Supporting Information

C8-modified CeO_2//SiO_2 Janus fibers for selective capture and individual MS detection of low-abundance peptides and phosphopeptides

Nan Lv,[a,b] Zhigang Wang,[a,b] Wenzhi Bi,[a,b] Guangming Li,[a,b] Jilin Zhang*,[a], and Jiazuan Ni[a]

a. State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Changchun 130022, P. R. China.

b. University of Chinese Academy of Sciences, Beijing 100049, P. R. China.

EXPERIMENTAL SECTION

Sample preparation of ZipTipC18:

Commercial Zip-TipC18 pipette tips for comparison were also employed to capture target peptides from diluted human serum. The sample preparation of peptides prior to MALDI-TOF MS using ZipTipC18 pipette tips was carried out according to the standard procedure provided by the technical note of MILLIPORE Corporation described in the reference [a].

Briefly, the preparation process included the following steps.

1. Prewet the tip by depressing pipettor plunger to a dead stop using the maximum volume setting of 10 μL. Aspirate wetting solution (50% ACN in TFA in Milli-Q water) into tip. Dispense to waste. Repeat.

2. Equilibrate the tip for binding by washing it twice with the equilibration solution (0.1% TFA in Milli-Q water).

3. Bind peptides to ZipTip by fully depressing the pipettor plunger to a dead stop. Aspirate and dispense 5 μL sample solution 8 cycles.

4. Wash tip and dispense to waste using at least 2 cycles of wash solution (0.1% TFA in Milli-Q water).

5. In this case, dispense 5 μL of elution solution (50% ACN/0.1% TFA in Milli-Q water) into a clean vial using a standard pipette tip. Carefully, aspirate and dispense eluant through ZipTip at least 3 times without introducing air.

6. The supernatant was collected and lyophilized to dryness.

[a] Chen, H. M.; Xu, X. Q.; Ning, Y.; Deng, C. H.;Yang, P. Y.; Zhang X. M. Facile synthesis of C8-functionalized magnetic silica microspheres for enrichment of low-concentration peptides for direct MALDI-TOF MS analysis. Proteomics.2008, 8, 2778–2784.

