Controlled Fabrication of Thin Silicon Nitride Membranes for Nanopore Sensing

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Abstract

Thin membranes are highly sought-after for nanopore-based single-molecule sensing. Fabrication of such membranes becomes challenging in the <10-20 nm thickness regime where a plethora of the work conducting nanopore sensing is reported. Silicon nitride (SiNₓ) is the ubiquitous choice of material in solid-state nanopore technology and in this work, we present a scalable method to fabricate SiNₓ membranes with thicknesses of ~5 to ~20 nm using standard silicon processing and chemical etching using hydrofluoric acid (HF). The well-characterized bulk etch rates of SiNₓ in HF conveniently extend the method to a range of desired thicknesses. The final thicknesses of the membranes were measured using ellipsometry and were in good agreement with the values calculated from the bulk etch rates. Rutherford backscattering spectrometry (RBS) measurements were used to characterize the membrane chemistry which revealed a stoichiometry and density of the membrane layer to be Si₃N₃.₉⁺±₀.₀₂ and 2.₉₇ ± 0.₀₂ g cm⁻³ respectively. Nanopores were fabricated using the controlled breakdown method with estimated pore diameters down to ~2 nm. The surface
charge density was determined by surveying the open pore conductance with changing pH and salt concentration which revealed the surface to be rich in silanol groups (pKa = ~8.0±0.4). Double-stranded DNA was used to probe the translocation characteristics using ~5.5 nm diameter pores. The membrane thicknesses estimated from the translocation measurements agree well with those obtained from ellipsometry.

Introduction

Registering information in the single-molecule realm transcends average ensemble approaches and boundaries are pushed even further in search of methods capable of delivering intramolecular details (e.g., DNA sequencing). Rapid, portable, low-cost methods and devices for single bio-molecule measurements have gained substantial traction and the current pandemic has recapitulated the need for such devices. Nanopores are often viewed as capable of satisfying these criteria with applications spanning, but not limited to, genomics\textsuperscript{1, 2}, proteomics\textsuperscript{3, 4}, glycomics\textsuperscript{5, 6}, lipidomics\textsuperscript{7, 8}, and virology\textsuperscript{9, 10} in its 25 years of research lifetime. The broad scope of the application spectrum of nanopores was greatly enhanced by advancements in nanopore fabrication\textsuperscript{1-3}, surface decoration\textsuperscript{4-5}, material development\textsuperscript{6-7}, signal processing algorithms\textsuperscript{8-9}, and electronics\textsuperscript{10}. A major challenge in nanopore technology is the membrane development and pore fabrication, and with the advent of controlled breakdown (CBD)\textsuperscript{1}, the economics of nanopore fabrication has become more affordable (and widespread) in terms of ease of production, as it does not require expensive electron/ion-microscope based methods. A multitude of auxiliary methods has now evolved from CBD expanding the available fabrication repertoire that can be tailored to user needs and available resources\textsuperscript{2, 11-13}. CBD is most suited for membranes that are thinner than ~30 nm. Thinner membranes provide a greater signal-to-noise ratio (SNR), larger capture radius compared to thicker membranes and are often perceived as a prerequisite for more captivating sequencing efforts. With nanopore
technology being driven more towards sequencing—may it be genomic or proteomic—fabricating thin membranes has become more desirable for high-resolution measurements.

Silicon nitride (SiNₙ) is the ubiquitous choice of material in most solid-state nanopore (SSN) studies (albeit having high capacitive noise) due to its mechanical, chemical and thermal stability, availability of thin-film deposition tools, compatibility with silicon-based microelectronics and surface modification approaches. In most studies, the membrane thickness \( L_0 \) exceeds \(~10 \text{ nm}\) with some notable examples of \( L_0 \leq 5 \text{ nm}\) in literature\(^{14-17}\). Generally, the fabrication involves methods where a thicker membrane is thinned down to reach a more desired thickness. Typical examples for thinning include laser-thinning\(^{17}\), electron-beam thinning\(^{18}\), and ion-beam thinning\(^{3,19-20}\). Etching methods have largely been used for etching electron/ion damaged tracks in polymer membranes and have mostly been overlooked for the fabrication of thin membranes for SSN studies partly due to the common usage of electron/ion-beam methods. However, etching methods are scalable, do not require cleanroom access, and deliver wafer-scale fabrication. Simplifying the thinning process in a reliable and reproducible manner could see widespread access to the coveted <10 nm thickness regime for sensing applications. The ability to tune the \( L_0 \) in a controllable manner is only a part of the solution since pores fabricated through these membranes should be conducive to analyte translocations. It has been shown previously that a simple change to the electrolyte chemistry during nanopore fabrication could change both nanopore surface chemistry and translocation characteristics\(^2\). Moreover, surface properties have been shown to influence translocation properties\(^4-5,21\).

