Supplementary Information

Ionic silver-infused peroxidase-like metal–organic frameworks as versatile "antibiotic" for enhanced bacterial elimination

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Experimental

Antibacterial experiments

We had optimized the concentrations of H_2O_2 for *E. coli* and *S. aureus*, respectively. Firstly, different concentrations of H_2O_2 (1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M) were used for treating *E. coli* and *S. aureus* cells. For *E. coli* cells and *S. aureus*, when the concentration of H_2O_2 was high than 10^{-3} M and 10^{-4} M, respectively, only H_2O_2 could kill bacteria; but actually, the concentration of H_2O_2 was out of the range of biologically relevant concentrations. For *E. coli* and *S. aureus* cells, when the concentration of H_2O_2 was lower than 10^{-4} M and 10^{-5} M, respectively, H_2O_2 exhibited almost no obvious antibacterial effect. Thus, the concentrations of H_2O_2 for *E. coli* and *S. aureus* cells were 10^{-4}-10^{-3} M and 10^{-5}-10^{-4} M in the antibacterial experiments. Then, different concentrations of H_2O_2 (10^{-4}, 2×10^{-4}, 4×10^{-4}, 6×10^{-4}, 8×10^{-4} and 10^{-3}M) were used to treated *E. coli* cells, and the results manifested that when the concentration of H_2O_2 was 6×10^{-4} M, the antibacterial system of NH_2-MIL-88B(Fe)-Ag exhibited the best antibacterial effect. At the same time, different concentrations of H_2O_2 (10^{-5}, 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5} and 10^{-4}M) were used to treated *S. aureus* cells, and the results manifested that when the concentration of H_2O_2 was 6×10^{-4} M, the antibacterial system of NH_2-MIL-88B(Fe)-Ag exhibited the best antibacterial effect. Thus the final concentrations of H_2O_2 were 6×10^{-4} M H_2O_2 for *E. coli* and 6×10^{-5} M H_2O_2 for *S. aureus*.

In vitro cytotoxicity experiments

Mouse embryonic fibroblast (NIH 3T3) cells were incubated in normal DMEM culture medium (Invitrogen by Life Technology, Carlsbad, CA) supplemented with 10% fetal bovineserum (FBS) and 1% antibiotics (100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin) in an incubator (37 °C, 5% CO_2). The cytotoxicity of our well-prepared nanoparticles was measured by the standard MTT assay. Briefly, NIH 3T3 cells were incubated in 24-well plates (2×10^4 cells $well^{-1}$) and cultured for 24 h at 37 °C. After washing each well with PBS (0.01 M, pH = 7.4) and replenishing the media, different concentrations (0, 10, 50, 100, 125 and 250 μg mL^{-1}) of NH_2-MIL-88B(Fe)-Ag solutions with or without 6×10^{-5} M H_2O_2 were added for further incubation of 24 h. Then, 50 μL of MTT solution (5 mg mL^{-1}) was added to each well of the microtiter plate and the plate was kept at 37 °C for an additional 4 h. Finally, the formazan crystals were dissolved by dimethyl sulfoxide (DMSO) and absorbance values at 490 nm were measured using a microplate reader. Cell viability was determined as a percentage of absorbance relative to the control cells.

Fluorescence microscopy observation of bacteria

Fluorescence-based live/ dead cell assay was also carried out to directly determine the bacterial viability. After the bacteria suspension was treated with PBS and NH_2-MIL-88B(Fe) + H_2O_2, both *S. aureus* and *E. coli* were obtained by centrifugation and washed with PBS. Then the bacteria stained with propidium iodide (PI, 2 μg mL^{-1}) for 30 min and counterstained with 4′-6-diamidino-2- phenylindole (DAPI, 5 μg mL^{-1}) for 15 min in the dark. Afterwards, bacteria were washed twice with PBS and observed by an inverted fluorescence microscope (Olympus IX71,

Tokyo, Japan).

Morphology observation of bacteria

After the assessment of antibacterial abilities, six typical groups of the bacterial suspensions: (1) PBS, (2) NH_2-MIL-88B(Fe) (50 μg mL^{-1}), (3) NH_2-MIL-88B(Fe)-Ag (50 μg mL^{-1}), (4) H_2O_2, (5) NH_2-MIL-88B(Fe) + H_2O_2, and (6) NH_2-MIL-88B(Fe)-Ag + H_2O_2 were collected by centrifugation and fixed with 4% paraformaldehyde for 4 h. Then, the microbes were dehydrated in ethanol solutions with a graded series (20-100%) for 10 min each time. Finally, the morphology of dried bacteria was observed under SEM after sputter-coating with gold.

