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Hydrophilic magnetic host-guest Ti-phenolic networks: a promising material for highly sensitive enrichment of glycopeptides/phosphopeptides.

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1. Materials

β-cyclodextrin (*β*-CD), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), titanium sulfate ($Ti(SO_4)_2$), zirconium chloride ($ZrCl_4$), zinc chloride ($ZnCl_2$), glutathione (GSH), 2,2'-azobis(2-methylpropionitrile) (AIBN), 1-adamantanecarboxylic acid (AdC), triethylamine (TEA), triflfluoroacetic acid (TFA), formic acid (FA), sodium acetate anhydrous (NaAc), and dopamine hydrochloride (DA·HCl) were purchased from Aladdin Reagents (Shanghai, China). Acetonitrile (ACN), ethanol, methanol anhydrous, sodium sulfate anhydrous (Na_2SO_4), ethylene glycol (EG), potassium carbonate (K_2CO_3), allyl bromide, *n*-hexane, hydrochloric acid, ethyl acetate, and acetone were obtained from Beijing Chemical Works (Beijing, China). *N*,*N*'-carbonyldiimidazole (CDI), *N*,*N*'-dimethylformamide anhydrous (DMF), and dimethyl sulfoxide (DMSO) were bought from Energy Chemical (Shanghai, China). Immunoglobulin G (IgG), bovine serum albumin (BSA), 2,5-dihydroxybenzoic acid (DHB), dithiothreitol (DTT), iodoacetamide (IAA), triethyl ammonium bicarbonate (TEAB), deuterium oxide (CD_2O), and trypsin from bovine pancreas were bought from Sigma-Aldrich (St. Louis, MO, USA). Human serum sample was obtained from China-Japan Hospital of Jilin University (Changchun, China) according to standard clinical procedures. Standard phosphopeptide with sequence of FQ(pSER)EEQQQTEDELQDK (2062 Da) was bought from GenScript company (Nanjing, China). Milli-Q water (typical resistivity of 18.2 MΩ cm^{-1}) was obtained from an inline Millipore RiOs/Origin water purification system.

2. Instruments

Scanning electron microscopic (SEM) images were obtained using a JEOL-JSM-6360LV scanning electron microscope (JEOL, Japan). Transmission electron microscopic (TEM) images were acquired on a TECNAI F20 instrument (FEI, USA) with the accelerating voltage of 200 kV. Fourier-transform infrared (FT-IR) spectra were determined on a Thermo Nicolet 670 FT-IR instrument (Thermo Nicolet Corporation, USA) operating at the wavelength range of 4000-500 cm^{-1}. An X-ray

photoelectron spectrometer (XPS, Thermo Electron, USA) was used to obtain XPS data. The magnetic properties were determined by a vibrating sample magnetometer (VSM) on a Superconducting Quantum Interference Device (SQUID XL-7, Quantum Design, USA). The water contact angle was measured with an OCA20 apparatus (DataPhysics, Germany) at a saturated humidity with the temperature controlled by a superthermostat (Julabo F25, Germany).

3. Sample Preparation

3.1 Tryptic digestion of standard proteins.

IgG (1 mg), *β*-casein (1 mg), and BSA (1 mg) were uniformly dispersed in NH_4HCO_3 buffer (1 mL, 50 mM, pH = 8.5), respectively, and then, the mixtures were denatured by boiling for 10 min. Thereafter, the trypsin (enzyme/substrate = 1/40, *w*/*w*) was added into the above mixtures at 37 °C overnight.

3.2 Tryptic digestion of human serum.

Human serum (2 μL) was added into the above NH_4HCO_3 buffer (20 μL) and incubated at room temperature for 1 h, and then centrifuged at 12000 rpm for 5 min. Subsequently, the supernatant was reduced with 5 mM DTT at 37 °C for 30 min and alkylated with 10 mM IAA at 25 °C in the dark for 30 min. Then, the mixtures were digested with trypsin (1/30, *w*/*w*) at 37 °C for 16 h.

3.3 Tryptic digestion of HeLa cell lysate.

HeLa cells (45 mg) were added into the mixed solution of RIPA (450 μL) and PMSF (4.5 μL), and the mixture was incubated at 0 °C for 10 min. After centrifuged at 10000 rpm for 10 min, the supernatant (25 mg) was added into 2500 μL aqueous solution containing urea (6 M) and NH_4HCO_3 (50 mM). The proteins extracted from Hela cell lysate were reduced with 200 mM DTT at 56 °C for 1 h and alkylated with 200 mM IAA at 37 °C in the dark for 30 min. Subsequently, the mixture was digested with trypsin (1/40, *w*/*w*) at 37 °C for 16 h. Finally, all the protein trypsin digests were reserved at -80 °C for further enrichment experiments.

4. Quantitative Recovery Study

A standard phosphopeptide with sequence of FQ(pSER)EEQQQTEDELQDK (2062 Da) was analyzed. The standard phosphopeptide was labeled with the method of stable isotope dimethyl labeling according to previous works[1 2]. The standard peptide (0.1 mg) was dispersed into 100 mM TEAB (500 μL), and then 100 μL above solution was added into centrifugal tube in duplicate. CH_2O (4%, *v*/*v*) and CD_2O (4 μL) were added into the above two tubes to conduct light and intermediate dimethyl labeling, respectively, and then the products were reduced with 0.6 M $NaBH_3CN$ (4 μL) at 22 °C for 1 h. The reaction was stopped with 1% $NH_3 \cdot H_2O$ (16 μL) and 5% FA (8 μL) at 0 °C.

Light dimethyl labeled samples (1 μL) and intermediate dimethyl labeled samples (1 μL) dispersed into H_2O (5 μL), and the above solution was analyzed by MALDI-TOF MS. The intensities of 2118 Da and 2126 Da are donated as A_1 and B_1 respectively, and the intensity ratio of two peaks was denoted as C_1 ($C_1 = A_1/B_1$).

Light dimethyl labeled samples (1 μL) was enriched with mTiCD@AG, and then all elute (5 μL) was mixed with intermediate dimethyl labeled samples (1 μL) followed by MALDI-TOF MS. The intensities of 2118 Da and 2126 Da were donated as A_2 and B_2 respectively, and the intensity ratio of two peaks was denoted as C_2 ($C_2 = A_2/B_2$). Three parallel experiments were conducted, and the C_2 of phosphopeptide enrichment was average C_2 of three parallel experiments. The quantitative recovery for phosphopeptides was calculated with the formula $R=(C_2/C_1)*100\%$.

5. MS Analysis

0.5 μL eluent was mixed with 0.5 μL DHB (25 mg/mL in ACN/H_2O/H_3PO_4, 70/29/1, *v*/*v*/*v*) on the MALDI plate and dried at room temperature. MALDI-TOF MS instrument (Autoflex speed TOF/TOF, Bruker Daltonik Gmbh, Germany) was operated in the reflection positive-ion mode with the *m/z* scan range of 1000-3500 Da and the laser pulse rate of 500 Hz. All MS signals were analyzed with the flexAnalysis software.

