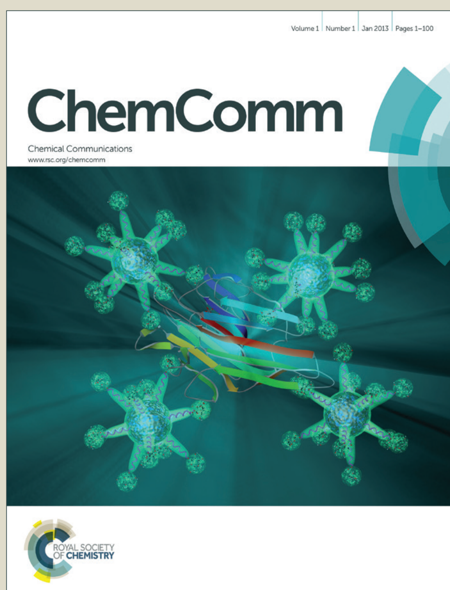
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm RSCPublishing

COMMUNICATION

Cite this: DOI: 10.1039/x0xx00000x

A Facilely Synthesized Amino-functionalized Metal-organic Framework for Highly Specific and Efficient Enrichment of Glycopeptides

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/chemcomm

Yi-Wei Zhang, Ze Li, Qiang Zhao, Ying-Lin Zhou, Hu-Wei Liu and Xin-Xiang Zhang*

A facilely synthesized amino-functionalized metal-organic framework (MOF) MIL-101(Cr)-NH_2 was first applied for highly specific glycopeptides enrichment based on the hydrophilic interactions. With the special characteristics of the MOF, the material performed well in selectivity and sensitivity for both standard glycoprotein samples and complex biological samples.

As one of the most predominant form, protein glycosylation is essential in protein folding,[1] cell recognition,[2] immune response,[3] and pathogenesis of many diseases,[4] which make the research of glycobiology a profound and intriguing field.[5] Although about 50% of all proteins are glycosylated,[6] the analysis on glycosylation are extremely complex because of the low abundance of glycoproteins, the heterogeneity of glycosylation sites and complicated glycan structures. Therefore, enrichment procedure is particularly important. The commonly adopted approaches for glycoconjugates enrichment include hydrophilic interaction liquid chromatography (HILIC),[7-9] lectin affinity chromatography,[10] hydrazide chemistry,[11] and boronic acid-based specific enrichment.[12, 13] Among them, the strategies based on HILIC are getting more and more attractive due to the unbiased enrichment towards different glycopeptides, remarkable reproducibility and good compatibility with mass spectrometry (MS). In a typical hydrophilic interaction retention process, analytes with different hydrophilicity could be separated by gradient elution according to the basic mechanism of HILIC, which provided the possibility of glycopeptides enrichment from high-abundance non-glycopeptides.

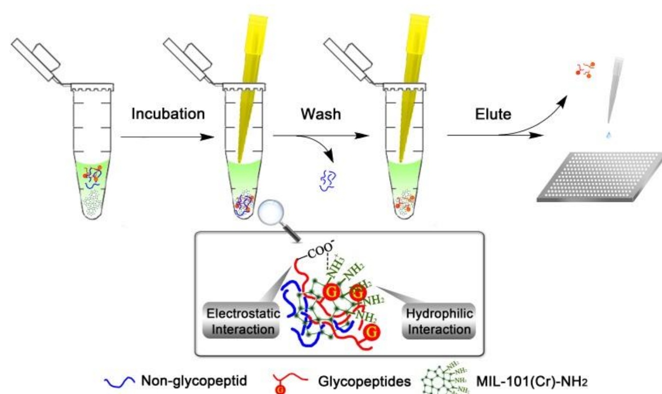
The materials in HILIC are a key point since the versatile stationary phase could improve binding strength and enrichment efficiency. Several researches had been carried out on bare silica,[14] derivatized silica,[15] underivatized mesoporous silica,[16] sepharose,[17] and magnetic nanoparticles.[7, 8, 18, 19] Among the diverse modifications, amide modified stationary phase is the most frequently used type for glycans and glycopeptides separation.[20]

Metal-organic frameworks (MOFs) are a new class of porous solid materials with some special properties, such as high surface area and permanent porosity.[21] The unique characteristics and possibilities of post-synthetic modification both in-pore and outer-surface make the applications of MOFs vary from gas absorption[22], sample separation[23-25], to catalysis[26] among the fields of chemistry and chemical engineering. *Gu et al.* reported the first adsorption and separation of large biomolecules which managed MOFs-based enrichment of peptides with simultaneous exclusion of proteins from complex biological samples utilizing the molecular sieving effect of MOFs.[27] *Messner et al.* later used a kind of Er(III) linked MOF as affinity material for the selective capturing of phosphopeptides.[28] It would be very significant to develop a novel application of MOFs for the glycopeptides enrichment by the combination of the trapping function of MOFs with selectivity towards glycopeptides.

