Supporting Information

Experimental section

1. Materials and reagents

Dopamine hydrochloride and zirconium chloride ($ZrCl_4$) were purchased from Aladdin Chemistry Co., Ltd. (USA). 1,4-benzenedicarboxylic acid was purchased from Ourchem Chemical Reagent Co., Ltd. (Shanghai, China). The NdFeB magnet was purchased from PCCW (Beijing), 2 cm long, 2 cm wide, 1 cm high, with surface magnetic field strength of 1000 Gauss. L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma Chemical (St. Louis, MO). Bovine β-casein and bovine serum albumin (BSA) were obtained from Bio Basic (Toronto, Canada). The human serum sample originated from a hepatocellular carcinoma patient was offered by Shanghai Zhongshan Hospital. Acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Millipore, Bedford, MA). All other chemicals and reagents are of the highest grade commercially available and used as received.

2. Synthesis of Fe_3O_4@PDA@Zr-MOF and Fe_3O_4@PDA@Zr^{4+}

Fe_3O_4 microspheres were synthesized via a hydrothermal reaction in the same way as our previous reports. Detailedly, $Fe_3O_4 \cdot 6H_2O$ (1.35 g) was dissolved in 75 mL of ethylene glycol under magnetic stirring. Then, 3.60 g of NaAc was added to this solution. After being stirred for 1 h, the resulting solution was transferred into a Teflon-lined stainless-steel autoclave. The autoclave was sealed and heated at 200 °C for 16 h. The obtained particles were washed with deionized water several times.

Fe_3O_4@PDA was prepared at room temperature. In brief, 40 mg of dopamine hydrochloride was dissolved in 40 mL of Tris buffer (10 mM) and sonicated for 5min, 10 mg of Fe_3O_4 was added afterwards and mechanically stirred for 10 h. The as-synthesized Fe_3O_4@PDA was isolated by magnetic separation and washed with deionized water.

0.1 g of Fe_3O_4@PDA was added to a N,N-dimethylformamide (DMF) solution of MOF precursors, which contained 78 mg of $ZrCl_4$ and 10 mg of 1,4-benzenedicarboxylic acid. The as-

prepared solution was heated at 140 ℃ for 20 min. Finally, the product was collected by magnetic separation and washed with ethanol.

To prepare its competitor Fe_3O_4@PDA@Zr^{4+} microspheres, the obtained Fe_3O_4@PDA composites were dispersed in 100 mM $ZrCl_4$ solution and mechanically stirred at 70 ℃ for 2-3 hours. The resulting Fe_3O_4@PDA@Zr^{4+} was gathered by magnetic separation and washed with ethanol.

3. Characterizations and measurements

Transmission electron microscopy (TEM) images were taken on a JEOL 2011 microscope (Japan) operated at 200 kV. Samples were dispersed in ethanol beforehand and collected by using carbon-film-covered copper grids for analysis. Scanning electronic microscope (SEM) images and energy dispersive X-ray (EDX) spectra were recorded on a Philips XL30 electron microscope (Netherlands) operating at 20 kV. A thin gold film was sprayed on the sample before measurements. Fourier transform infrared spectra (FT-IR) were collected on Nicolet Fourier spectrophotometer using KBr pellets (USA). The Raman spectra were recorded at room temperature on a LabRam-1B Raman spectrometer with a laser at an excitation wavelength of 632.8 nm. Zeta potential measurements were carried out on a Nano ZS90 zeta analyzer (Malvern Instruments Ltd.). Powder X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu K_α radiation (40 kV, 40 mA). Nitrogen sorption isotherms were measured at 77 K with a Micromeritcs Tristar 3000 analyzer (USA). The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface area.

4. Sample preparation

Bovine serum albumin (BSA) was reduced with dithiothreitol (DTT) and carboxamidomethylated with iodoacetamide (IAA). Bovine β-casein and BSA were dissolved in 25 mM ammonium bicarbonate (NH_4HCO_3) buffer at pH 8.3 respectively and treated with trypsin (40:1, w/w) for 16 h at 37 °C. The obtained peptide mixture was diluted with 25 mM NH_4HCO_3 for enrichment and MS analysis.

Human serum was diluted 10 fold with deionized water in advance.

Before the investigation into the enrichment efficiency of Fe_3O_4@PDA@Zr-MOF, the

microspheres were suspended in deionized water to prepare a dispersion (10 mg/mL). After being vibrated with the help of a vortex for a few seconds, a clear and homogeneous solution with no sedimentation was obtained (Fig. S7a), indicating PDA remarkably improved the dispersity of Fe_3O_4. Assisted by a magnetic field, the magnetic MOFs were immediately drawn from the solution to the sidewall of the vial within 2 s, which spared much time during magnetic separation.

5. Enrichment of phosphopeptides from β-casein tryptic digestions

10 mg of Fe_3O_4@PDA@Zr-MOF microspheres were suspended in 1 mL of deionized water with the help of a vortex. In a typical enrichment process, 10 μL of Fe_3O_4@PDA@Zr-MOF suspension was added to 200 μL of peptide solution (2μL of tryptic digest was spiked into 200 μL of 50%ACN/0.1%TFA buffer) in a 0.6 mL centrifuge tube, and the mixture was then vibrated in a shaker at 37 °C for 30 min to ensure equilibrium. After magnetic separation and removal of the supernatant, the magnetic particles were rinsed with 50% ACN/0.1% TFA buffer three times. Subsequently, 10 μL of 0.4 M ammonia was added into the tube and vibrated for 10 min to elute the captured peptides. The supernatant was pipetted onto a MALDI sample target and dried. Later on, 0.8 μL of DHB matrix was pipetted on it. The sample target was left at room temperature for evaporation of the solvent. At last, the substrates were subjected to MALDI-TOF MS for analysis.

6. Enrichment of phosphopeptides from the tryptic digest mixtures of β-casein and BSA

A suspension of Fe_3O_4@PDA@Zr-MOF microspheres (10 mg/mL, 10μL) was added into 200 μL of tryptic digest mixture of β-casein and BSA at a certain molar ratio. After similar enrichment, washing and elution protocol was followed, the eluent was deposited on a MALDI target using dried droplet method and 0.8 μL of DHB matrix was introduced.

