



# Boronic acid functionalized MOFs as HILIC material for N-linked glycopeptide enrichment

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## Abstract

Highly specific enrichment of N-linked glycopeptides from complex biological samples is crucial prior to mass spectrometric analysis. In this work, a hydrophilic metal–organic framework composite is prepared by the growth of UiO-66-NH<sub>2</sub> on graphene sheets, followed by its post-synthetic modification to attach boronic acid to form GO@UiO-66-PBA. The fabrication of graphene oxide-MOF composite results in enhanced surface area with improved thermal and chemical stability. The synthesized MOF nanocomposite is characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and BET. A crystalline structure with high porosity offering large surface area and good hydrophilicity of the nanocomposite assists as an enrichment tool in glycoproteomics. The GO@UiO-66-PBA nanocomposite selectively enriches N-linked glycopeptides from tryptic digests of horseradish peroxidase (HRP) and immunoglobulin (IgG). GO@UiO-66-PBA nanoparticles show a low detection limit (1 fmol) and good specificity (1:200), reusability and reproducibility for N-linked glycopeptide enrichment from IgG digest. The binding capacity of GO@UiO-66-PBA is 84 mg/g for protein concentration, with a good recovery of 86.5%. A total of 372 N-linked glycopeptides corresponding to different glycoproteins are identified from only 1 μL of human serum digest. Thus, the presented research work can be an efficient separation platform for N-linked glycopeptide enrichment from complex samples, which can be extended to cost-effective routine analysis.

**Keywords** N-linked glycopeptides · Enrichment · Metal–organic frameworks · Hydrophilic interaction chromatography (HILIC) · MALDI-MS · LC-MS

## Introduction

Protein glycosylation, one of the most important post-translational modifications (PTMs), plays a dynamic role in

biological processes [1, 2], including signal transduction [3], molecular recognition [4] and cell-cell interaction [5]. Abnormal glycosylation has close connection with extreme pathological disorders and neurotic conditions [6]. It is important to understand the phenomenon of protein glycosylation, glycosylation sites and relevant peptide grouping for investigating the connection between glycosylation and infections. In modern proteomic studies, mass spectrometry (MS) methods have been utilized for protein and peptide analyses because of their high throughput and high precision [7]. Glycopeptides are not distinguishable directly by MS, as their concentrations in test samples are low, and there are interferences from non-glycopeptides which suppress glycopeptide signals. It is therefore necessary to specifically enrich glycopeptides from intricate biological samples before MS studies [8].

Numerous enrichment strategies have been developed for glycosylated biomolecules, including lectin affinity

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chromatography [9], hydrophilic interaction chromatography (HILIC) [10], hydrazine chemistry [11], boronic acid chemistry [12] and metal/metal oxides [13]. Hydrazine chemistry requires commercial hydrazide resins which are costly and chemically bind glycopeptides in the enrichment process, making elution difficult. HILIC lacks selectivity, as some hydrophilic non-glycopeptides may be enriched [14]. Boronic acid-based affinity strategies show specific and reproducible enrichment [15]. Boronic acid in basic conditions can form five- or six-membered rings with cis-1,2-diols, whereas target molecules are released in acidic conditions [16]. Boronate affinity materials for the separation of glycoproteins [17], such as monoliths [18], magnetic nanoparticles [19], and mesoporous materials [20, 21], have been reported. The low surface area and limited functional groups in a majority of materials reduce the capacity for glycoprotein extraction.

Metal–organic frameworks (MOFs) have tunable porosity, high surface area and ease of functionalization [22]. They are comprised of organic ligands and metal ions [23], and are used in gas adsorption [24], separation [25], drug delivery [26] and as adsorbents [27]. MOFs have also been utilized in enzyme immobilization [28], proteomics [29], glycopeptides [30, 31], phosphopeptides [32, 33], endogenous peptides [34] and nucleoside [35] enrichment. Yang et al. produced pH-responsive magnetic MOF nanocomposites for glycoproteins [36]. MOFs, because of their low content or absence of functional ligands, have lower efficiency for selectively capturing glycopeptides and glycoproteins.

Functional groups such as carboxyl, hydroxyl and epoxide at the edges of the basal plane make graphene oxide (GO) highly soluble, which leads to the synthesis of functionalized graphene materials. GO has a good surface area; however, restacking of graphene sheets because of solvent removal limits the available surface [37]. Restacking can be overcome by fabricating its derivatives with other molecules or materials like MOFs [38, 39].

Graphene-MOF composite is prepared and functionalized with phenylboronic acid through amidation with UiO-66-NH<sub>2</sub> to specifically enrich glycoproteins. The synthetic strategy is simple because of amino groups on UiO-66-NH<sub>2</sub>. The higher surface area of GO and amine-functionalized MOF UiO-66-NH<sub>2</sub> with phenylboronic acid enhances the enrichment capability for glyco moieties.

## Experimental section

### Chemicals and reagents

Zirconium chloride (ZrCl<sub>4</sub>, 98%), 4-carboxy phenylboronic acid (CPBA, 98%), 2-amino terephthalic acid (H<sub>2</sub>BDC-NH<sub>2</sub>, 98%), 1-(3-dimethyl aminopropyl)-3-ethyl carbodiimide (EDC, 98%), N-hydroxy succinimide (NHS, 98%), N,N-

dimethylformamide (DMF, 99.9%), dimethyl sulfoxide (DMSO), graphite powder, NaNO<sub>3</sub>, sulfuric acid, KMnO<sub>4</sub>, 3% H<sub>2</sub>O<sub>2</sub>, 5% HCl, acetic acid, ethanol, urea, ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>), dithiothreitol (DTT), iodoacetamide (IAA), trifluoroacetic acid (TFA), bovine serum albumin (BSA, pI 4.7, MW 66.4 kDa), ammonium carbonate, trypsin, acetonitrile (can, 95%), 0.1% phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), 2,3-dihydroxybenzoic acid (DHB), TA-30 solution, horseradish peroxidase (HRP, pI 7.2, MW 44 kDa) and human serum immunoglobulin G (human IgG) were purchased from Sigma-Aldrich.

