A Metal-polyphenolic Nanosystem with NIR II Fluorescence-guided Combined Photothermal Therapy and Radiotherapy

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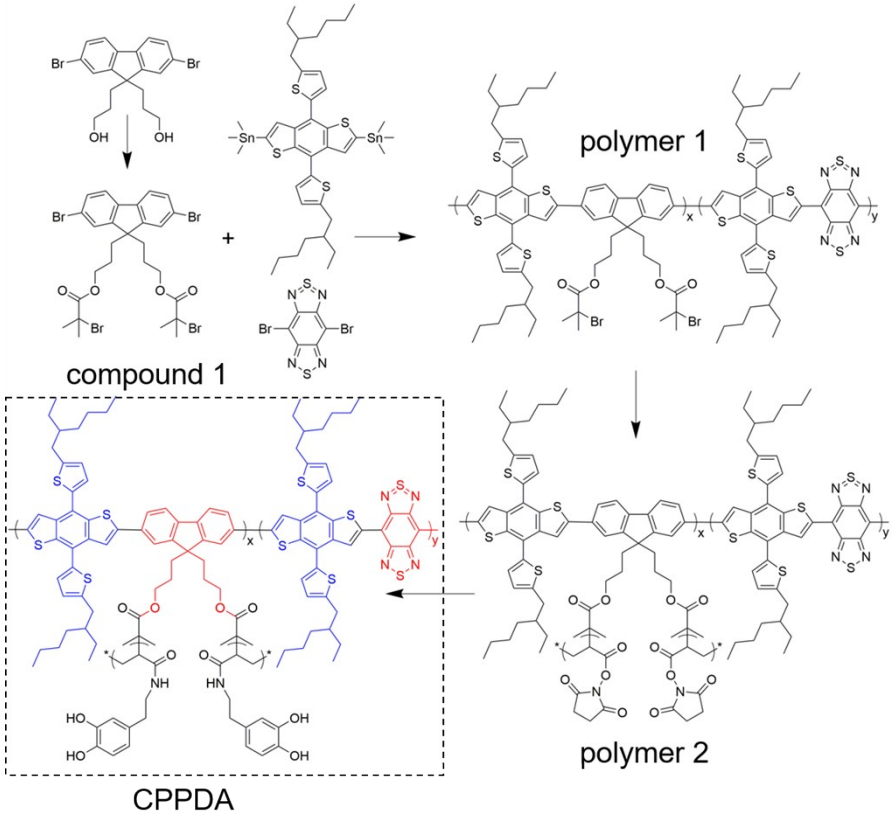
Materials and Characterization methods: All chemicals and regents were purchased from Sigma-Aldrich, Energy Chemical, and J&K. THF was purified by distillation from sodium in the presence of benzophenone. Other organic solvents were used without any further purification. All reactions were conducted under nitrogen atmosphere. The biological experiment materials, such as cells, and nude mice, were purchased from Neobioscience and Thermal Fisher: NMR spectra were recorded on a Bruker Ultra Shield Plus 400 MHz NMR. $CDCl_3$ was used as the solvent. The UV–Vis absorption spectra were recorded on a Shimadzu UV-3600 UV–Vis–NIR Spectrophotometer. Photoluminescent spectra were recorded at RT using a fluorescence spectrophotometer with excitation and emission slit widths of 5.0 nm. 1H NMR spectra were recorded on NMR (400 MHz) spectrometers, using tetramethylsilane as an internal standard. The particle diameters were measured by DLS using a 90 Plus particle size analyzer (Brookhaven Instruments). The MTT assay was measured by a microplate reader (BioTek, PowerWave XS/XS2, U.S.). GPC analysis of the polymers was conducted on a Shim-pack GPC-80X column with THF as the eluent at a flow rate of 1.0 mL/min at 35 °C and polystyrene as the standard. The data were analyzed by using the software package provided by Shimadzu Instruments. Thermal imaging was conducted on SC300 infrared camera (Fluke TiR, USA) with 808 nm and 1064 nm laser.

