This journal is © The Royal Society of Chemistry 2013

Supporting information

Materials and chemicals

Trifluoroaceticacid (TFA), β-casein, ammonium bicarbonate (NH_4HCO_3), bovine serum albumin, L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas). 3-(trihydroxysilyl)propyl methylphosphateand 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma Chemical (St. Louis, MO). Acetonitrile was purchased from Merck (Darmstadt, Germany). Dopamine hydrochloride was purchased from Aladdin Chemistry *Co*. Ltd. $Ti(SO_4)_2$ was purchased from Sinopharm. Chemical Regents Co. Ltd (Shanghai, China). The NdFeB magnet was purchased from PCCW (Beijing), 2cm long, 2cm wide, 1cm high, surface magnetic field strength of 1000 Gauss. All aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA). All other chemicals and reagents were of the highest grade commercially available.

Synthesis of Fe_3O_4@PD-Ti^{4+} microspheres

The magnetite particles (170 nm in diameter) were synthesized according to previous report [1]. Fe_3O_4@PD was prepared at room-temperature. Briefly, 40 mg of dopamine hydrochloride was dissolved in 40 mL of Tris buffer (10 mM) and sonicated for 5min, and then 10 mg of Fe_3O_4 was added and stirred for 10 h. The synthesized Fe_3O_4@PD was washed with water with a help of a magnet.

Second, the resultant Fe_3O_4@PD were incubated in a solution of $Ti(SO_4)_2$ (100 mM) for 2 h to immobilize Ti^{4+}cations. After incubation, the Fe_3O_4@PD-Ti^{4+} microspheres were collected by magnetic separation and washed thoroughly with 0.1% (v/v) FA and storage in 0.1% (v/v) FA. All

This journal is © The Royal Society of Chemistry 2013

of the modification processes were performed at room temperature.

Characterization and measurements

Scanning electron microscopy (SEM) images were obtained on a Philips XL30electron microscope (Netherlands) operating at 20 kV. Transmission electron microscopy (TEM) images were taken with a JEOL2011 microscope (Japan) operating at 200 kV. Wide-angle X-ray diffraction (WAXRD) patterns were recorded on a Bruker D4 X-ray diffractometer (Germany) with Ni-filtered Cu KR radiation (40 kV, 40 mA). Fourier-transform infrared (FT-IR) spectra were collected on a Nicolet Fourier spectrophotometer, using KBr pellets (USA).

Sample preparation

Bovine serum albumin was reduced with dithiothreitol [DTT] and carboxamidomethylated with iodoacetamide. Bovine serum albumin and bovine β-casein were dissolved in 25mM NH_4HCO_3 buffer at pH 8.3 and treated with trypsin (2%,w/w) for 16 h at 37°C respectively. Human serum was diluted with 50% acetonitrile and 0.1% trifluoroacetic acid [TFA] aqueous solution (v/v).

Enrichment of phosphopeptides from tryptic digestion of standard proteins

In a typical process, a suspension of Fe_3O_4@PD-Ti^{4+} microspheres (10 μL, 2 mg/mL) was added into 200 μL of a peptide mixture originating from tryptic digestion. The mixture was then vibrated at 37°C for 30 min. The phosphopeptide-loaded Fe_3O_4@PD-Ti^{4+} microspheres were collected by a magnet and washed with 200 μL of a 50% acetonitrile and 0.1% TFA water solution three times. Subsequently, an aqueous solution of NH_4OH (5 μL, 0.4 M) was added to elute the captured phosphopeptides, and the eluate was analyzed by MALDI–TOF MS.

This journal is © The Royal Society of Chemistry 2013

Enrichment of phosphopeptides from complex sample (the molar ratio of β-casein and BSA was 1:500)

In a typical process, a suspension of Fe_3O_4@PD-Ti^{4+} microspheres (10 μL, 2 mg/mL) was added into 200 μL of a peptide mixture (tryptic digests of β-casein and BSA mixture and the molar ratio of β-casein and BSA was 1:500). The mixture was then vibrated at 37°C for 30 min. The phosphopeptide-loaded Fe_3O_4@PD-Ti^{4+} microspheres were collected by a magnet and washed with 200 μL of a 50% acetonitrile and 0.1% TFA water solution three times. Subsequently, an aqueous solution of NH_4OH (5 μL, 0.4 M) was added to elute the captured phosphopeptides, and the eluate was analyzed by MALDI–TOF MS.