### Fabrication of GO@UiO-66-PBA nanocomposite

Graphene oxide was prepared following a reported method [40]. Twenty grams of graphite powder and 10 g NaNO<sub>3</sub> were suspended in 460 mL concentrated sulfuric acid at 0 °C (using an ice bath to maintain temperature), and the mixture was stirred for 1 h, after which 60 g of KMnO<sub>4</sub> was gradually added with continuous stirring. The suspension was heated to 35 °C, and the temperature was maintained for 30 min. Next, 920 mL of deionized water was added, and the temperature was further raised to 98 °C. After 1 h, the suspension was diluted to 1.4 L, and 50 mL 3% H<sub>2</sub>O<sub>2</sub> aqueous solution was added. Finally, the mixture was filtered and washed with 5% HCl aqueous solution to remove metal ions, followed by pure water to remove acid. ZrCl<sub>4</sub> (93.22 mg) and 2-amino terephthalic acid (72.46 mg) were dispersed in acetic acid solution (60 µL) and DMF (30 mL) by ultrasonication for 20 min. The obtained GO-COOH nanoparticles (20 mg) were added and sonicated for another 20 min. The prepared solution was heated at 130 °C for 4 h with stirring. The final product was then washed in sequence with deionized water and ethanol, and the obtained nanocomposite was dried in a vacuum oven at 60 °C. The hydrophilicity was increased by using 4-carboxy phenylboronic acid (200 mg) dissolved in DMSO (15 mL) by ultrasonication. EDC (40 mg), NHS (80 mg) and DMSO (5 mL) were added to the solution and stirred at ambient temperature for 20 min. GO@UiO-66-NH<sub>2</sub> nanocomposite (50 mg) was added, and the reaction continued for 24 h at room temperature. The surface-enhanced nanocomposite was washed successively with water and ethanol followed by drying overnight in a vacuum oven at 120 °C.

### Trypsin digestion of model glycoproteins

Human IgG (UniProt, accession no. P01857) and HRP (UniProt, accession no. P00433) are used as standards to estimate the enrichment performance of GO@UiO-66-NH<sub>2</sub> nanocomposite. 2 mg of IgG (or HRP) was dissolved in 500 µL ammonium bicarbonate (50 mM, pH 8.3), and then the protein solution was denatured by boiling at 90 °C for 10 min. The

solution was reduced in 10 mM DTT for 4 h at 37 °C and alkylated by 20 mM IAA in the dark for 1 h. The solution was then treated with trypsin (trypsin: protein = 1:40, w/w) at 37 °C for 16 h. The tryptic digest was lyophilized and stored for further use.

### Digestion of BSA

In the selectivity study, digested BSA was used to prepare complex non-glycoprotein background. 1 mg of BSA was dissolved in 1 mL 50 mM ammonium carbonate (pH 8.3), reduced by 10 mM DTT for 30 min at 56 °C and alkylated with 20 mM iodoacetamide at 30 °C for 1 h. Trypsin was added to the mixture at an enzyme-to-protein ratio of 1:30 for overnight hydrolysis at 37 °C. It was stored at -20 °C for further use.

### Enrichment of N-linked glycopeptides

200 µg GO@UiO-66-PBA was equilibrated three times with 100 µL loading buffer (95% ACN, 1% TFA), after which 1 pmol/µL dried peptide digest was redissolved in the loading buffer and 5 µL was pipetted onto the MOF material. After centrifugation for 10 min at 6000g, the MOF was washed twice with 50 µL loading buffer and once with 50 µL wash buffer II (85% ACN, 0.1% H<sub>3</sub>PO<sub>4</sub>). Bound peptides were eluted with 15 µL eluting buffer (30% ACN, 0.1% TFA) and spotted on a matrix-assisted laser desorption/ionization (MALDI) plate for MS analysis.

### Regeneration of GO@UiO-66-PBA

MOF was regenerated by washing with TA-30 (30% ACN, 0.1% TFA) three times (250 µL each time) and once with water, and then dried. To start another batch of enrichment, MOF was equilibrated with loading buffer. The cycle of regeneration and reuse of MOF for enrichment was carried out three times.

### Trypsin digestion of human serum

In-solution digestion was performed for the serum sample. For the digestion, 1.0 µL of serum was diluted with 16 µL of 25 mM ammonium carbonate (pH = 7.9). The serum solution was centrifuged at 12,000 rpm for 2 min. The reduction was performed with 10 mM dithiothreitol (DTT) for 30 min at 37 °C, followed by alkylation using 20 mM of iodoacetamide at 37 °C for 60 min. Finally, trypsin was added to the mixture at an enzyme-to-protein ratio of 1:30 for digestion overnight at 37 °C. The digestion was stopped by 1% TFA and stored at -20 °C before the enrichment.

### De-glycosylation of serum-enriched N-linked glycopeptides

Eluted serum N-linked glycopeptides were diluted by 50 µL of 50 mM ammonium bicarbonate. Then 1000 U of PNGase-F was added and incubated at 37 °C for overnight to release N-glycans. De-glycosylated were directly subjected to nano-liquid chromatography-mass spectrometry (nano-LC-MS) analysis.

### MALDI-TOF/TOF MS analysis

One microliter of enriched intact N-linked glycopeptides was deposited on a 600-µm AnchorChip target (Bruker Daltonics, Bremen, Germany), followed by 1 µL matrix solution prepared by dissolving 20 mg/mL of DHB in TA-30 solution (30% ACN in 0.1% TFA). Peptide Calibration Standard II mono was spotted for external calibration. MALDI-TOF/TOF MS analysis was carried out on an ultrafleXtreme mass spectrometer (Bruker Daltonics, Bremen, Germany). Spectra were acquired in positive reflection mode with 1000 laser shots per spectrum. Resolution was kept at 15000–20000. Spectra in a mass range of 1000–5500 were processed using flexAnalysis version 3.4 software supplied by Bruker Daltonics.

