Designed synthesis of a “One for Two” hydrophilic magnetic amino-functionalized metal-organic framework for highly efficient enrichment of glycopeptides and phosphopeptides

Supporting Information

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Experimental Section

1. Materials and reagents

Zircomiun tetrachloride ($ZrCl_4$), 2-aminoterephthalic acid ($H_2BDC-NH_2$), Phosphoric acid (H_3PO_4), N,N'- Dimethylformamide (DMF), Horseradish peroxidase (HRP), Immunoglobulin G (IgG), β-casein from bovine milk, Albumin from bovine serum (BSA), Trypsin from bovine pancreas, Formic Acid (FA) and Trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich. Iron (III) chloride hexahydrate ($FeCl_3•6H_2O$), Sodium acetate (CH_3COONa), Ethanol, Ethylene glycol, Ammonium hydroxide (NH3•H2O) and Ammonium bicarbonate (NH_4HCO_3) were purchased from Sinopharm Chemical Reagent Co., Ltd. Acetonitrile (ACN) was purchased from Merck. Tris(hydroxymethyl) aminomethane (Tris), and 2,5-dihydroxybenzoic acid (DHB) were purchased from J&K Scientific. Human serum was supplied by Zhongshan Hospital. All other chemicals are of the top grade commercially available. Distilled water was purified using Milli-Q system (Millipore, Bedford, MA).

2. Synthesis of Fe_3O_4@PDA@UiO-66-NH_2

Fe_3O_4 nanoparticles were prepared via a well-known solvo-thermal reaction.[1] In detail, 1.35 g $FeCl_3•6H_2O$ was dispersed in 75 mL ethylene glycol under stirring until completely dissolved. Then, 3.60 g smashed CH_3COONa was added into the above solution and under stirring for 30 min until a homogeneous solution was obtained. After that, the solution was transferred into a Teflon-lined stainless-steel autoclave and kept at 200C° for 16h. The obtained Fe_3O_4 was collected by magnetic separation, washed with deionized water and ethanol for several times and finally kept in vacuum oven at 50C°.

Fe_3O_4@PDA was prepared via the polymerization of dopamine in basic solution. In detail, 0.12 g Fe_3O_4 was added into 80 mL Tris buffer, which contained 0.05 g Tris and 0.32 g dopamine hydrochloride. The solution was kept vigorously stirring for 16 h at room temperature. The obtained Fe_3O_4@PDA was collected by magnetic separation, washed with deionized water and ethanol for several times and kept in vacuum oven at 50C°.

Fe_3O_4@PDA@UiO-66-NH_2 was prepared by a simple one-pot reaction. 0.10 g Fe_3O_4@PDA was dispersed in 75 mL DMF of MOFs precursors, containing 0.16 g $ZrCl_4$ (9mM) and 0.12 g (9mM) 2-aminoterephthalic acid (H_2BDC-NH_2) under 120C° for 45 min. The obtained Fe_3O_4@PDA@ UiO-66-NH_2 was collected by magnetic separation, washed with DMF and ethanol for several times and kept in vacuum oven at 50C°.

3. Characterization and measurements

Scanning electron microscopy (SEM) images and Energy dispersive X-ray (EDX) were operated on a Philips XL30 electron microscope (Netherlands) at 20 kV. Transmission electron microscopy (TEM) images were operated on a JEOL 2011 microscope (Japan) at 200 kV. Fourier transform infrared spectra (FT-IR) was operated on a Nicolet Fourier spectrophotometer (U.S.A.). Raman spectra (Raman) was operated on a LabRam-1B Raman spectrometer. Magnetization measurement was operated on a MPMS (SQUID) VSM (U.S.A.). Powder X-ray diffraction patterns (XRD) were operated on a Bruker D4 X-ray diffractometer at 40 kV, 40 mA. Nitrogen sorption isotherms were operated on a Micromeritcs Tristar 3000 analyzer (U.S.A.) at 77 K. Zeta potential measurements were operated on a Nano ZS90 zeta analyzer (Malvern Instruments Ltd.).

4. Sample preparation

The as-prepared Fe_3O_4@PDA@UiO-66-NH_2 was suspended in deionized water to make a dispersion of 10 mg/mL solution for further use.

For glycopeptide: 30 mg DHB was added into 1 mL 50%ACN to make a dispersion of 30 mg/mL solution for further use.

For phosphopeptide: 20 mg DHB was added into 1 mL 50%ACN (containing 1% H_3PO_4) to make a dispersion of 20 mg/mL solution for further use.

Horseradish peroxidase (HRP), Immunoglobulin G (IgG), β-Casein and Albumin from bovine serum (BSA) were dissolved in 25 mM NH_4HCO_3 buffer (pH=8.3), denatured at 100 C° for 10 min and incubated with trypsin (trypsin: protein is 1:50, w/w) at 37 C° for 16 h. The final concentration of HRP was 2 mg/mL, IgG was 4 mg/mL, β-Casein was 2.5 mg/mL and BSA was 5 mg/mL. The obtained tryptic digests were diluted with loading buffer (90%ACN/1%TFA for glycopeptide and 50%ACN/0.1%TFA for phosphopeptide) for further enrichment and analysis.

For glycopeptide enrichment: Human serum (2 μL) was diluted in 198 μL NH_4HCO_3 buffer and denatured for 10 min. The mixture was reduced by 10 mM DTT at 60 C° for 30 min and alkylated by 20 mM IAA at 37 C° for 1 h in the dark. Then the obtained mixture was incubated with trypsin (trypsin: protein is 1:50, w/w) at 37 C° for 16 h. The obtained tryptic digests were lyophilized for further enrichment and analysis.

For phosphopeptide enrichment: Human serum (2 μL) was diluted in 50%ACN/0.1%TFA by 10 fold without additional process.

5. Enrichment of glycopeptides or phosphopeptides from standard tryptic digests

The workflow of glycopeptide or phosphopeptide enrichment is illustrated in Scheme S1.

For glycopeptide enrichment: Detailedly, 200 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into 100 μL mixture of peptides (diluted by 90%ACN/1%TFA) and the obtained solution was vibrated in a vortex at 37 C° for 30 min. After removal of the supernatant under magnetic separation, the MOFs was rinsed with 90%ACN/1%TFA once and 80%ACN/1%H_3PO_4 twice to

remove non-glycopeptides. After that, 5 μL 30%ACN/0.1%FA was added into the MOFs and vibrated in the same vortex to elute the glycopeptides at 37 C° for 20 min. The elution was collected under magnetic separation.

For phosphopeptide enrichment: Detailedly, 200 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into 100 μL mixture of peptides (diluted by 50%ACN/0.1%TFA) and the obtained solution was vibrated in a vortex at 37 C° for 30 min. After removal of the supernatant under magnetic separation, the MOFs was rinsed with 50%ACN/0.1%TFA three times to remove non-phosphopeptides. After that, 10 μL 0.4 M $NH_3 \cdot H_2O$ was added into the MOFs and vibrated in the same vortex to elute the phosphopeptides at 37 C° for 20 min. The elution was collected under magnetic separation.

Finally, 1 μL of elution was deposited on a MALDI plate and let dry, followed by 0.5 μL DHB and let dry and analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

6. MALDI-TOF MS analysis

MALDI-TOF MS measurements were operated on a 5800 Proteomics Analyzer (Applied Biosystems, U.S.A.) in positive ion mode at the Nd:YAG laser of 355 nm, the repetition rate of 200 Hz and the acceleration voltage of 20 kV.

7. Enrichment of glycopeptides or phosphopeptides from tryptic digest mixtures of HRP/β-Casein and BSA

200 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into 100 μL tryptic digest mixtures of HRP/β-Casein and BSA at a given mass ratio. The enrichment process was the same as the section 5. The collected elution was analyzed by MALDI-TOF MS.

8. Enrichment of glycopeptides or phosphopeptides from healthy human serum

For glycopeptide enrichment: The lyophilized tryptic digests of human serum were redissolved in 100 μL loading buffer and then 400 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into the above solution. The enrichment process was the same as the section 5. The collected elution was lyophilized and redissolved in 50 μL 25 mM NH_4HCO_3 solution, and then 1 μL PNGase F was added at 37C° for 16 h to remove the glycans. The collected solution was lyophilized again for further LC-MS/MS analysis.

For phosphopeptide enrichment:

For human serum without treatment: It was the same as the standard phosphoprotein. Detailedly, 200 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into 200 μL serum dilution, containing 10 μL 10-fold-diluted human serum and 190 μL loading buffer. After enrichment, washing and elution, 1 μL of elution was deposited on a MALDI plate and let dry, followed by 0.5 μL DHB and let dry and analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

For human serum digests: The lyophilized tryptic digests of human serum were redissolved in 100 μL loading buffer and then 400 μg Fe_3O_4@PDA@UiO-66-NH_2 was added into the above solution. The enrichment process was the same as the section 5. The collected elution was lyophilized for further LC-MS/MS analysis.

9. Nano High Performance Liquid Chromatography- Mass Spectrometry (LC-MS/MS) analysis of glycopeptides and phosphopeptides

The peptide samples were resuspended with 10 μL solvent A respectively (A: water with 0.1% formic acid; B: ACN with 0.1% formic acid), separated by nanoLC and analyzed by

on-line electrospray tandem mass spectrometry. The experiments were performed on an EASY-nLC 1000 system (Thermo Fisher Scientific, Waltham, MA) connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an online nano-electrospray ion source. 4 μL peptide sample was loaded onto the analytical column (Acclaim PepMap C18, 75 μm x 50 cm) with a linear gradient, from 2% B to 40% B in 110 min. The column was re-equilibrated at initial conditions for 10 min. The column flow rate was maintained at 200nL/min. The electrospray voltage of 2.0 kV versus the inlet of the mass spectrometer was used.

