Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Selective enrichment of protein for MALDI-TOF MS analysis based on molecular imprinting

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1. Experimental

1.1. Materials

Lysozyme (Lyz) from egg white was purchased from J & K Chemical Technology Co., Ltd. (Beijing, China). Ribonuclease A (RNase A) was bought from Yuanye Biotech Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was acquired from Dingguo Biotech Development Center (Beijing, China). Trypsin was supplied by Fanke Biotech Co., Ltd. (Shanghai, China). Cytochrome C (Cyt C) was purchased from Sangon (Shanghai, China). Bovine hemoglobin (BHb) and dopamine hydrochloride were bought from Sigma-Aldrich. Tetraethyl orthosilicate (TEOS), Trifluoroacetic acid (TFA) were obtained from Beijing Chemical Works (Beijing, China). Acetonitrile (ACN) was from Amethyst Chemicals (Beijing, China) and of HPLC grade. Sinapic acid (SA) was from Bruker Daltonics (Bremen, Germany). Fresh chicken eggs were purchased from a local market. All other reagents were of analytical grade and used as received without further purification. Deionized water was used throughout the work.

1.2. Characterization

The morphology of samples was examined by a MERLIN (Zeiss, Germany) high resolution scanning electronic microscopy (HRSEM) operating at 5 kV. A thin carbon film was sprayed on the sample before measurements. Transmission electron microscopy (TEM) images were captured with an H-7650B (Hitachi, Japan) transmission electron microscope with an accelerating voltage of 80 kV. Ultraviolet visible (UV-vis) absorption spectra of proteins were recorded by a U-3010 UV-vis spectrophotometer (Hitachi, Japan). Elemental analyses were examined on a CE-440 Elemental Analyzer (EAI, USA). The powder X-ray diffraction (XRD) patterns were recorded by a D8 Advance X-ray diffractometer (Bruker, Germany) with Cu Kα radiation (λ = 1.5406 Å). The magnetic properties were analyzed with a 730T (Lakeshore, USA) vibrating sample magnetometer (VSM). Fourier transform infrared (FTIR) spectra were carried out using a Perkin-Elmer spectrometer in the frequency

range 4000-500 cm^{-1} with a resolution of 4 cm^{-1}. Thermogravimetric analysis (TGA, Q50, USA) was examined under nitrogen atmosphere at the rate of 10 °C per minute up to 900 °C. X-ray photoelectron spectroscopy (XPS) data were determined using a PHI-5300 ESCA X-ray photoelectron spectrometer (PHI, USA).

1.3. Preparation of Lyz-imprinted nanoparticles

The magnetic nanoparticles were synthesized through a solvothermal reaction.[1] Briefly, $FeCl_3 \cdot 6H_2O$ (2.70 g) and sodium acetate (7.20 g) were dissolved in ethylene glycol (80 mL) under magnetic stirring. Subsequently, the resultant homogeneous yellow solution was transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity), sealed, and heated at 200 °C. After reaction for 8 h, the autoclave was cooled to room temperature. The obtained black magnetite particles were washed five times with water and ethanol. Finally, the products were collected with a magnet and then dried in vacuum at 60 °C for 12 h.

The core-shell Fe_3O_4@SiO_2 nanoparticles were prepared as follow:[2] typically, Fe_3O_4 particles (600 mg) were ultrasonically treated with HCl aqueous solution (50 mL, 0.1 M) for 10 min. Then the magnetite particles were thoroughly washed with deionized water and redispersed in a mixture of ethanol (80 mL), deionized water (20 mL) and concentrated ammonia aqueous solution (10 mL, 28 wt.%) by ultrasonication for 30 min. Subsequently, TEOS (4 mL) was added dropwise to the above dispersion. After mechanically stirring at room temperature for 12 h, the Fe_3O_4@SiO_2 nanoparticles were separated and washed with ethanol and water, then the product was dried in vacuum at 60 °C for 24 h.

For the preparation of Lyz-imprinted nanoparticles, Fe_3O_4@SiO_2 nanoparticles (100 mg) were dispersed in 30 mL tris-buffer (10 mM, pH 8.0) by ultrasonication for 10 min, followed by the addition of Lyz (20 mg). After being stirred at room temperature for 7 h, dopamine (30 mg) was added, and the reaction was incubated for another 13 h at room temperature. Afterwards, the product was collected with a magnet and washed with the solution containing SDS (5%, w/v) and HAc (5%, v/v) for three times to remove the embedded template, then thoroughly with distilled water.