### Nano-LC-MS analysis

The N-linked glycopeptide enrichment was desalted using a µ-C18 ZipTip and dissolved in 10 µL of HPLC buffer A (0.1% (v/v) formic acid in water). A 5-µL aliquot of the sample was injected into a nano-LC system (EASY-nLC 1000, Thermo Fisher Scientific, Waltham, MA, USA). Chromatography was performed using an EASY-Spray nano-LC source with a 15 cm × 50 µm inner diameter column packed with 2 µm C18 particles. The flow rate was 300 nL/min, and a 45-min linear gradient from 2 to 35% HPLC buffer B (0.1% formic acid in ACN) developed. The organic content was increased to 50% over 10 min.

## Results and discussion

### Fabrication and characterization of GO@UiO-66-PBA

The synthesis procedure of GO@UiO-66-PBA is shown in Fig. 1. UiO-66-NH<sub>2</sub> grows on GO-COOH. UiO-66-NH<sub>2</sub> is modified on carboxylate-terminated GO by hydrothermal method. Boronic acid is immobilized on GO@UiO-66-NH<sub>2</sub> by reacting the -NH<sub>2</sub> group with CPBA to obtain GO@UiO-66-PBA.

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), BET surface area analysis, powder X-ray diffraction (XRD) and Fourier transform infrared

spectroscopy (FTIR) measurements are performed to characterize GO@UiO-66-PBA. SEM images of UiO-66-NH<sub>2</sub> and GO@UiO-66-PBA are presented in Fig. 2a and b, respectively. The UiO-66-NH<sub>2</sub> particle size is around 150 nm. UiO-66-NH<sub>2</sub> possesses a regular hexagonal morphology, while a slight change is observed in GO@UiO-66-PBA because of the presence of GO in the MOF structure. It can be seen that the oxygen-containing functional groups in GO combine with Zr<sup>4+</sup>, hindering the UiO-66-NH<sub>2</sub> crystal growth. TEM results are complementary to SEM results. The graphene sheets are visible with UiO-66-NH<sub>2</sub> crystals present evenly covering the entire surface. The sheet structure of GO enhances the surface area and exposed surface hydrophilic groups which helps to avoid saturation during enrichment.

BET surface area of UiO-66-NH<sub>2</sub> and GO@UiO-66-PBA is calculated by nitrogen absorption porosimetry. Using GO as a template has no adverse effect on nanocomposite material. Appropriately adding GO expressively improves the surface area of GO@UiO-66-PBA. The new pores generated on the interface between the layers of GO and the MOF “blocks” sufficiently improves the enrichment efficiency of nanocomposite. The increase in the surface area of GO@UiO-66-PBA (137.22 m<sup>2</sup>/g) as compared to UiO-66-NH<sub>2</sub> (78.54 m<sup>2</sup>/g) can be observed easily in Fig. 3a. The pore volume of the fabricated nanocomposite material is higher than UiO-66-NH<sub>2</sub> which allows efficient binding and elution capability of N-glycopeptides.

XRD analysis verifies the crystal structure of the fabricated nanocomposite. GO/UiO-66-NH<sub>2</sub> composite has the same diffraction peak position and a slightly stronger peak intensity when compared with UiO-66-NH<sub>2</sub>. It demonstrates that the main

component of GO/UiO-66-NH<sub>2</sub> composites is UiO-66-NH<sub>2</sub>, and GO/UiO-66-NH<sub>2</sub> composite still maintained a good crystal structure. The main diffraction peak of GO does not appear in GO/UiO-66-NH<sub>2</sub> composite, which is stable in the case of UiO-66/GO. Interaction of GO with UiO-66-NH<sub>2</sub> is carried out in polar solvent (DMF) which causes high dispersion of GO, although overall content of GO in composite is only 5%. Figure 3b shows the XRD patterns of UiO-66-NH<sub>2</sub> and GO@UiO-66-PBA. The overall pattern of XRD peaks is the same in the two materials, but the intensities of GO@UiO-66-PBA are slightly higher, indicating good crystallinity. Furthermore, the slight difference in the peak pattern is because of the attachment of boronic acid to GO@UiO-66-NH<sub>2</sub>.

FTIR analysis confirms the formation of MOF and attachment of boronic acid (Fig. 3c). A sharp band of an amine group is present at 3427 cm<sup>-1</sup> in UiO-66-NH<sub>2</sub> which disappears in GO@UiO-66-PBA, and a broad peak appears at 3400 cm<sup>-1</sup>, indicating hydroxyl groups of boronic acid. CH stretching vibrations are present at 3071 cm<sup>-1</sup>. Amide bond formation of 4-carboxy phenylboronic acid with GO@UiO-66-NH<sub>2</sub> and carbonyl at 1613 cm<sup>-1</sup> and 1690 cm<sup>-1</sup>, respectively, confirm the formation of GO@UiO-66-PBA.

### Assessment of enrichment performance of GO@UiO-66-PBA nanocomposite

#### Enrichment of protein-IgG

Few N-linked glycopeptides with low intensity are detected in direct analysis of IgG digest because of suppression with non-

