

# Manufacturable nanoscale membranes – material development and biological separations

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## ABSTRACT

We have developed a novel self-supporting ultrathin (15nm) nanoporous silicon membrane that will revolutionize molecular separations [1]. While commercial membranes are achieving the goal of defined pore sizes, these filters are still many microns thick and ineffective at separating proteins due to loss and transit time. Because of flow resistance and long diffusion times in thick membranes, the ideal molecular separation membrane would have a molecular thickness as well as pore size. We developed porous nanocrystalline silicon (pnc-Si) membranes using inexpensive and scalable silicon fabrication techniques. pnc-Si is formed from a deposited amorphous silicon thin film, where nanoscale pores are introduced through a thermodynamically-driven crystallization process. Our robust 15 nm thick membranes are free standing over thousands of square microns and can support >1 atm of differential pressure. The pore size and porosity are tunable and ideal for biomolecule separations.

**Keywords:** silicon, membrane, ultrathin, separation, protein

## 1 INTRODUCTION

Most commercial filters for biological materials use membranes made from sintered ceramic particles or netted polymers [2]. Separations occur in these materials because some components of a mixture are too large to enter the filter material (size exclusion separation) or because components have different degrees of solubility in the membrane material (solution-diffusion separation). These membranes are produced through sheet casting (polymer sheets are cast on flat glass and ceramic particles are cast in molds). The resulting membranes are tens of microns to millimeters in thickness with interiors that contain a tortuous path porosity reminiscent of a sponge. These membranes can be made more restrictive by increasing the density of the membrane material cast. Typically these membranes are used to separate species that are orders-of-magnitude different in size (desalinization, sterilization). Although some polymer membranes (*ex.* polyvinylidene fluoride (PVDF), cellulose, polyethylene sulfone (PES))

can be designed to exclude passage of materials at molecular weights ranging from 1kD to 100 kD, the lack of defined pore sizes means the molecular weight exclusion profiles are gradual declines rather than sharp cut-offs.

Track etched and anodized alumina membranes have more defined pore sizes in the nanometer range. Membranes with cylindrical pores as small as 10 nm in diameter have been created by bombarding dense polycarbonate or polyester sheets with high energy ions or particles. While the pores are well defined, they are not perfectly uniform because the effectiveness of etching will vary slightly from pore-to-pore and through the thickness of the film [3]. Additionally, porosities are kept small (< 0.5%) to ensure that pores do not have a significant chance of overlap [2]. Because these membranes are > 5  $\mu\text{m}$  thick, the resulting pores are cylindrical and predicted to give significant resistance to transport in both diffusion and fluid flow applications compared to thin (< 1  $\mu\text{m}$ ) membranes [4]. Higher porosity has been achieved with nanoporous alumina membranes. These membranes are formed by the controlled electrochemical anodization of thick aluminium films [5]. Anodization causes the transformation to alumina (aluminum oxide) and the subsequent self assembly of highly uniform cylindrical pores. By controlling the voltage during anodization, pore sizes can be varied between 20 and 200 nm with porosities of ~30%. While more porous than track etched membranes, alumina membranes must be even thicker (~60  $\mu\text{m}$ ) to maintain structural integrity. Since both of these membranes are many microns thick, their “pores” can be thought of more simply as tunnels rather than holes in a membrane filter.

Because of the flow resistance and long diffusion times created by thick membranes, the ideal membrane for molecular or nanoparticle separations would have molecular thickness as well as pore size. Additionally, a superior size exclusion membrane would have a pore distribution with an abrupt upper bound cutoff. The pore sizes would be controllable with nanometer precision so that any two species that varied in size could be separated. Lastly, such a thin membrane must be robust so that it can

stand up to the rigors of laboratory, medical and industrial applications.

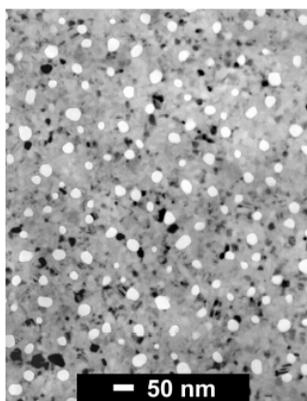
We developed porous nanocrystalline silicon (pnc-Si) membranes using inexpensive and scalable silicon fabrication techniques to meet the needs of molecular separation. pnc-Si is formed from a deposited amorphous silicon thin film, where nanoscale pores are introduced through a thermodynamically-driven crystallization process developed by our group (Figure 1) [1]. Our robust 15 nm thick membranes are free standing over thousands of square microns and can support >1 atm of differential pressure using only the surrounding silicon wafer framework as external support. The pore size and porosity are tunable (Table 1) and ideal for biomolecule separations. We demonstrate that pnc-Si membranes can retain proteins while permitting the transport of small molecules at rates an order of magnitude faster than existing materials (156 nmol/cm<sup>2</sup>-hr) [1]. Furthermore, we demonstrate that our membrane can effectively separate differently sized proteins (Figure 2) that are much closer in size than can be separated with existing membranes.

