Electronic Supplementary Information

Efficient Synthesis of Narrowly Dispersed Molecularly Imprinted Polymer Microspheres with Multiple Stimuli-Responsive Template Binding Properties in Aqueous Media

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Materials

4-Vinylpyridine (4-VP, Alfa Aesar, 96%), ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, Tianjin heowns Biochemical Technology Co., Ltd., China, 97%), methacrylic acid (MAA, Tianjin Jiangtian Chemicals, China, 99%), and *N,N*-dimethylformamide (DMF, Tianjin Jiangtian Chemicals, Analytical grade (AR)) were purified by distillation under vacuum. Methanol (Tianjin Jiangtian Chemicals, AR) was distilled prior to use. Tetrahydrofuran (THF, Tianjin Jiangtian Chemicals, 99%) was refluxed over sodium and then distilled. Acetonitrile (Tianjin Concord Chemicals, China, AR) was refluxed over calcium hydride (CaH$_2$) and then distilled. *N*-Isopropylacrylamide (NIPAAm, Acros, 99%) was recrystallized from hexane. Azobisisobutyronitrile (AIBN, Chemical Plant of Nankai University, AR) was recrystallized from ethanol. Cumyl dithiobenzoate (CDB) was prepared according to a literature procedure (T. P. Le, G. Moad, E. Rizzardo and S. H. Thang, *PCT Int. Appl. WO* 98/01478; *Chem. Abstr.* 1998, **128**, 115390). Methacrylic chloride was prepared by the reaction between methacrylic acid and the freshly distilled thionyl chloride (Tianjin Jiangtian Chemicals, 99%) following a literature procedure (Y. Feng, J. Huang and C. Li, *Chem. World* 1997, **38**, 258-260). Methacrylic anhydride was prepared by reacting methacrylic acid with methacrylic chloride in the presence of an aqueous solution of sodium hydroxide following a literature procedure (R. A. Barabashina and O. M. Sleptsova, *Z. Prikl. Fiz.* 1967, **40**, 696). 4-((4-Methacryloyloxy)phenylazo)benzoic acid (MPABA) was prepared following our previously reported procedure (Scheme S1. X. Li, R. Wen, Y. Zhang, L. Zhu, B. Zhang and H. Zhang, *J. Mater. Chem.* 2009, **19**, 236-245). (±)-Propranolol hydrochloride (Alfa Aesar, 99%) was converted into its free base form (Scheme S2) before use following the previously reported procedure (L. Schweitz, P. Spégel and S. Nilsson, *Analyst* 2000, **125**, 1899-1901). Atenolol (National Institute for the Control of Pharmaceutical and Biological Products, China, Chemical reference substance, Scheme S2) was dried

at 105 °C for 3 h before use. 4-(Dimethylamino)pyridine (DMAP, Merck, AR), triethylamine (TEA, Tianjin Jiangtian Chemicals, 99%), and all the other reagents were commercially available and used as received.
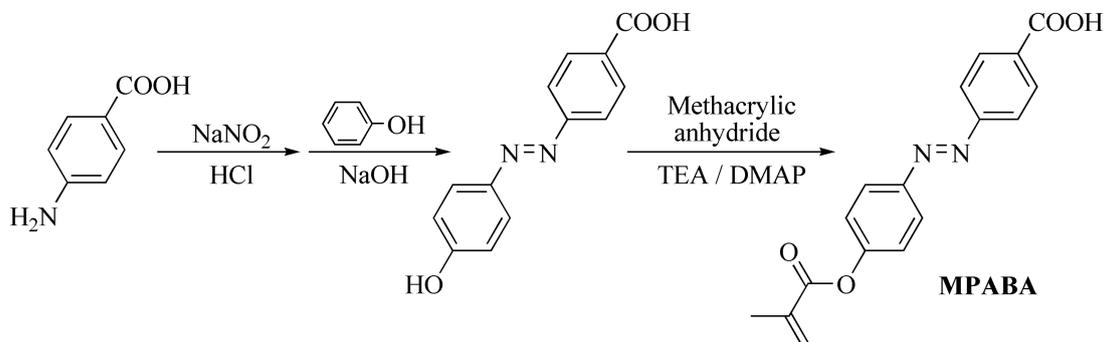
Scheme S1. Synthetic route for the azobenzene functional monomer 4-((4-methacryloyloxy)phenylazo)benzoic acid (MPABA).
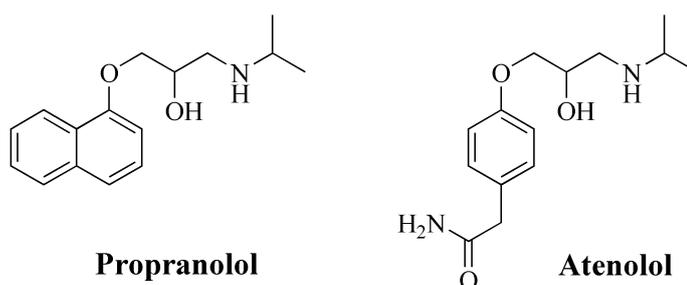
Scheme S2. The chemical structures of propranolol and atenolol.

Preparation of narrowly dispersed "living" core polymer microspheres with surface-immobilized dithioester groups via RAFT precipitation polymerization

4-VP (0.611 mmol), EGDMA (2.445 mmol), CDB (0.165 mmol), AIBN (0.055 mmol), and a mixture of methanol and water (4/1 v/v, 60 mL) were added into a one-neck round-bottom flask (100 mL) successively. A clear purple reaction mixture was obtained after 10 min of stirring at room temperature, which was then purged with argon for 30 min, sealed, and submerged in an oil bath. The temperature of the oil bath was heated from room temperature to 70 °C over 2 h and the polymerization was conducted at 70 °C for 24 h under stirring. The resulting polymer particles were collected by filtration and subsequently washed with methanol three times. After being dried at 40 °C under vacuum for 48 h, a light pink powder was obtained in a yield of 59%.