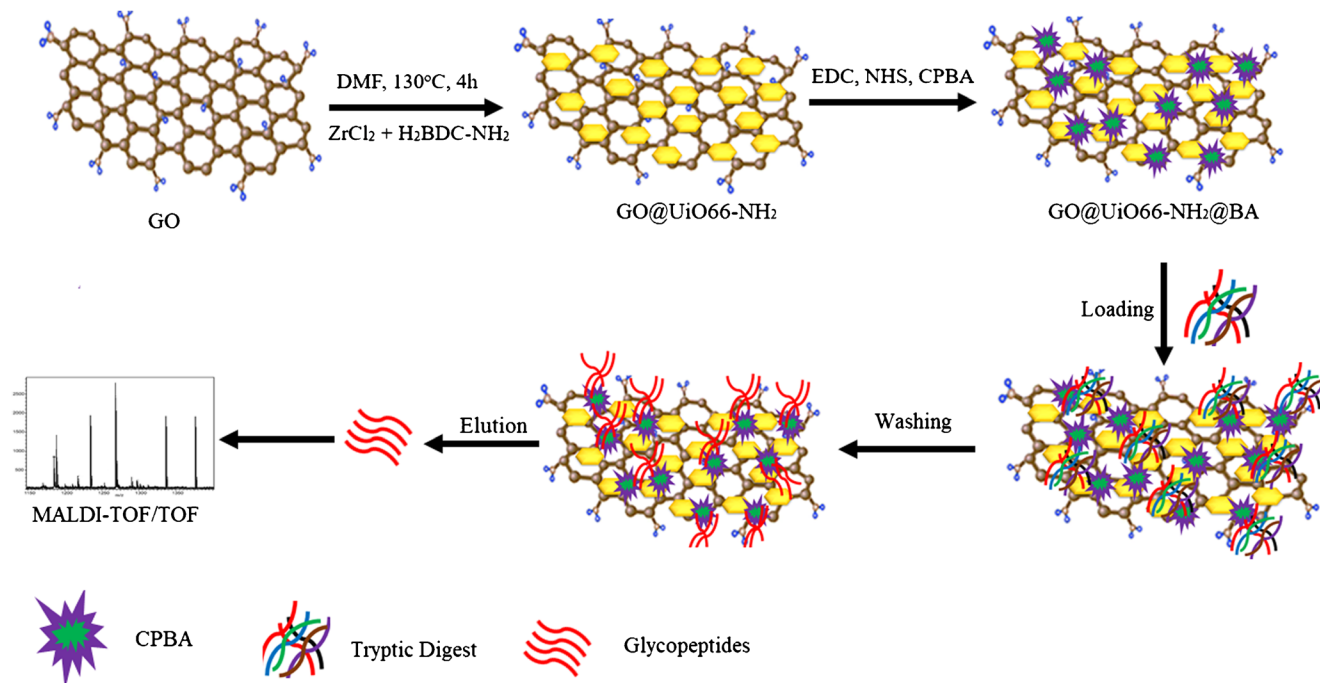


Fig. 1 Schematic representation of fabrication procedure for GO@UiO-66-PBA nanoparticles

glycopeptides. Hydrophilic enrichment by GO@UiO-66-PBA nanoparticles show a number of N-linked glycopeptides with high intensities in the MS spectrum. Nine N-linked glycopeptides without enrichment (Fig. 4a) and 19 N-linked glycopeptides with enrichment are detected (Fig. 4b). Modification with boronic acid on GO@UiO-66-NH<sub>2</sub> improves hydrophilicity. The detected N-linked glycopeptides from IgG digest by MALDI-TOF MS analysis are listed in Electronic Supplementary Material (ESM) Table S1. The glycosylation site has an amino acid sequence (EEQYN#STYR) at the 180 position. IgG (immunoglobulin heavy constant gamma 1, SwissProt accession number P01875) has a single glycosylation site at the amino acid position 180 sequence of EEQFN#STFR. The total sequence coverage of IgG is 2.727% with one reported glycosylation site detected successfully by GO@UiO-PBA nanoparticles, as shown in ESM Table S2.

#### Enrichment of N-linked glycopeptides from HRP digest

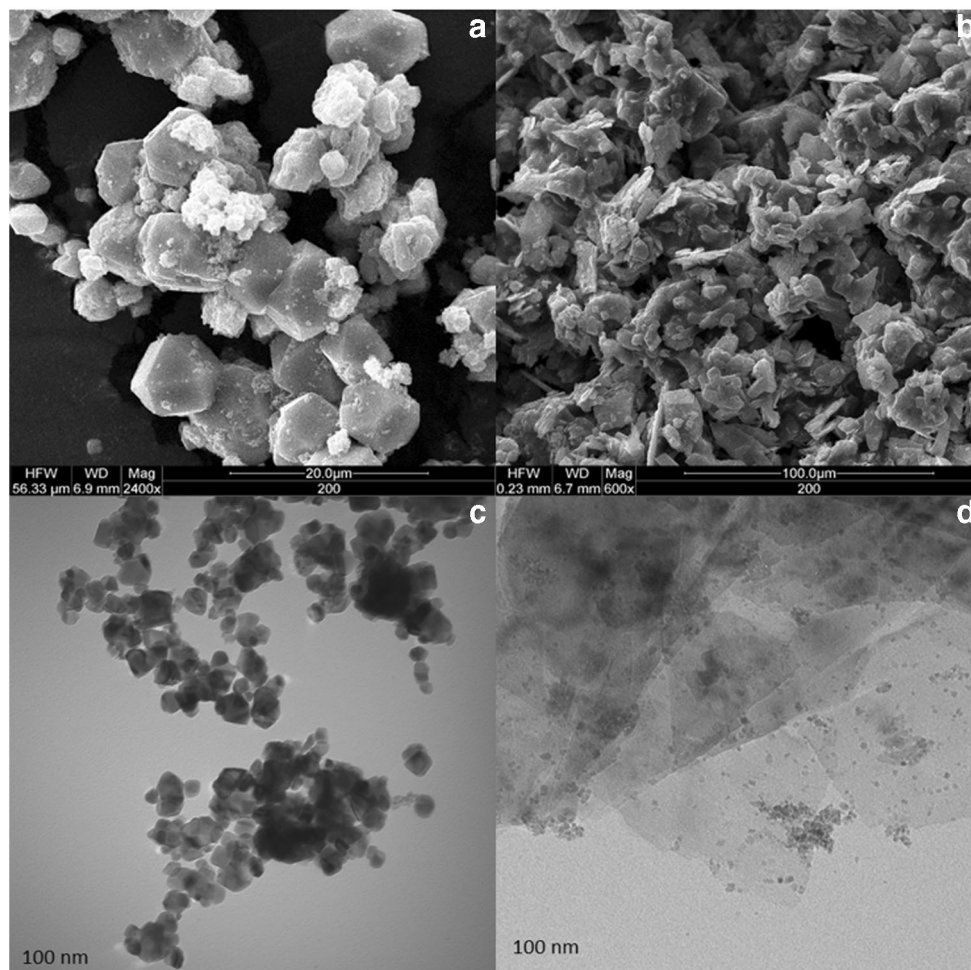
Glycopeptides are also enriched from HRP digest by GO@UiO-66-PBA. Five N-linked glycopeptides are

