Fabrication of a novel magnetic yolk-shell Fe_3O_4@mTiO_2@mSiO_2 nanocomposite for selective enrichment of endogenous phosphopeptides from a complex sample

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Experimental Section

Materials

Tetraethyl orthosilicate (TEOS, 99%), tetrabutyl titanate (TBOT, 97%), polyvinyl pyrrolidone (PVP, Mw=40000), hexadecyl trimethyl ammonium bromide (CTAB), 2, 5-dihydroxybenzoic acid (DHB), ammonium solution, sinapinic acid (SA), α- and β-casein, trypsin, bovine serum albumin (BSA) were purchased from Sigma-Aldrich and used as received. Human serum from healthy volunteers was provided by Dalian Medical University and stored at -80 ˚C before analysis. Acetonitrile (ACN), formic acid (FA) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Urea, dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from BioRad (Hercules, CA, USA). Deionized water used for all experiments was purified with a Milli-Q water system. All other chemicals were of analytical grade and purchased from Aladdin Corporation (Shanghai, China).

Preparation of Fe_3O_4@mTiO_2

Briefly, 50 mg citrate stabilized Fe_3O_4 synthesized through the solvothermal reaction[1] was dispersed in a mixture of 90 mL ethanol, 30 mL acetonitrile and 0.5 mL aqueous ammonia (25%). After sonication for 5 min, 1 mL TBOT was added dropwise, followed by 1.5 h reaction at room temperature. The obtained product (Fe_3O_4@TiO_2) was conducted through solvothermal reaction for the generation of mesopores and crystallization of amorphous TiO_2 shell. In detail, Fe_3O_4@TiO_2 was homogenously mixed with a solution of 40 mL ethanol and 20 mL water, which was then sealed within a Teflon-autoclave at 160 ˚C for 20 h. The final product was designated as Fe_3O_4@mTiO_2.

Preparation of YS Fe_3O_4@mTiO_2@mSiO_2

The obtained Fe_3O_4@mTiO_2 was then treated with PVP overnight for the facilitation of the subsequent deposition of dense SiO_2 shell. After separated by a magnet and washed with water, the Fe_3O_4@mTiO_2-PVP was mixed with a solution containing 23 mL ethanol, 4.3 mL H_2O, 0.62 mL aqueous ammonia and 0.86 mL TEOS. After 6 h reaction at room temperature, the Fe_3O_4@mTiO_2@nSiO_2 was obtained. Subsequently, the determined amount of Fe_3O_4@mTiO_2@nSiO_2 was added into another reaction system containing 25 mL ethanol, 15 mL H_2O and 0.275 mL aqueous ammonia, followed by 30 min mechanical stir at 300 rpm. Then 0.125 mL TEOS was dropwise added into this system for another 6 h reaction. The obtained product (Fe_3O_4@mTiO_2@nSiO_2@mSiO_2-CTAB) was washed by water several times and then treated with a solution of 10 mL H_2O, containing 212 mg Na_2CO_3 at 50 ˚C for 10 h. Finally, the CTAB and PVP were burnt out in the air at 450 ˚C for 6 h.

Tryptic digestion of BSA and β-casein

The 1 mg BSA was dissolved in a solution of 100 mM NH_4HCO_3 and 8 M urea (1 mL) followed by reduction with DTT at 60 ˚C for 1 h. After that, the BSA was alkylated by iodoacetamide at room temperature for 45 min in dark, followed by dilution with 100 mM NH_4HCO_3 (pH=8.1) to reduce the urea concentration below 1 M. Then, trypsin was added with a weight ratio of trypsin/BSA at 1:25 and incubated at 37 ˚C overnight. The 1 mg β-casein was dissolved in 100 mM NH_4HCO_3 and treated with the trypsin at a weight ratio of trypsin/β-casein at 1:25, followed by incubation at 37 ˚C overnight.

Selective enrichment of phosphopeptides from tryptic digests of β-casein and a tryptic digests mixture of β-casein and BSA by YS Fe_3O_4@mTiO_2@mSiO_2

1 mg YS Fe_3O_4@mTiO_2@mSiO_2 or commercial TiO_2 was diluted with 200 μL loading buffer (50% ACN/H_2O, 1% TFA) containing the determined amount of tryptic digests of β-casein. After incubation at room temperature for 1 h, the nanocomposite was separated by a magnet, followed by washed with 200 μL loading buffer for four times. Finally the adsorbed phosphopeptides were eluted from the nanocomposite with 10% aqueous ammonia. The process for selective enrichment of phosphopeptides from a tryptic digests mixture of BSA and β-casein at the determined molar ratio was the same as above described. After being lyophilized to dryness and redissolved in 20 μL 0.1% FA, the peptides were analysed by MALDI-TOF-MS.

