Supporting Information

Two-photon phosphorescence lifetime imaging of cells and tissues using a long-lived cyclometallated Npyridyl^Cphenyl^Npyridyl Pt(II) complex

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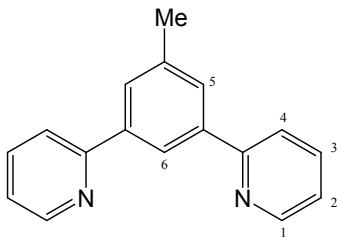
1. Synthesis and Characterisation

All reagents were purchased from commercial sources and used without further purification, with the exception of 2-(tri-n-butylstannyl) pyridine, which was synthesised according to literature methods[S1] and K_2PtCl_4 which was obtained from precious metals online (http://www.precmet.com.au/) and recrystalised from H_2O according to Keller.[S2] Dry solvents were obtained from the Grubbs dry solvent system at the University of Sheffield and deuterated solvents were purchased from Sigma Aldrich.

3,5-di(2-pyridyl)toluene



3,5-dibromotoluene (1.3 g, 5.2 mmol), lithium chloride (2.2 g, 51.9 mmol), $Pd(PPh_3)_2Cl_2$ (0.292 g, 0.416 mmol), 2-(Tri-n-butylstannyl)pyridine (4.6 g, 12.5 mmol) and toluene (16 mL) were added to an oven dried round bottomed flask. A condenser was fitted and the mixture degassed via five freeze-pump-thaw cycles, then refluxed for 48 hours under N_2. After cooling to room temperature a saturated aqueous solution of KF (12 mL) was added to the black mixture and stirred for 30 minutes, before filtering. The insoluble black solid was washed with toluene until the filtrate was clear. The combined filtrate and washings were dried under reduced pressure. The brown residue was re-dissolved in CH_2Cl_2 (150 mL) and washed with aqueous $NaHCO_3$ (5% by weight, 2 x 100 mL). Organic layers were combined, dried with anhydrous K_2CO_3 and filtered. Solvent was removed under vacuum to give pale yellow oil. Crude product was purified on silica using a gradient elution from hexane to 80:20, Et_2O:hexane. TLC solvent used was 80:20, Et_2O:hexane. Product isolated as a pale yellow oil (R_f = 0.27).

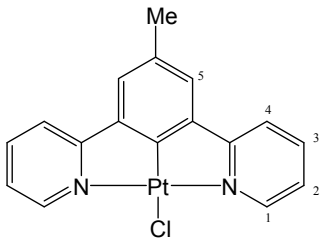
Yield: 59 % (0.760 g, 3.08 mmol).

Molecular formula: $C_{17}H_{14}N_2$. RMM: 246.31

^{1}H NMR (400MHz, $CDCl_3$): δ_H 2.54 (3H, s, Me), 7.26 (2H, ddd, *J* 7.7, 4.9, 1.2 Hz, H^2), 7.78 (2H, ddd, *J* 8.0, 7.8, 1.8 Hz, H^3), 7.85 (2H, dt, *J* 8.0, 1.0 Hz, H^4), 7.92 (2H, d, *J* 1.7 Hz, H^5), 8.39 (1H, t, *J* 1.7 Hz, H^6) and 8.73 (2H, ddd, *J* 4.9, 1.8, 1.0 Hz, H^1). $^{13}C\{^1H\}$ NMR (400MHz, $CDCl_3$): δ_C = 22.0 (1C, s, Me), 121.2 (2C, s, CH-Ar), 122.6 (2C, s, CH-Ar), 123.2 (1C, s, CH-Ar), 128.7 (2C, s, CH-Ar), 137.2 (2C, s, CH-Ar),

139.4 (1C, s, C^{quat}), 140.3 (2C, s, C^{quat}), 150.0 (2C, s, CH-Ar) and 157.8 (2C, s, C^{quat}). MS (EI): m/z = 246 $[M]^+$, 231 $[M\text{-}CH_3]^+$, 167 $[C_{12}H_{10}N]^+$.

3,5-di(pyridyl)toluene platinum (II) chloride



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Glacial acetic acid (6 mL) was added to a mixture of 3,5-di(2-pyridyl)toluene (0.161 g, 0.65 mmol) and K_2PtCl_4 (0.272 g, 0.65 mmol) in an oven dried, round bottomed flask. The mixture was degassed via four freeze-pump-thaw cycles then refluxed at 120°C, under N_2 for 72 hours. After cooling to room temperature the yellow heterogeneous mixture was filtered and the solid washed with water, ethanol and Et_2O (3 x 5 mL of each), then air dried. The solid was washed further with CH_2Cl_2 (50-100 mL) into another collection flask until the filtrate ran clear. CH_2Cl_2 was removed under vacuum to reveal a bright yellow crystalline solid.

