Supplementary Information

Magnetic metal-organic frameworks for selective enrichment and exclusion of proteins for MALDI-TOF MS analysis

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1. Experimental

1.1. Materials

Benzene-1,3,5-tricarboxylic acid (H3btc), tris (hydroxymethyl) aminomethane and lysozyme (Lyz) from egg white were bought from J & K Chemical Technology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) was acquired from Dingguo Biotech Development Center (Beijing, China). Bovine hemoglobin (BHb), myoglobin (Mb), papain (Pa) and sinapic acid (SA) were purchased from Sigma-Aldrich. Poly(acrylic acid) (PAA)，ribonuclease A (RNA) from bovine pancreas, horseradish peroxidase (HRP) and human serum were supplied by Keao Biotech Co., Ltd. (Beijing, China). Trifluoroacetic acid (TFA) was obtained from Beijing Chemical Works (Beijing, China). Acetonitrile (ACN) was from Amethyst Chemicals (Beijing, China) and of HPLC grade. 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 SU-8010 (Hitachi, Japan) field emission scanning electronic microscopy (SEM) operating at 10 kV. A thin Au 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-3900 UV-vis spectrophotometer (Hitachi, Japan). Thermogravimetric analysis (TGA, Q50, USA) was analyzed under nitrogen atmosphere at the rate of 10 °C per minute up to 900 °C. Energy dispersive spectrometer (EDS) was measured on a JEM-2100F (Japan) field emission electronic microscopy. The powder X-ray diffraction (PXRD) patterns were carried out by a D8 high resolution advance X-ray diffractometer (Siemens, Germany) with Cu target (2.2 kw). Fourier transform infrared (FTIR) spectra were recorded using a Perkin-Elmer spectrometer in the frequency range 4000-500 cm^{-1} with a resolution of 4 cm^{-1}. The

magnetic properties were analyzed with a 730T (Lakeshore, USA) vibrating sample magnetometer (VSM). Nitrogen sorption-desorption isotherms were obtained at 77 K on Micromeritics ASAP 2010C analyzer (Micromeritics, USA).

1.3. Preparation of Fe_3O_4@MIL-100(Fe) nanoparticles

PAA modified magnetic nanoparticles (Fe_3O_4-PAA) were synthesized through a solvothermal reaction.[1] Briefly, $FeCl_3 \cdot 6H_2O$ (2.16 g), sodium acetate (8.0 g) and PAA (200 mg) were dissolved in ethylene glycol (EG, 28 mL) and diethylene glycol (DEG, 52 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 10 h, the autoclave was cooled to room temperature. The obtained yellow 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 24 h.

For the preparation of Fe_3O_4@MIL-100(Fe) nanoparticles, Fe_3O_4-PAA nanoparticles (600 mg) were dispersed in $FeCl_3 \cdot 6H_2O$ ethanol solution (48 mL, 10 mM) for 15 minutes and then in H_3btc ethanol solution (48 mL, 10 mM) for 30 minutes at 70 °C. Between each step the nanoparticles were separated with a magnet and washed with ethanol. After 15 cycles, the product was washed with *N*,*N*-dimethylformamide and ethanol for five times. Finally, the obtained product was dried in vacuum at 70 °C for 12 h.

1.4. Enrichment and exclusion of proteins from standard proteins, protein mixture, chicken egg white and human serum

The standard proteins and protein mixture were both prepared in tris buffer (10 mM, pH 8.5). 2 mg of Fe_3O_4@MIL-100(Fe) were emerged with 1 mL of standard proteins or protein mixture. After shaking for 1h at room temperature, the particles were separated and washed three times with 1 mL of deionized water for 10 min to remove the nonspecific binding proteins. Finally, 20 μL of 30% ACN/0.1% TFA was used to elute the proteins for 20 min.

Egg white was isolated from fresh eggs and diluted by 4-fold with tris buffer (10

mM, pH 8.5). The diluted egg white was centrifugated at 10000 rpm for 10 min and the supernatant fraction was available for the same enrichment process as standard proteins or protein mixture.

Human serum was diluted 50 fold with tris buffer (10 mM, pH 8.5) and then spiked with 40 μg mL^{-1} RNA + 40 μg mL^{-1} Lyz, and applied to the enrichment procedure given above.

1.5. 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 ethanol solution of SA 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 80%, and the spectra were recorded in partial sampling mode at the center of dried droplets by summing 500 laser shots.

1.6. Sample preparation of ZipTipC18 and Nap-5 Columns (Sephadex G25)

The sample preparation of proteins prior to MALDI-TOF MS using ZipTipC18 pipette tips was according to the standard procedure provided by the technical note of MILLIPORE Corporation. Briefly, the tips were wetted with 10 μL of 50% ACN/0.1% TFA and equilibrated with 10 μL of 0.1% TFA twice. Then aspirate and dispense sample 5 times. Finally the tips were eluted by 2 μL 30% ACN/0.1% TFA and the eluted solution was employed with two-layer method described above.

