Electronic Supplementary Material (ESI) for Biomaterials Science.

Metalloporphyrin and Hydantoin Functionalized Nanozyme with Synergistic Enhanced Bacterial Inhibition

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S1 Characterization

The morphologies of $FePPOP_{Hydantoin}$ were observed by SEM (Hitachi S-4800 Japan) and Au (1-2 nm) was sputtered onto the solid to enhance conductivity and improve image sharpness. Transmission electron microscopy (TEM) images were carried out on a JEOL JEM-2010 transmission electron microscope. Fourier transform infrared spectra (FT-IR) were collected on an ALPHA-T spectrometer (Bruker, Germany) by using the KBr disks with tavenumber range of 400-4000 cm^{-1}. Thermogravimetric analysis (TGA) was performed on a Mettler Toledo TGA/SDTA analyzer using N_2 by heating to 800^oC at a rate of 10^oC min^{-1}. N_2 adsorption-desorption isotherms were measured using a Micromeritics ASAP 2020 automated sorption analyzer at 77.3 K and the Brunauer–Emmett–Teller (BET) and Langmuir method were applied to estimate the surface area of $FePPOP_{Hydantoin}$. To investigate the molecular level structures, solid-state ^{13}C CP/MAS NMR experiment was carried out by a 4 mm MAS probe and having a spinning rate of 12 kHz.

S2 Materials and Reagents

2,2'-bipyridyl was obtained from Shandong sozhili biotechnology Co. Ltd. 1,3-dibromo-5,5-dimethylhydantoin was purchased from Shanghai haohong biomedical technology Co. Ltd. Chloroform ($CHCl_3$), N, N-dimethylformamide (DMF) and tetrahydrofuran (THF) were all ordered from Jinan cyber instrument Co. Ltd. Bis(1,5-cyclooctadiene)nickel(0) ([$Ni(cod)_2$])was provided by Beijing Ouhe Technology Co. Ltd. And 1,5-cyclooctadiene (cod) was purchased from Shanghai alighting biochemical technology Co. Ltd. Peroxide (30% H_2O_2), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phosphate(K_2HPO_4), sodium chloride (NaCl), potassium chloride (KCl) were purchased from Sinopharm Chemical

Reagent Co. Ltd. (Shanghai, China). Tryptone and yeast extract were obtained from Shanghai haoran biotechnology Co. Ltd. Tetrahydrofuran (THF) was purchase from Sinopharm Chemical Reagent Co. Ltd. 2,4,6,8-Tetramethyl-2,4,6,8-tetravinyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane was obtained from Shanghai Bidde Medical Technology Co. Ltd. Mercaptopropylmethyldimethoxysilane and Dichlorodimethylsilane were provided by Beijing Jianqiang Weiye Technology Co. Ltd and Beijing Enokai Technology Co. Ltd, respectively. 2, 2-dimethoxy-2-phenylacetophenone were obtained from Tianjin Shinesopod Technology Co. Ltd. Iron (III) 5,10,15,20-tetrakis-(4'-bromophenyl) porphyrinate (FeTBrPP) was synthesized according to the previous methods [1]. All reagents and solvents in this work were of analytical grade and used as received.

S3 Synthesis of iron 5,10,15,20-tetrakis-(4'-bromophenyl)porphyrin (FeTBrPP)

H_2TBrPP (150 mg, 0.16 mmol) and anhydrous ferric chloride (30.24 mg, 0.16 mmol) were added to anhydrous DMF (75 mL) under a nitrogen atmosphere. After being stirred at 165°C for 4 h, DMF was distilled off under reduced pressure, the residue was subjected to chromatography on a silica gel column with dichlorom ethane/methanol [10:1(v/v)] as eluent. The crude product was purified by recrystallization from CH_2Cl_2/MeOH, giving dark purple FeTBrPP (126.0 mg, 80 %). MALDI-TOF MS: calcd. for $C_{44}H_{26}Br_4N_4Fe$ $[M+H]^+$: 983.06; found m/z 986.32.

