SI Appendix

Detouring of Cisplatin to Access Mitochondrial Genome for Overcoming Resistance

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Materials and Instrumentations: All chemicals were received and used without further purification unless otherwise noted. Cisplatin was purchased from Strem Chemicals, Inc. Dimethylaminopyridine (DMAP), K_2PtCl_4, KCl, N-hydroxysuccinimide (NHS), triethylamine, 5-bromopentanoic acid, 6-bromohexanoic acid, sodium azide, N,N'-dicyclohexylcarbodiimide (DCC), hydrogen peroxide solution (30 wt.% in H_2O), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Dibenzocyclooctynes (DBCO)-amine (Product No. A103) was procured from Click chemistry Tools Bioconjugate Technology Company. Carboxy terminated poly(lactic-*co*-glycolic acid) (PLGA) [dL/g, 0.15 to 0.25 (low molecular weight, LMW) and 0.55 to 0.75 (high molecular weight, HMW)] was procured from Lactel and OH-PEG-OH of molecular weight 3350 was purchased from Sigma Aldrich. Triphenyphosphine (TPP) was purchased from Sigma Aldrich. Bicinchoninic acid (BCA) protein assay kit (Pierce 23227) was purchased from Thermo Scientific. The mitochondrial isolation kit (catalog number PI-89874) for mammalian cells was purchased from Thermo Scientific. Tris(hydroxymethyl)aminomethane was purchased from Fischer Scientific. Sodium chloride, magnesium chloride, sucrose, potassium chloride, and ethylyenediaminetetraacetic acid (EDTA) were purchased from J.T. Baker. Oligomycin, rotenone, antimycin-A, and trifluorocarbonylcyanide phenylhydrazone (FCCP) were purchased from Sigma Aldrich. The protease inhibitor cocktail was purchased from Sigma Aldrich. Slide-A-Lyzer mini Dialysis Units (catalog number 69572) were purchased from Thermo Scientific. The mitochondrial DNA isolation Kit (ab65321) and nuclear DNA isolation kit (ab65358) were purchased from Abcam. 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide or JC-1 dye and the Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit were purchased from Invitrogen. The citrate synthase assay kit (ab119692) was purchased from Abcam.

Distilled water was purified by passage through a Millipore Milli-Q Biocel water purification system (18.2 MΩ) containing a 0.22 μm filter. ^{1}H, ^{13}C spectra were recorded on a 400 MHz; ^{31}P NMR and ^{195}Pt NMR spectra recorded on a 500 MHz Varian NMR spectrometer, respectively. Electrospray ionization mass spectrometry (ESI-MS) and high-resolution mass spectrometry (HRMS)-ESI were recorded on Perkin Elmer SCIEX API 1 plus and Thermo scientific ORBITRAP ELITE instruments, respectively.

Electrochemical measurements were made at 25 °C on an analytical system model CHI 920c potentiostat from CH Instruments, Inc. (Austin, TX). Cells were counted using Countess® Automated cell counter procured from Invitrogen life technology. Dynamic light scattering (DLS) measurements were carried out using a Malvern Zetasizer Nano ZS system. Optical measurements were carried out on a NanoDrop 2000 spectrophotometer. Transmission electron microscopy (TEM) images were acquired using a Philips/FEI Technai 20 microscope. Inductively coupled plasma mass spectrometry (ICP-MS) studies were performed on a VG PlasmaQuad 3 ICP mass spectrometer. Plate reader analyses were performed on a Bio-Tek Synergy HT microplate reader. Gel permeation chromatographic (GPC) analyses were performed on Shimadzu LC20-AD prominence liquid chromatographer equipped with a refractive index detector and Waters columns; molecular weights were calculated using a conventional calibration curve constructed from narrow polystyrene standards using tetrahydrofuran (THF) as an eluent at a temperature of 40 °C. Bioenergetic assays were carried out using a Seahorse XF24 analyzer (Seahorse Biosciences, North Billerica, MA, USA). Fluorescence imaging of cellular components was carried out on a Xenogen IVIS® Lumina system. Flow cytometry measurements were performed on a BD LSR II flow cytometer. Clinical chemistry analysis of the plasma samples was conducted at the University of Georgia Veterinary Teaching Hospital using a Hitachi P-Modular system.

Cell Lines and Cell Culture*:* Human prostate cancer cell line PC3 and neuroblastoma SH-SY5Y cells were procured from the American type culture collection (ATCC). Cisplatin resistant human ovarian carcinoma cell line A2780/CP70 was kindly provided by Prof. Thomas Hamilton (Fox Chase Cancer Center, Jenkintown, PA). Human bone marrow derived MSCs were purchased from Lonza. H9C2 cardiomyocytes was given as a generous gift from Prof. Mark Anderson, University of Iowa. The cardiomyocytes were grown in 90% Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, 4.5 g/L glucose, 1% penicillin/streptomycin, and 10% fetal bovine serum. PC3 and A2780/CP70 cells were grown at 37 °C in 5% CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, sodium pyruvate (100 mM),

HEPES buffer solution (1 M), and L-glutamine (200 mM). SH-SY5Y cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Human MSCs were grown in mesenchymal stem cell basal medium supplemented with 2% FBS, 1% penicillin/streptomycin, recombinant human fibroblast growth factor-basic (5 ng/mL), recombinant human fibroblast growth factor-acidic (5 ng/mL), and recombinant human epithelial growth factor (5 ng/mL). Cells were passed every 3 to 4 days and restarted from frozen stocks upon reaching pass number 20 for PC3, SH-SY5Y, A2780/CP70, H9C2 cells and 10 for MSC.

Synthesis of DBCO-TPP*:* A mixture of TPP-$(CH_2)_5$-COOH (1) (250 mg, 0.55 mmol) and NHS (75.40 mg, 0.66 mmol) in dry CH_2Cl_2 was stirred for 30 min at 0 °C. A solution of DCC (124 mg, 0.6 mmol) in CH_2Cl_2 was added drop wise to the reaction mixture. The reaction mixture was stirred from 0 °C to room temperature for 12 h. The precipitated N,N′-dicyclohexylurea (DCU) by-product was filtered off and the solution was evaporated using rotavap. This residue was dissolved in dry CH_2Cl_2. A solution of triethylamine (66.27 mg, 0.66 mmol) and DBCO-NH_2 (181 mg, 0.66 mmol) in CH_2Cl_2 was added slowly to the above reaction mixture. This reaction mixture was kept at room temperature for 24 h with vigorous stirring. The solvent was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and precipitated with diethyl ether (CH_2Cl_2:diethyl ether: 1:9). This process was repeated 5 times. Finally the product was purified by precipitation using a mixture of CH_2Cl_2:ethanol:diethyl ether (1:1:8). Yield, 266 mg, 68%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67-7.75 (m, 15H), 7.23-7.33 (m, 8H), 6.83 (t, 1H), 5.12 (d, 1H), 3.64 (m, 3H), 3.20 (t, 2H), 2.51 (m, 1H), 2.03 (t, 2H), 1.95 (m, 1H), 1.58 (m, 6H) (Fig. S1) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.94, 171.86, 151.17, 148.02, 134.98, 135.01, 133.67, 133.57, 133.53, 132.13, 130.60, 130.53, 130.40, 129.28, 128.69, 128.18, 128.13, 127.79, 127.01, 125.51, 123.00, 122.31, 118.69, 117.84, 114.74, 107.88, 65.82, 55.39, 35.85, 35.29, 34.65, 29.73, 29.56, 25.48, 24.62, 22.75, 22.25, 22.05, 22.01, 15.24 ppm (Fig. S2). ^{31}P NMR ($CDCl_3$) δ 24.65 ppm (Fig. S3). HRMS-ESI (m/z): $[M\text{-}Br]^+$ calcd. for $C_{42}H_{40}N_2O_2P^+$, 635.2822; found, 635.2823 (Fig. S4).

Synthesis of Platin-M*:* A solution of Platin-Az (2) (60 mg, 0.098 mmol) and DBCO-TPP (140 mg, 0.196 mmol) in 10 mL of dry dimethylformamide (DMF) was stirred at room temperature for 12 h. This reaction mixture was concentrated and the product was precipitated using diethyl ether. The crude product was suspended in CH_2Cl_2 and CH_3CN and precipitated with diethyl ether. Finally the product was isolated through precipitation with CH_2Cl_2:CH_3CN:diethyl ether (1:1:8) to get a light yellow solid. Yield, 140 mg, 71%. ^{1}H NMR (DMSO-d_6, 400 MHz): *δ* 7.78-7.90 (m, 30H), 7.27-7.66 (m, 18H), 6.57 (broad, 6H), 5.86-5.97 (m, 2H), 4.43-4.47 (m, 2H), 4.20-4.38 (m, 4H), 3.30-3.56 (m, 4H), 2.87-2.99 (m, 4H), 2.14-2.21 (m, 4H), 1.92 (m, 4H), 1.83 (m, 2H), 1.39-1.50 (m, 24H), 1.01-1.10 (m, 2H) ppm (Fig. S5), gCOSY (Fig. S6). ^{13}C NMR ($CDCl_3$, 100 MHz): δ δ 181.16, 181.13, 172.25, 172.08, 170.14, 169.79, 144.18, 142.64, 141.34, 140.46, 135.78, 135.33, 135.31, 134.27, 134.21, 134.08, 133.98, 132.36, 132.03, 131.72, 131.11, 130.74, 130.72, 130.62, 130.29, 129.98, 129.59, 129.11, 128.72, 127.94, 127.28, 124.70, 119.42, 119.40, 118.57, 118.55, 55.39, 52.26, 51.02, 48.89, 48.19, 40.60, 40.39, 40.18, 39.98, 39.77, 39.56, 39.35, 35.96, 35.70, 35.24, 35.18, 35.07, 33.91, 30.01, 29.84, 29.54, 28.48, 26.27, 26.16, 25.54, 25.40, 25.36, 25.19, 24.83, 24.72, 22.10, 20.86, 20.36 ppm (Fig. S7). ^{31}P NMR ($CDCl_3$) δ 24.10 ppm (Fig. S8). ^{195}Pt (DMSO-d_6, 107.6 MHz) *δ* 1108.94 ppm (Fig. S9). HRMS m/z Calcd. for $C_{96}H_{106}Cl_2N_{12}O_8P_2Pt^{2+}$: $(M)^{2+}$ 941.3390. Found 941.3378 (Fig. S10). Elemental analysis calcd (%) for $C_{96}H_{106}Br_2Cl_2N_{12}O_8P_2Pt.CH_3CN.CH_2Cl_2.H_2O$: C 54.35, H 5.21, N 8.32; found: C 54.10, H 5.44, N 8.55.

Electrochemistry of Platin-M: Electrochemical measurements were made at 25 °C on an analytical system model CHI 920c potentiostat from CH Instruments, Inc. (Austin, TX). A conventional three-electrode set-up comprising a glassy carbon working electrode, platinum wire auxiliary electrode, and Ag/AgCl (3M KCl) reference electrode was used for electrochemical measurements. The electrochemical data were uncorrected for junction potentials. KCl was used as a supporting electrolyte. Platin-M (1 mM) solutions were prepared in 20% DMF-phosphate buffered saline (PBS) of pH 6.4 and 7.4 with 0.1 M KCl and voltammograms were recorded at different scan rates

(Fig. S11). Redox potentials of Platin-M at pH 7.4 was found to be -0.376 V vs. Ag/AgCl; -0.275 vs. NHE and at pH 6.4 was found to be -0.369 V vs. Ag/AgCl; -0.269 vs. NHE.

Platin-M Ability to Form Micelles: Platin-M was dissolved in DMF to a final concentration of 1.5 mg/mL. This solution was added drop wise slowly into vigorously stirring nanopure water (10 mL) and stirred at room temperature for 3 h. This solution was then filtered and washed 3 times *via* centrifugation using Amicon centrifugal filters with a MW cutoff of 100 kDa in order to ensure removal of organic solvent. Finally, the micelles were resuspended in nanopure water (1 mL) and filtered through a 0.2 *μ*m filter. The resulting suspension was characterized by DLS.

Synthesis of $PLGA_{LMW}$-b-PEG-OH and $PLGA_{LMW}$-b-PEG-TPP: These two polymers were synthesized following methods previously reported by us (3). Spectral Data for $PLGA_{LMW}$-*b*-PEG-OH (Fig. S12): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.22 [m, 37 H (O*CH*CH_3C(O)], 4.81 [m, 74 H (O*CH*$_2$C(O)], 3.63 [s, 102 H (O*CH*$_2$)], 1.57 [m, 115 H (*CH*$_3$CH)] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.40, 166.33, 70.54, 69.08, 60.79, 16.66 ppm. Spectral Data for $PLGA_{LMW}$-*b*-PEG-TPP (Fig. S13): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.8-7.3 [m 15 H (PPh_3), 5.20 [m, 37 H (O*CH*CH_3C(O)], 4.81 [m, 73 H (O*CH*$_2$C(O)], 3.63 [s, 102 H (O*CH*$_2$)], 1.57 [m, 112 H (*CH*$_3$CH)] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 169.27, 166.32, 134.90, 133.84, 130.33, 118.79, 117.93, 70.54, 69.00, 60.80, 16.67 ppm. ^{31}P NMR ($CDCl_3$, 100 MHz): *δ* 24.62 ppm. GPC: M_n=14,420 g/mol, M_w=17,030 g/mol, M_z=20,320 g/mol, PDI = 1.18 (Fig. S16).

Synthesis of $PLGA_{HMW}$-b-PEG-OH: OH-PEG-OH (3.75 g, 1.1 mmol), PLGA-COOH (inherent viscosity of 0.55-0.75, 5.0 g, 0.38 mmol), and DMAP (0.045 g, 0.38 mmol) were dissolved in dry CH_2Cl_2 (50 mL). The reaction mixture was cooled to 0 °C while stirring. DCC (0.2 g, 1.1 mmol) was dissolved in CH_2Cl_2 (3 mL) and added drop wise to the polymer solution. The mixture was then warmed to room temperature and stirred overnight. Afterwards, DCU was filtered out and the resulting mixture was precipitated in a 50:50 mixture of cold diethyl ether:methanol (200 mL), repeatedly. The resulting solid was centrifuged at 5000 rpm for 10 min. The resulting solid was lyophilized to produce

the polymer with a 41% yield. Final product was analyzed by NMR and GPC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.21 [m, 574 H (O*CH*CH_3C(O)], 4.82 [m, 1132 H (O*CH*$_2$C(O)], 3.64 [s, 187 H (O*CH*$_2$)], 1.58 [m, 1868 H (*CH*$_3$CH)] ppm (Fig. S14). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.30, 166.33, 70.55, 68.96, 68.93, 60.80, 16.69 ppm (Fig. S14). GPC: M_n=47,280 g/mol, M_w=68,210 g/mol, M_z=92,900 g/mol, PDI = 1.36 (Fig. S16).

Synthesis of PLGA$_{HMW}$-b-PEG-TPP: PLGA$_{HMW}$-*b*-PEG-OH (1 g, 0.02 mmol), TPP-$(CH_2)_4$-COOH (0.045 g, 0.12 mmol), and DMAP (0.010 g, 0.08 mmol) were dissolved in CH_2Cl_2 for 30 min at 0 °C. A solution of DCC (12 mg, 0.06 mmol) was added drop wise. The solution was slowly returned to room temperature and stirred for 12 h. The resulting DCU was removed *via* gravity filtration. CH_2Cl_2 was removed *in vacuo* and the resulting polymer was dissolved in a 50:50 mixture of CH_2Cl_2/CH_3OH and precipitated with cold diethyl ether. The resulting solid was isolated by centrifugation (5000 rpm, 10 min, 4 °C) This process was repeated 4 times in order to remove the residual TPP-$(CH_2)_4$-COOH. Finally, the resulting polymer was lyophilized to produce the targeted polymer with a 59% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.6-7.7 [m, 15 H, (PPh_3)], 5.21 [m, 615 H (O*CH*CH_3C(O)], 4.82 [m, 1203 H (O*CH*$_2$C(O)], 3.64 [s, 187 H (O*CH*$_2$)], 1.57 [m, 2002 H (*CH*$_3$CH)] ppm (Fig. S15). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.30, 166.34, 135.17, 134.93, 130.52, 117.85, 116.59, 70.54, 69.05, 60.80, 16.69 ppm (Fig. S15). ^{31}P NMR ($CDCl_3$, 100 MHz): *δ* 24.37 ppm (Fig. S15). GPC: M_n=54,510 g/mol, M_w= 73,510 g/mol, M_z=94,380 g/mol, PDI = 1.35 (Fig. S16).

