Supporting Information

Experimental Details

Preparation of Fe_3O_4-GR-TiO_2

Fe_3O_4 nanoparticles were obtained by microwave-hydrothermal strategy in a microwave accelerated reaction system MARS-5 (CEM, USA). In detail, 1g precursor $FeCl_3 \cdot 6H_2O$, 2g anhydrous sodium acetate and 6.5g 1,6-hexanediamine were dissolved into 30ml ethylene glycol and stirred until transparency. After that, the mixture was transferred into the microwave system and treated at 200℃ for 30min. Then, it was washed with ethanol and water, separated by a permanent magnet and finally redispersed in de-ionized water for further use.

Graphene oxide (GO) was prepared from graphite powder (from Sigma) by a modified Hummers' method and dispersed in water to form a homogeneous solution with concentration of 0.5mg/ml. 4ml portion of Fe_3O_4 suspension (5mg/ml) was mixed with 40ml of GO solution and vibrated in a shaker for 2h. After magnetic separation, 5mg of intermediate Fe_3O_4-GO was dispersed in alcohol/water (140ml/10ml) mixture and heated to 70□. Then, 360μl $Ti(BuO)_4$ and 150μl H_2SO_4 were added and the solution was mechanically stirred for 12h at the same temperature. The product named as Fe_3O_4-GO-TiO_2 was washed with ethanol and water for three times and recovered by magnetic separation.

Hydrothermal treatment was required for the synthesis of Fe_3O_4-GR-TiO_2. As-prepared Fe_3O_4-GO-TiO_2 was dispersed in 30ml water and transferred into an autoclave for hydrothermal reaction at 200□ for 20h. Finally, the product was obtained and designated as Fe_3O_4-GR-TiO_2.

Tryptic digestion of proteins

1mg of α- and β-casein were respectively dissolved in 1ml 50mM ammonium bicarbonate buffer solution (pH=8.1), and incubated with trypsin at the ratio of enzyme-to-substrate of 1:40 (w/w) in a shaker at 37℃ for 16 h. 5mg of BSA was first denatured in 50mM ammonium bicarbonate solution containing 8M urea. After the addition of 5μl dithiothreitol (DTT) (1M), the solution was incubated in 60℃ water bath for 1 h to reduce the disulfide bonds. Then, 10μl iodoacetamide (IAA) (1M) was introduced and the mixture was incubated in ambient temperature in dark for 40min. Finally, the solution was diluted 8-fold with 50mM ammonium bicarbonate buffer solution (pH=8.1) and digested by trypsin at the ratio of enzyme-to-substrate of 1:40 (w/w).

Adsorption for tryptic digests of α-casein

The adsorption experiments were carried out at 25℃ in 50 mM ammonium bicarbonate buffer solution (pH=8.1). Known quantities of Fe_3O_4-GR-TiO_2 and commercial TiO_2 (0.5mg) were respectively added to 1ml tryptic digests of α-casein with varied concentrations ranging from 0.05 to 0.425mg/ml. The mixture was vibrated in shaker for 12 h, which allowed for adsorption to reach equilibrium. The concentration of peptides in the final solution was determined by monitoring the absorbance at 280nm.

The capture of phosphopeptides by Fe_3O_4-GR-TiO_2

Digests for phosphopeptide capture were diluted with 6% trifluoroacetic acid (TFA) in 50% (v/v) ACN. 0.1mg Fe_3O_4-GR-TiO_2 was dispersed into 200μl tryptic digests of α- and β-casein (2pmol), and vibrated in a vortex for 30min. The supernatant was removed by magnetic separation, and Fe_3O_4-GR-TiO_2 combined with phosphopeptides was rinsed with 200μl 0.1%TFA solution in 80% (v/v) ACN with and then without 200mM NaCl, respectively. The trapped phosphopeptides were eluted by 20μl 10% $NH_3 \cdot H_2O$ under sonication for 10min. After that, the supernatant was collected by magnetic separation. Consequently, 1μl resulting solution and 1μl 2,5-dihydroxybenzoicacid (DHB) solution (25mg/ml in 70% ACN) containing 1% H_3PO_4 (v/v) were deposited in turn onto MALDI target for MALDI-TOF analysis.