S4 Peroxidase-like activity

Peroxidase-like activity of $FePPOP_{Hydantoin}$ was measured through oxidizing a colorimetric substrate TMB to its oxidative product oxTMB, which has an intense absorbance at a wavelength of 652 nm. The concentration of $FePPOP_{Hydantoin}$, TMB and H_2O_2 were 10 μg/mL,

0.3 mM and 5 mM, respectively. pH and temperature were set at 3.77 and 30°C. The cycle experiment was carried out by oxidized TMB repeated, and $FePPOP_{Hydantoin}$ was extracted through the process of centrifugation. Moreover, the H_2O_2 concentration, pH and temperature dependent of $FePPOP_{Hydantoin}$ (10 μg·mL^{-1}) were also investigated. The pH in experiment of temperature dependent was set at 3.77. And the temperature in experiment of pH dependent was set at 30°C.

S5 Kinetics measurements

Kinetic assays of $FePPOP_{Hydantoin}$ were investigated by UV-vis spectroscopy at a wavelength of 652 nm with a three min interval. As shown in Figure S3, $FePPOP_{Hydantoin}$ and hydrogen peroxide concentration were settled and changing the concentration of TMB or vice versa. Then, a series of the initial reaction velocity was obtained. And the Michaelis constant (Km) and maximal reaction velocity (Vmax) were calculated by a Lineweaver-Burk double reciprocal plot shown in the following equation (1):

$$1/v = (K_m/V_{max}) \times (1/[C]) + 1/V_{max} \qquad \text{Eq. (1)}$$

In the equation, v is the initial velocity, C is the substrate's concentration, and V_{max} and K_m are the maximum reaction velocity and the Michaelis-Menten constant, respectively.

S6 The calculation of energy levels for $FePPOP_{Hydantoin}$

The cyclic voltammograms (CV) of $FePPOP_{Hydantoin}$ on an indium tin oxide (ITO) electrode in 0.1 M tetrabutylamonium hexafluorophosphate ($TBAPF_6$) acetonitrile solution are shown in Figure S4, the CV measurement of $FePPOP_{Hydantoin}$ exhibits distinct oxidation and reduction peaks at 0.77 eV and -0.71 eV vs normal hydrogen electrode (NHE). The Ag/AgCl reference electrode was calibrated against 1 mg/mL ferrocene in 0.1 M $TBAPF_6$ acetonitrile solution.

The energy level of ferrocene/ ferrocenium (Fc/Fc^+) is 4.80 eV on the zero vacuum level scale, and the reference potential [$E_{ref} = (E_{ox\text{-}Fc/Fc+}+E_{red\text{-}Fc/Fc+})/2$] of Fc/Fc^+ calibrated by using the bare ITO electrode in 1 mg/mL Fc solution was +0.04 eV. The HOMO and the LUMO can be obtained by using the formulas: $E_{HOMO} = -(4.8 - E_{ref} + Eox)$ (eV), $E_{LUMO} = -(4.8 - E_{ref} + Ered)$ (eV). The band gap of $FePPOP_{Hydantoin}$ was calculated by the equation: Eg=LUMO – HOMO.

S7 The measurement of photocurrent response for $FePPOP_{Hydantoin}$

To measure the photocurrent response, $FePPOP_{Hydantoin}$ solution (1 mg/mL, 20 μL) was first dripped onto an ITO electrode and dried under an infrared lamp. Then, the obtained electrode was soaked in PBS buffer solution, followed by NIR (808 nm) irradiation at 10 s intervals.

S8 Method of antibacterial efficiency.

The antibacterial efficiency of $FePPOP_{Hydantoin}$ was measured according to the previous reports. [2] The bacterial solutions deal with $FePPOP_{Hydantoin}$ at different condition was calculated by measuring optical density value at the wavelength of 600 nm before and after. Then, the antibacterial efficiency (AE) of *S. aureus* in 96-well plate was determined by equation 2.

$$AE = \frac{A_0 - A}{A_0} \times 100\% \qquad \text{equation (2)}$$

where A is the absorbance of redundant bacteria treated with $FePPOP_{Hydantoin}$, and A_0 is the absorbance of the bacteria without any treated.