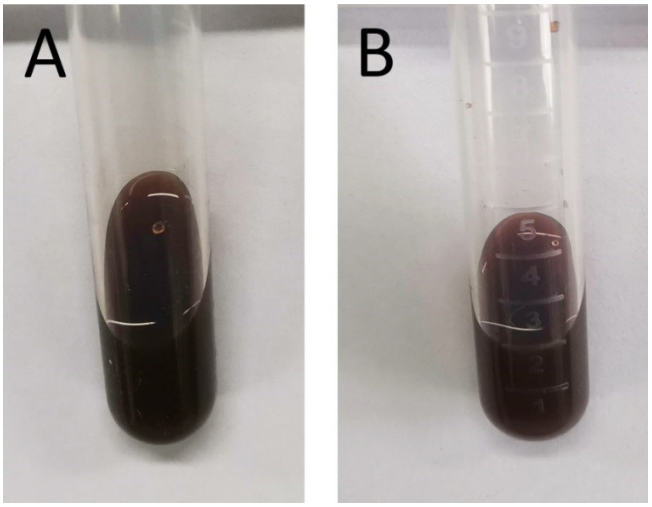

Figure S1 Photographs of A) NH_2-MIL-88B(Fe) and B) NH_2-MIL-88B(Fe)-Ag solutions.

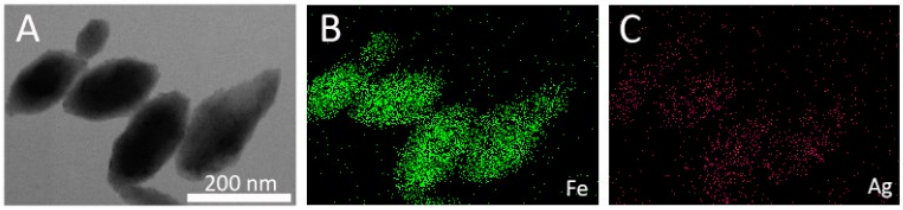

Figure S2 TEM images of NH_2-MIL-88(Fe)-Ag and corresponding elemental mappings of Fe and Ag.

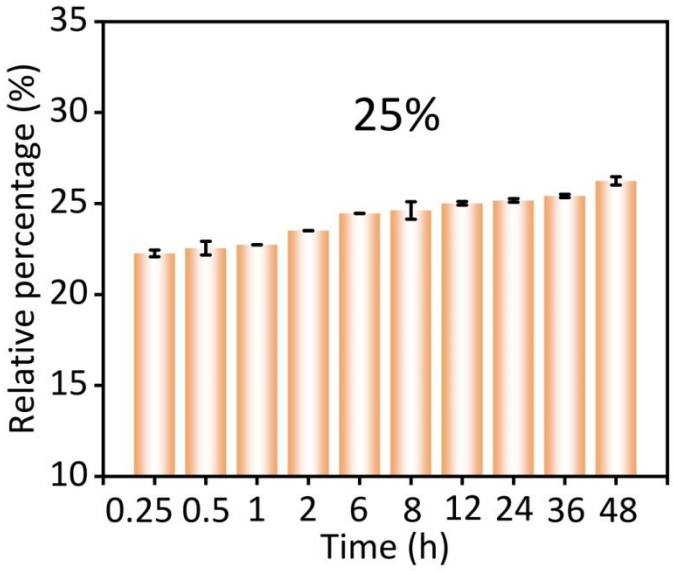

Figure S3 Release of Ag^{+} from NH_2-MIL-88B(Fe)-Ag under neutral condition. In the antibacterial experiments, the content of NH_2-MIL-88B(Fe) and NH_2-MIL-88B(Fe)-Ag is 0.05 mg mL^{-1}, thus the content of Ag^{+} in this system is 0.02385 μg mL^{-1}.

