Supporting Information

In-situ oxidative polymerization of platinum(IV) prodrugs in pore-confined spaces of $CaCO_3$ nanoparticles for cancer chemoimmunotherapy

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Experimental Procedures

Materials. Cisplatin, calcium chloride dihydrate, reduced glutathione, and distearyl phosphatidyl - polyethylene glycol$_{2000}$-Biotin (DSPE-PEG$_{2000}$-Biotin) were purchased from Sigma-Aldrich (USA). 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), N,N-diisopropylethylamine (DIEA), mercapto11-alkanoic acid (11-MUA), iodine, and ammonium bicarbonate were purchased from Aladdin (China). 30% hydrogen peroxide solution was obtained from Sigma-Aldrich (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,7-dichlorodihydro-fluorescein diacetate (DCFH-DA), and Annexin V-FITC apoptosis assay kit was purchased from Thermo Fisher Scientific (USA). GSH/GSSG Assay Kit and mitochondrial membrane potential assay kit were obtained Beyotime Biotechnology (China). All chemicals were of reagent grade and used without further purification. Ultrapure water (18.2 MΩ) was obtained from a Millipore Milli-Q Biocel purification system containing a 0.22 μm filter. $CaCO_3$ nanoparticles were synthesized by gas diffusion according to previous studies.[1] Di-hydroxy-cisplatin (cis,cis,trans-[$(Pt(NH_3)_2Cl_2(OH)_2$] was according to previous studies.[2]

General measurements. Microanalyses (C, H, and N) were performed using a Vario EL elemental analyzer. ^{1}H, ^{13}C, and ^{195}Pt NMR spectra were measured on a Bruker nuclear magnetic resonance spectrometer (Bruker Avance III, 600 MHz/500 MHz). Tetramethylsilane (TMS) was used as standard. High resolution electrospray ionization mass spectra (HRESI-MS) were obtained using a LCQ system (Q Exactive, Thermoscientific). Dynamic light scattering and zeta potential experiments were performed on a dynamic laser light scattering apparatus (BI-PALS03030131, Brookhaven Inst. Corp.). The platinum or calcium content was determined using an inductively coupled plasma mass spectrometer (iCAP RQ, Thermo Elemental Co., Ltd.). High-resolution TEM images, HADDF-STEM, and elemental mapping images were measured on a spherical aberration-corrected transmission electron microscope (JEM-ARM200P, Japan). Flow Cytometry measurements were performed on a BD FACS Canto II flow cytometer. Confocal microscopy images were recorded on LSM 880 NLO (Zeiss) microscope.

Synthesis of Pt(IV)-2SH. cis,cis,trans-[$Pt(NH_3)_2Cl_2(OH)_2$] (0.33 g, 1.0 mmol), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU, 1.14 g, 3.0 mmol), N,N-diisopropylethylamine (0.38 g, 3.0 mmol), and 11-mercaptoundecanoic acid (11-MUA, 0.65 g, 3.0 mmol) were dissolved in anhydrous N,N-dimethylformamide (6 mL). The mixture was stirred in the dark at room temperature for 24 h. After this time, the solvent was removed under reduced pressure. The crude product was purified by recrystallization from acetone and water (30:1) inside a freezer (-4 °C). The precipitate was collected by centrifugation (6000 rpm, 10 min) and washed three times with ethanol and dichloromethane. The product was recrystalized again by ethanol and toluene. As-obtained solid was dried under a vacuum with 30 °C. The product was obtained as a yellow powder (Yield = 65%). **Pt(IV)-2SH** was slightly soluble in water. ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.52 (s, 6H), 3.23 (s, 2H), 2.85 – 2.79 (m, 2H), 2.71 – 2.66 (m, 2H), 2.64 – 2.59 (m, 2H), 2.55 (s, 2H), 2.42 – 2.36 (m, 2H), 2.26 – 2.14 (m, 4H), 1.64 – 1.59 (m, 4H), 1.57 – 1.53 (m, 2H), 1.50 – 1.43 (m, 4H), 1.36 – 1.33 (m, 2H), 1.25 (s, 14H); ^{13}C NMR (151 MHz, DMSO- d_6) δ 181.42, 36.18, 33.87, 29.42, 29.38, 29.36, 29.09, 28.98, 28.22, 25.92, 24.23; ^{195}Pt NMR (108 MHz, DMSO-d_6) δ/ppm 1229.49; HRESI-MS (Q Exactive, Thermoscientific) [CH_3OH, m/z]: $[M-H]^-$ calcd. for $C_{22}H_{47}Cl_2N_2O_4PtS_2$, 733.1998; found, 733.1993.

Elemental analysis calcd (%) for $C_{22}H_{48}Cl_2N_2O_4PtS_2$ • 0.5 toluene, C: 39.23%, H: 6.71%, N: 3.59%, found : C: 39.49%, H: 6.67%, N: 3.63%.

Preparation of Pt(IV)-2SH@$CaCO_3$ nanoparticles. Amorphous $CaCO_3$ nanoparticles were synthesized by the gas-diffusion method according to previous studies.[1] **Pt(IV)-2SH@$CaCO_3$** were then synthesized via a approach of physical adsorption. Briefly, **Pt(IV)-2SH** (4.0 mg, 5.45 μmol) was dissolved in dimethyl sulfoxide (2 mL) and added to a suspension of $CaCO_3$ nanoparticles (5 mg/mL) in ethanol (3 mL). The mixture was stirred in the dark at room temperature for 24 h. After this time, the nanoparticles were isolated by centrifugation (8000 rpm, 15 min) and washed three times with anhydrous ethanol (3 × 5 mL) for purification. Then, **Pt(IV)-2SH@$CaCO_3$** nanoparticles was re-dispersed in anhydrous ethanol for further experiments. The platinum (195Pt) and calcium (44Ca) content of **Pt(IV)-2SH@$CaCO_3$** was determined upon digestion in nitric acid and analysis by inductively coupled plasma mass spectrometry. The yield of **Pt(IV)-2SH@$CaCO_3$** nanoparticles is 58.7% (based on Pt^{4+}).

Preparation of Pt(IV)SS@$CaCO_3$ nanoparticles. Pt(IV)-2SH (4.0 mg, 5.45 μmol) was dissolved in dimethyl sulfoxide (2 mL) and added to a suspension of $CaCO_3$ nanoparticles (5 mg/mL) in ethanol (3 mL). The mixture was stirred in the dark at room temperature for 24 h. After this time, the nanoparticles were isolated by centrifugation (8000 rpm, 15 min) and washed three times with anhydrous ethanol (3 × 5 mL) for purification. **Pt(IV)-2SH@$CaCO_3$** nanoparticles were suspended in anhydrous ethanol and a solution of iodine in dimethyl sulfoxide was added (1 μL, 10 M). The mixture was stirred in the dark at room temperature for 6 h. After this time, the nanoparticles were isolated by centrifugation (8000 rpm, 15 min) and washed three times with anhydrous ethanol (3 × 5 mL). Then, **Pt(IV)SS@$CaCO_3$** nanoparticles was re-dispersed in anhydrous ethanol for further experiments. The platinum (195Pt) or calcium (44Ca) content of **Pt(IV)SS@$CaCO_3$** was determined upon digestion in nitric acid and analysis by inductively coupled plasma mass spectrometry. The yield of **Pt(IV)SS@$CaCO_3$** nanoparticles is 46.8% (based on Pt^{4+}).

Previous studies have indicated that iodine could be used to form disulphides (not polymeric materials)[3] and iodine in combination with DMSO to polymerize dithiols at increased temperatures.[4] Further studies have suggested that the combination of DMSO and iodine could effectively produce disulphide polymers. [5-6]

Preparation of nano-assembled Pt(IV) nanoparticles (Pt(IV)SS NPs-1). Pt(IV)-2SH (4.0 mg, 5.45 μmol) was dissolved in dimethyl sulfoxide (2 mL) and a solution of iodine in dimethyl sulfoxide was added (1 μL, 10 M). The mixture was further stirred vigorously in the dark at room temperature for 6 h. After this time, the nanoparticles were isolated by centrifugation (8000 rpm, 15 min) and washed three times with anhydrous ethanol (3 × 5 mL) and re-dispersed in normal saline (5% DMSO) for further experiments. The platinum (195Pt) concentration was determined upon digestion in nitric acid and analysis by inductively coupled plasma mass spectrometry. The yield of **Pt(IV)SS NPs-1** nanoparticles is 85.8% (based on Pt^{4+}).

Preparation of localized in-situ oxidative polymerized Pt(IV) nanoparticles (Pt(IV)SS NPs-2). Pt(IV)SS@$CaCO_3$ nanoparticles (5 mg/mL, containing 0.25 mg Pt^{4+}) was resuspended at 5 mL anhydrous ethanol (pH 5.0) and further

stirred vigorously in the dark at room temperature for 24 h. **Pt(IV)SS NPs-2** was isolated by centrifugation (12000 rpm, 15 min) and washed three times with deionized water (3 x 5 mL) and dichloromethane (3 × 5 mL). The nanopartciles were further purified by dialysis. The nanopartciles were further re-dispersed in normal saline (5% DMSO) for further experiments. The platinum (195Pt) concentration was determined upon digestion in nitric acid and analysis by inductively coupled plasma mass spectrometry. The yield of **Pt(IV)SS NPs-2** nanoparticles is 98.2% (based on Pt^{4+}).

Encapsulation with DSPE-PEG_{2000}-Biotin to form Pt(IV)-2SH@$CaCO_3$ or Pt(IV)SS@$CaCO_3$ nanoparticles. DSPE-PEG_{2000}-Biotin (10 mg) was dissolved in chloroform (5 mL). A suspension of $CaCO_3$, **Pt(IV)-2SH@$CaCO_3$** or **Pt(IV)SS@$CaCO_3$** nanoparticles (0.4 mg/mL) in anhydrous ethanol (5 mL) was added and the mixture was stirred for 3 h at room temperature in the dark. After this time, ultrapure water (5 mL) was added and the solution was exposed to ultrasound radiation for 30 min (150 W). The organic solvent was removed under reduced pressure. The amphiphilic polymer-coated $CaCO_3$@Biotin, **Pt(IV)-2SH@$CaCO_3$@Biotin** or **Pt(IV)SS@$CaCO_3$@Biotin** nanoparticles were isolated by ultrafiltration (8000 rpm, 15 min) and washing with ultrapure water (3 × 5 mL). Then, all samples were re-dispersed in Milli-Q water for further experiments. The platinum (195Pt) or calcium (44 Ca) content was determined upon digestion in nitric acid and analysis by inductively coupled plasma mass spectrometry.

Pt loading capacity (weight %) = (weight of Pt in nanoparticles) / (weight of nanoparticles) x 100 %. The weight of Pt was obtained by ICP-MS, while the weight of lyophilized **Pt(IV)-2SH@$CaCO_3$@Biotin** or **Pt(IV)SS@$CaCO_3$@Biotin** was obtained using a digital balance.

pH and GSH-responsive behavior. To evaluate the pH-responsive Ca^{2+} releasing profiles and GSH-responsive Pt^{2+} releasing behavior, **Pt(IV)SS@$CaCO_3$@Biotin** nanoparticles (1 mL, 2 mg/mL) or **Pt(IV)SS NPs-2** nanoparticles (1 mL, 2 mg/mL) were loaded a dialysis bag (MWCO: 3000) and soaked in phosphate-buffered saline solutions with various pH levels (1. pH = 7.4, 2. pH = 6.0, 3. pH = 5.0, 4. pH = 5.0 + GSH (100 μM), 5. pH = 7.4 and 6. pH = 7.4 + GSH (100 μM)). At different time points, the metal content of the solution was determined. The released Ca^{2+} in the dialysate were analyzed by using calcium colorimetric assay kit (Beyotime Biotechnology), while the released platinum (195Pt) in the dialysate were analyzed by inductively coupled plasma mass spectrometer (iCAP RQ, Thermo Elemental Co., Ltd.).

Cell lines and culture conditions. Human lung cancer cells (A549), cisplatin-resistant human lung cancer cells (A549R) and mouse lung cancer cell (LLC) were purchased by the Experimental Animal Center at Sun Yat-Sen University (Guangzhou, China). The cells were maintained using Dulbecco's modified Eagle medium (DMEM, Gibco BRL) which was supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco BRL), 100 U/mL penicillin (Gibco BRL) and streptomycin (100 μg/mL) at 37°C with 5% CO_2 in a humidified atmosphere.

MTT assay. All solutions were freshly prepared in sterile phosphate-buffered saline . A549, A549R or LLC cells (10^4 cells/well) were seeded on a 96-well plate in DMEM media (180 μL) and incubated for 24 hours before adding increasing concentrations of phosphate-buffered saline, $CaCO_3$@Biotin**,** cisplatin, **Pt(IV)SS NPs-2**, **Pt(IV)-2SH@$CaCO_3$@Biotin**, or **Pt(IV)SS@$CaCO_3$@Biotin**. The cells treated with the compounds were incubated for 48 h. The media was removed, and

the cells were washed with phosphate-buffered saline and then incubated with fresh cell culture medium containing 20% MTT for 4 h. The medium was removed and dimethyl sulfoxide (150 μL) was added to each well to dissolve the formazan crystals generated in living cells. The optical density was recorded at 590 nm using a microplate reader. The background signal was subtracted from the measurements.

Mechanism of cell death. The cell viability upon pre-incubation with cell death inhibitors was evaluated. A549R cells were pre-incubated with Z-VAD-FMK (20 μM apoptosis inhibitor), 3-methyladenine (100 μM autophgy inhibitor), ferrostatin-1 (10 μM ferroptosis or peroxyradical scavenger), necrostatin-1 (60 μM necropotosis inhibitor) or deferoxamine (100 μM iron chelation and antioxidation inhibitor inhibitor) 1 h.[7] After this time, the cells were incubated with the IC_{50} value of $CaCO_3$@Biotin**,** cisplatin, **Pt(IV)SS NPs-2**, **Pt(IV)-2SH@$CaCO_3$@Biotin** or **Pt(IV)SS@$CaCO_3$@Biotin**. After 24 h, the media was removed, and the cells were washed with phosphate-buffered saline and then incubated with fresh cell culture medium containing 20% MTT for 4 h. The medium was removed and dimethyl sulfoxide (150 μL) was added to each well to dissolve the formazan crystals generated in living cells. The optical density was recorded at 590 nm using a microplate reader.

Cellular uptake. A549R cells were seeded on petri dishes (10 cm) with a density of 1×10^6 cells/dish and allowed to adhere for 24 h. After this time, the cells were treated with PBS, $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM Pt^{2+}), **Pt(IV)SS NPs-2** (10 μM Pt^{4+})**, Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+},0.14 mM Ca^{2+}) for 2, 4, 6, 8, 12, or 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were then counted, collected by centrifugation (1000 rpm, 5 min), and digested with concentrated nitric acid (0.5 mL) for 24 h. The samples were diluted with ultrapure water to set volume to 10 mL. The platinum (195Pt) or calcium (44 Ca) was determined using inductively coupled plasma mass spectrometry. Quantification was calculated by platinum or calcium standard curve.

Cellular distribution. A549R cells were seeded on petri dishes (10 cm) with a density of 1×10^6 cells/dish and allowed to adhere for 24 h. After this time, the cells were treated with **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}) for 12 or 24 h. The media was removed and the cells were counted after washing with phosphate-buffered saline (pH 7.4) three times. The nucleus, lysosome, cytoplasm, and mitochondria were isolated using the respective extraction kit (Pierce, Thermo). The organelles were digested with concentrated nitric acid (0.5 mL) for 24 h. The samples were diluted with ultrapure water to reach 3% nitric acid. The platinum content was determined using inductively coupled plasma mass spectrometry.

Intracellular DNA platination. A549R cells a density of 1×10^6 cells/dish were seeded on petri dishes (10 cm) and allowed to adhere overnight. When cells achieved 80% confluence, they were incubated with cisplatin (10 μM Pt^{2+}), **Pt(IV)SS NPs-2**(10 μM Pt^{4+})**, Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}) for 12 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were then counted and collected. A genomic DNA preparation kit (Beyotime, China) was used to extract the endo-nuclear

DNA. The DNA was digested with concentrated nitric acid (0.5 mL) for 24 h. The samples were diluted with ultrapure water to reach 3% nitric acid. The platinum content was determined using inductively coupled plasma mass spectrometry.

Fluo-4/AM staining for determination of intracellular Ca^{2+}levels. A549R cells were seeded on confocal dishes (3.5 cm) and allowed to adhere overnight. The cells were treated with the IC_{50} value of $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+})**, Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were incubated with Fluo-4/AM (5 μM) in cell media for 30 min. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. Fresh media was added to the cells. Confocal images (λ_{ex} = 488 nm, λ_{em} = 515 – 535 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

The intracellular Ca^{2+}levels induced by various samples were quantified by flow cytometry using the Fluo-4 AM (Calcium ion fluorescent probe) (Beyotime, China) in according with the manufacturer's protocol. Briefly, cells were seeded to a six-well plate in a density of 1×10^5 per well and incubated with or without the indicated tested $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The cell pellets were washed twice with phosphate-buffered saline and stained with Fluo-4/AM (5 μM) another 30 min. Then, the cells as well as residual attached cells were collected after washing with phosphate-buffered saline. All cells were resuspended in 300 μL phosphate-buffered saline and immediately examined using the flow cytometer.

Determination of intracellular glutathione levels. A549R cells were seeded on petri dishes (10 cm) and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were then counted and collected. The cells were lysed in a cell homogenizer at reduced temperature with an ice-water bath. The solution was centrifuged at 10000 rcf for 15 min. The the GSH levels of supernatant was determined using a commercially GSH/GSSG assay kit (Beyotime Biotechnology). Intracellular remaining GSH level (%) = Remaining GSH level / Intracellular GSH levels of control.

Determination of intracellular reactive oxygen species. A549R cells were seeded on 3.5 cm confocal dishes and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were incubated with DCFH-DA (2 μM) in cell media for 30 min. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. Fresh media was added to the cells. Confocal images (λ_{ex} = 488 nm, λ_{em} = 515 - 535 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

ROS generation assay was assessed by flow cytometry using DCFH-DA staining kit. Briefly, cells were seeded to a six-well plate or confocal dishes in a density of 1×10^5 per well and costained with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The cells were stained with DCFH-DA for 30 min. Floating cells as well as residual attached cells were collected and washed twice with phosphate-buffered saline.The cell pellets from the six-well plates were resuspended in 200 μL of phosphate-buffered saline and then examined using the flow cytometer.

Determination of changes in the mitochondrial membrane potential (MMP). A549R cells were seeded on 3.5 cm confocal dishes and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were incubated with JC-1 (200 μL, 10 μg/mL) in cell media for 30 min. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. Fresh media was added to the cells. Confocal images of the monomer (λ_{ex} = 488 nm, λ_{em} = 505 - 535 nm) and the aggregates (λ_{ex} = 488 nm, λ_{em} = 573 - 607 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

MMP was assessed by flow cytometry using a JC-1 staining kit following the manufacturer's protocol. Briefly, cells were seeded in a six-well plate at a density of 1×10^5 per well and incubated with or without the indicated tested $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. Floating cells as well as residual attached cells were collected and washed twice with phosphate-buffered saline. The cell pellets were resuspended in 500 μL of DMEM and 500 μL of 1× JC-1 working solution was added. After incubation for 20 min at 37 °C, stained samples were washed twice and resuspended by 1× binding buffer. The samples were examined using the flow cytometer.

Determination of intracellular ATP levels. A549R cells were seeded on white 96-well plates and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.28 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (20 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The intracellular ATP levels were determined using a CellTiter Glo® Luminescent Cell Viability Assay Kit (Promega, USA).

