# Analyst

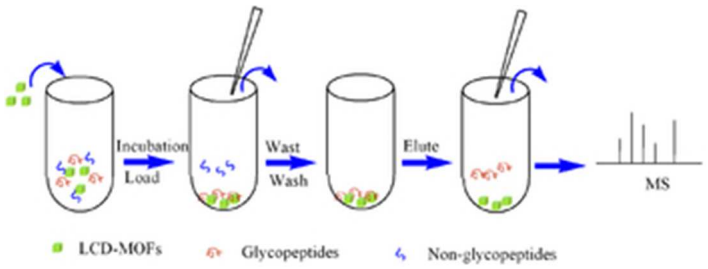
Accepted Manuscript






ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst




An efficient strategy of glycopeptides enrichment using metal-organic frameworks by hydrophilic interaction chromatography was demonstrated to analyze N-linked glycopeptides in mouse liver.
29x11mm (300 x 300 DPI)

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

# Efficient enrichment of glycopeptides using metal-organic frameworks by hydrophilic interaction chromatography

**Yongsheng Ji,[a,b] Zhichao Xiong,[b,c] Guang Huang,[a] Jing Liu,[a] Zhang Zhang,[a] Zheyi Liu,[a] Junjie Ou,[a] Mingliang Ye,[a] and Hanfa Zou[a]***

***Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX***
**DOI: 10.1039/b000000x**

Selective enrichment of glycopeptides from complicated biological samples is critical for glycoproteomics based on mass spectrometry (MS) technique to obtain the structure and glycosylation information of glycoproteins, which still remains a great challenge. Hydrophilic interaction chromatography (HILIC)-based strategies have been proposed for selective isolation of glycopeptides via the interactions between the glycan of glycopeptides and the matrices. However, the application of these methods is limited by the medium selectivity of HILIC matrices. In this study, a hydrophilic metal-organic frameworks (MOFs) was fabricated and used as HILIC-matrix. The desired MOFs named LCD-MOFs were facilely prepared with γ-cyclodextrin as ligand and possessed nano-sized cubic structure, superior hydrophilicity and bio-compatibility. The LCD-MOFs performance for the selective enrichment of glycopeptides from the complex biological samples was investigated with human immunoglobulin G (IgG) digested mixture as standard samples. In the selectivity assessment, the non-glycopeptides causing ion suppression to the glycopeptides were effectively removed, the signal of glycopeptides were enhanced significantly by LCD-MOFs, and twenty glycopeptides were identified with 67 fmol of IgG digest. In addition, the resulting LCD-MOFs presented the lower detection limit (3.3 fmol) and satisfied recovery yield (84-103 %) for the glycopeptides enrichment from digest of IgG. Furthermore, a promising protocol was developed for the selective enrichment of glycopeptides from mouse liver, and 344 unique N-glycosylation sites mapped to 290 different glycoproteins were identified in single MS run. The results clearly demonstrated that LCD-MOFs have great potential for enriching and identifying the low-abundant glycopeptides in complex biological samples by HILIC.

## Introduction

Glycosylation, one of the most important post-translational modifications (PTMs) of proteins, produces abundant and diverse glycoproteins which participate in many key biological processes such as cell adhesion, receptor activation, signal transduction and so on.[1] Aberrant glycosylation is a hallmark of cancer, and various biomarkers of diseases are glycoproteins.[2] Thus, developing new methodologies to discern glycoproteins will not only elucidate the physiological mechanism of living organisms but also accelerate biomarker discovery for disease diagnosis or prognosis. Currently, the primary strategy is to implement enzymatic digestion of the glycoprotein followed by glycopeptides sequencing through mass spectrometry (MS)for the comprehensive characterization of glycoproteins.[3] However, despite the advancements of MS technology, to direct MS analysis of glycoproteins is still a challenge due to the inherent low abundance, the low signal response, and the severe ion suppression caused by the co-existence of non-glycosylated peptides. Therefore, an efficient enrichment platform is indispensable for comprehensive analysis of glycoproteins by MS.

Several effective strategies including lectin affinity chromatography (LAC),[4, 5] hydrazide chemistry,[6, 7] boronic acid chemistry[8-10] and hydrophilic interaction chromatography (HILIC)[11-16] have been developed for the enrichment of glycoproteins and glycopeptides.[3] Among them, LAC based on the affinity interaction between lectin and specific glycan moieties is widely used, but the weak affinity and biased collection restricts its application for the global analysis of glycoproteins.[3] Besides, hydrazide chemistry shows high selectivity through the covalent bond formation. Unfortunately, the sample complexity is increased by the oxidation of carbohydrate cis–diol groups, and the structural information of glycan cannot be acquired due to the destruction and removal of the glycan segments.[13] The boronic acid chemistry can catch glycoproteins or glycopeptides with intact glycan structures through the reversible ester formation between boronic acid and cis–diol groups of glycan. However, the selectivity and sensitivity is unsatisfied in application of complex samples.[16]

