Supporting information

Carbon-gold hybrid nanoprobes for real-time imaging, photothermal/photodynamic and nanozyme oxidative therapy

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Methods

Investigating the effect of FA on the targeting ability of probes

Confocal microscopy could qualitatively investigate cellular uptake of OMCAPs@rBSA @IR780 and OMCAPs@ rBSA-FA@IR780. At first, MGC-803 cells were seeded in 14 mm glass coverslips and cultured for 24 h. After co-incubation with OMCAPs@rBSA@IR780 and OMCAPs@ rBSA-FA@IR780 (IR780 dosage: 6 μg/mL) for different times (1, 5 and 9 h), the MGC-803 cells were rinsed with PBS gently and fixed with 4% paraformaldehyde for 30 min at 37 °C. Finally, the cells were observed on a Leica TCS SP8 confocal laser scanning microscopy. Red fluorescence from IR780 was excited at 633 nm and emission was collected from 700 to 800 nm.

Determination of ROS generation of OMCAPs@rBSA-FA in cells at different concentrations

To investigate influence of concentration of OMCAPs@rBSA-FA on ROS generation in cells, the cells were firstly seeded in 14 mm glass coverslips and cultured for 24 h. After co-incubation with OMCAPs@rBSA-FA (dosage: 5 to 30 μg/mL) for 8 h, the MGC-803 cells were rinsed with PBS and further incubated with 500 mL carboxy-H_2DCFDA (25 μM) for 60 min. After washed with PBS, the cells were fixed with 4% paraformaldehyde for 30 min at 37 °C. Finally, the cells were observed on a Leica TCS SP8 confocal laser scanning microscopy with 488 nm excitation light and the corresponding emission was recorded at 525/50 nm.

Determination of ROS generation of various materials in cancer cells

Firstly, MGC-803 cells were seeded in 14 mm glass coverslips and cultured for 24 h. After interacted with PBS, free IR780 (6 μg/mL), OMCAPs@rBSA-FA (6 μg/mL) and OMCAPs@rBSA-FA@IR780 (18 μg/mL) for 8 h, the cells were incubated with 500 mL of carboxy-H_2DCFDA (25 μM) for 60 min. Afterwards, the cells were rinsed with PBS and treated with or without NIR laser irradiation (808 nm, 1 W/cm^2) for 5 min, and further cultured for 30 min at 37 °C. Following that, the cells were fixed with 4% paraformaldehyde for 30 min at 37 °C. After washed with PBS sufficiently to remove redundant paraformaldehyde, the cells were observed on a Leica TCS SP8 confocal laser scanning

microscopy with 488 nm excitation light and the corresponding emission was recorded at 525/50 nm.

Pharmacokinetics of the OMCAPs@ rBSA-FA@IR780 nanoprobes

The pharmacokinetics of constructed OMCAPs@ rBSA-FA@IR780 nanoprobes was investigated by measuring the IR780 fluorescence quantitatively in blood samples of BALB/c female mice after intravenously injected with OMCAPs@ rBSA-FA@IR780 (IR780 content: 5 mg/kg). In brief, 100 μL of blood was collected at 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h and detected by using a fluorescence spectrophotometer. Then concentration of probes in serum versus time was analyzed and used to calculate the blood circulation half-lives. Blood circulation data curve was plotted as shown in Fig. 7S.

Biodistribution investigation of OMCAPs@ rBSA-FA@IR780 nanoprobes

To study bio-distribution of nanoprobes, the MGC-803 tumor bearing mice were intravenously injected with 100 μL of OMCAPs@ rBSA-FA@IR780 probes (IR780 content: 5 mg/kg) and sacrificed at 1 h, 12 h, 24 h and 48 h after injection in respective. Then, the organ samples included heart, liver, spleen, lung and kidney were taken out and imaged at the same parameters by a Bruker In-Vivo F PRO imaging system (Billerica, MA, USA) with 720/20 nm excitation and 790/30 nm emission at 60 s exposure time.

