New GO/PEI/Au/L-Cys ZIC-HILIC composites: synthesis and selective enrichment of glycopeptides

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Chemicals and Materials

GO was purchased from Xianfeng Nanotech (Nanjing, China). Polyethylenimine (Mn: 10000, PEI). TPCK-treated trypsin, myoglobin (Myo), horseradish peroxidase (HRP), chicken avidin, human serum immunoglobulin G (human IgG), dithiothreitol (DTT), iodoacetamide (IAA), formic acid (FA), trifluoroacetic acid (TFA), formaldehyde (HCHO), formaldehyde-d_2 (DCDO) and urea were purchased from Sigma (St. Louis, MO, USA). 2, 5-Dihydroxybenzoic acid (DHB) was obtained from Bruker (Daltonios, Germany). HPLC grade acetonitrile (ACN) and SeQuant ZILIC-HIC (5 μm) were purchased from Merck (Darmstadt, Germany). L-Cysteine was obtained from J&K Chemical (Beijing, China). Peptide-N-glycosidase (PNGase F) was obtained from New England Biolabs (Ipswich, MA, USA). Multiple affinity removal column (4.6×50 mm, Hu-14) and buffer solution were obtained from Agilent Technologies (Agilent, CA, USA). Water was purified using a Milli-Q system (Millipore, Molsheim, France). All other reagents were of analytical grade purchased from China.

Synthesis of GO/PEI//Au/L-Cys composites

Thirty milligram PEI was added into 1 mL 0.1 mg/mL GO suspension under vigorous stirring for 60 min. After 2 mg $HAuCl_4.3H_2O$ was added, the mixture was heated at 70 ºC in a water bath for 60 min to generate GO/PEI/Au composites. After centrifugation and wash for four cycles at 13400 rpm (15 min/cycle), the resulted GO/PEI/Au composites were vacuum-dried for further use. Subsequently, 121 mg L-Cys dissolved in 2 mL H_2O was mixed with above GO/PEI/Au composites (0.5 mg) at room temperature for 24 h under stirring. Finally, the prepared GO/PEI/Au/L-Cys nanocomposites were centrifuged and washed with H_2O for three times to remove excess L-Cys, and vacuum-dried for further use.

High-abundant proteins removal from human plasma

The human plasma sample from healthy was thawed at -20 °C and ultrafiltrated at 134000 g for 60 min. A multiple affinity removal column was used to remove 14 high-abundant proteins from human plasma (4.6×50 mm, Hu-14) The obtained low-abundant proteins were desalted by a C8 trap column and dissolved in 50 mM NH_4HCO_3 (pH 8.0) containing 8 M urea. The concentration of the low-abundant proteins was determined as 1 mg/mL by BCA reagents.

Tryptic digestion of the glycoproteins and human plasma low-abundant proteins

Glycoproteins and human plasma low-abundant proteins were dissolved in 50 mM NH_4HCO_3 (pH 8.0) containing 8 M urea, and then reduced in 10 mM DTT for 1 h at 56 °C. When cooled to room temperature, cysteines were alkylated in the dark in 20 mM IAA for 30 min at 37 °C. After diluted ten-fold with 50 mM NH_4HCO_3 (pH 8.0), the solution was subsequently treated with trypsin at 37 °C (enzyme/protein ratio of 1:40, m/m) for 12 h. All digestion was stopped with FA to a final concentration of 1 %. The tryptic digestions were desalted on an SPE-C18 column with 2% and 98% ACN (v/v), containing 0.1% TFA (v/v), as the loading and eluting buffer, respectively. The eluent was lyophilized in a SpeedVac (Thermo Fisher, San Jose, CA, USA), and stocked at -20ºC for further use.

Enrichment of glycopeptides

In a typical procedure, 20 μg materials were added to 0.1 μg tryptic digests dissolved in 50 μL ACN/H_2O/FA solution (80: 20: 0.1, v/v/v, containing 5 mM NH_4HCO_3). The enrichment was carried out under gentle agitation at room temperature for 2.5 min. After incubation, the supernatant was discarded by centrifugation and washed three times with loading buffer to remove non-glycopeptides (20 μL/times). Glycopeptides were eluted with 20 μL ACN /H_2O/FA (60: 40: 0.1, v/v/v, containing 5 mM NH_4HCO_3) by continuously washing at room temperature for 2.5 min. For human plasma low-abundant proteins, 1 mg GO/PEI/Au/L-Cys nanocomposites were incubated with 5 μg tyrptic human plasma low-abundant proteins. ACN/H_2O/FA (75: 25: 0.1, v/v/v, containing 5 mM NH_4HCO_3) and ACN/H_2O/FA (50: 50: 0.1, v/v/v, containing 5 mM NH_4HCO_3) was used as loading and eluting solution, respectively. The enrich conditions of commercial were same to that of GO/PEI/Au/L-Cys nanocomposites.

Deglycosylation of N-linked glycopeptides by PNGase F

Each N-linked glycopeptides fraction collect from the enrichment was redissolved in50 μL 25 mM NH_4HCO solution, and incubated with 1000 U of PNGase F overnight at 37 °C. The obtained deglycosylated peptides were directly spotted on the MALDI target plate or analysis by *nano* -RPLC-ESI-MS/MS.

