Supporting Information

Titania coated magnetic mesoporous hollow silica microspheres: fabrication and application to selective enrichment of phosphopeptides

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Experimental details

Chemicals and reagents. Sodium silicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$), cetyltrimethylammonium bromide (CTAB), iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), ethanol (EtOH), ethylene glycol, ammonium hexafluorotitanate ($(NH_4)_2TiF_6$), boric acid (H_3BO_3) and other chemicals were supplied by Shanghai General Chemical Reagent Factory (Shanghai, China), HPLC grade acetonitrile (ACN) was obtained from Fisher Scientific (Pittsburgh, USA). Ammonia hydrate ($NH_3 \cdot H_2O$, 25%), phosphoric acid (H_3PO_4), trifluoroacetic acid (TFA), 2, 5-dihydroxybenzoic acid (2, 5-DHB), titanium isopropoxide (TTIP), bovine α-casein and bovine serum albumin were purchased from Sigma-Aldrich (St Louis, USA). Sequencing grade trypsin was obtained from Promega (Madison, WI, USA). Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Preparation of peptides mixture. Bovine α-casein and β-casein were originally made up into stock solutions of 1 mg/mL with purified water.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.

BSA (1 mg) 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. Iodoacetamide (3 μL of 500 mM stock) was added,

and the obtained 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. Subsequently, 9 µL of 100 mM CaCl2 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).

Preparation of hollow mesoporous silica spheres (HMSS). HMSS was prepared according to a modified procedure described previously. A total of 19.6 g of CTAB followed by 23.2 g of solid $Na_2SiO_3 \cdot 9H_2O$ were dissolved in 337 mL of water, resulting in clear solution at 30 °C. Afterwards 35 mL of ethyl acetate were quickly added, the mixture was stirred for 30 seconds. Then the mixture was aged at 90 °C for 48 h after allowing to stand at 30 °C for 5 h. Finally, the solid product was filtered and washed with purified water and EtOH. The filtered HMS was dried at room temperature and calcined at 550 °C for 5 h.

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As shown in Table 1, the as-prepared HMSS had high pore volume and low surface area, which seemed to be contradictory to other HMSS reported in some literatures.[1-3] In fact, our HMSS had a larger pore size (~ 15 nm) as compared with that of common HMSS (typically 2.5 nm), which led to the high pore volume and low surface area.[4]

Preparation of hollow mesoporous silica spheres with magnetic cores (MHMSS). MHMSS was prepared according to the procedure reported by Guo et al. with modification.[9] A certain amount of iron nitrate (0.24g /0.48 g/ 0.72g) was dissolved in 20 mL ethanol. Then the solution was added to 0.24 g hollow mesoporous silica

spheres. The suspension was subjected to vacuum under ultrasonic conditions. After certain minutes, the vacuum pump was turned off and air was allowed to enter the system till normal atmospheric pressure was achieved, while the ultrasonic treatment continued for 1 min. After that, the suspension was dried at 60 °C under vacuum. Subsequently, the product was washed with 5 mL ethanol twice and dried again. The product was impregnated with 1 mL ethylene glycol up to incipient wetness. The impregnated sample was then subjected to heat treatment under nitrogen up to a treatment of 450 °C at the rate of 5 K/min and kept at this temperature for 2 h. The different spheres with introducing different amount of iron nitrate were labeled MHMSS-1, MHMSS-2 and MHMSS-3.

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It is believed that ethylene glycol played an important role in the conversion of iron salt into magnetic ion oxide. Ethylene glycol is a versatile solvent, which possesses high boiling point, moderate reduction ability, good polarity, and can be acted as surfactant. Currently, ethylene glycol has been used widely in the preparation of magnetic iron oxide particles.[5-7] These results showed that magnetic particles could be easily formed even under mild conditions by using ethylene glycol as solvent.

