Electronic supporting information

Ring-opening of polythiolactones to construct protein imprinted nanospheres with high recognition and regulation capabilities

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1. Experimental Section

1.1 Preparation of Silica-coated Hollow Fe_3O_4 Nanospheres, $Fe_3O_4@SiO_2$ (MS)

Hollow Fe_3O_4 nanospheres were prepared according to our previous study.[1] Briefly, 300.0 mg of hollow Fe_3O_4 nanospheres were dispersed in a mixture of 100 mL of ethanol and 40 mL of distilled water. After ultrasonication for 10 minutes, 2 mL of ammonia aqueous solution (25 wt %) was injected into the mixture. Then, 20 mL of ethanol that contained 1 mL of tetraethyl orthosilicate was added dropwise under mechanical stirring at 30 ℃ for 8 h. After the reaction, the residue was washed with ethanol and distilled water for three times successively. Purified silica-coated Fe_3O_4 nanospheres (560.0 mg) were obtained by freeze-drying and labelled as $Fe_3O_4@SiO_2$ *or MS*.

1.2 Preparation of Bromine-coated Nanospheres, *MS@Br*

Amino-coated *MS* nanospheres were initially synthesized by 3-aminopropyltriethoxysilane (KH-550) hydrolysis on the surface of *MS*. Typically, 150.0 g of *MS* was dispersed in a mixture of 15 mL of water and 110 mL of ethanol. After ultrasonication for 10 minutes, 10 mL of ethanol that contained 1.5 mL of KH-550 was added dropwise under mechanical stirring at 40 ℃ for 6 h. After the reaction, the mixture was washed with ethanol and water for three times successively. The amino-coated *MS* nanospheres (140.0 mg), denoted as $MS@NH_2$, were finally obtained by freeze-drying and used to the next step.

138.0 mg of $MS@NH_2$ were dispersed in 70 mL of anhydrous THF. After ultrasonication for 10 minutes, 10 mL of anhydrous THF containing 1mL of 2-bromoisobutyryl bromide was added dropwise under mechanical stirring at 0 ℃ for 3 h. The reaction was stirred at 40 ℃ for another 12 h. After the reaction, the residue was washed with THF, ethanol and water for three times successively. The pure bromine-coated nanospheres (127.0 mg) were collected by freeze-drying and labelled as *MS@Br*.

1.3 Synthesis of monomer *TL*

The thiolactone-modified vinyl monomer *TL* was synthesized following the previous procedure.[2] 15.36 g (0.1 mol) of D, L-homocysteinethiolactone hydrochloride

and 18.90 g (0.225 mol) of sodium bicarbonate were dissolved in a mixture of water and ethyl acetate (1/1, v/v, 200 mL). The reaction mixture was stirred in an ice bath for 30 minutes. 10 mL of acryloyl chloride was added dropwise under stirring at 0 ℃ over 1 h. The reaction was stirred at room temperature for another 12 h. After the reaction, the mixture was washed with brine (200 mL). The aqueous layer was extracted with ethyl acetate for three times, the combined organic phases were dried over anhydrous sodium sulfate. The pure *TL* was obtained via evaporation of the solvent as a white solid (13.34 g, yield: 78%). ^{1}H NMR [400 MHz, (DMSO-d6)]: 2.14 ppm (m, 1H), 2.48 ppm (m, 1H), 3.32 ppm (m, 1H), 3.45 ppm (m, 1H), 4.72 ppm (m, 1H), 5.69 ppm (dd, 1H), 6.17 ppm (m, 1H), 6.28 ppm (m, 1H), 8.46 ppm (dd, 1H).

1.4 Rebinding Selectivity Experiments

1.5.1 Rebinding Selectivity of MIPs from the Protein Mixture

Typically, model proteins including BHb, HSA, Lyz and Cyt C were incubated with 5.0 mg of MIPs or NIPs at 25°C for 150 mins in Tris-HCl buffer (pH = 8.0, 50 mM), respectively. After magnetic separation, the protein content of the obtained supernatant was determined at 596 nm according to the Bradford assay[1] on a UV-2550 spectrophotometer.