Preparation of propranolol-imprinted azo-containing core-shell-structured polymer (i.e., core-shell MIP (or abbreviated as CS-MIP)) microspheres by using surface imprinting approach via surface-initiated RAFT polymerization

Surface-initiated RAFT polymerization was utilized to prepare propranolol-imprinted azo-containing core-shell polymer microspheres by using the above-obtained narrowly dispersed "living" core polymer microspheres with surface-immobilized dithioester groups as the immobilized RAFT agent, AIBN as the initiator, propranolol as the template, MPABA as the azo functional monomer, and EGDMA as the cross-linker according to the following procedure: MPABA (1.05 mmol), propranolol (1.05 mmol), and a mixture of THF and acetonitrile (1/2 v/v, 150 mL) were added into a one-neck round-bottom flask (250 mL). A clear solution was obtained after 30 min of stirring at room temperature, which was stored at 4 ^{o}C in the dark overnight to allow the formation of the MPABA-propranolol complex. The "living" core polymer microspheres with surface-immobilized dithioester groups (150 mg), EGDMA (4.72 mmol), and AIBN (0.01 mmol) were then added into the above solution. The obtained reaction mixture was degassed in an ultrasonic bath for 5 min, purged with argon for 10 min in an ice bath, and then sealed. The polymerization was carried out at 50 ^{o}C for 2 h and then at 60 ^{o}C for 20 h in an oil bath under stirring in the dark. The resulting polymer particles were collected by centrifugation and washed thoroughly with methanol/acetic acid (9/1 v/v) and methanol successively. After being dried at 40 ^{o}C under vacuum overnight, orange-yellow propranolol-imprinted core-shell polymer microspheres with an azo-containing MIP layer (i.e., CS-MIP microspheres) were obtained.

The corresponding core-shell-structured non-imprinted or control polymer (CP) (i.e., core-shell CP (or abbreviated as CS-CP)) microspheres (orange-yellow colour) were prepared and purified under the identical conditions except that the template was omitted.

It is worth mentioning here that a small amount of little particles were generated in the above polymerization systems during the surface imprinting processes, which could be totally removed by applying low-speed centrifugation (1000 rpm) for the resulting polymerization solutions.

Grafting poly(NIPAAm-*co*-DMAEMA) brushes onto the CS-MIP/CS-CP microspheres via surface-initiated RAFT polymerization

The propranolol-imprinted/non-imprinted core-shell polymer microspheres grafted with poly(NIPAAm-*co*-DMAEMA) brushes (i.e., the grafted CS-MIP/CS-CP microspheres) were prepared via surface-initiated RAFT copolymerization of NIPAAm and DMAEMA (DMAEMA/NIPAAm =

5/100, molar ratio) by using the above-obtained (ungrafted) CS-MIP/CS-CP microspheres as the immobilized RAFT agent according to the following procedure: The CS-MIP/CS-CP microspheres (50 mg), NIPAAm (13.27 mmol), DMAEMA (0.66 mmol), CDB (0.01 mmol), AIBN (0.003 mmol), and DMF (2.5 mL) were added into a two-neck round-bottom flask (25 mL) successively. After being degassed with five freeze-pump-thaw cycles, the flask was sealed and then immersed in a thermostatted oil bath at 70 ^{o}C and stirred for 24 h. The resulting polymer products were collected by centrifugation and thoroughly washed with methanol until no white sediment was detectable when ether was added into the washing solutions, which were then dried at 30 ^{o}C under vacuum to the constant weights, leading to orange-yellow grafted CS-MIP and CS-CP microspheres with a weight increase of 11.3 and 11.5% in comparison with the corresponding ungrafted ones, respectively.

It is important to stress here that the increased weights of the grafted CS-MIP/CS-CP particles should be mainly stemmed from the surface-grafted polymer brushes because the rather high cross-linking densities (around 80%) of the CS-MIP/CS-CP particles would prevent them from swelling in the reaction media and only allow the occurrence of surface polymerization, just as reported by Tirelli and coworkers (D. Bontempo, N. Tirelli, K. Feldman, G. Masci, V. Crescenzi and J. A. Hubbell, *Adv. Mater.* 2002, **14**, 1239-1241) and by our group (J. Jiang, Y. Zhang, X. Guo and H. Zhang, *Macromolecules* 2011, **44**, 5893-5904; G. Pan, Y. Ma, Y. Zhang, X. Guo, C. Li and H. Zhang, *Soft Matter* 2011, **7**, 8428-8439).

The addition of CDB into the above polymerization systems also led to the generation of free poly(NIPAAm-*co*-DMAEMA) in the reaction solutions, which were obtained by precipitating the supernatant solutions (after the centrifugation of the reaction mixtures) into pentane, filtered, and then dried at 40 ^{o}C under vacuum for 48 h, finally resulting in light pink polymer products.

Characterization of the morphologies, particle sizes, and size distributions of the polymer microspheres

The morphologies, particle sizes, and size distributions of the above-obtained "living" core polymer microspheres, the ungrafted CS-MIP/CS-CP microspheres, and the grafted CS-MIP/CS-CP microspheres were characterized with a scanning electron microscope (SEM, Shimadzu SS-550) (Table S1). All of the SEM size data reflect the averages of about 200 particles, which are calculated by the following formulas:

$$D_n = \sum_{i=1}^{k} n_i D_i / \sum_{i=1}^{k} n_i; \qquad D_w = \sum_{i=1}^{k} n_i D_i^4 / \sum_{i=1}^{k} n_i D_i^3; \qquad U = D_w / D_n$$

where D_n is the number-average diameter, D_w the weight-average diameter, k the total number of the measured particles, D_i the particle diameter of the ith polymer microsphere, and n_i the number of the microspheres with a diameter D_i, U the size distribution index.

Table S1. Characterization data for the "living" core polymer microspheres as well as the ungrafted and grafted CS-MIP and CS-CP microspheres.

Entry	Sample name	Sample colour	D_n (μm) [a]	U [a]	L (nm) [b]	$M_{n,GPC}$ [c]	PDI [c]	Contact angle (°) [d]
1	Core polymer microspheres	Light pink	2.694	1.019	-	-	-	119.2±1.4
2	Ungrafted CS-MIP	Orange-yellow	3.281	1.012	294	-	-	123.1±1.5
3	Ungrafted CS-CP	Orange-yellow	3.223	1.012	265	-	-	121.8±1.7
4	Grafted CS-MIP	Orange-yellow	3.311	1.012	15	42000	1.16	67.4±1.7
5	Grafted CS-CP	Orange-yellow	3.255	1.011	16	42000	1.15	66.5±1.1

[a] D_n and U refer to the number-average diameter and size distribution index of the polymer microspheres; [b] L is the thickness of the azo-containing MIP/CP layer for the ungrafted CS-MIP/CS-CP microspheres or that of the polymer brush layer for the grafted CS-MIP/CS-CP, which is equal to $\Delta D_n/2$ (where ΔD_n is the increased diameter after each modification step); [c] The number-average molecular weights ($M_{n,GPC}$) and polydispersity indices (PDI) of the free poly(NIPAAm-*co*-DMAEMA) (generated in the polymerization solutions during the surface-initiated RAFT copolymerization of NIPAAm and DMAEMA due to the addition of sacrificial RAFT agent) determined by gel permeation chromatography (GPC) with a solution of LiBr in DMF (0.05 M) as the mobile phase and polystyrene as standards; [d] The static water contact angles of the polymer films.