Preparation of TiO_2-1/MHMSS-2. The titania deposition of MHMSS-2 was accomplished by sol-gel method with titanium isopropoxide as Ti resource: 0.2 g titanium isopropoxide was dispersed in 2 mL ethanol. 1 mL of the solution was added dropwise into 0.2 g MHMSS-2. The Ti precursor was wet-impregnated into the pore of MHMSS-2 under vacuum condition at 60 °C until dryness. Hydrolysis of the procedure was accomplished with water vapor-induced internal hydrolysis.[19] In the

typical procedure, 25 mL H_2O was placed inside a Teflon-lined autoclave. The precursor-loaded MHMSS-2 was put in an open glass vial and kept inside the autoclave without direct contact between the solid and solution. The tightly closed autoclave was then heated to 100 °C for 4 h. The result solid was subjected to heat treatment under nitrogen up to a treatment of 300 °C at the rate of 1 K/min and kept at this temperature for 2 h.

Preparation of TiO_2-2/MHMSS-2. The titania deposition of MHMSS-2 was accomplished by liquid phase deposition method with ammonium hexafluorotitanate as Ti source: 0.5 g MHMSS-2 was added into a 50 mL solution containing of 0.1 M $(NH_4)_2TiF_6$ and 0.3 M H_3BO_3 in a PTFE container. After keeping under vacuum condition for 1 h, the mixture was heated at 35 °C for 12 h under continuous shaking. The resulting composite was washed with purified water thoroughly and dried at 100 °C for 4 h. The composites were then subjected to heat treatment under nitrogen up to a treatment of 300 °C at the rate of 1 K/min and kept at this temperature for 2 h.

Phosphopeptide enrichment procedure. 30 mg of titania coated 20-Fe-HMS (or commercial TiO_2) were suspended in 1 mL purified water. 30 μL of peptides mixture (in 1% trifluoroacetic (TFA) -50% acetonitrile (ACN)) was mixed with 5 μL magnetic materials suspension and incubated for 30 min. After successive washing with 50 μL 1% TFA-50% ACN and 0.1% TFA-50% ACN subsequently, the trapped peptides were eluted with 30 μL 2.5% ammonium hydroxide. Then, the eluted solution was lyophilized to dryness. During the procedure, magnetic material-target conjugate was separated from the sample solution by applying an external magnet. While

commercial TiO_2 were used as phosphopeptides enrichment material, centrifugation (15000 *g*) was used as separation method.

Instrumentation. The direct observations of spheres were displayed by a QUANTA-200 scanning election microscope (SEM, Eindhoven, The Netherlands). The composition of the spheres were determined by XSAM800 X-ray photoelectron spectroscopy (XPS, Kratos, UK), with Mg Kα radiation as the exciting source. The crystal structure of the spheres were determined with a Bruker SMART APEX II X-ray diffractometer (XRD, Billerica, German) using Cu Kα radiation and rotating anode operated at 40 KV and 30 mA. Raman spectra were collected using a Confocal Renishaw Raman Microspectroscopy RM-1000 (London, England). The 514.5 nm line from an Ar^+ laser was used as an excitation source. Nitrogen sorption measurement was performed at 77 K using a Beckman Coulter (Florida, USA) SA 3100 Plus surface area and pore size analyzer. The composites were activated by evacuating in vacuum and heating to 393 K for 6 h to remove any physically adsorbed substances before analysis. The specific surface area value was calculated according to the BET (Brunauer – Emmett – Teller) equation at P/P0 between 0.05 and 0.2. The pore parameters (pore volume and pore diameter) were evaluated from the desorption branch of isotherm based on BJH (Barrett – Joyner – Halenda) model.

All MALDI-TOF MS spectra of the peptides were recorded with a Voyager DE STR MALDI-TOF work station 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 μL of the mixture was used for MALDI-TOF analysis.