Preparation of Platin-M Encapsulated PLGA-b-PEG Polymeric Nanoparticles (NPs): Platin-M encapsulated targeted NPs (T-Platin-M-NP) and non-targeted (NT-Platin-M-NP) NPs were prepared by a nanoprecipitation method. Briefly, PLGA-*b*-PEG-TPP (3) or PLGA-PEG-OH were dissolved in DMF (50 mg/mL). Varying amounts of Platin-M (10 mg/mL in DMF) were added to the PLGA-*b*-PEG-TPP or PLGA-*b*-PEG-OH solution to a final polymer solution of 5 mg/mL. This was added drop-wise slowly into vigorously stirring nanopure water (10 mL) and stirred at room temperature for 2 h. This solution was then filtered and washed through centrifugal filters with a MW cutoff of 100 kDa 3 times at 3000 rpm and 4 °C in order to ensure the removal of all organic solvent.

Finally, the NPs were resuspended in nanopure water (1 mL) and filtered through a 0.2 *μ*m filter. The NPs were characterized by DLS (Fig. S19, Tables S2 and S3) for size and zeta potential and Platin-M content was analyzed by ICP-MS (Tables S2 and S3).

Preparation of PLGA-PEG-QD Encapsulated PLGA-b-PEG-TPP-NPs: $PLGA_{LMW}$-*b*-PEG-TPP-NPs and $PLGA_{HMW}$-*b*-PEG-TPP-NPs containing PLGA-PEG-QD (3) were synthesized using nanoprecipitation method. A mixture of $PLGA_{LMW}$-*b*-PEG-TPP or $PLGA_{HMW}$-*b*-PEG-TPP (5 mg/mL in DMF) and PLGA-PEG-QD (10 *μ*L, 8 *μ*M in DMF) was added drop wise to vigorously stirring water. The resultant T-QD-NPs were stirred for 2 h. Organic solvent was removed by 3 washes using centrifugal filters with a 100 kDa cut-off at 3000 rpm and 4 °C. NPs were resuspended in nanopure water.

Release Kinetics of Platin-M from NPs: In order to understand the rate at which Platin-M was released from the NP core, release kinetics of T-Platin-M-NPs and NT-Platin-M-NPs were studied. The NPs (T or NT) were synthesized according to the method above. The resulting NPs were subjected to dialysis using Slide-A-Lyzer mini dialysis devices with a 100 kDa MW cutoff of in 1X PBS at physiological conditions (pH 7.4 and 6.0, 37 °C). PBS was changed every 12 h. At different time points, the dialysis bag was removed and the amount of Platin-M remaining in the polymeric core was analyzed by ICP-MS.

Cellular Fractionation of Cells Treated with Platin-M-NPs, Platin-M, and Cisplatin: PC3 cells were plated at a concentration of 1.0×10^6 in 15 mL of media and allowed to grow overnight. Platin-M, T-Platin-M-NPs, NT-Platin-M-NPs, and cisplatin (1 *μ*M with respect to Pt) were added to cells and incubated for 12 h. After internalization, the mitochondria and the cytosol were isolated using a mitochondria isolation kit for mammalian cells. Cells were isolated by trypsinization and washed 3x with 1X PBS. Reagent A supplemented with protease inhibitors (10 mg/mL) was added followed by incubation on ice for 2 min. Reagent B was added and incubated on ice for 5 min with gentle vortexing every min. Following this, reagent C was added and the cells were centrifuged (700x*g* at 4 °C for 10 min). The resulting pellet yielded the nuclei and

cellular debris. The supernatant, containing the cytosolic and mitochondrial fractions, was removed and further centrifuged (12,000x*g* at 4 °C for 15 min). The resulting supernatant contained the cytosolic fraction and the pellet contained the impure mitochondrial fraction. This was further purified by washing with reagent C and centrifuging at 12,000 x*g* at 4 °C for 5 min. The isolated nucleus and cellular debris were further fractionated in order to obtain a pure nuclear fraction. The pellet was resuspended in 600 *μ*L of a modified Tris-HCl buffer (10 mM Tris-HCl, pH 7.0, 10 mM NaCl, 3 mM $MgCl_2$, 30 mM sucrose). This was incubated on ice for 10 min and centrifuged (3000 rpm, 4 °C). The resulting pellet was resuspended in 1 mL of pre-chilled $CaCl_2$ buffer (10 mM Tris-HCl of pH 7.0, 10 mM NaCl, 3 mM $MgCl_2$, 30 mM sucrose, 10 mM $CaCl_2$). This was repeatedly centrifuged and washed with the $CaCl_2$ buffer and the supernatant was discarded each time. The pellet was further purified by resuspending in a buffer containing 20 mM Tris-HCl of pH 7.9, 20% glycerol, 0.1 M KCl, and 0.2 mM EDTA and centrifuging at 14,000 rpm for 30 min at 4 °C. The resulting pellet yielded the purified nuclear fraction and was resuspended in H_2O. The amount of protein in each fraction was analyzed by a BCA assay and the Pt content of each fraction was quantified by ICP-MS.

Mitochondrial Sub-fractionation: PC3 cells were plated at a concentration of $1.0x10^6$ in 30 mL media and allowed to grow overnight. QD blended $PLGA_{HMW}$-*b*-PEG-TPP or $PLGA_{LMW}$-*b*-PEG-TPP NPs (0.5 mg/mL with respect to NP) were then internalized in PC3 cells for 6 h. After internalization, the mitochondria and the cytosol were isolated using a mitochondria isolation kit for mammalian cells. These fractions were further sub-fractionated. The freshly isolated PC3 mitochondria in PBS (1x) were incubated with protease inhibitor (0.125 mg/mL) and 0.6 % digitonin for 10 min on ice. Immediately after incubation, the mitochondria were centrifuged at 10,000xg for 10 min at 4 °C. The supernatant (SN-I) contained the outer mitochondrial membrane (OMM) fraction and the intermembrane space (IMS). The pellet was resuspended in 150 mmol/L KCl, protease inhibitor (0.125 mg/mL) and incubated on ice for 10 min. This was centrifuged at 10,000xg for 10 min at 4 °C. The supernatant, which contained the mitochondrial matrix, was collected. To this, 50 *μ*L of 1x cell lysis buffer (30 mM Tris-HCl, 0.1 mM EDTA, 20

% w/v sucrose) was added. This was subsequently sonicated and centrifuged at 10,000xg for 15 min at 4 °C. The supernatant (SN-II) containing the purified inner mitochondrial membrane (IMM) fraction and matrix was collected. SN-I and SN-II were centrifuged at 105,000xg for 60 min. The pellet from SN-I contained the OMM fraction and the supernatant contained the IMS. The pellet from SN-II was resuspended in PBS containing Lubrol WX (0.5 mg/mL), 37 % sucrose and incubated for 15 min on ice. This was once again centrifuged at 105,000 g for 60 min at 4 °C. The pellet containing the IMM fraction and the supernatant containing the matrix were collected. The collected fractions were analyzed for Cd concentration by ICP-MS. A BCA assay was performed on all the fractions in order to calculate the Cd (ng)/protein (pg). The collected fractions were imaged on a Xenogen IVIS® Lumina system with 570 excitation wavelength and a Cy5.5 emission channel with an exposure time of 0.5 s.

Cell MitoStress Analysis: Different parameters of respiration: basal respiration, coupling efficiency, and spare respiratory capacity were investigated by using Seahorse XF-24 cell MitoStress Test Kit. Prior to the assay, XF sensor cartridges were hydrated. To each well of an XF utility plate, 1 mL of Seahorse Bioscience calibrant was added and the XF sensor cartridges were placed on top of the utility plate, and kept at 37 °C incubator without CO_2 for a minimum of 12 h. PC3, A2780-CP70, SH-SY5Y, and H9C2 cells were cultured in XF24-well cell culture microplates (Seahorse Bioscience) at a density of 2.5×10^4 cells/well (except for H9C2, cell density of this cell line: 5×10^4 cells/well) (0.32 cm^2) in 200 μL growth medium and then incubated for 24 h at 37 °C in 5% CO_2 atmosphere. The cells were treated with Platin-M (10 μM), cisplatin (10 μM), DBCO-TPP (10 μM), empty-T-NPs, empty-NT-NPs, T-Platin-M-NPs, NT-Platin-M-NPs (10 μM with respect to Pt; ~0.5 mg/mL for empty NPs) for 12 h at 37 °C in 5% CO_2 atmosphere. After 12 h, all but 50 μL of the culture medium was removed from each well and the cells were rinsed two times with 500 μL of XF stress test glycolysis optimization medium pre-warmed to 37 °C and finally 450 μL of glucose depleted optimization medium was added to each well and the plate was placed at 37 °C without CO_2 for 1 h prior to assay. Different parameters of respiration were calculated by subtracting the average respiration rates before and after the addition of the electron

transport inhibitors oligomycin (1.0 μM), trifluorocarbonylcyanide phenylhydrazone or FCCP (1.0 μM), an ionophore that is a mobile ion carrier, and a mixture of antimycin-A (1.0 μM) which is a complex III inhibitor and rotenone (1.0 μM), a mitochondrial inhibitor that prevents the transfer of electrons from the Fe-S center in Complex I to ubiquinone. The parameters calculated included: basal respiration (baseline respiration minus antimycin-A post injection respiration), ATP turnover (baseline respiration minus oligomycin post injection respiration), maximal respiratory capacity (FCCP stimulated respiration minus antimycin-A post injection respiration) and reserve respiratory capacity (FCCP stimulated respiration minus baseline respiration). Test articles on each well had four replicates.

In Vivo Biodistribution (bioD) and Pharmacokinetics (PK): BioD and PK properties were determined using male Sprague Dawley rats weighing around ~300 g. Three rats per group, had T-QD-NPs injected *via* tail vein with ~1 mL of T-NPs (23 mg/kg with respect to NPs, 81 μg/kg with respect to Cd) or saline. In a separate experiment, two rats per group had Platin-M (~0.5 mL, 95.8±0.7 μg Pt), NT-Platin-M-NPs (~0.5 mL, 98.0±7.2 μg Pt; Size: 59.53±0.34 nm, Zeta Potential: -22.3±1.2 mV), and T-Platin-M-NPs (~0.14 mL, 25.7±2.1 μg Pt; Size: 57.23±0.52 nm, Zeta Potential: 34.2±0.5 mV) injected *via* tail vein or saline. At varying time intervals, blood samples were collected in heparinized tubes and centrifuged in order to collect blood plasma. The percentage of QD or Pt was calculated by taking into consideration that blood constitutes 7% of body weight and plasma constitutes 55% of blood volume (4). The amount of Cd from the QD or Pt from Platin-M was calculated in the blood plasma by ICP-MS. After 24 h, the animals were sacrificed and the vital organs were collected. The collective urine and feces were also collected over a 24 h period for T-QD-NPs. For Platin-M, NT-Platin-M-NPs, and T-Platin-M-NPs, urine samples (~100 μL) were collected at 24 h directly from the bladder and collective feces were collected over 24 h. The overall bioD was calculated by analyzing the amount of Cd or Pt in each organ as well as the feces and urine by ICP-MS. Before analysis, the organs and feces were dissolved with PerkinElmer solvable (Product number: 6NE9100) for 24 h with gentle heating and shaking. The calculations for AUC, C_{max}, T_{max}, and C_L (t=0) were performed in the

GraphPad Prism (Version 5.01). PK parameters were determined by fitting the data using a two-compartmental or one compartmental model equation (Tables S1 and S5).

Quantification of mtDNA-Pt and nDNA-Pt Adducts: The mitochondria and nuclei were isolated according to the protocols mentioned before. These fractions were further fractionated in order to isolate mitochondrial and nuclear DNA, respectively. For mitochondrial DNA (mtDNA), the freshly isolated mitochondria were re-suspended in 35 μL of mitochondrial lysis buffer. To this, 5 μL of the enzyme mix was added. This was incubated at 50 °C in water bath until the solution turned clear (~1 h). To this, 100 μL of absolute ethanol was added and the resulting solution was incubated for 10 min at -20 °C. The solution was then centrifuged at 14000 rpm for 5 min at room temperature. The resulting pellet was then purified by washing with 70% ethanol in nanopure H_2O. The resulting purified mtDNA was resuspended in tris-EDTA (TE) buffer. The resulting solution was quantified for the amount and purity of DNA by UV-Vis spectroscopy (260/280 nm) and the amount of Pt by ICP-MS. For nuclear DNA (nDNA), the freshly isolated nuclei were re-suspended in 40 μL of cell lysis buffer. To this, 5 μL of the enzyme mix was added. This was incubated in a 50 °C water bath until the solution turned clear (~1 h). To this, 100 μL of absolute ethanol was added and the resulting solution was incubated for 10 min at -20 °C. The solution was then centrifuged at 14000 rpm for 5 min at room temperature. The resulting pellet was then purified by washing with 70% ethanol in nanopure H_2O. Reprecipitation with 70% ethanol in nanopure H_2O was performed until the ratio of the absorbances at 260 and 280 nm was ≥1.75 and ≤2.1. The resulting purified nDNA was resuspended in TE buffer. The resulting solution was quantified for the amount of DNA by UV-Vis spectroscopy (260/280 nm) and the amount of Pt by ICP-MS.

Citrate Synthase Assay: SH-SY5Y cells were seeded at a density of $1x10^6$ cells/mL on each well of a six well plate and allowed to grow overnight. Cells were treated with 1.0 μM cisplatin, 1.0 μM Platin-M, 1.0 μM NT-Platin-M-NPs, and 1.0 μM T-Platin-M-NPs for 12 h at 37 °C. The cells were trypsinized and washed 3x with PBS. The isolated cell pellet was then solubilized using 500 μL of the extraction buffer and incubated on ice for

20 min. The cells were then centrifuged at 16,000xg at 4 °C for 20 min. The samples (100 μL) were then added to the pre-coated microplate strips, sealed, and incubated for 3 h at room temperature. The wells were aspirated and washed 3x with wash buffer. Activity buffer (100 μL) was then added to each well and the plate was transferred to the microplate reader. The plate was read every minute for 20 sec intervals for 10 min at a wavelength of 412 nm.

Cytotoxicity of Platin-M and Platin-M-NPs: The cytotoxicity of Platin-M, T-Platin-M-NPs, NT-Platin-M-NPs, and cisplatin was tested in PC3, A2780/CP70, SH-SY-5Y, and MSC by MTT assay (Fig. S20, Table S4). PC3 cells (2000 cells/well), A2780/CP70 cells (2000 cells/well), SH-SY-5Y (2000 cells/well), and MSC (2000 cells/well) were plated on a 96 well plate and allowed to grow overnight. The media was changed and increasing concentrations of each formulation was added. For T-Platin-M-NPs, NT-Platin-M-NPs, the media was changed after 12 h and further incubated for an additional 60 h. The free drugs were incubated for 72 h without further media changes. After the given incubation time, MTT was added (5 mg/mL, 20 μL/well) and incubated for 5 h in order for MTT to be reduced to purple formazan. The media was removed and the cells were lysed with 100 μL of DMSO. In order to homogenize the formazan solution, the plates were subjected to 10 min of gentle shaking and the absorbance was read at 550 nm with a background reading at 800 nm with a plate reader. Cytotoxicity was expressed as mean percentage increase relative to the unexposed control ± SD. Control values were set at 0% cytotoxicity or 100% cell viability. Cytotoxicity data (where appropriate) was fitted to a sigmoidal curve and a three parameters logistic model used to calculate the IC_{50}, which is the concentration of chemotherapeutics causing 50% inhibition in comparison to untreated controls. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent measurements that were reproducible and statistically significant. The IC_{50} values were reported at ±99% confidence intervals. These analyses were performed with GraphPad Prism (San Diego, U.S.A).

