This journal is (c) The Royal Society of Chemistry 2011

Supporting Information

Experimental details

1. Materials

Ferric chloride ($FeCl_3 \cdot 6H_2O$), Ethylene glycol (EG) and Ammonium hydroxide ($NH_3 \cdot H_2O$) were obtained from Beijing Chemical Regent Co. Ltd. (Beijing, China). Cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$), hexamethylenetetramine (HMT) Trifluoroacetic acid (TFA), Acetonitrile (ACN), Ammonium bicarbonate (NH_4HCO_3), Tetraethoxysilane (TEOS), 2,5-Dihydroxybenzoicacid (2,5-DHB) and Dithiothreitol (DTT) was purchased from Aladdin (Shanghai, China). Bovine β-casein, bovine serum albumin (BSA) and trypsin (from bovine pancreas, TPCK treated) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iodoaceticacid (IAA) was provided by Alfa Aesar (USA). Nonfat milk was purchased from a local grocery store. All the chemical agents were used without further purification.

2. Synthesis of the Fe_3O_4@SiO_2@$mCeO_2$ microspheres

2.1. Preparation of the Fe_3O_4@SiO_2 microspheres

The magnetic Fe_3O_4 submicrospheres were synthesized according to our previously reported method[1].

Fe_3O_4@SiO_2 core-shell particles were prepared via Stöber sol-gel process. Typically, 30 mg as-prepared Fe_3O_4 submicrospheres were ultrasonically dispersed in a solution containing 160 mL ethanol, 40 mL water and 10 mL concentrated ammonia (28 wt%). Then, 0.4 mL TEOS was added dropwise to the solution under sonication, followed mechanically stirring for 3 h at room temperature. Subsequently, the resulting particles were separated using an NdFeB magnet and washed with deionized water and ethanol. The step was repeated several times before drying at 60℃ for 12 h.

2.2. Preparation of Fe_3O_4@SiO_2@$mCeO_2$microspheres

Fe_3O_4@SiO_2@$mCeO_2$ microspheres were prepared by chemical precipitation method in accordance with the following procedure. The obtained Fe_3O_4@SiO_2 microspheres and 50 mg $Ce(NO_3)_3 \cdot 6H_2O$ was dispersed or dissolved in 30 mL ethanol through an ultrasonic treatment process for 15 min. Subsequently, 0.2 g HMT dissolved in 20 mL deionized water was added to the fully dispersed particle solution with ultrasonic vibrations for another 15min. The mixture was then stirred for 2 h in an oil bath maintained at 70 ℃. Final products were collected by the NdFeB permanent magnet, washing with deionized water and ethanol for several times to remove any possible ionic remnants, followed by drying overnight at 60℃ and

calcination at 400℃ for 2h. The Fe_3O_4@TiO_2 microspheres are prepared according to the previous report with slight modification[2].

3. Tryptic digests of proteins

1 mg β-casein was dissolved in 1 mL of 50 mM NH_4HCO_3 solution, and then trypsin was added into the solution with a ratio of 50:1 (substrate/trypsin) for 16 h at 37℃.The nonfat milk digest was prepared according to the previous report[2]. Briefly, 0.25 mL milk sample was mixed with the 0.25 mL of 50 mM NH_4HCO_3 solution, followed by vortex-mixing at 37℃ for 0.5h. Then, 50 μL of 100 mM DTT was added into the solution and mixed for an additional 1h at 50℃. After it was cooled to room temperature, 20 μL of 0.5M IAA was added into the mixture, followed by shaken for another 2.5 h at room temperature in the dark. Subsequently, the resulting solution was incubated with 10 μg trypsin in 50 mM aqueous ammonium bicarbonate at 37℃ for 18 h. Finally, the obtained tryptic digest was diluted to the target concentration with 50% acetonitrile and 0.15% TFA aqueous solution (v/v).

