Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

For

Click synthesis of boronic acid-functionalized molecularly imprinted silica nanoparticles with polydopamine coating for enrichment of trace glycoprotein†

Lixiang Sun,[a] Danhong Lin,[b] Guowei Lin,[b] Ling Wang,[a] Zian Lin[a]*

a: 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, 350108, China

b: Fujian Health College, Fuzhou, Fujian 350101,China.

- **Corresponding author:** Zi An Lin;
- **Postal address:** Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350108, China
- **Tel:** 86-591-22866165
- **Fax:** 86-591-22866135
- **E-mail:** zianlin@fzu.edu.cn (Z.A. Lin);

Characterization

The morphologies of the obtained silica NPs were examined by transmission electronic microscopy (TEM, Tecnai G2-20S-TWIN, FEI). Fourier transform infrared (FT-IR) spectra of the functionalized silica NPs were recorded using the AVATAR 360 FT-IR spectrophotometer (Nicolet, Waltham, MA, USA). The surface area, pore size and pore volume were determined with a physisorption analyzer (Micromeritics ASAP 2020 porosimeter, USA). The data of adsorption were obtained by using UV-2450 spectrophotometer (Shimadzu, Japan). Gel electrophoresis for protein separation was carried out by regular SDS-PAGE with 12% running gel and 5% stacking gel according to the manual introduction (Bio-Rad, Hercules, CA, USA). Proteins were stained with Coomasie Brilliant Blue R-250.

Binding experiments.

The isothermal binding and kinetic adsorption tests were performed in the same way as described in our previous work[1]. 1 mg of the HRP-MIPs and NIPs were individually incubated with 0.5 mL of HRP solution at different concentrations ranged from 0 to 11.5 μM for 12 h. Then the silica NPs were centrifuged at 12000 rpm for 1 min and the HRP concentration in the supernatant was determined by UV/Vis spectrometer at the wavelength of 403 nm. The binding amount (Q μmol/g) of HRP adsorbed by the HRP-MIPs and NIPs was calculated according to the

following formula:

$$Q = (C_0\text{-}C)\ V/m\ M \qquad (1)$$

Where C_0 is the initial HRP concentration (μM), C is the HRP concentration after adsorption. V is the volume of HRP solution (L), m is the weight of the HRP-MIPs or NIPs (g), and M is the protein molar mass.

The adsorption kinetics was investigated by changing the adsorption time from 0 to 360 min while keeping the initial concentration of HRP constant at 10.0 μM.

The selectivity of the HRP-MIPs and NIPs was studied by using glycoproteins (ConA and Trf) and nonglycoprotein (BSA and Try) as the competitive proteins with a known initial concentration (10.0 μM). The concentration of the competitive proteins in the supernatant was measured by UV/Vis spectrometer at the wavelength of 280 nm. In addition, the recognition ability of the HRP-MIPs was evaluated by imprinting factor (α), which is defined as the following formula:

$$\alpha = Q_{MIP} / Q_{NIP} \qquad (2)$$

Where Q_{MIP} and Q_{NIP} are the binding amount of the template or the competitive proteins on the HRP-MIPs and NIPs, respectively.

Langmuir equation was used to estimate the binding property of the HRP-MIPs and NIPs, respectively. The equation is expressed as:

$$C_e/Q = C_e/Q_{max} + 1/KQ_{max} \qquad (3)$$

Where Q and Q_{max} are the experimental adsorption capacity to the template and the theoretical maximum adsorption capacity (μM), respectively. C_e is the equilibrium concentration of HRP (μM), K is the rate constant of adsorption (L/μmol).

References

(1) Lin, Z. A.; Sun, L. X.; Liu, W.; Xia, Z.; Yang, H.; Chen, G. *J. Mater. Chem. B* **2013**, 637–643.

