Supplementary Information

Construction and Micellization of Noncovalent Double Hydrophilic Block Copolymer

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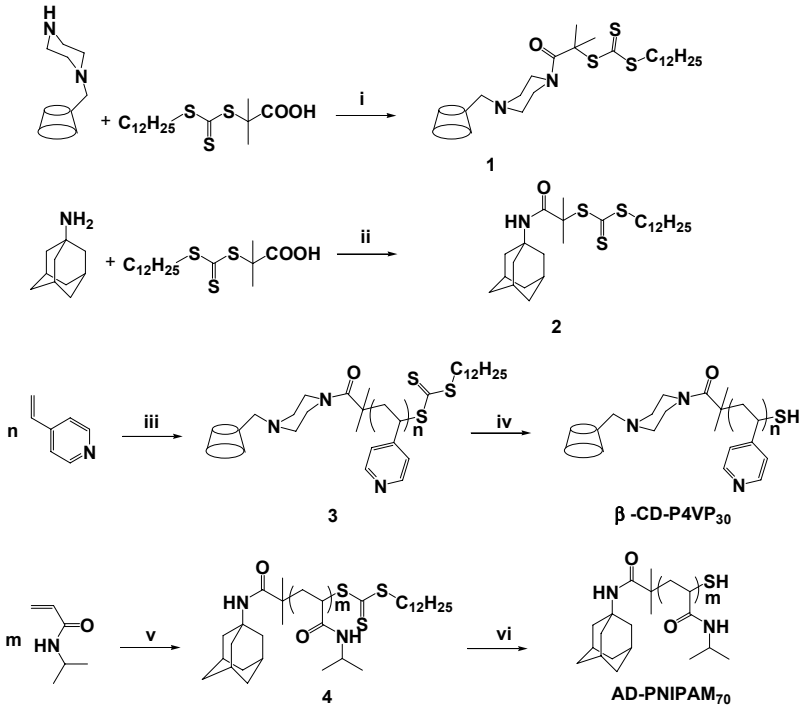
1. Materials

1-Dodecanethiol, acetone, chloroform, tetramethylammonium bromide, sodium hydroxide, ethanol, 2-propanol, hexane, carbon disulfide, 4-methylbenzene-1-sulfonyl chloride and anhydrous piperazine were commercially available and used as received, β-cyclodextrin (β-CD) was recrystallized from water and dried under vacuum at 90 ^{o}C for 24 h prior to use. 1-amantadine was refined from 1-amantadine hydrochloride (Alfa Aesar, 99%) by steam distillation. *N,N*-dimethylformamide (DMF) and 4-vinylpyridine (4VP, Alfa Aesar, 95%) were dried with calcium hydrate for 2 days and then purified by vacuum distillation before use. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized from ethanol. *N*-isopropylacrylamide (NIPAM) (Across, 99%) was recrystallized from hexane and dried at room temperature under vacuum prior to use.

2. Synthesis



Scheme S1. Synthetic route of β-CD-P4VP$_{30}$ and AD-PNIPAM$_{70}$

i) DMF, DCC/DMAP, 0 ^{o}C; ii) CH_2Cl_2, DCC/DMAP, r.t.; iii) DMF, AIBN, **1**, 70 ^{o}C; iv) DMF, hexylamine, 0.5 M $Na_2S_2O_4$, r.t.; v) DMF, AIBN, **2**, 70 ^{o}C; vi) DMF, hexylamine, 0.5 M $Na_2S_2O_4$, r.t.

