Electronic Supplementary Information

Facile synthesis of covalent organic framework derived composite Fe-COF as a peroxidase-mimicking artificial enzyme

Liya Zhou,[a] Xucong Luo,[a] Jing Gao,[a] Guanhua Liu,[a] Li Ma,[a] Ying He,[a] Zhihong Huang,[a] and Yanjun Jiang * [a b]

[a] School of Chemical Engineering and Technology, Hebei University of Technology, 8 Guangrong Road, Hongqiao District, Tianjin, 300130, P. R. China

[b] National-Local Joint Engineering Laboratory for Energy Conservation of Chemical Process Integration and Resources Utilization, Hebei University of Technology, Tianjin 300130, China

Corresponding Author

***** E-mail: yanjunjiang@hebut.edu.cn; jgao@hebut.edu.cn;

Fax: 86-22-60204294; Tel: 86-22-60204945

Experimental section

Materials

Hydrogen peroxide (H_2O_2, 30%) and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were purchased from Tianjin Chemical Reagent Company (Tianjin, China). 1,3,5-tris-l(4-aminophenyl)benzene (TAPB) was purchased from Shanghai Dibai Chemical Technology Co., Ltd. 1,3,5-Benzenetricarboxaldehyde (BTCA) was received from Shanghai Bide Pharmaceutical Technology Co., Ltd. (Shanghai, China). 3,3',5,5'-Tetramethylbenzidine (TMB) was obtained from Aladdin reagent Co., Ltd. Deionized water was produced by a Millipore Milli-Q water purification system. All other chemicals were bought from Sinopharm Reagent Co., Ltd (shanghai, China) and were analytical purity without further purification.

Characterization.

The microstructure and the morphology of the synthetic RT-COF-1 and Fe-COF nanocomposite were characterized by the scanning electron microscopy (SEM, Nova Nano SEM450). Ultraviolet−visible (UV–vis) absorption spectra were carried out on a cary 60 spectrophotometer (Agilent, USA). Fe contents of the samples were determined by inductively coupled plasma spectroscopy (ICP). ICP was carried out on a Prodigy (LEEMANLABS, USA). Fourier-transform infrared spectroscopy (FT-IR) spectra were performed with a Bruker VECTOR22 spectrometer. Thermogravimetric analyses (TGA) were obtained on a Netzsch TG 209 F3 in the temperature range of 50 to 800 °C with a heating ramp of 10 °C/min under the N_2 flow. An X-ray photoelectron spectrometer (XPS, Thermo Scientific K-Alpha) was used to determine the surface chemical states of elements. Fluoromax-4 spectrometer (Horiba, France) was used to measure fluorescence. Raman spectra were measured on a Renishaw InVia Raman Microscope, using a 532 nm excitation laser, between 100 and 3200 cm^{-1}. Nitrogen adsorption/desorption were measured using a Micromeritics ASAP 2020 analyzer. The samples were degassed at 150 °C for at least 12 h under vacuum conditions before the

test. The Brunauer-Emmett-Teller (BET) method was used to evaluate the specific surface areas.

Synthesis of RT-COF-1.

Inspired by Alejandro *et al*'s work[1], we prepared RT-COF-1 through minor modification. The following operations were carried out at room temperature unless otherwise stated. TAPB (100 mg, 0.285 mmol) and BTCA (46.1 mg, 0.285 mmol) were added into 5 mL dimethyl sulfoxide (DMSO) at room temperature with vigorous shaking. Both solutions were thoroughly mixed, and after that, 1 ml of 99.8% glacial acetic acid was slowly added under shaking condition. After acetic acid was added, the mixture turned to yellow gel immediately. The gel was repeatedly washed with methanol and tetrahydrofurane three times and dried under vacuum (50 mbar) overnight at room temperature. The obtained product was named RT-COF-1 and then thoroughly ground in a mortar prior.

Synthesis of Fe-COF.

$FeSO_4$•$7H_2O$ (75 mg, 0.3 mmol) and RT-COF-1 (60 mg) were mixed in 36 mL dichloromethane. The mixture was sonicated for 15 min and then kept under stirring condition for 36 h at room temperature. The produced precipitates were washed with dichloromethane three times and collected by centrifugation (10000 rpm for 5 min). Furthermore, the product was dried in vacuum at room temperature for five hours (named Fe-COF). Fe content was 21.75wt% from ICP analyses.

Peroxidase-like activity assay of Fe-COF. The peroxidase-like activity of the Fe-COF was evaluated through an oxidation of a chromogenic substrate (TMB) by H_2O_2. The standard experiment was described as follows: adding 10 μL Fe-COF (800 μg/mL) into a cuvette consisting of 930 μL of NaAc buffer solution (pH 5), 10 μL of H_2O_2 (100m M) together with 50 μL of TMB (1mM) and keeping the total volume at 1 mL. Then, after incubating at 25 °C for 20 minutes, the absorbance of the mixed solution was measured at 652 nm.

Colorimetric determination of H_2O_2. The detection of H_2O_2 was tested as follows: 10 μL Fe-COF (800 μg/mL) composite was added into a cuvette consisting of H_2O_2 (10 μL) with different concentrations, 50 μL TMB (1 mM) and 930 μL of buffer solution.

