Electronic Supplementary Information

Efficient synthesis of narrowly dispersed hydrophilic and magnetic molecularly imprinted polymer microspheres with excellent molecular recognition ability in a real biological sample

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Materials

4-Vinylpyridine (4-VP, Alfa Aesar, 96%) and ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%) were purified by distillation under vacuum. Methanol (Tianjin Jiangtian Chemicals, analytical grade (AR)) was distilled prior to use. *N*,*N*-Dimethylformamide (DMF, Tianjin Jiangtian Chemicals, AR) was first dried with magnesium sulfate ($MgSO_4$) overnight and then distilled under vacuum. 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). Glyceryl monomethacrylate (GMMA) was prepared following the literature method (W. N. E. van Dijk-Wolthuis, O. Franssen, H. Talsma, M. J. van Steenbergen, J. J. Kettenes-van den Bosch and W. E. Hennink, *Macromolecules*, 1995, **28**, 6317-6322). The standard fetal bovine serum (Beijing Solarbio Science & Technology Co., Ltd.) was stored at -20 ^{o}C prior to use and the thawed bovine serum was directly utilized in our study. 2,4-Dichlorophenoxyacetic acid (2,4-D, Alfa Aesar, 98%), phenoxyacetic acid (POAc, Acros, 98+%), and all the other reagents were commercially available and used as received.

Synthesis of the "living" 2,4-D-MIP/2,4-D-CP microspheres with surface-bound dithioester groups (or namely ungrafted 2,4-D-MIP/2,4-D-CP microspheres) via RAFT precipitation polymerization (RAFTPP)

The "living" 2,4-D-MIP microspheres with surface-bound dithioester groups were synthesized via RAFTPP following our previously described procedure (G. Pan, Y. Zhang, X. Guo, C. Li and H. Zhang, *Biosens. Bioelectron.*, 2010, **26**, 976-982), but with some modification in the reactant composition and experimental procedure: 4-VP (0.375 mmol), 2,4-D (0.0938 mmol), and a mixture of methanol and

water (4:1 v/v, 30 mL) were added into a one-neck round-bottom flask (50 mL) successively. The reaction mixture was agitated at ambient temperature to obtain a clear solution, to which EGDMA (1.880 mmol), CDB (0.0828 mmol), and AIBN (0.0414 mmol) were added. After being purged with argon for 30 min, the reaction mixture was sealed and then agitated at 25 ^{o}C for 2 h in order to allow the self-assembly of the functional monomer and template. The reaction flask was then attached to the rotor-arm of an evaporator, immersed into a thermostatic oil bath at 60 ^{o}C and rotated slowly (*ca.* 20 rpm) for 24 h. The resulting polymer particles in the reaction solutions were collected by centrifugation and they were purified through Soxhlet extraction with methanol/acetic acid (9:1 v/v, 48 h) and then methanol (24 h) successively until no template could be detected in the extraction solution. After being dried at 40 ^{o}C under vacuum for 48 h, a light pink MIP was obtained in a yield of 69% (entry 1 in Table S1).

The corresponding non-imprinted polymer or control polymer (CP, light pink color) microspheres with surface-bound dithioester groups were also prepared and purified under the identical conditions except that the template was omitted (yield: 73%) (entry 2 in Table S1).

Table S1 Characterization data for the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres.

Entry	Sample name	Sample color	ΔW [a] (%)	D_n [c] (μm)	U [c]	l [d] (nm)	$M_{n,GPC}$ [e] ($\times 10^{-4}$)	$Đ$ [e]	Contact angle (°) [f]
1	Ungrafted MIP	Light pink	-	2.655	1.030	-	-	-	124.9±1.5
2	Ungrafted CP	Light pink	-	2.752	1.022	-	-	-	122.3±1.5
3	Grafted MIP	Light pink	8.6	2.672	1.037	8.5	6.87	1.24	56.1±2.1
4	Grafted CP	Light pink	8.7	2.770	1.026	9.0	6.88	1.21	54.1±1.0
5	Grafted magnetic MIP	Dark brown	3.8 [b]	2.674	1.033	9.5	-	-	46.8±1.1
6	Grafted magnetic CP	Dark brown	4.0 [b]	2.773	1.028	10.5	-	-	44.3±1.3

