Electronic Supplementary Information

Thermally Controlled Wettability of Nanoporous Membrane Grafted with Catechol-tethered Poly(N-isopropylacrylamide)

Jee Seon Kim¹, Taek Gyung Kim², Won Ho Kong¹, Tae Gwan Park¹, and Yoon Sung Nam¹,³,⁴

¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea; ²Severance Hospital Integrative Research Institute for Cerebral & Cardiovascular Diseases, Yonsei University Health System, Seoul, 120-752, Republic of Korea; ³Department of Materials Science and Engineering and ⁴KAIST Institute for NanoCentury (KINC) and BioCentury (KIB), Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

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I.  Materials

N-isopropylacrylamide (NIPAAm) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were purchased from Tokyo Chemical Industry Co., tris(2-carboxyethyl)phosphine hydrochloride solution (Bond-Breaker® TCEP solution) and fluorescein-5-maleimide from Thermo Scientific, 2,2′-azobis(2-methylpropionitrile) (AIBN) from Junsei Chemical Co., and 4,4′-azobis(4-cyanopentanoic acid), sodium, sulfur, benzyl chloride, triethylamine (TEA), 3-hydroxytyramine hydrochloride, dimethyl sulfoxide (DMSO), ethyl acetate, dimethylformamide (DMF), FITC-dextran (MW 40 kDa), 1-hydroxybenzotriazole hydrate (HOBt), and 4-tert-butyl catechol from Sigma-Aldrich. All solvents and reagents were used as received. AAO membranes (Whatman Anodisc™) having a nominal pore diameter of 20 nm were obtained from Fisher Scientific.

II. Synthesis and Characterization

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\text{Synthesis of 4-Cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid.}^1 \text{ Elemental sodium (5.76 g, 0.25 mol) was dissolved in methanol (130 mL) under nitrogen, followed by rapid addition of elemental sulfur (6.4 g, 0.2 mol), turning the clear liquid into murky yellow one. Benzyl chloride (12.8 g, 10.1 mol) was dropwisely added to the solution through an addition funnel over 15 min. The resulting dark red solution was refluxed at 65°C for 10 h, allowed to cool to room temperature and finally placed into an ice bath. The reaction was filtered, and the}
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filtrates were concentrated in vacuo to give an oily sludge product. To the crude product dissolved in 100 mL water was added 5.0 N HCl, causing the color change of the solution to pink. The pink solution was extracted with diethyl ether and concentrated in vacuo to give a red oily product 1, dithiobenzoic acid. To the solution of 1 (8.60 g, 56 mmol) in ethyl acetate (25 mL) was added a catalytic amount of iodine in DMSO (2.19 mL, 28 mmol) in a dropwise manner. The reaction mixture was stirred at room temperature for 10 h in the dark to yield bis(thiocarbonyl) disulfide, 2. A mixture of 2 (17.11 g, 56 mmol) and 4,4′-azobis(4-cyanopentanoic acid) (12.71 mL, 34 mmol) in ethyl acetate (250 mL) was refluxed at 77°C for 16 h. The reaction was allowed to cool to room temperature and then concentrated under reduced pressure. The crude product was washed with n-hexane to give a red solid product, 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTPA). 1H NMR (400 MHz, CDCl3, δ/ppm): 1.92 (s, 3H); 2.58 - 2.61 (m, 2H); 2.69 - 2.75 (m, 2H); 7.36 - 7.40 (m, 2H); 7.53 - 7.57 (m, 1H); and 7.88 - 7.90 (m, 2H).
Fig S1. $^1$H NMR Spectrum (400 MHz, CDCl$_3$) of CTPA