Recovery evaluation of glycopeptides enrichment

According to the previous protocol [1], the tryptic digests of human IgG (10 μg) were respectively labeled with light and heavy isotopes. The heavy-tagged human IgG digest was enriched with GO/PEI/Au/L-Cys (2 mg), and the eluent was spiked into the light-tagged human IgG digests. The combined mixture was further enriched by GO/PEI/Au/L-Cys, and the eluant was deglycosylated, followed by MALDI-TOF MS analysis. The recovery was calculated by the peak intensity ratio of the heavy-tagged to the light-tagged deglycosylated peptides.

Characterization

Transmission electron microscopy (TEM) images were obtained by JEOL JEM-2000EX instrument operated at 120 kV (JEOL, Tokyo, Japan).High-resolution TEM (HR-TEM) images were collected on a Tecnai G^2 F30 S-Twin microscope operated at 300 kV (Tecnai G2, FEI, Eindhoven, Netherlands). X-ray photoelectron spectroscopy (XPS) measurements were conducted with Thermo ESCALAB250Xi spectrometer with Al Kα radiation as the X-ray source (Thermo, Waltham, USA). The size of gold nanoparticles were detected by Malven Nano-zs90 dynamic light scattering (Malven, Worcester, UK). Fourier-transformed infrared spectroscopy (FT-IR) characterization was performed on Perkin-Elmer Spectrum GX spectrometer (Perkin-Elmer, Waltham, USA). UV-vis analyses were performed on Agilent Cary 60 spectrometer (Agilent, California, USA). The contact angles were measured by DSA100 (Krüss, Hamburg, German). The nitrogen adsorption and desorption isotherms were measured by QuadrasorbSI (Quadraorb, Wisconsin, USA). The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface areas with adsorption data in a relative pressure range from 0.052 to 0.26. The BJH pore-size distribution curve was obtained by nitrogen adsorption results.

MS analysis and date research

All MALDI spectra were taken from a Bruker Ultraflex III MALDI-TOF/TOF MS instrument (Bruker, Daltonios, Germany). A total of 1 μL of elution was dropped onto a MALDI plate, to which 1 μL of DHB solution (20 mg/mL, 0.1% TFA in 60% ACN aqueous solution) was added. The laser intensity was kept constant for all samples. External calibration of MALDI-TOF/TOF MS spectra was performed with ten commercial peptides. The obtained human plasma glycopeptides were injected for *nano*-LC-MS/MS analysis. A packed C_{18} column (75 μmi.d.× 15 cm) was used for peptide separation, with the flow rate of 200 nL/min. Two percent (v/v) ACN with 0.1% (v/v) FA (buffer A) and 98% (v/v) ACN with 0.1% (v/v) FA (buffer B) were used to generate a 125 min gradient, set as follows: 0% B for 10 min, to 5% B in 15 min, to 35% B in 105 min, to 80% B in 115 min, and kept at 80% B for 10 min. The LTQ-Orbitrap instrument (Thermo-Fisher, San Jose, CA, USA) was operated at positive ion

mode. The spray voltage was 2.3 kV, and the heated capillary temperature was 200°C. Total ion current chromatograms and mass spectra covering the mass range from m/z 350 to 1800 were recorded with Xcalibur software (version 1.4). MS/MS spectra were acquired by data-dependent acquisition mode with 15 precursor ions selected from one MS scan. Precursor selection was based on parent ions intensity, and the normalized collision energy for MS/MS scanning was 35%.

The acquired MS/MS spectra were searched against the International Protein Index (IPI) human protein database (version 3.71) using MASCOT software (version 2.3.2). The search criteria were set as follows: variable modifications of methionine oxidation (+16Da),deamidation (N)and fixed modification of cysteine residues (+57 Da), at most two missed tryptic cleavage sites,10 ppm error tolerance in MS and 0.5 Da error tolerance in MS/MS. The search results were filtered by pBuild to control the peptide FDR≤1%. FDRs were calculated by using the following equation: FDR= n(rev)/n(forw), where n(forw) and n(rev) are the number of peptides identified in proteins with forward (normal) and reversed sequence, respectively. Since N-glycosylation occurred at a consensus N-X-S/T (X≠P), the remaining peptide sequences were additionally filtered to remove non-motif containing peptides.

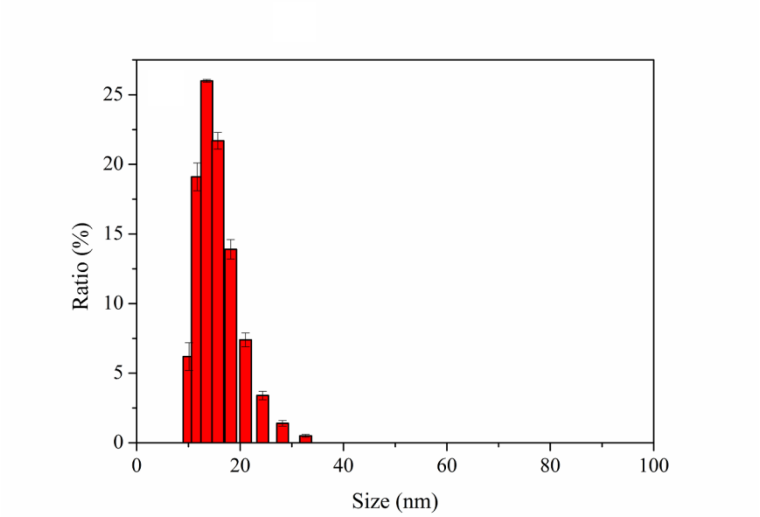

Fig. S1 Size of gold nanoparticles by dynamic light scattering.

