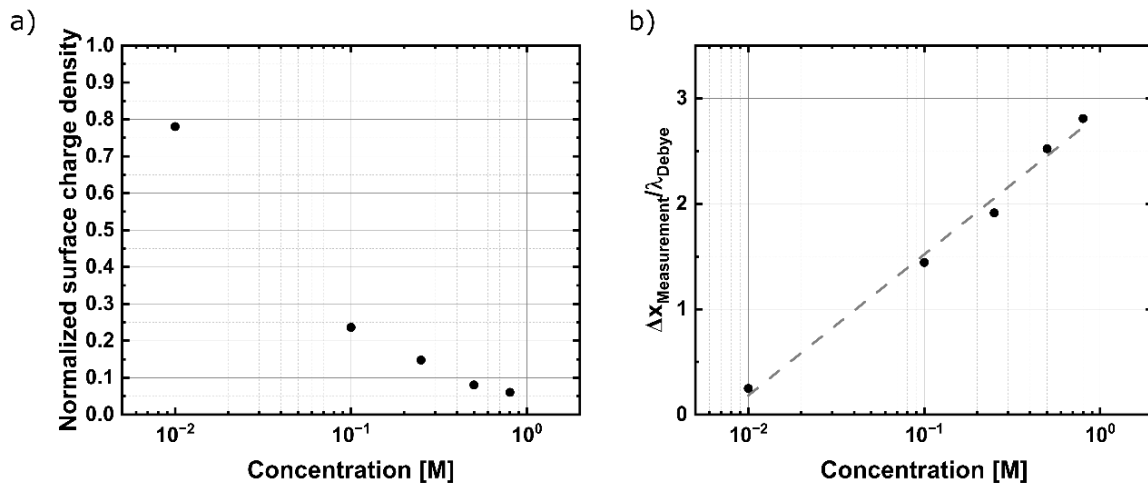


Figure 2.10 Impact of the measurement distance on the accuracy of the streaming current approximation for the surface charge density. a) Surface charge density calculated from the zeta potential measurements normalized by the surface charge density obtained from the conductance model approximation, as a function of the KCl concentration for an 11 nm SiN nanopore (20 nm in length) buffered at pH 8 with 10 mM HEPES. b) Approximated measurement distance between the zeta potential technique and the conductance model normalized by the Debye length over a KCl concentration sweep for an 11 nm SiN nanopore at pH 8. Dashed line is a guide to the eye.

We observe a good agreement between the two techniques at 10 mM KCl pH 8, and a steep exponential decay for higher KCl concentrations at fixed pH 8 (linear on a semi-log). To understand this discrepancy between the two measurement techniques, we fit the normalized surface charge densities to an exponential decay and plot the exponent of the fitted exponential over a KCl concentration sweep (see Figure 2.10(b)). From this panel, we can clearly observe that at 10 mM KCl the measurement distance from the nanopore wall for the streaming current method is below the Debye length at that

concentration. Hence, the two measurement techniques agree. With a constant measurement distance $\Delta x_{\text{Measurement}}$ calculated at 1.1 ± 0.2 nm between the reference point of the conductance model and the streaming current model, the increase in KCl concentration leads to a shorter Debye length. This is against the assumption made when performing streaming current measurements, where the measurement distance away from the wall is assumed to be within this salt concentration characteristic length scale. At KCl concentrations above 10 mM, we observe that this measurement distance is above the Debye length, highlighting the inaccuracy of the surface charge density from the streaming current method compared to the values extracted from the conductance model.

2.7.5 Sensing duration for coated membrane and pore interior during ssDNA experiments for CBD fabricated pores in 20 nm thick SiN membranes.⁵⁷

Table 2.1: Experiment duration and number of events for various coating types

Fabrication	Coating type	Pore size (nm)	Applied voltage (mV)	Experiment duration	Number of events
CBD	None	11	200	10 s	7
CBD	None	14	200	4 s	1
CBD	Pore interior	15	200	3,720 s	10,169
CBD	Pore interior	10	200	2,680 s	2,795
CBD	Membrane	11	200	2,708 s	3,560
CBD	Membrane	14	200	3,120 s	13,813

2.7.6 Dependence of the pore size on the translocation kinetics of ssDNA with coated membrane and pore interior

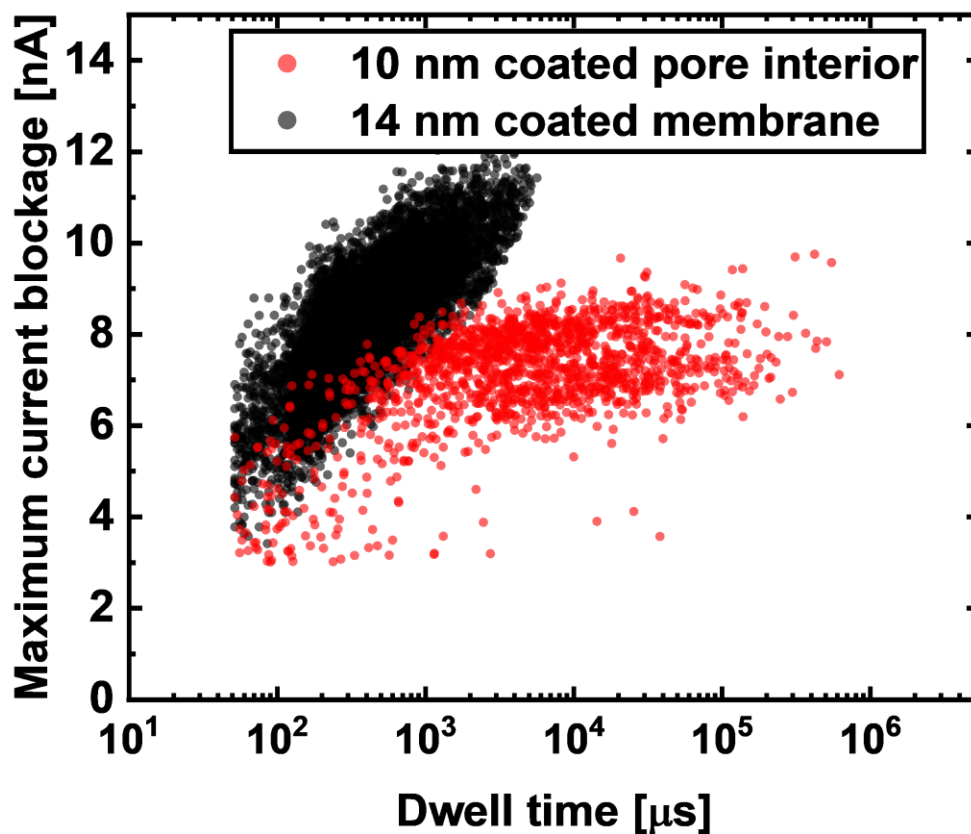


Figure 2.11 ssDNA translocation characteristics in functionalized nanopores in 3.6 M LiCl pH 8, -200 mV, sampled at 500 kHz and digitally low-pass filtered (8-pole Bessel) at 100 kHz, for a 10 nm pore with coated inner walls in red and 14 nm pore with outer membrane coating in black.

3. Pressure-voltage modulation of passage dynamics for enhanced nanopore biosensing

This chapter contains a submitted manuscript titled “Pressure-voltage modulation of passage dynamics for enhanced nanopore biosensing” (2026) by Mohamed Yassine Bouhamidi, Martin Charron, and Vincent Tabard-Cossa.

3.1 Abstract

Solid-state nanopores are established tools for single-molecule analysis, yet their specificity/sensitivity is often constrained by the limited temporal resolution of ionic current recordings, particularly for the short residence times of proteins and of nanostructured barcodes or targets bound to molecular carriers. To overcome this, we employ a platform that utilizes pressure gradients to actively control the forces acting on the analyte, thereby decoupling molecular transport speed from sensing voltage. Our results reveal that combined voltage-pressure forces reduce polymer folding probability and uniformly attenuate the instantaneous velocity profile throughout the translocation process, improving the accuracy of polymer information decoding. Similarly, slowing down low molecular weight proteins (<30 kDa) improves detection rate and provides rich event signatures, useful for their accurate fingerprinting. Furthermore, this platform provides novel insights into the origin of enhanced current blockages and an independent measurement of the effective charge of DNA under V-P trapping. Ultimately, this technique transforms nanopores into a highly tunable instrument for the study of complex protein signatures and barcoded DNA substructures, previously obscured by translocation speed.

3.2 Introduction

Nanopore resistive sensing has emerged as a versatile single-molecule technique for biophysical analysis, offering a label-free method to study molecular size, conformation, and dynamics.¹¹² The sensing mechanism relies on the resistive pulse principle, where a charged analyte, driven electrophoretically by an applied voltage bias, translocates through a fluidic channel, generating a transient, measurable blockage in the ionic current.³

Solid-state nanopores, with diameters in the 2–20 nm range fabricated in thin (10–20 nm) materials such as silicon nitride (SiN) membranes, offer a particularly advantageous platform, for multiple applications from disease biomarker detection to protein sizing and fingerprinting,^{4,26,134} given their stability across wide ranges of operating conditions (e.g., voltage, temperature, solution types) and ability to fine-tune their size to match the analyte of interest.

However, high-fidelity molecular characterization is frequently constrained by the limited temporal resolution of the measurement setup. The challenge originates from the fast passage speed of most biopolymers under the traditional electrophoretic driving force. This issue is especially acute for small proteins in the sub-35 kilodaltons (kDa) range, translocating too rapidly for even the state-of-the-art current amplifiers with ~1 MHz bandwidth, resulting in many events being missed or severely distorted due to instrument temporal resolution limitations.^{93,99} As many fast (<10 μ s) translocation events or sub-levels go undetected or attenuated, strategies for achieving precise control over molecular kinetics are greatly needed and continue to be an active research topic.¹⁴²

Research efforts to overcome these limitations fall into two principal categories: advancement of instrumentation temporal resolution and active control of molecular kinetics. On the instrumentation front, significant progress has been made through custom-designed, low-noise electronics, which minimize input capacitance and noise contributions. These high-bandwidth systems, together with

advanced chips and fluidic cells designed to minimize input capacitance,^{73,75,76} have led to ultra-high bandwidth recordings. For example, Shekar *et al.*⁶⁷ reached 5 MHz and resolved transient features of single-stranded DNA (ssDNA) lower than 200 ns, thereby extending the observable time scale significantly beyond the 10 μ s limit in off-the-shelf patch-clamp systems akin to the omnipresent Axopatch 200B. Although high-bandwidth instrumentation is preferred, a trade-off between signal amplitude and noise performance, the latter scaling quadratically at high frequencies,^{68,143} must be considered, and in practice most data sets acquired with MHz amplifiers are low-pass filtered at 200–500 kHz.

Complementary to electronic bandwidth enhancement are strategies focusing on physical and chemical manipulation of the analyte or nanopore environment to promote longer and better resolved translocations. Reducing the nanopore diameter, for instance, significantly increases pore-polymer interactions, and as such results in significantly longer passages.^{12,144–146} Furthermore, coating nanopores with fluid lipid bilayers has been shown to reduce protein translocation speed through ligand bindings.¹⁰² Environmental factors are modulated by manipulating parameters like temperature or viscosity,^{79,80,147,148} where high viscosity using organic-water mixtures successfully slows the kinetics of viral vectors, enhancing their discriminability based on single-nanometer size differences.⁸¹ However, increasing viscosity often results in a decreased conductivity and thus reduced signal-to-noise ratio (SNR). Another successful strategy involves applying gel media, specifically polyacrylamide, onto the *trans* side of the membrane to introduce mechanical and steric hindrance, shifting the mean DNA translocation time by an order of magnitude for short double-stranded DNA (dsDNA) fragments.⁸² Salt type or concentration manipulation can be used to slow down passage,² such as using Li⁺ ions, which reduce the net molecular charge compared to Na⁺ or K⁺ cations, albeit at the cost of lowering capture rates,¹⁵ or using asymmetric salt concentrations, which modulate the electric field distribution, although this time, enhancing capture rates.^{7,84,146} Trapping mechanisms are also relevant to improve residence time of analytes inside pores, such as optical tweezers and nanopore electroosmotic traps (NEOTrap).^{85,149,150}

Despite the utility of these methods, the inherent coupling of the electrophoretic driving force (voltage) to both molecular transport and signal readout fundamentally limits the specificity/sensitivity of solid-state nanopore sensors. The introduction of a pressure gradient (ΔP) across the membrane can resolve this issue by establishing a Voltage-Pressure (V–P) capture regime, which allows for the molecular speed to be tuned by the applied pressure, independently of the sensing voltage.⁹² Note that we use, as a convention, the acronym V–P when referring to a voltage-driving, pressure-opposing transport and vice-versa for the P–V acronym. This counter-force approach is useful in addressing detection limits for small analytes, exemplified by the detection and rate enhancement of small proteins with molecular weights as low as 6.5 kDa (Aprotinin).⁹¹ By balancing the pressure-derived force with a retarding voltage, a P–V system can significantly increase the passage duration, simultaneously improving the temporal resolution and modulating the capture rate. For dsDNA, the P–V method has been shown to achieve speed reductions of 2–3 $\times$, improving length discrimination.⁸⁷ Mechanistically, the balanced opposing forces establish a tunable P–V trap near the nanopore entrance, which facilitates the recapturing and trapping of single molecules for detailed dynamic study.⁸⁶

Despite the clear evidence supporting the efficacy of the P–V method in addressing the limitations of fast translocations and detection sensitivity for DNA and small proteins, a comprehensive study that quantitatively explores the capabilities of this approach across a spectrum of challenging analytes, including DNA nanostructures and differently sized proteins, remains necessary to improve our understanding of the transport properties under V–P and P–V regimes. In this work, we perform a detailed investigation of translocation kinetics, polymer folding, instantaneous velocity profiles of nanostructured DNA, and protein detection rates, while clarifying inherent effects of enhanced current blockages.

3.3 Results and Discussion

3.3.1 Forces at The Nanopore Interface

Understanding the forces governing biomolecular translocation is a prerequisite to precisely controlling capture dynamics in solid-state nanopores.^{25,84,149,151} Biomolecules such as DNA or proteins carry a net charge, the latter also more dependent on the solution pH. In response to the electric field E inside and outside a nanopore, charged biomolecules undergo electrophoretic (EP) motion (Figure 3.1(a).i) due to a linear force density F_{EP} :²²

$$F_{EP} = q_{eff}E, \quad (3.1)$$

where q_{eff} is the effective linear charge density of the biomolecule (*e.g.* the effective charge of a DNA base pair).

In parallel, nanopore walls are also charged, with a charge density dependent on the membrane material and solution conditions,^{100,103} inducing a non net-neutral distribution of counter-ions up to a distance referred to as Debye length, λ_D . The movement of the hydrated shielding counter-ions under an applied electrical field induces an electroosmotic (EO) flow (Figure 3.1(a).ii) whose fluid velocity at the pore center is expressed to first order as:²⁵

$$v_{EO} = -\frac{\varepsilon E \zeta}{\eta}. \quad (3.2)$$

Here ε is the salt solution permittivity, E is the applied electrical field, ζ is the zeta potential at the nanopore wall, and η is the solution viscosity. As the salt concentration increases, the decrease in λ_D leads to a diminishing number of free counter-ions near the nanopore surface within this distance, effectively reducing electrostatic interactions and the magnitude of the flow generated. EO flow profile is generally flat (plug-like) across the pore, unless the Debye length becomes comparable to the pore dimensions, and

the electric double layers overlapping leads to a curved profile. This EO flow induces a hydrodynamic drag force on the molecule.

In this work, a transverse pressure gradient is applied across nanopores to introduce voltage-independent molecular control. To first order, this pressure difference induces a Poiseuille flow (parabolic profile) inside the cylindrical nanopore with an average velocity defined as:⁸⁷

$$v_{\Delta P} = \frac{\Delta P \cdot d_{pore}^2}{32 \cdot \eta \cdot L_{pore}} \quad (3.3)$$

where ΔP is the applied pressure across the nanopore, and d_{pore} and L_{pore} are the nanopore diameter and length respectively, assuming laminar flow and a uniform cross-section along the length of the channel. Because of the continuity of the fluid flow, and similar to the electric field, a radially decaying liquid flow velocity is expected to extend outside the nanopore, and can thus promote or hinder molecular entry depending on the direction of the applied pressure (Figure 3.1(a).iii). The force generated by the pressure gradient on a DNA polymer of diameter d_{DNA} can be expressed as:

$$F_{\Delta P} = \frac{\Delta P \cdot \pi \cdot d_{DNA}^2}{4} \quad (3.4)$$

Furthermore, a viscous force arises from the interactions between the DNA polymer and the nanopore walls due to an opposing fluid motion. This viscous force is expressed as:

$$F_{viscous} = \frac{\pi}{\ln\left(\frac{d_{DNA}}{d_{pore}}\right)} \frac{\Delta P \cdot (d_{DNA}^2 - d_{pore}^2)}{8} \quad (3.5)$$

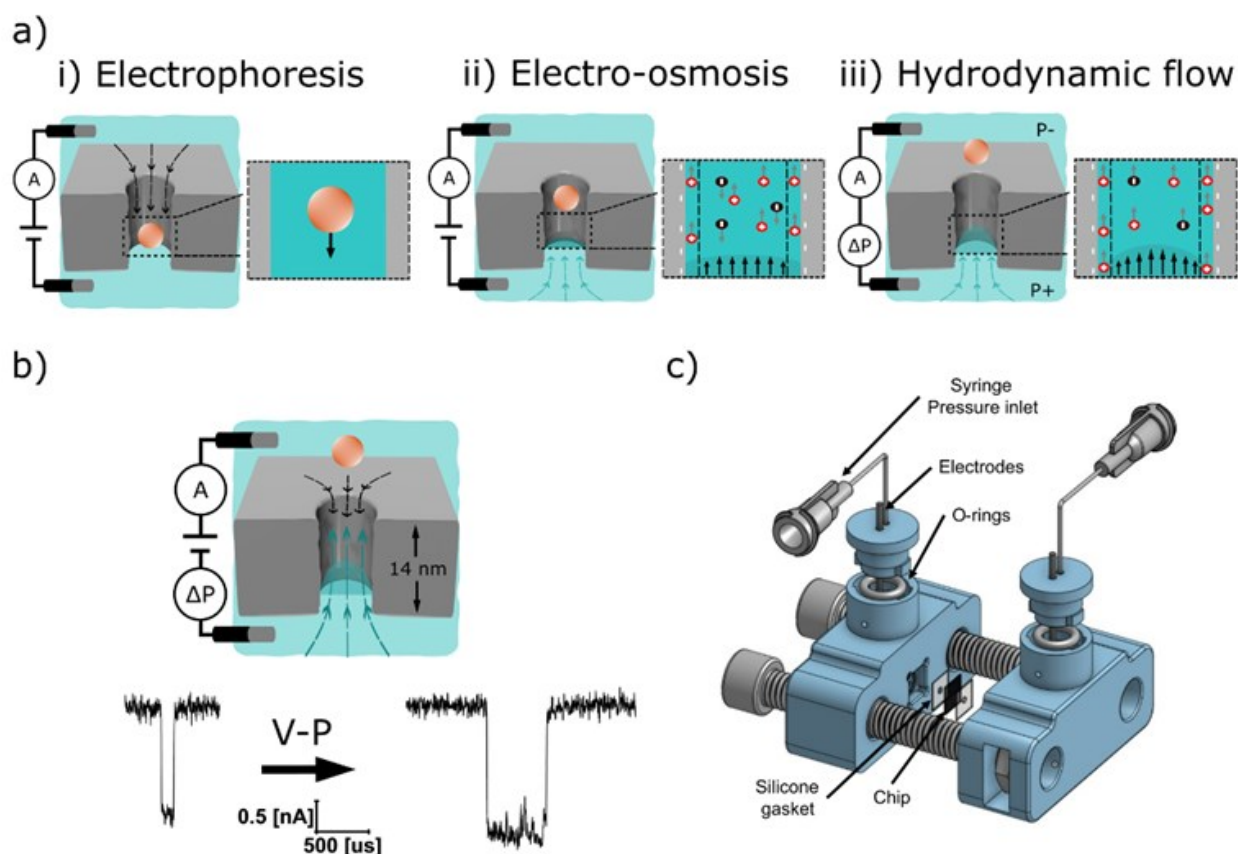


Figure 3.1 Forces at play during translocation and instrumentation schematic. a) Schematic of the forces dominating during solid-state nanopore sensing with the orange sphere as a model biomolecule: i) represents the electrophoretic driving force, ii) the electroosmotic flow opposing the applied electrical potential, when the biomolecule and pore have the same sign of surface charge, and iii) the hydrodynamic flow due to an applied pressure, hindering capture. b) Schematic highlighting the working principle of the V-P sensing regime inside a nanopore (top), where an increase in opposing pressure leads to an increase in temporal resolution (bottom). c) Drawing of the millifluidic flow cell used for V-P sensing and its 3D-printed components, housing a SiN chip.

Because pressure-driven flow and EO flow exhibit distinctly different velocity profiles, the net fluid velocity, $v_{net}(r)$, varies with radial distance from the pore wall, similar to the radial distribution of the electrical field.¹⁵² While a theoretical trapping condition can be found at the exact center of the pore where opposing forces balance, this state is spatially unstable. If the biomolecule drifts off-center, it can encounter a velocity gradient that disrupts the force balance and can cause the molecule to stretch or tumble as it translocates, depending on its rigidity.

Figure 3.1(b) illustrates the forces applied on a negatively charged spherical molecule translocating a nanopore under a voltage driving—pressure opposing (V–P) transport regime. The nanopore walls are negatively charged in the experimental conditions used herein and the *trans* side is taken as the reference for voltage and pressure. A pressure difference of 0 MPa here refers to uniform atmospheric pressure. To enable control on the pressure force in tandem with electrophoretic capture, we improved on the pressure-driven system previously reported,¹⁵³ by adopting the Luer-lock connector method,¹²⁹ leading to a reduction in sensing volume from 1.5 mL to 40 μ L while maintaining a similar accessible pressure range (± 0.3 MPa in steps of 0.1 MPa) as shown in Figure 3.1(c).

3.3.2 DNA Capture, Translocation Dynamics, Folding and Electrophoretic Mobility

To quantify the forces at play during nanopore sensing and validate the capabilities of our pressure setup to modulate molecular capture and translocation speed, a 2000 base pair (bp) double-stranded DNA (dsDNA) sample was introduced on the *cis* side of an 11 nm nanopore in 3.6 M LiCl pH 8 under a -200 mV potential and under different applied pressures ranging from -0.2 to 0.2 MPa. Figure 3.2(a).i-iii) specifically illustrate the modulation of dsDNA capture dynamics via current traces recorded under pressures of 0.1, 0, and -0.1 MPa, respectively. Under an applied potential (Figure 3.2(a).ii), capture is driven solely by electrophoresis. When an additive pressure is introduced (Figure 3.2(a).i), a net increase in capture rate is observed, as visualized by the increased frequency of transient current blockades. Conversely, an opposing pressure drastically hinders the capture rate (Figure 3.2(a).iii).

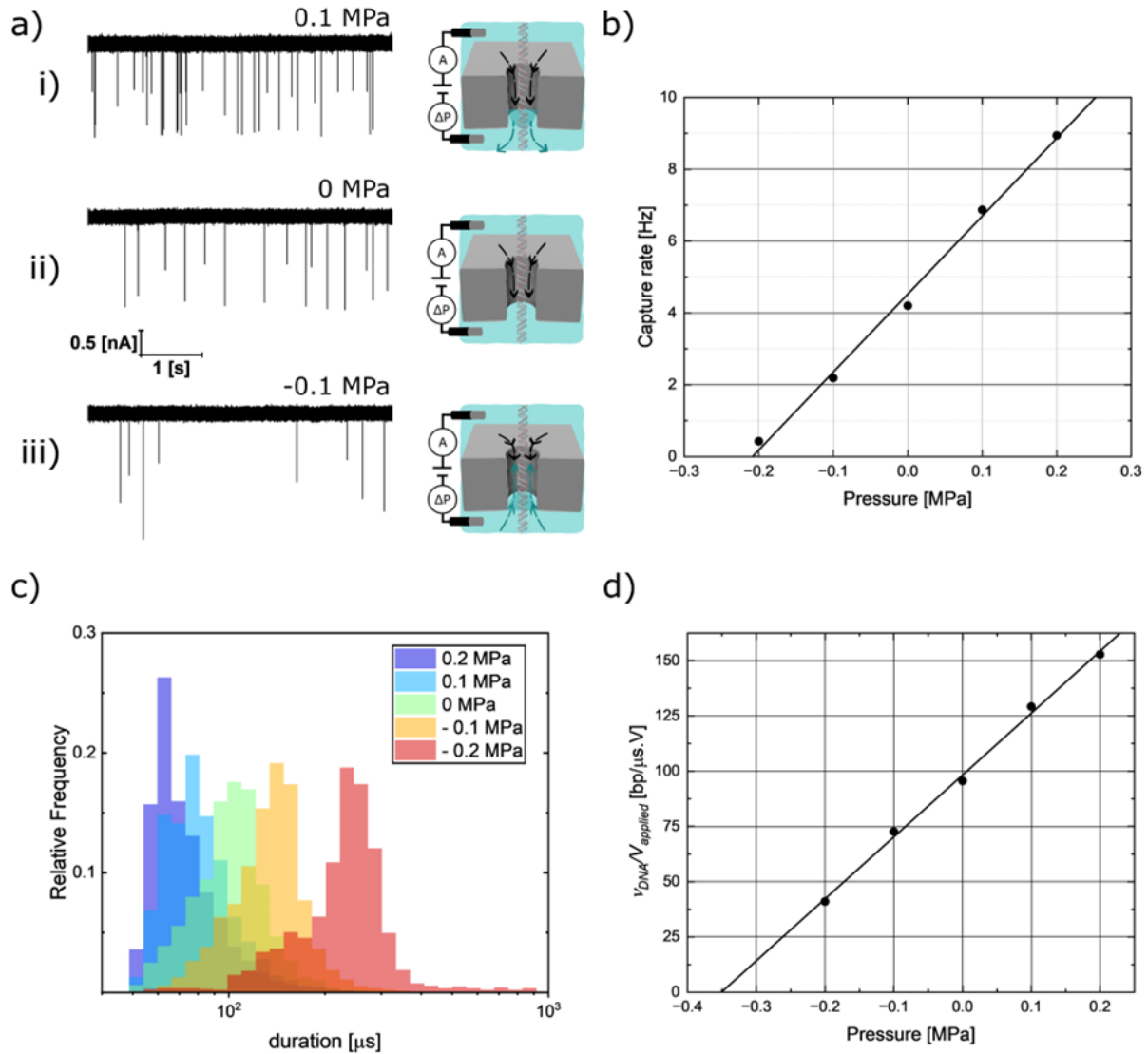


Figure 3.2 Effect of pressure on the dynamics of translocation of dsDNA under an applied voltage in SiN nanopores. a) Representative current traces of a 5 nM 2-kbp dsDNA sample in 3.6 M LiCl pH 8 at a fixed -200 mV applied voltage and varying pressure: i) enhancing capture rate by applying a driving 0.1 MPa pressure, ii) 0 MPa to provide a reference point, and iii) hindering capture rate by applying an opposing -0.1 MPa pressure in an 11 nm nanopore. b-d) Dependence of applied pressure ΔP on capture rate, translocation duration, where a negative pressure represents an opposing force to the applied electrical potential, and translocation speed. The linear fit in panel d) provides the intercept where the balance of forces should be reached at -0.35 ± 0.01 MPa, effectively trapping 2 kbp dsDNA.