Fourier Transform Infrared (FT-IR) analyses

Figure S1 shows the FT-IR spectra of the "living" core polymer microspheres, the (ungrafted) CS-MIP/CS-CP microspheres, and the grafted CS-MIP/CS-CP microspheres, which were obtained using a Nicolet Magna-560 FT-IR spectrometer. It can be seen clearly that in addition to the absorption peaks from the core poly(4-VP-*co*-EGDMA) microspheres such as those characteristic of the bonded EGDMA (i.e., 1722 (C=O stretching) and 1244/1145 cm^{-1} (C-O-C stretching)) and those corresponding to the C=N stretching (1590 and 1550 cm^{-1}) and C=C stretching (1448 cm^{-1}) from the bonded 4-VP, the CS-MIP and CS-CP microspheres showed the characteristic broad peak of the carboxylic acid group (around 3280 cm^{-1}, O-H stretching) from the bonded azo monomer MPABA, which suggested the successful grafting of the azo-containing MIP/CP layer onto the core polymer

microspheres after surface imprinting processes. Moreover, some new peaks characteristic of poly(NIPAAm) (i.e., the amide I band (around 1660 cm^{-1}, C=O stretching) and amide II band (1530 cm^{-1}, N-H stretching)) were also discernible in the FT-IR spectra of the grafted CS-MIP/CS-CP microspheres prepared by the surface-initiated RAFT copolymerization of NIPPAm and DMAEMA, demonstrating that hydrophilic polymer brushes have been efficiently grafted onto the CS-MIP and CS-CP microspheres after polymer brushes-grafting processes. Note that the absorption peaks corresponding to the bonded DMAEMA were not observed in the FT-IR spectra, which might be due to their overlap with the other peaks. Furthermore, all the above polymer microspheres proved to have the characteristic peaks of dithioester groups around 1040 cm^{-1} (i.e., C=S stretching) in their FT-IR spectra.
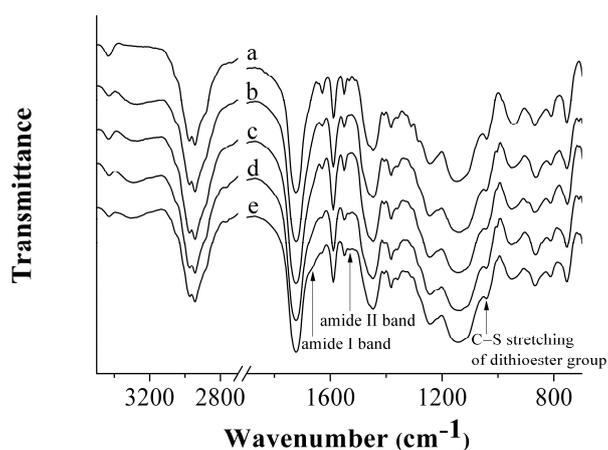
Figure S1. FT-IR spectra of the "living" core polymer microspheres (a), the ungrafted CS-MIP (b)/CS-CP (c) microspheres, and the grafted CS-MIP (d)/CS-CP (e) microspheres (The spectra are positioned in a row on a virtual third axes).

Measurement of the static water contact angles of the films prepared with the core polymer microspheres as well as the ungrafted and grafted CS-MIP/CS-CP microspheres

The films of the core polymer microspheres, the ungrafted CS-MIP/CS-CP microspheres, and the grafted CS-MIP/CS-CP microspheres were prepared by casting their suspension solutions in DMF (10 mg/mL, after ultrasonic dispersion) on clean glass surfaces. After the solvent was allowed to evaporate at ambient temperature under vacuum overnight, a KRÜSS FM40 Easy Drop contact angle equipment (Germany) was utilized to determine their static water contact angles. Two measurements were taken across each sample, with their average being used for analysis. All the obtained results are listed in Table S1.

Dispersion stability of the core polymer microspheres as well as the ungrafted and grafted CS-MIP/CS-CP microspheres in pure water

The dispersion properties of the core polymer microspheres, the ungrafted CS-MIP/CS-CP microspheres, and the grafted CS-MIP/CS-CP microspheres in pure water were studied. After their ultrasonic dispersion in pure water (1 mg/mL), the dispersed mixtures were allowed to settle down for different times at 25°C.

Figure S2 shows the typical photographs of the resultant solutions, from which it can be seen clearly that there was much faster sedimentation for the core polymer microspheres and the ungrafted CS-MIP/CS-CP microspheres in comparison with the grafted CS-MIP/CS-CP microspheres, confirming the presence of hydrophilic polymer brushes on the grafted CS-MIP/CS-CP microspheres.
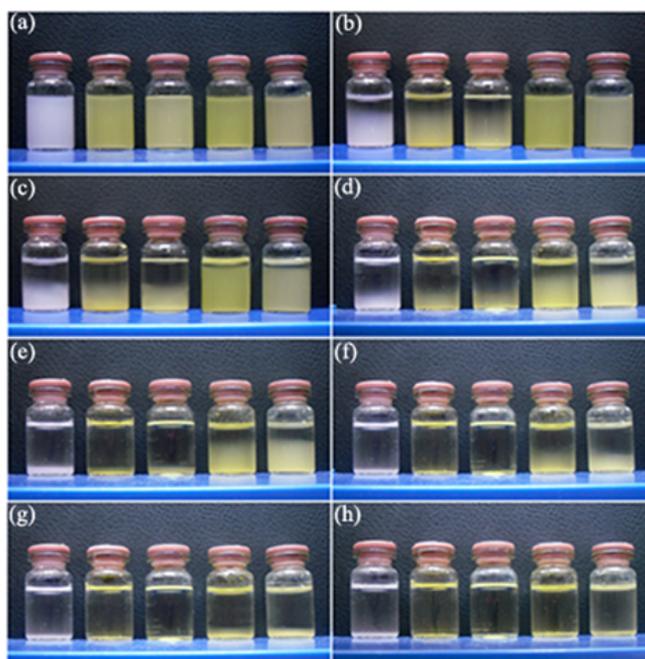
Figure S2. The detailed photographs for the dispersion stability of polymer microspheres in water (1 mg/mL) at 25 °C after their ultrasonically dispersed solutions being settled down for 0 (a), 0.5 (b), 1.0 (c), 1.5 (d), 2.5 (e), 4 (f), 5 (g), and 6 h (h), respectively. The samples located from left to right in each photograph are the core polymer microspheres, the ungrafted CS-MIP and CS-CP microspheres, and the grafted CS-MIP and CS-CP microspheres.

