CHEMISTRY OF MATERIALS

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pH Valve Based on Hydrophobicity Switching

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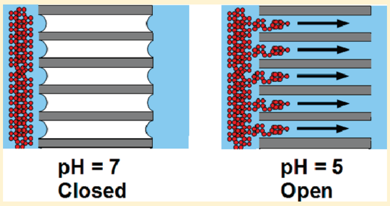
S *Supporting Information*

ABSTRACT: pH switchable valves were constructed using nanoporous membranes, the surface of which was modified by mixtures of aminopropyl trimethoxy silane and butyl trimethoxy silane. The modified membranes are dry at neutral and basic conditions because of their hydrophobicity but open to flux of aqueous solutions at slightly acidic pH because of protonation of amino groups. The resulting high contrast between the open and the closed states and a high flux in the open state because of large pore size make the approach attractive in applications where pH switching is employed, for example, in drug delivery applications.



KEYWORDS: pH sensitive valve, hydrophobicity

INTRODUCTION

One of the rapidly growing branches in material science deals with development of hybrid nanomaterials for applications in sensors, drug delivery, smart membranes, and others.[1−12] Smart gates represent a type of functional porous materials that control molecular transport by changing their properties in response to external stimuli. Reports on such nanostructures can be found in the literature, which include materials that are sensitive to such stimuli as light,[1] pressure,[2] biochemical analytes,[3−8] pH,[8−14] temperature,[8,15] and electrical potential.[16]

A number of studies have investigated pH sensitive surfaces and membranes. Among them, the most common approach employs variation of the pore size in membranes because of swelling/shrinking triggered by pH change. For example, in membranes made of polymer mixtures and copolymers, variation in the pore size because of pH change affects the mass flow through such pores.[9] Such pH sensitivity is achieved by the acidic or basic functional group in the polymer chains conformable to swelling because of electrostatic repulsion at the pH range where they are charged. By choosing appropriate groups, the flux enhancement at high or low pH can be achieved. A similar effect can be achieved when polymeric molecules are grafted on the surface of inert membranes acting as support.[9,10] In systems with small enough nanopores such as zeolites, more intricate supramolecular complexes with conformational changes induced by pH change were also employed.[12−14]

A less common approach is based on controlling the hydrophobicity of nanopores. Steinle et al.[11] reported that ionic current through nanoporous membranes modified by organic silanes with carboxylic ends dropped by 4 orders of magnitude when pH was raised from pH 7 to above pH 8. That was assigned to transition from the hydrophobic "closed" state at low pH to the hydrophilic "open" state brought about by deprotonation (ionization) of the carboxylic groups at high pHs. It remained unclear how sensitive the effect was to the density of carboxylic groups, and the dynamics of changes was not addressed.

In this paper we utilize a similar concept but proceed further in designing a pH sensitive valve, where not only ions but also the whole solution flow is altered by pH in the range suitable for applications in drug delivery. We achieve it by modifying the surface of inorganic nanoporous membranes with a mixed monolayer that changes surface wettability from hydrophobic to hydrophilic by lowering pH and thus allowing dramatic control of mass transport through the pores. We illustrate how such modifications can be optimized by evaluating contact angles on the similarly modified flat surfaces. This is an extension of the previously introduced concept of hydrophobicity switching, where wettability of hydrophobic surfaces of nanopores changes in response to various stimuli such as light and biochemical analytes.[1,6] Because the switching is induced by lowering the pH from neutral to slightly acidic, this platform can be employed in intracellular drug delivery systems where cargo is released from the hydrophobic pores because of pH drop in endosomes and lysosomes.

EXPERIMENTAL SECTION

Materials. All chemicals were of analytical grade and purchased from commercial suppliers: (3-aminopropyl) trimethoxysilane (APTS) and safranin O from Aldrich; *n*-butyl trimethoxysilane (BTS) from Polyscience. The buffers were either purchased, phosphate buffered saline (PBS) kit from Antibodies Incorporated (pH 7.4, 0.14 M NaCl), or prepared at 0.05 M concentration: pH 10.4 from triethylamine (Aldrich), pH 4.2 from succinic anhydride (Aldrich), pH 5.0 from acetic acid (Aldrich), and pH 7.4 from ethylenediamine (Fisher); 0.1 M NaCl was added to all of them. The microscope glass slides were from Fisher Scientific, and alumina nanoporous membranes Anopore (with a nominal diameter 0.2 μm) from Whatman.

