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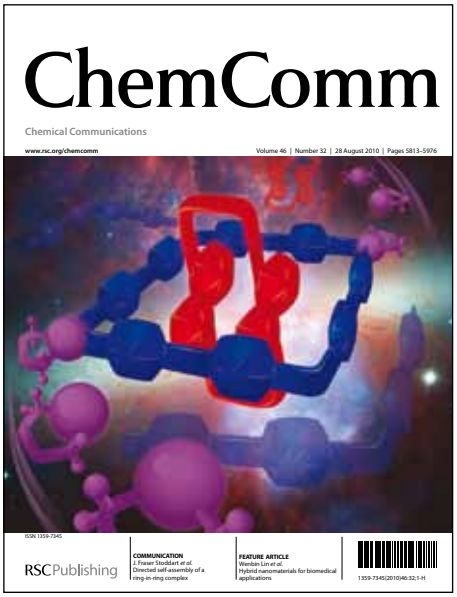

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ARTICLE TYPE

Water-Stable Metal-Organic Frameworks with Intrinsic Peroxidase-like Catalytic Activity as a Colorimetric Biosensing Platform

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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 accompanying 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 mostly 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, sensor, drug delivery, sorption and separation, and so on.[4-6] So far, very few work on MOF biosensing has been reported.[7] Moreover, to the best of our knowledge, there are only two MOFs, PCN-222 with porphyrinic Fe(III) centers[7a] and Fe(III)-based MIL-53[7e], 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 inhibition effect for the oxidation, thus resulting in the colorimetric biosensing for AA.

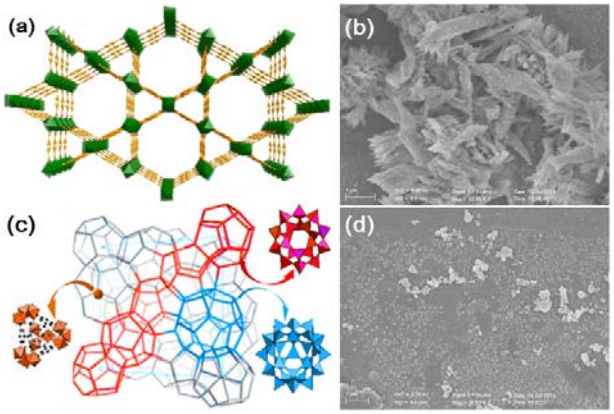

Fig. 1 (a) View of the structure of MIL-68 involving two types (hexagonal and trigonal) of channels running through the *c*-axis. (b) SEM image of MIL-68. (c) Topological view of MIL-100 with MTN type zeolitic architecture, in which each node represents supertetrahedron constructed by Fe^{III}_3O clusters 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.

The two Fe(III)-based MOFs have been prepared by a solvothermal reaction in Teflon-lined bomb according to the reported approaches with modifications (ESI†, Section 2).[8] Both MOFs are built up from inexpensive and biocompatible iron(III) and 1,4-benzenedicarboxylic acid (BDC, for MIL-68) / 1,3,5-benezenetricarboxylic 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 of $Fe_3O(H_2O)_2(BTC)_2F$ 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

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some extent of aggregation, while MIL-100 particles are well dispersed with sizes of ~200 nm (Fig. 1b, 1d; ESI†, Fig. S1, 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, respectively (ESI†, Fig. S3).

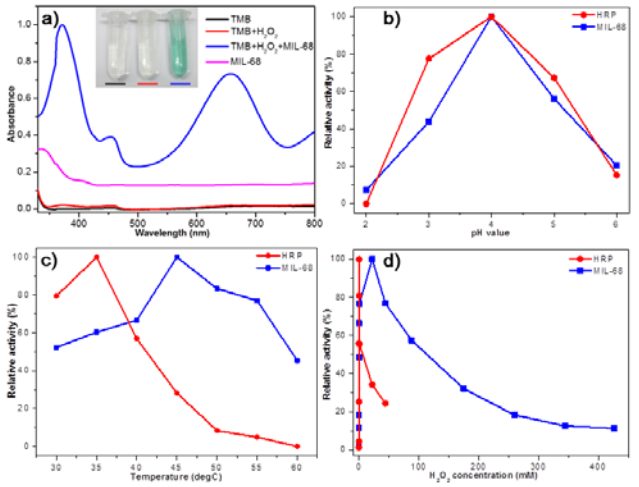

Fig. 2 (a) UV-vis adsorption spectra for TMB, TMB-H_2O_2, TMB-H_2O_2-MIL-68 and MIL-68 solutions in pH = 4.0 acetate buffer. Inset: typical photographs of three samples to the corresponding lines. Concentrations: TMB (0.1 mM), H_2O_2 (0.44 mM), MIL-68 (0.26 mg/mL). The peroxidase-like activity over MIL-68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

