

Supporting Information

Precisely-assembled Nanoparticles Against Cisplatin-Resistance via Cancer-specific Targeting of Mitochondria and Imaging Guided Chemo-Photothermal Therapy

Gang-Gang Yang,[†] Zheng-Yin Pan,[†] Dong-Yang Zhang, Qian Cao,
Liang-Nian Ji, and Zong-Wan Mao**

MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of
Chemistry, Sun Yat-Sen University, Guangzhou 510275 P. R. China

E-mail: caoqian3@mail.sysu.edu.cn (Q. Cao); cesmzw@mail.sysu.edu.cn (Z.-W Mao)

Experimental section

Materials: Cisplatin were purchased from energy chemical, 1-Adamantyl Isocyanate were buy from Beijing Enochai Technology Co., Ltd. Biotin polyethylene glycol amino group (Biotin-PEG-NH₂, M_w=5000) were Purchase from Shanghai Ponsure Biotech, Inc. IR780 iodide and 4-mercaptophenylacetic acid were Purchase from J&K Chemical Technology. Mono-(6-(1,6-hexamethylenediamine)-6-deoxy)-beta-Cyclodextrin [NH₂-(CH₂)₆-βCD] was supplied by Shandong Zhiyuan Biotechnology Co.,Ltd. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sangon Biotech, Shanghai), LTG and MTG (LysoTracker Green and Mitotracker Green, Life Technologies, USA), DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate, Beyotime Biotechnology) and Annexin V-FITC/PI apoptosis detection kit was bought from Beyotime Biotechnology. Other compounds were direct used without further purification.

General Instruments: ¹H NMR spectra were tested on 400 Nuclear Magnetic Resonance Spectrometer of Japan. ESI-MS was measured on LTQ XL system (Thermo, USA). UV-vis spectra were recorded by a UV-3600 spectrophotometer (USA). The NIR emission were tested on FLS 980 (Edinburgh Instrument, UK). The platinum element was determined by iCAP RQ. High performance liquid chromatography (HPLC) were measured by HP1100, USA. Morphology of the nanoparticles was measured by an atomic force microscope (AFM, Bruker Multimode). The picture of TEM was recorded by FEI Tecnai G2 (Holland).

Cell culture conditions: Cisplatin-resistant cancer cells A549R were purchased from Zhong Qiao Xin Zhou Biotechnology Co.,Ltd (Shanghai) and then cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Invitrogen) and cisplatin 5 μM. The cells were maintained in an atmosphere of 5 % CO₂ and 95 % air at 37 °C.

MTT assay: A549R cells were layed in 96-well plates. After 24 h of culture, different concentrations of cisplatin, Pt(IV)-NPs, Ad-Pt(IV)-PEG-Biotin, IR780-NPs and CD-IR780 were added for 44 h incubation, then MTT was mixed then incubate

for 4 h. For the PTT group, irradiation was conducted using an 808 nm source (1.0 W cm⁻², 10 min). Then 150 µL of DMSO was mixed to each well before removed the medium. Then measured the absorbance of 595 nm to calculated the cell viability (Infinite F200, Tecan, Switzerland). The drug combination index (CI) value of **Pt(IV)-NPs** was calculated by using the following equation¹:

$$CI = \frac{IC_{A+B}}{IC_A} + \frac{IC_{A+B}}{IC_B}$$

In which IC_{A+B} is the IC₅₀ value of **Pt(IV)-NPs** + light, IC_A and IC_B are the IC₅₀ values of **Pt(IV)-NPs** and **IR780-NPs** + light, respectively. CI value <1 means the presence of synergistic effect.

Statistical Analysis: The significance of several experimental results was analyzed by using the analysis of variance (ANOVA) test. Probabilities p < 0.05 (*) and p < 0.002 (**), ***P<0.001 were marked in Figure Sure Ss and 0.05 was chosen as the significance level.

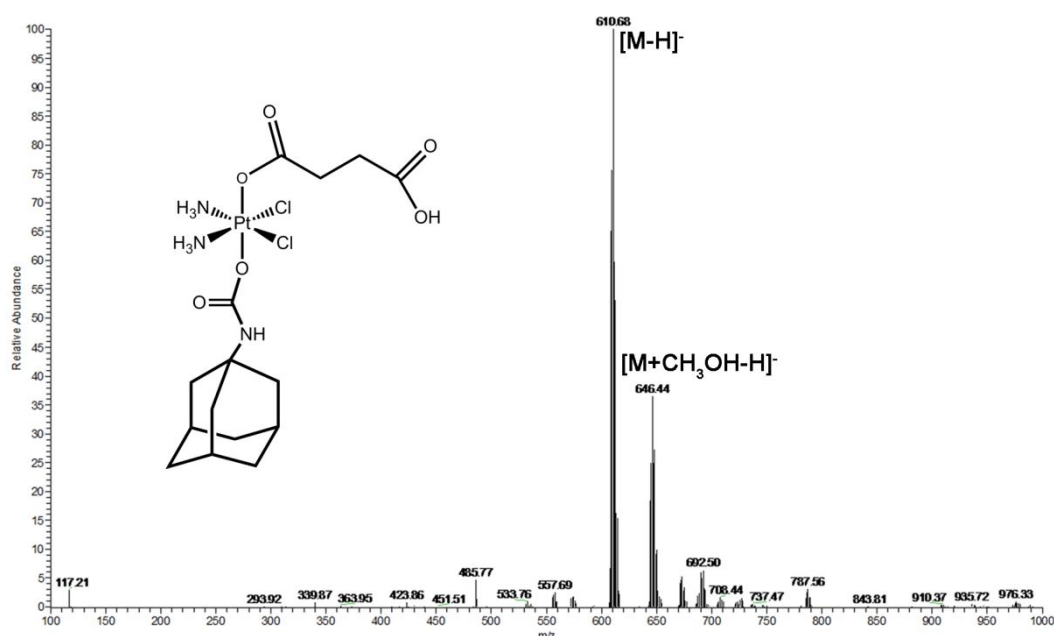
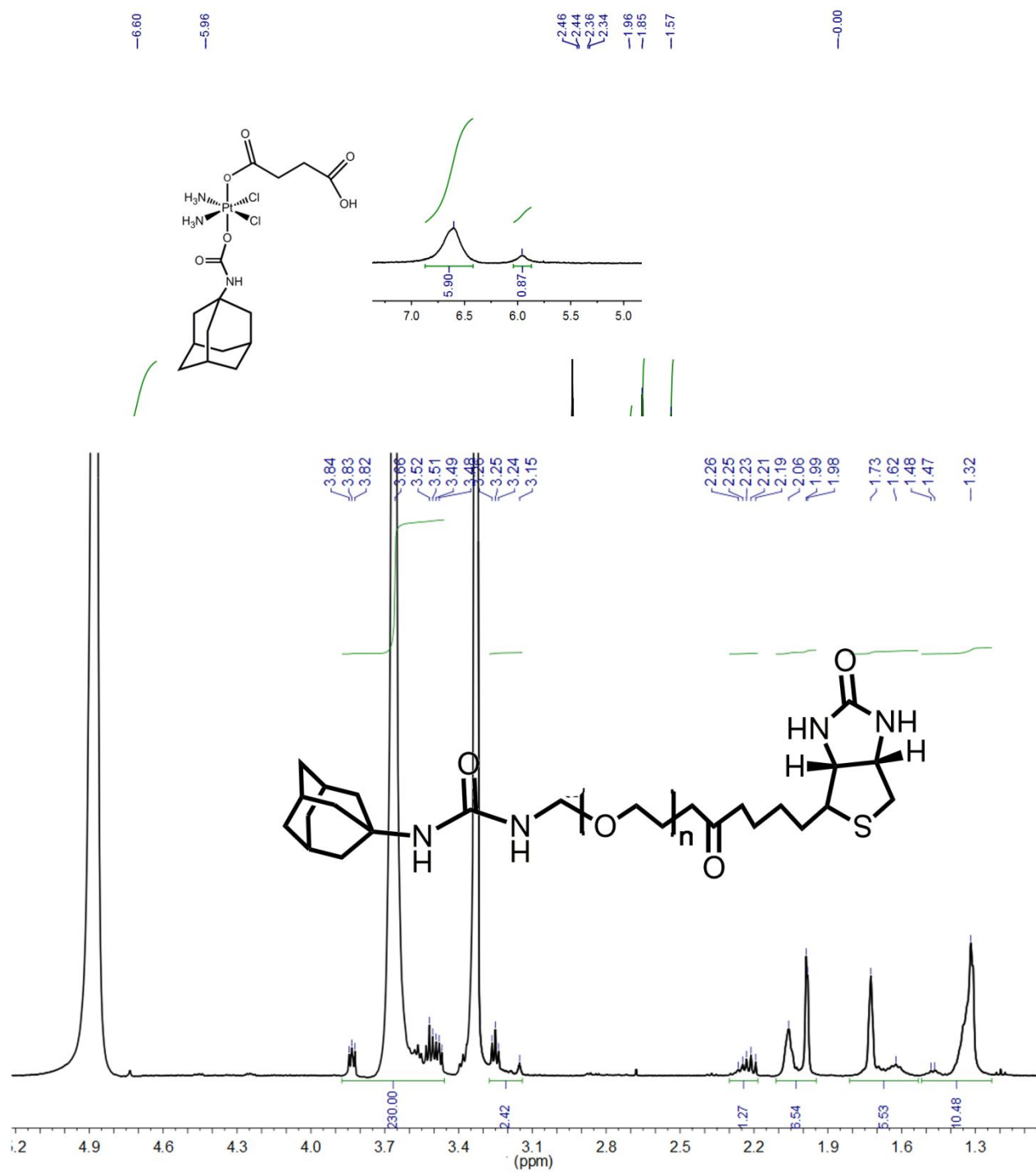


