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The synthesis and biological applications of photo-activated ruthenium anticancer drugs

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Author: Lameijer, L.N.

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Appendix I: General procedures

I.1.1 Singlet oxygen ($^1\text{O}_2$) and phosphorescence quantum yield measurements

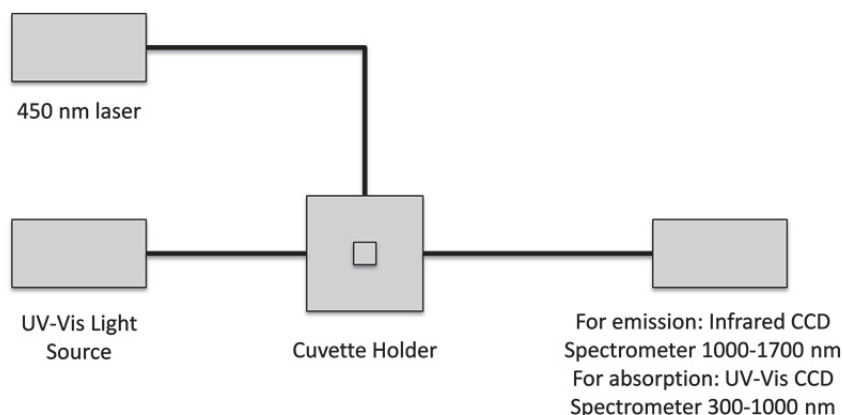


Figure S.I.1 Setup for $^1\text{O}_2$ quantum yield measurement and emission spectroscopy.

The quantum yield of singlet oxygen generation was determined in a custom-built setup (Figure S.I.1), in which both UV-VIS absorption and infrared emission spectroscopy could be performed. Emission experiments were carried out in the same setup. All optical parts were connected with optical fibers from Avantes (Apeldoorn, The Netherlands), with a diameter of 200-600 μm . For each measurement, 2 mL of sample, consisting of the compound in CD_3OD , was placed in a stirred 111-OS macro fluorescence cuvette from Hellma in a CUV-UV/VIS-TC temperature-controlled cuvette holder from Avantes. The sample was allowed to equilibrate at 293 K for 5 minutes. Emission spectroscopy was performed with a 450 nm fiber-coupled laser (Laser system LRD-0450 from Laserglow, Toronto, Canada), which was set to 50 mW at the cuvette (4 mm beam diameter; $0.4 \text{ W}\cdot\text{cm}^{-2}$) at a 90° angle with respect to the spectrometer. The excitation power was measured using a S310C thermal sensor connected to a PM100USB power meter (Thorlabs). The infrared emission spectrum was visualized from 1000 nm to 1700 nm with an Avantes NIR256-1.7TEC spectrometer, for the phosphorescence experiment the emission spectrum was visualized from 300 to 900 nm with an Avantes 2048L StarLine spectrometer with a Thorlabs FEL0500 500 nm long pass filter. The infrared emission spectrum was acquired within 9 seconds, after which the laser was turned off directly. UV-

Vis absorption spectra before and after emission spectroscopy were measured using an Avalight-DHc halogen-deuterium lamp (Avantes) as light source (turned off during emission spectroscopy) and an Avantes 2048L StarLine UV-Vis spectrometer as detector, both connected to the cuvette holder at a 180° angle. No difference in UV-Vis absorption spectrum was found due to exposure to the blue laser, showing that the singlet oxygen emission is that of the starting compound. All spectra were recorded with Avasoft software from Avantes and further processed with Microsoft Office Excel 2010 and Origin Pro software.

The quantum yield of singlet oxygen production was calculated using the relative method with [Ru(bpy)₃]Cl₂ as the standard (0.73 in CD₃OD)^[1], according to equation 1:

$$\Phi_{sam}^{\Delta} = \Phi_{std}^{\Delta} \times \frac{A_{std}^{450}}{A_{sam}^{450}} \times \frac{E_{sam}}{E_{std}}$$

Equation S.I.1

where Φ^{Δ} is the quantum yield of singlet oxygen generation, A^{450} is the absorbance at 450 nm (always kept below 0.1 for a 1 cm path length), E is the integrated emission peak of singlet oxygen at 1270 nm, and sam and std denote the sample and standard, respectively.

The quantum yield of phosphorescence was calculated using the relative method with [Ru(bpy)₃]Cl₂ as the standard (0.042 in water)^[2] according to equation 2:

$$\Phi_{sam}^{em} = \Phi_{std}^{em} \times \frac{A_{std}^{450}}{A_{sam}^{450}} \times \frac{E_{sam}}{E_{std}}$$

Equation S.I.2

where Φ^{em} is the quantum yield of phosphorescence, A^{450} is the absorbance at 450 nm (always kept below 0.1 for a 1 cm path length), E is the integrated emission range from 550 to 850 nm, and sam and std denote the sample and standard, respectively.

I.2.1 Cell culturing and cytotoxicity assay

I.2.1.1 General

Human cancer cell lines (A549, human lung carcinoma; MCF-7, human breast adenocarcinoma) were distributed by the European Collection of Cell Cultures (ECACC), and purchased through Sigma Aldrich. Dulbecco's Minimal Essential Medium (DMEM, with

and without phenol red, without glutamine), 200 mM Glutamine-S (GM), trichloroacetic acid (TCA), glacial acetic acid, sulforhodamine B (SRB), tris(hydroxymethyl)aminomethane (tris base), were purchased from Sigma Aldrich. Fetal calf serum (FCS) was purchased from Hyclone. Penicillin and streptomycin were purchased from Duchefa and were diluted to a 100 mg/mL penicillin/streptomycin solution (P/S). Trypsin and Opti-MEM[®] (without phenol red) were purchased from Gibco[®] Life Technologies. Trypan blue (0.4% in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution) was purchased from BioRad. Plastic disposable flasks and 96-well plates were purchased from Sarstedt. Cells were counted using a BioRad TC10 automated cell counter with Biorad Cell Counting Slides. UV-vis measurements for analysis of 96-well plates were performed on a M1000 Tecan Reader. Cells were inspected with an Olympus IX81 microscope.

1.2.1.2 Cell culturing

Cells were cultured in DMEM complete (Dulbecco's Modified Eagle Medium (DMEM) with phenol red, supplemented with 8.0% v/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 0.9% v/v Glutamine-S (GM)). Cells were cultured under humidified conditions, 37 °C atmosphere containing 7.0% CO₂ in 75 cm² flasks and sub-cultured (1:3 to 1:6 ratio) upon reaching 70-80% confluency (approximately once per week). Media was refreshed every second day. Cells were passaged for 4 - 8 weeks.

1.2.1.3 Cell irradiation setup

Cell irradiation setup. The cell irradiation system consists of a Ditabis thermostat (980923001) fitted with two flat-bottom microplate thermoblocks (800010600) and a 96.-LED array fitted to a standard 96-well plate. The 450 nm LED (OVL-3324), fans (40 mm, 24 V DC, 9714839), and power supply (EA-PS 2042-06B) were ordered from Farnell. Full description of the cell irradiation setup is given in Hopkins et al.^[3]

1.2.1.4 Cytotoxicity Assay

Cells were seeded at $t = 0$ in 96-well plates at a density of 5×10^3 (A549) and 8×10^3 (MCF7) cells/well in a volume of 100 μ L of Opti-MEM[®] complete without phenol red and incubated for 24 h at 37 °C and 7% CO₂. After this period, 100 μ L of six different concentrations (1 – 100 μ M for dark plates, 0.1 – 10 μ M for irradiated plates) of freshly prepared stock solutions of [D-2](PF₆)₂ and [L-2](PF₆)₂ in Opti-MEM were added to the wells in triplicate. Plates were incubated in the dark for an additional 24 h. After this period the media in each well was refreshed, and half of the plates were irradiated for 5 minutes with blue light (454 ± 11 nm, 10.5 ± 0.7 mW · cm⁻², $3.2 \cdot \text{J cm}^{-2} \pm 0.2$), while the other half was kept in the dark under otherwise identical conditions. After irradiation all plates were placed back in the incubator and incubated for an additional 48 hours. Then the cells were fixed by adding 100 μ L of cold trichloroacetic acid (TCA, 10% w/v) in each well. Next, TCA was removed from the wells, plates were gently washed five times with

water, air-dried, stained using 100 μL sulforhodamine B (0.6% w/v SRB in 1% v/v acetic acid) for 30-45 minutes, washed five times with ~ 300 μL acetic acid (1% v/v), and air-dried. The SRB dye was then solubilized using 200 μL of tris base (10 mM), and the absorbance in each well was read at 510 nm using a M1000 Tecan Reader.

The SRB absorbance data were used to calculate the fraction of viable cells in each well using Excel and GraphPad Prism[®] as follows. The absorbance data from three technical triplicate wells per concentration were averaged. Relative cell viabilities were calculated by dividing the average absorbance of treated wells by the average absorbance of the untreated wells. Three independent biological replicates were completed for each cell line (three different passage numbers per cell line). The average cell viability of the three biological replicates was plotted versus the logarithm of the concentration in μM , with standard deviation error of each point. Using the dose-response curve for each cell line in dark and irradiated conditions, the effective concentration EC_{50} (defined as the concentration of drug that gives a half-maximum effect) was calculated by fitting the curves using a non-linear regression function with fixed Y maximum (100%) and minimum (0%) (relative cell viability), and a variable Hill-slope, resulting in the simplified two-parameter Hill-slope equation 1.

$$\frac{100}{(1 + 10^{((\log_{10} EC_{50} - X) \times Hill\ Slope)})}$$

Equation S.I.3

1.2.3 Log P_{ow} determination

The partition coefficient between *n*-octanol and water (Log P_{ow}) were determined according to OECD guidelines^[4] according to the following method: Stock solutions of complexes (1×10^{-3} M) were prepared by dissolving the compounds in *n*-octanol saturated MilliQ water (MilliQ saturated *n*-octanol for complexes insoluble in water). Three aliquots (0.200 ml) of these stock solutions were transferred to 15 mL corning tubes and diluted with *n*-octanol saturated MilliQ to 1.00 ml resulting in $c_{final} = 0.20 \times 10^{-3}$ M. Then, 1.00 ml of MilliQ-saturated *n*-octanol (*n*-octanol saturated MilliQ for complexes insoluble in water) was added. The mixture was shaken in a GFL 3016 reciprocating shaker at 60 rpm for 1 h while protecting the compounds from light and then centrifuged for 10 minutes at 2000 rpm. 0.500 mL aliquots from each technical replicate were then transferred to 15 mL corning tubes and diluted with 5% HNO_3 until $V_{final} = 5.00$ mL. Ruthenium concentrations in the water phase (from both the stock solution and the dilution) were determined using a Varian Inc. Vista MPX Simultaneous ICP-OES. Partition coefficients were calculated using equation S.I.4.

$$\log P_{ow} = \log \frac{[\text{Ru}]_{\text{oct}}}{[\text{Ru}]_{\text{aq}}}$$

Equation S.I.4

Where $[Ru]_{\text{oct}}$ and $[Ru]_{\text{aq}}$ are the concentrations of Ru in the n-octanol and aqueous layers respectively and represent the mean \pm SD of three technical replicates.

