Supporting Information

Modulating Polymeric Amphiphiles Using Thermo- and pH-Responsive Copolymers with Cyclodextrin Pendant Groups through Molecular Recognition of Lipophilic Dye

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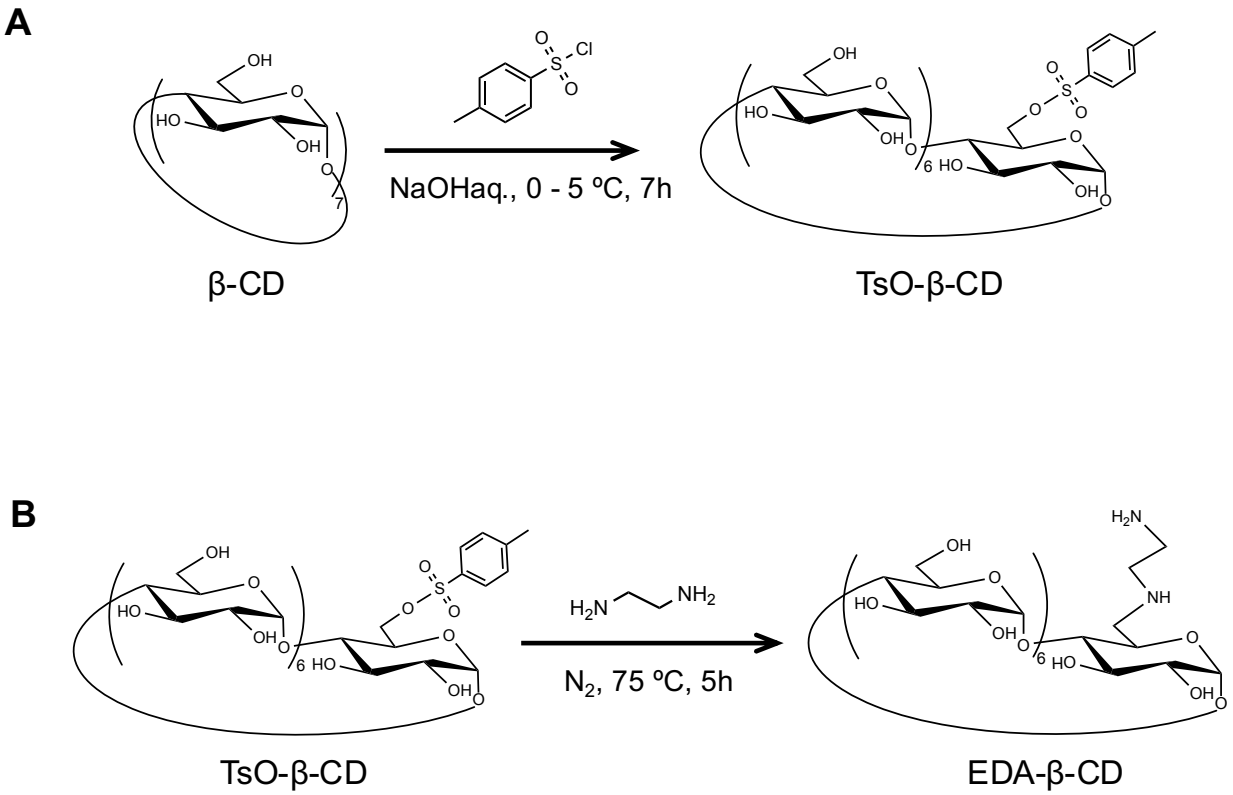
Materials.

All the reagents and solvents were commercially available and were used as received unless otherwise specified. All aqueous solutions were prepared with distilled water.

Measurements.

The product was characterized by proton nuclear magnetic resonance (^{1}H-NMR), Fourier-transform infrared spectroscopy (FT-IR), and matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS). The ^{1}H-NMR spectra (600 MHz) were obtained using a JEOL delta ECA system with DMSO-d_6 as the solvent for TsO-β-CD and EDA-β-CD. The ^{1}H-NMR spectra were analyzed by Delta NMR processing and control software v5.3 (JEOL RESONANCE, Inc). The FT-IR spectra of EDA-β-CD and AEA-β-CD were obtained using an FT/IR-4100 system (JASCO) with attenuated total reflectance (ATR). The molecular weights of the β-CD derivatives were determined by an AXIMA Confidence MALDI-TOF-MS spectrometer (Shimadzu Co. Ltd) with sodium trifluoroacetate as the ionizing agent and 2,5-dihydroxybenzoic acid as the matrix agent. DMSO and water were used as the solvents for TsO-β-CD and EDA-β-CD, respectively. Electrospray ionization-mass spectrometry (ESI-MS) was conducted by an LCMS-IT-TOF spectrometer (Shimadzu Corp.) to determine molecular weight of AEA-β-CD. 1%w/v sample dissolved in water was injected into the LC-MS-IT-TOF spectrometer.



Scheme S1. Synthetic routes for TsO-β-CD (A) and EDA-β-CD (B).

Synthesis and characterization of the β-cyclodextrin-substituted monomer.

