Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.
This journal is © the Partner Organisations 2023

Supporting Information

Facile synthesis of 2D Al-TCPP MOF nanosheets for efficient sonodynamic cancer therapy

Zhan Zhou,[1,#] Tao Wang,[2,#] Tingting Hu,[2,#] Chunhua Cheng,[1] Shilong Yu,[3] Hai Li,[3] Shuyan Liu,[4] Lufang Ma,[1,*] Meiting Zhao,[5,*] Ruizheng Liang,[2,*] Chaoliang Tan,[6,7,*]

[1]College of Chemistry and Chemical Engineering, Henan Key Laboratory of Function-Oriented Porous Materials, Luoyang Normal University, Luoyang, 471934, China

[2]State Key Laboratory of Chemical Resource Engineering, Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

[3]Institute of Advanced Materials (IAM) and Key Laboratory of Flexible Electronics (KLoFE), Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing 211816, China

[4]Department of Endocrinology, The First Affiliated Hospital of Henan Polytechnic University (Jiaozuo Second People's Hospital), Jiaozuo, China

[5]Department of Chemistry, Institute of Molecular Aggregation Science, Tianjin Key Laboratory of Molecular Optoelectronic Sciences, Tianjin University, Tianjin 300072, China

[6]Department of Chemistry and Center of Super-Diamond and Advanced Films (COSDAF), City University of Hong Kong, Kowloon, Hong Kong SAR 999077, China

[7]Shenzhen Research Institute, City University of Hong Kong, Shenzhen, 518057, China

# These authors contributed equally to this work.

*Corresponding author. Emails: chaoltan@cityu.edu.hk; liangrz@mail.buct.edu.cn; mtzhao@tju.edu.cn; mazhuxp@126.com

Experimental section

Chemicals

Aluminum chloride hexahydrate (99.99%), 5,5-dimethyl-1-pyrroline N-oxide (DMPO, 98%), 2,2,6,6-tetramethyl-4-piperidone (TEMP), polyethylene glycol (PEG), N,N-dimethylformamide (DMF, AR), N,N-dimethylacetamide (DMAC, AR), dimethyl sulfoxide (DMSO, AR), and N-methylpyrrolidone (NMP, AR) were supplied from Aladdin Reagent Company (China). Meso-tetra(4-carboxyphenyl) porphyrin (TCPP) was purchased from Beijing Huawei Ruike Chemical Co., Ltd. Annexin V-FITC & propidium iodide apoptosis detection kits, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), calcein acetoxymethyl ester (Calcein-AM), propidium iodide (PI) and methyl thiazolyl tetrazolium (MTT) were acquired from Sigma-Aldrich and Fisher (USA). Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) apoptosis detection kit and phosphate-buffered saline (PBS) were purchased from Solarbio Science &Technology Co, Ltd (Beijing, China). Streptomycin and penicillin, ciprofloxacin (CPFX), fetal bovine serum (FBS) and 0.25% trypsin-EDTA, high glucose Dulbecco's modified Eagles medium (DMEM) were purchased from Gibco (Invitrogen, Carlsbad, CA). 5,5′,6,6′-Tetrachloro-1,1′-3,3′-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1), singlet oxygen sensor green (SOSG) and acridine orange (AO) were obtained from Beyotime Biotechnology Co., Ltd.

Characterizations

A JEOL JEM-2100F transmission electron microscope was used to take the transmission electron microscope (TEM) images. A scanning electron microscope (ZEISS) was used to record the scanning electron microscopy (SEM) images. The atomic force microscopy (AFM, Dimension ICON, Bruker) was used for taking the AFM height image of the 2D Al-TCPP nanosheets. A Bruker D8 diffractometer with a Cu Kα (λ=1.54178 Å) X-ray source was used to record the X-ray powder diffraction (XRD) patterns. X-ray photoelectron spectra (XPS) were recorded on a Thermo Scientific K-Alpha+ instrument, in which the C 1 s peak was used as the reference to calibrate all the peaks. A PerkinElmer Lambda 950 UV/VIS/NIR spectrometer was

used to measure the absorption spectra. A Bruker electron spin resonance (ESR) spectroscopy (EMX1598) was used to monitor hydroxyl radical (·OH) and singlet oxygen ($^1O_2$) in the presence of different trapping agents. The contents of Al were characterized with inductively coupled plasma mass spectroscopy (ICP-MS, Thermo). Cellular fluorescence images were performed on a Leica confocal laser scanning microscopy (CLSM) (Leica). In vitro cell apoptosis and cellular fluorescence quantity was conducted by Flow cytometer (MoFlo XDP, Beckman Coulter).

Synthesis of 2D Al-TCPP nanosheets

The 2D Al-TCPP nanosheets were synthesized by the typical solvothermal method. Briefly, aluminum chloride hexahydrate (2.4 mg) was dissolved into deionized (DI) water (3 mL) and followed by adding 0.5 mL of DMF containing TCPP (4 mg). After stirring for 20 min, the mixture was transferred into a stainless-steel reactor and heated to 160 ℃ for 12 h. The precipitate was separated by high centrifugation (12000 rpm for 10 min), and washed with DMF and DI water for several times. The 2D Al-TCPP nanosheets was obtained by drying 70 °C for 10 h. Besides DMF/H_2O (1/6, v/v), the 2D Al-TCPP nanosheets were also prepared by other solution system including DMAC/H_2O (1/6, v/v), DMSO/H_2O (1/3, v/v), and NMP/H_2O (1/3, v/v) with the same synthesis procedures except changing the organic solution.