Selective enrichment of phosphopeptides from a mixture of α-casein protein and tryptic digests of β-casein

0.1 mg YS Fe_3O_4@mTiO_2@mSiO_2 or Fe_3O_4@mTiO_2 was spiked into 200 μL loading buffer containing α-casein and tryptic digests of β-casein. After a gentle vibration at 4 ˚C for 45 min, the nanocomposite was separated by a magnet and washed twice with the loading buffer. The nanocomposite was eluted by 150 μL 10% aqueous ammonia twice. The peptides and proteins were analysed by MALDI-TOF-MS.

Selective enrichment of endogenous phosphopeptides from human serum by YS Fe_3O_4@mTiO_2@mSiO_2

2 mg YS Fe_3O_4@mTiO_2@mSiO_2 was diluted with 200 μL loading buffer (50% ACN/H_2O, 1% TFA) and 10 μL human serum was spiked into this mixture. After incubation at room temperature for 1 h, the nanocomposite was separated by a magnet, followed by twice wash with 100 μL loading buffer and 100 μL washing buffer (30% ACN/H_2O, 0.1% TFA). Finally, the adsorbed endogenous phosphopeptides or proteins were eluted from the nanocomposite with 10% aqueous ammonia. After being lyophilized to dryness and redissolved in 20 μL 0.1% FA, the peptides and proteins were analysed by MALDI-TOF-MS.

Characterization

All MALDI-TOF-MS analysis results were obtained using a MALDI-TOF/TOF™ 5800 System (AB SCIEX, Foster City, CA) equipped with a 1 kHz OptiBeam™ on-axis laser. DHB (25 mg/mL in 70% ACN–H_2O solution containing 1% H_3PO_4) was used as the matrix for the analysis of peptides. Sinapinic acid (saturated in 50% ACN–H_2O solution containing 0.1% FA) was used for the analysis of proteins. Sample aliquots (0.5 μL) were first placed on a plate and dried at room temperature, and then the DHB matrix (0.5 μL) was added prior to MALDI-TOF-MS analysis. The analysis process of proteins was the same as peptides, except for sinapinic acid as the matrix. Transmission electron microscopy (TEM) was conducted on a JEOL 2000 EX electronic microscope with an accelerating voltage of 120 keV. Scanning electron microscope (SEM) was performed on a JEOL JSM-5600 (Tokyo, Japan). The nitrogen adsorption measurement was conducted at -196 ˚C (liquid nitrogen temperature) using a static-volumetric method on an ASAP 2010 (Micromeritics, USA). Powder X-ray diffraction patterns of

the samples were collected on a Bruker D8FOCUS X-ray diffractometer. The pore diameter and distribution curves were calculated by the BJH (Barrett–Joyner–Halenda) method from the adsorption branch. The saturation magnetization curve was obtained at room temperature on a Physical Property Measurement System 9T (Quantum Design, San Diego, USA). The EDS element mapping was conducted on the Inca X-Max80 EDS system (Oxford, England).

Reference

[1] J. Liu, Z. Sun, Y. Deng, Y. Zou, C. Li, X. Guo, L. Xiong, Y. Gao, F. Li and D. Y. Zhao, *Angew. Chem. Int. Ed.,* 2009, **48**, 5875-5879.