2.1 Synthesis of β-CD-containing trithiocarbonate (1).

A solution of mono-6-piperazin-β-cyclodextrin (1.2 g, 1 mmol) (S. F. Lincoln， J. H. Coates, C. J. Easton, S. J. Van Eyk, B. L. May, P. Singh, M. L. Williams, M. A. Stile, PCT Int. Appl. 1990, 118), S-1-dodecyl-S′-(α, α'-dimethyl-α"-acetic acid)trithiocarbonate (0.36 g, 1 mmol), dicyclohexylcarbodiimide (DCC, 0.25 g, 1.2 mmol) and 4-dimethylaminopyridine (DMAP, 24.5 mg, 0.2 mmol) in DMF (10 mL) was stirred at 0 ^{o}C for 72 h. The reaction mixture was filtered and the filtrate was concentrated and precipitated in diethyl ether. The precipitate was washed by water repeatedly, and the residue was dried under vacuum at 60 ^{o}C for 24 h to afford **1** as the pale yellow solid. Yield: 75%. ^{1}H NMR (DMSO-d$_6$, ppm) δ = 6.7 (14H, OH-2 and OH-3 for β-CD), 4.8 (7H, H-1 for β-CD), 4.5 (7H, OH-6 for β-CD), 2.5-3.6 (52H, 42H of H-2, H-3, H-4, H-5, and H-6a, b for β-CD, 8H for piperazine group and 2H for $C_{11}H_{23}C\mathit{H}_2$-$CS_3C(CH_3)_2CO$-), 1.6 (6H, $CH_3C_{10}H_{20}CH_2$-$CS_3C(C\mathit{H}_3)_2CO$-), 1.0-1.4 (20H, $CH_3C_{10}\mathit{H}_{20}CH_2$-$CS_3C(CH_3)_2CO$-), 0.85 (3H, $C\mathit{H}_3C_{10}H_{20}CH_2$-$CS_3C(CH_3)_2CO$-). FTIR (KBr, ν_{max}): 1655 cm^{-1} for C=O of –CO-N-.

2.2 Synthesis of AD-containing trithiocarbonate (2)

A solution of 1-amantadine (1.51 g, 10 mmol), S-1-dodecyl-S′-(α,

α'-dimethyl-α"-acetic acid)trithiocarbonate (4.38 g, 12 mmol), dicyclohexylcarbodiimide (DCC, 2.68 g, 13 mmol) and 4-dimethylaminopyridine (DMAP, 0.27 g, 2.2 mmol) in dehydrated CH_2Cl_2 (15 mL) was stirred at room temperature for 72 h. The reaction mixture was filtered and the filtrate was washed with 5% Na_2CO_3 aqueous solution and water. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel with CH_2Cl_2, where the second fraction was collected and evaporated to leave **2** as viscous oil. Yield: 83%. ^{1}H NMR ($CDCl_3$, ppm): δ = 6.22 (1H, $CH_3C_9H_{18}CH_2CH_2$-$CS_3C(CH_3)_2CO$-N*H*-) 3.3 (2H, $CH_3C_9H_{18}CH_2CH_2$--$CS_3C(CH_3)_2CO$-), 2.06 (3H, H-3 for 1-amantadine), 1.95 (6H, H-2 for 1-amantadine), 1.5-1.8 (14H, 6H of H-4 for 1-amantadine, 6H for $CH_3C_9H_{18}CH_2CH_2$-$CS_3C(CH_3)_2CO$-) and 2H for $CH_3C_9H_{18}CH_2CH_2$--$CS_3C(CH_3)_2CO$-), 1.2-1.5 (18H, $CH_3C_9H_{18}CH_2CH_2$-$CS_3C(CH_3)_2CO$-), 0.88 (3H, $CH_3C_9H_{18}CH_2CH_2$-$CS_3C(CH_3)_2CO$-). FTIR (KBr, ν_{max}): 3395 cm^{-1} for N-H of –CO-NH-), 1681 cm^{-1} for C=O of –CO-NH-).

