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Targeted delivery of photoactive diazido Pt^{IV} complexes conjugated with fluorescent carbon dots

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1 Chemicals and General Techniques:

All reagents were purchased commercially and used without further purification unless otherwise noted. Cisplatin was prepared according to literature.[S1]

^{1}H NMR spectra were recorded on Bruker AV400 spectrometer (400 MHz). ESI mass spectra were performed on Micromass LCTTM mass spectrometer. Transmission electron microscopy (TEM) was performed on a JEOL JEM-2011 transmission electron microscope operating at 100 kV. Powder X-ray diffraction (XRD) analysis was performed on Rigaku-2550 D/maxVB/PC X-Ray Diffractomer. XPS data were collected on a Thermo Escalab 250 XPS instrument with a monochromatic Al Kα X-ray source ($h\nu$=1486.6 eV). All binding energies were referenced to the C1s peak (284.6 eV) arising from adventitious carbon. FTIR spectra were recorded on a Shimadzu Fourier transform infrared spectrometer (IRPrestige-21). UV-vis absorption spectra were recorded on a Shimadzu UV-visible spectrophotometer (UV-2600). Zeta potential was measured by DLS on Malvern Zetasizer Nano ZS. Fluorescence studies were carried out on a Horiba Fluoromax-4 fluorescence spectrophotometer. Cyclic voltammetry (CV) were performed on a CH Instrument Model 760D potentiostat in a standard three-electrode cell, a glassy carbon electrode as working electrode, a platinum wire as the counter electrode, and a Ag/AgCl electrode as the reference electrode. Cyclic voltammograms were recorded at room temperature in PBS buffer (pH 7.0) with 0.1 M KCl as the electrolyte.

Light source: Asahi Spectra Max 303 Xe lamp equipped with a UV-VIS mirror module and 1.0 collimator lens. Ultraviolet and visible light were obtained through light guide fitting device fitted by UV filter (260 – 400 nm) and long-pass 400 nm filters, respectively. The intensity of light irradiation on the sample cell was measured by using a CEL-NP 2000 intensity meter. Fluorescence images were acquired by using a Leica TCS SP5 II inverted microscope with a Leica DMI 6000B confocal scanning system. Flow cytometry (FCM) was performed on a Beckman Coulter flow cytometer (Quanta SC, USA).

Cell culture: Human cervical carcinoma cells (HeLa cells) and human breast cancer cells (MCF-7 cells) were obtained from Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Science (CAS, China). HeLa cells and MCF-7 Cells were cultured in Roswell Park Memorial Institute medium (RPMI-1640, Thermo, USA) and Dulbecco′s Modified Eagle Medium (DMEM, Thermo, USA) at 37 °C under 5% CO_2 atmosphere, supplemented with 10% (v/v) fetal bovine serum (Gibco, USA) and 1% (v/v) penicillin/streptomycin.

2 Methods:

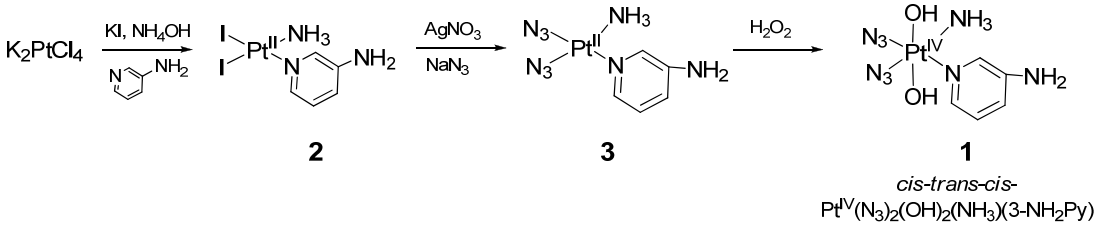
(1) Preparation of Carbon Dots (CDs)