7. Determination of phosphopeptides recovery on Fe_3O_4@PDA@Zr-MOF by using an internal standard

A standard multiply-phosphorylated peptide (Mr = 1219 Da) was added to both of 10^{-6} M β-casein tryptic digest without any treatment and the elution after enrichment with Fe_3O_4@PDA@Zr-MOF as an internal standard. We quantitatively evaluated the amounts of the three phosphopeptides derived from β-casein tryptic digest by comparing their S/N ratios to those

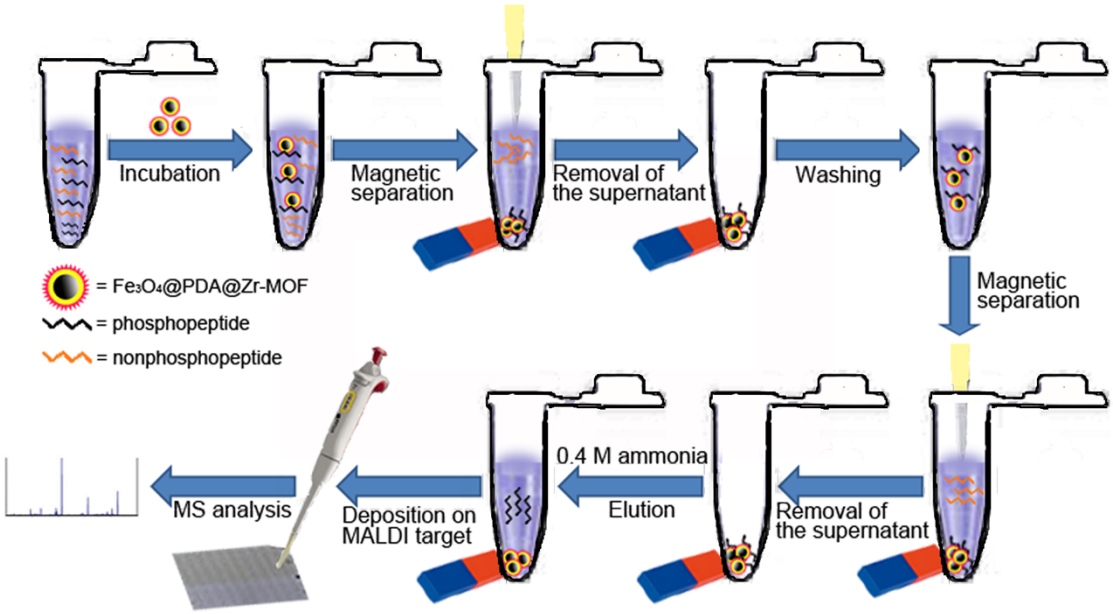
of the internal standard phosphopeptide. MALDI-TOF MS spectra of 10^{-6} M β-casein tryptic digest before and after enrichment with Fe_3O_4@PDA@Zr-MOF are shown in Fig. S11. The S/N ratio of phosphopeptides and the internal standard in the original solution and in the elution are listed in Table S2. Since the volume of the analyte solution was condensed from the initial 200 μL to 10 μL after elution, the recovery of phosphopeptides can be calculated as follows: Recovery = [phosphopeptide to internal standard ratio in the elution / (phosphopeptide to internal standard in the original solution*20)]. The recovery of the three phosphopeptides derived from β-casein tryptic digest was also given in Table S2.

8. Enrichment of phosphopeptides from human serum sample

A suspension of Fe_3O_4@PDA@Zr-MOF (10 mg/mL, 10μL) was added into 200 μL of a 50%ACN/0.1% TFA aqueous solution which contain 2 μL of human serum dilution. Similar enrichment and washing protocol described above was performed. After that, the obtained adducts were eluted by 0.4M ammonia. 1 μL of the elution was pippeted onto a MALDI target and dried, and 0.8 μL of DHB was introduced. The substrates were subjected to MALDI-MS for phosphopeptides identification eventually.

9. MALDI-TOF-MS analysis

Mass spectra were acquired in positive reflective mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd: YAG laser at 366 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV.



Scheme S1 Schematic illustration of phosphopeptides enrichment using Fe_3O_4@PDA@Zr-MOF microspheres.

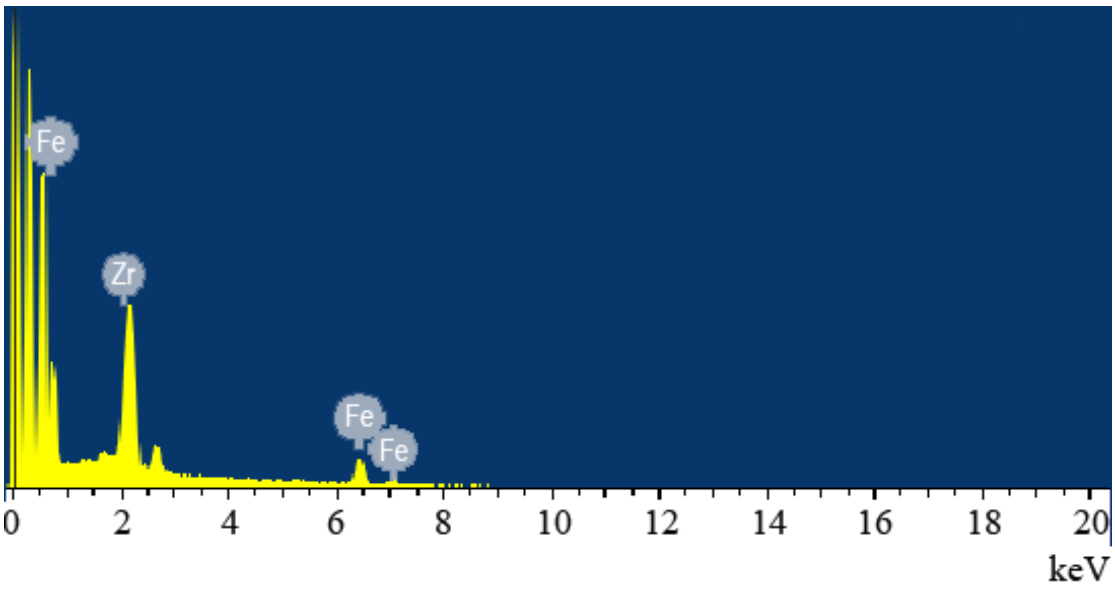


Fig. S1 The energy dispersive X-ray (EDX) spectrum of the as-synthesized Fe_3O_4@PDA@Zr-MOF microspheres.