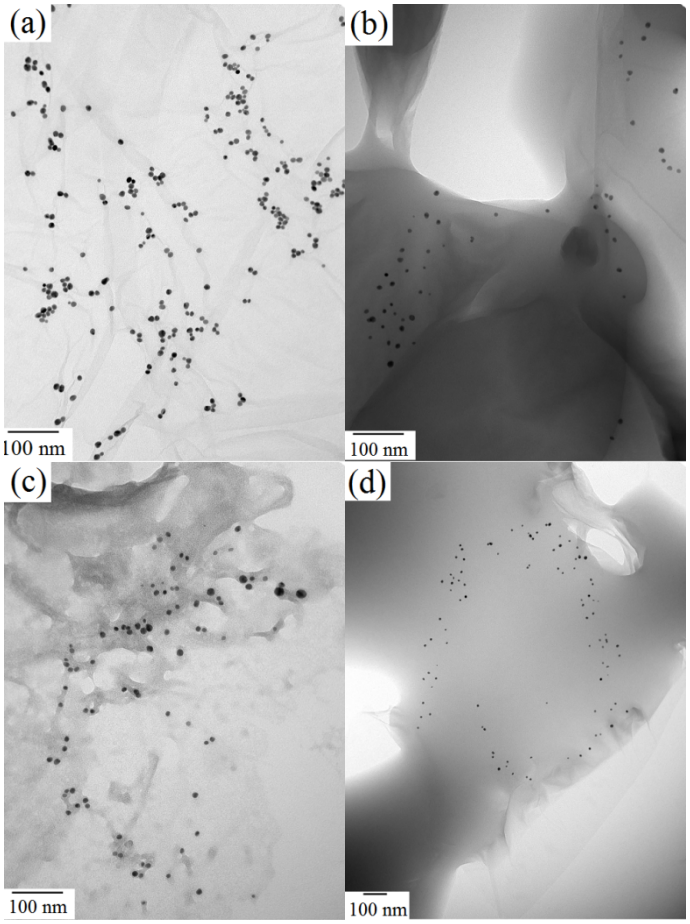

Fig. S2 TEM images of GO/PEI/Au/L-Cys composites prepared with different reaction time between L-Cys and Au NPs: (a) 2 h, (b) 4 h, (c) 16 h and (d) 24 h.

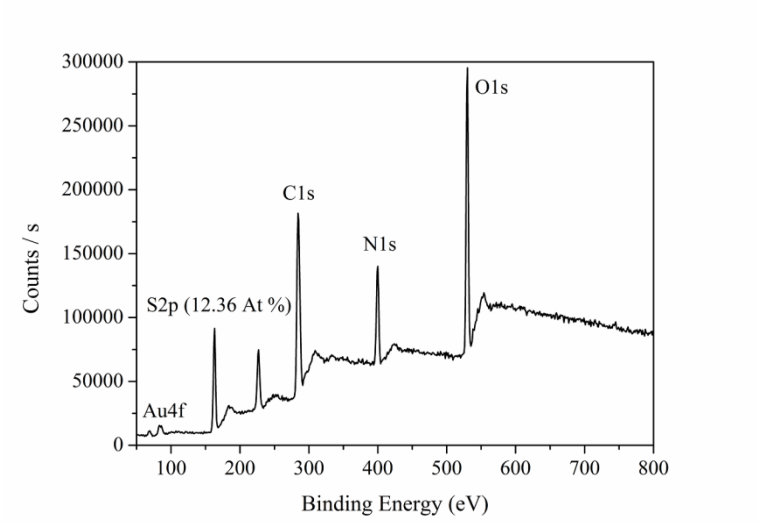

Fig. S3 XPS survey spectra of GO/PEI/Au/L-Cys composites.

