Supplementary Information for

Novel hydrophilic MOFs-303-functionalized magnetic probe for the highly efficient analysis of N-linked glycopeptides

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Experimental Section

Materials and Chemicals. Iodoacetamide (IAA), dithiothreitol (DTT), immunoglobulin G (IgG), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich. Hexane (≥97%), ethanol (≥99.7%), isopropanol (≥99.7%) and sodium hydroxide (NaOH, ≥96%) were purchased from Sinopharm Chemical Reagent Co. Ltd. PNGase F was acquired from New England Biolabs (Ipswich, MA). Ultrapure water used in all experiments was purified using the Milli-Q system (Millipore, Bedford, MA).

Preparation of the Enzymatic Hydrolysate from Human Serum (Healthy Controls and HCC Patients). Serum samples were collected from the healthy controls and HCC patients, and immediately centrifuged (14 000 rpm) at 4 °C for 20 min. The supernatants were transferred and collected. The protein concentration of the serum samples was measured by the bicinchoninic acid (BCA) assay. The proteins were diluted with 25 mM NH_4HCO_3 solution. Then, the proteins were denatured with 10 mM DTT for 30 min at 60 °C and alkylated in 20 mM IAA in the dark at 37 °C for 1 h. Finally, the samples were diluted with 50 mM NH_4HCO_3 and digested with trypsin (protein : enzyme w/w ratio was 50:1) overnight. The obtained tryptic digests were desalted with a Sep-pak C18 column (Waters, Milford, MA, U.S.A.) and stored at -20 °C for further analysis.

Measurements and Characterization. Transmission electron microscopy (TEM) images were obtained on a HT7800 (Hitachi) microscope operated at an acceleration voltage of 120 kV. Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy were recorded on a Zeiss Ultra-55 microscope operated at an accelerating voltage of 3 kV and 15 kV, respectively. Specific surface area and pore size distribution were measured by the Brunauer-Emmett-Teller (BET) method and the Barrett-Joyner-Halenda (BJH) model,

respectively. Fourier-transform infrared (FT-IR) spectra were recorded on a Nicolet Fourier spectrophotometer.

Nano-Liquid Chromatography Tandem Mass Spectrometry (Nano-LC-MS/MS) Analysis of Glycopeptides. Glycopeptides enriched from the serum of HCC patients and healthy people were resuspended with 10 μL solvent A (A: water with 0.1 % formic acid; B: 80% ACN with 0.1 % formic acid) and then 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 a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an online nano-electrospray ion source. 5 μL peptide sample was loaded onto the analytical column (Acclaim PepMap C18, 75 μm x 25 cm) and subsequently separated with a linear gradient, from 2 % B to 45 % B in 85 min. The column was re-equilibrated at initial conditions for 5 min. The column flow rate was maintained at 300 nL/min and the column temperature was maintained at 40 °C. The electrospray voltage of 2.3 kV versus the inlet of the mass spectrometer was used. The Q-Exactive HF 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 350-1600) were acquired in Orbitrap with a mass resolution of 60 000 at m/z 200. The AGC taget was set to 3000 000, and the maximum injection time was 50 ms. MS/MS acquisition was performed in Orbitrap with 3 s cycle time, the resolution was 15 000 at m/z 200. The intensity threshold was 50 000, and the maximum injection time was 100 ms. The AGC target was set to 10 000, and the isolation window was 1.6 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 21 seconds.

Database Retrieval and Data Analysis. Tandem mass spectra were processed by PEAKS Studio version X (Bioinformatics Solutions Inc., Waterloo, Canada). PEAKS DB was set up to search the Homo_sapiens_sp database (version 201007, 20428 entries) assuming trypsin as the digestion enzyme. PEAKS DB were searched with a fragment ion mass tolerance of 0.02 Da and a parent ion tolerance of 15 ppm. Carbamidomethylation (C) was specified as the fixed modifications. Oxidation (M), Deamidation (NQ) and Acetylation (Protein N-term) were specified as the variable modifications. Peptides were filter by 1% FDR. Only the N-glycosites match with the NX(S/T) motif, with a A score >13 is considered to be a potential glycosylation site.

Fig. S1. PXRD pattern of MOF-303.

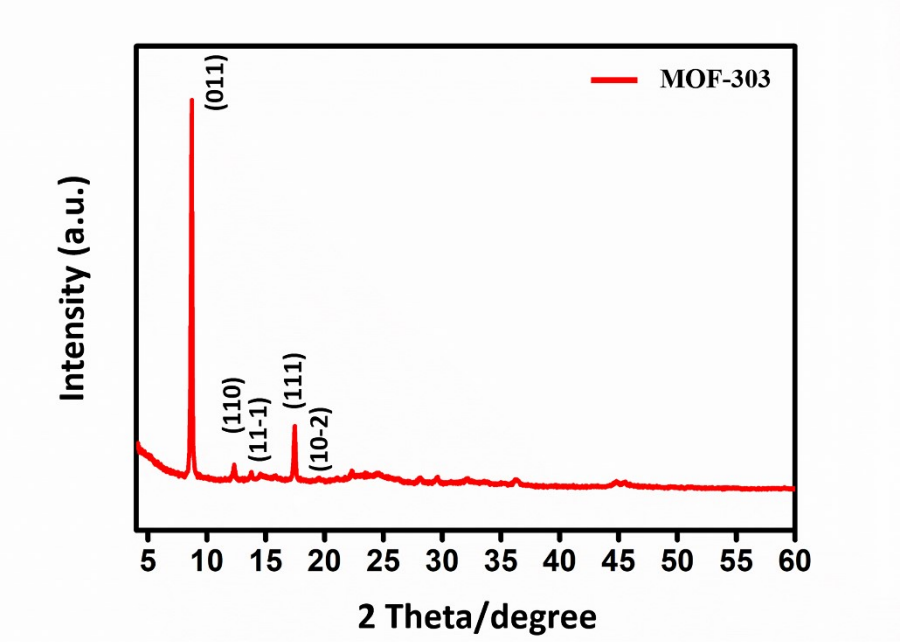


Fig. S2. Water contact angles of (a) GO@Fe_3O_4 and (b) GO@Fe_3O_4@MOF-303 probe.