Besides, the rebinding selectivity of MIPs was analyzed qualitatively by the protein gel electrophoresis experiment[3]. Typically, A protein mixture was prepared by dissolving the same amount of a competing protein and BSA (10.0 mg) in 20 mL of Tris-HCl buffer (pH = 8.0, 50 mM). Then, 6.0 mg of MIPs were added to the protein mixture (6 ml) and the solution was incubated at 25°C for 150 mins. After magnetic separation, the residual nanospheres were eluted with 6 mL of acetic acid (6.0 vt % in water) for 2 h and centrifuged. The supernatant was collected for further use. Then, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer, containing protease inhibitor and beta-mercaptoethanol, was heated at 95 °C for 10 min to denature the proteins. The acrylamide gels were run at 25 mA for 120 mins to resolve the samples along with a protein molecular weight marker (molecular weights: 14.4 –118 kDa) using a mini electrophoresis apparatus trophoresis (Bio-Rad, USA). Protein bands were visualized by staining the gel with a coomassie brilliant blue G-250.

1.5.2 Rebinding Selectivity of MIPs from Fetal Bovine Serum (FBS)

6.0 mg of *MS@PTLR-MIPs* was added to the 20-fold dilution of FBS or bovine whole blood sample (6 ml). The mixture was then incubated at 25 °C for 3 h. After magnetic separation, the nanospheres were eluted with 6 mL acetic acid (6.0 vt % in water) and the supernatant was collected to conduct the protein gel electrophoresis experiment with the same method as above.

1.5 Reusability Test

5.0 mg of MIPs and BSA were incubated in 5.0 mL Tris-HCl buffer (pH = 8.0, 50 mM) at 25 °C for 150 mins. After magnetic separation, the protein content of the obtained supernatant was determined according to the Bradford assay[1] on a UV-2550 spectrophotometer at 596 nm. The precipitates were collected and eluted repeatedly with the solution of acetic acid (6 mL, vt 6%) until no absorption peak were detected in the eluent. Then, the crude product was washed with distilled water for three times. The pure product was harvested by freeze-drying. Same experiments were repeated to characterize the reusability of the MIPs for 5 times.

1.7 Protein Conformation Measurement

4.0 mg of BSA and 4.0 mg of crosslinker or *MS@PTLR* nanospheres were co-incubated in 10 mL of Tris-HCl buffer (pH = 8.0, 50 mM) at 25 °C for 150 mins. Then, the solution was transferred to a quartz cuvette of 1 cm path length. The protein conformation was checked by a circular dichroism (CD) spectroscopy at 25 °C in the range of 200 - 260 nm with 1 nm resolution.

