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Supplementary Information

Switchable boronate affinity materials for thermally-modulated capture, separation and enrichment of *cis*-diol biomolecules

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Experimental section

1. Materials and methods

1.1 Materials

N-isopropylacrylamide (NIPAAm) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and purified by recrystallization from *n*-hexane and dried under vacuum. 2-Bromoisobutyryl bromide (97%) and acetyl chloride (98%) were provided by Acros Organics (USA). *N, N, N', N', N''*-pentamethyldiethylenetriamine (PMDETA, 99%) were purchased from J&K Chemical Ltd. (Beijing, China). 3-Aminopropyl silica (5μm; pore size, 300 Å; specific surface area, 0.1 m^2/mg) were obtained from Eka Chemicails (Sweden). Hydrocortisone and dexamethasone were kindly provided by National Institutes for Food and Drug Control (Beijing, China). Adenosine and deoxyadenosine were purchased from Beijing Bio-LAB Materials Institute (Beijing, China). 4-vinylphenylboronic acid (VPBA, 98%) was obtained from Alfa Aesar. Hemoglobin and horse radish peroxidase (HRP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The temperature-responsive PNIPAAm column (100 mm × 2.1 mm, 5μm, 120 Å) had been prepared in our laboratory.[5g] All other chemicals were obtained from Beijing Chemical Reagent Corp. (Beijing, China) and were analytical grade. Cuprous chloride (CuCl) was purified by stirring in acetic acid, washed with ethanol and acetone, and finally dried under vacuum. Triethylamine (TEA), tetrahydrofuran (THF), and 2-propanol were all dried over 4A molecular

sieves before distillation.

1.2 Preparation of 2-Bromoisobutyrate functionalized silica

4.0 g of anhydrous 3-Aminopropyl silica was dispersed into a solution of 4 mL of TEA in 32 mL of THF. After cooling to 0 ºC, the mixture was degassed under vacuum and nitrogen three times. A solution of 2-Bromoisobutyryl bromide (1.0 mL, 8.1 mmol) and acetyl chloride (2.18 mL, 24.3 mmol) in 8 mL of THF was added dropwise into the mixture. The mixture was stirred at 0 ºC for 1 h and then at ambient temperature for 12 h. 2-Bromoisobutyrate-functionalized silica was collected by centrifugation, rinsed with THF, water and ethanol, and dried under vacuum at 50 ºC for 8 h.

Elemental analyses (%): (1) 3-Aminopropyl silica: C, 0.77; H, 0.30; N, 0.27; (2) 2-Bromoisobutyrate-functionalized silica: C, 1.48; H, 0.39; N, 0.23.

1.3 Surface-initiated atom transfer radical polymerization of NIPAAm and VPBA

2-Bromoisobutyrate-functionalized silica (1.2 g), NIPAAm (5.196 g, 46 mmol), VPBA (0.34 g, 2.3 mmol) (the mol ratio of VPBA to NIPAAm was 5 %), PMDETA (405 μL, 1.94 mmol), and a mixture of 2-propanol and water (85:15, 48mL) were added to a round-bottomed flask. The mixture was deoxidated under vacuum and flushed by nitrogen for three times at 0 °C. CuCl (85.7 mg, 0.87 mmol) was added

under the protection of nitrogen flow. The reaction was allowed to proceed overnight at ambient temperature. The copolymer-modified silica were washed by repeated centrifugation and dispersion in ethanol, 50mM EDTA solution, and finally Milli-Q water, and then dried in a vacuum oven at 50 ºC for 5h. The above processes were repeated three times. Parts of modified silica were treated with hydrofluoric acid and then neutralized with sodium carbonate to cleave grafted copolymer for FTIR, ^{1}H NMR, and optical transmittance measurements.

Elemental analyses of grafted silica (%): C, 8.51; H, 1.36; N, 1.07.