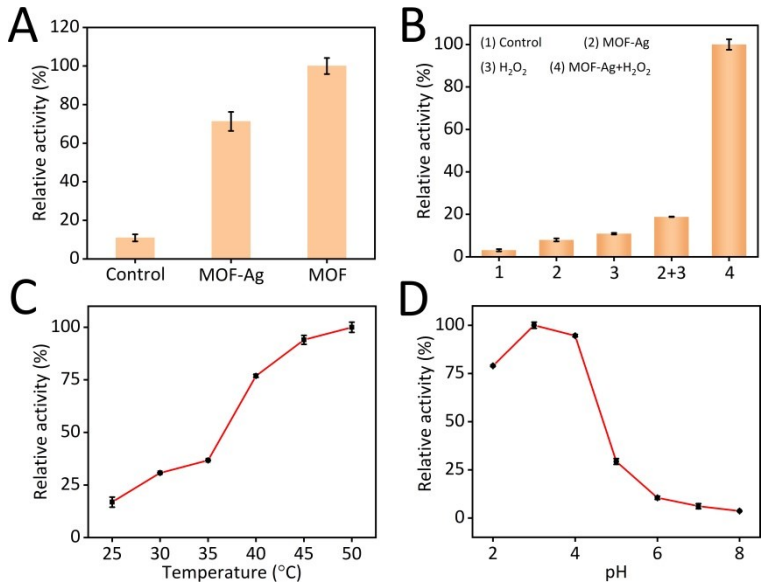

Figure S4 Peroxidase-like catalytic activity of NH_2-MIL-88B(Fe)-Ag. A) The relative activity for NH_2-MIL-88B(Fe)-Ag (20 μg mL^{-1}) reacted with TMB (1mM) and H_2O_2 (10 mM) after 10 min at 25 °C, B) The relative activity after 10 min: (1) control, (2) MOF-Ag, (3) H_2O_2, (4) MOF-Ag + H_2O_2. The catalytic activity dependent on C) temperature with TMB (1 mM), H_2O_2 (10 mM) and NH_2-MIL-88B(Fe)-Ag (50 μg mL^{-1}) at pH 3.0, D) pH with TMB (1 mM), H_2O_2 (10 mM) and NH_2-MIL-88B(Fe)-Ag (50 μg mL^{-1}) at 37 °C.

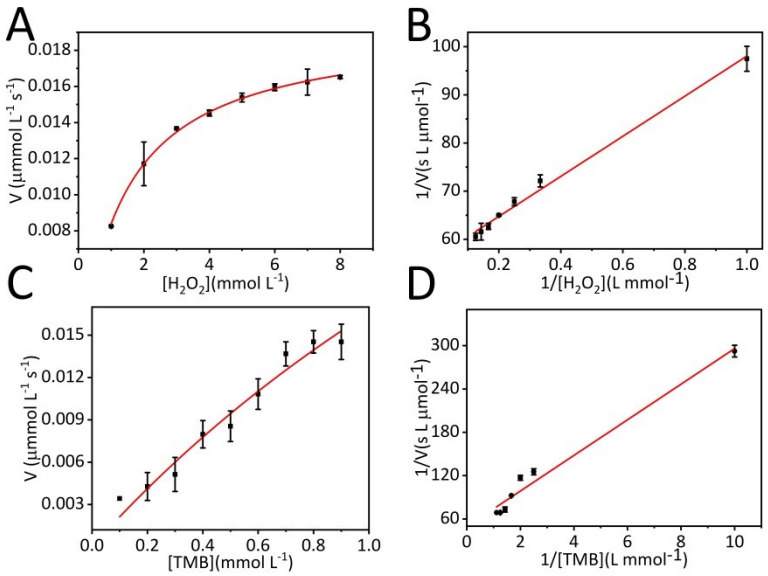

Figure S5 Steady-state kinetic assay of NH_2-MIL-88B(Fe)-Ag (10 μg mL^{-1}) at 37 °C with A) varied H_2O_2 concentration and B) varied TMB concentration. C) and D) Double-reciprocal plot generated from A) and B).

