Delivery and release of curcumin by a hypoxia-activated cobalt chaperone: a XANES and FLIM study

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Supplementary Information

Synthesis

Curcumin was obtained from Sigma-Aldrich as a mixture of curcumin, desmethoxycurcumin and bis-desmethoxycurcumin and purified by column chromatography (silica gel $CHCl_3$:MeOH 98:2-95:5). Acetylacetonoate was obtained from Sigma Aldrich and distilled before use. Dibenzoylmethane was obtained from Fluka and used as received. All other chemicals were used as received. tpa.*n*ClO_4,[1] $[RuCl_2(tpa)]ClO_4$,[2] $[CoCl_2(tpa)]ClO_4$,[3] $[Co(acac)(tpa)](ClO_4)_2$,[4] $[CoCl(H_2O)(tpa)](ClO_4)_2$,[5] $[Co(cys)(tpa)]ClO_4$,[6] $K[Co(cys)(H_2O)_2]$ and *fac*-$K_3[Co(cys)_3]$[7] were prepared according to literature procedures. All reactions involving ruthenium were carried out under nitrogen using standard Schlenk techniques. Caution! Many of the procedures involved the preparation of perchlorate salts of metal complexes. While no issues were experienced, such compounds should be handled with care, as they are potentially hazardous. Particular care was taken with the tris(2-methylpyridyl)amine perchlorate (tpa·nClO_4) ligand as organic perchlorate molecules are potentially explosive.

*General synthesis for complexes **1a – c***

Ruthenium complexes were prepared according to the following procedure. $[RuCl_2(tpa)]ClO_4$ (145 mg, 0.25 mmol) was refluxed in water (50 mL) for 30 min, during which the yellow suspension became a green or purple solution. 0.25 mmol of HL (where L corresponds to acetylacetone, dibenzolymethane or curcumin, respectively) and triethylamine (0.2 mL, 0.3 mmol) were added and the solution was heated at reflux for a further 16h during which a red precipitate formed. Excess $NaClO_4$ was added and the resulting precipitate was collected by suction filtration and washed with water (2 x 5 mL) and diethyl ether (2 x 10 mL).

*Ru(acac)tpa]ClO_4 (**1a**)*

Complex **1a** was recrystallised from 1:1 DCM:Hexane as orange/red microcrystals. Yield 126 mg (85%). Vapour diffusion of pentane into a DCM solution of **1a** yielded red needles suitable for X-ray diffraction. ^{1}H NMR ($(CD_3)_2CO$): δ 9.36 (d, ^3J = 5.4 Hz, 1H, tpa-pyridyl), 8.52 (d, ^3J = 5.4 Hz, 2H, tpa-pyridyl), 7.74 (t, ^3J = 7.8 Hz, 2H, tpa-pyridyl), 7.52 (t, ^3J = 7.8 Hz, 1H, tpa-pyridyl), 7.48 (d, ^3J = 7.8 Hz, 2H, tpa-pyridyl), 7.27 (t, ^3J = 6.8 Hz, 2H, tpa-pyridyl), 7.23 (t, ^3J = 7.8 Hz, 1H, tpa-pyridyl), 7.10(d, ^3J = 7.8 Hz, 1H, tpa-pyridyl), 5.30 (s, 1H, s, 1H, γ-hydrogen of acac), 5.08 (half of AB system, 2H, tpa-CH_2N), 4.99 (half of AB system, 2H, tpa-CH_2N), 4.66 (s, 2H, tpa-CH_2N), 2.15 (s, 3H, acac-CH_3), 1.75 (s, 3H, acac-CH_3). ESI-MS$^+$: m/z = 490.9 (M-ClO_4). IR: ν(C=O) 1567 s, 1508, ν(C=C) 1405 s, 764, ν(ClO) 621 s. Elemental analysis required for [Ru(acac)tpa](ClO_4) ($C_{23}H_{25}ClRuN_4O_6$): C, 45.43; H, 4.48; N, 9.21. Found: C, 45.59; H, 4.42; N, 9.08.

