Supporting information

An Ultra Hydrophilic Dendrimer-Modified Magnetic Graphene with a Polydopamine Coating for Selective Enrichment of Glycopeptides

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Experiment Section

Synthesis of magG

magG was synthesized via a solvothermal reaction. Firstly, grapheme (400 mg) was dissolved 50 mL concentration nitric acid, after magnetic stirring for 7 h, the dispersion was washed with distilled water for several times until it turned to be neutral, then it was centrifuged and dried in vacuum at 50 °C. Next, $Fe_3O_4 \cdot 6H_2O$ (405 mg) was dispersed into 40 mL ethylene glycol solution and the dried acid-treated graphene (150 mg) was added and also treated under magnetic stirring for 1 h. Then trisodium citrate (150 mg), sodium acetate (1.8 g) and poly (ethylene glycol) (PEG-20000) (1.0 g) were added into the solution. After another magnetic stirring for 0.5 h, the mixture was transferred into the airtight steel container and heated at 200 °C for 12 h. Lastly, the products were washed with distilled water and ethanol for several times, then dried at 50 °C in vacuum.

Preparation of protein digestion

The two standard protein bovine serum albumin (BSA) and horseradish peroxidase (HRP) were dissolved in ammonium bicarbonate buffer (NH_4HCO_3, 25 mM, pH =8.3). The concentration of HRP was 1 mg mL^{-1}, the BSA was 2 mg mL^{-1} and 10 mg mL^{-1}, then they were boiled at 100 °C for 5 min in order to be denatured. When the solution returned to room temperature, trypsin was added into the mixture in a proportion (trypsin:protein=1:40) and they were incubated at 37 °C for 16 h. Then they were stored at −20 °C for further use.

2 μL of human serum was diluted in denaturing buffer, containing 60 mM NH_4HCO_3

and 8 M urea. The proteins were reduced with 10 mM DTT at 37 °C for 1h and alkylated with 20 mM IAA at 37 °C for 1 h in the dark. After the protein solution was diluted with 50 mM NH_4HCO_3 to reduce the urea concentration below 1 M, trypsin was introduced at an enzyme-to-substrate ratio of 1:40 (w/w), and the mixture was incubated at 37 °C for 16 h.

The contrast experiments

In order to prvove our material has double hydrophilic abilities, another two materials were also synthesized to contrast. One is magG@PAMAM, and another is magG@PDA. Their enrichment conditions for HRP digestion are the same to magG@PDA@PAMAM.

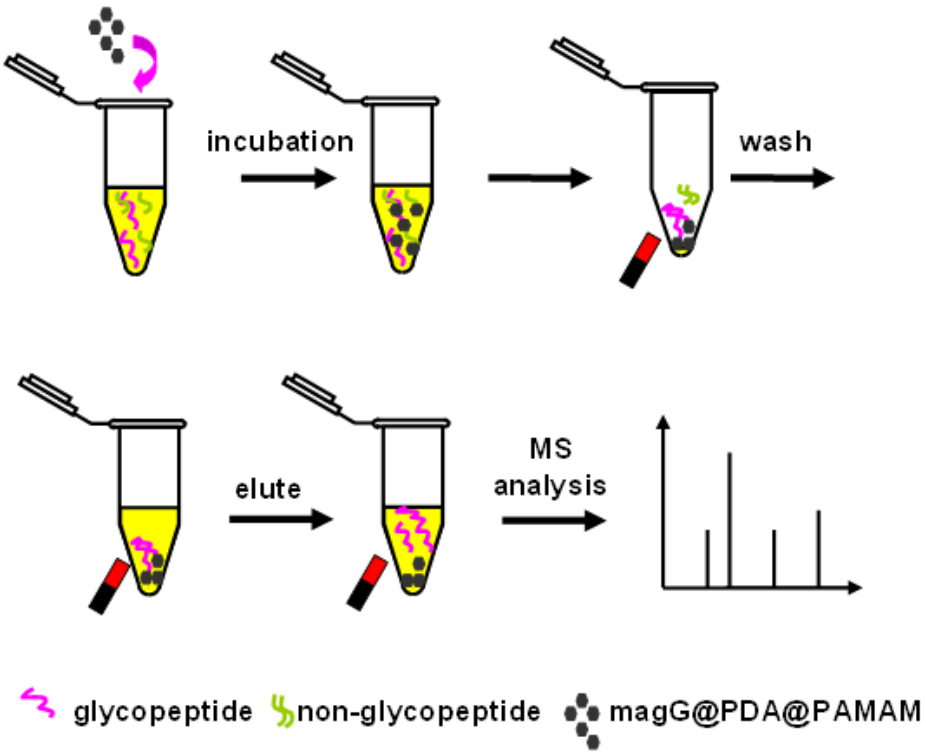
Nano-Liquid Chromatography Tandem Mass Spectrometry (Nano-LC−MS/MS) Analysis of Glycopeptides and Database Search

