Supplementary information

Figures and Table

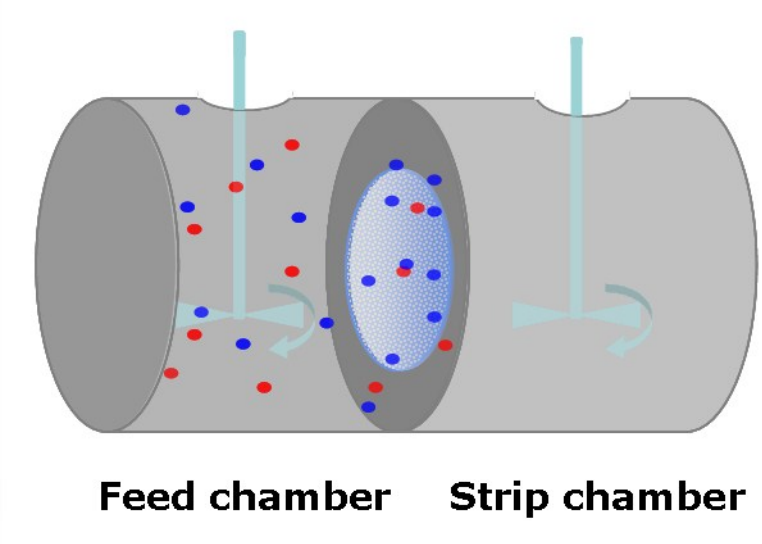

Figure S1 Schematic of the dialysis permeation cell with the molecularly imprinted membrane between the feed chamber and the strip chamber.

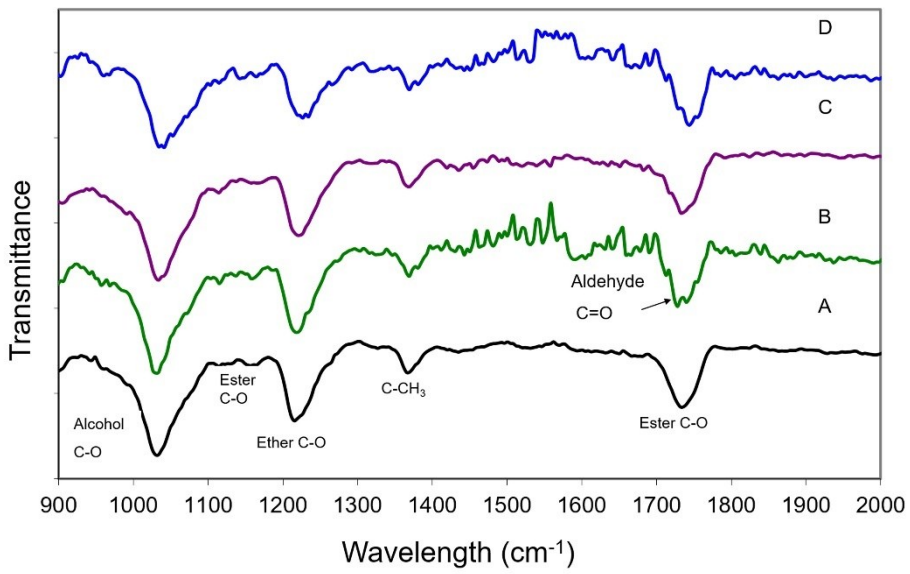

Figure S2 FTIR spectra of the original CA membrane and membranes after modification: (a) Original CA; (b) Aldehyde functionalization; (c) Surface polymerization of MIM_{BSA}; (d) Surface polymerization of MIM_{Lys}.

