Supporting Information

Magnetic nanofiber-based zwitterionic hydrophilic material for selective capture and identification of glycopeptides

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Biological sample preparation.

1 mg of human IgG or chicken avidin was dissolved in 1 mL of $NaHCO_3$ buffer (50 mmol L^{-1}, pH 8.3), and digested with 20 μg of trypsin for 18 h at 37 ^{o}C. 2 mg of BSA was dissolved 1 mL of $NaHCO_3$ buffer (50 mmol L^{-1}, pH 8.3) containing urea (8 mol L^{-1}) and denatured at 60 ^{o}C for 20 min. Subsequently, 20 μL of dithiothreitol (1 mol L^{-1}) was added, and the mixture was incubated at 60 ^{o}C for 1 h. Afterward, 7.4 mg of iodoacetamide was mixed and incubated at 25 ^{o}C for 1 h in darkness. Finally, after being diluted ten-fold with $NaHCO_3$ buffer (50 mmol L^{-1}, pH 8.3), the treated BSA was digested with 20 μg of trypsin for 18 h at 37 ^{o}C.

1 μL of human serum was dispersed in 200 μL of $NaHCO_3$ buffer (25 mmol L^{-1}, pH 8.3) and centrifugally separated at 12000 rpm for 5 min. The supernatant was collected and denaturized at boiling water for 10 min. After cooled to 25 ^{o}C, 10 μL of dithiothreitol (1 mol L^{-1}) was added and incubated at 60 ^{o}C for 1 h. Soon after, 7.4 mg of iodoacetamide was mixed, and the mixture was incubated at 25 ^{o}C for 1 h in darkness. Finally, 40 μg of trypsin was added and incubated at 37 ^{o}C for 18 h. The obtained tryptic peptides were lyophilized and stored at a refrigerator with a temperature of -20 ^{o}C.

Recovery evaluation of glycopeptide enrichment

Two aliquots of human IgG digests (3 μg) were labeled with light and heavy isotopes by the stable isotope dimethyl labeling approach, respectively. The heavy-tagged human IgG digest was enriched with magHN/Au-GSH according to the proposed procedure, and the eluted fraction was added into the light-tagged digests. The combined mixture was re-enriched with magHN/Au-GSH, and the eluted fraction was directly analyzed by MALDI-TOF MS. The enrichment recovery was calculated by the peak intensity ratio of heavy isotope-labeled glycopeptides to the light isotope-labeled glycopeptides.

Nano LC-MS/MS analysis.

The enriched glycopeptides after deglycosylation were lyophilized and re-dissolved in 20 μL of buffer A (water containing 0.1 % formic acid), and loaded onto the Thermo Scientific Acclaim PepMap C_{18} column (100 μm×2.5 cm, 5 μm), and then separated by the analytical column (Thermo Scientific Acclaim PepMap C_{18} column, 75 μm×30 cm, 100 Å) with a linear gradient from 2 % buffer B to 40 % buffer B in 100 min (buffer B, ACN containing 0.1 % formic acid). The flow rate is set as 300 nL min^{-1}. 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. The Orbitrap Fusion mass spectrometer was operated in a data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra (m/z 350—1500) were acquired with a mass resolution of 120 000. MS/MS acquisition was achieved in the Orbitrap with a cycle time of 3 s.

Database searching.

All the nano LC-MS/MS raw data was searched with Mascot version 2.3.2 against a database (Human UniProtKB/Swiss-Prot). The mass tolerance was 10 ppm for precursor ions and 50 mmu for fragment ions. The cut-off false discovery rates (FDRs) for all peptide identifications were lower than 1%. Trypsin was specified as the enzyme. Carbamidomethylation of cysteine was set as the fixed modification. Oxidation on methionine and deamidation on asparagine were set as the variable modifications. Only peptides with N-!P-[S/T] were identified as N-linked glycopeptides.

