

*Electronic Supplementary Information*

Effect of Sodium Thiosulfate on Cytotoxicity of a  
Diimine Re(I) Tricarbonyl Complex

*Miles S. Capper,<sup>a</sup> Alejandra Enriquez Garcia,<sup>a</sup> Barry Lai,<sup>b</sup> Owen Baiwen Wang,<sup>a</sup> Benjamin S.  
Gelfand,<sup>a</sup> Carrie S. Shemanko,<sup>c</sup> and Farideh Jalilehvand<sup>a,\*</sup>*

<sup>a</sup> Department of Chemistry, University of Calgary, Calgary, Alberta, T2N 1N4, Canada

<sup>b</sup> Advanced Photon Source, X-ray Science Division, Argonne National Laboratory, Argonne, USA

<sup>c</sup> Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada

## Table of Content

	<u>Page</u>
Physical measurements and methods (NMR, FT-IR, ESI-MS, TGA)	S-2
<b>Table S1.</b> Crystal data for $\{\text{Na}(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]) \cdot 1.75\text{H}_2\text{O} \cdot \text{C}_2\text{H}_5\text{OH}\}_4$ <i>i.e.</i> $(2 \cdot 0.75\text{H}_2\text{O} \cdot \text{C}_2\text{H}_5\text{OH})_4$ and $(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{H}_2\text{O})])(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]) \cdot 4\text{H}_2\text{O}$ ( <b>3</b> )	S-3
<b>Table S2.</b> Selected bond lengths for compound $(2 \cdot 0.75\text{H}_2\text{O} \cdot \text{C}_2\text{H}_5\text{OH})_4$	S-4
<b>Table S3.</b> Selected bond angles for compound $(2 \cdot 0.75\text{H}_2\text{O} \cdot \text{C}_2\text{H}_5\text{OH})_4$	S-5
<b>Table S4.</b> Selected bond lengths for compound <b>3</b>	S-6
<b>Table S5.</b> Selected bond angles for compound <b>3</b>	S-7
<b>Table S6.</b> Survey of Re(I) tricarbonyl compounds with an S-donor ligand in CSD	S-8
<b>Figure S1 (a).</b> $^1\text{H}$ and $^{13}\text{C}$ NMR spectra of $\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{H}_2\text{O})](\text{CF}_3\text{SO}_3)$ ( <b>1</b> )	S-9
<b>Figure S1 (b).</b> $^1\text{H}$ and $^{13}\text{C}$ NMR spectra of $\text{Na}(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]) \cdot \text{H}_2\text{O}$ ( <b>2</b> )	S-10
<b>Figure S2.</b> FT-IR spectra of <b>1</b> and <b>2</b>	S-11
<b>Figure S3.</b> ESI-mass spectra of <b>2</b> in (+) and (-) ion modes	S-12
<b>Table S7.</b> Assignments of mass peaks in the ESI-mass spectra shown in Figure S3	S-12
<b>Figure S4.</b> Thermal gravimetric analysis (TGA) of <b>2</b>	S-13
<b>Figure S5 (a-c).</b> XFM elemental distribution map of MDA-MB-231 cells treated with <b>1</b> , <b>2</b> or DMEM for 6 h	S-14
<b>Figure S6.</b> XFM spectra of MDA-MB-231 cells treated for 6 h with <b>1</b> , <b>2</b> or DMEM	S-17
<b>References</b>	S-18

## Physical Measurements and Methods

### *NMR Spectroscopy*

<sup>1</sup>H and <sup>13</sup>C-NMR spectroscopy measurements were carried out at room temperature by means of a Bruker AVIII-600 CFI-600 spectrometer using D<sub>2</sub>O as a solvent. <sup>1</sup>H NMR were internally referenced to the HOD/H<sub>2</sub>O peak at 4.80 ppm,<sup>1</sup> with a total of 64 scans being co-added. Using the UDEFT pulse sequence and broadband proton decoupling at 151 MHz, <sup>13</sup>C NMR spectra were recorded. <sup>13</sup>C NMR spectra were externally calibrated using CH<sub>3</sub>OH in D<sub>2</sub>O resonating at 49.15 ppm,<sup>2</sup> with a total of 4000 scans being co-added. A 30° pulse, 26.2 kHz sweep width, 1-s delay between scans, and 65 K data points were used.

### *FT-IR Spectroscopy*

500 scans were measured using an Agilent Cary 630 FTIR with a diamond ATR accessory.

### *ESI-Mass spectrometry*

Electrospray ionization (ESI) mass spectra were collected both in positive (+) and (-) modes by means of an Agilent 6520 Accurate-Mass Q-TOF. An aqueous solution of **2** was injected and mobilized using an injection flow rate of 0.2 mL/min. The source was held at 200 °C with a drying gas flow rate of 7 L/min and a nebulizer pressure of 12 p.s.i. The fragmentor, capillary, and skimmer voltages were set to 80, 4000 and 65 V, respectively. Peak assignments were confirmed using a high-resolution Isotope Distribution Calculator from Scientific Instrument Services.<sup>3</sup>

### *Thermogravimetric Analysis (TGA)*

TGA was performed using a Netzsch STA 409 PC Luxx instrument. The analyses were run from room temperature to 600 °C using a ramp rate of 2 °C min<sup>-1</sup> under a flow of N<sub>2</sub>.

## Crystallography Data

**Table S1.** Crystal Data and Structure Refinement for the Compounds:

{Na(*fac*-[(CO)<sub>3</sub>Re(bpy)(S<sub>2</sub>O<sub>3</sub>)]) · 1.75H<sub>2</sub>O · C<sub>2</sub>H<sub>5</sub>OH}<sub>4</sub> (**2** · 0.75H<sub>2</sub>O · C<sub>2</sub>H<sub>5</sub>OH)<sub>4</sub> and

(*fac*-[Re(CO)<sub>3</sub>(bpy)(H<sub>2</sub>O)])(*fac*-[Re(CO)<sub>3</sub>(bpy)(S<sub>2</sub>O<sub>3</sub>)])·4H<sub>2</sub>O (**3**).<sup>a</sup>

