Fabrication of Reusable Bifunctional Biomimetic Ti4+-Phosphorylated Cellulose Monolith with Coral-Like Structure for Enrichment of Phosphorylated and Glycosylated Peptides

Luwei Zhang[a], Yan Wang[a], Lei Pan[b], Wenrui Wu[b], Taka-Aki Asoh[a,*], Junjie Ou[b],

Hiroshi Uyama[a,*]

[a] Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita 565-0871, Japan

[b] CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China

[*] To whom correspondence should be addressed:

Prof. Taka-Aki Asoh

Tel: +81-6-6879-7365

Fax: +81-6-6879-7367

E-mail: asoh@chem.eng.osaka-u.ac.jp

Prof. Hiroshi Uyama

Tel: +81-6-6879-7364

Fax: +81-6-6879-7367

E-mail: uyama@chem.eng.osaka-u.ac.jp

Mass spectrometry analysis

MALDI-TOF MS experiments were carried out in positive ion mode on a Autoflex III (Bruker, Germany). The sample solution (0.5 μL) and 0.5 μL DHB solution (25 mg·mL^{-1}, ACN/H$_2$O/H$_3$PO$_4$, 70/29/1, v/v/v) were deposited in sequence on the MALDI plate for MS analysis.

The complete MS scan range was from m/z 400 to 2,000 and was obtained by Orbitrap at 70,000 resolutions in the mass spectrometer. The 20 strongest multi-charged ions were fragmented by higher energy collision dissociation. The normalized HCD collision energy was set to 27%, and the spray voltage was set to 2 kV. The temperature of the heated capillary was set at 250 °C. All MS and MS/MS spectra were collected in the data-dependent acquisition mode by Xcalibur software.

