Supporting Information

Ultrathin functionalized covalent organic framework nanosheets for tumor-targeted photodynamic therapy

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1. Reagents and Materials.

2,5-dihydroxyterephthalaldehyde (Dha) and Tetra (p-amino-phenyl) porphyrin (Tph) were obtained from Changchun Third Party Pharmaceutical Technology Co. Ltd. Hyaluronic acid (HA) was purchased from Shandong Freda Biotechnology Co., Ltd. 2-(3,6-Diacetoxy-2,7-dichloro-9H-xanthen-9-yl)benzoic acid was obtained from Macklin Biochemical Co., Ltd. 2′,7′-dichlorofluorescin diacetate (DCFH-DA) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1,3-Diphenylisobenzofuran (DPBF) were purchased from Tianjin Heowns Biochemical Technology Co. Ltd. The cancer cells were purchased from Procell (Wuhan, China). Fetal bovine serum (FBS) in cell culture medium was purchased from Gibco**.** Confocal dish was purchased from Cellvis, Mountain View, CA. All aqueous solutions were prepared using sartorius ultrapure water (18.2 $M\Omega \cdot cm^{-1}$). All the other chemical reagents were of analytical grade and used without further purification.

2. Instruments.

Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to characterize the infrared spectrum. Powder X-ray diffraction (XRD) pattern was obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å). Transmission electron microscopy (TEM, HT7700, Japan) was carried out to characterize the morphology of the nanoparticles. Scanning electron microscopy (SEM) micrographs were recorded on a Hitachi SU8010 Scanning Electron Microscope. UV-vis spectroscopy was achieved with UV-1700 (Shimadzu, Japan). Fluorescence spectra were obtained using a FLS-980 Edinburgh Fluorescence Spectrometer with a Xenon lamp. The absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) for the MTT assay. Confocal fluorescence imaging studies were performed using a TCS SP8 confocal laser scanning microscope (Leica, Germany). All pH measurements were performed with a digital pH-meter (pH-3e, LeiCi, China). Imaging flow cytometry was accomplished on Amnis ImageStream MarkII (Merck Millipore, Seattle, WA). In vivo fluorescence images were captured using live animal imaging system (IVIS Lumina III, US).

3. Experimental Section.

Preparation of COF and COF NPs.

The porphyrin-based COFs was prepared with Dha (19.9 mg, 0.12 mmol) and Tph (40.5 mg, 0.06 mmol) in dichlorobenzene/butyl alcohol/6 M acetic acid (5/5/1, v/v/v, 3.3 mL). After sonication for 10 min, the mixture was degassed in a Pyrex tube (20 mL) through freeze-pump-thaw cycles for three times and then sealed off. The tube was heated 3 days at 120 °C. After that, the product was collected and washed with THF, acetone. To prepare COF NPs, the as-synthesized COF was treated with a sonic probe for 1 h at the power of 1500 W. The COF NPs were collected via centrifugation (13000 rpm, 10 min) for subsequent use.

Preparation of HA-functionalized COF nanosheets (HA@COF NSs).

EDC (10 mg) and NHS (10 mg) were added to the pH=6.5 MES solution of HA (10 mg) and kept in dark place for 0.5 h. Then bulk COF (1 mg) was added in the above-mentioned solution and sonicated with a sonic probe for 1 hour. Then the HA@COF NSs were collected via centrifugation (13000 rpm, 10 min) and washed with water for subsequent use.

Preparation of HA-functionalized COF nanoparticles (HA@COF NPs).

EDC (10 mg) and NHS (10 mg) were added to the pH=6.5 MES solution of HA (10 mg) and kept in dark for 0.5 h. Then COF nanoparticles (1 mg) were added in the above-mentioned solution and stirred for 1 h. The obtained HA@COF NPs were collected via centrifugation (13000 rpm, 10 min) and washed with water for subsequent use.

ROS detection with DCFH.

To compare the ROS generation effects of COF NPs, HA@COF NPs and the HA@COF NSs (the concentration of COF were fixed to be 100 μg/mL), DCFH was added to the solutions, respectively, and the solutions were irradiated with 635 nm laser, the fluorescence signals of the solutions at different time points were recorded. Ex= 488 nm, Em=525 nm.

