

Electronic Supplementary Information (ESI)

Confocal and fluorescence lifetime imaging sheds light on the fate of a pyrene-tagged carbon monoxide-releasing Fischer carbene chromium complex

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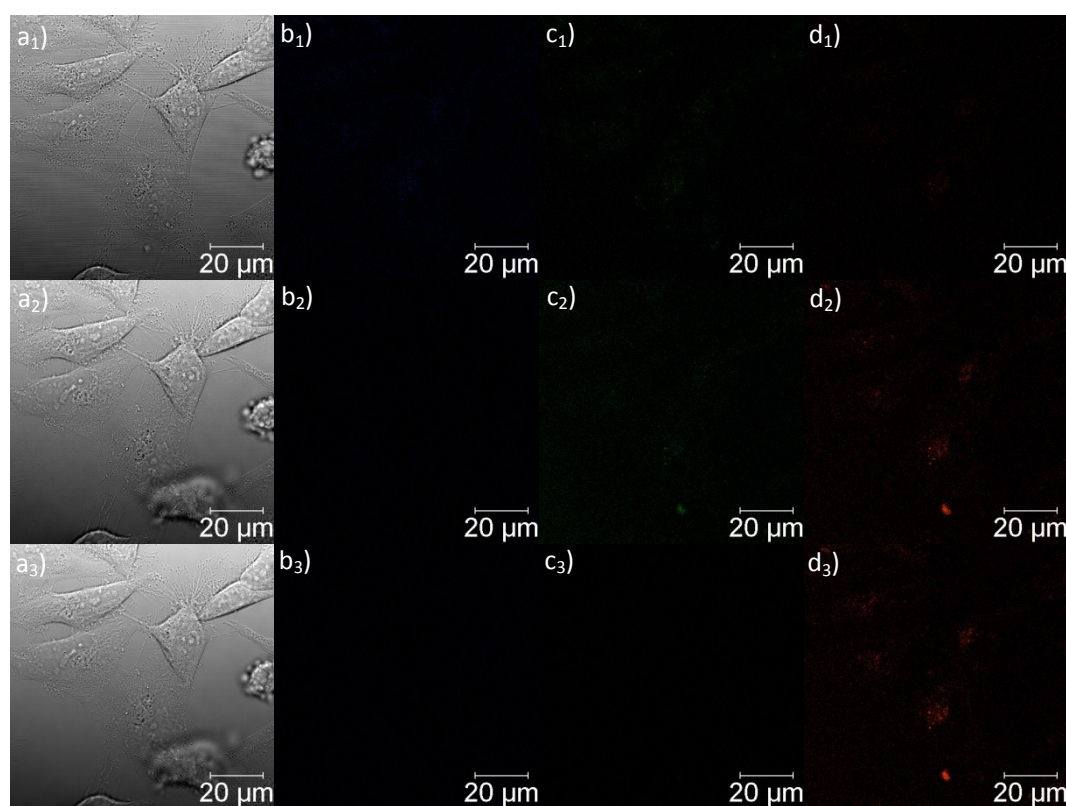


Figure S.1. Laser scanning confocal microscopy of $C_{16}H_9Br$ incubated in HeLa cells for 15 minutes at 50 μM (2% ethanol). a) DIC micrograph, b) blue channel, c) green channel d) red channel, with (1) $\lambda_{ex} = 405$ nm, (2) $\lambda_{ex} = 488$ nm and (3) $\lambda_{ex} = 543$ nm.

