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Electronic Supplementary Information

Design of Janus Black Phosphorus Quantum dots@Metal-Organic Nanoparticles for Simultaneously Enhancing Environmental Stability and Photodynamic therapy Efficiency

Da Zhang [b, c, d#], Ziguo Lin [a, b, d#], Shanyou Lan [a, b, d], Haiyan Sun [e*], Yongyi Zeng [a, b, c, d*], and Xiaolong Liu [b, c, d*]

[a.] Liver Disease Center, The First Affiliated Hospital of Fujian Medical University, Fuzhou 350005, P. R. China

[b.] The United Innovation of Mengchao Hepatobiliary Technology Key Laboratory of Fujian Province, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou 350025, P. R. China

[c.] Mengchao Med-X Center, Fuzhou University, Fuzhou 350116, P. R. China

[d.] The Liver Center of Fujian Province, Fujian Medical University, Fuzhou 350025, P. R. China

[e.] Department of Anesthesiology, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, PR China

*Corresponding authors: xiaoloong.liu@gmail.com; lamp1973@medmail.com.cn; 2254103187@qq.com;

Experiments

Materials and Reagents

Black phosphorus nanosheets were purchased from commercial supplier (XFNANO) (Nanjing, China). 1, 4, 5, 8-tetrahydroxyanthraquinone (organic dye, THQ) was purchased from Shenzhen Regent Biochemistry Technology CO., Ltd. 2, 7-dichlorodihy- drofluorescein diacetate (DCFH-DA), 1,3-Diphenylisobenzofuran

(DPBF), 4,6-diamidino-2- phenylindole (DAPI) and 3,3',5,5'-Tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (USA). Calcein-AM and propidium iodide (PI) were purchased from J&K Scientific Ltd (Beijing, China). Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies (Tokyo, Japan). N, N-dimethylformamide (DMF), Copper (II) Chloride Dihydrate ($CuCl_2$) and ethanol (EtOH) were obtained from Aladdin Reagents (Shanghai, China). Deionized water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) and used for all experiments. Unless specified, all the chemicals used in this study were obtained commercially and applied as received.

Cell Culture and animals

The hepatocellular carcinoma cells (HepG2) were purchased from ATCC (Manassas, VA) and cultured in RPMI 1640 medium supplemented with 10% FBS and penicillin-streptomycin (100 IU/mL, Cellgro, Manassas, VA) at 37°C in a humidified incubator containing 5%CO_2. Male BALB/c nude mice (20~22g) were purchased from China Wushi, Inc. (Shanghai, China). All animal procedures were approved by the Animal Ethics Committee of Mengchao Hepatobiliary Hospital of Fujian Medical University.

Synthesis of the J-MOPs

Firstly, the BPQDs were synthesized through previously reported literatures with slight modification [3, 9]. In short, 0.2 mg/mL black phosphorus nanosheets (BPNs) in 1-Methyl-2-pyrrolidinone (NMP) were treated by ultrasonic cell disruptor (SCIEWTZ-II D) with 70% output for 8hrs in ice bath. After that, the resultant

solution was treated by the ultrasonic cleaning system (KQ-500 DE) for another 4hrs. Afterwards, the resultant was centrifuged at 2000 rpm/min for 5 min to remove large BPNs, and then collected by centrifugation at 20000 rpm/min for 20 min. Finally, the precipitate products of BPQDs were re-suspended in deionized water for further usage. Secondly, the J-MOPs were prepared by one-step method. Briefly, a certain amount of BPQDs (0.2 mg/mL) was dispersed in deionized water and then mixed with $CuCl_2$ (10 mg/mL) for 10 min. Afterwards, the THQ aqueous solution (1 mg/mL) was added to the mixture and stirred for 1hr. Then, the mixture was centrifuged at 12000 rpm/min for 20 min, and washed by 50% ethanol to remove the excess THQ for twice. Subsequently, the J-MOPs were re-suspended in PBS buffer for further usage.