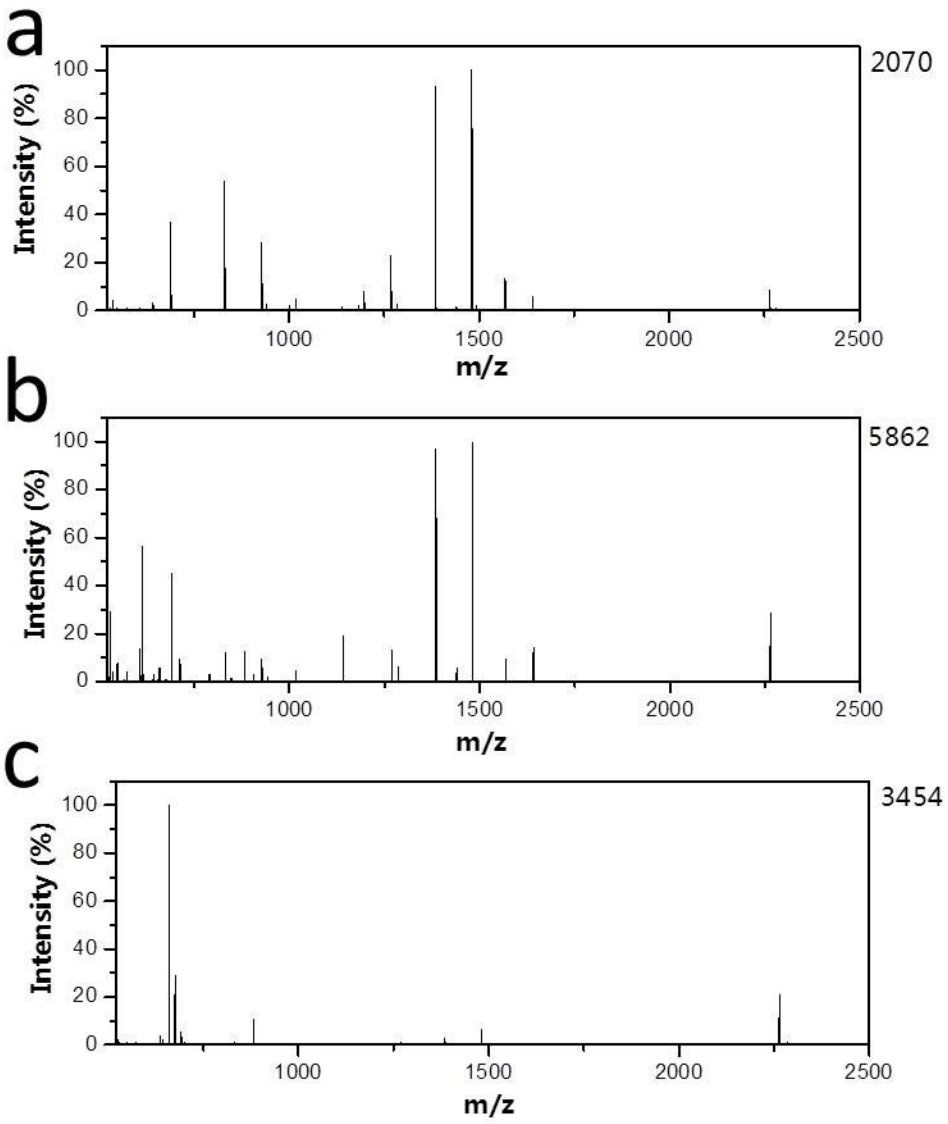

Fig. S1 MALDI-TOF mass spectra of low-abundance peptides enriched by the CeO_2//SiO_2-C8 probe from the tryptic digest mixture of β-casein (10 nM) and BSA (10 nM), and subsequently eluted by (A) 30%, (B) 50%, (C) 80% of ACN/H_2O (v/v) eluent, respectively. The number in the top right corner is the highest peak intensity. The experimental results indicate the concentration of 50% in the ACN/H_2O (v/v) eluent provides the optimum enrichment conditions.