The glycopeptides and phosphopeptides enriched from Hela cell lysate were separated by Easy-nLC 1000. Buffer A was 0.1% FA–H_2O (*v*/*v*) and B was 0.1% FA–84% ACN (*v*/*v*). After the chromatographic column was equilibrated with 95% Buffer A, the sample was loaded into the Trap column through an automatic sampler. After separated by chromatography, the peptides were analyzed by Q-Exactive mass spectrometry in the positive ion mode. The full-scan MS ranged from 300-1800 m/z. Resolution: 70000 at 200 *m*/*z*; Automatic gain control target: $1e^6$; Maximum IT: 50 ms; Number of scan ranges: 1; Dynamic exclusion: 30.0 s. Mass-to-charge ratio was collected as follows: 20 fragmentation patterns (MS2 scan) were acquired of after each full scan. Resolution: 17500 at 200 *m*/*z*; Activation SI-3 type: Higher energy collision induced dissociation (HCD); Normalized collision energy: 27 eV; Isolation window: 2 *m*/*z*; Microscans: 1; Maximum IT: 50 ms.

MS raw files were analyzed by search engine Mascot 2.2.2 with the Database of uniprot_Homo_sapiens_207393_20230103 for identifying phosphopeptides and glycopeptides from Hela cell lysate.

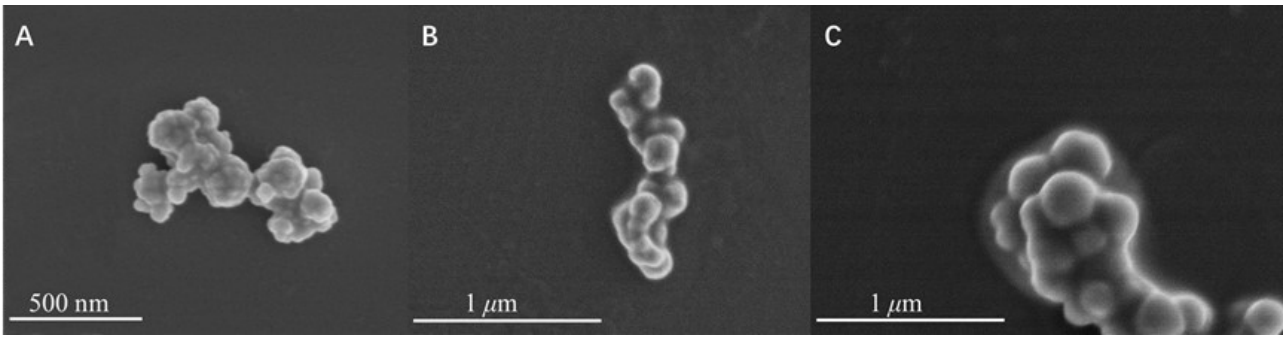

Fig. S1. SEM images of (A) MNPs, (B) mTiCD and (C) mTiCD@AG.

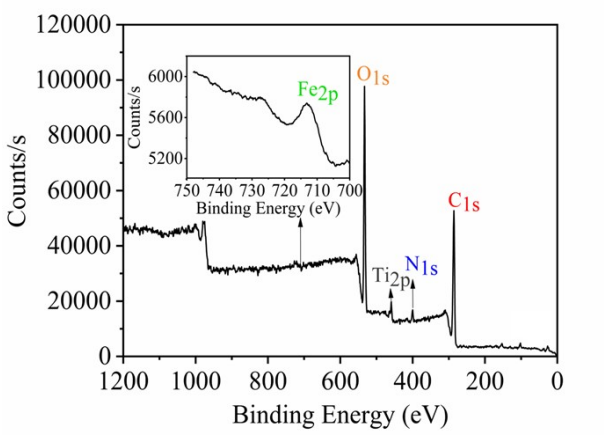

Fig. S2. XPS spectrum of mTiCD.

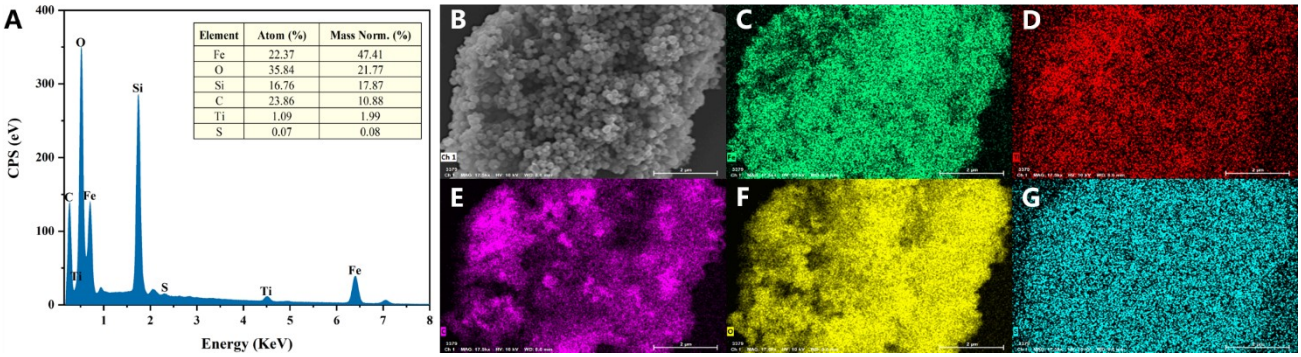

Fig. S3. (A) EDS spectrum of mTiCD@AG. (B) SEM image of mTiCD@AG and corresponding EDS element mapping of (C) Fe, (D) Ti, (E) C, (F) O and (G) S in mTiCD@AG.

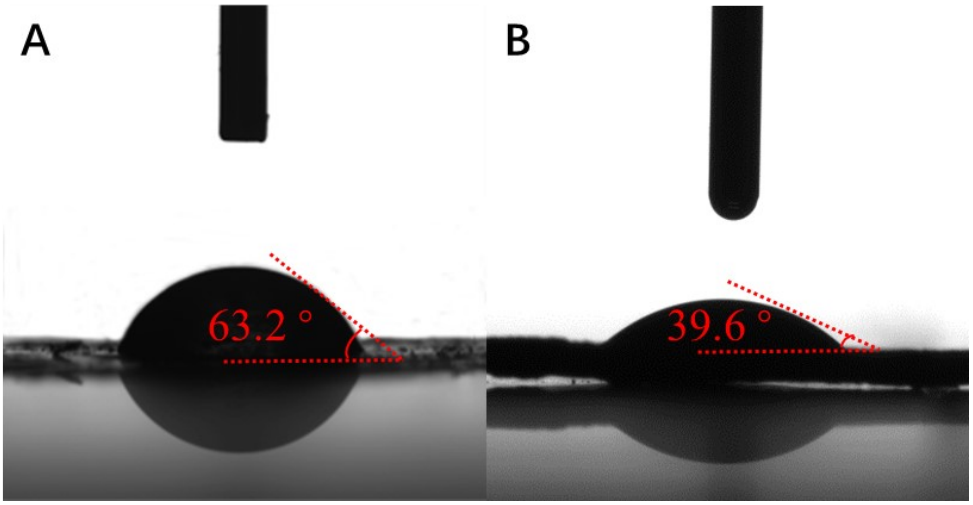

Fig. S4. Water contact angles of (A) mTiCD and (B) mTiCD@AG.