Apoptosis analysis by flow cytometry. A549R cells were seeded on 6-well plates with a density of 1×10^5 cells per well and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.28 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (20 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were incubated with the AnnexinV-FITC/ propidium iodide apoptosis double staining kit (BD Biosciences) in cell media for 15 min. The media was removed and the cells were washed with phosphate-buffered

saline (pH 7.4) three times. Fresh media was added to the cells. The samples were kept on ice and analyzed by flow cytometry for FITC (λ_{ex} = 488 nm, λ_{em} = 532 nm) and propidium iodide (λ_{ex} = 541 nm, λ_{em} = 610 nm).

Determination of Caspase 3/7 activation. A549R cells were seeded on white 96-well plates with a density of 1×10^4 cells per well and allowed to adhere overnight. Cells were treated with the IC_{50} concentration of different drugs for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The caspase activation was determined using a Caspase 3/7 activity detection kit.

Cell cycle analysis. A549R cells were seeded on 6-well plates with a density of 1×10^5 cells per well and allowed to adhere overnight. The cells were treated with the IC_{50} dose of various samples for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were collected by centrifugation at 400 × g for 5 min. The cell pellet was resuspended in 0.5 mL phosphate-buffered saline and fixed with 0.5 mL of ethanol (70%) at 4 °C overnight. The fixed cells were collected by centrifugation and washed twice with cold phosphate-buffered saline. The cells were resuspended in 1 mL propidium iodide solution (200 μg/mL RNase A, 0.1% Triton X-100, 20 μg/mL propidium iodide, pH 7.4). Cells were incubated at 37 °C for 15 min in the dark. The samples were kept on ice and analyzed by flow cytometry (λ_{ex} = 488 nm, λ_{em} = 612-652 nm).

Cellular lipid peroxidation assay. A549R cells were seeded on 3.5 cm confocal dishes and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were incubated with C11-BODIPY$^{581/591}$ in cell media for 30 min. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. Fresh media was added to the cells. Confocal images of C11-BODIPY$^{581/591}$ $_{green}$ (λ_{ex} = 488 nm, λ_{em} = 510-530 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

Western blot analysis. A549R cells were seeded on 10 cm dishes with a density of 5×10^6 cells per well and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The media was removed and the cells were washed with phosphate-buffered saline (pH 7.4) three times. The cells were then counted and collected. The cells were lysed in a cell homogenizer at reduced temperature with an ice-water bath. The protein concentration was determined using a BCA protein assay. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for 45 min at 120 V. The plot was then transferred on a polyvinylidene fluoride (PVDF) membrane (250 mA, ~40 min). The PVDF membrane was blocked with skim milk (5% in TBST) and then incubated with primary antibodies (anti-Tubulin (ab176560), anti-GPX4 (ab125066)) at 4 °C overnight. After this time, the membrane was further incubated with HRP conjugated secondary antibodies at

room temperature for 1.5 h. The Western Blot was detected using SuperSignal West Femto ECL (Thermo Science™ Emm™) and Omega Lum C Imaging System (Aplegen, USA).

Immunofluorescence staining of calreticulin. A549R cells were seeded on 2.5 cm confocal dishes and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The cells were washed, fixed, permeated and sealed. The cells were incubated with Calreticulin (D3E6) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) in according with the manufacturer's protocol at 4°C for 12 h, and then Hoechst for 10 min after being washed with phosphate-buffered saline three times. The cells were imaged using the confocal microscope. Confocal images of the antibody (λ_{ex} = 488 nm, λ_{em} = 505 - 535 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

For flow cytometry analysis, The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. After the incubation, the cells were trypsinized, collected, fixed, then stained with Anti-Calreticulin mAb (Alexa Fluor® 488 Conjugate) following the manufacture's protocol. Binned populations were expressed as % of total cells.

Immunofluorescence staining of extracellular HMGB1. A549R cells were seeded on 2.5 cm confocal dishes and allowed to adhere overnight. The cells were treated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The cells were collected, fixed, and sealed. The cells were incubated with HMGB1 Antibody and Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor®647 Conjugate) in according with the manufacturer's protocol, and Hoechst for 10 min after being washed with phosphate-buffered saline. The cells were imaged using the confocal microscope. Confocal images of the antibody (λ_{ex} = 514 nm, λ_{em} = 600 - 640 nm) were taken with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

Extracellular HMGB1 or ATP detection assay. A549R cells were seeded on 96-well plates at a density of 1.5×10^4 cells/well overnight to adhere. The cells were incubated with DMEM medium (10 % fetal bovine serum) containing $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h. The supernatant was transferred to the 96-well plates from HMGB1 Detection Kit, and then manufacturer's protocol was followed. The optical density of each well was measured on a microplate spectrophotometer at a wavelength of 450 nm (a 630 nm filter can be used as a reference). Relative HMGB1 contents of other groups were presented by their intensity ratios to control group and corrected by the cell viability in each incubation condition. Moreover, for extracellular ATP detection assay, the supernatants were transferred to the white-walled non-transparent bottomed 96-well plates. Upon completion, to each well was added an equal volume of ENLITEN®ATP Assay System Bioluminescence Detection Kit (Promega), the

plate was vigorously vibrated and then manufacturer's protocol was followed. The actual value of ATP contents in other groups were corrected by the cell viability in each incubation condition.

Tumor xenograft model. All procedures were approved by the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Institutional Animal Care and Use Committee (IACUC) at Sun Yat-Sen University, Guangzhou, China (Approval No. SYSU-IACUC-2021-000111). Nu/Nu mice (approximately 4-6 weeks old, ~15 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were given access to food and water ad libitum with 12 h light and dark cycles. 1×10^7 A549R cells in 100 μL of serum-free DMEM medium were injected subcutaneously into the right hip of Nu/Nu mice. After the tumor size had reached approximately about 75 mm^3, the mice were used in subsequent experiments.

Biodistribution in tumor xenograft model. When the tumor of the mice models reached approximately 75 mm^3, the animals (3 mice per group) were then intravenously injected with **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt) or **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). 4, 12, and 24 h after the injection, the mice were sacrificed. The major organs (heart, liver, spleen, lung, and kidneys) and tumors were dissected, rinsed with phosphate-buffered saline two times, weighted, and digested in concentrated nitric acid and hydrogen peroxide for 72 h. The biodistribution was analyzed upon determination of the amount of platinum per gram of tissue.

Pharmacokinetics in tumor xenograft model. When the tumor of the mice models reached approximately 75 mm^3, the animals (3 mice per group) were then intravenously injected with cisplatin(5 mg/kg, based on 1.67 mM Pt), **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), or **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). 0.5, 2, 4, 8, 20, 26, 48, 72, and 96 h after the injection, blood samples were obtained from the tail vein. The blood samples were digested with concentrated nitric acid (0.5 mL) for 24 h. The samples were diluted with ultrapure water to reach 3% nitric acid. The platinum content was determined using inductively coupled plasma mass spectrometry. The blood circulation half-life time of cisplatin, **Pt(IV)-2SH@$CaCO_3$@Biotin**, and **Pt(IV)SS@$CaCO_3$@Biotin** was determined using a double-compartment pharmacokinetic model. The eliminating rate curve was constructed by plotting ln(Cp) against time and fitting according to the two-compartment models.

Antitumor effect in tumor xenograft model. When the tumor of the mice models reached approximately 75 mm^3, the animals were randomly divided into 5 groups with 5 mice per group: Group I: saline (200 μL), Group II: cisplatin (5 mg/kg, based on 1.67 mM Pt), Group III: **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), Group IV: **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). The animals were intravenously injected in the tail vein on day 1, day 3 and day 5. The tumor volume and body weight of the animals were measured every two days for 20 days. The tumor volume (mm^3) was obtained according to $V = lw^2/2$, where w and l represent the width and length of the tumor. After this time, the mice were sacrificed and the tumor and major organs (heart, liver, spleen, lung, kidney, brain, intestines) obtained. The organs were fixed in 4% paraformaldehyde.

Histological analysis. The major organs and tumors of the treated mice were collected and fixed with paraformaldehyde. The major organs were analyzed with an H&E stain. The tumors were analyzed with an H&E stain and terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) stain. Images of the stained tissue slices were taken with a Leica TCS SP8 instrument and LSM 880 NLO (Zeiss) microscope.

Biosafety in tumor xenograft model. After the treatment with cisplatin (5 mg/kg, based on 1.67 mM Pt), **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt) and **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt), the blood of the animals was collected for hematological and serum biochemical analysis.

In vivo **vaccination experiment.** This study was performed with the Institutional Animal Care and Use Committee (IACUC) of Guangdong Pharmaceutical University (Approval No: gdpulacspf 2022037). Animals were treated as the guidelines of IACUC.

The C57BL/6J female mice (approximately 4-6 weeks old, ~15 g) were randomly divided into 4 groups with 5 mice per group, including saline group (n=5), cisplatin group (n=5), **Pt(IV)-2SH@$CaCO_3$@Biotin** group (n=5) and **Pt(IV)SS@$CaCO_3$@Biotin** group(n=5). The logarithmic growth LLC cancer cells (1×10^6 cells per mice) were incubated with cisplatin (5.96 mg/kg), **Pt(IV)-2SH@$CaCO_3$@Biotin** (1.95 mg/kg Pt^{4+}, 0.14 mM Ca^{2+}) and **Pt(IV)SS@$CaCO_3$@Biotin** (1.95 mg/kg Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h, respectively. All treated LLC cells (1×10^6 cells containing 100 μL free fetal bovine serum DMEM per mice) medium were subcutaneously injected into the right flanks of per mice. For saline group, 100 μL saline without LLC cells were subcutaneously injected into the right flanks of per mice. At day 7, C57BL/6 tumor-bearing mice in each group were euthanized, and tumor draining lymph nodes were harvested to prepare single cell suspension. The cells were first blocked with anti-mouse CD16/32, followed by live/dead staining for discriminating live cells from dead cells. Then, the cells were stained with anti-CD45, anti-CD11c, anti-CD80, anti-CD86 for 30 min at 4°C, and the stained cells were analyzed by flow cytometry for the maturation of DCs. At day 8, untreated LLC cells with logarithmic growth were subcutaneously injected at the same cell density into the left side of the mice, the mice were used in subsequent vaccination experiments.

The above experimental scheme is adopted and the left flanks of mice were subcutaneously injected with logarithmic growth LLC cells (1×10^6 cells containing 100 μL fetal free bovine serum DMEM per mice), recording as day 0. All mice was randomly divided into 4 groups with 5 mice per group, including saline group (n=5), cisplatin group (n=5), **Pt(IV)-2SH@$CaCO_3$@Biotin** group (n=5) and **Pt(IV)SS@$CaCO_3$@Biotin** group (n=5). The tumor volumes of both primary and distant tumors and body weights of were measured every 3 days. Tumor volumes at each measurement timepoint were calculated according to the formula: tumor volume = (tumor length) × (tumor width)^2/2. 27 days later, the mice of all groups were euthanized and the tumors and major organs were collected and washed with phosphate-buffered saline for subsequent H&E and flow cytometry analysis.

In vivo **evaluation of T cell population.**At day 26, C57BL/6 tumor-bearing mice in each group were euthanized, and the left side of tumors, spleens were harvested to prepare single cell suspension. All the single cell suspensions were stained with anti-CD45, anti-CD3, anti-CD4, anti-CD8, following the manufacturer's instructions, and the stained cells

were analyzed by flow cytometry for $CD4^+$ and $CD8^+$ T cell populations. For Treg analysis, the cells were stained with anti-CD45, anti-CD3, anti-CD4, anti-CD25 and anti-mouse Foxp3, following the manufacturer's instructions. For memory T cells evaluation, the single cell suspension was stained with anti-CD3, anti-CD4, anti-CD44 and anti-CD62L, following the manufacturer's instructions. The stained cells were detected using flow cytometry. The data were analyzed using FlowJo 10.0.

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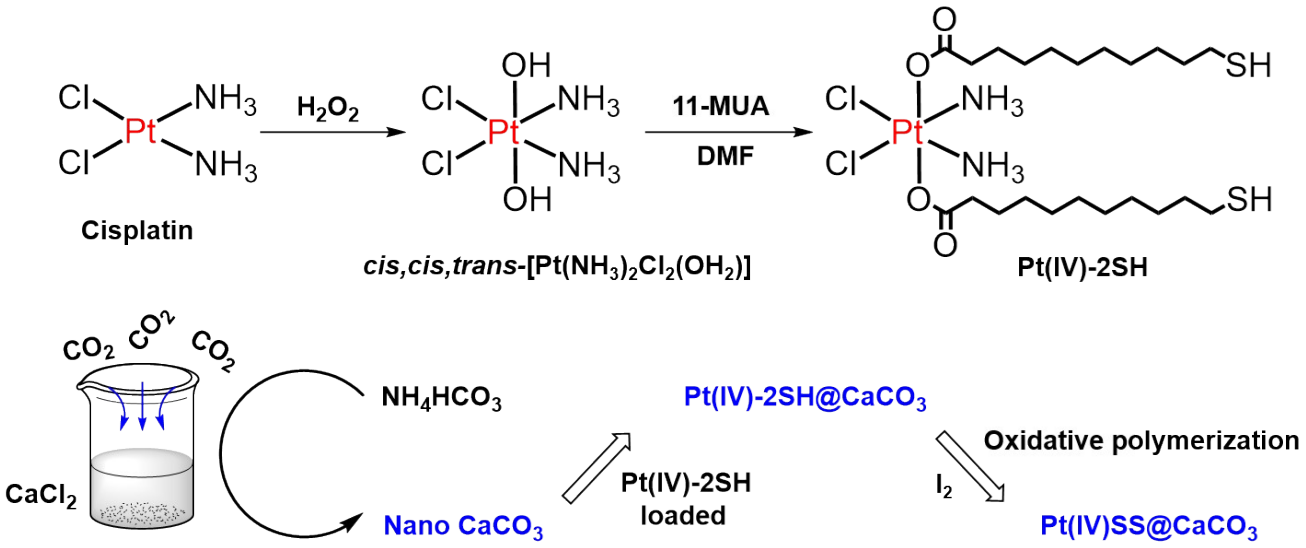
Supporting Figures and Tables



Scheme S1. Synthetic strategy for the preparation of **Pt(IV)-2SH**, $CaCO_3$ nanoparticles, **Pt(IV)-2SH@$CaCO_3$** and **Pt(IV)SS@$CaCO_3$**.

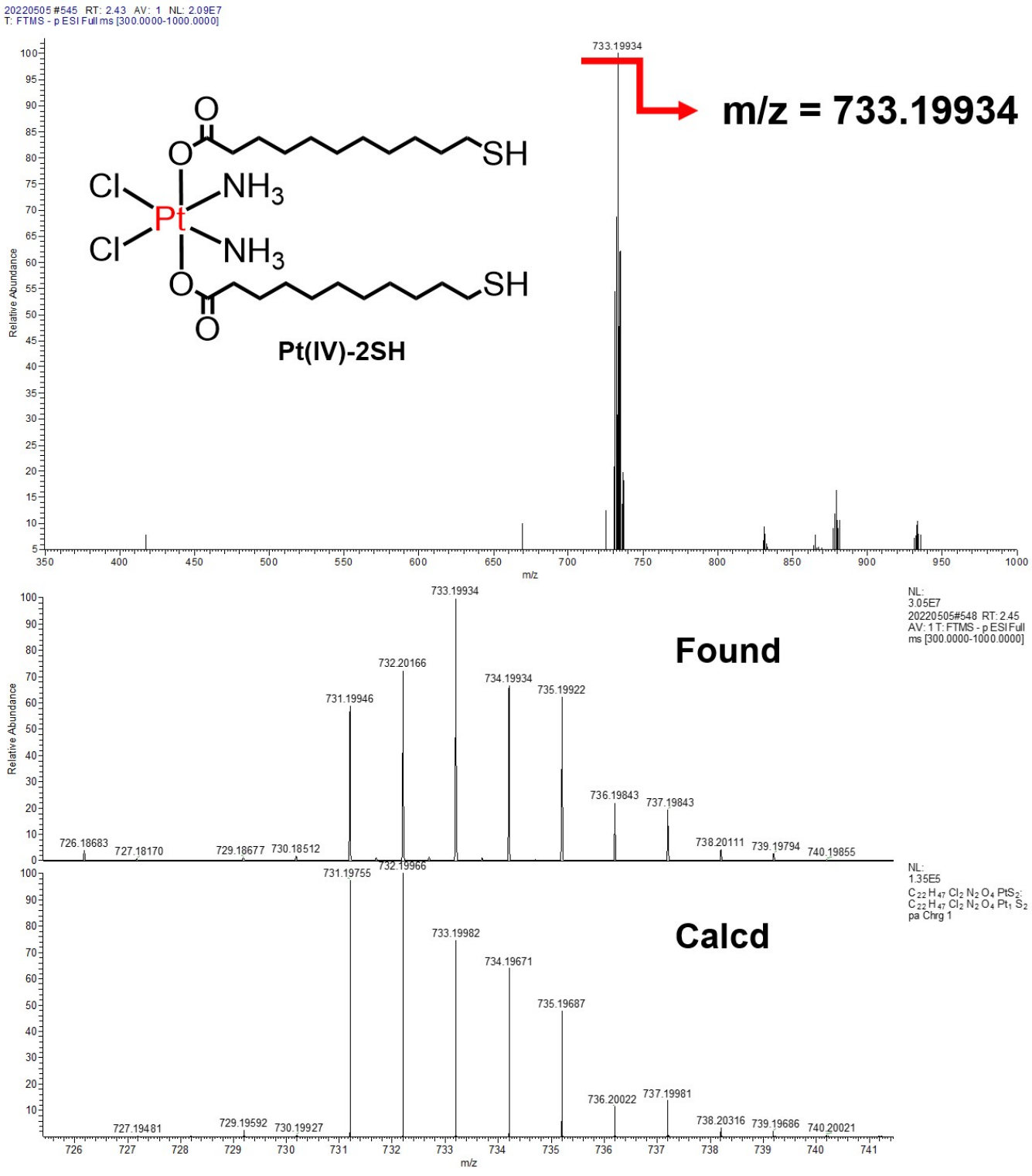


Figure S1. HR-MS spectrum of **Pt(IV)-2SH**.

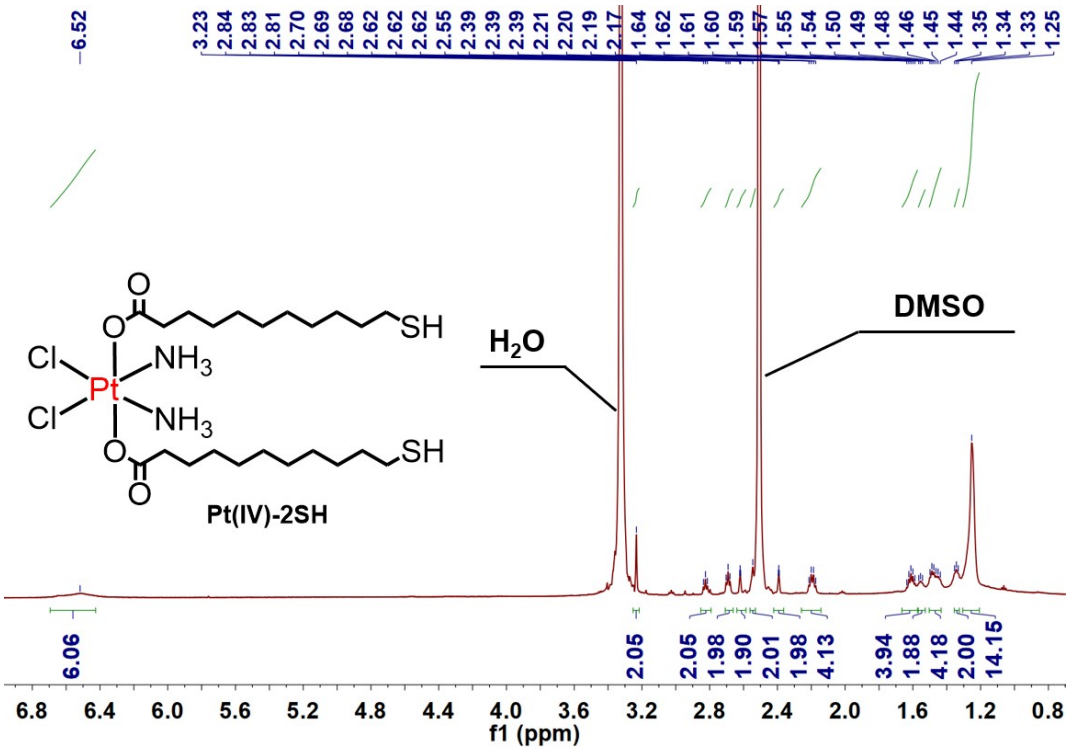


Figure S2. ^{1}H NMR (600 MHz) spectrum of **Pt(IV)-2SH** in DMSO-d_6.

