

Supporting Information

Magnetic Cellulose/TiO₂ Nanocomposite Microspheres for Highly Selective Enrichment of Phosphopeptides

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Experimental Section

Materials: Cellulose (cotton linter pulp) was provided by Hubei Chemical Fiber Group Ltd. (Xiangfan, China). Its viscosity-average molecular weight (M_η) was 10×10^4 determined in LiOH/urea aqueous solution at 25 ± 0.05 °C by Mark-Houwink equation.¹ Anhydrous ferric chloride (FeCl_3), tetrabutylorthotitanate (TBOT), anhydrous ethanol (EtOH), ethylene glycol, sodium citrate, sodium acetate (NaAc) and other chemicals were bought from Shanghai General Chemical Reagent Factory (Shanghai, China). HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Pittsburgh, USA). Ammonia hydrate ($\text{NH}_3 \cdot \text{H}_2\text{O}$, 25%), phosphoric acid (H_3PO_4), trifluoroacetic acid (TFA), 2, 5-dihydroxybenzoic acid (2, 5-DHB), commercial TiO_2 nanoparticles, bovine β -casein, bovine serum albumin (BSA) and trypsin were purchased from Sigma-Aldrich (St Louis, USA). Ultrapure water (15 M Ω cm) used for all experiments was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA). Fresh human serum was obtained from the Hospital of Wuhan University.

Preparation of highly water-dispersible MCNC: The magnetite Fe_3O_4 colloidal nanocrystal clusters (MCNC) were prepared through a modified solvothermal reaction.² Typically, 1.350g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 3.854g of NaAc, and 0.400g of sodium citrate were dissolved in 100 mL of ethylene glycol. The mixture was stirred vigorously for 1h at room temperature to form a homogeneous black solution, and then transferred into a Teflon-lined stainless-steel autoclave (150 mL capacity). The autoclave was heated at 200 °C for 10h, in which Fe^{3+} was partly reduced into Fe^{2+} ,

and then was cooled to room temperature. The black product was washed with ethanol and collected with the help of a magnet repeated several times. The final Fe_3O_4 product was dried in oven at 60 °C for further use. The SEM results indicated that MCNC exhibited mono-dispersed, uniform and spherical morphologies with an average diameter of about 320 nm (Fig. S10).

Preparation of MCMs: Magnetic cellulose microspheres (MCMs) were prepared as the follows. Firstly, 4 wt% of cellulose solution was obtained by dissolving 8.33g of cellulose in 200g of pre-cooled NaOH/urea/ H_2O (7:12:81) by weight under vigorous stirring for 2 min, and then 1.2g of the dried MCNC was dispersed into 200g of cellulose solution to form a uniformly brown colloidal solution with the assist of ultrasound. Subsequently, 200g brown colloidal solution was dropped into 2L of flask containing 1000 mL of paraffin oils and 20g of Span 80 under vigorous stirring at 1000 rpm for 30 min to form a cellulose/ Fe_3O_4 colloidal suspension. The resulted suspension was immediately placed into microwave oven (power, 250W) by heating for 2 min to form MCMs. The MCMs samples were separated from oil phase by introducing a magnetic field and washed with deionized water, and then with acetone three times to remove the residual paraffin oils and Span 80. Finally, the magnetic microspheres were stored in 20% alcohol at 5 °C for further use.

Preparation of MCTiMs: magnetic cellulose/ TiO_2 nanocomposite microspheres (MCTiMs) were synthesized by in-situ synthesizing TiO_2 on the surface and in inner pores of the MCMs microspheres by hydrolyzing TBOT in the presence of ammonia.

Briefly, about 100 mg of the as-prepared MCMs were dispersed into 100 mL of ethanol containing a different (1 mL, 2 mL, 3 mL, 5 mL) volume of TBOT. Subsequently, 1 mL of $\text{NH}_3 \cdot \text{H}_2\text{O}$ was added into the above suspension under stirring. After 2h at room temperature, the suspension was then transferred to a Teflon-lined stainless-steel autoclave (200 mL capacity). The autoclave was heated at 120 °C and maintained for 10h, and then was cooled to room temperature. Finally, the MCTiMs product was collected with the help of a magnet, and then washed with ethanol several times.