PEG modification of 2D Al-TCPP nanosheets

10 mg of 2D Al-TCPP nanosheets were poured into DI water (10 mL) and followed by ultrasound for uniform dispersion. Subsequently, 200 mg of PEG was added into the above solution, and then stirred at room temperature for 12 h. After washing with DI water, the as-prepared 2D PEG@Al-TCPP nanosheets were re-dispersed in DI water for further use. The PEG modification of TCPP was similar with that of Al-TCPP except changing Al-TCPP to TCPP.

Detection of singlet oxygen ($^1O_2$) by SOSG

The generation of $^1O_2$ of TCPP and Al-TCPP samples were determined using SOSG. After adding SOSG (200 μL, 1 mM) and TCPP or Al-TCPP (100 μL, 1 mg mL^{-1}) into 1700 μL H_2O, the obtained solution was irradiated by US (50 KHz, 1.6 W cm^{-2} for 8 min). The increased fluorescence intensity of SOSG at 530 nm indicated the $^1O_2$

generation and the fluorescence spectrum was recorded.

Detection of reactive oxygen species (ROS) by DCFH

100 μL of Al-TCPP nanosheets (1 mg mL^{-1}) was added into 1900 μL of DCFH solution (6 mM), and the mixed solution was then irradiated by US (50 KHz, 1.6 W cm^{-2} for 8 min). The production of ROS was monitored by recording the fluorescence intensity of DCFH at 525 nm.

Detection of ROS by ESR

To detect the generation of $^1O_2$, TCPP or Al-TCPP solution (100 μL, 50 μg mL^{-1}) containing 45 μM TEMP was exposed to US irradiation (50 KHz, 1.6 W cm^{-2}) for 8 min and then detected by ESR. Moreover, blank and US groups were also measured at the same conditions. The generation of ·OH was evaluated by a similar procedure except that TEMP (45 μM) was changed to DMPO (50 mM).

In vitro studies

4T1 cells were transferred into 96-well plates (1×10^4 cells/well) for evaluating the biocompatibility of PEG@Al-TCPP nanosheets. After growing for 24 h at 37 °C, the various concentrations (10, 30, 50 and 100 μg mL^{-1}) of PEG@Al-TCPP nanosheets were added into the wells, and cultured for another 24 h for determination of cell viability using MTT assay. For in vitro cytotoxicity assays, we divided the cells into six groups: blank, US (50 KHz, 1.6 W cm^{-2}, 6 min), PEG@TCPP, PEG@Al-TCPP, PEG@TCPP + US, PEG@Al-TCPP + US. After incubated under 37 °C for 24 h, the cells were carefully rinsed three times with PBS, and MTT solution (0.5 mg mL^{-1}) was added into each well for further 4 h. The cell viability of each well was measured using MTT assay. As for the cell imaging, 4T1 cells were pre-cultured with PEG@Al-TCPP (50 μg mL^{-1}), PEG@TCPP (50 μg mL^{-1}) or blank (PBS) for 24 h, and then the cells were treated with or without the irradiation of US (50 KHz, 1.6 W cm^{-2}) for 6 min. After co-staining with Calcein-AM and PI, cells were imaged by a confocal microscope.

Generation of intracellular ROS

4T1 cells (5×10^4 cells mL^{-1}) were pre-incubated for 24 h using 6-well plate, and then washed with PBS after removing the culture medium, followed by adding PEG@TCPP

(50 μg mL^{-1}) or PEG@Al-TCPP (50 μg mL^{-1}) DMEM solution with or without the irradiation by US (50 KHz, 1.6 W cm^{-2}, 6 min) for another 6 h culture. The cells were stained with 2 mL of DCFH-DA with the concentration of 10 μg mL^{-1} for further 0.5 h. After washing with PBS twice, the fluorescence photos of cells were captured *via* CLSM.

Cell apoptosis analysis

4T1 cells (1×10^5 cells mL^{-1}) were pre-incubated for 24 h using 6-well plate, and then washed with PBS after removing the culture medium, followed by co-culture with PEG@TCPP (50 μg mL^{-1}) or PEG@Al-TCPP (50 μg mL^{-1}). After incubation for 24 h, the cells were washed with PBS and then treated with US (50 KHz, 1.6 W cm^{-2}) irradiation for 6 min. After the collection of cells, we used Annexin V-FITC and PI Apoptosis Detection Kit to co-stain cells and measured the different fluorescent channels of cells by flow cytometry.

Testing mitochondrial membrane potential

4T1 cells (5×10^4 cells/mL) were pre-cultured using CLSM-exclusive culture disks for one day. After different treatments ((1) blank (PBS), (2) US (50 KHz, 1.6 W cm^{-2}, 6 min), (3) PEG@TCPP (50 μg mL^{-1}), (4) PEG@Al-TCPP (50 μg mL^{-1}), (5) PEG@TCPP + US, (6) PEG@Al-TCPP + US), we used PBS and JC-1 (10 μg mL^{-1}) to wash cells twice and stain cells for 20 min, respectively. Finally, the photos of cells were captured *via* CLSM.

