Supplementary Information

Shining light on the stability of metal thiosemicarbazonate complexes in living cells by FLIM

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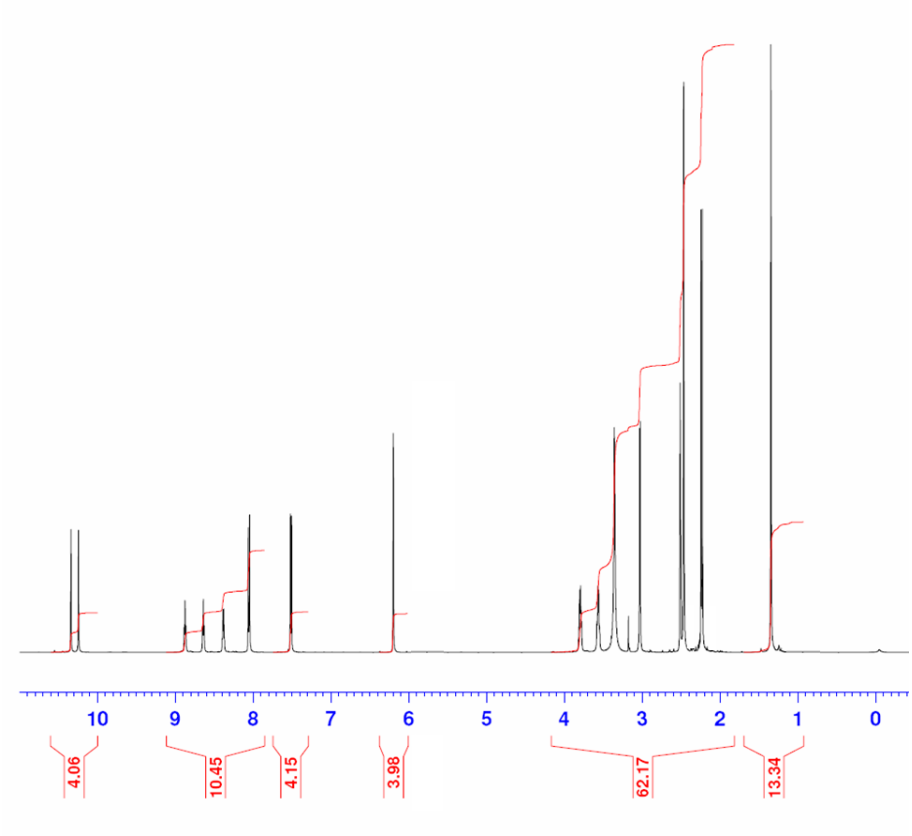
Contents

SI1: Experimental data

L1

^{1}H:



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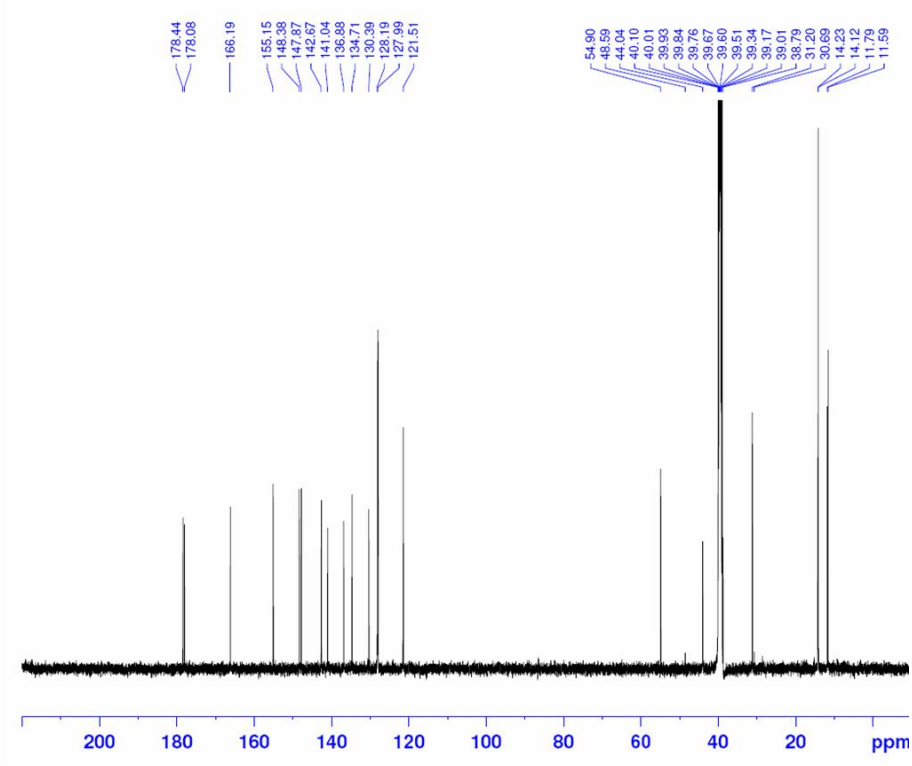

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Comment			

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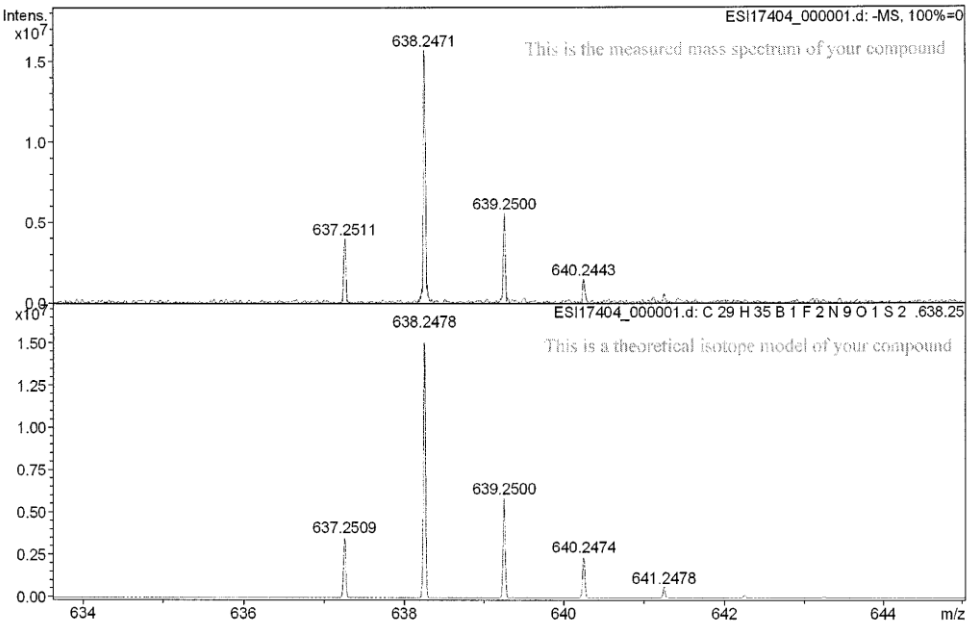

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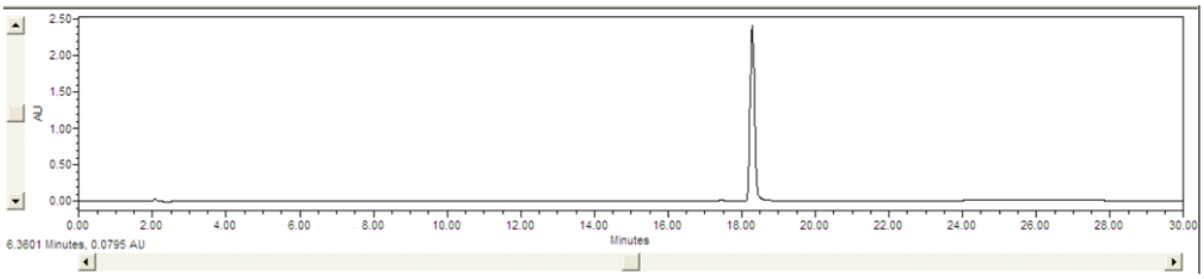

NiL1

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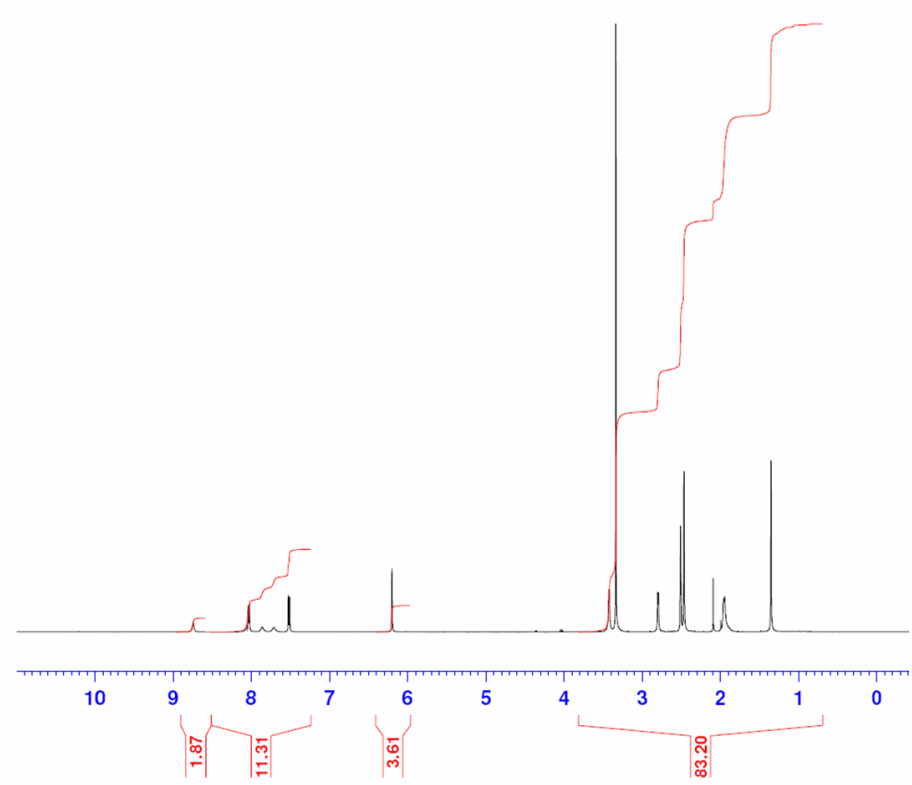

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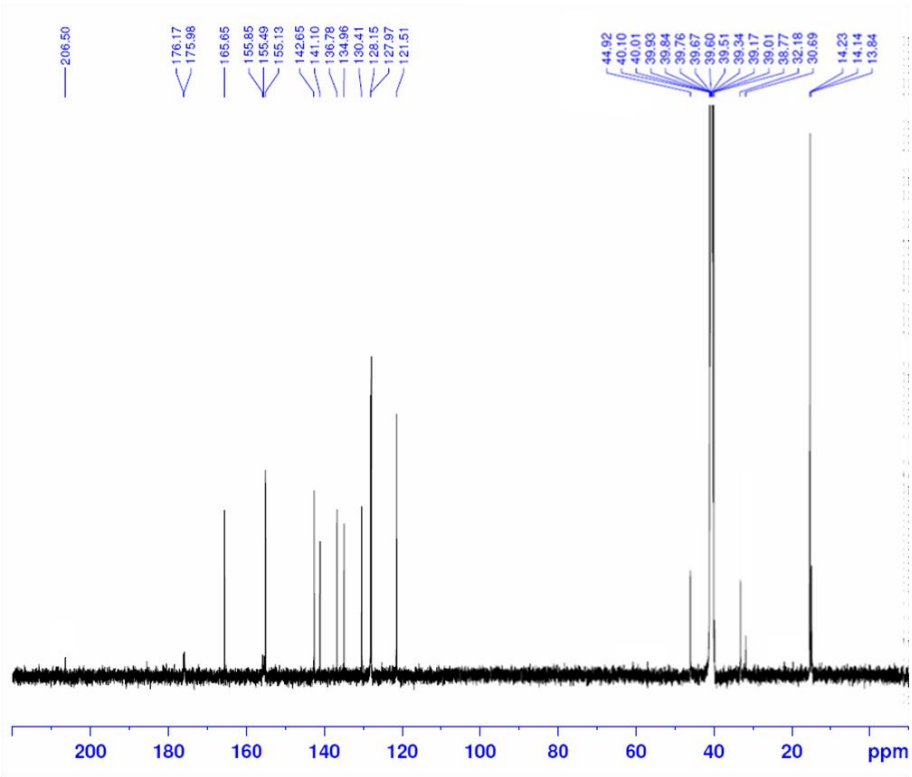

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Mass Spectrum SmartFormula Report

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Comment			

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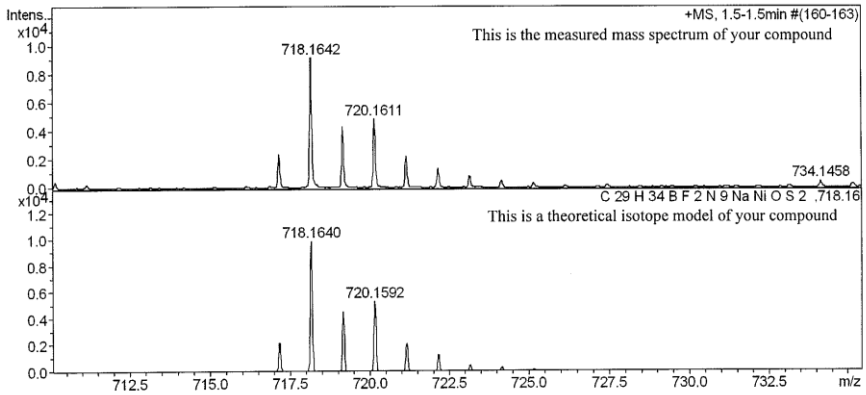

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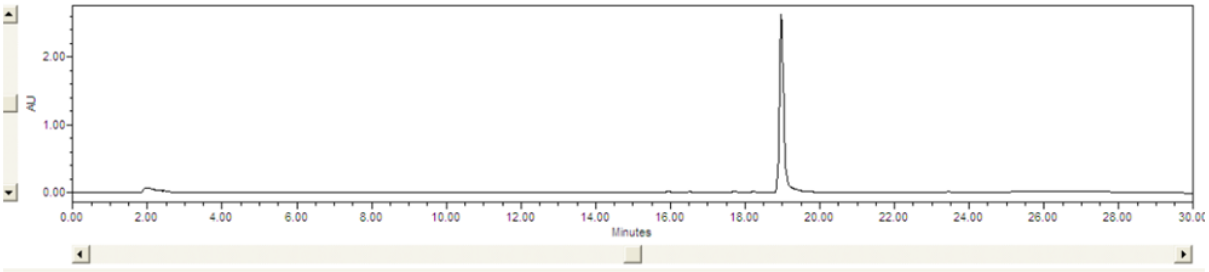

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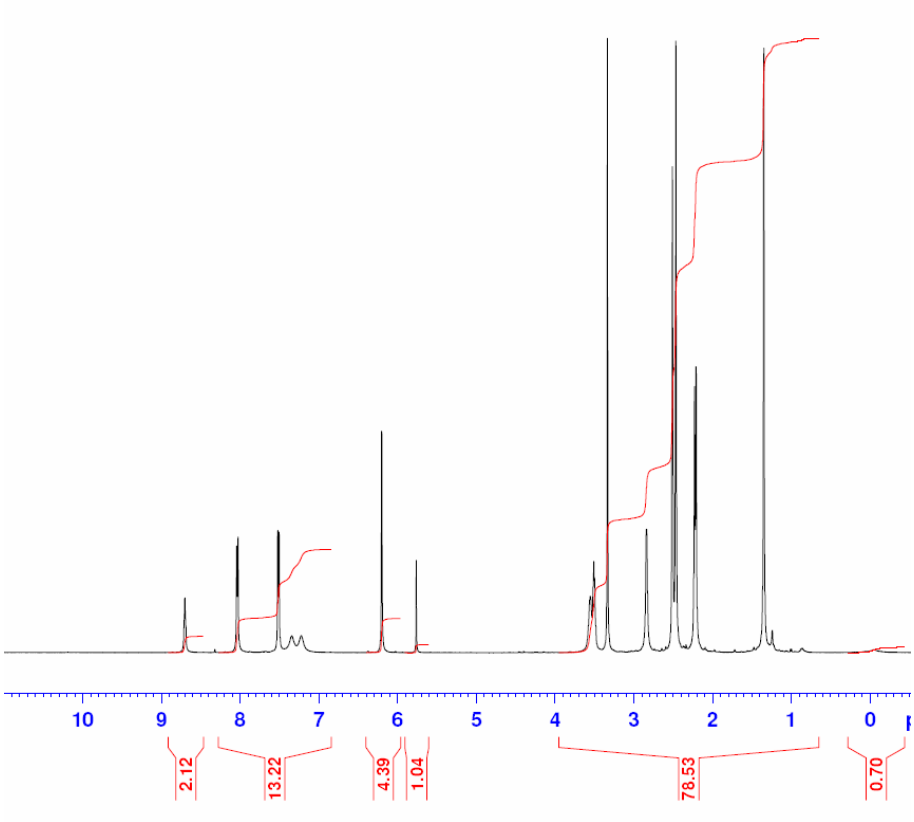