Recently, the HILIC enrichment methods depending on the partition mechanisms between the organic solvent and the aqueous layer on HILIC station phase have gained increasing popularity for the separation and purification of glycopeptides .[3] The HILIC-based tactics present advantages of a simple operation

process, a broad glycan specificity, and a good compatibility with MS analysis. Variety of hydrophilic matrixes such as sepharose,[17] cellulose,[18] zwitterionic polymer[11, 16] and click saccharide,[12, 19] have been exploited for the selective extraction of glycopeptides. The carbohydrate-based sorbents displayed superior performances in view of the previous works. However, the preparation protocol often suffers from harsh conditions, multistep reactions, and a tedious operation. Hence, it is interesting to cultivate a facile platform for producing the carbohydrate-associated materials, so that the HILIC technique utilizing the designed matrixes can perform their superiority for the enrichment of glycopeptides.

Metal-organic frameworks (MOFs), a fascinating class of cystalline materials consisting of organic linkers with bridging organic ligands and metal ions, have been utilized in gas adsorption, catalysis, drug delivery, and separation with the advantages of the high surface area, uniformly structured cavities, and available modification.[20] Recently, MIL-101[21] and magnetic MOFs[22, 23] have been used as sorbents to enrich peptides from biological samples. However, there is still a challenge of MOFs for the enrichment of peptides or proteins from biological samples. To the best of our knowledge, the MOFs-related enrichment of glycopeptides has not been reported. Generally, the vast majority of MOFs mentioned previously are composed of organic struts and transition metals,[20] which limits their application for purifying glycoproteins and glycopeptides. Surprisingly, an amazing MOFs with γ-cyclodextrin (γ-CD) as ligand were easily manufactured under mild experimental condition.[24, 25] Cyclodextrins, a cyclic oligosaccharide from starch, are comprised of six to eight glucose units bonded through α-(1, 4) linkages.[26] Owing to the truncated cone structure with hydrophilic rims and a hydrophobic cavity, the CD-modified matrixes are extensively involved in separation science[27] and especially, used for enrichment of glycopeptides via HILIC.[28] It will be a promising alternative to combine CD-MOFs with HILIC for the selective enrichment glycopeptides.

Herein, a MOFs-based HILIC strategy was planned for the highly efficient and selective enrichment of glycopeptides from complicated biological samples. To illustrate the proof-of-concept, CD-MOFs were selected as adsorbents. MOFs utilizing γ-CD, an inexpensive and green feedstock as ligand were facilely prepared. The as-prepared composite giving cubic structure possessed superior hydrophilicity, bio-compatibility and ability for selectively enriching glycopeptides. The performance of CD-MOFs was investigated for the selective enrichment of glycopeptides from a standard digested mixture, and then the desired platform was applied for analysis N-linked glycopeptides in mouse liver. The results indicated that the developed approach possesses easily fabrication, high specificity and expected capability in the selective enrichment of glycopeptides from complicated biological samples.

## Materials and methods

### Materials

Ethylene glycol diglycidyl ether (EGDE) and γ-cyclodextrin (γ-CD) were obtained from from J&K Scientific Ltd. (Beijing, China). Methanol ($CH_3OH$), acetonitrile (CAN), trifluoroacetic acid (TFA) and formic acid (FA) were supplied by Merck (Darmstadt, Germany). Human serum immunoglobulin G (human IgG), human α1-acid glycoprotein (AGP), human serotransferrin (TRF), bovine fetuin (BF), chick ovablumin (COV), trypsin (TPCK treated), potassium hydroxide (KOH), dithiothreitol (DTT), iodoacetamid (IAA), 2,5-dihydroxyl benzoic acid (DHB), cetyltrimethylammonium bromide (CTAB), concentrated ammonia aqueous solution ($NH_3{\cdot}H_2O$, 28-30 wt%) and sodium bicarbonate ($NaHCO_3$) were purchased from sigma-Aldrich (St, Louis, MO, USA). PNGase F was from New England Biolabs (Ipswich, MA). Pure water (18.4 MΩ cm) used in all experiments was purified by a Milli-Q system (Millipore, Milford, MA, USA). All other chemicals were of analytical grade.

### Preparation of CD-MOFs

CD-MOFs were synthesized as the previous protocol with minor revision.[25] γ-Cyclodextrin (163 mg, 0.126 mmol) was dissolved in 5.0 mL of 200 mM KOH aqueous solution in a glass bottle. The bottle was put into a jar, and methanol was allowed to vapor-diffuse into this solution at room temperature for 36 h. Then the solution was transferred to a tube and 5.0 mL of methanol containing CTAB (40 mg, 0.110 mmol) was added. The homogenous solution was incubated at room temperature for 3 h. The solution was centrifuged at 1000 rpm for 3 min and the supernatant was decanted. The obtained crystals were washed with ethanol to remove excess regents, and then dried under vacuum at room temperature for 12 h.

