Electronic Supplementary Material (ESI) for Dalton Transactions.

Responsive Fluorinated Nanoemulsions for ^{19}F Magnetic Resonance Detection of Cellular Hypoxia Supporting Information

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Experimental Methods

General

All solvents and chemicals were purchased from Sigma-Aldrich and Fisher Scientific and used as received. Reverse phase C18 chromatography was performed on a Biotage Isolera One. All water used in synthesis and purification was Milli-Q grade water. UV/Vis spectra were obtained using an Agilent Technologies Cary 6 UV-Vis Spectrophotometer. The ^{1}H, ^{13}C, and ^{19}F NMR spectroscopic measurements were conducted in deuterated solvents purchased from Cambridge Isotope Laboratories (Cambridge, MA), using an AGILENT MR 400 NMR spectrometer at 400, 100, 376 MHz, respectively. The chemical shifts for ^{1}H and ^{13}C NMR were calibrated to the solvent peak, while for ^{19}F NMR were calibrated to 5% TFA in D_2O (δ = -76.55 ppm) or 5-fluorocytosine (δ = -168 ppm). Walk-up LC-MS and high-resolution Electrospray Ionization (ESI) mass spectral analyses were performed by the Mass Spectrometry Facility of the Department of Chemistry at UT Austin. X-Ray crystallography was performed on an Agilent Technologies SuperNova Dual Source diffractometer using a μ-focus Cu Kα radiation source (λ = 1.5418 Å) with collimating mirror monochromators. Electrochemistry experiments were performed at the Center for Electrochemistry at UT Austin on a CHI 660D electrochemical workstation. EPR spectra were obtained with a Bruker Biospin EMXplus 114 X-band spectrometer equipped with a liquid nitrogen cryostat. Low-resolution Transmission Electron Microscopy (TEM) images were collected on a FEI Tecnai Transmission Electron Microscope operating at 80 kV. Energy Dispersion X-Ray Spectroscopy (EDS) 2-D elemental mapping were performed on JEOL 2010F Transmission Electron Microscope. Dynamic Light Scattering (DLS) was measured on Zetasizer Nano ZS from Malvern. Inductively coupled plasma optical emission spectrometry (ICP-OES) was performed on a Varian 710 Series ICP-OES in the Department of Civil, Architectural and Environmental Engineering at the University of Texas at Austin. MR images were collected on a 7T Bruker BioSpin (Karlsruhe, Germany) Pharmascan 70/16 magnet with a BioSpec two-channel console and BGA-9s gradient coil in the Imaging Research Center at UT Austin. Fluorescence microscopy images of MCF-7 cells were obtained on a Life Technologies EVOS FL Auto.

X-Ray Single Crystallography

Crystals grew as clusters of red plates by slow evaporation from ethanol. The data crystal was separated from a cluster of crystals and had approximate dimensions; 0.121 x 0.071 x 0.058 mm. The data were collected on an Agilent Technologies SuperNova Dual Source diffractometer using a μ-focus Cu Kα radiation source (λ = 1.5418Å) with collimating mirror monochromators. A total of 596 frames of data were collected using ω-scans with a scan range of 1° and a counting time of 7.5 seconds per frame for frames collected with a detector offset of -42.4° and 20 seconds per frame with frames collected with a detector offset of 111.0°. The data were collected at 100 K using an Oxford Cryostream low temperature device. Details of crystal data, data collection, and structure refinement are listed in Table S1. Data collection, unit cell refinement, and data reduction were performed using Rigaku Oxford Diffraction's CrysAlisPro V 1.171.40.67a.[1] The structure was solved by direct methods using SHELXT[2] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-2016/6.[3]

Structure analysis was aided by use of the programs PLATON,[4] OLEX2,[5] and WinGX.[6] The hydrogen atoms on the carbon atoms were calculated in ideal positions with isotropic displacement parameters set to 1.2xUeq of the attached atom (1.5xUeq for methyl hydrogen atoms).

Several of the trifluoromethyl groups showed signs of disorder with highly anisotropic displacement parameters. These groups were modeled for disorder using features available in OLEX2. The geometry of the trifluoromethyl groups was restrained to be equivalent throughout the final refinement stages. A large region of disordered solvent was observed in the unit cell. The solvent could not be satisfactorily modeled. The contributions to the scattering factors due to these solvent molecules were removed by using SQUEEZE.[7]

The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.0912*P)^2]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_w(F^2)$ refined to 0.195, with R(F) equal to 0.0738 and a goodness of fit, S = 1.05. Definitions used for calculating R(F), $R_w(F^2)$ and the goodness of fit, S, are given below.[8] The data were checked for secondary extinction effects, but no correction was necessary. Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography.[9] All figures were generated using SHELXTL/PC.[10] Tables of positional and thermal parameters, bond lengths and angles, torsion angles and figures are found elsewhere.

Cyclic voltammetry

Cyclic voltammetry (CV) measurements for a 5 mM solution of the compound were recorded at 100 mV/s in a glovebox. A three-electrode cell was used, including a platinum electrode as working electrode, an Ag/Ag^+ non-aqueous electrode as reference electrode (a 10 mM DMF solution of $AgNO_3$ was used as the source of Ag^+) and a platinum wire as auxiliary electrode. Bn_4NBF_4 (0.1 M) was used as the electrolyte and the spectra were calibrated by ferrocene ($E_{1/2}$ = 0.53 V in DMF vs. SCE).

Nanoemulsion Preparation

All nanoemulsions were prepared following published literature.[11] Taking **CuL_1** nanoemulsion as an example, lecithin (1.8 g) was weighed into a clean flat-bottom flask and allowed to disperse in 30 mL Milli-Q water for 30 minutes without stirring. Then, the flask was covered with a piece of aluminum foil and stirred at 80 C for an additional 30 minutes to better mix the lecithin with water. Safflower oil (1.8 mL) was added via a volumetric pipette to the mixture, which was further stirred at 80 ºC, with aluminum foil on top, for 1.5 hours to allow the formation of the pre-made emulsion (Scheme S2). **CuL_1** (2.7 mg) was weighed into a 15 mL falcon tube and dissolved in 100 μL DMSO. The hot pre-made emulsion (400 μL) was added straight into the **CuL_1** DMSO solution. The formed mixture was vortexed for 30 seconds to allow formation of crude **CuL_1** emulsion, which was further formulated into nanoemulsion (**NE CuL_1**) after ultra-sonication at 0 ºC using a probe-sonicator: 25% power, 10 second on-pulse, 10 second off-pulse, 20-min total sonication time. **NE CuL_1** prepared has a concentration of 5 mM **CuL_1** and appeared

as a clear brown suspension against light. All 1 mM samples were obtained by taking the 5 mM stock and diluting into Milli-Q grade water.

Transmission Electronic Microscopy (TEM) and Energy-Dispersive X-ray Spectroscopy (EDS)

NE CuL$_1$ (5 mM stock) was diluted into Milli-Q water for a final concentration of 100 μM Cu^{2+}. The diluted nanoemulsion (20 μL) was mixed with neutral ammonium phosphomolybdate (0.5% w/v, pH 6.7, 20 μL) and the mixed solution was drop-casting on a copper grid (200 mesh Cu/Formvar; Ted Pella, Inc.), and left to evaporate in air for 36 hours.

Cell Culture Media

Cell culture media (hereafter "DMEM" or "media") was prepared in a sterile fume hood by mixing 450 mL Dulbecco's Modified Eagle's Medium (DMEM, purchased from SigmaAldrich), 50 mL fetal bovine serum (FBS, purchased from SigmaAldrich), and 5 mL antibiotics (formulated with 5,000 U/mL penicillin and 2 mg/mL streptomycin, purchased from SigmaAldrich). The mixture was then sterile filtered and stored in the refrigerator (2-8 C).