2 Figure and Table

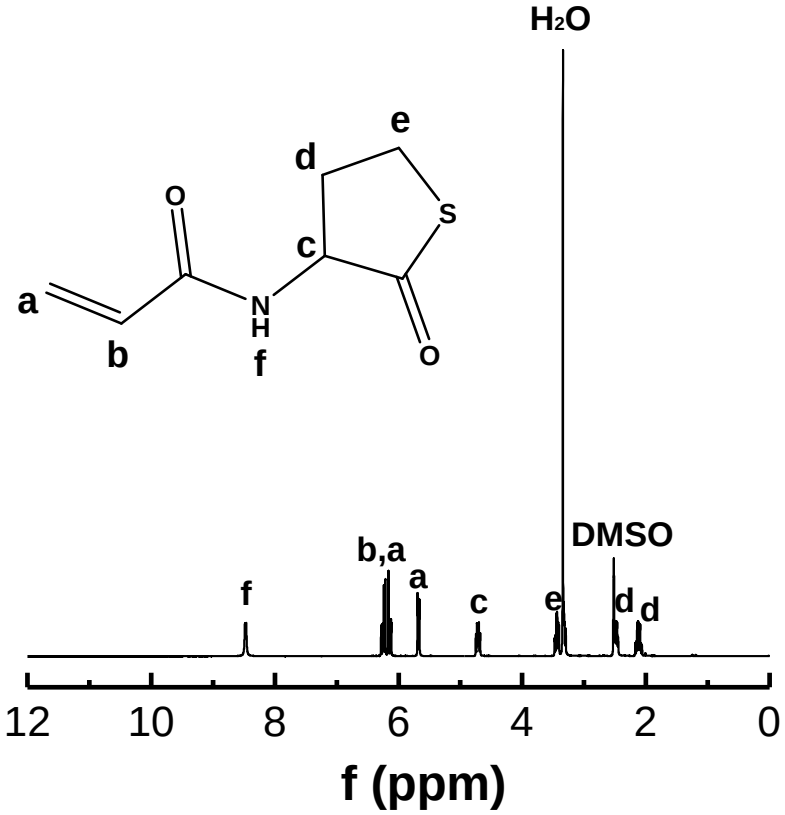

Fig. S1 ^{1}H NMR spectrum of *TL* monomer in DMSO-d_6

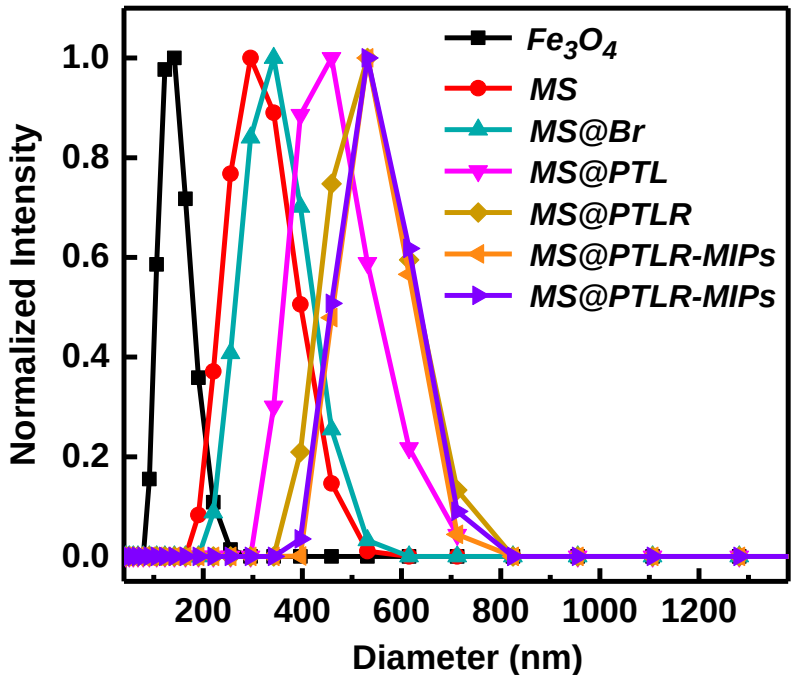

Fig. S2 Average size of each nanosphere Tris-HCl buffer (50 mM, pH = 8.0).

The average size of Fe_3O_4 was determined as 210.1 nm, after coated with silicon on the surface of Fe_3O_4, the average size of *MS* increased to 309.7 nm. A further increase of average size of *MS@Br* from 309.7 nm to 338.5 nm, indicating the successful grafting of ATRP initiation sites. After polymerization of thiolactones on the surface of *MS@Br*, the average size of obtained *MS@PTL* increased from 338.5 nm to 435.7 nm. The average size of MS@PTLR increased to 502.8 nm illustrated the ring-opening of thiolactone side groups of *MS@PTL,* and the imprinted nanospheres (*MS@PTLR-MIPs*) with average size of 541.1 nm were finally obtained by crosslinking of the thiols on the surface of *MS@PTLR* via a simple thiol-isocyanate click reaction. The non-imprinted nanospheres (*MS@PTLR-NIPs*) showed almost the same average size of *MS@PTLR-MIPs* and the valve was determined as 543.0 nm. However, compared with the results of TEM, the larger size of nanospheres measured by DLS was reasonable, because the hydrodynamic size measured by DLS was the hydration diameters.

