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**Supplementary Material**

# Correlation between bending angle and protein sensing properties of molecularly imprinted hydrogel strips with one-sided porous pattern

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## Materials and methods

### *Materials*

Sigma Aldrich Co. (St. Louis, MO, USA) supplied Polyvinylpyrrolidone (PVP, MW: 55,000) and ammonium persulfate (APS, regent grade 98%). Polystyrene (PS) particles were prepared using styrene monomer (99.5%, Samchun Chemical Co., Seoul, South Korea) and ethyl alcohol (99%, Sigma Aldrich Co.). N-[3-(dimethylamino) propyl] methacrylamide (DMAPMA, Sigma Aldrich Co.), acrylamide (AAm, Alfa Aesar Co., Ward Hill, MA, USA) and BSA, MW: 66 kDa, Roche Diagnostics GmbH Co., Mannheim, Germany) were used as functional monomers and target protein to create the hydrogel. N-isopropylacrylamide (NIPAm, Acros Organics Co., Geel, Belgium), N,N'-methylenebisacrylamide (MBAA, Sigma Aldrich Co.), and ketoglutaric acid (KGA, Acros Organics Co.) were used as backbone monomer, cross-linker, and photo-initiator. NIPAm was recrystallized in a (1:1, v/v) mixture of toluene and hexane. Acros Organics Co. provided sodium phosphate monobasic anhydrous (99%) and sodium phosphate dibasic dehydrate (98%). Sigma Aldrich Co. supplied hydrochloric acid (HCl, 36.5−38.0%). Other proteins used in the selectivity test included hemoglobin (Hb, MW: 64.5 kDa, Sigma Aldrich Co.) and lysozyme (Lyz, MW: 14.4 kDa, MP Biomedicals Co., Santa Ana, California, USA). Bradford reagent (5X concentrated) were made with phosphoric acid (85wt%, Sigma Aldrich Co.) and coomassie brilliant blue G-250 (CBBG-250, Fluka Chemical Co. Ronkonkoma, NY, USA). Calcium hydride was used to purify methanol (99.8%, Duksan Pure Chemicals Co. Ansan, South Korea) (94%, Junsei co. Chuo-ku, Tokyo, Japan). Without further purification, all other solvents of analytical reagent grade were used.

### *Synthesis of polystyrene colloidal particles*

The previously reported dispersion polymerization method was used to create the monodispersed PS colloidal particles. In a triangular flask, 0.01 g PVP stabilizer was dissolved in 25 mL of ethanol. The triangular flask was then filled with an initiator solution containing 6.5 mg APS in 3 mL of deionized (DI) water. PS colloidal particles were polymerized in an oil bath at 75–80°C for 12-h after stirring for 5 min and adding 2.2 mL of styrene monomer. Finally, the PS colloidal solution was centrifuged at 3800 rpm for 90 min and washed several times with ethanol to remove any remaining impurities.

### *Polystyrene colloidal opal template*

A convective self-assembly method was used to create PS colloidal opal templates.[17] Glass substrates were sonicated for 5 min with acetone, ethanol, and DI water before being treated for 50 min in a Piranha solution ($H_2SO_4$:$H_2O_2$, 3:1 v/v). After several rinses with DI water and drying with $N_2$ gas, the substrates were immersed in PS colloidal solution (1 vol% in ethanol, 30 mL) at a tilt angle of 30° under controlled ambient conditions (temperature: 22−23°C, humidity: 31−35%). After two days, the opal template was formed on the glass substrates using solvent evaporation from assembled particles.

### *Preparation of molecularly imprinted polymer hydrogel strip with one-sided inverse opal structure*

In a 5 mL vial, DMAPMA (0.1 mmol), AAm (0.28 mmol), BSA (0.1 μmol), and 0.5 mL phosphate buffer saline (PBS, 10 mM, pH = 7.0) solution were injected. NIPAm (1.75 mmol) and MBAA (0.1 mmol) were dissolved in 1.5 mL of PBS solution (10 mM, pH = 7.0) and poured into the complex solution. Finally, 0.03 mmol of KGA was added to the mixture solution.