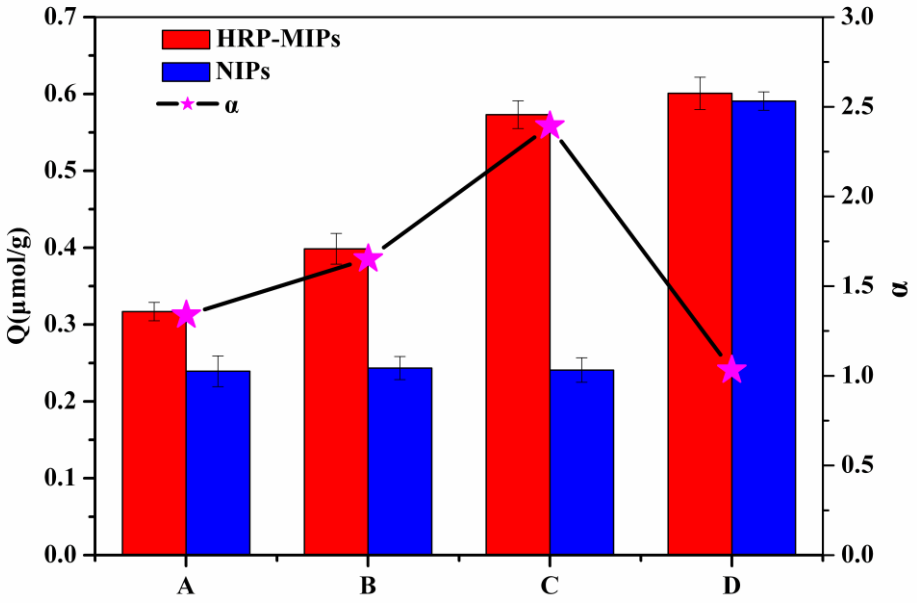

Fig.S1 Effect of DA concentrations on adsorption capacity and imprinting factor of HRP on the HRP-MIPs and NIPs.

Amount of silica NPs: 1 mg; volume: 0.5 mL; binding media: 0.01 M PBS (pH 9.0); incubation time: 6 h; C_{HRP}: 10.0 μmol L^{-1}. The points represent mean values of three measurements.

A: 6.67 mg mL^{-1} DA; B: 3.33 mg mL^{-1} DA; C: 1.67 mg mL^{-1} DA; and D:1.0 mg mL^{-1} DA

