

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

Sivasankar Kulandaivel, Chia-Her Lin * and Yi-Chun Yeh *

Department of Chemistry, National Taiwan Normal University, Taipei 11677,
Taiwan

*Corresponding authors: Chia-Her Lin and Yi-Chun Yeh

Email: chiaher@ntnu.edu.tw (Chia-Her Lin); Phone: +886277346221. Fax:
+886229324249.

E-mail: yichuny@ntnu.edu.tw (Yi-Chun Yeh). Phone: +886277346117. Fax:
+886229324249 ; ORCID: 0000-0002-8267-0360 (Yi-Chun Yeh)

Chemicals and materials

All chemicals were purchased from commercial suppliers without further purification unless otherwise mentioned. The iron chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was purchased from Alfa Aesar, and copper nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) was purchased from Showa chemicals. 4-Pyrazolecarboxylic ($\text{H}_2\text{-PyC}$) 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (0.1 M), 2.5 mL $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ 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 μ M), (2) Dopamine (250 μ M), (3) Epinephrine (250 μ M), and (4) Norepinephrine (250 μ M) 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 CuK α 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 N₂ 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.

Catalyst	Substrate fixed	Substrate Varied	K_m (mM)	V_{max} (10^{-8} M S ⁻¹)
MOF-919 (Fe-Cu)	-	OPD	8.99	29.60
	H ₂ O ₂	OPD	1.36	14.62
	OPD	H ₂ O ₂	0.24	4.15
MOF-818 (Zr- Cu)	-	OPD	49.87	84.65
	H ₂ O ₂	OPD	2.26	13.49
	OPD	H ₂ O ₂	0.17	0.26
HKUST-1 (Cu)	H ₂ O ₂	OPD	24.05	130
	OPD	H ₂ O ₂	2.201	3.01
MIL-100 (Fe)	H ₂ O ₂	OPD	1.06	2.92
	OPD	H ₂ O ₂	0.19	1.03
MOF-808 (Zr)	H ₂ O ₂	OPD	4.04	0.018
	OPD	H ₂ O ₂	10.02	0.75

Table S2: Comparison of epinephrine detection with previous studies

Catalyst	Linear range (μ M)	Detection limit (μ M)	Method	References
Au nanotube	10–150	2.8	Electrochemical	2
Au 4Mpy AuNPs	10–60	4.5	Electrochemical	3
Cu-tannic acid inorganic-organic nanohybrids (CTNs)	4.5-90	3.4	Colorimetric	4
Cu-Cys NLs	9-455	2.7	Colorimetric	5
laccase-mineral hybrid microflowers (La-HMFs)	1-400	0.6	Colorimetric	6
CMP-Pt/EG	4.55-172.98	0.66	Colorimetric	7
MOF-919 (Fe-Cu)	1-100	0.298	Colorimetric	This work

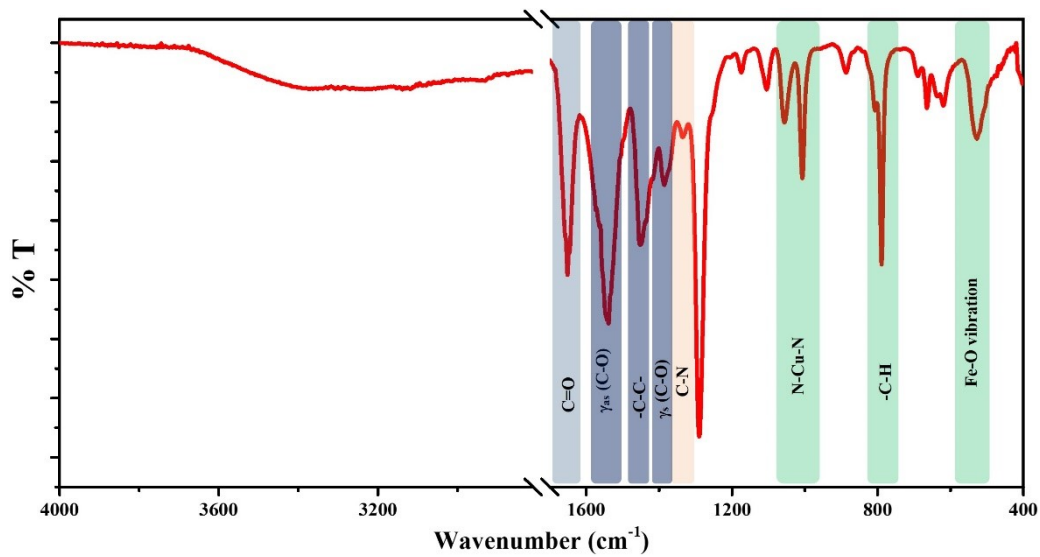


Figure S1. FT-IR spectrum of MOF-919 (Fe-Cu).

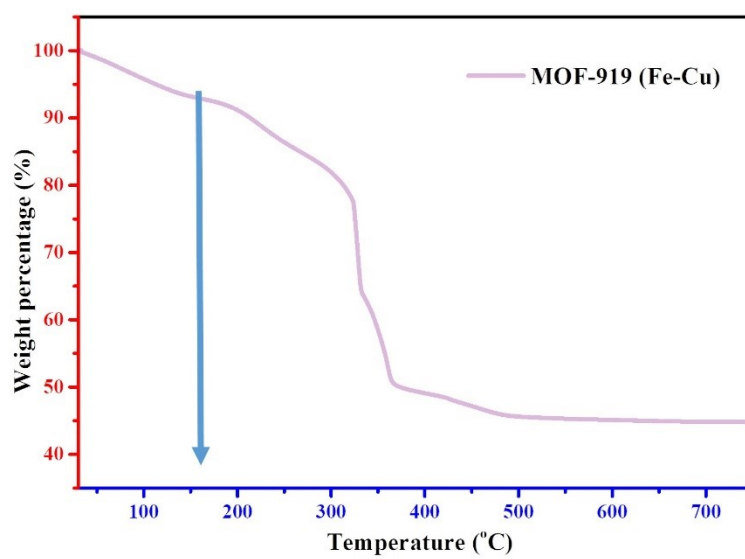


Figure S2. TGA spectrum of MOF-919 (Fe-Cu).

