Electronic Supplementary Information

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1 General Experimental

All solvents and reagents were purchased from standard commercial suppliers and were used as received. $^{64}CuCl_2$ solution (0.05 M HCl, or in water pH 6.0, no carrier added) was produced at The Austin Hospital through the $^{64}Ni(p,n)^{64}Cu$ nuclear reaction. ^{1}H, ^{13}C, COSY, HSQC, HMBC were all recorded using either a 500 MHz NEO system with cryoprobe (Bruker, USA) or FT NMR 500 spectrometer (Varian, California, USA).. All ^{1}H NMR spectra were acquired at 500 MHz and ^{13}C spectra were acquired at 126 MHz. The reported peaks were all referenced to solvent peaks in the order of parts per million at 25°C. ESI-QTOF MS was collected on an Exactive Plus Orbitrap Infusion mass spectrometer (Exactive Series, 2.8 Build 268801,

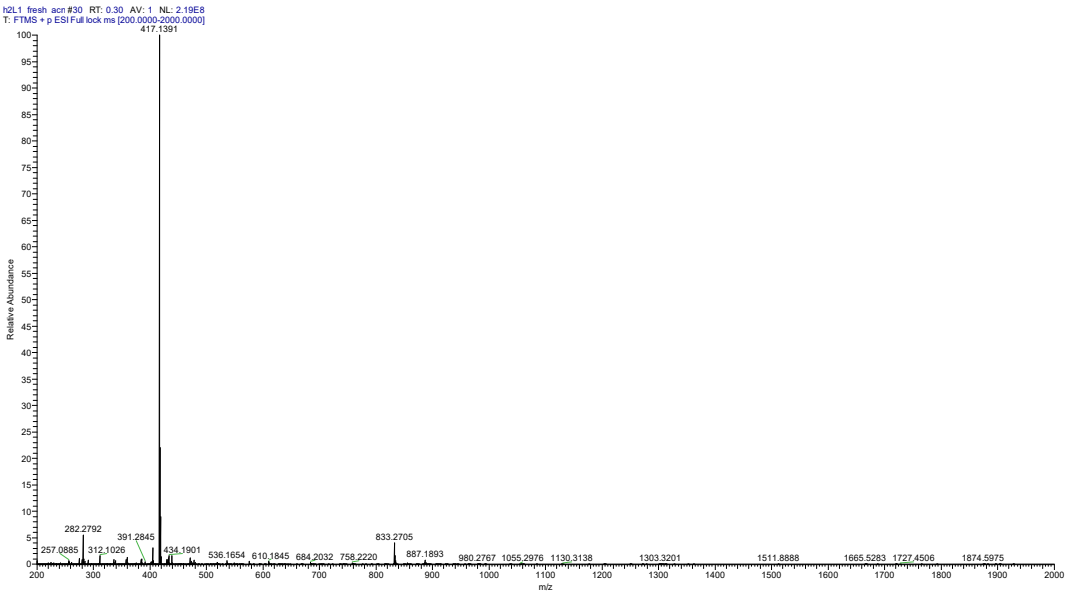
ThermoFisher Scientific). Analysis was performed using Xcalibur 4.0.27.10 (ThermoFisher Scientific). Protein samples were analysed on Agilent 6220 ESI-TOF LC/MS Mass Spectrometer coupled to an Agilent 1200 LC system (Agilent, Palo Alto, CA). All data were acquired, and reference mass corrected *via* a dual-spray electrospray ionisation (ESI) source. Acquisition was performed using the Agilent Mass Hunter Acquisition software version B.02.01 (B2116.30). Ionisation mode: Electrospray Ionisation; Drying gas flow: 7 L/min; Nebuliser: 35 psi; Drying gas temperature: 325°C; Capillary Voltage (Vcap): 4000 V; Fragmentor: 300 V; Skimmer: 65 V; OCT RFV: 250 V; Scan range acquired: 300–3200 m/z Internal Reference ions: Positive Ion Mode = m/z = 121.050873 & 922.009798. Protein desalting and chromatographic separation was performed using an Agilent Poroshell C18 2.1 x 75 mm, 5µm column using 5% (v/v) acetonitrile ported to waste (0-5min). Upon desalting of the sample, the flow was ported back into the ESI source for subsequent gradient elution with (5% (v/v) to 100% (v/v)) acetonitrile / 0.1% formic acid over 8 min at 0.25 mL/min. Analysis was performed using Mass Hunter version B.06.00 with BioConfirm software using the maximum entropy protein deconvolution algorithm; mass step 1 Da; Baseline factor 3.00; peak width set to uncertainty. Microwave assisted synthesis was performed using a Biotage Initiator+ (Stockholm, Sweeden) microwave reactor. Non-radioactive analytical HPLC were performed on Agilent 1200 series HPLC system fitted with a Phenomonex Luna C18(2) column (4.6 mm x 150 mm, 5 µm) and Phenomenex SecurityGuard^{TM} C18 guard cartridge (4 mm x 30 mm) with a 1 mL/min flow rate; system A: gradient elution of Buffer A = 0.1% TFA in H_2O and Buffer B = 0.1% TFA in acetonitrile (5 to 100% B in A over 20 min) and UV detection at λ 254, 280, and 350 nm. Radio-HPLC traces were acquired using a Shimadzu HPLC system with a Phenomonex Luna C18(2) column (4.6 mm x 150 mm, 5 µm) and Phenomenex SecurityGuard^{TM} C18 guard cartridge (4 mm x 30 mm) with a 1 mL·min^{-1} flow rate gradient elution with H_2O/MeCN/TFA of 95:5:0.1 to 0:100:0.1 over 15 minutes with 2