Figure S1. ESI-MS spectrum of **Ad-Pt(IV)-COOH** in CH₃OH.



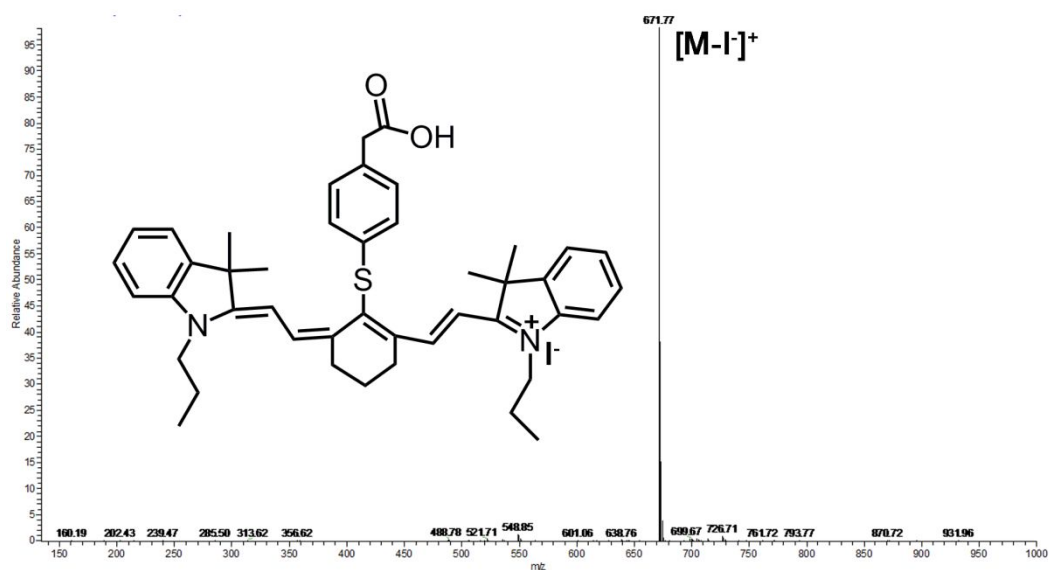


Figure S4. ESI-MS spectrum of IR780-COOH in CH₃OH.

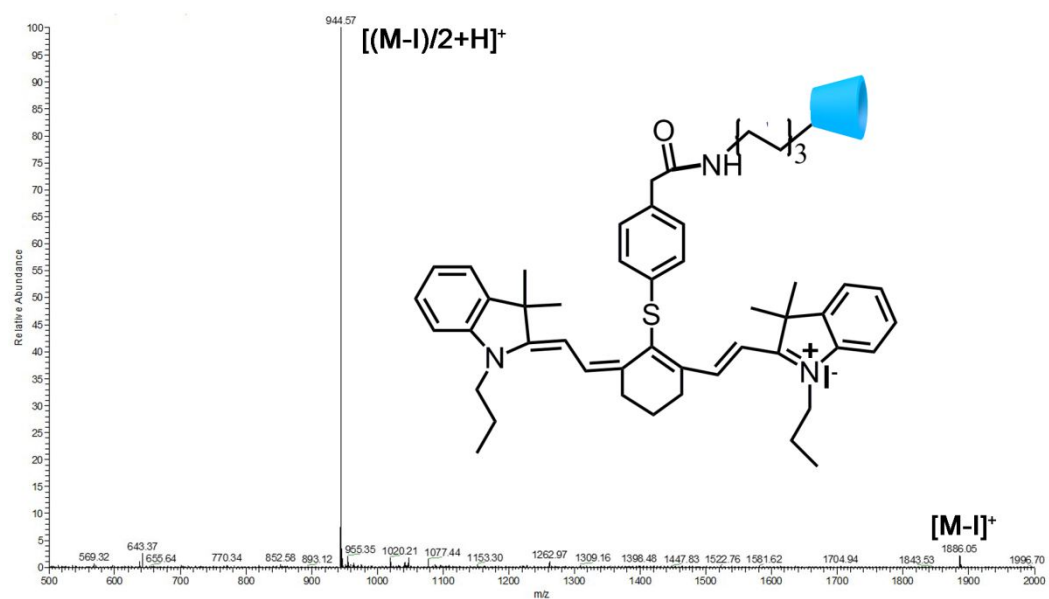


Figure S5. ESI-MS spectrum of CD-IR780 in CH₃OH.

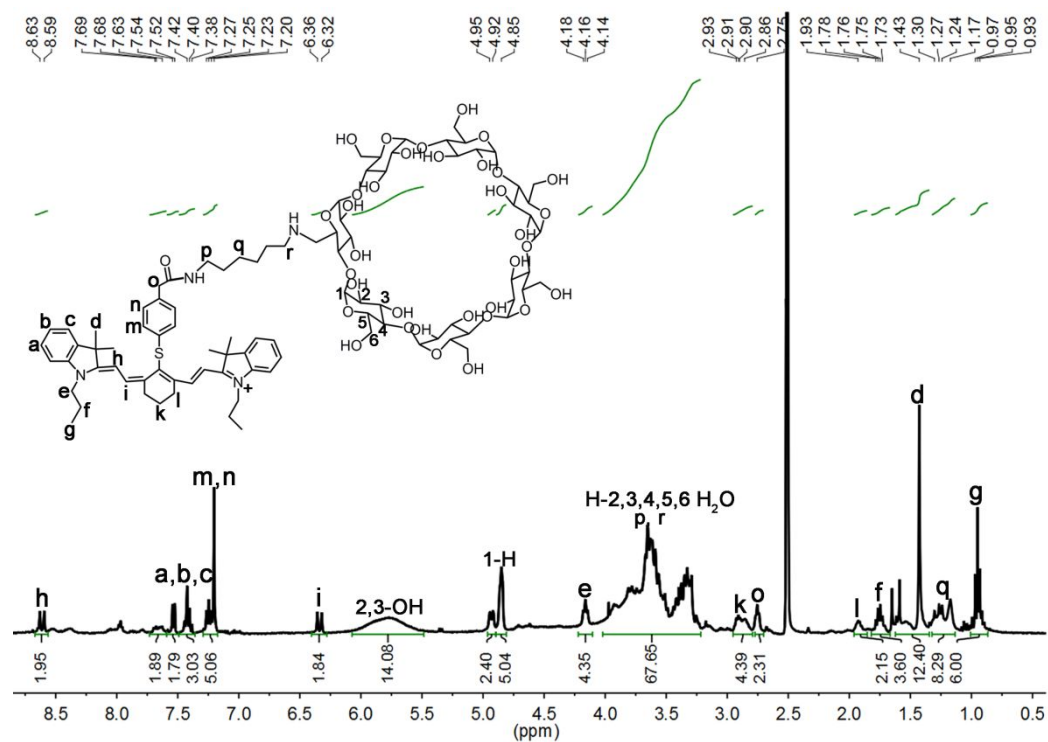


Figure S6. ^1H NMR spectrum of **CD-IR780** in $\text{DMSO}-d_6$.

