Supporting Information for

A multifunctional nanoprobe with targeting tumor and mitochondria for singlet oxygen generation and monitoring mitochondrion pH changes in cancer cells by ratiometric fluorescence imaging

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**Reagents**

High glucose medium (DMEM), trypsin digestive fluid, penicillin-streptomycin, NH$_2$-PEG-NH$_2$ (Formula Weight: 104.15088), fetal bovine serum (FBS) and oleic acid were purchased from Sigma company (USA). T24 cells (human bladder cancer cell line) and 7702 cells (human normal liver cell line) were purchased from Beijing Dingguo Biotechnology Co., Ltd. (China). Folic acid (FA) was purchased from Hefei Bomei Biotechnology Co., Ltd. (China). Annexin V-FITC Kit was purchased from Yingjie Co., Ltd. (China). All the other chemical reagents in the experiment were analysis pure, and the water used in the experiment was 18.2 MΩ·cm ultrapure water.

Preparation of culture medium: FBS (final content of 10%) and penicillin-streptomycin (final content of 1%) were added into DMEM high glucose culture medium, and the mixture was ready for use.

Preparation of 1-(3-dimethylaminopropyl)-3-ethyl carbon diimine hydrochloride (EDC) and N-hydroxybutyldiamide (NHS) solutions: 0.2 g EDC and 0.02 g NHS were dissolved in 2.5 mL ultrapure water and mixed evenly for later use.

**Instruments**

LS-55 fluorescence spectrometer (Perkin-Elmer, USA) was used for recording the fluorescence spectrum. Cary-60 UV-vis spectrophotometer (Agilent Technologies, USA) was used for recording the UV-vis absorption spectrum. A Perkin-Elmer Fourier transform infrared (FTIR) spectrometer (Perkin-Elmer Inc./Thermo Fishser Scientific, Waltham, MA, USA) was used for characterization of the BQDs surface groups. A Rigaku X-ray powder diffractometer (Rigaku Corp., Tokyo, Japan) was used for X-ray diffraction (XRD) analysis. A Philips transmission electron microscope (TEM) (Philips, Eindhoven, Netherlands) was used to characterize the particle size of the BQDs. An ESCALAB™ X-ray photoelectron spectrometer (Thermo Fisher, Waltham, MA, USA) was used for elemental analysis of BQDs by X-ray photoelectron spectroscopy (XPS). A laser scanning confocal microscopy (LSM710, Zeiss) was used for cells image.
**Preparation of BQDs**

Fresh *osmanthus* leaves were washed and dried in air, cut into pieces. About 22 g of leaf fragments were ground in an agate mortar. Then 100 mL mixed solution of ethanol and acetone (1:1) was added. After soaking for 30 min, the chlorophyll extract was obtained by filtering with gauze.

0.05 g NH$_2$-PEG-NH$_2$ and 20 mL oleic acid were added into a round-bottomed flask, stir and heat up to 250 °C in the atmosphere of nitrogen, stop heating when the solution is orange and cool to room temperature naturally. Then, 4 mL chlorophyll extract was added into above round-bottomed flask, and heat up to 180 °C, reflux and stir for 3 h, stop the reaction and cool down to room temperature naturally. 26.5 mL of HCl solution (6 M) was added and stirred violently at 25 °C for 12 h. The reaction solution was then transferred to a separating funnel and placed the solution for stratification. The underlying solution was collected, pH value of the solution was adjusted to neutral with 6 M NaOH solution, and the solution was filtered with a 0.22 μm filter membrane. Filtrate was transferred to a dialysis bag (MWCO: 1 kDa, aperture about 1.0 nm), and purified BQDs solution was obtained after dialysis for 24 h in ultrapure water.

**Preparation of BQD-FAs**

0.25 g FA was dissolved in 20 mL dimethyl sulfoxide (DMSO) solution, and ultrasound was performed on the ultrasonic instrument for 45 min. Then, 2.5 mL of NHS and EDC mixed solution (containing 0.2 g EDC and 0.02 g NHS) was added to the DMSO solution, and stirred for 12 h in a three-mouth flask filled with nitrogen at 28±2 °C. The activated FA could be obtained by filtering the reaction solution. Finally, 100 μL activated FA solution was added to 2 mL dilute BQDs solution (0.0053 mg/mL), and stirred for 24 h under the protection of nitrogen, dialysis in ultrapure water for 10 h to obtain pure BQD-FAs.

