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Supporting Information

Target Molecule-responsive Hydrogels Designed by Molecular Imprinting Using Bisphenol A as a Template

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Materials

p-Toluenesulfonyl chloride, *p*-toluenesulfonic acid monohydrate, β-cyclodextrin (β-CD), bisphenol A (BPA), sodium azide (NaN_3), triphenyl phosphine, 25 % ammonia solution, acryloyl chloride, acrylamide (AAm), *N, N′*-methylenebisacrylamide (MBAA), ammonium persulfate (APS), *N, N, N′, N′*-tetramethylethylenediamine (TEMED), dichloromethane, isopropyl ether, sodium hydroxide (NaOH), hydrogen chloride (HCl), acetone, dimethylformamide (DMF) and sodium hydrogen carbonate ($NaHCO_3$) were purchased from WAKO Pure Chemical Industries (Osaka, Japan). β-CD was used after recrystallized from deionized water. The other materials were used without further purification.

Synthesis of acryloyl-modified β-cyclodextrin (acryloyl-CD) (Scheme S1)

Acryloyl-modified β-cyclodextrin was synthesized *via* 5 step reaction as illustrated in Scheme S1.

(1) Synthesis of p-toluenesulfonic anhydride (Ts_2O)

p-Toluenesulfonyl chloride (16 g, 83.9 mmol) and *p*-toluenesulfonic acid monohydrate (4.0 g, 21.2 mmol) were dispersed in 100 mL of dichloromethane. The reaction mixture was stirred for overnight, and then the unreacted *p*-toluenesulfonyl chloride was removed by filtration. The filtrate was evaporated, and then the residue was recrystallized from isopropyl ether to yield Ts_2O as a white solid; yield, 10.8 g (78 %).

(2) Synthesis of 6-O-monotosyl-6-deoxy-β-cyclodextrin (TsO-CD)

β-CD (22.39 g, 19.7 mmol) and Ts_2O (9.43 g, 28.9 mmol) were dispersed in 200 mL of deionized water, and then the suspension was stirred for 2 hr. 100 mL of NaOH solution (2.5 M) was added to the reaction mixture, and after 10 min the unreacted Ts_2O was removed by filtration. The filtrate was neutralized by the addition of HCl, affording TsO-CD was collected after cooling at 4 °C for overnight;

yield, 11.12g (44 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.75 (d, J = 8.0 Hz, 2H, Ar–*H*), 7.44 (d, J = 8.0 Hz, 2H, Ar–*H*), 5.86–5.66 (m, 13H, O–*H* of CD), 4.84–4.19 (m, 14H, C–*H* of CD), 4.59–4.19 (m, 7H, O_6–*H* of CD), 3.36–3.48 (m, overlaps with HOD), 2.09 (s, 3H, C*H*$_3$–Ar).

(3) Synthesis of 6-deoxy-6-azide-β-cyclodextrin (CD-N_3)

TsO-CD (1.68 g, 1.30 mmol) and NaN_3 (1.07 g, 16.52 mmol) were dissolved in 25 mL of deionized water, and then stirred for 2 hr at 80 °C, followed by pouring into acetone to precipitate the CD-N_3 as a white powder. The obtained CD-N_3 was dried *in vacuo*; yield, 1.41 g (93 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.74 (br, 14H, O–*H*), 4.88–4.54 (m, 14H, C–*H* of CD), 3.85–3.29 (m, overlaps with HOD).

(4) Synthesis of 6-deoxy-6-amino-β-cyclodextrin (CD-NH_2)

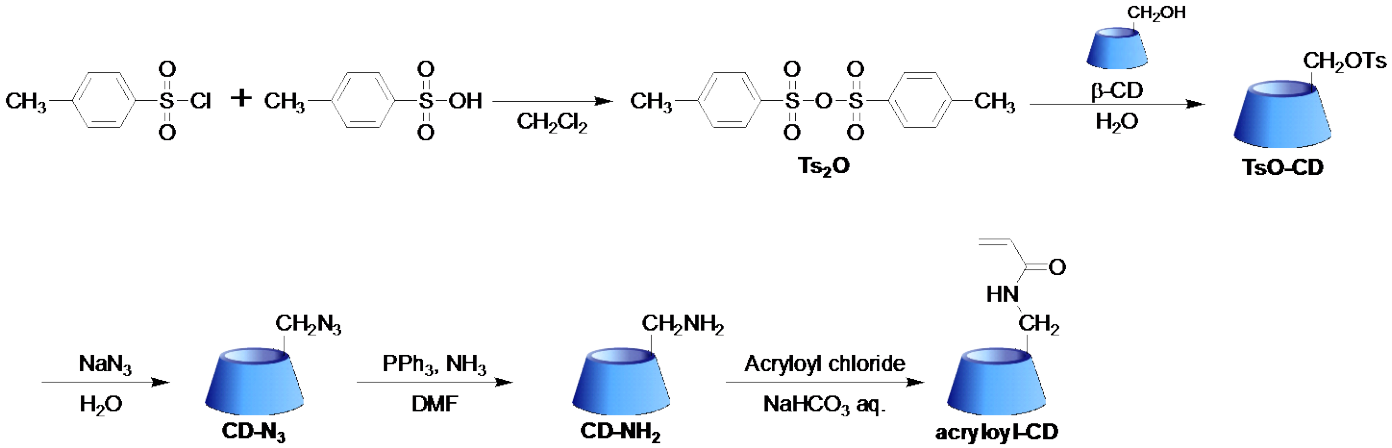
CD-N_3 (1.40 g, 1.21 mmol), triphenylphosphine (0.70 g, 2.68 mmol) and 5 mL of 25 % ammonia solution were dissolved in 25 mL of DMF, and then the reaction mixture was stirred for 4 hr. The reaction mixture was poured into acetone to precipitate CD-NH_2 as a white powder. The obtained CD-NH_2 was dried *in vacuo*; yield, 1.26 g (92 %). ^{1}H NMR (400 MHz, D_2O) δ ppm: 5.04 (s, 7H, C_1–*H* of CD), 3.93–3.55 (m, 42H, $C_{2,3,4,5,6}$–*H* of CD).

