Supporting Information

Effective Antibacterial Action of 2D Bimetallic Al/Fe-TCPP NMOF Loaded with Glucose Oxidase by Cascade Catalytic Reaction and Depleting Glutathione Simultaneously

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Abbreviations (in alphabetical order)

English abbreviation	Full english name
ABDA	9,10-anthracenediyl-bis(methylene)dimalonic acid
AFM	atomic force microscope
BCA	bicinchoninic acid
BMOFs	bimetallic-organic frameworks
Calcein-AM	calcein acetoxymethyl ester
CCK-8	cell-counting-kit-8
CFU	colony forming units
CTAB	hexadecyl trimethyl ammonium bromide
DLS	dynamic light scattering
DMPO	5,5-dimethyl-1-pyrroline N-oxide
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
E. coli	*Escherichia coli*
ESR	electron spin resonance
FT-IR	fourier transform infrared spectrometer
GOx	glucose oxidase
GSH	glutathione
H_2O_2	hydrogen peroxide
MIC	minimal inhibitory concentration
MOFs	metal-organic frameworks
NMOFs	nanoscale metallic-organic frameworks
$^1O_2$	singlet oxygen
•OH	hydroxyl radical
PBS	phosphate buffered saline
PDDA	poly dimethyl diallyl ammonium chloride
PI	propidium iodide
ROS	reactive oxygen species
S. aureus	*Staphylococcus aureus*
SBUs	second building units
SEM	scanning electron microscope
TA	1,4-dicarboxybenzene
TAOH	2-hydroxybenzene-1,4-dicarboxylic acid
TCPP	meso-tetra(4-carboxyphenyl)porphine
TEM	transmission electron microscope
TMB	3,3',5,5'-Tetramethylbenzidine
XRD	diffraction of x-rays

1. Materials and instruments

All reagents were purchased from commercial suppliers and used without further purification. 5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin (TCPP), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), iron chloride ($FeCl_3$), 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), 3,3',5,5'-tetramethylbenzidine (TMB), glutathione (GSH), β-D-glucose and Glucose oxidase (GOx, BC grade) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). N,N-dimethyl formamide (DMF), Dimethyl sulfoxide（DMSO）, hydrogen peroxide (30%, H_2O_2), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), sodium chloride (NaCl), acetic acid(CH_3COOH) and sodium acetate (CH_3COONa) were purchased from Xilong Scientific Co., Ltd. Poly dimethyl ammonium chloride (PDDA, 20 wt %) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich. The BCA protein assay kit, cell counting kit-8, GSH/GSSG assay kit, protein removal reagent was purchased from Beyotime. Trypsin, dulbecco's modified eagle's medium (DMEM), fetal bovine serums and phosphate buffer saline (PBS) were acquired from Gibco. NIH 3T3 cells (mouse embryonic fibroblast cells) and L929 cells (mouse epithelioid fibroblasts cells) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Calcein-AM/PI double stain kit was purchased from Jiangsu Solarbio Biotech Co., Ltd. LB broth agar (components: tryptone, yeast extract, NaCl, agar) was purchased from Dalian Meilun Biotech Co., Ltd. Ultrapure water was utilized throughout the experiments.

The UV-vis spectra were recorded by Purkinje T6 spectrophotometer (China). The fluorescence intensities were measured by Shimadzu RF-5301PC fluorescence spectrophotometer (Japan). SEM images were captured by a microscope (Scios 2 HiVac, FEI). TEM images and TEM element mapping were obtained using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. AFM measurement was analyzed using Nanoscope V multimode atomic force microscope. XRD measurement was carried out on a D8 Focus diffractometer (Bruker) using Cu Kα radiation. Fourier transform infrared (FT-IR) spectra were gained by FITR-8400S (Shimadzu Co., Ltd. Japan). Absorbance was measured on a microplate reader (Multiskan FC, Thermo Scientific, America) in the CCK-8 method. All pH measurements were performed with a pH-3c digital pH meter (Shanghai LeiCi Device Works, Shanghai, China) with a combined glass-calomel electrode. Inverted fluorescence imaging studies were performed with a IX71 inverted research microscope (Olympus., Ltd. Japan).

2. ESR Experiments for Hydroxyl Radical Determination

DMPO was used for hydroxyl radical scavenging, trapping radicals, and forming adducts that are detectable by an ESR spectroscope. In a typical assay, a mixture containing 200 $\mu g \cdot mL^{-1}$ Al/Fe-TCPP NMOF/GOx, 50 mM DMPO, and 10 mM glucose was prepared, and ESR signals were recorded immediately.