**Cell culture**
T24 cells and 7702 cells were grown in DMEM medium containing 10% fetal bovine serum, 100 IU/mL penicillin-streptomycin, respectively, and incubated at 37 °C in a 5% CO\textsubscript{2}/95% air humidified incubator.

**Cell viability assay (MTT method)**

T24 cells and 7702 cells were inoculated in a 96-well plate and cultured in a constant temperature and pressure incubator (37 °C, 5% CO\textsubscript{2}) for 24 h. When the relative cell density reached about 80%, BQD-FAs solution was added into the culture medium and the cells were incubated for 12 h under the same conditions. The cells were then irradiated with a 660 nm laser with a power less than 50 mW for 6 min and incubated for 24 h under the same conditions. Subsequently, 10 μL 3-(4, 5-dimethylthiazole-2)-2, 5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added to the medium and incubated for 4 h. Then, remove the culture medium and add 100 μL DMSO to dissolve MTT, and the mitochondrial succinic acid dehydrogenase in the living cells to form purple crystals. After shaking for 5 to 10 minutes, the absorbance value of the solution in each hole at 490 nm was detected by an enzyme marker to analyze the cell survival rate.

**Apoptosis experiment**

T24 cells and 7702 cells were inoculated in a 6-well plate and cultured in a constant temperature and pressure incubator (37 °C, 5% CO\textsubscript{2}) for 24 hours. When the relative cell density reached about 80%, a certain amount of BQD-FAs solution was added and the cells were incubated for 12 h. The cells were then irradiated with a laser less than 50 mW and 660 nm for 6 min, and incubated for 24 h under the same conditions. Subsequently, the cells were collected with trypsin without EDTA and centrifuged in a centrifuge tube for 3 min (2000 r/min), and then washed cells 2 time with PBS. The cells were then re-suspended in a 500 μL buffer solution (50 mM HEPES, 700 mM NaCl, 12.5 mM CaCl\textsubscript{2}, pH 7.4), 5 μL AnnexinV-FITC and 5 μL propidium iodide were added, and incubated for 10 min at room temperature and in dark. Then, the resulting cells solution was analyzed by flow cytometry.
Laser confocal fluorescence imaging analysis

T24 cells were inoculated in a laser-confocal cell culture dish and cultured in an incubator until the cell density was about 80%. Then a certain amount of BQD-FAs solution was added to the cell culture dish and the cells were incubated for 12 h. The cells were then washed with PBS 4 to 5 times.

For mitochondrial colocation experiment: 2 mL cell culture media was added to the cells washed with PBS, and then a certain volume of 1μM Mito-Tracker Rhodamine 123 solution was added. The cells were incubated at 37 °C and 5%CO₂ for 30 min, then the culture medium was removed, and the cells were thoroughly washed with PBS. Subsequently, 2 mL PBS was added for imaging analysis. During confocal fluorescence imaging, the fluorescence of cells in green channel (500-540 nm) and red channel (630-670 nm) were collected with a 514-nm laser as the excitation light.

For Intracellular mitochondrial pH imaging experiment: A certain volume of 10 μM Nigerian bacterin and 2 mL high K⁺ buffer solution with different pH values (1 mM CaCl₂, 120 mM KCl, 30 mM NaCl, 1 mM NaH₂PO₄, 0.5 mM MgSO₄, 20 mM HEPES, 5 mM glucose, 20 mM NaOAc) were added to the cells washed with PBS, and incubated for 30 min at 37 °C and 5%CO₂ for imaging analysis. During confocal fluorescence imaging, the fluorescence of cells in green channel (480-500 nm) and red channel (640-660 nm) were collected with a 405 nm laser as the excitation light.
Figure S1 (a) TEM image of BQD-FAs, inset is a high resolution TEM image. (b) Particle size distribution of BQD-FAs.

Figure S2 XRD diffractogram of BQD-FAs.
Figure S3. XPS spectrum of BQDs (a) and high resolution XPS spectra of C1s (b), N1s (c), O1s (d) in the BQDs.
Figure S4. FTIR spectra of BQDs, FA and BQD-FAs.
Figure S5. Uv-visible absorption spectrum of BQDs, FA and BQD-FAs.

Figure S6. The pH reversibility of BQD-FAs solution between pH 3.0 and 8.0.
Figure S7. Photographs of the BQD-FA$_s$ solutions at different pH values under the laser irradiation (excitation wavelength of 405 nm).