	<b>2</b> · 0.75H <sub>2</sub> O · C <sub>2</sub> H <sub>5</sub> OH	<b>3</b>
Empirical formula	C <sub>60</sub> H <sub>70</sub> N <sub>8</sub> Na <sub>4</sub> O <sub>35</sub> Re <sub>4</sub> S <sub>8</sub>	C <sub>26</sub> H <sub>26</sub> N <sub>4</sub> O <sub>14</sub> Re <sub>2</sub> S <sub>2</sub>
Formula weight (g mol <sup>-1</sup> )	2556.48	1055.03
Crystal system	monoclinic	monoclinic
Space group	P2 <sub>1</sub> /n	Pc
<i>a</i> (Å)	18.1076(4)	8.09140(10)
<i>b</i> (Å)	18.6534(4)	7.8966(2)
<i>c</i> (Å)	24.5013(6)	26.1412(5)
α (deg)	90	90
β (deg)	92.749(2)	98.6020(10)
γ (deg)	90	90
<i>V</i> (Å <sup>3</sup> )	8266.2(3)	1651.49(6)
<i>Z</i>	4	2
<i>D</i> <sub>calcd</sub> (g/cm <sup>3</sup> )	2.054	2.122
<i>T</i> (K)	173.0	173.0
λ (Å)	1.54178 (Cu Kα)	1.54178 (Cu Kα)
(μ / mm <sup>-1</sup> )	14.061	15.942
<i>F</i> (000)	4952	1008.0
2θ range for data collection (deg)	5.934 to 140.138	3.418 to 144.498
<i>R</i> <sub>1</sub> , <i>wR</i> <sub>2</sub> [ <i>I</i> ≥ 2σ( <i>I</i> )]	0.0700, 0.1631	0.0278, 0.0715

$$^a R_1 = \frac{\sum ||F_0 - F_c||}{\sum |F_0|}; wR_2 = \frac{[\sum w(F_0^2 - F_c^2)^2]}{[\sum w(F_0^2)^2]}^{1/2}$$

**Table S2.** Selected Bond Lengths (Å) for Compound (**2** · 0.75H<sub>2</sub>O · C<sub>2</sub>H<sub>5</sub>OH)<sub>4</sub>

Bond	Distance (Å)	Bond	Distance (Å)
Re(1)-S(1)	2.511(2)	Re(3)-S(5)	2.522(2)
Re(1)-N(1)	2.165(6)	Re(3)-N(5)	2.184(6)
Re(1)-N(2)	2.168(5)	Re(3)-N(6)	2.169(5)
Re(1)-C(11)	1.924(7)	Re(3)-C(37)	1.930(7)
Re(1)-C(12)	1.937(7)	Re(3)-C(38)	1.930(7)
Re(1)-C(13)	1.925(7)	Re(3)-C(39)	1.933(7)
C(11)-O(1)	1.143(9)	C(37)-O(13)	1.135(9)
C(12)-O(2)	1.137(9)	C(38)-O(14)	1.142(9)
C(13)-O(3)	1.145(9)	C(39)-O(15)	1.134(9)
S(1)-S(2)	2.036(3)	S(5)-S(6)	2.023(3)
Re(2)-S(3)	2.512(2)	Re(4)-S(7)	2.525(2)
Re(2)-N(3)	2.186(6)	Re(4)-N(7)	2.176(5)
Re(2)-N(4)	2.158(5)	Re(4)-N(8)	2.175(6)
Re(2)-C(24)	1.935(7)	Re(4)-C(50)	1.921(7)
Re(2)-C(25)	1.932(7)	Re(4)-C(51)	1.928(7)
Re(2)-C(26)	1.913(7)	Re(4)-C(52)	1.906(7)
C(24)-O(7)	1.141(9)	C(50)-O(19)	1.141(9)
C(25)-O(8)	1.138(9)	C(51)-O(20)	1.147(9)
C(26)-O(9)	1.154(9)	C(52)-O(21)	1.906(7)
S(3)-S(4)	2.030(3)	S(7)-S(8)	2.033(3)

**Table S3.** Selected Bond Angles (deg) for Compound ( $2 \cdot 0.75\text{H}_2\text{O} \cdot \text{C}_2\text{H}_5\text{OH}$ )<sub>4</sub>

Bond angle	Degree	Bond angle	Degree
N(1)-Re(1)-N(2)	75.3(2)	N(5)-Re(3)-N(6)	74.8(2)
N(1)-Re(1)-S(1)	78.3(1)	N(5)-Re(3)-S(5)	81.1(1)
N(1)-Re(1)-C(11)	97.4(3)	N(5)-Re(3)-C(38)	100.6(3)
N(1)-Re(1)-C(13)	95.3(3)	N(5)-Re(3)-C(39)	94.5(3)
C(12)-Re(1)-C(13)	89.6(3)	C(38)-Re(3)-C(39)	88.1(3)
Re(1)-S(1)-S(2)	109.77(9)	Re(3)-S(5)-S(6)	109.1(1)
N(3)-Re(2)-N(4)	74.6(2)	N(7)-Re(4)-N(8)	74.8(2)
N(3)-Re(2)-S(3)	78.4(1)	N(7)-Re(4)-S(7)	89.5(1)
N(3)-Re(2)-C(25)	99.4(3)	N(7)-Re(4)-C(51)	173.9(3)
N(3)-Re(2)-C(26)	95.5(3)	N(7)-Re(4)-C(52)	93.0(3)
C(25)-Re(2)-C(26)	87.5(3)	C(51)-Re(4)-C(52)	90.1(3)
Re(2)-S(3)-S(4)	108.43(9)	Re(4)-S(7)-S(8)	110.1(1)

**Table S4.** Selected Bond Lengths (Å) for Compound **3***(fac-[Re(CO)<sub>3</sub>(bpy)(H<sub>2</sub>O]))(fac-[Re(CO)<sub>3</sub>(bpy)(S<sub>2</sub>O<sub>3</sub>))]·4H<sub>2</sub>O.*

Bond	Distance (Å)	Bond	Distance (Å)
Re(1)-C(11)	1.948(12)	Re(2)-C(24)	1.937(11)
Re(1)-C(12)	1.915(10)	Re(2)-C(25)	1.929(11)
Re(1)-C(13)	1.941(11)	Re(2)-C(26)	1.889(11)
Re(1)-N(1)	2.157(10)	Re(2)-N(3)	2.169(9)
Re(1)-N(2)	2.169(9)	Re(2)-N(4)	2.166(9)
Re(1)-S(1)	2.481(3)	Re(2)-O(10)	2.253(7)
C(11)-O(4)	1.121(15)	C(24)-O(7)	1.120(14)
C(12)-O(5)	1.153(14)	C(25)-O(8)	1.144(15)
C(13)-O(6)	1.114(14)	C(26)-O(9)	1.170(14)
S(1)-S(2)	2.045(4)		
S(2)-O(1)	1.486(14)		
S(2)-O(2)	1.431(16)		
S(2)-O(3)	1.469(9)		