The following steps were taken to create MIP hydrogel (*hg*-MIP) strip: On the PS opal template, a silicon mold (internal groove dimension: 0.8 × 1.8 $cm^2$, external dimension: 1.5 × 2.5 $cm^2$) was placed, and a 300 ml precursor solution was carefully filled into the mold. After covering a glass substrate, photopolymerization was carried out at 5°C for 1-h under a certain pressure using a photolithography system (Labsys LIT-2000, Nextron, lamp wavelength: 352 nm, light intensity: 2 $mW/cm^2$, number of lamps: 9). To remove the PS colloids, the sandwich structure was immersed in toluene for 6-h. After the separation of the covered glass substrates, 1 mm thick *hg*-MIP strips with the width of 0.8 cm and the length of 1.8 cm were obtained. Based on the porous structure region with a thickness of 9 μm in the *hg*-MIP strip, the volume ratio of porous and nonporous region the strip was approximately 0.002. The *hg*-MIP films were immersed to remove the BSA template in NaCl aqueous solution (0.5 M, 100 mL) for 24-h and washed three times with PBS solution. Finally, the hydrogel strip was allowed to dry at room temperature for one day. In absence of template protein, the non-imprinted hydrogel (*hg*-NIP) strips was created using the same procedures as the *hg*-MIP strips. The *hg*-MIP(I) and *hg*-NIP strips were also dried at the room temperature for one day after removing colloids.

***Characteristics***

The topographies of PS opal template and hg-MIP film with one-sided inverse opal were investigated using field-emission scanning electron microscopy (FE-SEM, S-4800, Hitachi). The hydrogel strip was swollen in PBS (10 mM, pH = 7.0) for 12-h before being freeze-dried.

Three hydrogel strips, *hg*-MIP(I), *hg*-MIP(E), and *hg*-NIP, were incubated in 100 ml PBS solution (10 mM, pH = 7.0) to confirm the equilibrium mass swelling ratio. The swelling ratios of the strips were calculated using the equation below:

$$Swelling\ ratio\ (\%) = \frac{(W_S - W_d)}{W_d}, \qquad (1)$$

where $W_s$ and $W_d$ represent the weights of the hydrogel strip with swollen and dried state, respectively.

The 5X Bradford reagent was first prepared for the Bradford micro assay. 100 mg of CGGB-250 was dissolved in 47 mL of purified methanol. Phosphoric acid (100 mL) and DI water (200 mL) were added sequentially to the prepared mixture solution. Before use, the 5X Bradford reagent was kept in a dark bottle at 4°C. Diluted protein solutions were prepared using a protein stock solution of 1 mg/mL [in PBS solution (10 mM, pH = 7.0)] to plot a linear calibration curve in concentrations ranging from 1 to 20 μL. The absorbance of the mixture solution were measured using a UV-2401 PC

spectrophotometer (Shimadzu, Japan) at 595 nm after mixing the diluted protein solution and 5X Bradford reagent.

The *hg*-MIP and *hg*-NIP strips were incubated for 12-h at room temperature in each PBS solution (100 mL, 10 mM, pH = 7.0) containing BSA concentrations ranging from 2.6 to 15.8 μg $mL^{-1}$. The protein solutions of 800 μL were collected at specific times during the incubation process. Using a UV spectrophotometer, optical densities were measured at 595 nm after mixing the collected protein solution with a 5X Bradford reagent.