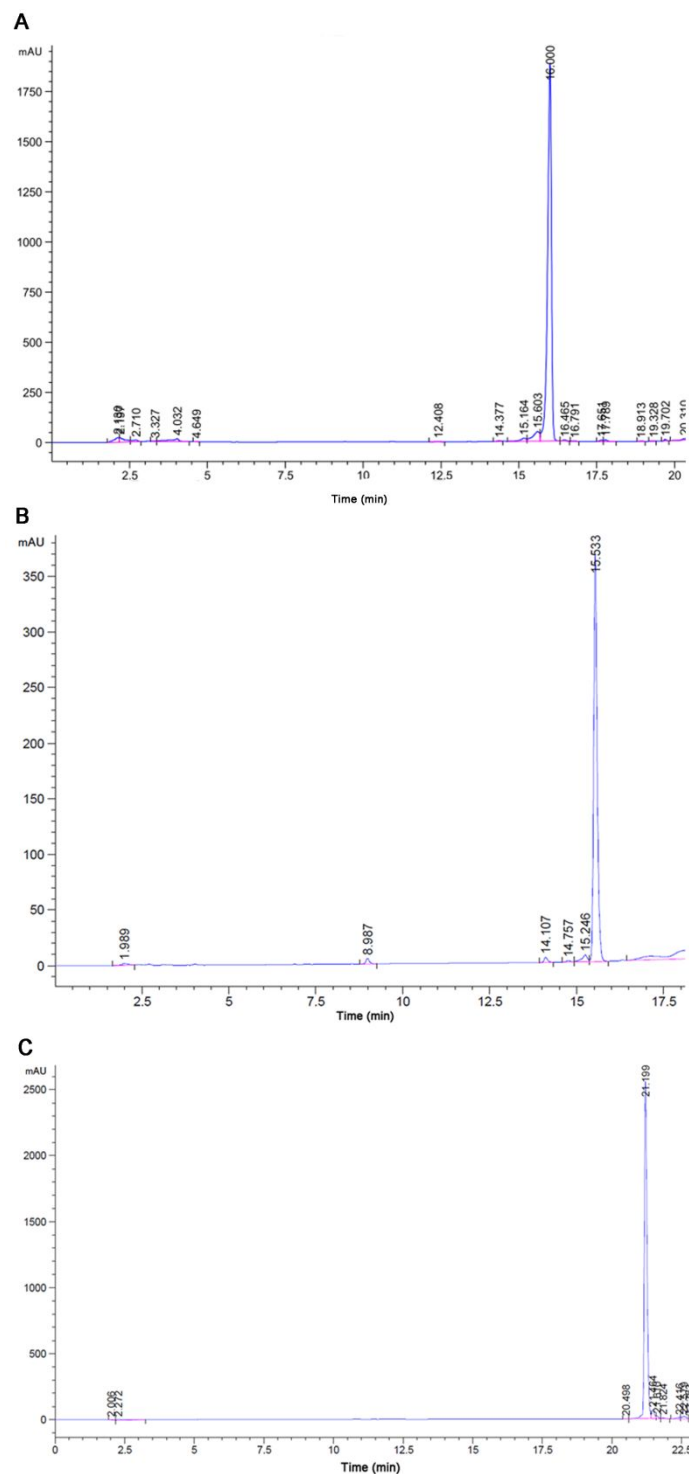


Figure S7. (A) HPLC (254 nm) chromatograms of **Ad-Pt(IV)-COOH**, (B) HPLC chromatograms of **Ad-Pt(IV)-PEG-Biotin** (254 nm) and (C) **CD-IR780** (790 nm). Solvent A ($\text{H}_2\text{O}+0.1\%$ TFA) and solvent B (methanol+0.1% TFA) were used for a gradient elution at a flow rate of 0.5 mL/min. The HPLC elution program was as follows: 5 % B (0 min)→90% B (linear increase in 15 min for **Ad-Pt(IV)-COOH** and **Ad-Pt(IV)-PEG-Biotin** and in 20 min for **CD-IR780**, respectively) →90% B (5 min). The injection volume was 10 μL .

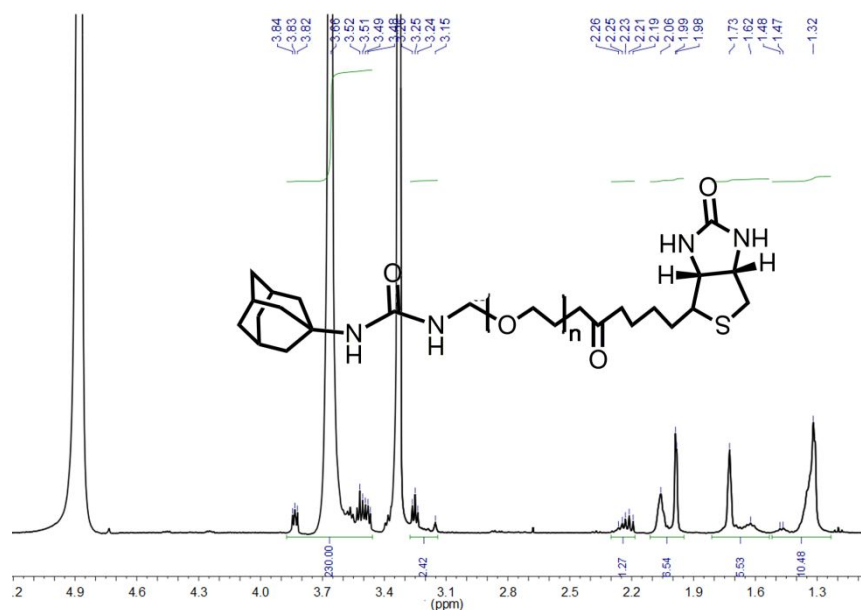


Figure S8. ^1H NMR spectrum of **Biotin-PEG-AD** in $\text{Methanol-}d_4$.

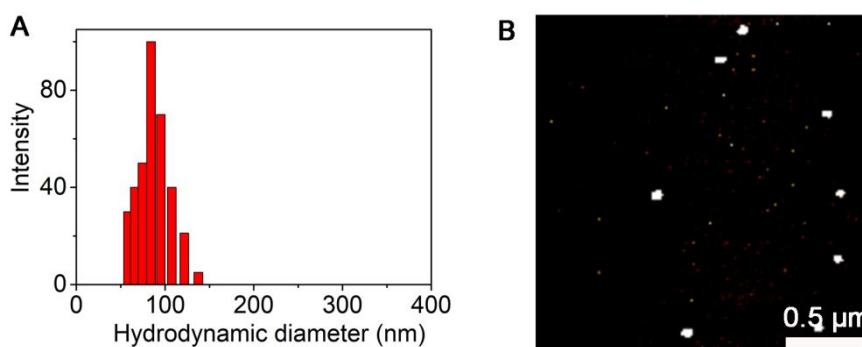


Figure S9. A-B) Dynamic laser scattering and atomic force microscopy of **IR780-NPs**.

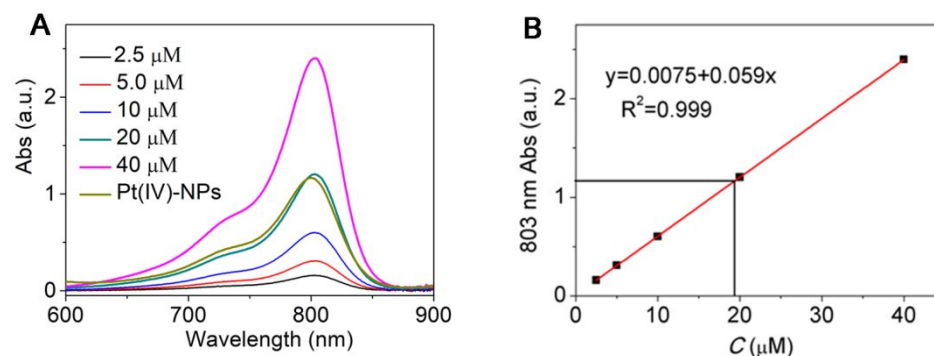


Figure S10. (A) The UV-Vis absorption spectra of **CD-IR780** at different concentrations and **Pt(IV)-NPs** (Pt concentration of $20\ \mu\text{M}$) in DMF solvent, (B) the linear fitting of absorbance at 803 nm. Accordingly, the **CD-IR780** content in **Pt(IV)-NPs** (Pt concentration of $20\ \mu\text{M}$) can be determined to be $19.3\ \mu\text{M}$, thus giving 1:1 molar ratio of IR780 : Pt in **Pt(IV)-NPs**.

