Bi-metallic MOF-919 (Fe-Cu) nanozyme capable of bifunctional enzyme-mimicking catalytic activity

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**Chemicals and materials**

All chemicals were purchased from commercial suppliers without further purification unless otherwise mentioned. The iron chloride hexahydrate (FeCl$_3$·6H$_2$O) was purchased from Alfa Aesar, and copper nitrate trihydrate (Cu(NO$_3$)$_2$·3H$_2$O) was purchased from Showa chemicals. 4-Pyrazolecarboxylic (H$_2$-PyC) acid was purchased from TCI chemicals. The o-phenylenediamine (OPD) specially prepared reagent was purchased from nacalai tesque Inc, Kyoto, Japan. The dopamine, L-DOPA, epinephrine, norepinephrine, tyramine, and ascorbic acid were purchased from ACROS. The 3,3',5,5'-Tetramethylbenzidine (TMB) and phenylethylamine were purchased from Sigma-Aldrich. The tyrosine was purchased from KYOWA and Merck. The hydrogen peroxide (30%, w/v) analytical reagent grade were purchased from Fisher chemical. The stock solution of 10 x PBS Buffer was purchased from Biokit Biotechnology Inc, Taiwan.

**Synthesis of MOF-919 (Fe-Cu)**

MOF-919 (Fe-Cu) was synthesized based on literature with minor modifications.$^1$ In summary, the stock solution was produced in DMF by combining 2.5 mL FeCl$_3$·6H$_2$O solution (0.1 M), 2.5 mL Cu(NO$_3$)$_2$·3H$_2$O solution (0.33 M), and 2.5 mL 4-pyrazolecarboxylic acid solution (0.385 M) in a Teflon reactor heated in an electric oven at 100 °C/12 h. After the reaction was completed, the reaction mixture was
washed three times using DMF (3x5 mL), and the sample was transferred to the vial. DMF solvent exchange was performed three times per day, followed by ethanol exchange three times per day, and ultimately rinsed three times with acetone (3x5 mL). After this process, the sample was activated by extracting the solvent from the MOF pores; the sample was transferred to a centrifuge tube and dried in a vacuum with heat (120 °C/12 h). Following this activation, the MOF sample was stored in the vial for future use.

**Oxidase–like and peroxidase-like catalytic activity of MOF-919 (Fe-Cu)**

PBSx1 pH 7.4 was used for the experiment. For oxidase-like activity, 0.1 mL 3.3 mM OPD was combined with MOF-919 (Fe-Cu) solutions (0.1 mg per 0.1 mL in PBSx1) and then reacted at 37 °C for 10 minutes. The reaction solution contains 0.1 mL 2 mM H₂O₂, which is also utilized for peroxidase-like activity investigations. The product was validated by measuring the UV-Vis absorbance at 420 nm (= 16300 M⁻¹ cm⁻¹). The oxidation and peroxidation reaction rates were calculated using the Michaelis-Menten equation.

\[ v = V_{max} \times \frac{[S]}{([S] + K_m)} \]

**Catechol oxidase-like activity of MOF-919 (Fe-Cu)**

The catechol oxidase-like activity of MOF-919 (Fe-Cu) in PBSx1 pH 7.4 was tested using catecholamines as the substrate, which could be oxidized by MOF-919
(Fe-Cu) to generate quinone derivatives. Each reaction tube containing MOF-919 (Fe-Cu) solutions (0.25 mg per 0.25 mL in PBSx1) with (1) L-DOPA (250 µM), (2) Dopamine (250 µM), (3) Epinephrine (250 µM), and (4) Norepinephrine (250 µM) was reacted at 37 °C for 30 minutes and the pictures were taken. The oxidation of catecholamines was investigated by adding MOF-919 (Fe-Cu) to PBSx1 at 37 °C for various time intervals, and the absorbance was measured 300-700 nm using a UV-Vis spectrophotometer.