4. Enrichment of phosphopeptides

200 μL (2.5×10^{-8}M) protein digest was mixed with 5 μL of 20 mg/mL Fe_3O_4@SiO_2@mCeO_2 microspheres solution and then shaken for 2 min. Subsequently, the particles trapped target peptides were collected and isolated from the mixture with the help of a magnet. To purify the obtained phosphopeptides, the particles were washed with 100 μL 1.5% and 0.15% TFA solution in acetonitrile/deionized water (1/1, v/v) for three times. After that, the trapped phosphopeptides were eluted with 30μL of 10% ammonium hydroxide, and the supernatant was collected after magnetic separation and lyophilized to dryness.

5. Sample Preparation for MALDI-TOF MS

The above products obtained from the elution step was dissolved in 2 μL of matrix solution containing 20 mg/mL DHB (in 50% acetonitrile aqueous solution, v/v) and 1% (v/v) H_3PO_4 aqueous solution by pipetting and 0.5 μL of mixture was deposited onto the MALDI target.

6. Characterization

Scanning electron microscopy (SEM) images were performed on a field emission scanning electron microscope (FESEM, S4800, Hitachi) equipped with an energy-dispersive X-ray spectrum (EDX, JEOLJXA-840). Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were taken with a FEITecnaiG2 S-Twin transmission electron microscope operated at 200 kV. Nitrogen

adsorption isotherms were measured at a liquid nitrogen temperature (77 K) with a Micromeritcs ASAP 2010M apparatus. The specific surface area was determined by the Brunauer-Emmett-Teller (BET) method. The total pore volume was evaluated by the t-plot method, and pore size distribution was analyzed with the supplied BJH software package from the desorption branches of the isotherms. Magnetization measurement was carried out with a VSM7300 magnetometer at 300 K.

MS analysis was carried out on Bruker Reflex III MALDI-TOF MS (Bruker Daltonics Bremen, Germany) equipped with a 337 nm nitrogen laser. The acceleration voltage and repetition rate were set at 20 kV and 200 Hz, respectively, while the laser power was optimized until the best quality spectra was obtained. For PMF data, 600 laser shots were accumulated for each spectrum, which was obtained in positive ion reflection mode and analyzed by Bruker Daltonics flex Analysis software.

7. Table and Figure

Table S1. The phosphopeptides and their labeling signals identified by MALDI-TOF MS from tryptic digest of β-casein.

AA	Peptide sequences	Observed *m/z*	Theoratical *m/z*	Labeling singals	Phosphorylation site
β/33-48	FQ[pS]EEQQQTEDELQDK	2061.8	2061.8	1981.8	1
β/33-52	FQ[pS]EEQQQTEDELQDKIHPF	2556.1	2556.0	2476.0	1
β/1-25	RELEELNVPGEIVE[pS]L[pS][pS] [pS]EESITR	3122.3	3122.2	3042.2/2962.2/2882.2/2802.2	4

Table S2. The phosphopeptides and their labeling signals identified by MALDI-TOF MS from tryptic digest of nonfat milk.

AA	Peptide sequences	Observed *m/z*	Theoratic al *m/z*	Labeling singals	Phosphorylation site
αS1/106-119	VPQLEIVPN[pS]AEER	1660.7	1660.8	1580.7	1
αS1/104-119	YKVPQLEIVPN[pS]AEER	1832.8	1832.8	1752.8	1
αS1/43-58	DIG[pS]E[pS]TEDQAMEDIK	1927.6	1927.6	1847.6	2
αS1/104-119	YKVPQLEIVPN[pS]AEER	1951.9	1951.9	1871.9	1
β/33-48	FQ[pS]EEQQQTEDELQDK	2061.8	2061.8	1981.8	1
β/33-52	FQ[pS]EEQQQTEDELQDKIHPF	2556.0	2556.0	2476.0	1
β/1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3121.9	3122.2	3042.0/2962.2/ 2882.0/2802.2	4

To confirm middle silica layer coated on the surface of the magnetite cores, FTIR spectra of the Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@CeO_2microspheres are shown in the Fig. 1S.The assignments of the strong bands Si-O-Si (1091, 798 cm^{-1}) and Si-OH (945 cm^{-1}) indicates existence of the silica.

