Supplementary Material

# Osmium (II) polypyridyl probe with a therapeutic twist: disrupting the mitochondrial membrane potential for targeted therapy

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## $^{1}$ H NMR spectra of tpybenzCOOH and Os(II) complexes

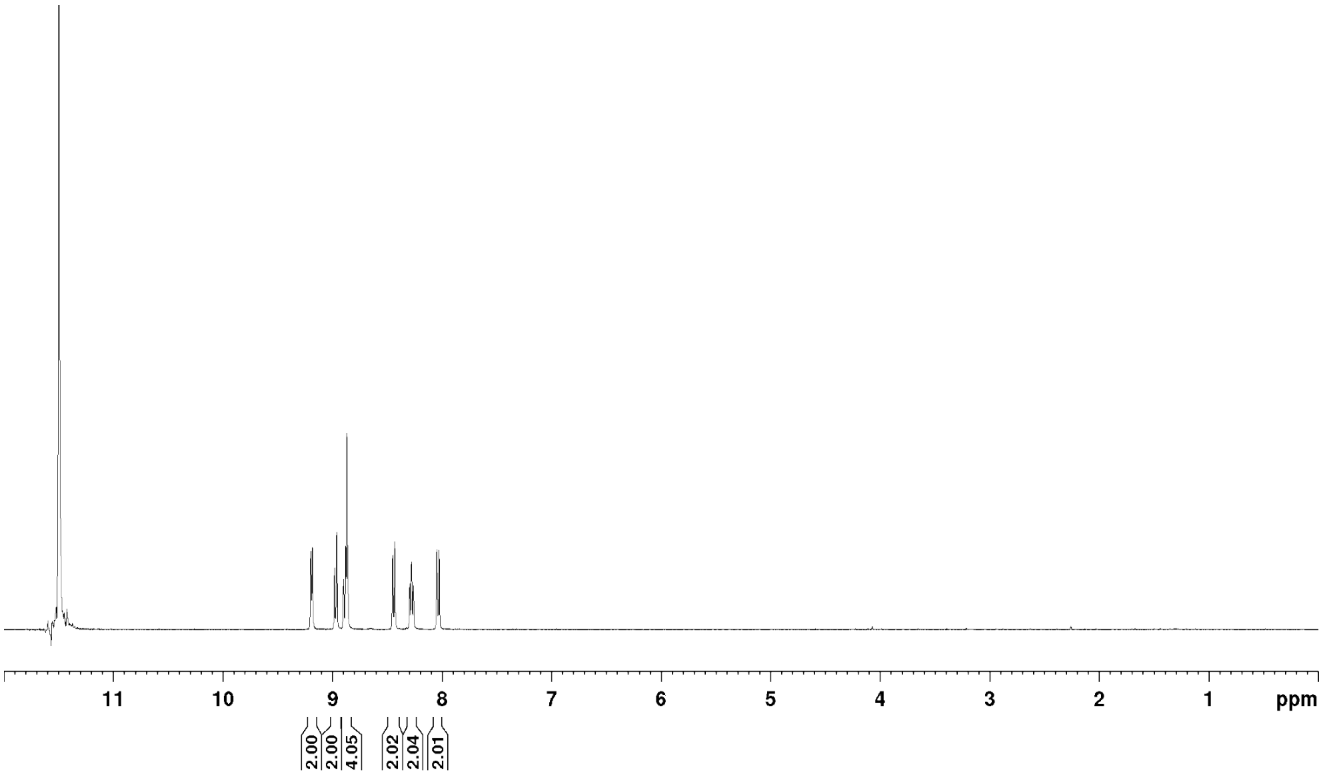



Figure S1: $^{1}$H-NMR spectra of tpybenzCOOH in DMSO-$d_6$ (top) and TFA-d, (bottom) 600 MHz

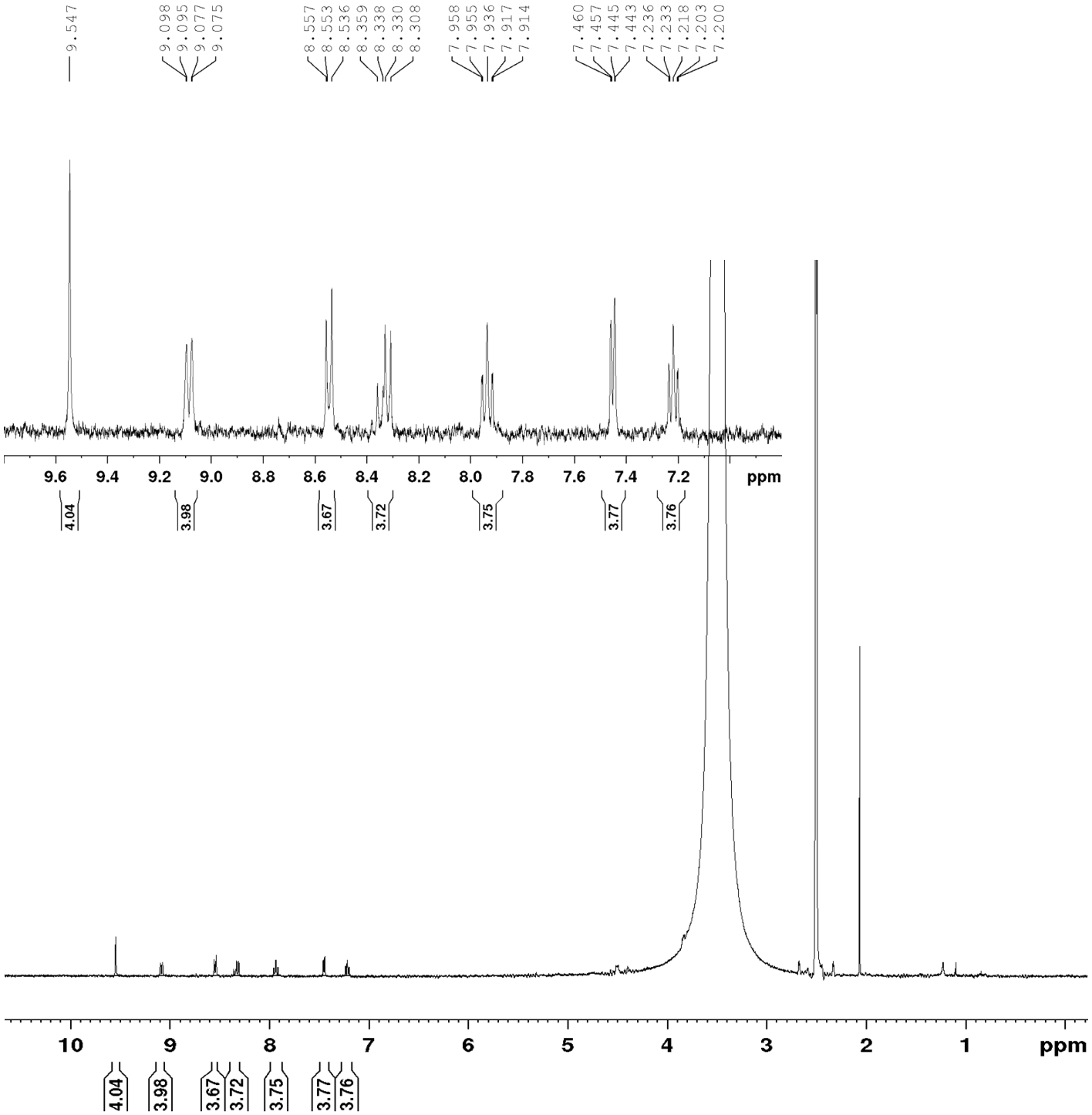



Figure S2: $^{1}H$ NMR of purified $[Os(tpybenzCOOH)_2]^{2+}$ in DMSO-$d_6$, 400 MHz

