Supporting information for

A platinum-based fluorescent "turn on" sensor to decipher the reduction of platinum(IV) prodrugs

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CONTENTS

Experimental Section

Materials and instruments. All the reagents were used as received without further purification. Carboplatin was purchased from Shandong Boyuan Pharmaceutical Co., Ltd. Amicon® Ultra-15 Centrifugal Filter Units with 3 kDa, 10 kDa, 50 kDa, and 100 kDa molecular weight cut off (MWCO) (UFC5003BK, UFC901008, UFC905008, UFC910008) were purchased from Merck Millipore Ltd. BCA Protein Assay Kit (P0012) and GSH and GSSG Assay Kit (S0053) were purchased from Beyotime Biotechnology. ^{1}H, ^{13}C, ^{19}F, and ^{195}Pt-NMR spectra were obtained on a Bruker AVANCE III 400 MHz, or Bruker Ascend AVANCE III HD 600 MHz NMR spectrometer with a BBO probe. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) spectra were obtained with a micrOTOF-Q II mass spectrometer (Bruker Daltonics). ESI-MS tests were performed on an Agilent API-3200 Q-Trap mass spectrometry. CHN elemental analysis was carried out on an elemental analyzer (Elementar vario MICRO cube). High-performance liquid chromatography (HPLC) experiments were conducted on a Shimadzu Prominence HPLC system. A Phenomenex column (Gemini, 5 μm, C18, 110 Å, 250 × 4.6 mm) was used. Absorbance spectra were recorded on a UV-Vis spectrophotometer (Agilent 8485). Fluorescence spectra were recorded by a spectrofluorometer (Fluormax-4). Pt contents were measured by an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 8000 spectrometer) or an inductively coupled plasma mass spectrometer (ICP-MS; PE Nexion 2000).

*Synthesis of compounds **1** and **2**.* Compounds **1** and **2** were synthesized following the procedure reported previously.[1]

*Synthesis of complex **3**.* Compound **1** (100 mg, 0.26 mmol, 1 eq.) was dissolved in 4 mL dimethyl sulfoxide (DMSO) before compound **2** (105 mg, 0.26 mmol, 1 eq.) was added. The reaction

mixture was stirred at room temperature for 48 h. The solvent was removed by lyophilization. The crude product was purified by column chromatography (DCM : MeOH = 10 : 1) in darkness to get 119 mg red solid **3**. Yield 68%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.67 (s, 1H), 7.07 (d, J = 4.0 Hz, 1H), 6.40 (d, J = 4.0 Hz, 1H), 6.28 (s, 1H), 6.07 – 5.75 (m, 6H), 3.09 – 3.00 (m, 2H), 2.59 – 2.53 (m, 4H), 2.48 (d, J = 8.1 Hz, 2H), 2.45 (s, 3H), 2.25 (s, 3H), 1.77 (dt, J = 16.0, 8.0 Hz, 2H), 1.74 (d, J = 6.6 Hz, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ 178.8, 176.6, 158.8, 158.3, 143.8, 134.3, 133.0, 129.1, 125.3, 120.1, 117.0, 55.7, 34.7, 32.3, 30.9, 24.6, 15.9, 14.5, 11.0; ^{19}F-NMR (565 MHz, DMSO-d_6) δ -143.2 (q, J = 66.8, 33.1 Hz); ^{195}Pt-NMR (129 MHz, DMSO-d_6) δ 1749.7; HR-ESI-MS: m/z $[M + Na]^+$ calculated for $C_{20}H_{27}BF_2N_4NaO_7Pt$: 702.1486, found 702.1441; Anal. Calcd. for $C_{20}H_{27}BF_2N_4O_7Pt \cdot 1.5H_2O$: C 34.01, H 4.28, N 7.93; found C 33.96, H 4.27, N 7.88; UV/Vis (PBS with 1% DMF): λ_{max} (ε) = 504 nm (74100 ± 1300 $cm^{-1}M^{-1}$); Fluorescence (PBS with 1% DMF): λ_{ex} = 470 nm; λ_{em} (quantum yield) = 512 nm (0.15 ± 0.02).

Solubility in water. 5 mg of complex **3** and carboplatin were added into 200 μL milli-Q water. The mixtures were under ultrasound for 10 min and then centrifuged at 14,000 rpm for 5 min. The Pt concentrations of the supernatants were determined by an ICP-OES after digestion by HNO_3 and dilution by milli-Q water.

Partition coefficient (Log P). A shake-flask method was utilized to measure the log P values of complex **3** and carboplatin. Octanol and PBS buffer (1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 137.9 mM NaCl, 2.7 mM KCl, pH 7.4) used in this experiment were pre-saturated by shaking this biphasic mixture overnight. Complex **3** and carboplatin were dissolved in 0.3 mL PBS before 0.3 mL octanol was added. The mixtures were vigorously shaken at room temperature for 3 h. The two layers were separated by centrifugation at 5000 rpm for 2 min. The Pt concentrations of the

two layers were determined by an ICP-OES after digestion by HNO_3 and dilution by milli-Q water. The log P values were calculated as Log_{10}(Pt in octanol / Pt in water).

Photophysical properties. Complex **3** and the BODIPY ligand were dissolved in DMF and diluted by 3 mL PBS buffer to 10 μM in a cuvette. The final concentration of DMF is 1%. The absorbance of these two compounds was recorded by a UV-Vis spectrophotometer. The molar extinction coefficient (ε) of the compounds was calculated as the absorbance at λ_{max} over the molar concentration of the compounds and the length of the cuvette. The fluorescence spectra of these samples were recorded by a spectrofluorometer with excitation at 470 nm. The BODIPY ligand with a known quantum yield (QY) of 0.81 was used as a standard to calculate the fluorescence quantum yield of complex **3**.[2] QY of complex **3** = 0.81 * [(Area under emission curve / Absorbance at 470 nm) of complex **3** / (Area under emission curve / Absorbance at 470 nm) of the BODIPY ligand].

Stability and reduction. Complex **3** was dissolved in PBS buffer (pH 7.4) with 1% DMF and injected into HPLC to check the purity. To test the stability and reduction properties, 10 μM of complex **3** was dissolved in 1 mL of the PBS buffer without or with 2 mM GSH or 1 mM sodium ascorbate and incubated in the dark at 37 ℃ or irradiated under a white light (4 mW/cm^2). 100 μL of the sample was injected into HPLC for analysis at different time points. 10 μM of complex **3** was also incubated in 1 mL of Roswell Park Memorial Institute (RPMI) 1640 full culture medium with 10% fetal bovine serum (FBS), 1% *L*-glutamine, and 100 units of penicillin and streptomycin in the dark at 37 ℃. The proteins in the samples were removed by adding 4 volumes of methanol and centrifugation before the samples were injected into HPLC. Phase A is H_2O with 0.1% HCOOH, and phase B is acetonitrile (ACN) with 0.1% HCOOH. The absorbance wavelengths were set at 254 nm and 504 nm. Flow rate was 1 mL/min. HPLC programs were set as follows:

gradient increased from 10% to 50% phase B in 3 min; gradient increased from 50% to 63% phase B in 13 min; gradient decreased from 63% to 10% phase B in 1 min; and isocratic 10% phase B until 20 min.

*Release of carboplatin from complex **3** by NMR.* Complex **3** was dissolved in a mixture of DMSO-d_6 (90.9%) and D_2O (9.1%) at a concentration of 10 mM. 100 mM sodium ascorbate was added into the NMR tube. The reduction of complex **3** was monitored by ^{1}H-NMR at time 0, 1, 3, and 5 h. Carboplatin and the BODIPY ligand were used as the controls for NMR tests. After the NMR tests, the sample was further analyzed by an ESI-MS.

Reduction and fluorescence change in PBS. 10 μM of complex **3** was dissolved in PBS buffer (pH 7.4) containing 1 mM sodium ascorbate and incubated at 37 ℃ in the dark. The emission of the sample was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. At the same time points, 100 μL of the sample was injected into HPLC to analyze the reduction of complex **3**. The percentage of intact Pt(IV) sensor at time 0 was determined to be 96.1% by HPLC (Figure 1a). The increased fold of fluorescence intensity at 512 nm and the percentage of remained complex **3** in HPLC at 504 nm were quantified and plotted. The experiment was repeated for three times.

Cell culture conditions. A2780 (human ovarian carcinoma), cisplatin-resistant A2780cisR, and 4T1 (mouse breast carcinoma) cells were cultured in RPMI 1640 medium with 10% FBS, 1% *L*-glutamine, and 100 units of penicillin and streptomycin. A549 (human lung adenocarcinoma), cisplatin-resistant A549cisR, MCF-7 (human breast carcinoma), SKOV3 (human ovarian carcinoma), and MDA-MB-231 (human breast carcinoma) cells were cultured in Dulbecco's

modified Eagle's medium (DMEM) containing 10% FBS and 100 units of penicillin and streptomycin. All cells were incubated at 37 ℃ with 5% CO_2.

Generation of standard curve in A2780 cell extract. The cell culture medium of A2780 cells in 10 cm dishes was removed. The cells were washed with 3 mL PBS buffer. 2 mL trypsin (25300-054, Gibco) was added into the dishes. The cells in the dishes were then incubated at 37 ℃ for 3 min. 4 mL fresh culture medium was added into the dishes. The cells were transferred to 15 mL tubes and centrifuged at 1,000 rpm for 3 min. The supernatants were discarded, and the cells were washed with PBS buffer for three times and then resuspended in 0.9 mL H_2O. The cells were transferred into 2 mL tubes and lysed by vortex for 1 min and ultrasonication for 12 × 5 s with intervals of 5 s on ice. After centrifugation at 12,000 rpm for 10 min at 4 ℃, the supernatant was collected. The protein concentration of the supernatant was determined by a BCA assay (P0012, Beyotime). The supernatant was diluted by water and 10X PBS to a protein concentration of 0.94 mg/mL. The pH of the diluted supernatant was determined to be 7.4. 10 μM of complex **3** was added into the diluted supernatant. The mixtures were incubated at 37 ℃ in the dark. The emission of these samples was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. At the same time points, the proteins in 50 μL of the sample were precipitated by 200 μL methanol and removed by centrifugation at 10,000 rpm for 3 min. 100 μL of the supernatant was injected into HPLC to analyze the reduction of complex **3**. The percentage of intact Pt(IV) sensor at time 0 was determined to be 97.9% by HPLC (Figure S26a). The increased fold of fluorescence intensity at 512 nm and the percentage of remained complex **3** in HPLC at 504 nm were quantified and plotted. The experiment was repeated for three times.

Fluorescence change in A2780, GSH-depleted A2780, and A2780cisR cell extracts. A2780 cells were treated with 250 μM BSO for 24 h to yield GSH-depleted A2780 cells. The A2780, GSH-

depleted A2780, and A2780cisR cell extracts were prepared as described above. 10 μM of complex **3** was added into the cell extracts. The protein concentration is 0.94 mg/mL. The mixtures were incubated at 37 ℃ in the dark. The emission of these samples was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. The increased folds of fluorescence intensity (y) were calculated in comparison to the fluorescence intensity at 0 h and fitted into the standard curve y = - 0.03652 x + 4.576 to quantify the percentages of remained sensor (x) at each time point. The experiment was repeated for four times.

Fluorescence change in HMW and LMW fractions of cell extracts. The A2780 and A2780cisR cell extracts were prepared as described above. The cell extracts were transferred to Centrifugal Filter Units (Millipore UFC5003BK) with molecular weight cut-off (MWCO) of 3 kDa and centrifuged at 14,000 g for 30 min at 4 ℃ to yield the HMW (> 3 kDa) and LMW (< 3 kDa) fractions. The two fractions were diluted by water and 10X PBS to the same volume. The protein and GSH concentrations of these samples were determined by a BCA Protein Assay Kit (Beyotime P0011) and a GSH and GSSG Assay Kit (Beyotime S0053), respectively. 10 μM of complex **3** was incubated in the HMW and LMW fractions of cell extracts at 37 ℃. The emission of these samples was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. The increased folds of fluorescence intensity were calculated and fitted into the standard curve y = - 0.03652 x + 4.576 to quantify the percentages of remained intact sensor at each time point. The experiment was repeated for four times.

Fluorescence change in the extracts of a panel of cancer cells. The extracts of A2780, A2780cisR, A549, A549cisR, MCF-7, MDA-MB-231, SKOV3, and 4T1 cells were prepared as described above. The protein concentrations of these cell extracts were determined by a BCA Protein Assay Kit (Beyotime P0011). Around 10 μM of complex **3** was incubated with these cell

extracts at 37 ℃ in the dark. The ratio of protein concentration to the concentration of complex **3** was kept at 138 times. The emission of these samples was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. The increased folds of fluorescence intensity were calculated and fitted into the standard curve y = - 0.03652 x + 4.576 to quantify the percentages of remained intact sensor at each time point. The experiment was repeated for three times.