The Orbitrap Fusion mass spectrometer was operated in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra (m/z 350-1500) were acquired in Orbitrap with a mass resolution of 120 000 at m/z 200. The AGC taget was set to 300 000, and the maximum injection time was 50ms. MS/MS acquisition was performed in Orbitrap with 3 s cycle time, the resolution was 30 000 at m/z 200. The intensity threshold was 50 000, and the maximum injection time was 150 ms. The AGC target was set to 200 000, and the isolation window was 2 m/z. Ions with charge states 2+, 3+, and 4+ were sequentially fragmented by higher energy collisional dissociation (HCD) with a normalized collision energy (NCE) of 30%, fixed first mass was set at 110. In all cases, one microscan was recorded using dynamic exclusion of 50 seconds.

10. Database Search

For glycopeptide search:

Tandem mass spectra were extracted by Proteome Discoverer software (Thermo Fisher Scientific, version 1.4.0.288). Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 2.3). Mascot was set up to search the human Uniprot-SwissProt database (release 2015_03_11, with 20199 entries) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.050 Da and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was specified in Mascot as fixed modifications. Oxidation of methionine and deamidation of asparagine were specified in Mascot as a variable modification. Percolator algorithm was used to control peptide level false discovery rates (FDR) lower than 1%.

Only the identified deamination sites which were confirmed to the N-glycosylation consensus sequence (n-!P-[S/T]) were considered as glycosylation sites.

For phosphopeptide search:

The raw mass spectrometry data files were extracted using the Proteome Discoverer software (Thermo Fisher Scientific, version 1.4.0.288), and the MS/MS results were analyzed using Mascot (Matrix Science, London, UK; version 2.3). The search parameters were as follows: Database was specified in Mascot as Uniprot-SwissProt (Taxonomy: human, 20199 entries), enzyme was specified as trypsin, a fragment ion mass tolerance was specified as 0.050 Da and a parent ion tolerance was specified as 10.0 PPM. Carbamidomethyl of cysteine was specified in Mascot as fixed modifications. Variable modifications of +80 Da for Ser, Thr, and Tyr, and +16 Da for Met. Peptide level false discovery rates (FDR) were controlled lower than 1% by the percolator algorithm. Using the PhosphoRS 3.0 software to calculate the probability of phosphorylation site.

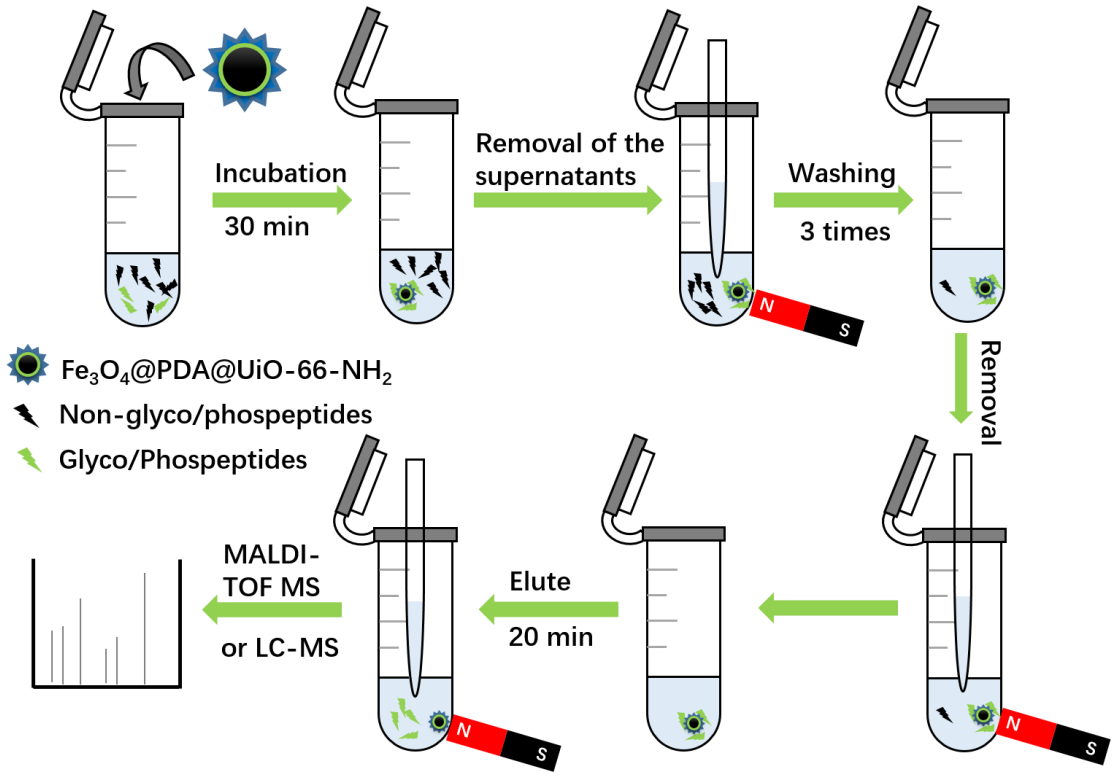
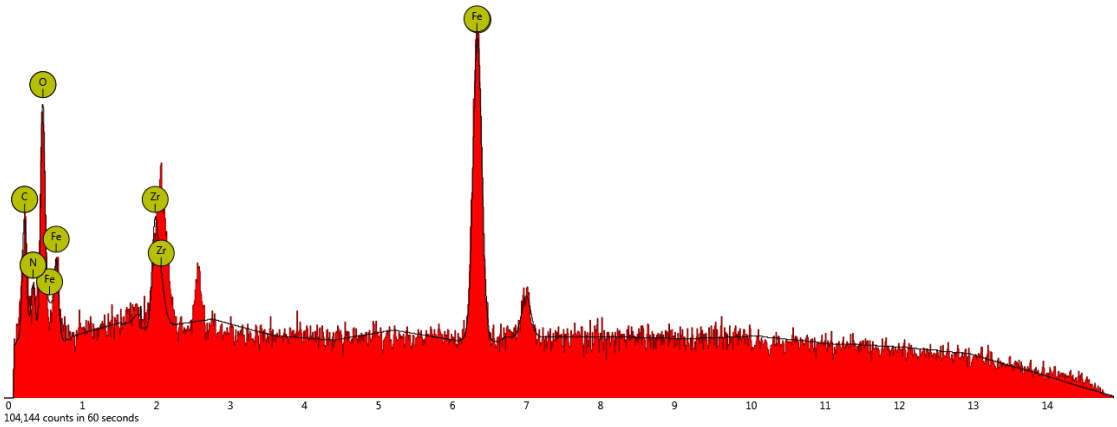


Figure S1. Workflow of glycopeptide or phosphopeptide enrichment from biological samples using Fe_3O_4@PDA@UiO-66-NH_2.



Element Number	Element Symbol	Element Name	Weight Concentration	Error
26	Fe	Iron	41.9	0.1
8	O	Oxygen	20.0	0.2
40	Zr	Zirconium	8.0	1.1
6	C	Carbon	18.2	1.0
7	N	Nitrogen	11.9	0.8

Figure S2. Energy dispersive X-ray (EDX) spectrum data of Fe_3O_4@PDA@UiO-66-NH_2.

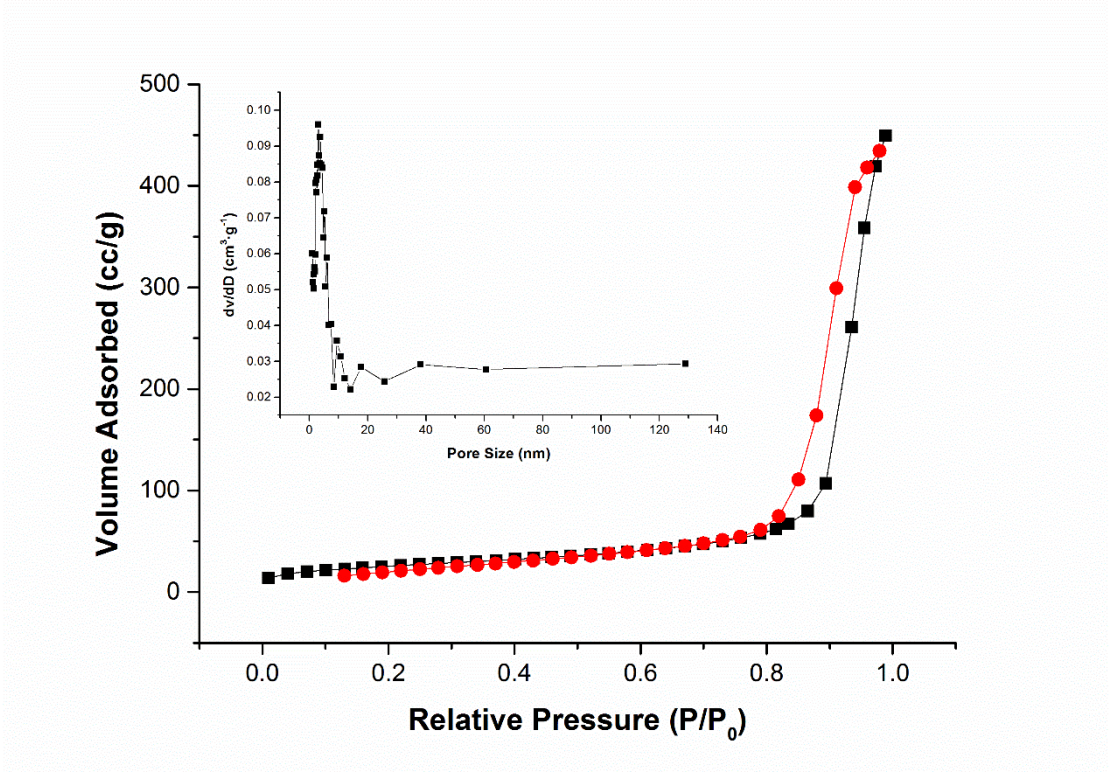


Figure S3. Nitrogen sorption isotherms and pore size distribution of Fe_3O_4@PDA@UiO-66-NH_2.

