Electronic Supplementary Information

Tailoring multifunctional magnetic cationic metal-organic framework composites for synchronous enrichment of glycopeptides and phosphopeptides

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Supplementary Experimental Section

Regents and materials

Iron trichloride ($FeCl_3·6H_2O$), zinc nitrate hexahydrate ($Zn(NO_3)_2·6H_2O$), sodium citrate tribasic dehydrate ($Na_3Citr·2H_2O$), ammonium bicarbonate (NH_4HCO_3), sodium acetate (NaAc), ethylene glycol (EG), concentrated hydrochloric acid (37%), ethanol (EtOH), and methanol (MeOH) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Methyl 4-bromobutyrate, 1-(trimethylsilyl)imidazole, titanium tetrafluoride (TiF_4), acetonitrile (ACN), urea, odoacetamide (IAA), trifluoroacetic acid (TFA), dithiothreitol (DTT), phosphoric acid (H_3PO_4), and formic acid (FA) were secured from Aladdin Reagents (Shanghai, China). Horseradish peroxidase (HRP), bovine serum albumin (BSA), trypsin, *β*-casein, and 2,5-dihydroxybenzoic acid (DHB) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Human serum and saliva samples were obtained from China-Japan Hospital of Jilin University (Changchun, China) according to standard clinical procedures.

Characterization

The morphologies of as-prepared materials were determined using an SU8020 ESEM microscope (Hitachi, Japan) and Hitachi H600 microscope (Hitachi, Japan). Brunauer-Emmett-Teller (BET) surface areas were determined with an ASAP 2420 gas adsorption instrument (Micromeritics, Atlanta, USA). Powder X-ray diffraction (PXRD) patterns were recorded in the range of 2θ = 5–80° using a D/Max-IIIC instrument (Rigaku, Japan) with Cu Kα radiation. Thermogravimetric analysis (TGA) was taken with a Q500 thermal gravimetric analyzer (TA, USA). Fourier-transform infrared (FT-IR) spectra were obtained using a Thermo Nicolet 670 FT-IR instrument (Thermo Nicolet Corporation, USA) with the recorded range of 4000−450 cm^{-1}. The magnetic properties were determined by a vibrating sample magnetometer (VSM) on a Superconducting Quantum Interference Device (SQUID XL-7, Quantum Design, USA). Zeta potential was calculated *via* dynamic light scattering (DLS, Zeta-sizer Nano ZS90, Malvern Company, USA). Mass spectra were obtained on a MALDI-TOF/TOF 5800 instrument (Applied Biosystems, Foster City, CA).

Preparation of Fe_3O_4 nanoparticles

3.24 g $FeCl_3·6H_2O$ and 0.6 g $Na_3Citr·2H_2O$ were dissolved into 50 mL EG and ultrasonically treated for 30 min to form yellow solution. Then, 3.6 g NaAc was added and the mixture was stirred for another 30 min to obtain a uniform brown solution. The above solution was transferred and sealed in a Teflon-lined stainless-steel autoclave. After heating at 200 ℃ for 12 h, taupe precipitates were obtained and washed with EtOH and H_2O.

Preparation of 1,3-bis(4-carboxybutyl)imidazolium bromide (ILI)

4.4 g 1-(trimethylsilyl)imidazole and 11.3 g methyl 4-bromobutyrate (11.3 g, 62.6 mmol) were mixed in a round-bottom flask. After agitating for 24 h at 60 ℃ under the protection of Ar, the product was thoroughly rinsed with ether for the purpose of discarding the excess reactants and 1,3-bis(4-methyl butyrate)imidazolium bromide was obtained. The obtained intermediate products were further dispersed into 7 mL concentrated hydrochloric acid followed by refluxing at 100 ℃ for 2 h to hydrolyze the diester groups. After washing by ether and acetone for three times sequentially, ILI was obtained.

Sample preparation

Standard proteins were dissolved in 50 mM NH_4HCO_3 (pH 8.3) to prepare 1 mg mL^{-1} sample solution. Then, the samples were heated to 100 ℃ and kept for 10 min to denature proteins. The denatured proteins were cooled to room temperature and trypsin was added to proteins solution with the mass ratio of 1:30 (*w/w*). Finally, the mixture was incubated at 37 ℃ for 18 h, followed by adding certain amount of FA to quench the digestion process. As for biological fluids like human serum and saliva, 50 μL of stock solution was diluted to 500 μL with 0.2% TFA. Then, the diluted biological fluids were stored at –20 ℃ for further use. For phosphopeptides identification from human serum digest, the eluent were collected and freeze-dried. Subsequently, lyophilized peptide sample were dispersed into 25 mM NH_4HCO_3 and incubated with certain PNGase F for 3 h to remove poly-saccharides. After that, the sample were freeze-dried and cryopreserved for LC-MS/MS analysis.