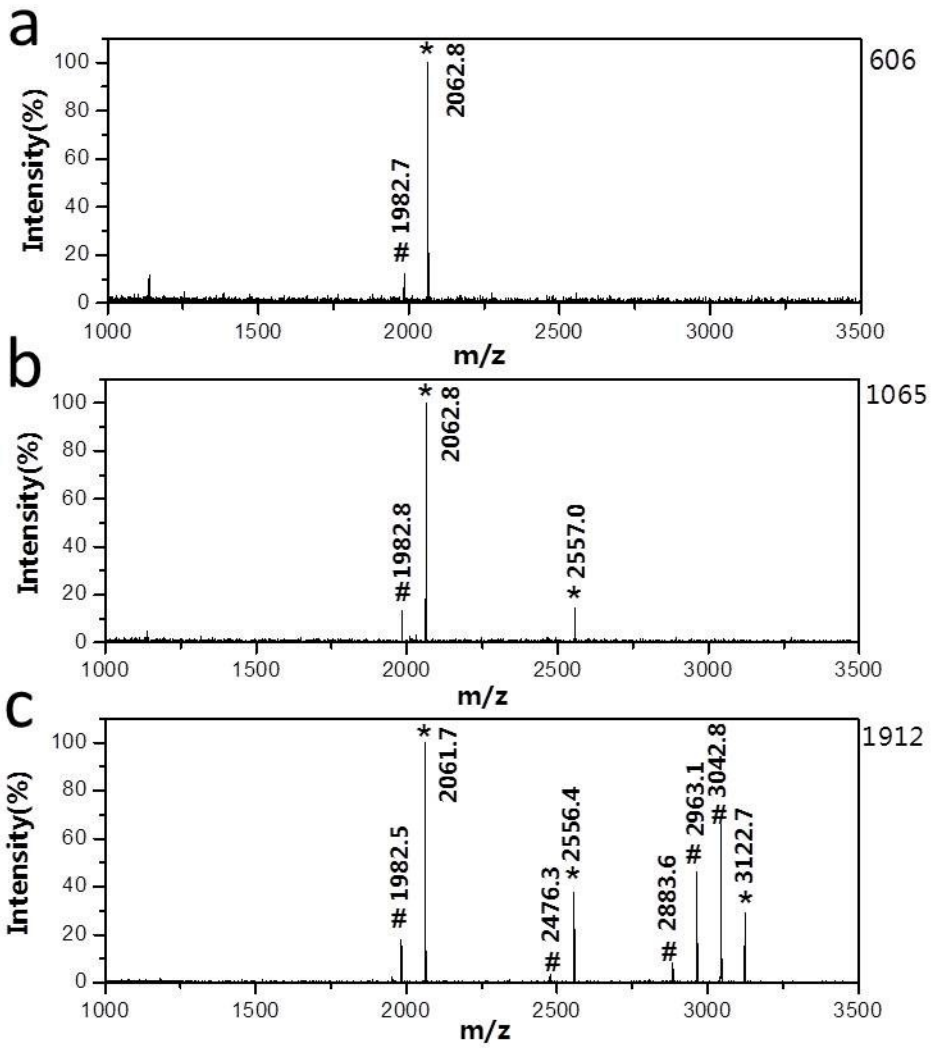

Fig. S2 MALDI-TOF mass spectra of phosphopeptides enriched by the CeO_2//SiO_2-C8 probe from the tryptic digest mixture of β-casein (10 nM) and BSA (10 nM) and subsequently eluted by 50% of ACN/H_2O (v/v) in the first step, and then eluted by (A) 1%, (B) 5%, (C) 10% of $NH_3 \cdot H_2O$ eluent in the second step respectively. '*' Indicates phosphopeptides, '#' indicates their dephosphorylated counterparts. The number in the top right corner is the highest peak intensity. The experimental results indicate the concentration of 10% in the $NH_3 \cdot H_2O$ eluent provides the optimum enrichment conditions.