S9 Hydantoin concentration-dependent antibacterial experiments

Staphylococcus aureus (*S. aureus*) was utilized as a model microorganism to explore the antibacterial characteristics of Hydantoin. All disks and materials were sterilized in an autoclave at 121°C over 30 minutes before experiments. Quantitative Hydantoin was dispersed

in sterilized PBS and suffered ultrasonic treatment for 30 min to get different concentration of Hydantoin suspension. The bacteria suspension (1×10^6 CFU mL^{-1}) was incubated with different concentrations of Hydantoin (18.5, 37, 55.5, 74 μg mL^{-1}) in 1.5 mL centrifuge tubes for 4 hours. Then, 10 μL of the above Hydantoin bacteria suspension was carefully pipetted out and incubated with LB medium in 96-well plates and cultured in a shaker (37°C, 12 h, 140 rpm). The antibacterial efficiency was estimated according to the optical density value (OD_{600}) before and after the treatment.

S10 Hemolysis Assay.

0.5 mL of new mice blood (1 mL) was collected by centrifugation with a 3000 rpm for 15 min to separate red blood cells (RBCs). Then RBCs washed with PBS three times was dispersed in PBS (10mL). After that, 100 μL of $FePPOP_{Hydantoin}$ dispersed in PBS with different concentrations (2–512 μg/mL) was mixed with 100 μL of RBCs suspension in PBS for 2h at 37°C. RBCs treated with Triton-X and PBS were used as the positive and negative controls, respectively. The OD_{545} of the supernatant was measured after centrifugation to evaluate the hemolysis ratio. The hemolysis ratio was calculated by the following equation:

$$\text{Hemolysis ratio (\%)} = (OD - OD_N)/(OD_P - OD_N) \times 100\% \quad \text{equation (3)}$$

where OD, OD_N, and OD_P are the OD values of samples, the negative control, and the positive control, respectively.

S11 Method of antibacterial efficiency by plate count method.

The colony forming units (CFU) of bacterial solutions before and after treatments with $FePPOP_{Hydantoin}$ at different condition were measured by plate count method, respectively. Then, the antibacterial efficiency (AE) of *S. aureus* was determined by equation 4.

$$AE = \frac{N_0 - N}{N_0} \times 100\% \qquad \text{equation (4)}$$

where N_0 and N are the colony forming units before and after treatments, respectively.