Characterization of the molecular weights and polydispersities of the grafted polymer brushes

It is generally accepted that the molecular weights and polydispersities of the free polymers generated in the surface-initiated RAFT polymerization systems (due to the addition of the sacrificial chain transfer agent) can be utilized to represent those of the grafted polymer brushes (M. D. Rowe, B. A. G.

Hammer and S. G. Boyes, *Macromolecules* 2008, **41**, 4147-4157). Therefore, the free polymers obtained in our study were characterized with gel permeation chromatography (GPC), from which the number-average molecular weights ($M_{n,GPC}$) of the grafted polymer brushes on both the CS-MIP and CS-CP microspheres were evaluated to be 42000 and their PDIs were found to be 1.16 and 1.15, respectively (Table S1).

A Waters GPC was utilized in the above measurements, which was equipped with a Waters 2414 series manual injector, a Waters 1515 high-performance liquid chromatography (HPLC) pump, a Waters 2414 refractive index detector, and three Waters UltraStyragel columns with 5 K-600 K, 500-30 K, and 100-10 K molecular ranges (the temperature of the column oven was set at 40 °C). A solution of LiBr in DMF (0.05 M) was used as the mobile phase with a flow rate of 1.0 mL/min, and the calibration curve was obtained by using polystyrene standards.

^{1}H NMR analyses of the chemical compositions of the grafted polymer brushes

The chemical compositions of the grafted polymer brushes were also evaluated by studying those of the free polymers generated in the polymerization solutions during the surface-initiated RAFT copolymerization of NIPAAm and DMAEMA (due to the addition of some sacrificial CDB into the polymerization systems) by ^{1}H NMR (Varian Unity plus-400 spectrometer, 400 MHz).

Figure S3 presents the ^{1}H NMR spectra of the free poly(NIPAAm-*co*-DMAEMA) generated in the polymerization solutions during the surface-grafting of polymer brushes onto CS-MIP and CS-CP microspheres, respectively, from which the molar ratios of the bonded DMAEMA unit to the bonded
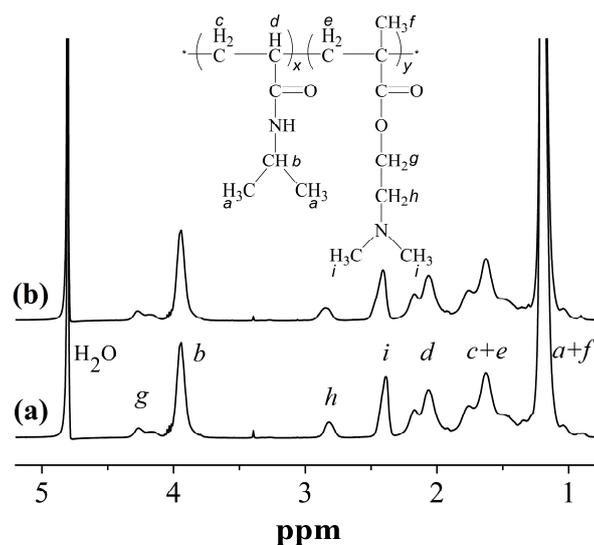
Figure S3. ^{1}H NMR spectra of the free poly(NIPAAm-*co*-DMAEMA) generated in the polymerization solutions during grafting of polymer brushes onto CS-MIP (a) and CS-CP (b) microspheres, respectively (solvent: D_2O).

NIPAAm unit in both poly(NIPAAm-*co*-DMAEMA) brushes grafted on CS-MIP and CS-CP microspheres were determined to be 8% by comparing the integral of the peak *h* around 2.76 ppm with that of the peak *b* around 3.93 ppm, which are close to the initial comonomer ratios (i.e., 5 mol%) in the surface-initiated RAFT copolymerization systems.

Determination of the lower critical solution temperatures (LCSTs) of the grafted polymer brushes in pure water and in Britton-Robison buffer (with different pH values)

The LCSTs of the grafted polymer brushes were evaluated by studying those of the free poly(NIPAAm-*co*-DMAEMA) generated in the polymerization solutions during surface-initiated RAFT polymerization processes. An UV-Vis scanning spectrophotometer (TU1900, Beijing Purkinje General Instrument Co., Ltd) equipped with a thermostatic cell holder (CH19-1) was utilized for this purpose. The polymer solutions (1.5 mg/mL) in the quartz sample cells were heated from 25 to 60 $^{\circ}$C at a rate of 0.2 $^{\circ}$C/min and the LCSTs of the polymers were determined at 50% of the transmittance of a 600 nm light beam through the polymer solutions (Y. Xia, N. A. D. Burke and H. D. H. Stöver, *Macromolecules* 2006, **39**, 2275-2283; C. Luo, Y. Liu and Z. Li, *Macromolecules* 2010, **43**, 8101-8108).

Figures S4a and b show the temperature-dependence of the optical transmittance at 600 nm for the pure aqueous solutions and Britton-Robison buffer solutions of the free poly(NIPAAm-*co*-DMAEMA) (1.5 mg/mL) generated in the polymerization systems during surface-grafting of polymer brushes onto CS-MIP and CS-CP microspheres, respectively, where the Britton-Robison buffer consists of a mixture of boric acid (0.04 M), phosphoric acid (0.04 M) and acetic acid (0.04 M) that has been titrated to the desired pH with sodium hydroxide aqueous solution (0.2 M). It can be seen clearly that the optical transmittance of the polymer solutions decreased quickly with increasing the temperatures of the pure aqueous solutions and those of the buffer solutions in the pH range of 7-10, while it only slightly decreased with an increase in the temperature from 25 to 60 $^{\circ}$C for the Britton-Robison buffer solutions at a pH value of 6, indicating that the free polymers experienced a significant conformation change from a hydrated (coiled and soluble) to a dehydrated (collapsed and insoluble) state with increasing the temperatures of the pure aqueous solutions and Britton-Robison buffer solutions in the pH range of 7-10, whereas no obvious thermo-responsive conformation change was observed for these polymers in Britton-Robison buffer solutions with their pH value being 6 in the temperature range of 25-60 $^{\circ}$C (which means that they might have a LCST higher than 60 $^{\circ}$C). The LCSTs of both free polymers were determined to be about 35 $^{\circ}$C in pure aqueous solutions, while the LCSTs of these polymers were found to increase from about 31 to 54 $^{\circ}$C with decreasing the pH values of the polymer