For the sample preparation of proteins prior to MALDI-TOF MS using Nap-5 Columns, the columns were equilibrated with 10 mL of deionized water. Then 0.5 mL of sample was added to the columns. Finally the columns were eluted by 1 mL of deionized water. The eluted solution was utilized with two-layer method described above.

2. Supporting Tables

Table S1. Isoelectric points (pI), molecular weights (M_w) and size of different proteins.[2]

Protein	pI	M_w (Da)	Size (nm)
RNA	9.6	13668.9	1.95~2.35[a]
Lyz	11.1	14280.8	4.5×3.0×3.0
Mb	7.1	16901.4	2.5×3.5×4.5
Pa	8.75	23847.4	3.7×3.7×5.0
HRP	7.2	42184.8	4.0×4.4×6.8
BHb	6.8	63782.0	5.3×5.4×6.5
BSA	4.8	66583.0	4.0×4.0×14.0
Ovalbumin	4.9	ca. 42700	4.0×5.0×7.0
Ovomucoid	4.1	ca. 28000	-----

[a] Stokes Radius from previous report[3] was used as the size of RNA.

Table S2. The concentrations of the supernatant and absorption capacities for different proteins on Fe_3O_4@MIL-100(Fe). Adsorption conditions: V = 1 mL, m = 2 mg, tris buffer (10 mM, pH 8.5), time 1h. The initial concentrations of different proteins were all 1 mg mL^{-1}. The concentrations of free proteins in the supernatant were measured by UV-vis absorption spectroscopy. The points shown here represent mean values of three measurements.

Protein	Protein concentration of the supernatant (mg mL^{-1})	Absorption capacity (μmol g^{-1})
RNA	0.666	12.23
Lyz	0.733	9.33
Mb	0.994	0.17
Pa	0.876	2.61
HRP	0.991	0.11
BHb	0.953	0.32

3. Supporting Figures

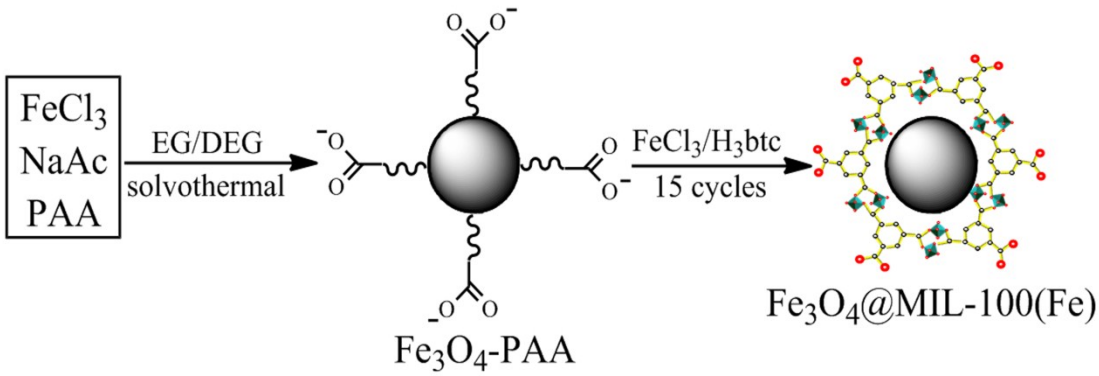


Figure S1. Schematic presentation of the fabrication of Fe_3O_4@MIL-100(Fe) nanoparticles over PAA modified Fe_3O_4 nanoparticles.