Lysosome disruption

4T1 cells (5×10^4 cells/mL) were pre-cultured using CLSM-exclusive culture disks for one day. The cells were exposed to the following treatments: (1) blank, (2) US (50 KHz, 1.6 W cm^{-2}, 6 min), (3) TCPP-PEG (50 μg mL^{-1}), (4) PEG@Al-TCPP (50 μg mL^{-1}), (5) PEG@TCPP (50 μg mL^{-1}) + US, (6) PEG@Al-TCPP (50 μg mL^{-1}) + US. After staining with AO (5 μM) for 0.5 h, the fluorescence photos of cells were captured by a CLSM using 488 nm laser as the excitation.

Animal experiments

All animal experiments were monitored and approved by Animal Care and Use Committee of China-Japan Friendship Hospital. 3-5 weeks old mice (female Balb/c-

nude mice with ~20 g body weight) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. 100 μL of 4T1 cells dispersion (1×10^7 cells) was subcutaneously injected into the right hind legs of mice. When the tumor volume reaches about 80 mm^3, we will start the following experiment in vivo.

Pharmacokinetic and biodistribution analysis

After intravenous injection of PEG@Al-TCPP for certain time, 10 μL of blood was extracted from the tail veins of mice. We used ICP-MS to monitor the content of Al^{3+} in the blood of mice. As for the biodistribution of PEG@Al-TCPP nanosheets, 200 μL of PEG@Al-TCPP (1 mg mL^{-1}) was intravenously injected to mice, and then mice were sacrificed at different times (4, 8, 12, 24 and 48 h) after injection. The tumor and main organs (heart, liver, spleen, lung, and kidney) of mice were extracted and fully digested using concentrated nitric acid. Finally, the content of Al^{3+} in the tissues was monitored by ICP-MS.

In vivo antitumor therapy

Mice were randomly divided into six groups (six mice per group): (1) PBS only (blank), (2) US (50 KHz, 1.6 W cm^{-2}) only, (3) PEG@TCPP only, (4) PEG@Al-TCPP only, (5) PEG@TCPP + US, and (6) PEG@Al-TCPP + US. The 4T1 tumor-bearing nude mice in group (1) and (2) were intravenously injected 200 μL PBS. Mice in group (3) and (5) were intravenously injected 200 μL PEG@TCPP (1 mg mL^{-1}). Group (4) and (6)'s mice received 200 μL PEG@Al-TCPP (1 mg mL^{-1}) injection. Next, the US (50 KHz, 1.6 W cm^{-2}) irradiation was performed on mice in group (5) and (6) for 6 min. Tumor volume and weight were measured every 2 days up to 16 days. The tumor volume was determined as $volume = 0.5 \times (tumor\ length) \times (tumor\ width)^2$. Relative tumor volume was defined as V/V_0 and measured on day t and day 0, respectively.

Histology examination and blood analysis

After 16 days of treatment, the mice were sacrificed, and tumors and major organs (heart, liver, spleen, lungs, kidneys) of mice were collected and immersed into 4% paraformaldehyde solution. All the tissues were embedded in paraffin, and then cut into thin slices by a cryomicrotome, and these slices were stained with hematoxylin and eosin (H&E). Moreover, the tumor slices were also stained by the anti-Ki-67 and

TUNEL, respectively. After intravenous injection of PBS and PEG@Al-TCPP for certain time (day 1 and day 16), blood of mice was extracted and analyzed for various indicators.

Statistical analysis

The significance of all data was analyzed using one-way analysis of variance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

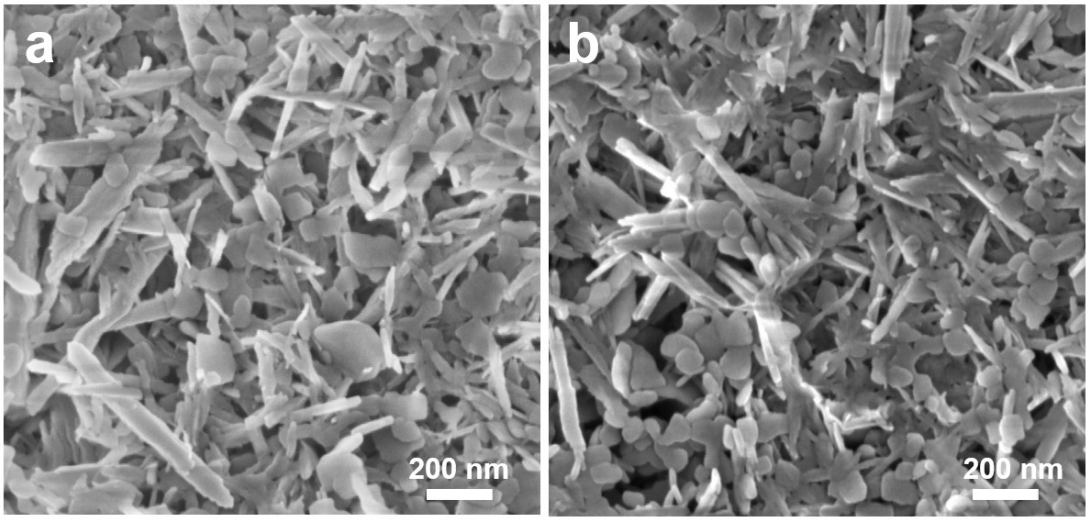

Figure S1. The SEM images of Al-TCPP MOFs synthesized by using different volumes DMF (a, 1 mL; b, 0.8 mL).