Surface Modification. Glass slides were first cleaned using piranha solution (35% H_2O_2-65% H_2SO_4. ***Caution!*** *piranha solution is explosive*)

Received: February 16, 2011
Revised: July 5, 2011
Published: July 21, 2011

for 20 min at 70 °C, washed with copious amount of deionized (DI) water and dried in an oven for 30 min at 115 °C. Silanization was performed overnight in 2% v/v toluene solution of silane mixtures (with different percentages of amino and butyl silanes).[1,17] Modified slides were washed with ethanol and methanol and cured for 3 h at 115 °C.

Anodized aluminum oxide (AAO) membranes were first cleaned by boiling in water for 20 min. After drying at 110 °C for 20 min, they were silanized using the same procedure as described above.

FTIR Characterization. Quality of modification of alumina membranes was evaluated with FTIR, for which the spectra were recorded using a Perkin-Elmer Spectrum One FT-IR spectrometer (0.1 cm^{-1} resolution); unmodified membrane was used as a reference.

Contact Angle Measurements. Contact angles, θ, were measured with an Attension Theta optical tensiometer from KSV Instruments LTD using the Sessile drop method. Droplets of approximately 1.5 μL volume were deposited on the surface enclosed in an environment chamber designed to minimize evaporation. Humidity close to 100% was maintained in the chamber by a container with liquid water. All measurements were performed at 25 °C. The value of θ was obtained by fitting the shape of the droplet using the circular approximation algorithm of the Attension Theta software.

Diffusion Experiments. Diffusion experiments were carried out at 25 °C in a diffusion cell made of two optical cuvettes connected via a membrane through complementary holes on each. The effective area of the membrane connecting the two compartments, reservoir and sink, was 12 mm^2. Solutions on both sides were buffered at the same pH and ionic strength (~0.1 M), but only the reservoir compartment initially contained safranin O dye at 1.4 mM concentration. The solutions were constantly stirred using magnetic stirrers, and the dye concentration in the sink was monitored using a CHEM2000 UV−vis spectrometer from Ocean Optics by measuring absorption at the wavelength of maximum (514 nm).

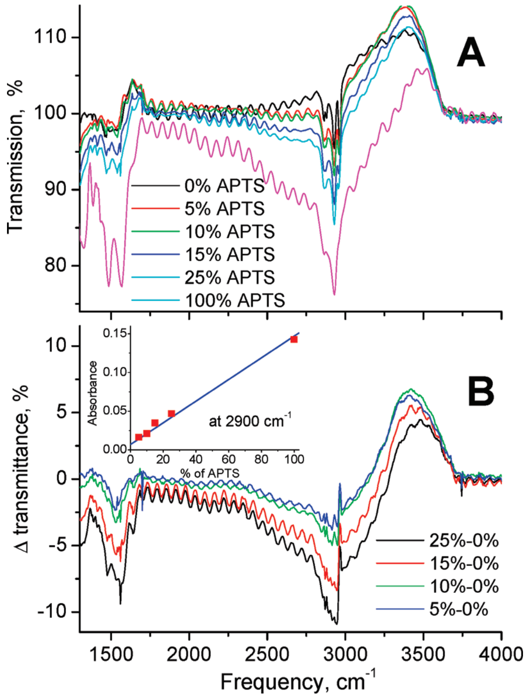


Figure 1. FTIR spectra of alumina membranes modified with different mixtures of butyl and aminopropyl silanes. **A.** The spectra are referenced with respect to an unmodified membrane. **B.** The spectra are referenced with respect to 0% of aminosilane in the modifying mixture. The inset illustrates that the amine concentration on the surface is close to that in the modifying solution.