**RAFT Polymerization of NIPAAm.** NIPAAm was recrystallized in cold n-hexane to remove MEHQ inhibitor in advance. A mixture of NIPAAm (3.93 g, 34.7 mmol), CTPA (88.1 mg, 0.32 mmol) and AIBN (12.9 mg, 0.08 mmol) ([NIPAAm]/[CTPA] = 110/1, [CTPA]/[initiator] = 4/1) was dissolved in 1,4-dioxane (16 mL). The solution was degassed for 10 min under nitrogen. RAFT polymerization was carried out at 80°C for 23 h. The product was precipitated in cold ether. $^1$H NMR (400 MHz, DMSO-d$_6$, δ/ppm): 1.05 (s, 6H); 1.22-1.44 (br, 2H); 1.90-1.96 (br, 1H); 3.83 (br, 1H).
**Conjugation of Catechol to PNIPAAm.** To the solution of PNIPAAm (600 mg, 0.05 mmol) in dichloromethane (5 mL) were added 3-hydroxytyramine hydrochloride (23.7 mg, 0.13 mmol), EDC (23.9 mg, 0.13 mmol), HOBt (16.8 mg, 0.13 mmol) and TEA (13 μL, 0.075 mmol). The reaction was stirred at room temperature for 12 h and then precipitated in cold ether. The obtained polymer was dissolved in deionized water and dialyzed with the membrane of MWCO 3,500 against deionized water at 4°C for 2 days, followed by lyophilization. The final solid product, PNIPAAm-ct, was pale pink. The conjugation yield was 94 % determined by the Waite & Benedict method.²,³

**Fluorescent Labeling of Catechol-conjugated PNIPAAm.** To the solution of PNIPAAm-ct (50 mg, 4 μmol) in deionized water (4 mL) was added 1 M sodium borohydride (20 μL). The
reaction was stirred at room temperature for 2 h, dialyzed against deionized water for 3 days, and then lyophilized. The dried solid product was dissolved in DMF, and 0.5 M TCEP solution in water was added to the solution at a 150:1 w/w ratio of PNIPAAm-ct and TCEP.\textsuperscript{4,5} The mixture was stirred at room temperature for 24 h. Fluorescein-5-maleimide was then added, and the coupling reaction was carried out with stirring at 50\textdegree C for 24 h. Unreacted dyes were eliminated by dialysis against deionized water. A pale yellow solid product was obtained by lyophilization.

**Surface Modification of AAO Nanopores.** An AAO membrane was cleaned in acetone and deionized water with sonication for 30 min, and then placed in deionized water for 12 h. Surface modification of the nanopores using PNIPAAm-ct (4) was performed by passing the polymer solution (1 mg mL\textsuperscript{-1}) through the AAO membrane for 10 min at 4\textdegree C. This procedure was repeated three times. The membranes were dried in air at room temperature for 12 h, and then an excess amount of deionized water and acetone were filtrated through the pores at 4\textdegree C to remove unbound and weakly-bound polymers. The PNIPAAm-grafted AAO membranes were kept in deionized water at 4\textdegree C until use. The atomic composition of untreated AAO and PNIPAAm-grafted AAO membrane was described in Table S1.

| Table S1. Atomic compositions of bare and PNIPAAm-grafted AAO membranes |
|------------------|-------|-------|-------|---|
|                  | % C   | % O   | % Al  | % N |
| Bare AAO         | 15.4  | 46.8  | 37.7  | NA\textsuperscript{a} |
| PNIPAAm-grafted AAO | 28.3  | 37.8  | 29.5  | 4.3 |

\textsuperscript{a} not applied
**Characterization.** The $^1$H NMR-spectra were measured with Varian VXR-4000 (400MHz) spectrometers. Chemical shifts were measured as part per million ($\delta$ values) from tetramethylsilane as an internal standard at probe temperature in CDCl$_3$ or corresponding deuterated solvents for neutral compounds, and the coupling constants were measured in Hz. The molecular weight distribution of PNIPAAm was determined using gel permeation chromatography equipped with a Waters$^{\text{TM}}$ 600 controller, a 410 differential refractometer, and a Phenomenex Phenogel 5 500Å column. Tetrahydrofuran was used as an eluent with a flow rate of 1.0 mL min$^{-1}$. Polystyrene standards were used for calibration. X-ray photoelectron spectroscopy (XPS) was carried out using an ESCALAB (SigmaProbe, Thermo Scientific, USA) with a monochromatized Al X-ray source and a take-off angle of 45°. Fourier transform-infrared (FT-IR) spectra were recorded using an IFS66V/S spectrometer (Bruker Optiks, Germany) in the transmission mode. Field-emission scanning electron microscopy (FE-SEM) was performed with a FEI Sirion SEM (FEI, Netherland), and confocal microscopy images were obtained using a Carl Zeiss LSM 510 microscope. Oil contact angle ($\theta$) in deionized water was measured using a drop of silicone oil (KF-54, Shin-Etsu Chemical Co.) loaded beneath the surface of membrane in a Phoenix 300 goniometer (Surface Electro Optics Co., Korea), and the obtained images were analyzed using Image J (NIH).