Preparation of peptides mixture: Bovine β -casein was originally made up into stock solutions of 1 mg/mL with purified water. Proteins were digested in trypsin by using an enzyme to substrate ratio of 1:50 (w/w) in 100 mM Tris-HCl pH 8.5, and incubated overnight at 37 °C. 1 mg of bovine serum albumin (BSA) was dissolved in 100 μL of denaturing buffer solution (8 M urea in 100 mM Tris-HCl pH 8.5). The obtained protein solution was mixed with 5 μL of 100 mM tri (2-chloroethyl) phosphate (TCEP) and incubated for 20 min at room temperature to reduce protein disulfide bonding. Subsequently, iodoacetamide (3 μL of 500 mM stock) was added, and the resulted solution was incubated for an additional 30 min at room temperature in the dark. The reduced and alkylated protein mixture was diluted with 100 mM Tris-HCl pH 8.5. Finally, 9 μL of 100 mM CaCl_2 was added, and the digestion mixture (~50 μL in volume) was digested by incubating overnight at 37 °C with trypsin at an enzyme to substrate ratio of 1:50 (w/w).

Selective enrichment of phosphopeptides with MCTiMs-2: The MCTiMs-2 microspheres were first washed with ethanol, and then suspended in deionized water at 50 mg/mL. Tryptic digests of β -casein and BSA or human serum sample (2 μ L) were dissolved in 200 μ L of loading buffer (50% ACN containing 3% TFA); then 10 μ L MCTiMs-2 was added, and the mixture was incubated at room temperature for 30 min. After that, MCTiMs-2 with captured phosphopeptides was separated from the mixed solutions via an external magnet. After washing with 100 μ L 3% TFA-50% ACN and 0.1% TFA-50% ACN subsequently to remove the nonspecifically adsorbed peptides, the trapped phosphopeptides were eluted with 50 mL of 5% $\text{NH}_3 \cdot \text{H}_2\text{O}$ for further MS analysis.

MALDI MASS Spectrometry: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a major and well-known tool in modern protein analysis due to its ultrahigh sensitivity, wide dynamic range and superior speed in analyzing mixtures. However, the identification and characterization of phosphoproteins by MALDI-TOF MS remains challenging due to their low abundance and low signal-to-noise ratio suppressed by nonphosphorylated peptides. Therefore, the highly selective enrichment of phosphopeptides from sample solution by special affinity probes is necessary before MS analysis. All MALDI-TOF MS spectra of the peptides were recorded with a Voyager DE STR MALDI-TOF workstation mass spectrometer (Applied Biosystems Inc., USA). During a typical analysis, 200 scans were accumulated, and were performed in positive ion reflector mode with an accelerating voltage of 20 kV and delayed extraction of 280 ns. Two microliters of

matrix solution (mixture of 20 mg/mL 2, 5-DHB in 50% (v/v) ACN, 1% (v/v) phosphoric acid) was introduced into the eluate and 1 μ L of the mixture was used for MALDI-TOF analysis.

Figures Captions

Fig. S1 SEM images of MCTiMs-1 (a), MCTiMs-2 (b), MCTiMs-3 (c) and MCTiMs-5 (d).

Fig. S2 Optical photomicrographs of MCMs (a), MCTiMs (b), and the size distribution of MCMs (c) and MCTiMs (d) obtained by the laser particle size analyzer

Fig. S3 Nitrogen adsorption-desorption isotherms (■ = adsorption, □ = desorption) (a) and BJH pore-size distribution curves for MCMs (b).

Fig. S4 TEM image of MCTiMs (a) and its EDS spectra corresponded to the marked locations (c). HRTEM image of an individual TiO₂ nanoparticle with the lattice fringes marked (b)

Fig. S5 TGA curves of MCMs and different MCTiMs (a), XRD patterns (b) and FT-IR spectra (c) of CMs, MCMs, MCTiMs-2.

Fig. S6 TEM images (a, b), high resolution TEM image (c) and EDX Spectrum of Fe₃O₄ nanocrystal cluster (MCNC) dispersed in MCTiMs-2.

Fig. S7 MALDI mass spectra of the tryptic digests of β -casein analysis after enrichment using MCMs (a), MCTiMs-1 (b), MCTiMs-3 (c), MCTiMs-5 (d).

★ indicates phosphorylated peptide, ● indicates their dephosphorylated counterparts.

Fig. S8 MALDI mass spectra the tryptic digest of β -casein and BSA with ratio of 1:1,

1:10, 1:100 direct analysis (a, b, c) and (d, e, f) analysis after enrichment using MCTiMs-2. ★ indicates phosphorylated peptides. β -casein was at concentration of 1.0×10^{-7} M (3 pmol).

Fig. S9 MALDI mass spectra the tryptic digest of β -casein and BSA with ratio of 1:1 (a), 1:10 (b) analysis after enrichment using commercial TiO_2 nanoparticles. ★ indicates phosphorylated peptides. β -casein was at concentration of 1.0×10^{-7} M (3 pmol).