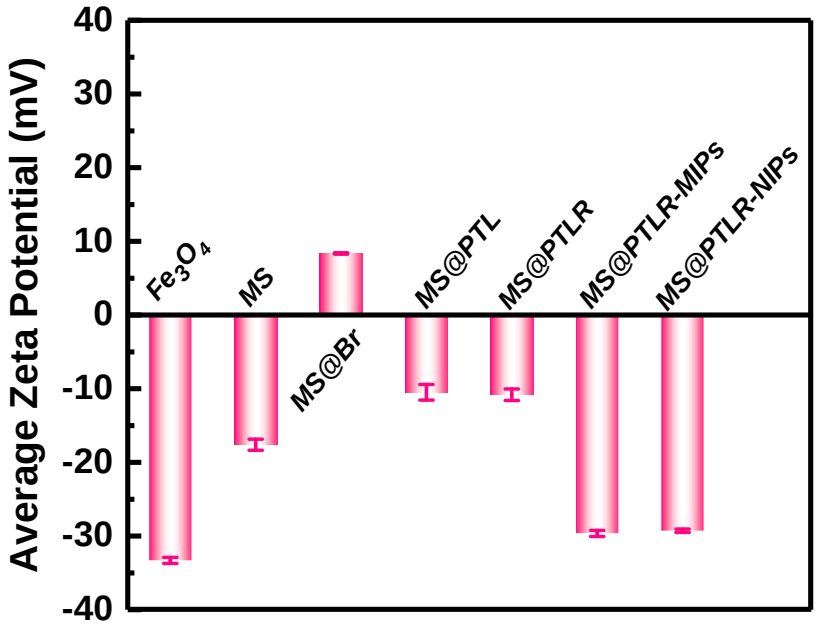

Fig. S3 Zeta potentials ζ for nanospheres in Tris-HCl buffer (50 mM, pH = 8.0)

The zeta potential (ζ) was measured to evaluate the surface charge and stability of nanospheres in Tris-HCl buffer (50 mM, pH = 8.0). As shown in Fig. S3, the negative surface charge of *Fe_3O_4* was attributed to the residual hydroxyl and carboxyl groups on the surface of *Fe_3O_4*. The high ζ value of *Fe_3O_4* (-33.3 ± 0.4 mV) illustrated strong electrostatic repulsion between *Fe_3O_4* nanospheres and the consequent stability of *Fe_3O_4* in Tris-HCl buffer (50 mM, pH = 8.0). As coated by silicon, the ζ value of *MS* decreased to -17.6 ± 0.7 mV. The positive ζ value of *MS@Br* (8.3 ± 0.1 mV) uncovered the grafting of the ATRP initiation sites. The ζ value of *MS@PTL* (-10.5 ± 1 mV) became negative again explained the success of ATRP and the similar ζ value of *MS@PTL* (-10.5 ± 1 mV) and *MS@PTLR* (-10.8 ± 0.7 mV) indicated that the ring opening reaction had no obvious effect on the surface charge of nanospheres. A further increase of ζ value for *MS@PTLR-MIPs* (-29.6 ± 0.4 mV) and *MS@PTLR-NIPs* (-29.3 ± 0.2 mV) was resulted from the repeated ethoxyl (–CH2CH2O-) segments of diisocyanate-terminated polyethylene glycol.

