Supporting information

for

Synthesis of a SiO_2/TiO_2 hybrid boronate affinity monolithic column for specific capture of glycoproteins

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Experimental sections

1 Materials and Instrumentation

1.1 Reagents and materials

Bovine serum albumin (BSA, M_w 66.4 kDa, p*I* 4.9), Cytochrome c (Cyt c, M_w 13.0 kDa, p*I* 9.8), myoglobin (Mb, M_w 16.7 kDa, p*I* 6.9), ovalbumin (OVA, M_w 46 kDa, p*I* 4.7) and lactoferrin (LF, M_w 80.4 kDa, p*I* 8.7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human immunoglobulin G was purchased from Bomei Biotechnology (Hefei, China). Monoclonal antibodies, WuTac（mouse anti-human T lymphocytes CD25） and WuT 4 （mouse anti-human T lymphocytes CD4), were obtained from Wuhan Institute of Biological Products (Wuhan, China). 4-Vinylphenylboronic acid (VPBA), adenosine and 2-deoxyadenosine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetraethoxysilane (TEOS), *γ*-methacryloxypropyltrimethoxy silane (*γ*-MAPS), *N*-(*β*-aminoethyl)-*γ*-aminopropyltriethoxysilane (AEAPTES) were products of Wuhan University Silicone New Material (Wuhan, China). 2, 2-Azobisisobutyronitrile (AIBN) was obtained from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized in methanol prior to use. Cetyltrimethylammonium bromide (CTMAB), sodium dihydrogenphosphate (NaH_2PO_4), disodium hydrogenphosphate (Na_2HPO_4), quinol, catechol, resorcinol, tetrabutyl orthotitanate (TBOT), HPLC grade methanol were obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were of analytical grade or better. Fused-silica capillaries (250 μm i.d. and 375 μm o.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

1.2 Instrumentation and Methods

All chromatographic experiments were performed on a LC-20AD miniaturized Liquid Chromatography (Shimadzu, Kyoto, Japan) with a UV Detector 2500 (Knauer, Berlin, Germany). A silica/titania hybrid affinity monolithic column with a total length of 25 cm was used. A flow rate of 0.01 mL/min was used and the UV absorbance was monitored at 214 nm unless otherwise stated. The signal was fed into an EC2000 chromatography workstation (Dalian Elite Analytical Instruments, Dalian, China). Samples were injected through an injection valve with a sample loop which was made of a total length of 10 cm fused-silica capillary (50 μm i.d. and 375 μm o.d.).Fourier transform infrared (FTIR) spectra were acquired on a Thermo Scientific Nicolet IS10 FT-IR spectrometer (Waltham, MA, USA). Morphology of the monolithic column (SEM) and the elemental species energy-dispersive X-ray spectroscopy (EDX) analysis were examined by Quanta 200 scanning electron microscopy system (Philips-FEI, Eindhoven, Netherlands). The surface area and mesopore size distribution were measured by nitrogen adsorption/desorption experiments with an ASAP-2020 Plus surface area and pore size analyzer (Micromeritics, Norcross, GA, USA). Prior to measurement, the silica/titania hybrid boronate affinity monolith was synthesized in a tube of 0.5 cm i.d. with the same procedure as that for the preparation of the capillary monolith. Then the monolithic materials were cut to 0.5-cm-long pieces, and the physically adsorbed substances, EtOH and porogens were extracted by MeOH using Soxhlet extraction. The specific surface area values were calculated according to the Brunauer-Emmett-Teller (BET) equation. The pore size was evaluated from the desorption branches of isotherms based on Barrett-Joyner-Halenda (BJH) model. X-ray diffraction (XRD) pattern was recorded on an X-ray diffractometer (XRD, PANalytical, Netherlands) with Cu Kα radiation (λ = 1.5406 Å) operated at 40 kV and 50 mA. The data were

collected in a step of 0.026° s^{-1} with the scattering angles (2θ) ranging from 10° to 80°. X-ray photoelectron spectroscopy (XPS) measurement was taken by a KRATOS XSAM 800 electron spectrometer (KRATOS, MAN, UK) using a radiation source of Mg Kα radiation with the energy of 1253.6 eV, 17 mA × 11.5 kV. The vacuum in the analysis chamber was always better than 7 × 10^{-7} Pa.

