

Supplementary material

Ti⁴⁺-immobilized chitosan coated magnetic graphene oxide for highly selective enrichment of phosphopeptides

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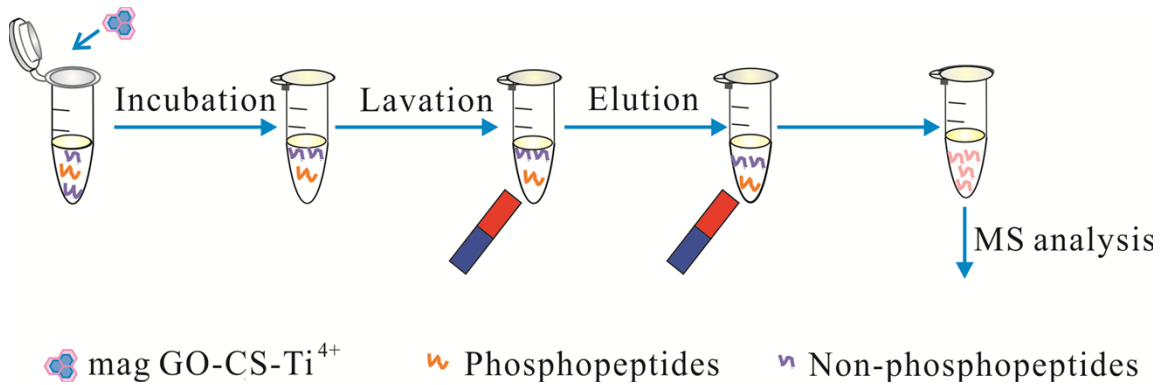
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Supplementary material



Scheme S1. Schematic illustration of the workflow of phosphopeptides enrichment from a biological sample by using mag GO-CS-Ti⁴⁺.

Table S1. Detailed information for the analysed phosphopeptides obtained from tryptic digests of β -casein after enrichment by mag GO-CS-Ti⁴⁺ in MALDI-TOF MS analysis.

No.	Observed m/z	Peptides sequence	Number of phosphorylation
β 1	2061.32	FQ[pS]EEQQQTEDELQDK	1
β 2	2556.09	FQ[pS]EEQQQTEDELQDKIHFP	1
β 3	3122.27	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4

[pS], phosphorylated site.

Table S2. Detailed information for the observed phosphopeptides obtained from tryptic digests of α -casein after enrichment by mag GO-CS-Ti⁴⁺ in MALDI-TOF MS analysis.

No.	Observed m/z	Peptides sequence	Number of phosphorylation
α 1	1237.07	TVDME[pS]TEVF	1
α 2	1337.28	HIQKEDV[pS]ER	1
α 3	1466.17	TVDME[pS]TEVFIK	1
α 4	1482.17	TVD[Mo]E[pS]TEVFTK ^b	1
α 5	1660.57	VPQLEIVPN[pS]AEER	1
α 6	1847.5	DIGSE[pS]TEDQAMEDIK	1

$\alpha 7$	1927.28	DIG[pS]E[pS]TEDQAMEDIK	2
$\alpha 8$	1943.45	DIG[pS]E[pS]TEDQA[Mo]EDIKa	2
$\alpha 9$	1951.72	YKVPQLEIVPN[pS]AEER	1
$\alpha 10$	2061.61	FQ[pS]EEQQQTEDELQDK	1
$\alpha 11$	2618.69	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4
$\alpha 12$	2677.47	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3
$\alpha 13$	2703.71	Q*MEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQ ^b	5
$\alpha 14$	2720.69	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5
$\alpha 15$	2934.98	KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ	3
$\alpha 16$	3007.85	NANEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4

[pS], phosphorylated site.