Data search of phosphopeptides and glycopeptides

The MS/MS spectra obtained were searched on the UniProt human database using the Mascot Daemon protein identification platform. The human database was downloaded from the website http://www.uniprot.org/. The obtained data were processed using ArMone v 2.0. The parent ion mass tolerance was set to 10 ppm and the fragment mass deviation was set to 0.05 Da. The enzyme specificity was selected as trypsin or elastase, and up to two missed digestion sites were allowed. The false discovery rate (FDR) was set at < 1%.
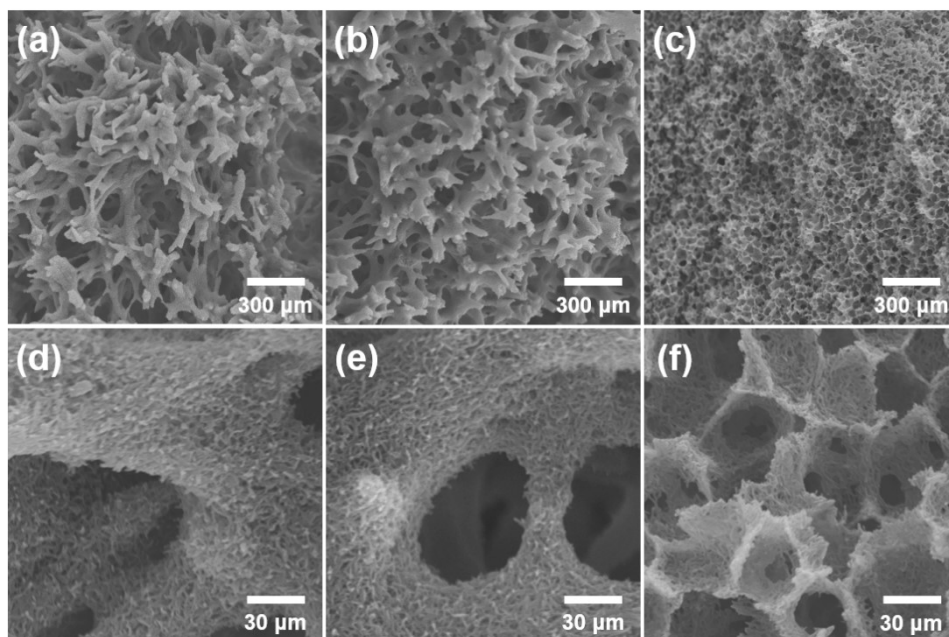
Figure S1. SEM images of (a, d) CM-1, (b, e) CM-2 and (c, f) CM-3 (at 500 ×, 5000 ×, 500 ×, 5000 ×, 500 × and 5000 × magnification, respectively).
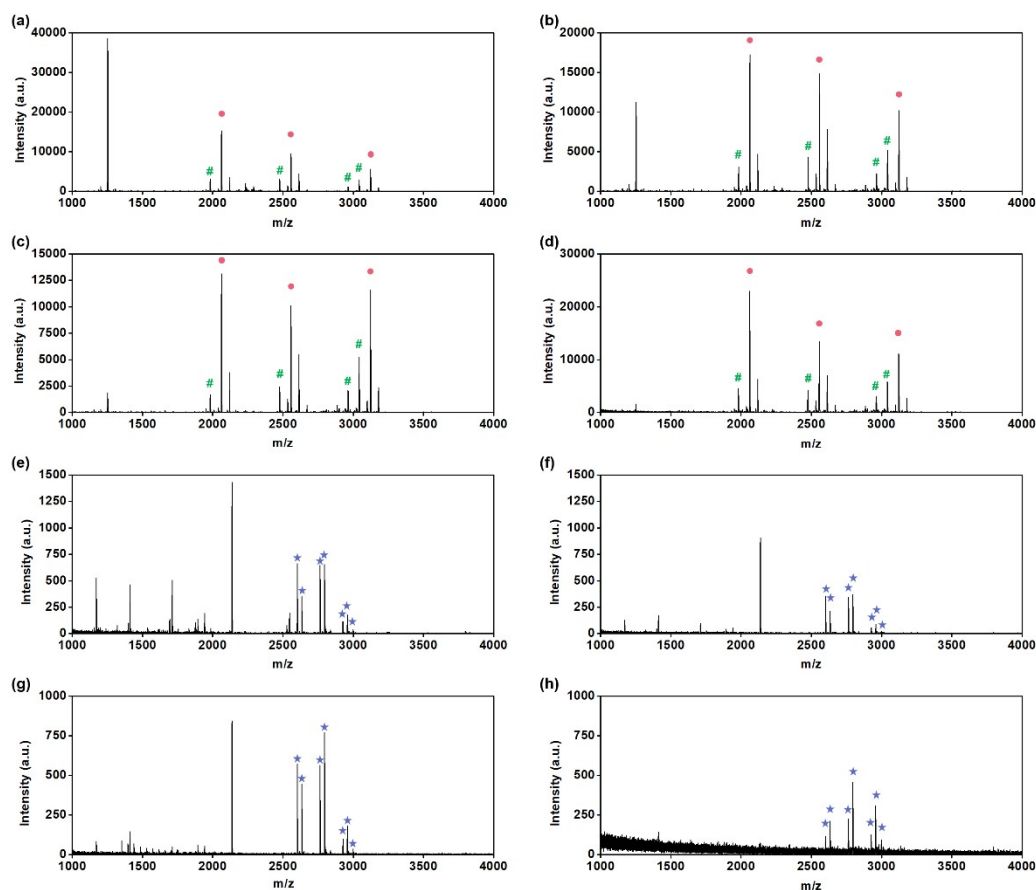
Figure S2. MALDI-TOF mass spectra of phosphopeptides from β-casein digest after enrichment by TiPCM with different ACN content. (a) 83% ACN + 3% TFA, (b) 81% ACN + 3% TFA, (c) 79% ACN + 3% TFA and (d) 77% ACN + 3% TFA. And MALDI-TOF mass spectra of glycopeptides from IgG digest after enrichment by TiPCM with different ACN content. (e) 83% ACN + 3% TFA, (f) 81% ACN + 3% TFA, (g) 79% ACN + 3% TFA and (h) 77% ACN + 3% TFA. Phosphopeptide peaks identified are marked with the symbol ●, dephosphorylated peptide peaks identified are marked with the symbol #, and glycopeptide peaks identified are marked with the symbol ★.
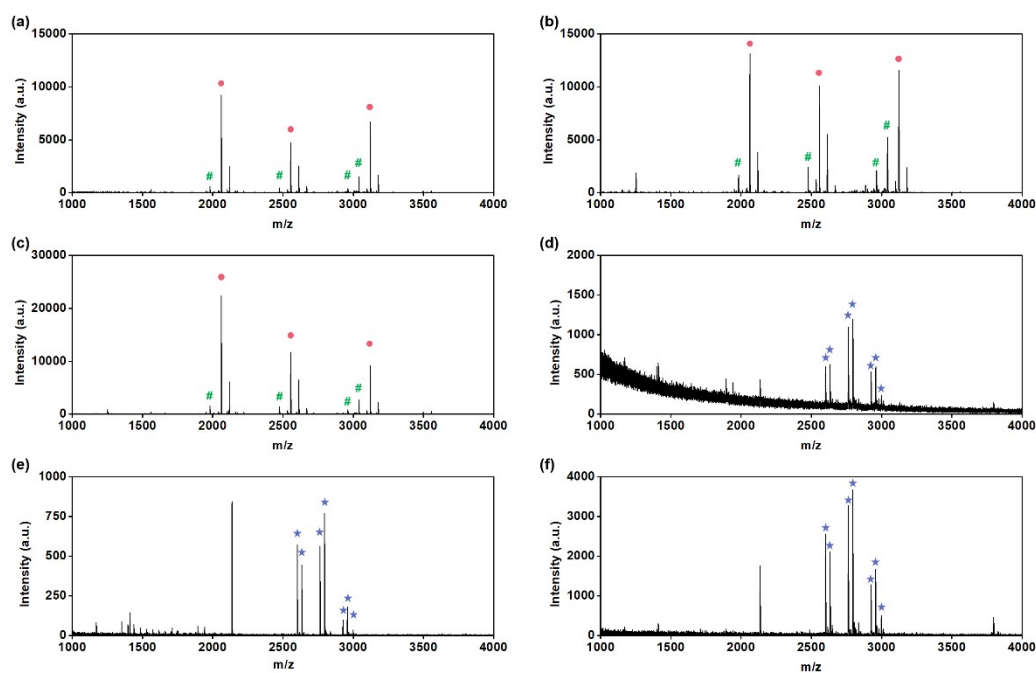
