Supporting Information

Hydrophilic magnetic covalent triazine frameworks fishing for differential N-glycopeptides in breast cancer plasma membrane

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Characterization.

A vibrating sample magnetometer (VSM, Model PPMS, Quantum Design Company, USA) was employed to measure the magnetization of the samples with field strength varying from 0 to 20000 Oe at 300 K. X-ray photoemission spectroscopy (XPS) was conducted using a Kratos XSAM 800 instrument equipped with a monochromatic Al anode X-ray gun (12 kV, 15 mA, 10^{-5} Pa). The mass loss of the samples was analyzed at temperatures ranging from 35 to 900 ℃ at the heating rate of 10 K min^{-1} by simultaneous thermal analysis (STA449 C Jupiter, NETZSCH). Fourier transform infrared spectra were obtained by a spectrometer (FTIR, PE spectrometer) with wavenumber in the range of 500-4000 cm^{-1}. The morphologies of the samples were observed by scanning electron microscopy (SEM, Hitachi S-4800, Japan) and transmission electron microscopy (TEM, JEOL, JEM-100CX, Japan). Surface area and pore size analyzer (Micromeritics, USA) was employed to study the surface and BJH pore size distribution at 77K. Elite-LC-MS/MS analysis by Easy-nLC nanoflow HPLC system connected to Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). MALDI-TOF/TOF analyzed by mass spectrometer (Bruker Daltonics, USA).

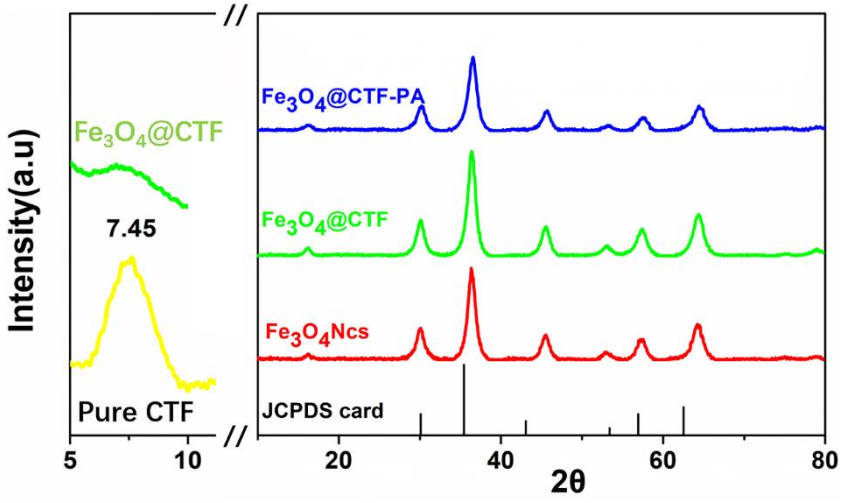

Fig. S1. The wide-angle and small-angle XRD profiles of materials.

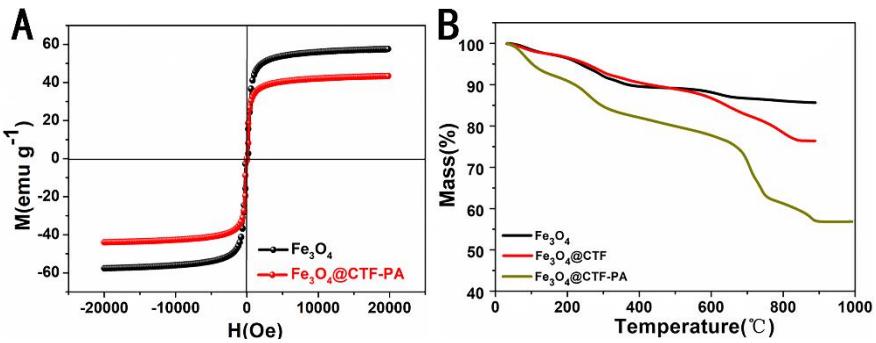

Fig. S2. (A) VSM curves of the Fe_3O_4 nanoparticles and Fe_3O_4@CTF-PA nanoparticles; (B) TGA curves of the Fe_3O_4 nanoparticles, Fe_3O_4@CTF nanoparticles and Fe_3O_4@CTF-PA nanoparticles.