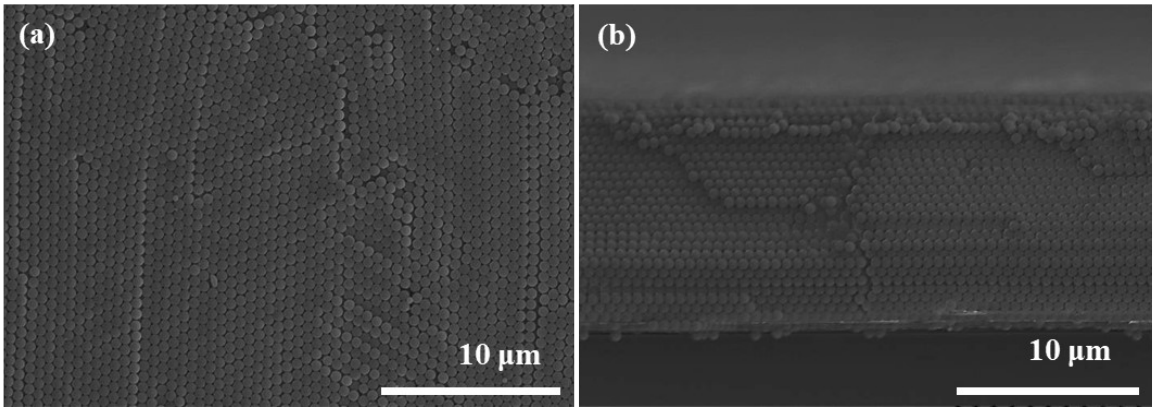


**Figure S1.** Scanning electron micrographs of three-dimensional (3D) photonic crystals with a thickness of 20 μm and a diameter of 600 nm made of polystyrene (PS) colloids. Top and cross-sectional views are shown in (a) and (b), respectively.

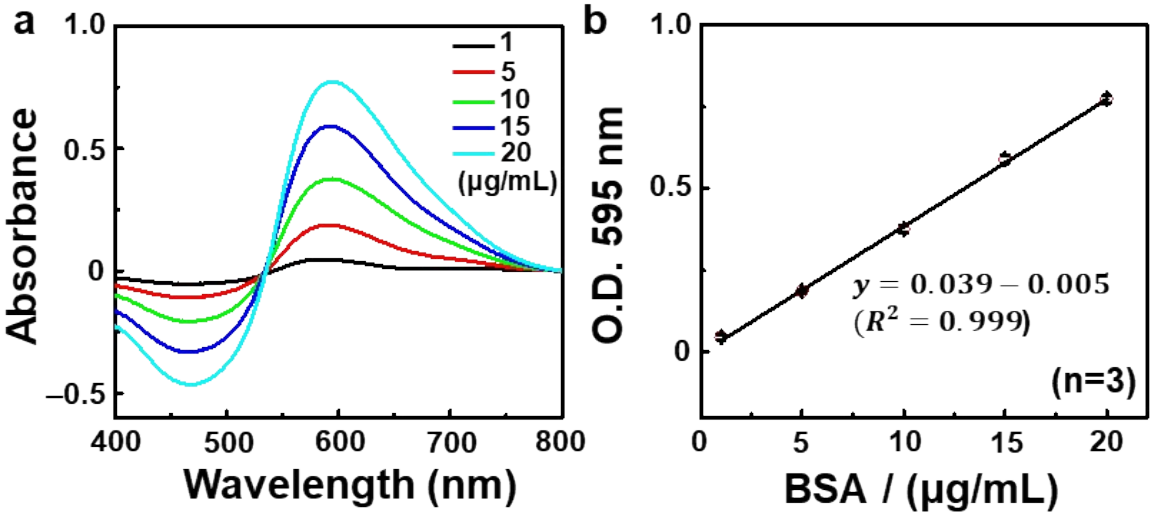


**Figure S2.** (a) UV-Vis spectra of BSA in PBS solution (1–20 μg/mL), recorded in the range of 400–800 nm and (b) a BSA calibration curve based on absorbance peaks at 595 nm wavelength.