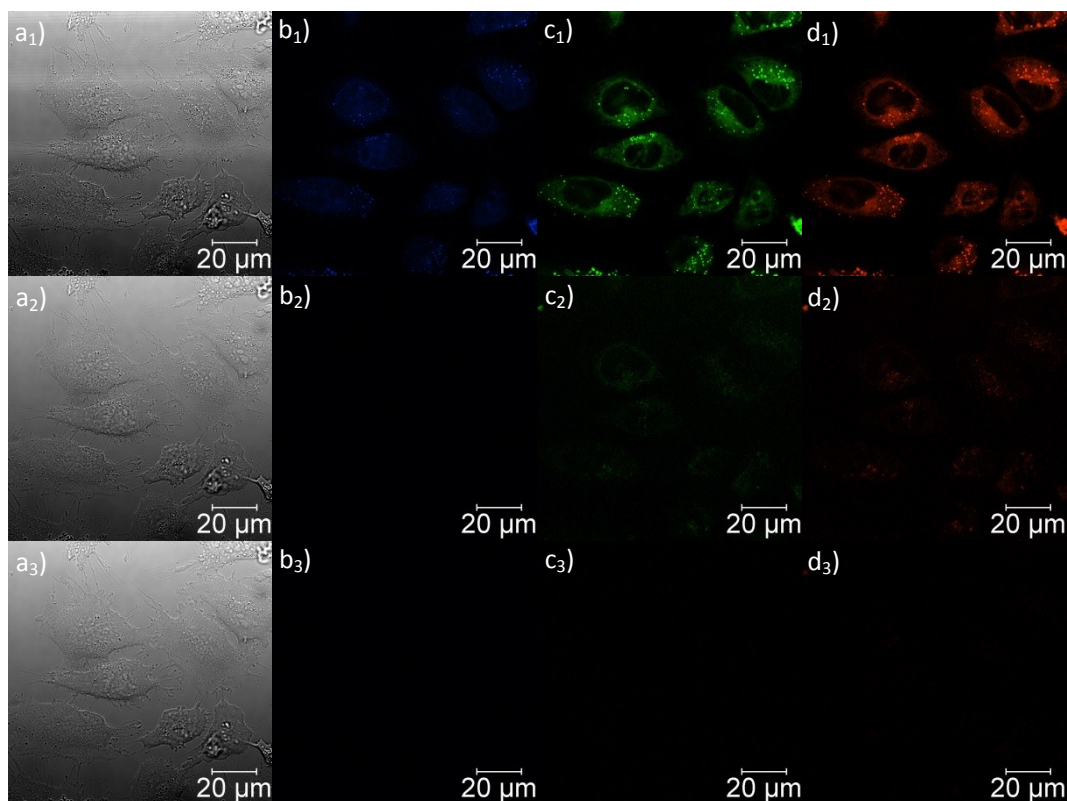


Figure S.2. Laser scanning confocal microscopy of complex **4** incubated in HeLa cells for 15 minutes at 50 μ M (2% ethanol). a) DIC micrograph, b) blue channel, c) green channel d) red channel, with (1) $\lambda_{\text{ex}} = 405$ nm, (2) $\lambda_{\text{ex}} = 488$ nm and (3) $\lambda_{\text{ex}} = 543$ nm.

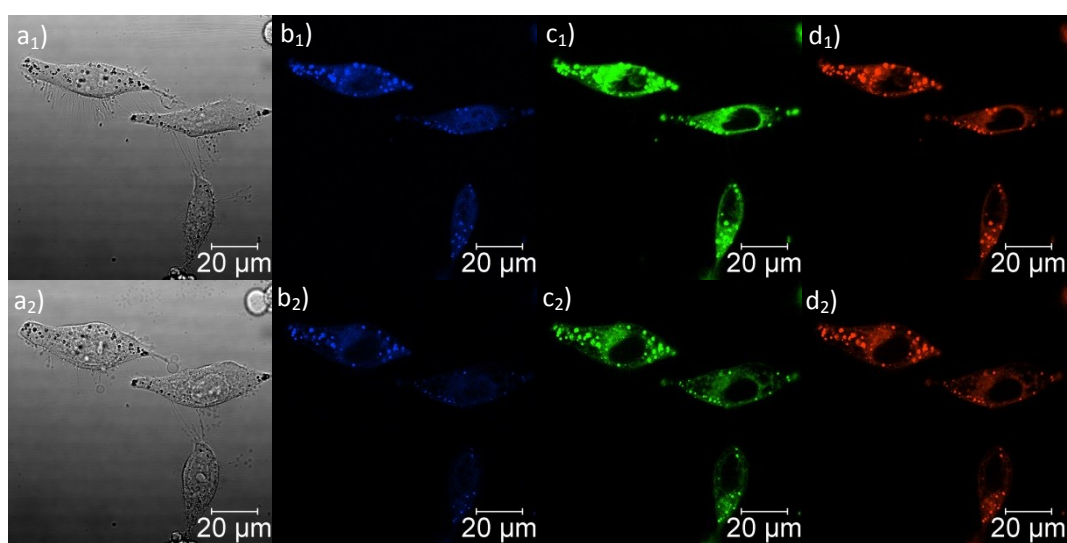


Figure S.3. Laser scanning confocal microscopy of complex **4** incubated in HeLa cells for 15 minutes at 50 μ M (2% ethanol). a) DIC micrograph, b) blue channel, c) green channel d) red channel, with (1) micrographs of cells prior to continuous illumination at 488 nm (2) micrographs of cells after continuous illumination at 488 nm.

