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 \( \text{H}_2\text{O}_2 \) accompanied with significant color change in the solution. With these findings, a simple and sensitive colorimetric assay to detect \( \text{H}_2\text{O}_2 \) and ascorbic acid has been established.

Hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), ascorbic acid (AA) and glucose are involved in many chemical, biological, pharmaceutical, clinical, and environmental processes. For their detection, optical, electrochemical and bioelectrochemical based sensing approaches are the most reported analytical techniques with certain advantages, 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, horse-radish 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 \( \text{Fe}_3\text{O}_4 \), \( \text{Au} \) nanoparticles, ceria nanoparticles, graphene oxide, carbon nanotubes, carbon nanodots, 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. So far, very few studies on MOF biosensing have been reported. Moreover, to the best of our knowledge, only two MOFs, PCN-222 and Fe(III)-based MIL-53, 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, 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 \( \text{H}_2\text{O}_2 \) and catalyze the oxidation of \( \alpha \)-phenylenediamine (OPD) with significant solution color change in the presence of \( \text{H}_2\text{O}_2 \), 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 solvo-thermal reaction in a Teflon-lined bomb according to the reported approaches with modifications (ESI, Section S2). Both MOFs are made from inexpensive and biocompatible iron(III) and 1,4-benzene-dicarboxylic 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(III)(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 \( \text{Fe}_3\text{O}_4(\text{H}_2\text{O})_2(\text{BTC})_2\text{F} \) 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† Figs. 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...
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 H₂O₂, indicating that no oxidation reaction occurs without an MOF catalyst. However, when MIL-68 or MIL-100 was introduced into the solution with H₂O₂ 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. 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 H₂O₂. It is proposed that the nature of peroxidase-like activity of the Fe-MOFs originates from their catalytic ability to decompose H₂O₂ into •OH radicals through electron transfer.⁹

Similar to HRP, the catalytic activity of both MOFs is dependent on temperature, pH value and H₂O₂ 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 H₂O₂ 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 H₂O₂ concentration than HRP.

Given the intrinsic peroxidase properties of both Fe(III)-based MOFs, a colorimetric method for detection of H₂O₂ using the MOF-catalyzed blue color reaction has been established. Since the catalytic activity of the MOFs is highly dependent on the concentration of H₂O₂ in the solution, the method could be used for the quantitative evaluation of H₂O₂. Fig. 3 shows the increase in absorbance at 652 nm upon increasing the H₂O₂ concentration in solution. A linear relationship (Fig. 3 inset) is observed between the absorbance and the H₂O₂ concentration ranging from 3.0 to 40 μM for both MOFs.
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$_2$O$_2$ concentration, similar to horseradish peroxidase. In the presence of H$_2$O$_2$ and the peroxidase substrate TMB, both MOFs can produce a blue color reaction, thus providing a colorimetric assay for H$_2$O$_2$. Moreover, the influence of AA introduction on oxidation of OPD catalyzed by the MOFs in the presence of H$_2$O$_2$ has been investigated. Based on AA-induced inhibition of the peroxidase-like activity of Fe(n)-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.

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Notes and references