Supporting Information:

Template-free synthesis of uniform mesoporous SnO_2 nanospheres for efficient phosphopeptide enrichment

Experimental Details

Materials

β-casein, trypsin, 2,5-dihydroxybenzoic acid (2,5-DHB) were purchased from Sigma-Aldrich. Ammonium hydroxide and ammonium bicarbonate were purchased from Fluka. Trifluoroacetic acid (TFA) was from J&K Technologies Inc. Acetonitrile (ACN) was obtained from Dikma Technologies Inc. Water used in MS was from Hangzhou Wahaha Group Co., Ltd.
Other reagents were obtained from Beijing Chemical Works. Nonfat milk was obtained from a local supermarket. All the chemicals were of analytical grade except TFA and ACN, which are of HPLC grade.

Preparation of $mSnO_2$ and $nSnO_2$

Synthesis of Mesoporous SnO_2 Nanospheres ($mSnO_2$)

In a typical synthesis, 0.175 g of $SnCl_4 \cdot 5H_2O$ (99.0%, Shantou Xilong Chemical Factory) was dissolved in a mixed solvent containing 8 mL of ethanol (99.7%, Beijing Chemical Reagent Co.) and 50 μL of concentrated aqueous HCl (GR, Beijing Chemical Reagent Co.). The obtained solution was transferred to a 20 mL Teflon-lined stainless-steel autoclave, which was then heated at 150 °C for 24 h. After the autoclave cooled to room temperature, the product was collected by centrifugation, washed with deionized water and ethanol for several times, and dried at 70 °C for 12 h.

Synthesis of SnO_2 Nanoparticles ($nSnO_2$)

SnO_2 nanoparticles were obtained by calcination of mesoporous SnO_2 nanospheres at 550 °C for 3 h.

Enrichment of phosphopeptides.

In a typical experiment, the tryptic digests of β-casein were diluted to a certain concentration by binding buffer (50% ACN, 0.2% TFA, pH ~2). Then suspensions of the AP materials (typically 2 μg/μL) were added into 200 μL diluted peptide mixture for selective enrichment. The mixed solutions were vibrated at room temperature for 30 min. After centrifugation (typically 10000 rpm, Eppendorf centrifuge 5417R), the supernatants were removed. After being washed with binding

buffer for three times, the remained nanomaterials were redispersed in 5 μL 1.6 M ammonia aqueous solution for MALDI-MS analysis. For phosphopeptide enrichment of peptide mixture digested from nonfat milk, the dilution, binding, washing and elution steps were similar to β-casein digests.

Instrumentation.

The products were characterized by scanning electron microscopy (SEM, Hitachi S4800, 5kV), X-ray diffraction (XRD, Rigaku Dmax-2000, Cu Kα radiation), transmission electron microscopy (TEM, FEI Tecnai T20, 200 kV), and high-resolution TEM (HRTEM, FEI Tecnai F30, 300 kV). Nitrogen adsorption-desorption measurements were performed using a Micromeritics ASAP 2010 instrument.

All MALDI-ToF MS spectra of the peptides were obtained by using a Bruker Daltonics ultraflex ToF mass spectrometer. The following voltage parameters were employed for MS analysis in our work: ion source 1, 25.00 kV; ion source 2, 22.35 kV; lens, 8.50 kV; reflector, 26.45 kV; reflector 2, 13.40 kV. The laser frequency was set on 200 Hz.

A mixture of 20 mg/mL 2, 5-DHB in 50% (v/v) ACN, 1% (v/v) phosphoric acid was introduced as the matrix. 0.5 μL of the washing buffer and 0.5 μL matrix solution were mixed on the plate, dried at room temperature for MALDI-ToF MS analysis.