*[Ru(dbm)(tpa)]ClO_4 (**1b**)*

Complex **1b** was recrystallised from 1:1 DCM:Hexane as dark red microcrystals. Yield 143 mg (80%). Vapour diffusion of diethylether into a DCM solution of **1b** yielded dark red plates suitable for X-ray diffraction. ^{1}H NMR ($(CD_3)_2CO$): δ 9.55 (d, ^3J = 5.7 Hz, 1H, tpa-pyridyl), 8.60 (d, ^3J = 5.4 Hz, 2H, dbm-phenyl), 8.28 (d, ^3J = 6.6 Hz 2H, dbm-phenyl), 8.16 (t, ^3J = 7.2 Hz, 2H, dbm-phenyl), 7.95 (d, ^3J = 7.2 Hz, 2H, dbm-phenyl), 7.85 (t, ^3J = 7.5 Hz, 1H, tpa-pyridyl), 7.80 (t, ^3J = 6.6 Hz, 1H, tpa-pyridyl), 7.75 (t, ^3J = 7.4 Hz, 2H, tpa-pyridyl) 7.64 (t, ^3J = 7.5 Hz, 2H, dbm-phenyl), 7.59 (t, ^3J = 6.9 Hz 2H, tpa-pyridyl) 7.55 (t, ^3J = 7.5 Hz, 2H tpa-pyridyl) 6.84 (s, 1H, γ-hydrogen of acac), 5.33 (half of AB system, 2H, tpa-CH_2N), 5.17 (half of AB system, 2H, tpa-CH_2N), 4.80 (s, 2H, tpa-CH_2N). ESI-MS$^+$: m/z = 615.0 (M-ClO_4). IR: ν(C=O) 1517 s, 1456, ν(C=C) 1402 s, 764, 758, 713, ν(ClO) 621 s. Elemental analysis required for [Ru(dbm)tpa](ClO_4) ($C_{33}H_{29}ClRuN_4O_6$): C, 55.50; H, 4.09; N, 7.85. Found: C, 55.48; H, 4.04; N, 7.74.

*[Ru(cur)(tpa)]ClO_4 (**1c**)*

Complex **1c** was recrystallised from 1:1 MeOH:Diethylether to give a dark red/brown microcrystals. Yield 163 mg (76%). ^{1}H NMR (MeOD): δ 9.46 (d, ^{3}J = 5.4 Hz, 1H, tpa-pyridyl), 8.52 (d, J = 5.8 Hz, 2H, tpa-pyridyl), 7.69 (t, J = 7.5 Hz, 2H, tpa-pyridyl), 7.54 (t, J = 6.8 Hz, 1H, tpa-pyridyl), 7.49 (t, J = 6.8 Hz, 1H, tpa-pyridyl), 7.44 (d, J = 7.2 Hz, 2H, tpa-pyridyl), 7.40 (t, J = 6.1 Hz, 2H, tpa-pyridyl), 7.38 (d, J = 7.2 Hz, 1H, tpa-pyridyl), 7.32 (d, $^3J_{trans}$ = 12.4 Hz, 2H, H_{alkene}), 7.23 (d, J_{meta} = 1.4 Hz, 2H, curcumin-phenyl), 6.86 (dd, J_{ortho} = 8.1 Hz, J_{meta} = 1.4 Hz, 2H, curcumin-phenyl), 6.75 (d, J_{ortho} = 8.1 Hz, 2H, curcumin-phenyl), 6.48 (d, $^3J_{trans}$ = 13.0 Hz, 2H, H_{alkene}) 5.68 (s, 1H, γ-hydrogen of acac), 5.12 (half of AB system, 2H, tpa-CH_2N), 5.06 (half of AB system, 2H, tpa-CH_2N), 4.79 (s, 2H, tpa-CH_2N), 3.98 (s, 3H, curcumin-OCH_3), 3.89 (s, 3H, curcumin-OCH_3). ESI-MS$^+$: m/z = 758.9 (M-ClO_4). IR: ν(C=O) 1504 vs, ν(C=C) 1425 s, 1274 s, 1177 br, 969, 825, 764 s, ν(ClO) 623, 525. Elemental analysis required for [Ru(cur)tpa](ClO_4)·H_2O ($C_{33}H_{29}ClRuN_4O_6$): C, 55.50; H, 4.09; N, 7.85. Found: C, 55.48; H, 4.04; N, 7.74.