The quantitative dependence of capture rate R on applied pressure ΔP under a fixed -200 mV potential is detailed in Figure 3.2(b), showing a linear relationship between R and ΔP . Under no applied

pressure, $\Delta P = 0$ MPa, the electrophoretically driven capture rate is measured at 4 Hz (0.8 Hz/nM). The superimposition of an additive pressure on the electrical potential increases the capture rate linearly to 7 Hz (0.1 MPa) and 9 Hz (0.2 MPa) respectively. As expected, the opposing pressure decreases the capture rate linearly, reducing R to < 0.43 Hz at a pressure of $\Delta P \leq -0.2$ MPa.

Figure 3.2(c) presents histograms detailing the temporal shift in the translocation duration (τ_{duration}) for 2 kbp dsDNA as pressure is varied and voltage is kept fixed. At neutral pressure ($\Delta P = 0$ MPa), the mean translocation duration is observed at $\tau_0 = 109 \pm 32$ μs , extracted as the mean of a Gaussian fit to the distribution of passage times. Applying an additive pressure (positive ΔP) accelerates the passage speed, reducing the mean duration to 81 ± 29 μs (0.1 MPa) and 68 ± 25 μs (0.2 MPa) respectively. On the other hand, the opposing pressure effectively reduces translocation speed, with a mean duration of 150 ± 34 μs (-0.1 MPa) and 257 ± 69 μs (-0.2 MPa). This reduction in translocation velocity for the V-P regime (-0.2 MPa) compared to electrophoretic transport (0MPa) represents an average $2.4\times$ enhancement in temporal resolution, extending the time available to sample the ionic current blockage signal.

Similarly, Figure 3.2(d) plots the average translocation speed of DNA normalized by the applied voltage *versus* the applied pressure, where average speed v_{DNA} is calculated by dividing the 2 kbp dsDNA length by the mean translocation duration $\tau_{\Delta P}$. Linear dependence is observed between the average DNA speed and the applied pressure, described by $v_{\text{DNA}} = 56 \times \Delta P + 20 \left[\frac{\text{bp}}{\mu\text{s}} \right]$ before normalizing by the -200 mV applied voltage, confirming the additive nature of the forces acting on captured and translocating molecules. By extrapolating the linear fit, our experimental data suggests that the stalling pressure ΔP_{stall} , *i.e.* the pressure resulting in a null DNA velocity, corresponds to -0.35 ± 0.01 MPa for a driving voltage of -200 mV. Interestingly, we note that by balancing the theoretical forces (Equations 3.1 & 3.4–3.5) assuming a DNA polymer at the center of the pore and solving for a null velocity, we can extract an effective dsDNA charge of $q_{\text{eff}} = 0.20 \pm 0.05 e^-/\text{bp}$, which matches the expected charge for the Li^+ ions, which screen DNA more effectively than K^+ cations.^{15,149,154} The effective DNA charge derivation is

available in section 3.7.5 of the SI. Although the focus here was placed on the average DNA speed, a non-uniform velocity profile is expected with the initial and final segments of the polymer translocating faster than the intermediate one.¹⁵¹ We note here that these fitted parameters are dependent on the generated pressure gradient, specifically for the pore size and salt condition adopted here.

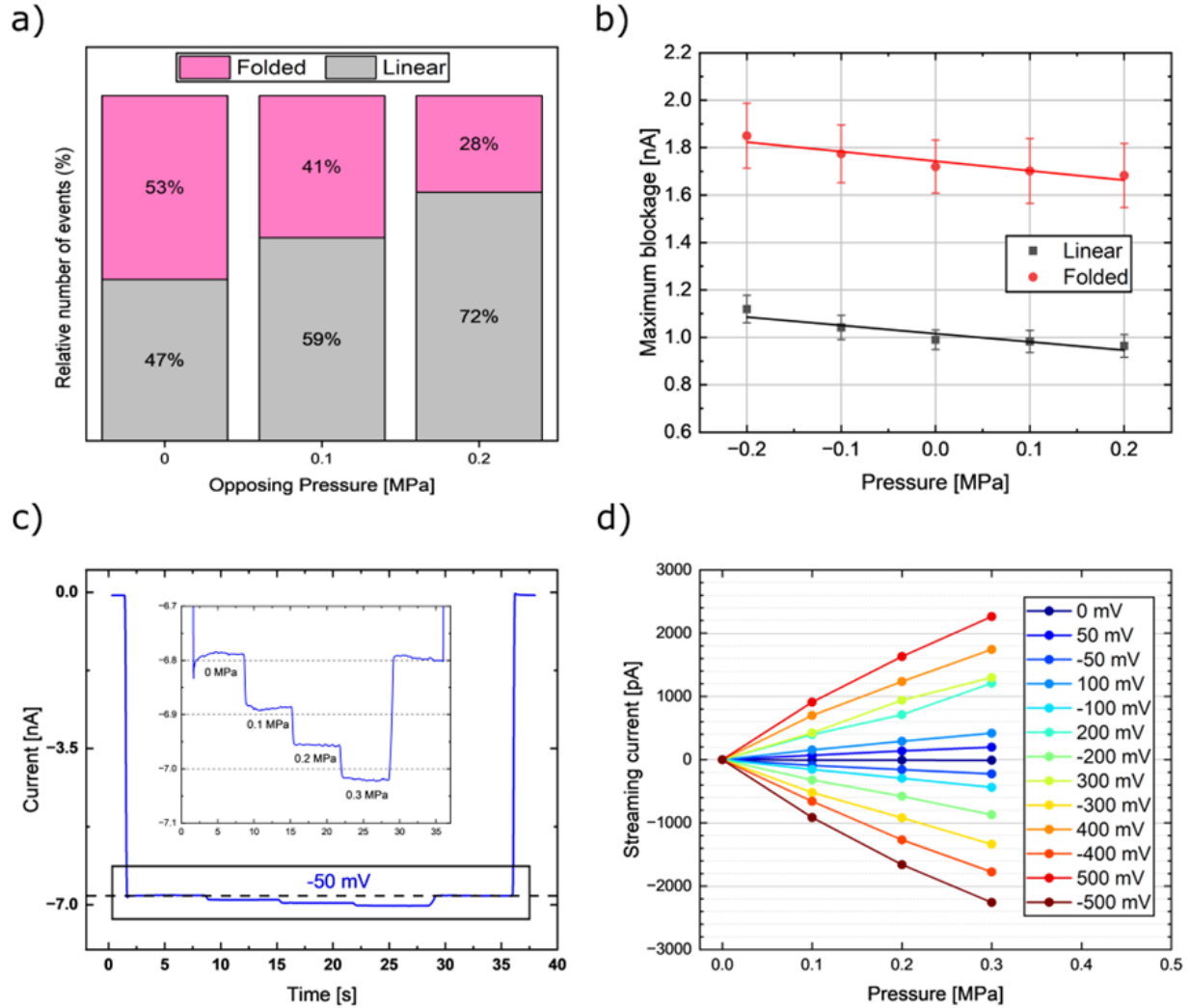


Figure 3.3 Effect of pressure on the folding and current blockages of dsDNA, and streaming currents under an applied voltage in SiN nanopores. a) Relative number of 2kbp dsDNA linear and folded events as a function of opposing pressure at a fixed -200 mV. b) Maximum blockage of 2kbp dsDNA linear and folded events as a function of applied pressure at a fixed -200 mV, with a 15% increase in fitted slope for the folded events compared to the linear ones. The points represent the mean of a Gaussian fit to the distribution of events with the error bars as the standard deviation. c) Current trace of streaming current measurements at -50 mV in a different 16 nm SiN nanopore. Inset: Zoom-in view of the streaming current contribution during 0.1 MPa pressure steps. d) Extracted streaming current measurement from panel c) with a 0-0.3 MPa sweep in net pressure, and 0-500 mV sweep in applied voltage.

While similar observations of speed reduction by opposing a counter pressure to the voltage driven passage have been reported previously by others,^{87,92,155} the changes in folding dynamics remain less explored.⁸⁸ It is established that lower salt concentrations increase the rate of passage of unfolded polymers (events that translocate in a single file configuration). This behavior was explained by an enhanced EO flow at low salt counteracting the EP force and by a reduction of counterions screening the charged nanopore surface and dsDNA.¹⁵⁶ Here, since an applied pressure can also generate a hydrodynamic flow, we investigated how pressure affects folding dynamics, by recording the fraction of unfolded events during V–P sensing of 2 kbp dsDNA through an 11 nm nanopore (Figure 3.3(a)). As a control, the EP driven transport of dsDNA in 3.6 M LiCl at –200 mV shows a 47% unfolded events fraction. Increasing the opposing pressure from 0 to 0.1 MPa increases the unfolded fraction to 59%, eventually reaching 72% at 0.2 MPa. This suggests that hydrodynamic forces, whether electroosmotic or pressure-driven, effectively stretch the extended polymer outside the nanopore, offering a method to linearize molecules without compromising SNR associated with low-salt buffers.

Next, interestingly, we observed a shift in maximum current blockage at –200 mV while varying the pressure. Starting at the reference condition ($\Delta P = 0$ MPa), the maximum current blockage for the unfolded level was measured at 990 ± 41 pA, extracted as the mean and standard deviation of a Gaussian fit to the distribution of unfolded current blockages. As shown in Figure 3.3(b), increasing the applied pressure (0.1 to 0.2 MPa) shifted the unfolded level's maximum current blockage upward under pressure opposing motion conditions (1042 ± 51 to 1119 ± 58 pA) and downward under pressure driving motion conditions (983 ± 47 to 964 ± 48 pA). Such pressure-dependent blockage amplitudes have been previously reported by Sharma *et al.* albeit with phenomenologically different behavior and dependences.^{88,157} Here, we postulate that the variation in current blockage during V–P sensing is primarily driven by a coupled electro-hydrodynamic effect.

Reducing salt concentration is known to reduce the blockage depth and even produce a conductance enhancement below a certain value for electrophoretically driven dsDNA, which is explained by surface potential effects.² At neutral electric potential, a streaming current is observed upon applying a pressure gradient which decays considerably at high salt concentration.¹¹⁵ Under V–P regime, this streaming current is thus combined with the sensing condition where a fixed voltage is required to drive molecular translocation.

It was also shown that under an applied bias in angstrom-scale slits, operated in the 1–300 mM KCl concentration range, the magnitude of the streaming current is enhanced.¹⁵⁸ For comparison, our sensing condition leads to a 100–200× higher streaming current magnitude under a voltage bias (200–500 mV) compared to the 0 mV control, pointing to increased counter-ion electrophoretic mobility under this coupled V–P biasing regime.

To explore this further, we used another 16 nm nanopore in 3.6 M LiCl pH 8 biased at –50 mV and swept the pressure with 0.1 MPa steps (Figure 3.3(c)). The streaming current was extracted by subtracting the voltage contribution from the ionic current and averaging the current magnitude for each pressure step. Figure 3.3(d) illustrates the streaming current measured under a wide range of applied voltages using the same pressure sweep. Below ± 100 mV, a symmetrical magnitude below 400 pA is measured. However, by extending the applied potential to the 200–500 mV range, we observe streaming current values >1 nA for this nanopore geometry. These observations support the interpretation that the variations in current blockage during V–P sensing are due to counter-ion dynamics along the DNA surface.

In this section, we clarified the impact of V–P sensing on key metrics such as capture rate, translocation dynamics, folding and electrophoretic mobility. Equipped with a clearer foundation for the physics of this pressure-controlled sensing regime, we can now move to explore the dynamics of barcoded nanostructures and subsequently proteins.

3.3.3 Instantaneous DNA Translocation Velocity

We now report on the effect of motion-opposing pressure on the instantaneous translocation velocity of electrophoretically driven DNA polymers. To achieve this, we utilized Velocity Profiling Molecules (VPMs), as recently described and reported in our previous work.¹⁵¹ VPMs are DNA origami nanostructures, assembled with a 7249 nucleotide-long single-stranded DNA and 171 40-nt oligomer staples, whose structure consists of seven dsDNA segments interspaced by six three-helix bundle (3HB) segments (Figure 3.4(a)). Due to different cross-sectional areas, the dsDNA and 3HB segments result in different blockage amplitudes. The duration of the easily identifiable and delimited segments within blockade traces can then be used to estimate the instantaneous translocation velocity of individual VPM translocations. This approach allows for the mapping of the polymer's instantaneous velocity as a function of its fractional contour distance (FCD), the profile and fluctuations of which are closely related to a polymer's segment at the onset and throughout the translocation process.^{151,159,160}

To assess the effect of pressure on electrophoretically driven translocation velocity profiles, VPM samples were first driven inside a 16 nm SiN nanopore by a -200 mV potential difference with no pressure. A representative current trace of a single VPM blockade is shown in Figure 3.4(b).i. Subsequently, a $\Delta P = -0.1$ MPa pressure was introduced to achieve a net deceleration of the nanostructure by superimposing an opposing hydrodynamic flow, as shown by the significantly longer representative current trace shown in Figure 3.4(b).ii.

Figure 3.4(c) quantitatively compares the averaged instantaneous velocity versus FCD measured for both transport regimes. In both conditions, two-step non-monotonic velocity profiles were observed wherein deceleration occurs at the start of translocation before accelerating when the tension front reaches the end of the polymer, as expected from tension propagation principles. Under purely electrophoretic transport ($\Delta P = 0$, red line), VPMs exhibited a velocity profile ranging from 11.6 bp/ μ s to 15.5 bp/ μ s as the polymer traversed the pore. Applying a $\Delta P = -0.1$ MPa counter-pressure (black line)

reduced the velocity profile across the entire polymer length, dropping the velocity of the initial and final segments to the 4.5 bp/ μ s–5.6 bp/ μ s range, a 3-fold reduction.

By slightly increasing the opposing pressure to 0.16 MPa to further slow the translocation velocity, a balance of forces is reached, and the DNA nanostructure is temporarily trapped inside the nanopore, effectively reaching a near-zero velocity until the pressure is removed (section 3.7.1 of the supplementary information). Nevertheless, before stalling the polymer, a $\sim$ 3-fold increase in the mean translocation time (τ) can be reached, which directly enhances the temporal resolution available for picking up structural information required for enhancing DNA barcode decoding.^{139,161}

To outline and compare the relative velocity fluctuations in both experimental conditions, Figure 3.4(d) plots the velocity profiles normalized by the end-segment velocities, which reveals an overlap between the control and pressure-hindered regimes. The overlapping of velocity profiles indicates that the nanostructure conformation is not significantly altered throughout the translocation process by the presence of the pressure-driven hydrodynamic flow.

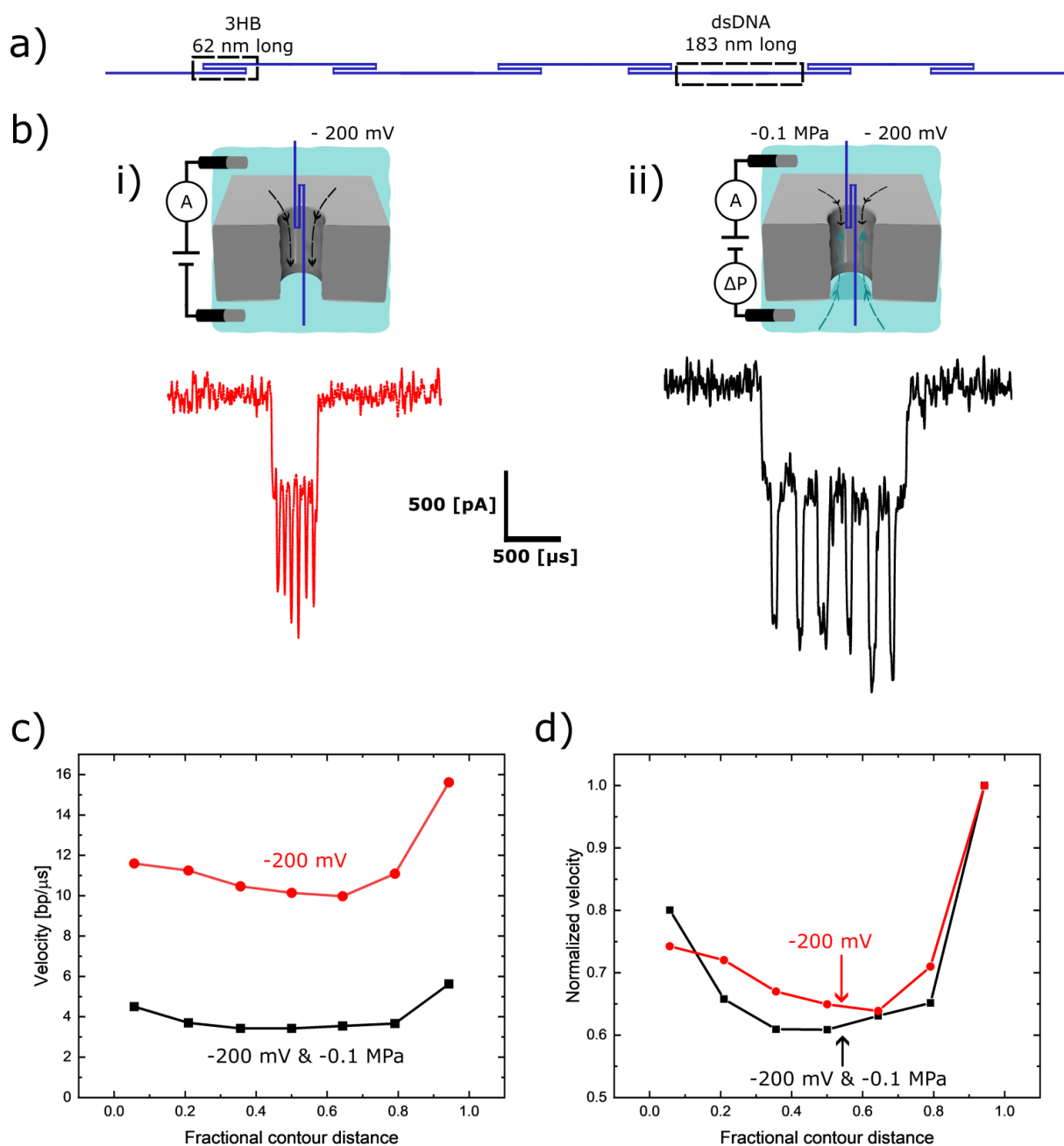


Figure 3.4 Characterization of Velocity Profiling Molecules under an opposing pressure in a 16 nm SiN nanopore. a) Schematic of the assembled VPM DNA nanostructure. b) Schematic of a VPM translocating through a nanopore under an applied electrical potential i) with no applied pressure and ii) a 0.1 MPa opposing pressure, and their associated representative event signatures. c) Velocity profile fluctuations of 5 kbp VPM in 3.6 M LiCl pH 8 at a fixed -200 mV, under an additional opposing pressure of 0.1 MPa (N = 135, 50 unfolded full length VPMs respectively). d) Normalized (by final velocity) dataset used in c).

3.3.4 Enhanced Detection of Proteins: GFP and Cytochrome C

Solid-state nanopores can be used for label-free protein analysis, specifically to determine their volume, shape and flexibility.^{95,100,101,112} However, the fast voltage-driven translocation velocities compromise the temporal resolution needed to extract accurate protein information.⁹³ By adopting a combination of higher bandwidth hardware and nanopore aspect ratios comparable to the protein of interest, detection of proteins such as proteinase K and RNase was achieved.⁹⁹ Nevertheless, most events were still translocating faster than the bandwidth limit of the hardware employed while minimizing amplitude error $<3\%$ ($t_{min} \cong 12 - 20 \mu\text{s}$ for a $f_c = 250 \text{ kHz}$), likely leading to distorted event signatures.^{143,162} Furthermore, by performing a voltage sweep, the observed capture rate decreased at a critical voltage, reaching a bandwidth limited regime. Previous attempts to improve protein analysis through pressure-voltage modulated capture led to similar results, where the majority of events remained faster than the bandwidth limit.^{90,91}

Our implementation of the V–P regime addresses these critical challenges in protein analysis, specifically for small proteins like green fluorescent protein (GFP, 27 kDa, with net negative charge of -6)¹⁰⁰ and cytochrome c (12 kDa, with a net positive charge of $+8$)¹⁶³ on nanopores with diameters similar to the sensed protein sizes, so as to optimize SNR. Figure 3.5 presents a detailed analysis of the impact of this opposing pressure on the dynamics of passage (capture rate and event duration) for these two proteins.

Figure 3.5(a) depicts sample current traces of GFP capture under electrophoretic force and the V–P regime, both at the same protein concentration. Qualitatively, from the number of transient blockades in the trace, a clear improvement in detection rate is observed in the pressure opposing condition, suggesting that a higher proportion of events are now slow enough to be detected by our system.

Representative current signatures of individual events highlight the significant increase in event duration for an opposing pressure of 0.3 MPa compared to 0 MPa (Figure 3.5(b)).

Figure 3.5(c) shows a scatter plot of maximum current deviation versus passage time, comparing the electrophoretic capture regime (-200 mV, 0 MPa, light green) to the V–P regime (-200 mV, -0.3 MPa, dark green). The maximum deviation is defined as the deepest point in the event (blockage level + noise). The dashed line here represents the bandwidth limit of our instrument assigned at $35\text{ }\mu\text{s}$ for a low-pass filter of 100 kHz , where events faster than this limit are assumed to be distorted and only partially resolved, or entirely missed.^{143,162} In Figure 3.5(d), the dwell time distribution of purely electrophoretic passages reveals fast events with a most probable translocation duration of $80\text{ }\mu\text{s}$. A 0.3 MPa opposing pressure extends the mean dwell time to $510\text{ }\mu\text{s}$, a six-fold increase in duration.

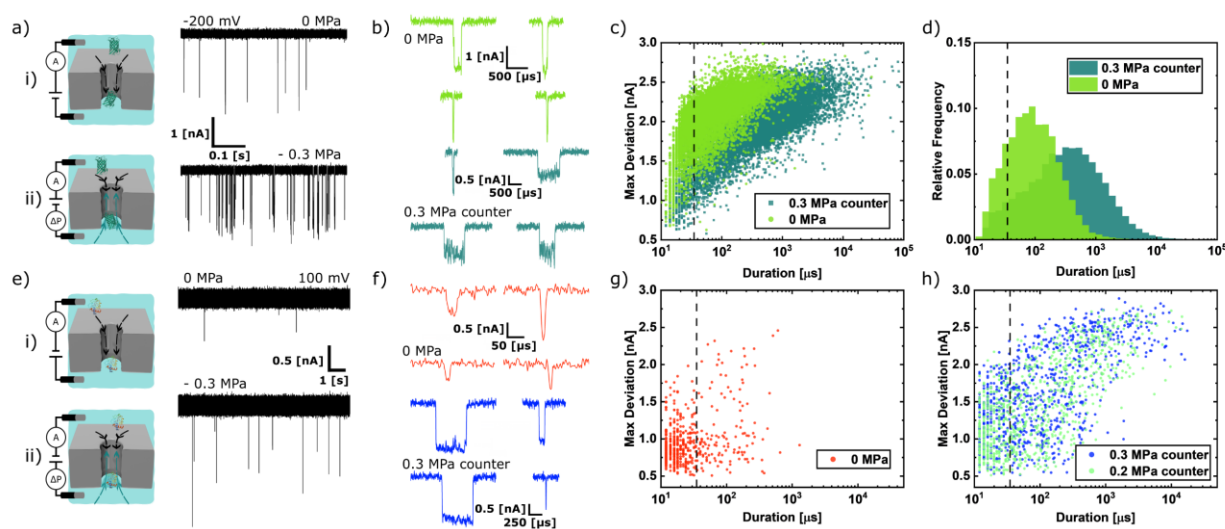


Figure 3.5 Protein translocation characteristics in SiN nanopores under opposing pressure. a) Schematic of Green Fluorescent protein (GFP) translocations: i) electrophoretically under an applied voltage, and ii) electrophoretically with an opposing net pressure force. b) Representative events of GFP showing the effect of the opposing pressure on the event shape. c) 2D scatter plot of maximum current deviation versus translocation duration (log scale) of $0.7\text{ }\mu\text{M}$ GFP in $3.6\text{ M LiCl pH }8$ in a 4 nm nanopore at -200 mV in light green, and an opposing 0.3 MPa pressure in dark green, showing the first $10,000$ events in the dataset. d) 1D histogram of translocation time (log scale) using the same dataset as in c). e) Schematic of cytochrome c translocations: i) electrophoretically under an applied voltage, and ii) electrophoretically with an opposing net pressure force. f) Representative events of cytochrome c showing the effect of the opposing pressure on the event shape. g-h) 2D scatter plot of maximum deviation versus translocation duration (log scale) of $2\text{ }\mu\text{M}$ cytochrome c in $3.6\text{ M LiCl pH }8$ in a 3.7 nm

nanopore at 100 mV in red, and an opposing 0.2-0.3 MPa pressure in green and blue respectively. The dashed lines in c–d) and g–h) represent the bandwidth limit of 35 μ s for a 100 kHz low-pass filter.

Similarly, Figure 3.5(e) displays the two sensing conditions for cytochrome c in a 3.7 nm pore, where an opposing pressure of 0 MPa (i) and 0.3 MPa (ii) is applied against a fixed +100 mV driving voltage. An increase in detection rate is readily observed from the representative current traces. Furthermore, the opposing pressure on cytochrome c led to better resolved events with longer durations, as depicted in Figure 3.5(f). The resulting scatter plot of maximum deviation versus passage time is shown in Figure 3.5(g–h). The electrophoretically driven cytochrome c results (red circles) exhibit shallow and fast deviations from the current baseline, with most events <1 nA in blockage and $\leq 35 \mu$ s in dwell time. However, the application of an opposing pressure in the 0.2–0.3 MPa range reveals deep events >1 nA with many translocations past the 35 μ s limit. Quantitatively, this fraction of events is 54% (486 out of 902) for 0.2 MPa, 60% (543 out of 900) for 0.3 MPa, compared to 11% (61 out of 533) of events for 0 MPa. This behavior is akin to the GFP V–P results where many translocating proteins are only slow enough under opposing pressure to be resolved.

