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Supporting information

A Facile Nanoagent Promoted by Photoelectrons Transfer and Consumption for Tumor Combination Therapy

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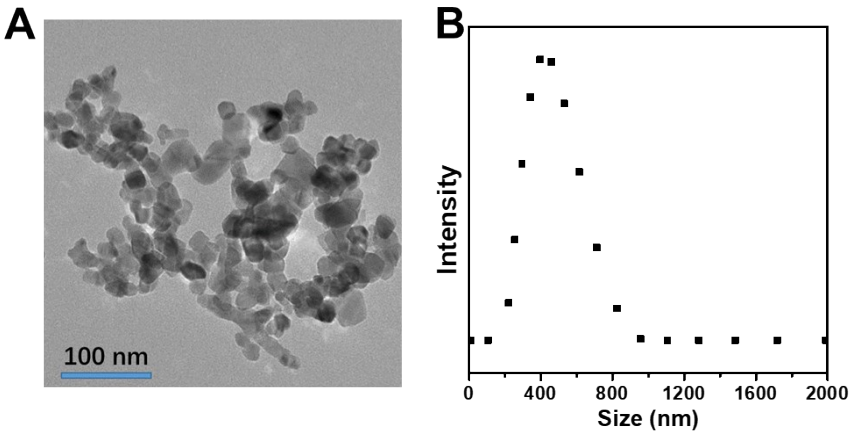

Figure S1. Characterizations of the BT NPs. (A)TEM image; (B)DLS.

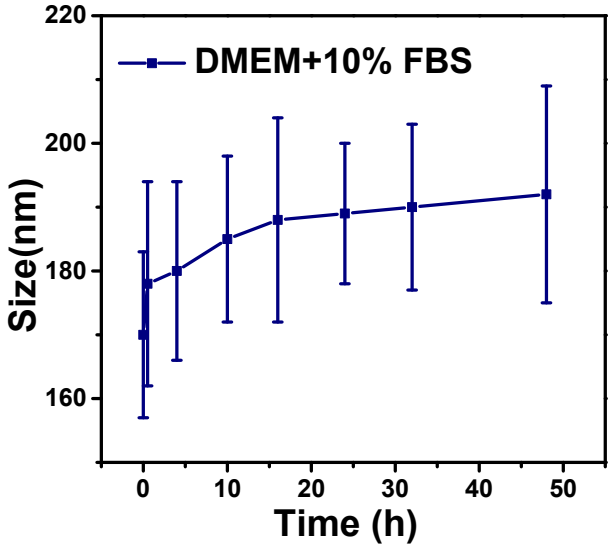

Figure S2. Characterizations of the in vitro stability of BTCu NPs.

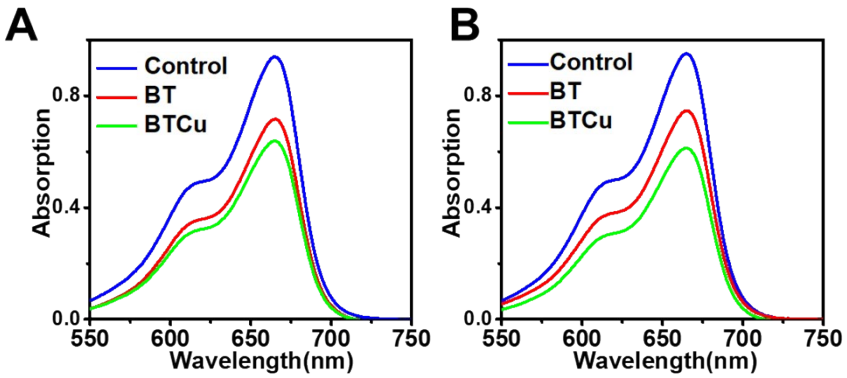

Figure S3. UV absorption spectra of MB degradation (A) under NIR;(B) Under NIR+0.1 mM H_2O_2.