Yield: 69% (0.216 g, 0.55 mmol).

Molecular formula: $C_{17}H_{14}N_2ClPt$. RMM: 475.83

^{1}H NMR (400MHz, DMSO): δ_H 2.33 (3H, s, Me), 7.54 (2H, ddd, *J* 7.8, 5.7, 1.5 Hz, H^2), 7.61 (2H, d, *J* 0.7 Hz, H^5), 8.10 (2H, ddd, *J* 8.1, 1.5, 0.7 Hz, (clear ^{195}Pt satellites observed in $CDCl_3$ but not it DMSO), H^4), 8.20 (2H, ddd, *J* 8.1, 7.8, 1.6 Hz, H^3) and 9.09 (2H, *J* (^{195}Pt) 40 Hz, *J* 5.7, 1.6, 0.7 Hz, H^1). $^{13}C\{^1H\}$ NMR (400MHz, $CDCl_3$): δ_C = 22.1 (1C, s, Me), 119.1 (2C, s, *J* (^{13}C-^{195}Pt) 25 Hz, CH-Ar), 123.1 (2C, s, *J* (^{13}C-^{195}Pt) 16 Hz, CH-Ar), 124.9 (2C, s, *J* (^{13}C-^{195}Pt) 19, CH-Ar), 132.4 (2C, s C^{quat}), 139.0 (2C, s, CH-Ar), 140.8 (2C, s, C^{quat}), 152.2 (2C, s, CH-Ar) and 167.4 (1C, s, C^{quat}). Remaining quaternary carbon not detected. MS (EI): m/z = 476 $[MH]^+$, 440 $[M\text{-}Cl]^+$, 246 $[C_{17}H_{14}N_2]^+$. NMR is shown in Figure S9.

Elemental analysis: Calculated: C 42.91% H 2.75% N 5.89% Cl 7.45%. Found: C 42.64% H 2.63% N 5.73% Cl 7.54%.

2. Cell culture, tissue preparation and Imaging

Cell culture:

Rat schwannoma cells (RN22) cells were cultured in a humidified 37°C, 5% CO_2/95% air (v/v) environment in Dulbecco's modified Eagle's medium (DMEM), purchased from Aldrich (500 mL), supplemented with 5 mL L-glutamine (200mM solution), 5 mL penicillin-streptomycin solution, 50 mL fetal calf serum (10%) and 1.25 mL fungizone. Cells were cultured as monolayers in T-75 flasks and passaged using trypsin-EDTA. For imaging experiments cells were seeded in to 6 well plates and cultured until 60% confluent

Cell staining with complex 1:

After removal of growth media cells were washed with PBS (phosphate buffered saline) (1 ml/well) before treating with a solution of **1**; *i)* 50 μM in PBS (5min at 37°C, 1ml/well). After incubation with **1**, cells were washed with more PBS (3 x 1 ml/well) to remove excess complex before imaging.

Tissue staining with complex 1:

Tissue sections, 10 mμ thickness, (embedded in paraffin wax) were re-hydrated, before staining with Pt-Me **1**, according to standard histology protocols. To re-hydrate, tissue samples were submerged

in 100% xylene (3 min), 100% methanol (2 min), 70% methanol (2 min) and 100% dH_2O (2 min), respectively. Tissue sections were then covered with a dilute solution of **1** (200-600 µL, 100 µM, 2:98% DMSO:PBS) for 5 minutes at room temperature; after which time excess platinum solution was removed. Tissue sections were washed in dH_2O to remove any excess, before water mounting glass cover slip.

Time-resolved imaging:

Live RN22 cells were imaged in PBS (in 6 well plates) using a x40 water dipping objective. Tissue sections were imaged on glass slides (with a water mounted coverslip) using x63 and x40 oil immersion objectives.

Time-resolved, phosphorescence lifetime imaging (PLIM) of cells and histological tissue sections was carried out using a Ti:Sa pulsed laser (λ_{ex}: 780 nm) on a LSM 510 upright confocal microscope connected to a Becker and Hickl combined FLIM/PLIM system, that is comprised of a SPC-150 TPSPC module and a DDG-210 pulse generator module.[S3] PLIM images were recorded using the 50 µs predefined set-up on the SPCM software (laser on time: 5 µs, PLIM decay window: 50 µs) and data processed using SPCImage software, with a pixel bin of 3 unless stated otherwise. Decay traces were best fit to a double exponential decay model (in all cases). Lifetimes maps are plotted as the intensity weighted average Ti. Reported emission lifetimes are that of the longer Pt emission component, T_2.