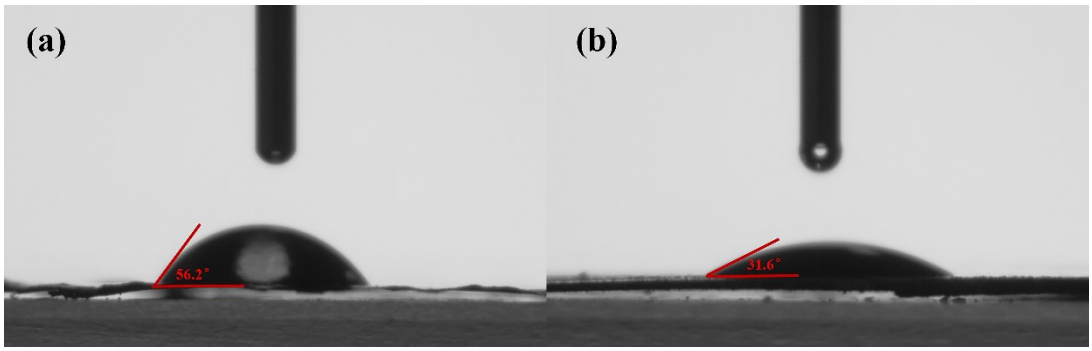


Fig. S3. The zeta potential distributions of (a) GO@Fe_3O_4 and (b) GO@Fe_3O_4@MOF-303 probe.

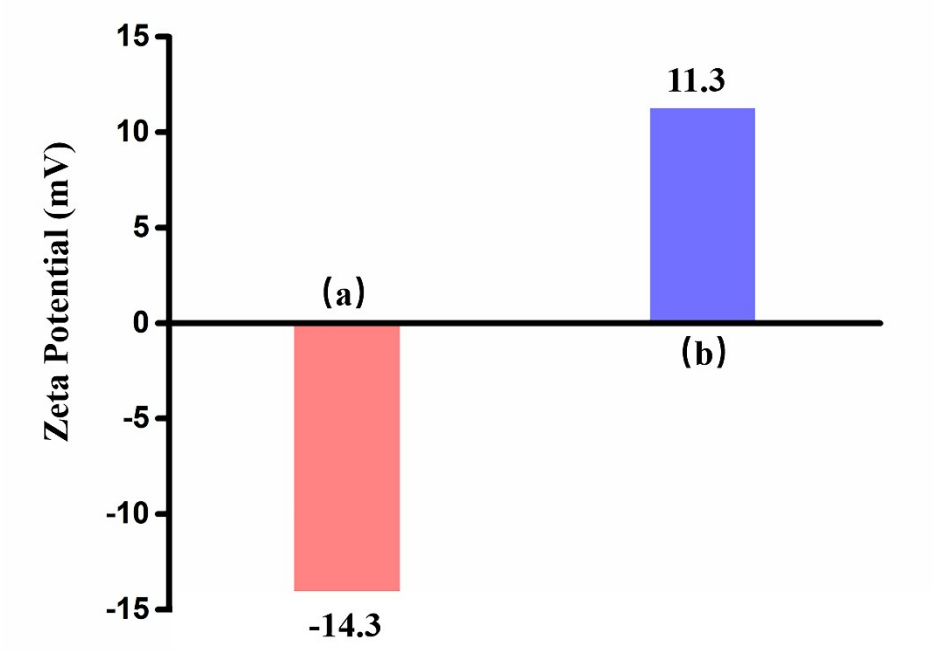


Fig. S4. GO@Fe_3O_4@MOF-303 dispersed in loading buffer solution and separated with an external magnetic field.

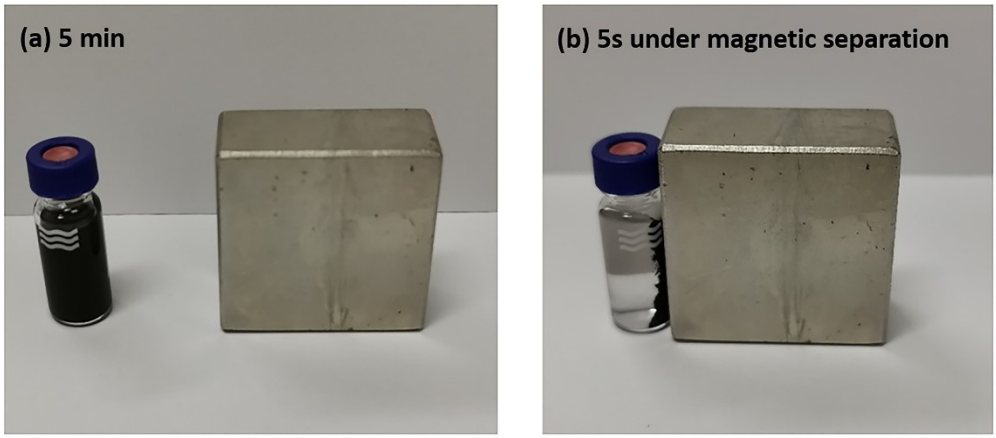


Fig. S5. MALDI-TOF-MS spectra of HRP tryptic digests after enrichment by GO@Fe_3O_4@MOF-303 with different loading buffers: (a) 95% ACN-1.0% TFA (v/v), (b) 95% ACN-0.1% TFA (v/v), (c) 85% ACN-1.0% TFA (v/v) and (d) 85% ACN-0.1% TFA (v/v). The peaks of N-linked glycopeptides are marked with "*".