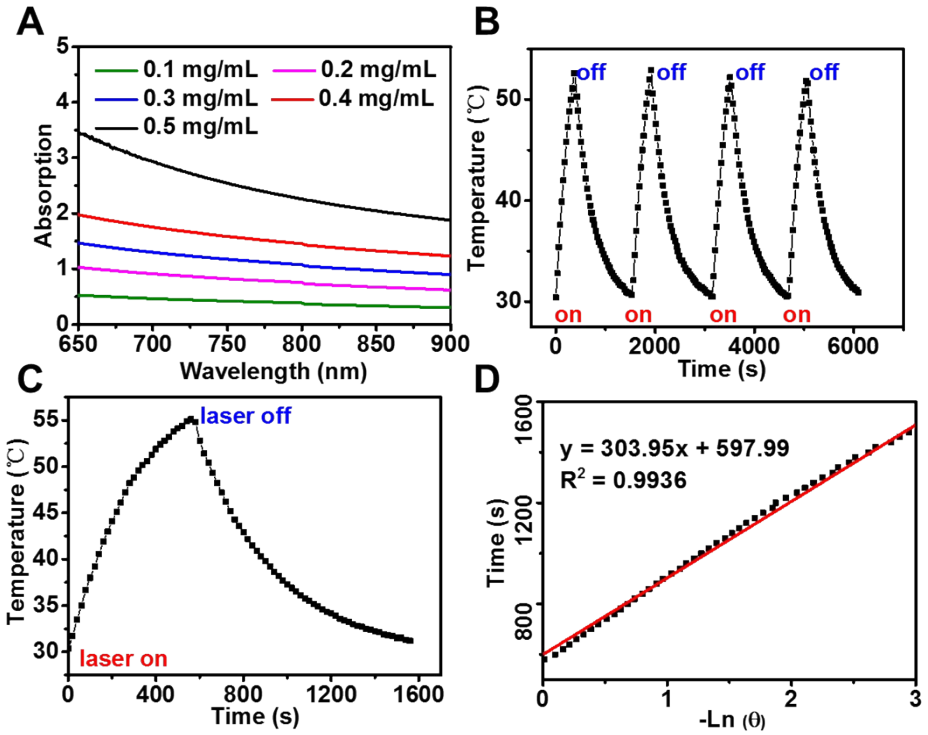

Figure S4. Characterizations of the photo-thermal performances of BTCu NPs. (A) UV absorption spectra of BTCu NPs; (B)Heating curve of BTCu dispersed in water for four cycles at a power of 1 W. (C) Heating and cooling performance of the BTCu aqueous dispersion for periods; (D) The time constant according to the BTCu performance in cooling period.

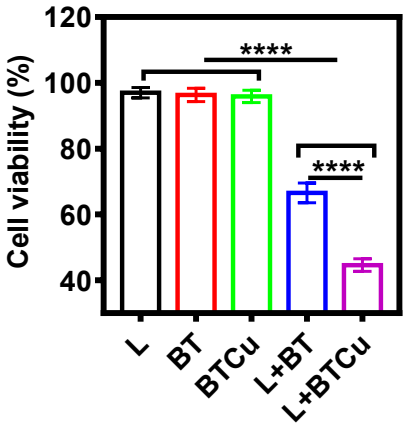

Figure S5. The photocytotoxicity of BTCu on HepG2 cancer cells under hypoxic environment (100 μM of $CoCl_2$).

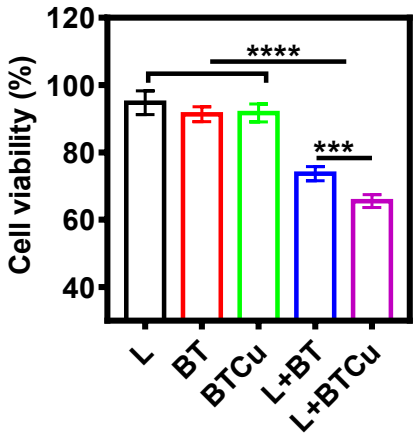

Figure S6. The photocytotoxicity of BTCu on LO2 normal cells.

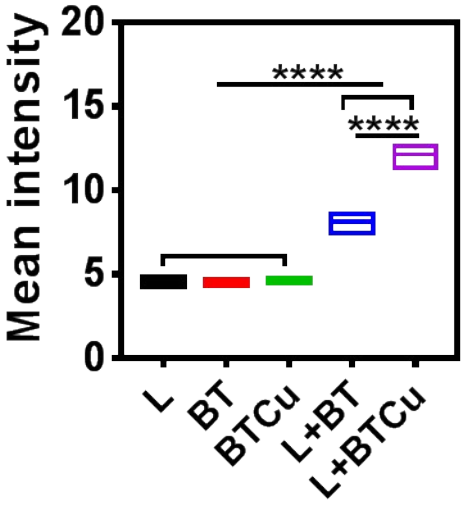

Figure S7. The quantification of ROS mean fluorescence intensity in Figure 4A by *image j*.