2.3 Synthesis of β-CD-P4VP$_{30}$ trithiocarbonate (3)

A solution of 4VP (4.05 g, 38.5 mmol), AIBN (19.8 mg, 0.12 mmol) and **1** (0.75 g, 0.48 mmol) in DMF (10.3 mL) was degassed by freeze-pump-thaw cycles three times, and then stirred at 70 ^{o}C for 30 h under N_2. The resulting mixture was precipitated in diethyl ether and the precipitate was dried under vacuum to give **3**. **3** has an M_n of 4800 and polydispersity, M_w/M_n, of 1.17 measured by GPC. On basis of the ^{1}H

NMR spectrum (Figure S1), the degree of polymerization was calculated to be 30, thus **3** was denoted as β-CD-P4VP$_{30}$ trithiocarbonate.

2.4 Synthesis of β-CD-P4VP$_{30}$

A solution of **3** (0.89 g, 0.2 mmol) in DMF (10 mL) was deoxygenated by freeze-pump-thaw cycles three times, then several drops of $Na_2S_2O_4$ aqueous solution (0.5 M) and hexylamine (2.0 g, 19.8 mmol) were added under N_2. The reaction mixture was stirred for 24 h at room temperature, then it was filtered and the filtrate was precipitated in diethyl ether and the precipitate was dried under vacuum to give **β-CD-P4VP$_{30}$**.

2.5 Synthesis of AD-PNIPAM$_{70}$ trithiocarbonate (4)

A solution of NIPAM (4.5 g, 40 mmol), AIBN (16.42 mg, 0.1 mmol) and **2** (0.25 g, 0.5 mmol) in DMF (10.8 mL) was degassed by freeze-pump-thaw cycles three times, and then stirred at 70 ^{o}C for 40 h under N_2. The resulting mixture was precipitated in hexane and the precipitate was dried under vacuum to give **4. 4** has an M_n of 5700 and polydispersity, M_w/M_n, of 1.10 measured by GPC. The degree of polymerization was calculated to be 70 from ^{1}H NMR spectrum (Figure S2), thus **4** was denoted as AD-PNIPAM$_{70}$ trithiocarbonate.

2.6 Synthesis of AD-PNIPAM$_{70}$

A solution of **4** (0.82 g, 0.1 mmol) in DMF (8 mL) was deoxygenated by

freeze-pump-thaw cycles three times, then several drops of $Na_2S_2O_4$ aqueous solution (0.5 M) and hexylamine (1.0 g, 9.9 mmol) were added under N_2. The reaction mixture was stirred for 24 h at room temperature, then it was filtered and the filtrate was precipitated in diethyl ether and the precipitate was dried under vacuum to give **AD-PNIPAM$_{70}$**.

2.7 Construction of noncolvalent DHBC, β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex, in aqueous solution

Noncolvalent DHBC, β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex, was obtained by mixing β-CD-P4VP$_{30}$ with equimolar amounts of AD-PNIPAM$_{70}$ in aqueous solution at pH 2.5 and 25 ^{o}C. ^{1}H NOESY spectrum was presented in Figure S3 to confirm the inclusion complexation between β-CD and AD groups.

3. Measurements

^{1}H NMR spectra of **1** and **3** in DMSO-d_6, **2** and **4** in $CDCl_3$ were recorded on a Varian UNITY plus-400 spectrometer (400MHz). Molecular weight and molecular weight distribution of **3** and **4** were assessed by a Waters 600E gel permeation chromatography (GPC) analysis system based on polystyrene standards, DMF and THF were used as eluent, respectively.

^{1}H NOESY spectrum of a mixed solution containing equimolar amounts of β-CD-P4VP$_{30}$ and AD-PNIPAM$_{70}$ in D_2O were recorded on a Varian Mercury VX300 spectrometer (300MHz).

Both pH- and thermo-responsive micellization were monitored on a VARIAN Cary Eclipse Fluorescence Spectrophotometer. The width of slits was set at 2.5 nm. The excitation wavelength was set at 335 nm, and the emission spectra were recorded in the range of 360-500 nm. Calculated volumes of pyrene solution in acetone were added into a series of little bottles. Acetone was removed by drying with a blower. Then an aqueous solution of β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex at a concentration of 0.2 mg/mL was added into the bottles, and the concentration of pyrene was fixed at 5×10^{-7} M. The pH value of the solution was adjusted by HCl solution. Samples were kept overnight before fluorescence measurements. β-CD-P4VP$_{30}$ was served as control for the investigation of the pH-responsive micellization.