References

- [1] D. Garcia-Fresnadillo, Y. Georgiadou, G. Orellana, A. M. Braun, E. Oliveros, *Helv Chim Acta* **1996**, *79*, 1222-1238.
- [2] J. Van Houten, R. J. Watts, *J Am Chem Soc* **1976**, *98*, 4853-4858.
- [3] S. L. Hopkins, B. Siewert, S. H. Askes, P. Veldhuizen, R. Zwier, M. Heger, S. Bonnet, *Photobiol Sci* **2016**, *15*, 644-653.
- [4] Oecd, *OECD Guidelines for the Testing of Chemicals*, The Organisation for Economic Co-operation and Development, **1994**.

Appendix II: Supporting information for Chapter 3

II.1 Photochemistry

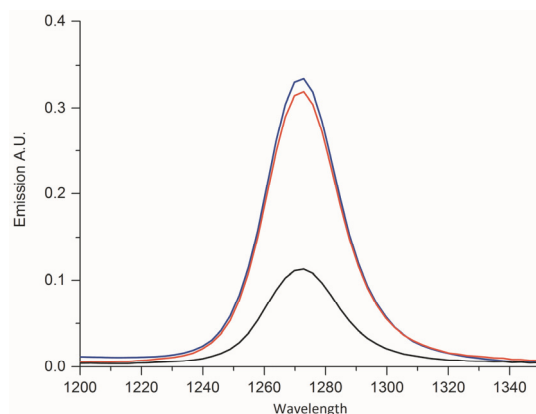


Figure S.II.2. Time-integrated emission spectra of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ (black), $[\text{D-2}](\text{PF}_6)_2$ (red) and the photoproduct of $[\text{D-2}](\text{PF}_6)_2 + 1$ eq. of ligand (black) in CD_3OD irradiated with blue light ($\lambda_{\text{exc}} = 450$ nm, 50 mW), stirred under air in CD_3OD at 298 K.

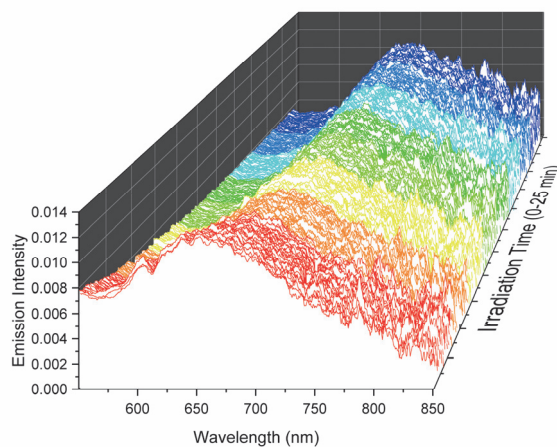


Figure S.II.3. Time evolution of the emission spectra of $[\text{D-2}](\text{PF}_6)_2$ (red, $\lambda_{\text{exc}} = 450$ nm, $\lambda_{\text{em}} = 648$ nm) irradiated with blue light (450 nm) to form $[\text{1}](\text{PF}_6)_2$ (blue, $\lambda_{\text{exc}} = 450$ nm, $\lambda_{\text{em}} = 690$ nm) in PBS.

II.2 Biology

II.2.2 DNA photointeraction studies

Agarose gel electrophoresis was used to assay the photoinduced binding and photocleavage of pUC19 plasmid using [D-2](PF₆)₂. A 5X tris-boric acid (TBA) buffer (45 mM tris(hydroxymethyl)aminomethane and 45 mM boric acid, pH = 7.4) was used in the gel and for the running buffer. Phosphate buffer (PB, 100 mM NaH₂PO₄, pH = 7.0) was used for DNA-MC interactions. A 0.8% w/w agarose gel (0.24 g agarose, 24 g DI H₂O, and 6 mL TBA) was made using the OWL B1A Easycast system. Each sample was prepared. The irradiated sample consisted of 20 μL pUC19 plasmid (20 μg, [DNA bp]_{final} = 1.9 × 10⁻³ M), 2 μL of 0.5 mM solution of complex [D-2](PF₆)₂ in PB ([[D-2](PF₆)₂]_{final} = 4.9 × 10⁻⁶ M) and 178 μL PB, for a total volume of 200 μL of a 400:1 base pair to metal complex ratio. A dark metal complex control was prepared using 4 μL pUC19 DNA, 0.4 μL of 0.5 mM solution of complex [D-2](PF₆)₂ in PB, and 35.6 μL PB. Dark and irradiated DNA controls were prepared using 4 μL pUC19 DNA and 36 μL PB. The dark and irradiated samples were run in parallel using the same LED irradiation setup above. Each sample was loaded into separate wells of two 96-well plates (dark and 455 nm irradiated). For the irradiated sample, 20 μL aliquots were removed at 1, 3, 5, 10, and 15 minutes correlating to doses of 0.6, 1.9, 3.2, 6.3, and 9.5 Jcm⁻², respectively. At the end of the experiment, 20 μL of the dark DNA control, irradiated DNA control, and dark DNA-metal complex control (t = 0 min irr) were removed. To each of the 20 μL aliquots, 4 μL of 6X loading dye was added. The λ DNA-*Hind*III digest molecular weight (MW) marker was prepared by adding 2 μL (1 μg) of the DNA MW marker, 18 μL PB, and 4 μL 6X loading dye. The gel electrophoresis chamber was filled with 50 mL TBA and 210 mL DI H₂O. To each well in the gel 12 μL (1 μg of DNA) of each sample were loaded according to the Table S.II.1. The gel was run at 105 V for 90 min. The gel was stained using 10 μL (10 mg/mL) ethidium bromide in 200 mL DI H₂O for 30 min with slight shaking and then destained in 200 mL DI H₂O for 20 min. Immediately following destaining, the gel was imaged on a BioRad ChemiDoc imaging system using the ethidium bromide setting. Using Image Lab, the volume of the OC and SC bands in each lane was determined for time 0-15 min irradiation of the 400:1 BP:MC ratio samples, Figure S.II.4.

Table S.II.1. Agarose gel electrophoresis loading description with irradiation times (min) and corresponding light doses (J x cm⁻²).

Lane	Description	Time of irradiation (min)	Light dose (Jcm ⁻²)
1	λ MW marker	0	0
2	DNA control, 37 °C, dark	0	0
3	DNA control, 37 °C, irradiated	15	9.5
4	400:1 BP:MC, 37 °C, dark	0	0
5	400:1 BP:MC, 37 °C, irradiated 1 min	1	0.6
6	400:1 BP:MC, 37 °C, irradiated 3 min	3	1.9
7	400:1 BP:MC, 37 °C, irradiated 5 min	5	3.2
8	400:1 BP:MC, 37 °C, irradiated 10 min	10	6.3
9	400:1 BP:MC, 37 °C, irradiated 15 min	15	9.5
10	λ MW marker	0	0

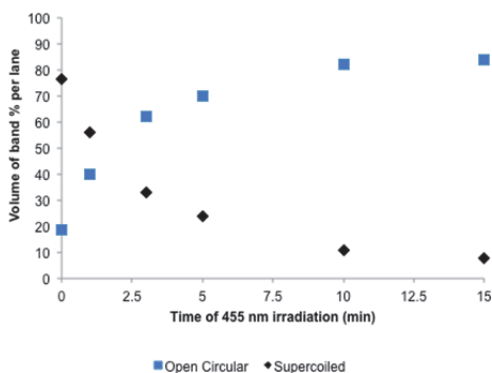


Figure S.II.4. Plot of volume of band per lane as a function of time of 455 nm irradiation (min).

II.2.3 Emission microscopy, 20x objective

All images using the 20x air objective were acquired using an Olympus IX81 inverted microscope system, Figures S.II.5-S.II.10. Fluorescence and emission imaging of the control cells and metal complex treated cells was performed using 488 nm excitation. Fluorescence imaging of DAPI stained cells was performed using the DAPI filter block (350 nm excitation/450 nm emission filter). The exposure time was 1 s, lamp intensity was 100% and gain was set to 100. For all images, cells were irradiated using 488 nm light for 30 s prior to capturing the image. Fiji ImageJ software was used to process images.^[1] The metal complex emission was visualized in yellow and DAPI in blue.

II.2.4 General preparation of cells samples for microscopy imaging

A549 cells were used for microscopy imaging experiments. Cells were seeded (100 μL OMEM-complete) at specified concentrations (see each experiment) in ibidi 1 $\mu\text{-slide}$ 8-well ibiTreat chamber. Cells were incubated for 24 h at 37 $^{\circ}\text{C}$ and 7% CO_2 . Following the 24 h incubation, cells were treated [D-2](PF₆)₂ and [L-2](PF₆)₂ (final concentration = 25 μM) and placed back in the incubator for a specified amount of time depending on experiment. Prior to imaging, media was removed from all wells, and refreshed with OMEM-complete.

II.2.5 Visualization (20x obj.) of the time-dependent incubation of [D-2](PF₆)₂ and [L-2](PF₆)₂

A549 cells were seeded at 1×10^5 cells/well in three ibidi 1 $\mu\text{-slide}$ 8-well ibiTreat chambers. Cells were incubated for 24 h (37 $^{\circ}\text{C}$, 7% CO_2) and then treated with a final concentration of 25 μM of [D-2](PF₆)₂ and [L-2](PF₆)₂. The cells were incubated in the presence of metal complexes for 4, 6, and 24 h, Figure S.II.5. Media was refreshed and cells were imaged. Control wells were imaged under the same conditions as the metal complex treated wells (30 s exposure to 488 nm irradiation, followed by imaging). For the

24 h metal complex treated samples, the nucleus was co-stained using DAPI. One well of [D-2](PF₆)₂ and [L-2](PF₆)₂ were stained with 2 μL of 0.1 mg/mL DAPI for one minute, Figure S.II.6.