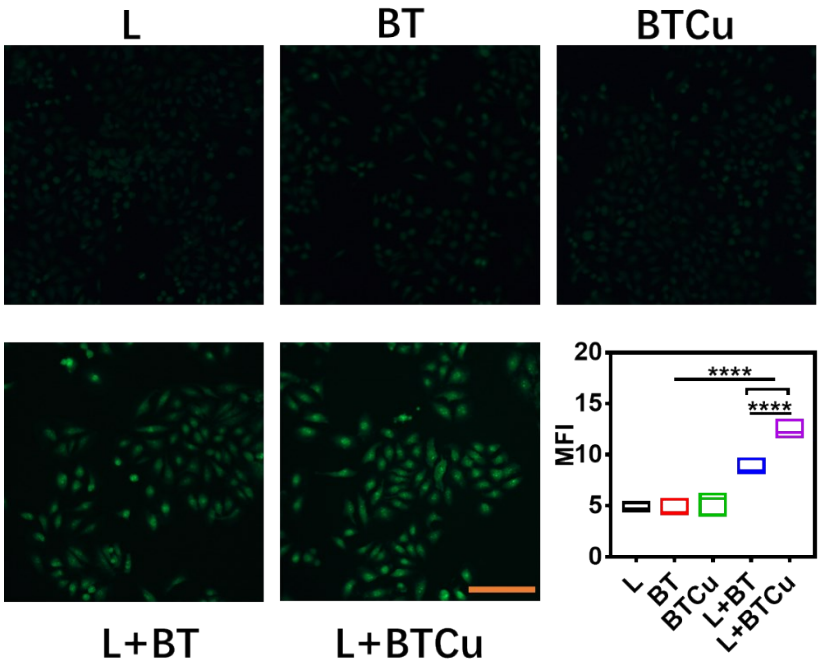

Figure S8. Images of cells stained with APF (green) for ·OH detection under different treatments. Scale bar= 50 μm.

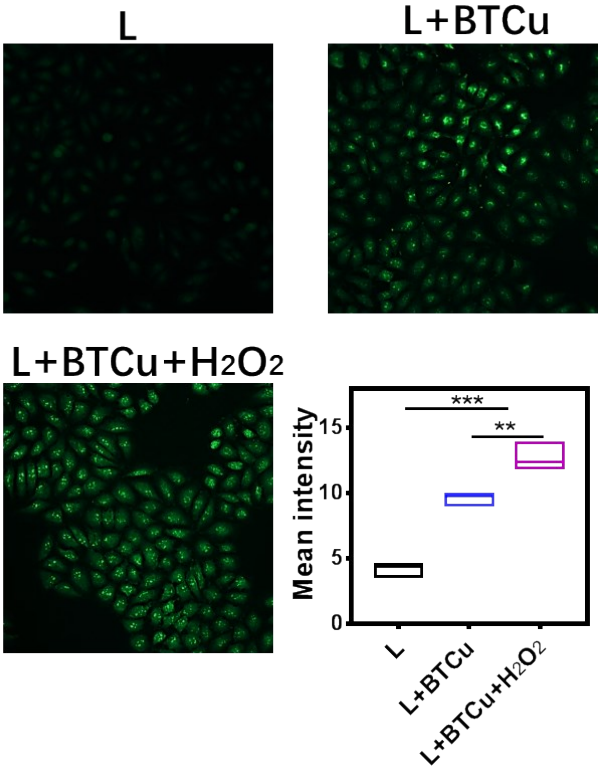

Figure S9. The ROS generation of LO2 cells with different treatments and the quantification of mean fluorescence intensity. Scale bar= 50μm.

