Electronic Supplementary Information

Covalent molecular imprinting made easy: a case study of mannose imprinted polymer

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1. Materials

Mannose, xylose, cellobiose, 3-aminophenylboronic acid monohydrate, acryloyl chloride and $(NH_4)_2S_2O_4$ were purchased from Beijing J&K Co., Ltd. (Beijing, China). N, N-methylenebisacrylamide (MBA) was obtained from Sigma Chem. Co. (St. Louis, USA). All of the reagents were purchased as the analytical grades and were used without further purification. The water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2. Synthesis of 3-acrylamidophenylboronic acid monomer (APBA)

3-aminophenylboronic acid monohydrate (3.4 g, 0.022 mol) was dissolved in a round bottom flask containing a 1:1 mixture of THF (40 mL) and water (40 mL). $NaHCO_3$ (3.7 g, 0.044 mol) and acryloyl chloride (3.6 mL, 0.044 mol) were added to

the flask at 0-5 °C. The solution was stirred for 4 h, and THF was subsequently evaporated. Then the product was stirred in ethyl acetate for 2 h. After filtering the solid materials, the ethyl acetate layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL), and brine (50 mL) successively. After that, the ethyl acetate layer was concentrated under reduced pressure. Finally the product was purified by recrystallization hot water (85 °C).

^{1}H NMR (300 MHz, DMSO) spectra of APBA was as follow: δ 10.10 (d, J = 16.3 Hz, 67H), 9.99 (s, 2H), 8.17 - 7.90 (m, 150H), 7.90 - 7.76 (m, 107H), 7.61 (d, J = 7.4 Hz, 11H), 7.51 (d, J = 7.3 Hz, 61H), 7.32 (dt, J = 15.4, 7.6 Hz, 70H), 6.58 - 6.38 (m, 69H), 6.37 - 6.18 (m, 69H), 5.74 (dd, J = 10.0, 2.1 Hz, 67H).

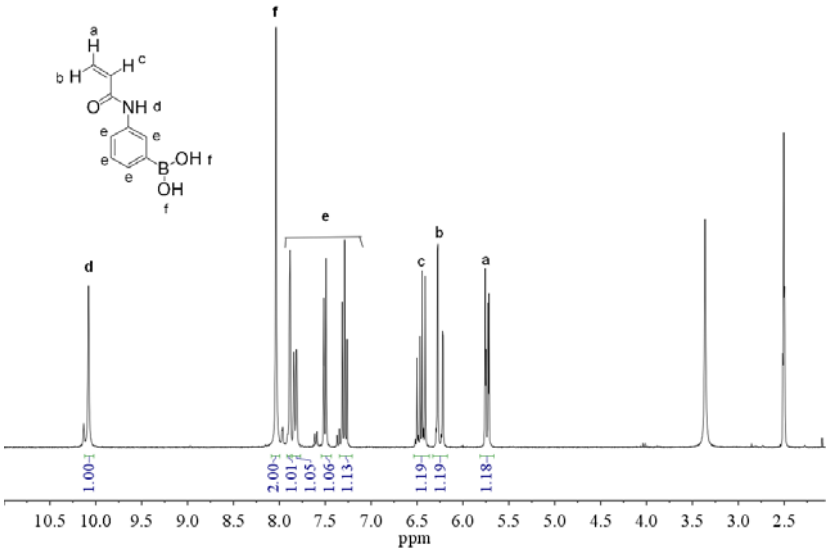

Fig. S1 ^{1}H NMR spectrum of APBA

3. Preparation of the mannose imprinted polymers

APBA (0.0573 g, 0.3 mmol) was dissolved in 25 mL of alkali aqueous solution (Na_2CO_3/$NaHCO_3$, pH 8.0). Then mannose (0.540 g, 3 mmol) was added. The

solution was stirred for 30 min, and MBA (0.231 g, 1.5 mmol) was added. The mixture was sonicated for 10 min and degassed 5 min with N_2. Free radical polymerization was initiated by $(NH_4)_2S_2O_4$ (20 mg) and carried out at 65 $^{\circ}$C for 8 h. The resultant microspheres were collected by centrifugation at 10,000 rpm for 5 min. Then the polymers were washed by acidic aqueous solution (glycine/HCl, pH 2.0) for three times to remove the template. The obtained polymers were finally rinsed with methanol to remove the remaining glycine/HCl (pH 3.0) and then dried under vacuum at 60 °C for 12 h. As a control, non-imprinted polymers (NIPs) were prepared according to the same procedure in the absence of the mannose.