$^1O_2$ detection with DPBF.

To detect the $^1O_2$ generation effects of COF NPs, HA@COF NPs and the HA@COF NSs, DPBF was added into the solutions (the concentration of COF were fixed to be

100 μg/mL), after irradiated with 635 nm laser with different times, the UV-Vis of the supernates were detected with UV/Vis spectrophotometer.

Cell culture.

HepG2 cells were incubated in cell culture dishes with a diameter of 10 cm containing DMEM supplemented with 10% (v/v) fetal bovine serum (FBS); 1% penicillin, and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. MCF-7 cells were incubated in cell culture dishes with a diameter of 10 cm containing DMEM supplemented with 10% (v/v) fetal bovine serum (FBS); 1% penicillin, and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. 4T1 cells were incubated in cell culture dishes with a diameter of 10 cm containing 1640 supplemented with 10% (v/v) fetal bovine serum (FBS); 1% penicillin, and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. MCF-7/Adr cells were incubated in cell culture dishes with a diameter of 10 cm containing 1640 supplemented with Dox of 1 μg/mL; 10% (v/v) fetal bovine serum (FBS); 1% penicillin, and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell targeting assay

Synthesis of Cy3-loaded HA@COF NSs. 1 mg HA@COF NSs were dispersed into in 2 mL aqueous solution and 20 μL of Cy3 solution (10 mg/mL) was added into the solution. Then the solution was stirred for 12 h and the product Cy3-loaded HA@COF NSs was obtained after centrifugation. HepG2 cells were cultured in confocal dishes overnight and divided into 3 groups: PBS, HA@COF NSs-Cy3+HA and HA@COF NSs-Cy3. HA@COF NSs-Cy3+HA groups was pretreated with HA. After incubated with each nanoparticle respectively for 1 hour, nanoparticles were removed and washed with PBS for 3 times. Finally, confocal imaging experiment was performed.

MTT assay.

HepG2/4T1, MCF-7 and MCF-7/ADR cells were cultured in 96-well plate and incubated for 24 h. The group (control, laser, HA@COF NPs, HA@COF NSs, HA@COF NPs+L and HA@COF NSs+L) of different concentrations (0, 20, 40, 60, 80 μg/mL) were injected into the well and incubated for 4 hours before irradiated with 635 nm laser with 8 minutes. After 12 hours, the media were removed and 150 μL of MTT

solution (0.5 mg/mL) was added to the well. After 4 hours, MTT solution was discarded and 150 μL DMSO were added, then the absorbance was monitored at 490 nm via a microplate reader.

Live/dead cell staining assay.

To detect the viable and dead cells, HepG2 cells were cultured in confocal dishes overnight and divided into 6 groups: I: PBS, II: Laser, Ⅲ: HA@COF NPs+L, Ⅳ: HA@COF NSs+L. For laser irradiation groups, 635 nm laser (0.2 W/cm^2) was utilized to irradiate the cells for 8 min. All the groups were further cultured for 12 hours. Finally the cells were stained with Calcein AM (λex=490nm, λem=515nm) / Propidium Iodide (PI) (λex=535nm, λem=617nm) for 15 min and analyzed with CLSM.

Tumor model establishment.

All animal experiments were conducted and agreed with the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. Female nude mice (6-8 weeks) were fed under normal conditions. The HepG2 cancer model was employed as an example to evaluate the therapeutic effect. 1×10^8 HepG2 cells in 100 μL of serum-free RPMI DMEM medium were injected subcutaneously into the right axillary region of nude mice. The mice were utilized in subsequent experiments after the tumor size had reached approximately 75-100 mm^3.

In vivo fluorescence imaging the distribution of the nanoreactor.

Synthesis of ICG-loaded COF NPs-IGC and HA@COF NSs-IGC. 1 mg COF NPs/HA@COF NSs were dispersed into in 2 mL aqueous solution and 20 μL of ICG solution (10 mg/mL) was added into the solution. Then the solution was stirred for 12 h and the product ICG-loaded COF NPs/HA@COF NSs were obtained after centrifugation. 50 μL of 4 mg/mL ICG-loaded COF NPs and HA@COF NSs were administrated into the tumor bearing nude mice, respectively. At different time points of 0, 12, 24, 36, 48, 60 hours post injection, the fluorescence in the mice was recorded with a live body imaging system.