*General synthesis for complexes **2b** and **2c***

[Co(Cl_2)tpa]ClO_4 (130 mg, 0.25 mmol) was dissolved in water (50 mL) and the pH adjusted to 7 using 1M NaOH. After refluxing for 10 minutes, 1 equivalent of HL and 1.2 equivalents of triethylamine added and the solution was heated at reflux for a further 3h, during which time an orange precipitate formed. The solution was cooled and excess $NaClO_4$ added to give an orange powder, which was collected by suction filtration and washed with water (2 x 5 mL) and diethyl ether (2 x 10 mL).

*[Co(dbm)(tpa)](ClO_4)$_2$ (**2b**)*

Complex **2b** was recrystallised from hot methanol to give an orange crystalline solid. Yield 148 mg (77%). Slow evaporation of an aqueous solution gave orange plates suitable for X-ray diffraction. ^{1}H NMR ($(CD_3)_2CO$): δ 9.62 (d, ^3J = 5.6 Hz, 1H, tpa-pyridyl), 8.69 (d, ^3J = 7.2 Hz, 2H, dbm-phenyl), 8.55 (d, ^3J = 6.6 Hz, 2H, dbm-phenyl), 8.20 (t, ^3J = 3.6 Hz, 2H, dbm-phenyl), 8.09 (t, ^3J = 7.2 Hz, 2H, tpa-pyridyl) 7.95 (d, ^3J = 7.2 Hz, 2H, dbm-phenyl), 7.85 (t, ^3J = 7.2 Hz, 1H, tpa-pyridyl), 7.80 (t, ^3J = 7.2 Hz, 1H, tpa-pyridyl), 7.68 (t, ^3J = 6.5 Hz, 2H, dbm-phenyl), 7.59 (t, ^{3}J = 6.1 Hz, 2H, tpa-pyridyl) 7.47 (t, ^{3}J = 7.4 Hz, 2H tpa-pyridyl) 7.39 (s, 1H, γ-hydrogen of acac), 6.04 (half of AB system, 2H, tpa-CH_2N), 5.69 (s, 2H, tpa-CH_2N), 5.51 (half of AB system, 2H, tpa-CH_2N). ESI-MS$^+$: m/z = 572.2 ([Co(dbm)tpa]$^{2+}$ - H^+), m/z =

287.7 ([Co(dbm)tpa]$^{2+}$). IR: ν(C=O) 1517 s, 1456, ν(C=C) 1402 s, 764, 758, 713, ν(ClO) 621 s. Elemental analysis required for [Co(dbm)tpa](ClO$_4$)$_2$ ($C_{33}H_{29}Cl_2CoN_4O_{10}$): C, 51.38; H, 3.79; N, 7.26. Found: C, 50.88; H, 3.76; N, 7.14.

[Co(cur)(tpa)](ClO$_4$)$_2$ (**2c**)