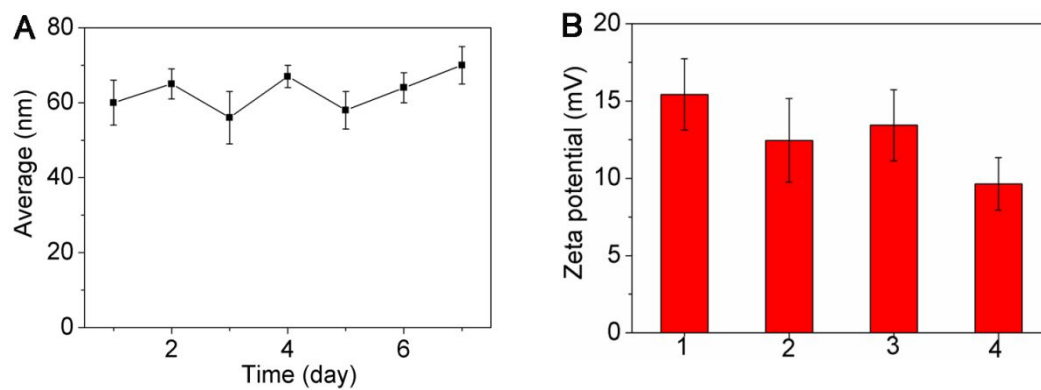


Figure S11. (A) Changes in the hydrodynamic diameters of **Pt(IV)-NPs** in water; (B) Zeta potentials of **Pt(IV)-NPs** in different solutions after 7 days, 1-water, 2-PBS, 3-FBS(10%)+PBS and 4-1640 culture medium.

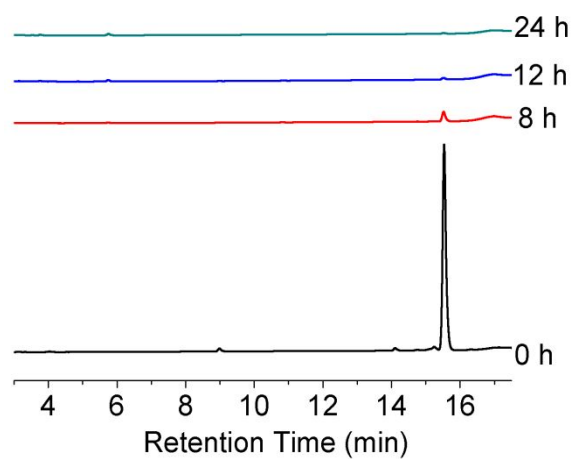


Figure S12. Reduction of **Ad-Pt(IV)-PEG-Biotin** in PBS/MeOH (9/1, v/v) in the presence of ascorbate (20 mM) monitored by HPLC, The injection volume was 5 μ L.

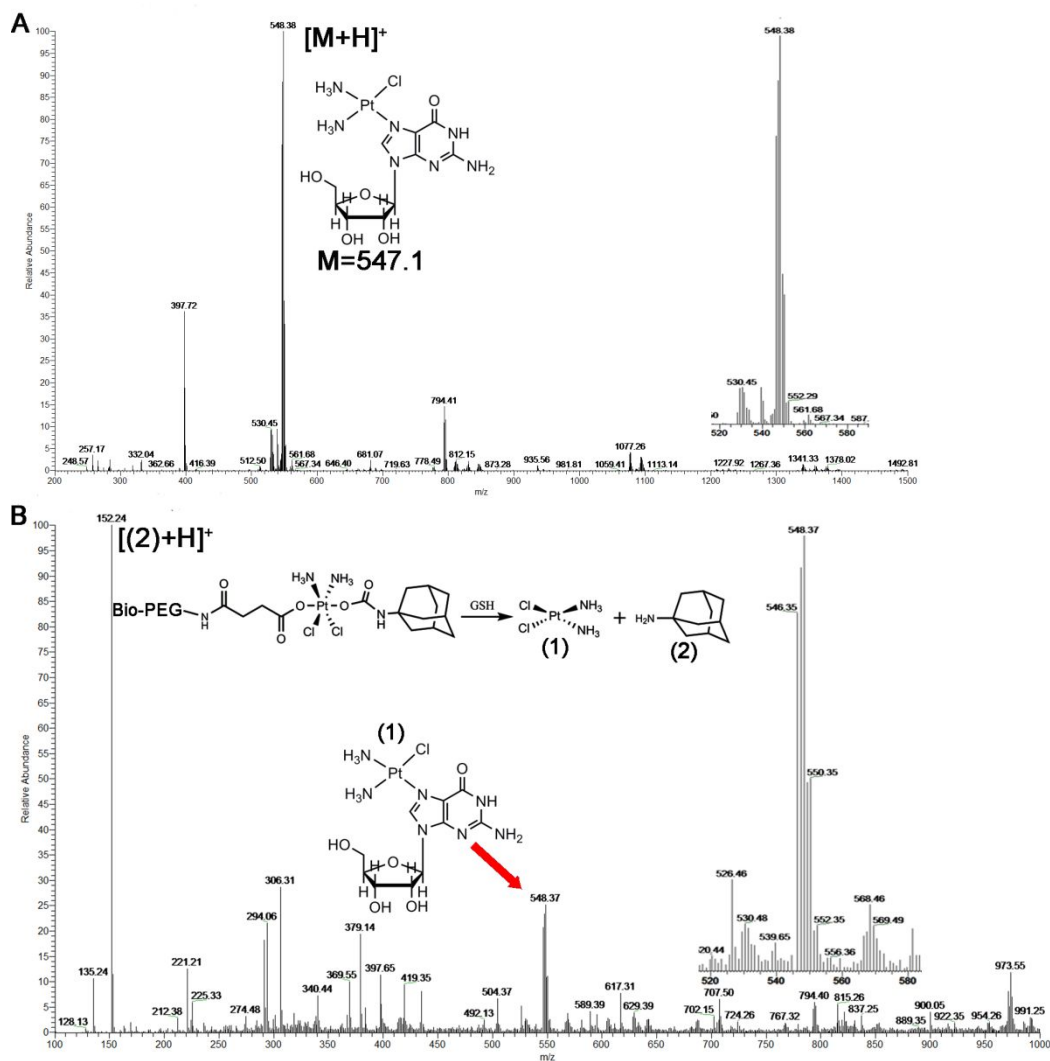


Figure S13. ESI-MS of (A) cisplatin and (B) Pt(IV)-NPs treated with glutathione (10 mM) after 48 h of incubation at 37 °C in water.

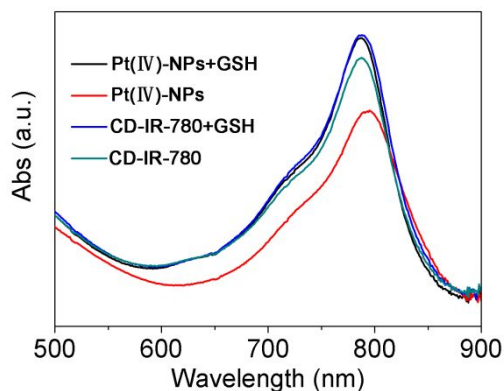


Figure S14. UV-Vis spectra of Pt(IV)-NPs and CD-IR780 measured in PBS with or without GSH (10 mM, 24 h).

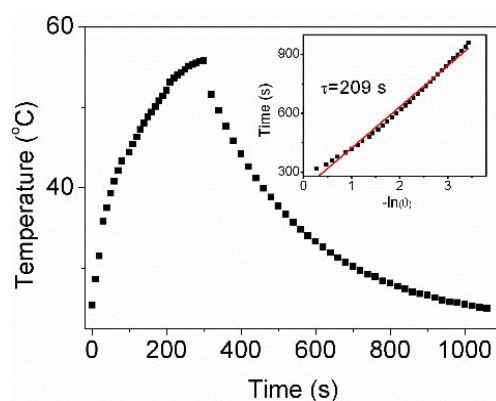


Figure S15. Photothermal conversion efficiency of Pt(IV)-NPs.

