Supporting Information

Multilayer Hydrophilic Poly(phenol-formaldehyde resin)-Coated Magnetic Graphene for Boronic Acid Immobilization as a Novel Matrix for Glycoproteome Analysis

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EXPERIMENTAL SECTION

Synthesis of Fe_3O_4-modified Graphene Sheets Nanoparticles. The Fe_3O_4-modified graphene sheets (magG) were synthesized via a hydrothermal reaction[32]. In brief, graphene (400 mg, Shanghai Boson Technology Co., Ltd) was added in HNO_3 under stirring at 60 ºC about 7 hours. The graphene treated by HNO_3 was washed thoroughly and then dried in vacuum at 50 ºC. After that, the $FeCl_3 \cdot 6H_2O$ (405 mg) and pretreated graphene (150 mg) were added in ethylene glycol (40 mL) containing 0.15 g of trisodium citrate, 1.8 g of sodium acetate and 1.0 g of poly(ethylene glycol)-20000 under vigorous agitating for 30 min. The pellucid mixture solution was sealed in a Teflon-lined stainless-steel autoclave and heated at 200 °C about 10 h. The product was collected by magnetic separation and washed using distilled water and anhydrous ethanol. The obtained magG was dried in vacuum at 50 ºC for further use.

Characterization. Transmission electron microscopy (TEM) images were taken with a JEOL2011 microscope. Fourier-transform infrared (FT-IR) spectra were tested on a Nicolet Fourier spectrophotometer. The composition of magG@PF@APB was identified by the energy dispersive X-ray spectroscopy (EDX). The Raman spectra were obtained at ambient temperature on a LabRam-1B Raman spectrometer. X-ray Powder diffraction (XRD) patterns were collected on a Bruker D4 X-ray diffractometer with Ni-filtered Cu KR radiation. Zeta potential measurements were carried out on a Nano ZS90 zeta analyzer (Malvern Instruments Ltd.).

Protein Sample Preparation. The Myo and HRP protein were dissolved in 50 mM NH_4HCO_3 buffer and heated at 100 °C for 10 min, respectively. Then trypsin was added to the reaction mixture to digest the protein at a ratio of 1 : 40 (enzyme-to-substrate w/w) overnight at 37 °C. 1 μL of human serum was diluted in denaturing buffer, containing 60 mM

NH_4HCO_3 and 8 M urea. The proteins were reduced with DTT and then alkylated with IAA (in the dark). After diluting the protein solution with 50 mM NH_4HCO_3 until the concentration of urea below 1 M. Then trypsin was introduced into the mixture, which was incubated at 37 °C about 16 h.

Nano-Liquid Chromatography Tandem Mass Spectrometry (Nano-LC−MS/MS) Analysis of Glycopeptides. In order to investigate the glycopeptides enriched from human serum protein mixture digestion, the eluate was lyophilized and then redissolved in 50 mM NH_4HCO_3 buffer (pH=9.0). For deglycosylation, 1 μL of PNGase F was added into the peptides solution and the proceeded at 37 °C for 16 h. The deglycosylated peptides were lyophilized and then redissolved in FA/H_2O (0.1:99.9,v/v). 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, with 20264 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 as variable 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.