Figures and tables

1 Characterization of the synthesized materials

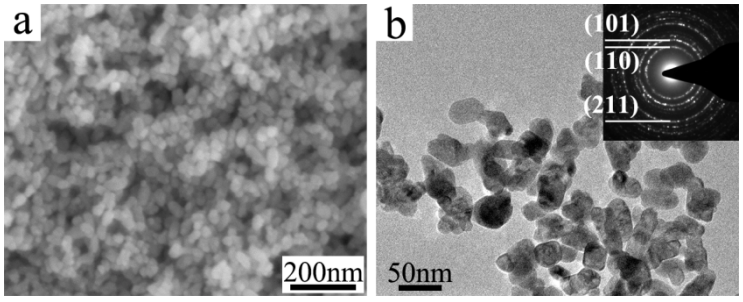

Fig. S1 SEM (a) and TEM (b) images of SnO_2 nanoparticles. The inset in (b) is the corresponding SAED pattern. The SnO_2 nanoparticles show an average diameter of ~30 nm.

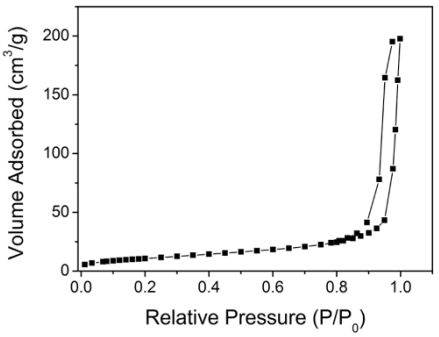

Fig. S2 N_2 adsorption-desorption isotherm of SnO_2 nanoparticles. The SnO_2 nanoparticles have a BET surface area of 39.9 m^2/g

2 Mass spectra

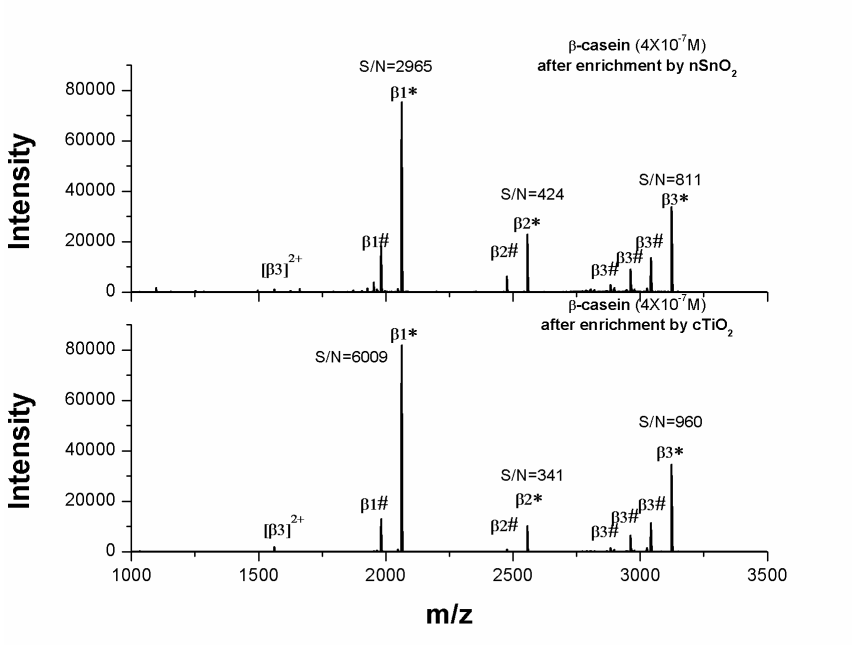

Fig. S3 MS spectra derived from β-casein (4×10^{-7} M) after enrichment by $nSnO_2$ and $cTiO_2$ ($nSnO_2$: non-porous SnO_2, $cTiO_2$: commercial TiO_2)