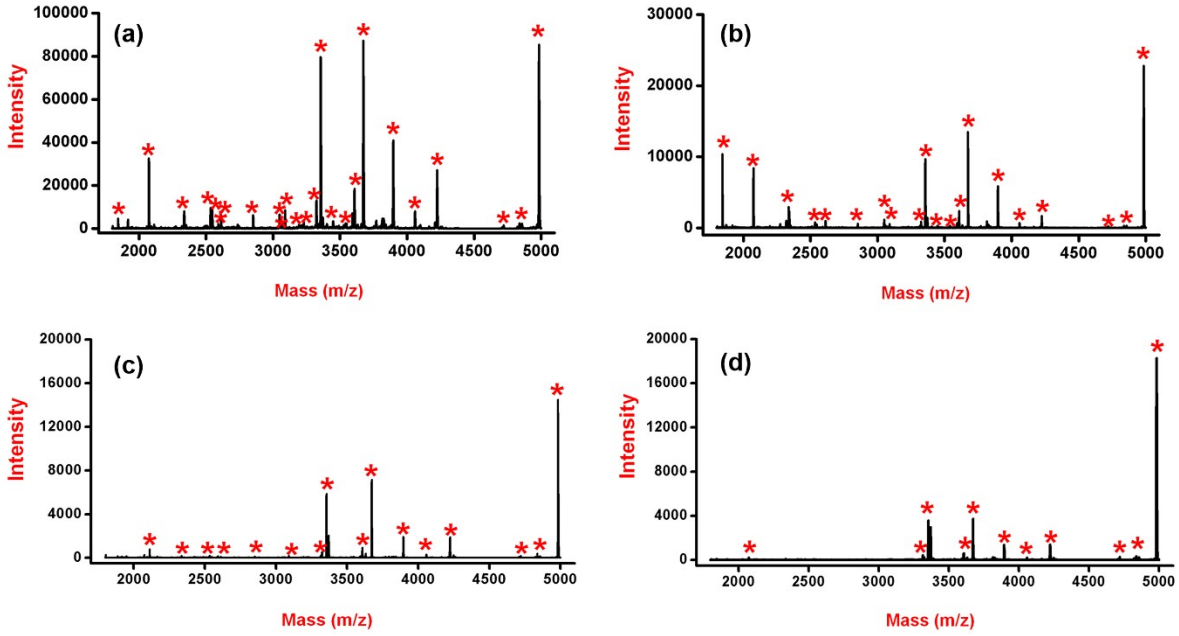


Fig. S6. The effect of different quantities of the GO@Fe_3O_4@MOF-303 probe (a) and different incubation time (b) in N-linked glycopeptides enriched from 10^{-6} M HRP tryptic digest through three parallel tests.

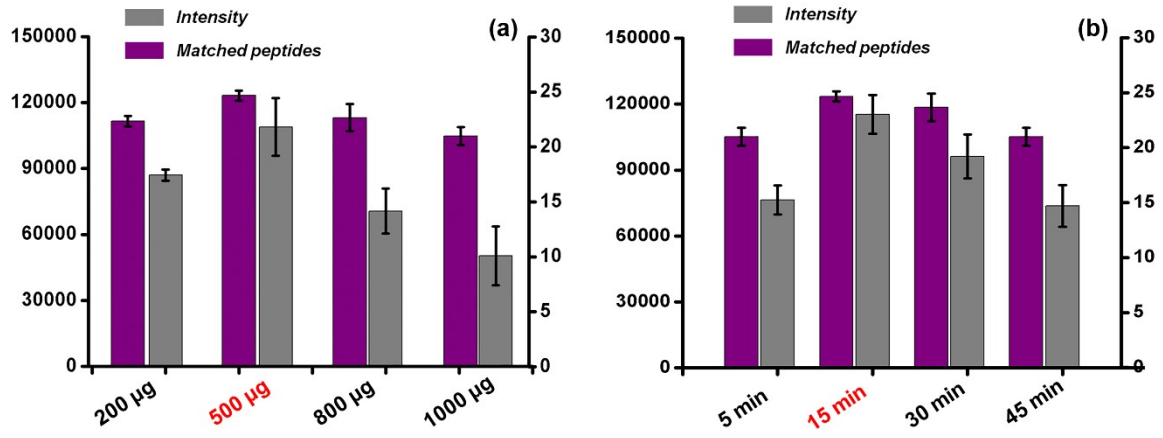


Fig. S7. MALDI-TOF MS spectra of different amounts of HRP tryptic digests after enrichment by GO@Fe_3O_4@MOF-303 probe: (a) 10 fmol/μL, (b) 1 fmol/μL, (c) 0.5 fmol/μL and (d) 0.1 fmol/μL. The peaks of N-linked glycopeptides are marked with "*".