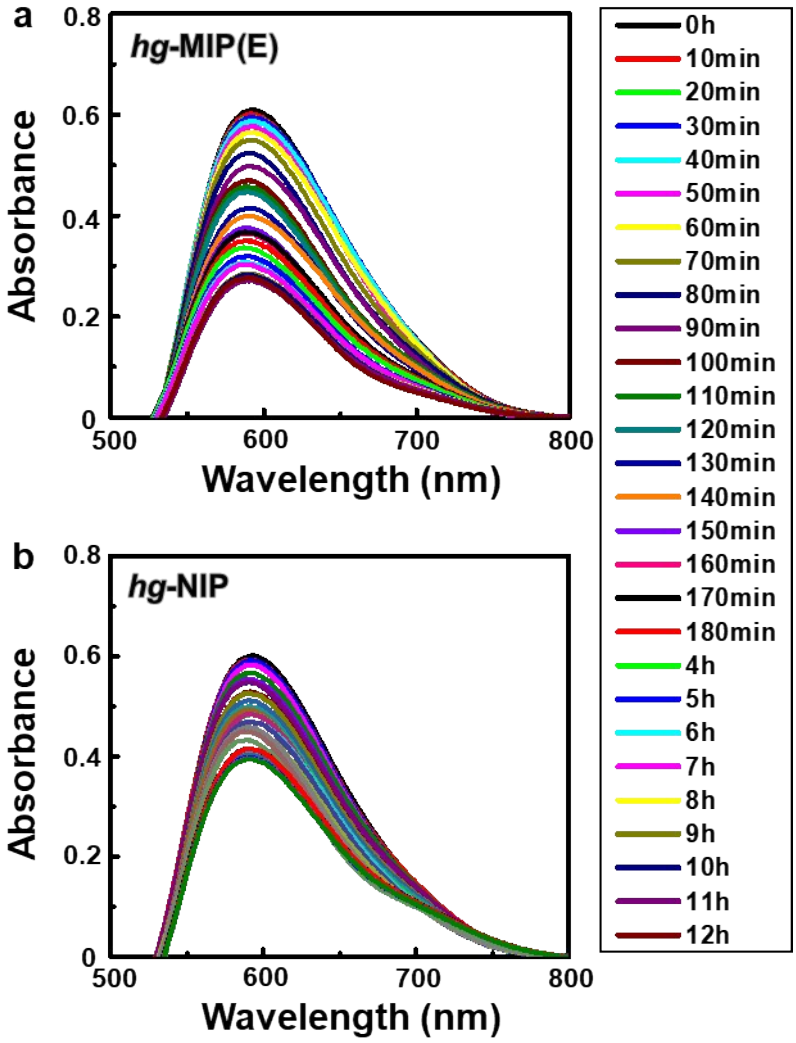


**Figure S3.** UV-Vis spectra of residual BSA, recorded in the 500–800 nm range after protein adsorption of the (a) *hg*-MIP(E) and (b) *hg*-NIP strips in 10 mM PBS solution (100 mL) with BSA (15.8 μg/mL) in the incubation time range between 0–12-h.

