Published on 21 November 2013. Downloaded by University of Science and Technology of China on 24/12/2013 11:54:27.

ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Water-stable metal–organic frameworks with intrinsic peroxidase-like catalytic activity as a colorimetric biosensing platform†

Cite this: *Chem. Commun.*, 2014, **50**, 1092

Received 2nd November 2013,
Accepted 20th November 2013

DOI: 10.1039/c3cc48398c

www.rsc.org/chemcomm

Jian-Wei Zhang,[ab] Hao-Tian Zhang,[a] Zi-Yi Du,[b] Xueqing Wang,[ac] Shu-Hong Yu[a] and Hai-Long Jiang*[a]

Two iron(III)-based metal–organic frameworks (MOFs) are found to behave as efficient peroxidase mimics and catalyze the oxidation of different peroxidase substrates by H_2O_2 accompanied with significant color change in the solution. With these findings, a simple and sensitive colorimetric assay to detect H_2O_2 and ascorbic acid has been established.

Hydrogen peroxide (H_2O_2), ascorbic acid (AA) and glucose are involved in many chemical, biological, pharmaceutical, clinical, and environmental processes.[1] For their detection, optical, electrochemical and bioelectrochemical based sensing approaches are the most reported analytical techniques with certain advantages,[2] while they suffer from serious drawbacks such as lack of sensitivity, rapidity, and/or specificity. Peroxidases are ubiquitous in nature and have great potential in many fields. Especially, horseradish peroxidase (HRP) with heme has caused widespread interest in immunoassays and possesses great potential as a diagnostic kit for hydrogen peroxide, glucose, ascorbic acid, *etc.* However, the enzymes readily get denatured upon heating or chemical changes and their preparation, purification and storage are relatively time-consuming. Therefore, many analogs as HRP mimics, such as Fe_3O_4,[3a] Au nanoparticles,[3b] ceria nanoparticles,[3c] graphene oxide,[3d] carbon nanotubes,[3e] carbon nanodots,[3f] *etc.*, have been reported to exhibit catalytic activity similar to that found in natural peroxidases, and have been successfully employed as enzyme mimics for the detection of these substrates.

Metal–organic frameworks (MOFs) are becoming a focus of great interest owing to their intriguing structural topologies and potential applications as functional materials in catalysis, sensors, drug delivery, sorption, separation, and so on.[4–6] So far, very few studies on MOF biosensing have been reported.[7] Moreover, to the best of our knowledge, only two MOFs, PCN-222 with porphyrinic Fe(III) centers[7a] and Fe(III)-based MIL-53,[7e] have been very recently reported to show intrinsic peroxidase-like catalytic activity for colorimetric biosensing.

In this communication, two previously reported Fe(III)-based MOFs, MIL-68 and MIL-100,[8] are found to be able to exhibit intrinsic peroxidase-like activity for colorimetric biosensing. Both MOFs catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) as peroxidase mimics to provide a colorimetric assay for H_2O_2 and catalyze the oxidation of *o*-phenylenediamine (OPD) with significant solution color change in the presence of H_2O_2, in the latter of which ascorbic acid (AA) induces an inhibitory effect on the oxidation, thus resulting in the colorimetric biosensing for AA.

The two Fe(III)-based MOFs have been prepared by a solvothermal reaction in a Teflon-lined bomb according to the reported approaches with modifications (ESI,† Section S2).[8] Both MOFs are made from inexpensive and biocompatible iron(III) and 1,4-benzenedicarboxylic acid (BDC, for MIL-68)/1,3,5-benzenetricarboxylic acid (BTC, for MIL-100) with very good water stability. The MIL-68 framework, formulated Fe(OH)(BDC), features a 3D network involving two types of 1D channels in triangular or hexagonal shape along the *c*-axis (Fig. 1a). The MIL-100 structure with formula $Fe_3O(H_2O)_2(BTC)_2F$ presents a 3D MTN-type zeolitic architecture, which delimits two types of mesoporous cages of free apertures of *ca.* 25 and 29 Å with microporous cage openings of *ca.* 5.5 and 8.6 Å, respectively (Fig. 1c). Powder X-ray diffraction (XRD) profiles have demonstrated the pure phases and good chemical stability of both MOFs, and scanning electron microscopy (SEM) images indicate that the MIL-68 particles with sizes of 0.1–1 μm have some extent of aggregation, while MIL-100 particles are well dispersed with sizes of ~200 nm (Fig. 1b and d; ESI,† Fig. S1 and S2). N_2 sorption isotherms confirm their permanent porosity and the BET surface areas of MIL-68 and MIL-100 are 393 and 1313 $m^2\ g^{-1}$, respectively (ESI,† Fig. S3).

