Supporting information

for

One-pot synthesis of organic-inorganic hybrid affinity monolithic

column for specific capture of glycoproteins

Zian Lin,* Jilei Pang, Huanghao Yang, Zongwei Cai, Lan Zhang,* and Guonan Chen

Ministry of Education Key Laboratory of Analysis and Detection for Food Safety,

Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food

Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350002, China

* To whom correspondence should be addressed: Dr. Zi An Lin, phone/fax
86-591-22866135, e-mail: zianlin@fzu.edu.cn;

EXPERIMENTAL SECTION

Materials and Chemicals. VPBA and poly (ethylene glycol) (PEG, M_n=10 000) were purchased from Alfa Aesar (Ward Hill, MA, USA). TMOS and γ-MAPS were products of Chemical Factory of Wuhan University (Wuhan, China). 2,2-Azobisisobutyronitrile (AIBN) was obtained from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized in methanol prior to use. Nonglycoproteins (bovine serum albumin (BSA; Mw 66.4 kDa, pI 4.9), bovine hemoglobin (BHb; Mw 64.5 kDa, pI 7.1), Cytochrome c (Cyt c; Mw 13.0 kDa, pI 9.8), lysozyme (Lyz; Mw 13.4 kDa, pI 10.7), and myoglobin (Mb; Mw 16.7 kDa, pI 6.9)) and glycoproteins (horseradish peroxidase (HRP; Mw 40 kDa, pI 5.5-9.0), ovalbumin (OVA; Mw 46 kDa, pI 4.7), and transferrin (TRF, Mw 80 kDa, pI5.5)) were obtained from Shanghai Lanji Co. Ltd. (Shanghai, China). Quinol, catechol, 2'-O-Methyladenosine (2'-OMe-A) and N6-Methyladenosine (m6A) were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were of analytical grade or better. Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA). Standard stock solutions of biomolecules and proteins at a concentration of 1.0 mg/mL were dissolved and diluted with deionized water, and then stored at 4 ℃ before use. Egg white sample was commercially available. Fused-silica capillaries (75 μm i.d. ×375 μm o.d.) were obtained from Reafine Chromatography Ltd (Hebei, China).

Apparatus and Measurements. An organic-inorganic hybrid affinity monolithic column with a total length of 50 cm (effective length 25 cm) was used unless otherwise stated. All chromatographic experiments were performed on a TriSep-2100 pressurized capillary electrochromatography (pCEC) (Unimicro Technologies, Pleasanton, CA, USA) with a UV

detector. Phosphate buffer/ACN at pH 8.0 (mobile phase A) and acetate buffer/ACN at pH 3.6

(mobile phase B) with different ratios and concentrations were prepared for gradient elution. A

flow rate of 0.05 mL/min was used unless otherwise stated and the UV absorbance was monitored

at 214 nm. Samples were injected through an injection valve with an internal 2 μL sample loop. A

four-port splitter was set between the injection valve and the monolithic column to split the flow

into a desirable and stable flow rate. Since the splitting ratio was set at 200:1, the actual injection

volume was about 10 nL. Gel electrophoresis for the separation of proteins was carried out by

regular SDS-PAGE system with 12% resolving gel and 5% stacking gel according to the operating

manual (Bio-Rad, Hercules, CA, USA)

Preparation of the VPBA-Silica Hybrid Affinity Monolithic Column. In order to covalently

anchor the silica matrix to the capillary wall, the inner surface of the capillary was treated with a

vinyl silanizing agent according to the previous procedure.[1] The schematic preparation of

VPBA-silica hybrid affinity monolithic column was illustrated in Figure 1A. A prehydrolyzed

mixture was prepared by mixing and stirring acetic acid (0.01 M, 5 mL), PEG10 000 (540 mg),

TMOS (1.8 mL), and γ-MAPS (0.3 mL) for 1 h at ice bath to form a homogeneous solution. Then,

20 mg of VPBA and 1 wt % AIBN dissolved with 80 μL diethylene glycol (DEG) were added into

0.5 mL of the resulting hydrolyzed mixture with 15 min sonication. Whereafter, the mixture was

manually injected into the pretreated capillary to an appropriate length with a syringe. When both

ends of the capillary were sealed with two pieces of rubbers, the capillary was incubated at 40 ℃

and 75 ℃ for 12 h, respectively. The obtained the VPBA-silica hybrid affinity monolithic column

was flushed with MeOH to remove the residual monomers and porogens.

