Supporting Information

A discrete platinum(II)-metallacycle with enhanced red emission for imaging-guided synergistic cancer photothermal-chemotherapy

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1. Materials and methods

Materials

All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature.

Measurements

NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AV400 spectrometer.

Fluorescence spectroscopy. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell peltier accessory to control temperature.

TEM microscopy. High-resolution Transmission electron microscopy (TEM) images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV.

ESI-MS spectroscopy. Electrospray ionization mass spectra (ESI-MS) were measured by Agilent 6520 Q-TOF-MS.

Cytotoxicity experiments. Human cervical cancer cells (HeLa cells) were incubated in Dulbecco's Modified Eagle's Medium (DMEM). The medium was supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin. HeLa cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37°C in 5% CO_2. Then the cells were incubated in different groups for 24 h. The relative cellular viability was determined by the MTT assay.

Confocal laser scanning microscopy. HeLa cells were seeded in 6-well plates (5×10^4 cell mL^{-1}, 2 mL per well) for 24 h at 37°C in 5% CO_2. The cells were incubated with the corresponding solution for 4 h. Then the medium was removed, and the cells were washed with phosphate buffer solution three times. Finally, the cells were subjected to observation by a confocal laser scanning microscope.

Prepare the working solution of PI/Calcein AM: Prepare the working solution by mixing the Calcein AM solution (1000X), PI (1000X) and cell culture medium at a ratio of 1 μL : 1 μL : 10 mL, and store it refrigerated at 4°C for later use.

Inoculate the cultured HeLa cells (4×10^5 cells/mL, 1 mL per well) onto four 6-well plates and culture them for 24 hours. After the cells adhere to the wall, replace the original culture medium of the cells in two plates with fresh culture medium containing AP1/MPt NPs (500 μg/mL), and replace the culture medium of the cells in the other two plates with fresh culture medium containing an equal amount of PBS. To explore the effect of photothermal therapy on tumor cells, for the two plates of cells with fresh culture medium containing AP1/MPt NPs, irradiate one portion of the cells with a 660 nm laser for 15 minutes (laser intensity of 1 W/cm²), and do not irradiate the other portion with the laser. After incubating the cells in the cell incubator for 24 hours, gently aspirate the old culture medium, wash the cells gently with PBS, and add 1 mL of the PI/Calcein AM working solution. After culturing in the cell incubator for 0.5 hours, wash the cells with PBS, and observe the fluorescence changes of live and dead cells through a fluorescence microscope.

DLS. Dynamic light scattering measurements were performed on a goniometer ALV/CGS-3 using a UNIPHASE He-Ne laser operating at 632.8 nm.

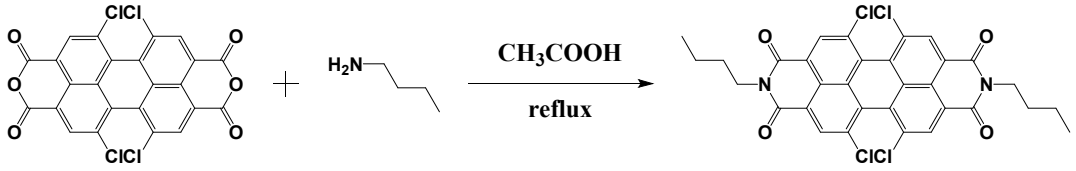
Infrared thermal image: The instrument used for photothermal imaging is FLIR—C2 (America) Infrared thermal imager.

In vivo study: All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Nantong University and approved by the Animal Ethics Committee of Nantong University.

The HeLa tumor-bearing mice with a tumor volume (~100 mm^3) were spontaneously assigned into four groups (n = 3), and then intravenously injected at 0 day with control, free Laser, **AP1/M_{Pt}** and **AP1/M_{Pt}** + Laser. As Laser and **AP1/M_{Pt}** + Laser groups, the tumor sites were irradiated with irradiation (660 nm, 1.0 W/cm2, 5 min) at 12 h post-injection. The tumor volume (V) is calculated according to the following equation: $V = 1/2 \times \text{length} \times \text{width}^2$. The tumor inhibitory rates (TIR) is calculated by the equation: TIR (%) = 100 × (mean tumor volume of the Control group - mean tumor volume of others)/(mean tumor volume of the Control group).

2. Synthesis of 2[S1]

Scheme S1. Synthetic route of **2**



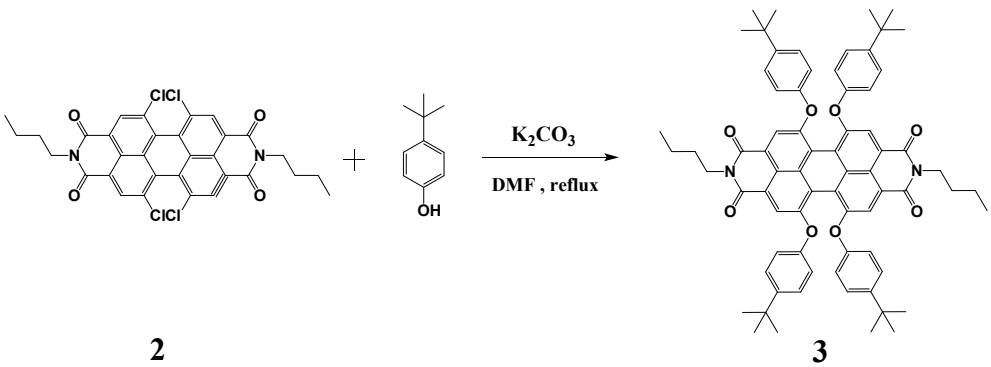
1 **2**

Synthesis of compound **2**: A mixture of compound **1** [S1] (10.00 g, 18.88 mmol) , acetic acid (20 ml) and n-Butylamine 7.50 mL (75.52 mmol) Butylamine was refluxed for 12 h under stirring. Then 200 mL water was added, the mixture was stirred for 1 h and filtered. The precipitate was washed with water and dried in vacuum, and then the compound **2** was obtained by recrystallization of ethanol to give a yellow solid. (10.50 g, yield 85%).

