Enzyme-immobilized metal-organic framework nanosheets as tandem catalysts for generation of nitric oxide

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Experimental

Materials and reagents. Cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$, 98%) and pyrazine (99%), were purchased from Alfa Aesar. N,N-dimethylformamide (DMF, 99.8%), ethanol (99.9%), glucose and hydrogen peroxide (H_2O_2, 30%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Fe(III) tetra(4-carboxyphenyl) porphine chloride (FeTCPP, 97%) and Tetrakis(4-carboxyphenyl)porphyrin (TCPP) were purchased from Frontier Scientific (Logan, Utah, USA). Polyvinylpyrrolidone (PVP, MW=40,000 g/mol), L-arginine (L-Arg), (3-aminopropyl)triethoxysilane (APTES), glucose oxidase (GOD) and fluorimetric hydrogen peroxide assay kit were obtained from Sigma-Aldrich Inc. Griess assay kit was purchased from Promega Corporation (USA). 3-amino,4-aminomethyl-2′,7′-difluorescein diacetate (DAF-FM DA) was obtained from beyotime Biotechnology (Nantong, China). Phosphate buffer saline (PBS, 0.1 M, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, and 1.41 mM KH_2PO_4. All other reagents were purchased from commercial vendors and used without further purification unless otherwise noted. All used solutions were prepared in Milli-Q water (18.2 MΩ·cm, Milli-Q System, Millipore, USA).

Apparatus. The Powder X-ray diffraction (pXRD) experiments were obtained on a Bruker D8 Advance X-ray powder diffractometer operating at 40 kV and 40 mA (CuKα radiation, λ = 1.5418 Å). Zeta potential was recorded on a ZetaPALS (90Plus zeta, Brookhaven, USA). The scanning electron microscope (SEM) images were conducted on an S-4800 scanning electron microscope (Hi-tachi, Japan). Transmission electron micrographs (TEM) were conducted on a JEOL JEM-2010 transmission electron microscope operating at an accelerating voltage of 200 kV. UV-vis spectra were performed with a lambda-35 UV-Vis spectrophotometer (PerkinElmer, USA). Fluorescence was measurement on a LS-55 (PerkinElmer, USA). The H_2O_2 in water was measured by a H_2O_2 assay kit (Sigma-Aldrich Inc). The NO in water was measured by a Griess assay kit (Promega Corporation, USA). All dates were done three times.

Preparation of Co-FeMOF and Co-MOF. The Co-FeMOF was synthesized based on the previous work[S1] with minor modifications. Typically, $Co(NO_3)_2 \cdot 6H_2O$ (0.015 mmol, 4.4 mg), pyrazine (0.01 mmol, 0.8 mg) and PVP (20 mg) were dissolved in DMF/ethanol (V:V = 3:1) in a 20 mL capped vial and sonicated for 10 min. Then, the FeTCPP (0.005 mmol, 4.4 mg) dissolved in 4 mL DMF/ethanol (V:V = 3:1) was mixed with the aforementioned solution. The mixture solution was sonicated for another 15 min. After that, the capped vial was heated at 80 °C for 24 h. After cooling to room temperature, the solid MOFs were washed with fresh ethanol for two times and re-dispersed in ethanol for the further using. Co-MOF was prepared only by TCPP (0.005 mmol) instead of FeTCPP.

Synthesis of GOD@Co-FeMOF. To prepare GOD@Co-FeMOF, GOD was firstly added into the mixture of 10 mg EDC and 15 mg (2.0 mL) and then added 10 μL APTES, followed by stirring for 4 h at room temperature to obtain the amino on the surface of the GOD. Then 200 μL Co-FeMOF, 50 μL amino-functionalized GOD was added into 1.0 mL H_2O. The above solution was shaking at room temperature overnight. Afterward, the mixture was washed three time with water by centrifugation and re-dispersed in 200 μL H_2O for the further use.

Fluorescence measurement. For the fluorescence spectra, 1.0 mg/mL GOD@Co-FeMOF was added into the mixture of 20 mM L-arginine and 1.0 mg/mL glucose in a pH 7.4 PBS buffer. Then, 5.0 mM DAF-FM DA was added to the reaction solution. After centrifuging, fluorescence spectra of the soultion were measured with the excitation wavelength of 485 nm and the emssion wavelength of 515 nm. The intensities of 515 nm were monitored continuously without centrifuging during the catalytic reaction.

Supporting figures

UV−vis absorption spectra of FeTCPP and Co-FeMOF.