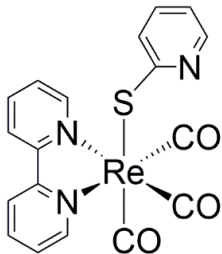
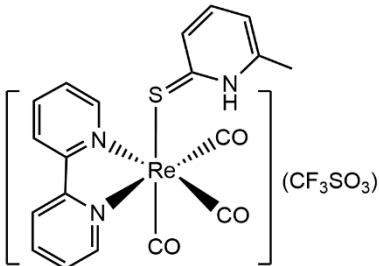
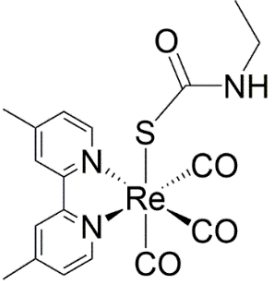
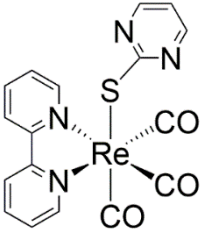
**Table S5.** Selected Bond Angles (deg) for Compound **3**

(*fac*-[Re(CO)<sub>3</sub>(bpy)(H<sub>2</sub>O)])(*fac*-[Re(CO)<sub>3</sub>(bpy)(S<sub>2</sub>O<sub>3</sub>]])•4H<sub>2</sub>O.

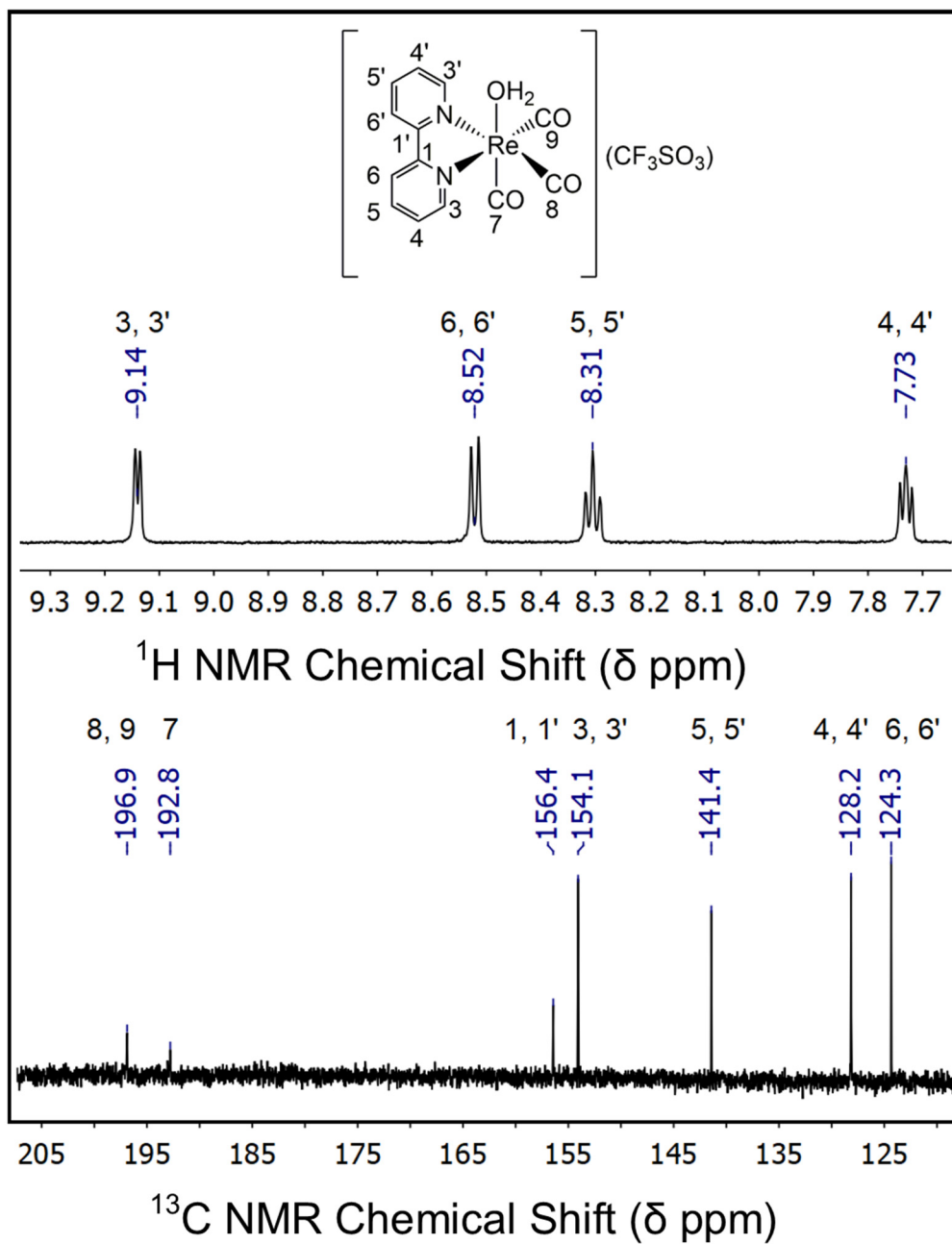
Bond	Degree	Bond	Degree
C(11)-Re(1)-N(1)	173.0(5)	C(24)-Re(2)-N(3)	172.4(4)
C(12)-Re(1)-N(1)	98.2(4)	C(25)-Re(2)-N(3)	97.8(4)
C(13)-Re(1)-N(1)	92.4(4)	C(26)-Re(2)-N(3)	95.7(4)
N(1)-Re(1)-N(2)	75.5(4)	N(4)-Re(2)-N(3)	74.6(4)
C(11)-Re(1)-N(2)	97.7(4)	C(24)-Re(2)-N(4)	99.0(4)
C(12)-Re(1)-N(2)	172.9(4)	C(25)-Re(2)-N(4)	172.1(4)
C(13)-Re(1)-N(2)	94.4(4)	C(26)-Re(2)-N(4)	93.6(5)
C(11)-Re(1)-S(1)	94.8(4)	C(24)-Re(2)-O(10)	90.0(4)
C(12)-Re(1)-S(1)	88.4(3)	C(25)-Re(2)-O(10)	92.9(4)
C(13)-Re(1)-S(1)	175.0(3)	C(26)-Re(2)-O(10)	177.6(4)
N(1)-Re(1)-S(1)	83.7(3)	N(3)-Re(2)-O(10)	85.6(3)
N(2)-Re(1)-S(1)	87.7(2)	N(4)-Re(2)-O(10)	84.8(4)
C(12)-Re(1)-C(11)	88.5(5)	C(25)-Re(2)-C(24)	88.5(5)
C(13)-Re(1)-C(11)	89.5(5)	C(26)-Re(2)-C(24)	88.6(5)
C(12)-Re(1)-C(13)	89.1(5)	C(25)-Re(2)-C(26)	89.0(5)
S(2)-S(1)-Re(1)	109.65(15)		