Cellular Apoptosis Detection by Flow Cytometry: SH-SY5Y cells were seeded at a density of $1x10^6$ cells/mL on each well of a six well plate and allowed to grow overnight. Cells were treated with 1.0 μM cisplatin, 1.0 μM Platin-M, 1.0 μM NT-Platin-M-NPs, and 1.0 μM T-Platin-M-NPs for 12 h at 37 °C. As positive controls, etoposide (100 μM, incubation time: 12 h) for apoptosis and H_2O_2 (1 mM, incubation time: 45 min) for necrosis were used. The cells were trypsinized, repeatedly washed with cold PBS, and centrifuged at 1800 rpm for 3 min, and the supernatants were discarded (Note: During trypsinization, media was centrifuged and to isolate the floating cells). Cell density was determined and cells were resuspended in 1X Annexin-binding buffer to ~1×10^6 cells/mL preparing a sufficient volume to have 100 μL per assay. To 100 μL of cell suspension, 5 μL Alexa Fluor® 488 Annexin V and 1 μL (100 μg/mL) PI working solution were added, and incubated for 15 min at room temperature. After the incubation period, 400 μL 1X Annexin-binding buffer was added to each sample, samples were gently mixed keeping the samples on ice and the samples were analyzed on the flow cytometer immediately.

Flow Cytometric Analyses of Mitochondrial Function and Mitochondrial Mass by *JC-1 Assay:* To evaluate the integrity of mitochondrial functions, we used the cationic dye JC-1 assay. SH-SY5Y cells were cultured on a six well plate at a density of $1x10^6$ cells/mL and allowed to grow overnight at 37 °C. Cells were treated with 1.0 μM cisplatin, 1.0 μM Platin-M, 1.0 μM NT-Platin-M-NPs, and 1.0 μM T-Platin-M-NPs for 12 h at 37 °C. A solution of JC-1 reagent (10 μg/mL in RPMI) was added and incubated for 10 min at 37 °C. The cells were washed 3 times with PBS and trypsinized. The cells were isolated and washed 3x by centrifugation with PBS (1,800 rpm for 3 min at 4 °C). The resulting cell pellet was resuspended in 100 μL PBS and analyzed by flow cytometry using 488 nm and 633 nm excitations with 530 nm and 660 nm bandpass filters.

Table S1. PK Parameters of T-NPs by Using a Two-compartment Model*						
		Central Compartment (Initial Phase, t = 0)			**Periphery Compartment (Terminal Phase, t = 24 h)**	
$AUC_{[0\text{-}24\ h]}$ (h.ng/mL)	C_{max} (ng/mL)	V_d (mL/kg)	C_L (mL/h.kg)	$t_{1/2}$ (h)	V_d (mL/kg)	C_L (mL/h.kg)
34784± 2117	3237± 128	25.2 ± 0.9	4.7 ± 0.8	2.4 ± 0.8	8.4 ± 0.9	0.05 ± 0.07
Least-square fit to model. $C = a \times \exp[(-k_1 \times t) + b \times \exp(-k_2 \times t)]$. AUC, area under curve; C_{max}, Peak plasma concentration; C_L, total body clearance; $t_{1/2}$, plasma half-life; V_d, volume of distribution. *Model used to extrapolate concentrations at t = 0.						

Table S2. Characterization of T-Platin-M-NPs*					
%Platin-M Feed	$Z_{Average}$ (nm)	PDI	Zeta Potential (mV)	%Loading	%EE
0	51.7 ± 1.9	0.194	28.5 ± 1.56	0	0
10	52.7 ± 2.7	0.108	32.8 ± 3.1	6.3±0.1	64.8±0.9
20	51.1 ± 1.1	0.190	30.2 ± 0.2	10.1±0.2	50.8±0.8
30	50.1 ± 0.8	0.154	37.1 ± 0.3	14.0±0.7	46.6±2.4
40	50.6 ± 0.1	0.184	34.5± 2.95	16.9±0.4	42.3±0.9
50	50.3 ± 0.6	0.157	35.8 ± 2.4	23.4±0.8	46.9±1.6
*During scale up for animal studies with 30% Platin-M feed, %loading varies between 4 to 11% based on the amount of polymer used and $Z_{Average}$ increases by 20 to 30 nm.					

Table S3. Characterization of NT-Platin-M-NPs*					
%Platin-M Feed	$Z_{Average}$ (nm)	PDI	Zeta Potential (mV)	%Loading	%EE
0	49.9 ± 0.9	0.156	-22.1 ± 4.2	0	0
10	50.1 ± 0.9	0.152	-31.6 ± 4.7	6.5±0.2	65.9±0.3
20	49.8 ± 1.2	0.159	-33.8 ± 2.6	10.5±1.4	50.9±5.0
30	50.6 ± 1.3	0.156	-31.1 ± 1.6	14.6±2.4	50.7±11
40	50.3 ± 2.5	0.175	-27.8 ± 1.1	18.10±0.02	45.3±0.1
50	48.9 ± 1.0	0.184	-24.5 ± 1.6	26.1±0.5	52.2±0.9
*During scale up for animal studies with 30% Platin-M feed, %loading varies between 4 to 11% based on the amount of polymer used and $Z_{Average}$ increases by 20 to 30 nm.					

Table S4. IC_{50} (μM) Values of Platin-M and NPs.			
	PC3	**SH-SY5Y**	**A2780/CP70**
Cisplatin	13.1±0.1	19±4.7	12.0±2.8
Platin-M	7.3±0.8	3.4±0.5	0.74±0.05
NT-Platin-M-NPs	3.1±0.1	2.7±0.6	2.2±0.7
T-Platin-M-NPs	0.19±0.01	1.1±0.2	0.14±0.04

Table S5. PK Parameters of Platin-M and its NPs by Using a One-compartment Model

	Platin-M	**NT-Platin-M-NPs**	**T-Platin-M-NPs**
Route of Administration	IV	IV	IV
Pt Dose Level, μg/animal	95.8 ± 0.7	98.0 ± 7.2	25.7 ± 2.1
Pt Dose Level, mg/kg	0.323 ± 0.000	0.322 ± 0.001	0.085 ± 0.002
AUC [0-24 h], ng.h/mL/mg/kg	4,756 ± 121	23,070 ± 9,823	40,807 ± 1,421
C_{max}, ng/mL	1,985 ± 180	2,313 ± 311	1,530 ± 18
V_d, L/kg	69.9 ± 7.3	77.6 ± 0.9	197.6 ± 24.7
C_L [t=0], L/h.kg	90.9 ± 19.9	27.1 ± 14.6	87.7 ± 14.9
$t_{1/2}$ (h)	0.5 ± 0.1	2.3 ± 1.3	1.6 ± 0.1

AUC, area under curve; C_{max}, Peak plasma concentration; C_L, total body clearance; $t_{1/2}$, plasma half-life; V_d, volume of distribution.

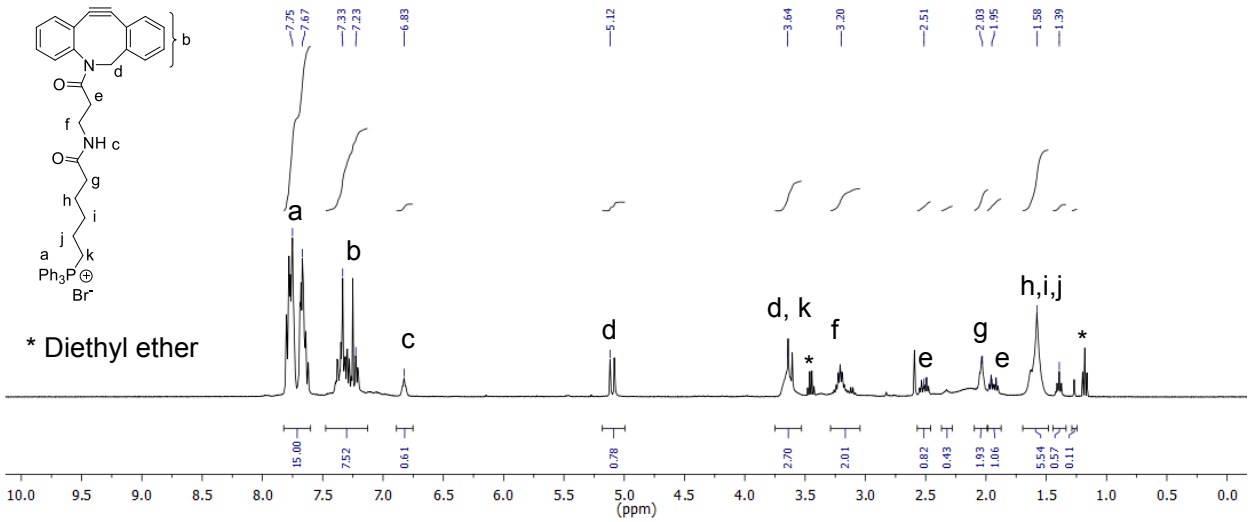


Fig. S1. ^{1}H NMR of DBCO-TPP in $CDCl_3$.

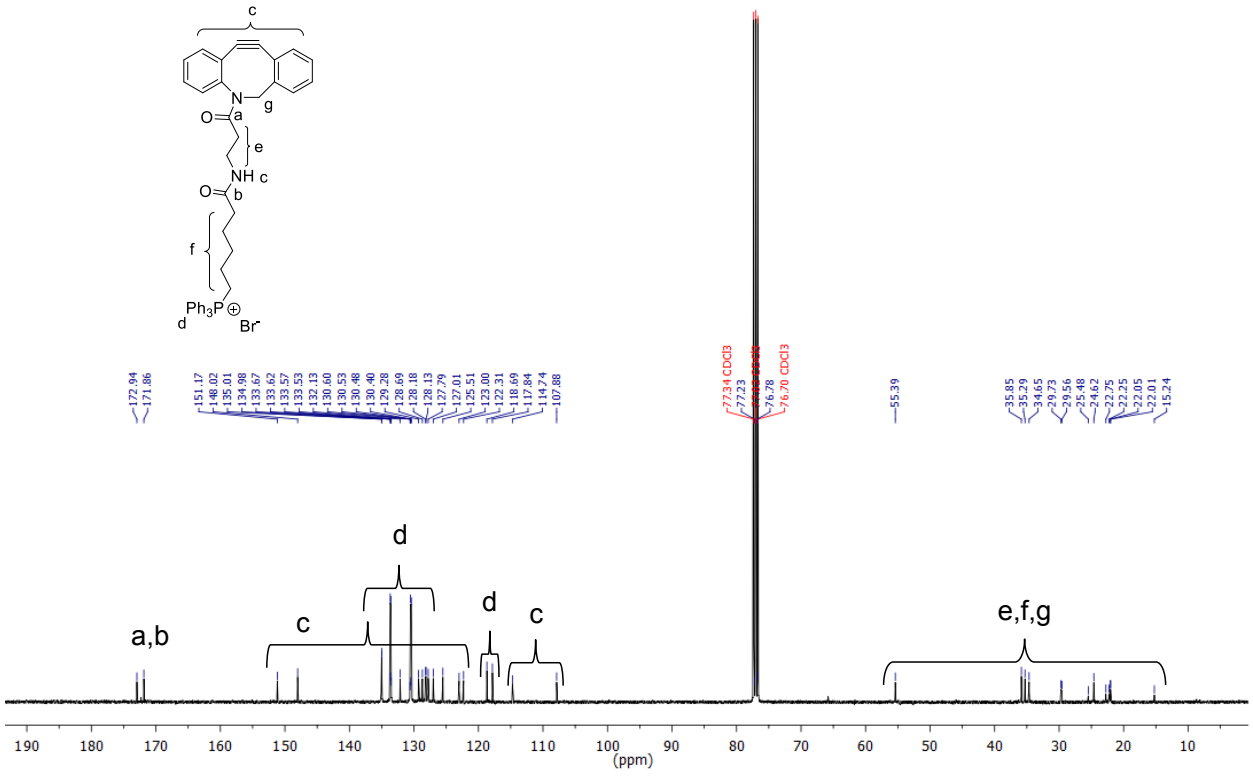


Fig. S2. ^{13}C NMR of DBCO-TPP in $CDCl_3$.

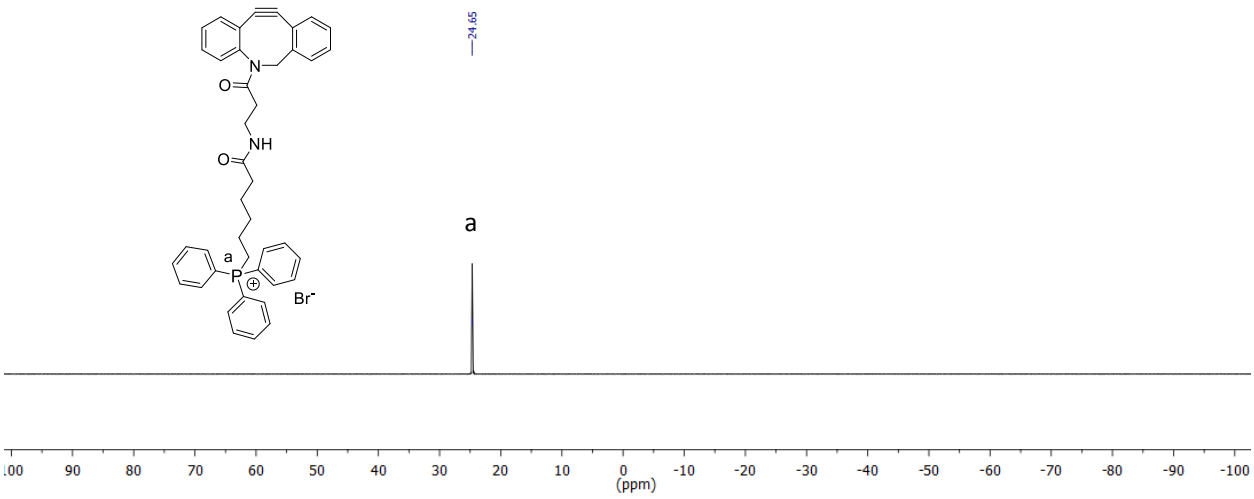


Fig. S3. ^{31}P NMR of DBCO-TPP in $CDCl_3$.

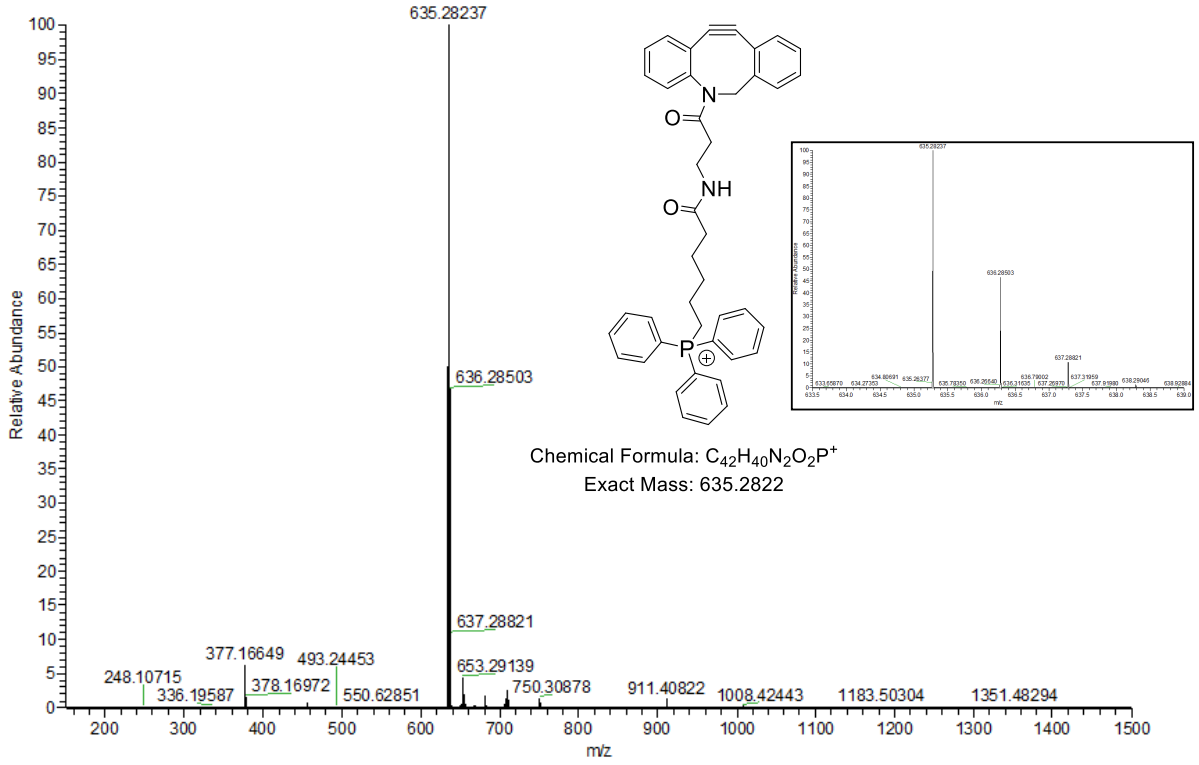


Fig. S4. HRMS-ESI of DBCO-TPP.