Human serum used here was collected from 100 healthy adults in Nanjing University Hospital according to their standard clinical procedures. In our case, 10μl of human serum was diluted into 200μl 6% trifluoroacetic acid (TFA) in 50% (v/v) ACN, and then treated with Fe_3O_4-GR-TiO_2 in the procedure elucidated above for phosphopeptide capture.

Mass spectrometry

MALDI-TOF MS experiments were performed on a Bruker Autoflex II time-of-flight mass spectrometer (Bruker, Bremen, Germany), equipped with a delayed ion-extraction device and a pulsed nitrogen laser operated at 337nm. The range of laser energy was adjusted slightly to obtain good resolution and S/N. The instrument was operated in the positive ion reflector mode. The MALDI uses a ground-steel sample target with 384 spots. Each spectrum was summed with 100 laser shots.

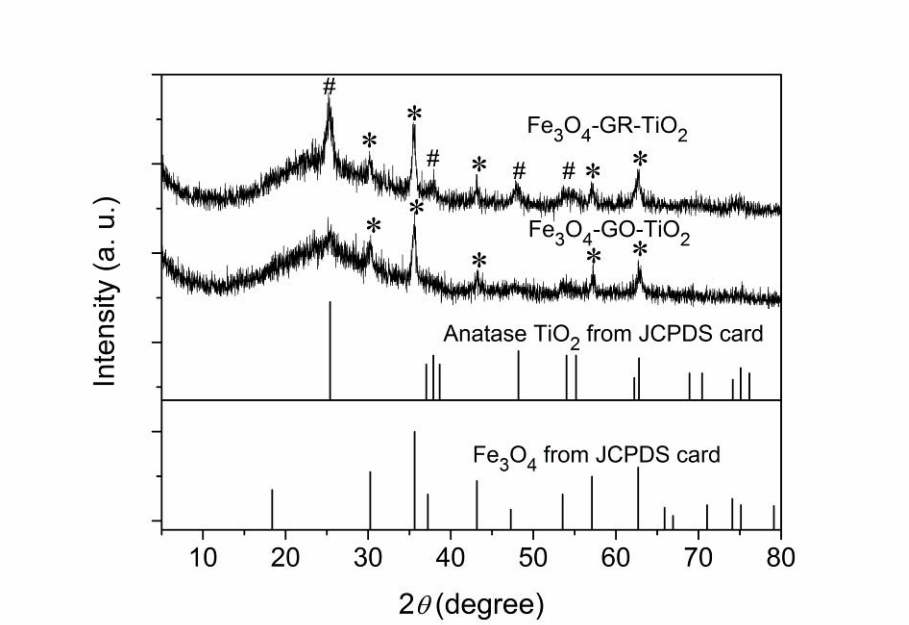

Fig. S1 XRD pattern of Fe_3O_4-GO-TiO_2 and Fe_3O_4-GR-TiO_2.

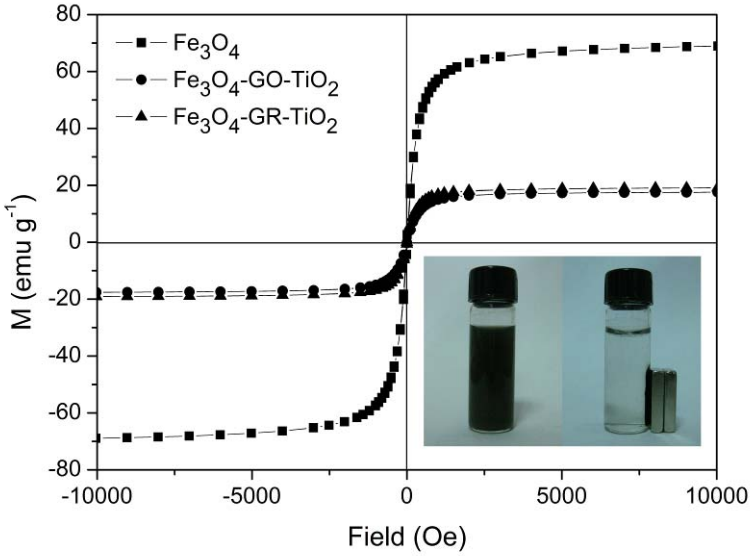

Fig. S2 Room-temperature magnetization curves of Fe_3O_4, Fe_3O_4-GO-TiO_2 and Fe_3O_4-GR-TiO_2. Inset shows the magnetic response of Fe_3O_4-GR-TiO_2 in the magnetic field generated from a magnet.