Enrichment of phosphopeptides from human serum

In a typical process, a suspension of Fe_3O_4@PD-Ti^{4+} microspheres (, 2 mg/mL) was added into 200 μL of a 50% acetonitrile and 0.1% TFA water solution which contain 2 μL human serum. The mixture was then vibrated at 37°C for 30 min. The phosphopeptide-loaded Fe_3O_4@PD-Ti^{4+} microspheres were collected by a magnet and washed with 200 μL of a 50% acetonitrile and 0.1% TFA water solution three times. Subsequently, an aqueous solution of NH_4OH (5 μL, 0.4 M) was added to elute the captured phosphopeptides, and the eluate was analyzed by MALDI–TOF MS.

MALDI-TOF MS analysis

The above phosphopeptides eluted from the Fe_3O_4@PD-Ti^{4+} microspheres were deposited on the MALDI target using the dried droplet method. 0.5 μL of washing buffer was deposited on the plate and then another 0.5μL of DHB aqueous solution (20 mg/mL, 50% acetonitrile and 1% H_3PO_4) was introduced as a matrix. MALDI–TOF MS experiments were performed in positive ion mode on a 5800 Proteomics Analyzer (Applied Biosystems, USA) with the Nd-YAG laser at 355 nm, a

repetition rate of 200 Hz and an acceleration voltage of 20 kV.

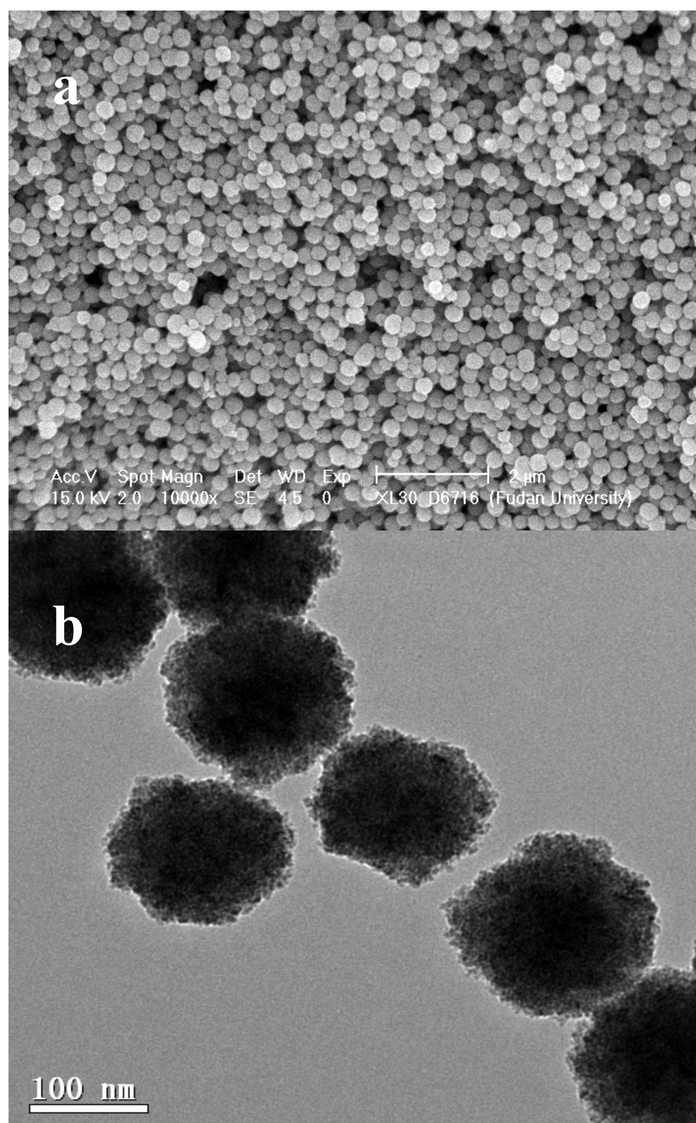

Figure S1. SEM (a) and TEM (b) images of the synthesized Fe_3O_4 particles.