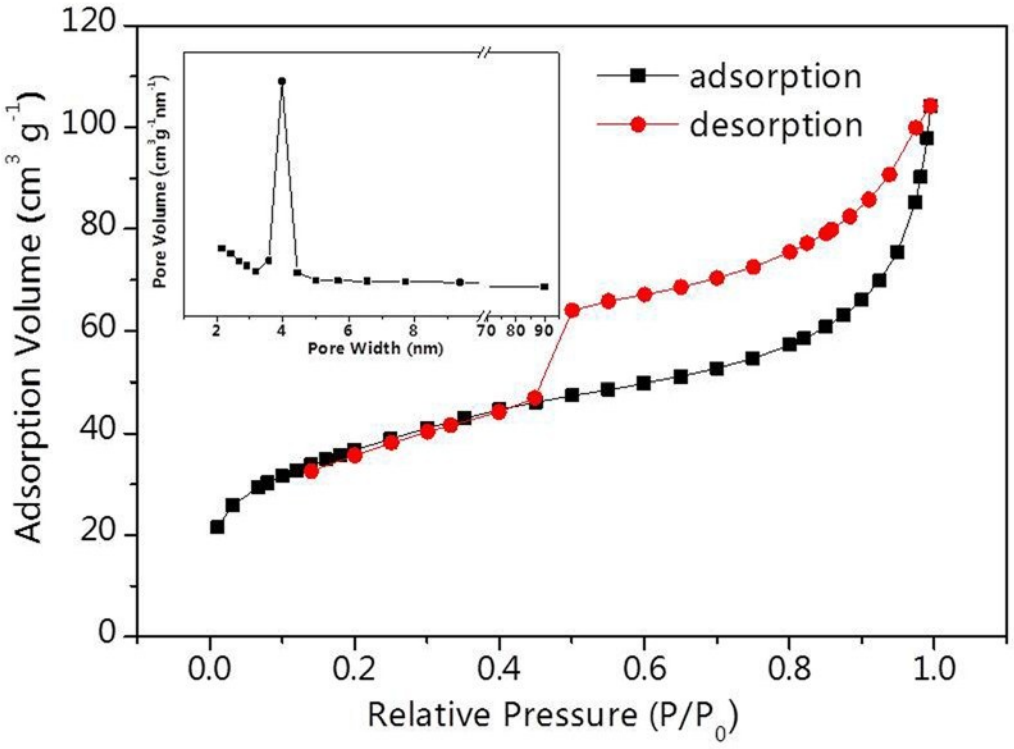

Fig. S3 N_2 adsorption-desorption isotherm and pore size distribution (inset) of CeO_2//SiO_2-C8 Janus fibers.

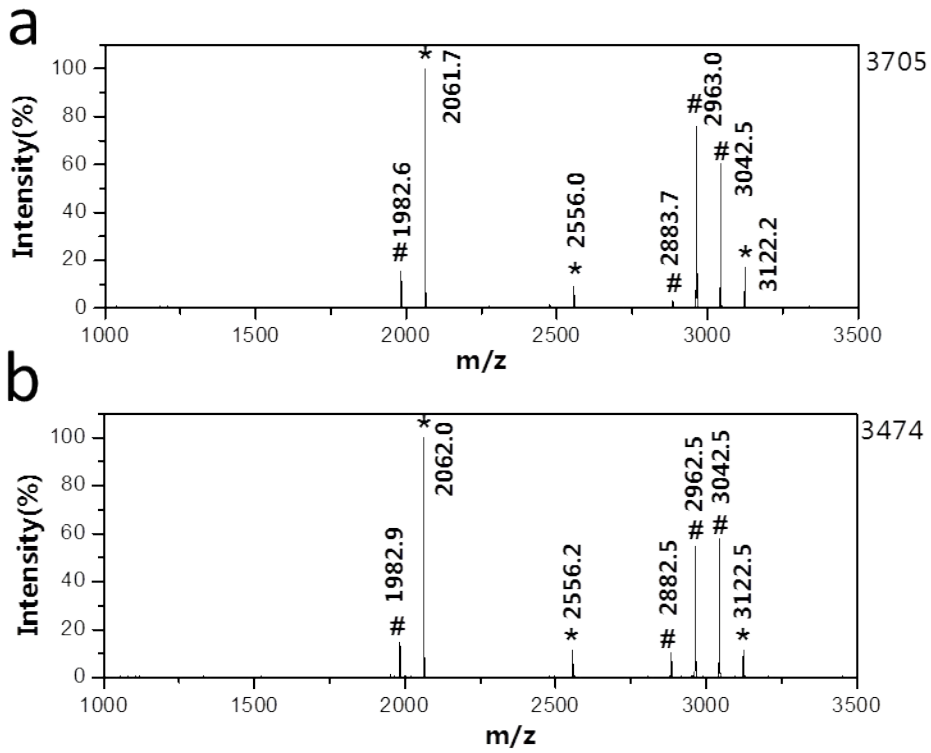

Fig. S4 MALDI-TOF mass spectra of phosphopeptides enriched from β-casein (100 nM) using CeO_2//SiO_2 fibers (a) and the CeO_2//SiO_2-C8 probe (b). '*' Indicates phosphopeptides, '#' indicates their dephosphorylated counterparts. The number in the top right corner is the highest peak intensity.