To investigate the peroxidase-like activity of these MOFs, the catalytic oxidation of the peroxidase substrate TMB in the presence

[a] *Hefei National Laboratory for Physical Sciences at the Microscale, Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, P.R. China. E-mail: jianglab@ustc.edu.cn; Fax: +86-551-63607861; Tel: +86-551-63607861*

[b] *College of Chemistry and Chemical Engineering, Gannan Normal University, Ganzhou 341000, P.R. China*

[c] *Department of Chemistry, University of British Columbia, Vancouver, BC V6T 1Z1, Canada*

† Electronic supplementary information (ESI) available: All experimental details and characterization data. See DOI: 10.1039/c3cc48398c

This journal is © The Royal Society of Chemistry 2014

Published on 21 November 2013. Downloaded by University of Science and Technology of China on 24/12/2013 11:54:27.

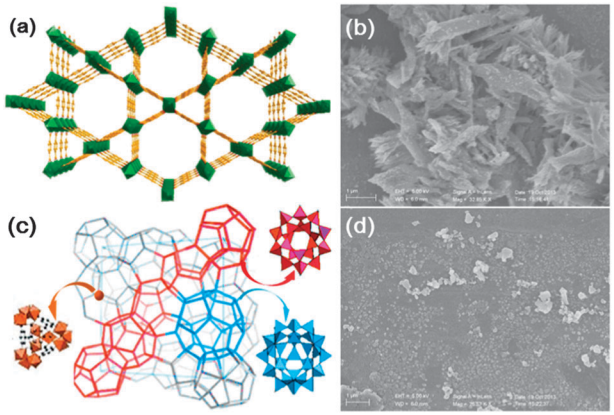

Fig. 1 (a) View of the structure of MIL-68 involving two types (hexagonal and trigonal) of channels running through the *c*-axis. (b) SEM image of MIL-68. (c) Topological view of MIL-100 with MTN-type zeolitic architecture, in which each node represents a supertetrahedron constructed by $Fe_3^{III}O$ clusters and BTC linkers. The structure involves two types of cages shown in red and blue with free diameters of *ca.* 25 and 29 Å, respectively. The FeO_x are shaded in the polyhedra. (d) SEM image of MIL-100. The scale bars in the SEM images are 1 μm.

of hydrogen peroxide was examined (Fig. 2). The photographs of TMB solutions under different conditions show that the solutions exhibit no color change both in the absence and presence of H_2O_2, indicating that no oxidation reaction occurs without an MOF catalyst. However, when MIL-68 or MIL-100 was introduced into the solution with H_2O_2 and TMB, a blue color was observed after incubation (Fig. 2a; ESI,† Fig. S4). The catalytic oxidation of TMB over MOFs accounts for the blue color of the solutions, which give intense characteristic absorbance at 369 and 652 nm in the UV-vis spectra. The phenomenon is similar to that observed for the commonly used horseradish peroxidase (HRP) enzyme,[3a] and the adsorption bands could be attributed to the charge-transfer complexes derived from the one-electron oxidation of TMB.[9a] All these observations reveal that both Fe(III)-based MOFs have peroxidase-like catalytic ability and they can catalyze the oxidation of TMB in the presence of H_2O_2. It is proposed that the nature of peroxidase-like activity of the Fe-MOFs originates from their catalytic ability to decompose H_2O_2 into $^{\bullet}OH$ radicals through electron transfer.[9] Similar to HRP, the catalytic activity of both MOFs is dependent on temperature, pH value and H_2O_2 concentration. The optimal pH is 4.0 for MIL-68 while 3.0 for MIL-100 and the best temperature for both MOFs is approximately 45 °C, which are similar to the values for other nanostructured peroxidase mimetics and HRP (Fig. 2b and c; ESI,† Fig. S4).[3] Thus, pH = 4.0 and 45 °C were adopted as standard conditions for subsequent activity analysis. We have found that both MOFs require much higher H_2O_2 concentrations than that of HRP to reach the maximal level of peroxidase activity (Fig. 2d and ESI†), revealing that the catalytic activity of the MOFs is more stable at high H_2O_2 concentration than HRP.