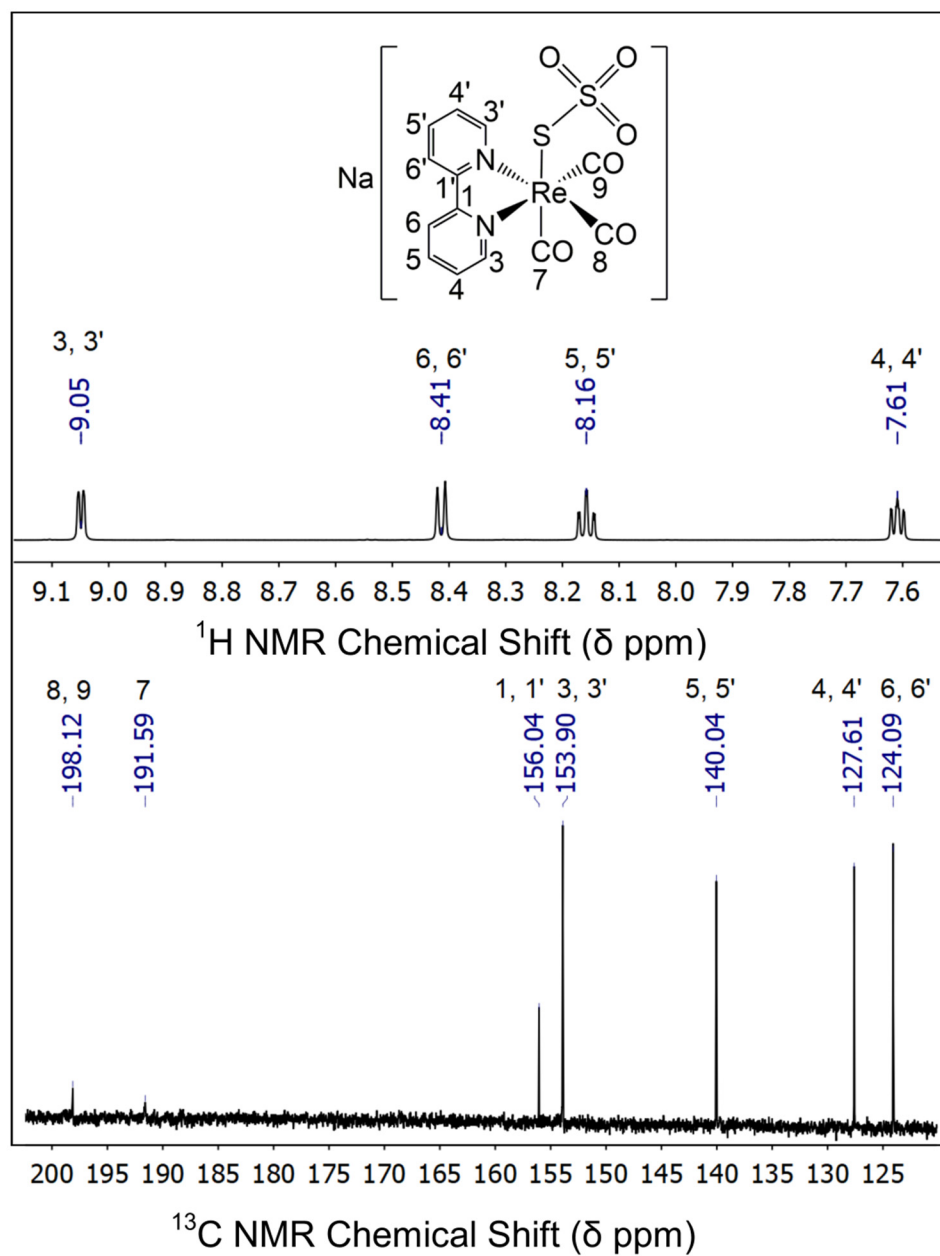
**Table S6.** Survey of Re(I) tricarbonyl complexes with an S-donor ligand in CSD version 5.38 (2017).

CSD Code	Reference	Structure	Re-S (Å)	Re-CO <sub>axial</sub> (Å)
ERUFII	<i>Inorg.Chim. Acta</i> , <b>2017</b> , 460, 127-133		2.4945(13)	1.924(6)
ERUFOO	<i>Inorg.Chim. Acta</i> , <b>2017</b> , 460, 127-133		2.5428(10)	1.931(3)
EXACAH	<i>Chem. Eur. J.</i> , 2004, <b>10</b> , 1765-1777.		2.496(2)	1.919(10)
RUMPUL	<i>Dalton Trans.</i> , 2009, 4458-4467.		2.5012(13)	1.927(5)
<b>Average Distance ± SD:</b>			2.509 ± 0.023	1.925 ± 0.005