40 mg of the dried CD-MOFs were added to 5 mL anhydrous ethanol containing EGDE (7.5 mmol), and the mixture was kept at 65 ℃ under nitrogen protection for 3 days. Then, the mixture were picked up and centrifuged at 1000 rpm for 3 min. The white products were collected and washed with ethanol and water respectively. The cross-linked CD-MOFs (LCD-MOFs) were dried under vacuum at room temperature for 12 h.

### Material characterization

Transmission electron microscopy (TEM) images were obtained by JEOL JEM-2000 EX transmission electron microscope (JEOL, Tokyo, Japan). Fourier-transformed infrared spectroscopy (FT-IR) characterization has been performed on Thermo Nicolet 380 spectrometer using KBr pellets (Nicolet, Wisconsin, USA). Water contact angles were measured with an OCA20 contact angle system (Dataphysics, Germany) at ambient temperature.

### Tryptic digests of proteins

An amount of 1 mg Human IgG was dissolved in 1 mL $NH_4HCO_3$ solution (50 mM, pH=8.3) and denatured by boiling for 15 min. After that, the samples were reduced with 20 mM DTT at 60 ℃ for 1 h and alkylated by 7.2 mg IAA at room temperature in the dark for 40 min. The solution was incubated with trypsin at an enzyme-protein ratio of 1:25 (w/w) at 37 ℃ for 16 h. The tryptic digests were stored at -20 ℃ until further use.

The extraction of proteins from mouse liver were following a literature procedure.[16] Briefly, the liver was cut into pieces and suspended in ice-cold homogenization buffer consisting of 8 M urea, 4% CHAPS (*w/v*), 65 mM DTT, 1 mM EDTA, 0.5 mM EGTA, a mixture of protease inhibitor (1 mM PMSF, 0.2 mM $Na_3VO_4$, 1 mM NaF) and 40 mM Tris-HCl at pH 7.4. Then, the samples were homogenized by a Potter-Elvejhem homogenizer,

sonicated at 100 W×30 s and centrifuged at 25,000 *g* for 1 h in turn. The total liver proteins in supernatant were recovered by precipitation and lyophilization. The obtained proteins were dissolved in denaturing buffer (8 M urea, 50 mM Tris-HCl at pH 8.3). The protein concentration was measured by Bradford assay. Thereafter, the proteins were digested with trypsin. Protein mixture (1 mg) were reduced by DTT (1 M, 20 μL) at 60 ℃ for 1 h and alkylated by IAA (7.2 mg) in dark at room temperature for 45 min. Thenceforth, the solution was diluted to 1 M urea with Tris-HCl solution (50 mM, pH= 8.3), and digested by trypsin at 37 ℃ for 16 h at an enzyme-protein ratio of 1:25 (*w/w*). The tryptic digests were desalted and lyophilized for the next use. The digestion of five-glycoproteins mixture (IgG, AGP, TRF, BF, COV) was similar to the operation of mouse liver.

**Enrichment of glycopeptides by LCD-MOFs**

An amount of 150 μg LCD-MOFs were washed with loading buffer (ACN/$H_2O$/TFA, 88:11.9:0.1, *v/v/v*) and dispersed in 200 μL of loading buffer containing 400 ng of IgG tryptic digests. After incubation for 30 min at room temperature, the suspension was centrifuged, and the supernatant was discarded by Eppendorf pipette, followed by rinsed three times with loading buffer (100 μL) to remove the non-glycopeptides. Then the captured glycopeptides were eluted by eluting buffer (20 μL, ACN/$H_2O$/TFA, 30: 69.9: 0.1, *v/v/v*) under gentle shaking at room temperature for 10 min. The collected peptides were analysis by MALDI-TOF MS.

For the glycopeptides enrichment from tryptic digests of mouse liver, 100 μg of the digests was dissolved in 200 μL loading buffer, incubated with 1.0 mg of LCD-MOFs for 30 min, and subsequently washed three times with 200 μL of loading buffer. Then, the trapped glycopeptides were eluted three times with 50 μL of eluting buffer under mild votexing for 10 min, and the elution was collected and lyophilized. The obtained glycopeptides were redissolved in 10 mM $NH_4HCO_3$, and 100 unites of PNGase F was added. After the mixture was incubated at 37℃ for overnight to remove the glycan moieties. The reaction was terminated by heating to 100℃ for 10 min, and then the mixture was lyophilized and redissolved prior to analysis by nano LC-MS/MS. The enrichment of glycopeptides from five-glycoproteins mixture was carried out as mentioned above.