CDs were prepared according to previous reports.[S2,S3] Activated carbon was chosen as an original carbon source,[S2] and 30% H_2O_2—HAc (V:V = 2:1) was used as oxidant.[S3] Activated carbon (1.2 g) were added into the solution of 30% H_2O_2—HAc (80 mL, V:V = 2:1). The mixture was heated to reflux for 24 h with stirring. The mixture was then cooled to RT, and the product was collected by sequential centrifugation and tube dialysis (MWCO 7000 and 1000). The product was finally dried by lyophilization. The amount of carboxyl groups on the CDs surface which was analyzed by acid-base titration was measured to be 6.28 ± 0.3 mmol/g. Spectroscopic characterization of as prepared CDs has been depicted in Fig. S1.

2) Synthesis of *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$

The platinum azide compound, *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$, was synthesized following procedures (Scheme S1) similar to previous reports.[S4,S5]

Caution: platinum azide complexes are potentially explosive and must be handled with care in small amount.



Scheme S1. Synthetic route for the platinum azide compound **1**.

Typically, $K_2[PtCl_4]$ (0.415 g, 1.0 mmol) and KI (4.0 g) were dissolved in deionized water (20 mL). After stirring at RT for several minutes, ammonium hydroxide (0.14 mL) was then added to the solution and kept stirring for 30 min. 3-aminopyridine (1.0 mmol) was added to the mixture. The solution was further stirred for 24 h at RT under shield of light. The precipitate was collected by filtration, washed with ice-cold water and ethanol, and dried under vacuum. Yield: ~93% (**2**).

Then, it was converted into the final product *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$ following reported procedures.[S5] The above precipitate was suspended in water and $AgNO_3$ (2.0 eq.) was added. The mixture was stirred at RT for 12 h in the absence of light. The precipitate of AgI was removed by centrifugation and then filtration with an inorganic membrane filter. NaN_3 (2 eq.) was added to the filtrate and the solution was stirred at RT overnight under dark. Yellow precipitates were obtained after the solution standing at 5 °C for 24 h. The precipitate was washed with ice-cold water, ethanol, diethyl ether, and dried under vacuum. Yield: ~35% (**3**). **^{1}H-NMR** (d_6-DMSO, ppm): 7.98 (d, 1H), 7.76 (d, 1H), 7.09 (dd, 1H), 7.02 (dd, 1H), 5.84 (s, 2H), 4.12 (s, 3H).

H_2O_2 (30%, 20 eq.) was added to an aqueous solution of **3**, and the solution was stirred overnight at RT in the dark. After that, the solution was concentrated and acetone was added. The precipitate was obtained by filtration, and washed by ice-cold water, ethanol, and diethyl ether. The product was finally dried under vacuum. Yield: ~ 63% (**1**). **ESI-MS**: *m/z* = 447.1 ($[M+Na]^+$). **^{1}H-NMR** (d_6-DMSO, ppm): 8.45 (d, 1H), 8.23 (d, 1H), 7.30 (dd, 1H), 7.17 (dd, 1H), 5.99 (s, 2H), 5.12 (s, 2H). **UV-vis** (H_2O)**:** λ_{max} = 253 nm. **FT-IR:** 2041 cm^{-1} (N_3^-). **CV**: $E_{Pt(IV/II)}$ = -0.79 V vs Ag/AgCl.

3) Preparation of Pt^{IV}-N_3-FA@CDs

The Diazido Pt^{IV} complexe **1**, *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$, was grafted onto CDs through amide bond formation. In a typical process, CDs (10 mg) were dissolved in water (10 mL) by sonication to form a 1.0 mg/mL solution, to which diluted ammonium hydroxide was added to adjust the pH to ~ 8. EDC (12.0 mg) was then added into the solution, and it was stirred at RT for several hours. **1** (30 mg) dissolved in water was added, and the mixture was stirred