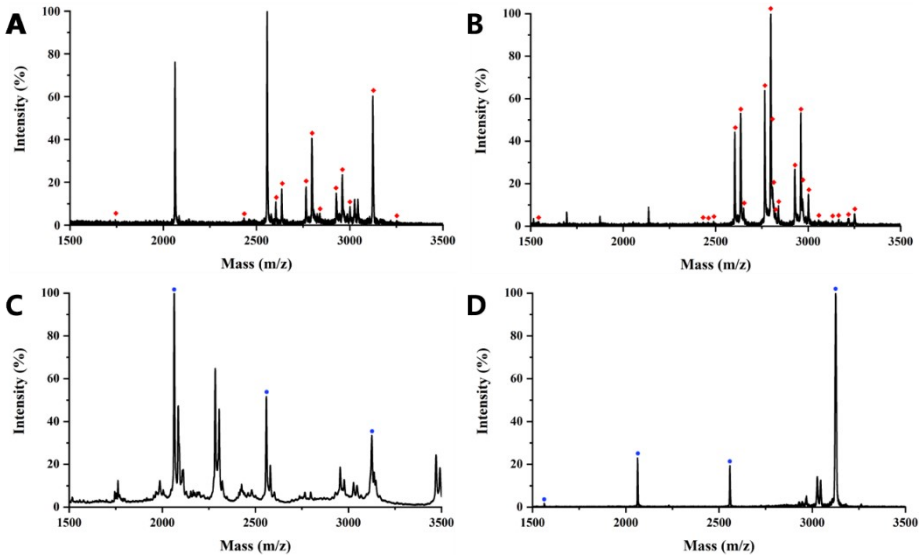

Fig. S5. MALDI-TOF MS analysis of (A-B) IgG digests and (C-D) *β*-casein digests: (A, C) after enrichment with Fe_3O_4 and (B, D) after enrichment with mTiCD. "◆" and "●" represent glycopeptides and phosphopeptides.

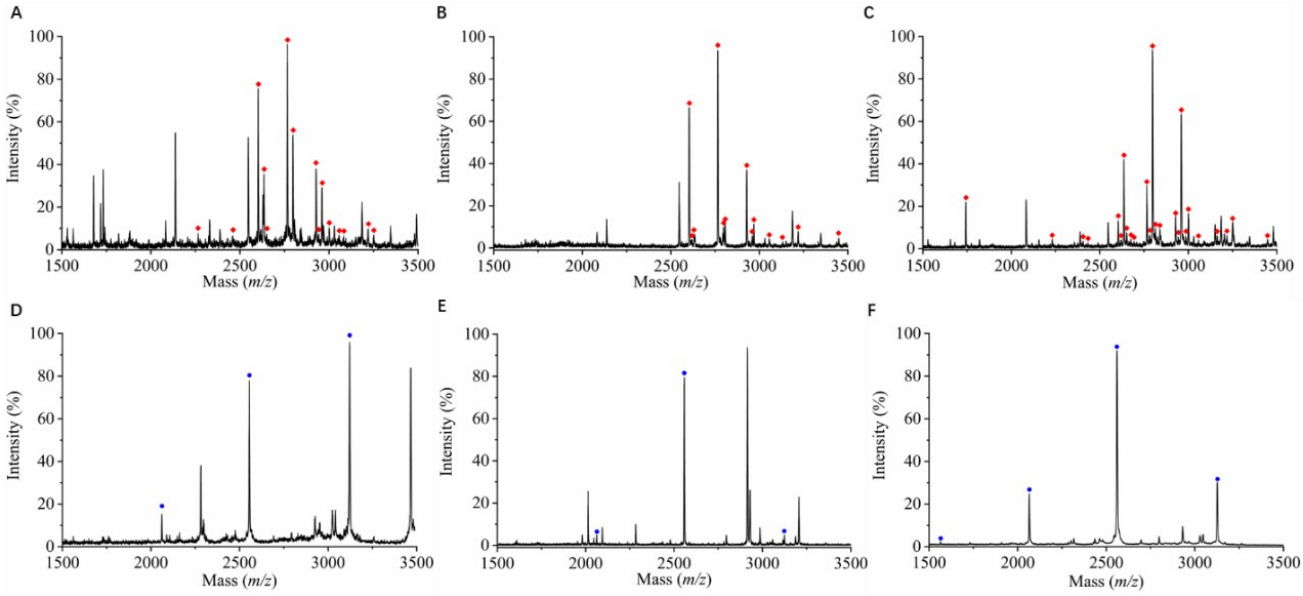

Fig. S6. MALDI-TOF MS analysis of (A-C) IgG digests and (D-F) *β*-casein digests: (A, D) after enrichment with mFeCD@AG, (B, E) after enrichment with mZrCD@AG and (C, F) after enrichment with mZnCD@AG. "◆" and "●" represent glycopeptides and phosphopeptides.