**Recovery estimation of glycopeptides enrichment**

The recovery yield of glycopeptides enrichment can be achieved by stable isotope dimethyl labeling according to the previous report.[15] Two of the same amounts of human IgG digests were firstly labeled with light and heavy isotopes. The heavy-tagged digests were enriched with LCD-MOFs as above-mentioned procedure, and the resulting elution was spiked into the light-tagged digests. The combined mixture was re-enriched with LCD-MOFs, and the eluted fraction was directly analyzed by MALDI-TOF MS. The recovery was calculated by the peak intensity ration of heavy isotope-labeled glycopeptides to the light isotope-labeled glycopeptides.

**Mass spectrometry analysis**

All MALDI-TOF mass analysis were fulfilled on AB Sciex 5800 MALDI-TOF/TOF mass spectrometer (AB Sciex, CA) equipped with a pulsed Nd/YAG laser at 355 nm. 0.5 μL of sample solution was deposited on the MALDI plate and dried at room temperature, then 0.5 μL of DHB (25 mg $mL^{-1}$, ACN/$H_2O$/$H_3PO_4$ , 70: 29: 1, *v/v/v*) matrix solution was loaded for MS performance.

A nano-LC-MS/MS system consisting of an Accela 600 HPLC (Thermo, San Jose, CA), LTQ-Orbitrap Velos mass spectrometer (Thermo, San Jose, CA) and a homemade spray tip packed with $C_{18}$ AQ beads (3 μm, 120 Å, Daison, Osaka, Japan) was equipped for the analysis of peptides. Buffer A ($H_2O$/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 gradient elution was programmed from 5% to 35% of the buffer B in 120 min with the flow rate of 150 nL $min^{-1}$. The MS/MS spectra were operated in a data-dependent collision induced dissociation (CID) mode, the full MS was acquired from *m/z* 400 to 2000 with resolution 60000, the collision energy was 35.0 %, and the activation time was 10 ms. The 20 most intense ions were selected for MS/MS. The lyophilized sample of 100 μg was redissolved in 50 μL of $H_2O$/FA (99.9:0.1, v/v) for the nano LC-MS/MS analysis.

**Database search and data analysis**

All the LC-MS/MS raw data files were searched with MASCOT software (version 2.3) against the target database of five glycoprotein or IPI mouse database (v3.82). For peptide identification, a mass tolerance of 20 ppm was permitted for parent ions and 0.5 Da for fragment ions, with allowance for two missed cleavages in the trypsin digests. Cysteine carboxamidomethylation was set as a static modification of 57.0215 Da. The variable modifications of methionine oxidation and asparagine deamination were stated as 15.9949 and 0.9840 Da, respectively. Besides, the false positive rates (FDRs) were controlled to <1 % for identification of peptides, proteins, and glycosylation sites.

## Results and discussion

**Preparation and characterization of CD-MOFs**

The desired CD-MOFs were facilely prepared by incubation of γ-CD and 8.0 equiv. of KOH in water under methanol vapor. The cubic crystals were formed between maltosyls of CD and K ions. After crystal-growth for 36 h, the micro-crystals were removed, and methanol containing CTAB were added to the mother liquor for accelerating the second crystal-growth. Then, the cross-linking reaction between the hydroxy groups of each γ-CD in the CD-MOFs was accomplished by EGDE possessing two epoxy groups. The morphologies of the obtained LCD-MOFs were characterized by TEM, and the photographs are shown in Fig. 1 (a, b). It can be seen that the cubic particle size is about 200 to 300 nm, and the LCD-MOFs display evident cross-link compared to CD-MOFs. The hydrophilicity of prepared materials was tested by the water contact angles. As shown in Fig. 1 (c, d), the angle of LCD-MOFs increases distinctly compared to CD-MOFs, indicating that the cross-link process was successful. However, the hydrophilic ability of LCD-MOFs can afford to the application in HILIC. Besides, the products were also determined by FT-IR and the spectra are listed in Fig. 1 (e). The typical peak of 2925 and 1125 $cm^{-1}$ are ascribed to the C-H and C-O-C stretching vibration, respectively.[25] The strong adsorption band within 3200 to 3600 $cm^{-1}$ contributes to hydroxy groups of γ-CD. There are no obvious differences between the spectrum of CD-