Complex **2c** was recrystallised from 1:1 MeOH:diethylether to give a dark red crystalline solid. Yield 156 mg (68%). ^{1}H NMR (MeOD): δ 9.47 (d, ^3J = 5.7 Hz, 1H, tpa-pyridyl), 8.32 (d, ^3J= 6.2 Hz, 2H, tpa-pyridyl), 8.11 (t, ^3J = 7.7 Hz, 2H, tpa-pyridyl), 8.03 (t, ^3J = 7.2 Hz, 1H, tpa-pyridyl), 7.85 (t, ^3J = 7.2 Hz, 1H, tpa-pyridyl), 7.79 (d, ^3J = 7.2 Hz, 2H, tpa-pyridyl), 7.60 (t, ^3J = 6.0 Hz, 2H, tpa-pyridyl), 7.53 (d, ^3J = 7.2 Hz, H, tpa-pyridyl), 7.46 (d, J_{trans} = 9.0 Hz, 2H, H$_{alkene}$), 7.27 (d, J_{meta} = 1.4 Hz, 2H curcumin-phenyl) 7.14 (d, $^3J_{ortho}$ = 8.1 Hz, 2H, curcumin-phenyl), 6.95 (d, $^3J_{trans}$ = 9.0 Hz 2H, H$_{alkene}$), 6.79 (d, J_{ortho} = 8.1 Hz, 2H, curcumin-phenyl), 6.26 (s, 1H, γ-hydrogen of acac), 5.80 (half of AB system, 2H, tpa-C*H*$_2$N), 5.35 (s, 2H, tpa-C*H*$_2$N), 5.09 (half of AB system, 2H, tpa-C*H*$_2$N), 4.04 (s, 3H, curcumin-O*CH*$_3$), 3.92 (s, 3H, curcumin-O*CH*$_3$). ESI-MS$^+$: m/z = 615.0 (M-ClO$_4$). IR: ν(C=O) 1517 s, 1456, ν(C=C) 1402 s, 764, 758, 713, ν(ClO) 621 s. ESI-MS$^+$: m/z = 715.8 ([Co(curcumin)tpa]$^{2+}$ - H$^+$), m/z = 357.7 ([Co(curcumin)tpa]$^{2+}$). IR: ν(C=O) 1504 vs, 1508, ν(C=C) 1425 s, 1274 s, 1177 br, 969, 825, 764 s, ν(ClO) 623, 525. Elemental analysis required for [Co(curcumin)tpa](ClO$_4$)$_2$·H$_2$O, ($C_{39}H_{39}Cl_2CoN_4O_{15}$): C, 50.17; H, 4.21; N, 6.00%. Found: C, 50.11; H, 4.21; N, 5.77%.

Methods and Instrumentation

^{1}H NMR spectra were collected at 300 K on a Bruker 300 MHz spectrometer using commercially available deuterated solvents. Isotopic impurities were used as internal reference signals. Signals for **1b**, **1c**, **2b** and **2c** were assigned from 2D COSY spectra. Mass spectrometry was performed using Electro-Spray Ionisation using a Finnigan LCQ-8 spectrometer. Elemental analyses (C, H, N) were conducted by the Chemical & MicroAnalytical Services Pty Ltd, Campbell Microanalytical Laboratory, at the University of Otago.

Electrochemistry
Cyclic voltammograms were recorded on a BAS100B instrument using a glassy carbon working electrode, a silver/silver chloride reference electrode and a platinum auxiliary electrode. Scans were performed at a scan rate of 100 mV s−1 and the solutions were degassed with argon for at least ten minutes prior to scanning. All experiments were performed at room temperature and were iR compensated. Scan were run in DMF, with 0.1 M tetrapropylammonium perchlorate as the supporting electrolyte, and the ferrocene/ferrocenium couple was used as an internal reference ([Fe(η^5-C$_5$H$_5$)$_2$]$^{0/+}$ = +0.72 V vs. NHE in DMF at 25 °C).

UV-vis spectra
UV-visible measurements were performed on a Cary 1E UVvisible spectrophotometer using a 1 cm x 1 cm quartz cuvette. Scans were run at room temperature as 25 x10^{-5} M solutions of 1:25 MeOH/TRIS buffer (pH 7.4).

Fluorescence spectra

Fluorescence measurements were performed using a Varian Cary Eclipse fluorescence spectrophotometer, using a 1 cm x 1 cm quartz cuvette. Scans were run at room temperature as 25 $x10^{-5}$ M solutions of 1:25 MeOH/TRIS buffer (pH 7.4) at 100 nm/min with excitation and emission slit widths of 10 nm. All solutions were prepared immediately prior to analysis. Emission scans were run between 460 and 700 nm using an excitation wavelength of 420 nm.

Reductions in situ

Emission scans were collected between 460 and 700 nm with an excitation wavelength of 420 nm every 30 minutes over 20 h of 25 $x10^{-5}$ M solutions of 1:25 MeOH/TRIS (pH 7.4) following the addition of 10 molar equivalents of (L)-ascorbic acid or (L)-glutathione (reduced). Deoxygenated solutions were purged by passing a stream of nitrogen through the solutions for 15 minutes prior to addition of the reducing agent and the cuvettes tightly sealed. The extent of fluorescence return was calculated with respect to the fluorescence emission intensity of free curcumin under the same conditions.