After incubating at 35 °C for 20 minutes, the absorbance of the mixed solution was measured at 652 nm and the standard curve of H_2O_2 was plotted.

Determination of H_2O_2 in milk. Milk samples were purchased from a local supermarket, and were treated as following steps: 5 ml raw milk was firstly diluted to 10 ml with water. Then the milk sample was centrifuged at 10000 rpm to remove the protein and other organic substances and separate the deposit. Thirdly, the supernatant was filtered through a 0.22 μm membrane (Whatman) to remove lipids. The milk samples containing different concentrations of H_2O_2 were prepared by adding different volumes of a stock solution of H_2O_2 (100 mM) to this supernatant. Then, 100 μL of milk sample and 10 μL of Fe-COF (800 μg/mL) were added to the mixture of 840 μL of buffer solution and 50 μL of TMB (1 mM). After incubating at 25 ℃ for 20 min, the absorbance of the mixed solution was measured at 652 nm.[2, 3]

Selectivity of catalytic determination of H_2O_2 under Fe-COF. For the selectivity of catalytic determination of H_2O_2, 50 μL of TMB (1 mM), 930 μL of buffer solution and 10 μL of stock solutions (100 mM) of glucose, urea, alanine, glycine, L-Cysteine, glutathione, ascorbic acid, K^+, Ca^{2+}, Na^+, Zn^{2+}, Cl^-, SO_4^{2-} and NO_3^- were added to 10 μL of Fe-COF (800 μg/mL). The blank solution was composed of 10 μL of Fe-COF (800 μg/mL), 50 μL of TMB (1 mM) and 930 μL of buffer solution. After incubating at 35 ℃ for 20 min, the absorbance of the mixed solution was measured at 652 nm.

Degradation of RhB in TMB-H_2O_2 System. The degradation of RhB was tested as follows: 50 μL Fe-COF (800 μg/mL) composite was added into 930 μL of buffer solution, 10 μL H_2O_2 (100 mM), 10 μL RhB (10 mg/mL). After incubating at 35 ℃, the absorbance of the mixed solution was measured at 554 nm at different times.

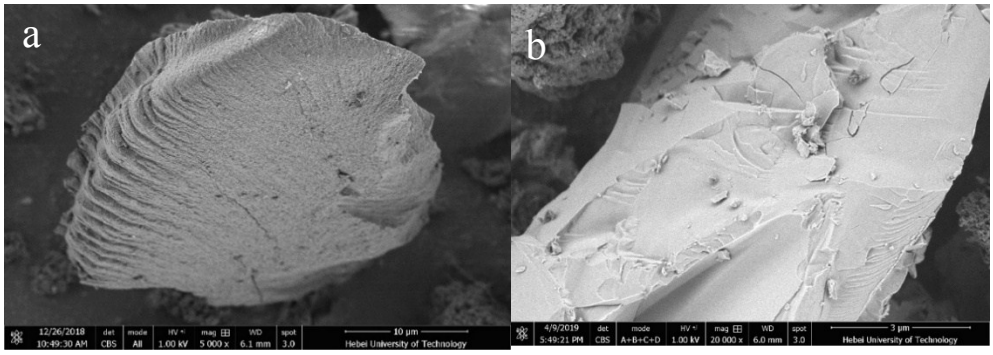

Fig. S1 SEM images of RT-COF-1 (a) and Fe-COF (b)

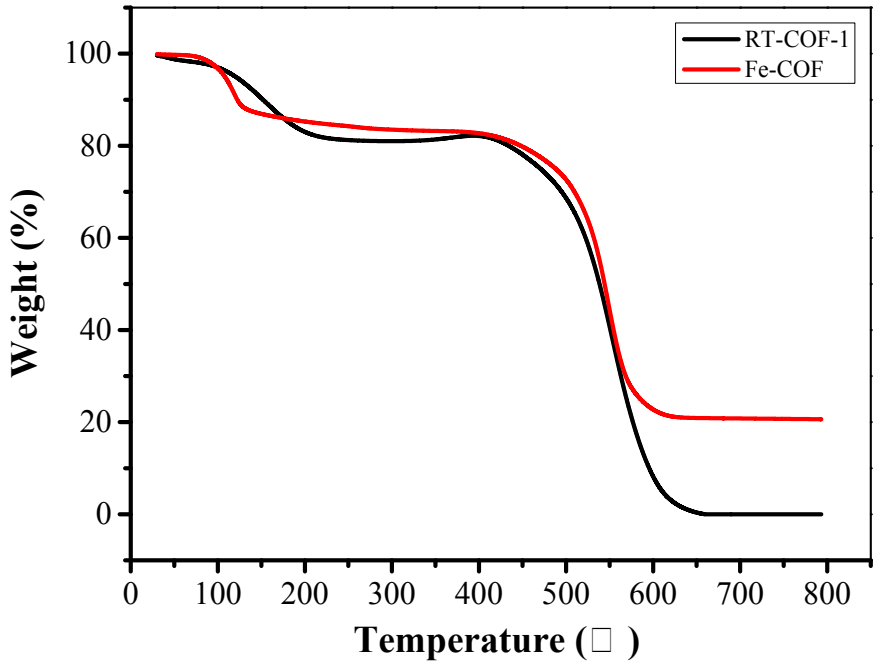

Fig. S2 TGA curves of RT-COF-1 (black) and Fe-COF (red) in air atmosphere.