The mono-6-*O*-(*p*-toluenesulfonyl)-β-cyclodextrin (TsO-β-CD) was synthesized as described in the literature[1]. The reaction is described in **Scheme S1**. The reaction of β-CD and TsCl was conducted at a molar ratio of 1: 5 in 750 mL of 0.4 M NaOH solution. The β-CD (34.1 g) was mixed with the NaOH solution at 0 °C for 1 h in a round-bottomed flask equipped with a magnetic stirring bar and a thermometer. The cooling bath was then removed from the system, and a portion of TsCl (18.7 g) was added to the mixed solution, which was stirred vigorously for 2 h. Another portion of TsCl (10.0 g) was added and the mixture was further stirred for 2 h. The mixture was filtered to remove the unreacted TsCl, and the filtrate was neutralized using 10% HCl solution to produce a pH of 6–7, then stored overnight in a fridge (< 4 °C). The white precipitate of TsO-β-

CD was filtered and dried under vacuum in an oven at 40 °C for 24 h. The product was recrystallized three times by dissolving in 300 mL of boiling water and cooling at < 4 °C. The purified product was dried under vacuum in an oven at 40 °C for 24 h to produce mono-TsO-β-CD at a yield of 19%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ (ppm) 7.76 (d, J = 8.2 Hz, 2H, Ar-*H*), 7.44 (d, J = 7.6 Hz, 2H, Ar-*H*), 5.84–5.64 (m, 14H, 2, 3-O*H* in β-CD), 4.84 (d, J = 46.1 Hz, 7H, 1-*H* in β-CD), 4.52–4.35 (m, 6H, 6-O*H* in β-CD), 4.20–4.17 (m, 2H, 6′-*H* in β-CD), 3.65–3.20 (m, 40H, 2, 3, 4, 5, 6-*H* in β-CD), 2.43 (s, 3H, Ar-CH_3); the purity was 97%. MALDI-TOF-MS: *m/z* calculated for $[M\text{-}Na]^+$ $C_{49}H_{76}O_{37}S^+$, 1311.16 found 1311.34.

The ethylene diamino β-CD **(**EDA-β-CD) was synthesized by mixing 5.16 g of TsO-β-CD in 30 mL of EDA at 75 °C under degassed N_2 for 5 h (**Scheme S1B**). To terminate the reaction, the mixed solution was cooled to room temperature, then added to cold acetone (300 mL). The resulting solid precipitate of EDA-β-CD was filtered under vacuum. Subsequently the precipitate was dissolved in a mixed solution comprising 40 mL of water and methanol (1:3), and was re-precipitated by adding dropwise to a solution of cold acetone. The reprecipitation and filtration processes were repeated three times, and the resulting EDA-β-CD was collected and dried at 60 °C under vacuum in an oven to produce the purified product (4.22 g, 90% yield). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 4.92 (s, 7H, 1-*H* in β-CD), 3.83–3.68 (m, 26H, 3, 5, 6-*H* in β-CD), 3.51–3.41 (m, 14H, 2, 4-*H* in β-CD), 3.32 (t, J = 9.3 Hz, 1H, -CH_2-CH_2-N*H*-CH_2-), 2.91 (dd, J = 12.7, 2.4 Hz, 1H, 6′-*H* in β-CD), 2.73–2.67 (m, 3H, 6′-*H* in β-CD and NH_2-CH_2-CH_2-NH-), 2.60 (t, J = 7.2 Hz, 2H, NH_2-CH_2-CH_2-NH-). MALDI-TOF-MS: *m/z* calculated for $[M\text{-}Na]^+$ $C_{44}H_{76}O_{34}N_2^+$, 1199.1 found 1200.4.

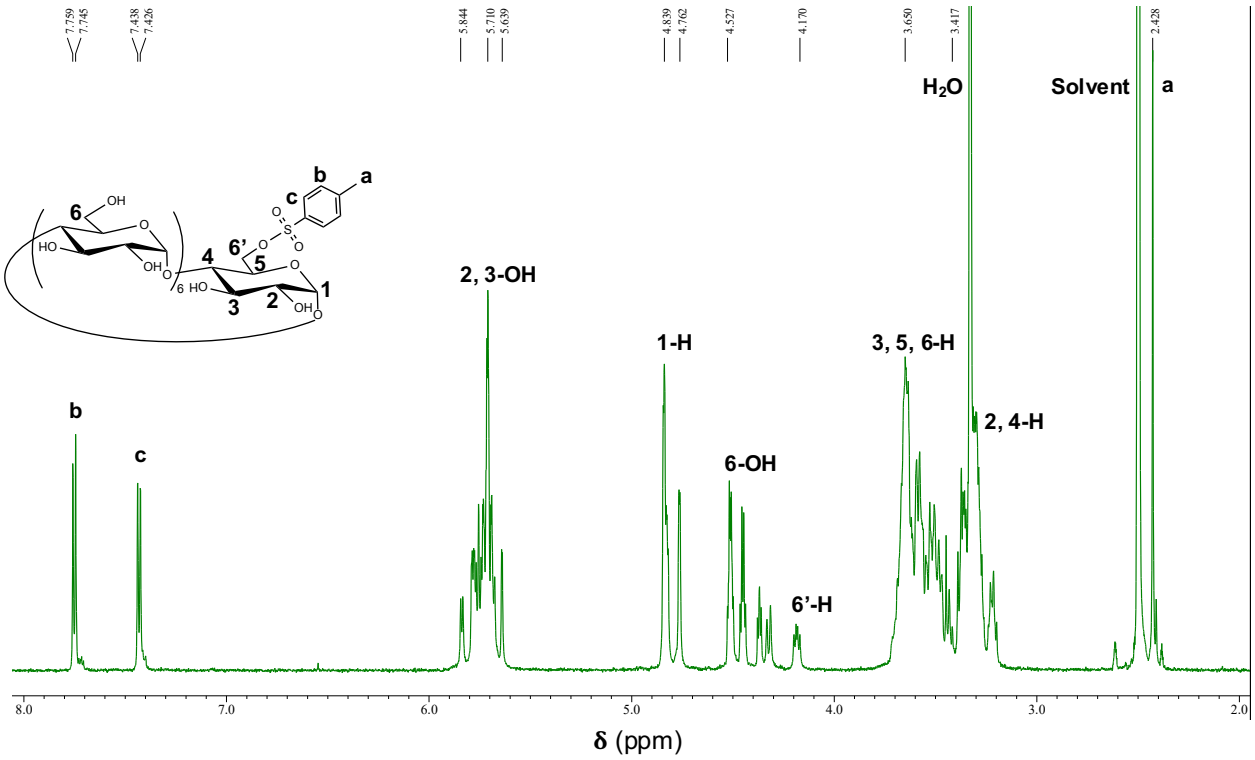


Figure S1. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6, 25 ºC) of TsO-β-CD.