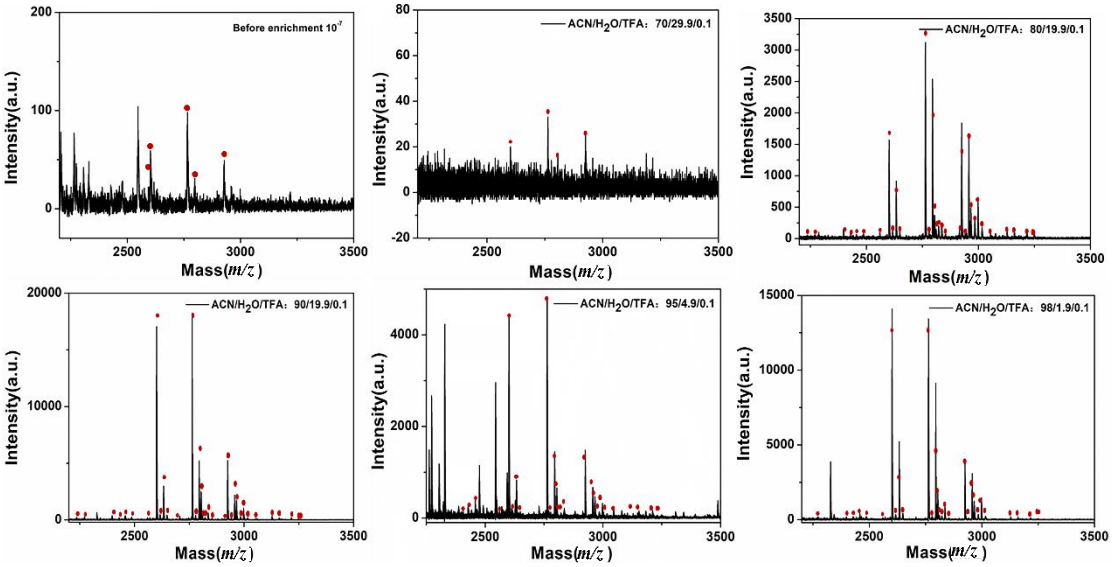

Fig. S3. The enrichment conditions screening of Fe_3O_4@CTF-PA nanoparticles selective adsorption N-glycopeptides, elution conditions ACN/$H_2$0/TFA:9.9/90/0.1, IgG digestion solution concentration of 100 fmol, N-glycopeptides were marked by red dots.

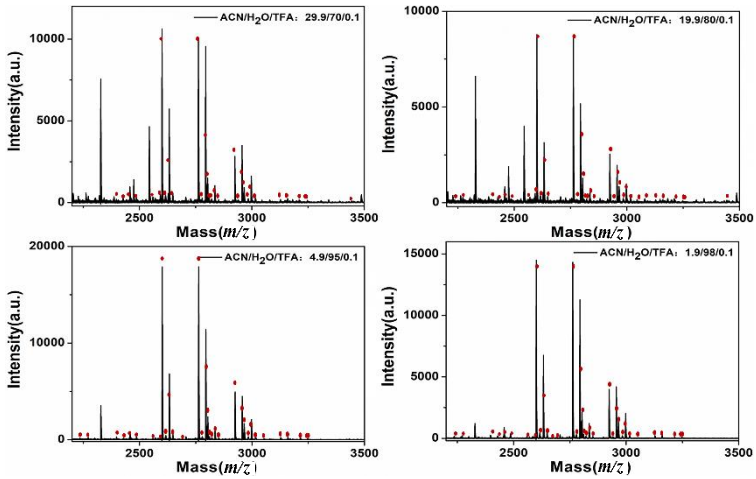

Fig. S4. The Fe_3O_4@CTF-PA nanoparticle selective adsorption N-glycopeptides elution conditions screening, N-glycopeptides were marked by red dots.

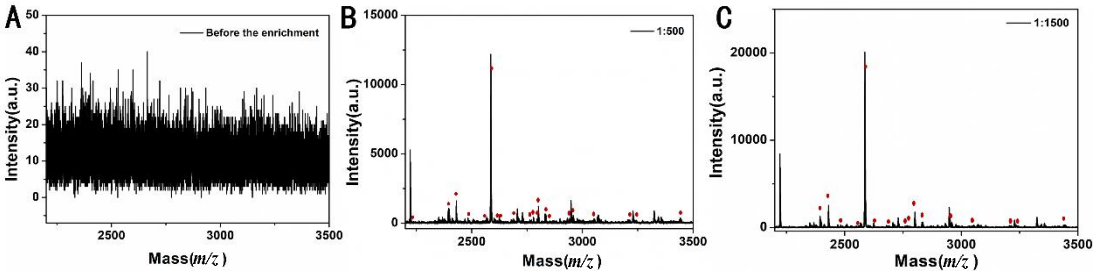

Fig. S5. MALDI-TOF mass spectrometry: Enrichment of IgG and BSA digestions (molar ratio1:500, 1:1500) with different proportions by Fe_3O_4@CTF-PA nanoparticle, N-glycopeptides were marked by red dots