Synthesis of compound 1: 2,7-dibromo-9,9'-hydroxypropylfluorene was prepared with the guidance of the previous work.[1] 2,7-dibromo-9,9'-hydroxypropylfluorene (2.06 g, 5 mmol) was dissolved in 30 mL dichloromethane and cooled in ice/water bath. 2-bromoisobutyryl bromide (4.6 g, 20 mmol) was dropwise added to the solution. After 2h stirring at room temperature, the reaction was terminated by adding 30 mL deionized water. Then, the raw mixture was extracted by ethyl acetate. The organic layer was collected and washed by deionized water for 3 times and desiccated with $NaSO_4$. After purification via SiO_2 column chromatography. The white powder of compound 1 was obtained with yield of 90% (1.83 g). 1H NMR (400 MHz, $CDCl_3$, ppm) was shown in Fig. S1.

Synthesis of polymer 1. 4,7-dibromobenzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) (17.6 mg, 0.05mmol), compound 1 (30.5 mg, 0.05 mmol) and 2,6-bis(trimethylstannyl)-4,8-bis(5-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-b:4,5-b']dithiophene (90.4 mg, 0.1 mmol) were mixed and dissolved with 5mL toluene. Then, under nitrogen protection, the system was heated to 90 ℃ using tris(dibenzylideneacetone)dipalladium(0) as a catalyst for 6 h. After cooling down, the crude product was filtered by AlO_3 to remove the solid catalyst, and then purified by sedimentation in methanol. CP was obtained with the yield of 60% (58.2 mg). Mn = 6483, PDI = 2.13 (5 repeat unit of each conjugated polymer). ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K, ppm) was shown in Fig. S2.

Synthesis of polymer 2. Polymer 1 (20 mg), N-succinimidyl acrylate (85 mg, 0.5mmol) and CuBr (50 mg) were dissolved in 2 mL methyl-phenoxide. Under nitrogen protection, 50 μL pentamethyldiethylene triamine (PMDETA) was added as ligand. The solution was stilled at 90 ℃ for 8 h. After cooling down, the crude product was filtered by AlO_3 to remove the solid catalyst, followed by the purification through precipitation in diethyl ether. Polymer 2 was obtained with the yield of 75 % (110 mg) Mn = 9871, PDI = 2.45 (20 N-Succinimidyl Acrylate repeat units in each conjugated polymer) ^{1}H NMR (Methanol-d4, 400 MHz, 298 K, ppm) was shown in Fig. S2.

Synthesis of CPPDA. Polymer 2 (50 mg) and dopamine hydrochloride (189.6mg, 1mmol) were mixed and dissolved in 10 methanol. Triethylamine (202 mg, 2 mmol) was added into the mixed solution and the reaction mixture was stirred at 60 ℃ under nitrogen protection overnight. After cooling down, the crude product purified by precipitation in diethyl ether. CPPDA was obtained with the yield of 43 mg. Mn = 10838, PDI = 2. 87 (18 dopamine repeat units in each conjugated polymer) ^{1}H NMR (Methanol-d4, 400 MHz, 298 K, ppm) was shown in Fig. S2.



Scheme S1. Synthetic routine of polyphenolic semiconducting polymer, CPPDA.

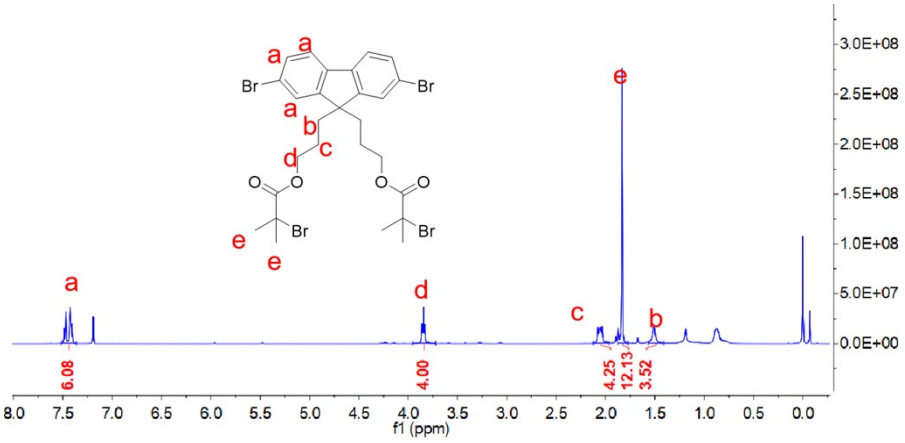


Fig. S1. ^{1}H NMR spectrum of compound 1.

