Supporting Information for

Cascaded enzymes-loaded Fe-Hemoporfin framework for synergistic sonodynamic-starvation therapy of tumors

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1.Experimental section

1.1 Chemicals and cells

Hemoporfin was brought from Shanghai Xianhui Pharmaceutical Co., Ltd. Iron chlorides anhydrous ($FeCl_3$), hydrogen peroxide (H_2O_2), methanol, 1.3-diphenyl isobenzofuran (DPBF), trimethylamine, and N.N-dimethylformamide (DMF) were received from Sinopharm Chemical Reagent Co., Ltd. Glucose oxidase (GOx), catalase (CAT), and amphiphilic PEGylated folic-acid-conjugated phospholipid (DSPE-PEG-FA, *M*w=5000) were acquired from Shanghai Yanyi Biotechnology Corporation. Murine breast cancer 4T1 cells (4T1), Human Umbilical Vein Endothelial Cells (HUVEC), a colon cancer cell CT26 (CT26), and Human ovarian cancer cell (SK-OV3) were originally purchased from Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China.

1.2 Cell test

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In vitro cytotoxicity assay. HUVEC, CT26, SK-OV3, and 4T1 were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in the presence of 5% CO_2 at 37°C. HUVEC CT26, SK-OV3, and 4T1 were seeded into 96-well plates at ~1×10^4/well under standard conditions for 12 h, and then the medium was replaced with fresh media containing FeHF-GOx/CAT at a series of final concentrations (0-150 μg/mL). After the incubation for 24 h, the medium was removed, and cells were washed with PBS to allow the addition of DMEM containing Cell Counting Kit-8 (CCK-8) assay. The cells were further cultured for 1 h, and the absorbance at 450 nm of each well was measured by using a microplate reader.

Cellular uptake of FeHF-GOx/CAT. 4T1 cells were incubated with a fresh medium containing fluorescein labeled FeHF-GOx/CAT (F-FeHF-GOx/CAT, 100 μg/mL) in a 12-well plate for 3 h, and then 4T1 cells were washed with PBS to remove free FeHF-GOx/CAT. Subsequently, the cells were stained by using DAPI for 15 min and imaged under CLSM.

2.Figures

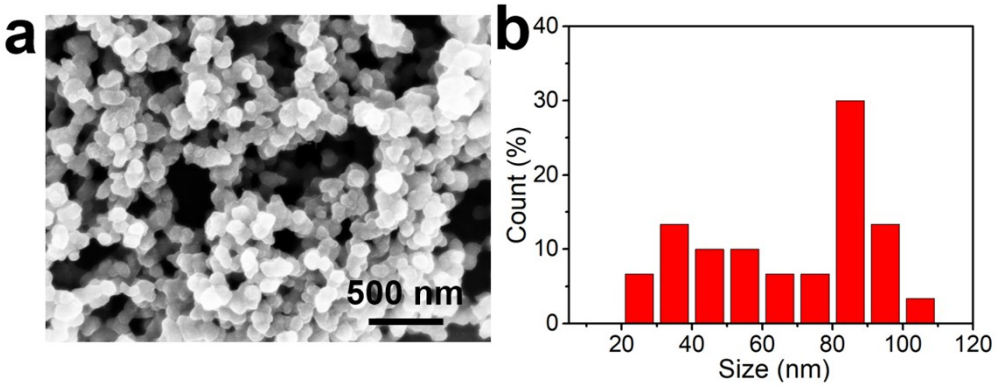

Fig. S1 (a) SEM image of FeHF. (b) Size distribution of FeHF.

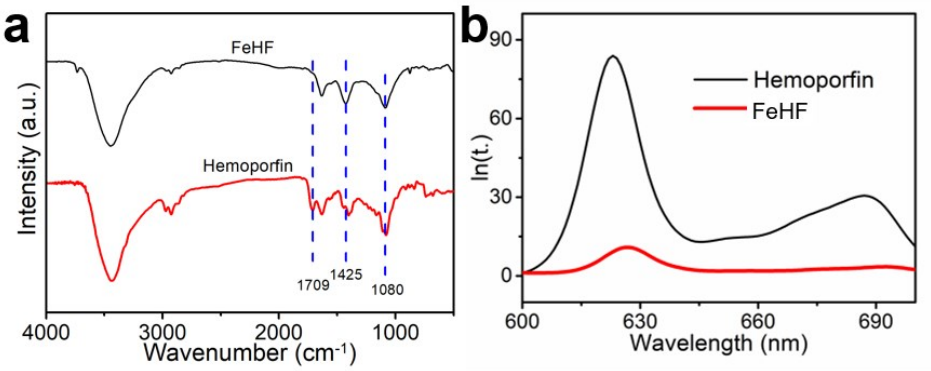

Fig. S2 (a) FTIR spectra of FeHF and Hemoporfin. (b) Fluorescence spectrum of FeHF and Hemoporfin.