Related Figures

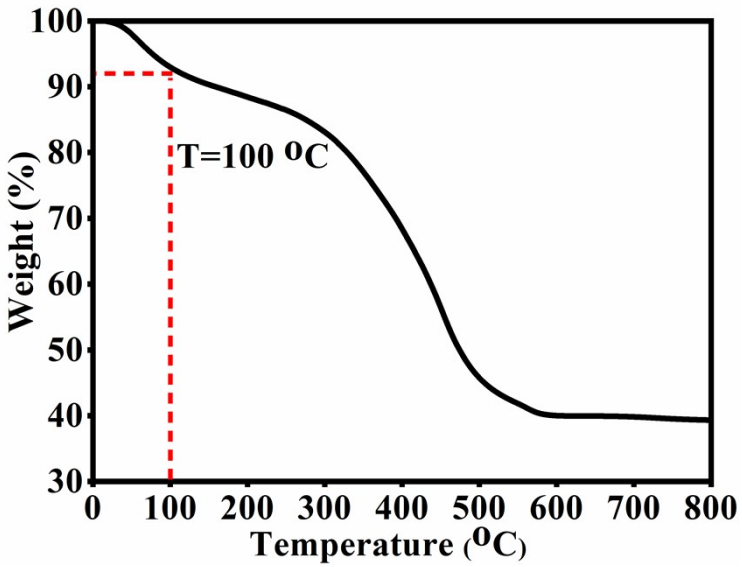

Figure S1. Thermogravimetric analysis (TGA) data of $FePPOP_{Hydantoin}$

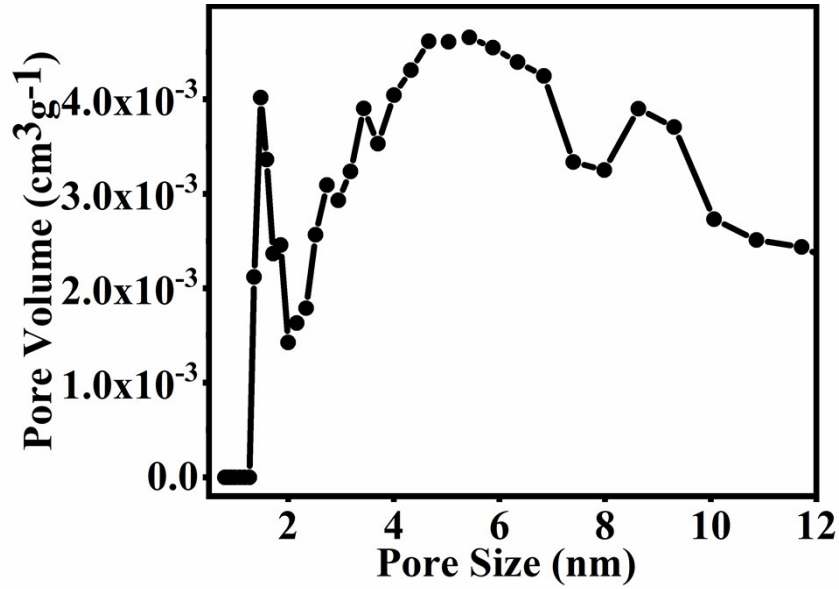

Figure S2. Pore size distribution of $FePPOP_{Hydantoin}$ by DFT modeling on the N_2 adsorption isotherms.

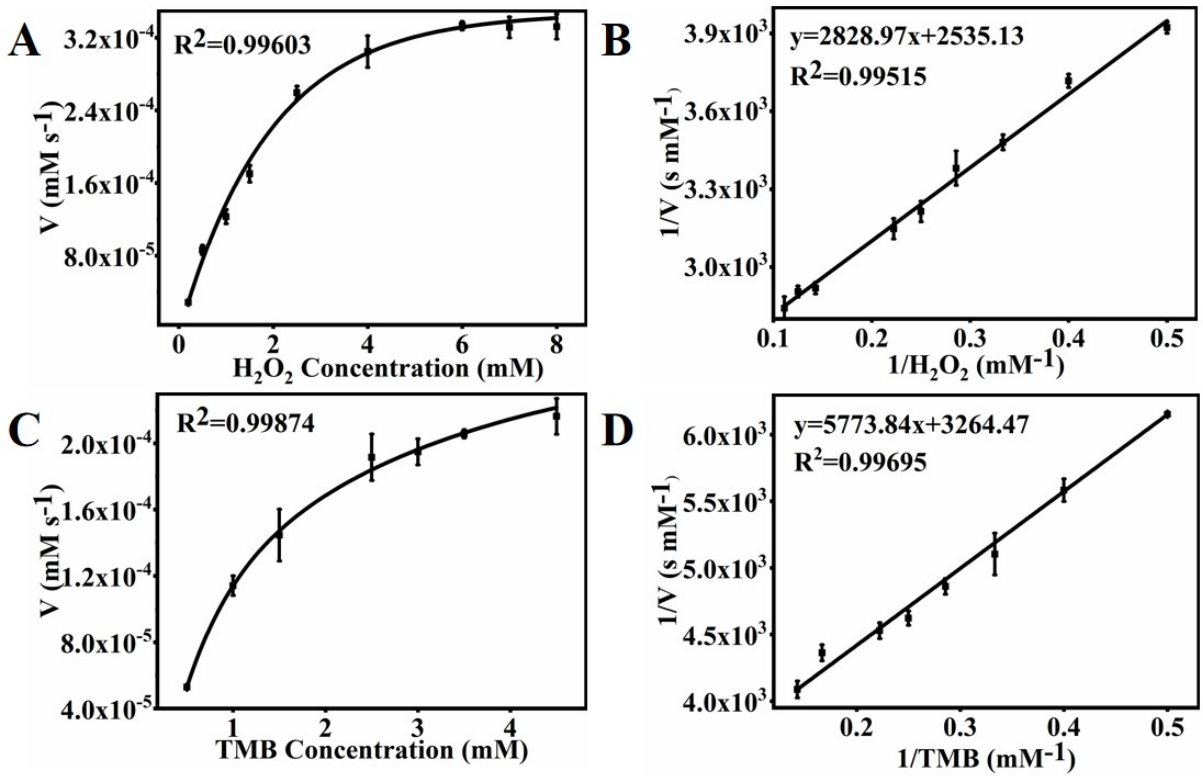

Figure S3. Steady-state kinetic analysis of $FePPOP_{Hydantoin}$. The concentration of $FePPOP_{Hydantoin}$ and H_2O_2 were 10 μg/mL and 5 mM, respectively in a 2.0 mL HAc-NaAc buffer (pH 3.77) with various concentrations of TMB (A and B). Then the concentration of TMB was fixed at 0.3 mM with various concentrations of H_2O_2 (C and D).