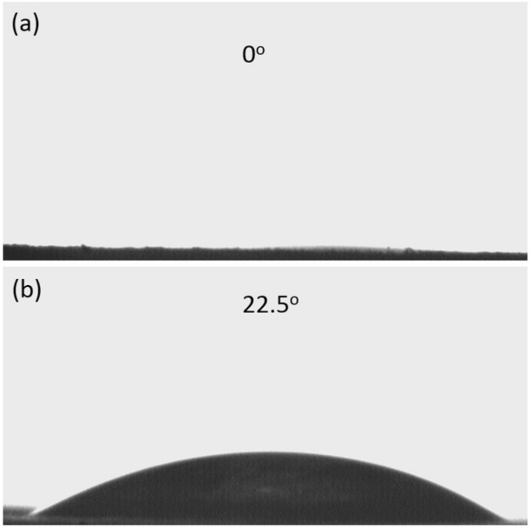

Fig. S1 The water contact angles: (a) HN and (b) magHN/Au-GSH.

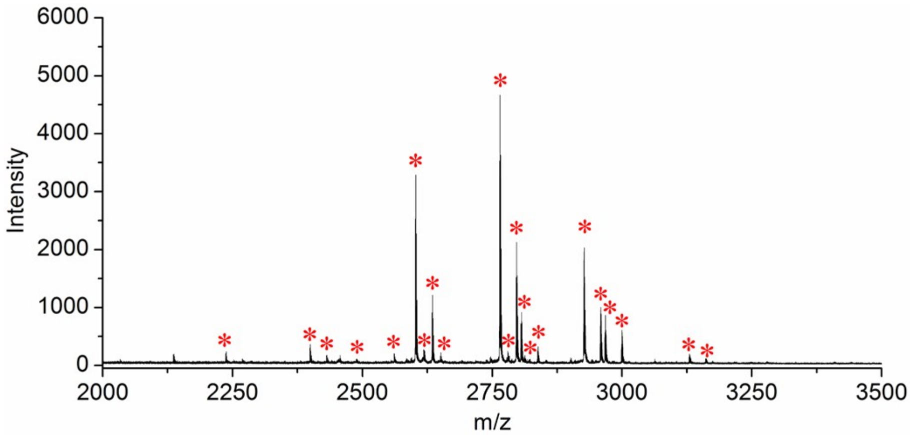

Fig. S2 The amplified spectrum of Fig. 5b with the *m/z* values range from 2000 to 3500.

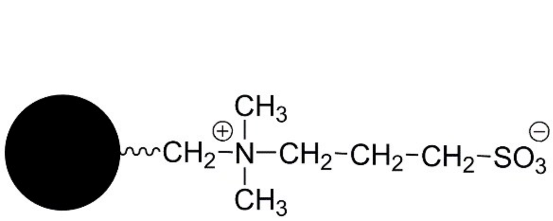

Fig. S3 The chemical structure of the commercial zwitterionic hydrophilic material.