S1. Queste M, Cadiou C, Pagoaga B. Synthesis and characterization of 1,7-disubstituted and 1,6,7,12-tetrasubstituted perylenetetracarboxy-3,4:9,10-diimide derivatives. *New Journal of Chemistry, **2010**, 34 (11) : 2537-2545.*

3. Synthesis of 3

Scheme S2. Synthetic route of **3**



2 **3**

Synthesis of compound **3**: According to previous report with some modification[S2],

compound 2 (10 g, 15.60 mmol), 4-(Tert-butyl)phenol (6.0 equiv , 93.60 mmol) and K_2CO_3 (4 equiv , 62.40 mmol) were added to 200 mL DMF. The mixture was stirred under N_2 and reflux for 12 h. After cooling to room temperature, the reaction mixture was poured under stirring into 200 mL water. A precipitate appeared, was washed with water and dried under vacuum to give compound **3** as a yellow solid (10.40 g, 95%). The ^{1}H NMR spectrum of compound 3 is shown in Figure S1.^{1}H NMR (400 MHz, chloroform-*d*, room temperature) δ (ppm): 8.22 (d, *J* = 1.0 Hz, 4H), 7.23 (dd, *J* = 8.8, 1.0 Hz, 8H), 6.85 – 6.80 (m, 8H), 4.11 (t, *J* = 7.5 Hz, 4H), 1.45 – 1.36 (m, 8H), 1.29 (d, *J* = 1.0 Hz, 36H), 0.98 – 0.90 (m, 6H).

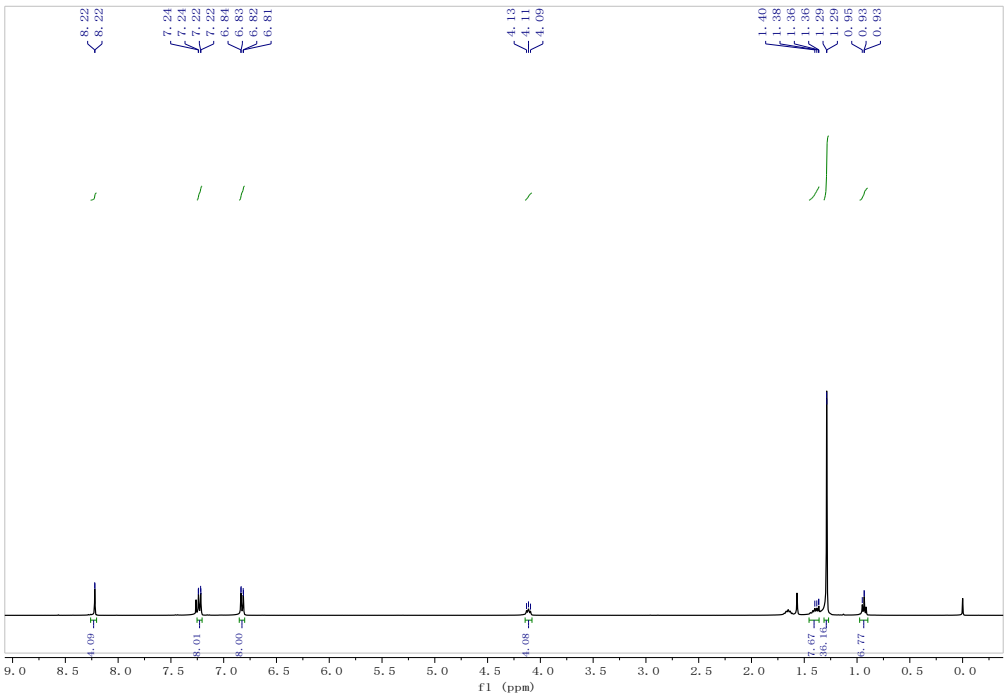
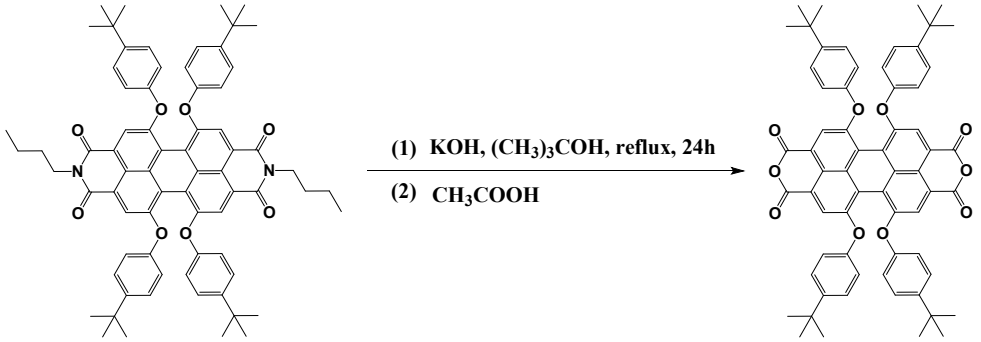


Figure S1 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **3**.

S2. Ding, Y.; Tong, Z.; Jin, L.; Ye, B.; Zhou, J.; Sun, Z.; Yang, H.; Hong, L.; Huang, F.; Wang, W.; Mao, Z. An NIR Discrete Metallacycle Constructed from Perylene Bisimide and Tetraphenylethylene Fluorophores for Imaging-Guided Cancer Radio-Chemotherapy. *Adv. Mater.*, ***2022***, *34*, 2106388.