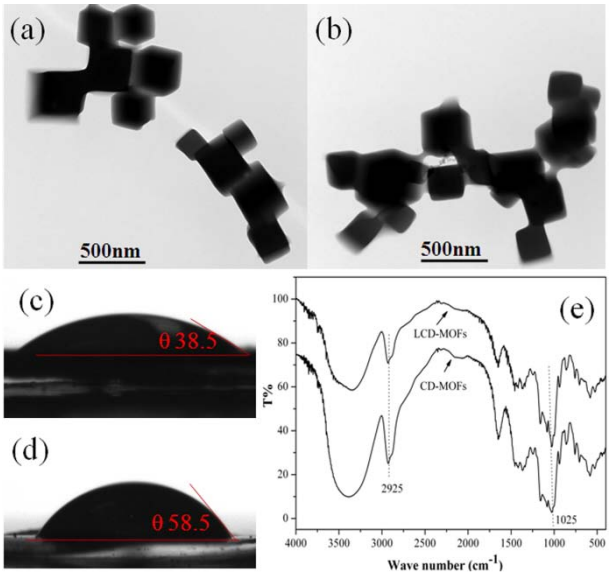
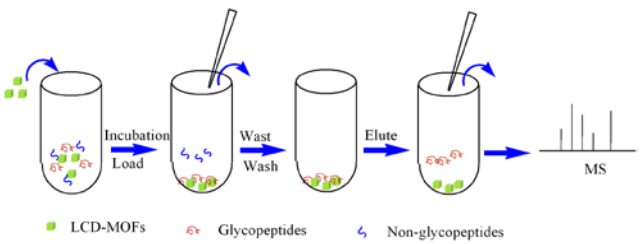



**Fig. 1** TEM image of (a) CD-MOFs, (b) LCD-MOFs, water contact angles of (c) CD-MOFs, (d) LCD-MOFs, and FT-IR spectra of (e).

MOFs and LCD-MOFs owing to the similar composition. The characterizations reveal that the designed products possess uniformly nano-sized structure and superior hydrophilicity.

### Glycopeptides enrichment from tryptic digest of human IgG

To evaluate the enrichment capacity of LCD-MOFs, tryptic human IgG digest was employed as the test standard sample. The protocol for glycopeptides enrichment is illustrated in Scheme 1. The tryptic digest was incubated with LCD-MOFs for capturing the glycopeptides, followed in turn the sorbents were collected and rinsed to remove non-glycopeptides, and then the captured glycopeptides on the LCD-MOFs were eluted for MALDI-TOF MS or nano LC-MS/MS analysis. Generally, it is assumed that the separation by HILC mode primarily relies on the partition mechanisms between the organic solvent and the aqueous layer on hydrophilic surface of solid matrix.[3, 29, 30] Typically, the hydration layer around polar matrices is formed while loading buffer with relatively low water content is used. More hydrophilic glycopeptides are concentrated in the water layer, and more hydrophobic non-glycopeptides remain in organic solvent, which is contributed to the multivalent intermolecular interactions such as hydrogen bonding, electrostatic and dipolar forces.[3, 30] The water content of loading buffer is the key factor for the selective enrichment of glycopeptides. Furthermore, the selectivity can be improved by adding ion-pairing reagent to the loading buffer which increases the hydrophobicity of non-glycopeptides.[31, 32] Therefore, the loading buffer including the ratio of ACN/water




**Scheme 1** Workflow of glycopeptides enrichment from a biological sample using LCD-MOFs.

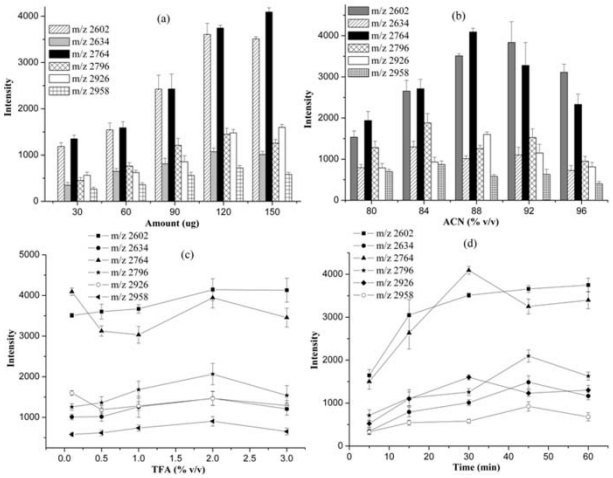



**Fig. 2** The effect of (a) amount of LCD-MOFs, (b) acetonitrile concentration, (c) the ratio of TFA in loading buffer and (d) incubation time influencing intensity on peaks of six chosen glycopeptides captured by LCD-MOFs from tryptic digest of human IgG (0.5 μL, 67 fmol).

and TFA percentage were investigated. As shown in Fig. 2, the little variation of the ratio (ACN/water) generated great influence on the enrichment of glycopeptides, but increasing of TFA volume fraction from 0.1 to 3.0 could not obviously intensify the enrichment efficiency. Besides, the incubation time and the amount of sorbents were optimized to gain higher enrichment efficiency, and the results are included in Fig. 2. According to the experimental results, 88% ACN containing 0.1% TFA (v/v) as the loading buffer, 150 μg of LCD-MOFs and incubation for 30 min were adopted for the next experiment.