Dynamic Light Scattering (DLS) at a fixed scattering angle of 90° and Static Light Scattering (SLS) at the scattering angles ranging from 40 to 140° for the micellar aqueous solution at a concentration of 0.2 mg/mL were performed by a light scattering spectrometer (BI-200SM).

Transmission electron microscopy (TEM) was conducted on a Philips T20ST electron microscopy at an acceleration voltage of 200 kV. The specimens for TEM observation were prepared by dropping a small drop of the micellar aqueous solution at a concentration of 0.2 mg/mL on a preheated carbon-coated copper grid at given temperature, air-dried at the same temperature and stained with OsO_4 for 30min.

Laser scanning confocal microscopy (LSCM) was conducted on a Leica TCS SP5 laser scanning confocal microscope. Water-soluble fluorescein sodium was used as

fluorescent probe and the excitation wavelength was set at 488 nm. At pH 2.5 and 25 ^{o}C, β-CD-P4VP$_{30}$ was mixed with equimolar amounts of AD-PNIPAM$_{70}$ in aqueous solution containing 5×10^{-6} mol/L fluorescein sodium. The pH value of the solution was adjusted to approximate 4.8 by dropping NaOH solution, leading to the formation of the micelles of β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex. Fluorescein sodium not encapsulated in the micelles was removed by dialysis (Mw cutoff 500) in a buffer solution at pH 4.8 for 2 days. Then the sample for LSCM observation was prepared by adding a drop of micellar solution on a piece of microscope slide.

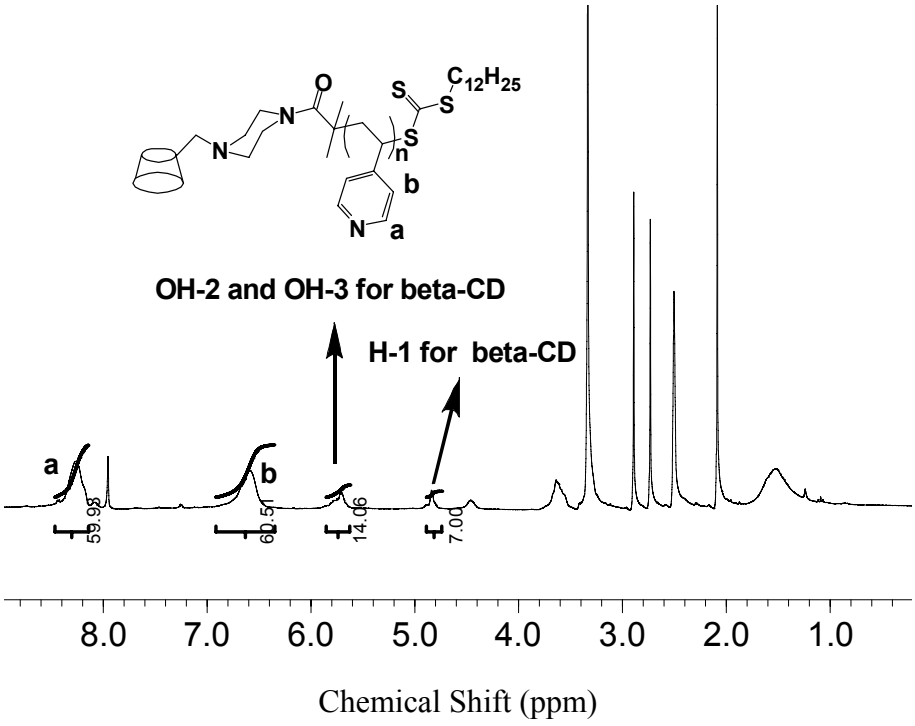


Figure S1. ^{1}H NMR spectrum of β-CD-P4VP$_{30}$ trithiocarbonate in DMSO-d$_6$.