4. Synthesis of 4

Scheme S3. Synthetic route of **4**



3 **4**

Synthesis of compound **4:** Following a modified procedure from reference S2. Compound **3** (10.00 g, 10.15 mmol) was added to alcoholic KOH (10equiv, 15.00 g KOH; 200 mL *tert*-butyl alcohol) and refluxed with stirring for 24 h. After cooling to room temperature, the liquid organic layer was separated, acidified with Acetic acid (40ml and 40ml water), the mixture was stirried overnight. The precipitate was filtered, washed with water to neutral pH and dried in vacuum to give compound **4** (0.65 g, 65%). The ^{1}H NMR spectrum of **4** is shown in Figure S2. ^{1}H NMR ($CDCl_3$, room temperature, 400 MHz) δ (ppm): 8.21 (s, 4H), 7.29 – 7.24 (m, 8H), 6.86 – 6.81 (m, 8H), 1.30 (s, 36H).

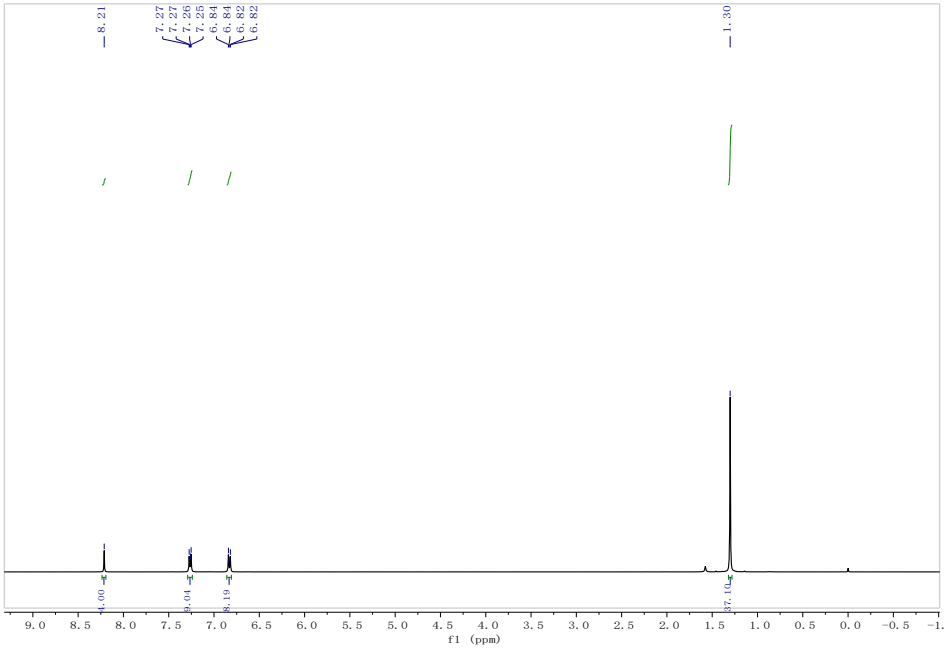
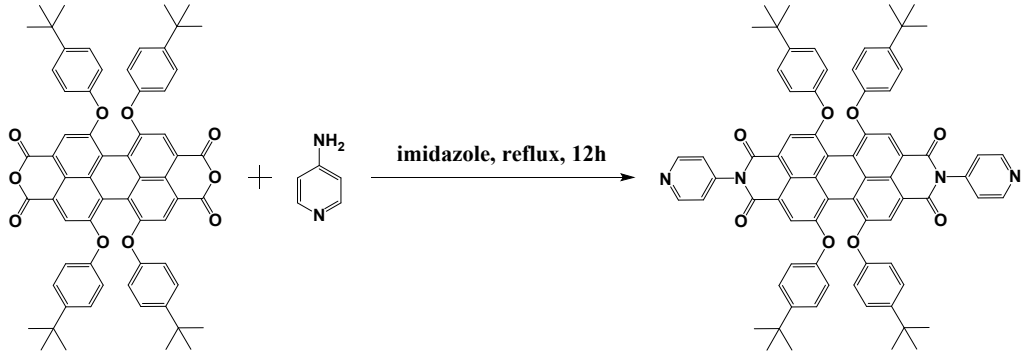


Figure S2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **4**.

5. Synthesis of PPY

Scheme S4. Synthetic route of **PPY**



4 **PPy**

Synthesis of **PPy**: Compound **4** (5.00 g, 4.50 mmol) and 4-aminopyridine (1.60 g, 18.00 mmol) were added to 20.0 mL imidazole. The mixture was stirred at 130 °C for 12 h. After cooling to room temperature, the reaction mixture was poured under stirring into 100ml water. A precipitate appeared, was washed with water and methanol, and dried under vacuum. Purification achieved by column chromatography on silica gel ($CHCl_3$/EtOAc 15:1) to give 4.60 g of product (90% yield) as a purple powder. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.74 (d, *J* = 5.3 Hz, 4H), 8.21 (s, 4H), 7.22 (d, *J* = 3.8 Hz, 10H), 7.20 (s, 2H), 6.81 (d, *J* = 8.6 Hz, 8H), 1.24 (s, 36H).