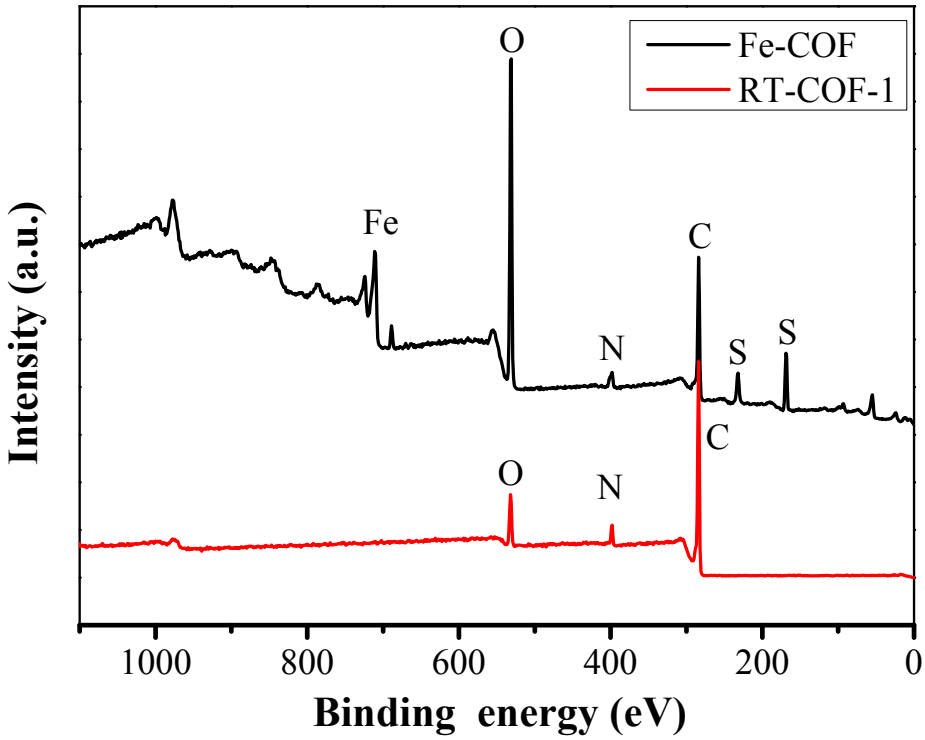

Fig. S3 XPS spectra of RT-COF-1 (red) and Fe-COF (black).

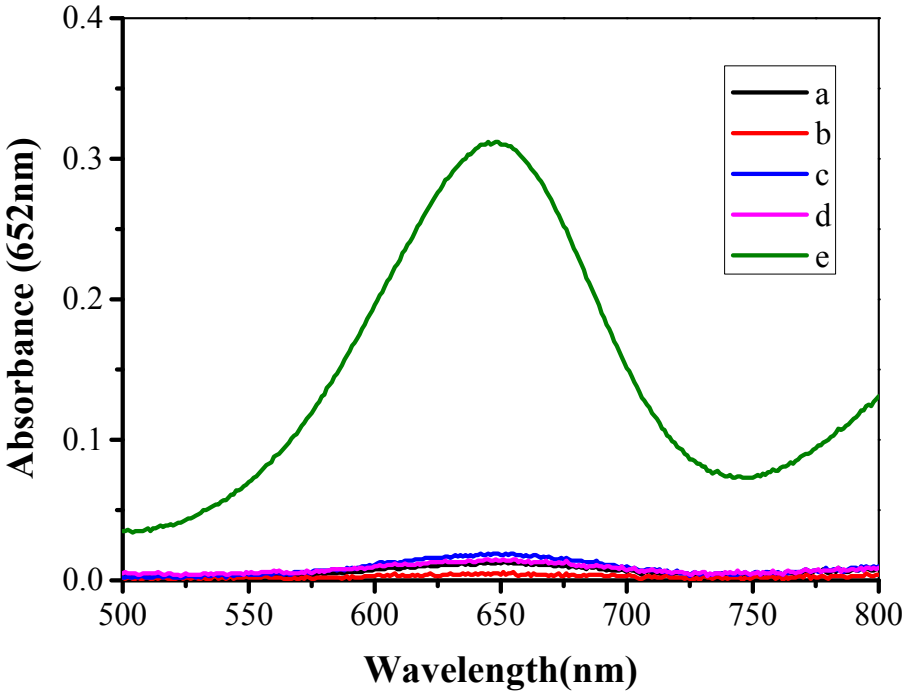

Fig. S4 UV–vis spectra of (a) TMB + RT-COF-1; (b) TMB + H_2O_2; (c)TMB + H_2O_2 + RT-COF-1; (d) TMB + Fe-COF and (e) TMB + H_2O_2 +Fe-COF. Conditions: 50 μL TMB (1mM, M=mol/L); 10 μL H_2O_2 (100 μM); 10 μL Fe-COF (800 μg/mL) and 930 μL NaAc buffer (0.2 M); incubation 10 min in pH 5.0 NaAc buffer at 25 °C.