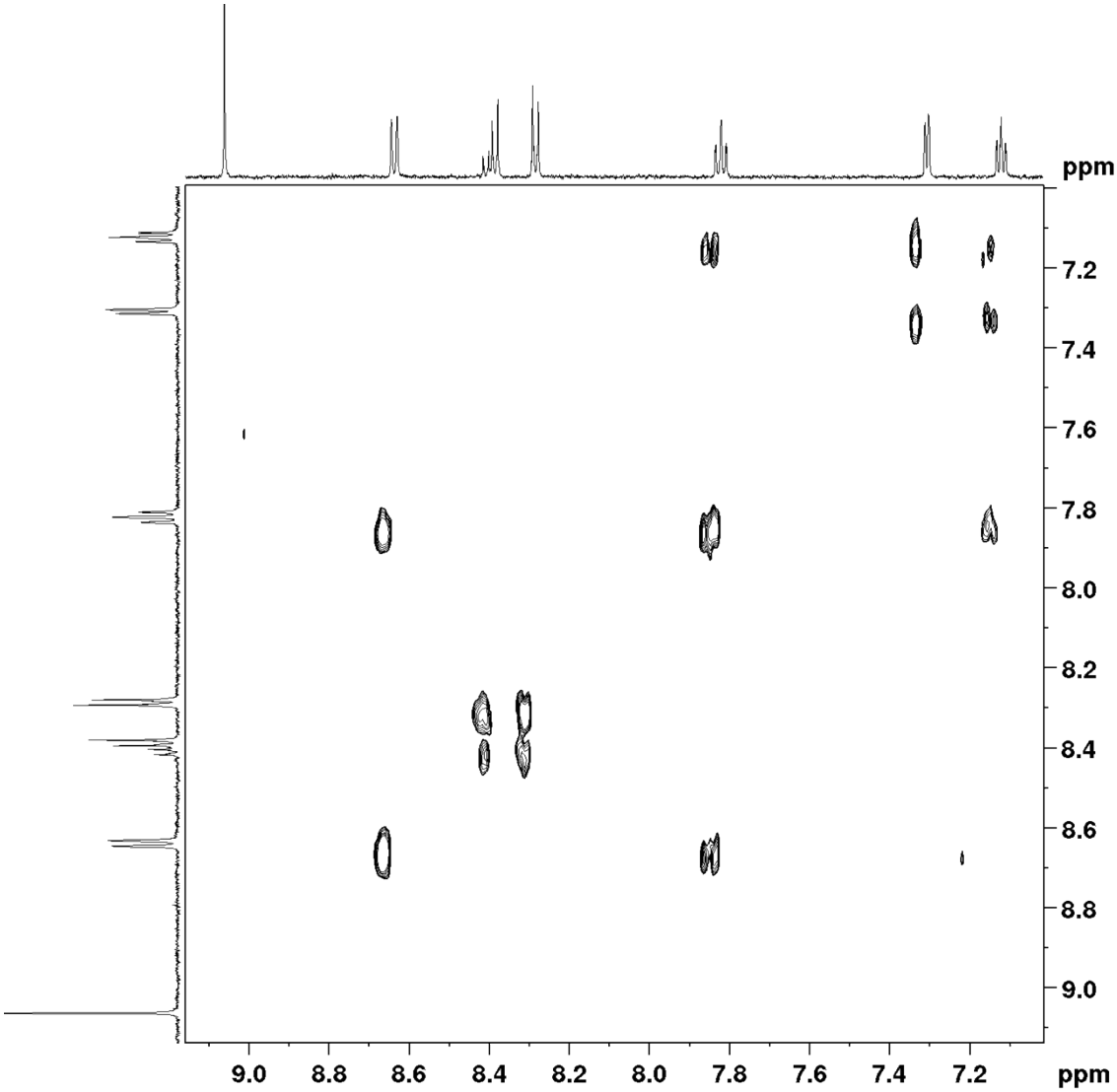



Figure S3: COSY spectrum of $[Os(tpybenzCOOH)_2]^{2+}$ in MeCN-$d_3$, 600 MHz

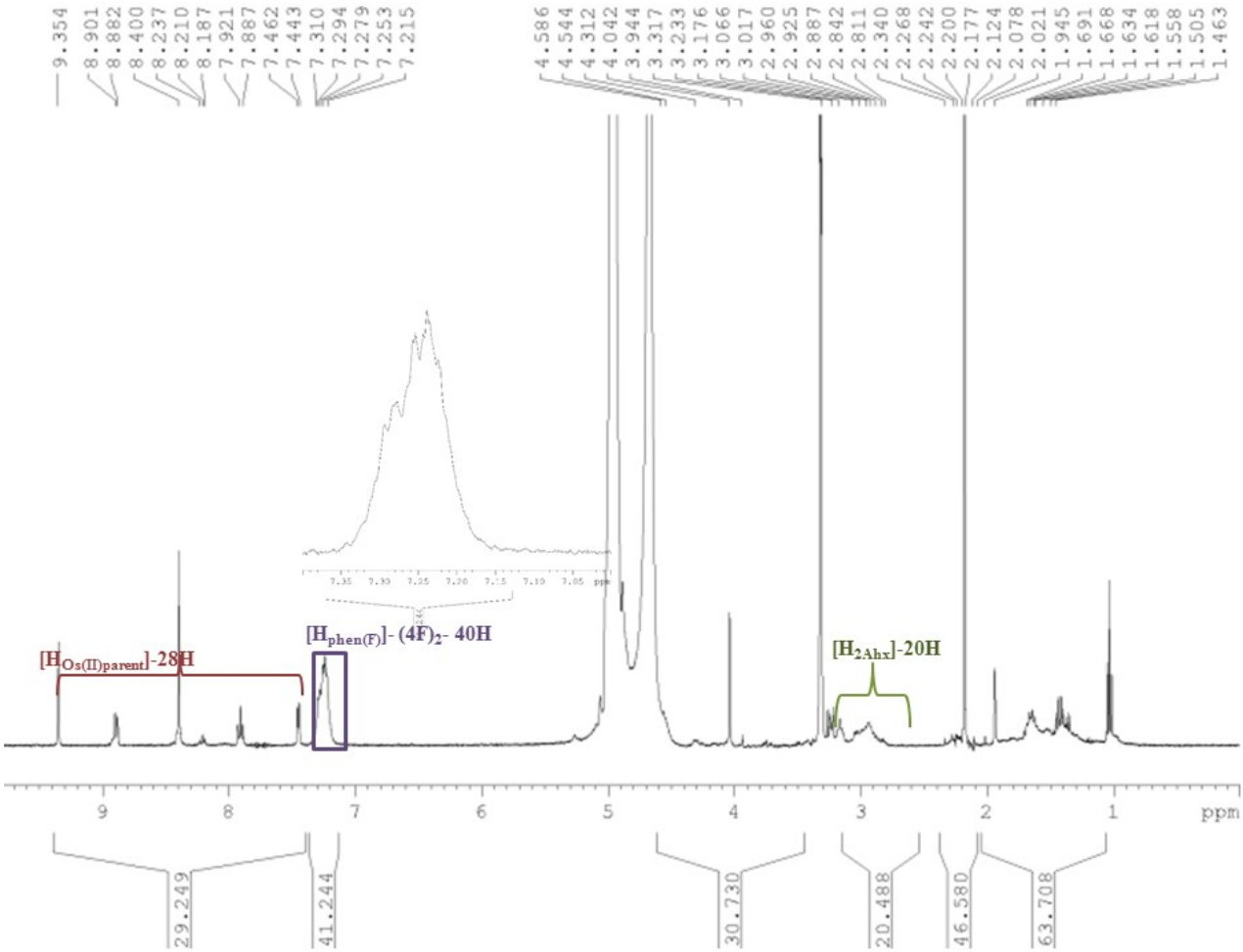



Figure S4: $^{1}$H NMR spectrum of $Os^{II}$MPP in MeOH-$d_4$, 400 MHz with key regions in the aromatic and aliphatic region highlighted