RESULTS AND DISCUSSION

IR Absorption. There is not enough sensitivity for measuring IR absorption of monolayers on glass slides, but FTIR spectra of alumina membranes modified with silanes of different proportions can be recorded because of a 3 orders of magnitude increase in the effective path length.[3] These spectra presented in Figure 1 have characteristic features that can be assigned to aminopropyl and butyl silanes. Membranes themselves present some artifacts such as lack of transmittance below 1300 cm^{-1} and the interference pattern due to variable membrane thickness that appears as oscillations of up to 1% in transmittance. A broad peak between 2700 and 3700 cm^{-1} because of hydroxyls appears as enhanced transmittance for all samples when compared to an untreated membrane.[17,18] Amine stretching appears as a broad absorption at lower frequencies, between 2300 and 3400 cm^{-1}, which increases with the concentration of aminated silane.[19] Both sharp peaks of methylene stretching, asymmetric near $\nu_a(CH_2)$ ~ 2920 cm^{-1} and symmetric near $\nu_s(CH_2)$ ~2860 cm^{-1},[17,20,21] practically do not change among the samples, as would be expected since aminopropyl and butyl silanes have the same number of methylenes. The effect is best seen in Figure 1B showing the spectra normalized to purely butyl silane modified membrane (0% of APTS). The intensity of methyl stretching at $\nu(CH_3)$ ~2965 cm^{-1}, on the other hand, declines with increasing proportion of APTS as the latter lacks the methyl. In Figure 1B it is seen as an increasing sharp feature at 2965 cm^{-1}. The range 1300−1700 cm^{-1} has multiple bending vibrations of CH, amines, and hydroxyls. The latter appear as enhanced transmittance near ~1650 cm^{-1} in the modified membranes while the former two are easier to recognize by comparison with membrane of 0% APTS (Figure 1B). The increasing absorption near ~1560 cm^{-1} with raising the amount of APTS agrees with the corresponding peak of NH bending,[22] while CH_x bending vibration near ~1370 cm^{-1} appears as a depression.

Plotting the absorbance at 2910 cm^{-1} versus the percentage of APTS in the modification mixture, shown in the inset of Figure 1B, illustrates a straightforward correlation, which suggests that the percentage of amines in the monolayer on the surface of alumina pores is the same as in the solution used for modification. Such a correlation is not universal as we have seen it to differ dramatically with longer aliphatic chains and longer amines on fused quartz as a substrate.[26] Nevertheless, at least for similarly short butyl and aminopropyl silanes, the percentage of amines in the monolayer on the walls of alumina nanoporous membrane is almost the same as in a modifying solution.

Since the thickness of a monolayer is almost 2 orders of magnitude less than the pore diameter, the pore clearance remains the same after the modification. SEM images confirm that (see Supporting Information). Because of such significant size dissimilarity, the monolayer behavior on the pore walls is not much different from that on a flat surface. Thus, if some segregation between aliphatic and aminated silanes takes place, its characteristic dimension on the pore walls should also be similar to such on flat surfaces. Hence, we can use pH sensitivity analysis of flat surfaces modified with different mixtures of the two silanes to mimic anticipated behavior inside the nanopores.

pH Sensitive Wetting of Flat Surfaces. Holmes-Farley et al.[23] have demonstrated that for some surfaces contact angles of aqueous solutions vary with pH. Ionizable groups such as carboxyls

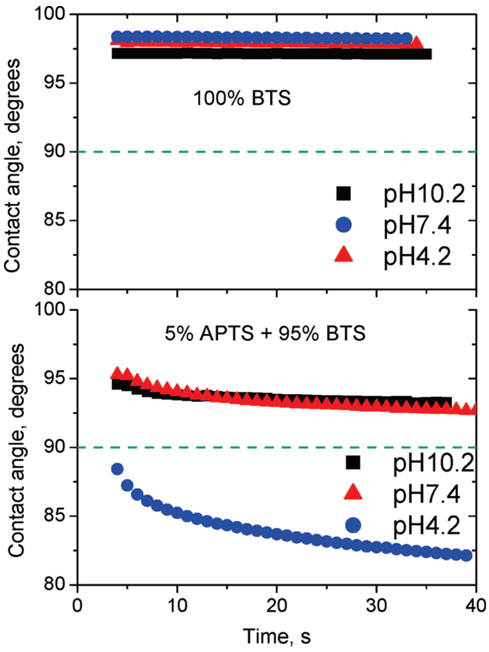


Figure 2. Variation of the contact angle θ with time at different pH. Top: purely aliphatic butyl silane modifier. Bottom: 5% of amino silane (APTS) in the mixture with butylsilane (BTS).

and amines, can make surfaces responsive to pH. For example, amines at low pH are charged because of protonation and thus are more hydrophilic. At high pH they are neutral and thus less hydrophilic.