This journal is © The Royal Society of Chemistry 2013

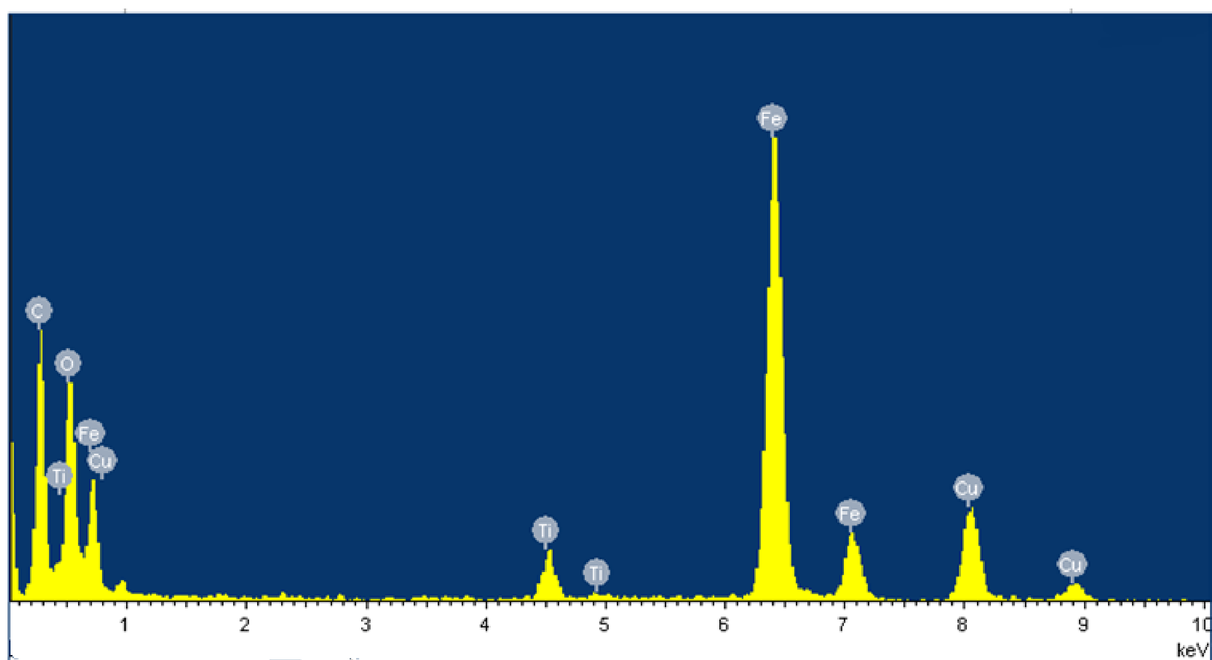

Figure S2. The energy dispersive X-ray (EDX) spectrum data of Fe_3O_4@PD-Ti^{4+} microspheres.

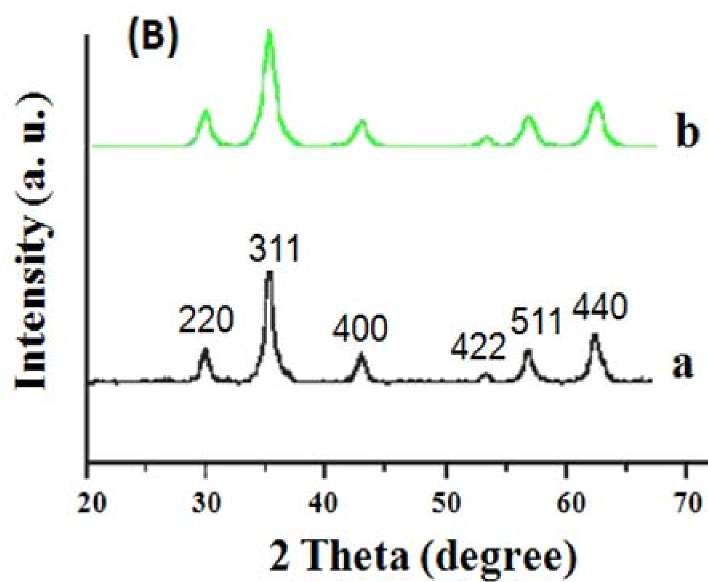

Figure S3. XRD patterns of (a) Fe_3O_4 particles and (b) Fe_3O_4@PD-Ti^{4+} microspheres.