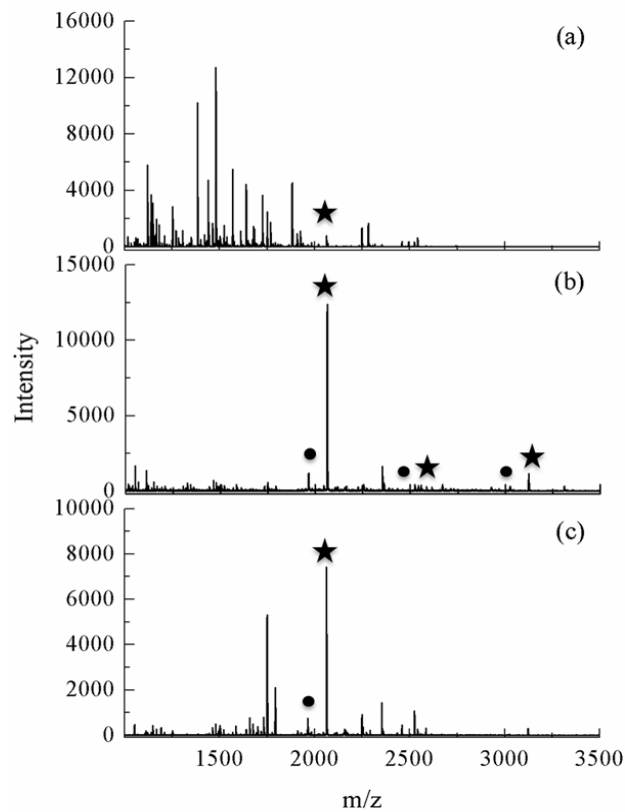


Figure S1. MALDI-TOF mass spectra of the tryptic digest mixture of β -casein (0.5 pmol) and BSA. (a) Direct analysis of the peptide mixture at a molar ratio of 1: 100; after enrichment by mag GO-CS-Ti⁴⁺ at molar ratios of (b) 1: 100, (c) 1: 400. ★ indicates phosphopeptides and ● indicates dephosphorylated peptides.

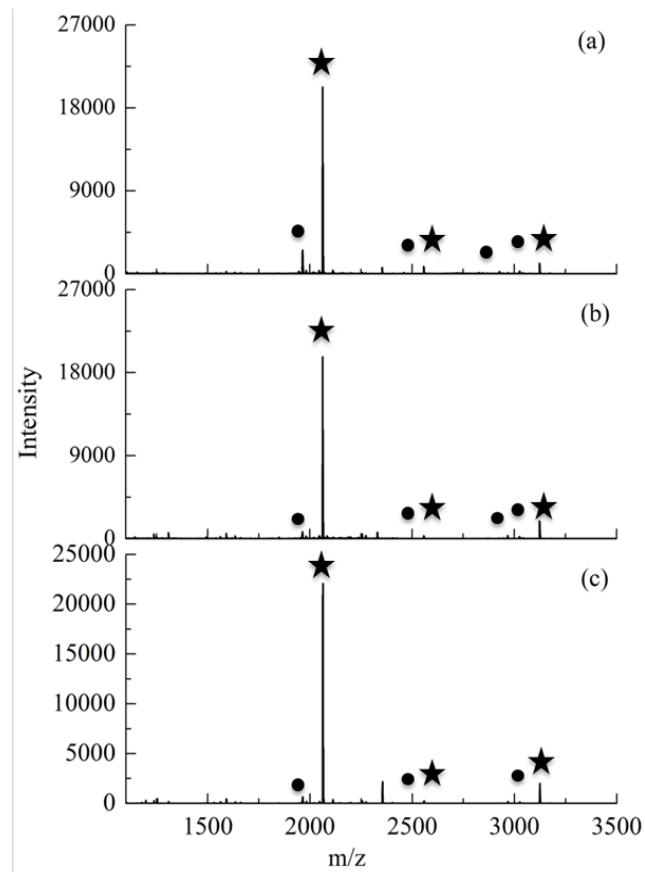


Figure S2. MALDI-TOF mass spectra of phosphopeptides enriched from β -casein digest (0.5 pmol) using mag GO-CS-Ti⁴⁺, (a) for the first time, (b) third time and c) for the sixth time. ★ indicates phosphopeptides and ● indicates dephosphorylated peptides.

