Supporting information

for

A unique boronic acid functionalized monolithic capillary for specific capture, separation and immobilization of cis-diol biomolecules

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EXPERIMENTAL SECTION

Materials. *N*, *N*'-methylenebisacrylamide (MBAA) was obtained from Alfa Aesar (Ward Hill, MA). Dimethyl sulfoxide (DMSO) and azobisisobutyronitrile (AIBN) were purchased from Sinopharm Chemical Reagent (Shanghai, China). HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). Human blood serum was kindly donated from Nanjing Railway Hospital. Human alpha1-acid glycoprotein (AGP), Horseradish peroxidase (HRP), lactoferrin from bovine milk, ribonuclease A (RNase A) and ribonuclease B (RNase B) from bovine pancreas, ovalbumin, myoglobin from horse heart, adenosine, guanosine, uridine, cytidine, dodecanol and γ-methacryloxypropyltrimethoxysilane (γ-MAPS) were all purchased from Sigma (St. Louis, MO). AIBN was recrystallized with methanol prior to use. Fused-silica capillaries of 150 μm I.D. and 375 μm O.D. were purchased from Yongnian Optic Plant (Hebei, China). 4-(3-Butenylsulfonyl) phenylboronic acid (BSPBA) was synthesized and characterized according to the methods reported by Li and co-authors.[1] Its selected ^{1}H NMR (500 MHz, CDCl3) data include: δ 8.44 (d, 2H, J=8 Hz), 8.09 (d, 2H, J=8 Hz), 5.78 (m, 1H, J_1=6 Hz, J_2=10.5 Hz, J_3=6.5 Hz, J_4= 10.5 Hz), 5.10 (m, 2H, J_1=10.5 Hz, J_2=6 Hz), 3.25(m, 2H, J_1=8 Hz, J_2=8.5 Hz), 2.54(m, 2H, J_1=7 Hz, J_2=9 Hz, J_3=7 Hz), which is consistent with the literature value. Its pKa value was determined to be 7.0±0.1 by the spectrometric method through monitoring the UV absorbance at 272 nm at a series of pH values, which is also consistent with the literature value.

Instruments and materials. All the chromatographic separations were performed on a TriSep 2100 system (Unimicro Technologies, Pleasanton, CA). Water used in all the experiments was purified by a Milli-Q system (Millipore, Milford, MA). A HPLC pump was utilized to flush the monolithic columns. Scanning electron microscopy (SEM) analyses were performed on a Hitachi FE-SEM S-4800 (Tokyo, Japan). The wetting contact angle of the poly (BSPBA-co-MBAA) layer was tested by the contact angle measurement device OCA30 (Dataphysics, GMb, Filderstadt, Germany).

In order to measure the pore size and surface area of the monolith, a monolithic column was prepared in an empty HPLC column. Then, the monolithic column was washed with methanol by using a HPLC pump for about 10 h until the residual reagents were flushed out. Subsequently, the polymers were flushed out from the columns, cut into small pieces, and dried under vacuum at 50 °C for 24 h. The porous properties of dry polymeric monolith were determined by the Brunauer-Emmett-Teller (BET) method with a accelerated surface area and porosimetry analyzer, Model ASAP 2020 (Micromeritics, Atlanta).

Poly(BSPBA-co-MBAA) layer for contact angle measurement. To evaluate the hydrophility of the poly (BSPBA-co-MBAA) monolith, a poly (BSPBA-co-MBAA) layer was prepared on a glass slide with a shallow fluohydric acid-etched groove by heating the glass slide at 75 °C on an electric hot plate for 6 h. To avoid the effect of the surface roughness of the polymer layers, a polymer layer was prepared without the presence of the poor solvent, dodecanol, in the porogen.

Column pretreatment. In order to covalently anchor the polymer to the capillary wall, the capillary was treated with a vinyl silanizing agent according to a method reported previously.[2] Briefly, the capillaries were rinsed by 0.1 M NaOH, water, 0.1 M HCl, water and methanol for 1 h, respectively, and then dried by passage of nitrogen gas for overnight. Subsequently, a 1:1 (v/v) mixture of γ-MAPS and methanol were flowed through the capillary and kept at 60 °C for 12 h. Finally, the capillaries were rinsed with methanol and water to flush out the residual reagents. Through the above pretreatments, the Si-O-Si-C bonds were formed between the capillary wall and the reactive methacryloyl groups, which were available for subsequent anchor of monolith to the wall during the polymerization reaction.

In situ polymerization. The boronate functionalized monolith was prepared by the thermal initiated polymerization of mixture of monovinyl monomers with a divinyl crosslinker using different systems. 15 mg BSPBA was mixed with 30 mg MBAA, in which binary porogens of 85 μL DMSO and 95 μL dodecanol were added. AIBN (1 wt% of total monomer amounts) was added as polymerization initiator. The mixture was ultrasonicated for 20 min. The remained homogeneous solution was purged with nitrogen for 10 min before it was injected into a pretreated capillary by a syringe. Then both ends

of the capillary were sealed with septa, and the capillary was submerged in a water bath and kept reacting for 12 h at 75 ºC. The resulting monolithic capillary was washed with methanol about 5 h using a HPLC pump to remove unreacted monomers and porogens.