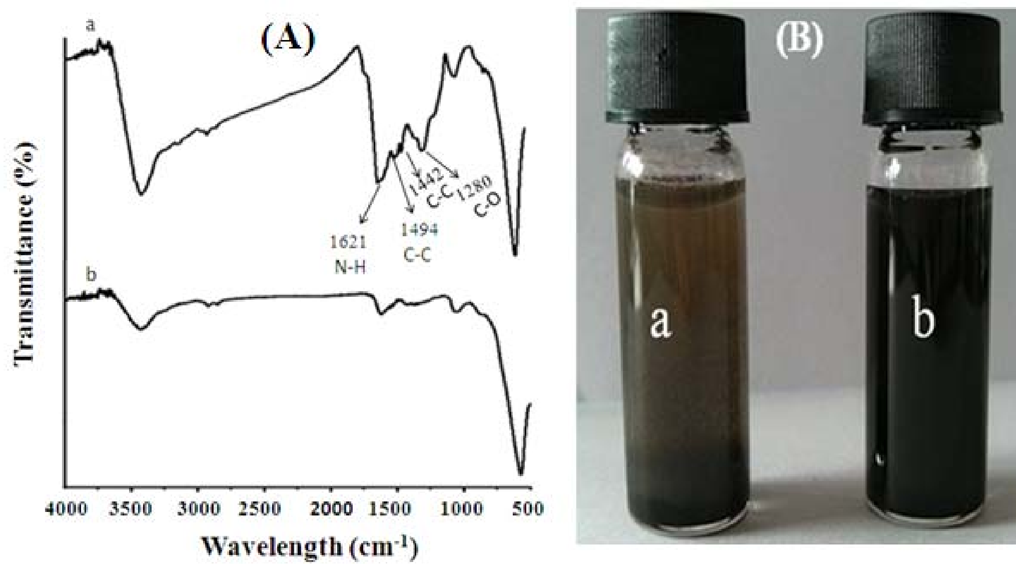

Figure S4. (a) ATR-FTIR spectra and (B) photographs of sample solutions after standing for 24h (a) Fe_3O_4 particles and (b) Fe_3O_4@PD-Ti^{4+} microspheres.

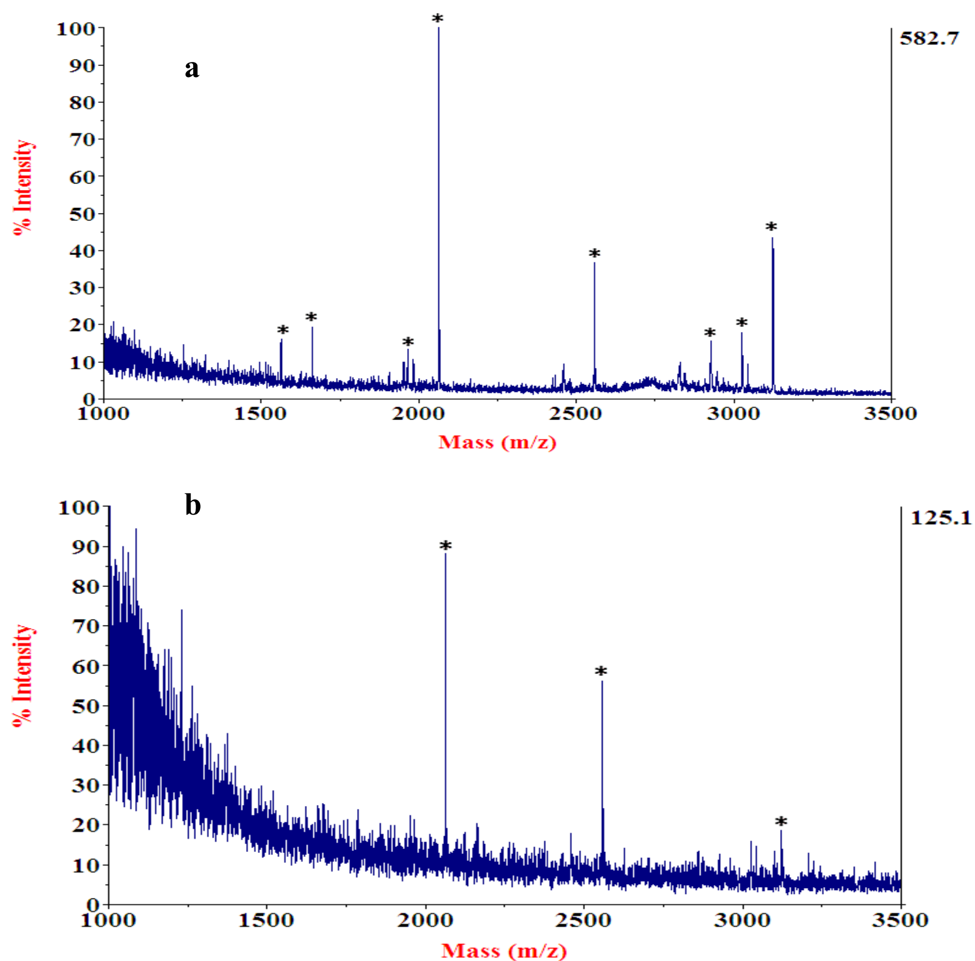

Figure S5. MALDI mass spectra of phosphopeptides enriched by Fe_3O_4@PD-Ti^{4+} microspheres, with the tryptic digests of β-casein concentration as (a) 20 fmol and (b) 2 fmol. Where the * indicates the phosphopeptides.