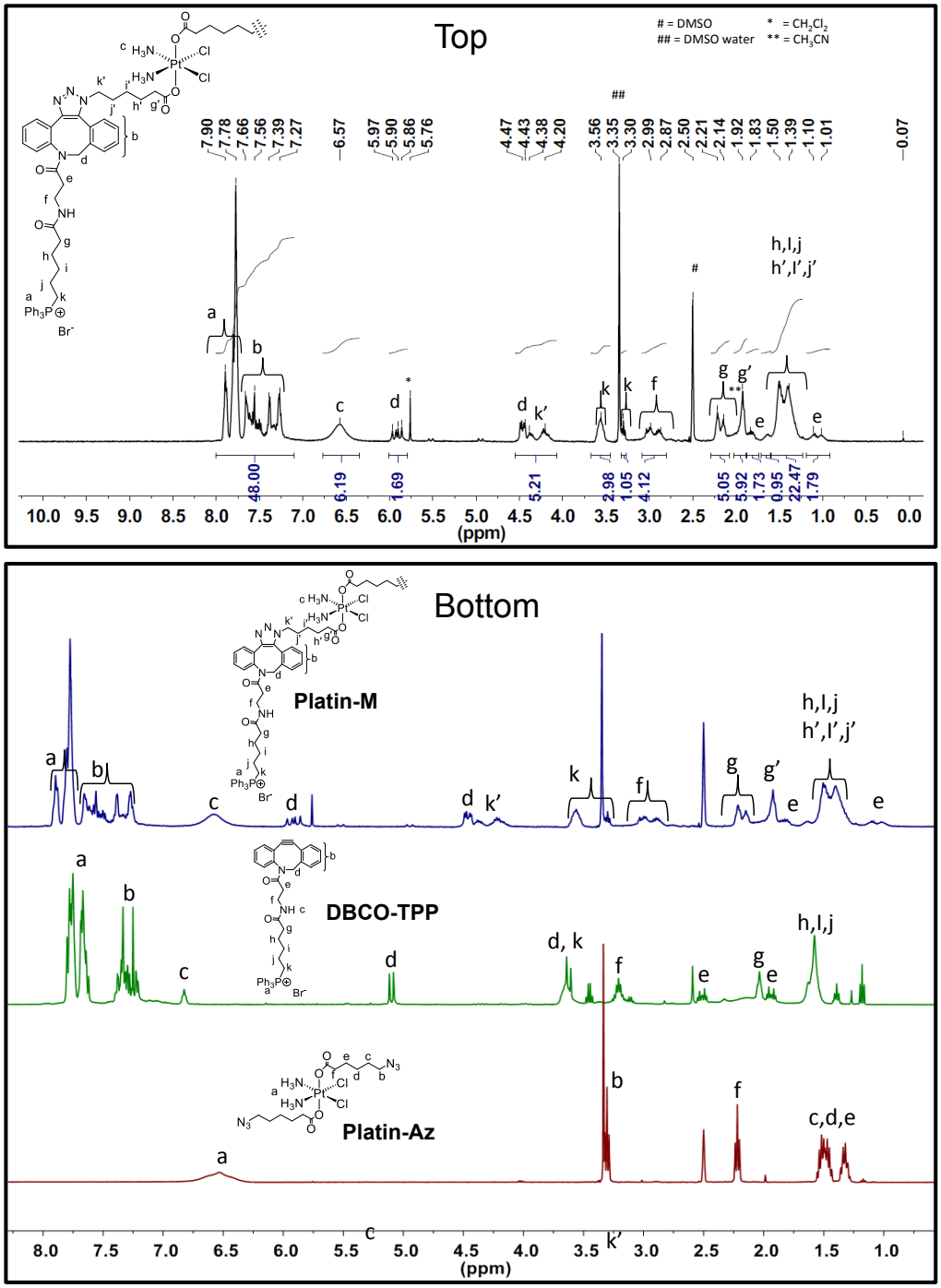


Fig. S5. (Top) ^{1}H NMR of Platin-M in DMSO-d_6. (Bottom) A comparison of ^{1}H NMR of Platin-M in DMSO-d_6 with the precursors used in SPAAC, Platin-Az and DBCO-TPP.

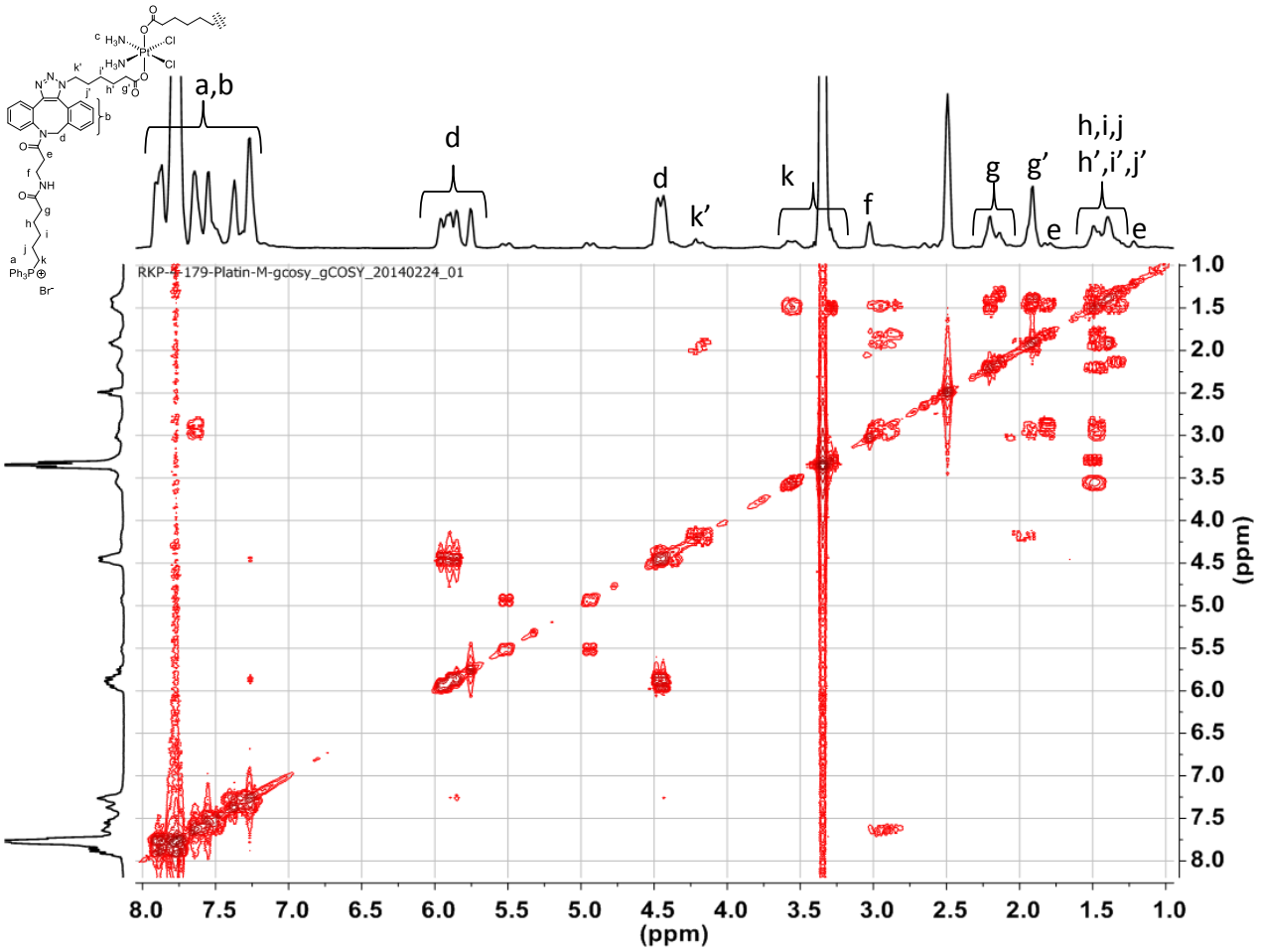


Fig. S6. gCOSY of NMR of Platin-M in DMSO-d_6.

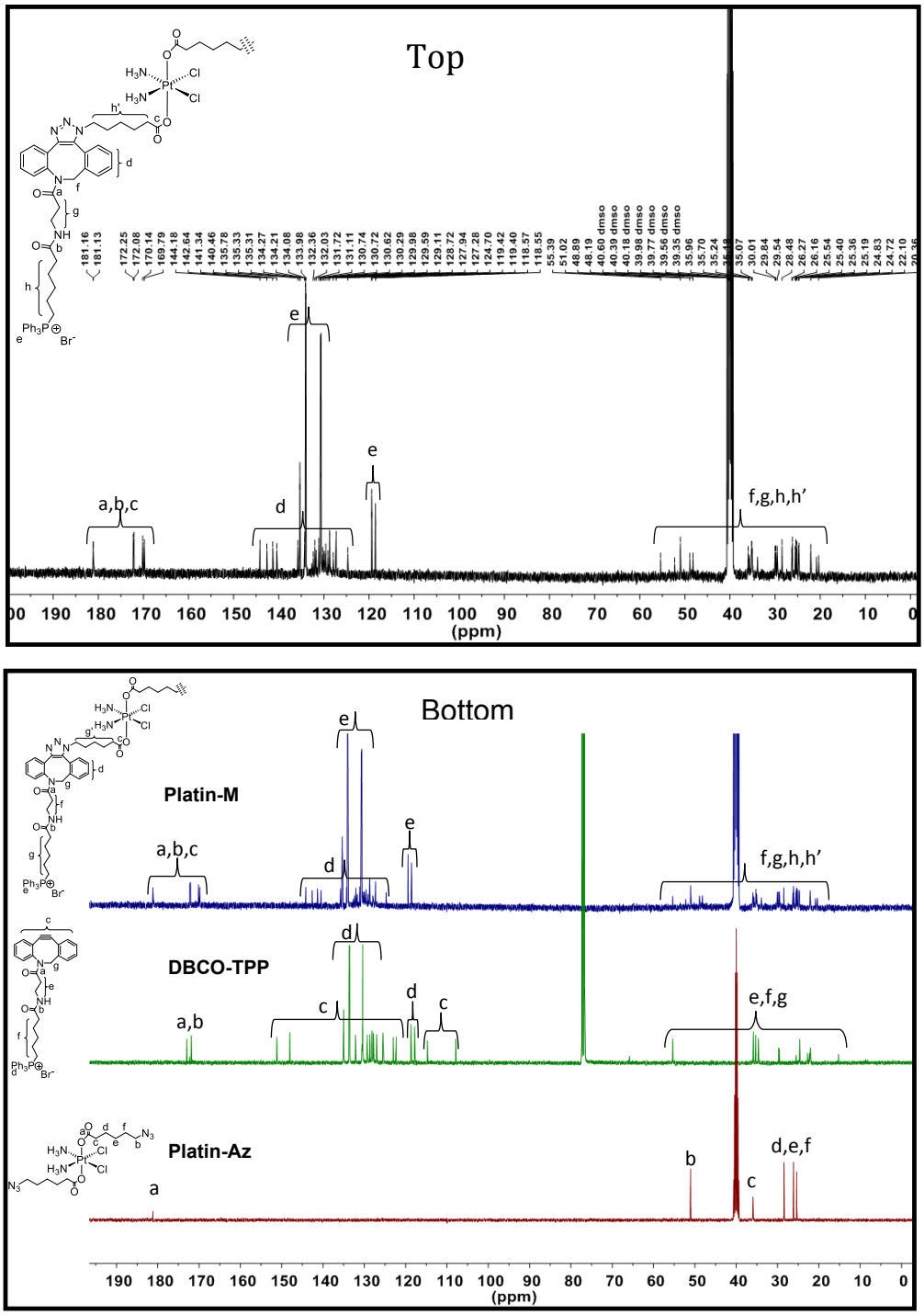


Fig. S7. (Top) ^{13}C NMR of Platin-M in DMSO-d_6. (Bottom) A comparison of ^{13}C NMR of Platin-M in DMSO-d_6 with the precursors used in SPAAC, Platin-Az and DBCO-TPP.

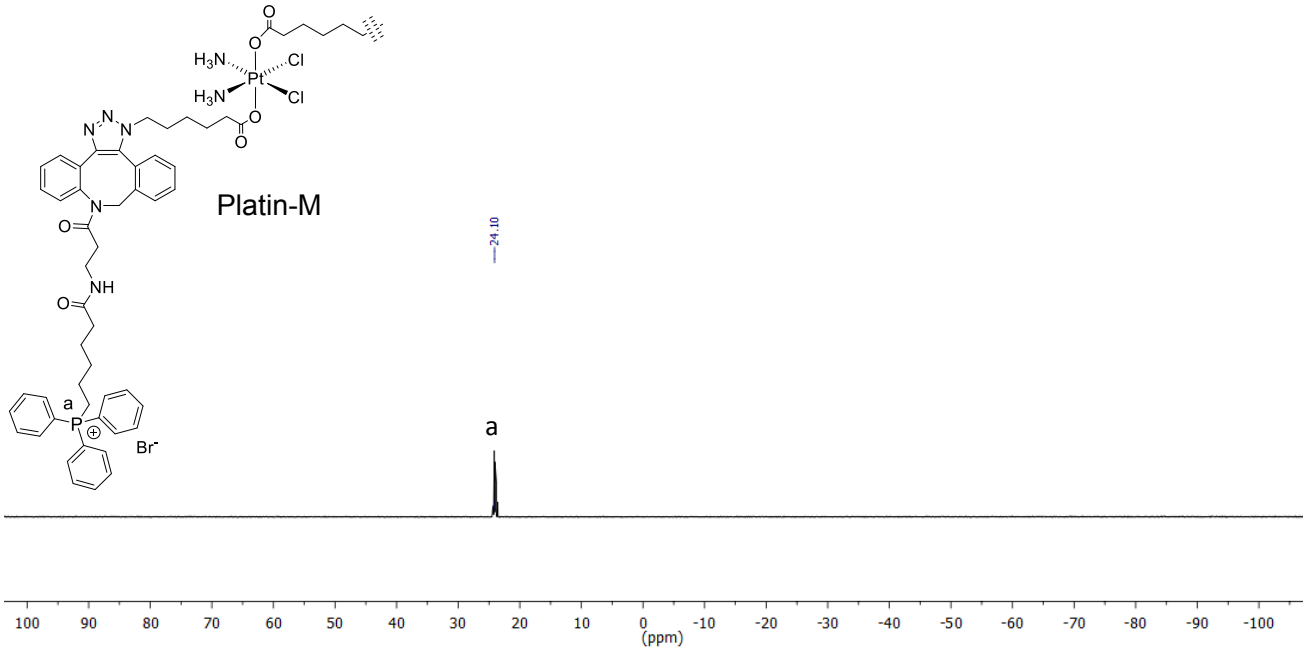


Fig. S8. ^{31}P NMR of Platin-M in DMSO-d_6.