# Mass Spectrometry Analysis

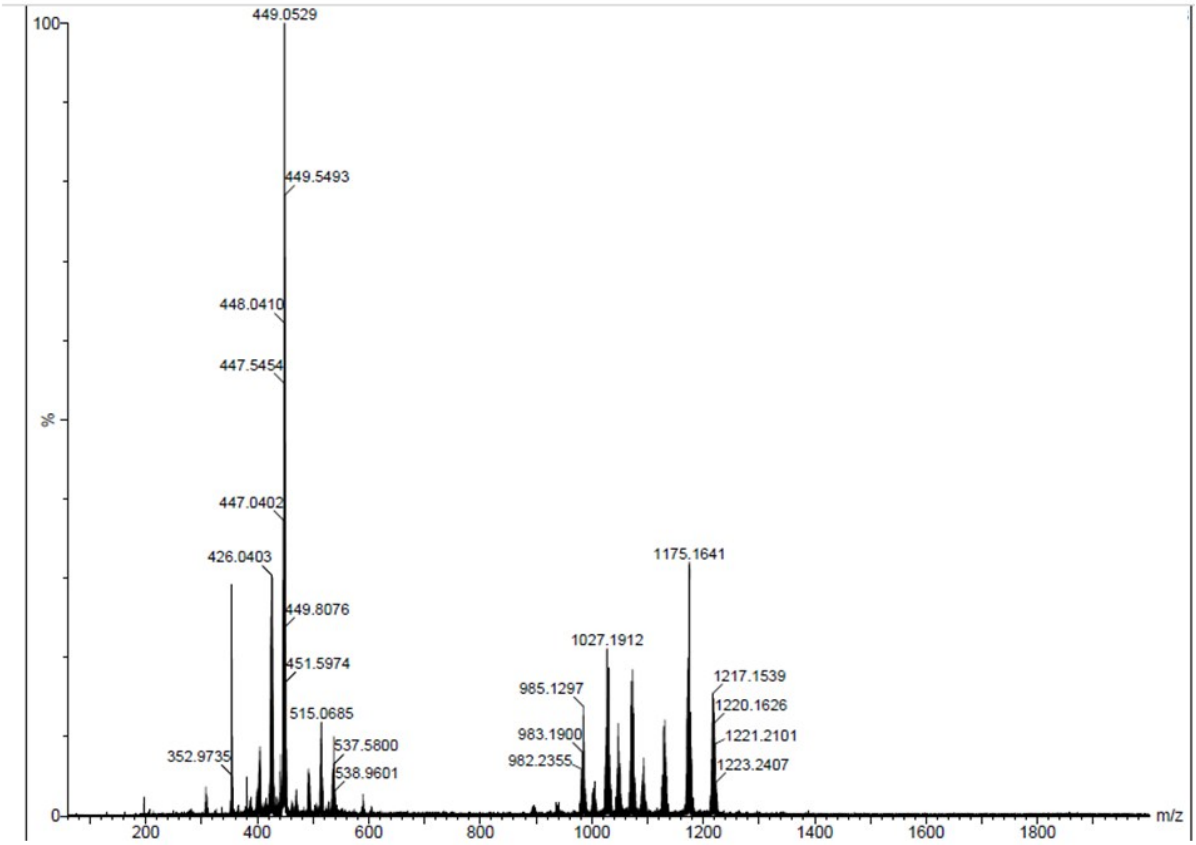



Figure S5: HRMS (TOF ES+) of $[Os(tpybenzCOOH)_2]^{2+}$

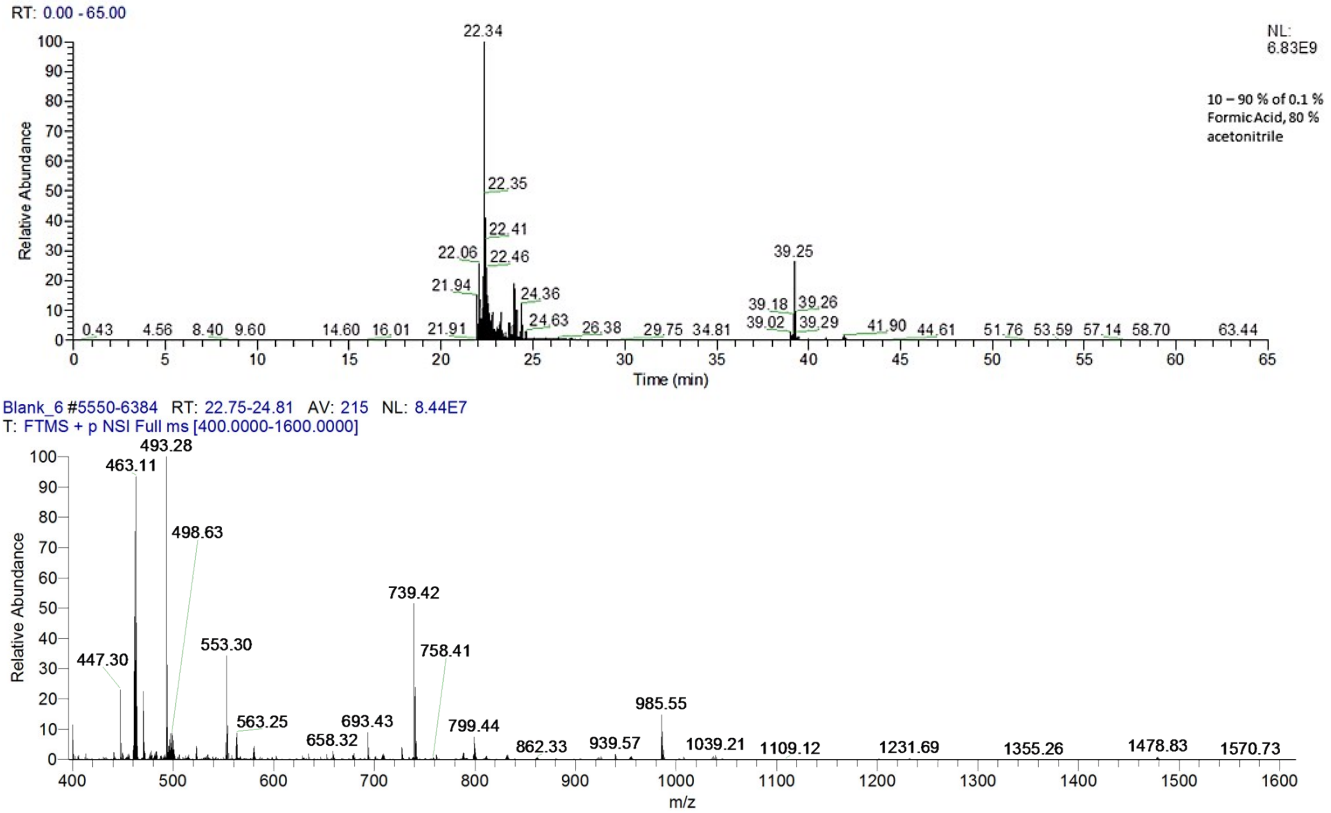



Figure S6: HPLC chromatogram (above) and Mass Spectrum (below) of $Os^{II}$ MPP complex following LC-MS analysis (Q-Exactive). Conditions; gradient extended from 10- 90% (Formic Acid 0.1%, 80% MeCN).