**General considerations of solution preparation**

MOF suspensions in PBSx1 pH 7.4 (MOF-919 (Fe-Cu), MOF-818 (Zr-Cu), MOF-808 (Zr), MIL-100 (Fe) and HKUST-1 (Cu)) were produced at a concentration of 1 mg/mL. The MOF suspensions were kept at room temperature and before use vortexed for 5 minutes. A 10 mM OPD stock solution was prepared by dissolving OPD in ddH₂O. Similarly, the 10 mM stock solution of L-DOPA, dopamine (DA), phenylethylamine, tyramine, tyrosine, and ascorbic acid (AA) solutions were prepared in ddH₂O, epinephrine (Epi), and norepinephrine (NE) 10 mM stock solution was prepared by dissolving the compound in DMSO. Further, all stock solution was diluted into various concentrations by ddH₂O. Aqueous solutions of 10 mM H₂O₂ and different concentrations were prepared by dilutions of purchased H₂O₂ (30%) in ddH₂O.
**Characterization**

The crystal structure of the MOFs was investigated by powder X-ray diffraction (PXRD), Bruker D8 Advance Eco using CuKα radiation $\lambda= 1.54178$. High-resolution scanning electron microscopy (HR-SEM, JEOL JEM-7600F) was used to investigate the morphology of the MOF samples, and elemental analysis of materials was conducted using an HR-SEM model JSM-7600F (JEOL) coupled with energy-dispersive X-ray spectroscopy (EDS) (OXFORD X-Max 80). FT-IR spectroscopic model FT/IR 4200 was used to record the FT-IR spectra of the MOF sample (Jasco, Japan). The BET surface area and pore volume were calculated using the N$_2$ sorption isotherms, which were evaluated in a Micrometrics ASAP 2020 surface area and a pore size analyzer. At 77 K, the experiments were conducted using liquid nitrogen. The pore size distribution was calculated using a non-linear density functional theory (NL-DFT) model. The samples were degassed for 6 hours before testing under a high vacuum at 80 °C. UV-Vis spectra were collected in a transparent 96-vial plate using a BioTek Synergy HT microplate reader (BioTek, Winooski, VT).
Table S1. Comparison of kinetic parameters of different MOFs.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate fixed</th>
<th>Substrate Varied</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (10^{-8} M S^{-1})</th>
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<tbody>
<tr>
<td>MOF-919 (Fe-Cu)</td>
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<td>OPD</td>
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<td>MOF-818 (Zr-Cu)</td>
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<td>84.65</td>
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<td>H$_2$O$_2$</td>
<td>OPD</td>
<td>2.26</td>
<td>13.49</td>
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<tr>
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<td>OPD</td>
<td>H$_2$O$_2$</td>
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<td>HKUST-1 (Cu)</td>
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<td>OPD</td>
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<td>OPD</td>
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<td>OPD</td>
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Table S2: Comparison of epinephrine detection with previous studies

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<tr>
<th>Catalyst</th>
<th>Linear range (μM)</th>
<th>Detection limit (μM)</th>
<th>Method</th>
<th>References</th>
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<tr>
<td>Au nanotube</td>
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<td>2.8</td>
<td>Electrochemical</td>
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<tr>
<td>Au 4Mpy AuNPs</td>
<td>10–60</td>
<td>4.5</td>
<td>Electrochemical</td>
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<td>Cu-tannic acid inorganic-organic nanohybrids (CTNs)</td>
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<td>3.4</td>
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<td>Cu-Cys NLs</td>
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<td>laccase-mineral hybrid microflowers (La-HMFs)</td>
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<td>0.6</td>
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<td>CMP-Pt/EG</td>
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<td>MOF-919 (Fe-Cu)</td>
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<td>0.298</td>
<td>Colorimetric</td>
<td>This work</td>
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**Figure S1.** FT-IR spectrum of MOF-919 (Fe-Cu).

**Figure S2.** TGA spectrum of MOF-919 (Fe-Cu).
Figure S3. (a) Nitrogen adsorption and desorption isotherms. (b) Pore size distribution curve of MOF-919 (Fe-Cu).
Figure S4. MOF-919 (Fe-Cu) crystal structure.
**Figure S5.** List of MOFs and their corresponding primary and secondary cluster type.

<table>
<thead>
<tr>
<th>MOF</th>
<th>Structure 1</th>
<th>Structure 2</th>
<th>Structure 3</th>
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<td>MIL-100 (Fe)</td>
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<td><img src="image2" alt="Structure" /></td>
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<td>$\text{Fe}_3(\mu_3-O)(\text{H}_2\text{O})_2\text{OH(BTC)}_2$</td>
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<td>HKUST-1 (Cu)</td>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td><img src="image9" alt="Structure" /></td>
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<tr>
<td>$\text{Cu}_3(\text{BTC})_2(\text{H}_2\text{O})_3$</td>
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<td>MOF-818 (Zr-Cu)</td>
<td><img src="image10" alt="Structure" /></td>
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<tr>
<td>$\text{Zr}_6(\mu_3-O)_4(\mu_3-\text{OH})_4(\text{OH})_6(\text{H}_2\text{O})_4[-\text{Cu}_3(\mu_3-O)(\mu-\text{PyC})_3(\text{H}_2\text{O})_6]_2$</td>
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<td>MOF-919 (Fe-Cu)</td>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
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<td>$[\text{Fe}_3(\mu_3-O)(\text{OH})_3]</td>
<td>\text{Cu}_3(\mu_3-O)(\mu-\text{PyC})_3(\text{H}_2\text{O})_6]_2$</td>
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Figure S6. PXRD pattern of MIL-100 (Fe).

