Supporting Information

Au-Based Conjugated Microporous Polymers for Combined Photodynamic and Radiation Therapy in Cancer Treatment

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Materials and General Methods

^{1}H-NMR spectra were recorded on Bruker BioSpin (400 MHz) spectrometer, and chemical shifts were reported as the delta scale in ppm relative to $CDCl_3$ (δ = 7.26 ppm). High resolution mass spectrometry (HR-MS) analyses were carried out using MALDI-TOF-MS techniques. XPS experiments were performed on an ESCALAB 250 (Thermo-VG Scientific). UV-vis absorption spectra were recorded on a DUV-3700 spectrophotometer (Shimadzu). Transmission electron microscopy (TEM) was conducted on a JEM-ARM 200F Atomic Resolution Analytical Microscope operating at an accelerating voltage of 200 kV. Dynamic light scattering (DLS) spectra were recorded on a Brookhaven Omni particle size analyzer. All chemicals were obtained from commercial suppliers (Innochem or Acros) and used without further purification. Air-sensitive reactions were all carried out under nitrogen or argon.

Synthesis of meso-Tetra (p-bromophenyl) porphine (P)

To a 100-mL flask containing a magnetic stirring bar were added 4-bromobenzaldehyde (1.48 g, 8 mmol) and propionic acid (20 mL). The mixture was heated to reflux with stirring, the freshly distilled pyrrole (0.54g, 8 mmol) was added dropwise into the above refluxing mixture. The reaction mixture was refluxed for approximately 1.5 h. The solution quickly changed color from yellow to black. After the reaction was stopped, the solution was cooled at -20 ℃ and left overnight to allow the precipitation of the meso-Tetra (p-bromophenyl) porphine (**P**). After filtration, the reaction solid was washed with methanol and dried under vacuum to afford **P** as purple solid (0.5 g, 27%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (s, 8H), 7.99 (d, J = 8.3 Hz, 8H), 7.83 (d, J = 8.3 Hz, 8H), -2.94 (s, 2H). IR (KBr): ν = 3321, 2988, 2900, 1523, 1474, 1391, 1349, 1070 ($^{\delta}$C-Br), 1012, 967 ($^{\delta}$N-H), 799, 729, and 702 cm^{-1}.

Synthesis of Au coordinated porphyrin (Au-P)

$K(AuCl_4)$ (406 mg, 1.1 mmol) and NaOAc (225 mg, 2.7 mmol) were added to a mixture of meso-Tetra (p-bromophenyl) porphine (250 mg, 0.27 mmol) and HOAc (20 mL). After refluxing with stirring for 5 h, deionized water (20 mL) was added to quench the reaction and the mixture was extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated with rotary evaporator. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 10/1) to afford **Au-P** as yellow solid (257 mg, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.26 (s, 8H), 8.18 (d, J = 8.2 Hz, 8H), 7.99 (d, J = 7.6 Hz, 8H). MALDI-TOF-MS (m/z), Calc. for $C_{44}H_{24}Br_4N_4Au$: 1124.8359; found: 1124.8362. IR (KBr): ν = 2988, 2900, 1583, 1482, 1395, 1232, 1074 ($^{\delta}$C-Br), 1026 ($^{\delta}$Au-N), 1010, 803, 720, and 704 cm^{-1}.

Synthesis of Au-P conjugated microporous polymers (Au-CMP)

1,5-Cyclooctadiene (39 mg, 0.36 mmol) was added to a DMF solution (10 mL) of bis(1,5-cyclooctadiene)nickel(0) (100 mg, 0.36 mmol) and 2,2'-bipyridyl (57 mg, 0.36 mmol) under argon atmosphere and the mixture was heated at 80 °C for 1 h. Au-P (101 mg, 0.09 mmol) was added to the mixture and stirred at 80 °C for 72 h to give a deep

purple suspension. After cooling to RT, acetic acid (10 mL) was added and the reaction mixture was stirred at RT for 10 h. After filtration, the solid was washed with $CHCl_3$ (30 mL × 5), THF (30 mL × 5), and water (30 mL × 5), followed by rigorous washing by Soxhlet extraction for 24 h with THF and $CHCl_3$, sequentially, and dried under vacuum to give **Au-CMP** as a brown powder (57 mg, 76% yield). IR (KBr): ν = 2988, 2900, 1600, 1444, 1394, 1026 ($^{\delta}$Au-N), 1026, 1006, and 880 cm^{-1}.

Synthesis of Au-CMP nanoparticles (Au-CMP NPs)

Au-CMP was loaded into nanoparticles composed of DSPE-PEG using a nanoprecipitation method. Briefly, 1 mL of Au-CMP chloroform solution was ultrasonic mixed with 20 mL chloroform containing 5 mg DSPE-PEG and stirred overnight at room temperature. After slow evaporation of solvent under low-pressure, a dry film of nanoparticles was obtained and then dispersed into distilled (DI) water. To remove the excess polymer, the solution was washed with DI water repeatedly, obtaining purified **Au-CMP NPs** sample which was re-dispersed in DI water.

Photodynamic performance of Au-CMP NPs

The generated reactive oxygen species (ROS) were measured indirectly using the DPBF chemical probe, whose absorption peak decreases in the presence of ROS. The mixture solution of Au-CMP NPs (20 μg/mL, 1 mL) and DPBF (37 mM, 1 μL) was irradiated by a 633 nm laser at a power intensity of 1 W/cm^2. A UV-vis spectrophotometer (Varian Inc., Palo Alto, CA, USA) was employed to record the absorbance (300 to 600 nm) and absorption peak of DPBF at 410 nm every 5 minutes.