2. Preparation of the silica/titania hybrid boronate affinity monolithic column

The capillary (250 μm i.d. and 370 μm o.d.) was rinsed with 0.1 M NaOH for 2 h, 0.1 M HCl for 2 h, water for 1 h, ethanol for 1 h, and dried under a stream of nitrogen for 3 h. Before synthesis, the inner surface of the capillaries was treated with γ-MAPS and then dried with nitrogen. The pretreated capillary was stored at 40℃ prior to use. The monolithic column was prepared as follows: 225 μL ethanol, 11.1 mg CTMAB and 6.0 mg 4-VPBA were mixed in 1.5 mL eppendorf tube and ultrasonicated for 5 min at 0℃. A volume of 160 μL TEOS, 40 μL AEAPTES, 40 μL TBOT and 10 μL γ-MAPS were added to the above mixture and ultrasonicated for 30 s at 0℃. 85 μL H_2O was then dripped into the mixture and ultrasonicated for 5 s at 0℃. Finally, the homogenous solution was filled into the pretreated capillary to an appropriate length with a syringe. After each end of the capillary was sealed with a piece of rubber, the capillary was incubated at 40℃ for 12 h and then 60℃ for 12 h. The obtained monolithic column was flushed with MeOH and water to remove the residual monomers and porogens.

In our previous study,[1] we prepared a boronate-functionalized silica hybrid monolithic column by 4-VPBA, γ-MAPS, TEOS and AEAPTES, in which an optimal ratio was found to give the best performance. In present approach, TBOT was added as one precursor. Because of the differences in rates of hydrolysis and condensation among different precursors,[2] the synthetic conditions for silica/tinania hybrid monolithic structures had to be optimized further. Various ratios of TBOT / H_2O were tested to prepare the monolith. Homogenous monolith with good permeability was obtained when the ratio of TBOT / H_2O was 40 : 85 v/v (shown in Table S1).

3. Permeability ($K_{p,F}$) of the silica/titania hybrid boronate affinity monolithic column

A linear relationship between pressure and mobile phase velocity was always observed. Permeability ($K_{p,F}$) was further determined by liquid chromatography in 100% MeOH. Calculations were made according to Darcy's equation for porous beds [3,4] in relation to the superficial velocity:

$$K_{p,F} = (F/S)(\eta L)/\Delta P \qquad (1)$$

where, F is the flow rate of mobile phase, S is the internal cross section of the capillary column, η is the viscosity of the mobile phase, L is the column length and ΔP is the column pressure drop. Mobile phase MeOH; η_{MeOH} =0.503 × 10^{-3} Pa·s (30℃); flow rate: 3 μL min^{-1}; L=25 cm; $S = \pi r^2$ (r=125 μm); ΔP =30 psi. The permeability ($K_{p,F}$) of the monolithic column was about 32.3×$10^{-14}$$m^2$.

4. Dynamic binding capacity measurement

4.1 Dynamic binding capacity for small molecule

To determine the dynamic binding capacity of the silica/titania hybrid boronate affinity monolithic column for small molecules in neutral media, frontal analysis of the monolith was carried out with

0.053 mg mL^{-1} quinol and 0.184 mg mL^{-1} catechol dissolved in 10 mM phosphate buffer (pH 7.0). The binding capacity (Q) was calculated by the equation (2).[5]

$$Q = (t_R - t_0)\, F \times C / V \qquad (2)$$

Quinol was not captured by the monolith and thus eluted first (t_0) as the dead time marker, but catechol was captured by the monolith and eluted later (t_R) until the monolith was saturated. C is the catechol concentration and V is the volume of monolithic column. F is the flow rate of elution buffer. In brief, the silica/titania hybrid boronate affinity monolithic column was equilibrated with a loading buffer. The sample solution containing quinol or catechol was pumped through the column. After equilibration, elution of catechol and regeneration of the monolith were carried out with the elution buffer. The dynamic binding capacity of the silica/titania hybrid affinity monolith for catechol was 20.20 μmol L^{-1}

