Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Novel Approach for the Synthesis of Fe_3O_4@TiO_2 Core-Shell Microsphere and Its Application to Highly Specific Capture of Phosphopeptides for MALDI-TOF MS Analysis

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Experimental section

1) Preparation and Characterization of Fe_3O_4@TiO_2 Core-Shell microspheres

Synthesis of Fe_3O_4@C magnetic microspheres. Fe_3O_4 microspheres with diameter of 280 nm were first synthesized *via* a solvothermal reaction as previously described.[8] In the next step, 0.05 g microspheres were ultrasonicated for 10 min in 0.1 M HNO_3, followed by washing with deionized water. Then, the treated Fe_3O_4 microspheres were redispersed in 0.5 M aqueous glucose solution. After vigorous stirring for 10 min, the suspension was transferred to autoclaves and kept at 180 ^oC for 4 h. After reaction, the autoclaves were cooled naturally in air, and the suspensions was isolated with the help of a magnet and washed with deionized water and alcohol three times, respectively. The final sample was obtained after oven-drying at 80 ^oC for more than 4 h.

Synthesis of Fe_3O_4@TiO_2 Core-Shell microspheres. Tetrabutyltitanate (5 mL) was dissolved in ethanol (35 mL) to form a clear solution. Fe_3O_4@C magnetic microspheres (~100 mg) were then dispersed in the freshly prepared solution with the aid of ultrasonication for 5 min. A 1:5 (v/v) mixture of water and ethanol was added dropwise to the suspension of Fe_3O_4@C magnetic microspheres with vigorous magnetic stirring over a period of approximately 15 min. Thereafter, the suspension was stirred for a further 1 h before separation and washing with ethanol. After five cycles of separation/washing/redispersion with ethanol, the powder obtained was oven-dried and calcined under nitrogen atmosphere at 500^oC for 3h.

Characterization. Transmission electron microscope (TEM) equipped with a nanoarea energy- dispersive X-ray spectroscopic analyzer (EDX) were employed to characterize all microspheres. The minimum size of the electron beam was ca. 0.7 nm. The FT-IR spectra of Fe_3O_4 microspheres, Fe_3O_4@C and Fe_3O_4@TiO_2 core-shell microspheres were recorded on FT-IR (NEXUS470, NICOLET).

2） Phosphopeptides Enrichment by Fe_3O_4@TiO_2 Core-Shell microspheres

Materials. Bovine β-casein and casein (from bovine milk), chicken egg albumin (ovalbumin), myoglobin horse heart, cytochrome C, bovine serum albumin (BSA), trypsin (from bovine pancreas, TPCK treated), phosphoric acid (H_3PO_4), ammonium bicarbonate (NH_4HCO_3) and 2,5-dihydroxybenzoic acid (2, 5-DHB) were purchased from Sigma Chemical (St. Louis, MO). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). All aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA). All other chemicals and reagents were of the highest grade commercially available.

Model Proteins and Peptide Mixtures. Bovine β-casein, casein (from bovine milk), chicken egg albumin (ovalbumin), myoglobin horse heart, cytochrome C, bovine serum albumin (BSA), Each protein was dissolved in 25mM ammonium bicarbonate buffer at pH 8.0 and treated with trypsin (2%, w/w) for 12 h at 37 ^{o}C respectively.

Peptide Mixture 1. Peptide mixture 1 contained peptides originating from a tryptic digestion of 2×10^{-8} M of β-casein.

Peptide Mixture 2. Peptide Mixture 2 contained peptides originating from a tryptic digestion of 5ng/ μL of commercial casein.

Peptide Mixture 3. Peptide mixture 3 contained peptides originating from tryptic digestions of 2×10^{-8} M of β-casein, ovalbumin, myoglobin horse heart, cytochrome C, bovine serum albumin (BSA).

All the Peptide mixtures were diluted with 50% acetonitrile and 0.1% TFA aqueous solution (v/v).

Enrichment of phosphopeptides by Fe_3O_4@TiO_2 Core-Shell Microspheres. Suspension of Fe_3O_4@TiO_2 Core-Shell Microspheres (5 μL of 10 mg mL^{-1}) was added into 200μL of Peptide mixture originating from tryptic digestions respectively. Then the mixed solutions were vibrated at 37 ^oC for 30s. After that, with the help of magnet, the peptides/ Fe_3O_4/TiO_2 Core/Shell Microspheres were collected by removal of the supernatant and washed with 50% acetonitrile and 0.1% TFA aqueous solution (v/v) for three times. Then the obtained peptides-loaded Fe_3O_4@TiO_2 microspheres were redispersed in 10 μL of 50% acetonitrile aqueous solution (v/v).