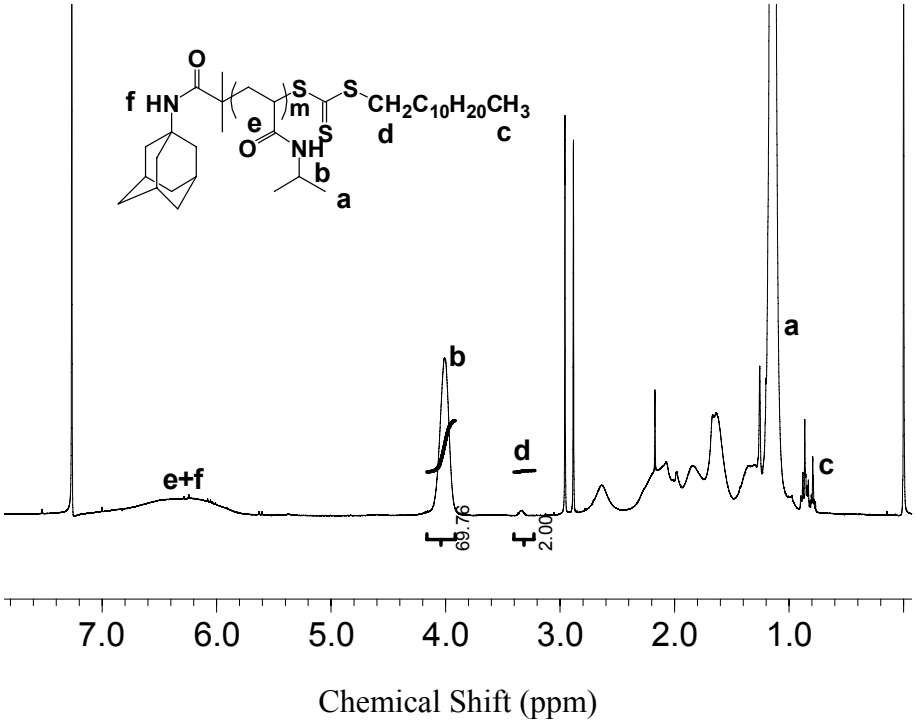


Figure S2. ^{1}H NMR spectrum of AD-PNIPAM$_{70}$ trithiocarbonate in $CDCl_3$.

