

1 **Dendrimer-assisted magnetic graphene-silica hydrophilic**
2 **composite for efficient and selective enrichment of**
3 **glycopeptides from the complex sample**

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11 **EXPERIMENTAL SECTION**

12 **Materials.** Tetraethyl orthosilicate (TEOS, 99%), PAMAM (G4), immunoglobulin G (IgG),
13 horse radish peroxidase (HRP), chloroauric acid ($\text{HAuCl}_4 \cdot 6\text{H}_2\text{O}$), 2, 5-dihydroxybenzoic acid (DHB),
14 ammonium solution (25 wt%) and cyanoborohydride were purchased from Sigma-Aldrich and used as
15 received. Mouse liver proteins were extracted following the previous protocol. Acetonitrile (ACN), formic
16 acid (FA) and triuoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Urea,
17 dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from BioRad (Hercules, CA, USA).
18 Deionized water used for all experiments was purified with a Milli-Q water system. All other chemical
19 agents were obtained from Aladdin Corporation (Shanghai, China).

20 **Synthesized protocol of hydrophilic component maltose-SH.** 1-Propargyl-O-maltose was
21 synthesized based on a method of the our previous publication.¹ In detail, in a dried flask under
22 nitrogen atmosphere, acetyl maltose (10.0 g) was dissolved in 150 mL dichloromethane, followed by
23 the addition of propargylalcohol (1.0 mL). The resulting solution was stirred and cooled to 0 °C and
24 then boron trifluoride/ether (3.0 mL) was added dropwise. The reaction was stirred for 1 h at 0 °C and
25 at room temperature for 4 h. After that, potassium carbonate (5.0 g) was added and the solution was
26 continuously stirred for another 30 min. The unreacted solid was filtered out and the product was
27 concentrated. 1-Propargyl-acetyl maltose (7.55 g, 76% yield) was obtained with m/z at 697.2 identified
28 by MALDI-TOF-MS. The obtained solid (1.0 g) was dissolved in 50 mL dry methanol solution, and a
29 freshly prepared sodium methoxide in methanol (12 mL, 1 mol/L) was slowly added dropwise.
30 Monitoring of the reaction by MALDI-TOF-MS indicated complete disappearance of 1-Propargyl-
31 acetyl maltose. The reaction was quenched by neutralization with Amberlite IR-120 resin (H⁺ form).
32 The solid was filtered out and the product was concentrated to get oily solid. 1-Propargyl-O-maltose
33 was confirmed with MALDI-TOF-MS (m/z at 402.8 [M⁺]). 430 mg 1-Propargyl-O-maltose was

1 homogenously mixed with 200 mg bis-2-azidyl-isobutyrylamide of cystamine (synthesized according
2 to a previously reported method²) in a solution of 4 mL THF and 6 mL methanol. The mixture was N₂
3 bubbled for 10 min to remove the trace amount of oxygen. Then, 200 μ L water solution containing 3.2
4 mg CuSO₄·5H₂O and 7 mg ascorbic acid was added into above mixture under the protection of N₂
5 flow. After reaction for 12 h at room temperature, the obtained product was purified with water for
6 three times. The 400 mg purified product was then treated with 100 mg tris(2-carboxyethyl)phosphine
7 hydrochloride in 8 mL water at ambient condition for the cleavage of disulfide bond. The final obtained
8 product was designated as maltose-SH and identified by ESI/MS.

9 **Synthesis of Fe₃O₄@nSiO₂-maltose.** The detailed synthesis protocol was based on our previous
10 study.¹

11 **Fabrication of Fe₃O₄ nanoparticles-decorated GO (Fe₃O₄-GO).** GO was obtained according to
12 previous studies. Then, 30 mg GO, 30 mL ethylene glycol, 160 mg FeCl₃·6H₂O and 30 mg citric acid
13 sodium were homogeneously sonicated for 2 h at the power of 160 W. After that, 700 mg CH₃COONa was
14 added into above mixture followed by mechanical stir for 30 min. The obtained yellowish solution was
15 transferred into a 50 mL Teflon autoclave encapsulated within a stain steel container for a solvothermal
16 reaction at 190 °C for 6 h. After wash with ethanol and water for three times and dry for further use, the
17 obtained product was designated as Fe₃O₄-GO.

18 **Fabrication of dendrimer-assisted magnetic graphene-silica hydrophilic composite.** 30 mg obtained
19 Fe₃O₄-GO was dispersed homogenously in a solution of 23 mL ethanol, 4.3 mL water and 0.62 mL aqueous
20 ammonia (28% wt) under sonication. Then, 0.86 mL TEOS was added dropwise into above mixture,
21 followed by 6 h reaction at room temperature for the assembly of a layer of silica onto Fe₃O₄-GO (Fe₃O₄-
22 GO@nSiO₂). After wash by water and isopropanol for three times, the obtained Fe₃O₄-GO@nSiO₂ was
23 treated with an isopropanol solution containing 1 mL APTEOS under Ar flow for 24 h (Fe₃O₄-GO@nSiO₂-
24 NH₂). Then, 30 mg Fe₃O₄-GO@nSiO₂-NH₂ was added into 40 mL anhydrous methanol containing 5%
25 glutaraldehyde and the mixture was mechanically stirred (400 rpm) for 12 h at room temperature. The
26 glutaraldehyde-activated product was coupled with PAMAM dendrimer in anhydrous methanol during
27 which 1% (w/w) sodium cyanoborohydride was added (Fe₃O₄-GO@nSiO₂-PAMAM). 35 mg above product
28 was mixed with HAuCl₄·6H₂O under sonication for 15 min. Then the reaction temperature was raised up to
29 100 °C as soon as the addition of 30 mg sodium citrate. After reaction for 15 min, the system was cooled
30 down to room temperature for another 120 min incubation. The obtained Fe₃O₄-GO@nSiO₂-PAMAM-Au
31 was then treated with the hydrophilic component maltose-SH at the room temperature overnight. After wash
32 with deionized water several times, the final product was dried at room temperature for further use.