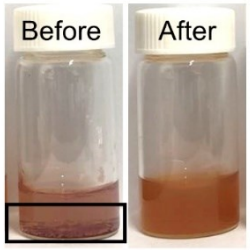

Fig. S3 Photographs of nanoparticles before and after coating DSPE-PEG-FA in deionized water for 24 h.

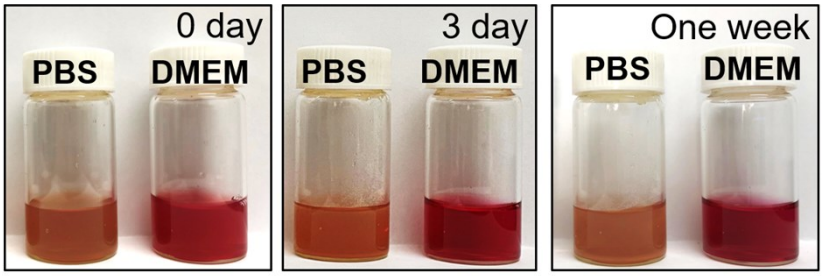

Fig. S4 Photographs of FeHF-GOx/CAT at long residence time in different biological fluids.

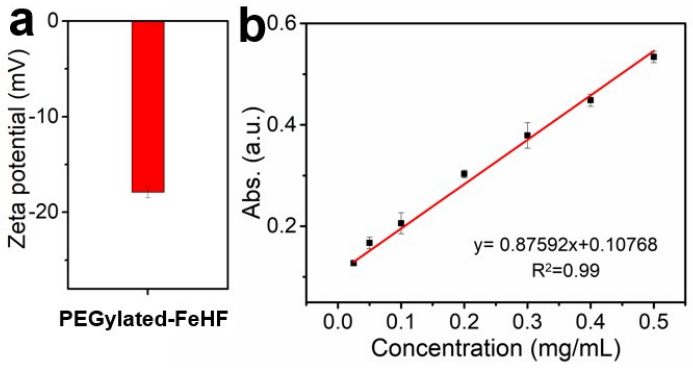

Fig. S5 (a) Zeta potential of PEGylated-FeHF. (b) Standard curve of BCA for BCA Protein Assay Kit.

The loading of the two enzymes (GOx and CAT) was evaluated by labelling GOx with FITC and CAT with TRITC. The fluorescence spectra of the resulting fluorophore-labelled FeHF-GOx/CAT (Fig. 2i) and appropriate calibration curves were used to evaluate the contents of the enzymes in the FeHF.

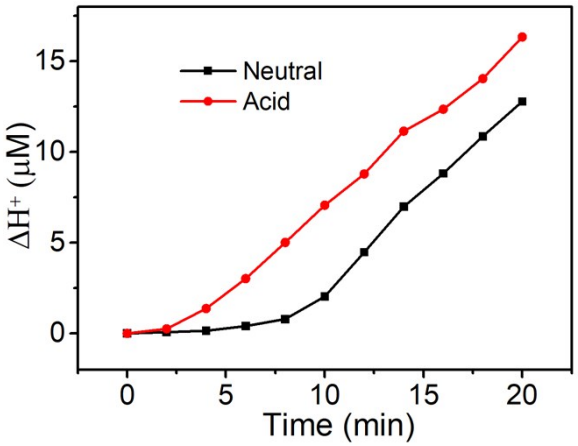

Fig. S6 The changes of H^+ concentration in the neutral or acid environment.