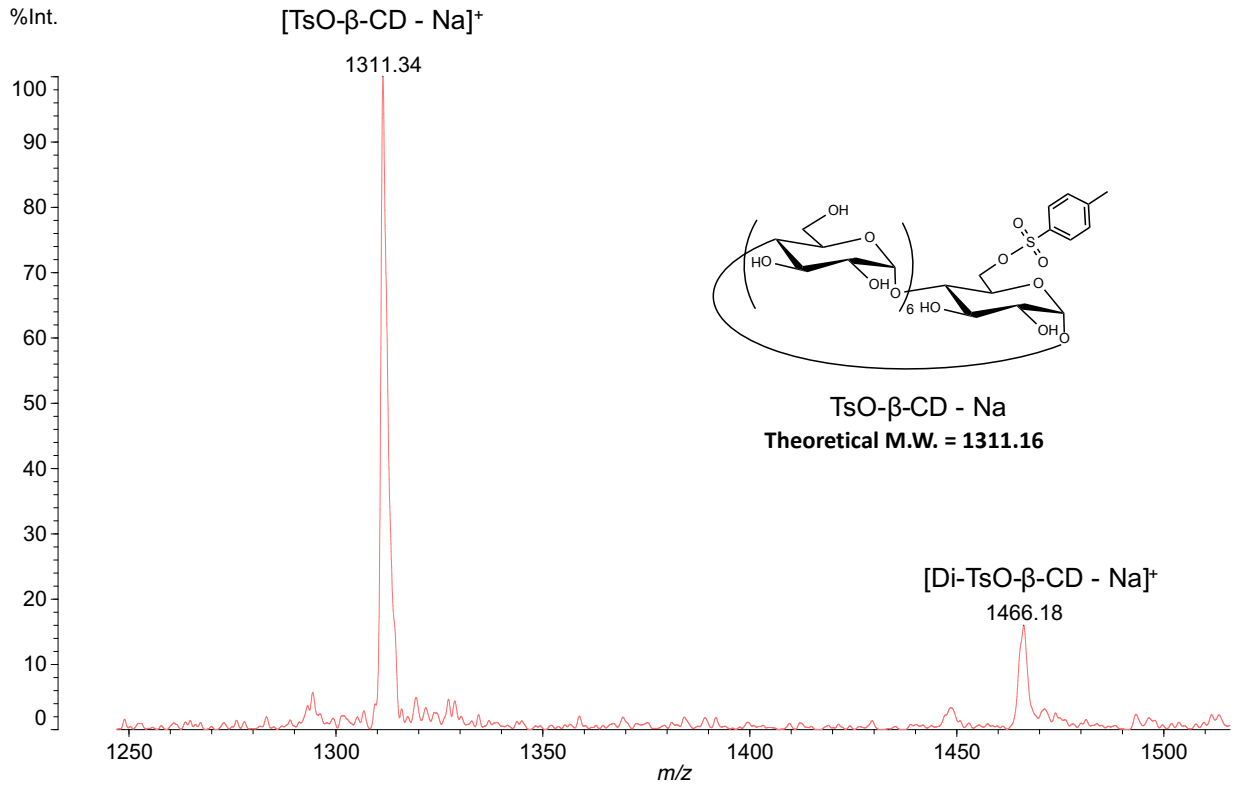


Figure S2. MALDI-TOF-MS of TsO-β-CD.