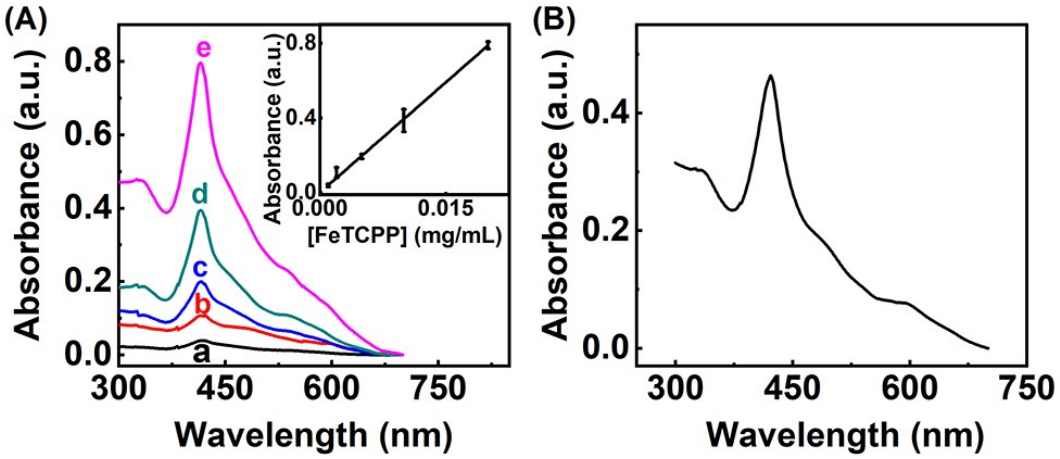
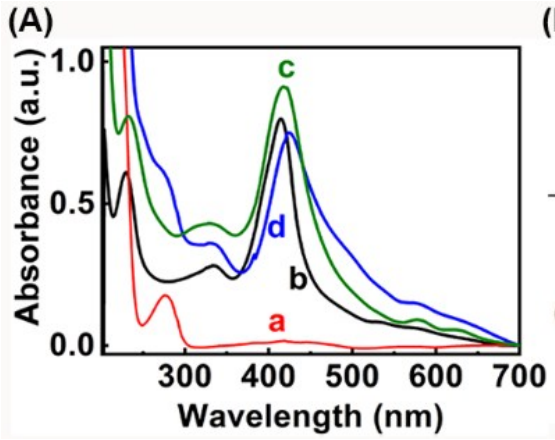


Fig. S1 (A) UV−vis absorption spectra of FeTCPP with different concentration, 0.001 (a), 0.002 (b), 0.005 (c), 0.01 (d) and 0.02 mg/mL (e). Inset: the UV-vis absorption of FeTCPP with a standard curve. (B) UV−vis absorption spectrum of Co-FeMOF.

UV−vis absorption spectra and Zeta potential of GOD, FeTCPP, Co-FeMOF and GOD@Co-FeMOF.



(B) Zeta potential

Materials	Zeta potential (mV)
GOD	-4.54
FeTCPP	-24.36
GOD@Co-FeMOF	-29.53
Co-FeMOF	-16.76

Fig. S2 (A) UV−vis absorption spectra of GOD (a), FeTCPP (b), Co-FeMOF (c) and GOD@Co-FeMOF (d). (B) Zeta potentials of GOD, FeTCPP, GOD@Co-FeMOF and Co-FeMOF.

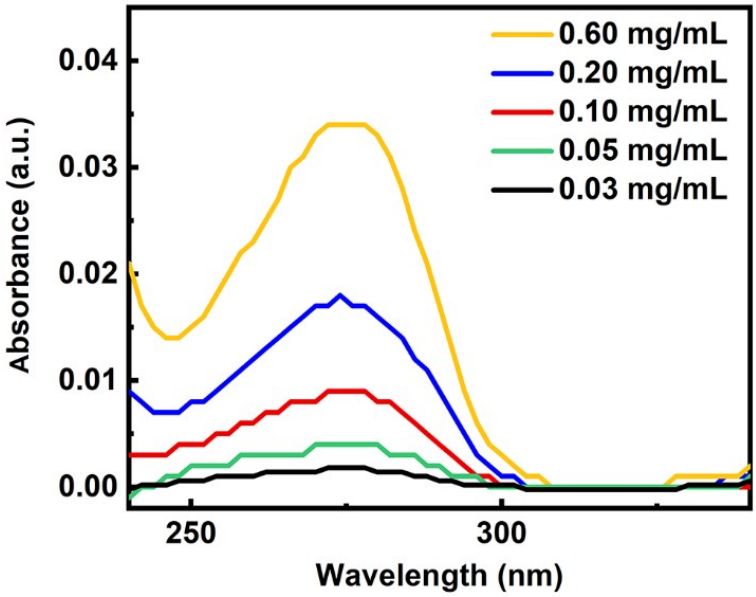


Fig. S3 UV−vis absorption spectra of GOD with different concentration.