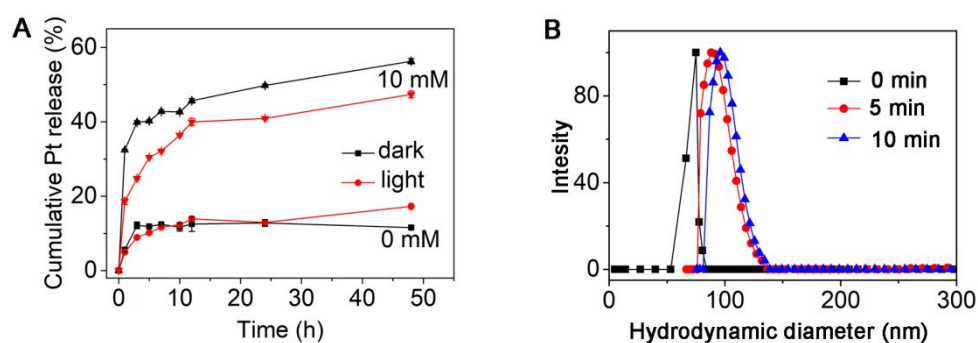


Figure S16. A) Pt release profiles of Pt(IV)-NPs in water with glutathione (GSH) after 808 nm light (1.0 W cm^{-2} 10 min); B) Dynamic laser scattering change of Pt(IV)-NPs with 808 nm light (1.0 W cm^{-2} 10 min).

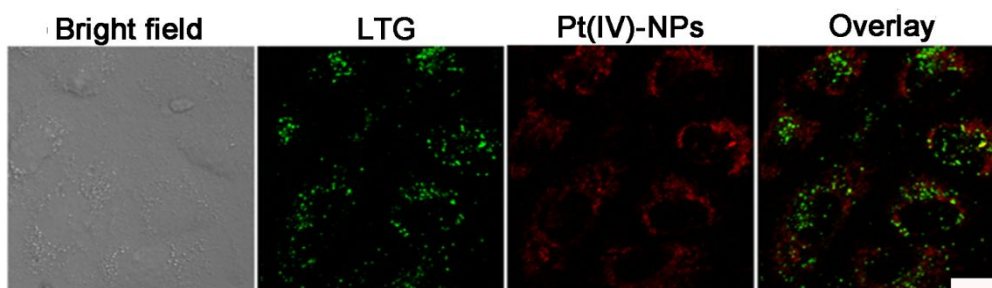


Figure S17. Co-localization of Pt(IV)-NPs with lysosome-specific stain LTG in A549R cells. LTG: $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 520 \pm 20 \text{ nm}$; Pt(IV)-NPs: $\lambda_{\text{ex}} = 633 \text{ nm}$, $\lambda_{\text{em}} = 720 \pm 20 \text{ nm}$. Scale bar: $20 \mu\text{m}$.

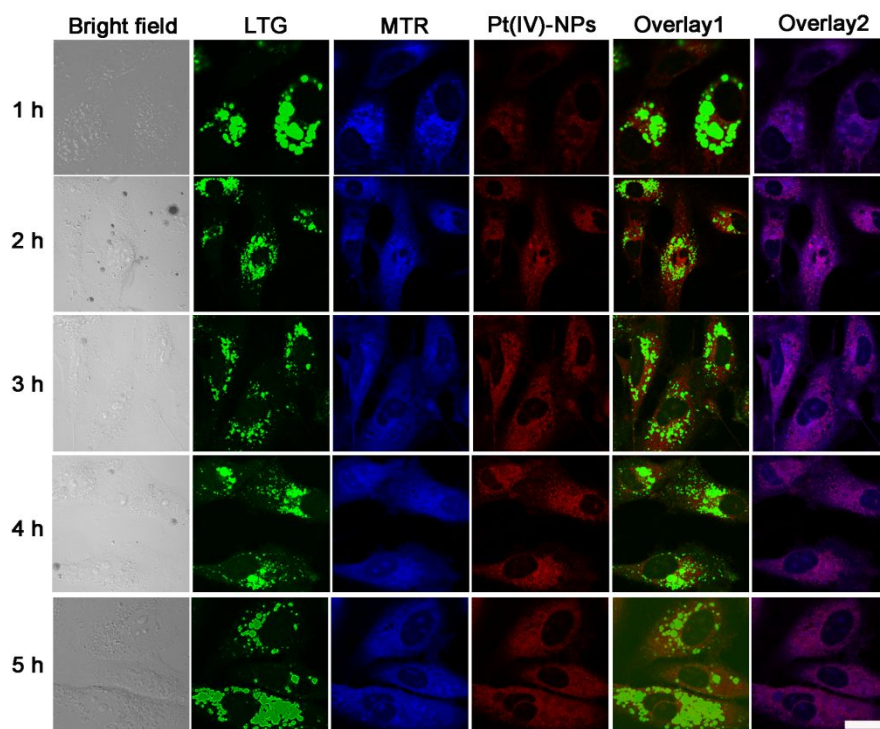


Figure S18. Real-time distribution of **Pt(IV)-NPs** in A549R cells and its colocalization with mitochondria-specific stain 0.5 h and lysosome-specific stain 0.5 h. LTG: $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 520 \pm 20 \text{ nm}$; MTR: $\lambda_{\text{ex}} = 575 \text{ nm}$, $\lambda_{\text{em}} = 600 \pm 20 \text{ nm}$; **Pt(IV)-NPs**: $\lambda_{\text{ex}} = 633 \text{ nm}$, $\lambda_{\text{em}} = 720 \pm 20 \text{ nm}$. Scale bar: 20 μm .

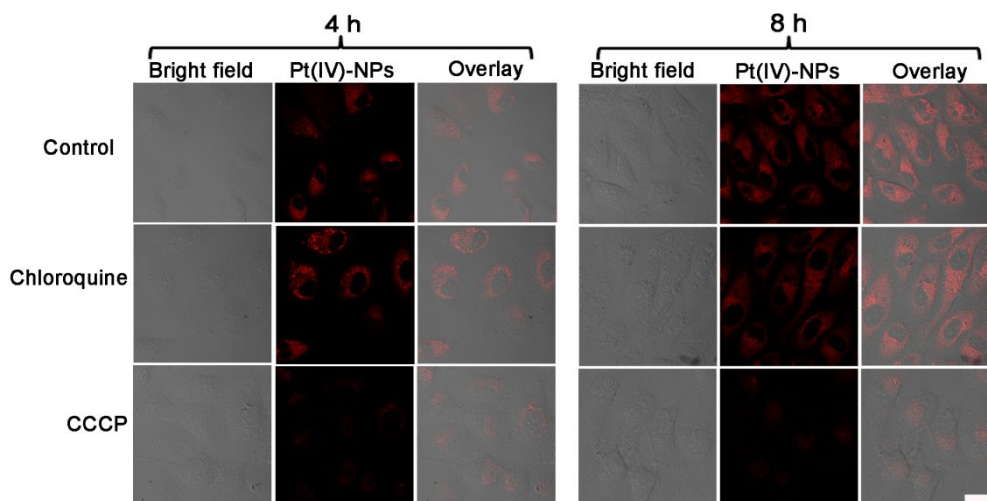


Figure S19. Cellular uptake of **Pt(IV)-NPs** (10 μM) in A549R cells pretreated with chloroquine (50 μM , 0.5 h) and CCCP chloroquine (10 μM , 1 h), respectively, Scale bar: 20 μm .

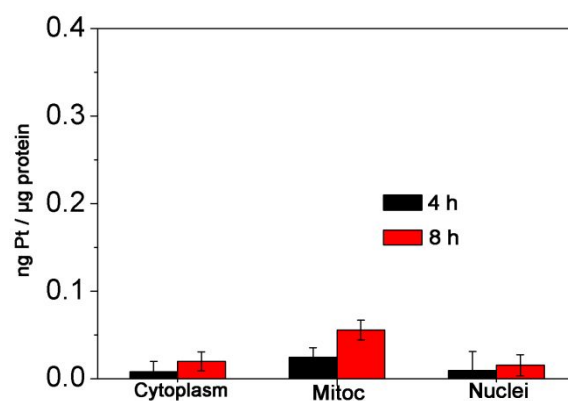


Figure S20. The accumulation of Pt contents in A549R cells after incubating with Ad-Pt(IV)-PEG-Biotin (10 μ M) for 4 and 8 h, respectively.