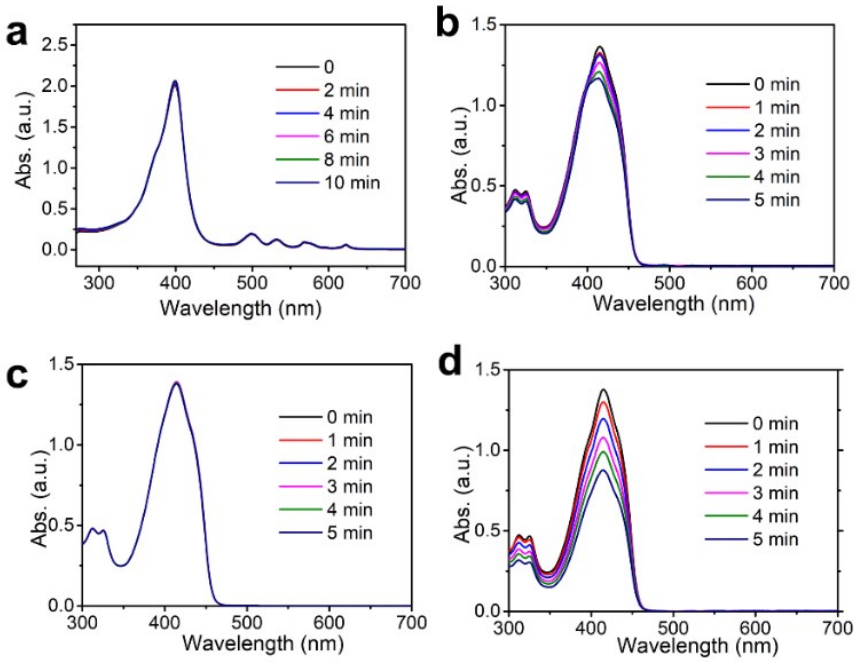

Fig. S7 (a)UV-vis spectra of pure FeHF under US irradiation. (b) Time-dependent oxidation of DPBF indicating US-triggered $^1O_2$ generation by FeHF (after deducting the peak of FeHF). (c) UV-vis spectra of DPBF under US irradiation. (d) Time-dependent oxidation of DPBF indicating US-triggered $^1O_2$ generation by FeHF-GOx/CAT after adding H_2O_2 and glucose (after deducting the peak of FeHF-GOx/CAT).

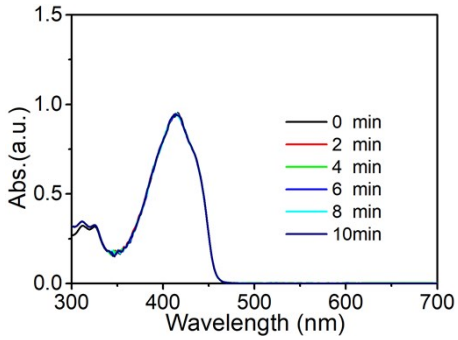

Fig. S8 UV-vis spectra of DPBF after addition of H_2O_2.

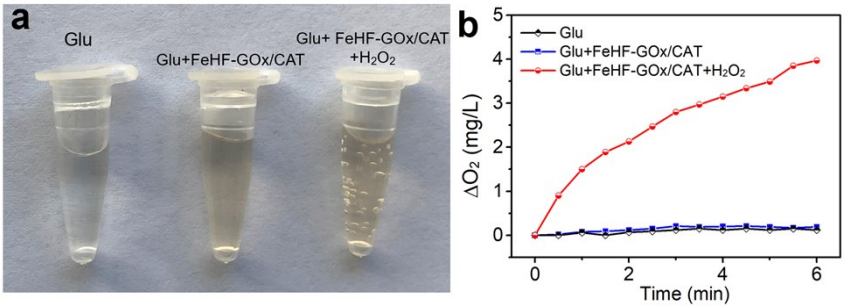

Fig. S9 (a) Typical photograph of different solutions. (b) The change of O_2 concentration for different solution over a period of 6 min.

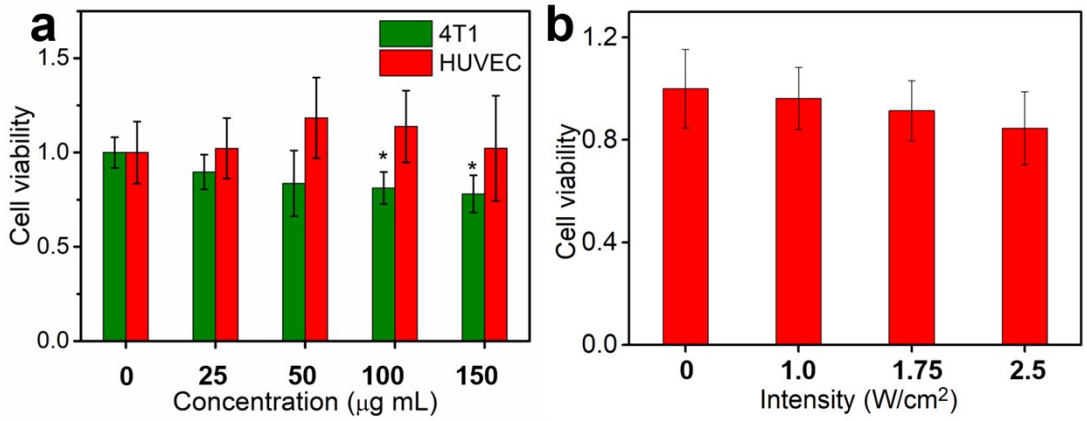

Fig. S10 (a) Cell viability of cells after treatment with FeHF-GOx/CAT at 0-150 μg/mL for 24 h. (b) Cell viability under different ultrasonic intensities.