In vivo antitumor experiment.

The tumor-bearing nude mice were divided into 6 groups: I: PBS only, II: Laser, III: HA@COF NPs, IV: HA@COF NSs, V: HA@COF NPs+L, VI: HA@COF NSs+L. For COF-survivin containing groups, 50 μL of 4 mg/mL materials were injected into the tumor bearing nude mice, respectively. 48 h later, the mice in laser irradiation groups were treated with 633 nm laser (0.2 W/cm^2) for 8 min. After that, the tumor growth and body weight change situations of the mice were recorded within 14 days. For H&E staining assay, at 7 days, the tumor tissue and the main organs of liver, heart, spleen, lung, kidney were collected for H&E staining.

4. Supplementary Figures.

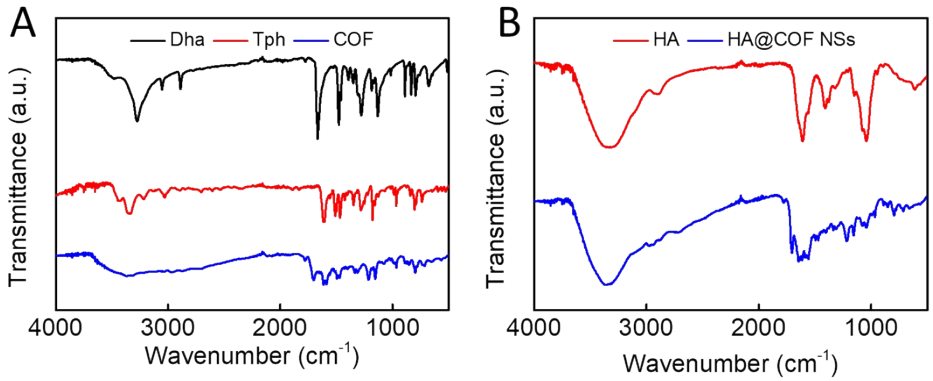

Figure S1. The FT-IR spectra of Dha, Tph, COF, HA and HA@COF NSs.

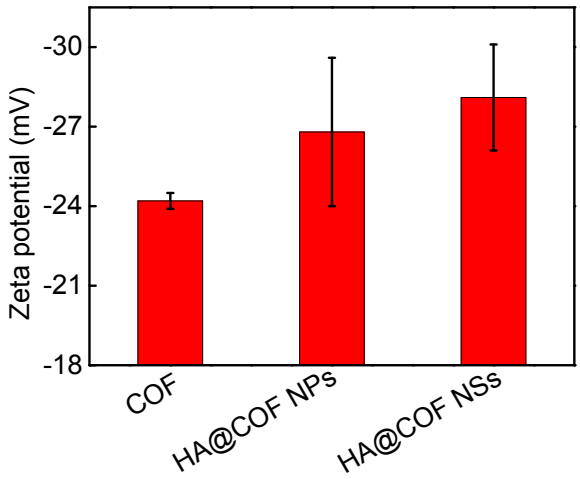

Figure S2. Zeta potential of COF, HA@COF NPs and HA@COF NSs.

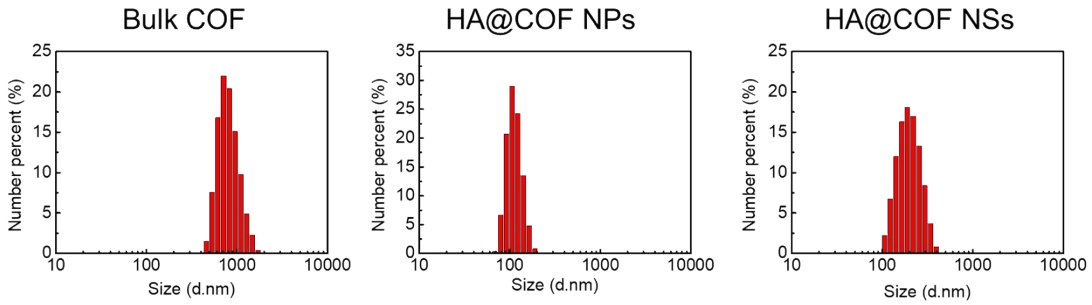

Figure S3. The DLS size distribution of Bulk COF, HA@COF NPs and HA@COF NSs.