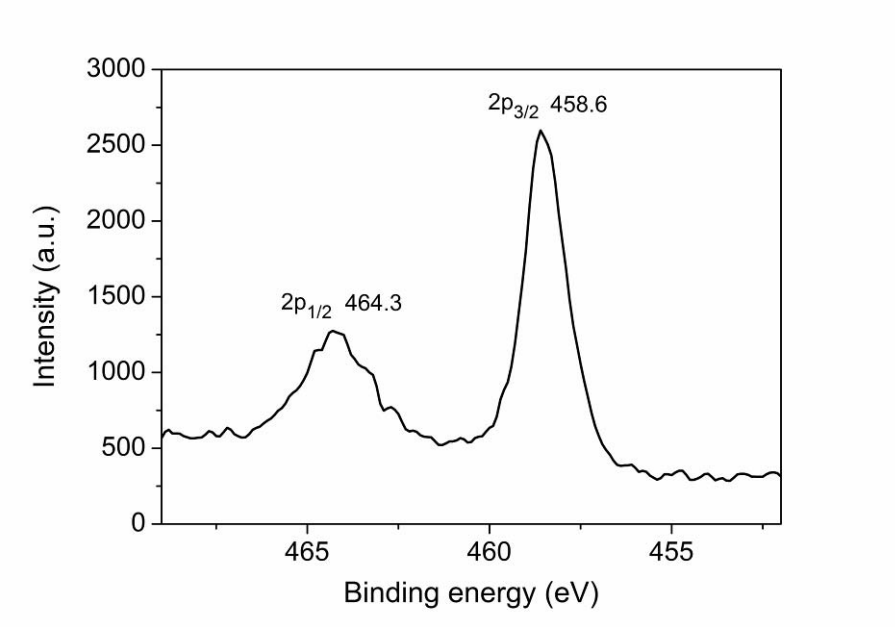

Fig. S3 XPS spectra of Ti2p in the Fe_3O_4-GR-TiO_2 networks.

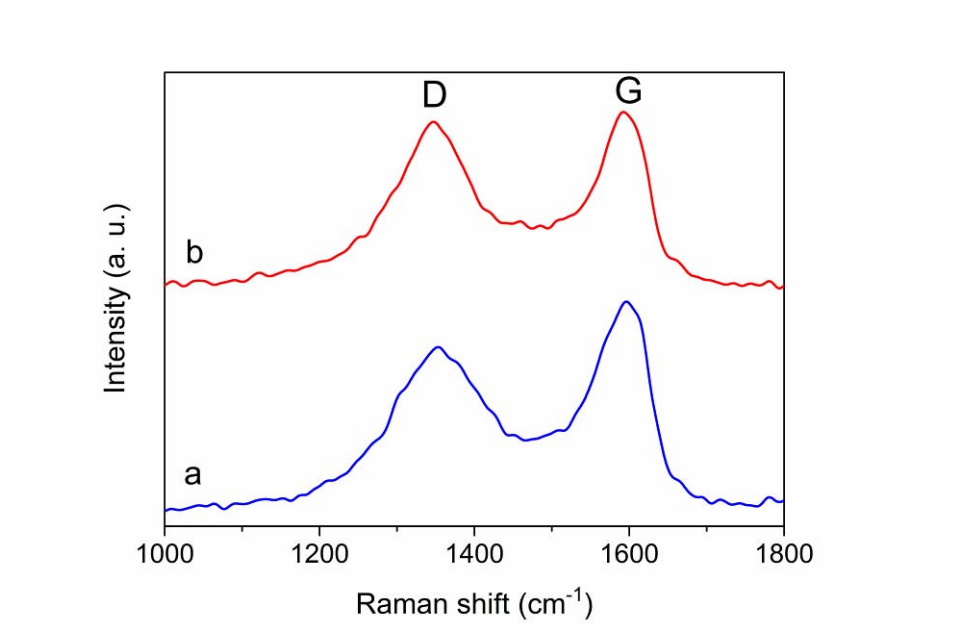

Fig. S4 Raman spectra of Fe_3O_4-GO-TiO_2 (Intensity ratio I_D/I_G = 0.784) (a) and Fe_3O_4-GR-TiO_2 (I_D/I_G = 0.944) (b).