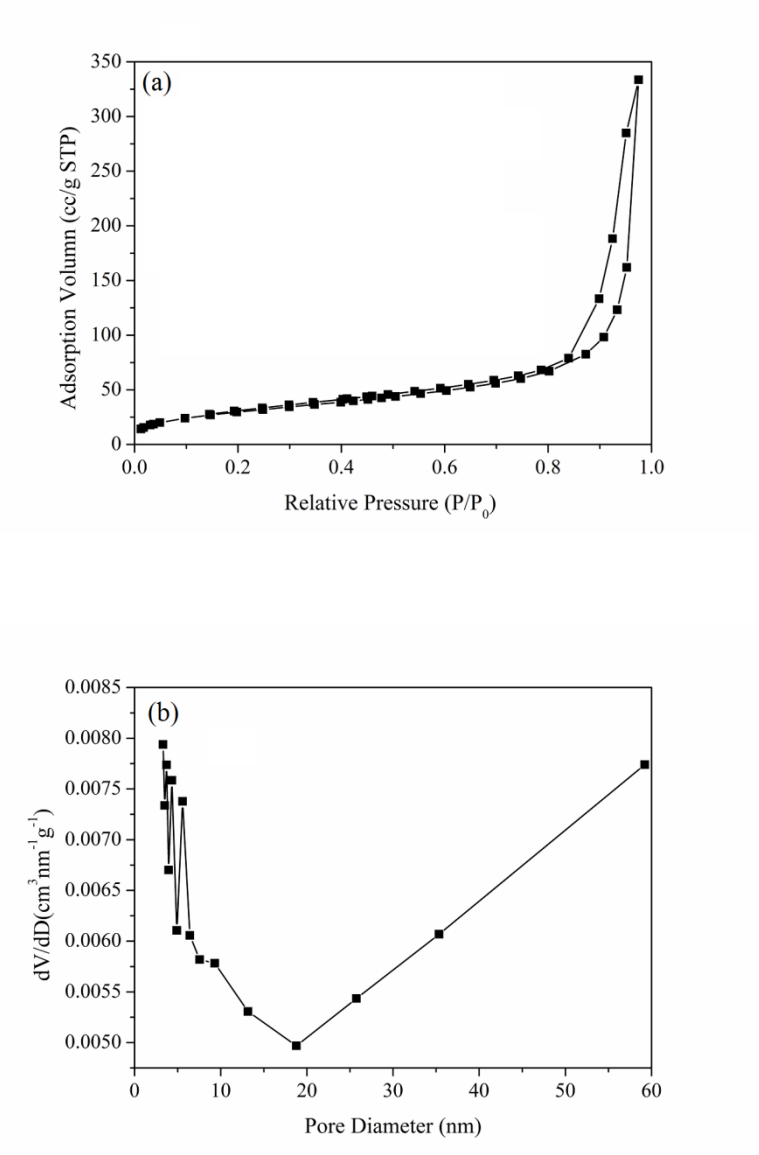

Fig. S4 (a) Nitrogen adsorption-desorption isotherms of GO/PEI/Au/L-Cys, (b) BJH pore-size distribution curve of GO/PEI/Au/L-Cys.

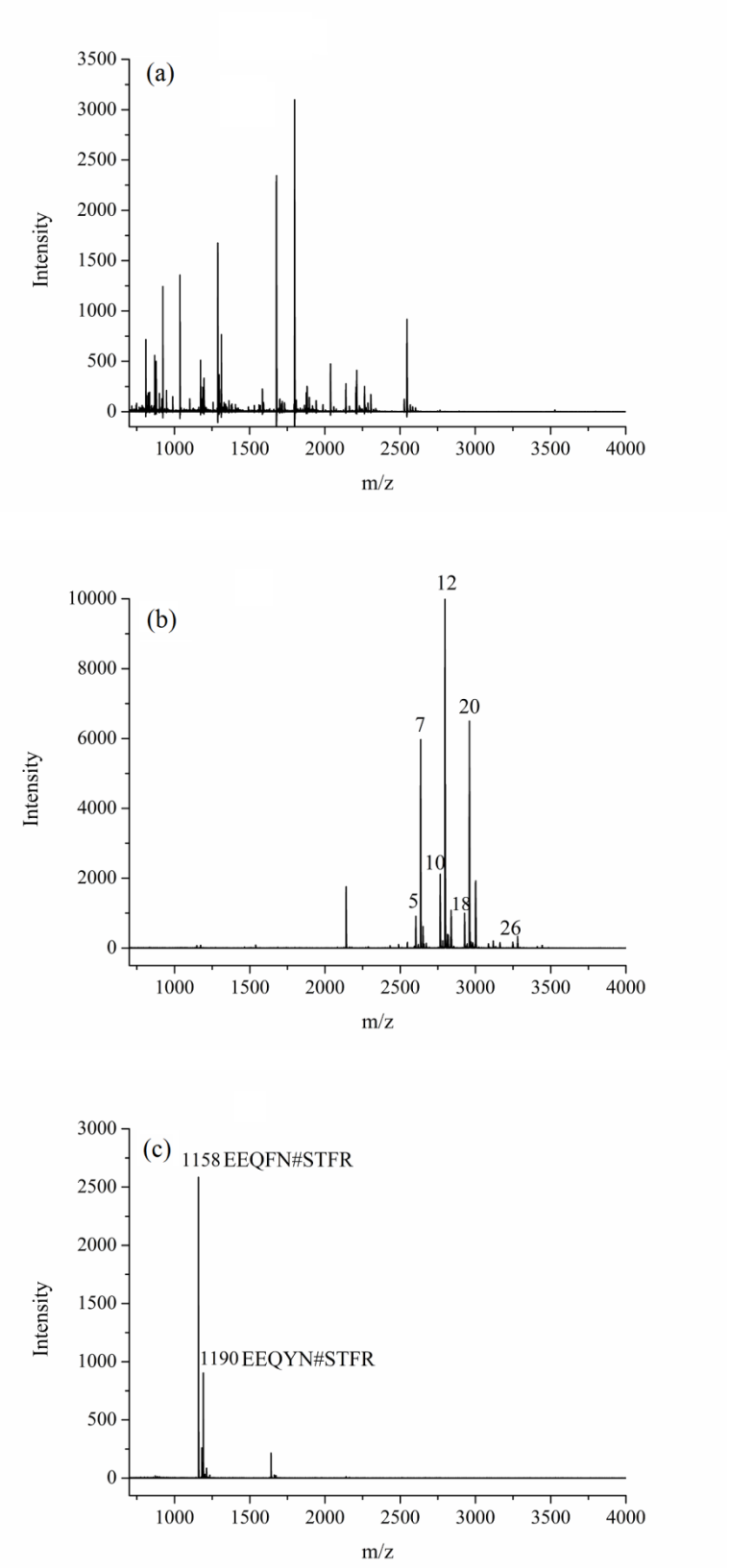

Fig. S5 MALDI-TOF MS spectra of human IgG (a) before enrichment, (b) after enrichment and (c) deglycosylated peptides of human IgG by PNGase F.