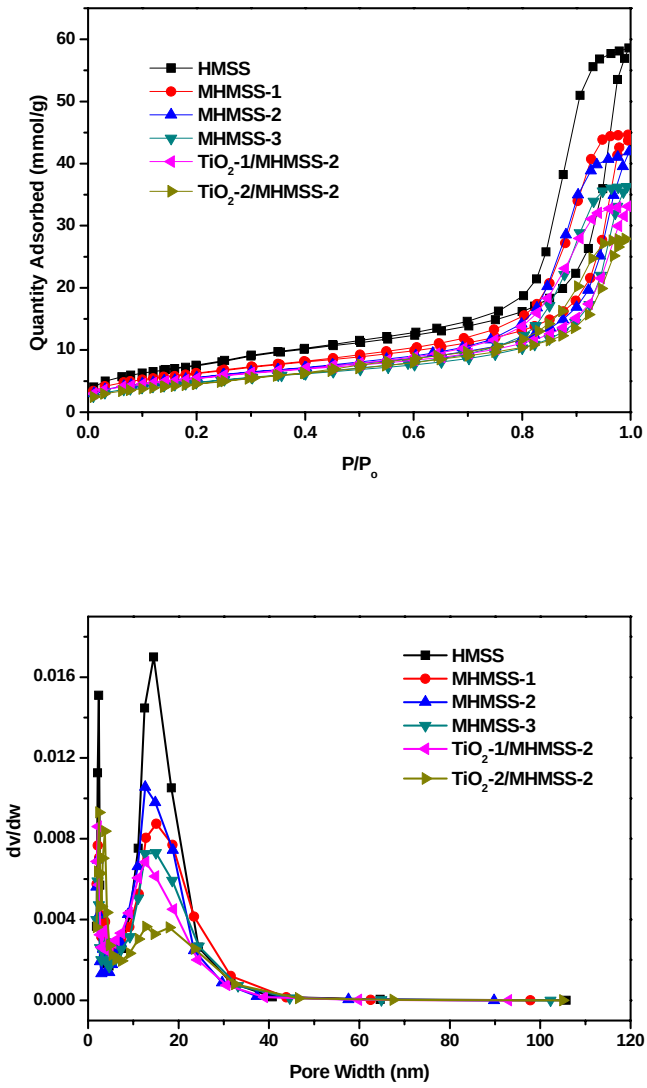

Figure S1. Isotherms and pore diameter distributions of the materials.

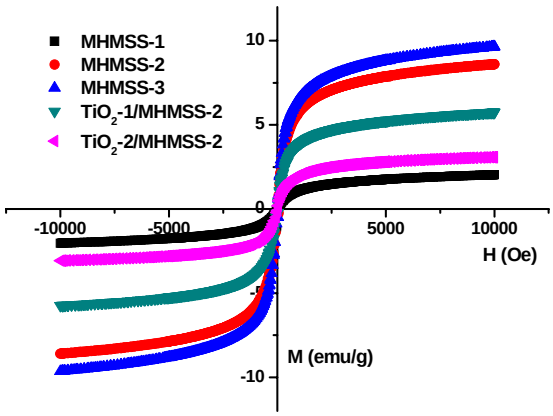

Figure S2. magnetization curves of the materials.

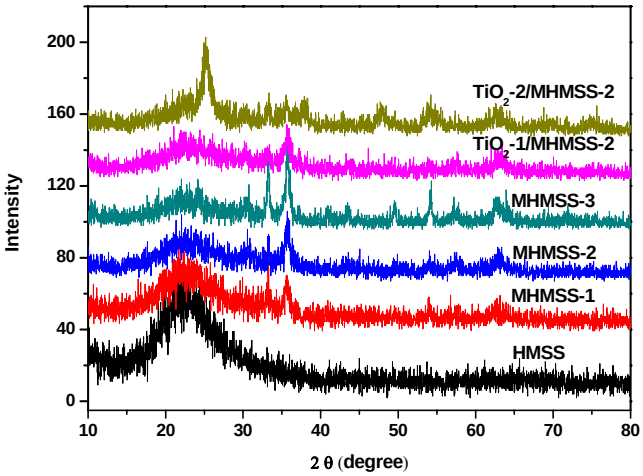

Figure S3. XRD patterns of the materials.

It is well known that the skin depth of the incident beam for XPS is a few nanometers, so it is difficult to directly examine the core iron oxides which are surrounded by relatively thick shell layers. Nevertheless, XPS signal could still be

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obtained for determining the components of core iron oxides since that a few iron oxides were deposited on the pore surface and external surface of HMSS.