Cell Culture

BEL-7402 cells (Human Hepatocellular Carcinoma Cells) were acquired from the cell bank of the Chinese Academy of Sciences and cultured in a humidified environment with 5% CO_2 at 37 °C. BEL-7402 cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

The cytotoxicity of Au-CMP NPs

To assess the potential cytotoxicity of Au-CMP NPs, cell viability was determined by CCK-8 assay. Exponentially growing BEL-7402 cells were seeded in 96-well cell culture plates at a density of 1×10^4 cells/well and cultured overnight until they reached about 70% confluence. The cells were then co-incubated with varying concentrations of Au-CMP NPs for 24 or 48 hours. After incubation, the medium containing Au-CMP NPs was aspirated, and the cells were washed three times with sterile PBS. 100 μL RPMI-1640 with 10% CCK-8 reagent was added to every well, and the incubation was continued for 2 h at 37 ℃ following the manufacturer's protocol. Then the absorbance at 450 nm characteristic peak was measured using a microplate reader. The experiment was conducted in triplicate.

For *in vitro* PDT and/or RT, BEL-7402 cells were seeded into 96-well plates. After overnight growth, the cells were co-incubated with or without Au-CMP NPs for 24 h. Thereafter, these cells were irradiated with or without 633 nm laser or X-ray (4Gy).

After another 12 h, the CCK-8 assay was conducted to assess the relative cell viability of each group.

Cellular Uptake Assays

Cellular uptake of Au-CMP NPs was evaluated using flow cytometry, confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) imaging. To quantify internalization, a red fluorescent probe-Cy7 was used to label Au-CMP NPs. Meanwhile, 2×10^5 BEL-7402 cells were seeded into CLSM culture dishes and cultured for 24 h. then 2 mL of fresh medium alone or freshly prepared Cy7-labeled Au-CMP NPs in medium (20 mg/mL) was added and co-incubated with BEL-7402 cells for an additional 24 h. The cells were then imaged and analyzed using confocal laser scanning microscopy and flow cytometry to assess the internalization capability of Au-CMP NPs qualitatively and quantitatively, respectively. To further analyze the intracellular distribution of Au-CMP NPs, transmission electron microscopy (TEM) was also performed on BEL-7402 cells co-incubated with Au-CMP NPs. Briefly, BEL-7402 cells were cultured in a 100 mm^2 cell culture dish and incubated for 24h, after which the growth medium was replaced with fresh medium containing Au-CMP NPs. After 24 h, the cells were digested with trypsin, collected via centrifugation, and fixed using 2.5% glutaraldehyde. The precipitate was subjected to a series of rinsing, pre-embedding, post-fixation, and dehydration processes before being embedded in resin. Finally, ultrathin sections of BEL-7402 cells were prepared, which were then placed on copper grids, and doubly stained. The TEM imaging of the sample was then observed and captured using a bio-TEM (HT7800 Hitachi, Japan).

Detection of Reactive Oxygen Species (ROS) *In Vitro*

BEL-7402 cells were seeded in four-chambered dishes with glass bottoms for 24 h and then co-incubated with or without Au-CMP NPs for a further 24 h. The cells were divided into eight groups: (a) control, (b) Au-CMP NPs, (c) NIR-only, (d) Au-CMP NPs + NIR, (e) X-ray only, (f) Au-CMP NPs + X-ray, (g) NIR + X-ray, (h) Au-CMP NPs + NIR + X-ray. Afterward, the cells in groups (c), (d), (g), and (h) were exposed to 633 nm NIR while those in groups (e), (f), (g), and (h) were subjected to X-ray irradiation. Subsequently, the cells were treated with 10 μM of DCFH-DA and incubated for 30 minutes at 37 °C in a cell culture incubator, protected from light. The cells were rinsed with serum-free culture medium twice to completely eliminate leftover H_2DCFDA. Finally, intracellular reactive oxygen species (ROS) production was analyzed and fluorescence images were captured using confocal laser scanning microscopy (TCS SP8 STED 3X; Leica; Germany).

JC-1 Assay

BEL-7402 cells were maintained in four-chambered dishes with glass bottoms for 24 h and divided into various groups. The cells were then administered with their respective treatments, as described earlier. Subsequently, 250 μL of JC-1 working solution was added to each chamber, mixed thoroughly, and incubated for 20 min in a cell culture incubator. Finally, the cells were washed twice with JC-1 Buffer (1 ×), and the

fluorescence images were captured using a confocal laser scanning microscopy (TCS SP8 STED 3X; Leica; Germany).

DNA breakage detection

BEL-7402 cells were plated in four-chambered dishes with glass bottoms for 24 h and incubated with or without Au-CMP NPs for another 24 h. Next, the cells were treated with or without NIR laser (633 nm, 1.0 W/cm^2) or X-ray (4Gy). After treatment for 1 h, the cells were fixed with 4% glutaraldehyde and permeabilized with 1% Triton X-100. Following this, a blocking buffer consisting of 2% BSA in PBS was added to avoid nonspecific protein adsorption. Subsequently, the cells were incubated with Anti-Human Phospho-H2AX (S139) Alexa Fluor 488 mouse mAb (eBioscience, USA) at 1:200 dilution in 0.1% BSA. overnight at 4 °C. Before staining the nuclei with DAPI, the cells were washed three times with PBS to remove excess antibodies. the formation of γ-H_2AX foci, indicative of DNA double-strand breaks, was observed and captured using a confocal laser scanning microscope (CLSM) (TCS SP8 STED 3X, Leica, Germany).