In order to investigate the glycopeptides enriched from human serum protein mixture digestion, the eluate was lyophilized and then redissolved in 50 mM loading buffer (95% ACN/H2O, 0.1% TFA). For deglycosylation, 1 μL PNGase F was added into the peptides solution and the proceeded at 37 °C for 16 h. The LC-MS/MS analysis was carried out using an high-performance liquid chromatography (HPLC) system composed of two LC-20AD nanoflow LC pumps, an SIL-20 AC autosampler, and an LC-20AB microflow LC pump (Shimadzu, Tokyo, Japan) connected to an LTQ-Orbitrap mass spectrometer (ThermoFisher, San Jose, CA).

Database Search

Thermo Scientific Proteome Discoverer software version 1.4 with the MASCOT

v2.3.2 search engine was used for all searches of the database. The database was the Human UniProtKB/Swiss-Prot database (Release 2014-04-10, with20264 sequences).The mass tolerance of the precursor ion was set to 10 ppm and that of the fragment ions was set to 50 mmu. The peptide false discovery rate (FDR) was set to 1%. Trypsin was chosen as the proteolytic enzyme and up to two miss cleavages were allowed. Carbamidomethyl on cysteine was set as a fixed modification. Oxidation on methionine and Deamidation on asparagine were set asvariable modifications. The Asn modification that did not occur in the N-X-S/T (X≠P) sequon was eliminated to ensure the false positive rate below 1% for the identified glycosylation sites.



Scheme S1 The selective enrichment process for the glycopeptides using magG@PDA@PAMAM

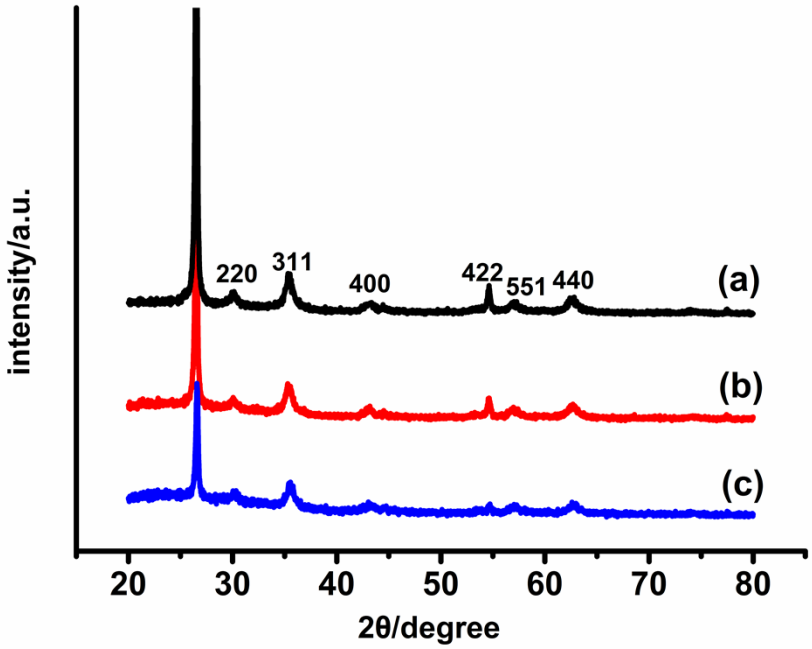


Fig. S1 XRD patterns of magG (a), magG@PDA (b), magG@PDA @PAMAM (c)

