Electronic Supplementary Information (ESI):

A tumor-cell biomimetic nanoplatform embedding biological enzymes for enhanced metabolic therapy

Ping Ji, Tian-Yang Wang, Wei-Hai Chen* and Xian-Zheng Zhang*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan 430072, PR China.
Email: chenweihai@whu.edu.cn; xz-zhang@whu.edu.cn

Materials. Zinc nitrate hexahydrate and 2-methylimidazole were obtained from Aladdin-Reagent Co. Ltd. Apyrase, Glucose oxidase (GOx) and Hoechst 33342 was purchased from Sigma-Aldrich and used without further purification. Phenylmethanesulfonyl fluoride (PMSF), Nuclear and Cytoplasmic Protein Extraction Kit and ATP Assay Kit were purchased from Shanghai Biyuntian Biotechnology Co., Ltd, China. Glucose Oxidase Activity Assay Kit and Na^+K^+-ATPase Activity Assay Kit was provided by Beijing Soleibao Technology Co., Ltd. Annexin V-FITC/PI Cell Apoptosis Kit was purchased from Yeasen, Shanghai, China. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, penicillin-streptomycin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), fetal bovine serum (FBS), trypsin and Dulbecco's PBS were provided by GIBCO Invitrogen Corp. All chemical reagents were used directly without further purification unless specified mentioned.

Cell line. 4T1 (Murine mammary carcinoma) cells and 3T3 (mouse embryonic fibroblasts) cells were purchased from the China Center for Type Culture Collection.

Characterization. Hydrodynamic diameter and zeta potential were measured by dynamic light scattering (DLS) of Malvern Zetasizer ZEN3600. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images were gained from JEM-2010HT (JEM Ltd., Japan) and Carl Zeiss sigma 500 (Germany), respectively. UV-Vis absorbance was measured by UV-Vis spectrophotometry Lambda 35 (Perkin-Elmer). The uptake ability of 4T1 cells and 3T3 cells were performed by confocal laser scanning microscopy (CLSM, PerkinElmer Ultra VIEW VoX). The flow cytometric analysis was performed by using a flow cytometer (BD Accuri C6). The GOx, Apyrase, ZIF, 4T1 tumor cell membrane (4M) were dispersed in SDS buffer to examine their protein contents by SDS-PAGE gel electrophoresis.

Preparation of 4M. The 4T1 cells were cultured in cell culture dishes in a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. Then, the cells were collected by

using a cell scraper,[1] and the collected cells were washed with cold PBS (pH=7.4, 10 mM) twice. After that, the obtained cell pellets were further suspended in a hypotonic lysine buffer containing phenylmethanesulfonyl fluoride (PMSF) and incubated in ice-bath for 15 min according to the manufacturer's instructions. Afterward, the cells in the above solution were broken using a repeated freeze-thaw method three times and further centrifuged at 700 g for 10 min at 4 ℃. The collected supernatant was further centrifuged at 14000 g for 30 min to collect the cracked cell membrane. The products of the cell membrane were lyophilized and stored at -80 ℃. The lyophilized membrane was rehydrated in ultrapure water before use.

Preparation of ZGA. Zeolitic imidazolate framework-8 (ZIF-8) was synthesized according to the reported method.[2] Briefly, $ZnNO_3{\cdot}6H_2O$ (100 mg), 2-methylimidazole (1970 mg), Apyrase (50 U) and GOx (2 mg) were dissolved in ultrapure water for stirring 5 min at 25 ℃. Then, the collected mixture was centrifuged at 12000 rpm for 10 min and thoroughly washed with ultrapure water three times. The obtained ZGA nanoparticles were preserved in 1 mL water at 4 ℃ for storage. ZIF-8/GOx (ZG) and ZIF-8/Apyrase (ZA) are prepared in the same way and stored at 4 ℃.