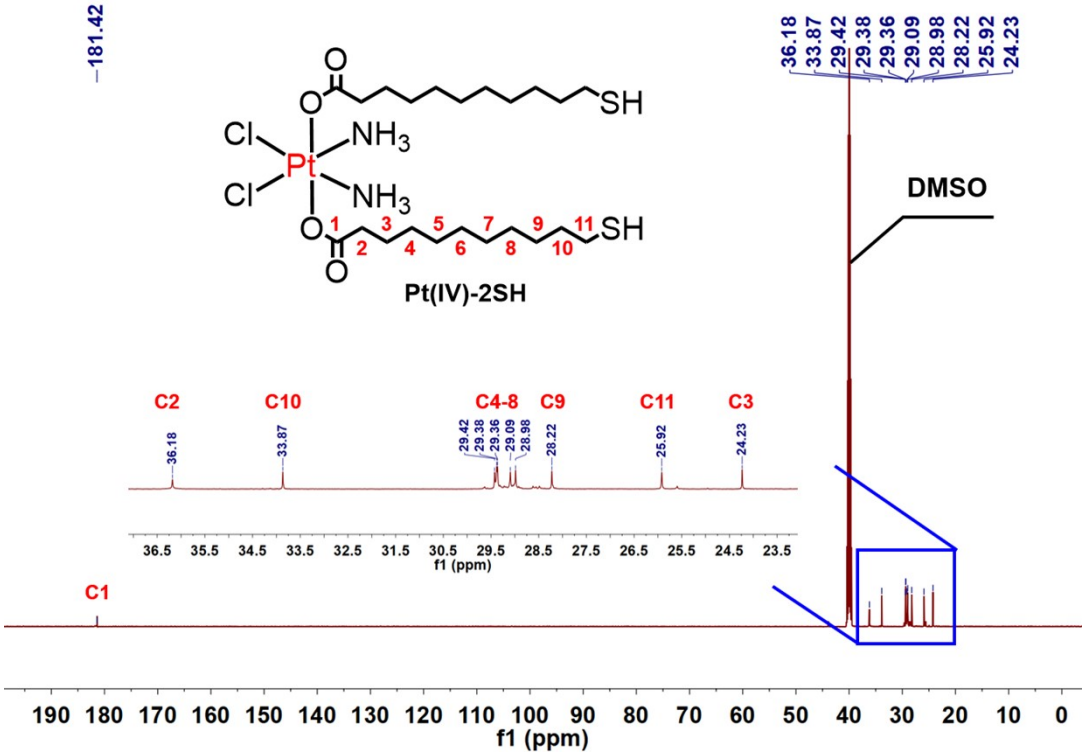


Figure S3. ^{13}C NMR spectrum of **Pt(IV)-2SH** in DMSO-d_6.

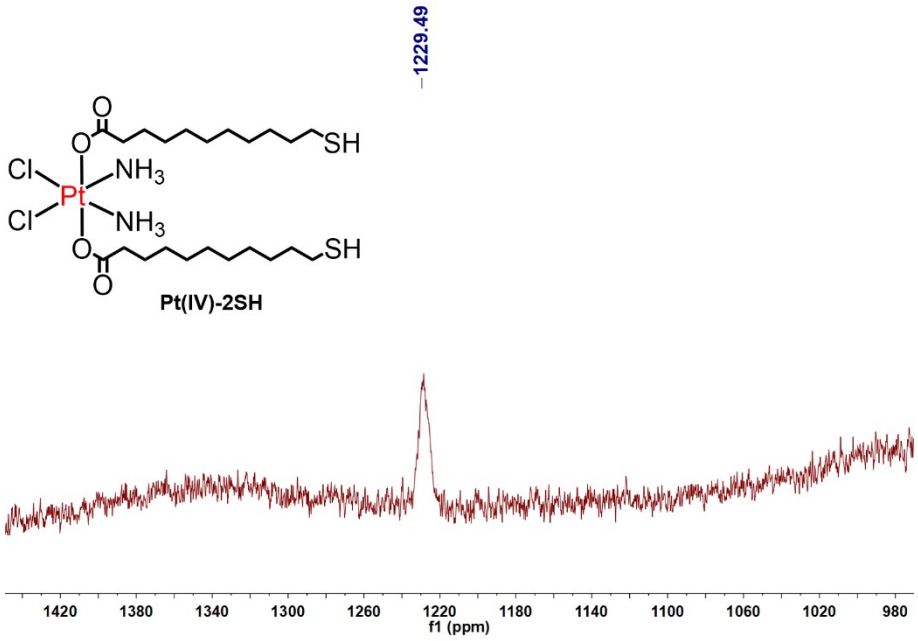


Figure S4. ^{195}Pt NMR spectra of **Pt(IV)-2SH** in DMSO-d_6.

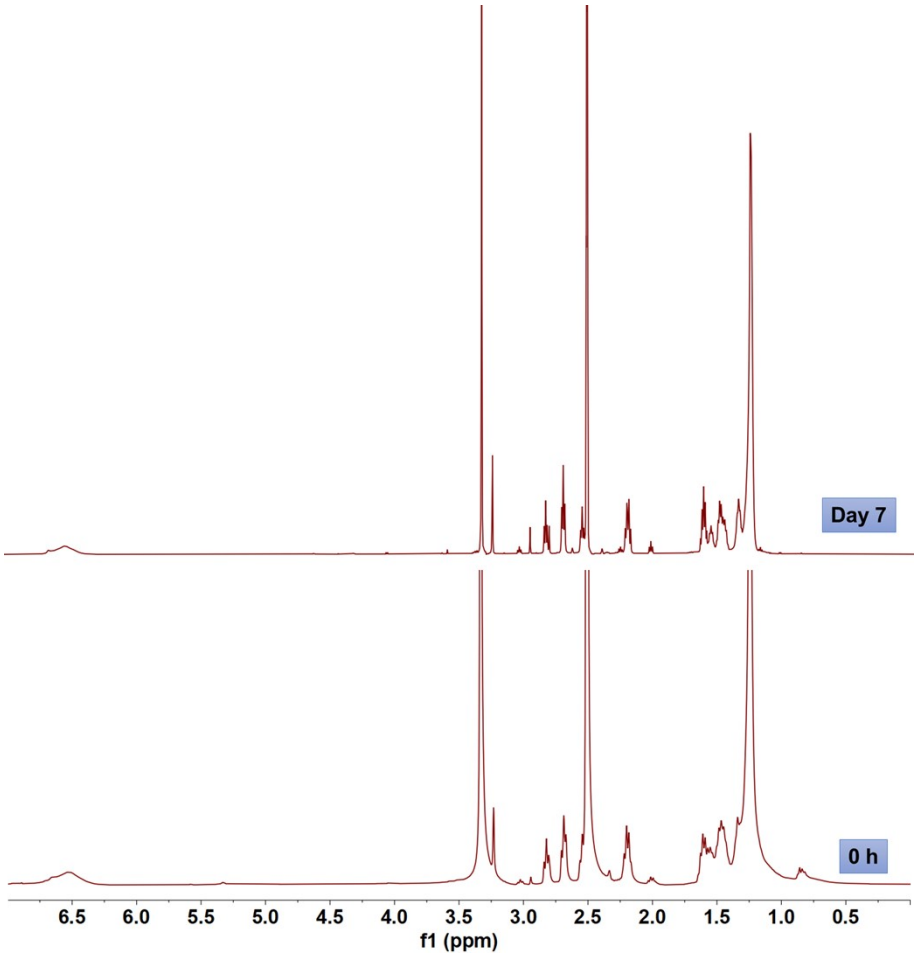


Figure S5. ^{1}H NMR (600 MHz) spectrum of **Pt(IV)-2SH** in DMSO-d_6 for 0 h and one week.

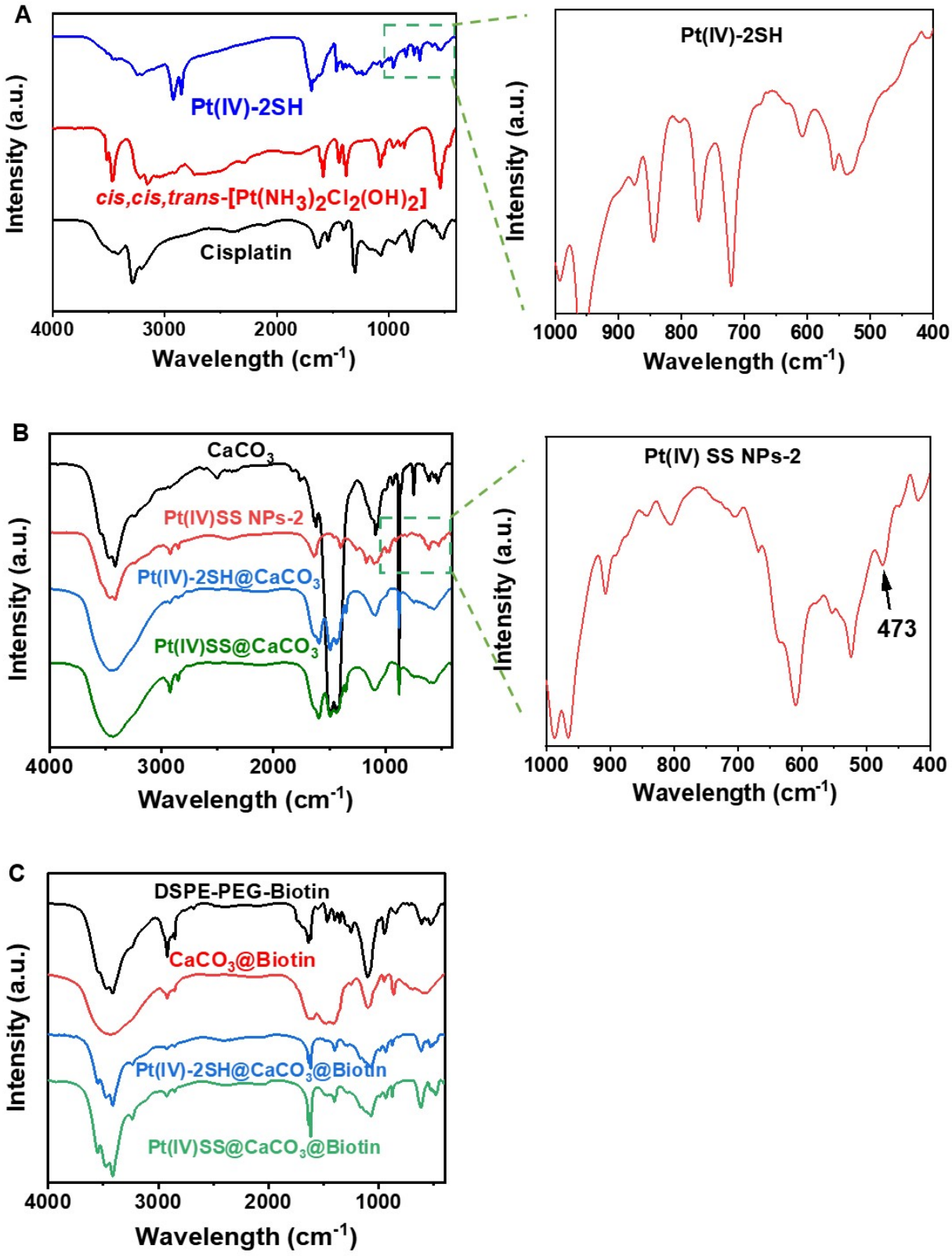


Figure S6. The FTIR spectrum of (A) cisplatin, cis,cis,trans-[$Pt(NH_3)_2Cl_2(OH)_2$], and **Pt(IV)-2SH**; (B)$CaCO_3$ nanoparticles, **Pt(IV)SS NPs-2**, **Pt(IV)-2SH@$CaCO_3$** and **Pt(IV)SS@$CaCO_3$**; (C) DSPE-PEG_{2000}-Biotin, $CaCO_3$@Biotin, **Pt(IV)-2SH@$CaCO_3$@Biotin** and **Pt(IV)SS@$CaCO_3$@Biotin**.

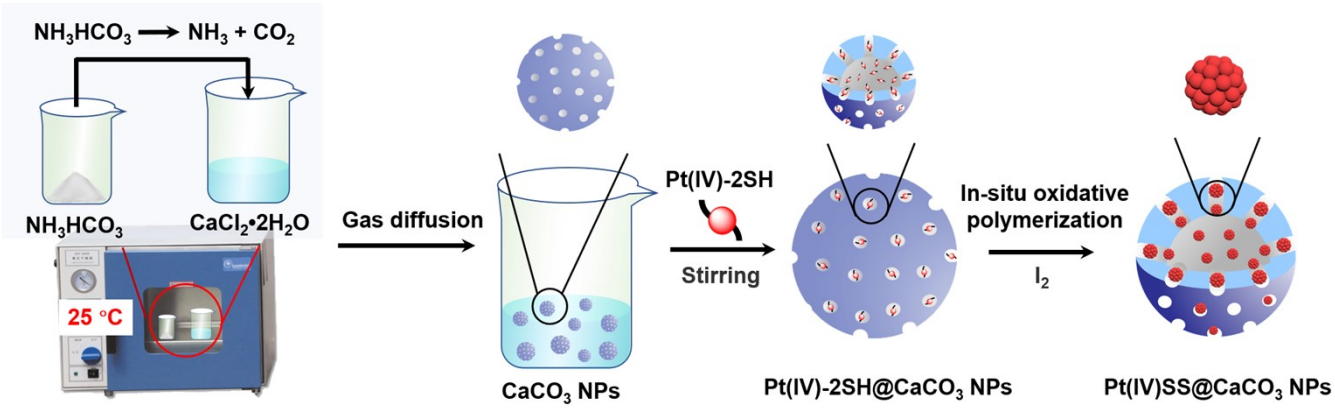


Figure S7. Schematic illustration of the preparation of **Pt(IV)SS@$CaCO_3$**.

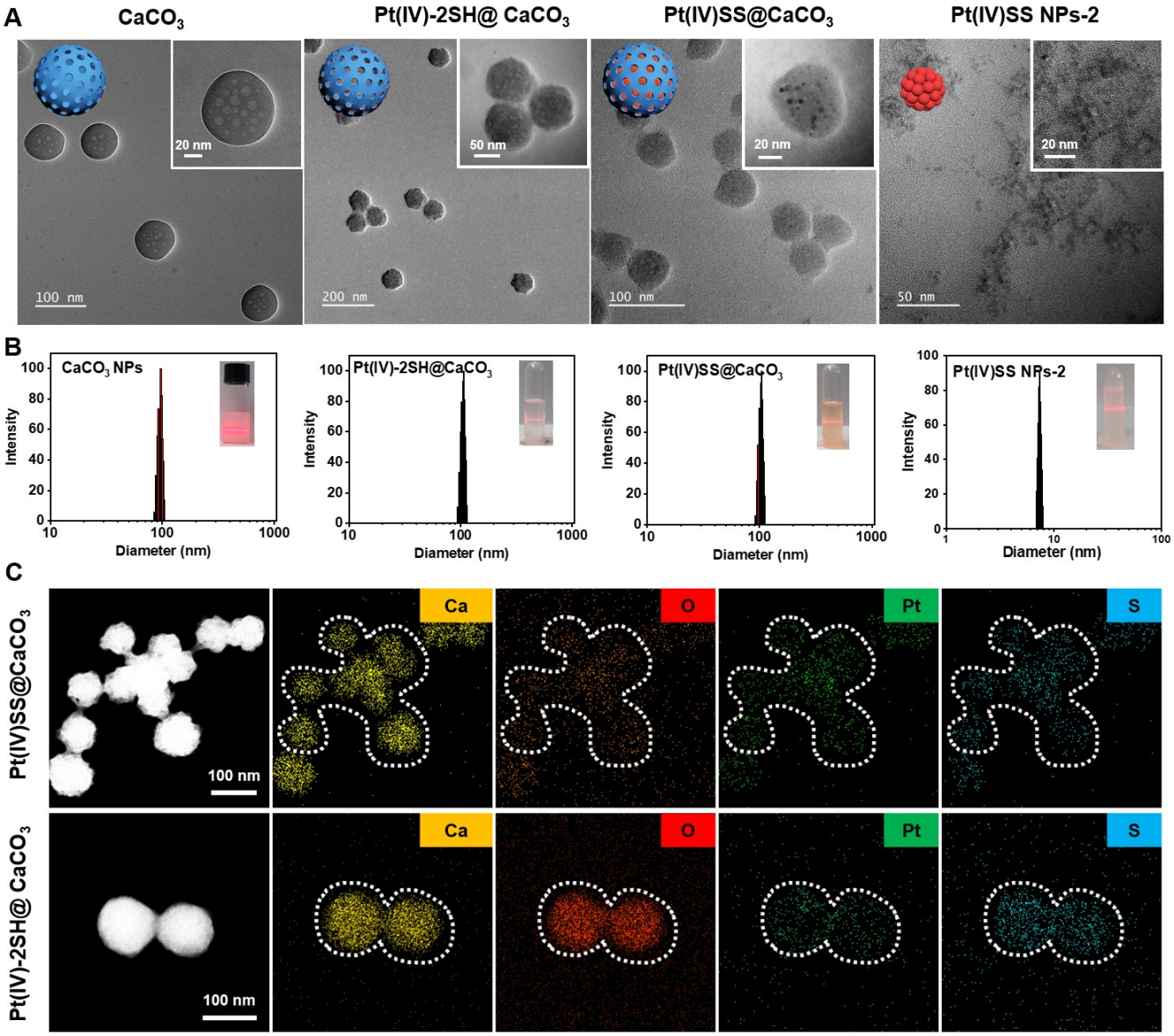


Figure S8. (A) Transmission electron microscopy image of $CaCO_3$, **Pt(IV)-2SH@$CaCO_3$**, **Pt(IV)SS@$CaCO_3$** and **Pt(IV)SS NPs-2**. (B) Dynamic light scattering spectra of $CaCO_3$, **Pt(IV)-2SH@$CaCO_3$**, **Pt(IV)SS@$CaCO_3$** and **Pt(IV)SS NPs-2**. Inset: Photograph of solutions of the nanoparticles within a laser beam. (C) Highly magnified high-angle annular dark-field scanning transmission electron microscopy image combined with elemental mapping of **Pt(IV)SS@$CaCO_3$** and **Pt(IV)-2SH@$CaCO_3$**.