[a]The weight increases of the grafted MIP/CP microspheres (in comparison with the ungrafted ones) expressed as the percentage values (i.e., the increased weights relative to the original weights); [b]The weight increases of the grafted magnetic MIP/CP microspheres in comparison with the grafted ones; [c]D_n and U refer to the number-average diameter and size distribution index of the polymer microspheres; [d]l refers to the thickness of the grafted polymer layer on the MIP/CP microspheres; [e]The number-average molecular weights ($M_{n,GPC}$) and dispersities ($Đ$) of the esterified form of PGMMA (generated in the polymerization solutions during the surface-initiated RAFT copolymerization due to the addition of sacrificial RAFT agent) determined by GPC with tetrahydrofuran (THF) as the mobile phase and polystyrene as standards; [f]The static water contact angles of the polymer films.

Synthesis of the 2,4-D-MIP/2,4-D-CP microspheres with surface-grafted PGMMA brushes (i.e., the grafted 2,4-D-MIP/2,4-D-CP microspheres)

The grafted 2,4-D-MIP/2,4-D-CP microspheres were synthesized via the surface-initiated RAFT polymerization of GMMA by using the above-obtained "living" 2,4-D-MIP/2,4-D-CP microspheres with surface-bound dithioester groups (or the ungrafted 2,4-D-MIP/2,4-D-CP microspheres) as the immobilized RAFT agent according to the following procedure: the "living" 2,4-D-MIP/2,4-D-CP microspheres with dithioester groups (200 mg), GMMA (18 mmol), CDB (0.036 mmol), AIBN (0.012 mmol), and methanol (20 mL) were added successively into a one-neck eggplant-shaped flask (50 mL) with a magnetic stirring bar inside. After being degassed with five freeze-pump-thaw cycles, the reaction flask was sealed and immersed into a thermostatted oil bath at 70 ^{o}C and stirred for 24 h. The resulting polymer particles 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 40 ^{o}C under vacuum to the constant weights, leading to light pink grafted 2,4-D-MIP and 2,4-D-CP microspheres with a weight increase of 8.6% and 8.7% in comparison with the corresponding ungrafted ones, respectively (entries 3 and 4 in Table S1).

The addition of some sacrificial CDB into the above polymerization systems also led to the generation of free PGMMA 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 (light pink polymers).

Esterification of the free PGMMA generated in the polymerization solutions during the surface-initiated RAFT polymerization systems

Since the above-obtained free PGMMA were insoluble in THF, they were reacted with benzoic anhydride to obtain the esterified PGMMA, which proved to be soluble in THF and could thus be characterized with gel permeation chromatography (GPC) by using THF as the eluent. The esterification process was carried out according to the following procedure: PGMMA (50.0 mg) was dissolved in dried DMF (1.5 mL) in a one-neck round-bottom flask (10 mL) at room temperature under ultrasound treatment. Benzoic anhydride (1.41 g, 6.24 mmol) and triethylamine (0.696 mL, 4.99 mmol) were then added into the above solution. The reaction was allowed to proceed under stirring at 25 ^{o}C for 24 h. The resulting reaction mixture was filtered and the obtained filtrate was precipitated into 20 mL ether to remove the unreacted benzoic anhydride. Finally the product was dried at 40 ^{o}C under vacuum for 48 h to provide the desired esterified PGMMA.

Synthesis of the grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres

The grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were synthesized by the chemical coprecipitation of Fe^{2+} and Fe^{3+} in a molar ratio of 1:2 in the presence of the grafted 2,4-D-MIP/2,4-D-CP microspheres according to the following procedure: the grafted 2,4-D-MIP/2,4-D-CP microspheres (100 mg), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 0.0359 mmol), and water/methanol (4:1 v/v, 10 mL) were added into a two-neck round bottom flask (25 mL) successively. After the mixed solution was dispersed with ultrasonic and purged with argon for 10 min, iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 0.0179 mmol) was added. The reaction mixture was again purged with argon for another 10 min and immersed into a thermostatted oil bath at 80 ^oC. Ammonium hydroxide (25%, 3 mL) was then added dropwise into the above solution under stirring and the resulting mixture was stirred at 80 ^oC in an argon atmosphere for 1 h. The resulting polymer particles were collected by centrifugation, washed thoroughly with water and methanol, and then dried at 40 ^oC under vacuum to the constant weights, leading to the dark brown grafted magnetic 2,4-D-MIP and 2,4-D-CP microspheres with a weight increase of 3.8% and 4.0% in comparison with the corresponding grafted 2,4-D-MIP and 2,4-D-CP microspheres, respectively (entries 5 and 6 in Table S1).

