Supporting Information Available

Self-accelerated biocatalyst for glucose initiated tumor starvation and chemodynamic therapy

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Experimental Section

1.Materials.

BSA was obtained from Sigma Aldrich Corp. Glutaraldehyde, tannic acid (TA), $FeCl.6H_2O$ and rhodamine B (RB) were bought from shanghai Aladdin. Glucose oxidase (GOx) was purchased from Shanghai Yuanye Biological Technology Co., LTD. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), Annexin V-FITC/PI, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), MitoTracker Green and Hoechst 33342 were provided by Beyotime Institute of Biotechnology (China). LysoTracker Green and Calcein-AM/PI Double Stain Kit were bought from Shanghai Yisheng Biotechnology Co., LTD. Cell culture medium and fetal bovine serum (FBS) were obtained from GIBCO Invitrogen Corp.

2. Preparation.

To synthesize the pure BSA and GOx@BSA nano-proteins, 20 g/L of BSA (1 mL) and the mixed solution (containing 750 μL of BSA and 250 μL of GOx) were respectively stirred for 30 min. After diluted with ethanol, 40 μL of glutaraldehyde (2%) was respectively added to stir over night. Nano-proteins were obtained after centrifugation (20000 g) for 20 min. Subsequently, BSA and GOx@BSA nano-proteins were washed with water and resuspended to preserve at 4 ℃. To prepare the biocatalyst (designated as Bio-Cat) and TA coated BSA (BSA@TA), 1 g/L of GOx@BSA (1 mL) or B (1 mL) was firstly stirred for 5 min. Then 20 μL of $FeCl_3.6H_2O$ (10 g/L) and 10 μL of TA (40 g/L) were successively added to stir for 5 min. Bio-Cat and BSA@TA were obtained after centrifugation (12000 g) for 20 min.

At last, they were washed with water and resuspended to preserve at 4 ℃.

3. Characterization.

The content of GOx@BSA in Bio-Cat was determined by acid degradation. In brief, pH value of Bio-Cat (1 g/L) was adjusted to 3.0 by 0.1 M of HCl. After stirring for 6 h, the solution was subjected to dialysis (3500 Da) for 12 h. Subsequently, the solution in dialysis tube was freeze-dried for weighting. 100 mg/L of Bio-Cat and RB-labeled Bio-Cat as well as 49.3 mg/L of GOx@BSA were prepared for the detections of size, zeta potential and TEM.

4. Activity detection.

The activity of Bio-Cat was detected by monitoring the pH changes and oxygen generation. 0.493 mg of GOx@BSA, 1 mg of BSA@TA and 1 mg of Bio-Cat were dispersed into 9 mL of ultrapure water. Then the pH values of these solutions were monitored every 1 min. At 5 min, 1mL of glucose (Glu, 200 mM) was added and the pH values were continue to be monitored for another 25 min. To test the generation of oxygen, 0.493 mg of GOx@BSA, 1 mg of BSA@TA and 1 mg of Bio-Cat were dispersed into 9 mL of ultrapure water. Then the contents of oxygen in these solutions were monitored every 10 s. At 60 s, 1mL of glucose (Glu, 200 mM) was added and the oxygen generation were continue to be monitored for another 240 s. Activity detection in 30 days was performed using the similar method.

5. Fenton reaction.

Above all, the mixed solutions containing methylene blue (MB, 8 mg/L), Bio-Cat (100 mg/L), GOx@BSA (49.3 mg/L), BSA@TA (100 mg/L), Glu (5 g/L) or ATP (1

g/L) were prepared. The absorbance at 664 nm was detected every 1 h. Fenton reaction efficiency was evaluated by detecting the remaining MB, which was calculated as A_t/A_0. In which, A_0 represented the initial absorbance and A_t represented the absorbance at 1, 2, 3, or 4 h.

6. Cellular uptake and Subcellular distribution.

4T1 cells were seeded and cultured for 24 h. After that, they were treated with 1, 5 or 10 mg/L of RB-labeled Bio-Cat for 2 h. Besides, 4T1 cells were also incubated with 3 mg/L RB-labeled Bio-Cat for 1, 2 and 4 h, respectively. Then, the cells were stained with Hoechst 33342 for CLSM observations. Furthermore, after incubation with RB-labeled Bio-Cat (3 mg/L) for 2 h, 4T1 cells were stained with Hoechst 33342, Lyso Tracker Green or Mito Tracker Green to detect the subcellular distribution by CLSM.

7. ROS detection.

Cellular ROS was detected by CLSM and flow cytometry using DCFH-DA as the sensor. Briefly, 4T1 cells were cultured with GOx@BSA (4.93 mg/L), BSA@TA (10 mg/L) or Bio-Cat (10 mg/L) for 2 h. Then the cells were treated by DCFH-DA for 30 min. After washed by PBS, the cells were observed by CLSM. Besides, the cells were also harvested for flow cytometry analysis.

