

## Supporting Information

# Gas-Stimuli-Responsive Molecularly Imprinted Polymer Particles with Switchable Affinity for Target Protein

Yukiya Kitayama\*, Manabu Isomura

Graduate School of Engineering, Kobe University, 1-1, Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

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## 1. Experimental Part

### 1-1. Materials

Styrene, divinylbenzene (DVB), *N,N'*-methylenebisacrylamide (MBAA), 2,2'-azobis[2-(2-imidazolin-2-yl)propane] (VA-061), 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044), sodium dihydrogenphosphate dihydrate, disodium hydrogenphosphate · 12H<sub>2</sub>O, human serum albumin (HSA), and cytochrome c (Cyt) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N*-Isopropylacrylamide (NIPAm) was purchased from Nacalai Tesque. (Kyoto, Japan). 2-Hydroxyethyl methacrylate (HEMA), cetyltrimethylammonium bromide (CTAB), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Di(ethylene glycol) methyl ether methacrylate (DEGMA),  $\gamma$ -globulin from human blood (IgG), and lysozyme (Lys) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). The microBCA protein assay kit was purchased from Thermo Fisher Scientific K.K. (U.S.A).

### 1-2. Characterization

<sup>1</sup>H-NMR spectra were measured using 300 MHz FT-NMR apparatus (JNM-LA300 FT NMR system, JEOL Ltd., Tokyo, Japan). UV-Vis spectra measurements were carried out using V560 (JASCO, Japan). The particle size distribution and zeta potential were measured using a dynamic light scattering system Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.). The logP values for comonomers were estimated by Pallas (Computational Chemistry List, Ltd).

### 1-3. CO<sub>2</sub>/N<sub>2</sub> responsive property of a functional initiator

VA-061 was dissolved in D<sub>2</sub>O (1 mg/mL). <sup>1</sup>H-NMR measurements were carried out with the VA-061 solution before gas treatment, after CO<sub>2</sub> bubbling for 30 min at 25°C, and after subsequent N<sub>2</sub> bubbling for 30 min at 25°C. VA-044 was also tested by same procedure.

### 1-4. Synthesis of P(S-DVB) seed particles

Styrene (960 mg, 9.21 mmol), DVB (51 mg, 0.38 mmol), VA-061 (26 mg, 0.103 mmol) were added to the CO<sub>2</sub> treated water (100 mL), where the CO<sub>2</sub> treated water was prepared by the CO<sub>2</sub> bubbling at *r.t.* for 30 min with needle. After CO<sub>2</sub>/degas cycles, an emulsifier-free emulsion polymerization was carried out under CO<sub>2</sub> atmosphere at 65 °C for 24 h with 1000 rpm stirring. The conversion was measured by gravimetry.

### **1-5. Synthesis of MIP and NIP particles**

The typical procedure is follow. HEMA (31.2 mg, 240 μmol), MBAA (11.1 mg, 60 μmol), HSA (66.4 mg, 1.0 μmol), and VA-061 (2.71 mg, 10 μmol) were added to the CO<sub>2</sub> treated emulsion containing P(S-DVB) seed particles (20 mL, solid content: 0.5 wt%), where the CO<sub>2</sub> bubbling was performed at *r.t.* for 30 min with needle to prepare the CO<sub>2</sub> treated emulsion. After CO<sub>2</sub>/degas cycles, and emulsifier-free seeded polymerization was carried out under CO<sub>2</sub> atmosphere at 40 °C for 24 h with 1000 rpm stirring. To remove the non-polymerized monomer species and template HSA, the obtained core-shell particles were washed by centrifugations with (i) aqueous solution of 0.5 wt% CTAB (thrice), (ii) 10 mM TCEP aqueous solution including 0.5 wt% CTAB and (thrice), and (iii) deionized water (ten times), resulting in the HEMA-VA061\_MIP particles. The other MIP particles bearing poly(OEGMA) and poly(NIPAm) matrix were synthesized by same procedures using OEGMA (45.2 mg, 240 μmol) and NIPAm (27.2 mg, 240 μmol) as comonomers, respectively. NIP particles were also synthesized by same procedure except for using template HSA during the emulsifier-free seeded polymerization.

### **1-6. CO<sub>2</sub>/N<sub>2</sub> responsive colloidal property of MIP particles**

MIP particles (100 μg/mL) were dispersed in a deionized water. The particle size and z-potential of the particles were measured by DLS as prepared, after CO<sub>2</sub> bubbling (for 90 min), and after subsequent N<sub>2</sub> bubbling (for 90 min). The measurements were carried out at each 30 min.

### 1-7. Rebinding Experiments

MIP particles (100 µg/mL) were dispersed in a deionized water and the dispersion were bubbled with CO<sub>2</sub> or N<sub>2</sub> gas for 90 min. The HSA aqueous solution (10 µL) was added to the MIP emulsion (1.0 mL) with 15.1, 30.2, 75.5, 151, 302 nM as final concentrations of HSA. After 90 min incubation at 25 °C, the MIP particles were removed by centrifugation, and the remained HSA concentration at the supernatant was measured by microBCA assay (λ=562 nm, n=3). The binding experiments for NIP particles were also demonstrated by same procedure.

The binding constant of the MIP particles toward HSA was determined by curve-fitting using DeltaGraph 5.4.5v. The fitting equation (1) is shown below, which was generally used for estimation of the binding constant of the 1: 1 complex formation.

$$Y = [(1 + KG + KH) - \sqrt{(1 + KG + KH) - 4K^2HG}] \times \frac{D}{2KG} \quad (1)$$

,where  $Y$  is binding amount,  $K$  is the affinity constant,  $H$  is found by fitting a theoretical curve to the raw data, which would result in the smallest deviation error,  $G$  is HSA concentration, and  $D$  is the maximum amount of HSA bound.