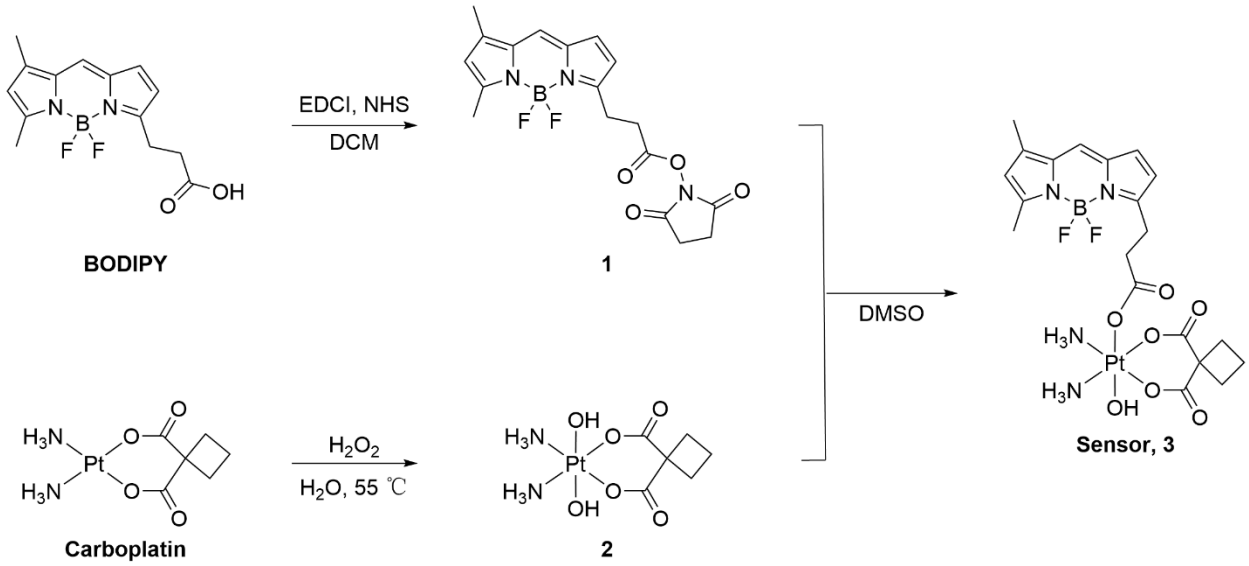
Fluorescence change in < 10 kDa, < 50 kDa, and < 100 kDa fractions of A2780 cell extract. The extract of A2780 cells was transferred to Centrifugal Filter Units (Millipore UFC901008, UFC905008, UFC910008) with MWCO of 10 kDa, 50 kDa, and 100 kDa, respectively, and centrifuged at 3,900 g for 30 min at 4 ℃ to yield the < 10 kDa, < 50 kDa, and < 100 kDa fractions of cell extract. These fractions of cell extract were concentrated by a lyophilizer. The protein concentrations of these concentrated samples were determined by a BCA Protein Assay Kit (Beyotime P0011) and adjusted to the same by water and 10X PBS buffer. 10 μM of complex **3** was incubated in each fraction of cell extract at 37 ℃. The protein concentration is 0.24 mg/mL. 10 μM of complex **3** was incubated in PBS buffer (pH 7.4) without or with 1 mM sodium ascorbate as the controls. The emission of these samples at 512 nm was monitored by a spectrofluorometer at different time points. The excitation wavelength is 470 nm. The increased folds of fluorescence intensity were calculated. The remained intact complex **3** was calculated based on the standard curve y = - 0.04545 x + 5.370. The low concentration (0.24 mg/mL) of proteins was found to not affect the fluorescence intensity of the sensor. The experiment was repeated for three times.

Protein separation efficiency of Centrifugal Filter Units by SDS-PAGE. The protein separation efficiency of the Centrifugal Filter Units was examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). 40 μL of different fractions of cell extract was mixed with 10

μL of SDS-PAGE Sample Loading Buffer (5X, Beyotime P0015L) or 13 μL of 4X loading buffer (0.2 M Tris-HCl, 0.4 M DTT, 8% SDS, 32% Glycerol, pH 6.8). 0.1 or 0.2 mg/mL of bovine serum albumin (BSA, 67 kDa) was set as a control. The samples were heated at 90 ℃ for 10 min. 40 μL of samples together with 6 μL of markers (Beyotime P0076) was loaded on a 6%, 10%, or 20% SDS-PAGE gel. The gels were under electrophoresis at 88 V for 100 ~ 120 min. The gels were stained by the Coomassie Brilliant Blue (Beyotime P0017) for 3 h and washed by water under shaking overnight. The gels were then photographed by a chemiDoc^{TM} Touch Imagine System.

Whole-cell accumulation. A2780, A2780cisR, A549, A549cisR, MCF-7, MDA-MB-231, SKOV3, and 4T1 cells were seeded in 6-well plates and incubated at 37 ℃ until the cell confluence reached 60%. The cells were treated with 10 μM complex **3** for 24 h. Afterward, cells were washed with ice-cold PBS for three times and harvested by trypsinization. The suspensions were spun at 1,000 g for 3 min, and the cell pellets were washed by PBS for three times. After counting the cell numbers, cells were digested by HNO_3 overnight. The lysates were diluted by milli-Q water to a final volume of 1 mL, and the Pt concentrations were measured by an ICP-MS.

Cytotoxicity. Cell viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A2780, A2780cisR, A549, A549cisR, MCF-7, MDA-MB-231, SKOV3, and 4T1 cells were seeded in 96-well plates at a density of 2,000 cells per well. After 48 h, complex **3** at different concentrations were added. 72 h later, media were removed and 200 μL MTT (1 mg/mL in serum-free media) solution was added. Cells were cultured for another 2 h at 37 ℃. MTT solution was removed carefully. Then 200 μL DMSO was added to dissolve the crystals. A microplate reader (Biotek Powerwave XS) was used to read the absorbances at 570 nm and 630 nm. The inhibition rate of the sensor at 100 μM was used to represent the cytotoxicity.



Scheme S1. Synthesis of the platinum(IV)-based sensor **3**. Reagents: EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysuccinimide; DCM, dichloromethane; DMSO, dimethyl sulfoxide.

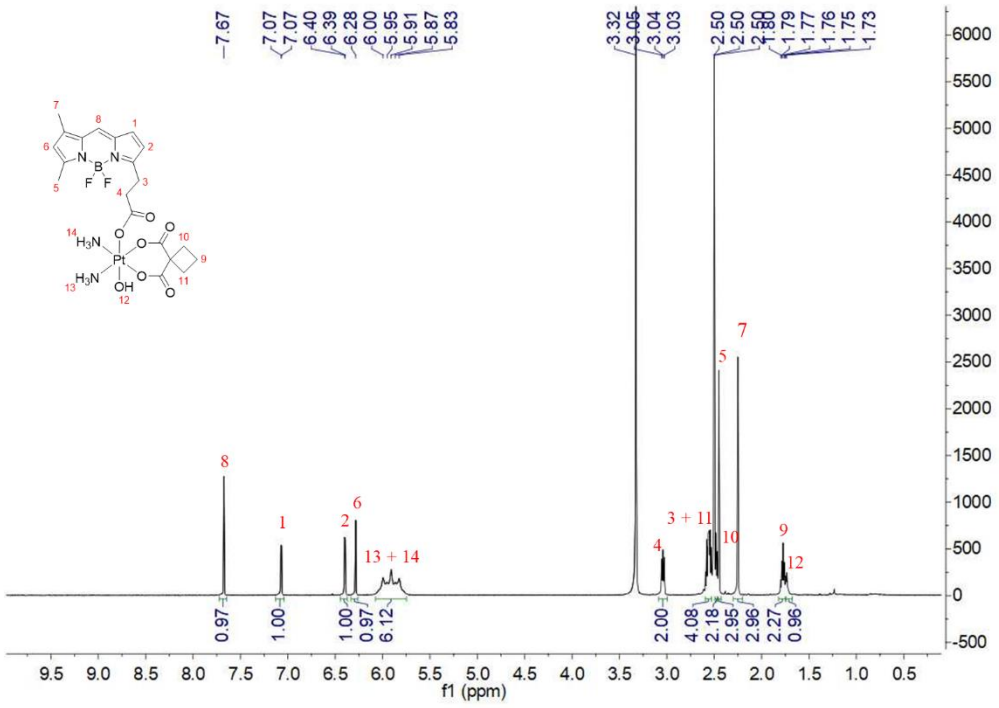


Figure S1. ^{1}H-NMR spectrum of complex **3** in DMSO-d_6.

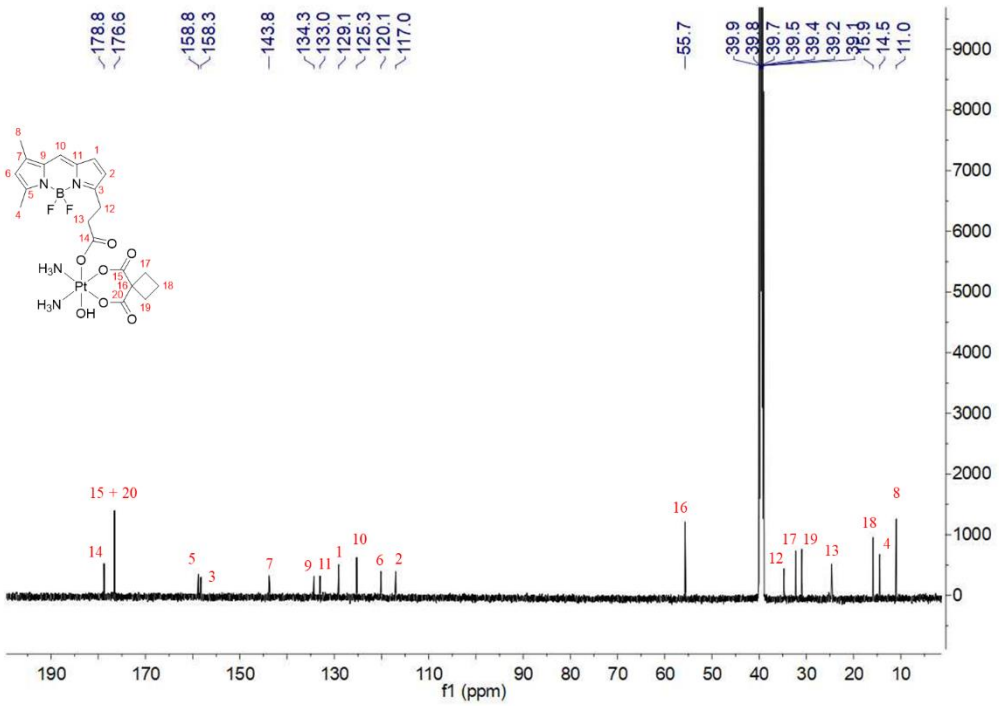


Figure S2. ^{13}C-NMR spectrum of complex **3** in DMSO-d_6.

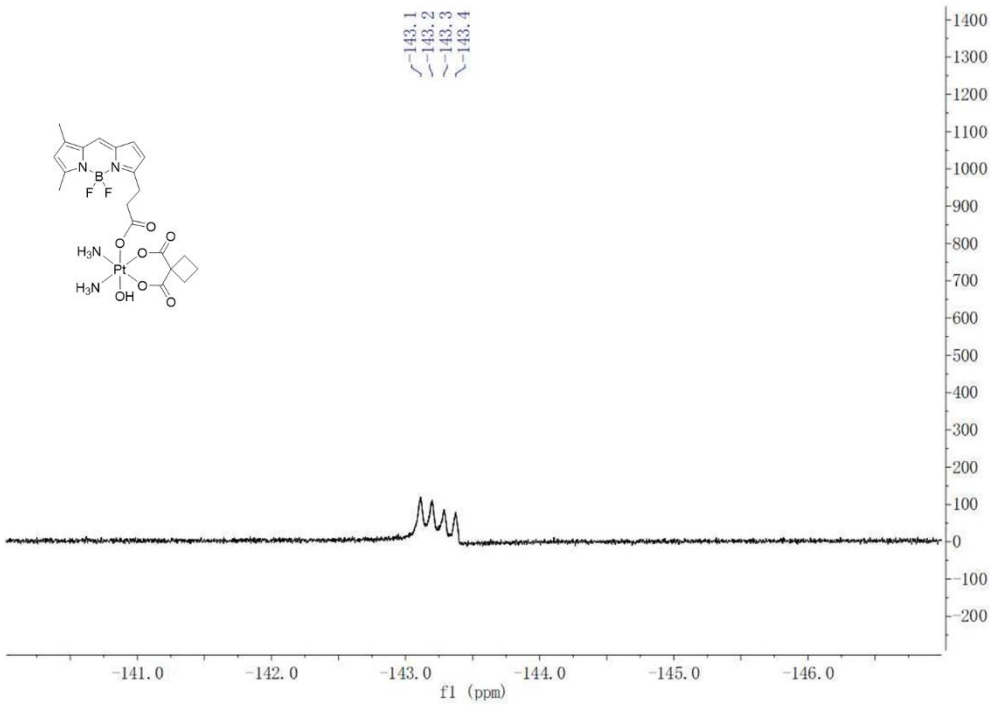


Figure S3. ^{19}F-NMR spectrum of complex **3** in DMSO-d_6.

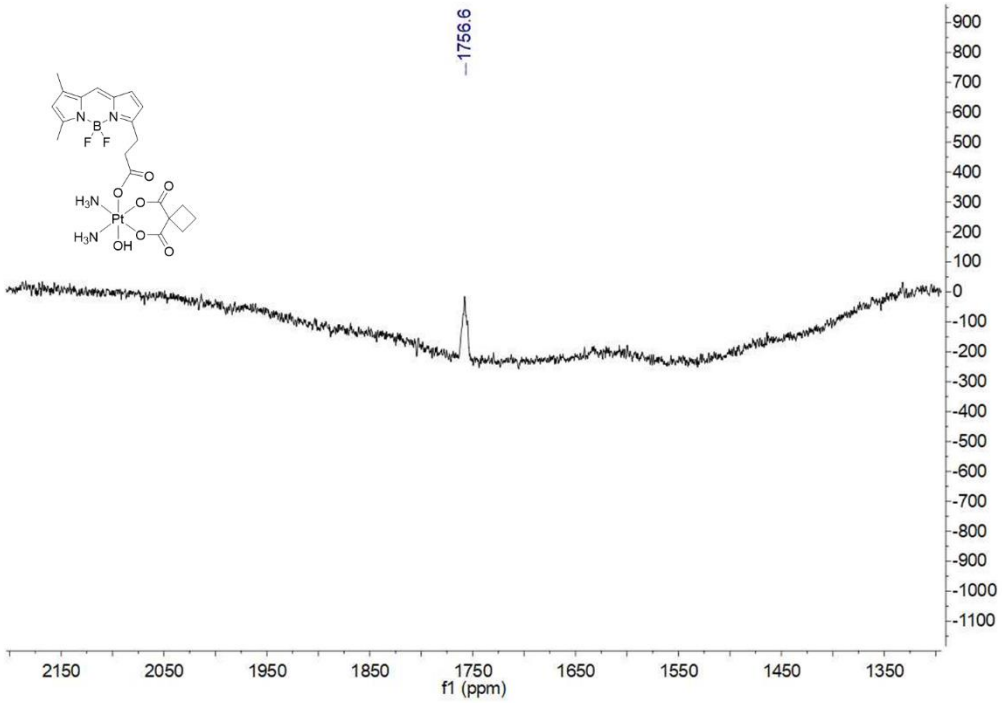


Figure S4. ^{195}Pt-NMR spectrum of complex **3** in DMSO-d_6.