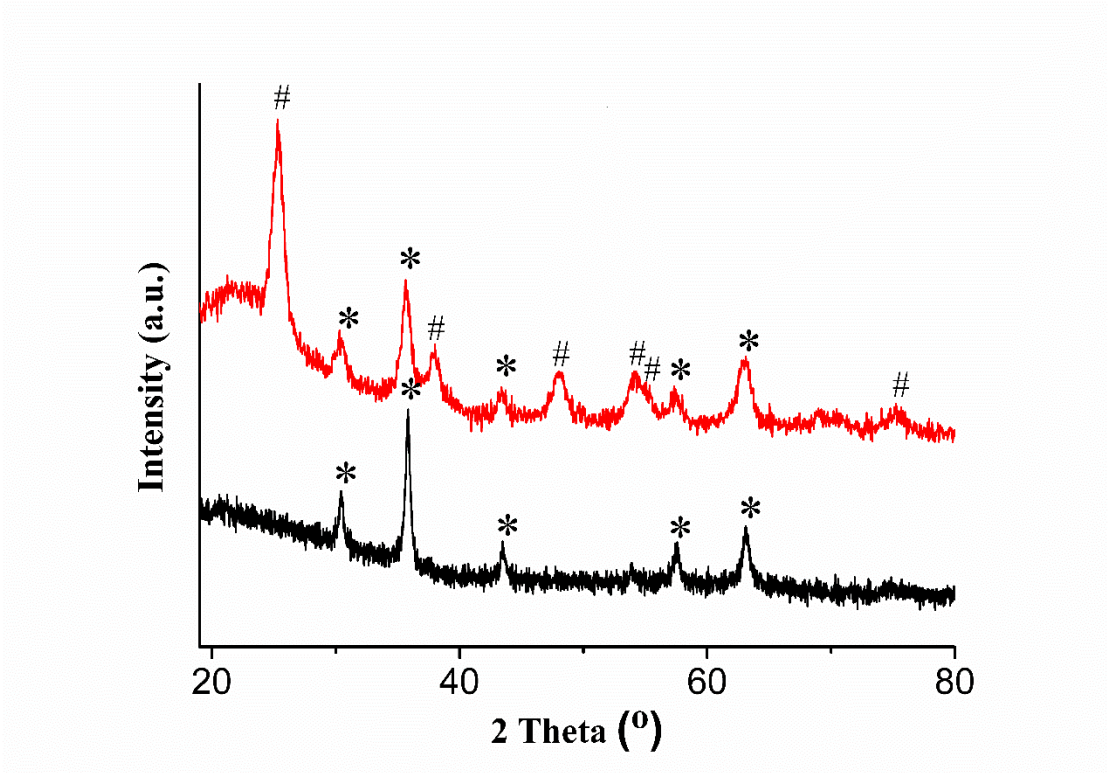

Fig. S1 XRD spectrum of Fe_3O_4 (black line) and YS Fe_3O_4@mTiO_2@mSiO_2 (red line). * and # indicate the diffraction peaks of magnetite phase of Fe_3O_4 and anatase TiO_2, respectively.