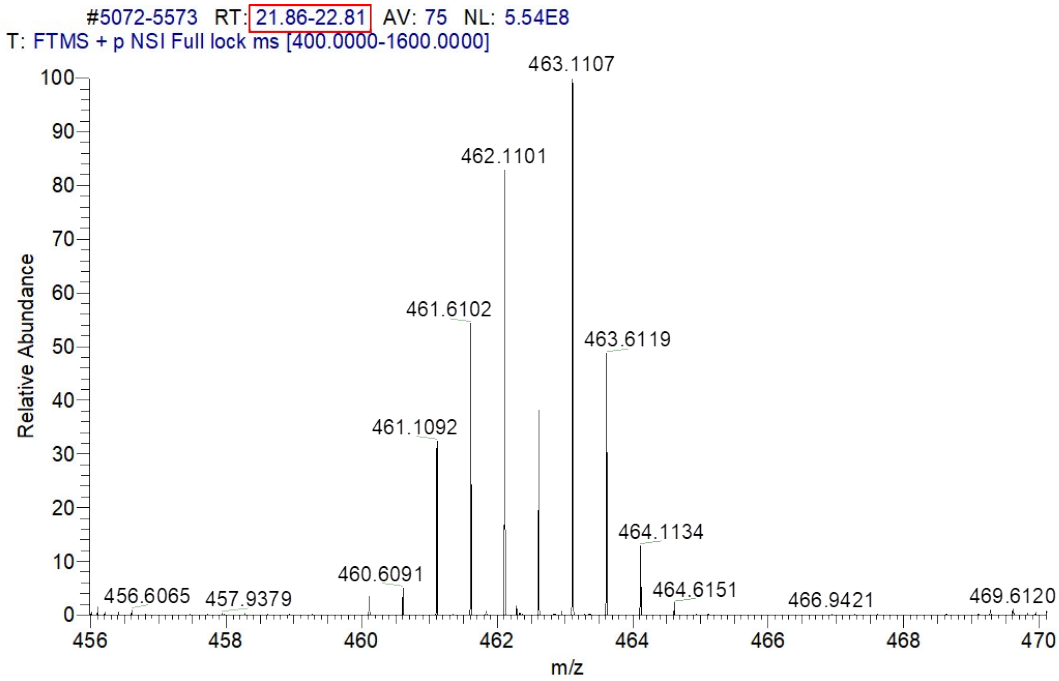



Figure S7: LC-MS analysis of $Os^{II}$MPP *Zoomed m/z 456-470 (RT 21-23 min)*

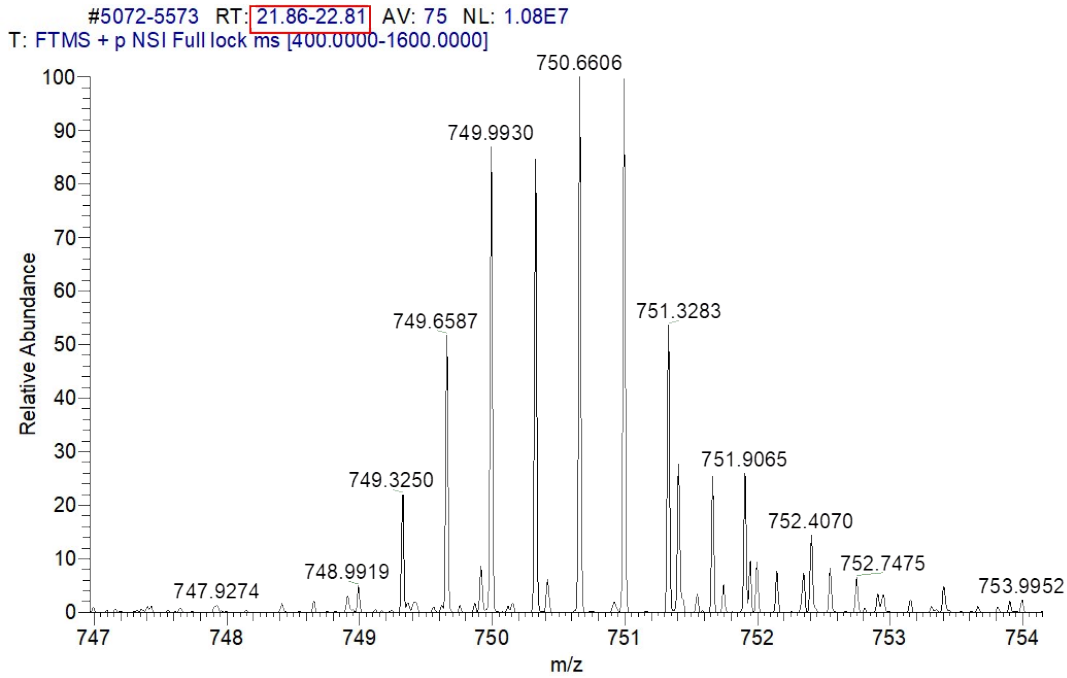



Figure S8: LC-MS analysis of $Os^{II}$MPP *Zoomed m/z 747-754 (RT 21-23 min)*

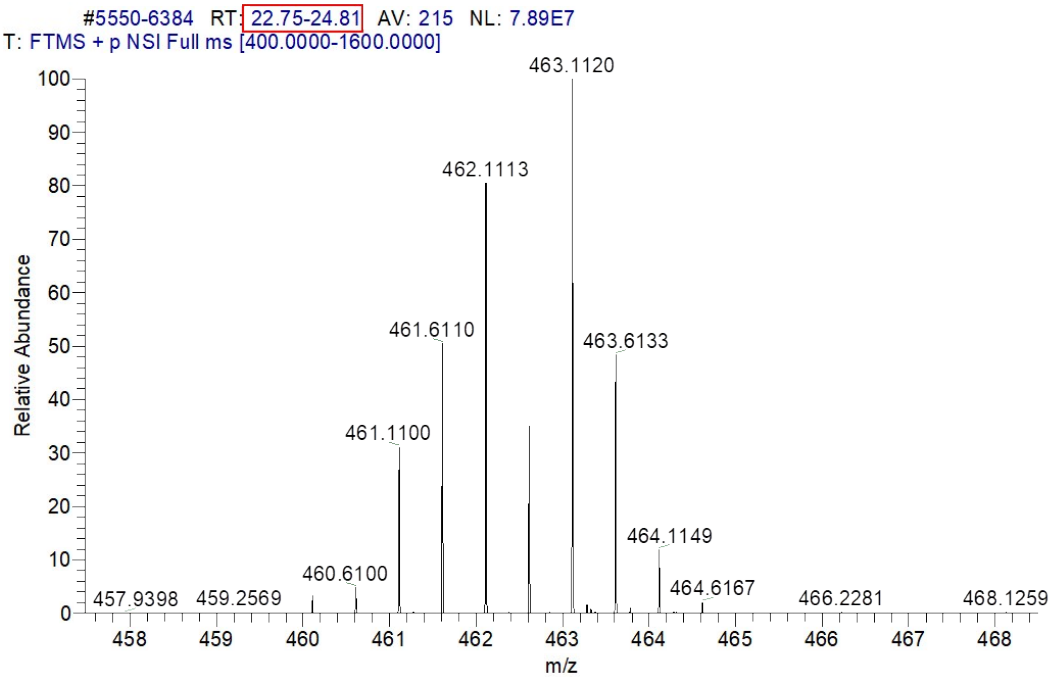



Figure S9: LC-MS analysis of Os$^{II}$MPP *Zoomed m/z 458-468 (RT 23-25 min)*

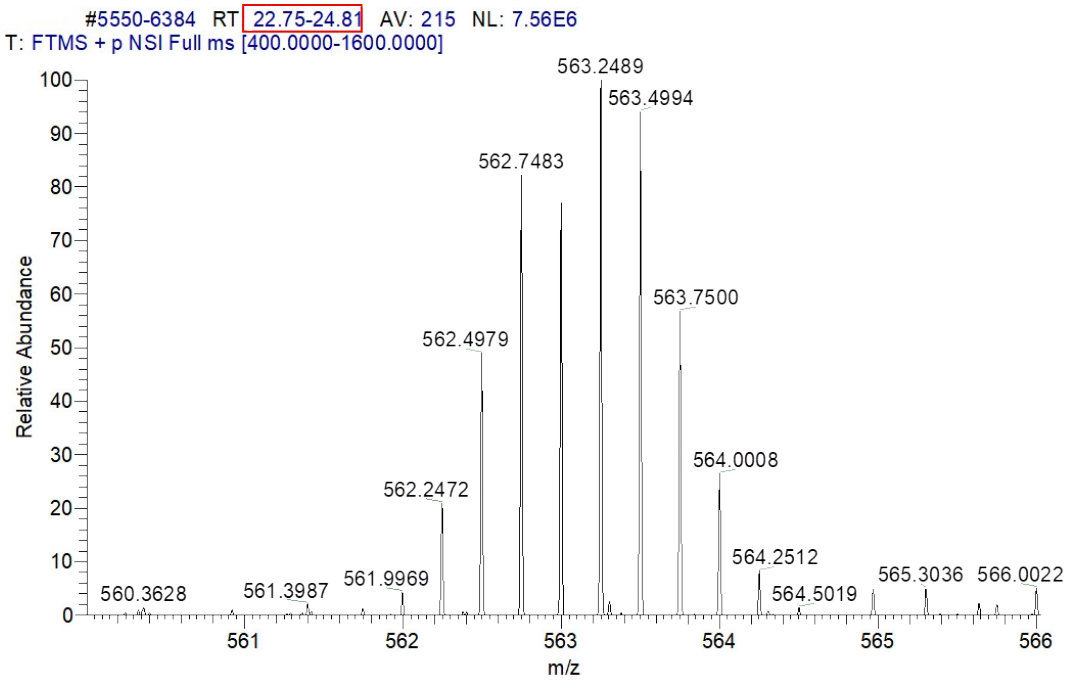



Figure S10: LC-MS analysis of Os$^{II}$MPP *Zoomed m/z 560-566 (RT 23-25 min)*

HPLC Analysis of parent and conjugate Os(II) complex

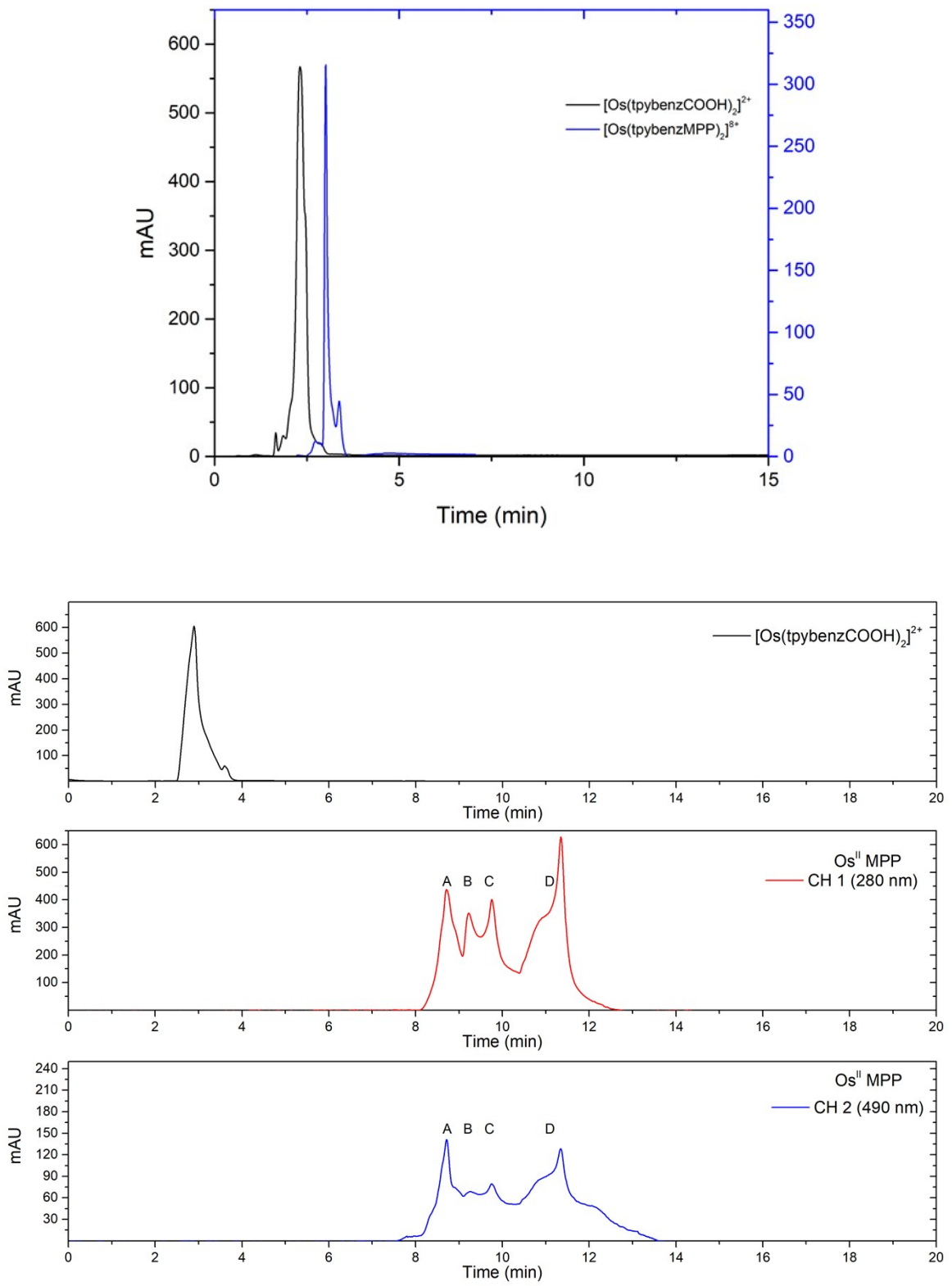



Figure S11: Top: HPLC Chromatogram of $Os^{II}$parent complex and $Os^{II}$MPP conjugate obtained RP-C18 HPLC with MeCN mobile phase. Bottom three traces show traces using gradient mobile phase detected at 280 nm. (Gradient MeCN/ $H_2O$ 0.1% TFA gradient, 1ml $min^{-1}$). Elution of $Os^{II}$MPP conjugate at 8.3 min (Channel 1: 280 nm and Channel 2: 490nm). Diode array detection was used and the HPLC UV-vis spectrum of each peak was obtained during $Os^{II}$ MPP analysis and is shown below.