3. Steady-State Kinetic Analysis

Steady-state kinetic analyses were carried out in a quartz cuvette using a UV-vis spectrophotometer.[1] For the colorimetric reaction, a mixture containing 100 $\mu g \cdot mL^{-1}$

Al/Fe-TCPP NMOF/GOx, 3.2 mM TMB, and 10 mM glucose was prepared, and the UV-Vis absorption spectra from 350 to 800 nm were recorded after the color of the mixture changed. The reaction of Al/Fe-TCPP NMOF, H_2O_2, and TMB was also investigated while the concentration of H_2O_2 was 100 μM.

Typically, the TMB solution (3.2 mM) was first added to the cuvette, followed by the addition of H_2O_2 or glucose with varied concentrations. Finally, Al/Fe-TCPP NMOF or Al/Fe-TCPP NMOF/GOx (100 μg·mL^{-1}) was added to trigger the reaction. The time-course absorbance (λ = 652 nm) was recorded for 10 min under the kinetic mode. The pH conditions were controlled by the sodium acetate (NaAc) buffer solution (20 mM). According to the Lambert−Beer law (eq 4), the initial velocities (v_0) can be calculated as eq 5. The initial velocities of concentration-dependent chromogenic curves can be summarized and fitted with the Michaelis-Menten equation (eq 6). The Michaelis-Menten constant (K_M) and maximal velocity (V_{max}) can be calculated via the Lineweaver-Burk plot.

$$A = kbc\#(4)$$

$$v_0 = \frac{\Delta A}{kb\Delta t}\#(5)$$

where A represents the absorbance values and k represents the molar extinction coefficient. In this case, k = 32 000 M·cm^{-1} and b = 1 cm. c represents the concentration of the chromogenic substances, while t represents time.

$$v_0 = \frac{V_{max} \cdot [S]}{K_M + [S]}\#(6)$$

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \#(7)$$

where V_{max} and K_M represent the maximum velocity and Menten constant of the chromogenic reaction. *[S]* represents the substrate concentration.

4. Live/Dead Bacteria Staining

Bacterial cells with different treatments were centrifuged (5000 rpm, 5 min) and washed with sterilized PBS 2-3 times. Then, the treated bacteria were put into a mixture solution of Calcein-AM (2 μM) and PI (1.6 μM), kept for 15min, then washed with sterilized PBS three times to remove the free dye, and observed by an inverted fluorescence microscope.

5. Protein Leakage

Bacterial cells were first washed twice with normal saline to remove secreted proteins. The bacterial suspension was centrifuged (5000 rpm, 5 min, 4 °C) to collect the supernatant after the bacterial cells were treated with different treatments. The collected upper liquids were added into a 96-well plate, and the concentration of proteins was measured using the BCA protein assay kit at 562 nm using a microplate reader.

6. Cell Culture

NIH 3T3 cells and L929 cells were cultured in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin, 1% streptomycin. The cultures were maintained in a humidified incubator in a 5% CO_2/95% air at 37 °C.

7. Cytotoxicity Test

NIH 3T3 cells and L929 cells harvested in a logarithmic growth phase were seeded in 96-well microtiter plates at a density of 1×10^4 cells/well and incubated in DMEM for

24 h in an atmosphere of 5 % CO_2, 95 % air at 37 ºC. The cells were sequentially incubated with different concentrations of Al/Fe-TCPP NMOF/GOx (0, 100, 200, 300, 400, 500 $\mu g \cdot mL^{-1}$) for another 24 h. Next, 100 μL CCK-8 solution (10%) was added to each well. After 2 hours, the absorbance was measured at 450 nm with microplate reader.

8. Statistical Analysis

Each experiment was performed three times. The statistical analysis and graphical presentation of experimental data were done by one-way analysis of variance (*P <0.05, **P < 0.01, ***P < 0.001; ns, not significant), and all data were presented as the means and standard deviations, indicated as mean ±SD, n = 3.

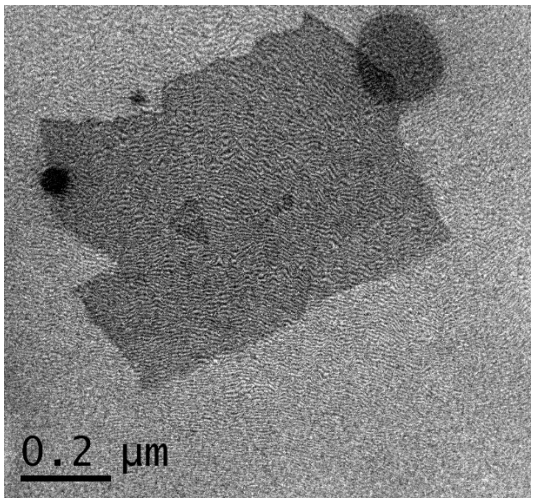

Figure S1. TEM image of Al-TCPP NMOF, scale bar = 200 nm.