Characterization of the J-MOPs

Transmission electron microscope image (TEM) was conducted by a JEM-2010 electron microscope (JEOL, Japan), and the chemical compositions of the J-MOPs were performed by EDS. The FT-IR spectra were collected on a FT-IR spectrometer (Perkin Elmer, USA). The UV-vis-NIR absorption spectra were measured by Spectro Max M5e, Germany. The fluorescence spectrum was investigated by Agilent Cary Eclipse fluorescence spectrophotometer (Santa Clara, USA). The Raman scattering was performed on Horiba Jobin-Yvon Lab Ram HR VIS high-resolution confocal Raman microscope with the 633 nm laser as the excitation source. The zeta potential measurements were performed at 25 °C on the NanoZS, using M3-PALS technology.

Evaluation of the stability and photodynamic effect of J-MOPs

To evaluate the stability of J-MOPs, the J-MOPs were dispersed in water-air condition at room temperature. Afterwards, the samples were imaged and measured by digital camera and UV-vis spectrometer (Molecular Devices, USA) at time points of 0, 12, 24, 36, and 48hrs. Next, to investigate the photodynamic properties of J-MOPs, DPBF as ROS indicator was used to confirm the ROS generation. Briefly, J-MOPs (0.2 mg/mL, BPQDs) and BPQDs (0.2 mg/mL) were added into DPBF (0.25 mM) PBS solution, and irradiated by 670 nm lasers for different times, and then the UV-vis spectrometer was used to measure the absorbance of DPBF ranging from 300 to 600 nm at different irradiation time points.

Cellular uptake

To investigate the HepG2 cell uptake of J-MOPs, confocal laser scanning microscope (CLSM, Carl Zeiss LSM 780, Germany) was performed. Briefly, the HepG2 cells were seeded into glass-bottom Petri dishes at a density of 5×10^5 cells at 37^oC in a 5% CO_2 atmosphere for 24 hrs. Then, the cells were washed by PBS buffer solution at room temperature (RT) and then incubated with J-MOPs for 1, 2 or 3hrs, respectively. Afterwards, the cells were washed with PBS buffer solution at 37^oC to remove the non-taken J-MOPs, and stained by DAPI. Afterwards, the cells were imaged by CLSM (Carl Zeiss LSM 780, Germany) with 561 nm laser excitation for THQ and 488 nm for DAPI.

Enhanced ROS generation of J-MOPs under 670 nm Laser irradiation in cells

According to our pervious works [12, 14, 28], DCFH-DA (ROS fluorescence probe) was used to detect the ROS generation from J-MOPs. Briefly, the HepG2 cells were

seeded in a 96 well plate at a density of 2×10^4 cells per well and incubated under a humidity atmosphere (with 5% CO_2) for 24hrs. Then, the original medium was replaced with fresh culture medium containing J-MOPs. After incubation for 3hrs, the cells were washed with fresh culture medium containing 100 μM of DCFH-DA for 30 min at 37°C. Then, the cells were irradiated by 670 nm laser for 5 min (0.1 W/cm^2). Fluorescence microscope (Zeiss microscope (Axio Lab.A1)) was used to detect the fluorescence DCFH-DA.

***In vitro* cytotoxicity assays**

The antitumor effect of J-MOPs was evaluated on HepG2 cells by using a Cell Counting Kit (CCK8). Briefly, the HpeG2 cells were first seeded in a 96-well plate at a density of 1×10^5 per well and incubated under a humid atmosphere (with 5% CO_2) for 24hrs. Then, the medium was replaced with a fresh medium containing of J-MOPs and further incubated for 3hrs. Afterwards, the cells were irradiated by 670 nm laser for 5 min (0.1 W/cm^2). The cells only incubated with the culture medium were taken as untreated control. Subsequently, 100 μL of fresh culture medium containing 10 μL of CCK8 were added to each well. After incubation for 2hrs at 37°C, the absorption was measured by a microplate reader. Cell viability was expressed as follows:

$$\text{Cell viability (\%)} = (OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) * 100. \qquad (1)$$

The OD_{sample} and $OD_{control}$ were the absorbance values of treated cells and the untreated control cells, respectively. OD_{blank} was the absorbance values of the CCK8 agent at 450 nm. All experiments were independent performed for five times.