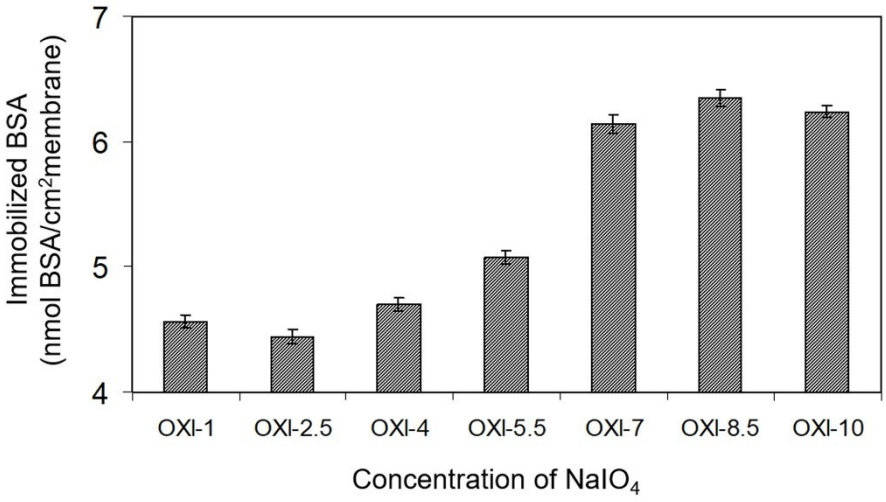

Figure S3 The profile of BSA immobilization density as a function of the concentration of $NaIO_4$ on the aldehyde functionalized CA membrane. (For all the samples, the initial BSA amount used for immobilization is about 380 nmol and the apparent surface area of the membrane is 20 cm^2).

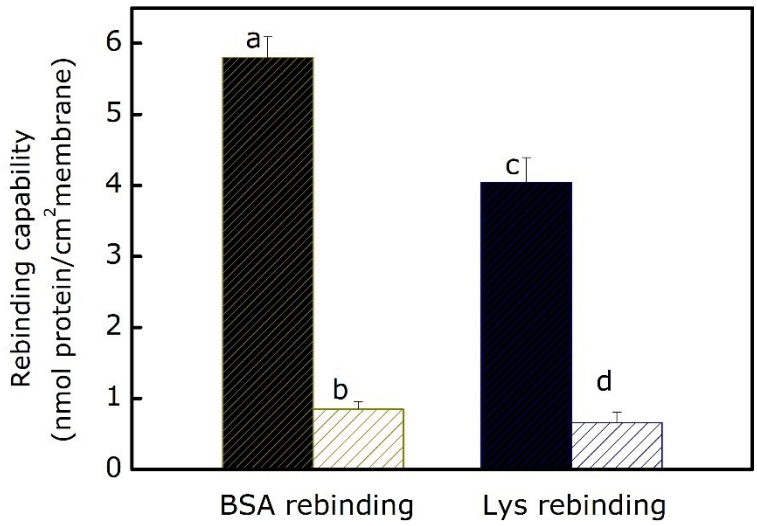

Figure S4 Protein rebinding capacity on MIMs and NIMs: BSA rebinding capacity on (a) MIM_{BSA} and (b) NIM_{BSA}; Lysozyme rebinding capacity on (c) MIM_{Lys} and (d) NIM_{Lys}.

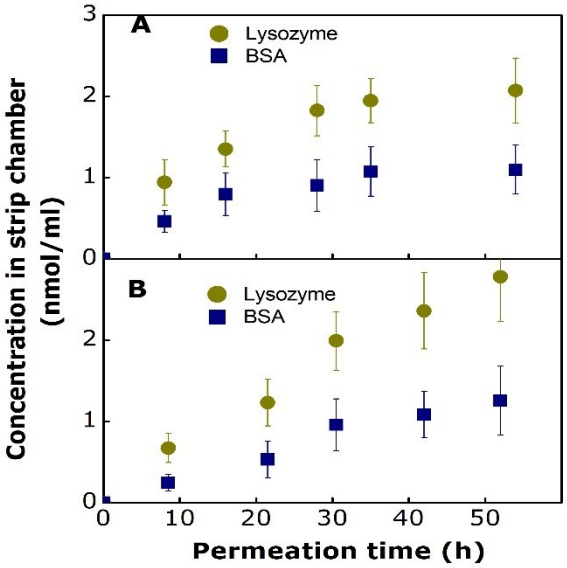

Figure S5. Protein concentration profile in the strip chamber using (A) NIM_{BSA} and (B) NIM_{Lys} in the permeation test.

