Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

An autophagy-inhibitory MOF nanoreactor for tumor-targeted synergistic therapy

Xiaohan Liu,‡ Peng Gao,‡ Mingwan Shi, Yuanyuan Chen, Wei Pan, Na Li* and Bo Tang

College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Institute of Molecular and Nano Science, Shandong Normal University, Jinan 250014, P. R. China.

E-mail: lina@sdnu.edu.cn

Experimental details:

Materials. $Zn(NO_3)_2 \cdot 6H_2O$, imidazole-2-carboxaldehyde (ICA), ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glucose oxidase (GOx), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (Shanghai, China). Chloroquine (CQ) was obtained from Energy Chemical Co., Ltd. (Shanghai, China). BCA Protein Assay Kit was purchased from Beyotime Biotechnology Company (Nantong, China). Calreticulin Rabbit Polyclonal antibody and CoraLite488-conjugated Affinipure Goat Anti-Rabbit lgG(H+L) were purchased from proteintech Co., Ltd. APC anti-mouse/human CD11b, FITC anti-mouse CD86, PE anti-mouse CD80, FITC anti-mouse CD206 (MMR) were purchased from BioLegend, Inc. Protease inhibitor was purchased from Medchemexpress (MCE), USA. Cell culture products, unless mentioned otherwise, were purchased from Biological Industries, Israel. The mouse breast cancer cell line (4T1) was purchased from Shanghai AOLU Biological Technology Co. Ltd, China. The Human hepatocellular liver carcinoma cell line (HepG2) were obtained from Procell Life Science & Technology Co., Ltd. All the aqueous solutions used in experiments were prepared using deionized water (18.2 MΩ cm^{-1}). All chemicals were of analytical grade and were used without further purification.

Instruments. TEM images were obtained from Transmission electron microscopy (TEM, HT7700, Japan). UV-Vis absorption spectra were measured on pharmaspec UV-1700 UV-Visible spectrophotometer (Shimadzu, Japan). MTT assay was carried out with a microplate reader (Synergy 2, Biotek, USA). The crystal structure of the materials was performed with powder X-ray diffraction (PXRD) patterns (Bruker D8, Germany). Zeta potential measurements were accomplished with Malvern Zeta Sizer Nano (Malvern Instruments). Centrifuge was performed with High Speed Refrigerated Centrifuge (Sigma 3K 15, USA). Confocal fluorescence imaging assay were performed with a TCS SP8 confocal laser scanning microscopy (Leica TCS SP8, Germany).

The preparation of CQ@ZIF-GOx. Firstly, ICA(15.3 mg) was dissolved in 2 mL water with ultrasound and stirred at room temperature. Then, 1 mg CQ was added and stirred until dissolved. 100 μL aqueous solution containing 11.9 mg $Zn(NO_3)_2 \cdot 6H_2O$

was rapidly added. After 1 h, 1 mg GOx was added and another 24 h was taken for the reaction. Finally, CQ@ZIF-GOx was separated by centrifugation and washed with water for three times. Both ZIF, CQ@ZIF and ZIF-GOx were synthesized by the same method.

The preparation of CQ@ZIF-GOx@C. 4T1 cells were collected and washed twice with Tris-HCl buffer (pH 7.4). Then the Tris-HCl buffer with 1% protease inhibitor was added to resuspend the cells. The cells were broken by mechanical membrane disruption. Organelles and large membrane fragments were removed by centrifugation (10,000 rcf, 4℃, 10 min). Subsequently, trivial membrane fragments were achieved through ultracentrifugation (100,000 rcf, 4 ℃, 1.5h). Finally, CQ@ZIF-GOx@C was prepared by mixing CQ@ZIF-GOx nanoparticles aqueous solution with trivial membrane fragments. The mixture was stirred for 24 h in an ice-water bath. Both ZIF@C, CQ@ZIF@C and ZIF-GOx@C were synthesized by the same method.