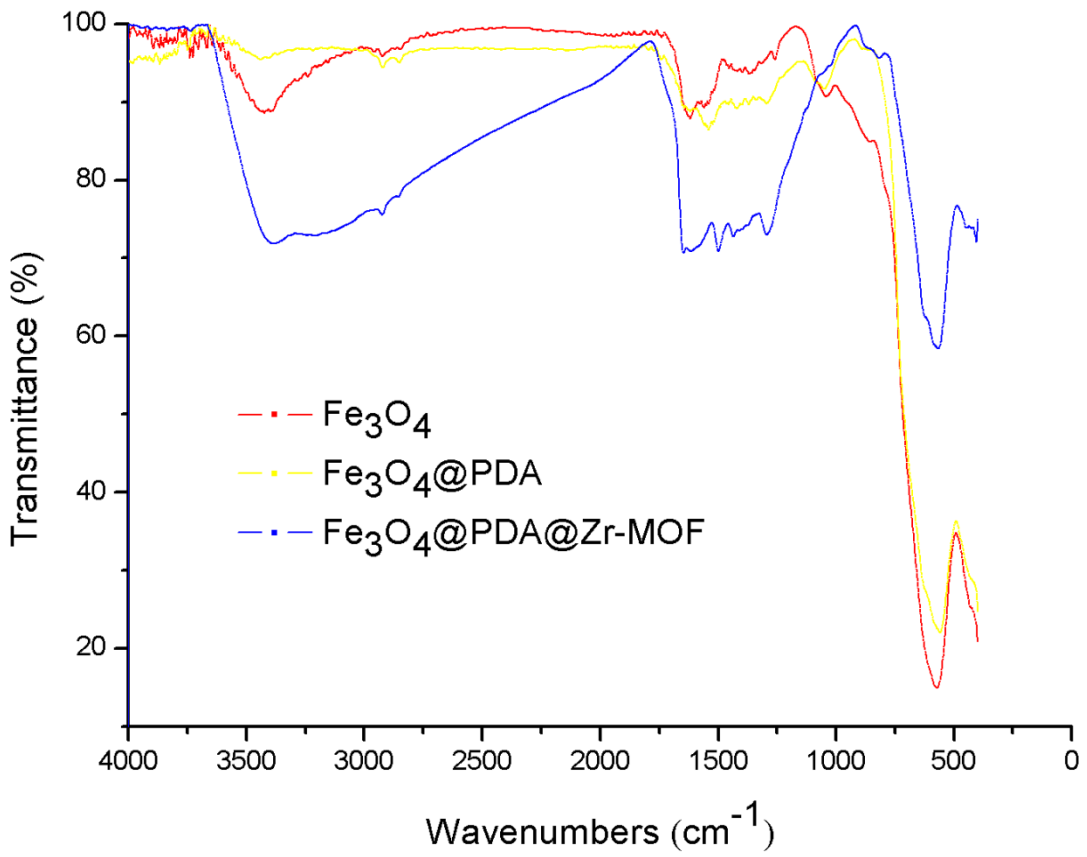


Fig. S2 The FT-IR spectra of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@Zr-MOF.

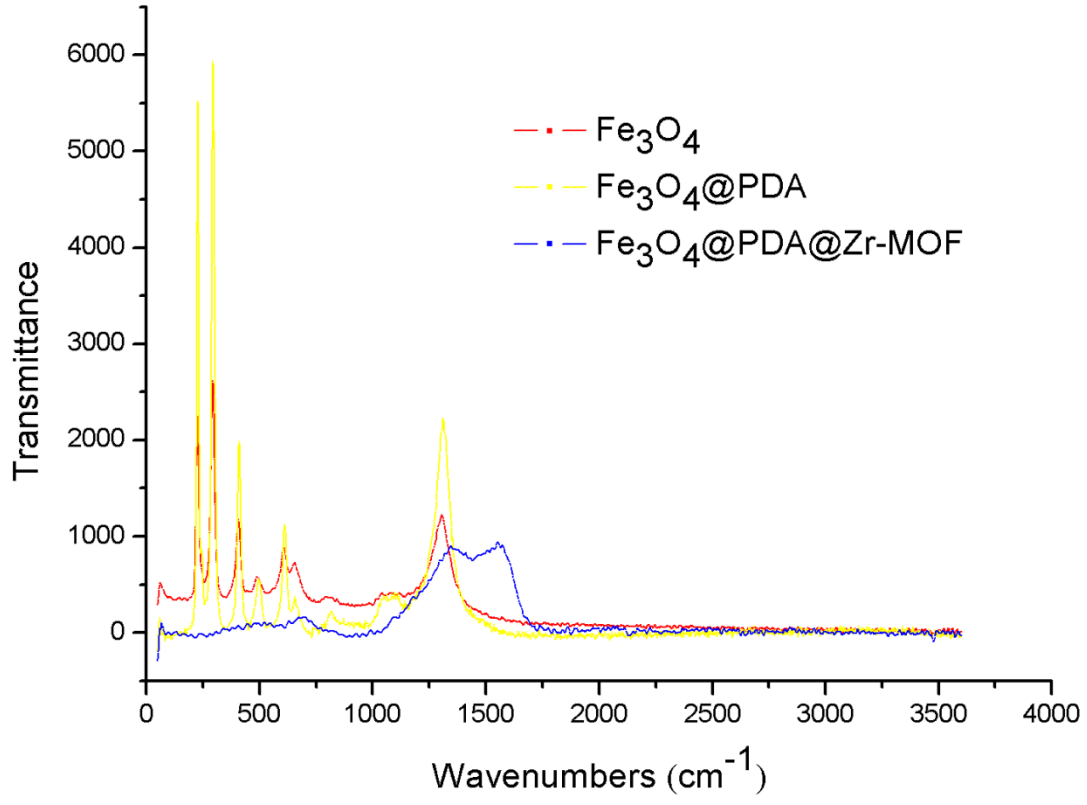


Fig. S3 The Raman spectra of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@Zr-MOF.

