Electronic Supplementary Information

for

Affinity-tunable dual-mode specific recognition of glycoproteins via boronate affinity-based controllable oriented surface imprinting

Shuangshou Wang, Jin Ye, Zijun Bie, and Zhen Liu*

*To whom correspondence should be addressed. E-mail:zhenliu@nju.edu.cn

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1. MATERIALS AND METHODS

1.1. Reagents and materials

γ-Methacryloxypropyltrimethoxysiliane (γ-MAPS), glycidyl methacrylate (GMA), ribonuclease A (RNase A), ribonuclease B (RNase B), ovalbumin (OVA), transferrin (TRF), myoglobin (Myo), sinapinic acid (SA) were purchased from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA) was from Shuangliu Zhenglong Chemical and Biological Research Laboratory (Sichuan, China). Horseradish peroxidase (HRP) and ammonium persulfate (APS) were from Shanghai Lingfeng Chemical Reagent (Shanghai, China) 3-Aminopropyltriethoxysilane (ATES), dopamine hydrochloride and 4-formylphenylboronic acid (FPBA) were purchased from J&K Chemical (Shanghai, China). 1-dodecanol, *N,N'*-methylenebisacylamide (MBAA), *N,N*-diisopropylethylamine (DIPEA), *o*-Benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), sodium cyanoborohydride, 1-hydroxy-7-azabenzotriazole (HOAt) and *m*-aminophenylboronic acid monohydrate (APBA) were purchased from Alfa Aesar (Tianjin, China). 3,3,5',5'-tetramethylbenzidine dihydrochloride (TMB) was from Sinopharm Chemical Reagent (Shanghai, China). Sodium dodecyl sulfate (SDS) was obtained from Bio-Rad (Hercules, CA, USA). 3-Carboxy-benzoboroxole was in-lab synthesized according to previously reported method.[1] All other chemicals were of analytical or HPLC grade. Water used in all experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA). Fused-silica capillaries of 150 μm i.d. and 375 μm o.d. were purchased from Yongnian Optic Plant (Hebei, China). Amino-modified sensors for binding kinetics assay were kindly provided by Pall Fortebio Analytics (Shanghai, China).

1.2. Instruments

Scanning electron microscopy (SEM) characterization was performed on a FE-SEM S-4800 instrument (Hitachi, Tokyo, Japan). Atomic Force microscopy (AFM) characterization was performed on a 5500 AFM instrument (Agilent Technologies, Santa Clara, CA, USA). All chromatographic separations, except repeatability test, were carried out on a TriSep-2100 pCEC (Unimicro Technologies, Pleasanton, CA, USA) system equipped with a UV-absorbance detector. The detection wavelength was set at 214 nm. Repeatability test was performed on an UltiMate 3000 nanoflow LC

system (Dionex, Sunnyvale, CA, USA) equipped with an LPG-3x00 micropump and a VWD-3400 variable-wavelength UV-vis absorbance detector with a 3 nL flow cell and a WPS-3000 automatic sampler. The detection wavelength was set at 214 nm.

MALDI-TOF MS analyses were carried out on a 4800 TOF/TOF Analyzer (AB Sciex, Darmstadt, Germany) equipped with a TOF/TOF ion optics, a 200-Hz Nd:YAG laser, controlled by the 4000 Series Explorer Software (V3.5.28193). Spectra were acquired in the positive linear ion mode between 10 000 and 200 000 m/z with fixed laser intensity (7000). Totally 500 laser shots per spot were accumulated for each spectrum. The accelerating voltage was 20 kV. The matrix used was 10 mg/mL SA in 0.1% trifluoroacetic acid:acetonitrile (70:30, v/v). Equivalent amounts (0.5 μL) of the sample and SA were sequentially dropped onto the MALDI plate for MS analysis. Data were processed using Data Explorer Software Version 4.9 (AB Sciex, Darmstadt, Germany).

The binding properties of HRP-imprinted layers were analyzed on an Octet Red96 instrument from FroteBio (Menlo Park, CA, USA), which is capable of reading signals from eight sensors simultaneously. HRP-templated MIP layers were prepared onto the sensor surfaces according to the proposed method. Binding parameters were obtained by fitting the signals for a series of template solutions of appropriate concentrations using the software associated with the instrument.