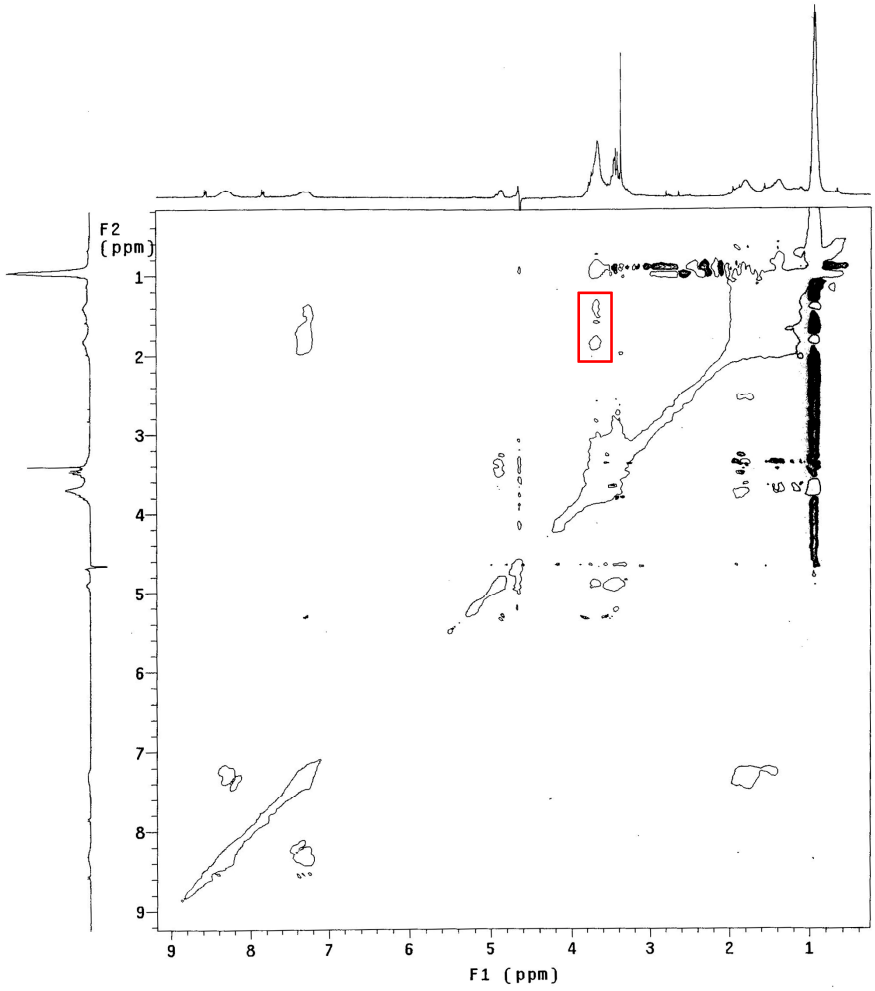


Figure S3. ^{1}H NOESY spectrum of a mixed solution of equivalent β-CD-P4VP$_{30}$ and AD-PNIPAM$_{70}$ in D_2O at pH 2.5 and 25 ^{o}C

(The NOE cross-peaks between the signals of the interior protons of β-CD and signals of AD is marked in the red square)

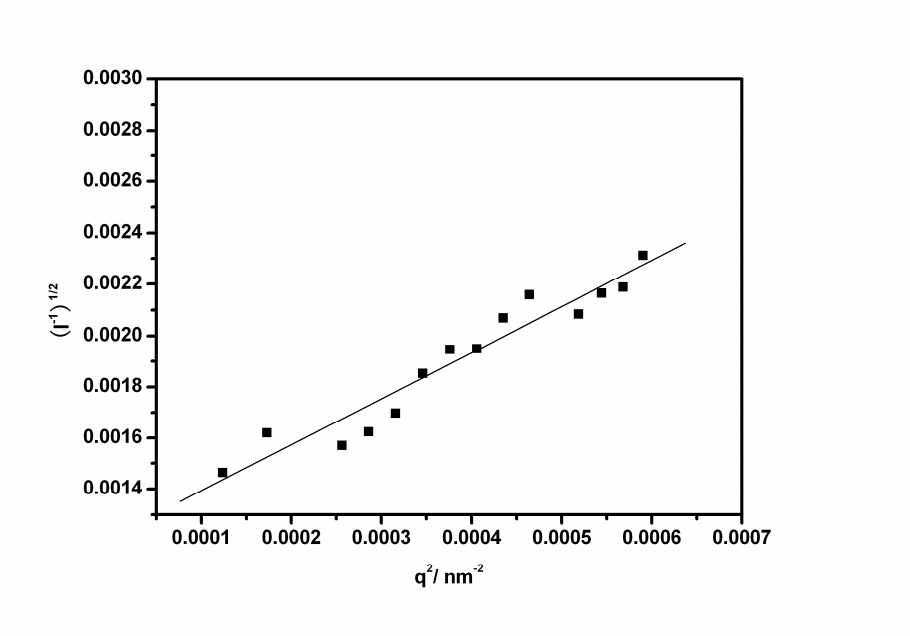


Figure S4. Berry plot of the pH-responsive micelles of the β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex in 0.2 mg/mL aqueous solution at pH 4.8 and 25 ^{o}C.