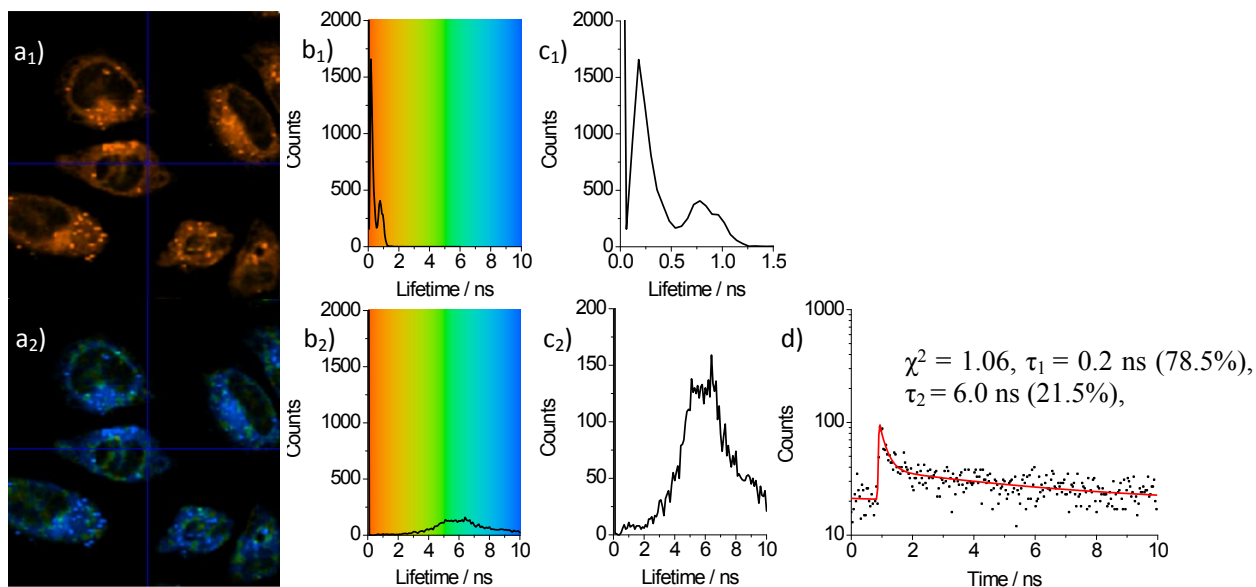


Figure S.4. Two-photon confocal fluorescence of complex **4** in HeLa cells (2% ethanol, 50 μ M, λ_{ex} = 810 nm) a) lifetime mapping b) corresponding lifetime distribution curve, $\tau_1 = 0.3 \pm 0.2$ ns (**b₁**), $\tau_2 = 6.5 \pm 2.8$ ns (**b₂**), x-axis 0 to 10 ns, y-axis 0 to 10 ns, **c₁**) corresponding lifetime distribution curve of τ_1 , x-axis is 0 to 1.5 ns and y-axis is 0 to 2000 counts, **c₂**) corresponding lifetime distribution curve of τ_2 , x-axis is 0 to 10 ns and y-axis is 0 to 200 counts, **d)** sample point decay trace.

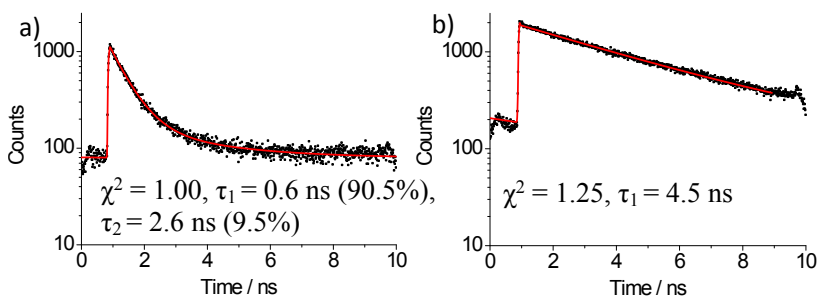


Figure S.5. TCSPC decay curves in DMSO at 2.5 mM of a) ligand precursor, b) complex **4**

Solution Stability Studies on Complex 4

A solution of complex **4** (1.28×10^{-4} mol dm⁻³) in EtOH was prepared and a UV-vis spectrum immediately acquired. Over the course of 2 hours, the bands assigned to the starting material were observed to decrease in intensity to be replaced by those which appear to be due to a new pyrene-containing compound (Figure S.6). A similar study was performed on a 9:1 H₂O:EtOH solution of **4**, however, significant baseline drift was observed in this case meaning that no quantitative analysis could be drawn (Figure S.7). In particular, bubbles were observed in the sample and, following shaking at 83 min a significant rise in the baseline was observed.

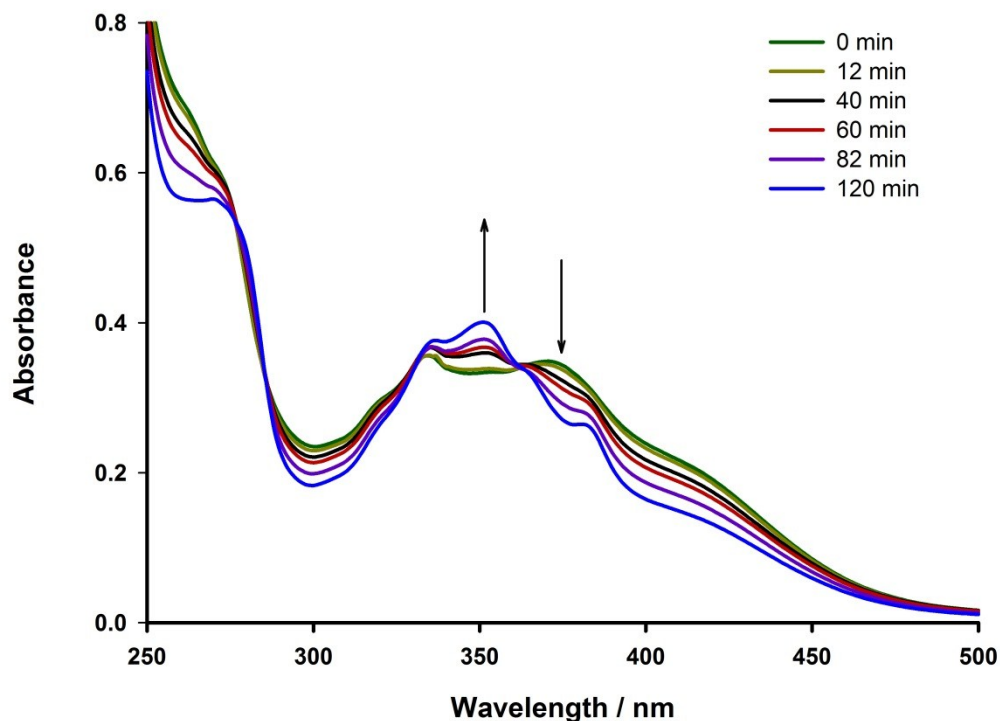


Figure S.6 Stability study of complex **4** in EtOH solution.