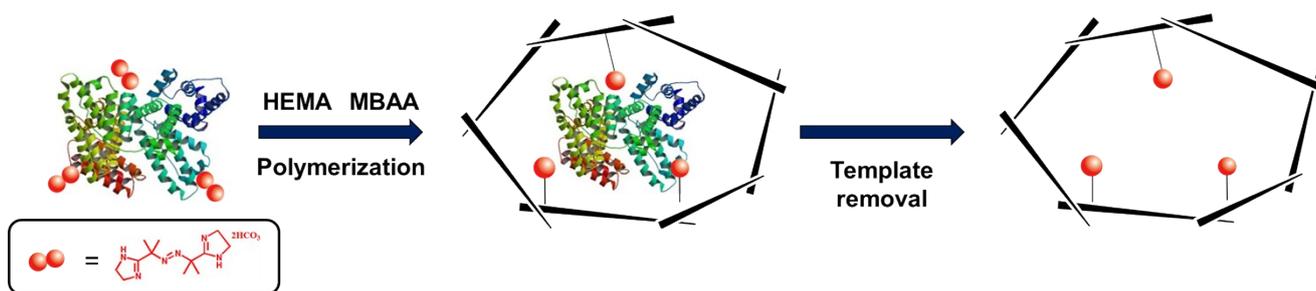
### 1-8. Selectivity test

IgG, Cyt, and Lys were selected as reference proteins. MIP particles (100 µg/mL) were dispersed in a deionized water and the dispersion were bubbled with CO<sub>2</sub> or N<sub>2</sub> gas for 90 min. The aqueous solution of the reference proteins (10 µL, final concentration: 151 nM) were added to the MIP emulsion (1.0 mL). After 90 min incubation at 25°C, the MIP particles were removed by centrifugation, and the remained HSA concentration at supernatant was determined by microBCA assay (λ=562 nm, n=3). The calibration curves for each protein were prepared for estimating the protein concentrations in microBCA assay. To evaluate the selective HSA binding capability for

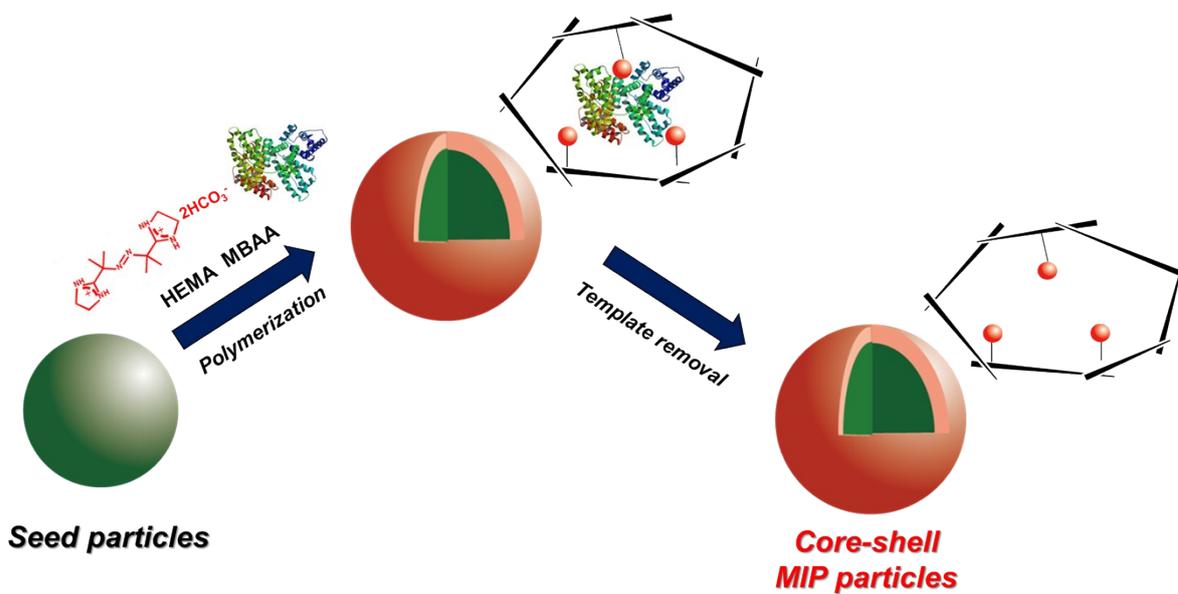
HEMA-VA061\_MIP particles, the selectivity factor (SF) values calculated from the following equation was adopted.

$$\text{Selectivity factor (SF)} = B_{\text{ref.}} / B_{\text{HSA}} \quad (1)$$

where  $B_{\text{ref}}$  and  $B_{\text{HSA}}$  are the amount for bound reference proteins and bound target HSA, respectively.



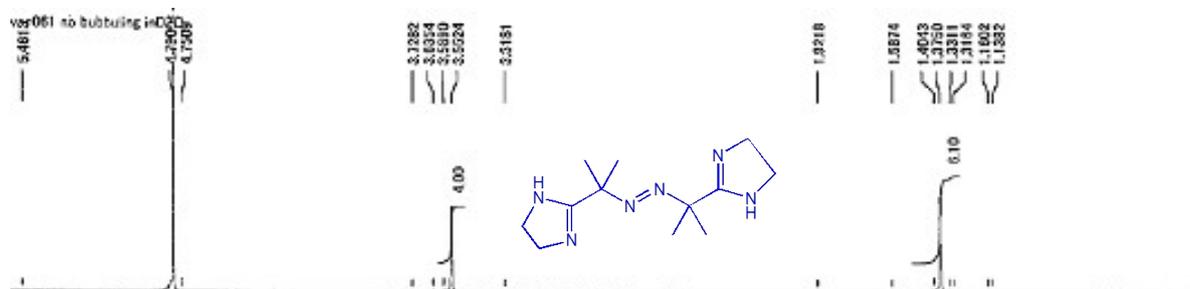
**Scheme S1.** Molecular imprinting with functional initiator



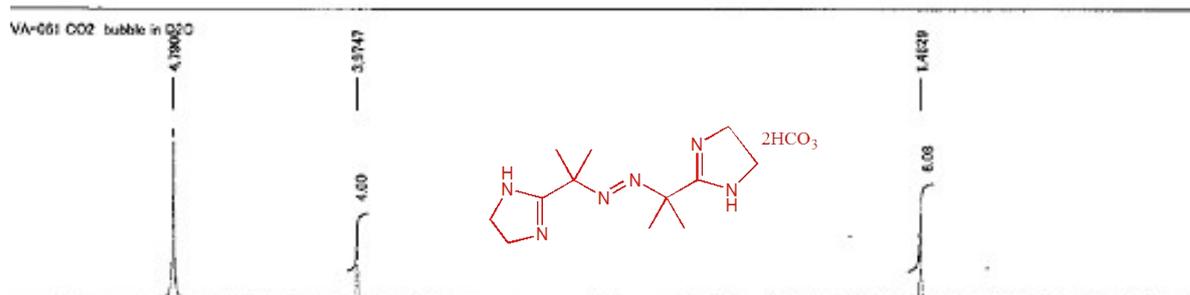
**Scheme S2.** Synthetic procedure of core-shell MIP particles

