Supplementary Information

Construction of magnetic covalent organic framework with synergistic affinity strategy for enhanced glycopeptide enrichment

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Preparation of standard protein tryptic digestions

First, the model proteins (IgG, HRP, or BSA) were dissolved in a buffer (8 M urea, 50 mM NH_4HCO_3). Next, DTT (final concentration of 20 mM) was added into the dissolved model protein (2 mg/mL), and then reacted at 60 °C for 1 hour. Then, IAA (final concentration of 40 mM) was added for 45 minutes at room temperature in the dark. Finally, trypsin was added to the alkylated protein solution for digestion, the mass ratio of trypsin to protein was 1:40 (w/w), and the digestion was carried out at 37 °C for 16 hours.

Preparation of tryptic digests of proteins extracted from rat liver and exosome

For rat liver, a Sprague-Dawley male rat was sacrificed. Remove the liver immediately, cut into small pieces, and wash with salt water. Grind the liver tissue into powder with a liquid nitrogen mortar. The sample was added to a new 1.5 mL EP test tube, and 400 μL SDT lysis buffer was added to each EP test tube. After that, the samples were mixed and heated at 100 °C for 10 minutes. The sample was then cooled to room temperature in an ice bath, sonicated and loaded onto a 0.22 μm membrane tube, and then centrifuged at 14,000 g for 20 minutes. Next, 100 μL of 50 mM iodoacetamide was added to the filter and then incubated at room temperature for 30 minutes. Then 100 μL of 25 mM ABC (Applied Biosystems, Foster City CA, USA) was added to each filter and centrifuged at 14,000 g for 30 minutes. The protein suspension was then digested with 40 μL trypsin and incubated at 37 °C for 18 hours. Finally, the filtrate was collected by centrifugation at 14000 g for 30 minutes at room temperature. All collected filtrate was lyophilized for further use. For exosome, 5 mL of fresh plasma from healthy

individuals (n=5) and patients diagnosed with renal failure (n=12) were centrifuged at 10,000×g at 4 °C for 1 h. The supernatants were collected and centrifuged at ultra-high speed of 100,000×g at 4 °C for 2 h. The pellets were washed with PBS and centrifuged at 100,000×g at 4 °C for 2 h again. The process of exosome lysis, protein extraction, and digestion were similar to the above.

MALDI-TOF MS analysis

The MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) was enployed to analyse all eluent samples from standard protein tryptic digests in reflector positive mode. Matrix DHB was dissolved in 70% ACN-H_2O containing 1% H_3PO_4 (25 mg mL^{-1}). A 0.5 μL aliquot of the eluent and 0.5 μL of DHB matrix were sequentially dropped onto the MALDI plate for MS analysis.

Elite-LC-MS/MS analysis

The samples were analyzed using Easy-nLC nanoflow HPLC system connected to Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). One microgram of each sample was loaded onto Thermo Scientific EASY column (two columns) at a flow rate of 200 nL/min. The sequential separation of peptides on Thermo Scientific EASY trap column (100 μm×2 cm, 5 μm, 100 Å, C18) and analytical column (75μm×25 cm, 5 μm, 100 Å, C18) was accomplished using a segmented 1 h gradient from 5% to 28% Solvent B (0.1% formic acid in 100% ACN) for 40 min, followed by 28-90% Solvent B for 2 min and then 90% Solvent B for 18 min. The mass spectrometer was operated in positive ion mode. The dynamic exclusion duration was 30s. The raw files were analyzed using the Proteome Discoverer 2.1 software (Thermo Fisher Scientific). The acquired data was searched against the uniprot rat protein sequence database (version 1.4.1.1). As for the exosome sample, the raw files were searched by Mascot (version 2.4.1) against a human protein database (20397 entries) from Uniprot. The following search parameters were used: monoisotopic mass, trypsin as the cleavage enzyme, two missed cleavages, carbamidomethylation of cysteine as fixed modifications, peptide charges of 2+, 3+, and 4+, and the oxidation of methionine and Delta: H(1)O(-1)18O(1) of Asn (glycosylated asparagine 18O labeling) were specified as variable modifications. The mass tolerance was set to 20 ppm for precursor ions and to 0.1 Da for the fragment ions. The results were filtered based on a false discovery rate (FDR) of no more than 1% both for protein and peptide.