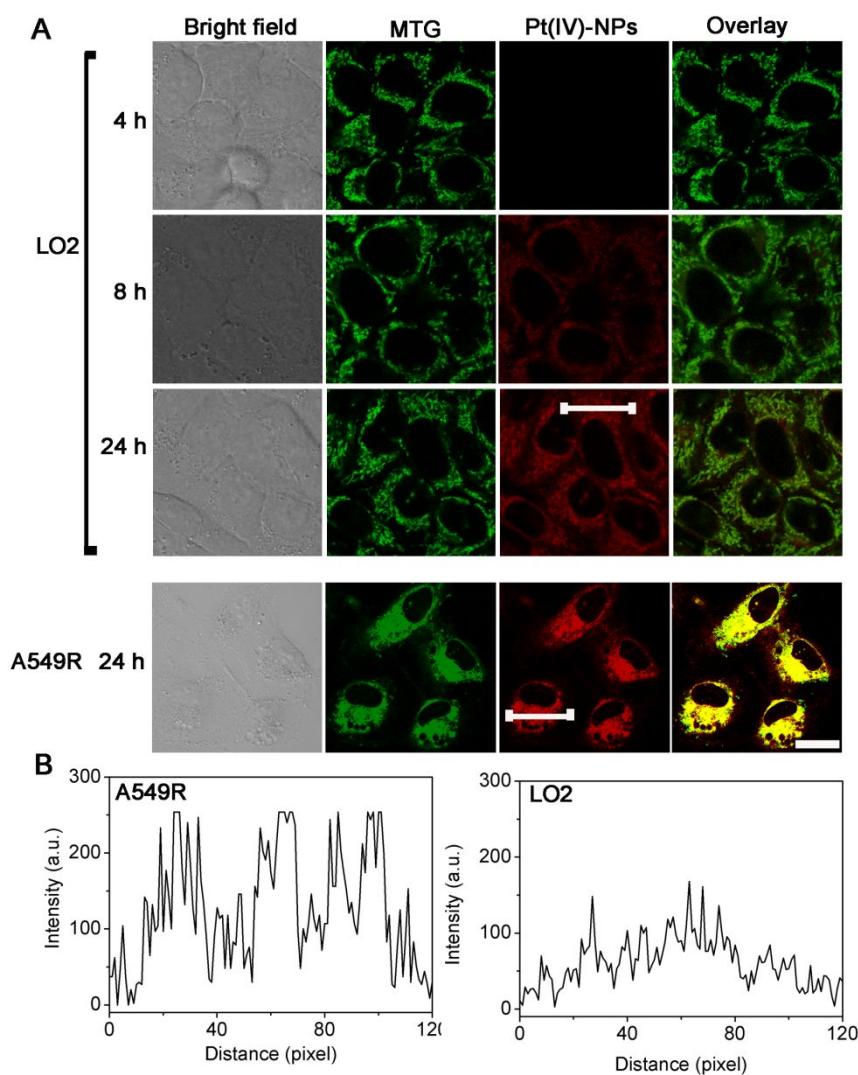


Figure S21. (A) Cellular uptake and distribution of Pt(IV)-NPs (10 μ M) in LO2 cells compared with that in A549R cells, (B) The fluorescence intensity of A549R and LO2 at 24 h, Scale bar: 20 μ m.

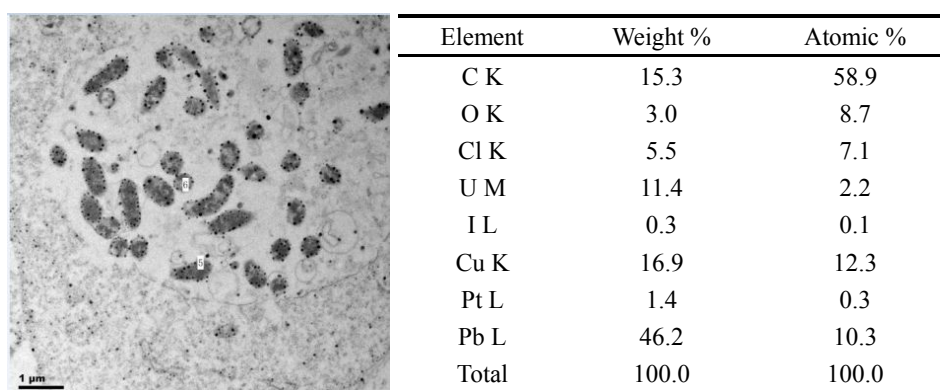


Figure S22. Bio-TEM image (left) and distribution of elements in energy spectrum analysis (right) of A549R cells treated with **Pt(IV)-NPs** for 12 h.

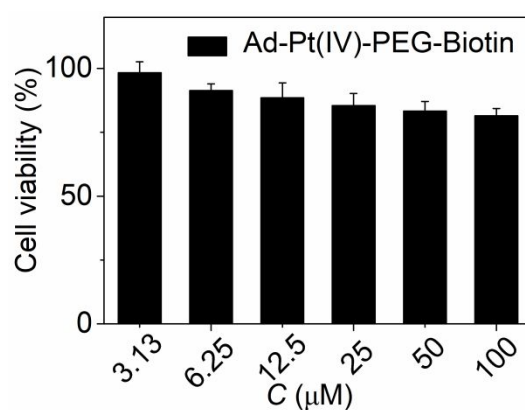


Figure S23. Cell viability of A549R cells after 48 h treatment with **Ad-Pt(IV)-PEG-Biotin** in the dark.

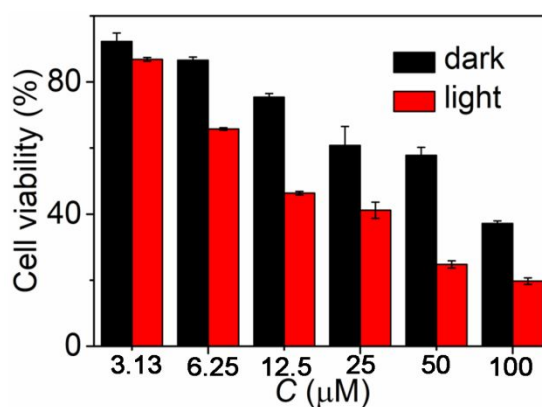


Figure S24. Cell viability of A549R cells after 48 h treatment with **IR780-NPs** with 808 nm (1 W/cm^2) light or without.

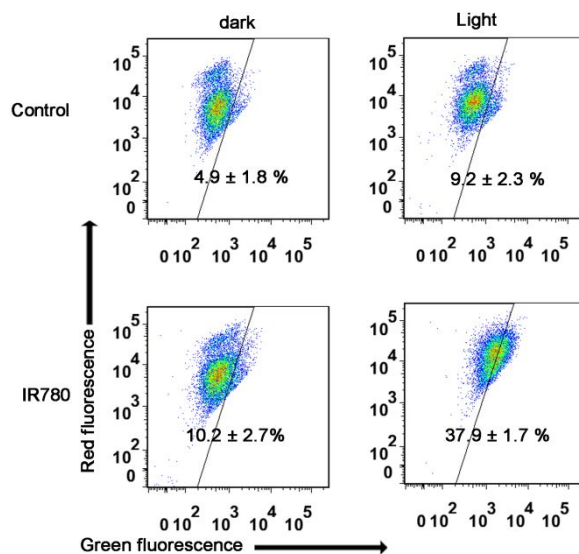


Figure S25. Mitochondrial membrane potential loss of A549R cells after treatment with IR780 (9 μ M, 36 h).

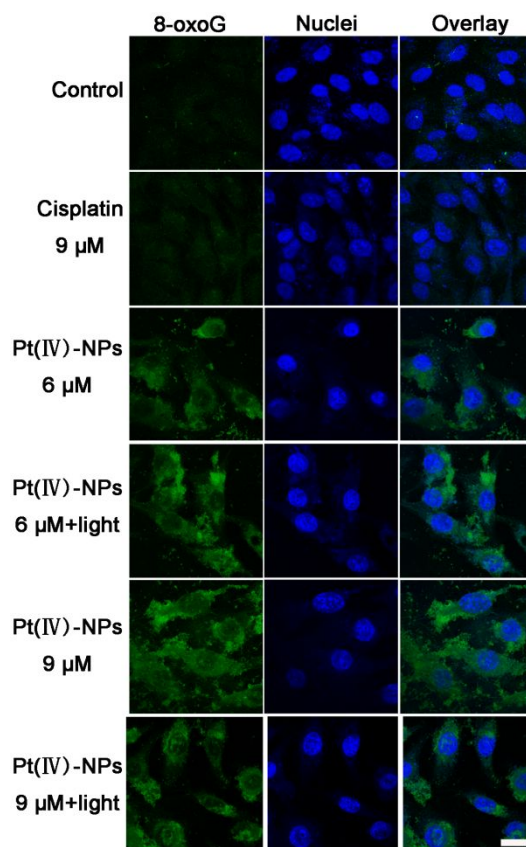


Figure S26. Immunofluorescence assay of 8-oxoG (8-oxoguanine as a DNA damage marker) in A549R cells treated with cisplatin and Pt(IV)-NPs at the indicated concentrations for 36 h with or without 808 nm irradiation (1.0 W cm^{-2} , 10 min). Green channel: $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 520 \pm 20 \text{ nm}$; Blue channel: $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 450 \pm 20 \text{ nm}$, Scale bar: 20 μ m.