1.3. Preparation of poly(dopamine), poly(APBA) or poly(APBA-co-dopamine) modified substrates for property characterization

Aqueous solutions consisted of 1) 2.0 mg/mL dopamine and 0.6 mg/mL APS, 2) 1.6 mg/mL APBA and 0.6 mg/mL APS, and 3) 2.0 mg/mL dopamine, 1.6 mg/mL APBA and 1.2 mg/mL APS, were separately used to prepared polymeric coatings on glass slides for property characterization. The self-polymerization reactions were sustained for 24 h at room temperature. After reaction, the glass slides were rinsed with water to remove remaining reagents and naturally dried in air.

1.4. Preparation and characterization of HRP-imprinted glass slides

The glass slides were first immobilized with boronic acid. The glass slides were treated with 0.1 M NaOH and 0.1 M HCl for 1 h each, followed by rinse with water until neutralization (pH 7.0), and then dried in a ventilated oven at 65°C for 30 min. Then the glass substrates were immersed in a 1:1 (v/v) mixture of ATES and THF at 80°C for 10 h, followed by rinse with methanol to remove residual reagents. After that, the amino-modified glass slides were immersed in a methanol solution containing 1 mg/mL FPBA at 25°C for 10 h, vibrated during reaction. After the reacting solution was removed, the glass slides were immersed into 1 mg/mL sodium cyanoborohydride methanol solution at 25°C for another 10 h and vibrated during reaction. Finally, the glass slides were washed with methanol and water to remove residual reagents, and then dried in an oven.

The boronic acid-functionalized glass slides were immersed into a solution containing 1 mg/mL HRP and 0.1 M phosphate buffer (pH 8.5) for 10 min to form a thin template layer, followed by rinsing with 0.1 M phosphate buffer, pH 8.5. Then the template-anchored glass slides were immersed into an aqueous mixture containing 2.0 mg/mL dopamine, 1.6 mg/mL APBA and 1.2 mg/mL APS at room temperature for 70 min. Finally, the glass sides were rinsed with 0.1 M HAc containing 10% SDS (w/v) to remove the template.

To prepare non-imprinted polymer (NIP) covered slides for comparison, the processing procedure was the same except that no template was immobilized onto the boronic acid-functionalized glass slides.

1.5. Preparation of poly(APBA-co-dopamine)-coated glass slides for thickness controllability characterization

Five groups of quartz glass slides were first washed with water and methanol several times to clean the surface and dried in oven. These glass slides were then immersed into a mixture of 2.0 mg/mL dopamine, 1.6 mg/mL APBA and 1.2 mg/mL APS, dissolved in 0.1 M phosphate buffer (pH 8.5) at room temperature for 0.5, 1.0, 1.5, 2.0, and 2.5 h, respectively. After self-polymerization reaction, all the glass slides were washed with water to remove the remaining reagents, and then dried at room temperature.

1.6. Colorimetric detection

To prevent solutions added to the detection spot from dispersing and cross-contaminating, detection spots were defined by printing a cycle array with hydrophobic ink on the target-imprinted slides and non-imprinted slides under investigation. In this way, solutions added to each spot were confined within the predefined area. 10 μL samples containing HRP at different concentrations were added to the spots to incubate for 10 min. After that, each spot was rinsed with 20 μL, 0.1 M phosphate buffer, pH 8.5. Then each spot was supplemented with 10 μL TMB staining solution. After reaction for 10 min, the array was recorded with a digital camera.

1.7. Preparation of poly(APBA-co-dopamine)-coated glycoprotein-imprinted monolithic capillaries

3-Carboxybenzoboroxole-functionalized monolithic capillaries were first prepared according a method reported previously [1] as base columns. The base capillaries were conditioned with 0.1 M phosphate buffer (pH 8.5) for 5 min. Then, 20 μL template solution containing 1 mg/mL template dissolved in 0.1 M phosphate buffer (pH 8.5) was injected into the base monolithic capillaries to allow incubation for 10 min at room temperature to covalently anchor the template. After that, the capillaries were filled with 20 μL imprinting solution containing 2.0 mg/mL dopamine, 1.6 mg/mL APBA and 1.2 mg/mL APS which dissolved in 0.1 M phosphate buffer (pH 8.5) and kept at room temperature for 30, 70 and 90 min for the templates RNase B, HRP and TRF, respectively. Finally, the capillaries were rinsed with 0.1 M HAc containing 10% SDS (w/v) to remove the templates. The procedure for non-imprinted monolithic columns was the same except that no templates were immobilized onto the benzoboroxole-functionalized monolithic capillaries.