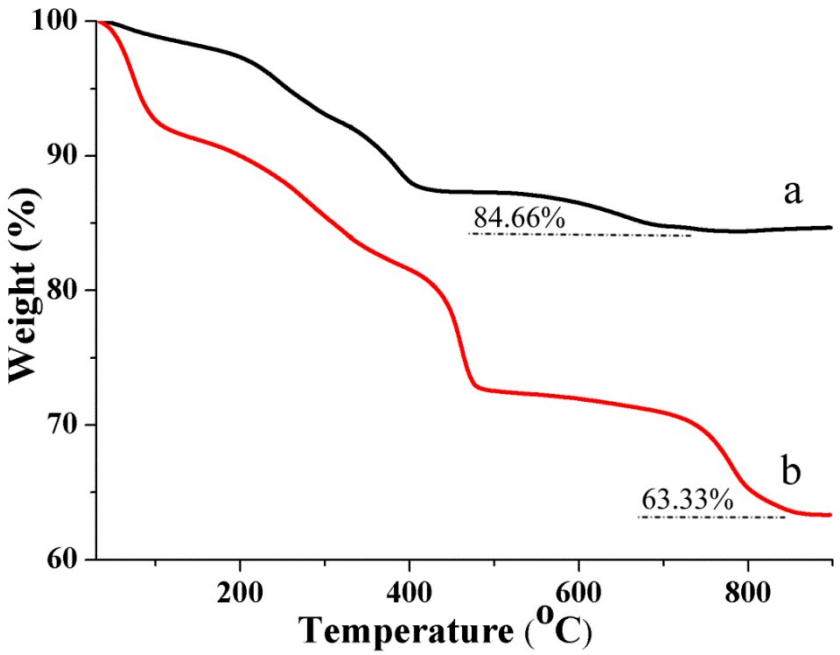
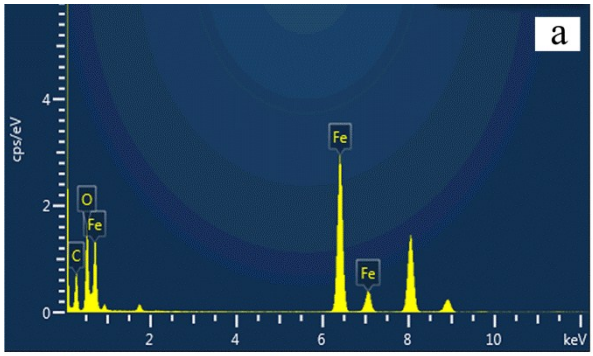
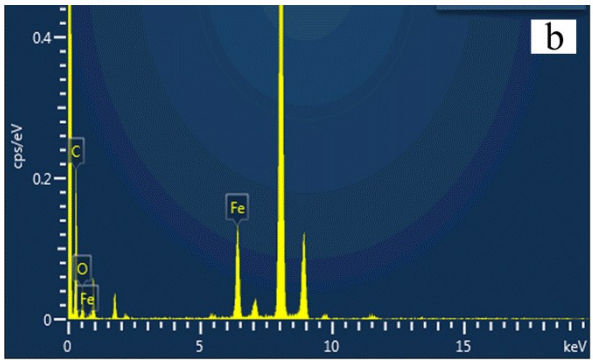


Figure S2. TGA curves of Fe_3O_4-PAA nanoparticles (a) and Fe_3O_4@MIL-100(Fe) nanoparticles (b).



Element	Wt %
C	14.47
O	25.50
Fe	60.03
Total	100.00



Element	Wt %
C	56.06
O	9.17
Fe	34.77
Total	100.00

Figure S3. EDS spectra at the centre of Fe_3O_4-PAA nanoparticles (a) and Fe_3O_4@MIL-100(Fe) nanoparticles (b).

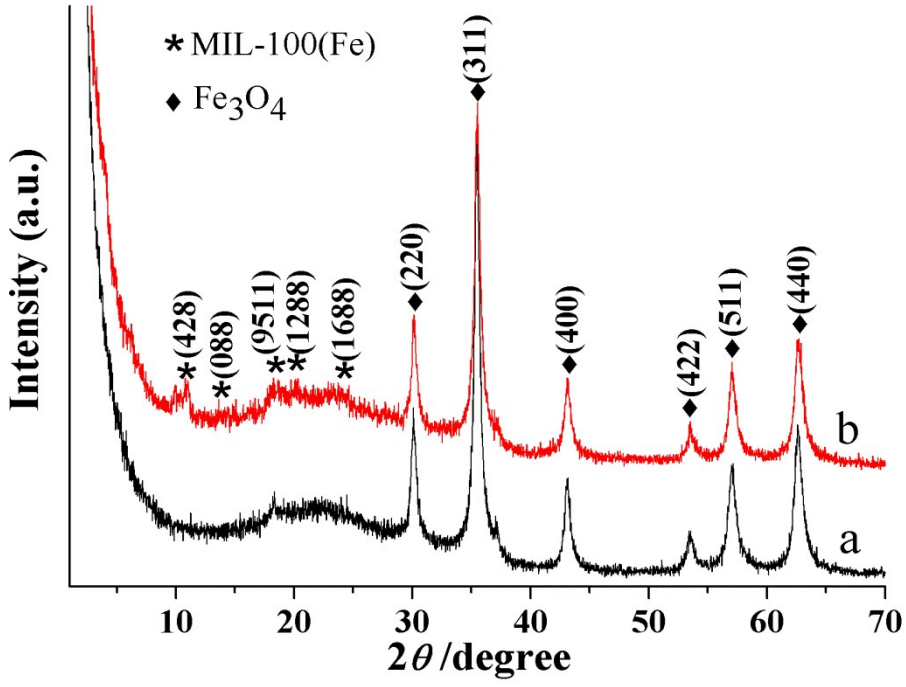


Figure S4. PXRD patterns of Fe_3O_4-PAA nanoparticles (a) and Fe_3O_4@MIL-100(Fe) nanoparticles (b).