buffer solutions from 10 to 7 (Figure S4c), which is easily understandable because the protonation of DMAEMA units at lower pH conditions improved the hydrophilicity of the polymer chains (M. S. Jones, *Eur. Polym. J.* 1999, **35**, 795-801; V. Bulmus, Z. Ding, C. J. Long, P. S. Stayton and A. S. Hoffman, *Bioconjugate Chem.* 2000, **11**, 78-83). Furthermore, it is worth noting that the LCSTs of both free polymers generated in the polymerization systems during the surface-grafting of polymer brushes onto CS-MIP and CS-CP microspheres were essentially the same for their pure aqueous solutions and aqueous buffer solutions in the studied pH range, which is in good consistency with their almost same chemical compositions, molecular weights, and polydispersities.
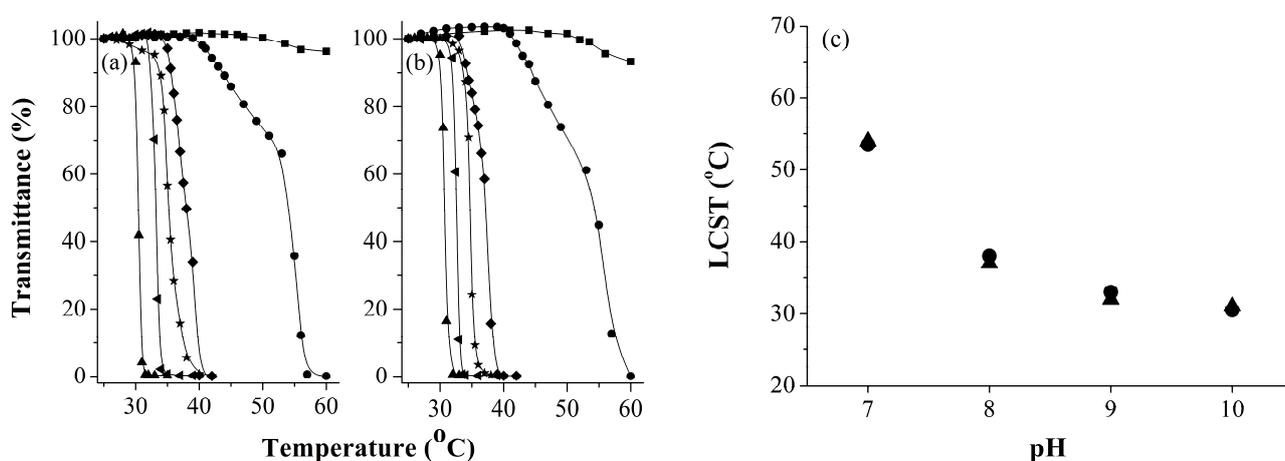
Figure S4. (a,b) Temperature-dependence of the optical transmittance at 600 nm obtained for the pure aqueous solutions (★) and Britton-Robison buffer solutions (pH 6 (■), 7 (●), 8 (◆), 9 (◀), and 10 (▲)) of the free poly(NIPAAm-*co*-DMAEMA) generated in the polymerization systems during surface-grafting of polymer brushes onto CS-MIP (a) and CS-CP (b) microspheres, respectively (polymer concentration: 1.5 mg/mL); (c) pH-dependence of LCSTs obtained for the Britton-Robison buffer solutions of free poly(NIPAAm-*co*-DMAEMA) generated in the polymerization systems during surface-grafting of polymer brushes onto CS-MIP (▲) and CS-CP (●) microspheres, respectively (polymer concentration: 1.5 mg/mL).

Equilibrium template binding experiments with the ungrafted and grafted CS-MIP/CS-CP microspheres

Equilibrium template binding experiments were performed by incubating a propranolol solution in acetonitrile (0.5 mL, 0.05 mM) or in pure water (0.5 mL, 0.05 mM) with different amounts of the ungrafted or grafted CS-MIP/CS-CP microspheres at 25 $^{\circ}$C for 16 h. After centrifugation, the amounts of the template remaining in the supernatants were then quantified by using a HPLC (Scientific System

Inc., USA) equipped with a UV-Vis detector, from which the amounts of the template bound to the ungrafted and grafted CS-MIP/CS-CP microspheres could be obtained (Figures 2a,b). The wavelength used for the determination of propranolol was 293 nm. A mixture of acetonitrile and 0.4% aqueous solution of triethylamine (7/3 v/v) was used as the mobile phase at a flow rate of 1 mL/min. All the above binding analyses were performed in duplicate and the mean values were used.

In this context, it is worth mentioning that the specific template bindings of the ungrafted and grafted CS-MIP microspheres (i.e., the template bindings by the imprinted binding sites of the studied MIPs) could be derived by using the following equation:

$$\text{Specific template binding} = B_{\text{MIP}} - B_{\text{CP}}$$

where B_{MIP} and B_{CP} are the equilibrium template bindings of the studied MIP and its corresponding CP, respectively.

Competitive binding experiments

The binding selectivity of the ungrafted and grafted CS-MIP/CS-CP microspheres was evaluated by measuring their competitive binding capacities towards propranolol and its structurally related compound atenolol as follows: 0.5 mg of ungrafted or grafted CS-MIP/CS-CP microspheres were incubated with 0.5 mL of a mixed solution of propranolol and atenolol in acetonitrile ($C_{\text{propranolol or atenolol}} = 0.05$ mM) or in pure water ($C_{\text{propranolol or atenolol}} = 0.05$ mM) at 25 $^{\text{o}}$C for 16 h and the amounts of propranolol and atenolol bound to the ungrafted and grafted CS-MIPs/CS-CPs were quantified by HPLC. The wavelength used for the determination of the mixed solution of propranolol and atenolol was 275 nm. A mixture of acetonitrile and 0.4% aqueous solution of triethylamine (7/3 v/v) was used as the mobile phase at a flow rate of 1 mL/min. All the above binding analyses were performed in duplicate and the mean values were used.