Colony Formation Assay

BEL-7402 cells were seeded in 6-well plates for 24 h at a density of 1.2 × 10^3 cells per well and incubated with or without Au-CMP NPs (20 μg/mL) for an additional 24 h. Next, the cells were treated with or without NIR laser (633 nm, 1.0 W/cm^2) or X-ray (4Gy) based on the grouping. After different treatment, the cells were rinsed with PBS and then cultured in fresh medium for 10 to 14 days to form colonies. The cell culture medium was replaced every 3 days throughout the growth of colonies. Finally, the cells were fixed with 4% paraformaldehyde followed by staining with crystal violet.

Live-Dead Assay

BEL-7402 cells were seeded in 48-well plates (1 × 10^4 cells/well) and allowed to attach for 24 h, followed by incubation with or without Au-CMP NPs (20 μg/mL) for additional 24 h. Subsequently, the cells were exposed to a 633 nm NIR laser (1.0 W/cm^2, 5 min) and/or X-ray irradiation (4 Gy). After 24 h incubation, the cells were stained with calcein-AM and propidium iodide (PI) in accordance with the manufacturer's instructions. Live (green) and dead (red) cells were differentiated using a confocal laser scanning microscope (CLSM) (TCS SP8 STED 3X; Leica; Germany).

Apoptosis Assay

BEL-7402 cells were cultured in 6-well plates for 24 h, followed by the addition of medium or Au-CMP NPs (20 μg/mL). After 24 h co-incubation, the cells were treated with or without 633 nm NIR laser (1.0 W/cm^2, 5 min) or X-ray irradiation (4 Gy). 24h after treatment, the cells were harvested using EDTA-free trypsin, collected via centrifugation, washed twice with PBS, and stained using an Annexin V-FITC/PI apoptosis detection kit. Finally, flow cytometry was performed to analyze the cell apoptosis and necrosis in each group.

Hemolysis

Fresh blood from BALB/c nude mice was used for the hemolysis experiment. Firstly, 1 mL of fresh blood was centrifuged at 2000 rpm/min for 10 min to collect erythrocytes and washed with PBS until the supernatant was colorless, and then it was diluted ten-fold with PBS. Then, 200 μL of erythrocyte dilution was added to 800 μL of PBS solution containing different concentrations of Au-CMP NPs for a 2 h-incubation. The same volume of ultrapure water was used as a positive control, and PBS served as a negative control. Finally, the solution was centrifuged at 9000 rpm/min for 5 min, the absorbance of the supernatant at 540 nm was measured and the hemolysis rate was calculated accordingly.

In Vivo PDT−RT Synergistic Therapy

All animal experimental procedures were performed according to the Guidelines for Care and Use of Laboratory Animals of Shanghai Jiao Tong University and approved by the Animal Ethics Committee of Shanghai Jiao Tong University. BEL-7402 cells (1×10^6) suspended in 100 μL of PBS were injected subcutaneously into the right hind limb of each BALB/c female nude mouse. When the tumor volume reached approximately 80 mm^3, these mice were randomly assigned to the following six groups with 5 mice per group: (a) control, (b) Au-CMP NPs, (c) Au-CMP NPs + NIR, (d) X-ray only, (e) Au-CMP NPs + X-ray, (f) Au-CMP NPs + NIR + X-ray and received various treatments. The mice in each group were injected intravenously with PBS (100 μL) or Au-CMP NPs (4 mg/mL, 100 μL) *via* tail vein. 12 h post-injection, the tumor sites of mice from groups (c), (f) were irradiated with a 633 nm NIR laser (0.75 W/cm^2) for 5 min, while mice in groups (d), (e), and (f) were exposed to X-ray radiation (6 Gy). During X-ray radiation, other parts of the whole body were shielded with a Pb cover. After the above treatments, the body weight and tumor volume of the mice were recorded every other day. Tumor volume was calculated using the formula $V=ab^2/2$. where a, b represented the longest and shortest diameter of the tumor, respectively. All mice were sacrificed 15 days after treatment, and the tumor tissue and major organs of each mouse were collected for further histological analysis.

Blood Hematology and Biochemistry Analyses

Blood samples were obtained using a standard retro-orbital vein plexus blood collection technique. Approximately 100 μL of the blood samples were placed into a potassium EDTA collection tube for hematology analysis. The remaining blood samples were allowed to rest in 1.5 mL EP tubes at room temperature for 3 h. After centrifugation at 2000 × g for 5 min, the serum was separated for biochemical analysis. All the tests were performed by Wuhan servicebio Biotechnology Co.

Statistics

For all *in vitro* and *in vivo* experiments, data were collected from at least three independent parallel experiments and presented as the mean ± SD. Statistical significance was assessed using a two-tailed Student's t-test using GraphPad Prism software, version 8.0 (San Diego, CA, USA), with P values less than 0.05 considered

statistically significant.