Herein we firstly introduce a convenient and highly-sensitive strategy based on hydrophilic interaction for glycopeptides enrichment with amino-functionalized MOF MIL-101(Cr)-NH_2. Compared with most other MOFs, MIL-101(Cr)-NH_2 has moisture and acid resistant stability and thermal stability under more than 200 ℃[29, 30]. The material had been facilely synthesized according to the previous literature[30] with a little modifications (ESI†). The synthesis process only took 24 h hydrothermal reaction under 130 ℃ from a suitable mixture molar ratio of low-cost reagents. MIL-101(Cr)-NH_2 has several superiorities for the enrichment of glycopeptides, especially most N-linked types. The extraordinarily high surface area (BET surface area of 2187.4 m^2/g) and highly ordered micropores structures (Fig. S5, ESI†) of the MOF provided nice support for peptides adsorption. The existences of glycan moiety of glycopeptides would cause stronger interactions than that of non-glycopeptides. Considering the extra electrostatic interaction between the negatively charged carboxyl group on the sialic acid and the amino group on the functionalized MOF, those relatively larger and polar N-linked types and sialylated glycopeptides might be captured more tightly by the material.[31] Meanwhile, the hydroxyl groups on the glycans and amino groups on the material could form complex network of hydrogen bonding among each other, thus enhanced the hydrophilic binding and improved the specificity and sensitivity to glycopeptides. In addition, the good stability against water and acid extended the material's application in bio-samples. As a consequence, the enrichment method would combine the advantages of easy, fast and universal to different glycopeptides together, thus open up a new field associated to glycoproteomics for the applications of MOFs materials.

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 2012



Scheme 1 Workflow of glycopeptides enrichment using MIL-101(Cr)-NH_2.

Standard glycoproteins including secreted form mouse IgG (anti-α-fetoprotein monoclonal) and horseradish peroxidase (HRP) were chosen to evaluate MIL-101(Cr)-NH_2 selective enrichment performance towards glycopeptides. Scheme 1 elucidated the whole enrichment procedure. By appropriate binding, washing and elution, glycopeptides could be efficiently enriched regardless the non-glycopeptides trapping effect belonging to the material itself. 90% acetonitrile (ACN) containing 0.1% formic acid (FA) was chosen as the loading buffer according to the basic principle of HILIC interaction. To efficiently remove most weakly bound non-glycopeptides, bovine serum albumin (BSA) tryptic digests that all consist of non-glycopeptides were chosen to optimize the washing condition. Table 1 shows the peptides matching ratio under different washing conditions. The results illustrated that after enrichment and two times washing by 80% ACN containing 1% phosphoric acid (H_3PO_4), nearly 50% sequence coverage could be achieved. Furthermore, 30% ACN (0.1% FA) was finally used to wash the peptides after two times washing by 80% ACN (1% H_3PO_4), no coverage was obtained. The phenomenon indicated that the previous two times washing had removed most BSA peptides successfully. Therefore 80% ACN containing 1% H_3PO_4 was applied for the washing step during the glycopeptides enrichment since the procedures could get out most trapped non-glycopeptides on the MOF. After optimized the final acetonitrile concentration (Fig. S6, ESI†), incubation time (Fig. S7, ESI†) and MOFs-to-protein ratio (Fig. S8, ESI†) influences on peak intensity, 30% ACN (0.1% FA), 5 minutes and 10 μg/pmol were adopted in the experiment, respectively.

Fig 1 compared the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) spectra of mouse IgG tryptic digests before and after enrichment by MIL-101(Cr)-NH_2. Fig. 1A was the direct analysis of 2.0 pmol samples without any obvious signal of glycopeptides even peptides at all. After enrichment, 22 typical N-linked glycopeptides including eight

Table 1 Effects of washing solvents on non-glycopeptides

Washing Conditions	Peptides matched[b]	Protein Sequence coverage[b]
80% ACN (1%H_3PO_4)-first time[a]	30	49%
80% ACN (1%H_3PO_4)-second time	23	44%
30% ACN (0.1%FA)	0	-

[a] Sample: 0.6 pmol/μL BSA digestions, [b] Search parameters: database, Swiss-Prot; digest used, trypsin; maximum number of missed cleavages, 2; mass tolerance, 1.0 Da; Fixed modifications, Carbamidomethyl (C); Variable modifications, Oxidation (M). Mascot from Matrix Science Ltd. (London, U.K.) was used to search all of the mass spectra.