Figure S5a shows the selective (or competitive) bindings of the ungrafted and grafted CS-MIP/CS-CP microspheres towards propranolol and atenolol in their mixed solution in acetonitrile. Both the ungrafted and grafted CS-MIPs were found to exhibit obviously lower binding capacities towards atenolol than towards propranolol. This, together with the rather similar binding capacities of the corresponding CS-CPs towards propranolol and atenolol (which means that the nonspecific bindings of the studied CS-MIPs towards propranolol and atenolol were similar because the binding capacities of the CS-CPs are generally considered to represent the nonspecific bindings of the corresponding CS-MIPs), demonstrates the high selectivity of both the ungrafted and grafted CS-MIP microspheres towards propranolol in acetonitrile solution.
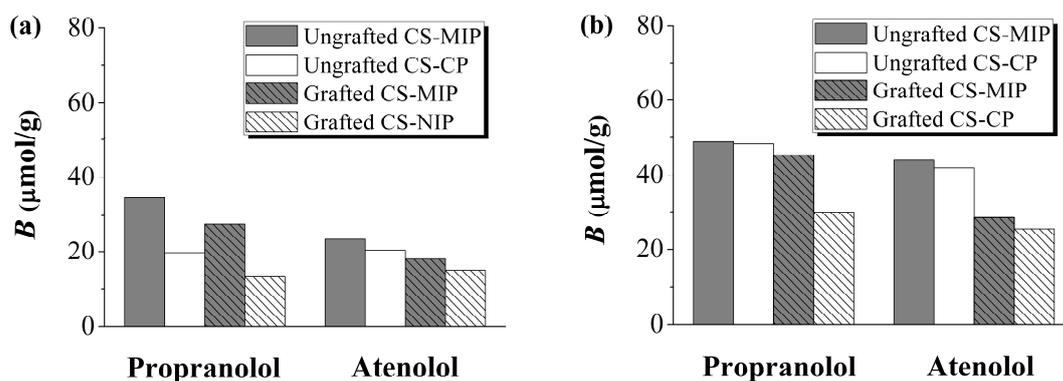
Figure S5. Selective bindings of the ungrafted and grafted CS-MIP/CS-CP microspheres towards propranolol and atenolol in their mixed solution in acetonitrile (a) and in pure water (b) ($C_{\text{Propranolol or atenolol}} = 0.05$ mM), respectively (polymer concentration: 1 mg/mL).

Figure S5b presents the selective bindings of the ungrafted and grafted CS-MIP/CS-CP microspheres towards propranolol and atenolol in their mixed pure aqueous solutions. It can be seen that although both the ungrafted and grafted CS-MIPs exhibited higher binding capacities towards propranolol than towards atenolol in pure aqueous solutions, the binding capacities of both the ungrafted and grafted CS-CPs towards propranolol were also higher than towards atenolol. The above results suggested that the nonspecific bindings of the studied CS-MIPs towards propranolol and atenolol were different, which makes it inappropriate to evaluate the selectivity (or specificity) of the ungrafted and grafted CS-MIPs by directly comparing their binding capacities towards propranolol and atenolol.

In the above case, the "imprinting-induced promotion of binding" (IPB) has proven to be an useful parameter for evaluating the MIPs' selectivity because the difference in the intrinsic nonspecific bindings of the MIPs towards different analytes is normalized (T. Hishiya, M. Shibata, M. Kakazu, H. Asanuma and M. Komiyama, *Macromolecules* 1999, **32**, 2265-2269; J. H. Zhang, M. Jiang, L. Zou, D. Shi, S. R. Mei, Y. X. Zhu, Y. Shi, K. Dai and B. Lu, *Anal. Bioanal. Chem.* 2006, **385**, 780-786; G. Pan, Y. Zhang, Y. Ma, C. Li and H. Zhang, *Angew. Chem. Int. Ed.* 2011, **50**, 11731-11734). IPB can be defined by the following equation:

$$\text{IPB} = (B_{\text{MIP}} - B_{\text{CP}})/B_{\text{CP}}$$

where B_{MIP} and B_{CP} are the equilibrium bindings of the studied MIP and its corresponding CP towards an analyte, respectively. The larger the IPB value of the MIP towards the analyte, the better the selectivity of the MIP.

The IPB values determined for both the ungrafted and grafted CS-MIPs towards propranolol and atenolol in pure aqueous solutions are listed in Table S2, which demonstrated clearly that the grafted CS-MIP microspheres showed obvious specificity towards propranolol in pure aqueous media, whereas no specificity towards propranolol was observed for the ungrafted CS-MIP microspheres under the pure aqueous condition.

Table S2. Selective binding properties of the ungrafted and grafted CS-MIPs/CS-CPs towards propanolol and atenolol in their mixed pure aqueous solutions.

Analyte	The ungrafted CS-MIP/CS-CP			The grafted CS-MIP/CS-CP		
	$B_{\text{CS-MIP}}$[a] (μmol/g)	$B_{\text{CS-CP}}$[a] (μmol/g)	IPB[b] (%)	$B_{\text{Grafted CS-MIP}}$[a] ($\mu$mol/g)	$B_{\text{Grafted CS-CP}}$[a] (μmol/g)	IPB[b] (%)
Propranolol	48.9	48.3	1.2	45.3	29.4	54.1
Atenolol	43.9	42.0	4.5	28.4	25.5	11.4

[a] $B_{\text{CS-MIP}}/B_{\text{CS-CP}}$ and $B_{\text{Grafted CS-MIP}}/B_{\text{Grafted CS-CP}}$ are the equilibrium binding capacities of the ungrafted CS-MIP/CS-CP and grafted ones towards propranolol and atenolol in their mixed pure aqueous solutions ($C_{\text{propranolol or atenolol}}$ = 0.05 mM), respectively (polymer concentration: 1 mg/mL), which are the same as those shown in Figures S5b; [b] IPB refers to the "imprinting-induced promotion of binding" value of the studied CS-MIPs.

Photoresponsive template binding properties of the grafted CS-MIP microspheres in pure aqueous solutions

The studies on the photoregulated release and uptake of the template propranolol by the grafted CS-MIP microspheres were performed by alternately switching on and turning off the UV light irradiation on the mixtures of the grafted CS-MIP microspheres and a pure aqueous solution of propranolol as follows: A series of samples were prepared by adding 0.5 mg of grafted CS-MIP microspheres and a solution of propranolol in pure water (0.5 mL, 0.05 mM) into plastic Eppendorf tubes (2 mL), respectively, which were subsequently sealed and put into an incubator equipped with a 365 nm UV lamp (16 W). After their incubation at 25 $^{\circ}$C in the dark for 10 h, one sample was taken out from the incubator and centrifugated to determine the amount of the template bound by the grafted CS-MIP microspheres (by HPLC). The UV light was then switched on in the incubator to irradiate the remaining samples immediately after the first sample was taken out. After 6 h of UV light irradiation with incubation for the samples at 25 $^{\circ}$C, the UV light was switched off and the second sample was taken out for the determination of the template binding. The remaining samples were then incubated at 25 $^{\circ}$C in the dark for another 18 h, and the third sample was taken out for the determination of the

template binding. The UV light was then switched on again to irradiate the remaining samples at 25 ^{o}C immediately after the third sample was taken out. The above photoswitching cycles (i.e., UV light on for 6 h and off for 18 h alternately) were repeated until all the other samples were measured.