overnight at RT in the dark. The mixture was purified by tube dialysis (MWCO =1kDa) for 2 days. The retentate was collected and lyophilized to afford the intermediate Pt^{IV}-N_3@CDs (18 mg). The surplus carboxyl groups on CDs were further activated by EDC (10 mg) and then reacted with excess amount of ethylenediamine (6.0 μL). Unreacted small molecule reactants were removed by dialysis (MWCO =1kDa) for 2 days. The obtained amine containing intermediate was further reacted with folic acid following the similar above procedures. The product Pt^{IV}-N_3-FA@CDs was further purified by tube dialysis (MWCO =1kDa) and finally lyophilized. The morphology of Pt^{IV}-N_3-FA@CDs was revealed by transmission electron microscope (TEM).The composition of Pt^{IV}-N_3-FA@CDs was analyzed by X-ray photoelectron spectroscopy (XPS), and the platinum content was accurately determined by atomic absorption spectroscopy (AAS).

4) Confocal laser scanning microscopy.

Fluorescence imaging was performed with a Leica DMI 6000B confocal scanning system. A 488 nm laser was used as the excitation source and the corresponding emissions were collected in the wavelength range of 500–550 nm. HeLa and MCF-7 cells were seeded on a plastic-bottomed μ-dish of diameter 35 mm, with a density of 10^4 cells and maintained at 37 °C in 5% CO_2 atmosphere for 24 h. The cells were then treated with the Pt^{IV}-N_3-FA@CDs solution (100 μg/mL) for 6 h. After incubation, the cells were washed twice with PBS and subjected to confocal fluorescence microscopy analysis.

5) MTT assay.

All the cells were seeded on a 96-well plate with a density of 5×10^4 cells per well and incubated in a humidified 5% CO_2 atmosphere for 24 h. The cell culture medium were removed and washed with PBS. Following that, different concentrations of Pt^{IV}-N_3-FA@CDs nanoplatform (0, 50, 100, 200, 400 μg/mL) in cell culture medium were added and incubated further for a period of 12 or 24 h at 37 °C in a humidified 5% CO_2 atmosphere. MTT (20 μL, 5.0 mg/mL) solution was added to each well. After 4 h of incubation at 37 °C, the cell culture medium was removed and the formazan crystals were lysed with 150 μL of DMSO. The absorbance was then measured at 490 nm using a microplate reader (Multiskan MK3, USA).

Visible light irradiation experiments: After incubation of the cells with different concentrations of the Pt^{IV}-N_3-FA@CDs nanoplatform (0, 50, 100, 200, 400 μg/mL) for 6 h, light irradiation was applied (λ > 400nm, 200 mW/cm^2, 20 min), and the cells were incubated for

another 12 or 24 h. Subsequently, the same procedures, as described above, were performed to obtain the final absorbance measurement at 490 nm using a microplate reader.

Control experiment with cisplatin: After incubation of the cells with different concentrations of cisplatin (0, 2, 4, 5, 10 μM) for 6 h, light irradiation was applied (λ > 400nm, 200 mW/cm^2, 20 min), and the cells were incubated for another 24 h. Subsequently, the same procedures, as described above, were performed to obtain the final absorbance measurement at 490 nm using a microplate reader.

6) Flow Cytometry (FCM).

Apoptosis assay: The HeLa cells were seeded in 12-well plates, at a concentration of 10^6 cells per well, and incubated in a humidified 5% CO_2 atmosphere for 24 h. Subsequently, the original medium was replaced with fresh RPMI 1640 medium containing the Pt^{IV}-N_3-FA@CDs nanoplatform (0, 50, 100, 200, 400 μg/mL). The cells were incubated at 37 °C for 6 h, followed by irradiation with visible light (λ > 400 nm, 200 mW/cm^2) for 20 min and incubation for 12 h. The treated cells were washed twice with PBS and harvested by trypsinization, followed by centrifugation at 1,500 rpm for 6 min. Cells were re-suspended in 500 μL binding buffer solution, to which 5.0 μL Annexin V-FITC and 5.0 μL propidium iodide (Keygen, China) were added and incubated for 15 min. Labeled cells were enumerated by FACS Beckman Coulter flow cytometer (Quanta SC, USA).