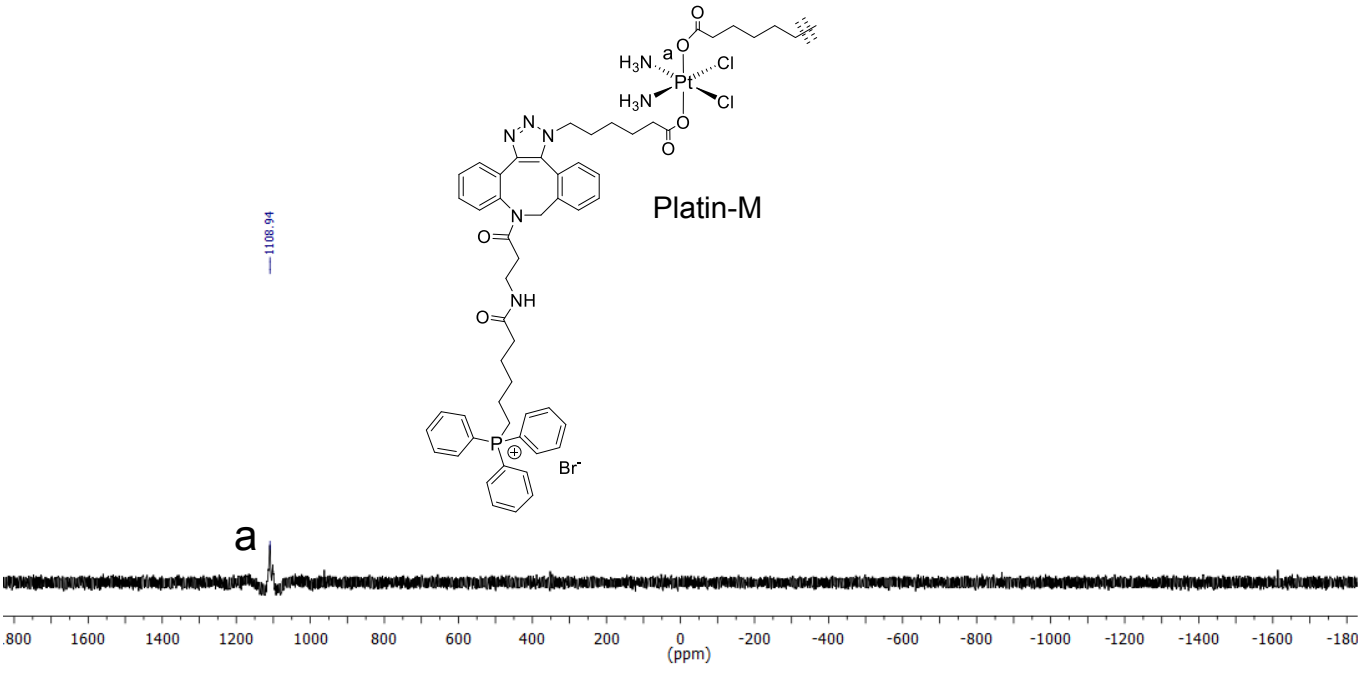


Fig. S9. ^{195}Pt NMR of Platin-M in DMSO-d_6.

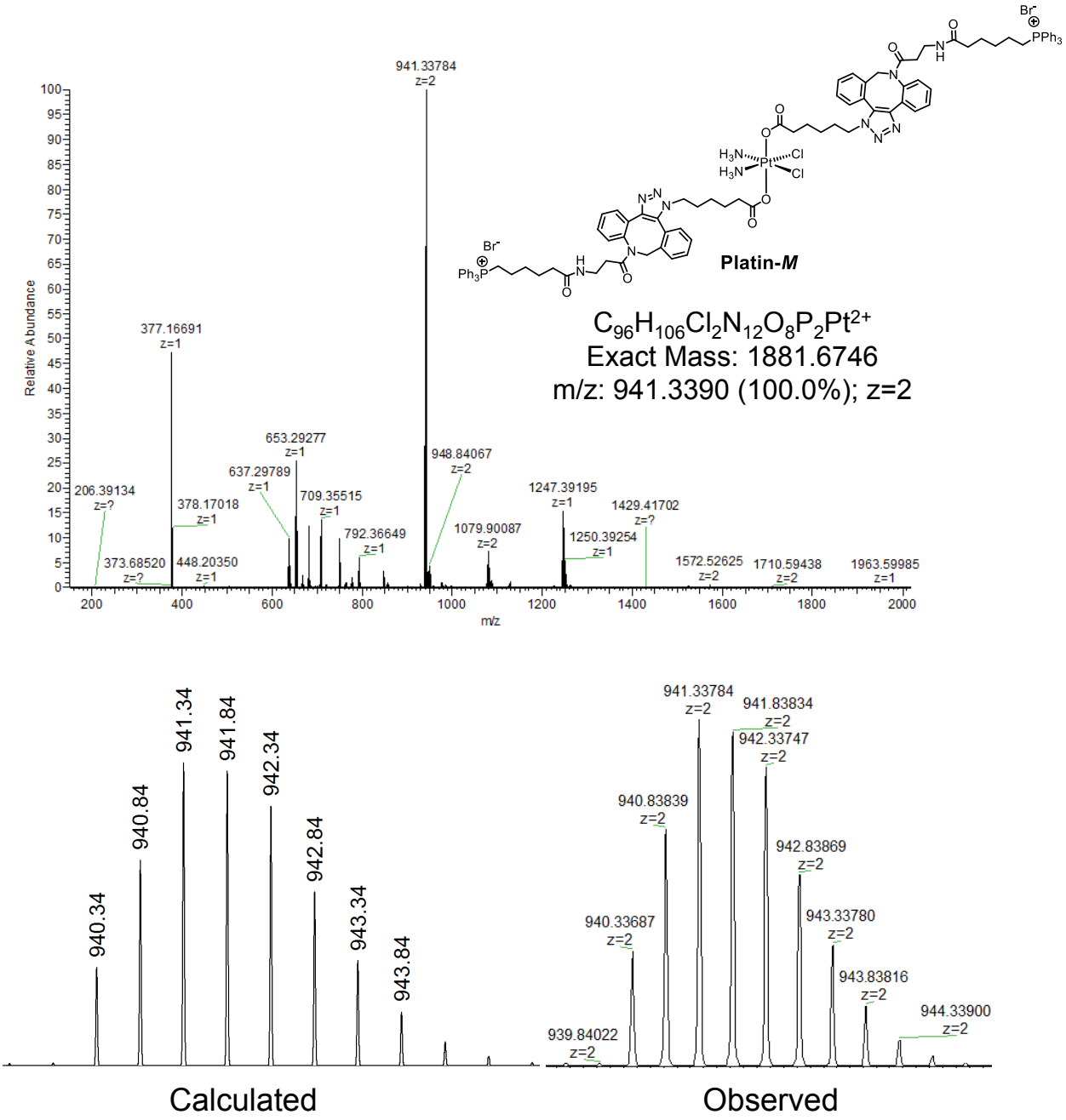
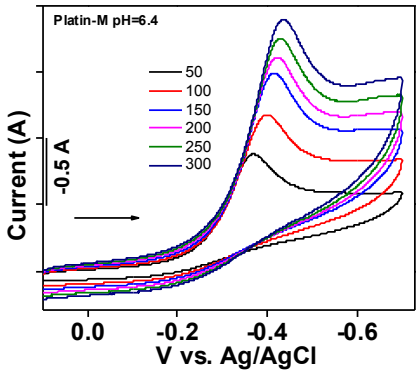
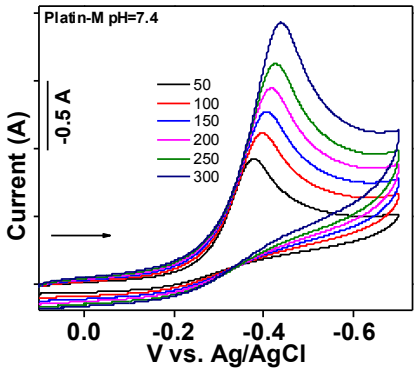


Fig. S10. HRMS-ESI of Platin-M.



Platin-M pH= 6.4; -0.369 V vs Ag/AgCl; -0.269 V vs NHE



Platin-M pH= 7.4; -0.376 V vs Ag/AgCl; -0.275 V vs NHE

Fig. S11. Electrochemistry data of Platin-M.

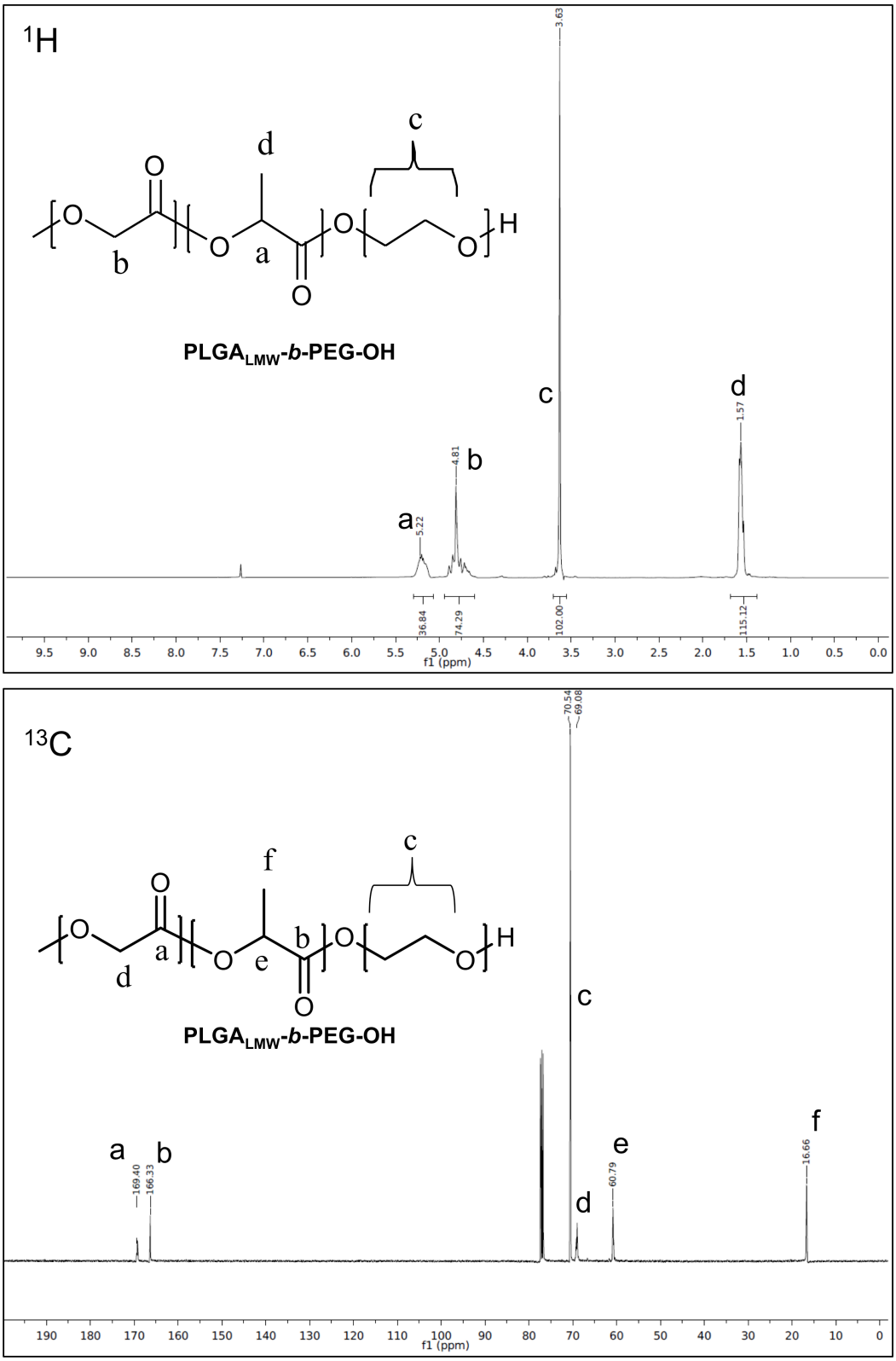


Fig. S12. ^{1}H and ^{13}C NMR of $PLGA_{LMW}$-*b*-PEG-OH in $CDCl_3$.

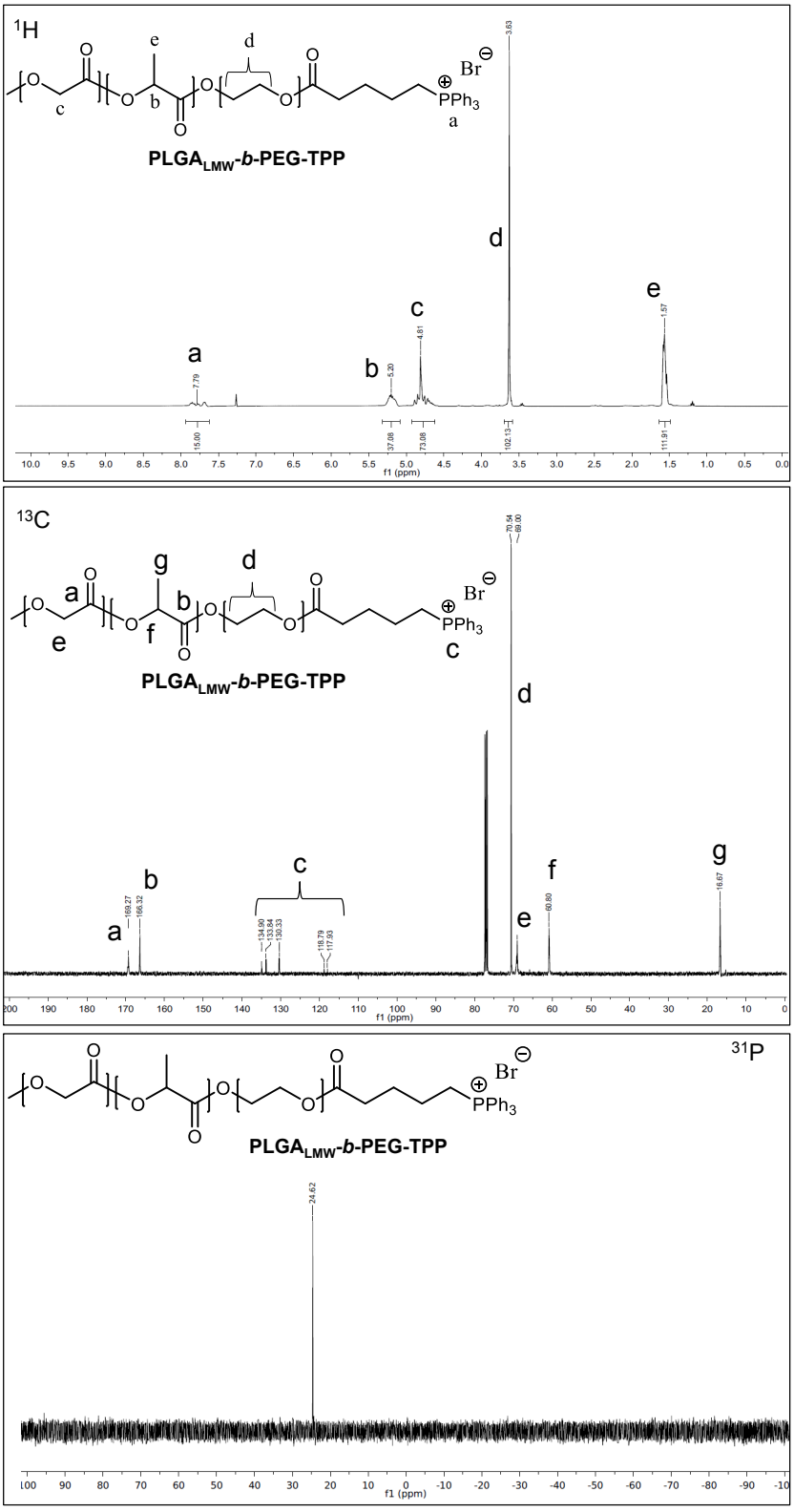


Fig. S13. ^{1}H, ^{13}C, and ^{31}P NMR of $PLGA_{LMW}$-*b*-PEG-TPP in $CDCl_3$.