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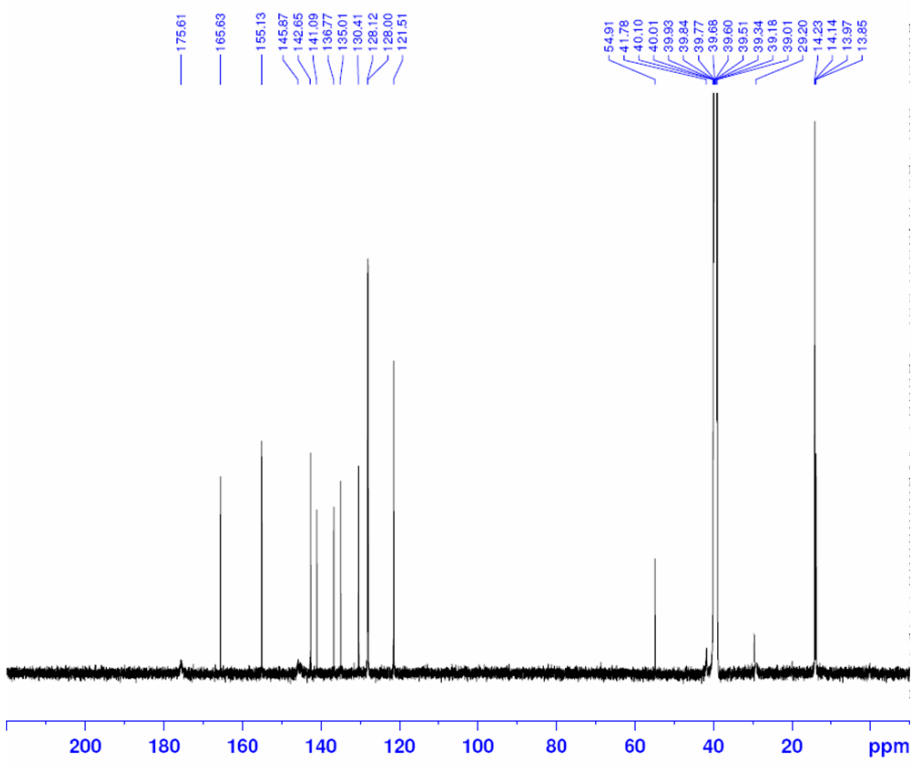

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ESI-MS Accurate Mass Report

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Comment			

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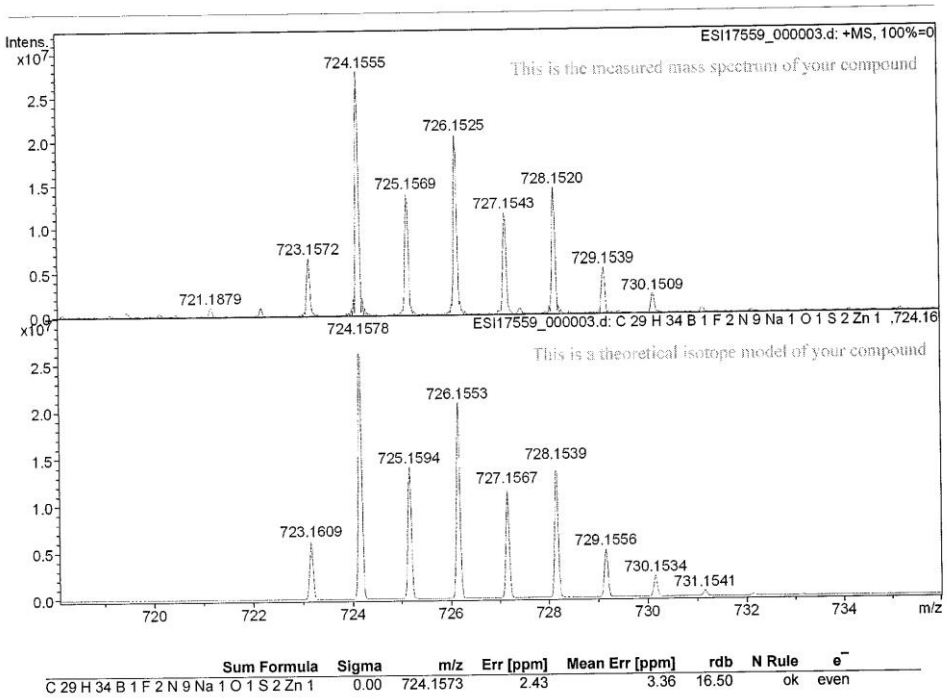

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
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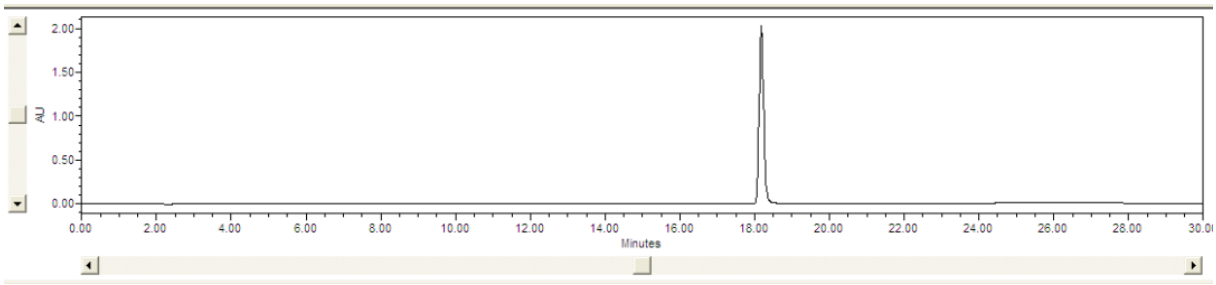

CuL1

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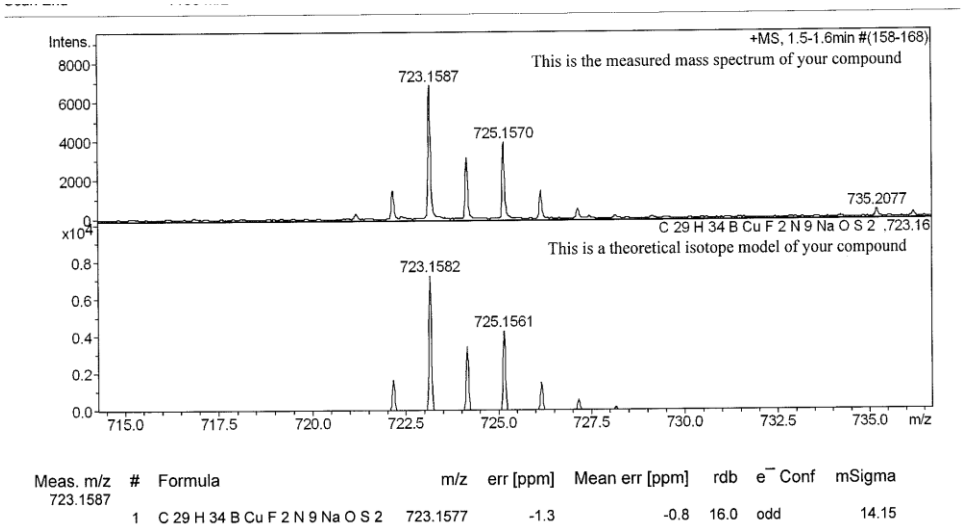

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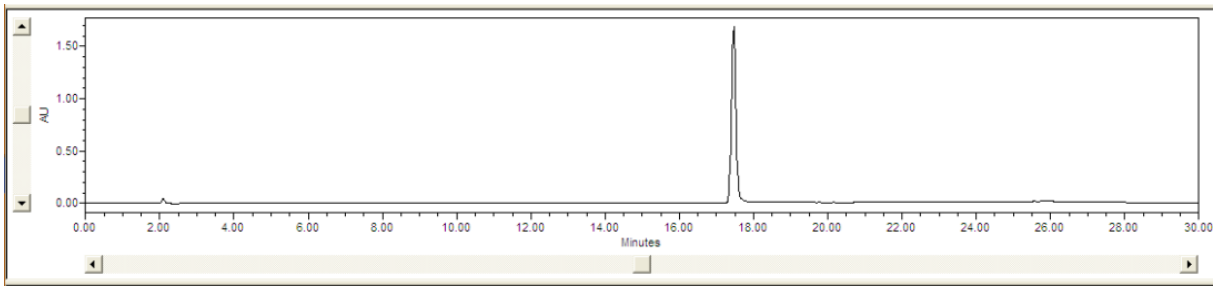
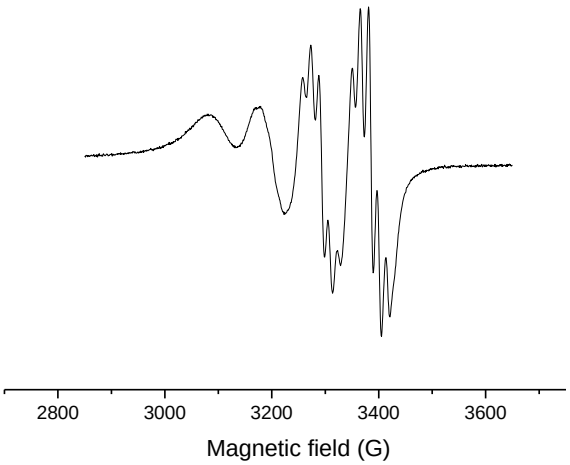


EPR:

Figure S1_1: EPR of **CuLI** at 295K at X-band and 1mmol concentration in DMSO/ethylene glycol (4:1).



L2:

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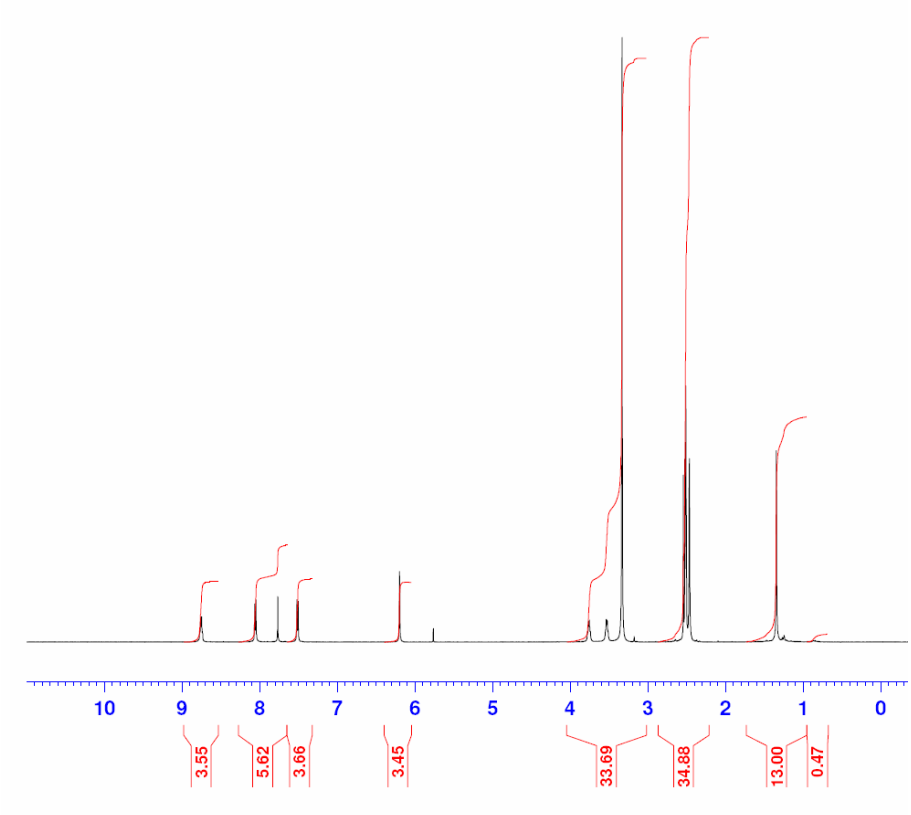

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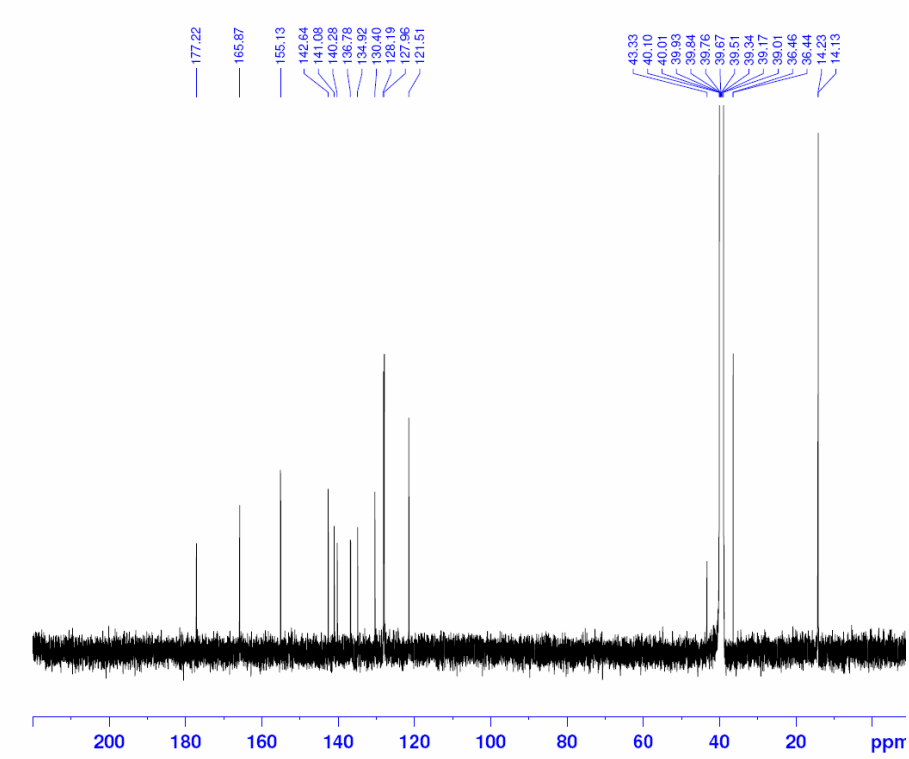