A quantitative analysis of the detection rates shows an observable increase by a factor of 4 $\times$ for GFP and 3 $\times$ for cytochrome c when comparing the no pressure control to the opposing pressure of 0.3 MPa (Section 3.7.2 of the SI). We attribute this detection rate increase to recovery of events missed by the limited bandwidth of our system, where the velocities of translocation of proteins are higher than the hardware’s ability to detect them. To validate this observation, we performed an opposing pressure sweep experiment at a fixed –100 mV driving voltage and calculated the detection rate (Section 3.7.2(c) of the SI). We note that, for a purely electrophoretic transport, the detection rate for GFP at –100 mV was higher than the value at –200 mV. This behavior is expected for bandwidth limited operation when fast events are missed. By sweeping the opposing pressure, a parabolic trend for the detection rate was observed, implying that a portion of missed events is recovered before reaching the point where the

opposing pressure force is overcoming the net driving electrophoretic force and fewer molecules are actually being captured. Therefore, this demonstrates that access to an opposing pressure force can extend the useful bandwidth during voltage driven nanopore-based protein sensing, but that the process remains a delicate balance between hydrodynamic and electrokinetic forces, which depends on the pore characteristics and the protein properties.

3.3.5 Balance of forces under pressure driven capture during protein sensing

Finally, we shift our focus to experiments taking advantage of the pressure-driven voltage-retarding (P–V) sensing regime to sense biomolecules irrespective of their net charge by capturing them by the pressure-driven hydrodynamic flow. The electrophoretically driven translocation of GFP in a 7 nm nanopore was first recorded at a –100 mV with no applied pressure, as illustrated by the current trace in Figure 3.6(a).i. Similarly, Figure 3.6(a).ii (0.2 MPa, +100 mV) and Figure 3.6(a).iii (0.3 MPa, +100 mV) depict current traces of the same pore now undergoing P–V sensing.

Events are represented as a 2D scatter plot of maximum deviation versus translocation duration in Figure 3.6(b). For electrophoretically driven GFP (–100 mV), we observed a geometric mean event duration of $45 \pm 3 \mu\text{s}$. Under P–V sensing ($\Delta P = 0.2\text{--}0.3 \text{ MPa}$, $V = +100 \text{ mV}$), the geometric mean event duration was recorded at $151 \pm 5 \mu\text{s}$ (0.2 MPa) and $77 \pm 4 \mu\text{s}$ (0.3 MPa), thus slowing the passage speed by a factor of up to 3.3 $\times$. Here, the geometric mean is taken since the distribution of events is not fully resolved. The error bars represent the geometric standard deviation, and the distributions of event duration are available in section 3.7.3(a) of the SI. Since the bandwidth limit of the instrument is 35 μs , a significant portion of events recorded for the electrophoretically driven GFP are in the attenuated sensing regime for a 7 nm nanopore.

In terms of event amplitude, electrophoretically driven GFP (–100 mV) had two populations at $729 \pm 107 \text{ pA}$ and $1201 \pm 346 \text{ pA}$, taken as the mean and standard deviation of a double Gaussian fit to the

distribution of event maximum deviation. On the other hand, two peaks at 684 ± 109 and 1012 ± 381 pA were recorded for P–V sensing ($\Delta P = 0.2$ MPa, $V = +100$ mV), with most events located at the lower peak value of 684 pA. We note here that the decrease in maximum deviation compared to the voltage driven condition is due to the electrohydrodynamic effects discussed in Figure 3.3. Furthermore, increasing the driving pressure to 0.3 MPa at a fixed +100 mV resulted in a single population at 1126 ± 289 pA, taken as the mean and standard deviation of a Gaussian fit to the distribution of events. We note here that a single Gaussian fit was selected since the two populations overlap strongly. By employing the P–V regime, we extend the mean duration of translocation past our hardware threshold of resolved events while maintaining event amplitudes, and thus SNR, comparable to the electrophoretically driven case.

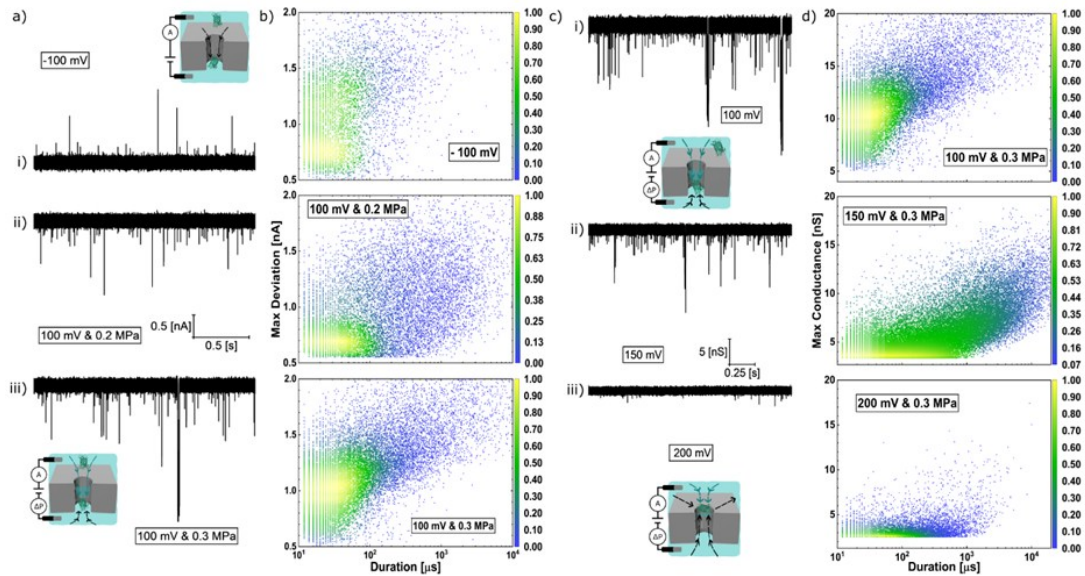


Figure 3.6 Protein translocation characteristics and balance of forces between voltage driven and pressure driven sensing in SiN nanopores. a) Representative current trace of GFP with (i) a fixed applied voltage of -100 mV, (ii–iii) a fixed opposing voltage of $+100$ mV and a 0.2 – 0.3 MPa driving net pressure for $0.7 \mu\text{M}$ GFP in 3.6 M LiCl pH 8 in a 7 nm nanopore. b) 2D scatter plots of maximum current deviation versus translocation duration (log scale) for the entire dataset of a), comparing -100 mV voltage driven to 0.2 – 0.3 MPa pressure driven events with a fixed opposing voltage of 100 mV. c) Representative current trace with a fixed driving net pressure of 0.3 MPa and opposing voltages of (i) $+100$ mV, (ii) $+150$ mV, and (iii) $+200$ mV for $0.7 \mu\text{M}$ GFP in 3.6 M LiCl pH 8 in a 7 nm nanopore. d) 2D scatter plots of maximum conductance blockage versus translocation duration (log scale) for the entire dataset of c), sweeping a fixed opposing voltage from $+100$ mV to $+200$ mV with a driving net pressure of 0.3 MPa.

We now shift our attention to the effect of a varying opposing electrical force on the translocation characteristics for a fixed pressure-driven hydrodynamic flow and report the resulting changes in transport kinetics. To achieve this, the driving pressure was fixed at $\Delta P = 0.3$ MPa and the opposing voltage was varied in 50 mV steps from +100 to +200 mV. Figure 3.6(c).i displays a representative current trace of GFP events under a driving pressure of 0.3 MPa and a counter voltage of +100 mV, characterized by well resolved deep events. Increasing the opposing voltage to +200 mV leads to a decreased blockage amplitude and a drastic reduction in capture rate, as shown in Figure 3.6(c).iii.

Figure 3.6(d) presents scatter plots of maximum conductance (i.e. maximum deviation divided by the applied voltage) versus translocation duration for GFP events. In terms of maximum conductance, the starting P–V condition of (0.3MPa, +100 mV) resulted in a single population of successful translocations centered at 11 nS. Increasing the opposing voltage by 50 mV shifts the maximum conductance to the 7–8 nS range. This reduction of blockage amplitude for an increased voltage is due to the electrohydrodynamic effects discussed earlier for dsDNA (Figure 3.3). A second population appears below 5 nS, characterized by shallow blockages that we attribute to incomplete translocation through the pore. Furthermore, once the opposing voltage extends to +200 mV, the maximum conductance for successful translocations is observed in the 3–5 nS range, with a greater proportion of failed translocations ≤ 3 nS. Indeed, we postulate that most GFP translocations fail to overcome the electrical field at the nanopore entrance for this P-V condition.

In terms of event dwell time, a P–V regime of (0.3 MPa, +100 mV) leads to a geometric mean duration of 77 ± 4 μ s. Increasing the opposing voltage to +150 and +200 mV enhances the geometric mean duration of successful translocations to 496 ± 6 μ s and 168 ± 3 μ s respectively, shifting the passage time by a factor of 2 to 6. The distributions of event duration are available in Section 3.7.4(a) of the SI.

Section 3.7.4(b) of the SI presents inter-event time distributions for GFP translocations under a P–V sensing regime. We observe similar capture rates for a driving pressure of 0.3 MPa and opposing

voltages in the 100–150 mV range. While maintaining the same driving pressure, increasing the opposing voltage by 50 mV leads to a dramatic decrease in capture rate of successful translocation events, further indicating the inability of GFP to overcome the electrical field at the nanopore entrance in this P–V condition (0.3 MPa driving and +200 mV opposing).

Similar results have been shown for P–V sensing systems in terms of capture rate, albeit with lower SNR and bandwidth. Briefly, a 615 bp dsDNA sample was driven through a 10 nm pore at a fixed –100 mV while the pressure was modulated between 0.17 MPa and 0.25 MPa (1.64 and 2.44 atm).⁸⁶ As the pressure increased, the fraction of attempts at translocation was drastically reduced, leading to mainly successful translocation at 0.25 MPa. A similar trend was recorded for aprotinin (6.5 kDa) and BSA (66.5 kDa), where a voltage sweep at a fixed driving pressure resulted in a reduction of capture rate once the proteins were unable to overcome the electric field in order to translocate through the nanopore.⁹¹

3.4 Conclusion

In this study, we presented an improved platform for performing voltage- and pressure-modulated sensing through solid-state nanopores, controlling translocation dynamics of biomolecules to better resolve event signatures and enhance sensing performance for a given finite bandwidth. Our developed voltage- and pressure-controlled setup enables pressure actuation in the 0–0.3 MPa range to enhance or hinder the applied electrokinetic forces. With this additional tunable force, we investigated the effects of V–P sensing on double-stranded and nanostructured DNA translocations, modulating capture rate and translocation velocity uniformly along the polymer length. Furthermore, the precise force control provided novel insights and elucidated processes underlying translocation dynamics, including polymer folding probability, pressure-induced enhancement in current blockage for DNA polymers, and an alternative measurement technique of the effective charge of DNA. Then, the successful, high-fidelity detection of previously fast and unresolved proteins (GFP and cytochrome c) was achieved by matching

their translocation duration to the finite bandwidth of the measurement instrument, while maintaining SNR levels comparable to or better than the electrophoretic capture regime. This result was demonstrated by an enhancement in dwell time and detection rate for these proteins, with most events now translocating slower than our system's bandwidth limit. Additionally, P–V sensing experiments for GFP were conducted, leading to better differentiation in protein translocation characteristics compared to voltage driven ones, albeit at the necessity of balancing the forces acting on the protein to promote successful capture and translocation. To conclude, we propose that this platform will enable the systematic analysis of complex protein signatures and densely assembled DNA multiplexed barcodes, thus providing the necessary resolution for structural characterization in single-molecule biophysics.^{139,164}

3.5 Methods

Biomolecular samples. Linear dsDNA fragments of 2000 base pairs were purchased from Thermo Fisher (NoLimits, SM1701) as our calibration sample. Samples were mixed with 3.6 M LiCl buffered with 10 mM HEPES at pH 8 to reach a sample concentration of 5 nM in an 80 μ L volume. VPM molecules were prepared following the protocol detailed in our previous work.¹⁵¹ Cytochrome c was purchased from Sigma-Aldrich (C2506), while GFP with an SsrA tag was grown by Emily Majaesic (University of Toronto). Protein samples were diluted to 0.7–2 μ M in 3.6 M LiCl pH 8 from a stock concentration of 100 μ M, following long-term storage in a –80 °C freezer.

Nanopore sensing. All nanopores used in this work were made on 5 mm square chips featuring 30×30 μ m free-standing membranes of 14 nm thin SiN, deposited at Addison Semiconductor Materials, Inc (Low stress LPCVD Nitride, film tensile stress $<250 \pm 50$ MPa) and manufactured in-house at the uOttawa NanoFab facilities. Controlled Breakdown (CBD) was used for nanopore formation and size tuning.^{17,56,57} Ionic current sensing was carried out on a single-channel patch-clamp amplifier (Axopatch 200B, Molecular Devices) at full-bandwidth (100 kHz hardware low-pass filter).

Data analysis. All event datasets were digitally low-pass filtered at 100 kHz with an 8-pole Bessel filter. On the other hand, streaming current datasets were digitally low-pass filtered at 10 Hz. A standard threshold crossing algorithm was used to detect events using Poriscope.^{165,166} Digital event filters were applied to account only for events with translocation durations greater than 10 μ s and a minimum SNR of 6.

3.6 Acknowledgment

The authors would like to acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC) [funding reference number RGPIN-2021-04304, I2IPJ 586238-23], and Oxford Nanopore Technologies. MYB would like to thank Emily Majaesic (University of Toronto) for the GFP samples.

3.7 Supporting Information

3.7.1 Trapping of Velocity Profiling Molecules under a voltage-pressure sensing regime

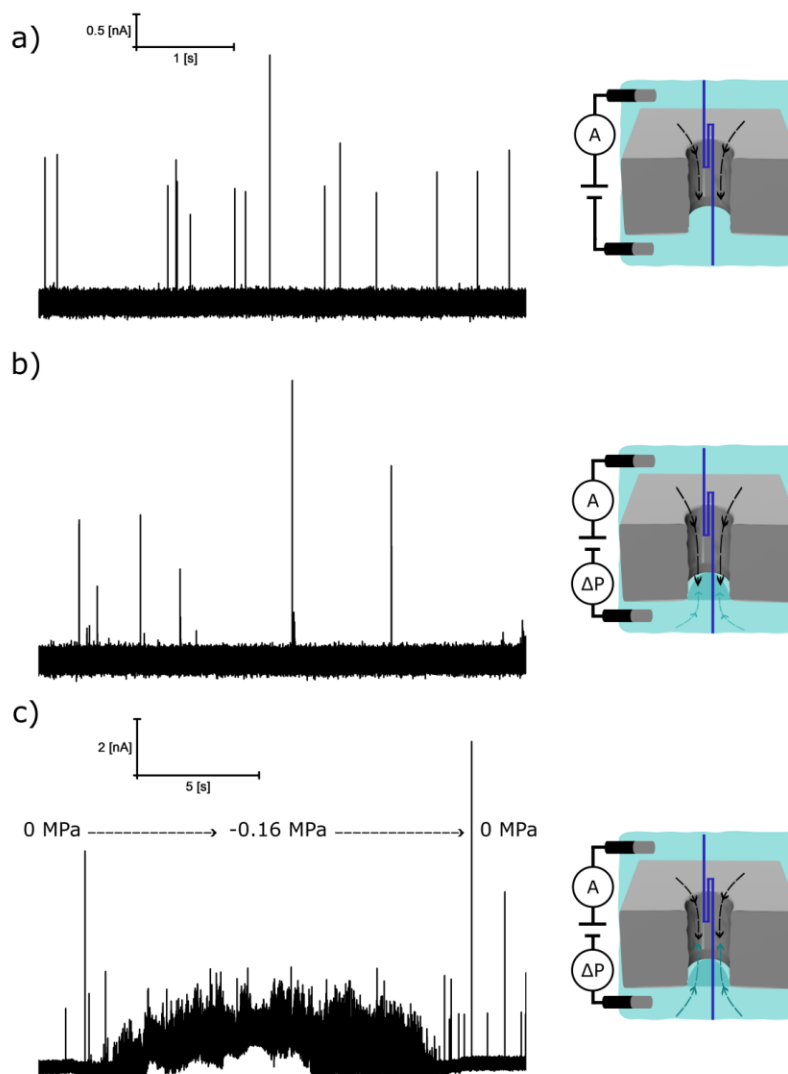


Figure 3.7 Effect of opposing pressure on the dynamics of translocation of Velocity Profiling Molecules (VPM) ¹⁵¹ for a 16 nm SiN nanopore in 3.6 M LiCl pH 8 at a fixed voltage of -200 mV. a) Current trace of the electrophoretically driven VPMs with a schematic detailing the forces at play on the right. b) Current trace of the electrophoretically driven VPMs opposed with a net pressure of 0.1 MPa, showing a decrease in capture rate and slower translocation velocities, with a schematic of forces depicting a stronger EP contribution compared to ΔP . c) Current trace of the electrophoretically driven VPMs opposed with a net pressure of 0.16 MPa, showing a clear trapping due to the balance of forces, with a schematic of forces depicting an equal contribution between EP and ΔP .

3.7.2 Enhanced detection of V–P sensed proteins: capture rate of GFP and Cytochrome C

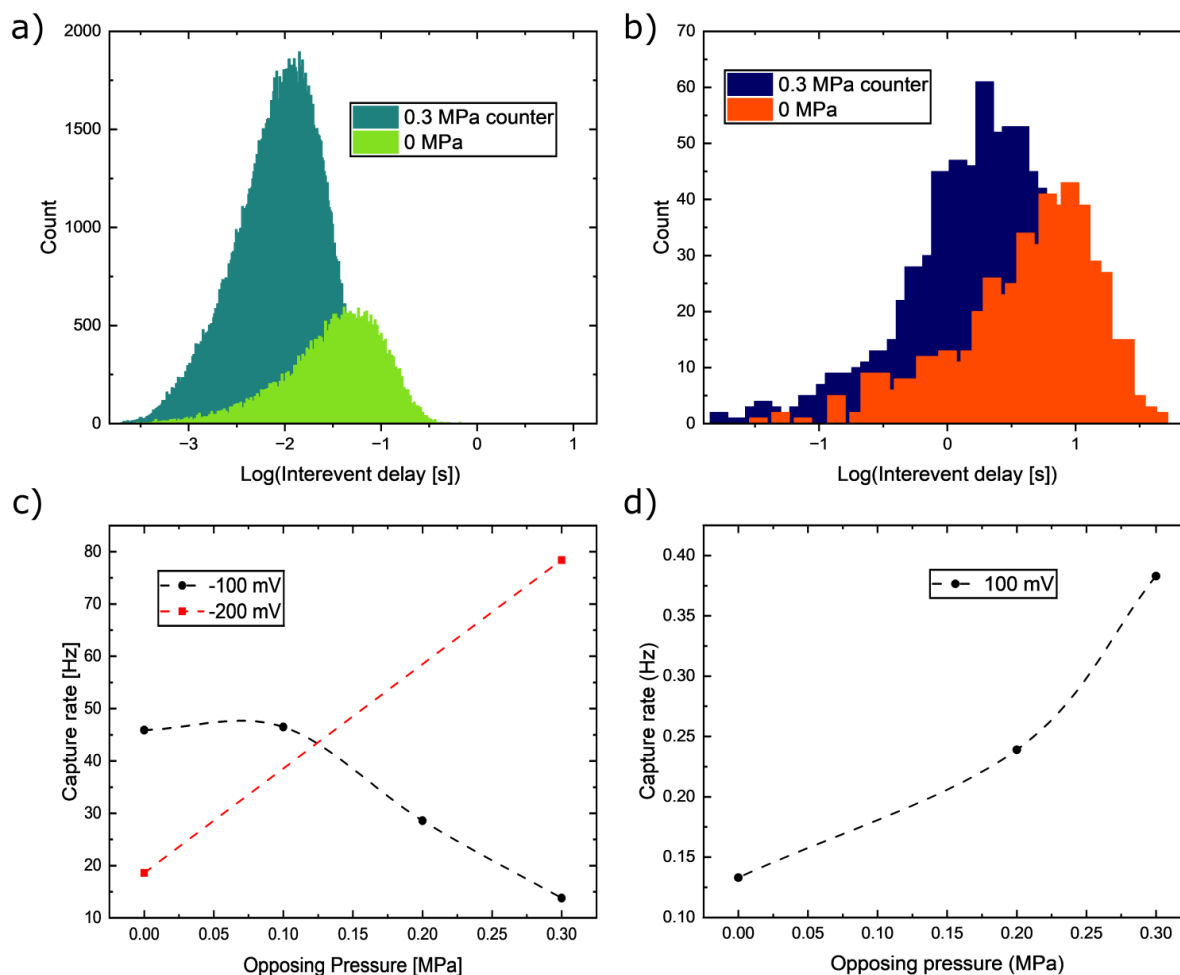


Figure 3.8 Effect of opposing pressure on the detection of electrophoretically driven proteins as measured by the capture rate. a) Inter-event time distribution of GFP translocations at a fixed driving voltage of -200 mV and opposing pressures (0 and 0.3 MPa) for a 4 nm SiN nanopore, showing a 4x increase in capture rate (from 18.6 ± 0.2 Hz to 78.4 ± 0.6 Hz), where capture rate is extracted by fitting the log-transform of a Poisson distribution. b) Inter-event time distribution of cytochrome c translocations at a fixed driving voltage of 100 mV and opposing pressures (0 and 0.3 MPa) for a 3.7 nm SiN nanopore, showing a 3x increase in capture rate (0.13 ± 0.01 Hz compared to 0.38 ± 0.02 Hz), where capture rate is extracted by fitting the log-transform of a Poisson distribution. c) Capture rate of GFP translocations for a 4 nm SiN nanopore at -200 mV in red and -100 mV in black versus opposing pressure. d) Capture rate of cytochrome c translocations for a 3.7 nm SiN nanopore at 100 mV versus opposing pressure. Dashed lines in c-d) are a guide to the eye.

3.7.3 Electrophoretically driven versus P–V sensed GFP

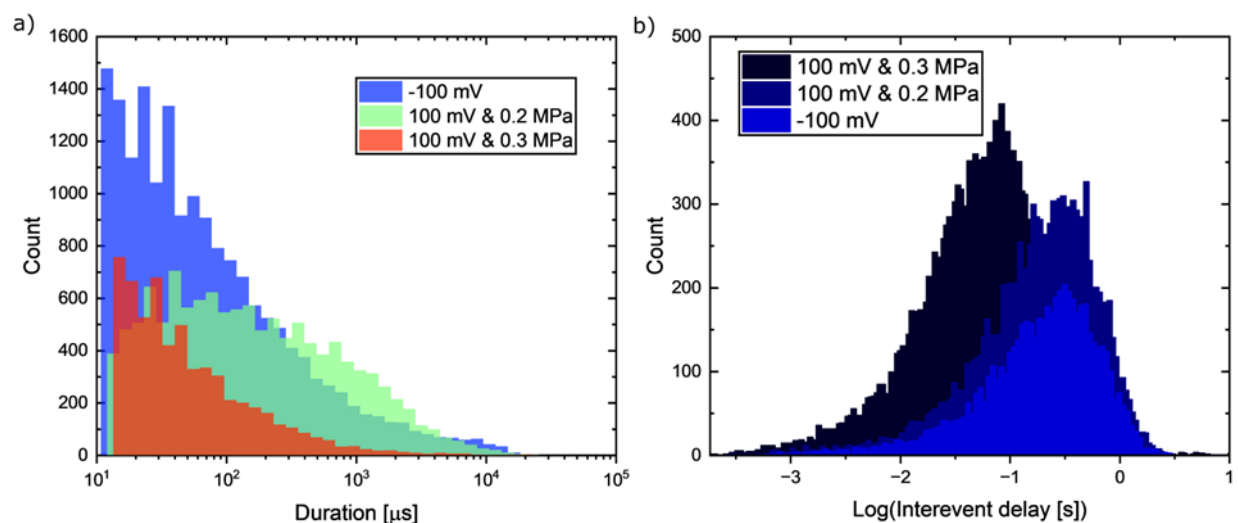


Figure 3.9 Effect of driving force on the event duration and capture rate of GFP for a 7 nm SiN nanopore. a) 1D histogram of translocation duration, comparing electrophoretically driven GFP at -100 mV and P–V driven GFP at a fixed opposing voltage of 100 mV and a 0.2–0.3 MPa driving pressure. b) Inter-event time distribution of GFP translocations, comparing electrophoretically driven to P–V sensed GFP events (3.2 ± 0.1 Hz for -100 mV, 3.3 ± 0.1 Hz for 100 mV & 0.2 MPa, and 13.2 ± 0.2 Hz for 100 mV & 0.3 MPa), where capture rate is extracted by fitting the log-transform of a Poisson distribution.