*NMR Spectroscopy*

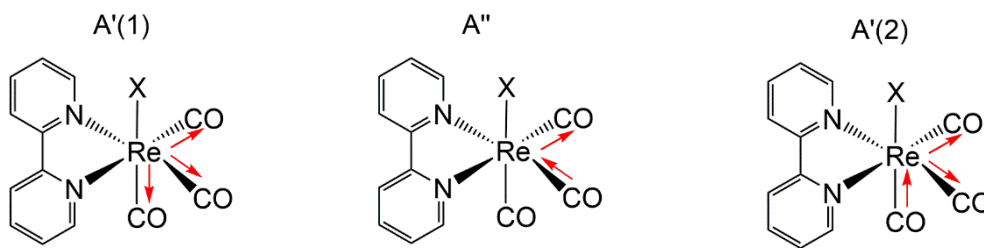
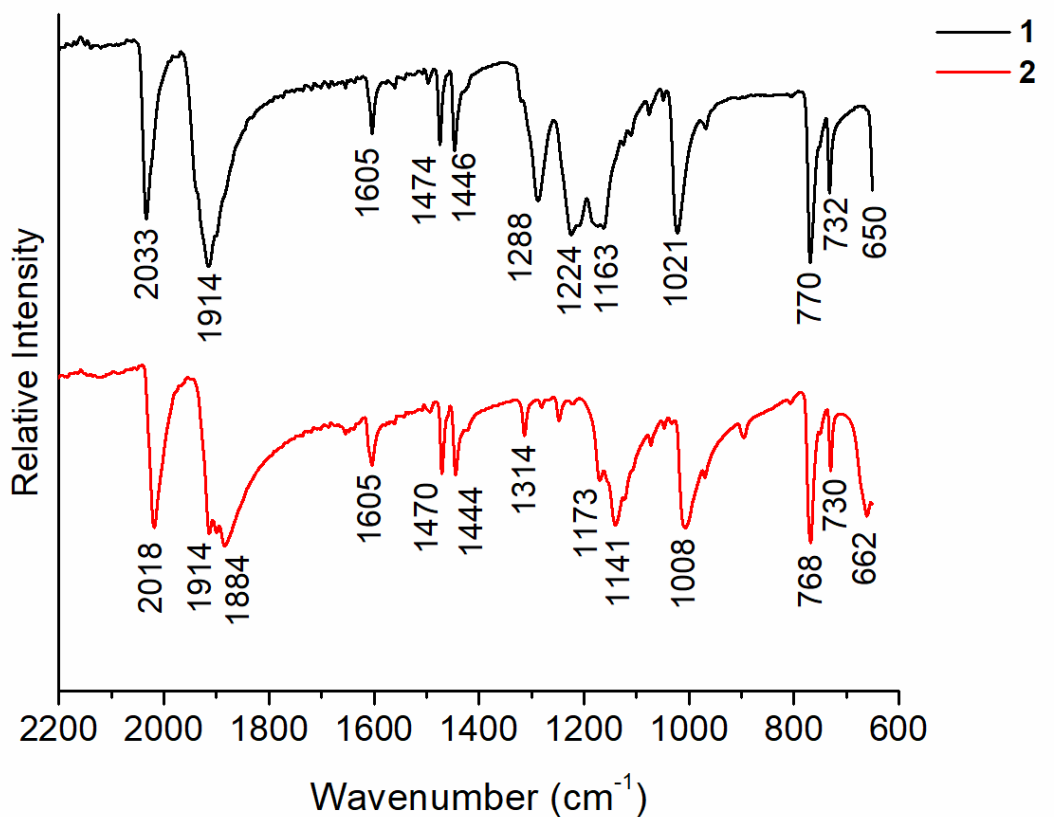


**Figure S1 (a)**  $^1H$  and  $^{13}C$  NMR of  $fac-[Re(CO)_3(bpy)(H_2O)](CF_3SO_3)$  (1) in  $D_2O$



**Figure S1 (b)**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of  $\text{Na}[\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]] \cdot \text{H}_2\text{O}$  (2) in  $\text{D}_2\text{O}$ .

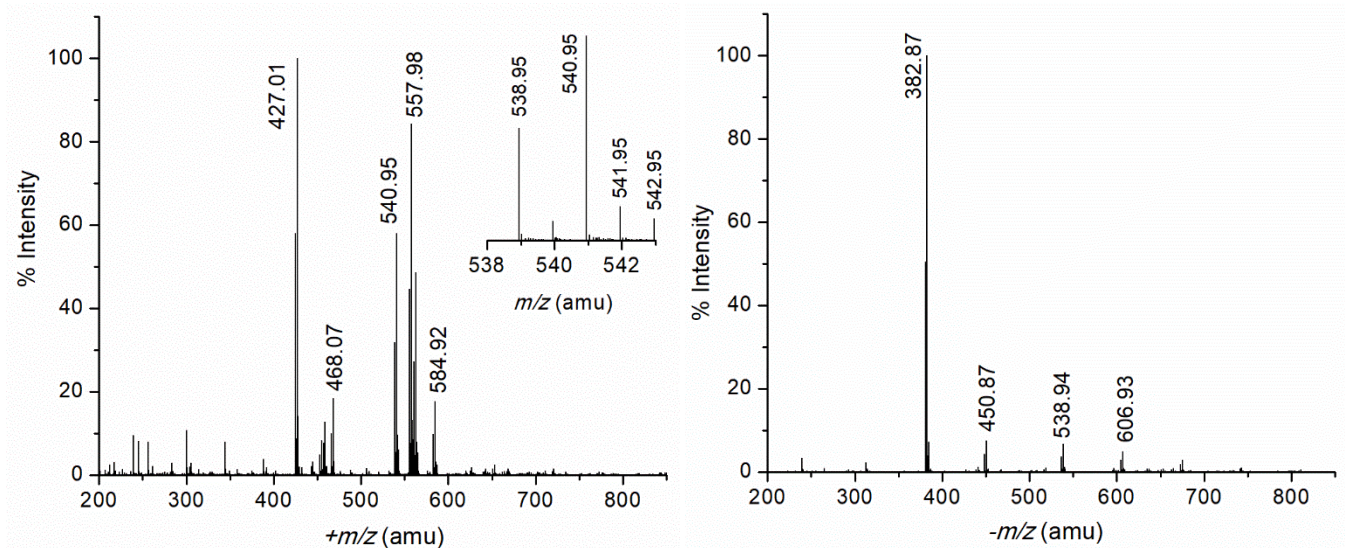
*FT-IR Spectroscopy*



Compound	A'(1) = totally symmetric in-phase $\nu(\text{CO})$ , $\text{cm}^{-1}$	A'' = asymmetric vibration of equatorial CO ligands, $\text{cm}^{-1}$	A'(2) = totally symmetric out-of-phase $\nu(\text{CO})$ , $\text{cm}^{-1}$
<b>1</b>	2033	1914	1914
<b>2</b>	2018	1914	1884

**Figure S2.** FT-IR spectra of *fac*-[Re(CO)<sub>3</sub>(bpy)(H<sub>2</sub>O)](CF<sub>3</sub>SO<sub>3</sub>) (**1**) and Na(*fac*-[Re(CO)<sub>3</sub>(bpy)(S<sub>2</sub>O<sub>3</sub>)]·H<sub>2</sub>O) (**2**) and corresponding  $\nu(\text{CO})$  vibrational modes.<sup>4,5</sup>