Finally, the obtained product was dried in vacuum at 60 °C for 12 h. The control non-imprinted nanoparticles were prepared using the same recipe by omitting the template. The imprinted and nonimprinted nanoparticles were denoted as Lyz-MIPs and NIPs, respectively.

1.4. Protein adsorption experiments

2 mg of the Lyz-MIPs or NIPs were incubated with 4 mL of Lyz solutions in tris buffer (10 mM, pH 8.0) for 1 h at room temperature. After separation by magnet, the concentration of free Lyz in the supernatant was measured by UV-vis absorption spectroscopy at 280 nm. The amount of Lyz adsorbed by the Lyz-MIPs or NIPs was calculated from the following formula:

$$Q = \frac{(C_0 - C)\,V}{m}$$

where Q (mg g^{-1}) is the mass of protein adsorbed by unit mass of particles; C_0 (mg mL^{-1}) is the initial protein concentration; C (mg mL^{-1}) is protein concentration of the supernatant; V (mL) is the volume of the initial solution; m (g) is the mass of the particles.

The adsorption kinetics was investigated by changing the adsorption time from 0 to 60 min with the initial concentration of Lyz at 0.4 mg mL^{-1}.

The selectivity of the Lyz-MIPs was studied using RNase A, BHb, BSA, trypsin and Cyt C as comparative proteins with initial concentrations of 0.4 mg mL^{-1}. The concentration of comparative proteins in the supernatant was measured by a UV/Vis absorption spectroscopy at the wavelength of 277 nm for RNase A, 406 nm for BHb, 278 nm for BSA, 260 nm for trypsin, and 410 nm for Cyt C.

1.5. Enrichment of Lyz from spiked protein mixture and chicken egg white

2 mg of Lyz-MIPs were emerged with 400 μL of spiked protein mixture. After shaking for 1h at room temperature, the particles were separated and washed three times with 1 mL of 2% ACN/0.1% TFA for 10 min to remove the nonspecific binding proteins. Finally, 20 μL of 30% ACN/0.1% TFA was used to elute Lyz for 20 min.

Egg white was isolated from fresh eggs and diluted by 10-fold with tris buffer (10

mM, pH 8.0). The diluted egg white was centrifugated at 10000 rpm for 10 min and the supernatant fraction was available for the same enrichment process as spiked protein mixture.

1.6. MALDI-TOF MS

MALDI-TOF MS analysis was performed on an Autoflex Speed TOF/TOF (Bruker Daltonics) equipped with a Nd:YAG laser (355 nm, 1 kHz). Linear mode was employed for the analysis of proteins. Two-layer method with SA as matrix was used. 1 μL of saturated solution of SA in ethanol was first placed on a MTP 384 ground steel (Bruker Daltonics) and air-dry to obtain the thin layer of matrix. Then 1 μL of saturated solution of SA in 30% ACN/0.1% TFA was mixed with 1 μL of sample, and 1 μL of the above mixture was spotted on the first layer. The laser power was adjusted to 70%, and the spectra were recorded in partial sampling mode by summing 500 laser shots. The data processing was analyzed with the FlexAnalysis 3.3 software.

2. Supporting Figures

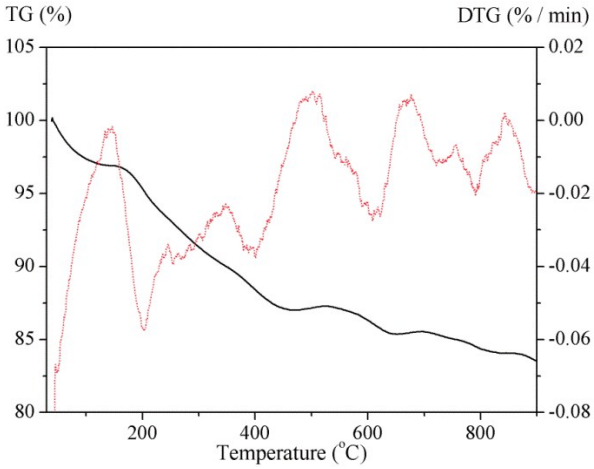

Figure S1. TGA curves of the Lyz-MIPs.