FL of Co-MOF and Co-FeMOF

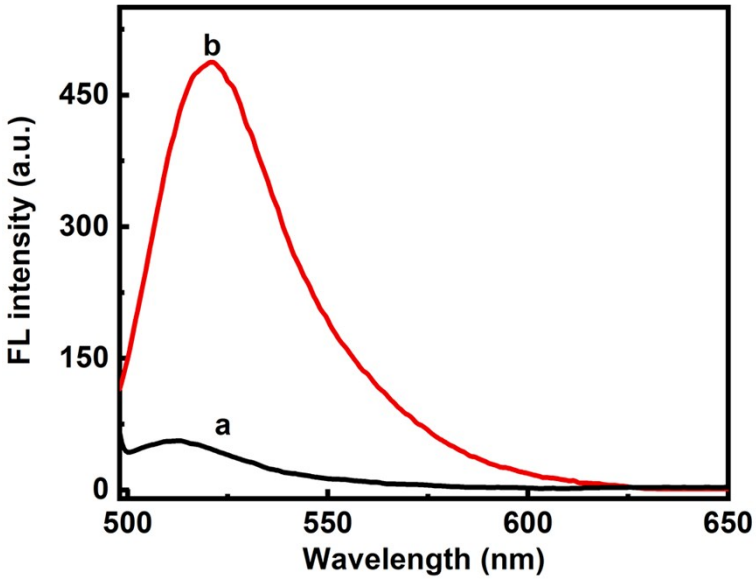


Fig. S4 FL of DAF-FM DA with 1.0 mg/mL Co-MOF (a) and Co-FeMOF (b) in 0.1 M PBS containing 5.0 mM H_2O_2 and 20 mM L-Arg. E_x=485 nm, E_m=515 nm.

H_2O_2 concentrations generated with GOD and Co-FeMOF@GOD.

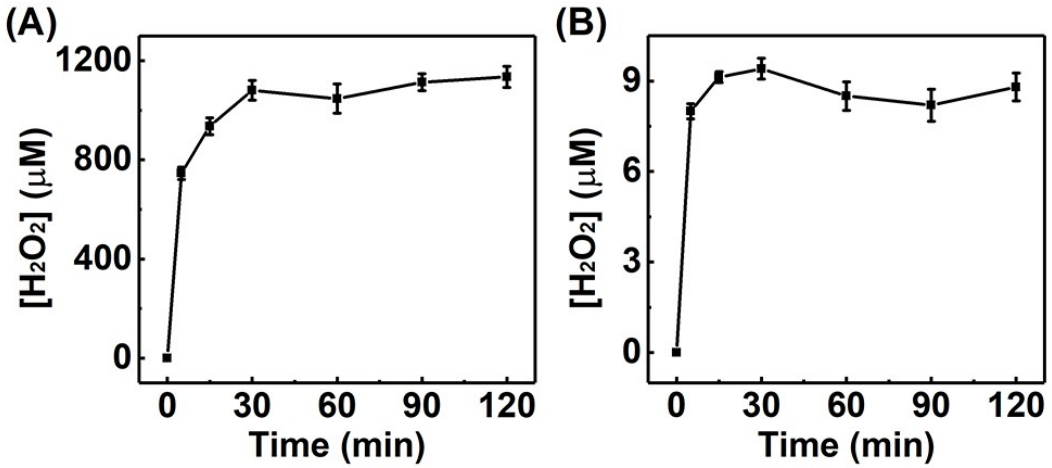


Fig. S5 The generated H_2O_2 concentrations at different time arising from (A) GOD (2.0 mg/mL) and (B) GOD@Co-FeMOF (1.0 mg/mL) catalyzed decomposition reaction of glucose (1.0 mg/mL).