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

The morphologies, particle sizes, and size distributions of the above-obtained ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were characterized with a scanning electron microscope (SEM, Shimadzu SS-550) (Fig. 2a-f, Table S1). All of the SEM size data reflect the averages of about 150 particles, which are calculated by using 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, U the size distribution index, 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.

Fourier Transform Infrared (FT-IR) analyses

Fig. S1 shows the FT-IR spectra of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-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 ungrafted 2,4-D-MIP/2,4-D-CP microspheres

(i.e., poly(4-VP-*co*-EGDMA) microspheres) such as those characteristic of the bonded EGDMA (i.e., 1728 (C=O stretching) and 1250/1152 cm^{-1} (C-O-C stretching)) and those corresponding to the C=N stretching (1595 and 1557 cm^{-1}) and C=C stretching (1454 cm^{-1}) from the bonded 4-VP, the grafted 2,4-D-MIP/2,4-D-CP microspheres also showed the characteristic broad peaks of the hydroxyl O-H stretching band around 3540 cm^{-1}, which suggested the successful grafting of PGMMA brushes onto the 2,4-D-MIP/2,4-D-CP microspheres after the polymer brushes-grafting processes. In addition, a new band around 584 cm^{-1} related to the Fe-O bond of the naked Fe_3O_4 was also discernible in the FT-IR spectra of the grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres prepared by the chemical coprecipitation of Fe^{2+}/Fe^{3+} (1:2 molar ratio) in the presence of the grafted 2,4-D-MIP/2,4-D-CP microspheres, demonstrating that Fe_3O_4 nanoparticles have been attached onto the grafted 2,4-D-MIP and 2,4-D-CP microspheres.

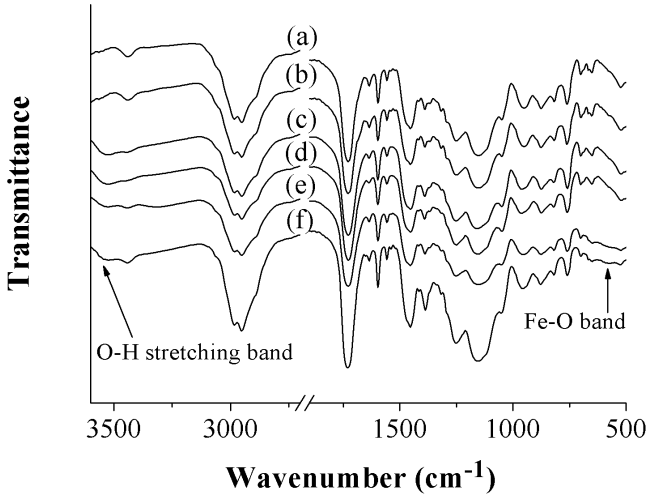

Fig. S1 FT-IR spectra of the ungrafted 2,4-D-MIP (a)/2,4-D-CP (b) microspheres, the grafted 2,4-D-MIP (c)/2,4-D-CP (d) microspheres, and the grafted magnetic 2,4-D-MIP (e)/2,4-D-CP (f) microspheres (The spectra are positioned in a row on a virtual third axes).

Measurement of the static water contact angles of the films prepared with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres

The films of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were prepared by casting their suspension solutions in DMF (10 mg/mL, after ultrasonic dispersion) onto clean glass surfaces. After the solvent was allowed to evaporate at ambient temperature and the resulting films were dried at 25 ^{o}C under vacuum overnight, a KRÜSS FM40 Easy Drop contact angle equipment (Germany) was used to determine their static water contact angles (Fig. 2g, Table S1). Two measurements were taken across each sample, with their average being used for analysis.

Dispersion stability of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in pure water

The dispersion properties of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-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 ^{o}C.