## 2. Gas-responsive property of VA-061

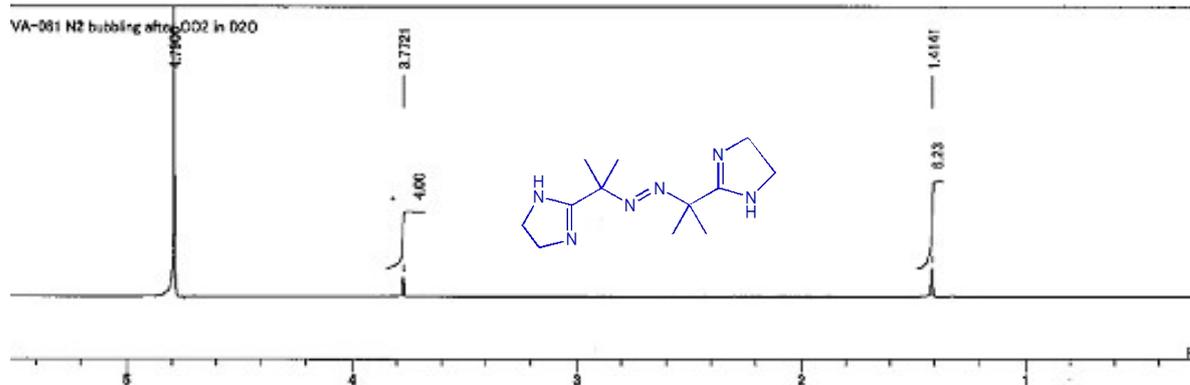
(a) VA-061 before treatment



(b) VA-061 after CO<sub>2</sub>



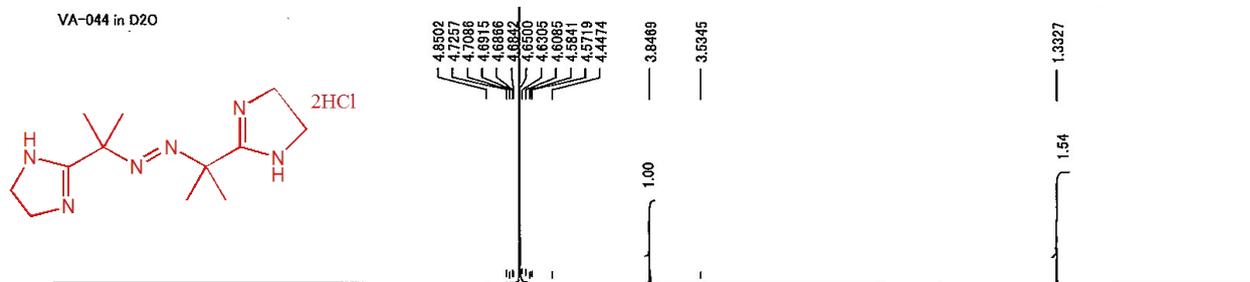
(c) VA-061 after CO<sub>2</sub> treatment, followed by N<sub>2</sub> treatment,



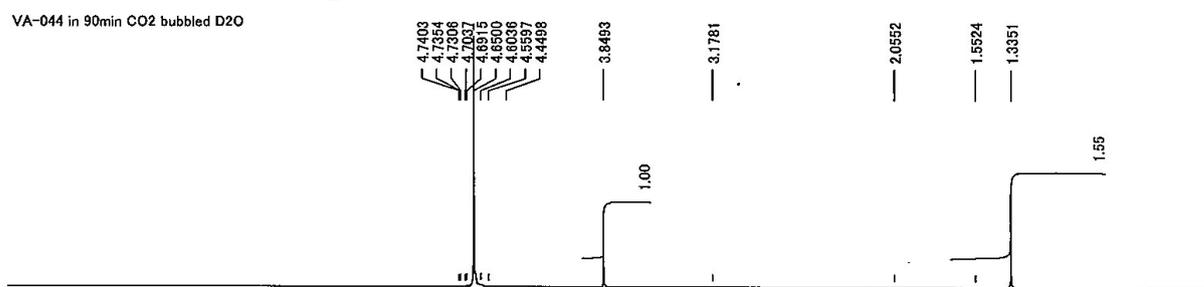
**Figure S1** <sup>1</sup>H-NMR spectra of VA-061 in D<sub>2</sub>O (a) before and (b) after CO<sub>2</sub> treatment for 30 min at room temperature, followed by (c) N<sub>2</sub> treatment for 30 min at room temperature.