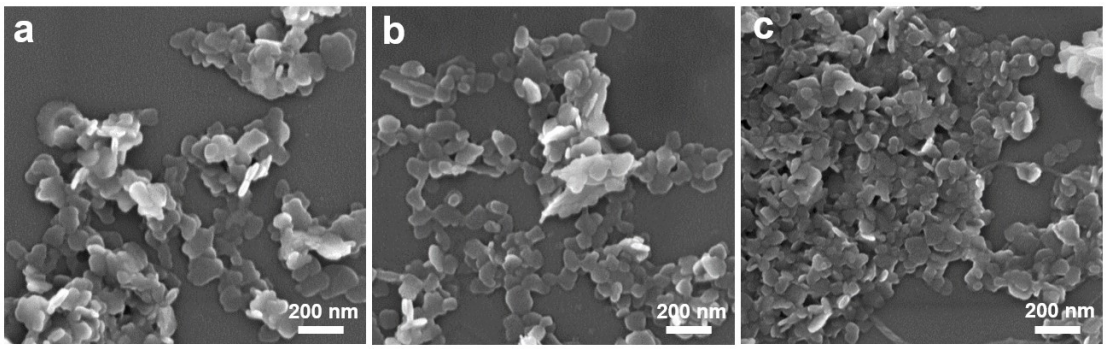

Figure S2. The SEM images of Al-TCPP nanosheets synthesized by using different organic solvents (a, DMAC/0.5 mL; b, DMSO/1 mL; c, NMP/1 mL).

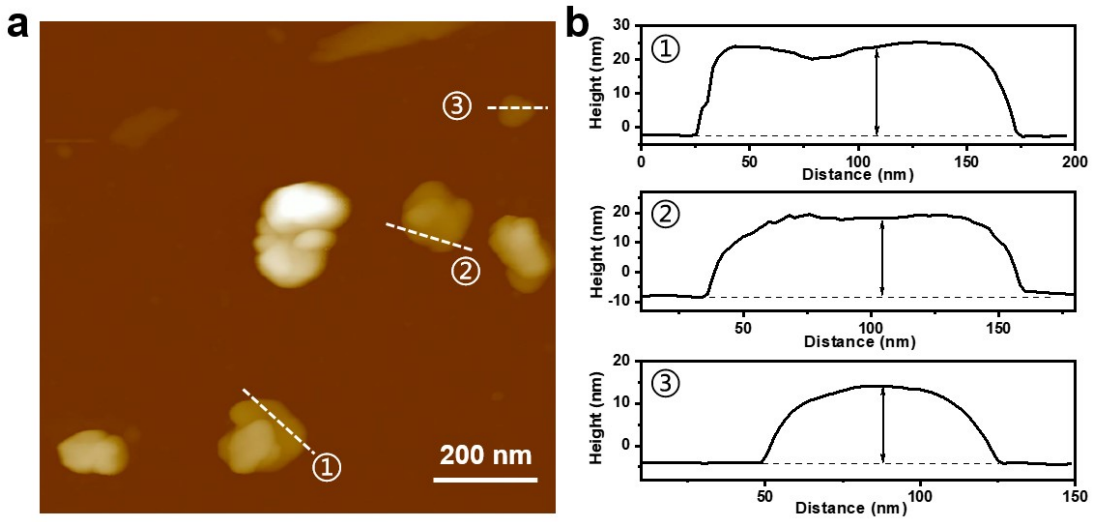

Figure S3. (a) AFM height image of 2D Al-TCPP nanosheets, and (b) its height profiles measured from 3 nanosheets.

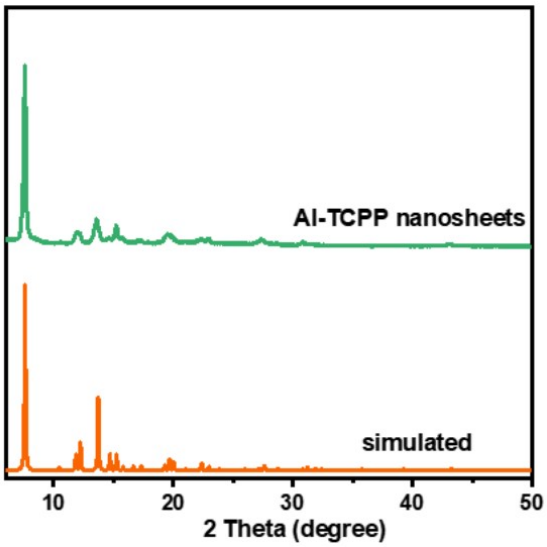

Figure S4. XRD patterns of Al-TCPP nanosheets.