Given the intrinsic peroxidase properties of both Fe(III)-based MOFs, a colorimetric method for detection of H_2O_2 using the MOF-catalyzed blue color reaction has been established. Since the catalytic activity of the MOFs is highly dependent on the concentration of H_2O_2 in the solution, the method could be used for the quantitative evaluation of H_2O_2. Fig. 3 shows the increase in absorbance at 652 nm upon increasing the H_2O_2 concentration in solution. A linear relationship (Fig. 3 inset) is observed between the absorbance and the H_2O_2 concentration ranging from 3.0 to 40 μM for both MOFs

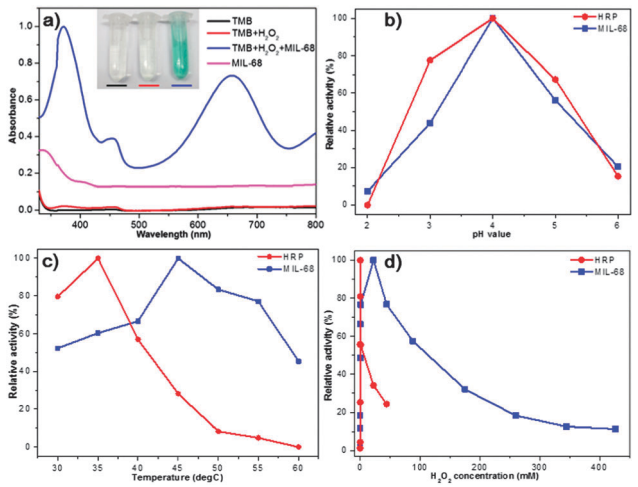

Fig. 2 (a) UV-vis adsorption spectra for TMB, TMB-H_2O_2, TMB-H_2O_2-MIL-68 and MIL-68 solutions in acetate buffer with pH = 4.0. Inset: typical photographs of the three samples corresponding to the lines. Concentrations: TMB (0.1 mM), H_2O_2 (0.44 mM), MIL-68 (0.26 mg mL^{-1}). The peroxidase-like activity over MIL-68 is dependent on (b) pH value, (c) temperature and (d) H_2O_2 concentration and the corresponding activity of HRP. Experiments were conducted using 80 μg MIL-68 or 0.3 μg mL^{-1} HRP in 1.6 mL buffer (pH = 4.0), and 0.4 mL of 1 mM TMB and 40 μL of 4.2 mM H_2O_2 were introduced into the system at pH = 4.0 and 45 °C unless otherwise stated.