Figure S3. MALDI-TOF mass spectra of phosphopeptides from β-casein digest after enrichment by TiPCM with different TFA content. (a) 79% ACN + 1% TFA, (b) 79% ACN + 3% TFA and (c) 79% ACN + 5% TFA. And MALDI-TOF mass spectra of glycopeptides from IgG digest after enrichment by TiPCM with different TFA content. (d) 79% ACN + 1% TFA, (e) 79% ACN + 3% TFA and (f) 79% ACN + 5% TFA. Phosphopeptide peaks identified are marked with the symbol ●, dephosphorylated peptide peaks identified are marked with the symbol #, and glycopeptide peaks identified are marked with the symbol ★.
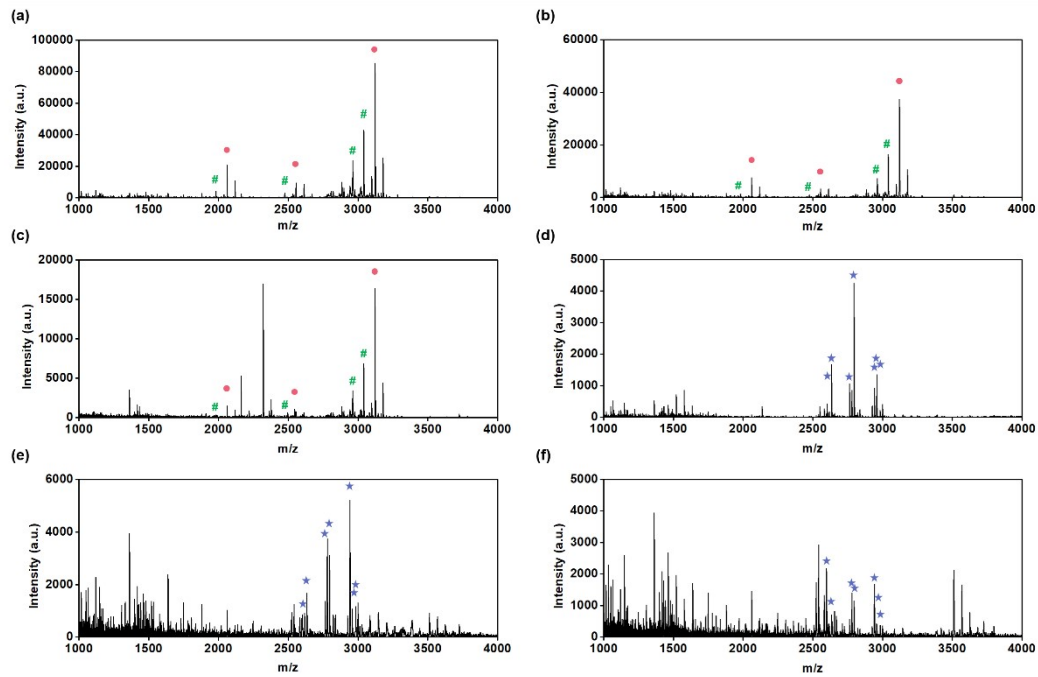
Figure S4. MALDI-TOF mass spectra of phosphopeptides from β-casein and BSA tryptic digest with molar ratios of (a) 1/500, (b) 1/1000 and (c) 1/5000 after enrichment by TiPCM. And MALDI-TOF mass spectra of glycopeptides from IgG and BSA tryptic digest with molar ratios of (d) 1/100, (e) 1/500 and (f) 1/1000 after enrichment by TiPCM. Phosphopeptide peaks identified are marked with the symbol ●, dephosphorylated peptide peaks identified are marked with the symbol #, and glycopeptide peaks identified are marked with the symbol ★.
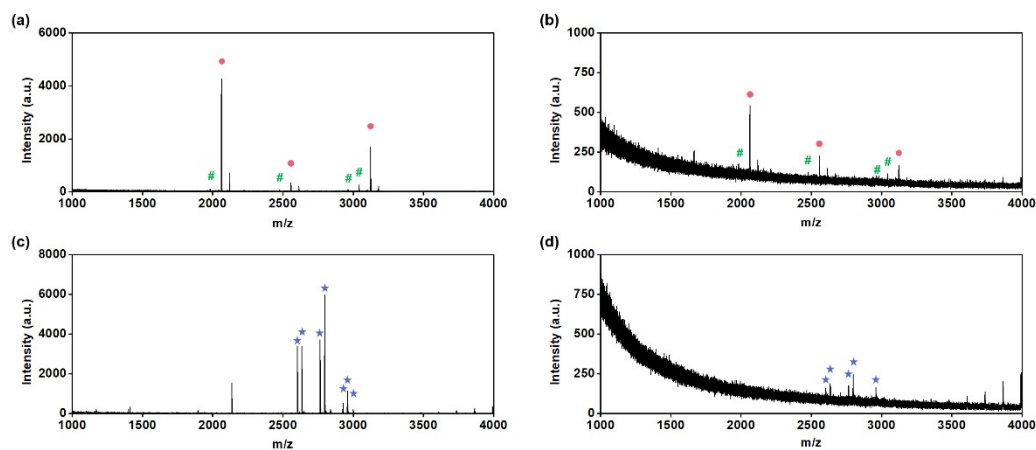
Figure S5. MALDI-TOF-MS spectra of (a) 50 and (b) 5 fmol of β-casein tryptic digest after enrichment by TiPCM. And MALDI-TOF-MS spectra of (c) 50 and (d) 5 fmol of IgG tryptic digest after enrichment by TiPCM. Phosphopeptide peaks identified are marked with the symbol ●, dephosphorylated peptide peaks identified are marked with the symbol #, and glycopeptide peaks identified are marked with the symbol ★.
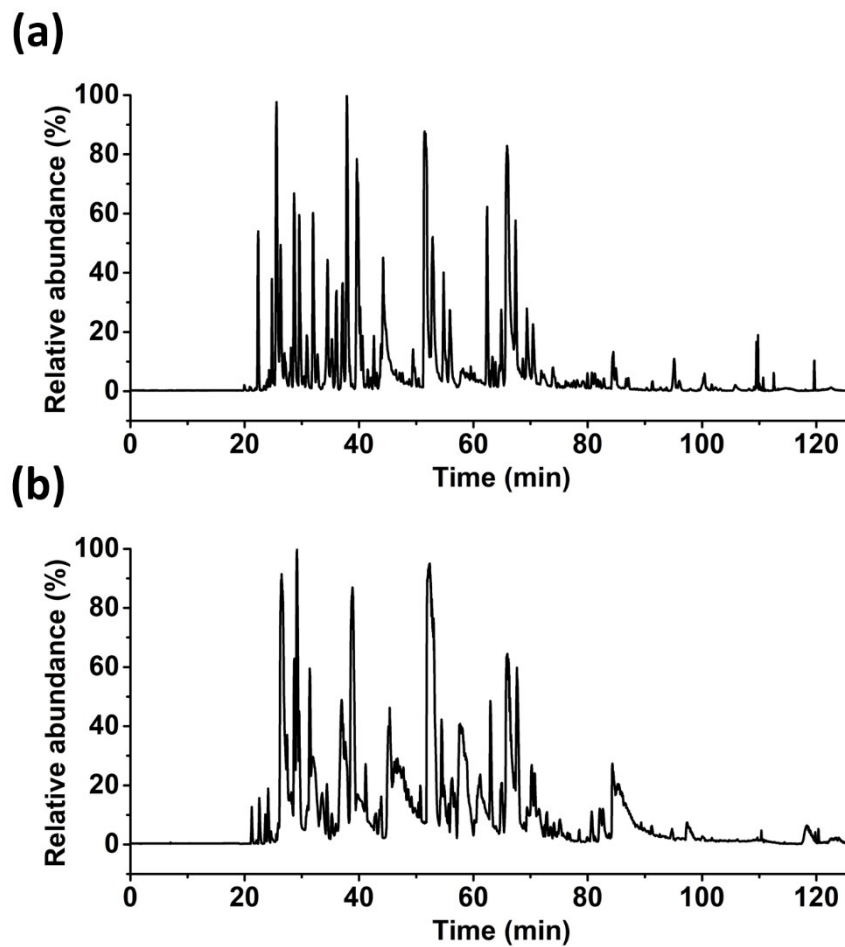
Figure S6. Chromatogram of tryptic digest of milk after enrichment with TiPCM by cLC-MS/MS, (a) glycopeptide and (b) phosphopeptides.