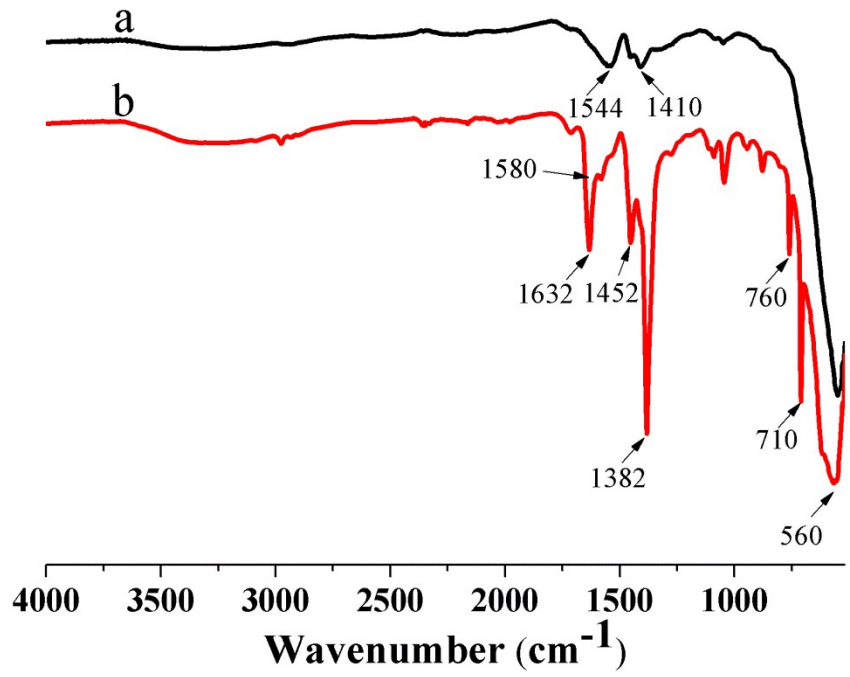


Figure S5. FTIR spectra of Fe_3O_4-PAA nanoparticles (a) and Fe_3O_4@MIL-100(Fe) nanoparticles (b).

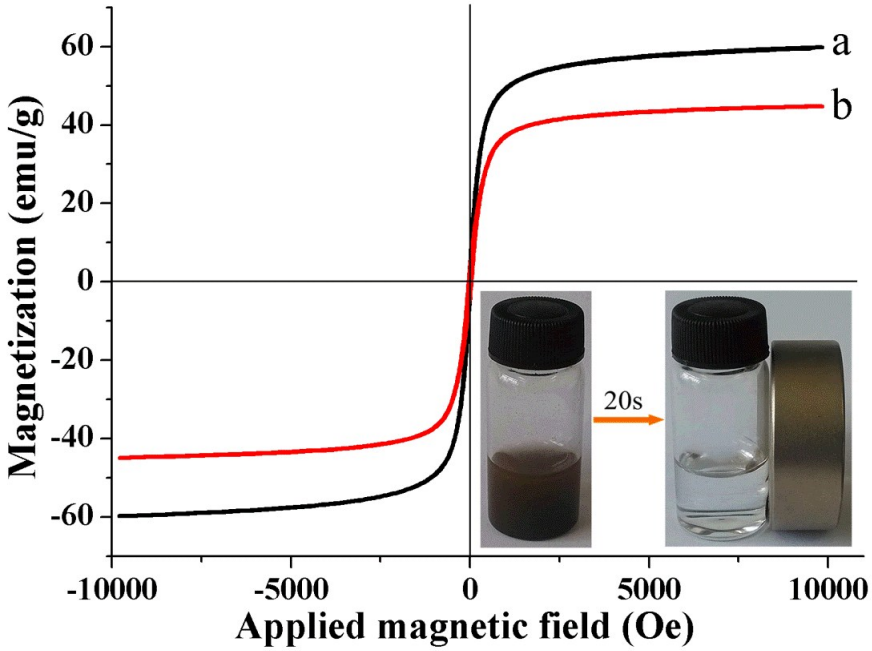


Figure S6. Magnetic hysteresis loops of Fe_3O_4-PAA nanoparticles (a) and Fe_3O_4@MIL-100(Fe) nanoparticles (b), and dispersion and separation process of Fe_3O_4@MIL-100(Fe) (inset).