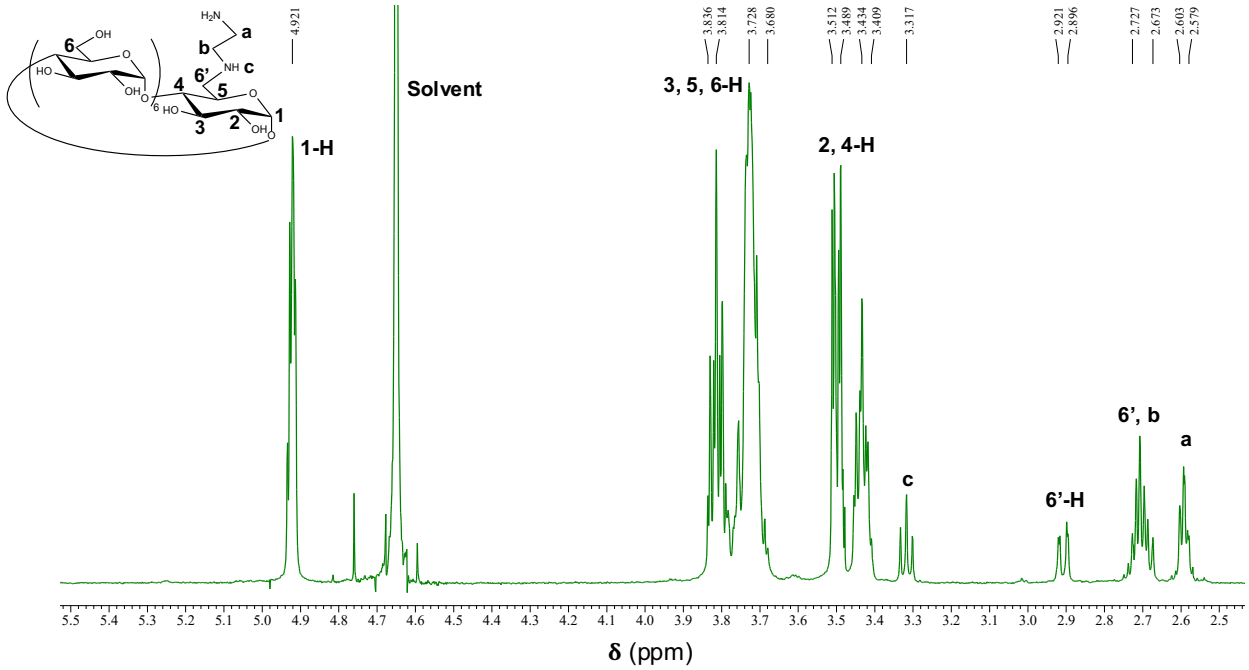


Figure S3. ^{1}H-NMR spectrum (600 MHz, D_2O, 25 ºC) of EDA-β-CD.

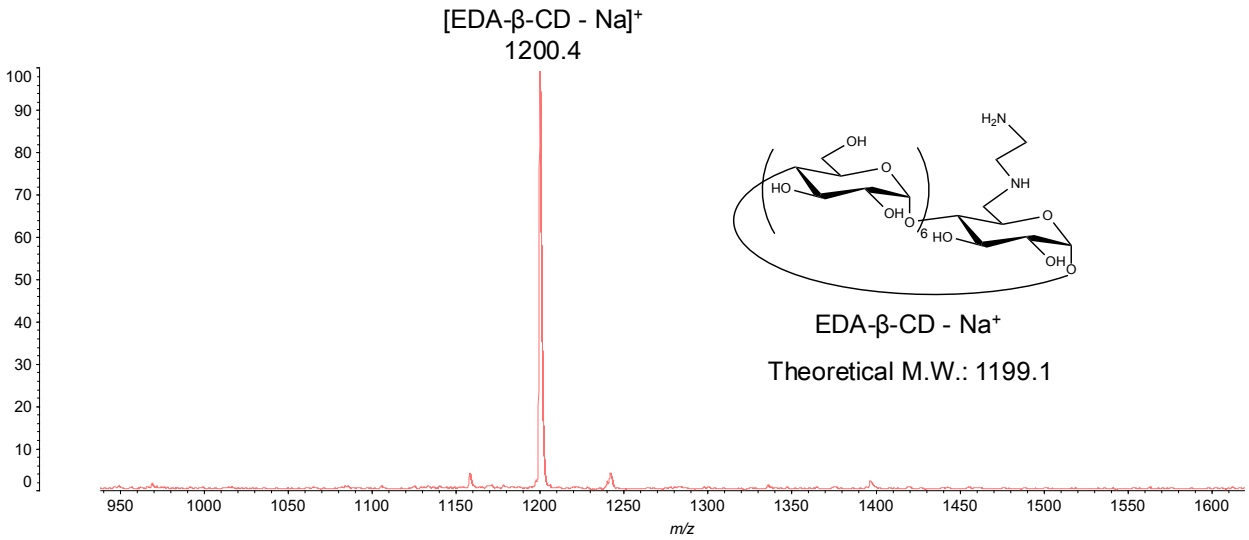


Figure S4. MALDI-TOF-MS of EDA-β-CD.

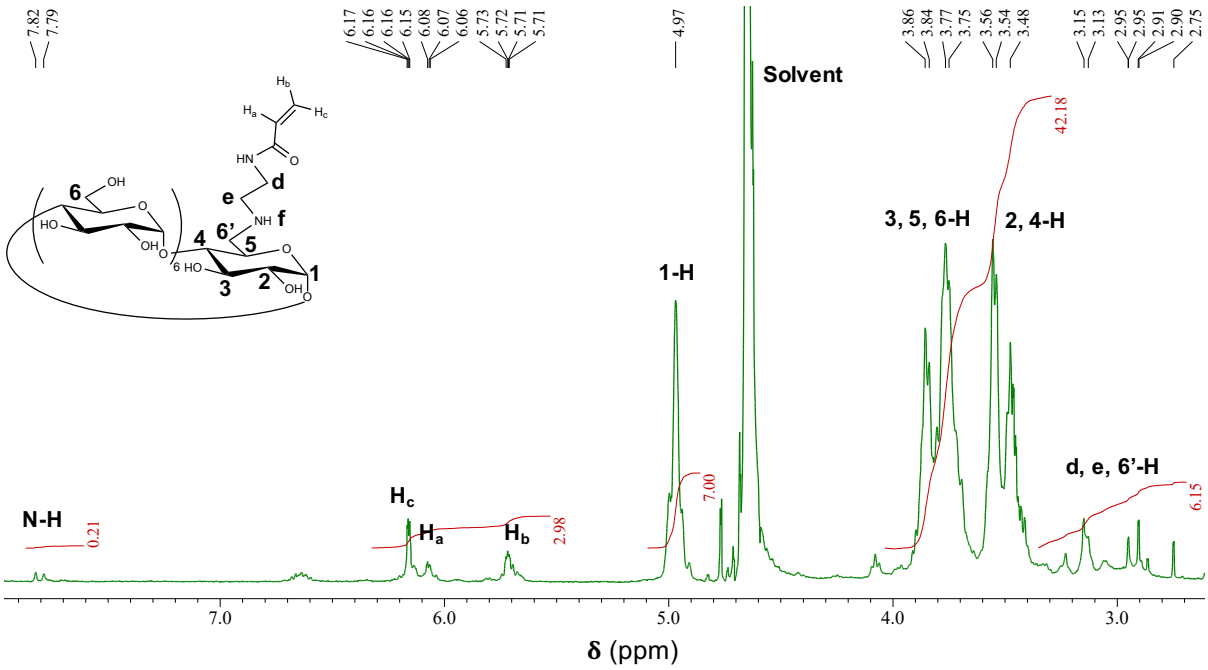


Figure S5. ^{1}H-NMR spectrum (600 MHz, D_2O, 25 ºC) of AEA-β-CD.