4.2 Dynamic binding capacity for macromolecule

To determine and compare the dynamic binding capacity of the silica/titania hybrid boronate affinity monolith for macromolecules, frontal analysis of the monolith was carried out with 0.1036 mg mL^{-1} BSA and 0.2357 mg mL^{-1} IgG dissolved in 10 mM phosphate buffer (pH 7.0). The binding capacity (Q) was calculated by the equation (2) as same as 4.1**.** Under neutral media, while BSA was not captured by the monolith and thus eluted first (t_0) as the dead time marker, IgG was captured by the monolith and eluted later (t_R) until the monolith was saturated. The molecular weight of IgG is about 150 kDa, and the dynamic binding capacity of the hybrid affinity monolith for IgG was calculated to be 0.013 μmol L^{-1}.

References

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Supporting Table and Figures

Table S1 Effect of synthesis parameters on the formation of silica/titania hybrid boronate affinity monolithic column

	TBOT (μL)	H_2O (μL)	State of column	Back pressure (psi)	Permeability K ($\times 10^{-14}$ m^2)
M 1	40	60	Sol state		
M 2	40	65	Slack		
M 3	40	70	Slack, slightly detached		
M 4	40	75	Shrunken		
M 5	40	80	Slightly shrunken		
M 6	40	85	Homogeneous	30	32.3
M 7	40	90	Homogeneous	Block	

Flushed with MeOH (η_{MeOH} =0.503×10^{-3} pa·s, 30℃); flow rate, 3 μL min^{-1}; L=25 cm; $S=\pi r^2$ (r=125 μm)

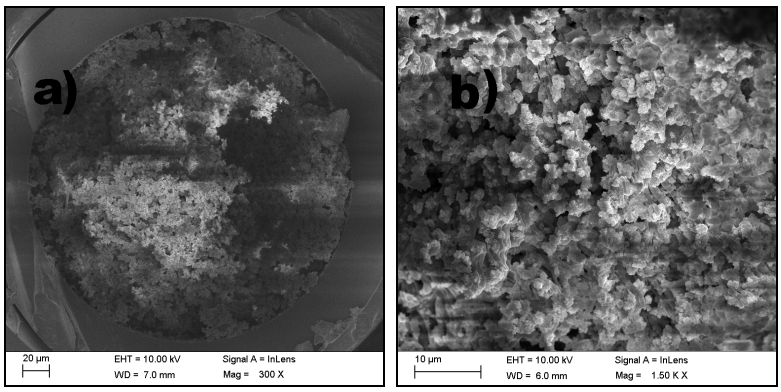

Fig. S1 SEM photographs of the cross-section of the monolithic column. (a) 300×, (b) 1500×

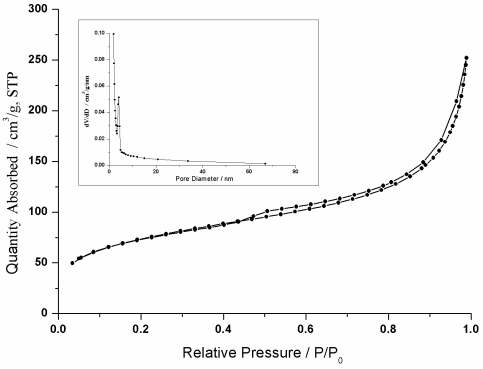

Fig. S2 N_2 adsorption-desorption isotherm of the monolith material and BJH pore-size distribution curve (inset) of monolith

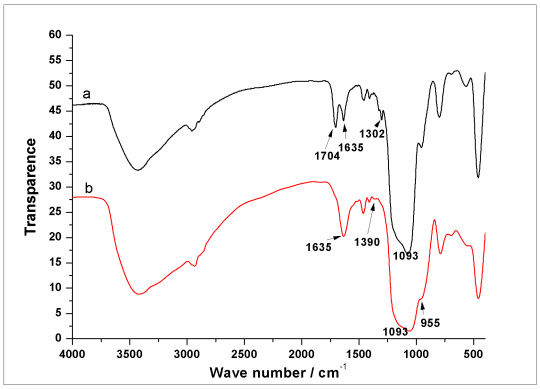

Fig. S3 FT-IR spectra of (a) the boronate-functionalized silica hybrid monolithic material and (b) the boronate-functionalized silica/titania hybrid monolithic material.