In this work, we demonstrate a scalable top-down method for the fabrication of thin membranes. Thicker membranes (~150 nm) are first fabricated followed by controlled etching through a series of different concentrations of hydrofluoric acid (HF) for the fabrication of <20 nm thick membranes reaching as low as ~5 nm. The stoichiometry, density, and thickness of the
fabricated membranes were investigated by Rutherford backscattering spectrometry (RBS) as those parameters can impact the surface charge as well as the pore fabrication characteristics. Typically, amorphous Si-rich SiNₓ membranes are used for CBD. Herein, we fabricated nanopores through nearly stoichiometric SiNₓ using CBD. The nanopores were characterized using pH-conductance (pH-$G_0$) and electrolyte conductivity-$G_0$ measurements to evaluate the pore surface chemistry and its surface charge density. Finally, double-stranded DNA (dsDNA) was used to explore the suitability of pores for single-molecule sensing. The $L_0$ was also calculated from the resistive pulse characteristics of dsDNA translocations and was found to be in excellent agreement with those obtained from the surface profilometry and ellipsometry.

**Materials and Methods**

**Fabrication and Characterization of Membranes:** The workflow for the fabrication of the membranes is shown in Figure 1a. As indicated in step (ii) of the workflow, ~150 nm thick SiNₓ layer was deposited on both sides of a double-sided polished Si wafer using low-pressure chemical vapor deposition (LPCVD). The deposition was performed at 775 °C and a gas flow of 30 sccm of dichlorosilane and 120 sccm of ammonia was maintained throughout the process to deposit near-stoichiometric SiNₓ ($x \sim 4/3$). The thickness of the nitride layer was measured by ellipsometry. As evident by Figure 1b showing 61 points of measurement on the wafer along with the measured thickness, almost uniform deposition was achieved with a variation of ~3 nm (standard deviation of 0.95 nm) across the 4-inch wafer. The next steps involve spinning a negative photoresist on the backside of the wafer (iii) and patterning a custom window (size of window varying from 430 µm x 430 µm to 550 µm x 550 µm for the case of 300 µm thick wafer) using UV lithography (iv). Afterwards, the silicon was exposed from the backside of the wafer in the window area by removing the SiNₓ layer using reactive ion etching (v). The photoresist was then removed, and the exposed
silicon was anisotropically etched by wet etching in 5% tetramethylammonium hydroxide (Sigma-Aldrich, 331635) solution at 85 °C (vi). This process leads to the parallel fabrication of 220 membranes of ~150 nm thickness on a 4-inch Si wafer. The material properties of the deposited SiNx layer such as density and stoichiometry were determined by RBS. A 2.0 MeV He ion beam was used to perform RBS on samples and RUMP code\textsuperscript{22} was used to simulate and fit the spectra.

**HF etching:** HF etching to thin down the nitride window was done using different concentrations (10\%, 5\%, and 1\%) of HF prepared by dilution of 48\% hydrofluoric acid (Sigma-Aldrich, 695068). The etching was performed in a custom-made etching cradle. To stop the etching, the membranes were rinsed in DI water and air-dried.

**Electrolyte preparation:** All electrolytes were prepared by first dissolving the as supplied salts (Sigma-Aldrich, KCl, P9333 and LiCl, L4408) in 18 M\(\Omega\).cm DI water (Sartorius Arium\textsuperscript{®} UV Ultrapure) with the HEPES buffer (Sigma-Aldrich, H0527) followed by filtering through a Millipore Express\textsuperscript{®} PLUS PES filter of 0.22 \(\mu\)m pore size. The pH was tuned to the desired value (\(\pm 0.1\) tolerance) through dropwise addition of either HCl (Ajax-Finechem, AJA1367, 36\%) or KOH (Chem Supply, PA161) and measured with an Orion Star\textsuperscript{TM} pH meter.