4. Chemical characterization

^{11}B NMR spectra were recorded on a Bruker DRX-500 spectrometer at 160.35 MHz. The chemical shifts of ^{11}B NMR spectra were given relative to external reference of BF_3-Et_2O. ^{1}H NMR spectra were recorded at room temperature in DMSO on BRUKER DPX (300 MHz). FT-IR spectra were recorded on a Nicolet NEXUS-470 Spectrometer (Thermo Fisher Scientific, USA) from KBr pellets at room temperature. Samples (2 mg) were thoroughly ground with KBr and pellets were prepared using a hydraulic press under a pressure of 600 kg cm^{-2}. All spectra were recorded with an accumulation number of 32 scans and a resolution of 8 cm^{-1}.

FT-IR spectra of MIPs containing bound template (MIP-T), MIPs lacking template (MIP) and NIPs are presented in Fig. S1. The fingerprint band (750-1120 cm^{-1}) in the spectrum of mannose template was appeared in the spectrum of the MIP precursor (MIP-T). The spectrum of the MIP was found to be similar to that of NIP

indicating the template had effectively been washed off the MIP precursor.

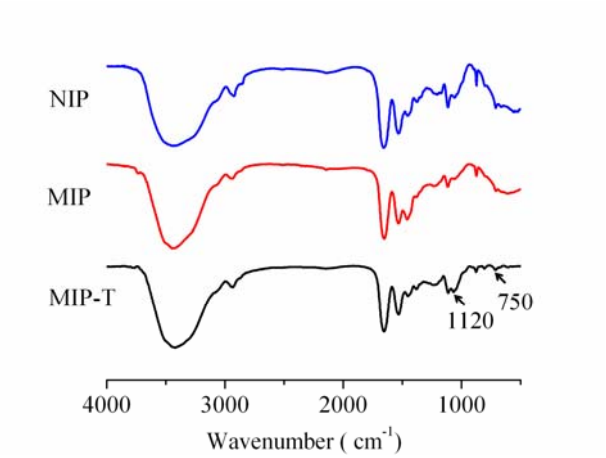

Fig. S2 FT-IR spectra of the MIP-T (without template removal), MIP (with template removal) and NIP.

5. Binding experiments

5 mg of polymer was suspended in 1 mL of sugar (1.1-8.8 mM) in alkali aqueous solution (Na_2CO_3/$NaHCO_3$, pH 10.0) and incubated for 5 min. Then the polymer was centrifuged at 10,000 rpm for 5 min, and the supernatant was used to determine the concentration of unbound sugar by 3,5-dinitrosalicylic acid (DNS) assay. The amount of sugar bound to the polymers was calculated by subtraction of the free fraction from the total amount added. All measurements were performed as triplicates and the error bars indicate the derived standard deviation.

Kinetic adsorption tests were carried out using the MIPs and NIPs (5 mg in each case), which were mixed with 1 mL of sugar solution (3 mM, pH 10.0). The samples were incubated at ambient temperature with agitation. The concentrations of sugar in the supernatants at certain time intervals (5 to 60 min) were monitored using a UV-vis spectrophotometer by DNS assay.

Effect of pH in rebinding buffer on the binding capacity of MIPs was

investigated and the result is shown in Fig. 3S (the initial concentration of the mannose was 10 mM). MIPs showed the maximum binding capacity at pH 10.0 for mannose in aqueous medium.

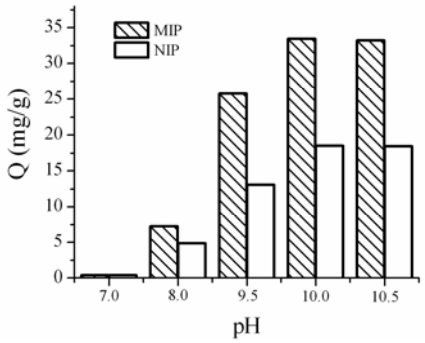

Fig. S3 Influence pH in loading buffer on the binding capacity of MIPs

2.5 Specific recognition experiments of MIPs and NIPs with mannose in the presence of xylose and cellobiose.

The MIPs and NIPs (20 mg in each case) were suspended in 5 mL of sugar mixtures (the concentration of each sugar was 8 mg g^{-1}, pH 10.0). The mixtures were agitated on a rocking table at ambient temperature for 10 min. The concentrations of different sugars in the supernatant were measured by HPLC-RID.

HPLC analysis was performed on a liquid chromatograph (Agilent 1260 Infinity) equipped with a vacuum degasser, quaternary pump (G1311B), and RID connected to a XB-NH_2 column (Ultimate XB-NH2, 250mm*4.6mm*5.0μm, Shanghai, China). The column temperature was 35 °C, and the mobile phase was 75% ACN/H_2O (v/v) with a flow rate at 0.5 mL min^{-1}.