Cytotoxicity

Cytotoxicity of **NE CuL$_1$** was tested in 6-well plate using ReadyProbes® Cell Viability Imaging Kit (Blue/Green) under both normoxic and hypoxic conditions. MCF-7 cells were grown in 6-well plate until 80% confluent. The cells were incubated with nanoemulsion (100 μM **NE CuL$_1$**, 0.4% DMSO) in 1.5 mL DMEM medium. For the normoxia group, MCF-7 cells were incubated under a 5% CO_2/95% air atmosphere for 4 hours. For the hypoxia group, MCF-7 cells were placed in a 0.1% O_2/5% CO_2/94.9% nitrogen atmosphere before incubation for 16 hours, followed by incubation of the nanoemulsion for 4 hours under the same hypoxic conditions. Post-incubation, the culture media was removed, and the cells were washed twice with 1 mL PBS buffer and incubated with cell viability imaging kit dyes (1 drop per mL for each dye) in a culture media without phenol red for 10 minutes. The cells were imaged directly under an EVOS® FL Auto inverted fluorescence microscope. Imaging parameters: total cell stain, DAPI light cube, Ex 360 nm/Em 447 nm; dead cell stain, GFP light cube, Ex 470nm/Em 525 nm. Percentage of dead cells was determined using an automated counting protocol in the EVOS software. Cytotoxicity studies were repeated with a 6 hour normoxic and hypoxic incubation as well.

Cell Uptake

The cell uptake of **NE CuL$_1$** was studied both quantitatively and qualitatively. For quantitative determination of the cellular uptake of the nanoemulsion, MCF-7 cells were grown in T-75 flask until 80% confluent and incubated with blank DMSO or **NE CuL$_1$** (100 μM, diluted from 5 mM stock, containing 0.4% DMSO) in 10 mL DMEM medium at 37 ºC. For normoxic group, MCF-7 cells were incubated under a 5% CO_2/95% air atmosphere for 2, 4, and 6 hours. For hypoxic group, MCF-7 cells were placed in a 0.1% O_2/5% CO_2/94.9% nitrogen atmosphere for hypoxic conditions for 16 hours, followed by incubation of the Cu complexes for 2, 4, 6 hours under hypoxic conditions. After incubation, the cells were washed twice with 5 mL PBS buffer to remove the extracellular

Cu complexes. Trypsin was used to help transfer the cells from T-75 flask into Eppendorf tubes. The tubes were centrifuged for 5 min at 0.2 rcf and the media was removed and discarded. The cell pellet was dried in oven, digested by conc. HNO_3 (143 μL) at 80 ºC for 30 minutes and diluted with Milli-Q water to 5 mL total (2% v/v HNO_3). The resulting mixture was subjected to inductively coupled plasma-optical emission spectrometry (ICP-OES) for determination of the whole copper content. The instrument was calibrated for copper by using standard solutions with copper level of 0, 10, 20, 50, 100, 200, 500, and 1000 ppb, respectively. The cell uptake level was also determined for the normoxia group at 4 ºC to help determine the uptake pathway. In this low-temperature cell uptake experiment, normoxic cells were incubated with the nanoemulsion for 4 hours. To qualitatively determine the cell uptake of the nanoemulsion, MCF-7 cells was plated on 6-well plates and incubated for 4 hours under a 5% CO_2/95% air atmosphere with (1) **NE CuL$_1$** (0.4% DMSO), (2) a lipid droplet targeting fluorescent dye (ZincSalen, 0.5 μM, 0.4% DMSO), and (3) **NE CuL$_1$** (100 μM, 0.4% DMSO) together with ZincSalen (2 μM). After incubation, cells were washed by PBS buffer for two times to remove extracellular nanoemulsion or fluorescent dyes and the cells were imaged on a confocal fluorescent microscope (Channel: excitation 532 nm, emission 600-660 nm). Intracellular fluorescent intensity was quantified by ImageJ.

^{19}F Relaxation Time Determination

T_1 and T_2 values were measured with an Agilent 400 NMR spectrometer using inversion-recovery sequence and Carr-Purcell-Meiboom-Gill (CPMG) sequence, respectively. The 90° pulse was calibrated for each sample individually. Samples (~500 uL) were dissolved in non-deuterated solvent systems at 5 mM within a 5 mm NMR tube. A 60 μL NMR coaxial tube (Wilmad glass) containing deuterated D_2O was inserted in the 5 mm NMR tube for locking. ^{19}F MR signals were observed at 18 different time points after excitation and the signal integrations were fitted to first-order exponential growth curve for T_1 measurement and first-order exponential decay for T_2 measurement. T_2* was determined by fitting the ^{19}F NMR peak to a Lorentzian line shape, where $T_2^* = 1/(\pi \cdot \Delta H)$ and ΔH is the full width at half height in Hz.

***in vitro* ^{19}F NMR**

Live cell ^{19}F NMR experiments were carried out for **NE CuL$_1$** at 100 μM concentration (0.4% DMSO) under both normoxic and hypoxic conditions. Roughly 40 million MCF-7 cells were used for this experiment. For the normoxic group, MCF-7 cells were grown in four T-150 flasks under a 5% CO_2/95% air atmosphere (20% O_2) and incubated with **NE CuL$_1$** (100 μM, 0.4% DMSO) for 4 hours in 12 mL DMEM medium. After incubation, cells were washed with PBS buffer twice and transferred into Eppendorf tubes after trypsin treatment. The tube was then centrifuged at 0.2 rcf for 5 min and the media was discarded. The cells were suspended in 400 μL DMEM media and the whole cell suspension was added to an NMR tube. A coaxial NMR capillary filled with 50 mM 5-fluorocytosine (5-FC) in D_2O solution was put into the NMR tube for locking and fluorine quantification. For the hypoxic group, MCF-7 cells (~40 million) were placed in a hypoxic chamber with 0.1% O_2/5.0% CO_2/94.9% nitrogen atmosphere for 16 hours, followed by incubation of **NE CuL$_1$** (100 μM, 0.4% DMSO) for 4 hours under same atmosphere. After incubation, the cells were kept inside the hypoxic chamber and the same treatment was

performed as the normoxic group. To keep the low-oxygen environment, the NMR tube of the hypoxic cell suspension was capped and sealed with Parafilm. Control experiments were performed by incubating cells with **NE H_2L_1** (100 μM, 0.4% DMSO) for 4 hours under normal oxygen tension (20% O_2).

^{19}F NMR was performed on an Agilent 400 NMR spectrometer at 376 MHz. For ^{19}F NMR spectrum acquisition, following parameters were set: relaxation delay, 0.6 seconds; acquisition time, 0.6 seconds; number of scans, 5000. For ^{19}F content quantification, the parameters were set as below: relaxation delay, 3 seconds; acquisition time, 3 seconds; number of scans, 2000. The spectra so obtained was processed on MestReNova.

To determine the reduced copper content inside the cells, the observable fluorine signal was first quantified through the following equation:

$$n\left(^{19}F_{obs}\right) = c\left(^{19}F_{obs}\right) \cdot V(cell\ sample)$$

$$c\left(^{19}F_{obs}\right) = \frac{V(coaxial)}{V(NMR\ tube)} \cdot \frac{c_{5-FC}}{18} \cdot \frac{I(\boldsymbol{CuL_1})}{I(5-FC)}$$

where $V(cell\ sample)$ is the volume of the live cell sample inserted into the NMR tube (~500 μL); $V(coaxial)$ and $V(NMR\ tube)$ are the volumes of the coaxial and the NMR tube scanned during the NMR experiment, respectively, and their ratio $\frac{V(coaxial)}{V(NMR\ tube)}$ is calculated as below; c_{5-FC} is the concentration of the 5-FC added to the coaxial (50 mM); "18" corresponds to the 18 equiv. of fluorine atoms in 1 equiv. of reduced **CuL_1**; $I(\boldsymbol{CuL_1})$ and $I(5-FC)$ are the integral values of the ^{19}F NMR peak of the reduced **CuL_1** (in the NMR tube) and 5-FC (in the coaxial), respectively, and their ratio $\frac{I(\boldsymbol{CuL_1})}{I(5-FC)}$ was calculated based on the spectra processed on MestReNova.