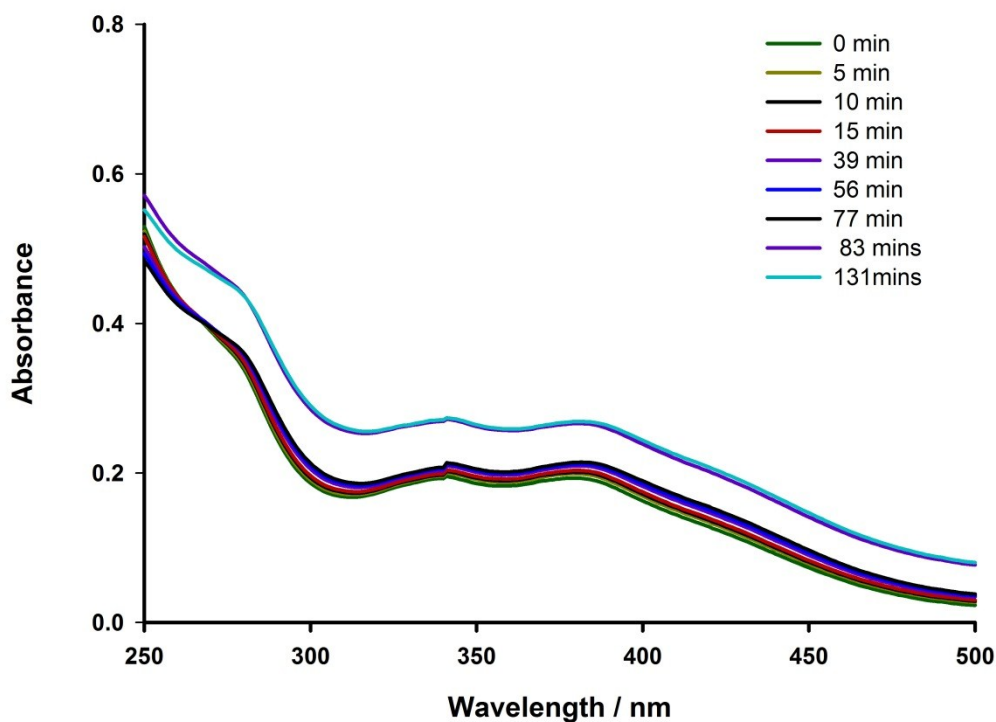
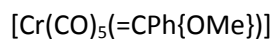


Figure S.7 Stability study of complex **4** in EtOH:H₂O (1:9) solution

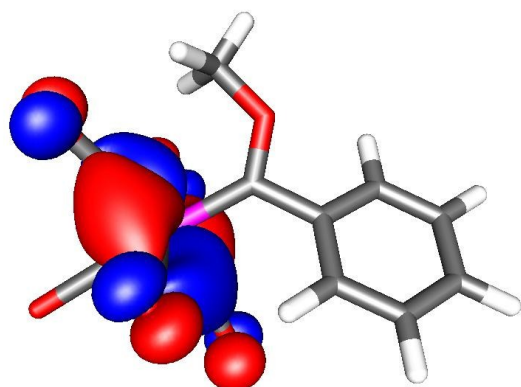
DFT Calculations

All calculations were performed using the TURBOMOLE V6.4 package using the resolution of identity (RI) approximation.^[1] Initial optimisations were performed at the (RI-)BP86/SV(P) level, followed by frequency calculations at the same level, minima were confirmed as such by the absence of imaginary frequencies. Single-point calculations on the (RI-)BP86/SV(P) optimised geometries were performed using the hybrid PBE0 functional and the flexible def2-TZVPP basis set. No symmetry constraints were applied during optimisations. TD-DFT calculations were performed using the *escf* module within TURBOMOLE. Orbitals were visualised using gOpenMol.

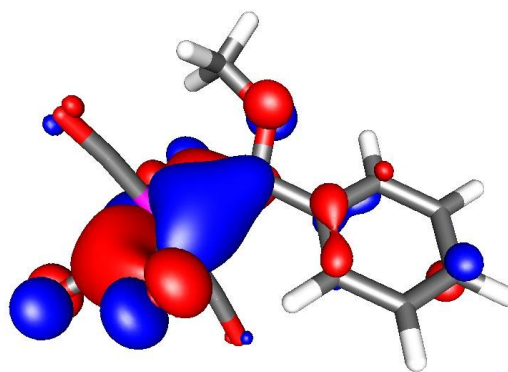
Time-Dependant DFT Studies.



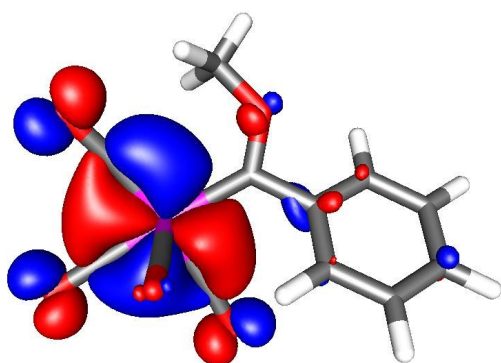
Transition Number	Energy / nm	Oscillator Strength	Orbitals
1	464	0.461×10^{-2}	79a \rightarrow 80a (93.4 %)
2	403	0.250×10^{-2}	77a \rightarrow 80a (93.4 %)
3	372	0.197×10^0	78a \rightarrow 80a (85.4%) 77a \rightarrow 80a (6.8%)