**Fabrication of Pores:** The membranes were first mounted between two custom fabricated Poly(methyl methacrylate) (PMMA) half cells followed by filling each of the reservoirs with 1 M KCl buffered with 10 mM HEPES at pH \(\sim 7\). Then an electric field of <1 V/nm was applied using a source meter unit (Keithley 2450) until a rapid surge of current was observed which is indicative of pore formation. Afterwards, to characterize the pore size, a current-voltage (I-V) curve was obtained using an Elements eNPR system. The diameter of the pore can be estimated from the slope of the I-V curve (i.e., open-pore conductance, \(G_0\)) with adequate knowledge of the membrane thickness \(L_0\) using the following equation:
\[ G_0 = K \left( \frac{1}{\frac{\pi r_0^2}{L_0} \mu |\sigma|} + \frac{2}{\alpha 2r_0 + \beta |\sigma|} \right)^{-1} \]  

(1)

where \( G_0, r_0, K, \sigma, \mu, \alpha \) and \( \beta \) are the open pore conductance, nanopore radius, electrolyte conductivity, nanopore surface charge density, mobility of counter-ions proximal to the surface and model-dependent parameters (both \( \alpha \) and \( \beta \) are set to 2)\textsuperscript{11,12}.

**Surface Characterization:** The open-pore conductance was measured as a function of the solution pH using 1 M KCl electrolyte and the data were fitted with eq. 1 using the following approximation for surface charge density (\( \sigma \)) which then permits the evaluation of the dissociation constant of surface head groups (\( pK_a \)),

\[ |\sigma| \cong \frac{C_{eff}}{\beta e} W \left( \frac{\beta e}{C_{eff}} \exp((pH - pK_a) \ln(10) + \ln(e\Gamma)) \right) \]  

(2)

where \( e, \Gamma, \beta, C_{eff}, \) and \( W \) are the elementary charge, number of surface chargeable groups, inverse of the thermal energy, effective Stern layer capacitance, and Lambert \( W \) function, respectively\textsuperscript{12,13}.

**DNA Sensing:** Double-stranded DNA (Thermo-fisher SM0311) was added to the \textit{cis} chamber to a final concentration of \( \sim 17 \) nM and driven across the nanopore in response to a positive voltage bias of 400 mV applied to the \textit{trans} chamber. Data were filtered at 10 kHz and sampled at 200 kHz. Collected data were then analyzed using the \textit{EventPro} analysis platform\textsuperscript{23}.

**Results and Discussion**

Membrane fabrication details are outlined in Figure 1a-b and discussed under \textit{Materials and Methods}. Figure 1c shows the RBS spectra recorded along with the fitted simulation from the RUMP code (solid red line). Fit to the data revealed the nearly stoichiometric composition of the membrane.
layer to be Si$_3$N$_3$ and density of 2.97 ± 0.02 g cm$^{-3}$. Electron transport is reduced in this material compared to more commonly used Si-rich SiN$_x$. After the fabrication of thick membranes, they were thinned down in a controlled manner through consecutive etching with hydrofluoric acid of different concentrations. The bulk etch rate calculations using ellipsometry measurements are shown in figure 1d. Solid lines represent the linear fit to the data revealing the etch rates of 3.78±0.03 nm/min, 1.88±0.04 nm/min, and 0.39±0.03 nm/min for 10% HF, 5% HF, and 1% HF respectively. The etch rate was also measured using a surface profiler (Bruker Dektak® Stylus Profiler) where half of the sample was covered with a non-etch medium (Polyimide film) before etching and the step height was measured after the etching process and removal of the etch barrier. The etch rate values from the surface profiler were 3.82±0.14 nm/min, 1.89±0.08 nm/min, and 0.43±0.04 nm/min for 10% HF, 5% HF, and 1% HF respectively (Figure 1d). These values agree well with the values obtained from the ellipsometry measurements. Figure 1e shows the process flow of thinning down of a ~150 nm thick membrane to ~5 nm thickness. In brevity, the membrane was etched using 10% HF to a thickness of ~40 nm. Then, it was etched with 5% HF to reach a thickness of ~15 nm and finally etched in 1% HF to reach a final thickness of ~5 nm. The small variation of the nitride thickness across the wafer was accounted for during the thinning process by measuring the thickness of the layer right next to the etched window. We fabricated membranes of different sizes using the above-defined method. Membranes as small as 10 µm x 10 µm and as large as 120 µm x 120 µm of ~5 nm thickness were fabricated. With this method, we can fabricate 220 chips/4-inch wafer (as thin as ~5 nm thick).
Figure 1: (a) Process flow showing the steps for the fabrication of ultrathin silicon nitride membranes. (b) 61 different points measured by ellipsometry on the wafer with thicknesses of the silicon nitride in nm. (c) Rutherford backscattering spectrum of the deposited film along with the simulation from the RUMP code (red solid line). (d) The thickness of the etched layer as a function...
of etching time for different concentrations of HF as measured by ellipsometry and surface profilometry. The solid lines represent the linear fit to the data depicting the etch rates for different concentrations. (e) Process flow shows the thickness reduction of the membranes using different concentrations of HF to obtain good control over the process. The process can be stopped at different points to obtain membranes of different thicknesses.