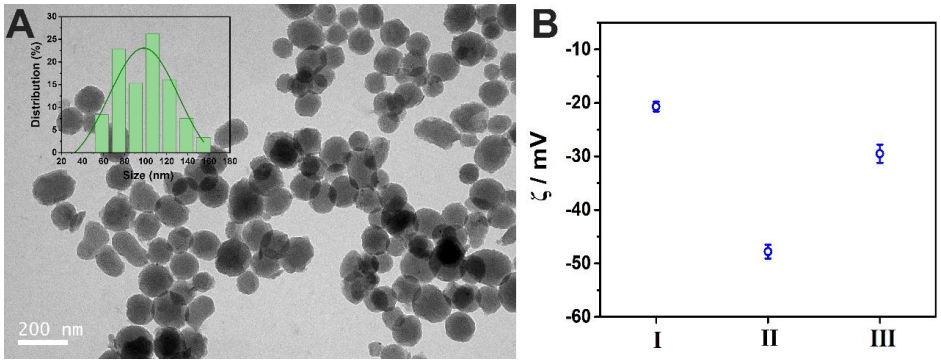

Fig. S1. (A) TEM image of OMCAPs. The inset: core size distribution of OMCAPs. (B) ζ -potential value of OMCAPs (I), OMCAPs@ rBSA-FA (II) and OMCAPs@ rBSA-FA@IR780 (III).

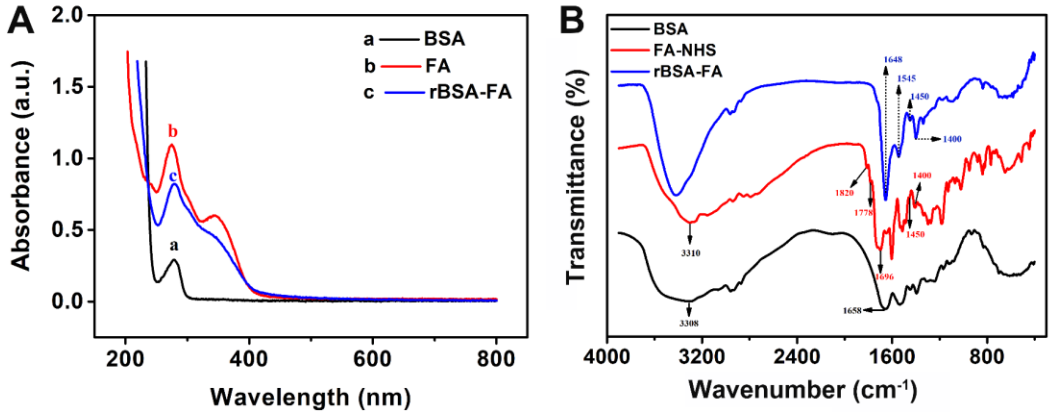

Fig. S2. (A) UV-vis spectra of various materials including (a) BSA, (b) FA, (c) rBSA-FA. (B) Fourier Transform Infrared Spectrum of different materials containing BSA, FA-NHS and rBSA-FA.

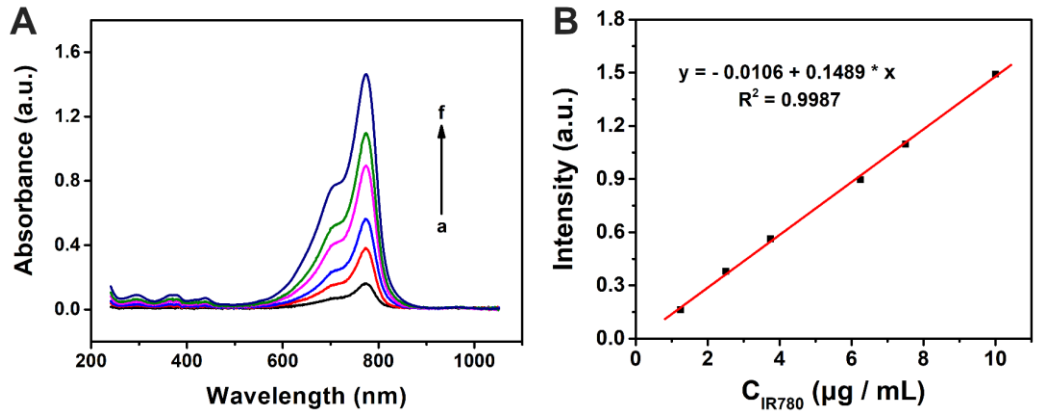

Fig. S3. (A) UV-vis spectra of IR780 under different concentrations. (B) Calibration curve

between absorbance intensity and IR780 concentrations.