The selectivity of LCD-MOFs for glycopeptides enrichment was also tested. Fig. 3 (a, b) shows MALDI mass spectra for direct analysis of 67 fmol human IgG digest and that with treatments by LCD-MOFs. It is clear that only two glycopeptides were barely detectable and non-glycopeptides peaks dominated the MS spectrum. However, after enrichment with LCD-MOFs, the non-glycopeptides were almost removed and the signals of glycopeptides were enhanced significantly, indicating that the unique structures and superior hydrophilicity of LCD-MOFs contributed to the performance of glycopeptides enrichment via multivalent hydrophilic interactions. Furthermore, the eluted fraction after enrichment was deglycosylated by PNGase F, and

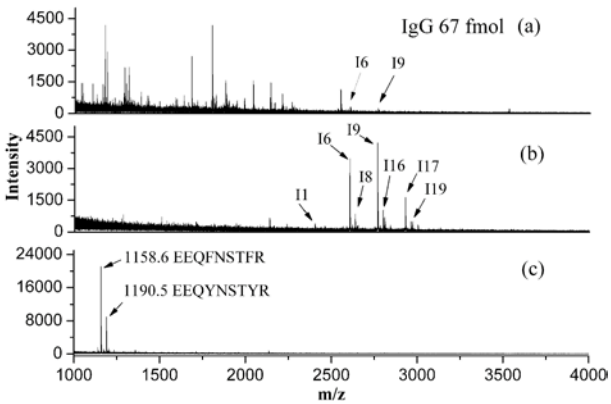



**Fig. 3** MALDI-TOF MS spectra of (a) direct analysis of 67 fmol tryptic digest of human IgG, (b) after enrichment by LCD-MOFs, and (c) deglycosylation by PNGse F.

the obtained product was further analyzed by MALDI-TOF MS (Fig. 3C). It can be seen that the MS peaks of presumed glycopeptides disappeared, which confirmed that the peaks in Fig. 3b can be attributed to glycopeptides. In addition, twenty glycopeptides from tryptic digest of human IgG were identified based on the MS data, and the detail sequences are listed in Table S1.

**Detection sensitivity of glycopeptides from tryptic human IgG digest**

The detection sensitivity of glycopeptides was measured for assessing the enrichment efficiency of LCD-MOFs. As shown in Fig. 4, tryptic human IgG digest with low amount of 13.4 pmol, 6.7 fmol and 3.3 fmol was tested by the treatment program mentioned above, respectively. Four glycopeptides with S/N $>3$ could still be detected while the amount of tryptic human IgG digest was as low as 3.3 fmol (Fig. 4c), which is lower than that enriched by zwitterionic HILIC materials (10 fmol)[16] and hydrophilic silica-based Click Maltose (30 fmol).[12] Such satisfactory results exhibited that LCD-MOFs can be used for glycopeptides enrichment with high sensitivity.

**Recovery yield test of LC-MOFs for glycopeptides enrichment**

Stable-isotope dimethyl labeling was used to study the recovery yield of LCD-MOFs for glycopeptides enrichment. The principle based on the previous protocol descript as the following. One of two equivalent digest labeled with light and heavy isotopes was enriched with LCD-MOFs and the resulting eluted fraction was combined with the other, followed in turn by enriching again and then analysis by MALDI-TOF MS. The recovery was calculated by the peak intensity ration of the heavy-tagged glycopeptides to light-tagged glycopeptides. Fig. 5 presents that the recovery yields of glycopeptides range from 84 % to 103 %, which confirmed that LCD-MOFs owned great potential for the enrichment of glycopeptides.

**Glycopeptides enrichment from five-glycoprotein mixture and mouse liver sample**

Furthermore, LCD-MOFs were employed to enrich glycopeptides from a semi-complex sample containing five standard glycoproteins. After enrichment and deglycosylation, the

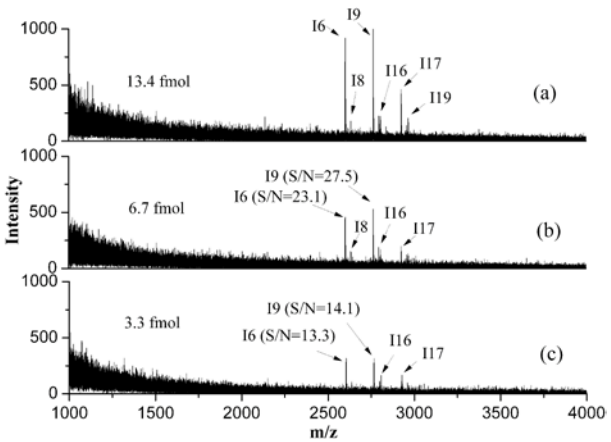



**Fig. 4** MALDI-TOF MS spectra of tryptic digest of human IgG analysis after enrichment by LCD-MOFs (a) 13.4 fmol (0.5 μL), (b) 6.7 fmol (0.5 μL) and (c) 3.3 fmol (0.5 μL).