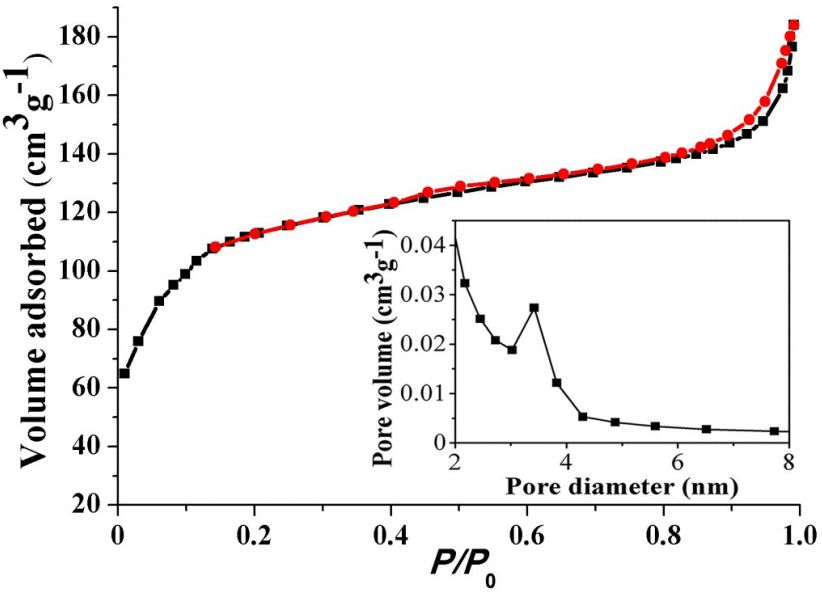


Figure S7. Nitrogen sorption-desorption isotherms of Fe_3O_4@MIL-100(Fe) nanoparticles at 77 K, and corresponding pore size distribution (inset) was estimated by the Barrett–Joyner–Halenda (BJH) method.

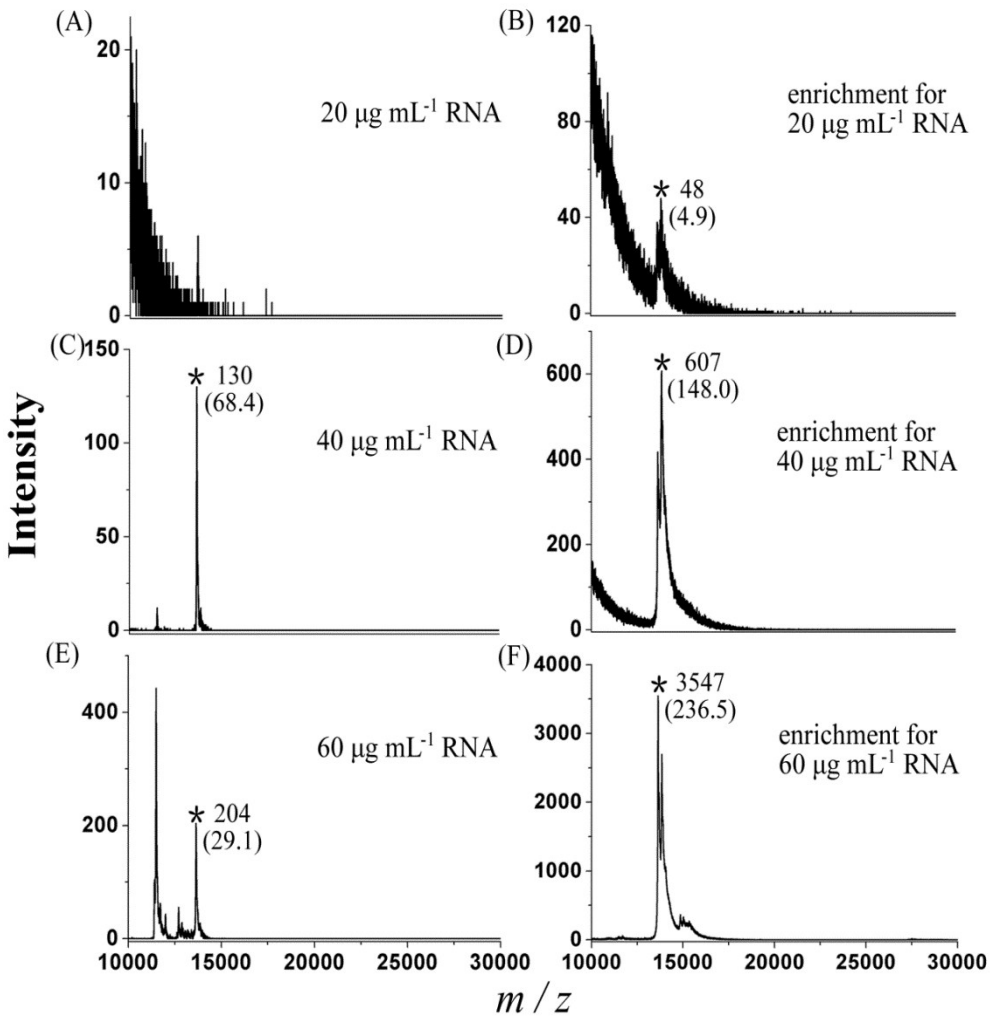


Figure S8. MALDI-TOF MS data for RNA before enrichment at concentrations of 20, 40, 60 μg mL^{-1} (A, C, E) and after enrichment with Fe_3O_4@MIL-100(Fe) nanoparticles (B, D, F). The peak intensity and S/N ratio (in parentheses) are labelled for the highest peaks.