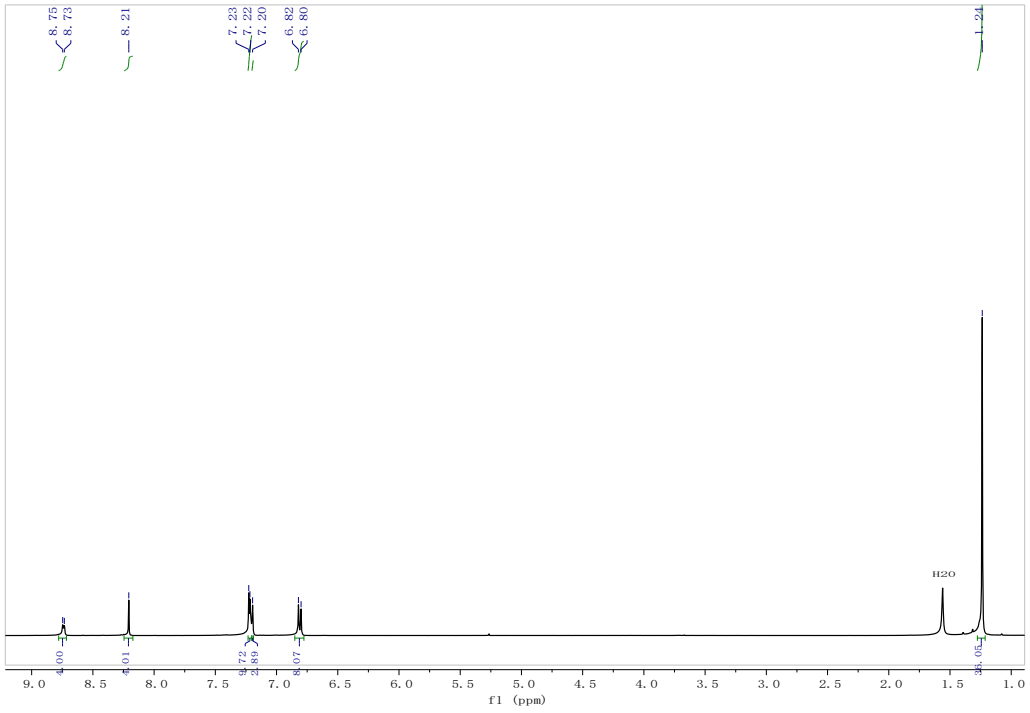
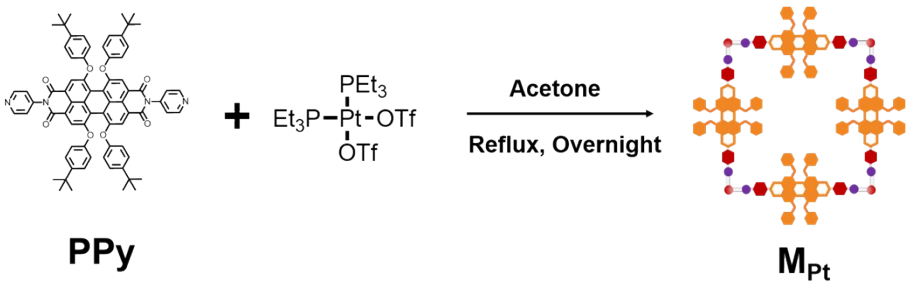


Figure S3 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **PPy**.

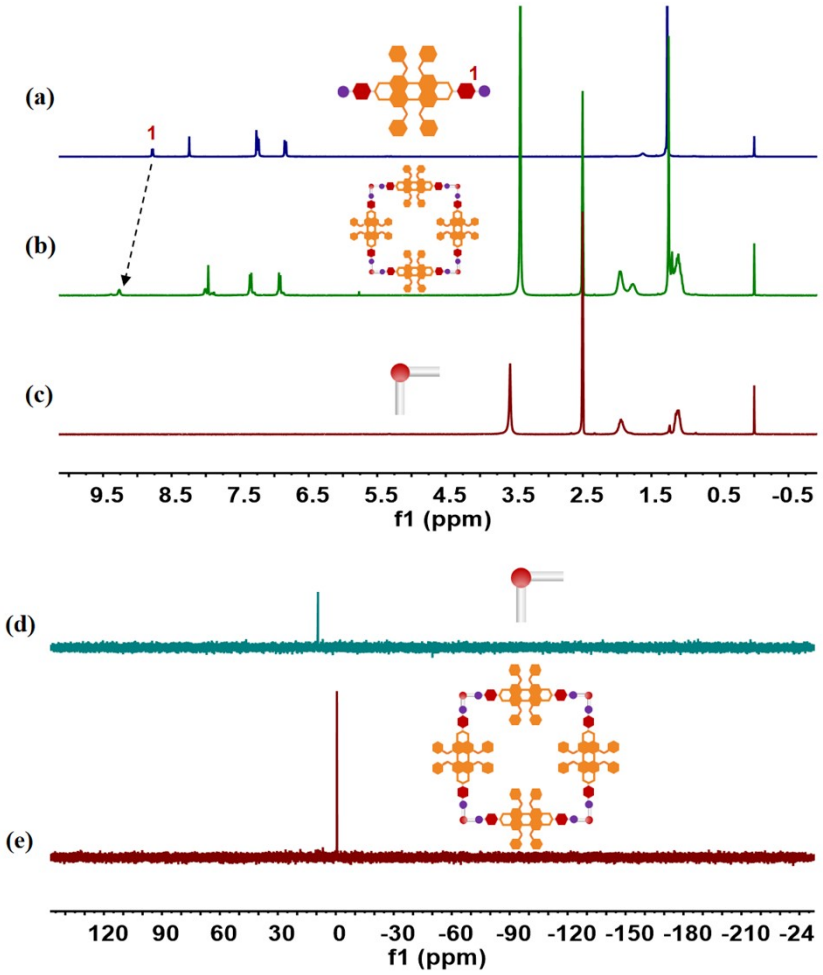
6. Synthesis of M_{Pt}

Scheme S5. Synthetic route of M_{Pt}



PPy **M_{Pt}**

Synthesis of $\mathbf{M_{Pt}}$: In a 1:1 molar ratio, PPy and 90° Pt (II) were placed in a 10-ml vial, followed by addition of acetone. After stirring overnight at 50°C, the mixture was filtered to remove insoluble materials. Then, the solvent was removed by N^2 flow and M_{Pt} was obtained by the addition of diethyl ether.



