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Selective separation of similarly sized proteins with tunable nanoporous block copolymer membranes

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The existing processes for bioseparations using conventional resin-based chromatography are time-consuming and highly expensive. Newly developed membrane separation processes offer substantial economic, environmental, and safety benefits.[1] Size-based separation of proteins can be carried out by ultrafiltration membrane systems when the proteins have significantly different molecular sizes. However, separation of similar-sized proteins with current commercially available membranes is not feasible.[2] Recently, Striemer *et al.*[3] have reported the fabrication of porous nanocrystalline silicon (pnc-Si) membranes using standard silicon fabrication techniques. This kind of ultrathin membrane has average pore sizes ranging from 5 to 25 nm and its thickness is only 15 nm. Using such membranes, they demonstrated for the first time the separation of bovine serum albumin (BSA, 66 kDa) and immunoglobulin-γ (IgG, 150 kDa), two proteins that only have a twofold difference in their MW. They found that BSA diffuses through the membrane 4 times more rapidly than IgG. However, the area of their membrane is only 0.04mm^2 and difficult to be up-scaled. Stroeve *et al.*[4] used the commercial poly (carbonate) track-etched (PCTE) membranes (6μm thickness) as template followed by electroless gold deposition to form the Au-coated membranes. The surface of the membrane was further modified and negatively charged with Self-Assembled Monolayers (SAMs) of functionalized thiols ($HSC_{10}H_{20}COOH$). Using this PCTE/Au/SAMs membrane, they separated two similar-sized proteins, BSA and bovine hemoglobin (BHb, 65 kDa), with an ionic strength difference (to cause osmotic flow) in a U-shaped diffusion cell. A separation factor of 4.2 was obtained at pH 4.7. Two years later, they used the same membrane for the separation of these two proteins again with a much lower ionic strength.[5] Although a higher separation selectivity of about 67 was achieved, the complicated procedures and low pore density (6/μm^2) could limit the use of the membranes.

We recently proposed the use of supramolecular assemblies of block copolymer micelles in solution in the presence of copper ions for the manufacture of skinned asymmetric membranes with a thin nanoporous top layer (400 nm thickness).[6, 7] In this work, we used a block copolymer with different block ratio and we used a different solvent system. An integral asymmetric membrane was obtained by combining the self-assembly of amphiphilic block copolymer (PS-*b*-P4VP) and non-solvent-induced phase separation. The membrane comprises a highly ordered nanostructured top thin layer with uniform pore size and a non-ordered sponge-like layer beneath. The diameter of the very regular pores was approximately 34 nm measured from SEM images at high magnification. It should be mentioned that the effective pore diameter is actually smaller, because the pores are lined with P4VP. Long-range order of regular monodispersed nanopores was also confirmed from SEM pictures (Fig. 1a). The determined extremely high surface pore density shown in Figure 1 is estimated to be around 2.2×10^{14} pores per square meter. The average cylinder length of the exceptionally well ordered top thin layer is around 100 nm as shown in the cross-section SEM image (Fig. 1b). As a separation layer, this is much thinner than the obtained 400 nm in our previous report. The free-standing membrane area in our experiment was more than 50 cm^2 and can therefore be easily handled and used. This very fast one-step manufacture of the membrane can be easily up-scaled. Due to the ultrahigh pore density and the thinner top layer, the water fluxes through the membranes have extremely large values of more than 3200 L m^{-2} h^{-1} bar^{-1}, which are almost 2 orders of magnitude higher than those of commercially available membranes with comparable pore size. Some medical processes like hemodialysis could be substantially shortened with high flux membranes that

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currently take patients hours for treatment each day. Another superior property of this membrane is that it is stimuli (pH)-responsive. The free pyridine groups at the membrane surface are protonated at low pH and the P4VP segments stretch to minimize charge repulsion, transforming the pores into a sensitive gate controlled by pH without any modification. The effective pore radius of this tunable membrane was estimated using Hagen–Poiseuille's law. These attractive characteristics make this membrane particularly well suitable for size-selective and charge-based separation applications of biomolecules.