In-tube SPME Procedures and SDS-PAGE Analysis. A simple in-tube SPME device was same

as described in our previous work.[2] Briefly, the as-prepared VPBA-silica hybrid affinity monolith (total length 25 cm) after cutting off the open part (from the detection window to the outlet end) was mounted on a six valve in the position where the loop was originally positioned. 0.1 mL egg white sample was diluted to 10 mL with 20 mM phosphate buffer (pH 8.0) unless otherwise stated. At the position A, the diluted sample solution was continuously pumped through the monolithic column at a flow rate of 0.005 mL/min for 40 min. Subsequently, the monolithic column was equilibrated with pH 8.0, 20 mM phosphate buffer for 30 min to remove the overloading glycoproteins and the nonspecifically adsorbed nonglycoproteins. After switching to the position B, the glycoproteins was eluted with a buffer containing 20% (v/v) ACN in 20 mM acetate buffer (pH 3.6). Finally, the eluate was collected and the total volume was about 50 μL for further SDS-PAGE analysis.

Dynamic binding capacity. To determine and compare the dynamic binding capacity of the VPBA-silica hybrid affinity monolith for nonglycoprotein and glycoprotein in basic (acidic) media, frontal analysis of the monolith was carried out with 0.2 mg mL^{-1} BSA and OVA dissolved in phosphate buffer (pH 8.0), respectively. The binding capacity (Q) was calculated by the equation[3]:

$$Q = (V_B - V_0) \times C / m \qquad\qquad (1)$$

Where V_B (mL) is the 10% background volume, V_0 is the dead volume of pCEC apparatus system, C is the protein concentration (mg mL^{-1}) and m is the weight of monolithic rod (g). In brief, the VPBA-silica hybrid affinity monolith was equilibrated with a loading buffer (elution buffer). A sample solution containing 0.2 mg mL^{-1} BSA (or 0.2 mg mL^{-1} OVA) was pumped through the column. After equilibration, elution of OVA and regeneration of the monolith were carried out

with the elution buffer.

Reference

(1) R. A. Wu, H. F. Zou, H. J. Fu, W. H. Jin, and M. L. Ye, Electrophoresis 2002, 23, 1239-1245.

(2) Z.A. Lin, J.L. Pang, Y. Lin, H. Huang, Z.W. Cai, L. Zhang, and G. N. Chen, Analyst, 2011,

DOI:10.1039/C1AN15180K.

(3) M.Y. Wang, J. Xu, X. Zhou, and T.W. Tan, J. Chromatogr. A, 2007, 1147, 24-29.

Supporting Table and Figures

**Table 1 Effect of Synthesis Parameters on the Formation of VPBA-Silica Hybrid Affinity Monoliths[a]**

Column	TMOS (mL)	γ-MAPS (mL)	The ratio of TMOS/γ-MAPS(v/v)	Polycondensation/ Copolymerization Temp (℃)	VPBA (mg)	Back Pressure (MPa)
A1[a]	1.8	0.2	9/1	40/75	20	10.4
A2[a]	1.8	0.3	6/1	40/75	20	16.6
A3[a]	1.8	0.6	3/1	40/75	20	>23.0
A4[a]	1.8	0.9	2/1	40/75	20	Too hard to pump
B1[b]	1.8	0.3	6/1	40/75	10	11.0
B2[b]	1.8	0.3	6/1	40/75	15	12.7
B3[b]	1.8	0.3	6/1	40/75	25	>23.0
C[c]	1.8	0.3	6/1	40/60	20	No copolymerization