For Brunauer-Emmett-Teller (BET) and Fourier transform infrared (FTIR) characterization of the monolith, a monolithic column was synthesized in a short HPLC column under otherwise identical conditions. The monolithic column was washed with methanol until the residual reagent flushed out. The monolithic bed was moved out of the column for characterization. The obtained monolith was dried under vacuum at 50 °C for 24 h. A small piece was cut from the monolithic rod and used for the BET characterization. A small portion of the monolithic rod was ground into powder and mixed with KBr and the resulting mixture was used for the FTIR characterization.

Breakthrough capacity measurement. The breakthrough capacity of the boronate affinity monolithic column was measured by the frontal chromatography method. A boronate functionalized monolithic column with a total length of 45 cm (effective length 30 cm) was used. First, the monolith column equilibrated with 10 mM phosphate buffer containing 50% (v/v) acetonitrile (pH 7.0) for 0.5 h. A sample solution containing 1 mg/mL catechol and 0.01 mg/mL quinol in the loading buffer was pumped through the column. Elution was carried out with 100 mM acetic acid solution containing 50% acetonitrile (v/v). The column was regenerated by equilibration with the loading buffer.

Chromatographic conditions. A boronate functionalized monolithic column with a total length of 45 cm (effective length 30 cm) was used for the enrichment and separation of small molecules. Samples were injected through an injection valve with an internal 600-nL sample loop. For experiments of small molecules, a three-port splitter was set between the injection valve and the monolithic capillary column, to split the flow into a desirable and stable flow rate. While for experiments of proteins, a boronate affinity monolithic column with total length of 55 cm and an effective length of 45 cm was used. The three-port splitter was set before the injection valve, to ensure a larger actual sample volume is injection and thereby better detection signal. The splitting ratio was set with 99:1. The flow rate from the pump was set at 0.10 mL/min, at which the backpressure was 16.0 MPa. For the detection of

nucleosides, the UV wavelength was set at 260 nm. While for the detection of proteins and other compounds, the wavelength was set at 214 nm.

Reference

1. X. Li, J. Pennington, J.F. Stobaugh, C. Schöneich, *Anal. Biochem.,* 2008, **372**, 227–236.
2. R. Wu, H. Zou, M. Ye, Z. Lei, J. Ni, *Anal. Chem.*, 2001, **73**, 4918-4923.

Supporting Figures

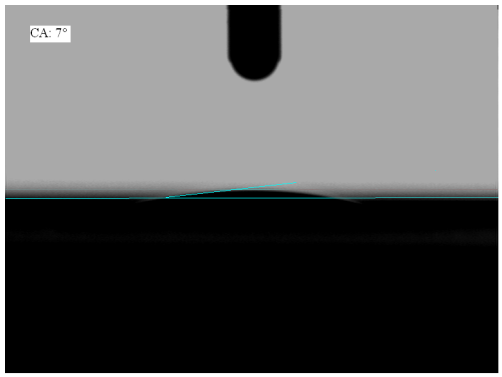

Fig. S1 The wetting contact angle of the poly (SPBA-co-MBAA) layer.

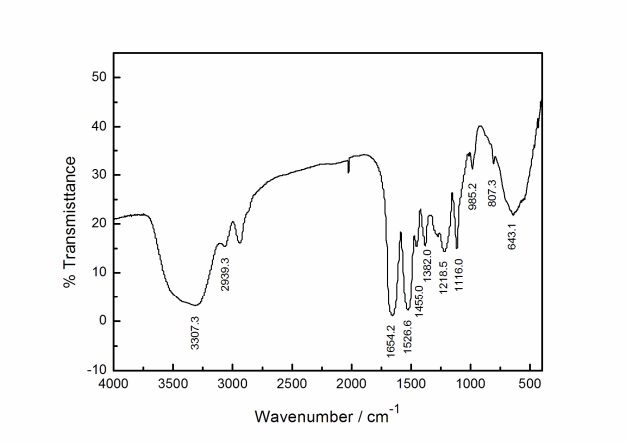

Fig. S2 FTIR spectrum of the poly(BSPBA-co-MBAA) monolith. The characteristic absorbance peaks for the sulfonyl group (1382.0, 1218.5 and 1116.0 cm-1) and the characteristic absorbance peaks for the phenyl group (807.3 and 643.1 cm-1) indicate successful polymerization of BSPBA with MBAA.