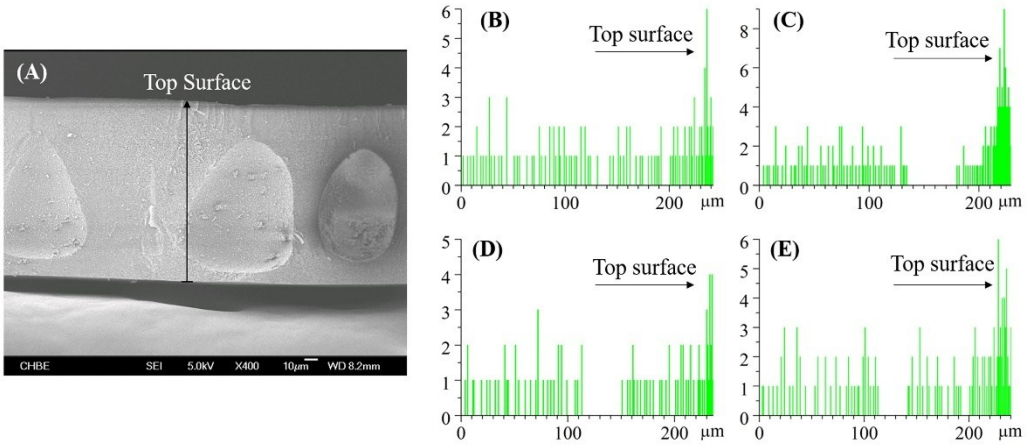

Figure S6. (A) Morphology of membrane cross-section with the dense skin layer as the top surface. Nitrogen distribution profiles in cross-sections of MIMs by EDX: (B) MIM_{BSA} after rebinding test for 48 h; (C) MIM_{BSA} after continuous permeation test for 48 h; (D) MIM_{Lys} after rebinding test for 48 h; (E) MIM_{Lys} after continuous permeation test of 48 h.

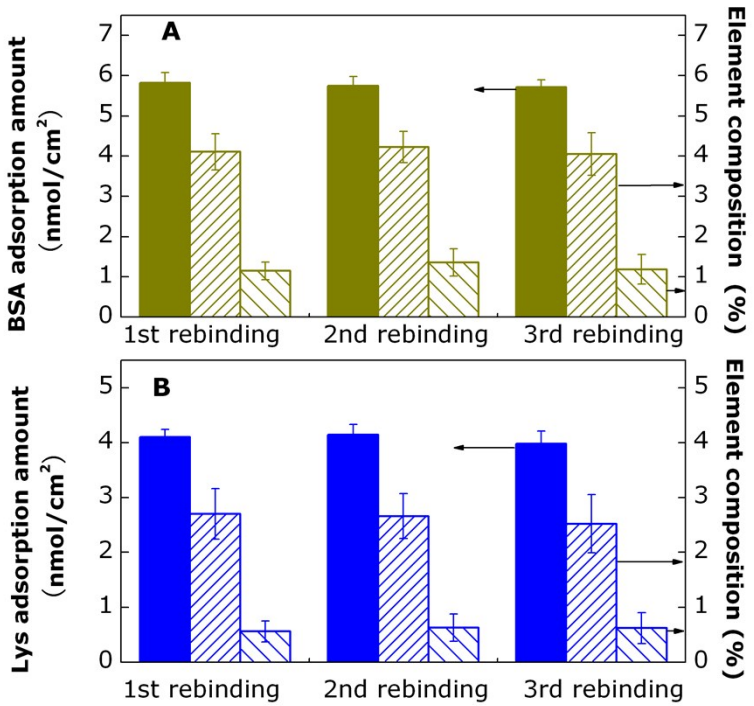

Figure S7 Protein adsorption amount (■) and Nitrogen element composition on the surface of MIMs after the first, second and third rebinding (▨), and Nitrogen element residue on the surface of MIMs after the first, second and third protein removal (▩): (A) MIM_{BSA}; (B)MIM_{Lys}.