Figure S7. PXRD pattern of MOF-808 (Zr).
Figure S8. PXRD pattern of HKUST-1 (Cu).

Figure S9. PXRD pattern of MOF-818 (Zr-Cu).
Figure S10. UV–Vis absorbance at 420 nm, (a) MOF-919 (Fe-Cu) with increasing concentration of OPD, (b) MOF-919 (Fe-Cu) with increasing concentration of OPD and 2 mM H$_2$O$_2$, (c) MOF-919 (Fe-Cu) with increasing concentration of H$_2$O$_2$ and 0.5 mM OPD.
Figure S11. Michaelis–Menten curves of steady-state kinetic assays. (a) Oxidase of increasing concentration of OPD without H$_2$O$_2$, (b) Peroxidase of increasing concentration of OPD with 2 mM H$_2$O$_2$, (c) Peroxidase of OPD with increasing concentration of H$_2$O$_2$. 
Figure S12. UV–Vis absorbance spectra, oxidase reaction condition: (a) MOF-919 (Fe-Cu) in PBS at pH=7.4, (b) MOF-919 (Fe-Cu) in 0.01M HEPES at pH 7.4, with increasing concentration of OPD after 10 min reaction time. Peroxidase reaction condition: (c) MOF-919 (Fe-Cu) in PBS at pH=7.4, (d) MOF-919 (Fe-Cu) in 0.01M HEPES at pH 7.4 and 2 mM H$_2$O$_2$, 1mg/mL of MOF-919 (Fe-Cu), and increasing concentration of OPD after 10 min reaction time.
Figure S13. (a) Schematic TMB oxidation reaction by MOF-919 (Fe-Cu). (b) TMB peroxidation by MOF-919 (Fe-Cu) in the presence of H$_2$O$_2$. Reaction conditions: 4 mM H$_2$O$_2$, 0.25 mM TMB, and MOF-919 (Fe-Cu) were incubated in 10 mM HAc–NaAc buffer (pH 5.0).
Figure S14. (a) Schematic representation of highly fluorescent molecule 2-hydroxyterephthalic acid (TA-OH) generation by MOF-919 (Fe-Cu). (b) Fluorescent spectrum of various reaction conditions (10 mM TA, 10 mM H$_2$O$_2$ and MOF-919 (Fe-Cu)), reaction medium in PBSx1 (pH 7.4).
Figure S15. UV–Vis absorption spectra of MOF-919 (Fe-Cu) reaction with catecholamine’s. (a). MOF-919 (Fe-Cu) in PBSx1 as blank, (b). MOF-919 (Fe-Cu) + 250 μM L-DOPA, (c). MOF-919 (Fe-Cu) + 250 μM Dopamine, (d). MOF-919 (Fe-Cu) + 250 μM Epinephrine, (e). MOF-919 (Fe-Cu) + 250 μM Norepinephrine, (f). MOF-919 (Fe-Cu) + 250 μM Phenylethylamine, (g). MOF-919 (Fe-Cu) + 250 μM Tyramine, (h). MOF-919 (Fe-Cu) + 250 μM Tyrosine, (i). MOF-919 (Fe-Cu) + 250 μM Ascorbic acid.
Figure S16. The selectivity of the MOF-919 (Fe-Cu) in the presence of various catecholamine and metabolite interferences at 250 μM concentration in PBSx1.
**Figure S17.** Reaction time optimization of MOF-919 (Fe-Cu) for the detection of 250 μM epinephrine based on UV–Vis absorbance at 480 nm.
Figure S18. UV–Vis absorbance intensity at 480 nm was compared in the presence of MOF-919 (Fe-Cu) reaction with Epi alone, Epi+L-DOPA, Epi+DA, and Epi+NE (25 μM concentration).
References