### 3. Gas-responsive property of VA-044

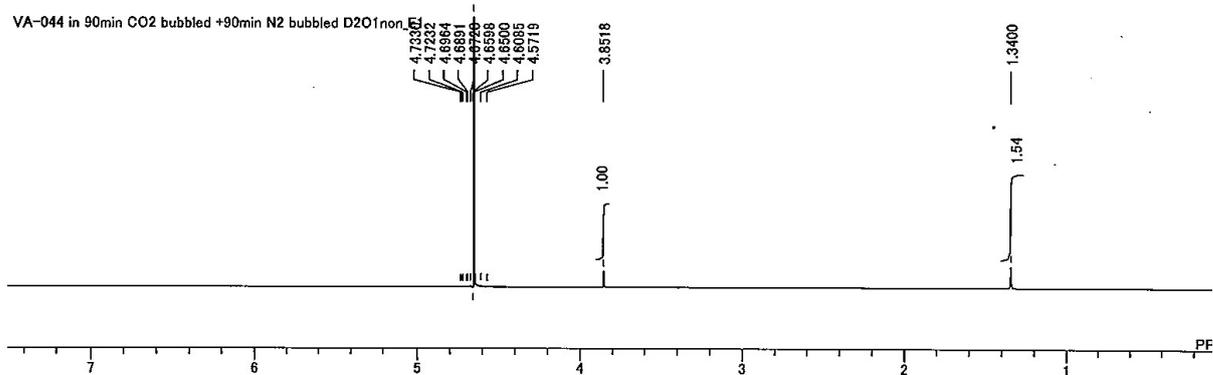
#### (a) VA-044 before treatment



#### (b) VA-044 after CO<sub>2</sub>

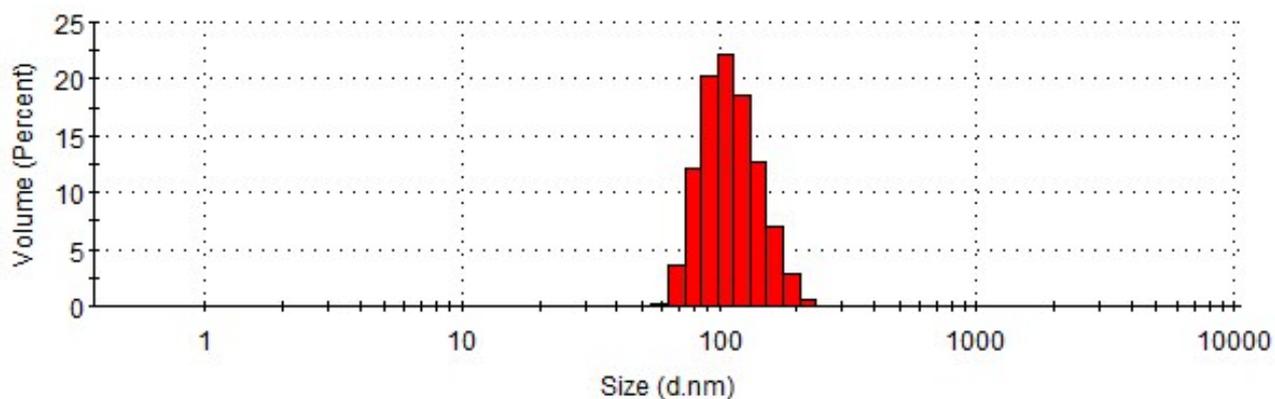


#### (c) VA-044 after N<sub>2</sub>



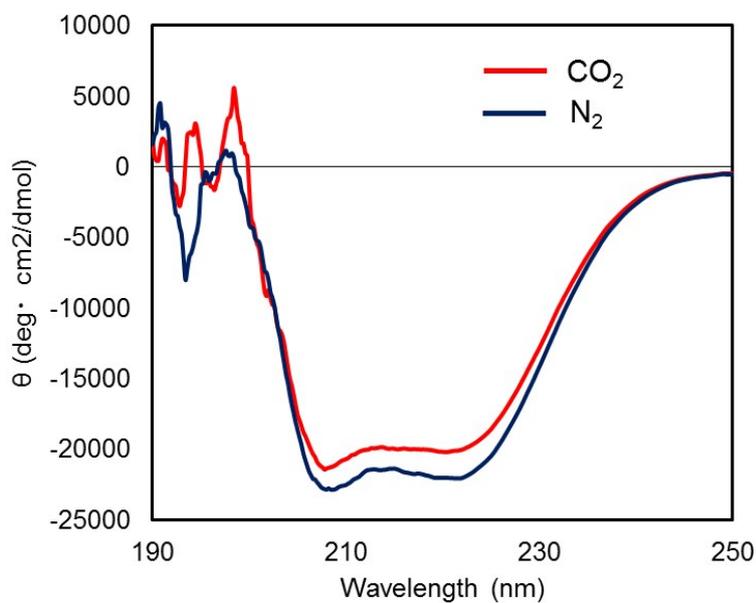
**Figure S2** <sup>1</sup>H-NMR spectra of VA-044 in D<sub>2</sub>O (a) before and (b) after CO<sub>2</sub> treatment for 30 min at room temperature, followed by (c) N<sub>2</sub> treatment for 30 min at room temperature.

#### 4. Particle size distribution of P(S-DVB) seed particles



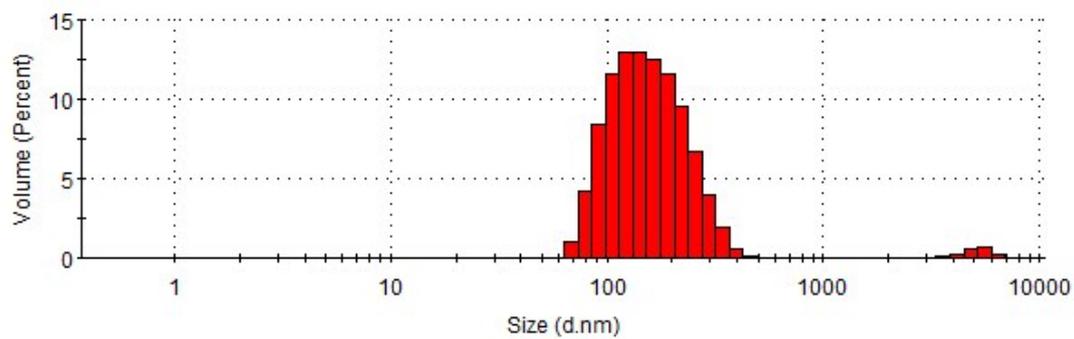
**Figure S3** Particle size distribution of P(S-DVB) seed particles prepared by emulsifier-free emulsion polymerization with VA-061 in CO<sub>2</sub>-treated water.

#### 5. CD spectra of HSA in gas-treated water



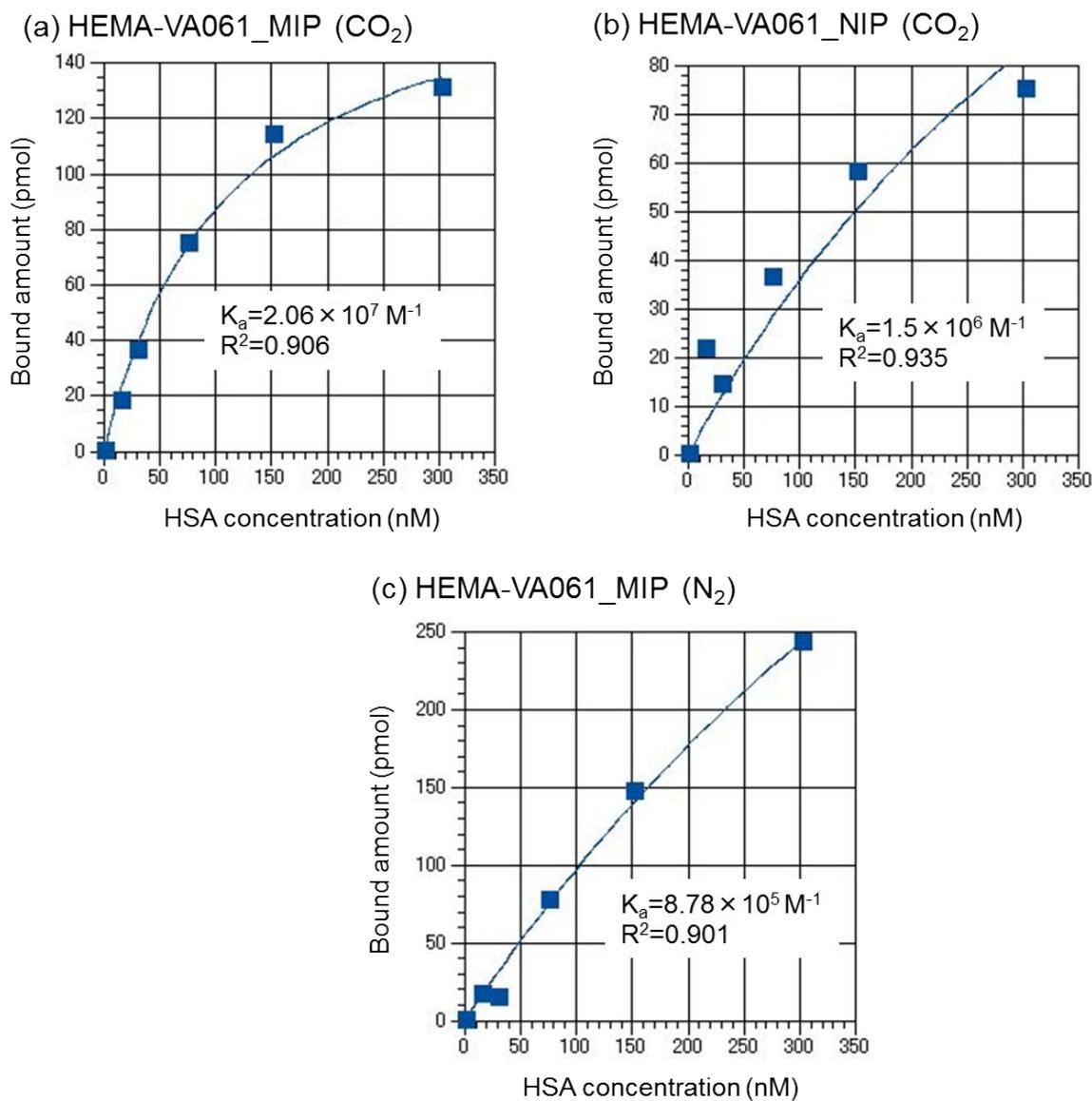
**Figure S4** CD spectra of HSA dissolved in CO<sub>2</sub>-treated and N<sub>2</sub>-treated water. HSA concentration was 66 μg/mL