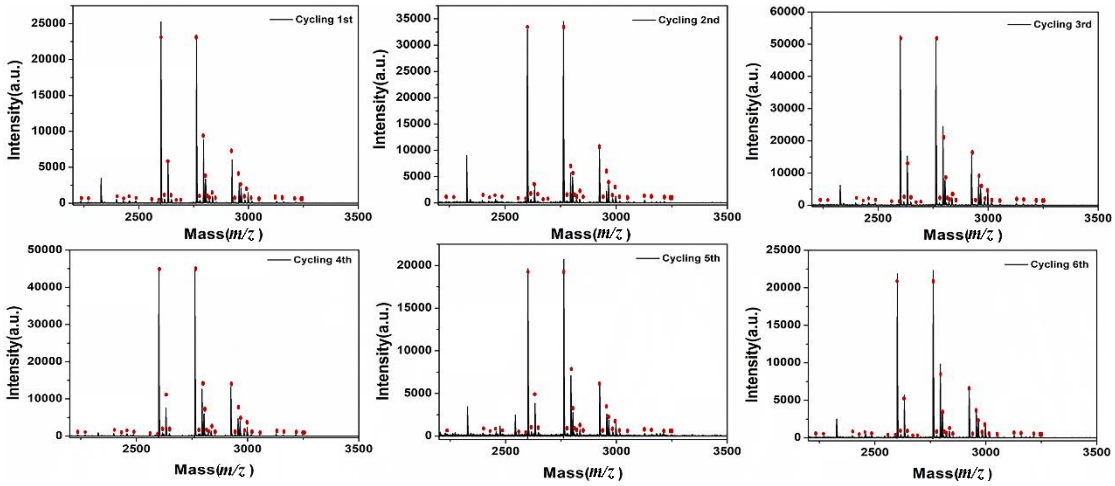

Fig. S6. MALDI-TOF mass spectra of N-glycopeptides with concentration of 100 fmol after enrichment by Fe_3O_4@CTF-PA nanoparticles, N-glycopeptides were marked by red dots.

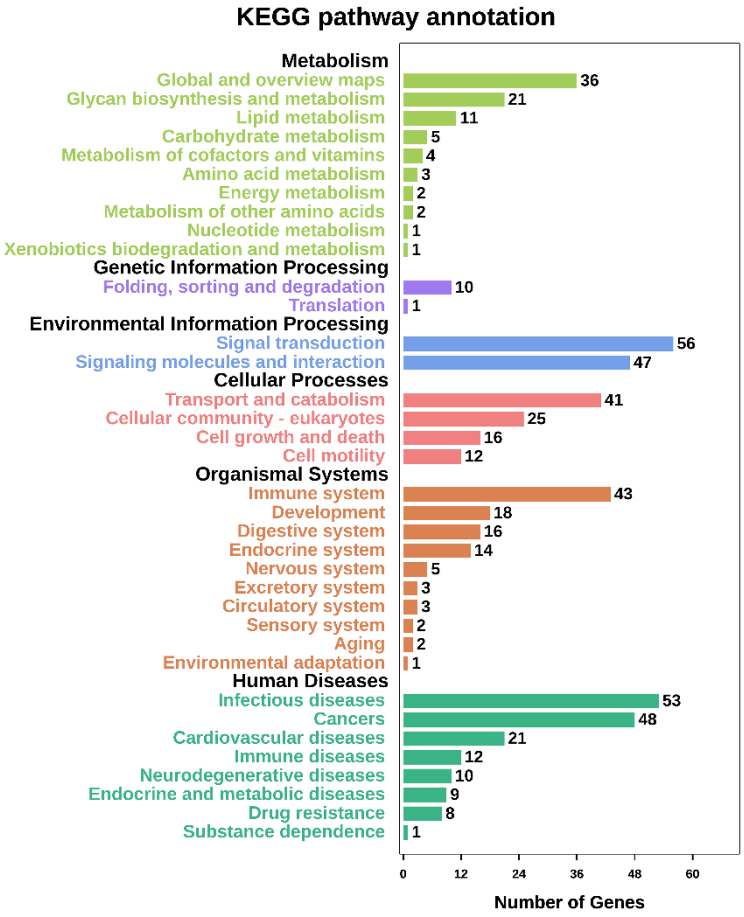

Fig. S7. KEGG pathway analysis mapping.