Fig. S10 SEM image of MCNC prepared by using a modified solvothermal route (a), the size distribution of MCNC obtained by the laser particle size analyzer (b). TEM image (c), HRTEM image (d) and EDS spectra (e) of MCNC.

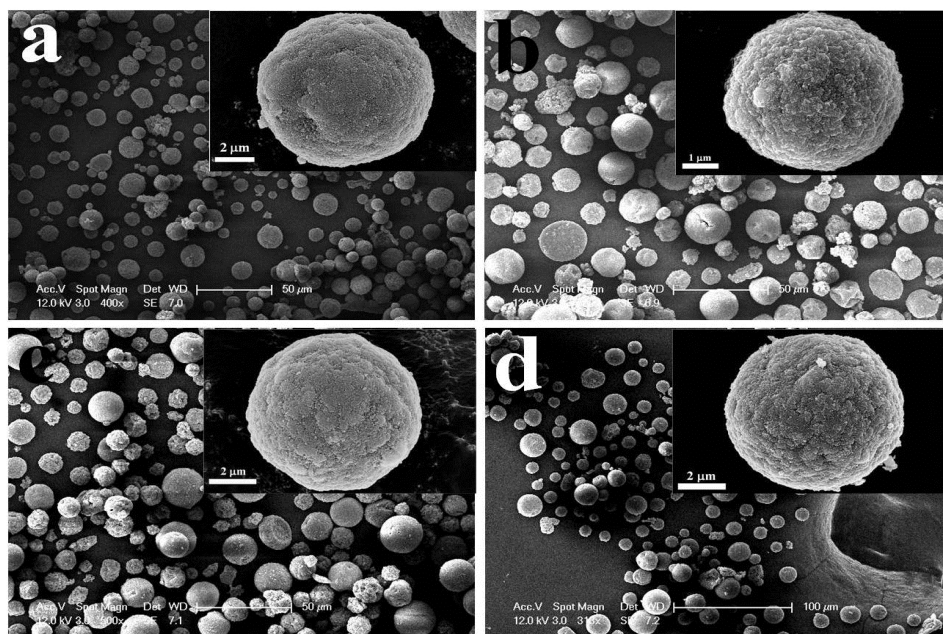


Fig. S1 SEM images of MCTiMs-1 (a), MCTiMs-2 (b), MCTiMs-3 (c) and MCTiMs-5 (d).