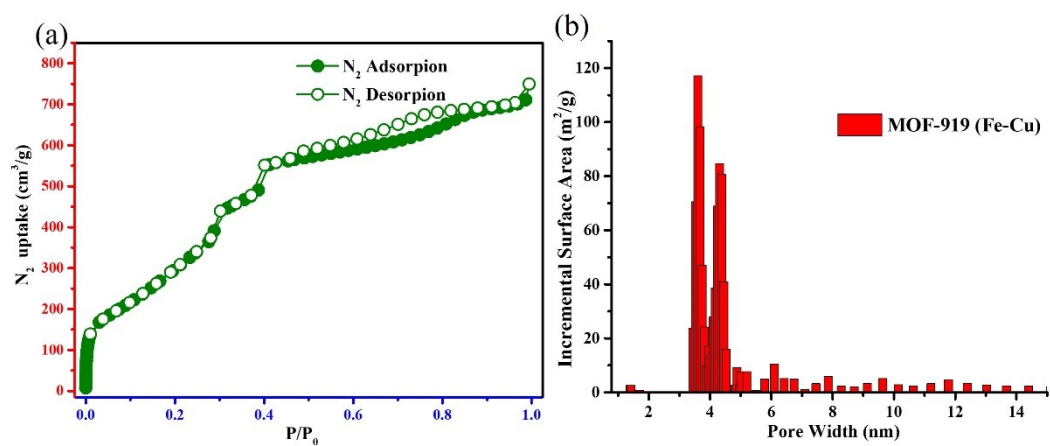


Figure S3. (a) Nitrogen adsorption and desorption isotherms. (b) Pore size distribution curve of MOF-919 (Fe-Cu).

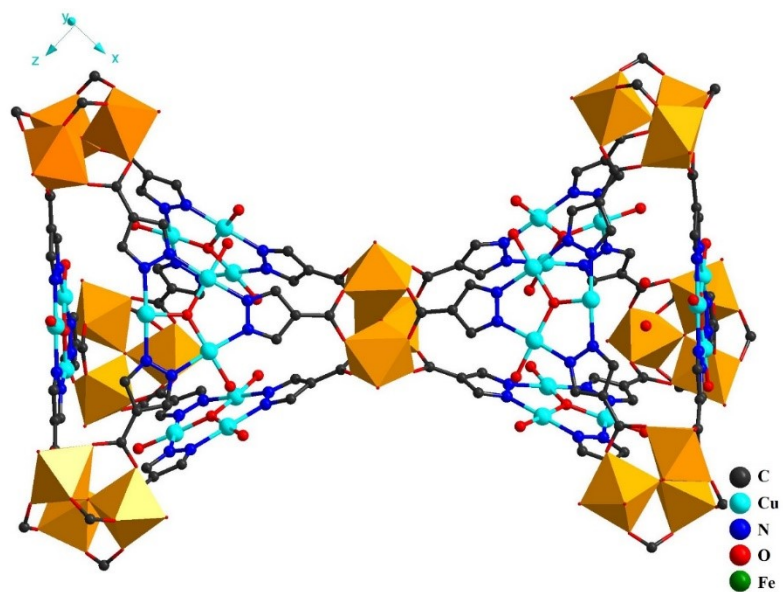


Figure S4. MOF-919 (Fe-Cu) crystal structure.

MIL-100 (Fe) $\text{Fe}_3(\mu_3\text{-O})(\text{H}_2\text{O})_2\text{OH}(\text{BTC})_2$			
MOF-808 (Zr) $\text{Zr}_6\text{O}_4(\text{OH})_4(\text{BTC})_2(\text{HCOO})_6$			
HKUST-1 (Cu) $\text{Cu}_3(\text{BTC})_2(\text{H}_2\text{O})_3$			
MOF-818 (Zr-Cu) $\text{Zr}_6(\mu_3\text{-O})_4(\mu_3\text{-OH})_4(\text{OH})_6(\text{H}_2\text{O})_6\text{-}[\text{Cu}_3(\mu_3\text{-O})(\mu\text{-PyC})_3(\text{H}_2\text{O})_6]_2$			
MOF-919 (Fe-Cu) $[\text{Fe}_3(\mu_3\text{-O})(\text{OH})_3][\text{Cu}_3(\mu_3\text{-O})(\mu\text{-PyC})_3(\text{H}_2\text{O})_6]_2$			

Figure S5. List of MOFs and their corresponding primary and secondary cluster type.

