Electronic Supporting Information

Soft-template Synthesis of Hydrophilic Metallic Zirconia Nanoparticles-Incorporated Ordered Mesoporous Carbon Composites and its Application in Phosphopeptides Enrichment

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Effect of incubation condition on the enrichment of phosphopeptides

In order to improve the enrichment efficiency of phosphopeptides, different incubation conditions were studied. Six different loading buffers were used to enrichment the tryptic digests of β-casein. The three MS peaks of phosphopeptides (2061.72 m/z, 2555.96 m/z, 3122.09 m/z) were used to evaluate the enrichment results. As shown in Fig. S3 ESI † and Table S1 ESI †, the MS signal intensities of three MS peaks were gradually increased with the enlargement of the ratio of acetonitrile. When the content of TFA was increased, the intensities were reduced. So, the best load buffer condition was ACN-H_2O-TFA (90 : 5: 5, v/v/v).

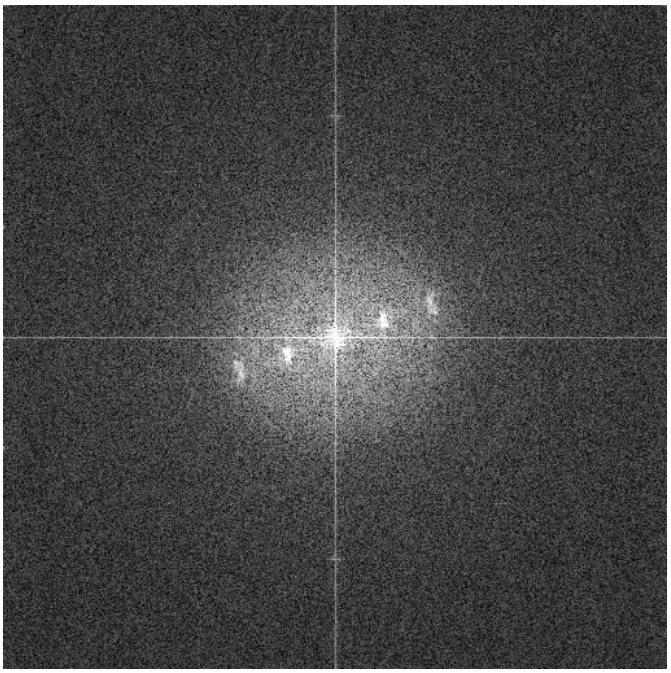

Figure S1. Selected Area Electron Diffraction of TEM of zirconia/OMC composites

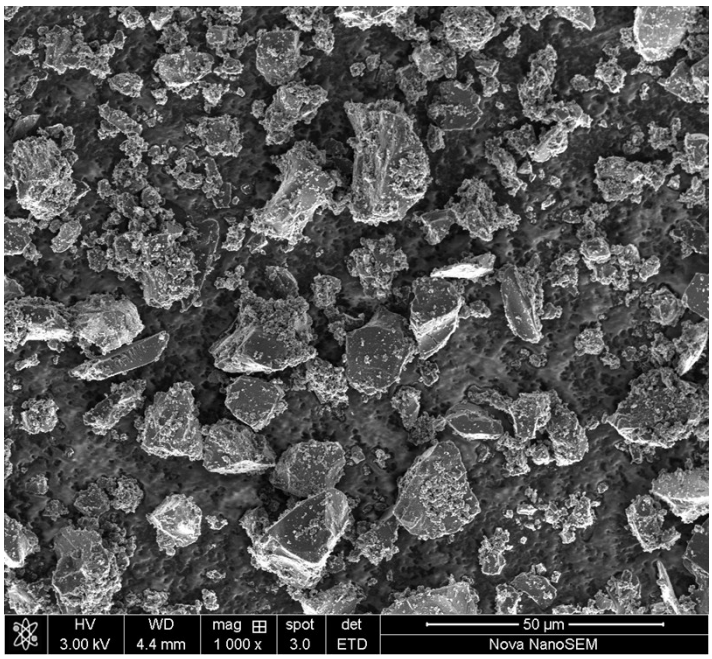

Figure S2. Ultrahigh Resolution Field Emission Scanning Electron Microscopy (UHRFESEM) of zirconia/OMC composites