Table S1 Relative content of elements on the surface of each nanosphere

Samples	Content (%)						
	C	O	N	S	Br	Si	Fe
Fe_3O_4	68.2	30.2	0	0	0	0	1.6
MS	33.9	44.7	0	0	0	21.4	0
MS@Br	30.8	45.0	1.9	0	3.4	18.9	0
MS@PTL	62.9	23.9	3.2	2.2	1.7	6.1	0
MS@PTLR	60.1	25.5	4.7	2.2	1.5	6.0	0
MS@PTLR-MIPs	59.2	26.1	5.3	2.2	1.4	5.8	0
MS@PTLR-NIPs	61.3	24.4	6.1	2.5	0.6	5.1	0

Apparently, the disappearance of Fe element peak and the characteristic peaks of Si on the surface of *MS* nanospheres uncovered the successful coated of SiO_2 layer. Furthermore, the appearance of Br characteristic peaks and the reduction of Si content on the surface of *MS@Br* nanospheres indicated the grafting of ATRP reaction sites. The appearance of S characteristic peaks, the increase of C and N contents and a further reduction of Si content on the surface of *MS@PTL* nanospheres revealed the polymerization of *TL* monomer as expected. Moreover, the increase of N content on the surface of *MS@PTLR and MS@PTLR-MIPs* nanospheres illustrated the success of ring-opening of polythiolactones and crosslinking reaction.

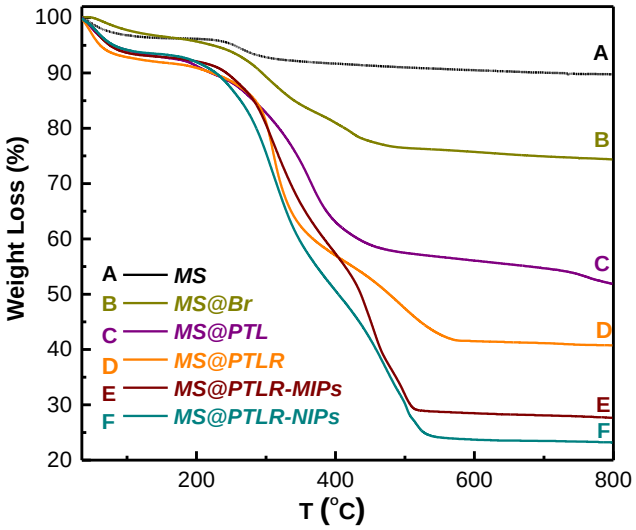

Fig. S4 TGA analysis of different nanospheres. As the Fe_3O_4 nanospheres were coated stepwise with more and more organic components, more and more weight loss was detected upon heating.

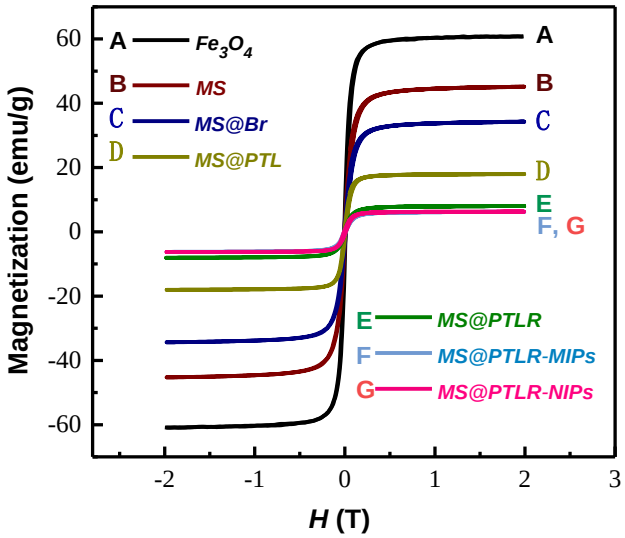

Fig. S5 Hysteresis curves (25 °C) of different nanospheres with magnified hysteresis curves from -2 T to 2 T.

The saturation magnetizations of different nanospheres were weakened compared with the Fe_3O_4 nanospheres, which illustrated the successful chemical modification of the nanospheres step by step. Besides, although the saturation magnetizations of *MS@PTLR-MIPs* (6.25 emu/g) was weaker than that of Fe_3O_4 nanospheres (60.72 emu/g), it still enabled the fast magnetic separation as needed.