Then, the localized photo-killing effects of J-MOPs were evaluated according to our previously published protocols [12, 14, 42]. Briefly, the HepG2 cells were first seeded into a plate at a density of 1×10^6 cells at 37°C in a 5% CO_2 atmosphere for 24hrs. Then, the cells were washed with PBS to remove dead cells, followed by incubation with J-MOPs in the presence or absence of 670 laser irradiation with the laser power intensity of 0.1W/cm^2 for 5 min. Afterwards, the treated cells were incubated with fresh culture medium at 37°C for 4hrs and stained with a DEAD Cytotoxicity Kit to visualize the dead cells by fluorescence microscope.

Antitumor Efficacy *in vivo*

The male BALB/c nude mice (about 5 weeks old) with body weight of ~22g were obtained from China Wushi, Inc. (Shanghai, China). All animal experiments were approved by the Animal Ethics Committee of Mengchao Hepatobiliary Hospital of Fujian Medical University. The mice with tumor-bearing were established by subcutaneously injection of HepG2 cells (1×10^7 cells) in sterilized PBS buffer. When the tumor size reached around ~5 to 7 mm, 50 μL J-MOPs (0.8 mg/mL) were intratumorally injected into each mouse. One group of mice treated with the same volume of sterilized PBS was taken as the control. The mice were segregated into following five groups:

(1) PBS treated mice without laser irradiation (n = 4);

(2) PBS treated mice with laser irradiation (670 nm, 0.1W/cm^2) for 5 min (n = 4);

(3) BPQDs treated mice with laser irradiation (670 nm, 0.1W/cm^2) for 5 min (n = 4);

(4) J-MOPs treated mice without laser irradiation (n = 4);

(5) J-MOPs treated mice with laser irradiation (670 nm, 0.1W/cm^2) for 5 min (n = 4);

For antitumor efficiency analysis, the irradiation was carefully conducted after 2hrs of injection. Then, the antitumor effects were evaluated by monitoring the tumor volume change and body weight change in each group every 2 days, and over 14days in total. The tumor size was calculated by the calipers every other day after indicated treatment. Finally, the volume of tumor (V) was calculated by the following equation:

$$V=A*B^2 / 2 \quad (2)$$

Here, the A is the longer diameter and B is the shorter diameter (mm) of tumor, respectively.

Histological Examination and Long-term Toxicity Assessment

To carefully evaluate the histological changes of tumors after indicated treatment, one tumor-bearing mouse in each group was sacrificed after 48hrs of irradiation; then, the tumors were collected, sectioned and stained with hematoxylin and eosin (H&E) for histopathology analysis and Ki67 for immunohistochemical analysis according to our previously published protocol [42, 45]. To assess the long-term toxicity of mice after different treatment, one tumor-bearing mouse in each group were sacrificed at the 14 day after indicated treatment, and major organs (heart, liver, spleen, lungs, and kidneys) of the mouse were collected, then fixed in 4% neutral formaldehyde, embedded in paraffin, stained with H&E, and finally observed under the Zeiss microscope (Axio Lab.A1).

Statistical analysis

Statistical analysis of data was performed using one-way of variance (ANOVA) method or the two-tailed paired Student's T-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. All the data were shown as means ± SD through at least three experiments.