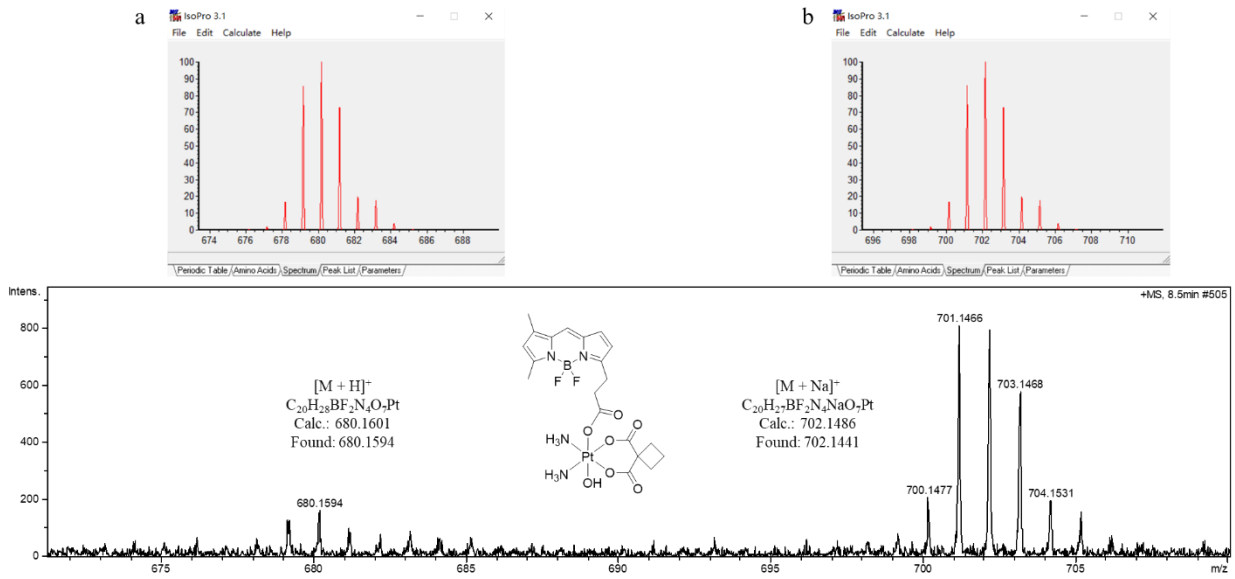


Figure S5. HR-ESI-MS spectrum of complex **3**, positive mode in methanol. Inserted (a) and (b) are the estimated $[M + H]^+$ and $[M + Na]^+$ spectrum of complex **3** by software IsoPro 3.1, respectively.

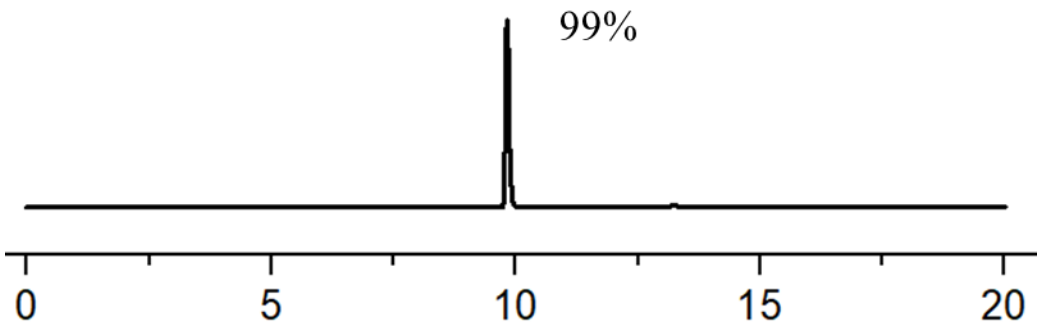


Figure S6. Purity of complex **3** by HPLC. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

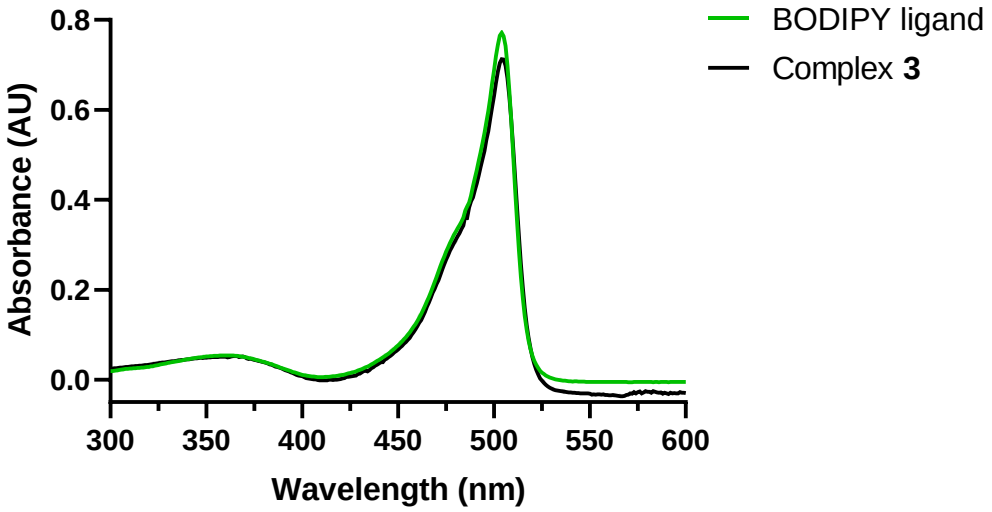


Figure S7. Absorbance of complex **3** and the BODIPY ligand recorded by a UV-Vis spectrophotometer. 10 μM compounds were dissolved in PBS buffer (pH 7.4) with 1% DMF.

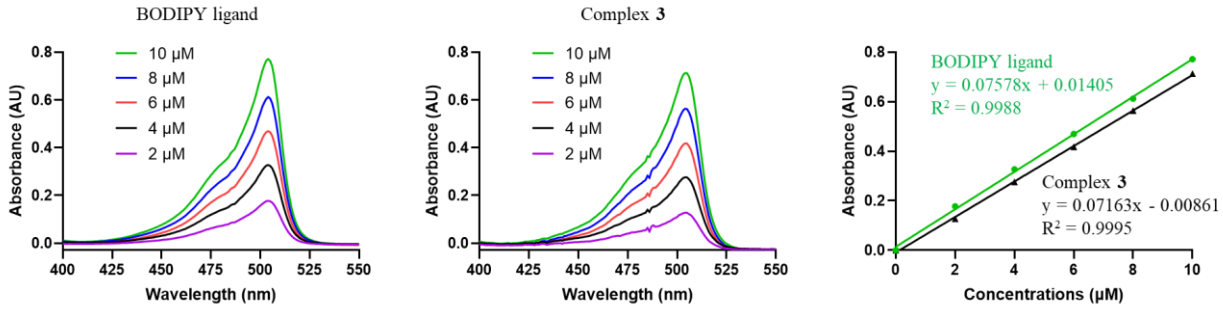


Figure S8. Molar extinction coefficient of complex **3** and the BODIPY ligand. 10, 8, 6, 4, and 2 μM of compounds were dissolved in PBS buffer (pH 7.4) with 1% DMF. The absorbances were recorded by a UV-Vis spectrophotometer. The maximum absorbances at each concentration were plotted with the concentrations of compounds.

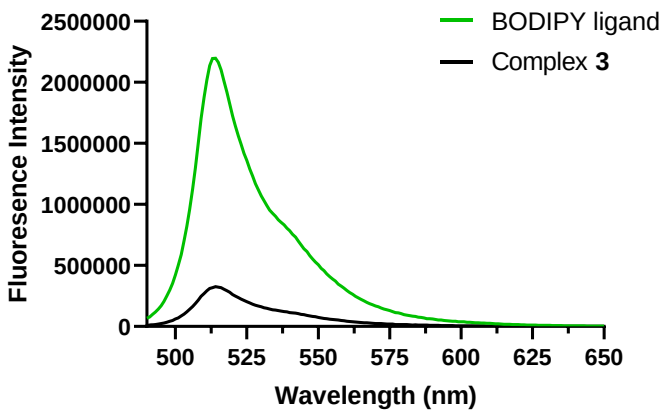


Figure S9. Emission of 10 μM complex **3** and the BODIPY ligand in PBS buffer (pH 7.4) with 1% DMF. The excitation wavelength is 470 nm.

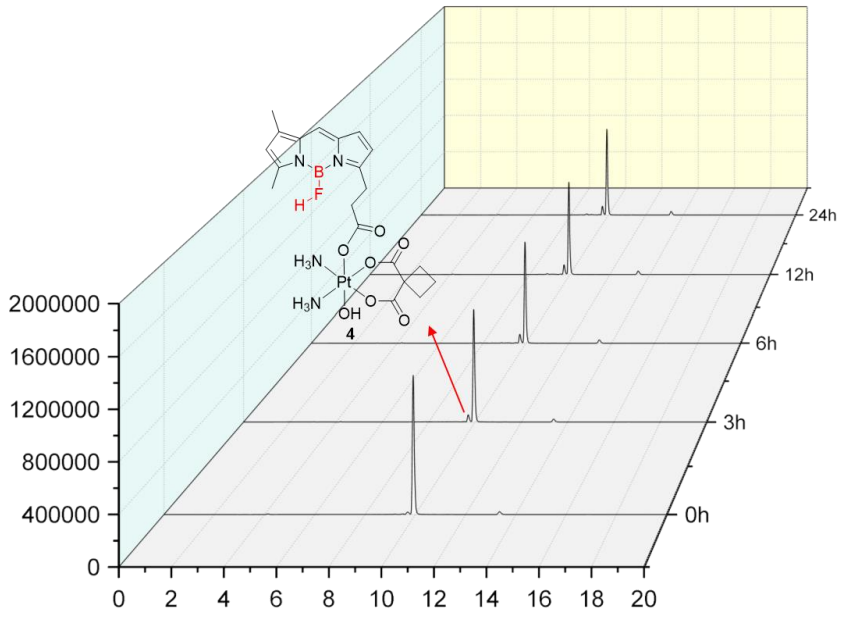


Figure S10. Stability of complex **3** in PBS buffer (pH 7.4) at 37 °C. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm. The red arrow indicates the newly formed product **4** that arises from the slight instability of the BODIPY ligand.

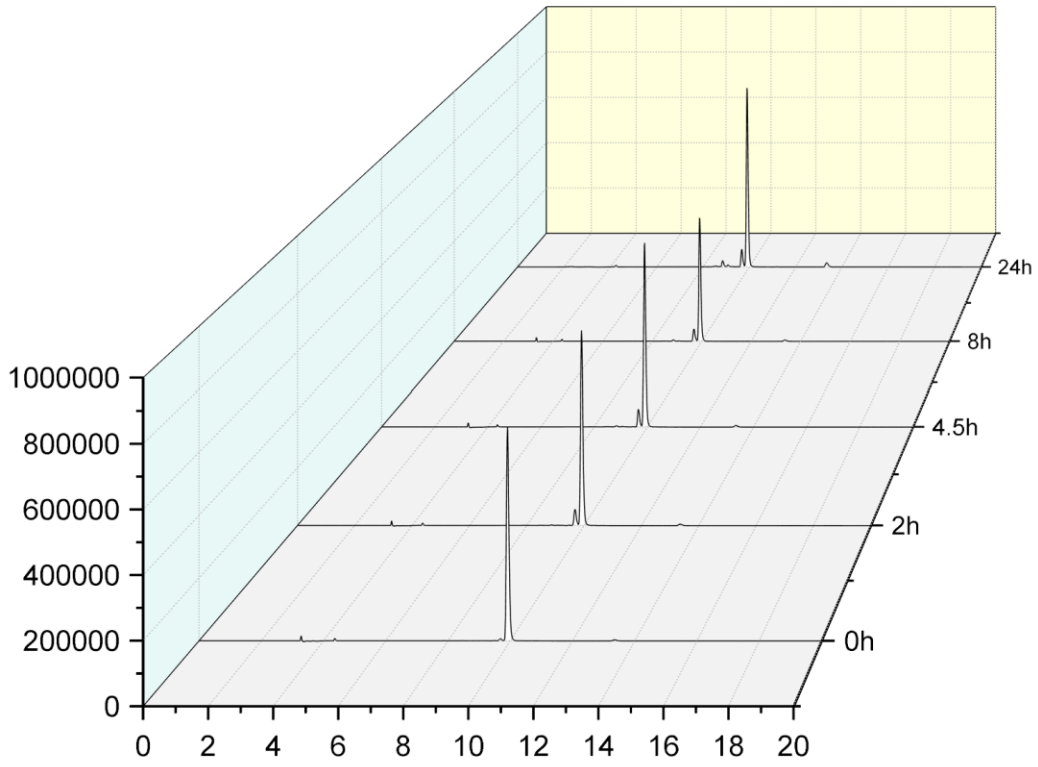


Figure S11. Stability of complex **3** in RPMI 1640 medium (pH 7.4) with 10% FBS, 1% L-glutamine, and 100 units of penicillin and streptomycin at 37 ℃. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

