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Profiling and Modification of Silicon Nitride Based Planar Substrates and Nanopores

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PROFILING AND MODIFICATION OF SILICON NITRIDE BASED PLANAR
SUBSTRATES AND NANOPORES

BY

YAPA MUDIYANSELAGE NUWAN DHANANJAYA YAPA BANDARA

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OF

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ABSTRACT

A nanopore—typically defined as a through-hole with dimensions <100 nm in all directions that functions as the sole path between two electrolyte reservoirs—is a robust single molecule sensor element which has enjoyed a wealth of applications spanning genomics and proteomics, with fledgling contributions to glycomics over the past two decades. Two classes of nanopores exist—biological and solid state. Biological nanopores, for example, α -hemolysin, are highly reproducible and precise—with nanopore lengths and critical constriction sizes that are well known and reproducible. This is not the case with solid state nanopores. Assuming total nanopore length is equal to the nominal thickness of the membrane provided by the manufacturer is a standard practice in the nanopore field. However, given fabrication tolerances, there is some room for error, in certain instances close to 60% of the provided nominal thickness. Any error in nanopore length will couple to errors in the radius calculation. Another two key assumptions are: i) the nanopore has a cylindrical shape unless (and often even if) the shape is otherwise known and ii) a single nanopore through the membrane is formed when one is intended. These issues were addressed by developing a framework that shows errors in harboring such geometric assumptions and eventual consequences for nanopore-based sensing experiments.

The focus of nanopore-based sensing has been predominantly on DNA and protein profiling with only fledgling contributions to glycomic profiling. Silicon nitride-based solid state nanopores were used to understand translocation conditions related to alginate and then to study source variability associated with alginates. The two alginates

used (from two different sources) gave two distinct signal patterns. Heparin, a common anticoagulant was contaminated in 2008 with over sulfated chondroitin sulfate (OSCS)—a structurally similar adulterant—which led to ~120 deaths in the United States. Nanopores, with sizes ranging from ~8.6 nm- ~13.6 nm were used to test the ability to flag the presence of OSCS in a contaminated heparin sample—all four unique nanopores used were able to flag the presence of the OSCS contaminant proving the diagnostic capability associated with nanopore sensing.

Surface modification techniques, for example, hydrosilylation, silane chemistry and electroless gold plating not only tune the size (minimum radius, r_0 , and total nanopore length, L) but also change the intrinsic surface chemistry. Hydrosilylation on planar silicon nitride—a less challenging and less volume-constricted environment compared to nanopore inner walls—has been shown to be possible photochemically and thermally. The photochemically driven hydrosilylation was scaled down to the nanopore level—decorating inner nanopore walls in a challenging zeptoliter volume—using a range of functional groups to potentially overcome unfavorable conditions such as analyte “sticking” problem while tuning analyte residence time favorably. Choice of molecule plays a significant role—one with a reactive terminal group such as hydroxyl or amine allowed for subsequent reactions, through condensation and click reactions, respectively, which are fast and facile, thus allowing for further modification of the size and surface chemistry of the pore. We observed the residence time of λ -DNA to increase with positive charge of the pore surface at pH 7, with bare, hydroxyl terminated, and amine terminated functional pores having peak residence times of ~250 μ S, ~450 μ S and ~1000 μ S respectively.

A carefully configured electroless plating procedure was used to deposit gold directly on silicon nitride. Since silicon nitride is an insulator, conventional electroplating would be futile—hence electroless plating. The plating was done at both 3 °C and 10 °C. The mean grain size of the gold grains plated at 3° C were found to be ~20 nm in radii. These nanostructured plated surfaces were also used to enhance the Raman signal of 4-nitrobenzothiol (test-molecule). The same plating method was extended to paper, nanocellulose, acrylate polymer grafted silicon nitride, nanoporous silicon nitride and Silmeco (a commercial substrate with a pillar like architecture) to create low cost surface enhance Raman active substrates. Enhancement values as high as $\sim 10^6$ for both acrylate polymer grafted silicon nitride and Silmeco was observed.

Patterned solution-phase gold depositions have great promise for electronics, photonics, and sensors such as nanopores as well—especially considering augmenting nanopore function with structures such as transverse electrodes. For nanopores and other fragile architectures, mechanical non-contact and cleaning ease (especially by simple rinsing) are key elements in designing modification and fabrication methods. Hydrosilylation meets these expectations as it can be guided and restricted to specific regions by manipulating the exposure of light (UV) to the surface. Hydrosilylated alkanes were used as a suppressing layer, for metal deposition in combination with electroless deposition to create spatial patterns of gold on silicon nitride. However, key modifications to the existing gold plating scheme had to be made. Key washing steps and replacement of Sn(II) chemistry with Pd(II) chemistry was done to increase the spatial selectivity of the plated patterns. Spatially selective patterns with lateral spacings as small as $\sim 30 \mu\text{m}$ have been fabricated using this method.

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I would like to sincerely thank my parents for constant motivation and guidance given to me throughout my life. None of this would have been possible if not for their sacrifices and words of wisdom. I am confident that I have done some justice to their long and hard hours at work to support me, my siblings and this thesis is a tribute for their life long efforts. I take this opportunity to thank my grandparents—all four them are instrumental pillars of my life. Two grandmothers, unarguably two of the strongest women I have seen in my life came through at some of my toughest times, helping me financially and by other means, most notably during the period of my undergraduate studies. Without them, this journey would have been impossible, and I doubt I would have taken the same path I took, to pursue a PhD in chemistry. Two grandfathers were my role models, each showed me, in their own way, how to prepare for the future. They are truly wonderful human beings and even today I deeply miss my late grandfather who always stressed the importance of school.

I found a life partner, a wonderful woman who was there during thick and thin. Backed me and encouraged me to go the extra mile. She was my strength at times of need and my mentor at times of desperation. No words would do justice to what she has done for me. She is a brilliant scientist, a wonderful family woman and a caring human being. This PhD journey was more than possible because I had her next to me 24x7. I am very happy that both of us would graduate at the same time as we did on two previous times.

I took the rudiments of chemistry from Wesley College, Colombo in Sri Lanka. My high school chemistry teacher is one of the best I have seen thus far. I would like to extend

my gratitude to the two undergraduate institutions—Institute of Chemistry, Ceylon (with special thanks to late Prof. J N O Fernando) and University of Colombo in Sri Lanka. The wonderful friends I made and the knowledge I gained from these two institutions paved the way for me to get to URI—a half way around the globe journey.

The family beyond home—friends I met at URI. All those Friday nights, travelling (if only our cars can speak), dining (we always had a good taste) and deep conversations (not to mention cricket) were a fulfilling experience. I think, it's safe to say, we were a big family. It was one for all and all for one. The game nights were always eventful and concentrated with too much fun at times. By the end of the PhD, these are the memories that I would cherish for a long time to come

Randall and Bethany Curtis—our host family. They taught us the way of American life to a great extent. We were regulars for their Thanksgiving, 4th of July celebration and other social activities. Randall was my first American car *guru* and helped me to get mobile. They are a happy family with five children. I thank you guys for all the help and support that was extended to our family.

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Last, but not least, my PI—Prof. Jason R. Dwyer—the smartest person I have seen. I think I made the right choice by joining his group. His guidance was very instrumental to get into nano science and the long technical conversations helped me to think about data in a scientifically critical manner. The value of scientific credibility and reproducibility of data is a regular topic in conversations and I believe it is only with such qualities the science would truly rise above the ashes of obstacles. I see the transformation in me as a young scientist and I frankly believe the training I got from him is greatly responsible for this positive change. I wish his group all the very best!

PREFACE

This thesis is written in manuscript format. Chapter 1 is an introduction to profiling and modification of silicon nitride based planar substrates and nanopores. Subsequent chapters and journals in which they were published are as follows,

Chapter 2: *ACS Appl. Mater. Interfaces*, 2013, 5 (19), pp 9330–9337

Chapter 3: *ACS Appl. Mater. Interfaces* 2016, 8 (44), 30583–30589

Chapter 4: *Electrophoresis* 2018, 39, 626-

Chapter 5: *ACS Appl. Mater. Interfaces* 2014, 6 (14), 10952–10957

Chapter 6: *ACS Appl. Mater. Interfaces* 2016, 8 (51), 34964–34969

Chapter 7: *ACS Appl. Nano Mater.*, 2018, 1 (2), pp 960–968

Chapter 8: *Submitted to Nature Communications*

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CHAPTER 1: BACKGROUND

NANOPORE FABRICATION AND CONDUCTANCE MODEL

(This sub-section is explored in detail in chapters 2, 3 and 4).

Fabricating nanopores was historically both time- and cost-strained as it required either charged-particle microscopes, for example, transmission electron microscopes (TEM)^{19,20}, scanning electron microscopes²¹ (SEM) and helium ion microscopes²² (HIM), or an accelerator facility, before the emergence of techniques such as dielectric breakdown²³. Microscopic inspection, for example, TEM, can determine the nanopore dimensions. However, from a practical standpoint, scanning every nanopore is not feasible and is expensive as well. Other disadvantages such as deposition of contaminants in vacuum chambers and fracture of nanopores during handling also exist. In the case of dielectric breakdown, fabrication takes place in the native sensing environment of a nanopore, mounted separating two electrolyte reservoirs. Such solution-based methods are well-complemented by using conductance based models to estimate size parameters of a nanopore^{24,25},

$$G = G_{\text{bulk}} + G_{\text{surface}} \quad (1)$$

These terms can be formulated by using Ohm's law for a conductor, resistance=resistivity · length/area. The first term, $G_{\text{bulk}} = K \cdot \left(\int_0^L \frac{dz}{\pi(r(z))^2} \right)^{-1} = K \cdot A$, uses the solution conductivity, K , to determine the passage of ions through the bulk of the nanopore. Radius along the z axis (vertical dotted lines along each nanopore profile in figure 3.1) of the nanopore is denoted by $r(z)$ with its initial minimum value being

r_0 . The second term, $G_{\text{surface}} = \mu|\sigma| \cdot \left(\int_0^L \frac{dz}{2\pi r(z)}\right)^{-1} = \mu|\sigma| \cdot B$, uses the surface charge density, σ , and the counterion mobility, μ , to determine the passage of ions along the surface of the nanopore. This model has the potential to allow for the real-time monitoring of the nanopore growth so that by setting a predetermined current threshold during the voltage-controlled dielectric breakdown, a nanopore with the size of interest could be fabricated²³.

NANOPORE CHARACTERIZATION

i) TOTAL NANOPORE LENGTH

(This sub-section is explored in detail in chapters 3 and 4).

In integral solved form of equation 1^{24,25}, there are two unknowns— r_0 and L —for a given nanopore shape and well-characterized surface chemistry and solution composition. From a single point measurement standpoint, to eliminate L as an unknown parameter, it is a customary practice to set it equal to the manufacturer provided nominal membrane thickness. However, manufacturing tolerances mean that there is some room for error, in certain instances close to 60% of the stated nominal thickness. For example, for an observed conductance of 200 nS ($L = 10$ nm, 1M KCl solution at pH 7), $r_0 \sim 6.7$ nm. However, if the actual L is 16 nm (Norcada Inc, NT-005Z, Lot #15)—a 60% error with respect to the assumed 10 nm nominal thickness—the calculated r_0 would be ~ 8.6 nm. Therefore, we let $\{r_0, L\}$ to be free parameters—so that an infinite number of $\{r_0, L\}$ probable combinations exist from a single conductance measurement standpoint²⁵. One approach to gain additional conductance data points to solve for the true $\{r_0, L\}$ combination would be to surface-modify the nanopore, for example by

electroless plating, hydrosilylation or silane chemistry—so that a minimum of two conductance data points can be generated²⁴. Another possible method would be to monitor pore formation with time—an array of real-time pore data would be generated. Since real-time/step-wise conductance data acquisition is experimentally possible, a framework that would simulate a set of conductance data to deduce the initial geometric parameters, $\{r_0, L\}$ was developed. This framework holds promise to be extended to experimentally observed conductance data.

ii) NANOPORE SHAPE

(This sub-section is explored in detail in chapters 3 and 4).

The values of the two integrals of equation 1, A (volume integral) and B (surface integral), are shape-dependent. It has also become a standard practice to assume the shape of the nanopore to be cylindrical ($A = \frac{\pi r_0^2}{L}, B = \frac{2\pi r_0}{L}$) unless the shape is clearly known, and even then the cylindrical approximation remains popular. Other nanopore shapes exist—double-conical, conical-cylindrical and hyperbolic are a few examples^{25–29}—which are both material and fabrication method dependent. For example, anisotropic etching of track-damaged silicon nitride produces conical or double-conical pores depending on whether the etching is done from a single side (conical) or from both the sides (double-conical) of the damaged track²⁶. In some instances, the possibility for conversion of one shape to another exists, if fabrication conditions are not controlled properly²⁹. If the initial conductance is assumed to be 200 nS ($L = 10$ nm, 1M KCl electrolyte at pH 7) for a silicon nitride nanopore, the calculated r_0 for cylindrical, double-conical, conical-cylindrical (assuming the inner cylindrical length to be $0.6L$)

and hyperbolic shapes would be ~6.4, 3.1, 5.5, and 4.0 nm respectively. There is, for example, an error greater than 50% in calculated r_0 , if a double-conical nanopore is wrongly assumed as a cylindrical nanopore or visa-versa. A need to deduce the shape of a nanopore therefore exists. Shape introduces another variable in addition to the two free geometric parameters, $\{r_0, L\}$. The same framework that was developed to solve for $\{r_0, L\}$ was used with critical modifications in the form of having additional simulated data points for robustness of the method and to solve the additional unknown, nanopore shape.

iii) NUMBER OF NANOPORES

(This sub-section is explored in detail in chapters 3 and 4).

As an added complexity to nanopore characterization, it is assumed that only one nanopore is formed when one was intended. However, recent work showed that this is not always the case: an unoptimized multilevel pulse voltage injection (MPVI) method yielded multiple pores when one was intended³⁰. A simple example yields valuable insight: a comparison of a single pore and two identical pores (double pores). For an initial conductance of 200 nS ($L = 10$ nm, 1M KCl electrolyte at pH 7) each profile gives the corresponding r_0 : cylindrical—6.4 vs. 4.5 nm; double-conical—3.1 vs. 1.7 nm; conical-cylindrical—5.5 vs. 3.8 nm; and hyperbolic—4.0 vs. 2.3 nm for single and double pore cases, respectively. If the number of pores is wrongly assumed to be one, a given molecule, depending on the actual size of the nanopore, would not translocate through the nanopore despite calculations (based on the wrong assumptions) saying otherwise. Hence, there exists a need to differentiate between a double pore and a single

pore case before precious analyte is spent/wasted in an incorrectly configured nanopore device. One of the methods to distinguish a single pore from its double pore counterpart is to use λ -DNA as a gauging molecule. That is to use, $\frac{\langle G \rangle - \langle G_b \rangle}{\langle G \rangle} \cong \left(\frac{r_{\lambda\text{-DNA}}}{r_0} \right)^2$ with $\langle G \rangle$, $\langle G_b \rangle$ and $r_{\lambda\text{-DNA}}$ the time-averaged conductances of open, and analyte-filled, nanopore and radius of λ -DNA respectively²³.

NANOPORE SURFACE MODIFICATIONS

(This sub-section is not explored in detail due to intellectual property filing).

In addition to analyte sticking, the charge of the pore sometimes decreases the translocation frequency by opposing the translocation by having electro-osmotic movement (in addition to electrostatic repulsion³¹) opposite to the direction in which the analyte is moving. This would require the experiment to be done over an extended period to collect an appreciable amount of data, or done at higher voltages risking voltage-driven electrode reactions. Switching the charge of the pore is possible through pH tuning if the surface contains an isoelectric point, which is the case for silicon nitride rich in hydroxy, amine and other nitrogen-based moieties³². However, the pH at which this switching occurs would sometimes be at a regime which can cause degradation of the analyte. A gentler approach would be to modify the nanopore surface with a surface terminal group that would produce the nanopore surface charge of interest at the desired experimental pH. Such changes would lead to changes in the direction of electro-osmotic flow. Careful attention, however, must be paid to the translocation velocity as it must be within the bandwidth limitation of the data acquisition electronics. Some of the recent surface modification efforts involve silane chemistry where an organosilane

molecule is initially reacted with a pristine silicon nitride nanopore surface³³. This requires the nanopore to be treated with piranha solution so that the nanopore surface would be clean and rich in hydroxyl groups. We carried out hydrosilylation on freshly fabricated nanopores to avoid such harsh surface treatments (e.g. piranha). Once the initial monolayer of molecules is photochemically laid, subsequent reactions, for example, condensation and even click, were carried out to further modify the nanopore surface. Such modification steps also provide the ability to tune the size of a nanopore—fabricating nanopores with diameters <5 nm is a challenging task and these modifications can allow one to shrink a pore that is initially made larger than expected back to the challenging <5 nm size regime.

ELECTROLESS GOLD PLATING, SPATIAL PATTERNING AND SERS

(This sub-section is explored in detail in chapters 5, 6 and 7).

Fabricating conductive patterns on nanofabrication compatible material such as silicon nitride could serve as, for example, recognition elements (electrode), signal amplifiers (hot spots for surface enhanced Raman) and circuitry elements. A mask to restrict conductive material to regions of interest is essential. Non-contact methods are preferred for fragile (ultra-thin) architectures. Photochemically driven hydrosilylation could be well-suited for such situations and area-selective exposure of UV light could lead to grafting of alkane molecules in a user-defined pattern. This step is followed by electroless plating and we anticipate this (alkane) molecular layer to arrest metal ion deposition on them and on the underlying silicon nitride. Thus, patterned metal formation is expected to prevail provided that non-specific adsorption does not lead to

plating, and that degradation of the alkane layer does not happen during the metallic structure fabrication process—modifications to the electroless method would be needed if these adverse effects take place. The density of Si-H sites—governed by the stoichiometric excess of Si in the silicon nitride—would determine how densely alkanes would be packed on the silicon nitride surface. One would desire a high coverage as these alkanes are expected to become an umbrella to the underlying silicon nitride—protecting it from the reactive elements of electroless baths.

The electroless gold plating method, with substrate specific modifications, can be extended to paper, nanocellulose, acrylate polymer grafted silicon nitride, nanoporous silicon nitride and Silmeco (a commercial substrate with a pillar like architecture) to create low cost surface enhance Raman (SER) active substrates. Coinage metals enhance the otherwise weak Raman signal. The presence of hot spots—a nanoscale region of especially high enhancement accessible by the analyte molecule—further contribute to signal enhancement. Silmeco and polymer grafted silicon nitride, due to their surface structure, are expected to have higher enhancement factors than other substrates. A distinct advantage of the cellulose based substrates is the ease of disposing.

POLYSACCHARIDE PROFILING

(This sub-section is explored in detail in chapter 8).

Polysaccharides have key roles in a multitude of biological functions, and they can be harnessed for therapeutic roles, with the clinically ubiquitous anticoagulant heparin being a standout example. Their complexity—*e.g.* >100 naturally occurring

monosaccharides with variety in linkage and branching structure—significantly complicates their analysis in comparison to other biopolymers such as DNA and proteins. More, and improved, analysis tools have been called for, and solid-state silicon nitride nanopore sensors and tuned sensing conditions can be used to reliably detect native polysaccharides and enzymatic digestion products, to differentiate between different polysaccharides in straightforward assays, to provide new experimental insights into nanopore electrokinetics, and to uncover polysaccharide properties. Nanopore sensing allows to easily differentiate between a clinical heparin sample and one spiked with the contaminant that caused deaths in 2008 when its presence went undetected by conventional assays. The work reported here lays the foundation to further explore polysaccharide characterization and develop assays using thin-film solid-state nanopore sensors.

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CHAPTER 2: PREFACE

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NANOPORE SURFACE COATING DELIVERS NANOPORE SIZE AND SHAPE
THROUGH CONDUCTANCE-BASED SIZING

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CHAPTER 2: NANOPORE SURFACE COATING DELIVERS NANOPORE SIZE AND SHAPE THROUGH CONDUCTANCE-BASED SIZING

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ABSTRACT

The performance of nanopore single-molecule sensing elements depends intimately on their physical dimensions and surface chemical properties. These factors underpin the dependence of the nanopore ionic conductance on electrolyte concentration, yet the measured, or modeled, dependence only partially illuminates the details of geometry and surface chemistry. Using the electrolyte-dependent conductance data before and after selective surface functionalization of solid-state nanopores, however, introduces more degrees of freedom and improves the performance of conductance-based nanopore characterizations. Sets of representative nanopore profiles were used to generate conductance data, and the nanopore shape and exact dimensions were identified, through conductance alone, by orders-of-magnitude reductions in the geometry optimization metrics. The optimization framework could similarly be used to evaluate the nanopore surface coating thickness.

INTRODUCTION

Nanopores are the core element of a powerful new class of methods and devices for single-molecule sensing and manipulation¹⁻⁹. A nanopore, at its most basic level, is

a nanometer-diameter through-hole in an insulating membrane. When such a membrane is used to divide an electrolyte-filled cell, and a transmembrane potential is applied, the flow of electrolyte ions through the nanopore can be readily measured. The presence of a single molecule in the nanopore can then be detected and identified if it perturbs the electrolyte-only, open pore current in a characteristic way. Experimental measurements of nanopore conductance in the absence of analyte show a rich behavior dependent upon the intricate interplay between nanopore geometry, nanopore surface chemistry, electrolyte composition and potential drop across the nanopore. This behavior is captured by theoretical treatments and simulations employing varying levels of sophistication¹⁰⁻¹⁶.

There are three broad classes of nanopores in routine use: proteinaceous pores such as α -hemolysin and MSPA, solid-state pores such as those fabricated in silicon nitride and silicon oxide using direct electron- and ion-beam milling, and solid-state pores formed by solution processing of ion-tracked polymer and silicon nitride films^{1-4, 7, 17}. These pore classes and fabrication conditions present quite different geometries and surface chemistries, and quite different challenges and opportunities. Protein pores offer self-assembly of reproducible pore structures with rich surface chemistries determined by the functional groups—amino acids in native pore structures, modifiable through complex formation and biochemical manipulation—lining the nanopore interior. Solid-state nanopores crafted in micro- and nanofabrication-compatible materials such as silicon nitride and silicon dioxide offer the prospect of streamlined fabrication of robust, complex nanopore devices for single molecule measurement and manipulation. The ability to create solid-state nanopores with a variety of sizes and shapes to accommodate

a wide range of target applications is also driving their increasing popularity. The surface chemistry of native solid-state nanopores is relatively simple, with silicon oxide nanopore surface chemistry, for instance, typically treated as being governed by the single chemical equilibrium¹⁰⁻¹¹



Advances in the surface chemical modification of nanopores, however, are dramatically blurring the boundaries between the rich surface chemistry of protein pores and the relatively straightforward chemistry of native solid-state pores. A variety of methods exists to tune nanopore surface chemistry, from direct covalent attachment to the use of physi- and chemisorbed layers¹⁸⁻²². Such surface modifications can be used to alter the nanopore surface chemistry and they can also be used to appreciably change the physical dimensions of the nanopore. Thus, what emerges is a design framework in which physical and molecular approaches can be used to tune the solid-state nanopore size and properties to suit applications as diverse as the fundamental investigation of receptor-ligand interactions²³ and rapid, low-cost DNA sequencing²⁴. The consequent challenge is the characterization of the resulting nanopore on a length scale that is challenging to access experimentally. Characterization approaches that rely on charged particle imaging place substantial demands on the user, and require access to facilities and expertise in methods beyond those required for nanopore use^{10, 25-26}. The development of characterization methods requiring routine nanopore operation, alone, thus continues, with the improved accessibility and efficiency of nanopore methods an attractive target^{10, 27}. Such methods would additionally promise benefits for advancing the foundations of nanopore technology by permitting, for example, nanopore size and

shape to be monitored and used for feedback during solution-based nanopore fabrication approaches^{19, 28-30}.

Given the central role of the nanopore ionic conductance in many nanopore experiments, and given that the conductance is determined by factors including the nanopore size and surface chemistry, it is common to use the ionic conductance to characterize the nanopore. Using a simple but experimentally supported model for nanopore conductance^{10-11, 19}, we have previously shown that the electrolyte-dependence of the conductance offers, in general, only a limited view of nanopore structure²⁷. In particular, the ability to determine at most two nanopore geometry parameters does not necessarily permit unambiguous identification, by conductance, of nanopore shape. Independent knowledge of some elements of the size or shape, though, can be used within that framework to allow the evaluation of conductance-derived parameters, or to impose constraints that allow the partial recovery of more geometric information from nanopores described by more than two geometric parameters²⁷. In this work, we show that by using the electrolyte-dependence of nanopore conductance before and after surface coating, we can more completely characterize nanopore size and shape without the need for independent geometry inputs. In particular, for experimentally realistic three-parameter pores, the augmented approach allows nanopore size and shape to be completely recovered from the conductance.

THEORY

We adopt a widely-used theoretical model for the nanopore conductance that has been successfully used to model experimental results^{10-11, 19}. We focus on nanopores

less than 20nm in diameter, for which the access resistance is a negligible contribution³¹, leaving two contributions to the nanopore conductance, $G^{10, 27}$

$$G_{\text{total}} = G_{\text{bulk}} + G_{\text{surface}} \quad (2)$$

The bulk term, G_{bulk} arises from the flow of ions through the pore, treated here as a uniform flow³²

$$G_{\text{bulk}} = K \left(\int \frac{dz}{\pi(r(z))^2} \right)^{-1} = K \cdot A \quad (3)$$

where K is the solution conductivity and $r(z)$ is the radius of the pore as a function of the distance into the pore, in a cylindrical coordinate system. The surface term, G_{surface} , accounts for the flow of counterions along the charged surface of the pore, which is especially significant in low bulk ionic strength solutions¹⁰⁻¹¹

$$G_{\text{surface}} = \mu|\sigma| \left(\int \frac{dz}{2\pi r(z)} \right)^{-1} = \mu|\sigma| \cdot B \quad (4)$$

where σ is the surface charge concentration, and μ is the mobility of the counter ions proximal to the surface. This surface term thus augments the conductance with additional information involving the geometry and the surface chemistry. For a nanopore with surface chemistry governed by the chemical equilibrium in equation 1, the surface charge will arise from the charged SiO^- groups on the surface, and the mobile counterions will be cations. By solving for the equilibrium concentration of H^+ ions at the surface, $[\text{H}^+]_0$, and applying the Nernst equation³³

$$[\text{H}^+]_0 = [\text{H}^+]_{\text{bulk}} \exp(-e\beta\psi_0) \quad (5)$$

where $[H^+]_{\text{bulk}} = 10^{-\text{pH}}$, one can obtain an expression for the diffuse layer potential proximal to a negatively charged surface³³

$$\psi_D(\sigma) = \frac{1}{\beta e} \ln \frac{-\sigma}{e\Gamma + \sigma} - (\text{pH} - \text{pK}_a) \frac{\ln 10}{\beta e} - \frac{\sigma}{C} \quad (6)$$

where e is the elementary charge, $1/\beta$ is the thermal energy (at 298K for all calculations), Γ is the total surface density of surface chargeable groups, pK_a is the acid dissociation constant for equation 1, pH is the bulk solution pH , C is the Stern layer capacitance, and σ is the surface charge density. Surface functionalization likely changes the pK_a , and if the surface becomes cationic, the argument of the first logarithm becomes $(e\Gamma - \sigma)/\sigma$, and the mobile surface counterions are anions. Coupling the appropriate expression for the diffuse layer potential with the Grahame equation³³

$$\sigma(\psi_D) = \frac{2\epsilon\epsilon_0\kappa}{\beta e} \sinh\left(\frac{\beta e \psi_D}{2}\right) \quad (7)$$

where $\epsilon\epsilon_0$ is the permittivity of the solution and κ^{-1} is the Debye screening length, calculated from $\kappa^2 = \beta e^2 n_{\text{KCl}}/\epsilon\epsilon_0$ where n_{KCl} is the numerical concentration of the potassium chloride electrolyte, allows one to find a solution for the surface charge concentration of the pore^{10-11, 33}.

The nanopore conductance in equation 2 can be expressed in a form that clarifies its geometrical and surface chemical underpinnings²⁷

$$G_{\text{total}} = A K + B \mu |\sigma| \quad (8)$$

where A and B are the volume and surface integrals, respectively, in equations 3 and 4. When a continuous coating of thickness δ is applied to the nanopore surface, the new conductance of the nanopore can be expressed as

$$G'_{\text{total}}(\delta) = A'(\delta) K + B'(\delta) \mu'|\sigma'| \quad (9)$$

where the prime denotes the parameter value after surface coating. Measurement of the nanopore conductance at a minimum of two electrolyte concentrations, each, before and after changing the surface coating (a dimension change, $\delta \neq 0$, is required, and a surface charge density change from σ to σ' is likely), formally allows for the unique determination of the geometry parameters A , $A'(\delta)$, B and $B'(\delta)$. These parameters can then be used to determine the values of the underlying geometric parameters such as the nanopore limiting radius.

The implementation of this approach is not restricted to experiments in which only changes in the solution electrolyte concentration are used to predictably change the solution conductivity, K , and the surface conductivities $\mu|\sigma|$ and $\mu'|\sigma'|$. Chemical and physical parameters both implicit and explicit in Equations (6) and (7) can be used instead, including: a direct change of solution pH, a change of solvent to drive changes in ion mobility or surface acid dissociation constants, or a change in temperature to affect the surface acid dissociations and ion mobilities. The method is quite general and relies only upon the explicit functional dependence of the conductance shown in Equations (8) and (9). It does not rely upon the particular chemical or physical parameter used experimentally to deliver the underlying functional dependence of K , $\mu|\sigma|$ and $\mu'|\sigma'|$.

METHODS

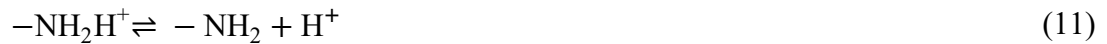
In all calculations where the parameters appear, the bulk solution pH was fixed at 7.5 and the nanopore membrane thickness, L , was held fixed at 30nm. The aqueous

electrolyte solution was composed of potassium chloride, so that the solution conductivity was calculated from

$$K = e n_{\text{KCl}}(\mu_{\text{K}} + \mu_{\text{Cl}}) \quad (10)$$

where $\mu_{\text{K}} = 7.6 \times 10^{-8} \text{m}^2/(\text{V} \cdot \text{s})$ and $\mu_{\text{Cl}} = 7.9 \times 10^{-8} \text{m}^2/(\text{V} \cdot \text{s})$ are the mobilities of the potassium and chloride ions, respectively¹¹. The solution permittivity was approximated as $\epsilon\epsilon_0 = 77.75\epsilon_0$ throughout. Native, uncoated nanopores had their surface chemistry described by the equilibrium in Equation 1, with a constant $\text{pK}_a=7.9$ ³⁴. The surface charge density, σ , of the uncoated nanopores was calculated as the simultaneous solution to equations 6 and 7, where Γ and C were held constant at $8 \times 10^{18} \text{m}^{-2}$, and $0.3 \text{F} \cdot \text{m}^{-2}$, respectively, and were not changed after surface coating³³⁻³⁴.

We selected a number of common nanopore radial profiles, listed in Table 1.1, to describe the shape of the nanopores. We chose to model an amine-terminated, covalently modified nanopore surface to give a surface coating involving the acid-base equilibrium



and described by $\text{pK}_a = 10.8$. The 1.7nm-thick coating was assumed to smoothly and uniformly coat the surface without changing the nanopore shape and with the monolayer chains orthogonal to the surface at the point of attachment. The surface coating did, however, change the sign of the charge on the nanopore surface and the identity of the mobile surface counterions, from cations in the native pore to anions in the coated pore.

To investigate the ability of the proposed method to recover the nanopore size and shape for nanopores with limiting radii, r_0 , between 2.5 and 10nm, we computed the integrals A , $A'(\delta)$, B and $B'(\delta)$, using $\delta = 1.7\text{nm}$ to account for the length of the silane-coupled monolayer, for each nanopore radial profile listed in Table 1.1. The lower limit was chosen to prevent the monolayer from sterically closing the pore, but must in practice be responsive to the onset of overlapping Debye layers. To generate the set of reference (ref) values, we varied the limiting radii, $r_{0,\text{ref}}$, and fixed the inner cylinder lengths, l_{ref} of the exponential-cylindrical and conical cylindrical models at 11nm, the slope parameter b_{ref} of the exponential-cylindrical model at 0.19nm^{-1} , and the outer radii, R_{ref} of the hyperbolic, conical and conical-cylindrical models at $r_{0,\text{ref}} + 10\text{nm}$ ^{10, 27, 31}. We then used these reference calculations to geometry-optimize all of the radial profiles at each limiting radius. For example, an $r_{0,\text{ref}} = 3\text{nm}$ exponential-cylindrical nanopore was used to geometry-optimize cylindrical, conical, hyperbolic, conical-cylindrical and exponential-cylindrical profiles, and an $r_{0,\text{ref}} = 7\text{nm}$ cylindrical nanopore was used to geometry-optimize cylindrical, conical, hyperbolic, conical-cylindrical and exponential-cylindrical profiles. All native geometry parameters, except for L , were varied during the geometry optimizations. The geometry optimizations were first performed with fixed monolayer thickness, $\delta = 1.7\text{nm}$, and then repeated in a separate trial with δ as a free parameter, in an attempt to recover the layer thickness. The optimization used the Nelder-Mead minimization algorithm, and involved varying the underlying geometry parameters (e.g. r_0 , l , etc.) of the radial profiles to minimize

$$\begin{aligned} & \text{RMSE}_{AB} \\ &= \sqrt{\frac{1}{4} \left(\left(\frac{A_{\text{fit}} - A_{\text{ref}}}{A_{\text{ref}}} \right)^2 + \left(\frac{B_{\text{fit}} - B_{\text{ref}}}{B_{\text{ref}}} \right)^2 + \left(\frac{A'_{\text{fit}} - A'_{\text{ref}}}{A'_{\text{ref}}} \right)^2 + \left(\frac{B'_{\text{fit}} - B'_{\text{ref}}}{B'_{\text{ref}}} \right)^2 \right)} \end{aligned} \quad (12)$$

where the subscript “ref” denotes the known, reference, parameter value, and the subscript “fit” denotes the corresponding value calculated using the trial values. Given the form of the conductance (equations 8 and 9), minimization of RMSE_{AB} delivers a weighted conductance-based geometry optimization. An error threshold of 10^{-12} was used in the optimization runs, and the optimized structure was the result of the trial with the lowest RMSE_{AB} . A similar metric expressed directly in terms of conductance requires an average across N potassium chloride concentrations

$$\text{RMSE}_G = \sqrt{\frac{1}{N} \sum_{[\text{KCl}]} \frac{1}{2} \left(\left(\frac{G_{\text{fit}}([\text{KCl}]) - G_{\text{ref}}([\text{KCl}])}{G_{\text{ref}}([\text{KCl}])} \right)^2 + \left(\frac{G'_{\text{fit}}([\text{KCl}]) - G'_{\text{ref}}([\text{KCl}])}{G'_{\text{ref}}([\text{KCl}])} \right)^2 \right)} \quad (13)$$

The potassium chloride concentrations used here ranged from 0.01M to 1M, with the i -th concentration calculated from $10^{-2+(i-1)0.01}$ M.

RESULTS AND DISCUSSION

In the most common implementation of conductance-based nanopore sizing, the nanopore conductance at a single electrolyte concentration is used to extract a radius, and nanopore surface charges may be either included or neglected in the calculation. We explore this canonical single-point approach as a prelude to the consideration of the more involved process outlined in the Methods section. The use of a single conductance value for geometry optimization permits only the use of single-parameter profiles—either those that are inherently single-parameter, such as the cylindrical profile, or those in which all parameters but one are fixed to particular values or fixed by functional

relationships that are either known or are assumed reasonable. In addition to this strictly geometric limitation, the use of a single conductance value, G_{total} , does not allow the separation of bulk and surface contributions to the conductance. Measurement in high ionic strength solutions, though, can minimize the effect of the surface term, albeit at the cost of information about the surface chemistry. Geometry optimization of a particular nanopore profile can produce dramatically different nanopore sizes when geometry parameters that satisfy Equation 8 are determined by either including or neglecting the surface charge. These single-point geometry optimizations produce exact agreement with the reference conductance, so that no error metrics exist to evaluate the suitability of the assumed nanopore shape. Figure 2.1 and the discussion that follows put the necessity to consider size, shape, surface chemistry and electrolyte composition in concert into relief.

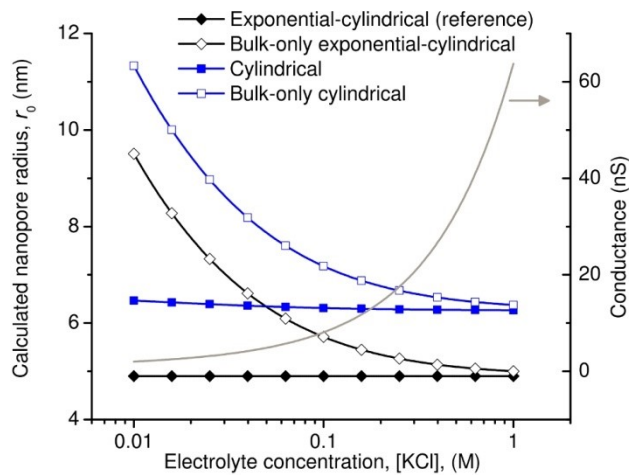


Figure 2. 1. The conductance of an uncoated, surface-charged exponential cylindrical reference pore ($r_{0,\text{ref}} = 4.9 \text{ nm}$, $l_{\text{ref}} = 1 \text{ nm}$, and $b_{\text{ref}} = 0.19 \text{ nm}^{-1}$) was calculated at a number of different electrolyte concentrations. All of the plotted radii were calculated by using

the single reference conductance at each electrolyte concentration to geometry optimize either the known reference radial profile (with fixed $l=11\text{nm}$, and $b=0.19\text{nm}^{-1}$), or the canonical cylindrical approximation. For each nanopore shape, the radial optimization was performed with the surface charge included and then neglected.

To generate Figure 2.1, the conductance of the uncoated reference nanopore was calculated using a realistic radial profile with three tunable geometric parameters (exponential-cylindrical, see Supplemental Table S1)¹⁰ and accounting for the surface charge established by the equilibrium described in Equation 1. At each electrolyte concentration considered, the single conductance value was used to determine the radius of a particular single-free-parameter nanopore profile—here, either the original reference profile with fixed $l=11\text{nm}$, and $b=0.19\text{nm}^{-1}$, or a cylindrical profile—by including or neglecting the surface charge. In solutions with high bulk conductivity and high ionic strength, omission of the surface charge had little effect on the best-fit nanopore radii. There was, however, a clear difference in the nanopore radii determined via assumption of the nanopore shape—a difference that persisted across solution electrolyte concentrations. At lower electrolyte concentrations, the profile-specific errors in best-fit radii were dramatically superseded by the errors arising from the neglect of surface charges in the geometry optimization. This tremendous sensitivity to the surface chemistry points both to the potential to profile the surface chemistry via conductance and to the necessity to consider it^{10-11, 14, 35}. It is moreover essential to emphasize that in addition to the visible differences in cylindrical and exponential-cylindrical best-fit radii shown in Figure 2.1, the two optimized versions of the same nanopore have dramatically different shapes—one has a cylindrical restriction of 11 nm

in length that then opens towards the membrane surfaces, the other a cylindrical restriction that spans the entire 30nm membrane thickness. These observations underscore the importance—and difficulty—of using conductance to determine nanopore shape and surface chemistry, together: a single conductance value can be exactly satisfied by nanopores of a host of different sizes and shapes. Extension of this basic, single-point optimization to use the electrolyte-dependence of the conductance—at minimum a two-point optimization, but more practically requiring more than two data points to improve the fit statistics—offers the possibility of determining the bulk and surface contributions. In addition, the extension delivers an additional degree of freedom for nanopore geometry optimizations: it permits the optimization of radial profiles with up to two free geometry parameters²⁷. Given that transmission electron microscope (TEM)-fabricated nanopore profiles can require description by no less than three free parameters, such a geometry optimization requires parameter constraints or reductions. This has the consequence of compromising the nanopore size determination and moreover prevents even the shape of pores from being determined without additional information²⁷. One of the substantial and myriad benefits conferred by coating nanopores with overlayers, then, is the additional degrees of freedom provided for conductance-based geometry optimizations.

Nanopores and nanopore surface functionalization are frequently characterized using a conductance-based method that does not involve variation of the electrolyte concentration, however. The approach is analogous to the single-point optimization of Figure 2.1 and uses the nanopore conductance at a single electrolyte concentration, before and after surface coating. The use of two conductance values provides a much-

needed additional degree of freedom compared to the single-point measurement, but the available information is still limited. In particular, one would perform a single measurement of the conductance before and after (') coating, $G_1 = A K_1 + B \mu_1 |\sigma_1|$ and $G'_1(\delta) = A(\delta) K_1 + B(\delta) \mu_1' |\sigma_1'|$, respectively, where the subscript “1” denotes the particular value of the parameter. Rewriting $A(\delta) = \alpha(\delta)A$ and $B(\delta) = \beta(\delta)B$ (with different values of $\alpha(\delta)$ and $\beta(\delta)$ for each nanopore size and shape), and defining effective (eff) values $\alpha(\delta)K_1 = K_{1,\text{eff}}$ and $\beta(\delta)\mu_1' |\sigma_1'| = (\mu_1' |\sigma_1'|)_{\text{eff}}$ yields two equations $G_1 = A K_1 + B \mu_1 |\sigma_1|$ and $G'_1(\delta) = A K_{1,\text{eff}} + B (\mu_1' |\sigma_1'|)_{\text{eff}}$ that makes this approach formally equivalent to the two-point nanopore geometry optimization that had previously been explored in detail²⁷. While delivering generally superior performance to a single-point optimization, it nevertheless has well-characterized performance limitations in comparison to the optimization method introduced here. For example, such a two-point approach cannot be used to uniquely geometry optimize nanopores requiring more than two free geometry parameters²⁷.

We now consider the nanopore optimization method outlined in the Theory and Methods sections, a method that requires knowledge of the nanopore conductance at a minimum of two electrolyte concentrations, before and after surface coating. The method therefore requires a minimum of four conductance values (a four-point optimization), but in practice more than these four conductance values would be used in order to improve the fit statistics, at least the first time that a pore was to be characterized. Equation (13) could be used to guide the geometry optimization using the conductance directly. In the conductance equations, Equations (8) and (9), however, the

physical pore dimensions and the surface chemical properties are separable contributions to the conductance. To highlight the performance of the optimization method in recovering nanopore size and shape, we used Equation (12) to perform the geometry optimizations, guided by the known values of A_{ref} , $A'_{\text{ref}}(\delta)$, B_{ref} and $B'_{\text{ref}}(\delta)$. The optimization results presented here using Equation (12) deal with geometry only, and are completely independent of the surface chemistry, which need not be specified. Experimentally, this geometry-based approach would have great utility if a two-step optimization were adopted. In the first step, the conductance versus electrolyte concentration curves (Equations 8 and 9) would be fit to extract best-fit values for A , $A'(\delta)$, B and $B'(\delta)$ —parameters that would be, at this stage, devoid of physical meaning because the core geometry parameters underlying their values would not yet be considered. Within the framework of the conductance model described by Equations (8) and (9), this first step would thus require no knowledge of nanopore geometry, but would require only knowledge of its surface chemistry. Minimization of RMSE_G to achievable $\sim 10^{-12}$ levels (*cf.* Figure 2.2) may require slight fine-tuning of surface parameters to optimize the fit to the conductance. The best-fit A , $A'(\delta)$, B and $B'(\delta)$ would then serve as the reference values to govern the subsequent determination of nanopore size and shape using Equation (12)—a geometry-only optimization.

Figure 2.2 summarizes geometry optimizations, using Equation (12), selected from the full set performed. In Figure 2.2A, exponential-cylindrical nanopores described by three underlying geometry parameters ($r_{0,\text{ref}}$, $l=11\text{nm}$, and $b=0.19\text{nm}^{-1}$)¹⁰ were used to calculate the reference A , $A'(\delta)$, B and $B'(\delta)$. Geometry optimizations of all the radial profiles listed in Table 1 were performed, without constraints on the values

of the geometry parameters (other than $L=30\text{nm}$ and $\delta = 1.7\text{nm}$, as outlined in Methods). The lowest values of the optimization metric RMSE_{AB} were for the exponential-cylindrical profile—the shape matching the reference nanopore shape—and were orders of magnitude lower, for all nanopore sizes considered, than the RMSE_{AB} for all of the other candidate nanopore shapes. The RMSE_{AB} metric was therefore clearly able to correctly identify the nanopore shape. The errors in conductance, RMSE_{G} , corresponding to all of the RMSE_{AB} -best-fit geometries, were also calculated, although they were not used for the optimization. While the RMSE_{G} are scaled by the solution and surface physicochemical parameters, they still showed the same relative trends and magnitudes as the RMSE_{AB} and the same performance in correctly identifying the nanopore shape from amongst the candidates. An examination of the best-fit limiting radii, r_0 , for each trial shape further emphasizes the merits of this conductance-based characterization approach. The cylindrical, conical and hyperbolic profiles rejected by the RMSE_{AB} metric yielded radii whose deviations from the reference radii were significant on the length scale of nanopore-based single-molecule sensing and manipulation. In spite of broad structural similarities (inner cylinders that widen towards the membrane surfaces) and limiting radii in very close agreement, the RMSE_{AB} metric was able to clearly differentiate between conical-cylindrical and exponential-cylindrical pore shapes. This inability of the conical-cylindrical pore to match the exponential-cylindrical nanopore conductance occurred in spite of the variation of $R - r_0$ from $\sim 3.5\text{nm}$ to $\sim 7\text{nm}$ with increasing $r_{0,\text{ref}}$, and l varying from 9.8 to 11nm versus the constant 11nm in the reference nanopores (not shown). This ability to distinguish between even structurally similar three-parameter (or fewer) nanopore

shapes using the present four-point method is in marked contrast to earlier reports using two-point conductance optimizations²⁷.

Figure 2.2B presents the results of the geometry optimizations of conical reference nanopores. Comparison of the $RMSE_{AB}$ and $RMSE_G$ for all best-fit trial profiles indicated, by several orders of magnitude difference in errors, that the reference nanopores were conical, and the best-fit radii r_0 and R for the conical trial profile matched the known reference values. Reassuringly, the greater parameter flexibility of the exponential-cylindrical and conical-cylindrical profiles (three parameters versus the two parameters of the conical model) could not overcome the large gap in $RMSE_{AB}$. The inability of the conical-cylindrical trial profile to match the conical reference conductances arises from its limiting behavior as $l \rightarrow 0$: the uncoated pore profile reduces to a conical profile, but the coated profile remains conical-cylindrical. Nevertheless, the optimized values of the conical-cylindrical profiles indicated strong conical character: limiting radii essentially matching conical reference limiting radii, and values of l nearing zero (not shown).

Four-point optimizations of hyperbolic and conical-cylindrical reference nanopores similarly allowed the correct determination of the reference nanopore shapes and their geometry parameters. A particularly interesting case of the ability of the four-point optimization to correctly determine the shape of reference nanopores with three free parameters or less occurred when using a cylindrical reference nanopore. All of the trial profiles listed in Table 1 and Supplemental Table S1 will reduce to a cylinder as a limiting case. It is therefore possible to fit a cylindrical reference pore with a conical-

cylindrical profile, for example, by satisfying either $R = r_0$, and $l = L$. It is necessary, therefore, to examine not only the $RMSE_{AB}$ or $RMSE_G$ for a particular trial profile, but also the resulting best-fit geometry parameters that could indicate a cylindrical reference nanopore even when using a conical-cylindrical trial, for example.