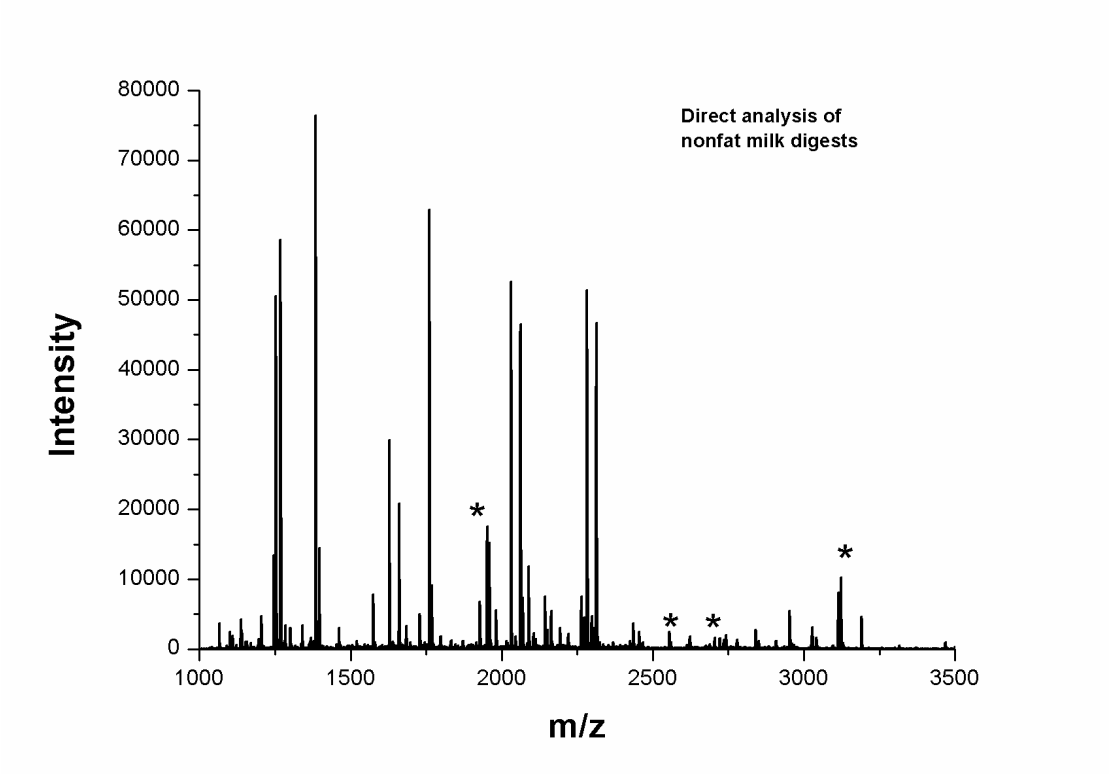

Fig. S4 MS spectra derived from nonfat milk digests without enrichment

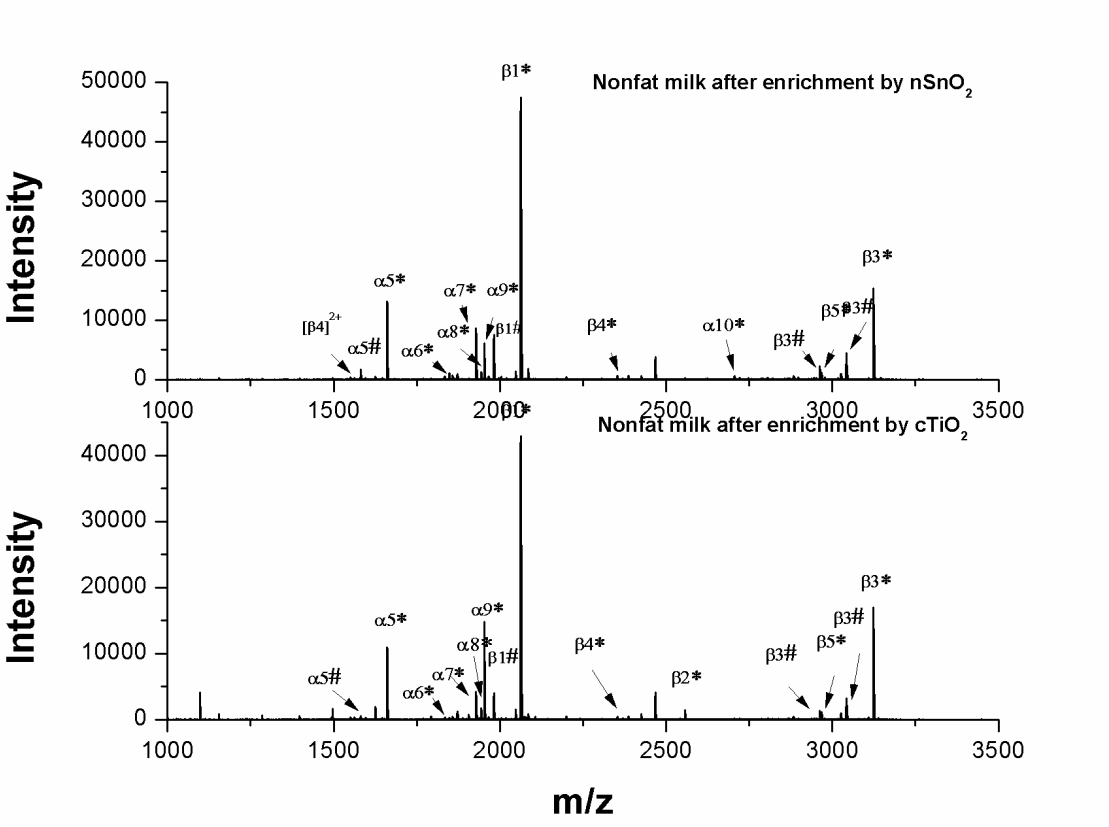

Fig. S5 MS spectra derived from nonfat milk after enrichment by $nSnO_2$ and $cTiO_2$

($nSnO_2$: non-porous SnO_2, $cTiO_2$: commercial TiO_2)

Table S1 Sequences of the detected phophopeptides

No.	m/z	Phosphorylation sites	Sequences	Protein	$mSnO_2$	SnO_2	TiO_2
α1*	1237.45	1	TVDMEpSTEVF	α-S2-casein	D	N	N
α2*	1253.56	1	TVD(oM)MEpSTEVF	α-S2-casein	D	N	N
α3*	1466.59	1	TVDMEpSTEVFTK	α-S2-casein	D	N	N
α4*	1594.85	1	TVDMEpSTEVFTKK	α-S2-casein	D	N	N
α5*	1660.77	1	VPQLEIVPNpSAEER	α-S1-casein	D	D	D
α6*	1833.83	1	YLGEYLIVPNpSAEER	α-S1-casein	D	D	D