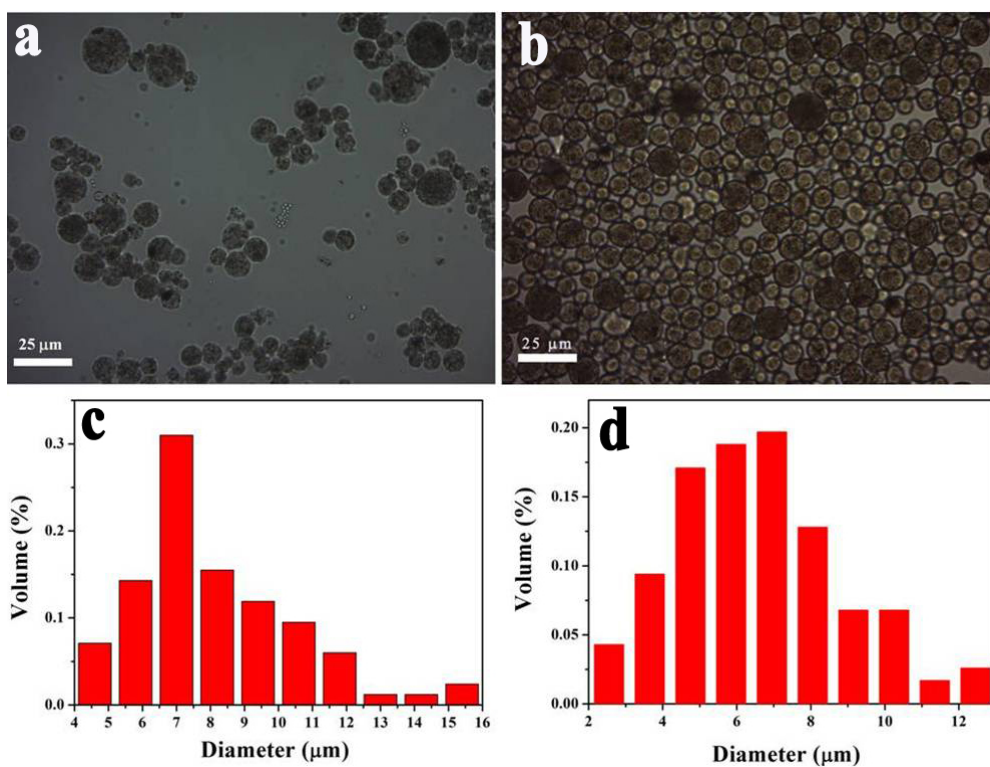


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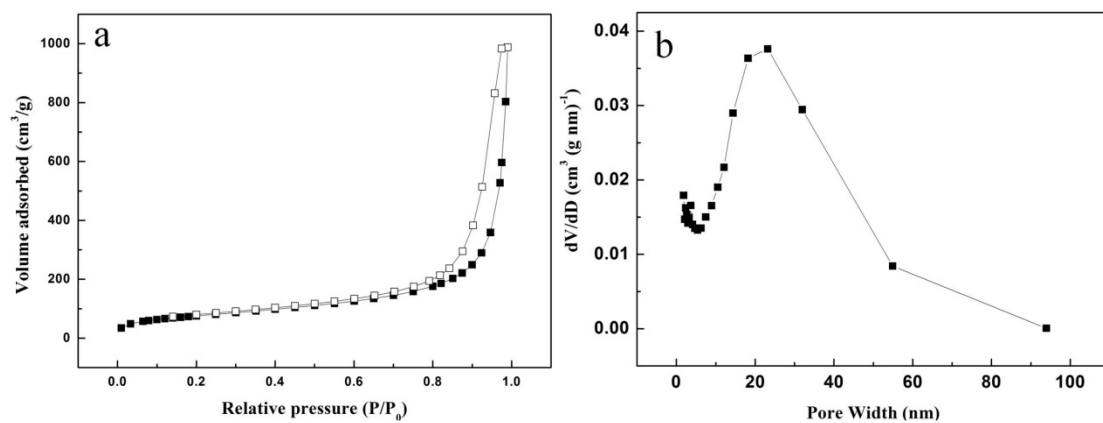


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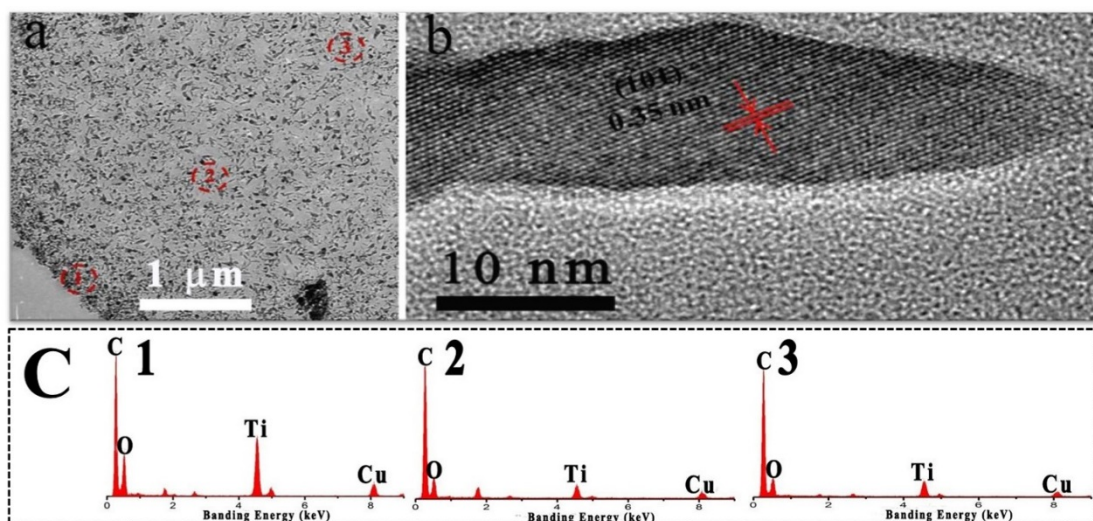


Fig. S4 TEM image of MCTiMs (a) and its EDS spectra corresponded to the marked locations (c).

HRTEM image of an individual TiO₂ nanoparticle with the lattice fringes marked (b)