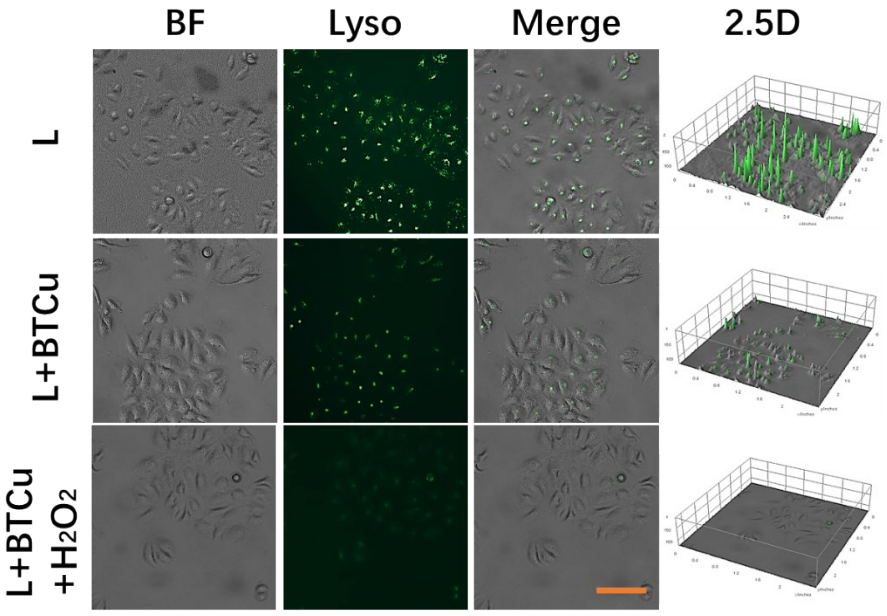

Figure S10. Images of LO2 cells stained with Lyso-tracker under different treatments and the corresponding 2.5D image produced by *image j*. Scale bar= 50 μm.

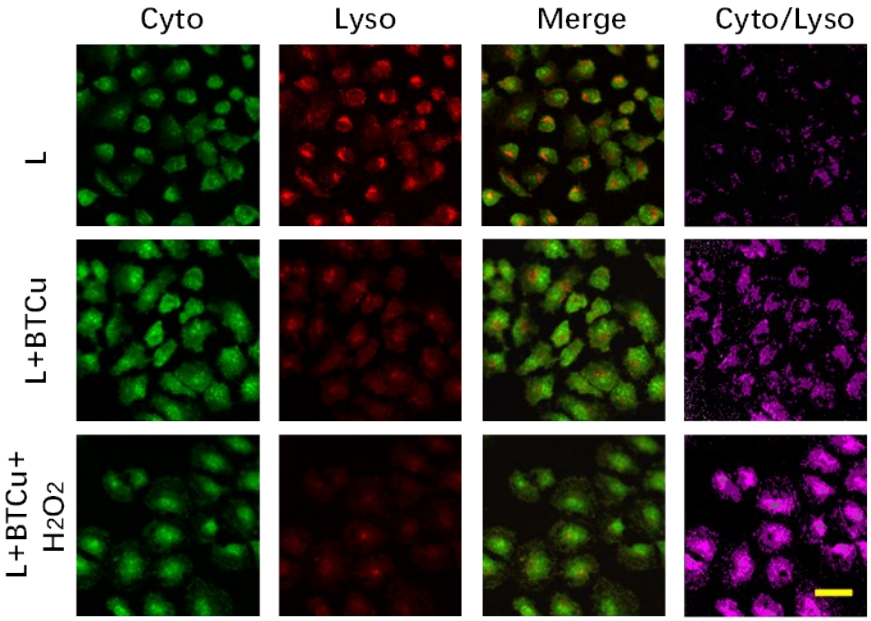

Figure S11. The AO staining images of LO2 cells with different treatments. The *image-pro Plus software* is used to calculate the ratio of the green fluorescence in the cytoplasm (cyto) and the red fluorescence in the lysosome(lyso), and the ratio (cyto/lyso) are displayed in purple pseudo-color. Scale bar =15 μm.