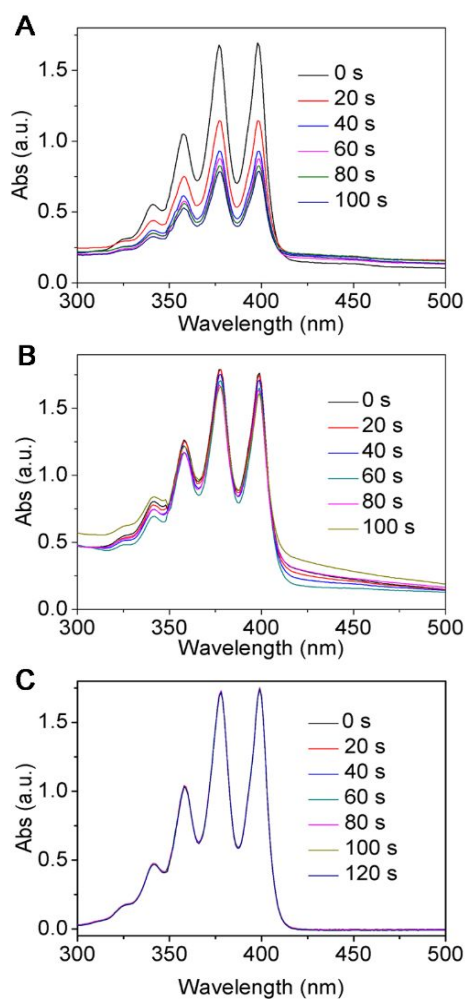


Figure S27. The ROS produced by (A) IR780, (B) **Pt(IV)-NPs** and (C) ABDA under 808 nm (1.0 W/ cm²) light.

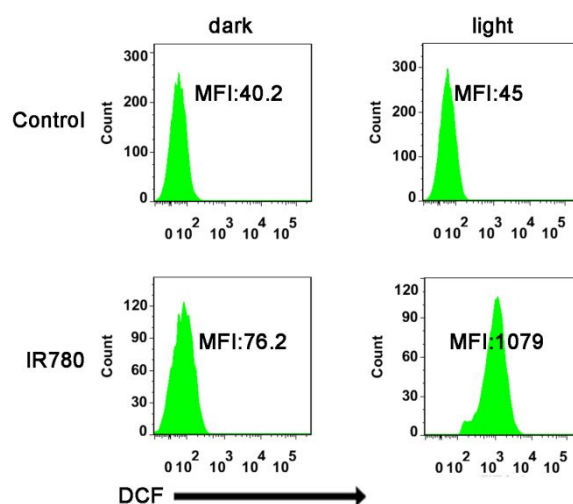


Figure S28. Flow cytometry of ROS levels detected by DCFH-DA staining with **IR780** (9 μM) for 36 h.

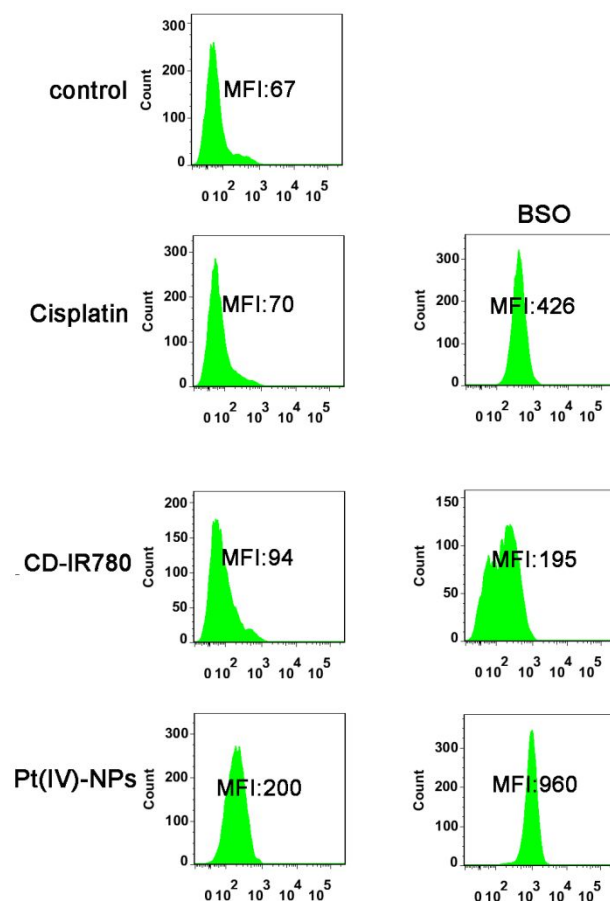


Figure S29. Flow cytometry of ROS levels detected by DCFH-DA staining with cisplatin, **CD-IR780** and **Pt(IV)-NPs** (5 μ M) for 36 h. A549R cells were pretreated with or without BSO (a GSH scavenger, 50 μ M, 48 h).

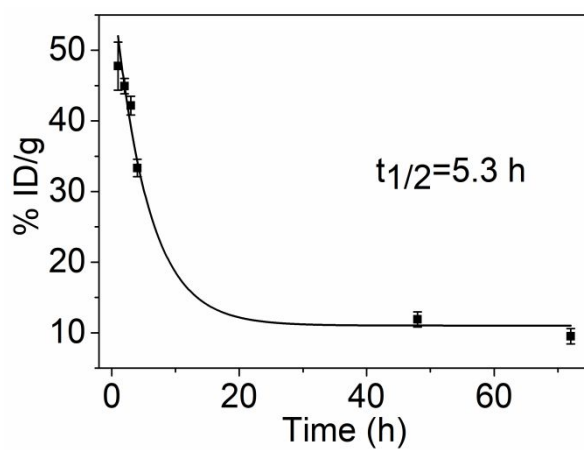


Figure S30. Blood circulation time of **Pt(IV)-NPs** after i.v. administration.