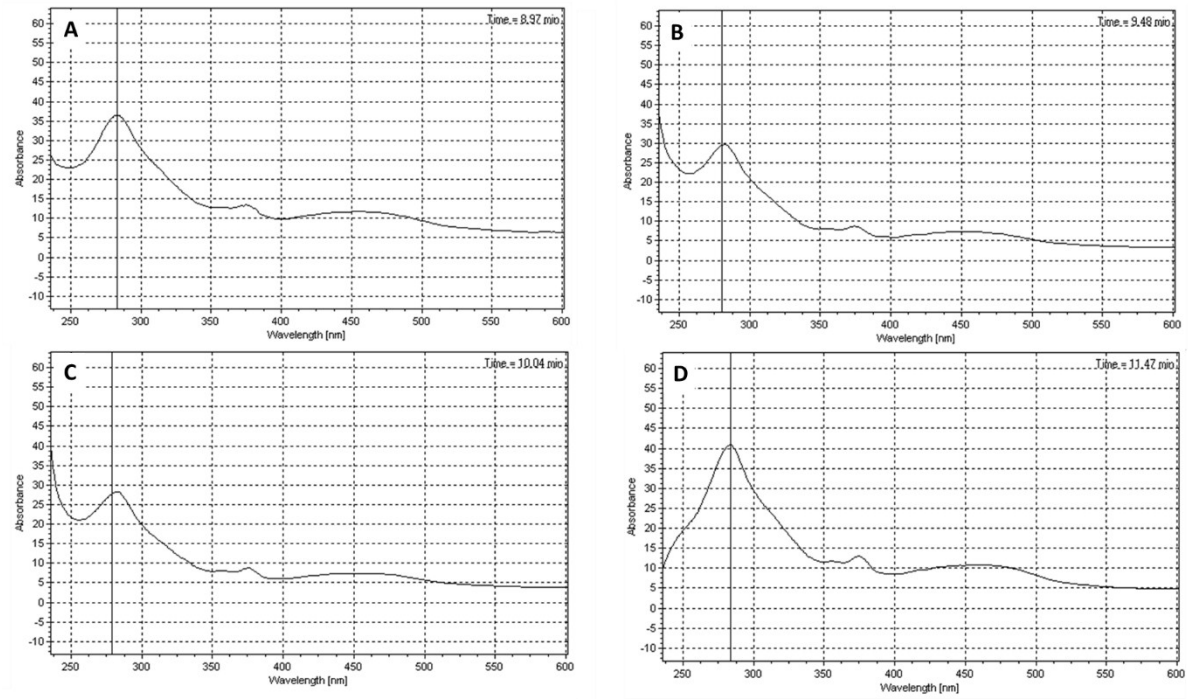



Figure S12: HPLC UV-vis spectra obtained for peaks observed during $Os^{II}$MPP HPLC analysis illustrating the absence of a component with absorbance only in the UV-vis region as the MLCT band at a longer wavelength confirms Osmium-coordinated complex for each peak. The distribution of peaks in the conjugate product is suspected to originate from different ionization states of the conjugate.

**Spectroscopic Measurements of Os(II) parent complex and Os(II) conjugate**

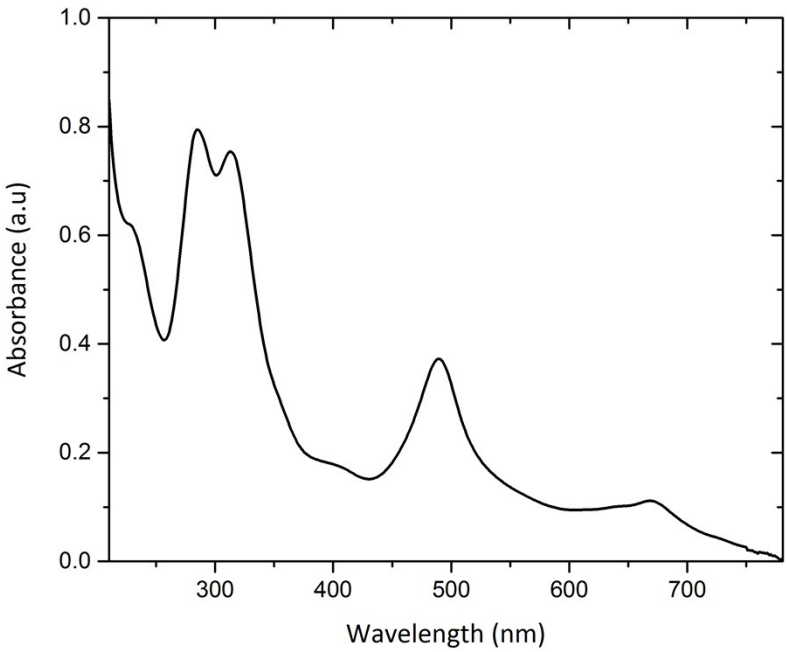



Figure S13: Absorbance spectrum of parent complex $[Os(tpybenzCOOH)_2]^{2+}$ **1** in acetonitrile solution (25µM).

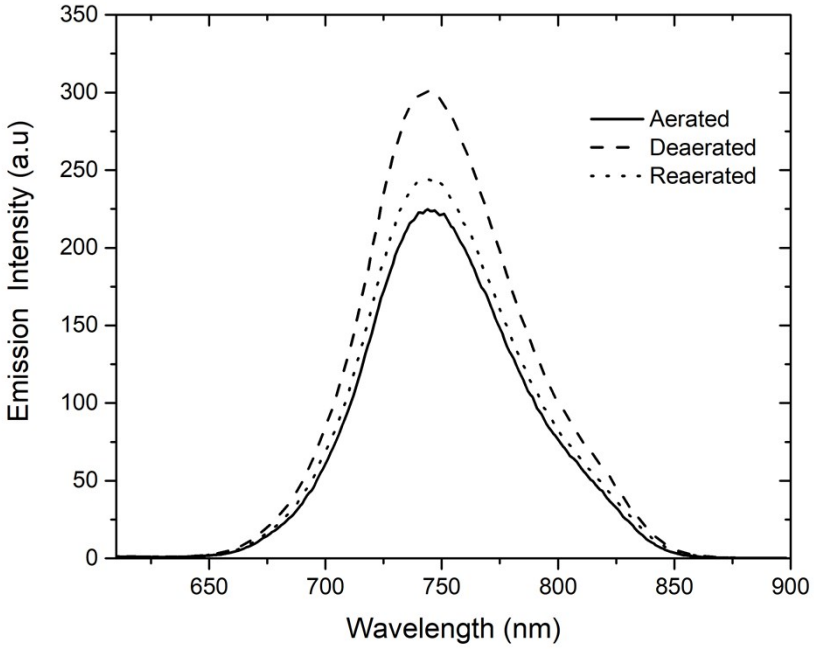



Figure S14: Emission spectra of parent complex $[Os(tpybenzCOOH)_2]^{2+}$ in acetonitrile (50µM) under aerated and deaerated conditions (slit widths 10 nm / 10 nm). Solution was deaerated using nitrogen gas and oxygen concentration was measured using PreSence $O_2$ sensor.