33 **Tryptic digestion of proteins.** For tryptic digestion, 1 mg human IgG or HRP was dissolved in 1 mL
34 NH₄HCO₃ solution (100 mM, pH=8.4) and denatured by boiling for 15 min. After that, samples were
35 reduced with 20 mM DTT at 37 °C for 2 h and alkylated by 7.2 mg IAA at room temperature in the dark for
36 40 min. The solution was incubated with trypsin at an enzyme-protein ratio of 1: 25 (w/w) in 37 °C water
37 bath for 16 h. Tryptic digests were stored at -20 °C for further use. Proteins from mouse liver were extracted
38 following a procedure as previously described. Briefly, liver was cut into pieces and suspended in ice-cold
39 homogenization buffer consisting of 8 M urea, 4% CHAPS (w/v), 65 mM DTT, 1 mM EDTA, 0.5 mM
40 EGTA, a mixture of protease inhibitor (1 mM PMSF, 0.2 mM Na₃VO₄, 1 mM NaF) and 40 mM Tris-HCl at
41 pH 7.4. Then, samples were homogenized by a Potter-Elvehjem homogenizer, sonicated at 100 W for 30 s
42 and centrifuged at 25000 g for 1 h in turn. The total liver proteins in the supernatant were recovered by

1 precipitation and lyophilization. The obtained proteins were dissolved in denaturing buffer (8 M urea, 50
2 mM Tris-HCl at pH 8.3). The protein concentration was measured by Bradford assay. Thereafter, the protein
3 mixture (1 mg) was reduced by DTT (1 M, 20 mL) at 60 °C for 1 h and alkylated by IAA (7.2 mg) in the
4 dark at room temperature for 45 min. The solution was diluted to 1 M urea with Tris-HCl solution (50 mM,
5 pH =8.4) and digested by trypsin at 37 °C for 16 h with an enzyme-protein ratio of 1: 25 (w/w). Tryptic
6 digests were desalted and lyophilized for the further use.

7 **Hydrophilic enrichment of glycopeptides from tryptic digests of glycoproteins.** 1 mg Fe₃O₄-
8 GO@nSiO₂-PAMAM-Au-maltose was diluted with 200 μL loading buffer (88% ACN/H₂O, 0.3% TFA)
9 containing a determined amount of tryptic digests of IgG or HRP. After incubation at room temperature for 1
10 h, the composite was separated by a magnet, followed by wash with 200 μL loading buffer for two times.
11 Finally adsorbed glycopeptides were eluted from the composite with 100 μL elution buffer (30% ACN/H₂O,
12 0.1% TFA) under wild vibration twice. After combination and being lyophilized to dryness and redissolved
13 in 20 μL 0.1% FA, glycopeptides were analyzed by MALDI-TOF-MS. The enrichment process of
14 glycopeptides by Fe₃O₄-GO@nSiO₂-Au-maltose was the same as described above. For enrichment of
15 glycopeptides from tryptic digests of mouse liver, 50 μg tryptic digests were dissolved in 200 μL loading
16 buffer and incubated with 1.0 mg Fe₃O₄-GO@nSiO₂-PAMAM-Au-maltose for 1 h, which was subsequently
17 washed with 200 μL loading buffer for three times to remove the non-specific adsorption. Then, trapped
18 glycopeptides were eluted from the composite twice with 100 μL elution buffer (30% ACN/H₂O, 0.1% TFA)
19 under wild vibration for 10 min and the elution was collected to be combined for lyophilization. The
20 obtained glycopeptides were redissolved in 100 μL 10 mM NH₄HCO₃ and 100 unites of PNGase F were
21 added. After the mixture was incubated at 37 °C overnight to remove glycan moieties. The peptides were
22 further lyophilized and redissolved in 20 μL 0.1% FA for LC-MS analysis.

23 **Evaluation of the recovery yield of glycopeptides enriched by Fe₃O₄-GO@nSiO₂-PAMAM-Au-
24 maltose.** Tryptic digests of IgG divided into duplicate groups were in parallel labelled with heavy and light
25 isotopes, respectively. The heavy-tagged group was first treated with 1 mg Fe₃O₄-GO@nSiO₂-PAMAM-Au-
26 maltose followed by the aforementioned enrichment process. Then, trapped glycopeptides were eluted twice
27 with 100 μL elution buffer (30% ACN/H₂O, 0.1% TFA) under wild vibration for 10 min and the eluent was
28 combined with the light-tagged group for another same enrichment process. After the same elution process,
29 the eluent was deglycosylated by PNGase F in the ammonium bicarbonate buffer overnight for the
30 subsequent MALDI-TOF-MS analysis. The recovery yield was calculated by the signal intensity ratio of
31 heavy isotope-labelled N-glycosylation motifs to light isotope-labelled N-glycosylation motifs.

32 **Mass spectrometry analysis.** All MALDI-TOF mass analyses were performed on an AB Sciex 5800
33 MALDI-TOF/TOF mass spectrometer (AB, CA) equipped with a pulsed Nd/YAG laser at 355 nm. The
34 sample solution (0.5 μL) was deposited on the MALDI plate and dried at room temperature. Then 0.5 μL
35 DHB (25 mg/mL, ACN/H₂O/H₃PO₄, 70: 29: 1, v/v/v) matrix solution was loaded for MS performance. LC-
36 MS/MS analysis was carried out using a Dionex UltiMate 3000 Nano LC system coupled with a Q-Exactive
37 mass spectrometer (Thermo Fisher Scientific, USA). The spray voltage was set at 2.0 kV. All MS and
38 MS/MS spectra were acquired in data-dependent acquisition mode with MS resolution of 70000 and MS/MS
39 resolution of 17500. The mass spectrometer was set to a scan range of 400-2000 m/z and the 15 most intense
40 precursor ions were selected for MS/MS analysis by higher-energy collision dissociation (HCD). Buffer A
41 (H₂O/FA, 99.9: 0.1, v/v) and buffer B (ACN/FA, 99.9: 0.1, v/v) were provided for the mobile phase, and the
42 gradient elution was programmed from 5% to 35% of buffer B in 150 min with a flow rate of 300 nL/min.