Cell culture. 4T1 cells were cultured in Roswell Park Memorial Institute medium (RPMI 1640) containing 10% fetal bovine serum and 1% penicillin/streptomycin. HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) medium containing 10 % fetal bovine serum and 1 % penicillin/streptomycin. They were all maintained at 37 °C in a 5% CO_2/95% air humidified incubator.

Cytotoxicity assay. 4T1 cells and HepG2 cells were cultivated in 96-well plates. After 24 h, they were incubated with different concentrations (0, 10, 50, 100 μg/mL) of different materials (ZIF, CQ@ZIF, ZIF-GOx, CQ@ZIF-GOx) for 4 h. Then, fresh medium was added to the plates and the cells were incubated for another 20 h. Subsequently, the culture medium was discarded. 150 μL MTT solution (0.5 mg/mL) was added to each plate. After incubated for 4 h, 150 μL DMSO was used to dissolve formazan crystal. The absorbance was measured at 490 nm with a microplate reader.

Live/dead cell staining assay. 4T1 cells and HepG2 cells were seeded in confocal dishes. When the cell density approached about 70%, the cells were treated with blank medium and medium containing ZIF, CQ@ZIF, ZIF-GOx, CQ@ZIF-GOx (50 μg/mL) for 4 h. Then, fresh medium was added to the plates and the cells were incubated for

another 20 h. Subsequently, the cells were stained with Calcein AM/Propidium Iodide (PI) for 15 min and analyzed with confocal fluorescence imaging.

Evaluation of calreticulin (CRT). The immunofluorescence staining was performed to evaluate the calreticulin (CRT) expression of 4T1 cells and HepG2 cells with different treatments. The cells were treated with blank medium and medium containing ZIF, CQ@ZIF, ZIF-GOx, CQ@ZIF-GOx (50 μg/mL) for 4 h. Then the cells were fixed and staining with CRT-related primary antibody, secondary antibody and Hoechst 33342. Finally, the cells were evaluated by confocal fluorescence imaging. All the antibodies in the experiment were used according to the instructions.

Monodansylcadaverine (MDC) staining experiment. The autofluorescent agent MDC was introduced as a specific autophagolysosome marker to analyze the autophagic process. 4T1 cells and HepG2 cells were seeded in confocal dishes. When the cell density approached about 70%, the cells were treated with blank medium and medium containing ZIF, CQ@ZIF, ZIF-GOx, CQ@ZIF-GOx (10 μg/mL) for 4 h. Then 1x Wash buffer was used to wash cells. Subsequently, the cells were stained with MDC for 30 min. Confocal laser scanning microscopy was applied to anaylst autophagy level.

Evaluation of the intracellular ROS. DCFH-DA probe was utilized to evaluate the intracellular level of ROS. 4T1 cells and HepG2 cells were seeded in confocal dishes. When the cell density approached about 70%, the cells were treated with blank medium and medium containing ZIF, CQ@ZIF, ZIF-GOx, CQ@ZIF-GOx (50 μg/mL) for 4 h. Then PBS buffer was used to wash cells. DCFH-DA probe was used to incubate cells for 20 min. Confocal laser scanning microscopy was applied to anaylst the ROS level.

Establishment of 4T1 subcutaneous tumor model. 4T1 cells were injected subcutaneously into the right axillary region of Balb/C mice. After the tumor size reached approximately 75-100 mm^3, the mice were divided into five groups: PBS, ZIF@C, CQ@ZIF@C, ZIF-GOx@C and CQ@ZIF-GOx@C. The intravenously injected dose was 20 mg/kg each time for 3 times in total. The tumor sizes and body weights were measured every other day for 15 days after treatment (Tumor Volume =

$W^2 \times L/2$, W = width, L = length). And after 15 days, the tumors and main organs were collected for H&E staining.