Table S1. Comparison of the kinetic parameters of $FePPOP_{Hydantoin}$ with other catalysts. K_m is the Michaelis constant and V_{max} is the maximal reaction velocity.

Catalyst	K_m (mM)		V_{max} (mM/s)		Reference
	TMB	H_2O_2	TMB	H_2O_2	
$FePPOP_{Hydantoin}$	1.77	1.12	3.06×10^{-4}	3.94×10^{-4}	This study
$FePPOP_{EPA}$	0.091	1.95	4.79×10^{-5}	2.35×10^{-5}	2
$FePPOP_{BFPB}$	0.0099	2.81	8.62×10^{-6}	6.70×10^{-5}	3
PtCNPs	0.0375	1595.3	1.49×10^{-4}	4.93×10^{-4}	4
CuCNPs	0.183	282.1	1.45×10^{-4}	1.68×10^{-4}	4
Fe_3O_4	4.84	2.74	4.69×10^{-5}	3.53×10^{-5}	5
GO-Fe_3O_4	0.71	0.43	5.31×10^{-5}	1.31×10^{-4}	6
CoO	-	92.1		0.11×10^{-4}	7
CeO_2		4.41		1.8×10^{-4}	7
HRP	0.43	1.21	1.87×10^{-5}	5.88×10^{-5}	8

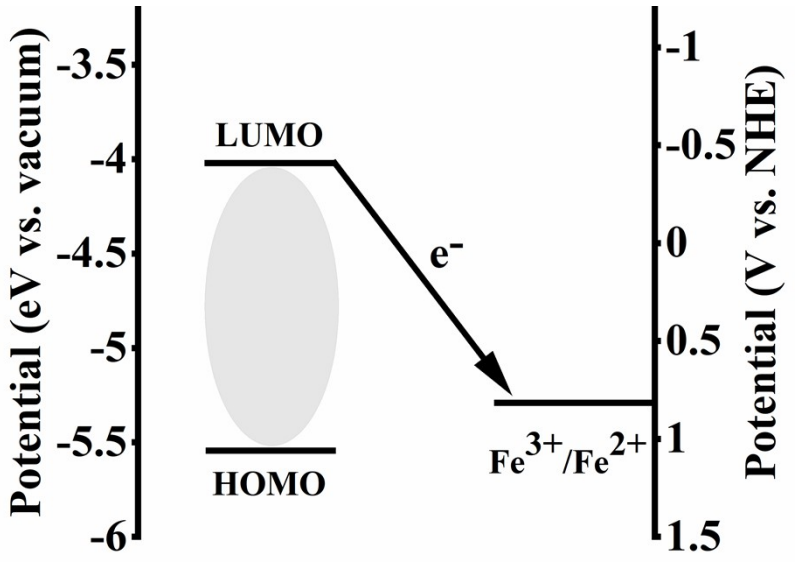

Figure S4. Comparison of the redox state of $FePPOP_{Hydantoin}$ and Fe^{3+}/Fe^{2+} to prove electron transfer process from $FePPOP_{Hydantoin}$ to Fe^{3+}.

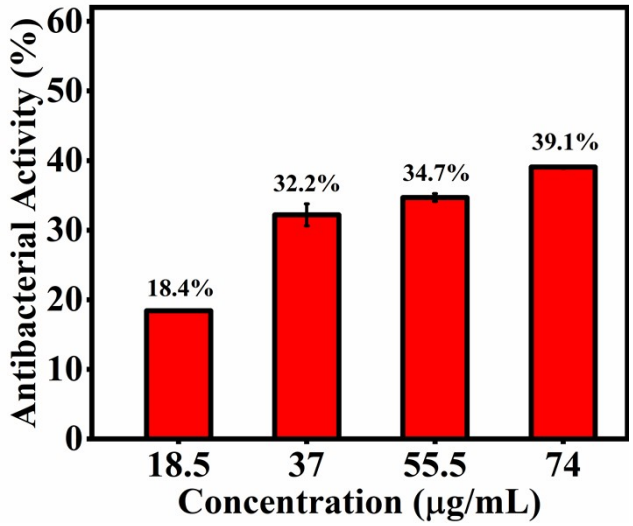

Figure S5. The cytotoxicity of Hydantoin with different concentrations.