minutes of isocratic flow at 0:100:0.1 then a gradient to 95:5:0.1 over 1 minute followed by 2 minutes of isocratic flow. The run was conducted at room temperature (~24 °C) with in-line UV spectroscopic detection at 254 nm and 280 nm and γ-detection with a Lab Logic Flow-RAM™ system controlled by the Laura 4.0 software. The radiation in each vial was counted over 60-240 seconds using a Multi-Wiper™ HE γ-radiation well-counter (2008 Laboratory Technologies, Inc.). Radio-iTLC were analysed using a Lab Logic Scan-RAM™ system controlled by the Laura 4.0 software. Electrochemistry was conducted using an AUTOLAB PGSTAT100 with GPES V4.9 software. A glassy carbon working electrode, a Pt/Ti wire counter electrode and a leakless miniature Ag/AgCl reference electrode were used. Ferrocene was used as an internal reference ($E°'(Fc/Fc^{+}) = 0$). X-ray crystallography of Cu(dtseTz) and $Cu[(H_2atsmTz)](ClO_4)_2$ were mounted in low temperature oil then cooled to 130 K using an Oxford low temperature device. Intensity data were collected at 130 K with an Oxford XCalibur X-ray diffractometer (Cu(dtseTz)) with Sapphire CCD detector using Cu Kα radiation (graphite crystal monochromators $\lambda = 1.5418$ Å) or Rigaku Oxford X-ray diffractometer ($Cu[(H_2atsmTz)](ClO_4)_2$). Data were reduced and corrected for absorption. The structures were solved by direct methods and difference Fourier synthesis using the SHELX - T program and refined within SHELX-L program as implemented within the Olex2 program. Thermal ellipsoid plots were generated using the programs ORTEP-3 integrated with the SHELX suite of programs according to the references; Sheldrick GM. Crystal structure refinement with SHELXL. Acta Crystallogr C 2015; 71: 3. doi:10.1107/S2053229614024218 and Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K. and Puschmann, H. (2009), *OLEX2*: a complete structure solution, refinement and analysis program. J. Appl. Cryst., 42: 339-341. https://doi.org/10.1107/S0021889808042726