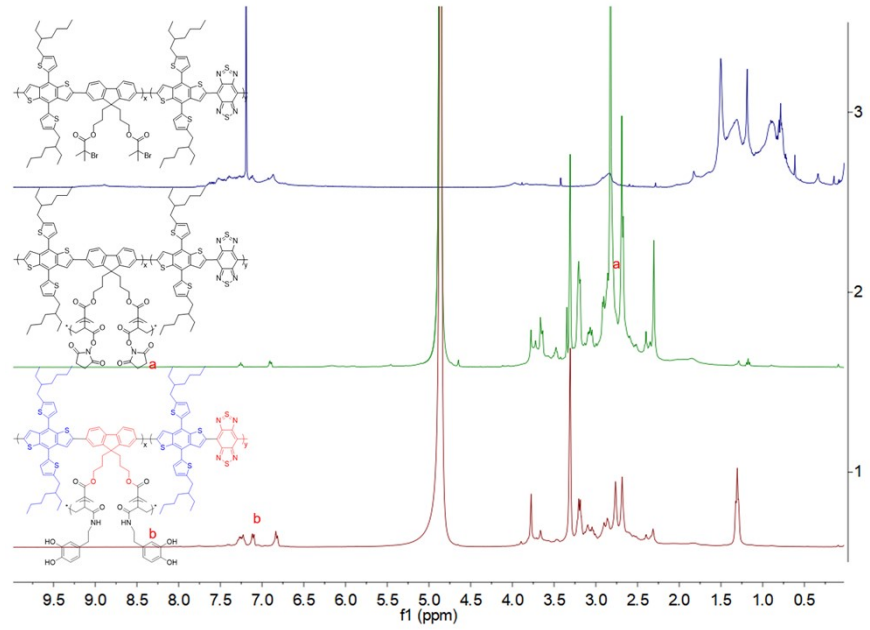


Fig. S2. ^{1}H NMR spectrum of polymer 1 (blue line), polymer 2 (green line) and CPPDA (red line).

Preperation of CPPDA-Hf@Poloxamer. CPPDA, Pluronic F127 were mixed (mass ratio = 1:10) and dissolved in tetrahydrofuran (THF). The 600 μg $HfCl_4$ was dissoved in water/THF solution. The 1 mL THF solution containing drugs was dropwise added into the $HfCl_4$ solution with a volume ratio 0.1 in an ultrasonic vibrator (100 Hz). Then, pure nitrogen was bubbled through the solution to remove THF completely. the CPPDA-Hf@Poloxamer NPs solution was washed and concentrated by ultrafiltration Millipore tube (30 kDa, Merck).

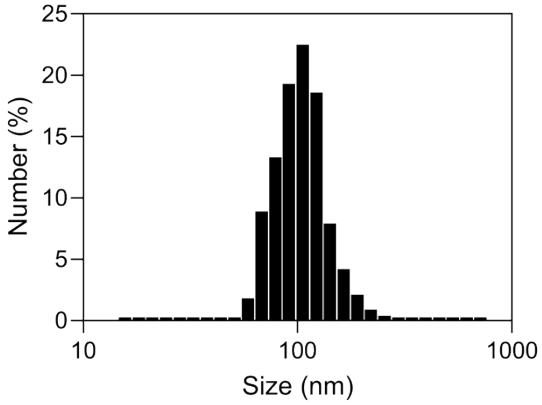


Fig. S3. Particle size of CPPDA-Hf@Poloxamer nanoparticles.