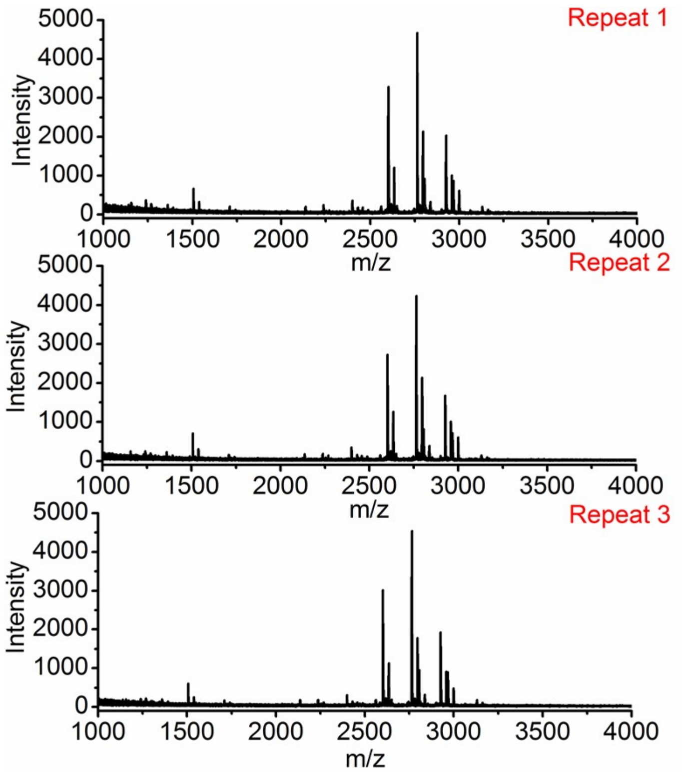

Fig. S4 MALDI-TOF MS spectra of 0.4 pmol of human IgG digest after enrichment with magHN/Au-GSH nanofiber during three consecutive repetition processes.

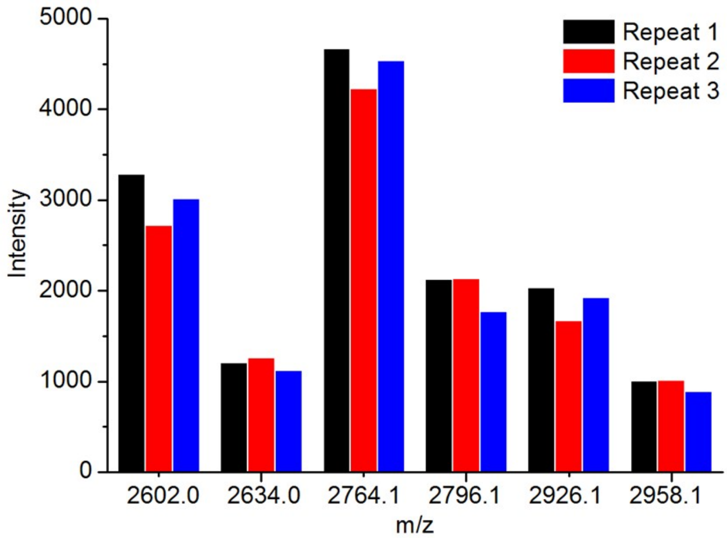

Fig. S5 The signal intensities of six selected N-linked glycopeptides in Fig. S4 during three consecutive repetition processes.

Table S1. Detailed information of the N-linked glycopeptides from human IgG digest after enrichment with magHN/Au-GSH nanofiber.
(N# denotes the N-linked glycosylation site)

No.	Glycan composition	Amino acid sequence	m/z
1	[Hex]3[HexNAc]2[Fuc]1	EEQFN#STFR	2236.8
2	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR	2398.9
3	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR	2430.9
4	[Hex]3[HexNAc]4	EEQYN#STYR	2487.9
5	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR	2560.9
6	[Hex]3[HexNAc]4[Fuc]1	EEQFN#STFR	2602.0
7	[Hex]4[HexNAc]4	EEQFN#STFR	2618.0
8	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR	2634.0
9	[Hex]4[HexNAc]4	EEQYN#STYR	2650.0
10	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR	2764.1
11	[Hex]5[HexNAc]4	EEQFN#STFR	2780.0
12	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR	2796.1
13	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STFR	2805.1
14	[Hex]5[HexNAc]4	EEQYN#STYR	2812.0
15	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR	2837.1
16	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR	2926.1
17	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR	2958.1
18	[Hex]4[HexNAc]5[Fuc]1	EEQFN#STFR	2967.1
19	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR	2999.1
20	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR	3129.1
21	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR	3161.1

Table S2. Detailed information of the N-linked glycopeptides from chicken avidin digest after enrichment with magHN/Au-GSH nanofiber.
(N# denotes the N-linked glycosylation site)

