Supplementary Information

Dopamine sensor development based on the modification of glassy carbon electrode with β-cyclodextrin-poly(N-isopropylacrylamide)

1. Experimental details

1.1 Materials and measurements

β-cyclodextrin, NaN₃, Al₂O₃, N, N'-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co. Ltd. 1-(p- toluenesulfonyl)imidazole and Triphenylphosphine were obtained from Shang Hai Jing Chun Reagent Co. Ltd. N-Isopropylacrylamide (97%, Aldrich) was recrystallized twice from benzene/hexane (10:1, v/v) prior to use. CuBr (Aldrich) was dissolved in glacial acetic acid with stirring, filtered and washed with anhydrous ethanol, anhydrous ether at room temperature, respectively, and then dried under vacuum for 24 h prior to use. Tris(2-(dimethylamino)ethyl)amine was obtained from Aldrich. Pyridine (99%) and tetrahydrofuran (THF, 99%) were distilled from calcium hydride immediately prior to use, respectively; All other chemical reagents used in this study were of analytical grade and used without further purification.

Fourier transform infrared (FTIR) spectra were collected using a Shimadzu 8400S FTIR spectrometer. The samples were ground with KBr and then pressed into disks. ¹H spectra were recorded in CDCl₃ or DMSO-d₆ at 25 °C on a Varian Unity 400 (¹H: 400 MHz). The number average molecular weight (Mₙ) and molecular weight distributions (Mₙ/Mₚ) of the polymers were measured by Waters 600 GPC system.

1.2 Synthesis of CD-PNIPAM polymer [1-5]

Synthesis of mono-6-(p-tolylsulfonyl)-β-cyclodextrin (CD-OTs)

β-cyclodextrin (20 g, 17.6 mmol) was suspended in 170 mL of water and heated to 60 °C under
vigorous agitation, then cooled to room temperature. 1-(p-toluenesulfonfyl)imidazole (Ts-Im, 15 g, 67 mmol) was added to the above solution. After 2 h of stirring at room temperature, 25 mL of 36% NaOH aqueous solution was added dropwise over 6 min, then unreacted Ts-Im was filtered off. NH₄Cl (24 g, 45 mmol) was added to the filtrate. The mixture was concentrated to about half of its original volume by blowing a stream of air over its surface overnight, the resulting precipitate was collected by suction filtration, the collected solid is washed with two 50 mL portions of ice water and one 100 mL portion of acetone and then dried to constant weight over calcium chloride in vacuum to yield 9 g (40%) of a white solid.

Synthesis of mono-6-azido-β-cyclodextrin (CD-N₃)

CD-OTs (7 g, 5.5 mmol) was suspended in water (60 mL), then NaN₃ (3.9 g, 0.06 mmol) was added. The mixture was stirred at 80 °C for 5 h, and then cooled to room temperature. The addition of acetone resulted in a white precipitate, which was filtered and dried overnight at 40 °C under vacuum, yielding a pure white solid (6.1 g). Yield: 95%.

Synthesis of mono-6-amino-β-cyclodextrin (CD- NH₂)

CD-N₃ (6 g, 5.2 mmol) was suspended in 100 mL of dry DMF. Triphenylphosphine (5.2 g, 20 mmol) was added, and after 1 h of stirring at room temperature, the solution was treated with 10 ml of concentrated NH₄OH. After 24 h of stirring, the reaction mixture was precipitated in 500 mL of acetone. The precipitate was separated by filtration, dried in vacuum. The obtained solid was dissolved with a small amount of water and precipitated in acetone, which was repeated three times, giving a white solid (5.3 g). Yield: 90%.

Synthesis of mono-6-(2'-bromine isobutyramide)-2,3,6-peracetyl-β-cyclodextrin (AcCDBr)

2-bromine isobutyric acid (0.88 g, 5.3 mmol) and 1-hydroxybenzotriazole (0.72 g, 5.3 mmol) were dissolved in 10 mL DMF under ice-water bath, then DCC (1.1 g, 5.3 mmol) was added. The mixture was stirred at 0 °C for 1 h, and then reacted for 1 h at room temperature. The produced
salts were filtered off. CD-NH₂ (5 g, 4.4 mmol), DMAP (0.064 g) and triethylamine (1 mL) were suspended in dry DMF and stirred at room temperature for 1 h, then added to the above obtained filtrate. The mixture was continuously stirred at room temperature for 25 h, and then the reaction mixture was filtered. After solvent was removed by reduced-pressure distillation, the obtained white solid was dried in vacuum.

The synthesized white solid and DMAP were dissolved in dry pyridine (60 mL) under ice-water bath, then 40 mL acetic anhydride was added. After three days of reaction, the reaction mixture was cooled to room temperature and added a certain amount of water, then extracted with chloroform. The residue was washed by 5 % HCl, 5 % NaHCO₃ and H₂O, respectively, and then dried by anhydrous MgSO₄. The obtained crude product was purified by column chromatography (SiO₂, n-hexane / ethyl acetate, 1:9 v/v), yielding a white solid. Yield: 72 %.

Synthesis of CD-PNIPAM

NIPAM (1.13 g, 10 mmol), CuBr (28.7 mg, 0.2 mmol), DMF (4 mL) and Me₆TREN (46.1 mg, 0.2 mmol) were deoxygenated by bubbling with nitrogen and combined. After three freeze-pump-thaw cycles were performed, the initiator AcCDBr (0.42 g, 0.2 mmol) dissolved in the mixed solution of DMF (2 mL) and double distilled water (0.5 mL), was added to begin polymerization. The reaction was carried out at 60 °C for a certain time under a nitrogen atmosphere. Polymerization was terminated by being exposed to air. The reaction mixture was diluted with DMF and passed through an Al₂O₃ column to remove the copper complex. The resulting polymer was purified by dialysis using a cellophane tube (MWCO, 3000) in DMF. After removing the solvents, the residue was dried in vacuum for 24 h to give a white AcCD-PNIPAM polymer.

AcCD-PNIPAM (0.2 g, Mₙ = 4360) was dissolved in dry THF (10 mL), then 5 drops of 28 wt % sodium methoxide in methanol was added under cooling condition. After 3 days of stirring at room temperature, the mixture was purified by dialysis using a cellophane tube (MWCO, 3000) in double distilled water, lyophilized to yield CD-PNIPAM (Mₙ = 3540) polymer.
1.3 Characterization of CD-PNIPAM polymer

Fourier transform infrared (FTIR) and $^1$H NMR measurements were carried to characterize the CD-PNIPAM polymer. Fig. 2 shows FT-IR spectra of AcCDBr (a), AcCD-PNIPAM (b) and CD-PNIPAM (c). As shown in Fig. 2c, the absorption peaks of CD-PNIPAM obviously enhanced at 3434 cm$^{-1}$ attributing to the characteristic adsorption of –OH in the structure of the deacetylated β-CD. In addition, its structure was further confirmed by the disappearance of the absorption peak of the C=O at 1747 cm$^{-1}$ and by the presence of strong bands at 1654 cm$^{-1}$ corresponding to the stretching vibration of secondary amide. These experiment results suggested that the acetyl group existing in polymer structure has been stripped, and the synthesized polymer still contains the structure of β-CD and repeating units of NIPAM. In order to further verify the structure of the CD-PNIPAM polymer, $^1$H NMR spectroscope measurements were employed for studying the structure of the polymer. Fig. 3 displays $^1$H NMR spectra of AcCDBr in CDCl$_3$ (a) and CD-PNIPAM in DMSO-$d_6$ (b). For the $^1$H NMR spectrum of CD-PNIPAM (Figure 3b), the signals appeared at 5.24-3.39 ppm should be attributed to the peaks of –H in the structure of β-CD,
and at 8.50, 1.24, 6.55, 2.93, 1.60 and 1.02 ppm attributed to the peaks of PNIPAM. Fig. 4 displays the GPC traces of AcCD-PNIPAM ($M_n=4360$, PDI=1.16) and CD-PNIPAM ($M_n=3540$, PDI=1.15). It can be seen from Fig. 4 that GPC traces were relatively symmetric and showed no tailing at either side, suggesting the absence of any residual small molecule in the synthesized polymer. Compared to that of AcCD-PNIPAM, the elution peak of CD-PNIPAM is shifted slightly to lower $M_w$ side, and both traces were monomodal and quite symmetric. These results confirmed that deacetylation of the polymer AcCD-PNIPAM was completed successfully.

Fig. S1. FT-IR spectra of AcCDBr (a), AcCD-PNIPAM (b) and CD-PNIPAM (c)
Fig. S2. $^1$H NMR spectra of AcCDBr in CDCl$_3$ (a) and CD-PNIPAM in DMSO-$d_6$ (b)

Fig. S3. GPC traces of AcCD-PNIPAM and CD-PNIPAM obtained though ATRP

2. Supplementary data
Fig. S4. Cyclic voltammograms of a bare GCE in 20ml phosphate buffer solution (pH 7.0) containing 0.048 g LiClO₄ and 50 mg CD-PNIPAM polymer by scanning between 0 and 1.0 V at a rate of 20 mV s⁻¹ for 10 cycles (a)-(j).

Fig. S5. (A) Cyclic voltammograms of 20 μM dopamine in phosphate buffer solution (pH=5): (a) bare GCE; (b) the activated electrode; From (c) to (g) the CD-PNIPAM modified GCE with 5, 7, 10, 15 and 20 cyclic potential sweeps. (B) Effect of the CD-PNIPAM polymer film modified GCE with different potential cycles on the anodic peak current for the oxidation of DA.
Fig. S6. (A) Cyclic voltammograms of the CD-PNIPAM film modified GCE with different scan rates in 5 mM Fe(CN)$_6^{3-/4-}$ containing 1 M KCl solution. (B) The relationship between the peak current and scan rate.

Fig. S7. Cyclic voltammograms of a bare GCE (a), the activated GCE (b), the CD-PNIPAM modified GCE (c) in phosphate buffer solution (pH=5.0) containing 20 μM DA and the CD-PNIPAM modified GCE in blank phosphate buffer solution (d). Scan rate: 50mV s$^{-1}$
Fig. S8. Cyclic voltammograms of 20 μM DA at the CD-PNIPAM modified GCE at different pH. From (a) to (f) pH = 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0.

Fig. S9. (A) Effect of pH on the peak current for the oxidation of 20 μM DA. (B) The linear relationship between the peak potential and pH. Scan rate: 50 mV s⁻¹.
Fig. S10. The long-term stability of CD-PNIPAM modified GCE by cyclic voltammograms scanning in PBS containing 20 μM DA with pH=5.0, curve (a) for one month before and curve (b) for one month later.

Fig. S11. The stability of the CD-PNIPAM modified GCE by cyclic voltammograms scanning in PBS containing 20 μM DA with pH=5.0 (A) for 100 cycles; (B) The first cycle (a) and the hundredth cycle (b) of (A). Scan rate: 50 mV·s⁻¹

Table S1 Results of determination of DA in dopamine hydrochloride injections (n = 10)

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Table S2 Results of determination of DA in human urine samples (n = 10)

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3. References