1.4 Assays for characterization of copolymer grafted silica and grafted copolymer

Elemental analyses were carried out using an Elemental Vario MICRO CUBE analyzer. Thermogravimetric analysis (TGA) was performed with a METTLER TOLEDO TGA/DSC STAR^{e} system at a heating rate of 10 ºC min^{-1} from ambient temperature to 800 ºC under nitrogen atmosphere. Differential scanning calorimetry (DSC) was measured with a TA DSC Q2000 at a heating rate of 1 ºC min^{-1} at 10-55 °C under nitrogen atmosphere. The silica particles were dispersed in 10mM HEPES (pH 9.8) at room temperature and allowed to reach the full hydrated state before DSC measurement. The hydrated silica was obtained by centrifugation and then placed in a silver sample pan and sealed. FTIR was recorded on a Perkin-Elmer Spectrum BX instrument using KBr pellets. ^{1}H NMR spectra were recorded at ambient temperature in DMSO-d6 by using a Bruke 600 NMR Spectrometer. The

optical transmittance of copolymer solution (0.4 mg mL^{-1}) was determined at 500 nm in water and 10 mM HEPES buffer (pH 9.8) by a UV-visible spectrometer (TU-1810, Pgeneral, China). LCST was defined as the temperature where 50% optical transmittance of copolymer aqueous solution was observed.

1.5 Chromatographic assays for separation and enrichment of steroids, adenosine, and proteins

The copolymer grafted silica was suspended in methanol and then packed into a stainless steel column (100 mm × 2.1 mm i.d.) under a maximum pressure of 50 MPa. The column was connected to a high performance liquid chromatography system, which consists of an Elite P230 pump and a DAD 230 detector. The column temperatures were controlled with a deviation of ±0.1 °C using a Yataikelong YT-15A thermostated water bath. Chromatographic separation of hydrocortisone and dexamethasone was carried out on P(NIPAAm-co-VPBA) column at various temperatures using 10 mM HEPES buffer (pH 9.8) as a mobile phase with a flow rate of 0.2 mL min^{-1}, and the UV detection was at 254 nm. The elution behavior of adenosine and deoxyadenosine was examined on P(NIPAAm-co-VPBA) column and PNIPAAm column, respectively. 10mM HEPES buffer (pH 9.8) was used as a mobile phase with a flow rate of 0.2 mL min^{-1} and UV monitoring at 260 nm. To confirm pH-controlled capture and release property of P(NIPAAm-co-VPBA) column, an additional mobile phase of 10mM HEPES buffer (pH 4.0) was used.

Temperature-modulated capture and release of hemoglobin and horse radish peroxidase on P(NIPAAm-co-VPBA) column were monitored at 420 nm with a flow rate of 0.2 mL min^{-1}.