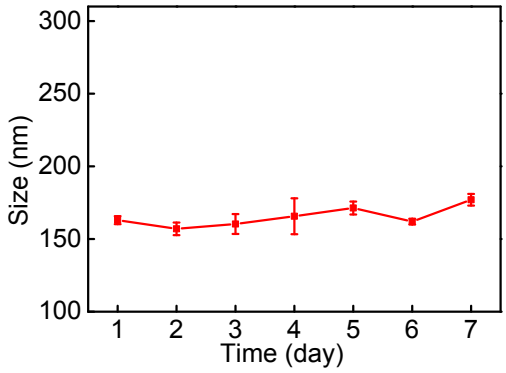

Figure S4. The average size of HA@COF NSs in aqueous solution for 7 days.

Figure S5. The digital photographs of the aqueous solutions of different materials stored at room temperature.

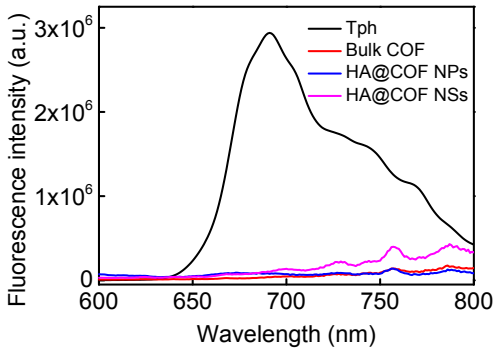

Figure S6. The fluorescence spectra of Tph, COF, HA@COF NPs and HA@COF NSs.

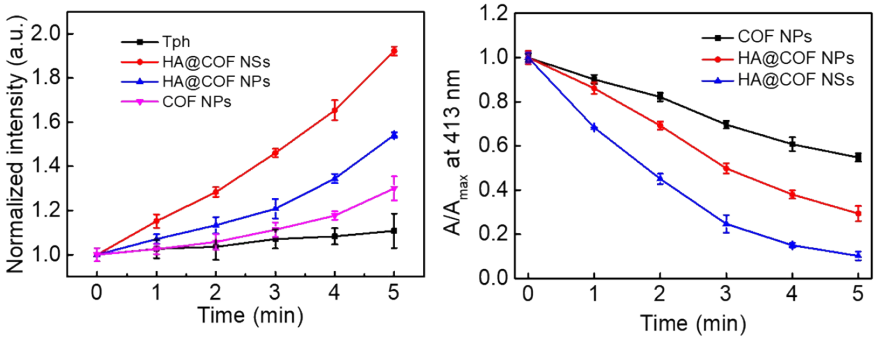

Figure S7. Left: The ROS generation effect of Tph, COF NPs, HA@COF NPs and HA@COF NSs evaluated by DCFH. The $^1O_2$ generation effect of COF NPs, HA@COF NPs and HA@COF NSs evaluated by DPBF.