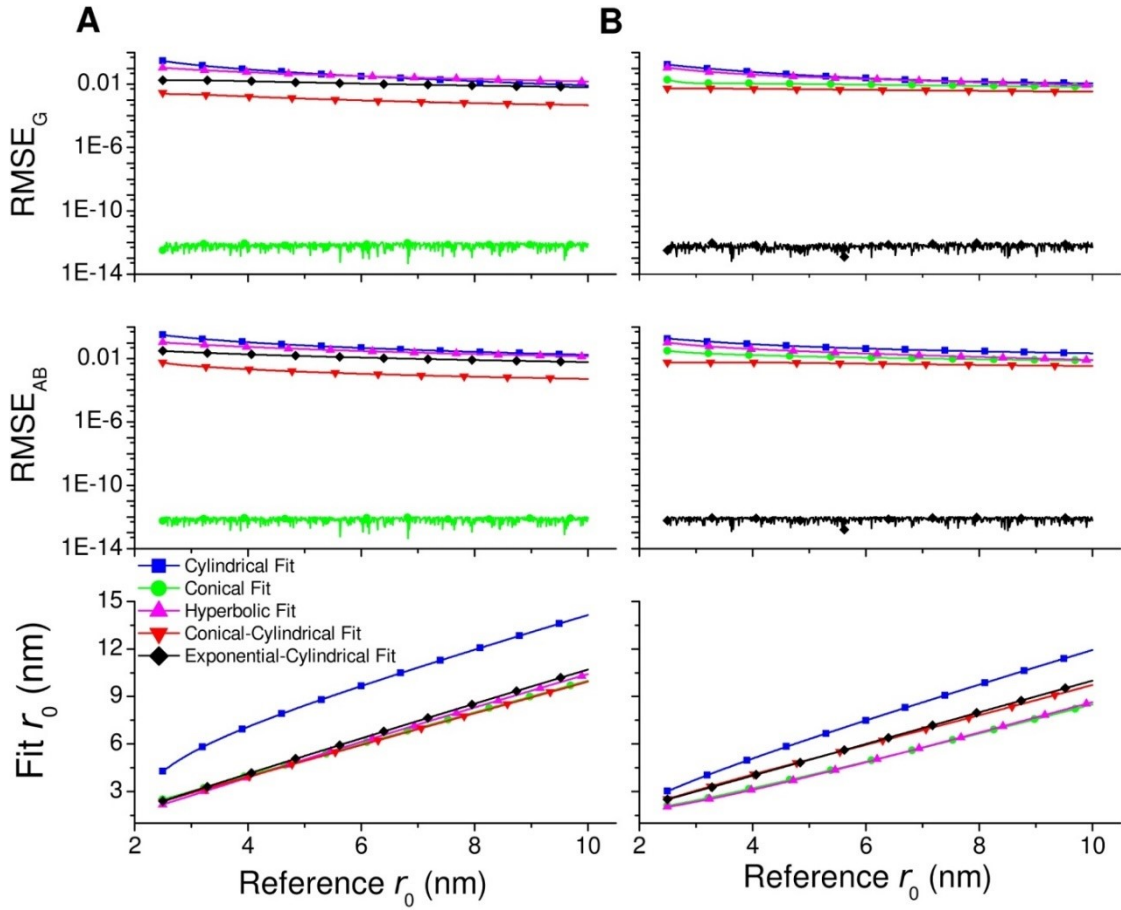


Figure 2. 2 (A) The electrolyte-dependence of the conductance of uncoated and amine-surface-decorated exponential-cylindrical nanopores ($r_{0,ref}$, $l = 11\text{nm}$, and $b = 0.19\text{nm}^{-1}$, $\delta = 1.7\text{nm}$) was used to geometry optimize, with fixed $\delta = 1.7\text{nm}$, the nanopore profiles in Table 1 using Equation 12. Upper panels denote the error in

the conductance calculated after optimization using RMSE_{AB} , shown in the middle panel. The lower panels denote the final limiting radius of the pore for each trial profile. (B) The reference nanopore was conical with $R_{\text{ref}} = r_{0,\text{ref}} + 10\text{nm}$, and also had $\delta = 1.7\text{nm}$.

The trial nanopore profiles span a range of experimentally representative nanopore shapes and, with a maximum of only three free geometry parameters, can nevertheless reproduce experimental conductance measurements^{10, 27}. The ease with which RMSE_{AB} and RMSE_{G} , when coupled with examination of the resulting best-fit parameters, determined the optimal radial profiles with fixed- δ hinged on the number of free parameters in the trial shapes compared to the degrees of freedom delivered by the functional form of the conductance. The four-point method should also be able to uniquely geometry-optimize four-parameter models, thereby allowing the nanopore surface coating thickness, δ , to be an additional free parameter of the optimization. Figure 2.3 shows the outcome of these free- δ geometry optimizations for an exponential-cylindrical reference nanopore. The RMSE metrics excluded the cylindrical, conical and hyperbolic trial profiles, identical to the behavior seen for the fixed- δ exponential-cylindrical reference nanopores characterization. The fit quality of exponential-cylindrical and conical-cylindrical profiles to the reference conductances, however, could not be distinguished on the basis of the RMSE metrics. In the four-point framework, the optimization of profiles with four free parameters is no longer overdetermined by the available conductance data, and such ambiguity can emerge. A conical profile artificially given four free parameters (r_0 , R , δ and the membrane thickness, L), for example, could also fit the conductance data with similarly low RMSE

values. Compared to prior two-point work in which the conductance could not distinguish between a variety of reasonable two-parameter nanopore profiles, however, the current uncertainty is rather benign and can be compensated for by judicious choice of trial profiles, careful examination of the optimized parameters or additional information²⁷. Best-fit parameters r_0 , l , and b from optimization of the exponential-cylindrical trial profiles exactly matched the reference native pore parameters, and the optimizations also yielded the correct surface coating thickness, δ . The radii of the conical-cylindrical pores were an excellent match to the reference radii, but the inner cylinder lengths, l , could be as much as 8nm larger than the 11nm reference value. The conical-cylindrical best-fit δ in Figure 2.3 consistently underestimated the 1.7nm reference value, but not unreasonably so. In general, though, the use of well-defined surface functionalization moieties allows the optimized values of δ to be used as an independent check on the nanopore conductance characterization. The best-fit δ values for the hyperbolic profiles, for example, exceeded the possible length of the monolayer, and can therefore be ruled out or, possibly, could motivate independent additional characterization of the surface decoration.

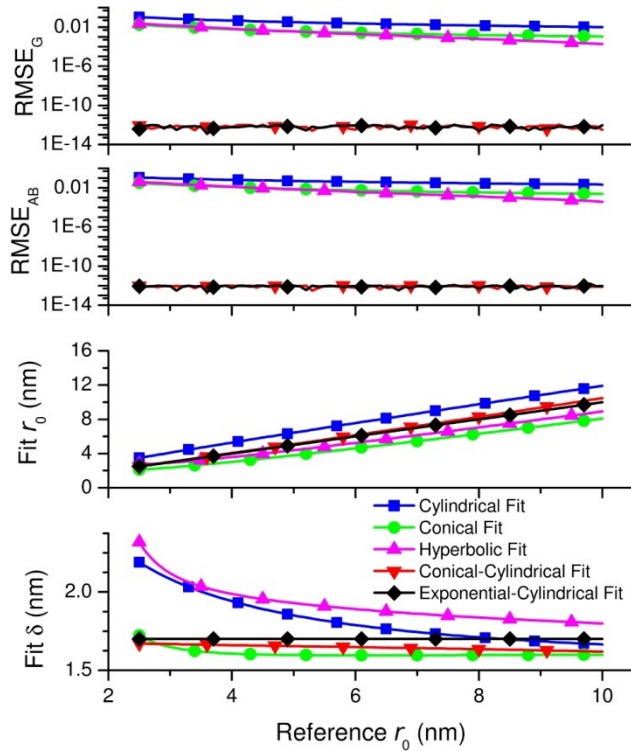


Figure 2. 3. The geometry optimizations of exponential-cylindrical reference nanopores in Figure 2.2 were repeated, but with δ as a free parameter of the optimization. The top three panels show the metrics and best-fit radii, while the bottom panel shows the corresponding values of the surface coating thickness, δ .

CONCLUSIONS

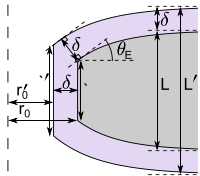
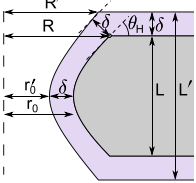
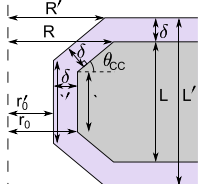
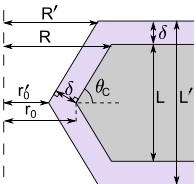
Surface-coated nanopores are receiving increasing attention for the ability of surface coatings to tune nanopore dimensions and surface chemistry, and to confer powerful performance capabilities on a host of nanopore single molecule sensing and manipulation schemes. Knowledge of a nanopore's size, shape and surface chemistry thus bears on nanopore creation, modification and application. While nanopore conductance is governed by the nanopore geometry and surface chemistry in concert with experimental parameters such as electrolyte composition and temperature, careful

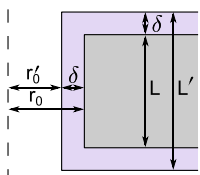
design is necessary if the measured conductance is to be used to reveal the underlying nanopore properties. The use of experimentally realistic trial nanopore profiles, coupled with consideration of the resulting best-fit parameters in the context of nanopore fabrication and surface functionalization details, is naturally essential to the success of this method. This is especially true when optimizing models with the full four degrees of freedom permitted by the method. The geometry optimization results were achieved using an experimentally-supported nanopore conductance model¹⁰⁻¹¹ that allows the effects of nanopore geometry on the conductance to be clearly separated from the effects of surface chemistry. In this context, the conclusions drawn regarding the quality of the geometry optimization results presented here are general and, so long as the surface modification changes the nanopore dimensions, are not restricted to a particular choice of surface chemical modification.

The four-point conductance framework introduced here was able to correctly identify nanopore shapes and to determine the correct magnitudes of all key geometry descriptors of realistic nanopores with greater structural complexity than had previously been possible by conductance, alone. This capability included the complete characterization of an elegant, experimentally-determined nanopore profile representative of TEM-manufactured nanopores¹⁰ without requiring constraint of its parameters²⁷. The performance capabilities thus dramatically exceed those of the more usual single-point conductance approach based on a cylindrical nanopore approximation, and of the more sophisticated two-point conductance approaches. Beyond recovering the native nanopore structure, the four-point method was able to also probe the thickness of the surface coating, δ . With the use of approaches that yield well-

defined surface coatings, the best-fit values for the coating thickness emerge as an additional metric for evaluating the conductance-based nanopore characterization. Straightforward measurements of the electrolyte-concentration-dependent conductance of nanopores can thus serve as a simple yet powerful foothold for peering into these bioinspired nanoscale environments.

Table 2. 1. Listing of nanopore radial profiles with the corresponding transformation of nanopore parameters after coating with a monolayer of thickness δ

<p>Exponential-cylindrical</p> 	<p>Hyperbolic</p> 	<p>Exponential-cylindrical</p> $r'_0 = r_0 - \delta$ $b' = \frac{2 \tan \theta_E}{L - l + 2\delta(1 - \cos \theta_E)}$ $l' = L + 2\delta - (L - l + 2\delta(1 - \cos \theta_E)) \exp\left\{\frac{2\delta \tan \theta_E (1 - \sin \theta_E)}{L - l + 2\delta(1 - \cos \theta_E)}\right\}$ $\tan \theta_E = \frac{L - l}{2} b$
<p>Conical Cylindrical</p> 	<p>Conical</p> 	<p>Hyperbolic</p> $r'_0 = r_0 - \delta$ $(R')^2 = (r_0 - \delta)^2 + \left(\frac{L + 2\delta}{L + 2\delta \cos \theta_H}\right)^2 [(R - \delta \sin \theta_H)^2 - (r_0 - \delta)^2]$ $\tan \theta_H = \frac{RL/2}{R^2 - r_0^2}$

<p>Cylindrical</p> 	All	Conical Cylindrical	Conical
	$L' = L + 2\delta$ Cylindrical $r'_0 = r_0 - \delta$	$r'_0 = r_0 - \delta$ $R' = R + \delta(\cot \theta_{CC} - \csc \theta_{CC})$ $l' = l + 2\delta(\sec \theta_{CC} - \tan \theta_{CC})$ $\tan \theta_{CC} = \frac{(L - l)/2}{R - r_0}$	$r'_0 = r_0 - \delta$ $R' = R + \delta(\cot \theta_C - \csc \theta_C)$ $\tan \theta_C = \frac{L/2}{R - r_0}$

Supporting Information. Table S1 listing nanopore radial profiles with the corresponding volume (A) and surface (B) integrals. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS

TEM, transmission electron microscope; ref, reference; eff, effective.

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CHAPTER 3: PREFACE

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REAL-TIME PROFILING OF SOLID-STATE NANOPORES DURING SOLUTION-PHASE NANOFABRICATION

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CHAPTER 3: REAL-TIME PROFILING OF SOLID-STATE NANOPORES DURING SOLUTION-PHASE NANOFABRICATION

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KEYWORDS: Nanopore; dielectric breakdown; electroless plating; nanopore conductance; silicon nitride nanopore; nanopore size; nanopore radius.

ABSTRACT

We describe a method for simply characterizing the size and shape of a nanopore during solution-based fabrication and surface modification, using only low-overhead approaches native to conventional nanopore measurements. Solution-based nanopore fabrication methods are democratizing nanopore science by supplanting the traditional use of charged-particle microscopes for fabrication, but nanopore profiling has customarily depended on microscopic examination. Our approach exploits the dependence of nanopore conductance in solution on nanopore size, shape, and surface chemistry in order to characterize nanopores. Measurements of the changing nanopore conductance during formation by etching or deposition can be analyzed using our method to characterize the nascent nanopore size and shape—beyond the typical cylindrical approximation—in real-time. Our approach thus accords with ongoing efforts to broaden the accessibility of nanopore science from fabrication through use: it is compatible with conventional instrumentation and offers straightforward nanoscale characterization of the core tool of the field.

INTRODUCTION

A nanopore is a nanofluidic channel, with dimensions in all directions generally less than 100 nm, that can be used to deliver a host of capabilities for single-molecule sensing.¹⁻¹⁰ High-profile nanopore sensing efforts have targeted sequencing single strands of DNA and RNA; protein conformational analysis; and characterization of other biomolecules, molecular complexes, and nanoparticles. In the most straightforward implementation of nanopore sensing, the nanopore is the sole path connecting two reservoirs containing electrolyte solutions. Electrodes in each reservoir establish a potential difference across the nanopore that drives ions through the nanopore: passage of a target molecule, nanoparticle, or complex through the nanopore perturbs that ionic current and provides molecular-level information. That information naturally depends on the target's dimensions and physicochemical properties and the ionic solution composition, but it is also profoundly affected by the size, shape, and surface chemistry of the nanopore. In the case of a (cylinder-like) double-stranded DNA polymer that fills the entire length of a cylindrical nanopore as it transits through, a simple geometric treatment considering only the displacement of bulk ions by the polymer gives a straightforward expression for the macromolecule-induced conductance change¹¹

$$\chi_B \equiv \frac{\langle G \rangle - \langle G_b \rangle}{\langle G \rangle} \cong \left(\frac{r_{\text{DNA}}}{r_0} \right)^2 \quad (1)$$

with $\langle G \rangle$ and $\langle G_b \rangle$ the time-averaged conductance through an unobstructed and DNA-containing nanopore, respectively, and r_{DNA} and r_0 the cross-sectional radii of the molecule and nanopore. The expression does not capture the panoply of complex

phenomena giving rise to conductance perturbations in nanopore sensing,¹²⁻¹³ but does, in convenient closed form, appropriately underscore the importance of nanopore dimension. This geometric basis of the conductance change has been used to infer biopolymer conformation, for example: a folded-over polymer presents a larger effective cross-section than a linear one.¹⁴ The more elusive dependence of current change on single-stranded DNA base sequence, for example, underpins efforts to sequence single strands of DNA using nanopores.^{2, 8} In a powerful implementation of nanopore force spectroscopy, details of interaction energetics can be revealed if, and only if, a nanopore size is properly engineered to sterically force the linearization of a folded moiety during passage, or rupture of an intermolecular complex by barring passage of one of the partners.¹⁵⁻¹⁷

The ionic conductance (G), alone, of a nanopore with a charged surface can be expressed as the sum of a bulk and surface conductance term¹⁸⁻²¹

$$G = G_{\text{bulk}} + G_{\text{surface}} = K \cdot A + \mu|\sigma| \cdot B \quad (2)$$

when access resistance is negligible.²² Overlapping Debye layers require a more sophisticated treatment, but need not be considered over a broad useful range of nanopore sizes and solution ionic strengths.²³⁻²⁴ This simple formulation for G has been supported by experimental measurements in which nanopore conductance was measured for nanopores that had size and shape interrogated by combinations of transmission electron microscopy and electron energy loss spectroscopy.^{13, 18} The bulk conductance is determined by the solution conductivity, K , and a volume integral, A ,

over the unique nanopore shape: $G_{\text{bulk}} = K \left(\int \frac{dz}{\pi(r(z))^2} \right)^{-1} = K \cdot A$ (with z-axis along

the length of the pore). The surface conductance is determined by the mobility of counterions proximal to the pore surface, μ , the density of surface chargeable groups, σ , and an integral, B , over the surface of the nanopore: $G_{\text{surface}} = \mu|\sigma| \left(\int \frac{dz}{2\pi r(z)} \right)^{-1} = \mu|\sigma| \cdot B$. The two defined quantities A and B therefore contain information about the size and shape of the nanopore, determined by the collection of geometric parameters, q_j , relevant for a particular shape: $A = A(\{q_j(t)\})$ and $B = B(\{q_j(t)\})$. Nanopore materials are usually chosen with mechanical and physicochemical properties to minimize the change in size and shape in time, t , absent deliberate action. Commonly reported parameter values, which may be only a subset of those needed to fully characterize a given nanopore profile, include the limiting radius (the minimum radius along the profile), r_0 , and total nanopore length, L , that can in some cases be equated with the supporting membrane thickness. The experimentally-supported^{13, 18} treatment of the nanopore conductance here assumes axially and cylindrically symmetric nanopores in a size regime where access resistance is negligible,²² and that any surface charge emerges from a singly ionizable surface species described by a characteristic pK_a

$$-A - H \rightleftharpoons -A^- + H^+ \quad (3)$$

Native or engineered nanopore surface chemistry is an important element in nanopore performance, and contributor to nanopore conductance. The conductance can be naturally exploited for nanopore characterizations in conjunction with solution-based nanopore fabrication methods, and is especially useful when more complex methods present barriers to use. Charged-particle milling is an established, but challenging and burdensome, approach for formation of the smallest, <10 nm nanopores in thin

membranes.²⁵⁻²⁸ The use of (scanning) transmission electron microscopes ((S)TEM), helium ion microscopes, and scanning electron microscopes (SEM) for fabrication imposes time and instrumentation costs; can expose the nanopore to possible surface contamination within the instrument and to risk of damage during handling, transfer, and charged particle beam exposure; and reveals little of the nanopore surface chemistry. In a purely imaging capacity, these microscopes are limited in their ability to characterize organic surface coatings, and without more involved measurements or image analysis,^{18, 29-34} yield only a nanopore limiting radius—not a fully characterized size and shape. Beyond the greater ease and technical benefits of a low-overhead, solution-based nanopore characterization, such an approach can more directly probe nanopore surface chemistry. The capabilities of solution-based nanopore fabrication make a strong case alone, however, for complementary solution-based characterization methods. The benefits and prospects of solution-based nanopore fabrication were demonstrated early-on in the field through the development and use of track-etched polymer nanopores.⁹ Formation of the etchant-susceptible ion-track requires a large-scale heavy ion accelerator facility which naturally imposes a barrier to widespread use of the fabrication method, although accessibility is improved by the ability to perform the solution-based chemical etching step in a standard chemistry lab well after the ion-track formation. Conformal metal coating of these often tortuous polymer nanopores by (solution-based) electroless plating was a vital development in the use of these polymer nanopores: the material deposition allows the nanopore dimensions to be fine-tuned after chemical etching, and the metal film provides a platform for subsequent chemical modification of the nanopore interior surface. Both etching and deposition steps

developed for polymer membrane nanopores have been extended to silicon nitride membranes which offer benefits such as the fabrication of smooth nanopores with lengths <100 nm.^{32, 35} More recently, dielectric breakdown (followed by voltage-assisted etching) of an impervious, insulating membrane, has emerged as a powerful new technique for nanopore fabrication.³⁶ It is an entirely solution-based approach, using essentially the same equipment required for conductance-based nanopore measurements, and quite readily produces nanopores in a wide range of sizes, including in the coveted <5 nm diameter range. The nanopore conductance can be measured during fabrication, providing an indication of the nanopore size at a given point in time. The dielectric breakdown approach allows nanopores to be fabricated in their native environment, in the same holder where they will be used for experiments, and without the contamination and damage risks associated with charged particle techniques. A conductance-based characterization will not damage a molecular surface coating suitable for conductance-based sensing, and can harness the natural and direct connection to the nanopore surface chemistry that makes it a valuable method for characterizing chemically-tailored nanopores.^{9, 23, 34, 37} The conductance model is equally useful when a pore is formed and enlarged, and when an initially large pore is resized by solution-based deposition, including film growth.^{9, 19, 35, 38} Etching and deposition may be used in concert, with a pore being initially etched larger than desired to accommodate an electroless gold film, for example, that may ease nanopore surface chemical modification. In this work we wanted to understand how the measured conductance during nanopore fabrication—by deliberate expansion, closure, or both in concert—could be used to profile the nascent nanochannel. Simulations will focus, for

expediency, on nanopores fabricated via deposition of surface coatings: the principles, however, are general.

THEORY

The algebraic structure of $G = K \cdot A + \mu|\sigma| \cdot B$, and its underlying dependencies, means that a single-point conductance measurement can provide enough information to size a nanopore only when the shape is known and the fitting involves only a single geometric degree of freedom. Measurement of G versus K —by changing the electrolyte solution conductivity—for a given nanopore can provide greater insight into the nanopore size, shape, and surface chemistry.^{18, 21-23} The conductance change after adding a monolayer of known thickness, for example, can provide similar information to what is provided after a solution conductivity change, and measuring G versus K for the nanopore before and after monolayer formation provides the richest description of the nanopore within this framework.²³ Changes of electrolyte solution are tedious, however, and disruptive to a solution-based nanopore fabrication approach. A simple ongoing measurement of the nanopore conductance during nanopore formation, however, can be done as part of the fabrication process, and is in fact performed routinely on a single-point measurement basis. Each fixed-time conductance is of course connected through Equation (2) to the instantaneous nanopore size and shape, where the applicability of the conductance model has been independently verified by electron-based imaging and spectroscopy.^{13, 18} A single conductance value, however, offers a limited ability to characterize a nanopore described by more than one free geometric parameter. Measurement and use of a series of conductance values at times t_i :

$G(t_0, \{q_j(t_0)\}), G(t_1, \{q_j(t_1)\}), \dots, G(t_n, \{q_j(t_n)\})$, can provide more information than the conductance at a single time-point since the changes in conductance are caused by underlying changes in the initial nanopore dimensions, $\{q_j(t_0)\}$, in time. We perform simulations consistent with the following conditions to demonstrate how to extract this information content. Nanometer-scale deposition or etching should not appreciably change the electrolyte solution conductivity, nor should the nanopore surface chemistry change (except through deliberate action) throughout either type of fabrication process. We make the reasonable assumption that material transfer will be uniform across the surface, so that the nanopore shape will remain unchanged. Silicon nitride, the most common membrane material in which to form nanopores, is amorphous, and so will not inherently be prone to anisotropic etching.³⁹ Electroless plating, a surface deposition method that has been used with great success in resizing nanopores,⁹ conformally coats even rough surfaces,⁴⁰ and film growth by polymer chain extension, for example, should be another effective route to reliably tune nanopore size.⁴¹ We can then write

$$\begin{aligned}
 \frac{dG}{dt} &= K \frac{dA(\{q_j(t)\})}{dt} + \mu|\sigma| \frac{dB(\{q_j(t)\})}{dt} = K \sum_j \left(\frac{\partial A}{\partial q_j} \right) \frac{dq_j}{dt} + \mu|\sigma| \sum_j \left(\frac{\partial B}{\partial q_j} \right) \frac{dq_j}{dt} = \\
 &K \sum_j f(\{q_j\}, \mathbf{v}_{mt}, t) + \mu|\sigma| \sum_j g(\{q_j\}, \mathbf{v}_{mt}, t)
 \end{aligned} \tag{4}$$

where the $\left(\frac{\partial A}{\partial q_j} \right)$ and $\left(\frac{\partial B}{\partial q_j} \right)$ depend on the nanopore profile, and the $\frac{dq_j}{dt}$ depend on the profile and the material transfer rate, \mathbf{v}_{mt} , whether by nanopore etching or coating by deposition. The material transfer rate is conveniently measured as the change in nanopore radius over time. While two nanopores with different shapes and sizes may have the same initial conductance, $\mathbf{G}(\mathbf{t}_0, \{q_j(\mathbf{t}_0)\}) = \mathbf{G}(\mathbf{t}_0, \{q'_j(\mathbf{t}_0)\})$, the rates of change

of the conductances will be different, and determined by the individual nanopore sizes and shapes (and identical material transfer rates). Measurement of several values of the experimental $G(t_i, \{q_j(t_i)\})$ can use this dependence to enhance real-time conductance-based nanopore characterization during fabrication. To present concrete examples of the general framework, we selected four representative nanopore profiles: cylindrical, double-conical, conical-cylindrical, and hyperbolic (Figure 3.1).^{18, 21-22, 29, 32} For all profiles, we limited the $\{q_j\}$ to two free parameters per shape: (r_0, L) —the limiting (minimum) radius and total nanopore length (see Tables S-1 and S-2 for notation and equations). Independent experimental studies of nanopore profiles^{18, 22} were used to guide the constraints and to make reasonable parameter value assignments to allow for numerical examples; the nanopore characterization method is general, however, and does not depend upon these particular numerical values.^{21, 23} We restricted the initial outer radius to be 10 nm greater than the initial limiting radius (not applicable to the cylindrical profile),²¹⁻²² and fixed the initial cylinder length of the conical-cylindrical pore to be 0.6 times its initial total length. The deposited coating was piecewise curved to maintain a uniform coating thickness across the entire nanopore surface (Figure 3.1 and Table S-2). Equation (4) then becomes

$$\frac{dG}{dt} = K \left(\left(\frac{\partial A}{\partial r_0} \right) \frac{dr_0}{dt} + \left(\frac{\partial A}{\partial L} \right) \frac{dL}{dt} \right) + \mu |\sigma| \left(\left(\frac{\partial B}{\partial r_0} \right) \frac{dr_0}{dt} + \left(\frac{\partial B}{\partial L} \right) \frac{dL}{dt} \right) = v_{mt} \left[K \left(\left(\frac{\partial A}{\partial r_0} \right) + 2 \left(\frac{\partial A}{\partial L} \right) \right) + \mu |\sigma| \left(\left(\frac{\partial B}{\partial r_0} \right) + 2 \left(\frac{\partial B}{\partial L} \right) \right) \right] \quad (5)$$

Parameter values used in calculations were typical of experiments and consistent with those in prior work with silicon nitride nanopores:²¹ for example, 1 M potassium

chloride electrolyte solution in water, $K=14.95 \text{ S}\cdot\text{m}^{-1}$ (calculated using ion mobilities), $\text{pH}=7.0$, and surface $\text{pK}_a=7.9$. The material transfer rate was kept constant, $\mathbf{v}_{mt} = dr_0/dt = 0.6 \text{ nm/h}$. More important than the particular parameter values, though, it is the form of equation (2) and its functional dependencies that are significant in this work.

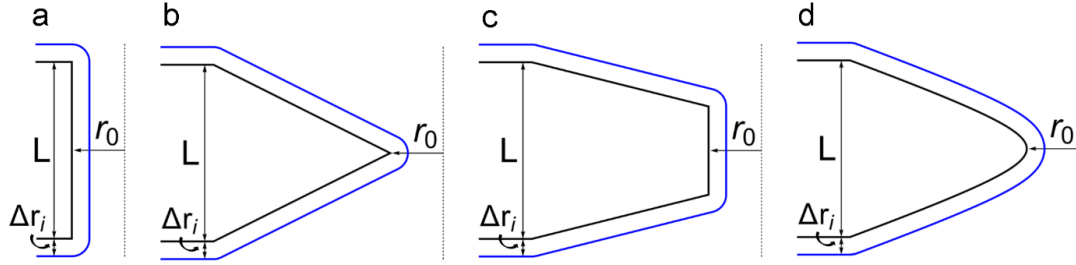


Figure 3. 1. (a) Cylindrical, (b) double-conical, (c) conical-cylindrical, and (d) hyperbolic nanopore half-profile cross-sections cylindrically symmetric about the vertical z -axis (dotted line) of the pore. Profiles are shown before (black line) and after (blue line) material deposition to decrease the limiting nanopore radius, r_0 , by an amount Δr_i determined by the deposition time and material transfer rate.

RESULTS AND DISCUSSION

The ability to characterize a nanopore in real-time, during its formation, using only its conductance, is an incredibly compelling goal. Its pursuit relies on the connection between the conductance of a nanopore and its size, shape, and surface chemistry, and its attainment hinges on properly exploiting the functional form of that connection. We will focus on nanopores fabricated by deposition of a coating onto the outer membrane surface and inner surface of an existing, larger pore, but similar arguments hold for a nanopore formed by etching of a smaller pore to create a larger pore. Figure 3.2 highlights a primary challenge of nanopore conductance-based

characterizations. The curves show the set of nanopore limiting radii and length, for each chosen nanopore shape, $\{r_{0,\text{shape}}, L_{\text{shape}}\}$, that generate a 200 nS conductance: there is not a unique solution. To use a single-point conductance value to characterize a nanopore by more than a broad range of possible shapes and sizes, or to provide better than an approximate size given an assumed profile, additional information is required.^{21,}
²³ Most commonly, knowledge of the particular fabrication method and conditions is used to choose an expected nanopore profile, and can often be used to constrain the nanopore length to an experimental parameter such as the thickness of the membrane in which it is formed. Measurement of the conductance of a nanopore in time, in an essentially single-point sense, has demonstrated utility as a monitor of nanopore evolution even if it cannot provide an unambiguous characterization. Yet the time-dependence provides a set of experimental data points that we seek to mine to more fully characterize the nanopore than is possible using a single-point measurement of the conductance

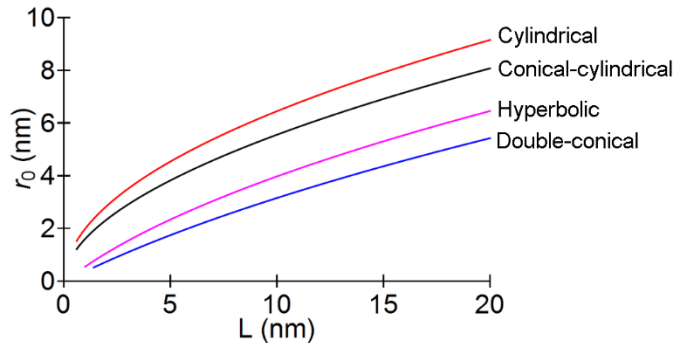


Figure 3. 2. The plotted lines denote the pairings of limiting nanopore radius, r_0 , and nanopore length, L , for each nanopore profile, that will produce a 200 nS conductance.

The most immediately striking consequence of a real-time measurement of the conductance is that, as shown in Figure 3.3, it reveals a clear distinction between different nanopore profiles. When different candidate profiles are used to fit experimental nanopore conductance data, the conductance versus time provides a means to determine nanopore shape and size. To produce the data plotted in Figure 3.3, we used the four representative nanopore profiles all with an initial 200 nS conductance and 10 nm total nanopore length. The initial nanopore limiting radii were ~6.4, 3.1, 5.5, and 4.0 nm, respectively, for the cylindrical, double-conical, conical-cylindrical, and hyperbolic nanopore profiles. We calculated the conductance for each profile as the radii were reduced at the same rate, $\mathbf{v}_{mt} = \mathbf{0.6}$ nm/h, during a simulated, deposition-based fabrication process. As shown below, the radius change after a given time must be known, but the method does not require a constant material transfer rate. We chose a constant rate, commonly observed in micromachining processing,³⁹ however, because it affords straightforward insights into the functional dependencies beyond what is revealed by the numerical results. Given the form of equation (5), it is perhaps unsurprising that even with constant \mathbf{v}_{mt} (and therefore identical absolute rates of change of the radii across profile type), $\frac{dG}{dt}$ is not linear and depends on profile type (inset of Figure 3.3). The quantitative details of this behavior provide a means of extracting nanopore size and shape information from the measured conductance changes. Figure S-3.2 reinforces the geometrical underpinnings of this profiling method, in plots of the geometry integrals, A and B , versus time.

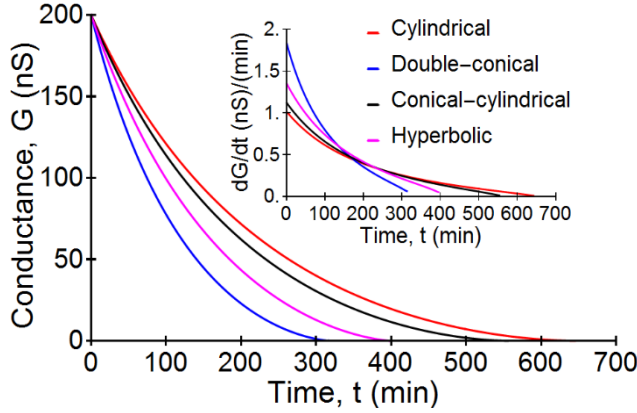


Figure 3. 3. Nanopores with an initial 200 nS conductance ($L(t_0) = 10$ nm, $r_0(t_0)$ from Figure 3.2) show a shape-dependent decrease in conductance due to material deposition at a constant rate, v_{mt} . The inset plots the rate of conductance change, calculated using nearest-neighbor differences, $\frac{dG}{dt} \cong \frac{G(t_{i+1}) - G(t_i)}{t_{i+1} - t_i}$.

Figure 3.4 illustrates the general approach we have adopted for extracting quantitative nanopore geometric parameters from $G(t)$ —an approach allowing for a nanopore characterization with the full geometric parameter flexibility outlined in Figure 3.2, and that emphasizes the minimal number of conductance values required. We chose to simulate the deposition-based fabrication of nanopores with an initial conductance, $G_{\text{shape}}^{\text{expt}}(t_0) = 200$ nS, and initial radius, $r_{0,\text{shape}}^{\text{expt}}(t_0) = 3.5$ nm (both values the same for all simulated experimental shapes); Figure 3.2 gives the corresponding initial nanopore lengths, $L_{\text{shape}}^{\text{expt}}(t_0)$, for each nanopore profile. For each nanopore profile, we set the initial nanopore size, $\left(r_{0,\text{shape}}^{\text{expt}}(t_0), L_{\text{shape}}^{\text{expt}}(t_0)\right)$, and used the progression of dimensions, $\left(r_{0,\text{shape}}^{\text{expt}}(t_0) - \Delta r_i(t_0, t_i), L_{\text{shape}}^{\text{expt}}(t_0) + 2\Delta r_i(t_0, t_i)\right)$, to simulate the post-deposition conductances $G_{\text{shape}}^{\text{expt}}(t_1)$ and $G_{\text{shape}}^{\text{expt}}(t_2)$. For a constant

material transfer rate, v_{mt} , $\Delta r_i = (t_i - t_0)v_{mt}$. While more generally $\Delta r_i = \Delta r_i(t_i, t_0, v_{mt}(t))$, the procedure implemented here relies on knowledge of this radius change only, not whether the material transfer rate is constant in time or not. We outline the conceptual framework for the characterization and provide a detailed step-by-step tutorial in the SI. The initial conductance, $G_{\text{shape}}^{\text{expt}}(t_0)$, was used in conjunction with Figure 3.2 to establish the set of candidate $\{(r_{0,\text{shape}}(t_0), L_{\text{shape}}(t_0))\}$, for each nanopore profile, whose members all have the initial conductance $G_{\text{shape}}(t_0) = G_{\text{shape}}^{\text{expt}}(t_0)$. The range of candidate sizes, for each candidate shape, is represented by the dotted lines in Figure 3.4a-d. Given $G_{\text{shape}}^{\text{expt}}(t_0)$, alone, neither size nor shape can yet be determined. Each of these possible candidate geometries (size and shape) was then modified by the deposition of material to provide sets of nanopore dimensions given by $\{(r_{0,\text{shape}}(t_0) - \Delta r_i, L_{\text{shape}}(t_0) + 2\Delta r_i)\}$ for times t_1 , t_2 , and t_3 , with corresponding sets of conductances $\{G_{\text{shape}}(t_1)\}$, $\{G_{\text{shape}}(t_2)\}$, and $\{G_{\text{shape}}(t_1)\}$ (solid curves in Figure 3.4a-d). We then used the post-deposition $G_{\text{shape}}^{\text{expt}}(t_i)$ to determine the nanopore size and shape. We found the initial limiting radius, $r_{0,\text{shape}}(t_0)$, for each nanopore shape, that gave a conductance $G_{\text{shape}}(t_1) = G_{\text{shape}}^{\text{expt}}(t_1)$. That is, when the experimental nanopore was cylindrical, we found the $r_{0,\text{shape}}(t_0)$ for cylindrical, double-conical, conical-cylindrical, and hyperbolic profiles that allowed the candidate pore conductance to match the experimental value, and plotted the radii in Figure 3.4e. Figure 3.4f-h are plots of the $r_{0,\text{shape}}(t_0)$ when the conductances of double-conical, conical-cylindrical, and hyperbolic experimental nanopores were equated to the conductances of the same

four candidate shapes. No matter the experimental profile, after two conductance values, all four candidate shapes—with different sizes—were equally viable conductance-based matches. By repeating this process by finding $r_{0,\text{shape}}(t_0)$ to satisfy $G_{\text{shape}}(t_2) = G_{\text{shape}}^{\text{expt}}(t_2)$, the experimental nanopore size and shape both emerge. When the candidate nanopore profile matches the simulated experimental profile, all extracted $r_{0,\text{shape}}(t_0)$ have the same value for all t_i , which essentially delivers a simultaneous solution of $G_{\text{shape}}(t_i, \{q_j(t_i)\}) = G_{\text{shape}}^{\text{expt}}(t_i, \{q_j(t_i)\})$ for all time-points. The curves in Figure 3.4e-h illustrate this successful characterization; the agreement is shown in terms of $r_{0,\text{shape}}(t_0)$, but $L_{\text{shape}}(t_0)$ has the same behavior. Figure 3.4e plots the $r_{0,\text{shape}}(t_0)$ when the simulated $G_{\text{cylindrical}}^{\text{expt}}(t_i)$ values were fit using cylindrical, double-conical, conical-cylindrical, and hyperbolic profiles: only the cylindrical candidate nanopore returns the same $r_{0,\text{shape}}(t_0)$ for different t_i . Figures 3.4f-h show, by the constancy of the correct $r_{0,\text{shape}}(t_0)$, the same successful capture of size and shape of double-conical, conical-cylindrical, and hyperbolic simulated experimental nanopores, respectively. Measurement of more conductance points does not provide more information, given the framework presented here, but can add numerical robustness to this approach. Alternatively, the formal need for only three conductance values allows one to piecewise repeat the shape-and size-profiling on independent sets of three conductance values throughout the duration of the fabrication, allowing for the possibility to extend this method to anisotropically-etching or -depositing materials. An extreme departure from the usual progression of conductance in time may signal the need for a more involved steady-state solution-based characterization of a pore after fabrication,²¹

although even in this case the present time-dependent method should provide bounds on the evolving nanopore size. We note again, for generality, that while we used a constant ν_{mt} , the plating rate must be known, but need not be constant. Fitting conductance values in time leverages the form of equation (2) to reveal the nanopore shape and extract dimensions from a solution-based nanopore fabrication method.

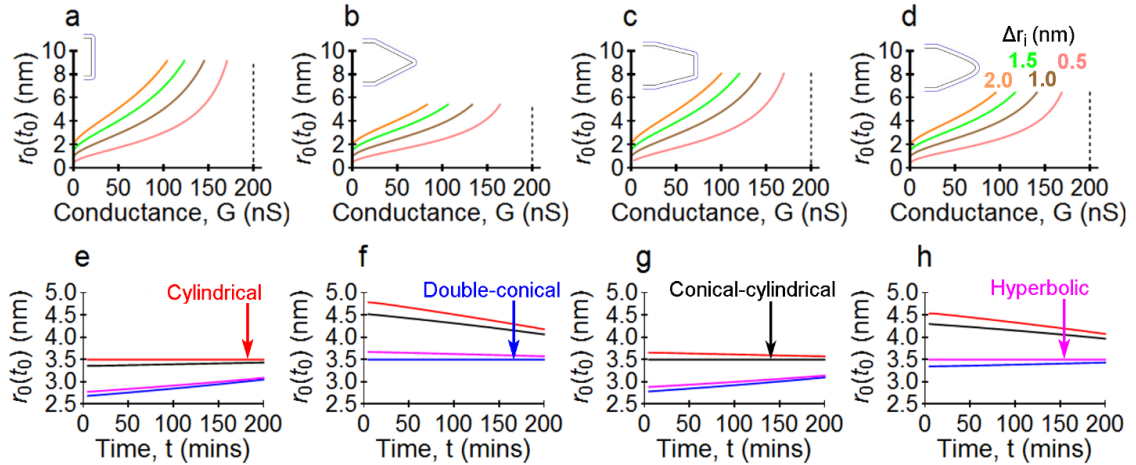


Figure 3. 4. The conductance of initially 200 nS (a) cylindrical, (b) double-conical, (c) conical-cylindrical, and (d) hyperbolic nanopores can be satisfied by a range of radii (dotted vertical lines). Fixed decreases of each possible radius (in time) generate characteristic conductance progressions that depend on the nanopore shape and initial size (conductance curves labelled with their particular Δr_i). Simulated experimental conductance data versus time for $G_{\text{shape}}^{\text{expt}}(t_0) = 200$ nS, $r_{0,\text{shape}}(t_0) = 3.5$ nm pores of each shape were compared to the plots in (a-d) to reveal the (e) cylindrical (red), (f) double-conical (blue), (g) conical-cylindrical (black), and (h) hyperbolic (magenta) experimental nanopore size and shapes by the constancy of the fitting $r_{0,\text{shape}}(t_0)$. The relevant experimental profiles for each column are inset in the top row.

CONCLUSIONS

The charged-particle, complex instrumentation approaches that dominated early nanopore fabrication methods allowed, in principle, for high-resolution nanopore characterizations, although such capability was rarely employed beyond determining a limiting radius. These instrumental approaches face limitations such as high likelihood of surface contamination and inability to probe soft (e.g. organic) nanopore coatings, and they add workflow steps that could be costly in time and instrumentation. Even so, since the nanopores were formed in these instruments, it was expedient to follow fabrication with the chosen degree of characterization in the same instrument. The ongoing development of completely solution-based methods—including the advent of new techniques—to fabricate nanopores has ushered in an exciting new area for nanofluidics, generally, and nanopore science in particular. Nanopores can now be formed in their native liquid environment, and without the instrument and workflow cost of charged-particle methods. We have modelled the nanopore conductance with a simple framework that nevertheless includes an explicit surface chemistry term and has demonstrated concordance with independent experimental characterizations of nanopore sizes and shapes of most importance for routine use in single molecule science.^{13, 18} We have presented theoretical examples that describe the creation of small nanopores by coating larger nanopores, so that fabrication involves a decrease in the nanopore radius and conductance. The results, however, are equally applicable to nanopore fabrication methods such as dielectric breakdown followed by voltage-assisted etching, or the chemical etching of ion-tracked membranes. The nanopore conductance is routinely measured during dielectric breakdown as a diagnostic, and

such a measurement can be readily implemented during nanopore fabrication by material deposition. We have shown here that by analyzing a series of conductance measurements in time, rather than only an instantaneous measurement, we are able to extract information on nanopore size and shape, and thereby enrich the execution and interpretation of nanopore experiments without increasing the experimental burden.

ASSOCIATED CONTENT

Supporting Information. Detailed descriptions of nanopore profiles and a step-by-step tutorial detailing the numerical nanopore characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS: min., minutes; h, hours.

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CHAPTER 4: PREFACE

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CHAPTER 4: CONDUCTANCE-BASED PROFILING OF NANOPORES: ACCOMMODATING FABRICATION IRREGULARITIES

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CHAPTER 4: CONDUCTANCE-BASED PROFILING OF NANOPORES: ACCOMMODATING FABRICATION IRREGULARITIES

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KEYWORDS: dielectric breakdown; nanopore; nanopore conductance; nanopore defect; pore density; silicon nitride nanopore.

ABBREVIATIONS: TEM-transmission electron microscopy; STEM-scanning transmission electron microscopy; EM-electron microscopy; MPVI-multilevel pulse-voltage injection

ABSTRACT

Solid-state nanopores are nanoscale channels through otherwise impermeable membranes. Single molecules or particles can be passed through electrolyte-filled nanopores by, e.g. electrophoresis, and then detected through the resulting physical displacement of ions within the nanopore. Nanopore size, shape, and surface chemistry must be carefully controlled, and on extremely challenging <10 nm-length scales. We previously developed a framework to characterize nanopores from the time-dependent changes in their conductance as they are being formed through solution-phase nanofabrication processes with the appeal of ease and accessibility. We revisited this

simulation work, confirmed the suitability of the basic conductance equation using the results of a time-dependent experimental conductance measurement during nanopore fabrication by Yanagi *et al.*, and then deliberately relaxed the model constraints to allow for (1) the presence of defects; and (2) the formation of two small pores instead of one larger one. Our simulations demonstrated that the time-dependent conductance formalism supports the detection and characterization of defects, as well as the determination of pore number, but with implementation performance depending on the measurement context and results. In some cases, the ability to discriminate numerically between the correct and incorrect nanopore profiles was slight, but with accompanying differences in candidate nanopore dimensions that could yield to post-fabrication conductance profiling, or be used as convenient uncertainty bounds. Time-dependent nanopore conductance thus offers insight into nanopore structure and function, even in the presence of fabrication defects.