### Electro-spray Ionization Mass Spectrometry (ESI-MS)



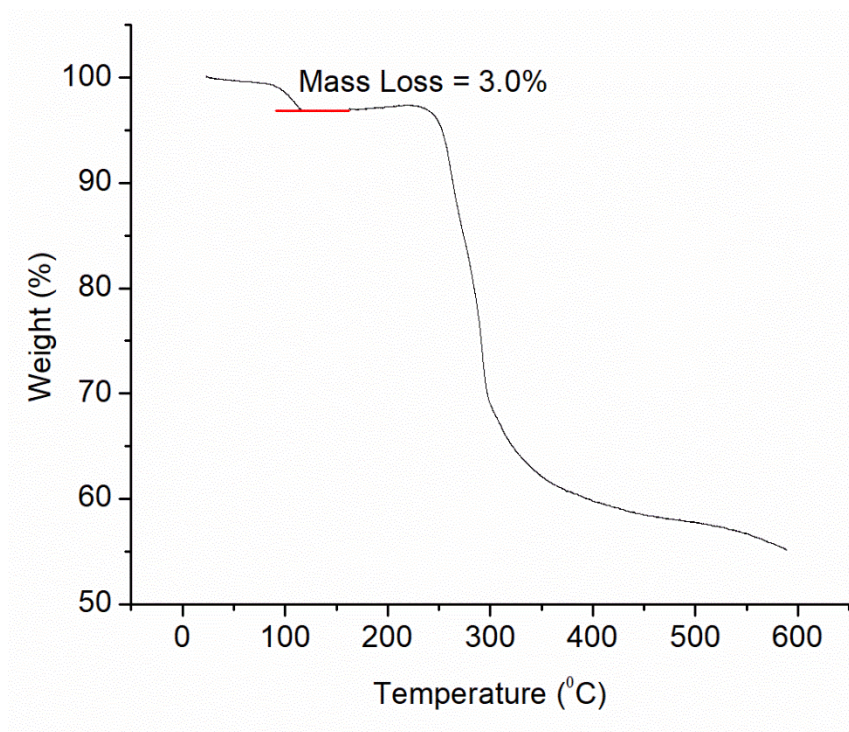
**Figure S3.** ESI mass spectra of  $\text{Na}(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]) \cdot \text{H}_2\text{O}$  (**2**) in (+) and (-) ion modes.

**Table S7.** Assignments of mass peaks in the ESI-mass spectra shown in Figure S3.<sup>a</sup>

+m/z	Assignment	-m/z	Assignment
<i>Complex 2</i>			
427.01	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy}]^+$	382.87	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{S}_2\text{O}_3^{2-}]^-$
468.07	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy} + \text{CH}_3\text{CN}]^+$ (Calc = 468.04)	450.87	$[\text{Re}^{\text{I}} + 4\text{CO} + \text{S}_2\text{O}_3^{2-} + \text{NaOH}]^-$ (Calc = 450.86)
540.95	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy} + \text{S}_2\text{O}_3^{2-} + 2\text{H}^+]^+$	538.94	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy} + \text{S}_2\text{O}_3^{2-}]^-$
557.98	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy} + \text{S}_2\text{O}_3^{2-} + \text{NH}_4^+ + \text{H}^+]^+$	606.94	$[\text{Re}^{\text{I}} + 4\text{CO} + \text{bpy} + \text{S}_2\text{O}_3^{2-} + \text{NaOH}]^-$ (Calc = 606.93)
584.92	$[\text{Re}^{\text{I}} + 3\text{CO} + \text{bpy} + \text{S}_2\text{O}_3^{2-} + 2\text{Na}^+]^+$		

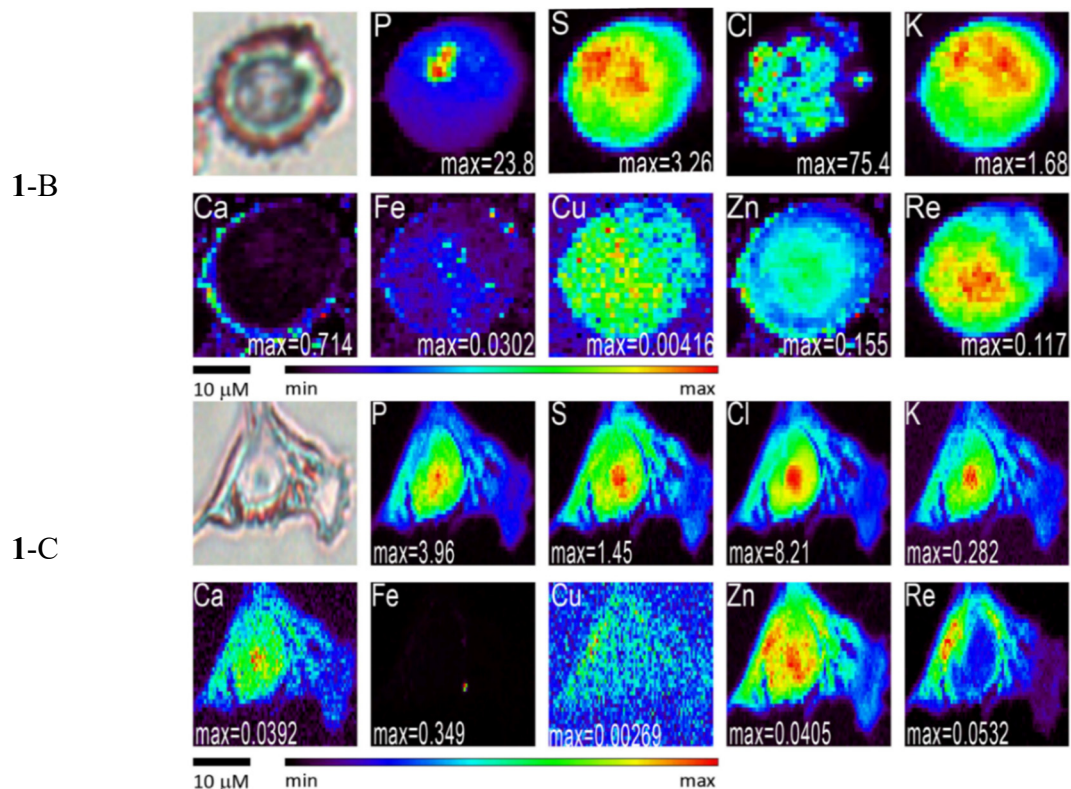
<sup>a</sup> bpy =  $\text{C}_{10}\text{H}_8\text{N}_2$