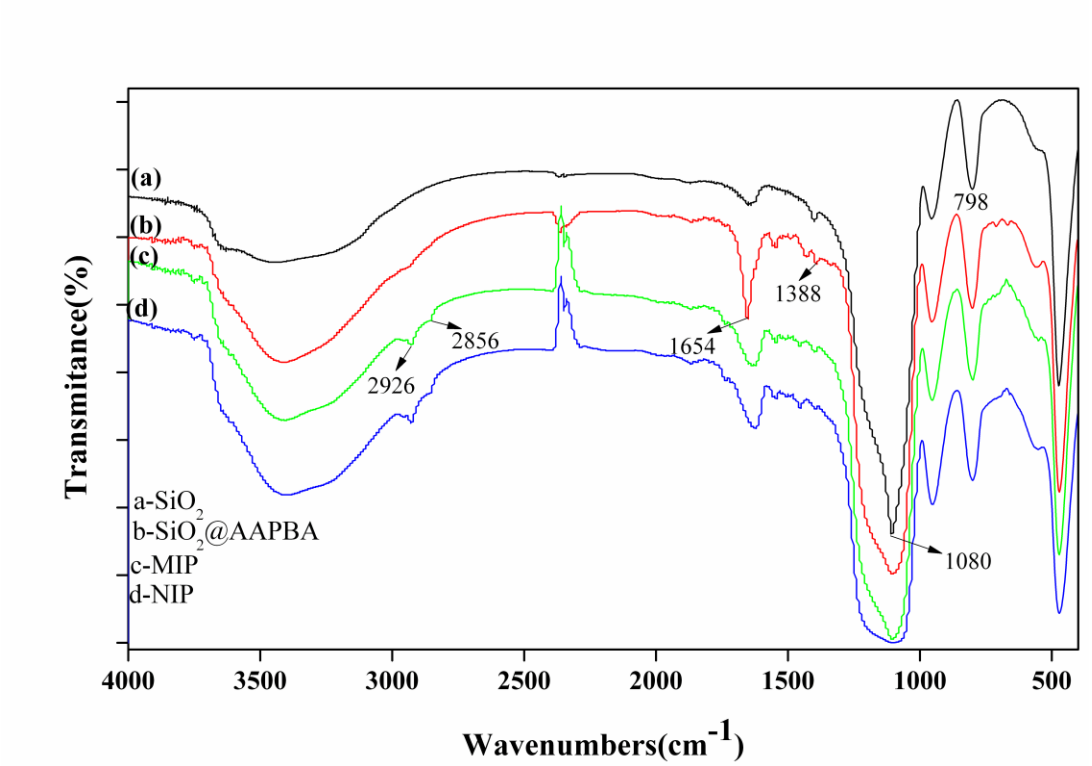

Fig.S2 FT-IR spectra of the (a) bare SiO_2; (b) SiO_2@AAPBA; (c) HRP-MIPs and (d) NIPs.

The strong peaks at 1080 and 798 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of Si–O–Si linkages (spectrum a). Some new peaks (1388 and 1654 cm^{-1}) appeared in the spectrum of SiO_2@AAPBA NPs (spectrum b), were assigned to B−O stretching and C=O stretching, demonstrating the existence of AAPBA. The HRP-MIPs and NIPs showed similar locations and appearances of the major bands (spectra c-d). The characteristic features of the both spectra were $–CH_3$ and $–CH_2$ bonds at 2856 and 2926 cm^{-1} confirming the successful coating of PDA.

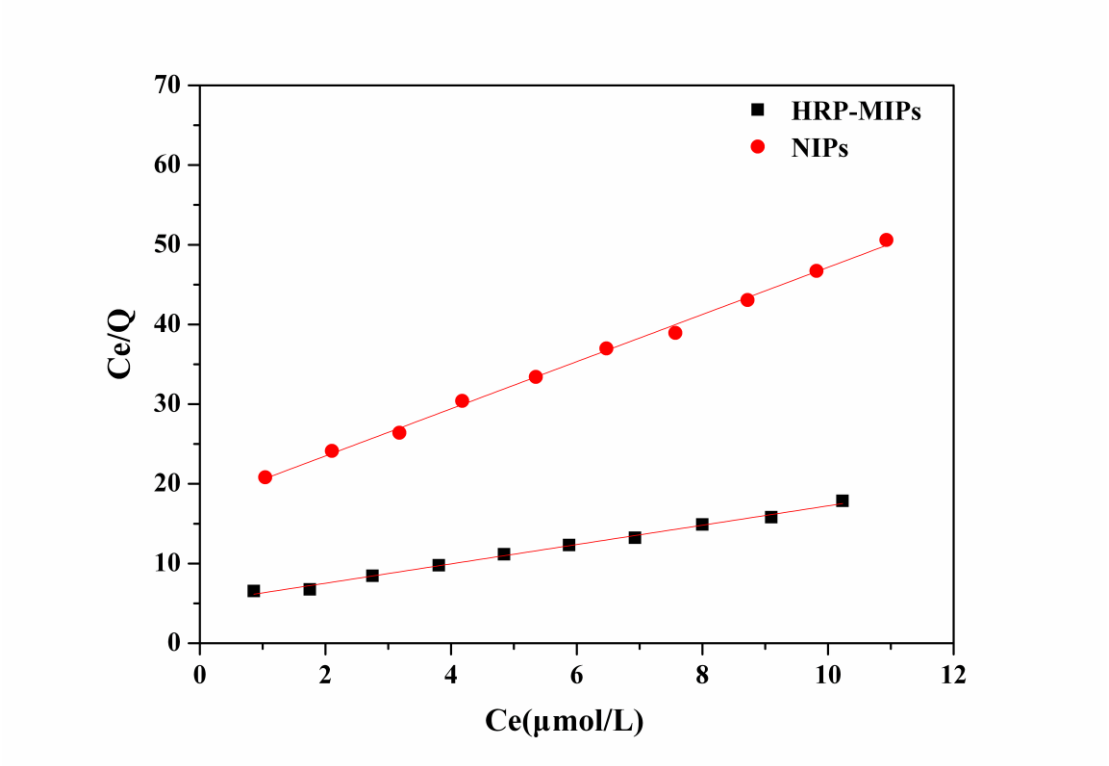

Fig.S3 The Langmuir adsorption equation for the HRP-MIPs and NIPs

HRP-MIPs: $C_e/Q=1.21358C_e+5.10249$ ($R^2= 0.9936$)