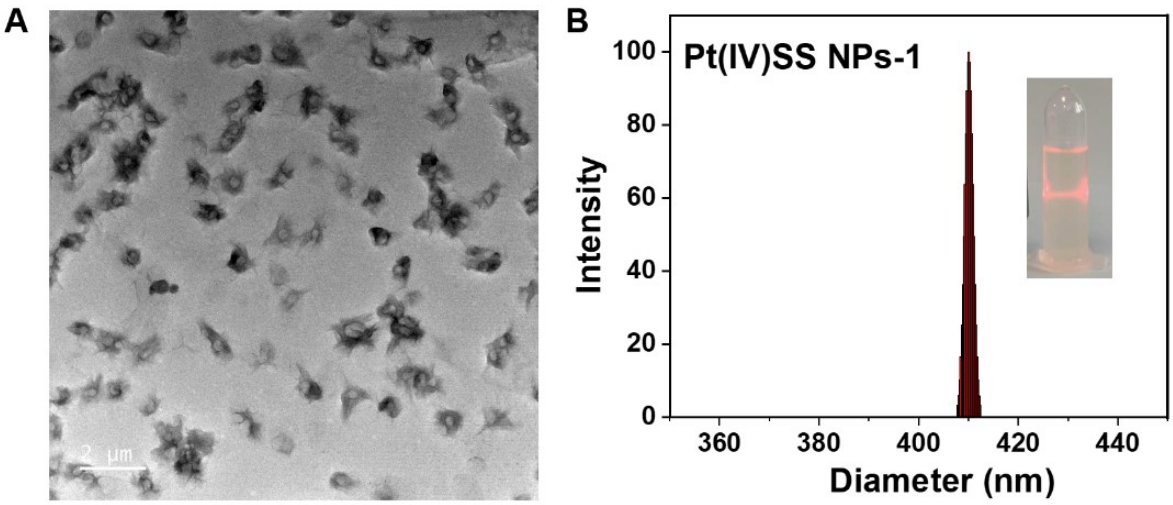


Figure S9. (A) Transmission electron microscopy images and (B) dynamic light scattering spectrum of **Pt(IV)SS NPs-1**. Inset: Photograph of solutions of the nanoparticles within a laser beam.

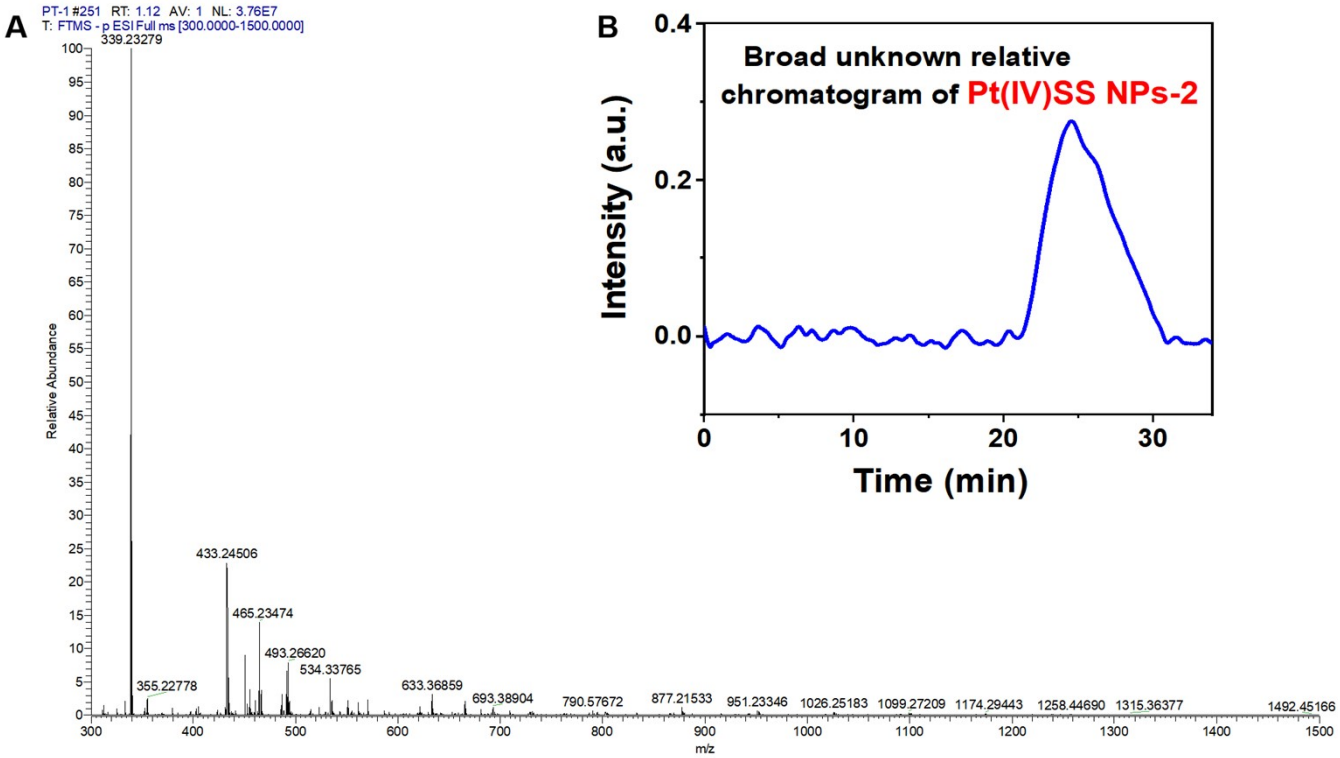


Figure S10. (A) **Pt(IV)SS@$CaCO_3$** was degraded by acid (pH 5.0) and purified using centrifugation, washing with dichloromethane, dialysis, and a HR-MS spectrum of the obtained **Pt(IV)SS NPs-2** recorded. (B) The broad unknown relative chromatogram of **Pt(IV)SS NPs-2** determined by gel permeation chromatography in tetrahydrofuran.

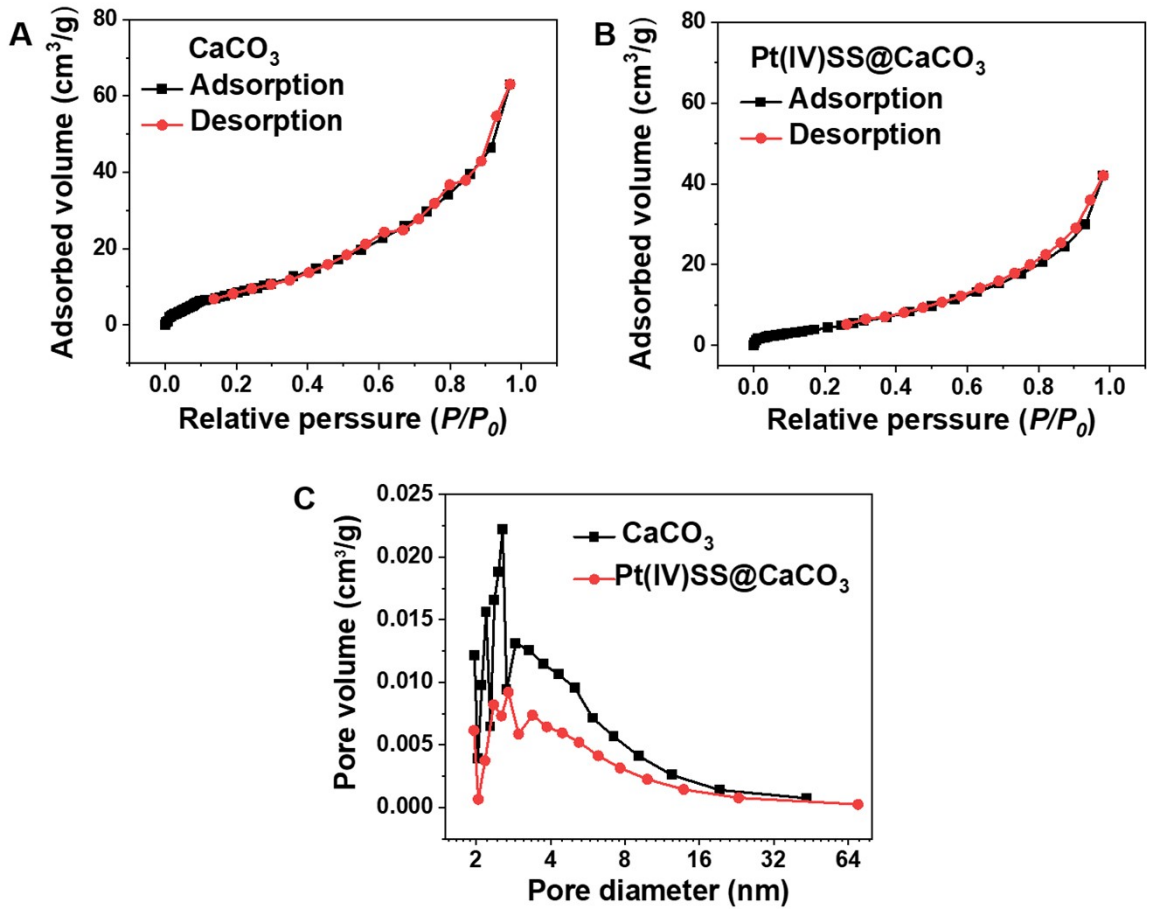


Figure S11. The N_2 adsorption/desorption isotherms of (A) bare $CaCO_3$ nanoparticles and (B) **Pt(IV)SS@CaCO$_3$**. (C) Pore-size distribution curve of $CaCO_3$ and **Pt(IV)SS@CaCO$_3$**.

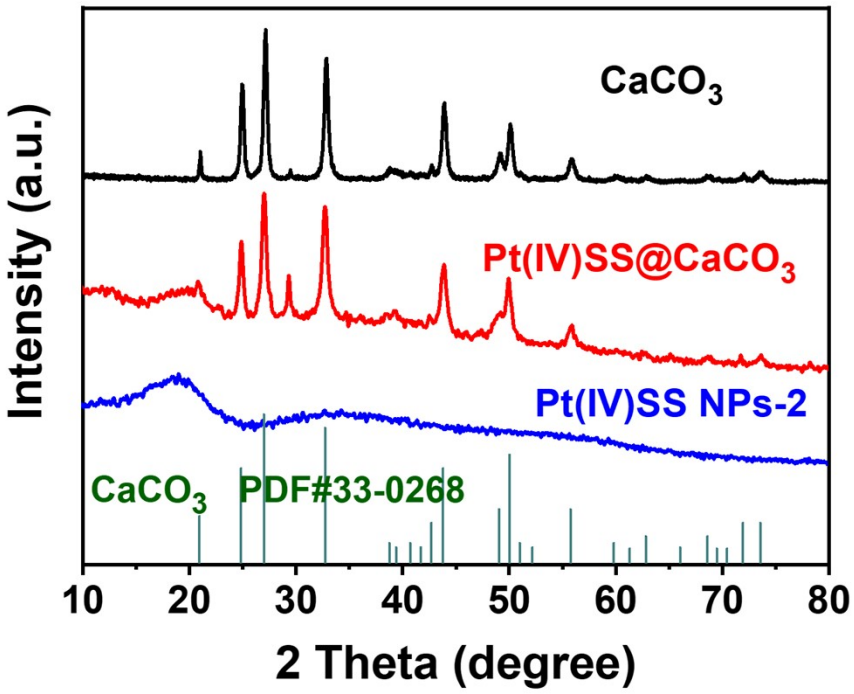


Figure S12. The X-ray diffraction (XRD) of $CaCO_3$, **Pt(IV)SS@$CaCO_3$** and **Pt(IV)SS NPs-2**.

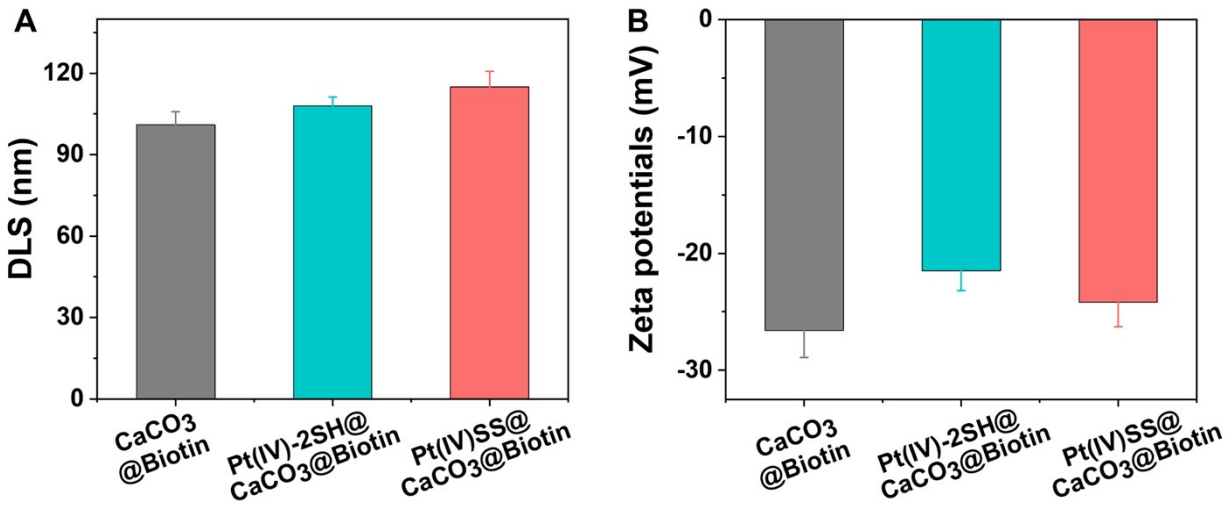


Figure S13. (A) Hydrodynamic diameter and (B) zeta potential of $CaCO_3$@Biotin, **Pt(IV)-2SH@$CaCO_3$@Biotin** and **Pt(IV)SS@$CaCO_3$@Biotin**.

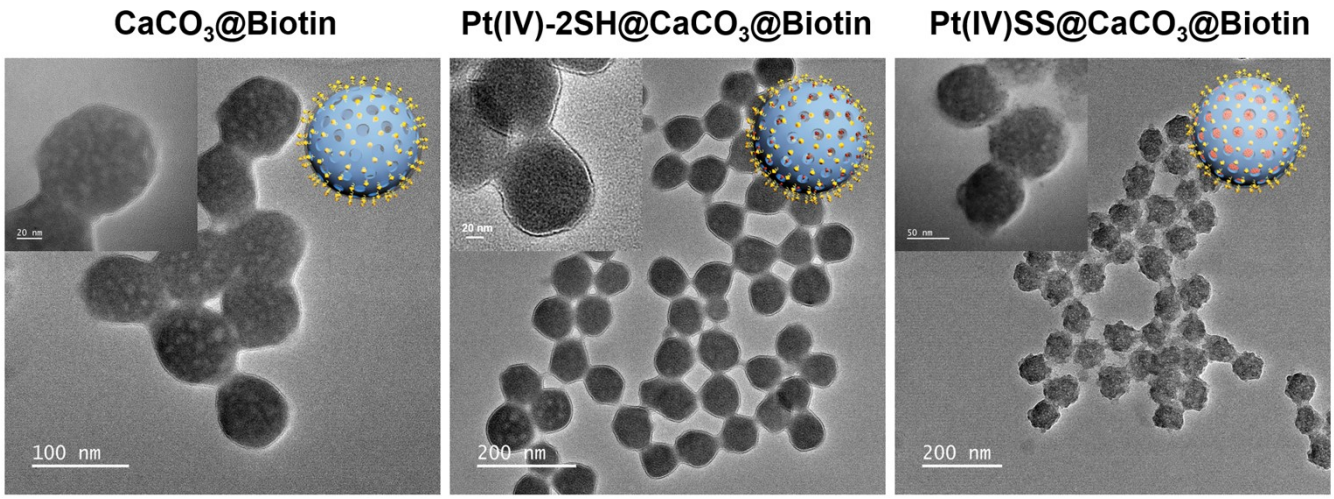


Figure S14. Transmission electron microscopy images of $CaCO_3$@Biotin, **Pt(IV)-2SH@$CaCO_3$@Biotin** and **Pt(IV)SS@$CaCO_3$@Biotin**.

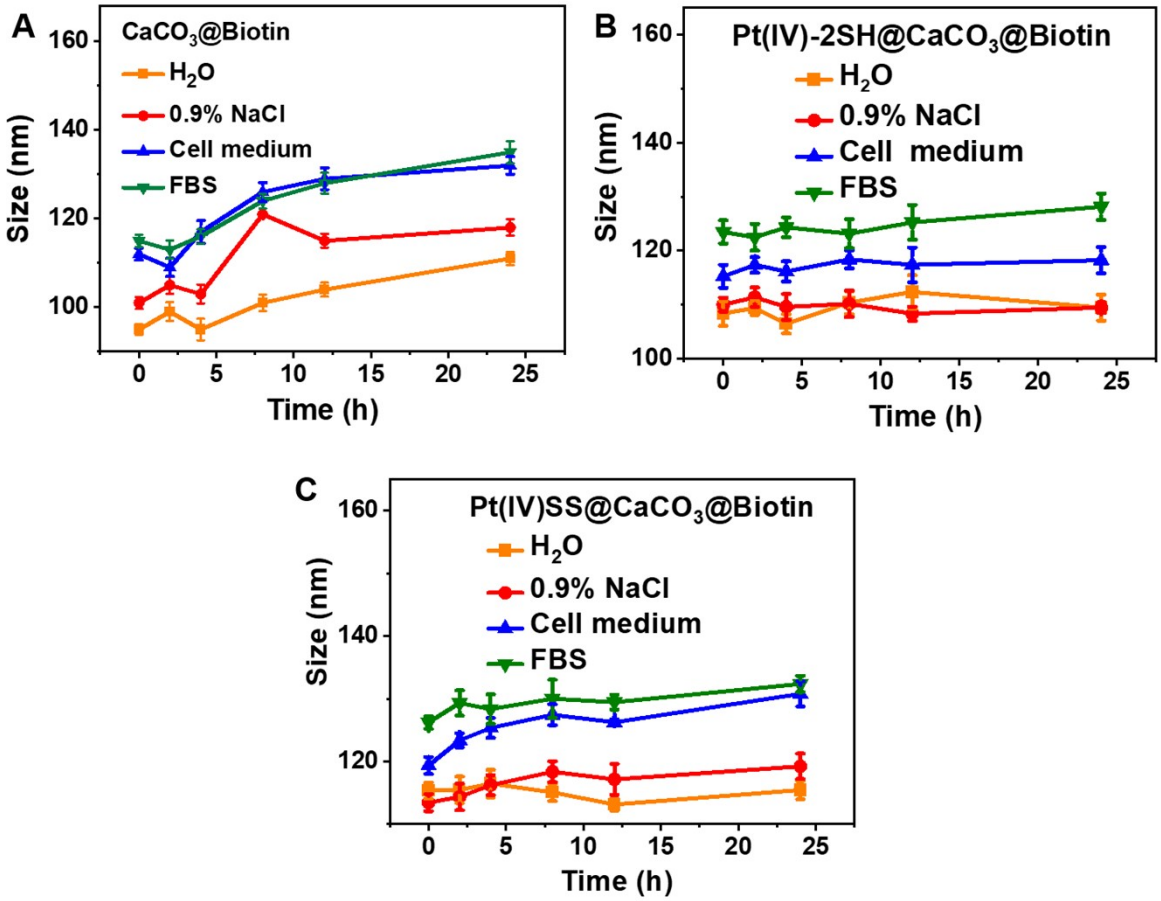


Figure S15. Time-dependent monitoring of the change in hydrodynamic diameter upon incubation of (A) $CaCO_3$@Biotin, (B) **Pt(IV)-2SH@$CaCO_3$@Biotin** and (C) **Pt(IV)SS@$CaCO_3$@Biotin** in water, an aqueous 0.9% sodium chloride solution, cell medium, or serum for 24 h.