Preparation of ZGA@4M (ZGAM). The ZGA solution was mixed with an equal weight of 4M in the ultrapure water. Afterward, the Avanti mini extruder and a 400 nm porous polycarbonate membrane were used to prepare ZGAM nanoparticles through multiple extrusion methods. The obtained ZGAM nanoparticles were further purified by centrifugation to remove the free 4M. ZG@4M (ZGM) and ZA@4M (ZAM) are obtained by the similar method.

Detection of the contents of enzymes. The glucose oxidase activity detection kit and Na^+ K^+ ATPase activity detection kit were used to detect the contents of GOx and Apyrase in the synthesized ZGA nanoparticles, respectively.

Cellular ATP assay. For the detection of intracellular ATP content, we seeded 4T1 cells in a 6-well cell culture plate at a density of 1.8×10^5 cells/well, and cultured them in RPMI 1640 medium at 37 ℃ and 5% CO_2 for 24 h. After 4T1 cells co-cultured with different nanocomposites for 4 h, the cells were washed several times with pre-cooled PBS, and the intracellular ATP content was detected by using an ATP detection kit.

Cytotoxicity assay. The in vitro cytotoxicity against 4T1 and 3T3 cells was detected by MTT assay. The 4T1 and 3T3 cells were seeded in 96 well plates and incubated in 100 μL of medium for 24 h at 37 ℃. Then, gradient concentrations of ZIF-8, ZGA, and ZGAM in 100 μL fresh medium were added. After 24 h, 20 μL of MTT (5 mg/mL) was added into each well and incubated for another 4 h. Subsequently, the culture medium containing MTT was replaced with DMSO (150 μL). The absorbance at 570 nm was determined using a microplate reader model. The relative cell viability

was calculated as follows: cell viability (%) = $OD_{570(sample)}/OD_{570(control)}\times100$, where $OD_{570(sample)}$ and $OD_{570(control)}$ were obtained in the presence and absence of samples, respectively.

In vitro cell uptake experiment. For the cellular uptake study, 4T1 cells were seeded in cell culture dishes for 24 h at 37 ℃. Then, Rhodamine B-labeled samples were added in 4T1 cells and incubated for 4 h. After that, 4T1 cells were washed by PBS three times and stained with Hoechst 33342 (blue fluorescence, nuclear dye) and then observed by CLSM.

For homotypic targeting study, 4T1 cells were seeded on 6-well plates and cultured for 24 h, and then incubated with 1640 mediums containing Rhodamine B-labeled ZGAM or Rhodamine B-labeled ZGA (50 μg mL^{-1}) for 4 h, respectively. After washed twice with PBS, the cells were digested by trypsin and collected in centrifuge tubes. Cells were resuspended with 0.5 mL PBS and analyzed by flow cytometry. The identical treatment was performed in 3T3 cells.

Cell apoptosis assay. The cell apoptosis assay of 4T1 cells was performed by flow cytometry analysis. 4T1 cells were seeded in a 6-well plate and cultured for 24 h at 37 ℃ in RPMI 1640. The medium was replaced with a fresh medium containing various samples. After incubation for 4 h, all the cells were washed by PBS for several times, then digested with trypsin (EDTA deplete) and collected by centrifugation. Subsequently, the cells were washed with PBS three times and stained with Annexin V-FITC/PI for 20 min. And then the cells were analyzed by flow cytometry.