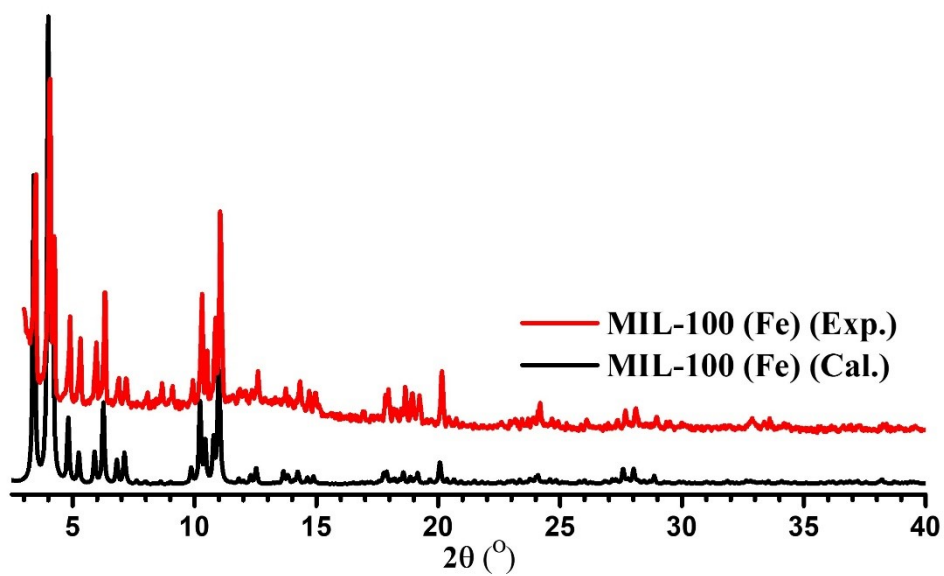


Figure S6. PXRD pattern of MIL-100 (Fe).

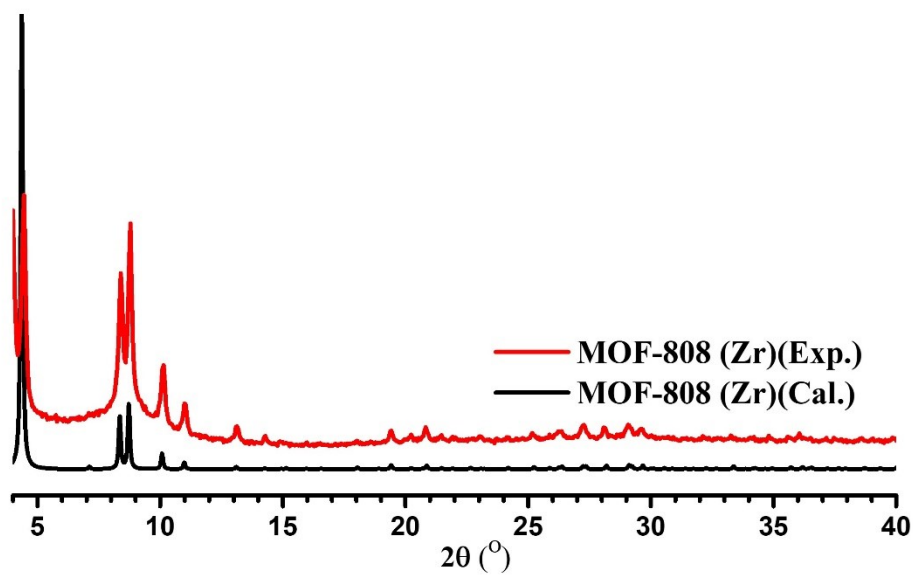


Figure S7. PXRD pattern of MOF-808 (Zr).

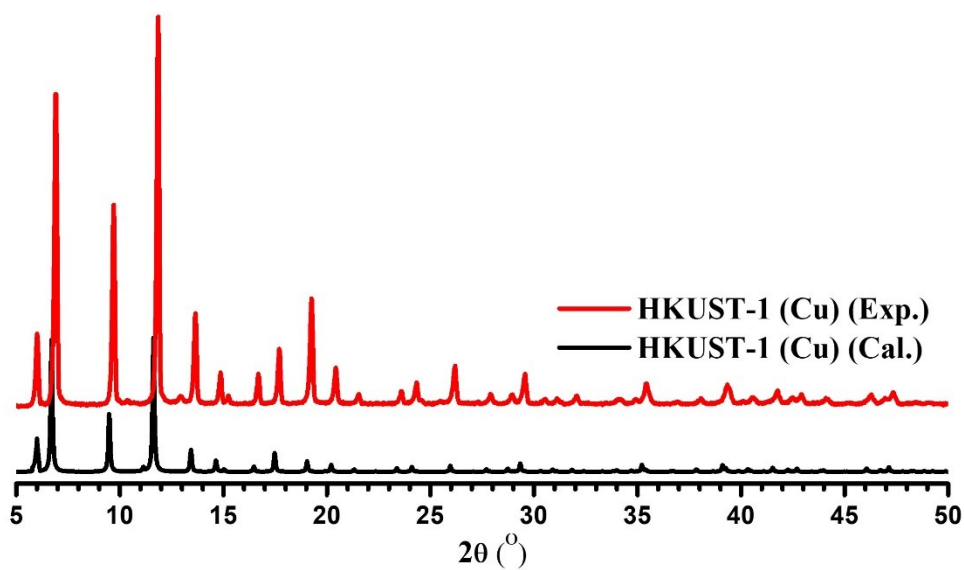


Figure S8. PXRD pattern of HKUST-1 (Cu).