## 2 MEMBRANE FABRICATION

Our pnc-Si membranes are produced using standard silicon microfabrication techniques which are scalable and highly reproducible. We grow a 500 nm thick SiO<sub>2</sub> layer on both sides of a silicon wafer. We pattern the backside to form an etch mask for the membrane. The front oxide is removed and replaced with a high quality three layer film stack consisting of: 20 nm SiO<sub>2</sub> / 15 nm amorphous Si / 20 nm SiO<sub>2</sub> using RF magnetron sputtering. Our processes [1] yield films with +/- 1% thickness accuracy and surface roughness less than 0.5 nm. The pnc-Si membrane is created by briefly exposing the material to high temperature (715-770C for 30s) which crystallizes the amorphous silicon layer. We then expose the membrane through two selective etches. First, the patterned backside is exposed to silicon selective etch, EDP (ethylenediamine pyrocatechol), which removes the silicon wafer along (111) crystal planes. The protective 20 nm SiO<sub>2</sub> layers prevent the EDP from reaching the pnc-Si membrane. Finally those protective layers are removed with buffered oxide etchant, leaving the pnc-Si membrane freely suspended across the wafer openings, which we design to span hundreds of microns.

**Our processes allow us to create pnc-Si films as thin as 5 nm with spans as great as 2mm x 2mm, but results shown here are from more robust 15 nm films.** These films are normally produced with spans limited to several hundred microns in one dimension to maintain integrity under differential pressure. We have reported cyclical testing at 15 psi on 200 x 200 μm membranes with no rupture or plastic deformation [1]. These properties allow us to create significant membrane surface area for filtration devices by using an array of narrow slits, with dimensions

of hundreds of microns by millimeters. This arrangement sacrifices minimal area while maintaining the material's robust properties.

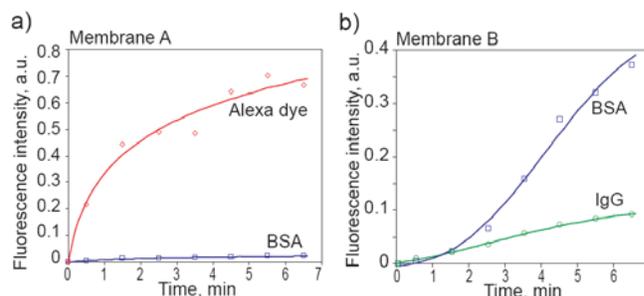


**Figure 1. pnc-Si membranes are fabricated with standard scalable silicon wafer technology.** A plan-view TEM image of the porous nanostructure of a 15 nm thick membrane. In this bright field image, pores appear as bright spots while nanocrystalline silicon is in gray or black contrast. Nanocrystals appearing black have a crystallographic orientation

that exhibits strong electron diffraction [1]. (Membrane C in Table 1)

## 3 BIOMOLECULE SEPARATIONS

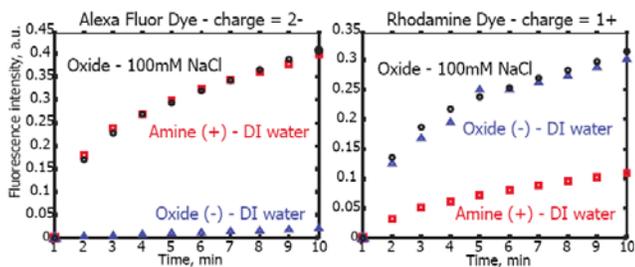
To demonstrate molecular level separations with pnc-Si membranes, we conducted several diffusion based experiments. In our first experiment, we mixed a free fluorescent dye molecule (Alexa 546, MW = 1kD, D ~ 1nm) with pre-labeled bovine serum albumin (BSA, MW = 67kD, D = 6.8 nm). Using an inverted fluorescent microscope, we were able to visualize nearly instantaneous separation of these two species using a pnc-Si membrane (Figure 2a). Typical membrane filters are commonly used for small contaminant removal, but not for protein separation because >10x difference in size is typically required.



**Figure 2. pnc-Si membranes are efficient at removing contaminants as well as separating middle weight proteins.** Highly efficient separation of bovine serum albumin (BSA), MW 67 kD, and free dye, MW 1kD, was observed through Membrane A (mean pore size 7.3nm – panel a). A 4-fold separation of proteins BSA, MW 67kD, and IgG, MW 150 kD, was observed though Membrane B using the same method (mean pore size 18nm – panel b) [1].

We chose to separate two common blood proteins, BSA and immunoglobulin (IgG, MW = 150k, D = 14 nm), which vary in size by less than 3x using our pnc-Si membranes. Within minutes, BSA diffused through the membrane >4 times faster than IgG, whose diffusion coefficient is only 25% smaller (Figure 2b). Quantitatively comparing the two separation plots demonstrates that the increased cutoff of membrane B over membrane A allows 15 times greater BSA diffusion, but nearly completely prohibits IgG diffusion. Additional refinement of pore size would likely lead to even greater separation of BSA and IgG.