MALDI-TOF-MS process. The above peptides/ Fe_3O_4@TiO_2 microspheres slurry was deposited on the MALDI target using dried droplet method. 0.5 μL of the slurry was deposited on the plate and then another 0.5 μL of a mixture of 20 mg mL^{-1} 2,5-dihydroxybenzoic acid (in 50% acetonitrile aqueous solution, v/v) and 1% (v/v) H_3PO_4 aqueous solution, 1:1(v/v) was introduced as a matrix. MALDI-TOF MS experiments were performed in positive ion mode on a 4700 Proteomics Analyzer (Applied Biosystems, USA) with the Nd-YAG laser at 355 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV. The intensities of the ion peaks in the mass spectra are varied according to the laser intensity, that is, higher laser intensity, higher peak intensity.

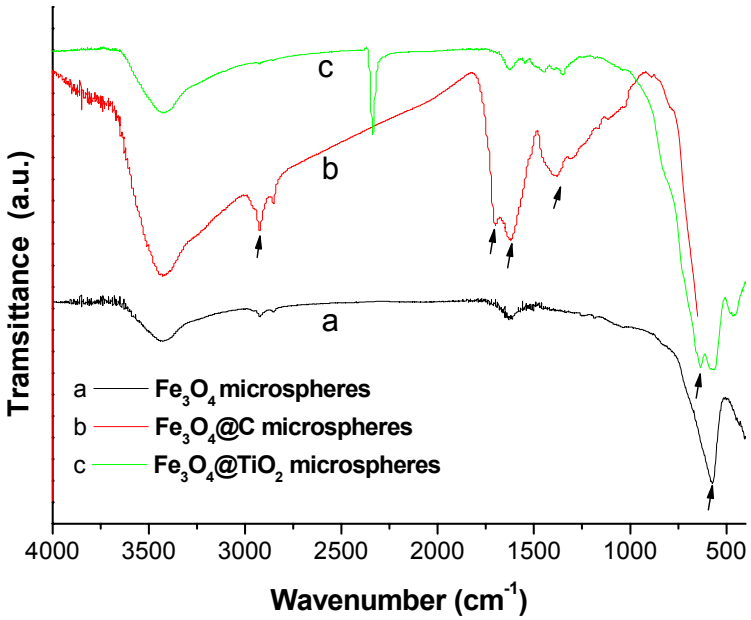

Figure S1. The FTIR spectra of (a) as-synthesized Fe_3O_4 microspheres (b)Fe_3O_4@C microspheres and (c) Fe_3O_4@TiO_2 core-shell microspheres