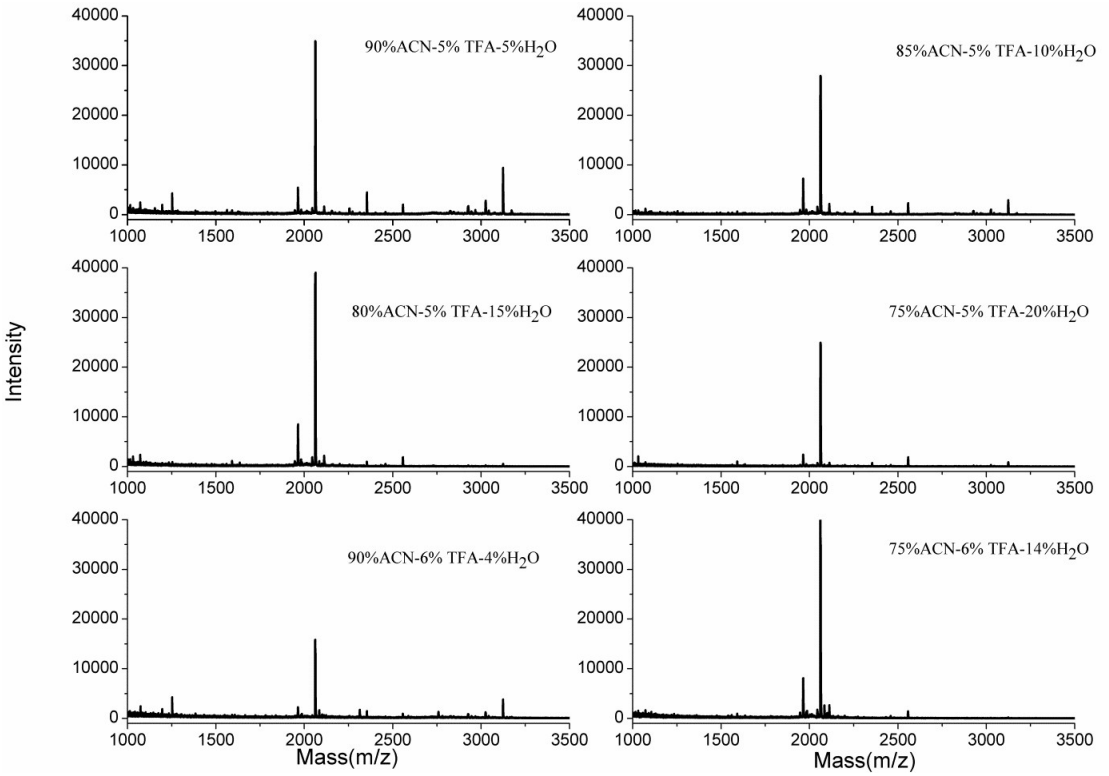

Figure S3. The effect of incubation condition on the enrichment of phosphopeptides

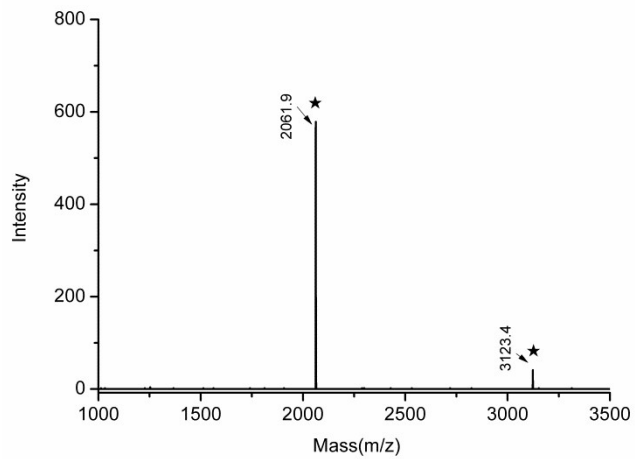

Figure S4. The fourth enrichment of phosphopeptides by zirconia/OMC composites which were stored in water about six months