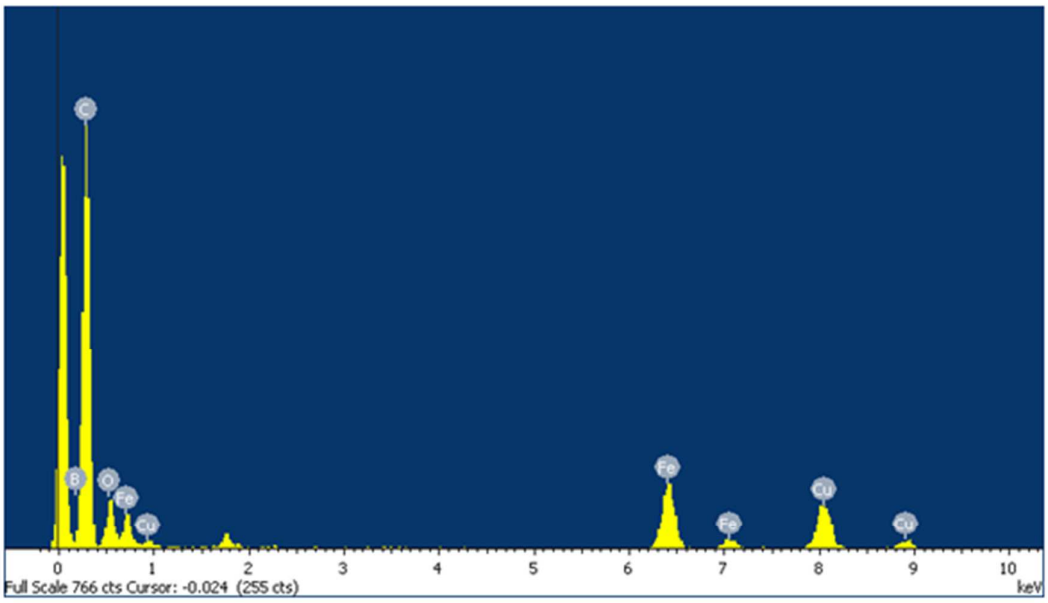

Figure S1. The energy dispersive X-ray (EDX) spectrum of magG@PF@APB.

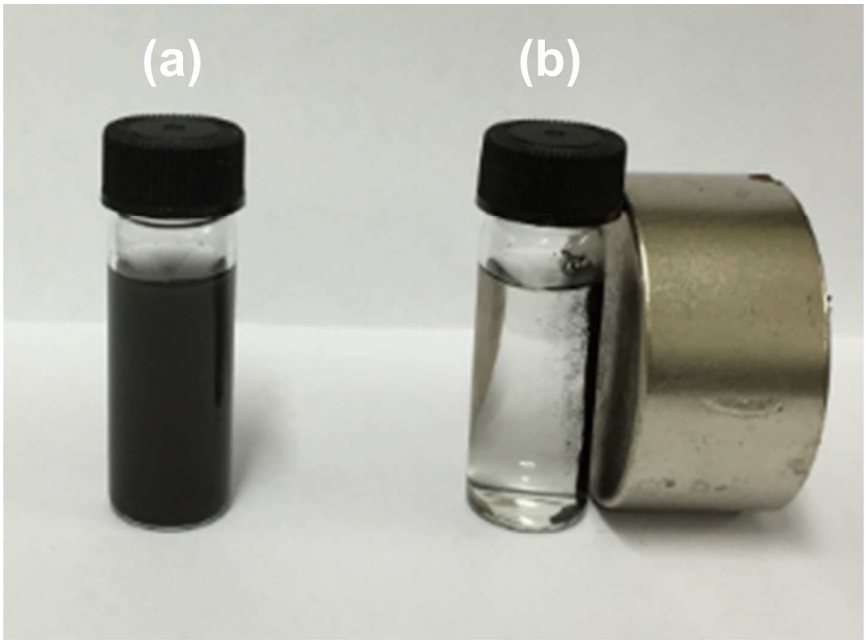

Figure S2. The photos of the aqueous dispersion of magG@PF@APB materials: (a) before and (b) after separation with a magnet within 5s.