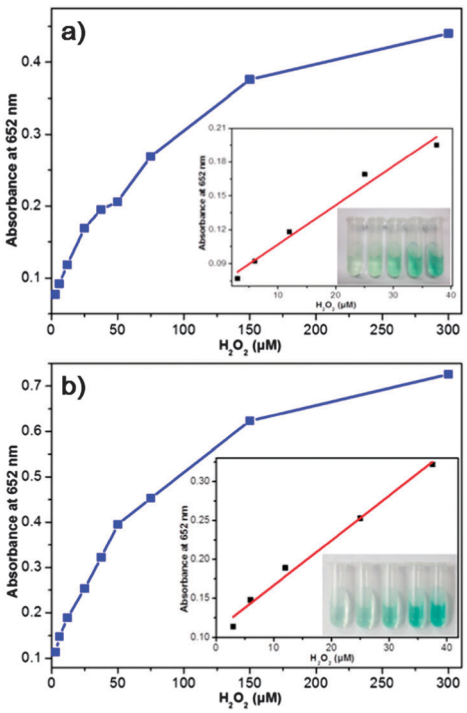

Fig. 3 Dose–response curves for H_2O_2 detection in the range of 3 to 300 μmol L^{-1} using (a) MIL-68 or (b) MIL-100 as a peroxidase mimic. Inset: linear calibration plot between the absorbance at 652 nm and concentration of H_2O_2 and the corresponding photographs for the formation of colored products with different concentrations of H_2O_2.

Published on 21 November 2013. Downloaded by University of Science and Technology of China on 24/12/2013 11:54:27.

(R^2 = 0.97 for MIL-68 and R^2 = 0.98 for MIL-100) with a detection limit of 0.256 μM and 0.155 μM, respectively, for MIL-68 and MIL-100, both of which are lower than those provided by Fe_3O_4 nanoparticles.[3a]

In addition, in the presence of H_2O_2, MIL-68 and MIL-100 have also been found to catalyze the oxidation of other peroxidase substrates such as OPD, presenting a remarkable color change to yellow-orange in the solution, indicating that the catalytic oxidation reaction occurs. Remarkably, the oxidation process can be effectively suppressed upon introducing a trace amount of ascorbic acid (AA) to afford the solution with very slight yellow-orange color (ESI,† Fig. S6). With this in mind, a colorimetric biosensor system for AA based on MIL-68 or MIL-100 can be readily developed. As shown in Fig. 4, the typical UV-vis absorbance intensity at λ = 450 nm *vs.* AA concentration indicates that more AA introduced weaker absorption peak intensity, which results in the corresponding calibration plot shown in the inset in Fig. 4. The linear detection range is estimated to be from 30 to 485 μM (R^2 = 0.96). The detection limit is estimated to be 6 μM.

In conclusion, our results indicate that two water-stable Fe(III)-based MOFs, MIL-68 and MIL-100, possess intrinsic peroxidase-like activity and their catalysis is strongly dependent on pH value, temperature, and H_2O_2 concentration, similar to horseradish peroxidase. In the presence of H_2O_2 and the peroxidase substrate TMB, both MOFs can produce a blue color reaction, thus providing a colorimetric assay for H_2O_2. Moreover, the influence of AA introduction on oxidation of OPD catalyzed by the MOFs in the presence of H_2O_2 has been investigated. Based on AA-induced inhibition of the peroxidase-like activity of Fe(III)-based MOFs, a simple colorimetric biosensing system for AA detection is developed. The MOFs as peroxidase mimics present several advantages over natural enzymes, such as ease of preparation, low cost, and stability, which allow MOFs to be used as enzymatic mimics for potential applications in immunoassays, medical diagnostics and biotechnology in future.