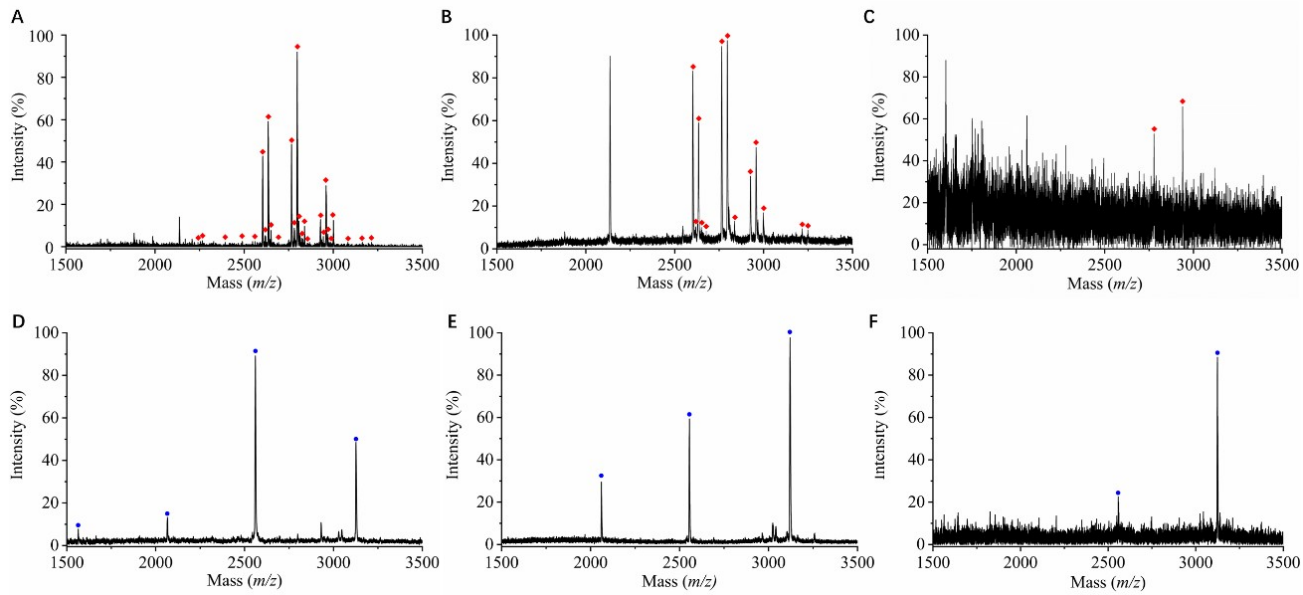

Fig. S7. MALDI-TOF MS analysis of IgG and *β*-casein with different concentrations after enrichment with mTiCD@AG: (A) 1 fmol IgG, (B) 0.1 fmol IgG, (C) 0.035 fmol IgG; (D) 1 fmol *β*-casein, (E) 0.1 fmol *β*-casein and (F) 0.01 fmol *β*-casein. "◆" and "●" represent glycopeptides and phosphopeptides.

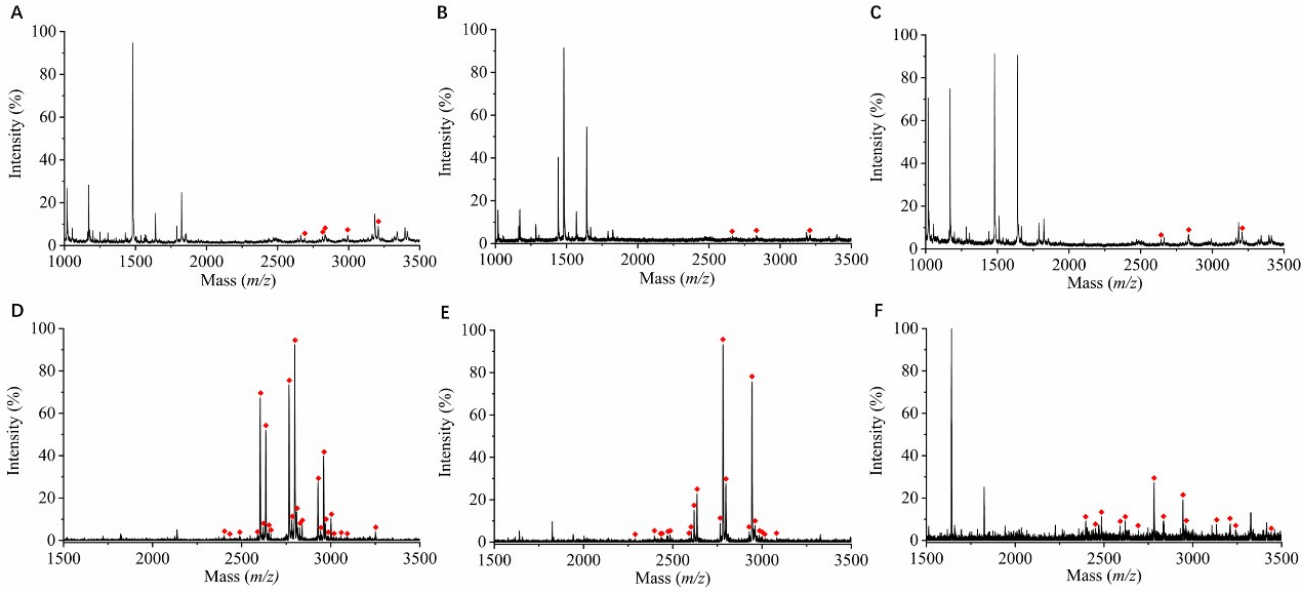

Fig. S8. MALDI-TOF MS analysis of IgG and BSA tryptic digest mixture: (A-C) Before enrichment and (D-F) after enrichment with mTiCD@AG. (A, D) BSA:IgG = 10:1 (*w*/*w*), (B, E) BSA:IgG = 100:1 (*w*/*w*) and (C, F) BSA:IgG = 500:1 (*w*/*w*). "◆" represents glycopeptides.

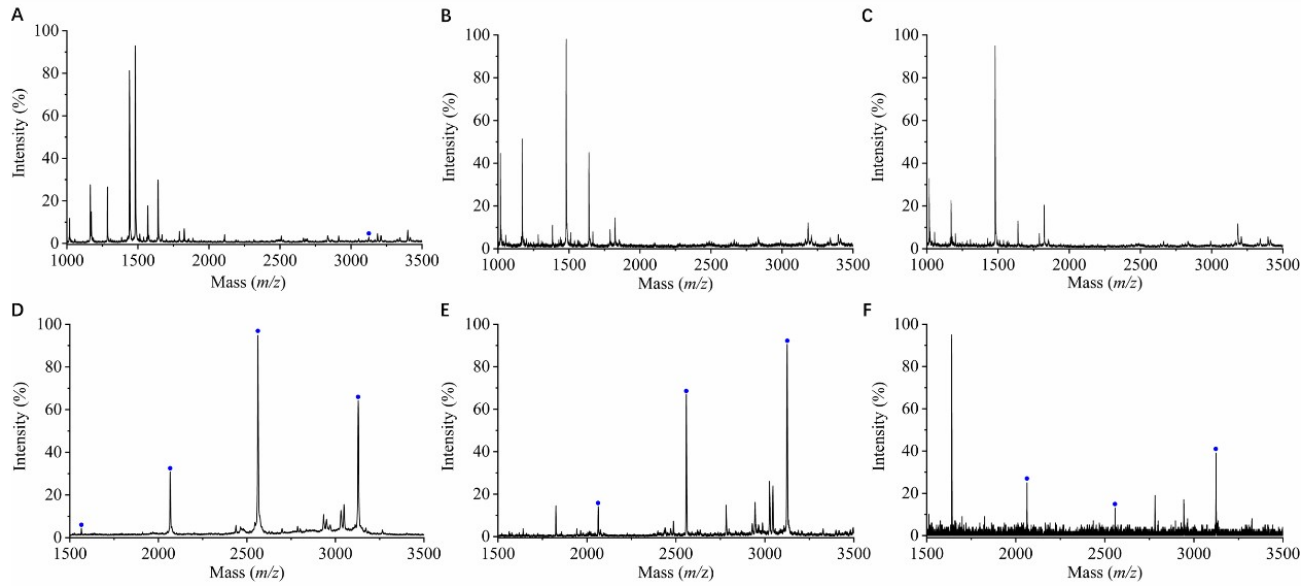

Fig. S9. MALDI-TOF MS analysis of *β*-casein and BSA tryptic digest mixture: (A-C) before enrichment and (D-F) after enrichment with mTiCD@AG. (A, D) BSA:*β*-casein = 10:1 (*w*/*w*), (B, E) BSA:*β*-casein = 100:1 (*w*/*w*) and (C, F) BSA:*β*-casein = 1000:1 (*w*/*w*). "●" represents phosphopeptides.