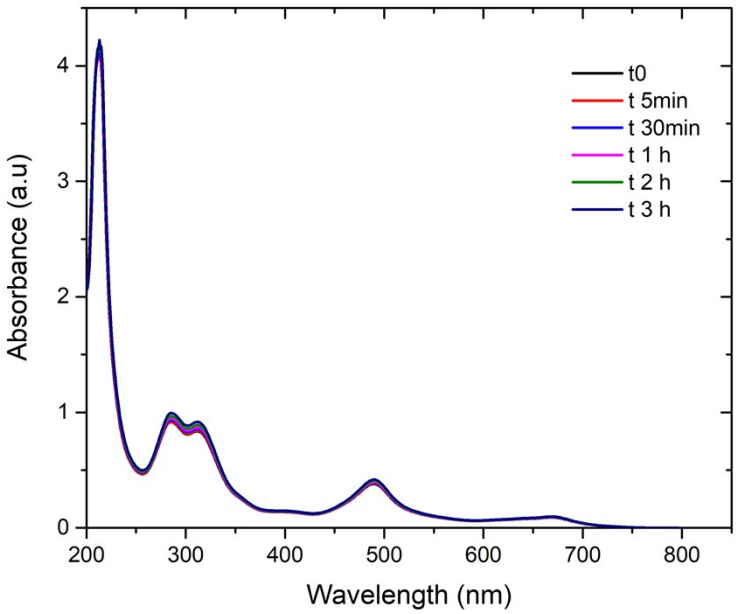
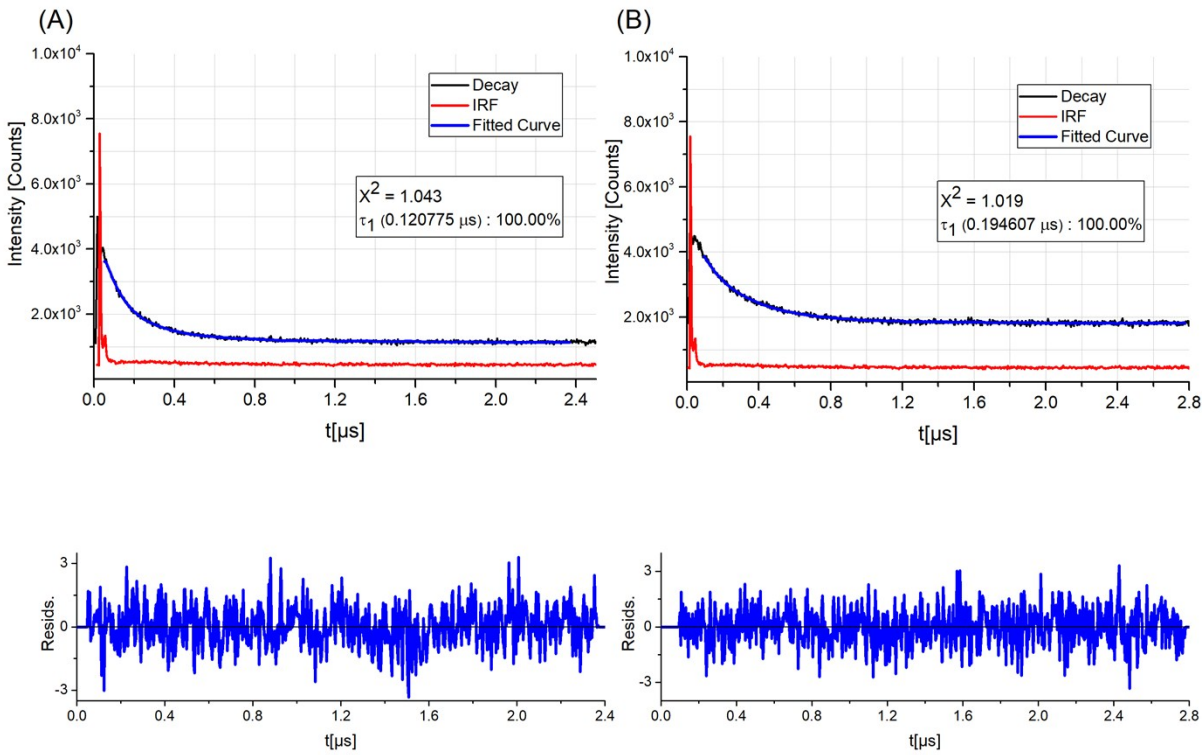


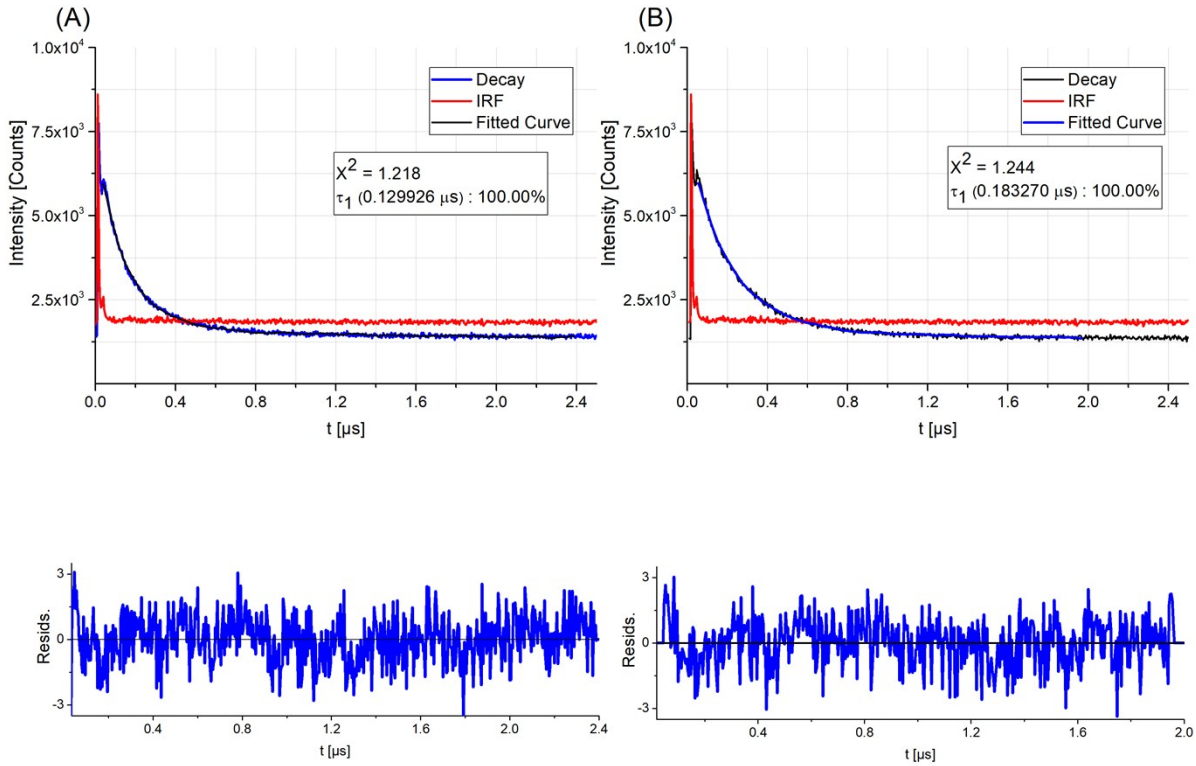

Figure S15: Absorbance spectra of parent complex $[Os(tpybenzCOOH)_2]^{2+}$ (50µM/ PBS pH 7.4) following continuous photo-irradiation with ARC Lamp 150W for 3 h at Room Temperature.

Emission Decays of Os (II) parent and $Os^{II}$ MPP complex – Time- correlated single photon counting (TCSPC) measurements using NanoHarp 2.1, FluoTime100 (PicoQuant) and mono-exponentially fitted using PicoQuant Fluofit software.



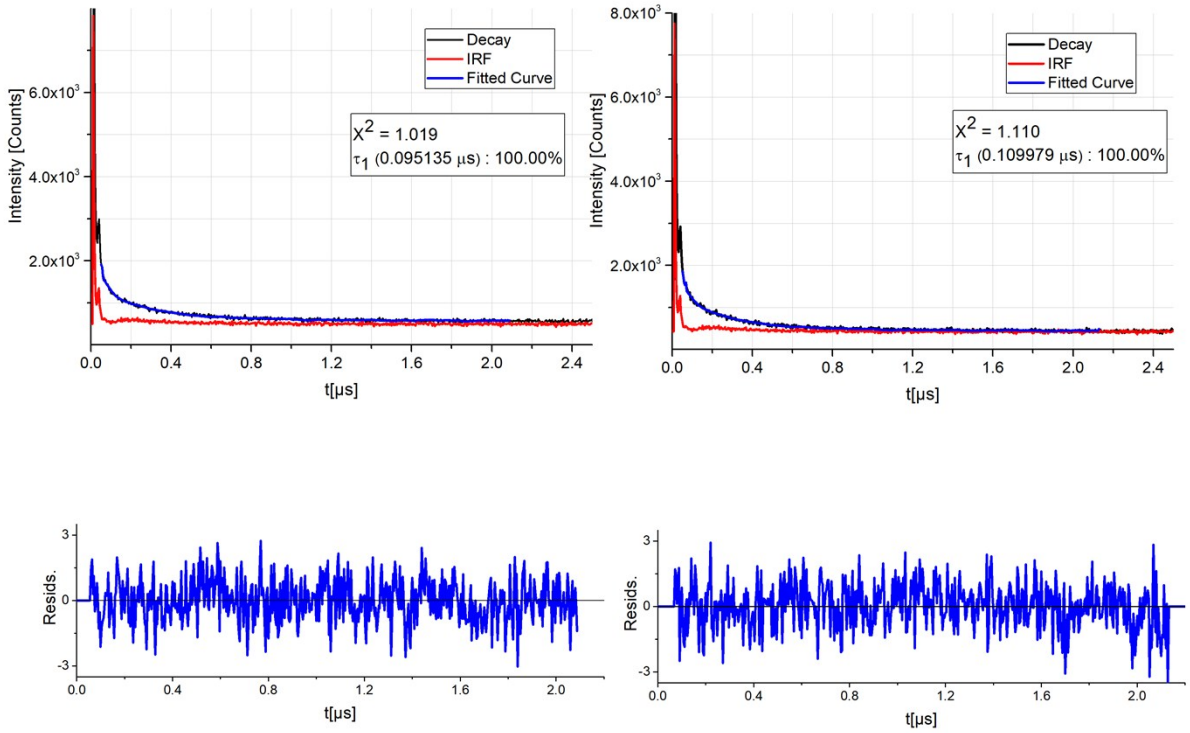

$$\int_{-\infty}^{t} IRF\,(t')\sum_{i-1}^{x} A_i e^{-\frac{t-t'}{t_1}}\,dt'$$

Figure S16: Emission Decays of parent complex $[Os(tpybenzCOOH)_2]^{2+}$ in aerated and deaerated acetonitrile (50µM); Residual plots for the exponential fitting of both curves are shown below each plot.