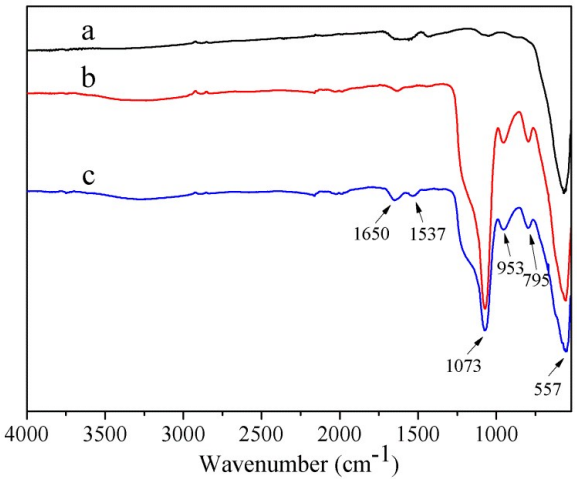

Figure S2. FTIR spectra of Fe_3O_4 nanoparticles (a), Fe_3O_4@SiO_2 nanoparticles (b), Lyz-MIPs (c).

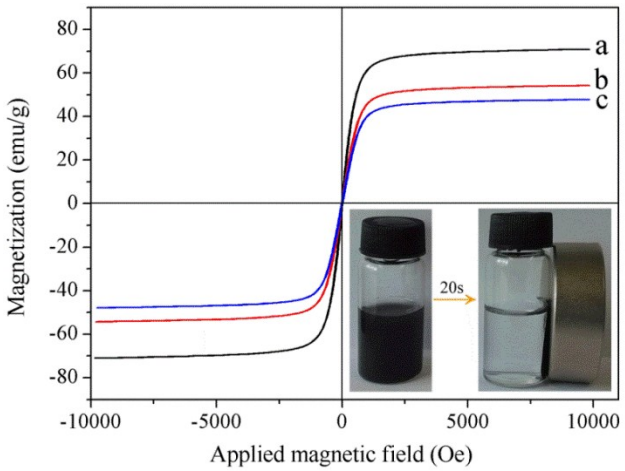

Figure S3. Magnetic hysteresis loops of Fe_3O_4 nanoparticles (a), Fe_3O_4@SiO_2 nanoparticles (b), Lyz-MIPs (c), and dispersion and separation process of Lyz-MIPs (inset)

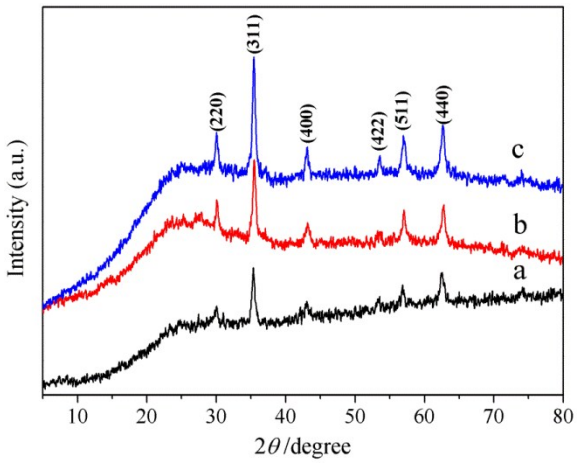

Figure S4. X-ray diffraction patterns of Fe_3O_4 nanoparticles (a), Fe_3O_4@SiO_2 nanoparticles (b), and Lyz-MIPs (c).

From XRD patterns in Fig. S4, Six characteristic peaks (2θ = 30.13°, 35.43°, 43.20°, 53.47°, 57.01°, 62.67°) for three samples could be indexed as (220), (311), (400), (422), (511), (440), respectively. Thus a face-centered cubic (fcc) Fe_3O_4 phase was confirmed (JCPDS Card: 19-629). After coating with silica, arched diffraction appeared in the 2θ range of 20-30°, which attributed to the superposition of amorphous SiO_2 on the Fe_3O_4 reflections. The XRD patterns of Lyz-MIPs were resemble that of Fe_3O_4@SiO_2 nanoparticles and no other crystal forms were obtained,

indicating that the amorphous structure presented in the PDA layers and had little influence to the Fe_3O_4 cores.