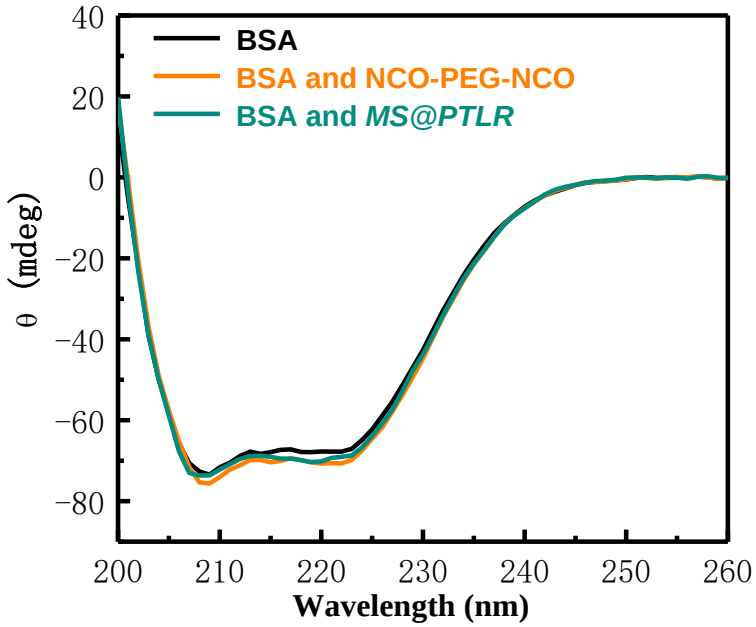

Fig. S6 CD spectra of BSA with different materials incubated in Tris-HCl (pH = 8, 50 mM). The CD signals at approximate 208 and 220 nm ascribing to the α-helical structure of BSA barely changed after co-incubation with the crosslinker and the nanospheres, indicating that BSA was not obviously damaged.

The α-helical of proteins plays a vital role in their performance, and the ideal protein imprinted materials should not destroy the secondary structure of proteins during the imprinting process. Here, the secondary structure of BSA was checked by CD measurement after co-incubation with the crosslinker and *MS@PTLR* nanospheres, respectively.

Determination of binding model of *MS@PTLR-MIPs*. The Langmuir and Freundlich adsorption isotherm models, which represented the monolayer and multi-layer adsorptions, respectively, were used to fit the experimental adsorption of *MS@PTLR-MIPs* (Table S2). The isothermal equations and related parameters were illustrated in Table S3. The R^2 of the Freundlich adsorption isotherm model (0.945) was slightly larger than that of the Langmuir adsorption isotherm (0.939). It suggested that the adsorption process of the imprinted nanospheres involved both monolayer and multi-layer adsorptions.

Table S2. Parameters of adsorption models of *MS@PTLR-MIPs* and *MS@PTLR-NIPs*

Sample	Langmuir			Freundlich		
	Q_{max} (mg/g)	K_L (L/g)	R^2	1/n	K_F (mg/g)	R^2
MS@PTLR-MIPs	294.98	1.81	0.939	0.53	199.38	0.945
MS@PTLR-NIPs	68.25	0.83	0.865	1.10	30.39	0.972

Table S3. Equations of adsorption isotherms

Isotherm model	Langmuir	Freundlich
Linear form equation	$C_e/Q_e = C_e/Q_{max} + 1/(K_L Q_{max})$	$\log Q_e = 1/n \log C_e + \log K_F$

Q_e = the equilibrium adsorption capacity, Q_{max} = the theoretical maximum adsorption capacity, C_e = the concentration of equilibrium solution, K_L = Langmuir adsorption equilibrium constant, n = adsorption intensity, K_F=adsorption capacity