According to Holmes-Farley et al.,[23] wettability of surfaces modified with only primary amines does not demonstrate significant variation with pH. We see some variation, which is not as essential because such surface remains hydrophilic ($\theta < 90°$) for both acidic and basic pH (see Figure 3). However, if amines are mixed with aliphatic molecules in an appropriate ratio, lowering pH can cause the surface switch from hydrophobic to hydrophilic. Figure 2 represents the typical kinetics of the contact angles for Sessile droplets of different pH on purely aliphatic surface and on a partially aminated surface prepared using a mixture of aminated (APTS) and aliphatic (BTS) silanes. On the aliphatic surface, contact angles have similar behavior for all pH: the angle initially decreases because of mechanical equilibration (first few seconds of which are not shown) and settles at an equilibrium value, $\theta \sim 97-98°$. Mixed partially aminated surfaces demonstrate a clear switch from hydrophobic ($\theta > 90°$) to hydrophilic ($\theta < 90°$) behavior upon decrease of pH. The equilibrium contact angle in Figure 2 for droplets with pH > 7 resembles that of the aliphatic surface with $\theta \sim 93°$, while the drop of pH 4.2 shows a smaller contact angle, $\theta \sim 82°$. The contact angle decrease is accompanied by spreading of the drop which illustrates that the wettability change in this case is not instantaneous. (See Supporting Information video)

Figure 3 represents the variation of θ with pH as a function of the amino silane portion (APTS) in the mixture used for surface modification. The graph confirms that a surface modified with only aliphatic silane (BTS) is not pH sensitive, while mixtures are. Because the droplet spreading on partially aminated surfaces is relatively slow and is obscured by water evaporation at longer times, the contact angles are given for a particular time, 50 s after droplet deposition, when evaporation is not noticeable yet.

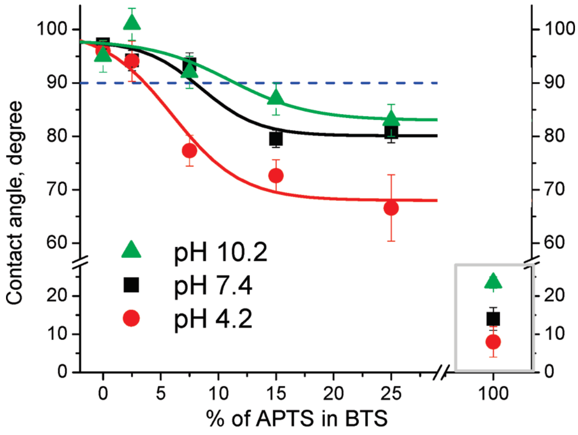


Figure 3. Contact angles for droplets of different pH on surfaces modified with different proportions of APTS in mixtures with BTS. The values were taken at 50 s after depositing the drop on the surface. The lines are guides to the eyes.

Partially aminated surfaces all have lower contact angles at pH = 4.2 than at pH = 7.4 and pH = 10.2 indicating their enhanced wettability by water at low pH. With increasing percentage of amines in the monolayer the contact angle decreases at both high and low pH, but the latter declines faster and thus increases the extent of pH induced variation. The trend of pH dependent contact angle variation remains even for 100% amine, but the contact angles indicate good wettability of this surface at all pHs.