3.7.4 Balance of forces for P–V sensed GFP at a fixed driving pressure

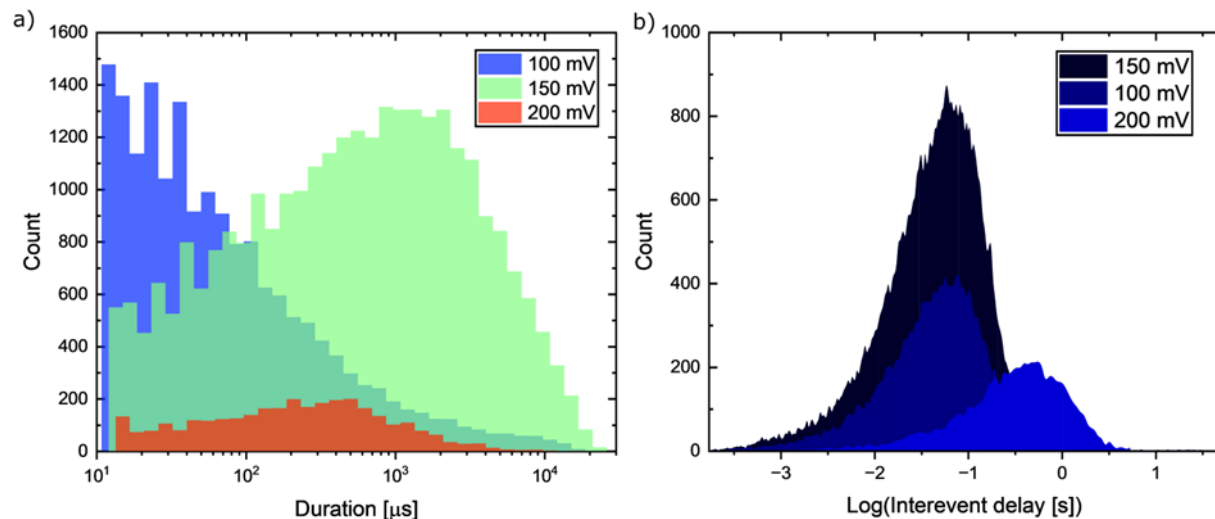


Figure 3.10 Effect of opposing voltage on the event duration and capture rate of pressure-driven GFP for a 7 nm SiN nanopore. a) 1D histogram of translocation duration, sweeping the opposing voltage (100–200 mV) at a fixed driving pressure of 0.3 MPa. b) Inter-event time distribution of GFP translocations, comparing P–V sensed GFP events for a fixed driving pressure of 0.3 MPa and an opposing voltage in the 100–200 mV range (13.2 ± 0.2 Hz for 100 mV, 12.7 ± 0.1 Hz for 150 mV, and 1.9 ± 0.1 Hz for 200 mV), where capture rate is extracted by fitting the log-transform of a Poisson distribution.

3.7.5 Derivation of an expression for the effective DNA charge in a V–P capture regime

Consider a solid cylinder of radius r_c , representing a DNA polymer and moving through a hollow cylindrical channel of radius r_p at a velocity v_c in response to a uniform electric field $E = \Delta V/L$ and applied pressure difference ΔP .

Let the pore walls and moving cylinder have non-zero surface charge densities with surface potentials $\phi(r = r_p) \equiv \phi_p$ and $\phi(r_c) \equiv \phi_c$, respectively. The radial dependence of the charge density $\rho(r)$ of counterions between the two charged surfaces is described by the Poisson equation as:

$$\nabla^2 \phi(r) = -\frac{\rho(r)}{\varepsilon}$$

Z-independence is assumed and ε is the bulk's permittivity.

Similarly, the radial dependence of the fluid velocity $v(r)$ between both cylindrical surfaces is described by the Navier-Stokes equation under an electric field and applied pressure with boundary conditions $v(r_p) = 0$ and $v(r_c) = v_c$, i.e. no slip conditions:

$$\eta \nabla^2 v(r) = -\rho(r)E + \nabla p$$

Solving the Navier-Stokes equation for the fluid velocity gives:

$$\eta \nabla^2 v(r) = \frac{\varepsilon \Delta V}{L} \nabla^2 \phi(r) + \frac{\Delta P}{L}$$

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{dv}{dr} \right) = \frac{\varepsilon \Delta V}{\eta L} \frac{1}{r} \frac{d}{dr} \left(r \frac{d\phi}{dr} \right) + \frac{\Delta P}{\eta L}$$

$$\frac{1}{r} \frac{d}{dr} \left(r \left(\frac{dv}{dr} - \frac{\varepsilon \Delta V}{\eta L} \frac{d\phi}{dr} \right) \right) = \frac{\Delta P}{\eta L}$$

$$\frac{d}{dr} \left(r \left(\frac{d}{dr} \left(v - \frac{\varepsilon \Delta V}{\eta L} \phi \right) \right) \right) = \frac{\Delta P}{\eta L} r$$

$$r \left(\frac{d}{dr} \left(v - \frac{\varepsilon \Delta V}{\eta L} \phi \right) \right) = \frac{\Delta P}{2\eta L} r^2 + c_1$$

$$\frac{d}{dr} \left(v - \frac{\varepsilon \Delta V}{\eta L} \phi \right) = \frac{\Delta P}{2\eta L} r + \frac{c_1}{r}$$

$$v - \frac{\varepsilon \Delta V}{\eta L} \phi = \frac{\Delta P}{4\eta L} r^2 + c_1 \ln r + c_2$$

$$v(r) = \frac{\varepsilon \Delta V}{\eta L} \phi(r) + \frac{\Delta P}{4\eta L} r^2 + c_1 \ln r + c_2$$

To find c_1 and c_2 , we apply the boundary conditions at the channel walls and the moving cylinder.

For $v(r_c) = v_c$, $\phi(r_c) = \phi_c$, and we get:

$$v_c = \frac{\varepsilon \Delta V}{\eta L} \phi_c + \frac{\Delta P}{4\eta L} r_c^2 + c_1 \ln r_c + c_2$$

Similarly, for $v(r_p) = 0$, $\phi(r_p) = \phi_p$, and we get:

$$0 = \frac{\varepsilon \Delta V}{\eta L} \phi_p + \frac{\Delta P}{4\eta L} r_p^2 + c_1 \ln r_p + c_2$$

By isolating c_1 and c_2 from the previous equations, we find an expression for the fluid velocity as:

$$\therefore v(r) = \frac{\varepsilon \Delta V}{\eta L} (\phi(r) - \phi_p) + \frac{\Delta P (r^2 - r_p^2)}{4\eta L} + \frac{\ln \frac{r}{r_p}}{\ln \frac{r_c}{r_p}} \left(v_c - \frac{\varepsilon \Delta V}{\eta L} (\phi_c - \phi_p) - \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) \right)$$

The drag force imparted by the fluid on the moving cylinder of length L can thus be calculated by integrating the viscous stress tensor over the inner cylinder's surface:

$$F_{drag} = 2\pi r_c L \eta \frac{dv}{dr} \Big|_{r_c}$$

We start by evaluating the derivative term at the inner cylinder's surface before replacing it in the drag force equation above.

The derivative of the fluid velocity is:

$$\frac{dv}{dr} = \frac{\varepsilon \Delta V}{\eta L} \frac{d\phi(r)}{dr} + \frac{\Delta P r}{2\eta L} + \frac{1}{r} \frac{1}{\ln \frac{r_c}{r_p}} \left(v_c - \frac{\varepsilon \Delta V}{\eta L} (\phi_c - \phi_p) - \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) \right)$$

When evaluated at the inner cylinder's surface, the expression becomes:

$$\frac{dv}{dr}|_{r_c} = \frac{\varepsilon \Delta V}{\eta L} \frac{d\phi(r)}{dr}|_{r_c} + \frac{\Delta P r_c}{2\eta L} + \frac{1}{r_c} \frac{1}{\ln \frac{r_c}{r_p}} \left(v_c - \frac{\varepsilon \Delta V}{\eta L} (\phi_c - \phi_p) - \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) \right)$$

Then, the drag force equation is:

$$F_{drag} = 2\pi r_c L \eta \frac{dv}{dr}|_{r_c}$$

$$F_{drag} = \frac{Q_{eff}}{L} \Delta V + \pi r_c^2 \Delta P + \frac{2\pi L \eta}{\ln \frac{r_c}{r_p}} \left(v_c - \frac{\varepsilon \Delta V}{\eta L} (\phi_c - \phi_p) - \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) \right)$$

To find the steady-state velocity of the cylinder in the channel, we set the drag force to zero and isolate v_c :

$$0 = \frac{Q_{eff} \Delta V}{L} + \pi r_c^2 \Delta P + \frac{2\pi L \eta}{\ln \frac{r_c}{r_p}} \left(v_c - \frac{\varepsilon \Delta V}{\eta L} (\phi_c - \phi_p) - \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) \right)$$

$$v_c = \frac{\varepsilon \Delta \phi}{\eta} \frac{\Delta V}{L} + \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) - \frac{\ln \frac{r_c}{r_p}}{2\pi L \eta} \left(\frac{Q_{eff} \Delta V}{L} + \pi r_c^2 \Delta P \right)$$

For a trapped DNA polymer, the velocity is null and we can isolate the effective DNA charge:

$$0 = (\mu_{EP} - \mu_{EO}) \frac{\Delta V}{L} + \frac{\Delta P}{4\eta L} (r_c^2 - r_p^2) - \frac{\ln \frac{r_c}{r_p}}{2\pi L \eta} \left(\frac{Q_{eff} \Delta V}{L} + \pi r_c^2 \Delta P \right)$$

$$\therefore \frac{Q_{eff}}{L} = \frac{2\pi}{\ln \frac{r_c}{r_p}} \left((\mu_{EP} - \mu_{EO}) \eta - \frac{\Delta P}{4\Delta V} (r_p^2 - r_c^2) \right) - \frac{\pi r_c^2 \Delta P}{\Delta V}$$

In our experimental conditions, the electrophoretic mobility from electroosmosis is negligible compared to the DNA electrophoretic mobility due to the high salt concentration (3.6 M LiCl). Furthermore, we want to extract an effective DNA charge per base pair ($L = 0.34$ nm). The other parameters were set to:

$$\mu_{EP} = -0.3 \times 3.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$$

$$\Delta P = -0.35 \times 10^6 \text{ Pa}$$

$$\Delta V = -0.2 \text{ V}$$

$$r_p = 5.5 \times 10^{-9} \text{ m}$$

$$r_c = 1.1 \times 10^{-9} \text{ m}$$

$$\eta = 0.001 \text{ Pa} \cdot \text{s}$$

The electrophoretic mobility of DNA is taken at 30% of its value in free solution due to the high salt concentration adopted here.¹⁶⁷

4. A method for fabricating CMOS back-end-of-line-compatible solid-state nanopore devices

This chapter is published in *Nanotechnology* as “A method for fabricating CMOS back-end-of-line-compatible solid-state nanopore devices” (2025) by Mohamed Yassine Bouhamidi, Chunhui Dai, Michel Stephan, Joyeeta Nag, Justin Kinney, Lei Wan, Matthew Waugh, Kyle Briggs, Jordan Katine, Vincent Tabard-Cossa, and Daniel Bedau.

4.1 Abstract

Solid-state nanopores (ssNPs), nm-sized holes in thin, freestanding membranes, are powerful single-molecule sensors capable of interrogating a wide range of target analytes, from small molecules to large polymers. Interestingly, due to their high spatial resolution, nanopores can also identify tags on long polymers, making them an attractive option as the reading element for molecular information storage strategies. To fully leverage the compact and robust nature of ssNPs, however, they will need to be packaged in a highly parallelized manner with on-chip electronic signal processing capabilities to rapidly and accurately handle the data generated. Additionally, the membrane itself must have specific physical, chemical, and electrical properties to ensure sufficient signal-to-noise ratios are achieved, with the traditional membrane material being SiN_x . Unfortunately, the typical method of deposition, low-pressure vapor deposition, requires temperatures beyond the thermal budget of complementary metal–oxide semiconductor back-end-of-line (BEOL) integration processes, limiting the potential to generate an on-chip solution. To this end, we explore various lower-temperature deposition techniques that are BEOL-compatible to generate SiN_x membranes for ssNP use, and successfully demonstrate the ability for these alternative methods to generate low-noise nanopores that are capable of performing single-molecule experiments.

4.2 Introduction

As the total volume of global data continues to soar, conventional storage methods such as tape, hard disk drives, and solid-state drives are struggling to keep pace.¹⁶⁸ This burgeoning demand has spurred considerable interest in exploring alternative data storage solutions. One such solution, molecular information storage (MIST), has emerged as a promising candidate, particularly for archival purposes, owing to its exceptional data density,^{169,170} long-term stability,¹⁷¹ and remarkable energy efficiency.¹⁷² However, in order for the promise of MIST to be realized, advancements in both the data writing element (i.e., molecular synthesis) and the data reading element (i.e. reading the sequence of data-encoded molecules) are required.¹⁶⁹ Among the various molecular sequencing techniques available, nanopores represent a paradigm shift due to their potential for long-read, label-free, and high-throughput bit detection^{10,11,56,173–175} and in particular, solid-state nanopores (ssNPs) offer additional advantages, including robustness and potential compatibility with large-scale electronics integration techniques^{56,175,11,10} when compared to their biological counterparts.

To date, however, integrating ssNPs into large-scale electronics has not yet been realized. One major reason for this is the manner through which the membrane film is deposited. Typically, ssNPs are fabricated in SiN_x membranes which are deposited on a substrate (typically Si) through low pressure chemical vapor deposition (LPCVD). This technique is based on the thermal decomposition of the gas-phase reactants (silane, SiH₄ and NH₃) on the wafer surface.³⁰ While the LPCVD process yields a slow deposition rate, it produces high-performance films with uniformity and low hydrogen content³⁰ and provides a reliable thin film (~10–20 nm) in which nanopores can be fabricated. However, in order for this reaction to proceed as described, the deposition process itself needs to be performed at 700–1000 °C.¹⁷⁶ This presents a problem for complementary metal–oxide semiconductor (CMOS) back-end-of-line (BEOL) integration as a low thermal budget (< 400 °C) is often required to protect the low-*k* dielectric and copper interconnects of the devices from undergoing dielectric breakdown and negatively impacting

reliability. Therefore, for ssNPs to leverage the signal processing power, system-level miniaturization, and low-cost mass production capabilities afforded by CMOS and BEOL integration, an alternative thin film deposition method, for making free-standing membranes, must be identified.

Fortunately, there are a number of deposition approaches that could provide suitable membranes for ssNP devices while also aligning with the thermal budget for BEOL integration (Figure 4.1). We identify three potentially suitable methods: (i) ion beam deposition (IBD), which operates at room temperature, (ii) inductively coupled plasma chemical vapor deposition (ICPCVD), which is a special form of plasma-enhanced chemical vapor deposition (PECVD) and operates at approximately $200\text{ }^{\circ}\text{C} - 400\text{ }^{\circ}\text{C}$,¹⁷⁷ and (iii) inductively coupled plasma-enhanced atomic layer deposition (ICPALD), which is a type of plasma-enhanced ALD (PEALD), which operates at approximately $200\text{ }^{\circ}\text{C}$ and achieves higher plasma densities and better control than other types of PEALD.

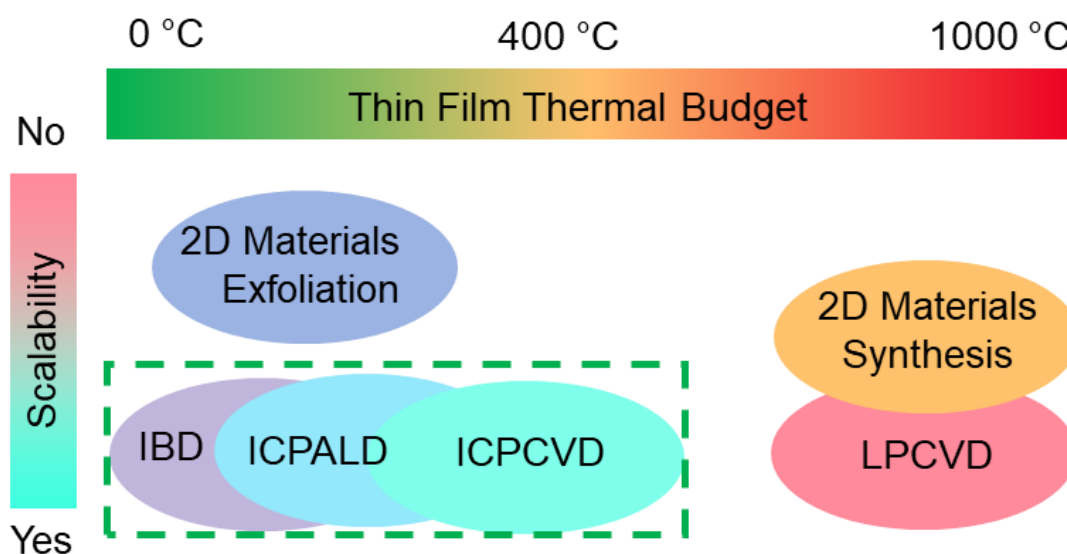


Figure 4.1 Assessment of the wafer-level process scalability and typical process temperatures of various film generation techniques. In particular, we highlight low-pressure chemical vapor deposition (LPCVD), 2D material exfoliation, 2D material synthesis, ion beam deposition (IBD), inductively coupled plasma chemical vapor deposition (ICPCVD), and plasma enhanced chemical vapor deposition (PECVD), specifically inductively coupled plasma enhanced ALD (ICPALD). The dotted rectangle highlights a regime that is both scalable and at a temperature low enough to be compatible with complementary metal–oxide semiconductor back-end-of-line integration.

However, as both ICPCVD and PEALD use plasma ionization to enable deposition at a much lower temperature than the temperatures at which the precursors (e.g. SiH_4 , NH_3 , N_2 , organic precursor such as bis(tert-butylamino)silane (BTBAS)) would naturally react, some of the precursors can remain unreacted or incompletely-reacted, leading to an elevated presence of carbon and hydrogen in the films.^{178,179} We have found these CVD and ALD films to be porous, rendering them less preferable for downstream processes for nanopore fabrication. In contrast, IBD deposits SiN_x films using nitrogen plasma to react with Si ions ejected from a pure Si target.¹⁸⁰ IBD is a physical vapor deposition (PVD) technique that produces thin films using inert gas plasma to dislodge film-forming particles from a desired target material onto a substrate with many desirable properties, for instance, improved adhesion, dense film structure, fewer defects, higher purity, and better controlled composition compared to films grown by other PVD techniques. This is related to various distinguishing features of IBD—(1) the working pressure is low, typically less than 10^{-1} Torr; (2) the ion generation and acceleration (ion beam source), generation of film-forming particles (target), and thin film growth (substrate) are spatially separated, thus geometrical parameters (ion incidence angle and emission angle) in addition to ion beam parameters (ion species and energy) can be varied; (3) another big difference between conventional IBD and magnetron sputtering is that for IBD there is no plasma between the substrate and the target, therefore IBD allows sensitive substrates to be deposited on and also the sputter gas inclusion in the deposit is minimal, increasing density of films.³⁴ In contrast to conventional sputtering, in IBD there is no bias between the substrate and the target, meaning both conducting as well as non-conducting targets and substrates can be used, avoiding the undesirable arcing during deposition altogether (arcing during depositions can cause significantly lower quality films). In comparison to CVD processes, in such a purely physical deposition process no organic precursor is involved and thus the presence of hydrogen and carbon are minimal in the film. As a result, we deemed IBD as the optimal process compatible with the needs of both ssNP use as well as BEOL.

We therefore present an analysis of the SiN_x film resulting from IBD on Si as well as a full characterization of its suitability for ssNP work including nanopore fabrication via controlled breakdown (CBD), electrical noise response, size stability, and the ability to detect single molecules for IBD membranes. Our findings pave the way for the continued development of a CMOS-integrated ssNP system for high-throughput sequencing, particularly for MIST applications.

4.3 Fabrication of nanopore devices

4.3.1 IBD of SiN_x

The IBD SiN_x film was prepared by first inserting the substrate, 100 mm intrinsic, undoped high resistivity ($>10\,000\ \Omega\ \text{cm}$) silicon wafers with a thickness of $300 \pm 20\ \mu\text{m}$, into an IBD system (Veeco) with its vacuum chamber pumped down to 10^{-8} Torr. A Si target (99.999% purity) was used as a source of ions for the deposition. Si atoms were liberated from the target by Ar ions and subsequently reacted with nitrogen ions (N₂ gas flow was fixed at 35 sccm). The SiN_x molecules then settled onto the substrate, forming a thin SiN_x film, with the thickness being determined by the duration of the deposition. For our purposes, 100 nm SiN_x was deposited for the membrane devices. The ion beam was operated at 550 V with a beam current of 200 mA. In parallel, we investigated the effect of thermal annealing as a potential method for improving the membrane for use with ssNPs. While realizing that subjecting the membrane to elevated temperatures and slowly allowing them to cool, may require temperature beyond the thermal budget of CMOS BEOL integration processes, we sought to explore how a reduction of the stress within the membrane itself, would aid in the formation of more stable, lower noise ssNPs. To anneal the SiN_x thin film, the wafer was loaded into a rapid thermal annealing system where the chamber was pumped down to 10^{-5} Torr and then purged with Ar gas with a flow rate of 40 sccm to maintain 5 Torr chamber pressure. The system temperature was then quickly ramped up (at $1\ ^\circ\text{C}\ \text{s}^{-1}$) to $800\ ^\circ\text{C}$ and maintained for

240 s. After annealing, the system was cooled to room temperature with a continuous Ar gas flow before the sample was removed from the chamber.

4.3.2 Membrane fabrication

Once the SiN_x deposition was complete, membrane devices were fabricated by standard photolithography and microfabrication procedures using two photomasks (soda-lime glass with chrome coating). The first photomask, used on the cleanest side of the wafer (referred to as *membrane side*), featured $5\text{ }\mu\text{m}$ circular patterns that were designed to be centred within the free-standing membranes that would be created. These circular features defined the region where the membrane would be locally thinned from 100 nm down to 20 nm to promote nanopore formation in that location. Additionally, the *membrane side* photomask contained the grid patterns necessary for dicing the wafer into $5 \times 5\text{ mm}$ square chips. The second photomask, which is used on the other side of the wafer (referred to as *etched pit side*), was designed to pattern specifically dimensioned square windows which would be centralized to the previously patterned local thinning circles. The square windows represent the region etched by potassium hydroxide (KOH) to produce the $40 \times 40\text{ }\mu\text{m}$ free-standing membranes. These two photomasks were each designed with the same coarse alignment marks which were used to align the wafer to the photomask to maximize the number of patterns that can fit on the surface of the wafer. Additionally, the photomasks featured different but complementary precision alignment marks that enabled double-sided alignment of the wafer and ensured the patterns on each side of its sides were centralized one to another.

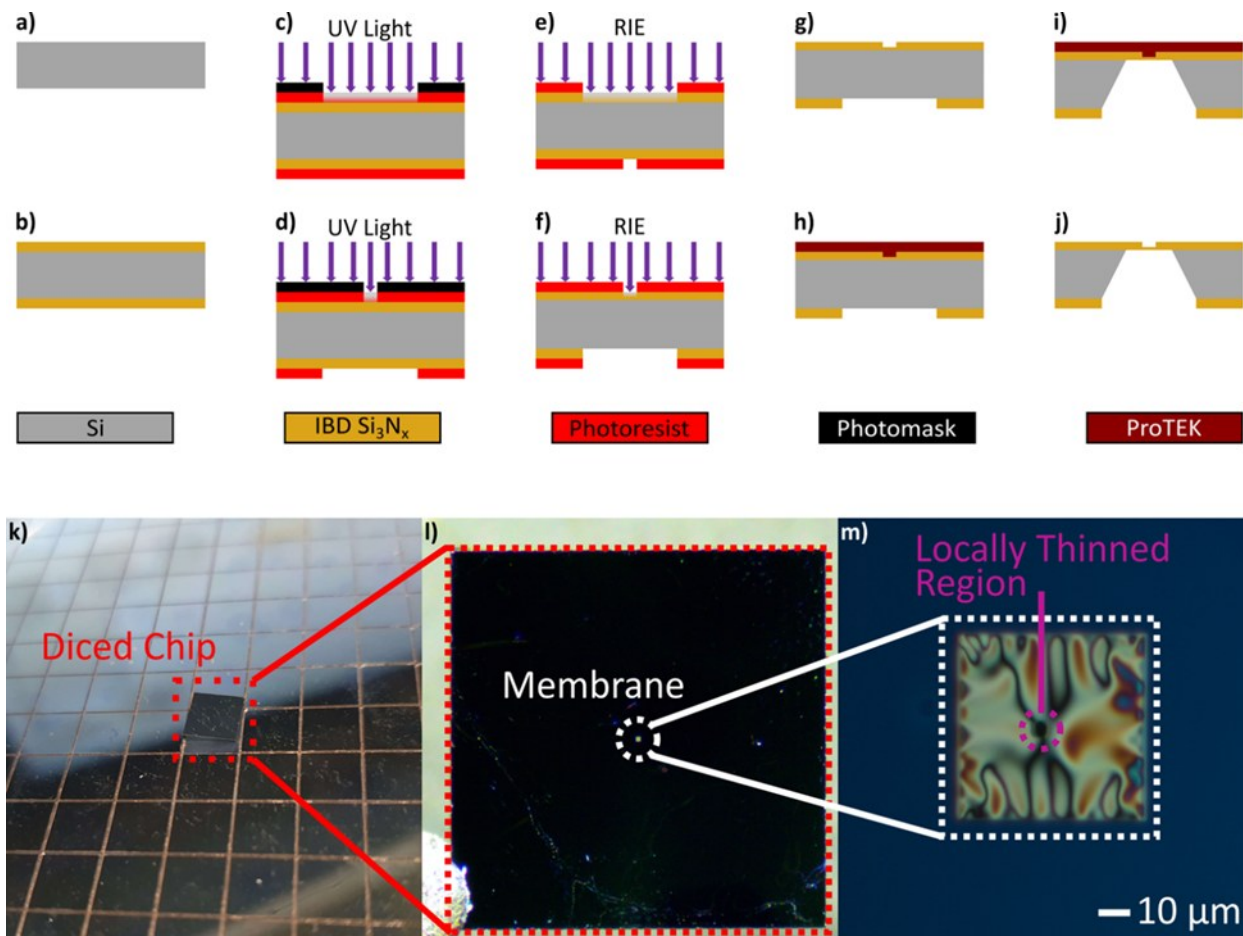


Figure 4.2 Overview of the IBD-based nanofabrication process to generate a SiN_x membrane (a)–(j) and associated resultant images (k)–(m). (a) Bare double-side polished silicon wafer with crystal orientation $\langle 100 \rangle$. (b) Ion beam deposition (IBD) of SiN_x on both sides of the wafer deposits a film of ~ 100 nm. (c) Alignment of the etched pit side of the wafer with its appropriate photomask and patterning, through UV photolithography, of the KOH etch windows. (d) Alignment of the membrane side of the wafer with its appropriate photomask and patterning, through UV photolithography, of the local thinning circular regions to be positioned in the centre of each membrane. (e) Reactive ion etching (RIE) of the entire thickness of the SiN_x exposed features on the etched pit side of the wafer to define square membrane opening for later KOH etching step. (f) RIE of a partial thickness of the SiN_x (until an approximate thickness of 10–20 nm remains) exposed features on the membrane side of the wafer to locally thin a portion of the membrane which will enforce nanopore fabrication by CBD to this region. (g) Photoresist stripping. (h) Spin coating of a protective polymer layer, ProTEK, on the membrane side of the wafer. (i) Hot KOH etching of the exposed silicon on the etched pit side of the wafer to form and release free stranding thin films. (j) Stripping of the protective polymer layer after the wafer is diced into individual 5×5 mm chips. (k) Optical microscope image of a diced wafer with a selected chip. (l) Close-up image of the chip and the membrane it contains. (m) Microscopy image of the IBD SiN_x membrane on the chosen chip.