Cell culture

DLD-1 human colon carcinoma cells were purchased from ATCC and used within 2 months of resuscitation. Cells were maintained in Advanced DMEM (Invitrogen) and supplemented with 2% FBS and 2mM Glutamine in a humidified environment at 37 ^{o}C and 5% CO_2.

Cytotoxicity assay

Cytotoxicity was determined using the MTT assay. 1 $x10^{5}$ cells per well were plated on to 96-well plates and allowed to adhere overnight. Freshly prepared media/DMSO (90:10) solutions of the complexes and free ligands were added to triplicate wells at concentrations spanning a 4-log range (final DMSO concentrations <0.5%) and incubated for 72 hrs. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (1.0 mM) was added to each well and the cells incubated for a further 4 hrs. The culture medium was removed and the resulting purple precipitate dissolved in DMSO (100 μL). The plate was shaken for 5 seconds and the absorbance measured immediately at 600 nm using a Victor^{3}V microplate reader (Perkin Elmer). IC_{50} values were determined as the drug concentration required to reduce the absorbance to 50% of that of the untreated control wells. At least three independent experiments were performed for each compound with triplicate readings in each experiment.

Preparation of monolayer cell samples for imaging

5 $x10^{3}$ cells were plated on to 2 mL Matek dishes and allowed to adhere overnight. The cells were treated with Spheroids were treated with media/methanol (90:10) solutions of curcumin, **1c** and **2c** to give a final concentration of 20 uM (0.5% methanol). After 24 h, the media was removed, cells were rinsed 3 times with PBS and imaged on a Leica TCS SP5 multiphoton microscope.

Preparation of spheroid samples for imaging

Spheroids were formed by plating 1.5 $x10^{4}$ cells onto 0.75% agarose coated 96 well plates and incubated without movement for 72 hrs in a humidified environment at 37 ^{o}C and 5% CO_2. Spheroids were treated with media/methanol (90:10) solutions of curcumin, **1c**, **2c**, $[RuCl_2(tpa)]ClO_4$, and $[CoCl_2(tpa)]ClO_4$ to give a final concentration of 20 uM (1% methanol). After 24 hrs, spheroids were transferred to a

Matek dish, washed 3 times with PBS and imaged on a Leica TCS SP5 multiphoton microscope using a HC PL APO 20x/0.70 IMM objective. The samples were excited with 800 nm light and the emission wavelengths collected between 540 - 750 nm.

Fluorescence Lifetime Imaging

Fluorescence lifetime images were collected on a Leica TCS SP5 MP FLIM system. The system had a tunable Mai Tai Deep See multiphoton laser with a repetition rate of 80 mHz (Spectra-Physics) attached to a Leica DMI6000B-CS inverted microscope. Samples were illuminated with 800 nm light and emitted light was collected in the descanned internal FLIM detectors over the 540-750 nm range using a HC PL APO 20x/0.70 IMM (glycerol) objective lens. The data was collected with the aid of the B&H SPCM software. Fluorescence lifetimes were determined using time correlated single photon counting (TCSPC) and analyzed with SPCImage software (version 3.1.0.0). The instrument response function was derived from the decay curve of a 0.5 % solution of Rose Bengal and the mono-exponential decay curve of a solution of FITC confirmed. 512 x 512 images were collected and 4X binning applied for analysis to ensure greater than 100 000 photons per pixel were analyzed. A minimum of 10 x 4 second accumulation scans were taken for each sample. For cell free analysis, 50 μM solutions of compounds in methanol at pH 7.4 (acidified with concentrated HCl) were analysed. Analysis of fluorescence lifetimes in spheroids required individually fitted bi-exponential curves to regions of interest defined by the outer edge of the spheroid to obtain average χ^2 values closest to 1. A total of 9 spheroids from 3 independent experiments were analyzed.

Preparation of cell samples for XANES

4×10^6 cells were seeded onto a 6 cm dish and allowed to adhere overnight. Cells were treated with a media/DMSO (90:10) solution of **2c** (prepared immediately prior to dosing) to give a final concentration of 30 μM (1% DMSO). Following incubation for 4 h, the growth medium was removed, the cells trypsinised and 5 mL of PBS solution added. The cells were centrifuged at 2000 rpm for 4 min, the supernatant removed, the washing repeated and then aqueous ammonium acetate (5 mL, 100 mM) added. After further centrifuging, the supernatant was again removed and 70% ethanol solution (0.5 mL) added, before the samples were centrifuged once more. The ethanol was removed and the resulting cell pellet freeze-dried for two days. The experiment was performed in triplicate. XANES analysis of the cell pellets was performed by packing the samples into polycarbonate sample holders (8 x 3 x 3 mm) which were secured with Kapton tape.