Supporting Figures

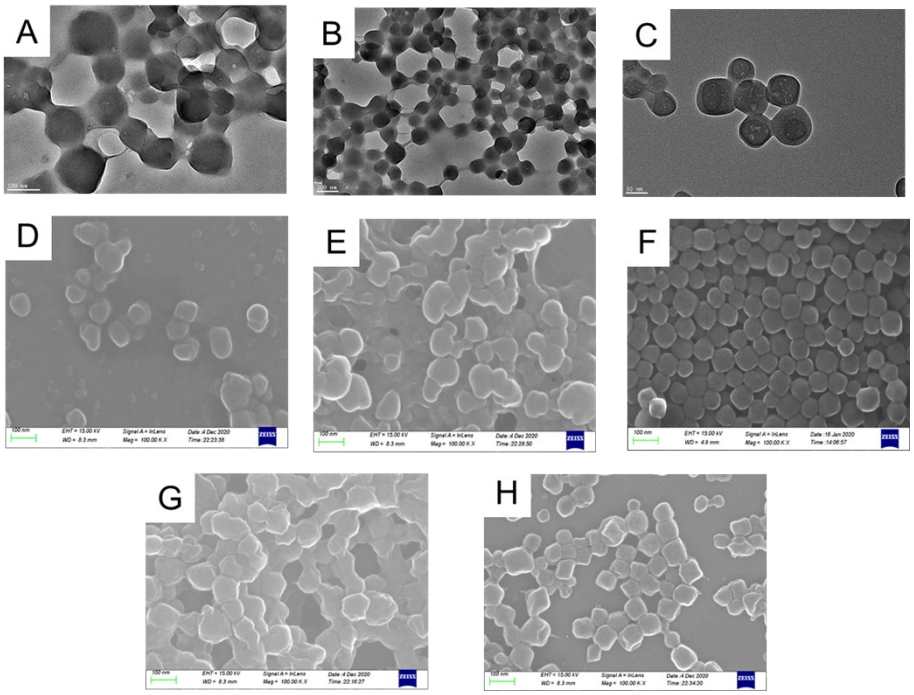

Fig S1. TEM images of A) ZAM, B) ZGM and C) ZIF-8 and SEM images of D) ZAM, E) ZGM, F) ZIF-8, G) ZGAM and H) ZGA.

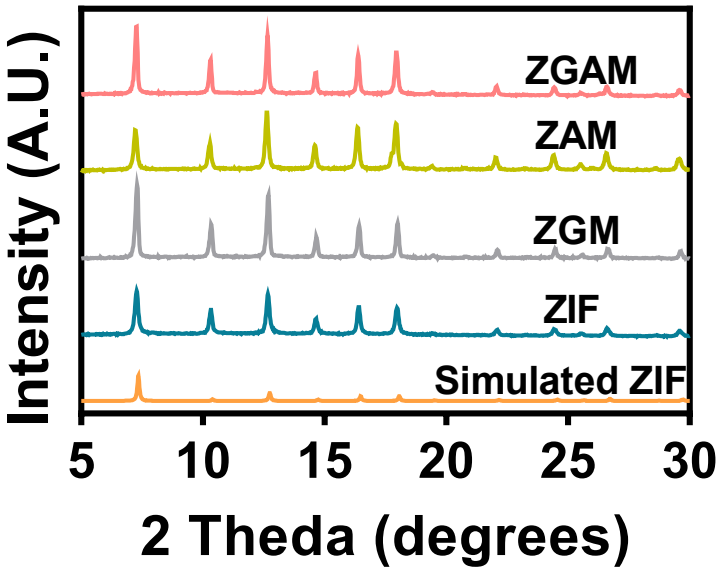

Fig S2. PXRD patterns of simulated ZIF, ZIF, ZGM, ZAM and ZGAM.