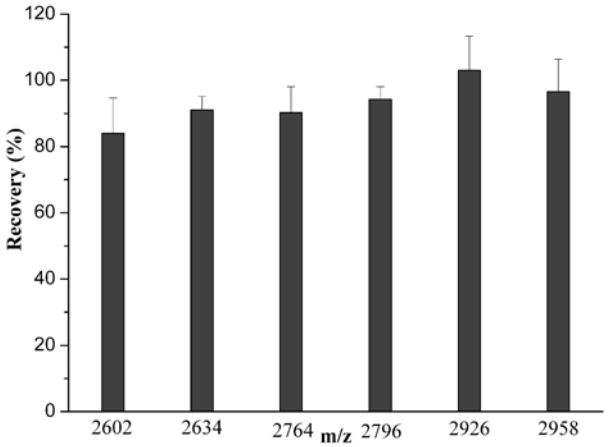



**Fig. 5** Recovery of six selected glycopeptides from human IgG digest by using LCD-MOFs.

resulting samples were analyzed by nano LC-MS/MS, and the results were listed in Table S2. It can be seen that 17 out of 23 unique N-glycosylation sites annotated in the UniProt Database were identified from 3 μg of the five-glycoprotein mixture. Five target glycoproteins were ascertained, and most of unique sites were covered. The above results revealed that LCD-MOFs contained remarkable ability for glycopeptides enrichment.

With the superior efficiency of enrichment glycopeptides in mind, LCD-MOFs were finally applied for the analysis of N-glycoproteome in complex mouse liver sample. The tryptic mouse liver digest of 100 μg were incubated with LCD-MOFs for enriching glycopeptides. The eluted glycopeptides were then deglycosylated by PNGase F and one third of the deglycosylated peptides were subjected to nano LC-MS/MS analysis. The MS data were processed as described above. In a single LC-MS/MS run, 344 unique N-glycosylation sites in 290 glycoproteins were identified, and the specified information was listed in Table S3. The identity of the N-glycosylated peptide was authenticated by the canonical discipline with a peptide sequence of NXS/T, in which X can be any amino acid except proline.[7] These results proved that LCD-MOFs showed the superior capability for the characterization of glycoproteome in complex biological samples.

## Conclusions

In summary, the uniformly cubic LCD-MOFs were facilely prepared. The resulting nano-sized LCD-MOFs composite possessed the desired hydrophilibity due to the hydrophilic γ-CD as ligand. It was confirmed that LCD-MOFs showed the superior selectivity, high detection sensitivity and satisfied recovery for enrichment of glycopeptides from glycoprotein digest with the help of the multivalent interactions between glycopeptides and LCD-MOFs. LCD-MOFs were further applied to analyze N-glycoproteome in complex mouse liver sample, and the results demonstrated that LCD-MOFs performed the great potential for the enrichment of low abundant glycopeptides and characterization of glycoproteome in real biological samples. This work was expected to open up a new promising strategy to design an efficient and sensitive HILIC MOFs for the analysis of

## Acknowledgements

This work is supported by the China State Key Basic Research Program Grant (2013CB911202, 2012CB910604), the Creative Research Group Project of NSFC (21321064), the National Natural Science Foundation of China (21275142， 21235006, 81161120540), National Key Special Program on Infection diseases (2012ZX10002009-011), Analytical Method Innovation Program of MOST (2012IM030900) to H.F. Zou. We also gratefully acknowledge the support of the Key Project of Henan Province Education Department Science and Technology Research (12A350002) and the Doctoral Research Fund of Henan Chinese Medicine (BSJJ-2010-23).

## Notes and references

[a] *Key Laboratory of Separation Sciences for Analytical Chemistry, National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 1160237, China. Fax: +86-411-84379620; Tel: +86-411-84379610*
*E-mail: hanfazou@dicp.ac.cn*
[b] *College of pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450000, China*
[c] *Shanghai Key Laboratory of Functional Materials Chemistry, Department of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China.*

† Electronic Supplementary Information (ESI) available: Table S1 Molecular masses and proposed oligosaccharide composition of the glycopeptides from human IgG after enrichment by LCD-MOFs. Table S2 N-Glycosylation sites identified from 3 g of the five-glycoprotein mixture with LCD-MOFs. Table S3 List of identified glycoproteins and unique N-glycosylation sites from mouse liver captured by LCD-MOF. See DOI: 10.1039/b000000x/