1.8. Preparation of poly(APBA-co-dopamine) coated HRP-imprinted monolithic column without template pre-immobilization

The procedure was nearly the same as the oriented imprinting approach except that HRP was not pre-immobilized onto the benzoboroxole-functionalized monolithic capillary and the imprinting solution contained not only dopamine, APBA and APS at the same concentrations as above but also 1 mg/ml HRP.

1.9. Preparation of poly(dopamine) or poly(APBA)-coated HRP-imprinted monolithic capillaries

The procedure was the same as the one using poly(APBA-co-dopamine) as imprinting coating, except that the imprinting solutions contained either dopamine or APBA.

1.10. Preparation of HRP-imprinted layers on the Fortebio sensors for binding kinetics assays

Amino-immobilized sensors were firstly immersed into a methanol solution containing 5 mg/mL 4-formylphenylboronic acid and 1 mg/mL sodium cyanoborohydride, then reacted at room temperature for 10 h. After that, the sensors were washed with water for several times to remove the remaining reagent, and then immersed into a 1 mg/mL HRP solution prepared with 0.1 M phosphate buffer (pH 8.5) and incubated for 30 min at room temperature. Then, the sensors were washed with 0.1 M phosphate buffer (pH 8.5) for several times. Consequently, the sensors were immersed into an imprinting solution containing 2.0 mg/mL dopamine, 1.6 mg/mL APBA and 1.2 mg/mL APS, which dissolved in 0.1 M phosphate buffer (pH 8.5), and kept at room temperature for 70 min. Finally, the sensors were washed with 0.1 M HAc solution containing 10% SDS (w/v) to remove the templates. Non-imprinted sensors were prepared as controls using the same procedure except that no templates were immobilized onto the sensors.

1.11. MALDI-TOF MS analysis of serum treated with TRF-imprinted monolithic capillary

The serum sample was diluted by 20 times with ultrapure water, then frozen immediately and stored at -20 °C. The samples were thawed at room temperature prior to analysis. A piece of TRF-imprinted monolithic capillary with effective length of 33 cm was used as an extraction column. 10 μl of diluted serum sample was pumped through the column slowly and the flow-out part was collected in a 200-μL centrifuge tube. Then, the column was washed with 20 μL ultrapure water to completely remove uncaptured species within the column. Finally, the column was rinsed with 20 μL 0.1 M HAc, and the flow out liquid was collected in a centrifuge tube for MALDI-TOF MS analysis.

1.12. Repeatability test of HRP-imprinted monolithic capillary

The repeatability of HRP-imprinted column was tested, results as shown in Fig. S7. RSD (n=10) of retention time and peak height was 0.11% and 0.23%, respectively, showing excellent repeatability of the HRP-imprinted monolithic capillary.

Reference:

1. Li, H., Wang, H., Liu, Y. & Liu, Z. A benzoboroxole-functionalized monolithic column for the selective enrichment and separation of cis-diol containing biomolecules. Chemical Communications 48, 4115-4117 (2012).

2. Supporting Data

Table S1. Relative content of C, O, B and N in different polymers measured by XPS (N as a standard).

Polymer	Relative content			
	C	O	B	N
Poly(APBA)	10.11	7.13	0.98	1.00
Poly(dopamine)	8.18	8.37	0.00	1.00
Poly(APBA-co-dopamine)	10.05	1.85	0.61	1.00