$K=5.38$ L μmol^{-1}, $Q_{max}=0.9$ μmol g^{-1}

NIP: $C_e/Q=2.95826C_e+17.587$ ($R^2= 0.9969$) ,
$K=3.79$ L μmol^{-1}; $Q_{max}=0.32$ μmol g^{-1}

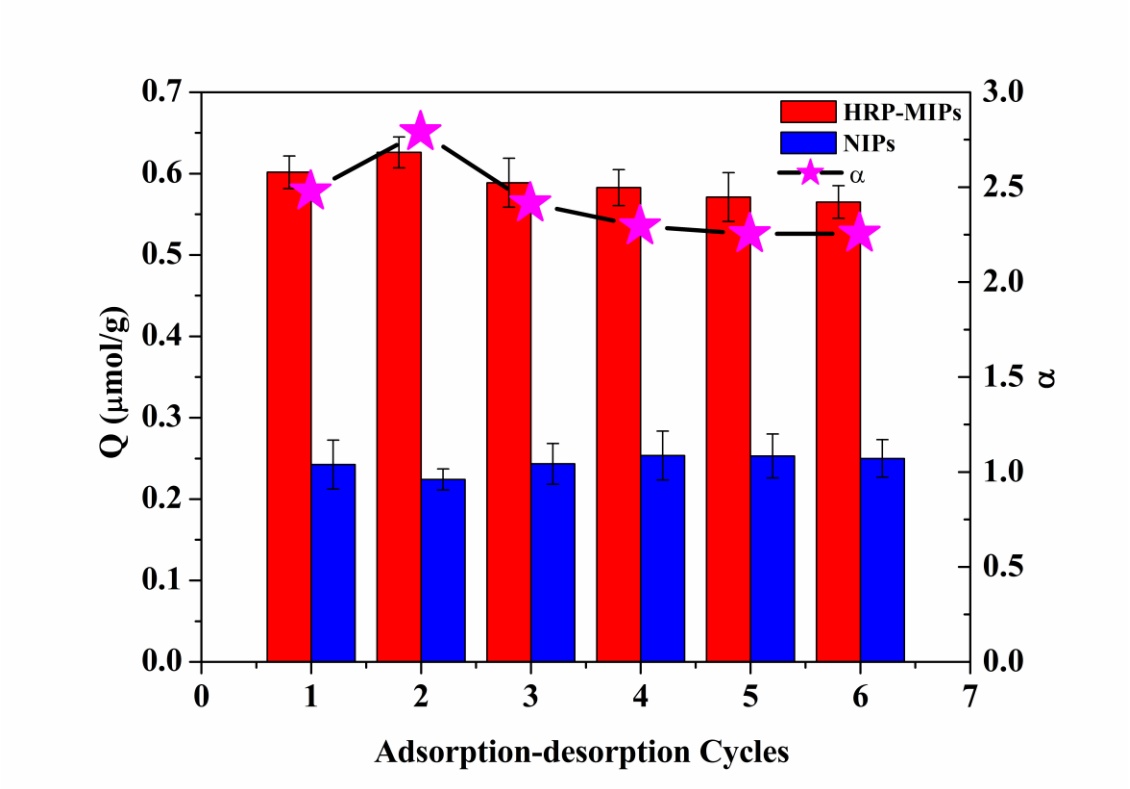

Fig.S4 Stability and regeneration of the HRP-MIPs and NIPs for six cycles. Amount of HRP-MIPs and NIPs: 5 mg; volume: 2.5 mL; binding media: 0.01 M PBS (pH 9.0); incubation time: 1h; C_{HRP}: 10.0 μM. The points represent mean values of three measurements.

Table.S1 UV/vis spectroscopy assay of human serums spiked with 10 ng/mL HRP and their corresponding eluate.*

*The experimental conditions are same as Fig.4

No	Absorbance	C_{HRP} (ng/mL)	Extraction (%)	Recovery (%)
Human serum spiked with HRP	3.016	10.0	--	--
HRP-MIPs	0.773	2.577	74.23	--
NIPs	2.189	7.258	27.23	
The eluate from HRP-MIPs	0.909	3.014	--	30.14
The eluate from NIPs	0.421	1.400	--	14.00