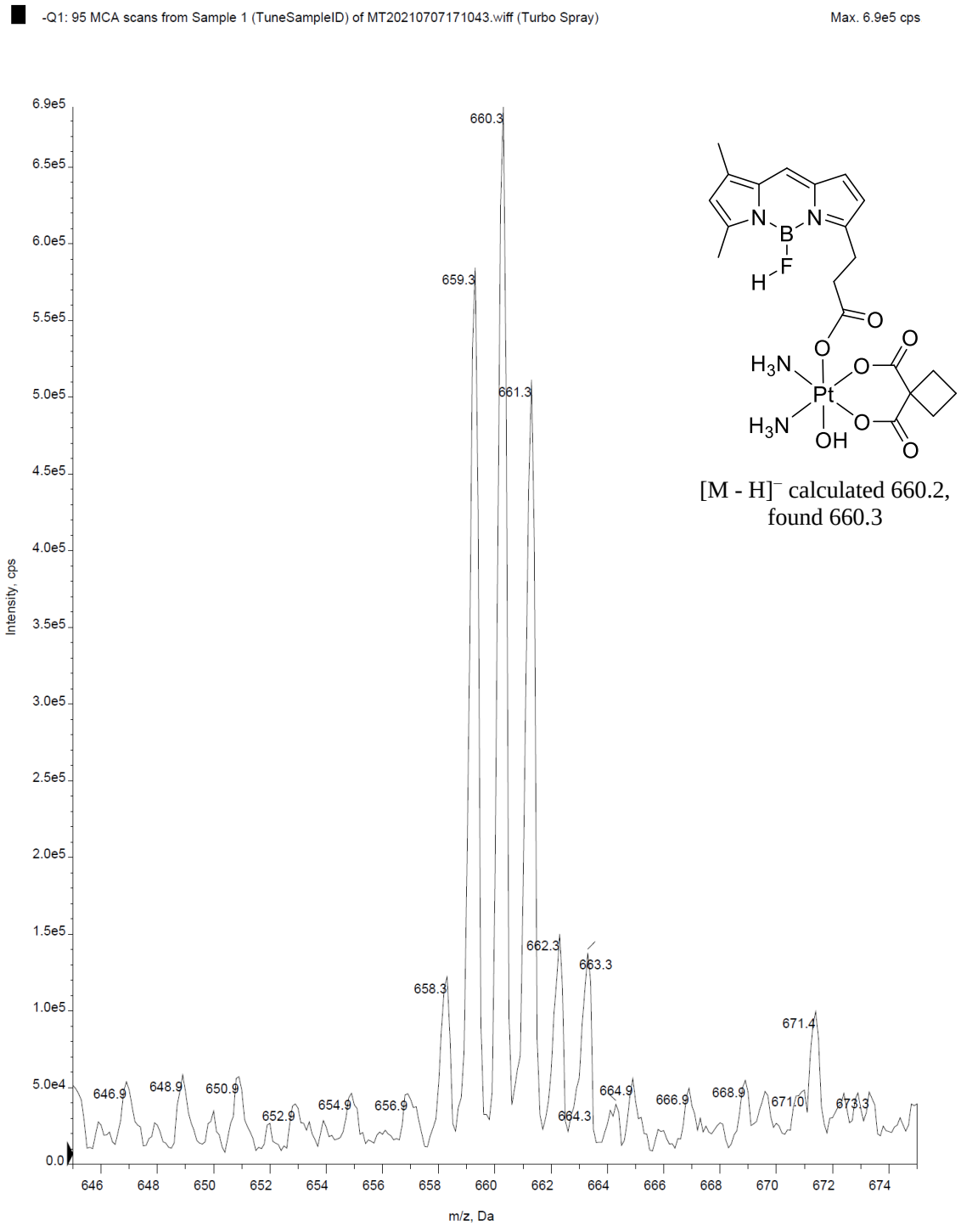


Figure S12. ESI-MS of the newly formed product **4** after the incubation of complex **3** in PBS buffer (pH 7.4) at 37 ℃ for 24 h.

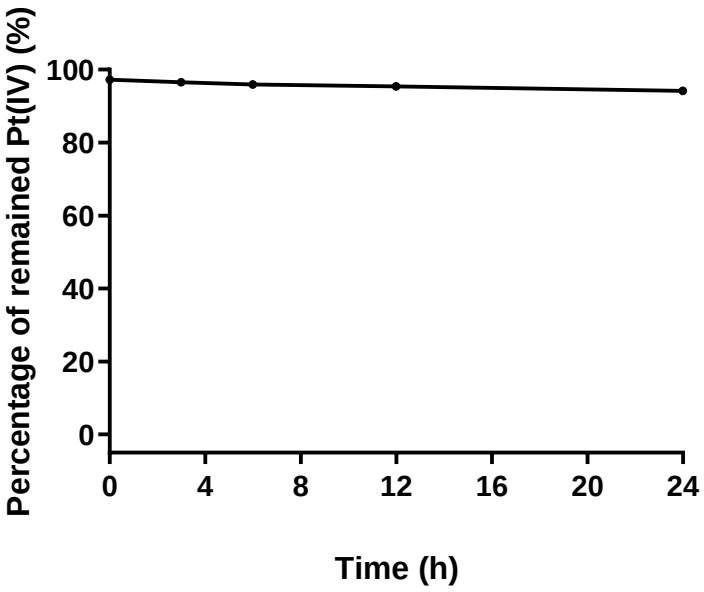


Figure S13. Percentage of remained Pt(IV) complexes from the HPLC analysis as shown in Figure S10. The Pt(IV) complexes include complex **3** and the BODIPY dissociation product **4**.

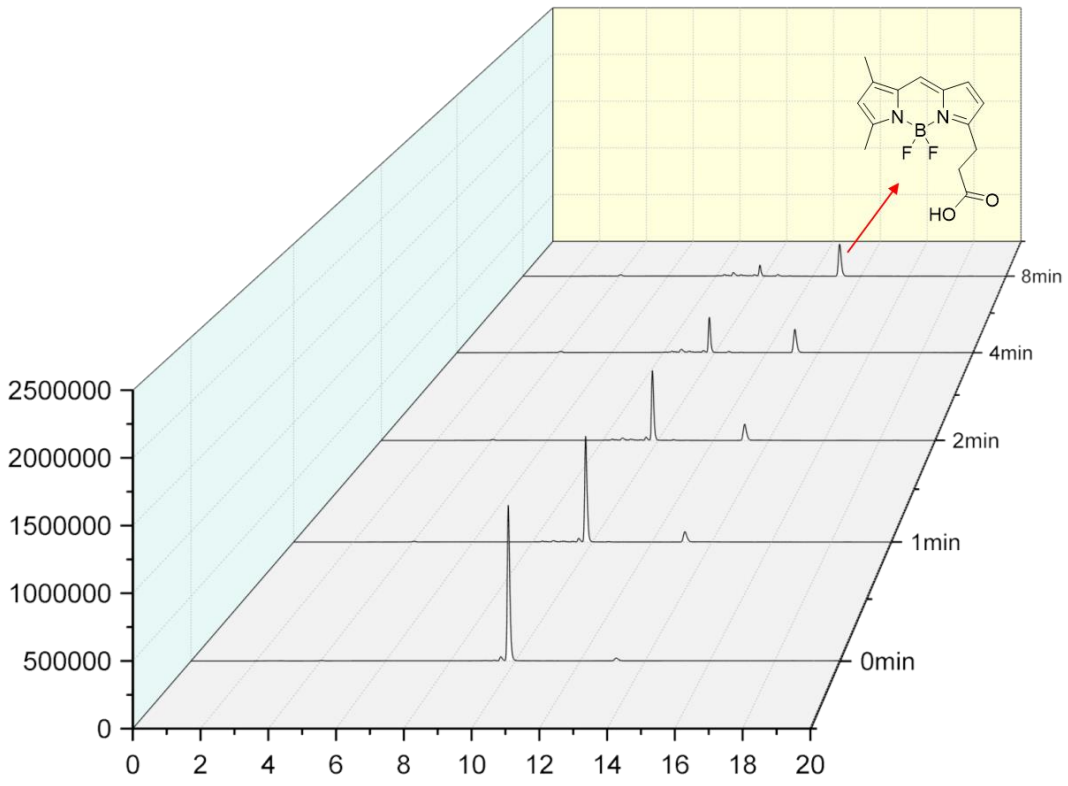


Figure S14. Stability of complex **3** under irradiation by white light (4 mW/cm^2) in PBS buffer (pH 7.4). HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

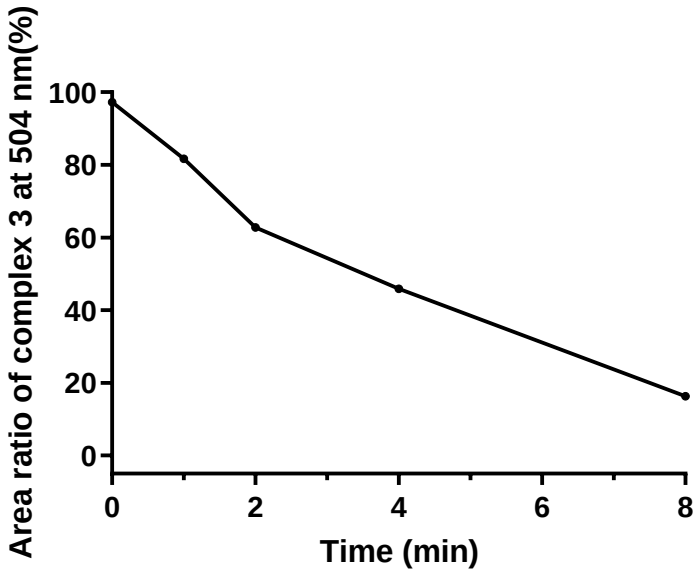


Figure S15. HPLC result of 10 μM complex **3** under white light (4 mW/cm^2) in PBS buffer (pH = 7.4).

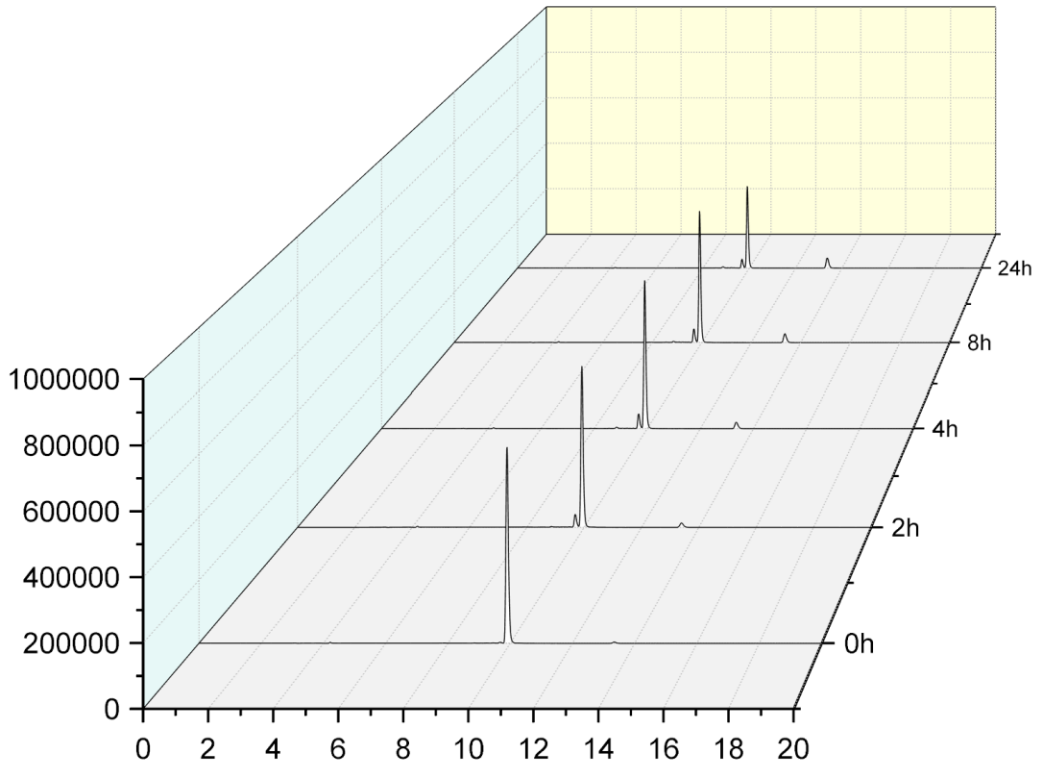


Figure S16. Reduction of complex **3** in PBS buffer (pH 7.4) with 2 mM GSH at 37 ℃. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

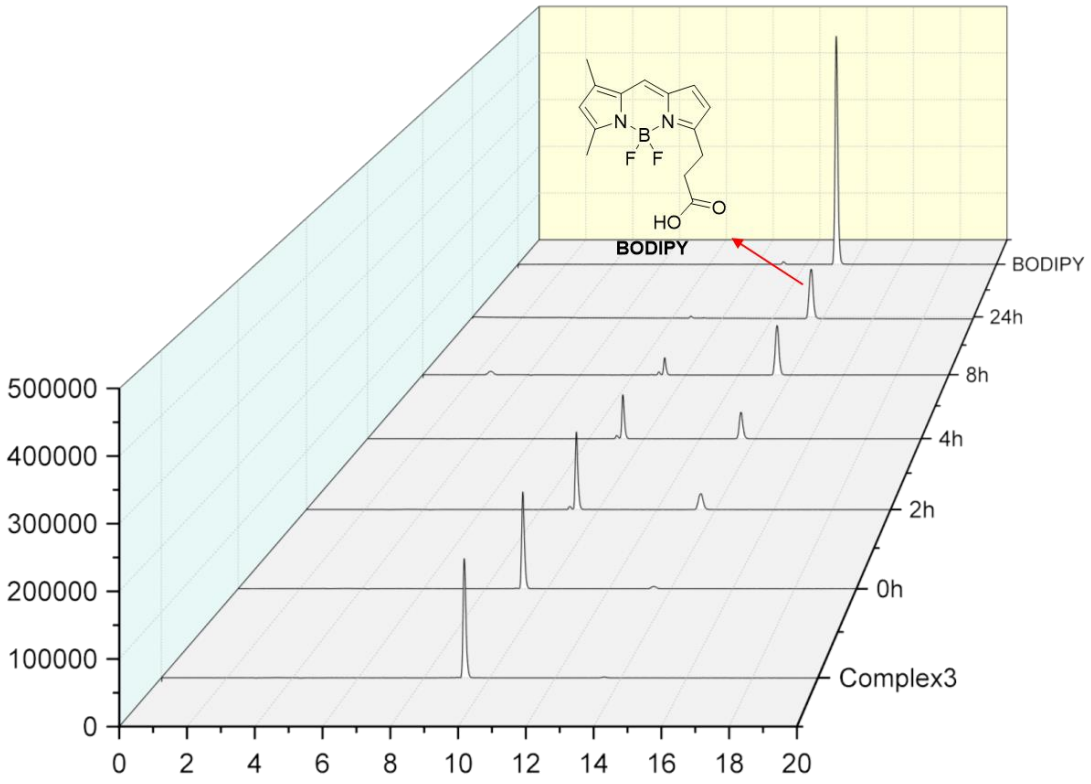


Figure S17. Reduction of complex **3** in PBS buffer (pH 7.4) with 1 mM sodium ascorbate at 37 ℃. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