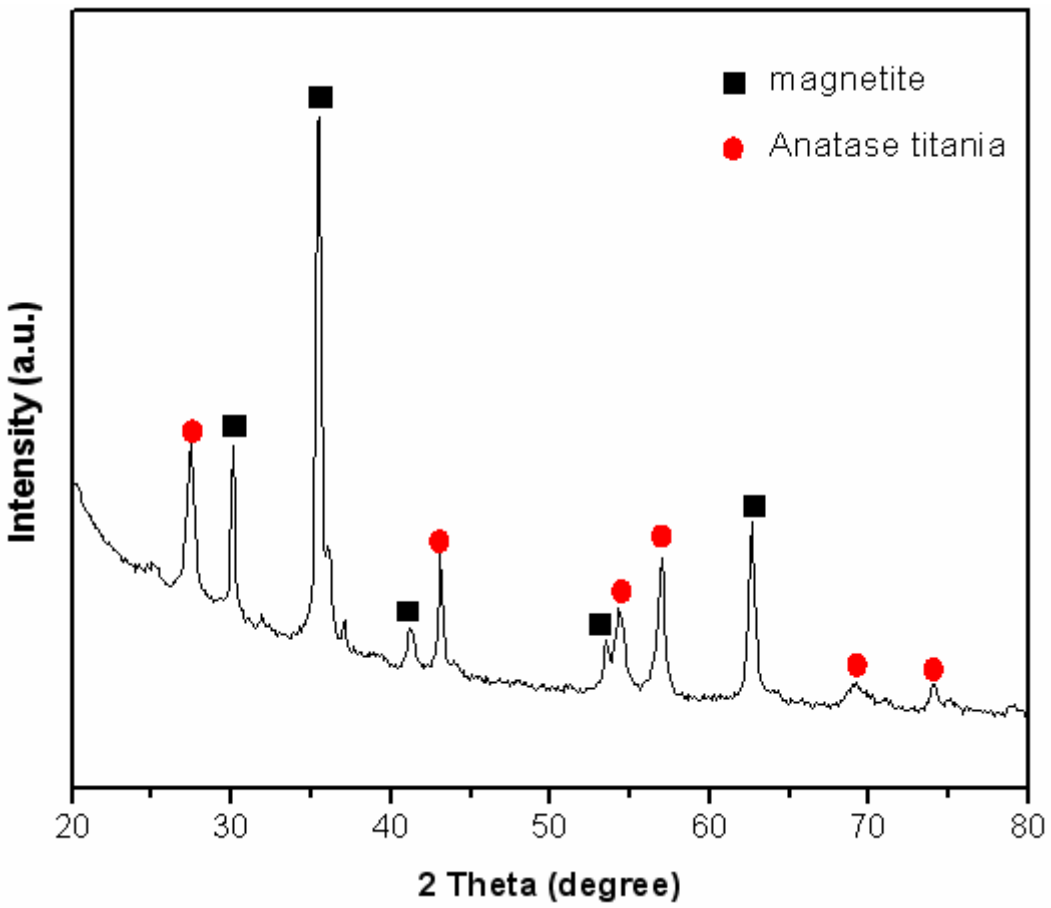

Figure S2. The wide-angle X-ray diffraction of the Fe3O4@TiO2 microspheres (red the magnetite.

A variety of core-shell structured magnetic metal oxide microspheres were synthesized through our proposed method, including Fe_3O_4@ZrO_2, Fe_3O_4@ Al_2O_3, Fe_3O_4@Ga_2O_3 and Fe_3O_4@In_2O_3. They synthesis predures are similar to that for Fe_3O_4@TiO2 by using zirconium isopropoxide, aluminum isopropoxide gallium, isopropoxide and indium isopropoxide as precursor, respectively.