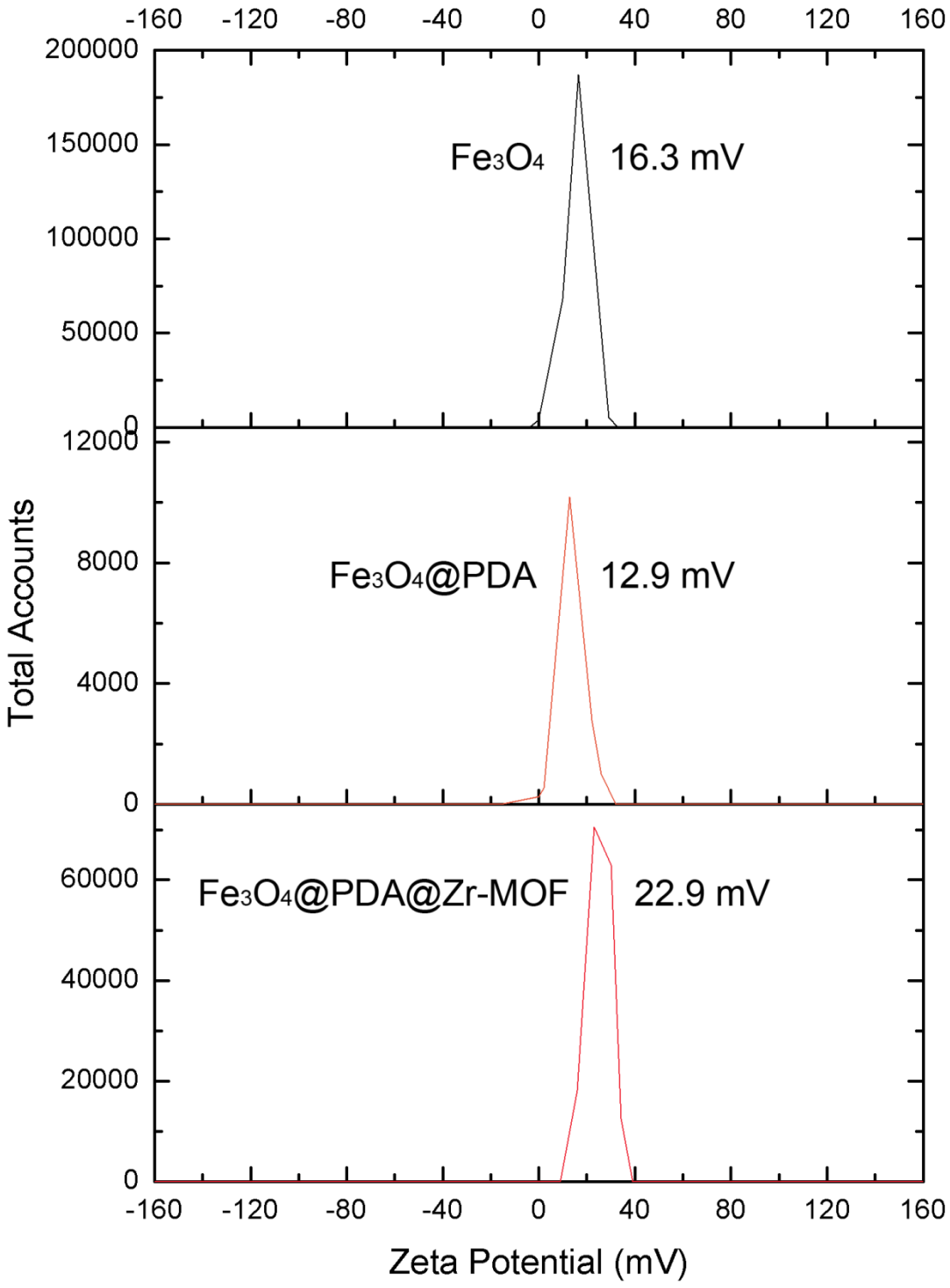


Fig. S4 The zeta potential distributions of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@Zr-MOF.

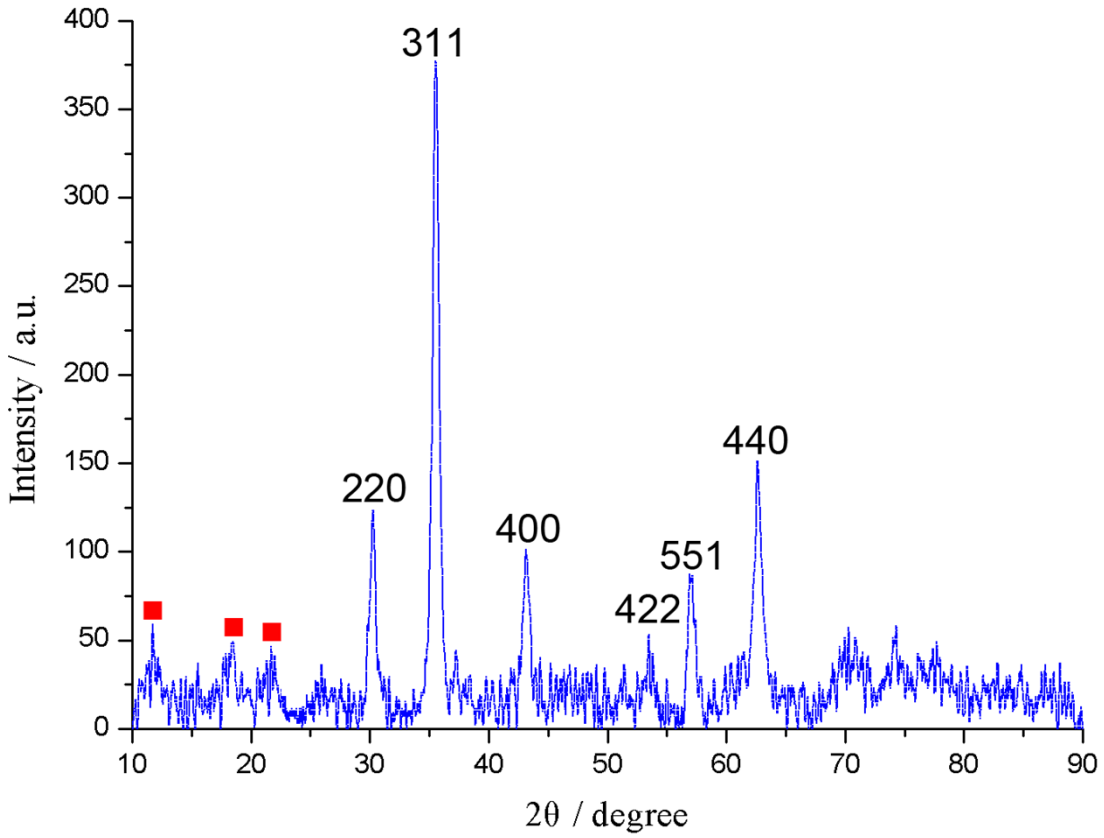


Fig. S5 XRD patterns of Fe_3O_4@PDA@Zr-MOF. Peaks originated from Zr-bdc MOFs are marked with the symbol ■ in red and those originated from Fe_3O_4 are marked with miller indexes.