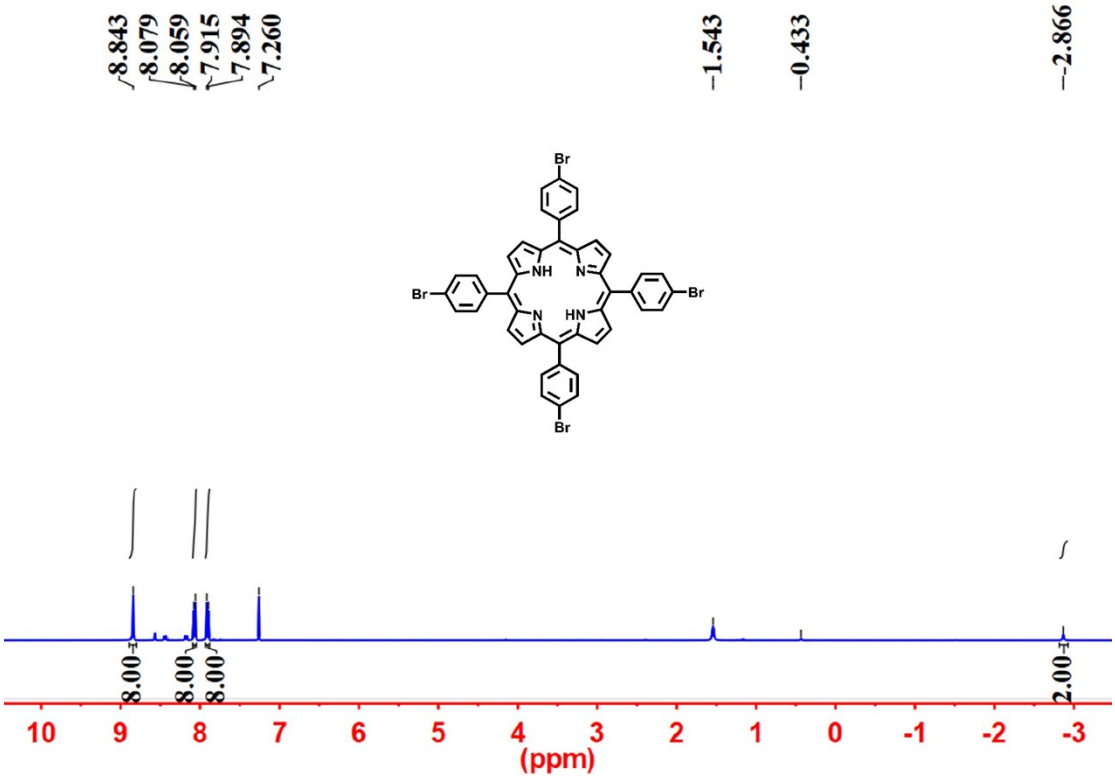

Figure S1. ^{1}H-NMR spectrum of meso-Tetra (p-bromophenyl) porphine (**P**) in $CDCl_3$.

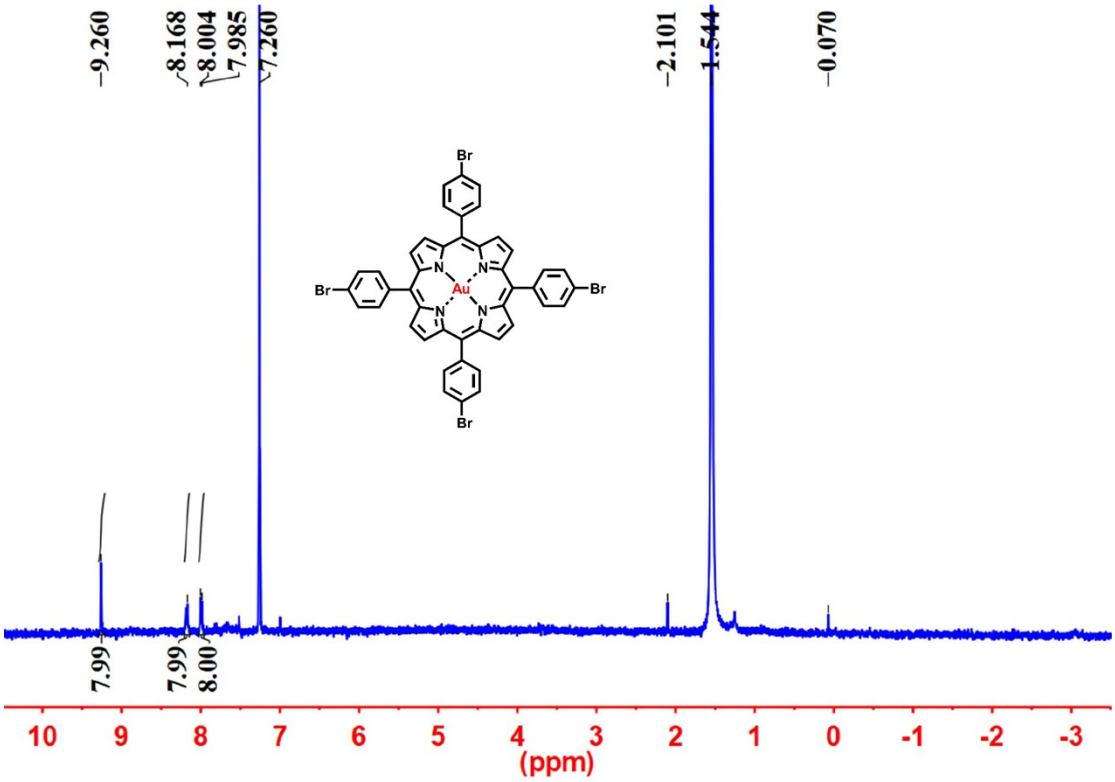

Figure S2. ^{1}H-NMR spectrum of Au coordinated porphyrin (**Au-P**) in $CDCl_3$.