6. Particle size distribution of HEMA-VA061\_MIP particles prepared at 40°C.



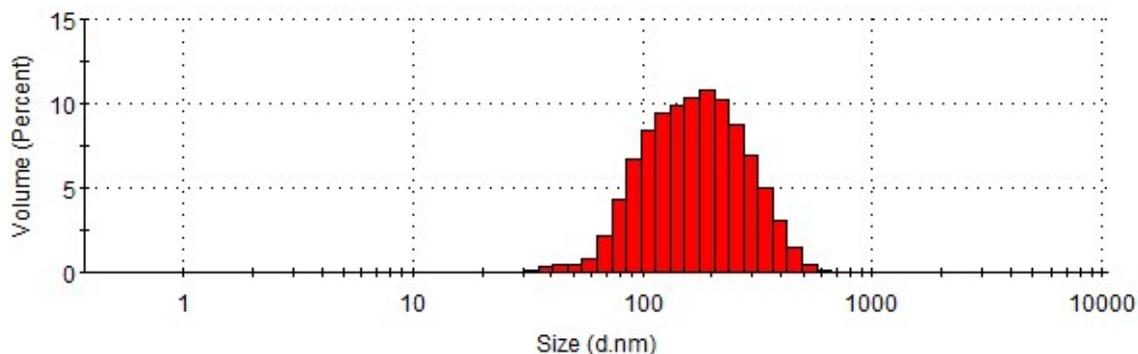
**Figure S5** Particle size distribution of HEMA-VA061\_MIP particles prepared by emulsifier-free emulsion polymerization with VA-061 in CO<sub>2</sub>-treated water at 40°C.

7. Curve fitting data of binding isotherms of HEMA-VA-061\_MIP and HEMA-VA-061\_NIP particles prepared at 40°C



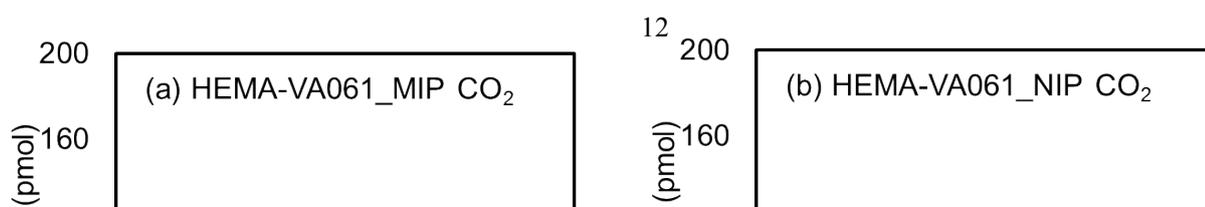
**Figure S6** Curve fitting data of binding isotherms of HSA towards HEMA-VA-061\_MIP particles (a) and HEMA-VA-061\_NIP particles (b) under CO<sub>2</sub>-treated condition, and for HEMA-VA-061\_MIP particles under N<sub>2</sub>-treated condition (c). The particles were prepared at 40°C.

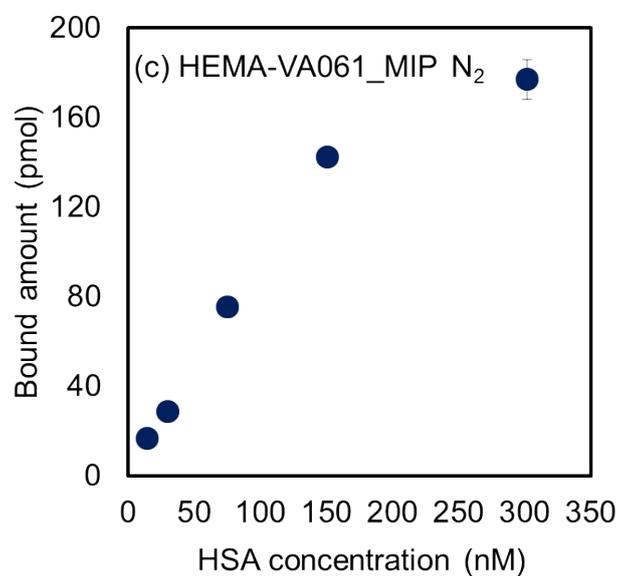
**8. Gas-responsive colloidal property of HEMA-VA061\_MIP particles prepared at 65°C**



**Figure S7** Particle size distribution of HEMA-VA061\_MIP particles prepared by emulsifier-free emulsion polymerization with VA-061 in CO<sub>2</sub>-treated water at 65°C.