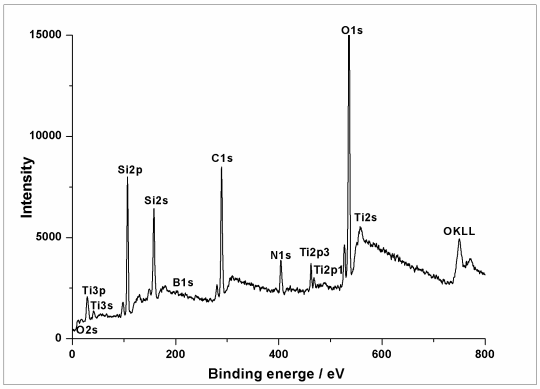

Fig. S4 XPS spectrum of the monolithic material

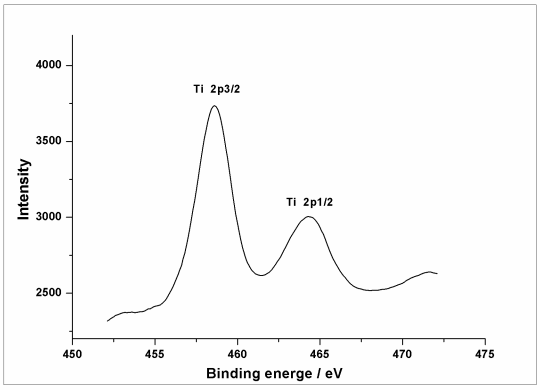

Fig. S5 XPS spectrum of Ti 2p3 of the monolithic material

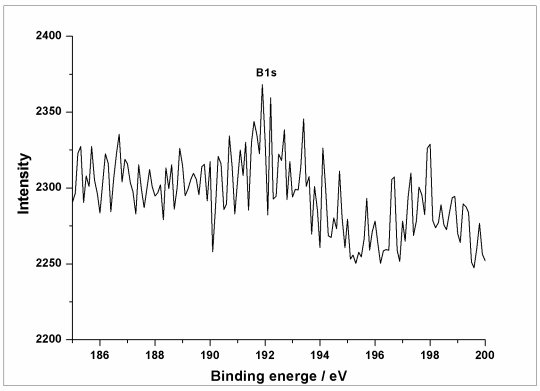

Fig. S6 XPS spectrum of B 1s of the monolithic material

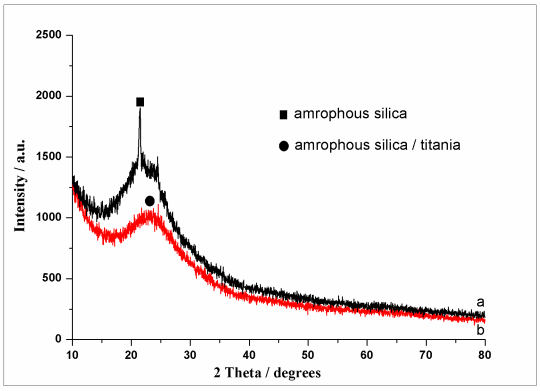

Fig. S7 The wide-angle XRD pattern of (a) the boronate-functionalized silica hybrid monolithic material and (b) the boronate-functionalized silica/titania hybrid monolithic material

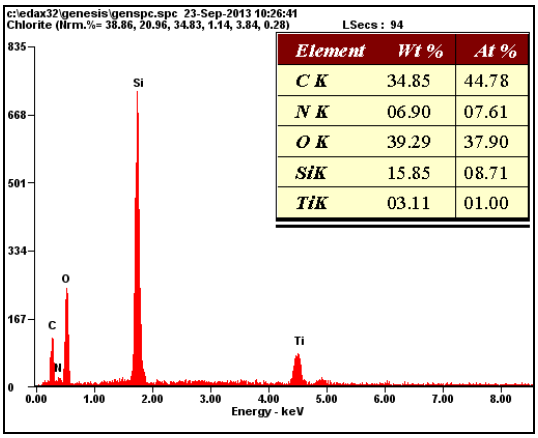

Fig. S8 The EDX spectrum and the elemental composition (the insert table) of the monolith. The Wt % and At % in the inset table referred to weight and atom percentage, respectively.