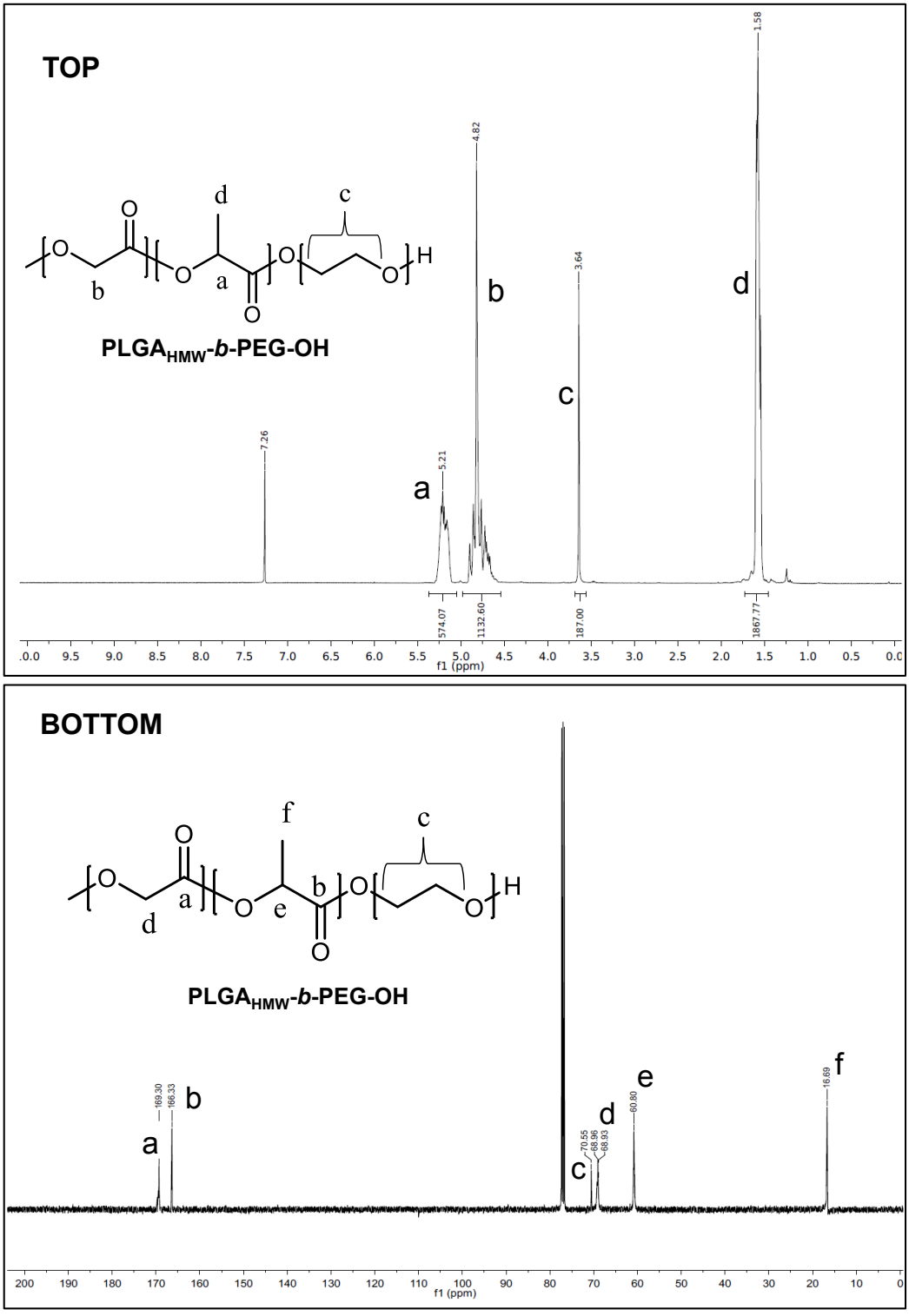


Fig. S14. ^{1}H (top) and ^{13}C (bottom) NMR of PLGA$_{HMW}$-*b*-PEG-OH in $CDCl_3$.

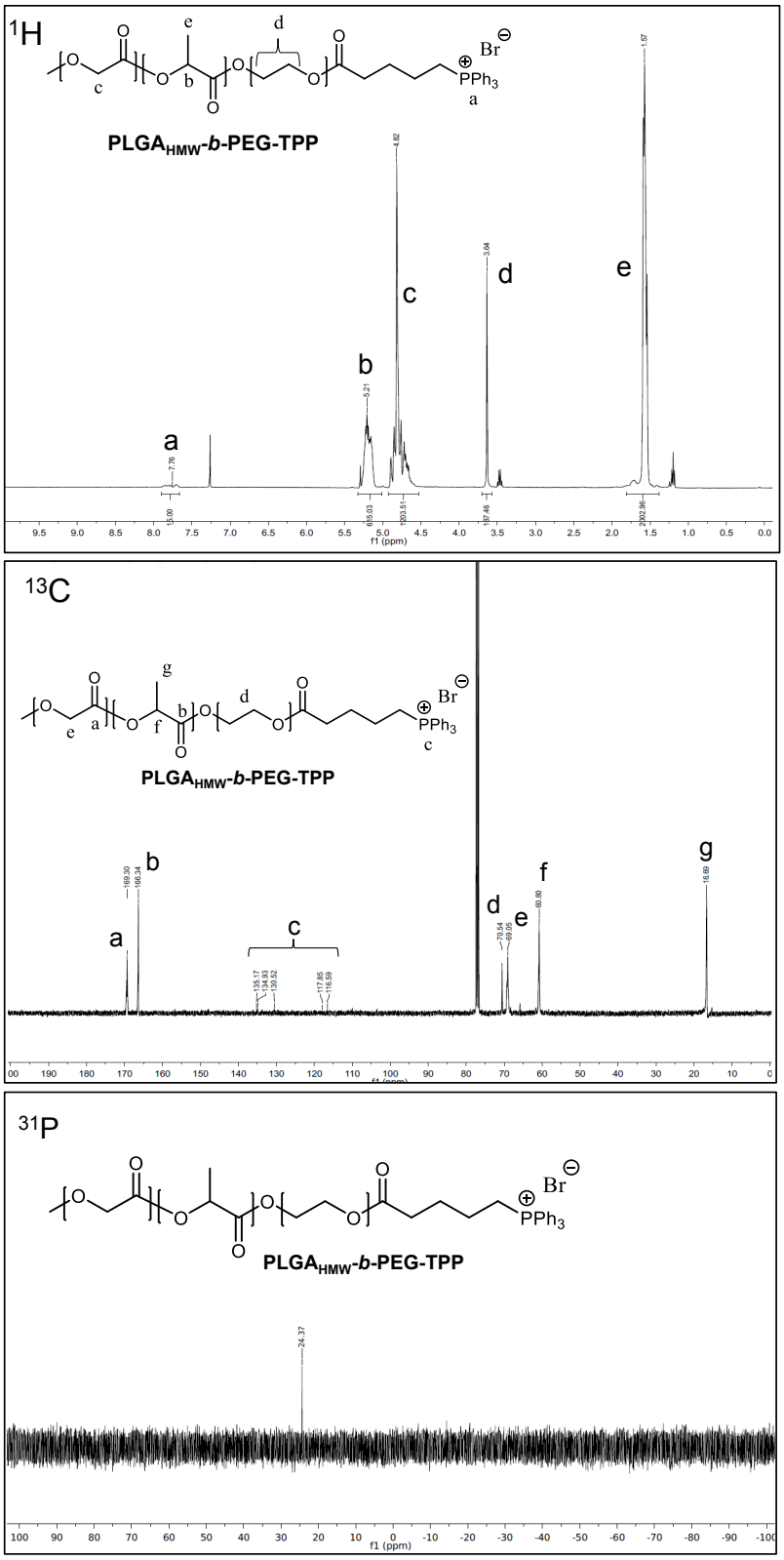


Fig. S15. ^{1}H, ^{13}C, and ^{31}P NMR of $PLGA_{HMW}$-*b*-PEG-TPP in $CDCl_3$.

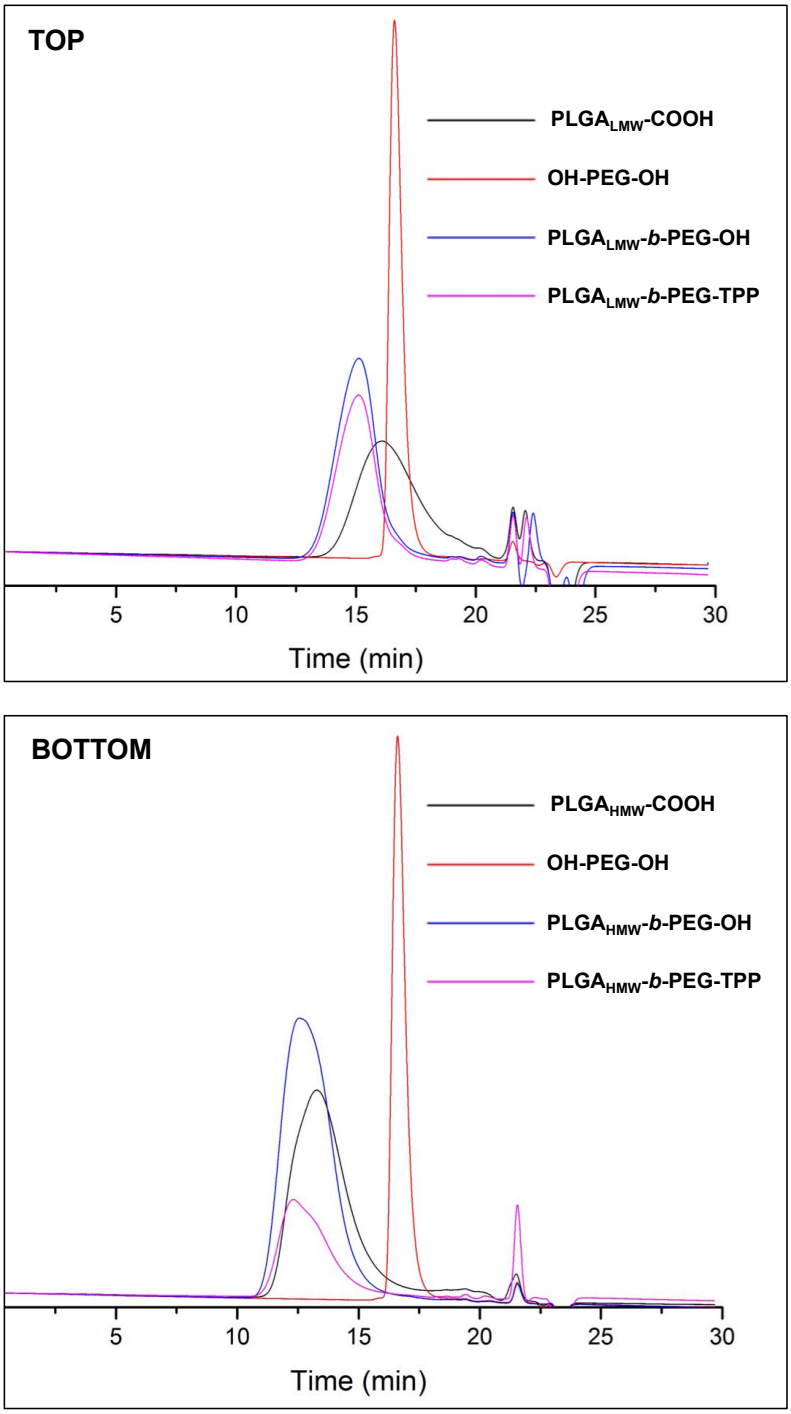


Fig. S16. GPC overlay of PLGA$_{LMW}$-COOH, OH-PEG-OH, PLGA$_{LMW}$-*b*-PEG-OH, PLGA$_{LMW}$-*b*-PEG-TPP (top) and GPC overlay of PLGA$_{HMW}$-COOH, OH-PEG-OH, PLGA$_{HMW}$-*b*-PEG-OH, PLGA$_{HMW}$-*b*-PEG-TPP (bottom).

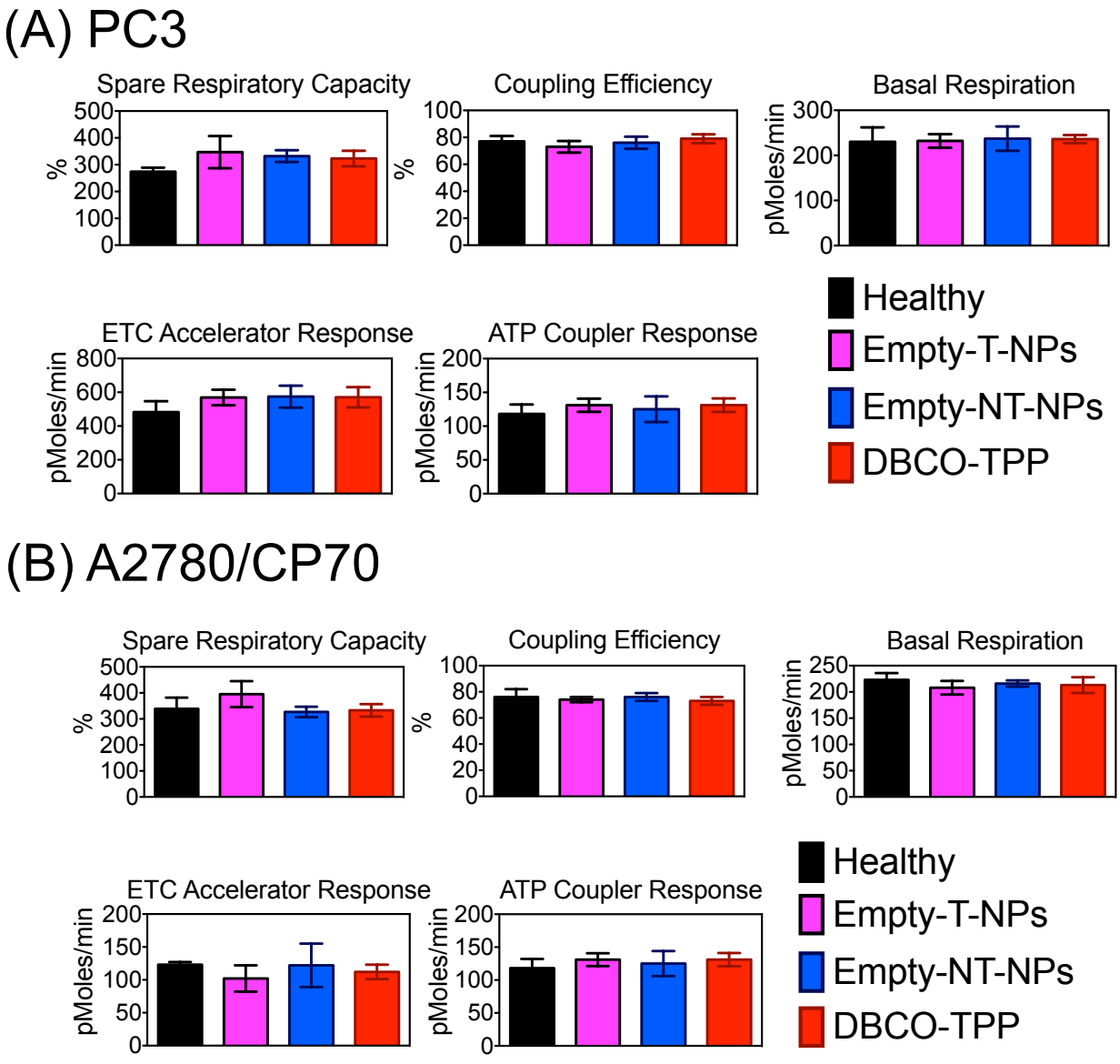


Fig. S17. Different parameters related to mitochondrial toxicity of Empty-T-NPs, Empty-NT-NPs, and DBCO-TPP in (A) PC3 and (B) cisplatin resistant A2780/CP70 cells by XF24 MitoStress assay.