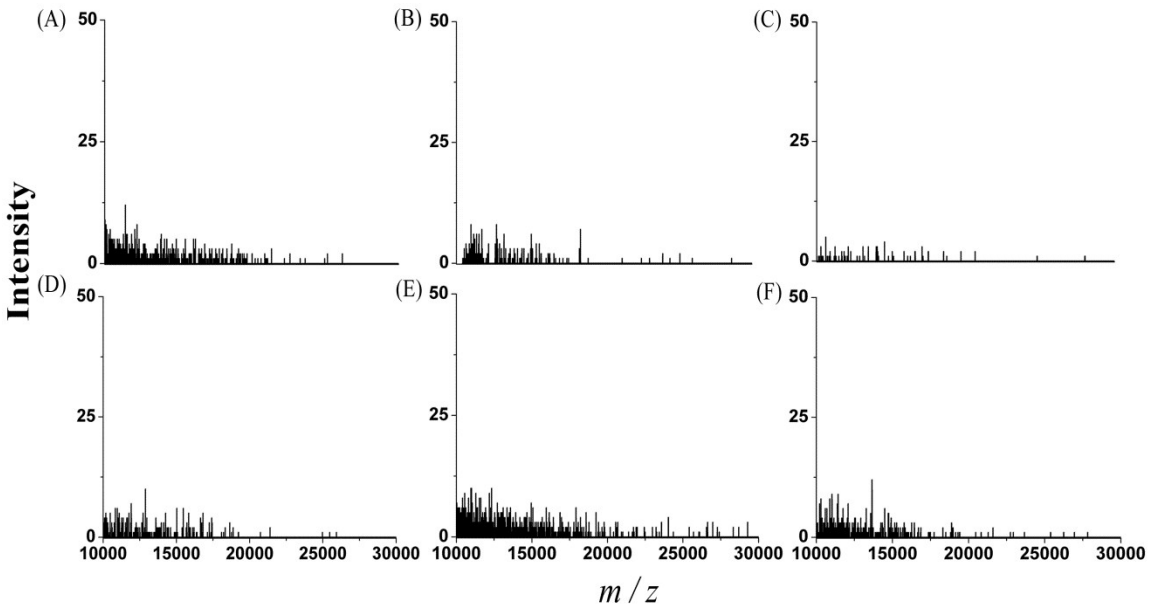


Figure S9. MALDI-TOF MS data for 5 μg mL^{-1} Lyz, 20 and 40 μg mL^{-1} RNA after treatment with ZipTipC18 (A, B, C) or Nap-5 Columns (D, E, F).