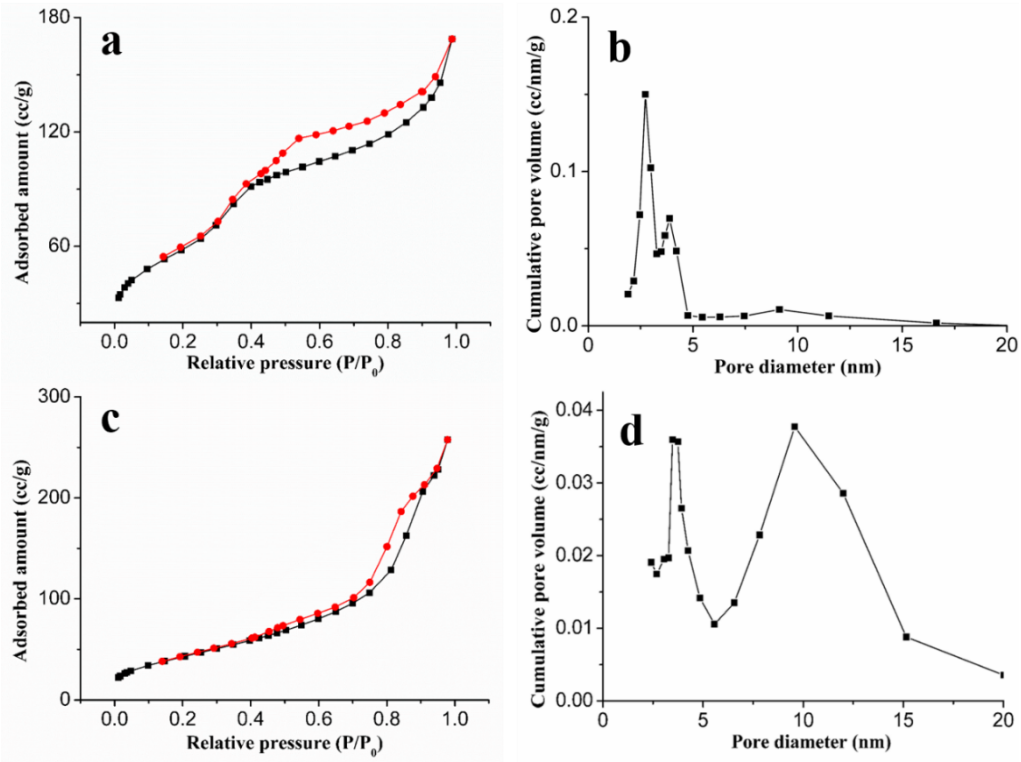

Fig. S2 The adsorption-desorption isotherms of (a) YS Fe_3O_4@mTiO_2@mSiO_2 and (c) Fe_3O_4@mTiO_2; Pore distribution of (b) YS Fe_3O_4@mTiO_2@mSiO_2 and (d) Fe_3O_4@mTiO_2.

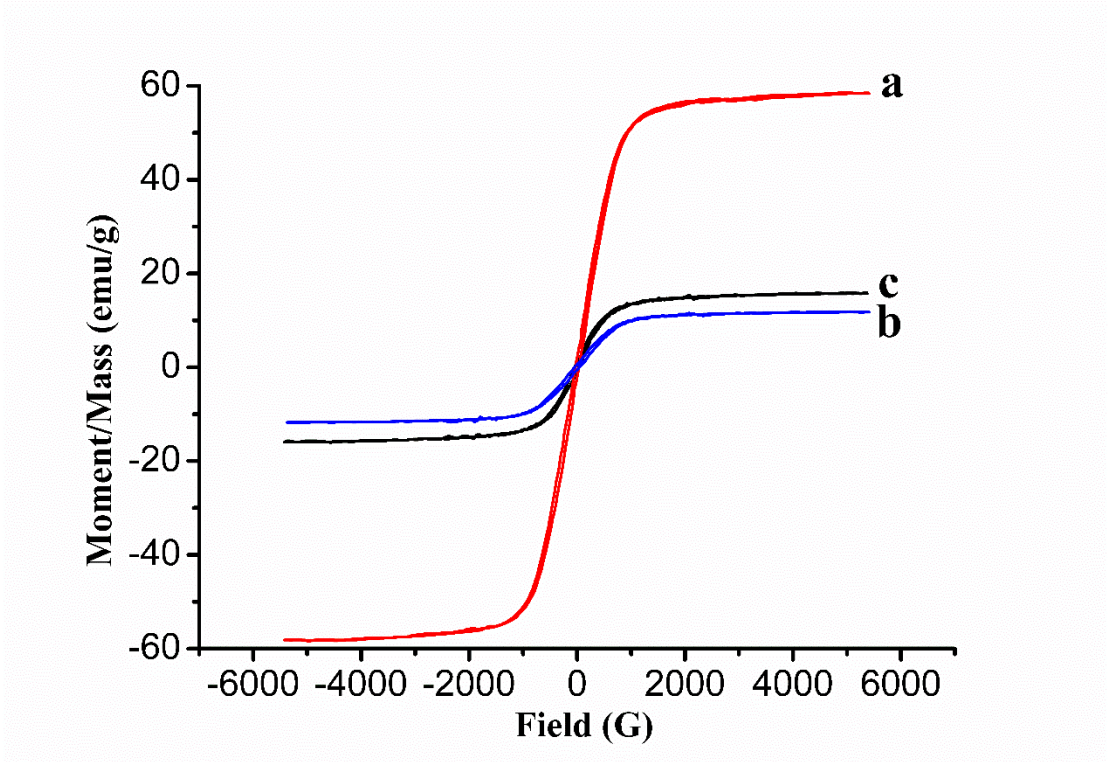

Fig. S3 Room-temperature magnetization curve of (a) Fe_3O_4, (b) Fe_3O_4@mTiO_2@nSiO_2@mSiO_2-CTAB and (c) YS Fe_3O_4@mTiO_2@mSiO_2.