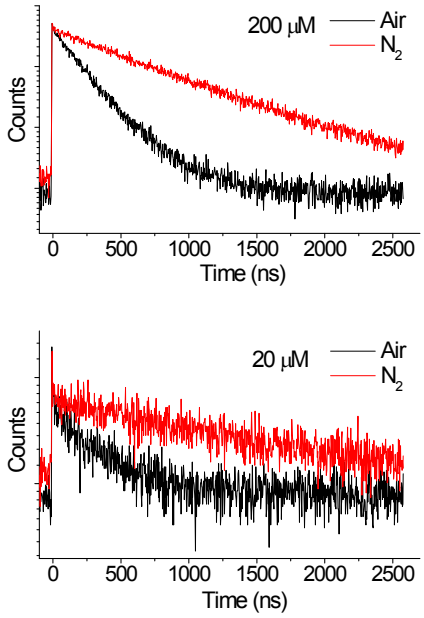
References

S1. Bianchini, C., Gatteschi, D., Giambastiani, G., Rios, I. G., Ienco, A., Laschi, F., Mealli, C., Meli, A., Sorace, L., Toti, A., Vizza, F., *Organometallics,* **2007**, 26, 726-739.

S2. Keller, R. N., *Inorg. Synth.,* **1946**, 2, 247-250.

S3. Becker & Hickl GmbH, Combined Fluorescence and Phosphorescence Lifetime Imaging. (FLIM / PLIM) with the Zeiss LSM 710 NLO Microscopes, Application note. Available on www.becker-hickl.com.

4. Supplementary figures



Concentration	*Lifetime (χ^2)*	
	Air	N_2
200 µM	320 ns (1.1)	1300 ns (1.3)
20 µM	390 ns (1.2)	2100 ns (1.3)

Figure S1: Emission decay traces and lifetime values of **1** in DMF (200 µM top, 20 µM bottom) demonstrating oxygen sensitivity of **1** using Becker and Hikl TCSCP module (and SPCM software) attached to a scanning confocal microscope. Lifetime and χ^2 values taken from fit in SPCImage (traces fit with to a single exponential). Air equilibrated solutions were degassed by passing a stream of nitrogen gas over the solution inside a sealed chamber on the microscope stage (laser power 0.05mW).

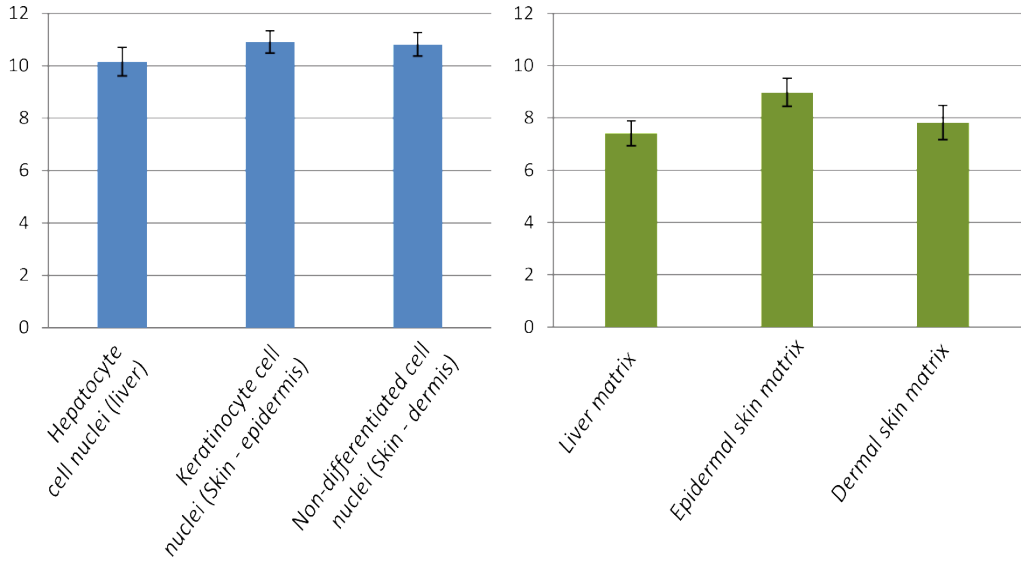


Figure S2: Comparison of average PLIM emission lifetimes (t2) of complex **1** from fixed liver and skin tissue. Data taken from 10 individual pixels at random in each data set (error bars represent the standard deviation of each 10 point data set).