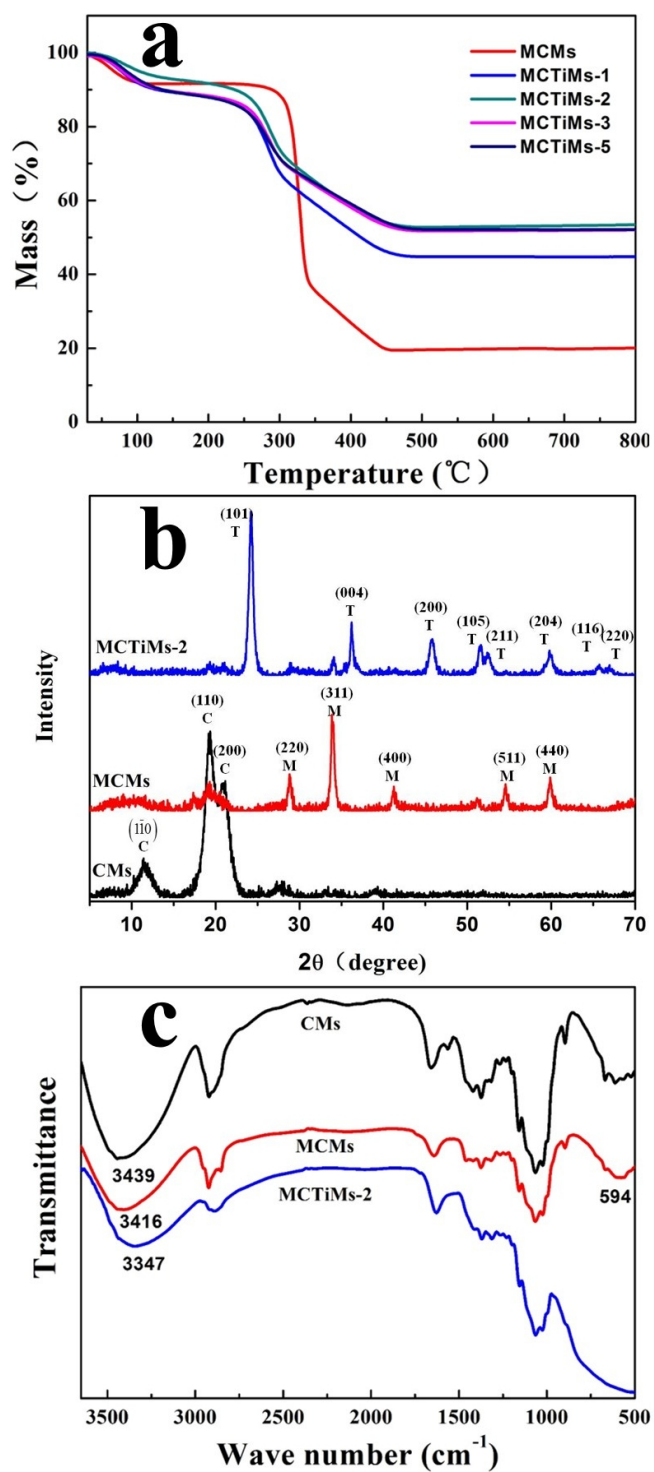


Fig. S5 TGA curves of MCMs and different MCTiMs (a), XRD patterns (b) and FT-IR spectra (c) of CMs, MCMs, MCTiMs-2.