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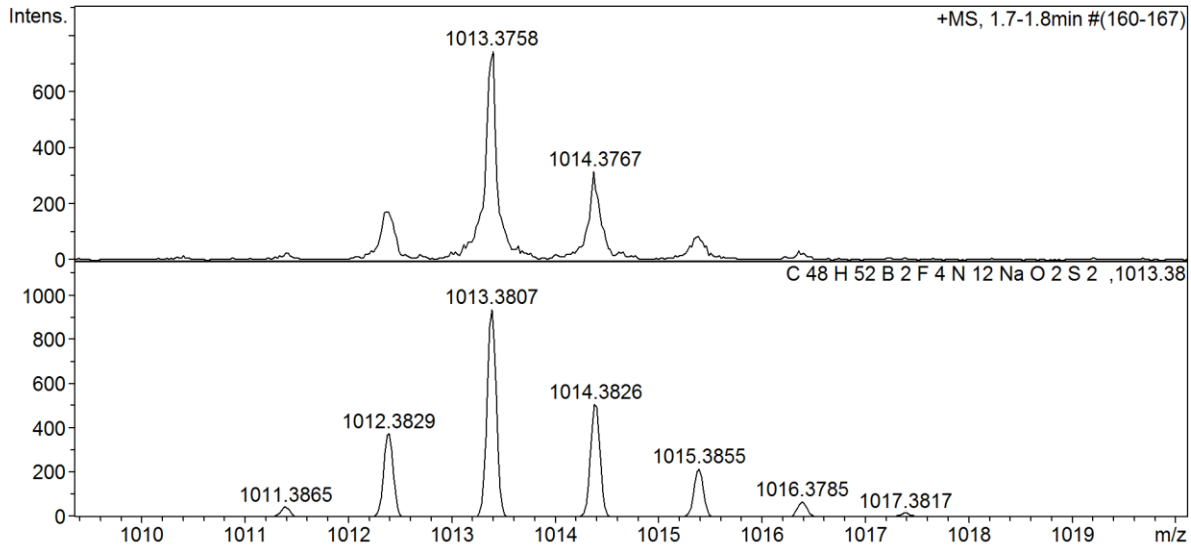
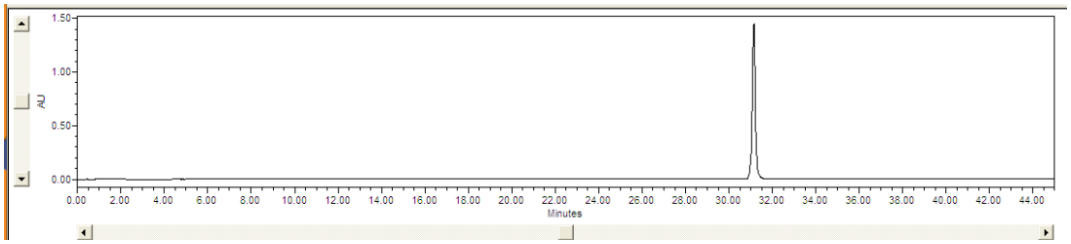
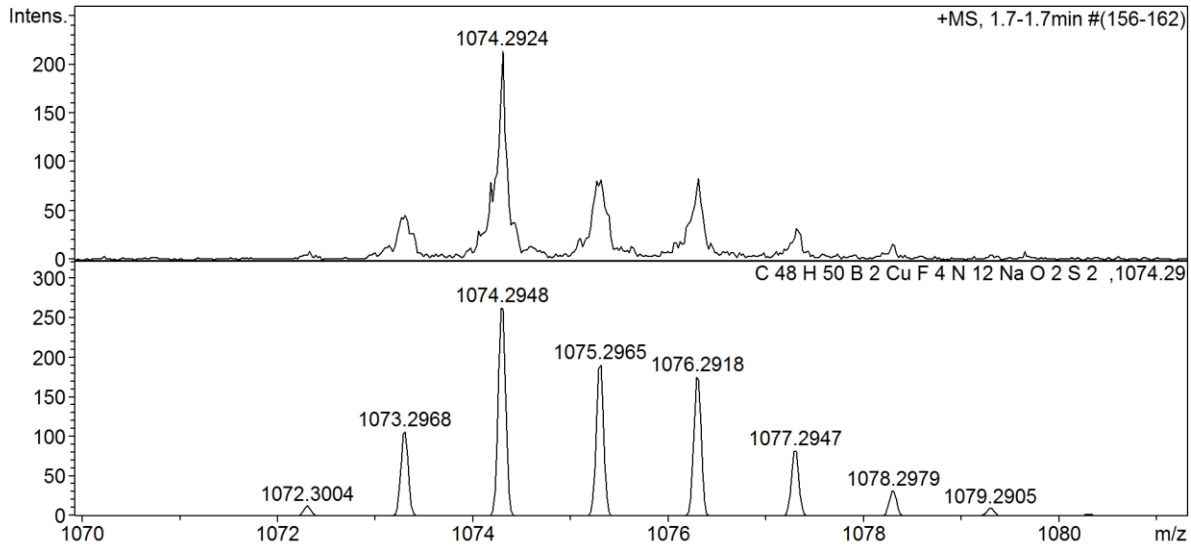

Intens.
+MS, 1.7-1.8min #(160-167)
1013.3758
1014.3767
C 48 H 52 B 2 F 4 N 12 Na O 2 S 2 ,1013.38
1013.3807
1014.3826
1012.3829
1015.3855
1011.3865
1016.3785
1017.3817
m/z

HPLC:


AU
Minutes

CuL2

MS:



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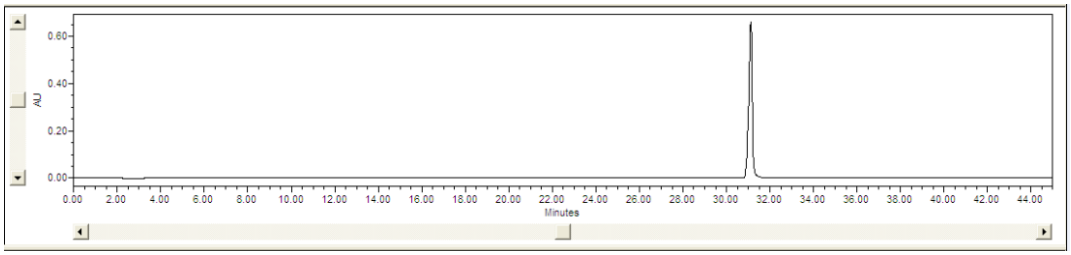


EPR

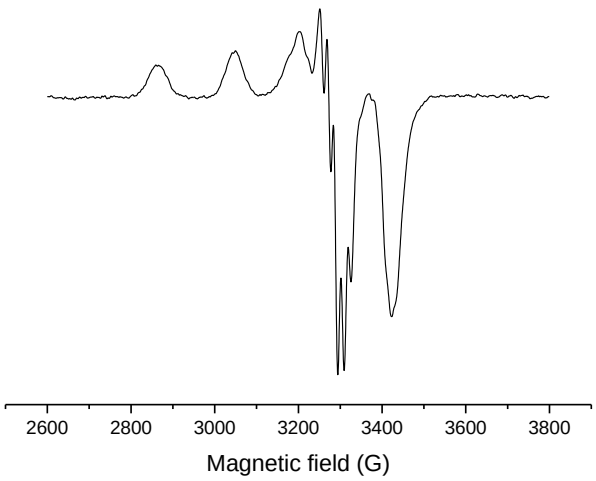


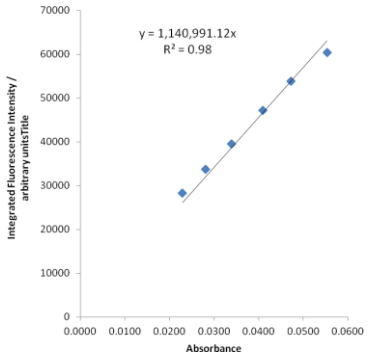
Figure S1_2 EPR of **CuL2** at 210K at X-band and 1mmol concentration in DMSO/ethylene glycol (4:1).

S2 a: Fluorescence quantum yields:

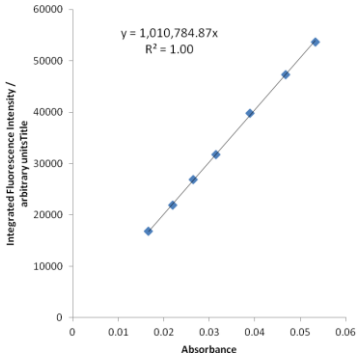
Standard plots used for calculating quantum yields of **L1**, **CuL1**, **NiL1**, **ZnL1**, **L2** and **CuL2** in DMSO, MeOH and H_2O by relating fluorescence intensity to UV absorption (λ_{ex} = 496nm), relative to fluorescein (Φ = 0.95) as a reference

L1

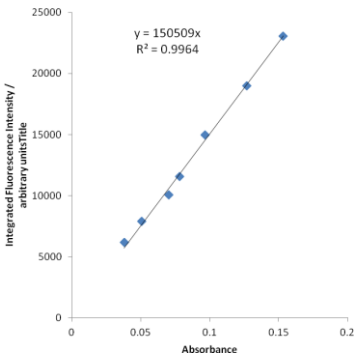
DMSO



MeOH

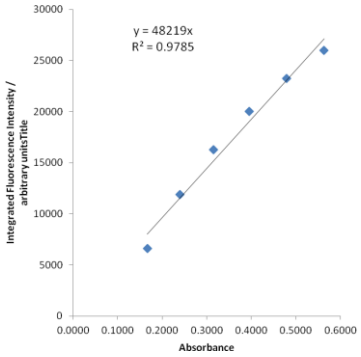


H_2O



CuL1

DMSO



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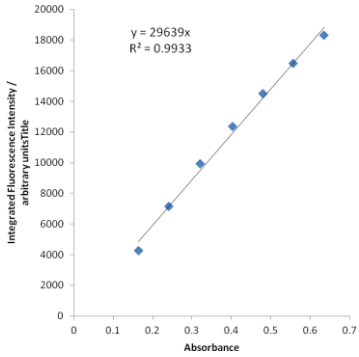

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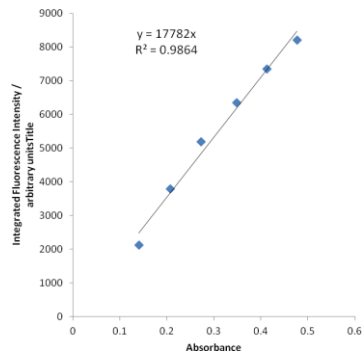
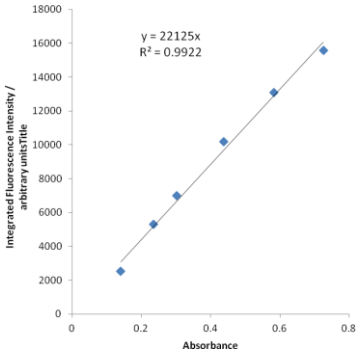


NiL1

DMSO



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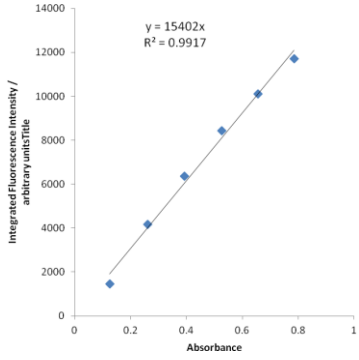

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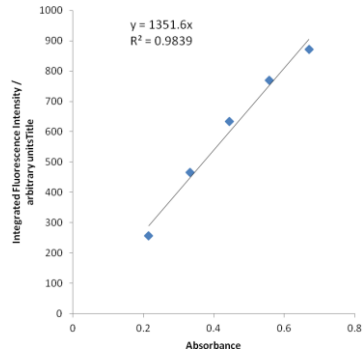
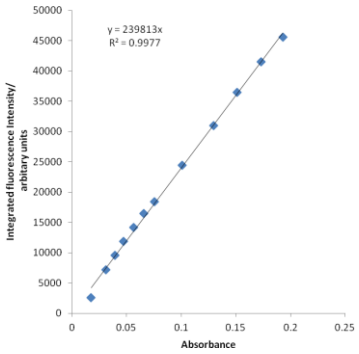


ZnL1

DMSO



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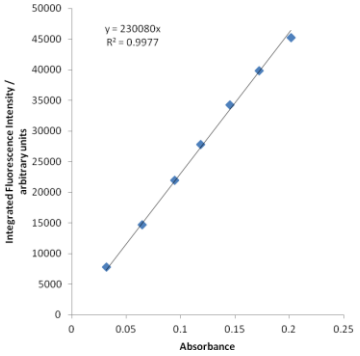

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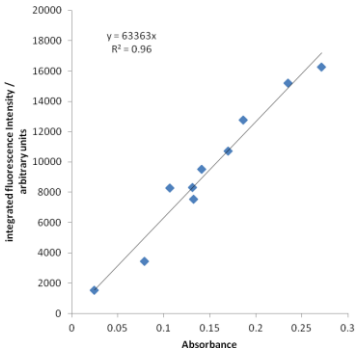
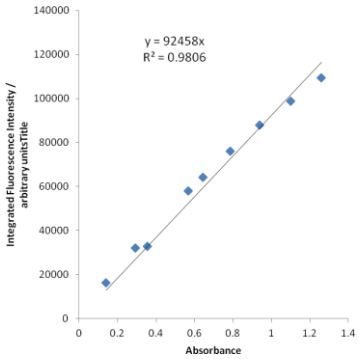


L2

DMSO



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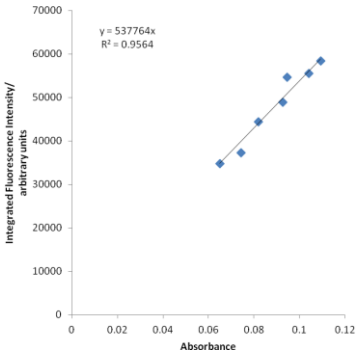

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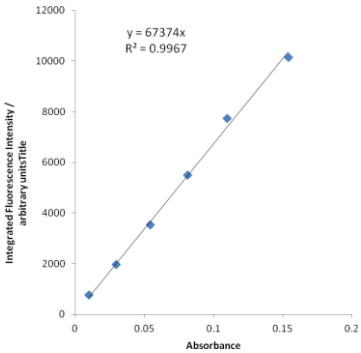
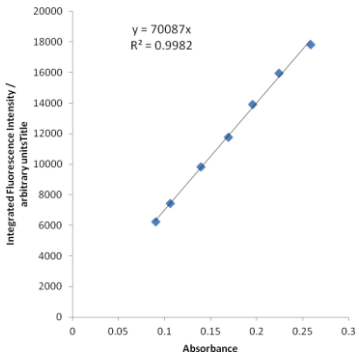


CuL2

DMSO



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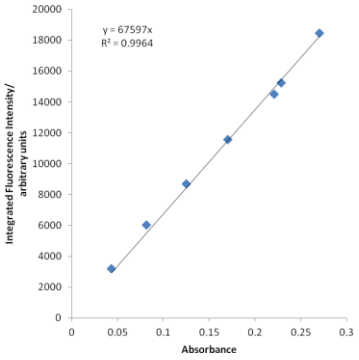

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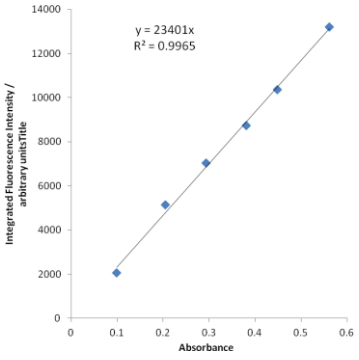


S2 b: Serum stability plots

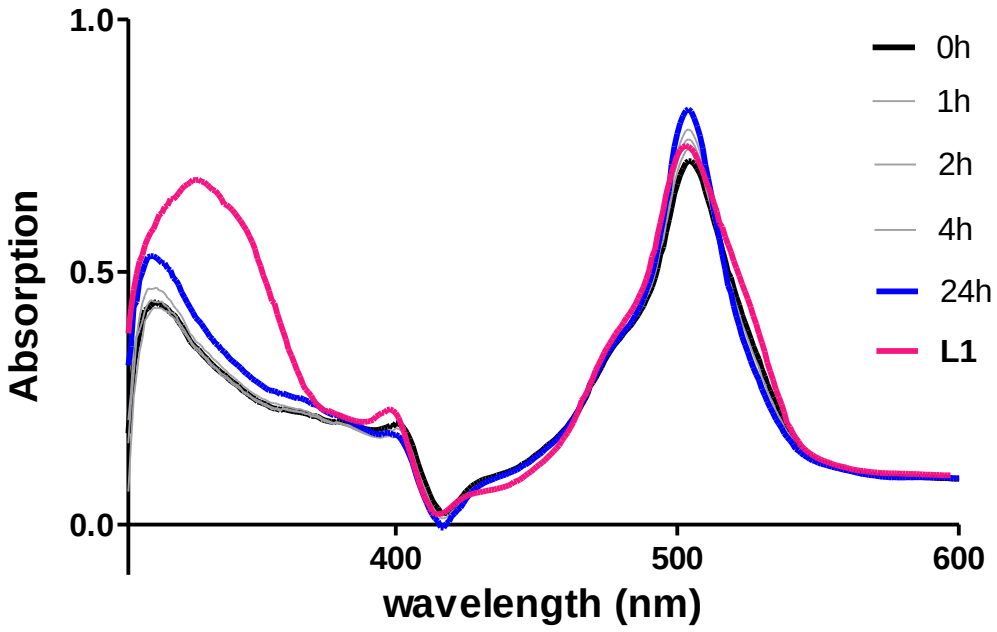


Figure S2_1: Overlay of UV/Vis spectra of **CuL1** in human serum after 0 (black), 1, 2, 4 and 24 h (blue) incubation in human serum. (Control: **L1** incubated in human serum for 24 h (pink))