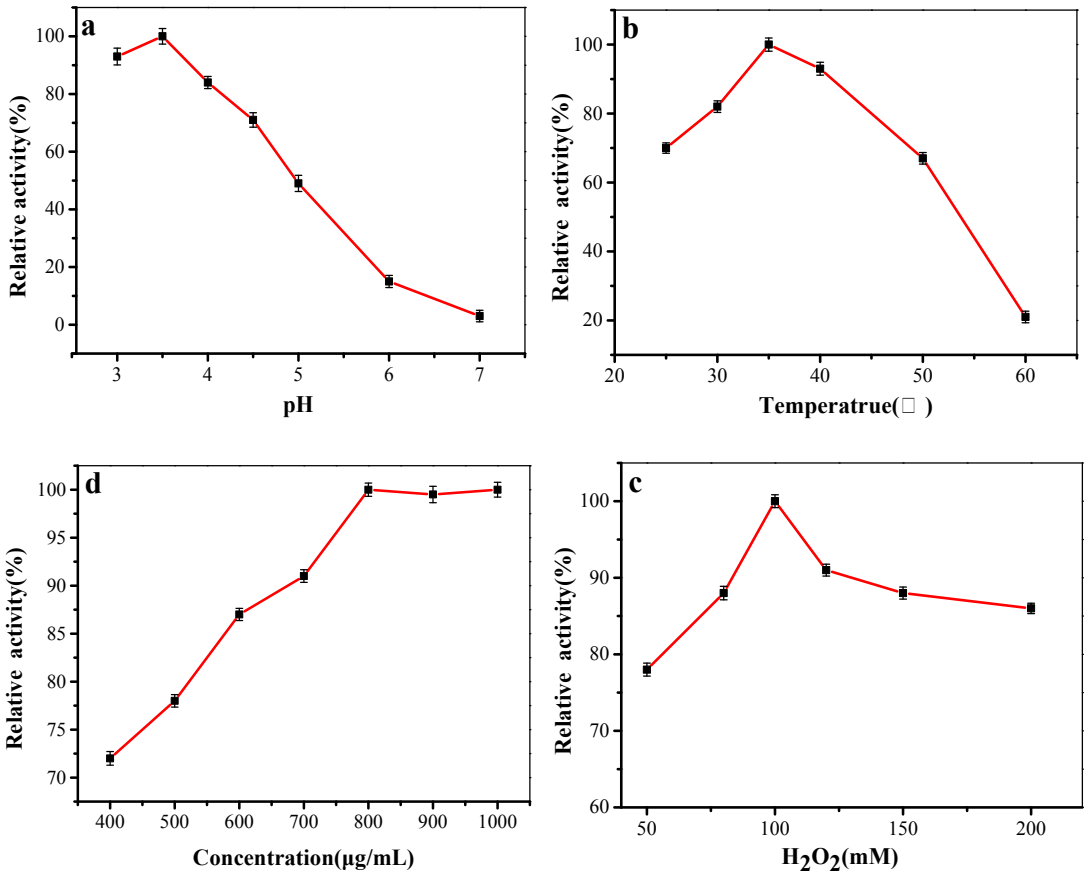

Fig. S5 Optimization of experimental conditions. (a) The pH of buffer from 3 to 7, (b) the temperature of reaction from 25 to 60℃, (c) the concentration of H_2O_2 from 50 mM to 200 mM, (d) the concentration of Fe-COF from 400 to 1000 μg/mL. The reactions included 10 μL Fe-COF (800 μg/mL), 50 μL TMB (1 mM), 10 μL H_2O_2 (100 mM) and 930 μL buffer (0.2 M, pH 5.0) unless otherwise stated. Error bars denote standard deviations based on three measurements. The maximum point in each curve was set as 100 %.

Table S1 The values of Km and Vmax of TMB and H_2O_2 compared with the natural enzyme HRP and other mimics.

Catalyst	K_m/mM		V_{max}/M s^{-1}		Ref
	TMB	H_2O_2	TMB	H_2O_2	
HRP	0.434	3.700	10.0×10^{-8}	8.71×10^{-8}	[4]
Fe-COF	0.026	0.196	3.88×10^{-8}	4.69×10^{-8}	This work
Fe_3O_4@MIL-100(Fe)	0.112	0.077	1.14×10^{-7}	1.80×10^{-7}	[5]
Fe_3O_4	0.098	3.440	154×10^{-8}	9.78×10^{-8}	[6]
FeS	0.008	9.360	87×10^{-8}	192×10^{-8}	[7]
Free-standing Ag@Fabric	0.190	7.610	15.1×10^{-8}	14.4×10^{-8}	[8]