The photoregulated release and uptake of propranolol by the grafted CS-CP microspheres were also studied similarly to provide control results for the grafted CS-MIP microspheres.

As can be seen clearly from Figure S6, the equilibrium template binding capacities of the grafted CS-MIP microspheres decreased upon exposure to 365 nm UV light, suggesting that the UV light irradiation led to the obvious release of the template from the grafted CS-MIP microspheres into the aqueous solution. The subsequent thermal back-isomerization of the above system in the dark caused re-uptake of the template by the grafted CS-MIP microspheres, resulting in an increase in the equilibrium template binding back to the original value (note that the visible light-induced back-isomerization of the grafted CS-MIP microspheres can also be used to cause the template uptake from the solutions). Repeating the photoswitching cycles (UV light on for 6 h and off for 18 h alternately) resulted in the release and uptake of propranolol in quantities very similar to those of the previous cycles, which clearly demonstrated the reversibility of the binding site configuration and substrate affinity in the course of photoswitching of azo chromophores in water (Scheme 1). It is worth pointing out here that the photoregulated release and uptake of propranolol were also observed for the grafted CS-CP microspheres under the repetitive photoswitching conditions, but they were in the opposite direction in comparison with the grafted CS-MIP microspheres. For example, the equilibrium bindings of the grafted CS-CP microspheres towards propranolol increased from 56 to 63% upon the UV light irradiation, while it dropped back to 57% upon switching off the UV light. The real cause of this phenomenon is not very clear yet, but might be due to the polarity difference between the *trans*- and *cis*-azo moieties on the surfaces of the grafted CS-CP microspheres under the photoswitching conditions, thus leading to their increased nonspecific template bindings upon UV light irradiation, similarly as reported in our previous work (L. Fang, S. Chen, Y. Zhang and H. Zhang, *J. Mater. Chem.* 2011, **21**, 2320-2329). To exclude the influence of these photoinduced nonspecific template binding changes, the dependence of the specific template bindings of the grafted CS-MIP microspheres on photoswitching conditions were also presented (Figure 3a and Figure S6b), which demonstrated the photoregulated release and uptake of the template by the grafted CS-MIP microspheres more clearly. Based on the above results, it can be concluded that the grafted CS-MIP microspheres indeed show obvious photoresponsive template binding properties in pure aqueous solutions and the affinity of the

binding sites in the grafted CS-MIP microspheres towards the template can be easily tuned by the simple photoswitching.
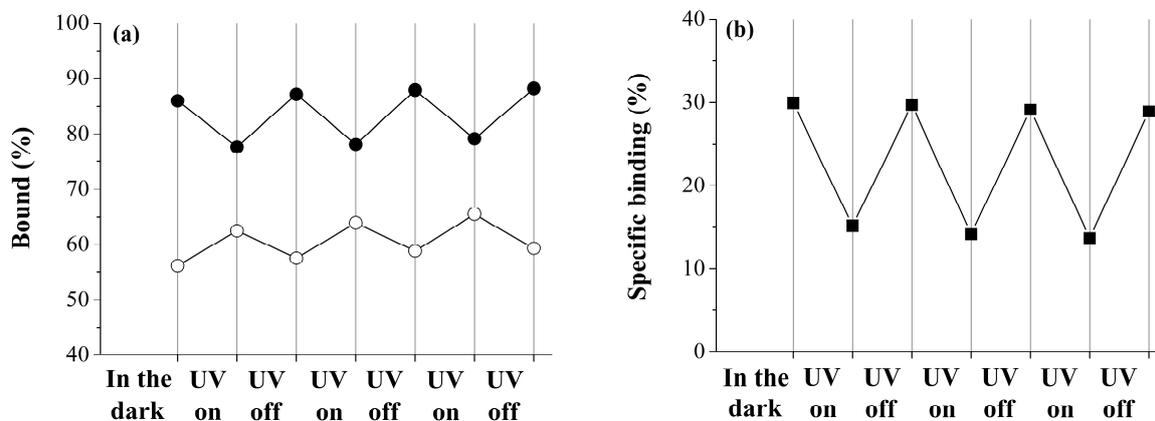
Figure S6. (a) Photoregulated release and uptake of propranolol by the grafted CS-MIP (filled circle)/CS-CP (open circle) microspheres in pure aqueous solutions under photoswitching conditions (UV light on for 6 h and off for 18 h alternately at 25 °C); (b) The photoresponsive specific template bindings of the grafted CS-MIP microspheres in pure aqueous solutions under photoswitching conditions. The concentration of the grafted CS-MIP/CS-CP microspheres was 1.0 mg/mL and the initial concentration of propranolol in the aqueous solution was 0.05 mM.

Thermo-responsive template binding properties of the grafted CS-MIP microspheres in pure aqueous solutions

Temperature-dependent equilibrium binding experiments were also performed by incubating 0.5 mL of a propranolol solution in pure water (0.05 mM) with 0.5 mg of grafted CS-MIP/CS-CP microspheres at different temperatures for 16 h. After centrifugation, the amounts of the template remaining in the supernatants were quantified with HPLC.

Since the grafted poly(NIPAAm-*co*-DMAEMA) brushes have proven to show obvious thermo-responsivity in pure water (LCST~35 °C) (Figures S4a and b), the CS-MIP/CS-CP microspheres grafted with such polymer brushes are expected to be also thermo-responsive in the aqueous solutions and might show thermo-responsive template binding properties. The experimental results presented in Figure S7 (or Figure 3b) indeed support this hypothesis. Figure S7a presents the temperature-dependent equilibrium template bindings of the grafted CS-MIP and CS-CP in pure aqueous solutions. It can be seen clearly that the template binding capacities of the grafted CS-CP microspheres increased largely with increasing the solution temperatures from 20 to 50 °C, which might be attributed to the increased hydrophobicity of the grafted CS-CP particle surfaces with the