***In vivo* distribution.** The dye IR808 was doped into ZIF (IR808@ZIF) and then coated with the cancer cell membrane (IR808@ZIF@C). 4T1 tumor-bearing Balb/C mice were intravenously administrated with 50 μL of 4 mg/mL nanoparticles (IR808@ZIF and IR808@ZIF@C). At different time points of 1, 4, 8, 12, 24 and 48 h post injection, the IR808 fluorescence in the mice was recorded with a live body imaging system.

Macrophage reeducation *in vivo*. 4T1 tumor-bearing Balb/C mice were divided into five groups and intravenously injected with PBS, ZIF@C, CQ@ZIF@C, ZIF-GOx@C, CQ@ZIF-GOx@C. At 7 days post injection, the mice were sacrificed and tumors were collected. Then tumors were divided into two parts. One part was for flow cytometry. In brief, the tumors were cut into pieces and put into a glass homogenizer containing PBS buffer (pH 7.4) with 2% heat-inactivated FBS. A single cell suspension was obtained by gentle pressure with the homogenizer. Then RBC lysis buffer was used to remove red blood cells (RBCs). Finally, the obtained single cells were stained with fluorescence-labelled antibodies: anti-CD11b-APC, anti-CD80-PE, anti-CD86-FITC, anti-CD206-FITC. The other part of the tumors was stored in 4 % paraformaldehyde and prepared for immunofluorescence staining of tissue sections.

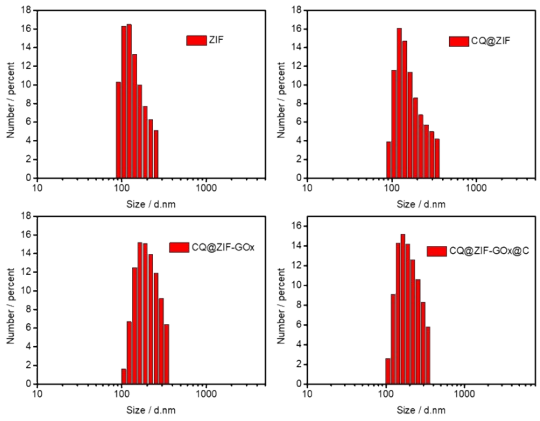

Figure S1. The hydrodynamic sizes of different materials.

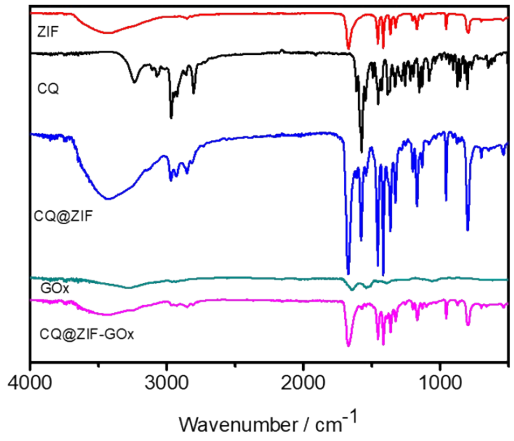

Figure S2. The FT-IR spectra of different materials.

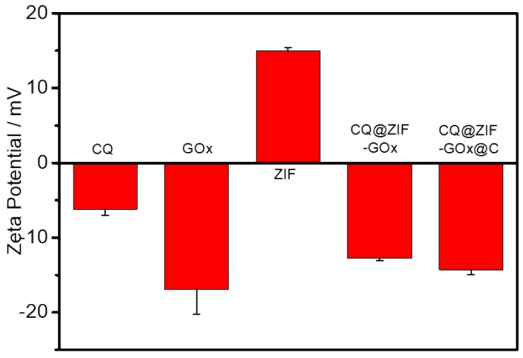

Figure S3. The Zeta potential of different materials.