$$\int_{-\infty}^{t} IRF\,(t')\sum_{i-1}^{x} A_i e^{-\frac{t-t'}{t_1}}\,dt'$$

Figure S17: Emission Decays of parent complex $[Os(tpybenzCOOH)_2]^{2+}$ in aerated and deaerated PBS pH 7.4 (50μM); The residual plots for the exponential fitting of both curves are shown below.




$$\int_{-\infty}^{t} IRF\,(t^{'}) \sum_{i-1}^{x} A_i e^{-\frac{t-t^{'}}{t_1}}\, dt^{'}$$

Figure S18: Emission decay of conjugate $Os^{II}$ MPP under aerated and deaerated conditions (25µM/ PBS pH 7.4); Residual Plots of the exponential fitting for both curves are shown below.

## Cytotoxicity Studies and determination of $IC_{50}$ of $Os^{II}$ MPP

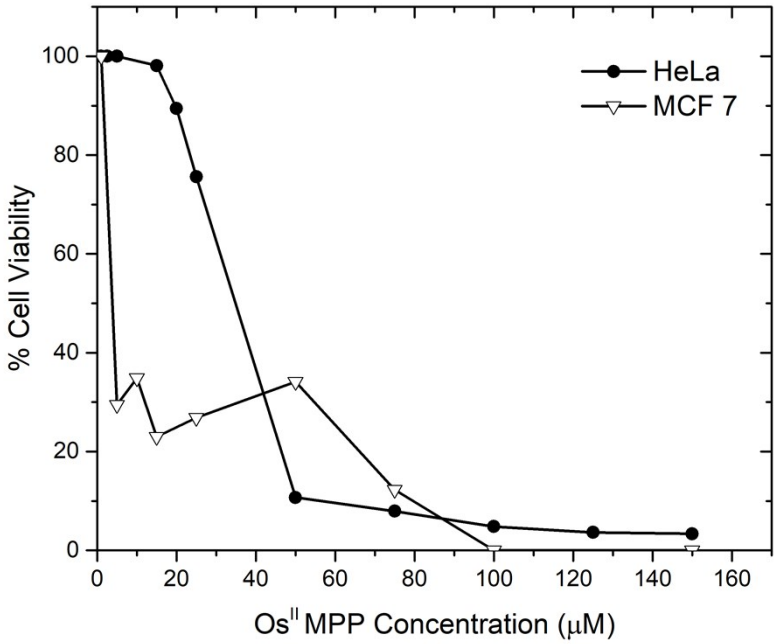



Figure S19: Cell Viability of HeLa and MCF 7 cells after 24hr exposure to $Os^{II}$ MPP probe. Live cells were treated with the probe followed by addition of Resazurin for 6 h. Absorbance was read at 570 nm with background at 600 nm subtracted (n=3).

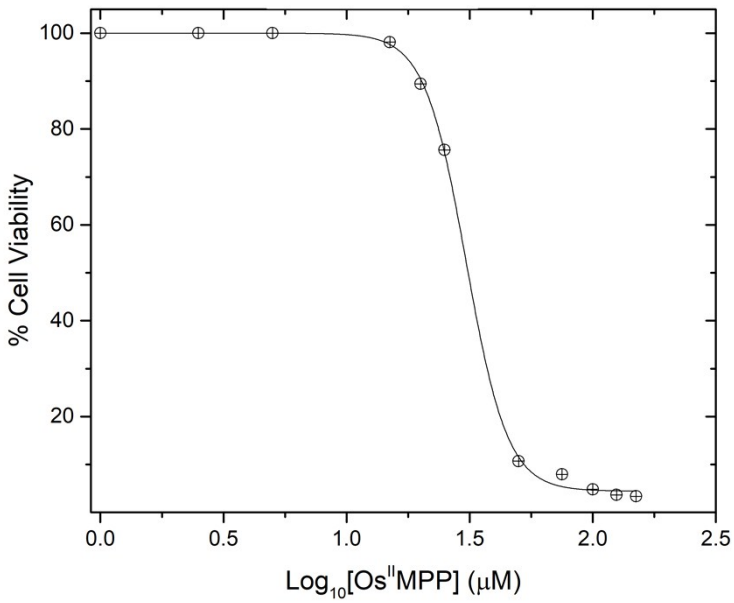



Figure S20: Determination of EC50. HeLa cells were incubated in the presence of $Os^{II}$ MPP (0.1µM to 150µM) for 24 h. Cell proliferation was assayed with Resazurin (n=3). The $IC_{50}$ value, the minimal amount of $Os^{II}$ MPP required to inhibit 50 % viability of HeLa cells was found to be 30.61µM.

## Confocal Imaging

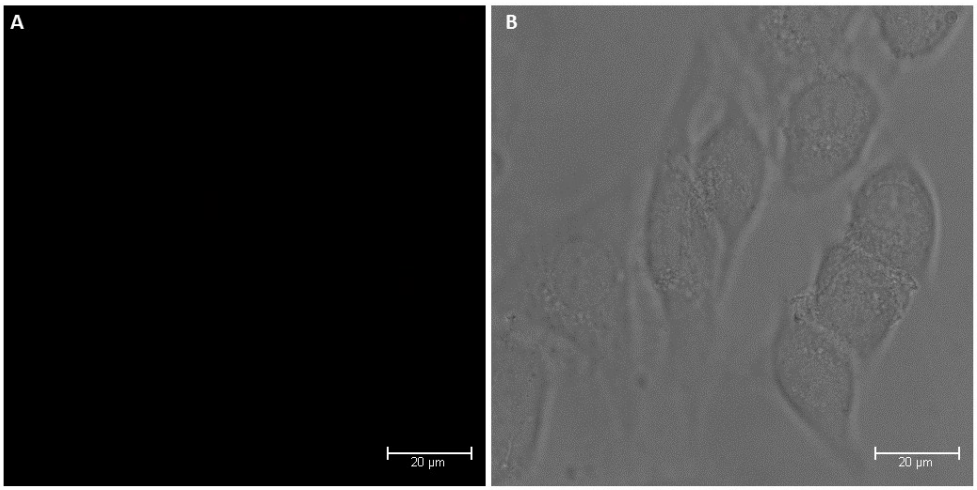



Figure S21: Confocal Imaging studies of $[Os(tpybenzCOOH)_2]^{2+}$. Cells were treated with $[Os(tpybenzCOOH)_2]^{2+}$ at 30µM for 2 h. (A) Osmium Channel: uptake of the parent complex was not evident; (B) Background channels showing HeLa cells. Cells were excited with 490 nm WLL and emission was collected between 650nm and 850 nm.