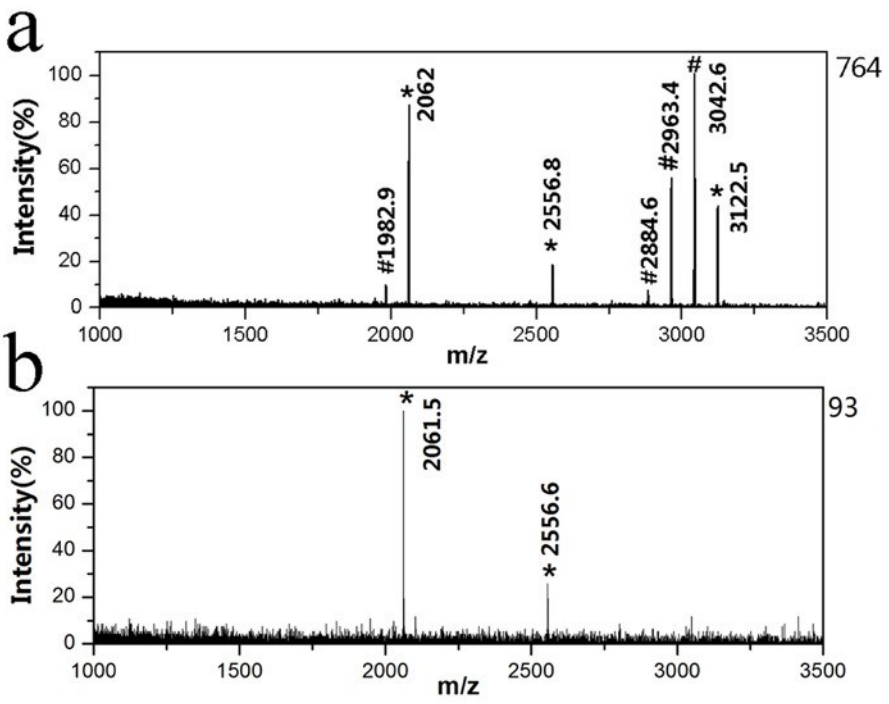

Fig. S5 MALDI-TOF mass spectra of phosphopeptides enriched from β-casein with different concentrations: 5 nM (a) and 1 nM (b) using the CeO_2//SiO_2-C8 probe. '*' Indicates phosphopeptides, '#' indicates their dephosphorylated counterparts. The number in the top right corner is the highest peak intensity.

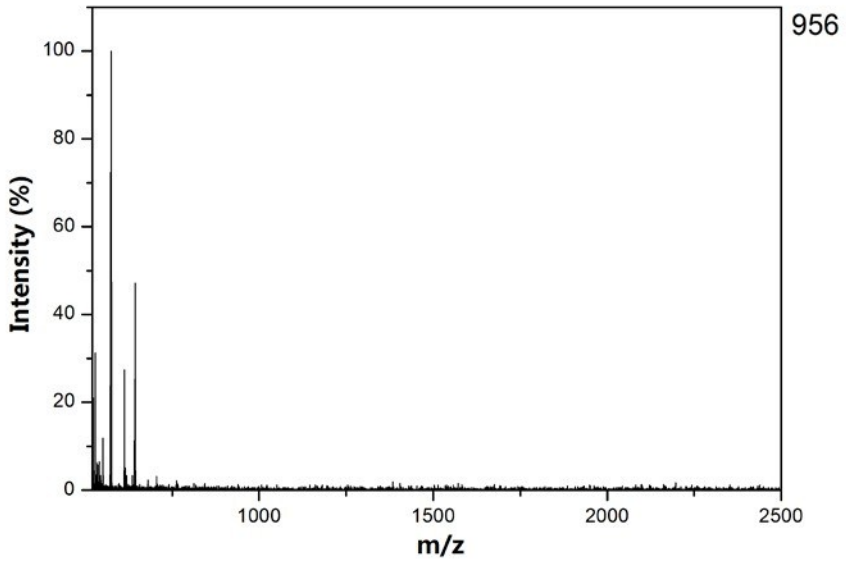

Fig. S6 MALDI-TOF mass spectrum of direct analysis of the tryptic digest mixture of β-casein and BSA with the molar ratio of 1:1, the concentration of β-casein was 10 nM. The number in the top right corner is the highest peak intensity.