After the controlled thinning of the membranes, pores were fabricated using the CBD method as shown in Figures 2a and 2b (see Fabrication of Pores under Materials and Methods for more details). Typically, it takes ~2-3 minutes and ~25-30 minutes for the initial breakdown to take place for a ~5 nm and ~12 nm thick membrane when 3-3.5 V and 7-7.5 V is applied across the membrane, respectively. Compared to Si-rich SiNₓ, this is about double the time required, which is not surprising given the near stoichiometric nature of the membranes used for this study. The slow breakdown facilitated the convenient fabrication of more coveted <5 nm diameter pores. Typical I-V curves of the pores are shown in Figure 2c which instantaneously exhibited Ohmic behavior without requiring overnight soaking or any pretreatment. This is especially advantageous since the fabricated pores could be instantly used for sensing applications. The pores depicted in Figure 2c ranged from ~1.8 nm (6 nS) to ~5.6 nm (35 nS) in diameter (determined using equation (1)). Figure 2d shows the thickness of 11 representative pores fabricated from the CBD method. The red ribbon in the figures depicts the target thickness (~5.5±0.5 nm). We see that all membranes were <8 nm in thickness with most membranes falling well within the expected thickness bracket with around ±2.5 nm deviation which is typical for commercial membranes as well. This further strengthens the application of our method to fabricate thin membranes in a controllable manner. The thickness control of the membranes can further be improved by precise control of the HF concentration and etching temperature.
Next, we looked at the surface chemical properties of the nanopore. The nanopore chemistry influences the translocation properties and can be tuned to slow down analyte transport\(^4\)\(^-\)\(^5\),\(^2\(^5\) and, selectively capture analytes\(^2\(^6\). The surface chemistry is also inextricably linked to properties such as the capture rate, open-pore stability, transient or incessant surface-analyte interactions, transport mechanism, and signal-to-noise ratio (SNR), to name a few. Although probing the inner nanopore surface is challenging due to the constricted volume, pH-conductance (pH-\(G_0\)) and electrolyte-conductance (K-\(G_0\)) can reveal significant information about the nature of surface head groups (e.g., pK\(_a\)) and surface charge (e.g., \(\sigma\)) of the inner nanopore surface. To this extent, \(G_0\) was evaluated as a function of pH as shown in Figure 2e. The raw data were then fitted with equations 1 and 2 which yielded a pKa of 8.0\(\pm\)0.4 (from 5 unique pores). The value suggests that the surface is rich with acidic head groups, unlike non-stoichiometric SiN\(_x\) which exhibits an amphoteric behavior with an isoelectric point closer to \(-4.1\)\(^4\)\(^,\)\(^2\(^7\). A similar trend (i.e., analogous to Figure 2e) was observed with pores fabricated in non-stoichiometric SiN\(_x\) using chemically tuned controlled dielectric breakdown (CT-CDB)\(^2\) and Tesla coil assisted method (TCAM)\(^1\(^1\) in which the electrolyte chemistry during fabrication was thought to play a key role for the observed results. Our pK\(_a\) is about 2 orders magnitude stronger than that reported for CT-CDB and about 2 orders magnitude weaker than that reported with TCAM. The pK\(_a\) calculated from Figure 2d is in good agreement with that reported for silanol groups which would imply that the surface hydroxyl groups are responsible for the observed value\(^2\(^8\). \(G_0\) was evaluated as a function of electrolyte conductivity (pH \(-8\)) as shown in Figure 2f. The observed pattern is typical and the deviation from the linear behavior at low electrolyte concentrations is attributed to the increasing contribution from the nanopore surface charge to the overall conductance. The dashed line is the fit to the raw data using equation 1 where \(L_0\) was constrained to a maximum of 10 nm. This yielded a \(\sigma\) of \(-4.6\pm1.0\) mC/m\(^2\) (2 unique pores).
Figure 2: (a) Schematic of nanopore fabrication by CBD using a source meter unit (SMU) where a voltage is applied until (b) a sudden rise in current is seen (in 1M KCl). (c) I-V curves for a range of pore sizes fabricated from the CBD method. They instantaneously showed Ohmic behavior, and the size ranged from ~ 1.8 nm (6nS) to ~ 5.6 nm (35 nS). Sizes were estimated using equation 1. SI Table S1 shows the complete set of pore diameters for the I-V curves shown in (c). (d) The thickness of 11 representative chips that were used for pore fabrication. The target thickness is represented by the red band (~5.5±0.5 nm). See SI table S1 for more details of these chips. Measurement of open-pore conductance ($G_0$) with (e) pH and (f) electrolyte conductivity (KCl buffered at pH ~8) with the fits corresponding to equation 1. In (e), $\sigma$ is substituted by equation 2.