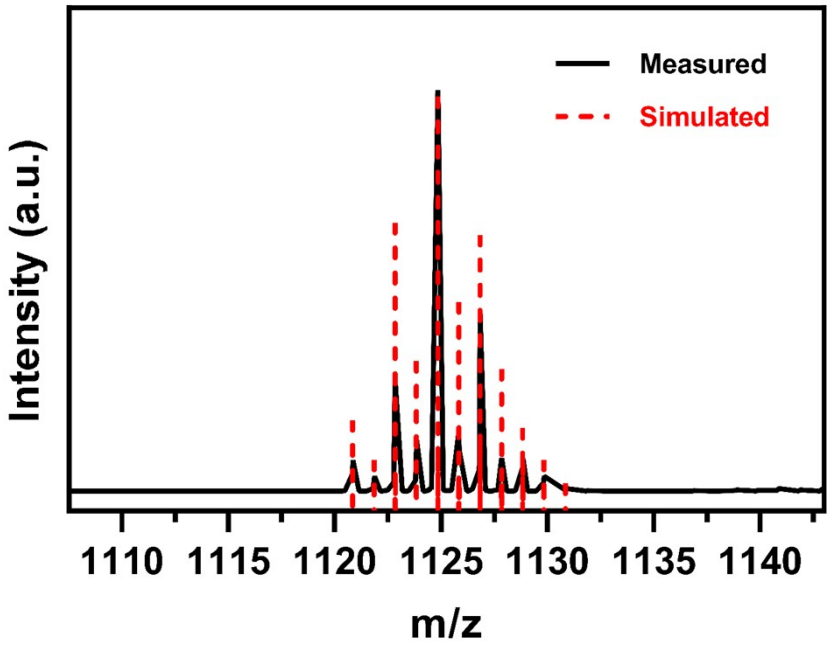

Figure S3. MALDI-TOF-MS and simulated data for Au coordinated porphyrin (**Au-P**).

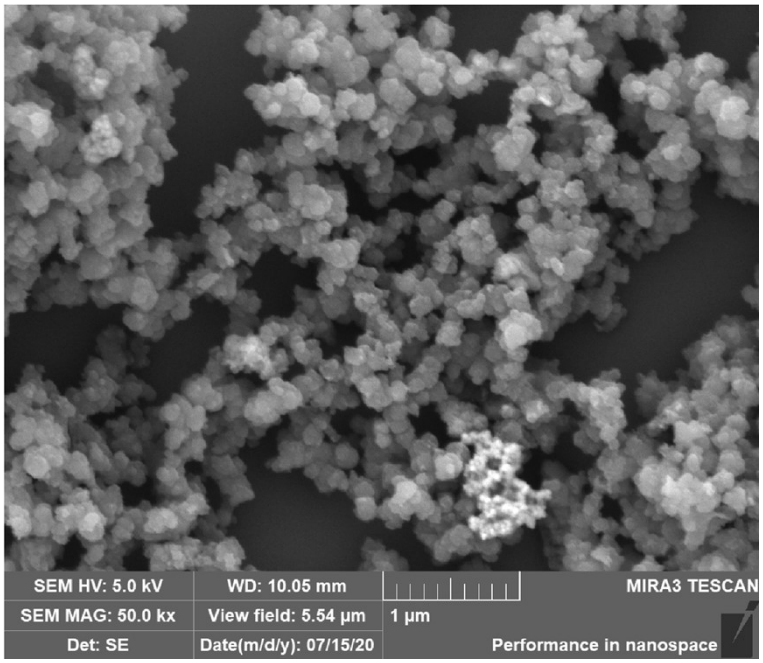

Figure S4. SEM image of Au-CMP.

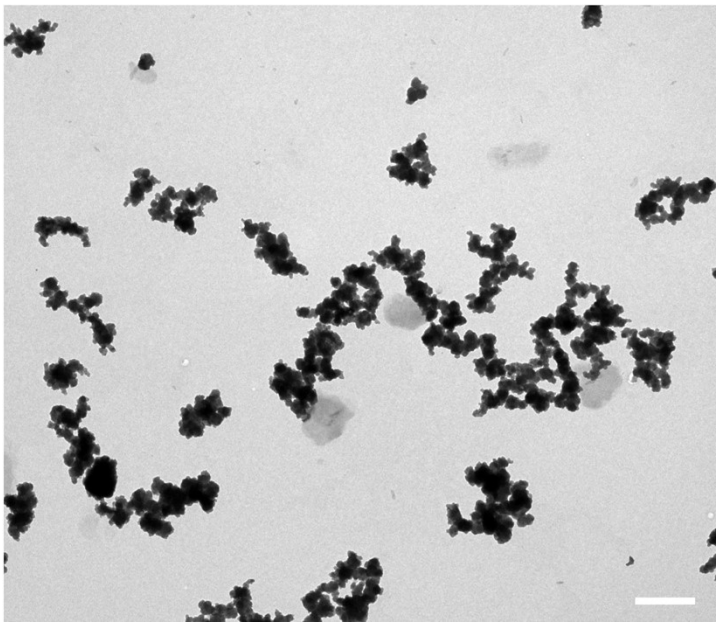

Figure S5. TEM image of Au-CMP NPs with a larger field of view. Scale bar: 1 μm.

Figure S6. A photograph of Au-CMP NPs in water, saline, serum and cell culture medium.