No.	Glycan composition	Amino acid sequence	m/z
1	[HexNAc]1	WTNDLGSN#MTIGAVNSR	2039.1
2	[Hex]3[HexNAc]2	WTNDLGSN#MTIGAVNSR	2728.3
3	[Hex]4[HexNAc]2	WTNDLGSN#MTIGAVNSR	2890.3
4	[Hex]2[HexNAc]3	WTNDLGSN#MTIGAVNSR	2931.4
5	[Hex]5[HexNAc]2	WTNDLGSN#MTIGAVNSR	3052.4
6	[Hex]3[HexNAc]3	WTNDLGSN#MTIGAVNSR	3093.4
7	[Hex]4[HexNAc]3	WTNDLGSN#MTIGAVNSR	3134.4
8	[Hex]6[HexNAc]2	WTNDLGSN#MTIGAVNSR	3214.5
9	[Hex]5[HexNAc]3	WTNDLGSN#MTIGAVNSR	3255.5
10	[Hex]4[HexNAc]4	WTNDLGSN#MTIGAVNSR	3296.5
11	[Hex]7[HexNAc]2	WTNDLGSN#MTIGAVNSR	3376.6
12	[Hex]6[HexNAc]3	WTNDLGSN#MTIGAVNSR	3417.6
13	[Hex]5[HexNAc]4	WTNDLGSN#MTIGAVNSR	3458.6
14	[Hex]6[HexNAc]4	WTNDLGSN#MTIGAVNSR	3620.6

Table S3. Enrichment recoveries of six selected N-linked glycopeptides from human IgG digest using magHN/Au-GSH nanofiber.

m/z	Recovery (%)
2602.0	85.3±3.6
2634.0	90.6±1.3
2764.1	94.1±0.9
2796.1	88.5±1.6
2926.1	87.7±0.8
2958.1	91.7±1.3

Table S4. Detailed information of N-linked glycopeptides from human serum digest after enrichment with magHN/Au-GSH nanofiber.

(N# denotes the N-glycosylation site; c denotes Carbamidomethyl; n denotes Deamidated; m denotes Oxidation)

No.	Protein Group Accessions	Sequence
1	P27169	SLDFNTLVDN#ISVDPETGDLWVGcHPNGMK
		VVAEGFDFANGIN#ISPDGK
		VTQVYAEN#GTVLQGSTVASVYK
2	P05543	VTAcHSSQPN#ATLYK
3	P55058	EGHFYYN#ISEVK
		N#WSLPNR
		VSN#VScQASVSR
		IYSN#HSALESLALIPLQAPLK
4	P06681	TMFPN#LTDVR
		QSVPAHFVALN#GSK
5	P01024	TVLTPATnHMGN#VTFTIPANR
		TVLTPATNHMGN#VTFTIPAnR
		TVLTPATNHMGN#VTFTIPANR
6	P01860	EEQYN#STFR
7	O95445	TELFSSScPGGIMLN#ETGQGYQR
8	P29622	SQILEGLGFN#LTELSESDVHR
		DFYVDEN#TTVR
9	P00734	SRYPHKPEIN#STTHPGADLQEN#FcR
		WVLTAAHcLLYPPWDKN#FTEnDLLVR
		YPHKPEIN#STTHPGADLQENFcR

10	P19652	QNQcFYN#SSYLNVQR
11	P01009	ADTHDEILEGLnFN#LTEIPEAQIHEGFQELLR
		YLGN#ATAIFFLPDEGKLQHLENELTHDIITK
		QLAHQSN#STNIFFSPVSIATAFAMLSLGTK
		YLGN#ATAIFFLPDEGK
		ADTHDEILEGLNFN#LTEIPEAQIHEGFQELLR
12	P02763	QDQcIYN#TTYLNVQR
13	P10643	N#YTLTGR
14	P00738	VVLHPN#YSQVDIGLIK
		VVLHPN#YSQVDIGLIKLK
		QLVEIEKVVLHPN#YSQVDIGLIK
15	P01008	WVSN#KTEGR
		LGAcN#DTLQQLmEVFK
		LGAcN#DTLQQLMEVFKFDTISEK
		SLTFN#ETYQDISELVYGAK
16	Q92954	N#GTLVAFR
17	P02790	SWPAVGN#cSSALR
		SLGPN#SSANGPGLYLIHGPnLYcYSDVEK
		ALPQPQN#VTSLLGcTH
		ERSWPAVGN#cSSALR
		cSDGWSFDATTLDDN#GTMLFFK
		N#GTGHGN#STHHGPEYMR
		SLGPN#SSANGPGLYLIHGPnLYcYSDVEKLNAAK
18	P01011	NVIFSPLSISTALAFLSLGAHN#TTLTEILK
		YTGN#ASALFILPDQDK
		YTGN#ASALFILPDQDKMEEVEAMLLPETLK
		LINDYVKN#GTR
		KLInDYVKN#GTR
		YTGN#ASALFILPDQDKMEEVEAMLLPETLKR
		APDKNVIFSPLSISTALAFLSLGAHN#TTLTEILK
		GLKFN#LTETSEAEIHQSFQHLLR
		FN#LTETSEAEIHQSFQHLLR
		TLN#QSSDELQLSMGNAMFVK
19	P15144	N#ATLVN#EADKLR
20	Q06033	NAHGEEKEN#LTAR
21	P10909	LAN#LTQGEDQYYLR
		MLN#TSSLLEQLNEQFNWVSR
		HN#STGcLR
		EDALN#ETR
		LKELPGVcN#ETMMALWEEcKPcLK
		EDALN#ETRESETK
22	P08603	MDGASN#VTcINSR
		ISEEN#ETTcYMGK
		IPcSQPPQIEHGTIN#SSR