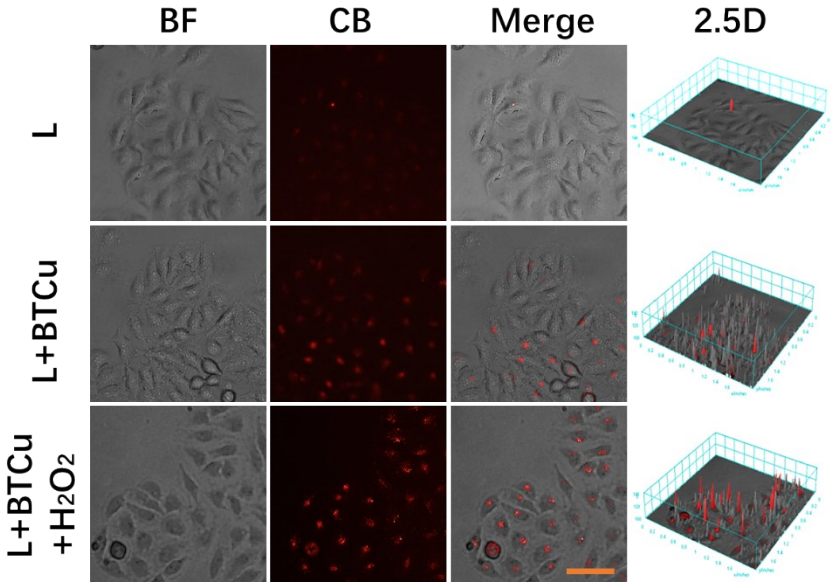

Figure S12. LO2 cells were treated with different groups, fixed and stained for immunofluorescence detection of cathepsin B (red). Scale bar=50 μm

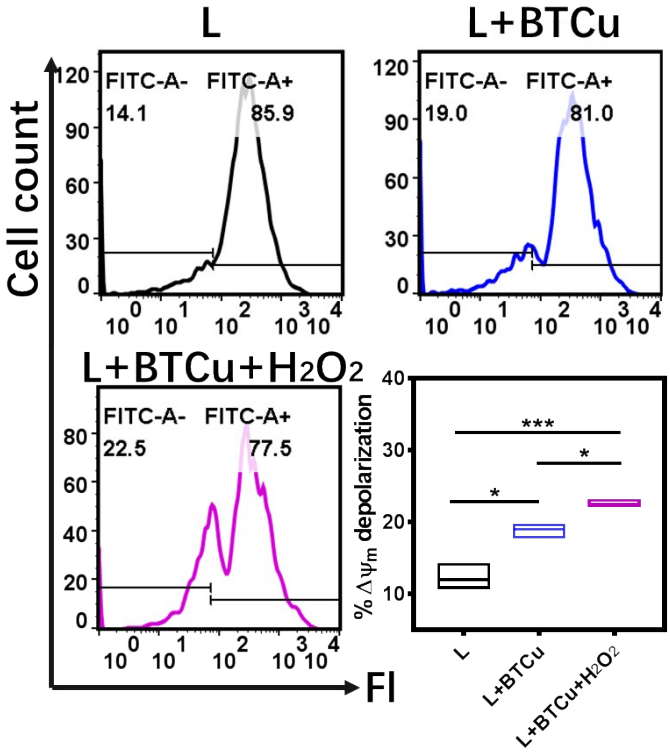

Figure S13. Flow cytometry histograms of Rh123 fluorescence 3 hours after different treatments in LO2 cells and percentage of mitochondrial membrane potential (Δψm) depolarization. *$p < 0.05$, ***$p < 0.001$.

Materials and Characterization

The chemicals applied in this study are P25 titanium white powder (AEROXIDE), $NaBH_4$ (Acros organics), Sodium polyacrylate (PAAS, Sigma-Aldrich), Neocuproine (Shanghai Dibai Biotechnology Co., Ltd.). The Rabbit Anti-Cathepsin B antibody (bs-1500R, Bioss) and Anti-rabbit IgG/Alexa Fluor 555 antibody(bs-0295G-AF555，Bioss) were all provided by Biosynthesis Biotechnology Inc. (Beijing, China). Cell counting kit-8 and the Hoechst 33342 / PI double staining kit were supported by Beijing Solarbio Science & Technology Co., Ltd. 3′-(p-aminophenyl) fluorescein (APF) was purchased from maokang-bio (Shang hai).

The UV-Vis absorption of materials was measured by UV-Vis spectrophotometer (SHIMADZU UV-2550 with an integrating Sphere). The morphology and dry size of the nanoparticles were observed by Transmission electron microscopy (JEM-2800). The DLS and zeta potential measurements were performed on a Nano-ZS90 instrument (Malvern). The crystal ratio of the materials was obtained by X-ray diffraction (XRD) (D/max 2550, Rigaku) with Cu-Kα (1.5406 Å) radiation. The surface electron spectroscopy was studied on an Axis Ultra DLD spectrometer (Kratos Analytical Ltd.). The Infrared spectroscopy was detected by Fourier transform infrared spectrometer (TENSOR II, Bruker).The Near-infrared photothermal experiments were completed by a semiconductor laser (Emission wavelength of 808 nm, WG1533D3) and a thermal imager (FOTRIC, US).