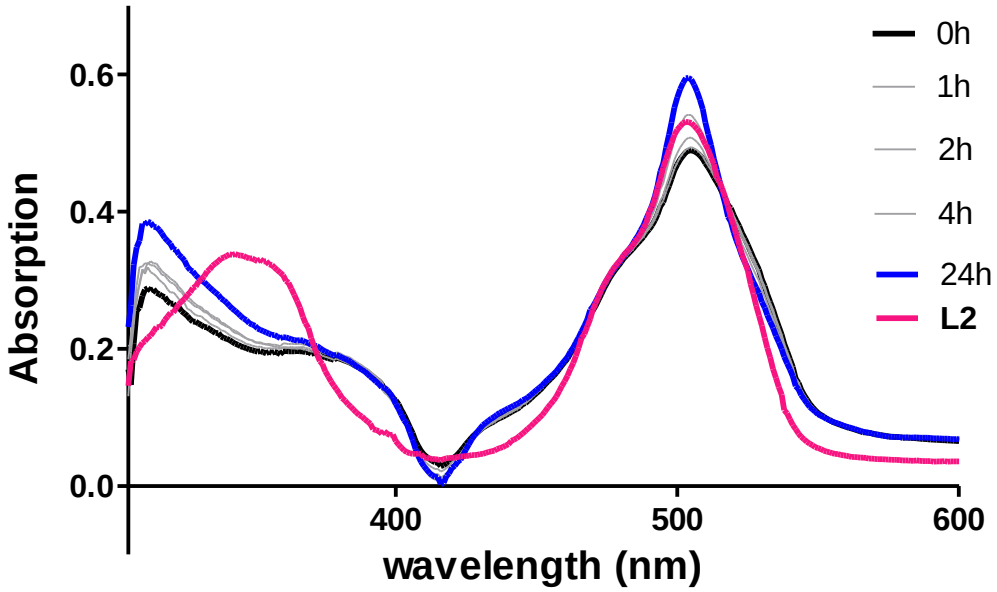


Figure S2_2: Overlay of UV/Vis spectra of **CuL2** in human serum after 0 (black), 1, 2, 4 and 24 h (blue) incubation in human serum. (Control: **L2** incubated in human serum for 24 h (pink))

S3: Confocal Fluorescence Colocalisation studies

NiL1

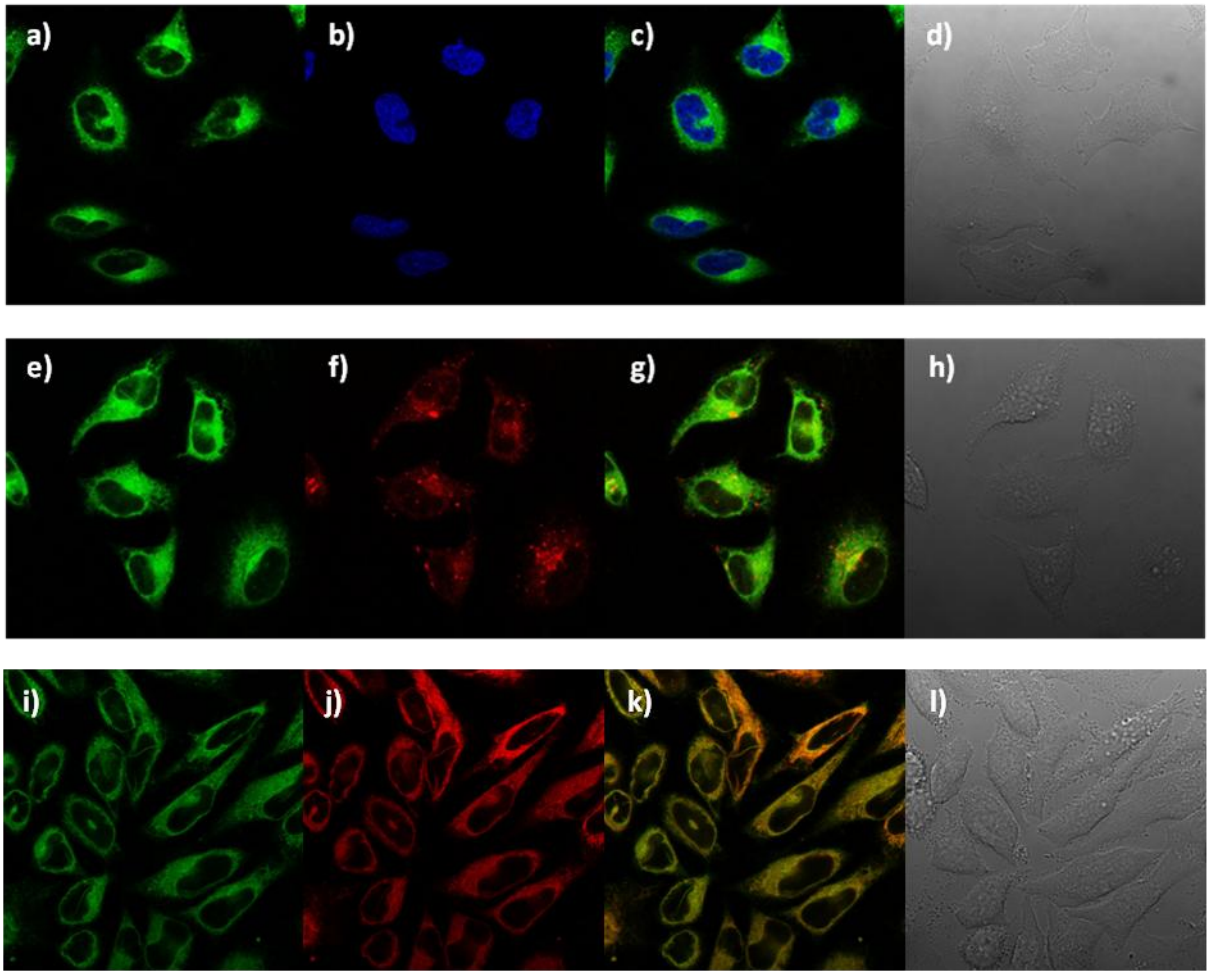


Figure S3_1: Confocal fluorescence images of uptake of a) **NiL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and b) Hoescht nucleic acid stain, 1 µg/mL, 30 min, λ_{ex} = 405 nm with c) overlay of **NiL1** and Hoescht and d) DIC image. Confocal fluorescence images of uptake of e) **NiL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and f) Lysotracker® Red DND-99, 200 nM, 60 min, λ_{ex} = 543 nm with g) overlay of **NiL1** and Lysotracker® Red and h) DIC image. Confocal fluorescence images of uptake of i) **NiL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and j) ER-tracker™ Red (BODIPY® TR Glibenclamide), 1 µM, 20 min, λ_{ex} = 543 nm; with k) overlay of **NiL1** and ER-tracker™ Red and l) DIC image.

ZnL1

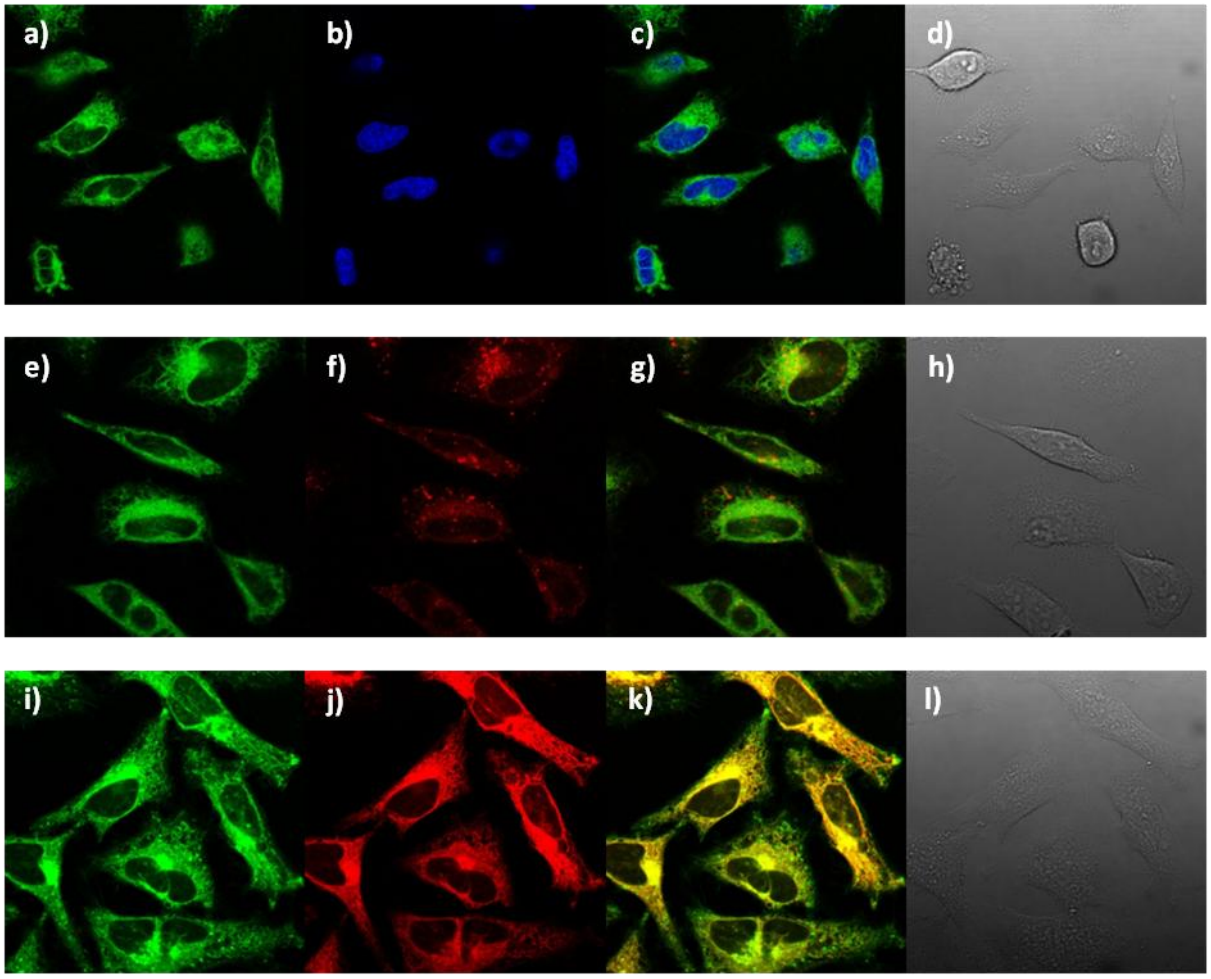


Figure S3_2: Confocal fluorescence images of uptake of a) **ZnL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and b) Hoescht nucleic acid stain, 1 µg/mL, 30 min, λ_{ex} = 405 nm with c) overlay of **ZnL1** and Hoescht and d) DIC image. Confocal fluorescence images of uptake of e) **ZnL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and f) Lysotracker® Red DND-99, 200 nM, 60 min, λ_{ex} = 543 nm with g) overlay of **ZnL1** and Lysotracker® Red and h) DIC image. Confocal fluorescence images of uptake of i) **ZnL1** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and j) ER-tracker™ Red (BODIPY® TR Glibenclamide), 1 µM, 20 min, λ_{ex} = 543 nm; with k) overlay of **ZnL1** and ER-tracker™ Red and l) DIC image.

L2

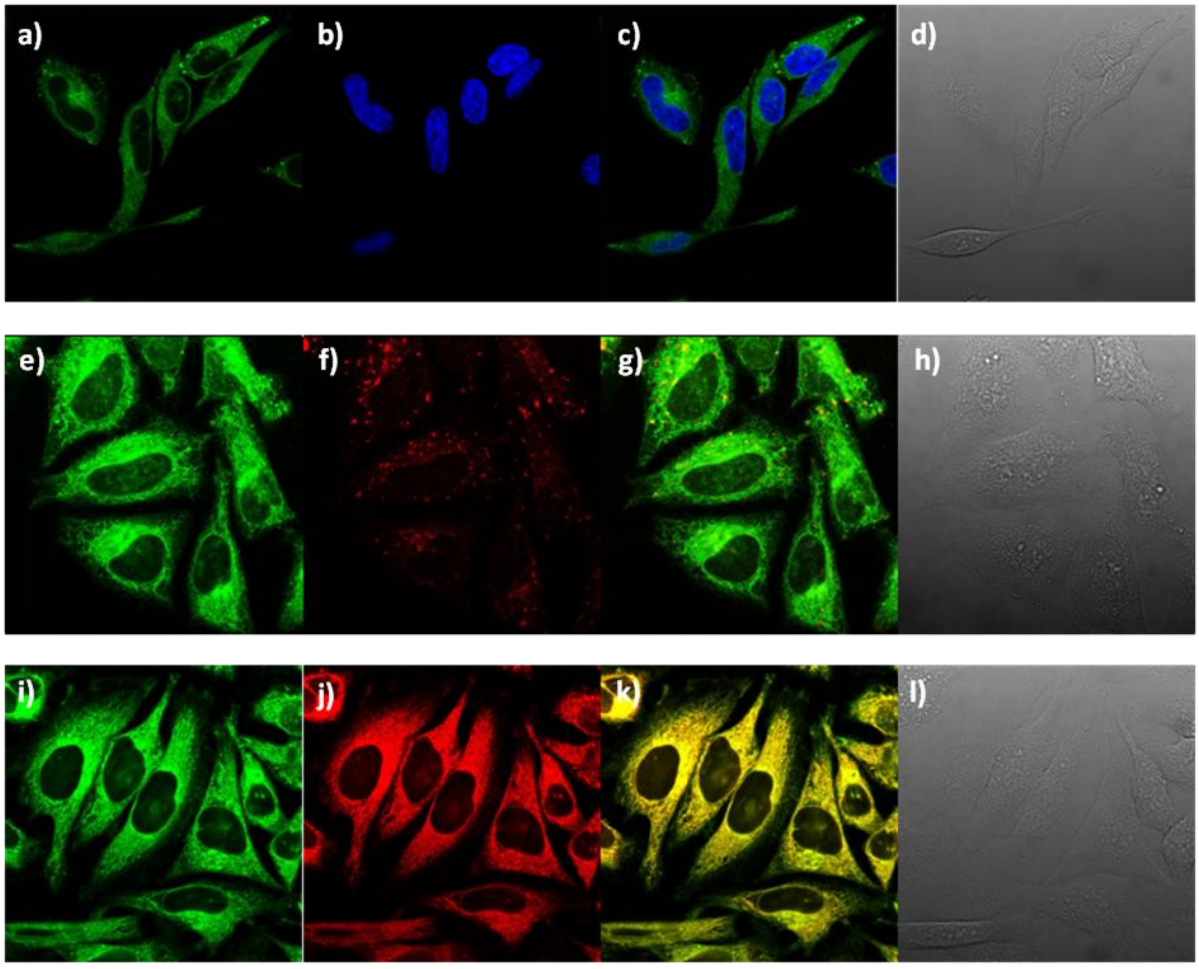


Figure S3_3: Confocal fluorescence images of uptake of a) **L2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and b) Hoescht nucleic acid stain, 1 µg/mL, 30 min, λ_{ex} = 405 nm with c) overlay of **L2** and Hoescht and d) DIC image. Confocal fluorescence images of uptake of e) **L2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and f) Lysotracker® Red DND-99, 200 nM, 60 min, λ_{ex} = 543 nm with g) overlay of **L2** and Lysotracker® Red and h) DIC image. Confocal fluorescence images of uptake of i) **L2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and j) ER-tracker™ Red (BODIPY® TR Glibenclamide), 1 µM, 20 min, λ_{ex} = 543 nm; with k) overlay of **L2** and ER-tracker™ Red and l) DIC image.