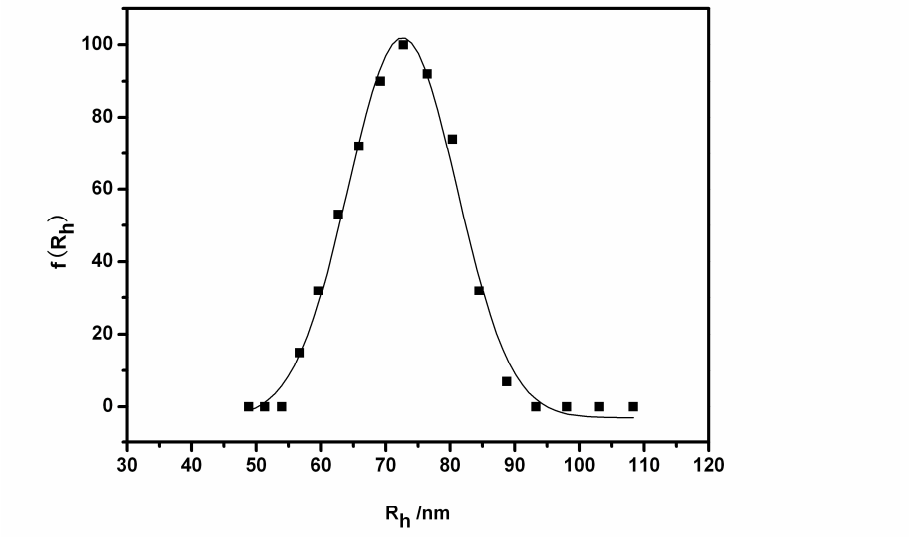


Figure S5. The hydrodynamic radius distribution of the pH-responsive micelles of the β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex in 0.2 mg/mL aqueous solution at pH 4.8 and 25 ^{o}C.

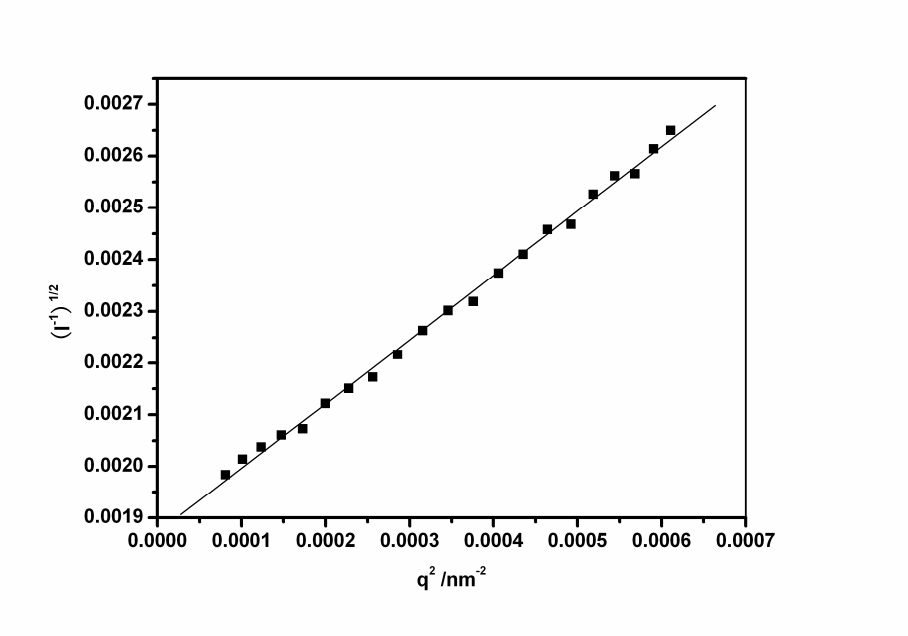


Figure S6. Berry plot of the thermo-responsive micelles of the β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex in 0.2 mg/mL aqueous solution at pH 2.5 and 60 ^{o}C.