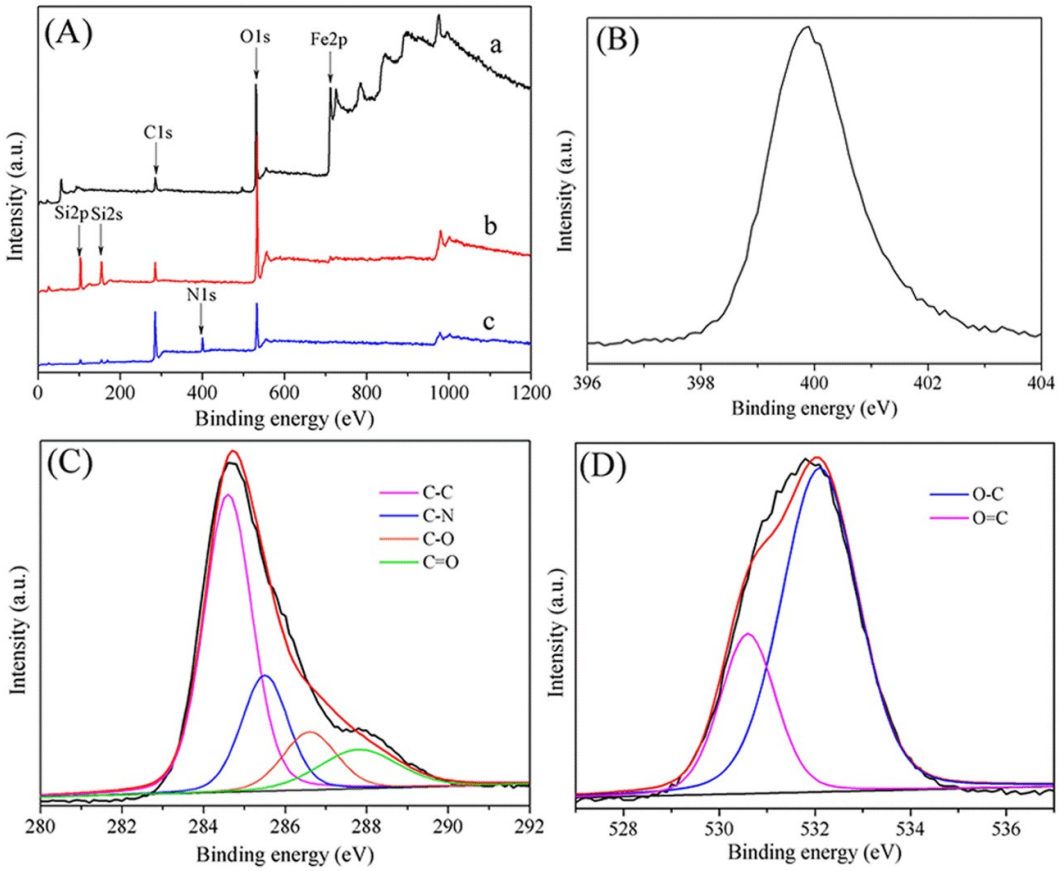

Figure S5. XPS spectra of (A) wide scan of Fe_3O_4 (a), Fe_3O_4@SiO_2 (b) and Lyz-MIPs (c), (B) N 1s, (C) C 1s and (D) O 1s spectra of Lyz-MIPs.

The peaks at approximately 285, 530 and 710 eV were attributed to C 1s, O 1s and Fe 2p, respectively (Fig. S5A(a)), illustrating the formation of magnetic materials. the Si 2p and Si 2s at 103 and 154 eV were consistent with the presence of SiO_2 layer. After the surface modification by PDA, a new peak of N 1s at 400 eV was observed with the increased intensity of C 1s and the disappearance of Si peaks (Fig. S5A(c) and Fig. S5B). Moreover, the signal ratio of nitrogen to carbon (N/C) peak areas was 0.142, which was close to the theoretical value of 0.125 of DA.[3] The C 1s spectra of Lyz-MIPs (Fig. S5C) could be curved into four peak components at about 284.6, 285.5, 286.6, 287.8eV, attributable to C-C, C-N, C-O, and C=O, respectively.[4] The appearance of C-N was the evidence of PDA layer.