MS Analysis

0.5 μL eluent was mixed with 0.5 μL DHB (20 mg mL^{-1} in 70% ACN-H_2O + 1% H_3PO_4) on the MALDI plate and dried at room temperature. MALDI-TOF MS instrument (Model 5800, Applied Biosystems, Foster City, CA) was operated in the linear positive-ion mode with the *m*/*z* scan range of 1000–5000 for glycopeptides and 1000–3500 for phosphopeptides.

Serum sample was separated by Easy-nLC HPLC system with nanoliter flow rate. Buffer A was 0.1% FA–H_2O (*v/v*) and B was 0.1% FA–84%ACN (*v/v*). The gradient elution condition was set as follows: 5-35% B for 22.00 min, 35-100% B for 5 min and 100% B for 3 min. Q-Exactive mass spectrometry was performed with positive ion mode. The full-scan MS ranged from 300-1800 m/z. Resolution: 70000 at 200 m/z; AGC target: $1e^6$; Maximum IT: 40 ms; Number of scan ranges: 1; Dynamic exclusion: 30.0 s. MS/MS data was collected as follows: 20 fragmentation patterns (MS2 scan) were acquired of after each full scan. Resolution: 17500 at 200 m/z; Activation

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 Proteome Discoverer 1.4 software with the database of uniprot_Homo_sapiens_203800_20220104.fasta for identifying phosphopeptides and glycopeptides from human serum tryptic digest.

Supplementary Figures

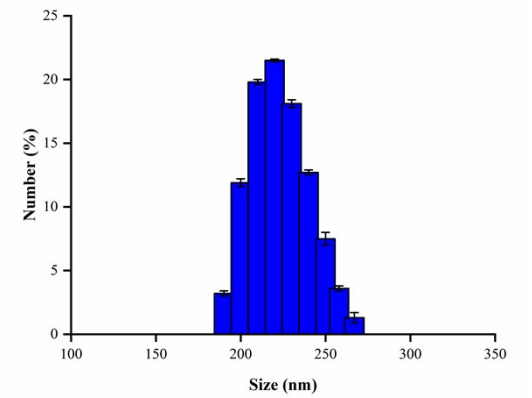

Fig. S1 DLS histogram of Fe_3O_4@ILI-01@Ti^{4+}.

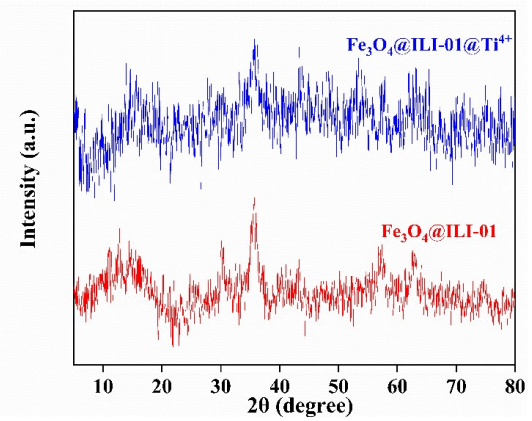

Fig. S2 XRD patterns of Fe_3O_4@ILI-01 and Fe_3O_4@ILI-01@Ti^{4+}.

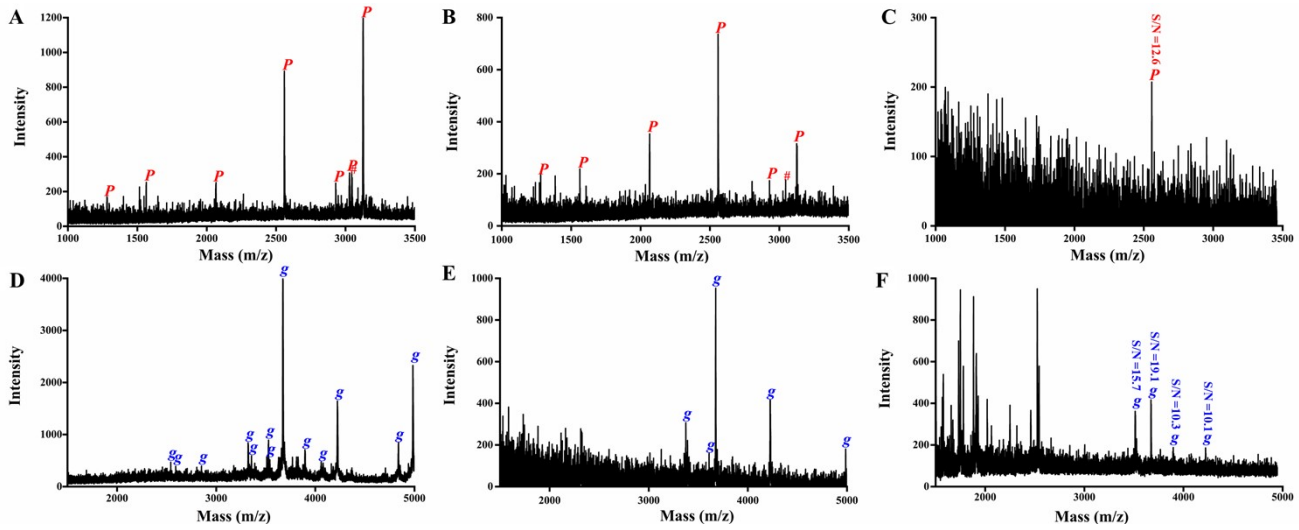

Fig. S3 MALDI-TOF mass spectrum of *β*-casein digests with various concentrations: (A) 10 fmol, (B) 1 fmol, and (C) 0.5 fmol. MALDI-TOF mass spectrum of HRP digests with various concentrations: (D) 10 fmol, (E) 0.5 fmol, and (F) 0.1 fmol.