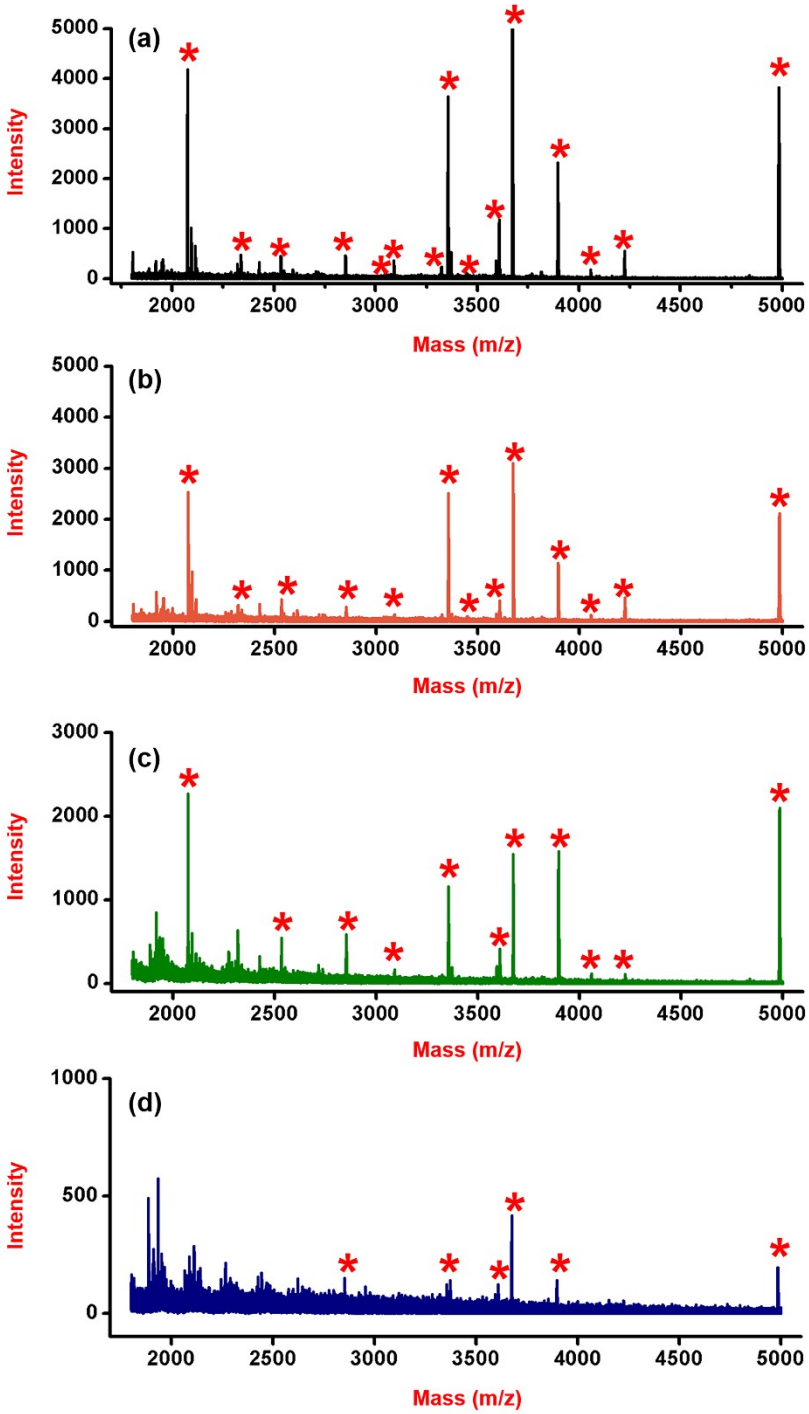


Fig. S8. MALDI-TOF MS intensity of four glycopeptides from 3 μg of HRP tryptic digest after enrichment by different amounts of the GO@Fe_3O_4@MOF-303 probe.

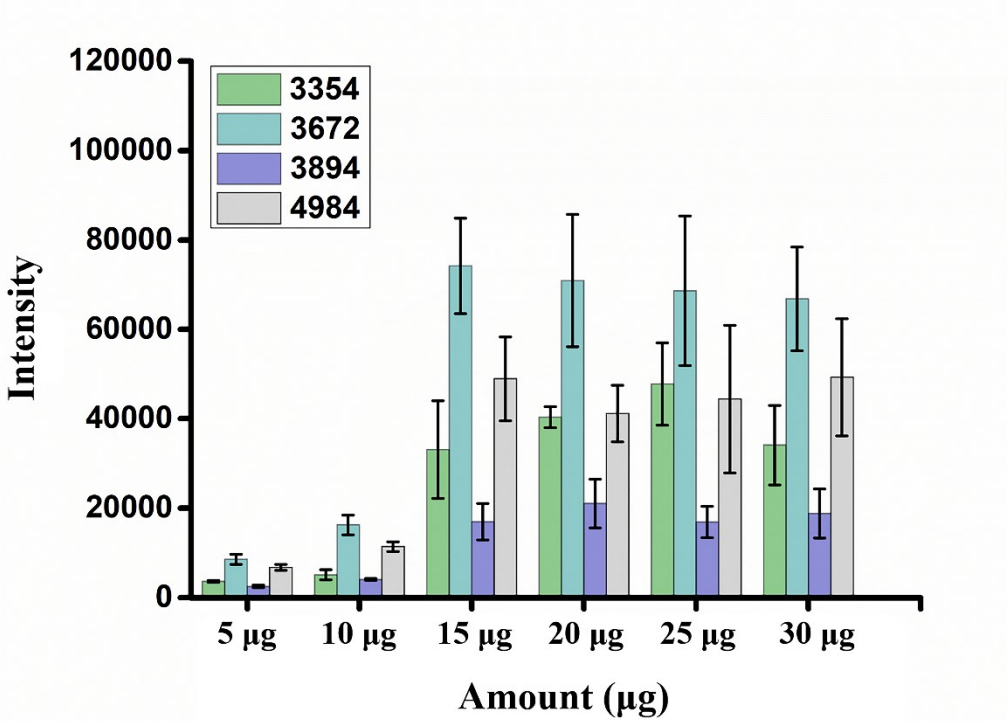


Fig. S9. Recovery of deglycosylated peptides from human IgG tryptic digest.