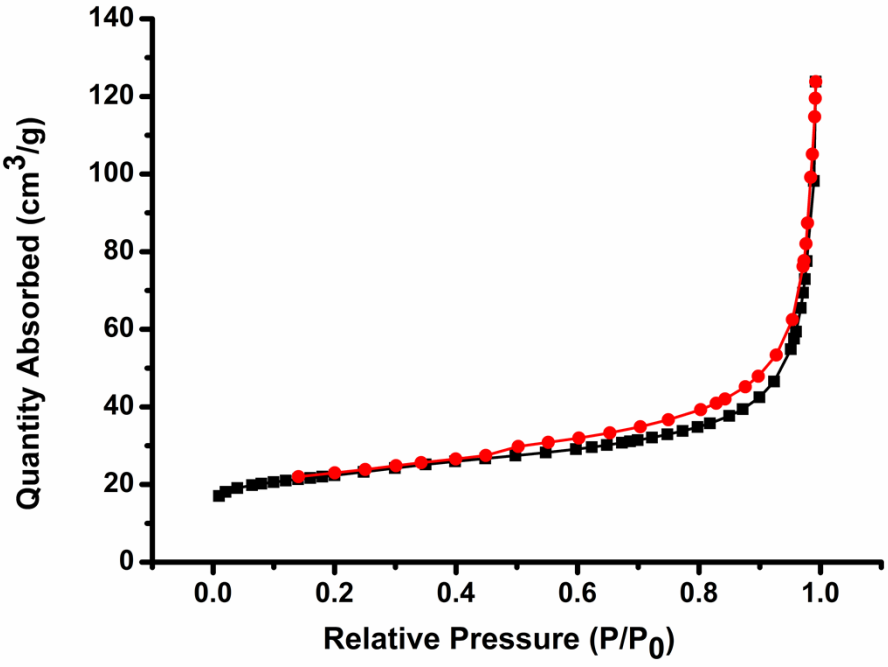


Fig. S2 N_2 sorption−desorption isotherms of magG@PDA@PAMAM

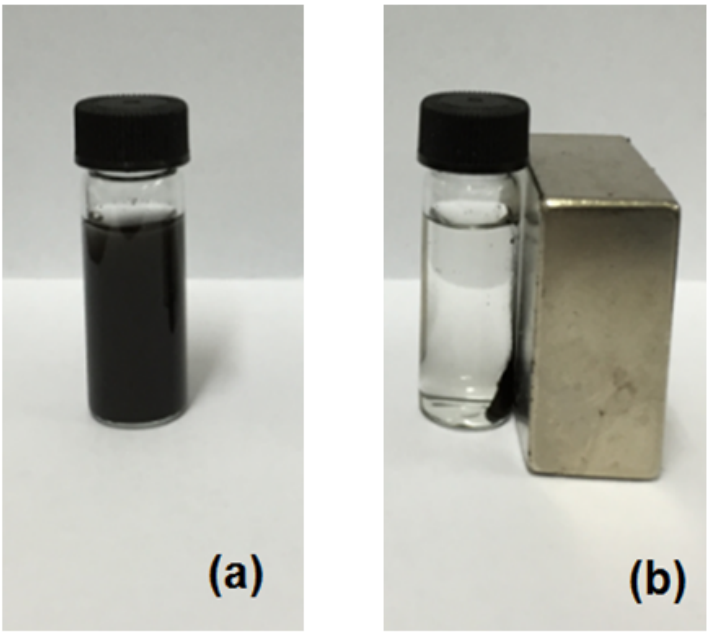


Fig. S3 The photo of magG@PDA@PAMAM in the aqueous solution (a), under magnetic field (b)