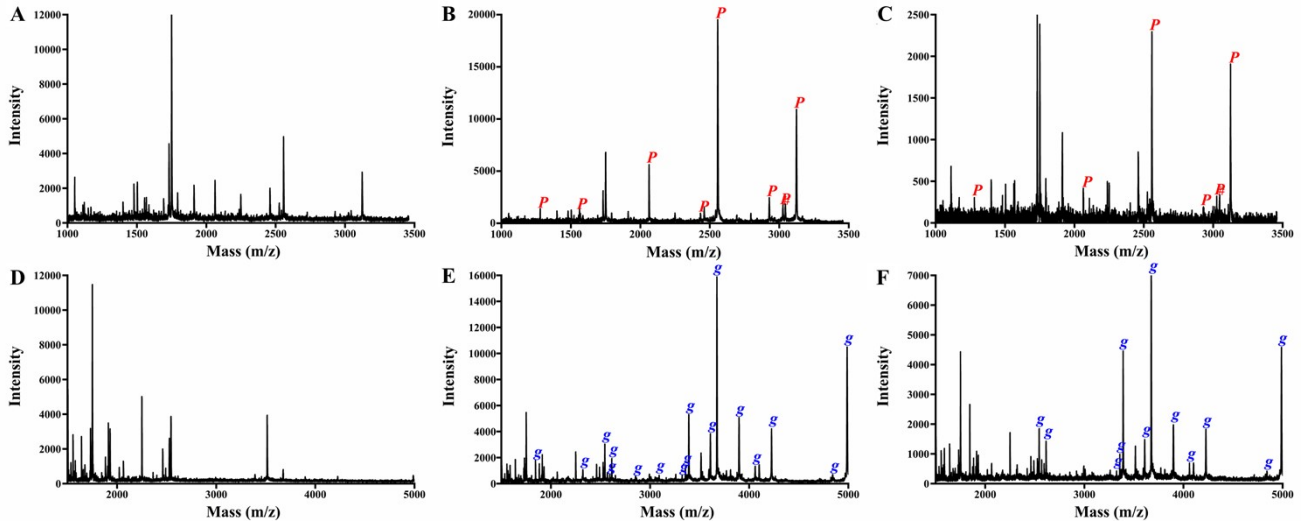

Fig. S4 MALDI-TOF mass spectrum of *β*-casein and BSA tryptic digests mixture with various mass ratios: (A) without enrichment, (B) 1:500, and (C) 1:5000. MALDI-TOF mass spectrum of HRP and BSA tryptic digests mixture with various mass ratios: (D) without enrichment, (E) 1:100, and (F) 1:500.

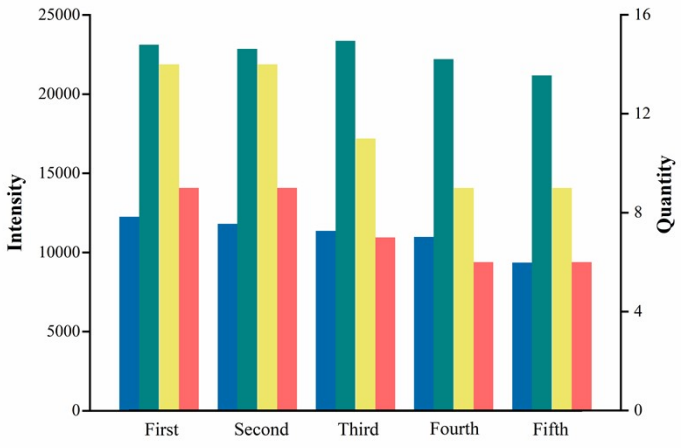

Fig. S5 Intensity and quantity of the glycopeptides/phosphopeptides simultaneously enriched during five repeated enrichment-elution processes: (Blue) highest intensity of glycopeptides, (Green) highest intensity of phosphopeptides, (Yellow) quantity of glycopeptides, and (Orange) quantity of phosphopeptides.

Supplementary Tables

Table S1. Detailed information of identified phosphopeptides or glycopeptides enriched from *β*-casein or HRP digests by Fe_3O_4@ILI-01@Ti^{4+}. [pS]:phosphorylation site. N#: N-glycosylation site.