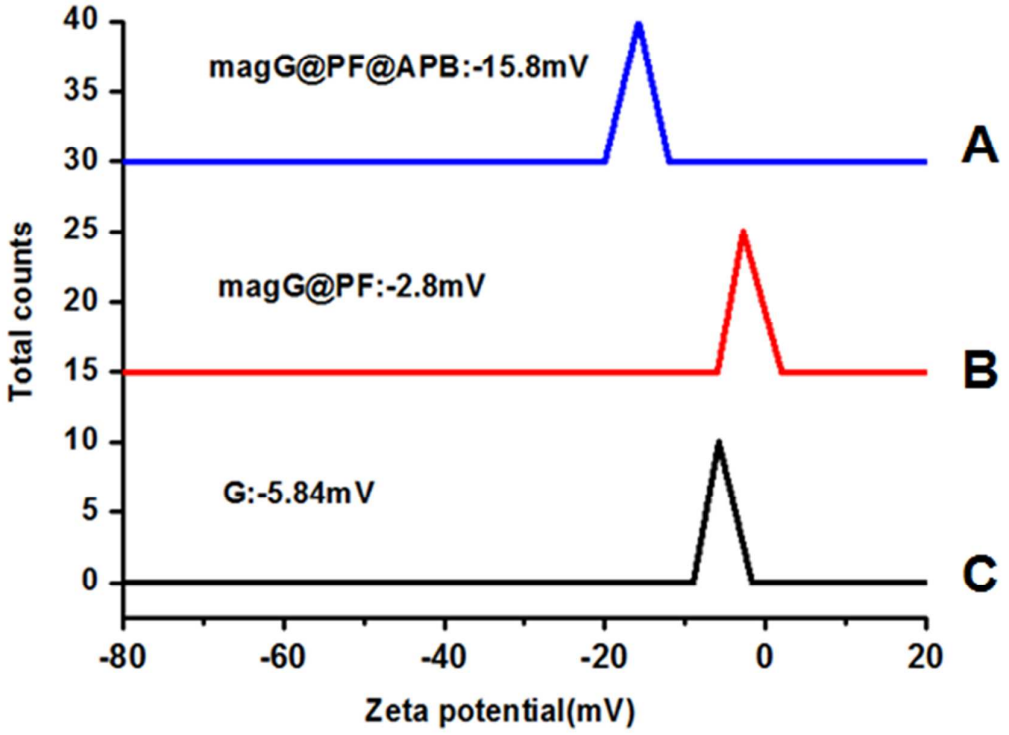

Figure S3. The zeta potential distributions of magG@PF@APB (A), magG@PF (B) and graphene (C).

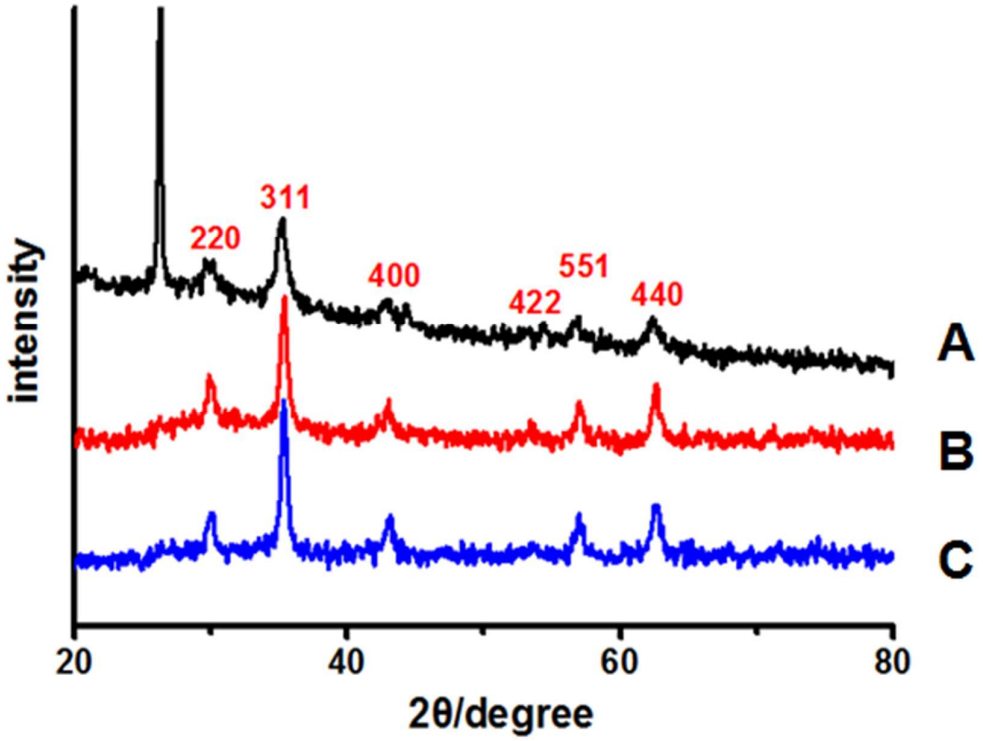

Figure S4. XRD patterns of (A) magG, (B) magG@PF and (C) magG@PF@APB materials.