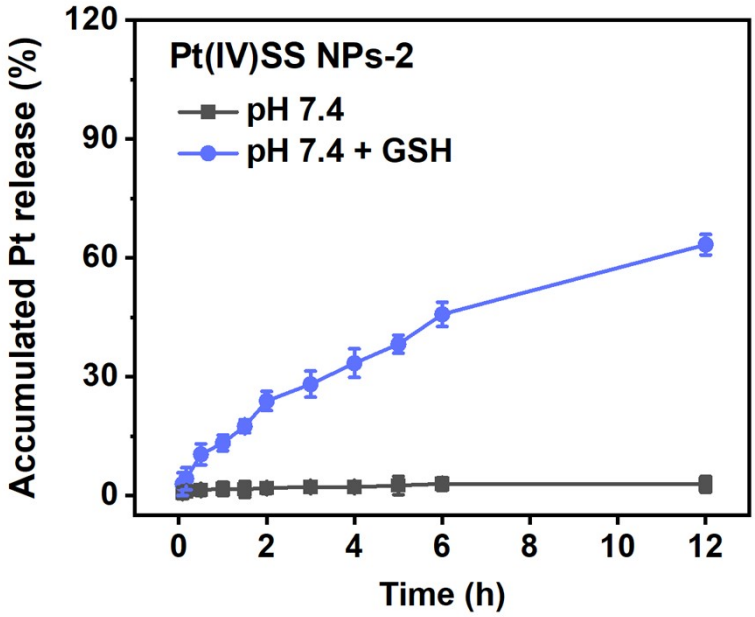


Figure S16. Time-dependent release Pt ion from **Pt(IV)SS NPs-2** by using ICP-MS under pH 7.4 without GSH, and pH 7.4 with GSH (100 µM).

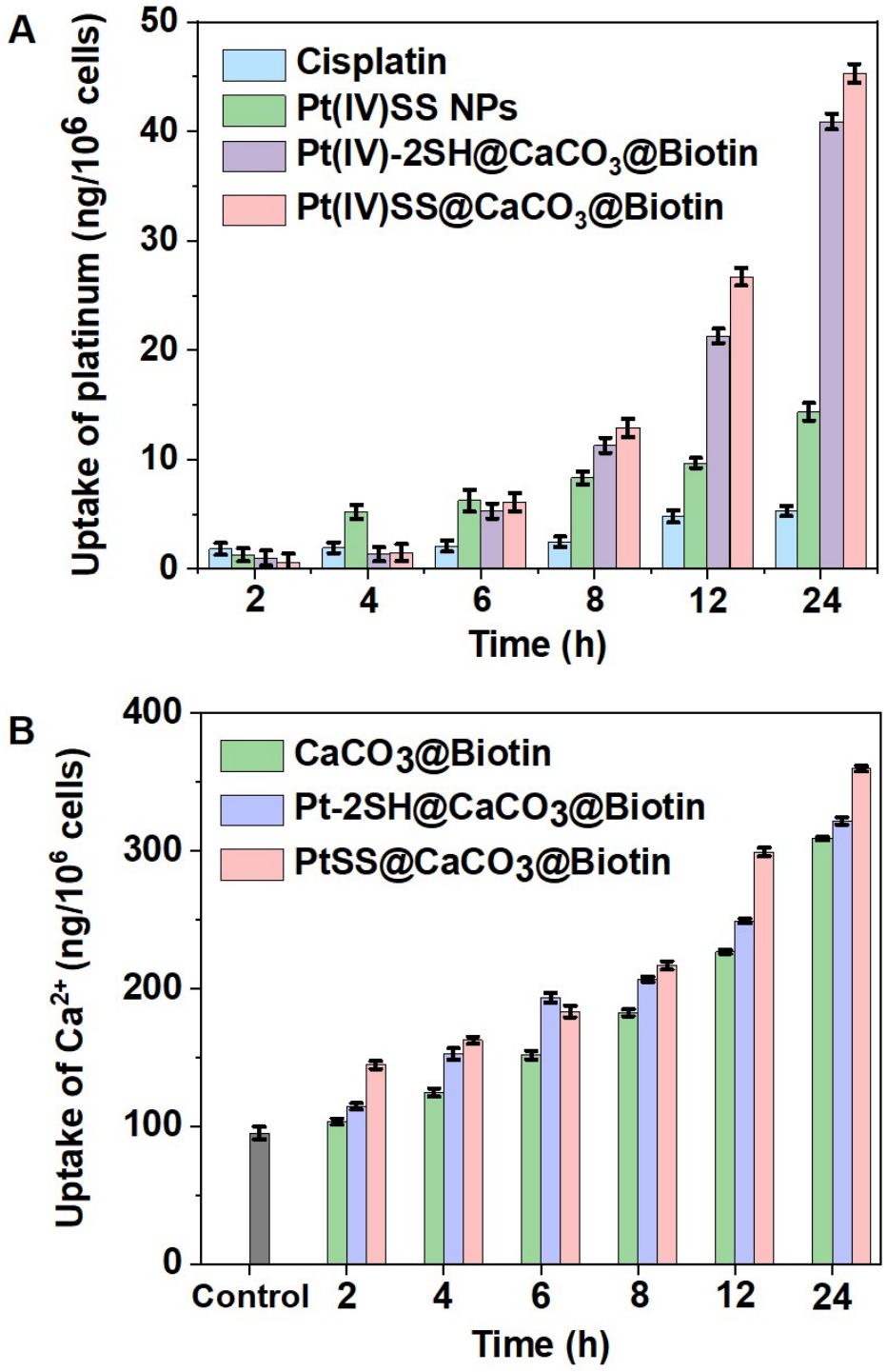


Figure S17. Cellular uptake of cisplatin (10 μM Pt^{2+}), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}), and **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}) upon incubation in A549R cells for various time intervals investigated through the determination of the (A) Pt and (B) Ca content by ICP-MS.

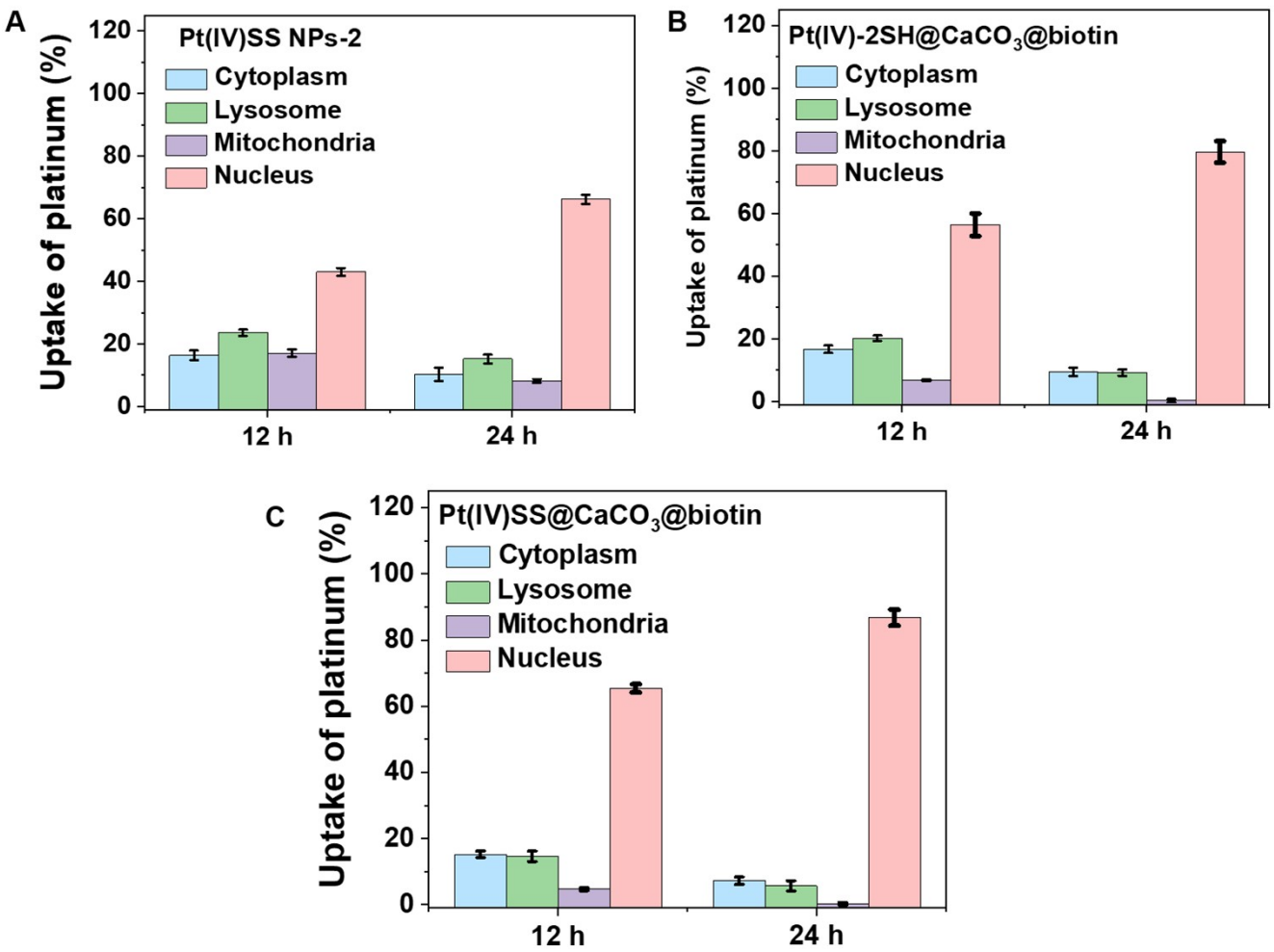


Figure S18. Subcellular distribution of (A) **Pt(IV)SS NPs-2** (10 μM Pt^{4+})**,** (B) **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}) and (C) **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}) upon incubation in A549R cells for 12 or 24 h investigated through determination of the platinum content by inductively coupled plasma mass spectrometry.

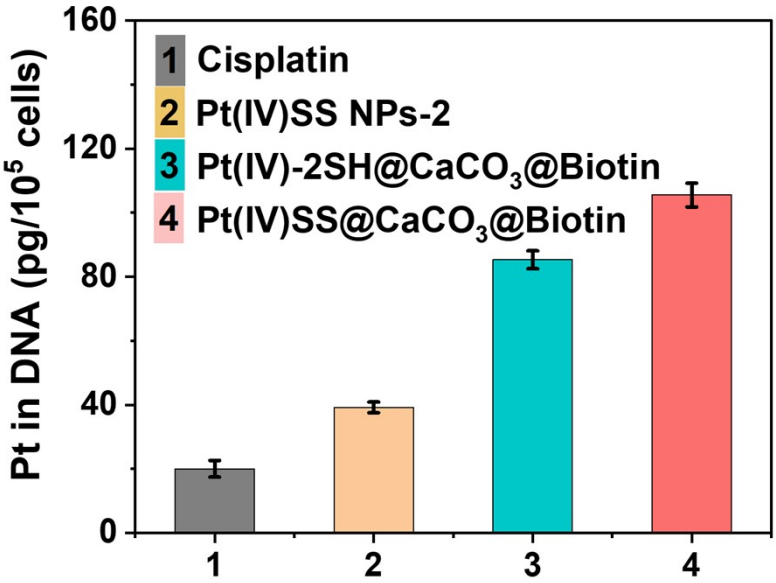


Figure S19. The DNA platination upon incubation of A549R cells with cisplatin (10 μM Pt^{2+}), **Pt(IV)SS NPs-2**, **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}), and **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}) for 12 h.

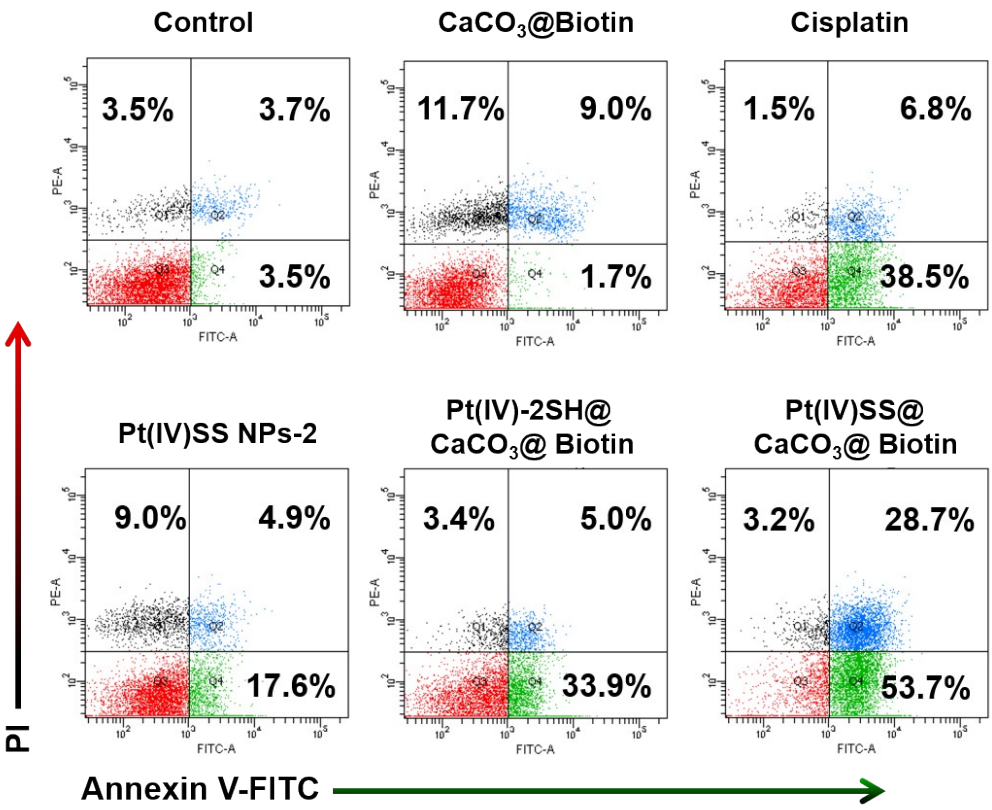


Figure S20. Flow cytometric analysis of A549R cells incubated with $CaCO_3$@Biotin (0.28 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (20 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}) for 24 h and stained with Annexin V-FITC/propidium iodide (PI).

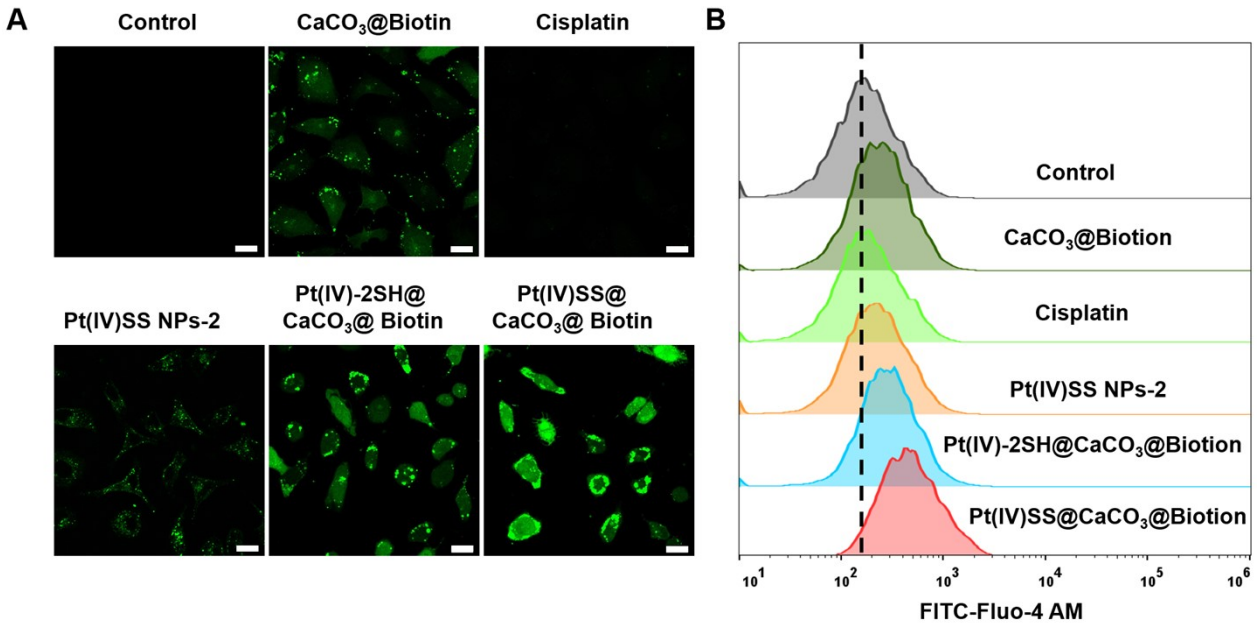


Figure S21. (A) Confocal laser scanning microscopy images and (B) flow cytometry data of A549R cells incubated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 µM), **Pt(IV)SS NPs-2** (10 µM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 µM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 µM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h and following incubation with the Ca^{2+} specific probe Fura-4-AM (5 µM). Fura-4-AM: $\lambda_{ex/em}$ = 488/520 ± 10 nm, Scale bars are 20 µm.

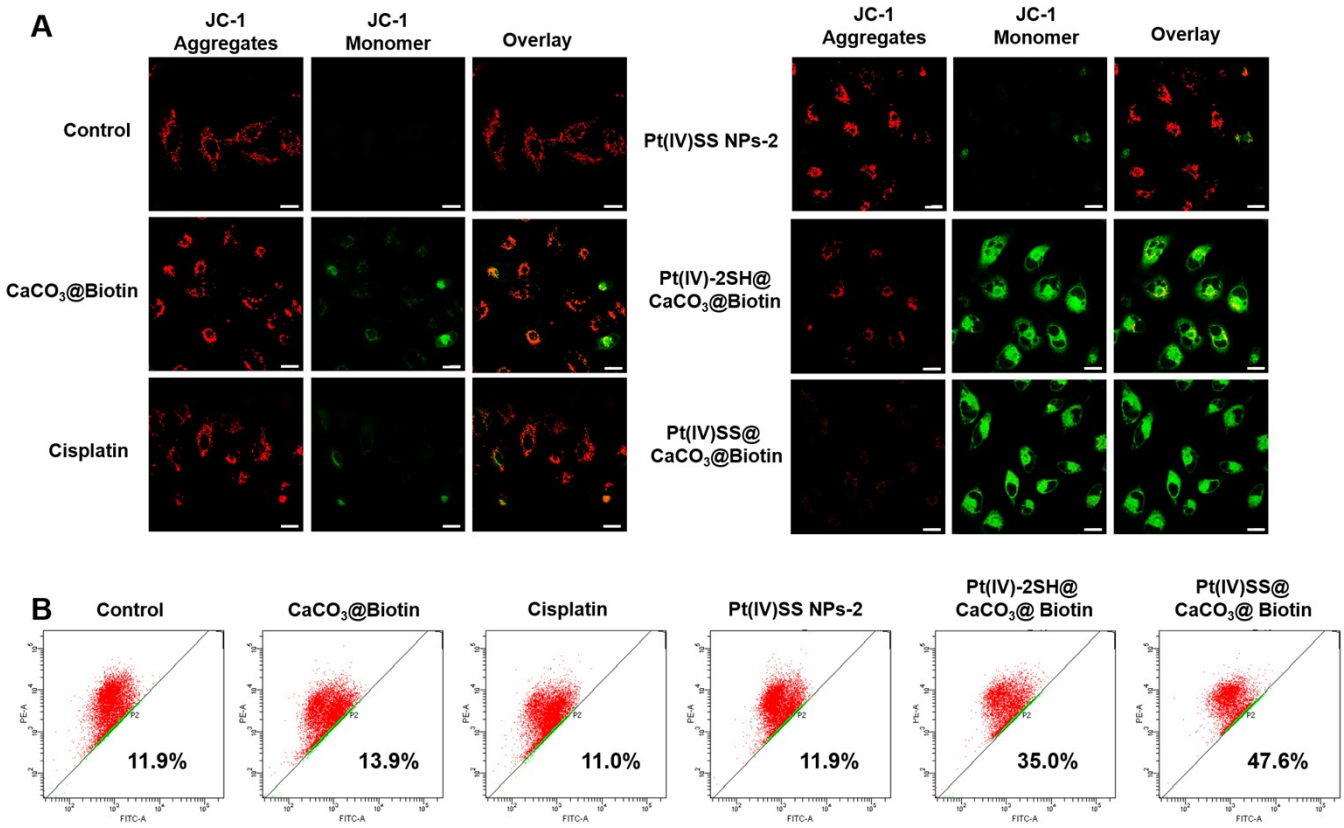


Figure S22. Mitochondrial depolarization of A549R cells was indicated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+})for 24 h by mitochondrial membrane potential kit staining (A) in the confocal imaging, JC-1,Aggregates: $\lambda_{ex/em}$ = 561/580 ± 10 nm, JC-1, Monomer: $\lambda_{ex/em}$ = 488/520 ± 10 nm, Scale bars are 20 μm, and (B) in the flow cytometry analysis.