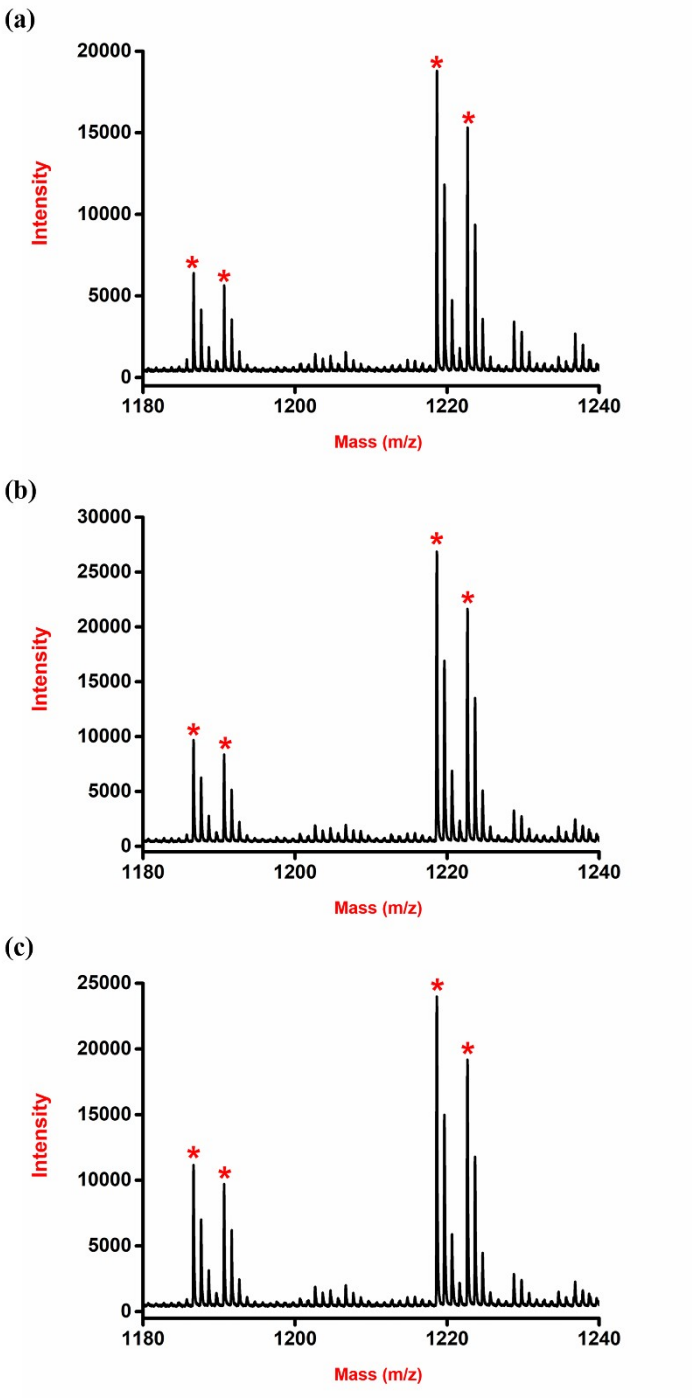


Fig. S10. (a) Cycling performance of GO@Fe_3O_4@MOF-303 probe for N-linked glycopeptides recognition. MALDI-TOF-MS for the N-linked glycopeptides derived from HRP tryptic digest: (b) after treatment with GO@Fe_3O_4@MOF-303 used for the first time and (c) after enrichment with GO@Fe_3O_4@MOF-303 probe recycled 8 times.