To test the performance of the membrane, diffusion experiments at the physiological pH of 7.4 in PBS solution were carried out with two proteins of different diameter, BSA and globulin-γ, which are too close in size (2-fold difference) to be efficiently separated through conventional dialysis membrane processes (proteins with at least a 10 times MW difference is recommended by the manufacturers). The passage of the two proteins through the membrane was monitored and the diffusion rate difference by a factor of 87 was measured indicating its potential use for membrane-based protein fractionation/chromatography. Furthermore, charge-based diffusive transport and separation with two proteins of similar molecular weight (BSA and BHb) through the unmodified membrane with pH-dependent pore size have also been studied as a function of external pH. BSA is of nearly identical molecular weight as BHb, but has a lower isoelectric point (pI=4.7) than BHb (pI=7.0). The pH of the phosphate buffer solutions was varied from 4.67 to 10.32, while the ionic strength of all the buffer solutions was maintained at 0.01M. We performed each experiment at different pH in triplicate to make sure the results are convincible. Figure 2 shows the results of the average flux of both BSA and BHb across the membrane as a function of the external pH. The achieved highest separation selectivity is about 10 at pH 4.7 (Fig. 3). The membrane was further modified and positively charged by quaternization resulting in extremely enhanced separation selectivity. The large protein molecules can be heavily charged (BSA net molecular charge is 13- at pH 7). Stronger electrostatic interactions between proteins and charged membranes are expected when the experiments were repeated with the modified membrane. BSA was completely blocked when the pH value was equal to 7.0, while BHb was completely blocked at pH 4.7. We successfully engineered this membrane that could completely exclude one protein but permit passage for another one. Separation experiments were all governed by diffusion in this study to allow us better understand the transport behavior and separation mechanism of the biomolecules through the membrane. Although the diffusion rates were not very high when ionic strength and pore sizes were reduced to enhance selectivity, substantial enhancement of the rate is expected in a centrifuge or in a pressure-driven system since our membrane can withstand up to 3 bars of differential pressure. Such systems are able to achieve a desirable separation performance with both high selectivity and high rate. Conclusively, this is the first study of protein transport and separation with PS-*b*-P4VP membranes in which the pore sizes and charges are tunable. These results highlight the perspective of the proposed new membrane for efficient separation applications of biological substances/pharmaceuticals in bioscience, biotechnology and biomedicine.

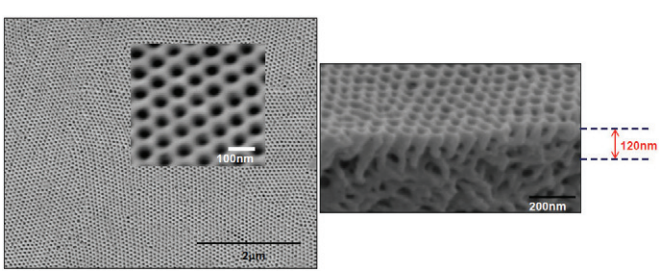

Figure 1. (a) Long-range ordering of the membrane surface, (b) cross-section SEM image.

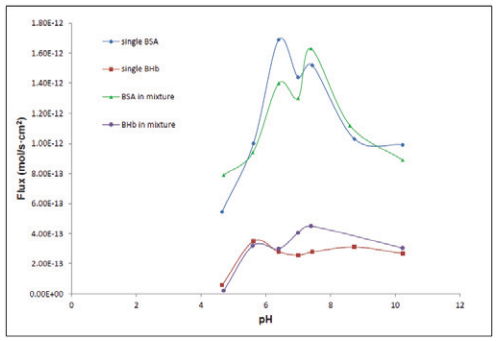

Figure 2. Average flux of replicates at each pH.

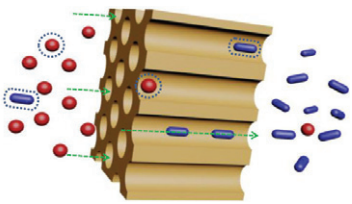

Figure 3. Cartoon picture shows the transport of BSA (red sphere) and BHb (blue ellipsoid) through the membrane at pH 4.7.

REFERENCES:

(1) de Vos, R. M.; Verweij, H. Science 1998, 279, 1710-1711.

(2) Osmanbeyoglu, H. U.; Hur, T. B.; Kim, H. K. Journal of Membrane Science 2009, 343, 1-6.

(3) Striemer, C. C.; Gaborski, T. R.; McGrath, J. L.; Fauchet, P. M. Nature 2007, 445, 749-753.

(4) Chun, K. Y.; Stroeve, P. Langmuir 2002, 18, 4653-4658.

(5) Ku, J. R.; Stroeve, P. Langmuir 2004, 20, 2030-2032.

(6) Peinemann, K. V.; Abetz, V.; Simon, P. F. W. Nature Material 2007, 6, 992-996.

(7) Nunes, S. P.; Behzad, A. R.; Hooghan, B.; Sougrat, R.; Karunakaran, M.; Pradeep, N.; Vainio, U.; Peinemann, K. V. ACS Nano 2011, 5, 3516-3522.

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