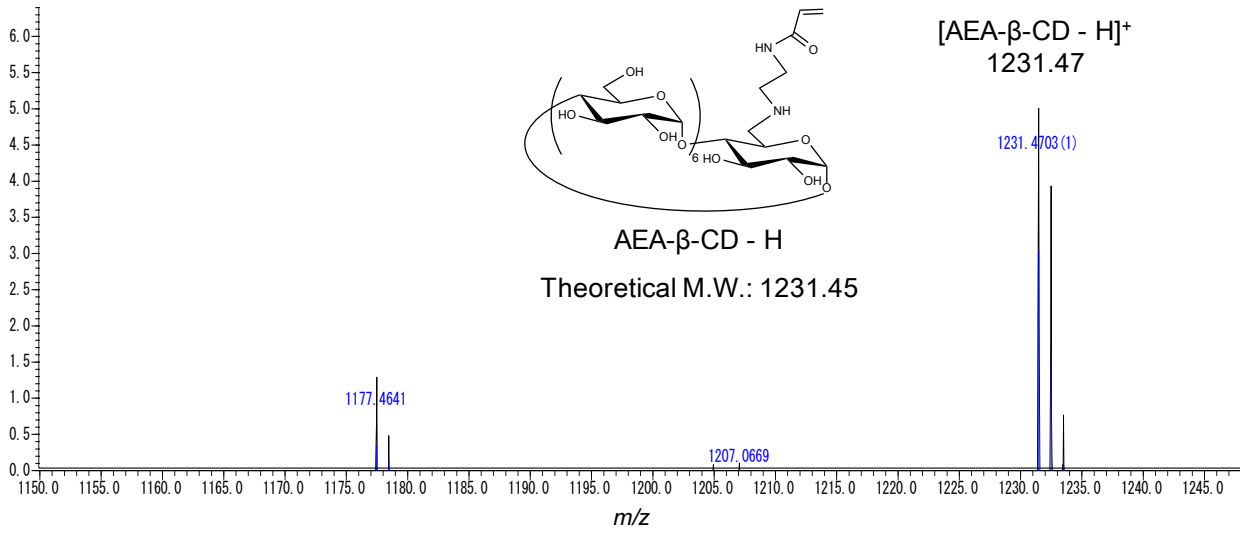


Figure S6. ESI-MS (positive) of AEA-β-CD.

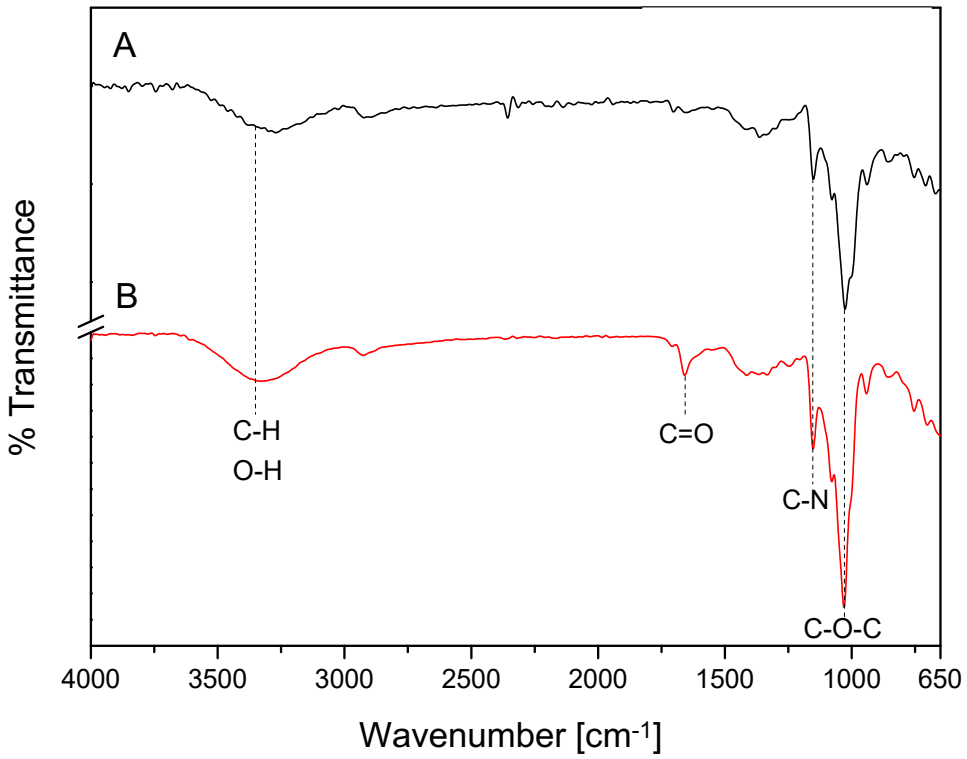


Figure S7. FT-IR spectra of EDA-β-CD (A) and AEA-β-CD (B).

Synthesis of poly (*N*-isopropylacrylamide-*co*-acrylic acid-*co*-AEA-β-CD) (poly(NIPAM-AA-β-CD)) and poly (*N*-isopropylacrylamide-*co*-acrylic acid) (poly(NIPAM-AA)).