23	P22792	LYLGSnNLTALHPALFQN#LSK
		AFGSNPN#LTK
24	P36980	LQNNENN#IScVER
25	Q13201	KPTVN#LTTVLIGR
		FNPGAESVVLSN#STLK
26	P03952	IYSGILN#LSDITK
		LQAPLN#YTEFQKPIcLPSKGDTSTIYTncWVTGWGFSK
		GVNFN#VSK
		IYPGVDFGGEELN#VTFVK
		IVGGTN#SSWGEWPWQVSLQVK
		LQAPLN#YTEFQKPIcLPSK
27	Q14624	LPTQN#ITFQTESSVAEQEAEFQSPK
		NQALN#LSLAYSFVTPLTSMVVTKPDDQEQSQVAEKPMEGESR
		AFITN#FSMIIDGMTYPGIIK
28	P20851	EWDN#TTTEcR
		TLFcN#ASK
		LGHcPDPVLVNGEFSSSGPVN#VSDK
		TLFcN#ASKEWDN#TTTEcR
29	P01876	LSLHRPALEDLLLGSEAN#LTcTLTGLR
		LAGKPTHVN#VSVVMAEVDGTcY
30	P01042	YNSQN#QSNNQFVLYR
		ITYSIVQTN#cSK
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33	O75882	IDSTGN#VTNELR
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34	P02787	QQQHLFGSN#VTDcSGN#FcLFR
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37	P43652	IcAMEGLPQKHN#FSHccSK
		N#NTENPPGcYR
		DIENFN#STQK

		YAEDKFN#ETTEK
38	P01033	FVGTPEVN#QTTLYQR
39	P35858	AGAFLGLTNVAVMN#LSGNcLR
40	Q08380	TVIRPFYLTN#SSGVD
		GLN#LTEDTYKPR
41	P05090	ADGTVNQIEGEATPVN#LTEPAKLEVK
		ADGTVnQIEGEATPVN#LTEPAK
		cIQAN#YSLMEnGK
42	P0DOX8	AN#PTVTLFPPSSEELQANK
43	P02766	ALGISPFHEHAEVVFTAN#DSGPR
44	Q04756	cFLGN#GTGYR
45	P00736	cN#YSIR
46	P17936	GLcVN#ASAVSR
47	P02748	AVN#ITSENLIDDVVSLIR
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		THTN#ISESHPNATFSAVGEASIcEDDWNSGER
		YKN#NSDISSTR
		STGKPTLYN#VSLVMSDTAGTcY
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		VHN#GSEILFSYFQDLVITLPFELR
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		TPLTAN#ITK
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		VSN#QTLSLFFTVLQDVPVR
		GcVLLSYLN#ETVTVSASLESVR

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		RN#HScEPcQTLAVR
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		VITVQVAN#FTLR
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		NLFLN#HSENATAK
		nLFLN#HSEnATAK
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