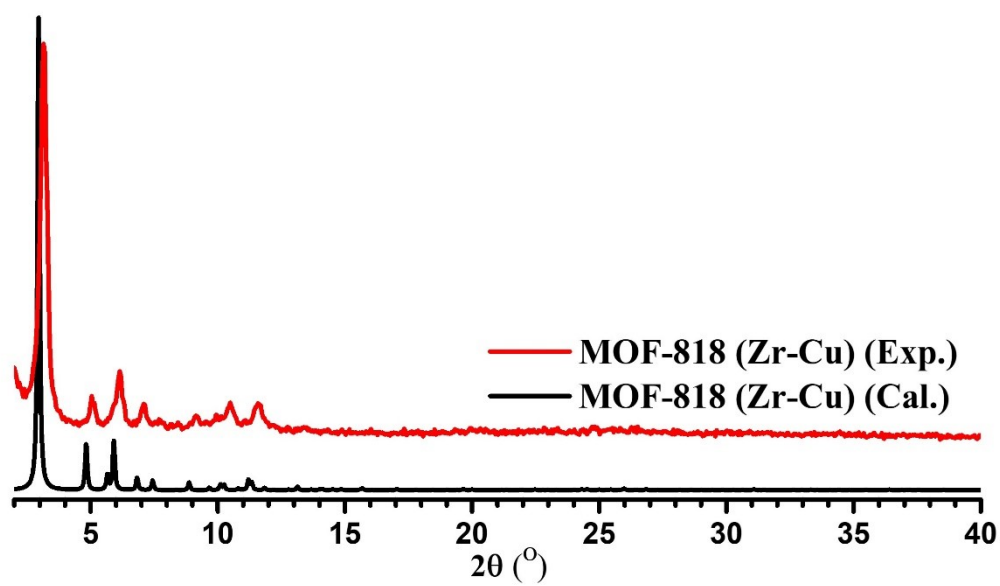


Figure S9. PXRD pattern of MOF-818 (Zr-Cu).

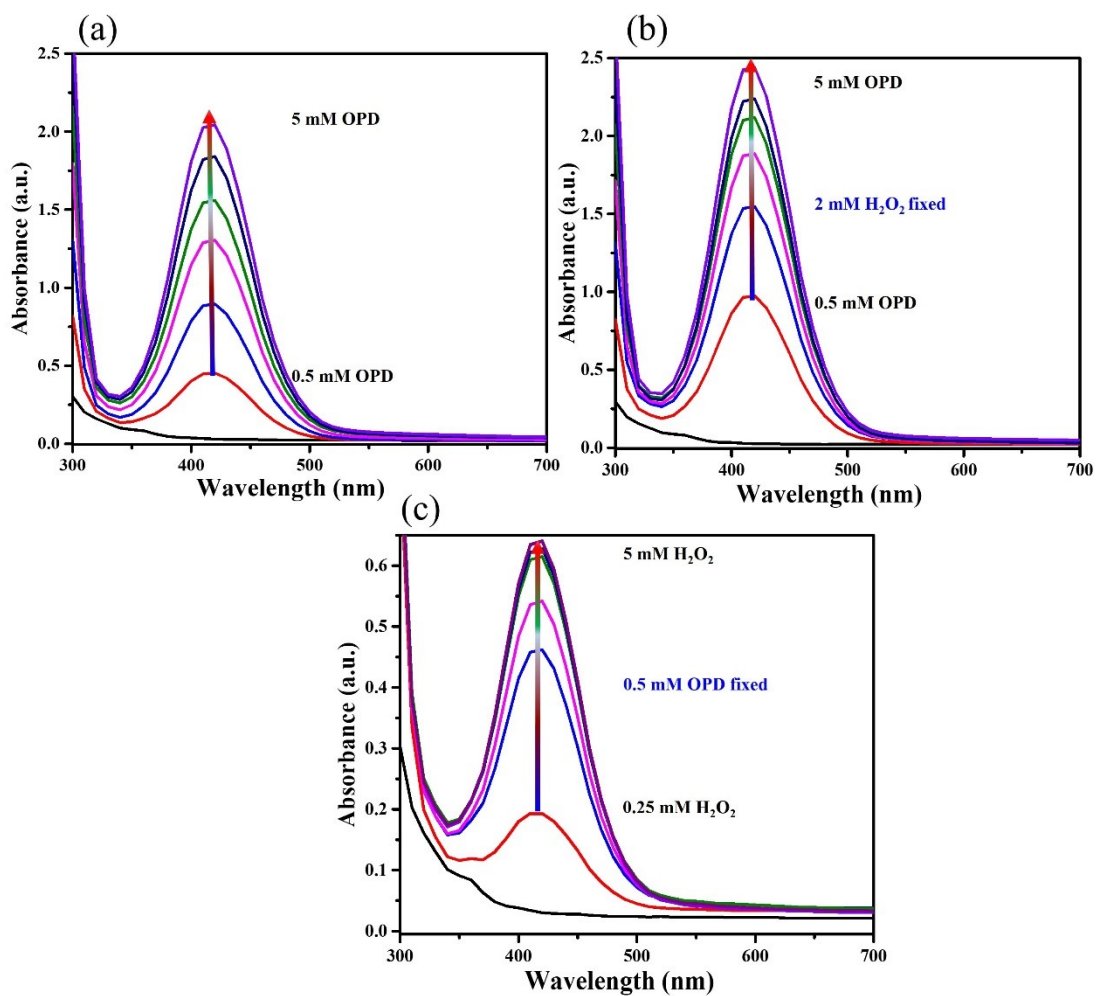


Figure S10. UV-Vis absorbance at 420 nm, (a) MOF-919 (Fe-Cu) with increasing concentration of OPD, (b) MOF-919 (Fe-Cu) with increasing concentration of OPD and 2 mM H₂O₂, (c) MOF-919 (Fe-Cu) with increasing concentration of H₂O₂ and 0.5 mM OPD.