It may seem strange at first that we observe switching for pH < 7 while pK_a of primary amines is near 10.5 but it should not be surprising. It is well-known that charge repulsion from proximal cations in polymers like polylysine can result in pK_a reduction to pK_a < 7.[24,25] The range for pH switching should depend on the density of amines. The pK_a value is also affected by the proximity of hydrophobic tails in the monolayer, which are of a similar length as the amine and provide enough amine access to solution. When silanes with longer aliphatic tails are used, they hide amines from access to water and thus prevent them from ionization and significant altering of the surface tension. For example, flat surfaces modified by 10% mixture of APTS with hexadecyl silane do not show noticeable pH dependence of the contact angle (Supporting Information). Different relative lengths of aliphatic and amino chains and the density of amines in the hydrophobic monolayer are the key factors that allow variation in the desired range of pH switching. One can recognize the effect of density in Figure 3, where the switching between hydrophobic and hydrophilic behavior (θ dropping below 90°) for pH = 7.4 is close to 8% of amines and for pH = 4.2 it happens at a much lower density ~3% of amines. These percentages may not coincide with those for the surface bound amines, as was reported before for other silane mixtures of amine/aliphatic tails,[21] but they are likely very similar for the short silanes used here, as was confirmed above using FTIR on alumina membranes. Nevertheless, because of the difference between silica and alumina surfaces, some deviations should be anticipated. One should also appreciate the two-dimensional nature of amines' assembly on the surface, which should lead to a much stronger variation of the resulting pK_a with their surface density than in a one-dimensional case like polylysine. Surfaces modified by carboxylic acids similarly show significantly altered pK_a > 8 greater than its pK_a ~ 4.5 in solution.[11]

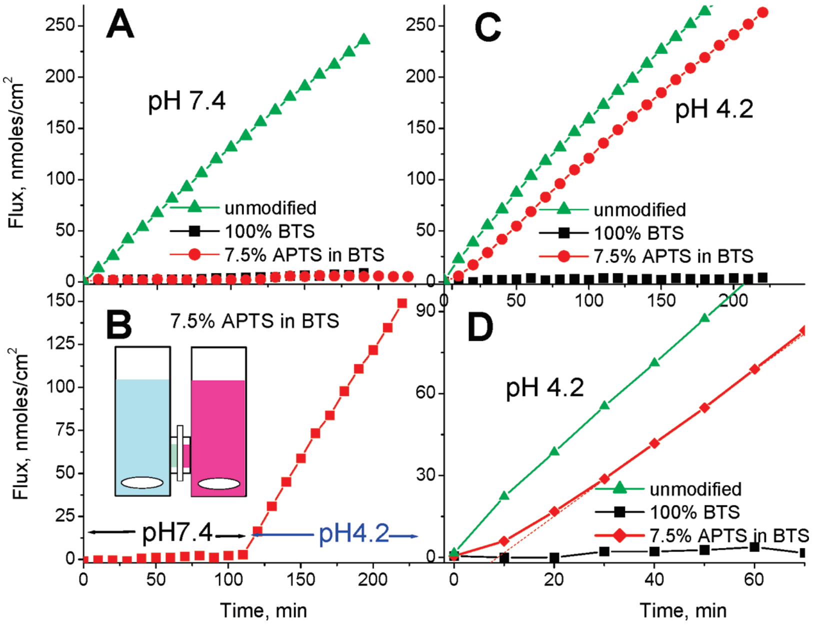


Figure 4. Kinetics of safranin dye flux through nanoporous membranes with different surface modifications and at different pH: (**A**) At pH = 7.4 only unmodified membrane is open while purely hydrophobic and partially aminated membranes are closed. (**B**, **C**) Partially aminated membrane opens up at pH = 4.2 with the flux similar to that of the unmodified membrane. (**D**) An expanded version of **C** illustrating a delay (~9 min) required for the membrane wetting (dashed line). The inset in **B** sketches the setup consisting of two optical cuvettes with complementary holes on the sides connected via the modified membrane. Only the reservoir (pink) side has safranin at the beginning. Solutions on both sides are have been stirred with magnetic bars (white).

In the pH sensitive valve application, as described below, it is imperative that at the pH of the desired closed state the contact angle is greater than 90° while it drops below 90° in the pH induced open state. One particular application of such an effect is in drug delivery, where release of cargo can be induced by a lower pH in lysosomes but should remain hydrophobic at pH 7.4 to retain the cargo outside the region of low pH. Figure 2 shows that such a condition can be realized using pH induced hydrophobicity switching in nanopores, the surface of which is modified with a mixture of aliphatic and aminated silanes. The optimum percentages of the latter should not exceed 7.5% for which we see that the contact angle exceeds 90° at pH 7.4 but drops below 90° for acidic pH.