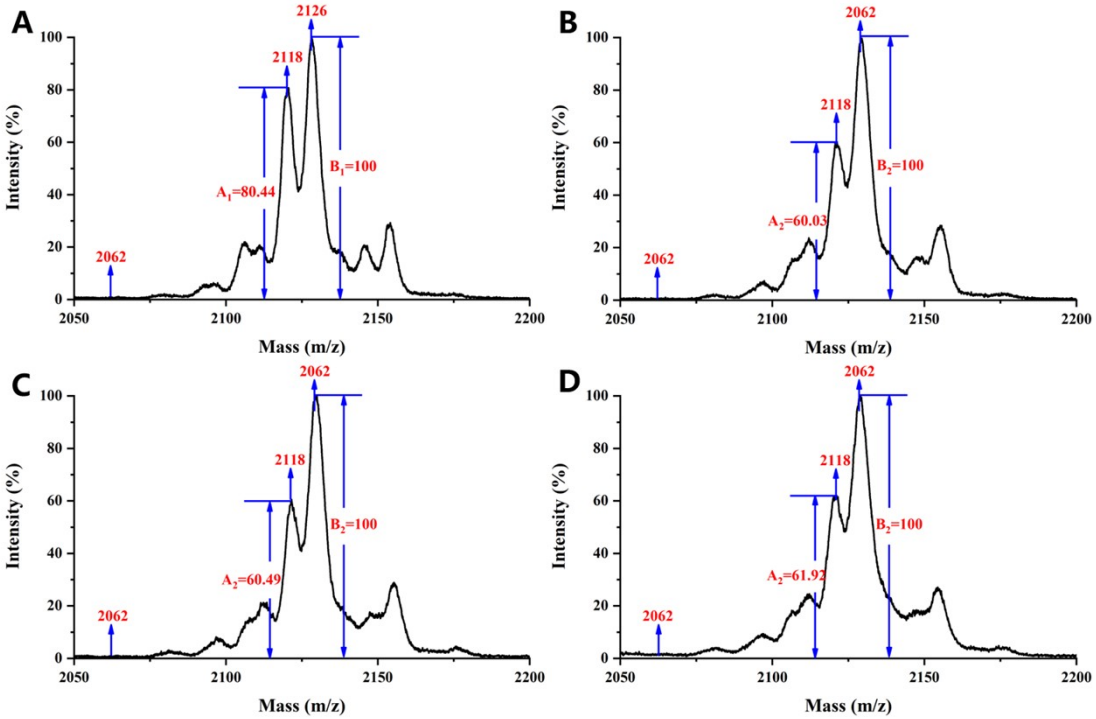

Fig. S10. MALDI-TOF MS analysis of (A) light and intermediate dimethyl labeled standard phosphopeptide before enrichment and (B-D) light dimethyl labeled standard phosphopeptide after enrichment with mTiCD@AG spiked with intermediate dimethyl labeled standard phosphopeptide.

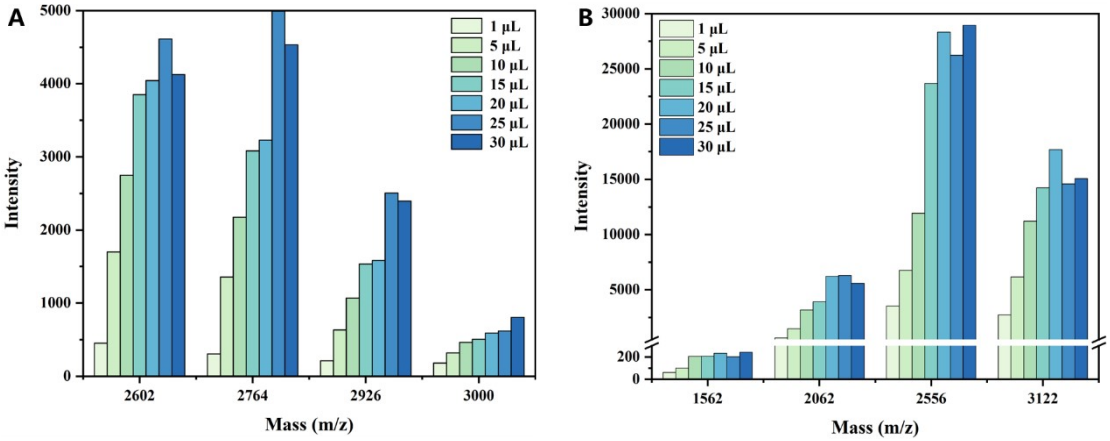

Fig. S11. Evaluation the loading capacity of mTiCD@AG towards (A) glycopeptides and (B) phosphopeptides. The concentration of IgG/*β*-casein digestion is 1 mg mL^{-1}, and the quality of mTiCD@AG is 0.5 mg.

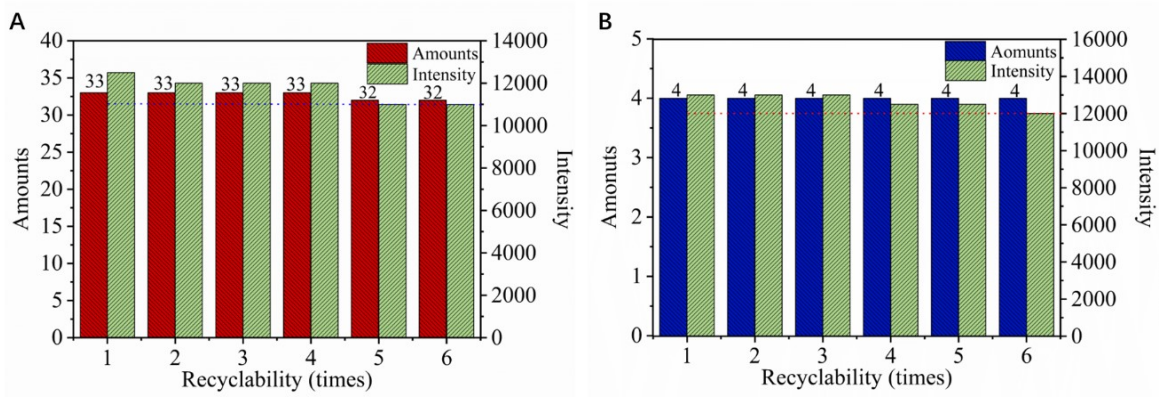

Fig. S12. Reusability of mTiCD@AG under the optimized experimental conditions: (A) IgG and (B) *β*-casein.