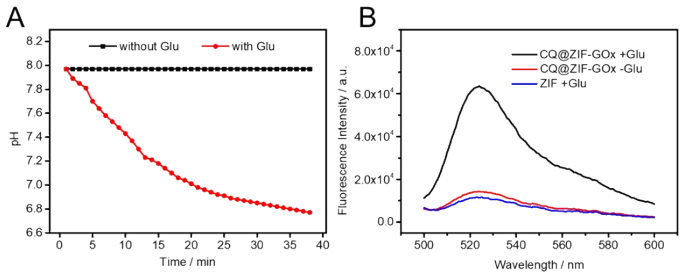

Figure S4. A) The pH values of CQ@ZIF-GOx solution with or without Glu. B) Fluorescence spectra of DCFH probe incubated with different materials with or without Glu.

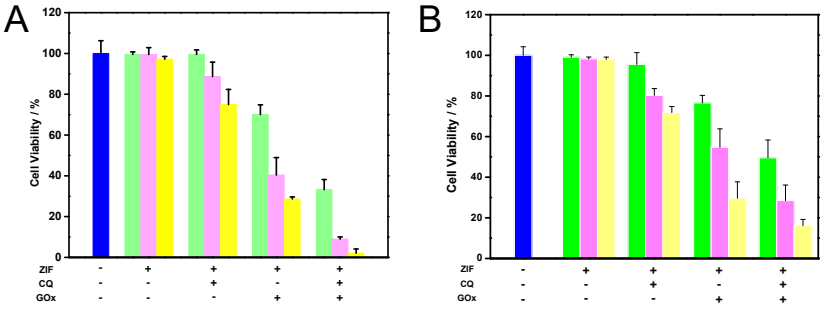

Figure S5. Cell viabilities of A) 4T1 and B) HepG2 post different treatments, the concentrations in each group are 10, 50 and 100 μg/mL.

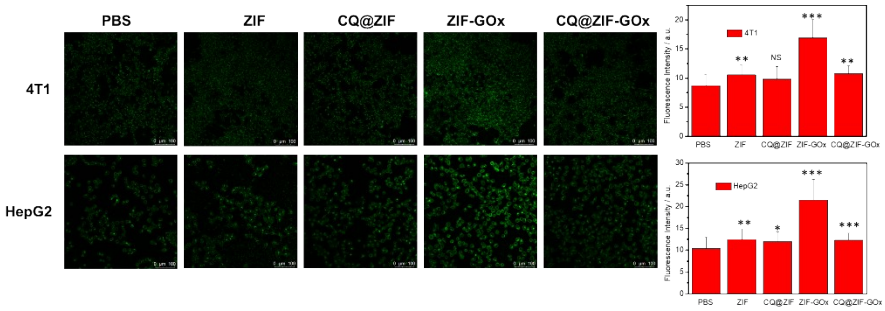

Figure S6. Confocal imaging of cell autophagy with a probe MDC and the quantitative analysis of the fluorescence intensities in different groups. The significance between two groups was analyzed by a two-tailed Student t-test (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $^{NS}p > 0.05$).

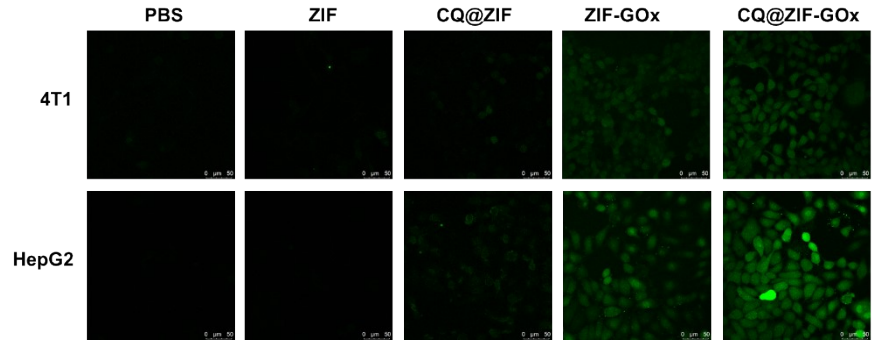

Figure S7. Evaluation of the intracellular ROS after different treatments.