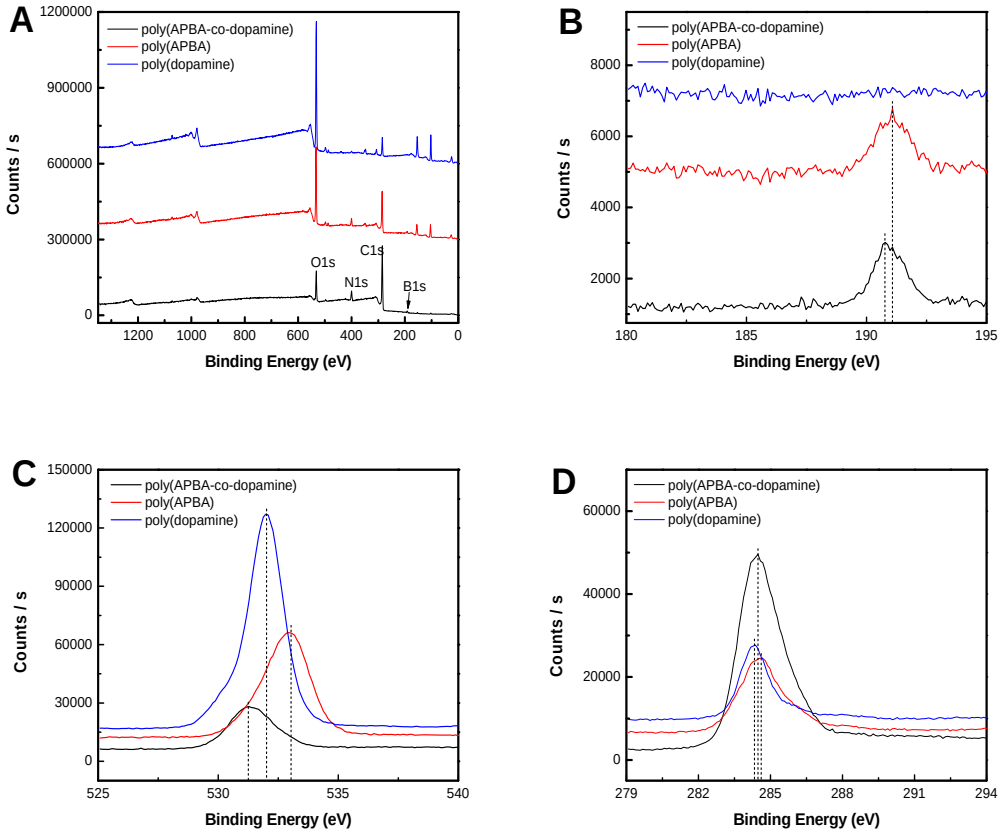

Figure S1. The X-ray photoelectron spectroscopy survey scan (A), the boron element (B), the oxygen element (C) and the carbon element (D) of the substrates modified with different monomers.

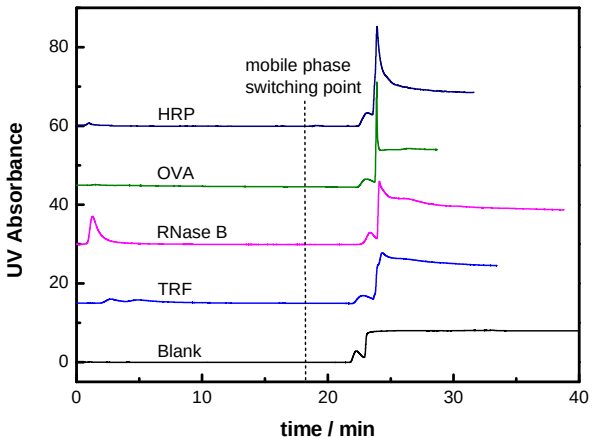

Figure S2. Chromatographic retention of glycoproteins on HRP-imprinted column without pre-immobilization of the template. Mobile phase: 0.1 M phosphate buffer, pH 7.4, switched to 0.1 M acetic acid at 18 min. Blank sample, 0.1 M phosphate buffer, pH 7.4. Sample: 1 mg/mL protein dissolved in 0.1 M phosphate buffer, pH 7.4.

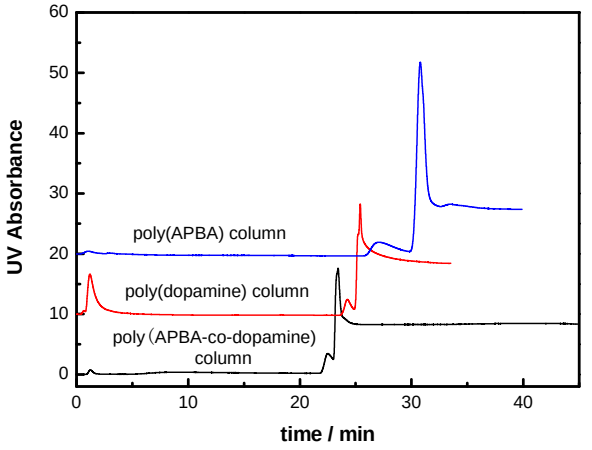

Figure S3. Chromatographic retention of HRP on HRP-imprinted monolithic columns using poly(APBA), poly(dopamine), and poly(APBA-co-dopamine) as the imprinting coating. Mobile phase: 0.1 M phosphate buffer, pH 7.4, switched to 0.1 M acetic acid at 22 min for poly(APBA)-coated column and 20 min for poly(dopamine)- and poly(APBA-co-dopamine)-coated columns. Sample: 1 mg/mL HRP dissolved in 0.1 M phosphate buffer, pH 7.4.