INTRODUCTION

Nanopores are a rising tool for single-molecule science, featuring prominently in DNA sequencing efforts, but with broader reach into biophysics, and bioanalytical and materials chemistry.[1-12] The nanopore heart of these techniques is a nanofluidic channel generally less than 100 nm in all dimensions, formed through a membrane or support, with the particular dimensions dictated by the analyte and method. The essential determinants of nanopore performance include the elements of three general nanopore-specific parameter groupings: nanopore size, shape, and surface chemistry.[13-19] Even the most basic nanopore operating configuration illustrates the

importance of these parameters, and also provides a means for assaying them. A nanopore is positioned as the sole fluid path between two wells of electrolyte solution. Application of suitable voltages, typically ≤ 200 mV, across the impermeable support membrane drives ion passage through the nanopore. The resulting open-pore ionic conductance, G , is determined by the bulk solution conductivity, K , by the size and shape of the nanopore (here captured in volume and surface integrals, $A = \left(\int \frac{dz}{\pi(r(z))^2}\right)^{-1}$ and $B = \left(\int \frac{dz}{2\pi r(z)}\right)^{-1}$, respectively), and by properties of the nanopore-solution interface[13, 16, 18, 20-23]

$$G = K \cdot A(r, L) + \mu|\sigma| \cdot B(r, L) = G_{\text{bulk}} + G_{\text{surface}} \quad (1)$$

where σ is the nanopore surface charge density that attract counterions of mobility, μ . The pore has a radius, $r(z)$, that can vary along length, L , of the pore (aligned with the z -axis as shown in Figure S4.1). More complex theoretical approaches exist—a formulation including the access resistance term (neglected here for simplicity) is discussed in the supporting information (see Equation S1, Figure S4.2 and associated discussion)—but this straightforward conductance model provides a tractable and useful framework with good agreement with the measured conductance of nanopores across a range of experimentally determined sizes and shapes.[13, 16, 18, 20, 21, 24] As a species of interest passes through the nanopore, or is entrained therein, it perturbs the open-pore flow of ions, and frequently generates an analyte-specific current blockage (or enhancement)[4, 10, 13, 17, 23]. A simple analytical model for the conductance blockage wrought by the extension of an analyte such as DNA, of radius r_{analyte} , through

the length of a uniformly cylindrical nanopore of radius r_0 , illustrates more directly the importance of nanopore dimensions:

$$\chi_B \equiv \frac{\langle G \rangle - \langle G_b \rangle}{\langle G \rangle} \cong \left(\frac{r_{\text{analyte}}}{r_0} \right)^2 \quad (2)$$

with $\langle G \rangle$ and $\langle G_b \rangle$ the time-averaged conductances of open, and analyte-filled, nanopore.[25] The more complex set of phenomena and parameters underpinning the current blockage explains the experimentally demonstrated ability to extract meaningful molecular information, such as detecting nucleotide sequence in such a strand of DNA.[2, 4, 8, 10, 17, 19, 26, 27] The details of nanopore surface charges are not only important in the context of conductance as in Equation 1, but extend to augmenting electrophoretic control over analyte motion through the nanopore with electroosmosis, and to allowing nanopores to analyte-select not only based on size, but also by charge.[9, 28-31] Conductance-based nanopore characterization is, in fact, uniquely positioned to provide geometric and chemical insights into nanopore properties. It is also exceedingly important in the context of solution-phase nanopore fabrication methods where post-fabrication microscopic characterizations are undesirable. The prevailing approach has been to assume formation of a single nanopore when one is intended, and to overlook possible structural defects. Inaccurate nanopore models will affect the quality of conductance characterizations, and other work has shown (and taken advantage of) the influence of internal nanopore structural irregularities on analyte current blockages.[32] While it is essential to control the size of isolated nanopores for single-molecule characterization and sensing applications; the use of arrays of nanopores as filters for

physical and chemical separations multiplies the challenges and underscores the need to detail the formation of even single nanochannels.[11]

The extreme, ~10 nm feature size has historically been challenging to nanopore fabrication (and characterization) efforts. Methods have tended to be instrumentation-intensive, using charged-particle microscopes such as scanning and (scanning) transmission electron microscopes (SEM and (S)TEM), and helium ion microscopes, or ion accelerator facilities to prepare membranes for subsequent chemical etching steps.[33-37] More recently, ~20 V potentials applied across thin membranes immersed in electrolytes conventionally used for nanopore experiments resulted in (controlled) dielectric breakdown of the films, and could produce size-tuned nanopores following voltage-assisted etching.[38] This truly low-overhead approach can yield <10 nm diameter nanopores, and produces them reliably wetted for use, without the risks of drying and surface contamination from steps such as TEM-based fabrication (or examination). A similarly all-solution-based approach uses deposition of largely conformal films to shrink suitable pores to the desired final dimension.[9, 39] By deliberately and beneficially removing high-magnification charged-particle microscopes from the fabrication workflow, however, the opportunity to immediately image the fabricated pores is lost. We therefore explored existing nanopore conductance formalisms[13, 18] and developed a framework to use conductance to characterize nanopore size, shape, and surface chemistry.[14-16] We most recently showed that the method could yield real-time insight into these nanopore properties during solution-phase fabrication processes such as those outlined above.[14] In all instances, however, the simulations assumed perfectly formed single nanopores. Here we (1) deliberately

introduce defects into the pore models, and we moreover (2) allow for the possibility that a measured conductance arises from two separate nanopores forming in the same membrane (denoted a double pore). The latter allowance arises from TEM observations, post-pore fabrication, showing that dielectric breakdown formation of nanopores using unoptimized multilevel pulse-voltage injection could yield more than one pore.[40] Conductance-based measurements should allow for these realities, at least through the setting of reasonable uncertainty levels. We focus here on nanopores formed in thin, free-standing silicon nitride membranes, so that our numerical simulations use parameter values from the most commonly used nanopore material platform. The films are amorphous and thus not inherently prone to anisotropic etching,[41] and silicon nitride is notably resistant to structural and chemical modification absent deliberate action.

METHODS

The form of Equation 1 means that a single measured conductance does not yield a single unique solution for the nanopore size and shape.[14-16] One can gain more degrees of freedom by measuring the conductances at two different solution conductivities, K , [15, 16] or after (or during) controlled structural modifications.[14, 15] A time-dependent framework was developed and examined conventionally in earlier work—without considering either defects or multiple pores.[14] During nanopore formation—by dissolution or deposition of material—the nanopore conductance is a function of time because the dimensions of the nanopore, $\{q_j(z, t)\}$, are changing in time, t :

$$\frac{dG}{dt} = K \sum_j \left(\frac{\partial A}{\partial q_j} \right) \frac{dq_j}{dt} + \mu |\sigma| \sum_j \left(\frac{\partial B}{\partial q_j} \right) \frac{dq_j}{dt}. \quad (3)$$

This particular implementation can determine geometries with two free parameters, and we chose the limiting (minimum) radius, $r_0(z, t)$, and the total nanopore length, $L(t)$. [14] The presence of a defect disrupts the usual cylindrical symmetry. For a membrane with more than one nanopore, the nanopores are conductors in parallel (with identical surface chemistries and electrolyte contents) so that their conductances would be added directly, $G = \sum_n G_n$. Using a single measurement of the conductance at a single time t_i , it is not possible to distinguish between a single large pore and two smaller pores, or between a pore with or without a defect, when $G(t_i, \{q_j(t_i)\}) = G(t_i, \{q'_j(t_i)\})$. [14] The size- and geometry-dependence of the conductance change in time, however,

$$\frac{dG}{dt} = \sum_n \left(K \sum_j \left(\frac{\partial A_n}{\partial q_j} \right) \frac{dq_j}{dt} + \mu |\sigma| \sum_j \left(\frac{\partial B_n}{\partial q_j} \right) \frac{dq_j}{dt} \right) \quad (4)$$

provides a much-needed degree of freedom to possibly differentiate between such configurations. The characterization method then has a very simple implementation: measurements of several sequential experimental conductance values at times $\{t_i, \dots\}$, $\{G(t_i, \{q_j(t_i)\}), \dots\}$, are the inputs to the geometry optimization of candidate nanopore profiles. We simulated the experimental conductances using the experimentally supported Equation 1 in conjunction with experimentally supported nanopore profiles, and then fit the data using candidate nanopore profiles. [16, 18] The focus was whether including either defects or double pores would negatively affect the feasibility of the approach augured by the formalism. To allow this emphasis, the effect of measurement

noise on the conductance was neglected. The change in nanopore radius in time, $\frac{dr}{dt} = v_{mt}$, occupies a privileged role as the material transfer rate (with opposite signs for etching and deposition). We used a constant $|v_{mt}| = 0.6 \text{ nm/h}$ to highlight the nonlinear dependence of conductance on geometry in Equations 1, 3, and 4, and in keeping with the linear etch rates common to micromachining, but the method does not depend on that particular magnitude or time-dependence.[14, 41] We chose four nanopore profiles finding widespread use: cylindrical, double-conical, conical-cylindrical, and hyperbolic (Figure S4.1), but the method does not hinge on these particular choices.[13, 16, 18, 37, 42] The label r_0 is used here to denote the radius of the cylindrical pores, and the minimum radius (at any given time) of the pores with radii varying with z ; “pinch” and “outline” labels will be introduced for the r_0 of cylindrical nanopores with defects. All profiles were conventionally restricted to two free parameters, each, (r_0 and L) with the outer radius of the three tapered profiles fixed to be 10 nm greater than their corresponding r_0 , and the initial length of the inner cylinder of the conical-cylindrical pore restricted to 0.6 times its overall length, $L(t_0)$, where t_0 is the starting time. To model the double pore case, the two pores were set to be identical. Parameter values and calculations were consistent with previous work:[14-16, 22] 1 M potassium chloride electrolyte solution in water, $K=14.95 \text{ S}\cdot\text{m}^{-1}$, pH 7.0, and silicon nitride surface $\text{pK}_a=7.9$, with σ calculated in the usual way.[16, 22] The influence of solution pH is outlined in Figure S4.3 and the discussion immediately preceding it. For the defect-free pores, surface-deposited films were treated in a piecewise curved manner to maintain a uniform surface coating thickness (Figure S4.1) across the entire nanopore surface.[14] For the case of the pores with defects (Figure 4.1a) the half-cylinder protrusions running

along the full length of the pore interior were centered on the pore outline, opposite each other. Simulations of $G(t_i)$ were performed using 0.01 nm step sizes in the nanopore radius (or 1 minute increments given v_{mt}), and fits to $r_0(t_0)$ versus t were plotted using 0.05 nm increments.

RESULTS AND DISCUSSION

Post-fabrication comparisons of electron microscopic and steady-state conductance measurements support the independent use of Equation 1 for nanopore characterization.[13, 16, 18, 20, 21, 24] Conductance measurements recorded during a fabrication process such as dielectric breakdown, however, occur in a different context than post-fabrication measurements.[38, 43] In Figure 4.2, we used experimental multilevel pulse-voltage injection (MPVI) nanopore formation measurements—both steady-state and time-dependent—by Yanagi *et al.*[43] to test whether a formalism such as Equation 1 would yield reasonable real-time size determinations using the time-dependent conductance of a forming nanopore. Yanagi *et al.*[43] measured the steady-state conductances, G , of post-fabrication pores and then used TEM imaging to determine their mean r_0 . With appropriate consideration of the usual caveats of EM nanopore characterization[14, 16], along with possible consequences of nanopore dewetting and handling, post-fabrication electron microscopy provides a valuable, albeit instrumentation- and expertise-intensive, measure of nanopore size. Unsurprisingly, we obtained good fits to post-fabrication data using Equation 1 (Figure 4.2a)—in particular with a conical-cylindrical profile with conventional constraints (see above)—and using Equation S1 (Equation 1 with an access resistance term—see discussion below) with

cylindrical models with effective or adjustable fitting parameters. To correlate Yanagi *et al.*'s[43] measured G and mean r_0 without biasing the fit with an explicit choice of nanopore shape, we modified the cylindrical model of Equation S1 by replacing G_{bulk} with αG_{bulk} , and G_{surface} with βG_{surface} . We optimized the parameters α and β using the fit to the experimental data (with known r_0 , L , and G) in Figure 4.2a to correlate experimental post-fabrication nanopore conductances and mean nanopore radii by TEM, $r_{0,\text{TEM}}^{\alpha,\beta}(G)$. We then used $r_{0,\text{TEM}}^{\alpha,\beta}(G)$ to convert Yanagi *et al.*'s[43] time-dependent measurements of the conductance into nanopore size as a function of time, $r_{0,\text{TEM}}^{\alpha,\beta}(t_i)$ (Figure 4.2b). In this context, the function $r_{0,\text{TEM}}^{\alpha,\beta}(G)$ is thus better thought of as simply a fit function relating nanopore conductance and TEM-based size, rather than representing a particular model choice for the nanopore conductance. Finally, for each $G(t_i)$ data point of Figure 4.2b, we calculated $r_{0,\text{candidate}}(t_i)$, with all other parameters fixed, for each of the candidate nanopore profiles, and compared the results with $r_{0,\text{TEM}}^{\alpha,\beta}(G)$ (Figure 4.2c). The experimental $G(t_i)$ of Yanagi *et al.*[43] was fit best, using Equation 1, by a conical-cylindrical model with overall length equal to the nominal membrane thickness. The cylindrical model using Equation S1 and with an effective length equal to a fraction of the nominal membrane thickness[43] did not fit as well as the conical-cylindrical model, but outperformed the remaining candidates. Overall, Equations 1 and S1 produce reasonable nanopore sizes when applied to conductance data recorded during nanopore fabrication. As discussed in earlier work[14], a time-dependent material-transfer rate, $v_{\text{mt}}(t)$, is no impediment to the time-dependent conductance profiling framework.[14]

As the first application of Equation 1 to more complex nanopore configurations, we investigated the effect of defects on our ability to extract reasonable geometric descriptions of nanopore sizes. Figure 4.1a shows a top-down view of defects in cylindrical nanopores ($L(t_0) = 10$ nm). Figure 1a also shows one of the key challenges of conductance-based nanopore characterizations: all of the different profiles shown have, by Equation 1, the same 200 nS conductance. With larger initial defect size, the initial radius of the cylindrical outline of the nanopore (the “outline radius”, $r_0^{\text{outline}}(t_0)$) must also be larger to compensate for the internal volume lost for ionic transport. Defects distort the circular symmetry of the nanopore and introduce “pinch points” (as illustrated in Figure 4.3, characterized by the radius of a cylinder just fitting between the two protrusions—the “pinch radius”, $r_0^{\text{pinch}}(t_0)$) that could preclude analyte passage where a defect-free pore of equivalent conductance could allow passage. Such a failure, of course, is diagnostic, but would require the addition of gauging molecules or particles (compatible with the fabrication conditions) if it were to be used for real-time monitoring of the fabrication. Such adjuncts could naturally be used post-fabrication.[44, 45] Figure 4.1b shows the evolution of a cylindrical nanopore with 1 nm-radius defects: as more material is added to the surface with time, the nanopore interior becomes increasingly anisotropic. Depending on defect size, shape, and position, depositing material onto the surface of a pore with defects could readily lead to overlapping Debye layers followed by physical scission of a single pore into two distinct pores. The comparison of single and double pore systems thus also overlaps with the consideration of fabrication defects. Figure 4.1c illustrates the heart of the method motivated by the form of Equations 1 and 3: it shows the time evolution, with

identical material transfer rates, of the nanopore profiles shown in Figure 4.1a. For small nanopore sizes where Debye layers overlap, more sophisticated treatments than Equation 1 are required, but as a guide to the eye we plotted the conductance until $r_0^{\text{pinch}} = 0$. [15, 46] From their identical initial value, the conductances of the different profiles differentiate in time, in spite of the constant material transfer rate changing all outline and pinch radii at the same rate.

When nanopore dimensions are changed during fabrication, the change in conductance with time is measured without knowledge of the presence or absence of defects. The question is whether the time-trace of the conductance can reveal the presence of defects or not—and if not, how serious the error in the resulting nanopore characterizations might be. To explore this, we chose to simulate (abbreviated to “sim” in labels) the time-dependent conductances, $G_{\text{case}}^{\text{sim}}(t_i)$ (case denotes defect size), for two cylindrical nanopores with $G_{\text{case}}^{\text{sim}}(t_0) = 200$ nS and $r_0^{\text{pinch}}(t_0) = 4$ nm: one with two 0.1 nm-radius defects, and the other with two 1.0 nm-radius defects (and lengths $L(t_0) \sim 4.1$ and ~ 5.9 nm, respectively, dictated by the conductance and radii). We attempted to fit these data by using the (known) material transfer rate and varying the dimensions of three candidate nanopore profiles: a defect-free cylindrical nanopore, and profiles with 0.1 and 1.0 nm-radii defects. The question was whether fitting to the $G_{\text{case}}^{\text{sim}}(t_i)$ would reveal the existence and size of defects. A step-by-step tutorial for this process is provided in earlier work, [14] which we abbreviate here to allow a suitable focus on fabrication irregularities. The initial conductance, $G_{\text{case}}^{\text{sim}}(t_0)$, was used to determine the (infinite) set of $\left\{ \left(r_{0,\text{candidate}}(t_0), L_{\text{candidate}}(t_0) \right) \right\}$ for which $G_{\text{candidate}}(t_0) = G_{\text{case}}^{\text{sim}}(t_0)$.

After the dimension changes from depositing material at the known rate (outline and pinch radii diminish at v_{mt} , whereas the cylinder length increases at $2v_{\text{mt}}$), only one pairing $(r_{0,\text{candidate}}(t_0), L_{\text{candidate}}(t_0))$ for each candidate also satisfied $G_{\text{candidate}}(t_1) = G_{\text{case}}^{\text{sim}}(t_1)$. This answer gave the unique initial nanopore size for each candidate with its specified defect size, but could not be used to identify the simulated defect size. That is, all three candidate profiles could exactly reproduce the two simulated conductances. After propagating the deposition one more time from the three different $(r_{0,\text{candidate}}(t_0), L_{\text{candidate}}(t_0))$, only one pair of initial nanopore dimensions gave $G_{\text{candidate}}(t_3) = G_{\text{case}}^{\text{sim}}(t_3)$. Figure 4.3 summarizes this behavior: the ordinate is the initial nanopore radius, $r_{0,\text{candidate}}(t_0)$, that, after deposition until time t_i , would give $G_{\text{candidate}}(t_i) = G_{\text{case}}^{\text{sim}}(t_i)$ (the dimensions at time t_i are readily calculated from the initial dimensions and the known material transfer rate). When the candidate profile (here, defect size) matches the simulated profile, then all the $r_{0,\text{candidate}}(t_0)$ from each t_i are equal to each other, and equal to $r_{0,\text{case}}^{\text{sim}}(t_0)$, and the line connecting the data is horizontal. When the candidate profile is incorrect, then the plotted data is no longer horizontal. Thus, in Figure 4.3a, when the simulated data is generated using a cylindrical pore with a 0.1 nm-radius defect, only the fit data using the 0.1 nm-defect candidate pore is perfectly horizontal. The defect-free nanopore fit data is close to horizontal and overlaps substantially with the outline radius of the simulated pore, but the 1 nm-defect fit data has a larger nonzero slope and is therefore the incorrect candidate. While $r_0^{\text{outline}}(t_0)$ of the 1 nm-defect candidate was not substantially larger than the true $r_0^{\text{outline}}(t_0)$, its small $r_0^{\text{pinch}}(t_i)$ would suggest an incorrect threshold for analyte size-exclusion. Figure 4.3b

shows that a 1 nm-defect simulated pore is successfully fit only with a 1 nm-defect candidate pore, and that radii for the remaining two candidates lie between limits set by the pore with the larger defect. In both fitting examples, the slopes of the fit data provide an indication of the correct defect magnitude, being positive when the candidate defect is too large, and negative when the candidate defect is too small. One might thus imagine a strategy in which a wider range of candidate defect sizes were used to more readily indicate the presence and provide bounds for the size of a defect. The feasibility of the method thus extends from the formalism to successful numerical examples, but these model calculations portend limitations in experimental implementation: $\Delta r_{0,\text{candidate}}(t_0) \sim 0.1 \text{ nm}$ for incorrect candidates, compared to the full 2 nm deposition thickness. In the presence of measurement noise, or with an unfavorable combination of defect size, v_{mt} , fabrication time, and number of conductance measurements, for example, even detection of defects may elude real-time analysis.

We extended this exploration of the effect of defects by considering the effect of candidate nanopore shape on the conductance-based geometry optimization. Figure 4.4a illustrates the underlying premise. At t_0 , the six listed nanopore profiles have identical 200 nS conductances and $L(t_0) = 10 \text{ nm}$, generated by different $r_0(t_0)$. As material deposition narrows the nanopore constrictions at a constant linear rate (inset), all of the conductances diverge from each other in time. This occurs in spite of, for example, the r_0^{pinch} of the 1.0 nm-defect cylindrical pore and the r_0 of the conical-cylindrical pore having essentially identical values over time. Figures 4.4b and c use this behavior quantitatively. The same procedure used for Figure 4.3 was used to fit the

simulated conductances of cylindrical nanopores with $r_0^{pinch}(t_0) = 5.0$ nm, and two defects of either 0.1 or 1.0 nm radius, with defect-free pores representing typical nanopore shapes. Even the smaller, 0.1 nm defects caused the defect-free cylindrical nanopore to be unable to fit the simulated conductance. The correct candidate profile—0.1 nm defects inside a cylindrical profile—gave a perfectly horizontal line when fit to the simulated 0.1 nm-defect data. Fitting with the conical-cylindrical nanopore, however, generated nearly horizontal data, likely because the distinct narrow and wide sections of the profile (including constraints) were able to approximate the defect-bearing cylinder’s balance of pinch and outline radii. The radius of the opening through the inner cylinder ($r_{0,conical-cylindrical}(t)$), however, was smaller than for the simulated profile. For the simulated cylindrical pore with the larger, 1.0 nm defect, the fitting procedure again returned the correct profile and defect size. Once again, the conical-cylindrical profile fit data was almost horizontal with the wrong radius, although lying between the pinch and outline radii of the defect model. Depending on the size, distribution, number of defects, and current noise, it may be difficult to use this conductance model to distinguish, in real-time during formation, between an ideal pore of a given shape, and a pore of a different shape, but with defects. It may be necessary to then resort to more involved post-fabrication approaches.[15, 16, 44, 45] Indeed, one may be forced to adopt a strategy of repeated cycles of incomplete fabrication—with real-time profiling—followed by more in-depth characterization. In such a case it is important to understand the inherent uncertainties—such as the error in r_0 —of these real-time characterization procedures to ensure that the fabrication cycles do not pass by the desired final size.

A second complication for nanopore formation is the formation of more than one pore when only one is intended. Microscopy can be used to directly enumerate the pore number, but at the cost of instrumentation and user burdens, and possible nanopore surface contamination, among other drawbacks. We wanted to determine if conductance could provide any insight into this possible problem of multipore formation. We explored the case of double pores of matching size and shape. Figure S4.4 illustrates that the conductance change in time provides the prospect of differentiating between single and double pore systems, just as it did for single pores of different shapes.[14]

To explore whether the conductance time trace could reliably determine the size and number of the pores during their fabrication, we simulated conductances for single and double pore configurations of the four profiles in Figure S4.1, choosing 200 nS as a convenient initial conductance. Double pores for each shape were identical in size to each other. The conductance fitting in Figure 4.5 mirrors that of Figure 4.3 and 4.4b,c. For each column, a given profile with a single (a-d) or double (e-h) pore was chosen and used to calculate a minimum of three simulated conductance values in time: $G_{\text{case}}^{\text{sim}}(t_0)$, $G_{\text{case}}^{\text{sim}}(t_1)$, and $G_{\text{case}}^{\text{sim}}(t_2)$, with additional $G_{\text{case}}^{\text{sim}}(t_i)$ providing added robustness (case here denotes profile and pore number). The broad outlines of the results detailed in Fig. 5a-d and e-h are that one-pore simulated conductances were fit by the one-pore candidate profiles of the correct shape (as revealed by the constancy of the corresponding $r_0(t_0)$), and double pore conductances were fit by the matching double pore candidate profiles. Interestingly from these examples, double pore cylindrical and conical-cylindrical profiles did a reasonable job of fitting single pore hyperbolic and double-conical conductance data, and single hyperbolic and double-conical candidates

did a reasonable job of fitting double pore cylindrical and conical-cylindrical conductance data. Exact agreement still only occurs for correct shape and pore number, but the wrong profile doesn't inherently produce a terribly inaccurate radius. While they returned the incorrect shapes, the nevertheless fairly accurate r_0 means the expectations of which sizes of molecules would fit through the candidate pores are unlikely to differ appreciably, although the double pore case would allow for twice the number of channels and have different analyte-induced current blockages. Sufficient attention should therefore be obtained to optimizing the nanopore fabrication conditions,[40] and more involved post-fabrication characterizations should be considered if analyte-induced blockages do not fall within the range expected for the relative sizes of analyte and pore.[15, 16, 44, 45]

CONCLUDING REMARKS

The performance of a nanopore used for applications such as single-molecule sensing, separations, and manipulations is dictated in large part by its size, shape, and surface chemistry. These three parameter groupings underpin the nanopore conductance and allow a suitable analysis framework to use straightforward measurements of the conductance as a means to gain insight into these nanopore properties. Nanopore conductance is routinely used to coarsely gauge nanopore size during use, typically with at least the assumption of a cylindrical shape, and then often with deliberately incorrect parameter constraints to ensure that reasonable numerical estimates of the radius are nevertheless produced. More sophisticated conductance formalisms have been developed and validated for use with more complicated nanopore shapes and to account

for additional considerations such as access resistance. Simple, analytical expressions allow for wider adoption of a characterization method that can easily accommodate a range of nanopore profiles, thereby providing both application flexibility and the possibility for using different model assumptions to explore the uncertainties in the extracted nanopore dimensions.[15, 16] New solution-based nanopore fabrication techniques have increased the importance of methods to characterize nanopores from their conductance. We tested the ability of a recently-developed method to characterize nanopores in real-time during fabrication by allowing for the possible formation of multiple pores or pores with defects. The simulations determined the correct nanopore number, size, and shape alongside the presence and size of any defects, but the numerical examples revealed challenges that await experimental applications of the approach. While the basic equations showed good agreement with experimental time-dependent conductance measurements, example characterizations that explicitly considered the possibility of nanofabrication defects yielded only very slight differences in the key metrics designed to identify nanopore profiles and determine their dimensions. Inadequate measurement statistics may therefore impede the ability to uniquely or correctly determine the correct nanopore shape, number, and size. In challenging cases, a selection of analyses using different assumptions could produce a set of parameter values whose spread could offer a measure of the uncertainty of the characterization. Such real-time estimates could be followed by post-fabrication characterizations where larger conductance changes than those accompanying nanoscale changes of nanopore dimension would be wrought by changes of solution concentration, thereby easing the conductance analysis.[16] Thus, in spite of the

limitations discussed here, the time-dependence of the nanopore conductance during fabrication remains a useful tool, given sufficient circumspection in application, for gaining insight into the evolving nanopore structure and for characterizing nanopores even without the usual assumptions of ideal formation.

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All authors declare no financial/commercial conflicts of interest.

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Figure Captions

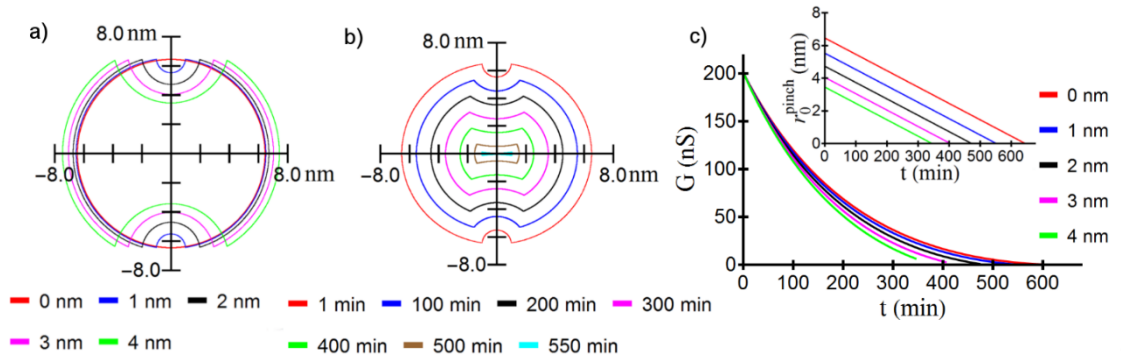


Figure 4. 1. a) Top view of $L(t_0) = 10$ nm cylindrical nanopores that yield a 200 nS conductance with the radii of the two inward-pointing defects given in the legend. b) Top view of the initially 1 nm-radius defect nanopore from (a), closing at $v_{\text{mt}} = 0.6$ nm/h with deposition time indicated. c) Progression of conductance (and r_0^{pinch} in inset) with time for the cylindrical nanopores from (a).

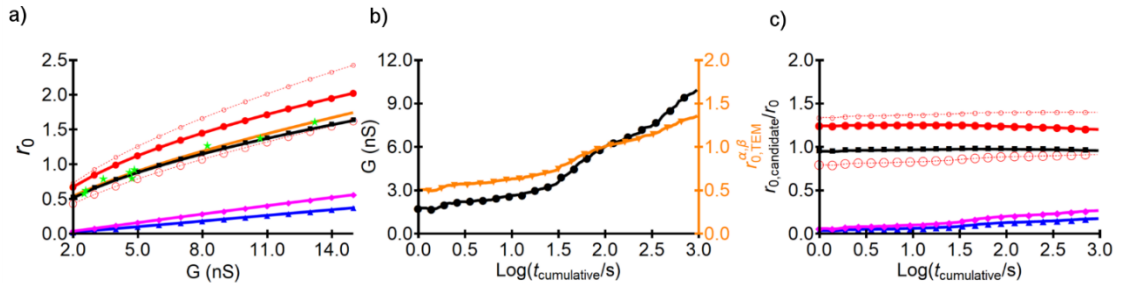


Figure 4. 2. (a) Experimental post-fabrication measurements of nanopore conductance and their corresponding TEM-based mean $r_{0,\text{TEM}}^{\text{expt}}$ (green stars)[43] were plotted versus several models: Equation 1 (solid markers) – cylindrical (red circles), double-conical (blue triangles), conical-cylindrical with an inner cylinder length of $0.6L$ (black squares), and hyperbolic (magenta diamonds); and with an added access resistance term, by Equation S1 (hollow markers) – cylindrical with length L (small circles) and

cylindrical with a $0.37L$ effective length [43] (large circles). To not bias further analysis with an explicit choice of nanopore profile, the $r_{0,\text{TEM}}^{\text{expt}}$ were fit to Equation S1 with G_{bulk} and G_{surface} from the cylindrical model weighted by fit parameters: αG_{bulk} and βG_{surface} (orange triangles— $r_{0,\text{TEM}}^{\alpha,\beta}(G)$). (b) Time-dependent conductance measurements were taken from the experimental work of Yanagi *et al.*[43] and were used with $r_{0,\text{TEM}}^{\alpha,\beta}(G)$ to determine $r_{0,\text{TEM}}^{\alpha,\beta}(t_i)$. (c) Candidate profiles matching those in (a) were used at each discrete value of $G(t_i)$ to calculate an $r_{0,\text{candidate}}(t_i)$. The figure compares the fit and experimentally-derived radii where the correct candidate size should result in a straight line at a ratio of 1. Selected data markers are shown for clarity.

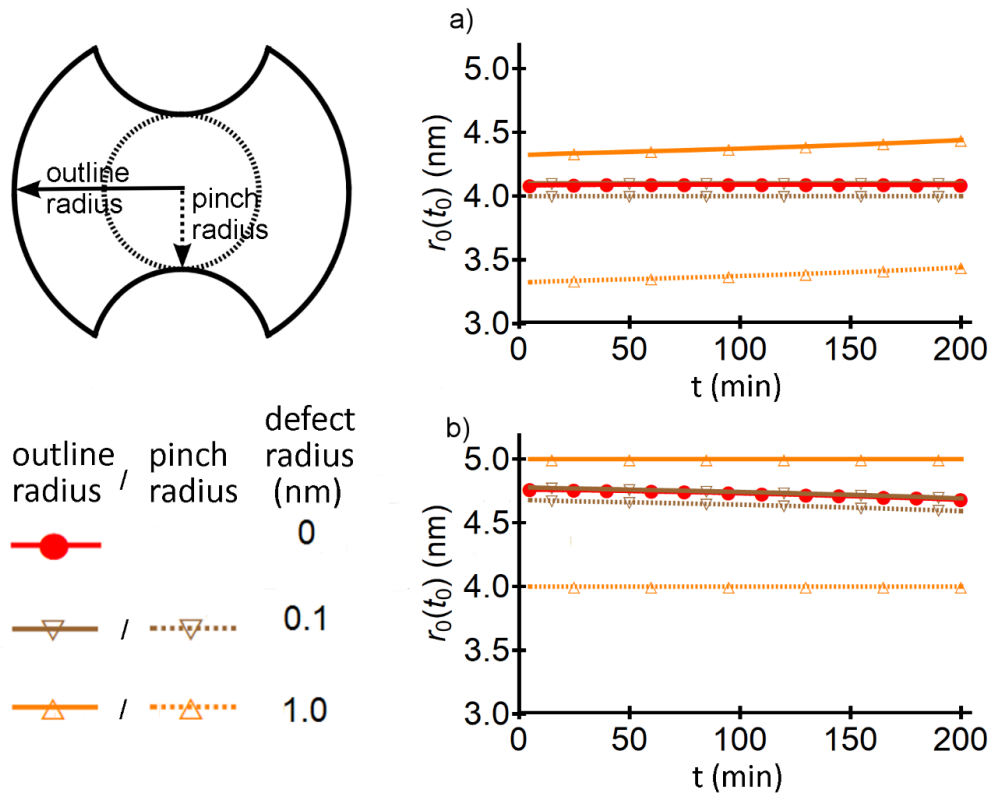


Figure 4. 3. Conductances during simulated material deposition onto nanopores with initial conductances of 200 nS, and $r_0^{\text{pinch}}(t_0) = 4$ nm, were fit with candidate cylindrical nanopores: a defect-free pore, and pores with 0.1 and 1.0 nm-radius defects. Dotted and solid lines denote the pinch and outline radii, respectively. a) 0.1 nm defect pore and b) 1.0 nm defect pore profiles were used to furnish the simulated conductance data. The correct candidate profile in each case was indicated by the horizontal slope of the fit data; the defect-free $r_0(t_0)$ nearly completely overlaps with $r_0^{\text{pinch}}(t_0)$ for the 0.1 nm defect pores. Selected data markers are shown for clarity.

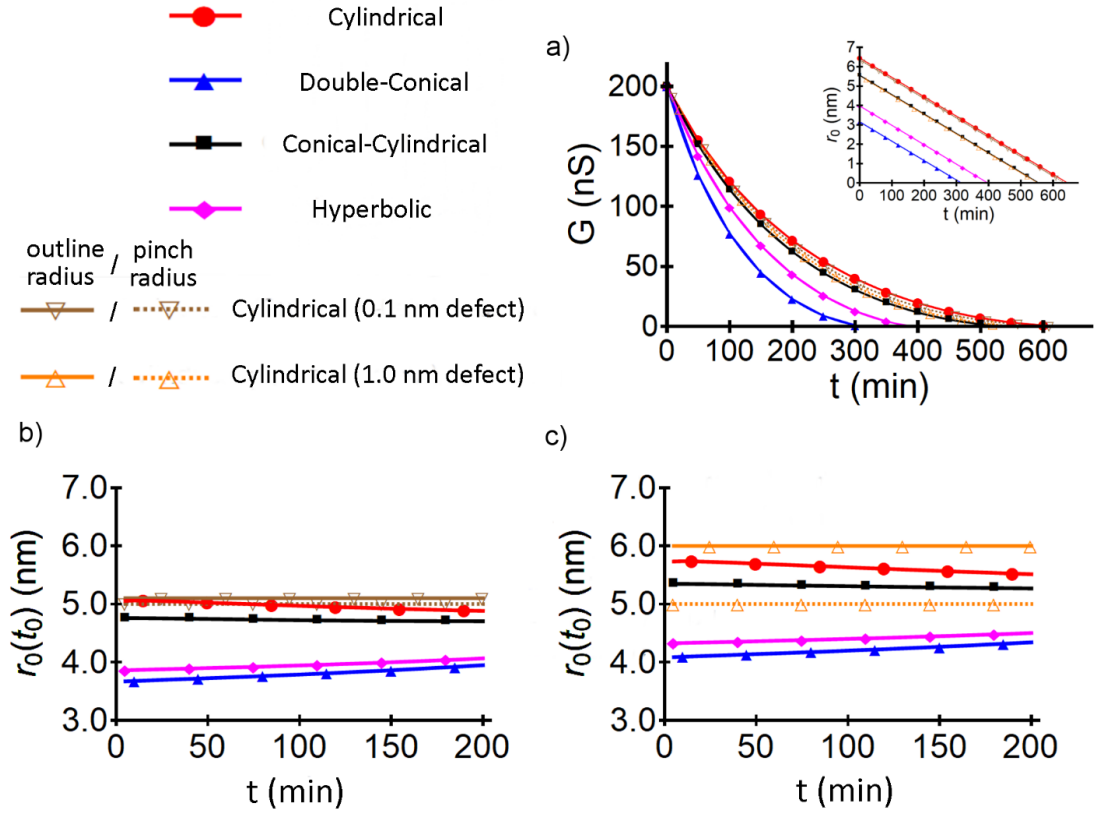


Figure 4. 4. a) Conductances and (inset) radii as a function of profile and time when simulating deposition onto surfaces of initially 200 nS, $L(t_0) = 10$ nm nanopores. Dotted curves in the conductance plots belong to the cylindrical pores with defects, and denote the corresponding r_0^{pinch} in the inset (solid line- r_0^{outline}) and in (b)-(c). Conductance versus time for b) 0.1 nm-defect and c) 1.0 nm-defect cylindrical pores were fit with each candidate profile in the legend; horizontal fit lines for each case indicated the correct simulated profile. Selected data markers are shown for clarity.

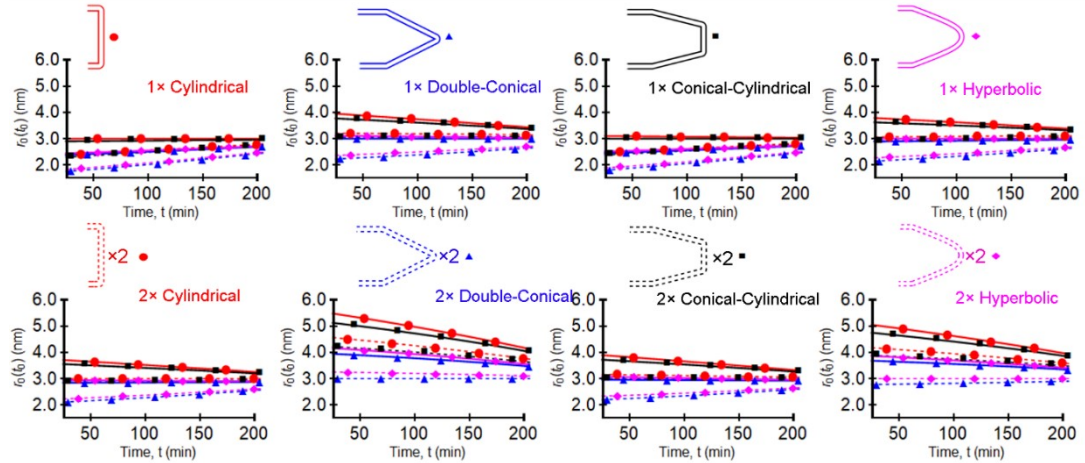


Figure 4. 5. Single (solid lines) and double (dotted lines)—left to right matching the half-profile sketches—cylindrical (red circles), double-conical (blue triangles), conical-cylindrical (black squares), and hyperbolic (magenta diamonds) profiles were used to simulate nanopore conductance values versus time. Eight candidate profiles (4 shapes, single and double) were used to fit (a-d) single pore simulated data and (e-h) double pore data from the 4 shapes. All experimental pores were initially 200 nS conductance. The correct nanopore shape was indicated by the constancy of the fit to $r_0(t_0)$ in time, and is labelled with the corresponding shape and number of pores. Selected data markers are shown for clarity.

CHAPTER 5: PREFACE

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ELECTROLESS PLATING OF THIN GOLD FILMS DIRECTLY ONTO SILICON NITRIDE THIN FILMS AND INTO MICROPORES

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CHAPTER 5: ELECTROLESS PLATING OF THIN GOLD FILMS DIRECTLY ONTO SILICON NITRIDE THIN FILMS AND INTO MICROPORES

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ABSTRACT

A method to directly electrolessly plate silicon-rich silicon nitride with thin gold films was developed and characterized. Films with thicknesses less than 100nm were grown at 3 and 10°C between 0.5 and 3 hours, with mean grain sizes between ~20-30nm. The method is compatible with plating free-standing ultrathin silicon nitride membranes, and we successfully plated the interior walls of micropore arrays in 200nm-thick silicon nitride membranes. The method is thus amenable to coating planar, curved, and line-of-sight-obscured silicon nitride surfaces.

KEYWORDS: Electroless plating; thin gold films; silicon nitride; micropores; surface enhanced Raman spectroscopy (SERS); tin sensitization.

INTRODUCTION

Thin gold films have widespread technological utility, from forming conductive elements and overlayers, to serving as a platform for chemical surface modification by molecular self-assembly¹. For gold films incorporated into conventional micro- and nanofabricated devices, silicon nitride is an appealing choice for a substrate. It is a

standard nanofabrication material, offering, in addition, favorable inherent properties such as mechanical strength²⁻³, chemical resistance, and dielectric strength⁴⁻⁵. Silicon nitride is thus ubiquitous as a structural and functional element in nanofabricated devices where it plays a variety of roles^{2, 5-8}. Its surface chemistry, however, presents especial challenges given the complex mixture of silicon-, oxygen-, and nitrogen-bearing surface species⁵. The nominal surface modification of silicon nitride is frequently carried out in practice using silane-based modification of a silica layer that may itself not be well-defined⁹. Thus, there remains both a need and opportunity to expand the suite of approaches useful for surface functionalizing silicon nitride directly. Electroless deposition is a particularly compelling approach to film formation: deposition proceeds from solution allowing the coating of three-dimensional surfaces, including surfaces hidden from line-of-sight deposition methods; no electrochemical instrumentation is required; no electrical power must be supplied nor must the substrate be conductive; there is no need for expensive vacuum deposition equipment; and a variety of classical physicochemical parameters such as reagent composition, solution properties such as pH and viscosity, and temperature, are available to tune the film properties¹⁰⁻¹¹. There is a wealth of familiar approaches for the electroless plating of substrates such as polymers, for example, but no established prior art for the direct metal-cation-mediated electroless plating of gold onto silicon nitride¹²⁻¹³. A particularly compelling sequence exists for the electroless gold plating of poly(vinylpyrrolidone)-coated polycarbonate substrates (Au/PVP)¹³: direct sensitization of the PVP surface with Sn²⁺, activation by immersion in ammoniacal silver nitrate to oxidize the surface Sn²⁺ to Sn⁴⁺ by reducing Ag⁺ to elemental silver (producing, also, a small amount of

silver oxide), and finally gold plating by galvanic displacement of the silver with reduction of Au(I) to Au(0) accompanied by the oxidation of formaldehyde. Amine and carbonyl groups in the PVP layer were proposed to complex the tin cation during sensitization¹³. Extending this approach, Sn²⁺ has been reported to complex effectively with oxygen-rich polymer surfaces¹² and with quartz and silica substrates^{10, 14-16}. Tin(II) sensitization has also been reported on NaOH-roughened surfaces¹⁷, suggesting that a specific chemical interaction may not be essential¹⁸, and underscoring the utility of electroless plating for rough and high-surface-area surfaces where physical deposition is challenged¹⁹. In principle, though, a smooth silicon nitride substrate with a well-defined silica surface layer should be amenable to direct tin sensitization. Yet, electroless deposition of gold on planar silicon nitride has been limited to routes requiring the use of a silica layer with organic linkers and metal layers between the silicon nitride and gold overlayer¹⁸. In the first case, covalent attachment of an organic monolayer using silane chemistry can be beneficial for film adhesion, but adds operational complexity¹⁸ and can constrain downstream processing conditions. In the second case, the intervening layers may lend beneficial properties, or may similarly introduce compositional constraints on applications, or morphological constraints on the final gold film nanostructure. Regardless of the ability to carry out a silica-based modification, it does not eliminate the benefits of a direct functionalization of silicon nitride. We present a dramatically simplified electroless gold deposition method in which we eliminate the initial covalent attachment of an organic monolayer to the substrate, and in which we do not need to initially mask the silicon nitride surface chemistry with a silica overlayer. Our method directly sensitizes the silicon nitride

substrate with a Sn^{2+} solution, followed by a series of metal ion treatments in which we exert control over the gold film thickness using process time and temperature. Film thicknesses ranged from 30 to 100nm for deposition times from 0.5-3h, and temperatures of 3 and 10°C.

Full details of materials and preparation are provided in the Supporting Information. In brief, polished silicon-rich low-pressure chemical vapor deposited (LPCVD) silicon nitride-coated silicon wafers were cleaved into $\sim 1\text{cm}^2$ chips. The chips were then electrolessly plated with gold deposited from solution as outlined in Scheme 5.1. Ultrasonic cleaning of the substrate²⁰ was strictly avoided so that straightforward extension of the scheme to ultrathin silicon nitride windows would not cause window fracture²⁻³. Each chip was plasma-cleaned and then briefly etched in a dilute hydrofluoric acid (HF) solution to remove unwanted native silicon oxide and expose the silicon nitride surface^{4, 20}. The prepared chips were immersed in a tin(II) chloride sensitizing solution, followed by a soak in ammoniacal silver nitrate solution^{10, 13}. The chips were carefully rinsed between each step of the process. Electroless gold plating was carried out by immersing chips in $\sim 1.5\text{-}3\text{mL}$ (0.75mL for micropores) of sodium gold sulfite plating solution²¹, with gentle rocking, in a refrigerator (3°C plating) or thermoelectric cooler (10°C plating). After plating for the desired time at the desired temperature, the chips were carefully rinsed, dried and then characterized. Gold film thicknesses were obtained by atomic force microscopy (AFM) measurements across an edge from the film to the substrate. Film morphology was examined by field-emission scanning electron microscopy (FE-SEM) and analyzed using a watershed analysis. Elemental analysis of the gold film was carried out by energy-dispersive x-ray

spectroscopy (EDS) and by x-ray photoelectron spectroscopy (XPS). Characterization details are provided in the Supporting Information.

Scheme 5. 1. Electroless plating of silicon nitride. The silicon nitride-coated substrates are plasma-cleaned of organics and HF-etched before the surface is exposed to Sn^{2+} ions which are oxidized during the redox-driven deposition of an elemental silver layer. Gold plating begins with galvanic displacement of the elemental silver.

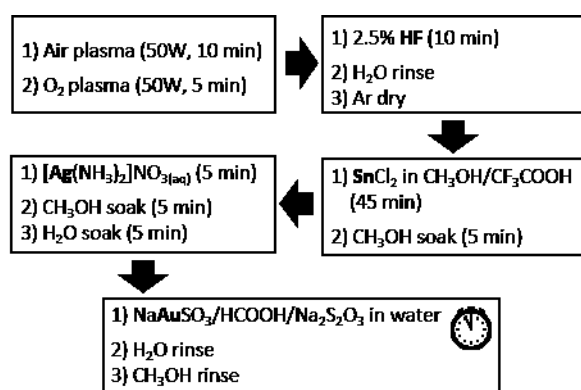
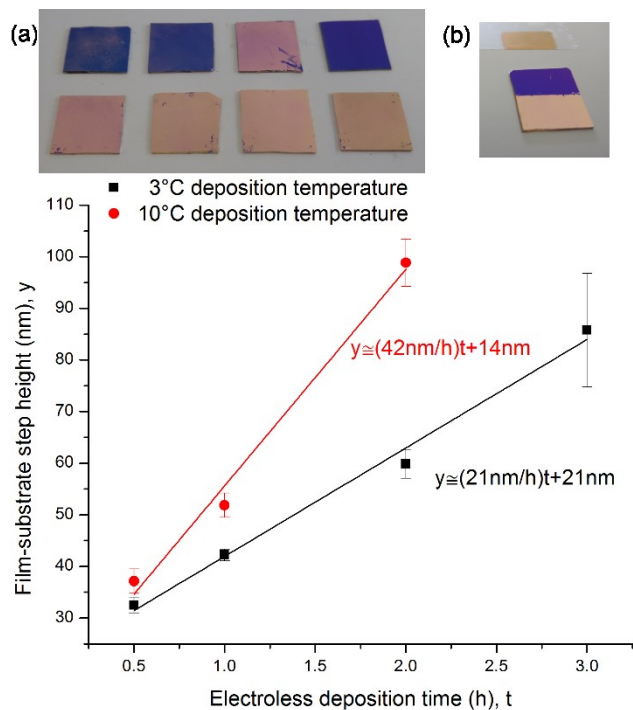


Figure 5.1 shows photographs of an array of silicon nitride-coated substrates subjected either strictly to the steps in Scheme 5.1, or to control experiment variations. Adherence to Scheme 5.1 produced gold films, evaluated by visual inspection, with good quality and excellent macroscopic surface coverage, and delivered these results reliably over many months of repeated trials. More detailed characterization of these films is provided below. Departures from the scheme, however, yielded generally poor or inconsistent results. We focused our attention on varying the surface preparation steps, specifically testing surface preparations that did not involve HF etching designed to remove the oxygen-containing overlayer. Tin(II) sensitization after sodium hydroxide surface roughening had been reported on silicon nitride powders of unknown

stoichiometry^{5, 17}. Indeed, surface roughening to improve film adhesion is a familiar preliminary process in electroless plating¹¹. Substituting 1, 4.5, or 9M NaOH treatments for the HF etching of Scheme 5.1, however, generated only gold smudges after 3 hours of plating at 3°C. The silicon-rich nature of our LPCVD films is a possible contributing factor to the poor plating quality after NaOH treatment in comparison to the published results¹⁷, given the general challenge that silicon nitride stoichiometry and available surface species—and thus functionalization opportunities²⁰—depend on the details of the silicon nitride synthesis⁵. Our use of large-area, planar substrates introduces another likely explanation: it provides a stringent test of film deposition quality, and easily reveals defects that may be more difficult to discern on a film coating a powder. Traditional silicon nitride surface modification schemes rely frequently on modification of a silica layer on the silicon nitride surface²²⁻²³ rather than of the silicon nitride, itself. Careful attention to the quality of the oxygen-containing surface layer can circumvent difficulties that stem from a lack of definition of this silica layer²². Holtzman and Richter used nitric acid to enrich the number of surface hydroxyl groups on silicon nitride so that they could use silane chemistry to provide an organic monolayer foundation for an overlying electrolessly deposited gold film¹⁸. While successful, the approach must contend with the acknowledged challenges of silane chemistry¹⁸ and with the persistence of the organic linker layer. Given the affinity of Sn²⁺ for such an oxygen-enriched silicon nitride surface, and given prior demonstrations of electroless gold plating on silica surfaces¹⁰, we replaced the HF etch in Scheme 5.1 with a 20 minute treatment in 10% (v/v) nitric acid at 80°C. The results, shown in Figure 5.1, were promising, with repeated, although not consistent, deposition of (visually inspected)

high-quality gold films. It is likely feasible to optimize this route to routinely deposit high-quality, uniform gold films, but our goal was to develop a simple route to electrolessly plate gold directly onto silicon nitride. Treatment of silicon-rich LPCVD silicon nitride surfaces with dilute hydrofluoric acid eliminates the native oxide^{4, 23} and leaves a H-terminated surface with Si-H, NH and NH₂ moieties²². Given the appeal of this surface for surface functionalizations^{20, 22}, we tested its compatibility with tin(II)-based sensitization. Scheme 5.1 thus follows the plasma-based cleaning steps with an HF etch step that removes oxide and H-terminates the surface²², and ends with the gold plating treatments¹³. We note that in the absence of the HF-etching step, chips would sporadically be coated with patchy gold layers, but no uniform high-quality gold films



were observed on these chips even after 3 hours in the gold plating solution.