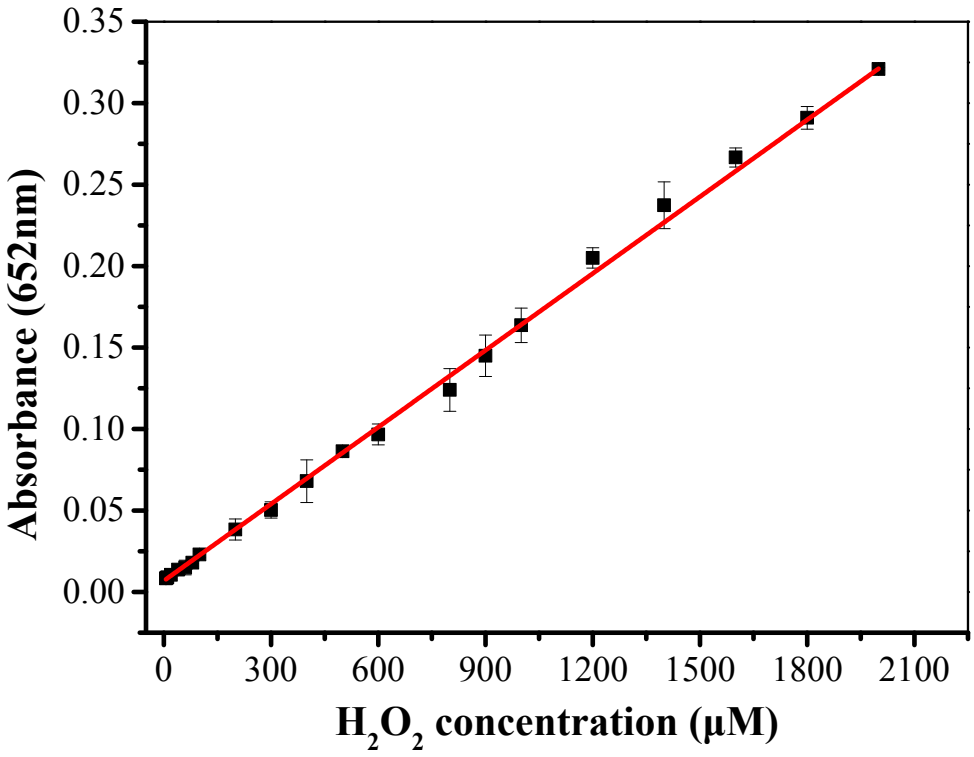

Fig. S6 The linear range for H_2O_2 detection using TMB and Fe-COF catalyst.

Table S2 List of linear range and limit of detection for H_2O_2 of various mimics.

Nanomaterials	Method	Linear range (μM)	LOD	Ref
DNA-CeO_2	Fluorescence	up to 1000	130 nM	[9]
rGO/Ag NPs	Electrochemical	100-100000	31.3 μM	[10]
PNEGHNs/GCE	Electrochemical	1-500	80 nM	[11]
Au@Pt NRs	Colorimetric	45-1000	45μM	[12]
Fe/$CuSn(OH)_6$	Colorimetric	30-1000	9.49μM	[13]
PtPdNDs/GNs	Colorimetric	0.5-150	0.1μM	[14]
H_2TCPP-Co_3O_4	Colorimetric	1-75	0.4μM	[15]
Fe-COF	Colorimetric	10-2000	5.6μM	This work