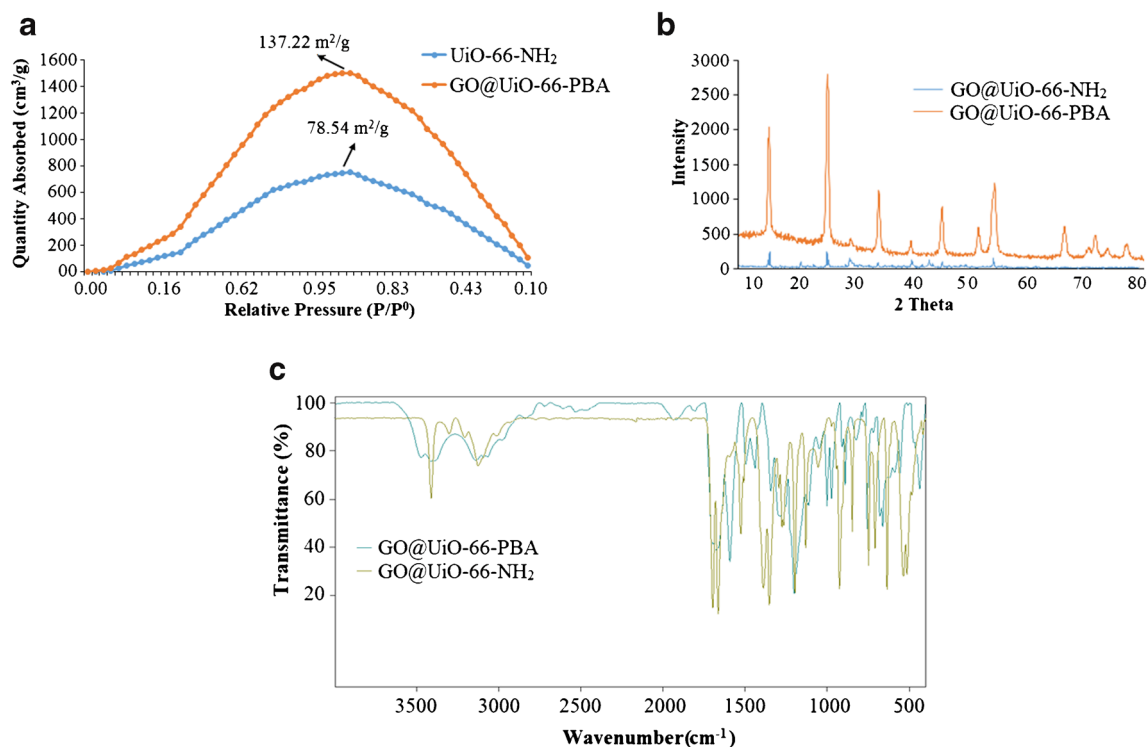
identified with a clear background (ESM Fig. S1). HRP contains eight glycosylation sites at 43, 87, 188, 216, 228, 244, 285 and 298. A total of 10 N-linked glycopeptides are enriched with eight reported glycosylation sites from the SwissProt database. Information of enriched N-linked glycopeptides from HRP digest is listed in ESM Table S3. The total sequence coverage of HRP is 21.714% with eight reported glycosylation sites detected successfully by GO@UiO-PBA nanoparticles, as shown in ESM Table S4.

#### Selectivity of GO@UiO-66-PBA

The selectivity of GO@UiO-66-PBA nanoparticles for N-linked glycopeptides is tested via IgG tryptic digest. Mixture of IgG digest and non-glycosylated BSA tryptic digest is taken as the sample. Volume ratios of 1:1, 1:50, 1:100, 1:150 and 1:200 (IgG: BSA) are applied for enrichment. Fifteen, 15, 11, 12 and 10 N-linked glycopeptides from volume ratios of 1:1, 1:50, 1:100, 1:150 and 1:200 (IgG tryptic digest and BSA), respectively, are detected after enrichment (Fig. 5). Good selectivity of GO@UiO-66-PBA for N-linked glycopeptides is observed even at a volume ratio of 1:200 (Fig. 5e). The

**Fig. 2** SEM images of **a** UiO-66-NH<sub>2</sub> and **b** GO@UiO-66-PBA. TEM images of **c** UiO-66-NH<sub>2</sub> and **d** GO@UiO-66-PBA