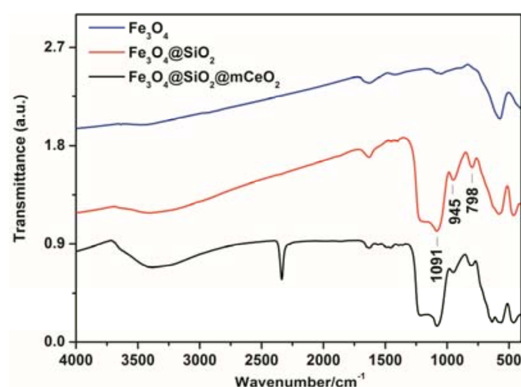

Figure S1. FTIR spectra of the as-synthesized Fe_3O_4 (a), Fe_3O_4@SiO_2 (b), and Fe_3O_4@SiO_2@$mCeO_2$ (c) microspheres.

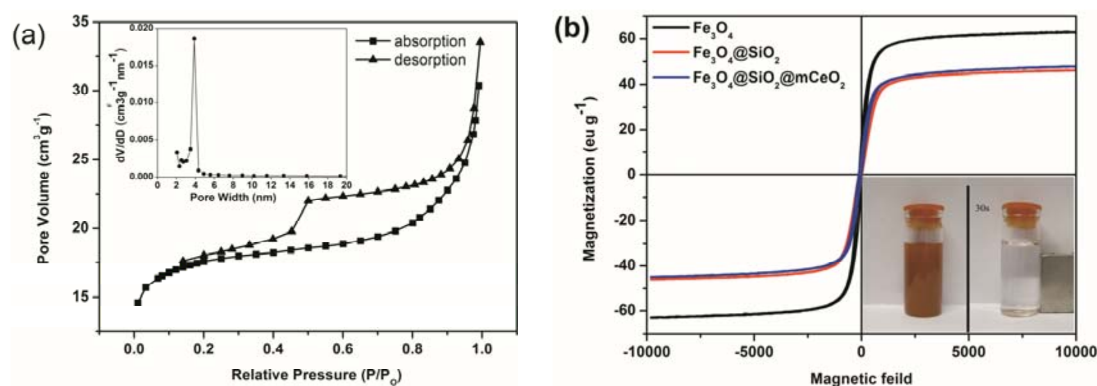

Figure S2. Nitrogen adsorption-desorption isotherm plots (a) and pore size distribution curves (inset in the Fig. a); hysteresis loops recorded at 300 K (b) and the dispersion and separation process of the Fe_3O_4@SiO_2@$mCeO_2$ microspheres (inset in the Fig. S2b).