Figure 5. 1. (a) Photograph array of plating results at 3°C. Top row, left-to-right – HF etch omitted, 1 h plating after HNO₃ preparation, HNO₃ step replicate, plasma-cleaned

only (subsequent steps omitted). Bottom row, left-to-right, Scheme 5.1 followed for plating times of 30 minutes, 1 hour, 2 hours, and 3 hours. The scratches in the film arose during handling of the chips. (b) Adhesive tape could lift most of the gold film to give an edge for (c) AFM measurements of electroless gold deposition film thickness as a function of time and temperature.

The row of visually high quality, high coverage gold films shown in Figure 5.1 were electrolessly plated at 3°C for increasing lengths of time, with strict adherence to Scheme 5.1. The gold films survived extensive handling including prolonged immersion in liquids interspersed with repeated rinsing and pressurized argon-drying steps, and moreover adhered well to free-standing films that we broke deliberately for imaging (Figure 5.4b). Certainly, in applications using gold-coated, freestanding silicon nitride membranes, consideration of membrane robustness will supersede gold adhesion in importance. The films could, however, be scratched with tweezers and mostly removed with adhesive tape (Figure 5.1b), and this afforded us the ability to perform AFM film thickness measurements. A swath of the gold film was removed and the mean difference in height between the film and the bare substrate was averaged across several representative line profiles and several independently plated chips for each plating time and temperature (see Supporting Information for details). Figure 5.1 plots the step height from plated film to bare substrate as a function of time: at 3°C a step height of ~30nm after 30 minutes with a linear fit yielding a ~20nm/h deposition rate thereafter, and at 10°C a step height of ~35nm after 30 minutes with a linear fit yielding a deposition rate of ~40nm/h thereafter. The intercept likely arises from residual silver nanoislands scattered across the substrate. Shorter plating times than those shown in Figure 5.1

typically produced chips with a purple-blue hue. Four-point film resistivities were measured for the films plated at 3°C for all the time points listed, and were in the range $\sim 3 - 5 \times 10^{-6} \Omega \cdot \text{cm}$; thin film resistivities higher than the known bulk gold resistivity ($2.2 \times 10^{-6} \Omega \cdot \text{cm}$)¹¹ are not surprising¹⁸. SEM micrographs afford a further detailed view of the film structure (Figure 5.2). Microscopic substrate coverage was high, but not complete, after 30 minutes of plating at 3°C, but was on par, after 30 minutes at 10°C and 1 hour at 3°C, with the coverage shown in the SEM micrograph shown in Figure 5.2. Micrographs for both temperatures and all plating times were subjected to watershed analysis (see Supporting Information for details) and yielded area-equivalent mean grain radii from 20-30nm. It is clear from the SEM images, however, that the film structure is more complex than can be represented in a single equivalent grain size. There were large agglomerates on the film surface, seen also in AFM line profiles, with radii of several hundred nanometers. EDS analysis of these larger features showed them to be gold (see Supporting Information, Figure S5.1). Many of these outcroppings had quite convoluted shapes; there is the potential for quite compelling applications arising from both the regular and irregular film grain structures²⁴⁻²⁵. Indeed, the films are useful as a platform for surface-enhanced Raman spectroscopy (SERS). Figure 5.3 shows a demonstration spectrum of 4-nitrothiophenol (NBT) taken from an electrolessly gold-coated silicon nitride substrate. Annealing of these films caused an attendant decrease in the SERS signal, and after annealing for 24 hours at 280°C, the mean grain size had increased to nearly 50nm.

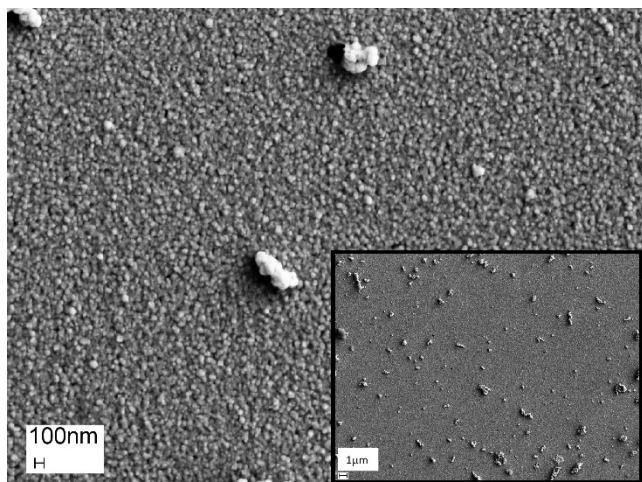


Figure 5. 2. SEM images of a film after 2h of gold plating at 3°C. The inset is of the same film at lower magnification.

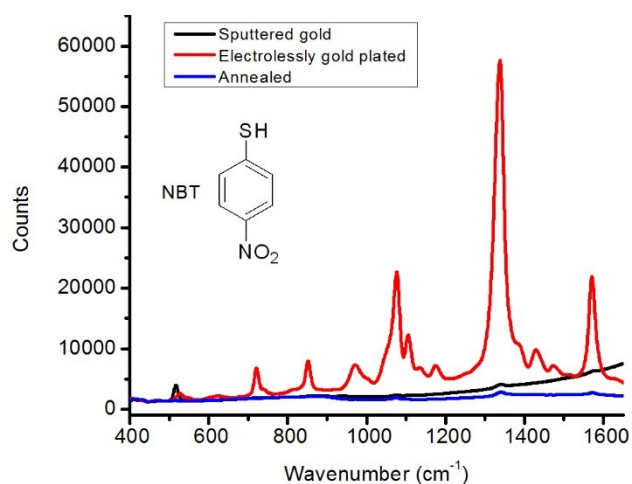


Figure 5. 3. Measured spectra from 1 cm² silicon nitride substrates soaked in 0.01M NBT for 5 minutes: from a substrate electrolessly gold-plated at 3°C for 3 hours (red), from the same chip plasma cleaned, annealed at 280°C for 20 minutes, and plasma cleaned again before NBT exposure (blue), and from a sputtered (30s) gold film (black).

While the electroless gold plating was strongly sensitive to the surface preparation of the silicon nitride, we note, for completeness, that the exposed silicon at

the edges of the chips was consistently gold-plated, regardless of whether the wafer was treated with HF, HNO₃ or NaOH. Polished ~1cm² silicon chips treated according to Scheme 5.1 developed uniform, high-quality gold films across the surface. This result suggests that the silicon-rich nature of our silicon nitride films may contribute to the electroless plating process in Scheme 5.1. Candidate mechanisms for tin-sensitizing silicon nitride thus extend beyond those involving nitrogen-containing surface species¹³. The prospect of definitive elucidation of the mechanism, however, must be weighed in the context of clear precedent in the literature that the complexity of silicon nitride surface chemistry makes it difficult to unravel surface attachment mechanisms²⁰. The chemical complexity of the reagents and supporting media involved in electroless plating further compounds the challenges, compared to physical deposition in vacuum or covalent attachment chemistry in solution. Nevertheless, the steps of various electroless plating approaches have a sound electrochemical basis and the method has a demonstrable outcome¹¹. XPS spectra were recorded from silicon nitride chips after each major step of Scheme 5.1. Selected spectra and details of the analysis are provided in the Supporting Information (Figure S5.2). XPS spectra were also recorded from silicon chips for use as a guide to unravelling the overlapping contributions to the Si2p region of the silicon nitride spectra, especially. HF treatment of the oxygen-plasma-cleaned silicon and silicon nitride caused a significant diminution of oxygen-related peaks at ~104eV (Si2p) and ~533eV (O1s), with the first component no longer evident. These spectral features—including the residual O1s peak that could indicate surface reoxidation generating a small number of surface hydroxyl groups, but has been principally attributed to presumably surface-inaccessible bulk oxygen—are consonant

with those recorded from silicon nitride substrates prepared for direct covalent chemical modification^{9, 20, 22}. The tin(II) treatment steps caused an appreciable widening of the residual, post-HF-etch O1s peaks of silicon and silicon nitride. We subjected silicon and silicon nitride substrates to two control treatments at this stage of Scheme 5.1: in the first, we omitted the hydrofluoric acid step prior to the introduction of the tin solution, and in the second, we prepared the tin sensitizing solution without adding tin. In none of the cases was the appreciable widening of the O1s peak observed. The broad, low-amplitude 102.5eV Si2p peak that appeared after Scheme 5.1 tin-sensitization of silicon also appeared after tin-free control processing, and it suggests submonolayer oxygen coverage that can arise from aqueous processing^{23, 26}. The analogous formation of silicon oxynitride²⁷⁻²⁸ on the silicon nitride substrate would be more difficult to discern from the main Si2p peak due to spectral overlap. Tin oxidation states can be difficult to definitively identify by XPS measurement^{16, 29}, but the shifts of the best-fit ~487eV Sn3d_{5/2} peak to lower binding energy after the addition of silver(I) ions to both substrates (by ~0.5eV for SiN_x and ~0.15eV for Si), would be consistent in direction with the oxidation of tin(II). The Sn3d_{5/2} peaks were affected by the substrate preparation, with ~0.2eV greater width on silicon and silicon nitride substrates that had not been treated with hydrofluoric acid, with an accompanying ~0.4eV shift to higher binding energy on the silicon substrate. Overall, the XPS spectra suggest complex roles for oxygen and tin in the surface sensitization steps and, while the detailed mechanism of sensitization remains unresolved, adherence to Scheme 5.1 exposed the silicon-rich LPCVD silicon nitride surface for direct surface modification and yielded high-quality gold films.

In fact, in spite of complex and challenging surface chemistry, the choice of silicon nitride as a substrate opens a panoply of possible applications for consideration, and the use of a solution-based gold plating method allows us to coat surfaces that are difficult or impossible to reach by line-of-sight metal coating methods. We paid special attention in our development to be able to coat free-standing thin silicon nitride membranes. As a final demonstration of the capabilities of this method, we electrolessly gold plated micropore arrays fabricated in thin (200nm) silicon nitride membranes. Figure 5.4 shows two representative gold-coated 2 μ m micropores, with the first plated into a free-standing portion of the membrane, and the second plated in a region of the silicon nitride pores overlapped with the underlying silicon support frame. Gold plating of the pore walls allows for the straightforward subsequent use of thiol chemistry for surface chemical functionalization. By choosing complementary pore dimensions and gold film thickness, either by fabricating pores with smaller initial sizes, or by increasing the plating time, this electroless plating process can also be used to physically tune the pore dimensions. This method thus provides access to surfaces that may not be accessible to line-of-sight methods, and it moreover provides control over both surface physicochemical properties and physical dimensions of surface and internal pores⁷. In addition, the method is well-suited for tuning and enhancing the properties and performance of thin film and pore-based devices.

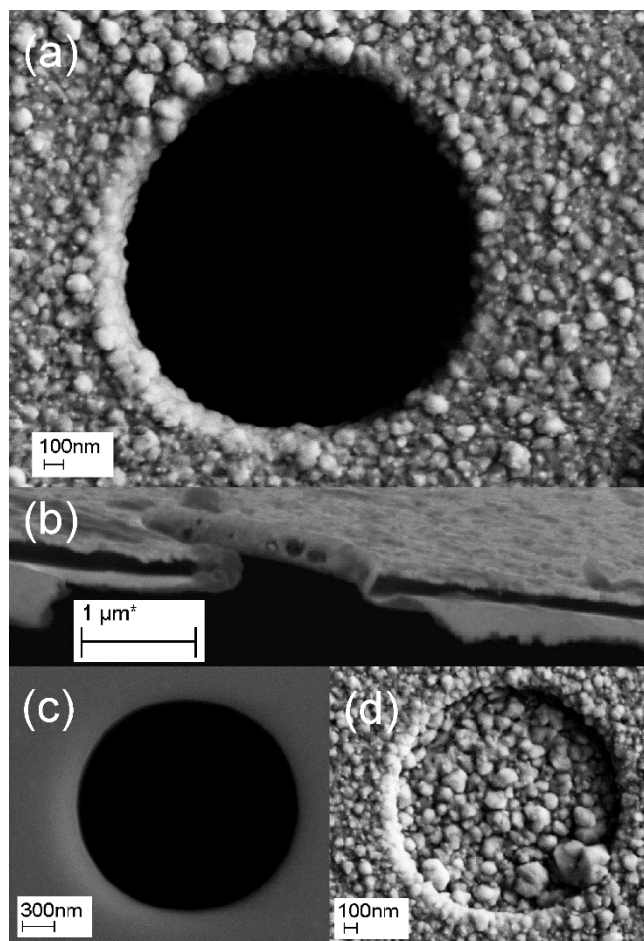


Figure 5. 4. Gold coating can be seen to cover (a) the planar membrane and curved inner pore surface of the free-standing membrane area, with its uncoated equivalent shown in (c). A purposefully fractured membrane in (b) shows the gold coating on the micropore surface and the silicon nitride membrane (dark line) with intact gold coating. In image (d), plating also occurred on the bottom of the 200nm-deep well where it intersects with the silicon substrate.

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AUTHOR CONTRIBUTIONS

All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

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ASSOCIATED CONTENT

Supporting Information Available: Experimental details, methods and sample characterizations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

ABBREVIATIONS

AFM, Atomic Force Microscopy; SEM, Scanning Electron Microscopy; FE-SEM, field-emission SEM; EDS, energy-dispersive X-ray spectroscopy; LPCVD, low-pressure chemical vapor deposition; XPS, x-ray photoelectron spectroscopy; PVP, poly(vinylpyrrolidone); SERS; surface-enhanced Raman spectroscopy.

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CHAPTER 6: PREFACE

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SOLUTION-BASED PHOTO-PATTERNED GOLD FILM FORMATION ON SILICON NITRIDE

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CHAPTER 6: SOLUTION-BASED PHOTO-PATTERNED GOLD FILM FORMATION ON SILICON NITRIDE

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ABSTRACT

Silicon nitride fabricated by low-pressure chemical vapor deposition (LPCVD) to be silicon-rich (SiN_x), is a ubiquitous insulating thin film in the microelectronics industry, and an exceptional structural material for nanofabrication. Free-standing <100 nm-thick SiN_x membranes are especially compelling, particularly when used to deliver forefront molecular sensing capabilities in nanofluidic devices. We developed an accessible, gentle, and solution-based photo-directed surface metallization approach well-suited to forming patterned metal films as integral structural and functional features in thin-membrane-based SiN_x devices—for use as electrodes or surface chemical functionalization platforms, for example—augmenting existing device capabilities and properties for a wide range of applications.

KEYWORDS: Patterned metallization; Photocontrolled metallization; Silicon nitride covalent photomasking; Silicon nitride surface functionalization; Silicon nitride membrane; Thin gold films; Electroless plating; Hydrosilylation.

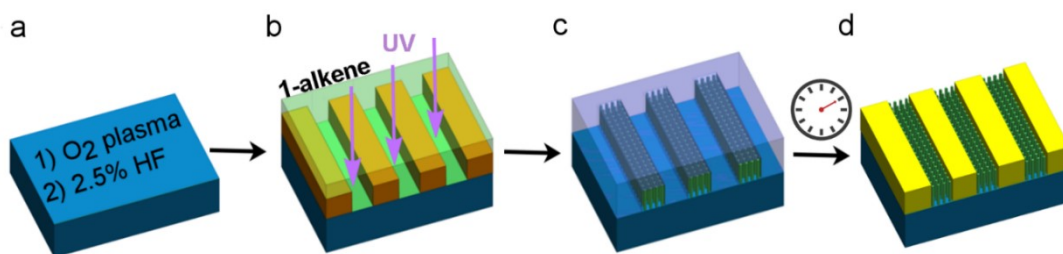
INTRODUCTION

Thin, silicon-rich silicon nitride films prepared by low pressure chemical vapor deposition (LPCVD SiN_x) are a prevalent element of micro- and nanofabricated devices and they can be used to confer mechanical and chemical robustness, diffusion inhibition, and dielectric strength.¹⁻³ Devices and applications exploiting these beneficial native features can be augmented and improved using designer metal overlayers that fulfill structural roles, serve as electrodes, and provide alternative surface chemistry options, including as a platform for subsequent thiol monolayer self-assembly. The field of nanopore single-molecule sensing offers compelling examples of the prospects of merging SiN_x thin films and designer metal layers into devices, and does this within a nanofluidic context where the need for versatile metallizing approaches is clear.³⁻⁷ The most common solid-state nanopores are <100 nm-diameter nanofluidic channels formed through <100 nm-thick, free-standing SiN_x films, and nanopore-integrated metal films can enhance sensing capabilities by serving as optical elements such as light shields and plasmonic films, as electrodes for tunneling and other molecular control and sensing functions, and as a means to tune nanopore size and surface chemistry.³⁻⁸ The nanoscale dimensions of the SiN_x film and pore can be significant barriers to efforts to incorporate such functional metal films, particularly when the interior of the pore must be metallized. Solution-based metallization routes offer an appealing route with natural compatibility with nanofluidic devices. Surface capture of nanoparticles—by specific and nonspecific attachment mechanisms—is a possible solution-based route to surface metallization.⁹⁻¹² Electroless plating is a compelling alternative: a solution-based process useful for metallizing a wide variety of materials, including nonconductive and

irregularly shaped materials.^{7, 13-14} Solution access, rather than line-of-sight as in physical vapor deposition, dictates where surface plating will occur, so that electroless plating is an appealing choice for fashioning nanofluidic devices where even irregular and concealed surfaces may require metallization. To fully exploit solution-based metallization as a tool for micro- and nanofabrication, however, requires control not just over the plated film composition, thickness, and grain size, but also over its spatial disposition, which must be at least partly independent of underlying substrate patterning.¹⁵ We wanted a patterning approach that did not need mechanical access to target surfaces, both to improve the generality of the approach, and to minimize the risk of damage that can accompany repeated handling of thin films—especially of free-standing thin-films. We sought to develop a gentle, solution-based patterned metallization approach¹⁶⁻¹⁷ capable of plating a range of even structured substrates, including inside existing (nano)fluidic channels.^{3, 7, 14-15, 18} The horizons of single-molecule science have recently been dramatically expanded by the development of simple methods for fabricating nanopores: entirely solution-based processes requiring only uncomplicated instrumentation are removing barriers to the widespread use of nanopore methods.¹⁹ To conserve the benefits of simple pore formation methods, our focus also included developing similarly widely-accessible, straightforward solution-based approaches to patterned metallization. We therefore wanted to avoid the instrumentation and processing overhead associated with traditional photoresist-based approaches and more exotic analogues and alternatives.^{11, 20-23} Instead, we chose to photo-pattern the covalent attachment of an organic monolayer to SiN_x ,²⁴ and to investigate its ability to then template the substrate metallization. By only attaching the

protective layer where it was desired, rather than removing portions of a patterned photoresist film, for example, we sought to simplify the processing compared to conventional approaches. With the use of an initially liquid patterning precursor (here, 1-octene), we sought to gain greater tolerance to irregularities—including the presence of engineered structures such as nanofluidic channels—of the SiN_x surface. For metallization, we initially adopted an electroless plating approach that had been specifically developed for gold-plating SiN_x .^{7, 25}

The approach is outlined in Scheme 6.1, and full details of materials, instrumentation, and safety precautions are provided in the Supporting Information (SI). We had previously developed a gold electroless plating approach for SiN_x that required a hydrofluoric acid (HF) etching step prior to surface metallization^{7, 25}. The HF etching step offered a natural point to incorporate patterned monolayer formation in an effort to guide the spatial extent of the substrate metallization. An alkane monolayer could be covalently linked to HF-etched SiN_x through the photochemically-driven hydrosilylation of a 1-alkene.²⁴ Tremendous care must be exercised in the use of HF, and we detail the precautions—including additional protective equipment and monitored work—in the SI. The UV (254 nm) photoirradiation was through copper transmission electron microscopy (TEM) grid masks, with different bar sizes and spacings (see SI for specifications), that had been placed directly on the wafer (without securing them or preventing liquid access underneath), with both wafer and mask then immersed in the 1-alkene. Plating selectivity depended on rigid adherence to the rinsing steps detailed in the SI, and, as in prior work, we ensured compatibility of the process with free-standing ultrathin SiN_x membranes by avoiding ultrasonic cleaning steps.²⁰



Scheme 6. 1: A SiN_x substrate is (a) plasma treated and hydrofluoric-acid etched, then (b) immersed in 1-octene for photopatterning (254 nm) through a TEM grid. The patterned substrate is then (c) immersed in a series of metallizing solutions to yield (d) a patterned gold film. A detailed description of solution compositions and process flow is provided in the SI.

We proposed to spatially pattern LPCVD SiN_x metallization by forming a physical barrier on the surface to control where the metal plating could take place. The first step of patterned plating thus involved the formation of this patterned protective layer. In our prior work to develop an electroless gold plating procedure for SiN_x, we found it was essential to first etch the SiN_x surface with dilute HF.⁷ This same initial etching step forms the starting point for the covalent attachment of 1-alkenes (or 1-alkynes) by photochemical (or thermal) hydrosilylation on silicon-rich SiN_x.^{2, 24} to form alkane monolayers that could potentially function as a barriers for electroless plating. Photoirradiation using a UV lamp (254 nm) proved convenient in transferring the spatial patterning offered by a selection of copper transmission electron microscopy (TEM) grids (Figure 6.1a) to the SiN_x surface. Figure 6.1b is a photograph of a representative substrate after patterned irradiation through a thin (<2 mm) layer of neat 1-octene held under a quartz plate in a specially constructed holder. This optical micrograph taken during the evaporation of a dichloromethane drop placed on the

surface reveals the transfer of the TEM grid pattern to the surface-functionalized substrate. Such patterned substrates were then electrolessly gold-plated, using the three-solution—Sn (II)/Ag (I)/Au (I)—process beginning with Sn (II) sensitization that had been proven successful for HF-etched SiN_x (see SI for complete details of metallization solutions and process flow).^{7, 25} While gold replicas of the TEM grid masks can be seen in Figure 6.1c, it is also apparent that the plating spatial selectivity was quite poor compared to its Pd (II)-initiated counterpart, Pd (II)/Ag (I)/Au (I) (*vide infra*, and calculation details in SI). Substrate tolerance of electroless plating, via substrate tolerance of the Sn (II) sensitization step, is one of the benefits of electroless plating:^{13, 23} it is clearly—in this instance, at least—detrimental to patterned metallization. Figure 6.1d provides a magnified view, by field emission scanning electron microscopy (FE-SEM), of a Sn (II)/Ag (I)/Au (I)-metallized substrate. We did not explore using ultrasonic cleaning steps to improve the plating selectivity,^{20, 26} because we wanted to remain compatible with plating free-standing SiN_x films that are a compelling structural element, especially for nanofluidic devices.³⁻⁷

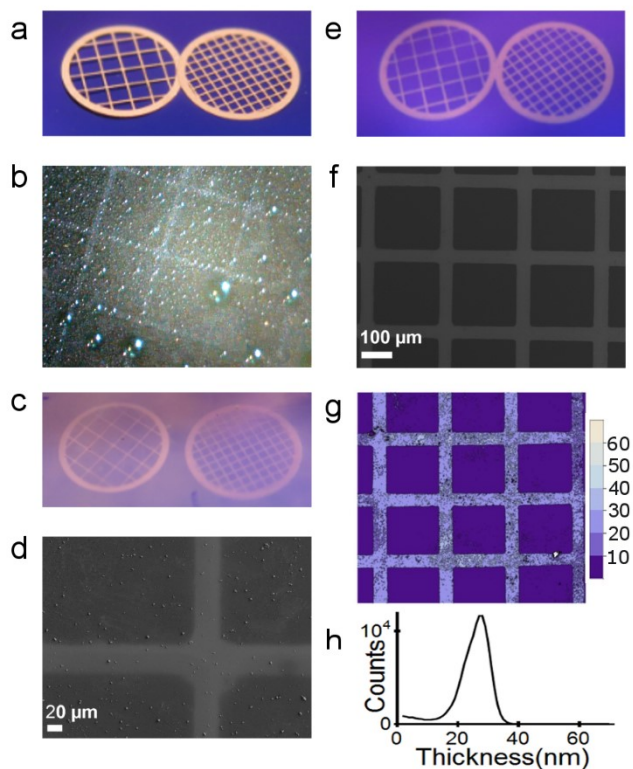


Figure 6. 1: (a) 50 and 100 mesh copper TEM grids on a SiN_x-coated silicon chip; (b) 50 mesh 1-octene replica during the evaporation of a dichloromethane drop from a photopatterned chip, with image contrast, gamma, and brightness adjusted for image clarity; (c) gold replicas after Sn (II) surface sensitization followed by 5 minutes of Ag (I) and 30 minutes of Au (I) at ~3°C, with corresponding (d) FE-SEM image of a 100 mesh pattern; (e) gold replica after Pd (II) surface treatment followed 5 minutes of Ag (I) and 30 minutes of Au (I) at ~3°C, with corresponding (f) FE-SEM, (g) DHM (5× magnification) images of a 100 mesh pattern, with color intensity legend denoting film thickness (nm), and (h) histogram giving the film thickness distribution measured inside the bars of the micrograph in (g).

We abandoned Sn (II)-sensitized electroless plating when efforts to improve the spatial selectivity by using different rinsing steps, for example, proved ineffective. We tested, instead, a palladium-based treatment²⁷ in place of the Sn (II) sensitization step to give an overall process flow of Pd (II)/Ag (I)/Au (I). The use of this Pd (II) surface treatment solution delivered extremely high pattern fidelity, as seen in Figures 6.1e and 6.1f. The rich chemistry of the native SiN_x surface, and of the palladium species, complicates the determination of the mechanism, and indeed may allow for multiple mechanisms to be simultaneously operational.^{3, 13, 23, 28} Figure S-6.1 shows the results of several process chemistry variations, all displaying lower metallized pattern quality than seen in Figures 6.1e and 6.1f. For example, substrate photopatterning through an air layer—likely through a photochemical oxidation route similar to that seen on silicon²⁹—instead of 1-octene (Figure S-6.1) yielded spatial selectivity degraded by smudges of gold across the surface. The patterned monolayer-templated route offers benefits beyond preserving pattern quality. Photohydrosilylation offers lower process overhead and better compatibility with fluidic channels than conventional photoresist-based approaches, and a suitable hydrosilylated monolayer confers some resistance to any subsequent HF etching, but can be readily removed if necessary (Figure S-6.2).^{2, 18,}
²⁴ The metal plating selectivity when using 1-octene with Pd (II) surface treatment as the first step was easily reproducible across scores of patterned gold depositions when scrupulous adherence to the rinsing steps was maintained. The results shown in Figures 6.1e and 6.1f are thus representative and reproducible.

We focus in this work on characterizing the spatial selectivity and the physical structure of the gold layers resulting from this successful initial Pd (II) surface

treatment. We present analyses of gold replicas produced after ~30 minute immersions in the Au (I) bath. This duration provides a balanced perspective of film nascence and degree of spatial selectivity. Examination of gold replicas using digital holographic microscopy (DHM; Figure 6.1g) allowed us to determine that the gold films were $\sim 23 \pm 1.5$ nm thick. Higher magnification scanning electron micrographs in Figure 6.2 upheld the quality of selectivity demonstrated in Figures 6.1e and f. There was only sparse gold coverage where the photoirradiation had installed the protective layer, between the mask grid lines. The gold grid lines, themselves, could be resolved into gold features with 28 ± 5 nm mean diameters providing $\sim 83\%$ surface area coverage (across 15 different grids, with a 13% standard deviation) after the 30 minutes of immersion in the gold plating bath at $\sim 3^\circ\text{C}$. This degree of infilling is high in the context of low-process-overhead patterned metallization steps,³⁰ and particularly when targeting suitability for use with structured surfaces incompatible with more involved conventional patterning, such as in enclosed nanofluidic channels.

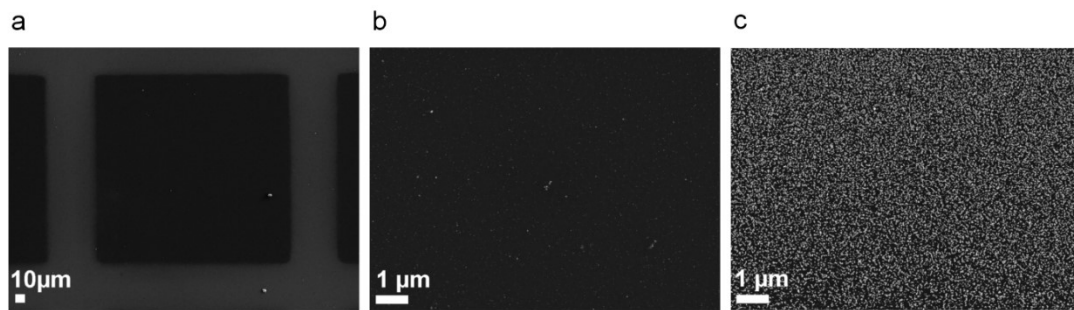


Figure 6. 2: FESEM image of a subsection of a 100 mesh pattern on a SiN_x chip processed with Pd (II), Ag (I), and then Au (I) baths, as detailed in the SI. Vertical and horizontal bars composed of lighter pixels correspond to gold-replicated grid lines on the chip. Zooming into regions outside the bars (b) reveals very little presence of gold grains, confirming the visually observed spatial selectivity as seen in Figure 6.1d.

Zooming into these bars at the same magnification (c) reveals the clear grain structure, and high infilling after only 30 minutes of gold plating.

To explore the spatial patterning in further detail, we focus on gold replicas of 100 mesh copper grids. The copper bars of these grid masks were $54.4 \pm 1.3 \mu\text{m}$ wide (measured by FE-SEM with analysis details in the SI), and they were placed on the SiN_x surfaces under 1-octene without securing them or attempting to prevent liquid access underneath. The spatial selectivity, defined in a classical signal-to-noise sense (details in the SI), was ~ 10.1 for the 1-octene-patterned Pd (II)/Ag (I)/Au (I) route that we focus on here, in contrast to ~ 2.7 for the 1-octene-patterned, Sn (II)-sensitized route, and ~ 3.2 for the former solution steps with air-patterning in place of 1-octene. In addition to FE-SEM micrographs, we collected elemental maps from representative gold replicas using energy-dispersive x-ray spectroscopy (EDS; also commonly abbreviated EDX). The maps and electron micrographs in Figure 6.3a,b are consistent with a thin gold overlayer on SiN_x that possesses a high degree of infilling and spatial selectivity. We used FE-SEM and EDS line profiles across the open spaces and grid lines to characterize the gold replica lines and the edge resolution, with procedural details provided in the SI. The mean line widths of the gold bars in the FE-SEM images of the gold replicas was $44.8 \pm 3.3 \mu\text{m}$, measured from more than 300 lines from each of 9 chips. To extract the edge resolution, we fit the Au-channel EDS intensity versus linear position to Boltzmann functions and recovered sub-micrometer ($0.92 \pm 0.24 \mu\text{m}$; 15 EDS line profiles) transition widths from metal-free to metallized segments.

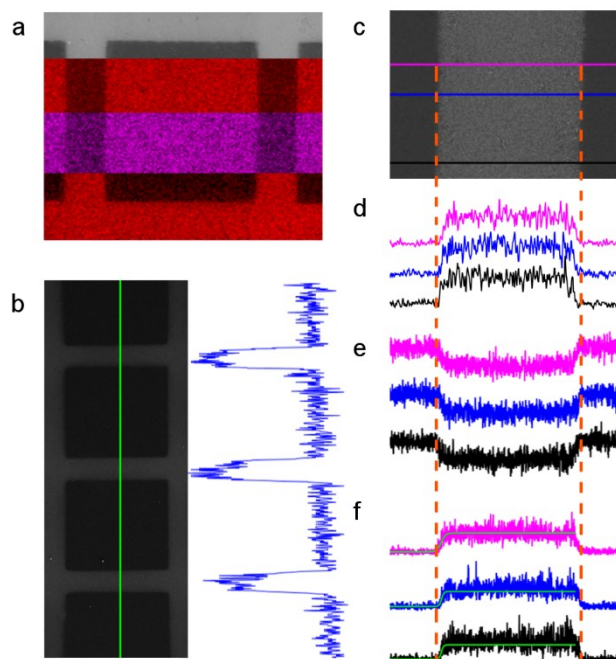


Figure 6. 3: (a) A composite of an electron image (top) and three EDS maps (descending from nitrogen to silicon to gold). (b) FESEM image of a patterned SiN_x chip (left) and pixel intensity (right) taken from the micrograph along the green line. (c) Electron image of a subsection of a 100 mesh pattern on a SiN_x chip. (d) Pixel intensity along each colored line in (c), along with line profiles of spatially-registered EDS maps corresponding to (e) silicon and (f) gold channels (Boltzmann fit is shown in green, with corresponding edge slopes, $dx = 0.81, 0.59, \text{ and } 0.87 \mu\text{m}$ from top to bottom).

We developed a solution-based method to form spatially patterned metal features on silicon-rich SiN_x thin films. This approach leverages the benefits of electroless plating and establishes a low-overhead surface-patterning approach suitable for SiN_x thin films. We ensured that spatial selectivity could be achieved without using ultrasonic excitation or other mechanically disruptive manipulations so that the

patterning approach would be compatible with free-standing thin SiN_x membranes useful in a host of other applications, particularly for nanofluidics. Photochemical hydrosilylation linkage of organic monolayers to SiN_x is a flexible and appealing route to surface-functionalize SiN_x, especially in conjunction with spatial patterning. The templating monolayer may serve as a permanent or removable coating, protecting the underlying SiN_x or being removed to expose it after metallization. The ability to readily modify the surface functional groups of these high quality monolayers using standard chemical transformations² dramatically widens the prospects of this simple patterned metallization approach. The already-excellent metallization selectivity could conceivably be further improved and prolonged by tuning the monolayer electrostatics and hydrophobicity, for example. Similarly, the monolayer surface chemistry could be tuned to promote metal layer adhesion if application needs permit the metal layer to rest on the monolayer, itself.^{9-12, 23} More tantalizingly, a base monolayer may be used as a platform for further chemical tuning of the surface, in which demonstrated properties and function² can be installed around the patterned gold layer. Thus, we contend that the patterned metallization strategy introduced here is promising and useful not only for delivering a spatially-selective solution-derived metal film, but one primed for further development.

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ABBREVIATIONS

SEM, Scanning Electron Microscopy; FE-SEM, Field-Emission SEM; EDS, Energy-Dispersive X-Ray Spectroscopy; LPCVD, Low-Pressure Chemical Vapor Deposition; SiN_x, (Silicon-rich) Silicon Nitride; XPS, X-Ray Photoelectron Spectroscopy; DHM, Digital Holographic Microscopy.

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CHAPTER 7: PREFACE

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A GENERAL STRATEGY TO MAKE AN ON-DEMAND LIBRARY OF
STRUCTURALLY AND FUNCTIONALLY DIVERSE SERS SUBSTRATES

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CHAPTER 7: A GENERAL STRATEGY TO MAKE AN ON-DEMAND LIBRARY OF STRUCTURALLY AND FUNCTIONALLY DIVERSE SERS SUBSTRATES

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KEYWORDS: Surface enhanced Raman spectroscopy; SERS; electroless plating; metallization.

ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) is a powerful technique for sensing molecules proximal to suitable coinage metal surfaces. The physical structure of the SERS-active metal layer and its support is a key design parameter inspiring considerable, and frequently specialized, efforts in substrate fabrication. The necessary gold film structure can arise from both the metallization process and the underlying support structure, and the structure of the support can deliver additional functions including analytical capabilities such as physical filtering. We used electroless plating as a general approach to create a library of SERS substrates: SERS-active gold films on a range of supports made from a variety of materials, made with a mixture of simple and complex fabrication histories, and offering a selection of structurally-derived functions. The result was that supports with existing functions had their capabilities enhanced by the addition of SERS sensing. Electroless plating thus offers a host of beneficial characteristics for nanofabricating multifunctional SERS substrates,

including: tolerance to substrate composition and form factor; low equipment overhead requirements; process chemistry flexibility—including compatibility with conventional top-down nanofabrication; and a lengthy history of commercial application as a simple metallization technique. We gold-plated standard nanofabrication-compatible silicon nitride support surfaces with planar and porous architectures, and with native and polymer-grafted surface chemistries. We used the same plating chemistry to form SERS-active gold films on cellulose fibers arrayed in commercial filter paper and formed into nanocellulose paper. In a functional sense, we used electroless plating to augment nanoporous filters, chromatography platforms, and nanofabrication building blocks with SERS capability.

INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) is a tool at the forefront of chemical analysis for analytes ranging from single molecules to bacterial cells.¹⁻⁵ Raman enhancement is engineered by tuning SERS substrate design parameters such as elemental composition; the size and shape of nanoscale elements; close-range interparticle spacing responsible for hot spots; and patterning of solid substrates that can include ordered and random hierarchies across short, long, and multiple length scales.^{1, 3, 6-10} Physical structure of the SERS-active metal layer—either its inherent structure or the structure imposed upon it by an underlying support layer—is a critical and performance-determining factor. Considerable effort has been devoted to crafting a host of solid-supported SERS substrates, with results that inspire further efforts to improve and expand fabrication options, sensing capabilities, and sensing performance.^{1, 3, 7-26}

Top-down nanofabrication using conventional and unorthodox approaches can produce exquisitely structured substrates, but can require substantial practitioner expertise along with expensive, specialized, and complicated instrumentation, and can moreover substantially limit the palette of fabrication materials. SERS substrates developed outside the material and processing constraints of conventional micro- and nanofabrication have been compelling. Both approaches and material sets hold promise. We sought, therefore, to develop a general route for nanofabricating SERS substrates that would bridge both paradigms—to draw on the strengths of each, and to be useful for both. Conventional micro- and nanofabrication approaches offer well-established, highly optimized, large-scale manufacturing capabilities for reproducibly fabricating nanoscale structures. A less conventional fabrication material such as paper offers a myriad of advantages that have driven its adoption as a material of choice for low-cost diagnostics for use in resource-limited settings.^{23, 27-28} The genesis for the present work was the discovery that gold films we had electrolessly plated onto silicon nitride as part of a nanofabrication effort were also capable, easily and without optimization, of generating reproducible SER spectra.²⁹ We wanted to take a variety of interesting and functional support materials and structures, and determine if a simple electroless plating process could make them SERS-active—thereby augmenting their core functions by creating multifunctional SERS substrates. This goal of multifunction does not exclude the conventional quest for maximum signal enhancement, but does require that SERS substrate evaluation be application-context dependent. Paper, for example, can support a SERS-active metal component, offers obvious advantages such as low-cost and

ubiquity, and has a pore structure that could improve sensing selectivity through separations by chromatography or by physical filtering.^{18-21, 23-26, 28, 30-42}

Electroless plating is a robust technique for surface metallization, well-established in commercial manufacturing applications for forming decorative, electrical, and optical elements, and with excellent substrate tolerance.^{17, 24, 29, 33, 41, 43-52} Objects are immersed in liquid baths, with solution access and homogeneity dictating the uniformity of the plating: rough and large-area surfaces can be coated without the geometric—including line-of-sight—constraints of physical vapor deposition. Equipment overhead is minimal, the surface being plated need not be conductive—allowing for support material tolerance—and the plating occurs without the need for external electrical power. Electroless plating is inherently different than the capture, by nonspecific or specific attachment protocols, of pre-formed, frequently ligand-coated solution-phase nanoparticles onto a surface:^{11-12, 15-16, 18, 30-32, 36-38, 40} the electrolessly plated metal film structure, properties, and composition can be controlled through surface pretreatment, plating bath formulation, and process conditions, and can occur on a timescale that can be measured in minutes. Vitrally important for our pursuit of a library of multifunctional SERS substrates, electroless plating is, in principle, compatible with coating sophisticated top-down nanofabricated, and low-cost bottom-up assembled structures and surfaces.

The term “electroless deposition” is used to describe a number of different plating mechanisms, including autocatalytic, substrate-catalyzed, and galvanic-displacement processes.⁵⁰ We adopted a single electroless plating process that had been

optimized for coating nonconductive porous plastic membranes.⁴⁹ In brief, a Sn (II) solution is used to sensitize the surface which, when treated with an ammoniacal silver nitrate solution, undergoes a redox reaction to produce a nanoscopic metallic silver layer. Gold plating is then accomplished by immersing this surface in a Au (I)-containing plating bath: the aurous ions galvanically displace silver, giving gold particles that catalyze the reduction of aurous ions by formaldehyde also present in the bath. Tin-based sensitizers provide fairly indiscriminate surface sensitization, which is beneficial since tolerance to surface composition is a desired goal of our SERS substrate fabrication explorations. There is also much flexibility in plating chemistry after sensitization, allowing full access to the metals typically used for SERS. While silver coatings can be produced through electroless plating, the chemical stability of gold motivates our testing of gold-coated substrates for SERS activity. The use of a conventional electroless plating protocol, with only minor material-specific modifications in washing steps, allowed us to focus on support material composition and physical structure—and thereby, function—in our exploration of whether electroless plating could be a general tool for incorporating SERS sensing capabilities into already functional and structured materials and platforms.

We selected a range of support structures and material compositions to explore the generality of using electroless plating to form a library of SERS substrates. Silicon-rich LPCVD silicon nitride (SiN_x) films on silicon were chosen for their ability to support a variety of nanofabricated structures and roles.⁵³⁻⁵⁵ Polished SiN_x films ensured the nanoscale gold grain structure would be the dominant substrate structural feature. Silicon nitride films with nanoscale through-channels introduced key structural

features (the individual nanochannels and the nanochannel array) underpinning designer filters and multifunctional chemical analysis platforms using plasmonic nanopores.⁵⁶⁻⁵⁷ Surface-grafting of an acrylate-based polymer generated a more subtle structural modification of the planar SiNx thin film, and was intended to increase the number of possible sensitizer interaction sites on the film. Our next selection was standard filter paper, a frequent actor in paper-based low-cost diagnostics.^{23, 27} We explored the effect of fiber dimensions and spacing, by electrolessly plating and attempting to record SER spectra from standard filter paper and nanocellulose fiber paper—the fourth and fifth choices of material and structure. We characterized a commercial substrate (Silmecco) based on a gold-coated nanopillar array architecture⁹ and etched away its gold coating to expose the sixth surface for examining electroless plating for SERS: a nanopillar array. Given the vastly different SERS substrate configurations, and the often severe approximations necessary to calculate enhancement factors,⁴⁶ we used a comparison framework designed to compare SERS performance across disparate substrates. The method yields a SERS enhancement value (SEV), which is defined as the ratio of the analyte concentrations that produce the same instrument response by normal Raman and SER measurements.⁵⁸ While spectral acquisition was formalized to allow comparisons between substrates, it nevertheless cannot account for the performance benefits of matching substrate function to a particular application.

EXPERIMENTAL

A detailed listing of materials and exposition of methods is provided in the Supporting Information. All substrates were electrolessly gold-plated by sequential

immersion in the same series of tin (II) chloride-, ammoniacal silver nitrate-, and sodium gold sulfite-containing solutions (Scheme S7.1), with appropriate rinsing steps in between immersions. The solutions were prepared as previously reported.^{29, 59} Immediately prior to direct plating of bare silicon or silicon nitride surfaces, they were oxygen-plasma-treated and then etched with dilute hydrofluoric acid. The severe chemical hazards presented by hydrofluoric acid require special precautions such as those detailed in the Supporting Information. A subset of cleaned and etched planar silicon nitride supports was polymer-coated by formation of a covalently-linked sodium polyacrylate film before electroless plating, and once polymer-coated, was treated neither with plasma nor hydrofluoric acid. Silmeco gold-coated nanopillar SERS substrates were used, as-supplied, for comparison measurements. These silicon nanopillar substrates were also immersed in iodide-based gold etchant and then, after plasma treatment and HF etching, electrolessly gold-plated. Whatman 1 filter paper was plated without modification. Nanocellulose fibers were formed between two glass slides into a crude paper-like mat ~1 mm thick (referred to as “nanocellulose paper”) before plating. Surface characterization of the plated metal films was performed by field emission scanning electron microscopy (FE-SEM), x-ray photoelectron spectroscopy (XPS), and surface enhanced Raman spectroscopy (SERS).

SER spectra were acquired at an excitation wavelength of 785 nm, with a ~100 μm diameter (full-width-half-maximum) beam, and at an excitation power of ~57 mW for cellulose and as-provided Silmeco, and ~250 mW for all other substrates. Standard solutions of 4-nitrobenzenethiol (NBT) in ethanol were prepared, covering a concentration range from 5×10^{-9} to 1×10^{-4} M. All measurements (save for replated

Silmeco) were performed with the substrates immersed in the standard solutions. Substrates were immersed in standard NBT solutions and SERS spectra were recorded every 2 minutes until saturation of the signal level. Following piecewise linear background subtraction (details provided in the SI), the data was analyzed according to a framework using receiver operating characteristic (ROC) curves and kinetic analysis to calculate the SEV.⁵⁸

RESULTS AND DISCUSSION

Figure 7.1a shows photographs of the complete set of materials before and after electroless gold plating: we use the term “support” to denote a material prior to gold plating, and the term “substrate” to denote a gold-plated support. All supports were successfully gold-plated by the series of baths of Scheme S7.1, as confirmed by visual inspection and XPS analysis (Figure S7.1). All plated substrates could be used to record SER spectra of 4-nitrobenzenethiol (NBT). The support composition, however, placed restrictions on the experimental parameters. Lower excitation power was required to avoid signal saturation using the as-supplied Silmeco substrates, and substrate damage using the cellulose-based substrates. The higher excitation power left a through-hole in the paper substrate, as shown in Figure 7.1b, and a hollow in the thicker nanocellulose substrate after 10 exposures (~60 s each) when both were irradiated when dry; fume evolution was observed when immersed in ethanol. No damage was apparent when unplated paper that had been soaked in NBT was irradiated, so that the damage mechanism is reasonably ascribed to photothermal transduction by the gold film. This susceptibility of paper to burning is a noted benefit of using paper diagnostics in

resource-limited settings where safe disposal options for biocontaminated devices may be limited.^{23, 27}

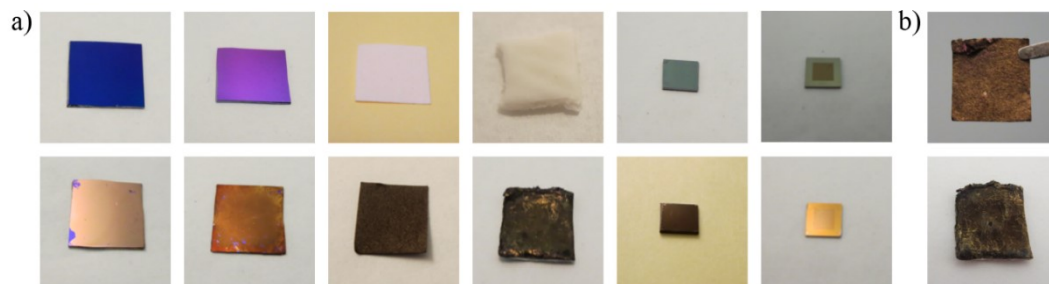


Figure 7. 1. a) Representative substrates before (supports, top row) and after (bottom row) electroless gold plating. Left to right: Silicon nitride, polymer-grafted silicon nitride, paper, nanocellulose paper, nanopillar silicon (Silmeco etched of its as-supplied gold coating), silicon nanoporous substrates. b) Laser-induced damage at 250 mW sets an excitation power limit for paper (top, showing a through-hole) and nanocellulose paper (bottom, showing a hollow in the thicker substrate).