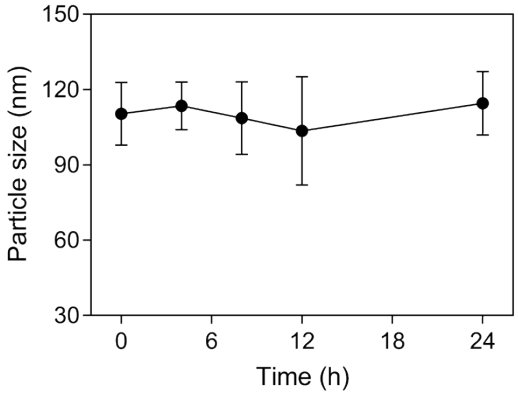


Fig. S4. Particle size of CPPDA-Hf@Poloxamer in serum from 0 to 24 h

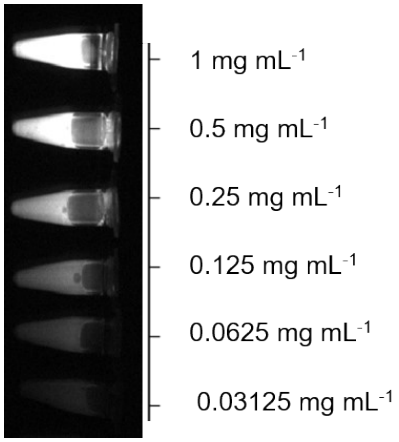


Fig. S5. The NIR II fluorescence of CPPDA-Hf@Poloxamer in different concentrations (λ_{ex}=1064 nm, 1 W cm^{-2})

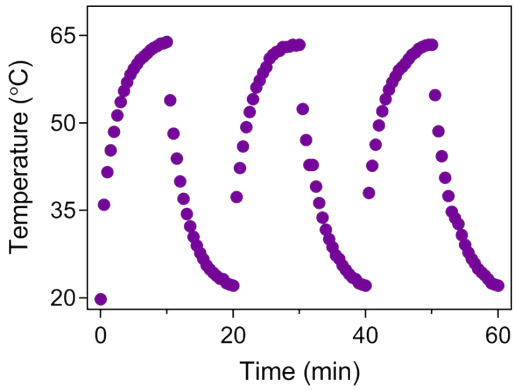


Fig. S6. The heating and cooling curve of CPPDA-Hf@Poloxamer (0.5 mg mL^{-1}, λ_{ex}=1064 nm, 1 W cm^{-2}).

In Vitro Cytotoxicity Study of CPPDA-Hf@Poloxamer nanoparticles. The viability of 4T1 cells were assessed by MTT assay and cultured in RPMI-1640 medium with 10% FBS in 96-well plates at a density of 5000 cells per well at 37 ℃ with 5% CO_2. As the cells were adherent, a series of CPPDA-Hf@Poloxamer solution were added for 2 h and removed for the certain treatment (X-ray irradiation) After the treatments, cells were incubated for 24 h. Then wells were washed with PBS buffer twice and incubated with MTT medium for 2 h. The cell viability was monitored by checking the absorbance on a Thermo Scientific Microplate Reader at 490 nm.

Animal Model. The mice (BALB/c, 6-8 weeks old, female) 4T1 cell line at 1×10^7 in 100 μL PBS were injected to the right axilla or back of the mice. The animal experimental procedures were conducted following an established protocol (UMARE-030-2018) that had previously been approved by the University of Macau Animal Ethics Committee.

Pharmacodynamic Evaluation. 5 groups of 4T1 bearing nude mice (n = 4) were treated with PBS and CPPDA-Hf@Poloxamer solutions under 1064 nm laser irradiation or X-ray irradiation every 2 days for 3 treatment circle. The control groups received saline only. The tumor growth and body weight changes of nude mice were monitored every 3 days until day 15 Tumor sizes were determined using caliper measurement. After 15-days treatment, tumor-bearing mice were sacrificed and the tissues (heart, liver, spleen, lung, kidneys) and the tumor were fixed in 10% formalin and embedded in paraffin.