Characterization

Scanning electron microscopy (SEM, Hitachi S-4800, Japan) and transmission electron microscopy (TEM, JEM- 2010, Japan electronic) were used to analysis the morphologies of the samples. The size distribution were calculated via dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Company). Crystal structure was tested by powder X-ray diffraction (XRD, X' Pert Pro MPD, Philips, Netherlands).

Surface area and pore size analyzer (QuadraSorb SI, America) was employed to study the surface and BJH pore size distribution at 77K. Fourier transform infrared spectra was obtained by spectrometer (FT-IR, PE spectrometer) with wave number range 500-4000 cm^{-1}. The mass loss of sample was analyzed at temperature ranging from 35 to 800 °C with the heating rate of 10K/min by simultaneous thermal analysis (STA449 C Jupiter, NETZSCH). Vibrating sample magnetometer (VSM, model BHV-525, Riken Japanese Electronics Company) was employed to measure the magnetization of the sample with field from 0 Oe to 18000 Oe at 300K. X-ray photoemission spectroscopy (XPS) was obtained using a Kratos XSAM 800 instrument equipped with a monochromatic Al anode X-ray gun (12 kV, 15 mA, 10^{-5} Pa).

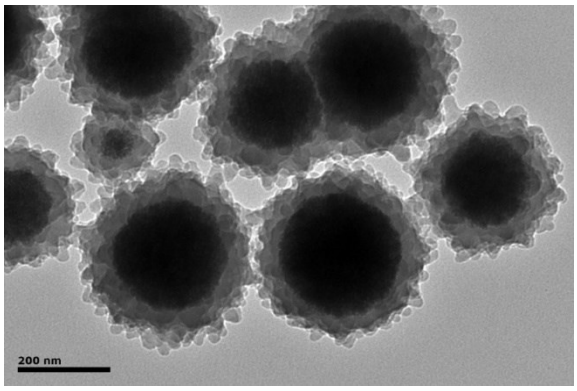
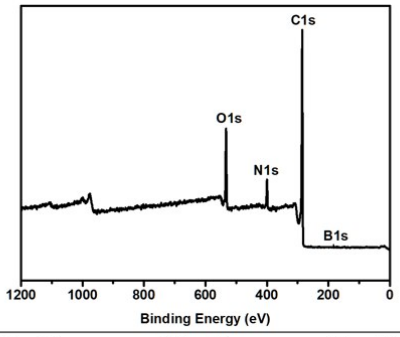


Fig. S1 TEM micrograph of the SPIOs@Polymer@COF nanocomposites.



Sample	C (at.%)	N (at.%)	O (at.%)	B (at.%)
SPIO@PVP/PEI@ COF(20%B)	82.05	4.72	12.83	0.4
SPIO@PVP/PEI@ COF(40%B)	77.57	6.58	15.27	0.58
SPIO@PVP/PEI@ COF(60%B)	79.66	7.08	12.44	0.82
SPIO@PVP/PEI@ COF(80%B)	76.76	6.88	15.41	0.95

Fig. S2 High-resolution XPS spectra of the SPIOs@Polymer@COF(80%B) nanocomposites. Determination of elemental ratio in SPIOs@Polymer@COF(B) nanocomposites containing different boric acid by XPS.