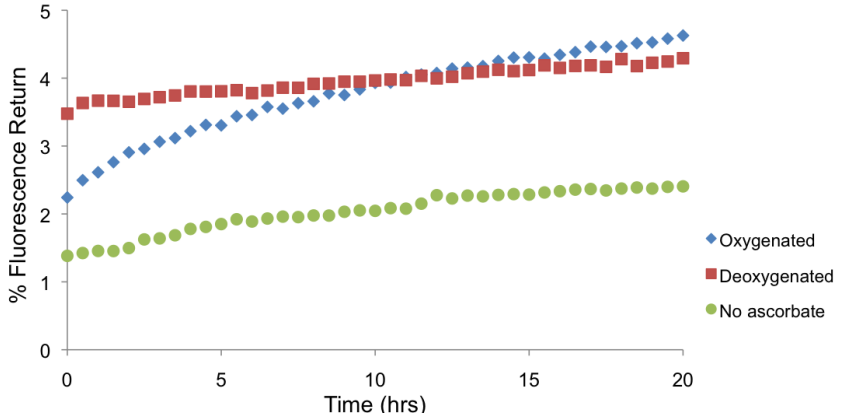
XANES spectra

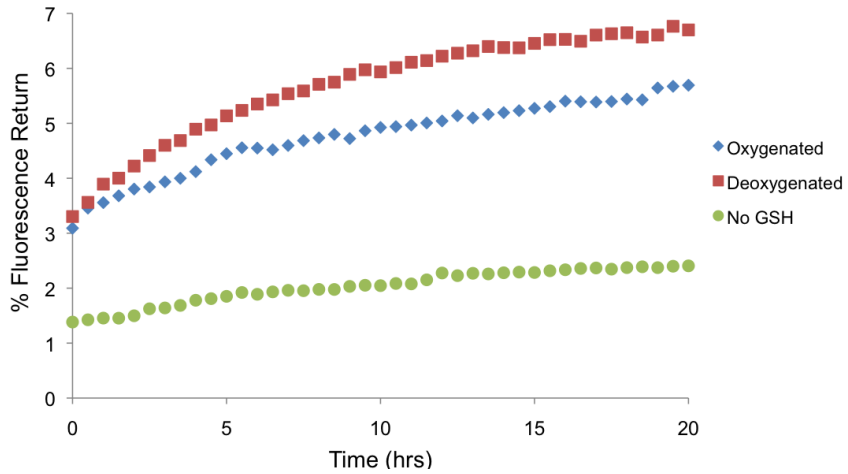
XANES spectra were collected at the X-ray Absorption Spectroscopy beamline at the Australian Synchrotron, using a Si(III) channel cut monochromator. This synchrotron light source operates the storage ring at 3 GeV with a ring current of 200 mA. Energies were calibrated with cobalt foil with the first inflection point being assigned as 7,709.5 eV. XANES spectra were recorded in fluorescence mode at the cobalt K-edge at temperature of 10 K. The scans were averaged using weights based on the signal-to-noise ratios, with each spectrum checked individually before averaging. The spectra were averaged with Average 2.0 and were normalized with Athena using appropriate pre-edge and post-edge values.

Supplementary Figures

Table S1 E_{pc} and E_{pa} values for all complexes in DMF vs. Fc/Fc^+ (mV).