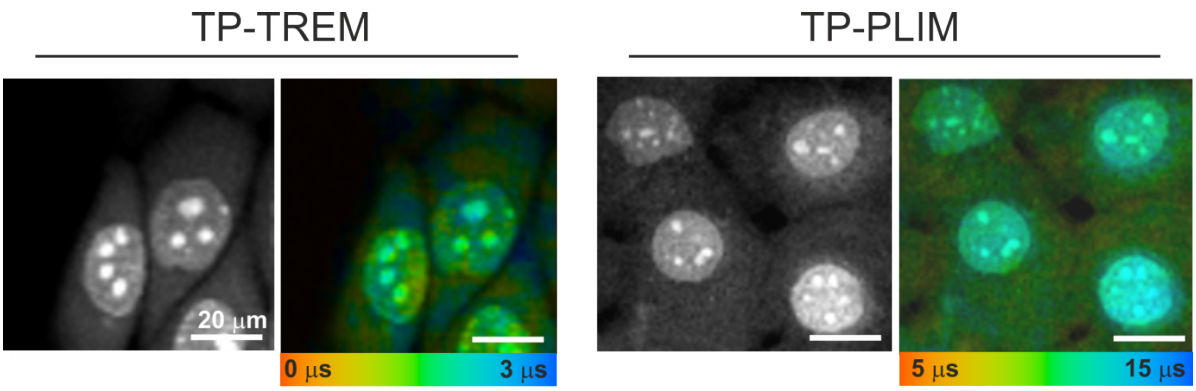


Figure S3: Intensity images (B&W) and lifetime maps of live HaCat cells labelled with Pt (100 µM) recorded using TP-TREM (left) and TP-PLIM (right) methodologies. The spatial resolution achieved is similar across both methods.

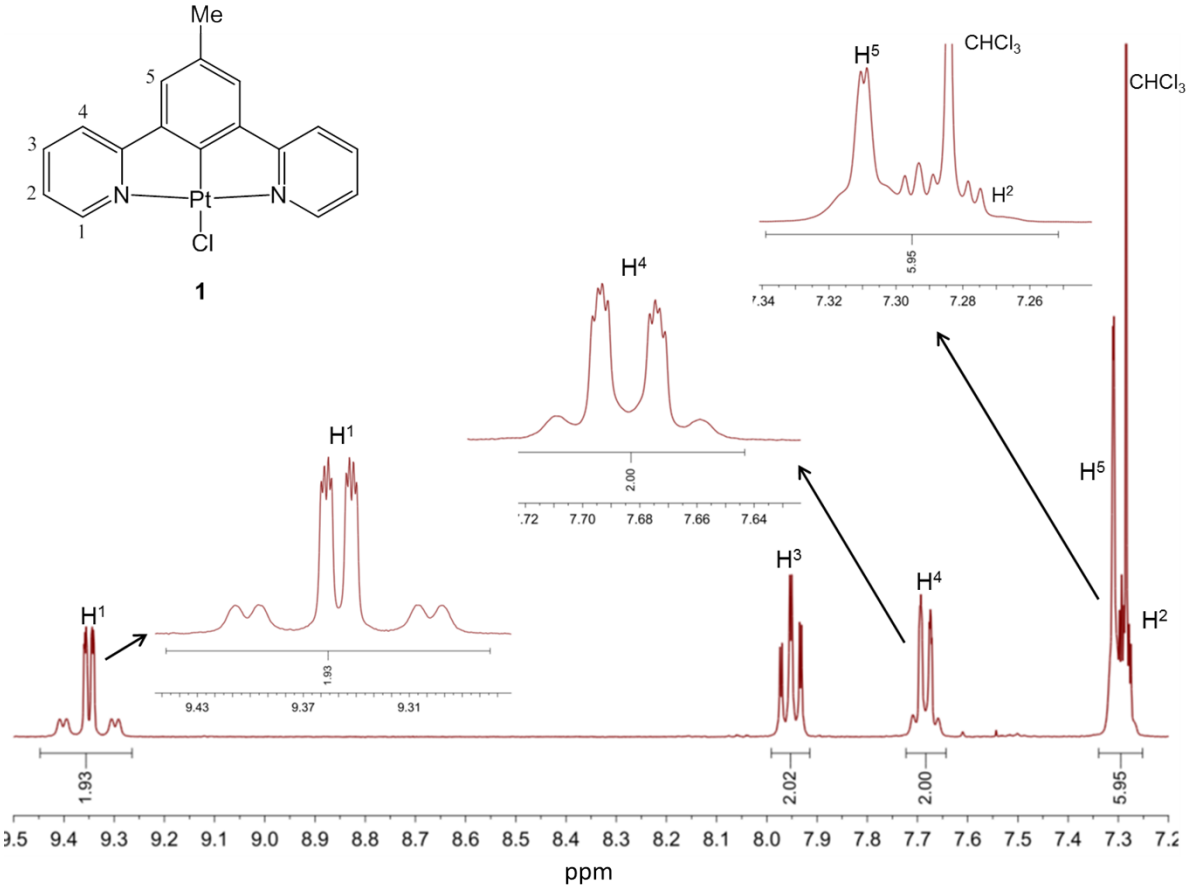


Figure S4: ^{1}H NMR spectrum (400MHz, 300K) of 3,5-dipyridyl toluene platinum (II) chloride **1** in $CDCl_3$, aromatic region only (7.2-9.5 ppm). Expanded spectra inserts show splitting of individual peaks.