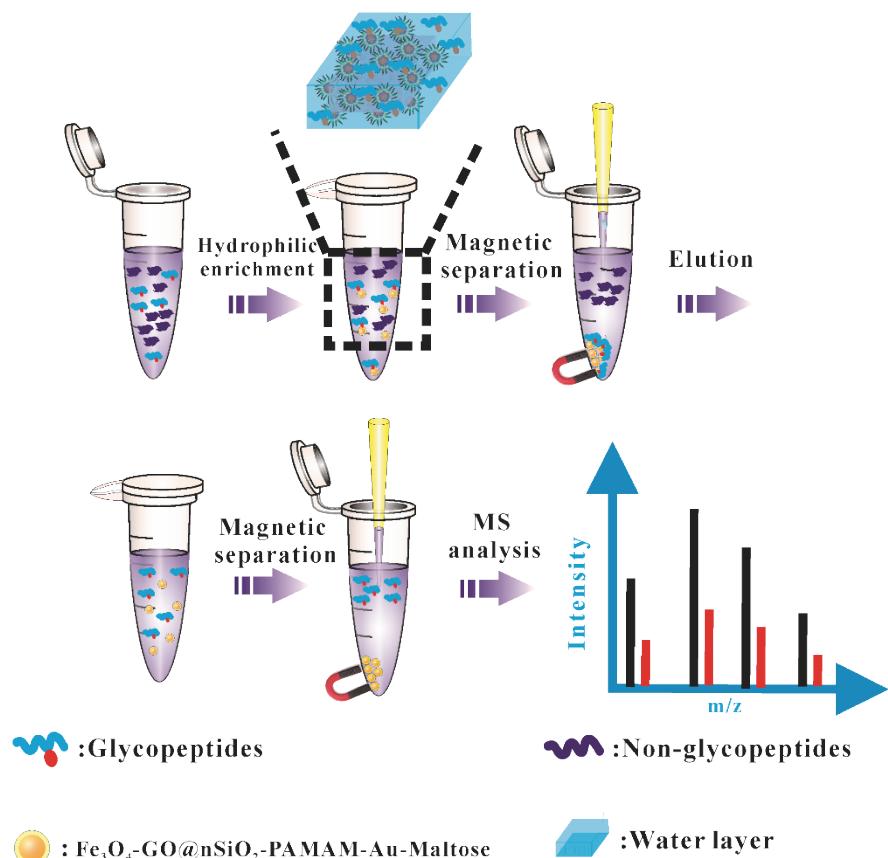
1 **Database search and data analysis.** All LC-MS/MS raw data files were searched with the MaxQuant
2 software (version 1.3.0.5) against the targeted database of the IPI mouse (v3.87, 59534 entries). For the
3 peptide identification, a mass tolerance of 20 ppm was permitted for parent ions and 0.5 Da for fragment
4 ions, with allowance for two missed cleavages in tryptic digests. Cysteine carboxamidomethylation was set
5 as a static modification of 57.0215 Da. The variable modifications of methionine oxidation, asparagine and
6 glutamine deamination are 15.9949, 0.9840 and 0.9840 Da, respectively. The peptide identification with the
7 false discovery rate ≤ 0.01 was accepted for protein identification. Only the identified deamination sites
8 which were conformed to the N-glycosylation consensus sequence (N-!P-[S/T]) were considered as
9 glycosylation sites.

10 **Characterizations.** Transmission electron microscopy (TEM) was conducted on a JEOL 2000 EX
11 electronic microscope with an accelerating voltage of 120 keV. Fourier transform infrared (FTIR)
12 spectroscopy characterization was performed on a Thermo Nicolet 380 spectrometer using KBr pellets
13 (Nicolet, Wisconsin, USA). X-ray diffraction (XRD) patterns of samples were collected on a Bruker
14 D8FOCUS X-ray diffractometer. The saturation magnetization curve was obtained at room temperature on a
15 Physical Property Measurement System 9T (Quantum Design, San Diego, USA). Raman spectra were
16 obtained on a ViaRelex with excitation from an argon ion laser (532 nm). Scanning electron microscope
17 (SEM) was performed on a JEOL JSM-5600 (Tokyo, Japan). Energy dispersive spectrometer (EDS) element
18 analysis was conducted on the Inca X-Max80 EDS system (Oxford, England)

19

20 **Reference**

- 21 1. Z. C. Xiong, L. Zhao, F. J. Wang, J. Zhu, H. Q. Qin, R. A. Wu, W. B. Zhang and H. F. Zou, *Chem.*
22 *Common.*, 2012, **48**, 8138-8140.
- 23 2. B. Y. Zhang, W. D. He, L. Y. Li, X. L. Sun, W. T. Li and K. R. Zhang, *J. Polym. Sci. A1* 2010, **48**,
24 3604-3612.



Scheme S1. The hydrophilic enrichment of glycopeptides from the complex sample.