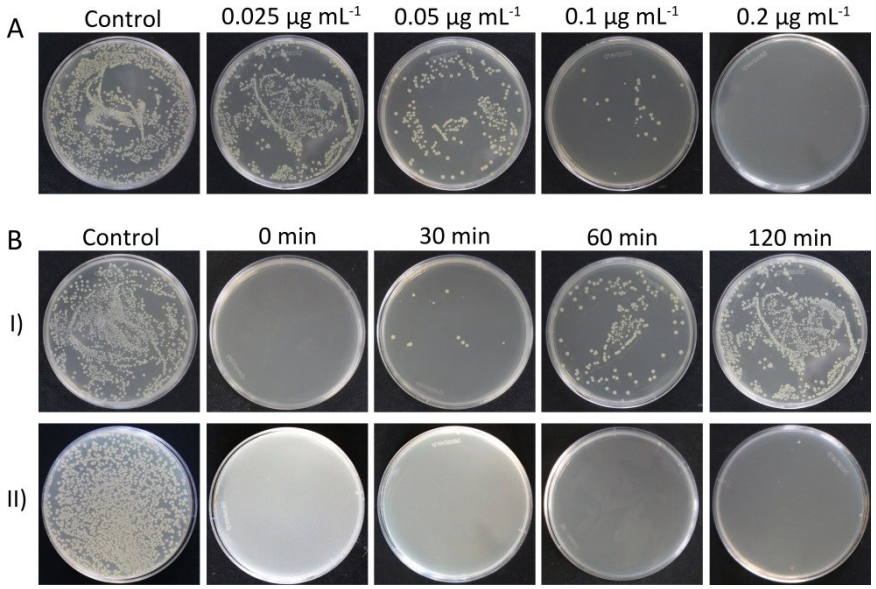

Figure S6. Comparing the efficiency and stability of free Ag^+ with NH_2-MIL-88B(Fe)-Ag. A) Activity of Ag^+ against bacteria cells. B) Activity of I) 0.2 μg mL^{-1} Ag^+ after light irradiation for different times and II) 50 μg mL^{-1} NH_2-MIL-88B(Fe)-Ag after light irradiation with the assistant of H_2O_2.

The concentration of Ag^+ was 0.477 μg mg^{-1} in NH_2-MIL-88B(Fe)-Ag and 50 μg mL^{-1} NH_2-MIL-88B(Fe)-Ag with the assistant of low concentration of H_2O_2 could kill the bacteria completely (about 0.024 μg mL^{-1} of Ag^+). As shown in Figure S6A, even at a concentration of 0.1 μg mL^{-1}, free Ag^+ still cannot kill the bacteria thoroughly, which indicated that NH_2-MIL-88B(Fe)-Ag compound showed better antibacterial efficiency than free Ag^+. This was due to the the combined function of NH_2-MIL-88B(Fe) and Ag^+, resulting in the improved antibacterial efficiency for NH_2-MIL-88B(Fe)-Ag. For stability examination, the antibacterial activity of Ag^+ and NH_2-MIL-88B(Fe)-Ag after light irradiation were compared. As shown in Figure S6B, the activity of free Ag^+ decreased with the increased light exposure time. In contrast, the system of NH_2-MIL-88B(Fe)-Ag + H_2O_2 remained strong antibacterial activity even after light irradiation for 2 h, indicating that NH_2-MIL-88B(Fe)-Ag showed better antibacterial stability than free Ag^+. This could be attributed to the fact that NH_2-MIL-88B(Fe) with remarkable optical absorptivity can serve as a photon harvester to improve its light stability and Ag^+ exhibiting more powerful activity than Ag(0).[1]