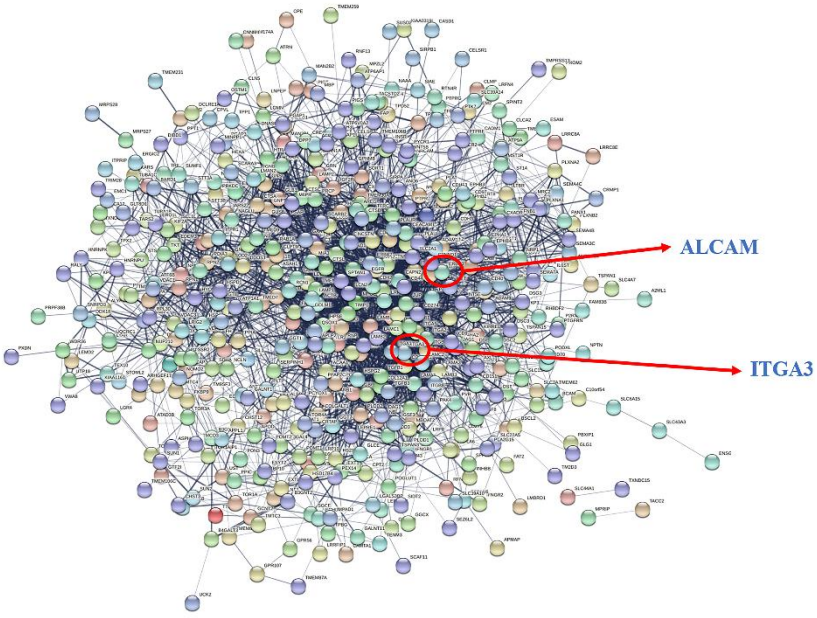

Fig. S8. PPI analysis mapping.

Table. S1. Observed molecular masses, proposed glycan compositions and peptide sequences of N-glycopeptides enriched from IgG tryptic digests by the Fe_3O_4@CTF-PA nanoparticle. Hex, HexNAc, Fuc and NeuAc are the abbreviations of hexose, N-acetylhexosamine, fucose and N-acetylneuraminic acid, respectively. N& denotes the glycosylation sites.

Number	m/z	Glycan composition	Peptide sequence
1	2236.4	[Hex]3[HexNAc]2[Fuc]1	EEQFN&STFR
2	2398.2	[Hex]3[HexNAc]3[Fuc]1	EEQFN&STFR
3	2430.3	[Hex]3[HexNAc]3[Fuc]1	EEQYN&STYR
4	2455.3	[Hex]3[HexNAc]4	EEQFN&STFR
5	2487.3	[Hex]3[HexNAc]4	EEQYN&STYR
6	2561.3	[Hex]4[HexNAc]3[Fuc]1	EEQFN&STFR
7	2592.3	[Hex]4[HexNAc]3[Fuc]1	EEQYN&STYR
8	2602.2	[Hex]3[HexNAc]4[Fuc]1	EEQFN&STFR
9	2617.2	[Hex]3[HexNAc]4[Fuc]1	EEQFN&STFR
10	2633.1	[Hex]3[HexNAc]4[Fuc]1	EEQYN&STYR
11	2649.2	[Hex]4[HexNAc]4	EEQYN&STYR
12	2691.3	[Hex]3[HexNAc]5	EEQYN&STYR
13	2764.1	[Hex]4[HexNAc]4[Fuc]1	EEQFN&STFR
14	2779.1	[Hex]5[HexNAc]4	EEQFN&STFR
15	2796.0	[Hex]4[HexNAc]4[Fuc]1	EEQYN&STYR
16	2804.3	[Hex]3[HexNAc]5[Fuc]1	EEQFN&STFR
17	2812.3	[Hex]5[HexNAc]4	EEQYN&STYR
18	2821.2	[Hex]4[HexNAc]5	EEQFN&STFR
19	2837.0	[Hex]3[HexNAc]5[Fuc]1	EEQYN&STYR
20	2853.2	[Hex]4[HexNAc]5	EEQYN&STYR
21	2908.0	[Hex]4[HexNAc]4[NeuAc]1	EEQFN&STFR
22	2925.9	[Hex]5[HexNAc]4[Fuc]1	EEQFN&STFR
23	2942.2	[Hex]5[HexNAc]4[Fuc]1	EEQFN&STYR
24	2958.0	[Hex]5[HexNAc]4[Fuc]1	EEQYN&STYR
25	2966.2	[Hex]4[HexNAc]5[Fuc]1	EEQFN&STFR
26	2983.3	[Hex]5[HexNAc]5	EEQFN&STFR
27	2999.0	[Hex]4[HexNAc]5 [Fuc]1	EEQYN&STYR
28	3016.0	[Hex]5[HexNAc]5	EEQYN&STYR
29	3054.3	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFN&STFR