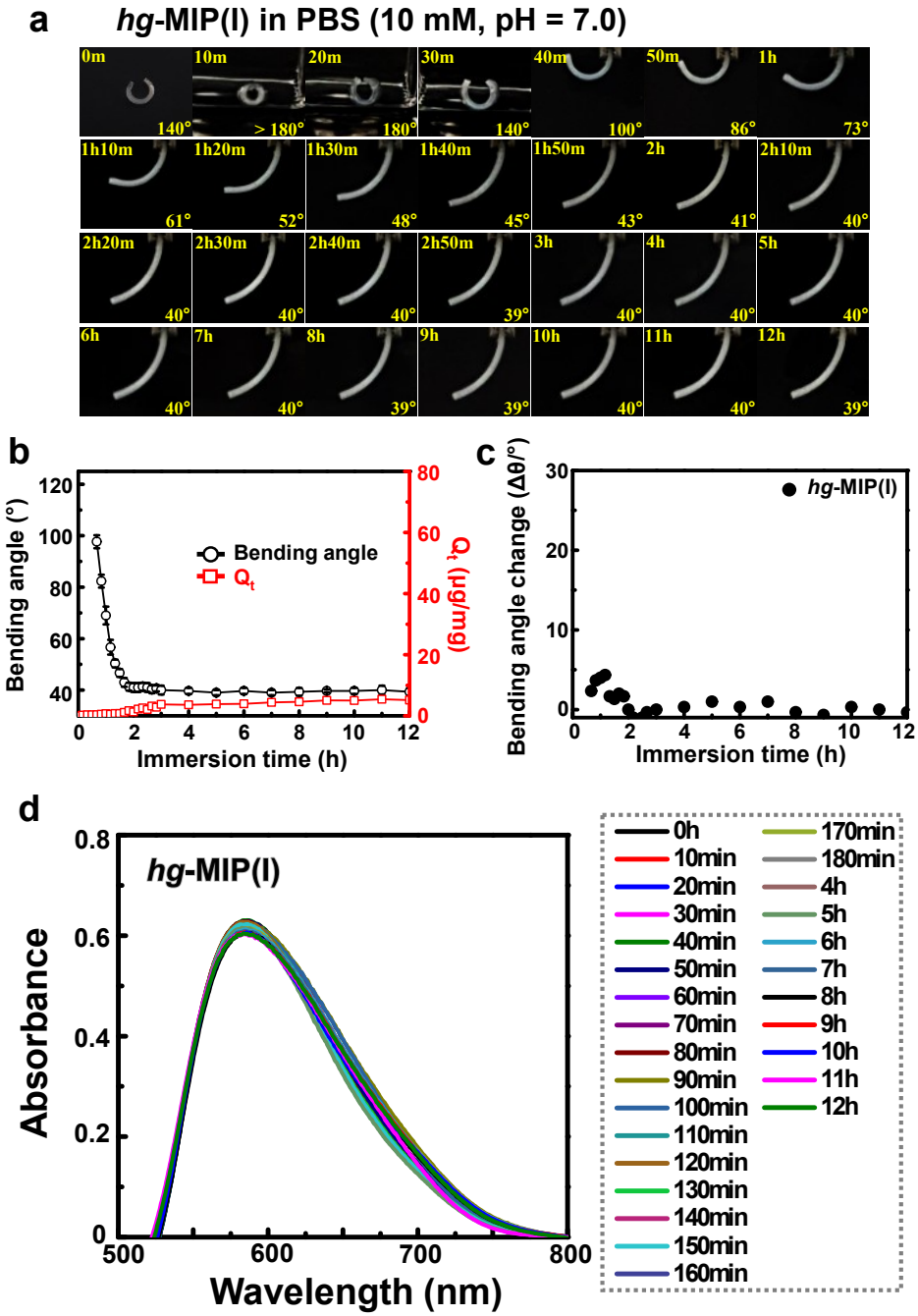


**Figure S4.** Photographs of the *hg*-MIP(I) strips demonstrating the changed bending angles after protein adsorption in 10 mM PBS solution (100 mL, pH = 7.0) with BSA (15.8 μg/mL) in the incubation times ranging from 0–12-h. (b) Bending angle and $Q_t$ of the *hg*-MIP(I) strip as a function of time incubated in 100 mL PBS solution containing 5 mM BSA. (c) Change in bending angle of the *hg*-MIP(I) strip as a function of incubation time after swelling in only PBS solution and