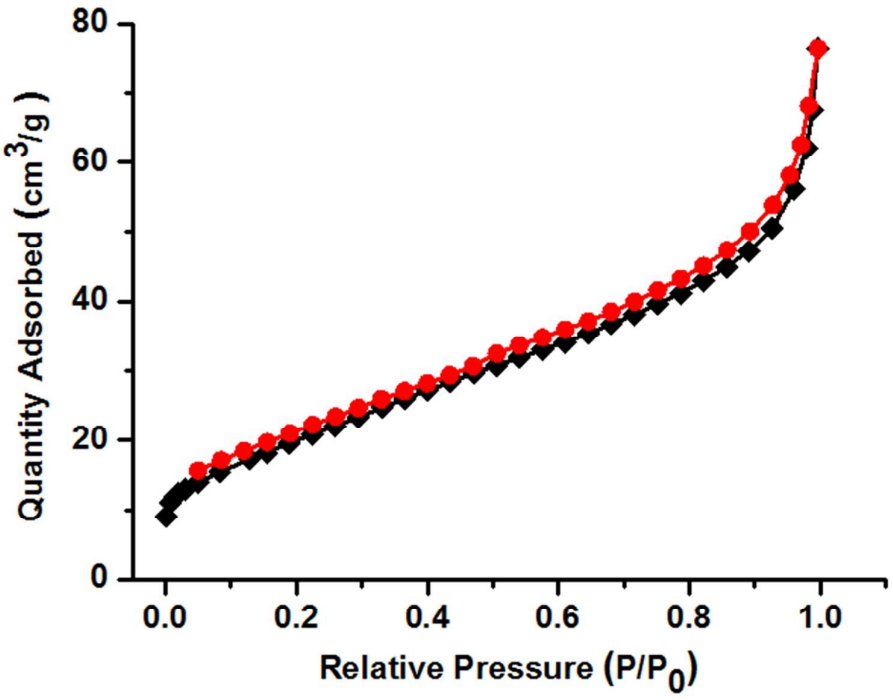

Figure S5. Nitrogen adsorption-desorption isotherms of magG@PF@APB composites.