Determination of L-citrulline

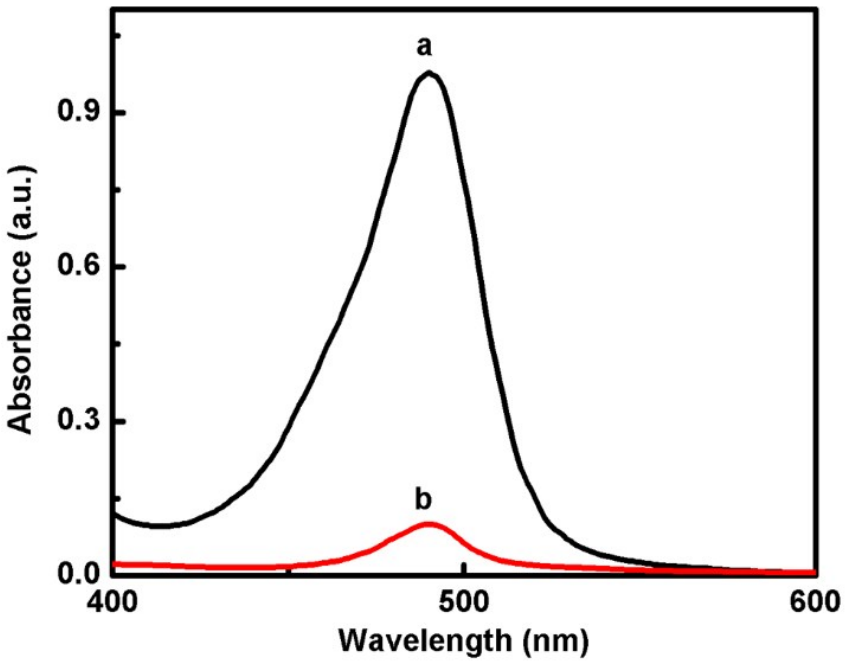


Fig. S6 UV−vis absorption spectra of 100 mM L-citrulline (a) and 20 mM L-Arginine + GOD@Co-FeMOF +1.0 mg/mL glucose (b) in 3.0 M H_2SO_4 solution containing 50 mM diacetylmonoxime.

Kinetic curves.

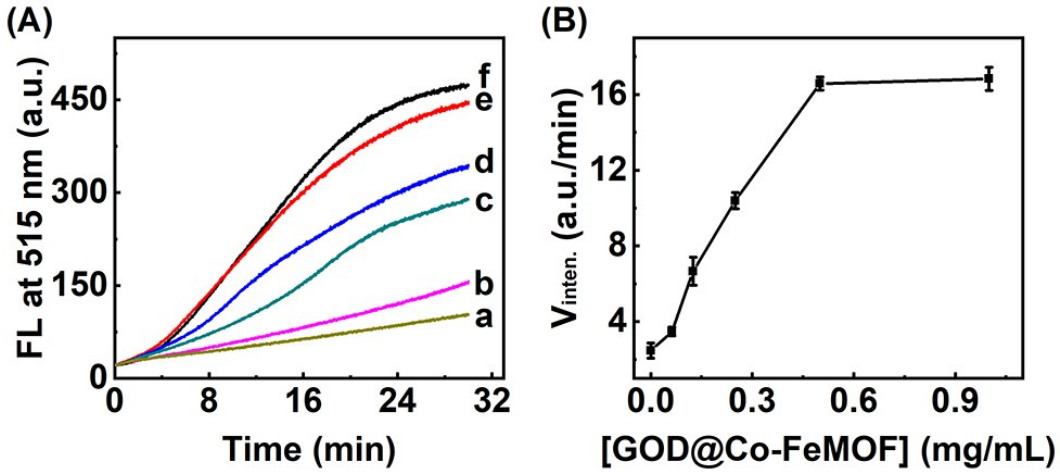


Fig. S7 (A) Kinetic curves plotting the time-dependent fluorescence emission intensity at 515 nm and (B) the rates of NO generation catalyzed by different GOD@Co-FeMOF concentrations, (a), 0.0625 (b), 0.125 (c), 0.25 (d), 0.5 (e) and 1.0 mg/mL (f) in the presence of glucose 1.0 mg/mL and L-Arg 20 mM. (E_x=485 nm).