PBS solution with BSA (15.8 μg/mL). (d) UV-Vis spectra of residual BSA, in the 500–800 nm range after protein adsorption of the *hg*-MIP(I) strip in 10 mM PBS solution (100 mL) with BSA (15.8 μg/mL) in the incubation time range between 0–12-h.

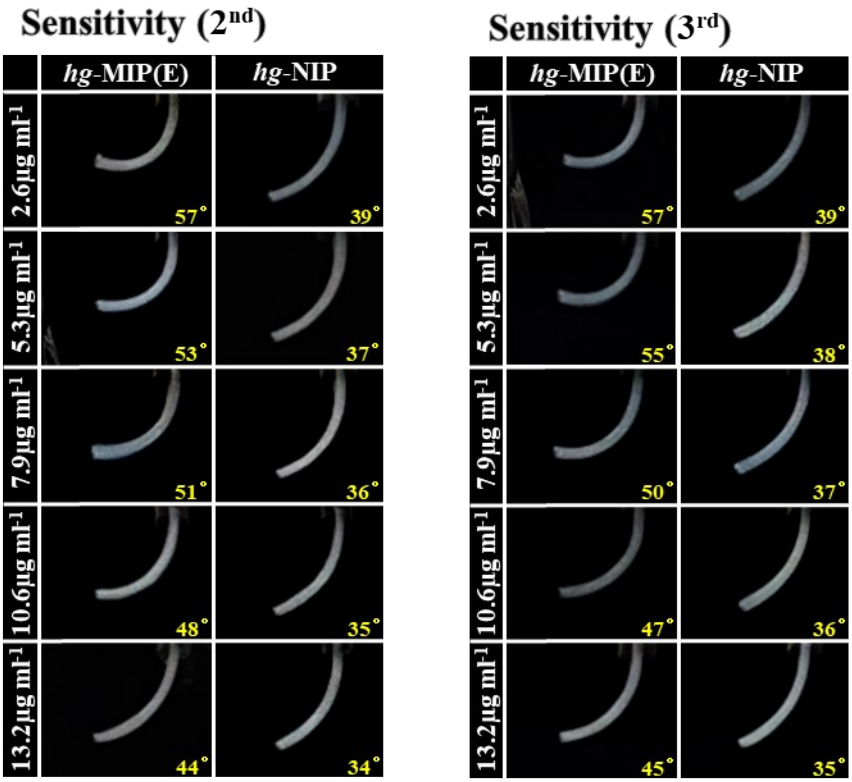


**Figure S5.** Photographs of the *hg*-MIP(E) and *hg*-NIP strips showing the changed bending angles after 12-h incubation in 10 mM PBS solution (100 mL) with various protein concentrations ranging from 2.6–13.2 µg/mL (2nd and 3rd trials).