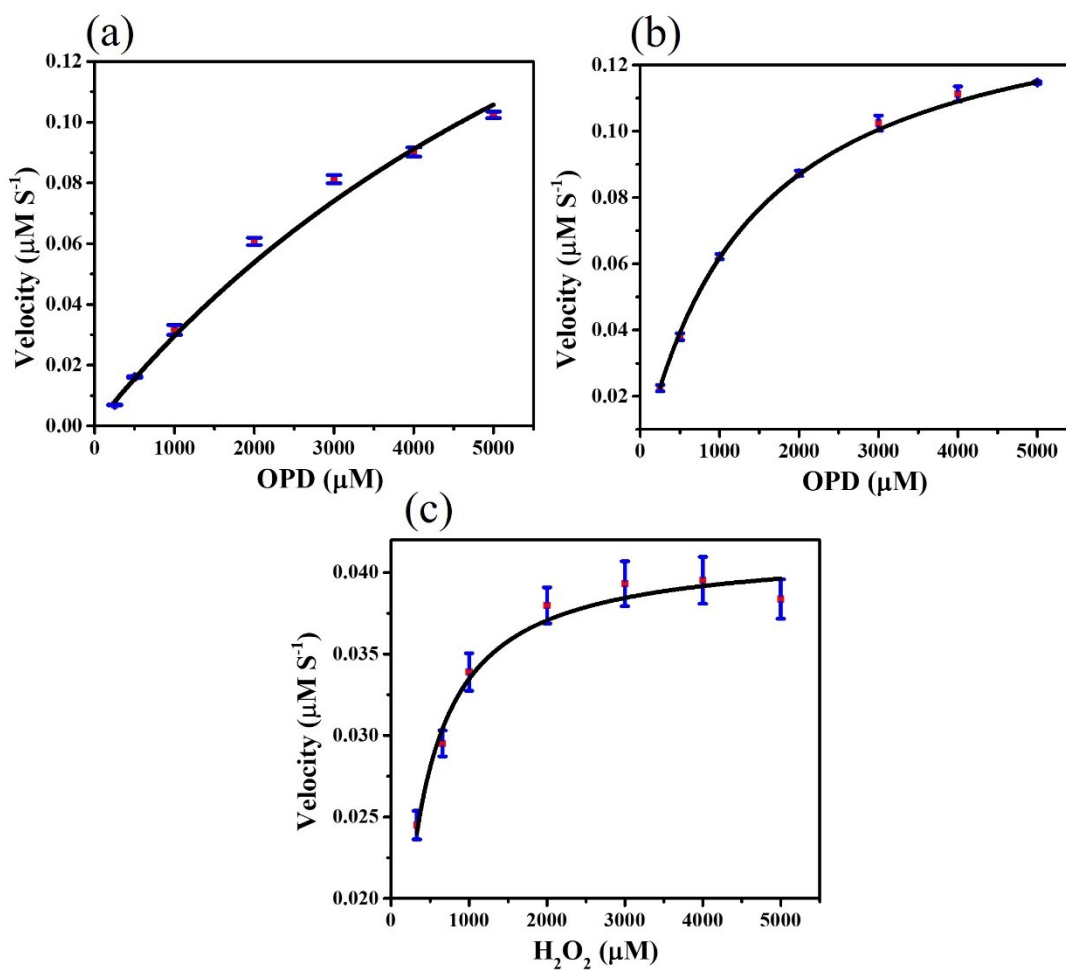


Figure S11. Michaelis–Menten curves of steady-state kinetic assays. (a) Oxidase of increasing concentration of OPD without H_2O_2 , (b) Peroxidase of increasing concentration of OPD with 2 mM H_2O_2 , (c) Peroxidase of OPD with increasing concentration of H_2O_2 .