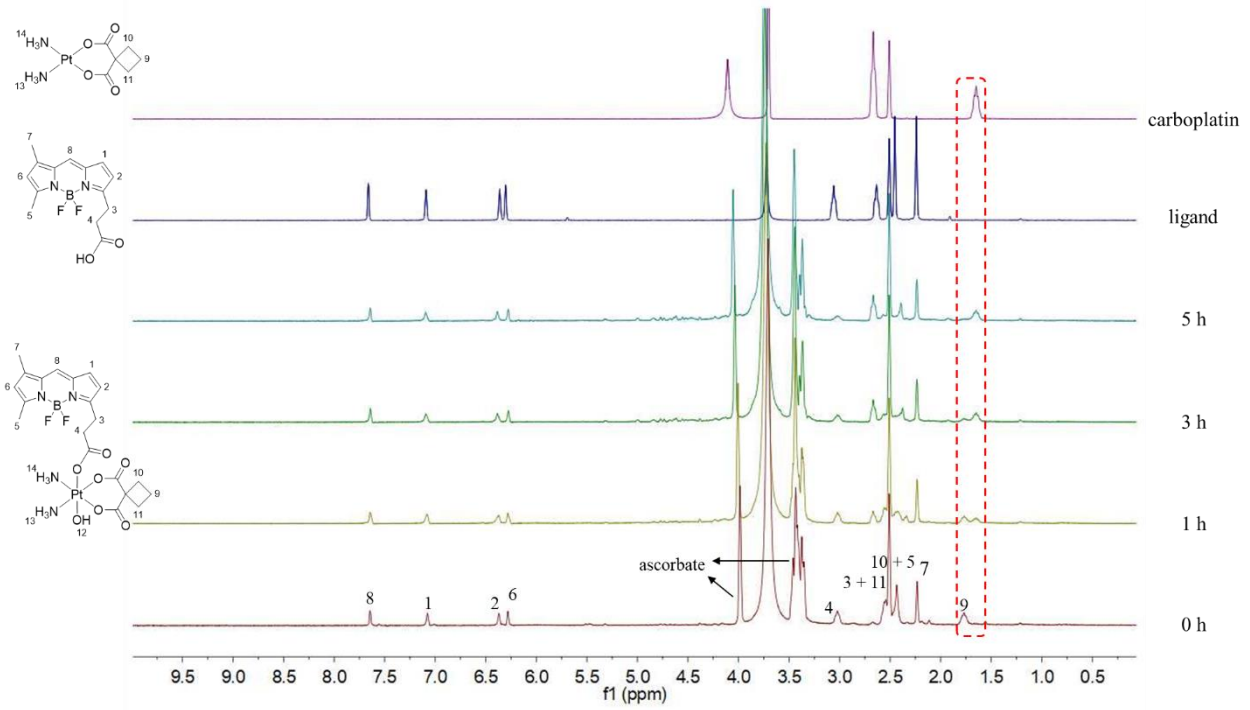


Figure S18. ^{1}H-NMR spectra of 10 mM complex **3** that was reduced by 100 mM sodium ascorbate in a mixture of DMSO-d_6 (90.9%) and D_2O (9.1%) at 37 ℃. Carboplatin and the BODIPY ligand were included as the controls.

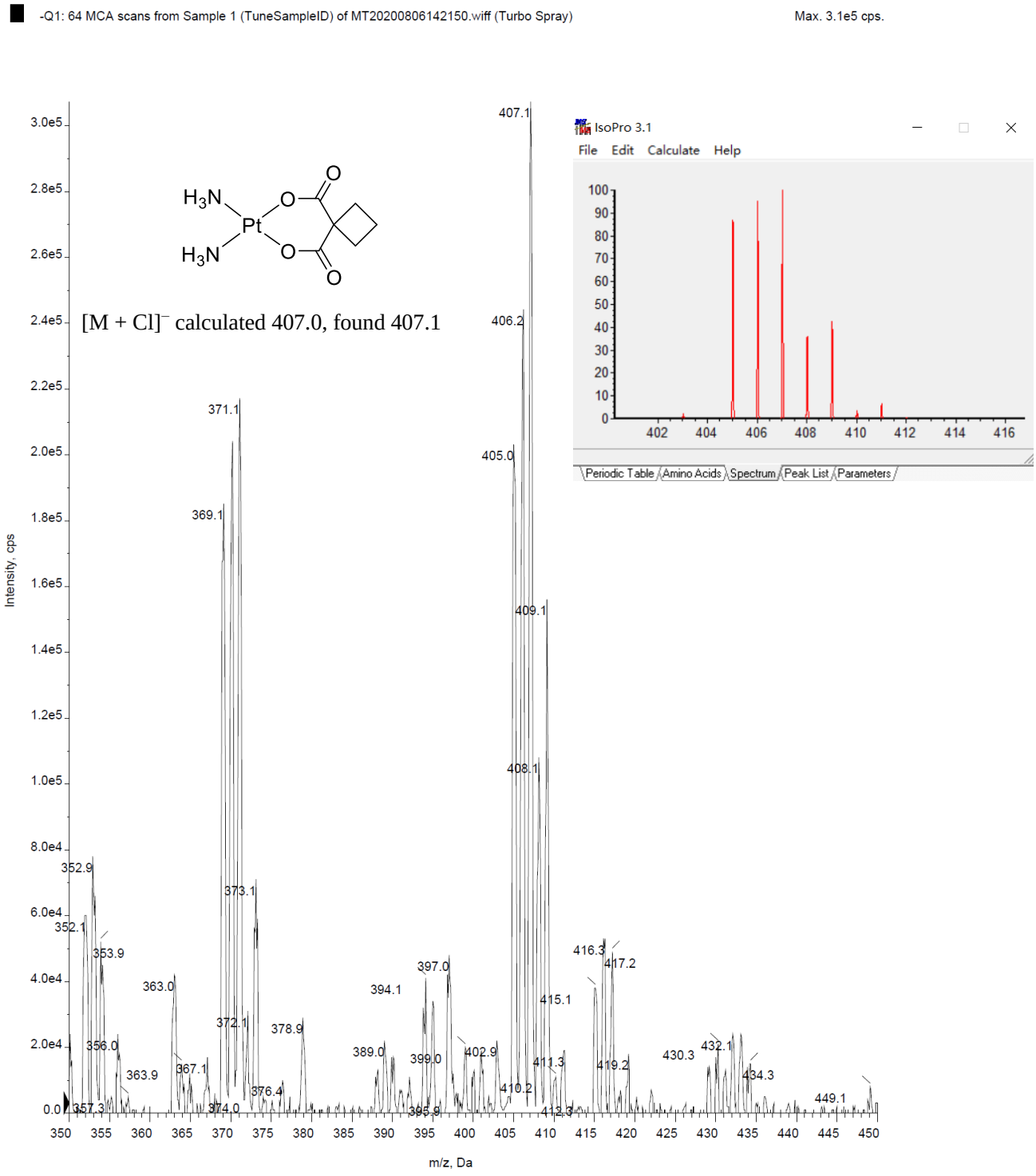


Figure S19. ESI-MS of carboplatin that is released from complex **3** that reduced by 100 mM sodium ascorbate in a mixture of DMSO-d_6 (90.9%) and D_2O (9.1%) at 37 °C. Inserted is the estimated $[M + Cl]^-$ spectrum of carboplatin by software IsoPro 3.1.

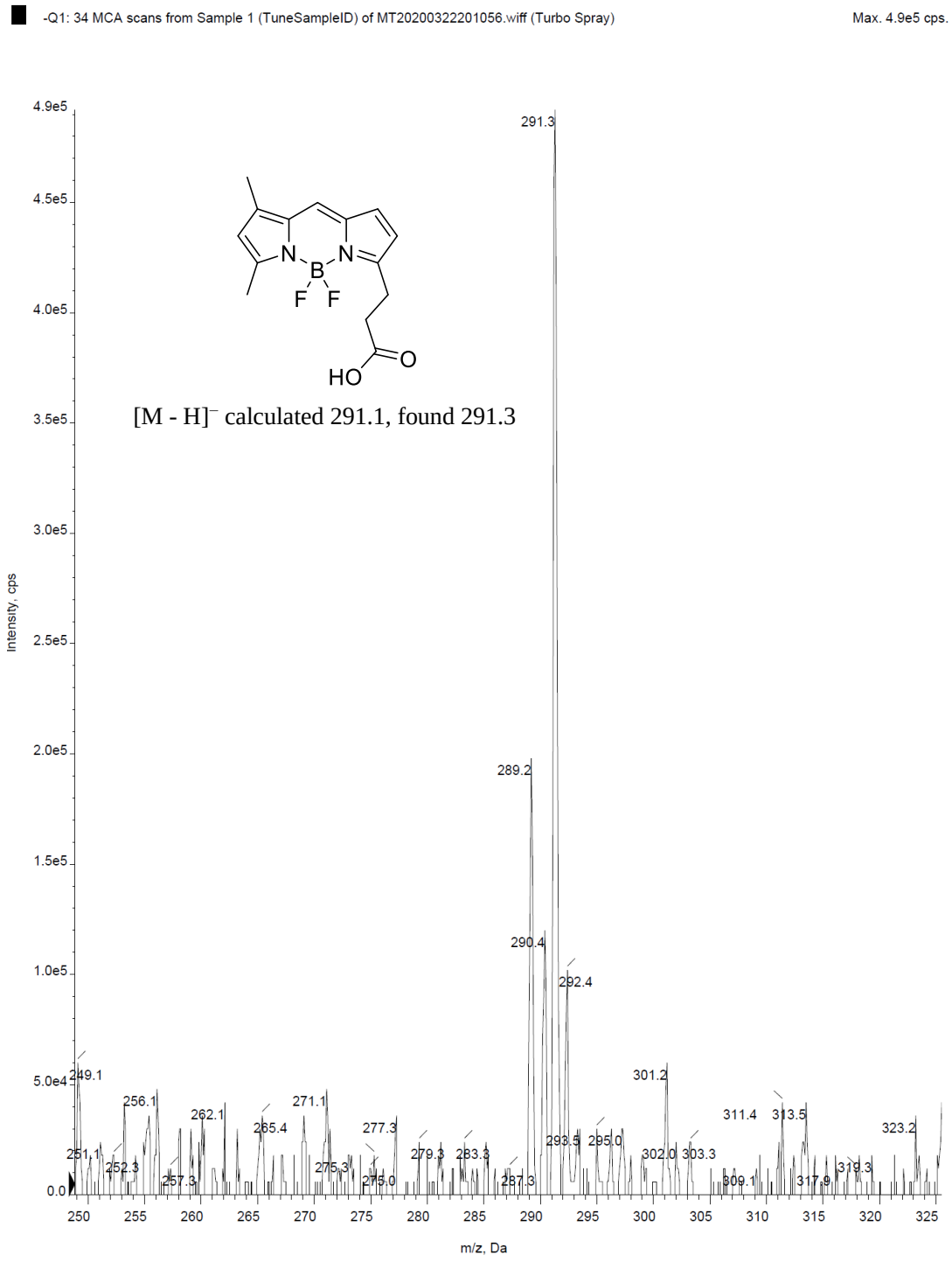


Figure S20. ESI-MS of the BODIPY ligand that is released from complex **3** after incubation in PBS buffer (pH 7.4) with 1 mM sodium ascorbate at 37 ℃ for 24 h.

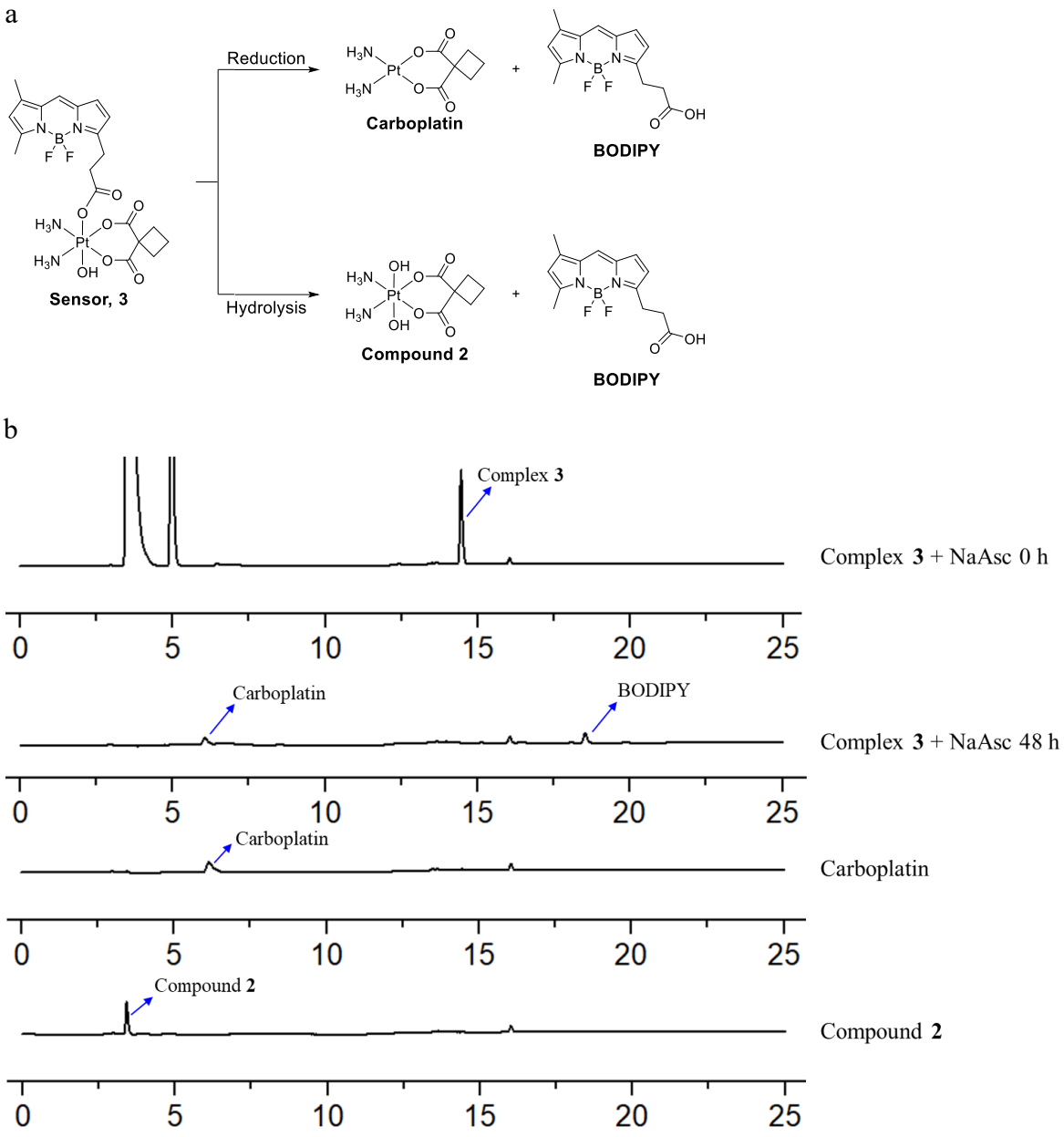


Figure S21 a. The reduction and hydrolysis pathways of the sensor. b. The release of BODIPY ligand was confirmed to arise from the reduction of the sensor by sodium ascorbate (NaAsc) to carboplatin rather than the hydrolysis of sensor to compound **2** by HPLC. 100 μM complex **3** was incubated in PBS buffer (pH 7.4) with 1 mM sodium ascorbate at 37 ℃ for 48 h. Carboplatin and compound **2** were used as controls. HPLC condition: 0 - 5 - 8 - 21 - 22 - 25 min, 1 - 1 - 50 - 63 - 1 - 1 % acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. Wavelength: 254 nm.