1. K. Ohtsubo and J. D. Marth, *Cell*, 2006, **126**, 855-867.
2. D. L. Meany and D. W. Chan, *Clin Proteomics*, 2011, **8**, 10.1186/1559-0275-8-7.
3. C.-C. Chen, W.-C. Su, B.-Y. Huang, Y.-J. Chen, H.-C. Tai, and R. P. Obena, *Analyst*, 2014, **139**, 688-704
4. Y.-W. Lu, C.-W. Chien, P.-C. Lin, L.-D. Huang, C.-Y. Chen, S.-W. Wu, C.-L. Han, K.-H. Khoo, C.-C. Lin and Y.-J. Chen, *Anal. Chem.*, 2013, **85**, 8268-8276.
5. C. Ma, X. Zhao, H. Han, W. Tong, Q. Zhang, P. Qin, C. Chang, B. Peng, W. Ying and X. Qian, *Electrophoresis*, 2013, **34**, 2440-2450.
6. H. Malerod, R. L. Graham, M. J. Sweredoski and S. Hess, *J. Proteome Res.*, 2012, **12**, 248-259.
7. J. Chen, P. Shah and H. Zhang, *Anal. Chem.*, 2013, **85**, 10670-10674.
8. L. Liu, Y. Zhang, L. Zhang, G. Yan, J. Yao, P. Yang and H. Lu, *Anal. Chim. Acta*, 2012, **753**, 64-72.
9. H. Wang, Z. Bie, C. Lü and Z. Liu, *Chem. Sci.*, 2013, **4**, 4298-4303.
10. H. Ju, R. Ma, J. Hu and Z. Cai, *Nanoscale*, 2013, **6**, 3150-3156.
11. L. Cao, L. Yu, Z. Guo, X. Li, X. Xue and X. Liang, *J. Chromatogr. A*, 2013, **1299**, 18-24.
12. J. Zhu, F. Wang, R. Chen, K. Cheng, B. Xu, Z. Guo, X. Liang, M. Ye and H. Zou, *Anal. Chem.*, 2012, **84**, 5146-5153.
13. W.-F. Ma, L.-L. Li, Y. Zhang, Q. An, L.-J. You, J.-M. Li, Y.-T. Zhang, S. Xu, M. Yu and J. Guo, *J. Mater. Chem.*, 2012, **22**, 23981-23988.
14. Z. Xiong, L. Zhao, F. Wang, J. Zhu, H. Qin, R. a. Wu, W. Zhang and H. Zou, *Chem. Commun.*, 2012, **48**, 8138-8140.
15. Z. Xiong, H. Qin, H. Wan, G. Huang, Z. Zhang, J. Dong, L. Zhang, W. Zhang and H. Zou, *Chem. Commun.*, 2013, **49**, 9284-9286.
16. G. Huang, Z. Xiong, H. Qin, J. Zhu, Z. Sun, Y. Zhang, X. Peng and H. Zou, *Anal. Chim. Acta*, 2014, **809**, 61-68.
17. Y. Wada, M. Tajiri and S. Yoshida, *Anal. Chem.*, 2004, **76**, 6560-6565.
18. Y. Shimizu, M. Nakata, Y. Kuroda, F. Tsutsumi, N. Kojima and T. Mizuochi, *Carbohydr. Res.*, 2001, **332**, 381-388.
19. J. Li, X. Li, Z. Guo, L. Yu, L. Zou and X. Liang, *Analyst*, 2011, **136**, 4075-4082.
20. C. M. Doherty, D. Buso, A. J. Hill, S. Furukawa, S. Kitagawa and P. Falcaro, *Acc. Chem. Res.*, 2014, **47**, 396-405.
21. Z.-Y. Gu, Y.-J. Chen, J.-Q. Jiang and X.-P. Yan, *Chem. Commun.*, 2011, **47**, 4787-4789.
22. M. Zhao, C. Deng, X. Zhang and P. Yang, *Proteomics*, 2013, **13**, 3387-3392.
23. Z. Xiong, Y. Ji, C. Fang, Q. Zhang, L. Zhang, M. Ye, W. Zhang and H. Zou, *Chem. Eur. J.*, 2014, **20**, 7389-7395.
24. R. A. Smaldone, R. S. Forgan, H. Furukawa, J. J. Gassensmith, A. M. Slawin, O. M. Yaghi and J. F. Stoddart, *Angew. Chem.*, 2010, **49**, 8630-8634.
25. Y. Furukawa, T. Ishiwata, K. Sugikawa, K. Kokado and K. Sada, *Angew. Chem.*, 2012, **51**, 10566-10569.
26. G. Chen and M. Jiang, *Chem. Soc. Rev.*, 2011, **40**, 2254-2266.
27. Y. Ji, X. Liu, M. Guan, C. Zhao, H. Huang, H. Zhang and C. Wang, *J. Sep. Sci.*, 2009, **32**, 2139-2145.
28. Y. Zhao, L. Yu, Z. Guo, X. Li and X. Liang, *Anal. Bioanal. Chem.*, 2011, **399**, 3359-3365.
29. P. Hemström and K. Irgum, *J. Sep. Sci.*, 2006, **29**, 1784-1821.
30. B. Buszewski and S. Noga, *Anal. Bioanal. Chem.*, 2012, **402**, 231-247.
31. L. Yu, X. Li, Z. Guo, X. Zhang and X. Liang, *Chem. Eur. J.*, 2009, **15**, 12618-12626.
32. W. Ding, J. J. Hill and J. Kelly, *Anal. Chem.*, 2007, **79**, 8891-8899.