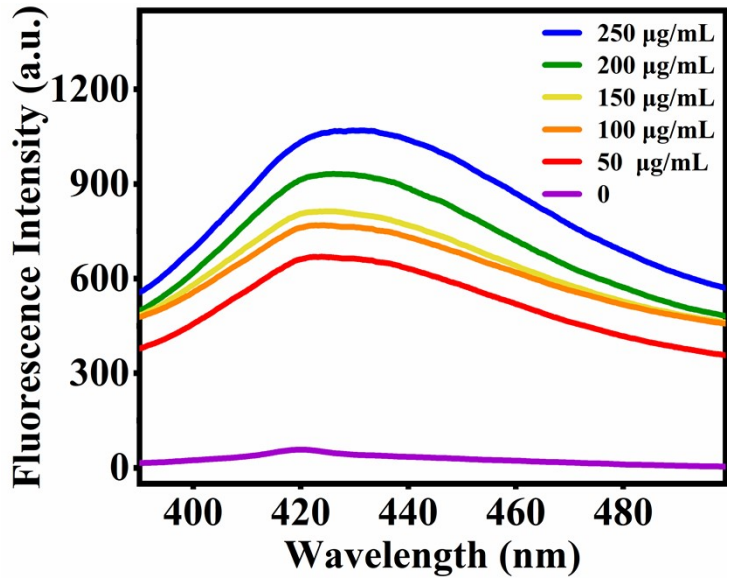

Figure S6. Fluorescence spectroscopy of the detection of ·OH with increased concentration of $FePPOP_{Hydantoin}$.

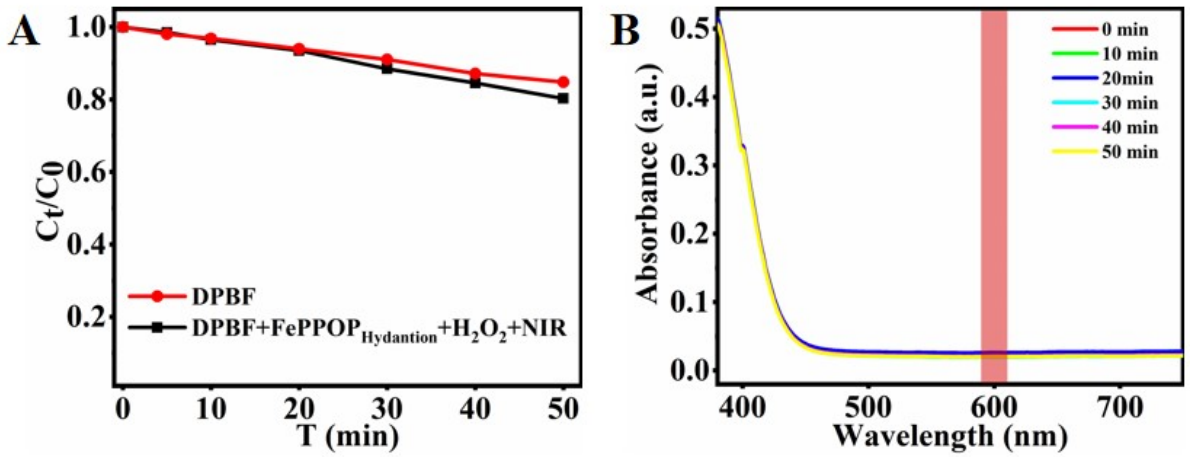

Figure S7 (A) Plot of DPBF with or without $FePPOP_{Hydantoin}$+H_2O_2+NIR system over varying intervals of time. (B) UV-Vis spectra of the $FePPOP_{Hydantoin}$+H_2O_2 +NBT+NADH system.