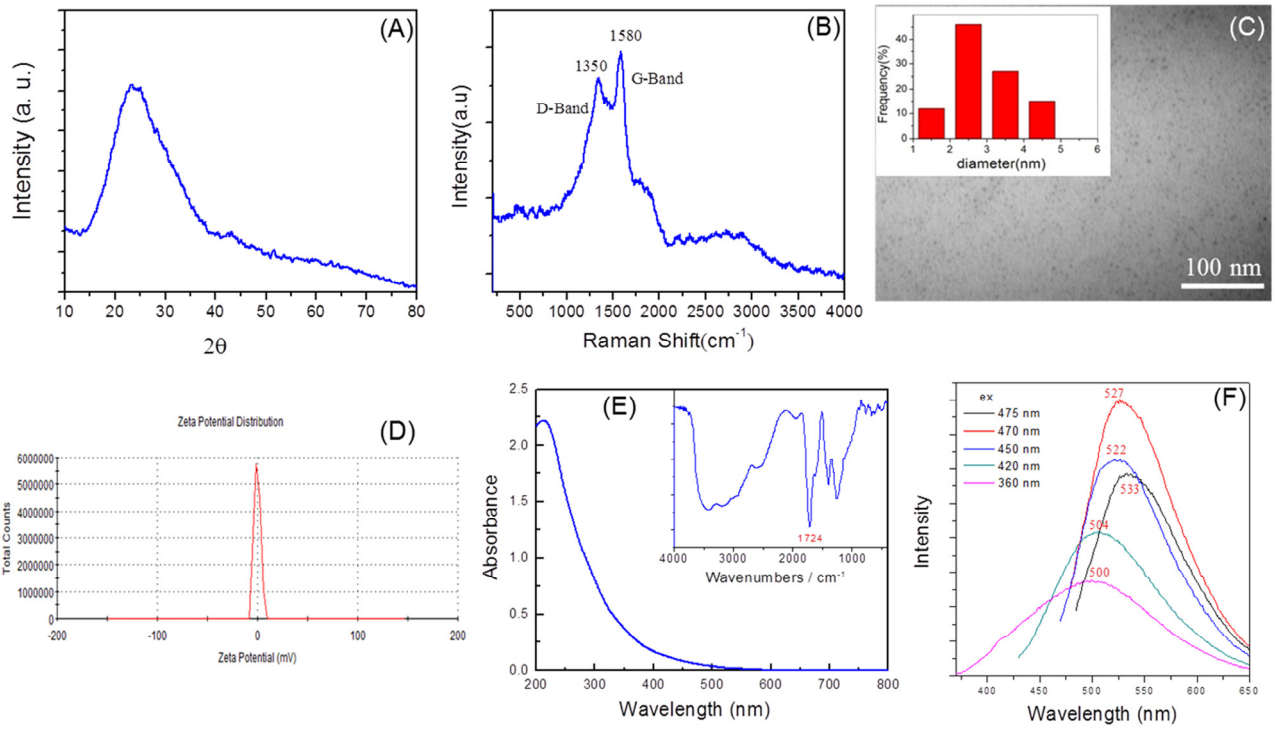


Fig. S1 Characterization of as-prepared CDs. (A) XRD pattern of CDs. (B) Raman spectra of CDs. Ex: 532 nm. (C) TEM image of CDs. The inset shows the size distribution of CDs. (D) Zeta potential of CDs. Surface potential of –0.24 mV was observed. (E) UV-vis spectra of CDs in water, [CDs] = 0.025 mg/mL. Inset shows the FT-IR spectra of CDs. (F) Photoluminescence of CDs under different excitation wavelength.