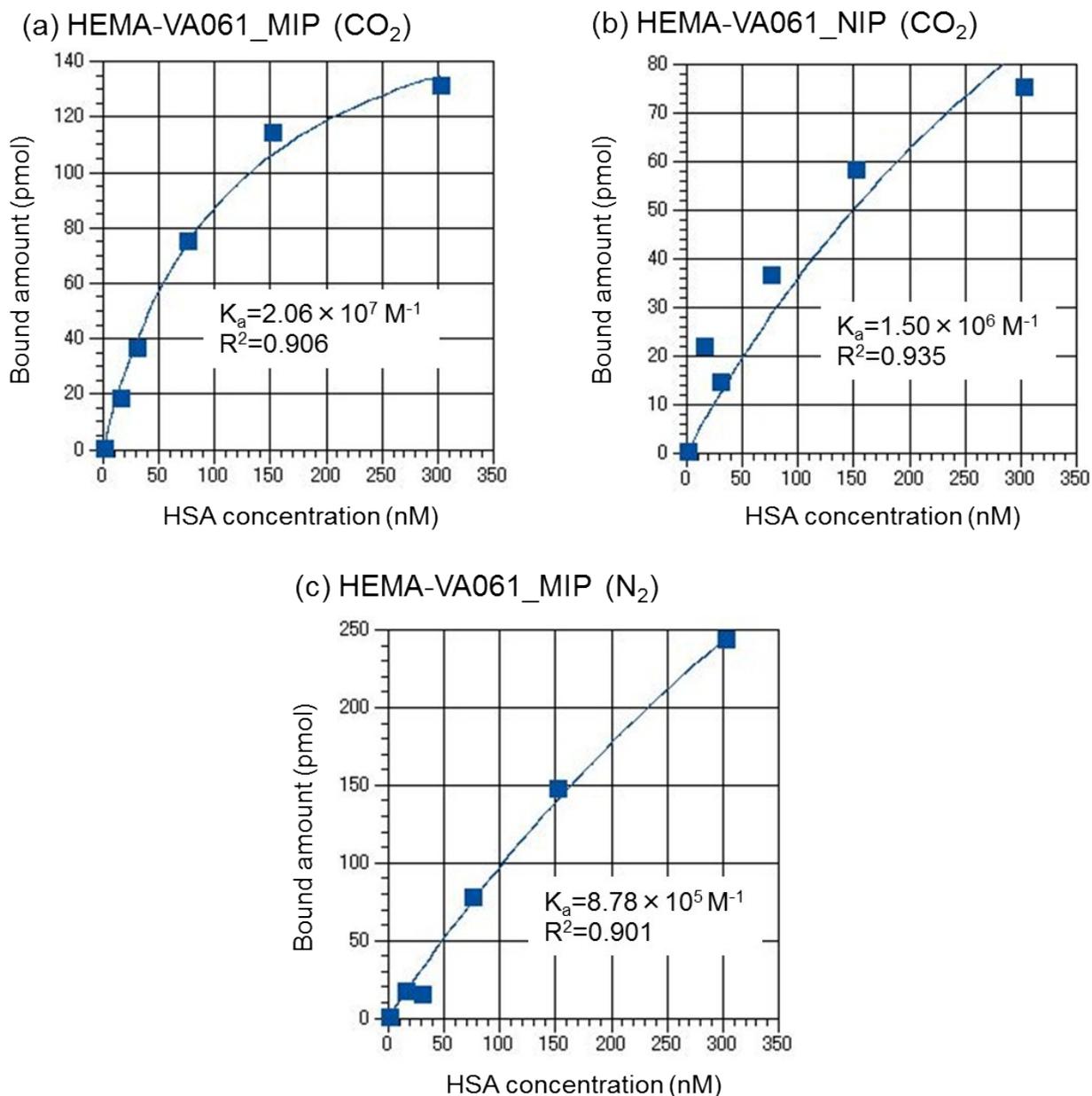
**9. Binding isotherms of HEMA-VA061\_MIP and HEMA-VA061\_NIP particles prepared at 65°C**





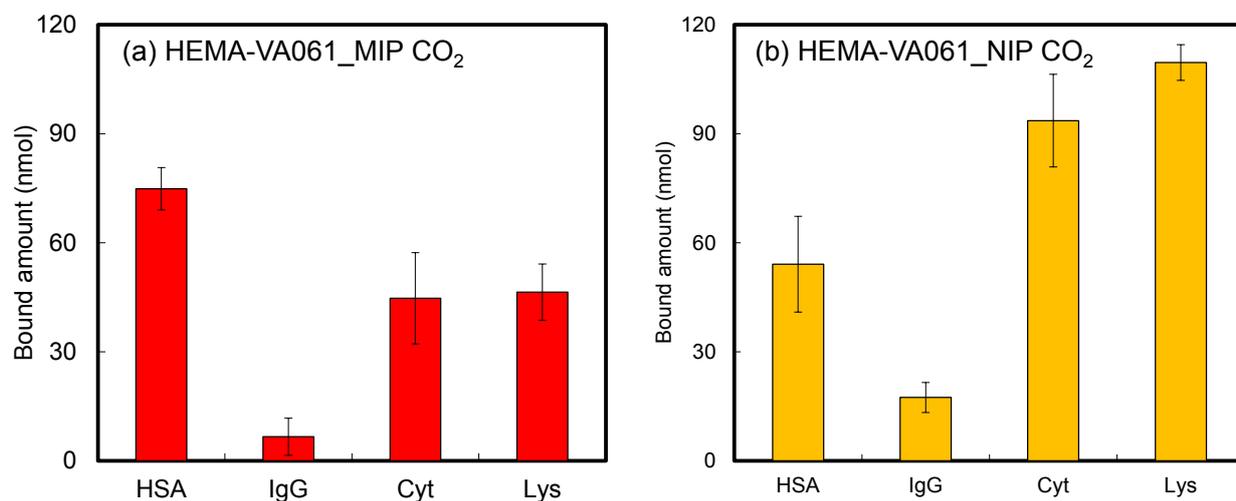
**Figure S8** HSA binding isotherms of HEMA-VA061\_MIP in CO<sub>2</sub>-treated aqueous phase (a), HEMA-VA061\_NIP in CO<sub>2</sub>-treated aqueous phase (b), and HEMA-VA061\_MIP in N<sub>2</sub>-treated aqueous phase (c). The particles were prepared at 65 °C.

