Supplementary Information

Enhanced In Vitro Photodynamic Performance under Hypoxia-Related Conditions by BP-Au@MnO_2-Ce6 Nanocomposites

1. Materials and methods

1.1 Materials and apparatus

Black phosphorus crystals were purchased from Nanjing Muke Nano Technology Co., Ltd. N-methyl-2-pyrrolidone (NMP), Gold chloride trihydrate ($HAuCl_4 \cdot 4H_2O$), Potassium permanganate ($KMnO_4$), Potassium carbonate (K_2CO_3), sodium citrate dihydrate, polyetherimide (PEI M_w=20000), 1,3-diphenylisobenzofuran (DPBF) and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from Aladdin (Shanghai, China). Chlorin e6 (Ce6) were obtained from Sigma-Aldrich. Cell Counting Kit-8 (CCK-8) was obtained from DOJINDO (Japan). All other chemicals and reagents were of the highest quality commercially available and used as received.

The transmission electron microscopic (TEM) images were recorded on a F-30 transmission electron microscope (Japan). Fourier transform Infrared (FT-IR) spectra were recorded on a Nicolet IS10 Fourier transform infrared spectrometer (USA). Zeta potential analysis and dynamic light scattering was obtained on a Zetasizer (Nano-Z, Malvern, UK). The UV–vis absorption spectra were acquired on a UV-3600 UV–Vis–NIR spectrophotometer (Shimadzu Co., Kyoto, Japan). Atomic force microscopic (AFM) images were performed with Dimension Icon (Bruker Co. USA).

CCK-8 assays were carried out on Varioskan Flash (Thermo Scientific, USA). The confocal cell imaging was gained on a Leica TCS SP5 laser scanning confocal microscope (Leica, Germany).

1.2. Synthesis of BP-Au@MnO_2-Ce6 nanocomposites (BAMC NCs)

BP nanosheets (BPNS) were prepared by ultrasonic liquid phase exfoliation of BP powder. 20 mg BP powder was added to 40 mL NMP dissolved with saturated NaOH in a sealed conical flask filled with argon. Then the mixture was ultrasonically exfoliated with 300 W ultrasonic machine for 10 h. The BPNS was obtained by centrifugation and washed with water. Subsequently, 10 μL PEI aqueous solution (20 mg/mL) was slowly added to BPNS solution under sonication. The mixture was sonicated for 10 min, stored overnight at 4 °C, and collected by centrifugation.

Au nanoparticles were synthesized according to previous literature. Briefly, 4.3 mg tannic acid and 21 mg K_2CO_3 was added to 150 mL deionized water dispersed with 97 mg sodium citrate in a four-necked flask. The mixture was heated to 70 °C on a magnetic heating stirrer. Then 10 mg $HAuCl_4 \cdot 4H_2O$ was quickly injected. The mixture quickly turned black-gray, and then turned orange-red within 1-2 min. Finally, the mixture was reacted at 70 °C for 10 min and cooled to room temperature for use.

Au@MnO_2 nanoparticles (AM NPs) were prepared by in situ reduction of $KMnO_4$. 5 mL $KMnO_4$ aqueous solution (1.4 mg/mL) was added dropwise to 20 mL Au nanoparticle solution with vigorous stirring. After stirring for 40 min, the mixture was stirred at 80 °C for 30 min in a water bath, and then cooled for use.

BAMC NCs were prepared through electrostatic interaction. 500 μL Au@MnO_2 nanoparticle was gradually added into 10 mL BP-PEI nanosheets (0.1 mg/mL) under sonication and the mixture was sonicated for 10 min. Subsequently, Ce6 was slowly added to the mixture and the mixture was stirred overnight in a sealed flask filled with Ar in the dark. BAMC NCs were collected by centrifugation for use.

1.3 Catalytic Activity Measurements of BAMC NCs

(1) O_2 generation

The catalytic O_2 production effect of various nanoparticles on H_2O_2 was measured using a dissolved oxygen meter. The three-necked flask was sealed with dissolved oxygen meter probe, three-way valve and glass stopper. Then the flask containing H_2O_2 solution of different concentration was evacuated for 10 min and slowly purged with Ar for 1 min under stirring. The solution was vigorously stirred for 0.5 h until the dissolved oxygen meter reading was stable. Subsequently, BAMC NCs at a final concentration of 25 μg/ml were rapidly added to H_2O_2 solution with a syringe, and the dissolved O_2 concentration were recorded. The catalytic performance of BAMC NCs on H_2O_2 was investigated by recording dissolved O_2 concentration of the mixture.