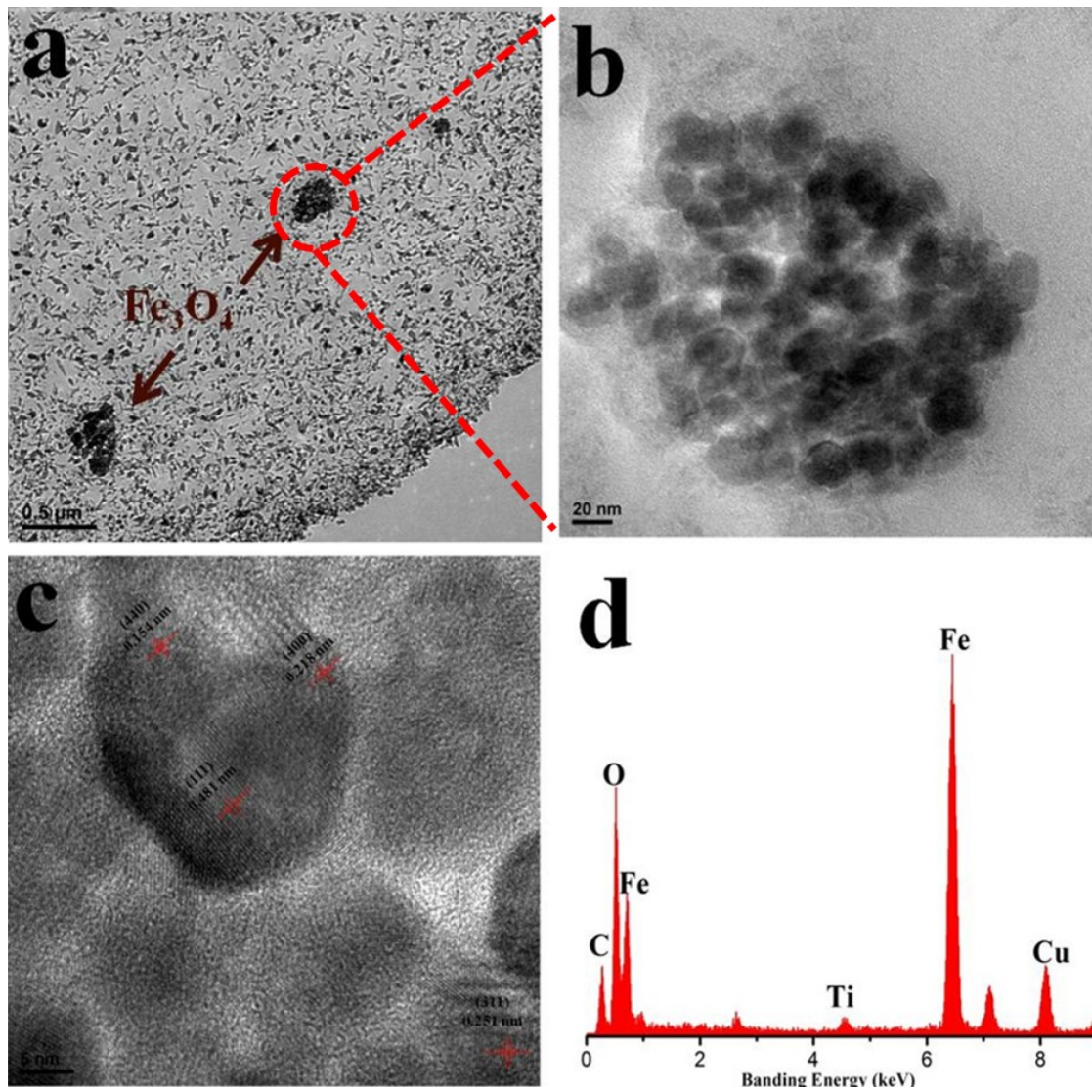


Fig. S6 TEM images (a, b), high resolution TEM image (c) and EDX Spectrum of Fe₃O₄ nanocrystal cluster (MCNC) dispersed in MCTiMs-2.

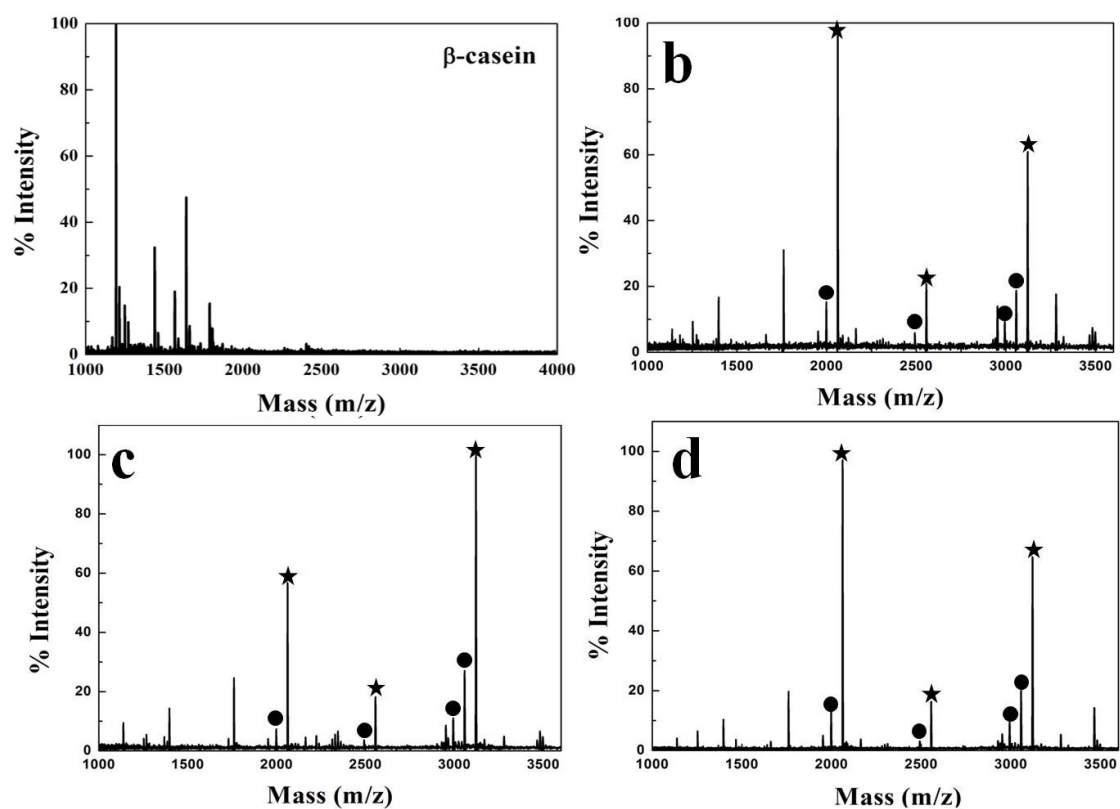


Fig. S7 MALDI mass spectra of the tryptic digests of β -casein analysis after enrichment using MCMs

(a), MCTiMs-1 (b), MCTiMs-3 (c), MCTiMs-5 (d). ★ indicates phosphorylated peptides, ● indicates their dephosphorylated counterparts.