To quantify the volume ratio of the coaxial tube and the NMR tube ($\frac{V(coaxial)}{V(NMR\ tube)}$), the coaxial was filled with 50 mM 5-fluorocytosine and the NMR tube was filled with 10 mM TFA. The NMR tube inserted with the coaxial was scanned using the above mentioned NMR scanner and the volume ratio was calculated as below:

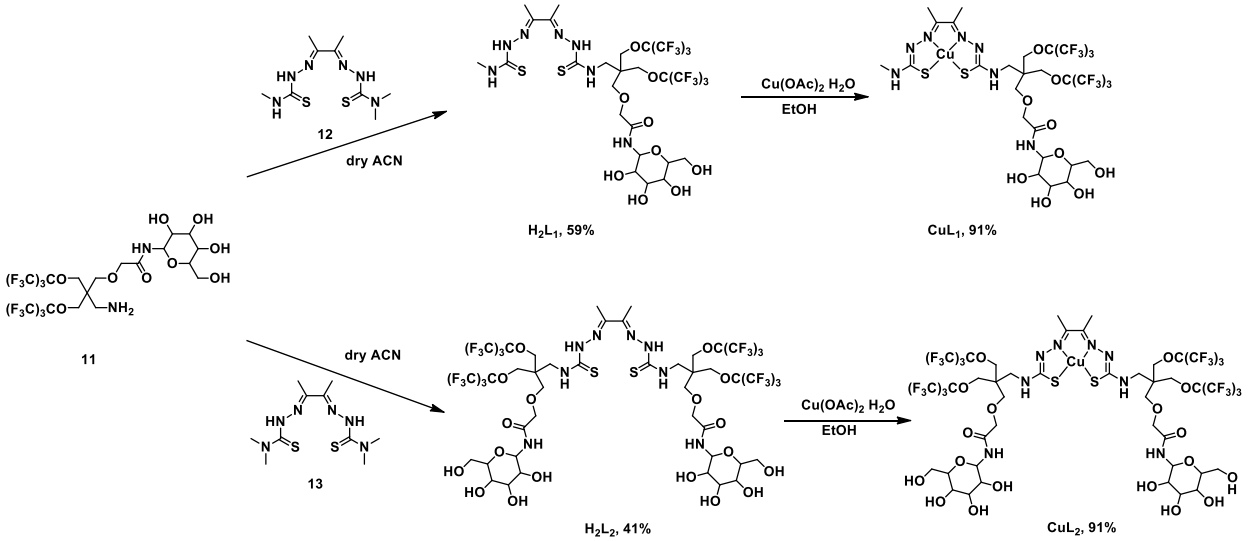
$$\frac{V(coaxial)}{V(NMR\ tube)} = 3 \cdot \frac{I(5-FC)}{I(TFA)} \cdot \frac{c(TFA)}{c(5-FC)}$$

where "3" corresponds to the 3 equiv. of fluorine atoms in 1 equiv. of TFA; $I(5-FC)$ and $I(TFA)$ are the integral of the ^{19}F NMR peak of 5-FC (in the coaxial) and TFA (in the NMR tube), respectively, and their ratio $\frac{I(5-FC)}{I(TFA)}$ was calculated based on the spectra processed on MestReNova; $c(TFA)$ and $c(5-FC)$ are the concentration of TFA and 5-FC and $c(TFA) = 10\ mM$ and $c(5-FC) = 50\ mM$.

^{19}F MR Imaging

In separate 500 μL Eppendorf tubes, 100 μL of stock 5 mM **NE H_2L_1** and **NE CuL_1** was diluted with 400 μL Milli-Q water to achieve a final concentration of 1 mM. The tubes were imaged using a flash-low-angle-shot (FLASH) pulse sequence with a 50 ms repetition time, a 1.563 ms echo time, a 28º flip angle, a 64x64 matrix size, a 40x40 mm^2 field of view, and an acquisition time of two hours. For the reduced sample, 3 eq. $Na_2S_2O_4$ was mixed in the tube prior to imaging.

Synthesis



Scheme S1. Synthetic route for $\mathbf{H_2L_1}$, $\mathbf{CuL_1}$, $\mathbf{H_2L_2}$, and $\mathbf{CuL_2}$. Ligands **1** and **2**[12] and fluorinated tag **3**[13] were synthesized from previously reported literature.

Compound $\mathbf{H_2L_1}$

1 (100 mg, 0.36 mmol) and **3** (317 mg, 0.40 mmol) were combined in 20 mL dry acetonitrile and refluxed at 75 ºC for 16 hours. The initially insoluble yellow solid **1** gradually dissolved as the reaction proceeded and the reaction mixture became bright yellow. Upon completion, the solvent was then evaporated under reduced pressure and the crude was purified by C18 reverse phase chromatography using 50% MeCN/50% H_2O/0.1% formic acid to remove all side produce for five minutes and then 70% MeCN/30% H_2O/0.1% formic acid for five minutes to obtain the ligand. All solvents were lyophilized to obtain 220 mg $\mathbf{H_2L_1}$ (off-white solid; 59%).

^{1}H NMR (400 MHz, d_6-DMSO) δ 10.47 (s, 1H), 10.14 (s, 1H), 8.48 (t, *J* = 6.1 Hz, 1H), 8.40 (t, *J* = 5.3 Hz, 1H), 8.38 (s, 1H), 5.01 (d, *J* = 4.6 Hz, 1H), 4.91 (dd, *J* = 4.9, 3.0 Hz, 2H), 4.73 (t, *J* = 9.1 Hz, 1H), 4.54 (t, *J* = 5.5 Hz, 1H), 4.25 (d, *J* = 8.9 Hz, 2H), 4.10 (t, *J* = 8.8 Hz, 2H), 4.04 (s, 2H), 4.00 – 3.90 (m, 2H), 3.69 (dd, *J* = 10.5, 5.2 Hz, 1H), 3.58 (q, *J* = 9.5 Hz, 2H), 3.47 – 3.39 (m, 2H), 3.23 – 3.06 (m, 3H), 3.02 (d, *J* = 4.4 Hz, 3H), 2.23 (s, 3H), 2.11 (s, 3H).

^{13}C NMR (101 MHz, d_6-DMSO) δ 179.40 (s), 178.82 (s), 170.01 (s), 120.23 (q, J = 292.3 Hz), 79.60 (s), 79.02 (s), 77.71 (s), 72.96 (s), 70.69 – 70.52 (m), 70.45 (s), 69.63 – 69.45 (m), 68.50 – 68.24 (m), 61.52 – 61.36 (m), 45.87 (s), 31.65 (s), 12.31 (s), 11.53 (s).

^{19}F NMR (376 MHz, d_6-DMSO) δ -70.0.

HR MS (MeOH, ESI$^+$): calculated for $[M+Na]^+$: $[C_{28}H_{35}F_{18}N_7NaO_9S_2]^+$ 1042.1542, found 1042.1546.

Compound $\mathbf{CuL_1}$

Compound $\mathbf{H_2L_1}$ (200 mg, 0.20 mmol) and $Cu(OAc)_2{\cdot}2H_2O$ (47 mg, 0.24 mmol) were combined and stirred in 20 mL ethanol at room temperature for 16 hours. The solvent was then removed under reduced pressure and the brown crude was purified by C18 reverse phase chromatography using 0% MeCN/100% H_2O/0.1% formic acid for five

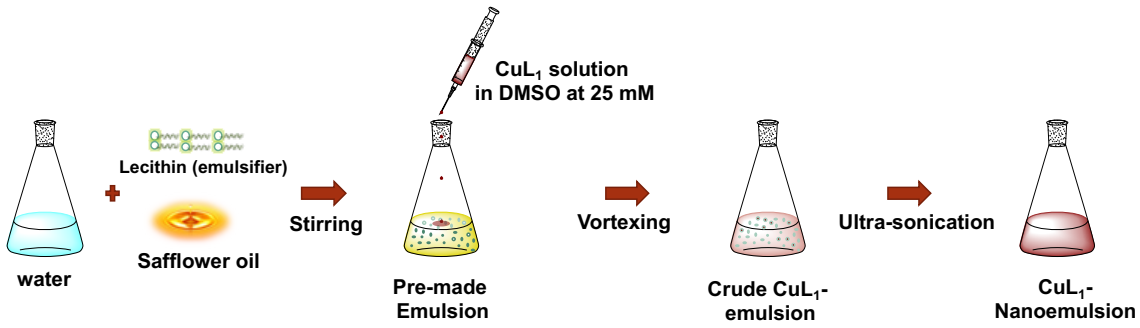
minutes to remove excess copper acetate, 65% MeCN/35% H_2O/0.1% formic acid for four minutes to remove all side products, and then 80% MeCN/20% H_2O/0.1% formic acid for fifteen minutes to obtain the complex. All solvents were lyophilized to obtain 193 mg **CuL_1** (brown powder; 91%). Single crystal of **CuL_1** was grown by slow evaporation of its ethanol solution at room temperature.
^{19}F NMR (376 MHz, d_6-DMSO) δ -69.9.
HR MS (MeOH, ESI^+): calculated for $[M+Na]^+$: $[C_{28}H_{33}CuF_{18}N_7NaO_9S_2]^+$ 1103.0682, found 1103.0685.
Elemental analysis calculated (%) for $C_{28}H_{33}CuF_{18}N_7O_9S_2 + H_2O$: C 30.59, H 3.21, N 8.92; found: C 30.54, H 3.13, N 8.65.