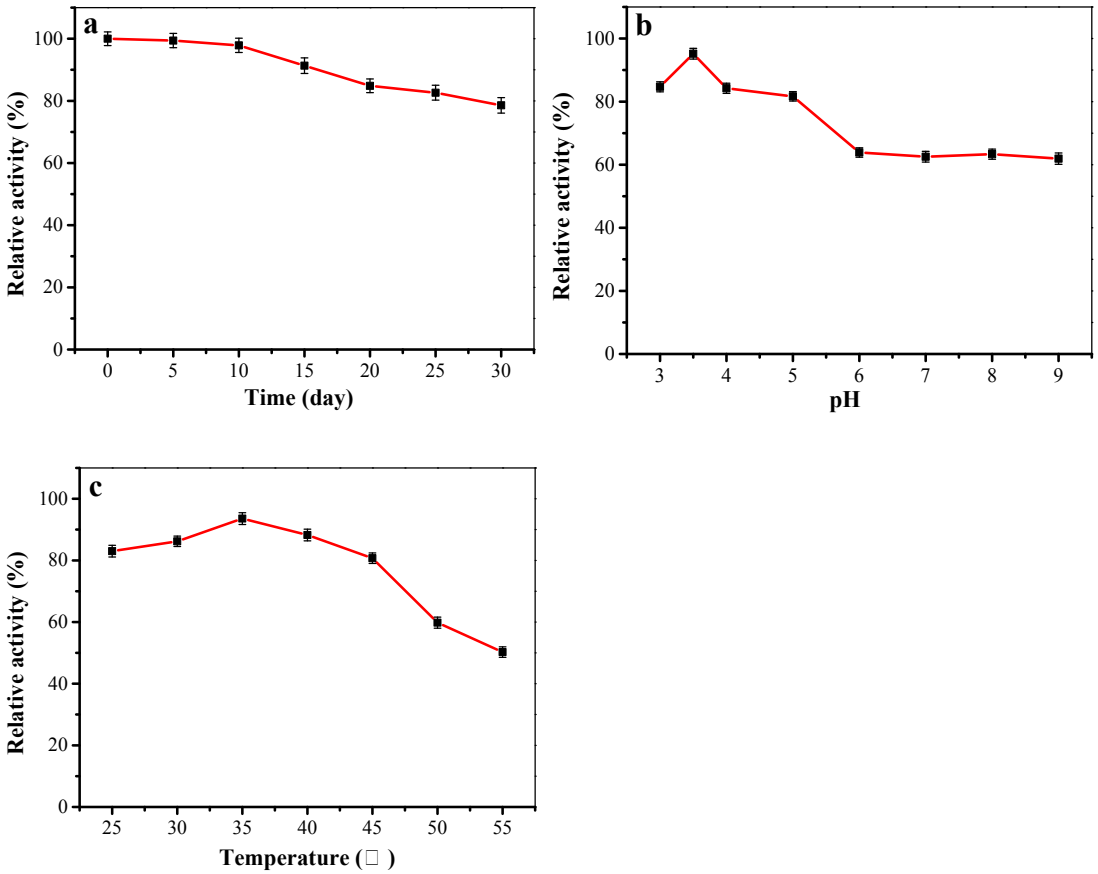

Fig. S7 Long-term stability (a), relative catalytic activity of Fe-COF after 2 h with incubation in different pHs (3−9) (b) and different temperatures (25−55°C) (c). Unprocessed was set as 100%.

Table S3 Determinations of H_2O_2 residue in milk.

Sample	Added H_2O_2 (μM)	Detected H_2O_2 (μM)	Recovery(%)	RSD(%)
Milk 1	100	96.27	96.27%	4.55%
Milk 2	500	503.49	100.70%	2.01%
Milk 3	1000	989.19	98.92%	0.71%

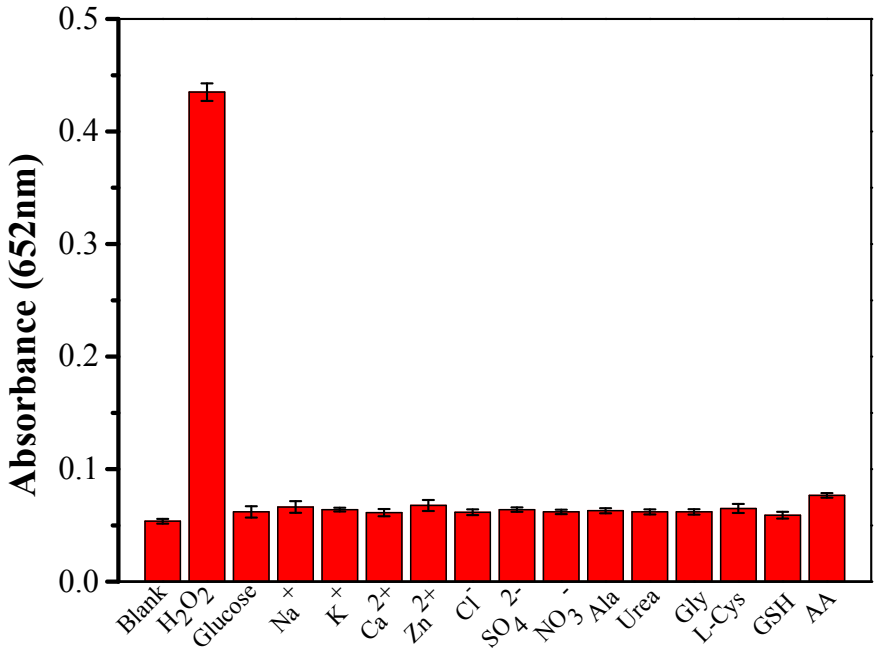

Fig. S8 The selectivity of the proposed H_2O_2 colorimetric sensor.