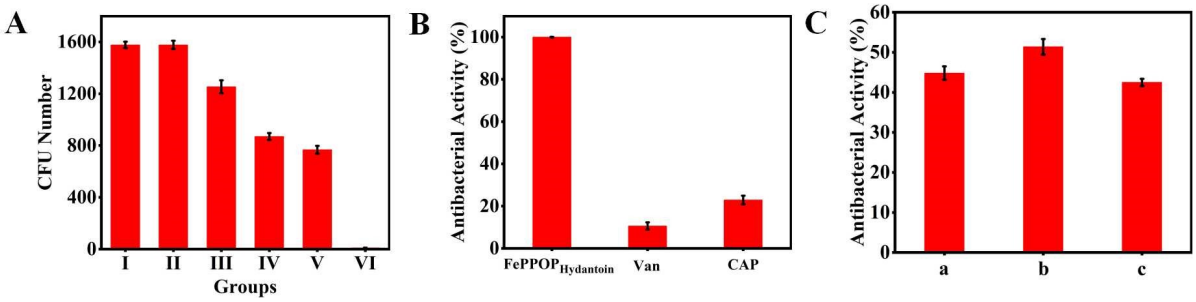

Figure S8 (A) The CFU number of bacteria treated with $FePPOP_{Hydantoin}$ estimated by plate count method (I: PBS; II: H_2O_2; III: NIR; IV: $FePPOP_{Hydantoin}$; V: $FePPOP_{Hydantoin}$+ H_2O_2; VI: $FePPOP_{Hydantoin}$+H_2O_2+NIR). (B) Antibacterial activity of $FePPOP_{Hydantoin}$ and conventional antibiotics, concentration: 200 μg mL^{-1}. (C) Antibacterial activity of $FePPOP_{Hydantoin}$ in the presence of ROS scavenger estimated by OD_{600}. (a) $FePPOP_{Hydantoin}$, (b) $FePPOP_{Hydantoin}$+H_2O_2, and (c) $FePPOP_{Hydantoin}$+H_2O_2+methanol.

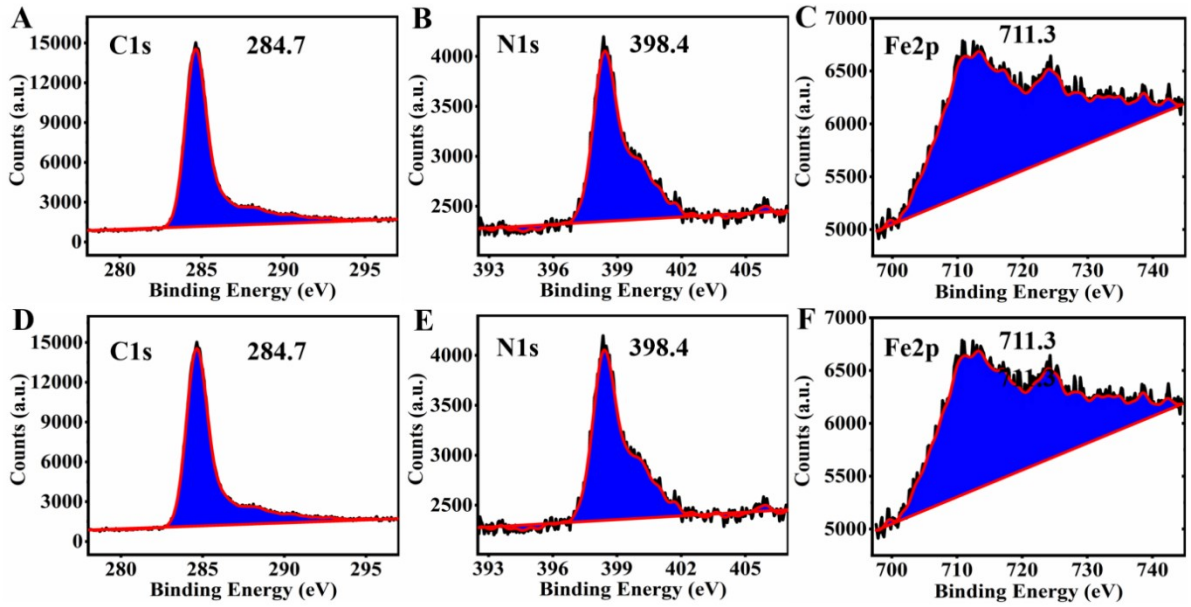

Figure S9. XPS spectra of C1s, N1s and Fe2p before (A, B, C) and after (D, E, F) antibacterial treatments for $FePPOP_{Hydantoin}$.