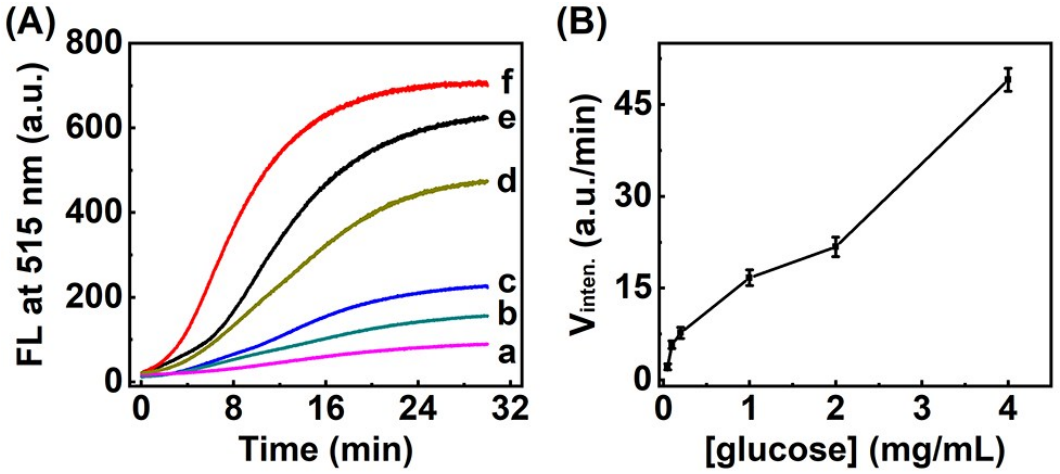


Fig. S8 (A) Kinetic curves plotting the time-dependent fluorescence emission intensity at 515 nm and (B) the rates of NO generation catalyzed by 1.0 mg/mL GOD@Co-FeMOF in the presence of 20 mM L-Arg, and variable concentrations of glucose, 0.05 (a), 0.1 (b), 0.2 (c), 1.0 (d), 2.0 (e) and 4.0 mg/mL (f). (E_x=485 nm).

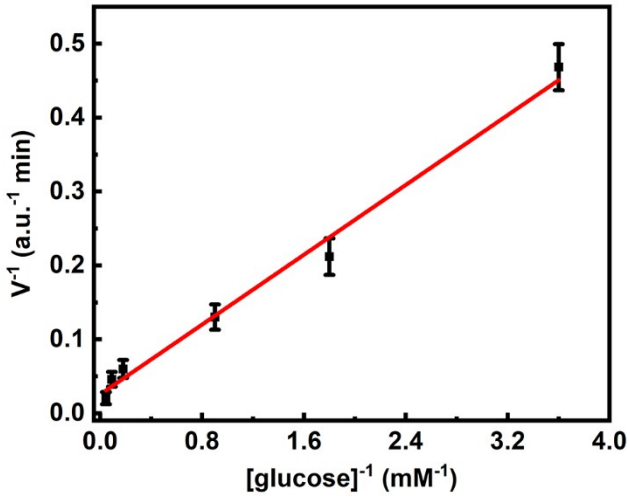


Fig. S9 Linear fitting of Michaelis-Menten curve for glucose oxidation catalyzed by 1.0 mg/mL GOD@Co-FeMOF in the presence of 20 mM L-Arg, and variable concentrations of glucose, 0.05 (a), 0.1 (b), 0.2 (c), 1.0 (d), 2.0 (e) and 4.0 mg/mL (f). (Ex=485 nm)