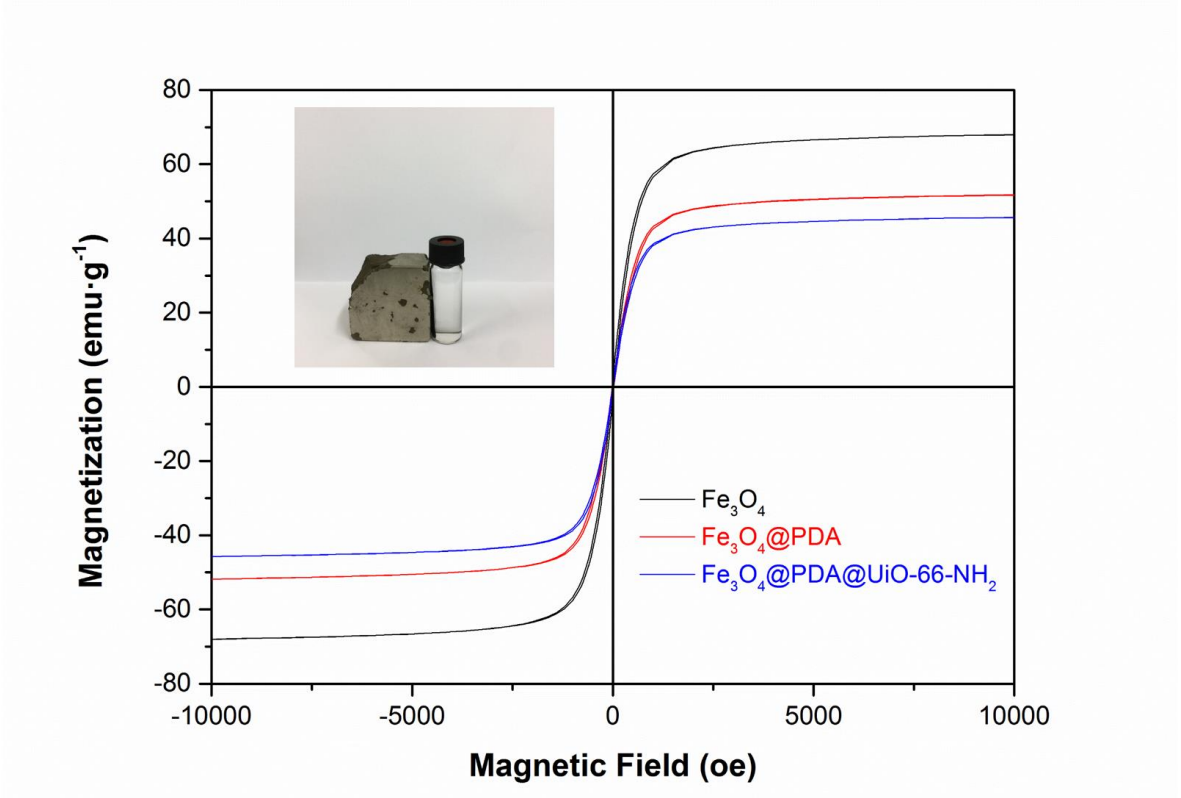


Figure S4. Magnetic hysteresis curves of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@UiO-66-NH_2. The saturation magnetization values of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@UiO-66-NH_2

were 67.9 emu·g^{-1}, 51.6 emu·g^{-1} and 45.6 emu·g^{-1} respectively.

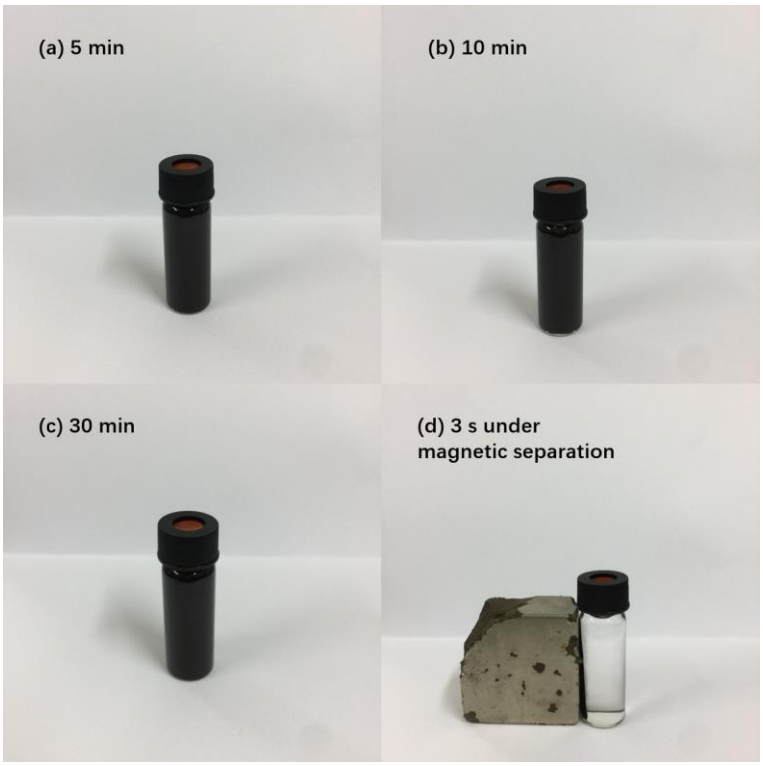


Figure S5. MOFs dispersion in water solution: (a) 5 min, (b) 10 min, (c) 30 min, and (d) 3 s under magnetic separation.

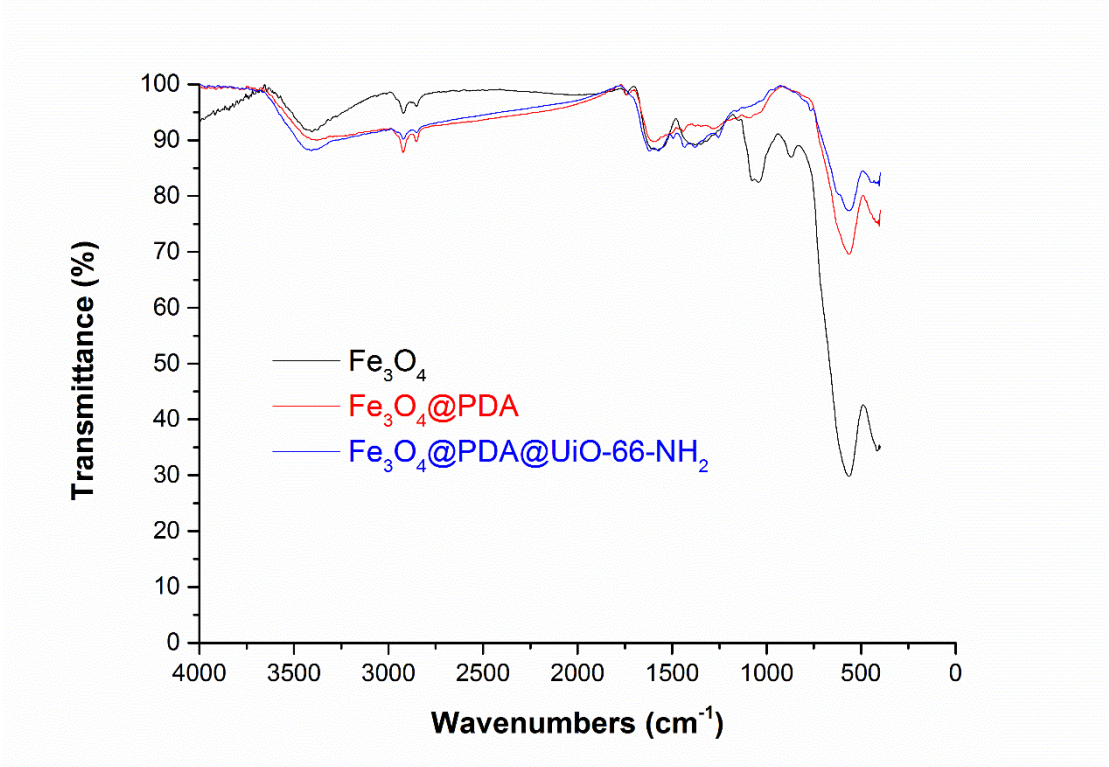


Figure S6. FT-IR spectra of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@UiO-66-NH_2.