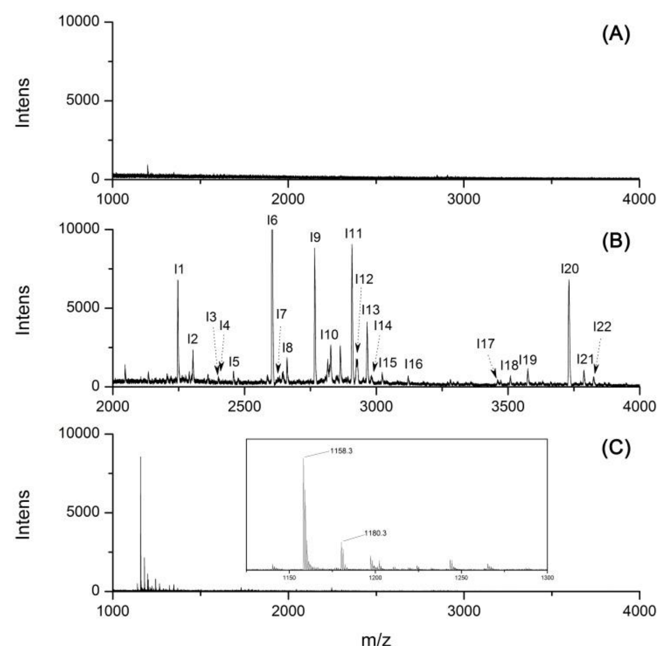


Fig. 1 MALDI-TOF-MS spectra of mouse IgG tryptic digest (2.0 pmol/μL) (A) by direct analysis, (B) after MIL-101(Cr)-NH_2 enrichment and (C) after MIL-101(Cr)-NH_2 enrichment then deglycosylated by PNGase F.

sialylated glycopeptides (Table S1, ESI†) were observed with strong intensities (Fig. 1B). Peptide-N-glycosidase (PNGase F) was then used to remove all the N-glycans after enrichment in situ, resulting in only one peak (Fig. 1C). Since there is only one glycosylation site (Asn[174] EEQF*N*#STFR) in secreted form mouse IgG, the results further confirmed the superior hydrophilicity of the simply synthesized MOF material and the highly selective enrichment performance towards glycopeptides. Similarly, 16 N-linked glycopeptides from 1.0 pmol HRP tryptic digests were successfully enriched and detected by the material (Fig. 2, Table S2, ESI†). Compared with lectin affinity chromatography, which are expensive and have specific affinity towards limited glycan structures, this MOF enrichment method could identify plentiful glycopeptides varied in glycan forms. In order to evaluate the discrimination performance between non-glycopeptides and glycopeptides, the mixture of HRP and BSA tryptic digests at a molar ratio of 1:10 were enriched and gradiently washed. As shown in Fig. S9 (ESI†), most detected glycopeptides in the pure HRP peptides sample were also appeared this time with declined peak intensity. It was due to the competitive effect of dominating non-glycopeptides, which occupied the binding sites and lowered the binding strength between glycopeptides and MOFs in some extent. The results suggested that this material had great potential to be a rapid and highly specific material for glycopeptides enrichment with suitable elution procedure.

The sensitivity was evaluated using mouse IgG digests. 9, 5, and 4 glycopeptides could be detected when the sample concentration reduced from 200 fmol to 100 fmol, and finally as low as 20 fmol, respectively (Fig. S10, ESI†). The results had similar sensitivities in femtomole level, even lower than that of most reported HILIC-based adsorbents[32] and boronic acid-based enrichment[33]. Under this case, the sensitivity was restricted to the detection limit in the reflect mode of MALDI-TOF-MS itself. Therefore, MIL-101(Cr)-NH_2 revealed great potential for trace amount glycopeptides sample enrichment thanks to the great adsorption ability and strong hydrophilicity towards polarity samples.