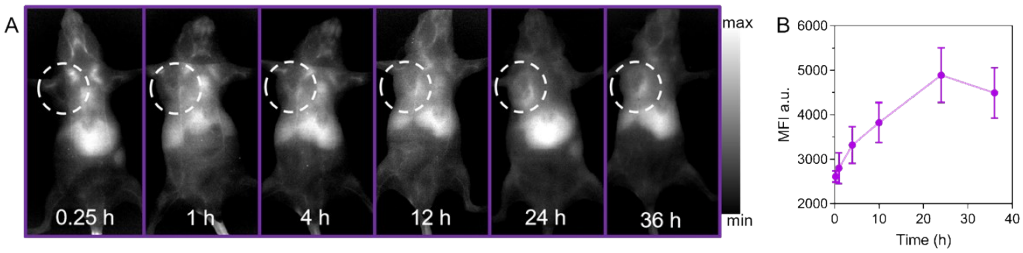


Fig. S7. NIR II imaging of CPPDA-Hf@Poloxamer in vivo, λ_{ex}=1064 nm (tumor region in dash circle). (A) The NIR II fluorescence signal distribution in mice model injected with CPPDA-Hf@Poloxamer at different time point. (B) The fluorescence intensity in tumor region.

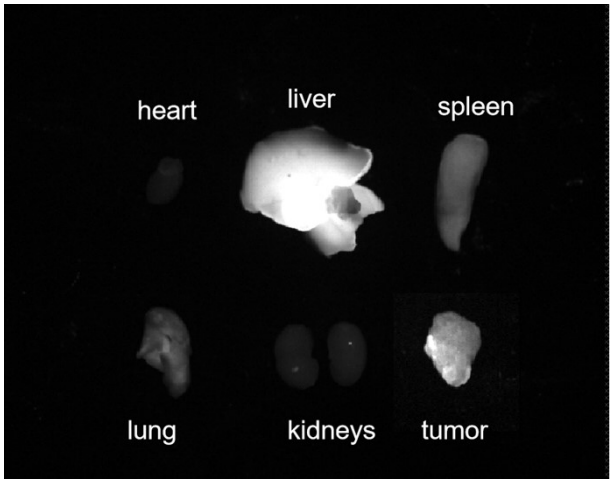


Fig. S8. NIR II fluorescence signal in main organs (heart, liver, spleen, lung, and kidneys) and tumor after postinjection of CPPDA-Hf@Poloxamer for 36h.

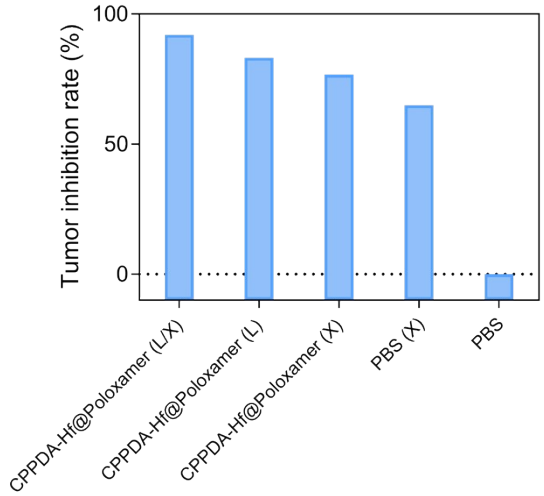


Fig. S9. The tumor inhibition rate after various treatments. (L: 1064 nm laser, X: X-ray)