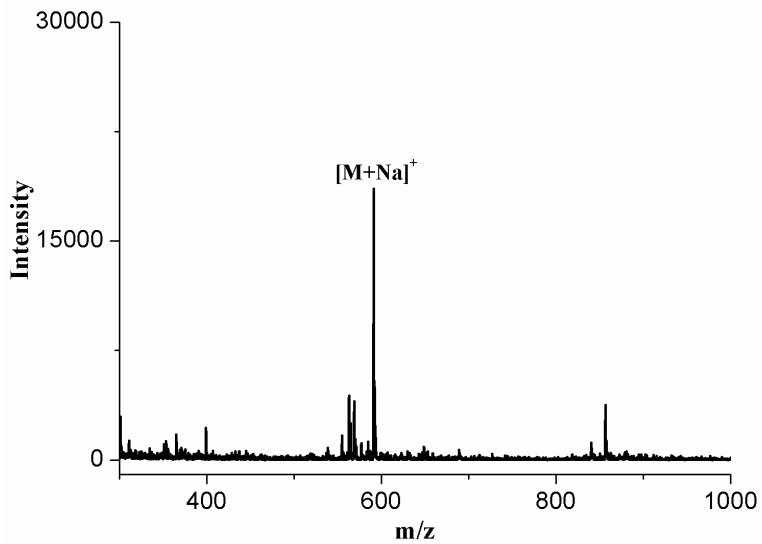
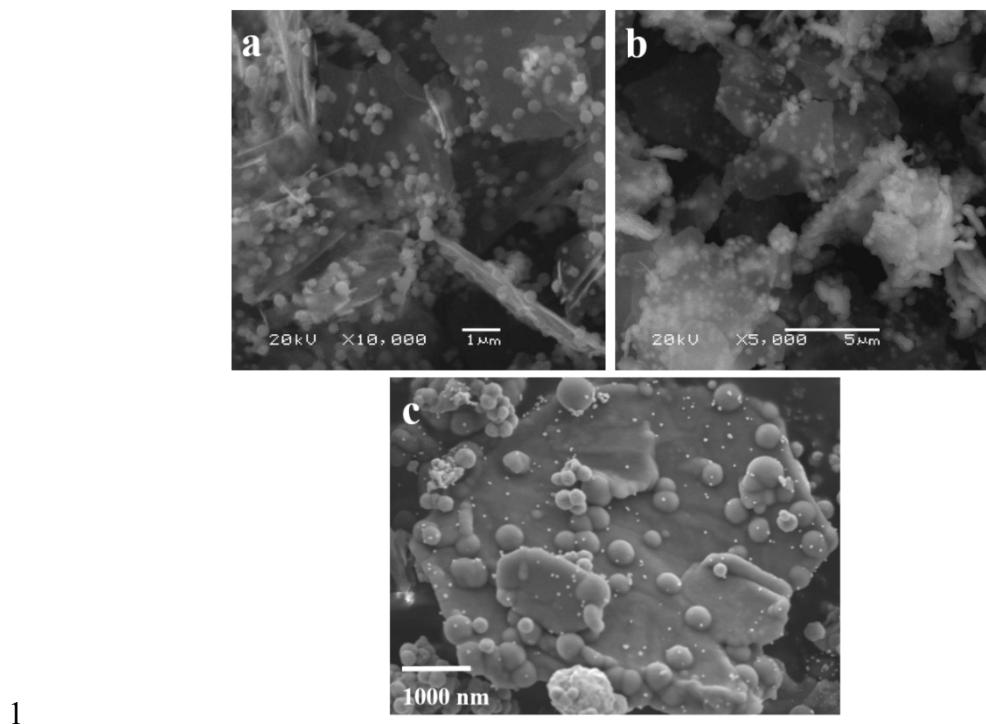
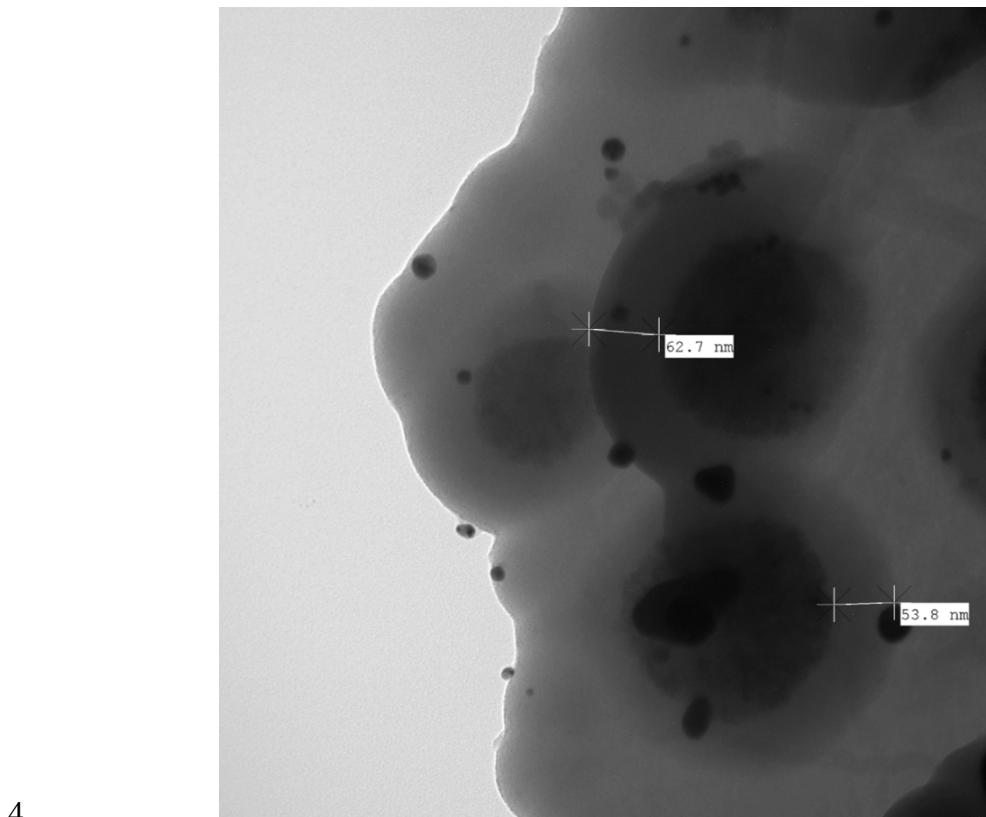


Fig. S1 The ESI/MS spectrum of maltose-SH.



