Supporting Information for:

Cisplatin Prodrug-Loaded Nanoparticles Based on Physalis Mottle Virus for Cancer Therapy

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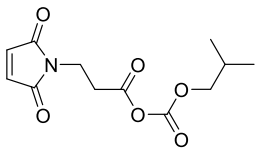
CisPt(IV) Maleimide synthesis and characterization:

(Isobutyl carbonic) 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic anhydride:



To a solution of maleimidopropanoic acid (215 mg, 0.80 mmol) in anh. THF (2.0 mL) was added 4-methylmorpholine (81 mg, 88 μL, 0.80 mmol). The reaction was cooled to 0 °C, and isobutylchloroformate (109 mg, 104 μL, 0.80 mmol) was added to this solution dropwise. The reaction was gradually warmed to room temperature and stirred for 30 min. Water (10 mL) was added to the reaction. The reaction mixture was extracted with EtOAc (20 mL × 3). The combined organic phase was dried over $MgSO_4$. The solvents were removed under vacuum. A slightly brown oil was obtained (482 mg, 89% yield). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (s, 2H), 4.04 (d, J = 6.7 Hz, 2H), 3.88 (t, J = 7.1 Hz, 2H), 2.83 (t, J = 7.1 Hz, 2H), 2.02 (nonet, J = 6.7 Hz, 1H), 0.96 (d, J = 6.8 Hz, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.25, 165.37, 148.73, 134.43, 75.90, 32.97, 32.83, 27.73, 18.88. EI-MS(+) m/z calcd for $[M+Na]^+$ 292.1, found 291.8.

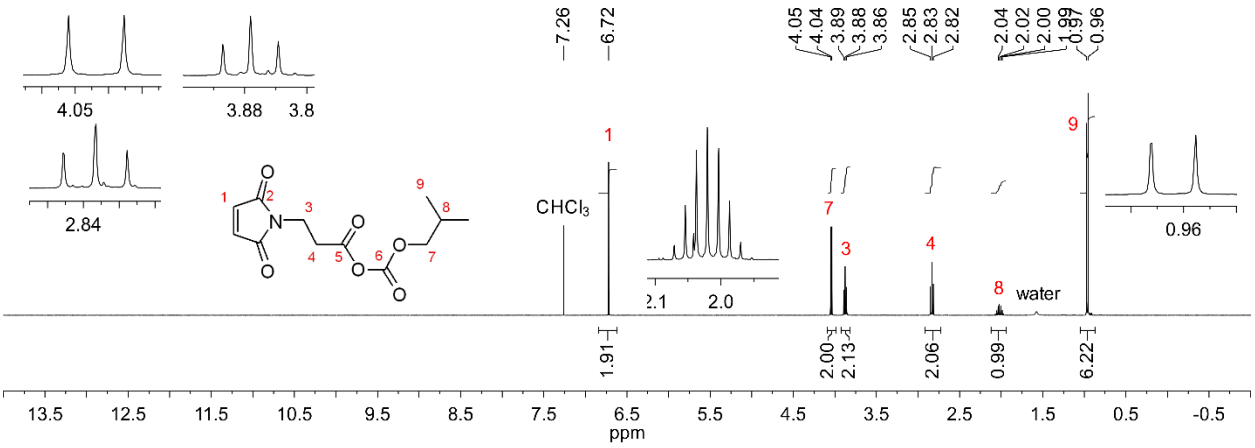


Figure S1. ^{1}H NMR spectrum of (isobutyl carbonic) 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic anhydride.

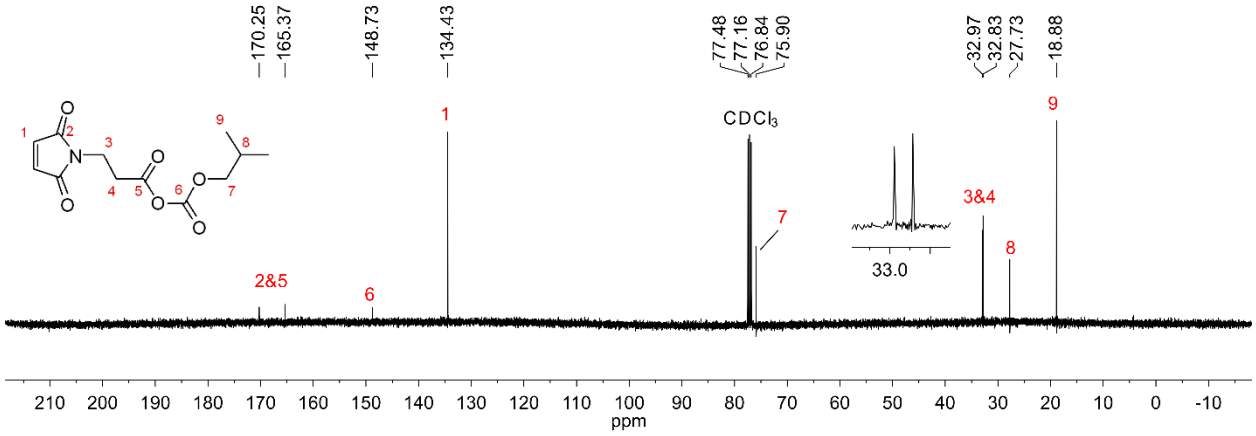
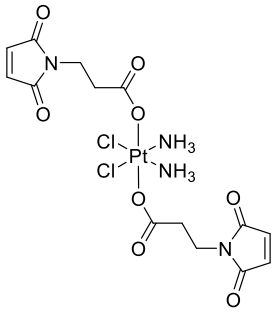