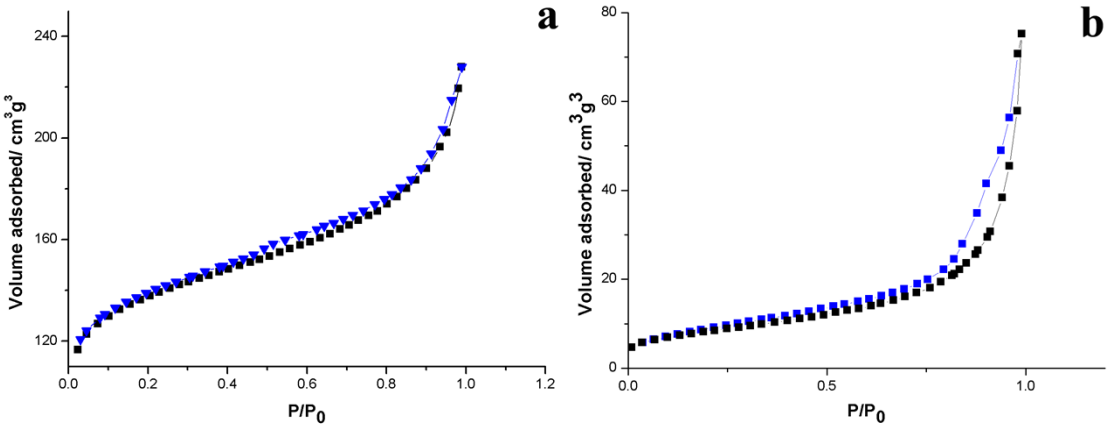


Fig. S6 N_2 adsorption-desorption isotherms of a) the as-synthesized Fe_3O_4@PDA@Zr-MOF microspheres and b) Fe_3O_4@PDA@Zr^{4+} microspheres measured at 77 K.

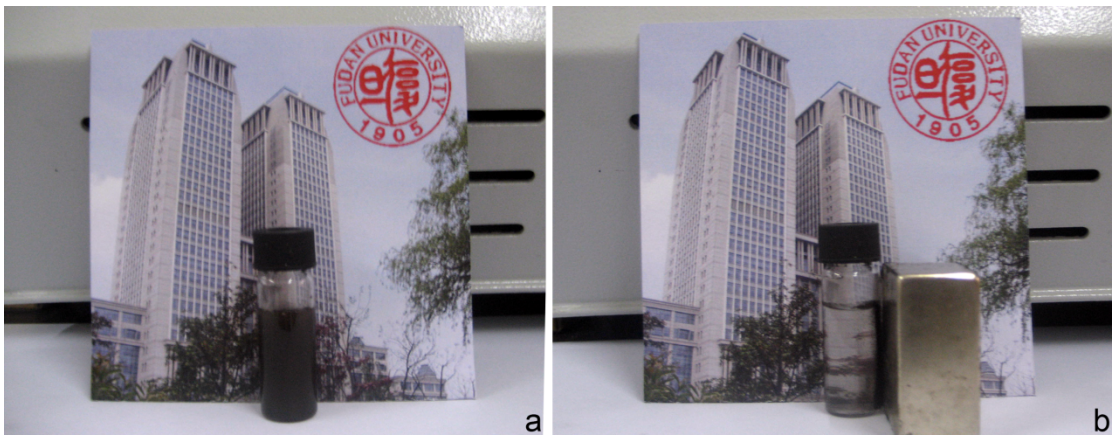


Fig. S7 The photos of the aqueous dispersion of Fe_3O_4@PDA@Zr-MOF microspheres: (a) before and (b) after separation with a magnet for 2 seconds.