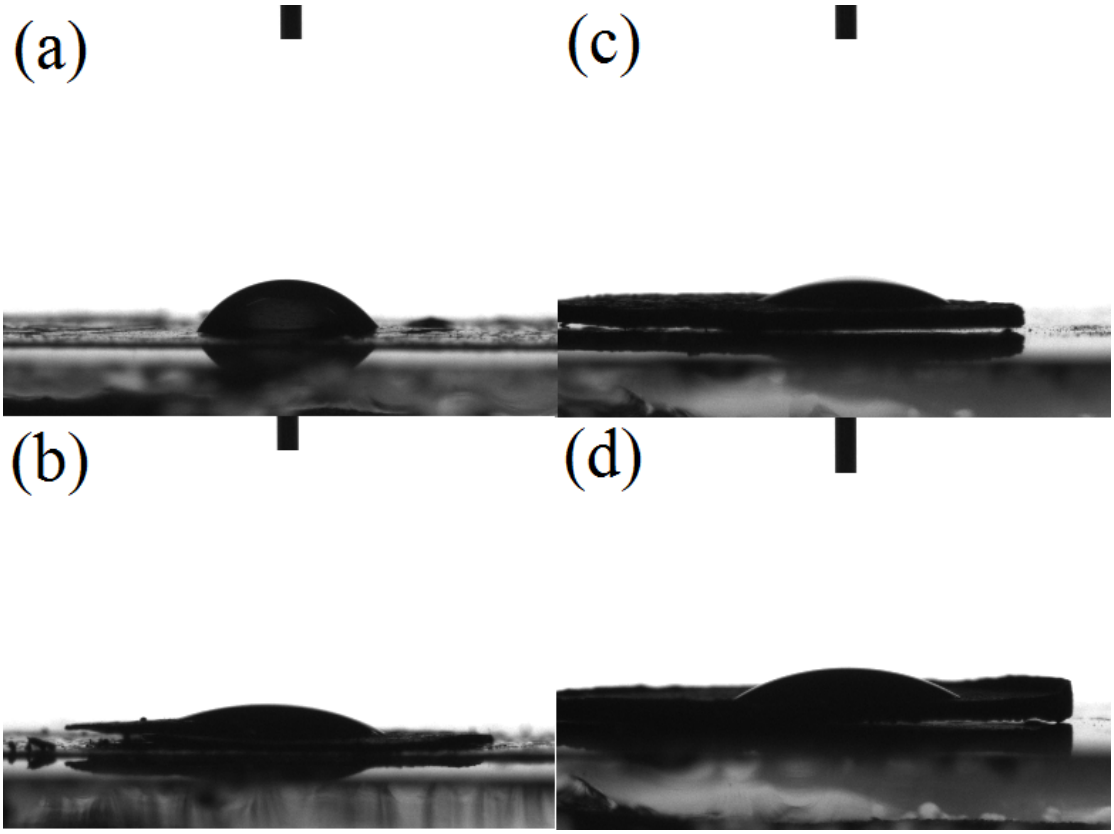

Fig. S6 Contact angles of (a) GO, (b) GO/PEI, (c) GO/PEI/Au and (d) GO/PEI/Au/L-Cys, detected when the drop just contacted the material.

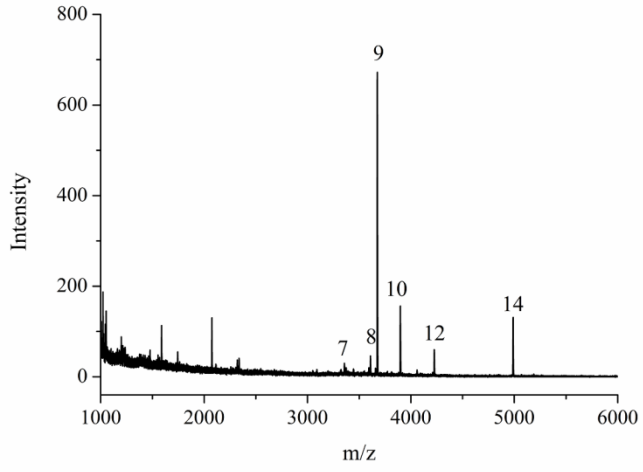

Fig. S7 MALDI-TOF spectra of 25 fmol tryptic digest of HRP after enrichment by

GO/PEI/Au/L-Cys composites. Glycopeptide peaks labeled with number.

Table S1 Molecular masses and proposed oligosaccharide composition of glycopeptides enriched from HRP digests. N# denotes the N-linked glycosylation site.