Peak	Observed *m/z*	Phosphoryl groups	Peptide sequence
β_1	1031.5	1	FQ[pS]EEQQQTEDELQDK
β_2	1278.6	1	FQ[pS]EEQQQTEDELQDKIHPF
β_3	1562.3	4	RELEELNVPGEIVESL[pS] [pS] [pS]EE[pS]ITR
β_4	2061.8; 1981.4#	1	FQ[pS]EEQQQTEDELQDK
β_5	2432.4	1	IEKFQ[pS]EEQQQTEDELQDK
β_6	2556.4; 2480.5#	1	FQ[pS]EEQQQTEDELQDKIHPF
β_7	2927.2	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
β_8	3027.1	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
β_9	3122.2; 3042.5#	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
α_1	1660.9	1	VPQLEIVPN[pS]AEER
α_2	1951.1	1	YKVPQLEIVPN[pS]AEER
Peak	**Observed *m/z***	**Glycan composition**	**Amino acid sequence**
H1	1842.0	$XylMan_3FucGlcNAc_2$	NVGLN#R
H2	2319.2	$Man_3GlcNAc_2$	PTLN#TTYLQTLR
H3	2539.7	$XylMan_3GlcNAc_2Fuc$	SSPN#ATDTIPLVR
H4	2590.9	$XylMan_3FucGlcNAc_2$	PTLN#TTYLQTLR
H5	2611.2	$XylMan_3GlcNAc_2$	MGN#ITPLTGTQGQIR
H6	2707.7	$XylMan_2GlcNAc_2$	SFAN#STQTFFNAFVEAMDR
H7	2850.3	FucGlcNAc	GLIQSDQELFSSPN#ATDTIPLVR
H8	3074.3	$FucGlcNAc_2$	LHFHDCFVNGCDASILLDN#TTSFR
H9	3088.8	$XylMan_3FucGlcNAc_2$	GLCPLNGN#LSALVDFDLR
H10	3222.0	$Man_3FucGlcNAc_2$	SFAN#STQTFFNAFVEAMDR
H11	3321.6	$XylMan_3GlcNAc_2Fuc$	QLTPTFYDNSCPN#VSNIVR
H12	3354.3	$XylMan_3GlcNAc_2Fuc$	SFAN#STQTFFNAFVEAMDR
H13	3371.1	$XylMan_3FucGlcNAc_2$	LYN#FSNTGLPDPTLN#TTYLQTLR
H14	3388.4	$XylHex_6HexNAc_4Fuc_2$	DSFRNVGLN#R
H15	3509.5	$XylMan_2FucGlcNAc_2$	GLIQSDQELFSSPN#ATDTIPLVR
H16	3526.4	$XylMan_3GlcNAc_2$	GLIQSDQELFSSPN#ATDTIPLVR
H17	3539.8	$Man_3FucGlcNAc_2$	GLIQSDQELFSSPN#ATDTIPLVR
H18	3605.7	$XylMan_3GlcNAc_2Fuc$	NQCRGLCPLNGN#LSALVDFDLR
H19	3672.2	$XylMan_3GlcNAc_2Fuc$	GLIQSDQELFSSPN#ATDTIPLVR
H20	3894.0	$XylMan_3GlcNAc_2Fuc$	LHFHDCFVNGCDASILLDN#TTSFR
H21	4056.9	$XylMan_4GlcNAc_2Fuc$	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
H22	4223.0	$XylMan_3GlcNAc_2Fuc$	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
H23	4822.9	$XylMan_2FucGlcNAc_2$, $XylMan_2GlcNAc_2$	LYN#FSNTGLPDPTLN#TTYLQTLR
H24	4839.7	$XylMan_3FucGlcNAc_2$, $XylMan_3GlcNAc_2$	LYN#FSNTGLPDPTLN#TTYLQTLR
H25	4984.7	$XylMan_3GlcNAc_2Fuc$	LYN#FSNTGLPDPTLN#TTYLQTLR

Table S2. Corresponding comparison of sensitivity toward phosphopeptides/glycopeptides enrichment based on different materials.

Material	Phosphopeptides, fmol	Glycopeptides, fmol	Ref.
MF@PDA@UiO-66-NH_2	0.5	0.5	47
CS@PGMA@IDA-Ti^{4+}	0.1	0.1	48
Fe_3O_4@SiO_2@(Zr–Ti-MOF)$_{10}$-NH_2	1	1	49
SiO_2-NH_2@TiO_2	0.16	2	50
Fe_3O_4@ILI-01@Ti^{4+}	0.5	0.1	This work

Table S3. Corresponding comparison of selectivity toward phosphopeptides/glycopeptides enrichment based on different materials.

Material	Glycopeptides	Phosphopeptides	Ref.
MF@PDA@UiO-66-NH_2	1:1000	1:200	47
CS@PGMA@IDA-Ti^{4+}	1:100	1:500	48
Fe_3O_4@SiO_2@(Zr–Ti-MOF)$_{10}$-NH_2	1:50	1:2000	49
SiO_2–NH_2@TiO_2	1:500	1:500	50
Fe_3O_4@ILI-01@Ti^{4+}	1:500	1:5000	This work

Table S4. Corresponding comparison of actual samples toward phosphopeptides/glycopeptides enrichment based on different materials.