2 **Fig. S2** SEM images of (a) Fe_3O_4 -GO, (b) Fe_3O_4 -GO@nSiO₂ and (c) Fe_3O_4 -GO@nSiO₂-PAMAM-Au-
3 maltose.



4
5 Fig. S3 High-magnification TEM analysis of Fe_3O_4 -GO@nSiO₂-PAMAM-Au-maltose

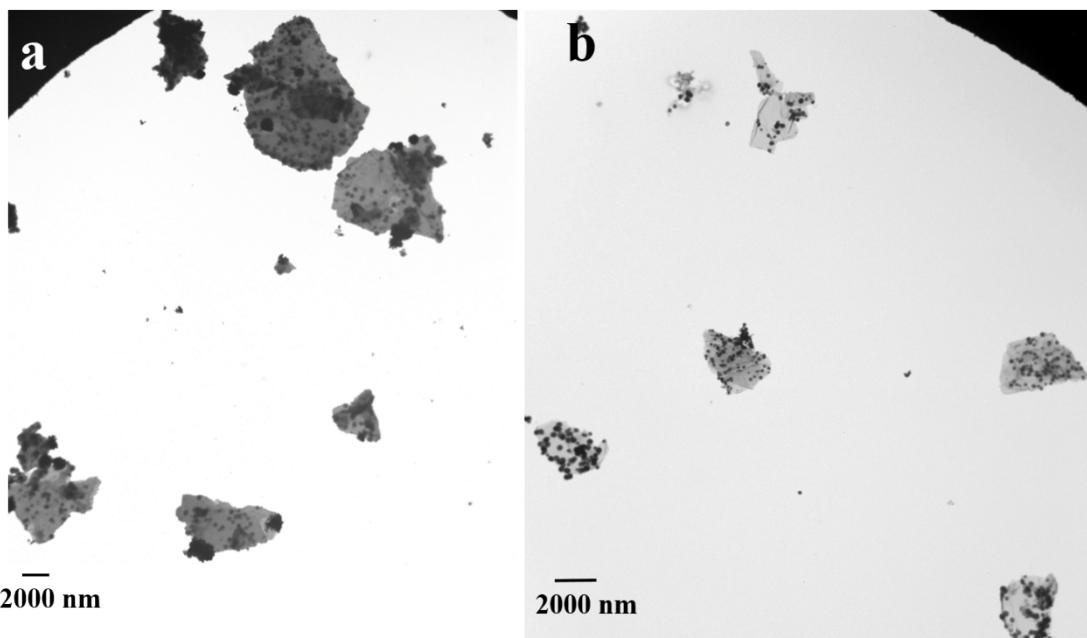
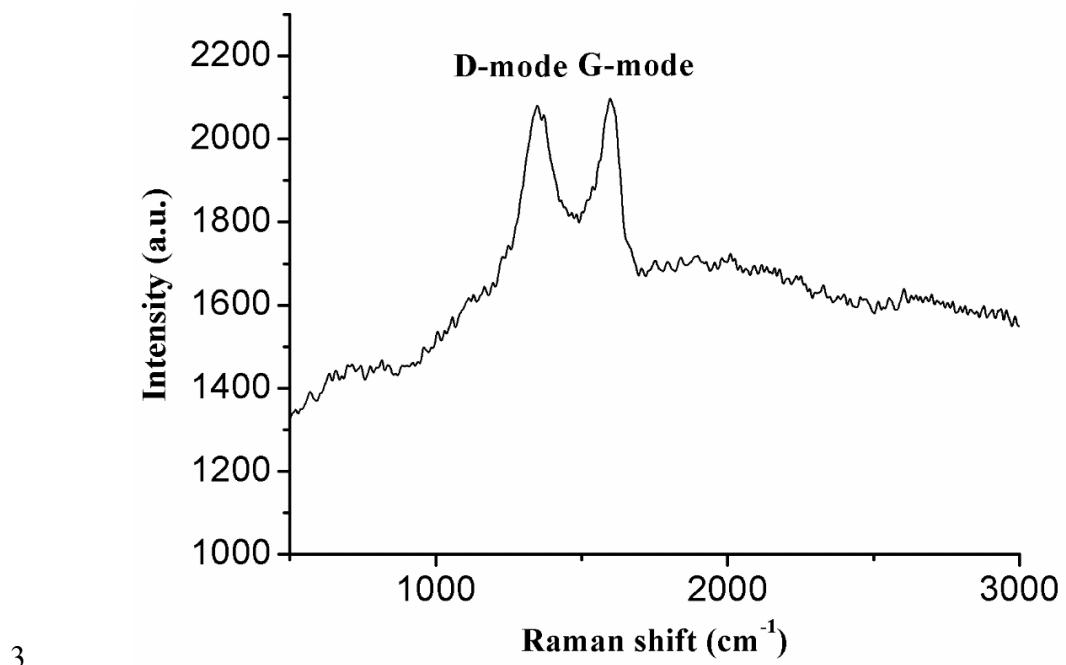
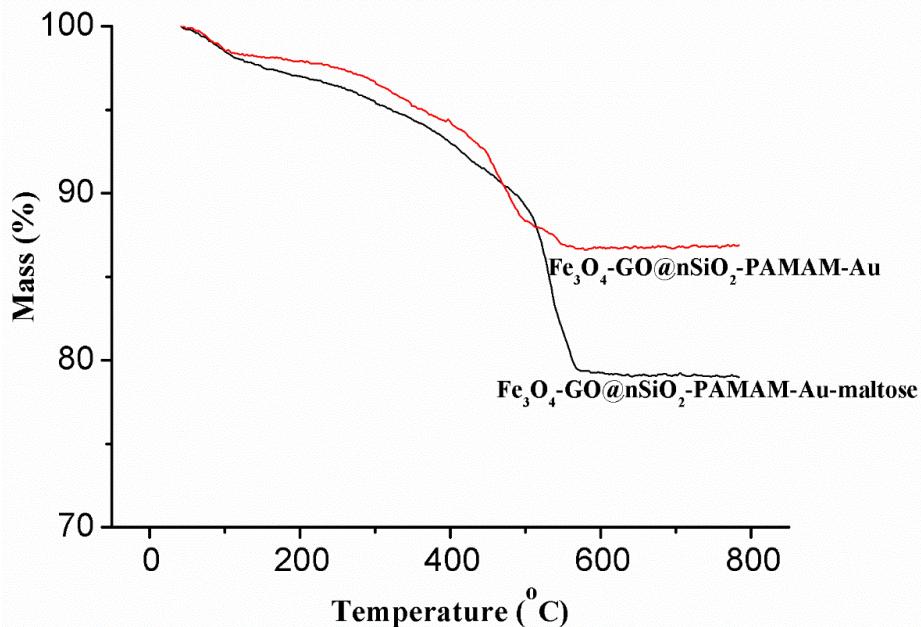