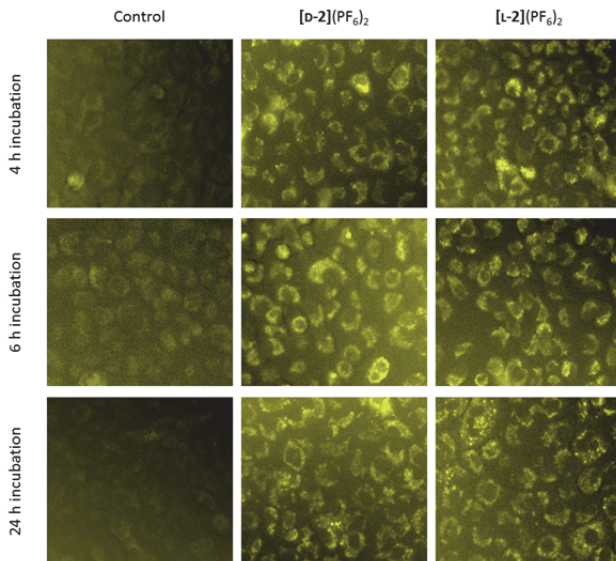


Figure S.II.5. Images of autofluorescence of A549 cells and emission of [D-2](PF₆)₂ and [L-2](PF₆)₂ treated A549 cells (20x obj.). Cells were treated at 25 μM final concentration for the metal complexes and then incubated for 4, 6, or 24 h at 37 °C and 7% CO₂. Images show that under the same conditions, the emission of the metal complexes is above the background autofluorescence observed in untreated cells.

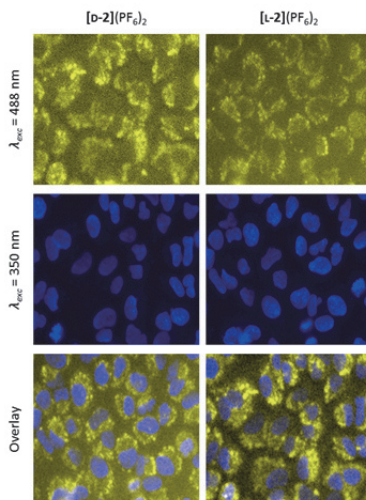


Figure S.II.6. Images of emission from [D-2](PF₆)₂ and [L-2](PF₆)₂ treated A549 cells, fluorescence of DAPI co-stained cells, and the overlay showing no nuclear co-localization of the metal complexes.

II.2.6 Visualization at 4 h incubation of [D-2](PF₆)₂ and [L-2](PF₆)₂ with lower seeding density

To determine if a higher ratio of metal complex to cell population influenced the localization of the complexes, A549 cells were seeded at 6×10^3 cells/well in an ibidi 1 μ -slide 8-well ibiTreat chamber. Cells were incubated for 24 h (37 °C, 7% CO₂) and then treated with a final concentration of 25 μ M of [D-2](PF₆)₂ and [L-2](PF₆)₂. The metal complexes for were incubated (37 °C, 7% CO₂) in the presence of the cells for 4 h, Figure S.II.7. Media was refreshed and cells were imaged. Control wells were imaged under the same conditions as the metal complex treated wells (30 s exposure to 488 nm irradiation, followed by imaging). Images were compared to those seeded at 1×10^5 cells/well, but no significant difference in localization was observed.

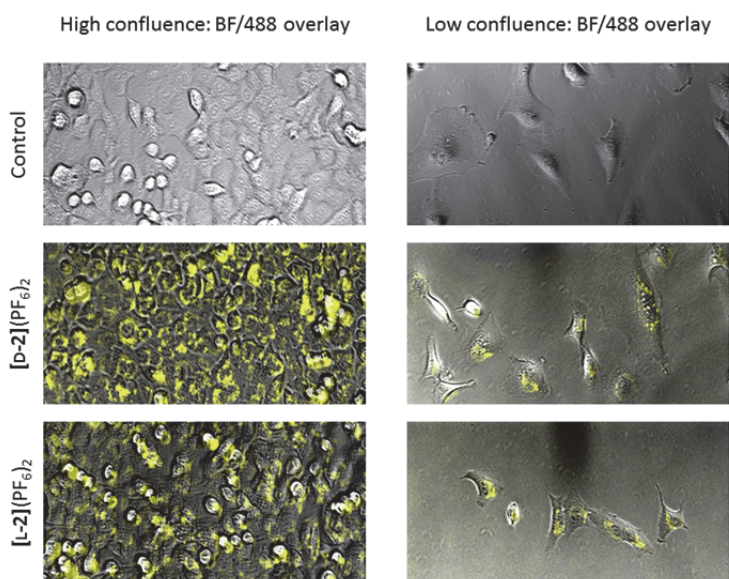


Figure S.II.7. Overlay images (BF and 488 nm excitation, 20x obj.) of cells only and [D-2](PF₆)₂ or [L-2](PF₆)₂ treated A549 cells after 4 h incubation at high and low seeding densities. The ratio of metal complex to cells does not influence metal complex localization.

II.2.7 Visualization at 4 h incubation of [D-2](PF₆)₂ and [L-2](PF₆)₂ in the presence of sodium azide

Sodium azide (NaN₃), an inhibitor of energy dependent uptake mechanisms, was used to determine whether the uptake of [D-2](PF₆)₂ or [L-2](PF₆)₂ was energy dependent. Cells were seeded at 6×10^3 cells/well in an ibidi 1 μ -slide 8-well ibiTreat chamber. Cells were incubated for 24 h (37 °C, 7% CO₂). Three wells were pretreated with NaN₃ (100 μ M final concentration) for one hour prior to treatment with media or [D-2](PF₆)₂ or [L-2](PF₆)₂. After one hour, control cells were incubated with 100 μ M NaN₃, and metal complex treated cells were incubated with 100 μ M NaN₃ and 25 μ M [D-2](PF₆)₂ or [L-2](PF₆)₂ for

four additional hours (37 °C, 7% CO₂). Media was refreshed and cells were imaged, Figure S.II.8. Control wells were imaged under the same conditions as the metal complex treated wells (30 s exposure to 488 nm irradiation, followed by imaging). All images were processed in the same fashion.

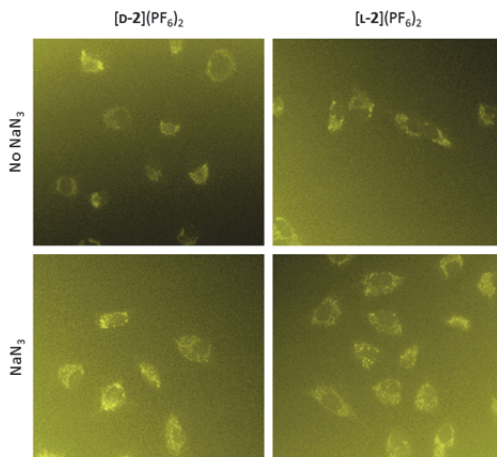


Figure S.II.8. A549 cells imaged (20x obj.) after no incubation or (pre)incubation with NaN₃ and [D-2](PF₆)₂ and [L-2](PF₆)₂. Images show negligible difference in the presence or absence of NaN₃ strongly supporting that the complexes utilize a facilitated diffusion uptake mechanism.

II.2.8 Emission microscopy, 100x objective

Emission and fluorescence imaging using a 100x objective was performed using a customized Zeiss Axiovert S100 Inverted Microscope setup. The microscope was fitted with a Zeiss 100 x Plan Apochromat 1.4 NA oil objective and an Orca Flash 4.0 V2 sCMOS camera from Hamamatsu, which together produced images with pixel size of 69 nm. The camera exposure time was 250 ms. For direct excitation and emission imaging of [D-2](PF₆)₂ and [L-2](PF₆)₂, a 488 nm laser was used. For fluorescence microscopy of MitoTracker Deep Red (MTDR), an LRD-0635-PFR-00200-01 LabSpec 635nm Collimated Diode Laser (Laserglow Technologies, Toronto, Canada) was used as excitation source, combined with a Chroma ZT405/532/635rpc dichroic beam splitter. All laser beam spots had a Gaussian intensity profile. Fiji ImageJ software was used to process images.^[1] The metal complex emission was visualized in yellow and MTDR in red.

II.2.9 Visualization (100x obj.) at 6 h incubation of [D-2](PF₆)₂ and [L-2](PF₆)₂ with MTDR

A549 cells were seeded at 3×10^3 cells/well in an ibidi 1 μ -slide 8-well ibiTreat chamber. Cells were incubated for 24 h (37 °C, 7% CO₂) and then treated with a final concentration of 25 μ M of [D-2](PF₆)₂ or [L-2](PF₆)₂. The metal complexes for were incubated for 6 h (37 °C, 7% CO₂). At 5.5 h cells were co-stained using MitoTracker Deep Red (1.1×10^{-3} M final concentration) and incubated for 30 min. Cells were washed 3 times with PBS buffer and

then 200 μL OMEM-complete was added to the wells for imaging. For each set of images, cells were initially imaged using the 635 nm channel, then 488 nm, and finally the 635 nm channel, Figure S.II.9.