Number	m/z	Glycan composite	Amino acid sequence
1	2543	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SSPN#ATDTIPLVR[
2	2591	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PTLN#TTYLQTLR
3	3089	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLCPLNGN#LSALVDFDLR
4	3146	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLCPLNGN#LSALVDFDLR.Oxide
5	3206	[Hex]3[HexNAc]2[Xyl]1	SFAN#STQTFFNAFVEAMDR
6	3322	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSCPN#VSNIVR
7	3354	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAMDR
8	3607	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	NQCRGLCPLNGN#LSALVDFDLR
9	3673	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
10	3895	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	LHFHDCFVNGCDASILLDN#TTSFR
11	4057	[Hex]3[HexNAc]2[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H2O
12	4224	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
13	4839	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
14	4985	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Fuc]1[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR

HexNAc=N-acetylglucosamine, Hex=mannose, Fuc=fuctose, Xyl=xylose.

Table S2 Molecular masses and proposed oligosaccharide composition of glycopeptides enriched from chicken avidin digests. N# denotes the N-linked glycosylation site.

Number	m/z	Glycan composite	Amino acid sequence
1	2039	[HexNAc]1	WTNDLGSN#MTIGAVNSR
2	2566	[Hex]2[HexNAc]2	WTNDLGSN#MTIGAVNSR
3	2728	[Hex]3[HexNAc]2	WTNDLGSN#MTIGAVNSR
4	2890	[Hex]4[HexNAc]2	WTNDLGSN#MTIGAVNSR
5	2931	[Hex]2[HexNAc]3	WTNDLGSN#MTIGAVNSR
6	3052	[Hex]5[HexNAc]2	WTNDLGSN#MTIGAVNSR
7	3093	[Hex]3[HexNAc]3	WTNDLGSN#MTIGAVNSR
8	3135	[Hex]4[HexNAc]3	WTNDLGSN#MTIGAVNSR
9	3214	[Hex]6[HexNAc]2	WTNDLGSN#MTIGAVNSR
10	3255	[Hex]5[HexNAc]3	WTNDLGSN#MTIGAVNSR
11	3296	[Hex]4[HexNAc]4	WTNDLGSN#MTIGAVNSR
12	3376	[Hex]7[HexNAc]2	WTNDLGSN#MTIGAVNSR
13	3417	[Hex]6[HexNAc]3	WTNDLGSN#MTIGAVNSR
14	3458	[Hex]5[HexNAc]4	WTNDLGSN#MTIGAVNSR
15	3620	[Hex]6[HexNAc]4	WTNDLGSN#MTIGAVNSR

HexNAc=N-acetylglucosamine, Hex=mannose.

Table S3 Molecular masses and proposed oligosaccharide composition of glycopeptides enriched from human IgG digests. N# denotes the N-linked glycosylation site.

Number	m/z	Glycan composite	Amino acid sequence
1	2286	[Hex]3[HexNAc]3	EEQYN#STYR
2	2432	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
3	2488	[Hex]3[HexNAc]4	EEQYN#STYR
4	2594	[Hex]4[HexNAc]3[Fuc]1	EEQYN#STYR
5	2603	[Hex]3[HexNAc]4[Fuc]1	EEQFN#STFR
6	2618	[Hex]4[HexNAc]4	EEQFN#STFR
7	2635	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR
8	2650	[Hex]4[HexNAc]4	EEQYN#STYR
9	2658	[Hex]3[HexNAc]5	EEQFN#STYR
10	2764	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
11	2780	[Hex]5[HexNAc]4	EEQFN#STFR
12	2797	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
13	2806	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STYR
14	2812	[Hex]5[HexNAc]4	EEQYN#STFR
15	2821	[Hex]4[HexNAc]5	EEQFN#STFR
16	2838	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR
17	2853	[Hex]4[HexNAc]5	EEQYN#STYR
18	2926	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
19	2958	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
20	2968	[Hex]4[Hex7NAc]5[Fuc]1	EEQFN#STFR
21	2983	[Hex]5[HexNAc]5	EEQFN#STFR
22	3000	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR

23	3087	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STFR
24	3129	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
25	3161	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR
26	3250	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR

HexNAc=N-acetylglucosamine, Hex=mannose, Fuc=fuctose, NeuAc=sialic.

Table S4 List of identified glycoproteins from digests of 5 μg low abundant proteins in human plasma captured by GO/PEI/Au/L-Cys composites.

Number	Protein	Description/Glycopeptides sequences
1	IPI00022463	Serotransferrin
		QQQHLFGSNVTDCSGNFCLFR
2	IPI00553177	Isoform 1 of Alpha-1-antitrypsin
		YLGNATAIFFLPDEGK
		ADTHDEILEGLNFNLTEIPEAQIHEGFQELLR
3	IPI00017601	Ceruloplasmin
		ELHHLQEQNVSNAFLDKGEFYIGSK
		EHEGAIYPDNTTDFQR
		ELHHLQEQNVSNAFLDK
		ENLTAPGSDSAVFFEQGTTR
4	IPI00291262	Isoform 1 of Clusterin
		MLNTSSLLEQLNEQFNWVSR
		LANLTQGEDQYYLR
		LKELPGVCNETMMALWEECKPCLK
		ELPGVCNETMMALWEECKPCLK
5	IPI00022431	cDNA FLJ55606, highly similar to Alpha-2-HS-glycoprotein
		AALAAFNAQNNGSNFQLEEISR
		KVCQDCPLLAPLNDTR
		VCQDCPLLAPLNDTR
6	IPI00555812	Isoform 1 of Vitamin D-binding protein
		LCDNLSTK
7	IPI00550991	cDNA FLJ35730 fis, clone TESTI2003131, highly similar to ANTICHYMOTRYPSIN
		TLNQSSDELQLSMGNAMFVK
8	IPI00019568	Prothrombin (Fragment)
		YPHKPEINSTTHPGADLQENFCR
		SEGSSVNLSPPLEQCVPDR
		NFTENDLLVR
		SRYPHKPEINSTTHPGADLQENFCR
9	IPI00022229	Apolipoprotein B-100
		VNQNLVYESGSLNFSK
		FNSSYLQGTNQITGR
		FVEGSHNSTVSLTTK
10	IPI00022488	Hemopexin
		ALPQPQNVTSLLGCTH
		NGTGHGNSTHHGPEYMR
		SWPAVGNCSSALR
11	IPI00032179	Antithrombin-III
		LGACNDTLQQLMEVFK