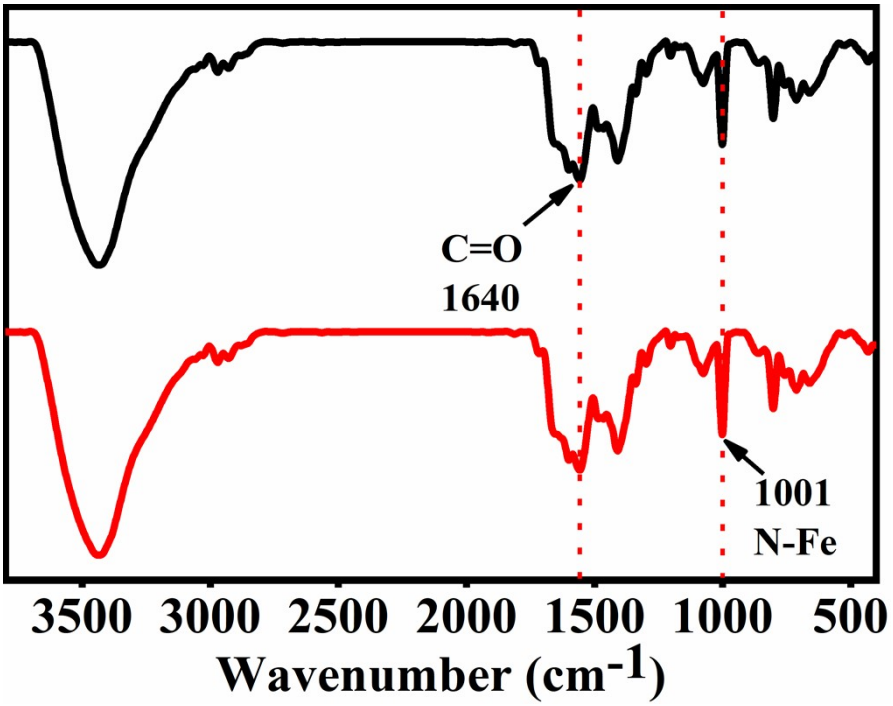

Figure S10. FT-IR spectra of $FePPOP_{Hydantoin}$ before (black) and after (red) disinfection.

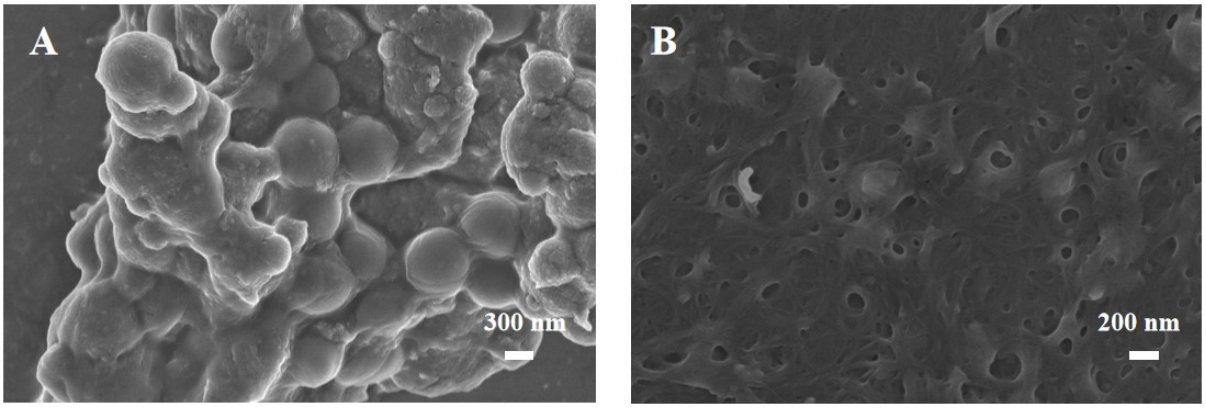

Figure S11. (A) SEM of *S. aureus* cultivated for 3 hours and 40 min and then treated with 808 nm irradiation for 20 min and (B) treated with 808 nm irradiation for 20 min and then cultivated for 3 hours and 40 min.

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