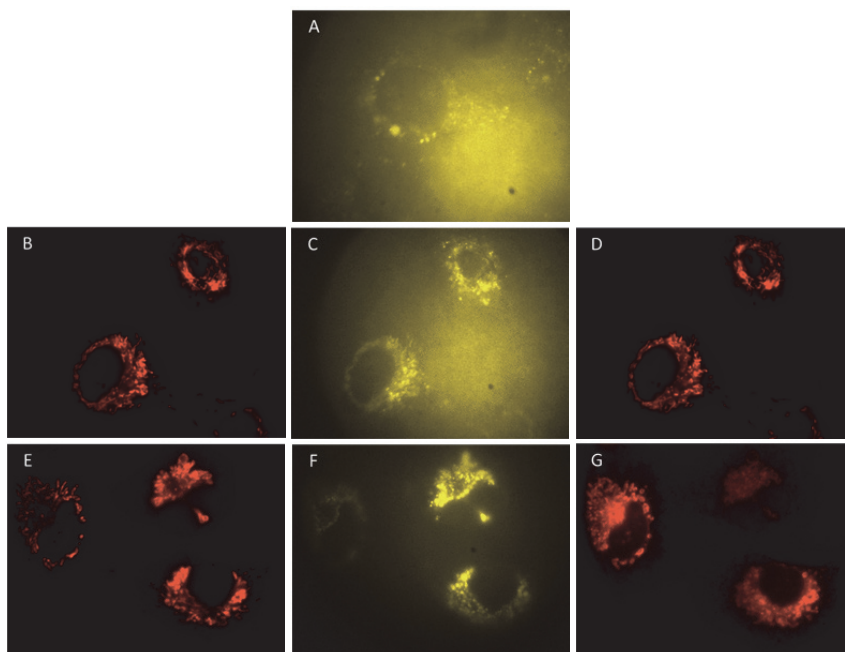


Figure S.II.9. A549 cells (A) untreated and unstained ($\lambda_{\text{exc}} = 488$ nm), (B) untreated and stained with MitoTracker Deep Red (MTDR) before 488 nm excitation ($\lambda_{\text{exc}} = 639$ nm), (C) untreated and stained with MTDR ($\lambda_{\text{exc}} = 488$ nm), (D) untreated and stained with MTDR after 488 nm excitation ($\lambda_{\text{exc}} = 639$ nm), (E) treated with $[\text{D-2}](\text{PF}_6)_2$ for 6 h and stained with MTDR before 488 nm excitation ($\lambda_{\text{exc}} = 639$ nm), (F) treated with $[\text{D-2}](\text{PF}_6)_2$ for 6 h and stained with MTDR ($\lambda_{\text{exc}} = 488$ nm), (G) treated with $[\text{D-2}](\text{PF}_6)_2$ for 6 h and stained with MTDR after 488 nm excitation ($\lambda_{\text{exc}} = 639$ nm). Images B-D and E-G were taken consecutively.

II.2.10 Visualization (100x obj.) at 24 h incubation of $[\text{D-2}](\text{PF}_6)_2$ and $[\text{L-2}](\text{PF}_6)_2$

A549 cells were seeded at 6×10^4 cells/well in an ibidi 1 μ -slide 8-well ibiTreat chamber. Cells were incubated for 24 h (37 $^\circ\text{C}$, 7% CO_2) and then treated with a final concentration of 25 μM of $[\text{D-2}](\text{PF}_6)_2$ and $[\text{L-2}](\text{PF}_6)_2$. The metal complexes for were incubated for 24 h (37 $^\circ\text{C}$, 7% CO_2). Media was refreshed and cells were imaged. A selection of single cell images are shown for untreated and $[\text{D-2}](\text{PF}_6)_2$ or $[\text{L-2}](\text{PF}_6)_2$ treated cells, Figure S.II.10.

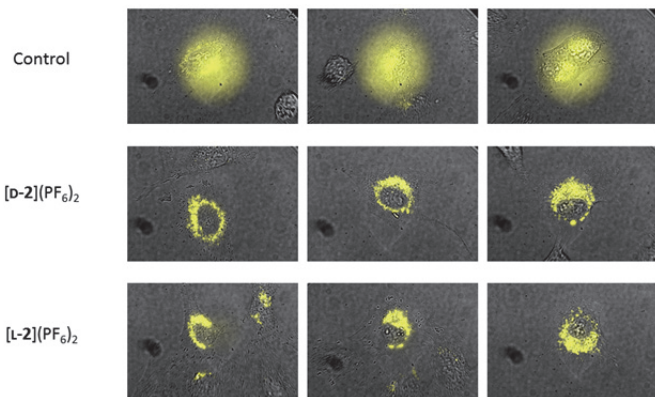


Figure S.II.10. Single cell examples of control images, [D-2](PF₆)₂ and [L-2](PF₆)₂ treated images at 488 nm.

II.2.11 Phase contrast microscopy images

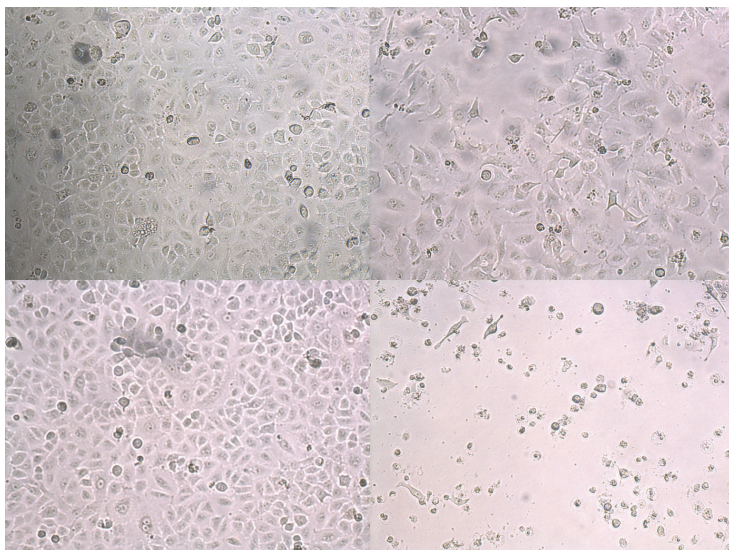


Figure S.II.11. A549 cells (10 x obj.). From top left to bottom right: A549 cells after 96 hours. Top left: A549 cells in the dark after 96 hours; Top right: A549 cells treated with 20 μM of [D-2](PF₆)₂ after 96 hours; Bottom left: A549 cells, irradiated with blue light (455 nm, 3.1 J.cm⁻²) after 96 hours; Bottom right: A549 cells treated with 1 μM of compound [D-2](PF₆)₂ and irradiated (455 nm, 3.1 J.cm⁻²), after 96 hours.

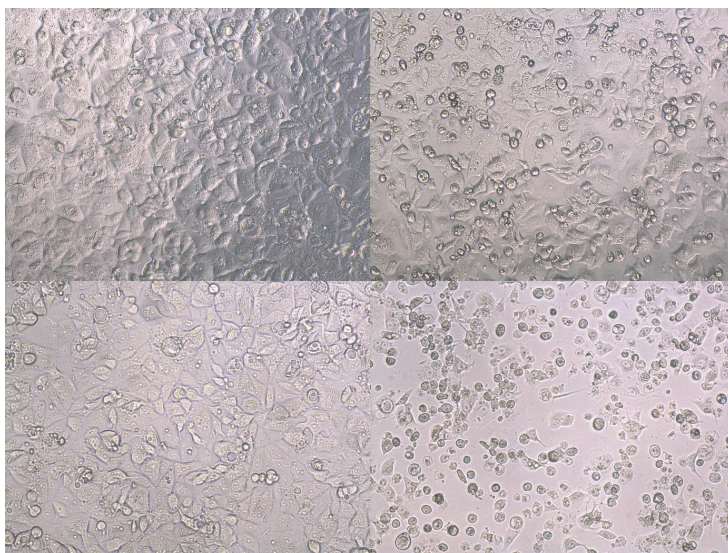


Figure S.II.12. MCF-7 cells (10 x obj.). From top left to bottom right: MCF-7 cells after 96 hours. Top left: MCF-7 cells in the dark after 96 hours; Top right: MCF-7 cells treated with 20 μM of [D-2](PF₆)₂ after 96 hours; Bottom left: MCF-7 cells, irradiated with blue light (455 nm, 3.1 J.cm⁻²) after 96 hours; Bottom right: MCF-7 cells treated with 1 μM of compound [D-2](PF₆)₂ and irradiated (455 nm, 3.1 J.cm⁻²), after 96 hours.

II.3 Chiral HPLC Traces of D-3 and L-3

Spectra were collected using an analytical Lux Cellulose-1 chiral HPLC column eluted with gradient: MeCN/MeOH from 0 to 30%.

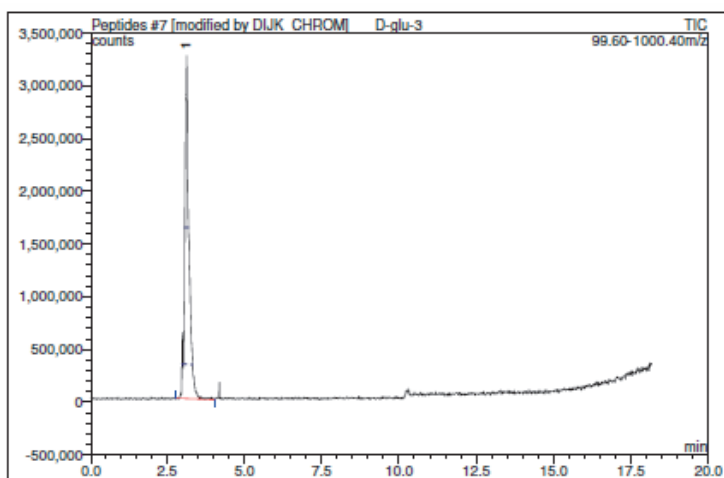


Figure S.II.13. Chiral HPLC trace of D-3. $R_t = 2.38$ min.

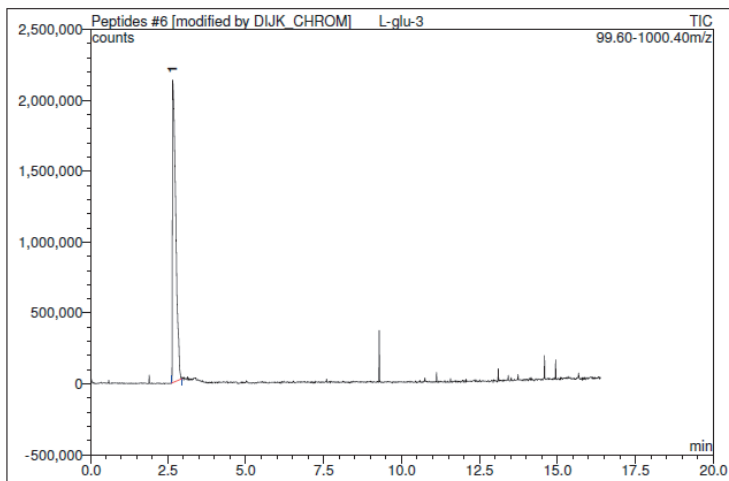


Figure S.II.14. Chiral HPLC trace of L-3. $R_t = 2.48$ min.

References

- [1] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, *Nat Methods* **2012**, *9*, 676-682.