			SLTFNETYQDISELVYGAK
12	IPI00019943	Afamin	
			DIENFNSTQK
			YAEDKFNETTEK
13	IPI00478003	Alpha-2-macroglobulin	
			GCVLLSYLNETVTVSASLESVR
			SLGNVNFTVSAEALESQELCGTEVPSVPEHGR
14	IPI00298828	Beta-2-glycoprotein 1	
			VYKPSAGNNSLYR
			LGNWSAMPSCK
15	IPI00032258	Complement C4-A	
			GLNVTLSSTGRNGFK
			FSDGLESNSSTQFEVK
			GLNVTLSSTGR
16	IPI00019591	cDNA FLJ55673, highly similar to Complement factor B	
			QSVPAHFVALNGSK
			TMFPNLTDVR
17	IPI00654888	Plasma kallikrein	
			LQAPLNYTEFQKPICLPSK
			IYSGILNLSDITK
			IYPGVDFGGEELNVTFVK
			IVGGTNSSWGEWPWQVSLQVK
18	IPI00218413	Biotinidase	
			DVQIIVFPEDGIHGFNFTR
			WNPCLEPHRFNDTEVLQR
			FNDTEVLQR
			NPVGLIGAENATGETDPSHSK
19	IPI00218192	Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H4	
			LPTQNITFQTESSVAEQEAEFQSPK
20	IPI00022395	Complement component C9	
			AVNITSENLIDDVVSLIR
21	IPI00423461	Putative uncharacterized protein DKFZp686C02220 (Fragment)	
			HYTNSSQDVTVPCR
			LSLHRPALEDLLLGSEANLTCTLTGLR
			TPLTANITK
22	IPI00298971	Vitronectin	
			NNATVHEQVGGPSLTSDLQAQSK
23	IPI00029739	Isoform 1 of Complement factor H	
			SPDVINGSPISQK
			MDGASNVTCINSR
			IPCSQPPQIEHGTINSSR
24	IPI00642017	Putative uncharacterized protein DKFZp686C02218 (Fragment)	
			LSLHRPALEDLLLGSEANLTCTLTGLR

			TPLTANITK
			LAGKPTHVNVSVVMAEVDGTCY
25	IPI00029061	Selenoprotein P	
			EGYSNISYIVVNHQGISSR
			CGNCSLTTLKDEDFCKR
			CGNCSLTTLK
26	IPI00025864	Butyrylcholinesterase, isoform CRA_b	
			DNNSIITR
			ENETEIIK
			WSDIWNATK
27	IPI00166729	Zinc-alpha-2-glycoprotein	
			DIVEYYNDSNGSHVLQGR
28	IPI00163207	Isoform 1 of N-acetylmuramoyl-L-alanine amidase	
			GFGVAIVGNYTAALPTEAALR
			LEPVHLQLQCMSQEQLAQVAANATK
29	IPI00022371	Histidine-rich glycoprotein	
			VIDFNCTTSSVSSALANTK
30	IPI00008556	Isoform 1 of Coagulation factor XI	
			LETTVNYTDSQRPICLPSK
			GINYNSSVAK
			VYSGILNQSEIK
31	IPI00020986	Lumican	
			LHINHNNLTESVGPLPK
			KLHINHNNLTESVGPLPK
32	IPI00012269	Isoform 1 of Multimerin-1	
			DEKLNQSNFQK
			VNESVVSIAAQQK
			LQNLTLPTNASIK
33	IPI00641737	Haptoglobin	
			VVLHPNYSQVDIGLIK
			NLFLNHSENATAK
34	IPI00012503	Isoform Sap-mu-0 of Proactivator polypeptide	
			TNSTFVQALVEHVKEECDR
			TNSTFVQALVEHVK
35	IPI00006114	Pigment epithelium-derived factor	
			VTQNLTLIEESLTSEFIHDIDR
36	IPI00027235	Isoform 1 of Attractin	
			CINQSICEK
			IDSTGNVTNELR
37	IPI00328609	Kallistatin	
			SQILEGLGFNLTELSESDVHR
			FLNDTMAVYEAK
38	IPI00027493	Isoform 2 of 4F2 cell-surface antigen heavy chain	