Material	Serum	Saliva	Ref.
MF@PDA@UiO-66-NH_2	-	52 GPs/41 PPs	47
CS@PGMA@IDA-Ti^{4+}	-	-	48
Fe_3O_4@SiO_2@(Zr–Ti-MOF)$_{10}$-NH_2	41 GPs-4 PPs	-	49
SiO_2–NH_2@TiO_2	-/4 PPs	-/17 PPs	50
Fe_3O_4@ILI-01@Ti^{4+}	37 GPs/4 PPs	23 GPs/16 PPs	This work

GPs: glycopeptides. PPs: phosphopeptid**es.** – represented that data was not provided.

Table S5. Detailed information on identified phosphopeptides/glycopeptides enriched from human serum by Fe_3O_4@ILI-01@Ti^{4+}. [pS]:phosphorylation site. N#: N-glycosylation site.

Peak	Observed *m/z*	Amino acid sequence
P1	1389	D[pS]GEGDFLAEGGGV
P2	1461	AD[pS]GEGDFLAEGGGV
P3	1545	D[pS]GEGDFLAEGGGVR
P4	1616	AD[pS]GEGDFLAEGGGVR
Peak	**Observed *m/z***	**Amino acid sequence**
G1	1076	WVSN#KTEGR
G2	1121	FN#DTEVLQR
G3	1138	GLcVN#ASAVSR
G4	1190	EEQYN#STYR
G5	1198	KKEDALN#ETR
G6	1226	EN#ISDPTSPLR
G7	1275	AAIPSALDTN#SSK
G8	1364	HIPGLIHN#MTAR
G9	1372	VVN#STTGPGEHLR
G10	1387	GN#ETLHYETFGK
G11	1400	FLN#DTMAVYEAK
G12	1405	SWPAVGN#cSSALR
G13	1423	KLI N#DYVKnGTR
G14	1435	GTA N#TTTAGVPcQR
G15	1443	mDGASN#VTcINSR
G16	1490	GAFISN#FSmTVDGK
G17	1494	N#LSmPLLPADFHK
G18	1512	AEPPLN#ASASDQGEK
G19	1530	YDFN#SSMLYSTAK
G20	1548	YDFN#SSmLYSTAK
G21	1554	KLPPGLLAN#FTLLR
G22	1598	KNAHGEEKEN#LTAR
G23	1613	FVEGSHN#STVSLTTK
G24	1628	LN#DSIQTLVNDNQR
G25	1640	TKPREEQFN#STFR
G26	1657	TKPREEQFN#STYR
G27	1677	TKPREEQYN#STYR
G28	1699	YKGLN#LTEDTYKPR
G29	1714	QVHFFVN#ASDVDNVK
G30	1817	CATPHGDN#ASLEATFVK
G31	1911	QDQCIYN#TTYLNVQR
G32	2037	VVLHPN#YSQVDIGLIKLK
G33	2044	AN#PTVTLFPPSSEELQANK
G34	2087	SEGSSVN#LSPPLEQCVPDR
G35	2164	VSN#QTLSLFFTVLQDVPVR
G36	2229	TLN#QSSDELQLSmGNAMFVK
G37	2273	TVLTPATNHmGN#VTFTIPANR

Table S6. Detailed information on identified phosphopeptides and glycopeptides enriched from human saliva by Fe_3O_4@ILI-01@Ti^{4+}. [pS]:phosphorylation site. N#: N-glycosylation site.

Peak	Observed *m/z*	Amino acid sequence
P1	1001.3	[pS]EEKFLR
P2	1021.0	ItFYEDR
P3	1076.2	[pS]SEEKFLR
P4	1136.9	GK[pS]PPPEFAK
P5	1154.8	[pS][pS]EEKFLR
P6	1214.8	LN[pS]EGMEEAR
P7	1233.0	[pS]EQFIDEER
P8	1270.8	D[pS][pS]EEKFLR
P9	1312.8	[pS][pS]EEKFLRR
P10	1426.8	D[pS][pS]EEKFLRR
P11	1443.6	GILAADESVG[pS]mAK
P12	1471.4	DVnSS[pS]PVMLAFK
P13	1539.5	FGQG[pS]GPIVLDDVR
P14	1663.6	SDGGD[pS]EQFIDEER
P15	1752.2	QPPQ[pS]StMGymGSQ
P16	1900.5	GPPGSRG[pS]PGAPGPPGPPGSH
Peak	**Observed *m/z***	**Amino acid sequence**
G1	1166.7	GPPPQEGN#KSR
G2	1222.8	DAGVVcTN#ETR
G3	1337.5	N#LTTSLTESVDR
G4	1395.2	KPERPPPQGGN#QS
G5	1524.3	LVNLN#SSYGLcAGR
G6	1572.6	N#HScEPcQTLAVR
G7	1664..0	HYTN#SSQDVTVPcR
G8	1677.2	KPEGPPPQEGN#KSRSA
G9	1732.8	RPGKPEGPPPQGGN#QSQ
G10	1769.2	PPQGGN#KSQGPPPPGKPQ
G11	1856.0	AAGAN#GSADDSPGHVPVPGA
G12	1867.6	VVLLDPKPVAN#VTcVNK
G13	1933.1	AGPPGPAGPAGPPGPIGN#VGAPGA
G14	2030.6	PGPPGLPGPQGPKGN#GSTGPAG
G15	2083.1	KPEGPPPQGGN#QSQGPPPRPG
G16	2161.7	GPPPHPGKPEGPPPQEGN#KSR
G17	2246.9	PPQGGN#KSQGPPPPGKPQGPPPQ
G18	2488.0	GKPEGPPPQGGN#QSQGPPPRPGKPE
G19	2526.3	QIGLYPVLVIDSSGYVNPN#YTGR
G20	2723.1	SPPGKPQGPPPQGGN#QPQGPPPPPGKPQ
G21	2795.0	DLMLLQLDREAN#LTSSVTILPLPLQ
G22	2879.7	GPPPPPGKPQGPPPQGGN#KSQGPPPPGKPQ
G23	2913.4	NVVDGQPFTNWYDN#GSNQVAFGRGNR