Table S1. Molecular masses and glycan composition of the glycopeptides from IgG tryptic digest after enrichment by TiPCM when 79% ACN + 3% TFA was used as loading solution.

No.	Observed m/z	Glycan composites	Peptide Sequence
1	2237.177	[Hex]2[HexNAc]3[Fuc]1	EEQFNSTFR
2	2268.528	[Hex]2[HexNAc]3[Fuc]1	EEQYNSTYR
3	2399.887	[Hex]3[HexNAc]3[Fuc]1	EEQFNSTFR
4	2431.148	[Hex]3[HexNAc]4	EEQYNSTFR
5	2456.623	[Hex]3[HexNAc]4	EEQFNSTFR
6	2489.21	[Hex]3[HexNAc]4	EEQYNSTYR
7	2561.447	[Hex]4[HexNAc]3[Fuc]1	EEQFNSTFR
8	2594.275	[Hex]4[HexNAc]3[Fuc]1	EEQYNSTYR
9	2602.008	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
10	2619.067	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
11	2634.022	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
12	2650.955	[Hex]4[HexNAc]4	EEQYNSTYR
13	2660.785	[Hex]3[HexNAc]5	EEQFNSTFR
14	2763.861	[Hex]4[HexNAc]4[Fuc]1	EEQFNSTFR
15	2781.085	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
16	2796.044	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
17	2806.939	[Hex]3[HexNAc]5[Fuc]1	EEQFNSTFR
18	2812.968	[Hex]5[HexNAc]4	EEQYNSTYR
19	2838.223	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
20	2853.347	[Hex]4[HexNAc]5	EEQYNSTYR
21	2925.811	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
22	2942.889	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
23	2958.013	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
24	2968.005	[Hex]4[HexNAc]5[Fuc]1	EEQFNSTFR
25	2982.18	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
26	2998.87	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR
27	3015.001	[Hex]5[HexNAc]5	EEQYNSTYR
28	3056.221	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFNSTFR
29	3084.293	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
30	3129.946	[Hex]5[HexNAc]5[Fuc]1	EEQFNSTFR
31	3218.684	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFNSTFR
32	3232.649	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTFR
33	3251.084	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTYR

Table S2. Molecular masses and peptides sequence from β-casein tryptic digest after enrichment by TiPCM when 79% CAN + 3% TFA was used as loading solution.

No.	Observed m/z	Peptide Sequence	Number of phosphoryl groups
1	2062.692	FQEEQQQTEDELQDK	1
2	2557.152	FQEEQQQTEDELQDKIHPF	1
3	3123.606	RELEELNVPGEIVELEESITR	4

Table S3. Molecular masses and glycan composition of the glycopeptides from IgG and β-casein tryptic digest after enrichment by TiPCM when 79% CAN + 3% TFA was used as loading solution.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2238.791	[Hex]2[HexNAc]3[Fuc]1	EEQFNSTFR
2	2269.57	[Hex]2[HexNAc]3[Fuc]1	EEQYNSTYR
3	2398.816	[Hex]3[HexNAc]3[Fuc]1	EEQFNSTFR
4	2431.881	[Hex]3[HexNAc]4	EEQYNSTFR
5	2456.753	[Hex]3[HexNAc]4	EEQFNSTFR
6	2488.774	[Hex]3[HexNAc]4	EEQYNSTYR
7	2533.538	[Hex]6[HexNAc]2	EEQFNSTFR
8	2601.81	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
9	2633.792	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
10	2649.78	[Hex]4[HexNAc]4	EEQYNSTYR
11	2779.932	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
12	2795.817	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
13	2805.872	[Hex]3[HexNAc]5[Fuc]1	EEQFNSTFR
14	2811.805	[Hex]5[HexNAc]4	EEQYNSTYR
15	2837.804	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
16	2853.019	[Hex]4[HexNAc]5	EEQYNSTYR
17	2925.875	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
18	2943.079	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
19	2957.904	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
20	2982.801	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
21	2998.894	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR

No.	Observed m/z	Glycan composites	Peptide Sequence
22	3015.098	[Hex]5[HexNAc]5	EEQYNSTYR
23	3056.772	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFNSTFR
24	3084.923	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
25	3160.875	[Hex]5[HexNAc]5[Fuc]1	EEQYNSTYR
26	3178.965	[Hex]6[HexNAc]3[Fuc]1[NeuAc]1	EEQFNSTFR
27	3217.738	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFNSTFR
28	3235.928	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTFR
29	3250.784	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTYR
30	3542.327	[Hex]5[HexNAc]4[Fuc]1[NeuAc]2	EEQYNSTYR

No.	Observed m/z	Peptide Sequence	Number of phosphoryl groups
1	2061.675	FQEEQQQTEDELQDK	1
2	2555.889	FQEEQQQTEDELQDKIHPF	1
3	3121.896	RELEELNVPGEIVELEESITR	4

Table S4. Molecular masses and glycan composition of the glycopeptides from IgG and BSA tryptic digest with the molar ratio of 1/100 after enrichment by TiPCM.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2432.634	[Hex]3[HexNAc]4	EEQYNSTFR
2	2448.804	[Hex]4[HexNAc]3	EEQYNSTYR
3	2488.808	[Hex]3[HexNAc]4	EEQYNSTYR
4	2537.991	[Hex]6[HexNAc]2	EEQFNSTFR
5	2594.917	[Hex]4[HexNAc]3[Fuc]1	EEQYNSTYR
6	2602.837	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
7	2619.853	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
8	2634.836	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
9	2657.119	[Hex]3[HexNAc]5	EEQFNSTFR
10	2780.908	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
11	2796.92	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
12	2806.825	[Hex]3[HexNAc]5[Fuc]1	EEQFNSTFR
13	2813.97	[Hex]5[HexNAc]4	EEQYNSTYR
14	2838.769	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
15	2855.066	[Hex]4[HexNAc]5	EEQYNSTYR
16	2927.033	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
17	2942.803	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
18	2959.017	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
19	2985.209	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
20	3000.047	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR

21	3015.17	[Hex]5[HexNAc]5	EEQYNSTYR
22	3131.071	[Hex]5[HexNAc]5[Fuc]1	EEQFNSTFR
23	3209.321	[Hex]6[HexNAc]3[Fuc]1[NeuAc]1	EEQYNSTYR
24	3251.053	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTYR
25	3263.99	[Hex]5[HexNAc]4[NeuAc]1	EEQYNSTYR
26	3269.442	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1[Na]1	EEQYNSTYR

Table S5. Molecular masses and glycan composition of the glycopeptides from IgG and BSA tryptic digest with the molar ratio of 1/500 after enrichment by TiPCM.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2399.287	[Hex]3[HexNAc]3[Fuc]1	EEQFNSTFR
2	2603.285	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
3	2619.336	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
4	2635.301	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
5	2765.327	[Hex]4[HexNAc]4[Fuc]1	EEQFNSTFR
6	2780.374	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
7	2796.365	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
8	2838.363	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
9	2854.796	[Hex]4[HexNAc]5	EEQYNSTYR
10	2925.258	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
11	2942.365	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
12	2959.383	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
13	2985.43	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
14	3001.276	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR
15	3015.53	[Hex]5[HexNAc]5	EEQYNSTYR
16	3130.24	[Hex]5[HexNAc]5[Fuc]1	EEQFNSTFR
17	3207.425	[Hex]6[HexNAc]3[Fuc]1[NeuAc]1	EEQYNSTYR
18	3249.587	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTYR
19	3266.319	[Hex]5[HexNAc]4[NeuAc]1	EEQYNSTYR
20	3441.854	[Hex]5[HexNAc]4[Fuc]1	TKPREEQYNSTYR