Figure S2. ^{13}C{^{1}H} NMR spectrum of (isobutyl carbonic) 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic anhydride.

***cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_2$CH$_2$CO-*N*-maleimide)$_2$]**:



To a solution of (isobutyl carbonic) 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic anhydride (269 mg, 1.0 mmol) in anh. DMF (5.0 mL) was added *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$] (84 mg, 0.25 mmol). The reaction was stirred at 55 °C for 48 h. The reaction mixture was filtered through a 0.20 μm PTFE syringe filter and diluted with water. The crude product was purified by HPLC using a C18 reverse stationary phase (Zorbax-SB C18 columns: 7 μm, 21.2 × 250 mm) and a mobile phase composed of two solvents (A: H_2O + 0.1% (v/v) CF_3CO_2H; B: CH_3CN + 0.1% (v/v) CF_3CO_2H). The following gradient was used: 0 – 3 min, solvent B = 10% – 22%; 3 – 9 min, solvent B = 22% – 28%; 9 – 12 min, solvent B = 28% – 100%; 12 – 15 min, solvent B = 100% – 10%; 15 – 16 min, solvent B = 10%. To remove the residual CF_3CO_2H, the combined product was lyophilized and passed through a Biotage C18 reverse phase column using a mobile phase composed of two solvents (A: H_2O + 0.1% (v/v) CH_3CO_2H; B: CH_3CN + 0.1% (v/v) CH_3CO_2H) using a linear gradient of solvent B from 0% to 40%. The purified product was lyophilized to give a slightly brown solid (41 mg, 26% yield). The purity of the product was assessed by analytical HPLC using a C18 reverse stationary phase (Zorbax-SB C18 columns: 5 μm, 4.6 × 250 mm) and a mobile phase composed of two solvents (A: H_2O + 0.1% (v/v) CF_3CO_2H; B: CH_3CN + 0.1% (v/v) CF_3CO_2H). The following gradient was used: 0 – 5 min, solvent B = 10%; 5 – 30 min, solvent B = 10% – 100%; 30 – 33 min, solvent B = 100%; 33 – 36 min, solvent B = 100% – 10%; 36 – 40 min, solvent B = 10%. T_R = 14.3 min. ^{1}H NMR (500 MHz, Acetone-d_6) δ 6.85 (s, 4H), 6.57 – 6.04 (m, 6H), 3.68 (t, J = 7.1 Hz, 4H), 2.50 (t, J = 7.1 Hz, 4H). ^{195}Pt NMR (108 MHz, Acetone-d_6) δ 1118. ^{13}C NMR (126 MHz, Acetone-d_6) δ 180.14 (m), 171.82, 135.25, 35.91, 35.29. MS (ESI+): m/z calcd for $[M+H]^+$ 636.0, found 636.8. MS (ESI−): m/z calcd for $[M-H]^-$ 634.0, found 634.6.