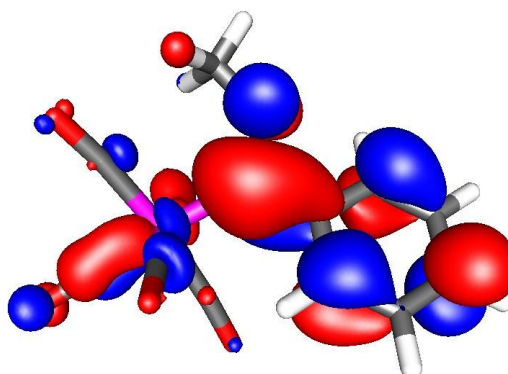
77a HOMO-2



78a HOMO-1



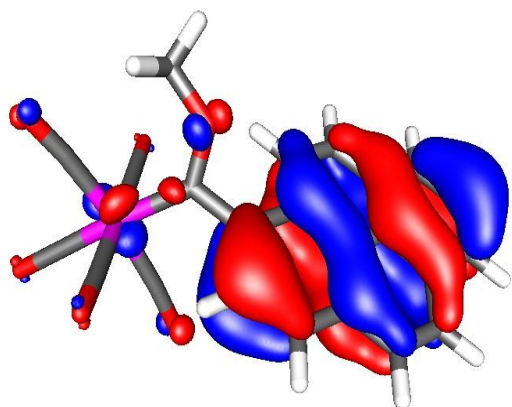
79a HOMO



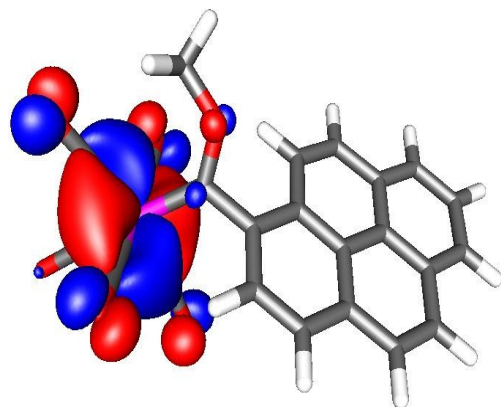
80a LUMO

Complex 4

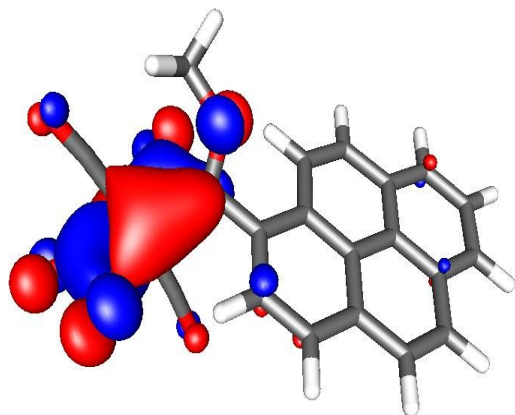
Transition Number	Energy / nm	Oscillator Strength	Orbitals
1	466	0.111×10^0	111a → 112a (43.3 %) 110a → 112a (41.0 %) 110a → 113a (8.1 %)
2	420	0.249×10^0	111a → 112a (43.8 %) 110a → 112a (42.8 %) 110a → 113a (3.2 %) 111a → 113a (3.2 %)
3	396	0.164×10^{-2}	108a → 112a (69.1 %) 109a → 112a (15.5 %) 108a → 113a (9.3 %)
4	359	0.719×10^{-2}	109a → 112a (62.3 %) 108a → 112a (12.1 %) 107a → 112a (10.8 %) 111a → 114a (4.3 %) 111a → 113a (3.5 %)
5	352	0.253×10^{-1}	107a → 112a (39.0 %) 111a → 113a (16.5 %) 111a → 114a (14.0 %) 109a → 112a (10.5 %) 110a → 113a (4.7 %) 108a → 112a (4.7 %) 111a → 112a (3.1 %)
6	339	0.105×10^0	111a → 113 a (52.9 %) 107a → 112 a (14.6 %) 111a → 114 a (12.0 %) 110a → 113 a (5.3 %) 111a → 112 a (5.1 %) 107a → 114 a (2.0 %)
7	316	0.200×10^{-1}	110a → 113 a (61.4 %) 111a → 113 a (10.1 %) 110a → 112 a (7.2 %) 110a → 116 a (5.1 %) 110a → 117 a (3.2 %) 107a → 114 a (2.6 %) 111a → 116a (1.2%)