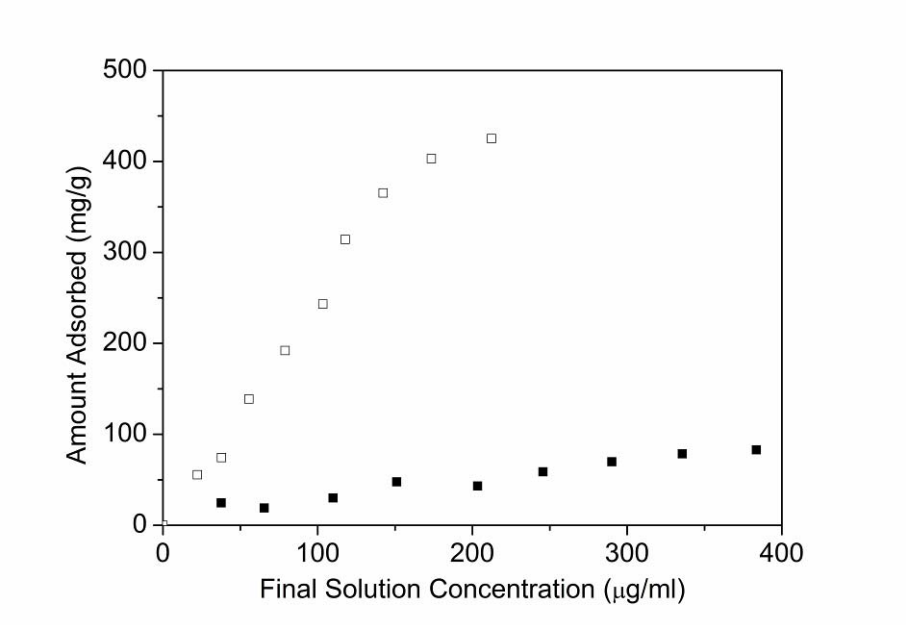

Fig. S5 Adsorption isotherm for tryptic digests of α-casein adsorbed on Fe_3O_4-GR-TiO_2 (□) and commercial TiO_2 (■).

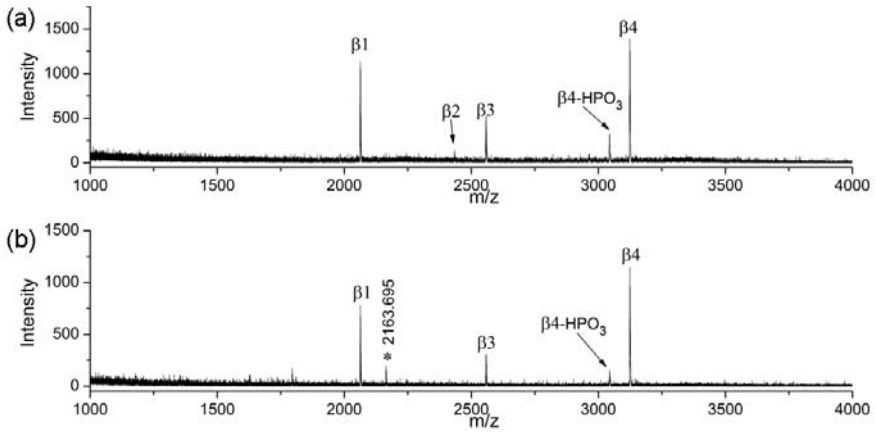

Fig. S6 MALDI-TOF spectra of the tryptic digests of a mixture of β-casein and BSA at mole ratio of 1:10 (a) and 1:100 (b) after enriched by Fe_3O_4-GR-TiO_2.