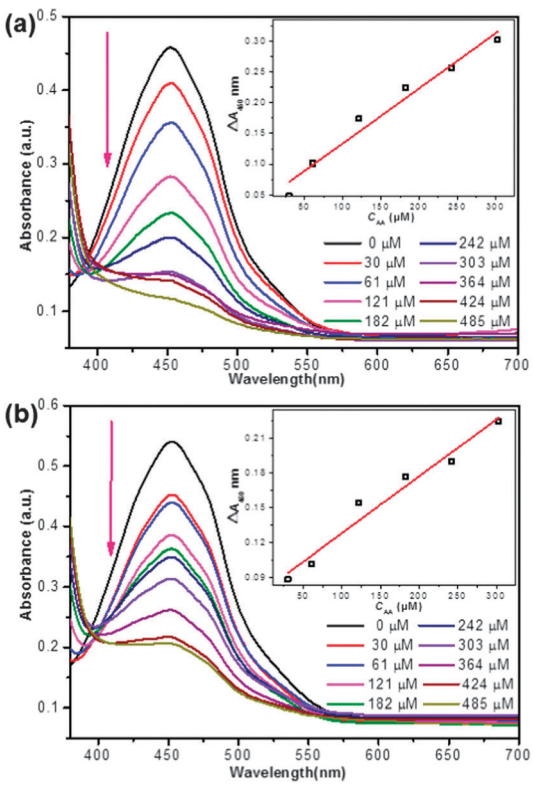

Fig. 4 UV-vis spectra of OPD oxidation catalyzed by (a) MIL-68 or (b) MIL-100 in the presence of AA as an inhibitor in a pH = 4.0 buffer solution under ambient conditions (OPD: 2.42 mM, H_2O_2: 0.24 M, MIL-68 or MIL-100: 0.04 mg mL^{-1}). Inset: linear relationship between absorbance at λ = 450 nm *vs.* concentration of AA. $\Delta A = A_0 - A_i$ (A_0 and A_i are the absorbance at 450 nm before and after the addition of AA with a concentration of *i*, respectively).

This work is supported by the National Natural Science Foundation of China (Grants 21371162 and 51301159), the National Key Basic Research Program of China (Grant 2014CB931803), Research Fund for the Doctoral Program of Higher Education of China (20133402120020), the "Hundred Talent" Program from the Chinese Academy of Sciences and start-up funding from the University of Science and Technology of China (USTC).