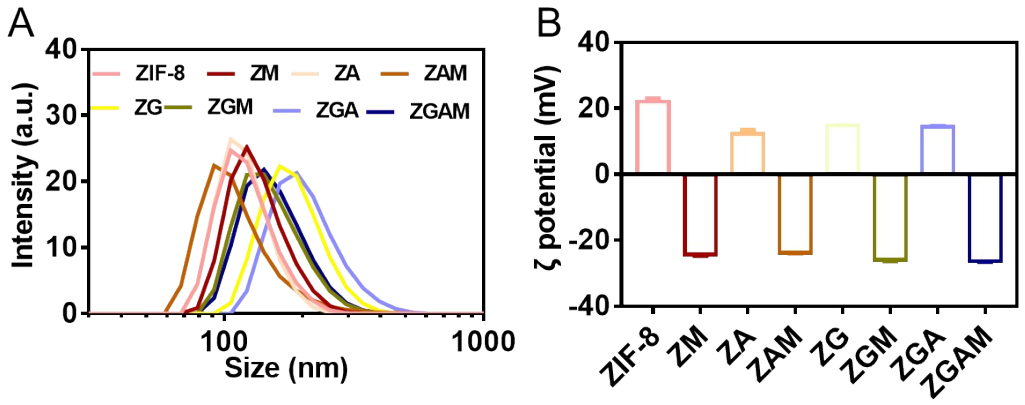

Fig S3. (A) Hydrodynamic sizes of ZIF-8, ZM, ZA, ZAM, ZG, ZGM, ZGA and ZGAM. (B) Zeta potential of ZIF-8, ZM, ZA, ZAM, ZG, ZGM, ZGA and ZGAM. Dynamic light scattering (DLS) measurements of all samples in ultrapure water.

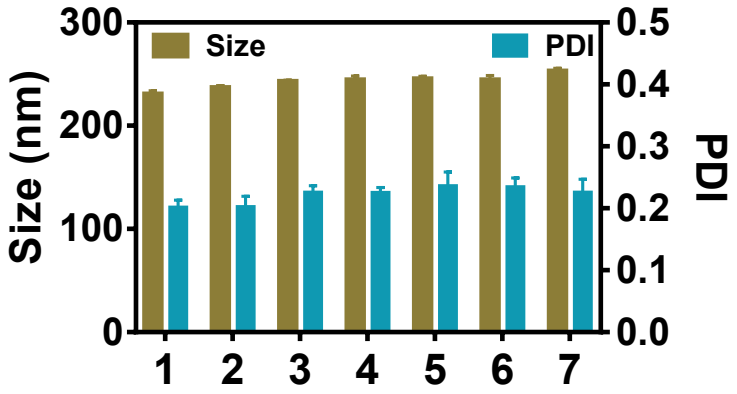

Fig S4. The hydrodynamic size distribution and PDI changes of ZGAM in 7 days in the serum.

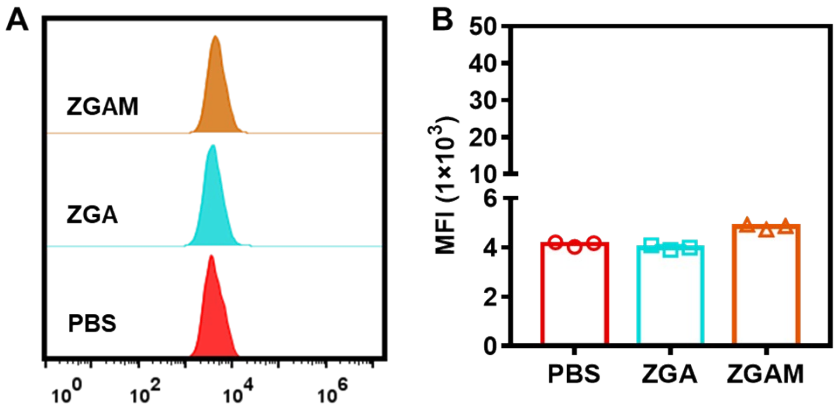

Fig S5. A) Flow cytometry analysis of 3T3 normal cells after treated with ZGA and ZGAM, and B) the corresponding mean fluorescence intensity (MFI) analysis.