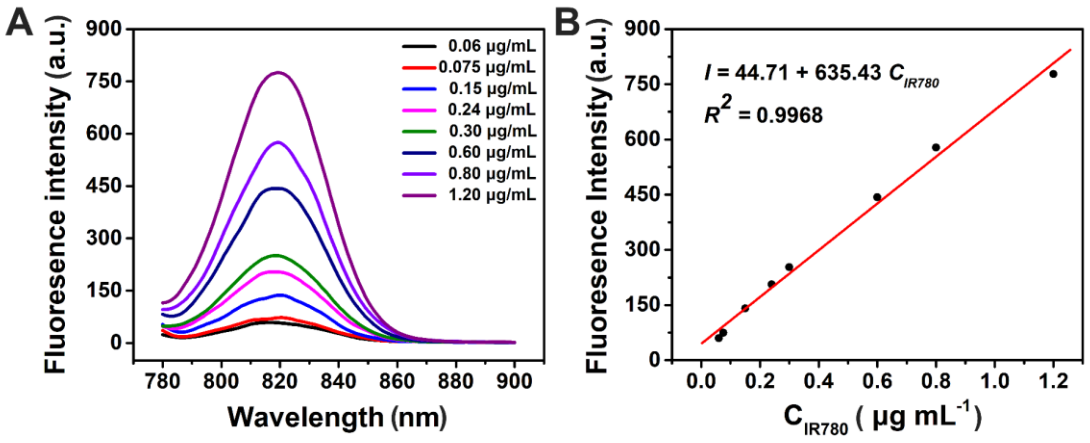

Fig. S4. (A) Fluorescence spectra of IR780 under different concentrations (λ_{ex}: 760 nm). (B) Calibration curve between fluorescence intensity and IR780 concentrations.

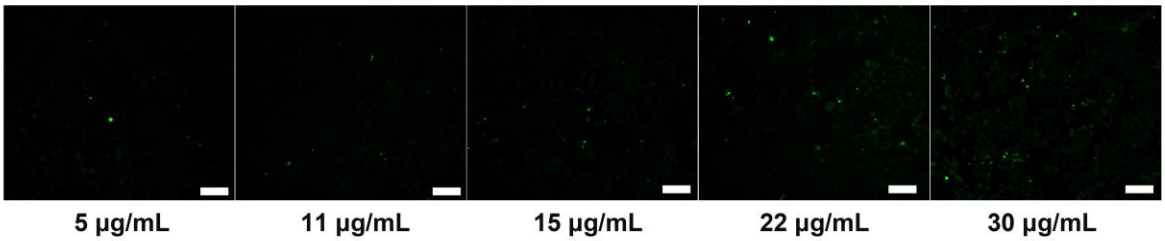

Fig. S5. Confocal images of MGC-803 Cells for ROS investigation after incubated with OMCAPs@rBSA-FA with various concentration. The green fluorescence was caused by carboxy-H_2DCFDA dye which revealed the existence ROS (scale bar, 100 mm).