8. MTT assay.

After cultured for 24 h, 4T1 cells were treated with the gradient concentrations of GOx@BSA, BSA@TA or Bio-Cat. 24 h later, 20 μL of MTT was added to incubation for another 4 h. Subsequently, the culture medium was replaced by 150 μL of DMSO.

The absorbance was detected by a microplate reader at 570 nm. Notably, gradient concentrations of GOx@BSA, BSA@TA or Bio-Cat were also incubated with 4T1 cells in sugar-free culture medium for MTT assay.

9. Live/dead cell staining analysis.

4T1 cells were cultured with GOx@BSA (4.93 mg/L), BSA@TA (10 mg/L) or Bio-Cat (10 mg/L) for 2 h. Then the cells were washed with buffer solution and stained with Calcein-AM/PI for 25 min. The fluorescence of 4T1 cells was observed by CLSM.

10. Cell apoptosis assay.

4T1 cells were cultured with gradient concentrations of GOx@BSA (0.493, 2.465, 4.930 mg/L), BSA@TA (1, 5, 10 mg/L) and Bio-Cat (1, 5, 10 mg/L) for 2 h, respectively. Then the cells were washed and harvested to treat with FITC and PI. After 15 min, the cells were analyzed by flow cytometry.

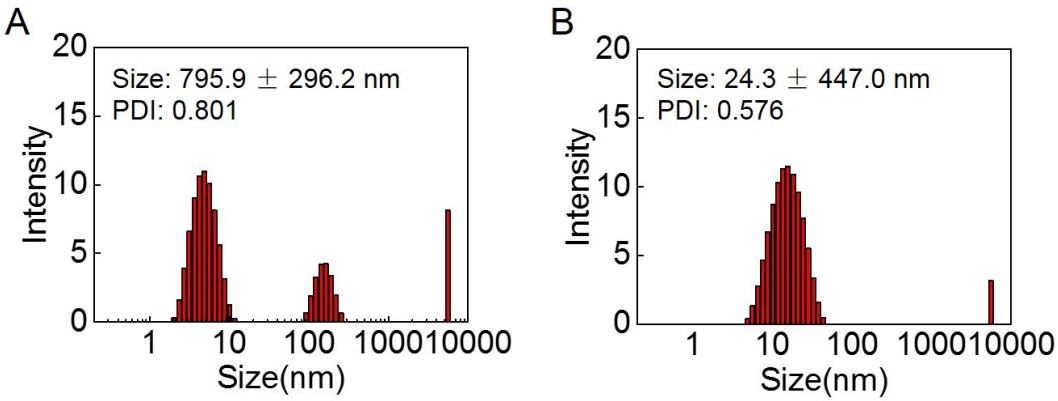

Fig. S1 The particle size distribution of (A) BSA and (B) GOx.

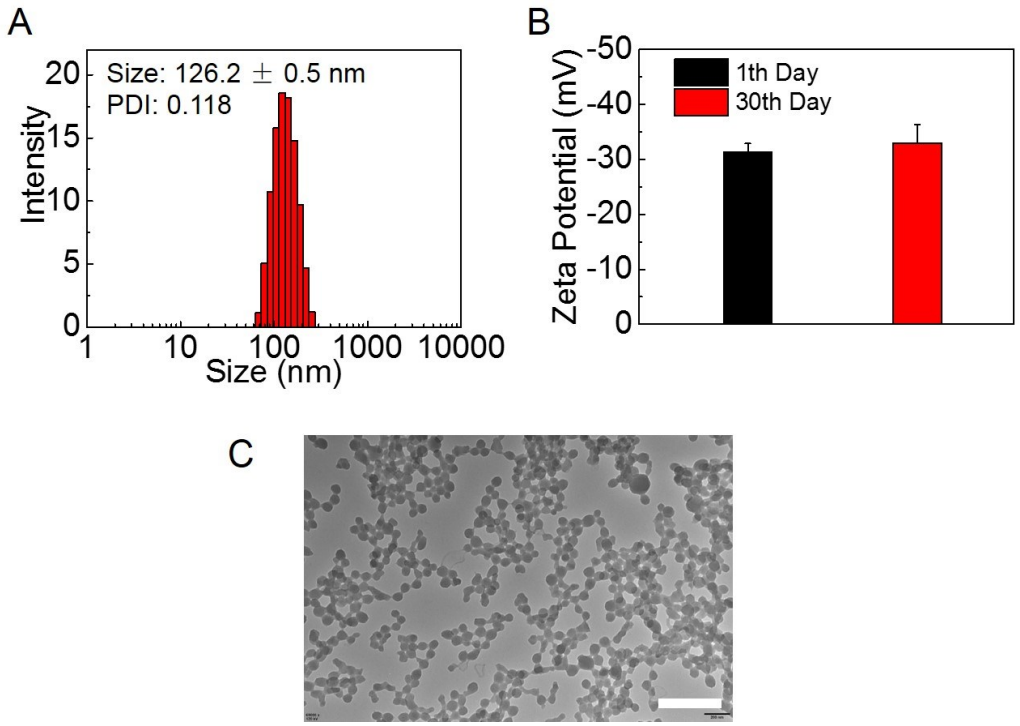

Fig. S2 (A) The particle size distribution, (B) the zeta potential and (C) the TEM image of GOx@BSA at 30th day. Scale bar: 500 nm.