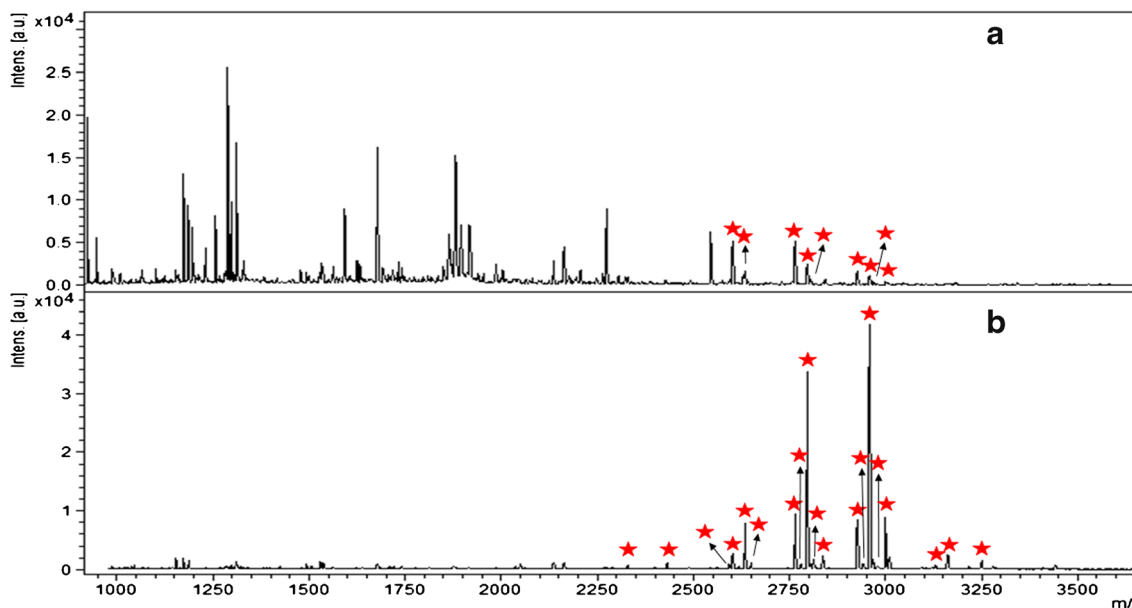
**Fig. 3** a BET surface area, b XRD and c FTIR of UiO-66-NH<sub>2</sub> and GO@UiO-66-PBA

numbers of identified N-linked glycopeptides from selectivity experiments are shown in ESM Fig. S2.

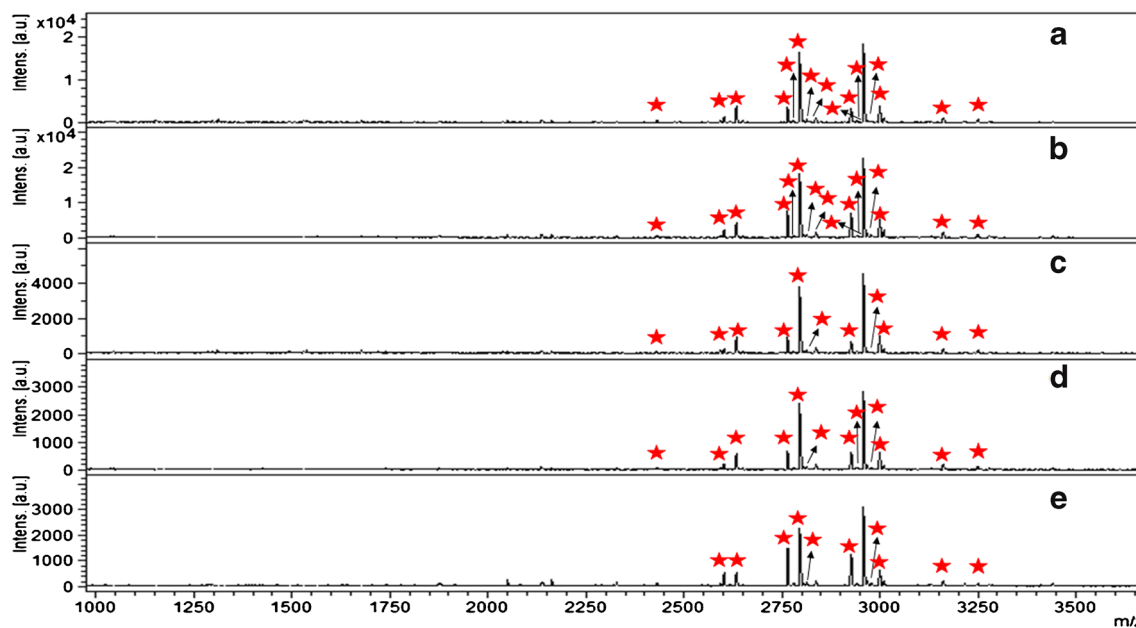
#### Sensitivity of GO@UiO-66-PBA

Human IgG tryptic digest is used to check the sensitivity for the capture of N-linked glycopeptides by GO@UiO-66-PBA.

The concentrations of IgG digest show a decreasing order of 10 pM/μL, 1 pM/μL, 100 fM/μL, 10 fM/μL and 1 fM/μL where 12, 14, 11, 10 and two N-linked glycopeptides are identified, respectively (Fig. 6). N-linked glycopeptides at m/z 2795 and 2956 are enriched even at a concentration of 1 fM/μL (Fig. 6e). This detection limit is better than previously reported methods for N-linked glycopeptide enrichment such



**Fig. 4** MALDI-TOF mass spectra of N-linked glycopeptides from IgG tryptic digest: a before enrichment, b after enrichment by GO@UiO-66-PBA. N-linked glycopeptides are marked as red stars



**Fig. 5** MALDI-TOF mass spectra for N-linked glycopeptide enrichment from a mixture of tryptic digests of IgG and BSA at volume ratios of **a** (1:1), **b** (1:50), **c** (1:100), **d** (1:150) and **e** (1:200). N-linked glycopeptides are marked as red stars

as boronic acid-functionalized  $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{PSV}$  (1.25  $\text{fmol}/\mu\text{L}$ ) [12] and magnetic carbon nanotubes (2.27  $\text{fmol}/\mu\text{L}$ ) [41].