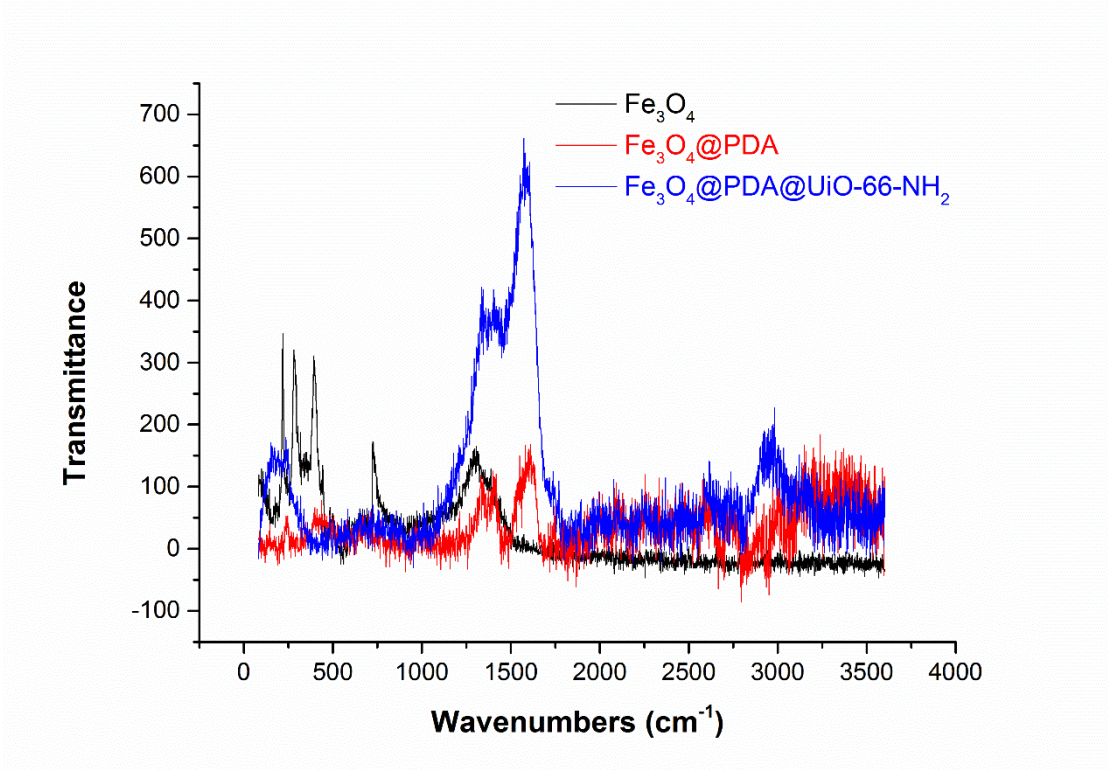


Figure S7. Raman spectra of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@UiO-66-NH_2.

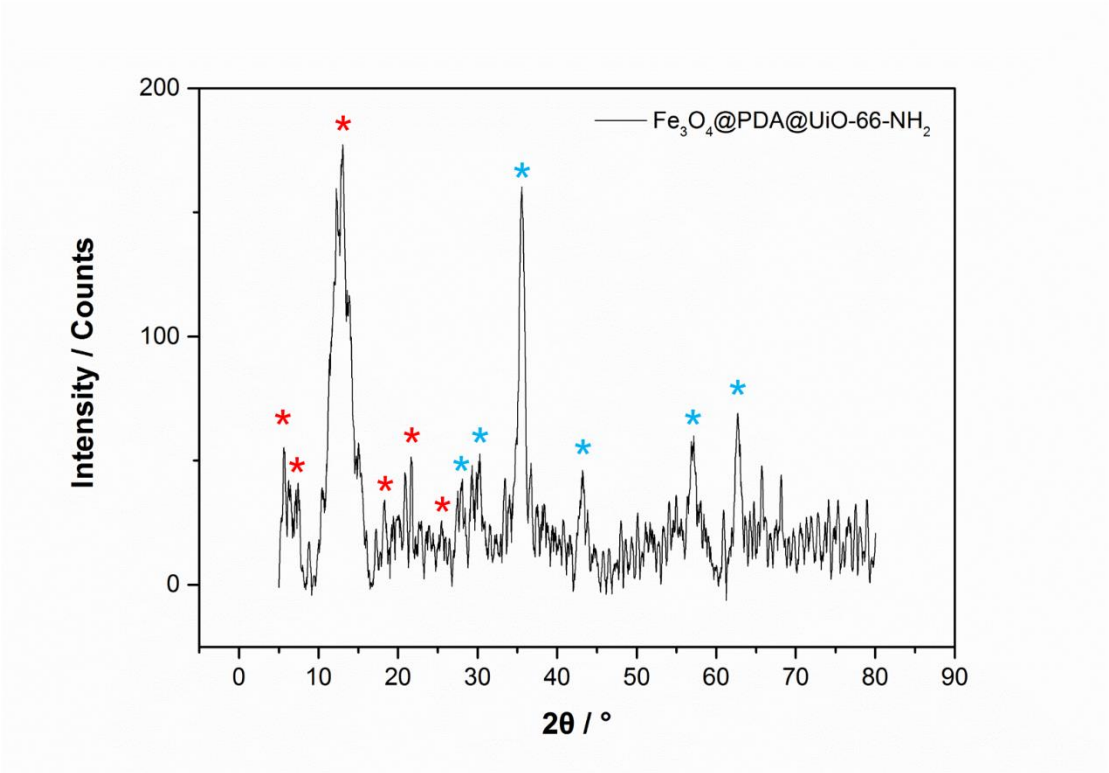


Figure S8. XRD patterns of Fe_3O_4@PDA@UiO-66-NH_2. Peaks from UiO-66-MOF were marked with * in red, while peaks from Fe_3O_4 were marked with * in blue.

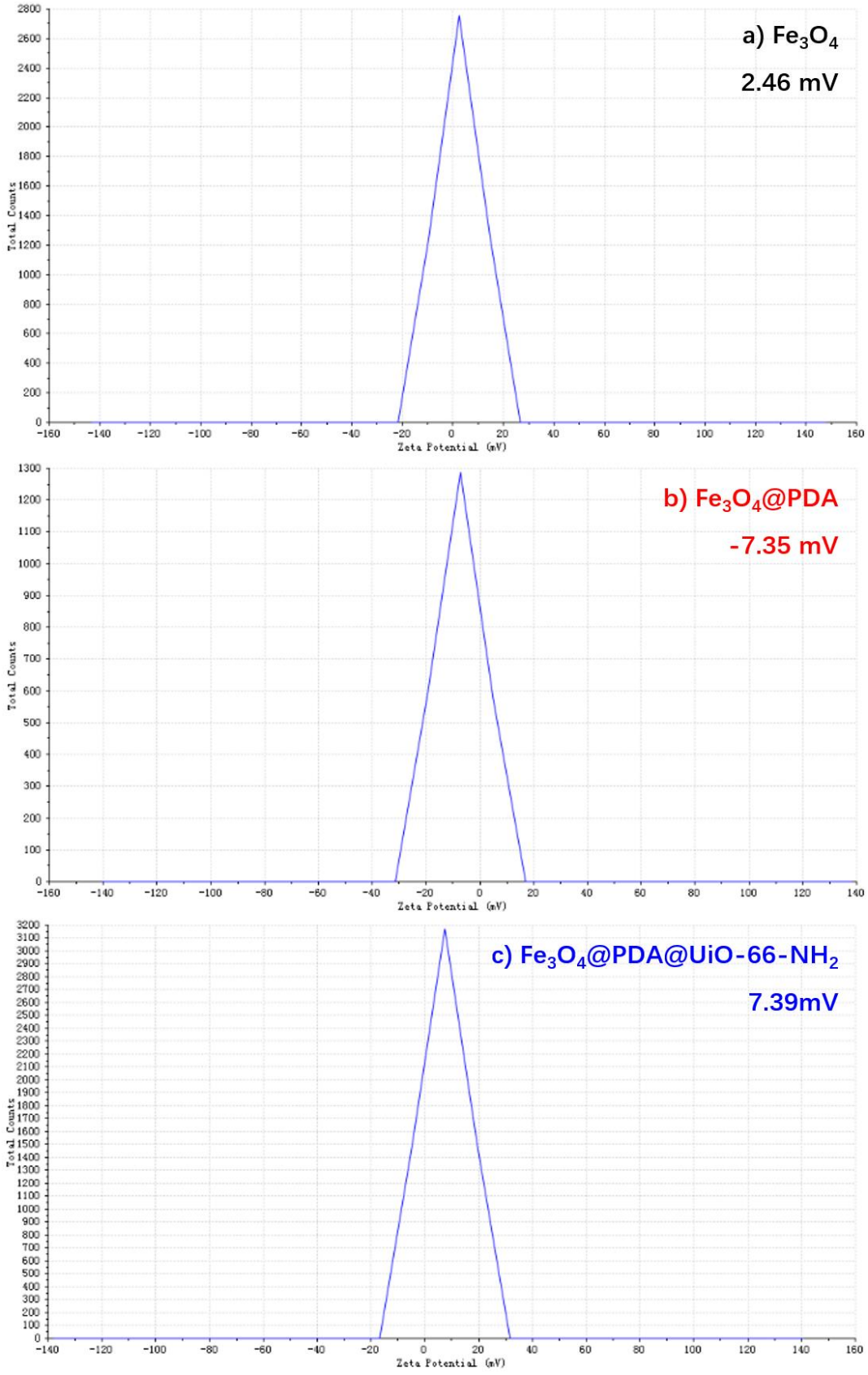


Figure S9. Zeta potential distributions of (a) Fe_3O_4, (b) Fe_3O_4@PDA and (c) Fe_3O_4@PDA@UiO-66-NH_2.