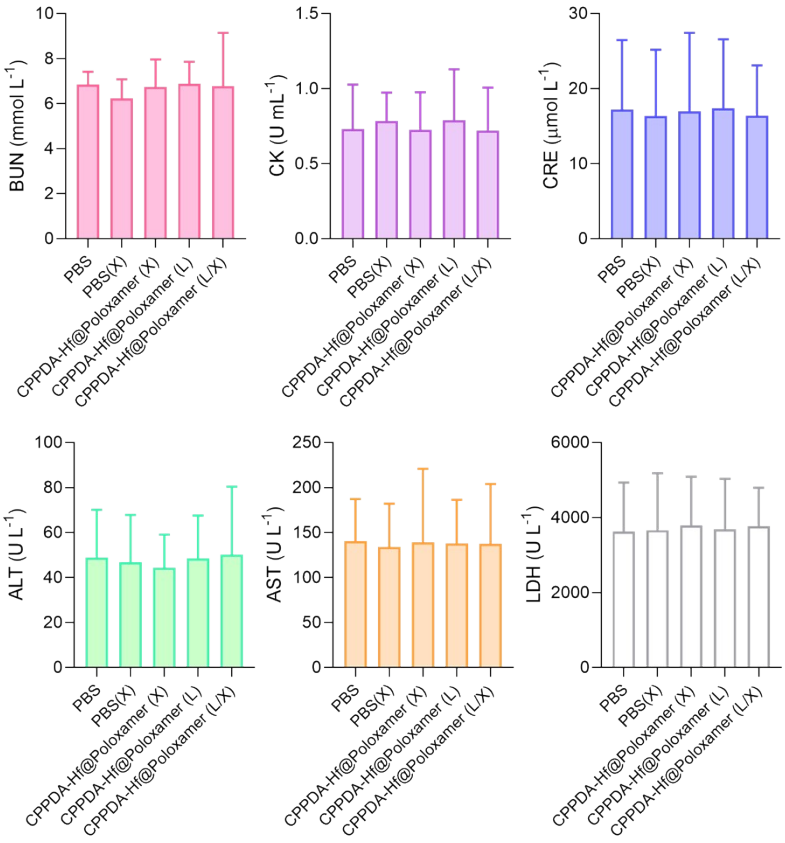


Fig. S10. Biochemical parameters including alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatine kinase (CK), creatinine (CRE), and lactate dehydrogenase (LDH) after various treatments. (L: 1064 nm laser, X: X-ray)

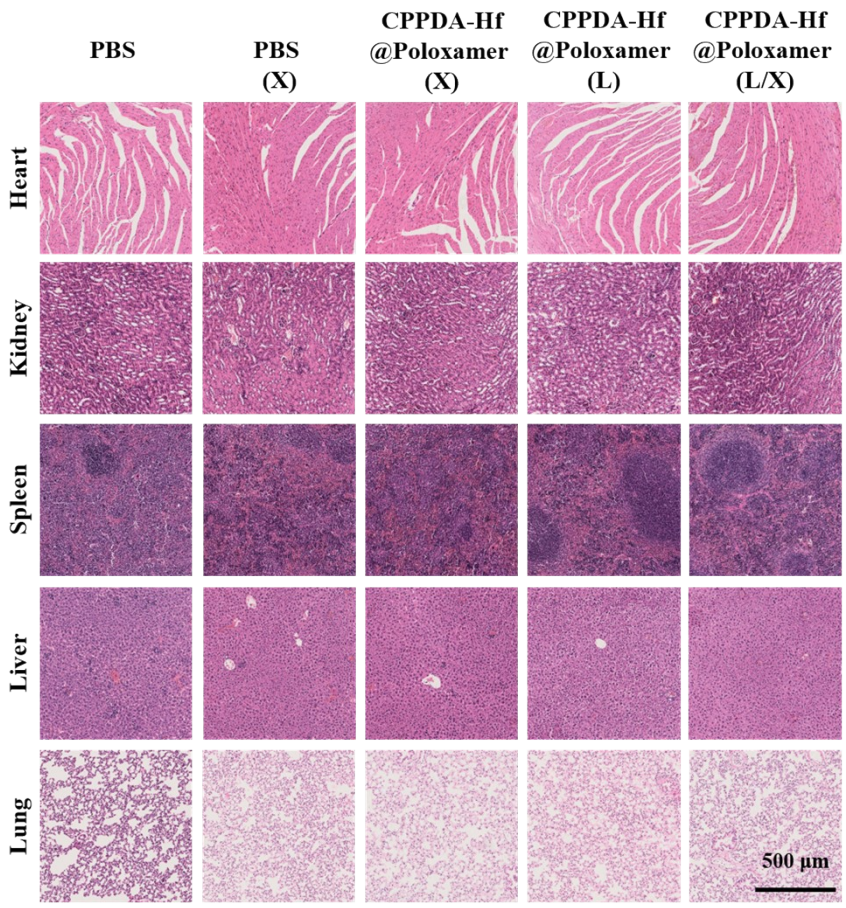


Fig. S11. Hematoxylin and eosin (H&E)-stained slice images of heart, liver, spleen, lung and kidney after various treatments. Scale bar: 500 μm. (L: 1064 nm laser, X: X-ray)

References

J. Li, L. Xie, B. Li, C. Yin, G. Wang, W. Sang, W. Li, H. Tian, Z. Zhang, X. Zhang, Q. Fan and Y. Dai, *Adv. Mater.*, 2021, **33**, 2008481.