a: Flushed with methanol; flow rate, 5 µL/min; (a) the effect of the ratio of TMOS/γ-MAPS; (b) the effect of VPBA content; (c) the effect of temperature.
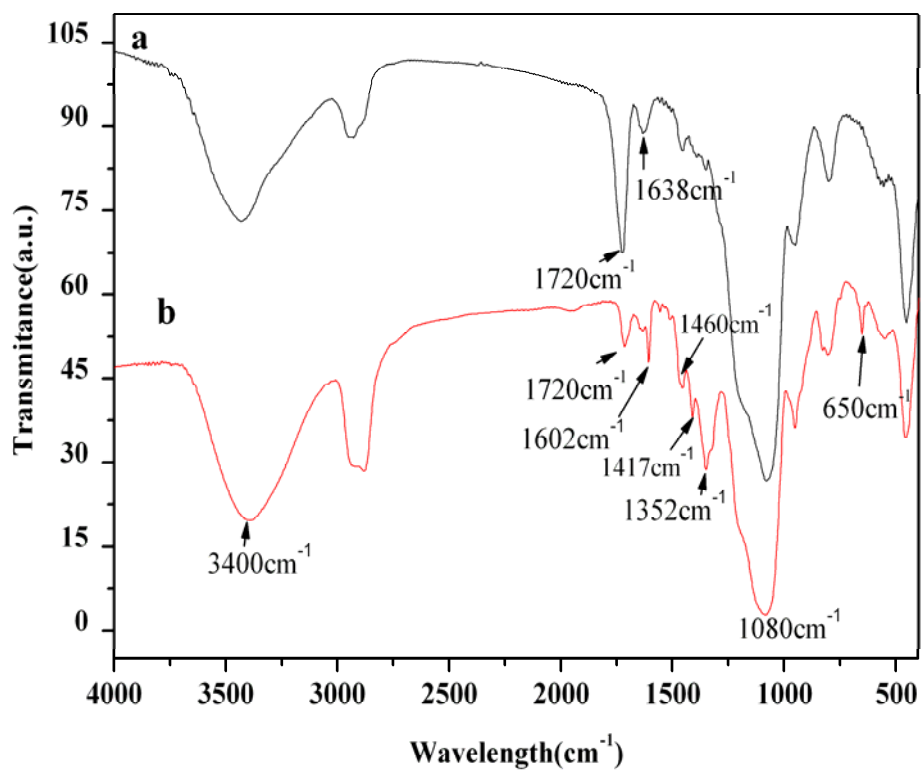
Fig. S1 FT-IR spectra of (a) the silica-based monolithic skeleton after polycondensation