(5) Synthesis of acryloyl-6-amino-6-deoxy-β-cyclodextrin (acryloyl-CD)

CD-NH_2 (2.1 g, 1.89 mmol) was dissolved in 30 mL of $NaHCO_3$ aq. (pH 11). Acryloyl chloride (1.75 g, 19.4 mmol) was added to the solution of CD-NH_2 on ice bath. The solution was stirred for 2 hr, and then the reaction mixture was poured into acetone. The precipitate was dried *in vacuo* to yield acryloyl-CD as a white powder; yield, 1.00 g (44 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm:

6.32–6.14 (m, 2H, CH_2=CH–), 5.80–5.75 (m, 1H, CH_2=CH–), 5.05 (s, 7H, C_1–H of CD), 3.94–3.29 (m, 42H, $C_{2,3,4,5,6}$–H of CD).

Scheme S1. Synthesis of acryloyl-CD.



Preparation of BPA-imprinted, non-imprinted and PAAm hydrogels

(1) Preparation of BPA-imprinted hydrogel

Acryloyl-CD (320 mg, 0.270 mmol), BPA (30.8 mg, 0.135 mmol), AAm (380 mg, 5.34 mmol) and MBAA (1.40 mg, 9.08 μmol) were dissolved in 4.37 mL of deionized water, and then the reaction mixture was incubated for 2 days at room temperature to form sandwich-like CD-BPA-CD complexes. After addition of 0.1 mL of 0.8 M aqueous TEMED solution and 0.1 mL of 0.1 M aqueous APS solution as redox initiators, the solution was injected into a glass capillary with an inner diameter of 3 mm, and polymerization was carried out at 25 °C for 8 hr. After formed hydrogels were taken out of the glass capillary, they were immersed in 30 % aqueous acetone solution for 2 weeks to remove template BPA and unreacted monomers from their networks. Then the hydrogels were immersed in deionized water to remove acetone and their swelling ratio achieved equilibrium in deionized water.

(2) Preparation of non-imprinted hydrogel

The non-imprinted hydrogel was synthesized by the preparation method similarly to that of the BPA-imprinted hydrogel without using BPA. Acryloyl-CD (320 mg, 0.270 mmol), AAm (380 mg, 5.34 mmol) and MBAA (1.40 mg, 9.08 μmol) were dissolved in 4.37 mL of deionized water, and then the reaction mixture was incubated for 2 days. After the addition of 0.1 mL of 0.8 M aqueous TEMED solution and 0.1 mL of 0.1 M aqueous APS solution as redox initiators, the solution was injected into a glass capillary with an inner diameter of 3 mm, and polymerization was carried out at 25 °C for 8 hr. After formed hydrogels were taken out of the glass capillary, they were immersed in 30 % aqueous acetone solution for 2 weeks to remove unreacted monomers. Then the hydrogels were immersed in deionized water to remove acetone and their swelling ratio achieved equilibrium in deionized water.

(3) Preparation of PAAm gel

AAm (700 mg, 5.34 mmol) and MBAA (1.40 mg, 9.08 μmol) were dissolved in 4.37 mL of deionized water, and then the reaction mixture was incubated for 2 days. After addition of 0.1 mL of 0.8 M aqueous TEMED solution and 0.1 mL of 0.1 M aqueous APS solution as redox initiators, the solution was injected into a glass capillary with an inner diameter of 3 mm, and polymerization was carried out at 25 °C for 8 hr. After formed hydrogels were taken out of the glass capillary, they were immersed in 30 % aqueous acetone solution for 2 weeks to remove unreacted monomers. Then the hydrogels were immersed in deionized water to remove acetone and their swelling ratio achieved equilibrium in deionized water.