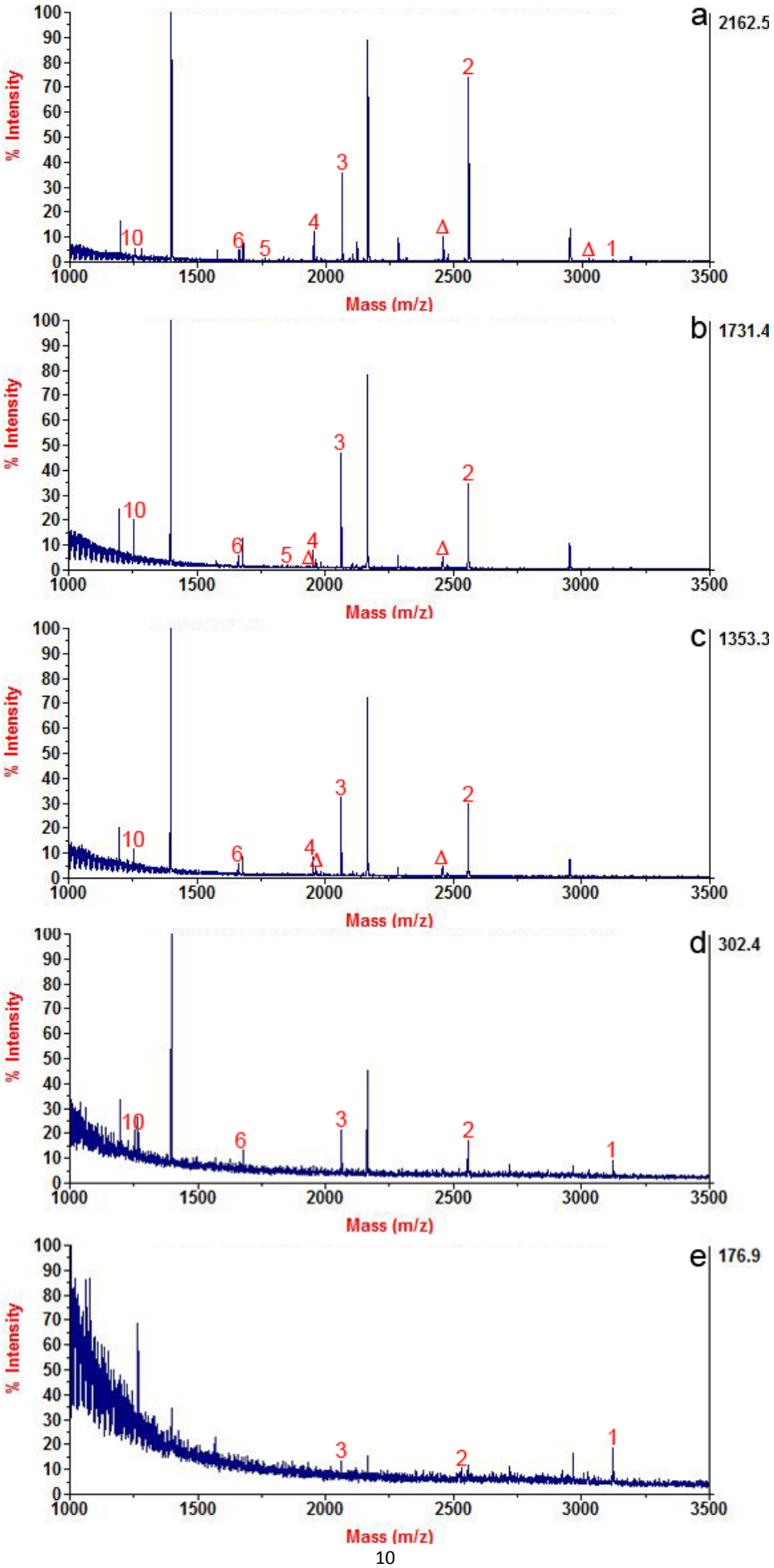

a
2162.5
b
1731.4
c
1353.3
d
302.4
e
176.9
% Intensity
Mass (m/z)
10
6
5
4
3
2
1
Δ

Fig. S8 MALDI-TOF mass spectra of the peptides derived from β-casein tryptic digest with various amount enriched by Fe_3O_4@PDA@Zr-MOF: (a) 10 pmol, (b) 1 pmol, (c) 100 fmol, (d)10 fmol and (e) 1 fmol. Phosphopeptide peaks identified are marked with numbers and dephosphorylated fragments of phosphopeptide peaks through loss of H_3PO_4 are marked with symbol Δ.

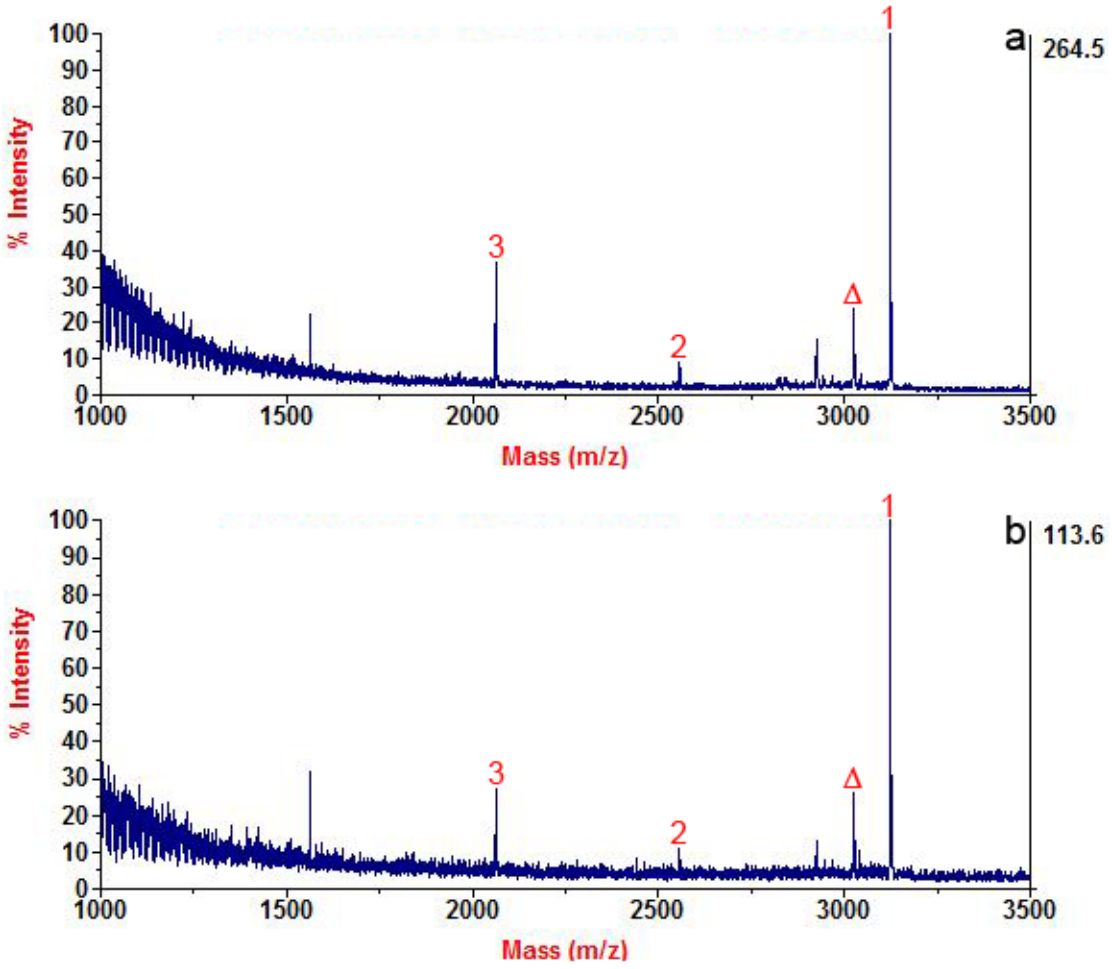


Fig. S9 MALDI-TOF mass spectra for the peptides derived from 1 nM β-casein tryptic digest: (a) after treatment with Fe_3O_4@PDA@Zr-MOF used for the first time and (b) after treatment with Fe_3O_4@PDA@Zr-MOF recycled 5 times. Phosphopeptide peaks identified are marked with numbers and dephosphorylated fragments of phosphopeptide peaks are marked with symbol Δ.