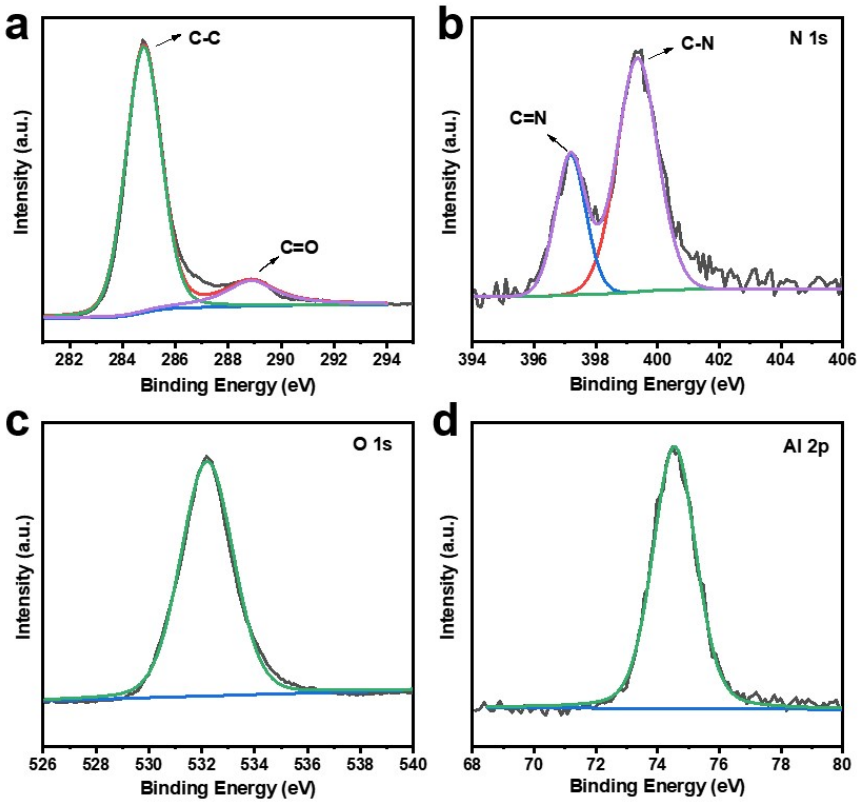

Figure S5. The high-resolution XPS spectra of the (a) C 1s, (b) N 1s, (c) O 1s, and (d) Al 2p.

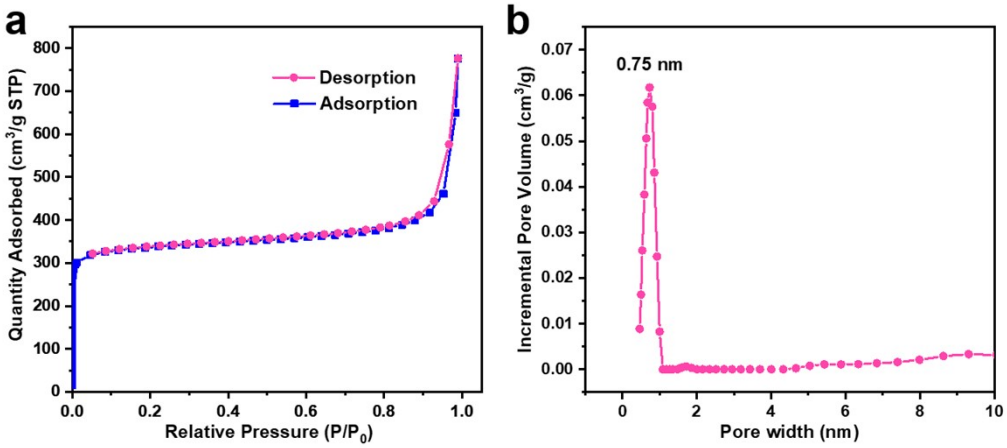

Figure S6. (a) N_2 adsorption-desorption isotherm and (b) pore size distribution of 2D Al-TCPP nanosheets.

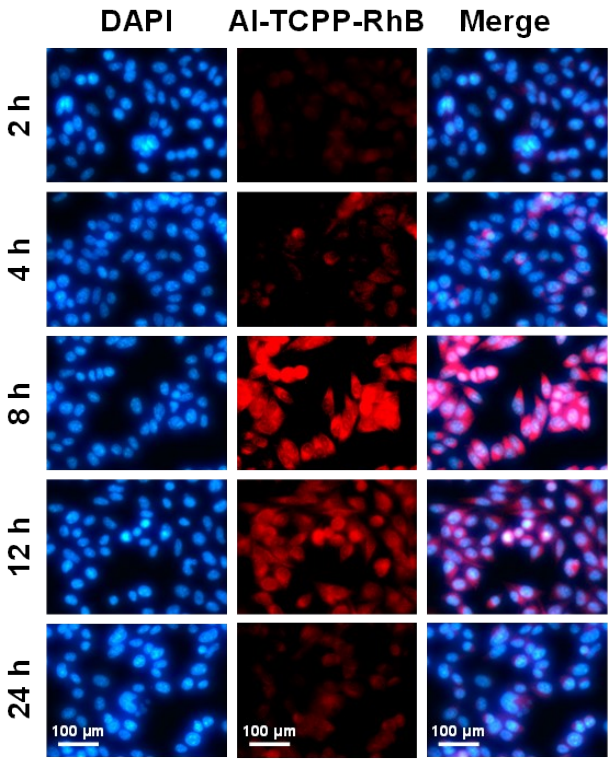

Figure S7. Fluorescence images of 4T1 cells treated with the RhB-labeled PEG@Al-TCPP at 2, 4, 8, 12, 24 h.