CuL2

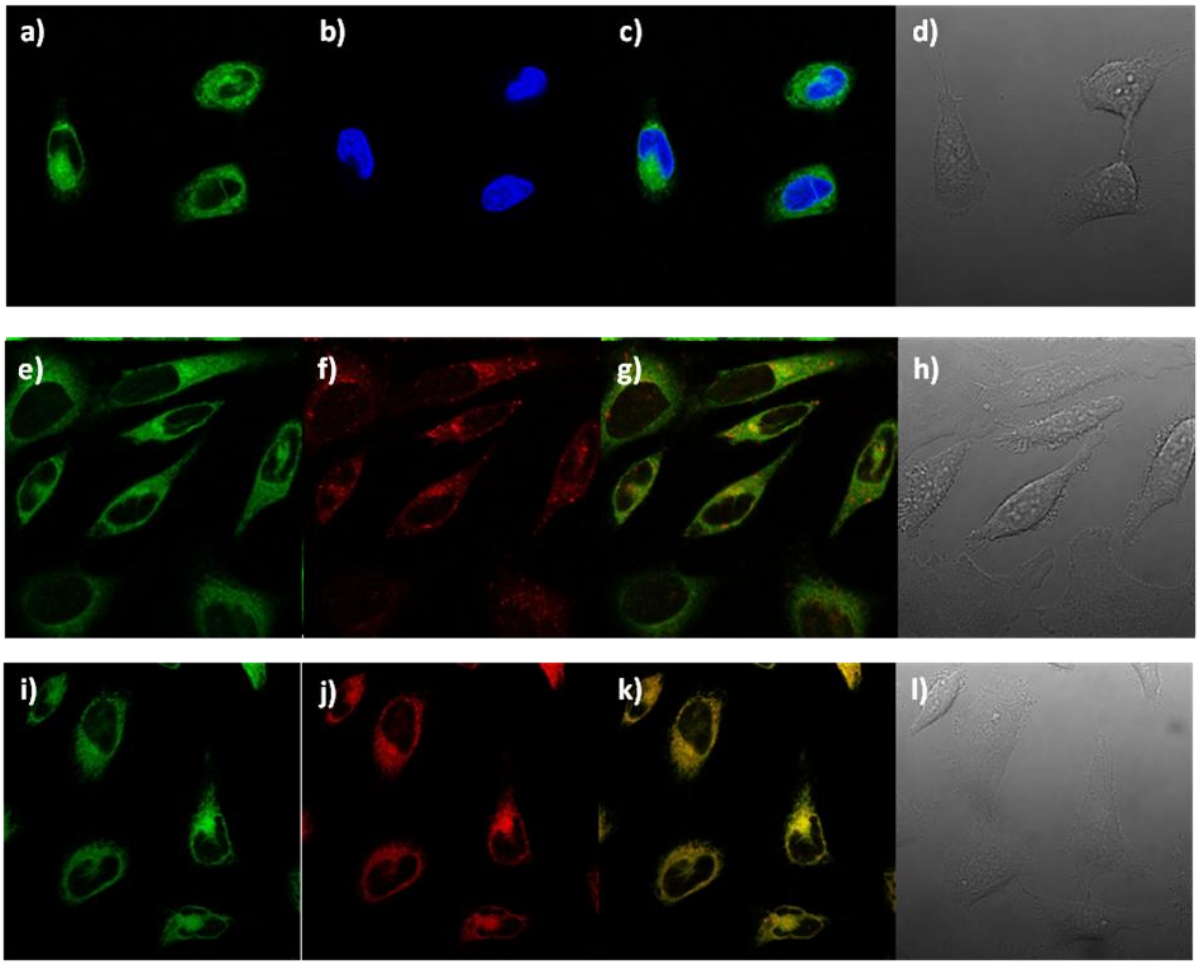


Figure S3_4: Confocal fluorescence images of uptake of a) **CuL2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and b) Hoescht nucleic acid stain, 1 µg/mL, 30 min, λ_{ex} = 405 nm with c) overlay of **CuL2** and Hoescht and d) DIC image. Confocal fluorescence images of uptake of e) **CuL2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and f) Lysotracker® Red DND-99, 200 nM, 60 min, λ_{ex} = 543 nm with g) overlay of **CuL2** and Lysotracker® Red and h) DIC image. Confocal fluorescence images of uptake of i) **CuL2** in HeLa cell line, at 10 µM, 20 min incubation, λ_{ex} = 488 nm and j) ER-tracker™ Red (BODIPY® TR Glibenclamide), 1 µM, 20 min, λ_{ex} = 543 nm; with k) overlay of **CuL2** and ER-tracker™ Red and l) DIC image.

S4: 2P FLIM solution lifetime data

Table S1: Solution lifetime decay values for **L1**, **ZnL1**, **CuL1**, **NiL1**, **L2** and **CuL2** in range of solvents. Lifetimes given are an average of three separate measurements and expressed as mean average with SD. χ^2 values and % weightings are mean values of three separate measurements.

	DMSO		**MeOH**		**DCM**		**Medium**	
L1	χ^2 1.17		χ^2 1.22		χ^2 1.30		χ^2 1.31	
	(τ_1)2.47± 0.13 ns	100%	(τ_1)2.01± 0.04 ns	100 %	(τ_1)2.52 ± 0.24 ns	100 %	(τ_3)8.49 ± 0.56 ns	21.4 %
	-	-	-	-	-	-	(τ_2)2.41 ± 0.21 ns	38.5 %
	-	-	-	-	-	-	(τ_1)0.37 ± 0.17 ns	40.1 %
ZnL1	χ^2 1.27		χ^2 1.25		χ^2 1.31		χ^2 1.24	
	(τ_2)1.91± 0.18 ns	65.7 %	(τ_2)1.79± 0.07 ns	68.5 %	(τ_2)2.19 ± 0.03 ns	59.6 %	(τ_3)7.18 ±0.42 ns	19.9 %
	(τ_1)0.65± 0.12 ns	34.3 %	(τ_1)0.51± 0.11 ns	31.5 %	(τ_1)0.78 ± 0.09 ns	41.4 %	(τ_2)2.04 ±0.22 ns	35.2 %
	-	-	-	-	-	-	(τ_1)0.32 ± 0.08 ns	44.9 %
CuL1	χ^2 1.28		χ^2 1.27		χ^2 1.31		χ^2 1.29	
	(τ_2)2.89 ± 0.31 ns	16.0 %	(τ_2)2.39± 0.11ns	21.5 %	(τ_2)2.49 ± 0.10 ns	21.6 %	(τ_3)7.49 ± 0.26 ns	12.4 %
	(τ_1)0.11± 0.04 ns	84.0 %	(τ_1)0.091± 0.02 ns	78.5 %	(τ_1)0.078 ± 0.02 ns	79.4 %	(τ_2)2.18 ± 0.41 ns	17.7 %
	-	-	-	-	-	-	(τ_1)0.11 ±0.02 ns	69.9 %
NiL1	χ^2 1.08		χ^2 1.17		χ^2 1.15		-	
	(τ_2)3.22 ± 0.12 ns	14.0 %	(τ_2)3.17 ± 0.15 ns	15.6 %	(τ_2)3.76± 0.25 ns	9.8 %	-	-
	(τ_1)0.19 ± 0.01 ns	86.0 %	(τ_1)0.17 ± 0.02 ns	84.4 %	(τ_1)0.19 ± 0.02 ns	90.2 %	-	-
L2	χ^2 1.04		χ^2 1.06		-		χ^2 1.31	
	(τ_1)2.84 ± 0.24 ns	100 %	(τ_1)2.13 ±0.15 ns	100 %	-	-	(τ_3)8.76 ± 0.41ns	14.2 %
	-	-	-	-	-	-	(τ_2)2.98 ± 0.25ns	41.3 %
	-	-	-	-	-	-	(τ_1)0.52 ± 0.11 ns	44.6 %
CuL2	χ^2 1.10		χ^2 1.21		-		χ^2 1.27	
	(τ_2)3.23 ± 0.17 ns	14.6 %	(τ_2)2.67 ± 0.41 ns	11.8 %	-	-	(τ_3)8.17 ±0.35 ns	5.4 %
	(τ_1)0.18 ± 0.01ns	85.4 %	(τ_1)0.11± 0.04 ns	88.2 %	-	-	(τ_2)2.01 ±0.11 ns	10.4 %
	-	-	-	-	-	-	(τ_1)0.07 ± 0.02 ns	84.2 %

Example screen shots of solution lifetime decay curves for each compound in each solvent are given below in Figures S4_1-S4_21:

L1

Figure S4_1: Solution lifetime decay plot and data fit for **L1** (10 µM) in DMSO:

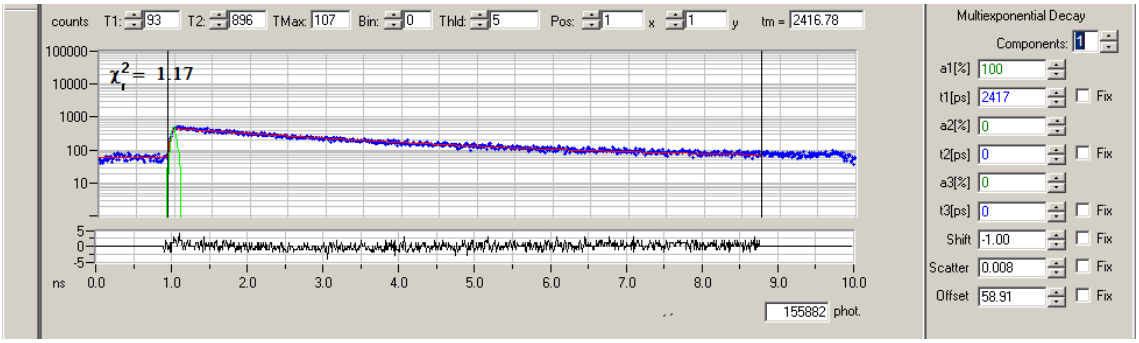


Figure S4_2: Solution lifetime decay plot and data fit for **L1** (10 µM) in MeOH:

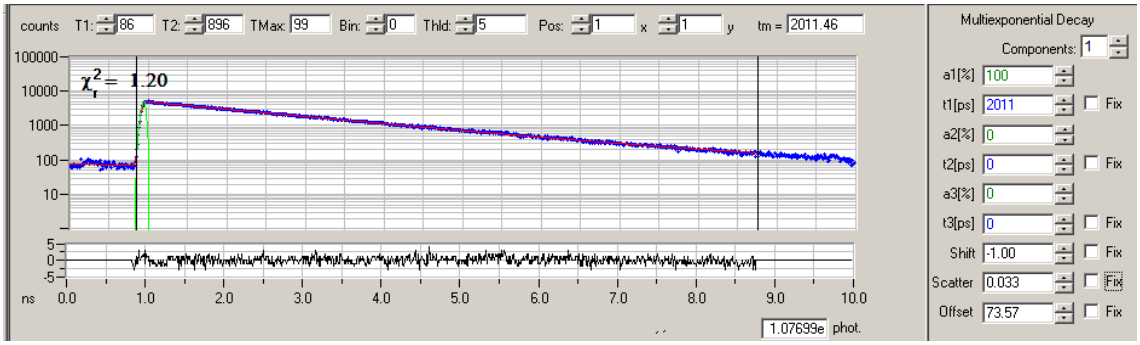


Figure S4_3: Solution lifetime decay plot and data fit for **L1** (10 µM) in DCM:

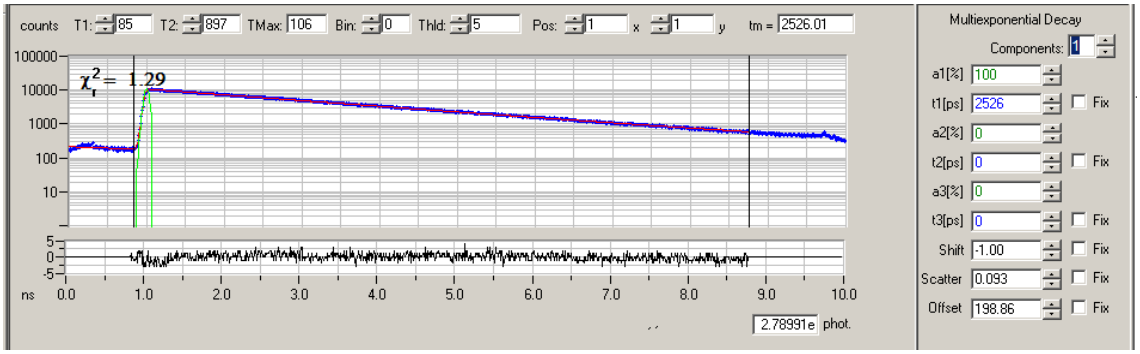
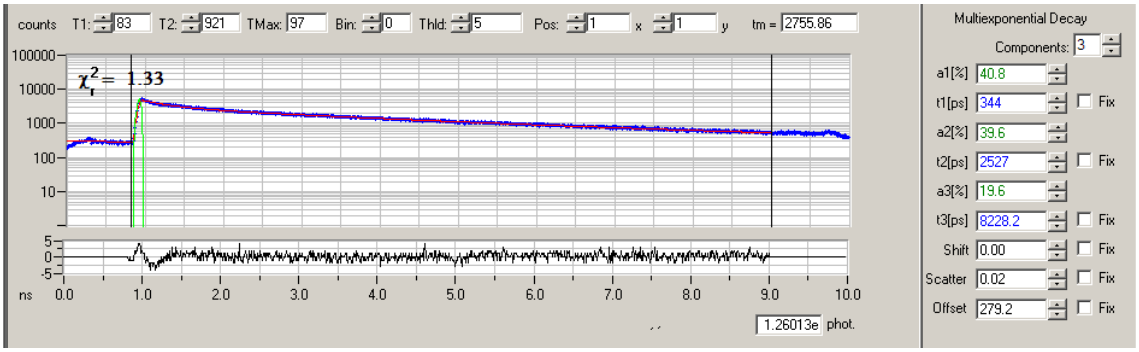


Figure S4_4: Solution lifetime decay plot and data fit for **L1** (10 µM) in Cell Medium:



ZnL1

Figure S4_5: Solution lifetime decay plot and data fit for **ZnL1** (10 µM) in DMSO:

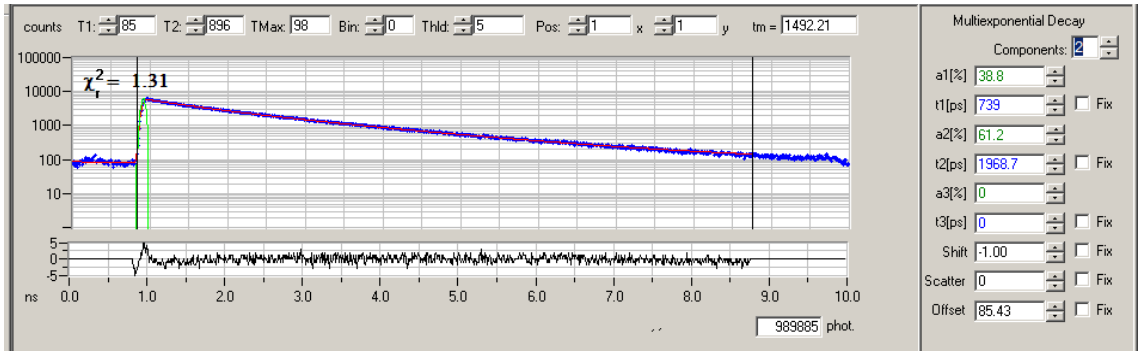


Figure S4_6: Solution lifetime decay plot and data fit for **ZnL1** (10 µM) in MeOH:

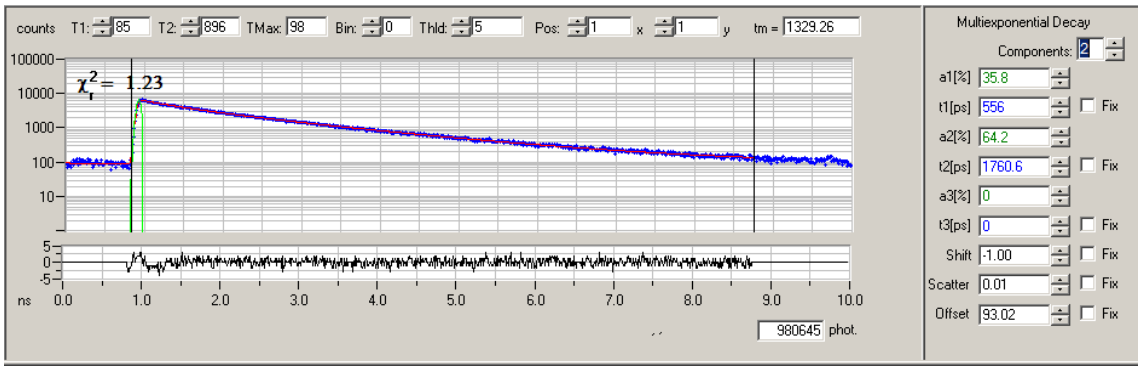


Figure S4_7: Solution lifetime decay plot and data fit for **ZnL1** (10 µM) in DCM:

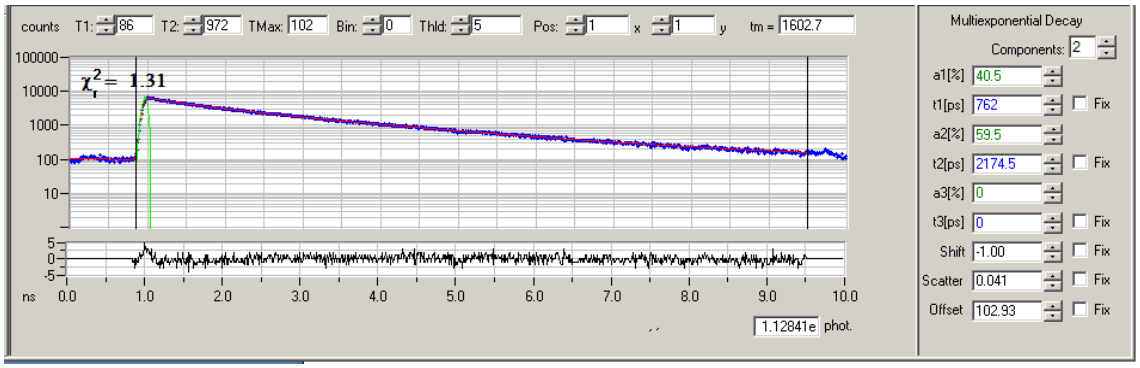
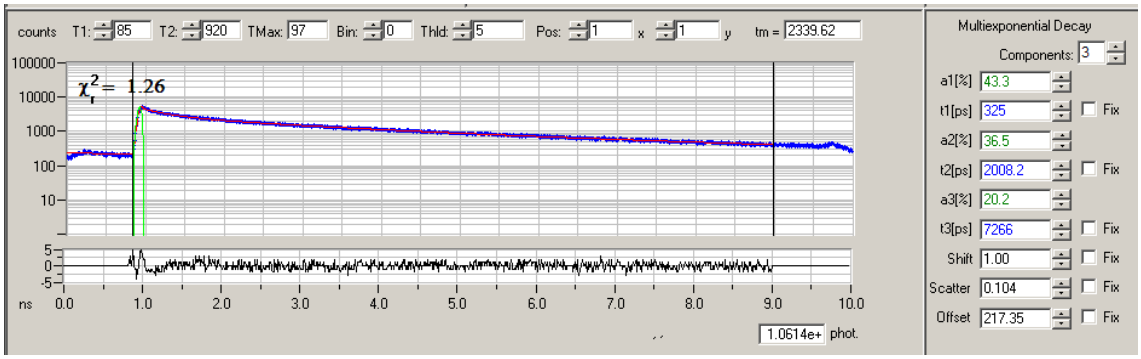


Figure S4_8: Solution lifetime decay plot and data fit for **ZnL1** (10 µM) in Cell Medium:



NiL1

Figure S4_9: Solution lifetime decay plot and data fit for **NiL1** (10 µM) in DMSO:

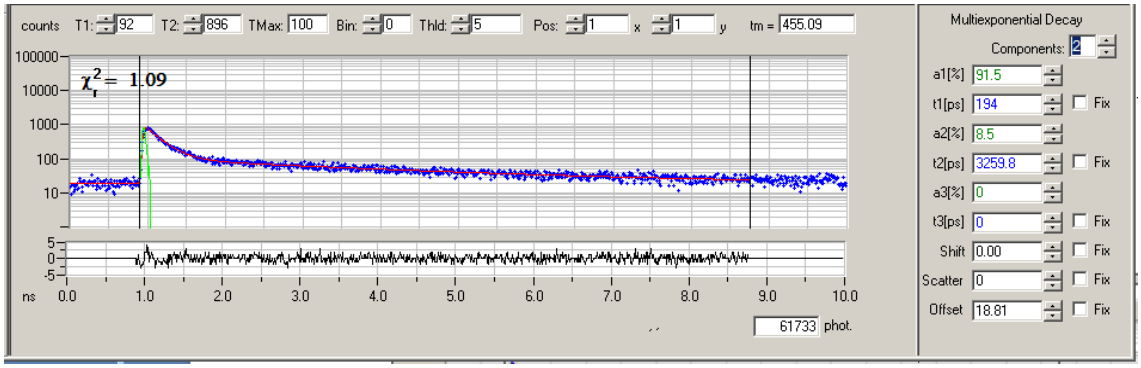


Figure S4_10: Solution lifetime decay plot and data fit for **NiL1** (10 µM) in MeOH:

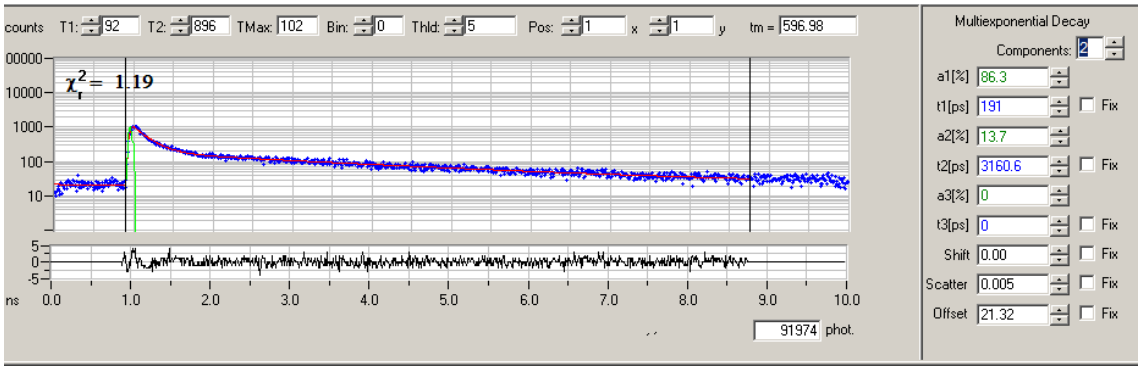
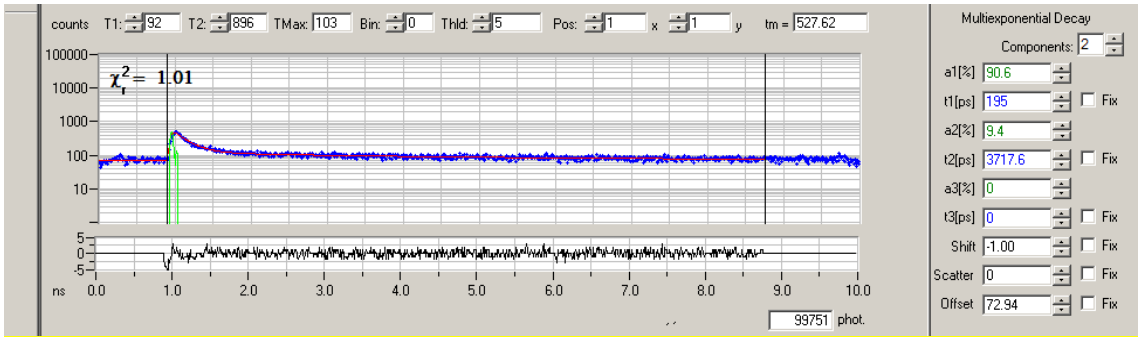


Figure S4_11: Solution lifetime decay plot and data fit for **NiL1** (10 µM) in DCM:



CuL1

Figure S4_12: Solution lifetime decay plot and data fit for **CuL1** (10 µM) in DMSO:

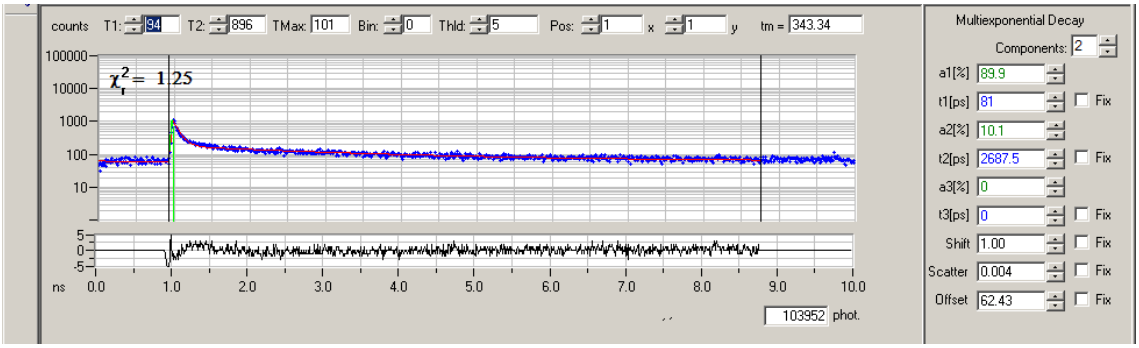


Figure S4_13: Solution lifetime decay plot and data fit for **CuL1** (10 µM) in MeOH:

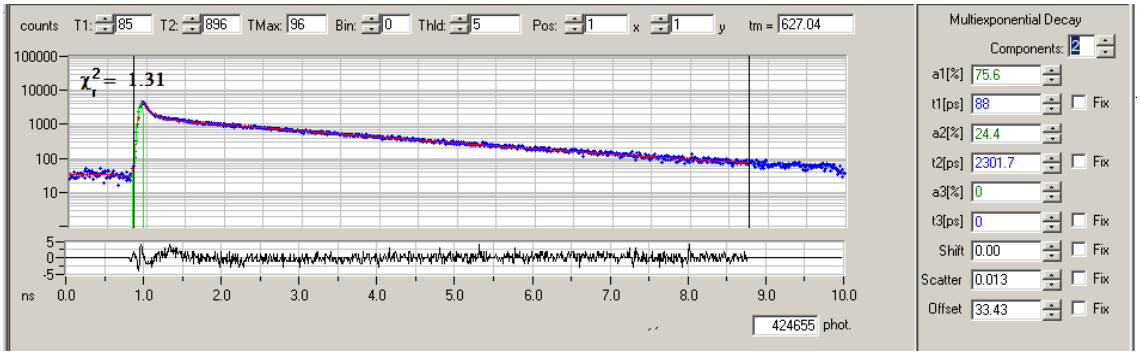


Figure S4_14: Solution lifetime decay plot and data fit for **CuL1** (10 µM) in DCM:

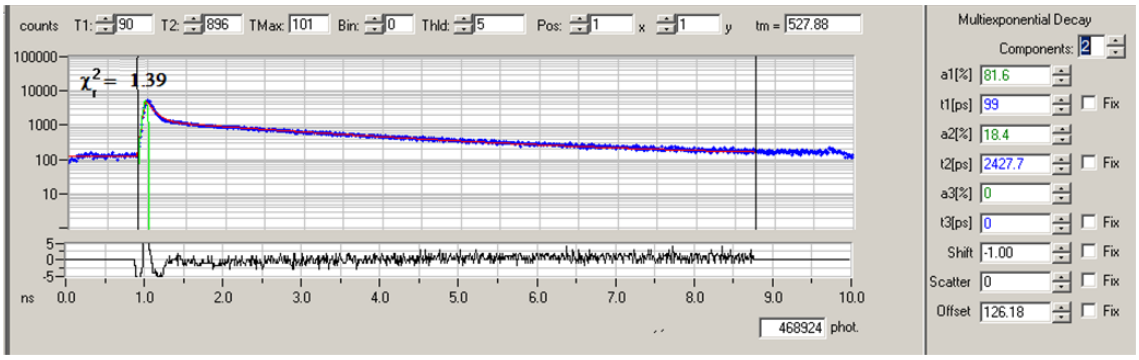
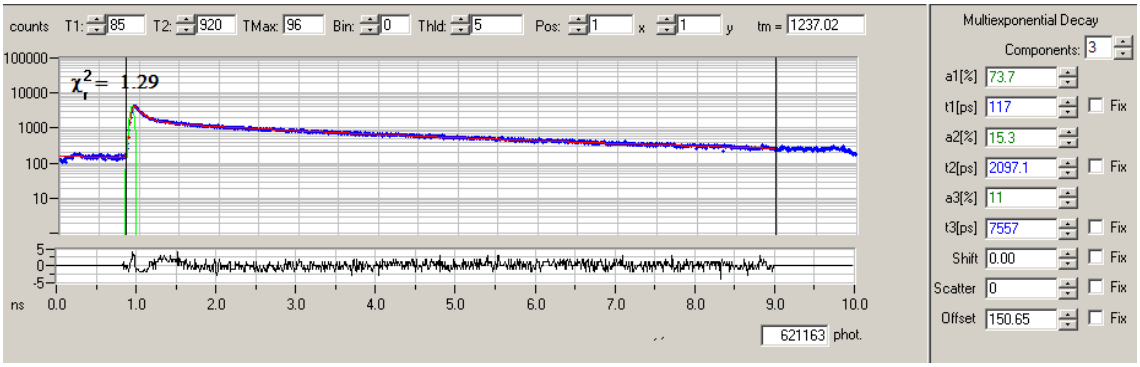


Figure S4_15: Solution lifetime decay plot and data fit for **CuL1** (10 µM) in Cell Medium:



L2

Figure S4_16: Solution lifetime decay plot and data fit for **L2** (10 µM) in DMSO:

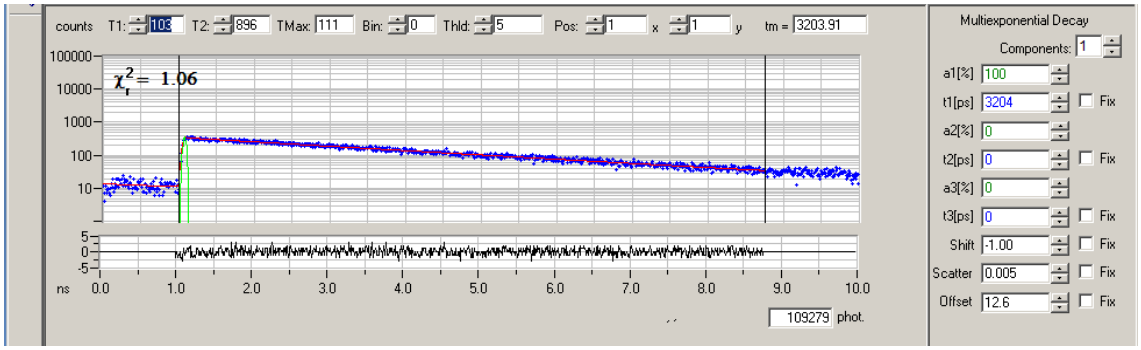


Figure S4_17: Solution lifetime decay plot and data fit for **L2** (10 µM) in MeOH:

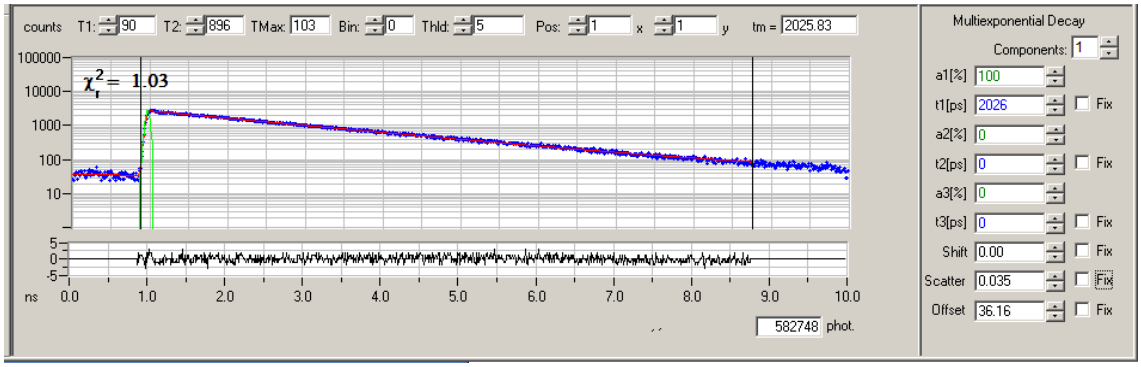
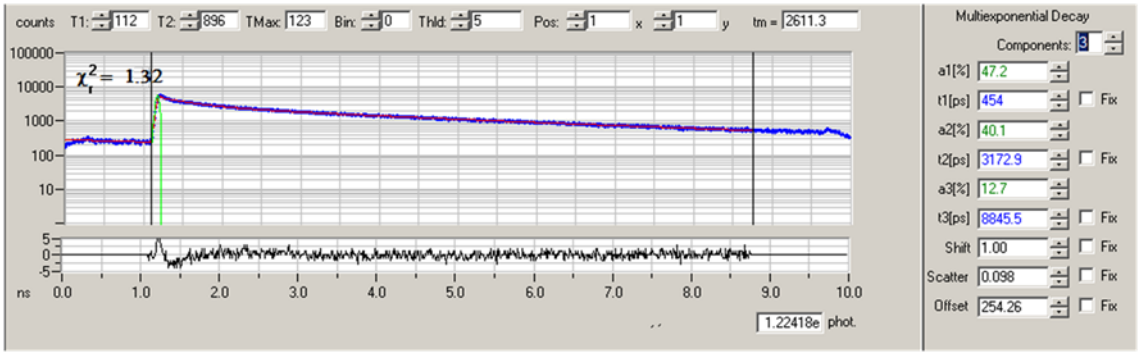