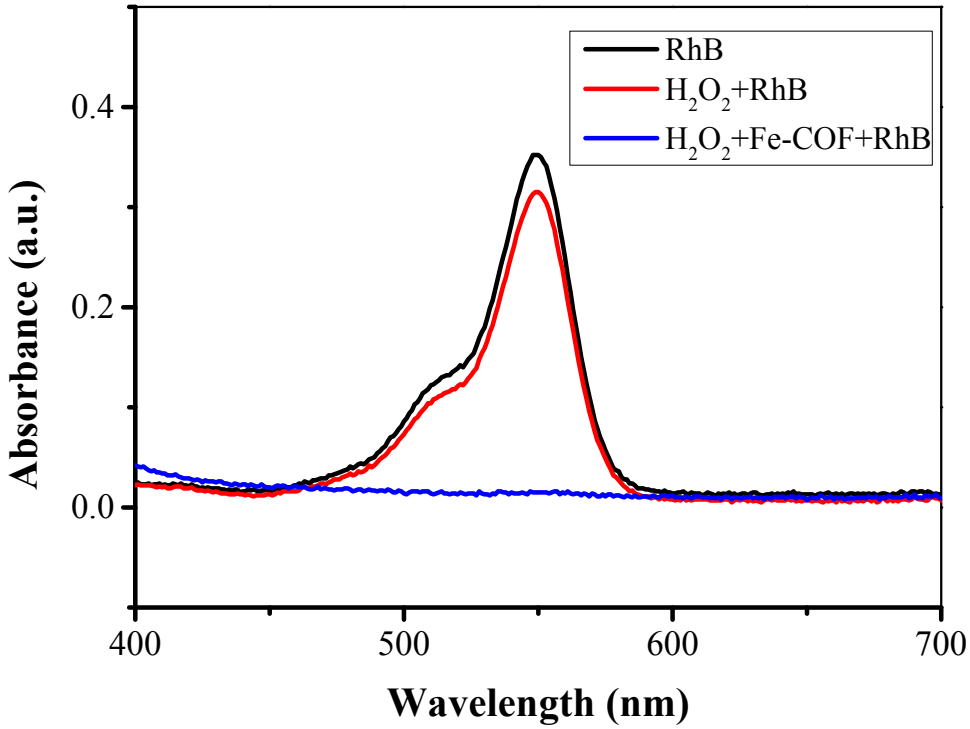

Fig. S9 UV–vis spectra of RhB (black); RhB + H_2O_2 (red) and RhB + H_2O_2 + Fe-COF (blue).

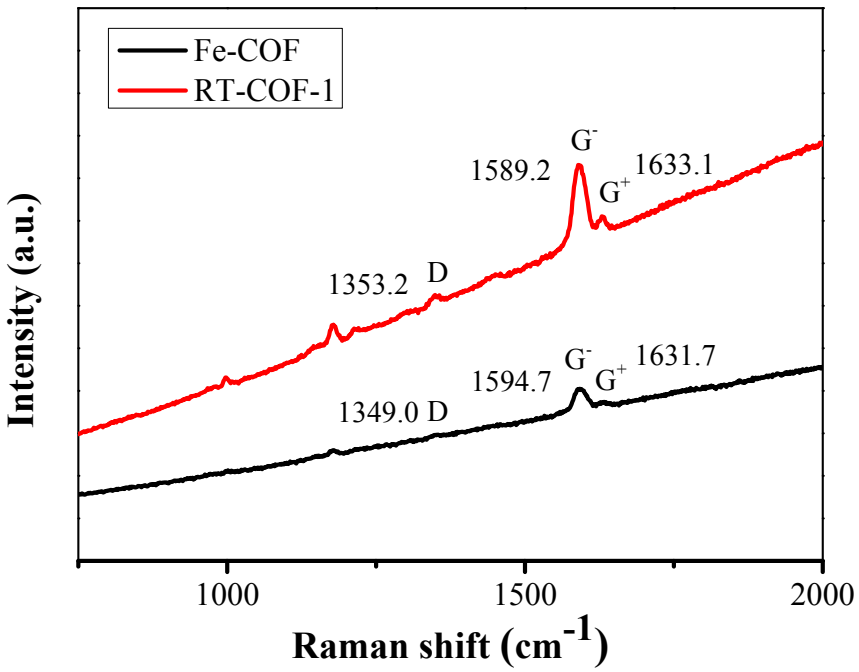

Fig. S10 Raman spectra of RT-COF-1 and Fe-COF.

Table S3 Comparison of response time between Fe-COF and other peroxidase mimics.

System	chromogenic substrate	Substrate concentration	Response time	Ref.
PtAg-MoS_2	TMB	50 mM	30 min	[16]
Au@Pt	TMB	6.3 mM	20min	[17]
PtPdNDs-GNs	TMB	8 mM	10min	[14]
Fe-COF	TMB	1mM	10min	This work

Table S4 Comparison Fe-COF and the other materials for dye degradation.

System	Dye	Degradation efficiency	Degradation time	Ref.
$ZnFe_2O_4$	RhB	94.2%	120 min	[18]
$Fe@Fe_2O_3$	RhB	100.0%	60min	[19]
Ce (IV)	RhB	80.0%	60min	[20]
Fe-COF	RhB	89.5%	30min	This work