Fig. 2h and Fig. S2 show the typical photographs of the resultant solutions, from which it can be seen clearly that there was much faster sedimentation for the ungrafted MIP/CP microspheres in comparison with the grafted and grafted magnetic MIP/CP microspheres, confirming the presence of hydrophilic polymer brushes on the grafted polymer microspheres.

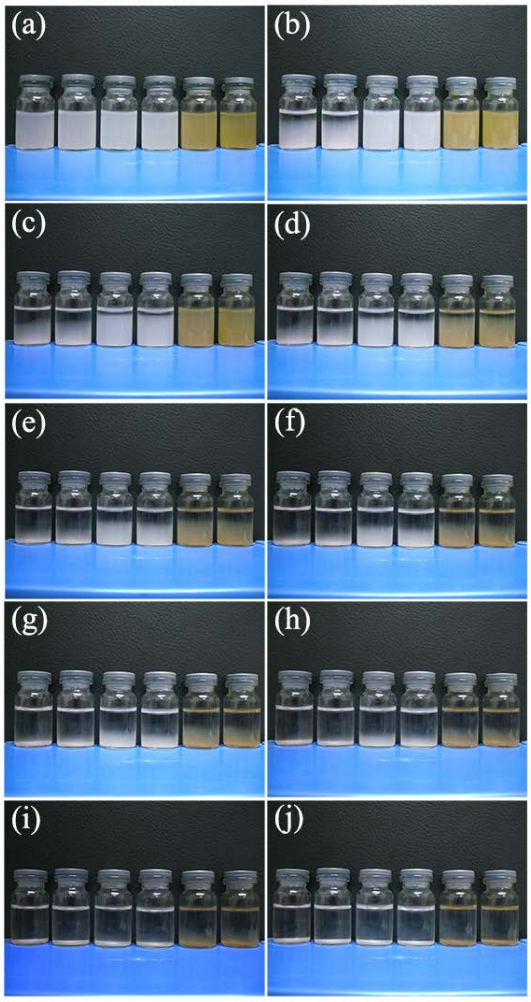

Fig. S2 The detailed photographs for the dispersion stability of polymer microspheres in water (1 mg/mL) at 25 ^{o}C after their ultrasonically dispersed solutions being settled down for 0 (a), 0.5 (b), 1.0 (c), 1.5 (d), 2.0 (e), 2.5 (f), 3.0 (g), 4.0 (h), 5.0 (i), and 6 h (j), respectively. The samples located from left to right in each photograph are the ungrafted 2,4-D-MIP and 2,4-D-CP microspheres, the grafted 2,4-D-MIP and 2,4-D-CP microspheres, and the grafted magnetic 2,4-D-MIP and 2,4-D-CP microspheres.

Characterization of the molecular weights and molar-mass dispersities of the grafted polymer brushes

It is generally accepted that the molecular weights and molar-mass dispersities (*Đ*) 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).

Since PGMMA was insoluble in THF, the PGMMA generated in the polymerization solutions during the surface-initiated RAFT polymerization processes were first esterified by their reaction with benzoic anhydride. The number-average molecular weights and *Đ* of the resulting esterified polymers were determined by using a GPC equipped with a Waters 717 autosampler, a Waters 1525 HPLC pump, three Waters UltraStyragel columns (with 5K-600K, 500-30K, and 100-10K molecular ranges) (the temperature of the column oven was 35 ^{o}C), and a Waters 2414 refractive index detector. THF (Tianjin Kangkede Chemicals, Chromatographic purity) was used as the eluent at a flow rate of 1.0 mL/min. The calibration curve was obtained by polystyrene (PS) standards.

Transmission electron microscope (TEM) analysis

The grafted magnetic 2,4-D-MIP microspheres were characterized with a TEM (Technai G2 20-S-TWIN) (Fig. 3a and b). The sample for the TEM characterization was first dispersed in ethanol, and a drop of the dispersed mixture was dropped onto the surface of a copper grid coated with a carbon membrane and then dried under vacuum at room temperature.

X-ray diffraction (XRD) analysis

The crystallographic analysis of the grafted magnetic 2,4-D-MIP microspheres was carried out with X-ray diffraction (XRD) by using a Philips D/Max-2500 diffractometer with Cu Kα radiation (1.5405 Å) generated at 18 kV and 100 mM from 20 to 70° (2θ value) at a scan rate of 4°/min (Fig. 3c).