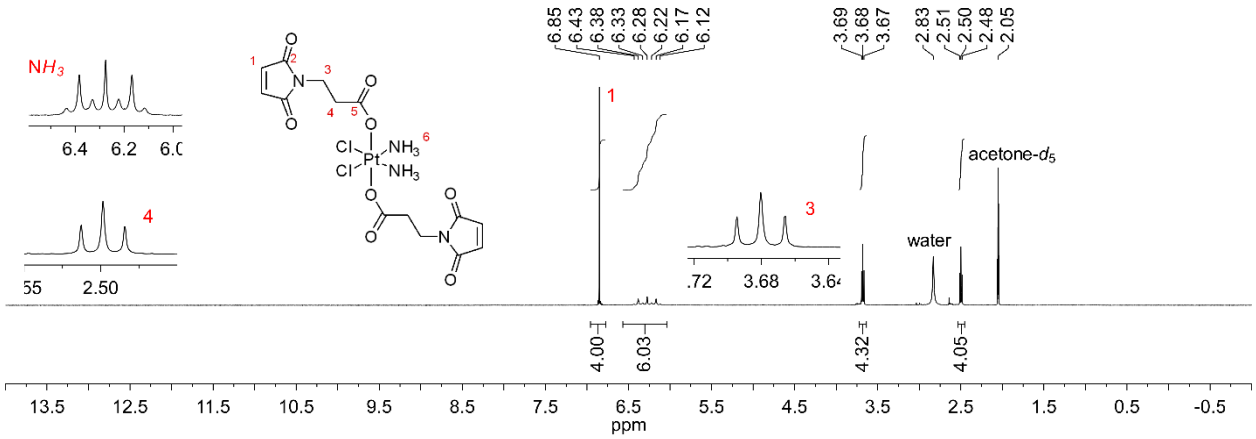


Figure S3. ^{1}H NMR spectrum of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_2$CH$_2$CO-*N*-maleimide)$_2$].

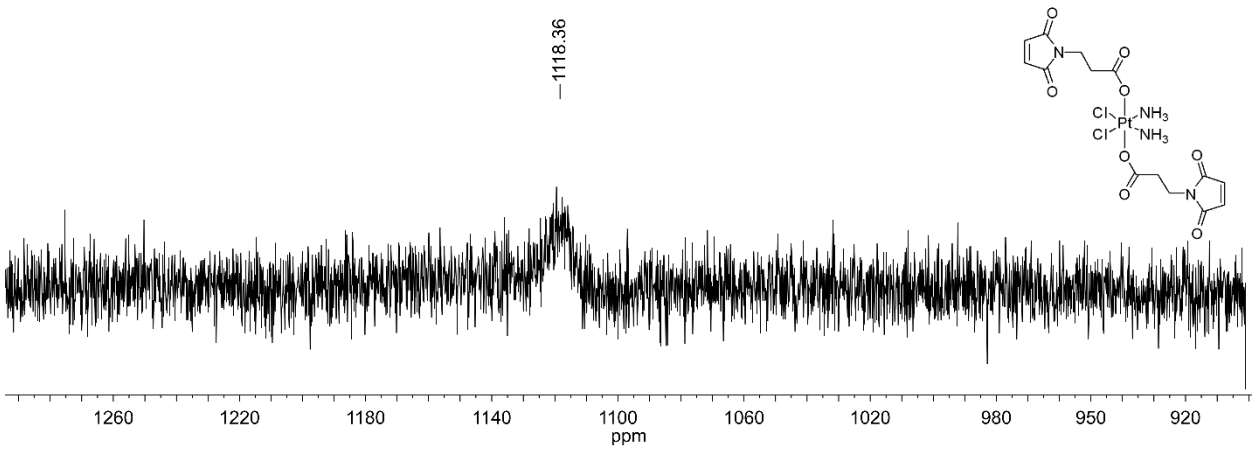


Figure S4. ^{195}Pt NMR spectrum of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_2$CH$_2$CO-*N*-maleimide)$_2$].