Poly(NIPAM-*co*-AA) without the CD moiety in the polymer segment was synthesized by the free radical polymerization of the NIPAM and AA monomers dissolved in toluene (the feed monomer ratio was 4:1 for NIPAM to AA). AIBN was added at a concentration of 0.5 mol% relative to the total concentration of the monomers. Another procedure was carried out in essentially the same manner as that described for the production of poly(NIPAM-AA-β-CD). After dialysis, the purified solution was freeze-dried for 3 days and a white solid was produced (78.1% yield). ^{1}H-NMR (600 MHz, D_2O). δ (ppm) 3.74 (br, 1H, -NH-C*H*-$(CH_3)_2$ in PNIPAM), 1.97–1.43 (br, 4H, -C*H*-CH_2- in the poly(NIPAM-*co*-AA) chain), 0.99 (br, 6H, -NHCH(CH_3)$_2$ in PNIPAM).

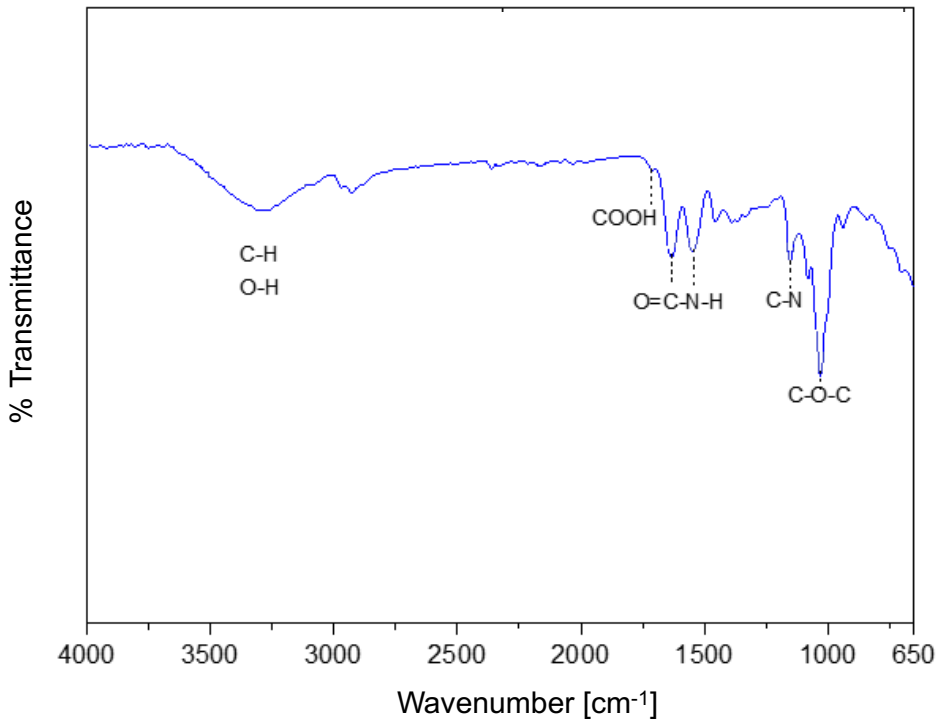


Figure S8. FT-IR spectra of Poly(NIPAM-AA-β-CD).

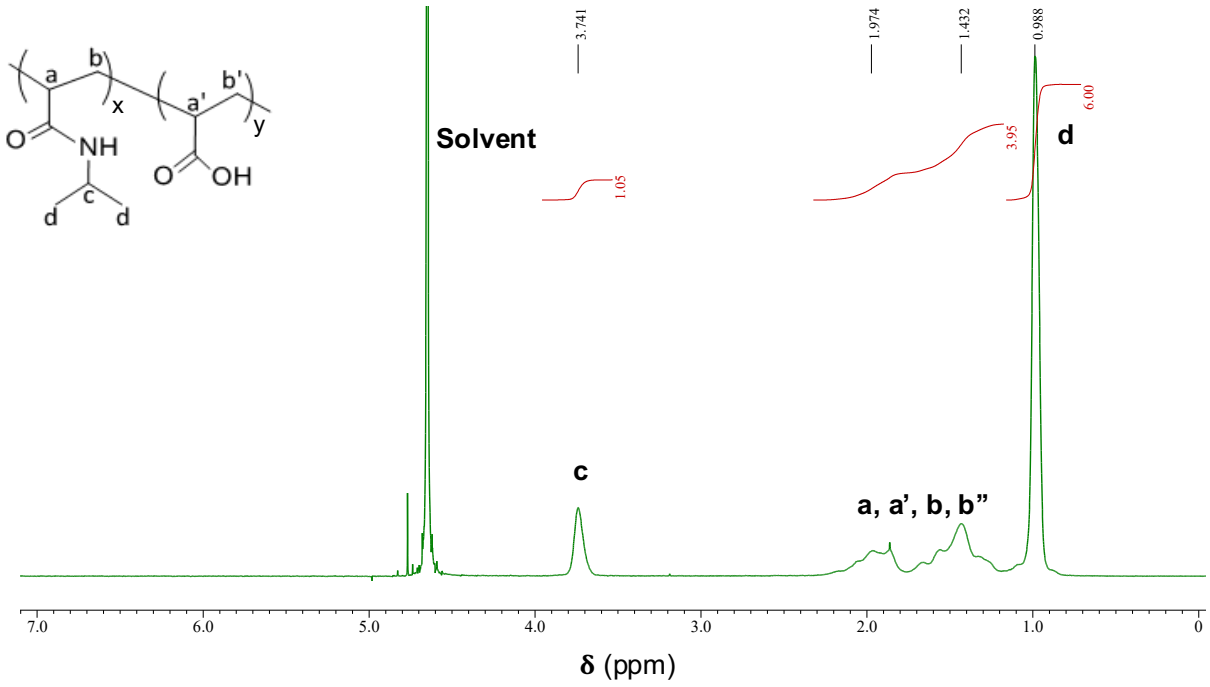


Figure S9. ^{1}H-NMR spectrum (600 MHz, D_2O, 25 ºC) of Poly(NIPAM-AA).