None of the (gold-free) supports produced detectable Raman spectra of NBT at a drop-cast $\sim 10^{-4}$ M test dose, and the (gold-plated) substrate analyte-free background spectra were, excepting a small ~ 1340 cm^{-1} peak in paper, flat and featureless in the key spectral regions used to benchmark the substrate performance (Figure S7.2). Figure 7.2 shows a representative background-subtracted SER spectrum from each substrate type using a 10^{-5} M NBT solution. The principal spectral features are consistent across substrate type, including the most intense signal from the NO_2 symmetric stretch, centered at ~ 1330 cm^{-1} in all spectra. The intensity ratio of this peak to the 880 cm^{-1} ethanol peak, $R_{\text{NBT}/\text{EtOH}}$, was used to construct the response versus concentration curve for each substrate type in Figure S7.3 in the Supporting Information. These response curves had

profiles typical for this class of experiment.^{58, 60} The Raman spectral intensity at a given analyte concentration was strongly dependent upon the support material and preparation, with a substantial penalty in signal strength imposed by the excitation power limitations required by the cellulose substrates. The use of polymer-grafted silicon nitride substrates resulted in the highest signal at all concentrations compared to all other electrolessly plated substrates, most notably when compared at low analyte concentrations. To quantify the SERS performance, representative ROC curves were constructed to calculate the SEV for each substrate: 0.646×10^3 (paper), 0.694×10^4 (porous silicon nitride), 2.34×10^5 (nanocellulose), and 5.91×10^5 (silicon nitride), and at least 9.33×10^5 for both polymer and Silmeco substrates. Following low signal intensities in the test measurement for replated Silmeco substrates in Figure 7.2, we pursued structural characterization (*vide infra*)—instead of further spectral characterization—in an effort to understand this lower response compared to as-supplied Silmeco substrates. For the Silmeco and polymer substrates, even the measurement at the lowest concentration demonstrated a better than 90% probability of detection for a 10% probability of false alarm and due to this, we can report only a minimum SEV.⁵⁸

These results emerged from proof-of-principle experiments of the general utility of electroless plating for SERS substrate creation rather than from longer-term substrate-specific optimizations. They are thus useful, when paired with the demands of a particular application, for indicating where efforts to gain additional enhancement might be warranted. The polymer-grafted silicon nitride is of note not simply for providing the largest SEV of our electrolessly plated substrates, but as an example of the benefits of nanoscale tailoring of SERS substrates, and for serving as a bridge

between substrates based on traditional, silicon-containing nanofabrication materials, and those based on larger organic polymer fibers. More broadly, the design of a SERS substrate type should balance, in an application-specific way, the SEV and any special capabilities, such as filtering, offered by a given substrate. For example, gold films electrolessly plated onto and into these membrane filters can be used to physically optimize filter performance by tuning pore dimensions; to chemically optimize filter performance by serving as a first step in surface functionalization; and to augment filter performance by adding SERS-sensing capabilities in addition to separation.^{29, 61} Ultrathin, nanofabricated membrane filters, such as nanoporous silicon and silicon nitride, offer significant advantages over conventional polymer ultrafiltration membranes.^{54, 62-70} Mechanically robust, unsupported ultrathin filters allow for high hydraulic and diffusive permeabilities. The material properties and ultrathin dimensions allow for the straightforward fabrication of smooth pores in controllable, well-defined sizes with narrow size distributions, and with high areal densities. The short, smooth walls do not suffer the drawbacks of flow resistance and sample losses due to the tortuosity and large surface area of conventional, thicker (polycarbonate) track-etched membranes. Such high-throughput, low-loss nanoporous membranes can be custom-fabricated with pore dimensions and characteristics optimized to filter micrometer-scale organisms such as bacteria, or even to separate macromolecules. Sensitivity might be enhanced by optimizing pore dimensions and distributions to form a nanoplasmonic array,⁵⁶ but at the cost of filtration performance (and selectivity).⁵⁷ A different example of the need to balance SEV and other application demands is illustrated in Figure S7.4: electrolessly gold-coated paper was used for the SERS readout of a crude paper-based

assay that performed physical filtration and chromatographic separation. This multifunction capability augments the spectral selectivity of SERS for greater ease of analysis of multicomponent samples, but by no means circumscribes the utility of SERS-active paper. Indeed, the development of paper-based diagnostics has been characterized by the incorporation—by a variety of approaches, sophisticated and simple—of ever-greater function into paper-based supports.^{23, 27-28, 42}

One means to create useful multifunctional SERS substrates—or even highly optimized SERS-only substrates—is through the deliberate incorporation of carefully selected structural features in the supports. The presence of pores, or voids, in a support has a number of consequences for SERS substrates: the available surface area for sensing can be diminished; the likelihood of hot spot formation can be affected, depending on the spatial extent and distribution of the voids; signal collection can be affected by scattering, line-of-sight access, and focal depth for three-dimensional and structured substrates; mismatches between the excitation volume and the surfaces bearing analyte can limit reproducibility or signal magnitude; plasmonic nanopores, especially in arrays, introduce new optical considerations; and if analyte is delivered by drop-casting, the open area can profoundly affect the spatial distribution of analyte during solvent evaporation. For SERS substrates fabricated using an electroless plating step, the pores can affect the electroless deposition nucleation and growth (by imposing boundaries, for example). These factors include effects that can be much stronger than simple geometric coverage, allowing for considerable parameter space for optimizing performance through the support geometry and through the electroless plating parameters. We recorded scanning electron micrographs, with representative examples

shown in Figures 7.3, 7.4, and 7.5, to gain preliminary structural insights, particularly with respect to the diversity of support structures that could be electrolessly plated. The set of micrographs showed consistently high coverage across the different replicates and substrate types.

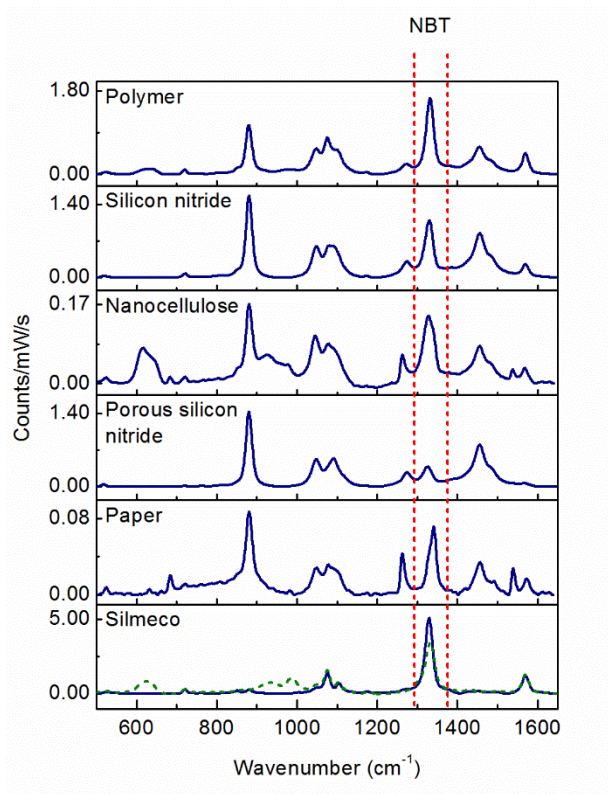


Figure 7. 2. Representative baseline-corrected spectra of each substrate at 10^{-5} M NBT in ethanol (~ 57 mW for cellulose and as-supplied Silmeco; ~ 250 mW for all others). The dotted spectrum in the bottom panel shows the signal (scaled $20\times$) at 250 mW from 5 μL of 1.6×10^{-5} M NBT in acetonitrile drop-cast onto the electrolessly-replated Silmeco. The vertical dotted lines denote the integration range for the NBT peak of interest.

Figure 7.3 provides a set of comparative micrographs of representative gold coatings on the silicon nitride-containing substrates. The uniform through-holes in the

nanoporous membrane are a captivating structural feature compatible with compelling functions,⁵⁶⁻⁵⁷ and the nanoporous membrane was moreover free-standing between support bars (not shown) so that it was electrolessly gold-plated within the pores and on both sides of the membrane. We avoided any ultrasonic cleaning steps that might cause rupture of this thin porous membrane, and we were consistent in this purposeful omission across all substrates. The three substrates were composed of nanostructured gold films with low- and high-aspect ratio grains, but the preponderance and character of the high-aspect ratio structures differed dramatically between the substrate types. The polymer-grafted silicon nitride gold film bore the greatest number of integral high-aspect ratio features, and with a unique grain structure characterized by the prevalence of larger, sharper, and more finely substructured gold flakes that projected from the surface. These flakes provide an increase in surface area for chemisorption of the NBT, and more significantly, are nanostructured on a length scale favorable for the existence of hot spots, and with an aspect ratio amenable to signal enhancement by the lightning rod effect.⁴ The nanoporous substrate imposed gaps between gold grains, although on length scales optimized, in this substrate, for filtering rather than hot spot formation.⁵⁷ The loss of planar substrate area might be compensated for by plating sufficiently long pores, but the nanochannel surface is normal to the conventional substrate surface, and longer pores would affect through-pore flow rates. Overall, detrimental decreases in sensitivity from surface area losses to pores may be quickly outpaced by beneficial gains to analytical performance through the selectivity and throughput that emerges from careful tuning of the pore geometry to support rapid and tuned sample filtering.

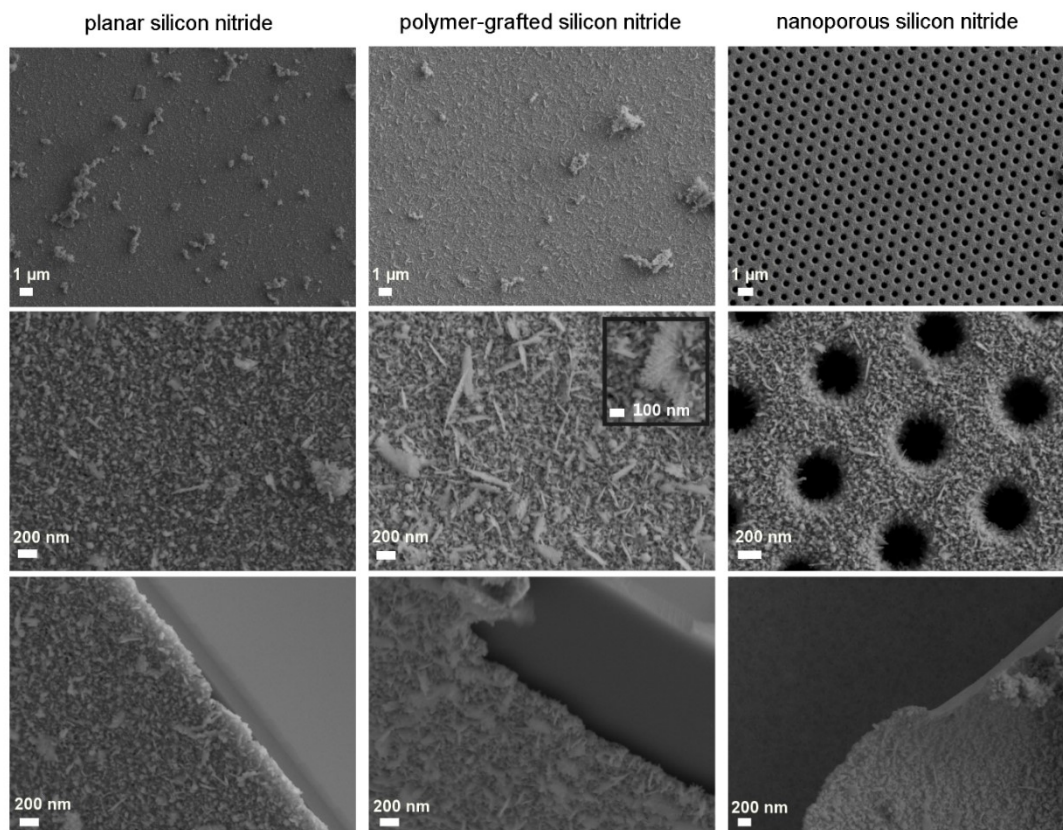


Figure 7. 3. SEM images of, from left-to-right by column, gold-plated silicon nitride, polymer-grafted silicon nitride, and nanoporous silicon nitride. The top two rows show top-down images while the bottom row shows an angled view of gold film cross-sections. The inset in the center micrograph more clearly shows a representative highly-structured flake.

Figure 7.4 shows scanning electron micrographs from electrolessly-plated paper and nanocellulose samples. The paper substrate was distinguished by voids between large fibers constructed of bundled nanoscale fibers. The presence of void spaces in a given layer of the paper is partially compensated by overlap with fibers in underlying layers. The pore, or void space, size distribution in paper can be controlled during its manufacture, and is an important metric when selecting commercial filter paper, for

example. The hand-fabricated nanocellulose substrate was highly textured and convoluted, without the fiber bundling, alignment, and low packing density that produced obvious microscale voids in the paper substrate. The ability of electroless plating to coat rough, nonplanar surfaces—beyond what was seen in the plating of the curved pore walls orthogonal to the planar upper surface of the porous silicon nitride film—is dramatically illustrated by the impressive surface coverage. Thick, porous supports such as the nanocellulose paper have a large surface area for plating—distributed throughout their interior—and require a greater minimum plating solution volume than a planar support. Similarly, most of the plated gold surfaces will be able to bind analyte but will be optically inaccessible, and must be considered when aliquoting samples. Even after addressing these issues, the available signal strength using the cellulose-supported substrates was limited by the lower allowable excitation intensity. The fiber-based construction of the cellulose substrates, however, is an intriguing structural design feature that can provide additional analytical capabilities such as swab sampling and chromatographic separation.^{35,44,71} The cellulose substrates are evocative of other fiber-mat platforms used for SERS,^{11-12, 14-22} with paper supports being available at scale and at low cost using well-established manufacturing methods. When the ability to filter or chromatographically separate a sample using a SERS-active porous substrate is desired in addition to SERS sensing, one must consider the effect of the pore size on each capability—and on the interplay between each capability. Pore size is tunable through support fabrication or through the plating time-dependent thickness—within the limits of cost and available gold in the plating bath—of the plated gold layer. The flexibility, simplicity, and ease-of-handling of these nanofiber-based substrates stand in

stark contrast to the more delicately engineered Silmeco nanopillar arrays, particularly for applications in resource-challenged settings.

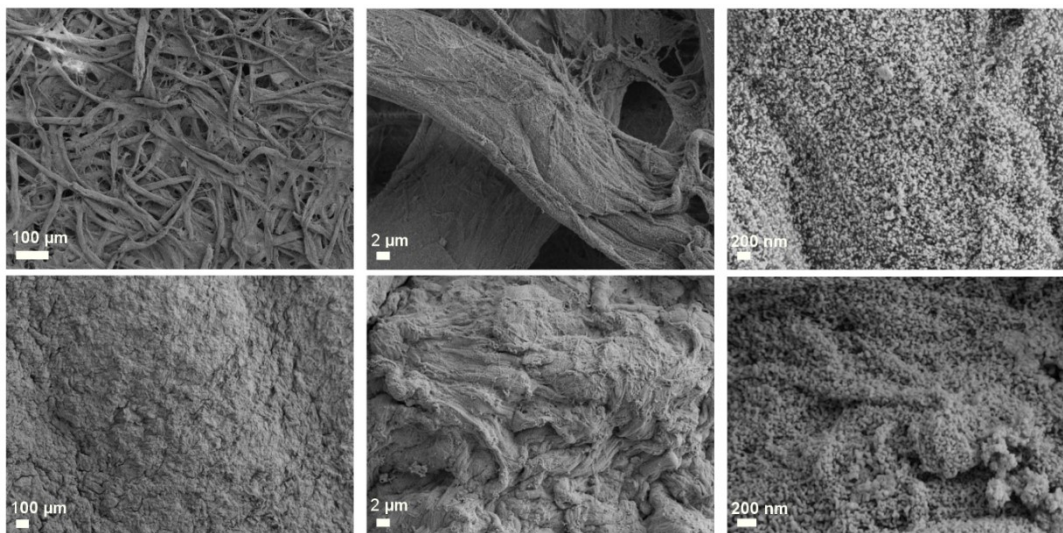


Figure 7. 4. SEM images of gold-plated paper substrates (top row) and gold-plated nanocellulose paper substrates (bottom row).

The superb Raman enhancement that the nanopillar substrates provided when used as-supplied, without modification, reinforces the utility of rationally patterning traditional micro- and nanofabrication materials to create SERS substrates. One must, however, be careful during handling and solution processing to prevent unwanted damage or modification of such high-aspect ratio features:⁹ the gold-etched surface shows some broken nanopillars. SEM images in Figure 7.5 show that our general process chemistry was able to successfully electrolessly gold-plate a nanopillar array. The figure shows a section of electrolessly plated gold film that had peeled back from the nanopillar array surface: the surface of the gold film formerly in contact with the nanopillar array clearly shows dark areas that are consistent with electroless gold plating around extant nanopillars of the array. The dominant structural motifs of as-supplied

Silmeco substrates—recognizable individual gold-encrusted nanopillars with limited numbers of contact points between nanopillars to yield likely hot spots—were not conspicuous in our top-down micrographs of the electrolessly plated substrates. This absence of a key SERS-associated (nano)structure is the most significant contributor to the dramatic loss of spectral intensity when using replated Silmeco. While several of the dark areas of the underside of the gold film are evocative of plating around nanopillars likely already leaning together⁹, optimization of the electroless plating for this nanopillar support would be necessary to deliver the engineered hot spots of the as-supplied substrate. The most reasonable starting point for such an optimization would be to plate pristine gold-free nanoarrays so that the distance between the gold regions of adjacent nanopillars could be controlled by the plating kinetics and time, and any post-plating drying-induced pillar leaning. Producing a nanoarray surface by etching gold from the as-supplied Silmeco handicaps the subsequent replating with the initial structural modification of hot spot formation and the likely damage to the nanoarray of the gold etching step. Nevertheless, the robust gold film formed around nanopillars in this particular micrograph is a compelling reminder of the ability of electroless plating to plate nanoscale structures, and its ability to create, without substantial equipment overhead, SERS substrates from highly engineered supports.

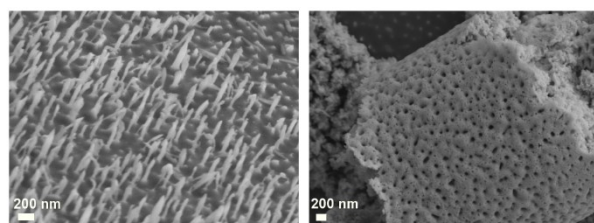


Figure 7. 5. SEM image of a nanopillar substrate after gold etch (left), and with an electrolessly plated gold film peeled off of the underlying nanopillar support (right).

CONCLUSIONS

Electroless plating is a robust method for fashioning a variety of materials, exhibiting a range of structural features and capabilities, into SERS-active substrates. The general electroless plating procedure we employed was able to successfully plate gold onto planar, porous, nanopillar, and fibrous surfaces; into well-defined nanochannels and variably-sized void volumes; onto traditional nanofabrication-compatible materials; and onto less conventional device platform materials such as paper that are important in the domain of low-cost diagnostics. All resulting substrates in our library were capable of generating SER spectra. This electroless plating approach produced nanostructured films where the size, shape, and position of the gold grains could be tuned by the particular material and form factor of the support material being plated, and this tuneability was evident from both microscopic imaging and SERS intensities. The underlying support structure for the gold plating did more than imprint structure on the gold film, though. Electroless plating of already functional structured supports created multifunctional SERS substrates. The force of the work presented here is thus both foundational and prospective: there is much promise in exploring electroless plating—including extensions such as patterned electroless plating^{51, 55}—as a straightforward, robust, and low-overhead method to create custom SERS-active substrates that augment the compelling material properties, structures, and capabilities of their supports. Multifunctional SERS substrates require a rich, and application-specific, context and framework for design and performance evaluation. The substrate must, of course, generate a useful Raman spectrum, but the particular implementation—from design and fabrication to end-use—dictates the balance between Raman

enhancement and other capabilities such as integral sample processing. This balance dictates how to tune the electroless plating process chemistry, and the support structure, to optimize the SERS substrate. We believe that electroless plating has great potential in the creation of multifunctional SERS substrates useful for answering a host of design and sensing challenges.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The following files are available free of charge.

Experimental details, methods and sample characterizations (PDF)

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AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

SERS, Surface enhanced Raman spectroscopy; LPCVD, low-pressure chemical vapor deposition; SiN_x, (silicon-rich) LPCVD silicon nitride; SEV, SERS enhancement value; FE-SEM, field emission scanning electron microscopy; XPS, x-ray photoelectron spectroscopy; ROC, receiver operating characteristic; NBT, 4-nitrobenzenethiol;

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CHAPTER 8: PREFACE

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TASTY, THERAPEUTIC, OR TOXIC? GAUGING THIN-FILM SOLID-STATE
NANOPORES FOR POLYSACCHARIDE SENSING

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CHAPTER 8: TASTY, THERAPEUTIC, OR TOXIC? GAUGING THIN-FILM SOLID-STATE NANOPORES FOR POLYSACCHARIDE SENSING

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ABSTRACT

Polysaccharides have key roles in a multitude of biological functions, and they can be harnessed for therapeutic roles, with the clinically ubiquitous anticoagulant heparin being a standout example. Their complexity—*e.g.* >100 naturally occurring monosaccharides with variety in linkage and branching structure—significantly complicates their analysis in comparison to other biopolymers such as DNA and proteins. More, and improved, analysis tools have been called for, and we demonstrate that solid-state silicon nitride nanopore sensors and tuned sensing conditions can be used to reliably detect native polysaccharides and enzymatic digestion products, to differentiate between different polysaccharides in straightforward assays, to provide new experimental insights into nanopore electrokinetics, and to uncover polysaccharide properties. Nanopore sensing allowed us to easily differentiate between a clinical heparin sample and one spiked with the contaminant that caused deaths in 2008 when its presence went undetected by conventional assays. The work reported here lays the foundation to further explore polysaccharide characterization and develop assays using thin-film solid-state nanopore sensors.

INTRODUCTION

Oligo- and polysaccharides are ubiquitous in nature, with a broad spectrum of roles that includes energy-storage and provision (including as a foodstuff), structural building block (e.g. cellulose), therapeutic function (e.g. the anticoagulant heparin), and a vital part in biological recognition processes.¹⁻¹¹ Conventional chemical analysis tools are frequently challenged by the daunting complexity of polysaccharide analysis:^{12, 13} identification of monomer composition (~120 naturally occurring monomers!) and sequence, monomer linkage types, stereochemistry, polymer length, and degree of polymer branching.¹³ These challenges were tragically driven home in 2008 when undetected contamination of the common anticoagulant heparin by a structurally similar adulterant, oversulfated chondroitin sulfate (OSCS), resulted in profoundly adverse clinical consequences in the United States, including ~100 deaths—underscoring the need for more sensitive sensing methods for contaminant flagging.¹⁴⁻¹⁹ Glycan samples can be challenged by heterogeneity and low abundance in addition to chemical and structural diversity, so while new analysis tools have been broadly called for,^{12, 13, 20} single-molecule-sensitive methods are a particularly compelling goal for glycomics—more so given the absence of sample amplification techniques analogous to PCR for DNA sequencing²¹.

Nanopore single-molecule methods have emerged as a powerful tool for characterizing DNA and proteins including aspects of sequence, structure, and interactions.²²⁻²⁸ Monomer-resolved length determinations of more prosaic

polyethylene glycol samples further buttress the potential of suitably configured nanopore assays for the analysis of polymers with biological utility.²⁹ The simplest implementation for nanopore measurements places the nanopore—a <100 nm-long nanofluidic channel through an insulating membrane—between two electrolyte solutions (Figure 8.1). Ion passage through the nanopore in response to a voltage applied across the pore gives the baseline “open pore” current, i_0 ; passage of a molecule into, across, or through the nanopore disrupts this ion flow to give a blocked-pore current, i_b . A discernible current perturbation reveals the presence of an analyte, and the sign, magnitude, and temporal structure of i_b depend strongly on size and shape of the analyte—and of the nanopore—and on the applied voltage and bulk and interfacial charge distributions. It thus provides insight into analyte presence, identity, and properties, including interactions between the analyte and pore interior or surface.²⁹⁻³² Analysis of the resistive-pulse characteristics of a sample offers the potential to glean molecular-level insights, but the i_b characteristics can also be used more simply as benchmarks in quality assurance assays where atypical i_b signal sample impurities.

Much groundwork must be laid, including proof-of-principle experiments, if nanopore methods are to emerge as a tool for glycan profiling—and by extension as a tool for –omics writ-large (spanning genomics, proteomics, and glycomics). Protein nanopores, polymer, and glass-supported nanopores have been used to detect sugar-pore binding, polysaccharides, and enzyme-digested oligosaccharides.³³⁻⁴² While solid-state nanopores in thin (~10 nm) membranes have been often portrayed as the preeminent nanopore platform, their use to profile classes of molecules beyond DNA and proteins is in its infancy. These nanopores can be size-tuned⁴³ to match analyte dimensions

(especially relevant for branched polysaccharides), and when fabricated from conventional nanofabrication materials such as silicon nitride (SiN_x),^{44, 45} offer resistance to chemical and mechanical insult alongside low barriers to large-scale manufacturing and device integration. The potential for integration of additional instrumentation components, such as control and readout electrodes, around the thin-film nanopore core, is especially compelling.^{28, 44, 45} Recent (nanopore-free) work on recognition electron tunneling measurements on polysaccharides, for example, has reaffirmed the importance of a nanopore development path that values augmented nanopore sensing capabilities.⁴⁶

A key question concerning the use of SiN_x nanopores for polysaccharide sensing is whether this fabrication material is compatible with sensing glycans. The often challenging surface chemistry of SiN_x (giving rise to a complex surface charge distribution)^{44, 45, 47} may lead to analyte-pore interactions that hinder or prevent its use. Variability in polysaccharide electrokinetic mobility arising from differences in molecular structures may exacerbate the effect of these interactions. These issues become particularly important when analyte translocation through a constricted pore is required, such as in transverse electron tunneling measurements.^{28, 46}

The aims of the present work were threefold: (1) to introduce and test the feasibility of SiN_x nanopores for sensing polysaccharides; (2) to explore the preliminary performance of this class of nanopores in this implementation; and (3) to gauge the prospects of a clinically relevant assay to detect a toxic impurity in the anticoagulant heparin. The broader implications of the successful use of SiN_x —a readily nanofabrication-compatible material—to form the nanopores would be to conceivably

smooth the path to large-scale production and to provide a platform amenable to modification for nanopore sensing configurations beyond resistive pulse sensing. We chose a set of polysaccharides with varied compositions to both gauge performance and challenge the SiN_x nanopores. Naturally occurring sodium alginate, with applications in biomedical and food industries, presents an overall negative, but unexceptional, formal charge in neutral pH aqueous solutions. We used samples from two different suppliers—*A1* (Alfa Aesar; $M_n \sim 74$ kDa based on viscosity measurements) and *A2* (FMC Corporation; $M_n \sim 18$ kDa based on viscosity measurements)—to explore the sourcing variability for a sample extracted from seaweed.⁴⁸ This variability can be as prosaic as molecular weight to more enticing changes in the relative abundances of alginate's constituent mannuronate (M) and guluronate (G) residues.⁴⁸ In contrast to alginate, heparin, the prevalent anticoagulant drug, is the most highly negative charge-dense biological molecule known.⁴⁹ This exceptional charge density couples with the demonstrated difficulty, by other methods, of detecting the negatively charged oversulfated chondroitin sulfate (OSCS; contaminant molecular weight ~ 17 kDa⁵⁰) in a heparin sample¹⁴⁻¹⁷ to make the analysis of heparin (~ 16 kDa) and OSCS by nanopore a compelling experimental test with clinical relevance.

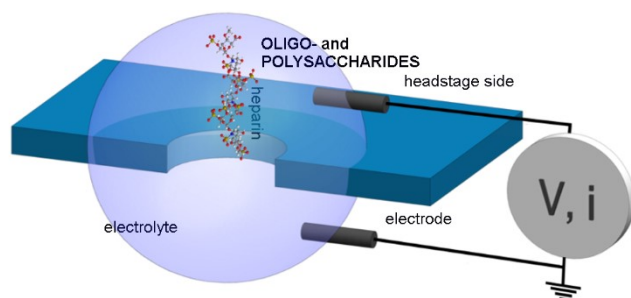


Figure 8. 1: Schematic of the nanopore setup. Analyte was added to the headstage side (“cis-” side, according to nanopore convention) unless otherwise noted, and applied voltages were referenced to the ground electrode (“trans-” side) on the other side.

RESULTS

Introduction of anionic alginate A1 ($M_n \sim 74$ kDa) into the headstage sample well failed to generate detectable transient current changes when a negative headstage voltage (the polarity consistent with purely electrophoretic motion for an anionic analyte) was applied with the analyte in the same well (Figure 8.1). Application of a positive potential, instead, generated transient current changes (here denoted “events”) that could be readily differentiated from the open current noise with $\sim 60:1$ event-to-noise frequency compared to analyte-free scans. Figure 8.2 shows a representative time trace of A1-induced events, with a characteristic event magnified. The frequency of discrete current blockages associated with the addition of A1 could be fit linearly over a reasonable range of analyte concentration (Supplementary Figure 8.1), so that regardless of mechanism, with appropriate measurement conditions, the event frequency can be used to determine the analyte concentration.

The mechanism of A1-induced signal generation was investigated in a series of experiments. Using a setup (Supplementary Figure 8.2) that physically separated

electrodes and nanopore, events were only detected when *AI* was injected into the well proximal to the nanopore, thus supporting a signal generation mechanism involving interaction with the nanopore and not with the electrodes. This result did not, however, distinguish between passage-free collision with the nanopore opening (“bumping” or “blocking”) or translocation through the pore.³² Either mechanism (including extending the idea of “bumping” or “blocking” to allow for transient interactions of the analyte with the pore mouth), though, has the potential to deliver analytically useful sensing performance.

Low analyte concentrations challenge the direct investigation of polysaccharide translocation through small, single nanopores. In one experiment to investigate this, a solution of *AI* was added to the headstage side of a ~22 nm-diameter nanopore and was left overnight with a +200 mV applied voltage. The initially analyte-free contents of the ground-stage side were then transferred to the headstage side of a fresh ~17 nm-diameter pore, and an appreciable number of *AI*-characteristic events (182 in 1 h) were detected again at +200 mV. Acid digestion was used as a signal generation and amplification technique (complete details in the Supplementary Information) to convert *AI* polymers to many smaller fragment-derived species absorbing at ~270 nm.^{51, 52} This spectrophotometric assay (Supplementary Figure 8.3) was used to confirm translocation of polysaccharide through a ~9 nm SiN_x nanopore.

The analyte-induced translocation blockage current, i_b , is expected to be determined by the properties of the analyte and its size relative to the nanopore, among other experimental factors (including interfacial phenomena).^{30, 32} For each individual

current blockage, we calculated the blockage duration, τ , and the fractional blockage current magnitude, $f_b = \langle i_b \rangle / \langle i_0 \rangle$, where $\langle \dots \rangle$ denotes a time-average, and i_0 is the current through the pore when unobstructed by analyte. Plots of number of events as a function of τ and f_b (Figure 8.3) provide an overarching summary of the total current trace. Given detectable differences as a function of analyte, such plots and other representations have the potential to function as analyte fingerprints in quality assurance assays. Fingerprints for *AI* are shown in Figure 8.3, acquired in 1 M KCl, pH ~7 solutions using a +200 mV applied voltage. Supplementary Figures 8.4 and 8.5 provide alternative presentations of the experimental measurements. The (most frequent) f_b increased in magnitude with increasing nanopore radius, r_{pore} (that is, the relative magnitude of the current perturbations due to the analyte were reduced). This parallels the behaviour observed in studies of DNA translocation that could be described using a simple volume-exclusion framework: $r_{\text{analyte}}^2 / r_{\text{pore}}^2 = 1 - f_b$.

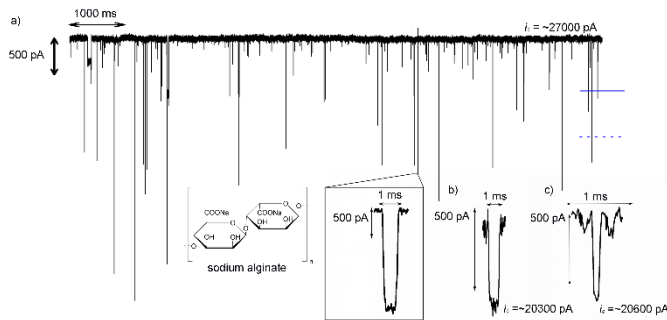


Figure 8. 2. Representative nanopore current trace and events from sodium alginate samples from two different sources. a) A representative segment of an *AI*-induced current trace using a ~22 nm-diameter pore; the solid blue line marks the most frequent event level, $\langle i_b \rangle$, and the blue dashed line is its mean across all events. The magnified

current event is from the same trace. b) *A2-* and c) enzyme-digested-*A2*-associated single events through a ~ 22 nm-diameter pore. All currents were measured in response to a 200 mV applied voltage.

For example, reducing the ion concentration from 1 to 0.1 M KCl increases the Debye layer thickness changing the electrostatic size of the pore with consequences for electrokinetic phenomena, and electroosmosis especially. Comparing Figures 8.3a and 8.3e, this change of concentration did not affect the voltage polarity needed to generate events, but decreased the f_b for the same experimental configuration, and appreciably lengthened the (most frequent) blockage duration. More profoundly, the 10-fold salt concentration decrease reduced the frequency of events 6-fold in the same size ~ 18 nm-diameter pore. We found, and exploited in a more general context for the sensing of heparin and OSCS (below), that such a simple change of electrolyte concentration is a powerful parameter for tuning our ability to sense polysaccharides. Changing the electrolyte pH offers a similar parameter for tuning the sensing performance of nanopores with ionizable surface groups. The surface charge of SiN_x nanopores can be tuned from negative through its isoelectric point ($\sim 4.3 \pm 0.3$) to positive,^{44, 53} and the consequence of this pH change is seen in Supplementary Figure 8.6: the voltage polarity for signal generation is opposite at pH 3 and 5 (and opposite to the electrophoretic direction for all pH values), and the event frequency is at its minimum nearest the isoelectric point and increases with increase and decrease in pH from this point.

After the initial exploratory and proof-of-principle experiments using *A1*, we turned to the second sodium alginate sample, *A2*, obtained from a separate supplier. In general, the interplay between analyte charge density, monomer chemical nature and polymer linkages, and electrolyte composition, is expected to influence nanopore sensing. Experiments showing the polarity-dependence of event occurrence, and its frequency, as a function of pH showed the same qualitative behaviour as for *A1* in Supplementary Figure 8.6, but with lower event frequencies overall. Both alginate samples were readily digested by alginate lyase (Supplementary Figure 8.3),⁵⁴ but infrared spectroscopy showed that *A2* contained a dramatically greater proportion of carboxylate groups than *A1* (Supplementary Figure 8.7), so that the overall charge density of this molecule was expected to be higher than *A1*. Further analysis was consistent with alginate *A1* having a ratio of guluronic (G) to mannuronic (M) residues exceeding that of *A2*, with values from IR spectroscopy of ~63%G/37%M and ~57%G/43%M, respectively.⁴⁸ Nanopore profiling of *A2* showed differences compared to *A1*. Using the same electrolyte for *A2* as for *A1*, measurements generated a ~7-fold lower event frequency with longer durations for *A2* compared to *A1*, despite the 75-fold higher *A2* concentrations required for reasonable measurement times. Enzymatic digestion of *A2* produced events at a higher frequency than for undigested *A2*, but still at lower frequency than for *A1*. The events for the digested sample of *A2* were ten-fold shorter-lived than for the *A2* polymer, but not appreciably different in terms of blockage depth (Figure 8.3).

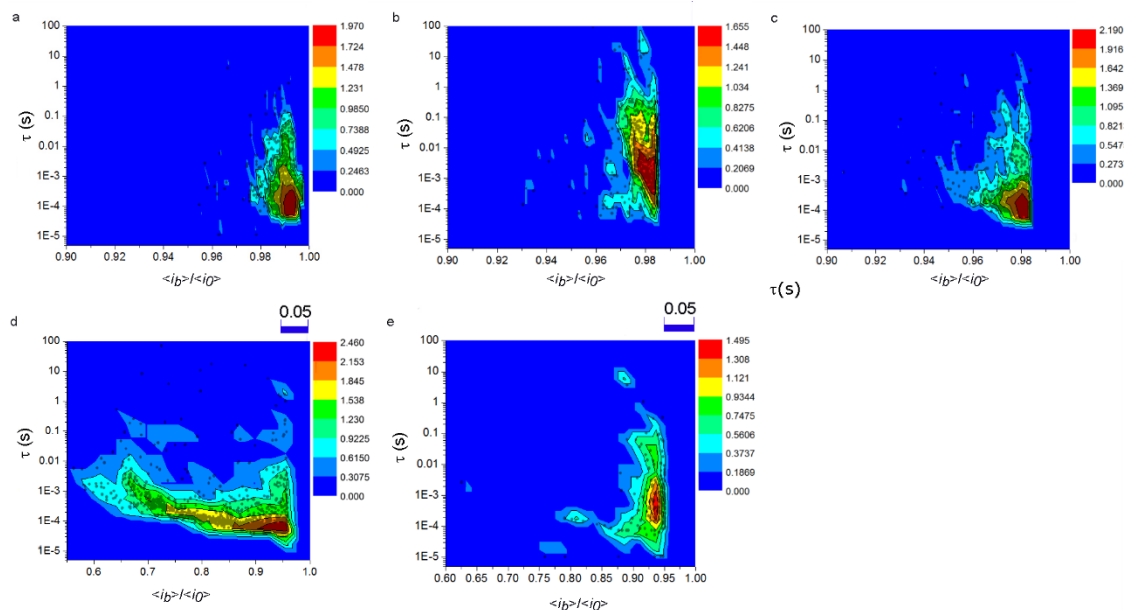


Figure 8. 3: Combination heat map-scatter plots of alginate-induced events. Event counts (plotted as \log_{10} on the colour axis) of a) 4 μL 0.2% (w/v) *A1* using a ~ 19 nm diameter pore (~ 0.321 events/s), b) 20 μL of 3% (w/v) *A2* using a ~ 22 nm (~ 0.046 events/s) and c) 20 μL of 10-minute enzyme digested 3% (w/v) *A2* using a ~ 23 nm diameter pore (~ 0.112 events/s), all in pH ~ 7 buffered 1 M KCl. The experiment in (a) was repeated d) using a ~ 5 nm nanopore (~ 0.403 events/s), and e) an ~ 18 nm-diameter pore, but in 0.1 M KCl (vs. 1M KCl in (a)) electrolyte buffered at pH ~ 7 (~ 0.0527 events/s).

These initial survey experiments showed measurement outcomes with strong sensitivity to analyte identity, with the number of anionic carboxylate moieties being a compelling differentiator between *A1* and *A2*. We then turned to the pressing specific challenge of (anionic) heparin sensing and (anionic) OSCS impurity detection. The first change, from the earlier work, was that the signal generation voltage polarity now corresponded with the conventional electrophoretic direction for an anionic species.

Acid digestion experiments akin to those in Supplementary Figure 8.3 confirmed that heparin could translocate through the pore in response to an applied voltage. As with *AI*, heparin could be detected in 1 M KCl electrolyte, but the heparin event blockage magnitude and event frequency were both greater in 4 M KCl, and so measurements were performed at this higher salt concentration (see Supplementary Figure 8.8 for representative events and a heat map). Plots of event frequency versus heparin concentration were linear (Figure 8.4), with a limit of detection of 0.379 USP heparin units/mL (in a 500 μ L well). In comparison, clinical dosage levels of $\sim 10^4$ units/day using $\sim 10^3$ units/mL stock solutions are not uncommon. Heparin and alginate fingerprints differed in appearance from each other, but also through the profoundly different measurement configuration—opposite applied voltage polarity and fourfold higher electrolyte concentration for heparin—used to acquire them. We were more keenly interested, though, in whether an OSCS impurity in heparin could be detected. We performed measurements on unadulterated USP samples of either heparin or OSCS under identical experimental conditions. On the level of individual events, heparin and OSCS differed in their apparent interaction with the nanopore, with OSCS having a greater propensity to permanently block the pore unless a ~ 1.3 V (“zap”) pulse—a common approach leveraging the electrokinetic basis of analyte motion—was quickly applied when indications suggesting an impending permanent blockage arose. In addition, events associated with the heparin and OSCS samples differed appreciably in the current fluctuations during individual current blockages: OSCS current blockages exhibited $\sim 2\text{--}3\times$ greater current noise, $\sigma(f_b)$, than heparin-induced events. Overall, in spite of considerable overlap in the most frequent event f_b and τ , the distribution of

event characteristics revealed a key difference between heparin and OSCS samples (Figure 8.5 and Supplementary Figure 8.9). Namely, events measured using heparin samples exhibited a longer duration tail in the total event duration distribution, while events measured using OSCS samples exhibited a longer tail in f_b . Measurements of mixtures of heparin and OSCS (16 ppm each) yielded event distributions showing both tails, consistent with the presence of both the heparin therapeutic and its contaminant. We developed an automatic thresholding procedure based on event distribution statistics in f_b and τ (details in the Supplementary Information) to collapse the event distribution fingerprints into recognition flags denoting the presence or absence of each component. In brief, OSCS was declared present when events occurred with $f_{b,\text{sample}} \lesssim \text{mode}(f_{b,\text{USP heparin}}^{\text{binned}}) - 3\sigma(f_{b,\text{USP heparin}}^{\text{binned}})$, and heparin was declared present when events occurred with $\tau_{\text{sample}} \gtrsim \text{mode}((\log_{10} \tau_{\text{USP OSCS}})^{\text{binned}}) - 3\sigma((\log_{10} \tau_{\text{USP OSCS}})^{\text{binned}})$. Figure 8.5 shows the correct recognition of USP heparin, USP OSCS, and a mixture of both, across four trials using nanopores of slightly different sizes. The OSCS contaminant levels detected here were fourfold lower (without efforts to explore a lower bound) than the OSCS detection limit reported in the work that examined and quantified the contaminant in suspect heparin lots.¹⁸

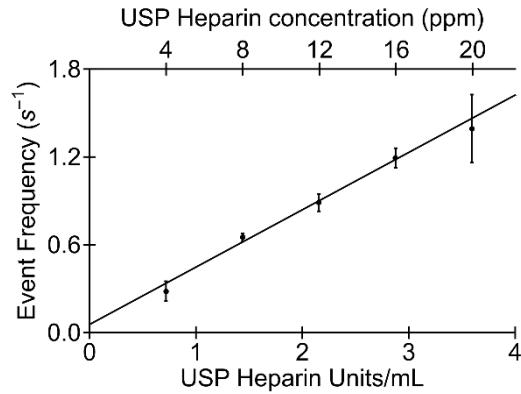


Figure 8. 4: Heparin calibration curve. Three trials were performed, with at least 500 events per run extracted from 900 s-long measurements in a ~ 9 nm pore at -200 mV applied voltage after consecutive addition of $1 \mu\text{L}$ aliquots to the head-stage side of the same nanopore. Error bars are the standard deviation for the three trials.

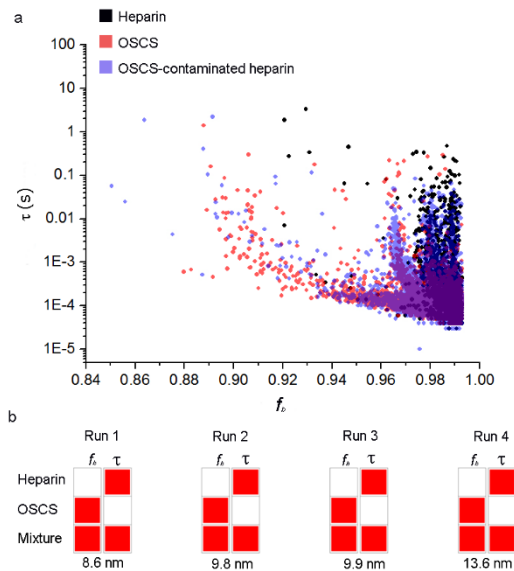


Figure 8. 5: Nanopore resistive-pulse analysis of heparin, OSCS, and their mixture. a) Superimposed scatter plots of $4 \mu\text{L}$ heparin, OSCS and OSCS-contaminated heparin added to 4 M potassium chloride at -200 mV and measured using a ~ 14 nm pore. The colours in the legend correspond to the listed sample, and are blended (using transparency) in the plot where events from different samples overlap. b) Recognition

flags of heparin, OSCS and their mixture from four independent trials accurately identify the presence of the OSCS aliquot in the mixture.

DISCUSSION

We demonstrated the feasibility of using SiN_x nanopores to characterize glycans exhibiting a variety of chemical compositions, including a prevalent therapeutic, heparin. The extremely high charge density carried by heparin poses a particular challenge to a nanoscale sensor element that can, itself, be charged. More generally, unwanted interactions between analyte and nanopore—and the ease and feasibility of ameliorative steps—can imperil nanopore-based experiments: that none of the diverse polysaccharides considered here catastrophically clogged the nanopore—even when subjected to the stringent test of translocation through the pore—was salutary.⁴⁷ Indeed, nanopore sensing was successful over a number of electrolyte concentration ranges, from 0.1 to 4 M KCl, for which shielding of the charged nanopore surface would be quite different in degree. With translocation possible through SiN_x nanopores, even with their charged surface, a rich set of nanopore-based sensing configurations should be within reach.

In this work, we used a straightforward resistive-pulse sensing paradigm to readily detect and differentiate between different polysaccharides, including enzymatic digestion products and two separate alginate samples differing in relative monomer composition. We used voltage polarity and electrolyte composition alongside the distribution of events as a function of f_b and τ to construct fingerprints and recognition flags characteristic of each sample. Linear calibration curves show that these

measurements easily support concentration determinations in addition to analyte recognition.

From a fundamental perspective, nanopores can be a powerful tool for exploring molecular, interfacial, and intermolecular phenomena, often arising from only simple changes of experimental conditions. Electrolyte-dependent interfacial interactions—at nanopore and molecule surfaces—are complex, and treatments of widely varying levels of sophistication have emerged from decades of experimental and theoretical studies of the canonical nanopore-DNA system, in particular.³² For example, changes of electrolyte concentration have been observed to reverse the sign of the current perturbation in DNA translocations through solid-state nanopores, and to decrease dextran sulfate blockage frequencies while increasing their durations using ~1.3 nm-diameter pores where the Debye length was comparable to the pore dimensions.^{42, 55} With the larger pores used here, overlapping Debye layers would not be expected in 0.1 M KCl solutions, leaving three expected principal effects of lowering the electrolyte concentration from 1 M KCl: a lowering of the potential across the pore and thus of the overall electrophoretic force on an analyte near the pore; a reduction in the available number of bulk ions displaced by the analyte volume; and a change in the ion distribution around charged interfaces—the nanopore and analyte surfaces—that influences the nanopore signal through a complex overall mechanism within a given experimental configuration.

Blockage magnitudes measured here in the more conventional 1 M KCl would be consistent with, in a simple volume exclusion sense ($r_{\text{analyte}}^2 / r_{\text{pore}}^2 = 1 - f_b$), translocation of linearized polysaccharides. Deeper blockages would be expected from

the polysaccharides here with hydrodynamic radii on par with the nanopore diameters. Polysaccharide translocation was independently confirmed and signals were generated only when the analytes had access to the nanopores, so these events either arose from analyte interactions with the pore mouth rather than from complete translocation, or the blockage magnitude analysis must include additional factors such as charge density carried by the analyte, itself, and mobile charge at the analyte-solution and solution-nanopore interfaces.^{55, 56} The effects of these and more complex interfacial phenomena emerged in one of the more startling observations in this work: that the voltage polarity for signal generation with both alginate samples was opposite to that expected for electrophoretic motion of an anionic polymer, whereas for heparin the voltage polarity was consistent with electrophoresis.