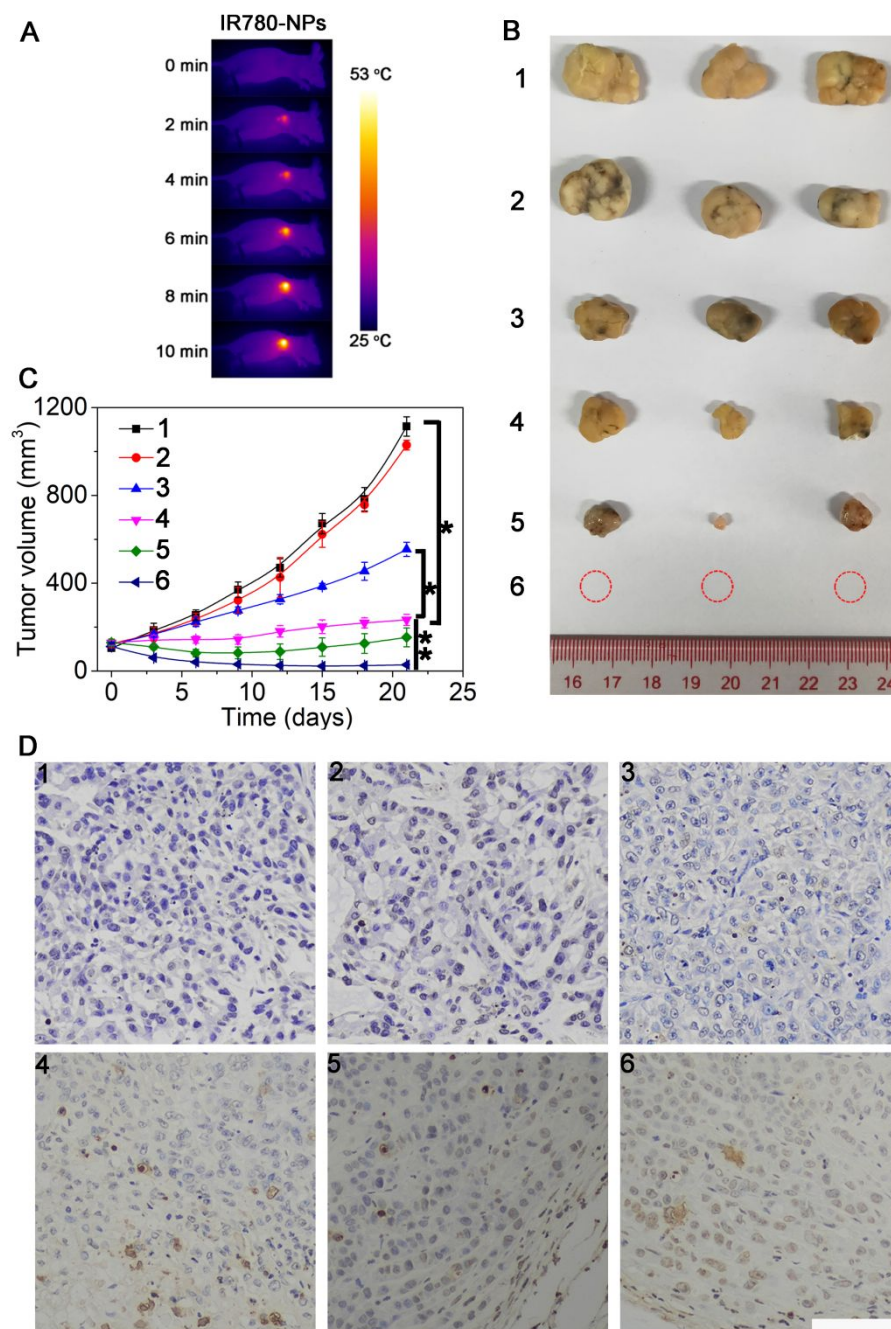


Figure S31. A) Photothermal picture of mice bearing A549R tumors under 808 nm light (1.0 W cm^{-2}); B) Digital photos of resected tumors after 21 days treatment C) Changes in the tumor volumes of mice bearing A549R tumors after i.v. injection of (1) PBS, (2) PBS + light, (3) cisplatin, (4) Pt(IV)-NPs, (5) IR780-NPs + light, (6) Pt(IV)-NPs + light; D) TUNEL staining of resected tumors after 21 days treatment. Light: 808 nm light (1.0 W cm^{-2} , 10 min); Scale bar: 100 μm .

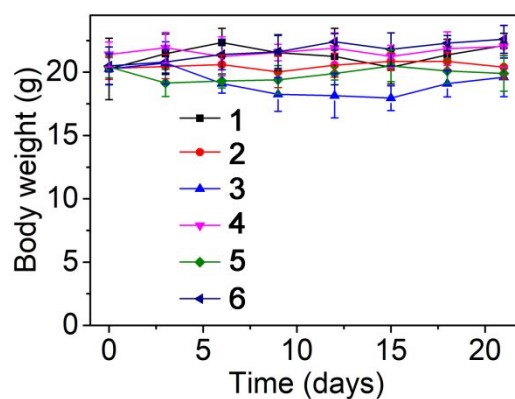


Figure S32. Changes in the body weights of mice bearing A549R tumors after i.v. injection of (1) PBS, (2) PBS + light, (3) cisplatin, (4) **Pt(IV)-NPs**, (5) IR780-NPs + light, (6) **Pt(IV)-NPs** + light;

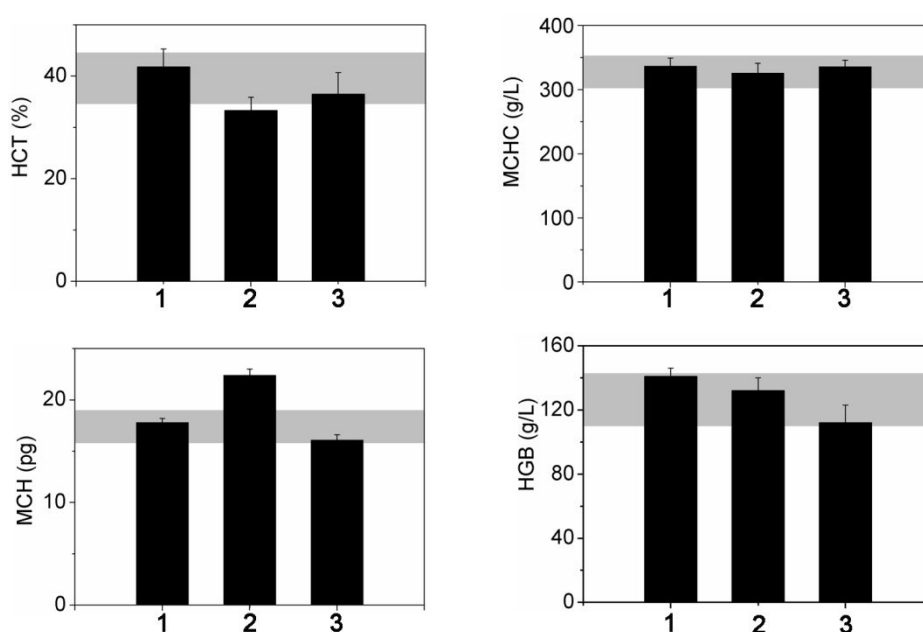


Figure S33. Blood biochemistry/complete blood panel analysis data of mice bearing A549R tumors conducted 21 days post PBS (1), cisplatin (2), **Pt(IV)-NPs** (3) injection.

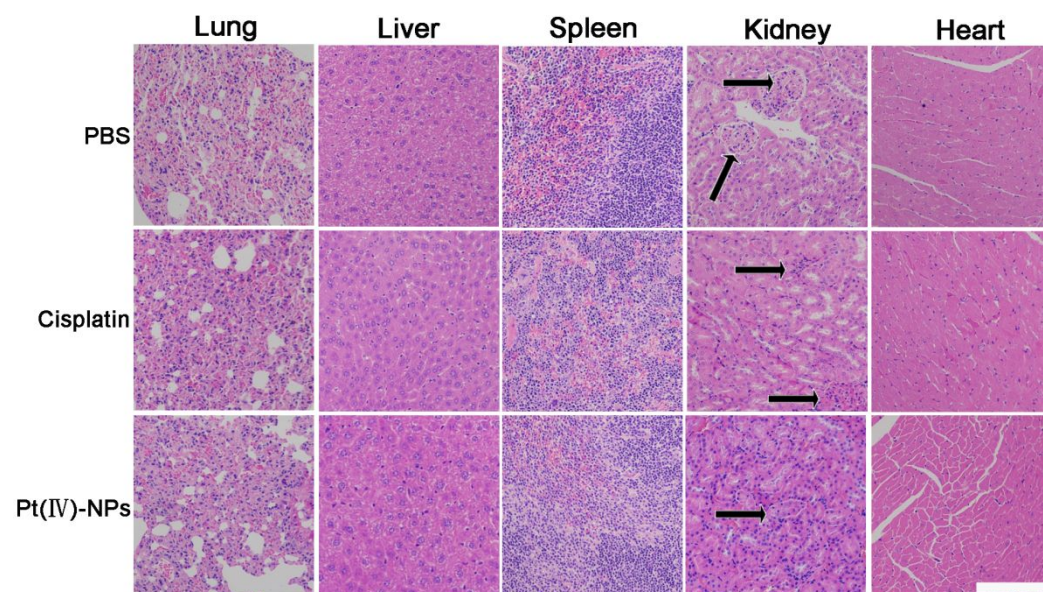


Figure S34. Haematoxylin and eosin staining of major organ sections harvested from mice 21 days post PBS, cisplatin, or **Pt(IV)-NPs** treatment. The black arrows indicate glomerular injury. Bar represents 200 μm .

Table S1. The levels of albumin (ALB), total protein (TP), creatinine (CRE) and blood urea nitrogen (BUN) in the serum of mice 21 days post PBS, cisplatin, or **Pt(IV)-NPs** treatment.

Drugs	ALB(U/L)	TP (U/L)	CRE (U/L)	BUN (mg/dl)
PBS	25.13	48.21	15.59	36.51
Cisplatin	41.70	108.19	415.26	1126
Pt(IV)-NPs	26.43	65.56	81.16	147.59

References

(1) Hu, Q.; Sun, W.; Wang, C.; Gu, Z., Recent Advances of Cocktail Chemotherapy by Combination Drug Delivery Systems. *Adv. Drug Deliv. Rev.* **2016**, 98, 19-34.