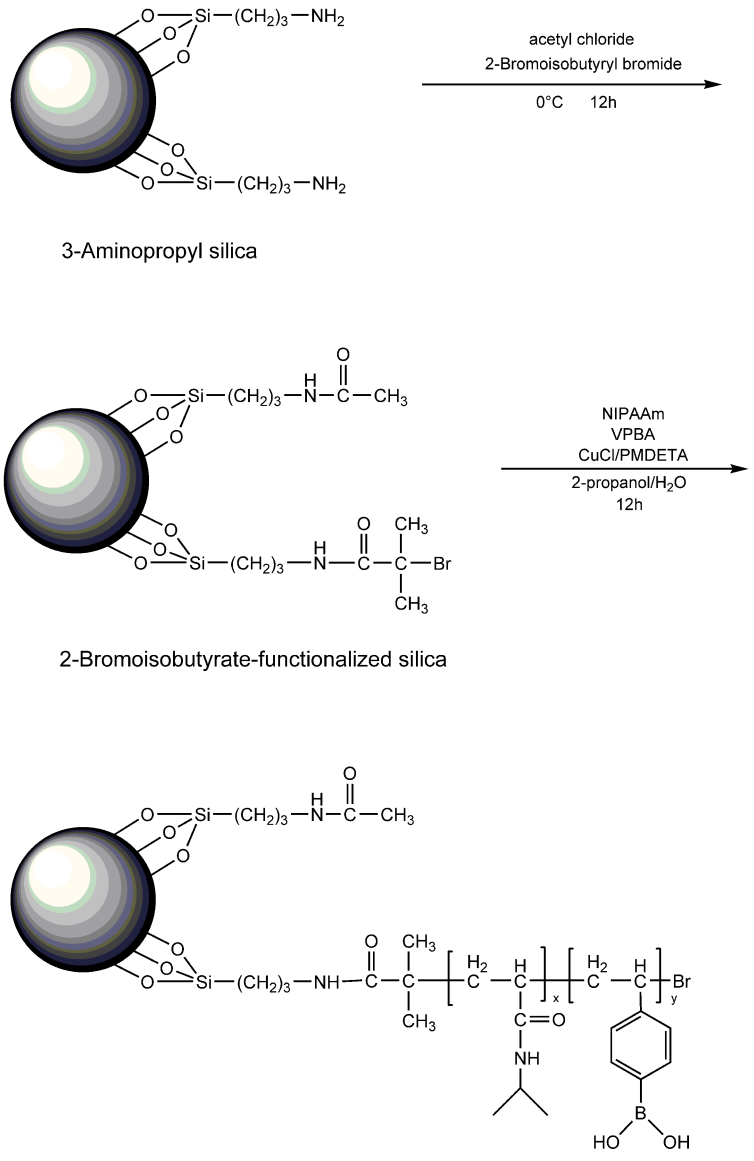

Fig. S1 Schematic preparation of P(NIPAAm-co-VPBA) grafted silica via surface-initiated atom transfer radical polymerization.

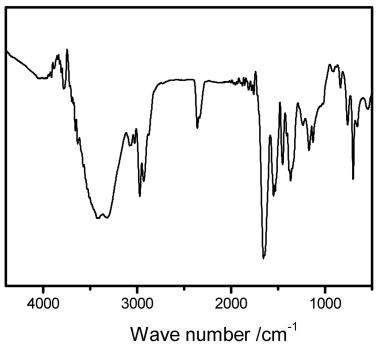

Fig. S2 FTIR spectrum of the grafted P(NIPAAm-co-VPBA) cleaved from silica surfaces.

Fig. S2 shows the FTIR spectrum of the grafted P(NIPAAm-co-VPBA) cleaved from silica surfaces. The main characteristic peaks of NIPAAm unit and end groups are at 1657 cm^{-1} (amide I band, C=O stretching vibration) and 1550 cm^{-1}(amide II band, N-H bending vibration). For VPBA unit, we can also clearly observe the phenyl ring skeletal vibration at 1529 cm^{-1} and C-H stretching vibration at 3027, and 3080 cm^{-1}.

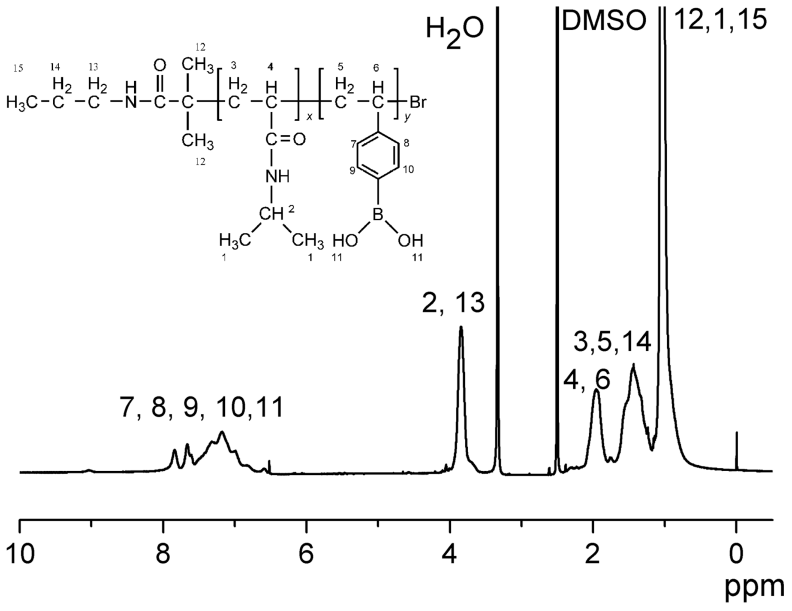

Fig. S3 [1]HNMR spectrum of the grafted P(NIPAAm-co-VPBA) in DMSO. The copolymer was

cleaved from silica surfaces.