Table S7 LC-MS/MS identification results of phosphopeptides from tryptic digest of human serum after enrichment by Fe_3O_4@ILI-01@Ti^{4+}.

Index	Protein ID	Peptides Sequence
1	P04114	R.VRES#DEETQIK.V
2	P02768	K.T#CVADESAENCDKSLHTLFGDK.L
3	P02768	K.TCVADES#AENCDK.S
4	P02768	R.YT#KKVPQVSTPTLVEVSR.N
5	P02768	R.Y#TKKVPQVSTPTLVEVSR.N
6	P02647	R.THLAPYS#DELR.Q
7	P00747	K.KQLGAGS#IEECAAK.C
8	P00747	K.QLGAGS#IEECAAK.C
9	P00747	R.AFQY#HSKEQQCVIMAENR.K
10	P00747	R.AFQYHS#KEQQCVIMAENRK.S
11	P01009	K.TDTS#HHDQDHPTFNK.I
12	P01042	K.ES#NEELTESCETK.K
13	P01042	K.ES#NEELTESCETKK.L
14	P01042	R.ETT#CSKESNEELTESCETK.K
15	P01042	R.ETTCS#KESNEELTESCETK.K
16	P01042	R.ET#TCSKESNEELTESCETK.K
17	P01042	R.ETTCSKES#NEELTESCETKK.L
18	P01008	K.KATEDEGS#EQKIPEATNR.R
19	P02649	R.GEVQAMLGQS#TEELR.V
20	P02765	K.CDS#SPDSAEDVRK.V
21	P02765	K.CDSSPDS#AEDVR.K
22	A8MZA4	K.IREQEEM*T#QEQEEK.M
23	O14791	K.HLHEGAKS#ETAEELKK.V
24	O14791	R.VTEPIS#AESGEQVER.V
25	O14791	VTEPISAES#GEQVER.V
26	O15520	K.LFS#FTK.Y
27	O75122	R.KPGS#AGGPK.V
28	O75829	K.QSISS#KLEGK.I
29	O95294	M.AKS#S#SLNVR.V
30	O95859	K.KM*Y#SFLRGTK.Q
31	P02647	R.THLAPYS#DELR.Q
32	P02652	K.VKS#PELQAEAK.S
33	P02671	K.M*ADEAGS#EADHEGTHSTK.R
34	P02671	K.MADEAGS#EADHEGTHSTK.R
35	P02671	K.MADEAGS#EADHEGTHSTKR.G
36	P02765	K.CDSSPDS#AEDVR.K
37	P02765	K.CDSSPDS#AEDVRK.V
38	P02765	R.HTFM*GVVSLGSPS#GEVSHPR.K
39	P02765	R.HTFMGVVSLGSPS#GEVSHPR.K
40	P08151	R.RSSSSSS#ISSAYTVSR.R
41	P08833	K.AQETS#GEEISK.F