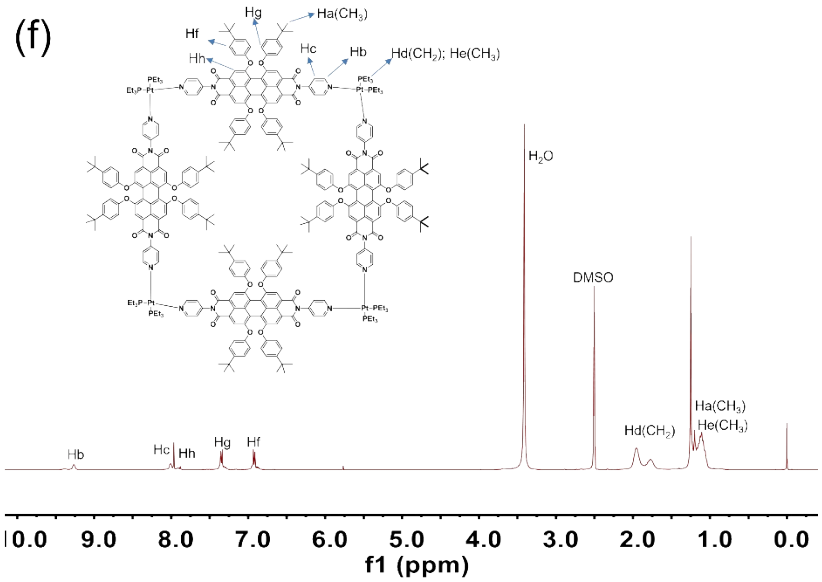


Figure S4 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **PPy** (a), $\mathbf{M_{Pt}}$ (b), and **Pt** (c). ^{13}P NMR spectra (DMSO-d_6, 298 K) of **Pt** (d) and $\mathbf{M_{Pt}}$ (e). (f) ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of $\mathbf{M_{Pt}}$.

Figure S5. Full ESI-TOF-MS spectra of $\mathbf{M_{Pt}}$ $[M - 4OTf]^{4+}$ and $[M - 5OTf]^{3+}$

7. Self-assembly of M_{Pt} in water

First, we prepare solutions of PPy and M_{Pt} with a concentration of 10^{-3} mol/L using acetone, and then dilute them with water to the corresponding concentrations. Subsequently, we add a certain amount of AP1 to the M_{Pt} solution with a concentration of 2.5×10^{-6} mol/L. After ultrasonic treatment for 30 minutes, we let it stand to obtain the AP1/M_{Pt} solution.

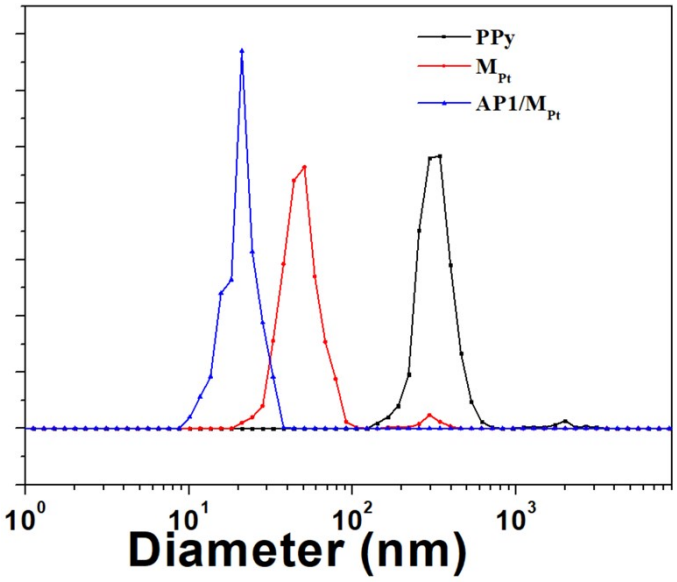


Figure S6. DLS studies of **PPy** (10^{-5} mol/L), **M_{Pt}** (2.5×10^{-6} mol/L), and **AP1/M_{Pt}** (**AP1**: 10^{-4} mol/L; **M_{Pt}**: 2.5×10^{-6} mol/L) self-assembly in water.

Photothermal experiment

To measure the photothermal effect of the obtained particles, various concentrations of the products were suspended in quartz cuvettes and irradiated by a 660 nm laser with an output power of 1.0 W cm^{-2}. The temperature change profiles of the solution were recorded by a digital thermocouple with a thermocouple probe every 30 s.

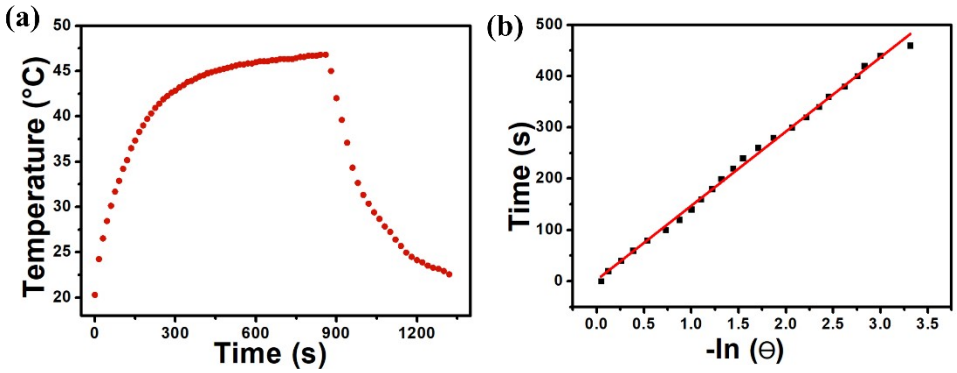


Figure S7. (a) Photothermal response of the **AP1/M_{Pt}** under 660 nm laser irradiation (1.0 W·cm^{-2}) for 15 min and then the laser was turned off. (e) Plot and linear fitting of cooling time versus–ln(θ) received from cooling section of photothermal effect curve in (d). The photothermal conversion efficiency (η) was calculated to be 36.9%.