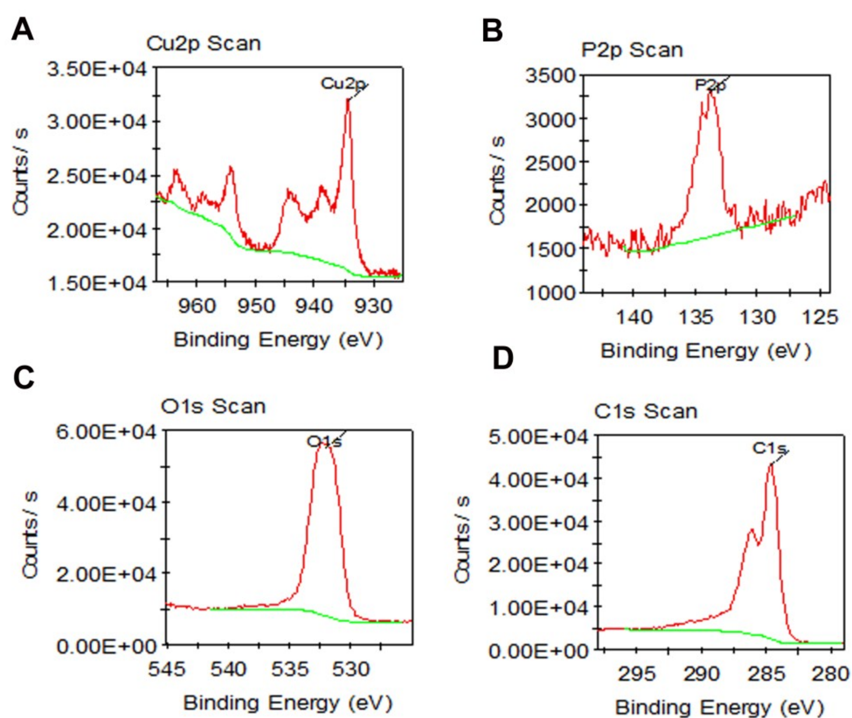

Fig. S1. XPS spectrum of J-MOPs.

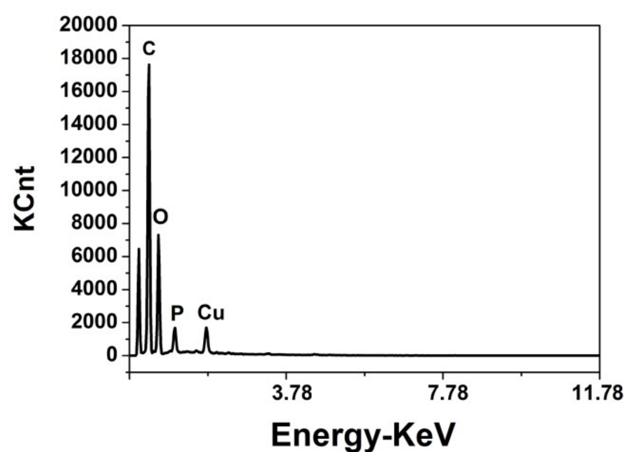

Fig. S2. EDS spectra of J-MOPs.