*Thermal Gravimetric Analysis (TGA)*

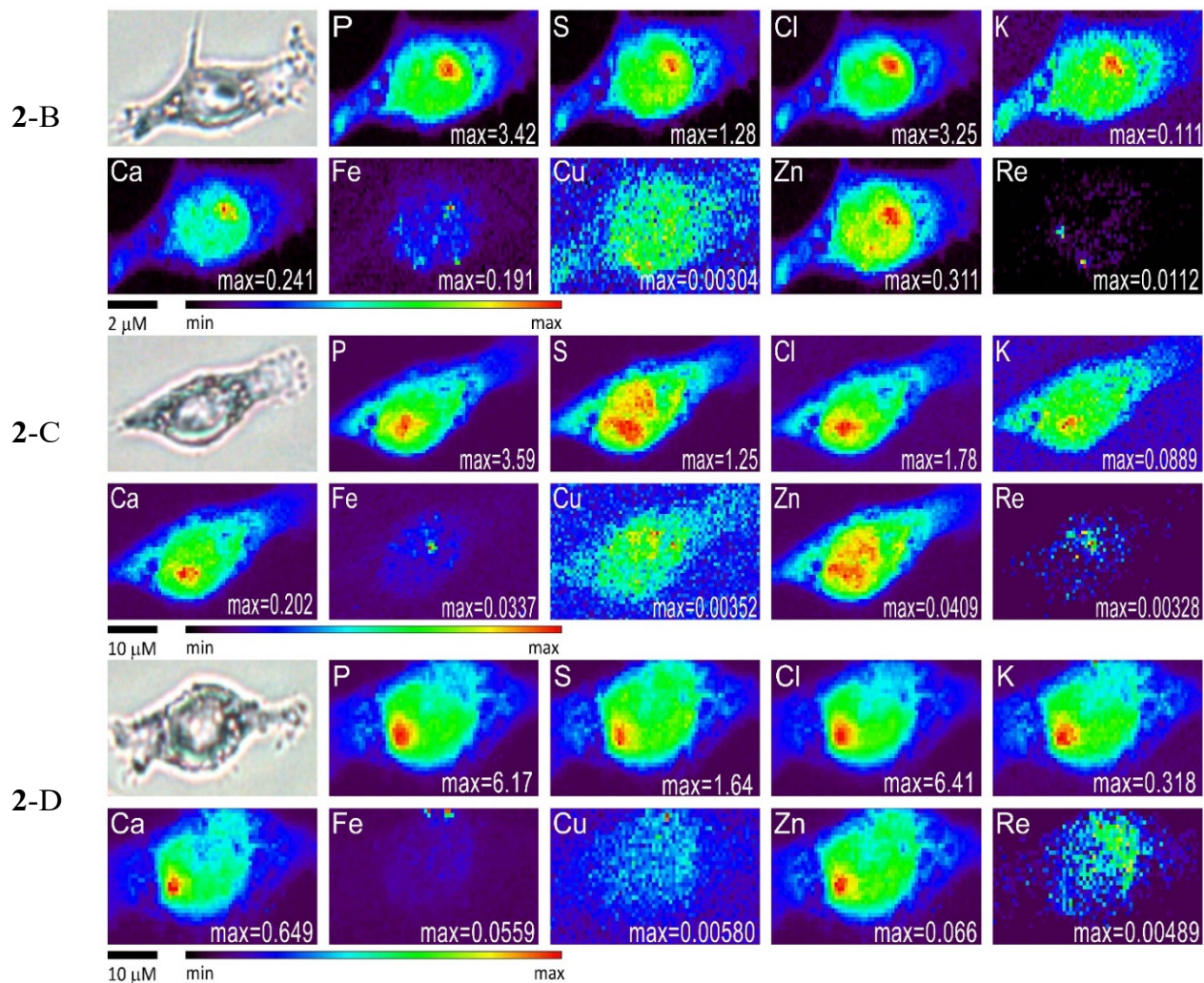


**Figure S4.** Thermogravimetric analysis (TGA) of  $\text{Na}(\text{fac}-[\text{Re}(\text{CO})_3(\text{bpy})(\text{S}_2\text{O}_3)]) \cdot \text{H}_2\text{O}$  (**2**).