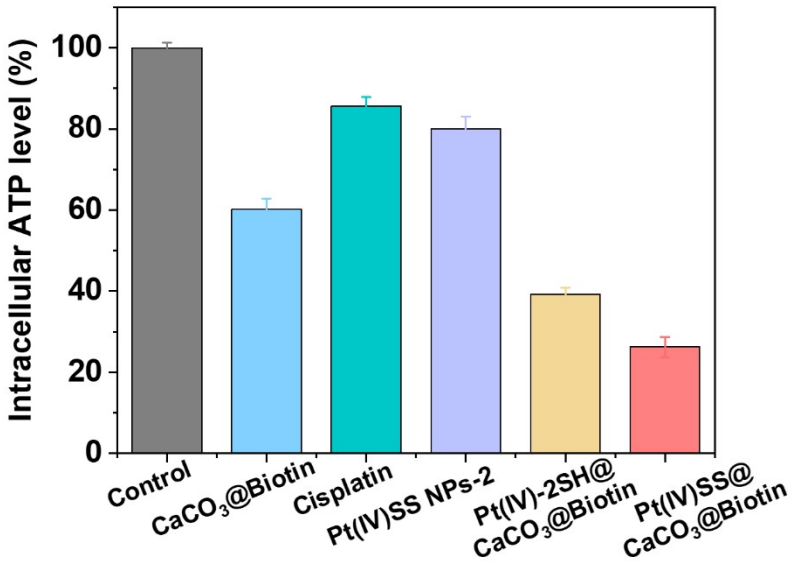


Figure S23. Changes in intracellular adenosine triphosphate levels of A549R cells upon treatment with $CaCO_3$@Biotin (0.28 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (20 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (20 μM Pt^{4+}, 0.28 mM Ca^{2+})for 24 h. Data is represented as mean ± standard deviation based on independent triplicate measurements.

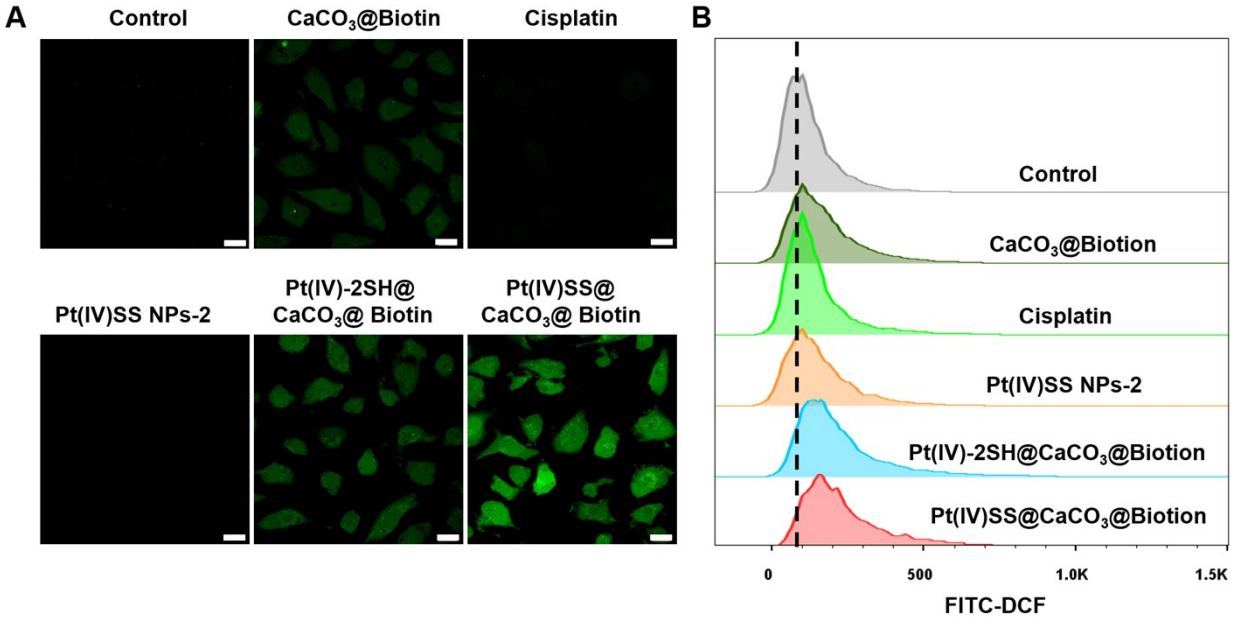


Figure S24. (A) Confocal laser scanning microscopy images and (B)Flow cytometry analysis of A549R cells incubated with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h and following incubation with ROS dye 2′,7′-dichlorodihydrofluorescein diacetate (10 μM). 2′,7′-Dichlorodihydrofluorescein: $\lambda_{ex/em}$ = 488/520 ± 10 nm, Scale bars are 20 μm.

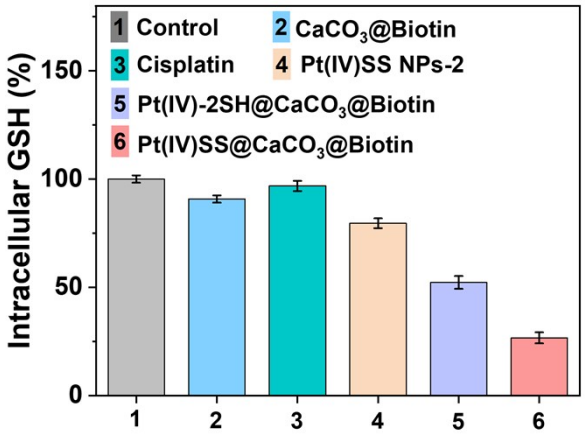


Figure S25. Depletion of GSH in A549R cells upon incubation with $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h.

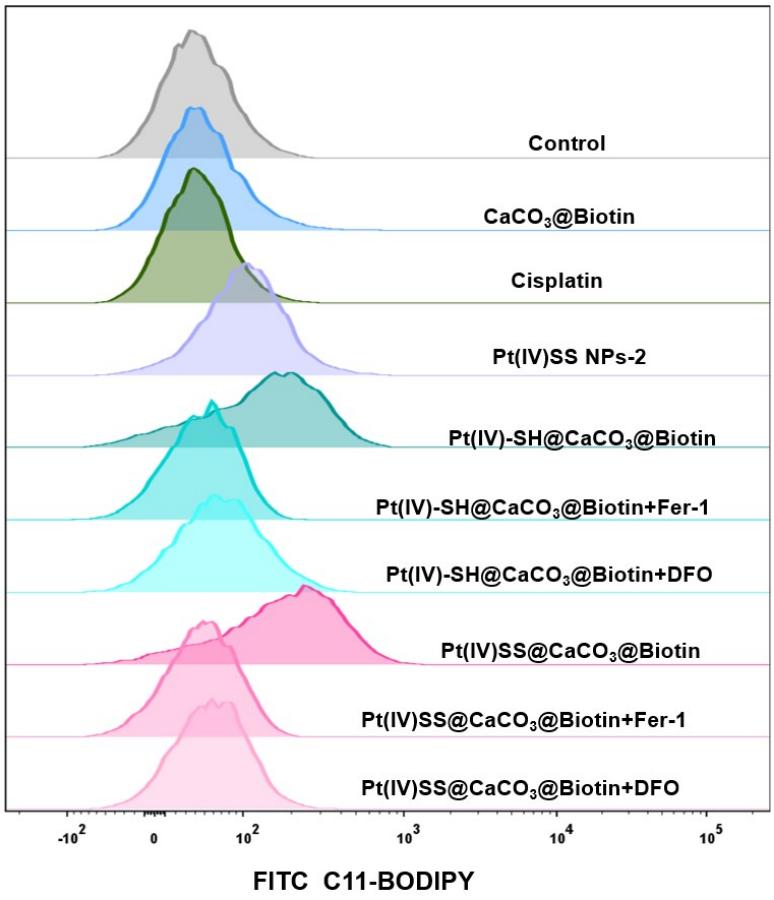


Figure S26. Flow cytometric assay of the generation of lipid peroxides in A549R cells. The cells were co-incubated with the IC_{50} of $CaCO_3$@Biotin, cisplatin, **Pt(IV)SS NPs-2**, **Pt(IV)-2SH@$CaCO_3$@Biotin**, or **2SH@$CaCO_3$@Biotin** for 24 h in the presence or absence of ferroptosis inhibitors deferoxamine (DFO, 100 μM) or ferrostatin (Fer-1, 10 μM). The cells were stained by C11-BODIPY (5 μM, 30 min).

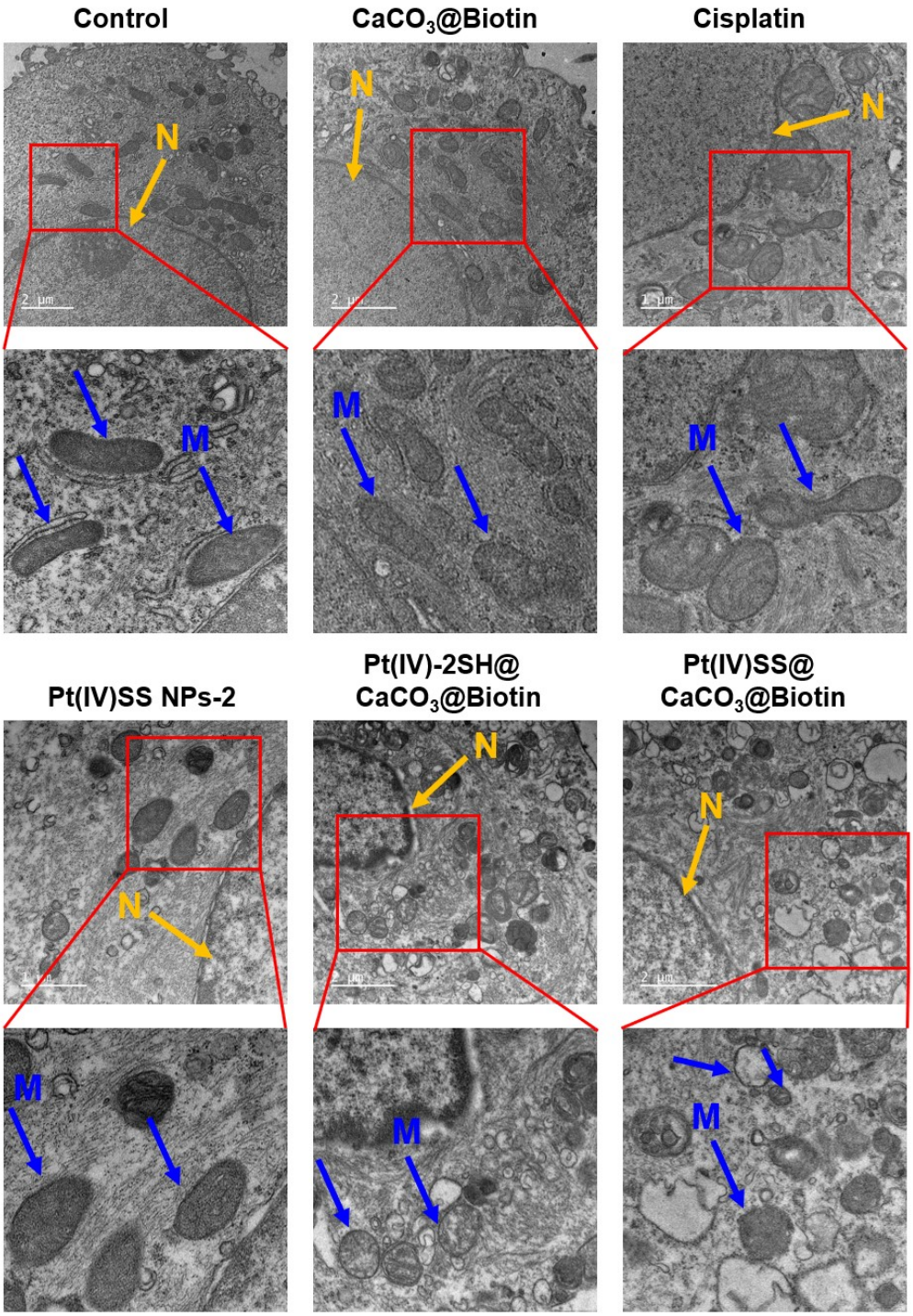


Figure S27. Bio-TEM images of micromorphological changes of A549R cells treated with phosphate-buffered saline, $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (10 μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 12 h (blue and orange arrows indicate the mitochondria and nucleus, respectively).

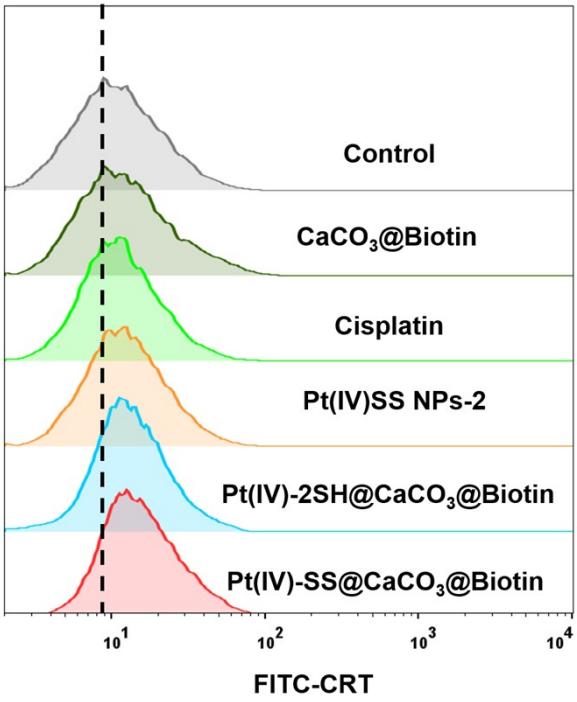


Figure S28. Flow cytometry results of CRT expression in drugs-treated A549R cells. A549 cells were treated with phosphate-buffered saline, $CaCO_3$@Biotin (0.14 mM Ca^{2+}), cisplatin (IC_{50} μM), **Pt(IV)SS NPs-2** (10 μM Pt^{4+}), **Pt(IV)-2SH@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}), or **Pt(IV)SS@$CaCO_3$@Biotin** (10 μM Pt^{4+}, 0.14 mM Ca^{2+}) for 24 h.

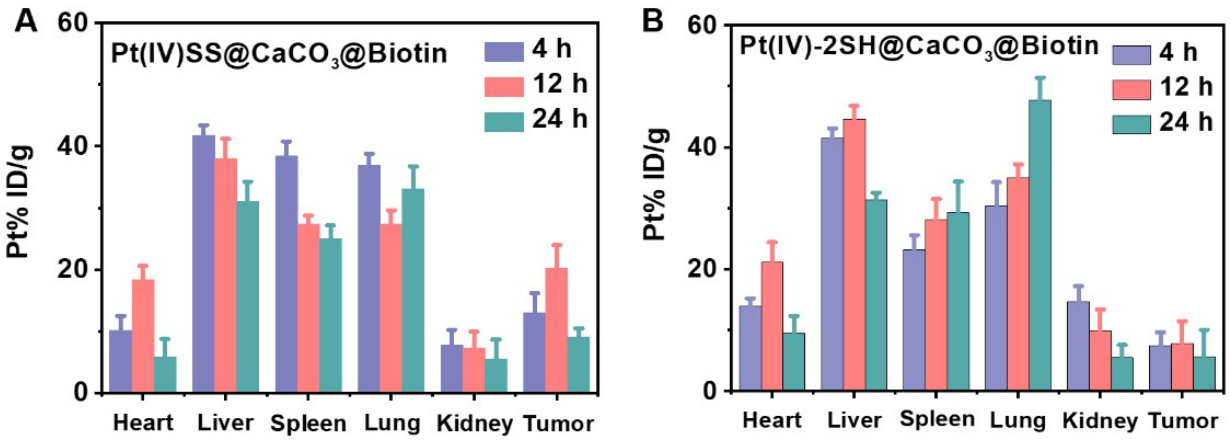


Figure S29. Biodistribution of **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt) and **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt) in the major orangs 4, 12, or 24 h after intravenous injection determined by inductively coupled plasma mass spectrometry. Data is represented as mean ± standard deviation based on independent triplicate measurements.

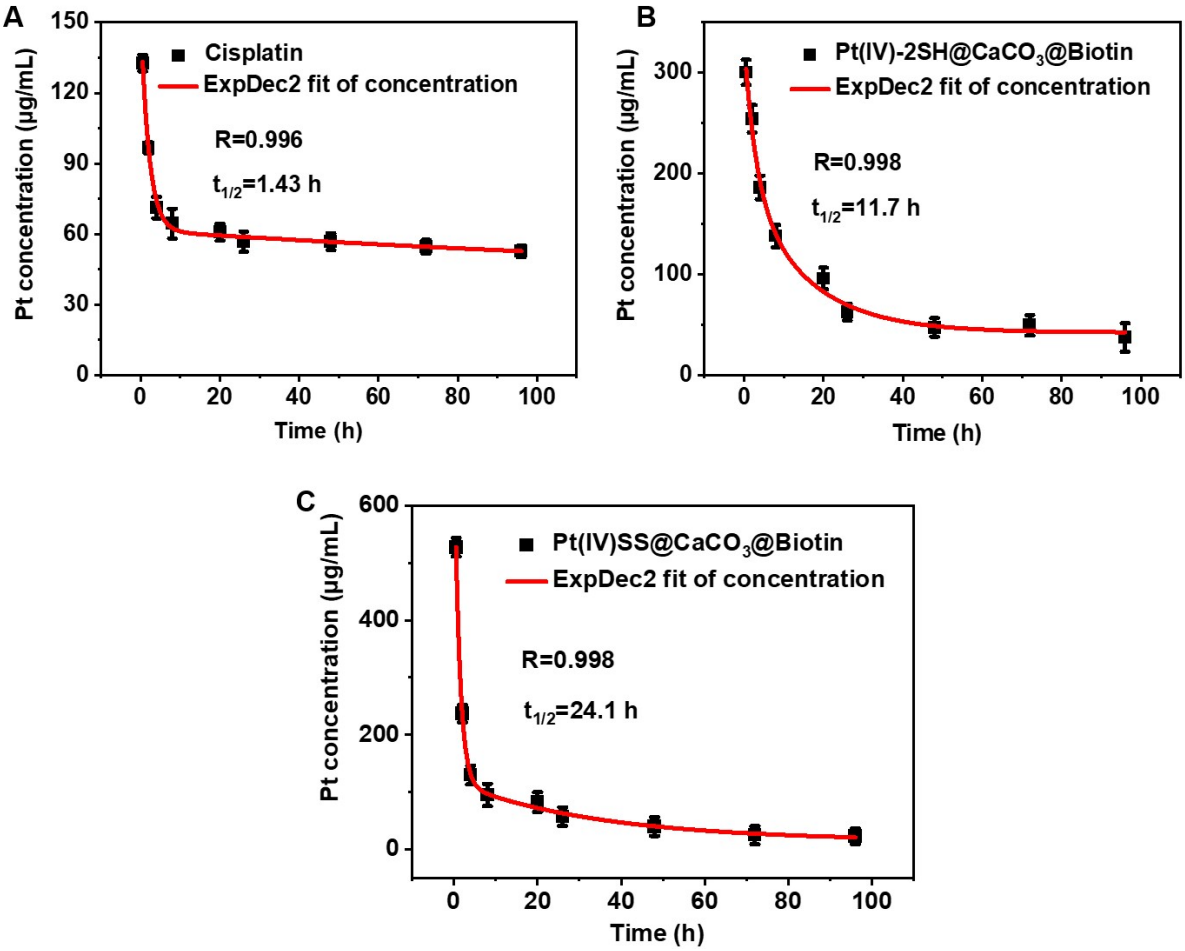


Figure S30. Blood-circulation half-time of (A) cisplatin (5 mg/kg, based on 1.67 mM Pt), (B) **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), and (C) **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt) after intravenous injection determined by the analysis of the platinum content in the blood of the mice by inductively coupled plasma mass spectrometry. Data is represented as mean ± standard deviation based on independent triplicate measurements.