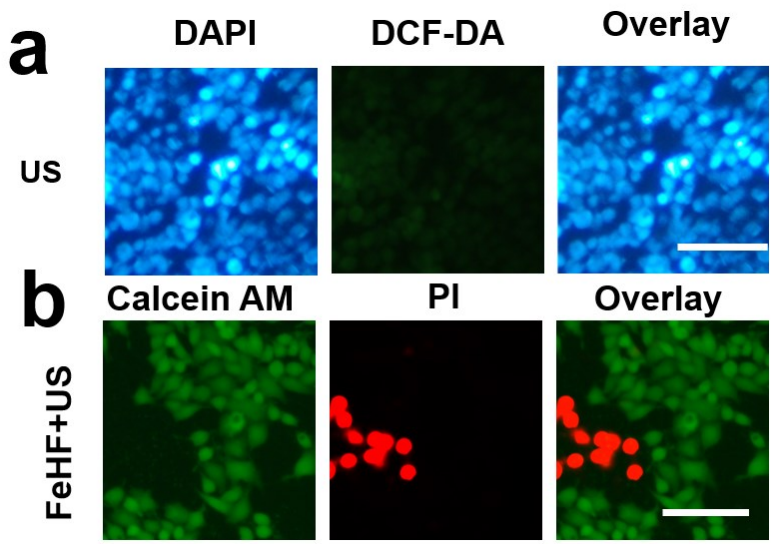

Fig. S11 (a)Fluorescence images of DCF-DA-stained 4T1 cells after US treatment. The scar bar is 50 μm. (b)The fluorescence images of Calcein-A/PI-stained 4T1 cells after FHF+US treatment. The scar bar is 50 μm.

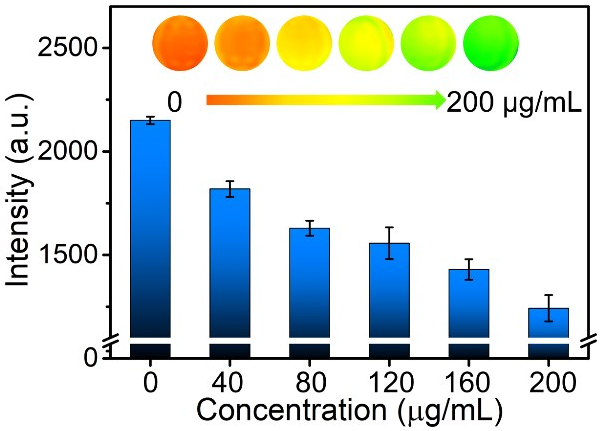

Fig. S12 MR images and signal intensities of FeHF-GOx/CAT dispersions at a series of concentrations.

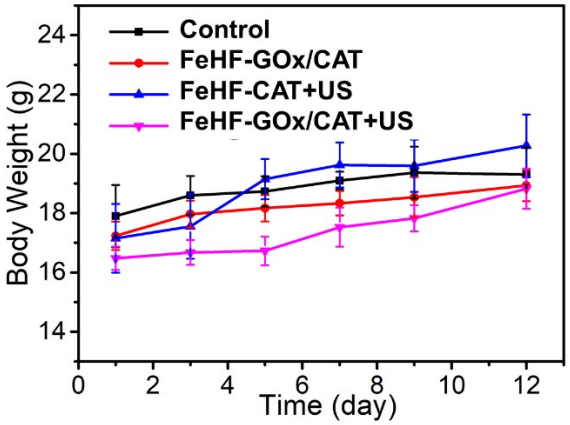

Fig. S13 Body weight of CT26 tumor-bearing mice in different therapies.

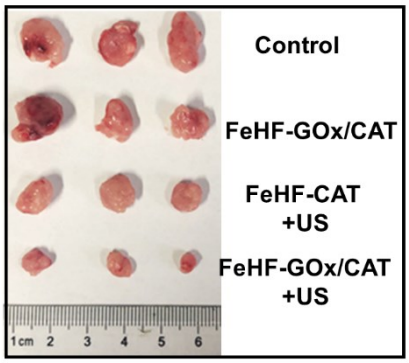

Fig. S14 Tumor photographs of CT26 tumor-bearing mice in different therapies.