Fig. S4 Low-magnification TEM analysis



4 **Fig. S5** The Raman spectrum of $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au-maltose}$. The final composite showed
5 two obvious peaks corresponding to G and D modes of the carbon framework, demonstrating the
6 existing GO.



1

2 **Fig. S6** TGA analysis of $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au}$ and $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au-}$
3 maltose.

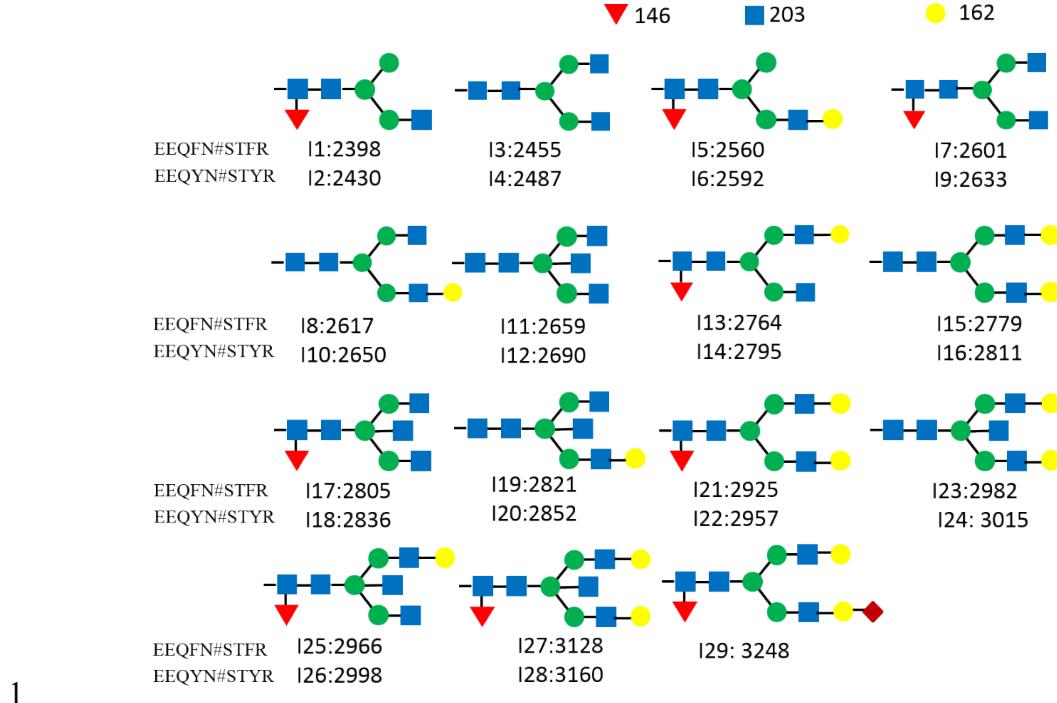
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5 The graft density of maltose on the $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au-maltose}$ was calculated based on
6 the following the equation:

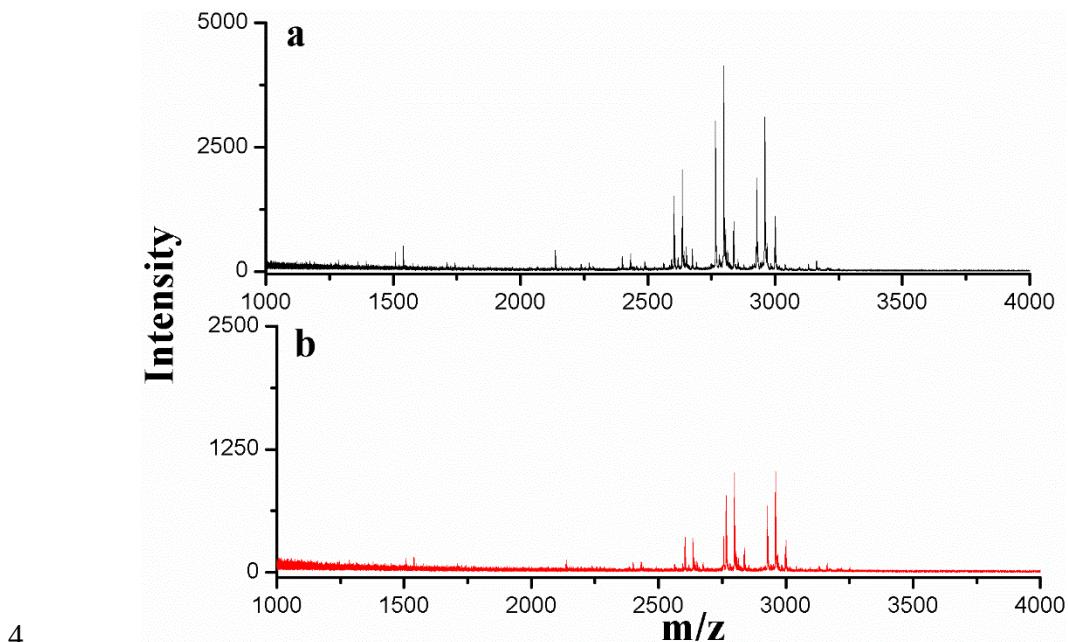
7
$$\chi_{\text{maltose}} (\mu\text{mol}/\text{m}^2) = \frac{\%X * m * 10^6}{MW * m * S} \quad \text{Equation S1}$$