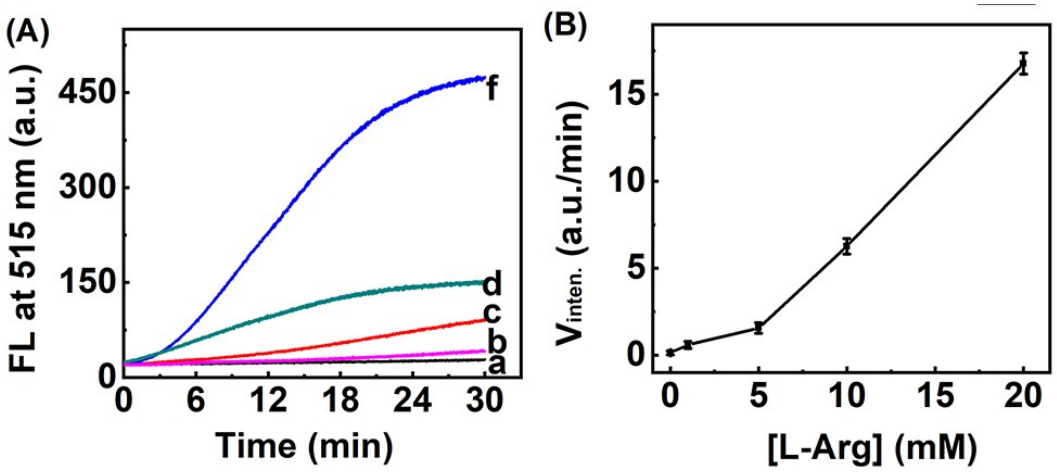


Fig. S10 (A) Kinetic curves plotting the time-dependent fluorescence emission intensity at 515 nm and (B) the rates of NO generation catalyzed by 1.0 mg/mL GOD@Co-FeMOF in the presence of 1.0 mg/mL glucose, and variable concentrations of L-Arg, 0 (a), 1.0 (b), 5.0 (c), 10 (d), and 20 mM (e). (Ex=485 nm)

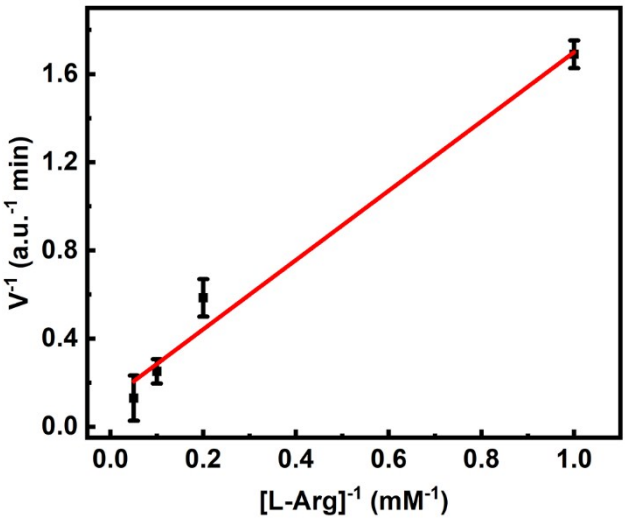


Fig. S11 Linear fitting of Michaelis-Menten curves for L-Arg oxidation catalyzed by 1.0 mg/mL GOD@Co-FeMOF in the presence of 1.0 mg/mL glucose, and variable concentrations of L-Arg, 0 (a), 1.0 (b), 5.0 (c), 10 (d), and 20 mM (e). (Ex=485 nm)

The generated NO concentrations.

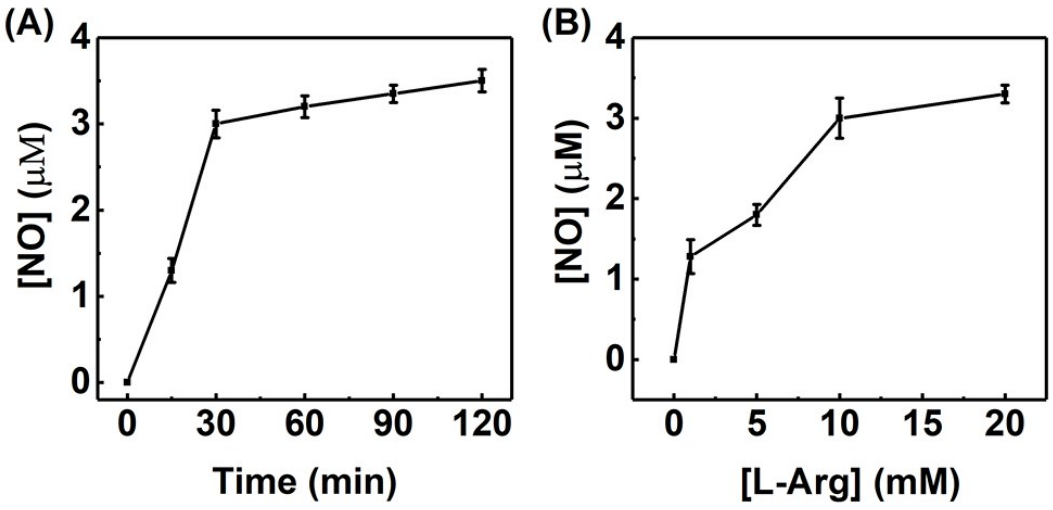


Fig. S12 The generated NO concentrations (A) at different time points and (B) arising from different concentrations of L-Arg, 0, 1.0, 5, 10 and 20 mM in the presence of 1.0 mg/mL GOD@Co-FeMOF and 1.0 mg/mL glucose.

The stability of GOD@Co-FeMOF.