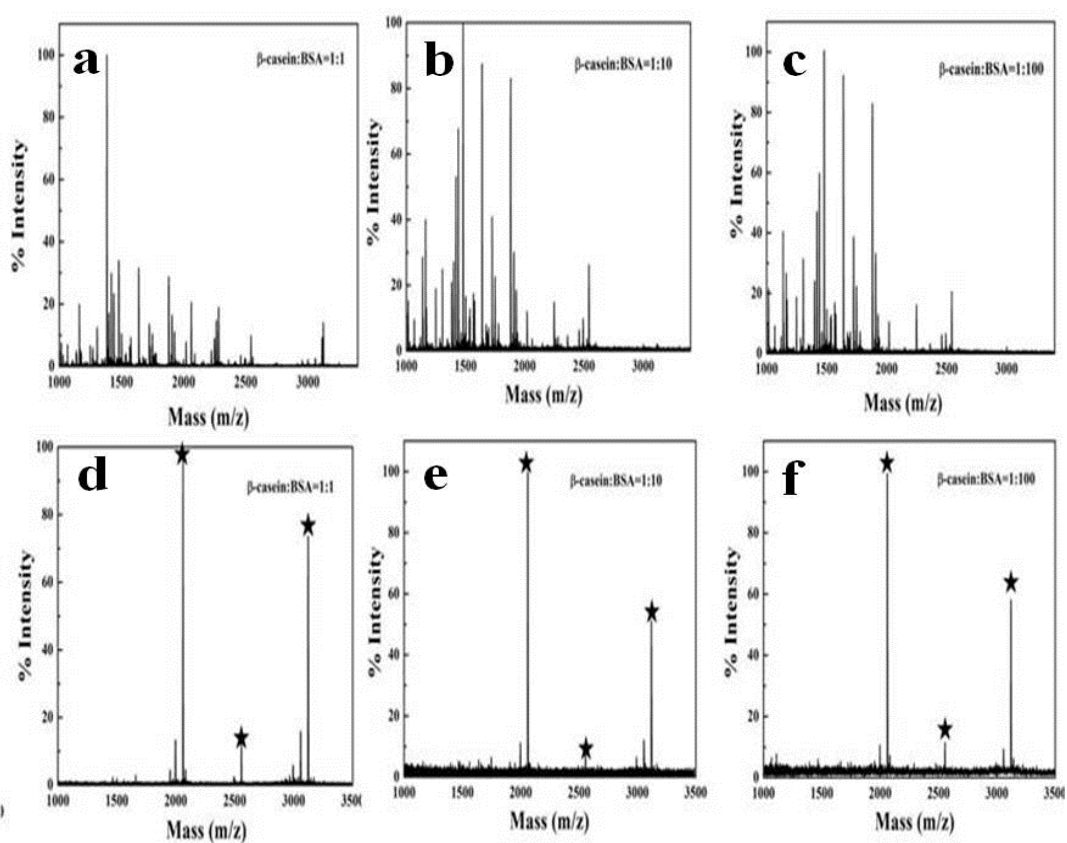


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direct analysis (a, b, c) and (d, e, f) analysis after enrichment using MCTiMs-2. ★ indicates phosphorylated peptides. β -casein was at concentration of 1.0×10^{-7} M (3 pmol).