Notes and references

1 A. Tanaka, Y. Takeda, T. Nagasawa and K. Takahashi, *Solid State Commun.*, 2003, **126**, 191.
2 (*a*) A. McNeillie, D. H. Brown, W. E. Smith, M. Gibson and L. Watson, *Dalton Trans.*, 1980, 767; (*b*) D. G. Duff, A. Baiker and P. P. Edwards, *Langmuir*, 1993, **9**, 2301; (*c*) G. Liu and Y. Lin, *Electrochem. Commun.*, 2006, **8**, 251; (*d*) B. C. Dickinson and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 9638; (*e*) M. Zhou and S. Dong, *Acc. Chem. Res.*, 2011, **44**, 1232.
3 (*a*) L. Z. Gao, J. Zhuang, L. Nie, J. B. Zhang, Y. Zhang, N. Gu, T. H. Wang, J. Feng, D. L. Yang, S. Perrett and X. Yan, *Nat. Nanotechnol.*, 2007, **2**, 577; (*b*) Y. Jv, B. X. Li and R. Cao, *Chem. Commun.*, 2010, **46**, 8017; (*c*) A. Asati, S. Santra, C. Kaittanis, S. Nath and J. M. Perez, *Angew. Chem., Int. Ed.*, 2009, **48**, 2308; (*d*) Y. Song, K. Qu, C. Zhao, J. Ren and X. Qu, *Adv. Mater.*, 2010, **22**, 2206; (*e*) Y. Song, X. Wang, C. Zhao, K. Qu, J. Ren and X. Qu, *Chem.–Eur. J.*, 2010, **16**, 3617; (*f*) W. Shi, Q. Wang, Y. Long, Z. Cheng, S. Chen, H. Zheng and Y. Huang, *Chem. Commun.*, 2011, **47**, 6695.
4 (*a*) S. Horike, S. Shimomura and S. Kitagawa, *Nat. Chem.*, 2009, **1**, 695; (*b*) J. R. Long and O. M. Yaghi, *Chem. Soc. Rev.*, 2009, **38**, 1213; (*c*) H.-C. Zhou, R. J. Long and O. M. Yaghi, *Chem. Rev.*, 2012, **112**, 673.
5 (*a*) L. Ma, C. Abney and W. Lin, *Chem. Soc. Rev.*, 2009, **38**, 1248; (*b*) H.-L. Jiang and Q. Xu, *Chem. Commun.*, 2011, **47**, 3351; (*c*) B. Chen, S. Xiang and G. Qian, *Acc. Chem. Res.*, 2010, **43**, 1115; (*d*) P. Horcajada, R. Gref, T. Baati, P. K. Allan, G. Maurin, P. Couvreur, G. Férey, R. E. Morris and C. Serre, *Chem. Rev.*, 2012, **112**, 1232; (*e*) K. Sumida, D. L. Rogow, J. A. Mason, T. M. McDonald, E. D. Bloch, Z. R. Herm, T.-H. Bae and J. R. Long, *Chem. Rev.*, 2012, **112**, 724; (*f*) M. P. Suh, H. J. Park, T. K. Prasad and D.-W. Lim, *Chem. Rev.*, 2012, **112**, 782; (*g*) J.-R. Li, J. Sculley and H.-C. Zhou, *Chem. Rev.*, 2012, **112**, 869; (*h*) P. Nugent, Y. Belmabkhout, S. D. Burd, A. J. Cairns, R. Luebke, K. Forrest, T. Pham, S. Ma, B. Space, L. Wojtas, M. Eddaoudi and M. J. Zaworotko, *Nature*, 2013, **495**, 80.
6 (*a*) F. Wang, Z. S. Liu, H. Yang, Y. X. Tan and J. Zhang, *Angew. Chem., Int. Ed.*, 2011, **50**, 450; (*b*) Z. Wang and S. M. Cohen, *Chem. Soc. Rev.*, 2009, **38**, 1315–1329; (*c*) D. Q. Yuan, D. Zhao, D. F. Sun and H.-C. Zhou, *Angew. Chem., Int. Ed.*, 2010, **49**, 5357; (*d*) G.-S. Yang, Z.-L. Lang, H.-Y. Zang, Y.-Q. Lan, W.-W. He, X.-L. Zhao, L.-K. Yan, X.-L. Wang and Z.-M. Su, *Chem. Commun.*, 2013, **49**, 1088.
7 (*a*) D. Feng, Z.-Y. Gu, J.-R. Li, H.-L. Jiang, Z. Wei and H.-C. Zhou, *Angew. Chem., Int. Ed.*, 2012, **51**, 10307; (*b*) S. Liu, L. Wang, J. Tian, Y. Luo, G. Chang, A. M. Asiri, A. O. Al-Youbi and X. Sun, *ChemPlusChem*, 2012, **77**, 23; (*c*) X. Zhu, H. Zheng, X. Wei, Z. Lin, L. Guo, B. Qiu and G. Chen, *Chem. Commun.*, 2013, **49**, 1276; (*d*) X. Wei, L. Zheng, F. Luo, Z. Lin, L. Guo, B. Qiu and G. Chen, *J. Mater. Chem. B*, 2013, **1**, 1812; (*e*) L. Ai, L. Li, C. Zhang, J. Fu and J. Jiang, *Chem.–Eur. J*, 2013, DOI: 10.1002/chem.201303051.
8 (*a*) A. Fateeva, P. Horcajada, T. Devic, C. Serre, J. Marrot, J.-M. Grenèche, M. Morcrette, J.-M. Tarascon, G. Maurin and G. Férey, *Eur. J. Inorg. Chem.*, 2010, 3789; (*b*) P. Horcajada, S. Surblé, C. Serre, D.-Y. Hong, Y.-K. Seo, J.-S. Chang, J.-M. Grenèche, I. Margiolakid and G. Férey, *Chem. Commun.*, 2007, 2820.
9 (*a*) P. D. Josephy, T. Eling and R. P. Mason, *J. Biol. Chem.*, 1982, **257**, 3669; (*b*) X.-Q. Zhang, S.-W. Gong, Y. Zhang, T. Yang, C.-Y. Wang and N. Gu, *J. Mater. Chem.*, 2010, **20**, 5110; (*c*) Y. Chen, H. Cao, W. Shi, H. Liu and Y. Huang, *Chem. Commun.*, 2013, **49**, 5013.

 This journal is © The Royal Society of Chemistry 2014