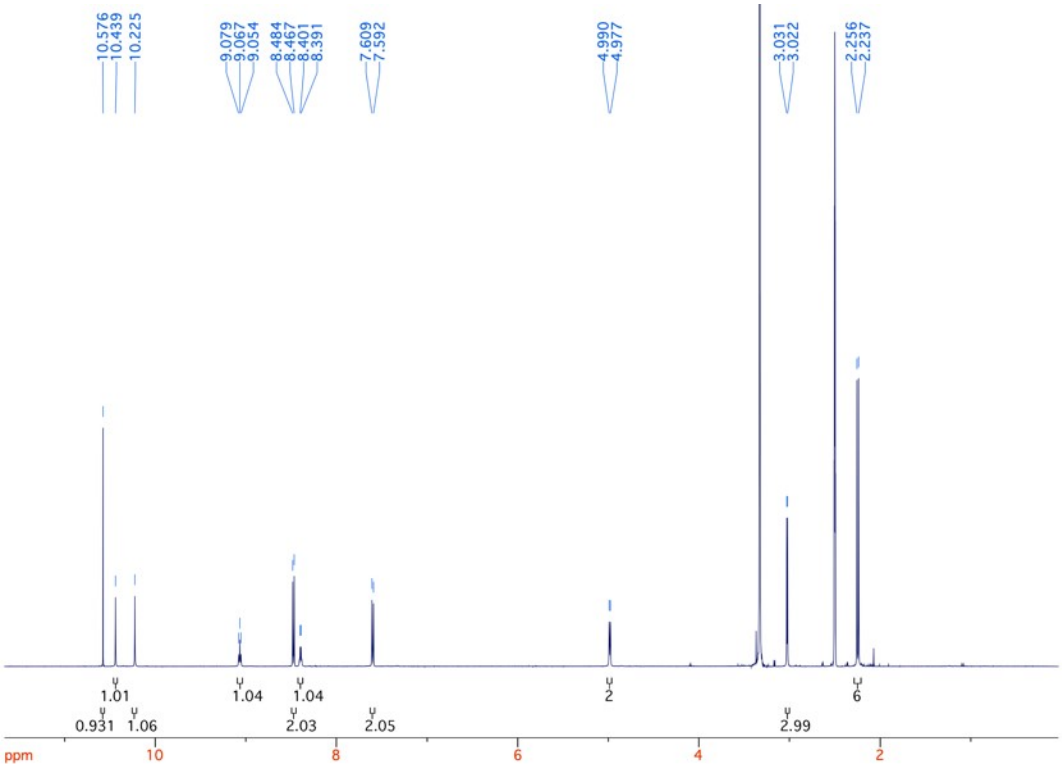
2 Procedures & Characterisation

2.1 H_2atsm-Tz

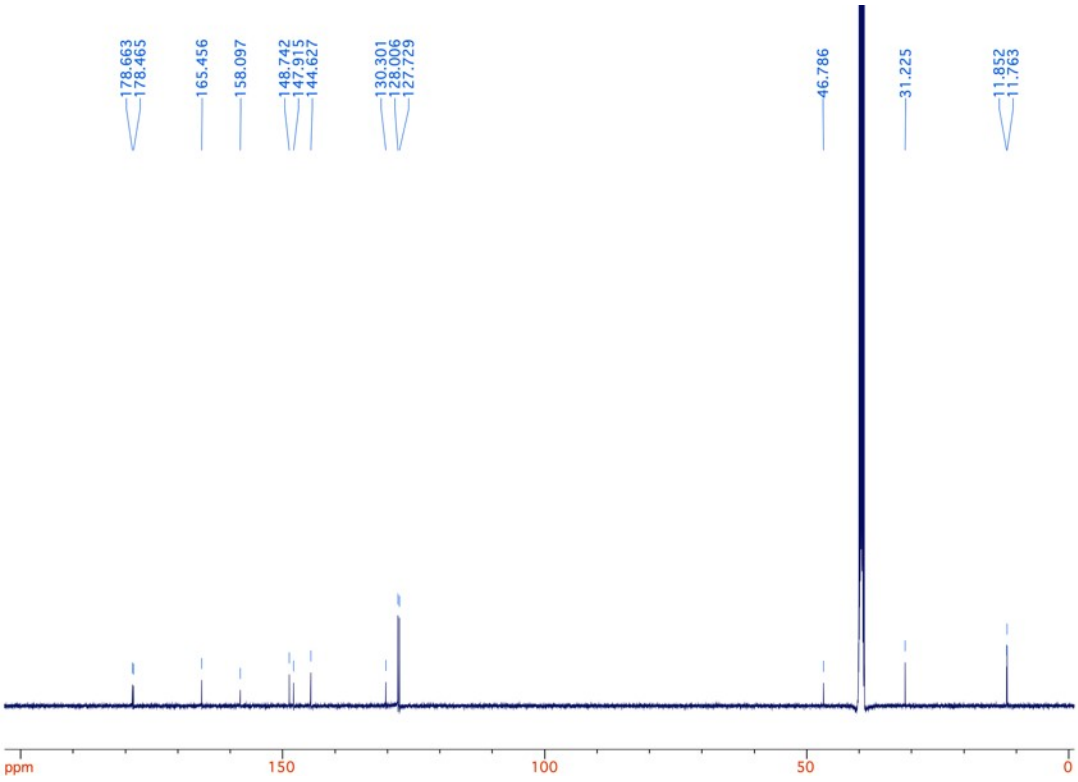
Diacetyl-4,4-dimethyl-4'-methyl*bis*(thiosemicarbazone) (22.0 mg, 0.08 mmol), (4-(1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride (22.1 mg, 0.096 mmol) and triethyl amine (13 μL, 0.096 mmol) were suspended in acetonitrile (1.5 mL) and irradiated with μW radiation for 5 minutes at 90°C with high absorbency. Upon cooling a bright pink precipitate formed and the reaction suspension was centrifuged, washed with acetonitrile (2 x 1 mL), H_2O (2 x 1 mL), cold ethanol (2 mL) and diethyl ether (2mL) then dried *in vacuo* to yield a pink powder **(**21.2 mg, 0.051 mmol, 64%). R_t: 11.65 min (system A). ESI MS[M+H^+]: 417.1391 calculated for $(C_{16}H_{20}N_{10}S_2)^+$: 417.1392 ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.58 (s, 1H), 10.43 (s, 1H), 10.22 (s, 1H), 9.06 (s, 1H), 8.48 (d, J = 8.4 Hz, 2H), 8.39 (d, J = 4.6 Hz, 1H), 7.60 (d, J = 8.5 Hz, 2H), 4.98 (d, J = 6.3 Hz, 2H), 3.03 (d, J = 4.6 Hz, 3H), 2.25 (d, J = 9.5 Hz, 6H), 2.08 (s, 1H).^{13}C NMR (126 MHz, DMSO) δ 178.5, 178.3, 165.3, 157.9, 148.6, 147.7, 144.5, 130.1, 127.8, 127.6, 46.6, 31.0, 11.7, 11.6.