**III. Diffusivity of FITC-dextran through an AAO Membrane.**

**Experimental Determination.** The aqueous solution of FITC-dextran (40 kDa) was allowed to diffuse through the PNIPAAm-grafted AAO membrane using a flow-type diffusion cell (PermGear, USA). The donor compartment was filled with the solution of FITC-dextran (8

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D = -K \frac{LV_AV_S}{A(V_V + V_A)}
\]  

Eq. (S1)
mg, 200 nmol) in deionized water (4 mL), and the receptor was charged only with degassed
deionized water (11 mL). The AAO membrane having an effective thickness of 1 μm and a
diffusion area of 0.50 cm$^2$ was located between the donor and receptor compartments.\textsuperscript{6} To
maintain the sink condition, the solution in the receptor was circulated with a peristaltic pump
at a flow rate of 0.36 mL min$^{-1}$. FITC-dextran was excited at 490 nm, and its emission was
measured at 519 nm. The diffusivity was calculated from Eq. S1 derived from the Fick’s first
law of diffusion.\textsuperscript{7}

where $D$ is the diffusivity (cm$^2$ s$^{-1}$); $V_D$ and $V_R$ are the volumes of solutions in the donor and
receptor compartments, respectively (cm$^3$); $L$ is the thickness of the membrane (cm); $A$ is the
diffusion area of the membrane (cm$^2$); and $K$ is defined as $t^{-1} \ln[1 - (1 + V_R/V_D)\frac{C_{R,t}}{C_{D,0}}]$ where $C_{D,0}$ and $C_{R,t}$ are the initial and intermediary (at time $t$) concentrations of dextran in the
donor and receptor compartments, respectively.

**Calculation from the ‘Hindered Diffusion’ Model.** The diffusivity of FITC-dextran through
the nanopores was calculated using a ‘hindered diffusion’ model equation (Eq. S2) to clarify
the contribution of the partial unblocking on the increased diffusivity.\textsuperscript{8} The effective
diffusion coefficient is determined by two major factors: the steric restriction resulted from
the pore blocking and the interaction between the surface of pore wall and solutes.

$$D_{\text{eff}} = \Phi K^{-1} D_{\infty}$$  \hspace{1cm} \text{Eq. (S2)}

where $\Phi$ is the partition coefficient caused by steric restriction. $\Phi$ is the production of
membrane porosity ($\varepsilon$) and available area in a pore ($\varphi$), where $\varphi = (1-\lambda)^2$ and $\lambda$ is the ratio of
the diameter of solute to the diameter of pore. The diameter of solute is 7.5 nm. The diameter
of pore depends on its surface: 1) 20 nm for the bare membrane, 2) 16 nm for the PNIPAAm-
grafted membrane at 15 °C, and 3) 18 nm for the PNIPAAm-grafted membrane at 42 °C.

Based on the pore size of membranes, the value of $\varphi$ was calculated as follows; 1) $\varphi = 0.391$ for the bare membrane, 2) $\varphi = 0.282$ for the PNIPAAm-grafted membrane at 15 °C, and 3) $\varphi = 0.340$ for the PNIPAAm-grafted membrane at 42 °C. $K^{-1}$ is a hydrodynamic factor due to the surface interaction between the pore wall and solutes. $D_\infty$ is the bulk diffusivity of solutes depending on the temperature. As for $\Phi$, it was assumed that the membrane porosities ($\varepsilon$) of all of the samples in our experiments were identical. Therefore, the pore size was the only factor for the $D_{\text{eff}}$ in the view of steric hindrance.