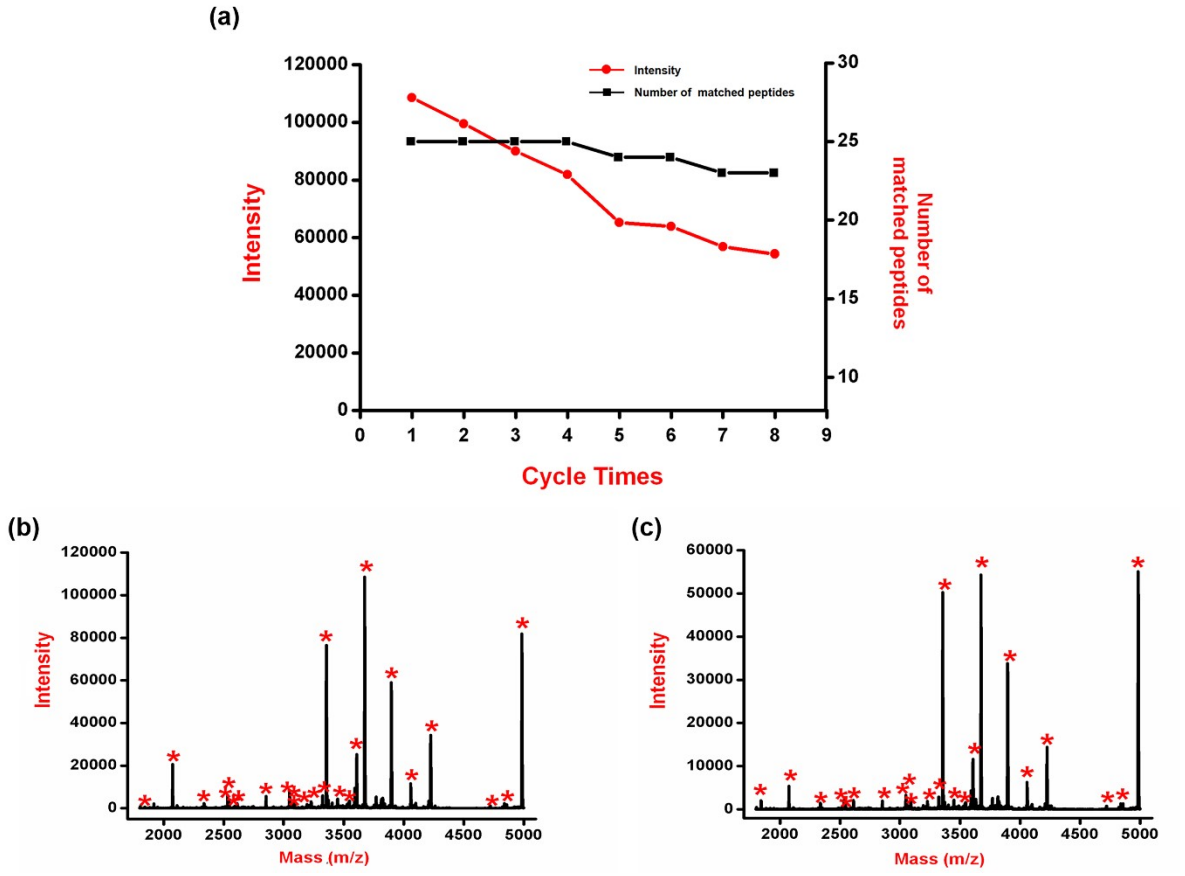


Fig. S11. MALDI-TOF-MS for the N-linked glycopeptides derived from HRP tryptic digest: (a) after treatment with the GO@Fe_3O_4@MOF-303 probe used for the first time and (b) after enrichment with the GO@Fe_3O_4@MOF-303 which had been placed for three months.

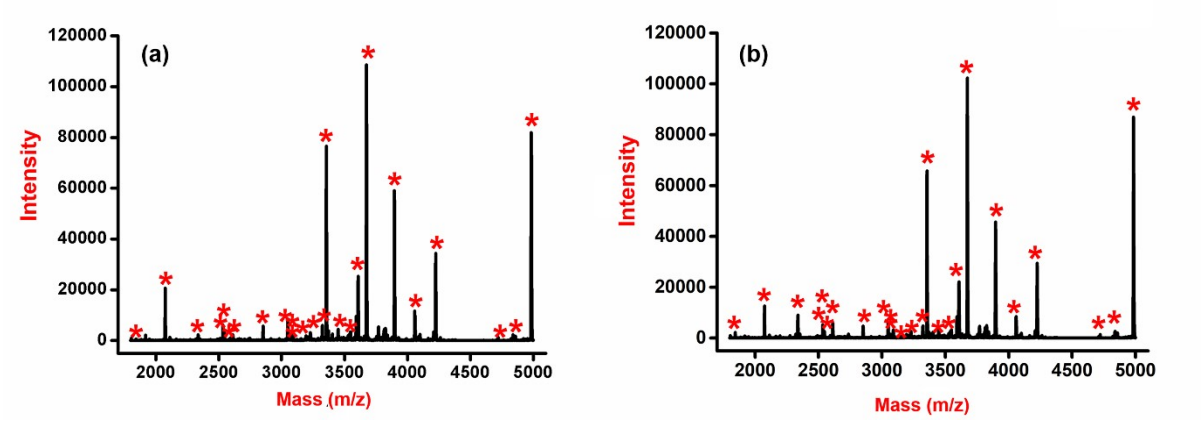


Fig. S12. MALDI-TOF-MS analysis of the N-linked glycopeptides derived from the mixture of the HRP digests containing different amounts of the glycosylated protein (HRP) and non-glycosylated protein (BSA) (at mass ratio of 1 : 500 : 500). Supernate before enrichment (a) in positive mode and (b) in linear mode and eluate after enrichment by GO@Fe_3O_4@MOF-303 probe (c) in positive mode and (d) in linear mode. The peaks of glycopeptides are marked with red asterisks and the peaks of proteins are marked with blue asterisks.