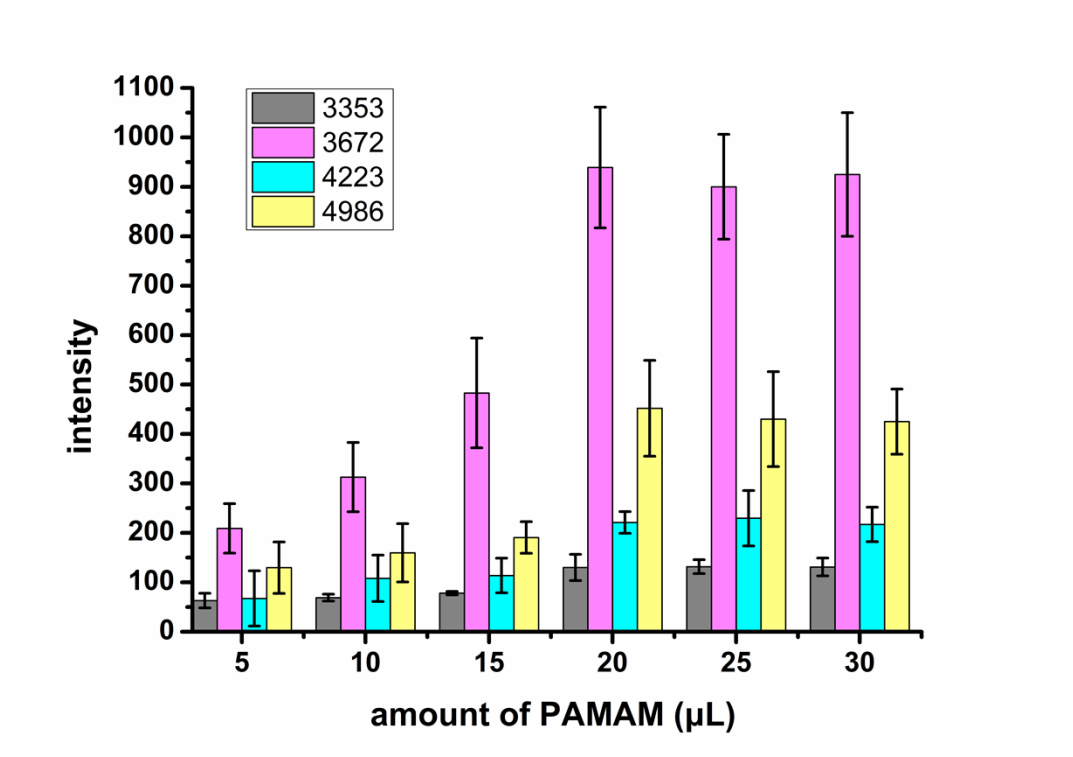


Fig. S4 Intensity of four selected N-linked glycopeptides from HRP digestion after