8. MTT studies

Inoculate the cultured HeLa cells (5×10^4 cells/mL, 0.2 mL per well) into two 96-well plates, and incubate them in a cell incubator (with a CO_2 concentration of 5% at 37°C) for 24 hours until the HeLa cells adhere completely to the wall. Replace the original culture medium with fresh culture medium containing different concentrations of AP1/M_{Pt} NPs (500, 250, 80, 20, 5 μg/mL). For the control group, replace the original culture medium with fresh culture medium containing an equal amount of PBS. Incubate one plate in the incubator for 48 hours. For the other plate, after incubating for 4 hours, irradiate each well with a 660 nm laser (laser intensity of 1 W/cm^2) for 15 minutes, and then put it back into the cell incubator and incubate for up to 48 hours. Gently aspirate the old culture medium, wash it slowly with PBS, and then add 0.2 mL of fresh culture medium. Prepare a 5 mg/mL MTT solution, add 10 μL of the solution to each well of the 96-well plate, and continue to incubate in the cell incubator for 4 hours. Gently aspirate the culture medium, add 0.15 mL of DMSO, shake on a shaker for 15 minutes, detect the absorbance at 490 nm with a microplate reader, and calculate the viability of HeLa cells under different conditions. The entire experiment is carried out in the dark.

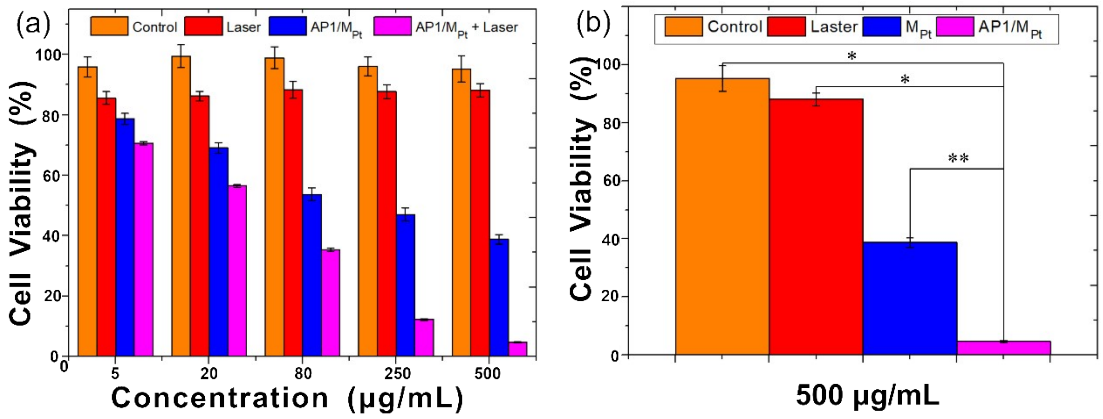


Figure S8. (a) The viabilities of HeLa cells in Control, Laser, **AP1/M$_{Pt}$** NPs, and **AP1/M$_{Pt}$** + Laser group, respectively. (Laser: 1 W cm^{-2} and 660 nm). (b) The viabilities of HeLa cells in Control, Laser, **AP1/M$_{Pt}$** NPs, and **AP1/M$_{Pt}$** + Laser group when concnetration is 500 μg/mL. Statistical significance: *P < 0.1, **P < 0.01, and ***P < 0.001.

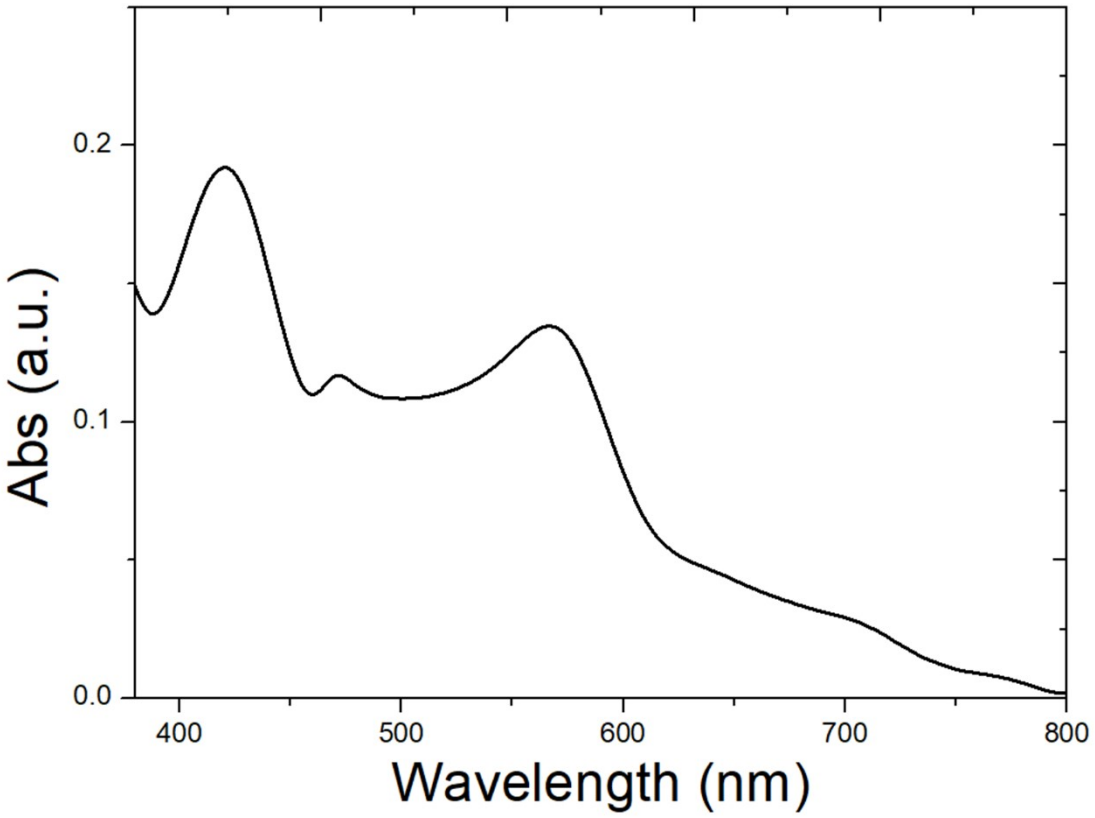


Figure 9. UV-Vis absorption spectrum of **AP1/M$_{Pt}$** in water, where above 600 nm also has absorption.