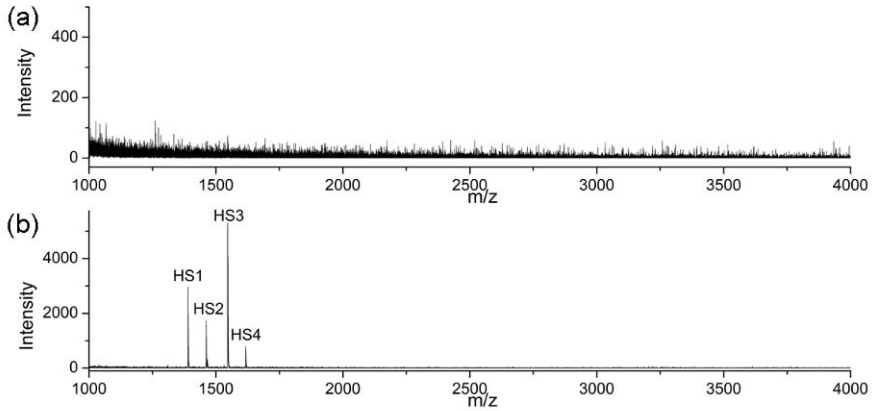

Fig. S7 MALDI-TOF spectra of human serum by direct analysis (a) and enriched by Fe_3O_4-GR-TiO_2 (b).

Table S1. The phosphopeptides identified from tryptic digests of β-casein after enriched by Fe_3O_4-GR-TiO_2 in MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Observed m/z
β1	FQ[pS]EEQQQTEDELQDK	1	2061.828
β2	IEKFQ[pS]EEQQQTEDELQDK	1	2432.050
β3	FQ[pS]EEQQQTEDELQDKIHPF	1	2556.092
β4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.266

[pS]: phosphorylated site.

Table S2. The non-phosphopeptides identified from tryptic digests of β-casein after enriched by Fe_3O_4-GO-TiO_2 in MALDI-TOF MS analysis.

Protein source	Label	Peptide sequence	Observed m/z
β-casein	#	VKEAMAPKHK	1138.023
trypsin autolysis	*	LGEDNINVVEGNEQFISASK	2163.738

Table S3. The phosphopeptides identified from tryptic digests of α-casein after enriched by Fe_3O_4-GR-TiO_2 in MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Observed m/z
α1	TVDME[pS]TEVF	1	1237.267
α2	TVD[Mo]ME[pS]TEVF	1	1253.142
α3	TVDME[pS]TEVFTK	1	1466.779
α4	TVD[Mo]E[pS]TEVFTK	1	1482.654
α5	EQL[pS]T[pS]EENSKK	2	1538.978
α6	VPQLEIVPN[pS]AEER	1	1660.949
α7	YLGEYLIVPN [pS]AEER	1	1833.197
α8	DIGSE[pS]TEDQAMEDIK	1	1848.213
α9	DIGSE[pS]TEDQA[Mo]EDIK	1	1864.366
α10	DIG[pS]E[pS]TEDQAMEDIK	2	1927.885
α11	DIG[pS]E[pS]TEDQA[Mo]EDIK	2	1943.829
α12	YKVPQLEIVPN[pS]AEER	1	1952.109
α13	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4	2619.391
α14	NT[Mo]EHV[pS][pS][pS]EESII[pS]QETYK	4	2635.332
α15	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3	2678.944
α16	VNEL[pS]KDIG[pS]E[pS]TEDQA[Mo]EDIK	3	2695.032
α17	Q*MEAE[pS]I[pS][pS] [pS]EEIVPN[pS]VEAQK	5	2703.758
α18	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5	2720.822
α19	KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ	3	2936.202
α20	KEKVNEL[pS]KDIG[pS]E[pS]TEDQA[Mo]EDIKQ	3	2952.388
α21	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4	3008.618
α22	NANEEEY[pS]IG[pS][pS][pS]EE[pS]AEVATEEVK	5	3088.415

[pS]: phosphorylated site;

[Mo]: oxidation on methionine;

*Pyroglutamylation on the N-terminal Q.

Table S4. The phosphopeptides identified from human serum after enriched by Fe_3O_4-GR-TiO_2 in MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Observed m/z
HS1	D[pS]GEGDFLAEGGGV	1	1389.588
HS2	AD[pS]GEGDFLAEGGGV	1	1460.677
HS3	D[pS]GEGDFLAEGGGVR	1	1545.750
HS4	AD[pS]GEGDFLAEGGGVR	1	1616.738

[pS]: phosphorylated site.