1. Preparation of BTCu nanoparticles

Take 1g of P25 titanium dioxide powder and 0.4g of $NaBH_4$, and grind and mix in an agate mortar.Then, the mixed powder was transferred to an enamel boat and placed in a tube furnace with argon gas for burning. After heating to 300 ° C at a rate of 10 ° C per minute, keep this temperature for 1 h and finally cool to room temperature. The burned product was dissolved in 200 mL of deionized water and stirred for 12 h to fully remove the remaining $NaBH_4$. Centrifuge the above nanoparticle solution (8000 rpm, 30 min), take the precipitate and dissolve in 50 mL of deionized water. After sonicating the solution for 2 min, centrifuge, and repeat this washing-centrifugation step 3 times, The precipitate obtained by the last centrifugation was ultrasonically dissolved in 10 mL of deionized water, then lyophilized to obtain blue titanium dioxide nanoparticles(BT).

Take 50 mg of the prepared BT powder and dissolve it in 30 mL of deionized water, and disperse it ultrasonically for 10 min. Add 50 mg of sodium polyacrylate (PAAS) powder and stir at room temperature for 4 h. Next, the reaction solution was centrifuged at a low temperature (10000 rpm, 20 min, 10 ° C), the resulting precipitate was dispersed again in deionized water, and the washing-centrifugation operation was repeated 3 times. After the last wash, the precipitate was dispersed in 25 mL of deionized water. Add 5 mL of $Cu(NO_3)_2 \cdot 3H_2O$ solution (15 mg / mL) to the dispersion, magnetically stir at room temperature for 10 min; then add 5 mL of $Na_2HPO4 \cdot 12H_2O$ solution (30 mg / mL) , and continue stirring at room temperature for 20 min. After the inorganic salts added were fully dissolved, the reaction solution was adjusted to pH = 7.0 with 0.1 M NaOH aqueous solution. Finally, the above reaction solution was transferred to a 50 mL high-pressure polytetrafluoroethylene-

lined reactor, placed in a temperature-controlled oven, and reacted at 120 ° C for 6 h. After the reaction, the reaction solution was sucked out and centrifuged at low temperature (10000 rpm, 20 min, 10 ° C); then the resulting centrifugal precipitate was dispersed in water again, and the washing-centrifugation step was repeated 3 times. The obtained precipitate was washed with ethanol and ethyl acetate successively and centrifuged, and finally dispersed in 5 mL of ethyl acetate. After the organic solvent naturally volatilized, a powdered BTCu nanoparticle sample was obtained.

2. Photothermal performance of BTCu nanoparticles

The BTCu nanoparticle powder was dissolved in deionized water to obtain sample solutions with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 mg / mL.Take 1 mL of the above sample solution in a 1 cm transparent quartz sample cell and place it under an 808 nm near infrared laser (1W, 5 min), and continuously record the temperature change of the sample. In addition, the BTCu nanoparticle solution with a concentration of 0.5 mg / mL was selected and irradiated under a 808 nm near-infrared laser, and the sample temperature changes were continuously recorded under the conditions of irradiation power of 0.6, 0.8, 1, 1.25, and 1.5 W.

In addition, the BTCu solution (0.5 mg / mL) was irradiated with a 808 nm near-infrared laser (1 W), and the light source was turned off after 360 s. After the sample temperature has cooled down to room temperature, turn on the laser again. Circulate the above operation 4 times, and continuously record the temperature change of the sample with an infrared camera.

The procedure of the photothermal attenuation experiment of the sample is: after irradiating the BTCu solution (0.5 mg / mL) with an 808 nm near-infrared laser (1W, 560 s), the sample solution is naturally cooled to room temperature, and the temperature change is recorded throughout.