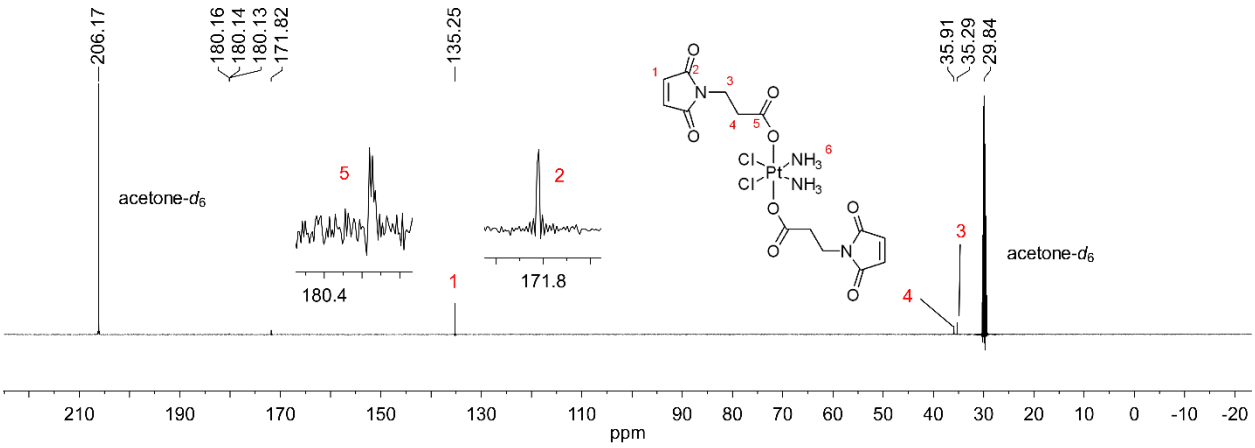


Figure S5. $^{13}C\{^1H\}$ NMR spectrum of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_2$CH$_2$CO-*N*-maleimide)$_2$].

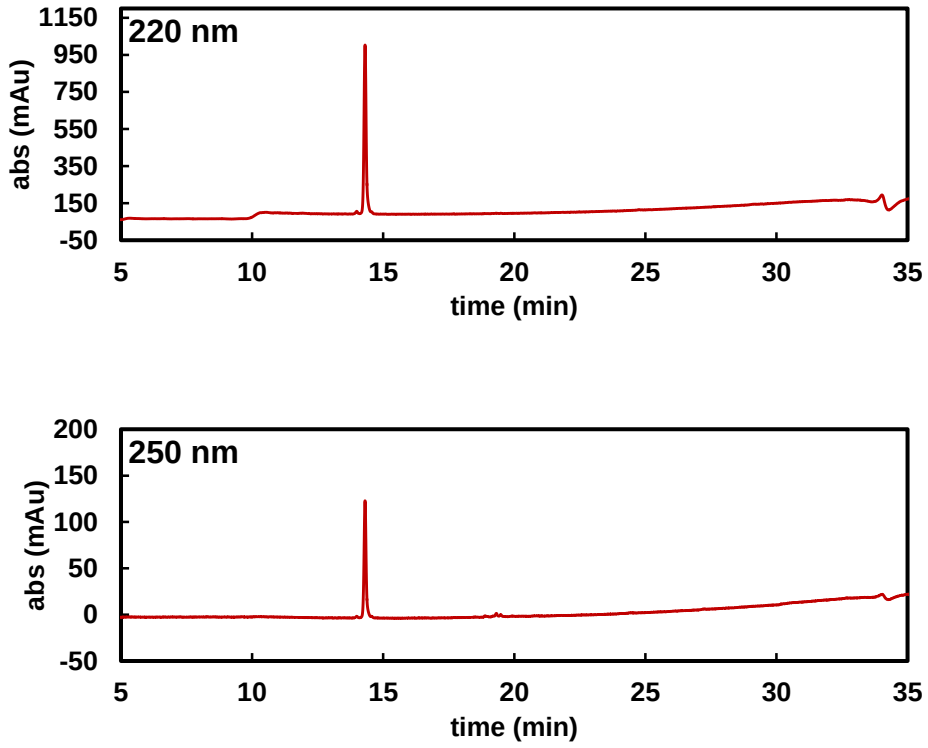


Figure S6. Analytical HPLC chromatogram of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(O$_2$CCH$_2$CH$_2$CO-*N*-maleimide)$_2$]. Absorbance monitored at 220 nm (top) and 250 nm (bottom).

Characterization methods:

UV/vis spectroscopy: The protein concentration in the nanoparticle formulations was determined using the Pierce BCA protein quantitation assay kit (Thermo Fisher Scientific). The dye load was determined by measuring UV/vis absorbance on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and using the protein concentration combined with the Beer–Lambert law and the dye-specific extinction coefficient for Cy5.5-maleimide of $\varepsilon(673\ \text{nm}) = 209{,}000\ M^{-1}\ cm^{-1}$.

Native and denaturing gel electrophoresis: Native and modified VLPs were analyzed by 1% (w/v) agarose native gel electrophoresis (10–20 μg per lane) in 0.1 M Tris-maleate running buffer (pH 6.5). Denatured protein subunits (10 μg per lane) were analyzed by SDS-PAGE using 4–12% NuPAGE gels in 1x MOPS buffer (Thermo Fisher Scientific). Samples were denatured by boiling in SDS loading dye for 10 min. Gels were photographed under UV or white light before staining with Coomassie Brilliant Blue, and under white light after staining, using an AlphaImager system (ProteinSimple, Santa Clara, CA, USA).