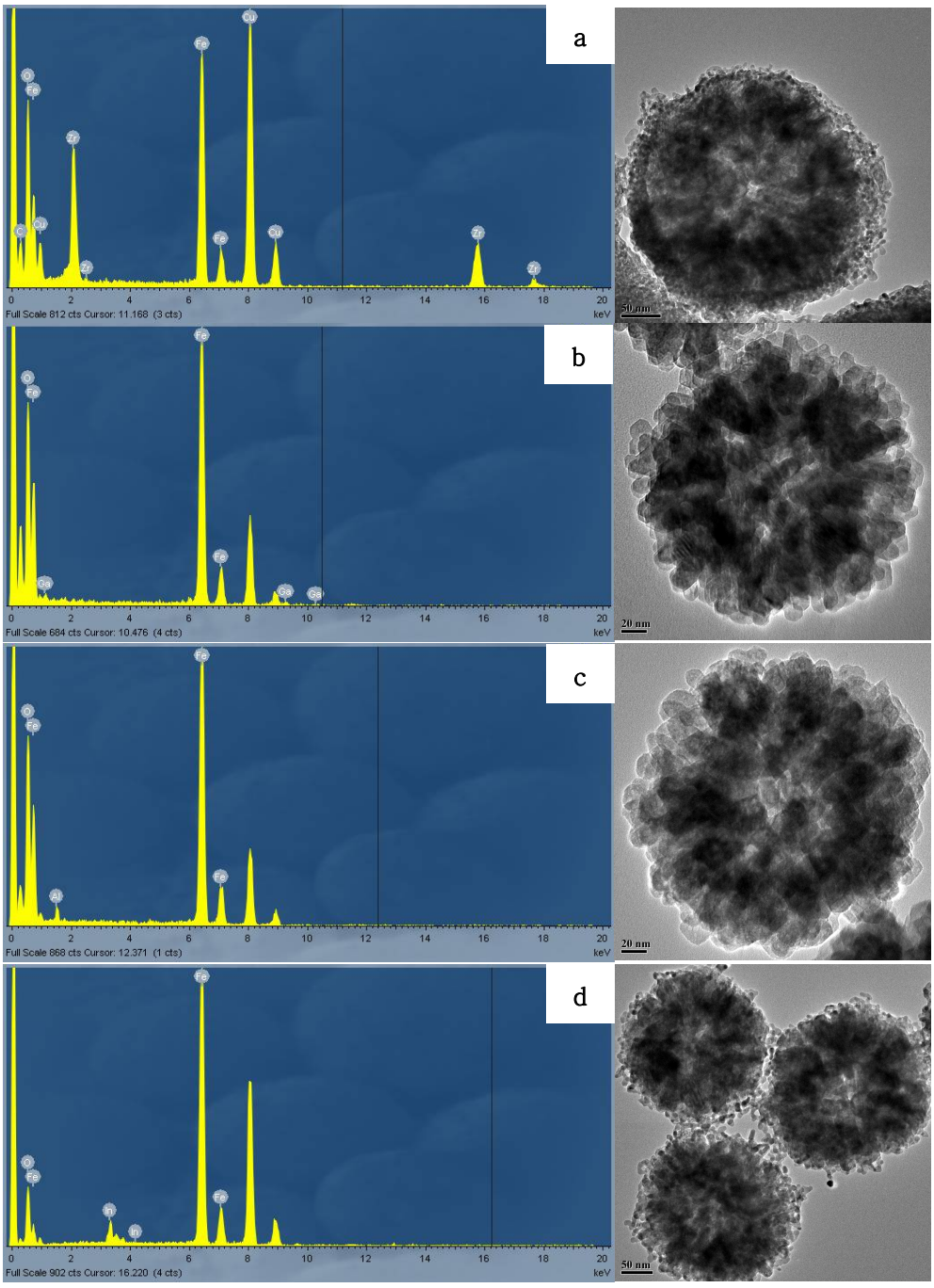

Figure S3 the EDX spectrum data (left) and TEM images (right) of ovarious core-shell magnetic metal oxide microspheres (a)Fe_3O_4@ZrO_2.(b) Fe_3O_4@Ga_2O_3 (a) Fe_3O_4@TiO_2 core-shell microspheres. (c) Fe_3O_4@Al_2O_3 (d) Fe_3O_4@In_2O_3