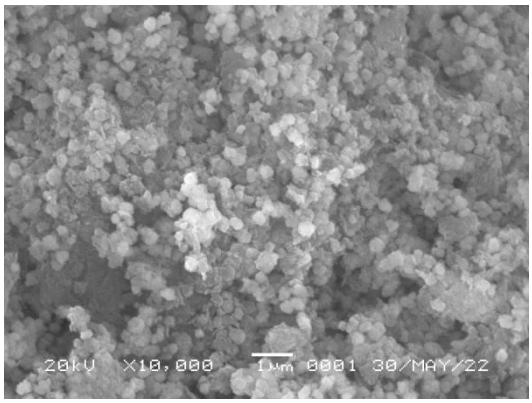

Figure S2. SEM image of Al/Fe-TCPP NMOF, scale bar = 1 µm.

Figure S3. Images of water dispersions of Al/Fe-TCPP NMOF (left) and Fe-TCPP NMOF (right).

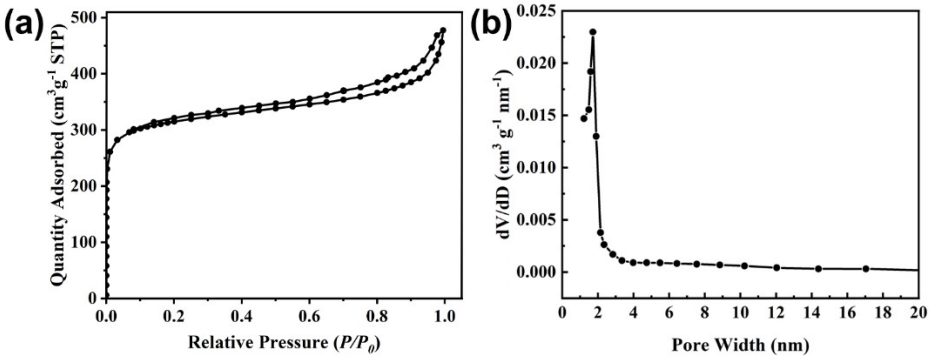

Figure S4. (a) Nitrogen-sorption isotherms and (b) pore distribution of Al/Fe-TCPP NMOF.

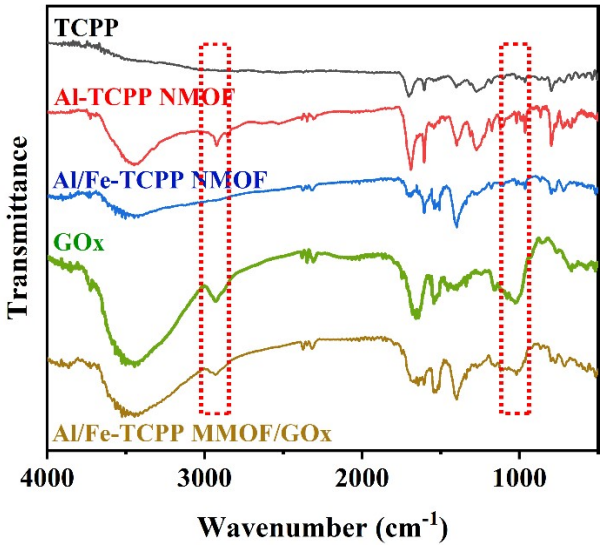

Figure S5. FTIR spectra of TCPP, Al-TCPP NMOF, Al/Fe-TCPP NMOF, GOx and Al/Fe-TCPP NMOF/GOx, respectively.

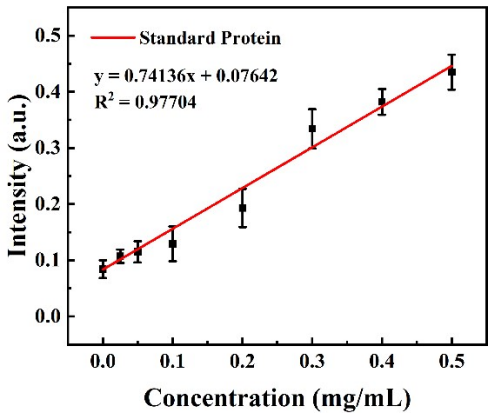

Figure S6. Standard curve of the standard protein applied in BCA protein test. (mean ± SD, n=3).