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 2012

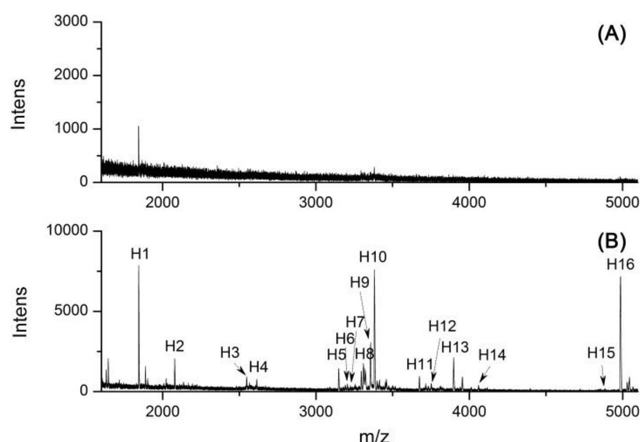


Fig. 2 MALDI-TOF-MS spectra of HRP tryptic digest (1.0 pmol/μL) (A) by direct analysis and (B) after MIL-101(Cr)-NH_2 enrichemnt.

Human serum sample was then applied as a real biological sample to demonstrate the material performance and enrichment method. Since the existence of high abundance proteins and the inherent low concentration of glycoproteins in serum always perplex the analysis, high specificity and sensitivity based enrichment methods are crucial in diagnostic and therapeutic biomarkers discovery, which always to be concerned with N-glycans in serum glycoproteins. Herein, a 10 μL human serum sample was used in analysis without any pretreatment. After enzymolysis and enrichment by the powerful MOF, the enriched glycopeptides were further deglycosylated by PNGase F before analyzed by LC-MS/MS. A total of 42 different glycoproteins and 116 glycopeptides were finally identified (Table S3, ESI†). Thus we could demonstrate that the amino-functionalized MOF MIL-101(Cr)-NH_2 exhibited remarkable capability in specific enrichment towards N-linked glycopeptides in complex matrix. The analysis was fast, convenient and feasible with excellent selectivity and sensitivity at the same time. The HILIC-based strategy would have huge potential in glycoprotein related biomarkers research associated with variety of diseases.

Conclusions

In summary, an extremely easily synthesized metal-organic framework MIL-101(Cr)-NH_2 was first applied in glycopeptides enrichment research. Due to the special characteristics such as large surface area, porous structures and strong hydrophilicity, the material had miraculous ability in trapping peptides and highly specific enrichment function to N-linked glycopeptides under optimized eluting conditions. The wonderful performance in both selectivity and sensitivity made enrichment of glycopeptides from limited volume of complex biological sample without any pretreatment, like a 10 μL human serum sample, successfully accomplished despite the dominating high abundance proteins' suppression effect, revealing the potential applications of MOFs in glycoproteomics and glycomics research.

This work was supported by the National Natural Science Foundation of China (No. 21275009).

Notes and references

Beijing National Laboratory for Molecular Sciences (BNLMS), MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry, Peking University, Beijing 100871, China.
E-mail: zxx@pku.edu.cn; Fax: +86-10-62754112; Tel: +86-10-62754112

† Electronic Supplementary Information (ESI) available: Experimental details, figures and tables. See DOI: 10.1039/c000000x/