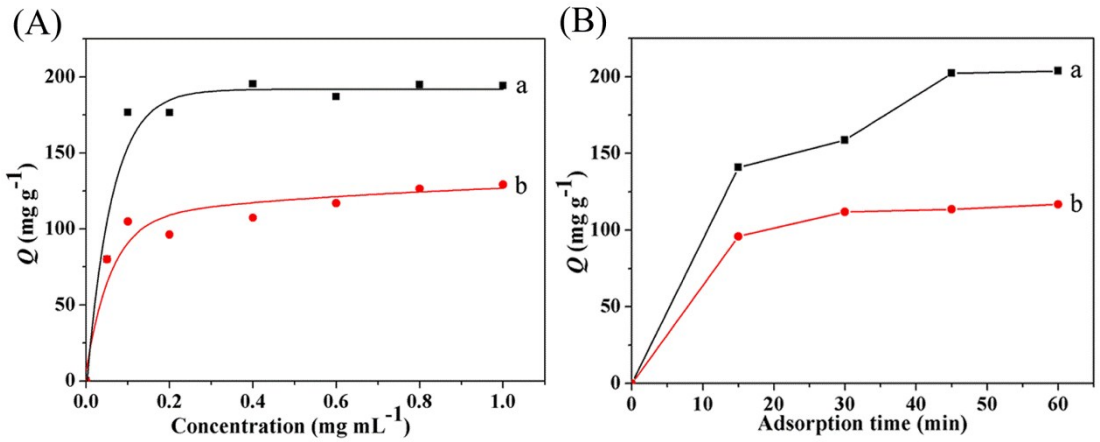

Figure S6. (A) Adsorption isotherms of Lyz on the Lyz-MIPs (a) and NIPs (b). Adsorption conditions: V = 4 mL, m = 2 mg, time 1 h, tris buffer (10 mM, pH 8.0). (B) Adsorption kinetics of Lyz on the Lyz-MIPs (a) and NIPs (b). Adsorption conditions: V = 4 mL, m = 2 mg, C_0 = 0.4 mg mL^{-1}, tris buffer (10 mM, pH 8.0).

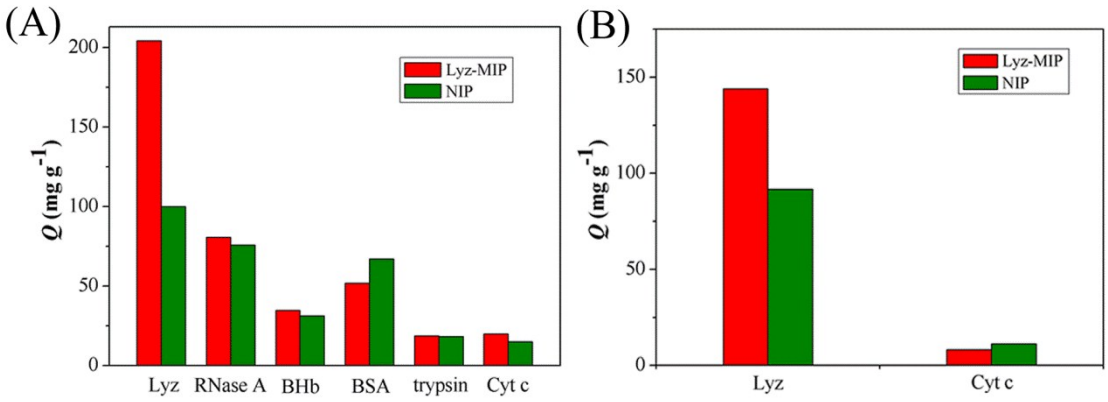

Figure S7. (A) Binding amounts of different proteins on the Lyz-MIPs and NIPs. Adsorption conditions: V = 4 mL, m = 2 mg, concentration of analytes: 0.4 mg mL^{-1}, tris buffer (10 mM, pH 8.0). (B) Competitive binding of Lyz and Cyt C to the Lyz-MIPs and NIPs. Adsorption conditions: V = 4 mL, m = 2 mg, $C_{0,\mathrm{Lyz}}$ = $C_{0,\mathrm{Cyt\ C}}$ = 0.2 mg mL^{-1}, tris buffer (10 mM, pH 8.0). The points shown here represent mean values of three measurements.

References

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