Size exclusion chromatography: Native and modified VLPs were analyzed by SEC using the AkTA Explorer system fitted with a Superose-6 column (GE Healthcare, Chicago, IL, USA). The column was loaded with 100-μL samples (1 mg/mL) at a flow rate of 0.5 mL/min in KP buffer. Absorbance was measured at 260 and 280 nm, and the Cy5.5 dye was monitored at 673 nm.

Transmission electron microscopy: Native and modified VLPs (1 mg/mL) were suspended in 20 μL KP buffer and deposited onto Formvar carbon-coated copper TEM grids (Electron Microscopy

Sciences, Hatfield, PA, USA) for 2 min at room temperature. The grids were then washed twice with deionized water for 45 s and stained twice with 2% (w/v) uranyl acetate in deionized water for another 30 s. A Tecnai F30 transmission electron microscope was used to analyze the samples at 300 kV.

Laser scanning confocal microscopy: Cell lines were grown for 24 h on glass coverslips (25,000 cells per well) in an untreated 24-well plate in 200 µL of the appropriate medium. The cells were washed twice with Dulbecco's PBS (DPBS) before adding the VLPs at a final concentration of ~2 µg/mL normalized to native PhMV particles (equivalent to 2×10^6 PhMV particles per cell) and incubating for 3 h. The cells were washed twice in DPBS to remove unbound particles and fixed for 5 min at room temperature in DPBS containing 4% (v/v) paraformaldehyde and 0.3% (v/v) glutaraldehyde. Cell membranes were stained with 1 µg/mL wheat germ agglutinin conjugated to AlexaFluor-555 (Invitrogen. Thermo Fisher Scientific) diluted 1:1000 in DPBS containing 5% (v/v) goat serum, and the cells were then incubated for 45 min at room temperature in the dark. Finally, the cells were washed three times with DPBS, and the coverslips were mounted on glass slides using Fluroshield with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St Louis, MO, USA) and sealed using nail polish. Confocal images were captured on a Nikon A1R confocal microscope and the images were processed using Nikon A1R confocal microscope workstation (Nikon, Tokyo, Japan).

Zeta potential analysis: The zeta potential (ζ) of the VLPs was determined by placing 0.25 mg/mL solutions of each VLP in a 90Plus Zeta potential analyzer (Brookhaven Instruments) and conducting five measurements, each comprising six runs.

Supporting Data:

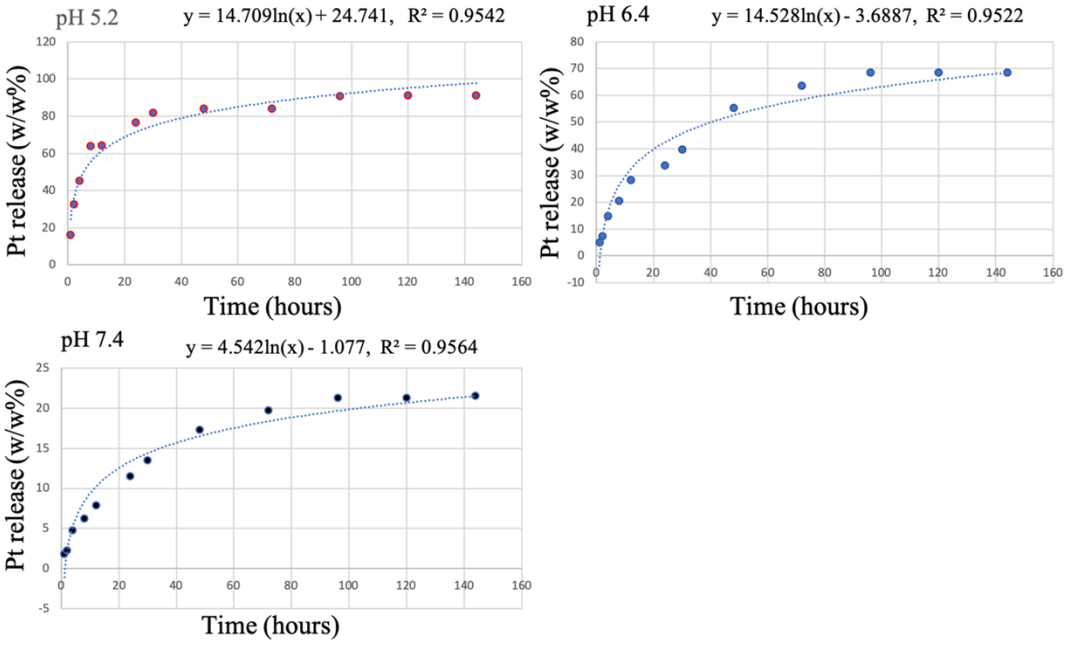


Figure S7. The logarithmic increment fitting of the kinetics of Pt(IV) reduced to Pt(II) at different pH.