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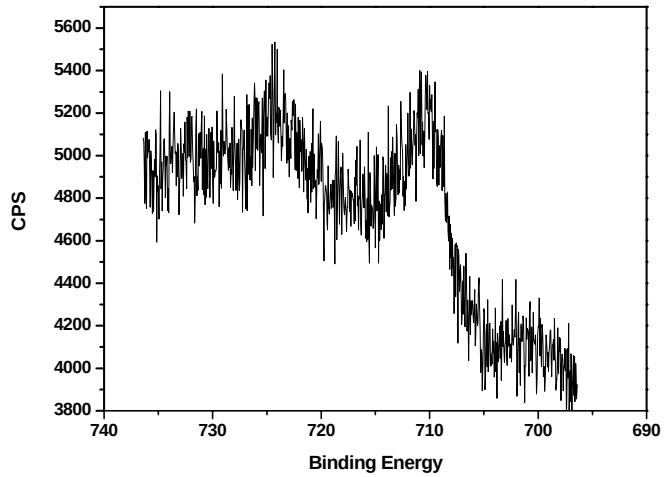

Figure S4. XPS pattern of MHMSS-2.

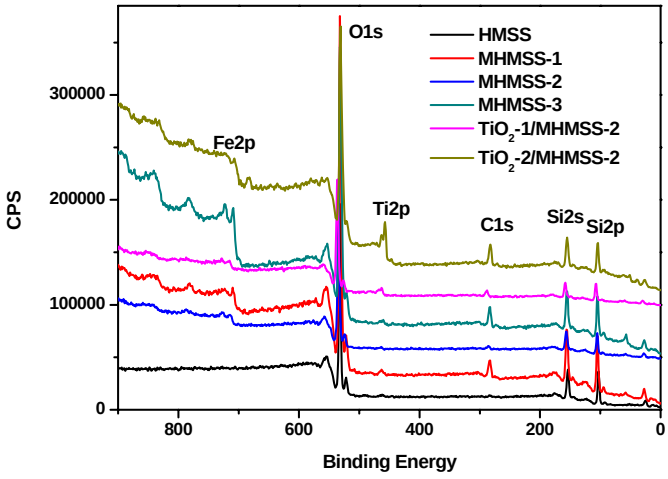

Figure S5. XPS patterns of the materials.

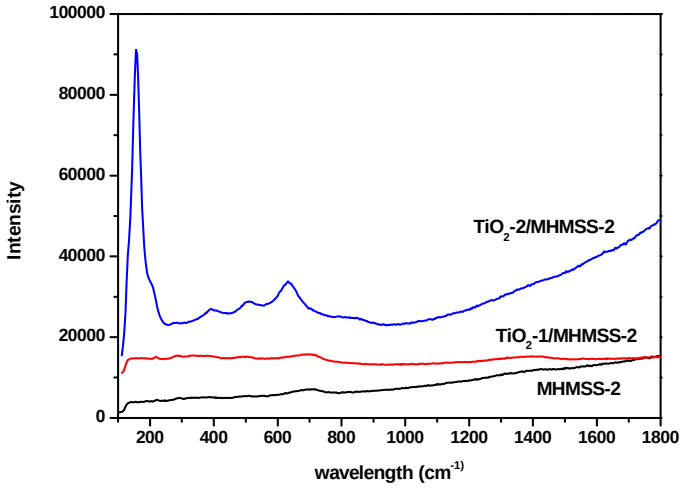

Figure S6. Raman patterns of the materials.

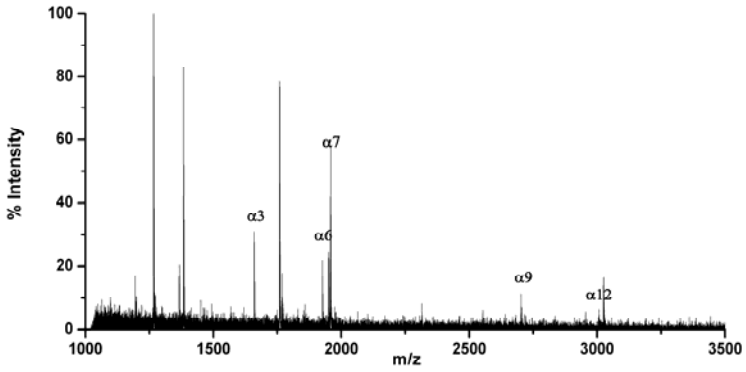

Figure S7. MALDI-TOF mass spectrum of the tryptic digests of α-casein at concentration of 1.0×10^{-7} M obtained by direct analysis.