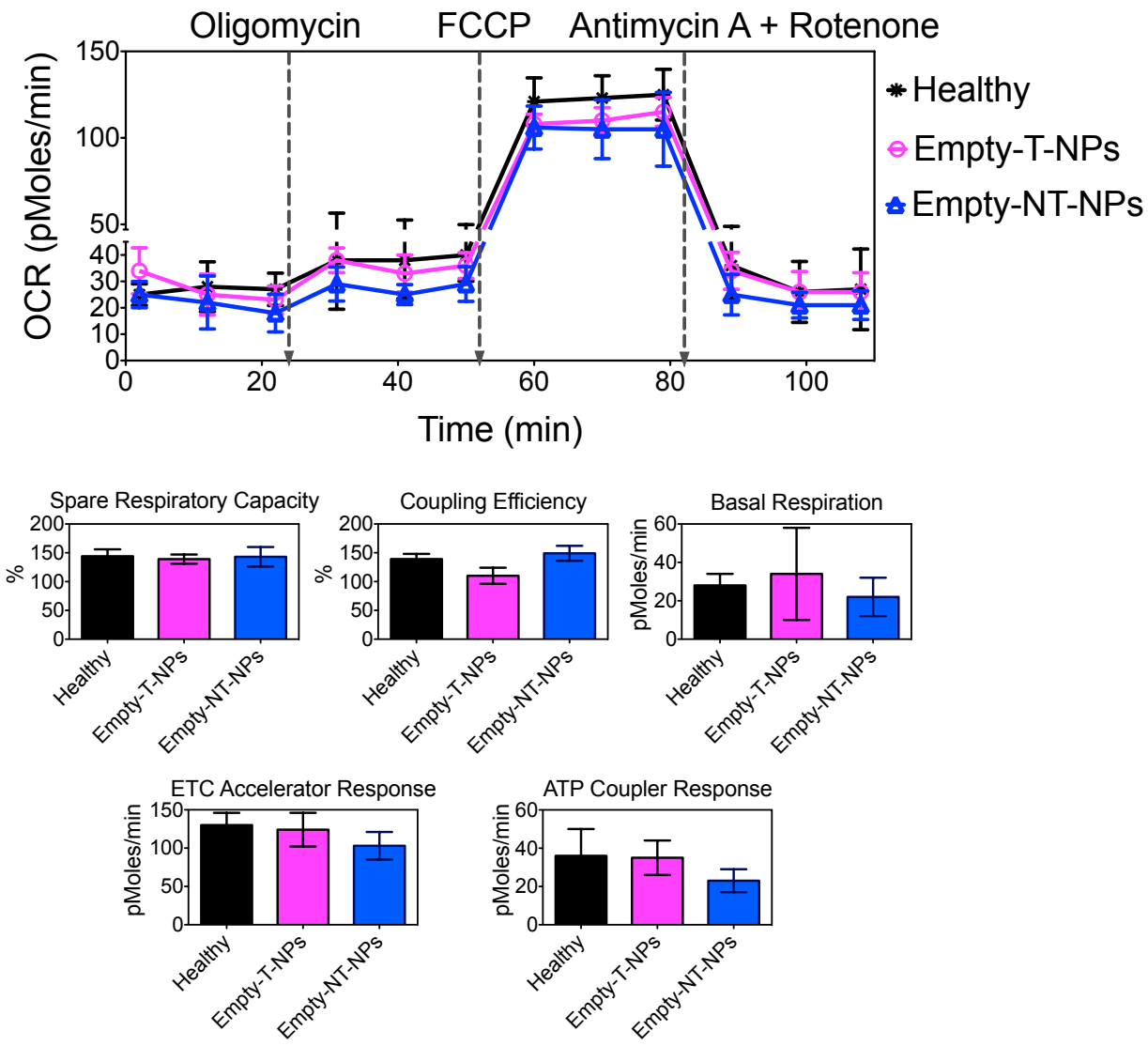


Fig. S18. Effect of Empty T and NT-NPs in H9C2 cardiomyocytes by Mito-Stress assay. The cells were treated with empty-T-NPs and empty-NT-NPs (0.5 mg/mL) for 12 h at 37 °C in 5% CO_2 atmosphere.

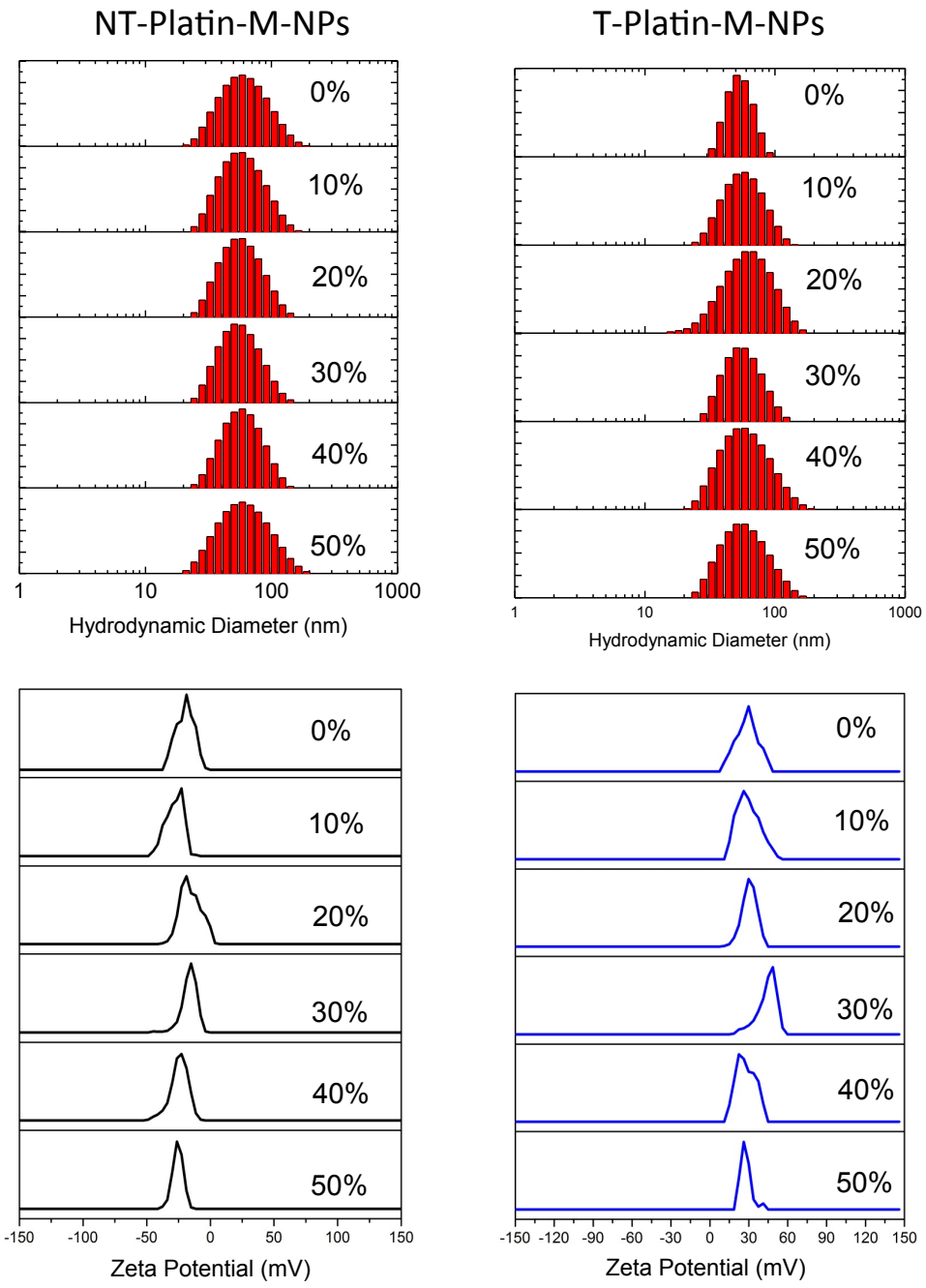


Fig. S19. Size and Zeta potential of T and NT-Platin-M-NPs.

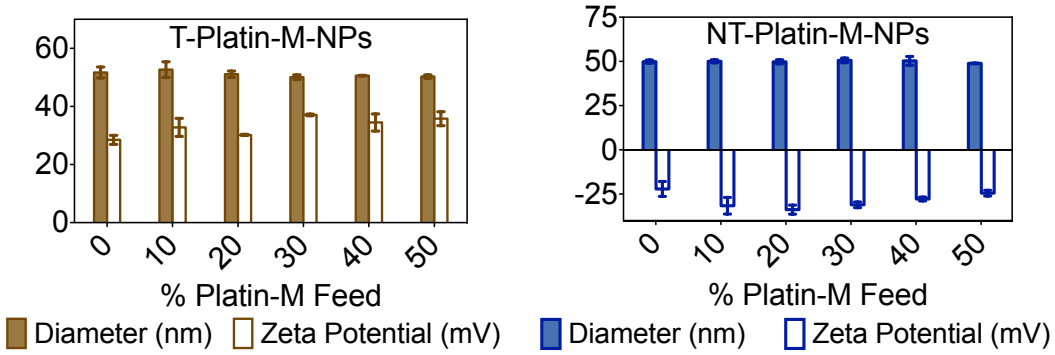
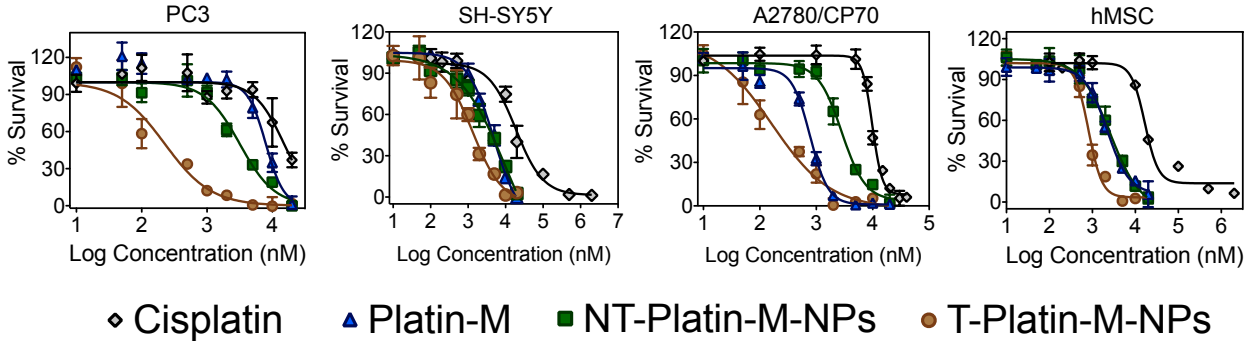


Fig. S20. Size and zeta potential of T-Platin-M and NT-Platin-M-NPs.



IC_{50} (μM) Values of Platin-M and NPs.				
	PC3	**SH-SY5Y**	**A2780/CP70**	**hMSC**
Cisplatin	13.1±0.1	19±4.7	12.0±2.8	16.4±0.5
Platin-M	7.3±0.8	3.4±0.5	0.74±0.05	2.4±0.3
NT-Platin-M-NPs	3.1±0.1	2.7±0.6	2.2±0.7	2.5±0.3
T-Platin-M-NPs	0.19±0.01	1.1±0.2	0.14±0.04	0.81±0.01

Fig. S21. Cytotoxicity profiles of cisplatin, Platin-M, NT-Platin-M-NPs, and T-Platin-M-NPs in prostate cancer PC3, neuroblastoma SH-SY5Y, cisplatin-resistant ovarian cancer A2780/CP70, and human mesenchymal stem cells hMSC cells as determined by the MTT assay.

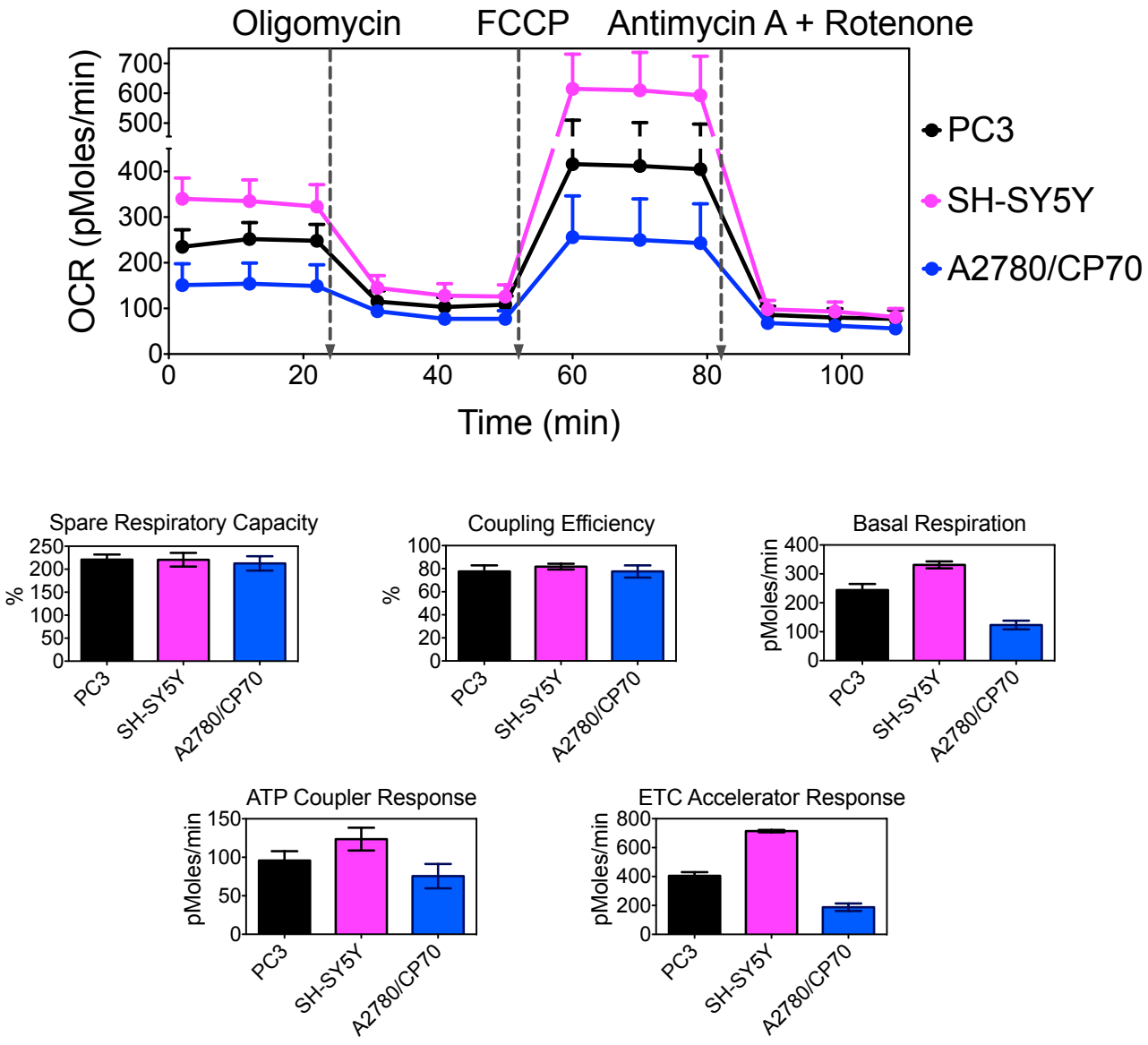


Fig. S22. A comparison of OCR levels in different cancer cells by MitoStress analyses.

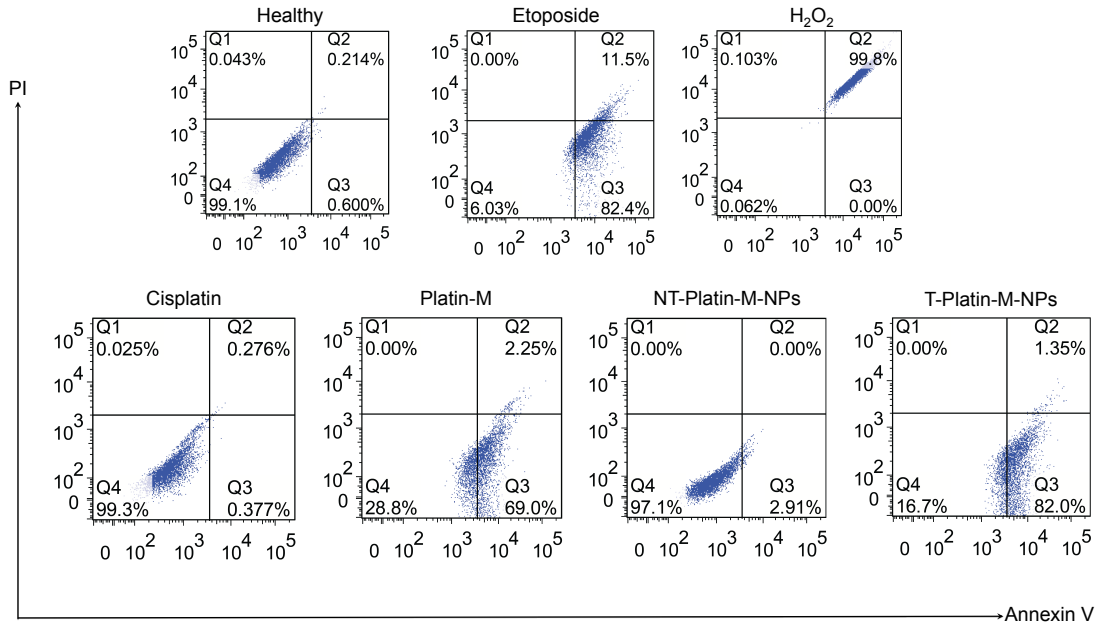


Fig. S23. FACS analysis using Annexin V-Alexa Fluor/PI staining for apoptosis detection in SH-SY5Y cells treated with 1 μM cisplatin, 1 μM Platin-M, 1 μM NT-Platin-M-NPs, and 1 μM T-Platin-M-NPs for 12 h at 37 °C. As positive controls, etoposide (100 μM, incubation time: 12 h) for apoptosis and H_2O_2 (1 mM, incubation time: 45 min) for necrosis were used. Cells in the lower right quadrant indicate Annexin V-positive/PI negative, early apoptotic cells. The cells in the upper right quadrant indicate Annexin V-positive/PI positive, late apoptotic or necrotic cells.