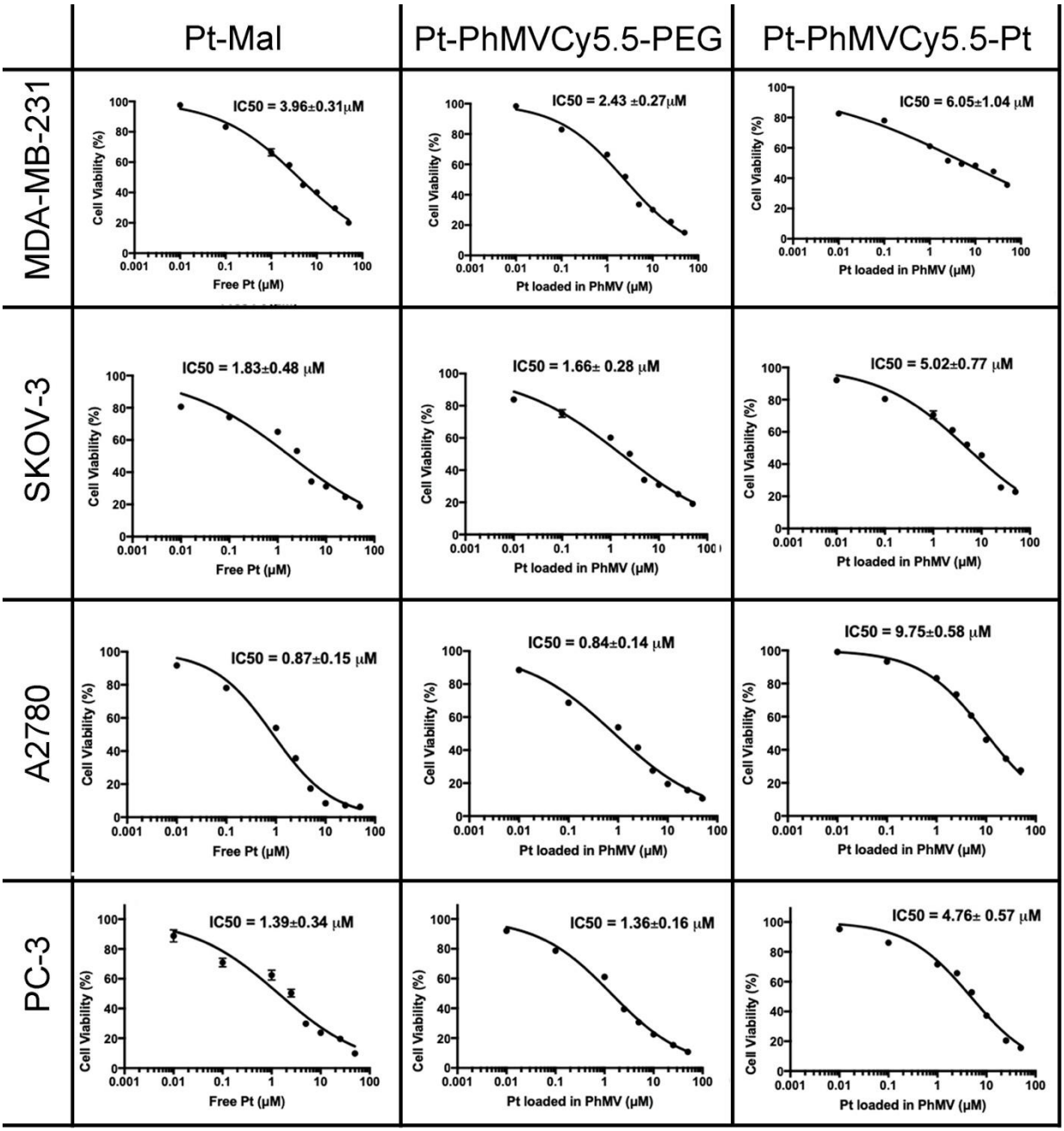


Figure S8. The IC_{50} profiles of free cisplatin, Pt-Mal, and Pt-loaded VLPs against a panel of cancer cells. Data from triplet experiments and standard deviations are shown.