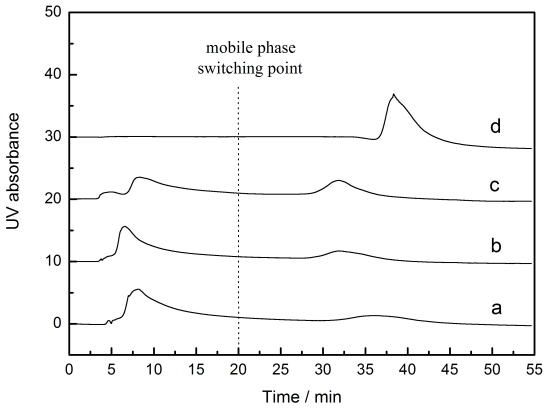

Fig. S3 Effect of salt concentration in the loading buffer on the retention of AGP on the monolithic capillary. When the salt concentration was inadequate, only a portion of AGP was captured by the monolithic capillary. Sample: 1 mg/mL AGP dissolved in 100 mM phosphate buffered solution (pH 7.4) with 500 mM NaCl. Mobile phase: 100 mM phosphate buffer (pH 7.4) containing 0 (a), 100 (b), 250 (c) and 500 (d) mM NaCl, switched to 100 mM HAc at 20 min.

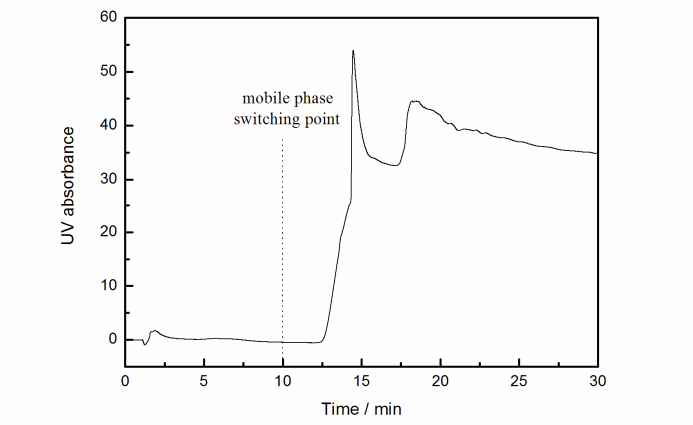

Fig. S4 Secondary separation of lactoferrin on the monolithic capillary. Sample, lactoferrin, 1 mg/mL, dissolved in 50 mM ammonium bicarbonate buffer (pH 7.4). Mobile phase: 50 mM ammonium bicarbonate (pH 7.4), switched to 100 mM HAc (pH 2.7) containing 3 M urea at 15 min.

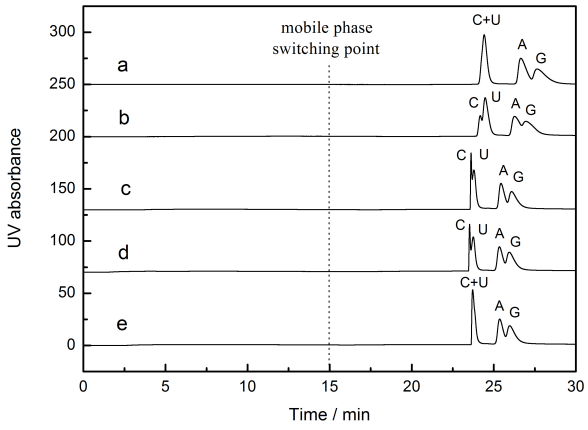

Fig. S5 Secondary separation of nucleosides on the monolithic capillary and the effect of urea concentration in the elution solution. Sample: cytidine (C), uridine (U), adenosine (A), guanosine (G) (0.05 mg/mL each) dissolved in 50 mM ammonium bicarbonate (pH 7.4). Mobile phase: 50 mM ammonium bicarbonate buffer (pH 7.4), switched to 100 mM HAc (pH 2.7) containing different concentration urea at 15 min; urea concentration, a) 0, b) 1, c) 2, d) 3, and e) 4 M.

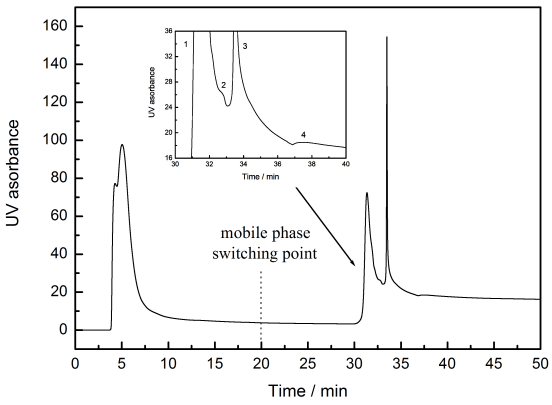

Fig. S6 2D separation of human serum on the monolithic column. Mobile phase: 100 mM phosphate buffer (pH 7.4) switched to 100 mM HAc (pH 2.7) containing 3 M urea at 15 min; Sample: human serum was diluted ten times by 100 mM phosphate buffer solvent (pH 7.4).