Figure S4_18: Solution lifetime decay plot and data fit for **L2** (10 µM) in Cell Medium:



CuL2

Figure S4_19: Solution lifetime decay plot and data fit for **CuL2** (10 µM) in DMSO:

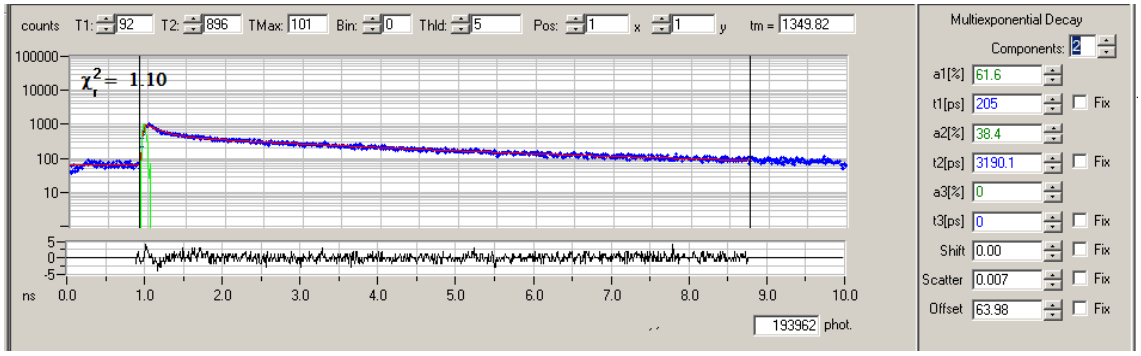


Figure S4_20: Solution lifetime decay plot and data fit for **CuL2** (10 µM) in MeOH:

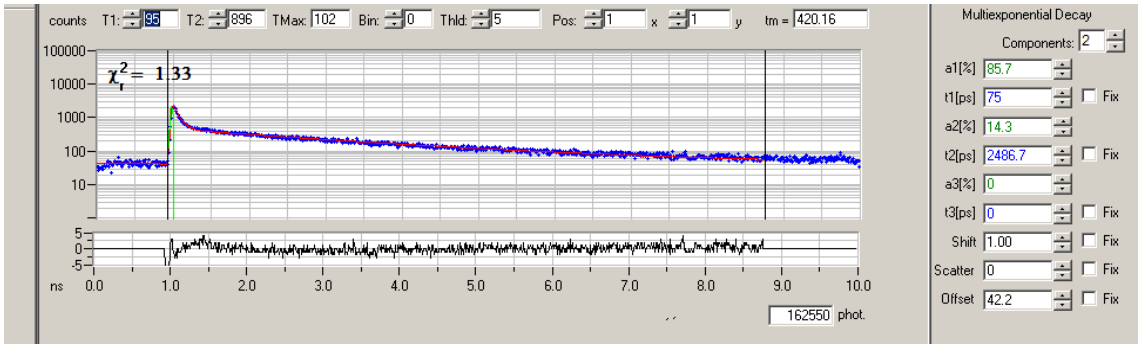
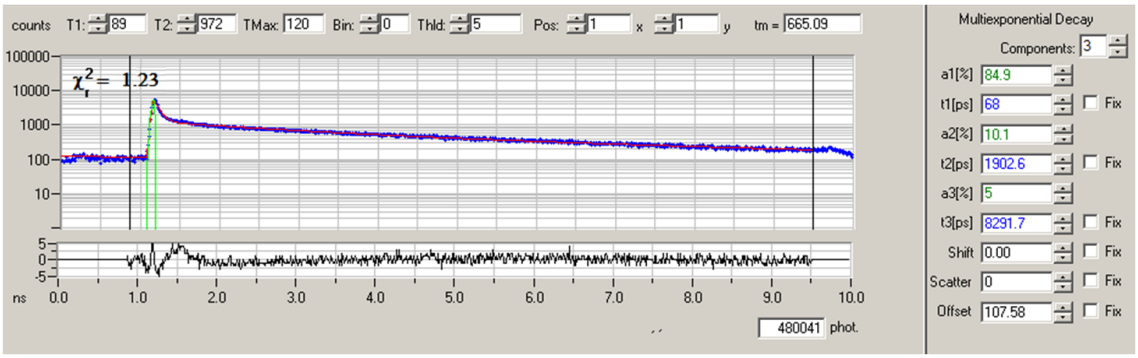


Figure S4_21: Solution lifetime decay plot and data fit for **CuL2** (10 µM) in Cell Medium:



SI5: 2P FLIM cell image data

Example screen shots of *in vitro* lifetime decay curves for each compound are given below along with GLD maps

Figure S5_1: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **L1** (10 µM) after 20 min incubation in HeLa cells:

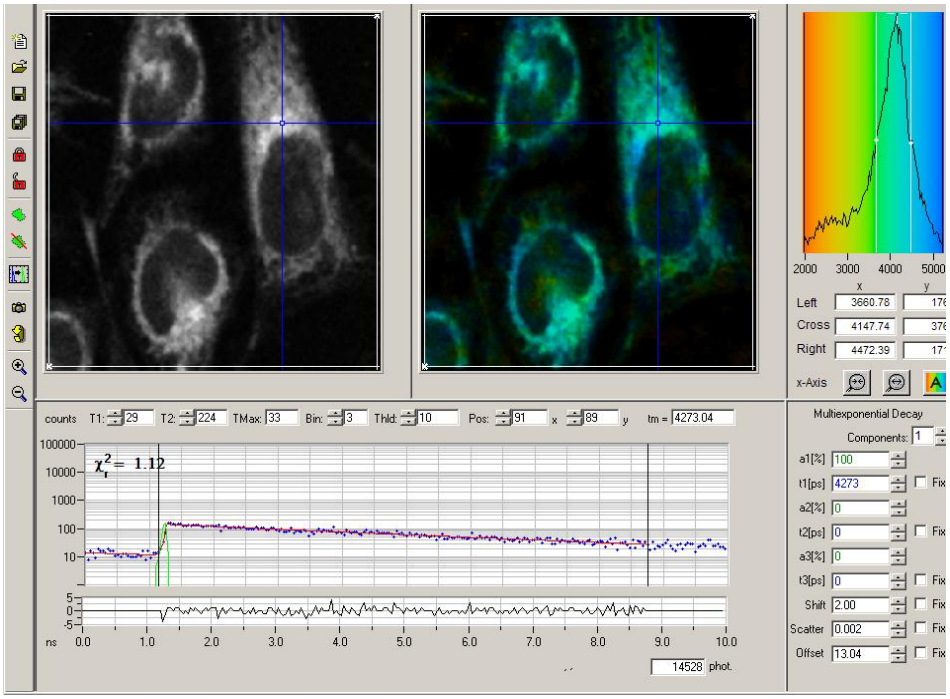


Figure S5_2: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **ZnL1** (10 µM) after 20 min incubation in HeLa cells:

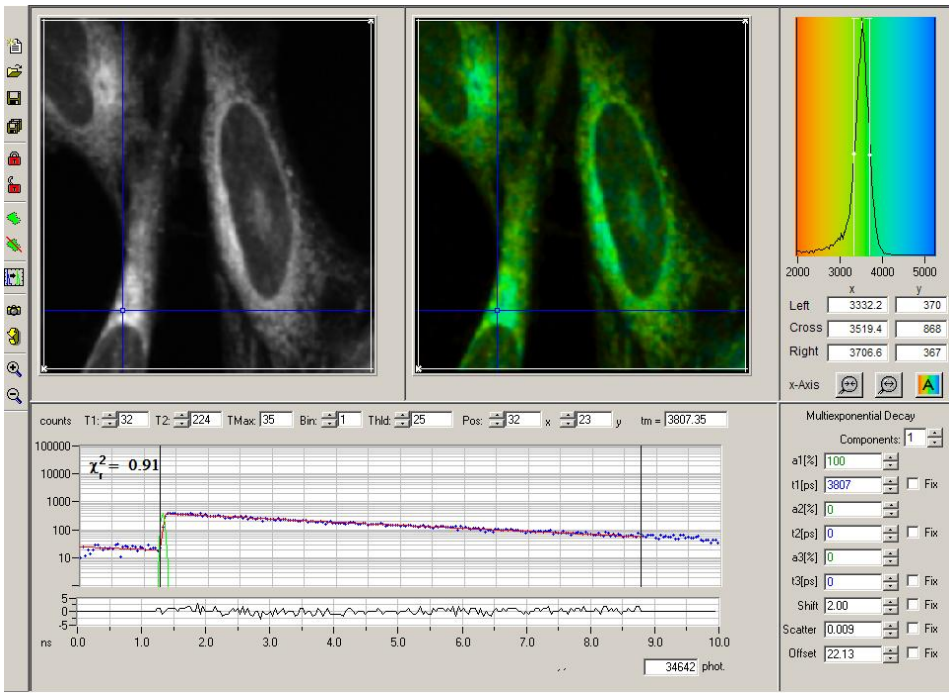


Figure S5_3: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **NiL1** (10 µM) after 20 min incubation in HeLa cells:

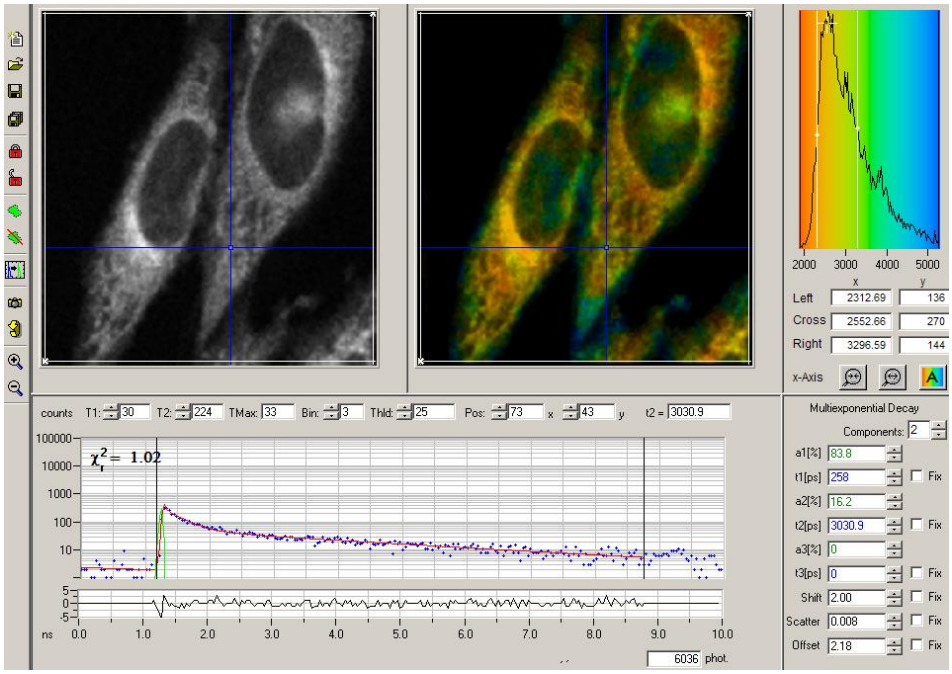


Figure S5_4: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 1):

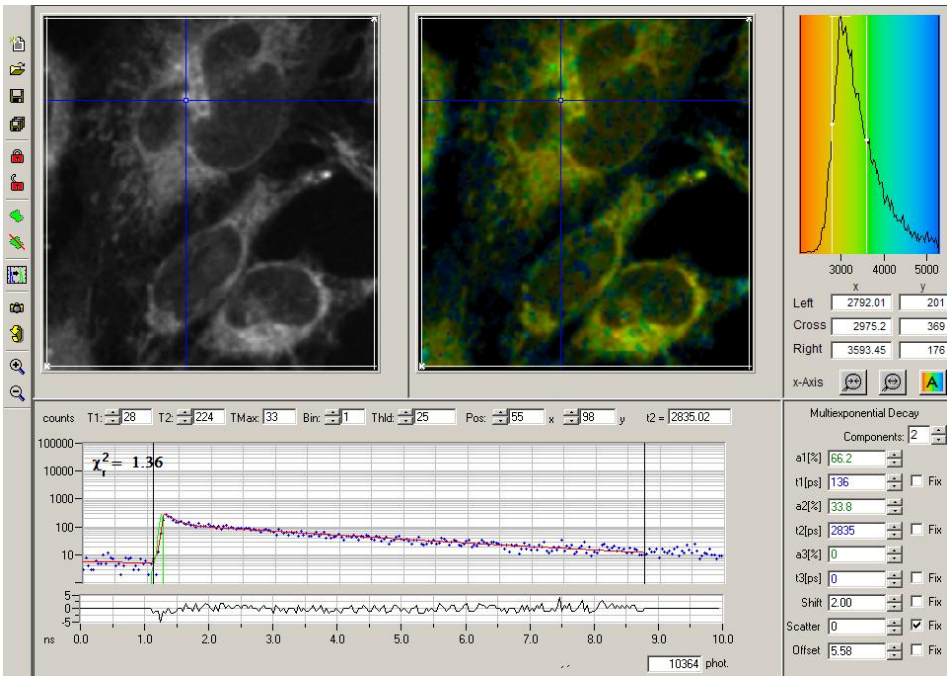


Figure S5_5: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 2):

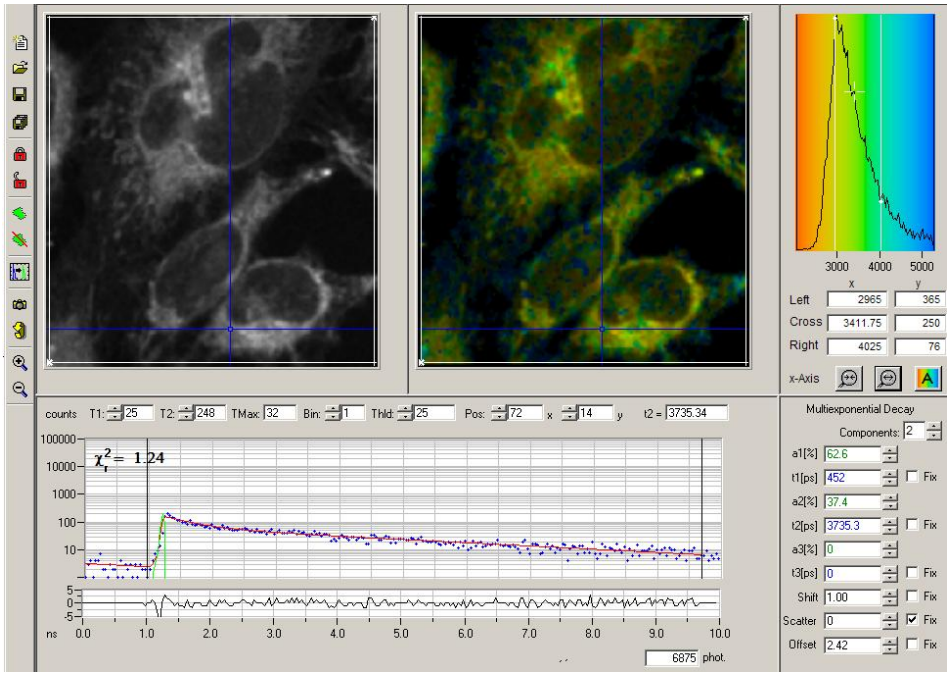


Figure S5_6: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 3):