pH Gated Membranes. Hydrophobic pores of a small enough diameter remain dry in water and can withstand a high pressure difference, ΔP, defined by the Laplace equation:

$$\Delta P > 4|\Delta\gamma|/D_{\text{pore}} = 4|\gamma\cos\theta|/D_{\text{pore}} \qquad (1)$$

where D_{pore} is the pore diameter. The surface energy difference, $\Delta\gamma$, between the wall/vapor surface tension, γ_{wv}, and the wall/liquid surface tension, γ_{wl}, can be related to the contact angle on a flat surface, θ, and the surface tension of liquid/vapor interface, γ, via the Young equation:

$$\Delta\gamma \equiv \gamma_{wl} - \gamma_{wv} = \gamma\cos\theta \qquad (2)$$

The critical pressure exceeds 1 bar even for the very modest contact angles, $\theta \sim 93°$, in quite large pores with $D_{\text{pore}} \sim 0.2\ \mu\text{m}$, creating an effective natural plug against water intrusion.[2,5] When the contact angle becomes smaller than $\theta < 90°$, this plug disappears and opens the valve.

On the basis of the data from the flat surface, 0.2 μm AAO membranes were modified with 7.5% mixture of aminated (APTS) silane along with aliphatic (BTS) silanes and investigated their performance as pH triggered valves. The transport behavior was evaluated using safranin O dye that has a higher solubility in water with a strong absorbance that does not significantly depend on pH.[27] Placing concentrated safranin solution into the reservoir allows convenient optical monitoring of its diffusion into the sink solution in real time. Both reservoirs had solutions buffered at the same pH and with high concentration of salt (>0.1 M NaCl) to minimize possible surface wall charge effects on transport of charged molecules through the nanopores.[5,7,28]

Figure 4 shows that at pH 7.4 only the unmodified membrane is open to flux of safranin molecules while the BTS modified membrane and the partially aminated membrane prevent the dye transport because of their hydrophobicity. At pH 4.2 the partially aminated membrane also opens up, as expected, allowing dye to diffuse through to the sink solution. Note that the solely aliphatic modification retains membrane hydrophobicity and blocks the dye transport. The rates of transport for both, unmodified and modified, membranes are very similar as can be seen from the slopes of their accumulation curves, with a short delay for the partially aminated membrane because of a slow wetting of the pores by the solution (~ 9 min in Figure 4). Changing pH from 4.2 back to neutral pH 7.4 does not automatically regain the blockage because water filled hydrophobic pores do not spontaneously dewet.[1,2,5] Formation of a bubble is the first step of dewetting and is associated with a large activation barrier (>10^6 kT for the 0.2 μm pore diameter) that makes it kinetically unfeasible, as was discussed previously.[29,30] Drying off the membrane is required for the recovery of its water repelling property at higher pHs.

The described switching effect is due to pH and not due to sensitivity to other ions as the same behavior was observed with other buffers of similar pH, for example, pH 7.4 with ethylenediamine buffer behaves as PBS and pH 5.0 with acetic acid buffer behaves similar to pH 4.2 succinic anhydride buffer (see Supporting Information).

We wish to emphasize the benefits of our pH induced hydrophobicity switching membranes in performing as valves when compared to other pH sensitive membranes such as those employing pH induced swelling. These benefits are as follows: (i) dry pores do not allow water intrusion and thus prevent any diffusion through in the closed state, and (ii) in the open state, the flux is very large and is the same as in the pores with no modification. The overall large contrast in controlling the flux should be particularly useful in drug delivery systems as well as in other possible applications.

CONCLUSIONS

Hydrophobically modified nanoporous membranes prevent aqueous solutions from passing through in both acidic and basic conditions. Mixed modifications with 5−8% of aminosilane (APTS) along with aliphatic (BTS) silane make surfaces pH responsive, and the corresponding membranes perform as pH switchable valves. These membranes retain hydrophobicity at neutral and basic conditions but open to passage of molecules at slightly acidic conditions, which makes this platform suitable for drug delivery application. The high contrast between the open and closed states, as well as high fluxes in the open state because of large pore size can probably be useful in other applications for pH switchable valves.

ASSOCIATED CONTENT

Ⓢ **Supporting Information.** SEM images of the membranes before and after modification, contact angles with different buffers and with a longer aliphatic silane; also two movies illustrating pH dependent droplet spreading and transport through the membrane. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This work was partially supported by a grant from the National Science Foundation (NSF DMR 0900238). The authors are grateful to J. Curtiss and P. Cook for help with SEM imaging.

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