The fabrication of the membrane devices for nanopore use was performed as outlined in Figure 4.2(a)–(j).⁴⁸ It began by spin-coating a thin (~ 650 nm) layer of positive photoresist (PR) (*Microposit S1805*) on each side of the wafer, which was then soft baked on a hot plate ($115\text{ }^{\circ}\text{C}$ for 60 s). Next, with a mask aligner, rough alignment of the *membrane side* of the wafer to the first photomask was conducted using the patternable bottom alignment marks and the wafer primary flat. Once aligned, the wafer was exposed to UV light that rendered the PR soluble where patterns were desired. The photolithographed wafer was then put into a developer solution (*Microposit MF-321*) that dissolved the exposed PR and visually defined the patterned features and the precision alignment marks (Figure 4.2(d)). At that point, the wafer was turned over and the *etched pit side* and loaded in the mask-aligner where the second photomask would rest on top of it. The initial alignment was performed as before, using the bottom alignment marks and wafer primary flat. Once rough alignment was achieved, greater precision was reached by using an infrared lamp and aligning the patterned alignment marks on the *membrane side* of the wafer with the complementary alignment marks on the second photomask. The wafer was then exposed and developed as previously done (Figure 4.2(c)). Reactive ion etching (RIE) of the *etched pit side* of the wafer was then executed following a standard recipe (5 sccm of O_2 , 25 sccm of CF_4 , 100 W, 8 Pa, 45 s) to ablate the exposed SiN_x patterns through their entire thickness, until the silicon substrate was reached. This step produced exposed silicon windows, to be etched by KOH to release free-standing membranes in the upcoming steps (Figure 4.2(e)). Next, with the objective of retaining a very thin SiN_x layer for the future thin film structure, a low-power and slower RIE step (10 sccm of CF_4 , 25 W, 8 Pa, 4.5–6 min) was performed on the *membrane side* of the wafer to obtain a locally thinned region that was targeted to be below 20 nm in thickness on average (Figure 4.2(f)). Remaining PR was then stripped of both sides of the wafer by sequentially submerging it into separate solutions of acetone, isopropyl alcohol, and deionized water (Figure 4.2(g)). A primer and protective polymer (*Brewer Science ProTEK B3-Primer* and *B3-25*) were spin coated and baked ($205\text{ }^{\circ}\text{C}$ for 60 s for the primer and $120\text{ }^{\circ}\text{C}$ for 120 s followed by $205\text{ }^{\circ}\text{C}$ for 60 s for the protective

polymer) on the membrane side of the device to prevent formation of pinholes during the following wet etch (Figure 4.2(h)). To release the SiN_x thin film structures, the wafer underwent a wet etch step by being submerged for 4 h in a 75 °C KOH solution with a weight-by-weight concentration of 30%. In this solution, any exposed silicon surface undergoes an anisotropic etch with a characteristic angle of 54.7° between the wafer crystalline plane <100> and <111> (Figure 4.2(i)).¹⁸¹ The wafer was then diced in individual 5 × 5 mm chips as shown in Figure 4.2(k). The precisely diced chips were then subjected to a cleaning step involving a sequential Piranha at 90 °C for 1 h and an RCA1 procedure¹⁸¹ to fully remove the *ProTEK* protective film and any surface contamination (Figure 4.2(j)). Finally, the cleaned chips were stored in 0.6 mL microcentrifuge tubes filled with a 1:1 solution of ethanol and DI water until they were used.

4.3.3 Nanopore fabrication and use

Nanopores were fabricated in-situ by the CBD method.^{56,57} To do this, the chips were first removed from their storage buffer, carefully dried by using a weak vacuum or a soft stream of N₂ gas and mounted into 3D-printed flow cells (Northern Nanopore Instruments). Isopropyl alcohol was then flushed through each side of the flow cell to ensure proper wetting of the membrane, followed by DI water and then a solution of 1 M KCl + 10 mM HEPES pH 8. Electrical contact was then made via a pair of Ag/AgCl electrodes of 1 mm in diameter that were immersed in the salt solution. The electrodes were held in place by caps that seal the fluidic chambers to reduce evaporation of the solution. The flow cell was finally placed into the Spark-E2 unit (Northern Nanopore Instruments) for automated nanopore fabrication, and the associated software commanded to fabricate a nanopore of the size desired using the standard protocol that involves increasing the applied voltage across the membrane at a rate of 3 V/min until 5 V, and at 0.75 V/min until pore formation or until the maximum voltage of 18 V of the Spark-E2 is reached. Once the nanopore was fabricated via CBD, 3.6 M LiCl + 10 mM HEPES pH 8 was flushed into the flow cell and the software commanded to enter the conditioning stage where it enlarged the nanopore to the

desired size using a series of pulses at 40% of the breakdown voltage for 3 s each, or no greater than 5 V. Once completed, a power spectral density (PSD) at ± 200 mV was performed to characterize the electrical noise of the nanopore using a low-noise current amplifier (Axopatch 200B, Molecular Devices). For nanopores with sufficiently low $1/f$ noise, defined here for most pores as $<10 \text{ pA}^2 \text{ Hz}^{-1}$ at 1 Hz on the PSD, molecular translocation experiments were performed at -200 mV with this same amplifier by introducing the 2 kbp DNA at 5 nM in the same 3.6 M LiCl + 10 mM HEPES pH 8 mentioned previously. Results of the DNA experiments were subsequently analyzed using the Nanolyzer (Northern Nanopore Instruments) software.

4.4 Results and discussion

4.4.1 IBD SiN_x film analysis

The properties of the IBD SiN_x films (non-annealed) were characterized by ellipsometry, X-ray reflectometry, laser-based stress measurement, and wet etch rate (WER) tests to study the thickness, density, stress, and chemical stability, respectively (Figure 4.3). These measurements, coupled with the nanopore results themselves, are crucial in helping us understand which metrics are important for obtaining a SiN_x film that is suitable for ssNP work, and will help inform the optimization of future depositions. To match the thickness of the membrane in the locally thinned regions, we deposited 20 nm thin films. The ellipsometry results show that the IBD film tested had an average thickness of 20.8 ± 0.1 nm with a surface roughness of 0.36 nm and percentage of non-uniformity of 1.2% (Figure 4.3(a) and (b)). In comparison, traditional 20 nm films deposited via LPCVD have a thickness of 19.6 nm with a uniformity of $\pm 0.2\%$, as per a recent deposition. While the uniformity of the IBD film is slightly less than that of the LPCVD, this difference represents such a small variation in thickness that the nanopore fabrication and sensing will be unaffected. Thus, in terms of absolute thickness, the IBD films seem to be on par with those deposited by LPCVD. In contrast, the stresses of the SiN_x films are quite different; while LPCVD generates

membranes with tensile stress that optically look flat, the nature of the IBD method generates a compressive stress within the SiN_x films, measured to be – 1.89 GPa for our deposition. As a result, the membranes look heavily rippled (Figure 4.2(m)). The density of the films also presented a difference, with the IBD SiN_x film measured at 3.07 g cm⁻³, 96% of the density of stoichiometric LPCVD SiN_x films, 3.2 g cm⁻³.¹⁸² This measurement suggests that the IBD SiN_x membrane is Si-rich, which could have an impact during the nanopore fabrication as the conductive silicon may provide increased leakage current and/or a path for fabrication to occur. It should be noted, however, that the IBD method allows for the tuning of the Si and N ratio within the thin film, which could be an avenue for optimization in the future.

Lastly, we conducted a KOH wet-etch test on the SiN_x films deposited by IBD and LPCVD SiN_x as well as ICPCVD and ICPALD, the two other BOEL-compatible methods previously identified, by subjecting each film to a 2 h KOH bath at 80 °C. While the final workflow for generating BEOL integrated ssNP devices may use a membrane release technique other than KOH, the wet etch process can nonetheless reveal the chemical stability of the membrane, which is pivotal for device fabrication and characterization.

After etching, the SiN_x films were examined under an optical microscope. The films deposited by IBD and LPCVD appeared smooth and uniform without obvious defects (Figure 4.3(c) and Figure 4.7), indicating high quality and good chemical stability. However, high-density square defects were observed on the ICPCVD SiN_x film (where the ICPCVD SiN_x had been etched through, partially affecting the Si substrate). Additionally, the presence of scattered defects indicates nonuniform film quality, which could pose challenges for nanopore device fabrication and performance. The ICPALD fared even worse, as the SiN_x film was fully removed during the 2 h wet etch test, leaving only a rough surface of Si wafer (Figure 4.7). The rate of the etch was also determined for each deposition method via ellipsometry. The WER of the IBD film was the lowest, at 0.17 nm h⁻¹, with the LPCVD film nearly an order of magnitude greater at 1.47 nm h⁻¹ (Figure 4.3(d)). The ICPCVD SiN_x displayed a relatively high and non-uniform etch rate, ranging from 8.38 nm h⁻¹ to above 10.00 nm h⁻¹ and the ICPALD SiN_x, having been etched all the way

through, could only be assigned a minimum value of $10.00 \text{ nm } h^{-1}$. The high WER observed in ICPALD and ICPCVD SiN_x membranes stems from impurity incorporation in the films. From these analyses, we conclude that of the SiN_x deposition methods that have a low thermal budget, the accuracy and precision of the deposition thickness, the tunability of the Si content, and the high purity of the film all combine to make the IBD SiN_x the best candidate for BEOL-integrated ssNPs.

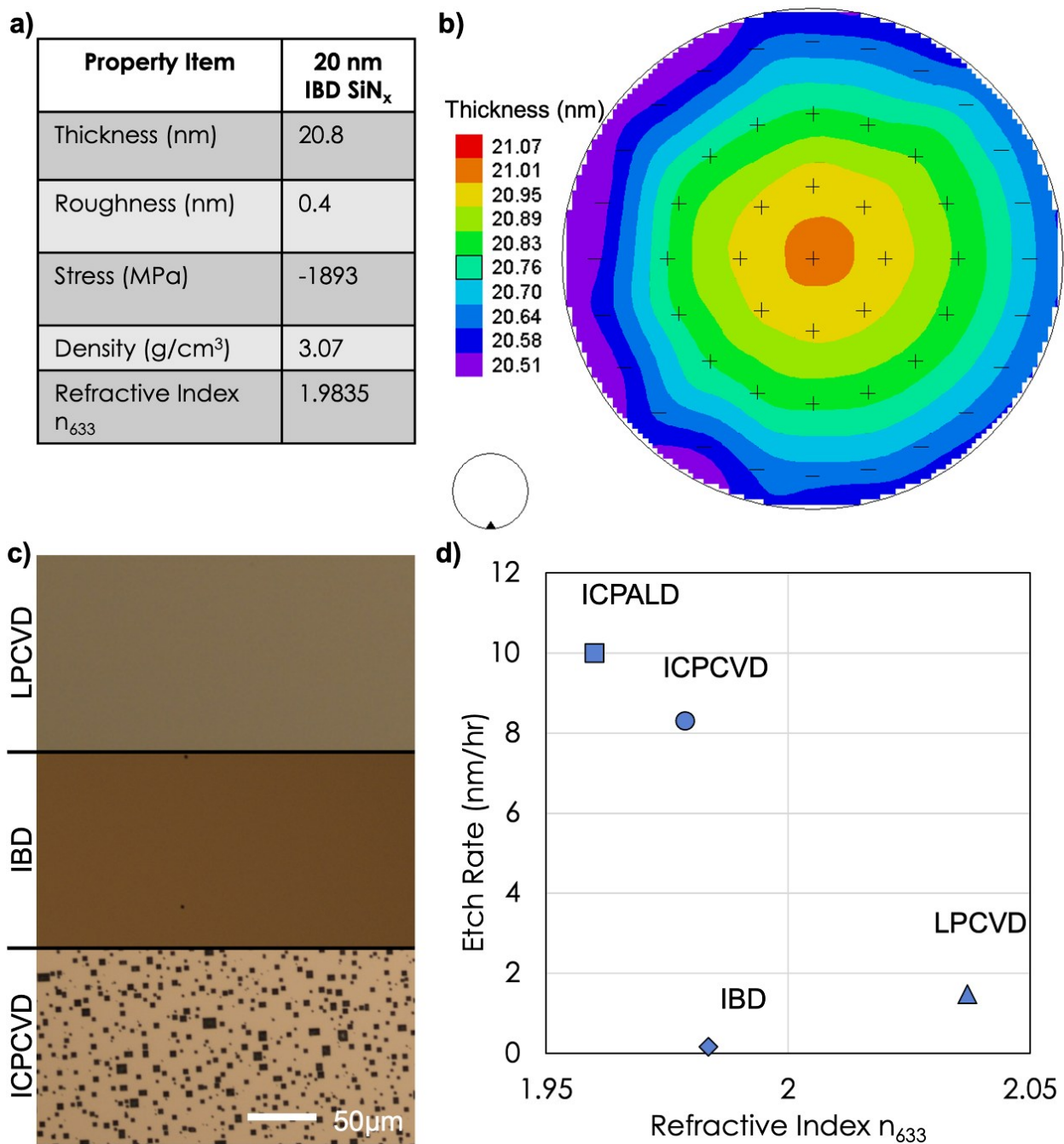


Figure 4.3 Characterization of 20 nm thick, non-annealed, SiN_x membranes deposited by ion beam deposition (IBD). (a) Film properties including thickness, roughness, stress, density, and refractive index. (b) Ellipsometry result showing the wafer scale thickness and uniformity of the film deposited on a six-inch wafer. (c) Optical microscopy images comparing the results of a 2-hour-long hot KOH wet etch (80 °C, 30 wt.% potassium hydroxide) conducted on SiN_x thin films deposited by LPCVD, IBD, and ICPCVD. No obvious or few defects are observed for IBD and LPCVD films, in contrast to ICPCVD. (d) Plot showing the etch rate and refractive index (at wavelength of 633 nm) comparison of IBD SiN_x membrane with membranes deposited by other techniques.

4.4.2 ssNPs

Once mounted in fluidic solution, both IBD non-annealed and IBD annealed membranes were easy to wet and proved to be insulating, as evidenced by the resistance across the chip measuring $> 1 \text{ G}\Omega$. Once the resistance was validated, the membranes underwent fabrication via CBD (Figure 4.4(a) and (b)). As shown, in both types of membranes the voltage ramp generates the expected and characteristic leakage current response prior to the formation of the nanopore (i.e. the breakdown event). While the magnitude of the leakage current is slightly different between the non-annealed and the annealed IBD chips, it is not possible to conclude that the type of film deposition is the cause of this difference, as several variables can contribute to this current including the resistivity of the particular wafer, the thickness of the SiN_x layer, the area of chip exposed to liquid, etc. Additionally, the fabrication voltage is significantly different, but this too depends on extraneous variables, principally the thickness of the thinned region, which itself is dependent on the RIE which has considerable margins of error. As such, we do not draw direct comparisons regarding the breakdown voltage or leakage current, but rather that both exhibit typical pre-fabrication curves which culminate in a sudden increase in current, indicating the transition from leakage and capacitive current to ionic current, and thus a nanopore.

After fabrication, the nanopores were enlarged to their target size of 8 nm via the conditioning protocol, and as shown in Figure 4.4(c) and (d), nanopores in both non-annealed IBD and annealed IBD films were capable of controlled enlargement (triangles). However, both membrane types also generated pores that were relatively large immediately after fabrication (squares). In this case, the target size was set to 20 nm and as shown, the conditioning steps enlarged the pores much more rapidly. It should be noted that this uncontrolled pore enlargement behavior is rare in typical LPCVD membranes.

Post enlargement, all nanopores underwent a $\pm 200 \text{ mV}$ voltage sweep while the current was measured. Characteristic linear I–V curves are shown in Figure 4.4(e), for each pore shown in Figure 4.4(c)

and (d), demonstrating that pores in both non-annealed IBD and annealed IBD membranes can behave ohmically, and that the rapid pore growth does not preclude linear I–V curves.

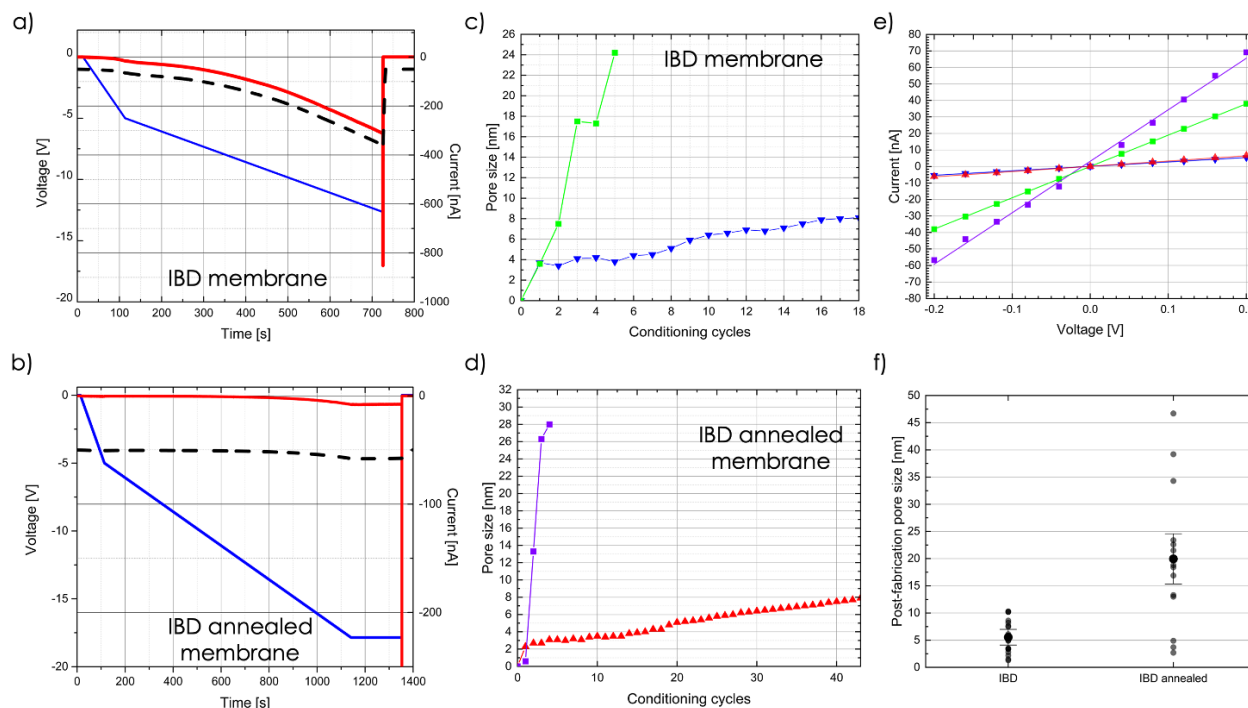


Figure 4.4 Solid-state nanopore fabrication by controlled breakdown and enlargement by voltage-pulse method on locally thinned ~ 20 nm thick annealed and non-annealed SiN_x membranes deposited by IBD. (a), (b) Representative example of the fabrication curve of a solid-state nanopore for non-annealed and annealed IBD membranes, respectively. The membranes were submerged in 1 M KCl, pH 8 solution and the voltage increased until nanopore formation. The red line represents the current, the black dashed line the threshold the software uses to detect nanopore formation, and the blue line the applied voltage. (c), (d) Growth behaviors observed during the enlargement phase for non-annealed and annealed SiN_x membranes deposited by IBD. Squares represent a nanopore that grew fast while triangles represent a nanopore that grew more controllably. Each nanopore was enlarged in a 3.6 M LiCl pH 8 solution with a square voltage pulse of 40% of the breakdown voltage and no greater than ± 5 V for 3 s. (e) Typical I–V curves for annealed and non-annealed SiN_x membranes formed by IBD after conditioning, with colors conserved from (c), (d). Red and blue triangle data points overlap. Note that the observed conductance is also a function of the effective membrane thickness / pore length for each pore. (f) Distribution of post fabrication pore sizes annealed and non-annealed SiN_x membranes formed by IBD.

In total, 19 nanopores were fabricated on non-annealed IBD chips and 14 on annealed IBD chips. While all these samples underwent successful nanopore fabrication, the distribution of the initial pore sizes of the non-annealed IBD chips was 5.5 ± 3 nm while the distribution of the initial pore sizes for the

annealed IBD chips was 20 ± 13 nm (Figure 4.4(f)). This large difference in initial pore size was unexpected and may be due to how the annealing modifies the previously amorphous SiN_x structure (see AFM roughness measurement in Figure 4.8 for an IBD annealed membrane).

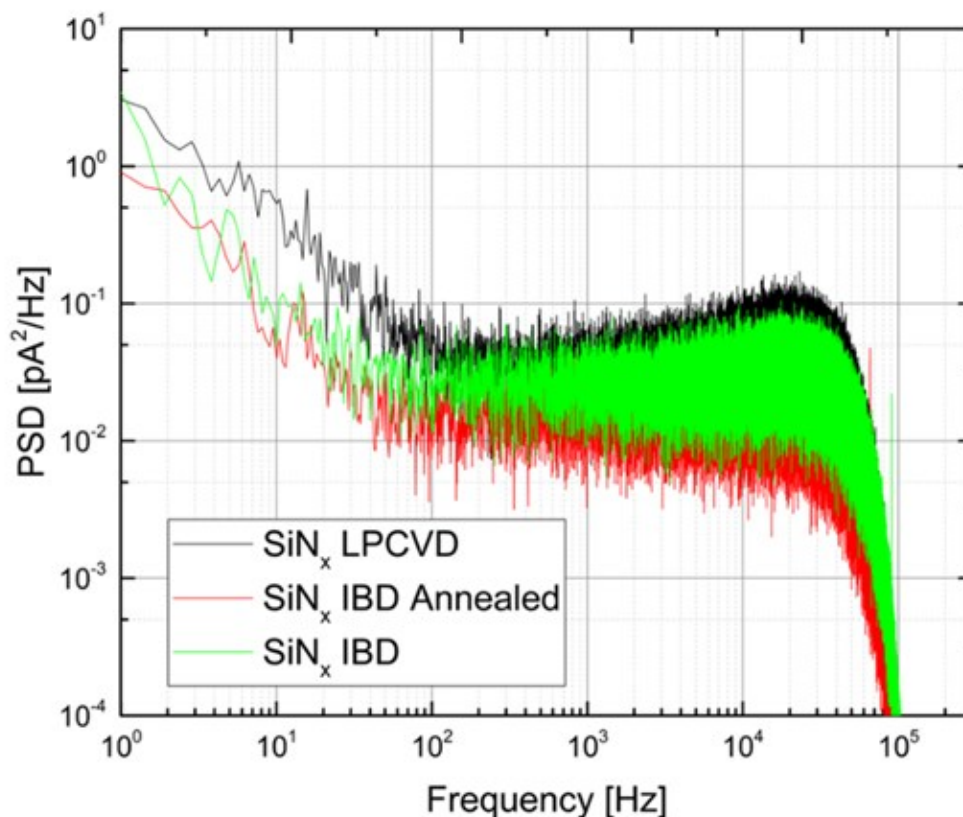


Figure 4.5 Power spectral density (PSD) of the current trace at the sensing voltage (-200 mV) in 3.6 M LiCl pH 8 for nanopore devices fabricated on 3 types of membrane devices (IBD, IBD annealed, and LPCVD), recorded with an Axopatch 200B with the low-pass filter set to 100 kHz.

To demonstrate the ability of these nanopores to perform single-molecule measurements, representative power spectral densities (performed in 3.6 M LiCl + 10 mM HEPES (pH 8) and at -200 mV) are presented in Figure 4.5. For benchmarking, a PSD of a nanopore fabricated in a traditional LPCVD low-stress, silicon-rich, SiN_x membrane, and mounted in the same type of flow cells, is included. Variability in the magnitude of low-frequency $1/f$ noise is indicative of a stable pore conductance over time (in the

seconds timescale), which is generally a good indicator for the viability of the pore for molecular sensing.⁶⁸ Here, the nanopores from non-annealed IBD and annealed IBD membranes exhibit a noise profile in the low-frequency range similar to that of nanopores formed in traditional LPCVD. Interestingly, we observed that the annealed IBD film exhibited a lower high-frequency noise contribution than the non-annealed film (see section 4.7.3 and 4.7.4 of the supporting information for the noise performance distribution between LPCVD, IBD and IBD annealed nanopores).

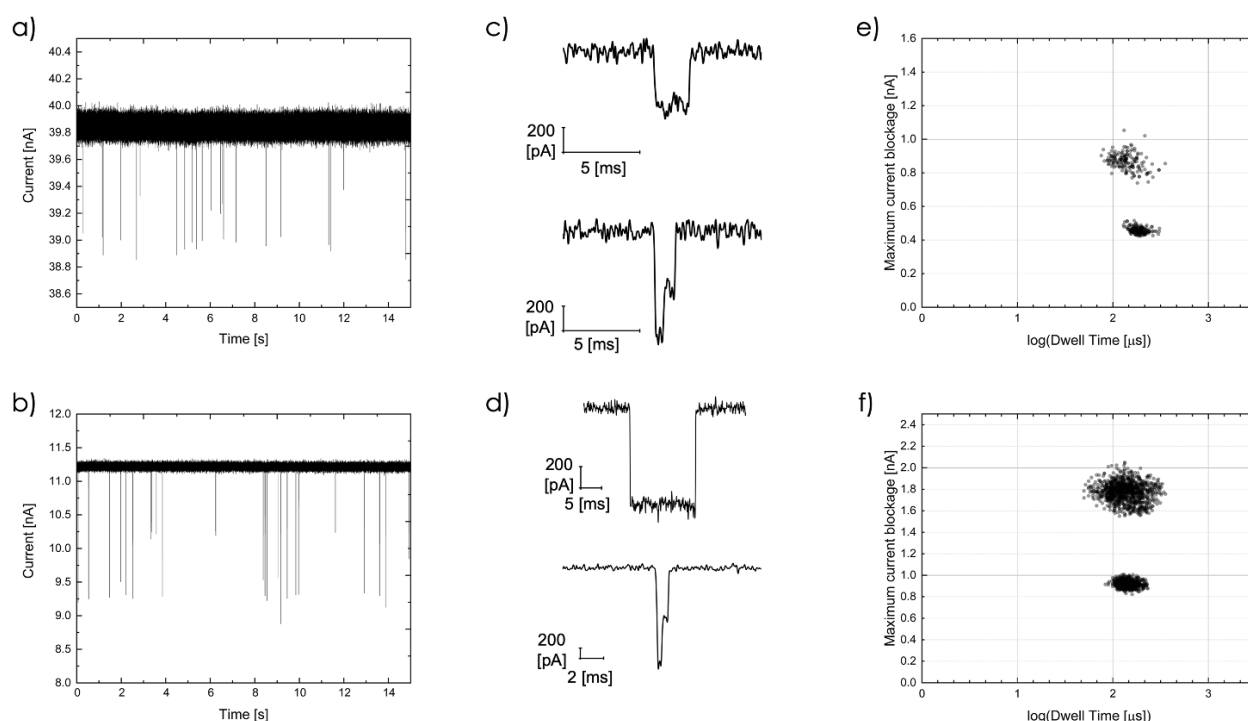


Figure 4.6 DNA translocation experiments (5 nM 2 kbp no-limit DNA in 3.6 M LiCl in pH 8 at -200 mV, low-pass filtered at 100 kHz) using annealed and non-annealed SiN_x membranes formed by IBD. (a), (b) Baseline current trace showing several molecular translocations using non-annealed and annealed SiN_x membranes formed by IBD, respectively. (c), (d) Current traces of typical DNA translocation events observed in non-annealed and annealed SiN_x membranes formed by IBD, respectively. (e), (f) Scatter plots of a typical maximum blockage current versus dwell time for non-annealed and annealed SiN_x membranes formed by IBD, respectively.