Table S6. Molecular masses and glycan composition of the glycopeptides from IgG and BSA tryptic digest with the molar ratio of 1/1000 after enrichment by TiPCM.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2399.244	[Hex]3[HexNAc]3[Fuc]1	EEQFNSTFR
2	2601.365	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
3	2616.113	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
4	2635.212	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR

No.	Observed *m/z*	Glycan composites	Peptide Sequence
5	2657.401	[Hex]3[HexNAc]5	EEQFNSTFR
6	2765.327	[Hex]4[HexNAc]4[Fuc]1	EEQFNSTFR
7	2780.365	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
8	2796.336	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
9	2853.347	[Hex]4[HexNAc]5	EEQYNSTYR
10	2927.293	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
11	2942.434	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
12	2959.288	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
13	2986.625	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
14	3000.365	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR
15	3013.416	[Hex]5[HexNAc]5	EEQYNSTYR
16	3084.39	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
17	3127.45	[Hex]5[HexNAc]5[Fuc]1	EEQFNSTFR

Table S7. Molecular masses and glycan composition of the glycopeptides from 50 fmol IgG tryptic digest after enrichment by TiPCM.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2234.083	[Hex]2[HexNAc]3[Fuc]1	EEQFNSTFR
2	2458.384	[Hex]3[HexNAc]4	EEQFNSTFR
3	2486.32	[Hex]3[HexNAc]4	EEQYNSTYR
4	2603.346	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
5	2620.48	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
6	2635.35	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
7	2652.385	[Hex]4[HexNAc]4	EEQYNSTYR
8	2765.476	[Hex]4[HexNAc]4[Fuc]1	EEQFNSTFR
9	2782.721	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
10	2797.472	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
11	2807.474	[Hex]3[HexNAc]5[Fuc]1	EEQFNSTFR
12	2813.273	[Hex]5[HexNAc]4	EEQYNSTYR
13	2838.57	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
14	2927.588	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
15	2941.759	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTYR
16	2959.597	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
17	2969.498	[Hex]4[HexNAc]5[Fuc]1	EEQFNSTFR
18	2984.301	[Hex]5[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
19	3000.638	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR
20	3015.843	[Hex]5[HexNAc]5	EEQYNSTYR
21	3055.624	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFNSTFR
22	3250.554	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYNSTYR
23	3270.944	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1[Na]1	EEQYNSTYR

Table S8. Molecular masses and glycan composition of the glycopeptides from 5 fmol

IgG tryptic digest after enrichment by TiPCM.

No.	Observed *m/z*	Glycan composites	Peptide Sequence
1	2487.63	[Hex]3[HexNAc]4	EEQYNSTYR
2	2536.184	[Hex]6[HexNAc]2	EEQFNSTFR
3	2604.421	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTFR
4	2618.912	[Hex]3[HexNAc]4[Fuc]1	EEQFNSTYR
5	2635.689	[Hex]3[HexNAc]4[Fuc]1	EEQYNSTYR
6	2658.112	[Hex]3[HexNAc]5	EEQFNSTFR
7	2781.704	[Hex]4[HexNAc]4[Fuc]1	EEQYNSTFR
8	2798.577	[Hex]4[HexNAc]4[Fuc]1[Na]1	EEQYNSTYR
9	2839.468	[Hex]3[HexNAc]5[Fuc]1	EEQYNSTYR
10	2855.206	[Hex]4[HexNAc]5	EEQYNSTYR
11	2927.488	[Hex]5[HexNAc]4[Fuc]1	EEQFNSTFR
12	2960.586	[Hex]5[HexNAc]4[Fuc]1	EEQYNSTYR
13	3001.297	[Hex]4[HexNAc]5[Fuc]1	EEQYNSTYR
14	3017.669	[Hex]5[HexNAc]5	EEQYNSTYR
15	3127.741	[Hex]5[HexNAc]5[Fuc]1	EEQFNSTFR

Table S9. Glycopeptides identified from milk tryptic digest after enrichment by

TiPCM.

Site	Protein	Modified Sequence
N120	F1N726	R.CQTSAPLWLN*GTHPGLGEGIVN*R.T
N132	F1N726	R.CQTSAPLWLN*GTHPGLGEGIVN*R.T
N63	F1N726	K.DLDCGAPGTPEAQLCFDPCQN*YTLLNEPFR.S
N257	F1N726	R.DGNWN*CSSLR.Q
N252	B9VPZ5	K.ADRDQYELLCLN*NSR.A
N564	B9VPZ5	K.N*DTVWENTNGESTADWAK.N
N93	B6V3I5	K.DDQNPHSSNICN*ISCDK.F
N231	F1MXX6	K.IFIGNVN*N*SGLK.I
N55	E1BHI7	R.LSPN*VSAK.G
N215	E1BHI7	K.N*VSCCIR.N
N83	G3MXZ0	K.IVGYLDEEGVLDQN*R.S
N229	G3MXZ0	K.KPSPCEFIN*TTAR.V
N320	G3MXZ0	K.WIPPYQGYN*NSVDPR.I
N468	P81265	K.N*VTAWLGEPLK.L
N95	P80195	R.N*ATLGSEETTEHTPSDASTTEGK.L
N321	P26201	K.N*CTLYGVLDIGK.C
N79	P26201	R.QFWIFDVQNPDEVTVN*SSK.I