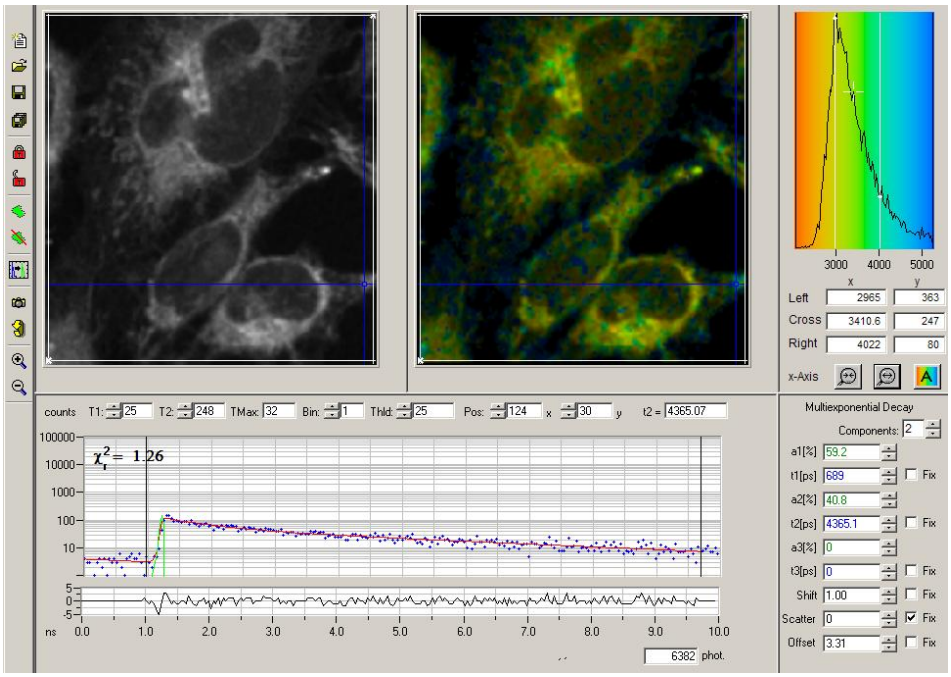


Figure S5_7: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 4):

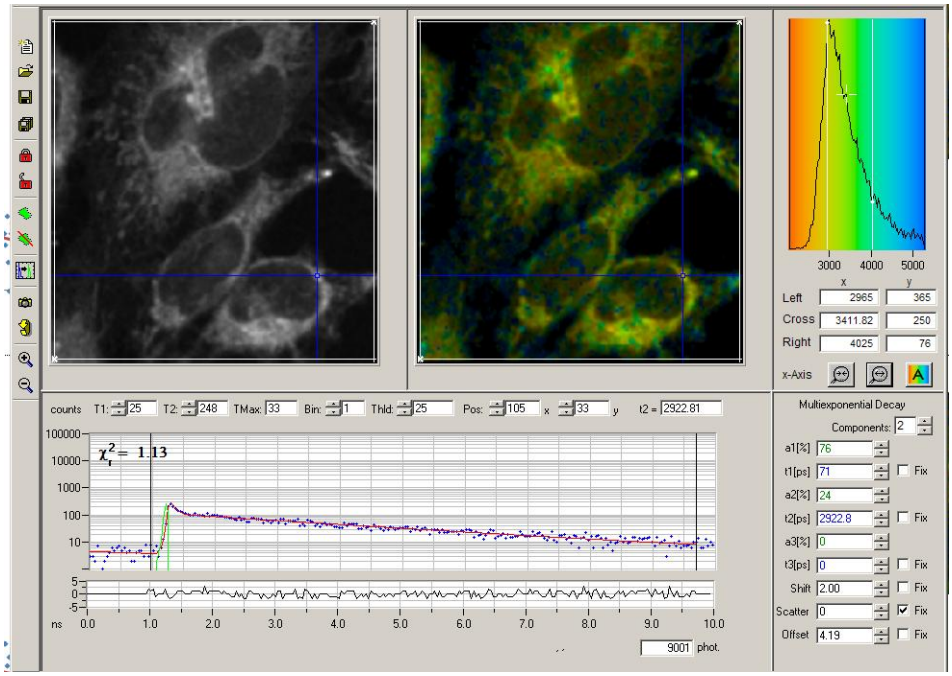


Figure S5_8: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 5):

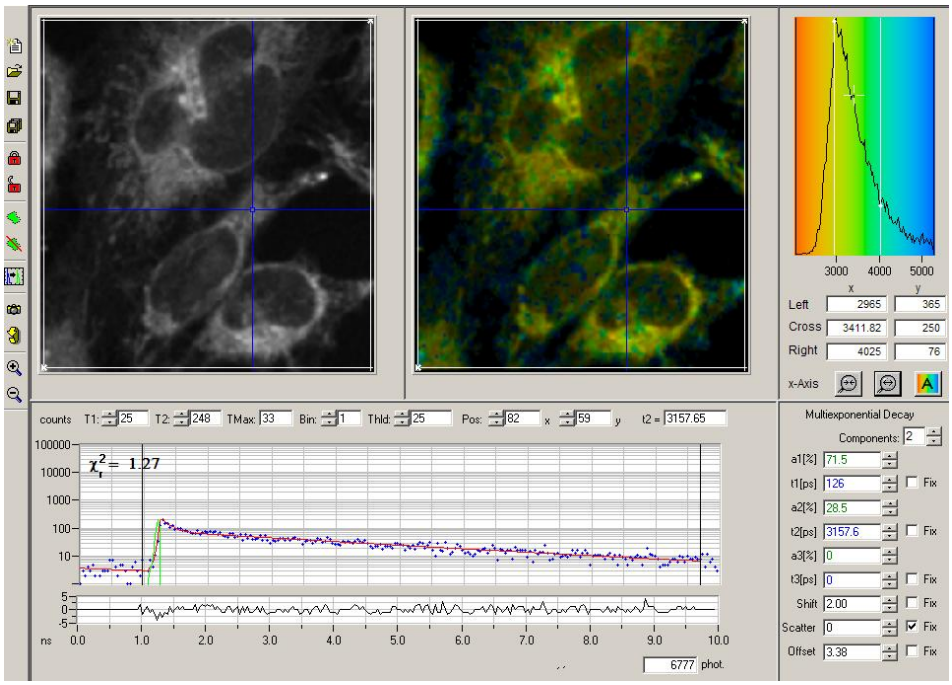


Figure S5_9: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 20 min incubation in HeLa cells (Point 6):

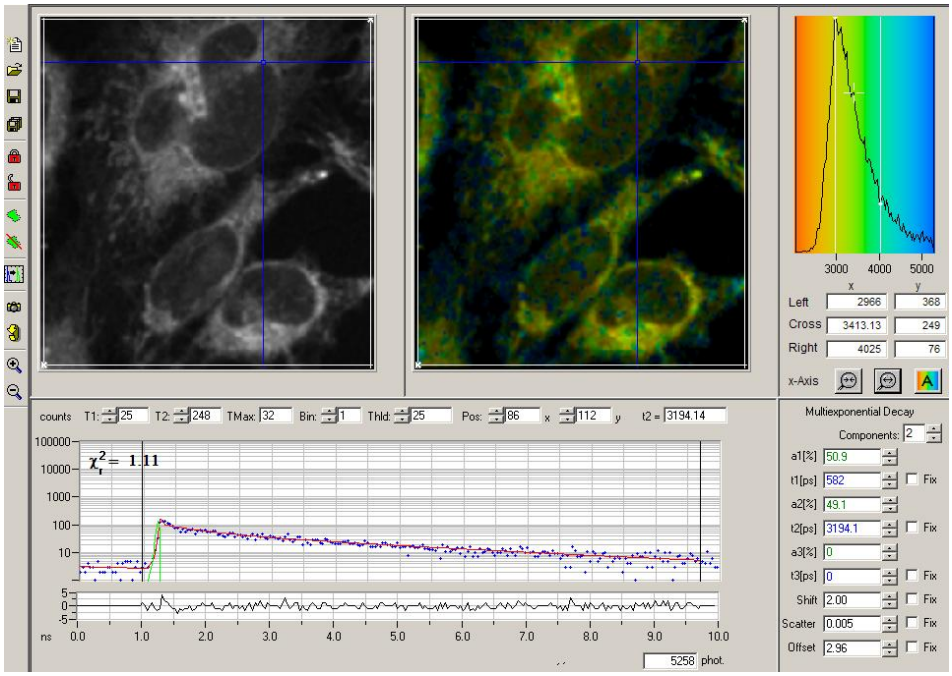


Figure S5_10: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL1** (10 µM) after 1 h incubation in HeLa cells:

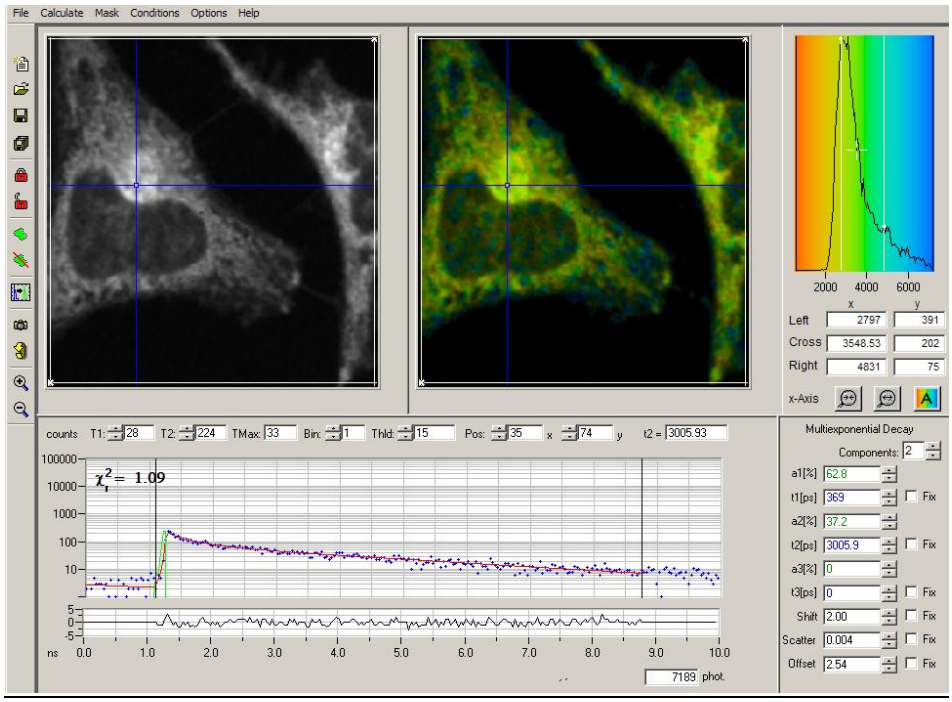


Figure S5_11: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **L2** (10 µM) after 20 min incubation in HeLa cells:

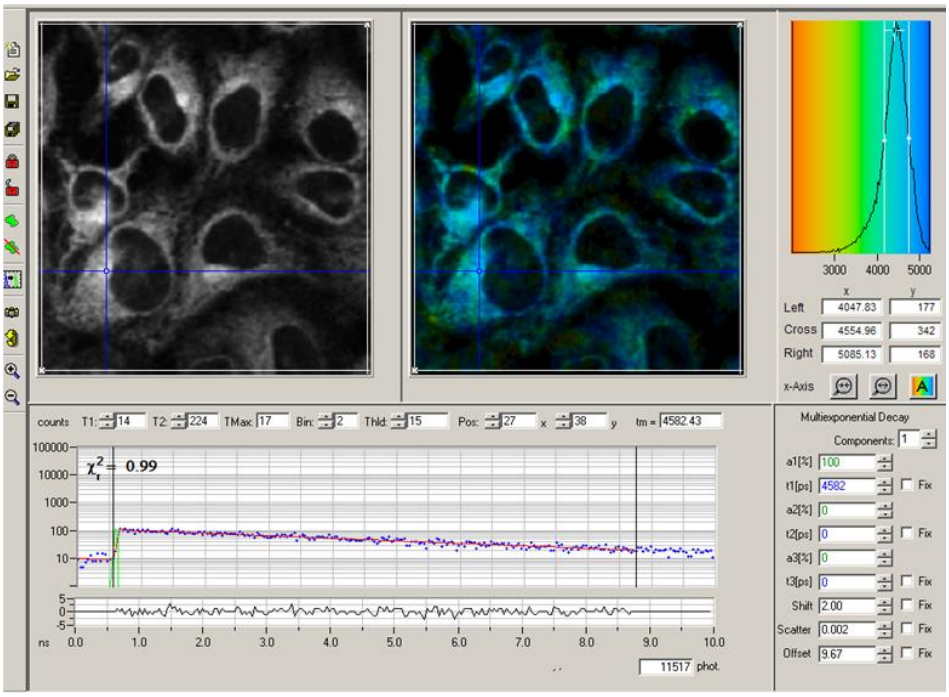


Figure S5_12: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for **CuL2** (10 µM) after 20 min incubation in HeLa cells:

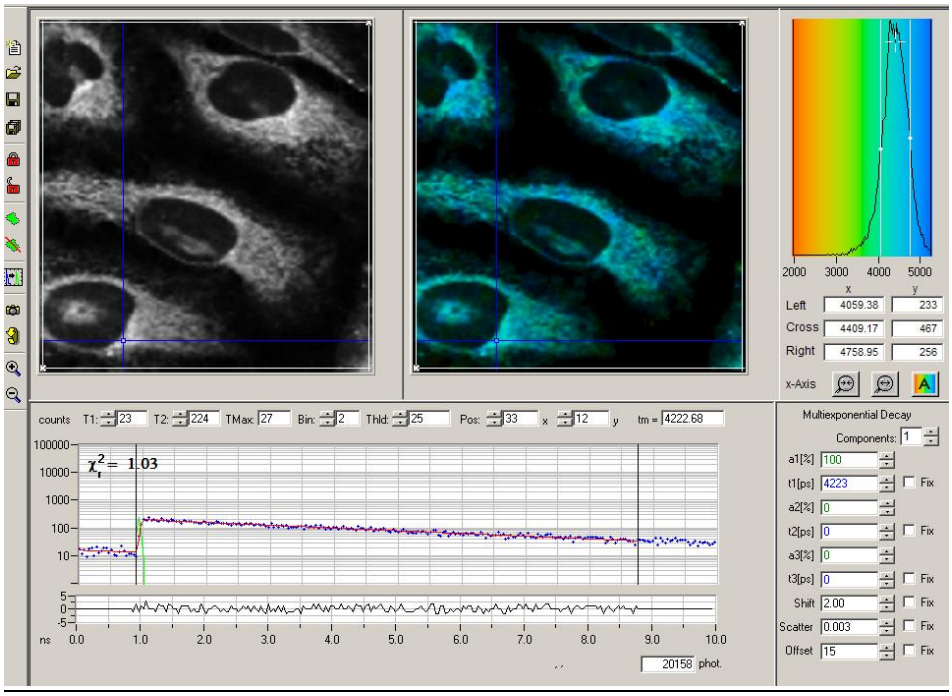


Figure S5_13: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for 1:1 mix of **L1:CuL1** (10 μM) after 20 min incubation in HeLa cells:

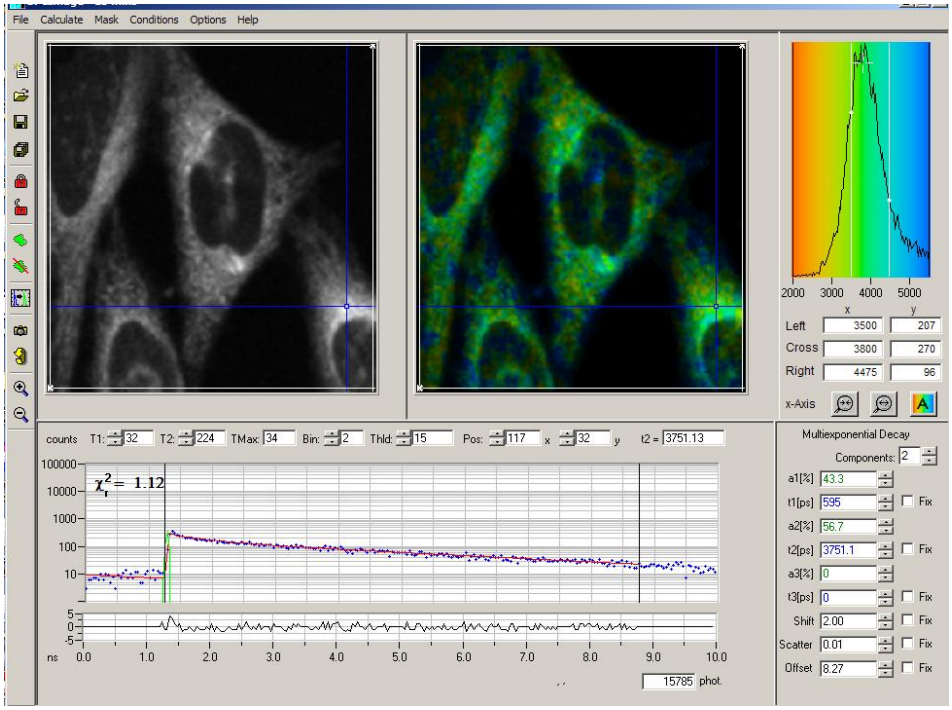


Figure S5_14: Cellular fluorescence intensity and lifetime maps, selected lifetime decay plot with data fit and GLD for 1:3 mix of **L1:CuL1** (10 μM) after 20 min incubation in HeLa cells: