Electronic supplementary information (ESI)

Well-defined biological sample-compatible molecularly imprinted polymer microspheres by combining RAFT polymerization and thiol-epoxy coupling chemistry

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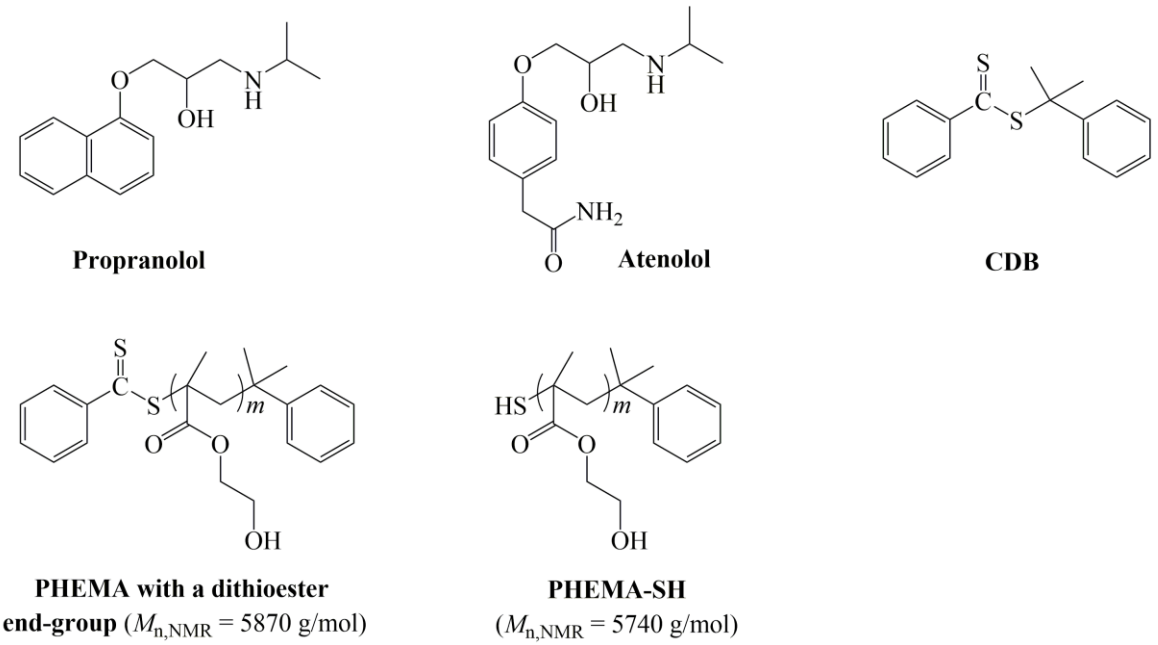

Scheme S1. Chemical structures of some reagents used in the study.

Synthesis of a poly(HEMA) (or PHEMA) with a dithioester end-group

A PHEMA with a dithioester end-group was synthesized via the RAFT polymerization of HEMA following our previously reported method as shown below: HEMA (0.91 mL, 7.50 mmol), AIBN (2.57 mg, 15.65 μmol), CDB (34.02 mg, 0.12 mmol), and methanol (7.5 mL)

were added into a one-neck round-bottom flask (25 mL) successively. A clear purple solution was obtained after 5 min of stirring at room temperature, which was then purged with argon for 30 min in an ice-water bath, sealed, immersed into a thermostatted oil bath at 60 °C, and magnetically stirred for 48 h. The resulting reaction mixture was diluted with methanol (2.5 mL) and precipitated into ethyl ether twice to obtain a light pink polymer, which was dried at room temperature under vacuum to the constant weight to provide the product in a yield of 68 % ($M_{n,NMR}$ = 5870 g/mol).

Synthesis of the thiol-terminated PHEMA (i.e., PHEMA-SH)

The above-obtained PHEMA with a dithioester end-group (100.03 mg) was dissolved in ethanol (5 mL). After the resulting solution was purged with argon for 10 min, *n*-butylamine (12 μL, 0.12 mmol) and tributylphosphine (15 μL, 0.06 mmol) were added into the reaction system. After 3 h of magnetic stirring of the above reaction mixture (200 rpm) at 25 °C for 3 h, the light pink color of the polymer solution disappeared. The resulted reaction mixture was precipitated into ethyl ether twice and then dried at 40 °C under vacuum to the constant weight, leading to the white PHEMA-SH in a yield of 80% ($M_{n,NMR}$ = 5740 g/mol).

The number-average molecular weights of both PHEMA with a dithioester end-group and PHEMA-SH were derived from their ^{1}H NMR spectra (Fig. S1a,b) by using the equation:

$$M_{n,NMR} = x \times [(S_c + S_d)/S_h] \times M_{HEMA} + M_{end\text{-}groups}$$

Where S_c, S_d, and S_h refer to the integral of the peak *c* around 3.90 ppm, that of the peak *d* around 3.60 ppm, and that of the peaks *h* between 7 and 8 ppm, M_{HEMA} is the molar mass of HEMA, $M_{end\text{-}groups}$ is the total molar mass of the end-groups of the resulting polymers (e.g., for PHEMA with a dithioester end-group, $M_{end\text{-}groups} = M_{CDB}$), and *x* is 2.5 and 1.25 for PHEMA with a dithioester end-group and PHEMA-SH, respectively.

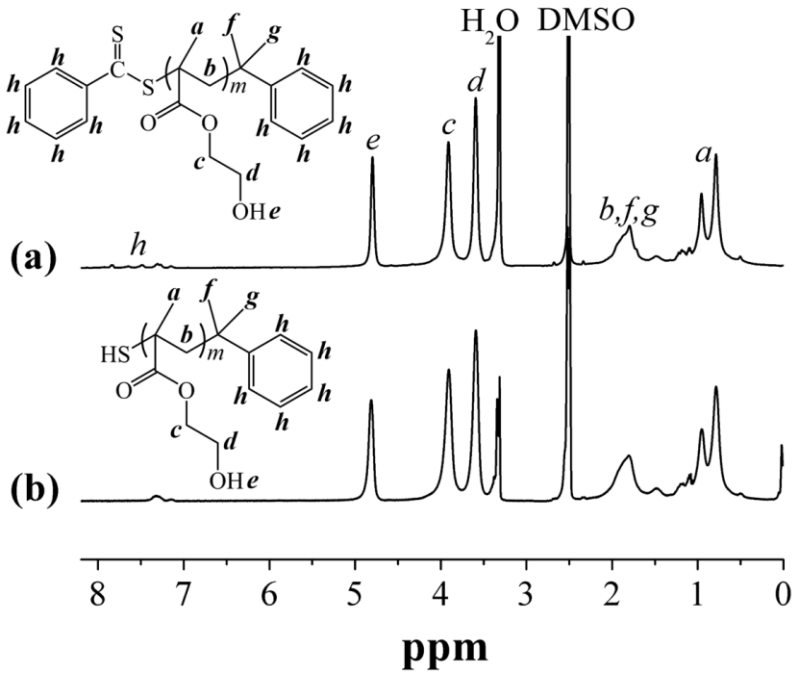

Fig. S1. ^{1}H NMR spectra of PHEMA with a dithioester end-group (a) and PHEMA-SH (b) (DMSO-d6).

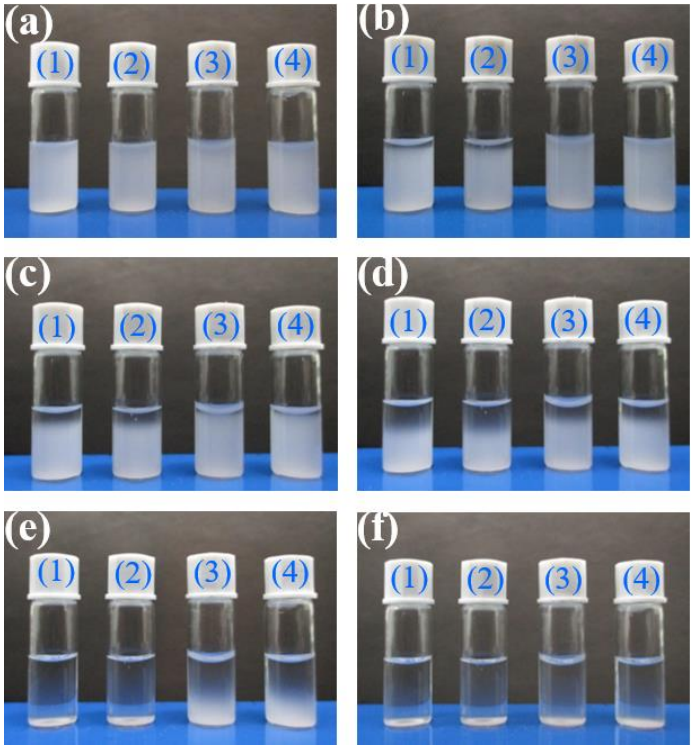

Fig. S2 Dispersion photographs of MIP-PHEMA/CP-PHEMA obtained from two repetition experiments (i.e., MIP-PHEMA (1)/CP-PHEMA (3) from the first experiment; MIP-PHEMA (2)/CP-PHEMA (4) from the second experiment) in pure water (1 mg/mL) at 20 ^{o}C after settling down for 0 h (a), 1.5 h (b), 2.5 h (c), 4 h (d), 6 h (e), and 10 h (f), respectively (Note that CP-PHEMA particles showed somewhat higher aqueous dispersion stability than MIP-PHEMA particles because of their relatively smaller sizes).

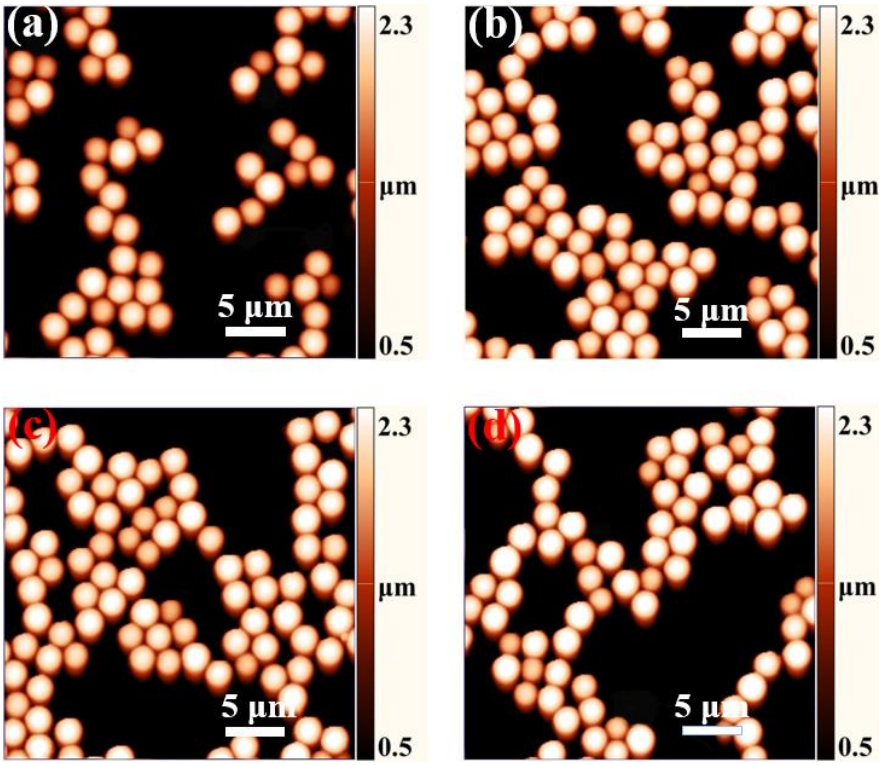

Fig. S3 AFM height images of CP (a), CP-EP (b), CP-OH (c), and CP-PHEMA (d) (samples a, b, c, and d correspond to entries 2, 4, 6, and 8 in Table 1, respectively).

Table S1 Particle sizes and size distribution indices of the unmodified CP and its surface-modified ones determined by using AFM

Entry	Polymer particles	D_n (μm) [a]	U [a]
1	CP	2.115	1.017
2	CP-EP	2.127	1.013
3	CP-OH	2.130	1.009
4	CP-PHEMA	2.136	1.013

[a] D_n and U refer to the number-average diameter and size distribution index of CPs, respectively, as derived from their AFM height images.

Fluorescent labelling of MIP-EP microspheres

The fluorescent labeling of MIP-EP microspheres was achieved by the reaction between the epoxy groups on MIP-EP microspheres and the amino group of the fluorescent dye

7-amino-4-trifluoromethylcoumarin (AFC) as shown below: MIP-EP microspheres (2.0 mg) were added into a solution of AFC (0.5 mg, 0.0022 mmol) in ethanol (0.5 mL). The above mixture was first ultrasonically dispersed, purged with argon for 5 min, sealed, and then magnetically stirred (180 rpm) at 60 ^{o}C for 24 h. After centrifugation, the resulting solid product were thoroughly washed with ethanol to remove the excessive AFC until no fluorescence was detectable for the centrifuged supernatant, which was then dried at 40 ^{o}C under vacuum overnight, leading to the fluorescently labeled product quantitatively.

The unmodified MIP microspheres were also submitted to the same labeling condition as a control experiment.

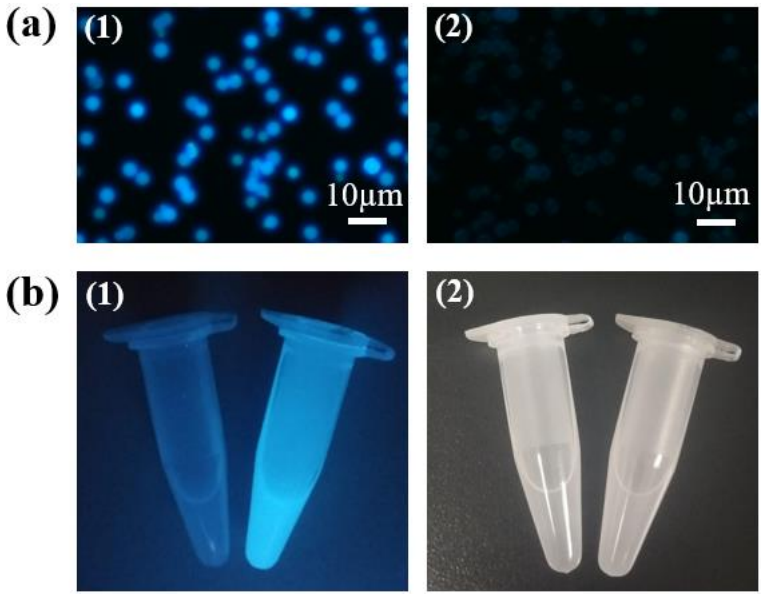

Fig. S4 (a) Fluorescence microscopy images of MIP-EP (1) and MIP (2) microspheres after their treatment with AFC (obtained by using a Nikon Eclipse Ts2-FL inverted fluorescent microscope equipped with a Nikon DS-Fi3 microscope camera and a 385 nm LED lamp); (b) Photographs of the dispersed solutions (in ethanol, polymer concentration: 2 mg/mL) of MIP-EP (in the right tube in each image) and MIP (in the left tube in each image) microspheres after their treatment with AFC, which were taken under the irradiation of 365 nm UV light (1) and normal light (2), respectively.