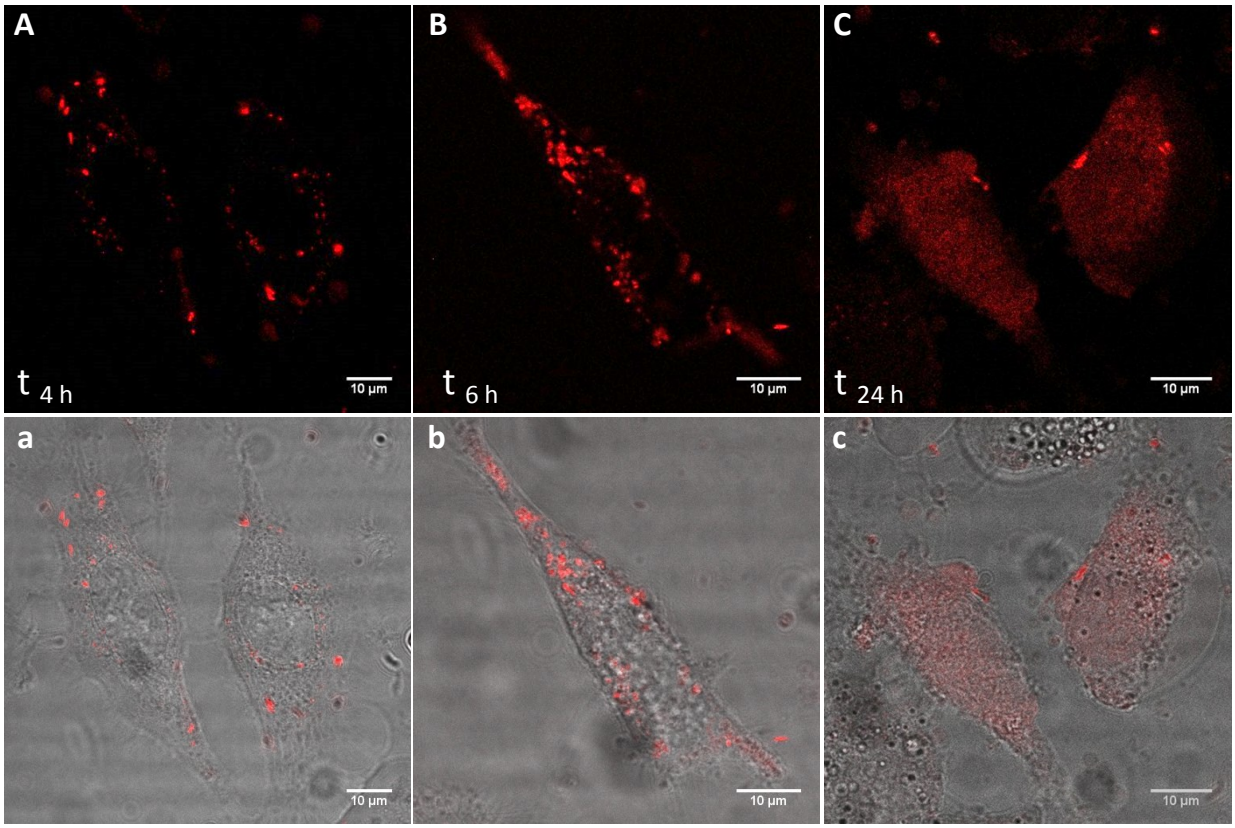



Figure S22: Confocal microscopy of HeLa cells incubated with $Os^{II}$ MPP at 30µM for (A) 4 h, (B) 6 h and (C) 24 h in cell media at 37 °C in the absence of light. (a-c) Overlay images with the background channel. $Os^{II}$MPP was excited using a 490 nm white light laser and emission was collected between 650nm and 850 nm.

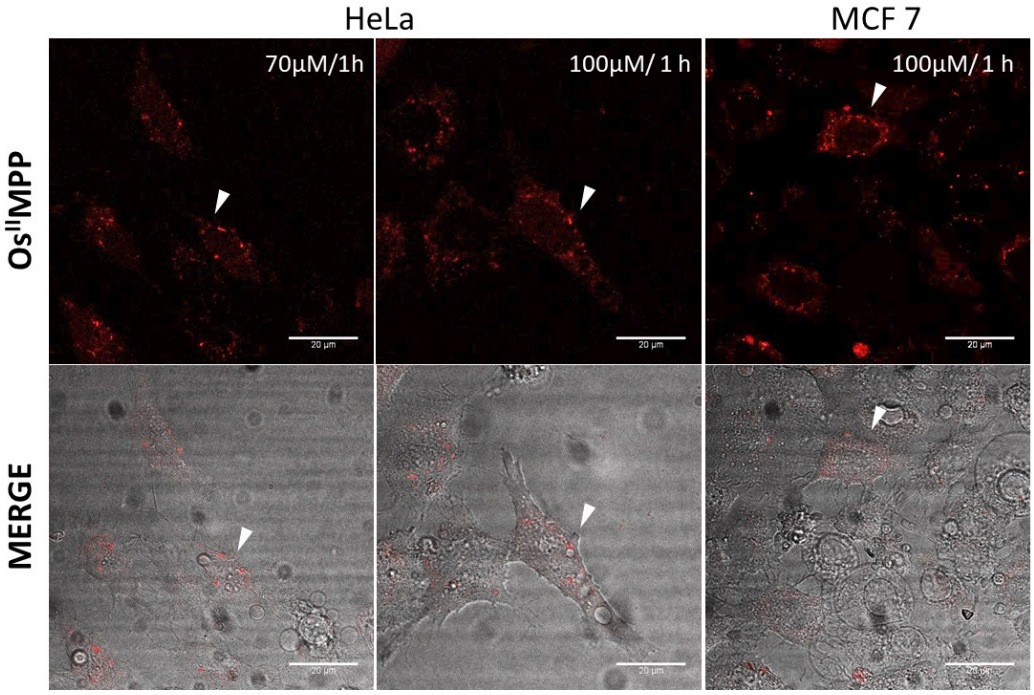



Figure S23: Confocal imaging of $Os^{II}$ MPP in HeLa and MCF 7 cells at increased concentrations; Uptake of $Os^{II}$MPP probe is evident but changes are observed in the morphological features of cells indicative of cell death.

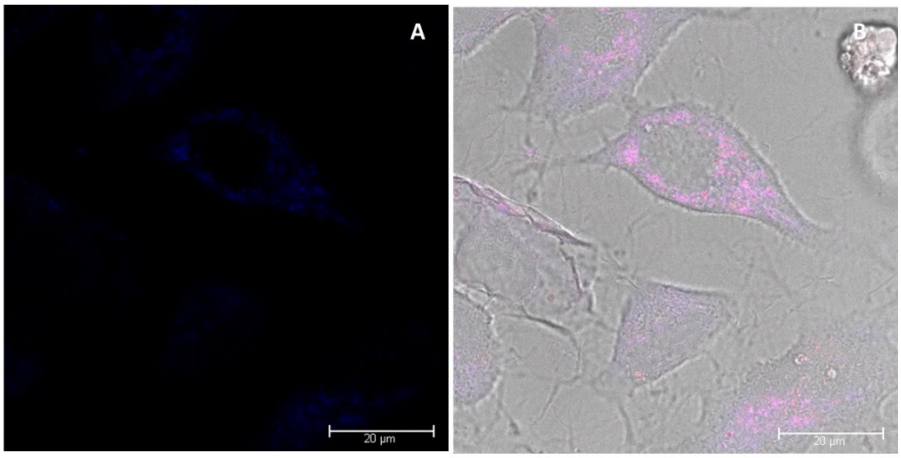



Figure S24: Co-staining with DRAQ7 following $Os^{II}$MPP 30uM/2h; (A) Absence of nuclear staining confirms cells are viable; observed in blue is the emission of MitoTracker Deep Red due to co-excitation of MitoTracker Deep Red at 633 nm. (B) Overlay of all channels

## Polycaspase FAM-FLICA and MitoPT TMRE Assay

Table 1: MitoPT and FLICA Assay: Cell Population Experimental Conditions

| Assay | Negative Control Populations | Positive Control Population | Experimental Populations |
|---|---|---|---|
| Mito PT | (1) Non- exposed population<br>(2) DMSO (100µM/ 1 h) | CCCP (20µM/ 2 h) | $Os^{II}MPP$ 30µM/ 2 h<br>$Os^{II}MPP$ 100µM/ 1 h |
| FLICA | (1) Non- exposed population<br>(2) DMSO (100µM/ 1 h) | Staurosporine (1µM/ 3 h) | $Os^{II}MPP$ 30µM/ 2 h<br>$Os^{II}MPP$ 100µM/ 1 h |

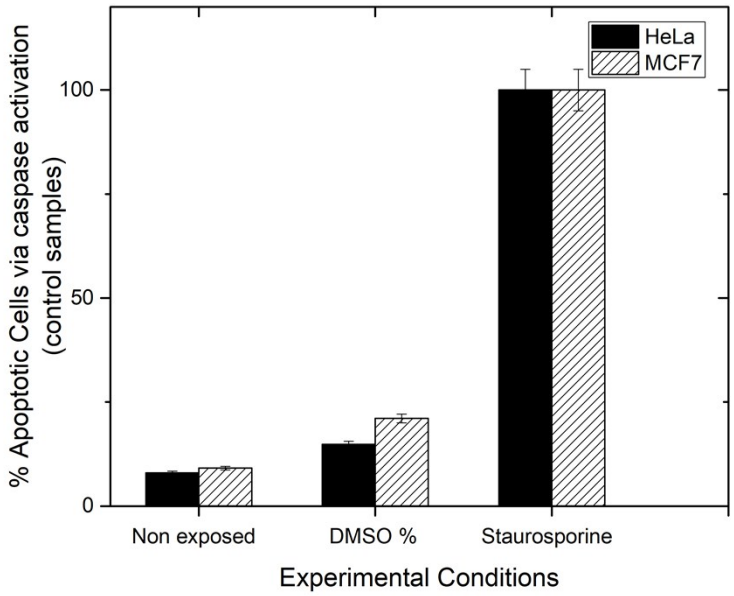



Figure S25: Control studies for the FLICA assay (n=3) in HeLa and MCF 7 cell line. Cells were cultivated at 3 x $10^5$ cells/well and were exposed to negative control 1 (non-treated) (B) negative control 2 DMSO (10% v/v) (C) positive control (staurosporine 1µM/ 3 h). Cells were spiked with 30X FAM-FLICA reagent (v/v ratio of 1:30) and incubated for 45 minutes at 37°C. Samples were analyzed in triplicate (3 x 100 µl) in a black bottomed 96-well plate using Tecan Plate fluorescence plate reader set at 488 nm excitation and 520 nm emission.