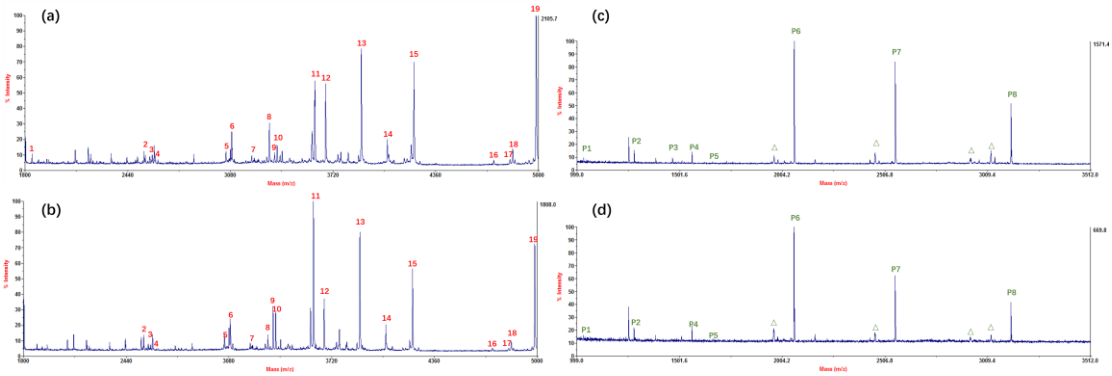


Figure S10. MALDI-TOF mass spectra for the glycopeptide enrichment from 250 fmol/μL HRP tryptic digest: (a) after treatment with the first-time Fe_3O_4@PDA@UiO-66-NH_2; (b) after treatment with the five-times-recycled Fe_3O_4@PDA@UiO-66-NH_2; for the phosphopeptide enrichment from 200 fmol/μL β-Casein tryptic digest: (c) after treatment with the first-time Fe_3O_4@PDA@UiO-66-NH_2; (d) after treatment with the five-times-recycled Fe_3O_4@PDA@UiO-66-NH_2, where glycopeptides were marked with Arabic Numerals in red, phosphopeptides were marked with Arabic Numerals in green, and △ indicates the losses of phosphoric acid.

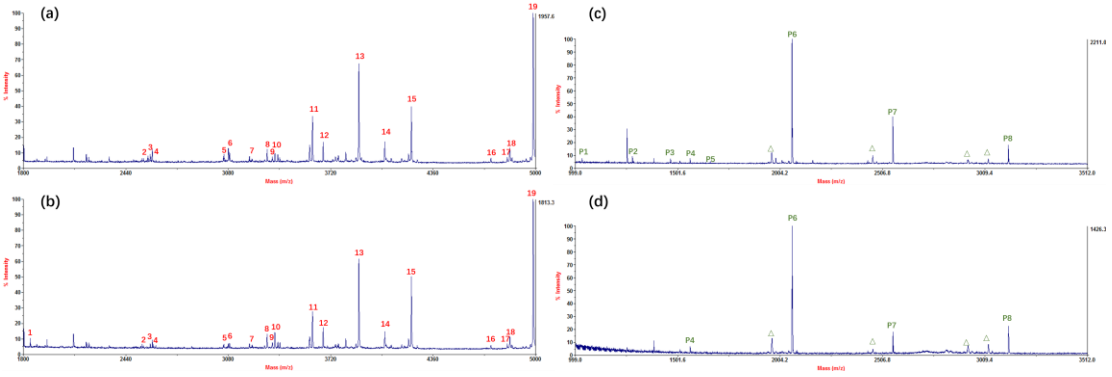


Figure S11. MALDI-TOF mass spectra for the glycopeptide enrichment from 250 fmol/μL HRP tryptic digest: (a) after treatment with the freshly-made Fe_3O_4@PDA@UiO-66-NH_2; (b) after treatment with the Fe_3O_4@PDA@UiO-66-NH_2 kept at -20C° for a month; for the phosphopeptide enrichment from 200 fmol/μL β-Casein tryptic digest: (c) after treatment with the freshly-made Fe_3O_4@PDA@UiO-66-NH_2; (d) after treatment with the Fe_3O_4@PDA@UiO-66-NH_2 kept at -20C° for a month, where glycopeptides were marked with Arabic Numerals in red, phosphopeptides were marked with Arabic Numerals in green, and △ indicates the losses of phosphoric acid.

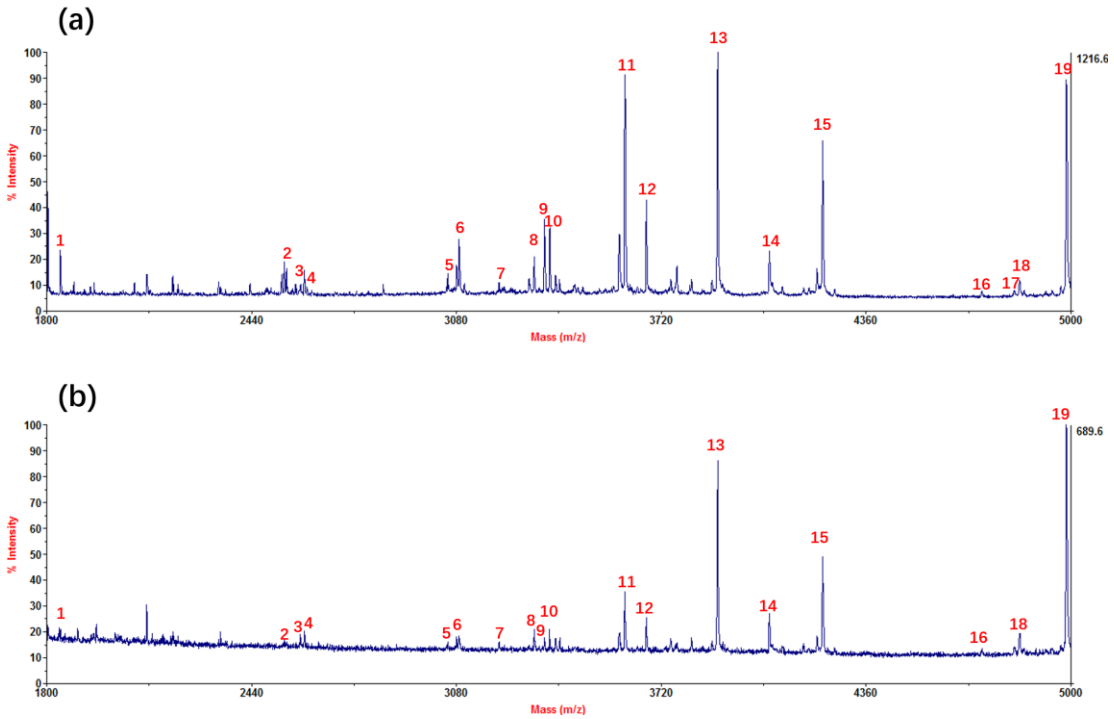


Figure S12. MALDI-TOF mass spectra for the glycopeptide enrichment from a mixture of HRP and BSA at a mass ratio of 1: 50: (a) after enrichment; 1: 100: (b) after enrichment, where glycopeptides were marked with Arabic Numerals in red.

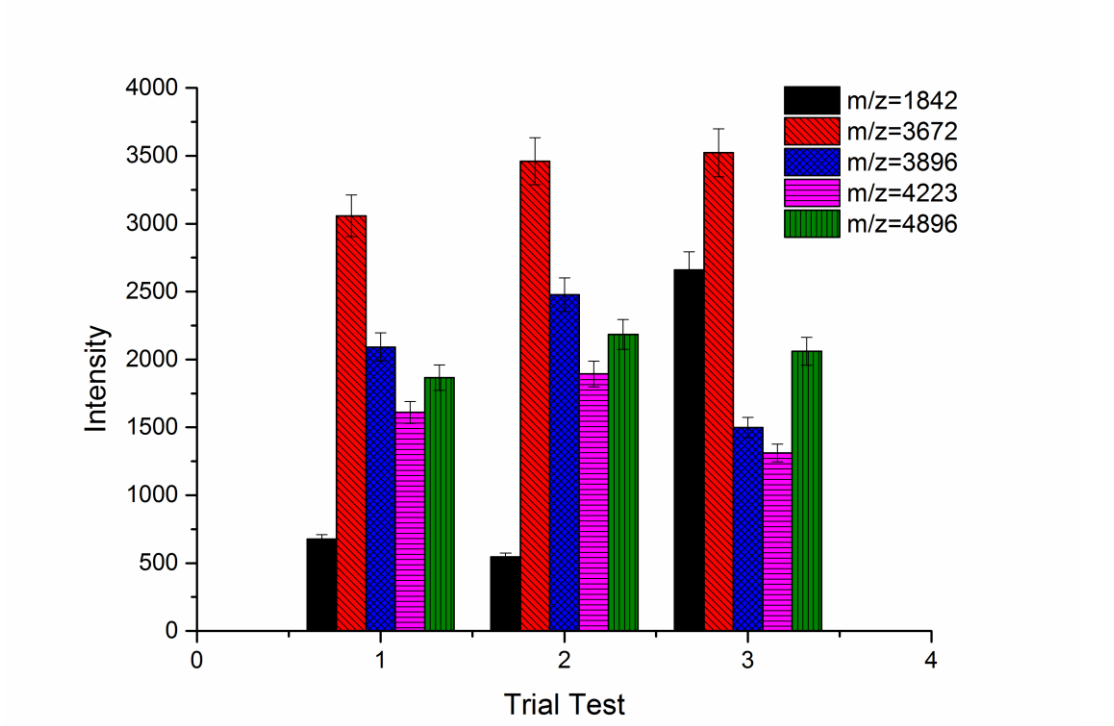


Figure S13. Recovery of the as-prepared MOFs through trial tests.