After fabrication of the pores in the thin membranes and the chemical characterization of the nanopore surface, we conducted translocation experiments using dsDNA. The purpose of using dsDNA in this study is twofold: (i) to show that the fabricated pores through the thinned down membranes are conducive for analyte translocation and (ii) to corroborate the membrane thickness
from methods discussed previously using dsDNA translocation characteristics. DNA (mostly dsDNA) is often used to benchmark nanopores due to the well-known physiochemical properties of DNA under the typical conditions used for nanopore studies.

**Figure 3:** (a) Typical operational paradigm of a solid-state nanopore where the analyte (dsDNA in this case) is added to the *cis* side and a suitable voltage bias (+400 mV this case) is applied to the *trans* side to drive the analyte (dsDNA) through the nanopore. (b) I-V curves corresponding to ~5.3±0.1 nm diameter pores in membranes of ~11.9 nm (blue), ~8.8 nm (red), and ~5.3 nm (green) thickness. The thicknesses were evaluated using a molecular capillary method that uses dsDNA translocation characteristics. (c) Representative current traces, (d) Scatter plots and histograms, corresponding to (e) change in conductance (ΔG) and (f) translocation time (Δt)
resulting from the translocation of dsDNA through ~11.9 nm (first row) ~8.8 nm (second row) and ~5.3 nm (third row) thick membranes. All experiments were conducted with 17 nM dsDNA in 3.6 M LiCl buffered at pH ~8 with a bias of +400 mV.

A schematic of analyte translocation is shown in Figure 3a where the analyte is added to the cis side and a voltage of +400 mV is applied to the trans side to facilitate the electrophoretic transport of DNA through the nanopore. We used 3.6 M LiCl buffered at pH ~8 for the translocation experiments. Under such high electrolyte conditions, electroosmosis would be meager. We opted for LiCl instead of KCl since it is known to slow down the translocation of DNA. Representative current traces from three different pores (~5.3, ~8.8, and ~11.9 nm in thickness and ~5.3±0.1 nm diameter) are shown in Figure 3c. The thicknesses were evaluated using a molecular capillary method which uses dsDNA translocation characteristics (explained later in the manuscript and in Figure S2).

The scatter plots and histograms corresponding to dsDNA translocations are shown in Figures 3d-3f. Histograms corresponding to the change in conductance due to dsDNA translocation (ΔG) were fitted with a Lorentzian mixture model. While the dsDNA translocations through the thicker pore favored single-file translocations (Figure 3e, first row), the two thinner pores displayed multiple translocating configurations: the two thinner pores had three peaks in the distribution, unlike the thicker pore. The first ΔG distribution (ΔG₀) is often attributed to collisions while the other two (in the order of increasing ΔG) are attributed to single file (ΔG₁) and folded over (ΔG₂) conformations of dsDNA. For further analysis, the single file and folded-over translocations were separated using Gaussian Mixture clustering (see SI figure S1 for more information). For dsDNA, the ratio of the third and second peaks (i.e., ΔG₂/ΔG₁) is typically ~2. From the fits corresponding to ΔG₁ and ΔG₂ (SI figure S1), the ratios ΔG₂/ΔG₁ corresponding to ~8.8 nm and ~5.3 nm thick pores were computed to be ~1.94 and ~1.97, respectively, which is in close agreement with the ideally anticipated value
of 2 for dsDNA. Here we note, without the clustering of the single file and folded over conformations, the ratio $\Delta G_2 / \Delta G_1$ from the fits shown in Figure 3e corresponding to $\sim 8.8$ nm and $\sim 5.3$ nm thick pores (middle and bottom rows) would yield $\sim 1.87$ and $\sim 1.94$ which is still in good agreement with the ideally expected value of $\sim 2$. The improvement due to the Gaussian Mixture clustering is attributed to the separation of conformations which reduces the parametric constraints associated with the fitting.