8 where %X is the mass percent of maltose-SH as determined by TGA analysis, m is the mass of the
9 $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au-maltose}$, MW is the molecular weight of maltose-SH, S is the specific
10 surface area of $\text{Fe}_3\text{O}_4\text{-GO@nSiO}_2\text{-PAMAM-Au-maltose}$.

11



2 **Fig. S7** The detailed information of glycopeptides enriched from tryptic digests of IgG by Fe_3O_4 -
 3 GO@nSiO₂-PAMAM-Au-maltose.



5 **Fig. S8** MALDI-TOF-MS analysis of glycopeptides enriched from tryptic digests of IgG by (a) Fe_3O_4 -
 6 GO@nSiO₂-PAMAM-Au-maltose and (b) $\text{Fe}_3\text{O}_4@SiO_2$ -maltose. A simple structure $\text{Fe}_3\text{O}_4@SiO_2$ -
 7 maltose was fabricated and compared its hydrophilic enrichment performance with that of Fe_3O_4 -
 8 GO@nSiO₂-PAMAM-Au-maltose at the same experimental condition where tryptic digests of IgG
 9 were used as the model. Seen from the result, Fe_3O_4 -GO@nSiO₂-PAMAM-Au-maltose demonstrated
 10 better enrichment performance than $\text{Fe}_3\text{O}_4@SiO_2$ -maltose, further identifying the superiority of this
 11 novel structure in glycoproteomics.

1 **Table S1** The detailed information of glycopeptides enriched from tryptic digests of HRP by Fe₃O₄-
 2 GO@nSiO₂-PAMAM-Au-maltose.

Peak number	Observed m/z	Glycan composition	Sequence
1	1844	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SPN#ATDTIPLVR
2	2543.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SSPN#ATDTIPLVR
3	2612.6	[Hex]3[HexNAc]2[Xyl]1	MGN#ITPLTGTQGQIR
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	QLTPTFYDNSCP#VSNIVR
7	3354	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAMDR
8	3673	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
9	3895	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	LHFHDCFVNGCDASILLDN#TTSFR
10	4057	[Hex]3[HexNAc]2[Xyl]1	QLTPTFYDNC(AAVESACPR)PN#VSNIVR-H ₂
11	4224	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNC(AAVESACPR)PN#VSNIVR
12	4839	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
13	4985	[Hex]3[HexNAc]2[Fuc]1[Xyl]1 [Hex]3[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR

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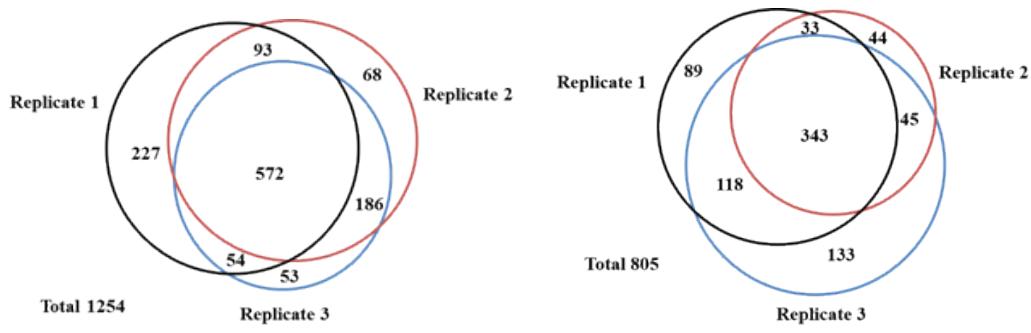
1 **Table S2.** The recovery yield of N-glycosylation motifs from tryptic digests of IgG enriched by Fe₃O₄-
2 GO@nSiO₂-PAMAM-Au-maltose.

N-glycosylation motif	Recovery 1	Recovery 2	Recovery 3
1	86.02%	84.76%	85.20%
2	84.31%	83.84%	80.37%

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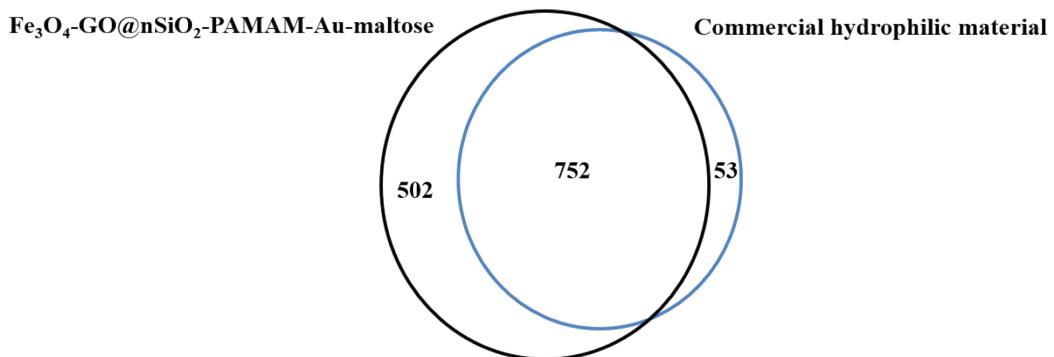


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7 **Fig. S9** Data of three independent LC/MS runs of N-glycosylation sites enriched by Fe₃O₄-GO@nSiO₂-
8 PAMAM-Au-maltose (left) and commercial hydrophilic materials from tryptic digests of mouse liver (right).