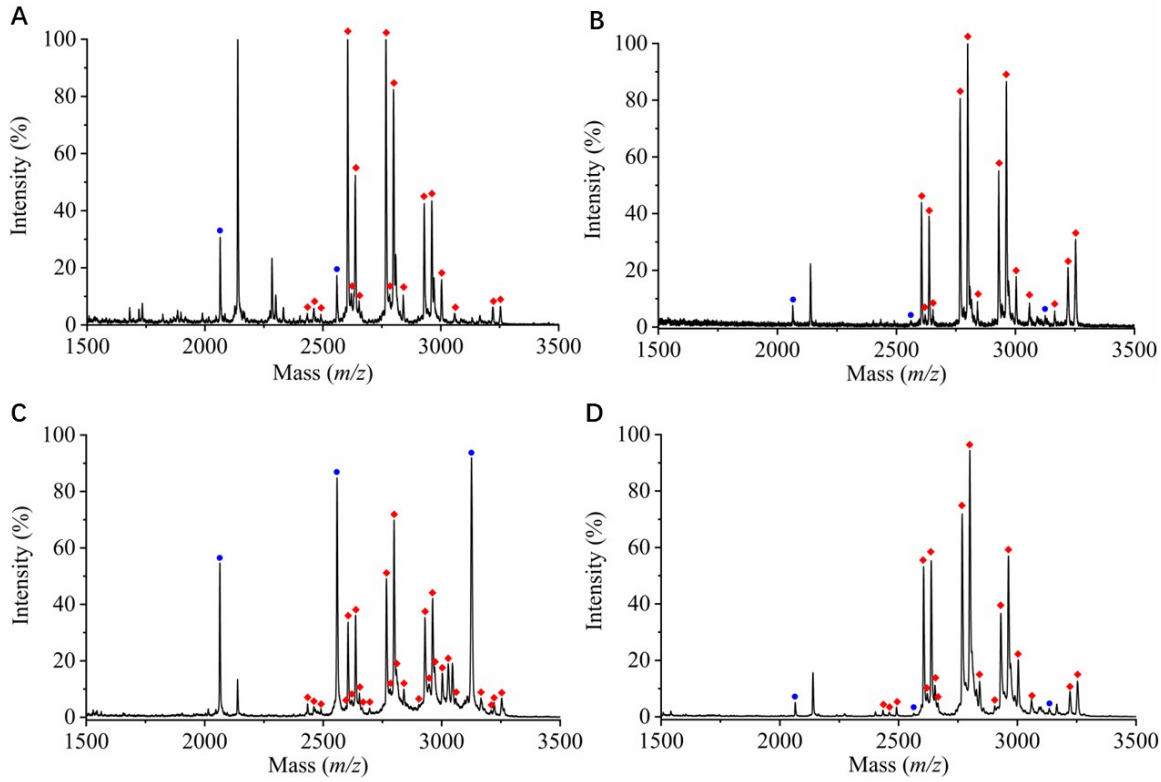

Fig. S13. Enrichment efficiency of 1 pmol IgG and 1 pmol *β*-casein digests with mTiCD@AG versus loading buffer: (A) ACN/H_2O/FA (80/17/3, *v/v/v*), (B) ACN/H_2O/FA (85/12/3, *v/v/v*), (C) ACN/H_2O/FA (90/7/3, *v/v/v*) and (D) ACN/H_2O/FA (90/5/5, *v/v/v*). "◆" and "●" represent glycopeptides and phosphopeptides.

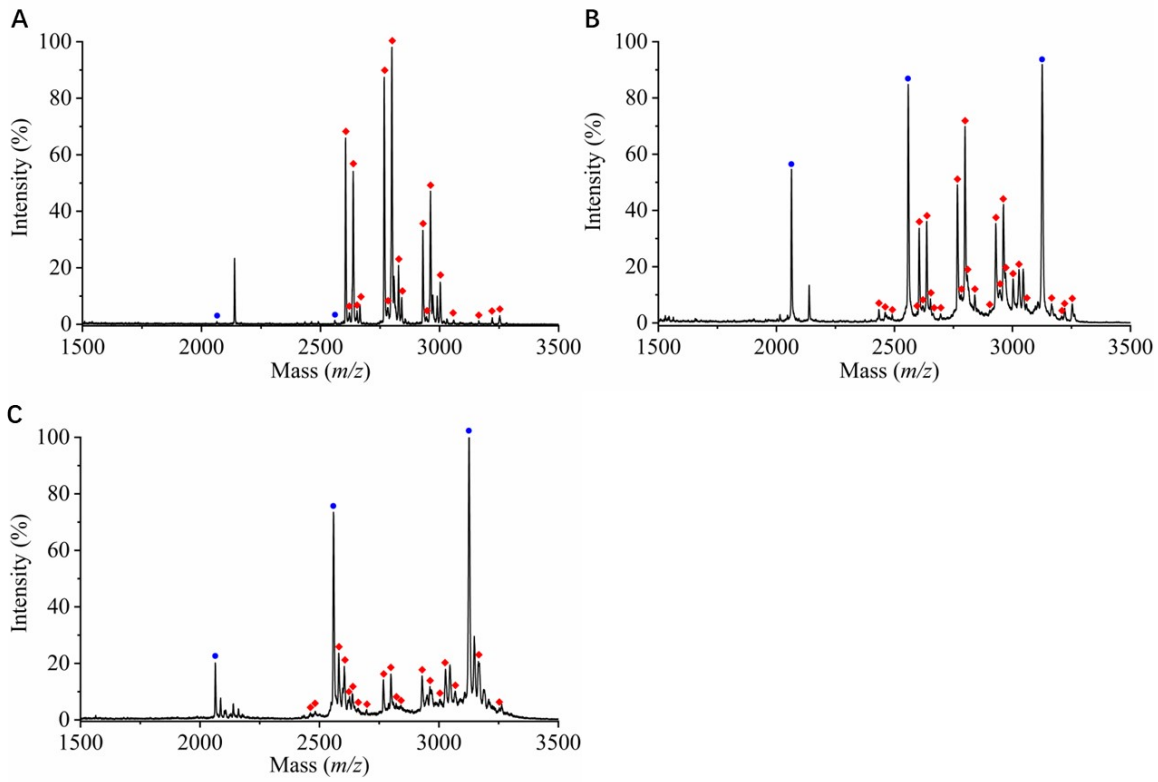

Fig. S14. Enrichment efficiency of 1 pmol IgG and 1 pmol *β*-casein digests with mTiCD@AG versus elution: (A) 1% $NH_3 \cdot H_2O$, (B) 3% $NH_3 \cdot H_2O$ and (C) 5% $NH_3 \cdot H_2O$. "◆" and "●" represent glycopeptides and phosphopeptides.