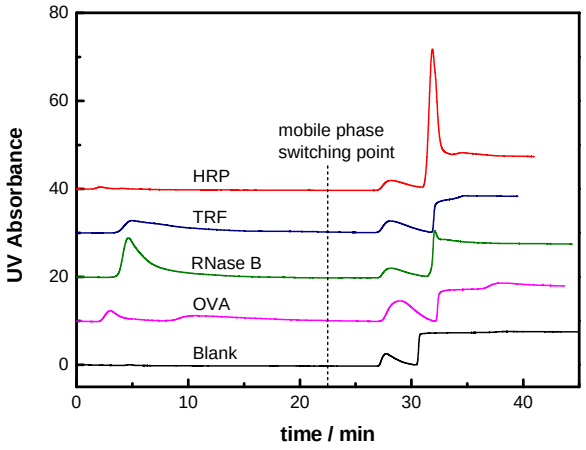

Figure S4. Chromatographic retention of glycoproteins on HRP-imprinted monolithic column with poly(APBA) as the imprinting coating. Mobile phase: 0.1 M phosphate buffer, pH 7.4, switched to 0.1 M acetic acid at 22.5 min. Blank sample, 0.1 M phosphate buffer, pH 7.4. Sample: 1 mg/mL protein dissolved in 0.1 M phosphate buffer, pH 7.4.

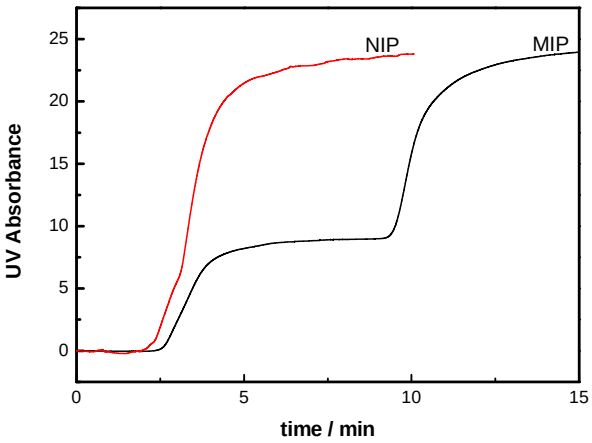

Figure S5. Breakthrough curve of HRP-imprinted monolithic column. Mobile phase: 0.1 M phosphate buffer (pH 7.4) containing 1 mg/mL HRP and 1 mg/mL RNase A.