42	P12259	R.LLSLGAGEFKS#QEHAK.H
43	P17936	R.YKVDYESQSTDTQNFS#SESKR.E
44	P31948	R.EDY#RQIAK.A
45	P35579	R.KGAGDGS#DEEVDGK.A
46	P35712	R.FENLGPQLT#GK.S
47	P46013	K.SSEPVVIM*KRS#LR.T
48	P46100	K.KIKVDS#EK.S
49	P48067	R.GVKSS#GK.V
50	P49908	R.DM*PAS#EDLQDLQK.K
51	P49908	R.DMPAS#EDLQDLQKK.L
52	P50479	R.RPSGTGT#GPEDGRPSLGSPYGQPPR.F
53	P50479	R.RPSGT#GTGPEDGRPSLGSPYGQPPR.F
54	P57740	R.QS#QLVVDWLESIAK.D
55	P61026	K.SFENIS#K.W
56	P85037	R.QS#PGPALARLEGR.E
57	Q13103	R.S#LGIMR.R
58	Q13443	R.NFSS#CSAEDFEK.L
59	Q13535	R.KDAES#RR.R
60	Q14202	R.KCTTPRGT#TK.V
61	Q14515	K.S#KEESHEQSAEQGK.S
62	Q14515	K.SKEES#HEQSAEQGK.
63	Q14515	R.AEAEENEKETAVS#TEDDSHHK.A
64	Q14515	K.SSS#QELGLK.D
65	Q14974	K.T#VSPDR.L
66	Q2TAY7	R.LIMQY#LK.E
67	Q5HYC2	K.KS#VSKK.A
68	Q5SZK8	R.EGGAPET#LLMDCK.A
69	Q5T1R4	R.KHS#LTK.N
70	Q7RTP6	K.Y#M*ATQLLAK.F
71	Q7Z407	K.QRT#APK.T
72	Q7Z7A1	K.VSSHSSQAT#K.D
73	Q86V59	MS#KT#M*AM*NLLEDWCR.G
74	Q86YS7	R.QSSCGVKFFCTTSIPKCY#R.A
75	Q8N0Z2	K.EVSKT#VVSK.T
76	Q8N7Z5	R.KT#QHKR.T
77	Q8TCC3	K.NIPS#VNAKLK.V
78	Q8TF74	K.LKKVT#NINDR.S
79	Q8WZ42	R.QTDSTTWVELAT#T#VIR.T
80	Q96ME7	R.S#T#RIVGAKNSR.T
81	Q96MT7	R.KDGDLTTRDS#ISR.S
82	Q99578	R.KKESM*PS#LM*EK.K
83	Q99961	K.LTMLNT#VSK.I
84	Q9BVQ7	R.LGSAVKIS#LPDGGS#CLCT#AWPR.R
85	Q9P270	K.KKLT#PMQK.S
86	Q9UK53	R.QFQAAS#LLTR.G

87	Q9UK55	K.EEEEDEQEAS#EEK.A
88	Q9ULH0	R.DMGGWTALMWACY#KGR.T
89	Q9UQ03	K.RLLTT#GVSR.W
90	Q9Y485	K.QDM*YLSS#K.E
91	Q9Y6W5	K.SS#LPAVSDAR.S
92	A0A0A0MTS7	K.KPEAPAVTVPEVPQEAT#EKEIPVAPPK.K
93	A0A0G2JIT5	R.RPLS#SSAPR.D
94	A0A0J9YWI3	K.IISGSSGS#LLSSGSGAR.R
95	A0A126LAY2	K.QLS#VALNIDR.R
96	A0A1W2PQW7	K.GTRES#GQKAK.T
97	A0A5C2GEV4	EVQLAVS#GGGLVQPGGSLR.L
98	A0A5C2GEX6	DLQLVES#GGGLVKPGGSLR.L
99	A0A5C2GF59	K.DT#HPVTTR.G
100	A0A5C2GF59	K.DT#HPVTTR.G
101	A0A5C2GF59	K.DT#HPVTTR.G
102	A0A5C2GJK3	K.FGTSASLAIS#GLR.S
103	A0A5C2GLK7	K.EVQVS#KSGGGLVQPGGSLR.L
104	A0A5C2GQ09	K.AVQMT#QSPSSLSASVGDR.V
105	A0A7S5C359	R.LSCAASGFTFT#R.Y
106	A0A7S5C359	R.LSCAASGFTFT#R.H
107	A0A804HI41	R.DPS#PVSDR.F
108	B2RBZ5	R.EEEEDEQEAS#EEKAGEEEK.A
109	D6RG00	R.FQFIY#LNLL.-
110	E9PFE3	K.KM*PT#LLPR.L
111	F5H6I8	K.WVGSSS#.-
112	H0Y5I1	R.QDAM*VVS#SRPK.A
113	H0Y7L6	R.X#PEKPVK.Q
114	H0Y7Y5	R.AAT#FR.L
115	H0Y7Y5	R.AAT#FR.L
116	H0YHH4	K.XPESS#RSEASR.I
117	H0YJB2	K.XT#LAPER.W
118	H7C1Y7	K.T#PLKDR.G
119	K7ERQ3	K.XWMLLT#GK.L
120	Q9NSI4	K.SQYVPGST#MRLIKTK.S
121	Q9NSI9	K.LFKLES#KNR.W
122	S4R325	R.QEPGGPST#R.K

phosphor (STY); * Oxidation (M)

Table S8 LC-MS/MS identification results of glycopeptides from tryptic digest of human serum after enrichment by Fe_3O_4@ILI-01@Ti^{4+}.