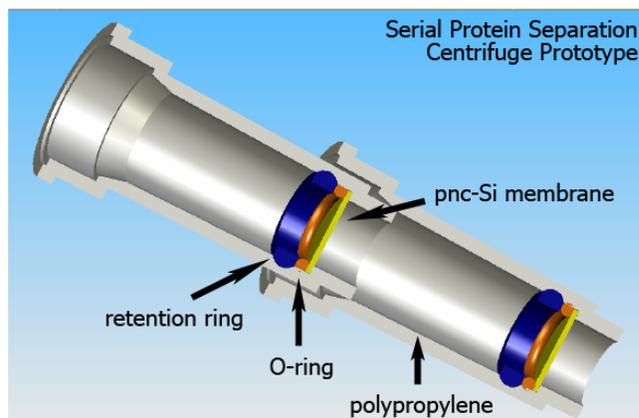
In addition to size based separation, pnc-Si membranes are also efficient at separating molecules based on charge. To test this effect, we induced net positive and negative charges on the membrane. We used these surface modifications to separate small molecules based on charge rather than size (Alexa Fluor 488: charge 2- and rhodamine 6G: charge 1+). In the first experiment, oxidation of the membrane induced a net negative charge at neutral pH. In low ionic strength solution, only positively charged molecules freely passed. We also induced a net positive charge through covalent attachment of aminosilane to the membrane surface. In these experiments, negatively charged small molecules passed freely, while positively charged molecule passage was retarded. In both cases, the presence of high ionic strength buffer (100 mM NaCl) eliminated the charge repulsion and both species passed freely (Figure 3).



**Figure 3. Surface charge restricts transport of like charged molecules through pnc-Si membranes.** pnc-Si membranes were surface modified with either aminosilane (+) or oxide (-). Transport of dye molecules 20x smaller than pore size was significantly affected by the surface charge at low ionic strength for both membranes, with like charged molecules being retarded, while oppositely charged molecules freely passed. The plot on the left shows how negatively charged Alexa Fluor dye passed freely through a positively charged membrane, but was retarded by a negatively charged membrane. The opposite was true for positively charged rhodamine 6G shown on the right. In high ionic strength buffer the Debye length and the electrostatic repulsion was reduced, resulting in similar transport for both charged species [1].

## 4 DEVICE DEVELOPMENT

Current methods to fractionate complex proteins mixtures while retaining biological function utilize chromatography columns. Column separation results in dilution with protein loss and is prohibitive for small volume samples. Loss is compounded by subsequent concentration techniques. These methods require hours of process time and considerable optimization. Our goal is to develop a device that can fractionate protein mixtures with volumes ranging from 10-500  $\mu$ L without loss and within minutes. To this end, we have developed a polypropylene centrifuge tube prototype for the pnc-Si membranes (Figure 4). The silicon insert is circular with narrow (400  $\mu$ m) membrane slits. Theoretical flow rates at 10 psi would result in separation in fewer than 10 minutes (Table 1) [6]. These devices would be invaluable to those seeking to isolate and identify proteins. Mass spectroscopy typically requires upstream purification to eliminate abundant proteins, while retaining proteins of interest. Future integration of our novel nanomaterial into microfluidic systems will also enable the development of rapid and high-efficiency blood analyzers as well as hemodialysis devices.



**Figure 4. pnc-Si membranes are easily integrated into separation devices.** Polypropylene centrifuge inserts were developed with Harbec Plastics (Ontario, NY). pnc-Si membranes are sealed in the prototype with a silicon O-ring and plastic retention ring. Larger species are retained behind the membrane, while smaller species pass through. Intermediate sized species can be trapped with serial separation. This device is also amenable to vacuum separation

**Table 1. Theoretical flow rate at 10 psi (mL/cm<sup>2</sup>-sec)**

material	Thick-ness	Porosity	Mean pore size	Water flow rate
pnc-Si (A)	15 nm	0.2 %	7.3 nm	0.26
pnc-Si (B)	15 nm	1.3 %	18 nm	4.0
pnc-Si (C)	15 nm	5.7 %	25 nm	46.8
track etched <sup>1</sup>	6000 nm	0.047 %	10 nm	0.00045
track etched <sup>1</sup>	6000 nm	0.42 %	30 nm	0.036
anodized alumina <sup>2</sup>	60000 nm	25 %	20 nm	0.10

Theoretical discussion of flow through nanopores was presented by Tong et. al. [6].

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<sup>1</sup> Polycarbonate track etched membrane characteristics from Sterlitech Corp.

<sup>2</sup> Nanoporous anodized alumina membrane characteristics from Whatman.

### *Disclosure:*

TRG, CCS, PMF and JLM are also co-founders of SiMPore Inc., a private company established to commercialize the pnc-Si membranes technology.