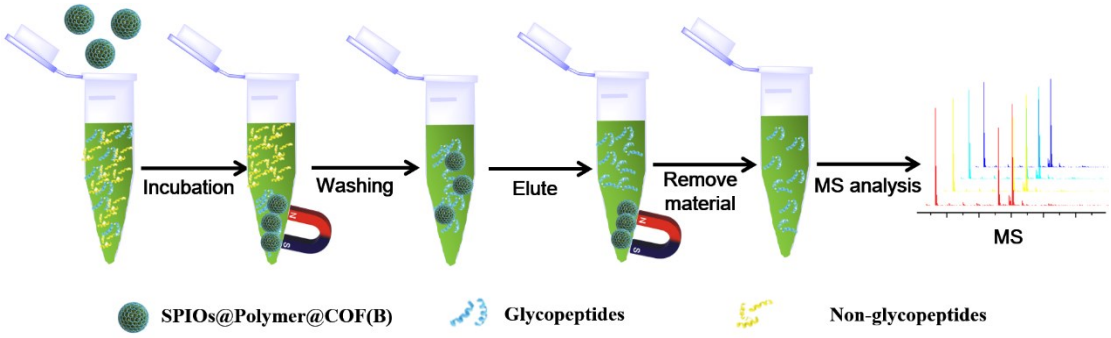


Fig. S3 Workflow of glycopeptide enrichment by the SPIOs@Polymer@COF(B) nanocomposites.

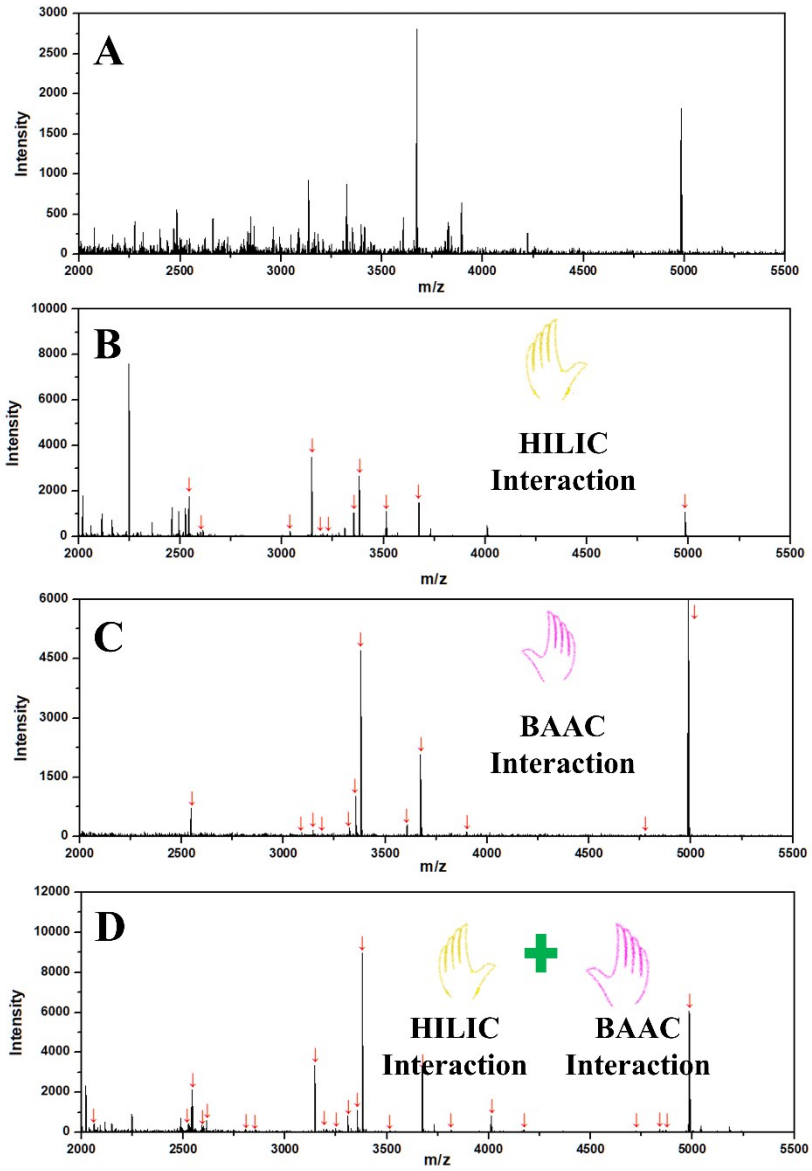


Fig. S4 MALDI-TOF mass spectra of HRP digests (A) before enrichment and after enrichment by SPIOs@Polymer@COF(B) nanocomposites (B) under the HILIC mode, (C) under BAAC mode,

and (D) under HILIC and BAAC modes. Glycopeptides are marked with red arrows.