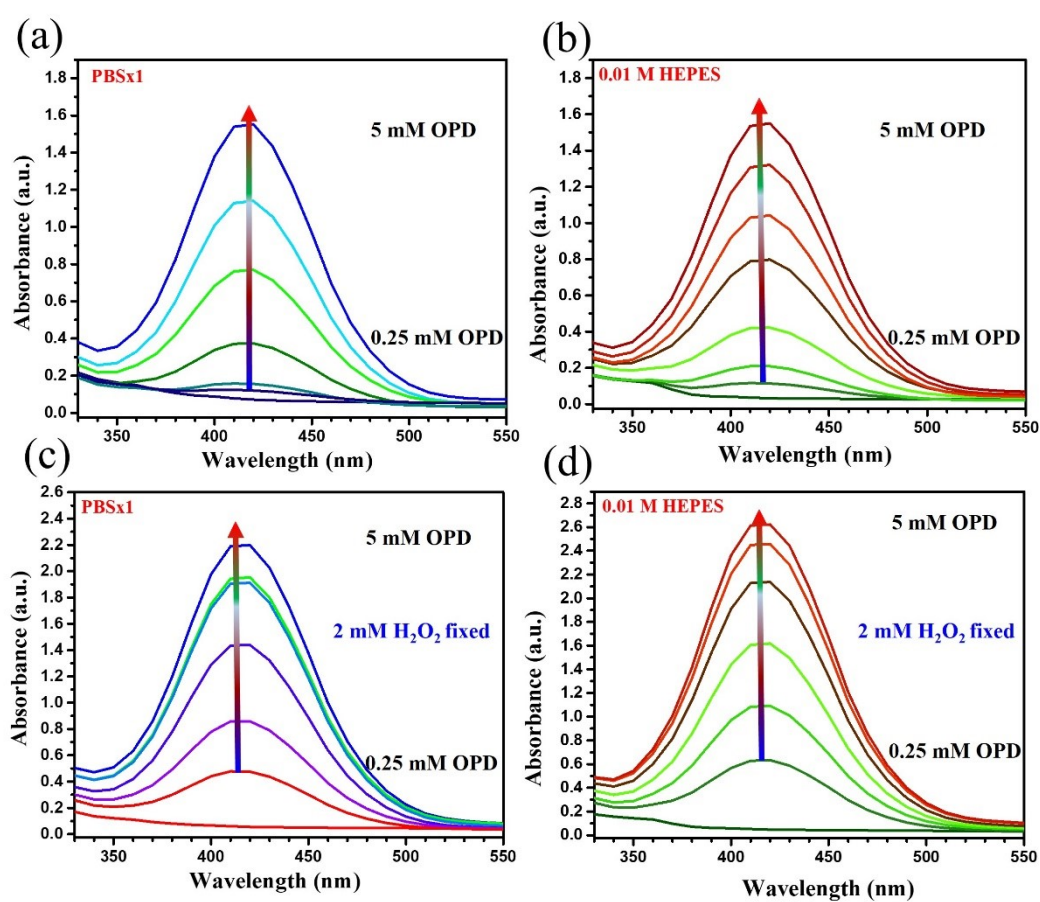


Figure S12. UV-Vis absorbance spectra, oxidase reaction condition: (a) MOF-919 (Fe-Cu) in PBS at pH=7.4, (b) MOF-919 (Fe-Cu) in 0.01M HEPES at pH 7.4, with increasing concentration of OPD after 10 min reaction time. Peroxidase reaction condition: (c) MOF-919 (Fe-Cu) in PBS at pH=7.4, (d) MOF-919 (Fe-Cu) in 0.01M HEPES at pH 7.4 and 2 mM H₂O₂, 1mg/mL of MOF-919 (Fe-Cu), and increasing concentration of OPD after 10 min reaction time.

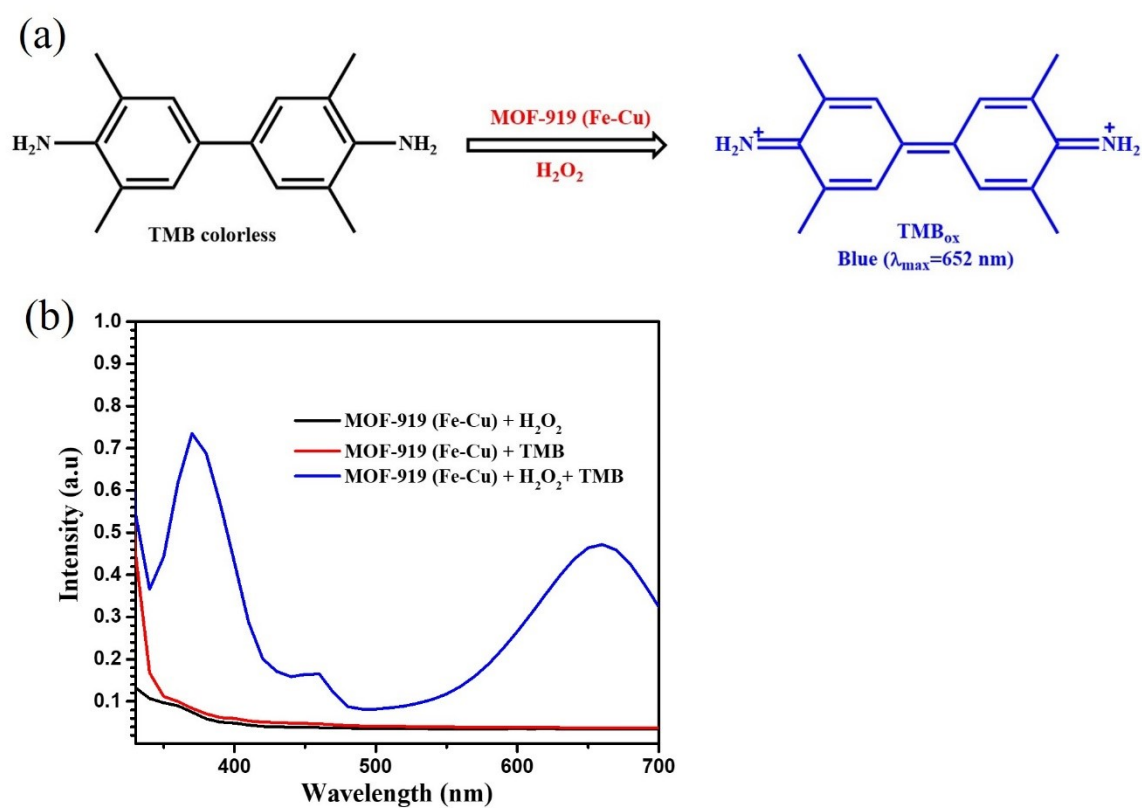


Figure S13. (a) Schematic TMB oxidation reaction by MOF-919 (Fe-Cu). (b) TMB peroxidation by MOF-919 (Fe-Cu) in the presence of H₂O₂. Reaction conditions: 4 mM H₂O₂, 0.25 mM TMB, and MOF-919 (Fe-Cu) were incubated in 10 mM HAc–NaAc buffer (pH 5.0).