Compound **H_2L_2**
2 (66 mg, 0.23 mmol) and **3** (400 mg, 0.51 mmol) were combined in 30 mL dry acetonitrile and refluxed at 75 ºC for 12 hours. The reaction was monitored by LC-MS every two hours and was cooled down to room temperature after full conversion of the starting material **2**. The initially insoluble orange solid **2** gradually dissolved as the reaction proceeded and the reaction mixture became dark yellow. The solvent was then evaporated under reduced pressure and the crude was purified by C18 reverse phase chromatography using 80% MeCN/20% H_2O/0.1% formic acid for six minutes to remove side products and then 88% MeCN/12% H_2O/0.1% formic acid for ten minutes to obtain ligand. All solvents were lyophilized to obtain 170 mg **H_2L_2** (off-white solid; 41%).
^1H NMR (400 MHz, d_6-DMSO) δ 10.38 (s, 2H), 8.43 (s, 2H), 8.35 (d, *J* = 9.1 Hz, 2H), 4.99 (d, *J* = 4.5 Hz, 2H), 4.88 (d, *J* = 5.0 Hz, 4H), 4.70 (t, *J* = 9.0 Hz, 2H), 4.48 (t, *J* = 5.5 Hz, 2H), 4.20 (d, *J* = 8.4 Hz, 4H), 4.07 (t, *J* = 8.7 Hz, 4H), 3.99 (d, *J* = 7.6 Hz, 4H), 3.92 (q, *J* = 14.0 Hz, 4H), 3.70 – 3.60 (m, 2H), 3.54 (dd, *J* = 14.9, 9.7 Hz, 4H), 3.38 (dd, *J* = 11.2, 5.5 Hz, 2H), 3.23 – 2.97 (m, 8H), 2.10 (s, 6H).
^{13}C NMR (101 MHz, d_6-DMSO) δ 179.34 (s), 169.95 (s), 148.77 (s), 120.22 (q, *J* = 292.6 Hz), 79.62 (s), 79.01 (s), 77.71 (s), 72.95 (s), 70.77 – 70.57 (m), 70.42 (s), 69.77 – 69.54 (m), 68.51 – 68.14 (m), 61.55 – 61.30 (m), 45.81 (s), 11.72 (s).
^{19}F NMR (376 MHz, d_6-DMSO) δ -70.0.
HR MS (MeOH, ESI^+): calculated for $[M+Na]^+$: $[C_{48}H_{54}F_{36}N_8NaO_{18}S_2]^+$ 1801.2315, found 1801.2321.

Compound **CuL_2**
Compound **H_2L_2** (100 mg, 0.056 mmol) and $Cu(OAc)_2 \cdot 2H_2O$ (17 mg, 0.084 mmol) were combined and stirred in 20 mL ethanol at room temperature for 16 hours. The solvent was then removed under reduced pressure and the brown crude was purified by C18 reverse phase chromatography using 0% MeCN/100% H_2O/0.1% formic acid for four minutes to remove excess copper acetate and then 92% MeCN/8% H_2O/0.1% formic acid for ten minutes to obtain the complex. All solvents were lyophilized to obtain 94 mg **CuL_2** (brown powder; 91%).
^{19}F NMR (376 MHz, d_6-DMSO) δ -70.0.
HR MS (MeOH, ESI^+): calculated for $[M+Na]^+$: $[C_{48}H_{52}CuF_{36}N_8NaO_{18}S_2]^+$ 1862.1454, found 1862.1418.
Elemental analysis calculated (%) for $C_{48}H_{52}CuF_{36}N_8O_{18}S_2 + 2H_2O$: C 30.72, H 3.01, N 5.97; found: C 30.22, H 2.81, N 5.91.

Table S1. Crystal data and structure refinement for **CuL_1**.

Empirical formula	C_{28} H_{33} Cu F_{18} N_7 O_9 S_2
Formula weight	1081.27
Temperature	100.15 K
Wavelength	1.54184 Å
Crystal system	orthorhombic
Space group	P 21 21 2
Unit cell dimensions	a = 38.4703(7) Å; α = 90° b = 32.8730(7) Å; β = 90° c = 11.9611(3) Å; γ = 90°
Volume	15126.4(5) $Å^3$
Z	12
Density (calculated)	1.424 Mg/m^3
Absorption coefficient	2.448 mm^{-1}
F(000)	6540
Crystal size	0.121 x 0.071 x 0.058 mm^3
Theta range for data collection	2.662 to 66.600°
Index ranges	-45<=h<=41, -38<=k<=39, -14<=l<=10
Reflections collected	50033
Independent reflections	25049 [R(int) = 0.0752]
Completeness to theta = 66.600°	99.90%
Absorption correction	Gaussian and multi-scan
Max. and min. transmission	1.00 and 0.255
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	25049 / 1936 / 2108
Goodness-of-fit on F^2	1.043
Final R indices [I>2sigma(I)]	R1 = 0.0738, wR2 = 0.1796
R indices (all data)	R1 = 0.0930, wR2 = 0.1950
Absolute structure parameter	0.01(2)
Extinction coefficient	N/A
Largest diff. peak and hole	0.775 and -0.653 $e.Å^{-3}$



Scheme S2. Preparation of all nanoemulsions, using **CuL_1** as an example.

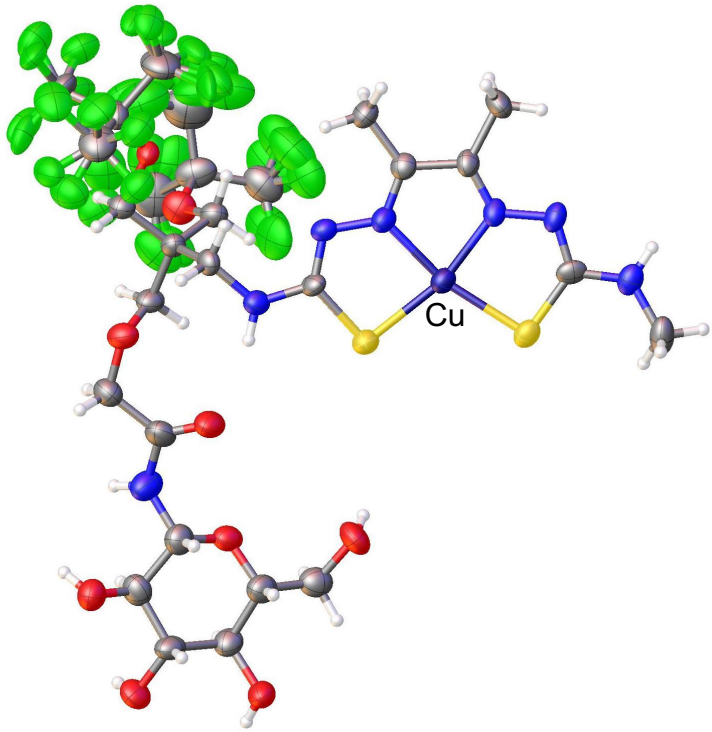


Figure S1. Molecular structures of **CuL_1** from single crystal X-Ray diffraction showing the heteroatom labeling scheme. Displacement ellipsoids are scaled to the 30% probability level. We note that the trifluoromethyl groups were disordered. Solvent molecules and weak interactions were omitted for clarity. Color assignments: carbon (gray), oxygen (red), nitrogen (blue), sulfur (yellow), hydrogen (white), fluorine (green), and copper (purple; labeled).