10. Curve fitting data of binding isotherms of HEMA-VA-061\_MIP and HEMA-VA-061\_NIP particles prepared at 65°C



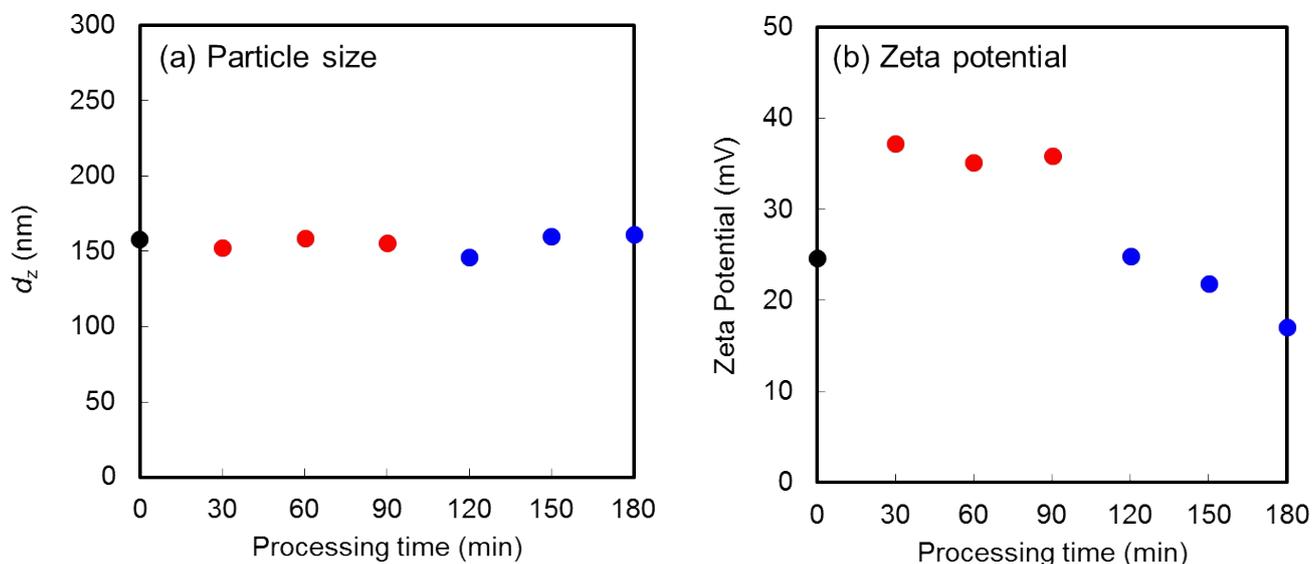
**Figure S9** Curve fitting data of binding isotherms of HSA towards HEMA-VA-061\_MIP particles (a) and HEMA-VA-061\_NIP particles (b) under CO<sub>2</sub>-treated condition. The particles were prepared at 65°C.

## 11. Selectivity test of HEMA-VA061\_MIP and HEMA-VA061\_NIP particles prepared at 65°C

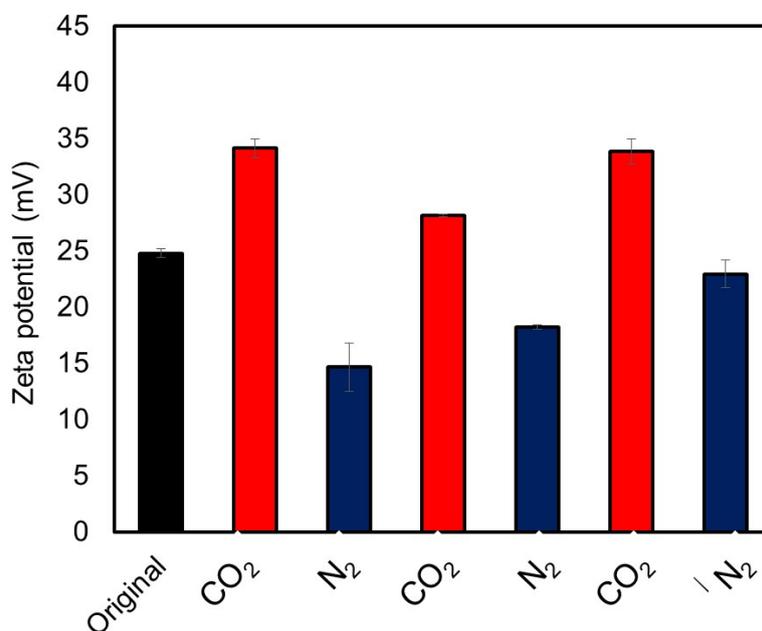


**Figure S10** Selectivity test of HEMA-VA061\_MIP in CO<sub>2</sub>-treated aqueous phase (a) and HEMA-VA061\_NIP in CO<sub>2</sub>-treated aqueous phase (b). The protein concentration was 150 nM. The particles were prepared at 65 °C.

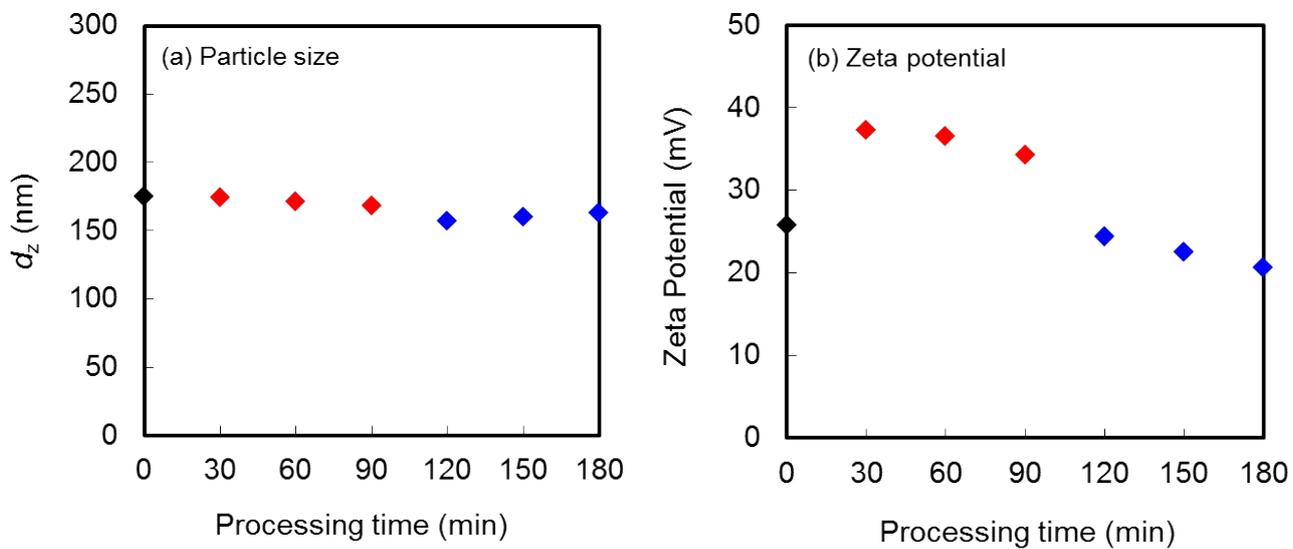
## 12. Gas-responsive colloidal property of HEMA-VA061\_MIP particles



**Figure S11.** Particle size (a) and zeta potential profiles (b) of HEMA-VA061\_MIP particles prepared at 40°C after CO<sub>2</sub> treatment (for 90 min), followed by N<sub>2</sub> treatment (for 90 min)