The change in conductance because of dsDNA passage ($\Delta G_{dsDNA}$) was used as an independent metric to estimate $L_0$ (i.e., molecular capillary method). $\Delta G_{dsDNA}$ can be modeled using,

$$
\Delta G_{dsDNA} = G_0 - K \left( \frac{1}{\pi r_0^2 \text{with dsDNA}} + \frac{2}{\pi \alpha^2 r_0 \text{with dsDNA}} \right)^{-1}
$$

where $r_{dsDNA}$ and, $r_{0, with \text{ dsDNA}}$ are the radius of dsDNA (set to 1.1 nm) and open-pore radius when dsDNA is inside the pore, $\left( \sqrt{r_0^2 - r_{dsDNA}^2} \right) / 2$, respectively. For this, we disregarded the surface contributions, both from the nanopore surface and dsDNA due to the high salt concentration in the experiments. Thus, $G_0$ of equation 1 reduces to $K \left( \frac{1}{\pi r_0^2} + \frac{2}{\pi \alpha^2 r_0} \right)^{-1}$ (see equation 1 for the full form).

Both $G_0$ and $\Delta G_{dsDNA}$ are isolines of $L_0$ and $r_0$ as shown in SI Figure S2. Thus, the knowledge of $\Delta G_{dsDNA}$ can be used to find $L_0$ (or $r_0$) and by extension $r_0$ (or $L_0$) as shown in SI Figure S2c. The $\Delta G_1$ values (i.e., single file translocation conformation of dsDNA) were found to be $\sim 3.7$ nS, $\sim 4.5$ nS, and $\sim 5.6$ nS respectively for the pores shown in Figures 3d-f. As expected, the thinner the membrane, the higher the $\Delta G_1$. Then using the isoline intersection method, the $L_0$ were found to be $\sim 11.9$ nm, 8.8 nm, and $\sim 5.3$ nm respectively. The values estimated from the ellipsometry measurements were $(12.9 \pm 1.1)$ nm, $(8.8 \pm 0.9)$ nm, and $(5.3 \pm 1.4)$ nm respectively which are in excellent agreement
with the values calculated using the isoline intersection method (i.e., *molecular capillary* method). The translocation time profiles were fitted with the first passage model in the form 
\[ f(\tau) = \frac{l}{\sqrt{4\pi D\tau}} \exp \left( -\frac{(l-v\tau)^2}{4D\tau} \right) \]
where \( D, l \) and \( v \) are the diffusion coefficient, effective sensing length and drift velocity of dsDNA. The peaks of the fits (i.e., \( \tau_p \)) were found to be \( \sim 149 \) µs, \( \sim 88 \) µs, and \( \sim 103 \) µs respectively for the \( \sim 11.9 \) nm, \( \sim 8.8 \) nm, and \( \sim 5.3 \) nm thick membranes. While the \( \sim 11.9 \) nm thick pore produced the highest \( \tau_p \) as anticipated (i.e., thicker the membrane, higher the translocation time for a given pore size), the values from \( \sim 8.8 \) nm and \( \sim 5.3 \) nm thick membranes were somewhat peculiar and opposite of the anticipated trend. While the aspect ratio in the case of \( \sim 11.9 \) nm, \( \sim 8.8 \) nm thick membranes were \( >1 \) (\( \sim 2.2 \) and \( \sim 1.7 \) respectively), in the case of the \( \sim 5.3 \) nm thick membrane, it reaches unity. The observed \( \tau_p \) trends could be a result of an interplay of the aspect ratio which would need further investigation and is beyond the scope of the current study.