As shown in Fig. S3, The chemical shifts of one proton of CH $(CH_3)_2$ in NIPAAm unit and two protons of methylene adjacent to nitrogen in chain ends are found to be located at ~3.8 ppm. The chemical shift of 7.8 ppm corresponds to two protons in $B(OH)_2$ unit. The broad peaks, 6.5-7.7 ppm are assigned to four protons of CH in phenyl group of VPBA unit. The chemical shifts of six protons of CH $(CH_3)_2$ in NIPAAm, six protons of $C(CH_3)_2$ in chain ends, and methyl protons in chain ends are at ~1.0 ppm. The CH (unit x and unit y) in backbone appears at ~1.9 ppm. The chemical shifts of methylene protons in backbone and methylene protons adjacent to methyl in chain ends are at 1.2-1.5 ppm.

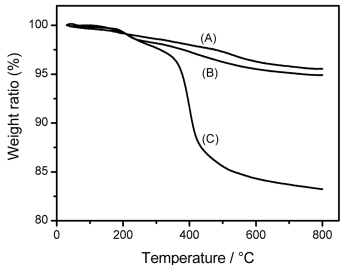

Fig. S4 Thermogravimetric analysis (TGA) curves of silica: (a) 3-Aminopropyl silica, (b) 2-Bromoisobutyrate functionalized silica, and (c) P(NIPAAm-co-VPBA) grafted silica. TGA was performed in nitrogen atmosphere at a heating rate of 10 °C/min.

The ratio of P(NIPAAm-co-VPBA) grafted on silica can be quantitatively

determined by TGA. The weight losses of 2-Bromoisobutyrate functionalized silica, and P(NIPAAm-co-VPBA) grafted silica at 750 °C were 5.03% and 16.55%，respectively. According to previous reports,[5b,f] the ratio of grafted copolymer was calculated to be ~11.52%.

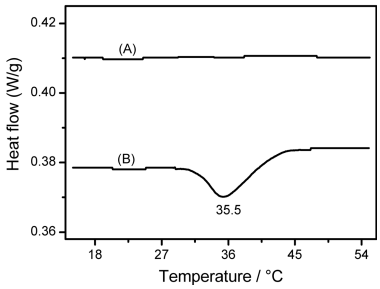

Fig. S5 DSC curves of (a) 2-Bromoisobutyrate functionalized silica and (b) P(NIPAAm-co-VPBA) grafted silica in 10mM HEPES buffer at pH 9.8.

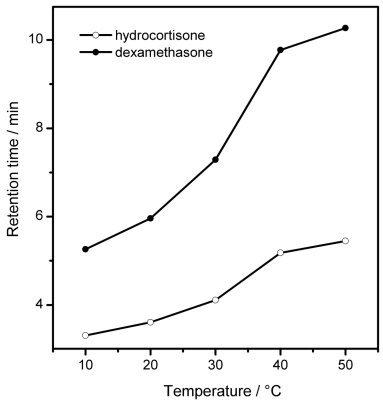

Fig. S6 Temperature-dependent retention time changes of steroids on P(NIPAAm-co-VPBA) grafted silica.

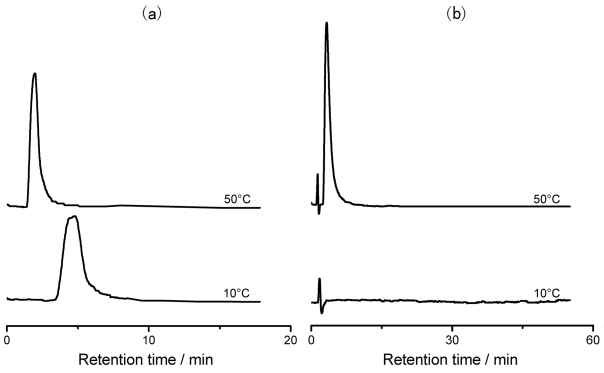

Fig. S7 Temperature-responsive elution profiles of (a) hemoglobin and (b) horse radish peroxidase on P(NIPAAm-co-VPBA) grafted silica column at 10 and 50 ºC. Mobile phase: 10 mM HEPES buffer at pH 9.8; flow rate: 0.2 mL min^{-1}; detection at 420 nm.