N172	P26201	K.SSM#FQN*R.T
N417	P26201	K.HNYIVPILWLN*ETGTIGDEK.A
N30	E1BGW1	K.TN*TTESTAEDLK.T
N44	E1BGW1	K.TM#EN*QSVPLESK.A
N221	E1BGW1	K.IFPN*TSDPQEENR.N
N71	E1BGW1	K.ASN*FSFEDPSN*K.T
N79	E1BGW1	K.ASN*FSFEDPSN*K.T
N54	E1BGW1	K.AN*LTSDKENR.E
N444	G5E513	K.STGKPTLYN*VSLVLSDTASTCY.-
N48	G5E513	K.FNN*STVSSER.F
N47	G5E513	K.FN*NSTVSSER.F
N283	G5E513	R.HLN*DTFSAR.G
N307	F1N6D4	K.TFTN*VTER.N
N294	F1N6D4	K.SILNQ*IAMNDESVQN*K.S
N80	P17697	K.EDALN*DTR.D
N320	P17697	R.QQLN*ASLQLAEK.F
N346	P17697	K.MLN*TSALLK.Q
N68	P02702	K.NACCSVN*TSIEAHK.D
N160	P02702	K.GWN*WTSGYNQCPVK.A
N97	F1N514	K.IFIQDVN*CN*GTEDELIECDR.V
N269	F1N514	K.AGWN*LSAAK.V
N510	G3N2D8	R.LHNQLLPN*TTVLEK.G
N336	G3N2D8	K.FVN*VTEVVR.N
N362	E1BHY6	K.APAHLSLLDLGAVEGDVPCDN*VTSCPSSTTCCR.L
N260	E1BHY6	K.EN*ATDLLTK.L
N73	P11151	R.TPEDTAEDTCHLIPGVTESVANCHFN*HSSK.T
N318	A2I7N0	K.SNYELN*DTLSQMGIK.K
N230	A2I7N0	K.HTEQAEFHVSDN*K.T
N174	G3N0V0	R.EEQFN*STYR.V
N216	A0A140T8C6	R.TN*STFVEALVDHAK.E
N336	A0A140T8C6	K.LIDNN*RTEEEILHALDK.V
N101	A0A140T8C6	R.TCDWLPKPN*MSASCK.E
N221	A0A0A0MPA0	K.TPFNPN*HTYESEFHVSKN*ER.V
N344	G3X6M9	K.N*HTLEVSAPQK.D
N246	G3X6M9	R.GTAN*LSETIR.V
N499	Q29451	K.LN*ISICPLTQTAER.F
N369	Q29451	K.AN*LSWSVK.K
N78	B1H0W7	K.SVVAPAADGGLN*LTSTFLR.K
N51	B1H0W7	R.WFTSGLASN*SSWFLEK.K
N596	E1B748	K.EN*GTDTVQEEEEGPAEGSK.D
N515	E1B748	R.VFGSQN*LTTVK.L
N188	Q3SZV7	R.SWPAVGN*CSSAIR.W
N218	Q3SZV7	R.FDPVTGEVN*STYPR.D
N109	F1MUL0	K.SDAVLGN*YTCEVTELSR.E

N50	F1MUL0	K.N*ITELYVR.W
N642	F1MGR1	R.N*TTNVVSCCQGLLSSAVVK.G
N384	Q8WML4	R.ITNLQFN*SSLENPQTSYYQELQR.S
N177	F1MX65	R.YHSQTYGN*GSK.C
N71	Q3SYR8	R.EN*ISDPTSPM#R.T
N92	G3N342	R.VLHAETNSNLN*K.T
N103	A0A0A0MP89	K.FN*LTETPER.E
N1325	E1BH06	K.GLN*VTLSSLGR.S
N136	Q3SZR3	R.TFMLAASWN*GTK.N
N83	G3MZC1	K.LDN*YSTQELGK.T
N337	F1MPE1	R.N*ASSNVFFK.Q
N602	Q28065	K.N*VTCDFNPE.-
N271	A6QP79	K.VQSLQTLAAN*NSALAK.A
N677	G8JKZ1	R.VAPSESQ*N*CSFSLADKN*ITHGFLYPPAN*N*R.T
N686	G8JKZ1	R.VAPSESQ*N*CSFSLADKN*ITHGFLYPPAN*N*R.T
N698	G8JKZ1	R.VAPSESQ*N*CSFSLADKN*ITHGFLYPPAN*N*R.T
N100	A2I7M9	K.FN*LTEIQEK.E
N140	P09487	R.SQCN*TTQGNEVTSILR.W
N152	Q2KIU3	K.N*DTQILEK.E
N155	Q9MZ06	K.LVN*STLIR.I
N3763	F1MER7	R.SLTQGSLIVGSLAPVN*GTSQGK.F
N146	Q5E9E3	K.VITNQENVYQN*NTGR.F
N238	F1MGK8	K.N*FSCETQDLK.T
N60	F1MLR4	R.VN*GTDLAPDLLN*GSQLVLR.S
N70	F1MLR4	R.VN*GTDLAPDLLN*GSQLVLR.S
N98	F1MS32	R.ADGTVNQIEGEATPEN*ITEPAK.L
N253	A0A140T843	K.FGN*WSAQPSCK.A
N108	A0A140T8A8	R.SN*DTLYIPDWEGR.A
N72	A6QPK0	K.QCFLQQSN*ETLANFK.Q
N288	P50291	K.SEEPVCASDN*ATYASECAMK.E
N103	E1BJ49	R.APGN*DTFYSPGSSLDVTFR.S
N1394	F1MD77	R.IPAIN*QTIIEANEK.T
N219	F1N1G1	K.NYN*ISWELGNEPNSFQR.K
N122	P81187	R.AAYYN*LSDEISFR.C
N33	Q3ZBS7	R.CTEGFN*ATR.K
N96	Q2YDH0	K.SYCSDN*STAGDCK.V
N208	F1MMD7	K.LAEALTTSQN*K.T
N217	F1MX50	K.IVTPAEYYN*ATSQ.-
N1087	F1MRA6	K.N*GTQLQN*FTLDR.N
N1093	F1MRA6	K.N*GTQLQN*FTLDR.N
N514	G3X6N3	R.N*SSLCALCIGSEK.G
N114	F1N720	R.LSN*LSSSWFR.S
N90	P08037	R.GVAPPPPLQN*SSKPR.S
N398	F1MAV0	K.GTAGNALIEGASQLVGEN*R.T