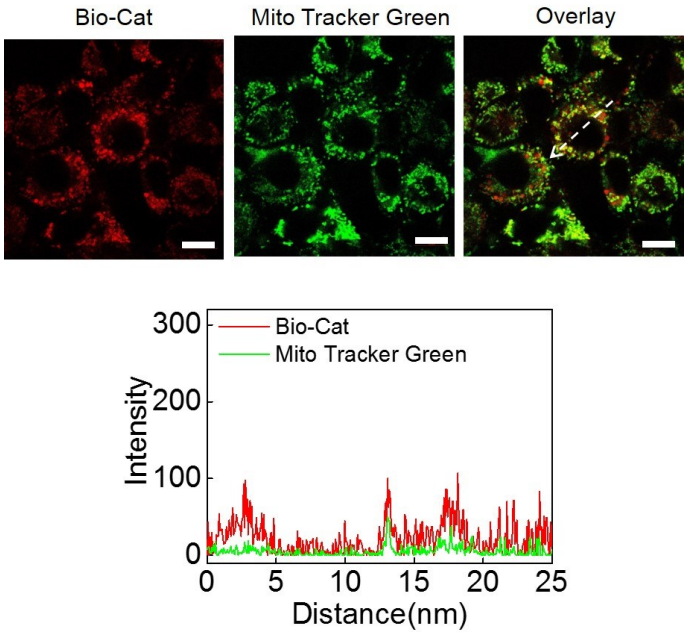

Fig. S3 CLSM images and fluorescence intensity profile analysis of 4T1 cells after treated with RB-labeled Bio-Cat and stained by Mito Tracker Green. Scale bar: 10 μm.

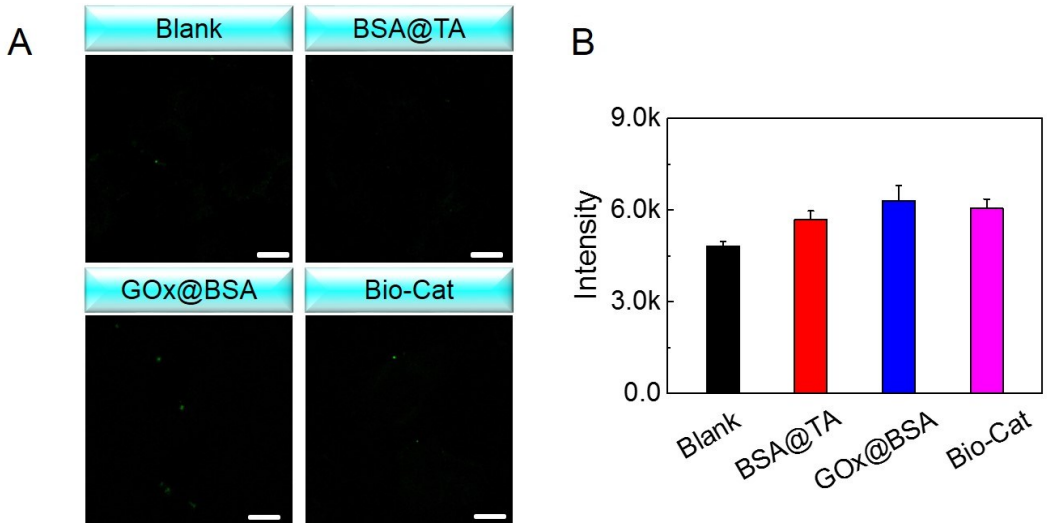

Fig. S4 (A) CLSM images and (B) quantitative fluorescence analysis of 4T1 cells after treated with BSA@TA, GOx@BSA or Bio-Cat and stained by DCFH-DA in the absence of glucose. Scale bar: 10 μm.

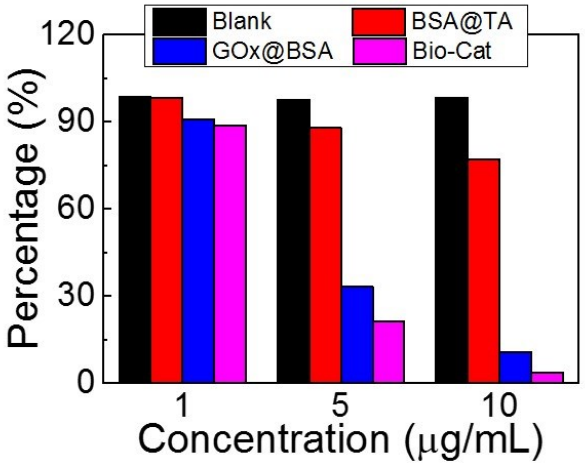

Fig. S5 Percentages of apoptotic 4T1 cells after treated with BSA@TA, GOx@BSA or Bio-Cat. 4T1 cells without any treatments were used as the blank control.