Characterization of copolymer, solution property and the functionality.

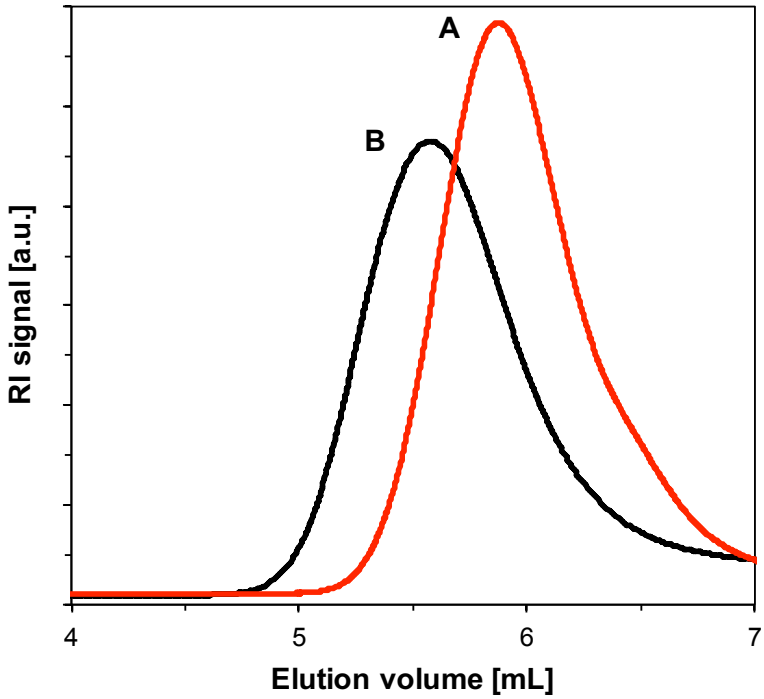


Figure S10. GPC curves of (A) Poly(NIPAM-AA-β-CD) and (B) Poly(NIPAM-AA).

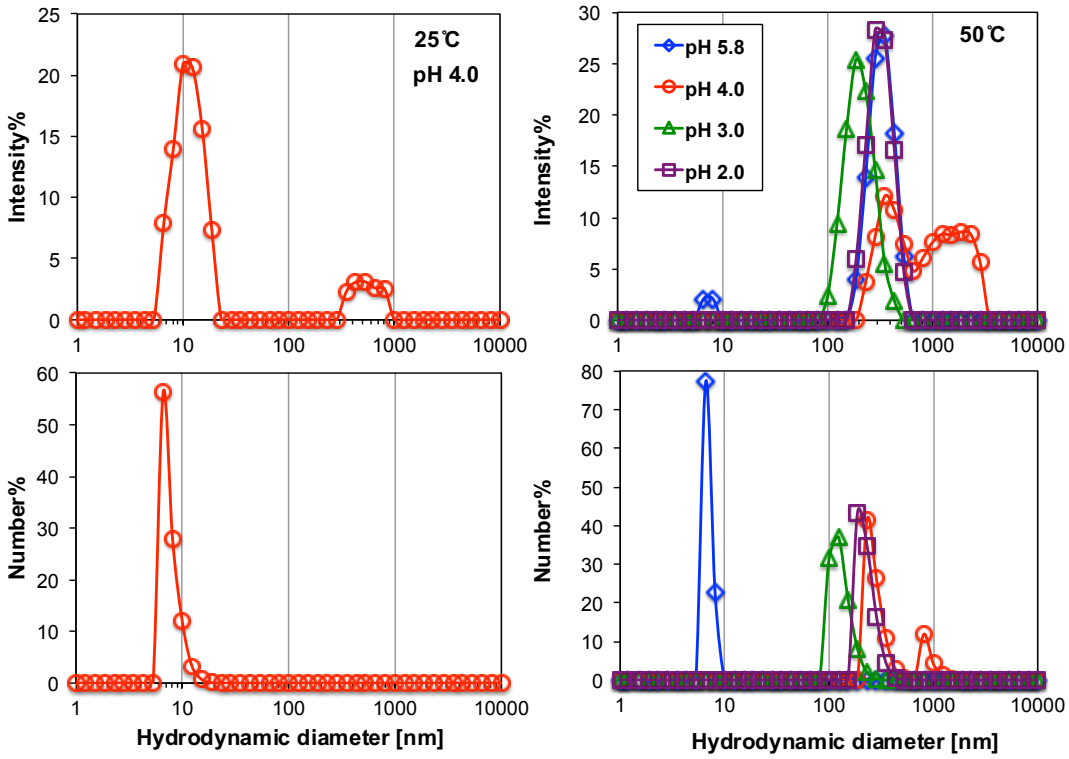


Figure S11. DLS size distributions (intensity% and number%) of Poly(NIPAM-AA-β-CD) at each pH as function of temperature at 25 (pH 4.0) and 50 ˚C.