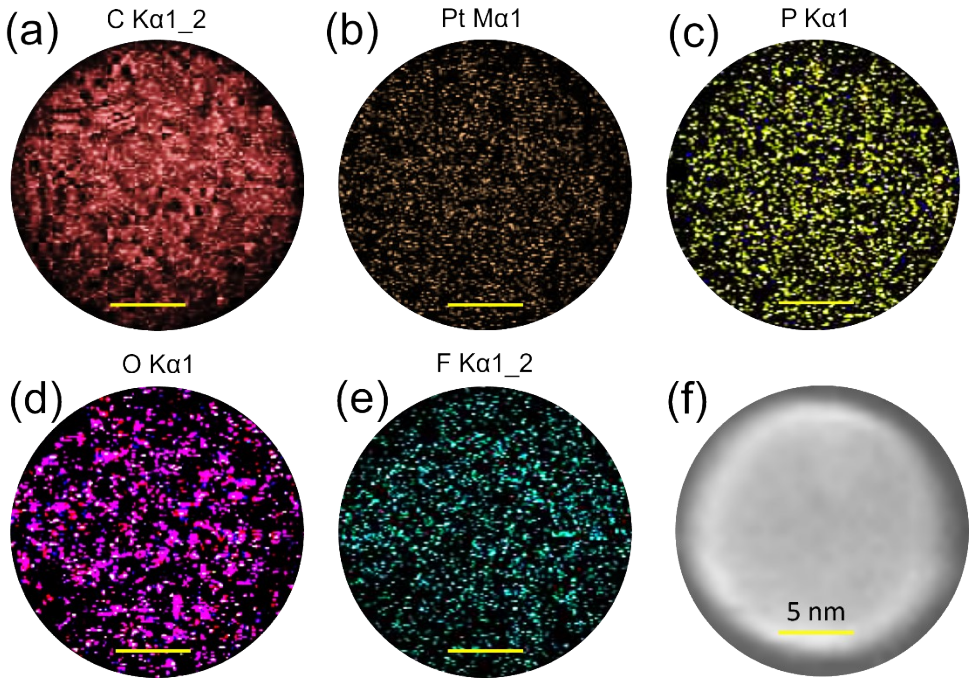


Figure S10. (a)-(e) EDS mapping images of **AP1/M$_{Pt}$** NPs. (f) TEM image of of **AP1/M$_{Pt}$** NPs. Scale bar = 5 nm.

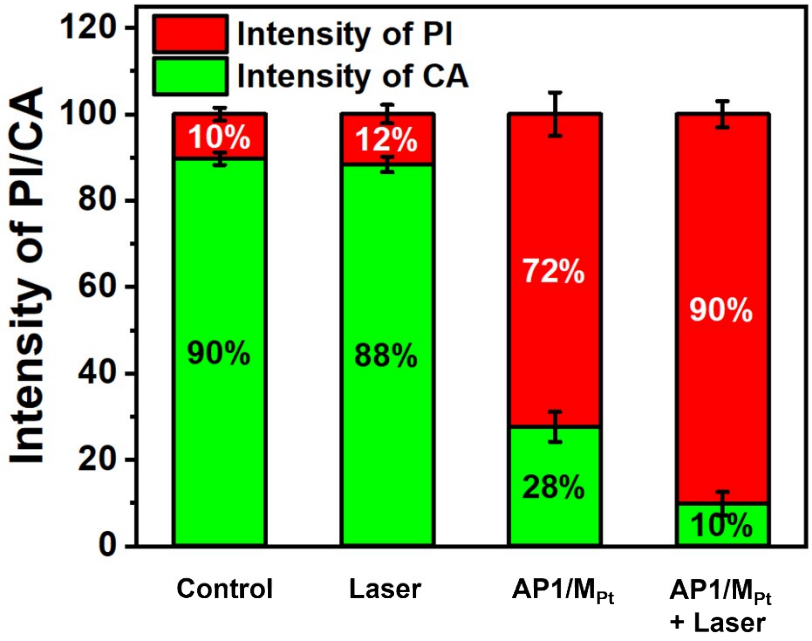


Figure S11. Fluorescence Intensity of Calcein AM (live cells, green) and PI (dead cells, red) contained HeLa cells after different treatments.