reaction and (b) the VPBA-silica hybrid affinity monolith after polymerization reaction.
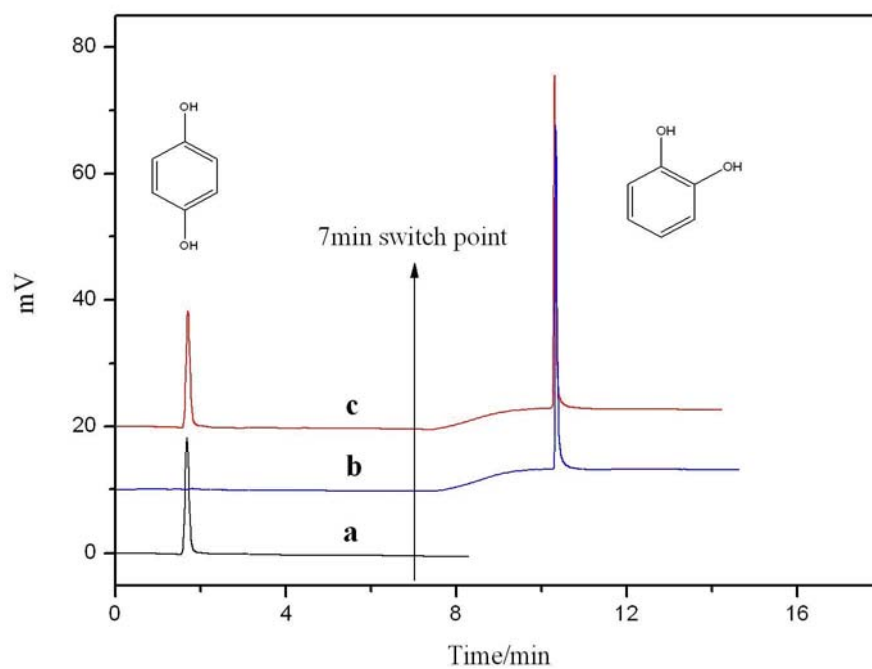
Fig. S2 Chromatographic retention of quinol and catechol on the VPBA-silica hybrid
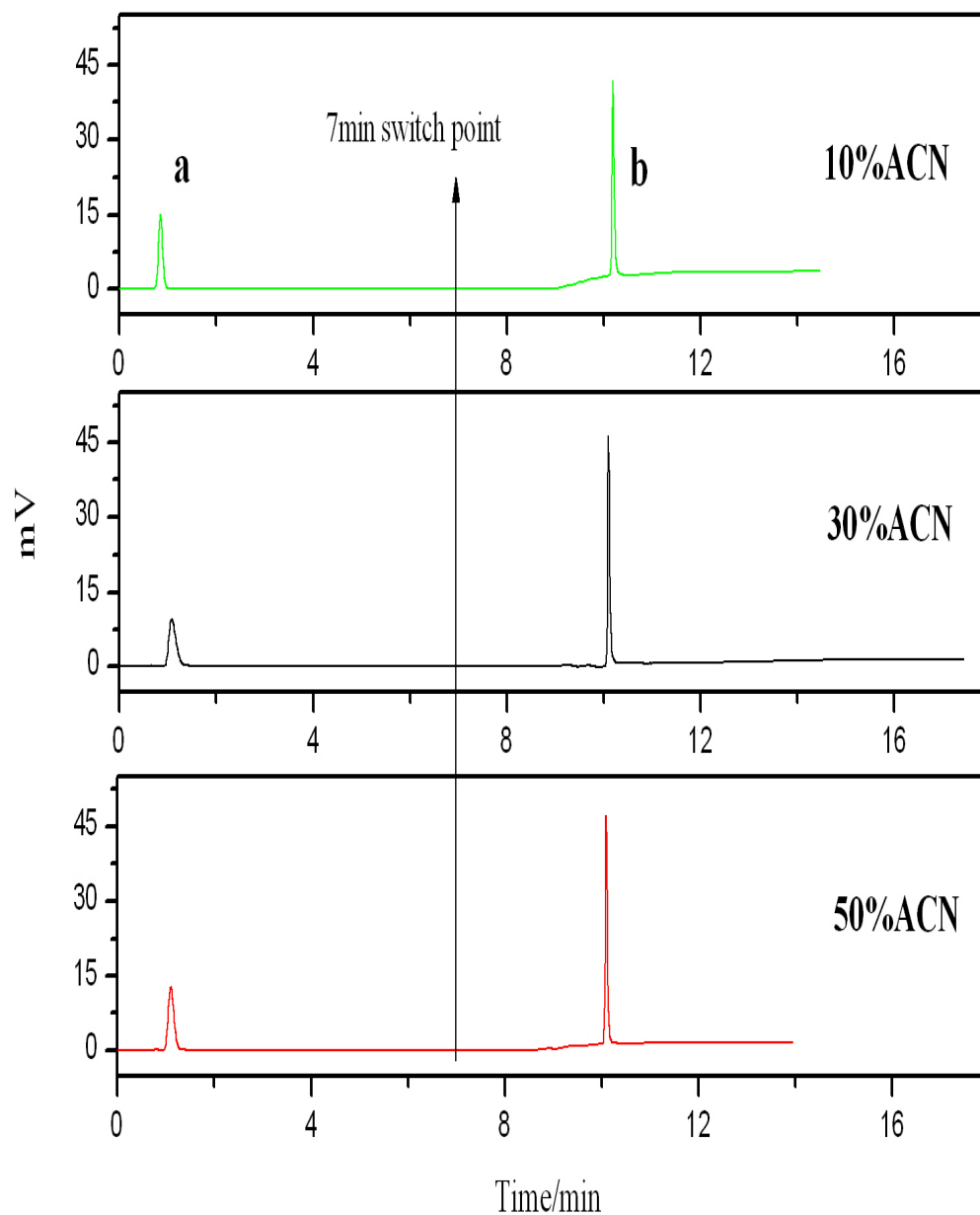
affinity monolith