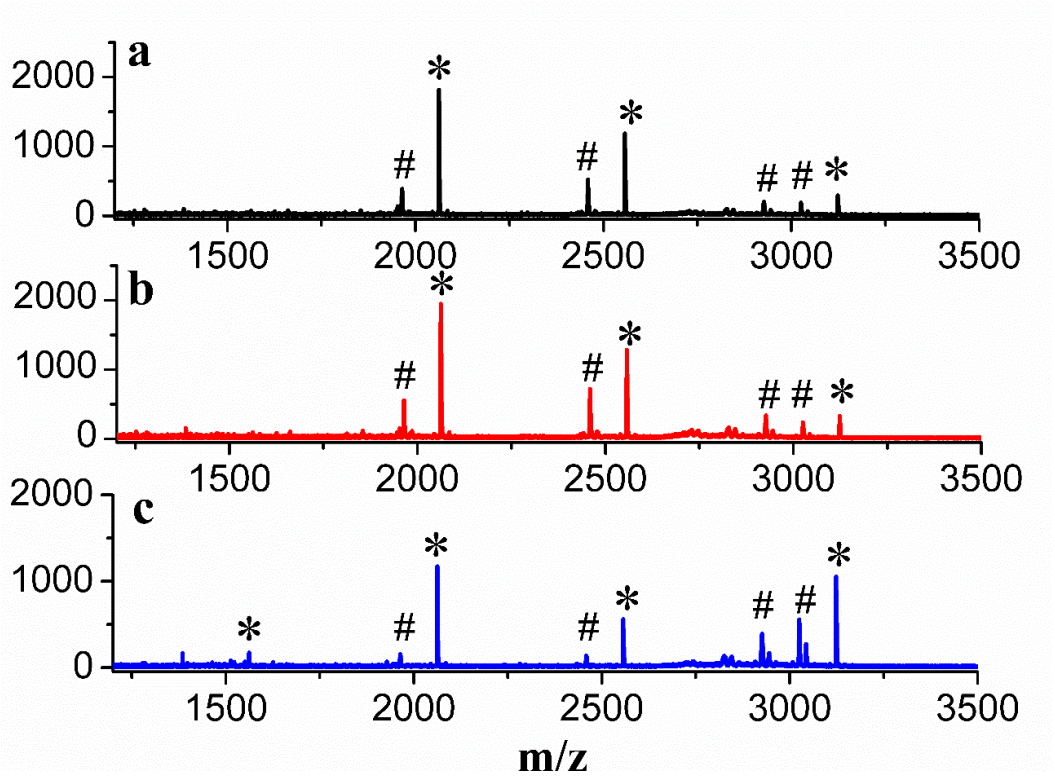

Fig. S4 The MALDI-TOF-MS analysis of a tryptic digests mixture of β-casein and BSA after enrichment with YS Fe_3O_4@mTiO_2@mSiO_2 at the molar ratio of (a) 1:10, (b) 1:100 and (c) 1:1000. * indicates phosphopeptides and # indicates dephosphorylated peptides.

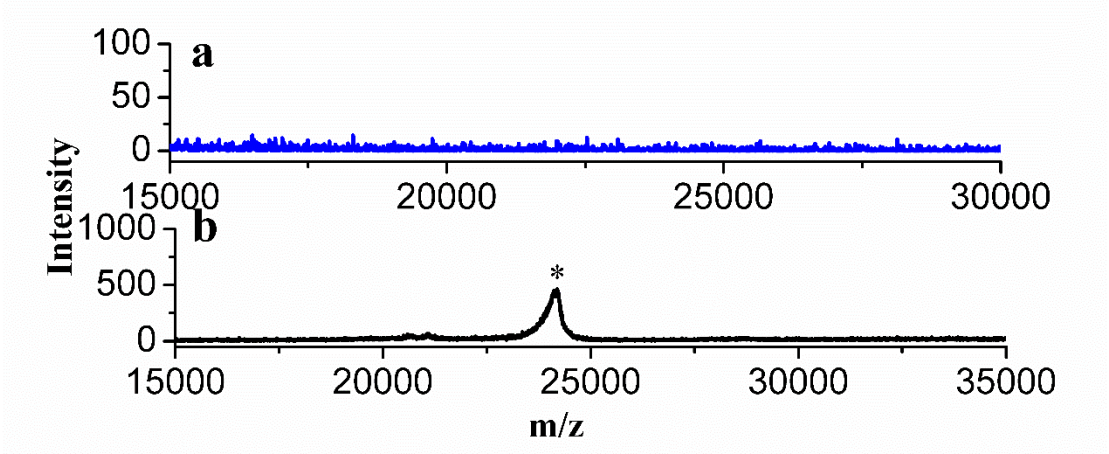

Fig. S5 The MALDI-TOF-MS analysis of the elution after enrichment of β-casein with (a) YS Fe_3O_4@mTiO_2@mSiO_2 and (b) Fe_3O_4@mTiO_2. The size of β-casein (24200 Da, radius of gyration is about 4.6 nm) was too large to penetrate through the narrow mesoporous channels (2.7 nm) distributing within the outmost mSiO_2 shell on the YS Fe_3O_4@mTiO_2@mSiO_2, resulting in the size-exclusion effect.