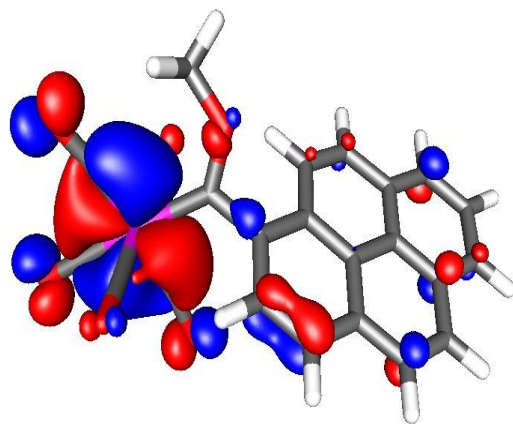
107a HOMO-4



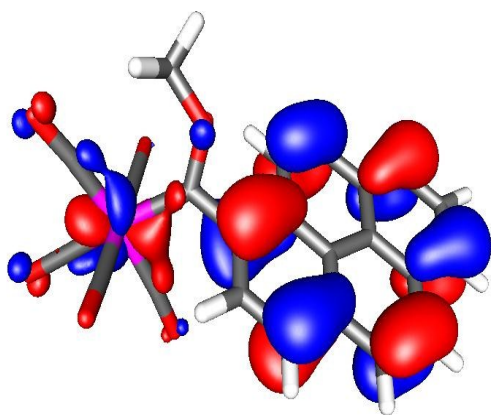
108a HOMO-3



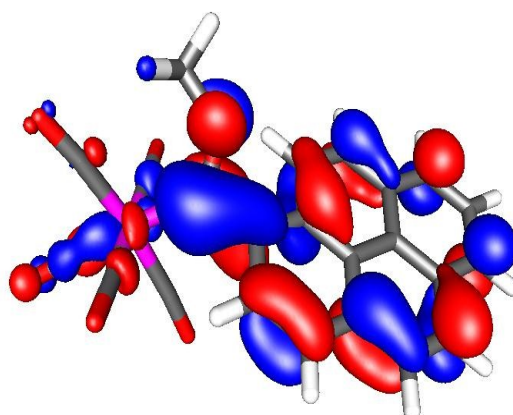
109a HOMO-2



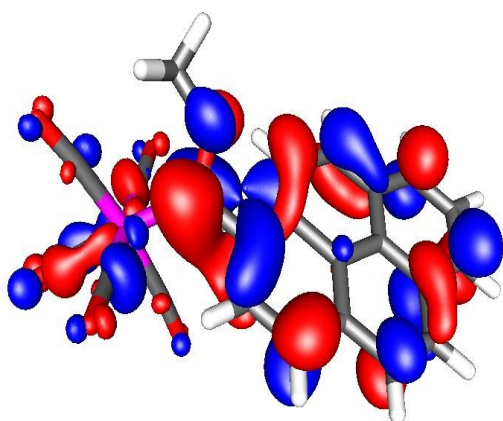
110a HOMO-1



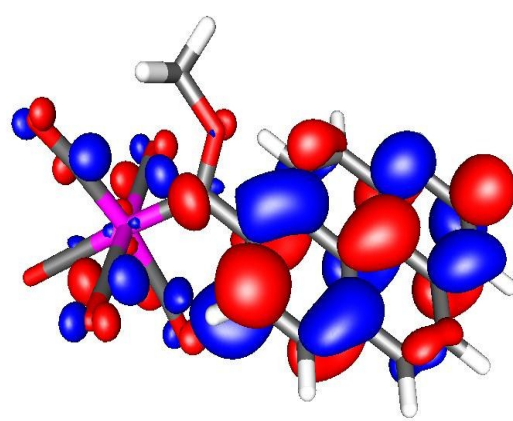
111a HOMO



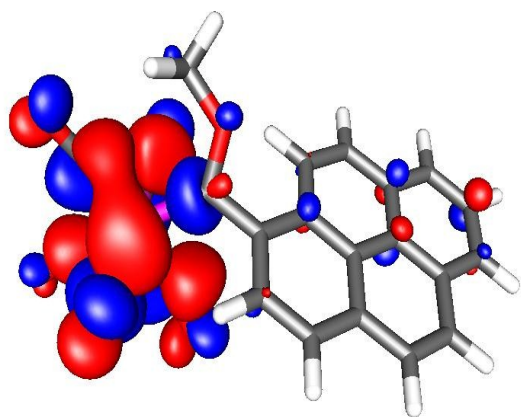
112a LUMO



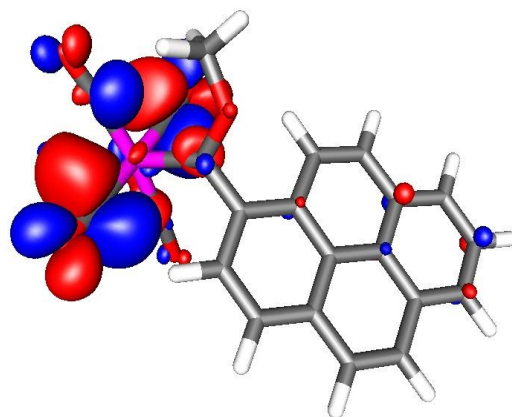
113a LUMO+1



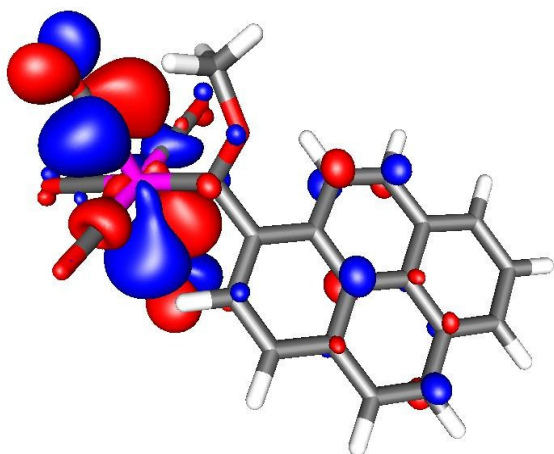
114a LUMO+2



115a LUMO+3

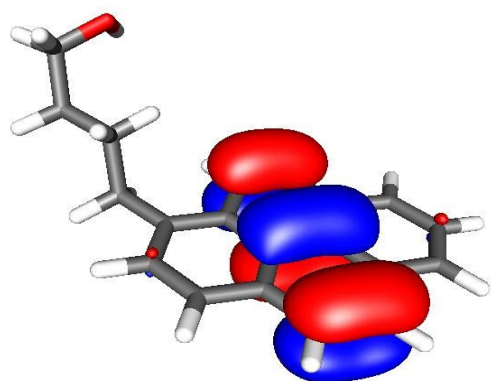


116a LUMO+4

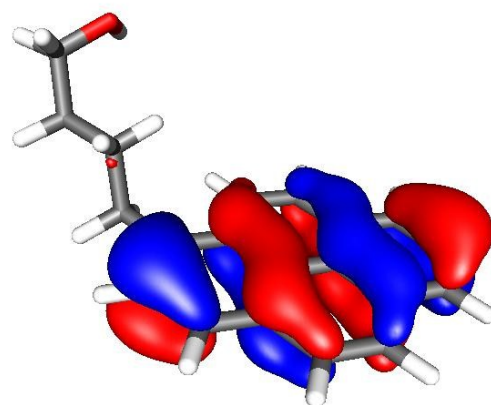


117a LUMO+5

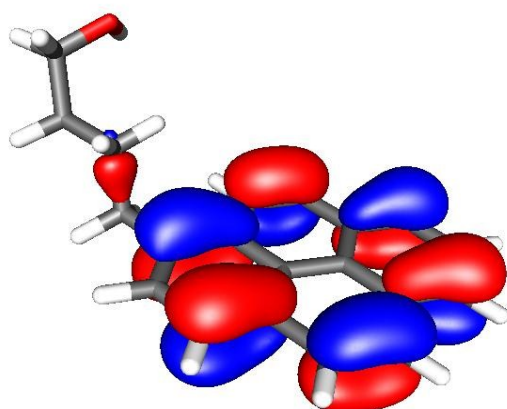
Transition Number	Energy / nm	Oscillator Strength	Orbitals
1	350	0.329×10^0	73a \rightarrow 74a (90.7%)
2	337	0.345×10^{-2}	73a \rightarrow 75a (57.7%) 72a \rightarrow 74a (41.2%)
3	292	0.112×10^{-2}	73a \rightarrow 76a (97.2%)
4	274	0.300×10^0	72a \rightarrow 74a (53.7%) 73a \rightarrow 75a (37.9%)
5	271	0.338×10^{-2}	71a \rightarrow 74a (96.6%)



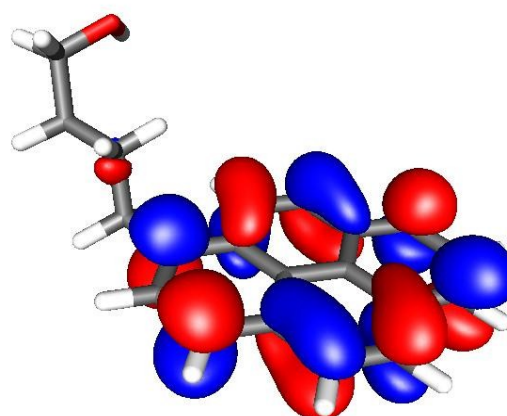
71a HOMO-2



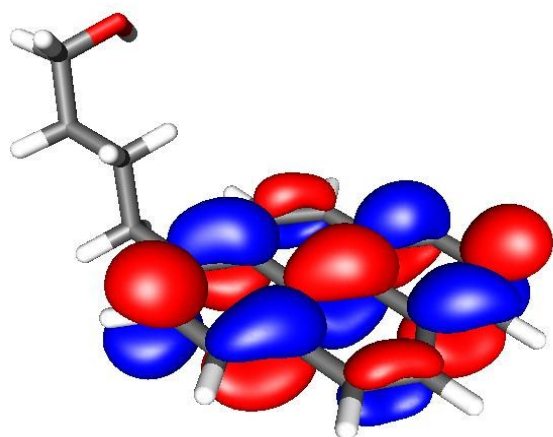
72a HOMO-1