Table S1. Detailed information of glycopeptides identified from HRP digest.

Peak No.	Observed m/z	Glycan composition	Glycopeptide Sequence
1	1843.0	XylMan3FucGlcNAc2	NVGLN#R
2	2541.4	XylMan3FucGlcNAc2	SSPN#ATDTIPLVR
3	2591.4	XylMan3FucGlcNAc2	PTLN#TTYLQTLR
4	2611.4	XylMan3GlcNAc2	MGN#ITPLTGTQGQIR
5	3074.5	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
6	3087.7	XylMan3FucGlcNAc2	GLCPLNGN#LSALVDFDLR
7	3222.9	Man3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
8	3321.8	XylMan3FucGlcNAc2	QLTPTFYDNSCPN#VSNIVR
9	3353.7	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
10	3369.7	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAM*DR
11	3605.0	XylMan3FucGlcNAc2	NQCRGLCPLNGN#LSALVDFDLR
12	3672.1	XylMan3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
13	3894.1	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
14	4056.2	XylMan3GlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H_2O
15	4222.4	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
16	4719.6	Man3FucGlcNAc2, Man3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
17	4821.7	XylMan2FucGlcNAc2, XylMan2GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
18	4838.7	XylMan3FucGlcNAc2, XylMan3GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
19	4984.7	XylMan3FucGlcNAc2, XylMan3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR

Table S2. Detailed information of glycopeptides identified from IgG digest.

Peak No.	Observed m/z	Glycan composition	Glycopeptide Sequence
I1	2399.3	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR
I2	2431.3	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
I3	2457.3	[Hex]3[HexNAc]4	EEQFN#STFR
I4	2488.3	[Hex]3[HexNAc]4	EEQYN#STYR
I5	2561.4	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR
I6	2602.4	[Hex]3[HexNAc]4[Fuc]1	EEQFN#STFR
I7	2618.4	[Hex]4[HexNAc]4	EEQFN#STFR
I8	2634.4	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR
I9	2650.4	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR
I10	2764.5	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I11	2781.5	[Hex]5[HexNAc]4	EEQFN#STFR
I12	2796.5	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
I13	2805.5	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STFR
I14	2837.5	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR

I15	2853.5	[Hex]4[HexNAc]5	EEQYN#STYR
I16	2926.6	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
I17	2958.6	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
I18	2967.6	[Hex]4[HexNAc]5[Fuc]1	EEQFN#STFR
I19	3000.0	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR
I20	3130.0	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
I21	3161.7	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR

Table S3. Detailed information of phosphopeptides identified from β-Casein digest.

Peak No.	Observed m/z	Protein	Phosphopeptide Sequence
P1	1031.3785	β/33-48	FQpSEEQQQTEDELQDK
P2	1279.1156	β/33-52	FQpSEEQQQTEDELQDKIHPF
P3	1466.6918	α-S2/138-149	TVDMEpSTEVFTK
P4	1561.2365	β/1-25	RELEELNVPGEIVEpSLpSpSpSEESITR
P5	1660.9084	α-S1/106-119	VPQLEIVPNpSAEER
P6	2061.9438	β/33-48	FQpSEEQQQTEDELQDK
P7	2556.2473	β/33-52	FQpSEEQQQTEDELQDKIHPF
P8	3122.4907	β/1-25	RELEELNVPGEIVEpSLpSpSpSEESITR

Table S4. Detailed information of glycopeptides identified from human serum.

Peak No.	Protein Group Accessions	Sequence	MH+ [Da]
1		VTPAcnTSLPAQR	1415.6951 2
2		EIKNnQTEK	1104.5535 1
3		THTnISESHPnATFSAVGEASIcEDDWDSGER	3521.4865 5
4		DGQLLPSSnYSNIK	1536.7543 2
5		nISLQLmSNMNISnK	1724.8188 8
6	O00187	VVINSnITPIcLPR	1596.8781 0
7	O75882	ISnSSDTVEcEcSENWK	2045.8062 0
8		IDSTGnVTNELR	1319.6438 5
9		nHScSEGQISIFR	1535.6901 6
10		cInQSIcEK	1152.5010 2
11		GIcnSSDVR	1008.4411

			5
		AATcINPLnGSVcERPAnHSAK	2369.0978
12			0
	O95445	TELFSSScPGGIMLnETGQGYQR	2533.1357
13			8
	O95497	LTGVAGnYTVcQK	1411.6899
14			9
	P00450	EnLTAPGSDSAVFFEQGTTR	2127.9832
15			5
		ELHHLQEQnVSNAFLDK	2022.9906
16			5
		EHEGAIYPDnTTDFQR	1893.8263
17			4
		ELHHLQEQnVSnAFLDK	2023.9727
18			5
	P00734	SEGSSVnLSPPLEQcVPDR	2071.9641
19			6
20		GHVnITR	797.42632
21	P00736	cnYSIR	813.35637
	P00738	VVLHPnYSQVDIGLIK	1795.9957
22			8
		VVLHPnYSQVDIGLIKLK	2037.1755
23			7
		QLVEIEKVVLHPnYSQVDIGLIK	2635.4671
24			8
	P00739;P00738	NLFLnHSEnATAK	1460.7019
25			5
		NLFLnHSENATAK	1459.7179
26			4
		nLFLnHSEnATAK	1461.6869
27			4
		MVSHHnLTTGATLINEQWLLTTAK	2680.3734
28			3
		mVSHHnLTTGATLINEQWLLTTAK	2696.3773
29			4
		mVSHHnLTTGATLInEQWLLTTAK	2697.3746
30			5
31	P00742	GDnnLTR	791.35204
32		GDNnLTR	790.36858
	P00748	RnHScEPcQTLAVR	1728.7913
33			5
	P00751	IVLDPSGSmNIYLVLDGSDSIGASnFTGAK	3058.4862
34			4
35	P01008	LGAcnDTLQQLMEVFK	1867.8914

			1
		LGAcnDTLQQLmEVFK	1883.8915
36			3
		SLTFnETYQDISELVYGAK	2179.0455
37			1
		LGAcnDTLQQLMEVFKFDTISEK	2688.2898
38			3
		WVSnKTEGR	1077.5322
39			6
	P01009	YLGnATAIFFLPDEGK	1756.8804
40			2
		ADTHDEILEGLNFnLTEIPEAQIHEGFQELLR	3692.8084
41			9
	P01011	TLnQSSDELQLSMGNAMFVK	2214.0454
42			6
		YTGnASALFILPDQDK	1753.8652
43			8
		FnLTETSEAEIHQSFQHLLR	2401.1797
44			2
		TLnQSSDELQLSmGNAMFVK	2230.0361
45			7
		LInDYVKnGTR	1294.6644
46			8
	P01019	LQAILGVPWKDKncTSR	1987.0412
47			9
48		DKncTSR	881.37828
	P01023	GcVLLSYLnETVTVSASLESVR	2398.2209
49			9
		VSnQTLSLFFTVLQDVPVR	2164.1643
50			5
		SLGNVnFTVSAEALESQELcGTEVPSVPEHGR	3414.6152
51			1
		SLGNVnFTVSAEALESQELcGTEVPSVPEHGRK	3542.7115
52			7
		SLGnVnFTVSAEALESQELcGTEVPSVPEHGR	3415.6136
53			7
		SLGnVnFTVSAEALESQELcGTEVPSVPEHGRK	3543.7155
54			5
	P01024	TVLTPATNHMGnVTFTIPANR	2256.1449
55			9
		TVLTPATNHmGnVTFTIPANR	2272.1426
56			3
		TVLTPATnHMGnVTFTIPANR	2257.1282
57			7

		TVLTPATnHmGnVTFTIPANR	2273.1183
58			2
	P01033	FVGTPEVnQTTLYQR	1753.8795
59			6
	P01042	YNSQnQSNNQFVLYR	1875.8635
60			7
		LnAENnATFYFK	1433.6580
61			1
		LNAENnATFYFK	1432.6747
62			3
	P01591	EnISDPTSPLR	1229.6012
63			4
		IIVPLNNREnISDPTSPLR	2149.1588
64			5
		IIVPLnNREnISDPTSPLR	2150.1456
65			0
	P01833	AnLTNFPENGTFVVnIAQLSQDDSGR	2809.3282
66			2
		LSLLEEPGnGTFTVILNQLTSR	2403.2744
67			5
		AnLTnFPEnGTFVVNIAQLSQDDSGR	2810.3315
68			9
	P01857	EEQYnSTYR	1190.4952
69			9
		TKPREEQYnSTYR	1672.7929
70			4
	P01859	EEQFnSTFR	1158.5064
71			0
		TKPREEQFnSTFR	1640.8029
72			5
	P01860	EEQYnSTFR	1174.5011
73			5
	P01861	EEQFnSTYR	1174.5013
74			9
	P01871	GLTFQQnASSMcVPDQDTAIR	2340.0530
75			3
		STGKPTLYnVSLVMSDTAGTcY	2366.0876
76			9
		GLTFQQnASSmcVPDQDTAIR	2356.0586
77			4
		THTnISESHPnATFSAVGEASIcEDDWNSGER	3520.4833
78			0
79		nNSDISSTR	994.44359
80		STGKPTLYnVSLVmSDTAGTcY	2382.0855