Table S1 Surface elemental compositions of membranes from each modification step tested by XPS

Atomic concentration (%)	**O**	**N**	**C**
Original CA	39.22	0	60.78
Aldehyde functionalization	37.68	0	62.32
BSA immobilization	32.44	4.49	63.07
Surface polymerization (BSA)	42.76	4.11	53.13
BSA removal	45.84	0.71	53.45
Lysozyme immobilization	37.56	2.70	59.74
Surface polymerization (Lys)	41.25	2.01	56.74
Lysozyme removal	41.96	0.52	57.52

Table S2 Pore diameter and pure water permeability of MIMs and NIMs.

Membrane	Pore diameter on the skin layer (nm)	Pure water permeability (L m^{-2} h^{-1} bar^{-1})
NIM_{lys}	22.9	62.5±3.3
NIM_{BSA}	18.0	37.9±3.4
MIM_{lys}	19.6	42.2±4.5
MIM_{BSA}	14.5	24.2±3.4

Optimization of aldehyde functionalization degree for protein immobilization

As shown in Figure S2, the appearance of the characteristic peak of aldehyde group at ~1720 cm^{-1} in the FTIR spectrum demonstrates that the modification of CA membrane using $NaIO_4$ generated aldehyde groups from the hydroxyl groups of the CA polymer. These aldehyde groups could then be used as binding sites for subsequent covalent protein immobilization. Therefore, it is desirable to generate as many aldehyde groups on the CA membrane surface as possible to maximize the density of immobilized protein. Different concentrations of $NaIO_4$ ranging from 1-10% were thus investigated on the immobilized BSA density at constant reaction duration of 6 h.

It was observed from Figure S3 that the immobilized BSA density on CA membrane reached a plateau at ~7 wt% $NaIO_4$. The plateau shows that there may be a limit to the BSA attachment space on the CA membrane, thus increasing $NaIO_4$ beyond 7 wt% did not result in further increase of immobilization. In addition, BSA has 60 lysine residues[1] which are capable of binding with the aldehyde functional group to form Schiff base imine linkages. Thus, each BSA molecule might bind to several aldehyde functional groups on the membrane, reducing the capacity of the CA membrane. It is also worth noting at this point that overly high $NaIO_4$ concentration and/or prolonged

reaction durations may result in extensive oxidation that breaks the polymer chains of the CA membrane. Therefore, an optimized concentration of 7 wt% $NaIO_4$ was used for the subsequent tests. In Table S1, an increase in nitrogen content at the protein immobilization step indicated that protein molecule had been immobilized. The physical adsorption of protein by CA membrane is assumed to be negligible, pristine CA is very hydrophilic (contact angle of 58 ± 0.5˚).

Permeation study

By comparing Figure S5c and S5e, it was revealed that some nitrogen residues and thus the proteins were retained within the membrane substrate just beneath the skin layer after permeation. By comparing Figure S5c and S5b with previous competitive binding tests, it can be deduced that the adsorbed BSA molecules were in a higher quantity in the MIM_{BSA} after permeation than after just adsorption. The same phenomenon was also found in MIM_{Lys} as shown in Figure S5d and S5e.

Methods

Membrane substrate preparation

Raw Cellulose acetate (CA) (CA-389-30, Eastman Chemical Company, USA) powder was dried overnight at 120°C under vacuum. A CA solution was prepared by dissolving the dried CA (15 wt%) in N-Methyl-2-pyrrolidone (NMP, 99.5%, Merck, USA) (85 wt%) under constant mechanical stirring (100 rpm) in a flask for 14 h at 40°C. The obtained homogeneous solution was allowed to stand at room temperature

for a day to remove bubbles. The polymer solution was poured onto a glass plate at ambient temperature and casted with an automatic film applicator (TB1000-1, BESV) to obtain a designed thickness of 250 μm. The glass plate was then immediately immersed in a water bath at ambient temperature for precipitation and solvent exchange for 48 h.

Aldehyde functionalization on the membrane

Each CA membrane had an apparent surface area of 20 cm^2 and was fixed onto a Petri dish to ensure that oxidation by 15 ml of sodium periodate solution occur mainly on the top of the membrane surface. Periodate solutions with the concentrations of 1, 2.5, 4.0, 5.5, 7.0, 8.5 and 10 wt% were used to produce different density of aldehyde groups on the membrane surface. The oxidization was conducted for 6 h at room temperature in the dark. The oxidized membranes were named OXI-1.0, OXI-2.5, OXI-4.0, OXI-5.5, OXI-7.0, OXI-8.5 and OXI-10.0, respectively.