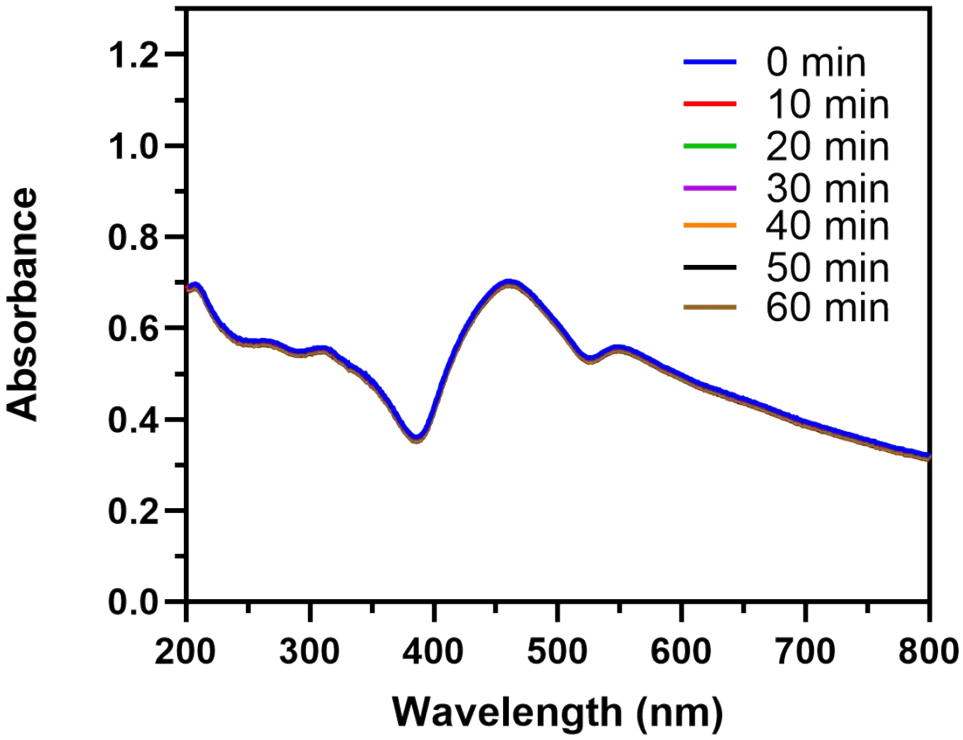

Figure S7. The absorption spectra of Au-CMP NPs after different 633 nm laser irradiation (1 W/cm^2) durations.

Table S1. Summary of nanomaterials used in PDT and RT.

Nanomaterial	Surface modification	Size/nm	Experiment parameter	Cell type	Ref.
PDA-PEG/Cur/Ce6	-	100 (SEM, DLS)	Laser: 660 nm, 5mW/cm^2 Dose: 6 Gy X-ray	A549 cells	1
Hf-TCPP	PEG	80-150 (TEM)	Laser: 661 nm, 5mW/cm^2 Dose: 6 Gy X-ray	4T1, Hela, NIH3T3 cells	2
UCNP-$SnWO_4$	-	~490 (DLS)	Laser: 980 nm, 0.7W/cm^2 Dose: 6 Gy X-ray	4T1 cells	3
$SrAl_2O_4:Eu^{2+}$	MC540, $mSiO_2$	73.5±26.9 (TEM)	Dose: 5 Gy X-ray	H1299 cells	4
CeF_3	VP	9±2	Dose: 6 Gy X-ray	Panc1 cells	5
$LaF_3:Ce^{3+}$	PPIX, DMSO, PLGA	~2 μm	Dose: 3 Gy X-ray	PC3 line	6
ZnS:Cu,Co	TBrRh123	4 (TEM)	Dose: 2 Gy X-ray	PC3 line	7
SiC/SiO_x	H2TPACPP	~60 (TEM)	Dose: 2 Gy X-ray	A549 cells	8
Hf-based MOLs	-	20-220	Dose: 2 Gy X-ray	CT26, MC38 cells	9
Cu-Cy	-	50-100	Dose: 5 Gy X-ray	MCF-7 cells	10
$LiYF_4:Ce^{3+}$	ZnO, SiO_2, PEG	33.8 (TEM)	Dose: 8 Gy X-ray	Hela cells	11
Au-CMP NPs	PEG	~252.3 (DLS)	Laser: 633 nm, 1W/cm^2 Dose: 4 Gy X-ray	BEL-7402 cells	This work