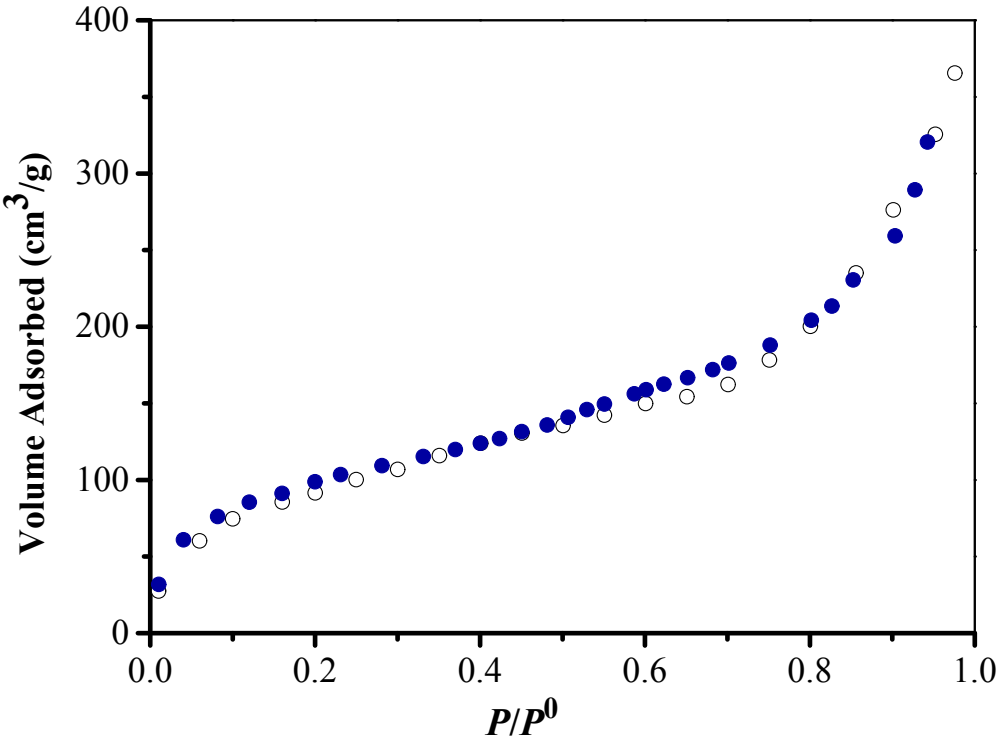

Fig. S11 N_2 adsorption–desorption isotherms of RT-COF-1

REFERENCES

1. A. de la Pena Ruigomez, D. Rodriguez-San-Miguel, K. C. Stylianou, M. Cavallini, D. Gentili, F. Liscio, S. Milita, O. M. Roscioni, M. L. Ruiz-Gonzalez, C. Carbonell, D. Maspoch, R. Mas-Balleste, J. L. Segura and F. Zamora, Chemistry, 2015, **21**, 10666-10670.
2. Z. Qi, L. Wang, Q. You and Y. Chen, Biosens. Bioelectron. 2017, **96**, 227-232.
3. Y. Lu, W. Ye, Q. Yang, J. Yu, Q. Wang, P. Zhou, C. Wang, D. Xue and S. Zhao, Sensor. and Actuat. B: Chem. 2016, **230**, 721-730.
4. Y. Dong, J. Zhang, P. Jiang, G. Wang, X. Wu, H. Zhao and C. Zhang, New J. Chem. 2015, **39**, 4141-4146.
5. J. Tian, J. Xu, F. Zhu, T. Lu, C. Su and G. Ouyang, J. Chromatogr. A, 2013, **1300**, 2-16.
6. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, Nat. Nanotechnol. 2007, **2**, 577-583.
7. A. K. Dutta, S. K. Maji, D. N. Srivastava, A. Mondal, P. Biswas, P. Paul and B. Adhikary, ACS Appl. Mater. Interfaces, 2012, **4**, 1919-1927.
8. M. N. Karim, S. R. Anderson, S. Singh, R. Ramanathan and V. Bansal, Biosen. Bioelectron. 2018, **110**, 8-15.
9. B. Liu, Z. Sun, P. J. Huang and J. Liu, J. Am. Chem. Soc. 2015, **137**, 1290-1295.
10. S. Liu, J. Tian, L. Wang and X. Sun, Carbon, 2011, **49**, 3158-3164.
11. S. Guo, D. Wen, Y. Zhai, S. Dong and E. Wang, ACS Nano. 2010, **4**, 3959-3968.
12. J. Liu, X. Hu, S. Hou, T. Wen, W. Liu, X. Zhu, J.-J. Yin and X. Wu, Sensor. Actuat. B: Chem. 2012, **166-167**, 708-714.
13. H. Liu, Y.-N. Ding, B. Yang, Z. Liu, X. Zhang and Q. Liu, ACS Sustain. Chem. Eng. 2018, **6**, 14383-14393.
14. X. Chen, B. Su, Z. Cai, X. Chen and M. Oyama, Sensor. Actuat. B: Chem. 2014, **201**, 286-292.
15. Q. Liu, R. Zhu, H. Du, H. Li, Y. Yang, Q. Jia and B. Bian, Mater. Sci. Eng. C Mater. Biol. Appl. 2014, **43**, 321-329.
16. S. Cai, Q. Han, C. Qi, Z. Lian, X. Jia, R. Yang and C. Wang, Nanoscale, 2016, **8**, 3685-3693.
17. W. He, Y. Liu, J. Yuan, J. J. Yin, X. Wu, X. Hu, K. Zhang, J. Liu, C. Chen, Y. Ji and Y. Guo, Biomater. 2011, **32**, 1139-1147.
18. Y. Guo, Y. Guo, D. Tang, Y. Liu, X. Wang, P. Li and G. Wang, J. Alloy. Compd. 2019, 781, 1101-1109.
19. Z. Ai, L. Lu, J. Li, L. Zhang, J. Qiu and M. Wu, The J. Phys. Chem. C, 2007, 111, 4087-4093.
20. S. Wang, Y. Jia, L. Song and H. Zhang, ACS Omega, 2018, 3, 18456-18465.