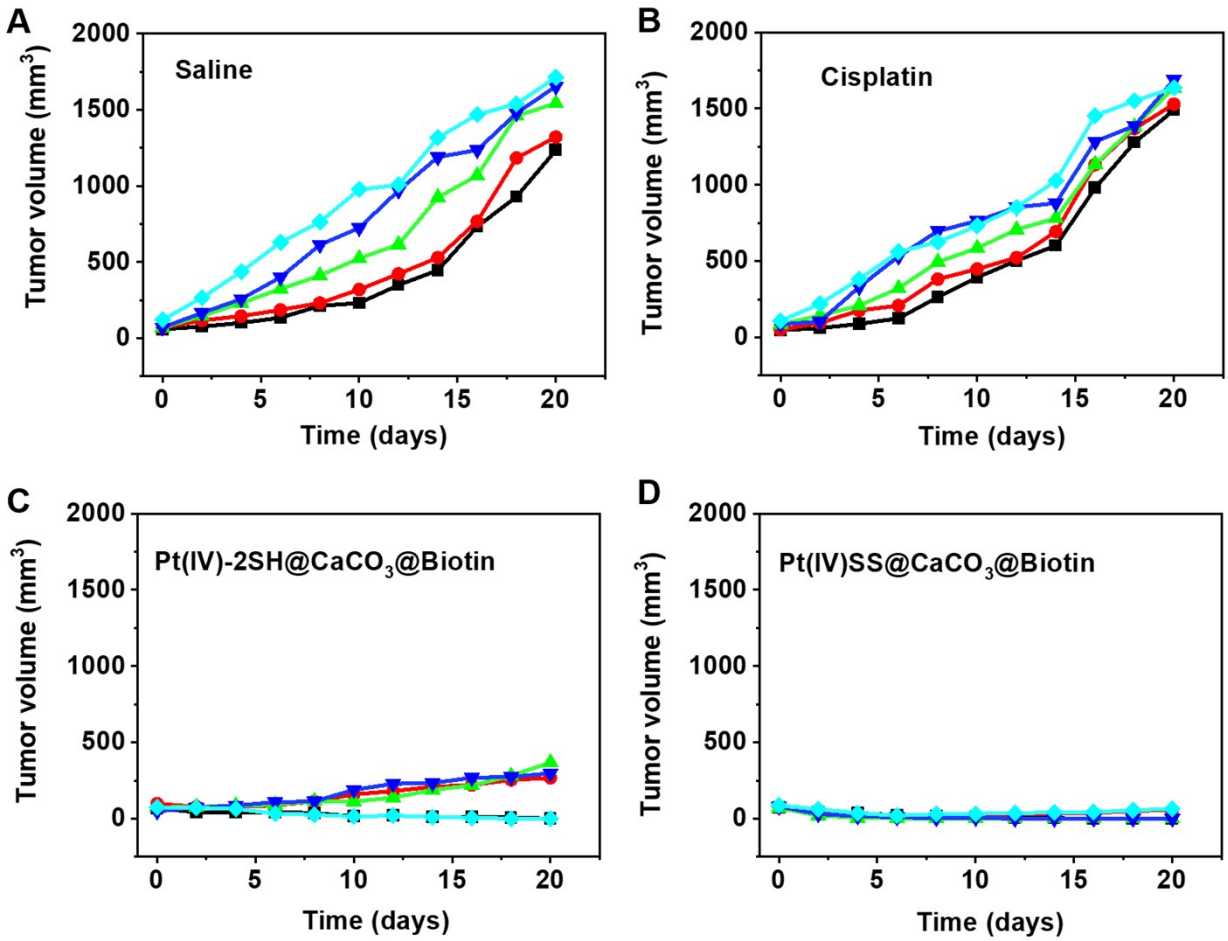


Figure S31. Tumor growth curves of each mouse model upon respective treatment. Group I: saline (200 μL), Group II: cisplatin (5 mg/kg, based on 1.67 mM Pt), Group III: **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), Group IV: **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). The animals were intravenously injected in the tail vein on day 1, day 3, and day 5.

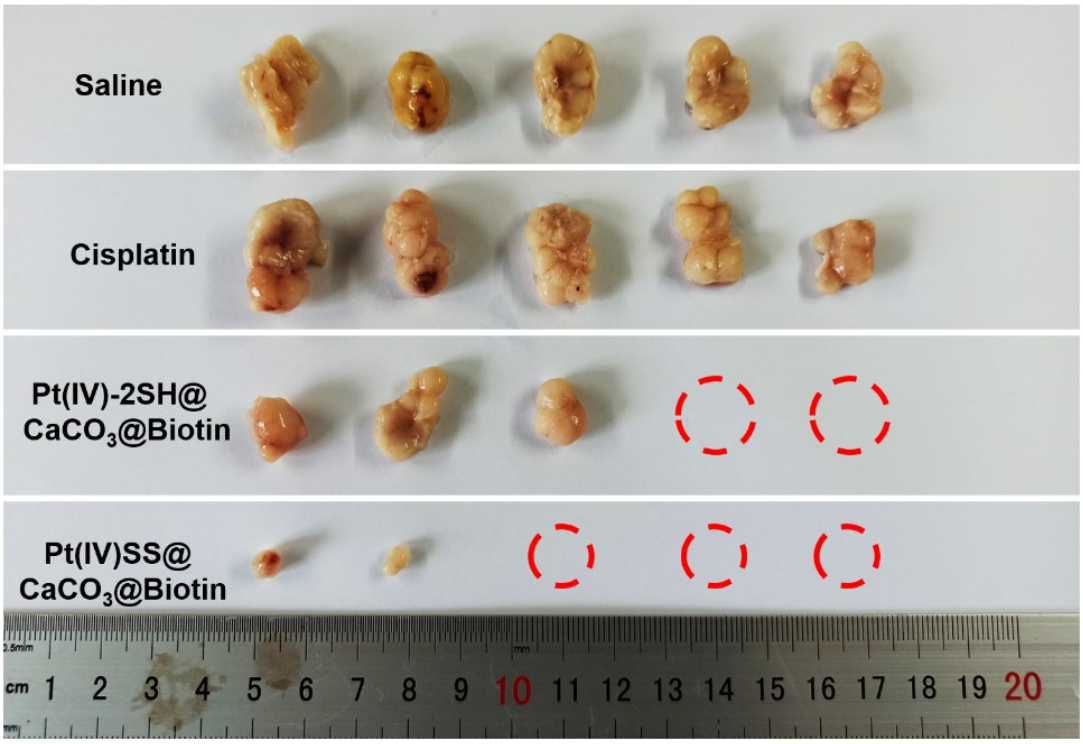


Figure S32. Digital photographs of the isolated tumors from each group at the end of treatments (day 20).

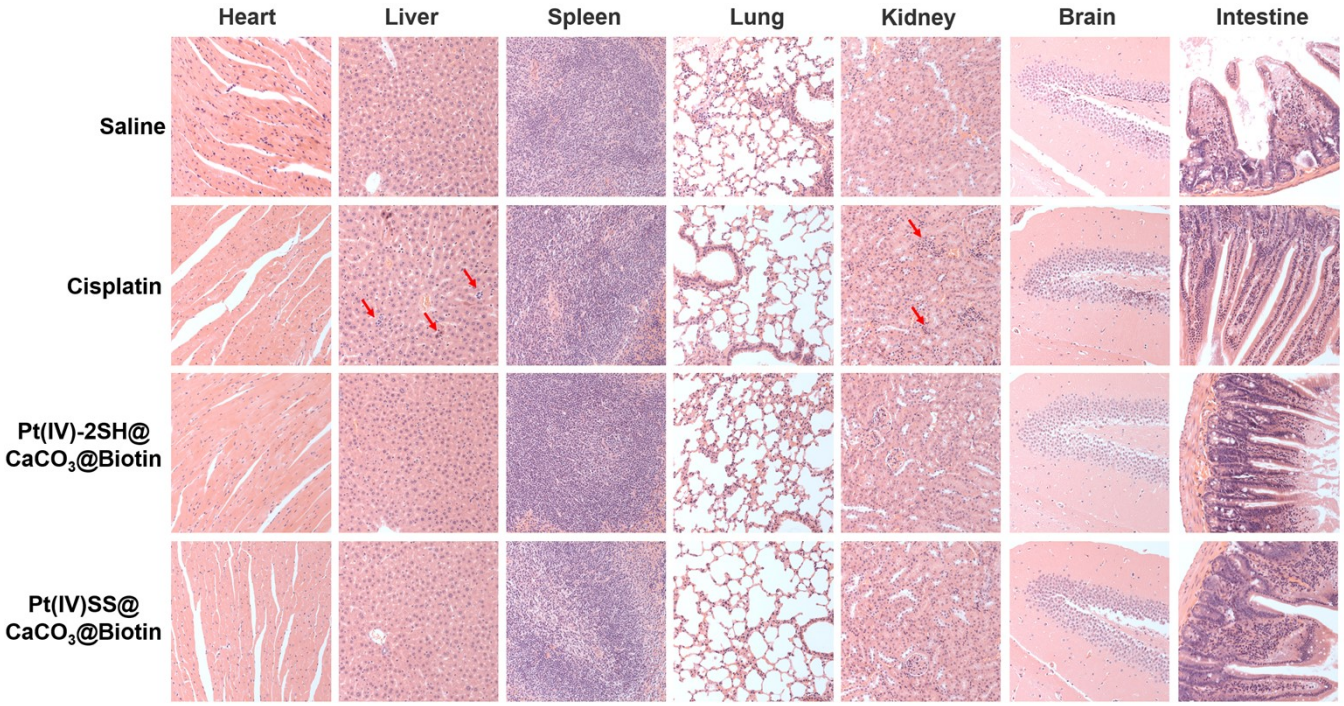


Figure S33. Histological analysis of the main organs using a hematoxylin and eosin stain upon treatment of the mice on day 1, day 3, and day 5 and collection of the organs on day 20. Tissue damage was marked with red arrows. Group I: saline (200 µL), Group II: cisplatin (5 mg/kg, based on 1.67 mM Pt), Group III: **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), Group IV: **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). Scale bar = 50 µm.

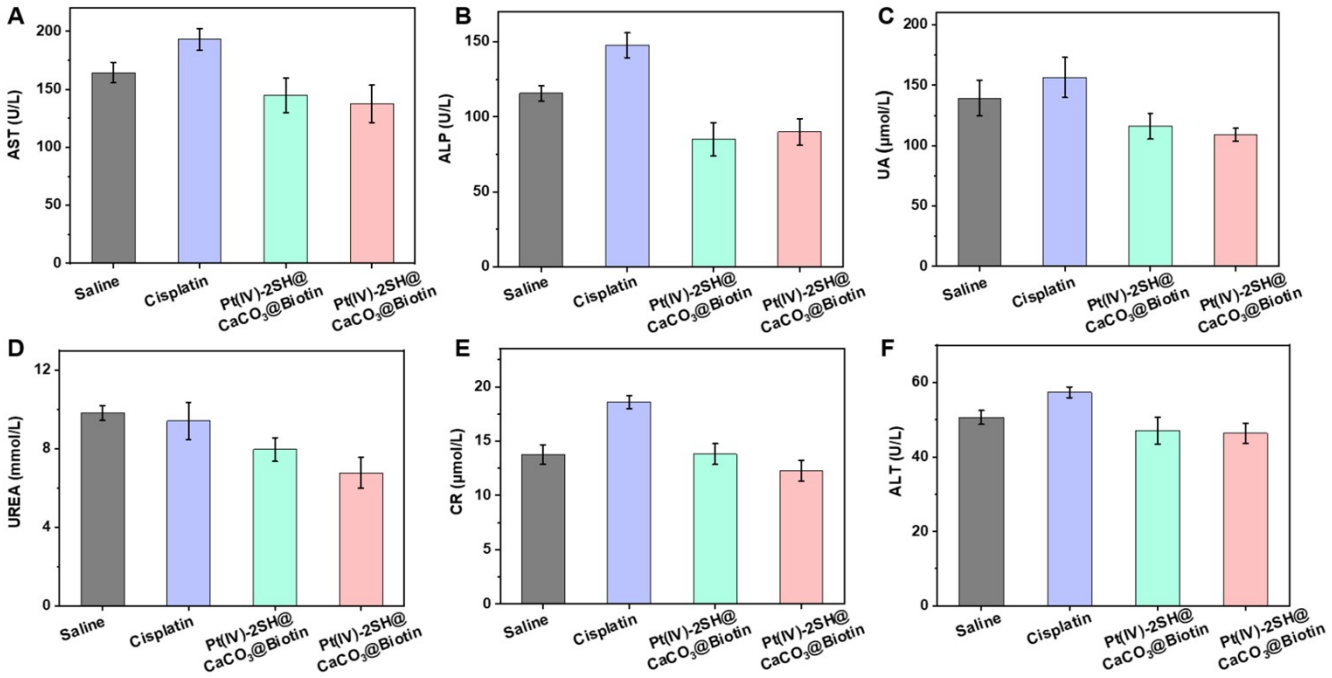


Figure S34. A-C) Hepatic function markers and D-F) renal function markers upon treatment of the mice on day 1, day 3, and day 5 and collection of the samples on day 20. Group I: saline (200 µL), Group II: cisplatin (5 mg/kg, based on 1.67 mM Pt), Group III: **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), Group IV: **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt). Data is represented as mean ± standard deviation based on independent triplicate measurements.

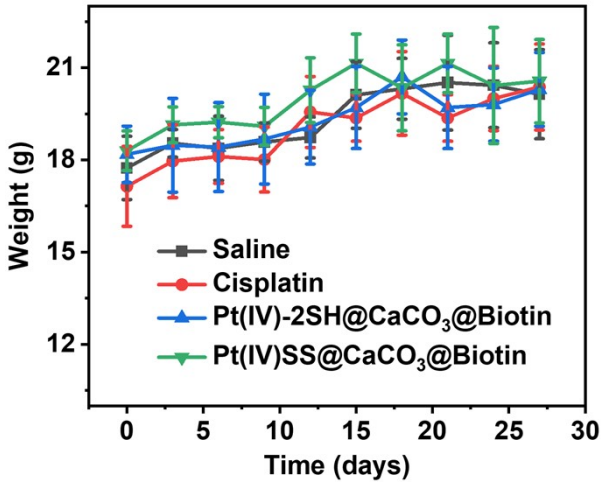


Figure S35. The weight of the C57BL/6 mice throughout the follow-up period. Data is represented as mean ± standard deviation (n=5).

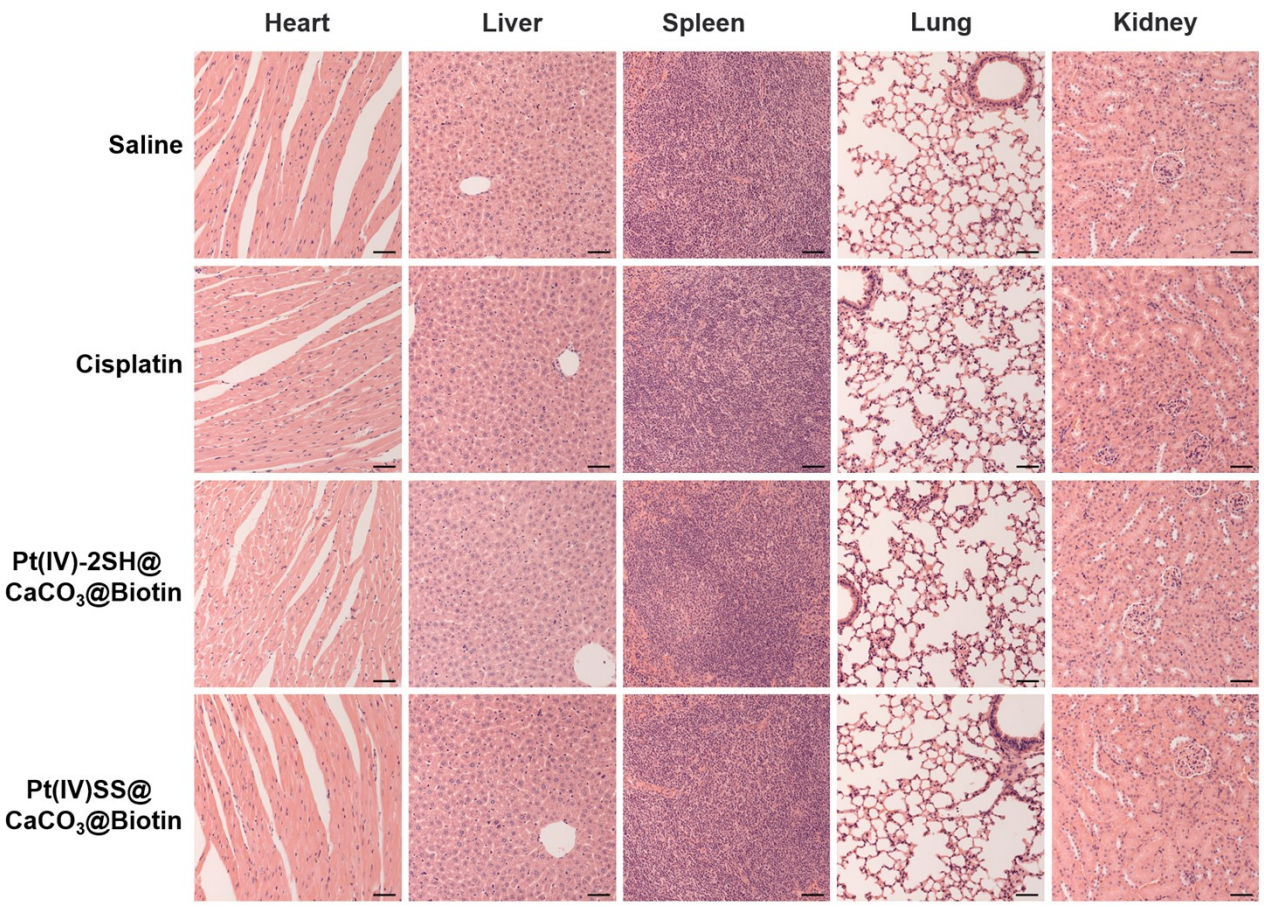


Figure S36. Histological analysis of the main organs (Heart, Liver, Spleen, Lung and Kidney) using a hematoxylin and eosin stain upon treatment of the C57BL/6J mice and collection of the organs on day 27.