N125	A7E3W2	K.DASVICTN*ETR.G
N182	Q2KJF1	R.AGN*YSCSYR.T
N199	A5D7E6	K.GFCEVPFGN*K.T
N211	F1N726	R.QDLN*VSDVHSLQPQLDCGDTEIK.V
N252	B9VPZ5	K.ADRDQYELLCLN*N*SR.A
N79	E1BGW1	K.ASNFSFEDPSN*K.T
N212	P80025	K.IVGYLDEEGVLDQN*R.S
N106	P80025	R.DTTLTN*VTDPSLDLTALSWEVGCGAPVPLVK.C
N358	P80025	K.KPSPCEFIN*TTAR.V
N449	P80025	K.WIPPYQGYN*NSVDPR.I
N47	G5E513	K.FN*N*STVSSER.F
N73	P11151	R.TPEDTAEDTCHLIPGVTESVAN*CHFN*HSSK.T
N101	A0A140T8C6	R.TCDWLPKPN*M#SASCK.E
N97	F1N514	K.IFIQDVNCN*GTEDELIECDR.V
N94	Q3SZR3	K.CVYN*CSFIK.I
N221	A0A0A0MP92	K.TPFNPN*HTYESEFHVSQNER.V
N386	A6QP79	K.HTDDLTSLN*NTLANIR.L
N677	G8JKZ1	R.VAPSESQN*CSFSLADK.N
N698	G8JKZ1	K.NITHGFLYPPANN*R.T
N152	Q3ZBX0	K.ESESGDQVITN*STQSK.F
N86	E1B6Z6	K.EDGSYN*VTSILLK.D
N234	F1MAU4	K.IVTTDFSQSGPN*CSESIR.R
N256	E1BNR9	R.EDN*PDKNPEAPLN*VSR.V
N503	A6QM01	R.SVYN*CSGEECR.G
N331	F1MJM4	R.NPPGLISAGN*ATGK.T
N138	F1N076	K.EHEGAIYPDN*TTDFQK.A
N225	F1MSZ6	R.LTINQWISN*K.T
N87	E1BJ75	R.N*CSAAPQPEPTAGLAR.Y
N195	F1MVJ8	R.N*MTLLVEQLETLDK.N
N307	F1MNJ2	R.QLQ*DLDCSSSDDEGAAQ*N*STKPSATK.G
N156	B0JYN6	K.LCPDCPLLAPLN*DSR.V
N282	A6QNL0	R.SLN*LSFAGLEQVPK.G
N184	Q2TBX4	R.N*STIQAANLAGLK.I
N130	B0JYM4	K.DN*QTASILDK.M
N87	A0A140T8C8	K.EGDCPFQSN*K.T
N564	B9VPZ5	K.N*DTVWEN*TNGESTADWAK.N
N97	P17697	K.ASQGVCN*ETMTALWEECKPCLK.Q
N346	P17697	K.M#LN*TSALLK.Q
N231	F1MXX6	K.IFIGNVN*NSGLK.I
N294	F1N6D4	K.SILNQIAMNDESVQN*K.S
N318	A2I7N0	K.SNYELN*DTLSQM#GIK.K
N87	A0JNP2	K.ILLN*CTVTDVK.A
N130	B0JYM4	K.NYPKDN*QTASILDK.M
N147	A0A140T891	K.SCN*ASDPQCPPDGR.Y

N493	E1BNX4	K.DVGGN*STGPDFSHPK.E
N221	A0A0A0MP92	K.TPFN*PN*HTYESEFHVSQN*ER.V
N118	F1MPE1	R.AQDEILFSN*STR.L
N792	A5D7C4	R.DAN*CSVM#R.F
N170	G3N2K4	K.LN*LTTDPK.F
N1348	F1MNT4	R.VN*ASTTSSN*STVEESALTR.D
N1355	F1MNT4	R.VN*ASTTSSN*STVEESALTR.D
N132	F1MJM4	R.FN*DTEVLQR.L
N48	Q3ZC83	R.LDMSQN*MSLGPAEVSK.D
N194	Q32KV6	K.FN*SSSSSLEEK.I
N307	F1MNJ2	R.QLQDLDCSSSDDEGAAQN*STKPSATK.G
N46	E1BPQ9	R.FN*CTISQGWK.L
N1093	F1MRA6	K.NGTQ*LQN*FTLDR.N
N109	E1BLA8	K.AVLVNN*ITTGER.L
N295	F1ML75	R.LN*ISAVESK.F
N86	F1MGC2	R.TFAYVAGICNTPN*VTCSKPGR.M
N225	F1N2B5	R.DVQNLTN*PSSFLAEWQN*ITK.S

Table S10. Phosphopeptides identified from milk tryptic digest after enrichment by TiPCM.

Protein	Peptide sequence	Variable modification
P02662	EDVPSER	Phospho (ST)
P02662	EKVNELSK	Phospho (ST)
P02662	VPQLEIVPNSAEER	Phospho (ST)
P02662	DIGSESTEDQAMEDIK	Phospho (ST)
P02662	DIGSESTEDQAMEDIK	Oxidation (M); Phospho (ST)
P02662	DIGSESTEDQAMEDIK	2 Phospho (ST)
P02662	DIGSESTEDQAMEDIK	Oxidation (M); 2 Phospho (ST)
P02662	YKVPQLEIVPNSAEER	Phospho (ST)
P02662	DIGSESTEDQAMEDIK	3 Phospho (ST)
P02662	DIGSESTEDQAMEDIK	Oxidation (M); 3 Phospho (ST)
P02662	QMEAESISSSEEIVPNSVEQK	2 Phospho (ST)
P02662	QMEAESISSSEEIVPNSVEQK	3 Phospho (ST)
P02662	QMEAESISSSEEIVPNSVEQK	4 Phospho (ST)
P02666	FQSEEQQQTEDELQDK	Phospho (ST)
P02666	FQSEEQQQTEDELQDK	2 Phospho (ST)
P02666	IEKFQSEEQQQTEDELQDK	Phospho (ST)
P02666	ELEELNVPGEIVESLSSSEESITR	Phospho (ST)
P02666	ELEELNVPGEIVESLSSSEESITR	2 Phospho (ST)

P02666	ELEELNVPGEIVESLSSSEESITR	3 Phospho (ST)
P02666	ELEELNVPGEIVESLSSSEESITR	4 Phospho (ST)
P02666	ELEELNVPGEIVESLSSSEESITR	5 Phospho (ST)
P02663	NMAINPSK	Phospho (ST)
P02663	NMAINPSK	Oxidation (M); Phospho (ST)
P02663	EQLSTSEENSK	Phospho (ST)
P02663	EQLSTSEENSK	2 Phospho (ST)
P02663	EQLSTSEENSKK	Phospho (ST)
P02663	TVDMESTEVFTK	Phospho (ST)
P02663	TVDMESTEVFTK	Oxidation (M); Phospho (ST)
P02663	EQLSTSEENSK	3 Phospho (ST)
P02663	EQLSTSEENSKK	2 Phospho (ST)
P02663	TVDMESTEVFTK	Oxidation (M); 2 Phospho (ST)
P02663	KTVDMESTEVFTK	Phospho (ST)
P02663	TVDMESTEVFTKK	Phospho (ST)
P02663	EQLSTSEENSKK	3 Phospho (ST)
P02663	NMAINPSKENLCSTFCK	Phospho (ST)
P02663	NTMEHVSSSEESIISQETYK	3 Phospho (ST)
P02663	NTMEHVSSSEESIISQETYK	3 Phospho (ST); Phospho (Y)
P02663	NTMEHVSSSEESIISQETYK	4 Phospho (ST)
P02663	NANEEEYSIGSSSEESAEVATEEVK	3 Phospho (ST)
P02663	NANEEEYSIGSSSEESAEVATEEVK	4 Phospho (ST)
P02663	NANEEEYSIGSSSEESAEVATEEVK	3 Phospho (ST); Phospho (Y)
P02663	NANEEEYSIGSSSEESAEVATEEVK	4 Phospho (ST); Phospho (Y)
P02663	NANEEEYSIGSSSEESAEVATEEVK	5 Phospho (ST)
P02663	NANEEEYSIGSSSEESAEVATEEVK	5 Phospho (ST); Phospho (Y)
F1MI46	QNTLPSK	Phospho (ST)
F1MI46	ETNSSELSK	Phospho (ST)
F1MI46	TSQLTDHSK	Phospho (ST)
F1MI46	HSNLIESQENSK	Phospho (ST)
F1MI46	ISHELDSASSEVN	Phospho (ST)
F1MI46	ISHELDSASSEVN	2 Phospho (ST)
F1MI46	ISHELDSASSEVN	3 Phospho (ST)
F1MI46	IRISHELDSASSEVN	3 Phospho (ST)
F1MI46	TSQLTDHSKETNSSELSK	3 Phospho (ST)
F1MI46	QTFLAPQNSVSSEETDDNK	3 Phospho (ST)
F1MI46	SNVQSPDATEEDFTSHIESEEMHDAPK	2 Phospho (ST)
P31098	QNTLPSK	Phospho (ST)
P31098	HSNLIESQENSK	Phospho (ST)
P31098	ISHELDSASSEVN	Phospho (ST)
P31098	ISHELDSASSEVN	2 Phospho (ST)
P31098	ISHELDSASSEVN	3 Phospho (ST)
P31098	IRISHELDSASSEVN	3 Phospho (ST)