Complex	E_p vs Fc/Fc^+ (mV)	E_a vs Fc/Fc^+ (mV)	Δ_E (mV)
1as	-266	-200	56
1b	-190	-52	138
1c	-288	-	-
2a	-794	-694	100
2b	-722	-612	110
2c	-955	-	-





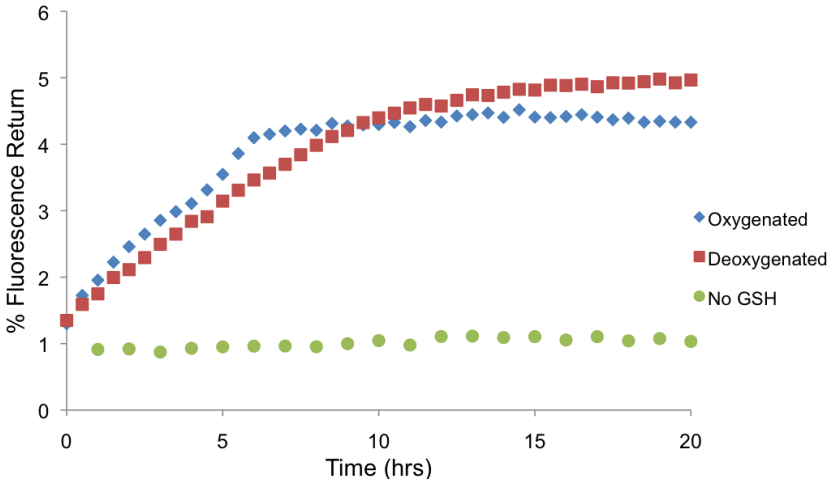


Figure S1 Increase in fluorescence emission over time of solutions of **1c** in the presence of 10 equivalents of ascorbate (top) and glutathione (centre) and **2c** in the presence of 10 equivalents of glutathione (bottom), (25 uM 1:25 MeOH/TRIS buffer, λ_{ex} = 420 nm).

Table S2 Selected crystallographic data for **1a**, **1b**, and **2b**.

Parameter	**1a**	**1b**	**2b**
Formula	$C_{24.5}H_{28.5}Cl_{1.5}N_4O_6Ru$	$C_{34}H_{31}Cl_3N_4O_6Ru$	$C_{66}H_{58}Cl_4Co_2N_8O_{21}$
Fw (g mol^{-1})	629.26	799.05	1558.86
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/c$(#14)	$C2/c$(#15)	$P1$(#2)
a (Å)	8.9060(9)	23.297(2)	9.1898(3)
b (Å)	17.7086(17)	12.4626(12)	11.3905(4)
c (Å)	17.0210(17)	25.803(3)	16.2931(6)
α (°)	90	90	78.018(3)
β (°)	91.073(4)	115.977(4)	82.142(3)
γ (°)	90	90	76.846(3)
Volume (Å^3)	2684.0(5)	6734.8(11)	1617.39(10)
Z	4	8	1
Density (g cm^{-3})	1.557	1.576	1.600
Crystal size (mm)	0.196x0.178x0.120	0.526x0.373x0.090	0.174x0.101x0.026
Crystal colour	Red	Dark Red	Orange
Crystal habit	Block	Plate	Plate
μ (mm^{-1})	1.541	1.770	0.675
Temp (K)	150(2)	150(2)	150(2)
λ(MoKα)(Å)	0.71073	0.71073	0.71073
μ(MoKα) (mm^{-1})	0.779	0.755	0.765
T(SADABS)$_{min,max}$	0.687, 0.746	0.670, 0.747	0.912, 1.000
$2\theta_{max}$ (°)	52.0	72.5	52.0
hkl range	-9 10/21 21/20 20	-38 38/20 20/42 42	-11 11/-14 14/-20 20
N	35996	102834	27884
N_{ind}	5246(R_{merge} 0.0285)	16222(R_{merge} 0.0238)	6332(R_{merge} 0.0357)
N_{obs}	4717(I > 2σ(I))	13519(I > 2σ(I))	5460(I > 2σ(I))
N_{var}	375	460	460
Residuals * $R1(F)$, $wR2(F^2)$	0.0382, 0.1040	0.0296, 0.0793	0.0489, 0.1268
GoF(all)	1.215	1.058	1.019
Residual Extrema (e- Å^{-3})	-0.697, 1.048	-0.598, 0.989	-1.594, 1.194

* $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for $F_o > 2\sigma(F_o)$; $wR2 = (\Sigma w(F_o^2 - F_c^2)^2/\Sigma(wF_c^2)^2)^{1/2}$ all reflections
$w=1/[\sigma^2(F_o^2)+(0.055P)^2+3.7P]$ where $P=(F_o^2+2F_c^2)/3$