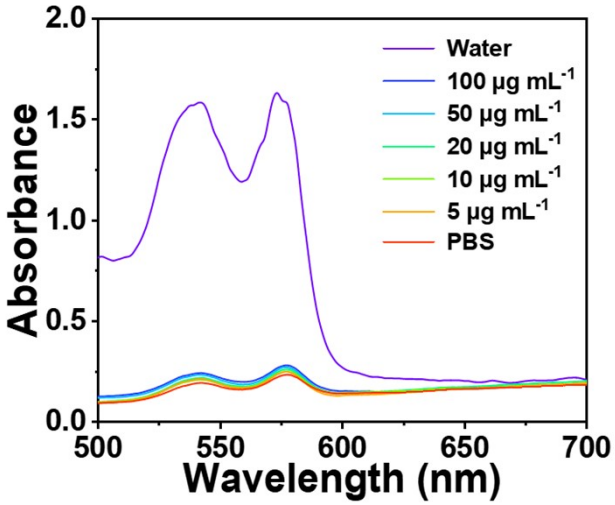

Figure S8. The absorption of the RBCs supernatant after being treated by different concentrations of the PEG@Al-TCPP, H_2O and PBS.

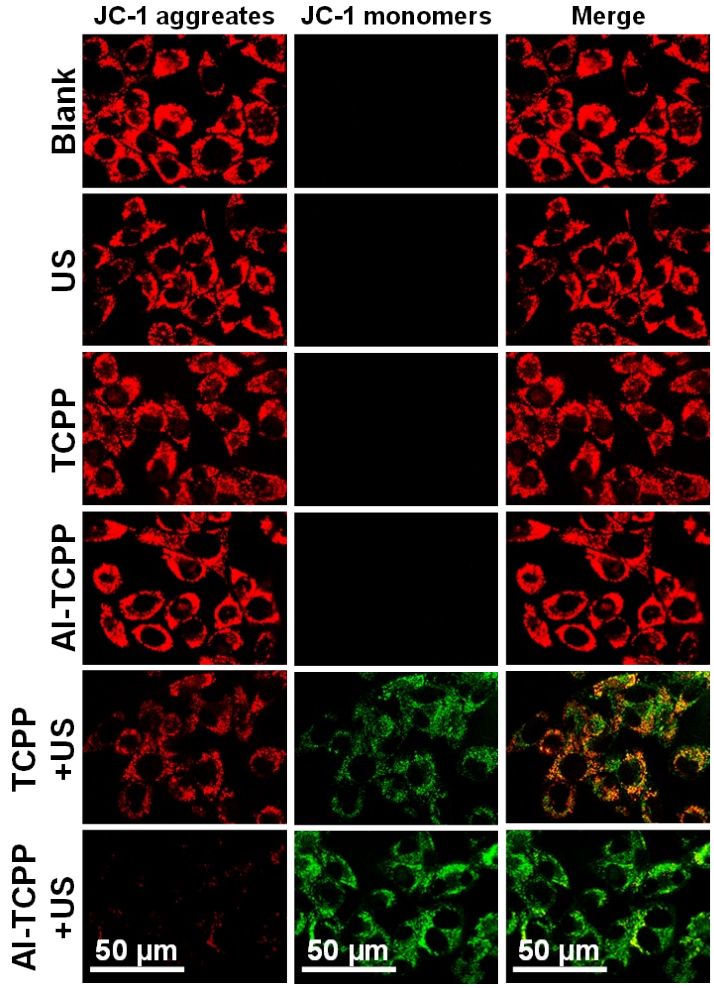

Figure S9. CLSM images of JC-1 staining for mitochondrial membrane potential after different treatments.