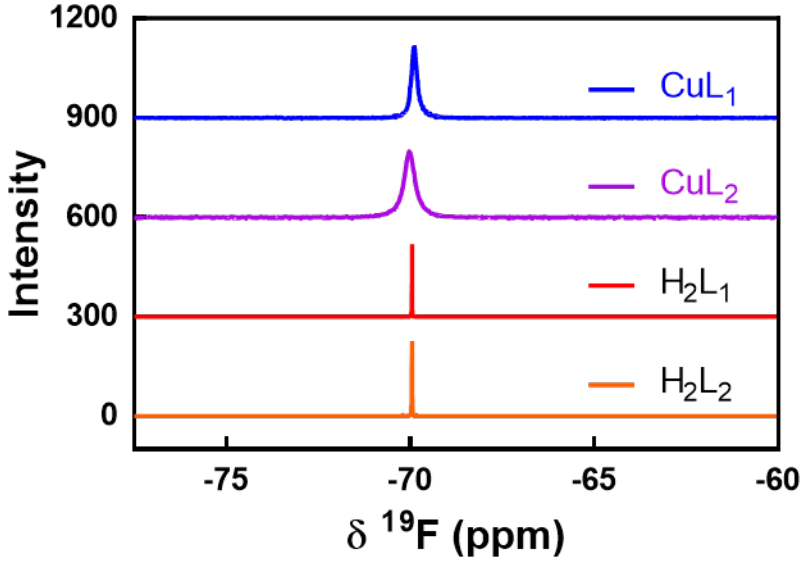


Figure S2. ^{19}F NMR spectra of 3 mM **H_2L_1**, **CuL_1**, **H_2L_2**, and **CuL_2** in d_6-DMSO at room temperature.

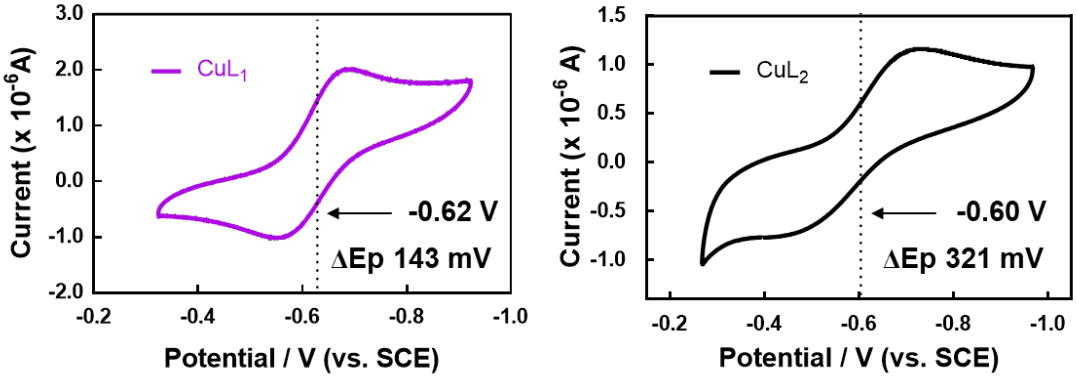


Figure S3. Cyclic voltammograms of **CuL_1** and **CuL_2** (*vs.* SCE in DMF).

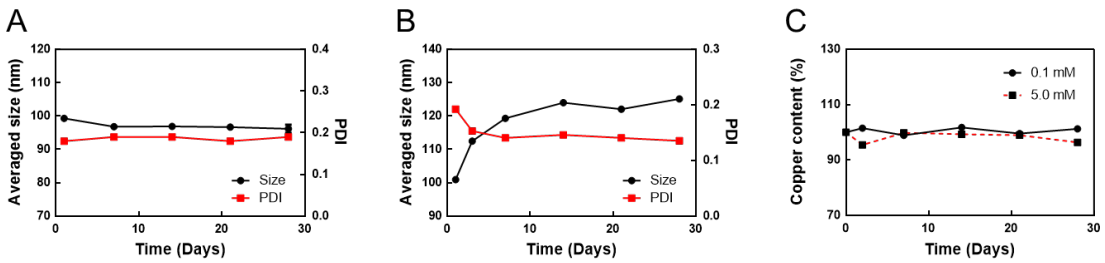


Figure S4. Stability of **NE CuL$_1$** monitored over 4 weeks. (A, B) The change of size distribution of **NE CuL$_1$** at (A) 0.1 mM and (B) 5.0 mM, assessed by dynamic light scattering. (C) The loss of copper content from **NE CuL$_1$** determined by inductively coupled plasma optical emission spectroscopy.

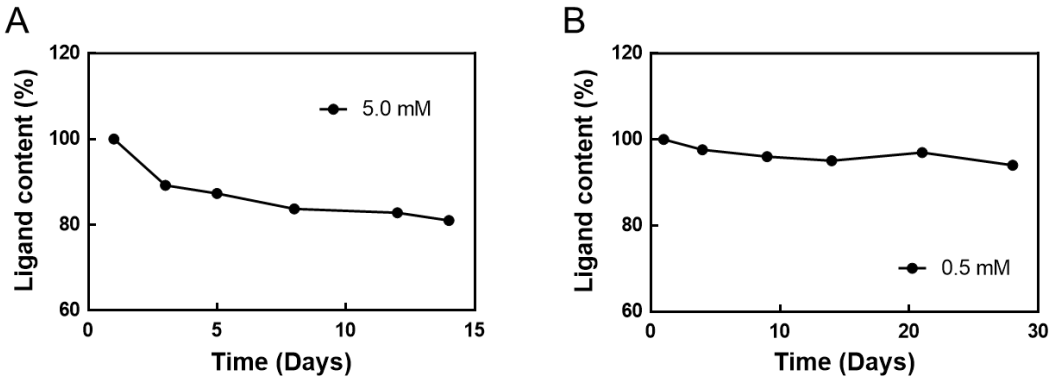


Figure S5. Stability of **NE H$_2$L$_1$**. Loss of **H$_2$L$_1$** within **NE H$_2$L$_1$** at (A) 5.0 mM and (B) 0.5 mM level determined by ^{19}F NMR spectroscopy.

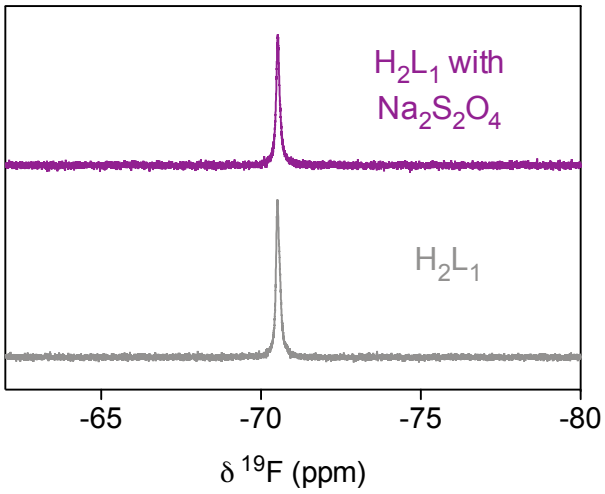


Figure S6. Control ^{19}F NMR of 1 mM **NE H$_2$L$_1$** with and without the addition of excess $Na_2S_2O_4$.