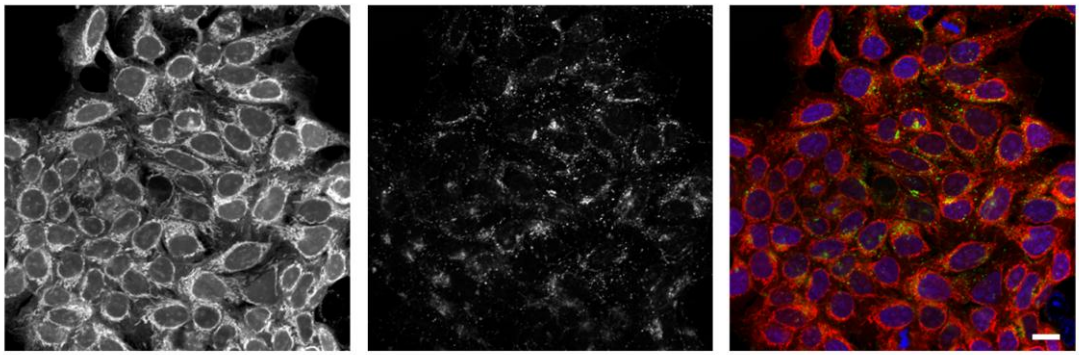


Figure S2 DLD-1 cells stained with Mitotracker (left), Lysotracker (centre) and an overlay of the two images (right) Scale bar = 10 μm.

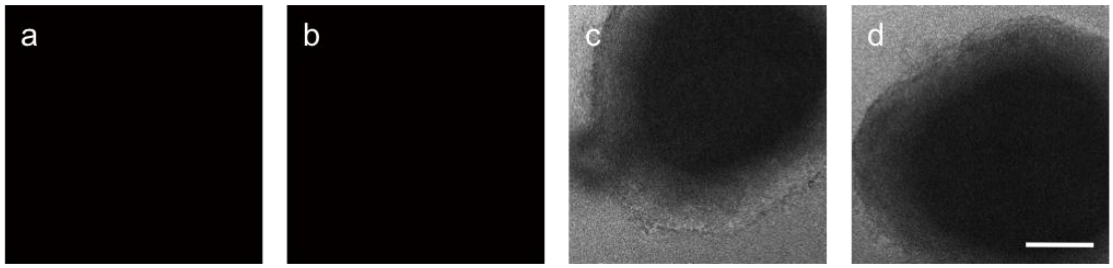


Figure S3 DLD-1 spheroids treated with a) $[RuCl_2(tpa)]ClO_4$, b) $[CoCl_2(tpa)]ClO_4$ and greyscale images c) $[RuCl_2(tpa)]ClO_4$, d) $[CoCl_2(tpa)]ClO_4$. Scale bar = 20 μm.

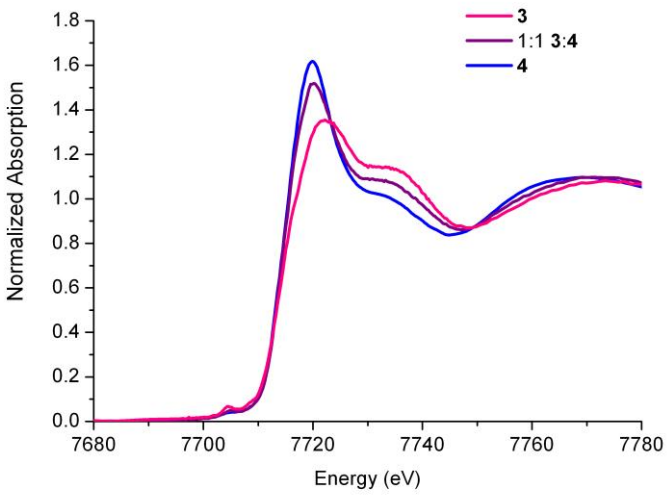


Figure S4 XANES spectra of cobalt(III) complex **3** (pink), cobalt(II) complex **4** (blue) and a 1:1 mixture of **3** and **4** (pink).

Supporting References

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