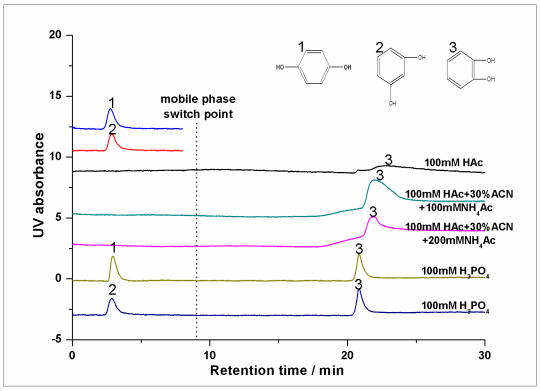

Fig. S9 Chromatographic retention of quinol, resorcinol and catechol on the silica/titania hybrid boronate affinity monolithic column. Mobile phase: 10 mM sodium phosphate buffer (pH 7.0), the mobile phase was changed to the eluted solution at 9 min. Flow rate: 0.01 mL min^{-1}; Detection wavelength: 275 nm. Sample: quinol, resorcinol and catechol were dissolved in the 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.1 mg mL^{-1} each

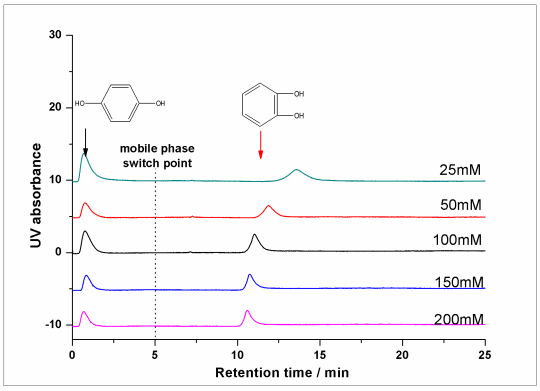

Fig. S10 Chromatographic retention of quinol and catechol on the silica/titania hybrid boronate affinity monolithic column. Mobile phase: 10 mM sodium phosphate buffer (pH 7.0), the mobile phase was changed to H_3PO_4 at 5 min. Flow rate: 0.01 mL min^{-1}; Detection wavelength: 275 nm. Sample: quinol and catechol were dissolved in the 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.1 mg mL^{-1} each.

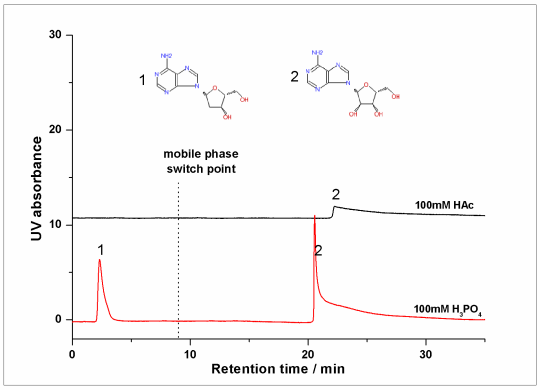

Fig. S11 Chromatographic retention of adenosine and deoxyadenosine on the silica/titania hybrid boronate affinity monolithic column. Mobile phase: 10 mM sodium phosphate buffer (pH 7.0), the mobile phase was changed to HAc or H_3PO_4 at 9 min. Flow rate: 0.01 mL min^{-1}; Detection wavelength: 254 nm. Sample: adenosine and deoxyadenosine were dissolved in the 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.2 mg mL^{-1} each.