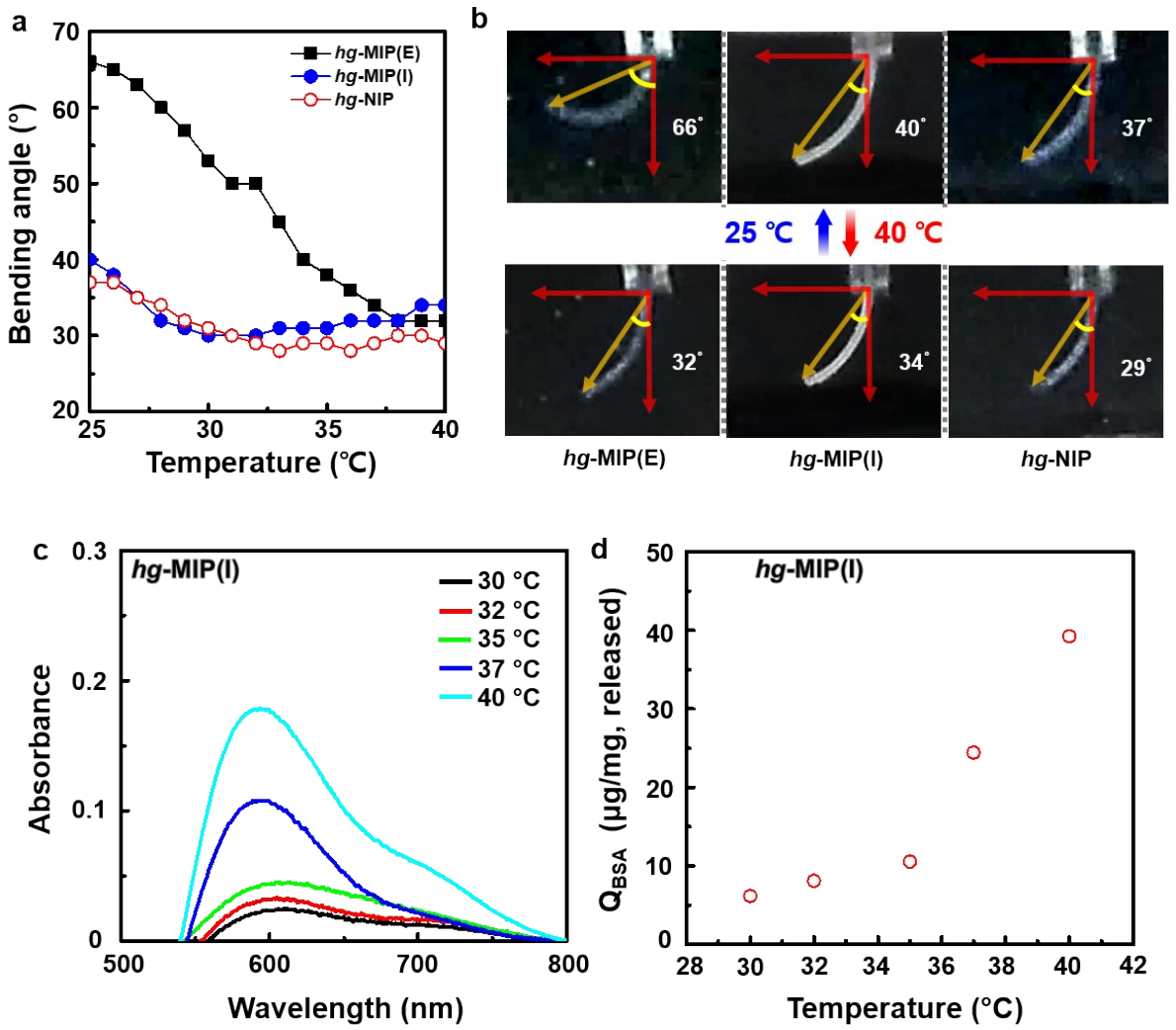


**Figure S6.** (a) Temperature dependence and (b) a change in bending angle of three hydrogel strips [*hg*-MIP(E), *hg*-MIP(I), and *hg*-NIP strips] in 10 mM PBS solution (100 mL) at the temperature of 25°C and 40°C . (c) UV-Vis spectra of BSA protein released from the *hg*-MIP(I) at the temperature range of 30–40°C, measured in the 500–800 nm range and (d) released BSA mass from the *hg*-MIP(I) strip as a function of temperature.

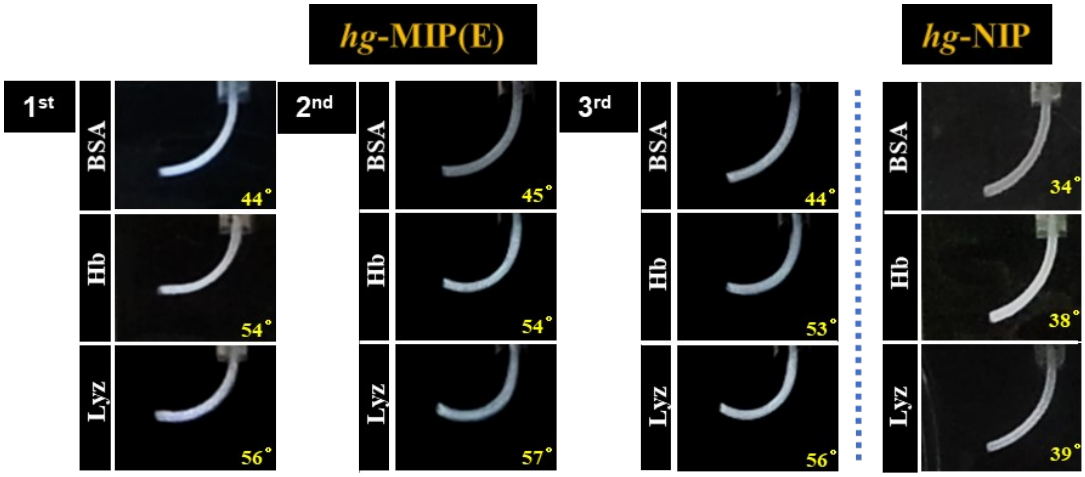


**Figure S7.** Photographs of the *hg*-MIP(E) (n=3) and *hg*-NIP strips showing the changed bending angles after 12-h incubation in 10 mM PBS solution (100 mL) with each protein (15.8 μg/mL) (BSA, Hb, or Lyz).