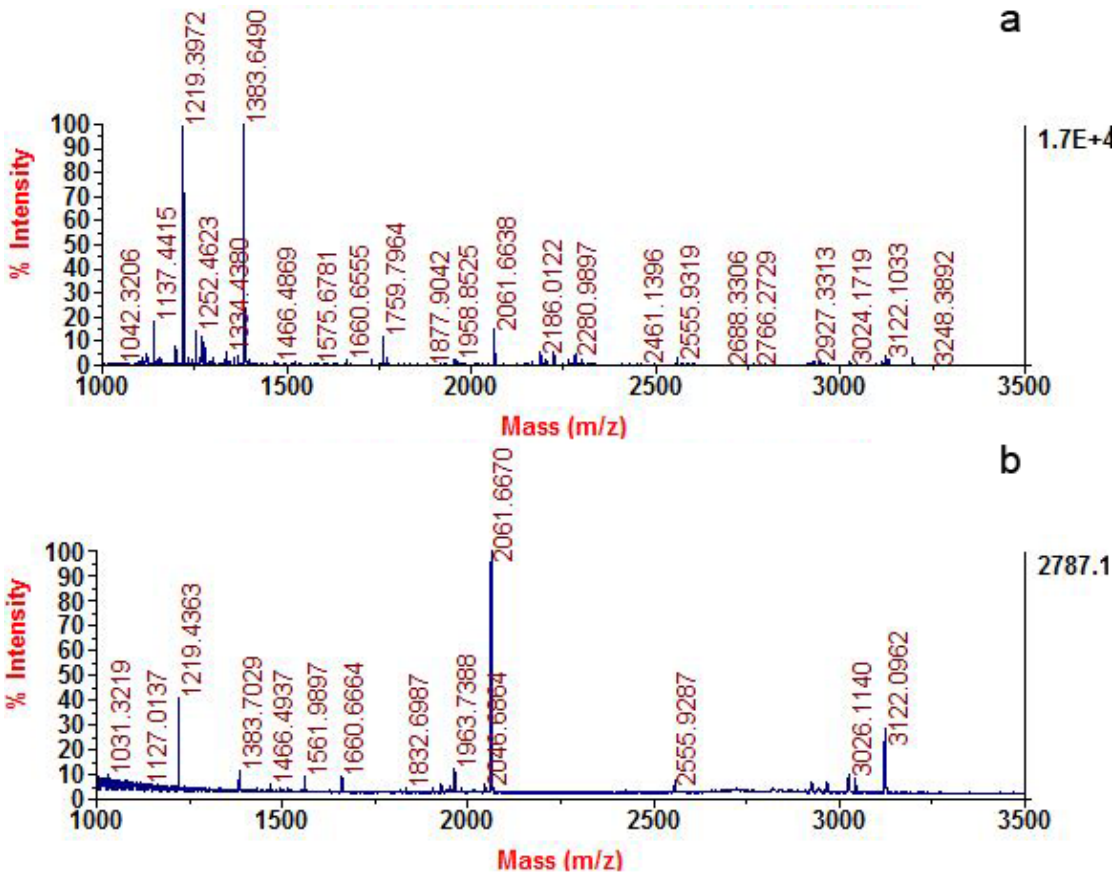


Fig. S10 MALDI-TOF mass spectra for the peptides derived from 10^{-6} M β-casein tryptic digest (a) before; (b) after enrichment with Fe_3O_4@PDA@Zr-MOF.

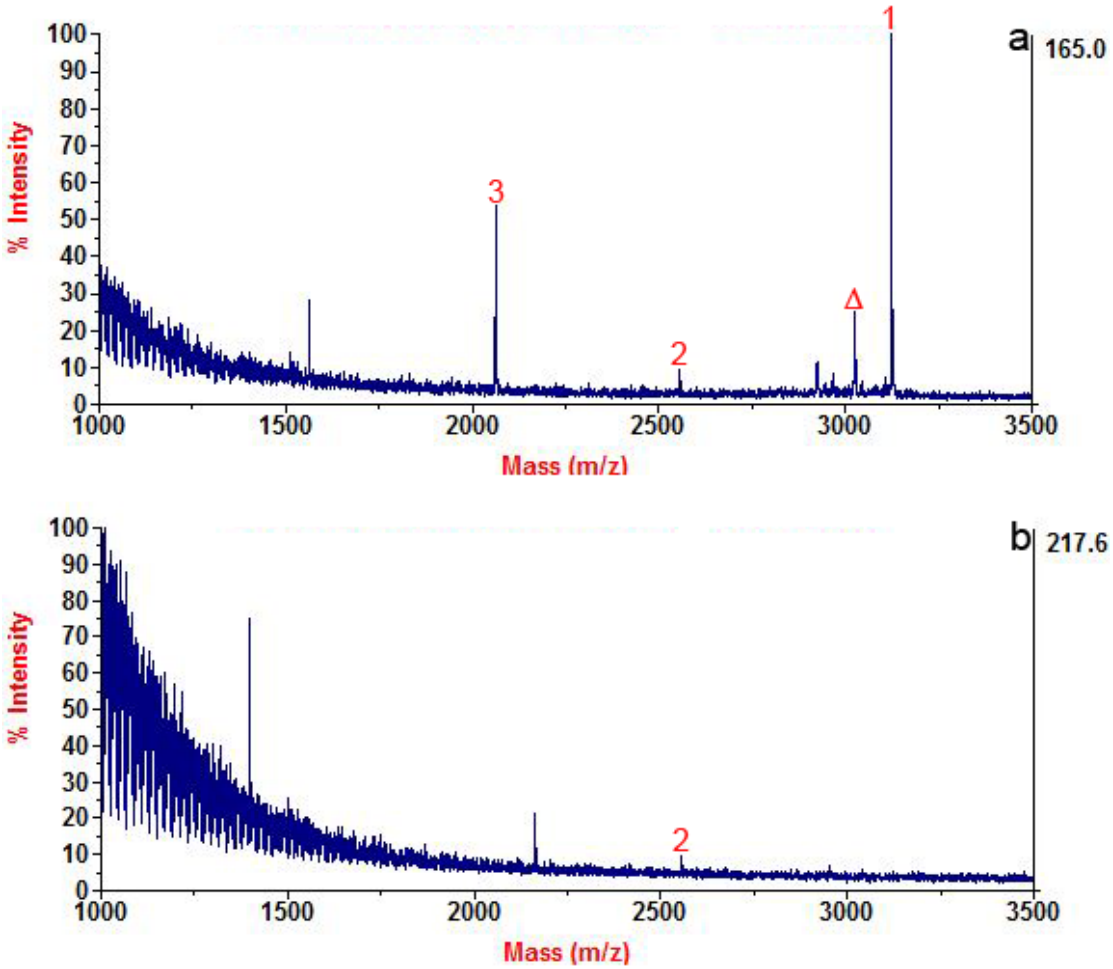


Fig. S11 MALDI-TOF mass spectra for the peptides derived from 1 nM β-casein tryptic digest: (a) after enrichment with Fe_3O_4@PDA@Zr-MOF and (b) after enrichment with Fe_3O_4@PDA@Zr^{4+}. Phosphopeptide peaks identified are marked with numbers and dephosphorylated fragments of phosphopeptide peaks are marked with symbol Δ.