In addition, when comparing the two alginates, the more charge-rich *A2* was detected at a lower event frequency than *A1*. Nanopore-based studies with polyethylene glycol polymers point to a change of effective analyte charge by sorption of electrolyte ions (K^+ for those studies) with the resultant analyte motion then being electrophoretic for the voltage polarity and the sign of the sorbed charge.²⁹ The results of Supplementary Figure 8.6, however, point to pH-dependent changes in the voltage polarity required for sensing alginates, with the polarity having opposite signs on either side of the isoelectric point of SiN_x . Mirroring this change in the voltage polarity is the SiN_x surface charge that is positive at lower pH and negative at higher pH. This change in surface charge sign causes a reversal in the direction of electroosmotic motion for a fixed voltage polarity (and thus fixed electrophoretic direction).^{44, 45}

The apparent mobility of an analyte in response to electrolyte flow through the surface-charged nanochannel is the sum of its electrophoretic and electroosmotic mobilities. Changes of solution pH can then tune the apparent analyte mobility and even overall direction of analyte motion. Changes of solution pH can also affect the charge density and sign of analytes (and thus the voltage polarity required for electrophoresis in a given direction) containing at least one acidic or basic functional group as determined by the balance of acid-base equilibria (determined by functional group abundance and pK_a). Given the acidic functional groups in the analytes here, the changes in nanopore surface chemistry should dominate the effective mobility and its voltage polarity dependence.

The event frequency and voltage polarity behaviours are consistent with the distinct physicochemical properties of each analyte in a signal generation method in which both electrophoresis and electroosmosis occur simultaneously. Alginate *A1* has the lowest charge density, and thus its electrophoretic response is dominated by electroosmosis with the electrophoretic and electroosmotic driving forces being in opposition in the negatively charged SiN_x pores at $\text{pH} \sim 7$. Alginate *A2* is more negatively charged and so one would anticipate a stronger electrophoretic driving force; the direction of signal generation is still consistent with electroosmosis. The lower event frequency compared to *A1* can be understood as arising from opposing electrophoretic and electroosmotic driving forces, but with the electrophoretic force on *A2* being greater than on *A1*. More detailed exploration of the differences between *A1* and *A2* must also contend with their different molecular weights and their different chain flexibilities arising from their different M/G ratios. In the case of heparin, the charge density is

sufficiently high so that events are detected using a voltage polarity that would drive the anionic polymer towards the nanopore.

The experimental investigations including and beyond the ones presented here, exploring the underpinnings of the nanopore-generated signal using (polysaccharide) biopolymers with greater chemical and structural complexity than the canonical nanopore test molecule, DNA, or than homopolymers such as polyethylene glycol, should also provide fertile ground for high-level simulations. Interfacial effects will require additional study in the context of polysaccharides, but hold possibilities for tuning sensing selectivity and sensitivity. Indeed, explicit consideration of sensing conditions—including nanopore size, electrolyte composition, and voltage polarity—already augments the ability to compare nanopore molecular fingerprints as shown in Figure 8.3.

The failure in 2008 to detect an OSCS contaminant in clinical heparin samples had previously led to patient morbidity and mortality—stressing the need for more sensitive sensing methods for contaminant flagging,¹⁴⁻¹⁸ so that our ability to use a simple nanopore-based assay to quantify heparin levels and detect OSCS at clinically meaningful contamination levels, is itself significant. In a broader sense, we expect that these initial results exploring polysaccharide structure can, by analogy with earlier nanopore DNA and protein sensing supporting genomics and proteomics, spotlight the potential of using nanopores as a tool for glycomics. The demonstration of polysaccharide translocation through nanofabrication-compatible SiN_x nanopores portends the development of more sophisticated sensing schemes as seen in the use of nanopores for genomics. Similarly, the successful use of chemical tuning—of

electrolyte composition and by enzyme addition—to alter the nanopore signal generated by diverse polysaccharides suggests that nanopore glycomics might borrow from and extend upon similar approaches developed for nanopore genomics. There is an ongoing need in glycomics for new tools to cope with the analytical challenges caused by the structural and physicochemical complexity of polysaccharides, and by the often inherently heterogeneous nature of naturally derived carbohydrates. The demonstrations of nanopore sensing here provide a beachhead for ongoing efforts to develop solid-state nanopores as a promising platform technology for glycomics.

METHODS

A full listing of the experimental details is available in the Supplementary Information. Nanopores were formed via dielectric breakdown⁴³ in nominally 10 nm-thick silicon nitride (SiN_x) membranes. Nanopore sizes were inferred from their conductance, G , determined from Ohmic current-voltage data. Nanopores used for measurements produced stable open-pore (analyte-free) currents in the electrolyte solutions used. Polysaccharides were commercially obtained: sodium alginate samples from two different sources - *A1* (Alfa Aesar, Ward Hill, MA) and *A2* (FMC Corporation Health and Nutrition, PA, USA); USP heparin sodium salt; and USP OSCS. For routine measurements, sample aliquots were added to the headstage side (Figure 8.1), leaving the ground side free of initially added analyte. Current blockages were extracted using a current-threshold analysis. All applied voltages are stated with the polarity of the electrode on the headstage side relative to ground on the ground side of the sample cell.

Code Availability. Labview source code to view the current event files can be supplied upon request.

Data Availability. The datasets generated during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

All authors have contributed to, and approve, the manuscript.

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SUPPORTING INFORMATION

SUPPORTING INFORMATION FOR CHAPTER 2: NANOPORE SURFACE COATING DELIVERS NANOPORE SIZE AND SHAPE THROUGH CONDUCTANCE-BASED SIZING

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Table S2. 1. Listing of nanopore radial profiles with the corresponding volume (A) and surface (B) integrals from equations (3) and (4).

Nanopore radial profile	A	B
Exponential-cylindrical	$\frac{\pi r_0^2}{L} \frac{1}{y + b r_0(1 - y)(1 - b r_0 e^{b r_0} \Gamma(0, b r_0))}$	$\frac{2\pi r_0}{L} \frac{1}{y + b r_0(1 - y)e^{b r_0} \Gamma(0, b r_0)}$
Conical-cylindrical	$\frac{\pi r_0^2}{L} \frac{x}{y(x - 1) + 1}$	$\frac{2\pi r_0}{L} \frac{x - 1}{y(x - 1) + (1 - y) \ln x}$
Cylindrical	$\frac{\pi r_0^2}{L}$	$\frac{2\pi r_0}{L}$
Conical	$\frac{\pi r_0^2}{L} x$	$\frac{2\pi r_0}{L} \frac{x - 1}{\ln x}$
Hyperbolic	$\frac{\pi r_0^2}{L} \frac{\sqrt{x^2 - 1}}{\tan^{-1} \sqrt{x^2 - 1}}$	$\frac{2\pi r_0}{L} \frac{\sqrt{x^2 - 1}}{\ln(\sqrt{x^2 - 1} + x)}$

where $\Gamma(a, s) \equiv \int_s^\infty t^{a-1} e^{-t} dt$, the incomplete gamma function, $x = R/r_0$, and $y = l/L$. Rearranging the expressions above after substitution of x and y reproduces the equations in Ref. 27, save the absorption of the constants 2 and π into A and B in equation (8).

SUPPORTING INFORMATION FOR CHAPTER 3: REAL-TIME PROFILING OF SOLID-STATE NANOPORES DURING SOLUTION-PHASE NANOFABRICATION

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Notation	Definition
r_0	limiting nanopore radius
R	pore opening radius ($R = r_0 + 10$ nm, except for the cylindrical
z-axis	principal rotation axis of the nanopore along its length
$r(z)$	radius of the pore at a given location along the z axis of the nanopore
$L_{a-b}, L_{b-c} \dots$	length of a region of the nanopore surface along the z-axis of the nanopore between the subscripted points
L	total nanopore length
l	inner nanopore length of conical-cylindrical profile
α and β	angles defining the curved sections of the coating deposited onto the
Δr_i	thickness of the deposited nanopore coating

Table S3. 1. Definitions of notation used in describing the nanopore profiles.

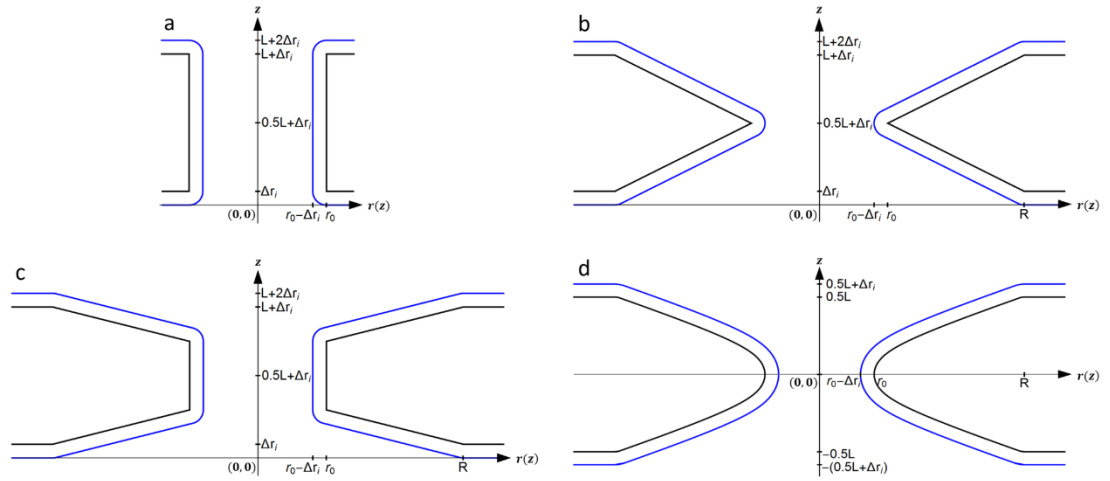


Figure S3. 1. 2D cross-sections of pristine (black lines) (a) cylindrical, (b) double conical, (c) conical-cylindrical and (d) hyperbolic nanopore profiles modified uniformly across their surfaces by a thickness of Δr_i (blue lines).

Region	Cylindrical	Double conical	Conical cylindrical	Hyperbolic
Profiles	$\beta = \frac{\pi}{2}$	$R = r_0 + 10 \text{ nm}$ $\beta = \tan^{-1}\left(\frac{L}{2 \cdot (R - r_0)}\right)$ $\alpha = \pi - 2\beta$	$R = r_0 + 10 \text{ nm}$ $\beta = \tan^{-1}\left(\frac{L - l}{2 \cdot (R - r_0)}\right)$ $\alpha = \frac{\pi}{2} - \beta$	$R = r_0 + 10 \text{ nm}$ $\beta = \tan^{-1}\left(\frac{R \cdot L}{2 \cdot (R^2 - r_0^2)}\right)$ $b = \sqrt{\left(\frac{L}{2}\right)^2 \cdot \frac{r_0^2}{R^2 - r_0^2}}$
a-b	$r(z) = r_0$	$r(z) = R$	$r(z) = R$	$r(z) = R$
b-c	$r(z) = r_0 - \Delta r_i \cdot \cos \theta$ $\theta \rightarrow \beta \text{ to } 0$ $L_{b-c} = \Delta r_i$	$r(z) = R - \Delta r_i \cdot \sin \theta$ $\theta \rightarrow 0 \text{ to } \beta$ $L_{b-c} = \Delta r_i \cdot (1 - \sin \beta)$	$r(z) = R - \Delta r_i \cdot \sin \theta$ $\theta \rightarrow 0 \text{ to } \beta$ $L_{b-c} = \Delta r_i \cdot (1 - \sin \beta)$	$r(z) = R - \Delta r_i \cdot \sin \theta$ $\theta \rightarrow 0 \text{ to } \beta$ $L_{b-c} = \Delta r_i \cdot (1 - \sin \beta)$

c-d	$r(z) = r_0 - \Delta r_i$ $L_{c-d} = L$	$r(z) = (R - \Delta r_i \cdot \sin \beta) - y \cdot \tan \beta$ $y \rightarrow 0 \text{ to } \frac{L}{2}$ $L_{c-d} = \frac{L}{2}$	$r(z) = (R - \Delta r_i \cdot \sin \beta) - y \cdot \tan \beta$ $y \rightarrow 0 \text{ to } \frac{(L-l)}{2}$ $L_{c-d} = \frac{(L-l)}{2}$	$r(z) = r_0 \cdot \sqrt{\left(1 + \frac{y^2}{b^2}\right)} + \Delta r_i$ $\cdot \sin \left(\tan^{-1} \left(\frac{r_0 \cdot \sqrt{\left(1 + \frac{y^2}{b^2}\right)} \cdot b}{y \cdot r_0^2} \right) \right)$ $y \rightarrow -\frac{L}{2} \text{ to } 0$ $L_{c-d} = \frac{L}{2}$
d-e	$r(z) = r_0 - \Delta r_i \cdot \cos \theta$ $\theta \rightarrow 0 \text{ to } \beta$ $L_{d-e} = \Delta r_i$	$r(z) = r_0 - \Delta r_i \cdot \cos \left(\frac{\alpha}{2} - \epsilon \right)$ $\epsilon \rightarrow 0 \text{ to } \alpha$ $L_{d-e} = 2 \cdot \Delta r_i \cdot \sin \frac{\alpha}{2}$	$r(z) = r_0 - \cos \theta$ $\theta \rightarrow \alpha \text{ to } 0$ $L_{d-e} = \Delta r_i (1 - \sin \alpha)$	$r(z) = r_0 \cdot \sqrt{\left(1 + \frac{y^2}{b^2}\right)} - \Delta r_i$ $\cdot \sin \left(\tan^{-1} \left(\frac{r_0 \cdot \sqrt{\left(1 + \frac{y^2}{b^2}\right)} \cdot b}{y \cdot r_0^2} \right) \right)$ $y \rightarrow 0 \text{ to } \frac{L}{2}$ $L_{d-e} = \frac{L}{2}$
e-f	$r(z) = r_0$	$r(z) = (R - \Delta r_i \cdot \sin \beta) - y \cdot \tan \beta$ $y \rightarrow \frac{L}{2} \text{ to } 0$ $L_{e-f} = \frac{L}{2}$	$r(z) = r_0 - \Delta r_i$ $L_{e-f} = l$	$r(z) = R - \Delta r_i \cdot \sin \theta$ $\theta \rightarrow \gamma \text{ to } 0$ $L_{e-f} = \Delta r_i (1 - \sin \beta)$
f-g	-	$r(z) = R - \Delta r_i \cdot \sin \theta$ $\theta \rightarrow \beta \text{ to } 0$ $L_{f-g} = \Delta r_i \cdot (1 - \sin \beta)$	$r(z) = r_0 - \cos \theta$ $\theta \rightarrow 0 \text{ to } \alpha$ $L_{f-g} = \Delta r_i \cdot (1 - \sin \alpha)$	$r(z) = R$
g-h	-	$r(z) = R$	$r(z) = (R - \Delta r_i \cdot \sin \beta) - y \cdot \tan \beta$ $y \rightarrow 0 \text{ to } \frac{(L-l)}{2}$ $L_{g-h} = \frac{(L-l)}{2}$	-

h-i	-	-	$r(z) = R - \Delta r_i \cdot \sin$ $\theta \rightarrow \beta \text{ to } 0$ L_{h-i} $= \Delta r_i(1 - \sin \beta)$	-
i-j	-	-	$r(z) = R$	-

Table S3. 2. Geometric profiles and equations describing nanopore shapes before (black line) and after (blue line) a uniform surface modification of thickness of Δr_i over the entire pore surface. We provide the equations that determine the nanopore profile, $r(z)$, for the piecewise integration, between points labelled with undercase letters, of volume (A) and surface (B) integrals.

METHOD OF CALCULATING VOLUME (A) AND SURFACE (B) INTEGRALS

Integrals were calculated using Mathematica 10.3.1 (Wolfram Research, Champaign, IL) in the following manner,

$$A = \left(\int_{z_{\text{initial}}}^{z_{\text{final}}} \frac{dz}{\pi(r(z))^2} \right)^{-1} \cong \left(\int_{z_{\text{initial}}}^{z_{\text{final}}} \frac{dz}{\pi(r_{\text{int}}(z))^2} dz \right)^{-1}$$

$$B = \left(\int_{z_{\text{initial}}}^{z_{\text{final}}} \frac{dz}{2\pi \cdot r(z)} \right)^{-1} \cong \left(\int_{z_{\text{initial}}}^{z_{\text{final}}} \frac{dz}{2\pi \cdot r_{\text{int}}(z)} dz \right)^{-1}$$

where $r_{\text{int}}(z)$ is a 3rd-order polynomial interpolation of $r(z)$ sampled with a step height, $\Delta z = 0.0001$ nm, along the z -axis from z_{initial} to z_{final} . Here, z_{initial} and z_{final} are 0 and L for all profiles except the hyperbolic profile for which they are set to $-L/2$ and $L/2$, respectively.

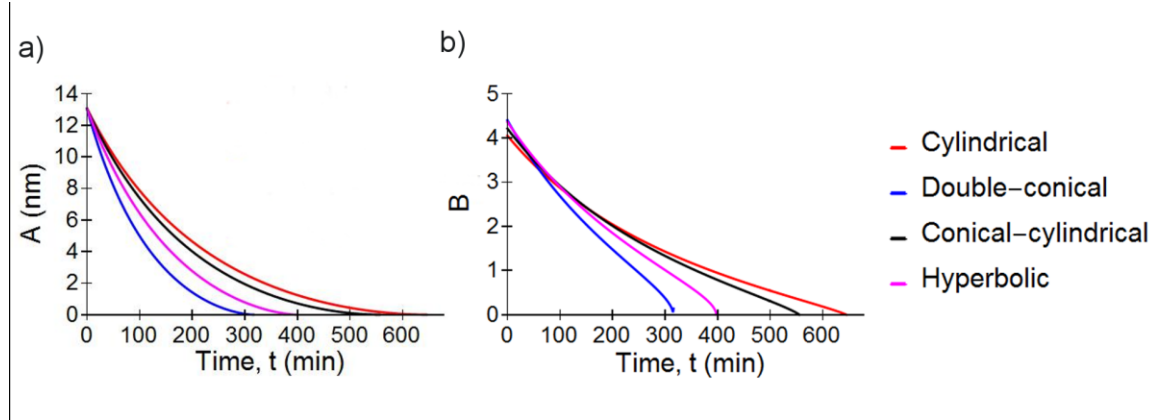


Figure S3. 2: As 10 nm-long nanopores of different shapes, all with initial conductances of 200 nS are progressively reduced in size due to material deposition, the profile-dependent decreases in the conductances are caused by profile-dependent changes in the underlying geometry integrals, A and B .

TUTORIAL: Stepwise Construction of Figure 3.4.

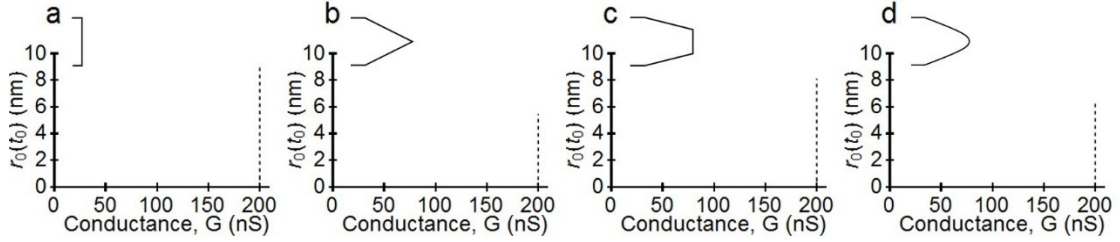
Generating the experimental data for a cylindrical experimental nanopore.

An experimental first conductance, $G_{\text{cylindrical}}^{\text{expt}}(t_0) = 200 \text{ nS}$ is simulated using a cylindrical model with $(r_{0,\text{cylindrical}}^{\text{expt}}(t_0), L_{\text{cylindrical}}^{\text{expt}}(t_0)) = (3.5 \text{ nm}, 3.8 \text{ nm})$. We calculate $G_{\text{cylindrical}}^{\text{expt}}(t_1) = \sim 114.5 \text{ nS}$ after a $\Delta r_1 = 0.5 \text{ nm}$ decrease in the pore radius. Similarly, $G_{\text{cylindrical}}^{\text{expt}}(t_2) = \sim 67.3 \text{ nS}$ is calculated after a $\Delta r_2 = 1.0 \text{ nm}$ change in the pore radius.

Step 1: First conductance value, $G_{\text{cylindrical}}^{\text{expt}}(t_0) = 200 \text{ nS}$

This conductance could be generated equally well by any appropriate combination of nanopore shape and geometric parameters, $(r_{0,\text{shape}}(t_0), L_{\text{shape}}(t_0))$, plotted in Figure

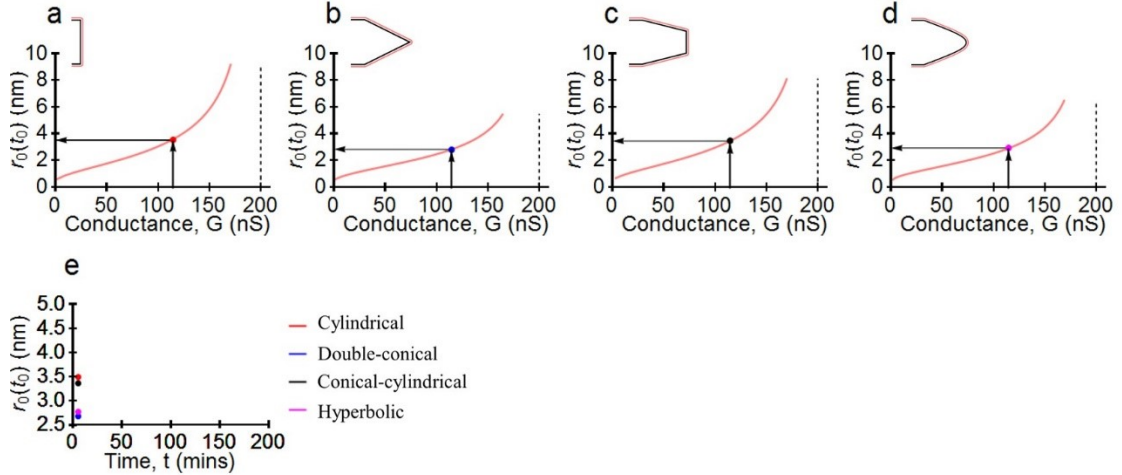
3.2. The dotted lines in Panels a-d below show the range of possible $r_{0,\text{shape}}(t_0)$ for each shape given the 200 nS initial conductance.



Step 1 in construction of Figure 3.4: Plots of $r_0(t_0)$ versus conductance for (a) cylindrical, (b) double-conical, (c) conical-cylindrical, and (d) hyperbolic nanopore shapes for an initial conductance of 200 nS.

Step 2: Second conductance value, $G_{\text{cylindrical}}^{\text{expt}}(t_1) = \sim 114.5$ nS

Knowing the change in radius, $\Delta r_1 = 0.5$ nm, we take each possible $(r_{0,\text{shape}}(t_0), L_{\text{shape}}(t_0))$ from Step 1 and calculate the conductance for each profile given $(r_{0,\text{shape}}(t_0) - \Delta r_1, L_{\text{shape}}(t_0) + 2\Delta r_1)$. The ordinate of the $G_{\text{shape}}(t_1)$ point shows that the initially (but now smaller) 200 nS conductance pore must have had an *initial* limiting radius, $r_{0,\text{shape}}(t_0)$, of 3.5 nm (if cylindrical); ~ 2.7 nm (if double-conical); ~ 3.3 nm (if conical-cylindrical); and ~ 2.7 nm (if hyperbolic), plotted in panel e, below. Figure 2 gives us the corresponding $L_{\text{shape}}(t_0)$: ~ 3.8 nm (if cylindrical); ~ 8.3 nm (if double-conical); ~ 3.8 nm (if conical-cylindrical); and ~ 6 nm (if hyperbolic).

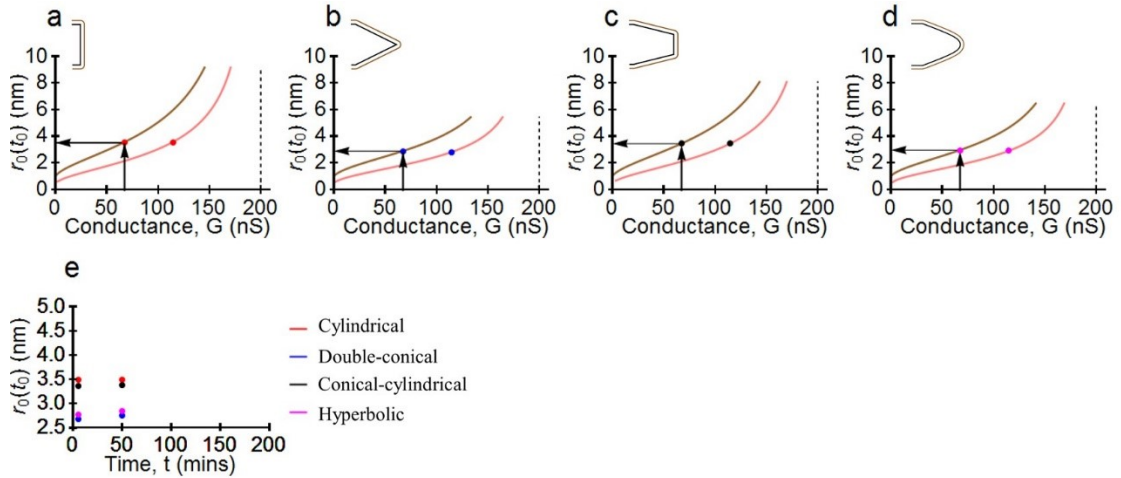


Step 2 in construction of Figure 3.4: Plots of $r_0(t_0)$ with conductance for (a) cylindrical, (b) double-conical, (c) conical-cylindrical and (d) hyperbolic nanopore profiles with $\Delta r_1 = 0.5$ nm, and (e) the corresponding $r_0(t_0)$ for each candidate profile.

Step 3: Third conductance value, $G_{\text{cylindrical}}^{\text{expt}}(t_2) = \sim 67.3$ nS

Knowing the change in radius, $\Delta r_2 = 1.0$ nm, we take each possible $(r_{0,\text{shape}}(t_0), L_{\text{shape}}(t_0))$ from Step 1 and calculate the conductance for each profile given $(r_{0,\text{shape}}(t_0) - \Delta r_2, L_{\text{shape}}(t_0) + 2\Delta r_2)$. The ordinate of the $G_{\text{shape}}(t_2)$ point shows that the pore must have had an *initial* limiting radius, $r_0(t_0)$, of 3.5 nm (if cylindrical); ~ 2.8 nm (if double-conical), ~ 3.4 nm (if conical-cylindrical), and ~ 2.8 nm (if hyperbolic), plotted in panel e below. Figure 2 gives us the corresponding $L(t_0)$: ~ 3.8 nm (if cylindrical); ~ 8.6 nm (if double-conical); ~ 4 nm (if conical-cylindrical); and ~ 6.3 nm (if hyperbolic).

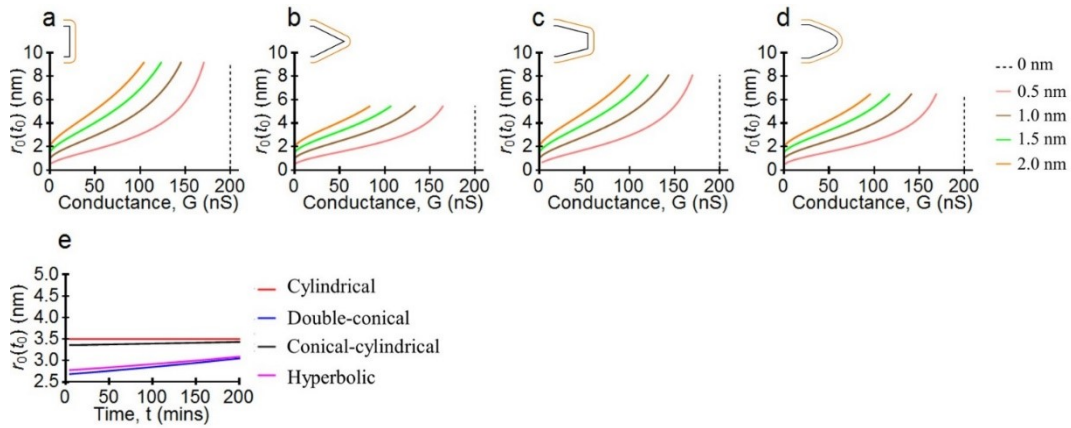
The consistent value of $r_0(t_0)$ in panel e (and of the $L(t_0)$ that we don't show) for the cylindrical trial profile tells us that the simulated pore was cylindrical, and that its initial size was $\left(r_{0,\text{cylindrical}}^{\text{expt}}(t_0), L_{\text{cylindrical}}^{\text{expt}}(t_0)\right) = (3.5 \text{ nm}, 3.8 \text{ nm})$.



Step 3 in construction of Figure 3.4: Plots of $r_0(t_0)$ with conductance for (a) cylindrical, (b) double-conical, (c) conical-cylindrical and (d) hyperbolic nanopore profiles with $\Delta r_2 = 1.0 \text{ nm}$, and (e) the corresponding $r_0(t_0)$ for each candidate profile.

Step 4: Additional conductance values, $G_{\text{cylindrical}}^{\text{expt}}(t_i)$

Additional conductance values can be collected and used to, for example, improve the robustness of the $r_0(t_0)$ determinations.



Step 4 in construction of Figure 3.4: $r_0(t_0)$ with time for a large pool of Δr_i (only 4 shown for clarity) for (a) cylindrical, (b) double-conical, (c) conical-cylindrical and (d) hyperbolic nanopore profiles. Only for the experimental model (cylindrical profile), is $r_0(t_0)$ constant for all time-dependent conductance values, as plotted in (e).

To generate Fig. 4f-h, we repeated this process by respectively simulating the experimental conductances as double-conical, conical-cylindrical, and hyperbolic profiles.

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SUPPORTING INFORMATION FOR CHAPTER 4: CONDUCTANCE-BASED PROFILING OF NANOPORES: ACCOMMODATING FABRICATION IRREGULARITIES

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SUPPORTING INFORMATION.

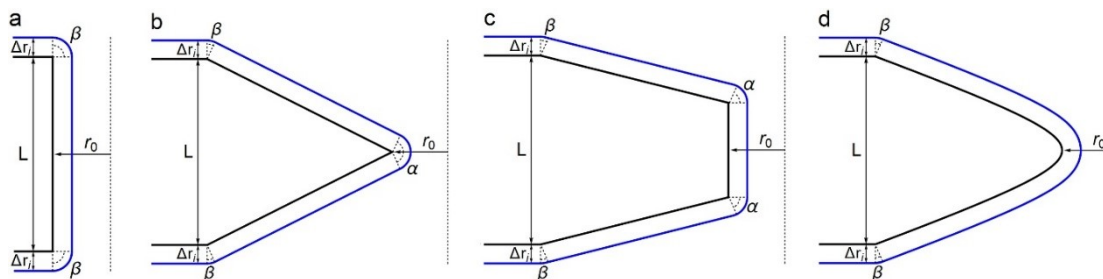


Figure S4. 1. (a) Cylindrical, (b) double-conical, (c) conical-cylindrical, and (d) hyperbolic nanopore half-profile cross-sections cylindrically symmetric about the vertical z -axis (dotted vertical line) of the pore. Profiles are shown before (black line) and after (blue line) material deposition to decrease the limiting nanopore radius, r_0 , by an amount Δr_i determined by the deposition time and material transfer rate. Reprinted with permission from [1]. Copyright 2016 American Chemical Society.

Nanopore Access Resistance. Departures from the cylindrical profile, or from bulk-only access resistance formulations, can make arriving at closed-form solutions for the access resistance of a nanopore difficult or intractable.[2-6] A conventional formulation for the access resistance of a cylindrical nanopore, here with a surface conductance term included in parallel with the bulk conductance, gives

$$G = K \left(\frac{1}{\frac{\pi r_0^2}{L} + \frac{\mu|\sigma| 2\pi r_0}{K}} + \frac{1}{2r_0} \right)^{-1} \quad (\text{S1})$$

where the second fraction arises from a common formulation of the nanopore access resistance, $2/G_{\text{access}}$ (where there is a $1/G_{\text{access}}$ contribution from each open side of the nanopore).[2-6] More complex treatments exist that also include a surface term in the access resistance, and others have noted the difficulty of treating the access resistance of other nanopore shapes.[2, 3] To investigate the effect of including the access resistance into the conductance modelling, we used equation (S1) to calculate the conductances of nanopores with selected aspect ratios, $L(t_0)/r_0(t_0)$, and then fit the results to the cylindrical conductance model of equations (1) and (S1), where access resistance is neglected in equation (1). Simulation results are shown in Figure S4.2.

If one rewrites equation (S1) more generally, $G = \left(\frac{1}{G_{\text{bulk}} + G_{\text{surface}}} + \frac{1}{G_{\text{access}}^{\text{total}}} \right)^{-1}$, it can

then be rearranged to

$$G = (G_{\text{bulk}} + G_{\text{surface}}) \left(1 + \frac{G_{\text{bulk}} + G_{\text{surface}}}{G_{\text{access}}^{\text{total}}} \right)^{-1} \quad (\text{S2})$$

that is, to equation (1) multiplied by a term containing the total contribution (*i.e.* from both openings of the pore) to the nanopore conductance provided by the access

resistance: $G = G_{\text{eqn1}} G_{\text{access}}^{\text{scaled}}$. In the limit of low access resistance when $\frac{G_{\text{bulk}} + G_{\text{surface}}}{G_{\text{access}}^{\text{total}}} \ll 1$, a first-order expansion gives $G \cong (G_{\text{bulk}} + G_{\text{surface}}) \left(1 - \frac{G_{\text{bulk}} + G_{\text{surface}}}{G_{\text{access}}^{\text{total}}}\right)$, so that for sufficiently low access resistance, equation (1) is recovered from equation (S2). Constructing a more general analytic formulation of $\frac{2}{G_{\text{access}}}$, beyond that shown in equation (S1) for a cylindrical nanopore, remains challenging, especially if nanopore surface contributions are to be included.[2, 6] Scaling arguments and earlier work,[2] however, offer a possible approach in which setting $G_{\text{access}} = \alpha K r_0$ is followed by numerical calculations of α , a parameter dependent on nanopore shape.

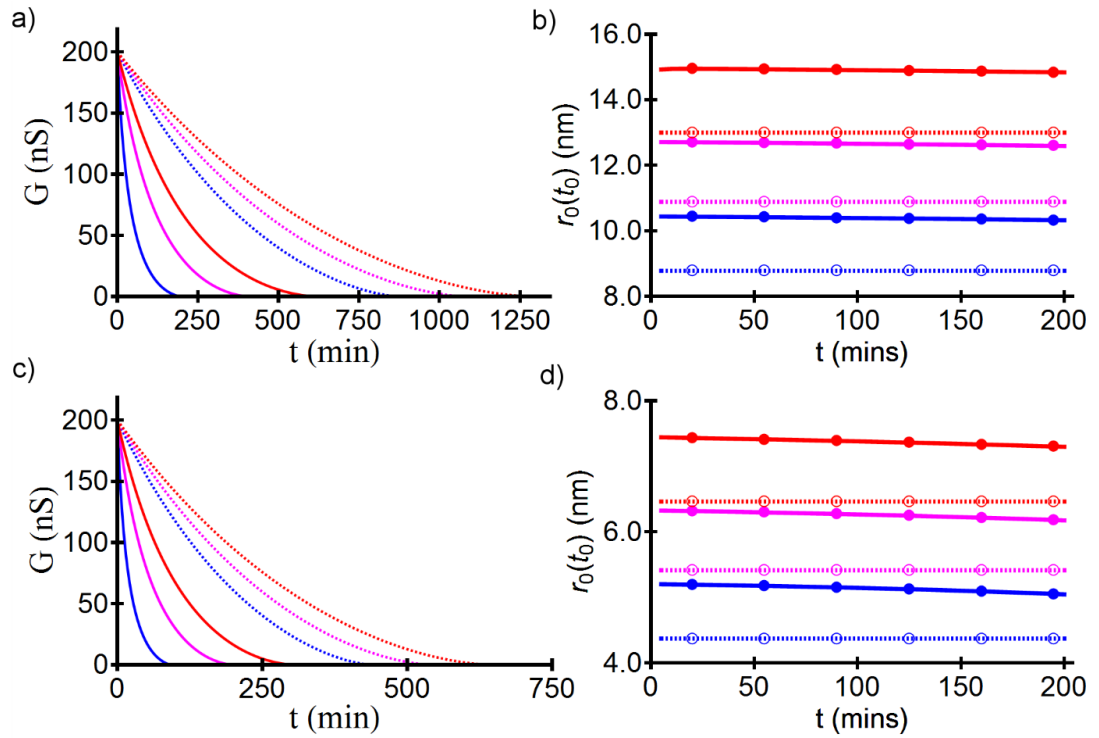


Figure S4. 2. Simulations of conductance versus time for initially 200 nS pores with $L(t_0)/r_0(t_0)$ ratios of 0.5 (blue), 1.0 (magenta), and 1.5 (red) for (a) single and (c) double pores, with (dotted lines) and without (solid-lines) the access resistance term

in Equation S1. In (b) and (d), we fit candidate pore models with and without access resistance using the conductance data in (a) and (c) that included the access resistance. There are three correct fits in (b) and (d)—one for each $L(t_0)/r_0(t_0)$ —that are indicated by the horizontal slope of the fit $r_0(t_0)$ versus t data. Neglecting the access resistance when fitting the conductance-versus-time simulations results in a ~ 2 nm overestimate of the nanopore dimensions and a nonzero slope that indicates the incorrect fit. The simulations used step sizes in the nanopore radius of 0.01 nm to calculate G versus t , and 0.05 nm to determine $r_0(t_0)$.

The dependence of nanopore conductance show in Equation (1) is explicitly on solution conductivity, K , and implicitly on solution pH through its effect on the surface charge density, σ (and, where a surface can carry a solution-pH-dependent charge of either polarity, through the mobility of the counterion, μ). Here we take the reasonable step of treating the case where the solution conductivity is not itself dependent on pH. Thus, without change of either nanopore dimension or solution conductivity, a change of solution pH can change the nanopore conductance—especially at lower solution conductivities.[7, 8] This behavior is shown in Figure S4.3, and can be expressed by rewriting Equation (1) as

$$G(\text{pH}) = K \cdot A(\mathbf{r}, L) + \mu |\sigma(\text{pH})| \cdot B(\mathbf{r}, L) = K \cdot A(\mathbf{r}, L) + \chi(\text{pH}) \cdot \mu |\sigma(\text{pH}_{\text{ref}})| \cdot B(\mathbf{r}, L) \quad (\text{S3})$$

where the parameter $\chi(\text{pH})$ is used to explicitly carry the pH-dependence of the nanopore conductance (calculated relative to a particular chosen reference pH). In this form, with $\mu |\sigma(\text{pH}_{\text{ref}})|$ and $\chi(\text{pH})$ constant in time for a given fixed solution composition as for Equation (1), the consequence of solution pH is simply a reweighting

of the surface contribution to the conductance, relative to the behavior at the reference pH. Figure S4.3 shows the time-dependence of the conductance of the nanopore conductance at several pH values, and their successful use to correctly recover the nanopore size.

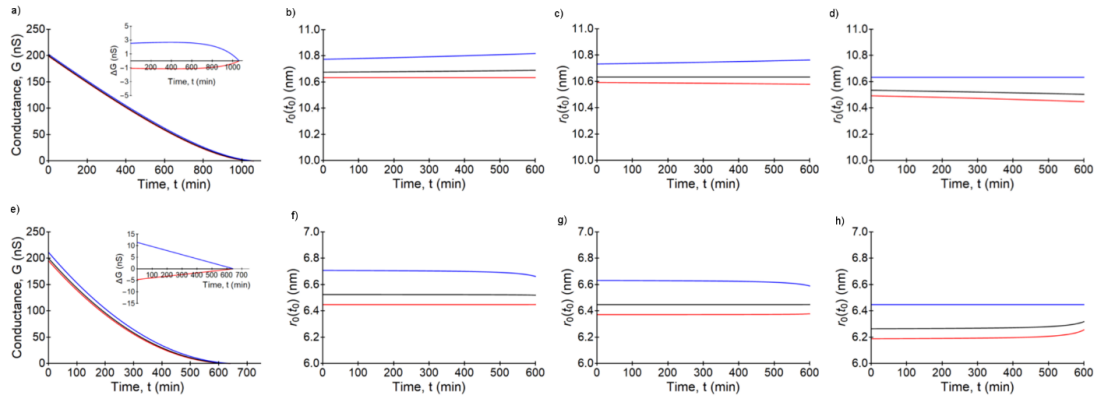


Figure S4. 3. Plots of nanopore ($L=10$ nm, $r_0=6.45$ nm) conductance in time at pH 4 (red), 7 (black), and 10 (blue), showing the effect of pH on initial conductance (200 nS at pH 7) and on the time-evolution of the nanopore conductance, (a) with and (e) without access resistance. The influence of the solution pH is through the nanopore surface charge density, σ (equation (1)), and so pores of identical shape and size immersed in solutions of different pH may have different conductances. The inset shows the difference between the curves at all pH values, relative to the curve at pH 7. Geometry determinations (b-d) with and (f-h) without access resistance included in the candidate cylindrical profile were performed using the data in (a) and (e), using values of 4, 7, and 10 for the solution pH, respectively.

Figure S4.4a reinforces that for a given experimental conductance value and even a given candidate nanopore profile, unless the nanopore length is known, then one

must contend with an infinite set of $\{(r_{0,\text{candidate}}, L_{\text{candidate}})\}$ that deliver that single conductance value through Equation 1. This figure furthermore illustrates that the presence of multiple pores further expands the combinations of the possible nanopore dimensions delivering that single conductance value. Figure S4.4a gives single vs. double pore values of r_0 for a 200 nS pore. Choosing a 10 nm-long nanopore for each profile gives the corresponding r_0 : cylindrical—6.4 vs. 4.5 nm; double-conical—3.1 vs. 1.7 nm; conical-cylindrical—5.5 vs. 3.8 nm; and hyperbolic—4.0 vs. 2.3 nm. For translocation-based experiments, this physical pore size is vital: the 200 nS single pore double-conical profile could allow intact passage of a species too large to fit through the smaller pores of its 200 nS double pore equivalent. Figure S4.4b shows that, as established for single pores,[1] the conductance change in time provides the prospect of differentiating between single and double pore systems. As an example of the complexity introduced by more than one nanopore, the double pore conductance of the cylindrical pore here lies close to the single pore conductance of the hyperbolic profile. Such time traces thus reveal insights into the type and number of pores, but also suggest practical challenges.

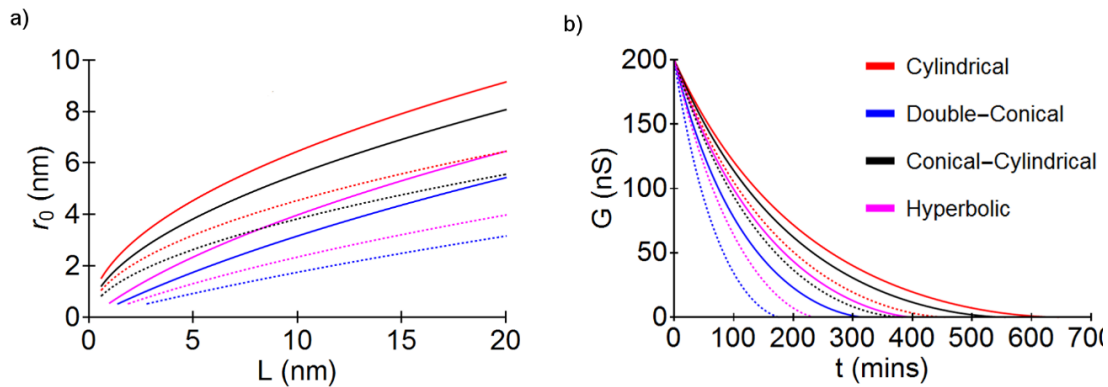


Figure S4. 4. a) Pairings of r_0 and L for a given nanopore shape and number (solid line-single pore; dotted line-double pore) giving a nanopore with 200 nS conductance. b) Change in conductance with time for 10 nm-long profiles with single and double pore configurations. The simulations used step sizes in the nanopore radius of 0.01 nm to calculate G versus t .

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SUPPORTING INFORMATION FOR CHAPTER 5: ELECTROLESS PLATING OF THIN GOLD FILMS DIRECTLY ONTO SILICON NITRIDE THIN FILMS AND INTO MICROPORES

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MATERIALS

The following chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), identified by (product number, specifications), and used as-supplied: methanol (34860, CHROMASOLV® for HPLC $\geq 99.9\%$), tin(II) chloride (208256, Reagent Grade 98%), trifluoroacetic acid (6508, ReagentPlus® 99%), silver nitrate (S6506, ReagentPlus® $\geq 99.0\%$), ammonium hydroxide solution (320145, ACS Reagent 28.0-30.0% NH₃ basis), sodium tetrachloroaurate(III) dihydrate (298174, 99%), barium hydroxide octahydrate (B2507, $\geq 98\%$), sodium hydroxide (S5881, reagent grade $\geq 98\%$), sodium sulfite (S0505, $\geq 98\%$), and formaldehyde (252549, ACS reagent, 37 wt% in water, methanol-stabilized). A 5% solution of hydrofluoric acid (C4354) was purchased from Science Lab Supplies (St. Augustine, FL) and diluted prior to use. All aqueous dilutions and washes were performed using 18M Ω ·cm ultrapure water (Millipore Synergy UV, Billerica, MA). Silicon nitride-coated wafers were purchased from Rogue Valley Microdevices, Inc. (Medford, OR), and consisted of 200nm-thick, low-stress (<250 MPa Tensile; silicon-rich), LPCVD silicon nitride films deposited on

3” diameter, <100> polished silicon wafers. A diamond scribe was used to create ~1cm² sample chips. The silicon nitride micropore arrays had 2µm diameter pores in 200nm-thick membranes and were purchased from Protochips (DTM-25231, Raleigh, NC). The efficacy of Scheme 1 for electrolessly plating gold onto silicon was examined using polished <111> silicon wafers (University Wafer, product number 1080).

ELECTROLESS PLATING

Each chip was plasma-cleaned prior to use in a Glow Research (Phoenix, AZ) Autoglow plasma cleaner with 10 minutes of 50W air plasma (0.8-1.2Torr pressure) followed by 5 minutes of 50W O₂ plasma (0.8-1.2Torr pressure). Each chip was then etched for 10 minutes in 2mL of a 2.5% aqueous HF solution to remove unwanted silicon oxide from the silicon nitride surface¹⁻², followed by 3 immersion rinses in water and then drying under an argon stream. The prepared chips were immersed for 45 minutes in 2mL of a 50/50 methanol/water solution that was 0.025M tin(II) chloride and 0.07M trifluoroacetic acid, followed by a methanol rinse and 5 minute methanol soak, a 5 minute soak in 2mL of ammoniacal silver nitrate solution³, 5 minutes in methanol and finally 5 minutes in water³. Electroless gold plating involved submersing the chips in aqueous plating baths comprised of 7.9×10⁻³M sodium gold sulfite⁴, 0.127M sodium sulfite and 0.625M formaldehyde⁵. The chips were plated in 1.5-3mL of plating solution in small plastic beakers with gentle rocking in a refrigerator (3°C plating) or thermoelectric cooler (10°C plating). After plating for the desired time at the desired temperature, the chips were thrice rinsed in alternating methanol and water, and dried in an argon stream (Airgas PP300). For comparison, we additionally sputter-coated

(Denton Vacuum Desk II, Moorestown, NJ) a plasma-cleaned silicon nitride-coated wafer with gold.