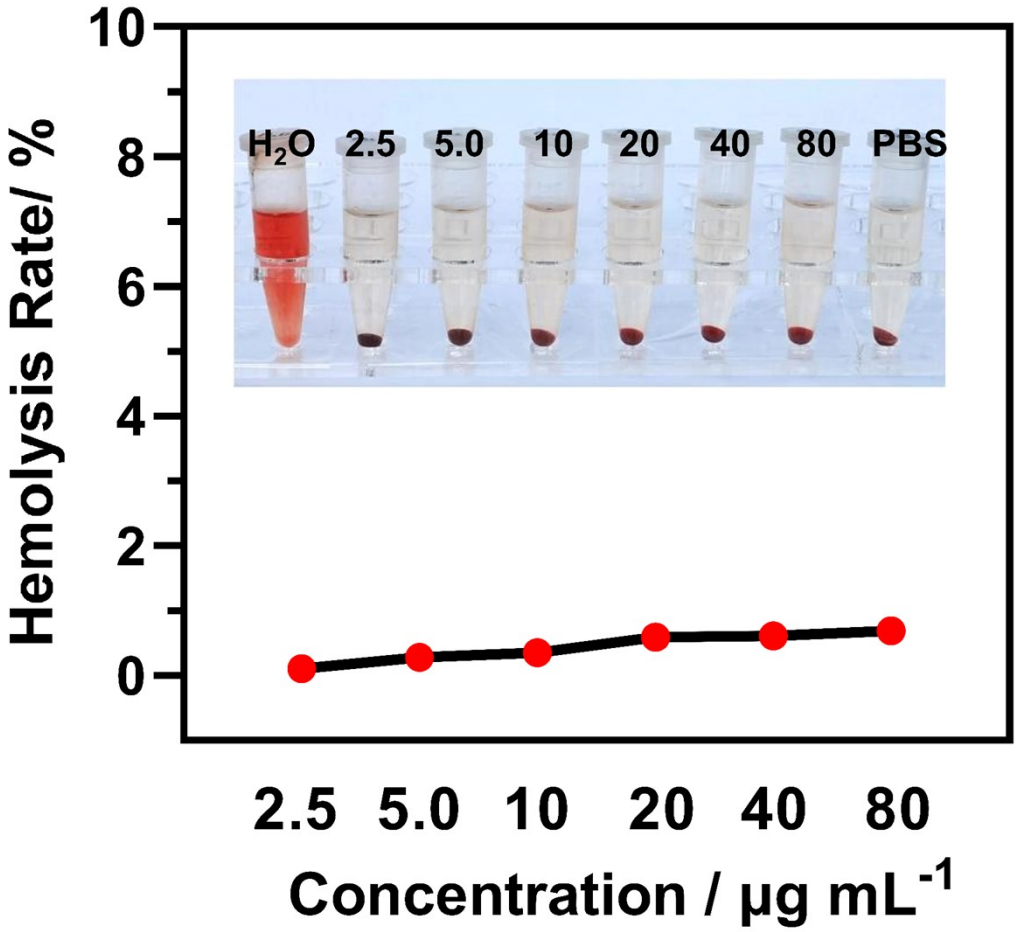

Figure S8. Hemolytic activity of Au-CMP NPs at various concentration with mouse red blood cells. H_2O and PBS were served as positive and negative control, respectively.

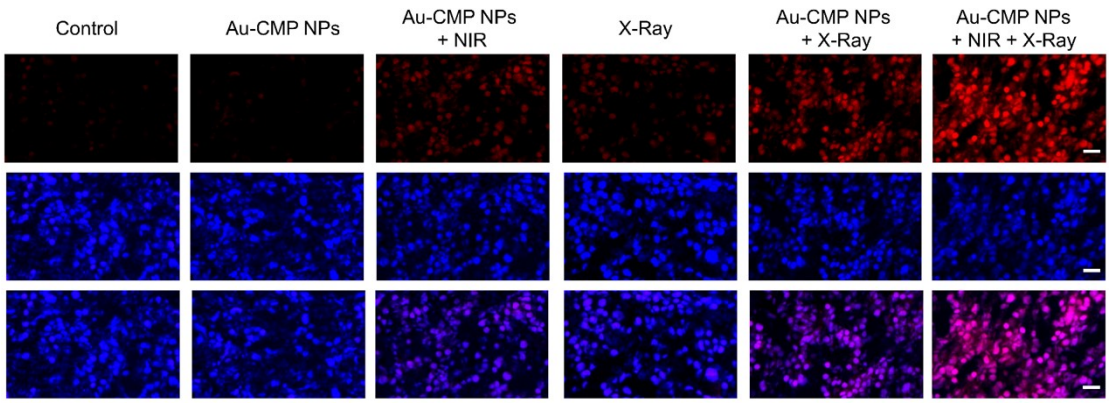

Figure S9. ROS staining images of tumors after different treatments with DHE as the ROS probe. Scale bar = 20 μm.

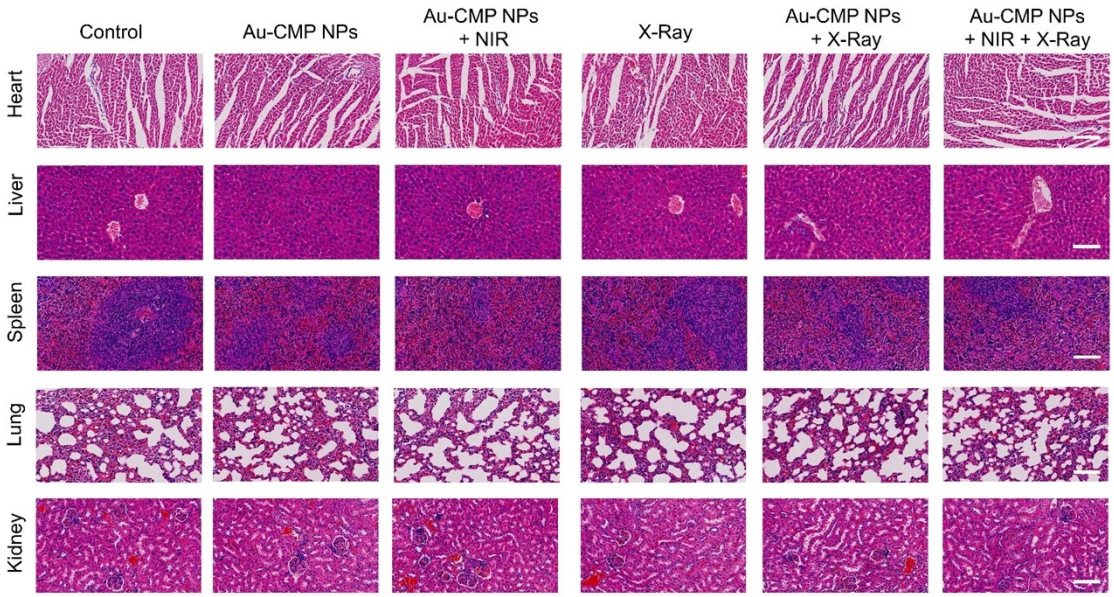

Figure S10. Representative images of H&E staining for main organs (heart, liver, spleen, lung, and kidney) of mice in each treatment group at 14 days from the treatments started. Scale bar = 100 μm.