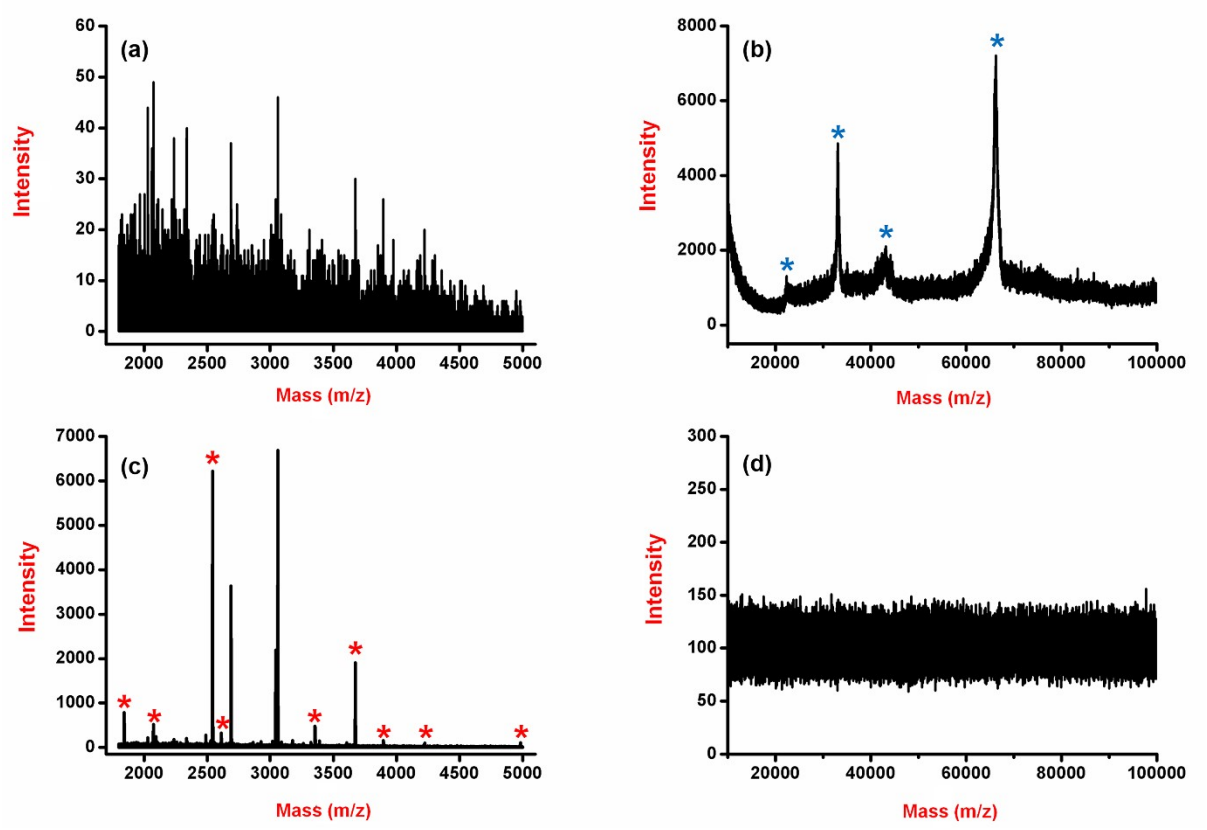


Fig. S13. Glycoproteomics Venn diagram analysis of N-linked glycopeptides profiling. (a) Venn diagram depicting a general view of the overlap of glycopeptides quantified in five samples of glycoproteomics profiling (S1-S5, healthy controls); (b) Venn diagram depicting a general view of the overlap of glycopeptides quantified in five samples of glycoproteomics profiling (S6-S10, HCC patients serum).

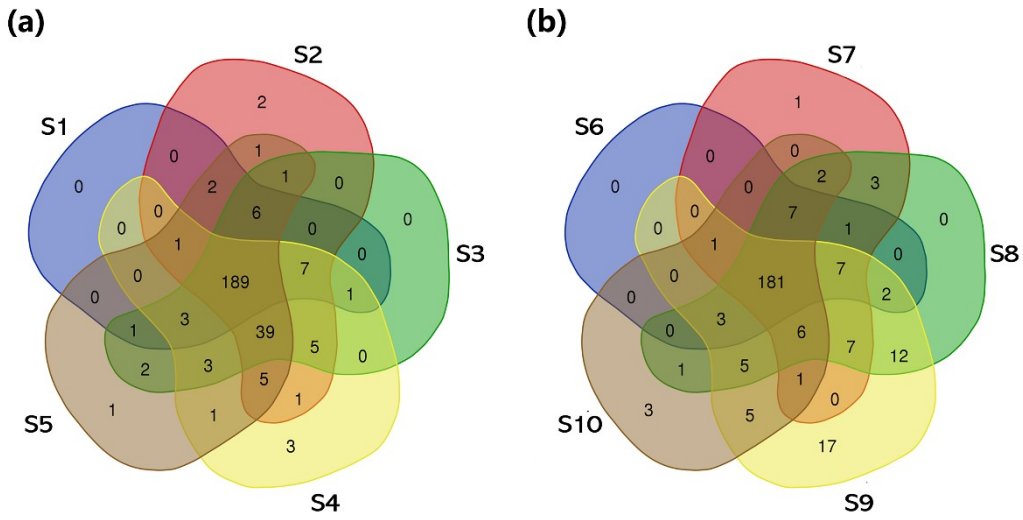


Fig. S14. Glycoproteomics Venn diagram analysis of glycoproteins profiling. (a) Venn diagram depicting a general view of the overlap of glycoproteins quantified in five samples of proteomic profiling (S1-S5, healthy controls); (b) Venn diagram depicting a general view of the overlap of glycoproteins quantified in five samples of proteomic profiling (S6-S10, HCC patients serum).

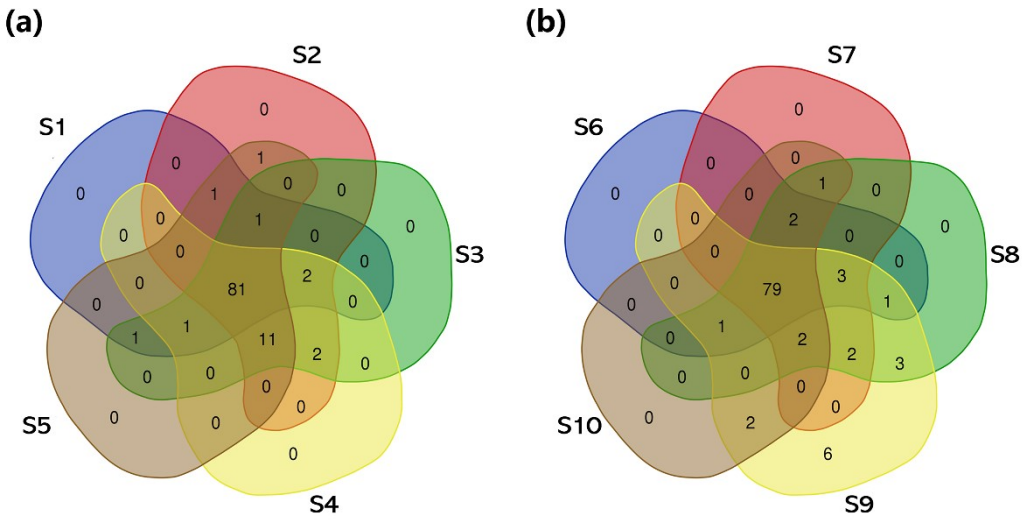
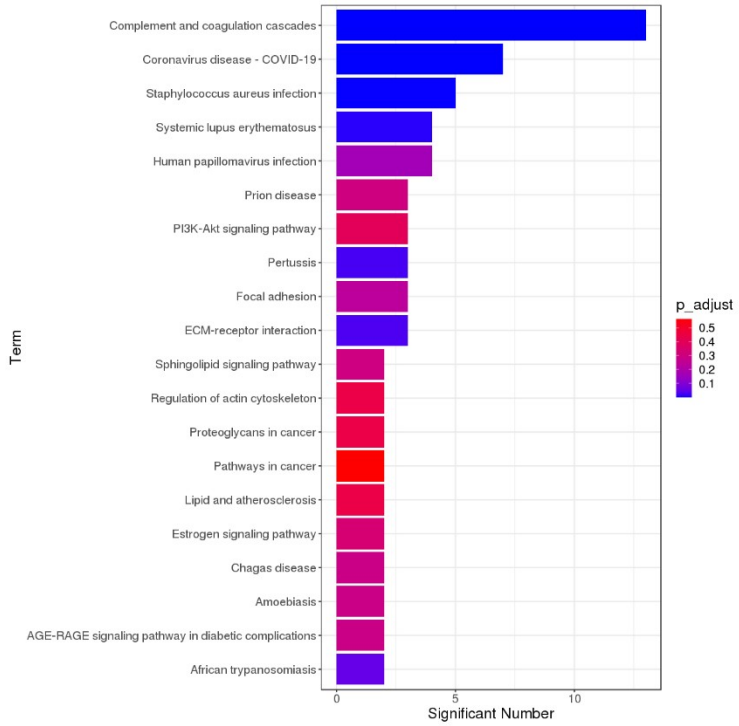