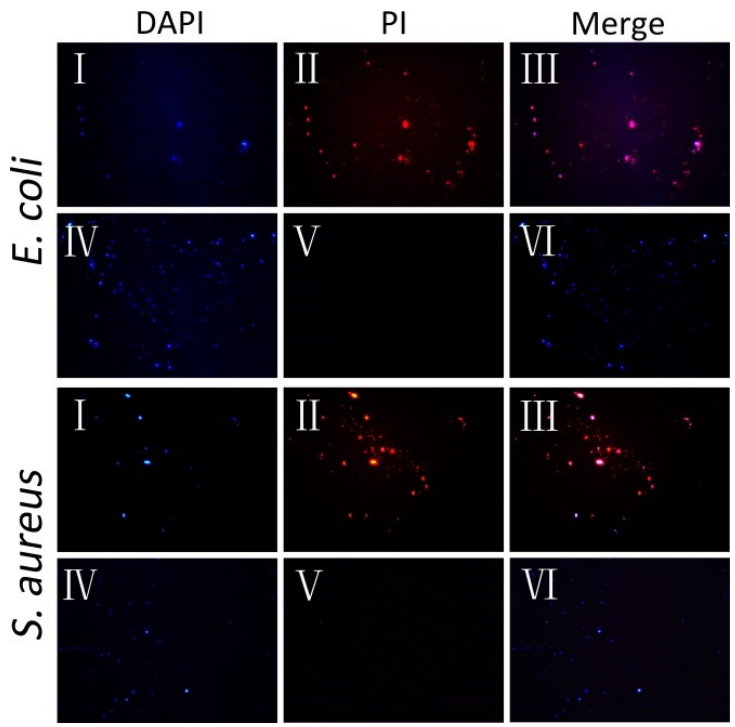

Figure S7 Typical fluorescence images of *E. coli* and *S. aureus* treated by: Ⅰ), Ⅱ) and Ⅲ) NH_2-MIL-88B(Fe)-Ag (50 μg mL^{-1}) + H_2O_2; Ⅳ), Ⅴ) and Ⅵ) PBS alone.

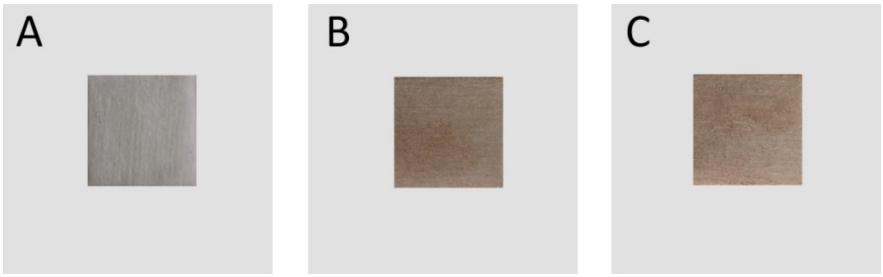

Figure S8 The cotton fabric absorbed with PBS, NH_2-MIL-88B(Fe) and NH_2-MIL-88B(Fe)-Ag.

Table S1 The Michaelis-Menten (K_m) constant and maximum reaction rate (V_{max}) of NH_2-MIL-88B(Fe)-Ag.

Catalyst	Substance	K_m (mmol L^{-1})	V_{max} (mol L^{-1} S^{-1})
HRP[2]	TMB	0.172	41.8×10^{-8}
HRP[2]	H_2O_2	10.9	58.5×10^{-8}
NH_2-MIL-88B(Fe)	TMB	0.729	3.82×10^{-8}
NH2-MIL-88B(Fe)	H_2O_2	0.57	5.27×10^{-8}
NH_2-MIL-88B(Fe)-Ag	TMB	0.5	2.02×10^{-8}
NH_2-MIL-88B(Fe)-Ag	H_2O_2	0.738	1.77×10^{-8}

Table S2 Comparison of the Ag(I) loading content of NH_2-MIL-88B(Fe)-Ag with other materials reported in previous articles.

Samples	Initial Concentration of Ag^+ (mg mL^{-1})	Bioassay	Silver-loading Content (mg g^{-1})	Reference
Silver- zeolite	0.5	*E. coli* and *S. aureus*	365.73	Y. Zhou et al.[3]
[Ag(Pen)(CH_3OH)]n	0.08	*S. epidermidis*, *S. aureus* and *P. aeruginosa* PAO1	227.9	I. Ketikidis et al.[4]
Ag/Zn–SPEEK	1.7	*E. coli* and *S. aureus*	42.82	Y. Deng et al.[5]
PDDA-Ag+-Cys-MoS_2	1.0	*E. coli* and *S. aureus*	30.48	F. Cao et al.[6]
Ag-infused lignin	8.5	*E. coli* BL21, P. aeruginosa and *S. epidermidis*	8.7	A. P. Richter et al.[7]
PCN-224-Ag-HA	0.5	methicillin-resistant *S. aureus*	5	Y. Zhang et al.[8]
Zirconium Titanium Phosphate-Ag	0.78	*E. coli*	0.5	N. Biswal et al.[9]
NH_2-MIL-88B(Fe)-Ag	0.1	*E. coli* and *S. aureus*	0.477	This work

References

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