Finally, single-molecule translocation data using nanopores formed in both non-annealed IBD and annealed IBD membranes is shown in Figure 4.6. A 15 s trace of a 24 nm non-annealed IBD and 8 nm diameter annealed IBD nanopore is shown in Figure 4.6(a) and (b) respectively, where the flow cells

contain 3.6 M LiCl + 10 mM HEPES (pH 8) and 5 nM of 2 kbp DNA (NoLimits 2000 bp, Thermo Fisher). The signal is obtained by applying 200 mV with an Axopatch 200B, and sampled at 500 kHz before being digitally filtered with an 8-pole Bessel at 100 kHz. Examples of individual events are presented in Figure 4.6(c) and (d) respectively, and the resultant maximum blockage versus dwell time plots are shown in Figure 4.6(e) and (f), respectively, validating successful translocation of both single-file and folded DNA molecules. The observed discrepancy between the blockage depths between the non-annealed IBD and annealed IBD nanopores is attributed to a difference in membrane thickness, which could have arisen during the local thinning process by RIE or is indicative of a variation in the effective pore thickness of the CBD pores, assuming a cylindrical pore geometry.¹⁷ Future work may need to be done to improve the membrane fabrication workflow to more reliably achieve the desired membrane thickness and to determine the 3D shape of solid-state nanopores fabricated by CBD.

4.5 Conclusion

Our investigation focused on evaluating the quality of SiN_x membranes deposited by IBD onto Si substrates and their suitability for ssNP biosensing. We showed SiN_x films prepared by IBD show high density, good uniformity, and wet etch resistance, and that low $1/f$ noise nanopores can be successfully fabricated by CBD. These resulting nanopores were then used for single-molecule experiments where 2 kbp DNA molecules translocations showed a signal-to-noise ratio comparable to nanopore formed in the gold standard LPCVD SiN_x membranes. These findings are promising for the integration of ssNP systems on CMOS platforms as this represents a BEOL compatible method of merging ssNPs with CMOS technologies which could significantly advance the development of DNA sensing and storage technologies.

4.6 Acknowledgment

This work was supported by Mitacs through the Mitacs Accelerate program (IT30712), and Natural Sciences and Engineering Research Council of Canada (NSERC) [funding reference I2IPJ 586238-23]. We would also like to thank Vanmaly Phanousith and Simon Riberolles for support with the X-ray measurements.

4.7 Supplementary Information

4.7.1 Optical characterization of thin film deposition technique after KOH wet etching

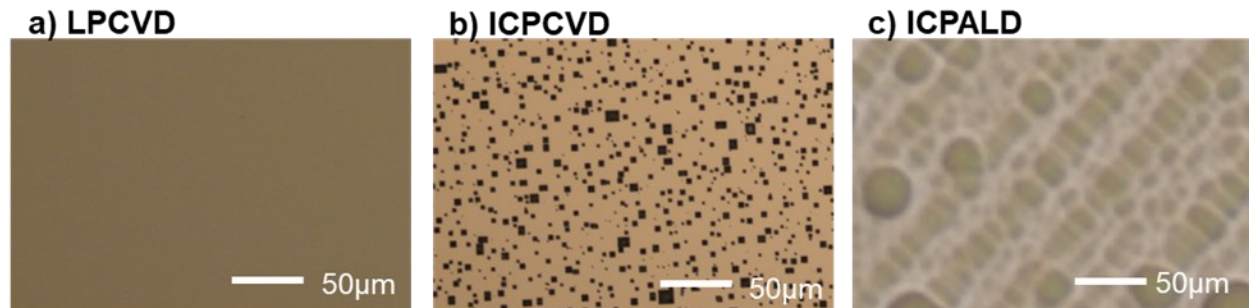


Figure 4.7 Evaluation of the membrane quality. 20 nm silicon nitride (SiN_x) membranes are deposited on both sides of silicon wafers by different techniques. The samples are further etched in (80 °C) 30 wt.% potassium hydroxide (KOH) bath for 2 hours. a) The SiN_x membranes deposited by low pressure chemical vapor deposition (LPCVD) remain smooth and continuous after etching test. No obvious defects are observed. b) The SiN_x membrane deposited by inductively coupled plasma chemical vapor deposition (ICPCVD) shows a high density of defects with different sizes across the sample. The SiN_x membrane at these defect sites is fully removed. c) The SiN_x membrane deposited by inductively coupled plasma atomic layer deposition (ICPALD) is fully etched after the 2-hour test. The image shows the rough etched surface of the exposed silicon.

4.7.2 Roughness measurement of IBD annealed free-standing membrane

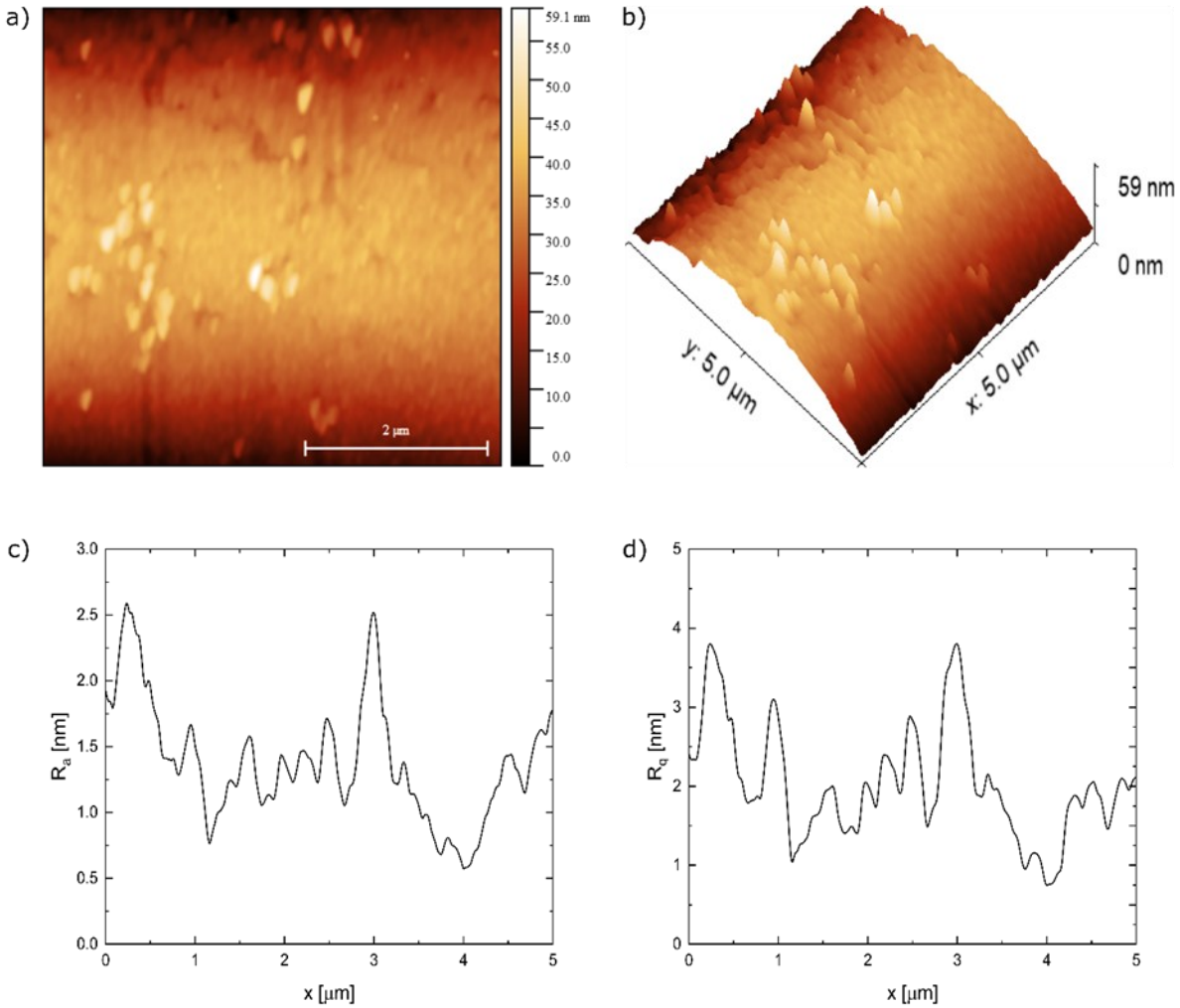


Figure 4.8 AFM roughness measurement on IBD annealed free-standing membrane, with a $5 \times 5 \mu\text{m}$ scanning window at 0.75 Hz. a) AFM topography of the membrane. b) 3D view of the AFM topography. c) R_a (arithmetic average) roughness measurement of the panel presented in (a), with a recorded value of $1.4 \pm 0.4 \text{ nm}$. d) R_q (root mean square) roughness measurement of the panel presented in (a), with a recorded value of $2.2 \pm 0.8 \text{ nm}$.

4.7.3 Stability of IBD and IBD annealed CBD-fabricated nanopores

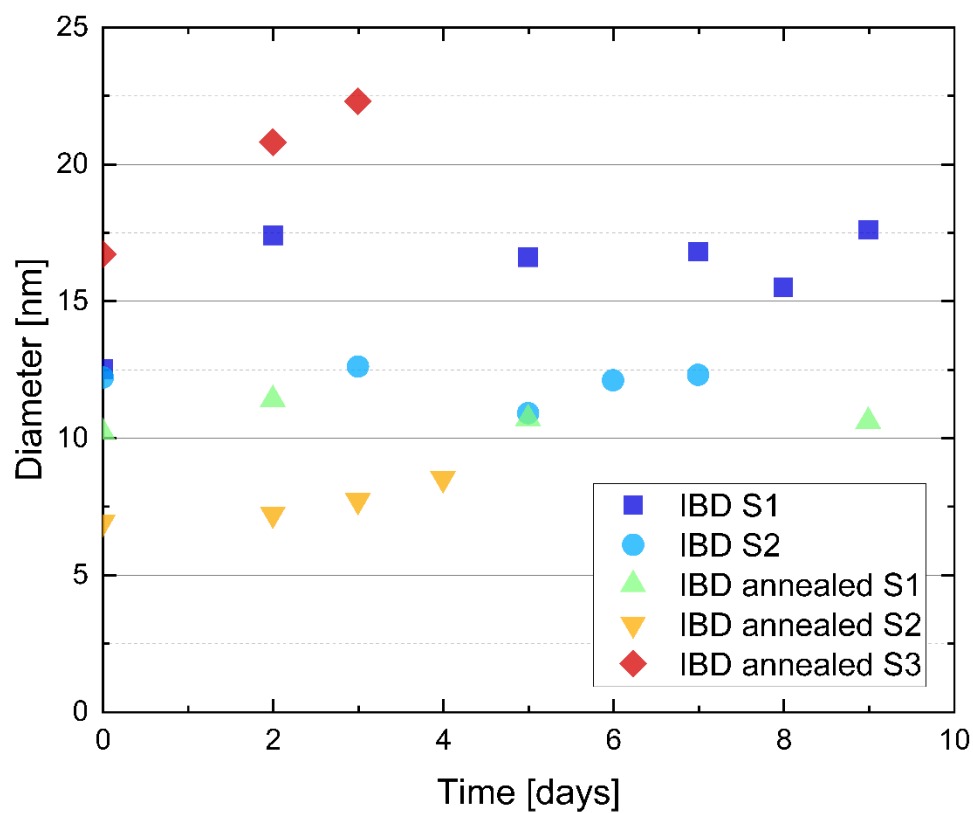


Figure 4.9 Stability study of IBD and IBD annealed CBD fabricated nanopores stored in 3.6 M LiCl pH 8. IBD nanopores show minimal growth (~ 0.4 nm/day) over an inspection period of 7–9 days. IBD annealed nanopores show mostly minimal growth (~ 0.4 – 2 nm/day) over an inspection period of 4–9 days, comparable to LPCVD SiN_x nanopores.⁶²

4.7.4 Noise characteristics of LPCVD, IBD and IBD annealed CBD fabricated nanopores

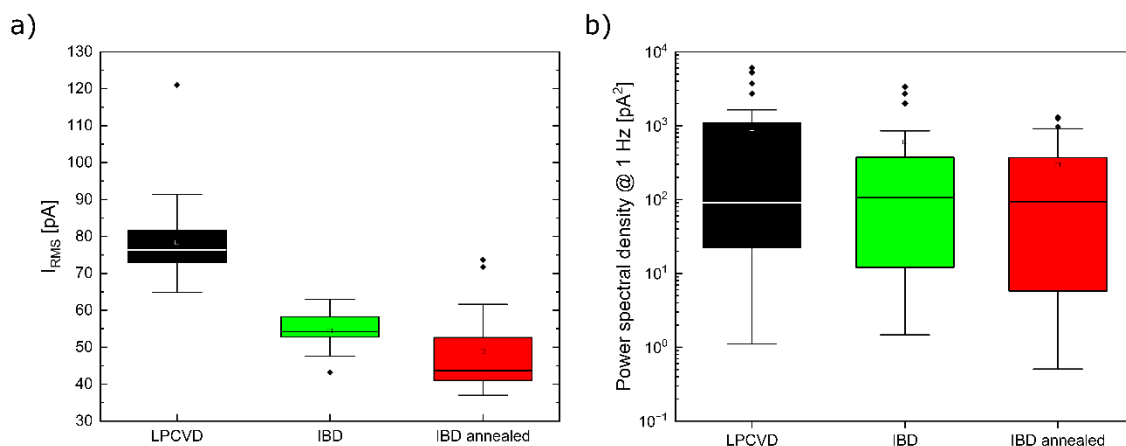


Figure 4.10 Noise performance characterization of SiN_x nanopores in 3.6 M LiCl pH 8 on the Axopatch 200B amplifier with a 100 kHz hardware low-pass filter. a) Root mean square of the recorded current at 0 mV. LPCVD, IBD and IBD annealed nanopores show decreasing high-frequency noise with a sample size of 18, 14 and 9 respectively. b) Power spectral density of SiN_x nanopores with an applied bias voltage of ± 200 mV, characterizing flicker noise at 1 Hz. LPCVD, IBD and IBD annealed nanopores show comparable flicker noise with a sample size of 29, 17 and 22 respectively. Note here that the same testing conditions are used for all samples.

5. Microfabrication of thin, stable, low noise and high throughput nanopore membranes

Silicon nitride (SiN) has been the predominant membrane material for nanopore fabrication due to its established microfabrication and insulating electrical properties. Solid-state nanopores are generally praised for their robustness over a wider range of operating conditions compared to their biological protein pore counterparts. However, nanopores in 10-20 nm thick SiN membranes exhibit significant instability in aqueous environments used during biosensing, with diameter expansion rates as high as many nanometers per day depending on membrane fabrication batch, salt concentration and type, and other operating conditions (e.g. applied voltage, flushing). The high pore growth rate compromises measurement reliability when complete studies require multiple experiments over many hours, and the sensing scheme depends on a precise range of pore size. It should be noted that some batches of SiN do exhibit better long-term stability, with expansions as little as a few angstroms per day, and much of the progress in the field has relied on using the “good” batch of SiN membranes.⁶²

Hafnium oxide (HfO₂) presents a compelling alternative due to its superior chemical stability. The suitability of HfO₂ as a membrane material is greatly enhanced by its compatibility with ALD techniques. ALD grants superior control over film thickness with single-digit atomic layer precision (angstrom level), compared to the standard LPCVD SiN technique, which cannot produce uniform, defect and pinhole free films below 10 nm, and which has a >10× precision in the film thickness achievable. As discussed in Chapter 1, the film thickness impacts the absolute signal strength (i.e. depth of recorded current blockages), with a tendency to favor lower thicknesses for higher sensitivity.^{65,76,78} While previous research focused on SiN chips coated with ALD hafnia and nanopore fabrication via TEM-drilling, either forming nanopores in a 4.5 nm thick HfO₂ free standing membrane or coating pre-drilled ones with a 2.5

nm HfO₂ layer,^{11,62} or CBD nanopores through 5 nm SiN encapsulated by 1-3 nm HfO₂,¹⁸³ a robust microfabrication workflow on a wafer scale with ultra-thin HfO₂ membranes is yet to be demonstrated. To address this gap, this chapter investigates two novel nanopore membrane architectures. The first architecture employs a locally thin annealed 5 nm HfO₂ membrane supported by a 100 nm LPCVD SiN film (see section 5.1), while the second utilizes an annealed 6 nm HfO₂ membrane protected by a 4 nm Al₂O₃ and encapsulated by a 17 nm LPCVD SiN film (see section 5.3). The annealing step here is important as it not only increases the HfO₂ thin film's resistance to chemical etching,⁴⁴ but also reduces the mismatch in deposition temperatures relative to the supporting LPCVD SiN layer. Additionally, the aluminum oxide layer serves as an etch stop during the RIE process, significantly simplifying microfabrication complexity.⁴¹

5.1 Annealed HfO₂ membranes supported by LPCVD SiN

Device microfabrication was performed following the sequential process flow detailed in Figure 5.1. The process began with 4-inch high-resistivity (intrinsic) silicon wafers (purchased from Addison Engineering Inc) with the following specifications:

Table 5.1: Silicon wafer specifications as purchased from Addison Engineering Inc

Property	Value
Diameter	100 ± 0.5 mm
Growth method	Fz (float zone)
Type/Dopant	Intrinsic
Resistivity	> 10,000.0 Ω-cm
Thickness	300 ± 25 µm
Orientation	[100]
Front/Back Finish	Polish (5 Å)
Total Thickness Variation	≤ 10 µm
Bow/Warp	≤ 30 µm

First, a 5 nm layer of HfO_2 was deposited by ALD on one side of the wafer at Western Digital (a collaborator on this project), arbitrarily selected as the membrane side. Then, the wafer underwent a Rapid Thermal Annealing (RTA) process at 800 °C for four minutes. Next, a layer of 100 nm LPCVD SiN encapsulated the wafer via the LSN1 recipe performed at the uAlberta Nanofabrication facilities.¹⁸⁴ Finally, a local-thinning workflow is followed to create free-standing HfO_2 membranes at the uOttawa Nanofabrication facilities,³⁵ prior to precise wafer dicing at the uAlberta Nanofabrication facilities.

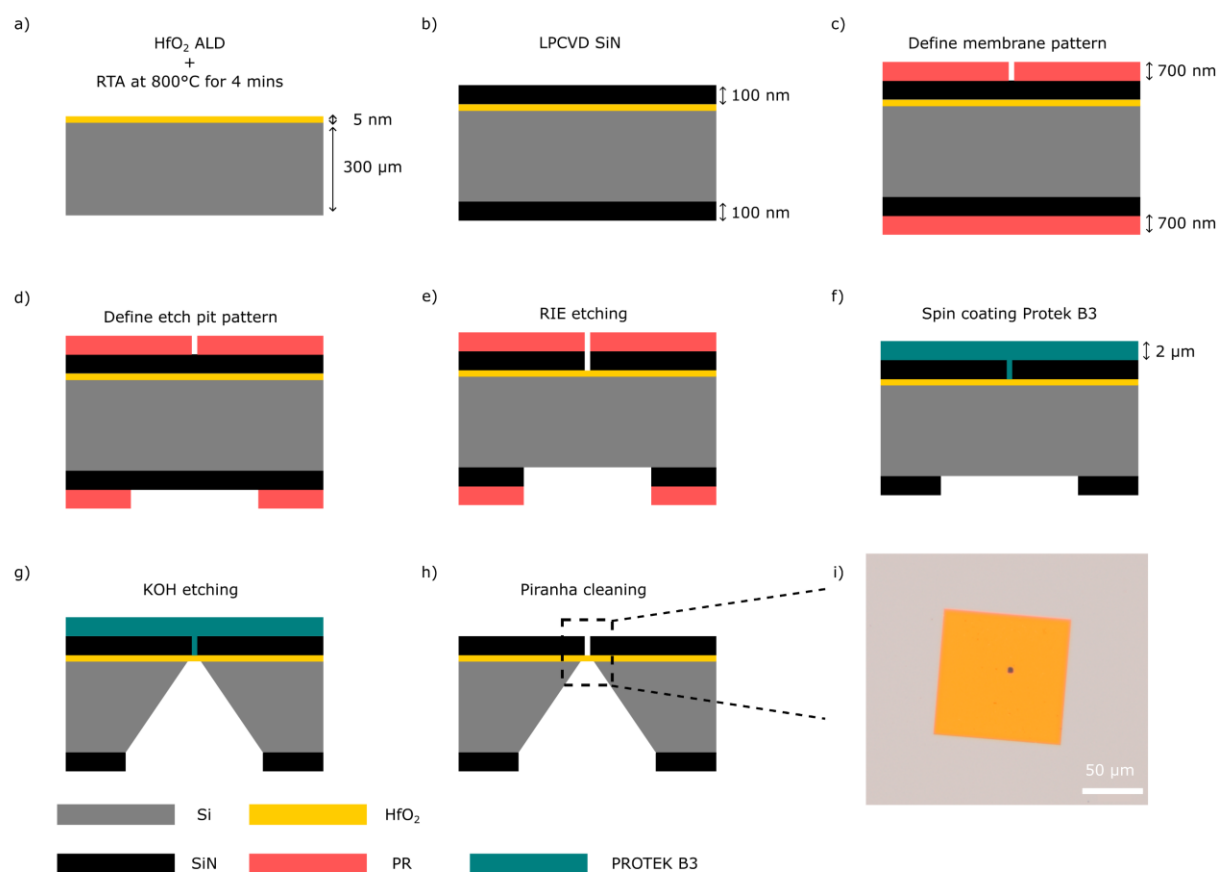


Figure 5.1 Microfabrication process flow for HfO₂ devices. a) ALD of a 5 nm HfO₂ film on the membrane side of the wafer, followed by a Rapid Thermal Annealing step at 800 °C for four minutes. b) Deposition of 100 nm LPCVD SiN, encapsulating the wafer. c) Membrane-side patterning to define the local thinning area, where a photoresist (S1805) is spin-coated, soft-baked, exposed to UV during contact-lithography, and developed using an alkaline solution (MF321). d) Back-side patterning to define the etch pit, where a photoresist (S1805) is spin-coated, soft-baked, exposed to UV during contact-lithography after IR front-to-back alignment, and developed using an alkaline solution (MF321). e) Sequential RIE etching of SiN to thin the local thinning area and expose the silicon on the etch pit side of the wafer. f) Spin-coating and baking of the ProTEK Primer and B3 layer to protect the membrane during subsequent wet etching. g) KOH etching of the silicon wafer to create free-standing membranes. h) Wafer dicing to form 5×5 mm dies and piranha cleaning of the chip prior to membrane characterization. i) Microscopy image of the local thinning area following piranha cleaning, showing a ~100 μm free-standing membrane (yellow), and the locally thin 5 μm opening (black spot).

To ensure device integrity and mitigate risks associated with varying RIE etch rates for SiN, the initial thinning protocol was conservatively executed. Due to the lack of established etch rates for HfO₂ films under the specific RIE conditions employed (10 sccm CF₄ gas flow, 25 W, 8 Pa), and to ensure minimal damage to the ALD deposited layer, the initial 100 nm SiN film was etched down to $\sim 15 \pm 5$ nm by running

the process for 430 seconds with periodic AFM inspections of the etch depth, resulting in a membrane thickness in the 15-25 nm range (accounting for the 5 nm HfO₂).

Characterization of the etch depth for the specific RIE etch conditions above was achieved using a Park NX10 AFM and a TAP300-G tip (resonant frequency of 300 kHz, 40 N/m force constant, and a beam shape of 125 (L) × 30 (w) × 4 (h) μm). This characterization was performed across 2 different wafers containing designated dicing streets (~ 20 μm die separations), which allowed for profile analysis without compromising the membrane integrity. Briefly, photoresist was removed from an approximately 2 mm circle with an acetone-soaked cleanroom swab, following RIE etching (Figure 5.1(e)). Then, a contact-mode scan in a 45×10 μm window was performed in the Park SmartScan operating software, while the resulting profile was analyzed using the Gwyddion analysis software. Figure 5.2 presents etch profiles for both wafers (WD11 and WD14 respectively). The wafers underwent RIE in different runs, but on the same day. The measured etch depth was in the 80-85 nm range with the inset showing the extracted topography of the dicing streets.