3. Determination of reduction of Cu^{II}-Cu^{I} by neocuproine reagent

First, prepare an ethanol solution of neocuproine at a concentration of 5 mM, and then dilute to 2 mM with water. Next, 0.272 g of KH_2PO_4 was weighed and dissolved in 20 mL of deionized water, and then the pH was adjusted to 6.2 with 0.1 M NaOH solution. In the sample cell, add 0.5 mL of the nanoparticle solution to be tested (0.25 mg / mL), 0.4 mL of KH_2PO_4 buffer solution, and then 0.4 mL of neocuproine solution (2 mM). The sample cell was placed under the 808 near infrared laser (1 W, 10 min). After the irradiation, the ultraviolet absorption of the solution at 450 nm was measured.

4. In vitro photodynamic effect of BTCu nanoparticles

0.2 mL (0.008 mM) of methylene blue solution was added to 0.8 mL of nanoparticle solution (0.2 mg / mL), and the mixture was placed under 808 near infrared laser irradiation (1 W, 10 min). After the irradiation, the above solution was centrifuged to take the supernatant, and the ultraviolet absorption intensity of methylene blue (MB) in the supernatant was measured. Take 0.8 mL of nanoparticle solution (0.2 mg / mL) containing H_2O_2 (0.1 mM), add 0.2 mL (0.008 mM) of MB solution, and irradiate the mixture under 808 near infrared laser (1 W, 10 min). After the irradiation, the ultraviolet absorption of MB was measured.

0.2 mL (0.05 mM) of APF solution was added to 0.8 mL of nanoparticle solution

(0.2 mg / mL), and the mixture was placed under 808 NIR irradiation (1 W, 10 min). After the irradiation, the above solution was centrifuged to take the supernatant, and the fluorescence of the supernatant was measured (Ex=490 nm). Take 0.8 mL of nanoparticle solution (0.2 mg / mL) containing H_2O_2 (0.1 mM), add 0.2 mL (0.05 mM) of APF solution, and irradiate the mixture under 808 NIR light (1 W, 10 min). After the irradiation, the fluorescence of the supernatant was measured (Ex=490 nm).

5. Determination of intracellular ROS

Spread the well-grown human liver cancer cells HepG2 and human normal liver cells LO2 into 12-well plates (1.2 * 10^5 cells / well), and after 24 hours, aspirate the supernatant. The 0.2 mg / mL BTCu and BT diluted in culture medium were added to HepG2 cells, respectively. The 0.2 mg / mL BTCu and BTCu + H_2O_2 (0.1 mM) diluted in culture medium were added to LO2 cells, respectively. After continuing the culture for 24 h, the supernatant was aspirated and the cells were washed twice with PBS. After adding DCFH-DA (20 μM) diluted in culture medium and continuing the culture for 0.5 h, the solution was aspirated and washed twice with PBS. Next, the cells were irradiated with 808 nm laser (1 W, 10 min). After the irradiation, the fluorescence intensity was observed under an inverted fluorescence microscope. The excitation wavelength was 485 nm and the emission wavelength was 530 nm. Besides, APF (0.001 mM) was also used to detect the ·OH generation, and the operation steps are the same as DCFH-DA staining. The excitation wavelength was 490 nm and the emission wavelength was 515 nm.

6. The photo-killing ability of BTCu nanoparticles on HepG2 cells

Spread well-grown HepG2 cells in a 96-well plate (8000 cells / well). After 24 hours, aspirate the supernatant and add 0.2 mg / mL BTCu and BT diluted in culture medium respectively. After continuing the culture for 24 h, the supernatant was aspirated and the cells were washed twice with PBS. Next, the cells were irradiated with 808 nm near infrared laser (1 W, 10 min). After the irradiation, continue to incubate in the incubator for 24 h, and use Cell counting kit-8 (Solarbio life sciences) to detect cell viability. Using a method similar to the above operation, the toxicity of nanoparticles to LO2 cells was also measured. Besides, 100 μM of $CoCl_2$ was used to simulate the hypoxic environment, and the experiment process is the same as above.

Spread well-grown HepG2 cells into a 12-well plate (1.2 * 10^5 cells / well). After 24 hours, aspirate the supernatant and add 0.2 mg / mL BTCu and BT diluted in culture medium, respectively. After incubation for 24 h, the supernatant was aspirated and the cell were washed twice with PBS. Next, irradiate with 808 nm near infrared laser (1 W, 10 min). After the irradiation, after incubating for 10 h in an incubator, Hoechst 33342 / PI double staining was performed.