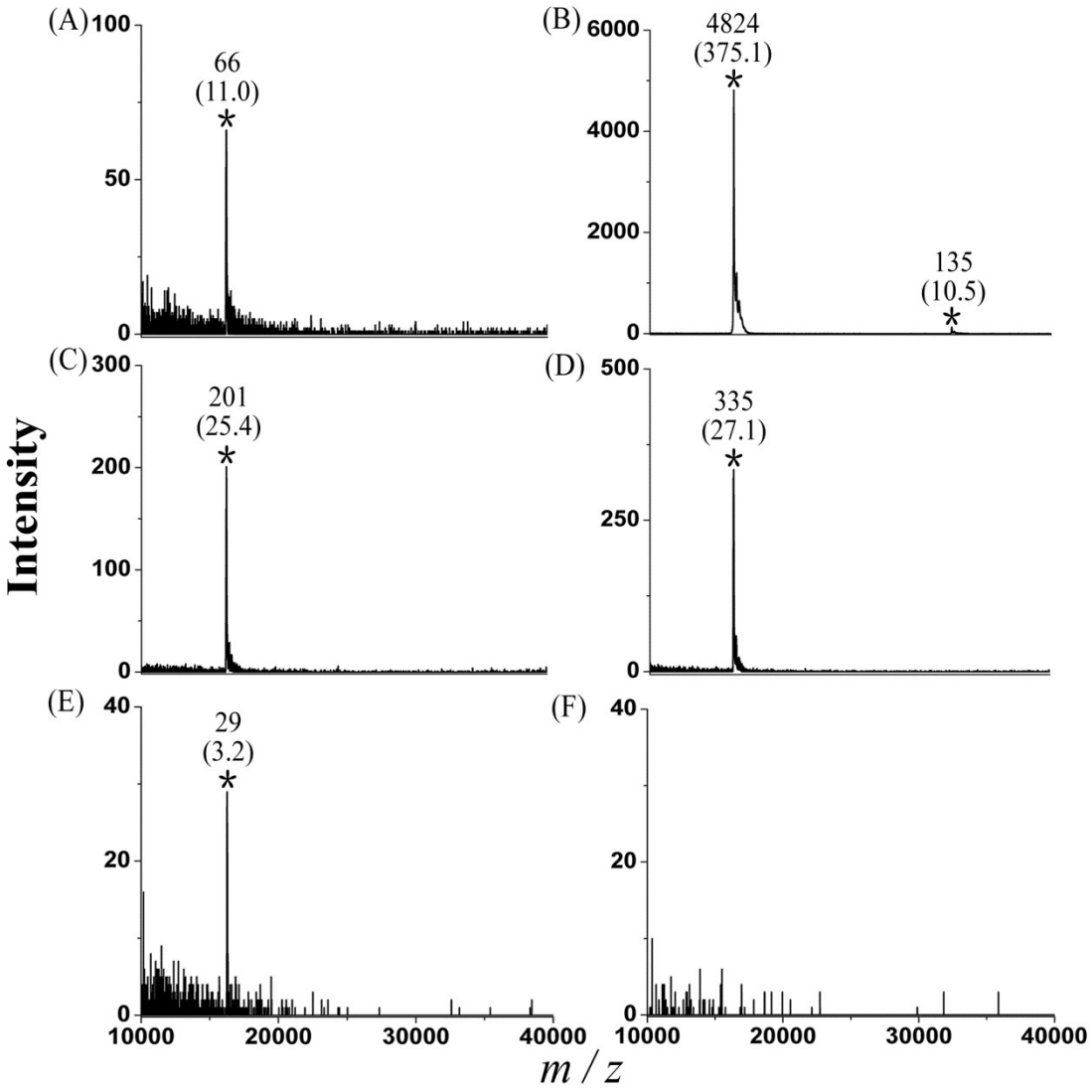


Figure S10. MALDI-TOF MS data for 50 μg mL^{-1} Mb at pH 5.5, 7, 8.5 (A, C, E) and after enrichment with Fe_3O_4@MIL-100(Fe) nanoparticles (B, D, F). The peak intensity and S/N ratio (in parentheses) are labelled for the highest peaks.