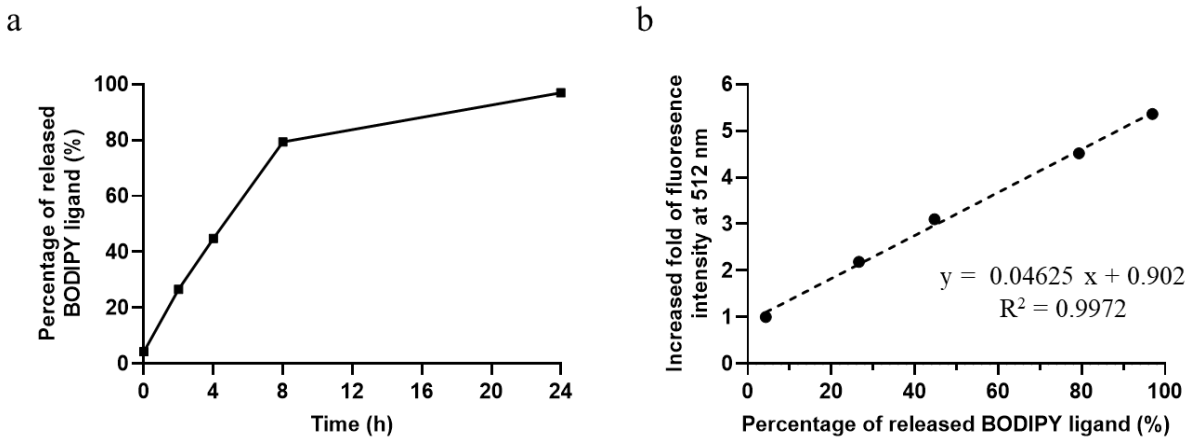


Figure S22. a. Percentage of released BODIPY ligand from complex **3** at 504 nm by HPLC. 10 μM complex **3** was incubated in PBS buffer (pH = 7.4) with 1 mM sodium ascorbate at 37 ℃. b. Plot of the increased fold of fluorescence intensity of complex **3** at 512 nm with the percentage of released BODIPY ligand at 504 nm in HPLC at the same time points. The data are presented as the mean from three independent assays.

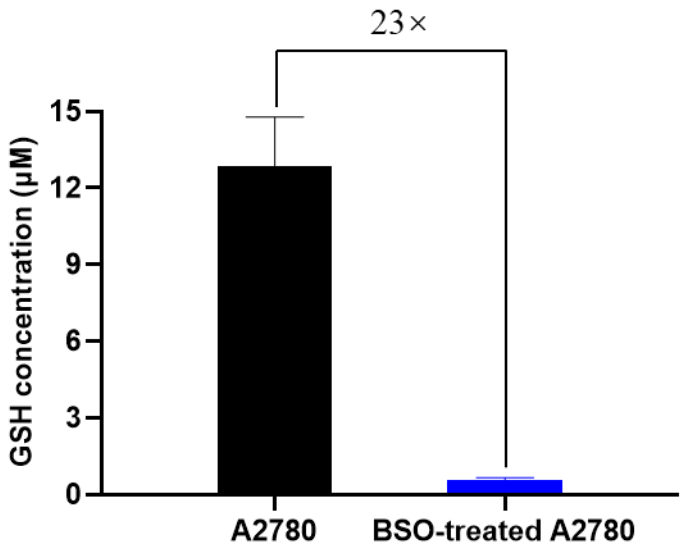


Figure S23. The concentration of GSH in the extracts of A2780 and BSO-treated A2780 cells. A2780 cells were treated with 250 μM buthionine sulfoximine (BSO) for 24 h to yield the BSO-treated A2780 cells. Mean ± SD, n = 3.

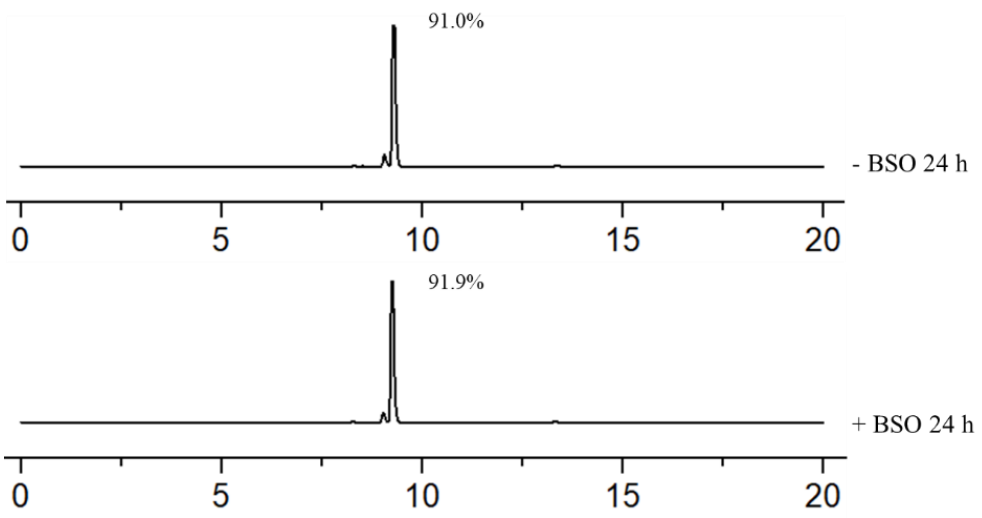


Figure S24. Stability of 50 μM complex **3** in PBS buffer (pH 7.4) without (-) or with (+) 250 μM buthionine sulfoximine (BSO) after 24 h. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

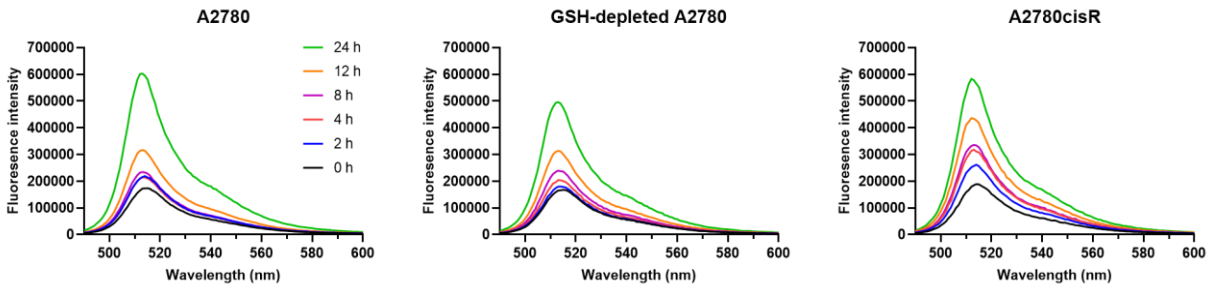


Figure S25. The time-dependent emission of 10 μM complex **3** in the extracts of A2780, GSH-depleted A2780, and A2780cisR cells containing 0.94 mg/mL proteins and 1% DMF at 37 ℃. The excitation wavelength is 470 nm.

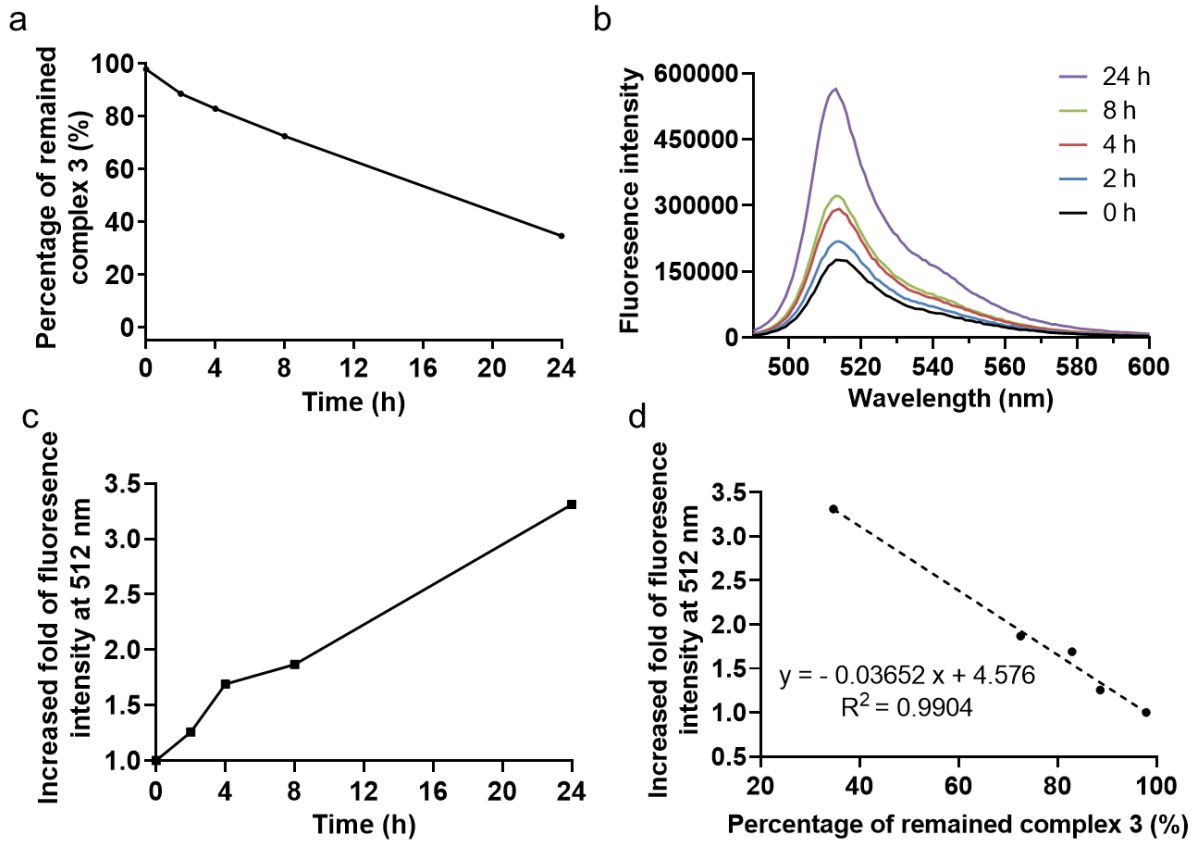


Figure S26. a. HPLC result of 10 μM complex **3** in A2780 cell extract with 0.94 mg/mL proteins and 1% DMF in the dark at 37 ℃. b. The time-dependent emission of 10 μM complex **3** in A2780 cell extract containing 0.94 mg/mL proteins and 1% DMF at 37 ℃. c. The increased fold of fluorescence intensity of complex **3** after incubation in A2780 cell extract with 0.94 mg/mL proteins for different time points compared with the fluorescence intensity at time 0 h at 512 nm. d. Plot of the increased fold of fluorescence intensity of complex **3** at 512 nm with the percentage of remained complex **3** at the same time points. The data are presented as the mean from three independent assays.

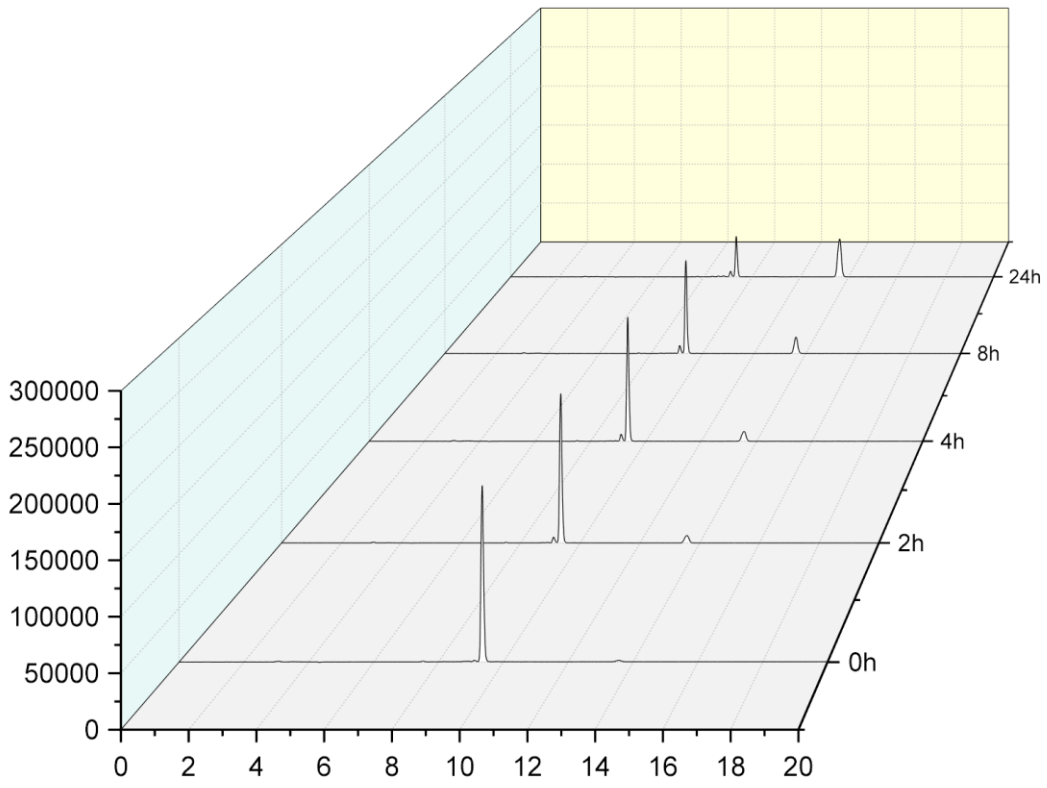


Figure S27. Reduction of complex **3** in A2780 cell extract containing 0.94 mg/mL proteins and 1% DMF at 37 ℃. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 10 - 50 - 63 - 10 - 10% acetonitrile with 0.1% HCOOH. Flow rate: 1 mL/min. wavelength: 504 nm.