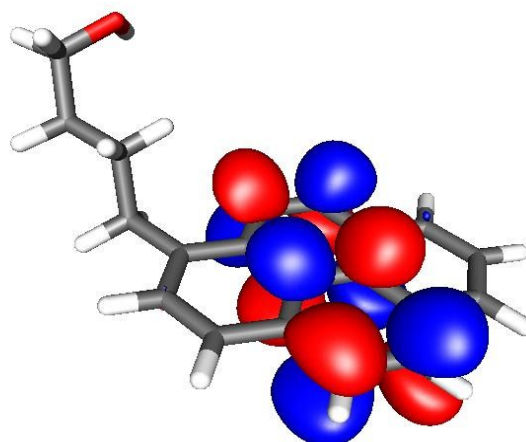
73a HOMO



74a LUMO



75a LUMO+1



76a LUMO+2

Cell culture Studies

Murine RAW264.7 monocyte macrophages (European Collection of Cell Cultures, Wiltshire, UK) and cultured in medium consisting of: Dulbecco's modified Eagle's medium (DMEM) supplementation with 10% foetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 0.1 mg/mL streptomycin. The cells were incubated at 37°C with a 5% CO₂ atmosphere.

Subculture of cells into 24 wells

Passaging the cells

The media in the flask was disposed of and the cells were washed with 10ml of 1x PBS pH 7.2. 10 ml of fresh media was added and the cells were re-suspended in the media using a plastic cell scraper. It is important to be gentle at this stage. 2 ml of the cell suspension was added to a new T-25 cell flask and 8 ml of fresh media was added and the cells were incubated as before. The remaining 8 ml of cell suspension could be used for cell studies. Cells were not used in studies further than 10 passages to prevent false results due to mutation/contamination.