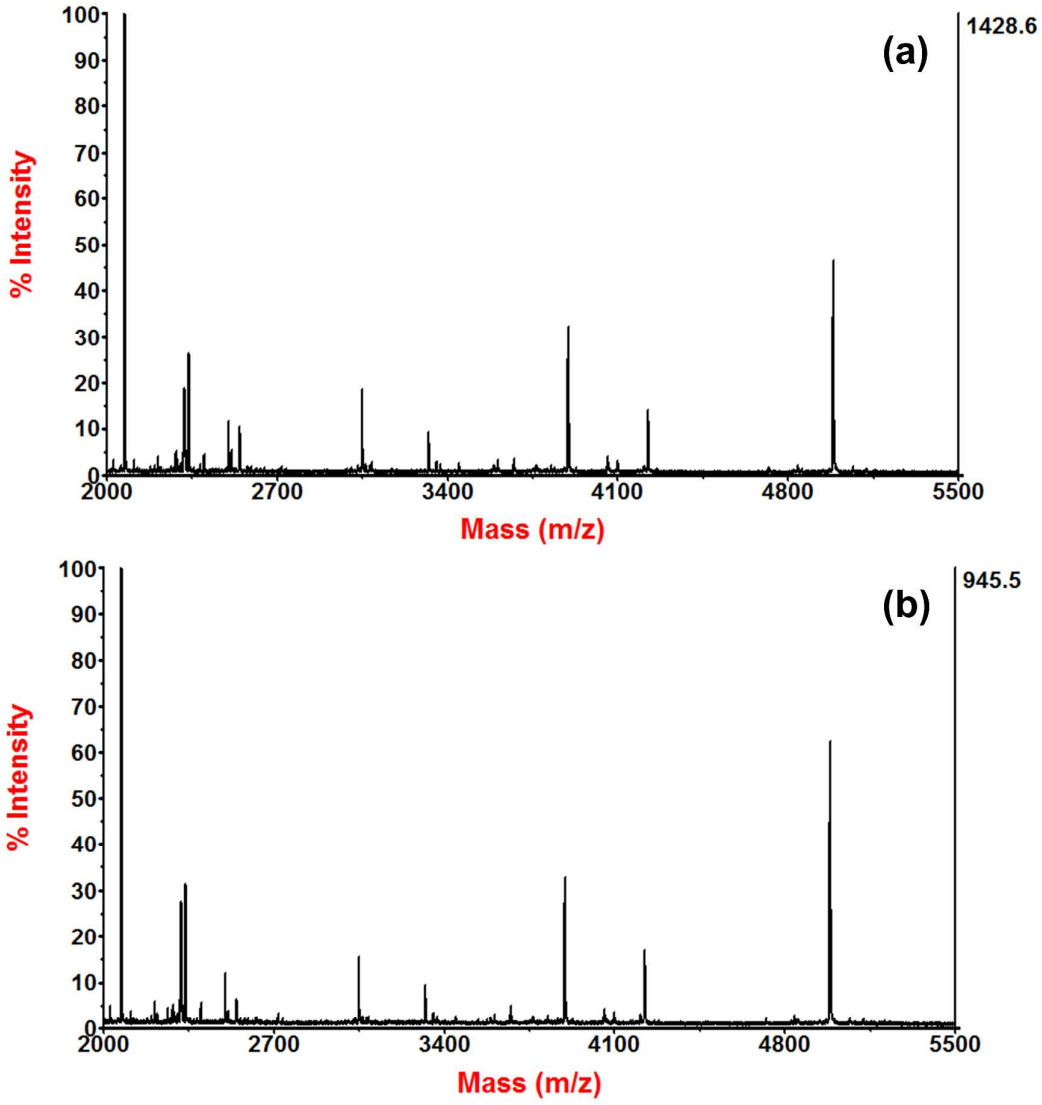

Figure S6. MALDI mass spectrum of glycopeptides enriched from HRP using magG@PF@APB, (a) for the first time and (b) for the third time.

Detailed information of glycopeptides

Table S1. Detailed information of the observed glycopeptides in HRP tryptic digest**.** N# denotes the N-linked glycosylation site.

Observed m/z	Glycan composition	Amino acid sequence
2074	XylMan3GlcNAc2	PN#VSNIVRRR
2290	XylMan2GlcNAc2	SILLDN#TTSFR
2321	Man2GlcNAc2	MGN#ITPLTGTQGQIR
2543	XylMan3FucGlcNAc2	SSPN#ATDTIPLVR
3048	XylMan2GlcNAc2	SFAN#STQTFFNAFVEAMDR
3074	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
3323	XylMan3FucGlcNAc2	QLTPTFYDNSCPN#VSNIVR
3354	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
3672	XylMan3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
3894	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
4057	XylMan3GlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H2O
4222	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
4839	XylMan3FucGlcNAc2, XylMan3GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
4984	XylMan3FucGlcNAc2, XylMan3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
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 1 μL human serum after solid phase extraction with magG@PF@APB, N# denotes the N-linked glycosylation site.

NO.	Protein	Description	Peptide sequence
1	P02787	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [TRFE_HUMAN]	QQqHLFGSN#VTDcSGNFcLFR
2	P02787	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [TRFE_HUMAN]	QQQHLFGSN#VTDcSGNFcLFR
3	P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]	TKPREEQYN#STYR
4	P05155	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2 - [IC1_HUMAN]	VGQLQLSHN#LSLVILVPQNLK
5	P01011	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1	KLINDYVKN#GTR

		SV=2 - [AACT_HUMAN]	
6	P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 - [ANT3_HUMAN]	AAINKWVSN#KTEGR
7	P01011	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2 - [AACT_HUMAN]	LINDYVKN#GTR
8	P02790	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2 - [HEMO_HUMAN]	SWPAVGN#cSSALR
9	P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]	EEQYN#STYR
10	P05155	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2 - [IC1_HUMAN]	DTFVN#ASR
11	P01859	Ig gamma-2 chain C	EEQFN#STFR

		region OS=Homo sapiens GN=IGHG2 PE=1 SV=2 - [IGHG2_HUMAN]	
12	P05155	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2 - [IC1_HUMAN]	VLSN#NSDANLELINTWVAK
13	P01009	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 - [A1AT_HUMAN]	YLGN#ATAIFFLPDEGK
14	P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 - [ANT3_HUMAN]	WVSN#KTEGR
15	P05546	Heparin cofactor 2 OS=Homo sapiens GN=SERPIND1 PE=1 SV=3 - [HEP2_HUMAN]	DFVN#ASSKYEITTIHNLFR
16	P04114	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2 -	FN#SSYLQGTNqITGR

		[APOB_HUMAN]	
17	P02766	Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1 - [TTHY_HUMAN]	ALGISPFHEHAEVVFTAN#DSGPR