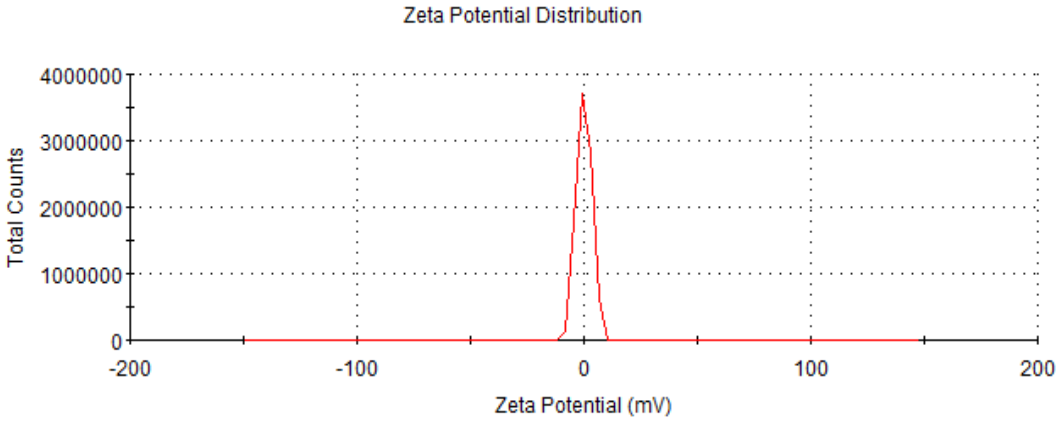


Fig. S2 Zeta potential of the Pt^{IV}-N_3-FA@CDs namoplatform. Surface potential of –0.05 mV was observed.

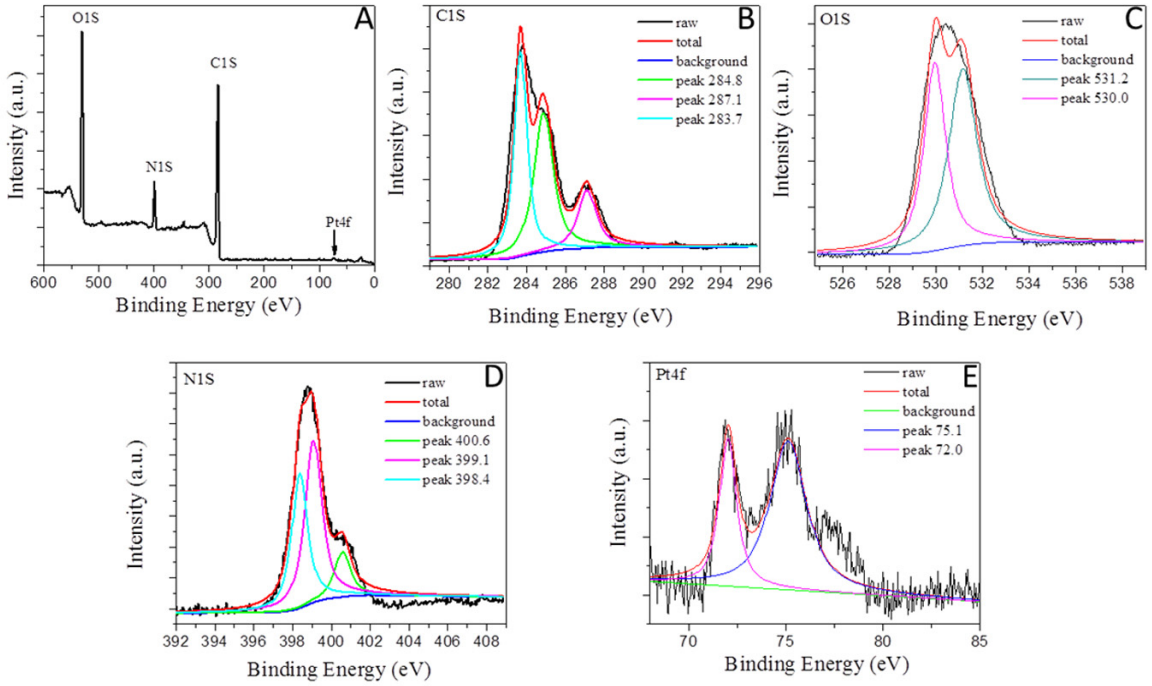


Fig. S3 XPS spectra of the Pt^{IV}-N_3-FA@CDs nanoplatform. (A) Survey spectrum. (B) High-resolution of C1s spectrum. (C) High-resolution of O1s spectrum. (D) High-resolution of N1s spectrum. (E) High-resolution of Pt4f spectrum.