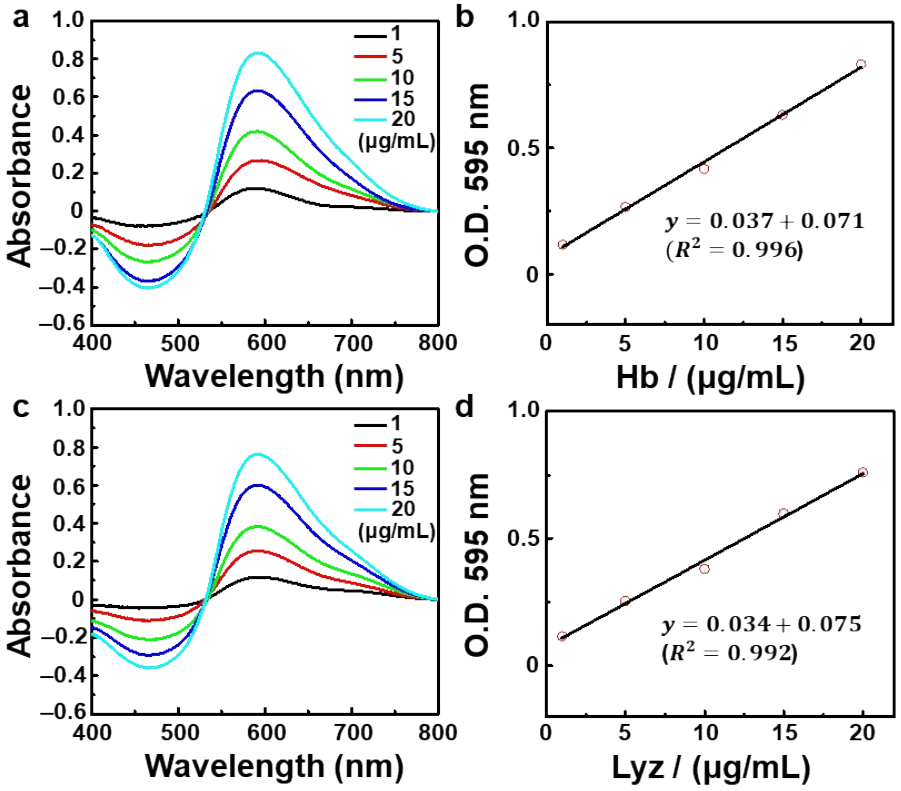


**Figure S8.** (a,c) UV-Vis spectra of Hb or Lyz in PBS solution (1–20 μg/mL), recorded in the range of 400–800 nm and (b,d) a calibration curve of Hb or Lyz based on absorbance peaks at 595 nm wavelength.

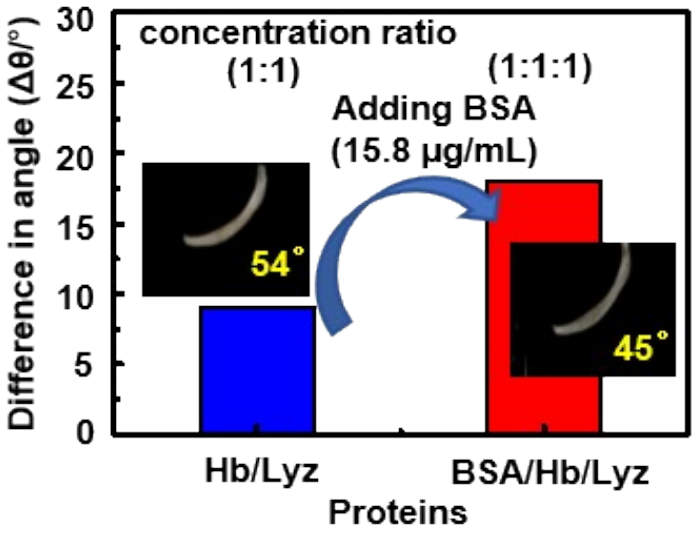


**Figure S9.** Bending angle difference and photographs of the *hg*-MIP(E) strip showing the changed bending angles (a) after 6-h incubation time in 10 mM PBS solution (pH = 7.0) with Hb and Lyz (15.8 μg/mL each) and (b) by an additional incubation process (6-h) after adding BSA (15.8 μg/mL) in the mixed protein solution.

**Table S1**. Comparison of sensitivity and selectivity on various BSA-imprinted hydrogels

| Configuration[a] | Sensitivity[b] | Selectivity coefficients[c] | Ref. |
|---|---|---|---|
| MIP(CS-g-PAM)-3 | – | 1.6 (Ova), 2.4 (Hb), 2.8 (Lyz) | 1 |
| [VAFMIM]Cl MIP | – | 2.5 (Ova), 4.6 (Hb), 7.5 (Lyz) | 2 |
| PP-CA/PAM MIP | – | 3.2 (Ova), 3.1 (Hb), 1.6 (Lyz), 5.8 (Glo) | 3 |
| *hg*-MIP(E) strip | 4.426 | 2.6 (Hb), 5.8 (Lyz) | This work |

[a] CS-g-PAM: chitosan-g-polyacrylamide; [VAFMIM]Cl: 1-vinyl-3-aminoformylmethyl imidazolium chloride; PP: polypropylene; CA/PAM: calcium alginate/polyacrylamide; Ova: ovalbumin; Hb: bovine hemoglobin; Lyz: lysozyme; Glo: bovine γ-globulin

[b] sensitivity unit: (μg/mg)/(μg/mL)

[c] Selectivity coefficients ($k$*) = adsorption mass$_{(BSA)}$/adsorption mass$_{(others)}$

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[3] B. Kan, B. Lin, K, Zhao, X. Zhang, L. Feng, J. Wei, Y. Fan, RSC Adv., 2014, 4, 55846.