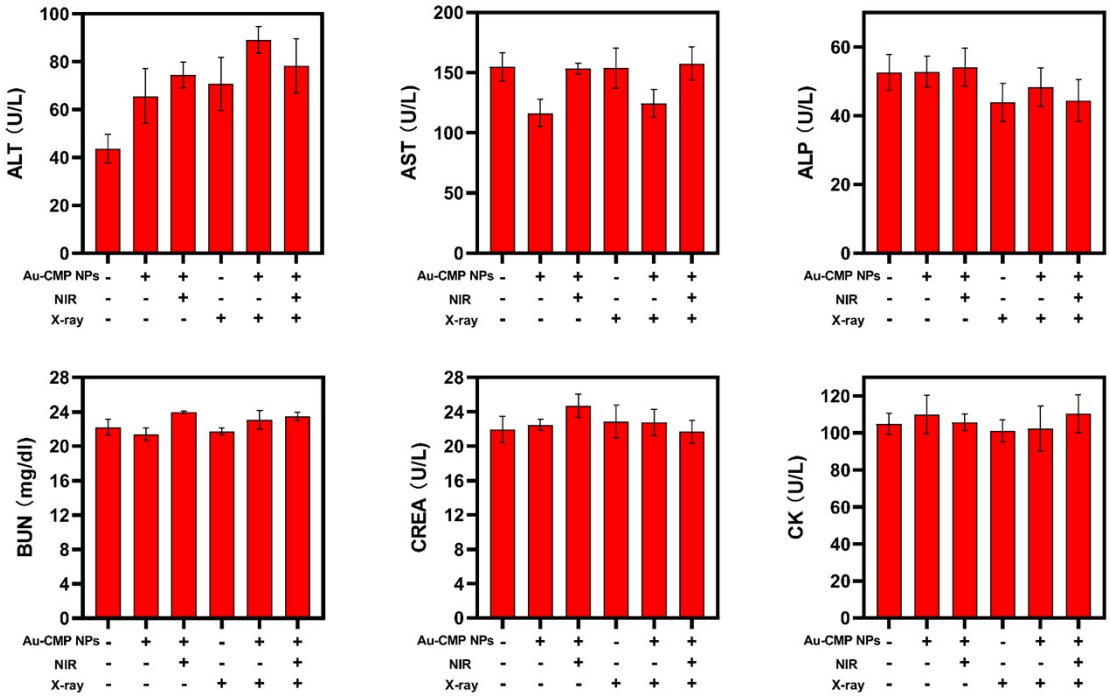

Figure S11. Biochemical levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CREA), creatine kinase (CK) from mice in each group.

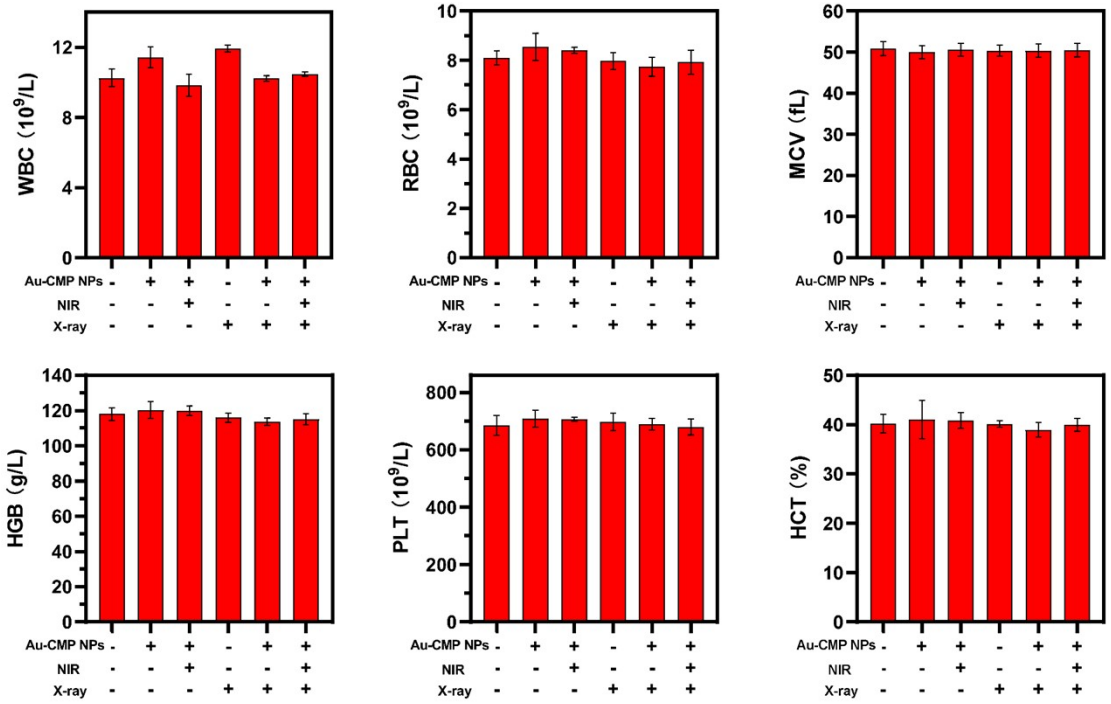

Figure S12. Hematology of white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), hemoglobin (HGB), platelets (PLT), erythrocyte compact (HCT) from mice in each group.

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