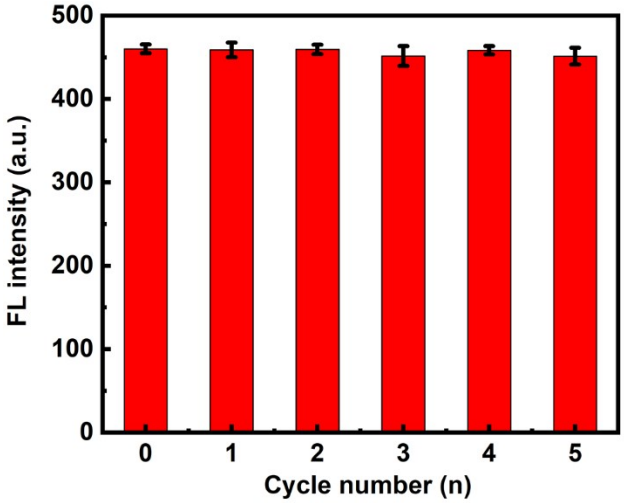


Fig. S13 Fluorescence intensity of DAF-FM DA with 1.0 mg/mL GOD@Co-FeMOF for different cycle in 0.1 M pH =6.9 PBS containing 5.0 mM H_2O_2 and 20 mM L-Arg. E_x=485 nm, E_m=515 nm.

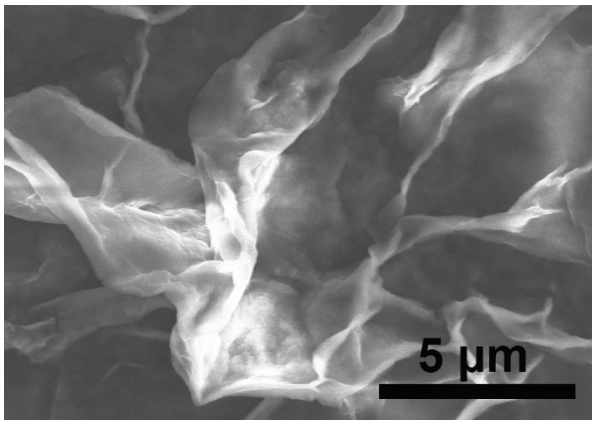


Fig. S14 SEM image of GOD@Co-FeMOF after five cycles in 0.1 M pH 6.9 PBS.

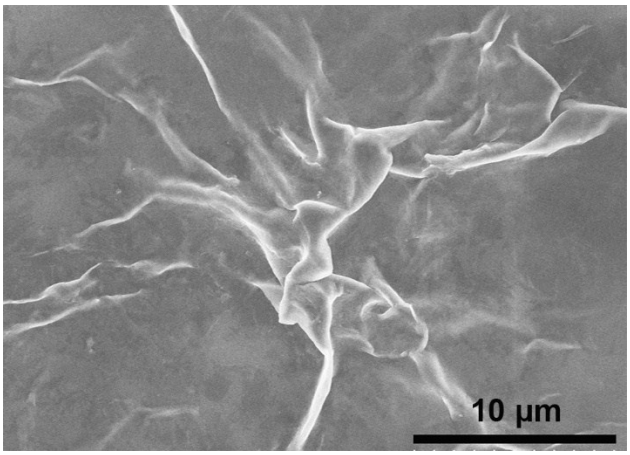


Fig. S15 SEM image of GOD@Co-FeMOF after five cycles in 0.1 M pH 7.4 PBS.

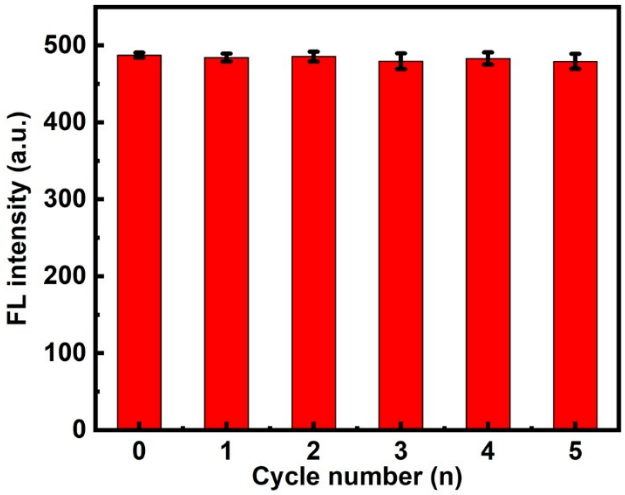


Fig. S16 Fluorescence intensity of DAF-FM DA with 1.0 mg/mL GOD@Co-FeMOF for different cycles in 0.1 M pH =7.4 PBS containing 5.0 mM H_2O_2 and 20 mM L-Arg. E_x=485 nm, E_m=515 nm.