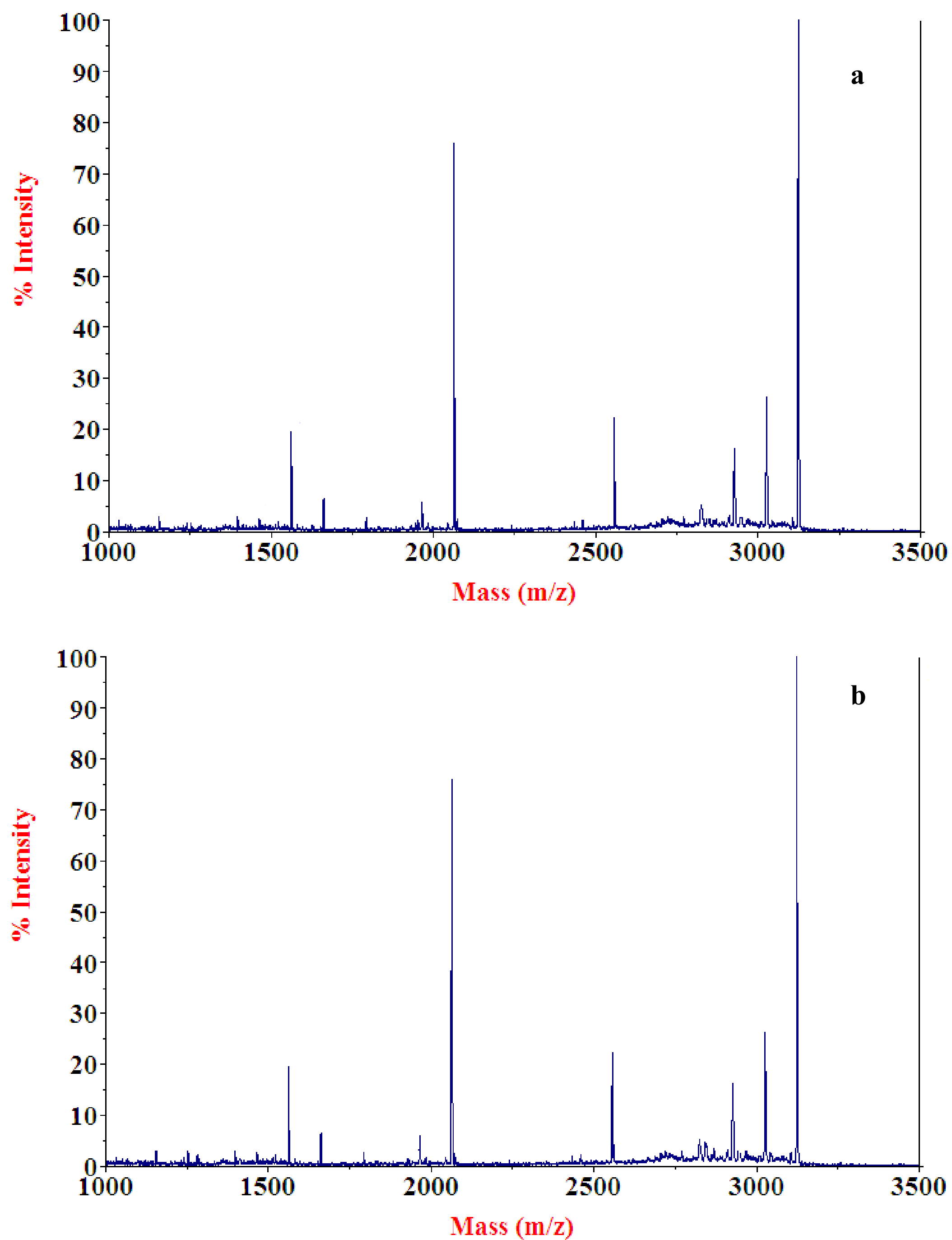

Figure S6. MALDI mass spectrum of phosphopeptides enriched from β-casein using Fe_3O_4@PD-Ti^{4+} microspheres, (a) for the first time and (b) for the fifth time.