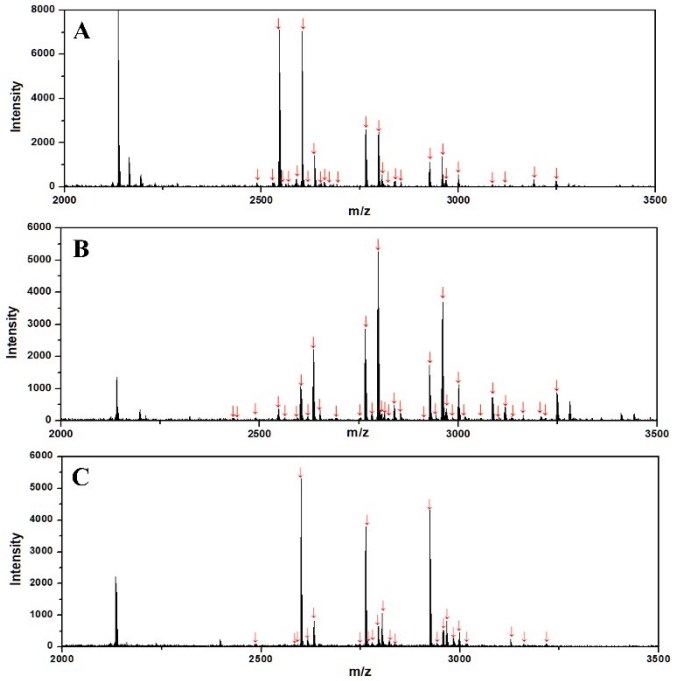


Fig. S5 MALDI-TOF mass spectra of IgG digests after enrichment by (A) SPIOs@Polymer@COF(40%B) nanocomposites, (B) SPIOs@Polymer@COF(60%B) nanocomposites, and (C) SPIOs@Polymer@COF(80%B) nanocomposites. Glycopeptides are marked with red arrows.

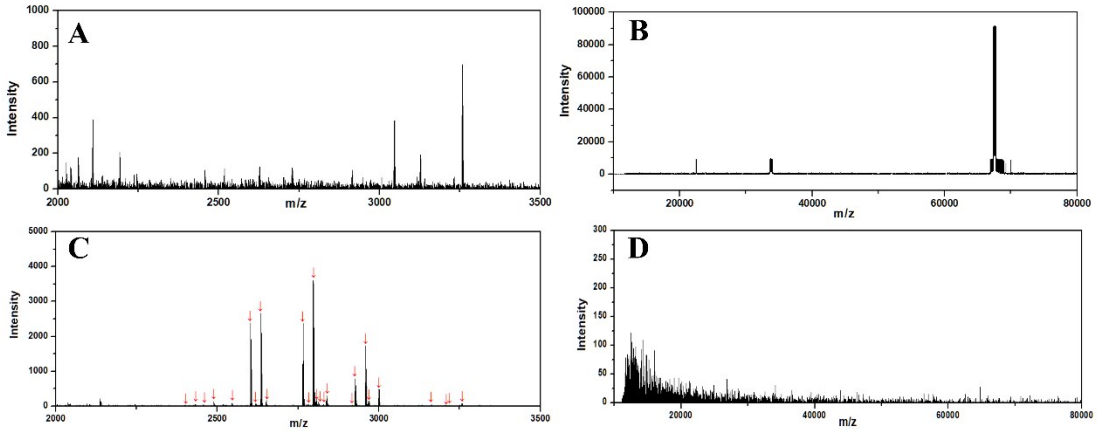


Fig. S6 MALDI-TOF mass spectra analysis of glycopeptides enriched from the mixture of IgG digests and nonglycoprotein BSA at the molar ratio of 1:500. Supernatant before enrichment (A) in reflection positive mode and (B) in high linear mode, eluate after treatment with SPIOs@Polymer@COF(B) (C) in reflection positive mode and (D) in high linear mode. Glycopeptides are marked with red arrows.