Appendix III: Supporting information for Chapter 4

III.1 Single Crystal X-ray Crystallography

All reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Cu $K\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$) for [3a]Cl and [5a]Cl and with Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) for [4a]Cl under the program CrysAlisPro (Versions 1.171.36.32 or 1.171.37.35, Agilent Technologies, 2013-2014). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2014/7^[1] and was refined on F^2 with SHELXL-2014/7.^[1] Analytical numeric absorption correction using a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions using the instructions AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 U_{eq} of the attached C atoms.

[3a]PF₆: The structure is mostly ordered. The contribution of a very disordered acetone lattice solvent molecule (at least 4 different orientations) has been removed from the final refinement using the Squeeze procedure in Platon.^[2]

[4a]PF₆: The structure is partly disordered. The PF₆⁻ counterion is found disordered over two orientations, and the occupancy factor of the major component of the disorder refines to 0.768(7).

[5a]Cl: The structure is mostly ordered. The crystal that was mounted on the diffractometer was twinned. The twin relationship corresponds to a twofold axis along the [0.9830 0.1273 0.1321] reciprocal axis. The BASF scale factor refines to 0.2154(6). The crystal lattice contains a fair amount of partially occupied and disordered lattice ethanol molecules. As the crystal is twinned and diffracted poorly, the contribution of those lattice solvent molecules was removed in the final refinement *via* the Squeeze procedure^[2] in order to keep the data-to-parameter ratio to an acceptable level.

Table SIII.1 Experimental details for [3a]PF₆

Crystal data	
Chemical formula	C ₂₉ H ₁₉ ClN ₇ Ru·F ₆ P
<i>M_r</i>	747.00
Crystal system, space group	Triclinic, <i>P</i> -1
Temperature (K)	110
<i>a</i> , <i>b</i> , <i>c</i> (Å)	11.6962 (3), 11.7752 (4), 13.0474 (4)
α , β , γ (°)	66.304 (3), 73.164 (2), 77.418 (3)
<i>V</i> (Å ³)	1564.34 (9)
<i>Z</i>	2
Radiation type	Cu <i>K</i> α
μ (mm ⁻¹)	5.94
Crystal size (mm)	0.12 × 0.07 × 0.03
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Analytical <i>CrysAlis PRO</i> , Agilent Technologies, Version 1.171.36.32 (release 02-08-2013 <i>CrysAlis171</i> .NET) (compiled Aug 2 2013,16:46:58) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (Clark, R. C. & Reid, J. S. (1995). <i>Acta Cryst.</i> A51, 887-897)
<i>T</i> _{min} , <i>T</i> _{max}	0.630, 0.858
No. of measured, independent and observed [<i>I</i> > 2 σ (<i>I</i>)] reflections	20417, 6125, 5665
<i>R</i> _{int}	0.032
($\sin \theta/\lambda$) _{max} (Å ⁻¹)	0.616
	Refinement
<i>R</i> [<i>F</i> ² > 2 σ (<i>F</i> ²)], <i>wR</i> [<i>F</i> ²], <i>S</i>	0.029, 0.074, 1.05
No. of reflections	6125
No. of parameters	406
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{\max}$, $\Delta\rho_{\min}$ (e Å ⁻³)	0.49, -0.71

Table 2. Experimental details for [4a]PF₆

Crystal data	
Chemical formula	C ₂₃ H ₂₁ ClN ₇ Ru·F ₆ P·C ₃ H ₆ O
<i>M_r</i>	855.13
Crystal system, space group	Monoclinic, <i>C2/c</i>
Temperature (K)	110
<i>a</i> , <i>b</i> , <i>c</i> (Å)	32.2377 (8), 13.6587 (3), 15.3929 (4)
β (°)	94.373 (2)
<i>V</i> (Å ³)	6758.1 (3)
<i>Z</i>	8
Radiation type	Mo <i>K</i> α
μ (mm ⁻¹)	0.67
Crystal size (mm)	0.16 × 0.12 × 0.04
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Gaussian <i>CrysAlis PRO</i> , Agilent Technologies, Version 1.171.36.32 (release 02-08-2013 <i>CrysAlis171</i> .NET) (compiled Aug 2 2013,16:46:58) Numerical absorption correction based on gaussian integration over a multifaceted crystal model
<i>T</i> _{min} , <i>T</i> _{max}	0.840, 1.000
No. of measured, independent and observed [<i>I</i> > 2 σ (<i>I</i>)] reflections	22327, 6645, 5405
<i>R</i> _{int}	0.038
($\sin \theta/\lambda$) _{max} (Å ⁻¹)	0.617

Refinement	
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.034, 0.080, 1.06
No. of reflections	6645
No. of parameters	532
No. of restraints	219
H-atom treatment	H-atom parameters constrained
	$w = 1/[\sigma^2(F_o^2) + (0.0273P)^2 + 16.154P]$ where $P = (F_o^2 + 2F_c^2)/3$
$\Delta\rho_{\max}, \Delta\rho_{\min}$ (e Å ⁻³)	0.56, -0.47

Table 3. Experimental details for [5a]Cl

Crystal data	
Chemical formula	C ₃₇ H ₂₃ ClN ₇ Ru-Cl
M_r	737.59
Crystal system, space group	Triclinic, <i>P</i> -1
Temperature (K)	110
a, b, c (Å)	13.6493 (4), 20.4870 (5), 28.9153 (8)
α, β, γ (°)	69.435 (2), 86.421 (2), 85.182 (2)
V (Å ³)	7539.0 (4)
Z	8
Radiation type	Cu K α
μ (mm ⁻¹)	4.94
Crystal size (mm)	0.17 × 0.07 × 0.02
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Analytical CrysAlis PRO, Agilent Technologies, Version 1.171.37.35 (release 13-08-2014 CrysAlis171 .NET) (compiled Aug 13 2014, 18:06:01) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (Clark, R. C. & Reid, J. S. (1995). Acta Cryst. A51, 887-897) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{\min}, T_{\max}	0.643, 0.904
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	67801, 29948, 16371
R_{int}	0.071
$(\sin \theta/\lambda)_{\text{max}}$ (Å ⁻¹)	0.598
Refinement	
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.049, 0.099, 0.77
No. of reflections	29948
No. of parameters	1694
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{\max}, \Delta\rho_{\min}$ (e Å ⁻³)	0.93, -0.77

References

- [1] G. M. Sheldrick, *Acta Crystallogr C Struct Chem* **2015**, *71*, 3-8.
- [2] A. L. Spek, *Acta Crystallogr C Struct Chem* **2015**, *71*, 9-18.

Appendix IV: Supporting information for Chapter 5

S.IV.1 Optimized structures of [1]PF₆ to [5]PF₆ by DFT (COSMO).

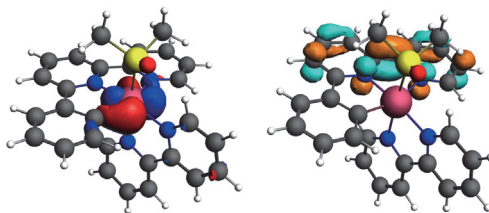


Figure S.IV.1. HOMO (left) and LUMO (right) of [1]PF₆ optimized by DFT (COSMO).

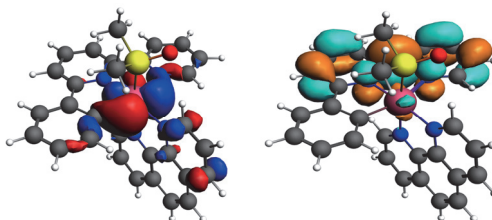


Figure S.IV.2. HOMO (left) and LUMO (right) of [2]PF₆ optimized by DFT (COSMO).

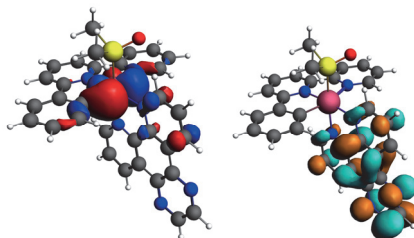


Figure S.IV.3. HOMO (left) and LUMO (right) of [3]PF₆ optimized by DFT (COSMO).

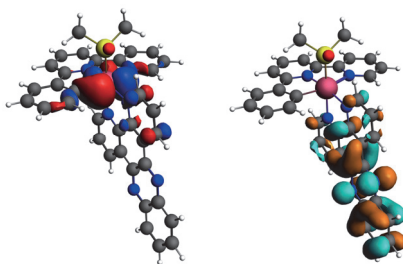


Figure S.IV.4. HOMO (left) and LUMO (right) of [4]PF₆ optimized by DFT (COSMO).

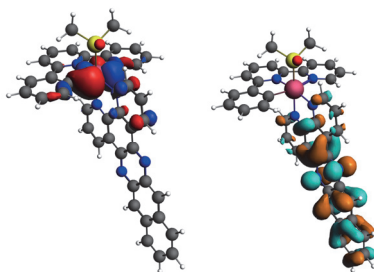


Figure S.IV.5. HOMO (left) and LUMO (right) of [5]PF₆ optimized by DFT (COSMO).