treatment with different amounts of PAMAM during the synthesis of magG@PDA@PAMAM

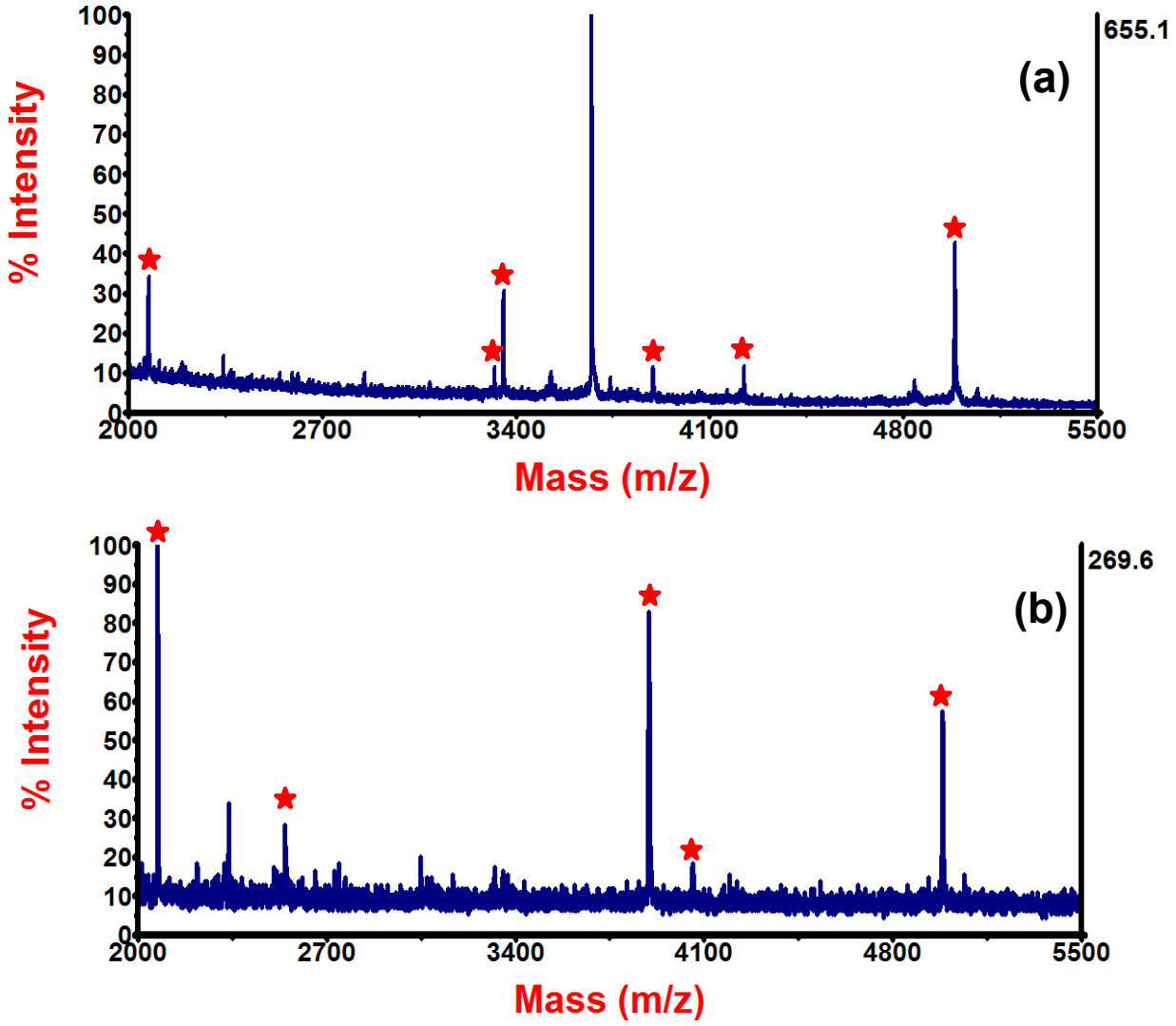


Fig. S5 MALDI-TOF mass spectrum of the tryptic digested HRP 1 μg μL^{-1}: after enrichment by magG@ PAMAM (a), magG@PDA (b), the glycopeptides was marked with ★

Table S1 Detailed information of the observed glycopeptides in HRP tryptic digest.

No.	m/z	Glycan composition	Amino acid sequence
1	2073	XylMan3FucGlcNAc2	PN#VSNIVR
2	2850	FucGlcNAc	GLIQSDQELFSSPN#ATDTIPLVR
3	3047	XylMan2GlcNAc2	SFAN#STQTFFNAFVEAMDR
4	3074	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
5	3087	XylMan3FucGlcNAc2	GLCPLNGN#LSALVDFDLR
6	3323	XylMan3FucGlcNAc2	QLTPTFYDNSCPN#VSNIVR
7	3353	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
8	3672	XylMan3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
9	3747	XylMan3GlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
10	3894	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
11	4058	XylMan3GlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H2O
12	4223	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
13	4836	XylMan3FucGlcNAc2, XylMan3GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
14	4986	XylMan3FucGlcNAc2, XylMan3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR

The N-glycosylation sites are marked with N#. GlcNAc = N-acetylglucosamine, Fuc = fuctose, Man = mannose, Xyl = xylose.

Table S2 List of identified glycoproteins from 2 μL of human serum after solid phase extraction with magG@PDA@PAMAM after three parallel runs, N# denotes the glycosylation site.

No.	protein	Description	Pepide sequence
1	P02647	Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 - [APOA1_HUMAN]	LLDN#WDSVTSTFSK
2	P02671	Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2-[FIBA_HUMAN]	NPSSAGSWN#SGSSGPGSTGNR
3	P02760	Protein AMBP OS=Homo sapiens GN=AMBP PE=1 SV=1 - [AMBP_HUMAN]	WYN#LAIGSTcPWLK
4	P19823	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2-[ITIH2_HUMAN]	KFYN#QVSTPLLR

5	P02749	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3-[APOH_HUMAN]	cPFPSRPDnGFVN#YPAKPTLYYK
6	P01042	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2-[KNG1_HUMAN]	YN#SQNQSNNQFVLYR
7	P02787	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3-[TRFE_HUMAN]	APN#HAVVTRK
8	P07358	Complement component C8 beta chain OS=Homo sapiens GN=C8B PE=1 SV=3 - [CO8B_HUMAN]	YYAGGcSPHYILN#TR

9	P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 - [ALBU_HUMAN]	YIcEN#QDSISSK
10	P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]	TKPREEQYN#STYR
11	P05155	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2 - [IC1_HUMAN]	DTFVN#ASR