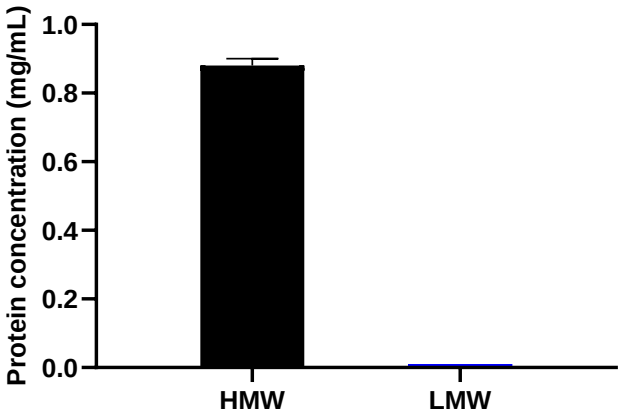


Figure S28. The concentration of proteins in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780 cell extract. A2780 cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions. Mean ± SD, n = 3.

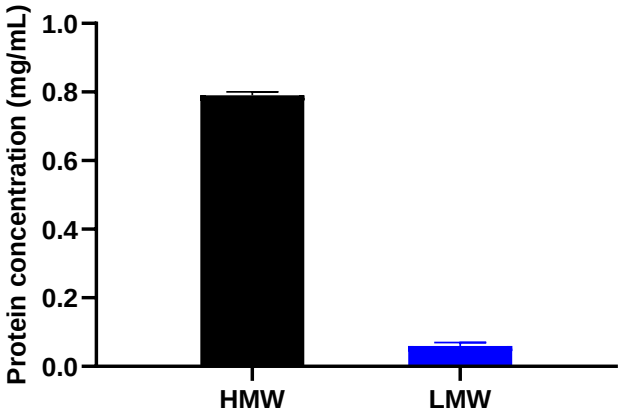


Figure S29. The concentration of proteins in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780cisR cell extract. A2780cisR cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions. Mean ± SD, n = 3.

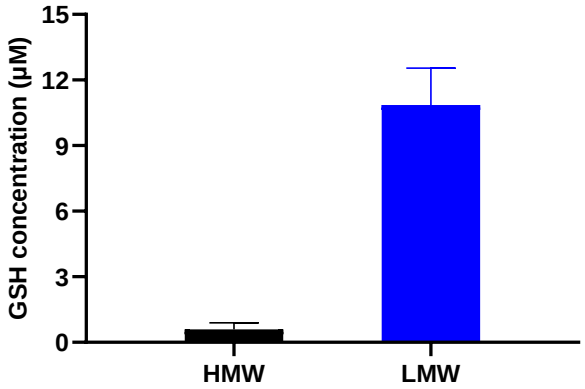


Figure S30. The concentration of GSH in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780 cell extract. A2780 cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions. Mean ± SD, n = 3.

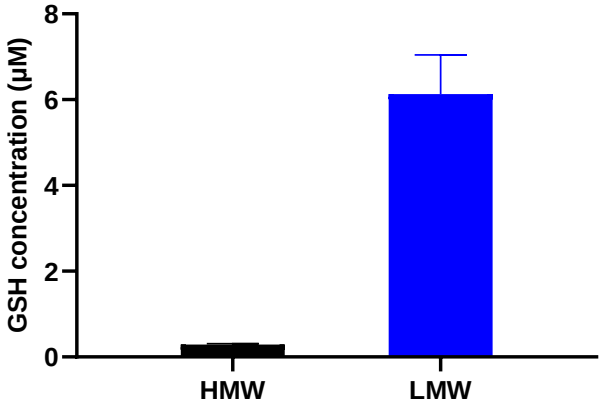


Figure S31. The concentration of GSH in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780cisR cell extract. A2780cisR cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions. Mean ± SD, n = 3.

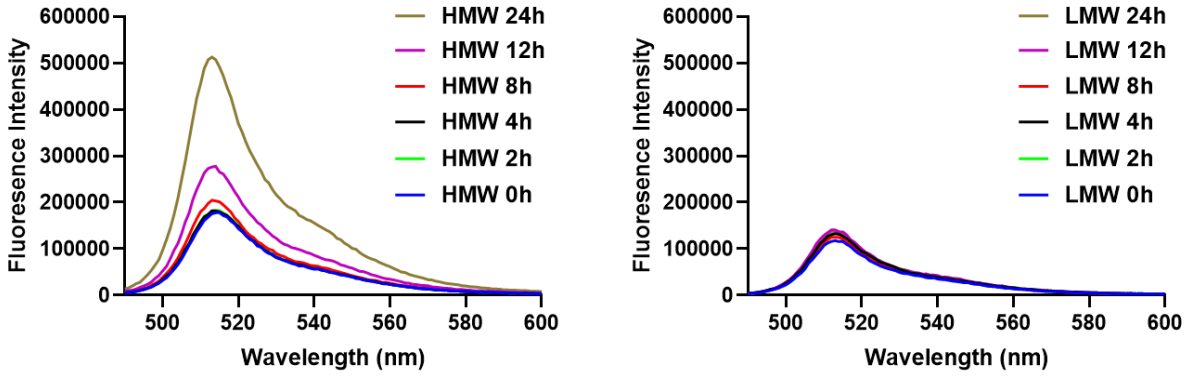


Figure S32. The time-dependent emission of 10 μM complex **3** in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780 cell extract at 37 ℃. The excitation wavelength is 470 nm. A2780 cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions.

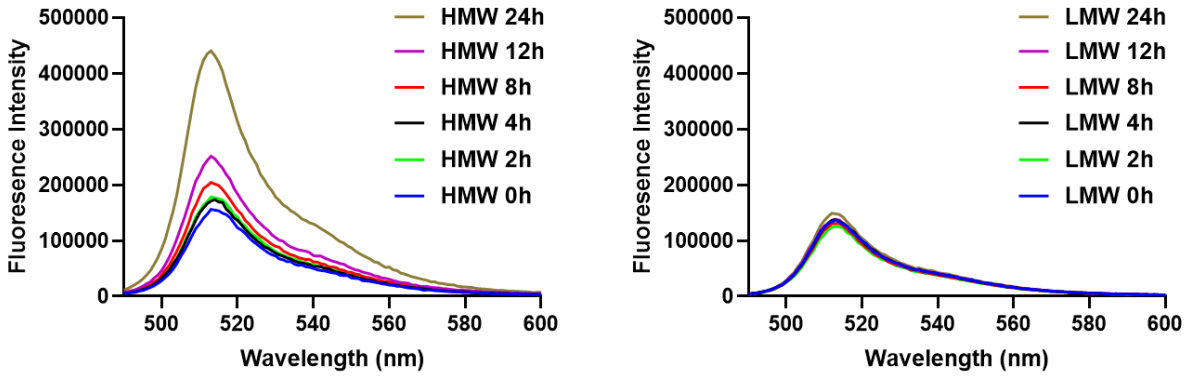


Figure S33. The time-dependent emission of 10 μM complex **3** in the high molecular weight (HMW) and low molecular weight (LMW) portions of the A2780cisR cell extract at 37 ℃. The excitation wavelength is 470 nm. A2780cisR cell extract was separated by a Centrifugal Filter Unit with 3 kDa molecular weight cut off (MWCO) to yield the HMW (> 3 kDa) and LMW (< 3 kDa) portions.

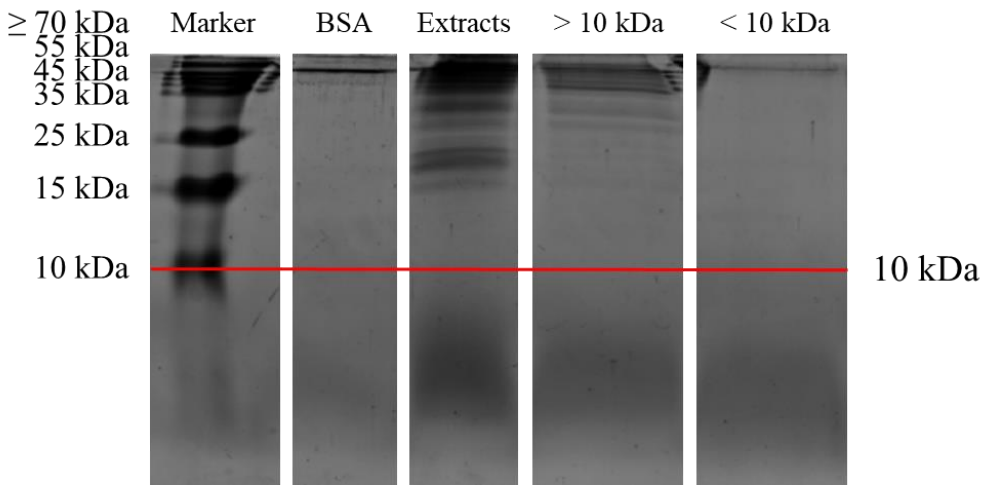


Figure S34. SDS-PAGE gels of A2780 cell extract, > 10 kDa, and < 10 kDa portions of cell extract. 0.1 mg/mL BSA (67 kDa) was included as a control. The loading buffer contains no bromophenol blue. The samples were under electrophoresis on a 6% SDS-PAGE gel at 88 V for 120 min, stained by the Coomassie Brilliant Blue for 3 h, and photographed by a chemiDoc™ Touch Imagine System.

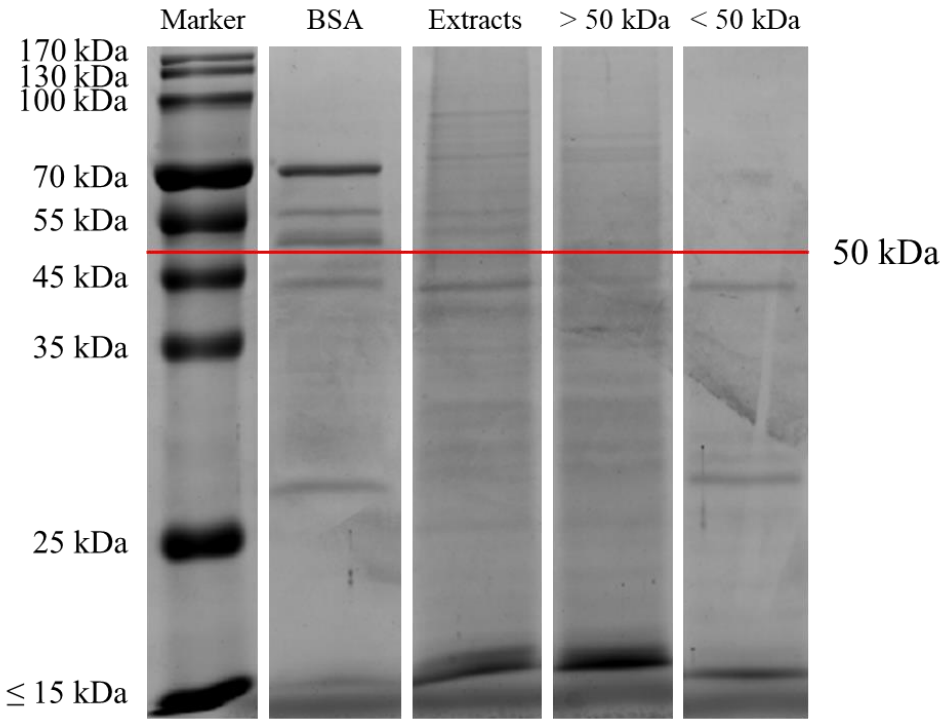


Figure S35. SDS-PAGE gels of A2780 cell extract, > 50 kDa, and < 50 kDa portions of cell extract. 0.1 mg/mL BSA (67 kDa) was included as a control. Samples were under electrophoresis on a 10% SDS-PAGE gel at 88 V for 120 min, stained by the Coomassie Brilliant Blue for 3 h, and photographed by a chemiDoc^{TM} Touch Imagine System.

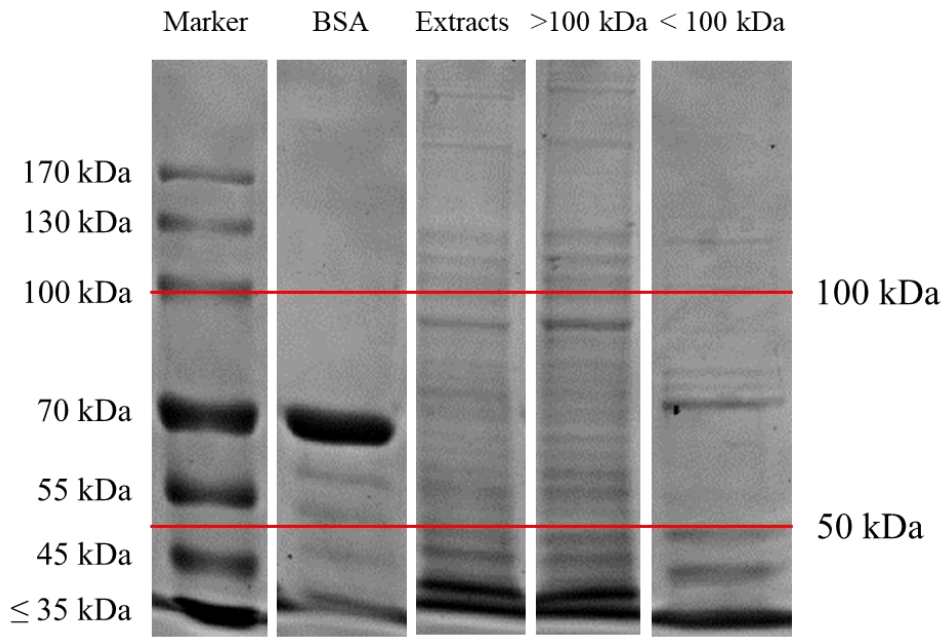


Figure S36. SDS-PAGE gels of A2780 cell extract, > 100 kDa, and < 100 kDa portions of cell extract. 0.2 mg/mL BSA (67 kDa) was included as a control. Samples were under electrophoresis on a 20% SDS-PAGE gel at 88 V for 100 min, stained by the Coomassie Brilliant Blue for 3 h, and photographed by a chemiDoc^{TM} Touch Imagine System.