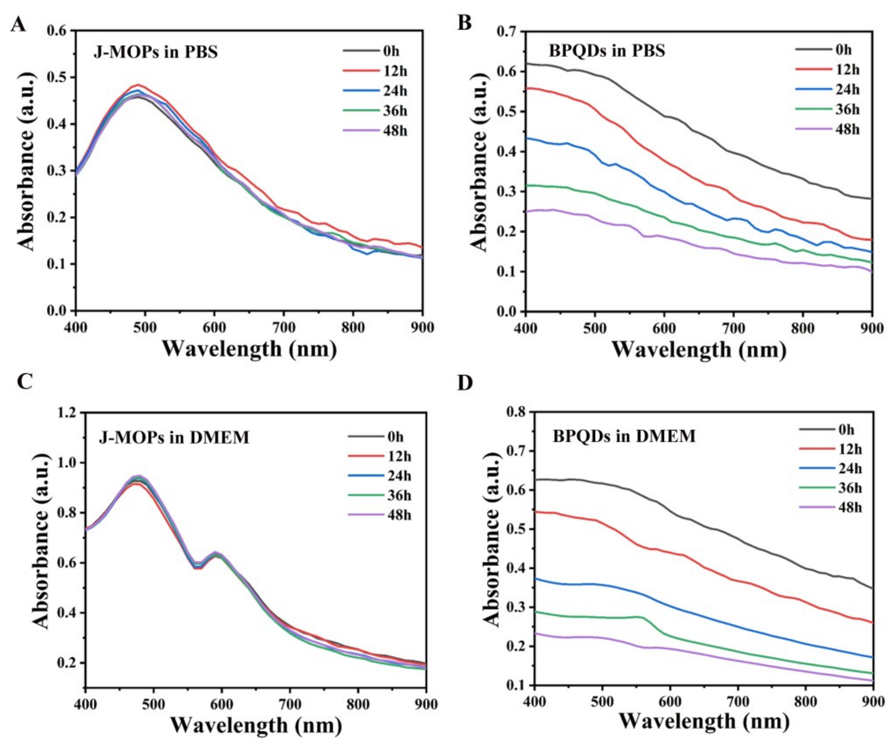

Fig. S3. Vis-NIR absorption spectra of J-MOPs and BPQD dispersed in PBS buffer and culture medium (containing 10% FBS) with different incubation times.

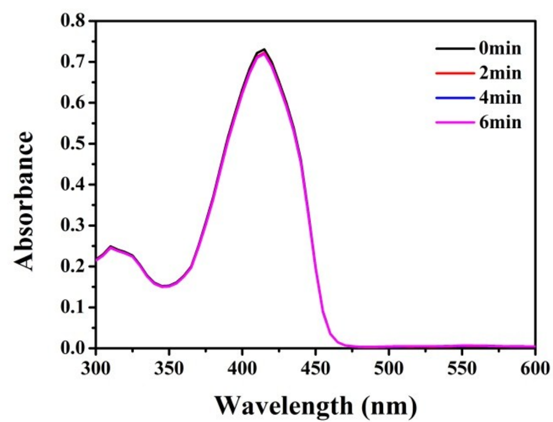

Fig. S4. Absorption of DPBF after photodecomposition by ROS generation of J-MOPs at different times without laser irradiation.

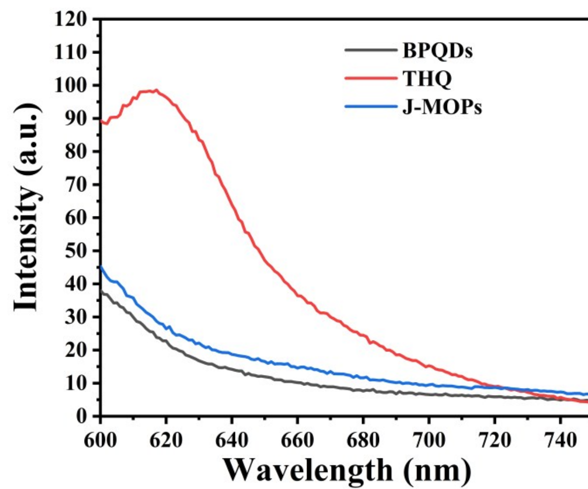

Fig. S5. The fluorescence spectra of THQ, BPQDs and J-MOPs, excited at 550 nm, respectively.