30	3128.3	[Hex]5[HexNAc]5[Fuc]1	EEQFN&STFR
31	3161.1	[Hex]5[HexNAc]5[Fuc]1	EEQYN&STYR
32	3217.1	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFN&STFR
33	3247.0	[Hex]4[HexNAc]4[Fuc]1	TKPREEQFN&STFR
34	3250.0	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN&STYR
35	3441.3	[Hex]5[HexNAc]4[Fuc]1	TKPYEEQYN&STYR

Table. S2. The number of unique glycoproteins of the two cell membranes involved in the top 20 CC entries of significance.

Number of GO	**CC**	**SK-BR-3**	**MCF-10A**
GO:0031224	intrinsic component of membrane	73	168
GO:0016021	integral component of membrane	73	167
GO:0101003	ficolin-1-rich granule membrane	5	35
GO:0044425	membrane part	75	168
GO:0030667	secretory granule membrane	16	72
GO:0031233	intrinsic component of external side of plasma membrane	9	30
GO:1903561	extracellular vesicle	60	124
GO:0043230	extracellular organelle	60	124
GO:0070062	extracellular exosome	60	122
GO:0065010	extracellular membrane-bounded organelle	60	122
GO:0012505	endomembrane system	70	148
GO:0005604	basement membrane	15	50
GO:0098588	bounding membrane of organelle	63	134
GO:0043083	synaptic cleft	11	18
GO:0030659	cytoplasmic vesicle membrane	35	92
GO:0031226	intrinsic component of plasma membrane	44	123
GO:0009986	cell surface	47	118
GO:0005887	integral component of plasma membrane	43	121
GO:0009897	external side of plasma membrane	27	91
GO:0005788	endoplasmic reticulum lumen	34	58

Table. S3. The number of unique glycoproteins of the two cell membranes involved in the top 20 BP entries of significance.

Number of GO	BP	SK-BR-3	MCF-10A
GO:0007157	heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	17	43
GO:0035924	cellular response to vascular endothelial growth factor stimulus	11	36
GO:0038084	vascular endothelial growth factor signaling pathway	5	39
GO:0031290	retinal ganglion cell axon guidance	14	3
GO:0008038	neuron recognition	16	53
GO:0097374	sensory neuron axon guidance	6	19
GO:0007413	axonal fasciculation	4	42
GO:1900746	regulation of vascular endothelial growth factor signaling pathway	10	24
GO:1902547	regulation of cellular response to vascular endothelial growth factor stimulus	8	23
GO:0048333	mesodermal cell differentiation	10	36
GO:0042661	regulation of mesodermal cell fate specification	11	2
GO:0042476	odontogenesis	15	54
GO:0048846	axon extension involved in axon guidance	14	33
GO:1902284	neuron projection extension involved in neuron projection guidance	12	34
GO:0010669	epithelial structure maintenance	9	31
GO:0016525	negative regulation of angiogenesis	21	36
GO:2000181	negative regulation of blood vessel morphogenesis	17	39
GO:0007424	open tracheal system development	23	51
GO:0099560	synaptic membrane adhesion	10	24
GO:0090598	male anatomical structure morphogenesis	12	25

Table. S4. The number of unique glycoproteins of the two cell membranes involved in the top 20 MF entries of significance.

Number of GO	MF	SK-BR-3	MCF-10A
GO:0001618	virus receptor activity	13	51
GO:0043236	laminin binding	12	36
GO:0005518	collagen binding	16	44

GO:0050840	extracellular matrix binding	16	40
GO:0019838	growth factor binding	20	59
GO:0005178	integrin binding	18	64
GO:0070051	fibrinogen binding	2	22
GO:0043184	vascular endothelial growth factor receptor 2 binding	1	16
GO:0071936	coreceptor activity involved in Wnt signaling pathway	5	15
GO:0005172	vascular endothelial growth factor receptor binding	1	16
GO:0070700	BMP receptor binding	8	17
GO:1904929	coreceptor activity involved in Wnt signaling pathway, planar cell polarity pathway	4	14
GO:0098631	protein binding involved in cell adhesion	10	38
GO:0038085	vascular endothelial growth factor binding	3	15
GO:0001846	opsonin binding	6	18
GO:0086080	protein binding involved in heterotypic cell-cell adhesion	2	18
GO:0038064	collagen receptor activity	2	15
GO:1990405	protein antigen binding	5	20
GO:0015026	coreceptor activity	9	29
GO:0001540	beta-amyloid binding	17	37