**Conclusion**

In this study, we have demonstrated a controllable HF-based etching method to fabricate thin SiNx membranes on wafer-scale. Moreover, membranes of <20 nm thickness can be fabricated conveniently with the ability to reach thicknesses as low as \( \sim 5 \) nm. Membranes were stoichiometric and confirmed through RBS. Single nanopores were then formed using the CBD method and the surfaces of the fabricated pores were probed using pH-\( G_0 \) and K-\( G_0 \) curves. From the former, the surface was found to be rich in acidic head groups which are unlike those formed from non-stoichiometric (Si-rich) membranes. The pKa was found to be in close agreement with that of a silanol-rich surface. The conductivity for analyte translocations was measured using dsDNA with a range of pores which produced translocation characteristics commensurate with the structure of DNA and dimensions of the pore. The \( L_0 \) calculated from dsDNA translocations further corroborated the
thickness predicted by the surface profiler and ellipsometry techniques. Our findings would be beneficial for the widespread adoption of nanopore technology as it presents a convenient and scalable membrane fabrication method conducive for nanopore fabrication by CBD and analyte translocations.

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Disclaimers

YMNDYB is currently employed at the Department of Bioengineering, University of California, Riverside, California, USA.

Data Availability

Data will be available upon reasonable request.

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**Supporting Information**

**Controlled Fabrication of Thin Silicon Nitride Membranes for Nanopore Sensing**

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**Table S1:** Membrane thickness (estimated from the surface profiler, with 3\(\times\) the relative error), \(G_0\) (from I-V curves in Figure 2c), estimated pore diameter (using equation 1). All I-V curves were measured using 1M KCl (K~12.69 S/m) buffered at pH ~7.

| Membrane thickness (nm) | \(G_0\) (nS) | Calculated Pore Diameter |
|-------------------------|--------------|--------------------------|
| 4.8 ± 0.5               | 5.8 ± 0.1    | 1.8 ± 0.1                |
| 5.1 ± 0.4               | 7.7 ± 0.1    | 2.3 ± 0.1                |
| 5.0 ± 0.5               | 10.4 ± 0.1   | 2.7 ± 0.2                |
| 6.3 ± 0.2               | 13.0 ± 0.3   | 3.4 ± 0.1                |
| 6.8 ± 0.9               | 14.3 ± 0.1   | 3.7 ± 0.5                |
| 4.6 ± 1.2               | 17.4 ± 0.2   | 3.5 ± 0.6                |
| 6.6 ± 0.5               | 19.7 ± 0.1   | 4.4 ± 0.3                |
| 7.2 ± 0.6               | 20.8 ± 0.1   | 4.7 ± 0.4                |
| 5.5 ± 0.6               | 23.0 ± 0.2   | 4.5 ± 0.3                |
| 6.2 ± 0.6               | 28.8 ± 0.1   | 5.4 ± 0.4                |
| 4.7 ± 0.6               | 35.1 ± 0.1   | 5.6 ± 0.3                |
Figure S1: (a) single-level events corresponding to ~8.8 nm thick membrane of Figure 3. Two populations can be seen since not only single files but also looped conformations are detected as single-level events. (b) The scatter plot is then subjected to clustering using the “Gaussian-Mixture” method of Mathematica. (c) Populations with similar ΔG are combined to get two populations with the lower one corresponding to single file translocations (blue) and the upper one signifying looped conformations (magenta). (d) The histograms corresponding to single file translocations showed two populations with the lower ΔG population assigned to collisions and the higher ΔG to true translocations. The histogram was then fitted using a Gaussian Mixture Model to extract ΔG\(_1\). (e) multi-level events corresponding to ~8.8 nm thick membrane of Figure 3. Two populations can be seen since events with shallow steps are grouped into the multi-level category.\(^1\) (f) The scatter plot is then subjected to clustering using the “Gaussian-Mixture” method of Mathematica. (g) Populations with similar ΔG are combined to get two populations with the lower one corresponding to events with shallow steps (blue) and the upper one signifying folded-over conformations (magenta). (h) The histograms corresponding to folded-over translocations showed a single population and was fitted with Gaussian model extract ΔG\(_2\).
Figure S2: (a) The relationship between membrane thickness and pore diameter for a pore with a conductance ($G_0$) of ~19.7 nS (electrolyte conductivity ~12.69 S/m) calculated using equation 1. (b) The relationship between thickness and pore diameter that satisfies $\Delta G_1 \sim 4.5$ nS (single file translocations of dsDNA) calculated using equation 3. (c) The overlay of figure S2a and S2b with the intersection point corresponding to $L_0$ and $2r_0$. The calculated values through the intersection point were ~8.8 nm and ~5.3 nm for $L_0$ and $r_0$, respectively.

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