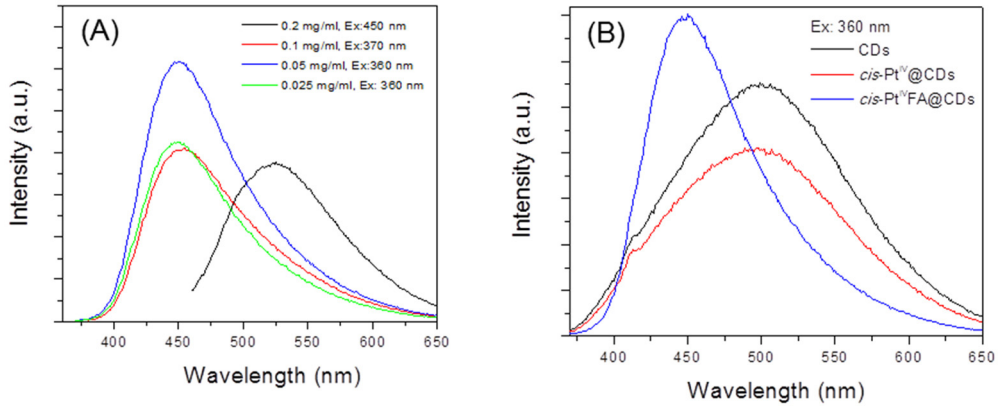


Fig. S4 (A) Fluorescence spectra of Pt^{IV}-N_3-FA@CDs with varied concentrations under different excitation wavelength. (B) Fluorescence spectra of Pt^{IV}-N_3-FA@CDs, Pt^{IV}-N_3@CDs and CDs under an excitation wavelength of 360 nm.

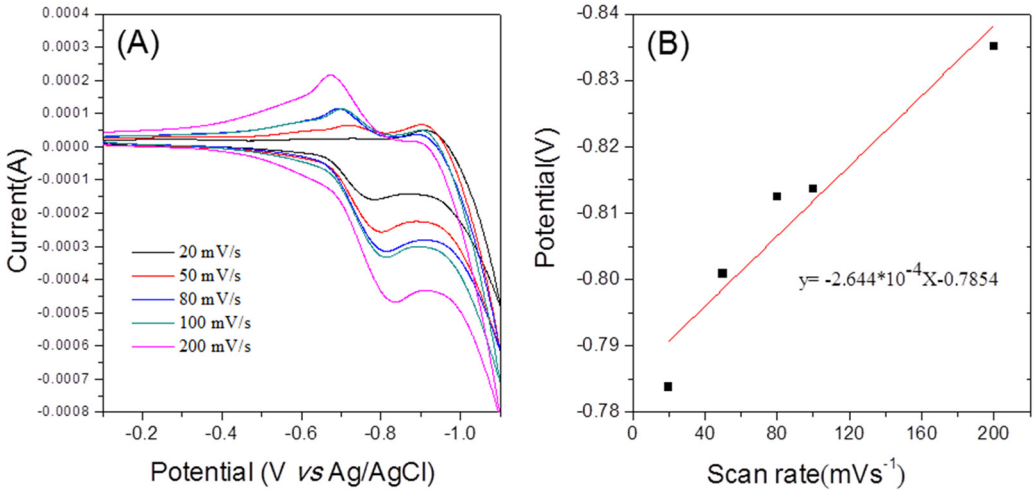


Fig. S5 (A) CV curves of *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$ in PBS solution (pH 7.0). (B) Reduction potential—scan rate plot for *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$. The $Pt^{IV/II}$ reduction potential of -0.79 V (*vs* Ag/AgCl) was obtained following extrapolation to a scan rate of 0.0 mV s^{-1} to account for the irreversible behavior of the reduction processes.[S6]