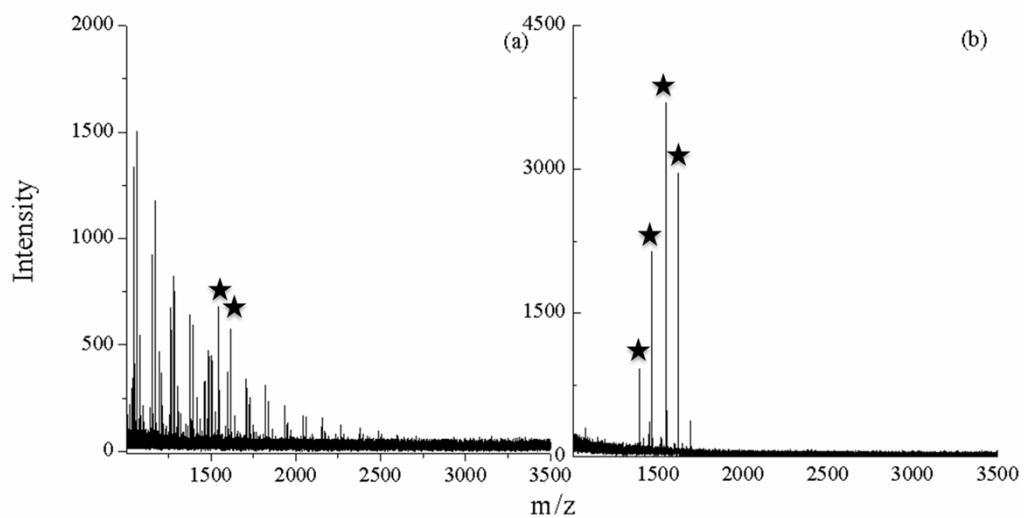


Figure S3. (a) and (b) MALDI-TOF spectrum of serum from an endometrial cancer pepople before and after enrichment by mag GO-CS-Ti⁴⁺, respectively. ★ indicates phosphorylated peptides.

Table S3. List of intensities of the selected phosphopeptide peak in β -casein captured by three batches of mag GO-CS-Ti⁴⁺.

Selected Peak	Intensity			RSD(%)
β -casein m/z 2061.32	19938	21548	19709	4.91

Table S4. List of phosphopeptides from tryptic digests of proteins extracted from nonfat milk after enrichment by mag GO-CS-Ti⁴⁺ in MALDI-TOF MS analysis.

No.	Observed m/z	Peptides sequence	Number of phosphorylation
1	1466.24	TVDME[pS]TEVFIK	1
2	1563.71	TVD[Mo]E[pS]TEVFTK ^b	1
3	1661.39	VPQLEIVPN[pS]AEER	1
4	1854.21	YLGEYLIVPN [pS]AEER	1
5	1927.28	DIG[pS]E[pS]TEDQAMEDIK	2
6	1952.01	YKVPQLEIVPN[pS]AEER	1
7	2062.61	FQ[pS]EEQQQTEDELQDK	1
8	2080.37	KKYKVPQLEIVPN[pS]AEERL	1
9	2555.65	FQ[pS]EEQQQTEDELQDKIHPF	1
10	2720.69	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5
11	2965.46	ELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4
12	3026.33	NANEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4
13	3122.98	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4

[pS], phosphorylated site.

Table S5. List of phosphopeptides enriched by mag GO-CS-Ti⁴⁺ from human serum.

No.	Observed m/z	Peptides sequence	Number of phosphorylation
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1	1389.33	D[pS]GEGDFLAEGGGV	1
2	1460.35	AD[pS]GEGDFLAEGGGV	1
3	1545.43	D[pS]GEGDFLAEGGGVR	1
4	1616.45	AD[pS]GEGDFLAEGGGVR	1

[pS], **phosphorylated site.**