7. lysosomal membrane permeability (LMP) effect of BTCu nanopraticles

Spread well-grown HepG2 cells into a 12-well plate (1.2 * 10^5 cells/well). After 24 hours, aspirate the supernatant and add 0.2 mg / mL BTCu and BT diluted in culture medium, respectively. After incubation for 24 h, the supernatant was aspirated and the cell were washed twice with PBS. Next, irradiate with 808 nm near infrared laser (1 W, 10 min). After the irradiation, after incubating for 4 h in an incubator, the AO staining, Lyso tracker staining and Cathepsin B Immunofluorescence staining were performed.

The specific steps of AO staining are: aspirate the supernatant, wash the cells twice

with PBS, and add 100 μL of AO staining solution (5 μg/mL) to each well. After incubating for 15 min in the incubator, the liquid was aspirated, the cells were washed three times with PBS, 200 μL of PBS was added, and the fluorescence was observed with a laser confocal microscope. When the excitation wavelength is 555 nm and the emission wavelength is 617 nm, red fluorescence is emitted; when the excitation wavelength is 490 nm and the emission wavelength is 528 nm, green fluorescence is emitted.

The specific steps of Lyso tracker staining are: aspirate the supernatant and wash the cells twice with PBS. Add 100 μL lyso Tracker Green staining solution (50 nM, L7526, Thermo Fisher) to each well. After incubating for 5 min in the incubator, the liquid was aspirated, washed three times with PBS, and 200 μL of PBS was added to each well. Finally, it was placed under an inverted fluorescence microscope for observation, with an excitation wavelength of 488 nm.

The specific steps of Cathepsin B immunofluorescence staining are: aspirate the medium, wash the cells twice with PBS, and fix with paraformaldehyde for 10 min. Aspirate the liquid, wash the cells three times with PBS, add 250 μL TritonX-100 (w% = 0.25%), and incubate at room temperature for 20 min. Aspirate the liquid, wash three times with PBS, add 200 μL of 10% goat serum, block at room temperature for 30 min. Discard the blocking solution, add the primary antibody Rabbit Anti-Cathepsin B antibody (bs-1500R, Bioss), incubate for 5 h at 4 ℃ in the dark; after washing three times with PBS, add the secondary antibody Goat Anti-rabbit IgG / Alexa Fluor 555 antibody (bs-0295G-AF555, Bioss), incubate at room temperature in the dark for 1 h. Aspirate the liquid, wash three times with PBS, add 200 μL PBS, and finally place it under an inverted fluorescence microscope for observation.

Spread well-grown LO2 cells into a 12-well plate ($1.2 * 10^5$ cells/well). After 24 hours, aspirate the supernatant and add 0.2 mg / mL BTCu and BTCu+H_2O_2(0.1 mM) diluted in culture medium, respectively. After incubation for 24 h, the supernatant was aspirated and the cell were washed twice with PBS. Next, irradiate with 808 nm near infrared laser (1 W, 10 min). After the irradiation, after incubating for 4 h in an incubator, the AO staining, Lyso tracker staining and Cathepsin B Immunofluorescence staining were performed. The staining method are the same as above.

8. Effect of BTCu on mitochondrial membrane potential

Spread well-grown HepG2 cells into a 12-well plate ($1.2 * 10^5$ cells/well). After 24 hours, aspirate the supernatant and add 0.2 mg / mL BTCu and BT diluted in culture medium, respectively. After incubation for 24 h, the supernatant was aspirated and the cell were washed twice with PBS. Next, irradiate with 808 nm near infrared laser (1 W, 10 min). After the irradiation, after incubating for 4 h in an incubator, the Rhodamine 123 (Rh123) staining is performed. The specific steps are as follows: the cells are digested with trypsin, centrifuged to obtain a cell pellet, and washed twice with PBS. The cells were re-suspended in a pointed centrifuge tube with 200 μL of PBS, and 2 μL of Rh 123 staining solution (0.5 mg / mL) was added. After 20 minutes of staining, the cells were centrifuged and washed twice with PBS, and the fluorescence intensity was detected by flow cytometry (excitation wavelength 511 nm, emission wavelength 534 nm). Rhodamine 123 staining procedure for LO2 cells is the same as above. The

nanoparticles added to the medium are 0.2 mg / mL BTCu and BTCu + H_2O_2 (0.1 mM).