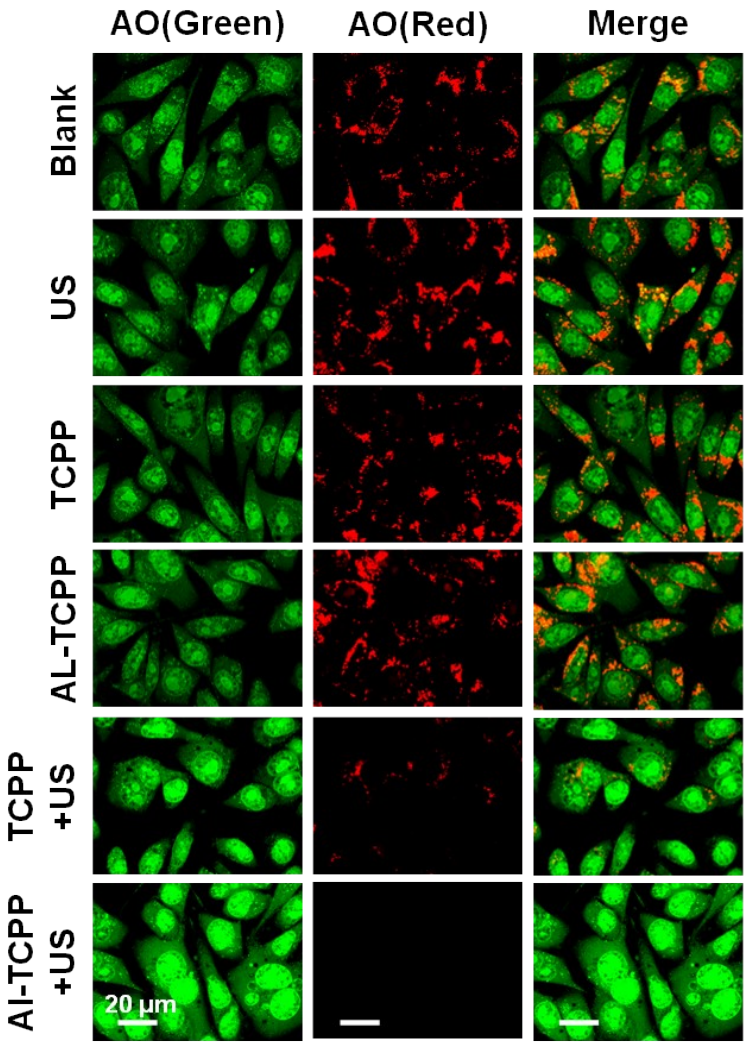

Figure S10. Cell images of AO staining for lysosomal integrity with different treatments.

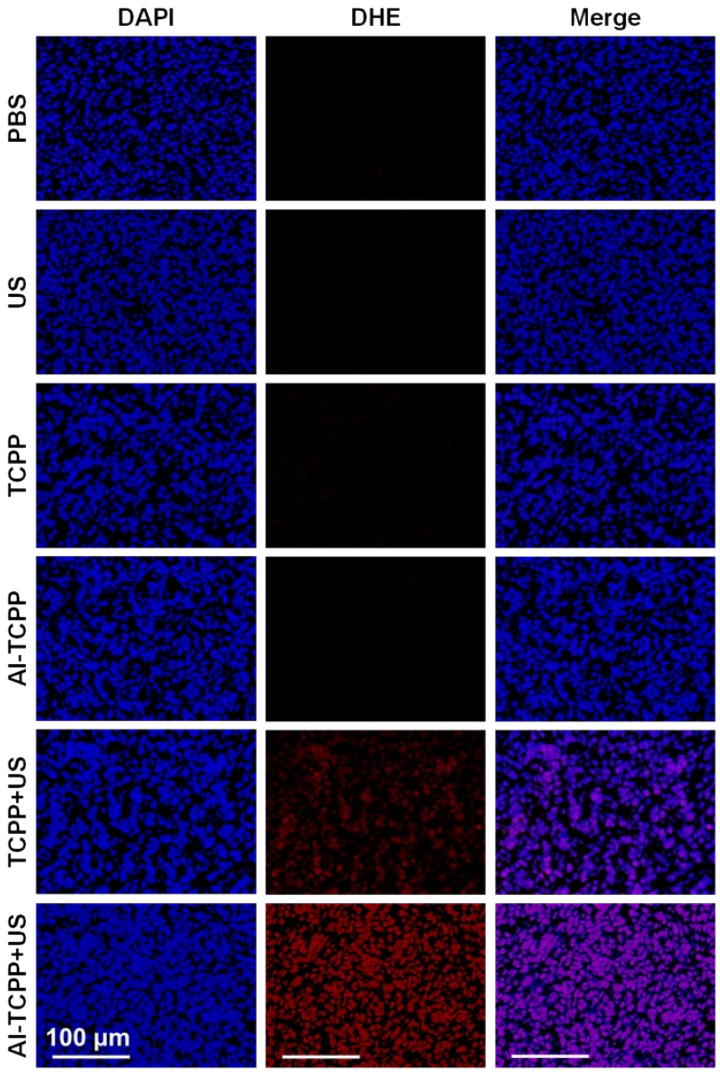

Figure S11. DHE staining assay of tumor tissue slices after different treatments at day 16.

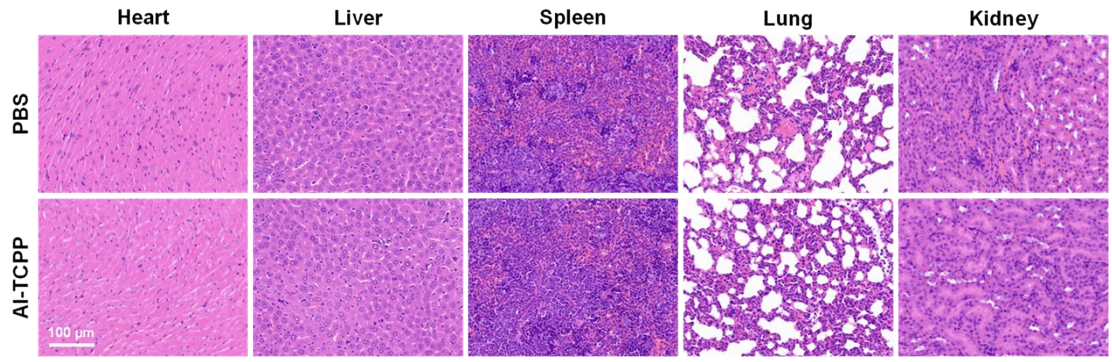

Figure S12. Histological images of major organs collected on Day 16 treated with PBS and PEG@Al-TCPP, respectively.