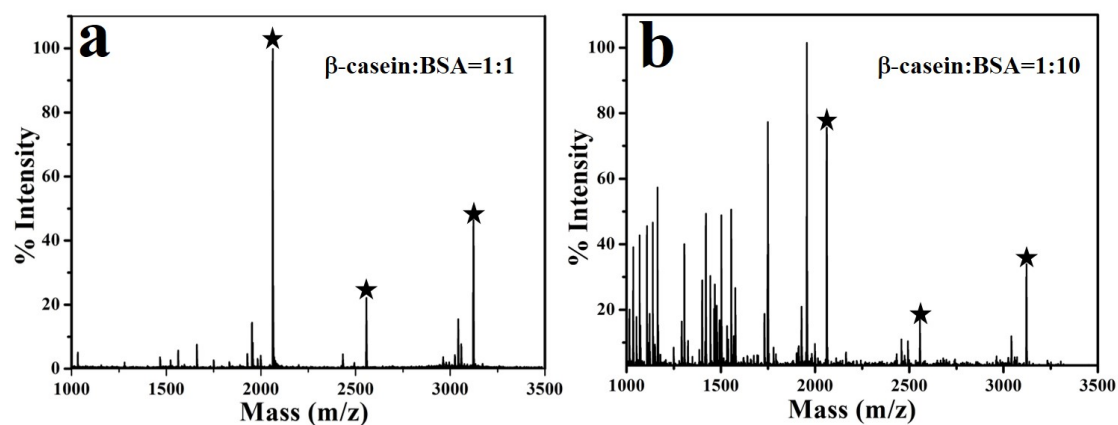


Fig. S9 MALDI mass spectra the tryptic digest of β -casein and BSA with ratio of 1:1 (a), 1:10 (b)

analysis after enrichment using commercial TiO_2 nanoparticles. ★ indicates phosphorylated peptides.

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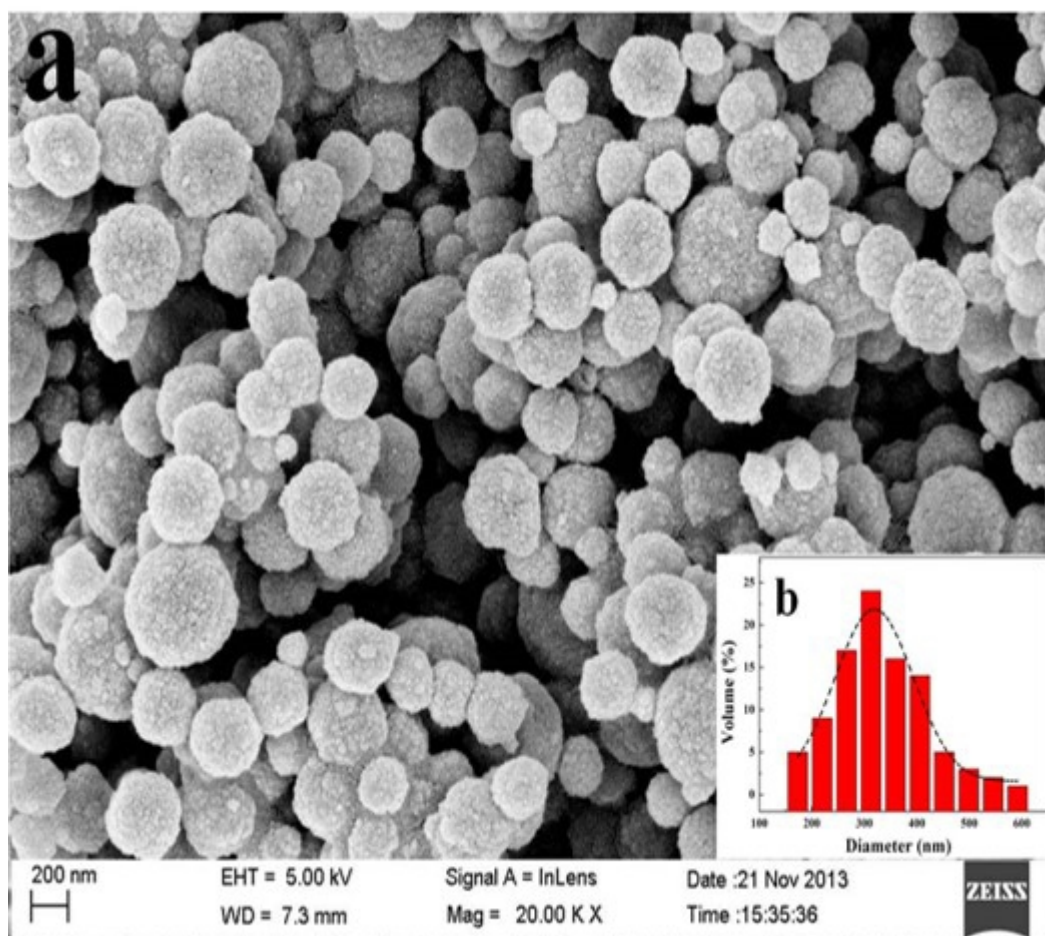


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- 1 J. Cai, Y. Liu and L. Zhang, *J. Polym. Sci., Part B: Polym. Phys*, 2006, **44**, 3096.
- 2 J. Liu, Z. Sun, Y. Deng, Y. Zou, C. Li, X. H. Guo, L. Xiong, Y. Gao, F. Li and D. Zhao, *Angew. Chem. Int. Ed*, 2009, **48**, 5875.