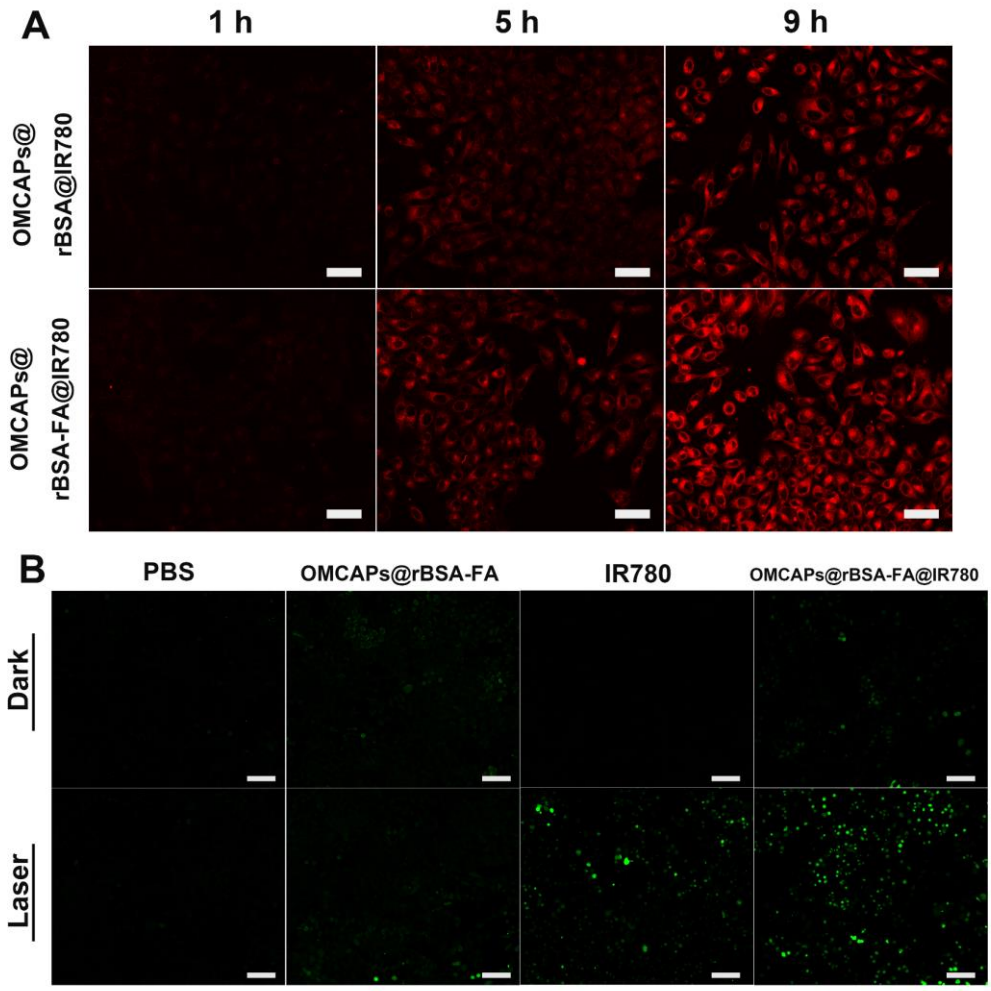

Fig. S6. (A) Confocal images of MGC-803 cells incubated with OMCAPs@rBSA@IR780 and OMCAPs@rBSA-FA@IR780 nanoprobes at various time point (1, 5 and 9 h). The red fluorescence revealed the existence of IR780 dye. Scale bar: 50 μm. (B) Confocal images of MGC 803 Cells for ROS investigation after incubated with various agents and then irradiated without or with 808 nm laser (1 W/cm^2 for 5 min). The green fluorescence of cells staining by carboxy-H_2DCFDA dye revealed the existence ROS (scale bar: 100 mm).

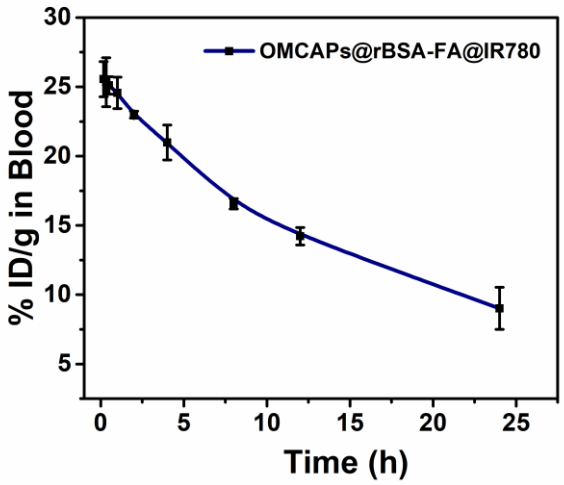

Fig. S7. Pharmacokinetics of the OMCAPs@ rBSA-FA@IR780 after intravenous injection (data presented as the mean ± standard deviation, n = 3).