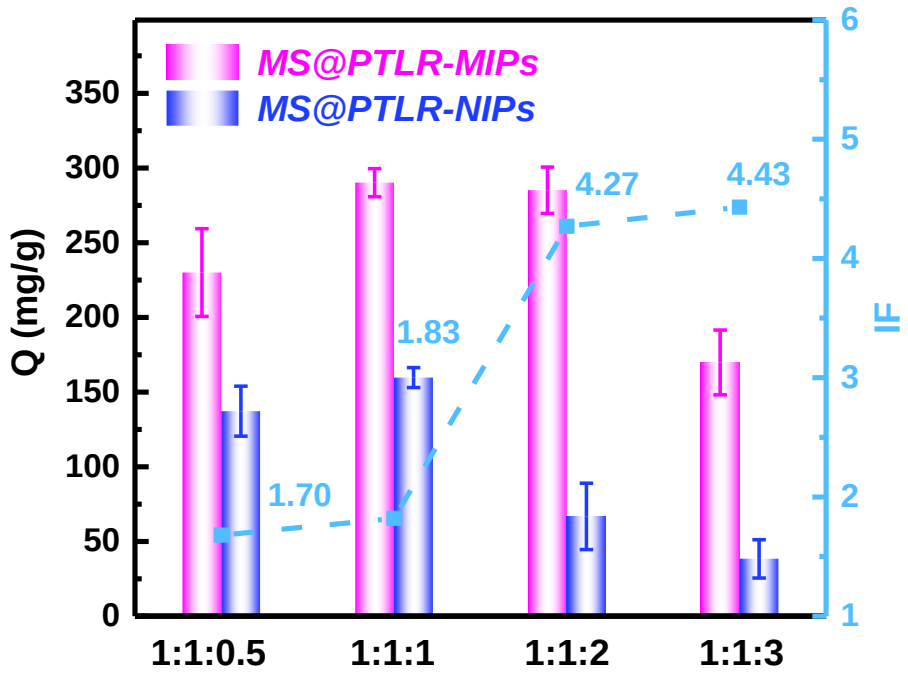

Fig. S7 Rebinding capacity for BSA of *MS@PTLR-MIPs* prepared with different molar ratio of βA, ELA and PLA and their imprinting factors. The error bars represent ± standard deviations, n = 3.

By increasing the ration of PLA, the adsorption of the MIPs was decreased, while the specificity was improved. In contrast, more βA and ELA ligands enhanced the adsorption capacity of MIPs and decreased the specificity. When the molar ratio of βA, ELA and PLA was 1:1:2, the prepared MIPs showed satisfactory adsorption capacity (285 ± 15 mg/g) and the highest IF of 4.27, which indicated that the hydrophobic interactions and hydrogen-bonds between *MS@PTLR-MIPs* with BSA had a synergetic effect at this stage. Therefore, the formed imprinting cavities had closer complexation with BSA and the prepared MIPs could better recognize template BSA.

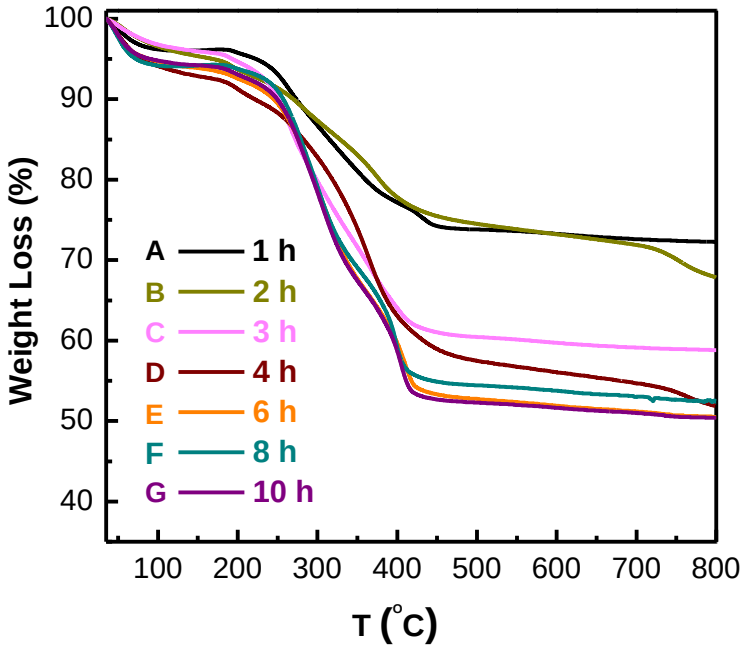

Fig. S8 TGA analysis of *MS@PTL* prepared with different polymerization time.