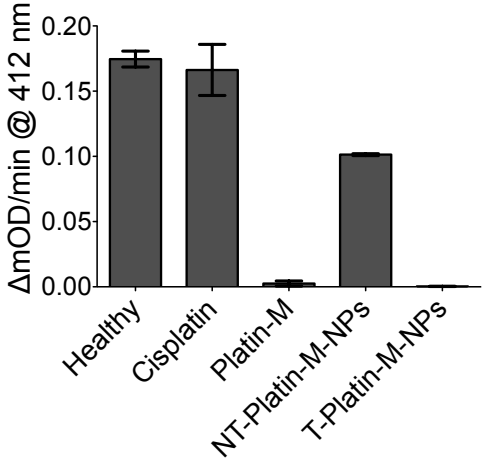


Fig. S24. Citrate synthase activity in SH-SY5Y cells treated with 1 μM cisplatin, 1 μM Platin-M, 1 μM NT-Platin-M-NPs, and 1 μM T-Platin-M-NPs for 12 h at 37 °C.

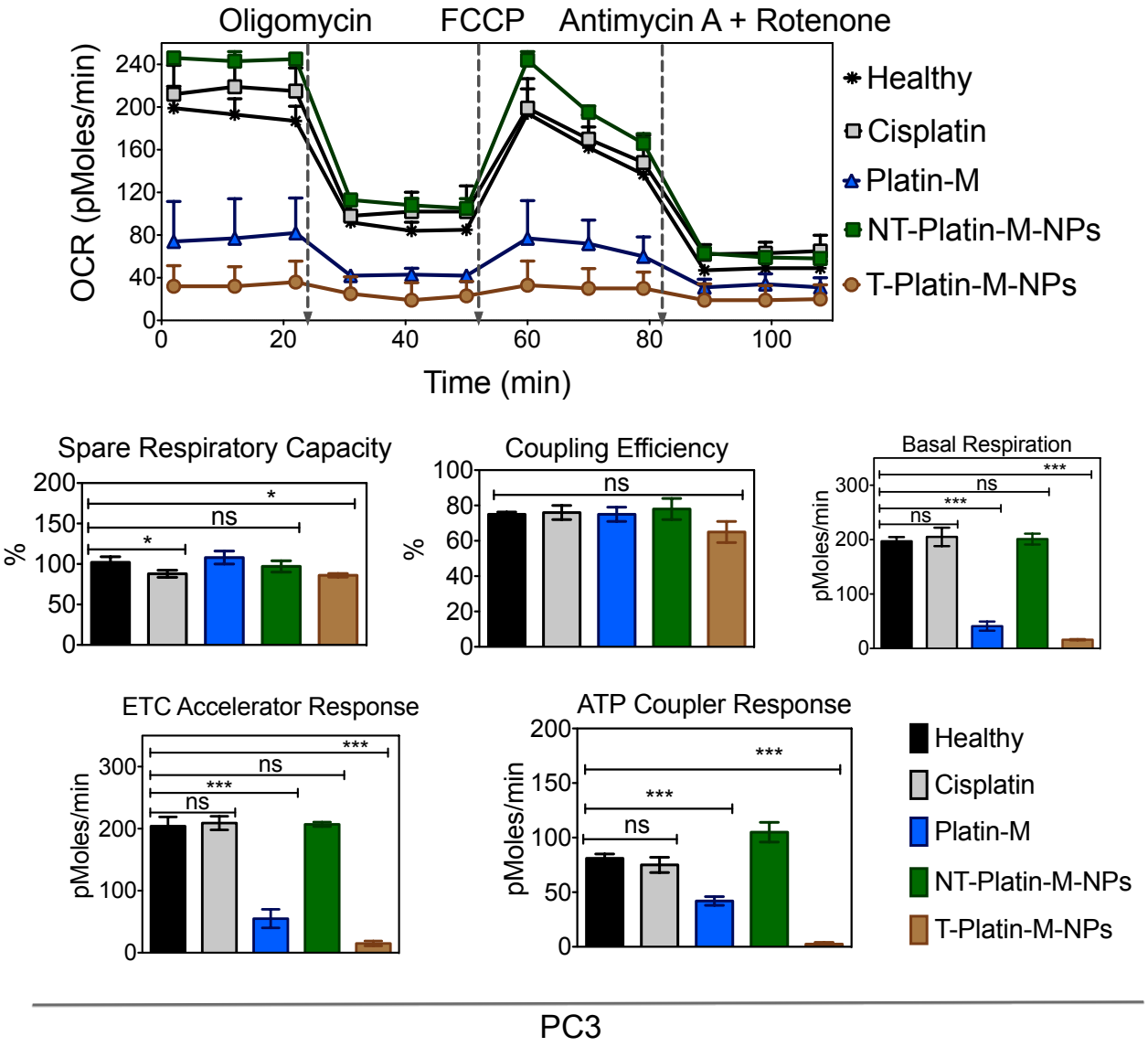


Fig. S25. Effect of cisplatin, Platin-M, and its NPs in PC3 cells by Mito-Stress assay. The cells were treated with Platin-M (10 μM), cisplatin (10 μM), T-Platin-M-NPs (10 μM with respect to Pt), NT-Platin-M-NPs (10 μM with respect to Pt) for 12 h at 37 °C in 5% CO_2 atmosphere. ***, P<0.001; **, P=0.001-0.01; *, P=0.01-0.05; non significant (ns), P>0.05.

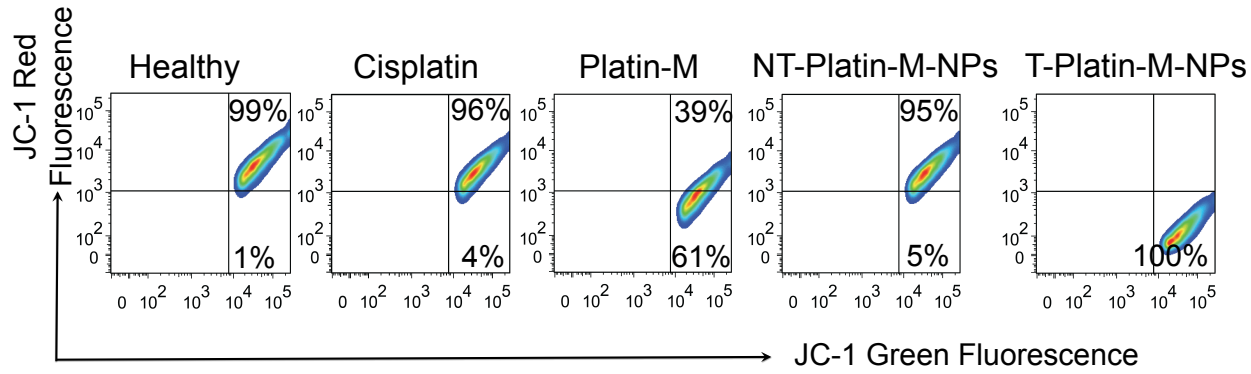


Fig. S26. Changes in the $\Delta\psi_m$ and mitochondrial mass loss by JC-1 assay. Treatment of SH-SY5Y cells with 1 μM Platin-M, 1 μM NT-Platin-M-NPs, and 1 μM T-Platin-M-NPs for 12 h at 37 °C dramatically caused the collapse of $\Delta\psi_m$ in these cells compared to 1 μM cisplatin. Cells were stained with JC-1. Cells were analyzed on a flow cytometer using 488 nm and 633 nm excitations with 530 nm and 660 nm band-pass emission filters. Green fluorescence, depolarized mitochondria (J-monomer); red fluorescence, hyperpolarized (J-aggregates). The shift in $\Delta\psi_m$ observed by disappearance of red-orange-stained mitochondria (large negative $\Delta\psi_m$) and an increase in fluorescent green-stained mitochondria (loss of $\Delta\psi_m$).

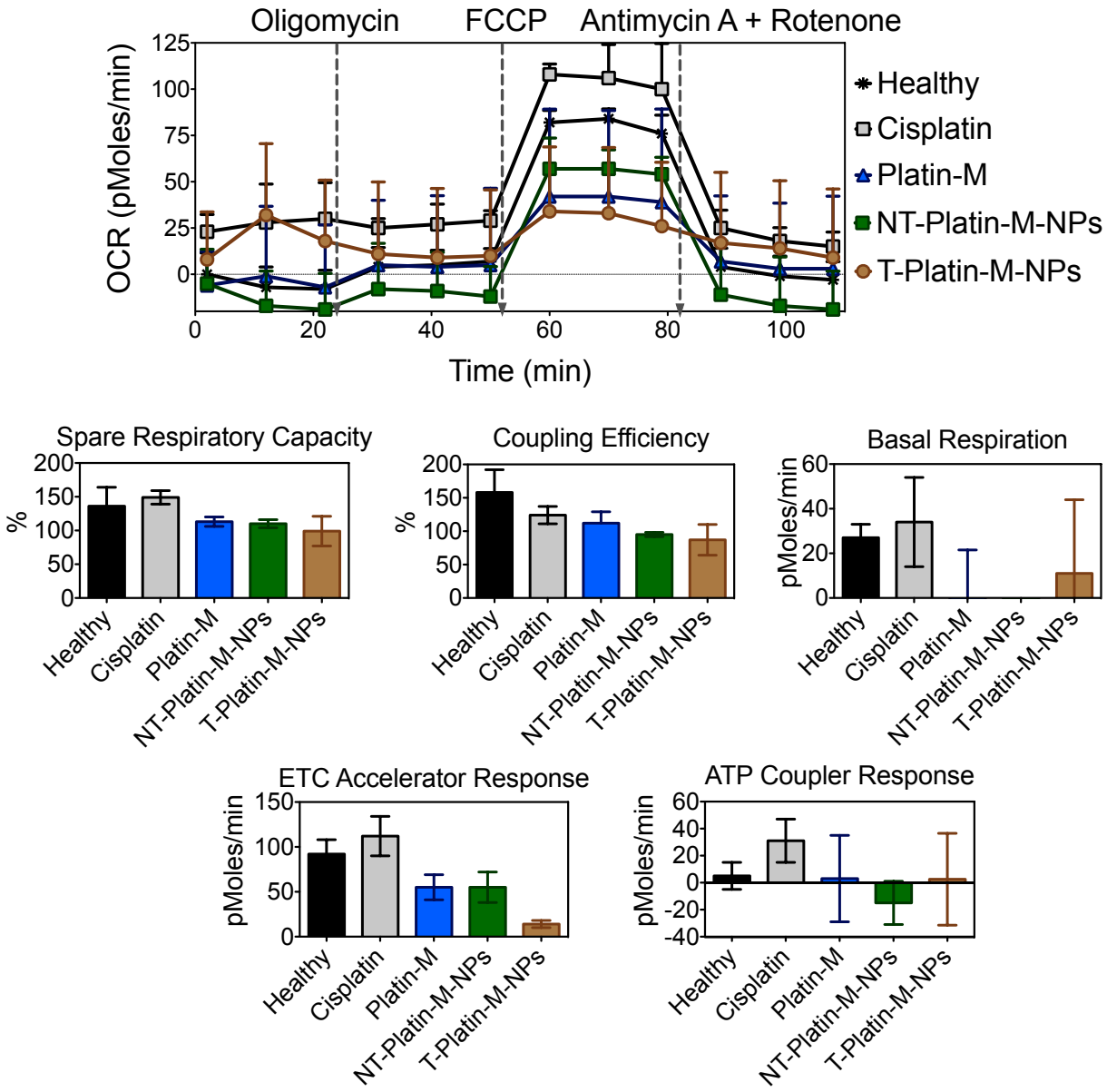
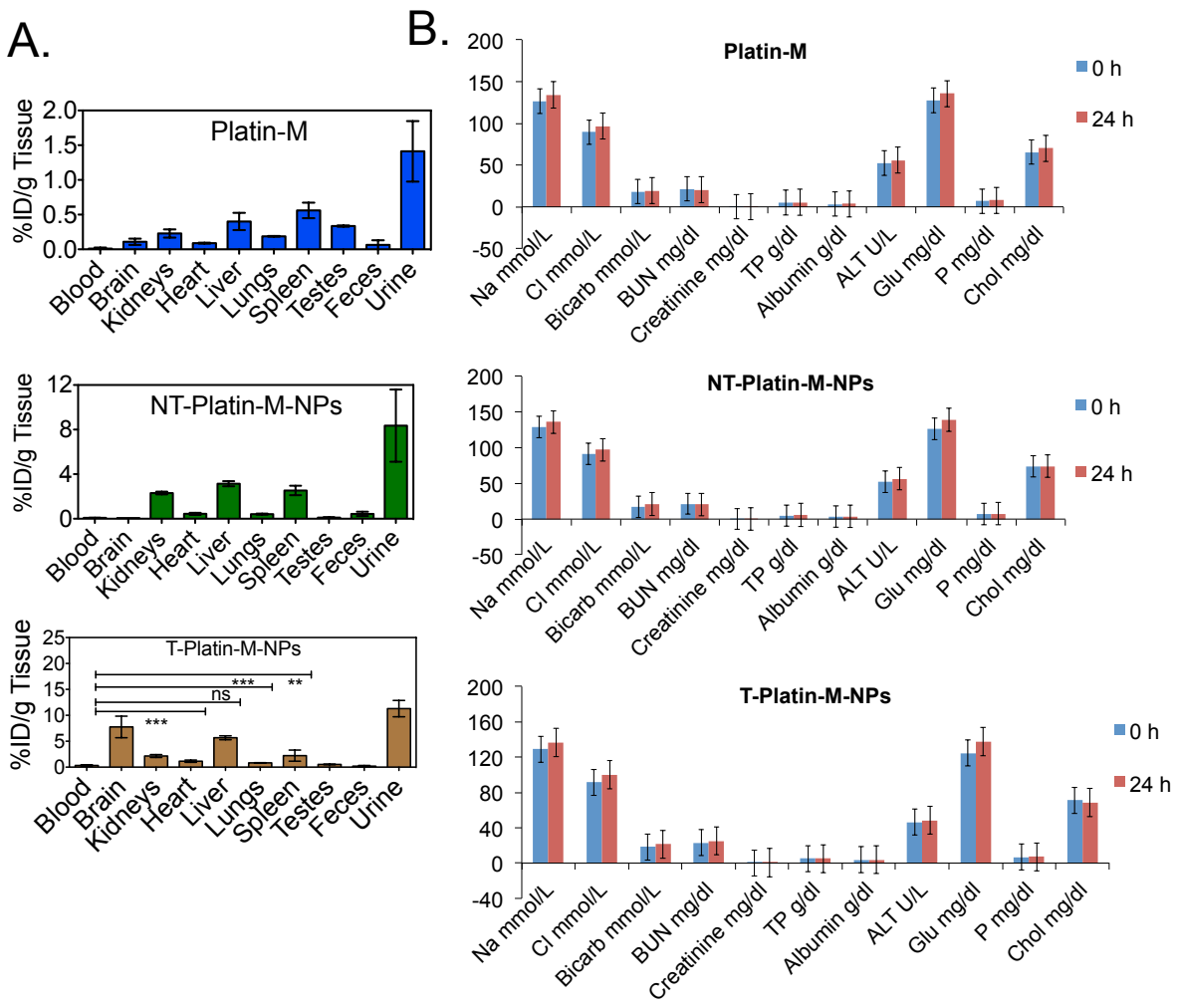


Fig. S27. Effect of cisplatin, Platin-M, and its NPs in H9C2 cardiomyocytes by Mito-Stress assay. The cells were treated with Platin-M (10 μM), cisplatin (10 μM), T-Platin-M-NPs (10 μM with respect to Pt), NT-Platin-M-NPs (10 μM with respect to Pt) for 12 h at 37 °C in 5% CO_2 atmosphere.



Na: Total Sodium; Cl: Total Chloride; Bicarb: Total Bicarbonate
BUN: Blood urea nitrogen; TP: Total Protein; ALT: Alanine aminotransferase; Glu: Glucose; P: Phosphorous; Chol: Cholesterol

0 h: Samples predose; **24 h:** samples 24 h after dosing

Fig. S28. (A) A comparison of bioD and excretion profiles of Platin-M, NT-Platin-M-NPs, and T-Platin-M-NPs in male rats. ***, *P*<0.001; **, *P*=0.001-0.01; *, *P*=0.01-0.05; non significant (ns), *P*>0.05. (B) Clinical chemistry of plasma samples from the rats treated with Platin-M, NT-Platin-M-NPs, and T-Platin-M-NPs.

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