Determination of stoichiometry of inclusion complex between CD and BPA

The complexation between CD and BPA were investigated by UV-Vis spectroscopy[1]. The 20 mL of 4 mM aqueous β-CD solution and 20 mL of 0–4 mM aqueous BPA solution were mixed, and then the UV-Vis spectra were measured using UV2550PC (Shimadzu Co., Ltd., Kyoto, Japan).

1. F. Cramer, W. Saenger and H. C. Spatz,, *J. Am. Chem. Soc.*, 1967, **89**, 14.

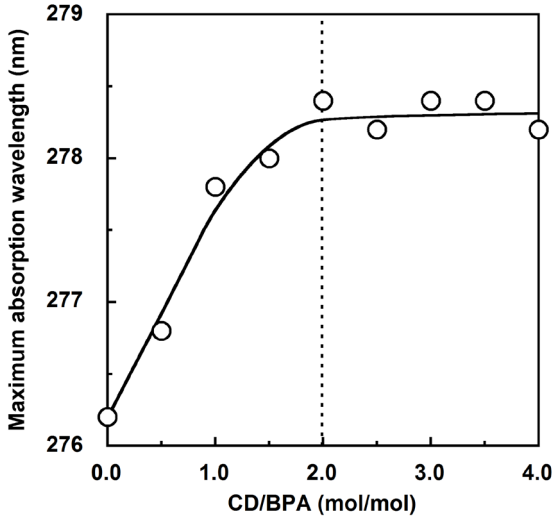


Fig. S1. Effect of the CD/BPA ratio on the maximum absorption wavelength of BPA in an aqueous BPA solution containing 2 mM of β-CD.

Measurements of swelling ratio

The BPA-imprinted, non-imprinted and PAAm hydrogels were kept immersed in deionized water until equilibrium was achieved at 25 °C. After that, the hydrogels were transferred and kept immersed in an aqueous BPA solution at 25 °C. The swelling ratio (V/V_0) of hydrogels was determined from the ratio of their diameters by Eq. 1. The diameters of hydrogels that achieved equilibrium swelling in deionized water (d_0) and an aqueous BPA solution (d) were measured using an optical microscope.

$$Swelling\ ratio = \frac{V}{V_0} = \left(\frac{d}{d_0}\right)^3 \qquad (1)$$

Measurements of crosslinking density

Compressive modulus of hydrogels was determined using a rheometer (Rheology Co., DVE Rheospectoler DVE-V4). The hydrogel that achieved equilibrium swelling was compressed by the

crosshead of the apparatus, and then the relationship between the compressive stress and strain of the hydrogel was recorded. The compressive modulus can be obtained by Eq. 2 from the compressive stress and strain of hydrogels. Moreover, the apparent crosslinking density of the hydrogel can be calculated by Eq. 3.

$$\tau = G(\alpha - \alpha^{-2}) \quad (2)$$

$$G = RT\nu_e V_0^{2/3} V_2^{1/3} \quad (3)$$

where τ is compressive stress (Pa), G is compressive modulus (Pa), R is the gas constant, T is absolute temperature (K), α is the ratio of the thickness of the gel before and after compression, ν_e is the effective crosslinking density (mol/L), V_0 is the volume fraction of the polymer during network formation and V_2 is the volume fraction of the polymer in the hydrogel, which is obtained from the swelling ratio using Eq. 4:

$$\frac{1}{V_2} = 1 + (swelling\ ratio) \times \frac{\rho_g}{\rho_s} \quad (4)$$

where ρ_g and ρ_s are the density of the dried gel and solvent, respectively.

The effective crosslinking densities determined by equation (3) are the numbers of crosslinks per a dried network without water. As the compressive modulus are influenced by both the number of crosslinks and swelling ratio, we compared changes in the number of crosslinks between BPA-imprinted and non-imprinted hydrogels by the crosslinking densities based on the number of crosslinks per a dried network. The comparison in the crosslinking densities that mean the number of crosslinks per a dried network enables us to discuss the mechanism for the BPA-responsive behaviour by changes in the number of crosslinks. Compressive modulus and crosslinking density of the BPA-imprinted and non-imprinted hydrogels in water with and without BPA are shown in Table S1

Table S1. Compressive modulus (G) and crosslinking density of BPA-imprinted, non-imprinted and PAAm hydrogels in an aqueous solution with or without BPA.

	Water		BPA solution[a]	
	G (kPa)	ν_e (mol/m^3)	G (kPa)	ν_e (mol/m^3)
BPA-imprinted hydrogel	2.33	30.9	4.91	65.1
Non-imprinted hydrogel	1.68	22.3	3.17	42.1
PAAm hydrogel	0.38	5.0	0.38	5.0

[a]Concentration of BPA solution was 0.12 mg/mL