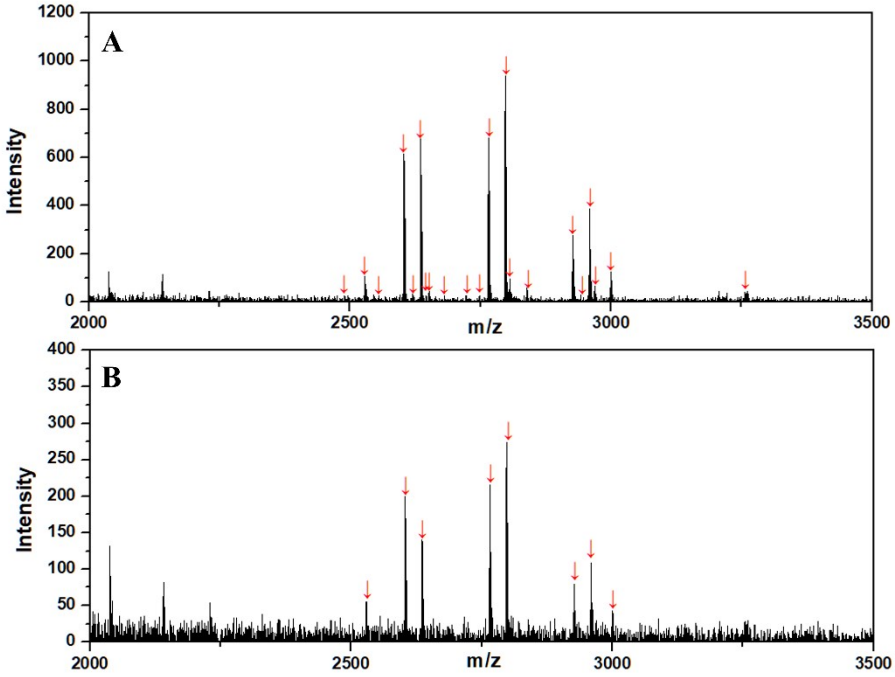


Fig. S7 MALDI-TOF-MS analysis of the tryptic digest mixture of IgG with different concentrations after enrichment with the SPIOs@Polymer@COF(B) nanocomposites: (A) 0.5 fmol/μL and (B) 0.05 fmol/μL. Glycopeptides are marked with red arrows.