9. In vivo study

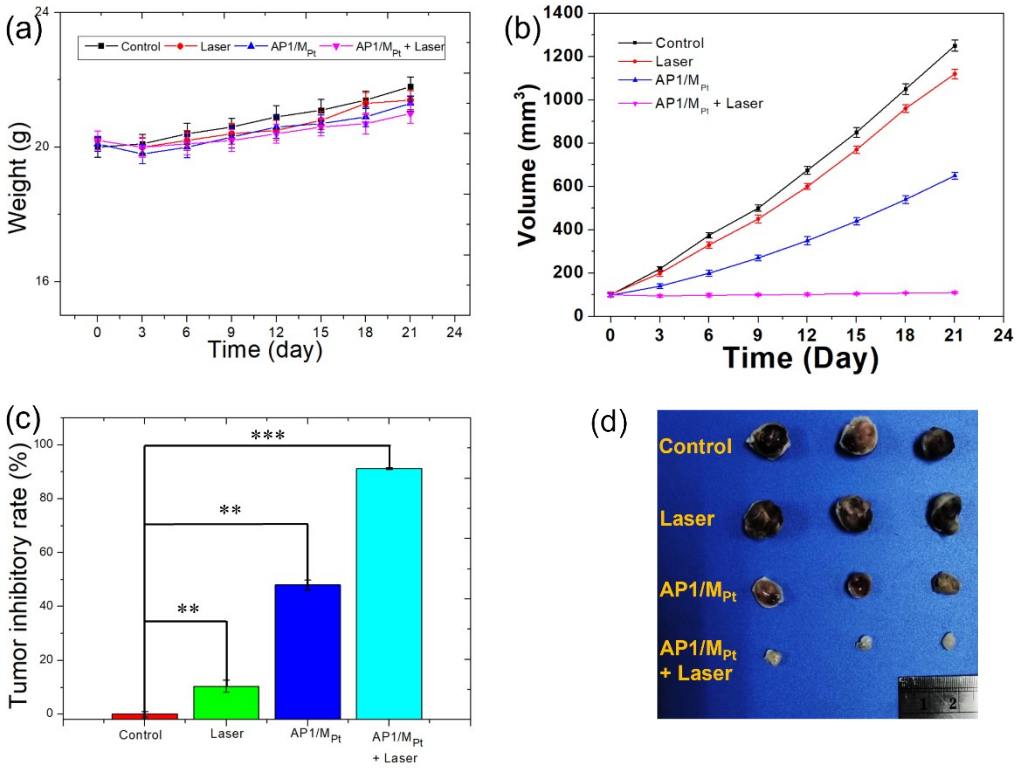
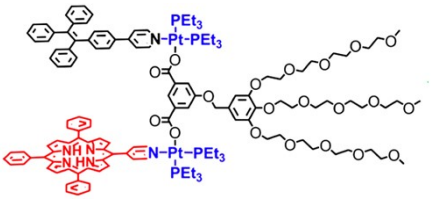
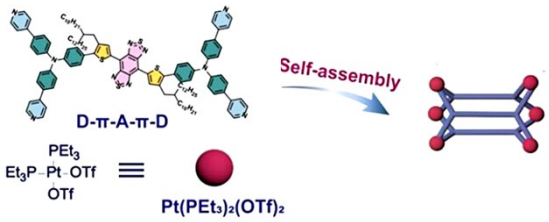
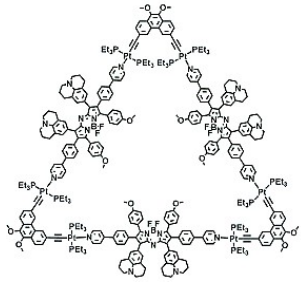
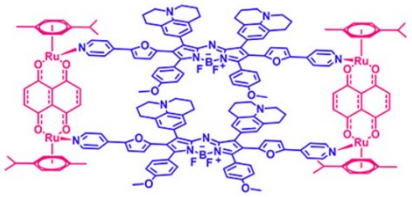
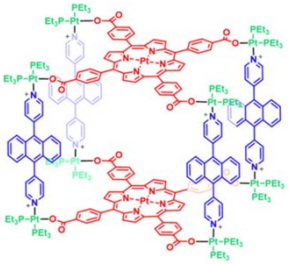
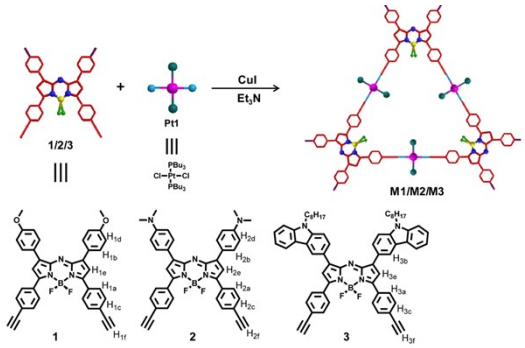


Fig. S12 (a) Body weight changes of mice intravenously injected with different treatments. (b) Time-dependent tumor growth profiles under different treatments. (c) Tumor inhibitory rates (TIR) after different treatment. (d) Ex vivo tumor images isolated from HeLa-tumor-bearing mice at end of the treatments. *$P < 0.1$, **$P < 0.01$, and ***$P < 0.001$.

Table S1

Structure	property	Ref
	Advantages: amphiphilic, AIE, CT and PDT. Disadvantages: difficult to synthesis.	S3
	Advantages: NIR-II imaging, PTT, CT, PAI and PDT. Disadvantages: difficult to synthesis.	S4
	Advantages: NIR-II emission, activates deep tumor immunity. Disadvantages: The formation of a uniform assembly is rather difficult.	S5
	Advantages: A Lysosome-Targeted, NIR-Light-Activated. Disadvantages: The formation of a uniform assembly is rather difficult.	S6
	Advantages: Sensitive Oxygen Response for Imaging of Hypoxia and Imaging-Guided Chemotherapy. Disadvantages: No PTT.	S7
	Advantages: PTT, PDT, formation of uniform assemblies. Disadvantages: No CT, The fluorescence is relatively weak.	S8

S3. Yang Wang, Ruowen Tang, Di Wang, Jian Wang, Yuying Huang, Yue Ding, Bing Lu, Yan Sun, Peter J. Stang, and Yong Yao, *Inorg. Chem.*, **2023**, *62*, 1786–1790.

S4. Jingpei Zhang, Wei Ma, Boyu Yang, Tingyu Shi, Shenglong Liao, Yang Li, and Shouchun Yin, *ACS Appl. Mater. Interfaces*, **2024**, *16*, 69028–69044.

S5. Chonglu Li, Le Tu, Yuling Xu, Meiqin Li, Jiaxing Du, Peter J. Stang, Yan Sun, Yao Sun, *Angew. Chem. Int. Ed.*, **2024**, *63*, e202406392.

S6. Le Tu, Chonglu Li, Qihang Ding, Amit Sharma, Meiqin Li, Junrong Li, Jong Seung Kim, and Yao Sun, *J. Am. Chem. Soc.*, **2024**, *146*, 8991–9003.

S7. Huangtianzhi Zhu, Qi Li, Bingbing Shi, Fujing Ge, Yuezhou Liu, Zhengwei Mao, Hong Zhu, Sheng Wang, Guocan Yu, Feihe Huang, Peter J. Stang, *Angew. Chem. Int. Ed.*, **2020**, *59*, 20208-20214.

S8. Guo Li, Xiangxiang Zhang, Weili Zhao, Weiwei Zhao, Feiyang Li, Kang Xiao, Qi Yu, Shujuan Liu, and Qiang Zhao, *ACS Appl. Mater. Interfaces*, **2020**, *12*, 20180–20190.