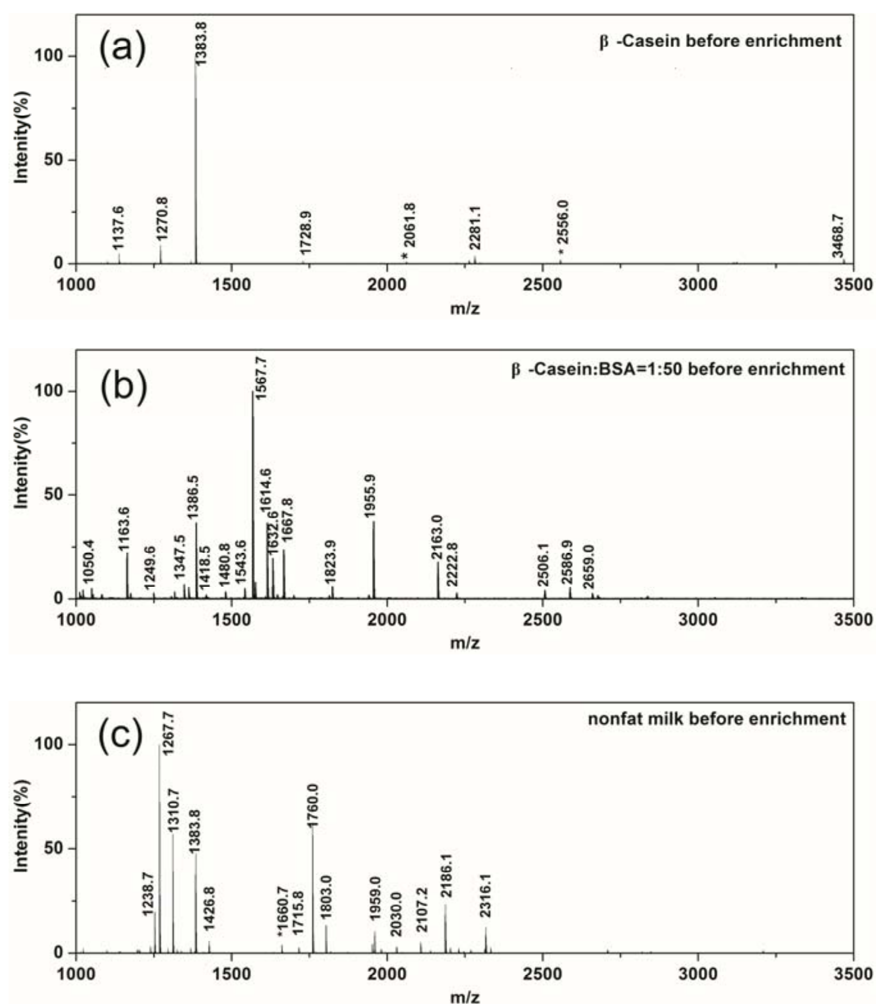

Figure S3. Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF MS) of peptides from β-casein digest (a), the digest mixtures of β-casein and BSA (1:50) (b) and nonfat bovine milk digest (c) before enrichment.

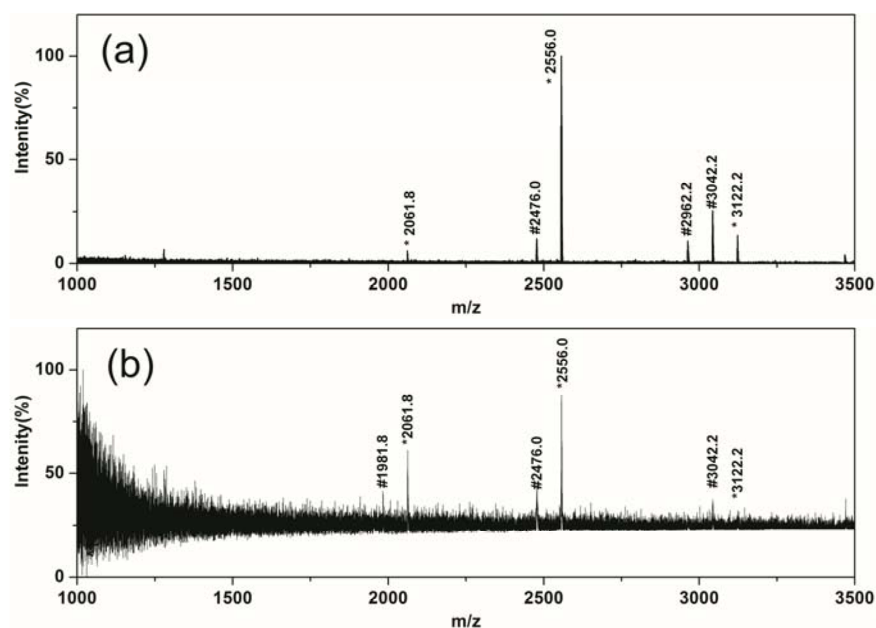

Figure S4. Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF MS) of phosphopeptides enriched from β-casein with different concentrations: 5×10^{-9} M (a) and 10^{-9} M (b), using Fe_3O_4@SiO_2@mCeO_2 microspheres.

Reference:

1. P. Lu, J.-L. Zhang, Y.-L. Liu, D.-H. Sun, G.-X. Liu, G.-Y. Hong and J.-Z. Ni, *Talanta*, 2010, **82**, 450-457.
2. C. T. Chen and Y. C. Chen, *Analytical Chemistry*, 2005, **77**, 5912-5919; C. T. Chen, W. Y. Chen, P. J. Tsai, K. Y. Chien, J. S. Yu and Y. C. Chen, *J. Proteome Res.*, 2006, **6**, 316-325.