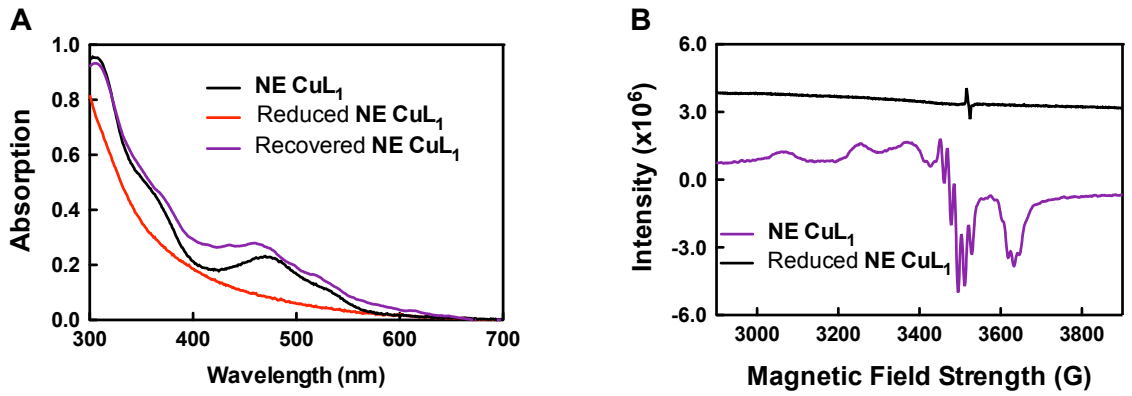


Figure S7. Reduction of **NE CuL$_1$** and reformation of **NE CuL$_1$** illustrated by (A) UV-vis absorption and (B) EPR spectroscopy.

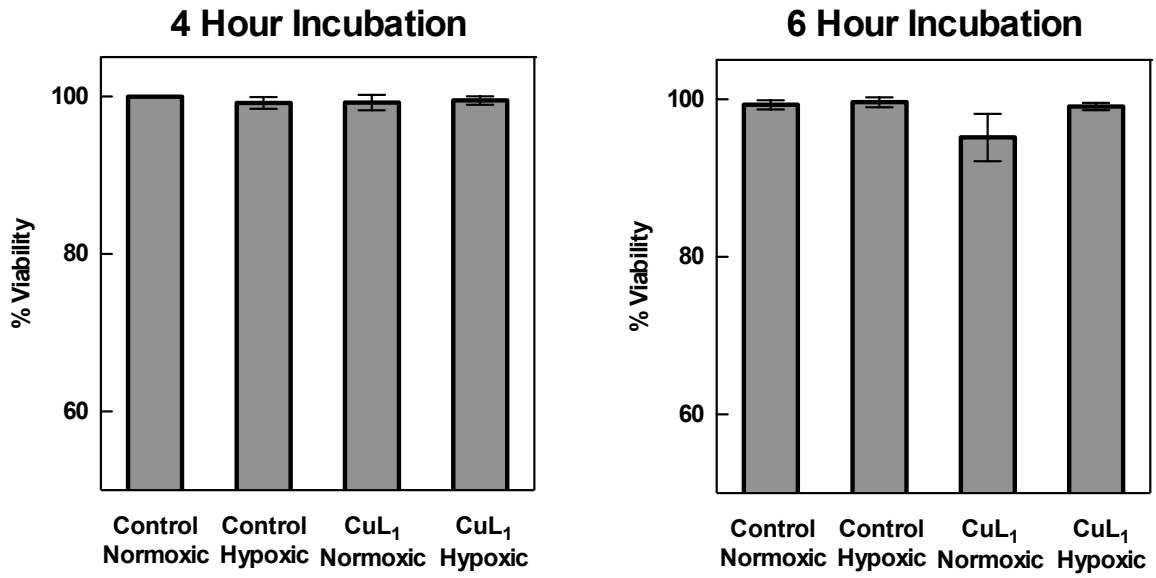


Figure S8. Viability studies after 4 (left) and 6 (right) hour incubation of MCF-7 cells without complex (control) and cells with **NE CuL$_1$** in both normoxic and hypoxic environments.

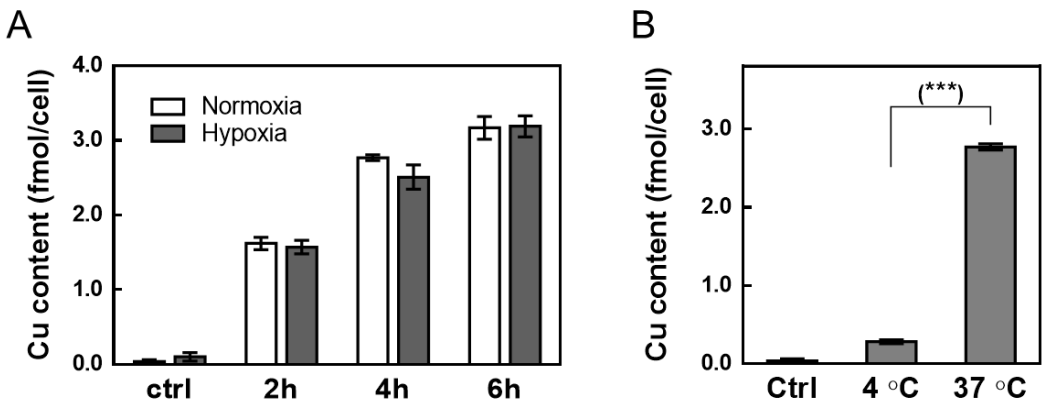


Figure S9. (A) Uptake levels of **NE CuL_1** in MCF-7 cells under both normoxic (20%, white bars) and hypoxic (0.1 %, grey bars) conditions at 2, 4, and 6 hours. (B) Uptake levels of **CuL_1** in MCF-7 cells for 4 hours at 4 °C and 37 °C. MCF-7 cells were incubated with **NE CuL_1** (100 μM Cu^{2+} level) at different conditions before the intracellular Cu content was analyzed by ICP-OES. The control group represents cells that were not incubated with a copper source.