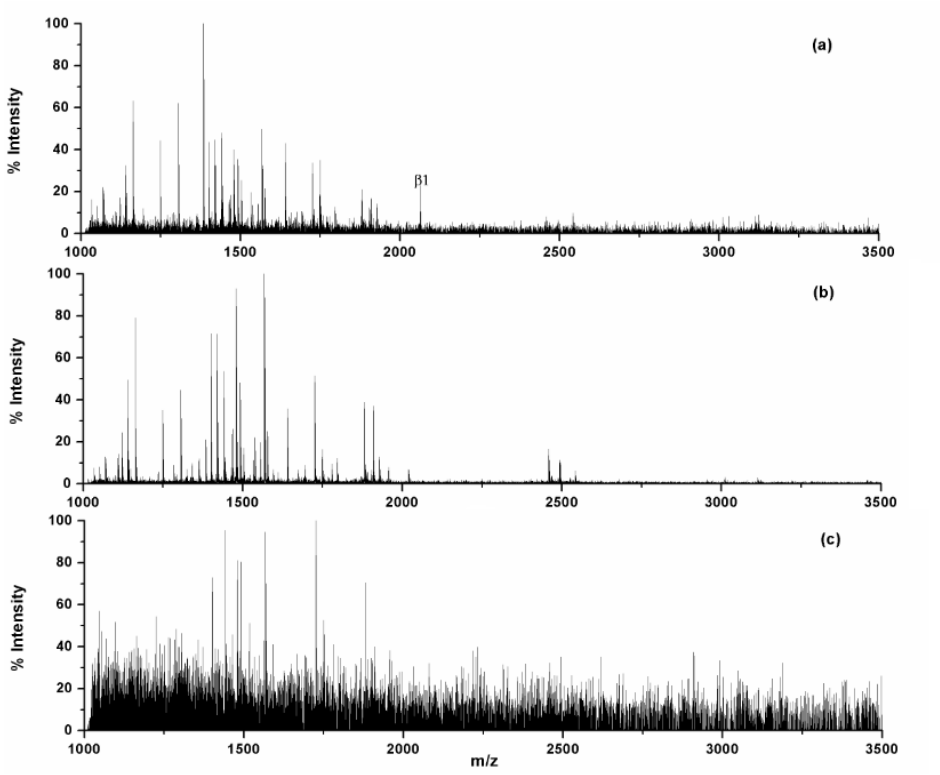

Figure S8. MALDI-TOF mass spectrum of the tryptic digests of the mixture of β-casein and non-phosphoprotein BSA with a molar ratio of 1:1 (a), 1:10 (b) and 1:100 (c) obtained by direct analysis. β-casein was at concentration of 1.0×10^{-7} M.

Table S1. Details information of the observed phosphopeptides obtained from tryptic digestion of α-casein and β-casein.

No.	$[M+H]^+$	Phosphorylation site	Amino acid sequence
α_1	1466.6	1	TVDME<u>S</u>TEVFTK
α_2	1660.8	1	VPQLEIVPN<u>S</u>AEER
α_3	1832.9	1	YLGEYLIVPN<u>S</u>AEER
α_4	1847.7	1	DIGSE<u>S</u>TEDQAMEDIK
α_5	1927.7	2	DIG<u>S</u>E<u>S</u>TEDQAMEDIK
α_6	1952.0	1	YKVPQLEIVPN<u>S</u>AEER
α_7	2619.0	4	NTMEHV<u>SSS</u>EE<u>S</u>IISQETYK
α_9	2703.5	1	LRLKKYKVPQLEIVPN<u>S</u>AEERL
α_9	2720.9	5	QMEAE<u>S</u>I<u>SSS</u>EEIVPN<u>S</u>VEQK
α_{10}	2747.1	4	NTMEHV<u>SSS</u>EE<u>S</u>IISQETYKQ

α_{11}	3008.0	4	NANEEEYSIGSSSEESAEVATEEVK
β_1	2061.8	1	FQSEEQQQTEDELQDK
β_2	2966.2	4	ELEELNVPGEIVESLSSSEESITR
β_3	3122.3	4	RELEELNVPGEIVESLSSSEESITR

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