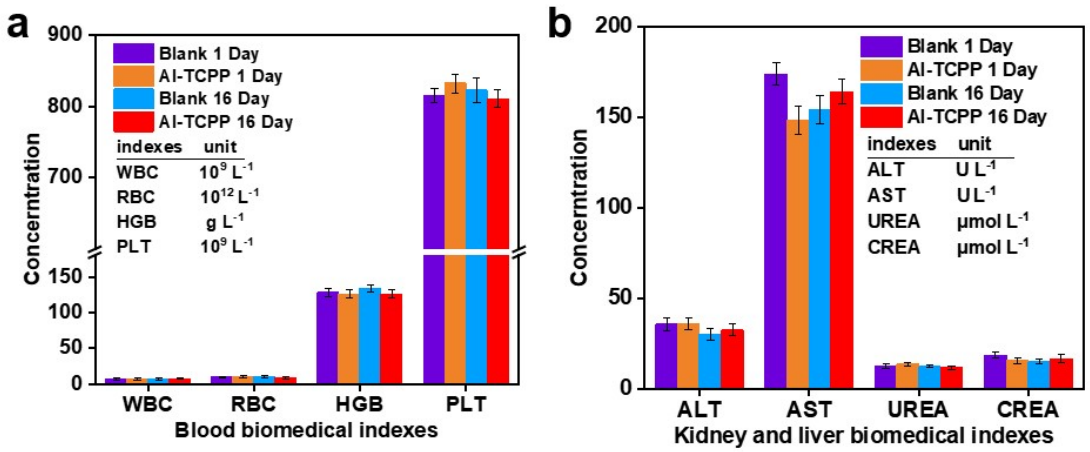

Figure S13. (a) Blood, (b) kidney and liver biochemical indexes of nude mice bearing 4T1 tumors detected after the injection of PBS (blank) and PEG@Al-TCPP at Day 1 and Day 16. Error bars stand for ± s.d. (n = 3).

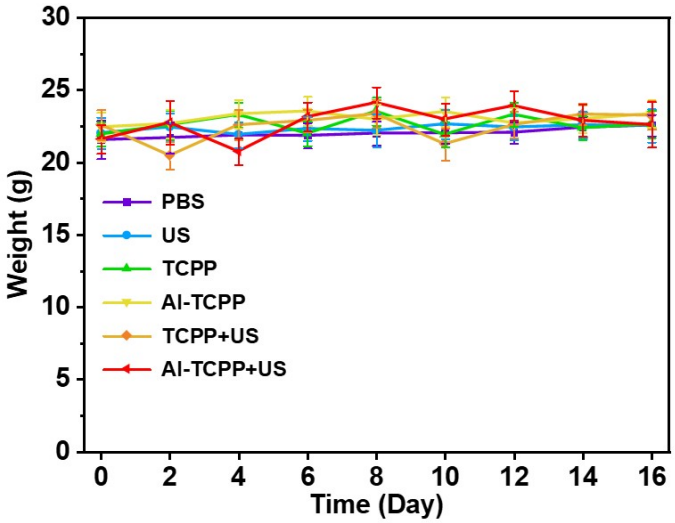

Figure S14. Bodyweight change of 4T1 tumor-bearing mice after various treatments as a function of time.

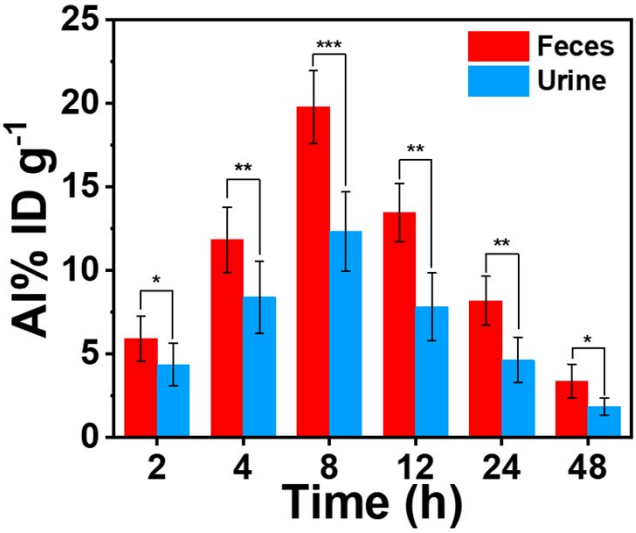

Figure S15. Excretion of the PEG@Al-TCPP nanosheets quantified by Al concentration (*p < 0.05, **p < 0.01, ***p < 0.001).