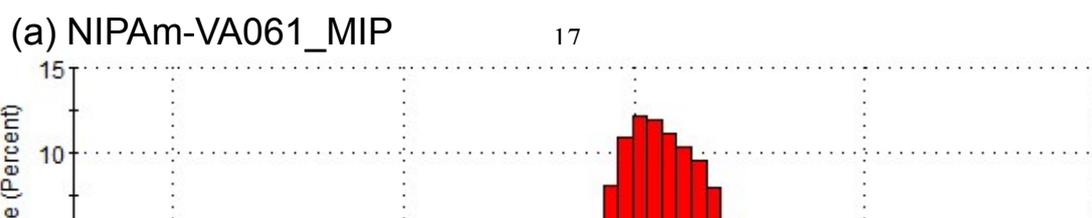


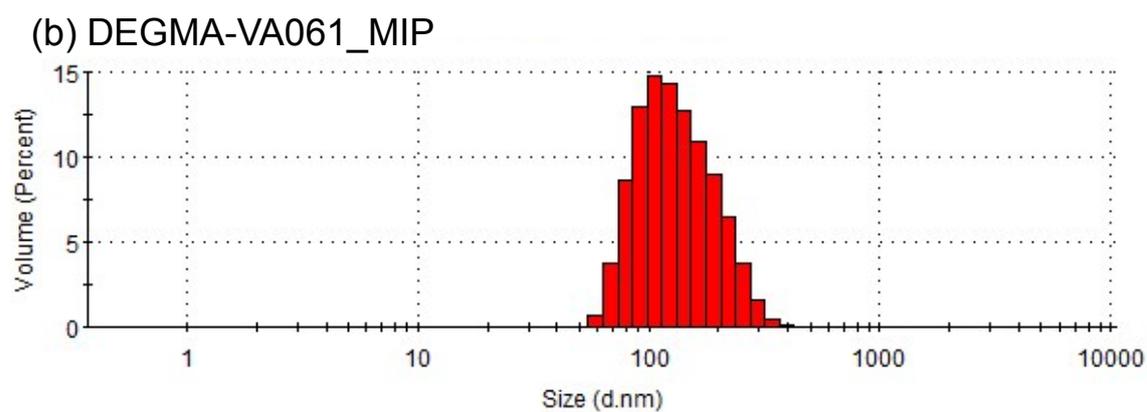
**Figure S12.** Reversible gas-responsive property of the zeta potential of HEMA-VA-061\_MIP particles. The gas-treatment was carried out for 90 min.



**Figure S13** Particle size (a) and zeta potential profiles (b) of HEMA-VA061\_MIP particles prepared at 65°C after CO<sub>2</sub> treatment (until 90 min), followed by N<sub>2</sub> treatment (until 180 min)

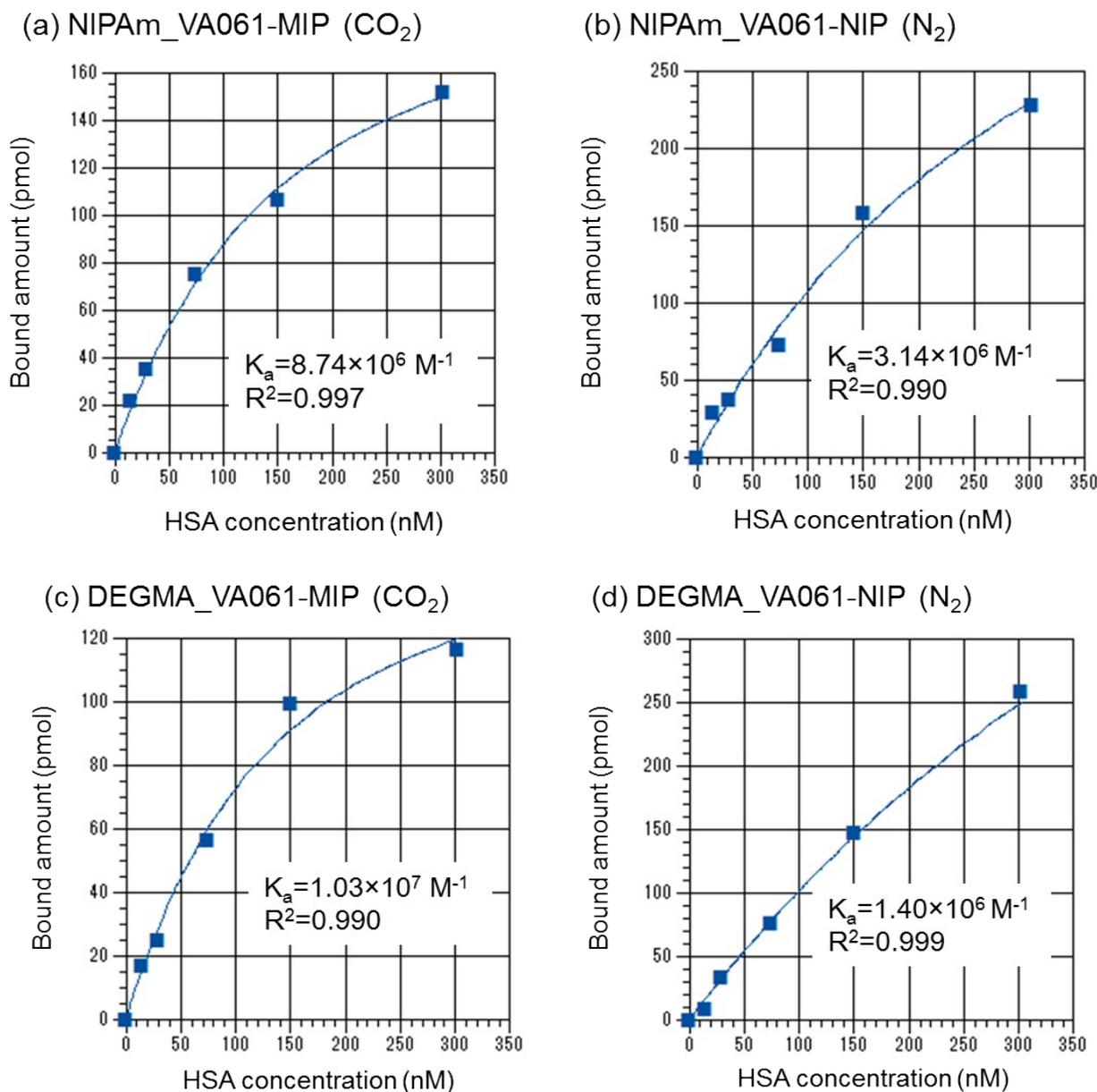
**13. Particle size distributions of NIPAm-VA061\_MIP and DEGMA-VA061\_MIP particles**





**Figure S14** Particle size distributions of NIPAM\_VA-061-MIP (a) and DEGMA\_VA-061-MIP (b) particles prepared by emulsifier-free emulsion polymerization with VA-061 in CO<sub>2</sub>-treated water at 40°C.

14. Curve fitting data of binding isotherms of NIPAm-VA-061\_MIP and DEGMA-VA-061\_NIP particles prepared at 40°C



**Figure S15** Curve fitting data of binding isotherms of HSA towards NIPAm-VA-061\_MIP particles (a, b) and DEGMA-VA-061\_NIP particles (c, d) under CO<sub>2</sub>-treated condition (a, c) and N<sub>2</sub>-treated condition (b, d). The particles were prepared at 40°C.