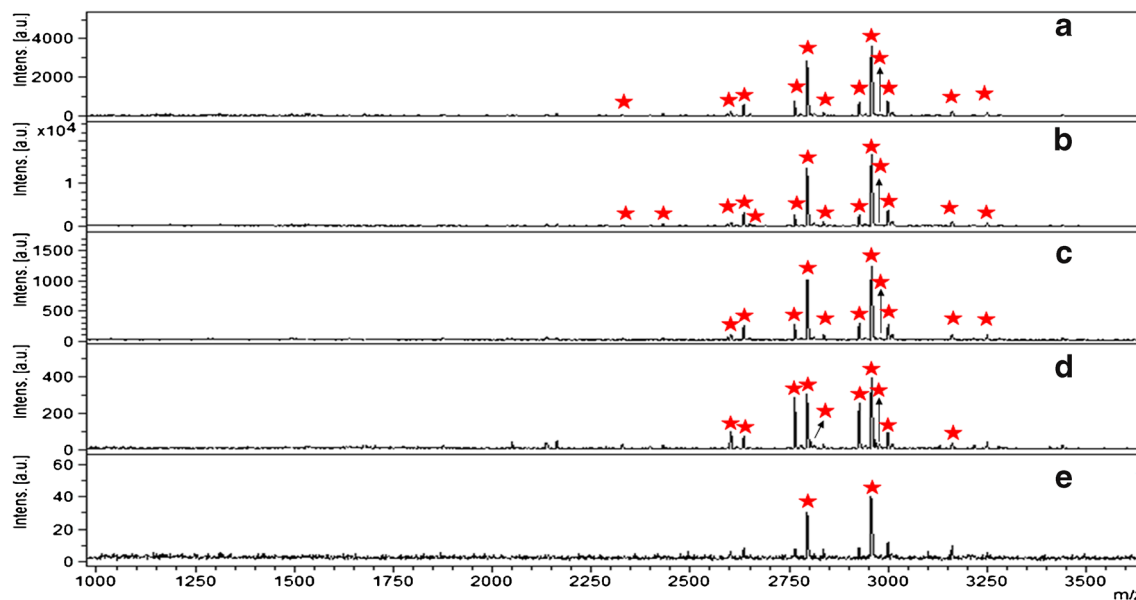
#### Reproducibility of GO@UiO-66-PBA

The reproducibility of enrichment by GO@UiO-66-PBA is determined using IgG digest (Fig. 7). 20, 19 and 18 N-linked glycopeptides are detected in the first, second and third runs, respectively. Reproducibility is also calculated through

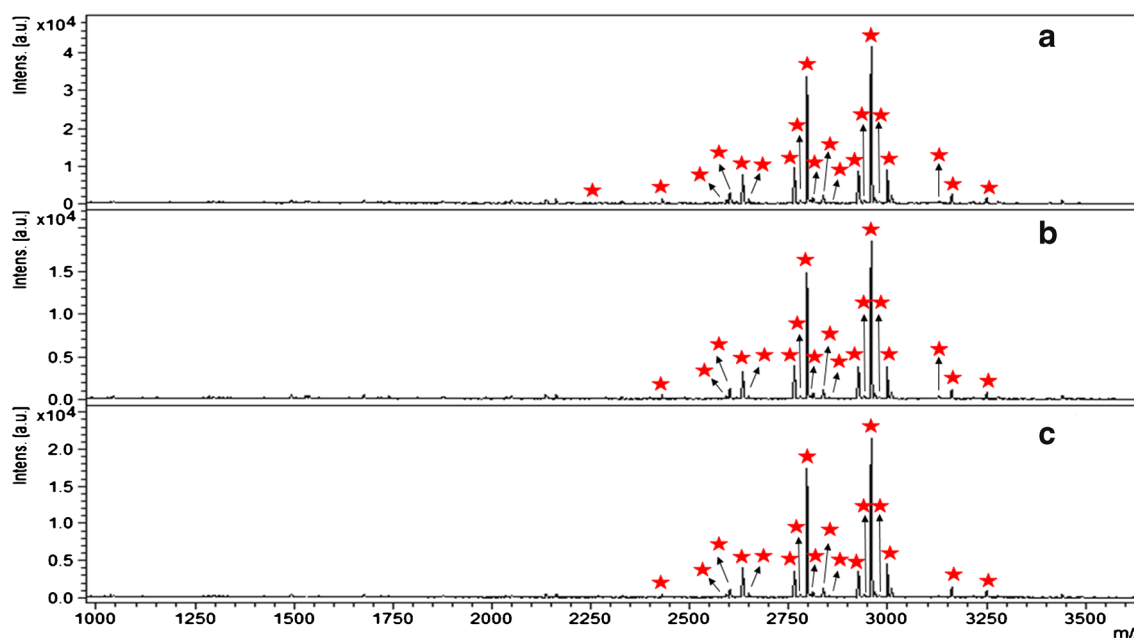
standard deviation (SD), which is presented in Table 1. The relative standard deviation (RSD) of all peptides is less than 1, which shows excellent enrichment reproducibility.

#### Reusability, binding capacity and recovery of GO@UiO-66-PBA

Reusability of the material is tested by IgG tryptic digest under similar conditions. MOF nanoparticles are regenerated with loading buffer to remove the residues for its multiple usage.



**Fig. 6** MALDI-TOF mass spectra for N-linked glycopeptide enrichment from IgG tryptic digest by GO@UiO-66-PBA nanoparticles: **a** 10  $\text{pM}/\mu\text{L}$ , **b** 1  $\text{pM}/\mu\text{L}$ , **c** 100  $\text{fM}/\mu\text{L}$ , **d** 10  $\text{fM}/\mu\text{L}$  and **e** 1  $\text{fM}/\mu\text{L}$ . Peaks of N-linked glycopeptides are marked as red stars



**Fig. 7** MALDI-TOF mass spectra for N-linked glycopeptides batch-to-batch reproducibility by GO@UiO-66-PBA in the **a** first, **b** second and **c** third runs

The enrichment process is repeated three times, and MS spectra show minor changes (ESM Fig. S3). A comparison of enrichment performance of GO@UiO-66-PBA for N-linked glycopeptides with previously reported materials in terms of selectivity, sensitivity and real sample analysis is given in Table 2.

The binding capacity of the MOF was determined through enrichment of 5  $\mu\text{g}$  of IgG digest by different amounts of GO@UiO-66-PBA (20–100  $\mu\text{g}$ ). The binding capacity of the MOF nanocomposite was calculated to be 84 mg/g, which may be attributed to the higher amount of surface hydroxyl groups resulting in enhanced hydrophilic interactions.