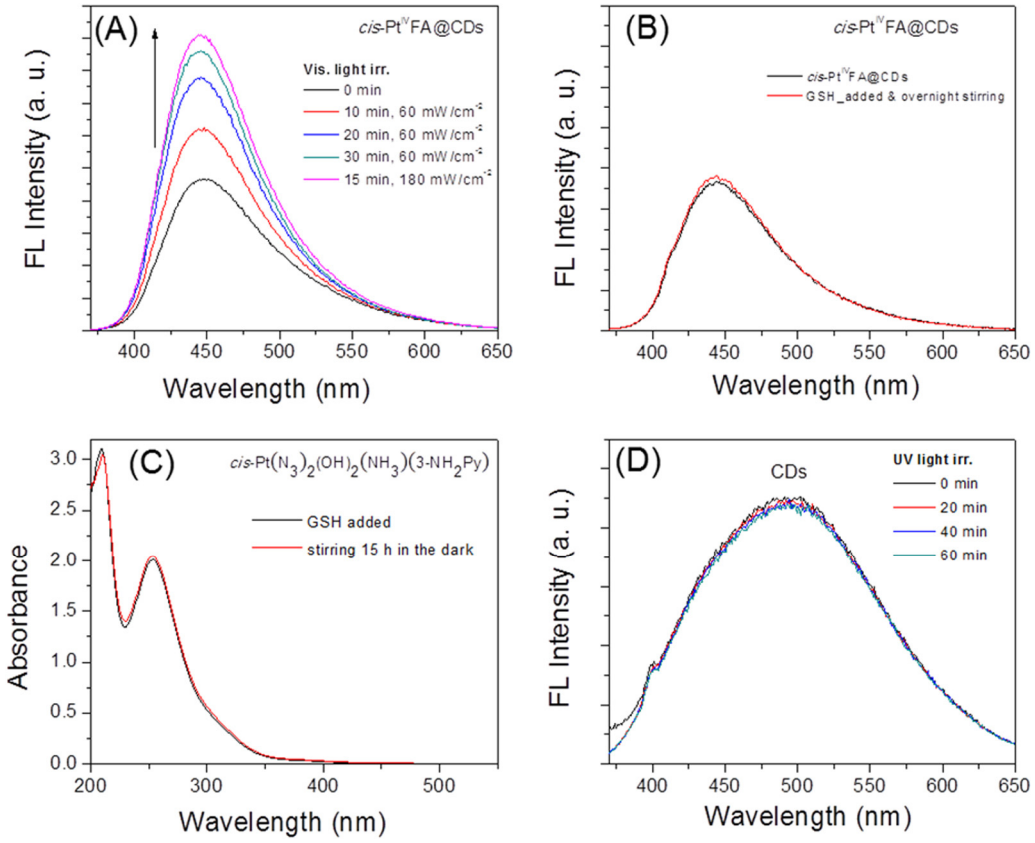


Fig. S6 (A) Fluorescence spectra of Pt^{IV}-N_3-FA@CDs (0.025 mg/mL) under visible light irradiation (λ > 400 nm). Light intensity: 60 or 180 mW/cm^{-2}. Ex: 360 nm. (B) Fluorescence spectra of Pt^{IV}-N_3-FA@CDs (0.025 mg/mL) in the presence of GSH (1.0 mM). Ex: 360 nm. (C) UV-vis spectra of *cis*, *trans*, *cis*-$Pt^{IV}(N_3)_2(OH)_2(NH_3)(3\text{-}NH_2Py)$ in the presence of added GSH. (D) Fluorescence spectra of CDs under UV light irradiation. Light intensity: 3.3 mW/cm^{-2}. Ex: 360 nm.