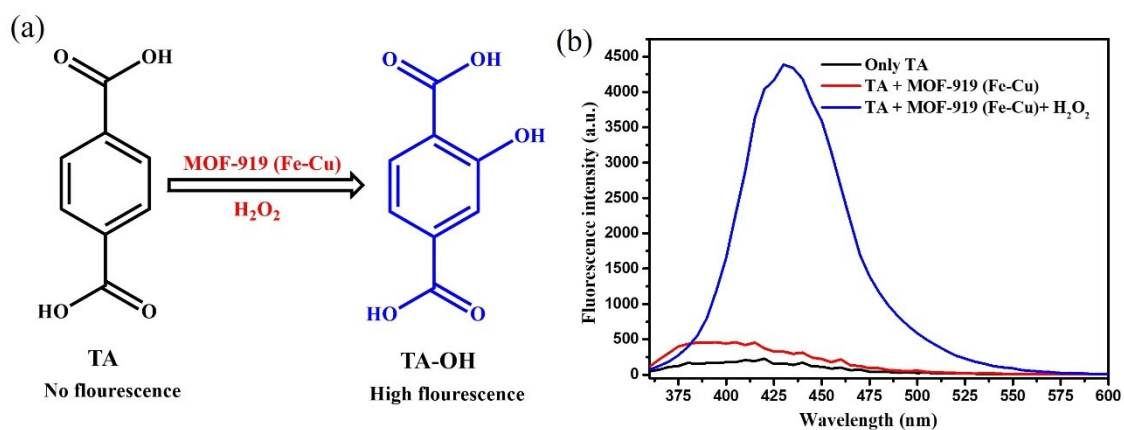


Figure S14. (a) Schematic representation of highly fluorescent molecule 2-hydroxyterephthalic acid (TA-OH) generation by MOF-919 (Fe-Cu). (b) Fluorescent spectrum of various reaction conditions (10 mM TA, 10 mM H₂O₂ and MOF-919 (Fe-Cu)), reaction medium in PBSx1 (pH 7.4).

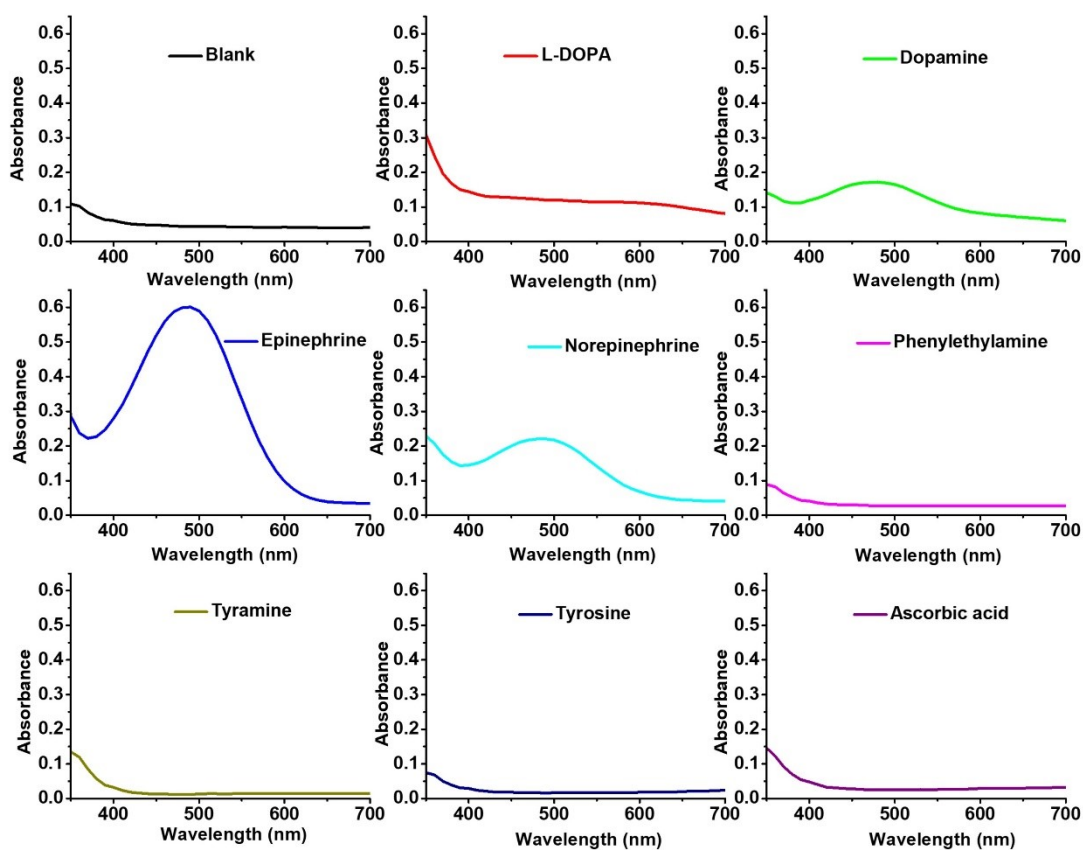


Figure S15. UV-Vis absorption spectra of MOF-919 (Fe-Cu) reaction with catecholamine's. (a). MOF-919 (Fe-Cu) in PBSx1 as blank, (b). MOF-919 (Fe-Cu) + 250 μ M L-DOPA, (c). MOF-919 (Fe-Cu) + 250 μ M Dopamine, (d). MOF-919 (Fe-Cu) + 250 μ M Epinephrine, (e). MOF-919 (Fe-Cu) + 250 μ M Norepinephrine, (f). MOF-919 (Fe-Cu) + 250 μ M Phenylethylamine, (g). MOF-919 (Fe-Cu) + 250 μ M Tyramine, (h). MOF-919 (Fe-Cu) + 250 μ M Tyrosine, (i). MOF-919 (Fe-Cu) + 250 μ M Ascorbic acid.