Even dilute hydrofluoric acid presents significant chemical hazards upon operator exposure, requiring special working precautions. All beakers for HF containment were polypropylene, instead of glass which can be etched and rendered permeable. Dilute (5%) stock solutions were purchased to avoid handling concentrated solutions and Calgonate (Port St. Lucie, FL) 2.5% calcium gluconate gel was kept at hand in case of accidental skin exposure. To minimize exposure risk, all personnel wore a full faceshield, a disposable polypropylene apron and thick neoprene long-sleeved gloves over standard chemical safety glasses, laboratory coat and long-sleeved nitrile gloves, respectively. Finally, we employed a “buddy system” so that one researcher monitored the other’s work with HF. All labware and gloves were thoroughly rinsed with water after use.

PREPARATION OF AMMONIACAL SILVER NITRATE³

This solution was prepared by adding 4 drops of 1M sodium hydroxide solution to 0.010g of silver nitrate. Ammonium hydroxide was slowly added, dropwise, until all traces of dark precipitate had dissolved. The solution was then diluted to a final volume of 10mL using ultrapure water.

Ammoniacal silver nitrate solution can form explosives if allowed to dry. This solution should be prepared on only a scale sufficient for immediate use, and should preferably be deactivated by precipitation by the addition of dilute hydrochloric acid or sodium chloride prior to disposal⁶.

PREPARATION OF SODIUM GOLD SULFITE^{4,7}

The synthesis of the gold plating solution was in accordance with the Abys *et al.* patent⁴ modified by the addition of a drying step⁷, as described here. 0.275g sodium tetrachloroaurate dihydrate was added to approximately 15 mL ultrapure water at 80°C with stirring. To this solution were added 1.500g barium hydroxide octahydrate and 54µL of 50% w/w sodium hydroxide to yield an orange-yellow precipitate. The solution was boiled until all visible water had evaporated, and then allowed to cool to room temperature. The precipitate was slurried with approximately 10mL of ultrapure water and filtered through a medium porosity Buchner funnel. The precipitate was slurried with approximately 10mL of ultrapure water, heated to 60-65°C with stirring, cooled, and then filtered (*bis*). The precipitate was then slurried with approximately 20mL of ultrapure water, and 0.500g sodium sulfite was added to the solution. The solution was heated to 60-65°C with stirring until the precipitate turned blue-purple. This solution was filtered while still warm, and the resulting filtrate was diluted to a final volume of 25mL. The pH was adjusted with 1M sodium hydroxide to a final pH above 10.

CHARACTERIZATION

Gold film depositions were carried out in triplicate at each temperature and time point, and the 3°C trial was repeated so that each film thickness was based on deposition and measurements from between 3-6 different silicon nitride chips (allowing for occasional chip breakage). A step edge from gold film to exposed silicon nitride substrate was created by selectively removing gold film with adhesive tape (Scotch® 810 Magic™ tape) or, when film adhesion to the substrate was stronger, with a gentle

pass of plastic tweezers across the substrate. AFM measurements of gold film thickness were performed in tapping mode at 0.1Hz across $10\mu\text{m} \times 10\mu\text{m}$ segments of the step edge with an AFM Workshop (Signal Hill, CA) TT-AFM (equipped with SensaProbesTM190-A-15, 190kHz, aluminum-coated probes with tip radius $<10\text{ nm}$). Line profiles at several points across the step edge were analyzed, using the planar silicon nitride surface as a reference for quadratic background subtractions. For each background-subtracted profile, the means of the coated and uncoated sides were calculated (omitting large particle outliers from the statistics), and averaged for each chip over several profiles. These mean step heights were then averaged over each deposition time and temperature point, propagating the standard deviation as an uncertainty to yield the final reported step heights (Figure 5.1).

Gold film morphology was examined using a Zeiss Sigma VP FE-SEM at an electron energy of 8keV (Oberkochen, Germany), and elemental analysis by EDS was performed on the same instrument equipped with an Oxford Instruments X-MaxN 50mm^2 silicon drift detector (Concord, MA). Custom code was written in Mathematica 9 (Wolfram Research, Champaign, IL) to yield gold film grain size estimates via watershed analysis. X-ray photoelectron spectroscopy was used for the majority of the elemental analysis. XPS spectra were acquired using a PHI 5500 system (Physical Electronics, Inc., Chanhassen, MN) using unmonochromatized Al $K\alpha$ radiation (1486.6 eV) and an aperture size of $600 \times 600\mu\text{m}^2$. Survey scans were performed with 0.8eV step sizes and 20ms per step, with a pass energy of 187.85eV and 10 scans per spectrum. High resolution spectra were recorded with 50 scans per spectrum, 0.1eV step sizes, 40ms per step and a pass energy of 23.50eV. Spectra were analyzed initially with

Multipak 6.1 (Physical Electronics). All curve fitting was performed using XPSPeak 4.1⁸ using linear baselines and the minimum meaningful number of fixed 90% Gaussian-10% Lorentzian peak profiles per peak, with all other peak parameters free. To compensate for substrate charging, we aligned the N1s peak from silicon nitride substrates to 398.00eV, and the lower binding energy Si2p peak from silicon substrates to 99.25eV⁹, shifting spectra by up to 0.49eV. The particular choice of reference precludes analysis based on the binding energy, alone, of that component of the XPS spectrum. We chose these peaks, rather than the commonly used C1s peak¹⁰, because they had better signal-to-noise ratios; the peak fitting reliability would be less frequently compromised by the presence of multiple contributing features; and the C1s binding energy, itself, has been shown to be variable, notably in response to the particular surface treatment of silicon^{9, 11}. To gain a measure of the binding energy uncertainties useful for guiding the interpretation of binding energy shifts, and of the consistency of the reference alignment, we fit the main, shifted, C1s peak centers, yielding a range of values between 284.61 and 285.49eV that arises from a combination of the shortcomings of multicomponent peak fitting and any real shifts in binding energy. As an additional check on the silicon nitride alignment, we also aligned the spectra using the Si2p region by fixing its principal component at 102.5eV. For silicon-rich silicon nitride, the Si2p peaks include overlapping contributions from hydrogen-, oxygen-, silicon- and nitrogen-bound silicon, with magnitudes weighted by the substrate processing conditions; the N1s binding energies, referenced to the 102.5eV components of fits of the Si2p peaks, were 398.35, 398.48, 398.53 and 398.43eV after plasma, HF, tin and silver treatments, respectively. These results of these referencing sensitivity

studies helped to guide the interpretation of Si2p-referenced silicon XPS spectra and N1s-referenced silicon nitride XPS spectra.

Gold film conductivity was measured using an Alessi 4-point probe head with spring-loaded contacts, mounted on a translation stage. Voltages of ~3-6mV were applied with an HP 6115a precision power supply and measured with a Keithley 196 DMM (Cleveland, OH); the current was measured using a Hewlett-Packard 973a multimeter.

SERS measurements were performed on an R3000QE Raman Systems spectrometer using 290mW laser excitation at 785 nm. Substrates were submerged in a 0.01M solution of NBT for 5 minutes before 3× rinsing in acetonitrile and argon drying. Spectra were collected at three random locations for each substrate and averaged together after correcting to a zero baseline at ~494cm⁻¹.

Figure S5. 1. Elemental analysis of gold films. At left, XPS scans comparing a sputtered gold film with an electrolessly plated gold film. The curves are vertically offset for clarity. At right, EDS profiling confirms the gold composition of one of the larger surface particles.

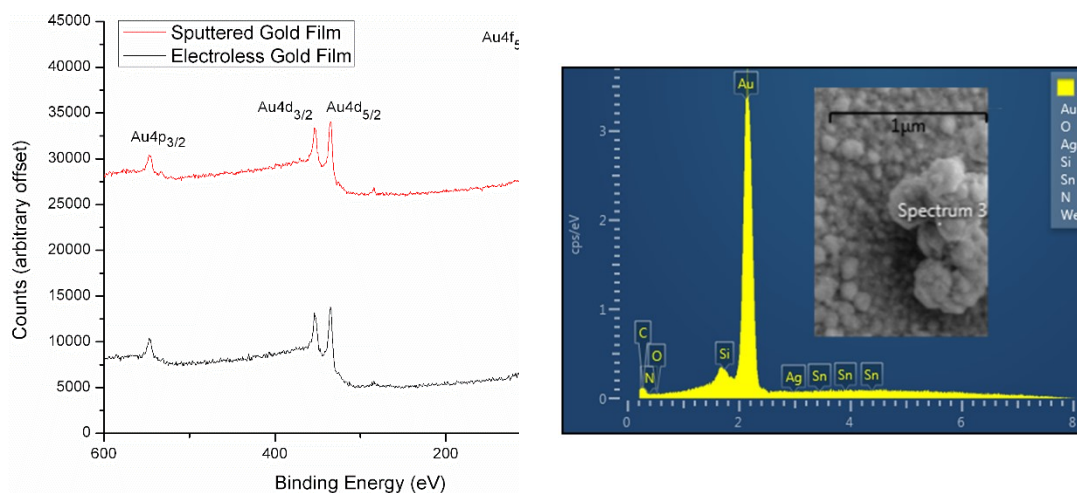
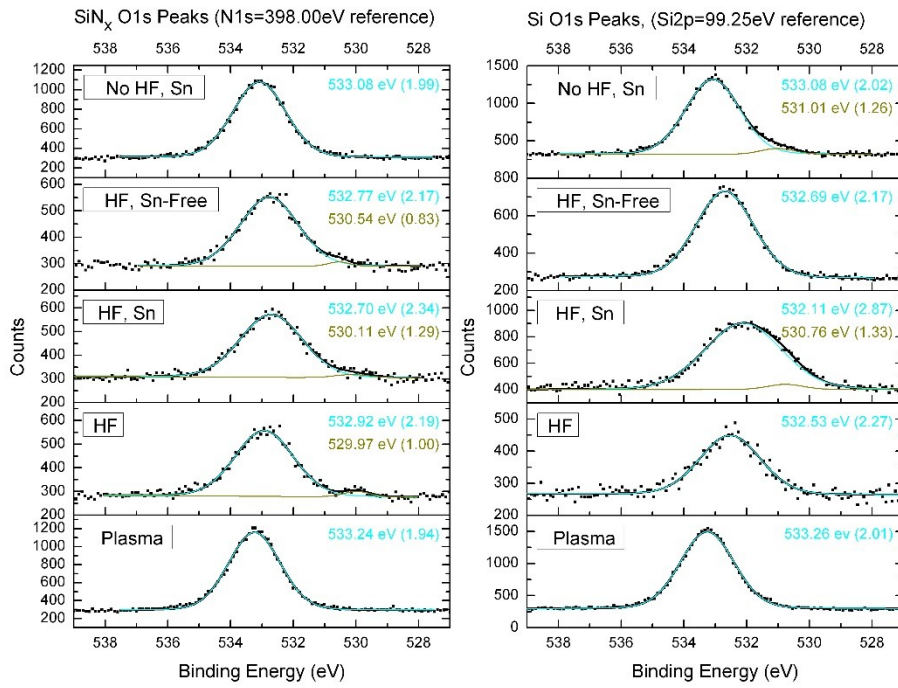
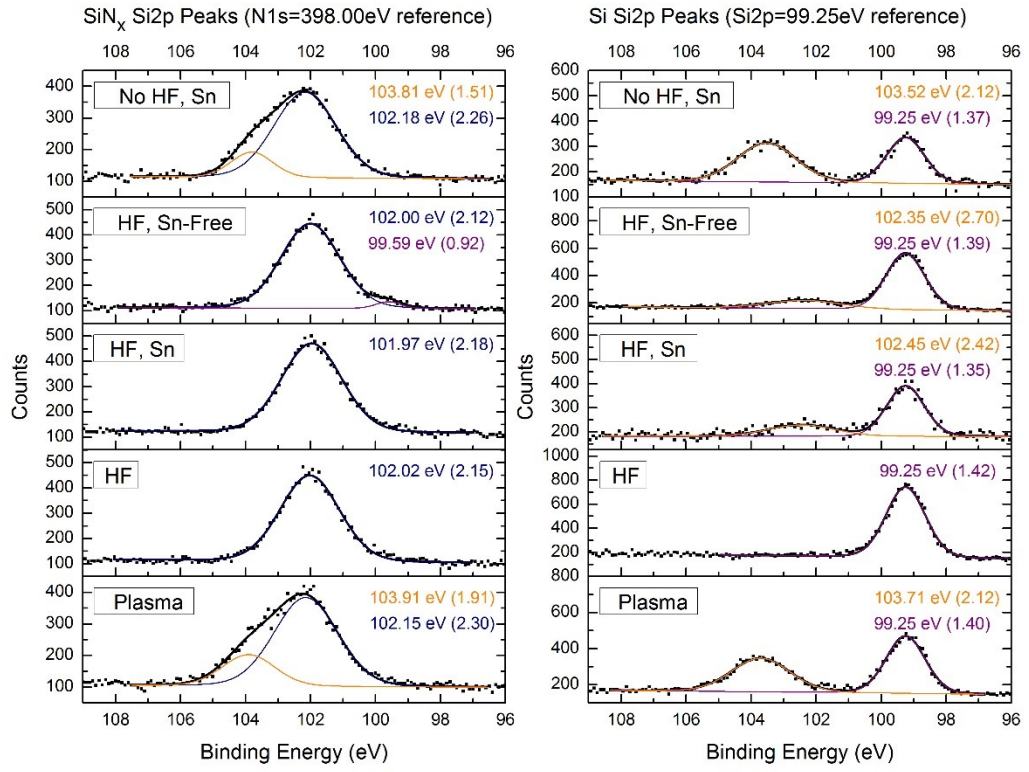


Figure S5. 2. XPS spectra at key steps in the application of Scheme 1, and after selected control experiments. The label given to each spectrum indicates the terminal steps of Scheme 1 (or control experiment variation) that were performed on the substrate. The control data center on the effect of HF etching (performed or omitted) and tin sensitization (with standard solution or tin-free control). The scattered points are experimental data, and solid lines are used for the fit to the data (individual components and their sum). Each plot includes the center value and (width) of each component used to fit the experimental spectrum.

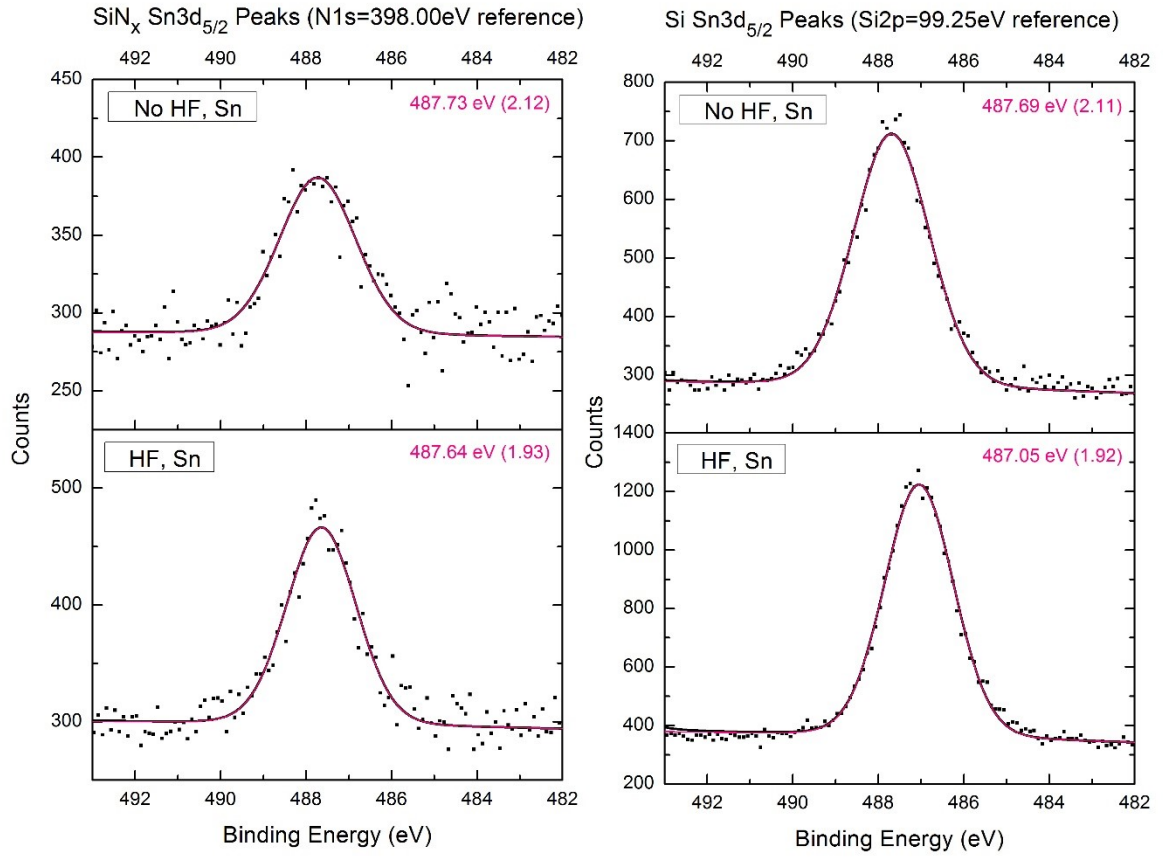
(a)



(b)



(c)



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SUPPORTING INFORMATION FOR CHAPTER 6: SOLUTION-BASED PHOTO-PATTERNED GOLD FILM FORMATION ON SILICON NITRIDE

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SUPPORTING INFORMATION

MATERIALS AND EQUIPMENT

To photoprotect the LPCVD SiN_x films, we purchased 1-octene (O4806, 98%) and 11-bromo-1-undecene (467642, 95%) from Sigma-Aldrich (St. Louis, MO, USA), and the following 3.05 mm diameter, 0.8 mil thick copper Veco Specimen Grids from Electron Microscopy Sciences (Hatfield, PA, USA):

Type	Catalog #	Pitch (μm)	Hole (μm)	Bar (μm)
50 mesh	0050-Cu	500	450	50
100 mesh	0100-Cu	250	200	50

The general framework for metallization follows that of earlier electroless plating work,¹⁻² and is fully detailed here, for completeness, alongside the new procedures necessary to achieve spatial selectivity. The following chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), identified by (product number, specifications), and used as-supplied: methanol (34860, CHROMASOLV® for HPLC ≥99.9%), tin (II) chloride (208256, Reagent Grade 98%), palladium (II) chloride (205885, ReagentPlus®, 99%), trifluoroacetic acid (6508, ReagentPlus®

99%), silver nitrate (S6506, ReagentPlus® ≥99.0%), ammonium hydroxide solution (320145, ACS Reagent 28.0–30.0% NH₃ basis), sodium tetrachloroaurate (III) dihydrate (298174, 99%), barium hydroxide octahydrate (B2507, ≥98%), sodium hydroxide (S5881, reagent grade ≥98%), sodium sulfite (S0505, ≥98%), dichloromethane (270997, anhydrous, ≥99.8%, contains 50–150 ppm amylene as stabilizer), isopropanol (W292907, ≥99.7%, FCC, FG), 3,4,5-trihydroxy benzoate (274194, 98%), polyethylene glycol (81227, BioUltra, 3,000; M_r 2700–3300), phosphoric acid (695017, ACS reagent, ≥85 wt % in H₂O), hydrochloric acid (320331, ACS reagent, 37%), and formaldehyde (252549, ACS reagent, methanol-stabilized). A 5% solution of hydrofluoric acid (C4354) was purchased from Science Lab Supplies (St. Augustine, FL) and was diluted with water by 50% prior to use. All aqueous dilutions and washes were performed using 18 MΩ·cm ultrapure water (Millipore Synergy UV, Billerica, MA). Silicon nitride-coated wafers were purchased from Rogue Valley Microdevices, Inc. (Medford, OR), and consisted of 200 nm-thick, low-stress (<250 MPa Tensile; silicon-rich), LPCVD SiN_x films deposited on 3” diameter, <100> polished silicon wafers. A diamond scribe was used to create ~ (1 cm)² sample chips.

PRECAUTIONS FOR WORKING WITH HYDROFLUORIC ACID

Even dilute hydrofluoric acid (HF) presents significant chemical hazards upon operator exposure, requiring special working precautions. All beakers for HF containment were polypropylene, instead of glass which can be etched and rendered leaky. Dilute (5%) stock solutions were purchased to avoid handling concentrated solutions and Calgonate (Port St. Lucie, FL)—2.5% calcium gluconate gel—was kept at hand in case of accidental skin exposure. To minimize exposure risk, all personnel

wore a full faceshield over standard chemical safety glasses, a disposable polypropylene apron, thick neoprene long-sleeved gloves over extended cuff nitrile gloves, and a laboratory coat. Finally, we employed a “buddy system” so that one researcher actively monitored the other’s work with HF. All labware and gloves were thoroughly rinsed with water after use.

PREPARATION OF REAGENTS

PALLADIUM SOLUTIONS³

0.014 M PALLADIUM (II) STOCK SOLUTION

0.050 g of palladium (II) chloride was added to a solution consisting of 1.50 mL of 0.9 M hydrochloric acid and 18.50 mL of water. The solution was shaken well, and, to prevent possible degradation, was covered with aluminum foil and stored overnight at 3°C so that all solids dissolved.

PALLADIUM SURFACE TREATMENT SOLUTION

To 1120 µL of water were added: 80 µL of 0.014 M palladium (II) stock solution, 600 µL of 0.014 M 3,4,5-trimethylbenzoate, 100 µL of phosphoric acid and 100 µL of 43 wt % polyethylene glycol.

0.014 M 3,4,5-TRIMETHYLBENZOATE STOCK SOLUTION

To 0.10 g of 3,4,5-trimethylbenzoate, 40.00 mL of water was added and shaken well for about 10–15 minutes until all solids dissolved. The vial containing the solution was covered with aluminum foil and stored in a dark and cool place.

43 WEIGHT % POLYETHYLENE GLYCOL STOCK SOLUTION

To 15.00 g of polyethylene glycol, 20.00 ml of water was added and stirred vigorously until all solids dissolved. The solution vial was covered with aluminum foil and stored at 3°C.

AMMONIACAL SILVER NITRATE⁴

This solution was prepared by adding 4 drops of 1 M sodium hydroxide solution to 0.010 g of silver nitrate. Ammonium hydroxide was slowly added, dropwise, until all traces of dark brown precipitate had dissolved. The solution was then diluted to a final volume of 10 mL with water.

HAZARD NOTIFICATION

Ammoniacal silver nitrate solution can form explosives if allowed to dry. This solution should be prepared only on a scale sufficient for immediate use, and should preferably be deactivated by precipitation by the addition of dilute hydrochloric acid or sodium chloride prior to disposal⁵.

SODIUM GOLD (I) SULFITE⁶⁻⁷

The synthesis of the gold plating solution was in accordance with the Abys et al. patent⁷ modified by the addition of a drying step⁶, as described here. 0.275 g sodium tetrachloroaurate (III) dihydrate was added to approximately 15 mL water at 80°C with stirring. To this solution, 0.15 g barium hydroxide octahydrate and 54 µL of 50% w/w sodium hydroxide were added to yield an orange-yellow precipitate. The solution was boiled until all visible water had evaporated, and then allowed to cool to room temperature. The precipitate was slurried with approximately 10 mL of water and filtered through a medium porosity Büchner funnel. The precipitate was slurried with

approximately 10 mL of water, heated to 60–65°C with stirring, cooled, and then filtered. The precipitate was then slurried with approximately 20 mL of water, and 0.500 g sodium sulfite was added to the solution. The solution was heated to 60–65°C with stirring until the precipitate turned blue-purple. This solution was filtered while still warm, and the resulting filtrate was diluted to a final volume of 25 mL. If necessary, the pH was adjusted with 1 M sodium hydroxide to a final pH above 10.

METALLIZATION

Each chip was plasma-cleaned at least one day prior to the subsequent hydrosilylation and metallization steps using a Glow Research (Phoenix, AZ) Autoglow plasma cleaner with 10 minutes of 50 W N₂ plasma (0.8–1.2 Torr pressure) followed by 5 minutes of 50 W O₂ plasma (0.8–1.2 Torr pressure). Each chip was then etched for 10 minutes in 2.5% aqueous HF solution, followed by 3 immersion rinses in water and then drying under an argon stream. The chips were placed in a custom holder under <2 mm of 1-octene, sealed under a quartz plate (Fisher, CGQ-0620-09), and irradiated for 24 hours by a 15 W UV lamp operating at 254 nm (Model XX-15S, Part # 95-0042-05; UVP, LLC, Upland, CA, USA). The chips were rinsed with dichloromethane, allowed to dry, rinsed by isopropanol, and then processed in the metal-ion-containing solutions.

SN (II) / AG (I) / AU (I): ELECTROLESS GOLD PLATING PROCESS FLOW FOR LPCVD SiN_x¹⁻²

The patterned (HF-etched, then patterned) chips were immersed in a series of custom electroless plating bath solutions⁴ that had been successfully used to gold-plate suitably prepared SiN_x.¹⁻² The first immersion was for 45 minutes in 2 mL of a 50/50 methanol/water solution that was 0.025 M tin (II) chloride and 0.07 M trifluoroacetic

acid, followed by a methanol rinse and 5 minute methanol soak. The next step was a 5 minute soak in 2 mL of ammoniacal silver nitrate solution, with a methanol rinse, and 5 minute soak in methanol and then 5 minutes in water. The chips were then submerged in aqueous plating baths comprised of 7.9×10^{-3} M sodium gold (I) sulfite,⁷ 0.127 M sodium sulfite and 0.625 M formaldehyde.²⁻³ The chips were plated in 1.5 mL of plating solution in small plastic beakers with gentle rocking in a refrigerator (3°C plating) for 30 minutes. The chips were then thrice-rinsed in alternating methanol and water, and dried in an argon stream.

PD (II) / AG (I) / AU (I)

Similar to the previous procedure, but with the Sn (II) step replaced with a Pd (II)-based treatment. The patterned chips were immersed in 1 M hydrochloric acid for 5 minutes, washed with isopropanol, and then immersed for 1 hour in 2 mL of the palladium surface treatment solution, followed by 3 rinses, each, of 1 M hydrochloric acid and water, a 5 minute soak in 2 mL of ammoniacal silver nitrate solution, one rinse with methanol and three rinses with water. The chips were then submerged in the Au (I) bath as described in the previous section.

AG (I) / AU (I)

The patterned SiN_x chips were immersed in 1 M hydrochloric acid for 5 minutes, washed with isopropanol, and then immersed for 5 minutes in 2 mL of ammoniacal silver nitrate solution followed by one rinse with methanol and three rinses with water. The chips were then submerged in the Au (I) bath as described in the two previous sections.

CHARACTERIZATION

Optical micrographs of SiN_x patterning were taken with a Digiscope DS-300 (Motic, Hong Kong; controlled with Motic Educator, 2004 ed. software). Gold film morphology was examined using a Zeiss Sigma VP FE-SEM at an electron energy of 8 keV (Oberkochen, Germany), elemental analysis by EDS was performed on the same instrument equipped with an Oxford Instruments X-MaxN 50 mm² silicon drift detector (Concord MA). XPS (Phi 5500 Al K α) was used for additional elemental analyses. A DHM-R 2200 (Lyncée Tec SA, Lausanne, Switzerland) operating at 666 nm, 680 nm, and 794 nm, was used to extract gold film thicknesses; all DHM measurements were courtesy of Lyncée Tec SA staff. Custom codes were written in Mathematica 10.3.1 (Wolfram Research, Champaign, IL) to analyze gold film properties.

GRID RECOGNITION

To distinguish between grid and grid-free zones of an FE-SEM or DHM contour image, each image was first filtered using a median filter with an appropriate pixel value threshold (usually 5), followed by image binarization (with automatic thresholding) and color-negation.

THICKNESS OF DEPOSITED GOLD

ImageJ⁸ was used to extract raw gold film thickness data from a DHM image at 5 \times magnification, provided by Lyncée Tec, of a gold replica of a 100 mesh grid. The grid recognition algorithm was used to distinguish between grid and grid-free zones of a given contour plot. The mean film thickness with standard deviation ($\sim 23 \pm 1.5$ nm) was calculated by averaging across 10 such grid images each with metal-plated grid lines containing at least 35,000 pixels.

WIDTH OF GOLD AND COPPER (TEM) GRID LINES

Regions of interest of grid-recognized FE-SEM micrographs were chosen so that the grid lines we analyzed were distant from the curved sections (from the as-supplied Cu mesh) at grid line intersections. At least 300 line profiles were sampled from each micrograph, and used to calculate a mean grid line width and standard deviation ($54.4 \pm 1.3 \mu\text{m}$ for copper grids provided by the supplier and $44.8 \pm 3.3 \mu\text{m}$ for the gold plated mesh grids on SiN_x).

SURFACE AREA COVERAGE

FE-SEM micrographs of grid lines were taken at $25,000\times$ magnification and the grid recognition algorithm was used to subdivide the image into regions with and without metal particle coverage. This delineated image was then binarized using the “Automatic” thresholding setting in Mathematica. The surface area coverage was calculated using the following equation,

Surface area coverage

$$= \frac{(\# \text{ of total pixels}) - (\# \text{ of zero valued pixels})}{\# \text{ of total pixels}} 100\%$$

and the mean surface area coverage across micrographs of 15 gold replica grids, with standard deviation, was $\sim 83 \pm 13\%$.

BOLTZMANN FIT TO EDS LINE PROFILES

EDS line profiles of the gold thin-film grid replicas were made by acquiring data for ~ 7.5 minutes per line with readings taken every 59 nm, and 15 lines from each of 5 chips were used in the analysis. Each line profile was then fit to a Boltzmann function to quantify the transition from open-area to gold-filled lines

$$f(x) = \frac{A_{min} - A_{max}}{1 + e^{(x-x_0)/dx}} + A_{max}$$

where A_{min} and A_{max} are the initial and final values, and x_0 and dx are the center and slope (spatial resolution) of the edge transition. These were set as free parameters for fitting the EDS line profiles using the “Automatic” setting of the nonlinear-model-fit in Mathematica. The mean spatial resolution (as the mean dx , with standard deviation) from the EDS line profiles was $0.92 \pm 0.24 \mu\text{m}$.

SELECTIVITY

Pixel values corresponding to grid and grid-free regions of grid-recognized FE-SEM images were used to build histograms for each region. A single Gaussian fit was made to each of the histograms using the following equation,

$$g(x) = A^2 \cdot e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

where A^2 , μ , σ , and x are the amplitude coefficient, mean, standard deviation, and pixel intensity, respectively. All parameters were left free during the fit to the histogram, using Mathematica’s nonlinear-model-fit method with “Automatic” setting. The selectivity was then defined, in a classical signal-to-noise sense, as

$$selectivity = \frac{\mu_{grid\ region} - \mu_{grid-free\ region}}{\sigma_{grid-free\ region}}$$

so that 0 is the lower bound and larger values represent superior selectivity. Figure S-6.1 shows photographs of the results of various spatially selective metallization approaches. The selectivity using photopatterned 1-octene masking was ~ 2.7 using Sn (II) (single chip), and ~ 10.1 (8 chips) when begun with Pd (II). With air-based

photopatterning followed by Pd (II) as the first metallization step, the selectivity was ~3.2 (2 chips).

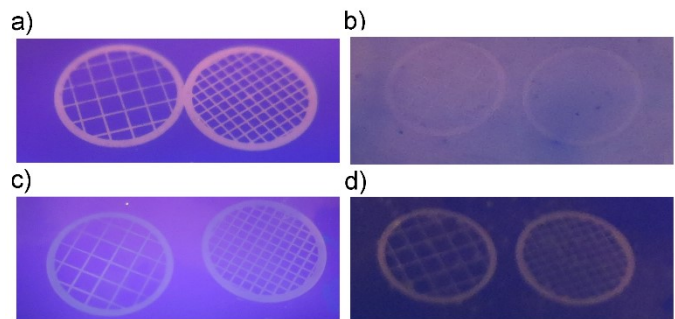


Figure S6. 1: (a) Use of the standard Pd (II) surface treatment solution produced excellent spatial selectivity and pattern quality for the process flow Pd (II)/Ag (I)/Au (I). The pattern quality was sensitive to the solution preparation, as shown by the example in (b) for which we omitted phosphoric acid from the Pd (II) solution. (c) Metallization begun with the Ag (I) solution, as a Ag (I)/Au (I) process flow, produced marginal pattern quality, (d) as did replacing 1-octene with an air layer during the patterning step.

INSTALLATION AND REMOVAL OF 1-ALKENE-DERIVED MONOLAYER

A bromine-terminated 1-alkene, 11-bromo-1-undecene, was photolinked to an HF-etched SiN_x surface. The bromine label allowed straightforward examination of XPS spectra (Figure S-6.2) to confirm (a) surface attachment (black spectrum), and (b) successful intentional removal after 18 hours of UV irradiation in air (red spectrum).

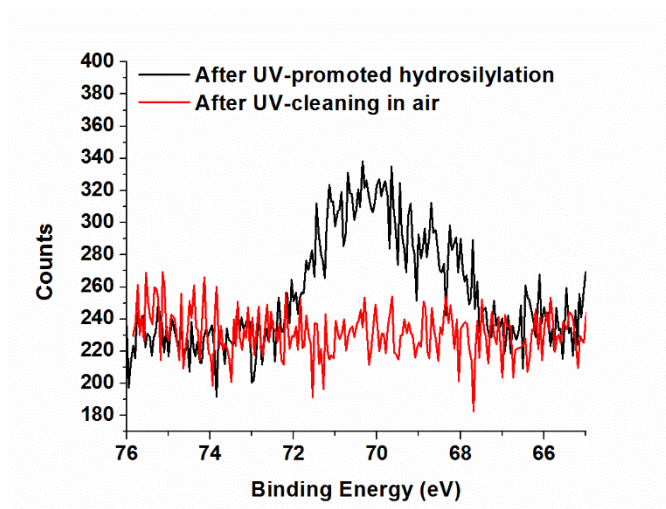


Figure S6. 2: XPS peaks corresponding to Br 3d region. (a) Photo-attachment of 11-bromo-1-undecene to the surface (black spectra) was followed by (b) removal of the alkane monolayer through prolonged exposure (18 hours) to UV in air (red spectra).

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SUPPORTING INFORMATION FOR CHAPTER 7: A GENERAL STRATEGY TO MAKE AN ON-DEMAND LIBRARY OF STRUCTURALLY AND FUNCTIONALLY DIVERSE SERS SUBSTRATES

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MATERIALS

The following materials, identified by their product number and specification, were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA): allyl 2-bromo-2-methylpropionate (381756, 98%); sodium acrylate (408220, 97%); copper (I) bromide (254185, 99.999% trace metals basis); copper (II) bromide (221775, 99%); 2,2'-bipyridyl (D216305, ReagentPlus[®], ≥99%); methanol (34860, CHROMASOLV[®], for HPLC, ≥99.9%); ethanol (34852, CHROMASOLV[®], for HPLC, absolute, ≥99.8%); gold etchant (651818, “standard gold etchant”: iodine and potassium iodide basis); 4-nitrobenzenethiol (NBT; N27209, technical grade, 80 %); acetonitrile (34998, CHROMASOLV[®] Plus, for HPLC, ≥99.9%). Ethanol (200CSPTP, 200 proof ACS/USP grade) was purchased from Ultra-Pure LLC (CT, USA). A 5% solution of hydrofluoric acid (C4354) was purchased from Science Lab Supplies (St. Augustine, FL) and diluted to 2.5% with water. Dichloromethane (390700010, 99.5%); chloroform (326820010, 99.9%, Extra Dry, stabilized, AcroSeal[®]); and 4-aminothiophenol (ATP; 104680, 96%)

were purchased from Acros Organics (NJ, USA). Planar, 200 nm-thick, low-stress (<250 MPa tensile) LPCVD silicon nitride thin films on 356 ± 25 μm -thick polished <100> silicon wafers (P/Boron doped, 1-20 $\Omega\cdot\text{cm}$ resistivity) were purchased from Rogue Valley Microdevices, Inc. (Medford, OR). The following materials, identified by their product number and specification, were purchased from Fisher Scientific (Pittsburgh, PA, USA): $2\times 2\times \frac{1}{4}$ "-thick quartz plate (CGQ062009); acetone (A16P, histological grade, $\geq 99.5\%$); hexane (H303, Optima™); ethyl acetate (E145, certified ACS, $\geq 99.5\%$); Whatman Grade 1 qualitative filter paper (1001-055 and 1001-110, GE Healthcare Bio-Sciences, Pittsburgh, PA); Whatman™ Grade 1 Chr Cellulose Chromatography Paper (3001-672); vacuum filtration system (SCVPU11RE, Stericup-VP, 0.10 μm pore size in polyethersulfone membrane) from EMD Millipore Corporation (MA, USA). Nitrogen (NI HP200), oxygen (OX UHP300), and argon (AR PP300) were purchased from Airgas Inc. (PA, USA). A UV lamp (Model XX-15S, Part # 95-0042-05) was acquired from UVP, LLC (CA, USA). Nanoporous silicon nitride substrates with 450 nm-diameter pores in 100 nm-thick membranes were purchased from Innosieve Diagnostics (custom-provided, reference number ID12200; Wageningen, Netherland). Commercial silicon nanopillar substrates (item ID 15G, gold on nanostructured Si with a SERS active area of 5×5 mm^2) were purchased from Silmeco ApS (Copenhagen, Denmark). For easier handling for the drop-casting spectral acquisition, nanopillar substrates were mounted at the center of a 1 cm \times 1 cm plain silicon nitride chip with carbon tape (16084-6; Ted Pella, Inc., Redding, CA) after electroless plating. Nanocellulose fibers of (declared) nominal 50 nm diameter and hundreds of micrometers length, were obtained as a slurry (University of Maine: The

Process Development Center Nanocellulose Facility, Orono, Maine). No special precautions were taken during processing to avoid potentially breaking nanocellulose fibers. All aqueous dilutions and washes were performed using 18 M Ω ·cm ultrapure water (Millipore Synergy UV, Billerica, MA). For the laser power measurements, an 842-R-USB power meter with 919P-040-50 thermopile sensor was used (Newport Corporation, CA, USA).

ELECTROLESS PLATING

Electroless plating baths were prepared as previously reported¹ (note: a mass of 0.1500 g of barium hydroxide octahydrate was incorrectly reported previously² as 1.500 g). Material-specific preliminary processing steps preceding the electroless plating method are detailed below, before a more general discussion of the electroless plating steps outlined in Scheme S1.

MATERIAL-SPECIFIC SURFACE PREPARATION

Hydrofluoric acid presents significant chemical hazards, so that we adopted special operating procedures when working with it. All containers used were polypropylene because HF can etch glass containers and render them porous and at risk of leaking. To reduce the risk of handling concentrated HF, dilute (5%) stock solutions were purchased and Calgonate (Port St. Lucie, FL) 2.5% calcium gluconate gel was kept at hand in case of accidental skin exposure. To minimize the risk of exposure, all personnel wore a full face shield over chemical safety glasses, a disposable polypropylene apron over a standard laboratory coat, and thick neoprene long gloves over extended-cuff nitrile gloves. We also used a “buddy system” so that

one researcher supervised the other's work with HF. All labware, gloves, and working areas were thoroughly rinsed with water after use.

POLYMER-GRAFTED SILICON NITRIDE

A subset of purchased planar silicon nitride films (with films on silicon supports cut to 1 cm×1 cm) was polymer-grafted, as described briefly here, before electroless plating. The as-supplied silicon nitride-coated substrates were exposed first to 10 minutes of a nitrogen plasma, and then to 5 minutes of an oxygen plasma, using a Glow Research Autoglow plasma cleaner (Phoenix, AZ) set to 50 W and with operating pressures held between 0.8-1.2 Torr during the flow of each process gas. The chips were then etched in 2.5% hydrofluoric acid for 10 minutes, rinsed 3 times in water, argon-dried, and submerged in 50 μ L of allyl 2-bromo-2-methylpropionate to a depth of \sim 100 μ m in a custom holder, and therein irradiated with UV light through a ¼"-thick quartz plate, for 5 hours using a 15 W, 254 nm UV lamp.³ Post-irradiation, they were rinsed at least three times with alternating washes of dichloromethane and acetone before being dried under an argon stream. In a glass vial, 1.88 g of sodium acrylate; 57.4 mg of copper (I) bromide; 9.0 mg of copper (II) bromide; and 137.4 mg of 2,2'-bipyridyl were dissolved in 4 mL of argon-purged methanol and stirred (1000 rpm) under argon for 10 minutes at 30°C, followed by filtering into a Schlenk flask containing four of the silicon nitride substrates that had been pretreated with allyl 2-bromo-2-methylpropionate. The wafers were gently stirred (300 rpm) in this solution at 30°C, under argon, for 2 hours.⁴ After this polymerization step, the substrates were alternately washed with water and ethanol at least three times, then dried under an argon stream.

SILICON NANOPILLAR ARRAY (GOLD-ETCHED SILMECO)

A number of the commercial gold-coated silicon nanopillar SERS substrates were immersed in gold etchant under vacuum (to remove any initial air layer and any generated bubbles preventing full etching solution access between the pillars) for 30 minutes and then washed with copious amounts of water. A gold coating was no longer visible, and while x-ray photoelectron spectroscopy (XPS) analysis showed low residual amounts of gold, there was no measurable SERS response from the gold-etched Silmeco substrates before they were electrolessly plated according to Scheme S1.

CELLULOSE

Whatman 1 filter paper substrates were used without modification. Nanocellulose fibers were formed into a crude paper-like mat by filtering the as-supplied slurry of nanocellulose in water with a polyethersulfone membrane with 0.1 μm pores. When most of the water had filtered through, the resulting paper-like mat (hereafter referred to as “nanocellulose paper”) was compressed to ~ 1 mm thickness (thickness chosen for fabrication convenience) between two glass slides in a custom-designed, 3D printed holder and left to dry under vacuum in a desiccator for two days before plating.

SILICON- AND SILICON NITRIDE SURFACES

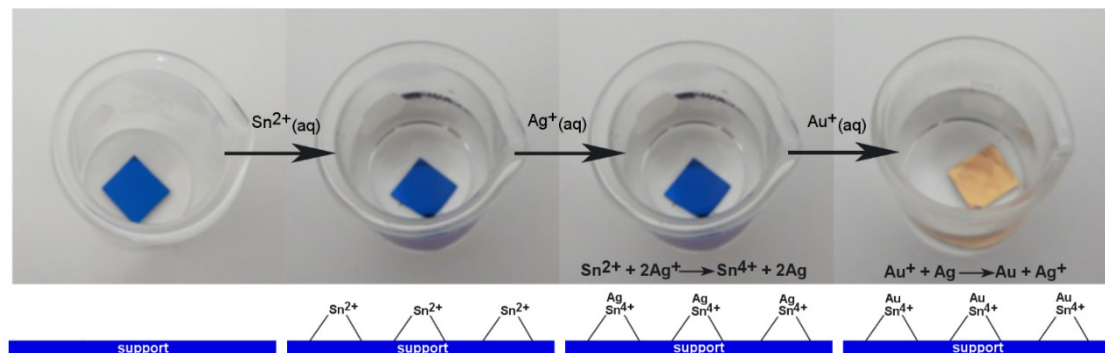
Prior to plating, the planar and nanoporous silicon nitride chips, and the gold-etched silicon nanopillar array, were subjected to cleaning and etch steps. Nitrogen and oxygen plasma treatment were used to remove organic contaminants and hydrofluoric acid etching was used to remove surface oxide layers, as described above and also in

reference 1. Plasma-based surface pretreatments were not performed for the surfaces bearing organic moieties.

ELECTROLESS PLATING SCHEME

Scheme S1 illustrates the general electroless plating process which followed the previous material-specific surface preparation steps, and consisted of sequential plating bath immersions interleaved with rinsing steps. Electroless plating of planar and porous silicon nitride, polymer-grafted silicon nitride, and gold-etched Silmeco was carried out for 2 hours at $\sim 3^{\circ}\text{C}$ with gentle rocking of the plating baths. Whatman 1 filter paper substrates and nanocellulose paper were electrolessly plated at room temperature for 2 hours with gentle rocking using a BenchRocker 3D (Benchmark Scientific, Edison, NJ, USA), and then vacuum dried (~ 15 minutes) as the final step. Plating bath volumes were 2 mL, 2 mL, and 1.5 mL for tin-, silver-, and gold-containing solutions for all substrates except for nanocellulose paper for which the volumes were tripled. Solvent washes between metal ion baths were identical for all plated materials: after tin, rinsing and 5 minutes of soaking in methanol followed by drying; after silver, soaking in methanol for 5 minutes and in water for 5 minutes; and after gold, alternate rinses with methanol and then water at least three times.

Scheme S7. 1. Process flow for the electroless plating steps common to the plating of each support type.



SURFACE CHARACTERIZATION OF ELECTROLESSLY PLATED FILMS

Gold film morphology was examined using a Zeiss Sigma VP FE-SEM at an electron energy of 3-8 keV (Oberkochen, Germany). Elemental analysis was performed using a Thermo Scientific K-Alpha-X-ray Photoelectron Spectrometer System used with monochromator micro-focused Al $K\alpha$ x-rays with a spot size of 400 μm and source energy of 486.6 eV. The energy step was 0.050 eV, dwell time was 50 ms, and pass energy was 20.000 eV, with a charge-neutralizing flood gun used during each acquisition. The number of scans varied from 5-30 depending on the sensitivity factor for each element.

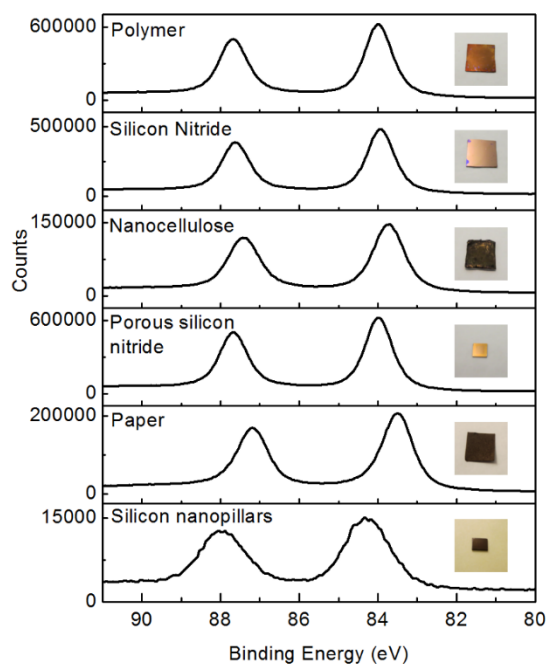


Figure S7. 1. Au_{4f} peaks of X-ray photoelectron spectroscopy data confirm gold deposition on the surface of each substrate. Photographs of gold-coated substrates are shown as insets.