Table S1. The effect of incubation condition on three peaks of the enrichment of phosphopeptides

Incubation condition	Peak of 2061.72 m/z	Peak of 2555.96 m/z	Peak of 3122.09 m/z
90 % ACN-5 % TFA-5 % H_2O	35112	2077	9380
85 % ACN-5 % TFA-10 % H_2O	27996	3202	2929
80 % ACN-5 % TFA-15 % H_2O	38971	1884	594
75 % ACN-5 % TFA-20 % H_2O	25033	1868	818
90 % ACN-6 % TFA-4 % H_2O	12587	967	3821
75 % ACN-6 % TFA-14 % H_2O	4008	1394	2128

Table S2. Phosphopeptides identified in tryptic digests of β-casein after enriched by metallic zirconia incorporated Ordered Mesoporous Carbon

No.	Protein	Peptide sequence	Number of phosphoryl groups	Observed m/z
1	β-casein	FQ[pS]EEQQQTEDELQDK	1	2061.72
2	β-casein	FQ[pS]EEQQQTEDELQDKIHPF	1	2555.96
3	β-casein	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.09
4	β-casein[a]	IEKFQ[pS]EEQQQTEDELQDK	1	2353.39
5	α-casein	TVD[Mo]ME[pS]TEVF	1	1252.57

β-casein[a] : dephosphopeptide of m/z : 2432.05

Table S3. Phosphopeptides identified in tryptic digests of proteins extracted from nonfat milk after enriched by metallic zirconia incorporated Ordered Mesoporous Carbon

No.	Protein	Peptide sequence	Number of phosphoryl groups	Observed m/z
1	α-casein	TVD[Mo]ME[pS]TEVF	1	1252.57
2	β-casein	FQSEEQQQTEDELQDKIHPF	1	1277.57
3	α-casein	TVD[Mo]E[pS]TEVFTK	1	1482.53
4	α-casein	EQL[pS]T[pS]EENSKK	2	1539.49
5	β-casein	RELEELNVPGEIVESLSSSEESITR	1	1561.20
6	α-casein	VPQLEIVPN[pS]AEER	1	1660.71
7	α-casein	YLGEYLIVPN [pS]AEER	1	1831.56
8	α-casein	DIG[pS]E[pS]TEDQAMEDIK	2	1927.59
9	α-casein	DIG[pS]E[pS]TEDQA[Mo]EDIK	2	1943.58
10	β-casein	FQ[pS]EEQQQTEDELQDK	1	2061.72
11	α-casein	Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK	5	2703.83
12	α-casein	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5	2720.76
13	α-casein	ELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	2966.00
14	α-casein	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4	3007.74
15	β-casein	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.04

Table S4. Identified phosphopeptides from nonfat milk enriched by Zirconia/OMC or other materials cited from the literatures using MAIDI-TOF MS

Materials	Detection limit	Selectivity (ratios of β-casein and BSA)	Phosopho peptides identified	Ref
Zirconia/OMC	1.5 fmol	1:300	15	This work
ZrO_2-MSN	2.5 fmol	1:100	16	1
ZrO_2-NP	-	-	9	1
α-ZrO_2-NP	2 fmol	-	13	2
SiO_2- ZrO_2	-	-	14	3

[1] X. L. Zhang, F. Wang, Y. Xia, *J. Chromatogr. A.*, 2013, **1306**, 20-26.
[2] S. K. Kailasa, H. F. Wu, *Anal. Bioanal. Chem.*, 2010, **396**, 1115-1125.
[3] H. H. Wan, J. Y. Yan, L. Yu, Q. Y. Sheng, X. L. Zhang, X. Y. Xue, X. L. Li, X. M. Liang, *Analyst*, 2011, **136**, 4422-4430.