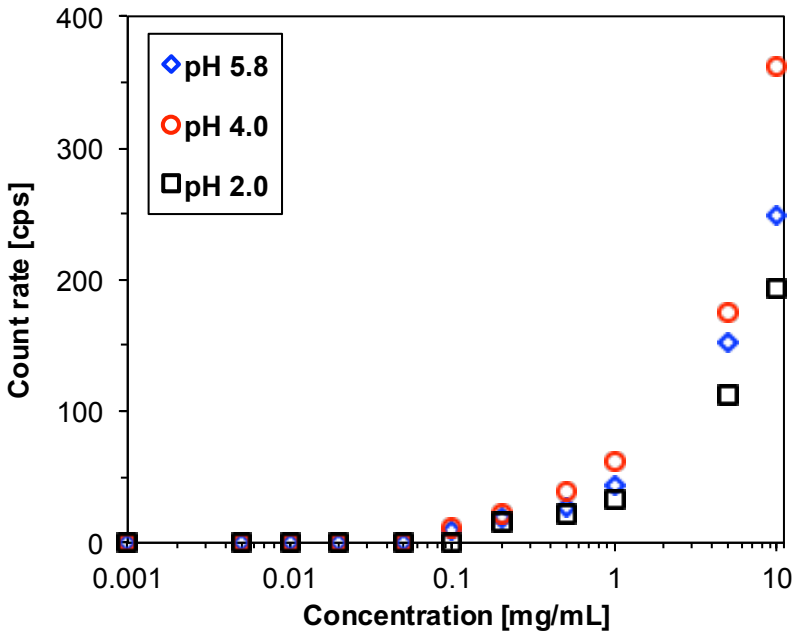


Figure S12. DLS count rates versus the concentrations of the Poly(NIPAM-AA-β-CD) solutions at each pH (25 °C).

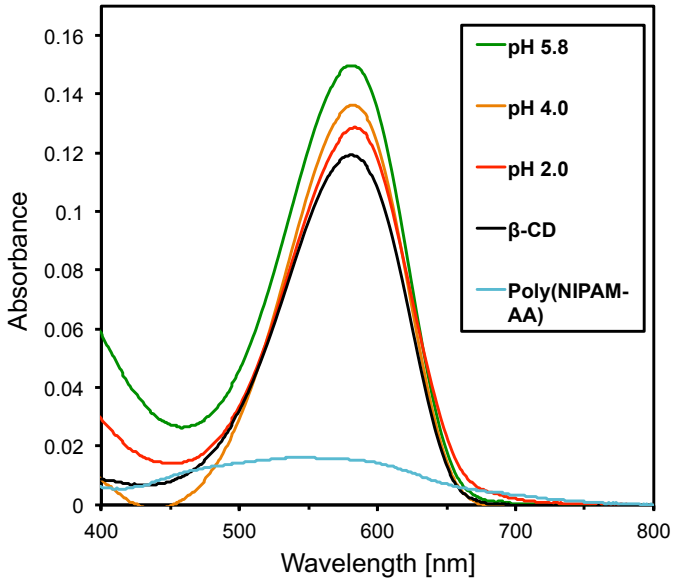


Figure S13. UV-Vis absorption spectra of NR-incorpolated complex solutions of Poly(NIPAM-AA-β-CD) at each pH, β-CD and Poly(NIPAM-AA) (< pH= 4.0) after subtracting the absorption base line of each naked sample.

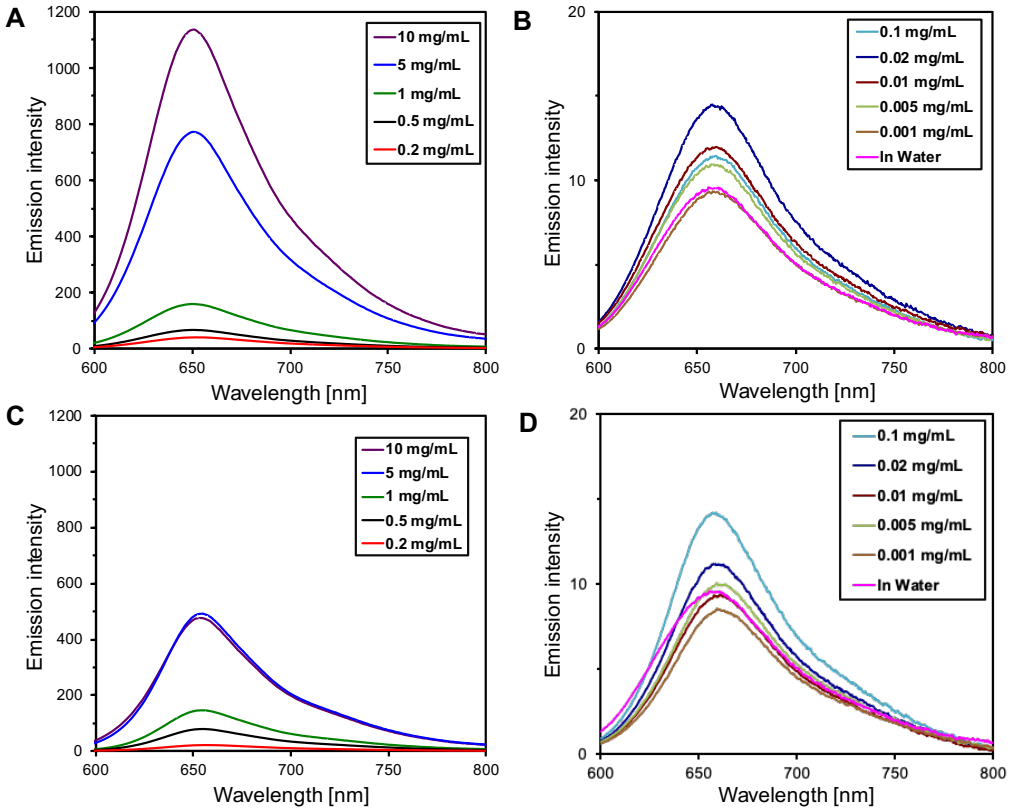


Figure S14. Fluorescence emission spectra of the NR-incorporated complex solutions of Poly(NIPAM-AA-β-CD) (pH 5.8) (A, B) and β-CD (C, D). Spectra B and D show the magnified images at the concentrations from 0.001 to 0.1 mg/ mL.

REFERENCE

1) Brady, B.; Lynam, N.; O'Sullivan, T.; Ahern, C. ; Darcy, R. 6A-*O*-*p*-Toluenesulfonyl-β-Cyclodextrin. *Org Synth*. **2000**, *77*, 220-222.