S.IV.2 Chiral HPLC trace of [11-C]PF₆ and [11-A]PF₆

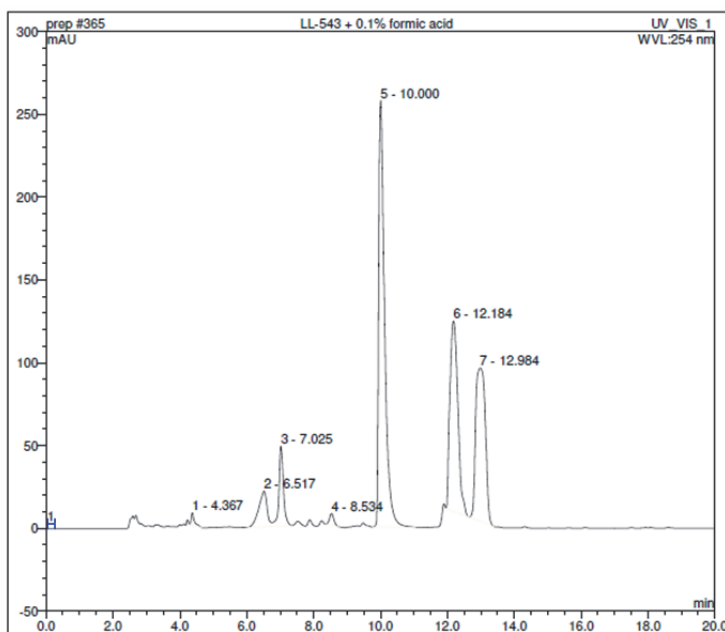
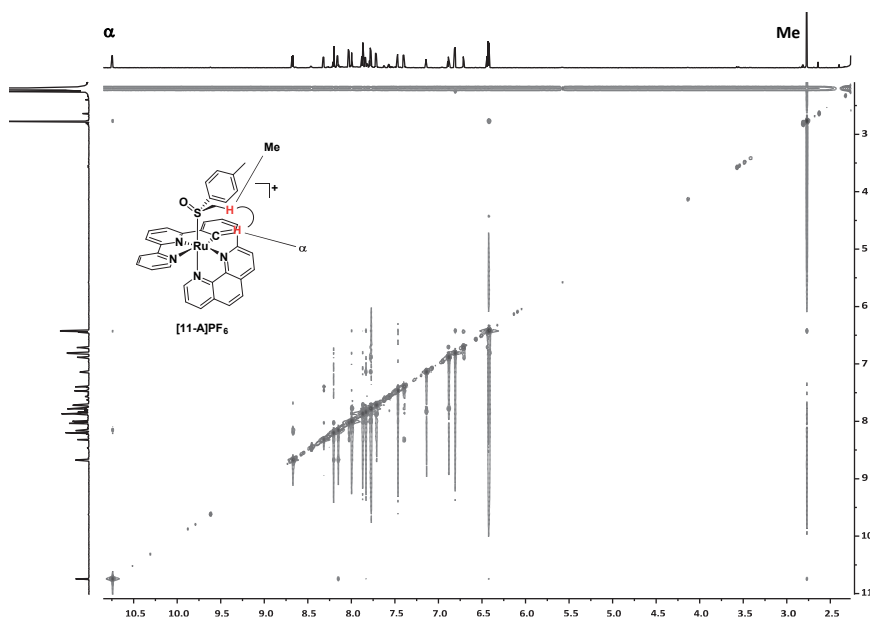
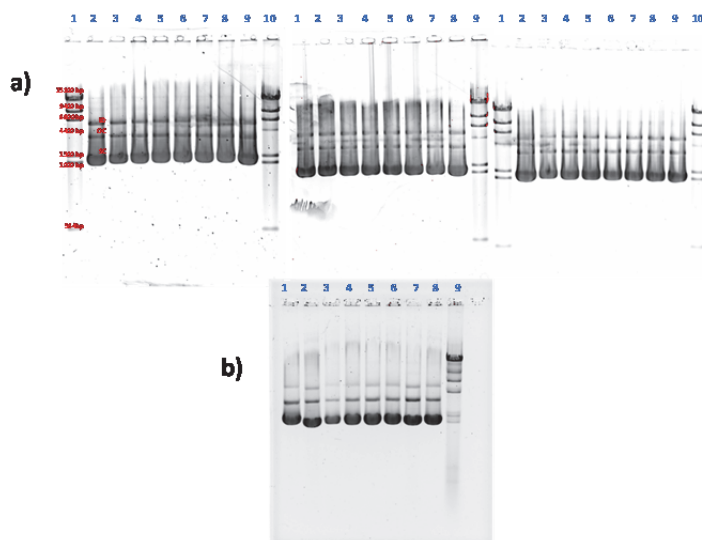


Figure S.IV.6. HPLC trace of [11-C]PF₆ (6, R_t = 12.184 min) and [11-A]PF₆ (7, R_t = 12.984 min).

S.IV.3 NOESY [11-A]PF₆

S.IV.4 Agarose DNA gels



Appendix V: Supporting information for Chapter 6

V.1 ^1H NMR evolution spectra in D_2O

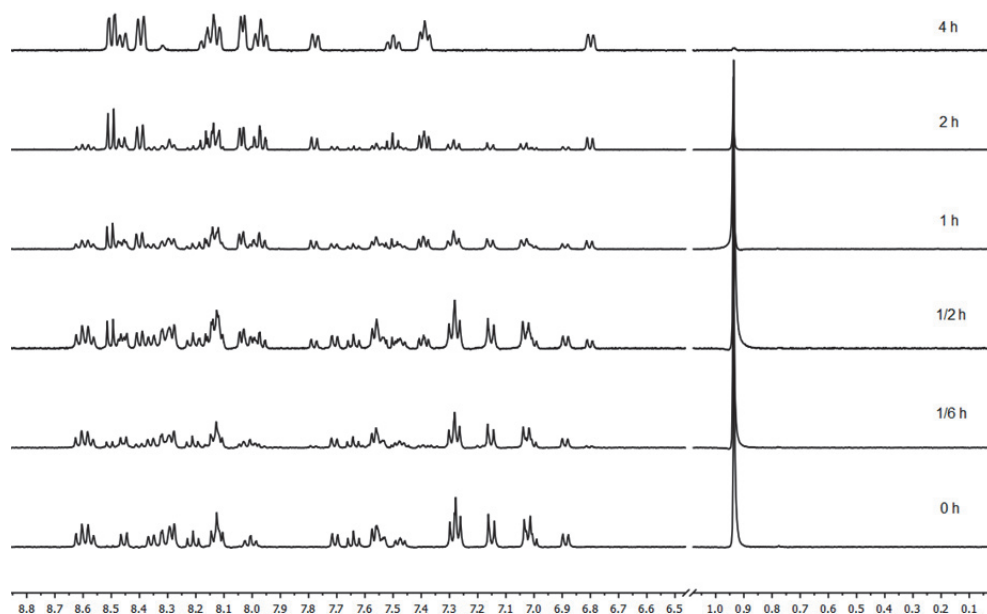


Figure S.V.1. ^1H NMR evolution spectrum of $[\text{1}]\text{Cl}_2$ in D_2O (1 mg in 0.6 mL) irradiated with white light using a 1000 W Xenon arc lamp fitted with a combined 400 nm cutoff filter, and a 610 nm long-pass filter 30 cm from the light source at $T = 298$ K. Spectra were taken at time 0, 10, 30, 60, 120 and 240 minutes on a Bruker 400 NMR.

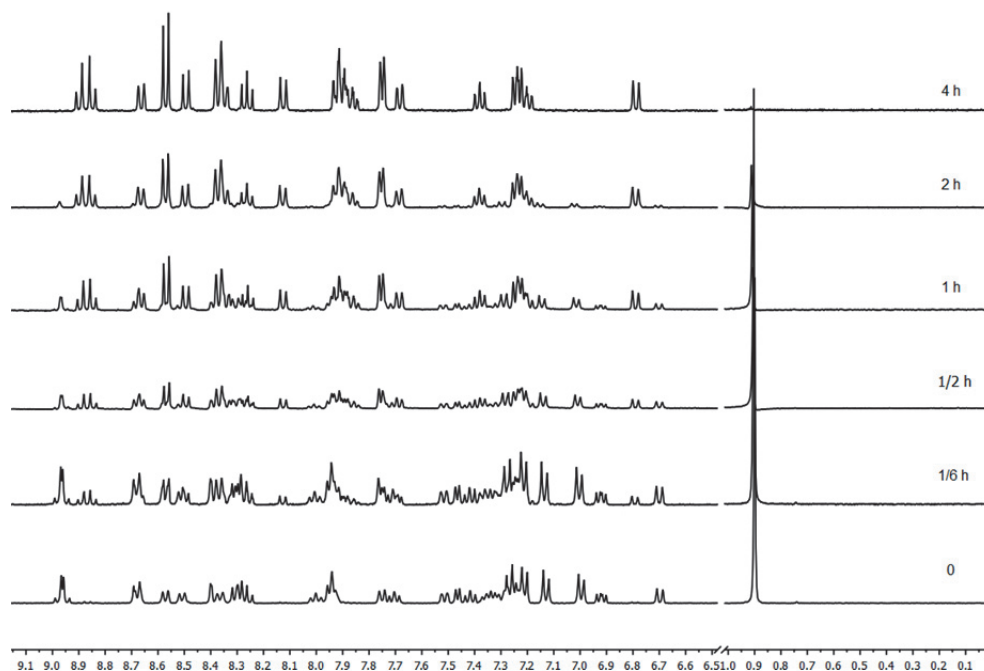


Figure S.V.2. ^1H NMR evolution spectrum of 1 mg of $[2]\text{Cl}_2$ in 0.6 mL D_2O irradiated using a Xenon arc lamp fitted with a combined 400 nm cutoff filter, and a 610 longpass filter 30 cm from the light source at $T = 298$ K. Spectra were taken at intervals $t = 0, 10, 30, 60, 120$ and 240 minutes on a Bruker 400 NMR.

V.2 Cell culturing

General

Human cancer cell lines (A549, human lung carcinoma; MCF-7, human breast adenocarcinoma, A431, human epidermoid) and the non-cancerous cell line MRC-5 (fetal lung fibroblasts) were distributed by the European Collection of Cell Cultures (ECACC), and purchased through Sigma Aldrich. Dulbecco's Minimal Essential Medium (DMEM, with and without phenol red, without glutamine), 200 mM Glutamine-S (GM), trichloroacetic acid (TCA), glacial acetic acid, sulforhodamine B (SRB), tris(hydroxymethyl)aminomethane (tris base), were purchased from Sigma Aldrich. Fetal calf serum (FCS) was purchased from Hyclone. Penicillin and streptomycin were purchased from Duchefa and were diluted to a 100 mg/mL penicillin/streptomycin solution (P/S). Trypsin and Opti-MEM® (without phenol red) were purchased from Gibco® Life Technologies. Trypan blue (0.4% in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution) was purchased from BioRad. Plastic disposable flasks and 96-well plates were purchased from Sarstedt. Cells were counted using a BioRad TC10 automated cell counter with Biorad Cell Counting Slides. UV-vis measurements for analysis of 96-well plates were performed on a M1000 Tecan Reader. Cells were inspected with an Olympus IX81 microscope.

Cell culturing under normoxia and hypoxia

Cells were cultured in DMEM complete (Dulbecco's Modified Eagle Medium (DMEM) with phenol red, supplemented with 8.0% v/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 0.9% v/v Glutamine-S (GM)). Cells were cultured under humidified conditions, 37 °C atmosphere, 21% O₂ and 7.0% CO₂ in 75 cm² flasks and sub-cultured (1:3 to 1:6 ratio) upon reaching 70-80% confluency (approximately once per week). Media was refreshed every second day. Cells were passaged for 4 - 8 weeks. Cells under hypoxia were cultured under similar conditions, but under an atmosphere of 1.0% O₂ with 7.0% CO₂ in a hypoxic incubator (New Brunswick Galaxy 170R).