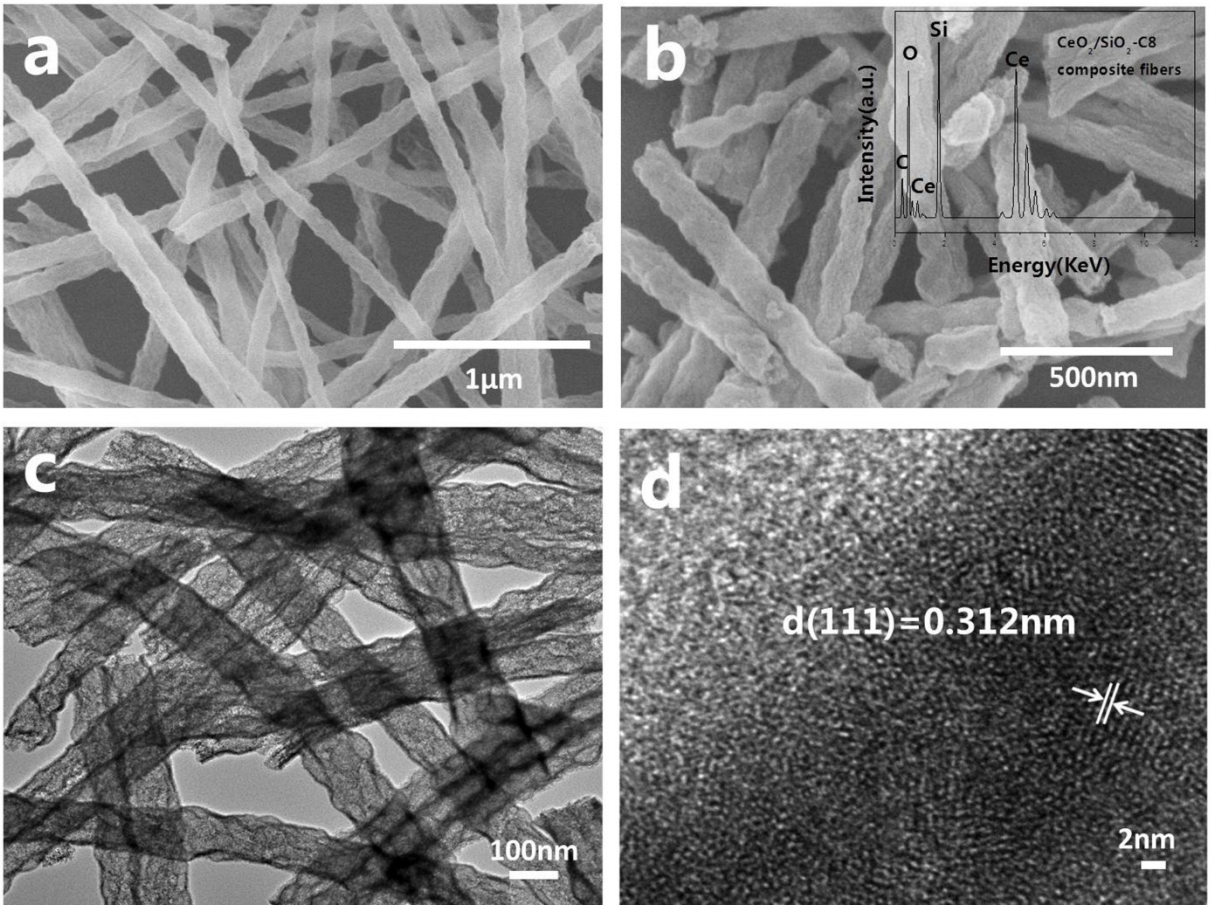

Fig. S7 SEM (a), TEM (c) and high resolution HRTEM (d) images of the CeO_2/SiO_2 composite fibers, SEM (b) image of and EDS spectrum (inset) of CeO_2/SiO_2-C8 composite fibers.