## Synchrotron Based X-ray Fluorescence Microscopy (XFM)

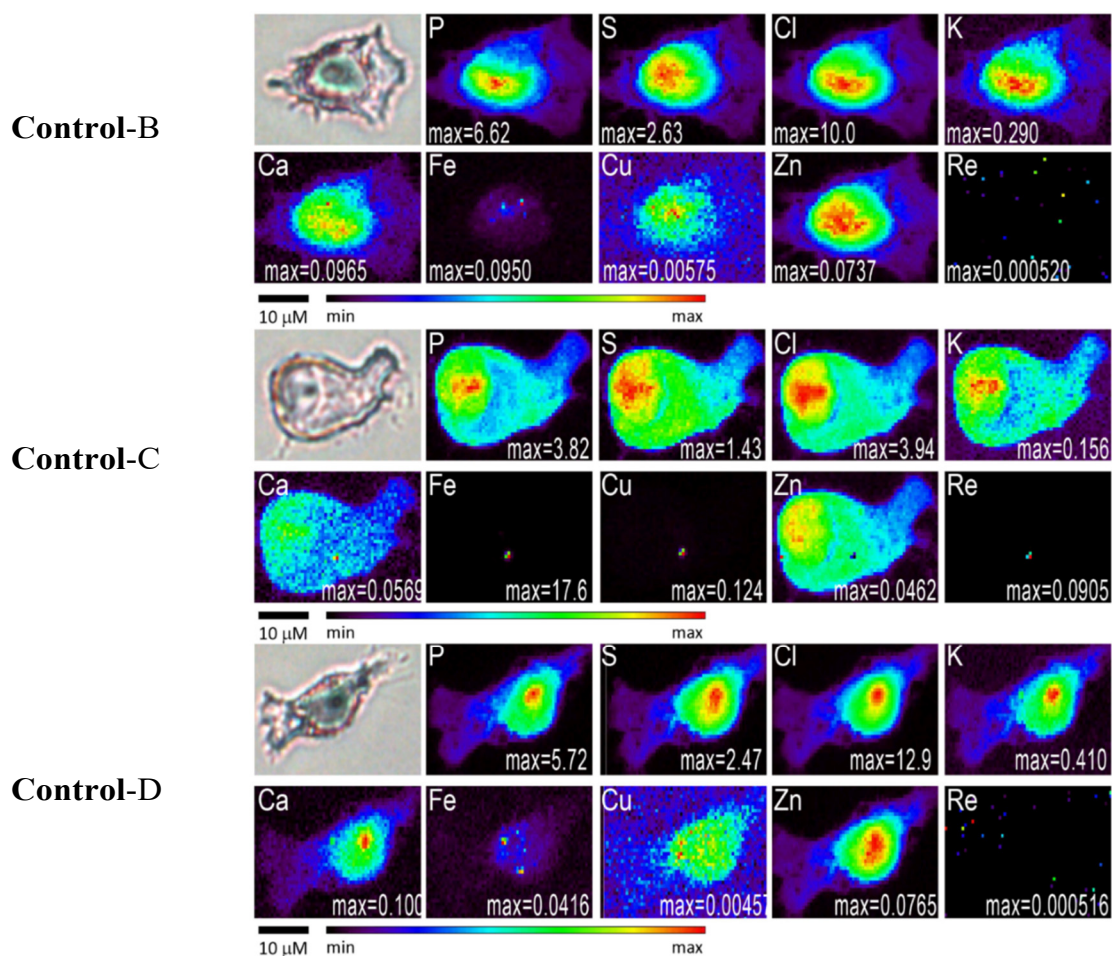


**Figure S5 (a).** Optical micrographs (*top left*) and XFM elemental distribution map of two MDA-MB-231 cells (**1-B** and **1-C**) treated with **1** for 6 h. The maximum elemental area densities (quantified from standards and expressed in  $\mu\text{g cm}^{-2}$ ) are given in the bottom of each map. The scale bar represents 10  $\mu\text{M}$  unless otherwise indicated. Images were reproduced by permission from Springer: Capper, M. S. et al. Cytotoxicity, cellular localization and photophysical properties of Re(I) tricarbonyl complexes bound to cysteine and its derivatives. *J. Biol. Inorg. Chem.* 2020, [25, 759](#).<sup>5</sup>

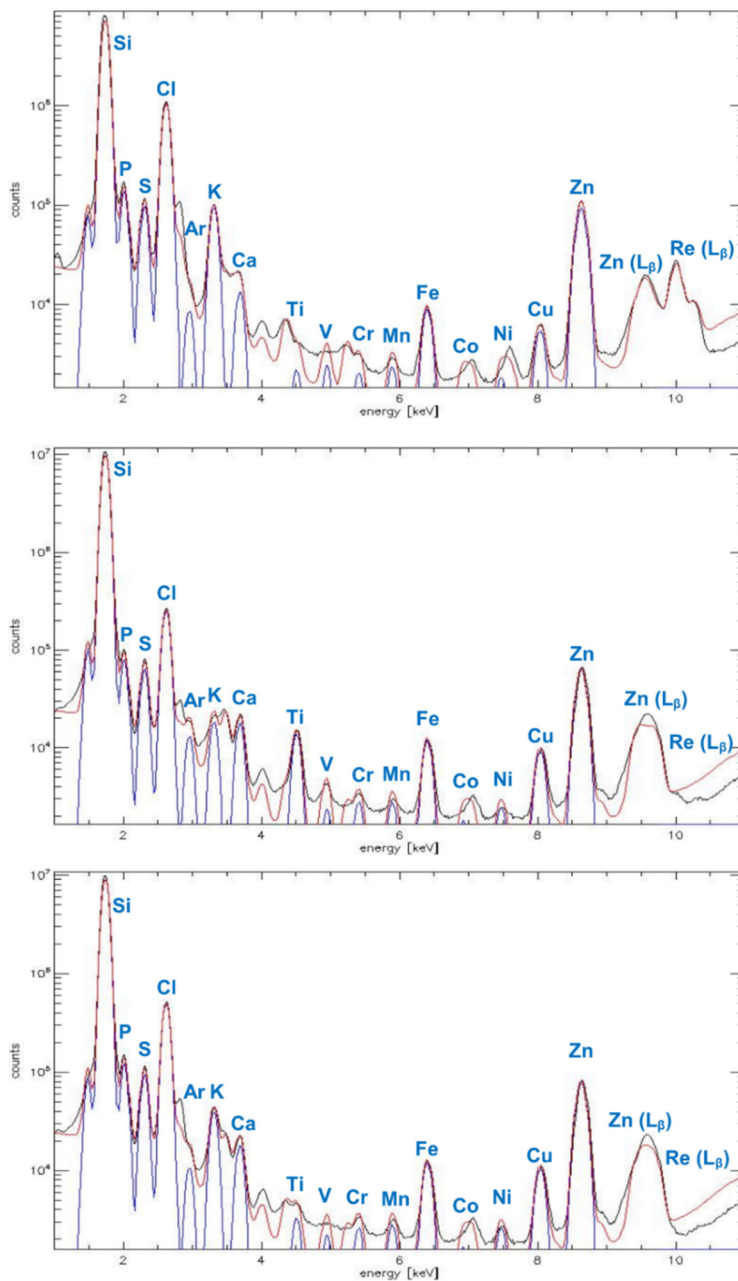


**Figure S5 (b).** Optical micrographs (*top left*) and XFM elemental distribution map of three MDA-MB-231 cells (2-B, 2-C and 2-D) treated with 2 for 6 h. The maximum elemental area densities (quantified from standards and expressed in  $\mu\text{g cm}^{-2}$ ) are given in the bottom of each map. The scale bar represents 10  $\mu\text{m}$  unless otherwise indicated.





**Figure S5 (c).** Optical micrographs (*top left*) and XFM elemental distribution map of three MDA-MB-231 cells (**Control-B**, **Control-C** and **Control-D**) treated with DMEM for 6 h. The maximum elemental area densities (quantified from standards and expressed in  $\mu\text{g cm}^{-2}$ ) are given in the bottom of each map. The scale bar represents 10  $\mu\text{m}$  unless otherwise indicated. Images were reproduced by permission from Springer: Capper, M. S. et al. Cytotoxicity, cellular localization and photophysical properties of Re(I) tricarbonyl complexes bound to cysteine and its derivatives. *J. Biol. Inorg. Chem.* 2020, 25, 759. <sup>5</sup>



**Figure S6.** XFM spectra (black) of MDA-MB-231 sample cells treated for 6 h with 20  $\mu$ M solutions of **1** (*top*) and **2** (*middle*) or in DMEM (*bottom*). Each figure shows the fit to the spectrum (red) and the contribution of the  $K_{\alpha}$  and  $L_{\beta}$  peaks used in the fitting (blue) with their corresponding labelling. Images for **1** and control were reproduced by permission from Springer: Capper, M. S. et al. Cytotoxicity, cellular localization and photophysical properties of Re(I) tricarbonyl complexes bound to cysteine and its derivatives. *J. Biol. Inorg. Chem.* 2020, 25, 759.<sup>5</sup>

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