V.3 Cell irradiation setup for normoxia and hypoxia

96-well plates were irradiated using the setup described in detail earlier.^[1] For irradiation under hypoxic conditions a Tokai Hit® stage top incubator (INUBG2ETFP-WSKM) with sensor lid for multi-well plate (W-200F) was coupled to a GM-8000 digital gas mixer (Figure S.V.4). 96-well plates were placed in the incubator and the red LED-array described earlier^[1] was placed on top of the sensor lid (give ref van the LEDs). The power-intensity of the red LED array was determined using a custom-built integrating sphere setup and was found to be 23.0 ± 1.5 mW cm⁻² using the setup under hypoxic conditions. Under normoxic conditions the same LED array was placed directly on top of the well plates (with lid), leading to slightly higher power density at the level of the cell monolayer, as reported earlier (34.4 ± 1.7 mW cm⁻²).^[1] In such conditions, 15 min of red light irradiation under hypoxia corresponded to the same dose as 10 min irradiation under normoxia (20.6 J.cm⁻²).

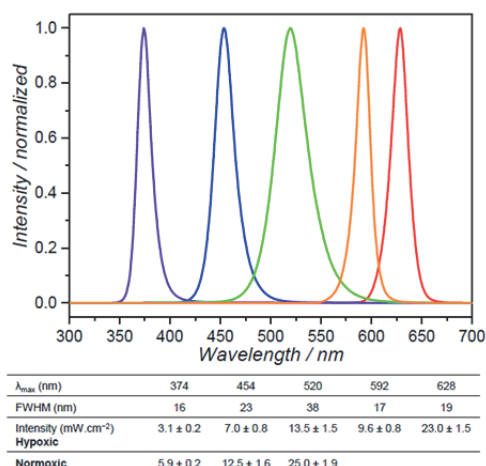


Figure SV.3. Spectroscopic characteristics and light intensities (mW cm⁻²) for each LED at normoxic and hypoxic conditions.

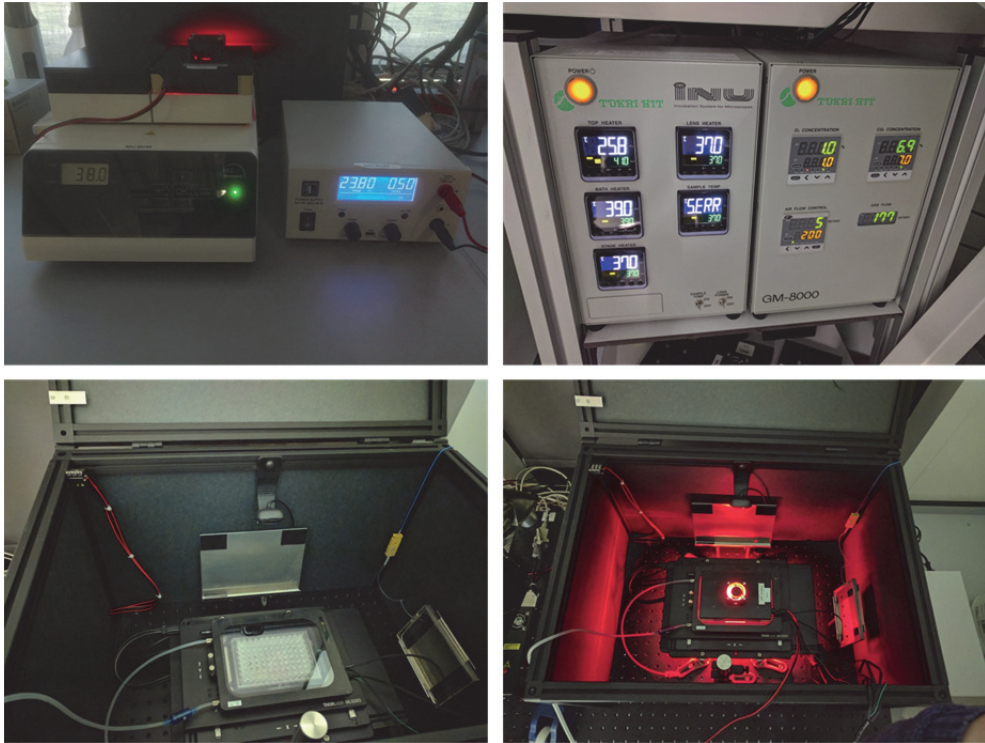


Figure S.V.4. Top left: LED irradiation system fitted with red LED array. Top right: Hypoxic digital gas mixer and stage top incubator temperature controller. Lower left: Stage top incubator with 96-well plate. Lower right: Stage top incubator with 96-well plate fitted with red LED array (on).

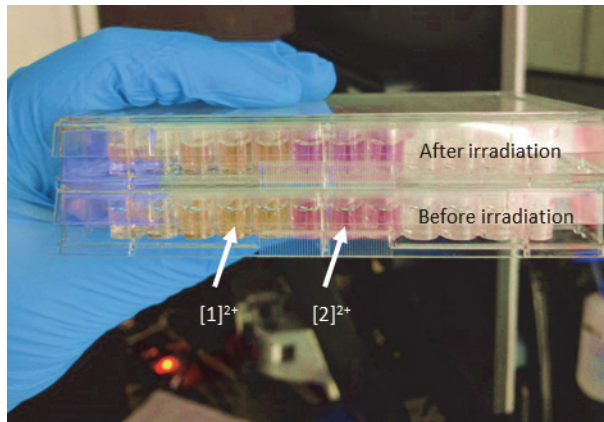


Figure S.V.5. $[1]Cl_2$ and $[2]Cl_2$ before (bottom, orange and pink) and after (top, brown and purple) red light irradiation. Top: $[1]Cl_2$ and $[2]Cl_2$ left in the dark. Bottom: $[2]Cl_2$ and $[2]Cl_2$ after irradiation with 625 nm for 10 minutes in the normoxia setup.

V.4. Cytotoxicity Assay

Treatment under normoxia and hypoxia

The treatment protocol was carried out as described in our previous paper^[2] with the following modifications: Cells were treated with aliquots of test compounds dissolved in OMEM with the exception of STF-31 which was dissolved in 1% DMSO in OMEM, with final concentration not exceeding 0.5% DMSO. Cells were incubated for 6 hours after which one plate was treated with red light (628 ± 19 nm, 34.4 mW $\pm 1.7 \cdot \text{cm}^{-2}$, 10 minutes, $20.6 \cdot \text{J cm}^{-2} \pm 1.02$), while the other was kept in the dark at 37 °C. For treatment under hypoxia, one plate was kept in the hypoxic incubator (New Brunswick Galaxy 170R, 1.0% O₂, 7.0% CO₂) while the other plate was transferred quickly from the hypoxic incubator to the stage top incubator allowing to settle for 15 minutes to reach 37 °C and an atmosphere of 1% O₂ and 7.0% CO₂, followed by irradiation with red light (628 ± 19 nm, 23.0 ± 1.5 mW $\cdot \text{cm}^{-2}$, 15 minutes, $20.6 \cdot \text{J cm}^{-2} \pm 2.03$). After 18 hours medium was replaced with OMEM, and after a total of 96 hours after seeding cells were fixed by adding cold TCA (10% w/v; 100 mL) in each well. The SRB assay was carried out as described before.^[2] All values were determined as the mean of three independent biological experiments, with three technical triplicates for each biological replicate. Results are summarized in table S.V.1.

Table S.V.1. (Photo)cytotoxicity of [1]Cl₂, [2]Cl₂ and STF-31 expressed as effective concentrations (EC₅₀ in μM) in the dark and after irradiation with red light (628 ± 19 nm, 34.4 mW $\pm 1.7 \cdot \text{cm}^{-2}$, 10 minutes, $20.6 \cdot \text{J cm}^{-2} \pm 1.02$) under normoxic (21% O₂) conditions versus A549, A431, MCF-7 and MRC-5 cells. And versus A549 and A431 cells under hypoxic (1.0% O₂) conditions with red light (628 ± 19 nm, 23.0 ± 1.5 mW $\cdot \text{cm}^{-2}$, 15 minutes, $20.6 \cdot \text{J cm}^{-2} \pm 2.03$).

Cell line	% O ₂	Light dose (J cm ²)	[1]Cl ₂			[2]Cl ₂			STF-31		Cisplatin	
			EC ₅₀	CI	PI	EC ₅₀	CI	PI	EC ₅₀	CI	EC ₅₀	CI
A549	20	0	8.9	+2.7	0.93	20.3	+2.9	2.6	4.4	+2.1	4.8	+0.89
				-2.1			-2.5			-1.5		-0.76
	1	20.6	9.6	+2.1		7.7	+1.4		4.2	+1.7	-	
				-1.8	1.2	45.6	+9.1	2.4	10.8	+5.7	7.5	+1.7
A431	20	0	20.6	+9.4		45.6	+9.1		10.8	+5.7	7.5	+1.7
				-6.0			-7.3			-3.7		-1.4
	1	20.6	17.9	+6.4		18.7	+5.8		-		-	
				-4.6			-4.3					
MCF-7	20	0	5.9	+2.1	0.67	23.6	+4.2	3.3	2.9	+0.79	6.6	+0.94
				-1.7			-3.5			-0.66		-0.81
	1	20.6	8.8	+2.4		7.1	+2.0		3.2	+0.73	-	
				-1.9	1.1	34.6	+3.8	3.6	21	+7.2	11.6	+2.2
MRC-5	20	0	10.7	+4.2		34.6	+3.8		21	+7.2	11.6	+2.2
				-3.0			-3.5			-5.1		-1.8
	1	20.6	9.9	+3.3		9.6	+2.1		-		-	
				-2.5	1.3	20.5	+3.6	1.6	4.8	+2.6	10.6	+1.9
MCF-7	20	0	12.7	+4.8		20.5	+3.6	1.6	4.8	+2.6	10.6	+1.9
				-3.6			-3.1			-1.8		-1.5
	1	20.6	16.8	+4.3		13.1	+1.5		6.2	+2.5	-	
				-3.4	1.3	45.8	+11.9	2.4	11.8	+4.0	5.9	+1.4
MRC-5	20	0	26.1	+10.2		45.8	+11.9	2.4	11.8	+4.0	5.9	+1.4
				-6.9			-9.3			-3.0		-1.1
	1	20.6	20.5	+7.1		18.8	+6.3		10.5	+3.4	-	
				-5.2			-4.7			-2.6		