Characterization of the magnetic properties of the grafted magnetic 2,4-D-MIP microspheres

The magnetic properties of the grafted magnetic 2,4-D-MIP microspheres were characterized with a Quantum Design MPMS XL-5 SQUID magnetometer by measuring the applied magnetic field (*H*) dependence of their magnetization (*M*) between +10000 and -10000 Oe at 300 K (Fig. 3d).

The magnetic separation of the grafted magnetic 2,4-D-MIP microspheres from their dispersed solution in water (1 mg/mL) was also performed at 25 ^{o}C under the external magnetic field. As shown in Fig. S3, the grafted magnetic 2,4-D-MIP microspheres could be collected easily from the solution

when they were exposed to a magnet, which again demonstrated the successful immobilization of Fe_3O_4 nanoparticles on the grafted 2,4-D-MIP microspheres.

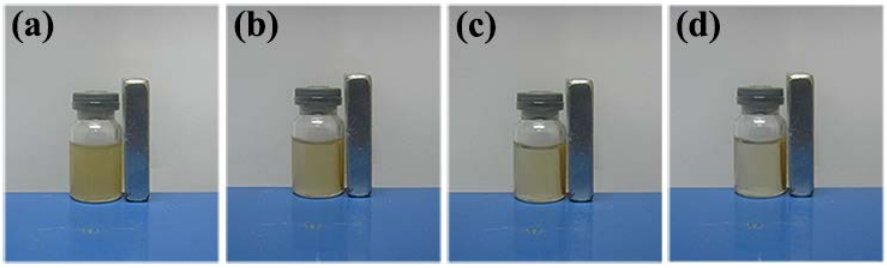

Fig. S3 Magnetic separation of the grafted magnetic 2,4-D-MIP microspheres from their suspensions in water (1 mg/mL) at 25 ^{o}C upon their exposure to a magnet for 0 (a), 1 (b), 2 (c), 3 min (d), respectively.

Equilibrium template binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in different media

Equilibrium template binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in methanol/water (4:1 v/v) and in pure water, respectively

Equilibrium template binding experiments were performed by incubating a 2,4-D solution (0.02 mM) in methanol/water (4:1 v/v, 0.4 mL) or in pure water (0.4 mL) with different amounts of the ungrafted, grafted, or grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres at 25 ^{o}C for 16 h. After centrifugation, the amounts of the template remaining in the supernatants were then quantified with a high-performance liquid chromatography (HPLC, Scientific System Inc., USA) equipped with a UV-vis detector and a Lanbo Kromasil C18 column (250 mm × 4.6 mm), from which the amounts of the template bound to the studied MIP/CP microspheres could be obtained. The wavelength used for the determination of 2,4-D was 284 nm. A mixture of methanol and 0.5% aqueous solution of acetic acid (4:1 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 (Fig. S4a,b).

In this context, it is worth mentioning that the specific template bindings of the studied 2,4-D-MIP microspheres (i.e., the template bindings by the imprinted binding sites of the studied MIPs) could be derived by using the following equation:

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

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

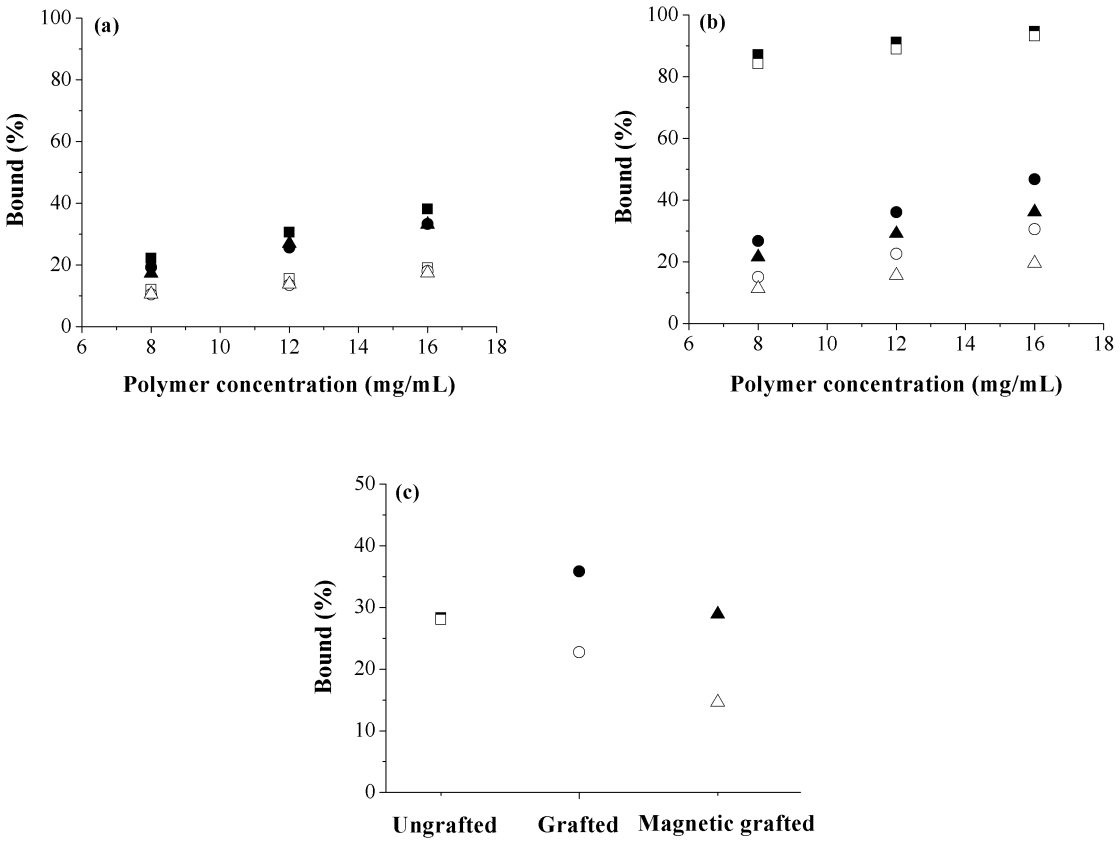

Fig. S4 (a,b) Equilibrium bindings of 2,4-D on different amounts of the ungrafted (square), grafted (circle), and grafted magnetic (triangle) 2,4-D-MIP (filled symbols)/2,4-D-CP (filled symbols) microspheres in its solution (0.02 mM) in methanol/water (4:1 v/v) (a) and in pure water (b) at 25 ^{o}C, respectively; (c) Equilibrium bindings of 2,4-D on the ungrafted (square), grafted (circle), and grafted magnetic (triangle) 2,4-D-MIP (filled symbols)/2,4-D-CP (open symbols) microspheres in its solution (0.02 mM) in bovine serum at 25 ^{o}C (polymer concentration: 12 mg/mL).

Equilibrium template binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in bovine serum

Equilibrium template binding experiments of the ungrafted, grafted, or grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were also performed in bovine serum following our previously reported procedure (Y. Ma, G. Pan, Y. Zhang, X. Guo and H. Zhang, *Angew. Chem. Int. Ed.*, 2013, **52**, 1511-1514): a 2,4-D solution in the undiluted bovine serum (0.02 mM, 0.4 mL) was incubated with 4.8 mg of the ungrafted, grafted, or grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres at 25 ^{o}C for 16 h. After centrifugation, 320 μL of the supernatants were collected, to which 480 μL of acetonitrile was added to precipitate all the proteins in the samples. After 5 min of ultrasonic treatment of the above mixtures, the samples were centrifuged and all the supernatants were collected, to which some

acetonitrile/water (3:2 v/v) was added to reach a constant volume of 800 μL. An aliquot of the obtained solutions were then injected into HPLC to measure the 2,4-D concentrations there, from which the amounts of the template in the original 320 μL of the supernatants as well as those bound to the MIP/CP particles were derived. The wavelength used for the determination of 2,4-D was 284 nm. A mixture of methanol and 0.5% aqueous solution of acetic acid (4:1 v/v) was utilized 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 (Fig. S4c).