polymer brushes changing from the hydrophilic soluble state to hydrophobic insoluble state following the temperature changes, thus leading to their higher nonspecific template bindings with the increase in the solution temperatures. In comparison, the template binding capacities of the grafted CS-MIP microspheres showed much less increase with an increase in the solution temperatures. The above results demonstrated that the specific template bindings of the grafted CS-MIP microspheres (which could be derived by subtracting the binding capacities of the grafted CS-CP from those of the grafted CS-MIP) decreased obviously with increasing the solution temperatures (Figure S7b, Figure 3b), which might be ascribed to the collapse of the polymer brushes at higher temperatures, thus resulting in the blocking of the imprinted binding sites in the grafted CS-MIP (Scheme 1), just as reported by the groups of Tatsuma and Hoffman in a peptide electrode and a protein system, respectively (K. Komori, K. Takada and T. Tatsuma, *Anal. Sci.* 2005, **21**, 351-353; P. S. Stayton, T. Shimoboji, C. Long, A. Chilkoti, G. Chen, J. M. Harris and A. S. Hoffman, *Nature* 1995, **378**, 472-474).
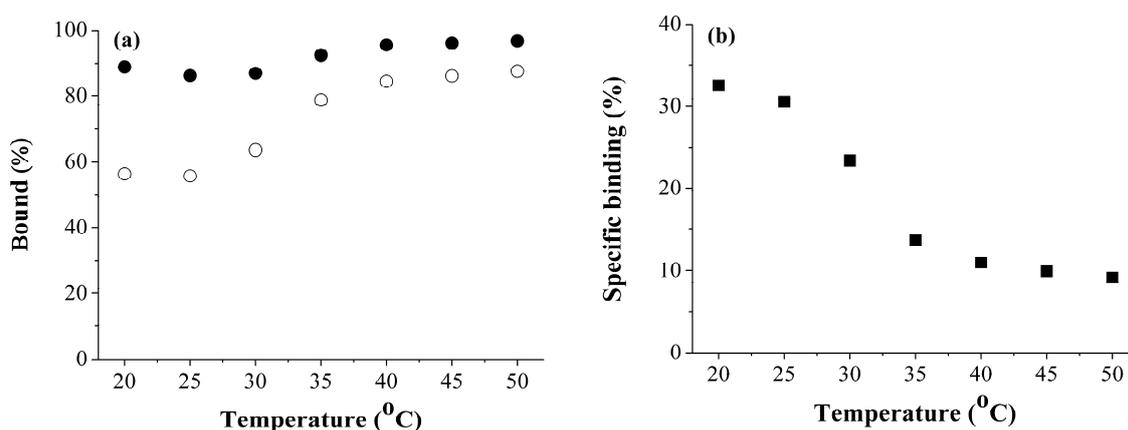
Figure S7. (a) Temperature-dependent equilibrium bindings of the grafted CS-MIP (filled circle)/CS-CP (open circle) microspheres towards propranolol in pure water; (b) Temperature-dependent specific bindings of the grafted CS-MIP microspheres towards propranolol in pure water. The concentration of the grafted CS-MIP/CS-CP microspheres was 1.0 mg/mL and the initial concentration of the propranolol solution in pure water was 0.05 mM.

pH-responsive template binding properties of the grafted CS-MIP microspheres in Britton-Robison buffer solutions

Equilibrium binding experiments were further carried out by incubating 0.5 mg of grafted CS-MIP/CS-CP microspheres with a propranolol solution in Britton-Robison buffer with different pH values (0.5 mL, 0.05 mM) at 40 ^{o}C for 16 h. After centrifugation, the amounts of the template remaining in the supernatants were then quantified with HPLC. A temperature of 40 ^{o}C was chosen

here on the basis of the experimental results shown in Figure S4c in order to allow the occurrence of the conformation change of the polymer brushes between a hydrated (coiled and soluble) and a dehydrated (collapsed and insoluble) state by changing the pH values of the buffer solutions.

Figure S8a shows the dependence of the equilibrium template bindings of the grafted CS-MIP and CS-CP microspheres on the pH values of the propranolol buffer solutions, from which the pH-dependent specific template bindings of the grafted CS-MIP microspheres were derived (Figure S8b, Figure 3c). It can be seen clearly that the specific template bindings of the grafted CS-MIP microspheres decreased dramatically with increasing the pH values of the propranolol buffer solutions from 6 to 10, which might be attributed to the blocking of the imprinted binding sites in the grafted CS-MIP microspheres because of the conformation transition of the polymer brushes from a hydrated soluble state to a dehydrated insoluble state when their LCSTs were lower than 40 ^{o}C at certain pH values (the LCSTs of the polymer brushes have proven to decrease with an increase in the pH values of the buffer solutions (Figure S4c)). It is worth mentioning here that this pH-responsive template binding properties should be capable of taking place at any temperature between 31~54°C (i.e., the LCST range of the polymer brushes with the change of the pH values of the buffer solutions from 10 to 7 (Figure S4c)) for the studied grafted CS-MIP microspheres (e.g., we also observed obvious pH-responsive template binding properties for the grafted CS-MIP at 37 ^{o}C (Figure not shown)).
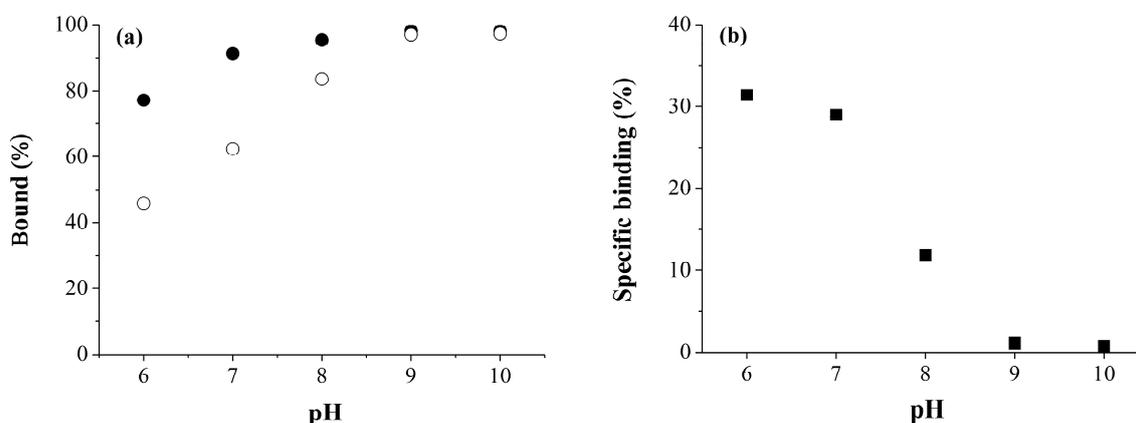
Figure S8. (a) pH-dependent equilibrium bindings of the grafted CS-MIP (filled circle)/CS-CP (open circle) microspheres towards propranolol in Britton-Robison buffer; (b) pH-dependent specific bindings of the grafted CS-MIP microspheres towards propranolol in Britton-Robison buffer. The equilibrium template bindings of the grafted CS-MIP/CS-CP microspheres were determined by incubating them (0.5 mg) with a series of propranolol buffer solutions (0.05 mM, 0.5 mL) at different pH values at 40 ^{o}C for 16 h.