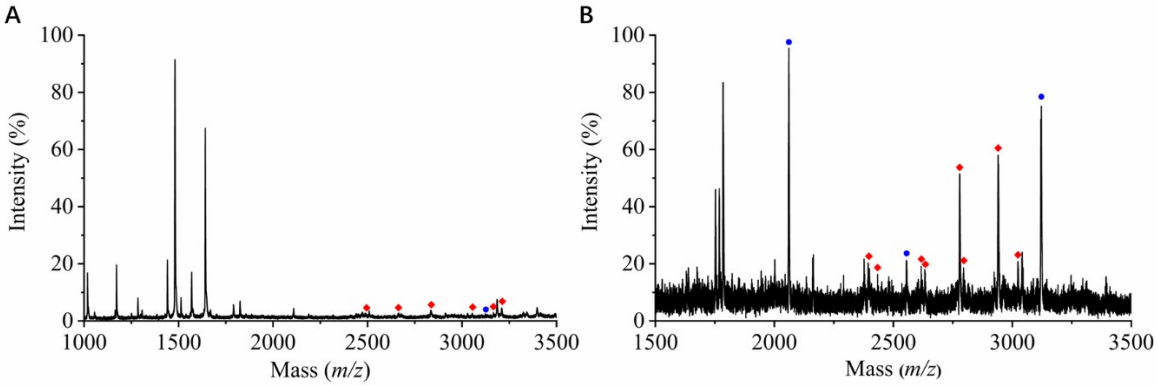

Fig. S15. MALDI-TOF MS analysis of BSA, IgG, and *β*-casein tryptic digest mixture at BSA:IgG:*β*-casein = 500:1:1 (*w*/*w*/*w*): (A) Before enrichment and (B) after enrichment with mTiCD@AG. "◆" and "●" represent glycopeptides and phosphopeptides.

Table S1. Detailed information of the detected glycopeptides and phosphopeptides from IgG and *β*-casein tryptic digests after enrichment with mTiCD@AG (N#: glycosylation site; s: phosphorylated site.)

No.	Observed *m/z*	Glycan composition	Peptide sequence
I_1	1740.9	[HexNAc]2[Fuc]1	EEQYN#STYR
I_2	2400.5	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR
I_3	2430.8	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
I_4	2488.7	[Hex]3[HexNAc]4XylMan3GlcNAc2	EEQYN#STYR
I_5	2561.9	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR
I_6	2595	[Hex]4[HexNAc]3[Fuc]1	EEQYN#STYR
I_7	2602.3	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I_8	2618.7	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STYR
I_9	2634.7	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
I_{10}	2650.7	[Hex]4[HexNAc]4	EEQYN#STYR
I_{11}	2691.4	[Hex]3[HexNAc]5	EEQYN#STYR
I_{12}	2764.8	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I_{13}	2780.2	[Hex]5[HexNAc]4	EEQFN#STFR
I_{14}	2795.9	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
I_{15}	2805.8	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STFR
I_{16}	2812.2	[Hex]5[HexNAc]4	EEQYN#STYR
I_{17}	2822.3	[Hex]4[HexNAc]5	EEQFN#STFR
I_{18}	2837.3	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR
I_{19}	2853.8	[Hex]4[HexNAc]5	EEQYN#STYR
I_{20}	2908.9	[Hex]4[HexNAc]4[NeuAc]1	EEQFN#STFR
I_{21}	2926.8	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
I_{22}	2943.1	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STYR
I_{23}	2958.8	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
I_{24}	2967.5	[Hex]4[HexNAc]5[Fuc]1	EEQFN#STFR
I_{25}	2983.8	[Hex]5[HexNAc]5	EEQFN#STFR
I_{26}	3000.3	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR
I_{27}	3057.1	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I_{28}	3086.5	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR
I_{29}	3130.9	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
I_{30}	3161.8	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR
I_{31}	3218.7	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I_{32}	3252.1	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR
I_{33}	3280.1	[Hex]4[HexNAc]4[Fuc]1	TKPYEEQYN#STYR
No.	**Observed m/z**	**Phosphoryl groups**	**Peptide sequence**
β_1	1562.3	4	RELEELNVPGEIVESLsssEEsITR
β_2	2061.9	1	FQsEEQQQTEDELQDK
β_3	2556.9	1	FQsEEQQQTEDELQDKIHPF
β_4	3122.5	4	RELEELNVPGEIVEsLsssEESITR

HeNac = N-acetyglucosamine, Hex = mannose, Fuc = fuctose, Xyl = xylose.

Table S2. Comparison of the detection limit of glycopeptides based on different materials

Material	Detection method	LOD (fmol)	Ref.
MoS_2-Fe_3O_4-Au/NWs-GSH	MALDI-TOF-MS	0.5	[3]
PEIZIF-8@Au@GSH	MALDI-TOF-MS	2.0	[4]
CS@PGMA@IDA-Ti^{4+}	MALDI-TOF-MS	0.1	[5]
Mag-MSMs@PEI-PA-Ti^{4+}	MALDI-TOF-MS	0.5	[6]
MCDC	MALDI-TOF-MS	0.05	[7]
mTiCD@AG	MALDI-TOF-MS	0.035	This work

Table S3. Comparison of the detection limit of phosphopeptides based on different materials

Material	Detection method	LOD (fmol)	Ref.
CS@PGMA@IDA-Ti^{4+}	MALDI-TOF-MS	0.1	[5]
Mag-MSMs@PEI-PA-Ti^{4+}	MALDI-TOF-MS	0.2	[6]
TA-Ti-PA@Fe_3O_4	MALDI-TOF-MS	4.0	[8]
Fe_3O_4@TiTA	MALDI-TOF-MS	0.5	[9]
Fe_3O_4@TAPTDHTA-Ti^{4+}	MALDI-TOF-MS	0.05	[10]
mTiCD@AG	MALDI-TOF-MS	0.01	This work

Table S4. List of glycopeptides and phosphopeptides enriched with mTiCD@AG from human serum (N#: glycosylation site, s: phosphorylated site.)

Glycosyl	Observed *m/z*	Sequence
1	1582.76	WFSAGLASN#SSWLR
2	1642.61	N#ccNTENPPGcYR
3	1656.80	TKPREEQFN#STYR
4	1690.79	ERSWPAVGN#cSSALR
5	1775.82	FSDGLESN#SSTQFEVK
6	1853.76	N#GTGHGN#STHHGPEYMR
7	1884.03	LHINHNN#LTESVGPLPK
8	1903.89	FSDGLESN#SSTQFEVKK
9	2022.99	ELHHLQEQN#VSNAFLDK
10	2041.94	LSDLSIN#STEcLHVHcR
11	2149.16	IIVPLNNREN#ISDPTSPLR
12	2161.15	VVGVPYQGN#ATALFILPSEGK
13	2180.12	AGAFLGLTnVAVMN#LSGNcLR
14	2205.10	DVQIIVFPEDGIHGFN#FTR
15	2214.04	TLN#QSSDELQLSMGNAMFVK
16	2273.14	TVLTPATnHmGN#VTFTIPANR
17	2345.17	SQILEGLGFN#LTELSESDVHR
18	2356.05	GLTFQQN#ASSmcVPDQDTAIR
19	2398.22	GcVLLSYLN#ETVTVSASLESVR
20	2452.21	ALGISPFHEHAEVVFTAN#DSGPR
21	2536.26	VYIHPFHLVIHN#ESTcEQLAK
22	2601.05	EEQFN#STFR
23	2618.37	AELSN#HTRPVILVPGcLGnQLEAK
24	2635.47	QLVEIEKVVLHPN#YSQVDIGLIK
25	2763.20	EEQFN#STFR
26	2795.02	EEQYN#STYR
27	2842.05	FVEGSHN#STVSLTTK
28	2925.02	YVN#MSN#HHR
29	2957.10	EEQYN#STYR
30	2998.06	EEQYN#STYR
31	3040.00	FVEGSHN#STVSLTTK
32	3051.55	N#LSSLESVQLDHnQLETLPGDVFGALPR
33	3216.10	YVNMSN#HHR
34	3373.63	FSLLGHASIScTVEN#ETIGVWRPSPPTcEK
Phosphoryl	**Observed m/z**	**Sequence**
1	1388.25	DsGEGDFLAEGGGV
2	1465.70	DsGEGDFLAEGGGVR
3	1616.84	ADsGEGDFLAEGGGVR