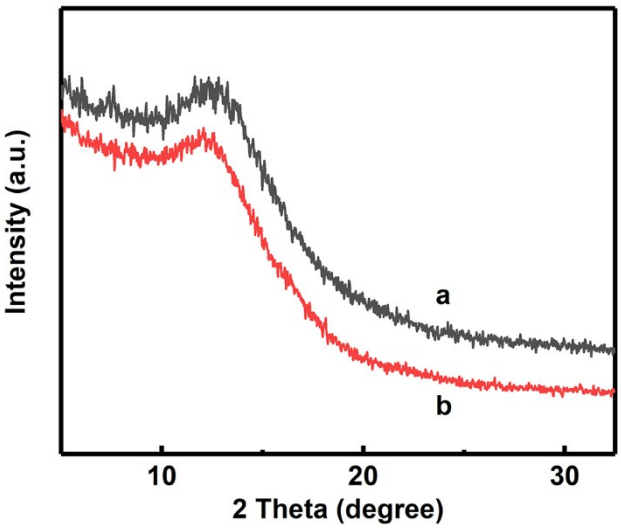


Fig. S17 Powder X-ray di□raction patterns of the GOD@Co-FeMOF for five catalytic cycles in 0.1 M pH =7.4 PBS (a) and 0.1 M pH=6.9 PBS (b) containing 5.0 mM H_2O_2 and 20 mM L-Arg.

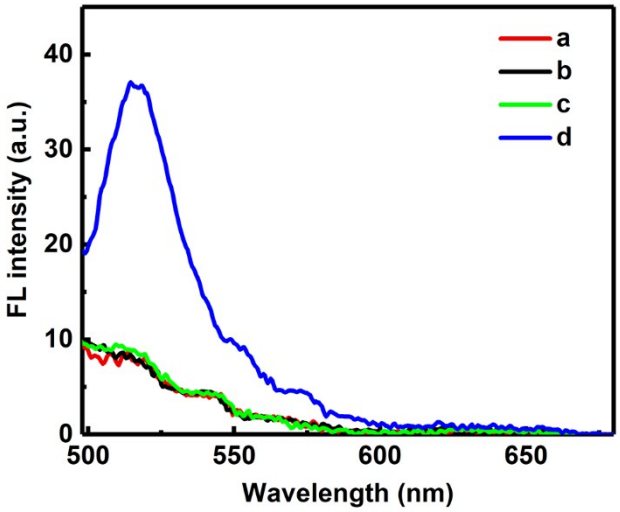


Fig. S18 FL of DAF-FM DA with serum (a), serum + L-Arg (b), GOD@Co-FeMOF + serum (c) and GOD@Co-FeMOF + serum + L-Arg (d) for 30 min, respectively. 20 mM L-Arg, 5.0 μM DAF-FM DA, and 0.4 mg/mL Co-FeMOF@GOD, in 0.1 M PBS. E_x=485 nm, E_m=515 nm.

Table S1. The obtained elemental ratio of Co-FeMOF and GOD@Co-FeMOF by EDS.

	Co-FeMOF		GOD@Co-FeMOF	
Element	Wt%	At%	Wt%	At%
C	68.12	75.95	65.66	72.60
N	10.39	9.93	12.52	11.87
O	15.09	12.63	17.28	14.34
Fe	2.17	0.52	1.65	0.39
Co	4.22	0.96	2.88	0.79

Table S2. ICP-OES analysis of Co-FeMOF and Co-FeMOF during the catalysis.

	Co-FeMOF		Co-MOF	
Element	Before catalysis mg/L	After filtrating mg/L	Before catalysis mg/L	After filtrating mg/L
Fe	0.339	0.009	0.000	0.000
Co	0.654	0.017	0.537	0.015

Supporting references

S1 Y. X. Wang, M. T. Zhao, J. F. Ping, B. Chen, X. H. Cao, Y. Huang, C. L. Tan, Q. L. Ma, S. X. Wu, Y. F. Yu, Q. P. Lu, J. Z. Chen, W. Zhao, Y. B. Ying and H. Zhang, *Adv. Mater.*, 2016, **28**, 4149–4155.