			0
		THTnISESHPnATFSAVGEASIcEDDWnSGER	3521.4865
81			5
	P01876	LAGKPTHVnVSVVmAEVDGTcY	2364.1221
82			2
		LAGKPTHVnVSVVMAEVDGTcY	2348.1265
83			6
	P01876;P01877	LSLHRPALEDLLLGSEAnLTcTLTGLR	2964.5821
84			7
85	P01877	TPLTAnITK	959.54088
	P02743	ESVTDHVNLITPLEKPLQnFTLcFR	2972.5206
86			5
	P02748	AVnITSENLIDDVVSLIR	1972.0605
87			9
	P02749	VYKPSAGnNSLYR	1469.7380
88			4
		LGnWSAmPScK	1267.5442
89			4
		LGnWSAMPScK	1251.5503
90			4
		DTAVFEcLPQHAMFGnDTITcTTHGnWTK	3354.4615
91			7
		DTAVFEcLPQHAmFGnDTITcTTHGnWTK	3370.4476
92			5
	P02750	mFSQnDTR	1015.4148
93			4
		LPPGLLAnFTLLR	1425.8485
94			6
	P02751	LDAPTNLQFVnETDSTVLVR	2233.1409
95			0
		DQcIVDDITYNVnDTFHK	2197.9724
96			6
		NSITLTnLTPGTEYVVSIVALnGR	2534.3569
97			8
	P02763	QDQcIYnTTYLNVQR	1916.8814
98			0
		QDQcIYnTTYLnVQR	1917.8683
99			3
100		EnGTISR	777.37401
	P02765	KVcQDcPLLAPLnDTR	1900.9207
101			0
		AALAAFNAQNnGSNFQLEEISR	2366.1387
102			2
103		AALAAFNAQnnGSNFQLEEISR	2367.1272

			4
		VcQDcPLLAPLnDTR	1772.8339
104			1
105	P02774	LcDnLSTK	951.44542
	P02786	DFEDLYTPVnGSIVIVR	1937.9877
106			2
		KDFEDLYTPVnGSIVIVR	2066.0824
107			3
	P02787	QQQHLFGSnVTDcSGNFcLFR	2516.1099
108			0
		QQQHLFGSnVTDcSGnFcLFR	2517.0946
109			4
	P02790	ALPQPQnVTSLLGcTH	1736.8638
110			2
		SWPAVGncSSALR	1405.6532
111			5
		cSDGWSFDATTLDDnGTMLFFK	2529.0574
112			2
		LNAAKALPQPQnVTSLLGcTH	2234.1635
113			5
		nGTGHGnSTHHGPEYmR	1869.7582
114			0
	P03951	LETTVnYTDSQRPIcLPSK	2223.0977
115			0
		VYSGILnQSEIK	1351.7114
116			7
		LETTVnYTDSQRPIcLPSKGDR	2551.2450
117			9
		GINYnSSVAK	1053.5209
118			2
	P03952	IYPGVDFGGEELnVTFVK	1984.9900
119			4
		IYSGILnLSDITK	1437.7838
120			6
		LQAPLnYTEFQKPIcLPSK	2248.1685
121			0
		IVGGTnSSWGEWPWQVSLQVK	2359.1726
122			6
123		GVNFnVSK	865.44145
	P04003	LSVDKDQYVEPEnVTIQcDSGYGVVGPQSITcSGnR	3973.8152
124			8
		LSVDKDQYVEPEnVTIQcDSGYGVVGPQSITcSGNR	3972.8268
125			8
126	P04004	NnATVHEQVGGPSLTSDLQAQSK	2382.1563

			5
		nnATVHEQVGGPSLTSDLQAQSK	2383.1442
127			0
	P04070	EVFVHPnYSK	1220.5947
128			7
	P04114	FnSSYLQGTNQITGR	1686.8085
129			2
		FnSSYLQGTnQITGR	1687.7964
130			3
		FVEGSHnSTVSLTTK	1607.7916
131			7
		YDFnSSMLYSTAK	1527.6676
132			5
		QVLFLDTVYGncSTHFTVK	2230.0803
133			0
		YDFnSSmLYSTAK	1543.6611
134			8
		FEVDSPVYnATWSASLK	1914.9183
135			8
		QVFPGLNYcTSGAYSnASSTDSASYYPLTGDTR	3551.5572
136			2
		IQSPLFTLDAnADIGnGTTSANEAGIAASITAK	3234.6265
137			6
	P04180	AELSnHTRPVILVPGcLGNQLEAK	2617.3763
138			6
	P04196	VIDFncTTSSVSSALANTK	2015.9597
139			6
		VIDFncTTSSVSSALAnTK	2016.9436
140			5
	P05090	ADGTVNQIEGEATPVnLTEPAK	2255.1123
141			5
		ADGTVnQIEGEATPVnLTEPAK	2256.0894
142			0
		ADGTVNQIEGEATPVnLTEPAKLEVK	2724.3943
143			8
		clQAnYSLMENGK	1528.6774
144			2
		ADGTVnQIEGEATPVnLTEPAKLEVK	2725.3760
145			1
	P05155	VLSnNSDANLELINTWVAK	2102.0789
146			1
147		DTFVnASR	910.42650
		VGQLQLSHnLSLVILVPQNLK	2314.3492
148			8

	P05156	FLNnGTcTAEGK	1312.5839
149			1
		FLnnGTcTAEGK	1313.5684
150			1
		LSDLSInSTEcLHVHcR	2041.9449
151			7
		nGTAVcATNR	1064.4795
152			4
		nGTAVcATNRR	1220.5803
153			0
	P05160	EHETcLAPELYnGnYSTTQK	2357.0262
154			2
		EHETcLAPELYNGnYSTTQK	2356.0380
155			0
156	P05362	AnLTVVLLR	999.61992
		LNPTVTYGnDSFSAK	1614.7657
157			9
	P05543	VTAcHSSQPnATLYK	1677.7904
158			5
		TLYETEVFSTDFSnISAAK	2124.0039
159			5
	P05546	DFVnASSKYEITTIHNLFR	2256.1266
160			1
		nLSMPLLPADFHK	1483.7610
161			8
162		DFVnASSK	868.40434
163	P06276	EnETEIIK	976.48265
		DnYTKAEEILSR	1439.7006
164			9
165		DnNSIITR	933.46343
	P06681	QSVPAHFVALnGSK	1455.7595
166			7
		LTDTIcGVGnMSAnASDQER	2140.9092
167			3
		LGSYPVGGnVSFEcEDGFILR	2317.0896
168			5
		LTDTIcGVGnmSAnASDQER	2156.9077
169			6
		TmFPnLTDVR	1210.5774
170			4
	P07602	TnSTFVQALVEHVKEEcDR	2263.0663
171			0
	P07996	VVnSTTGPGEHLR	1367.6915
172			3

		VScPIMPcSnATVPDGEccPR	2407.9844
173			7
	P08185	AVLQLNEEGVDTAGSTGVTLnLTSKPIILR	3110.6927
174			2
		AQLLQGLGFnLTER	1560.8376
175			9
	P08603	ISEEnETTcYMGK	1562.6356
176			7
		MDGASnVTcINSR	1425.6104
177			0
		IPcSQPPQIEHGTInSSR	2021.9713
178			6
		ISEEnETTcYmGK	1578.6290
179			8
		MDGASnVTcInSR	1426.5930
180			7
		SPDVInGSPISQK	1342.6854
181			7
	P0C0L5;P0C0L4	FSDGLESnSSTQFEVK	1775.7980
182			2
		nTTcQDLQIEVTVK	1649.8097
183			4
		GLnVTLSSTGRnGFK	1552.7969
184			2
		FSDGLESnSSTQFEVKK	1903.8969
185			0
		GLnVTLSSTGR	1105.5850
186			1
187	P10643	nYTLTGR	825.40990
188	P10721	SEnESNIR	949.42266
	P10909	LAnLTQGEDQYYLR	1684.8182
189			8
		mLnTSSLLEQLNEQFNWVSR	2426.1709
190			5
		MLnTSSLLEQLNEQFNWVSR	2410.1705
191			7
		KKEDALnETR	1204.6176
192			0
		KEDALnETR	1076.5221
193			4
194		EDALnETR	948.42717
		EDALnETRESETK	1522.6871
195			8
196		ELPGVcnETMMALWEEcKPcLK	2696.1917

			5
197		HnSTGcLR	945.42052
198	P11362	HIEVnGSK	884.44713
	P11597	SIDVSIQnVSVVFK	1535.8312
199			2
200	P12259	TNInSSR	792.38402
		NSVLnSSTAEHSSPYSEDPIEDPLQPDVTGIR	3455.6095
201			9
	P13473	VASVININPnTTHSTGScR	2028.9780
202			7
203		LnSSTIK	763.41954
	P13598	GnETLHYETFGK	1396.6392
204			1
205	P13671	VLnFTTK	823.45543
206		LSSnSTK	737.36778
	P14151	DnYTDLVAIQNK	1394.6788
207			8
		FcRDnYTDLVAIQNK	1857.8799
208			8
	P15144	nATLVNEADKLR	1344.7117
209			2
	P17936	AYLLPAPPAPGnASESEEDR	2084.9785
210			6
		GLcVnASAVSR	1134.5574
211			2
		VDYESQSTDTQnFSSESKR	2208.9475
212			4
	P19652	QNQcFYnSSYLNVQR	1921.8510
213			0
		QnQcFYnSSYLNVQR	1922.8357
214			4
215		EnGTVSR	763.35808
		EnGTVSRYEGGR	1325.6084
216			0
	P19823	GAFISnFSMTVDGK	1474.6886
217			5
		GAFISnFSmTVDGK	1490.6837
218			6
	P20851	LGHcPDPVLVNGEFSSSGPVnVSDK	2612.2325
219			8
		LGHcPDPVLVnGEFSSSGPVnVSDK	2613.2110
220			9
		EWDnTTTEcR	1312.5111
221			6