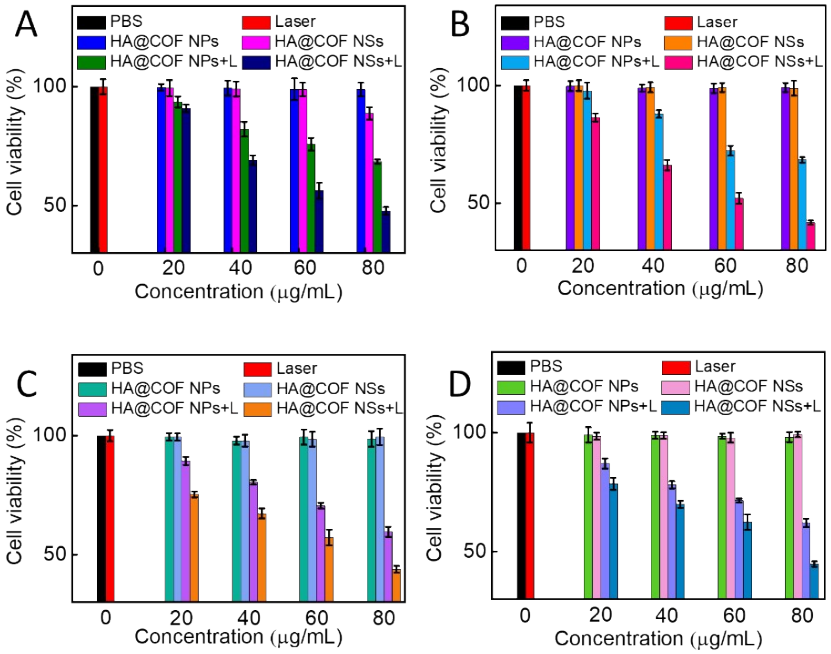

Figure S8. Evaluation of the broad-spectrum cancer cell inhibition effect of HA@COF NSs. The cell viabilities of (A) HepG2, (B) 4T1, (C) MCF-7 and (D) MCF-7/Adr cancer cells after different treatments.

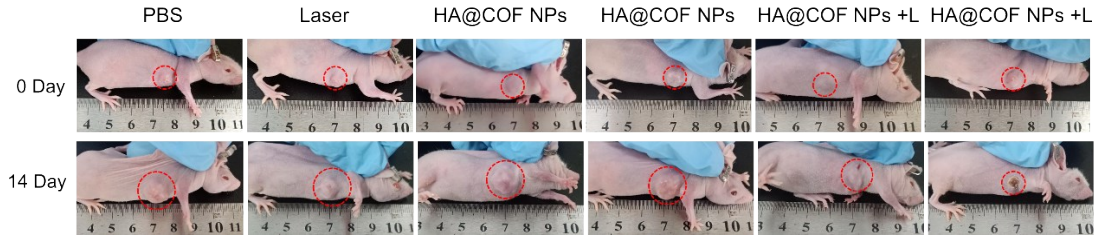

Figure S9. Digital photographs of HepG2 tumor-bearing nude mice receiving different treatments at 0 and 14th day.

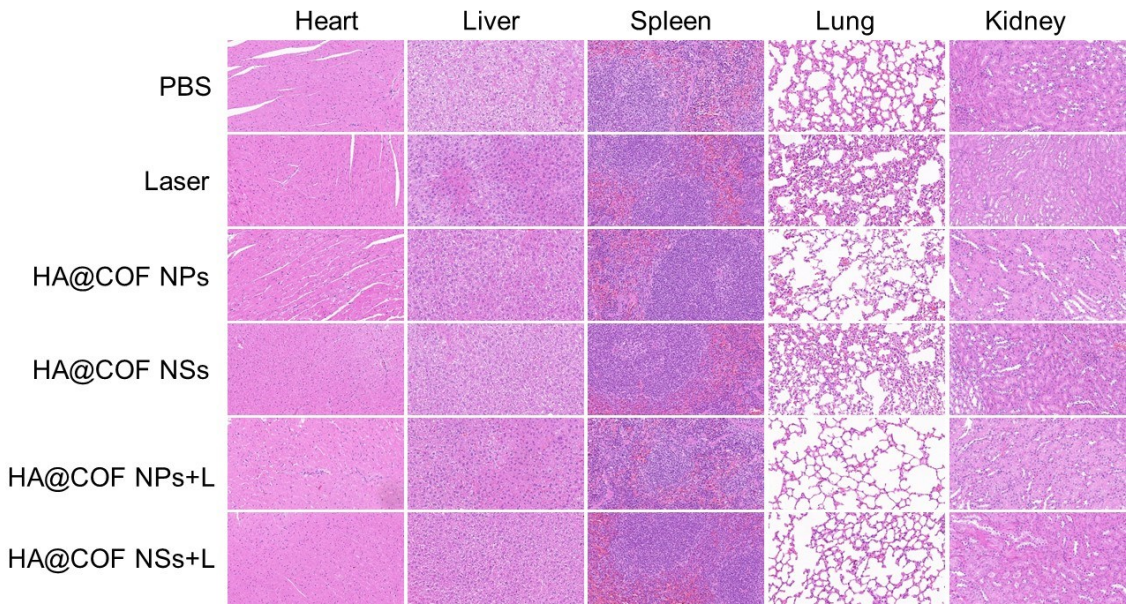

Figure S10. The H&E staining of main organs from tumor-bearing mice receiving different treatments.