		SLVTQYLNATGNR
		DASSFLAEWQNITK
39	IPI00879936	29 kDa protein
		HRDDPRPAKTEISEMNWNMSQLQAETEGLK
40	IPI00296165	cDNA FLJ54471, highly similar to Complement C1r subcomponent
		CNYSIR
41	IPI00291867	Complement factor I
		LSDLSINSTECLHVHCR
		FLNNGTCTAEGK
42	IPI00299503	Isoform 1 of Phosphatidylinositol-glycan-specific phospholipase D
		NLTTSLTESVDR
		LGTSLSSGHVLMNGTLK
43	IPI00384938	Putative uncharacterized protein DKFZp686N02209
		EEQYNSTYR
44	IPI00292946	Thyroxine-binding globulin
		TLYETEVFSTDFSNISAAK
		VTACHSSQPNATLYK
45	IPI00019581	Coagulation factor XII
		RNHSCEPCQTLAVR
		NHSCEPCQTLAVR
46	IPI00020091	Alpha-1-acid glycoprotein 2
		QNQCFYNSSYLNVQR
47	IPI00022429	Alpha-1-acid glycoprotein 1
		QDQCIYNTTYLNVQR
48	IPI00006662	Apolipoprotein D
		ADGTVNQIEGEATPVNLTEPAKLEVK
		ADGTVNQIEGEATPVNLTEPAK
49	IPI00023014	von Willebrand factor
		ASPPSSSCNISSGEMQK
50	IPI00418153	Putative uncharacterized protein DKFZp686I15212
		EEQYNSTFR
51	IPI00025862	Isoform 1 of C4b-binding protein beta chain
		EWDNTTTECR
		LGHCPDPVLVNGEFSSSGPVNVSDK
52	IPI00292218	Hepatocyte growth factor-like protein
		GTANTTTAGVPCQR
53	IPI00293057	Isoform 2 of Carboxypeptidase B2
		QVHFFVNASDVDNVK
54	IPI00296099	Thrombospondin-1
		VVNSTTGPGEHLR
55	IPI00292950	Serpin peptidase inhibitor, clade D (Heparin cofactor), member 1
		NLSMPLLPADFHK
56	IPI00479116	Carboxypeptidase N subunit 2
		AFGSNPNLTK

57	IPI00395488	Vasori n LHEITNETFR
58	IPI00383150	PRAME family member 19 CSNLTTFCFHGNDTSMDGLKDLLR
59	IPI00299547	Neutrophil gelatinase-associated lipocalin SYNVTSVLFR
60	IPI00299435	apolipoprotein F precursor QGGVNATQVLIQHLR
61	IPI00012792	Cadherin-5 EVYPWYNLTVEAK
62	IPI00025753	Desmoglein-1 TGEINITSIVDR
63	IPI00023673	Galectin-3-binding protein ALGFENATQALGR
64	IPI00022733	45 kDa protein VSNVSCQASVSR
65	IPI00022417	Leucine-rich alpha-2-glycoprotein MFSQNDTR
66	IPI00022331	Phosphatidylcholine-sterol acyltransferase AELSNHTRPVILVPGCLGNQLEAK
67	IPI00017696	Complement C1s subcomponent NCGVNCSGDVFTALIGEIASPNYPKPYPENSR
68	IPI00013179	Prostaglandin-H2 D-isomerase WFSAGLASNSSWLR
69	IPI00026240	ADP-ribosylcyclase 2 NKNCTAIWEAFK
70	IPI00009477	Intercellular adhesion molecule 2 GNETLHYETFGK
71	IPI00008494	Intercellular adhesion molecule 1 LNPTVTYGNDSFSAK
72	IPI00007921	Isoform 1 of Neurexin-2-alpha SLQLSVDNVTVEGQMAGAHMR
73	IPI00007240	Coagulation factor XIII B chain KEHETCLAPELYNGNYSTTQK
74	IPI00007199	Protein Z-dependent protease inhibitor ETFFNLSK
75	IPI00006173	Isoform 1 of Cholesteryl ester transfer protein NVSEDLPLPTFSPTLLGDSR
76	IPI00006154	Isoform Long of Complement factor H-related protein 2 LQNNENNISCVER
77	IPI00003813	Isoform 1 of Cell adhesion molecule 1 FQLLNFSSSELK
78	IPI00003590	Isoform 1 of Sulfhydryl oxidase 1

		NGSGAVFPVAGADVQTLR
79	IPI00003351	Isoform 1 of Extracellular matrix protein 1
		HIPGLIHNMTAR
80	IPI00218732	Serum paraoxonase/arylesterase 1
		VTQVYAENGTVLQGSTVASVYK
81	IPI00163446	Isoform 2 of Ig delta chain C region
		TLLNASR
82	IPI00291866	Plasma protease C1 inhibitor
		DTFVNASR
83	IPI00000877	Hypoxia up-regulated protein 1
		VINETWAWK
84	IPI00030871	Pantetheinase
		MTGSGIYAPNSSR
85	IPI00030739	Apolipoprotein M
		TELFSSSCPGGIMLNETGQGYQR
86	IPI00027482	Corticosteroid-binding globulin
		AQLLQGLGFNLTER
87	IPI00027410	Platelet glycoprotein V
		LLDLSGNNLTHLPK

Reference

1 Z. C. Xiong, H. Q. Qin, H. Wan, G. Huang, Z. Zhang, J. Dong, L. Y. Zhang, W. B. Zhang and H. F. Zou, *Chem. Commun.*, 2013, **49**, 9284.