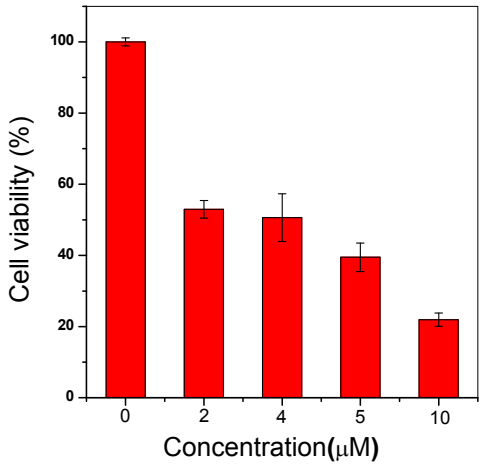


Fig. S7 Cell viability of HeLa cells treated with different concentrations of cisplatin and incubated for 24 h, as determined by MTT assay.

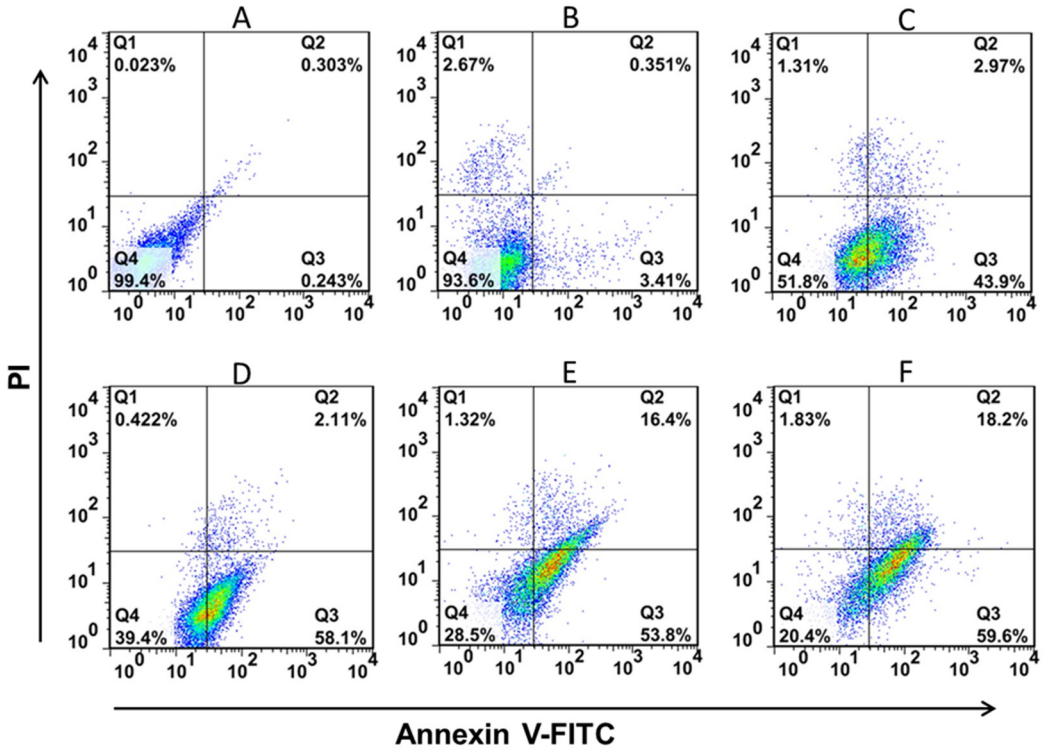


Fig. S8 Flow cytometric analysis for early and late apoptotic cells. (A) Control: HeLa cells incubated for 12 h in the dark. (B) Control: HeLa cells were irradiated by visible light (> 400 nm, 200 mW/cm^{-2}, 20 min) in the absence of the nanoplatform and incubated for 12 h. From (C—F): HeLa cells were treated with the Pt^{IV}-N_3-FA@CDs nanoplatform in the concentration of 50 (C), 100 (D), 200 (E), and 400 (F) μg/mL, followed by visible light irradiation (> 400 nm, 200 mW/cm^{-2}, 20 min), and then incubated for 12 h, respectively.

References:

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