Competitive binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in different media

Competitive binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in methanol/water (4:1 v/v) and in pure water, respectively

The binding selectivity of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres was first evaluated by measuring their competitive binding capacities towards 2,4-D and its structurally related compound POAc (which have the same functionality (i.e., carboxyl group) but differ in the numbers of substitutents on the benzene ring) in methanol/water (4:1 v/v) and in pure water as follows: 6.4 mg of the ungrafted, grafted, or grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were incubated with 0.4 mL of a mixed solution of 2,4-D and POAc ($C_{2,4\text{-D or POAc}}$ = 0.02 mM) in methanol/water (4:1 v/v) or in pure water at 25 ^{o}C for 16 h and the amounts of 2,4-D and POAc bound to the MIPs/CPs were quantified by HPLC. The wavelength used for the determination of the mixed solution of 2,4-D and POAc was 272 nm. A mixture of methanol and 0.5% aqueous solution of acetic acid (3:2 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.

Fig. S5a and S5b present the selective bindings of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres towards 2,4-D and POAc in their mixed solution in methanol/water (4:1 v/v) and in pure water, respectively. It can be seen that although all the studied 2,4-D-MIPs exhibited higher binding capacities towards 2,4-D than towards POAc in both methanol/water (4:1 v/v) and in pure water in all cases, the binding capacities of their corresponding 2,4-D-CPs towards 2,4-D were also higher than towards POAc in some cases. The above results suggested that the nonspecific bindings of the studied 2,4-D-MIPs towards 2,4-D and POAc were different in some conditions, which makes it inappropriate to evaluate the selectivity (or specificity) of the 2,4-D-MIPs by directly comparing their binding capacities towards 2,4-D and POAc.

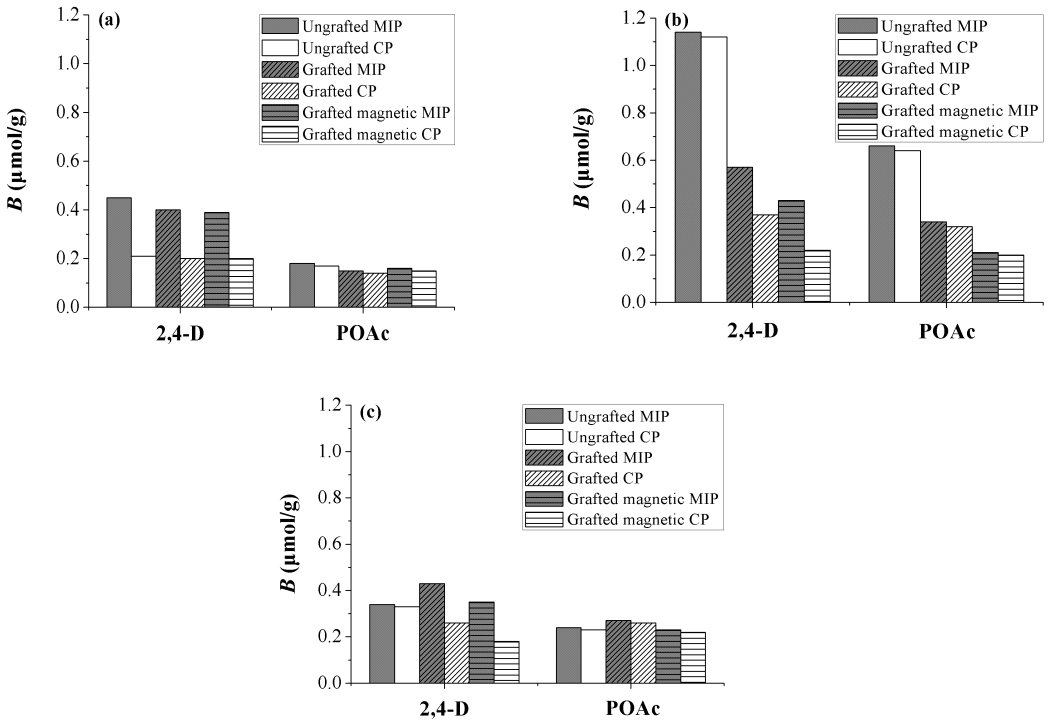

Fig. S5 Selective bindings of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres towards 2,4-D and POAc in their mixed solution ($C_{2,4\text{-D or POAc}}$ = 0.02 mM) in methanol/water (4:1 v/v) (a), in pure water (b), and in bovine serum (c), respectively (the polymer concentration is 16 mg/mL for a and b and 12 mg/mL for c).