To investigate the peroxidase like activity of these MOFs, the catalytic oxidation of peroxidase substrate TMB in the presence of hydrogen peroxide was examined (Fig. 2). The photographs for TMB solutions under different conditions have shown the solutions exhibit no color change both in the absence and presence of H_2O_2, indicating that no oxidation reaction occurs without 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 horse radish 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 decomposition of H_2O_2 into •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; 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,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 property of both Fe(III)-based MOFs, a colorimetric method for detection of H_2O_2 using the MOFs-catalyzed blue color reaction has been established. Since the catalytic activity of the MOFs is highly dependent on the concentration of the H_2O_2 in the solution, thus the method could be used for the quantitative evaluation of H_2O_2. Fig. 3 displays the increase in absorbance at 652 nm upon increasing the H_2O_2 concentration in solution. A linear relationship (inset to Fig. 3) between the absorbance and the H_2O_2 concentration ranging from 3.0 to 40 μM for both MOFs (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 that provided by Fe_3O_4 nanoparticles.[3a]

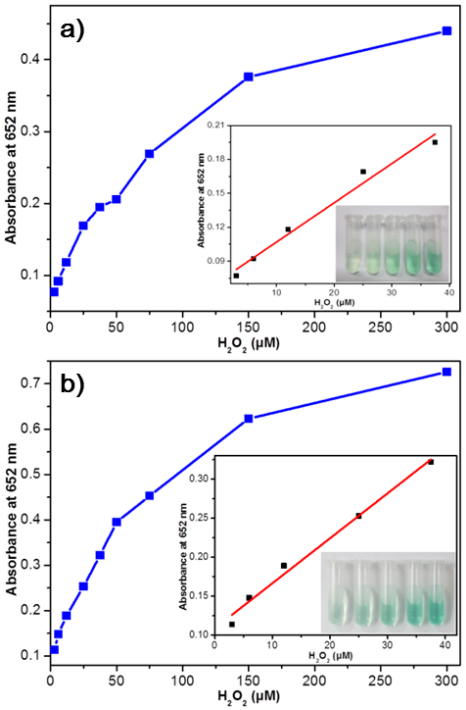

Fig. 3 Dose-response curves for H_2O_2 detection in the range of 3 to 300 μmol/L using (a) MIL-68 or (b) MIL-100 as a peroxidase mimic. Inset: linear calibration plot between the absorbance at 652 nm and concentration of H_2O_2 and corresponding photographs for the formation of colored products with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

In addition, in the presence of H_2O_2, MIL-68 and MIL-100 have also been found to catalyze the oxidation of the other peroxidase substrate such as OPD, presenting remarkable color change to yellow-orange in the solution, indicating 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 displayed in Fig. 4, the typical UV-vis absorbance intensity at λ = 450 nm *vs* AA concentration indicates more AA introduced, weaker absorption peak intensity, which

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results in the corresponding calibration plot shown as 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.

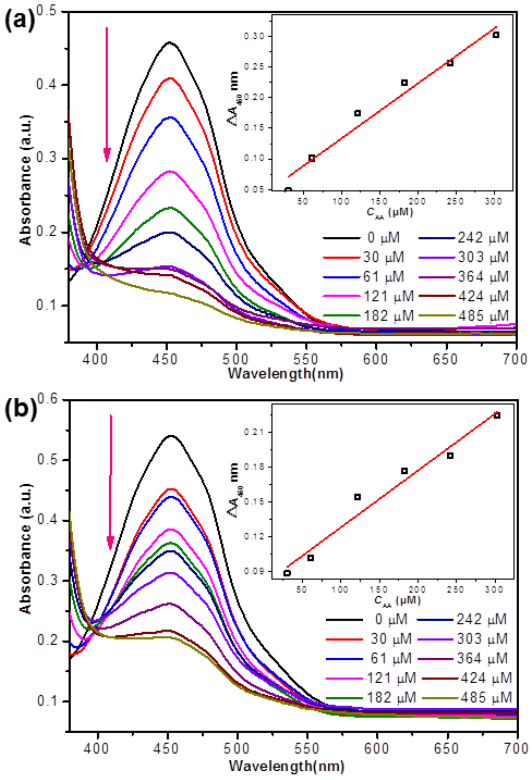

Fig. 4 UV-vis spectra of OPD oxidation catalyzed by (a) MIL-68 or (b) MIL-100 in the presence of AA as an inhibitor in a pH = 4.0 buffer solution under ambient conditions ([OPD]: 2.42 mM, [H_2O_2]: 0.24 M, [MIL-68] or [MIL-100]: 0.04 mg/mL). Inset: linear relationship between absorbance at λ = 450 nm *vs* concentration of AA. ΔA = 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).

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 peroxidase substrate TMB, both MOFs can produce a blue color reaction, thus to provide 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 mimic peroxidases present several advantages over natural enzymes, such as ease of preparation, low-cost, and stability, which and allow MOFs as enzymatic mimics for potential applications in immunoassays, medical diagnostics and biotechnology in future.

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† Electronic Supplementary Information (ESI) available: All experimental details and characerization data. See DOI: 10.1039/b000000x/

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