P31098	QTFLAPQNSVSSEETDDNK	3 Phospho (ST)
Q58DM6	QNTLPSK	Phospho (ST)
Q58DM6	QTFLAPQNSVSSEETDDNK	3 Phospho (ST)
P80195	NLQISNEDLSK	Phospho (ST)
P80195	NLQISNEDLSK	2 Phospho (ST)
P80195	EPSISREDLISK	2 Phospho (ST)
P80195	EPSISREDLISK	3 Phospho (ST)
P80195	NLQISNEDLSKEPSISR	2 Phospho (ST)
P80195	NLQISNEDLSKEPSISR	3 Phospho (ST)
P80195	NLQISNEDLSKEPSISR	4 Phospho (ST)
P80195	NLQISNEDLSKEPSISREDLISK	3 Phospho (ST)
P80195	NLQISNEDLSKEPSISREDLISK	4 Phospho (ST)
P80195	NLQISNEDLSKEPSISREDLISK	5 Phospho (ST)
B0JYN6	CDSSPDSAEDVR	Phospho (ST)
E1BC95	HSGALDTK	Phospho (ST)
Q2TA12	AGDTILNSDHIGRMAVAAREGDAHPLGALP VGTLINNVESEPGR	Oxidation (M); Phospho (ST)
G3N1L4	DTPKGAGRAAPPISSSATTPR	2 Phospho (ST)
P02662	VNELSKDIGSESTEDQAMEDIK	2 Phospho (ST)
P02663	TVDMESTEVFTK	2 Phospho (ST)
P02663	TVDMESTEVFTKK	Oxidation (M); Phospho (ST)
P02663	KTVDMESTEVFTK	Oxidation (M); Phospho (ST)
P02663	NTMEHVSSSEESIISQETYK	Oxidation (M); 4 Phospho (ST)
P02663	NANEEEYSIGSSSEESAEVATEEVK	6 Phospho (ST)
F1MI46	TSQLTDHSKETNSSELSK	2 Phospho (ST)
F1MI46	SNVQSPDATEEDFTSHIESEEMHDAPK	Oxidation (M); 2 Phospho (ST)
P80025	TTLSSEAPTTQQLSEYFK	Phospho (ST)
E1BKG4	TVTAAGAENIQQKTDEK	3 Phospho (ST)
A5PJK3	SDSFYSSSASMYMPPPSADMGTYGMQTCG LLPSLAKR	6 Phospho (ST); 2 Phospho (Y)
Q0V8M0	KTCMYRSEQEELQDK	Phospho (ST); Phospho (Y)
Q0V8M0	KTCMYRSEQEELQDK	2 Phospho (ST); Phospho (Y)
Q0V8R5	QAGGSTVK	Phospho (ST)
E1BNK2	RGRSTVTK	Phospho (ST)
E1B9Z9	MPTSSHATRTTAGLTVPEVMASTPTPWATS RVTSQPADR	Oxidation (M); 10 Phospho (ST)
A6QPR4	FMTVGQVSARSSK	Oxidation (M); 2 Phospho (ST)
A1A4R9	SNSNAVSAIPINK	Phospho (ST)
F1MRQ2	SPSMPFLGEPLMEK	2 Oxidation (M); 2 Phospho (ST)
E1BQ34	GNSGSLPEGPGR	Phospho (ST)

F1MER1	KAQNGMENGDAGSEK	Oxidation (M); Phospho (ST)
Q0V8L2	LQRLPSSSSEMGSQDGSPLR	Oxidation (M); 5 Phospho (ST)
P02662	QMEAESISSSEEIVPNSVEQK	5 Phospho (ST)
P02662	QMEAESISSSEEIVPNSVEQK	Oxidation (M); 5 Phospho (ST)
P00760	DSCQGDSGGPVVCSGK	Phospho (ST)
P02663	NTMEHVSSSEESIISQETYK	5 Phospho (ST)
F1N0Q2	DKLNEISK	Phospho (ST)
F1MTE8	EKITSDENSNEK	Phospho (ST)
F1MTE8	EKITSDENSNEK	2 Phospho (ST)
A5D973	DCEAEVRAAASHK	Phospho (ST)
Q0VD52	ETHSHLSSEVTK	Phospho (ST)
F1MY54	GAGITRKK	Phospho (ST)
F1MLY7	KPIYLSK	Phospho (Y)
F1MLY7	KPIYLSK	Phospho (ST); Phospho (Y)
G3N1X7	TPEALLQVGGLRADQR	Phospho (ST)
A8E4P2	KGGYAIK	Phospho (Y)
G3N1K9	SSSLMVK	Phospho (ST)
F1MKY5	KAISFGGCLTQIFFIHDIGGTEMVLLIAMAFDR	2 Oxidation (M); 2 Phospho (ST)
F1MNM6	SNLSALEHK	Phospho (ST)
F1MSL9	MYLSGYGVELAIK	Oxidation (M); Phospho (ST); Phospho (Y)
F1MME5	MSLPKSSIADNSGIQMK	Phospho (ST)