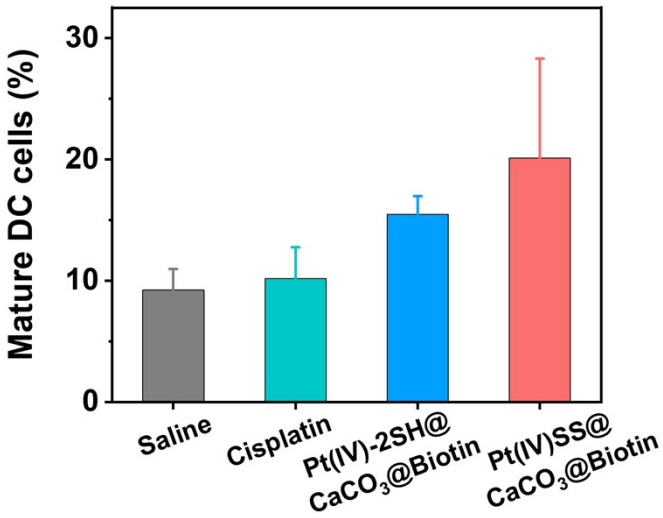


Figure S37. Representative flow cytometric quantification of mature DC cells on tumor-draining lymph node. Data is represented as mean ± standard deviation (n=5).

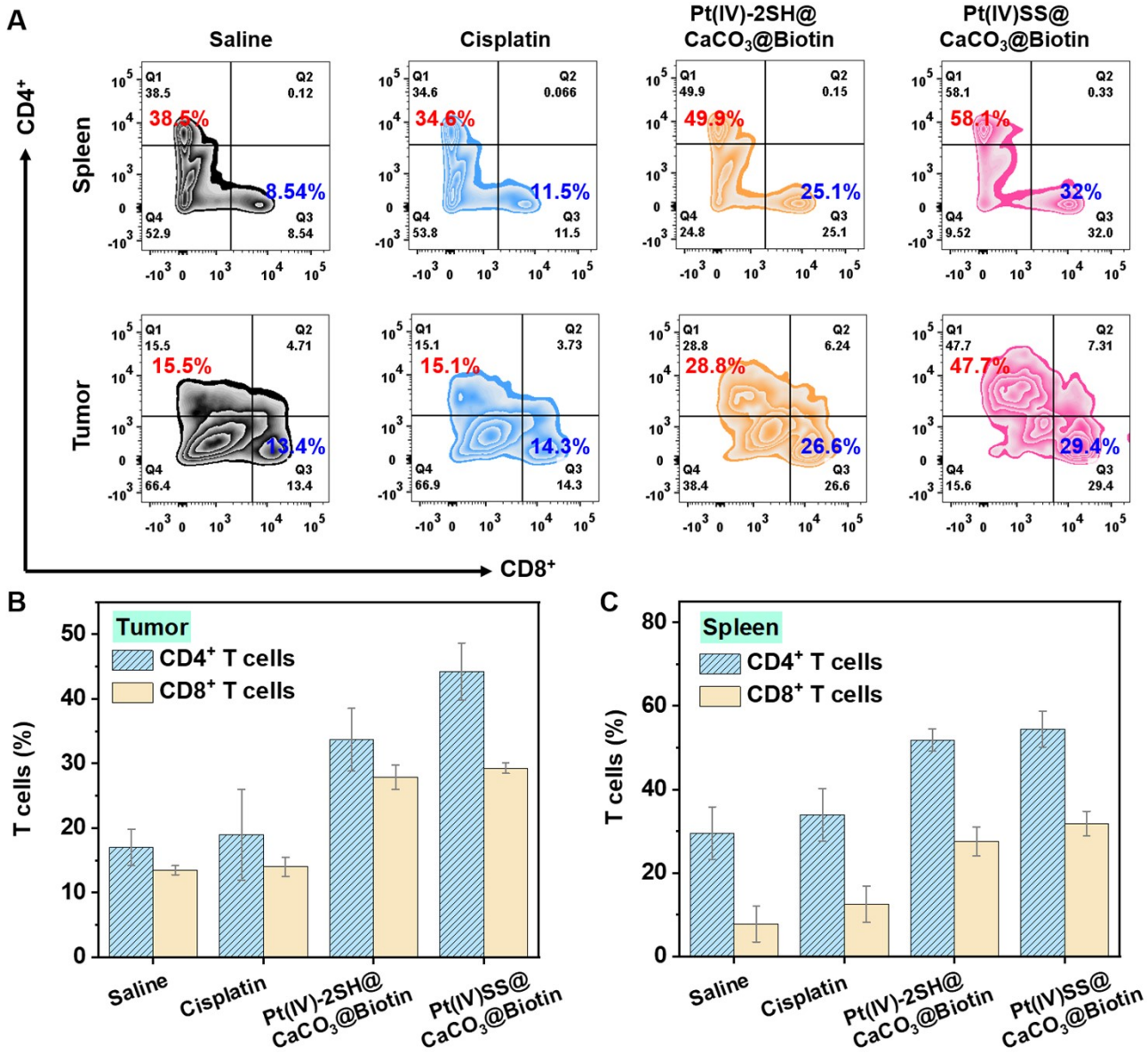


Figure S38. (A) Flow cytometric analysis of $CD4^+$ and $CD8^+$ T cells (gating on $CD3^+$ T cells) in the spleen and tumors. Representative flow cytometric quantification of $CD4^+$ and $CD8^+$ T cells (gating on $CD3^+$ T cells) in tumor (B) and spleen (C). Data is represented as mean ± standard deviation (n = 4).

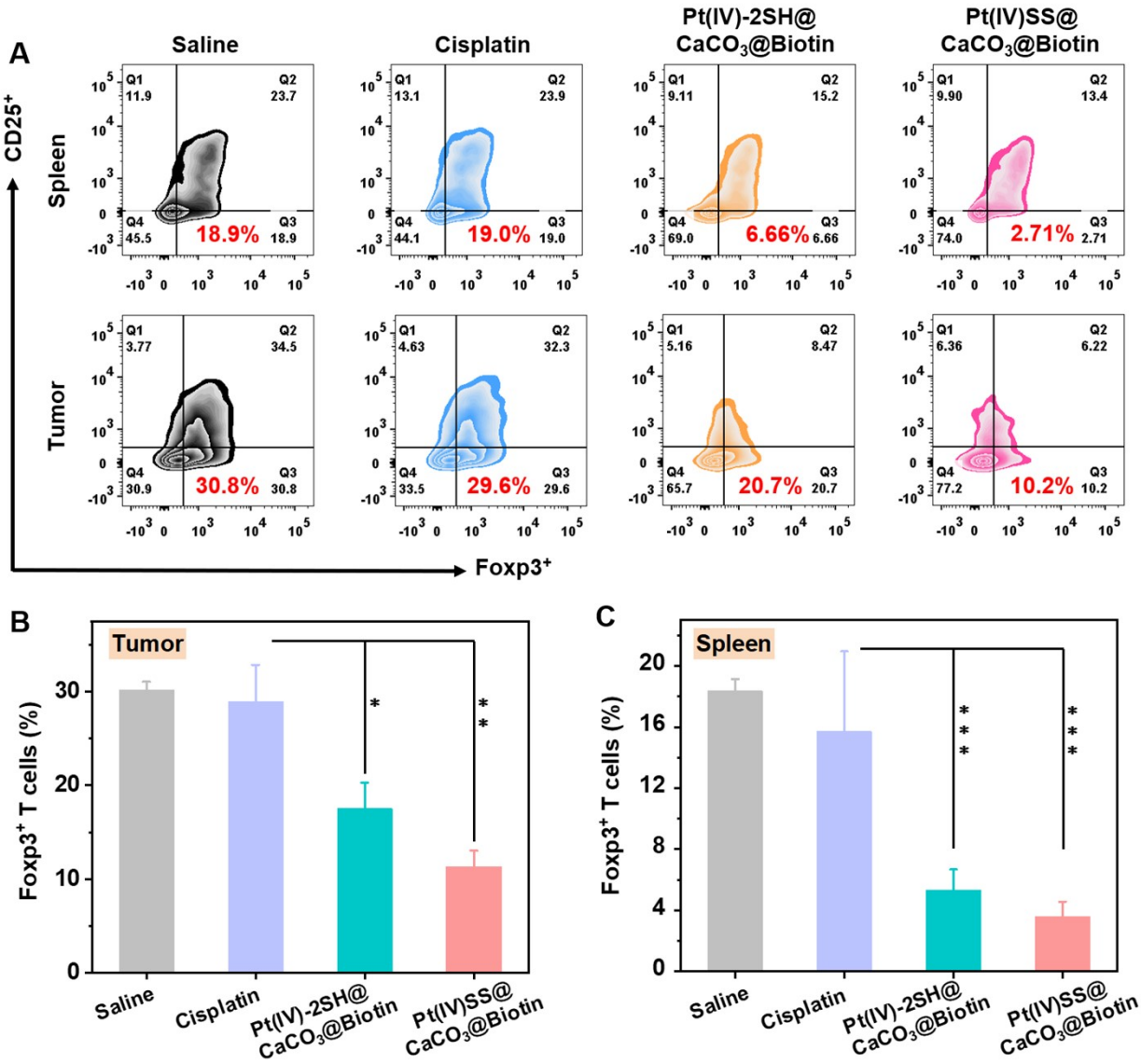


Figure S39. (A) Flow cytometric analysis of Foxp3$^+$ T cells (gating on CD3$^+$CD4$^+$ T cells) in the spleen and tumors. Representative flow cytometric quantification of Foxp3$^+$ T cells (gating on CD3$^+$CD4$^+$ T cells) in tumor (B) and spleen (C). Data is represented as mean ± standard deviation (n = 4).

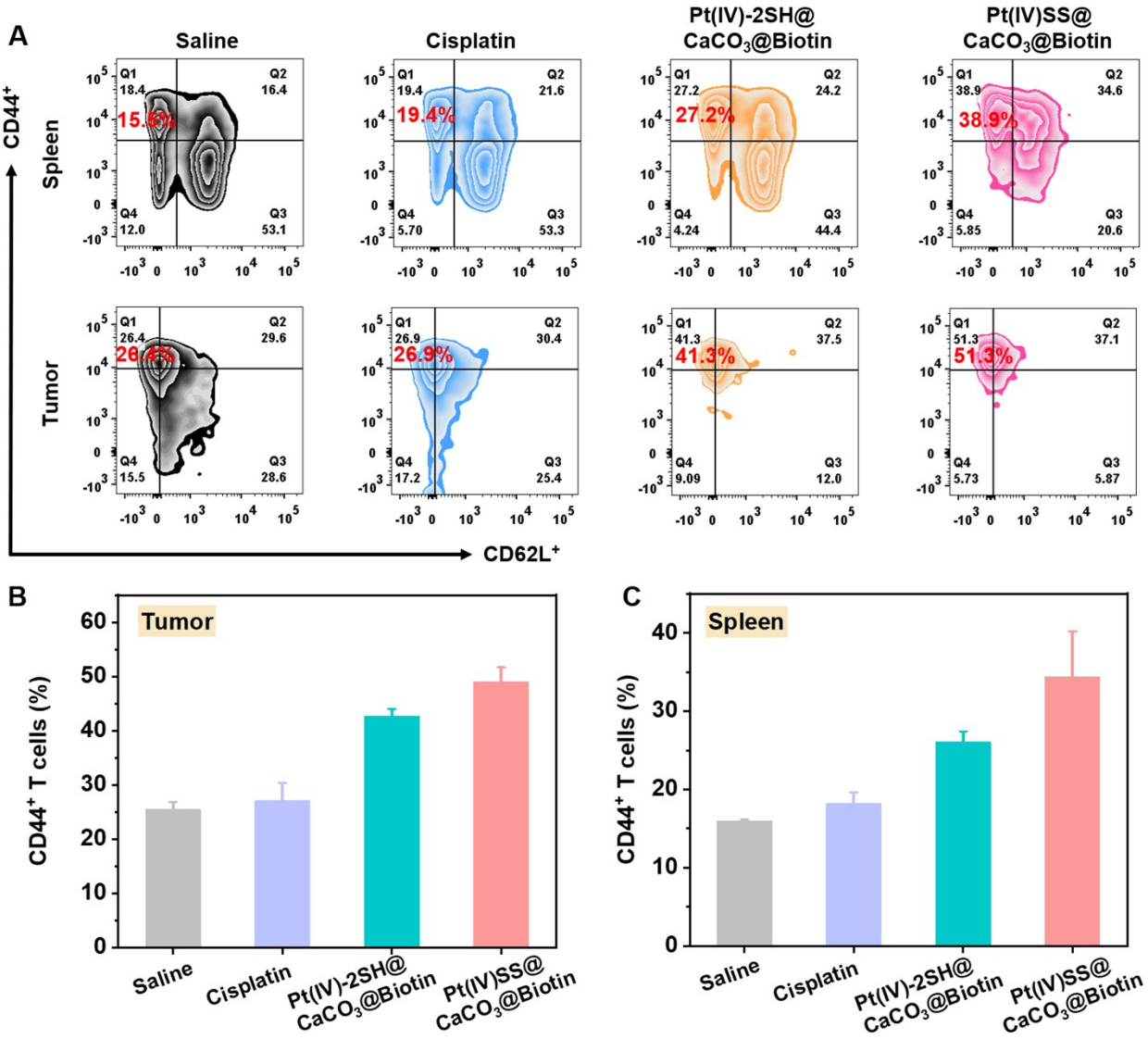


Figure S40. A) Flow cytometric analysis of memory T cells ($CD44^+$, $CD62L^+$, gating on $CD4^+$ T cells) in the spleen and tumors. Representative flow cytometric quantification of memory T cells ($CD44^+$, $CD62L^+$, gating on $CD4^+$ T cells) in tumor (B) and spleen (C). Data is represented as mean ± standard deviation (n = 4).

Table S1. Inductively coupled plasma mass spectrometry analysis of the platinum content in **Pt(IV)-2SH@$CaCO_3$@Biotin** or **Pt(IV)SS@$CaCO_3$@Biotin**. Data is represented as mean ± standard deviation based on independent triplicate measurements.

Samples	Content (%)
Pt(IV)-2SH@$CaCO_3$@Biotin	21.4 ± 0.92
Pt(IV)SS@$CaCO_3$@Biotin	19.8 ± 1.08

Table S2. Cytotoxicity (μM) of **$CaCO_3$@Biotin**, cisplatin, **Pt(IV)SS NPs-2, Pt(IV)-2SH@$CaCO_3$@Biotin** or **Pt(IV)SS@$CaCO_3$@Biotin** towards A549, A549 and LLC cells. Data is represented as mean ± standard deviation based on independent triplicate measurements.

	$CaCO_3$@Biotin (Based on $CaCO_3$)	Cisplatin	Pt(IV)SS NPs-2 (Based on Pt)	Pt(IV)-2SH@ $CaCO_3$@Biotin (Based on Pt)	Pt(IV)SS@ $CaCO_3$@Biotin (Based on Pt)
A549	233.4± 3.2	16.3 ± 1.2	6.4 ± 0.81	1.71 ± 0.54	0.77 ± 0.18
A549R	315.2± 9.2	86.1 ± 3.4	14.9 ± 1.4	4.87 ± 0.98	2.82 ± 0.87
LLC	267.6± 8.7	16.8 ± 3.1	7.2 ± 0.34	2.9 ± 0.88	1.2 ± 0.31

The cytotoxicity was obtained after incubation of the cells with the compound for 48 h.

Table S3. Level of various biomarkers after treatment. Data is represented as mean ± standard deviation based on independent quintuplet measurements (Group I: saline (200 µL), Group II: cisplatin (5 mg/kg, based on 1.67 mM Pt), Group III: **Pt(IV)-2SH@$CaCO_3$@Biotin** (5 mg/kg, based on 0.548 mM Pt), Group IV: **Pt(IV)SS@$CaCO_3$@Biotin** (5 mg/kg, based on 0.507 mM Pt).

		Saline	Cisplatin	Pt(IV)-2SH@$CaCO_3$@Biotin	Pt(IV)SS@$CaCO_3$@Biotin
WBC	10^9/L	6.79 ± 1.61	8.13 ± 1.05	3.18 ± 0.84	3.23 ± 0.75
RBC	10^{12}/L	12.724 ± 0.29	9.63 ± 0.74	11.6 ± 0.98	11.49 ± 0.28
HGB	g/L	168.6 ± 3.51	145.5 ± 3.54	170.8 ± 0.43	169.2 ± 5.01
HCT	%	51.68 ± 0.54	45.4 ± 3.71	50.7 ± 1.75	49.94 ± 1.43
MCV	fL	43.66 ±0.89	44.24 ± 0.39	43.5 ± 0.48	43.48 ± 0.61
MCH	pg	14.38 ± 0.39	14.74 ± 0.29	14.66 ± 0.67	14.72 ± 0.28
MCHC	g/L	329.4 ± 3.57	333.6 ± 5.5	342.6 ± 0.57	338.8 ± 2.86
PLT	10^9/L	1192.3 ± 35.1	1865 ± 3.18	1017.6 ± 237.7	1013.6 ± 81.9
RDW-SD	fL	25.58 ± 3.18	26.92 ± 2.06	23.3 ± 0.93	23.12 ± 0.64
RDW-CV	%	21.46 ± 1.97	21.14 ± 0.73	20.6 ± 0.84	20.04 ± 0.31
PDW	fL	11.73 ± 6.45	7.96 ± 0.65	7.7 ± 3.44	8.97 ± 0.76
MPV	fL	8.88 ± 0.68	7.3 ± 0.35	7.32 ± 0.61	7.56 ± 0.34
P-LCR	%	20.03 ± 4.36	7.84 ± 2.56	5.93 ± 0.73	9.9 ± 2.01
PCT	%	1.55 ±0.28	1.995 ± 0.43	1 ± 0.23	1.06 ± 0.29
NRBC#	10^9/L	0.218 ± 0.17	0.074 ± 0.023	0.048 ± 0.02	0.058 ± 0.029
NRBC%	%	4.37 ± 1.05	2.2 ± 0.991	1.9 ± 0.66	1.5 ± 0.36
NEUT#	10^9/L	4.81 ± 1.16	2.65 ± 1.81	1.72 ± 0.99	2.38 ± 0.68
LYMPH#	10^9/L	1.94 ± 0.63	0.84 ± 0.31	0.69 ± 0.23	0.69 ± 0.17
MONO#	10^9/L	0.224 ± 0.06	0.32 ± 0.21	0.106 ± 0.06	0.072 ± 0.003
EO#	10^9/L	0.082 ± 0.03	0.044 ± 0.039	0.042 ± 0.027	0.042 ± 0.013
BASO#	10^9/L	0.006 ± 0.004	0.008 ± 0.013	0.004 ± 0.0054	0.004 ± 0.005
NEUT%	%	67.2 ± 3.2	67.47 ± 8.76	68.8 ± 11.6	73.05 ± 6.7
LYMPH%	%	28.4 ± 5.2	24.03 ± 9.54	29.6 ± 11.6	27.38 ± 6.28

MONO%	%	3.42 ± 0.71	7.96 ± 1.79	3.57 ± 1.11	2.6 ± 0.68
EO%	%	1.2 ± 0.34	1 ± 0.41	1.5 ± 0.53	1.6 ± 0.87
BASO%	%	0.08 ± 0.071	0.26 ± 0.47	0.24 ± 0.34	0.22 ± 0.30
RET%	%	6.18 ± 042	6.51 ± 1.01	3.93 ± 0.76	4.24 ± 0.74
RET#	10^9/L	709.166 ± 54.0	705.4 ± 141.1	457.5 ± 87.4	446.1 ± 21.7
IRF	%	61.34 ± 1.52	56.2 ± 1.93	56.7 ± 3.64	60.07 ± 1.16
LFR	%	38.66 ± 1.52	43.76 ± 1.93	43.2 ± 4.96	39.93 ± 1.16
MFR	%	12.42 ± 1.01	12.68 ± 1.25	12.02 ± 0.45	11.67 ± 0.61
HFR	%	48.92 ± 1.21	43.06 ± 2.77	44.86 ± 5.03	47.27 ± 0.73
RET-He	pg	16.68 ± 0.64	16.64 ± 0.86	17.64 ± 0.88	17.72 ± 0.42
IPF	%	0.566 ± 0.12	0.42 ± 0.25	0.78 ± 0.38	0.58 ± 0.19