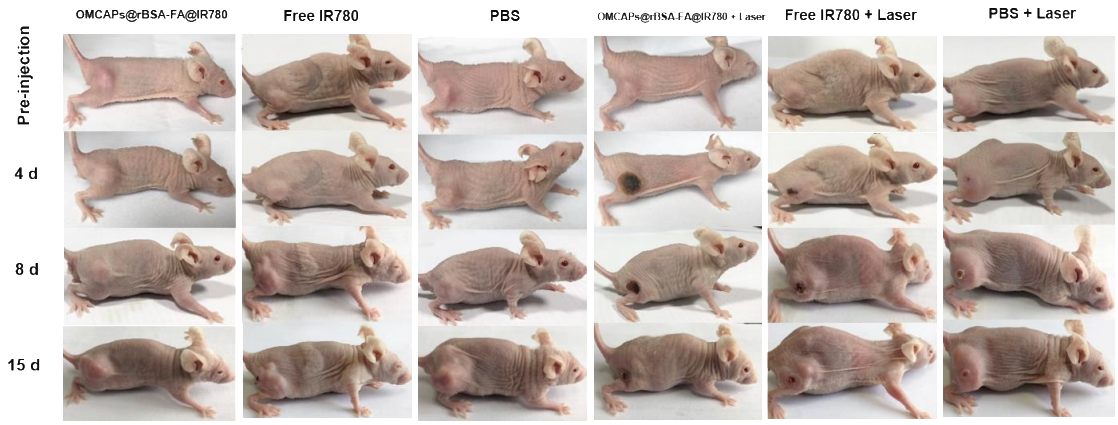

Fig. S8. Tumor images of various materials treated mice recorded by camera after laser irradiation at 0, 4, 8 and 15 d respectively.

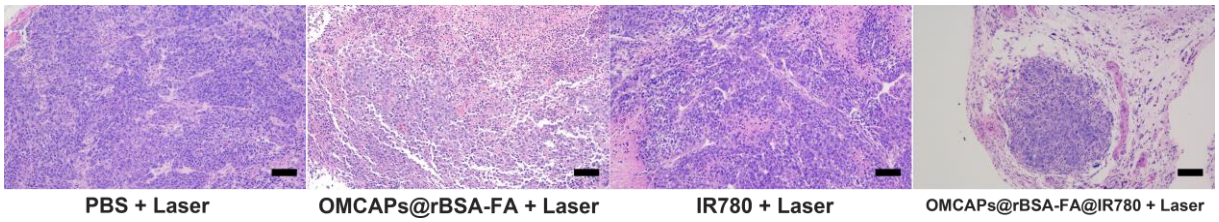

Fig. S9. H&E staining of the tumor sections treated with various materials under irradiation after 15 days (Scale bar: 100 μm).

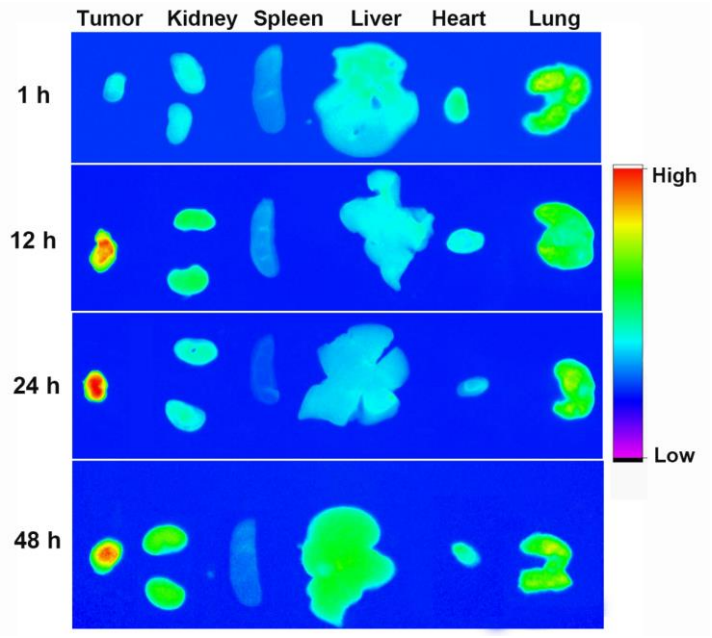

Fig. S10. The fluorescence images of organs from MGC803-tumor bearing mice after intravenous injection of OMCAPs@rBSA-FA@IR780 after various time points (1 h, 12 h, 24 h, 48 h).

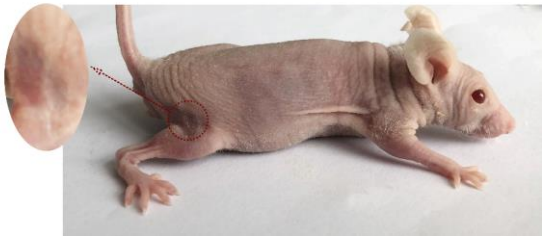

Fig. S11. Photograph of mice treated with probe OMCAPs@rBSA-FA@IR780 under irradiation after 30 days.