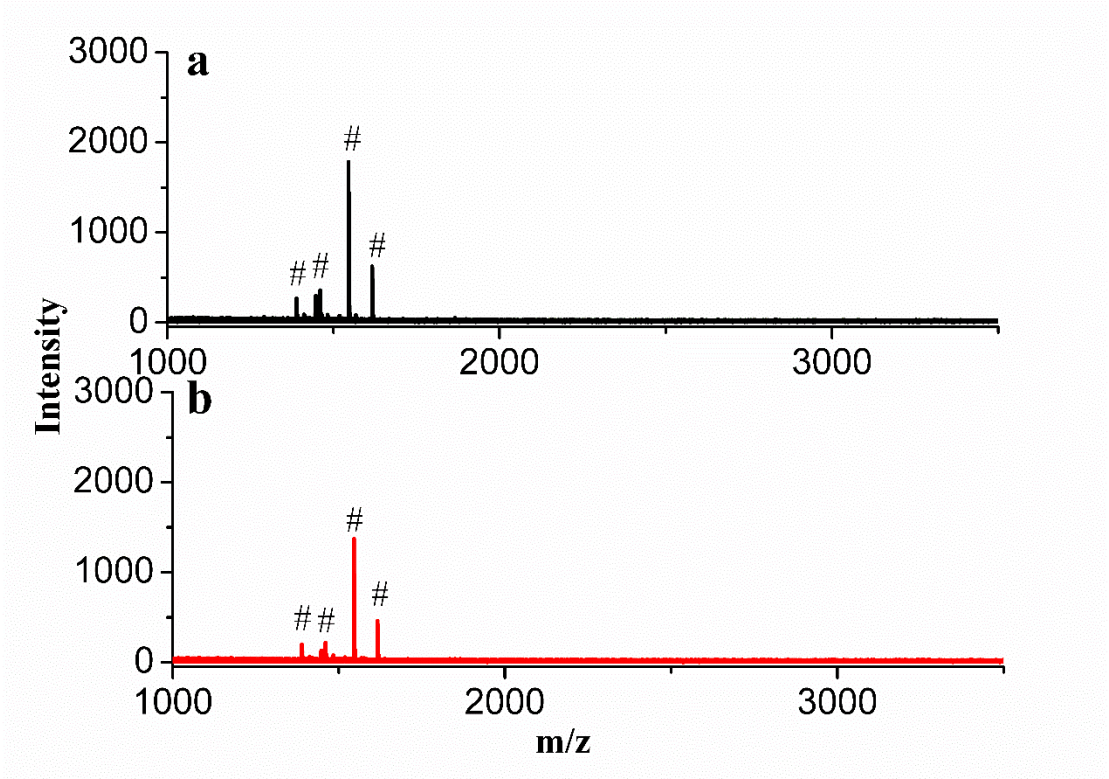

Fig. S6 The MALDI-TOF-MS analysis of human serum after enrichment with YS Fe_3O_4@mTiO_2@mSiO_2 when the loading buffer was (a) 50% ACN, 1.5% TFA and (b) 50% ACN, 2% TFA. # indicates endogenous phosphopeptides.