Fig. S15. Dysregulated pathways of the differential glycoproteins in the serum by enriched KEGG pathways analysis.



Detailed information of N-linked glycopeptides.

Table S1. Detailed information of the observed glycopeptides in HRP tryptic digest**.** N# denotes the N-glycosylation site.

No.	m/z	Glycan composition	Amino acid sequence
1	1843	XylMan3FucGlcNAc2	NVGLN#R
2	2074	XylMan3GlcNAc2	PN#VSNIVR
3	2321	Man2GlcNAc2	MGN#ITPLTGTQGQIR
4	2532	FucGlcNAc	SFAN#STQTFFNAFVEAMDR
5	2545	XylMan3FucGlcNAc2	SSPN#ATDTIPLVR
6	2591	XylMan3FucGlcNAc2	PTLN#TTYLQTLR
7	2612	XylMan3GlcNAc2	MGN#ITPLTGTQGQIR
8	2850	FucGlcNAc	GLIQSDQELFSSPN#ATDTIPLVR
9	3048	XylMan2GlcNAc2	SFAN#STQTFFNAFVEAMDR
10	3074	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
11	3087	XylMan3FucGlcNAc2	GLCPLNGN#LSALVDFDLR
12	3206	XylMan3GlcNAc2	SFAN#STQTFFNAFVEAMDR
13	3221	Man3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
14	3323	XylMan3FucGlcNAc2	QLTPTFYDNSCPN#VSNIVR
15	3354	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
16	3526	XylMan3GlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
17	3539	Man3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
18	3606	XylMan3FucGlcNAc2	NQCRGLCPLNGN#LSALVDFDLR
19	3672	XylMan3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
20	3894	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
21	4057	XylMan3GlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H_2O
22	4222	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
23	4721	Man3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
24	4839	XylMan3FucGlcNAc2 XylMan3GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
25	4984	XylMan3FucGlcNAc2 XylMan3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR

Table S2. Enrichment recovery yields of two deglycosylated peptides enriched from IgG tryptic digest using GO@Fe_3O_4@MOF-303 probe by MALDI-TOF MS.

Ratio (%)	EEQFN#STFR	EEQYN#STYR
D/H 1	90.6%	82.1%
D/H 2	86.4%	83.8%
D/H 3	88.3%	81.4%
Average recovery ± S. D. (%)	88.4%±2.1%	82.4%±1.2%

Table S3. The comparison of GO@Fe_3O_4@MOF-303 probe and other published hydrophilic composites for the enrichment of glycopeptides.

Materials	Standard Glycopeptides	LOD	Selectivity	Binding Capacity (mg/g)	Repeatability	Ref.
GO@Fe_3O_4@MOF-303	**25 (HRP)**	**0.1 fmol/μL (HRP)**	**HRP digests: HRP:BSA 1:1000:1000**	**200**	**8 times**	**this work**
mMOF@Au@GSH	19 (HRP)	0.5 fmol/μL (HRP)	HRP : BSA 1：100	140	5 times	[1]
Ce-MOF@PA	39 (IgG)	1 fmol/μL (IgG)	IgG digest: IgG:BSA 1:1000:1000	300	5 times	[2]
MUiO-66-NH_2/PA	21 (HRP)	1 fmol/μL (HRP)	HRP/BSA 1:1000	40	5 times	[3]
MG@Zn-MOFs	17 (HRP)	0.8 fmol/μL (HRP)	HRP digests: HRP: BSA 1:800:800	-	5 times	[4]
MIL-101(Cr)-maltose	15 (HRP)	1 fmol (IgG)	-	150	5 times	[5]
MIL-101-NH_2@PAMAM	14 (HRP)	1 fmol/μL (HRP)	HRP:BSA 1:100	-	-	[6]
MagG@Mg-MOFs-1C	23 (HRP)	0.1 fmol/μL (HRP)	HRP digests: HRP: BSA 1:500:500	150	8 times	[7]
UiO-66-COOH(20)	13 (HRP)	0.5 fmol/μL (HRP)	HRP:BSA 1:20	-	5 times	[8]
Fe_3O_4@Mg-MOF-74	17 (HRP)	0.5 fmol/μL (HRP)	HRP digests: HRP:BSA 1:800:800	-	5 times	[9]
magMOF@Au-maltose	24 (HRP)	10 fmol (HRP)	HRP digests: BSA digest 1:200	83	5 times	[10]

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