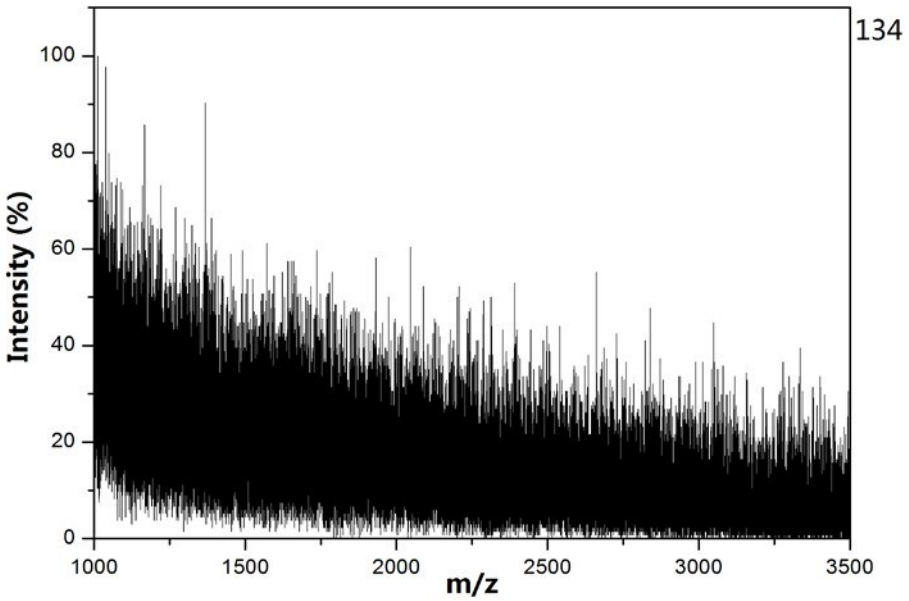

Fig. S8 MALDI-TOF mass spectrum of the diluted human serum solution without treatment. The number in the top right corner is the highest peak intensity.

Table S1 The search results of the tryptic digest of BSA before and after enrichment by the CeO_2//SiO_2-C8 probe.

Start-End	Calculated (m/z)	Peptide sequences	Before enrichment	After enrichment
24 – 28	655.273	R.RDTHK.S	√	√
29 – 34	712.451	K.SEIAHR.F		√
35 – 44	1249.836	R.FKDLGEEHFK.G		√
37 – 44	974.644	K.DLGEEHFK.G		√
66 – 75	1163.834	K.LVNELTEFAK.T		√
118 – 122	658.2510	K.QEPER.N	√	√
152 – 156	589.3070	K.ADEKK.F	√	√
156 – 160	665.399	K.KFWGK.Y		√
156 – 160	537.192	K.FWGK.Y	√	√
161–167	927.7	K.YLYEIAR.R		√
198 – 204	700.26	K.GACLLPK.I	√	√
219 – 222	572.249	R.QRLR.C		√
233 – 241	1001.617	R.ALKAWSVAR.L		√
236 – 241	689.412	K.AWSVAR.L		√
242 – 248	846.493	R.LSQKFPK.A		√
257 – 263	788.461	K.LVTDLTK.V		√
347 – 359	1567.962	K.DAFLGSFLYEYSR.R		√
360 – 371	1440.047	R.RHPEYAVSVLLR.L		√
402 – 412	1305.937	K.HLVDEPQNLIK.Q		√
421 – 433	1480.013	K.LGEYGFQNALIVR.Y		√
437 – 451	1640.176	R.KVPQVSTPTLVEVSR.S		√
548 – 557	1142.878	K.KQTALVELLK.H		√
549 – 557	1014.768	K.QTALVELLK.H		√
Peptides matched			5	23
Sequence coverage (%)			4	26

Table S2 The phosphopeptides identified by the CeO_2//SiO_2-C8 probe from tryptic digest of β-casein.

Start-End	Peptide sequences	Observed (m/z)	Theoretical (m/z)	Labeling signals	Phosphorylation site
33-48	FQ[pS]EEQQQTEDELQDK	2061.7	2061.8	1982.8	1
33-52	FQ[pS]EEQQQTEDELQDKIHPF	2555.9	2556.0	2476.8	1
1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3122.1	3122.2	3042.2/2962.6/2883.2	4

[pS], phosphorylated site