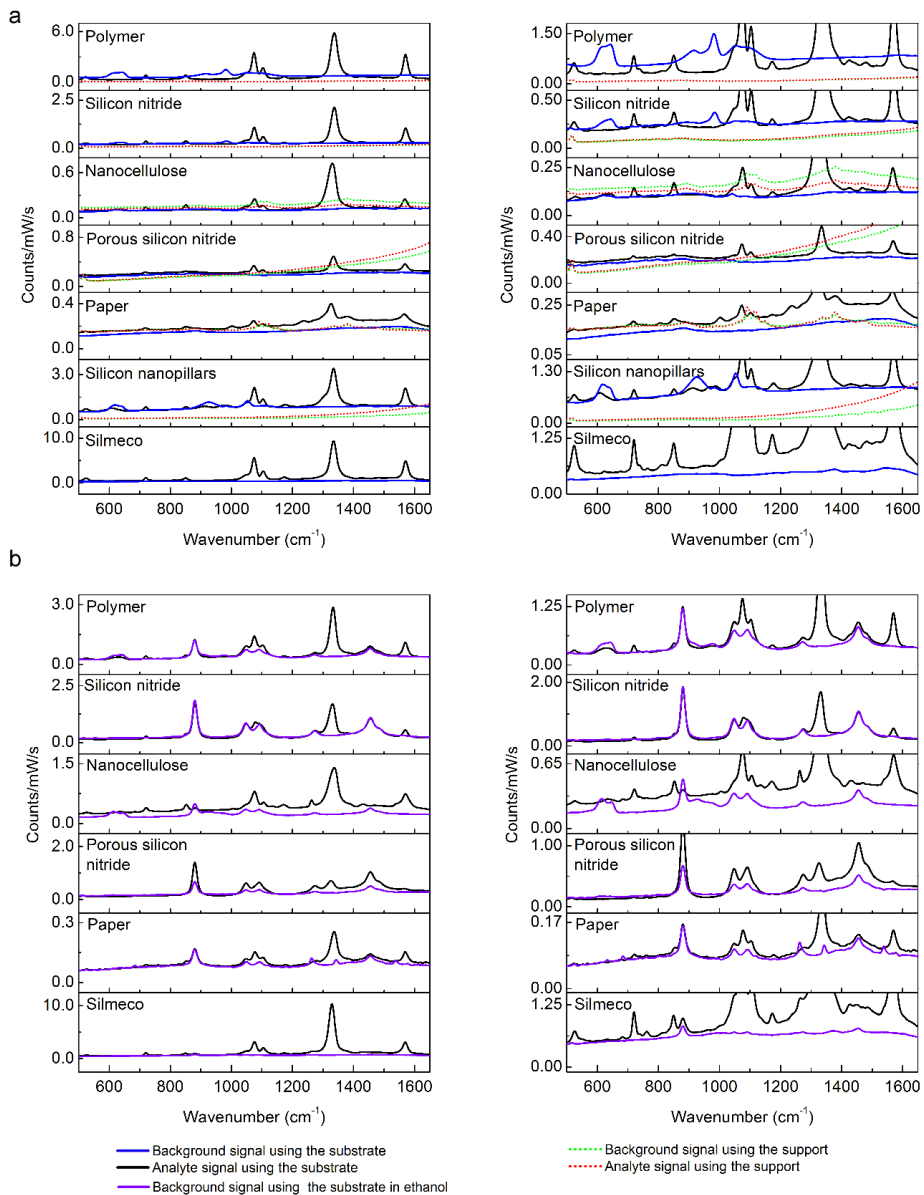


Figure S7. 2. As-acquired spectra of support materials, substrates, and analyte. Spectra are displayed at full vertical range at left, and scaled at right to more clearly reveal the details of the baseline. (a) 1.67×10^{-4} M NBT in acetonitrile was added to each element (drop-casting followed by 5 minutes of air-drying: 20 μ L aliquots for silicon- and silicon-nitride-containing elements; 5 μ L aliquots for commercial silicon nanopillar and nanoporous silicon nitride; and by soaking for 5 minutes followed by 5 minutes of

vacuum drying: 1 mL for paper and 10 mL for nanocellulose paper), with the solvent allowed to dry before spectral acquisition. (b) Elements were immersed in 10^{-4} M solutions of NBT in ethanol and spectra were recorded after signal level saturation in time.

SURFACE ENHANCED RAMAN SPECTROSCOPY

SPECTRAL ACQUISITION

Standard solutions of 4-nitrobenzenethiol (NBT) in ethanol were prepared by serial dilution, covering a concentration range from 5×10^{-9} - 1×10^{-4} M. Solutions were covered in aluminum foil to minimize any photodamage and stored around 3°C in the refrigerator when not in use. Solutions were allowed to reach room temperature before use. An R3000QE Raman Systems spectrometer was used for all SERS measurements, with an excitation laser wavelength of 785 nm set to a power of 57 mW on cellulose and as-provided Silmeco substrates, and 250 mW power on all other substrates. The full-width-half-maximum excitation spot size was ~ 100 μm , measured at the substrate surface with the reader head placed at a slight stand-off of ~ 2.0 mm from the substrate. Each substrate was placed in a glass beaker and a spectrum was acquired at this point to ensure that the substrate was not contaminated. The substrate was then immersed in ethanol and spectra were collected every 2 minutes for about 20 minutes. Once this ethanol-only blank experiment was done, the substrate was removed from solution and dried under nitrogen before being immersed in the standard NBT solution. A spectrum was recorded every 2 minutes until equilibrium was reached, and then the rinsing, drying, immersion, and signal acquisition were repeated for all NBT standard

solution from lowest to highest concentration. To provide (unenhanced) Raman spectra for the SEV analysis,⁵ the same procedure was repeated using a gold-free silicon nitride substrate, using NBT concentrations in the range of 2×10^{-4} M to 2.5×10^{-3} M.

SPECTRAL ACQUISITION FOR DRIED SAMPLES

A 1.67×10^{-5} M solution of NBT in acetonitrile was prepared and a 5 μ L aliquot was pipetted onto the Silmeco substrate. The substrate was allowed to air-dry for about 5 minutes before spectral acquisition, and the Raman spectrometer read head was aligned with the center where the pipette tip had been for drop-casting. There was a slight ~ 1.2 mm stand-off between the SERS substrate and the pipette tip and read head to prevent mechanical damage to the SERS substrate (the nanopillar substrates were especially susceptible to scratches). Excitation power was 250 mW. This alignment of pipette tip and read head was repeated for the other drop-cast spectra in Figure S7.2a, and additional details specific to each substrate are provided in the figure caption.

SPECTRAL ANALYSIS

All spectra were analyzed by custom programs written in Mathematica 11.2 (Wolfram Research, Champaign, IL). Acquired spectra were background-subtracted using piecewise linear fitting between local minima that were selected using a relative thresholding approach to bracket known spectral peaks. To obtain the SEV for all substrates, the remainder of the analysis was performed according to Guicheteau *et al.*⁵ For each spectrum we calculated the ratio of the area of the ~ 1330 cm^{-1} peak of NBT to the area of the ~ 880 cm^{-1} peak of ethanol, $R_{\text{NBT}/\text{EtOH}}$. For a given substrate and concentration, the plot of $R_{\text{NBT}/\text{EtOH}}$ versus time, t , was fit to the equation $R_{\text{NBT}/\text{EtOH}} = R_{\text{NBT}/\text{EtOH}}^{\text{max}} A t / (1 + A t)$, with A and $R_{\text{NBT}/\text{EtOH}}^{\text{max}}$ as free parameters, using the Levenberg-

Marquardt method implemented in Mathematica. The standard error of the fit, $\sigma([\text{NBT}])$, used for subsequent calculation of the SEV for each substrate, was determined in this step. The best-fit value for $R_{\text{NBT}/\text{EtOH}}^{\text{max}}$ (here, representing the surface adsorption equilibrium value) for each concentration was then plotted against $[\text{NBT}]$ for each substrate, as shown in Figure S7.3. For each substrate and analyte concentration, we used $R_{\text{NBT}/\text{EtOH}}^{\text{max}}([\text{NBT}])$ and $\sigma([\text{NBT}])$ as the mean and standard deviation of a Gaussian distribution, $\rho(r, [\text{NBT}]) = \exp\left(-\left(r - R_{\text{NBT}/\text{EtOH}}^{\text{max}}\right)^2 / (2\sigma^2)\right)$, to calculate detection thresholds. Using the ethanol-only (NBT-free) samples, we calculated $r_{90\%,\text{blank}}$, the limit of integration capturing 90% of the distribution's area, $\int_{-\infty}^{r_{90\%,\text{blank}}} \rho(r, \emptyset) dr = 0.9 \int_{-\infty}^{\infty} \rho(r, \emptyset) dr$, for each substrate. For each analyte-containing sample for each substrate, we then calculated $\text{PD}([\text{NBT}]) = \int_{r_{90\%,\text{blank}}}^{\infty} \rho(r, [\text{NBT}]) dr / \int_{-\infty}^{\infty} \rho(r, [\text{NBT}]) dr$, where PD is the probability of detection with a 10% probability of false alarm (PFA). Subsequently, receiver-operator characteristic (ROC) curves were constructed for each substrate by plotting PD versus $[\text{NBT}]$. The concentration, C_{SER} , at which $\text{PD}=0.9$ was found for each substrate by linearly extrapolating between the two experimental concentration values bracketing the PD threshold: $C_{\text{SER}}=7.89 \times 10^{-9}$ M for SiN_x , 6.72×10^{-7} M for porous silicon nitride, 7.23×10^{-6} M for paper, and 2×10^{-8} M for nanocellulose. For the commercial Silmeco and custom polymer-coated SiN_x substrates, even the lowest concentration measured better than 90% PD for a 10% PFA, and so the lowest concentration we used provides an upper bound for C_{SER} (and a lower bound for the SEV, below). The same procedure was repeated for Raman spectra (in the absence of substrate) to get $C_{\text{RS}}=0.00467$ M, the

concentration at which the PD became 0.9. The SERS enhancement value, $SEV = C_{RS/SER}$, was developed by Guicheteau *et al.*,⁵ to provide a representative metric for comparing Raman enhancement between often widely different SERS substrate types.

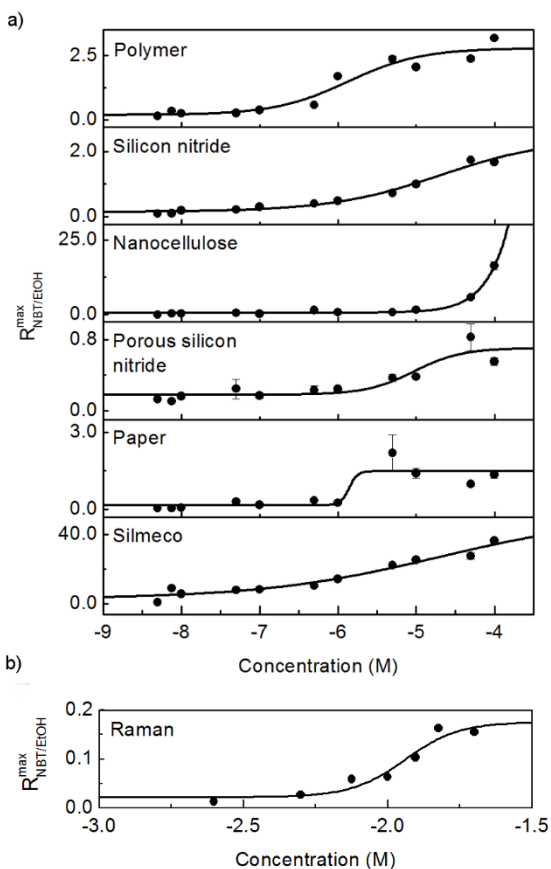


Figure S7. 3. Peak area ratio as a function of concentration for a) SERS and b) normal Raman measurements, with solid lines to aid the eye. Spectra were acquired using 250 mW excitation, except as noted: for cellulose substrates and commercial substrate, excitation was limited to 57 mW. Limits of detection ($LOD = 3s_{blank}/sensitivity$) were estimated by fitting the first 3–4 data points of each response curve to a straight line. The sensitivity was equated to the linear slope and the standard deviation of the blank,

S_{blank} , was calculated from experimental measurements. The LOD, in matching order to the substrates, were 2.58×10^{-10} , 2.7×10^{-10} , 2.13×10^{-10} , 1.08×10^{-9} , 1.16×10^{-8} and 3.62×10^{-11} M, but these should be understood, along with the data below, as providing a benchmark for optimizing the *application-specific* substrate preparation.

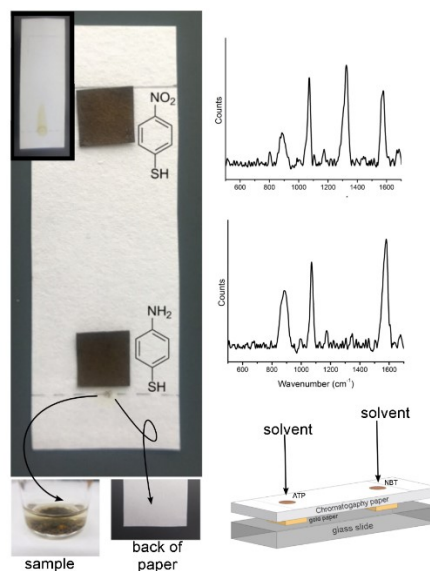


Figure S7. 4. We constructed a crude paper-based assembly to demonstrate the prospects of using electrolessly gold-plated supports as multifunction SERS substrates. This assembly incorporated physical filtration of a heterogeneous sample, chromatographic separation of a multicomponent mixture, and SERS readout. The sample was constructed from NBT in acetonitrile and 4-aminothiophenol (ATP) in ethanol, with dirt added to the mixture. The mixture was spotted onto chromatography paper (7.5 cm \times 2.5 cm), which physically filtered the dirt (a view of the back shows the dirt did not fully penetrate through the paper). A separation was run in 4% (v/v) ethyl acetate in hexane. Iodine staining allowed visual determination of the ATP retention time (photograph shown as an inset), but SERS was needed to localize the NBT spot. After

sampling then separation, squares of electrolessly gold-coated paper were placed on a glass slide underneath the two individual analyte spots. Transfer of the separated analytes was achieved using 10–40 μL drops of ethanol and SER spectra were then recorded from each piece of electrolessly gold-plated readout paper.

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SUPPORTING INFORMATION FOR CHAPTER 8: TASTY, THERAPEUTIC, OR TOXIC? GAUGING THIN-FILM SOLID-STATE NANOPORES FOR POLYSACCHARIDE SENSING

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REAGENTS AND MATERIALS

The following materials, identified by their product number and specification, were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA): potassium chloride (60130, puriss. p.a., $\geq 99.5\%$ (AT)); sodium chloride (S7653, BioXtra, $\geq 99.5\%$ (AT)); HEPES potassium salt (H0527, $\geq 99.5\%$ (titration)); sulphuric acid (339741, 99.999%); alginate lyase (A1603, $\geq 10,000$ units/g); and hydrochloric acid (320331, ACS reagent, 37%). Polysaccharides were commercially obtained: sodium alginate A1-B25266 (~75-120 kDa, 40-90 centipoise (1% solution); Alfa Aesar [Ward Hill, MA, USA]) and A2- PROTANAL® LFR5/60 (120kDa, 300-700 centipoise (10% solution); FMC Corporation Health and Nutrition, PA, USA); heparin sodium salt (USP, 1304038, Rockville, MD; mol. wt. ~16 kDa by lot certificate) and over sulfated chondroitin sulfate (OSCS) (USP, 1133580; est. mol. wt. ~17 kDa by porcine origin¹; from Sigma Aldrich Corporation (St. Louis, MO, USA)). The potency of the USP heparin samples was 180 USP heparin units according to Pharmacopeial Forum Vol. 35(5) [Sept.–Oct. 2009].

Silicon-rich LPCVD silicon nitride (nominally) 10 nm-thick membranes on 200 μm -thick silicon frame (NT001Z and NT005Z; with reported membrane thicknesses for Lot # L8 10.5 ± 0.3 nm, L15 16 ± 2 nm, L31 14 ± 2 nm, L68 12 ± 2 nm) were purchased from Norcada, Inc. (Alberta, Canada). All aqueous solutions were prepared using Type I water (~ 18 $\text{M}\Omega\cdot\text{cm}$ resistivity from either a Millipore Synergy UV [Billerica, MA], or American Aqua Maxicab system [Narragansett, RI, USA]); all dilutions and washes also used this water. Stericup-VP vacuum filtration systems were used to filter electrolyte solutions after preparation, and water to prepare alginate solutions (SCVPU11RE 0.10 μm pore size in polyethersulfone membrane; EMD Millipore Corporation [MA, USA]).

Ag/AgCl electrodes were made from 1.0 mm-diameter silver wire (Alfa Aesar 11434, annealed, 99.9% (metals basis)) by soaking overnight in sodium hypochlorite (Alfa Aesar 33369, 11-15% available chlorine). Electrodes were insulated using shrink-wrap PTFE tubing (McMaster-Carr, 7960K21, high-temperature harsh environment tubing, moisture seal, heat-shrink, 0.07" ID before; and 7564K67, high-temperature harsh environment tubing, heat-shrink, 0.08" ID before, 0.05" ID after) and connected to electronics using pins (Connectivity TE Connectivity / AMP 205090-1 D sub circular connector contact, AMPLIMITE 109 Series, Socket, Crimp, 20-24 AWG). Nanopore chips were compressed between silicone gaskets (McMaster-Carr, 86435K43, high-temperature silicone rubber sheet, ultra-thin, 12" x 12", 0.015" thick, 35A durometer) in custom-machined PTFE holders with ~ 500 μL sample wells.² Silicone tubing with ID 1.0 mm x OD 3.0 mm was obtained from Nanion Technologies GmbH, Munich, Germany.

INSTRUMENTAL DETAILS

Measurements of solution pH and conductivity were with an Orion Star™ pH meter and Orion™ ROSS Ultra™ Refillable pH/ATC Triode™ Combination Electrodes and Orion™ DuraProbe™ 4-Electrode Conductivity Cells (Thermo Fisher Scientific Inc, MA, USA). Nanopore formation by dielectric breakdown was performed using programmable DC power supplies (Model 9121A, B&K Precision Corporation, CA, USA) interfaced to a home-built circuit;³ real-time current measurements were by a 428-Programmable Current Amplifier (Keithley Instruments, Cleveland, OH, USA) interfaced to NI USB 6351 DAQ card using custom LabView-based (National Instruments Corp., TX, USA) software to control the applied voltage. All nanopore measurements were performed using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) in voltage clamp mode. The amplifier was interfaced to a computer system using a data acquisition card (779512-01 NI PCIE-6251 M Series with 777960-01 NI BNC-2120 shielded connector block) and control software written in LabView. Current-versus-time measurements were typically acquired for 1 h (3× 20 min) at 100 kHz acquisition rates with the 4-pole low pass Bessel filter built-in to the Axopatch 200B set to 10 kHz. Measurements of nanopore conductance were acquired at a rate of 10 kHz, with the filter set to 1 kHz.

Infrared spectra of the powder were acquired by FTIR-ATR (Bruker Tensor 27 equipped with a Ge crystal) averaged over 256 scans with 4 cm⁻¹ spectral resolution. All measurements done inside a nitrogen filled glovebox.

UV/Vis spectra were collected using a Varian Cary 50 Bio UV/Visible Spectrophotometer with a quartz cuvette with a 1 cm pathlength. Single run

measurements were taken from 200 to 400 nm at a scan rate of 300 nm/min and 0.50 nm intervals.

All 3D printed components were designed in Solid Works 2014 Professional Edition (Dassault Systems SolidWorks Corporation, Waltham, MA) and printed by Makerbot Replicator (MakerBot Industries, Brooklyn, NY) using PLA plastic (MP06103, MakerBot Industries, Brooklyn, NY).

GENERAL NANOPORE SENSING PROCEDURE

Nanopores in the ~10 nm-thick silicon nitride membranes were fabricated by controlled dielectric breakdown using 11-15.5 V DC applied potentials.³ The nanopore formation was carried out in 1 M KCl electrolyte, HEPES-buffered to pH ~7, and the membranes and pores were secured in custom-machined PTFE holders with ~500 μ L sample wells. Nanopore conductances, G , were the slope of the linear fit to the experimental Ohmic current-voltage data, measured in 1 M KCl electrolyte buffered with HEPES at pH ~7. The corresponding nominal nanopore diameters were calculated using a conductance model (including bulk, surface, and access resistance terms) and cylindrical nanopore shape suitable for this salt concentration and fabrication method,

$G = \left(\frac{1}{G_{\text{bulk}} + G_{\text{surface}}} + \frac{1}{G_{\text{access}}} \right)^{-1}$.³⁻⁶ Nanopores used for measurements produced stable open-pore (analyte-free) currents at the salt concentrations used.

All electrolyte solutions were HEPES-buffered (10 mM) to pH ~7 unless otherwise noted (adjusted with dropwise addition of concentrated hydrochloric acid), and measurements were carried out using filtered solutions with 0.1, 1.0, and 4.0 M KCl

concentrations. Solutions of 0.2% (w/v) sodium alginate, 0.2% (w/v) heparin, and 0.2% (w/v) OSCS were made by dissolving the solids in filtered Type I water. For routine measurements and unless otherwise specified, 4 μ L aliquots were added to the headstage side (Figure 8.1), leaving the ground side free of initially added analyte. Calibration curves for each nanopore were constructed by repeated cycles of measurement followed by the addition of another analyte aliquot. Current blockages were extracted using a current-threshold analysis. Any current blockages exceeding 100 s (\approx 0.1%) were not included in analyses.

POLYSACCHARIDE VISCOSITY MEASUREMENTS

Apparent viscosity measurements were carried out on aqueous sodium alginate solutions (0.15-1.0 g/dL) in 0.1 M sodium chloride solutions using a capillary viscometer (SI Analytics Ubbelohde Viscometer, Thermo Fisher Scientific, Inc., MA, USA) immersed in a water bath at \sim 23°C. Triplicate measurements of the apparent viscosity were made at each solution concentration to yield the intrinsic viscosity, $[\eta]$, from a plot of⁷

$$\frac{\eta_{sp}}{C} = [\eta] + k[\eta]^2 C$$

where C is the macromolecule's concentration in g/dL, k is a constant characteristic of the solute-solvent system, $\eta_{sp} = \frac{\eta_{solution}}{\eta_{solvent}} - 1$ is the specific viscosity calculated from the apparent viscosities. The weight- and number-average molecular masses, M_w and M_n , of the polymers in kDa were calculated according to⁸

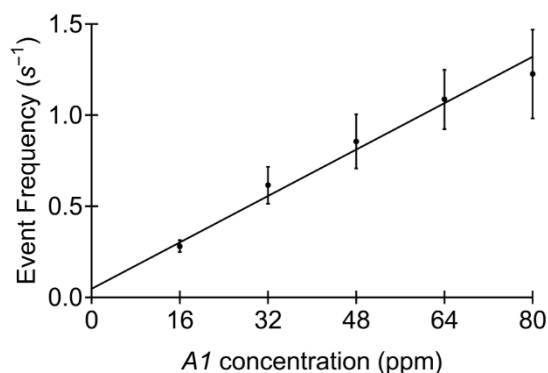
$$[\eta] = 0.023(M_w)^{0.984}$$

$$[\eta] = 0.095(M_n)^{0.963}.$$

The respective molecular masses of the two alginate samples were determined by this method to be ~286 kDa and ~74 kDa for *A1*, and ~71 kDa and ~18 kDa for *A2*. Using a polymer's molecular weight, M , we can calculate the hydrodynamic radius (N_A is Avogadro's number)⁹

$$R_h = \left(\frac{3[\eta]M}{10\pi N_A} \right)^{1/3}$$

to be ~19 nm for *A1* and ~8 nm for *A2* (using M_n as the molecular weight). The corresponding root-mean-squared end-to-end distance, $\langle r^2 \rangle^{1/2}$ for each sample is



equal to $3.1R_h$.

Figure S8. 1: Calibration curve of sodium alginate event frequency versus concentration of *A1*. Three trials were performed, with each data point including at least 1000 events extracted from at least 1 h long measurements at 200 mV applied voltage after consecutive additions of 4 μ L aliquots to the headstage side of the same nanopore. Error bars represent the standard deviation across the trials.

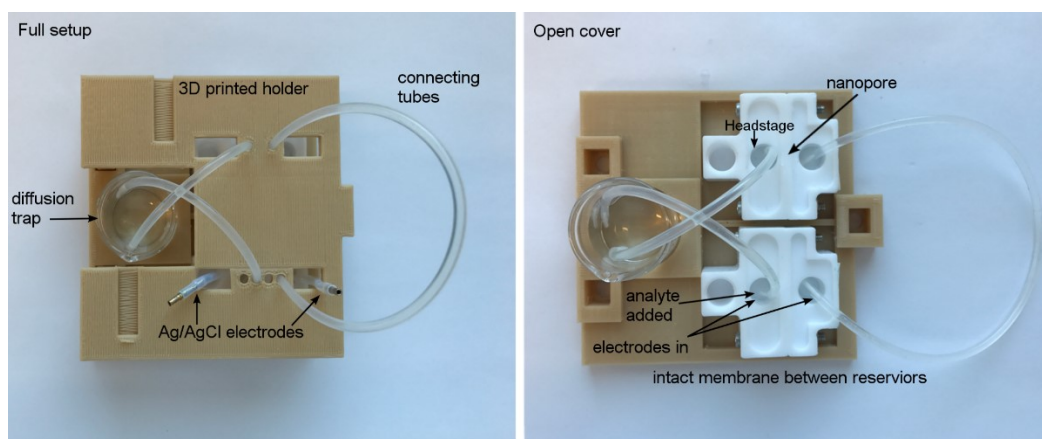


Figure S8. 2: A special nanopore configuration in which the electrolyte wells proximal to the electrodes and to the nanopore were physically separated. The purpose of this configuration was to determine if the current blockages arose from analyte interaction with the electrodes, or with the nanopore, itself. The electrolyte wells in the lower PTFE cell held the electrodes and were separated by an intact SiN_x membrane that did not allow ionic flow. These wells were connected through electrolyte-filled silicone tubing and an electrolyte-filled beaker (acting as a diffusion trap), to a second electrolyte-filled PTFE cell in which the wells were separated by a SiN_x nanopore. With analyte injected into the bottom cell, the only possible mechanism of current blockage was either by direct interaction with the electrodes, or by the passage of analyte through the tubing and beaker of solution until it could interact with the nanopore. When a $4 \mu\text{L}$ aliquot of the alginate was added to the head

stage side of the lower cell, only 18 appreciable current transients were detected in a 1 hour measuring period, contrasted with 561 events in 1 hour when the alginate was directly injected adjacent to the head stage side of the nanopore. The additional electrolyte between electrodes and nanopore reduces the cross-pore applied potential compared to the usual single-cell sensing configuration.

ACID AND ENZYMATIC DIGESTION PROCEDURES

ACID DIGESTION POST-NANOPORE MEASUREMENT

A ~9 nm nanopore was mounted in the PTFE sample holder. A 200 μL amount of 0.2% (w/v) *A1* was added to the head stage side in 5 μL aliquots per hour throughout the work day during 4 days of application of a +200 mV cross-membrane voltage. For overnight voltage applications, the electrode polarity was maintained, but the electrodes were placed in the opposite wells. The head-stage and initially analyte-free ground side solutions were extracted, individually mixed with 1 mL of 75% sulphuric acid and heated overnight (16 h) at 80°C. Samples were diluted with 3 mL of water before spectral acquisition. For comparison, 500 μL aliquots of 0.2% (w/v) *A1* and *A2* were each subjected to the same acid digestion and dilution before spectral acquisition.

ENZYMATIC DIGESTION FOR SPECTROSCOPIC MEASUREMENTS

A 2250 μL aliquot of 0.2% (w/v) *A1* was added to a 150 μL aliquot of 1 unit/mL alginate lyase and heated in a water bath at 37°C for 30 minutes. The procedure was repeated for sample *A2*, but the sample was diluted with 10 mL H₂O before spectral acquisition.

ENZYMATIC SAMPLE PREPARATION FOR NANOPORE SENSING

For enzymatic digestion, samples of 3% (w/v) *A2* were mixed with alginate lyase (1:1 (v/v) mixture with 1 unit/mL enzyme) for 10 minutes at 37°C. 20 μ L of this mixture was added to the headstage side and events were detected with the application of +200 mV on the head stage side. Measurements in the presence of 20 μ L of 1 unit/mL of alginate lyase, alone, in the headstage side support that the detected events in the presence of analyte originated from enzymatic digestion products.

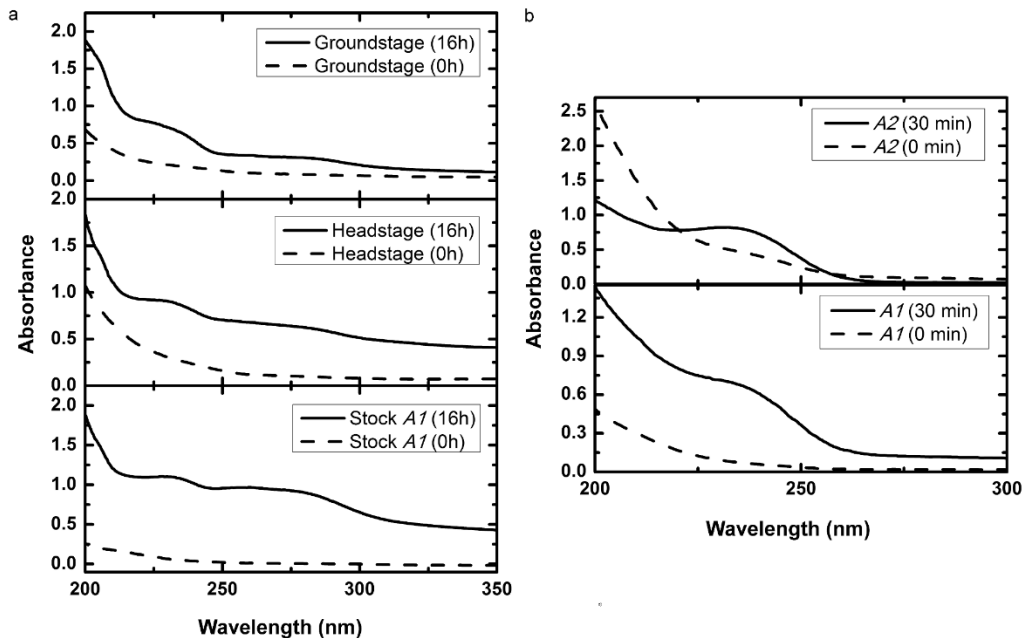


Figure S8. 3. UV/Vis spectra of acid and enzymatic digestion products. a) Stock *A1* subjected to 16 h of sulphuric acid digestion generated a \sim 270 nm absorption band characteristic of the digested polysaccharide^{10, 11} that was replicated in the samples taken from the headstage and from the groundstage sample wells after 4 days of a translocation experiment (200 μ L aliquot). The dashed lines denote the UV/Vis spectra of the sample before digestion, and the solid lines denote the spectra after

digestion. b) Alginate lyase digestion of alginate is expected to introduce chromophores with a peak absorption at ~232 nm, consistent with observations here.¹²

PREPARATION OF HEAT MAPS BY HISTOGRAMMING INDIVIDUAL EVENTS

Heat maps were prepared in Origin (Originlab Corporation, MA) from event data sorted into bins by paired f_b and τ . The bin width along the f_b axis was set equal to $W_{\text{bin}} = 3.49\sigma(f_b)N^{-\frac{1}{3}}$, where $\sigma(f_b)$ is the standard deviation across all events, and N is the total number of events.¹³ Bin size along the τ axis was set to $\sqrt{10}$. Heat maps are plotted using \log_{10} of the number of events in each bin.

The distributions of event counts by f_b in Supplementary Figure 8.4 were fit using the function

$$\phi_{f_b} = \frac{1}{2}(1 + \theta) \sum_{i=1}^M A_i \cdot \exp\left(-\frac{(f_b - \mu_i)^2}{2\sigma_i^2}\right) \quad (\text{S8.1})$$

where the parameters of the unmodified Gaussian function are as conventional - A_i , μ_i , and σ_i are the magnitude scaling, expected value, and standard deviation. The step function, $(1 + \theta)$, was set to 1 for $f_b < f_b^{\text{cutoff}} + W_{\text{bin}}$, and 0 otherwise, so that the fit function covers only the accessible experimental data (f_b^{cutoff} was the threshold for event extraction). The fit parameters are outlined in table S8.1

Panel	A_1	μ_1	σ_1
a	364	0.971	0.0624
	$A_2=76$	$\mu_2=0.773$	$\sigma_2=0.0992$
b	240	0.991	0.00274
c	150	0.98	0.00558
d	100	0.974	0.0041
	$A_2=304$	$\mu_2=0.979$	$\sigma_2=0.002$
e	312	0.991	0.00635
f	500	0.985	0.0077
	$A_2=2120$	$\mu_2=0.989$	$\sigma_2=0.0016$

Table S8. 1: Fit parameters for fits shown in left column of figures S8.4a-f (red curves) using equation S8.1.

The distributions of the log of event counts by duration were fit to a log-normal distribution, $\phi_\tau = \frac{A}{\tau} e^{-(\ln \tau - M)^2 / (2S^2)}$ (S8.2)

where the parameters had conventional meanings, and the event duration was expressed in μs . The event duration corresponding to the peak of the event count distribution, τ_p , was found by taking the first derivative of the curve.

Panel	<i>A</i>	<i>M</i>	<i>S</i>	τ_p (μs)
a	5.49	1.01	0.57	98.91
b	5.93	1.07	0.55	143.98
c	6.95	1.38	0.51	1102.32
d	5.43	1.11	0.67	89.31
e	6.62	1.15	0.55	218.69
f	6.85	0.81	0.50	57.27

Table S8. 2: Fit parameters for fits shown in right column of figures S8.4a-f (red curves) using equation S8.2.

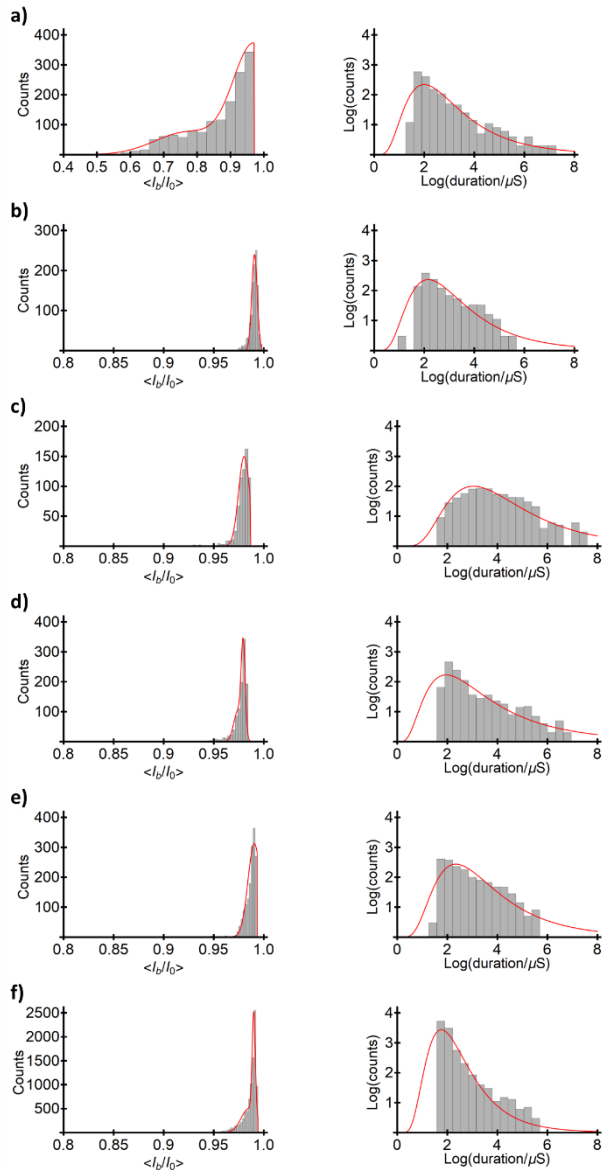


Figure S8. 4: Histograms of (left column) $\langle i_b \rangle / \langle i_0 \rangle$ (right column) duration in \log_{10} of $A1$ alginate in (a) ~ 5 nm and (b) ~ 19 nm pore, $A2$ in (c) ~ 22 nm, (d) 10-min enzyme digested $A2$ in ~ 23 nm pore, (e) heparin and (f) OSCS in the same ~ 14 nm pore with the bin size set automatically by the measurement statistics as described above.

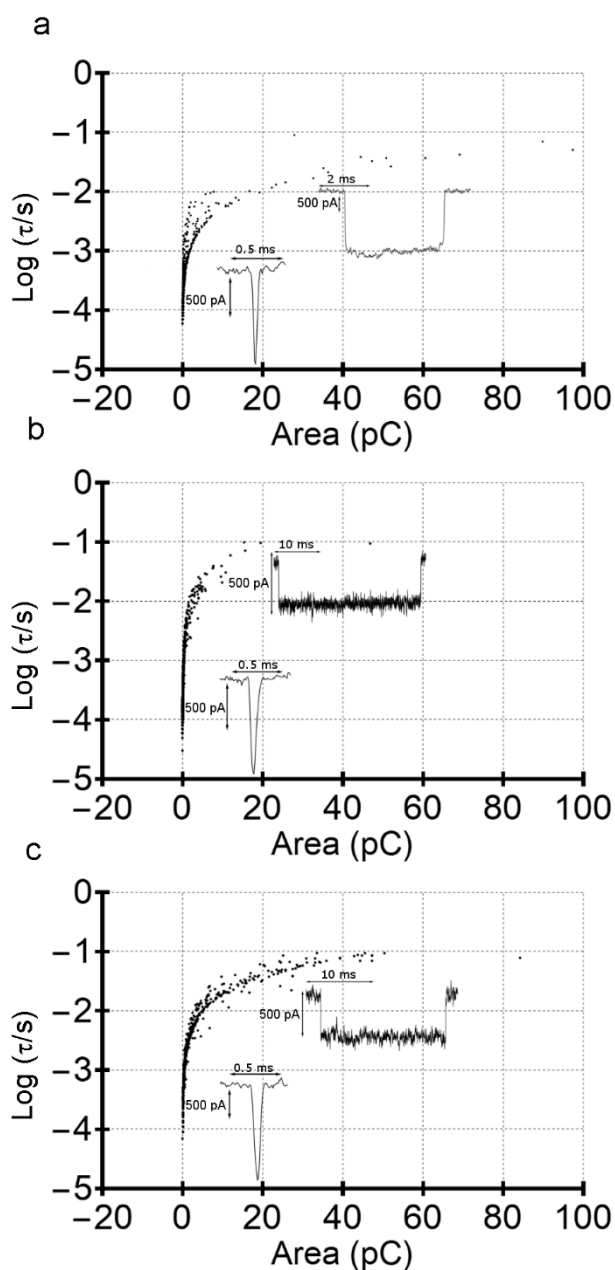


Figure S8. 5: Plots of \log_{10} of event duration (τ) versus area under each event for alginate *A1* in a) ~ 5 nm and b) ~ 19 nm diameter pores and c) for alginate *A2* in a ~ 22 nm diameter pore recorded for 1 hour in 1 M KCl at pH ~ 7 . Two distinct event distribution tails are visible corresponding to short-lived spike-like pulses and longer-lived rectangular blockages. The longer-lived tail for *A2* is more prominent as a

percentage of total events than for *AI*, consistent with the appearance of the combined heat and scatter plots in Figure 8.3. The shorter events could be attributed to either “bumps” or fast translocations, and longer-lived events could be attributed to slower translocations or longer-lived interactions with the pore (in both cases, complementary measurements independently confirmed alginate translocation). The low molecular weight and high M/G ratio (more G is attributed to stiffness) of A2 meant, it has a greater probability of translocating through a given pore hence tails seen in the figure above are not surprising. Area under each event was calculated by integrating the interpolation function (interpolation order of 1) of each event in Mathematica.

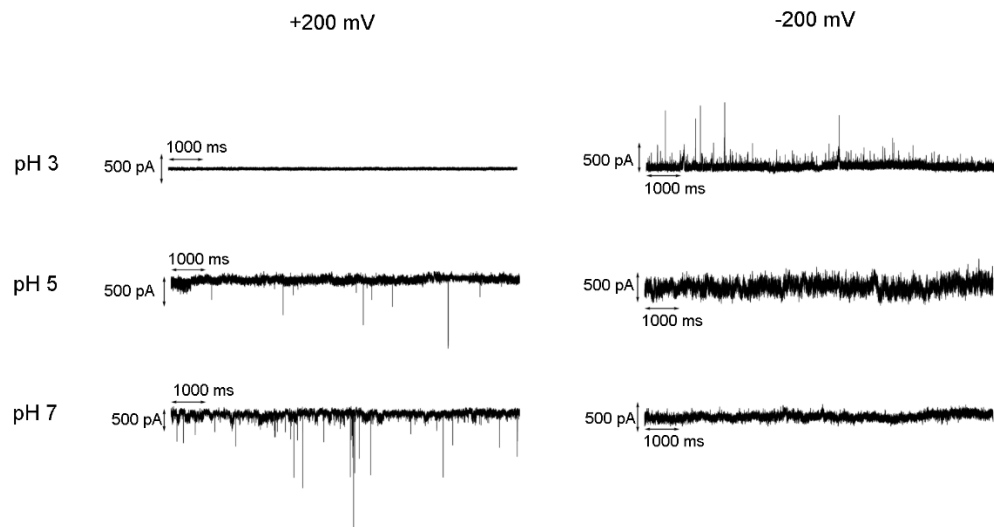


Figure S8. 6. Representative current events of *AI* alginate at pH 3,5 and 7 at negative and positive 200 mV applied on the head stage side for 1-hour each in the same ~8 nm diameter pore at 1M KCl.

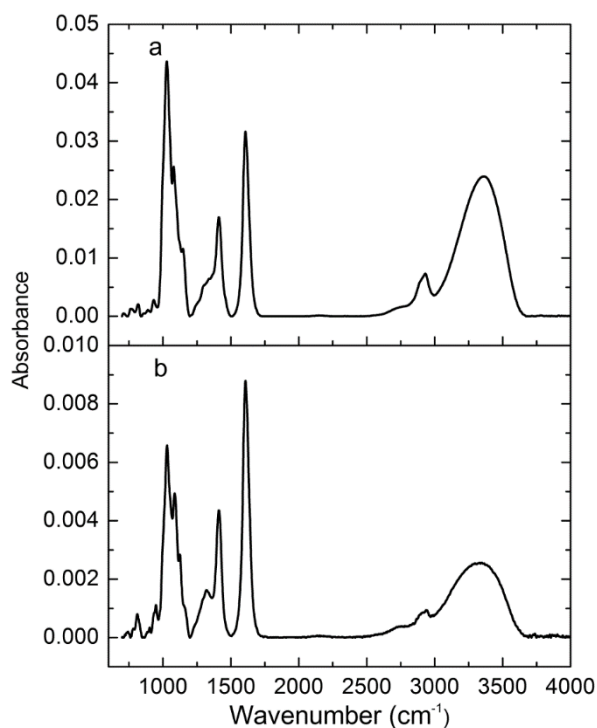


Figure S8. 7. Infrared spectra of alginate samples. The intensity of the peaks near 1400 and 1600 cm^{-1} , relative to the remainder of the spectrum, are consistent with a lesser proportion of carboxylic acid salt residues in (a) *A1* than in (b) *A2*. Comparison of the intensity of the guluronic (G) unit absorption at $\sim 1025 \text{ cm}^{-1}$ to the mannuronic (M) unit absorption at $\sim 1100 \text{ cm}^{-1}$ allows calculation of the M/G ratio that varies with particular alginate source.¹⁴ Using this approach, alginate *A1* was determined to be $\sim 63\% \text{G}/37\% \text{M}$, and alginate *A2* was $\sim 57\% \text{G}/43\% \text{M}$. These relative proportions were supported by additional analysis: in Supplementary Figure 8.3b, the particular alginate lyase was a mannuronic lyase, so that the greater absorption from the digestion of *A2* than *A1* was consistent with a greater proportion of *M* in *A2*.

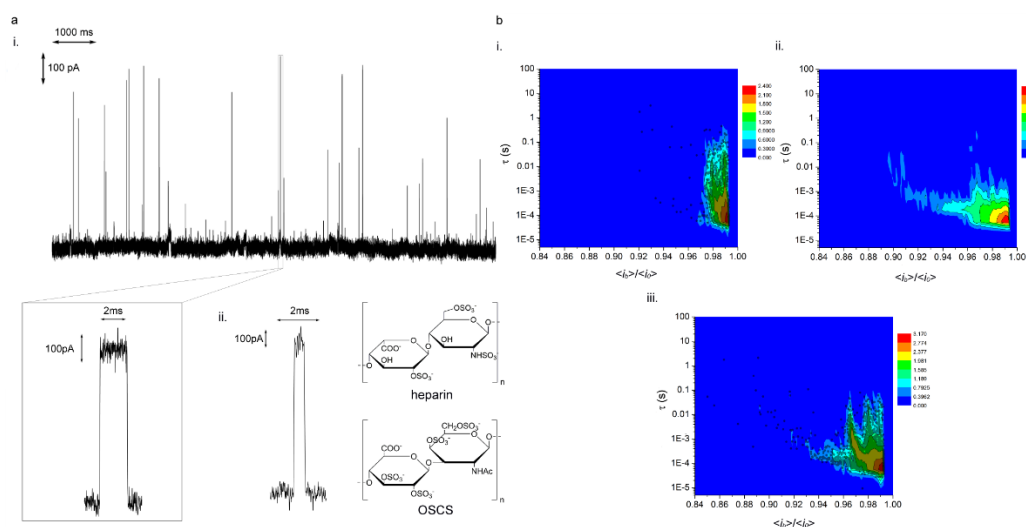


Figure S8. 8. Heparin and OSCS events. A representative a) i) segment of a heparin induced-current trace using a ~ 10 nm-diameter pore with a magnified current event from the same trace, and from ii) OSCS through the same pore in response to a -200 mV applied voltage in 4 M KCl at pH ~ 7 . b) Contour+scatter plots of i) heparin, ii) OSCS and iii) heparin contaminated with OSCS through a ~ 14 nm diameter pore.

RECOGNITION FLAG GENERATION

Recognition flag generation was done using custom codes written in Mathematica 11.0.1.0 (Wolfram, Champaign, IL). (1) A histogram of all individual events were created with respect to f_b using a bin width of 0.0025 (using nanopores with diameters from ~ 8 -14 nm, and determined using the USP heparin data). (2) Any bin with counts below 0.5% of the maximum bin count were removed, and all counts were then normalized. (3) The OSCS identification threshold was taken to be at the nearest bin at the distance of three standard deviations (after the 0.5% filter) from the

bin with the maximum number of counts. (4) When events had been detected at f_b below this threshold, the recognition flag was set to red to signal the presence of OSCS; it was otherwise left white. (5) A histogram of all individual events were created with respect to the logarithm (\log_{10}) of the event duration (τ) using a bin width of 0.25 (here, determined using the USP OSCS data). (6) The same 0.5% filter was applied to these histograms, which then had their counts normalized. (7) The event duration threshold was taken to be the nearest bin at the distance of three standard deviations (after the 0.5% filter) from the bin with the maximum number of counts. (8) When events had been detected at $\log_{10} \tau$ above this threshold, the recognition flag was set to red to signal the presence of heparin; it was otherwise left white.

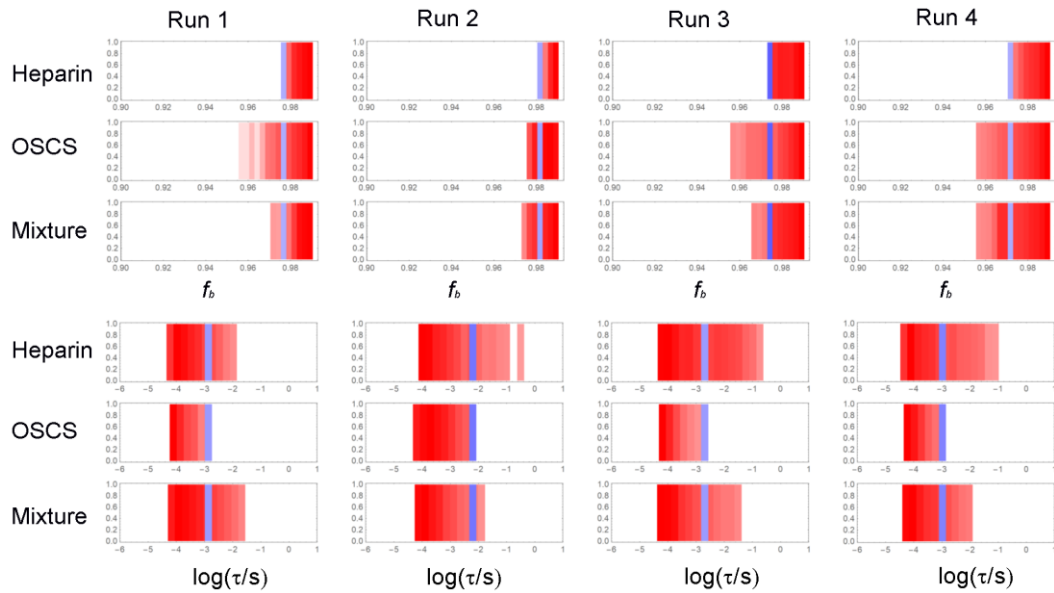


Figure S8. 9. Hue plots of show the outcomes of recognition flag generation (and measurement statistics—see procedure detailed above) after steps 3 (top) and 7 (bottom), based on $f_b = \langle i_b \rangle / \langle i_0 \rangle$ and $\log_{10} \tau$ of the individual events. The

identification threshold, determined by the measurement statistics of each run, is given by the blue line. The corresponding final recognition flags, showing successful detection of the toxic OSCS impurity across four independent trials in ~8.6, 9.8, 9.9, and 13.6 nm (left to right), are shown in Figure 8.5.

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