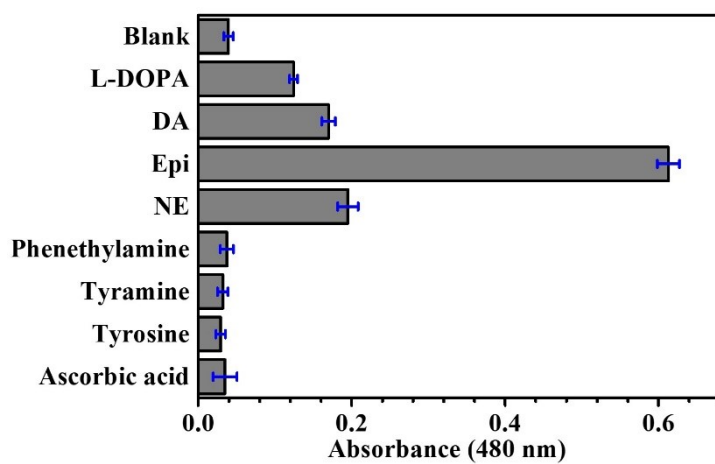


Figure S16. The selectivity of the MOF-919 (Fe-Cu) in the presence of various catecholamine and metabolite interferences at 250 μM concentration in PBSx1.

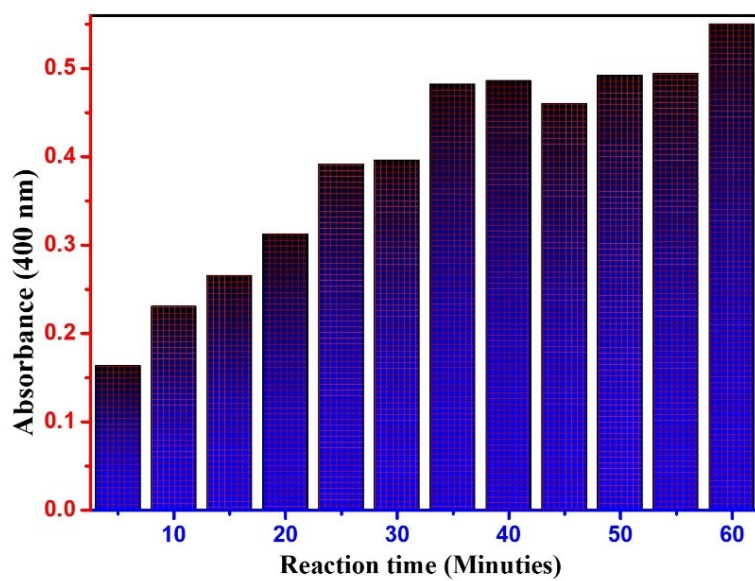


Figure S17. Reaction time optimization of MOF-919 (Fe-Cu) for the detection of 250 μM epinephrine based on UV-Vis absorbance at 480 nm.

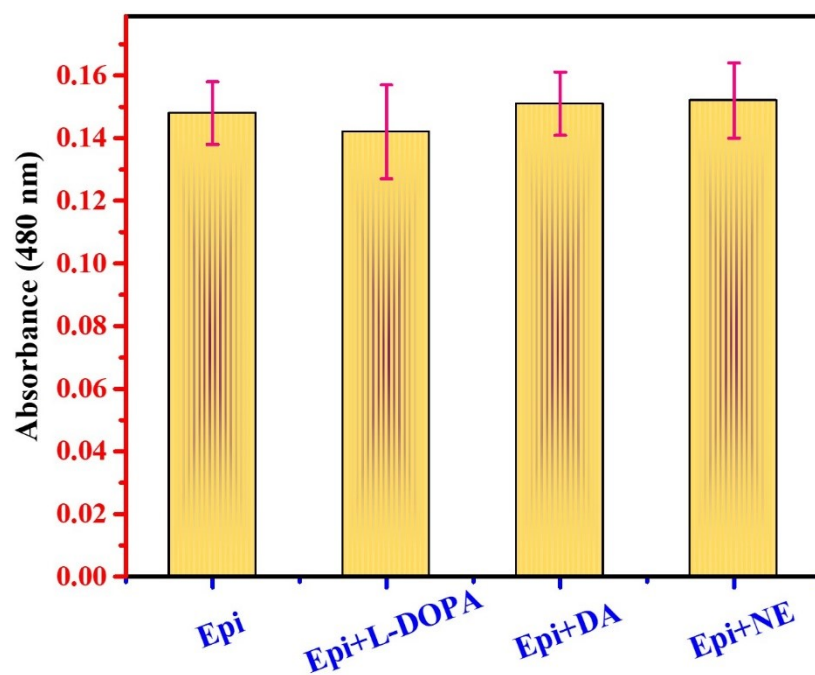


Figure S18. UV–Vis absorbance intensity at 480 nm was compared in the presence of MOF-919 (Fe-Cu) reaction with Epi alone, Epi+L-DOPA, Epi+DA, and Epi+NE (25 μ M concentration).

References

1. Q. Liu, Y. Song, Y. Ma, Y. Zhou, H. Cong, C. Wang, J. Wu, G. Hu, M. O’Keeffe and H. Deng, *Journal of the American Chemical Society*, 2018, **141**, 488-496.
2. E. Wierzbicka, M. Szultka-Młyńska, B. Buszewski and G. D. Sulka, *Sensors and Actuators B: Chemical*, 2016, **237**, 206-215.
3. S. Ramirez, N. Silva, M. P. Oyarzun, J. Pavez and J. F. Silva, *Journal of Electroanalytical Chemistry*, 2017, **799**, 349-357.
4. H. Ma, N. Zheng, Y. Chen and L. Jiang, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2021, **613**, 126105.
5. M. Guan, M. Wang, W. Qi, R. Su and Z. He, *Frontiers of Chemical Science and Engineering*, 2021, **15**, 310-318.
6. M. Zhang, Y. Zhang, C. Yang, C. Ma and J. Tang, *Talanta*, 2021, **224**, 121840.
7. Y. Gao, G. Wang, H. Gu, J. Zhang, W. Li and Y. Fu, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2021, **613**, 126070.