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 the ungrafted, grafted, and grafted magnetic 2,4-D-MIPs towards 2,4-D and POAc in methanol/water (4:1 v/v) and in pure water are listed in Table S2, which demonstrated clearly that the grafted and grafted magnetic 2,4-D-MIP microspheres showed obvious

specificity towards 2,4-D in both methanol/water (4:1 v/v) and pure water, whereas the ungrafted 2,4-D-MIP microspheres only showed specificity towards 2,4-D in methanol/water (4:1 v/v) and no specificity towards 2,4-D was observed under the pure aqueous condition.

Table S2 Selective binding properties of the ungrafted, grafted, and grafted magnetic 2,4-D-MIPs/2,4-D-CPs towards 2,4-D and POAc in different media.

Solvent	Analyte	The ungrafted MIP/CP microspheres			The grafted MIP/CP microspheres			The grafted magnetic MIP/CP microspheres		
		B_{MIP}[a]	B_{CP}[a]	IPB (%)[b]	B_{MIP}[a]	B_{CP}[a]	IPB (%)[b]	B_{MIP}[a]	B_{CP}[a]	IPB (%)[b]
Methaol/ Water	2,4-D	0.45	0.21	**114**	0.40	0.20	**100**	0.39	0.20	**95**
	POAc	0.18	0.17	**6**	0.15	0.14	**7**	0.16	0.15	**7**
Pure water	2,4-D	1.14	1.12	**2**	0.57	0.37	**54**	0.43	0.22	**96**
	POAc	0.66	0.64	**3**	0.34	0.32	**6**	0.21	0.20	**5**
Bovine serum	2,4-D	0.34	0.33	**3**	0.43	0.26	**65**	0.35	0.18	**94**
	POAc	0.24	0.23	**4**	0.27	0.26	**4**	0.23	0.22	**5**

[a] B_{MIP} and B_{CP} are the equilibrium binding capacities of the studied MIP and its corresponding CP towards 2,4-D and POAc in their mixed solution ($C_{2,4\text{-D or POAc}}$ = 0.02 mM) in methanol/water (4:1 v/v), in pure water, and in bovine serum, respectively, which are the same as those shown in Fig. S5 and have a unit of μmol/g; [b] IPB refers to the "imprinting-induced promotion of binding" value of the studied MIP.

Competitive binding experiments with the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in bovine serum

The binding selectivity of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres in bovine serum was also evaluated by measuring their competitive binding capacities towards 2,4-D and POAc as follows: 4.8 mg of the ungrafted, grafted, or grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres were incubated with 0.4 mL of a mixed solution of 2,4-D and POAc in bovine serum ($C_{2,4\text{-D or POAc}}$ = 0.02 mM) at 25 °C for 16 h. After centrifugation, 320 μL of the supernatants were collected, to which 480 μL of acetonitrile was added to precipitate all the proteins in the samples. After 5 min of ultrasonic treatment of the above mixtures, the samples were centrifuged and all the supernatants were collected, to which some acetonitrile/water (3:2 v/v) was added to reach a constant volume of 800 μL. An aliquot of the obtained solutions were then injected into HPLC to measure the 2,4-D concentrations there, from which the amounts of the template in the original 320 μL

of the supernatants as well as those bound to the MIP/CP particles were derived. The wavelength used for the determination of the mixed solution of 2,4-D and POAc was 272 nm. A mixture of methanol and 0.5% aqueous solution of acetic acid (3:2 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.

Fig. S5c shows the selective bindings of the ungrafted, grafted, and grafted magnetic 2,4-D-MIP/2,4-D-CP microspheres towards 2,4-D and POAc in their mixed solution in bovine serum, from which the IPB values of the studied 2,4-D-MIPs towards 2,4-D and POAc in bovine serum can be obtained and listed in Table S2. It can be seen clearly that while the ungrafted 2,4-D-MIP showed no selectivity towards 2,4-D in bovine serum, both the grafted and grafted magnetic 2,4-D-MIPs exhibited good selectivity towards 2,4-D in this complex biological medium.