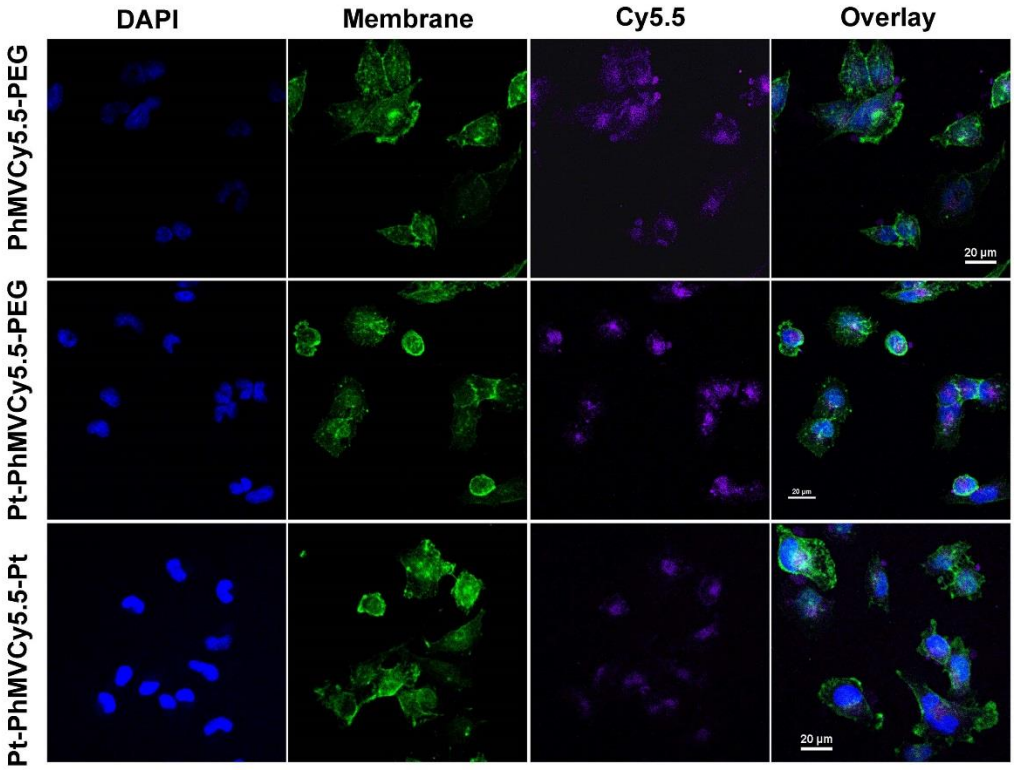


Figure S9. Cell trafficking of PhMV within MDA-MB-231 cells after 3 h incubation at the particle concentration of 10 µg/mL by confocal laser scanning microscopy (CLSM) (nuclei stained with DAPI in blue, membrane stained with A555-labeled wheat germ agglutinin in green, PhMV conjugated with Cy5.5 in purple, with scale bar = 20 µm).