Experimental conditions: loading buffer (pH8.0, 20 mM phosphate buffer containing

30% ACN) was adopted from 0~6 min, and then switched to elution buffer (pH3.6, 20

mM acetate buffer containing 30% ACN) at 7 min; Flow rate: 0.05 mL/min;

Detection wavelength: 214 nm. a: 1.0 mg/mL quinol; b: 1.0 mg/mL catechol; c: 1.0

mg/mL quinol + catechol.
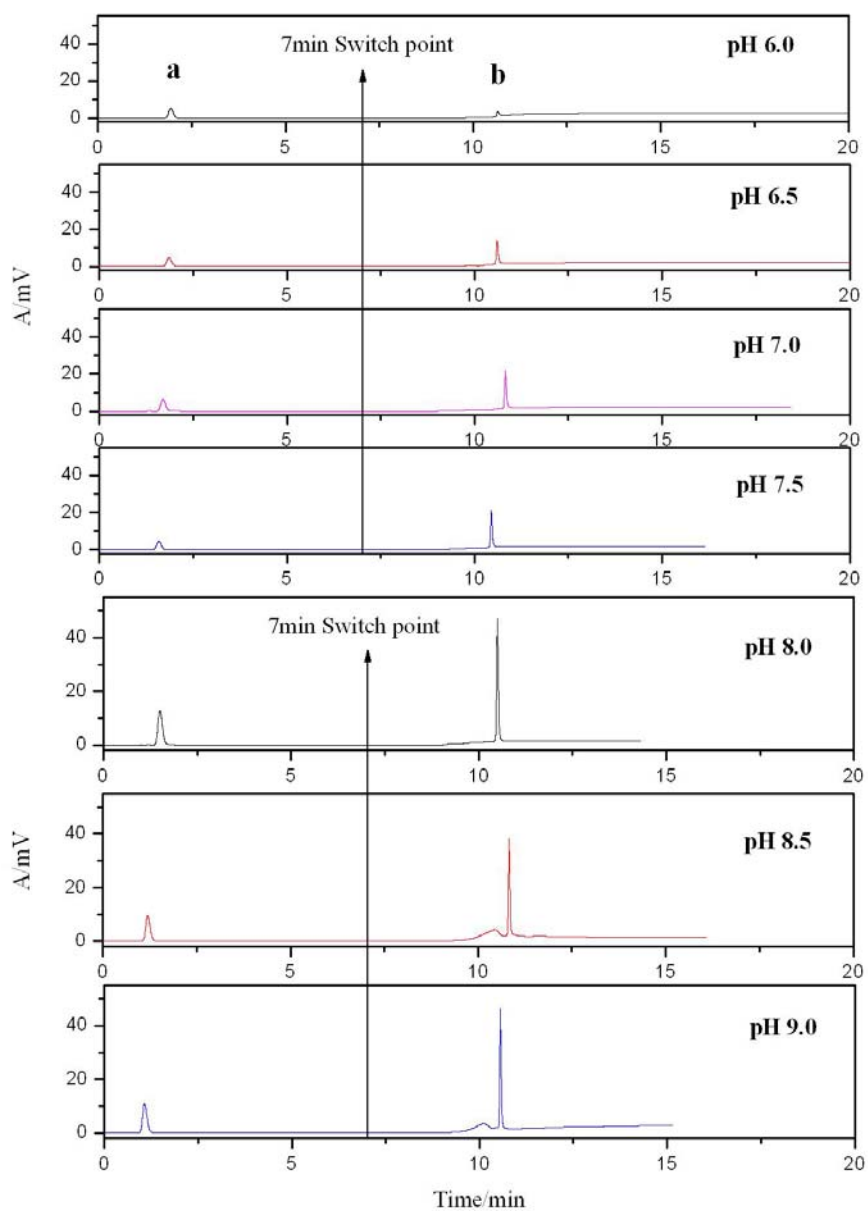
Fig. S3 Effect of pH value of loading buffer on the retention of quinol and catechol on the VPBA-silica hybrid affinity monolith