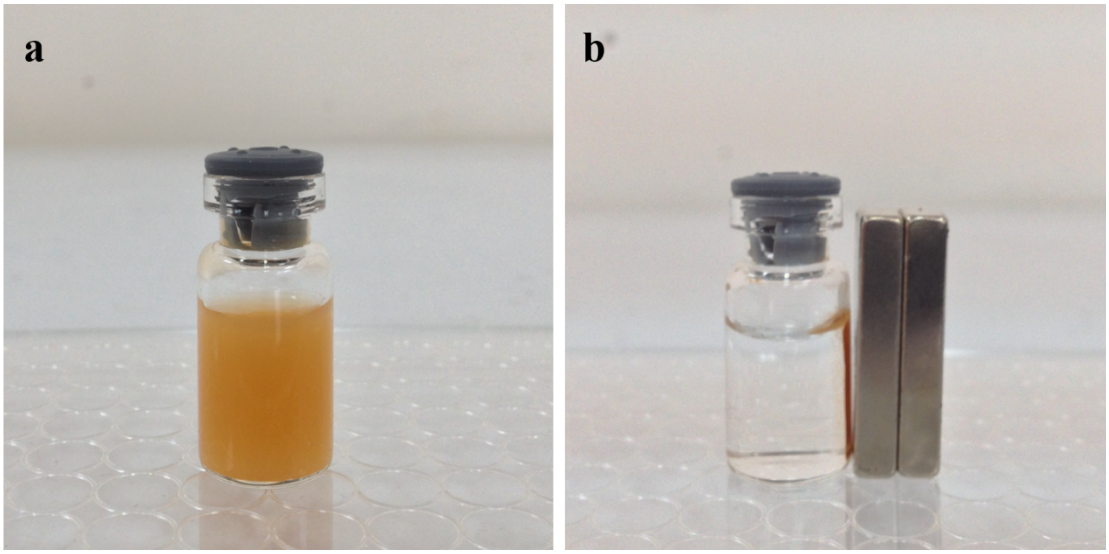

Fig. S7 The images of a water dipersion of YS Fe_3O_4@mTiO_2@mSiO_2 (a) before and (b) after magnetic separation.

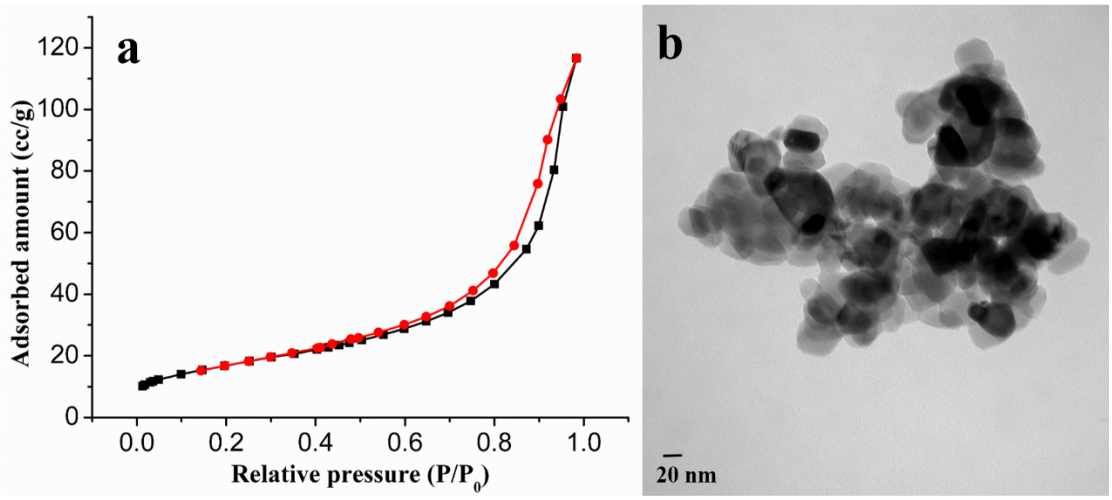

Fig. S8 The adsorption-desorption isotherms and TEM image of commercial TiO_2.

Table S1 The phosphopeptides identified from tryptic digests of β-casein after enrichment with YS Fe_3O_4@mTiO_2@mSiO_2

No.	Peptide sequence	Observed m/z
β1	FQ[pS]EEQQQTEDELQDK	2061.828
β2	FQ[pS]EEQQQTEDELQDKIHPF	2556.092
β3	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3122.266

Table S2 The endogenous phosphopeptides identified from human serum after enrichment with YS Fe_3O_4@mTiO_2@mSiO_2

No.	Peptide sequence	Observed m/z
HS1	D[pS]GEGDFLAEGGGV	1389.588
HS2	AD[pS]GEGDFLAEGGGV	1460.677
HS3	D[pS]GEGDFLAEGGGVR	1545.750
HS4	AD[pS]GEGDFLAEGGGVR	1616.738