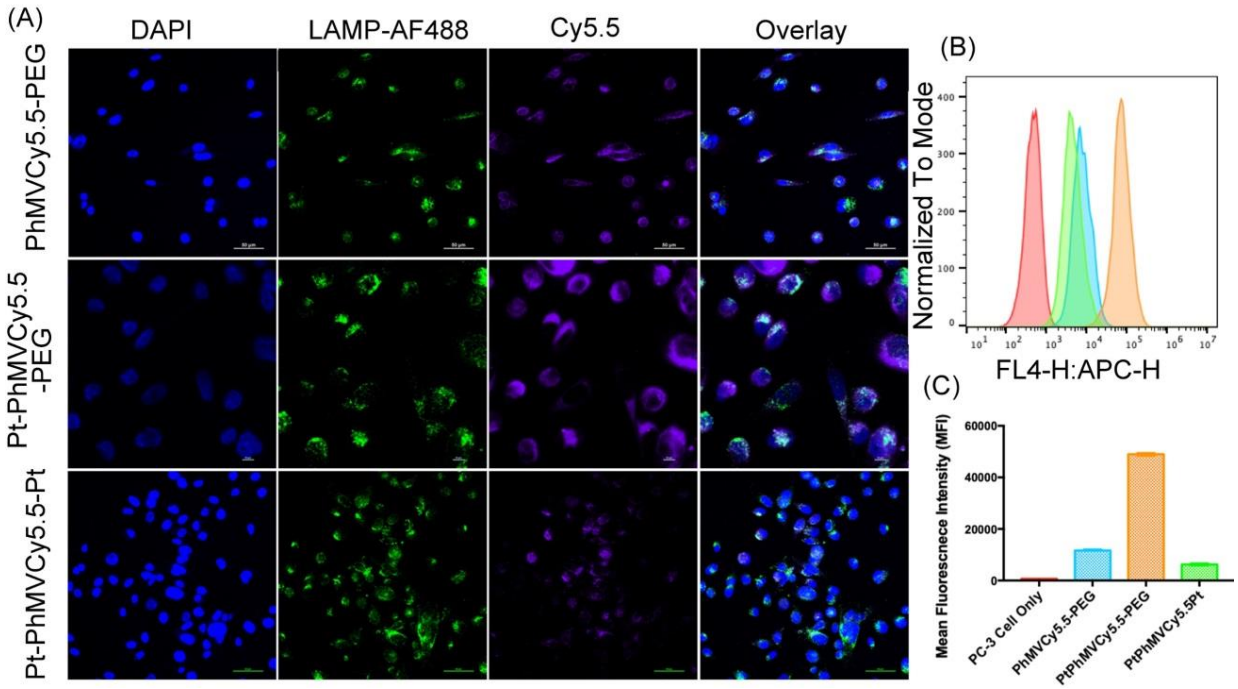


Figure S10. Analysis of VLP uptake and intracellular distribution by PC-3 cells. (A) Trafficking of PhMV-derived VLPs in PC-3 cells after incubation with 10 µg/mL VLPs for 3 h, observed by confocal laser scanning microscopy. Nuclei are stained with DAPI (blue), membranes are stained with A555-labeled wheat germ agglutinin (green) and Cy5.5 conjugated to the VLPs is shown in purple. Scale bar = 20 µm. (B) Quantitative analysis by flow cytometry and (C) mean fluorescence intensities (MFIs) of cells in each sample (n = 3 ± standard deviations, $p < 0.001$ using Student's T-test). Data were analyzed using FlowJo v10.

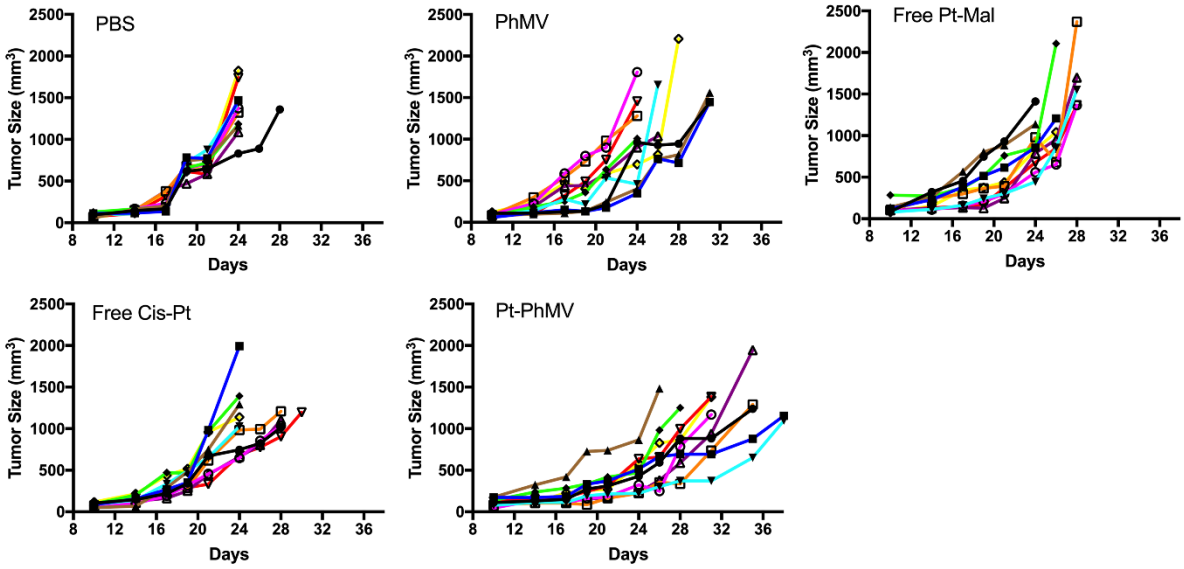


Figure S11. Inhibition of tumor growth in an athymic mouse model (n = 10) with MDA-MB-231 xenografts after treatment with different formulations. The treatment began when tumors reached a volume of ~150 mm^3 and involved a twice-weekly intravenous bolus of 1.0 mg Pt/kg in the form of cisplatin (cis-Pt), Pt-Mal or Pt-PhMVCy5.5-PEG (Pt-PhMV). PBS or PhMVCy5.5-PEG (PhMV) particles were administered as controls, with PhMVCy5.5-PEG particle dosage normalized to Pt-PhMVCy5.5-PEG. Tumor volumes and body weight were measured before each injection. Tumor growth curves of individual mice in each group.