Table S5. List of glycopeptides and phosphopeptides enriched with mTiCD@AG from Hela cell lysate (n: glycosylation site, #: phosphorylated site, *: oxidized site.)

Glycosyl	Protein ID	Peptides Sequence
1	H0YA55	K.KVPQVSTPTLVEVSR.n
2	H0YA55	K.VPQVSTPTLVEVSR.n
3	P0DOX7	K.VDNALQSGnSQESVTEQDSK.D
4	Q8TCD0	K.VDNALQSGnSQESVTEQDSK.D
5	Q96E61	K.AnPTVTLFPPSSEELQANK.A
6	A6XGL3	K.TLnNDIM*LIK.L
7	A6XGL3	K.TLnNDIMLIK.L
8	Q6PJF2	K.VDNALQSGnSQESVTEQDSK.D
9	A0A5C2G1F5	R.LLIYDASnR.T
10	A0A5C2G2T3	R.LLIYDASnR.T
11	A0A5C2G8I7	R.LLIYDASnR.T
12	A0A5C2GJH3	R.LLIYDASnR.T
13	S6AWF4	R.LLIYDASnR.T
14	O60287	K.nITKT#QK.V
15	P08254	R.IVnY#TPDLPK.D
16	P48547	R.nGTQVR.Y
17	Q14593	R.SSnLTR.H
18	Q8TBG4	K.EnPS#R.K
19	Q8WYK1	K.nGSLQVR.Y
20	Q96EN8	R.QLnTSDENGK.E
21	A0A0A0MRU9	K.KFLILnPS#K.R
22	A0A0A0MSW0	R.TM*QLnLSR.S
23	A0A5C2GU23	R.VTISCSGnSSNIGR.N
24	A0A7P0MW50	R.NnNTDLMILK.N
25	B3KXA5	K.ENNLKNVEELnK.S
26	B4DVG9	K.KDnLTAVEK.W
27	B7ZM71	K.T#GnLAQTR.A
28	C9J9V9	K.EnDTLKALLK.A
29	D6RGX6	R.AnMSQVR.Q
30	H0Y5D0	-.X#CFLEnK.S
31	H0YL13	-.XAKTInK.V
32	J3QLS5	-.XSWnPR.L
33	Q7Z4E0	K.STnIIS#TIK.Y
34	Q86YK2	-.M*DIRPnHTIYINNMNDKIK.K
35	Q9ULR4	K.QFnKS#GDLSM*nLLR.H
36	H0YA55	K.KVPQVSTPTLVEVSR.n
Phosphoryl	**Protein ID**	**Peptides Sequence**
1	P02765	K.CDSSPDS#AEDVRK.V

2	Q8IYH3	R.RCS#GLARR.V
3	P42684	R.GAT#ALPLR.T
4	H0Y9X0	-.XGYLY#K.W
5	H7C063	R.HS#LGIQVR.G
6	Q9Y5I7	R.KAY#SAAGVSMAK.S
7	Q9Y5I7	R.KAYS#AAGVSMAK.S
8	H7BZI1	R.T#KLDELK.R
9	A5YKK6	K.QLFS#FPIK.H
10	A8MTJ3	K.KDIFQEKVT#K.V
11	O15520	R.KLFSFT#K.Y
12	O60287	K.nITKT#QK.V
13	P08254	R.IVnY#TPDLPK.D
14	P17540	K.HTT#DLDASK.I
15	Q6N063	R.QRLGQES#AAR.K
16	Q6NSI8	R.QSSSEKNT#RSK.S
17	Q6P4R8	K.SGPS#TVS#EPAK.S
18	Q6ZSJ9	K.TNPS#K.Y
19	Q7Z695	K.TEM*AMLVT#QAR.K
20	Q8TBG4	K.EnPS#R.K
21	Q8TCS8	R.ALT#QLQVR.A
22	A0A087WYM2	K.AGQAKLT#K.S
23	A0A0A0MRU9	K.KFLILnPS#K.R
24	A0A0F7WAP3	R.LCLLRQRT#R.R
25	A0A0U1RQX8	K.QSPKANLRSPDLAAEEIS#R.V
26	A0A1S5UZ27	R.KRY#SVDK.S ! R.KRY#SVDK.T
27	A0A3B3ITJ0	-.XQDVVFT#DEM*AHFDR.E
28	A0A5F9ZHW6	R.EY#GRPEVLK.E
29	A0A5H1ZRP3	K.APLLHS#VS#R.D
30	A0A8I5QJU2	K.T#SAAAGSSS#R.K
31	A0A8Q3WL55	K.KMLQT#NLK.V
32	A0A8V8TLX3	R.IDLS#RVPGISK.D
33	A0A8V8TMC2	K.TEDGQAS#QSRYSK.R
34	B4DM78	R.GGKS#PISS#VLK.D
35	B7Z8Z8	K.ARKSVFIT#R.K
36	B7ZM71	K.T#GnLAQTR.A
37	F8W883	R.IYTY#LLEK.S
38	F8WEI8	R.LGSGGTQDGS#DTPLLK.T
39	H0Y5D0	-.X#CFLEnK.S
40	H0YAN3	-.XKT#GHAEVVR.V
41	H0YAN9	R.T#ASGKSK.K
42	H0YJG5	-.XS#SHVK.I
43	H0YMC7	K.KLESS#VSTGR.K
44	H3BMD8	R.KPS#LVASK.L
45	I3L1P8	R.LT#GADGTPPGFLLK.A
46	Q53SN6	R.IST#EHDPVLK.Q

47	Q6NWR3	R.QPS#PVPSFR.V
48	Q7Z4E0	K.STnIIS#TIK.Y
49	Q9ULR4	K.QFnKS#GDLSM*NLLR.H
50	V9GYS1	K.VKS#PELQAEAK.S

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