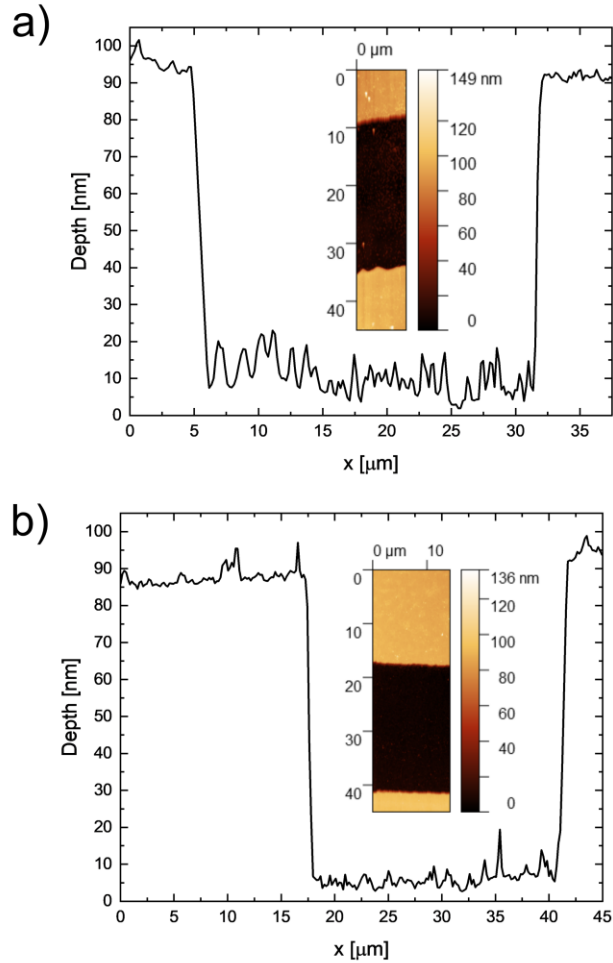


Figure 5.2 AFM etch depth measurement of the local-thinning process across 2 different wafers, with a recorded value of 83.9 ± 5.6 nm for WD11 in panel a) and 82.5 ± 4.5 nm for WD14 in panel b). Insets show the dicing streets over which the AFM scan is performed for each wafer.

Following full device microfabrication and piranha cleaning, the free-standing membranes were initially inspected via optical microscopy on a Zeiss Axio Imager 2, utilizing 20× magnification over the local thinning region. This initial examination confirmed the membrane window size and the successful front-to-back alignment of the local thinning process, ensuring the thinned SiN region lay atop the KOH-released membrane (Figure 5.1(i) – black dot on image).

Subsequently, the focus shifted to the characterization of nanopores formed by CBD on these devices. Figure 5.3 outlines the nanopore characterization workflow on the HfO_2 membranes. Initial pore

fabrication was conducted in 1 M KCl pH 8 as is typically done for CBD pore fabrication in SiN membranes. However, unlike in 10-20 nm SiN, these membranes required prolonged fabrication times, as the fabrication ramp voltage (0.75 V/min) reached the maximum 18 V applied voltage of our custom fabrication instrument, and sustained that 18 V for minutes before the film experienced breakdown and the opening of a pore. From previous CBD studies,¹⁸⁵ we know that time to breakdown is exponentially related to the applied voltage, so prolonged fabrication at 18 V implies, either a thick film (reducing the electric field for a given voltage), or a high dielectric strength material or both. For pore fabrication by TEM drilling with an energetic beam of electrons, it was shown that HfO₂ membranes require much higher irradiation (2.5×10^8 e/nm²) to form nanopores compared to SiN ($\sim 10^6$ e/nm²).¹¹ This supports our observation that hafnia membranes are more difficult to breakdown than SiN of similar thicknesses.

Once the fabrication was completed, the salt solution was replaced by 3.6 M LiCl pH 8 for subsequent ionic conduction characterization. The pore was enlarged to the desired size (set to 12 nm, assuming a nominal membrane thickness of 20 nm) through repetitive application of five square voltage pulses per cycle. These pulses were sustained for 1 second with an amplitude corresponding to 45% of the breakdown voltage (Figure 5.3(b)). After each cycle, the pore diameter was then estimated by performing an I-V sweep within the ± 200 mV range, which simultaneously allowed for the determination of the pore's rectification ratio or ohmic behavior (Figure 5.3(c)). To assess the quality for biosensing of the formed pore on the Axopatch 200B current amplifier, a PSD measurement was taken at a sensing voltage of -200 mV to characterize $1/f$ noise. A threshold criterion of ≤ 50 pA² Hz⁻¹ at 1 Hz was enforced for pore quality, taken as the y-intercept derived from the fitted slope in the 1-100 Hz range (Figure 5.3(d) – blue line). Finally, the root mean square (RMS) noise was calculated at hardware full-bandwidth with a 100 kHz digital 8-pole low-pass Bessel filter, yielding an RMS noise of 45 pA at 100 kHz (note that this data was recorded with regular silicone elastomer gaskets, exposing the nanopore chip to liquid with an opening of 500 μ m diameter).

The final characterization step involved a DNA sensing experiment to validate performance and pore size, in the same 3.6 M LiCl pH 8 solution. 5 nM of 2 kbp dsDNA was injected on the membrane side of the nanopore, and -200 mV was applied (Figure 5.3(e-f)). Event analysis was conducted on Poriscope,¹⁶⁵ employing a threshold crossing algorithm for event extraction and the CUSUM algorithm for event fitting.^{141,186}

A resulting 2D scatterplot of maximum current blockage as a function of event duration (log) was generated. The unfolded DNA level was recorded at 574 ± 20 pA, with a mean event duration of 145 ± 45 μ s, extracted from the Gaussian fit to the distributions of unfolded level current blockages and event durations.

Based on the recent model proposed by Charron *et al.*,¹⁷ calculations utilizing an open pore conductance of 77.5 nS and an unfolded level conductance blockage of 2.9 nS yielded an estimated pore diameter of 9 nm and an effective thickness of 6.9 nm. This effective thickness suggests two possible interpretations: either the membrane produces a cylindrically shaped pore with a length equal to that of the thickness within the local thinning area etched during device microfabrication, or the resulting pore had an hourglass shape with a height of ~ 21 nm, approximately three times the calculated effective membrane thickness. At this stage, our conductance model does not allow us to distinguish between the two, however, given our AFM measurement, the latter is more likely. In fact, this hourglass-shaped pore with an effective length of 1/3 the membrane thickness is often used to report TEM-drilled pores.¹⁸⁷

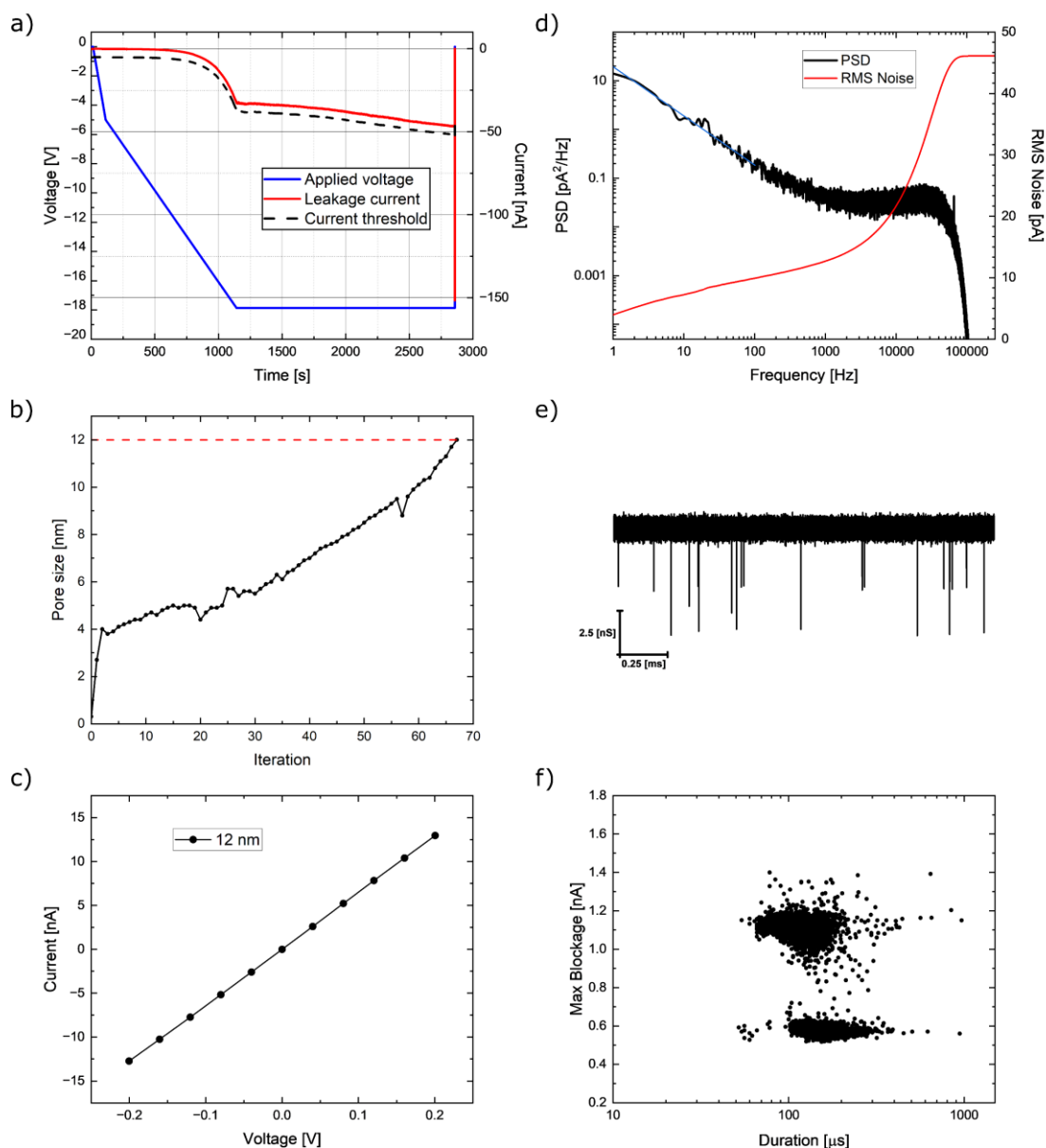


Figure 5.3 CBD-fabricated HfO_2 nanopore characterization workflow. **a)** Typical fabrication curve for HfO_2 nanopores in 1 M KCl pH 8. The applied voltage is represented in blue, the leakage current in red, while the nanopore fabrication threshold is represented by the black dashed line. **b)** Conditioning curve for the fabricated pore, using 1 s square voltage pulses in 3.6 M LiCl pH 8, to reach the target diameter (the red dashed line). **c)** I-V sweep of the conditioned pore to extract a final diameter of 12 nm assuming a nominal membrane thickness of 20 nm and cylindrical geometry. **d)** Noise characteristics of the conditioned pore at an applied voltage of -200 mV in 3.6 M LiCl pH 8. The PSD of the recorded current is presented in black and the RMS of the current in red. The blue line is used to extract the 1-100 Hz fit intercept for the PSD. **e)** A representative current trace of dsDNA translocations in this representative nanopore. **f)** 2D scatterplot of 5 nM 2 kbp dsDNA translocation events in 3.6 M LiCl pH 8 at -200 mV.

Because our RIE was conservative, some SiN should remain on the HfO₂ membrane, and we characterized the long-term stability of this SiN-HfO₂ heterostructure membrane architecture. To this end, an aging study was performed over a period of up to 11 days. During this period, the chips were stored in 3.6 M LiCl pH 8 and subjected to daily flushing prior to I-V measurements. Figure 5.4 presents the results from three distinct SiN-HfO₂ membranes tested under identical conditions and selected from separate wafers. All tested membranes exhibited minimal growth, less than 1 nm increase over the 11-day period. These findings corroborate previous literature reports,⁶² confirming that the SiN-HfO₂ hybrid architecture leads to stable pores in these experimental conditions.

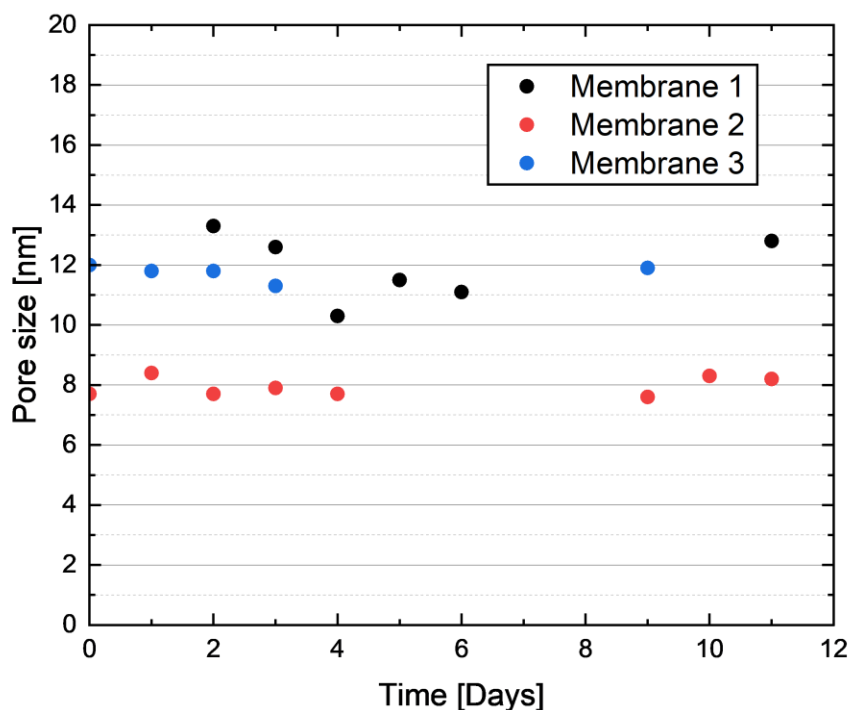


Figure 5.4 Aging study of SiN-HfO₂ hybrid membranes in 3.6 M LiCl pH 8 over the span of 9-11 days, recorded as pore diameter from an I-V sweep. Pore growth was below 1 nm on average over the recording span.

Despite the proven stability of the hybrid membrane, its primary limitation stems from the added thickness contributed by the unetched SiN supporting layer. Our ultimate goal was to use ultra-thin membranes (<5 nm), and the current SiN-HfO₂ membranes produced have a membrane thickness similar to the pure SiN membrane we otherwise use in the 10-20 nm range. Removing this residual SiN above the ALD material layer, and further reducing the ALD film thickness, is crucial for minimizing the nanopore fabrication time and further enhancing the signal obtained from DNA current blockages. Although the RIE etching process itself could be optimized in terms of etch duration and wafer-to-wafer variations, fundamental simplification of the microfabrication process necessitates the integration of an etch stop layer. Consequently, the following section details the study of an ALD Al₂O₃ film as a reliable RIE etch stop.

5.2 Aluminum oxide as an RIE etch stop

An etch stop layer serves as a passivation layer, protecting the underlying film stack during RIE etching processes. For fluorine-based plasma etching chemistries (CF₄ in this work), aluminum oxide is widely adopted in the semiconductor industry for its chemical resistance,^{188,189} coupled with a readily available removal step based on wet etching procedures such as Piranha or KOH mixtures.⁴¹

An initial 5.3 nm layer of ALD Al₂O₃, exhibiting a non-uniformity of 4.4%, was deposited onto a bare 6-inch Si wafer at Western Digital. To assess the etch resistance of this Al₂O₃ layer, a process flow was implemented, involving dry etching conditions of 100 W, CF₄:O₂ (with a sccm ratio of 25:5), conducted at 8 Pa for 90 seconds (Figure 5.5).

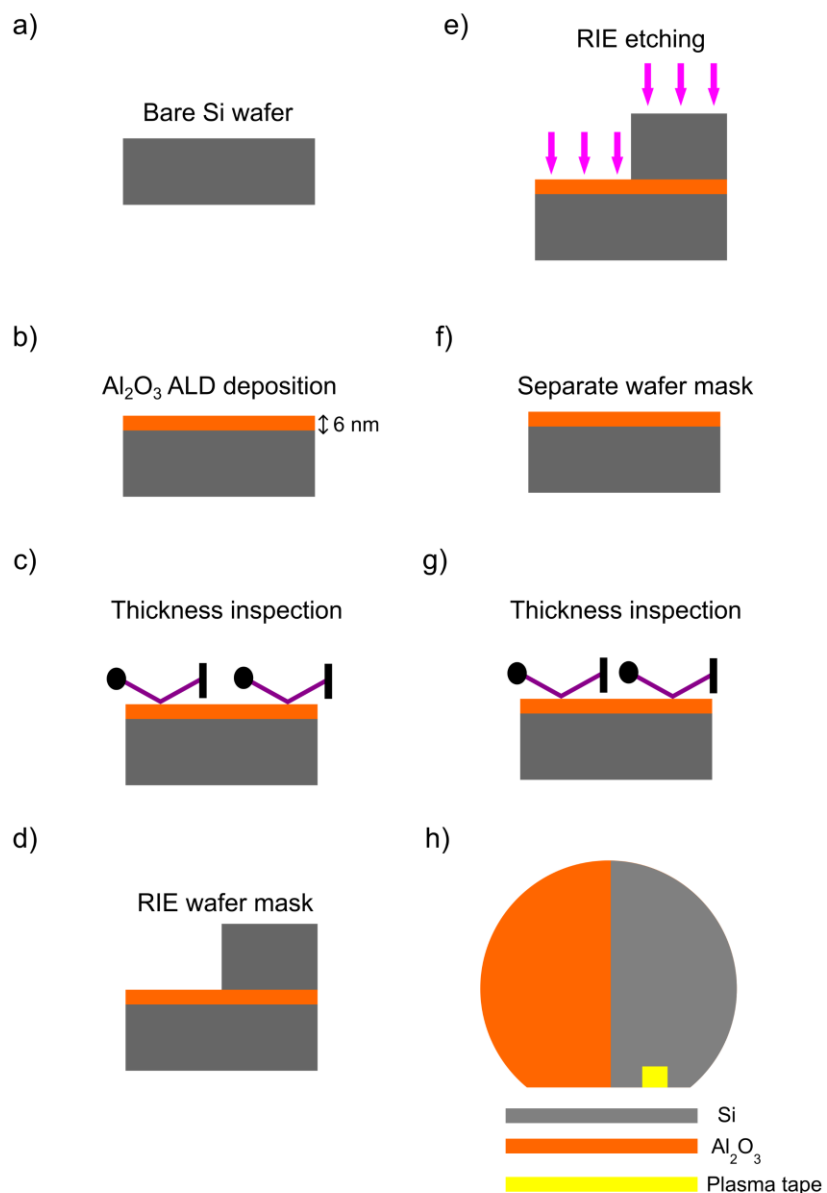


Figure 5.5 RIE etch stop characterization workflow. a) A pristine 6-inch bare silicon wafer is selected. **b)** ALD deposition of 6 nm Al_2O_3 on one side of the wafer. **c)** Film thickness inspection using Ellipsometry. **d)** A second pristine 6-inch bare silicon wafer is cleaved in half and used as a hard mask to prepare for RIE etching of the ALD Al_2O_3 wafer. **e)** RIE etching of the combined wafers at 100 W for 90 s. **f-g)** The hard mask wafer is removed, and the ALD wafer is subsequently inspected using ellipsometry to extract the thickness heatmap. **h)** Top view of the ALD and hard-mask wafer assembly during the RIE etching step in panel e).

Subsequently, the Al_2O_3 -coated wafer underwent inspection using ellipsometry. Figure 5.6 illustrates the position of the dummy wafer compared to the coated one, while providing information about the thickness of the Al_2O_3 layer. The region protected by the dummy wafer exhibited a measured growth of

~1 nm compared to the initial deposition measurement. This growth may be attributed to the formation of a contamination layer leading up to the characterization process. Conversely, on the exposed side of the wafer the alumina layer measured as little as 4.5 nm at the edges, over a 90-second etch period, approximately 1 nm lower than the initial deposition (Figure 5.6(b)). This reveals an average etch rate less than 1.3 nm/min under our RIE condition.

Based on these measurements, the Al_2O_3 layer proves suitable for acting as an etch stop. Specifically, the data indicate that a thickness of 2 nm is sufficient to protect the underlayer of the desired material while allowing for complete removal of the supporting SiN layer. This conclusion assumes an etch rate of 85 nm/min for the tested RIE recipe, measured from previous LPCVD SiN depositions outsourced to Addison Engineering Inc.

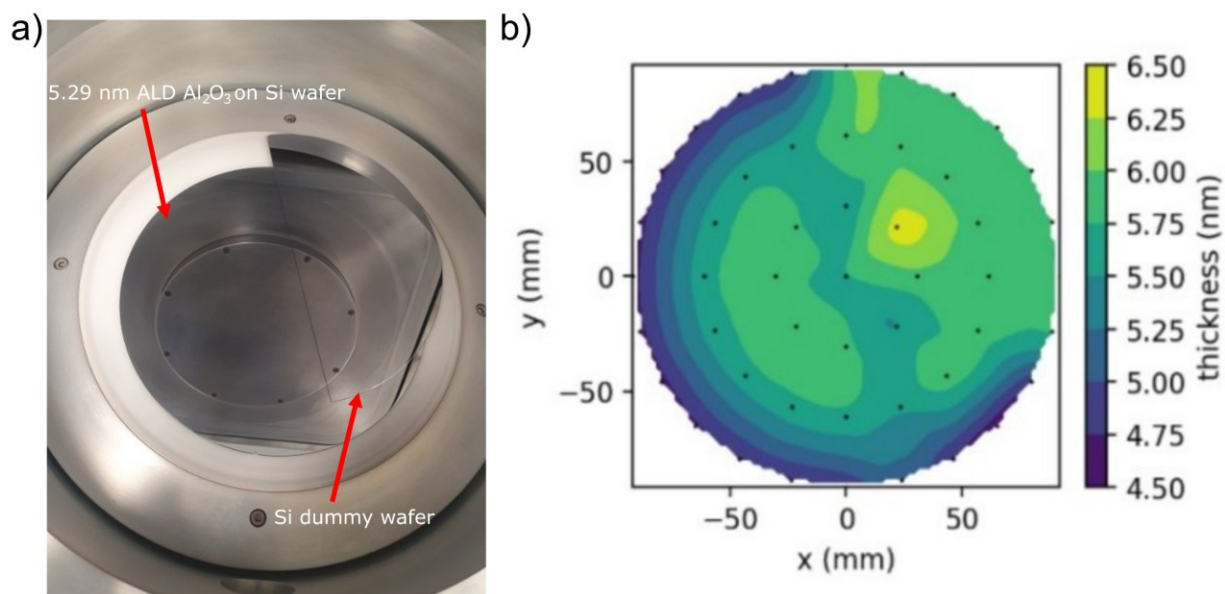


Figure 5.6 RIE etch stop experiment. a) Image of the RIE chamber post-etching, housing the ALD Al_2O_3 wafer covered with a Si dummy wafer. b) Ellipsometry data, taken by our collaborators at Western Digital, of the RIE-etched ALD Al_2O_3 wafer.

5.3 Annealed HfO₂ membranes protected by Al₂O₃ and encapsulated by LPCVD SiN

The initial microfabrication process flow was revised to incorporate the ALD Al₂O₃ layer as a dedicated etch stop, thereby enabling complete removal of the supporting SiN layer in the local-thinning region.

Figure 5.7 provides a detailed representation of this improved process flow.

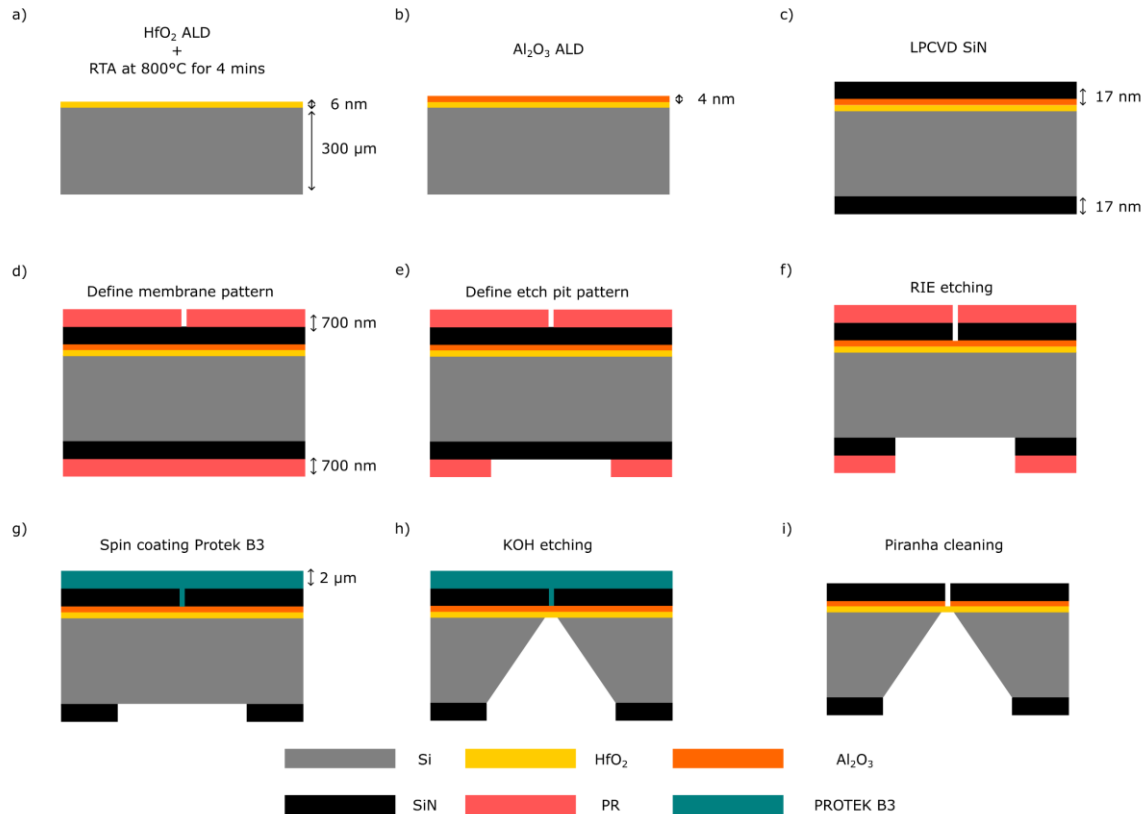


Figure 5.7 Microfabrication process flow for Al₂O₃-HfO₂ devices. a) ALD deposition of a 6 nm HfO₂ film on the membrane side of the wafer, followed by a Rapid Thermal Annealing step at 800 °C for four minutes. **b)** ALD deposition of a 4 nm Al₂O₃ film on the membrane side of the wafer, to protect the underlying HfO₂ layer during RIE etching. **c)** Deposition of 17 nm LPCVD SiN, encapsulating the wafer. **d)** Membrane-side patterning to define the local thinning area, where a photoresist (S1805) is spin-coated, soft-baked, exposed to UV during contact-lithography, and developed using an alkaline solution (MF321). **e)** Back-side patterning to define the etch pit, where a photoresist (S1805) is spin-coated, soft-baked, exposed to UV during contact-lithography after IR front-to-back alignment, and developed using an alkaline solution (MF321). **f)** Sequential RIE etching of SiN to thin the local thinning area and expose the silicon on the etch pit side of the wafer. **g)** Spin-coating and baking of the ProTEK Primer and B3 layer to protect the membrane during subsequent wet etching. **h)** KOH etching of the silicon wafer to create free-standing membranes. **i)** Wafer dicing to form 5×5 mm dies and piranha cleaning of the chip prior to membrane characterization.