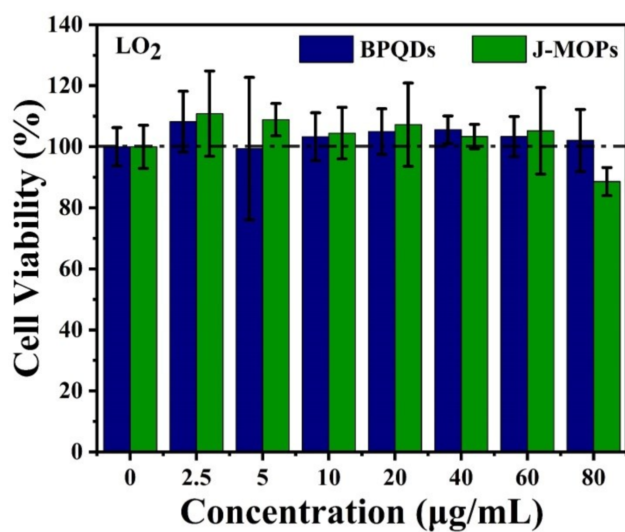

Fig. S6. Cell viability of BPQDs and J-MOPs treated normal liver cells (LO2) for 48hrs at different concentration without laser irradiation (n = 5).

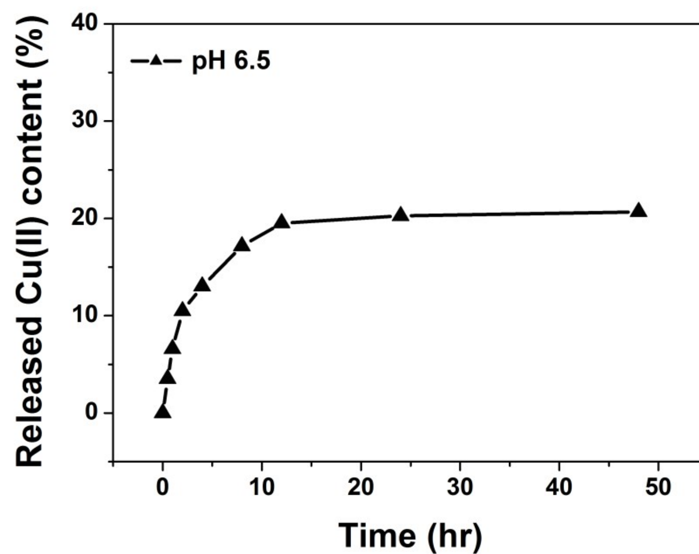

Fig. S7. Percentage of released Cu(II) from J-MOPs under tumor-specific acid conditions.

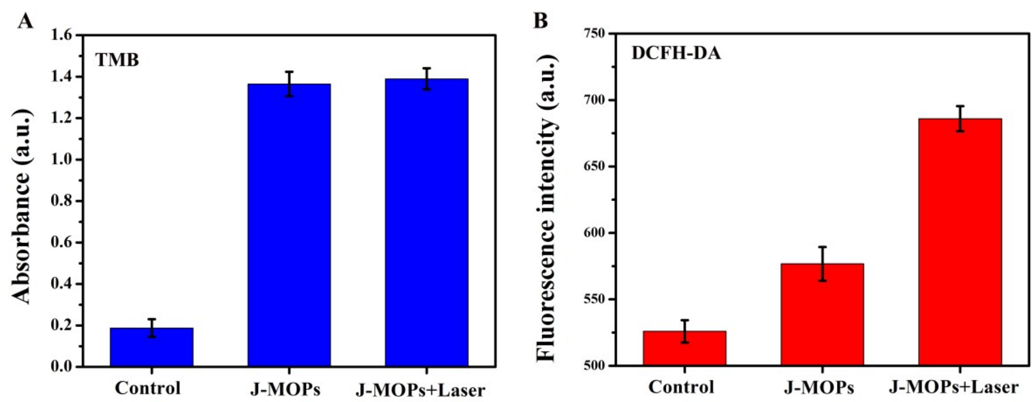

Fig. S8. (A) Absorbance of TMB after photodecomposition by ·OH generated from J-MOPs in tumor microenvironment with or without laser irradiation. (B) The total ROS generation of J-MOPs in tumor microenvironment with or without laser irradiation.