(2) $^1O_2$ generation

All experiments were performed in dark conditions. BPNS and BAMC NCs were separately added into 2 mL DMSO containing DPBF, respectively. Then the mixture was irradiated by 660 nm laser (0.5 W/cm^2) and the absorbance at 416 nm was measured at 1 min intervals. To examine $^1O_2$ generation ability of the sample under hypoxic conditions, 2 mL DMSO containing DPBF and various nanoparticles was evacuated for 30 min and slowly purged with Ar for 1 min. Then 100 μL H_2O_2 (5×10^{-3} M) was rapidly injected into the mixture and incubated for 30 min. Finally, the mixture was irradiated by 660 nm laser (0.5 W/cm^2) and the absorbance at 416 nm was measured at 1 min intervals.

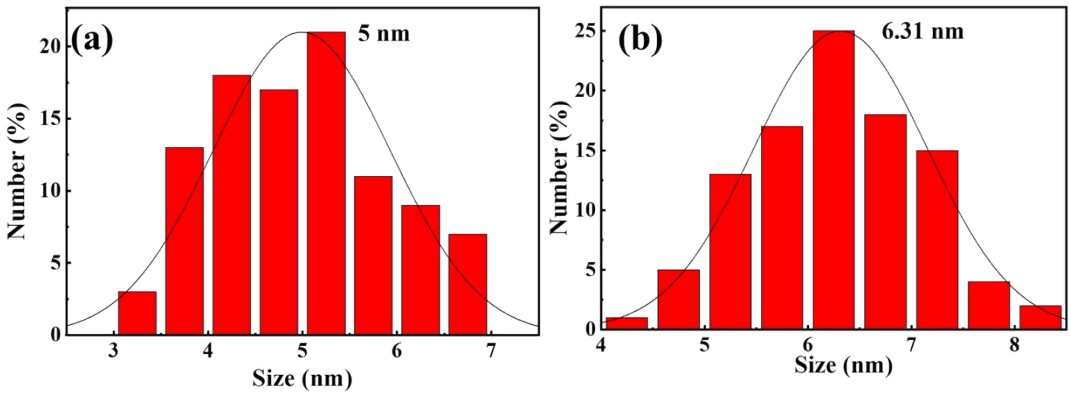

Figure S1 The average particle size of Au nanoparticles and Au@MnO_2 nanoparticles.

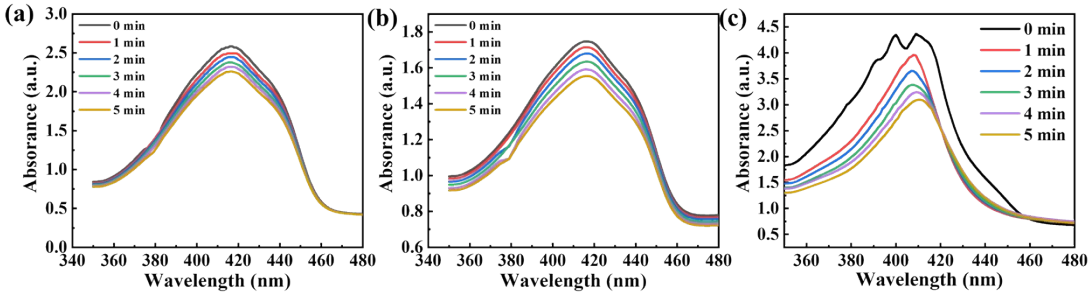

Figure S2. Time-course absorbance spectra of DPBF mixed with BPNS and BAM NCs under hypoxia conditions.

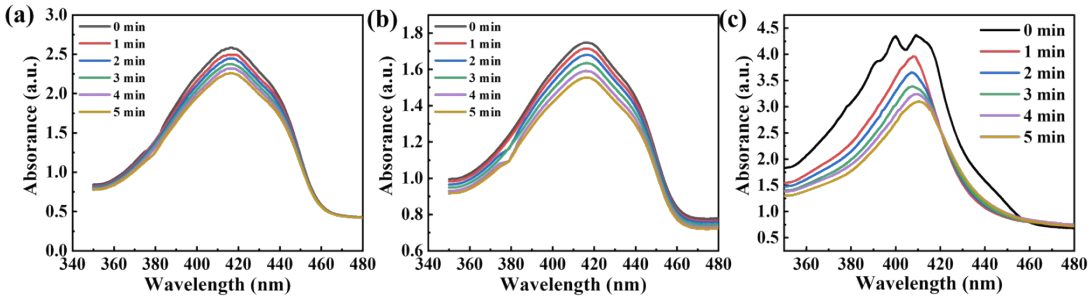

Figure S3. Time-course absorbance spectra of DPBF mixed with BPNS, BA NCs and BAMC NCs under normoxic conditions