1. S. J. Wijeyesakere, S. M. Rizvi and M. Raghavan, *J. Biol. Chem.*, 2013, **288**, 35104-35116.
2. R. Kleene and M. Schachner, *Nat. Rev. Neurosci.*, 2004, **5**, 195-208.
3. C. C. Wang, J. R. Chen, Y. C. Tseng, C. H. Hsu, Y. F. Hung, S. W. Chen, C. M. Chen, K. H. Khoo, T. J. Cheng, Y. S. E. Cheng, J. T. Jan, C. Y. Wu, C. Ma and C. H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 18137-18142.
4. I. Mitra, W. R. Alley, Jr., J. A. Goetz, J. A. Vasseur, M. V. Novotny and S. C. Jacobson, *J. Proteome. Res.*, 2013, **12**, 4490-4496.
5. R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683-720.
6. J. Li, X. L. Li, Z. M. Guo, L. Yu, L. J. Zou and X. M. Liang, *Analyst*, 2011, **136**, 4075-4082.
7. Z. C. Xiong, L. Zhao, F. J. Wang, J. Zhu, H. Q. Qin, R. A. Wu, W. B. Zhang and H. F. Zou, *Chem. Commun.*, 2012, **48**, 8138-8140.
8. Z. C. Xiong, H. Q. Qin, H. Wan, G. Huang, Z. Zhang, J. Dong, L. Y. Zhang, W. B. Zhang and H. F. Zou, *Chem. Commun.*, 2013, **49**, 9284-9286.
9. B. Jiang, Y. Liang, Q. Wu, H. Jiang, K. G. Yang, L. H. Zhang, Z. Liang, X. J. Peng and Y. K. Zhang, *Nanoscale*, 2014, **6**, 5616-5619.
10. H. Kaji, H. Saito, Y. Yamauchi, T. Shinkawa, M. Taoka, J. Hirabayashi, K.-i. Kasai, N. Takahashi and T. Isobe, *Nat. Biotech.*, 2003, **21**, 667-672.
11. S. J. Yang and H. Zhang, *Anal. Chem.*, 2012, **84**, 2232-2238.
12. Y. Y. Qu, J. X. Liu, K. G. Yang, Z. Liang, L. H. Zhang and Y. K. Zhang, *Chem. Eur. J.*, 2012, **18**, 9056-9062.
13. G. B. Xu, W. Zhang, L. M. Wei, H. J. Lu and P. Y. Yang, *Analyst*, 2013, **138**, 1876-1885.
14. Y. M. An and J. F. Cipollo, *Anal. biochem.*, 2011, **415**, 67-80.
15. A. J. Shen, Z. M. Guo, X. M. Cai, X. Y. Xue and X. M. Liang, *J. Chromatogr., A*, 2012, **1228**, 175-182.
16. P. Pompach, K. B. Chandler, R. Lan, N. Edwards and R. Goldman, *J. Proteome. Res.*, 2012, **11**, 1728-1740.
17. L. Yu, X. L. Li, Z. M. Guo, X. L. Zhang and X. M. Liang, *Chem. Eur. J.*, 2009, **15**, 12618-12626.
18. G. Huang, Z. Sun, H. Q. Qin, L. Zhao, Z. C. Xiong, X. J. Peng, J. J. Ou and H. F. Zou, *Analyst*, 2014, **139**, 2199-2206.
19. W. F. Ma, L. L. Li, Y. Zhang, Q. An, L. J. You, J. M. Li, Y. T. Zhang, S. Xu, M. Yu, J. Guo, H. J. Lu and C. C. Wang, *J. Mater. Chem.*, 2012, **22**, 23981-23988.
20. G. Zauner, A. M. Deelder and M. Wuhrer, *Electrophoresis*, 2011, **32**, 3456-3466.
21. J. R. Li, J. Sculley and H. C. Zhou, *Chem. Rev.*, 2012, **112**, 869-932.
22. M. Anbia and V. Hoseini, *J. Nat. Gas Chem.*, 2012, **21**, 339-343.
23. Z. Y. Gu, C. X. Yang, N. Chang and X. P. Yan, *Acc. Chem. Res.*, 2012, **45**, 734-745.
24. Z. Y. Gu and X. P. Yan, *Angew. Chem., Int. Ed.*, 2010, **49**, 1477-1480.
25. N. Chang, Z. Y. Gu and X. P. Yan, *J. Am. Chem. Soc.*, 2010, **132**, 13645-13647.
26. D. Farrusseng, S. Aguado and C. Pinel, *Angew. Chem., Int. Ed.*, 2009, **48**, 7502-7513.
27. Z. Y. Gu, Y. J. Chen, J. Q. Jiang and X. P. Yan, *Chem. Commun.*, 2011, **47**, 4787-4789.
28. C. B. Messner, M. R. Mirza, M. Rainer, O. M. D. Lutz, Y. Güzel, T. S. Hofer, C. W. Huck, B. M. Rode and G. K. Bonn, *Anal. Methods*, 2013, **5**, 2379-2383.
29. Y. C. Lin, C. L. Kong and L. Chen, *RSC Advances*, 2012, **2**, 6417-6419.
30. D. Jiang, L. L. Keenan, A. D. Burrows and K. J. Edler, *Chem. Commun.*, 2012, **48**, 12053-12055.
31. C. W. Kuo, I. L. Wu, H. H. Hsiao and K. H. Khoo, *Anal. Bioanal. Chem.*, 2012, **402**, 2765-2776.
32. J. Zhu, F. J. Wang, R. Chen, K. Cheng, B. Xu, Z. M. Guo, X. M. Liang, M. L. Ye and H. F. Zou, *Anal. Chem.*, 2012, **84**, 5146-5153.
33. L. J. Zhang, Y. W. Xu, H. L. Yao, L. Q. Xie, J. Yao, H. J. Lu and P. Y. Yang, *Chem. Eur. J.*, 2009, **15**, 10158-10166.

ChemComm Accepted Manuscript