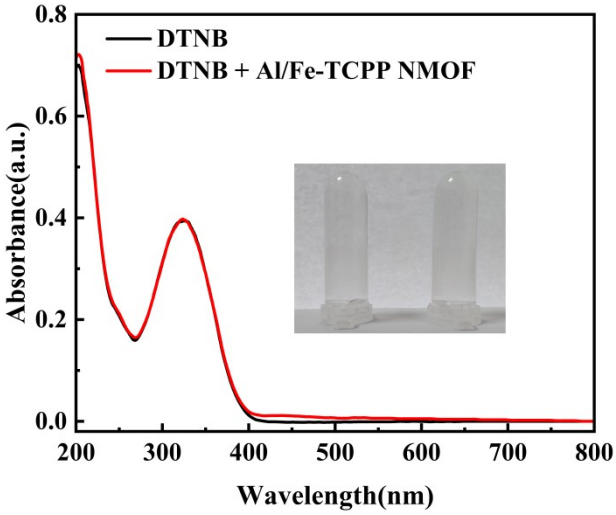

Figure S7. Control experiment for the GSH depletion assay. The concentrations of DTNB and Al/Fe-TCPP NMOF were 180 μg·mL^{-1} and 0.167 mg·mL^{-1}.

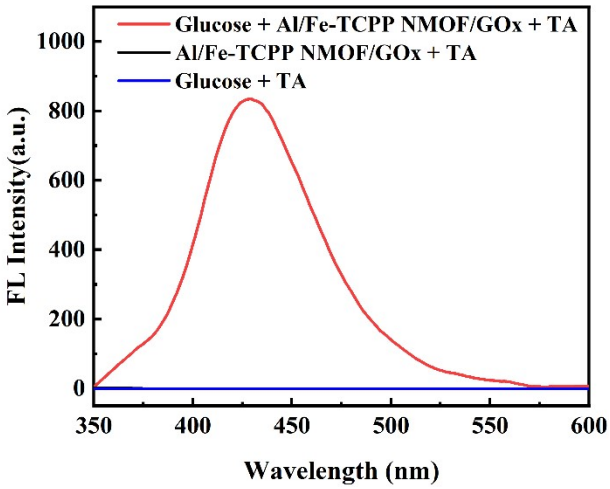

Figure S8. Fluorescence spectra of different reaction systems in PBS buffer (pH = 7.4) after 1h reaction. The concentrations of TA, glucose, and Al/Fe-TCPP NMOF/GOx were 0.5 mM, 5 mM, and 20 μg·mL^{-1}, respectively.

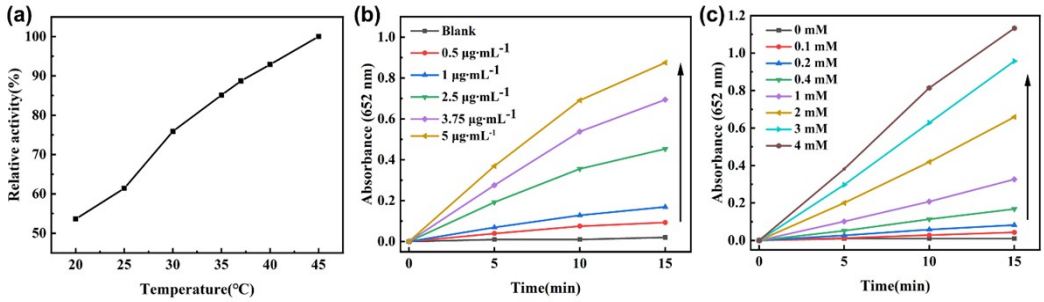

Figure S9. Peroxidase-like catalytic activity of Al/Fe-TCPP NMOF/GOx composite is dependent on temperature (a), Al/Fe-TCPP NMOF/GOx (b) and H_2O_2 concentration (c). (a) Experiments were performed using 5 μg·mL^{-1} Al/Fe-TCPP NMOF/GOx in a reaction volume of 500 μL in 25 mM PBS buffer (pH=4.0), and 4 mM H_2O_2, with 1.2 mM TMB as substrate under different temperature. (b) Experiments were performed using different concentration of Al/Fe-TCPP NMOF/GOx in a reaction volume of 500 μL in 25 mM PBS buffer (pH=4.0, 37°C), and 4 mM H_2O_2, with 1.2 mM TMB as substrate. (c) Experiments were performed using 5 μg·mL^{-1} Al/Fe-TCPP NMOF/GOx in a reaction volume of 500 μL in 25 mM PBS buffer (pH=4.0, 37°C), different concentration of H_2O_2 with 1.2 mM TMB as substrate.

REFERENCES

[1] X. Zhou, S. Zhang, Y. Liu, J. Meng, M. Wang, Y. Sun, L. Xia, Z. He, W. Hu, L. Ren, Antibacterial cascade catalytic glutathione-depleting MOF nanoreactors, ACS Applied Materials & Interfaces 14(9) (2022) 11104-11115.