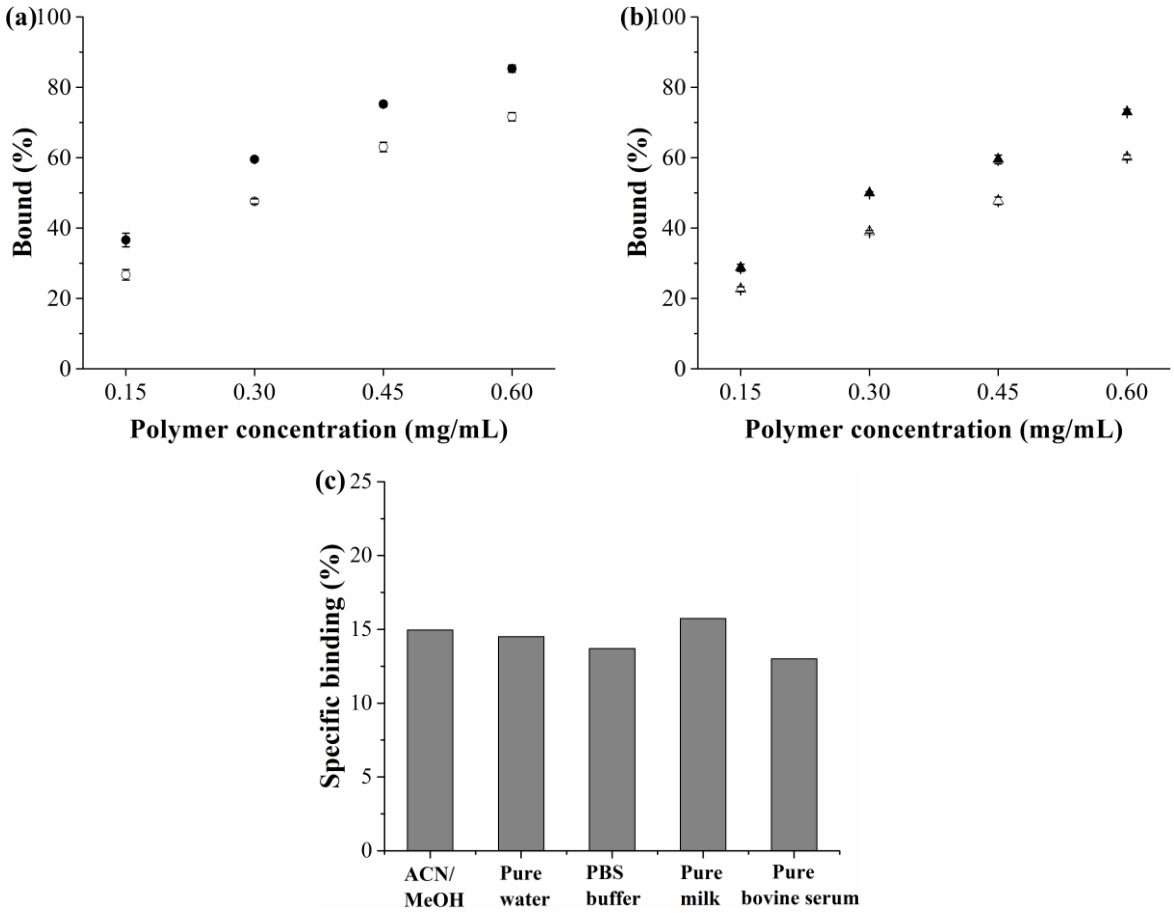

Fig. S5 (a,b) Equilibrium template bindings of MIP-PHEMA (filled symbols)/CP-PHEMA (open symbols) in propranolol solutions (0.1 mM) in PBS buffer (a) or the undiluted pure bovine serum (b); (c) Specific template bindings of MIP-PHEMA in propranolol solutions (0.1 mM) in different media at 25 ºC (polymer concentration: 0.6 mg/mL).

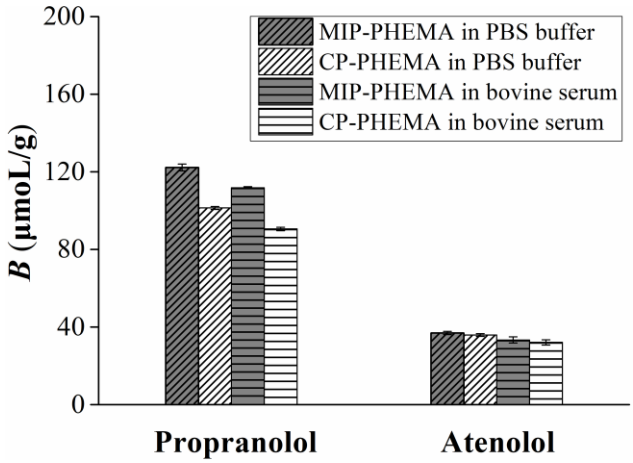

Fig. S6 Selective bindings of MIP-PHEMA/CP-PHEMA toward propranolol and atenolol in their mixed solution ($C_{\text{propranolol or atenolol}}$ = 0.1 mM) in PBS buffer or the undiluted pure bovine serum (polymer concentration: 0.6 mg/mL).

Table S2 Selective bindings of MIP-PHEMA/CP-PHEMA toward propranolol and atenolol in their mixed solutions in different media

Solvent	Analyte	B_{MIP}[a]	B_{CP}[a]	IPB (%)[b]
PBS buffer	Propranolol	122.3	101.4	20.6
	Atenolol	37.0	35.9	3.1
Undiluted pure bovine serum	Propranolol	111.8	90.6	23.4
	Atenolol	33.4	32.1	4.1

[a] B_{MIP} and B_{CP} refer to the equilibrium binding capacity of the studied MIP-PHEMA and CP-PHEMA toward propranolol and atenolol in their mixed solutions in different media ($C_{propranolol\ or\ atenolol}$ = 0.1 mM), respectively (polymer concentration: 0.6 mg/mL), which have a unit of μmol/g and are the same as those shown in Fig. S6. [b] IPB refers to the "imprinting-induced promotion of binding" value of the studied MIP-PHEMA.