222		TLFcnASK	941.43877
		TLFcnASKEWDnTTTEcR	2234.9305
223			8
		KTLFcnASK	1069.5334
224			8
	P22792	LYLGSNnLTALHPALFQnLSK	2316.2200
225			2
		AFGSNPnLTK	1049.5264
226			1
		LYLGSnnLTALHPALFQnLSK	2317.2095
227			7
	P23142	cATPHGDnASLEATFVK	1818.8312
228			7
	P25311	DIVEYYNDSnGSHVLQGR	2066.9485
229			3
		DIVEYYnDSnGSHVLQGR	2067.9280
230			3
	P26927	GTAnTTTAGVPcQR	1434.6661
231			9
		GTGnDTVLNVALLNVISNQEcNIK	2587.3036
232			8
	P27169	VTQVYAEnGTVLQGSTVASVYK	2315.1785
233			1
		HAnWTLTPLK	1181.6304
234			2
	P29622	SQILEGLGFnLTELSESDVHR	2345.1609
235			4
		FLnDTMAVYEAK	1402.6564
236			2
		DFYVDEnTTVR	1359.6080
237			8
	P35858	AGAFLGLTNVAVmnLSGNcLR	2195.1021
238			0
		AGAFLGLTNVAVMnLSGNcLR	2179.1002
239			5
	P36955	VTQnLTLIEESLTSEFIHDIDR	2574.2903
240			3
	P36980	LQNNENnIScVER	1590.7179
241			4
		LQNNEnnIScVER	1591.7036
242			6
	P40197	LLDLSGNnLTHLPK	1535.8425
243			0
244	P41222	SVVAPATDGGLnLTSTFLR	1920.0063

			8
	P43251	YQFNTNVVFSNnGTLVDR	2088.9988
245			3
		NPVGLIGAEnATGETDPSHSK	2094.9942
246			4
		FnDTEVLQR	1122.5432
247			6
		DVQIIVFPEDGIHGFnFTR	2205.0983
248			0
	P43652	YAEDKFnETTEK	1475.6576
249			4
		DIENFnSTQK	1196.5433
250			8
		nccNTENPPGcYR	1642.6071
251			0
		DIEnFnSTQK	1197.5259
252			3
253		FnETTEK	869.38860
254	P48740	NnLTTYK	854.42546
	P49908	EGYSnISYIVVNHQGISSR	2124.0366
255			7
	P51884	LHINHNnLTESVGPLPK	1883.9974
256			1
		AFEnVTDLQWLILDHNLLENSK	2613.3193
257			1
		LSHNELADSGIPGnSFnVSSLVELDLSYNK	3221.5737
258			0
	P55058	VSnVScQASVSR	1294.6053
259			9
		EGHFYYnISEVK	1486.6855
260			9
		IYSnHSALESLALIPLQAPLK	2279.2644
261			4
	P55103	EQEcEIISFAETGLSTInQTR	2427.1356
262			5
	P55290	InNTHALVSLLQNLNK	1792.9917
263			9
	P80108	LGTSLSSGHVLMnGTLK	1715.9008
264			0
		nLTTSLTESVDR	1336.6578
265			8
266		NInYTER	910.42656
		LGTSLSSGHVLmnGTLK	1731.8946
267			3

	Q06033	NAHGEEKEnLTAR	1469.6982
268			2
269		EnLTAR	704.35698
	Q08380	TVIRPFYLTnSSGVD	1669.8439
270			2
		ALGFEnATQALGR	1348.6851
271			1
		GLnLTEDTYKPR	1407.7118
272			4
		DAGVVcTnETR	1222.5372
273			8
	Q12913	TPEQGSnGTDGASQK	1477.6410
274			4
		IHVAGETDSSNLnVSEPR	1925.9212
275			4
276	Q12981	AnLTcK	707.33843
	Q13201	LnDSIQTLVNDNQR	1630.8032
277			7
		FNPGAESVVLSnSTLK	1663.8561
278			3
		VnESVVSIAAQQK	1373.7275
279			9
		LQnLTLPTnASIK	1414.7771
280			5
		KIDnISLTVNDVR	1487.8046
281			1
	Q14624	LPTQnITFQTESSVAEQEAEFQSPK	2810.3347
282			6
	Q15063	EVnDTLLVNELK	1387.7340
283			6
	Q6EMK4	LHEITnETFR	1260.6222
284			4
	Q6UXB8	SLPNFPnTSATAnATGGR	1777.8362
285			3
	Q6YHK3	TQDEILFSnSTR	1411.6692
286			4
287	Q7Z7G0	EnGSFSGK	826.35741
	Q8IZF2	YEEQQLEIQnSSR	1624.7444
288			3
289	Q92954	nGTLVAFR	878.47283
	Q96IY4	QVHFFVnASDVDNVK	1719.8346
290			4
	Q96KN2	LVPHmnVSAVEK	1340.6879
291			1

		LVPHMnVSAVEK	1324.6936
292			1
	Q96PD5	LEPVHLQLQcmSQEQLAQVAAnATK	2824.3990
293			8
		LEPVHLQLQcMSQEQLAQVAAnATK	2808.4031
294			7
		GFGVAIVGnYTAALPTEAALR	2092.1075
295			8
	Q9BY67	FQLLnFSSSELK	1413.7264
296			9
	Q9HDC9	AGPnGTLFVADAYK	1424.7046
297			4
298	Q9NQ38	nGTLIcTR	935.46178
299	Q9UGM5	YNNEnTSK	970.40746
	Q9UK55	LPYQGnATMLVVLMEK	1807.9321
300			8
301		ETFFnLSK	986.48314
		ETFFnLSKR	1142.5841
302			5
	Q9Y5Y7	KANQQLnFTEAK	1392.7114
303			7
		ANQQLnFTEAK	1264.6169
304			9
	Q9Y6R7	YLPVnSSLLTSDcSER	1841.8571
305			0
		VVTVAALGTnISIHKDEIGK	2066.1493
306			1
		LLISSLSESPASVSILSQADnTSK	2448.2782
307			9

Table S5. Detailed information of phosphopeptides identified from human serum.

Peak No.	Observed m/z	Phosphopeptide
1	1389.7030	ADpSGEGDFLAEGGGV
2	1460.7516	DpSGEGDFLAEGGGV
3	1545.8314	DpSGEGDFLAEGGGVR
4	1616.8799	ADpSGEGDFLAEGGGVR

Table S6. Detailed information of phosphopeptides identified from human serum.

Peak No.	Protein Group Accessions	Sequence	MH+ [Da]
1		AAFEcMyTLLDscLDR	2124.78476
2		GEQGDGLRELNKQEsASDmTSTFPVAQsLtPGsMEER	4318.72702
3		NLFHFGEsTtGsNFSFK	2159.79253

4		NVyMLAttVSSK	1553.57512
5		QQQQDsIDPsSR	1548.57584
6		RtVLTTQPNGLTTVGK	1765.92363
7		tFsFAIPLIEK	1425.64286
8		tVVsSPGPGSGPGPGTTsGAssPARPAtPLVPcR	3639.39145
9	A6NKC4	tNISHNGTYHcSGK	1655.65337
10	O14791	VTEPIsAESGEQVER	1710.76006
11	O95294	AKssSLNVR	1121.47209
12	P00747	QLGAGsIEEcAAK	1413.60808
13	P01008	ATEDEGsEQKIPEATNR	1954.83891
14	P01042	DIPTNsPELEETLTHTITK	2219.04856
15		DIPTNsPELEETLTHTITK	2219.03647
16		EsNEELTEScETK	1635.61089
17		ETTcSKEsNEELTEScETK	2341.90093
18		ETtcSKESNEELTEScETK	2341.90702
19		ETTcSKEsNEELTEScETKK	2469.99826
20	P02649	GEVQAMLGQsTEELR	1727.77031
21	P02751	TNTNVNcPIEcFMPLDVQADREDsRE	3190.33152
22	P02765	cDSSPDsAEDVR	1417.49126
23		cDSSPDsAEDVRK	1545.59490
24	P02768	ADDKEtcFAEEGKK	1707.68852
25		EtYGEMADccAK	1514.49040
26		TcVADEsAENcDK	1578.54607
27	P04114	VREsDEETQIK	1413.62639
28	P05546	ENtVTNDWIPEGEEDDDYLDLEK	2819.13132
29		GGEtAQSADPQWEQLNNK	2052.86724
30	P10909	VtTVASHTSDSDVPSGVTEVVVK	2394.14444
31	P49908	DMPAsEDLQDLQK	1569.65190
32	Q05084	TEDGKSILsALDK	1456.69768
33	Q9H5V7	tTPTGGLPR	979.46288

References

1 Sun, S. & Zeng, H. Size-Controlled Synthesis of Magnetite Nanoparticles. *J. Am. Chem. Soc.* **124**, 8204-8205 (2002).