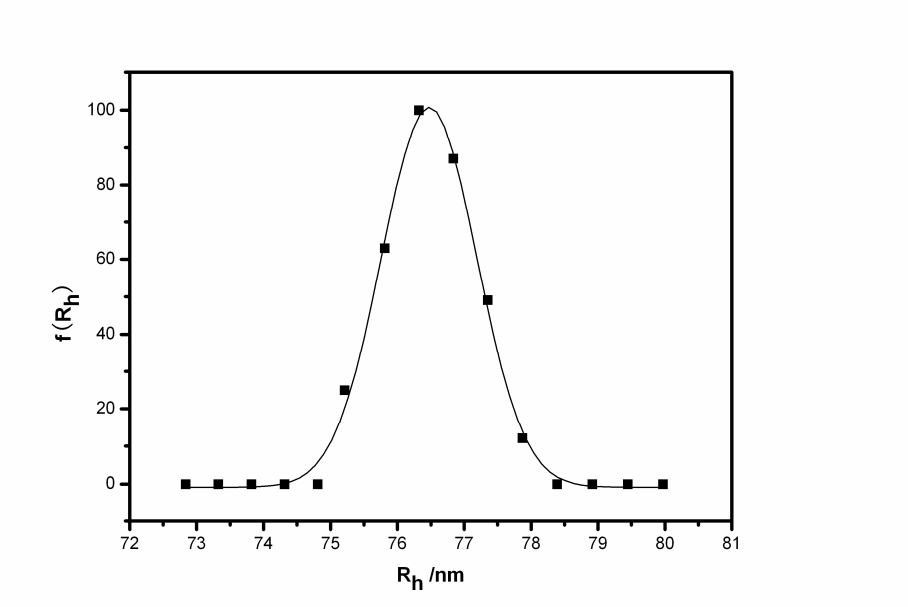


Figure S7. The hydrodynamic radius distribution of the thermo-responsive micelles of the β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex in 0.2 mg/mL aqueous solution at pH 2.5 and 60 ^{o}C.

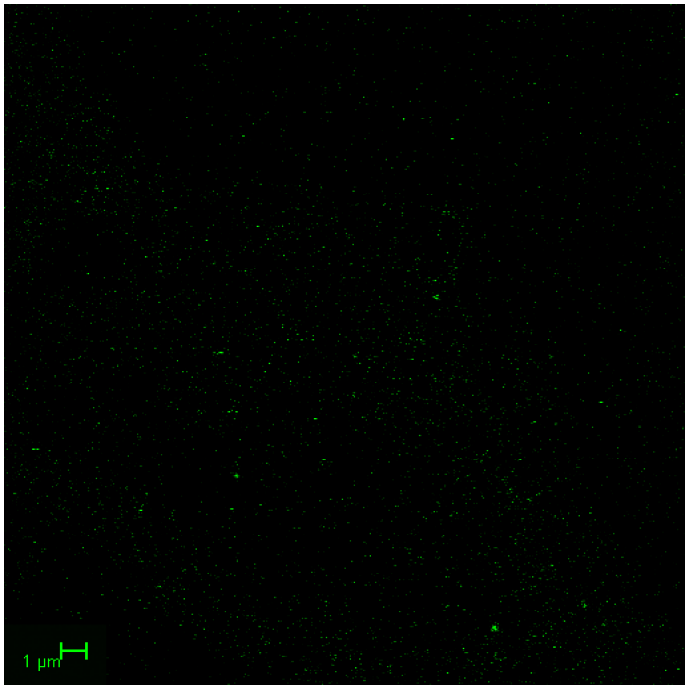


Figure S8. LSCM image of the micelles of β-CD-P4VP$_{30}$/AD-PNIPAM$_{70}$ inclusion complex formed at pH 4.8 and 25 ^{o}C (0.2 mg/mL, water-soluble fluorescein sodium as fluorescent probe)