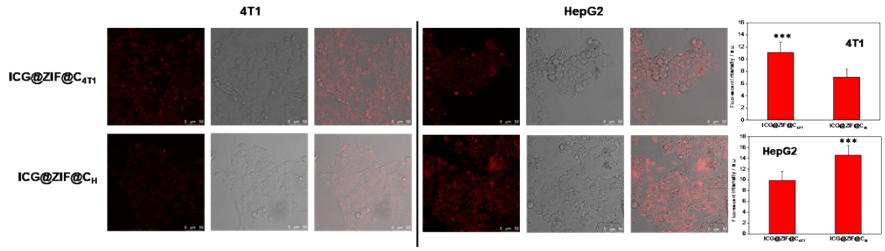

Figure S8. Confocal imaging of cell uptake of different materials (C_H: HepG2 cell membrane; C_{4T1}: 4T1 cell membrane).

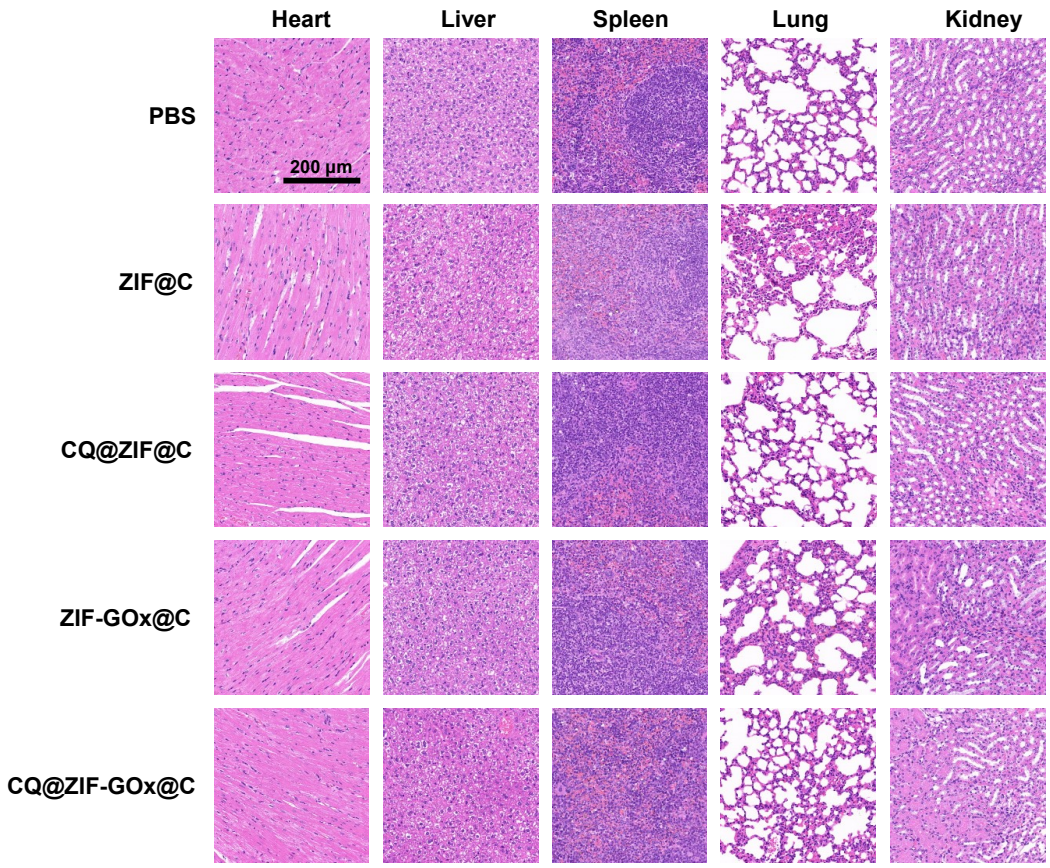

Figure S9. H&E staining of the main organs of mice in different groups post different treatments.