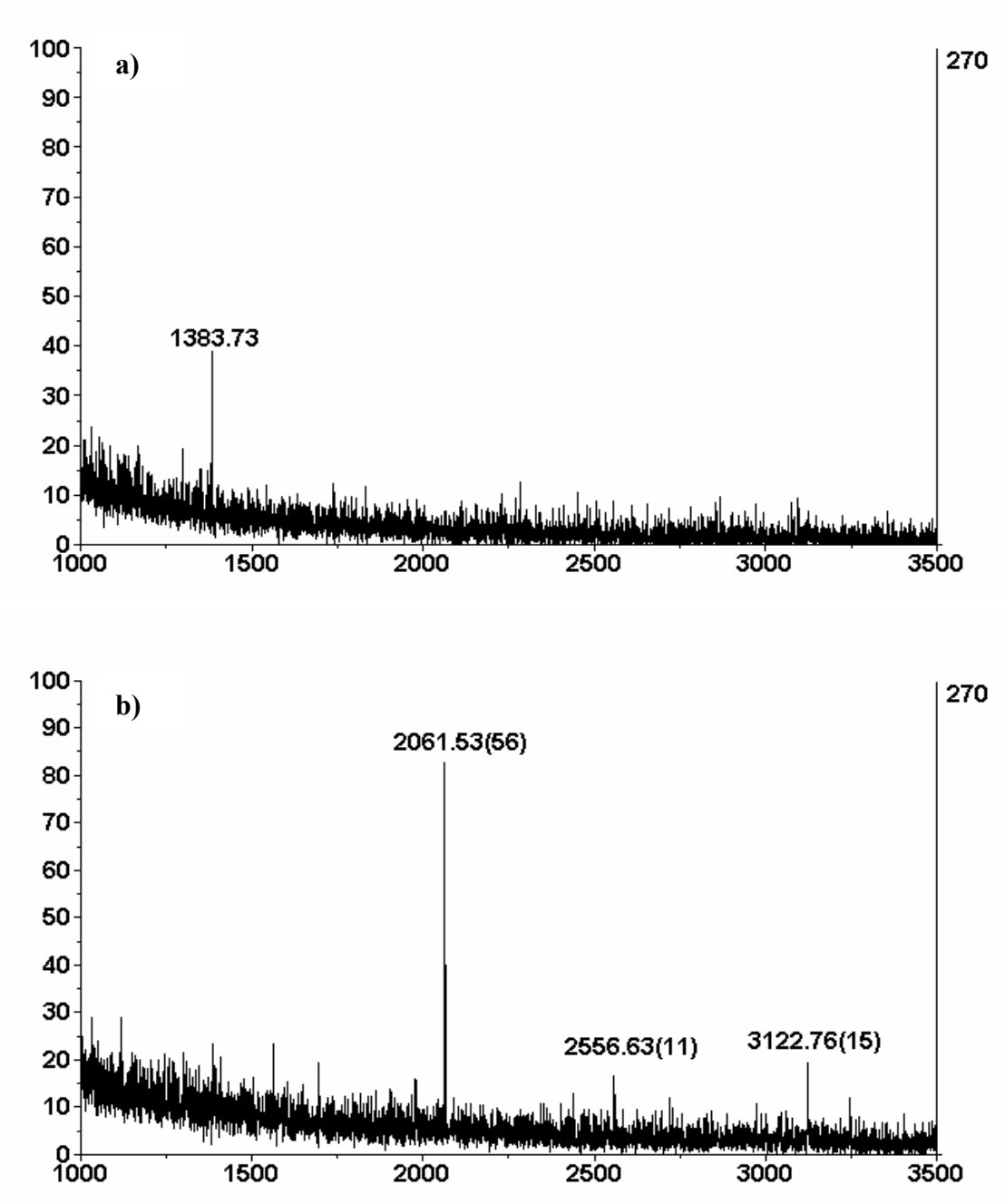

Figure S4. MALDI mass spectra of (a): the tryptic digest product of β-casein without any pre-treatment, 2×10^{-9} M, 0.5 μL; (b): on bead analysis of phosphopeptides obtained when using Fe_3O_4/TiO_2 microspheres to selectively trap target peptides from the tryptic digest product of β-casein at the following concentrations and extraction volumes, 2×10^{-9}M, 200 μL. The data in parentheses are S/N of the corresponding peptides.

Table S1. Phosphopeptides Ion Peaks Observed in the MALDI Mass Spectrum of Tryptic Digest of casein

No.	AA	Peptide sequenceS	$[M+H]^+$
1	α-S2/138-147	TVDME[pS]TEVF	1237.42
2	α-S2/138-149	TVDME[pS]TEVFTK	1466.51
3	α-S2/126-137	EQL[pS]T[pS]EENSKK	1562.04*
4	α-S1/106-119	VPQLEIVPN[pS]AEER	1660.661
5	α-S1/104-119	YKVPQLEIVPN[pS]AEER	1832.66
6	α-S1/43-58	DIG[pS]E[pS]TEDQAMETIK	1927.49
7	α-S1/104-119	YKVPQLEIVPN[pS]AEER	1951.76
8	β/33-48	FQ[pS]EEQQQTEDELQDK	2061.64
9	β/33-52	FQ[pS]EEQQQTEDELQDKIHPF	2555.86
10	α-S2/2-21	NTMEHV[pS] [pS] [pS]EESII[pS]QETYK	2618.84
11	α-S1/99-120	LRLKKYKVPQLEIVPN[pS]AEERL	2703.60
12	α-S2/2-22	NTMEHV[pS] [pS] [pS]EESII[pS]QETYKQ	2746.59
13	β/1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3121.70

The asterisk means Phosphopeptides Ion Peaks is $[M+Na]^+$.

To demonstrate the reproducibility of the MS technique, we deposited the same sample (0.5 μL of peptides/ Fe_3O_4@TiO_2 microspheres slurry after enrichment of 200 μL of 5 ng/μL β-casein) on three wells of the MALI plate and performed MS analysis under the same condition. The S/N of the three phosphopeptides is reproducible with relative standard deviation (RSD) of 5.6% (at *m/z* 2061), 3.9% (at *m/z* 2556), 4.7% (at *m/z* 3122), respectively.