V.5 NBDG uptake

A549 cells were seeded in 6.5 cm² dishes at a density of 2.5×10^5 per well in OMEM. After 24 hours, reaching a confluency of ~80%, the media was aspirated and the cells were washed 3 x times with 1 mL PBS. Vehicle control, STF-31 (50 μ M) and phloretin (250 μ M) dissolved in phenol-red and glucose-free DMEM[®] media were then added to each well (V = 1.0 mL). After incubating for one hour at 37 °C, 0.5 mL of a stock solution (300 μ g/mL) of 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG, Cayman Chemical Company) in MilliQ was added. At t = 3 h, media was removed and each well was washed with cold PBS twice and cells were trypsinized (500 μ L), glucosefree medium with 2.5 % FCS was added (500 μ L) and the content of the well were transferred to 1.5 mL Smart-Lock Eppendorf[®] Cups. After centrifugation (5 min, 1200 RCF, rt), the supernatant was gently removed, cells were resuspended in 0.5 mL DMEM glucosefree medium and transferred to a 96-well plate with V = 200 μ L per well. One out of two groups were stained with propidium iodide (PI) with $c_{final} = 1 \mu$ g/mL. Flow cytometry analysis was performed within 30 minutes on a FACSCanto II (Becton Dickinson, Franklin Lakes, NJ). The cells were examined on 4 different parameters: relative size (Forward Scatter), granularity or internal complexity (Side Scatter), and FITC-A ($\lambda_{ex} = 488 \text{ nm}$, $\lambda_{em} = 530 \pm 15 \text{ nm}$) and APC-A ($\lambda_{ex} = 633 \text{ nm}$, $\lambda_{em} = 660 \pm 10 \text{ nm}$) for 2-NBDG and PI, respectively. Ten thousand events were recorded in the gated region within 100 seconds, and data were analyzed using FlowJo software (Treestar, Ashland, OR). Healthy cells were defined as propidium iodide (PI)-negative, and necrotic cells were defined as PI-positive.

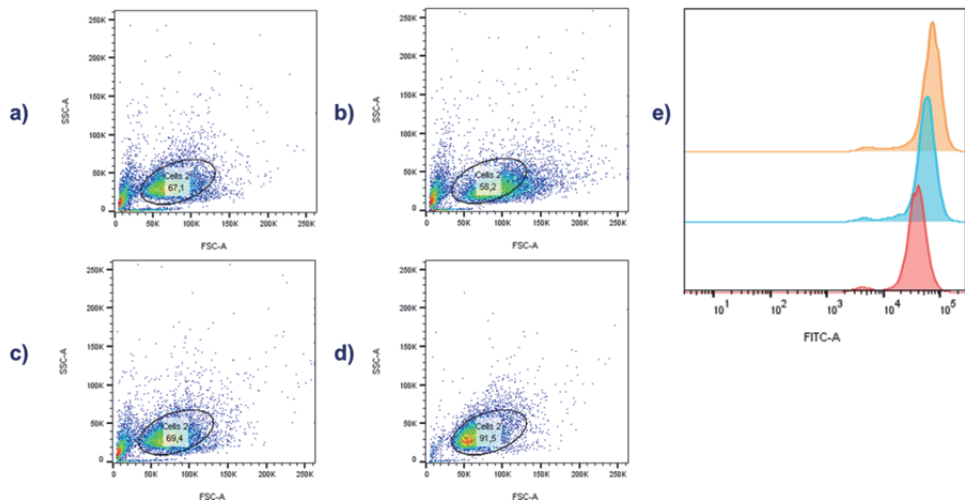


Figure S.V.6. Dot plots of a). Control with only NBDG. b). Phloretin (250 μ M). c). STF-31 (50 μ M) and negative control containing only medium (DMEM glucosefree). Dot plots are representative of three independent experiments. e). Histograms showing fluorescence of 2-NBDG ($\lambda_{ex} = 488 \text{ nm}$) after 3 hour treatment with control (top), 50 μ M STF-31 (middle) and 100 μ M phloretin (lower).

V.6 NAMPT assay

The Cyclax[®] NAMPT assay (Sanbio BV Biologicals) was carried out as described in the company manual using method II with the following modifications: Half the concentration of each component in the kit was used to allow measurements over a longer period of time and to prevent oversaturation of the absorbance for the control wells. The IC₅₀ of compound [2]Cl₂ and STF-31 were determined by measuring the reaction rate for four different concentrations of compound. Stock solutions for STF-31 and [2]Cl₂ were diluted in DMSO to four different concentrations. For [2]Cl₂ an aliquot of the stock solution was irradiated for 10 minutes in the normoxia setup and further diluted in DMSO. Slopes were determined using linear regression during the linear range (0-10 minutes) and then the slope of regression lines was plotted against compound concentration. Using the dose-response curve the inhibition concentration IC₅₀ (defined as the concentration of drug that inhibits the enzyme activity by 50%) was calculated by fitting the curves using a non-linear regression function with fixed Y maximum (100%) and minimum (0%) (relative cell viability), and a variable Hill-slope, resulting in the simplified two-parameter Hill-slope equation 2. All graphs were plotted using Graphpad Prism 7, Graphpad Software Inc.

$$\frac{100}{(1 + 10^{((\log_{10} IC_{50} - X) \times Hill\ Slope)})}$$

Equation V.1

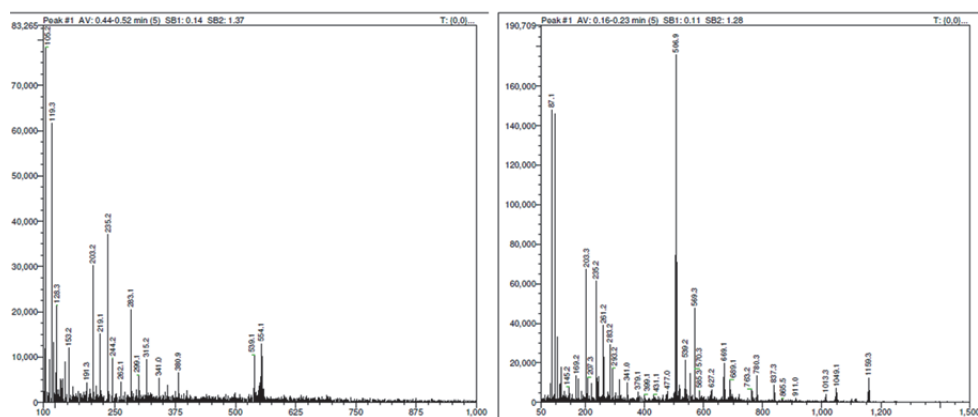


Figure S.V.7. Left: MS spectrum showing conversion of [1]Cl₂ to [Ru(tpy)(dmbpy)Cl]⁺ after 48 hours exposure to OMEM. Right: [2]Cl₂ after 48 hours.

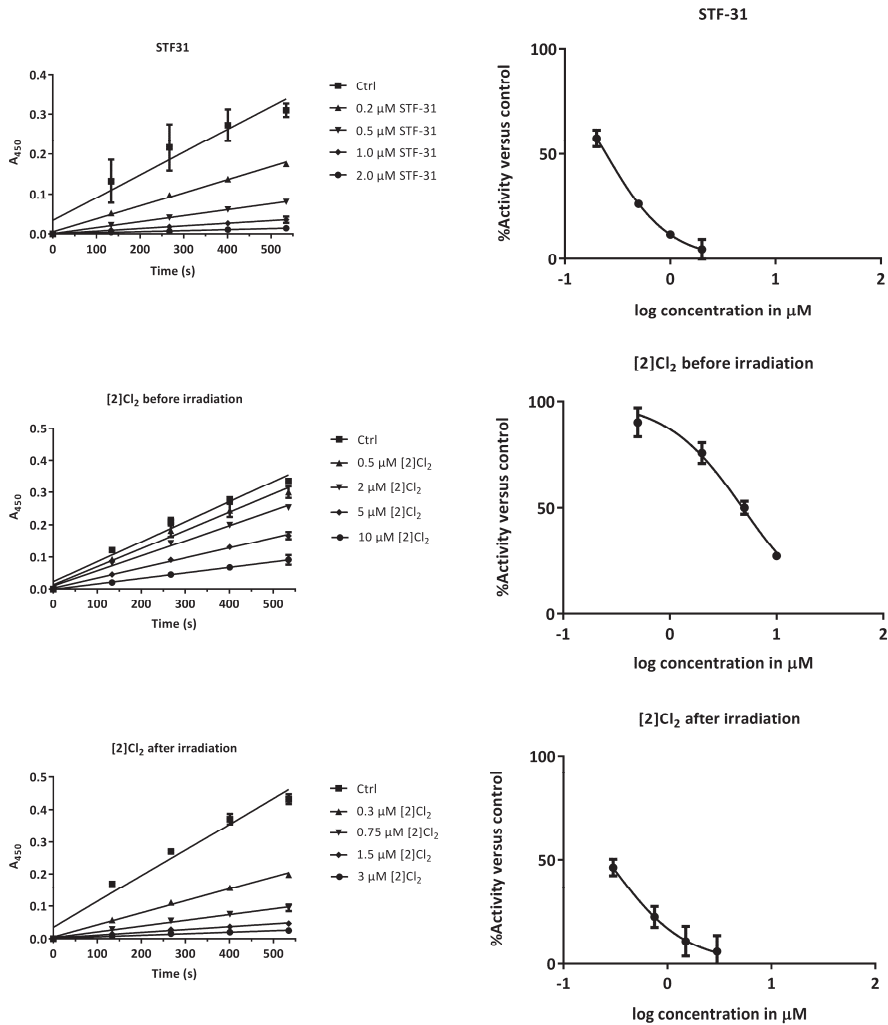


Figure S.V.8. Left: A_{450} of formazan against time (s) at different concentrations of STF-31, $[2]Cl_2$ -dark and $[2]Cl_2$ -light. $[2]Cl_2$ before and after irradiation with red light versus absorbance of formazan at 450 nm. Right: Dose response curves of %activity of enzyme versus the logarithm of concentration of substrate in μM .

References

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