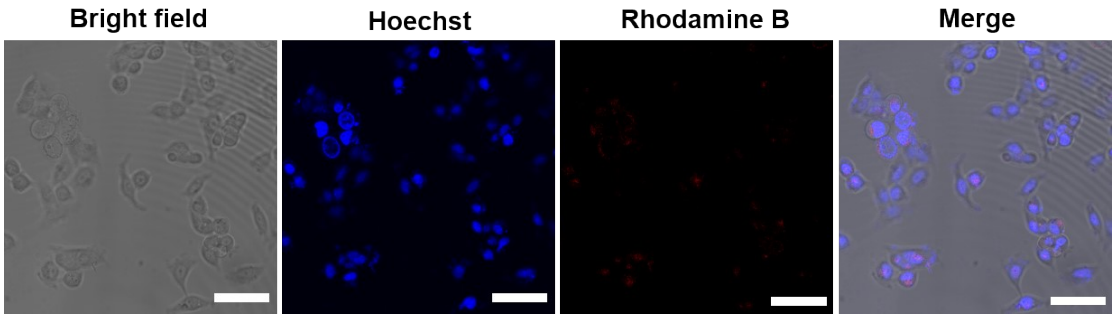

Fig S6. Confocal images of MCF-7 breast tumor cells cells treated with the ZGAM nanoparticles for 4 h. Scale bar: 50 μm.

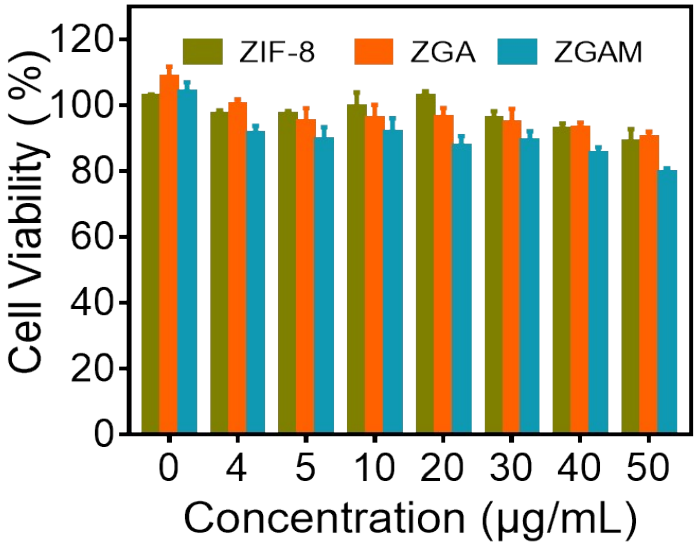

Fig S7. In vitro cytotoxicity of ZIF-8, ZGA, and ZGAM towards 3T3 normal cells. The mean values and s.d. were presented and measurements were taken from distinct samples (n = 5).

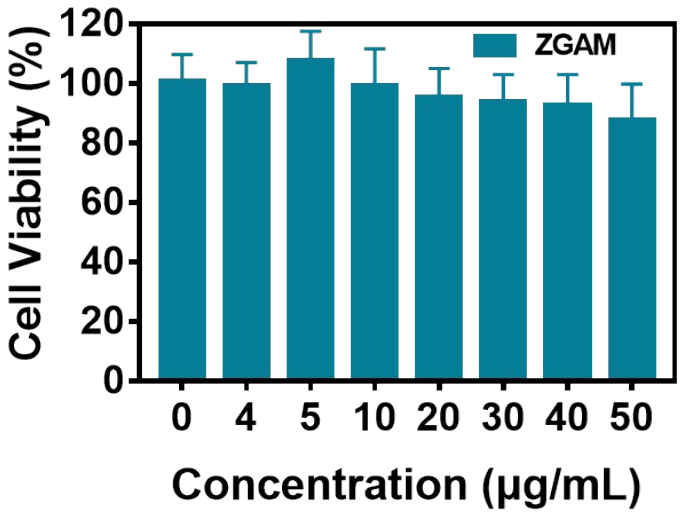

Fig S8. In vitro cytotoxicity of ZGAM towards MCF-10A normal breast cells. The mean values and s.d. were presented and measurements were taken from distinct samples (n = 5).

References

1 H. Cheng, J.-Y. Zhu, S.-Y. Li, J.-Y. Zeng, Q. Lei, K.-W. Chen, C. Zhang and X.-Z. Zhang, *Adv. Funct. Mater.*, 2016, 26, 7847.

2 J. Y. Zhu, D. W. Zheng, M. K. Zhang, W. Y. Yu, W. X. Qiu, J. J. Hu, J. Feng and X. Z. Zhang, *Nano Lett.*, 2016, **16**, 5895.