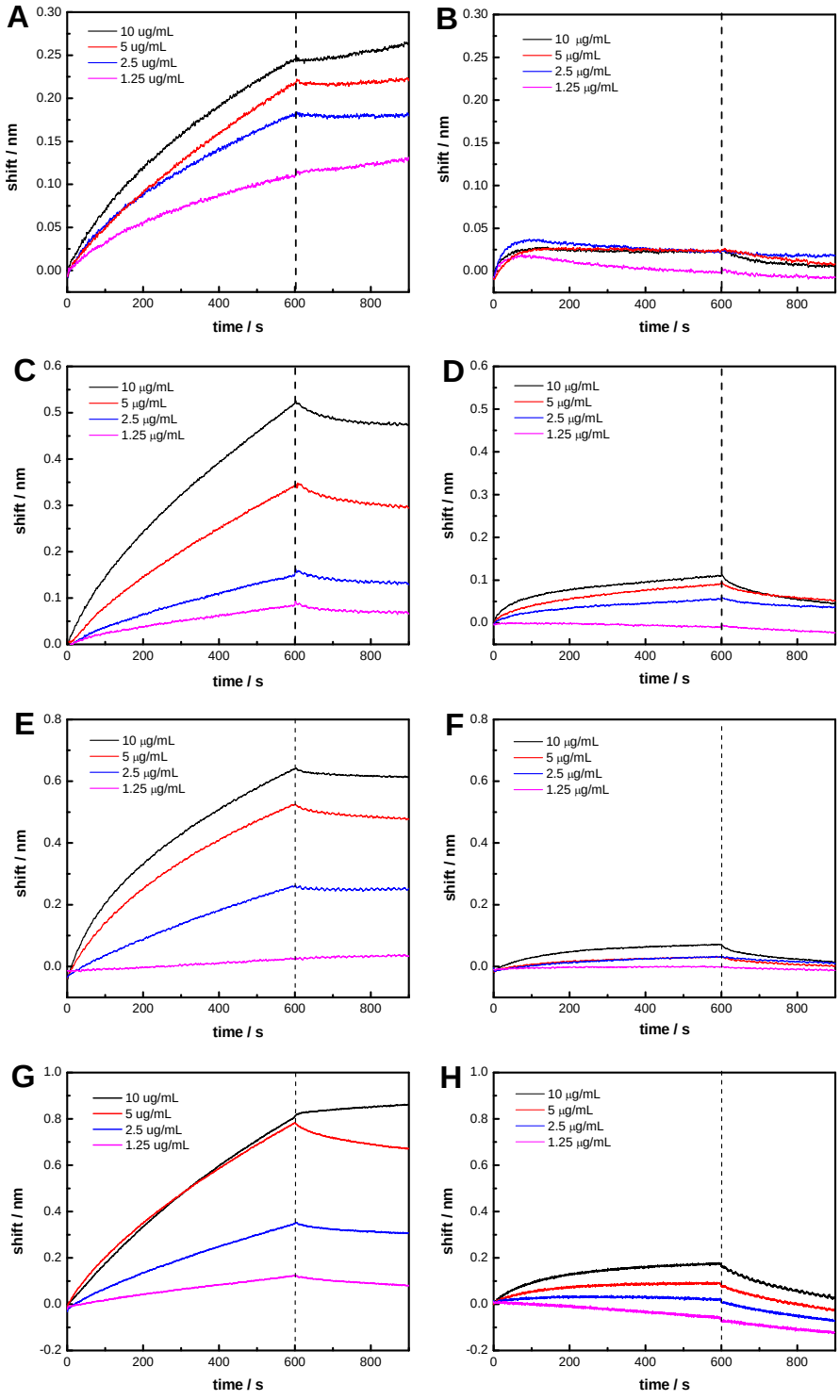

Figure S6. Binding curves for HRP-imprinted layers (A, C, E and G) and non-imprinted (B, D, F and H) layers on the sensors at binding pH. Buffer for association: 0.1 M phosphate containing different HRP concentrations at different pH. Buffer for dissociation: 0.1 M phosphate. pH: 3.0 for A and B; 5.5 for C and D; 7.4 for E and F; 9.0 for G and H.

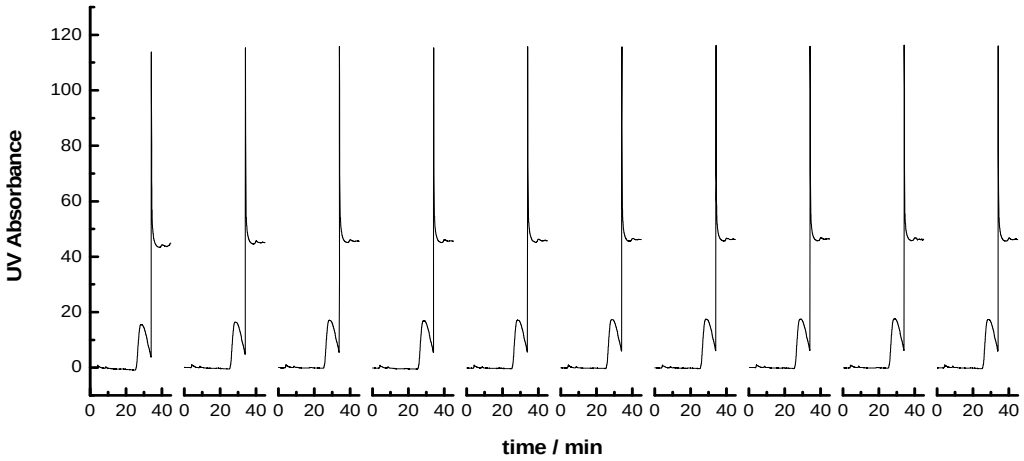

Figure S7. Repeatability test of HRP-imprinted column (n = 10). Mobile phase: 0.1 M phosphate buffer, pH 7.4, switched to 0.1 M acetic acid at 20 min. Sample: 1 mg/mL HRP dissolved in 0.1 M phosphate buffer, pH 7.4.