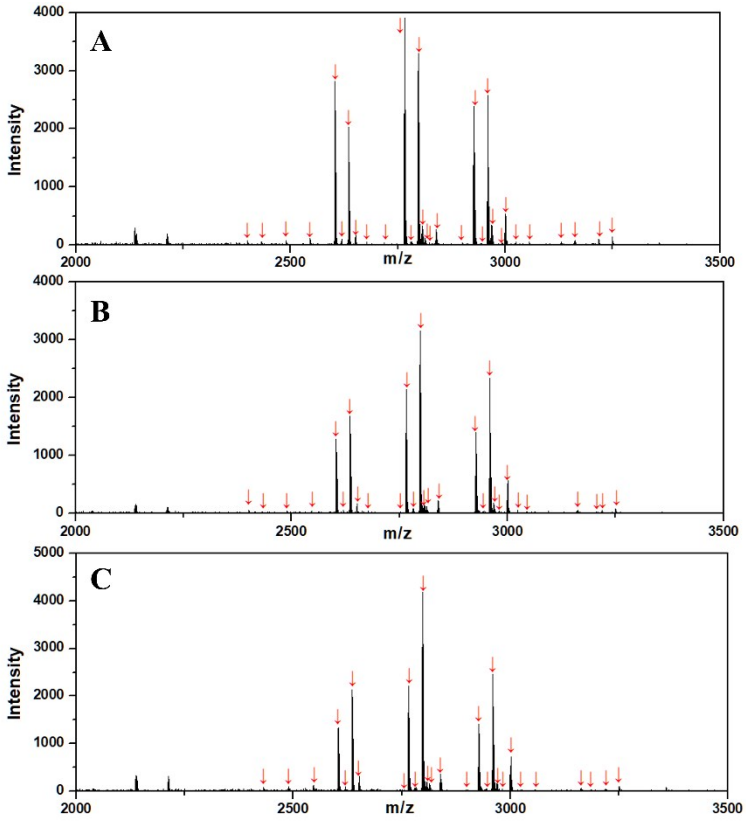


Fig. S8 MALDI-TOF-MS analysis for the glycopeptides derived from IgG tryptic digest after treatment with the SPIOs@Polymer@COF(B) nanocomposites used for the (A) first time, (B) third time, and (C) fifth time. Glycopeptides are marked with red arrows.

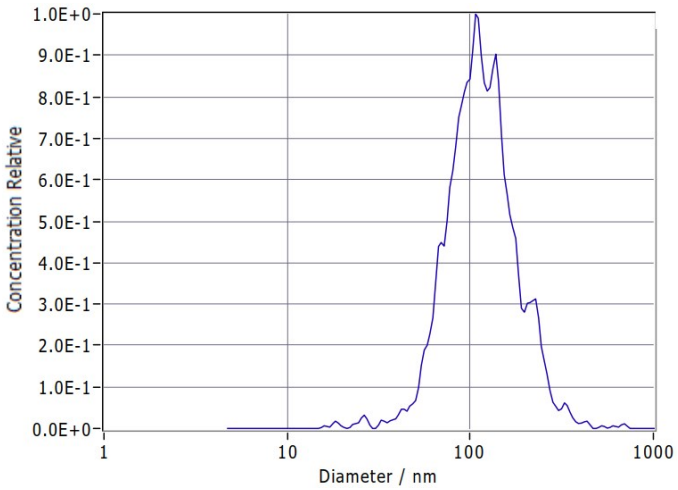


Fig. S9 The nanoparticle tracking analysis of the exosomes.

Table S1 Observed molecular masses, proposed glycan compositions and peptide sequences of N-linked glycopeptides enriched from human IgG tryptic digests by the SPIOs@Polymer@COF(B) nanocomposites. Hex, HexNAc, Fuc and NeuAc are the abbreviations of hexose, N-acetylhexosamine, fucose and N-acetylneuraminic acid, respectively. N# denotes the glycosylation sites.

Peak number	m/z	Glycan composition	Peptide sequence
I1	2236.9	[Hex]2 [NAc]3 [Fuc]1	EEQFN#STFR
I2	2269.2	[Hex]2[HexNAc]3[Fuc]1	EEQYN#STYR
I3	2399.6	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR
I4	2432.3	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
I5	2456.9	[Hex]3[HexNAc]4	EEQFN#STFR
I6	2489.2	[Hex]3[HexNAc]4	EEQYN#STYR
I7	2562.2	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR
I8	2593.8	[Hex]4[HexNAc]3[Fuc]1	EEQYN#STYR
I9	2603.3	[Hex]3[HexNAc]4[Fuc]1	EEQFN#STFR
I10	2619.4	[Hex]4[HexNAc]4	EEQFN#STFR
I11	2635.1	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR
I12	2650.9	[Hex]4[HexNAc]4	EEQYN#STYR
I13	2660.2	[Hex]3[HexNAc]5	EEQFN#STFR

I14	2692.6	[Hex]3[HexNAc]5	EEQYN#STYR
I15	2765.2	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I16	2781.6	[Hex]5[HexNAc]4	EEQFN#STFR
I17	2797.2	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
I18	2806.4	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STYR
I19	2813.2	[Hex]5[HexNAc]4	EEQYN#STFR
I20	2822.5	[Hex]4[HexNAc]5	EEQFN#STFR
I21	2838.2	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR
I22	2854.3	[Hex]4[HexNAc]5	EEQYN#STYR
I23	2910.7	[Hex]4[HexNAc]4 [NeuAc]1	EEQFN#STFR
I24	2927.6	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
I25	2942.4	[Hex]4[HexNAc]4 [NeuAc]1	EEQYN#STYR
I26	2959.3	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
I27	2968.2	[Hex]4[Hex7NAc]5[Fuc]1	EEQFN#STFR
I28	2984.1	[Hex]5[HexNAc]5	EEQFN#STFR
I29	3000.6	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR
I30	3016.5	[Hex]5[HexNAc]5	EEQYN#STYR
I31	3056.7	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I32	3104.2	[Hex]5[HexNAc]4[NeuAc]1	EEQYN#STYR
I33	3129.8	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
I34	3162.3	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR
I35	3205.1	[Hex]5[HexNAc]3[Fuc]1	TKPREEQFN#STFR
I36	3219.6	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I37	3250.5	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR

Table S2 Observed molecular masses, proposed glycan compositions and peptide sequences of N-linked glycopeptides enriched from HRP tryptic digests by the SPIOs@Polymer@COF(B) nanocomposites. Hex, HexNAc, Fuc and Xyl are the abbreviations of hexose, N-acetylhexosamine, fucose and xylose, respectively. N# denotes the glycosylation sites.

Peak number	m/z	Glycan composition	Peptide sequence
H1	2068.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PNVSN#IVR
H2	2533.2	[HexNAc]1[Fuc]1	SFAN#STQTFFNAFVEAMDR
H3	2542.3	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SSPN#ATDTIPLVR
H4	2592.1	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PTLN#TTYLQTLR
H5	2612.1	[Hex]3[HexNAc]2[Xyl]1	MGN#ITPLTGTQGQIR
H6	2803.6	[Hex]2[HexNAc]2	MGN#ITPLTGTQGQIRLNCR
H7	2851.4	[HexNAc][Fuc]1	GLIQSDQELFSSPN#ATDTIPLVR
H8	3146.4	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLCPLNGN#LSALVDFDLR.Oxide
H9	3190.4	[HexNAc]1[Fuc]1	LHFHDCFVNGCDASILLDN#TTSFR.Oxide
H10	3222.2	[Hex]3[HexNAc]2[Fuc]1	SFAN#STQTFFNAFVEAMDR
H11	3322.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSCPN#VSNIVR
H12	3354.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAMDR
H13	3379.2	[Hex]2[HexNAc]2[Fuc]1	GLIQSDQELFSSPN#ATDTIPLVR
H14	3510.1	[Hex]2[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
H15	3672.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
H16	3812.7	[Hex]2[HexNAc]2	LHFHDCFVNGCDASILLDN#TTSFRTEK
H17	4057.6	[Hex]4[HexNAc]2[Fuc]1[Xyl]1	LHFHDCFVNGCDASILLDN#TTSFR
H18	4222.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
H19	4720.2	[Hex]3[HexNAc]2[Fuc]1 [Hex]3[HexNAc]2[Fuc]1	LYN#FSNTGLPDPTLN#TTYLQTLR
H20	4839.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
H21	4852. 7	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Fuc]1	LYN#FSNTGLPDPTLN#TTYLQTLR

H22	4984.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Fuc]1[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR

Table S3 The comparison of the SPIOs@Polymer@COF(B) nanocomposites with the other reported affinity materials.

No.	Materials	sample source	Performance	Journal
1	magOTfP5SOFGa^{3+}	Rat liver	1006	Anal. Chem. , 2020, 92, 2680-2689
2	GO-PEI-Carr	Rat liver	149	Anal. Chem. , 2019, 91, 4047-4054
3	GO-Fe_3O_4/SiO_2/AuNWs/L-Cys	Rat liver	793	Analytica Chimica Acta, 2017, 970, 47-56
4	MAR@MOP	Rat liver	811	Analytica Chimica Acta, 2018, 1030, 96-104
5	SPIOs@SiO_2@MOF	Rat liver	152	ACS Sustainable Chem. Eng., 2019, 7, 6043-6052
6	MAR@(HA/CS)	Rat liver	1353	Journal of Chromatography A, 2017, 1498, 72-79
7	Fe_3O_4@Polymer@COF(B)	Rat liver	1921	This work