Experimental conditions: loading buffer (pH6.0-9.0, 20 mM phosphate buffer containing 30% ACN) was adopted from 0~6 min, and then switched to elution buffer (pH3.6, 20 mM acetate buffer containing 30% ACN) at 7 min; Flow rate: 0.05 mL/min; Detection wavelength: 214 nm. Sample: 1.0 mg/mL quinol + catechol.

Fig. S4 Effect of organic modifier content on retention of catechol and quinol on the hybrid affinity monolithic column

Experimental conditions: loading buffer (pH8.0, 20 mM phosphate buffer containing

10-50% ACN) was adopted from 0~6 min, and then switched to elution buffer (pH3.6,

20 mM acetate buffer containing 10-50%(ACN) at 7 min; Flow rate: 0.05 mL/min;

Detection wavelength: 214 nm. Sample: 1.0 mg/mL quinol + catechol.
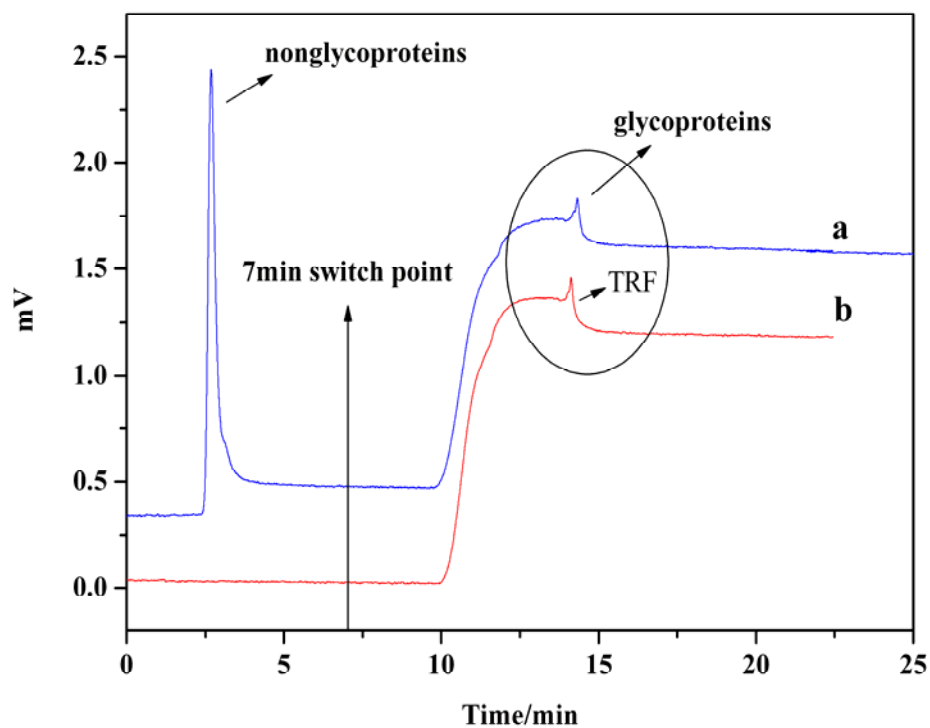
Fig.S5 Specific capture of glycoproteins from egg white sample by the VPBA-silica

hybrid affinity monolith

Experimental conditions are same as Fig.4; (a) egg white sample diluted to 1000-fold;

(b) 0.2 mg/mL TRF standard;