9

10



11

12 **Fig. S10** Overlap of identified N-glycosylation sites from tryptic digests of mouse liver after enrichment by
13 Fe₃O₄-GO@nSiO₂-PAMAM-Au-maltose and commercial hydrophilic material.

14

15

1 **Table S3.** Data of three independent LC/MS runs of the N-glycosylation sites enriched by Fe₃O₄-
2 GO@nSiO₂-PAMAM-Au-maltose.

3 Replicate 1

Protein	Sequence window
IPI00123342	PRPKDKNGTRAEPPLNASAGDQEEKVIPPAG
IPI00314141	DEELSCTVVELKYTGNASALFILPDQGRMQQ
IPI00131830	DEELSCSVLELKYTGNASALLILPDQGRMQQ
IPI00330632	AKVVVDCKEVGQKAINASANITSQDGVEVLGR
IPI00463492	NFKAGLFVDKGVKTTNASASDPREYLCLDNS
IPI00849192	KLIYSYTGEHIPRITNASDSTKRNNLDFSIR
IPI00459432	SSALVFTRLLEFDSTNASEGAQPPGKPYPY
IPI00123223	LSCLESSWKTIEQERNASFVYTKALMAYAFA
IPI00130661	QFLKSSSHLPPSSYFNASGRAYPDVAALSDG
IPI00226430	KLPSVFKKDGTVTAGNASGVSDGAGAVIIAS
IPI00454033	FTLQHLQHHGSHLKANASHDQPQKHGLELAS
IPI00387362	GLFVEYETPQNASKQKNASHQNASQQGNTSSS
IPI00473190	QISYFQDNHHVRHQGNASLGKLLHTLEGPS
IPI00762091	CLEAQGVQVRVTPVCNASLPAQRWKWSRNR
IPI00122523	RKGATLEPEELLRAGNASLTLPNLTALKDEGN
IPI00119063	EEDCSIDPKLTSCATNASMCGDEARCVTEK
IPI00757771	SYWTRNGVELTATRKNASNMMEYRINKPRAED
IPI00114044	NLSESVEDGPRGSPGNASQGSTIHLHSPQLAL
IPI00129526	EVVQREEEAIQLDGLNASQIRELREKSEKFA
IPI00134585	ILGFACKMGDREALGNASQLFDWLKGSAI
IPI00130573	HTNAHGKDLDTDFTSNASQPETKAIENLIQ
IPI00387362	YETPQNASKQKNASHQNASQQGNTSSAKKDQ
IPI00338785	TSIESETAASETLTNASQRISKLERNVEEL
IPI00119065	AEEAQQRQAQALDKANASRGQVEQANQELRE
IPI00113227	THEEVHSLHFRDFVNASSKYEVTTIHNLFR
IPI00762812	HPLDLQEDFNNATLVNASSLIPKEAGSKETF
IPI00338785	ITKYFQMSLEAEKRVNASTTDPNSTVEQSAL
IPI00742278	QSSCCRLYNVTHQFCNASVMDPTCVRCRPLT
IPI00132189	CSCLAINRNTQADVINASWSVLSNSTRHELE
IPI00396840	NLWEHNQAVIKHLLENATASVSEAERKAQVY
IPI00944095	AKTEDLTEGNKSISENATATTEPKMTESTRV
IPI00608063	DVPSGPPRKVEVEAVNATAVKVSWRSPVPNK
IPI00314443	NCQFETAQKKCQEAINATCKGSYCTGNSSE
IPI00119063	LCDGDNDCGNSEDESNATCSARTCPPNQFSC
IPI00119063	DRTHANGSIKRGCKDNATDSVPLRTGIGVQL
IPI00321190	TCQFVMNKFSELIVNNATEELLVKGLSNACA
IPI00114844	NINKMTSGDPGETANATEVKKCLTQPLKNL

IPI00122272	TNYLRNVALVAGDTGNATGLGEQGPTRGTD
IPI00122293	LQNNFITEPLESFQNATGLRWVNLDNNRIR
IPI00742414	VPKEKRFPKIRRHDVNATGRFQEEVKIPRVN
IPI00124221	IPDGYPQISCLPKNEENATIATYPEFGVLDLK
IPI00757771	GEYHCYHFVSAPKANATIEVKAAPDITGHK
IPI00876558	VFIYRSLGQFLSTENATIKLGADLMGRNST
IPI00348266	EQRRNVIRTIPHQYNATINKYSHDIALLEL
IPI00876028	TLVIPIRYYVRWRLRNATITQALTNKDSPFI
IPI00130010	PVCKDNSCVDPPHPVNATIVTRTKNKYLHGD
IPI00123342	MTTLEKVINDTWAWKNATLAEQAKLPATEKP
IPI00116913	RLKEALQWKQELSQDNATLQATLQAASLILG
IPI00109996	EGERCVELHRYLKNGNATLLRTDSPKAHVTH
IPI00227508	ARLAPYLCGRDPNCNATLPQAPCNKQKVEM
IPI00762812	IRMSQHPLDLQEDFNNATLVNASSLIPKEAG
IPI00319509	FGGEEEWNFAWEQFRNATLVNEADKLRSALA
IPI00408495	ISNLDVNDPGTYVCNATNAQGTTRETISLR
IPI00625262	THRLPLLGEKQEAKENATNVTFTGDKIAMHE
IPI00742278	EKALGLLCGRDARACNATNWIEYMFNKDNGQ
IPI00119809	NLLDAHVVCRALGYENATQALGRAAFGPGKG
IPI00321190	ICKTVVTEAGNLLKDNTQEEILHYLEKTCE
IPI00749655	FCILDVMSTGSSSGVGNA TRIAHQLHQHRLKS
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1 Replicate 2

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1

2 Replicate 3

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