Immobilization of template BSA on the membrane

The original CA, OXI-1.0, OXI-2.5, OXI-4.0, OXI-5.5, OXI-7.0, OXI-8.5 and OXI-10.0 membranes were immersed in the BSA solution (2.5 mg/ml in 0.1 M PBS) for 12 h at 4°C with gentle shaking. The BSA-immobilized membranes were then washed thoroughly with de-ionized water. The amount of BSA immobilized on the membrane surfaces were determined by measuring the difference between the initial and final BSA concentrations in the solution using UV-Vis spectrophotometry at 220 nm. The density of the immobilized protein was calculated using:

$$Q = \frac{(C_i - C_f)V}{A}, \text{ (S1)}$$

where Q (mg of protein / cm^2 of membrane) is the mass of protein immobilized per cm^2 of molecular imprinted membrane, C_i (mg/ml) and C_f (mg/ml) are the initial and final protein concentration in the solution respectively. V (ml) is the total volume of the solution, and A (cm^2) is the apparent surface area of the molecularly imprinted membrane.

Membrane Pore size Characterization

The pore size of the substrate CA membrane, MIMs and NIMs were characterized via neutral solute rejection by using a dead-end permeation cell. Polyethylene glycol (PEG) with MWs of 20 and 35 kDa and polyethylene oxide (PEO) with MWs of 100, 200, 300, 600 kDa were used as neutral solutes for the preparation of feed solutions. Concentrations of the feed and the permeate solutions were determined using total organic carbon analyzer (Shimadzu ASI-5000A). The single solute rejection was calculated as follows:

$$R = \left(\frac{C_f - C_p}{C_f} \right) \times 100\% \quad \text{(S2)}$$

where C_f and C_p (ppm) are the single solute concentrations in the feed and permeate solution respectively. The solute rejection data as a function of molecular weights were also used for the estimation of mean pore size of the membranes[2].

Pure water permeability (PWP) of membranes were tested with an argon-pressurized dead-end permeation cell under operation pressure of 5 bars. PWP was calculated from equation S(3).

$$P_w = \frac{W}{d_w \cdot \Delta t \cdot A \cdot \Delta P} \quad \text{(S3)}$$

where P_w is the pure water permeability (L m^{-2} h^{-1} bar^{-1}), W is the weight of water penetrating through the membrane (g), d_w is the water density at room temperature (g/L), Δt is the filtration time (h), A is the effective membrane area (m^2), ΔP is the hydraulic pressure in the permeation cell (bar).

The PWP and the pore diameter on the membrane skin layer of NIMs and MIMs are listed in the table S2.

Labeling of proteins

In order to visualize the adsorption and desorption performance of the MIM, fluorescein isothiocyanate (FITC, Sigma Aldrich, USA) was used to label BSA. The conjugation of FITC-BSA was prepared as recommended by Sigma Aldrich. The labeling molar ratio of FITC to BSA was 4 to 1. The resultant solution was mixed with Lys for the preparation of binary solution with a concentration of 1 mg/ml of FITC-BSA and Lys respectively. The BSA-labeled binary mixture was subjected to the same permeation test as the BSA-lysozyme binary solution. After the permeation test, the membrane was washed with de-ionized water to remove any residual of protein solution on the membrane surface. The membrane was then observed using a confocal laser scanning microscope (Nikon, C1, Tokyo, Japan). The excitation wavelength used was 495nm and the emitted fluorescence was detected at a wavelength of 520 nm. For evaluation of the images, the EZ-C1 3.60 software (Nikon, Tokyo, USA) was used.

Reference

1. Ma, Z. & Ramakrishna, S. Electrospun regenerated cellulose nanofiber affinity

membrane functionalized with protein A/G for IgG purification. *J. Membr. Sci.* 319, 23-28 (2008).

2. K. Y. Wang and T.-S. Chung. The characterization of flat composite nanofiltration membranes and their applications in the separation of Cephalexin. *J. Membr. Sci.*, 247, 37–50 (2005).