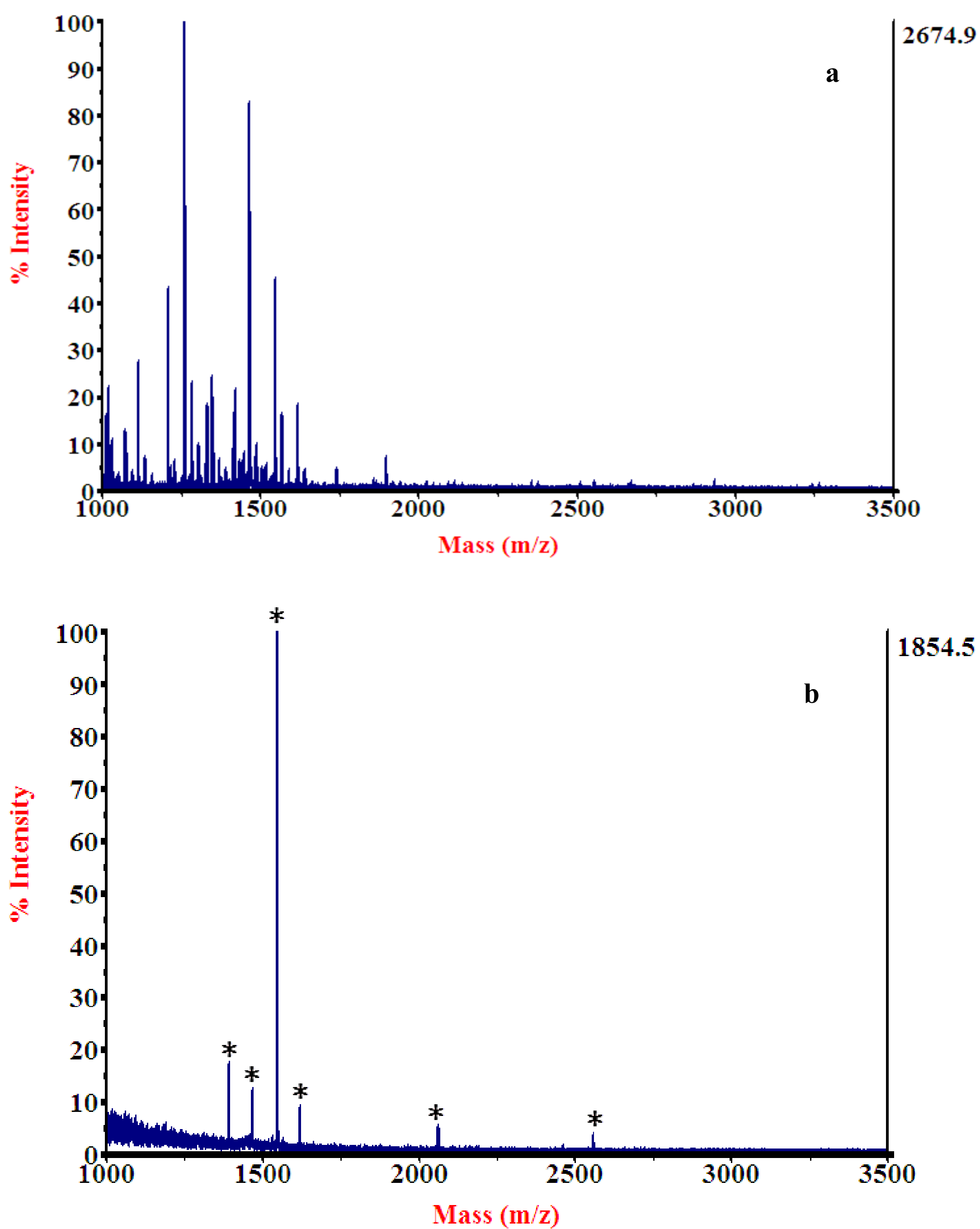

Figure S7. MALDI mass spectrum of peptides derived from human serum (a)without enrichment and (b) enriched by Fe_3O_4@PD-Ti^{4+} microspheres, where the * indicates the phosphopeptides.

This journal is © The Royal Society of Chemistry 2013

[1] J. Liu, Z. K. Sun, Y. H. Deng, Y. Zou, C. Y. Li, X. H. Guo, L. Q. Xiong, Y. Gao, F. Y. Li and D. Y. Zhao, *Angew. Chem. Int. Ed.* 2009, **48**, 5875.