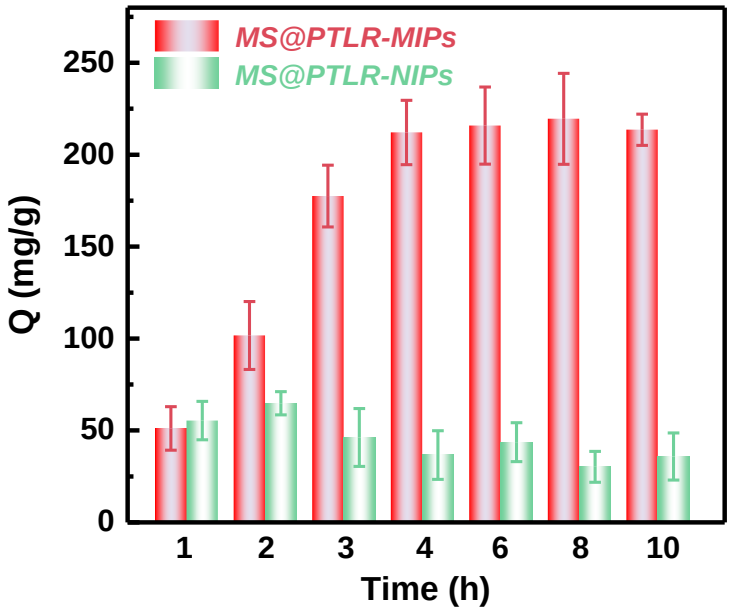

Fig. S9 Rebinding capacity for BSA of *MS@PTLR-MIPs* prepared with different polymerization time of ATRP.

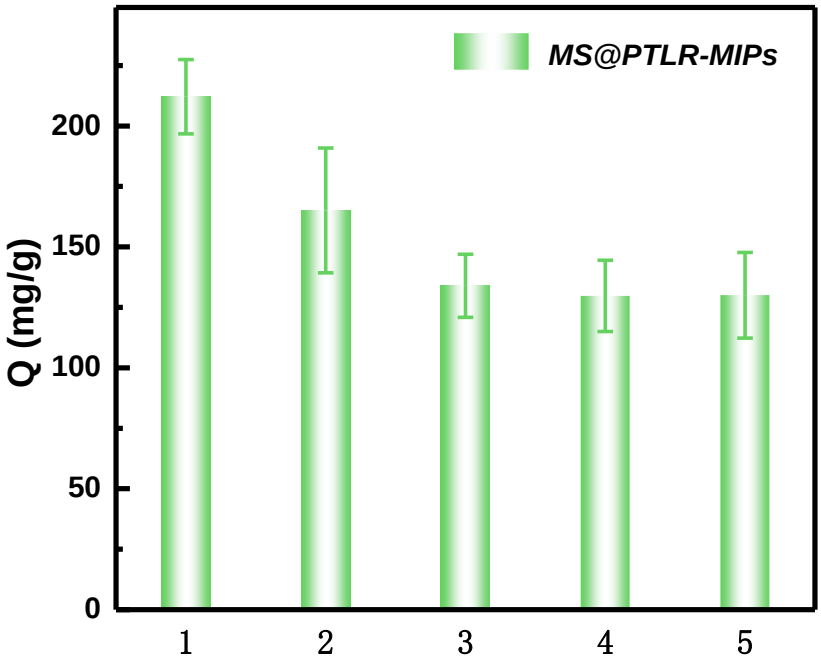

Fig. S10 Reusability of *MS@PTLR-MIPs*. The error bars represent ± standard deviations, n = 3.

3. Reference

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