The measured diffusivities of FITC-dextran through the bare membranes were $8.48 \times 10^{-9}$ cm$^2$ s$^{-1}$ at 15°C and $1.02 \times 10^{-8}$ cm$^2$ s$^{-1}$ at 42°C. If we take into account of only temperature and pore size, the calculated $D_{\text{eff}}$ of FITC-dextran through the PNIPAAm-grafted membrane is $6.11 \times 10^{-9}$ cm$^2$s$^{-1}$ at 15°C and $8.87 \times 10^{-9}$ cm$^2$s$^{-1}$ at 42°C. The measured $D_{\text{eff}}$ was much larger than the calculated one at both of the temperatures: $7.94 \times 10^{-9}$ cm$^2$s$^{-1}$ at 15°C and $2.03 \times 10^{-8}$ cm$^2$s$^{-1}$ at 42°C. If we consider the thermal effect, the calculated $D_{\text{eff}}$ is $6.66 \times 10^{-9}$ cm$^2$s$^{-1}$ at 42°C, so the contribution by the pore enlargement was only $2.21 \times 10^{-9}$ cm$^2$s$^{-1}$ ($= 8.87 \times 10^{-9} - 6.66 \times 10^{-9}$ cm$^2$s$^{-1}$) at 42°C. The contribution by the decreased wettability was $13.64 \times 10^{-9}$ cm$^2$s$^{-1}$ ($= 2.03 \times 10^{-8} - 6.66 \times 10^{-9}$ cm$^2$s$^{-1}$) at 42°C, which was about 6 times more significant as compared to the one by the pore enlargement.

The change of diffusivity by the coatings of PNIPAAm at 15°C also provides the importance of surface wettability. The calculated $D_{\text{eff}}$ of FITC-dextran through the PNIPAAm-coated membrane is $6.11 \times 10^{-9}$ cm$^2$s$^{-1}$ at 15°C when only thermal and pore size effects were considered, while the experimentally measured one was $7.94 \times 10^{-9}$ cm$^2$s$^{-1}$, which was much higher than the calculated one. This phenomenon can be also explained by the change of surface wettability originated from the PNIPAAm coating of the AAO pores.
The static oil contact angle of the PNIPAAm-coated membrane was 102° at 15°C, while the untreated AAO membrane had a contact angle of 123° at the same temperature (see Fig. 2c). This indicates that the increased hydrophobicity of the nanopores was responsible for unexpected large increase of diffusivity. This effect cannot be explained simply by the change of pore sizes. Therefore, our results indicate that the hydrodynamic effect ($K^{-1}$) from the change of surface wettability is much more important for the dramatically increased diffusivity in PNIPAAm-coated membrane at 42°C.

**IV. Determination of Grafting Density of PNIPAAm-ct on AAO membrane**

The grafting density ($\sigma$) is given by Eq. S2.

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\sigma = \frac{a \times N_A}{S}
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where $a$ is the weight average grafting density of PNIPAAm chain on AAO membrane (mol g$^{-1}$), $S$ is BET surface area (9.02 m$^2$ g$^{-1}$) and $N_A$ is Avogadro’s number ($6.02 \times 10^{23}$ mol$^{-1}$). To obtain the value $a$, an AAO membrane modified with PNIPAAm-ct was prepared as described above. Then, the PNIPAAm-ct modified AAO membranes were grond to powder. Dithiobenzoate of PNIPAAm-ct on AAO powder surface was reduced to free thiol using TCEP and NaBH$_4$ as described above. Briefly, AAO powder (50 mg) was dispersed in deionized water (4 mL) and 1 M sodium borohydride (20 μL) was added. A mixture was stirred at room temperature for 2 h, dialyzed against deionized water for 3 days. To the mixture was added excessive amount of 0.5 M TCEP solution in water (1 mL) and stirred at room temperature for 24 h. The resultant was purified by dialysis against deionized water.
The concentration of product (5) was quantified using Ellman’s assay, which indicated $a$ as $3.9 \times 10^{-6}$ mol g$^{-1}$.

V. References