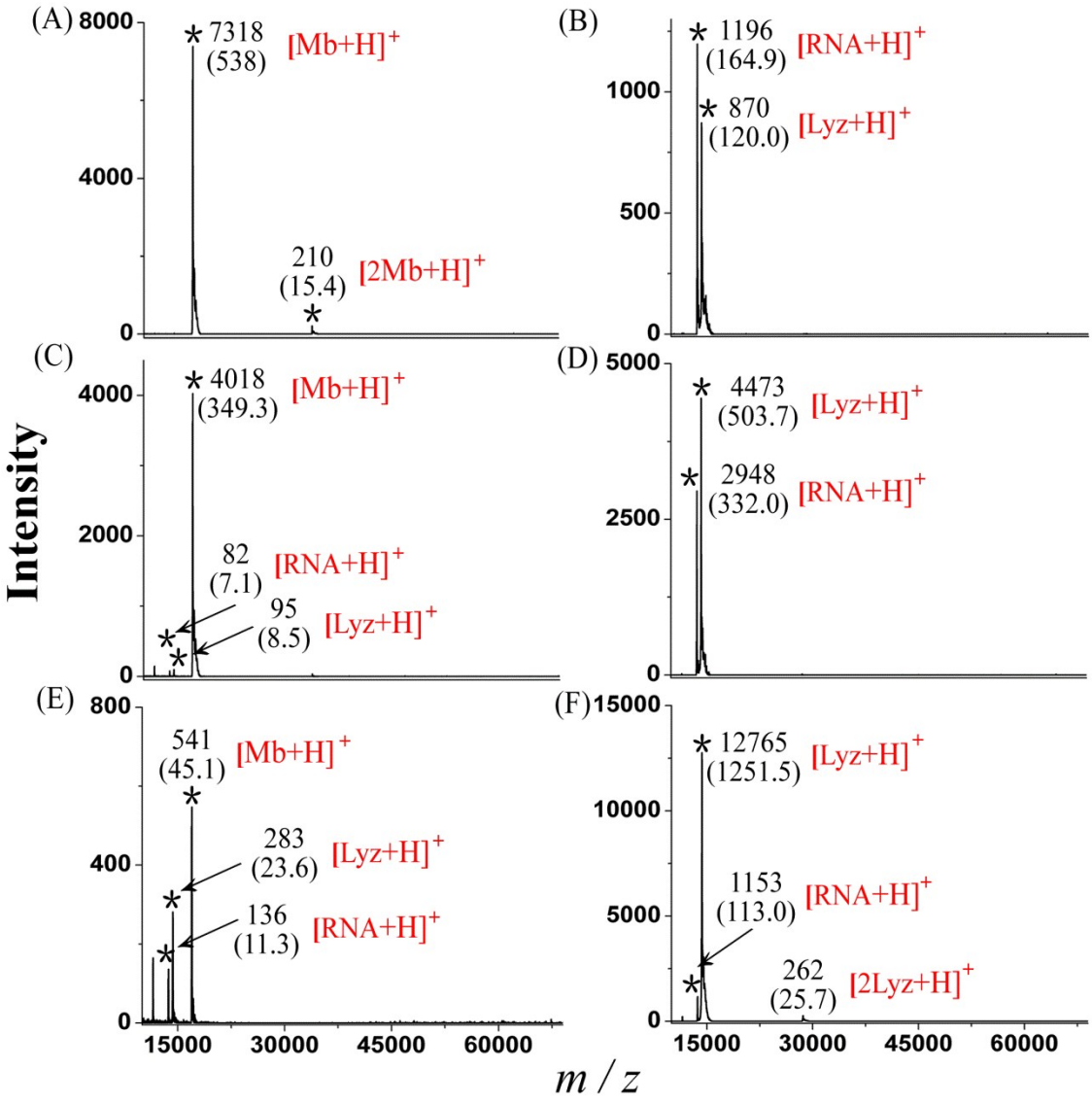


Figure S11. MALDI-TOF MS data for protein mixture **2** (30 μg mL^{-1} RNA + 80 μg mL^{-1} Lyz + 100 μg mL^{-1} Mb + 100 μg mL^{-1} BSA) before (A) and after enrichment (B), protein mixture **3** (30 μg mL^{-1} RNA + 80 μg mL^{-1} Lyz + 50 μg mL^{-1} Mb + 50 μg mL^{-1} BSA) before (C) and after enrichment (D), and protein mixture **4** (30 μg mL^{-1} RNA + 80 μg mL^{-1} Lyz + 20 μg mL^{-1} Mb + 20 μg mL^{-1} BSA) before (E) and after enrichment (F). The peak intensity and S/N ratio (in parentheses) are labelled for the highest peaks.

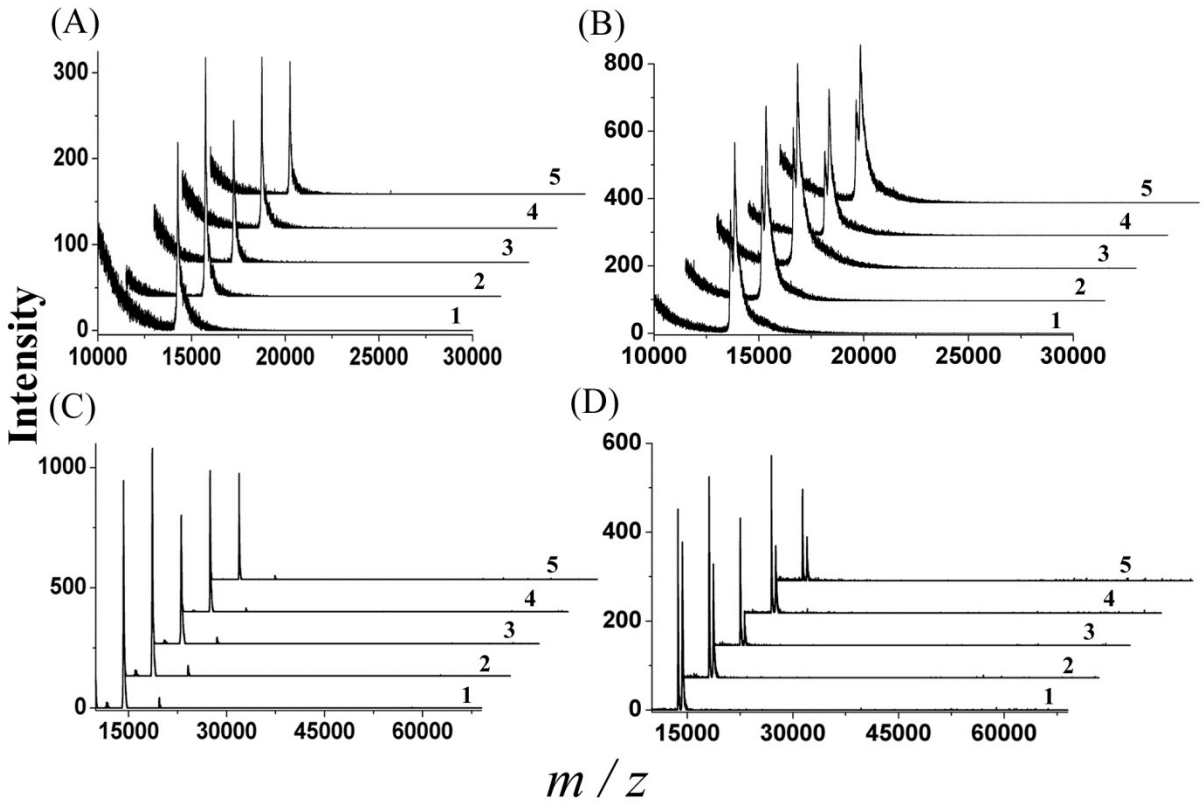


Figure S12. MALDI-TOF MS data for reproducibility test of Fe_3O_4@MIL-100(Fe) nanoparticles in enrichment of 5 μg mL^{-1} Lyz (A), 40 μg mL^{-1} RNA (B), diluted egg white samples (C) and human serum (D) for five cycles. After each use, the nanoparticles were recycled by washing with 500 μL of 30% ACN/0.1% TFA and 1 mL of water. The peak intensity and S/N ratio (in parentheses) are labelled for the highest peaks.

References

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