Beginning with a 4-inch intrinsic Si wafer, the fabrication process involved several stages: first, a 6 nm HfO₂ layer was deposited onto one side of the wafer (the membrane side), followed by an RTA step at 800 °C for 4 minutes. Then, a 4 nm layer of ALD Al₂O₃ was deposited atop the HfO₂ layer to function as the etch stop during the dry etching step. The remaining steps mirrored the procedure detailed earlier in Figure 5.1, with the only modification being the reduction of SiN thickness from 100 nm to 17 nm. This reduction was meant to facilitate both the minimization of the dry etching duration and the improvement of wetting of the locally etched region, given the decrease in the local-thinning area's aspect ratio. All material depositions and wafer dicing steps were executed at the uAlberta Nanofabrication facilities.

Upon completion of the optimized device microfabrication, the architecture underwent pore fabrication, and DNA translocation was performed to validate performance. Figure 5.8 presents a proof-of-concept characterization of these fabricated devices. CBD pore fabrication was conducted in 1 M KCl pH 8 using the standard protocol. The fabrication voltage ramp reached 18 V before the membrane experienced breakdown. Assuming a membrane thickness of 10 nm due to the difficulty in confidently confirming the removal of the ALD Al₂O₃ layer, particularly during the piranha cleaning process as documented in the literature,⁴¹ the electric field strength of 1.8 V/nm for breakdown is much greater than for pure 10 nm SiN in similar salt conditions.¹⁹⁰ The resulting pore size was determined via an I-V sweep in 3.6 M LiCl pH 8 and calculated to be 9 nm, assuming a cylindrical geometry. Because the low-frequency noise performance as determined by the PSD measurement in Figure 5.8(b) was adequate, a DNA experiment was performed to validate sensing performance. 2 kbp dsDNA was injected onto the membrane side, and a -200 mV voltage was applied while the current was recorded on an Axopatch 200B.

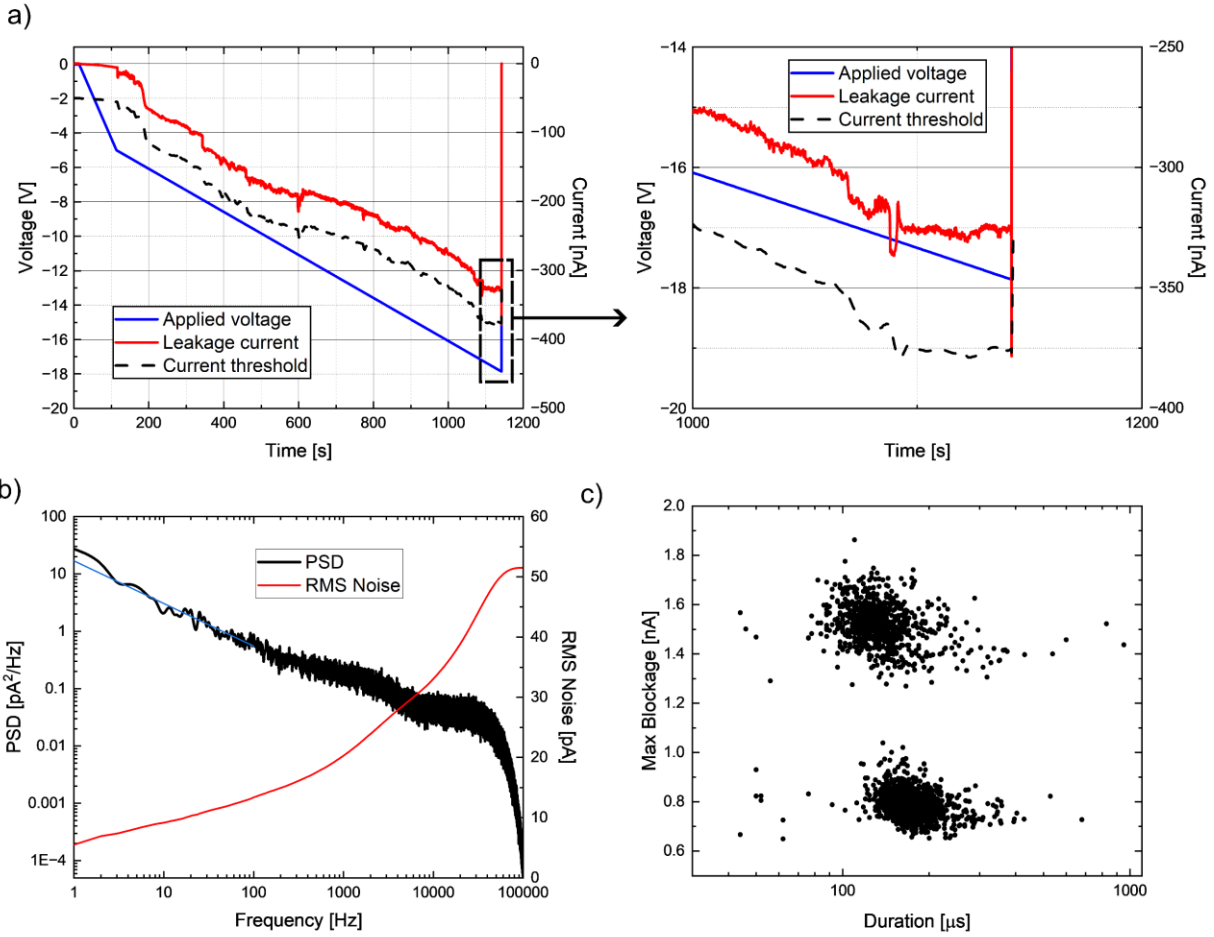


Figure 5.8 $\text{Al}_2\text{O}_3\text{-HfO}_2$ proof of concept CBD fabrication and characterization. a) Nanopore fabrication following a voltage ramp in 1 M KCl pH 8 (left), with a zoom-in on the breakdown event (right). b) Noise characteristics of the fabricated ~9 nm pore at an applied voltage of -200 mV in 3.6 M LiCl pH 8. The PSD of the recorded current is presented in black and the RMS of the current in red. The blue line is used to extract the 1-100 Hz fit intercept for the PSD. c) 2D scatterplot of 5 nM 2 kbp dsDNA translocation events in 3.6 M LiCl pH 8 at -200 mV.

As depicted in Figure 5.8(c), two distinct dsDNA translocation levels were observed slightly deeper than the previous architecture, with the unfolded level appearing at 783 ± 50 pA, extracted from the Gaussian fit to the distributions of unfolded level current blockages. Using this recorded current blockage value and the open pore conductance of 87 nS, we recalculated the effective pore dimensions. An effective thickness (as described earlier for the HfO_2 -only membranes) of 2.3 nm and a 7.2 nm nanopore diameter were extracted. Interestingly, the calculated effective pore thickness is smaller than the 6-nm nominal HfO_2

deposited thickness. However, by adopting the 1/3 correction factor applied during TEM-drilling for hourglass-shaped pores, the derived thickness is recovered to ~6 nm, matching the nominal deposited membrane thickness (Figure 5.9).

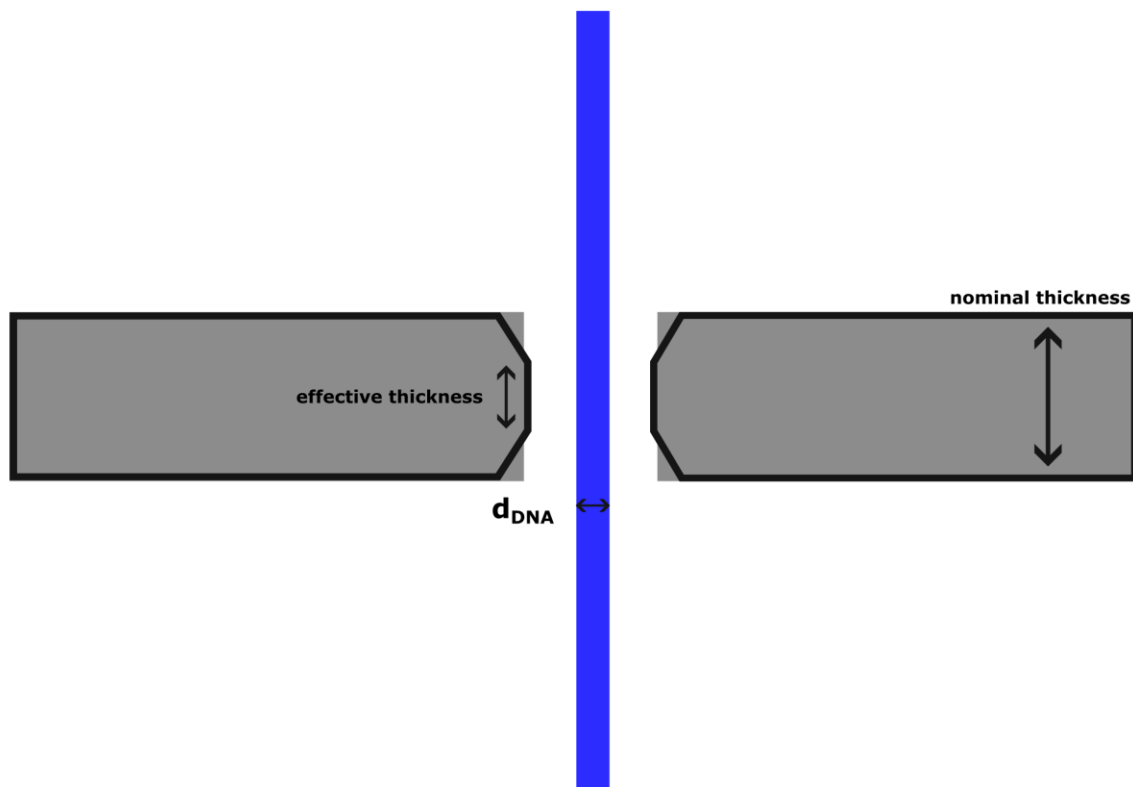


Figure 5.9 Extracted nanopore shape comparing the cylindrical model in grey and the effective dimensions calculated in black.

These results show that it is possible to make CBD nanopores in these hafnia/alumina membranes. However, given the dielectric strength of hafnia, fabrication can be long and would potentially require 18 V for a breakdown event in standard salt conditions.

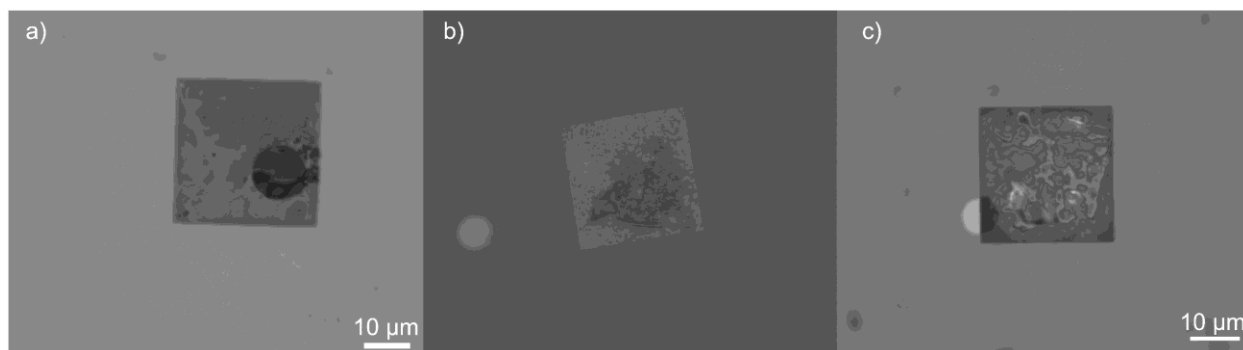


Figure 5.10 Optical microscope images of the local-thinning area on the microfabricated $\text{Al}_2\text{O}_3\text{-HfO}_2$ devices prior to piranha cleaning. a) Representative image of a successful front-to-back alignment on the microfabricated devices. b-c) Representative images of unsuccessful front-to-back alignment on the microfabricated devices.

Another key technical challenge encountered during the microfabrication process was ensuring the front-to-back alignment of the $\sim 5\ \mu\text{m}$ local-thinning on the $40\times 40\ \mu\text{m}$, free-standing, membrane area. Figure 5.10 displays $20\times$ microscopy images of the membranes taken immediately prior to the piranha cleaning step to strip the Protek layer from the surface. Ideal alignment, depicted in panel a), places the local thinning circle entirely within the free-standing membrane area. Conversely, non-optimal alignments, depicted in panels b-c), position the local-thinning cylinder near the membrane edge or on the chip frame. This variability is primarily attributable to a reduction in the final membrane area from $\sim 100\ \mu\text{m}$ to $\sim 30\ \mu\text{m}$ while maintaining the same tolerances for the alignment marks established during the contact-lithography process; as a result, 90% of the inspected devices had a misaligned local thinning area, making them non-usable for this study.

Given the fabrication challenges discussed above, this device architecture still requires optimization to improve performance. Future steps would involve reducing the fabrication duration by minimizing the thickness of the deposited architecture to 2 nm of Al_2O_3 and 4 nm of HfO_2 , and refining the alignment process to minimize the possibility of the patterned area falling outside the released membrane, by

adopting e-beam lithography to form 250–500 nm openings for the local thinning area (outsourced to the uAlberta Nanofabrication Facilities).

5.4 High throughput approach to nanopore fabrication

Having shown a path toward optimized ultra-thin membrane architectures, parallel efforts focused on the scalable integration of membrane arrays on a single chip, to accelerate the pace of research. The goal is to have an array of nanopore membranes that are electrically and fluidically separated on both sides, while achieving the highest density possible on the single chip. The initial design used so far in this thesis involved a single window located in the center of a 5×5 mm chip. The fluidic circuit to address that single membrane is described in Chapter 2 based on a previous design.⁵⁷ Increasing the number of membranes per chip is not only limited by the microfabrication process but also by the design of the fluidic flow cell. Although prior research has explored increasing nanopore density on a single membrane,^{62,64,118,123,187,191–193} demonstrating individual fluidic access for electrochemical readout remained a significant technological gap. To overcome this constraint, I collaborated closely with research engineers in the T.-Cossa lab in redesigning the current platform. Specifically, Michel Stephan designed photomasks to incorporate 1, 4, 8 and 16 membranes on individual 5×5 mm chip dies. As illustrated in Figure 5.11(a), a microfabricated chip was optically inspected from the etch pit side, showing 2 rows of 4 individual membranes with defined spacing of 2 mm between rows and 1 mm between columns. Complementing the microfabricated chips, the fluidic flowcell and gasket interface was developed by Dmytro Lomovtsev (Figure 5.11(b)). Based on 3D printing technology, a miniaturized flow cell was developed, allowing independent access to the membrane array. This micro-3D-printed cell was mated to the nanopore array chip using micro-molded PDMS gaskets. The 3D-printed molds to produce these gaskets were engineered to leverage capillary effects, ensuring a uniform gasket thickness and established

fluidically accessible interfaces between the nanopore membrane array and the channels on the micro-3D-printed cell.

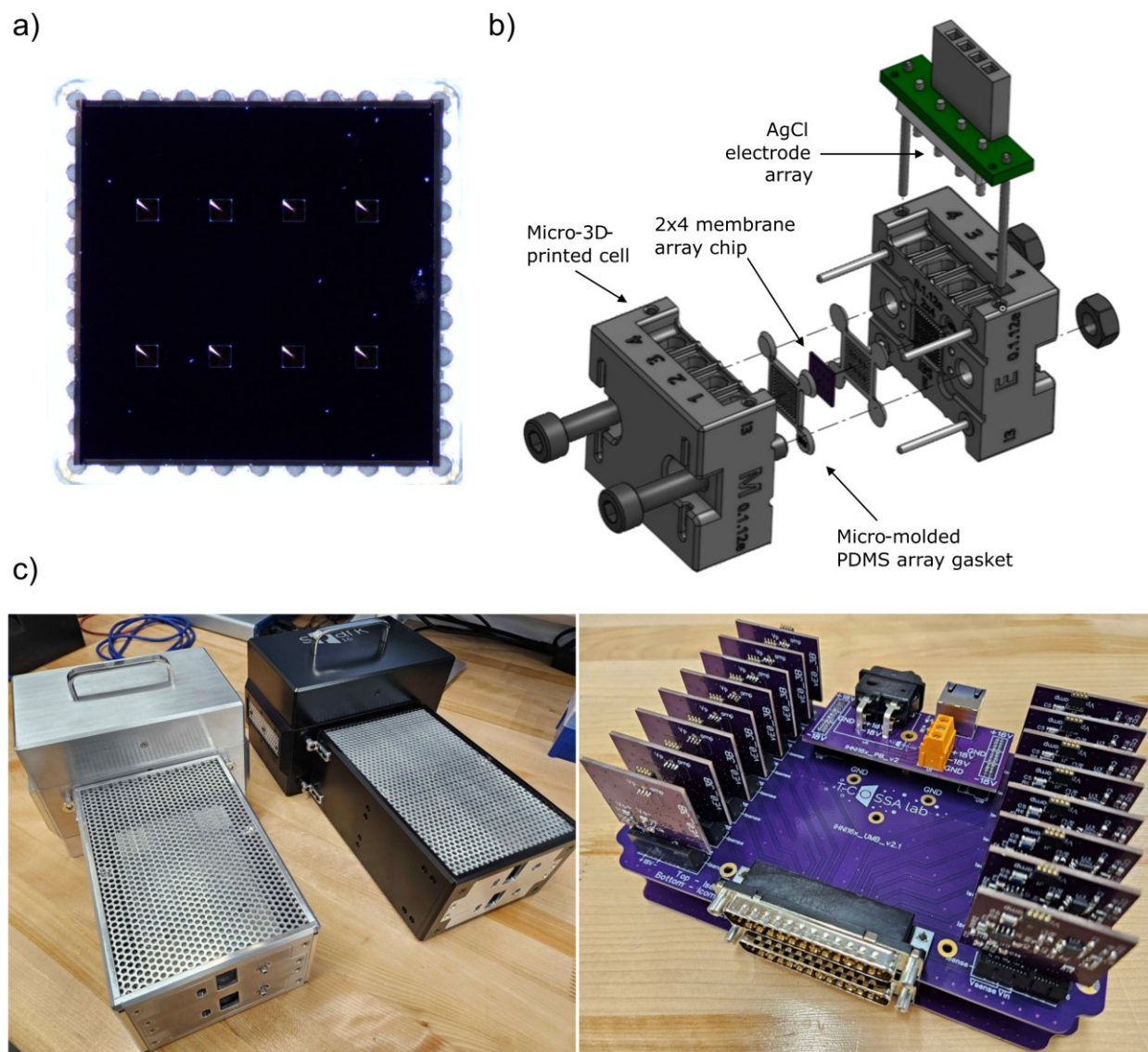


Figure 5.11 Nanopore membrane array chip, fluidics and fabrication instrumentation. a) Optical microscope image of a 5×5 mm multi-membrane chip from the etch pit side containing 2 rows of 4 membranes, all of them individually addressable. b) Exploded view of the fluidic cell assembly to interface with the 2×4 nanopore chip. c) Custom-built sixteen-channel fabrication hardware.

Next, the fluidic cell was connected to a custom-built, sixteen-channel fabrication hardware (Figure 5.11(c)), through integrated Ag/AgCl electrode arrays. Michel Stephan and Mathieu Gibeault (ONT

Canada consultant) collaborated closely to develop a software interface, which facilitates control of the multi-channel hardware to fabricate nanopores by CBD and characterize their ionic current response.

Despite successful parallelization, key challenges remain to increase nanopore density on a single chip. The KOH etch profile of the silicon wafer, inherently following a 54.7° angle, limits the pitch between free-standing membranes. While reducing the wafer thickness from 300 to 150-200 μm partially relaxes this limit, a workaround would involve connecting all etch pits on the etch pit side to create a common-ground interface. However, this shifts the challenge to the 3D-printing resolution, which must miniaturize both the channels and PDMS interface to maintain individual membrane addressability. Overcoming this constraint necessitates considering alternative technologies beyond current commercially available 3D printers.

6. Conclusion and Outlook

6.1 Thesis summary

In summary, the work presented in this thesis endeavored to minimize the experimental knowledge gap present in nanopore research and expand the understanding of fundamental nanopore properties. These findings pave the way for versatile applications in the fields of proteomics, data storage and diagnostics.

In more detail, this thesis explored nanopore sensors in terms of surface properties, biomolecular transport and microfabrication approaches, while attempting to address limitations in terms of robustness, stability, translocation velocity and spatial resolution.

In Chapter 2, we developed a surface characterization platform capable of modulating pressure gradients across the membrane and resolving streaming currents in the picoampere range, thus facilitating the study of surface properties such as zeta potential and surface charge density, and enabling the characterization of novel membrane materials. We found that the zeta potential has a non-monotonic trend with increasing salt concentration in the 0.01-0.8 M KCl range, and the isoelectric point of CBD-fabricated SiN nanopores is 4.4 ± 0.1 as extracted from a power law fit to zeta potentials varied from pH 3 to 12. Next, we extended nanopore longevity by improving surface functionalization protocols to selectively target the pore interior or the membrane and allow the sensing of challenging sticky samples, while examining the impact of these surface modifications on ion transport at the nanopore interface. We found that a polymer coating applied on a SiN membrane before CBD fabrication was an effective antifouling film, extending the pore lifetime from seconds to about an hour (from a handful of translocations to thousands) without increasing $1/f$ noise for the notoriously sticky single-stranded ~ 7 knt long DNA polymer from M13 bacteriophage. These results were published in “Unraveling clogging of solid-state nanopores by selective polymer coating: surface potential measurements and sensing of long single stranded DNA”.¹⁵³

In Chapter 3, we expanded on the developed pressure-regulated platform to understand biomolecular transport and overcome bandwidth limitations, with an emphasis on balancing the voltage-pressure (V–P) coupled forces to modulate the sample’s translocation velocity, and on the effect of these forces on capture rate, signal amplitude, polymer folding probability and provided an independent measurement of the effective charge of DNA. We found that the V–P regime extends translocation duration by a factor 2 to 6 for DNA nanostructures and proteins respectively, the latter experiencing a detection rate enhancement of 3-4×. These results are available as a preprint in “Pressure-Voltage Modulation of Passage Dynamics for Enhanced Nanopore Biosensing”.¹⁹⁴

In Chapter 4, we characterized a novel thin film deposition approach to form solid-state nanopores compatible with CMOS microfabrication requirements, highlighting the ability to control membrane thickness validated by DNA translocations. We found that IBD SiN nanopores provided comparable results to LPCVD SiN nanopores in terms of nanopore fabrication and $1/f$ noise performance, with IBD annealed SiN nanopores offering reduced RMS noise compared to LPCVD and IBD SiN ones at 100 kHz. These results were published in “A method for fabricating CMOS back-end-of-line-compatible solid-state nanopore devices”.³⁵

In Chapter 5, we explored novel membrane materials to enhance nanopore stability and spatial resolution, with an emphasis on optimizing microfabrication workflows and increasing sensing throughput based on nanopore density. We found that HfO₂ nanopores were stable with minimal pore growth (< 1.3 nm/day) for an aging duration up to 11 days, and an etch stop layer such as Al₂O₃ offers a passivation layer during RIE etching to further optimize film thickness fidelity and reach ultra-thin <3 nm HfO₂ membranes. These results remain unpublished so far, but depending on the results of the latest device fabrication batch underway, sections of this chapter could be converted to a manuscript and submitted for peer-review publication. The rest of this chapter will focus on ongoing work and potential avenues for investigation building on the results presented in this thesis.

6.2 Outlook

These findings serve as a foundation for ongoing applications of solid-state nanopores in the community, some of which are already underway. First, Ben Hyatt, a PhD student in the group, is adopting the platform detailed in Chapter 3 during protein sensing experiments, extending their residence time inside the nanopore, and facilitating the extraction of relevant event signatures crucial to volume and shape estimation while improving on existing conductance models, and extending volume estimation from ensemble measurements to single event volume/shape approximations. This paper is currently in preparation, and preliminary results show good agreement with state-of-the-art volume measurements. Secondly, DNA barcodes as molecules to encode digital information would benefit greatly from this developed platform. Precisely, the demonstrated reduction in translocation velocity with a counter pressure facilitates the extraction of structures (encoded information) along the polymer, opening the possibility to increase information density and complexity beyond a binary bit system.^{96,195} Furthermore, DNA folding reduction under the effect of an opposing pressure could be tuned to more reliably decode stored information. A key drawback to this platform is the linear effect of pressure on capture rate, which could impact sensing time to reach statistical significance (up to a 10× increase in sensing time depending on nanopore dimensions).

Overcoming this obstacle involves ongoing work to move toward nanopore arrays to increase experimental throughput as detailed in Chapter 5. Furthermore, by adopting nanopore arrays on the same chip, experimental reproducibility should be enhanced while pore-to-pore variations would be more readily studied. To this effect, work in collaboration with Michel Stephan and Dmytro Lomovtsev, research engineers in the group, is underway to reach the next generation of solid-state nanopores featuring arrays of 16 individually addressable nanopores. Moving from silicon wafers to fused silica substrates reduce channel crosstalk and high-frequency noise.⁷⁷ We are currently exploring femtosecond laser micromachining to locally modify glass, enable selective etching and overcome geometry constraints

enforced by the silicon substrate during KOH etching. By adopting a fused silica substrate, the nanopore noise performance will also be drastically improved in the high-frequency noise regime, extending the sensing bandwidth to extract a more detailed image of the samples as they translocate through the pore. Once the HfO₂ membranes are tuned for optimal thickness on Si wafers, the same membrane process could be applied to glass substrates, allowing for dense DNA barcodes and complex protein samples to be studied with unmatched spatial and temporal resolutions.

Building on the work presented in Chapter 2, the surface properties of these novel HfO₂-only membranes need to be extensively characterized in various conditions of salt concentration and pH, informing researchers on the optimal experimental conditions for sensing. Furthermore, the developed membrane surface functionalization protocol is now routinely performed in the group to overcome clogging behavior during sensing experiments, with no impact on noise performance. Possible improvements on the protocol could involve successive surface modifications to passivate the inner pore surface without the increase in $1/f$ noise that currently limits nanopore usability. These coated nanopores are now readily equipped for clinically oriented applications involving diagnostics of complex biomarkers that could potentially damage the longevity of a bare nanopore.

Priority should be given to $1/f$ noise suppression and high-throughput instrumentation, addressing two fundamental nanopore limitations while driving development of next-generation solid-state nanopore sensors. These different experimental next steps represent a range of ongoing projects that promise to impact the nanopore field.

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