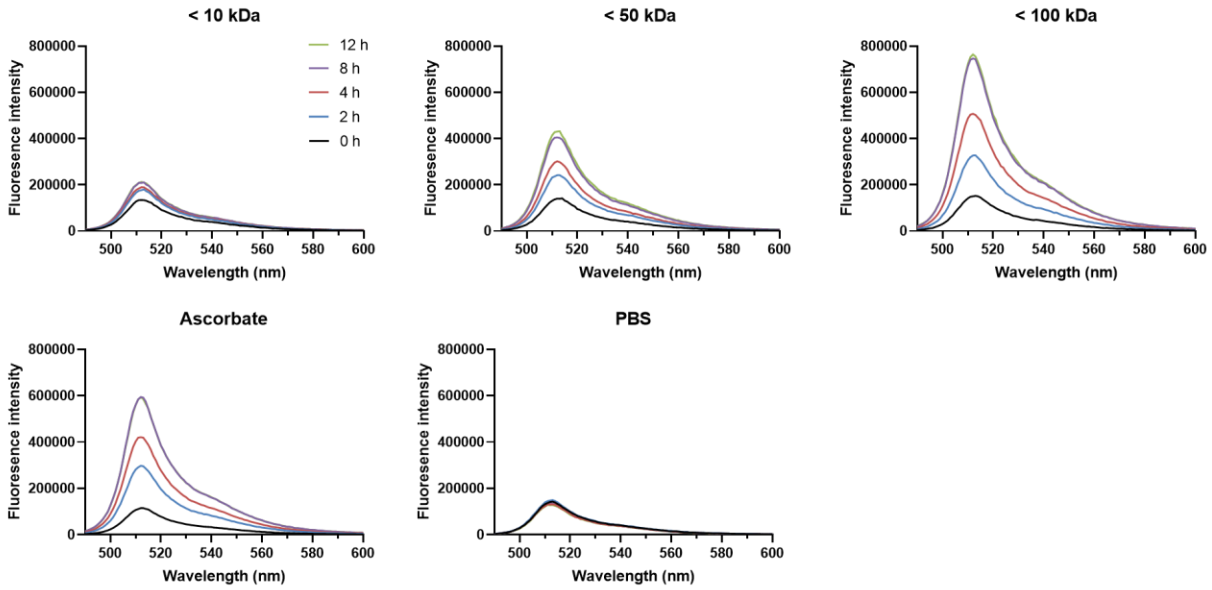


Figure S37. The time-dependent emission of 10 μM complex **3** in different portions of the A2780 cell extract at 37 ℃. A2780 cell extract was separated by Centrifugal Filter Units with 10 kDa, 50 kDa, and 100 kDa molecular weight cut off (MWCO) to yield the < 10 kDa, < 50 kDa, and < 100 kDa portion, respectively. Complex **3** was incubated in PBS buffer (pH 7.4) without or with 1 mM sodium ascorbate as the controls. The excitation wavelength is 470 nm.

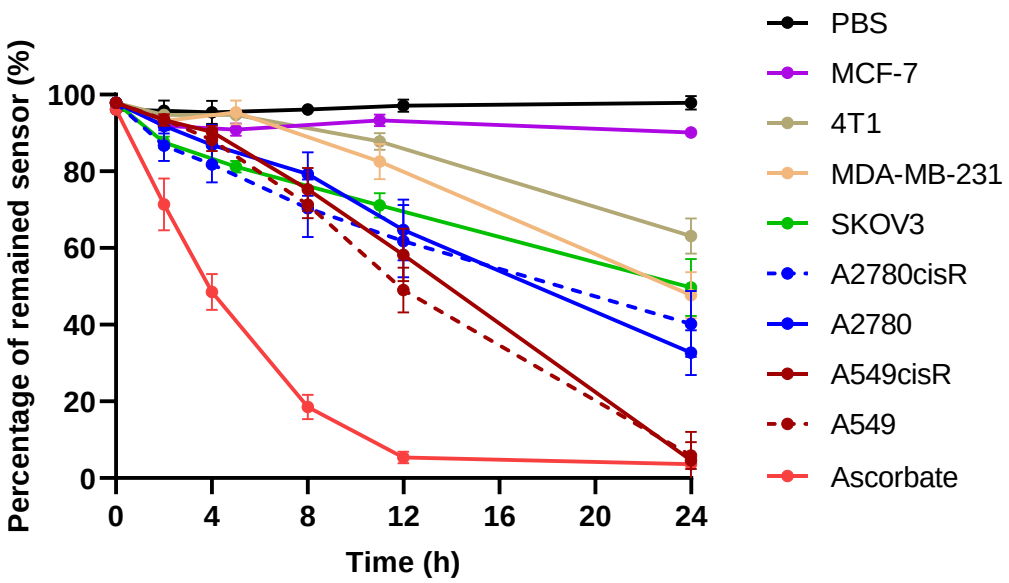


Figure S38. The percentage of remained platinum(IV) sensor in different cell extracts. Complex **3** was incubated in PBS buffer (pH 7.4) without or with 1 mM sodium ascorbate as the controls. Mean ± SD, n = 3.

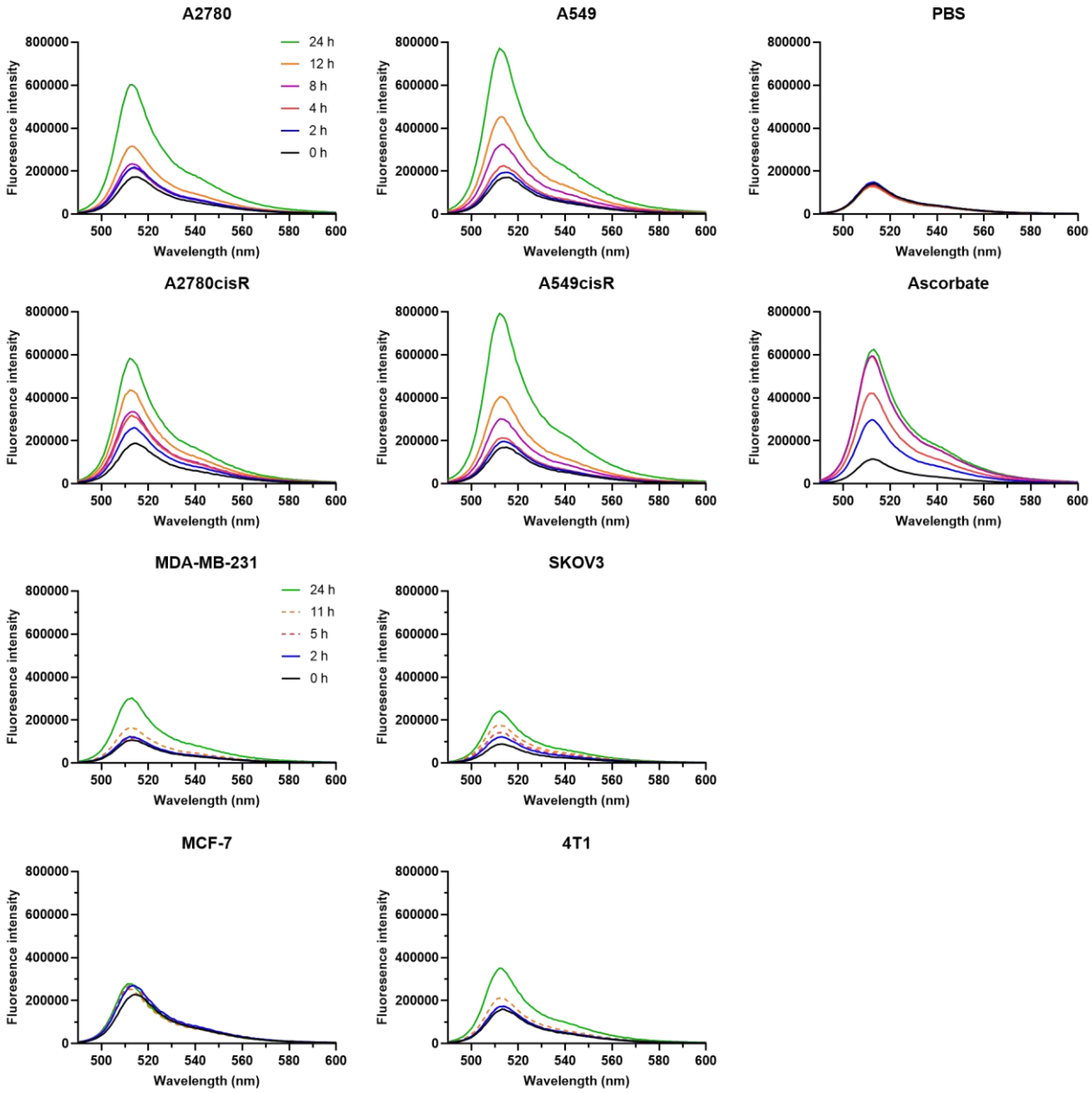


Figure S39. The time-dependent emission of complex **3** in different cell extracts at 37 ℃. Complex **3** was incubated in PBS buffer (pH 7.4) without or with 1 mM sodium ascorbate as the controls. The excitation wavelength is 470 nm.

Table S1. Water solubility, log P value, and photophysical properties of complex **3**, BODIPY ligand, and carboplatin.[1]

Compounds	Water Solubility (mM)	Log P	λ_{max} of Absorbance (nm)	Molar Extinction Coefficient (ε) (10^4 cm^{-1} M^{-1}) [a]	λ_{max} of Emission (nm) [b]	Fluorescence Quantum Yield [c]
Complex **3**	0.45 ± 0.03	0.92 ± 0.02	504	7.41 ± 0.13	512	0.15 ± 0.02
BODIPY	-	-	504	7.35 ± 0.20	512	0.81 [d]
Carboplatin	41.91 ± 0.55	- 1.15 ± 0.01	-	-	-	-

[a] Molar Extinction Coefficient (ε) = Absorbance at λ_{max} / (molar concentration of compounds * pathlength). Here, the molar concentration of compounds is 10 μM. The path length is 1 cm. Mean ± SD, n = 3.

[b] Excitation at 470 nm

[c] Fluorescence Quantum Yield = 0.81 * [(Area under emission curve / Absorbance at 470 nm) of compounds / (Area under emission curve / Absorbance at 470 nm) of BODIPY ligand]. Mean ± SD, n = 3.

[d] a known fluorescence quantum yield of the BODIPY ligand.[2]

Table S2. Reduction ratio, cellular accumulation level, and cytotoxicity of complex **3** in different cancer cells.

Cell lines	Reduction ratio (%)	Reduction (%)	Accumulation (ng Pt/10^6 cells)	Accumulation (%)	Reduction (%) + Accumulation (%)	(Reduction + Accumulation) (%)	Inhibition rate (%)	Cytotoxicity (%)
A549cisR	95.4 ± 7.4	100.0	16.9 ± 1.7	30.2	130.2	100.0	82.9 ± 3.6	100.0
SKOV3	50.3 ± 7.4	52.7	38.5 ± 7.8	68.9	121.6	93.4	80.3 ± 2.8	96.9
MCF-7	9.9 ± 1.3	10.4	55.9 ± 6.4	100.0	110.4	84.8	70.1 ± 1.3	84.6
A549	94.1 ± 3.5	98.6	13.0 ± 1.6	23.2	121.8	93.6	61.4 ± 1.3	74.1
A2780cisR	59.8 ± 8.6	62.7	27.7 ± 0.8	49.6	112.3	86.2	61.1 ± 2.9	73.7
MDA-MB-231	52.3 ± 6.0	54.8	21.6 ± 2.4	38.6	93.4	71.8	59.9 ± 4.3	72.3
A2780	67.3 ± 5.8	70.5	19.1 ± 1.0	34.1	104.6	80.4	43.7 ± 3.7	52.7
4T1	36.9 ± 4.6	38.7	2.7 ± 0.1	4.8	43.5	33.4	20.0 ± 2.1	24.1

Reduction ratio (%): the reduction ratios of the sensor in the extracts of different cancer cells for 24 h. Mean ± SD, n = 3.

Reduction (%): The highest Reduction ratio 83.2% was set as 100%.

Accumulation (ng Pt/10^6 cells): Cells were treated with 10 μM complex **3** for 24 h. Mean ± SD, n = 4.

Accumulation (%): The highest Accumulation 55.9 ng Pt/10^6 cells was set as 100%.

Reduction (%) + Accumulation (%): An addition of Reduction (%) and Accumulation (%)

(Reduction + Accumulation) (%): The highest Reduction (%) + Accumulation (%) 130.2 % was set as 100%.

Inhibition rate (%): Cells were treated with 100 μM complex **3** for 72 h. The cell viability was then determined by a MTT assay. Cells treated with media only were used as a control. A (Absorbance) = A_{570} - A_{630}. Inhibition rate (%) = [1 – (mean of $A_{complex}$ – mean of A_{blank}) / (mean of $A_{control}$ – mean of A_{blank})] * 100%. Mean ± SD, n = 4.

Cytotoxicity (%): The highest inhibition rate 82.9% was set as 100%.

References

(1) Yao, H.; Gunawan, Y. F.; Liu, G.; Tse, M.-K.; Zhu, G. Optimization of axial ligands to promote the photoactivation of BODIPY-conjugated platinum(IV) anticancer prodrugs. *Dalton Trans.* **2021**, *50*, 13737-13747.

(2) Bittel, A. M.; Davis, A. M.; Wang, L.; Nederlof, M. A.; Escobedo, J. O.; Strongin, R. M.; Gibbs, S. L. Varied length stokes shift BODIPY-based fluorophores for multicolor microscopy. *Sci. Rep.* **2018**, *8*, 4590.