The recovery of glycopeptides enriched by the GO@UiO-66-PBA is investigated using HRP digest using the standard addition method. Using peak area, the recovery calculated for GO@UiO-66-PBA is 86.5%. This shows that minimal loss of glycopeptides is observed, and the enrichment protocol adopted provides suitable binding/elution conditions that are

compatible with the designed material. High recovery and binding capacity are significant factors contributing to the enhanced efficiency of the MOF nanocomposite.

#### N-linked glycopeptide enrichment from a real sample

A healthy human serum is applied as a real biological sample to demonstrate the performance and enrichment of GO@UiO-PBA followed by LC-MS analysis. Analyzing a complex serum sample containing a high abundance and low concentration of glycoproteins is a difficult task. A 1- $\mu\text{L}$  sample of digested human serum is used in analysis without any pretreatment. After enzymolysis and enrichment by the GO@UiO-PBA composite, the enriched N-linked glycopeptides are deglycosylated by PNGase F before analysis by LC-MS. A total of 33 different glycoproteins, and especially serotransferrin, transthyretin, trypsin-1, haptoglobin, alpha-2-macroglobulin and immunoglobulin gamma-1 heavy chain, are identified

**Table 1** Statistically represented batch-to-batch reproducibility data after enrichment. The peak abundance (mass-to-charge ratio) is used to calculate the standard deviation

Selected N-linked glycopeptides	[M + H] <sup>+</sup>	Peak abundance (m/z ratio)			SD value	RSD value
		Batch 1	Batch 2	Batch 3		
G-1	2430	16.2	12.8	12.6	2.023198787	0.1459 $\pm$ 13.867
G-2	2592	13.8	10.0	10.8	2.00333056	0.17368 $\pm$ 11.534
G-3	2649	16.7	13.6	12.9	2.022374842	0.14044 $\pm$ 14.4
G-4	2778	12.6	11.5	8.2	2.289832599	0.2126 $\pm$ 10.767
G-5	2811	23.0	20.6	17.1	2.967041175	0.1466 $\pm$ 20.234
G-6	2941	13.8	13.0	9.3	2.400694344	0.1945 $\pm$ 12.033
G-7	2966	21.0	18.5	12.3	4.47921124	0.2594 $\pm$ 17.267



**Table 2** Literature survey for functionalized materials used for enrichment of low-abundance glyco peptides/proteins

Sl. no.	Material composition	Selectivity	Sensitivity	Standard protein	No. of N-linked glycopeptides	Real sample	No. of N-linked glycopeptides in real sample	Reference
1	Fe <sub>3</sub> O <sub>4</sub> /ZIF-8/APBA	1:50	10 ng mL <sup>-1</sup>	HRP	6	N/A	N/A	[42]
2	magG@PDA@UiO-66-NH <sub>2</sub>	1:200	10 fmol	HRP	12	N/A	N/A	[43]
3	(MOF) MIL-101(Cr)-maltose		1 fmol	IgG, HRP	33 by IgG, 9 by HRP	Human serum	111 N-linked glycopeptides from 5 $\mu$ L	[44]
4	(MOF) MIL-101 (Cr)-NH <sub>2</sub>	1:10	20 fmol	IgG, HRP	18 by HRP	Human serum	116 N-linked glycopeptides from 10 $\mu$ L	[45]
5	mMOF@Au@GSH	1:100	0.5 fmol/ $\mu$ L	HRP	19 by IgG, 10 by HRP	Human serum	273 N-linked glycopeptides from 2 $\mu$ L	[46]
6	GO@UiO-66-PBA	1:200	1 fmol	IgG, HRP		Human serum	372 N-linked glycopeptides from 1 $\mu$ L	Present work

using a bottom-up approach, and 372 N-linked glycopeptides are identified (ESM Table S5). These glycoproteins have a significant role in different cancers, and their glycosylation has been reported as a potential highlight of cancer related to these glycoproteins (ESM Table S6). Thus, the GO@UiO-PBA nanoparticles presented significant efficiency in specific enrichment towards N-linked glycopeptides in a complex human serum sample. This would have huge potential in glycoprotein-related biomarker research associated with a variety of diseases.

## Conclusion

In summary, a new composite was prepared and functionalized with phenylboronic acid on hydrophilic MOFs via post-synthetic modification. The material showed good crystallinity and enhanced surface area (137.22 m<sup>2</sup>/g). After a series of characterizations, the material was applied for N-linked glycopeptide enrichment from IgG and HRP digests. Nineteen N-linked glycopeptides and five N-linked glycopeptides with a clear background were detected from IgG and HRP digest, respectively. The material showed excellent selectivity at a volume ratio of 1:200, sensitivity down to 1 fM/ $\mu$ L and reproducibility with RSD less than 1. With the combination of MOF and boronate affinity, the glyco content was enriched, with good characteristics, revealing that GO@UiO-66-PBA has the potential to capture glycoproteins from biological samples. By comparing the present work with previous work, it was demonstrated that the present material is superior in both selectivity and sensitivity. Using the MS analysis of serum, a number of abundant glycoproteins were identified, particularly serotransferrin, transthyretin, trypsin-1, haptoglobin, alpha-2-macroglobulin and immunoglobulin gamma-1 heavy chain. These glycoproteins are reported as potential biomarkers for different cancers. Therefore, the GO@UiO-66-PBA material showed improved performance in N-linked glycopeptide enrichment from complex biological samples such as human serum, thus exhibiting great potential for glycoproteome profiling.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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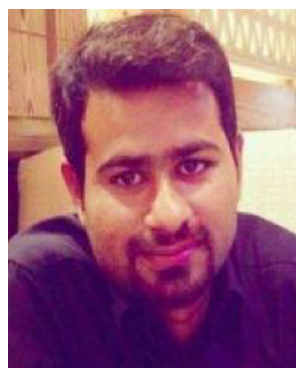
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