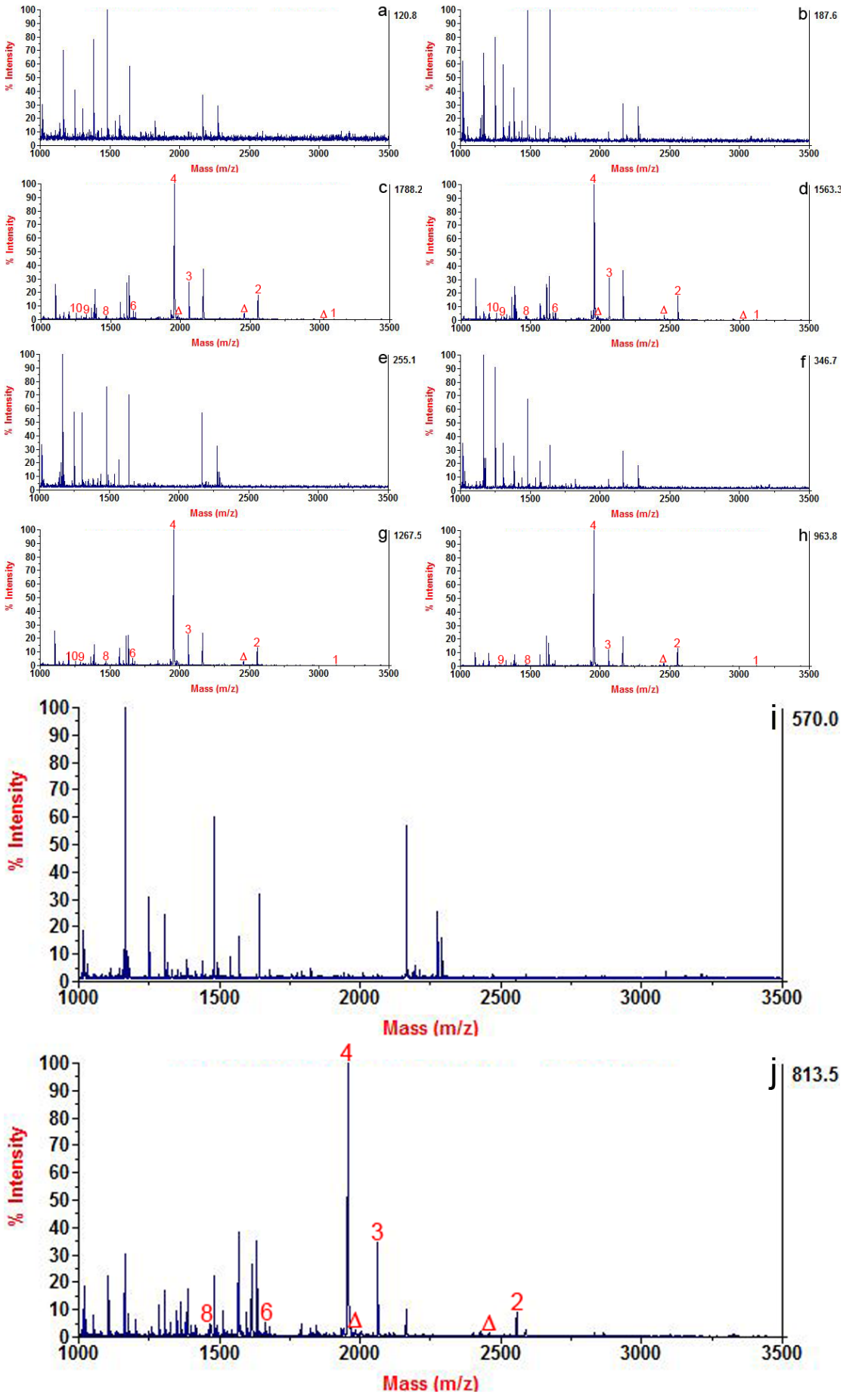

a
120.8
b
187.6
c
1788.2
d
1563.3
e
255.1
f
346.7
g
1267.5
h
963.8
% Intensity
Mass (m/z)
i
570.0
j
813.5

Fig. S12 MALDI-TOF mass spectra for the peptides derived from the tryptic digest mixture of β-casein and BSA (at molar ratio of 1:50, 1:100, 1:200, 1:400 and 1:500): (a), (b), (e), (f) and (i) before; (c), (d), (g), (h) and (j) after enrichment with Fe_3O_4@PDA@Zr-MOF. Phosphopeptide peaks identified are marked with numbers and dephosphorylated fragments of phosphopeptide peaks are marked with symbol Δ.

Table S1. Phosphopeptides identified in tryptic digests of β-Casein after enrichment with Fe_3O_4@PDA@Zr-MOF core-shell-shell microspheres.

Peak No.	Theoretical *m/z*	aa	Peptide Sequence
1	3122.27	*β*/1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
2	2556.09	*β*/33-52	FQ[pS]EEQQQTEDELQDKIHPF
3	2061.83	*β*/33-48	FQ[pS]EEQQQTEDELQDK
4	1951.95[a]	α-S1/104-119	YKVPQLEIVPN[pS]AEER
5	1832.66[a]	α-S1/104-119	YLGEYLIVPN [pS]AEER
6	1660.79[a]	α-S1/106-119	VPQLEIVPN[pS]AEER
7	1561.70	*β*/1–25	RELEELNVPGEIVESLSSSEESITR
8	1466.51[a]	α-S2/138-149	TVDME[pS]TEVFTK
9	1278.60	*β*/33–52	FQSEEQQQTEDELQDKIHPF
10	1253.11[a]	α-S2/138-147	TVD[Mo]ME[PS]TEVF[b]

a Phosphopeptides derived from α-casein.

The amino acid sequences are cited from Zou et al[1].

Table S2 The S/N ratios of phosphopeptides and the internal standard peptide in 10^{-6} M β-casein tryptic digest without any treatment and the elution after treatment with Fe_3O_4@PDA@Zr-MOF and recovery of the phosphopeptides on Fe_3O_4@PDA@Zr-MOF.

m/z of the peptide	S/N ratio in 10^{-6} M β-casein tryptic digest	S/N ratio in the elution	Recovery / %
1219 (the internal standard)	17000	1145.1	/
2061	2565.9	2787.1	80.63
2556	556.1	203.5	27.16
3122	626.3	802.7	95.14

Reference

1 L. Zhao, R. Wu, G. Han, H. Zhou, L. Ren, R. Tian, H. Zou, *J Am Soc Mass Spectrom*, 2008, **19**, 1176.