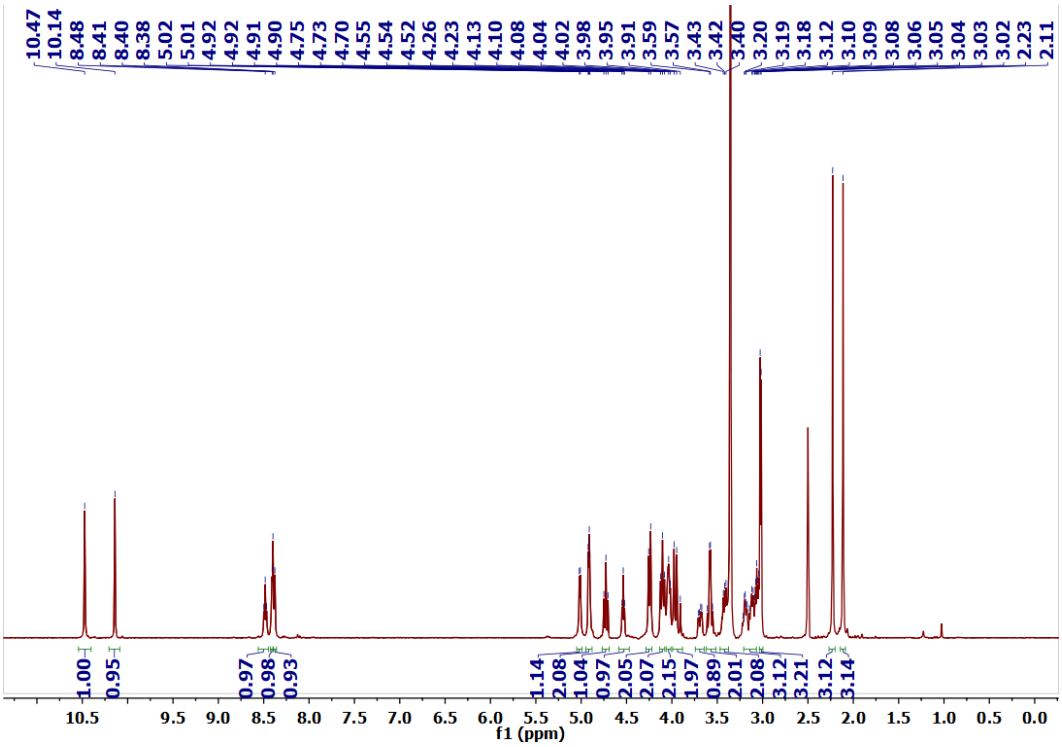


Figure S10. ^{1}H NMR of $\mathbf{H_2L_1}$ taken in d_6-DMSO at room temperature

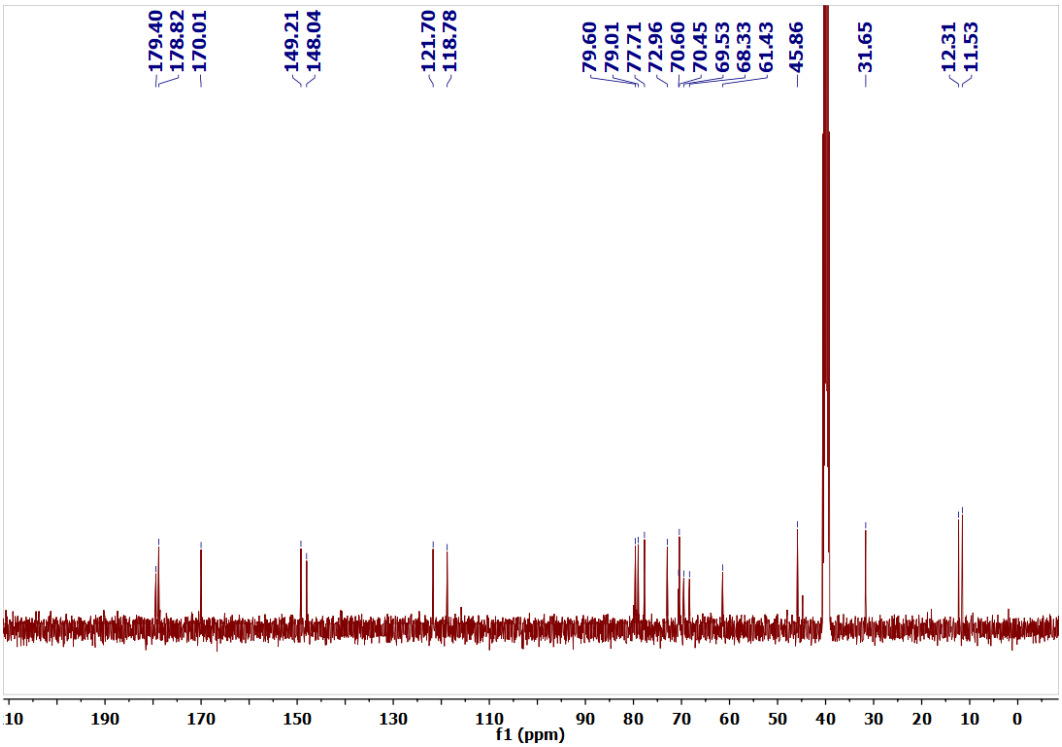


Figure S11. ^{13}C NMR of $\mathbf{H_2L_1}$ taken in d_6-DMSO at room temperature

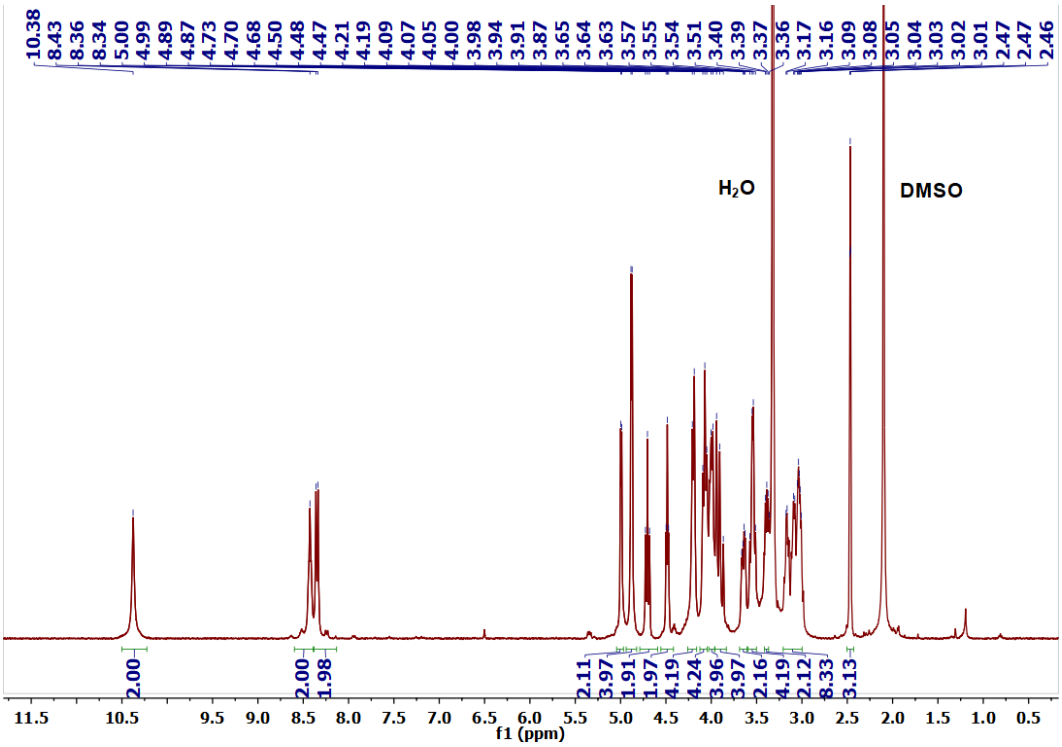


Figure S12. ^{1}H NMR of $\mathbf{H_2L_2}$ taken in d_6-DMSO at room temperature

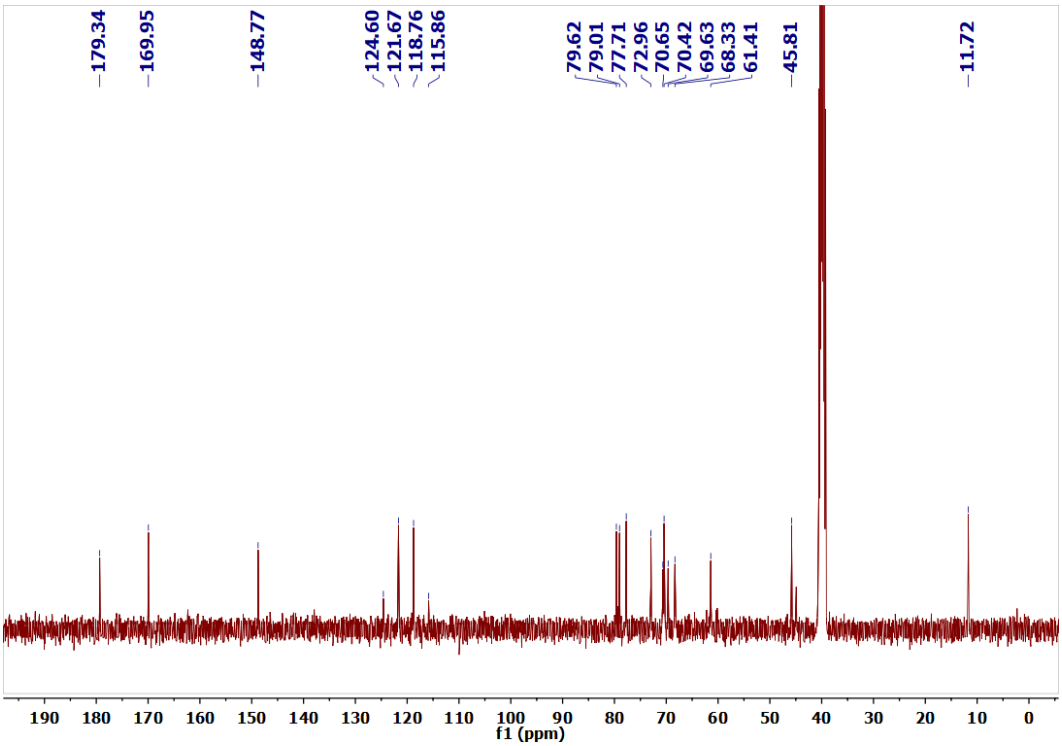
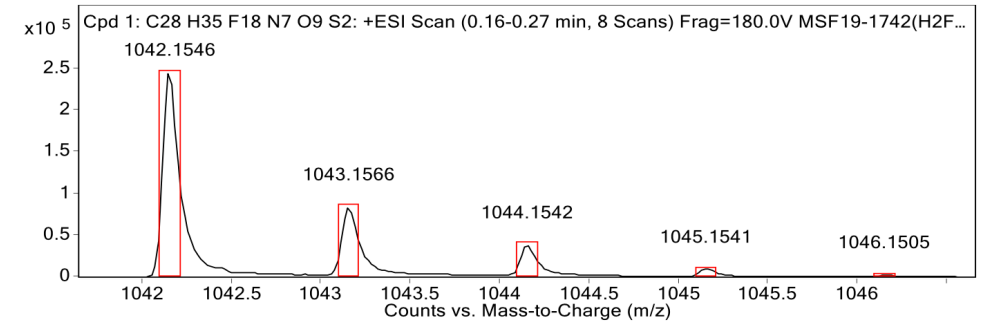