α7*	1927.67	2	DIGpSEpSTEDQAMEDIK	α-S1-casein	D	D	D
α8*	1943.66	2	DIGpSEpSTEDQA(oM)MEDIK	α-S1-casein	D	D	D
α9*	1952.94	1	YKVPQLEIVPNpSAEER	α-S1-casein	D	D	D
β1*	2061.81	1	FQpSEEQQQTEDELQDK	β-casein	D	D	D
β4*	2353.87	4	NVPGEIVEpSLpSpSpSEESITR	β-casein	D	D	D
β2*	2557.06	1	FQpSEEQQQTEDELQDKIHPF	β-casein	D	N	D
α10*	2704.87	1	LRLKKYKVPQLEIVPNpSAEEERL	α-S1-casein	D	D	N
α11*	2721.86	5	QMEAEpSIpSpSpSEEIVPNPNpSVEQK	α-S1-casein	D	N	N
α12*	2952.05	3	EKVNELpSKDIGpSEpSTEDQAMEDIK*	α-S1-casein	D	N	N
β5*	2966.11	4	ELEELNVPGEIVEpSLpSpSpSEESITR	β-casein	D	D	D
β3*	3123.22	4	RELEELNVPGEIVEpSLpSpSpSEESITR	β-casein	D	D	D
Total					17	10	10

"oM" represents oxidation on methionine; D:detected N: non-detected