Culturing cells in a 24 well plate

The cells from the 8 ml suspension described previously were counted using a hemocytometer and approximately 5×10^5 cells were added to each well. The total volume of media in each well was 1 ml. The cells were incubated at 37 °C overnight and were 80-90% confluent the next day.

Alamar blue assay^[2]

Cell metabolism was determined by the use of an Alamar Blue assay kit and carried out according to the manufacturer's instructions (SigmaAldrich). The medium from CO-RM treated wells was removed from the wells on the plate and replaced by 1 mL of a 10% AlamarBlue: 90% complete cell culture medium mixture. The plates were incubated for 6 h at 37°C with 5% CO₂, after which 200 µL from each well was loaded onto a 96 well plate to be read on a plate reader at 570 nm (subtracting any background absorbance at 630 nm). The intensity of the red colour is proportional to the metabolism of the cells, which is calculated as the difference in absorbance between 570 and 630 nm and expressed as a percentage of a 1% Triton-X114 control. The resulting data are shown in Figure S.8.

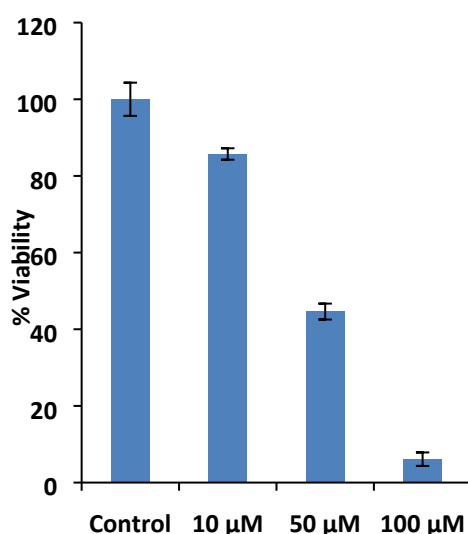


Figure S.8 Alamar blue assay on complex 4.

Lactate dehydrogenase (LDH) assay^[2]

The LDH based cytotoxicity detection kit (Sigma Aldrich) gives a method for the colourimetric assay for the quantification of cell death and cell lysis based on the measurement of LDH activity released from the cytosol of damaged cells into the supernatant. The assay was carried out according to the manufacturer's instructions. After incubation with the CO-RM, 50 µl of supernatant was removed from each well and was added to a flat bottomed 96 well plate. 100 µl of LDH reaction mixture (Sigma Aldrich) was then added to each well across the plate using a multi-channel pipette (this is essential to ensure comparable absorbances). The plate was protected from light and after 30 minutes 15 µl of 1M HCl was added to each well with a multi-channel pipette. Any bubbles were popped with a needle and the plate was read on a plate reader at 490 nm with a background subtraction at 630 nm. Results were expressed as a percentage scaled between the difference in absorbance of a 1% triton-X-114 control (dead cells) and

a live control with no compound. Blank media was also ran in this assay and the absorbance from this was subtracted from all wells. Samples were run in triplicate and the resulting data are shown in Figure S.9.

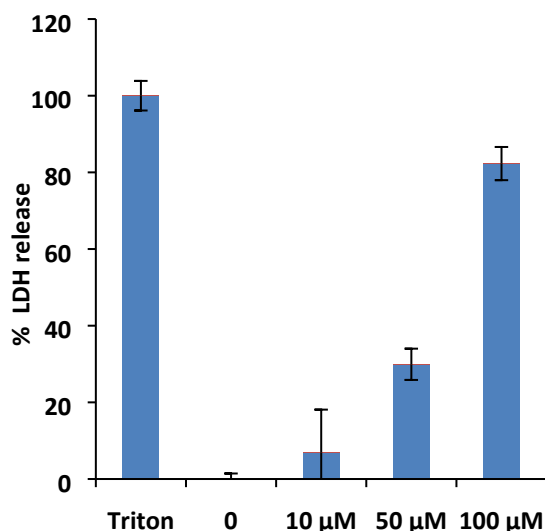


Figure S.9 LDH assay on complex 4.

References

- [1] (a) P. Csaszar and P. Pulay, *J. Mol. Str.*, 1984, **114**, 31;(b) R. Ahlrichs, M. Bar, M. Haser, H. Horn and C. Kolmel, *Chem. Phys. Lett.*, 1989, **162**, 165;(c) K. Eichkorn, O. Treutler, H. Ohm, M. Haser and R. Ahlrichs, *Chem. Phys. Lett.*, 1995, **240**, 283;(d) O. Treutler and R.Ahlrichs, *J. Chem. Phys.*, 1995, **102**, 346;(e) K. Eichkorn, F. Weigend, O. Treutler and R. Ahlrichs, *Theo. Chem. Acc.*, 1997, **97**, 119;(f) M. Arnim and R. J. v. Ahlrichs, *Chem. Phys.*, 1999, **111**, 9183;(g) P. Deglmann and F.Furche, *J. Chem. Phys.*, 2002, **117**, 9535;(h) P. Deglmann, F. Furche and R. Ahlrichs, *Chem. Phys. Lett.*, 2002, **362**, 511;(i) P. Deglmann, K. May, F. Furche and R. Ahlrichs, *Chem. Phys. Lett.*, 2004, **384**, 103.
- [2] D. Scapens, H. Adams, T. R. Johnson, B. E. Mann, P. Sawle, R. Aqil, T. Perrior and R. Motterlini, *Dalton Trans.*, 2007, 4962.