Figure S13. ^{13}C NMR of $\mathbf{H_2L_2}$ taken in d_6-DMSO at room temperature



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
1042.1546	1042.1542	1	246666	C28H35F18N7O9S2	(M+Na)+	-0.34
1043.1566	1043.1570	1	84161	C28H35F18N7O9S2	(M+Na)+	0.4
1044.1542	1044.1544	1	38398	C28H35F18N7O9S2	(M+Na)+	0.28
1045.1541	1045.1556	1	9082	C28H35F18N7O9S2	(M+Na)+	1.39
1046.1505	1046.1549	1	2492	C28H35F18N7O9S2	(M+Na)+	4.28
1047.0830	1047.1556	1	869	C28H35F18N7O9S2	(M+Na)+	69.33

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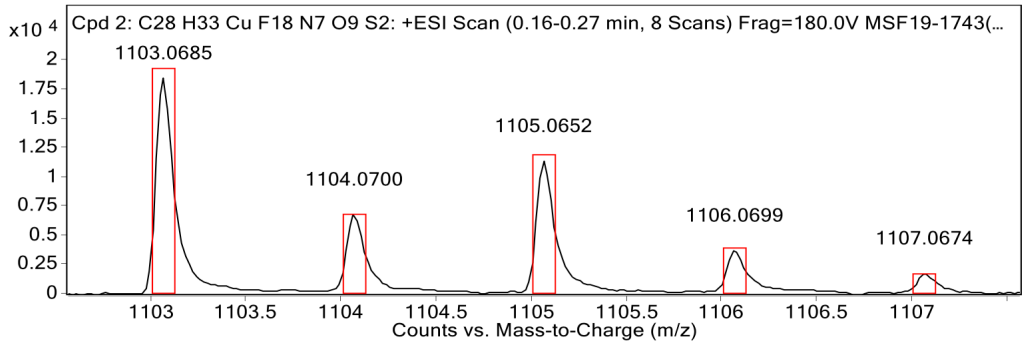
Figure S14. Flow injection analysis (FIA) ESI$^+$ HRMS of $\mathbf{H_2L_1}$.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
1103.0685	1103.0682	1	18555	C28H33CuF18N7O9S2	(M+Na)+	-0.24
1104.0700	1104.0710	1	6878	C28H33CuF18N7O9S2	(M+Na)+	0.9
1105.0652	1105.0669	1	11452	C28H33CuF18N7O9S2	(M+Na)+	1.55
1106.0699	1106.0692	1	3794	C28H33CuF18N7O9S2	(M+Na)+	-0.59
1107.0674	1107.0669	1	1843	C28H33CuF18N7O9S2	(M+Na)+	-0.47
1108.0712	1108.0679	1	626	C28H33CuF18N7O9S2	(M+Na)+	-3.02
1109.1039	1109.0673	1	706	C28H33CuF18N7O9S2	(M+Na)+	-33.03

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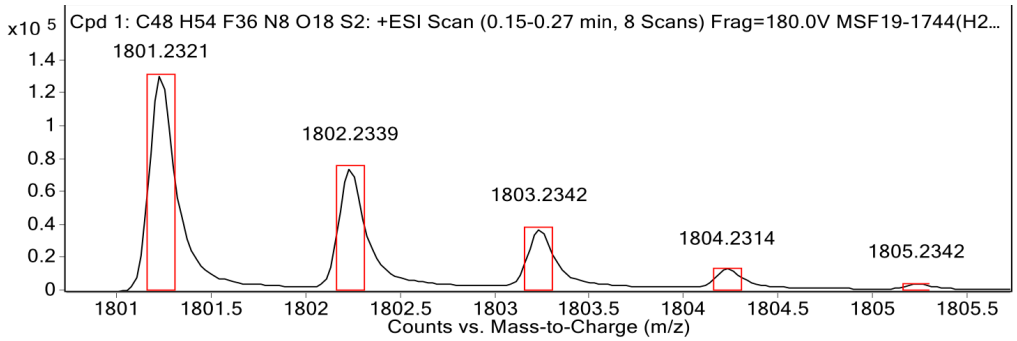
Figure S15. FIA ESI$^+$ HRMS of $\mathbf{CuL_1}$.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
912.1137			223651			
1801.2321	1801.2315	1	130790	C48H54F36N8O18S2	(M+Na)+	-0.32
1802.2339	1802.2345	1	74666	C48H54F36N8O18S2	(M+Na)+	0.33
1803.2342	1803.2341	1	37632	C48H54F36N8O18S2	(M+Na)+	-0.08
1804.2314	1804.2350	1	11191	C48H54F36N8O18S2	(M+Na)+	2
1805.2342	1805.2355	1	3216	C48H54F36N8O18S2	(M+Na)+	0.74
1806.2415	1806.2364	1	757	C48H54F36N8O18S2	(M+Na)+	-2.82
1807.2035	1807.2374	1	284	C48H54F36N8O18S2	(M+Na)+	18.76

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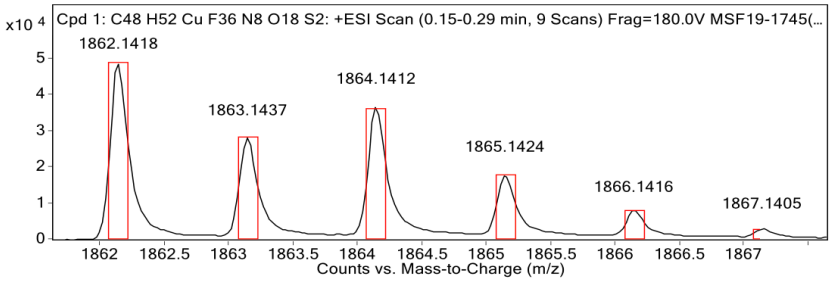
Figure S16. FIA ESI$^+$ HRMS of $\mathbf{H_2L_2}$.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
1862.1418	1862.1454	1	48711	C48H52CuF36N8O18S2	(M+Na)+	1.94
1863.1437	1863.1484	1	28055	C48H52CuF36N8O18S2	(M+Na)+	2.5
1864.1412	1864.1454	1	36745	C48H52CuF36N8O18S2	(M+Na)+	2.21
1865.1424	1865.1473	1	17956	C48H52CuF36N8O18S2	(M+Na)+	2.61
1866.1416	1866.1468	1	6868	C48H52CuF36N8O18S2	(M+Na)+	2.8
1867.1405	1867.1476	1	2259	C48H52CuF36N8O18S2	(M+Na)+	3.8
1868.1470	1868.1481	1	802	C48H52CuF36N8O18S2	(M+Na)+	0.57
1869.1498	1869.1489	1	229	C48H52CuF36N8O18S2	(M+Na)+	-0.51
1870.2279	1870.1498	1	172	C48H52CuF36N8O18S2	(M+Na)+	-41.73

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Figure S17. FIA ESI$^+$ HRMS of $\mathbf{CuL_2}$.

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   $R(F) = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$ for reflections with $F_o > 4(\sigma(F_o))$.
   $S = [\Sigma w(|F_o|^2 - |F_c|^2)^2/(n - p)]^{1/2}$, where n is the number of reflections and p is the number of refined parameters.
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