Index	Protein ID	Peptides Sequence
1	Q96K17	LAVNNIAGIEEVnMIKDDGTVIHFNNPK
2	Q8IVF2	TECSTDLQPPEGVPTSQAESHSGPLnSMIPVSLGQVSFPK
3	B3KY79	TLNETELTELQSQISDTSVVLSMnNSR
4	P04075	YASICQQnGIVPIVEPEILPDGDHDLK
5	A8MXP9	GDADQASnILASFGLSAR
6	B3KN30	HVMNFTnWAIASGSSTALLYSK
7	A0A4P8J3Z9	InCGGNNIGSK
8	Q96JA1	RAFSGLEGLEHLNLGGnAIR
9	P02765	AALAAFNAQNnGSNFQLEEISR
10	P02765	VcQDcPLLAPLnDTR
11	P01834	VDnALQSGnSQESVTEQDSK
12	S6BAQ4	SLSLQMHnLR
13	Q9Y520	KNADLnAQTVVK
14	B4DI38	QVAYIYKCVnTTLQIK
15	P0DOX7	VDnALQSGnSQESVTEQDSK
16	B3GQS7	DMAIATGGAVFGEEGLTLnLEDVQPHDLGK
17	D6RBL1	NIDQTMLSILLFFHSASGASVVAIDnK
18	P01011	TLnQSSDELQLSMGNAMFVK
19	P01011	FnLTETSEAEIHQSFQHLLR
20	P01011	KLInDYVKnGTR
21	E7EX73	GGEELLPPESTPIPANLSQnLEAAAATQVAVSVPK
22	C9JIZ6	DnGDVCQDCIQMVTDIQTAVR
23	P00738	VVLHPnYSQVDIGLIKLK
24	P00738	NLFLnHSENATAK
25	P00738	MVSHHnLTTGATLINEQWLLTTAK
26	A0A2X0SFE1	QASVAAEnSVICSFLHYMEKGGK
27	Q6ZTR5	HDDDMSSSGSDTDQGCSDSPnVLHTSIK
28	P01042	LNAENnATFYFK
29	A0A2H4G3R8	DYIALnEDLSTWTAADTAAQITQR
30	P02749	VYKPSAGnNSLYR
31	P54802	LLLTAAPnLTTSPAFR
32	P25391	TVLAnVTHLLIR
33	P00739	MVSHHnLTTGATLINEQWLLTTAK
34	P00739	NLFLnHSENATAK
35	P02768	MPCAEDYLSVVLnQLCVLHEK
36	P02768	ETFFnLSKR
37	B4E1Z4	QSVPAHFVALnGSK
38	P01591	IIVPLNNREnISDPTSPLR
39	P01591	EnISDPTSPLR
40	Q8IUP8	TNGNTYMLLTnATLDR
41	Q9UK55	LPYQGnATMLVVLMEK

42	Q9UK55	ETFFnLSKR
43	Q9Y490	ALCGFTEAAAQAAYLVGVSDPnSQAGQQGLVEPTQFA R
44	K4DI92	EQFMENHnPINSATSISNIISIETPNTAPSSK
45	Q06033	KNAHGEEKEnLTAR
46	Q06033	NAHGEEKEnLTAR
47	A0A140TA32	GHLFLQTDQPIYnPGQR
48	P32004	LLFPTnSSSR
49	P02751	NSITLTnLTPGTEYVVSIVALnGR
50	Q13201	FNPGAESVVLSnSTLK
51	O75882	IDSTGnVTNELR
52	P25311	DIVEYYnDSnGSHVLQGR
53	Q02985	FVQGnSTEVACHPGYGLPK
54	P08603	MDGASnVTcINSR
55	P08603	SPDVInGSPISQK
56	F6KPG5	SHCIAEVEnDEMRADLPSLAADFVESK
57	O75882	ISnSSDTVECECSENWK
58	Q8TAX7	ITTLPnVTFLPQnATTISSR
59	P01877	nFPPSQDASGDLYTTSSQLTLPATQCPDGK
60	P01877	TPLTAnITK
61	P09871	NCGVnCSGDVFTALIGEIASPnYPKPYPEnSR
62	A0A5F9ZH15	NPVGLIGAEnATGETDPSHSK
63	B2RMS9	LALDnGGLAR
64	O75882	IDSTGnVTNELR
65	O75882	ISnSSDTVEcEcSENWK
66	P0CG04	AnPTVTLFPPSSEELQANK
67	P05546	nLSMPLLPADFHK
68	P22792	LYLGSnnLTALHPALFQnLSK
69	P22792	LSnNALSGLPQGVFGK
70	Q6UXB8	SLPNFPnTSATANATGGR
71	P10909	LAnLTQGEDQYYLR
72	P00739	MVSHHnLTTGATLINEQWLLTTAK
73	P00739	TEGDGVYTLnDKK
74	P43251	FnDTEVLQR
75	Q08380	AAIPSALDTnSSK
76	Q08380	ALGFEnATQALGR
77	P43251	YQFNTNVVFSNnGTLVDR
78	Q15818	ALPGGTDnASAASAAGGSGPQR
79	P10253	GVFITnETGQPLIGK
80	P10253	LEnLSSTESGYTATLTR
81	P32004	VPGnQTSTTLK
82	P32004	THnLTNLNPDLQYR
83	Q9Y6R7	VVTVAALGTnISIHKDEIGK

n: N-glycosylation site