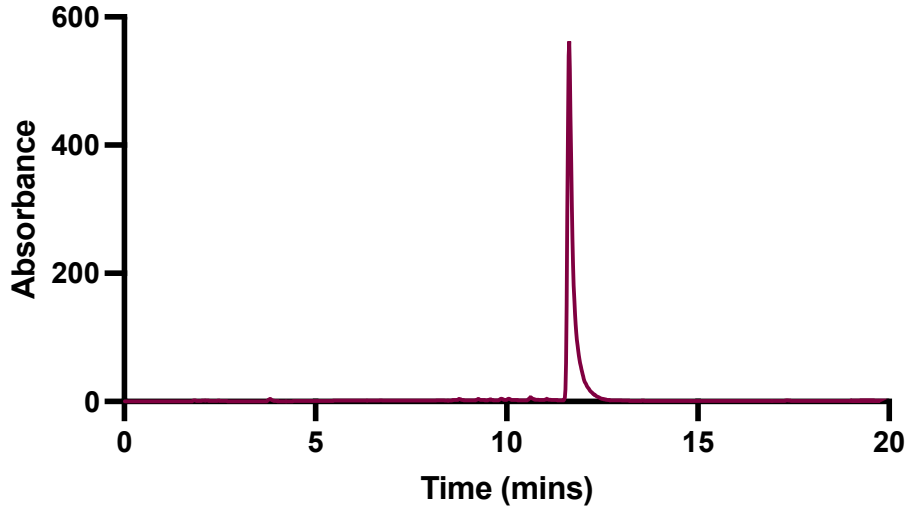
S 1 Electrospray ionisation high resolution mass spectrum of H_2atsm-Tz.



S 2 ^{1}H NMR spectrum of H_2atsm-Tz. 500 MHz, d_6-DMSO.



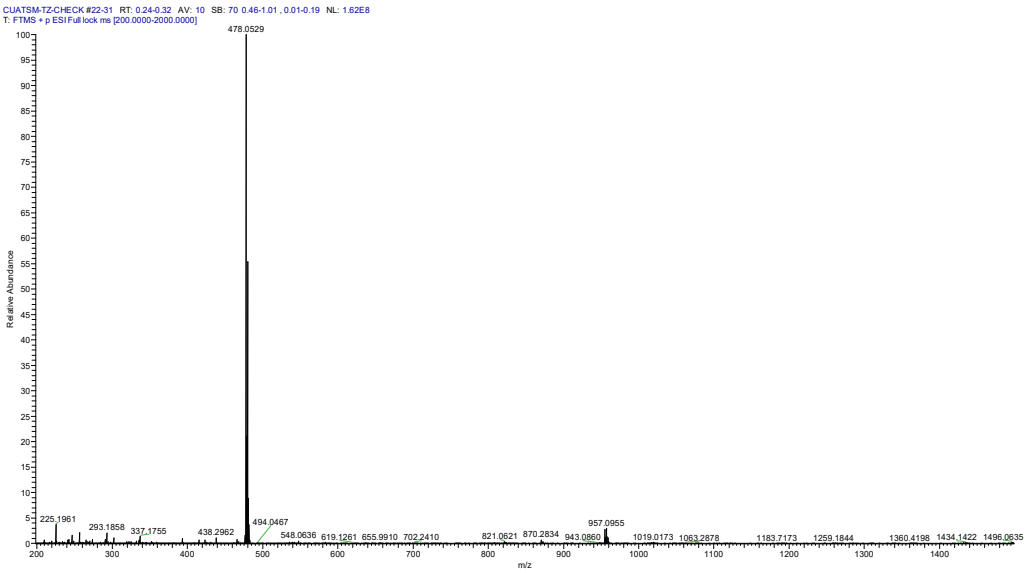
S 3 ^{13}C NMR spectrum of H_2atsm-Tz. 126 MHz, d_6-DMSO.



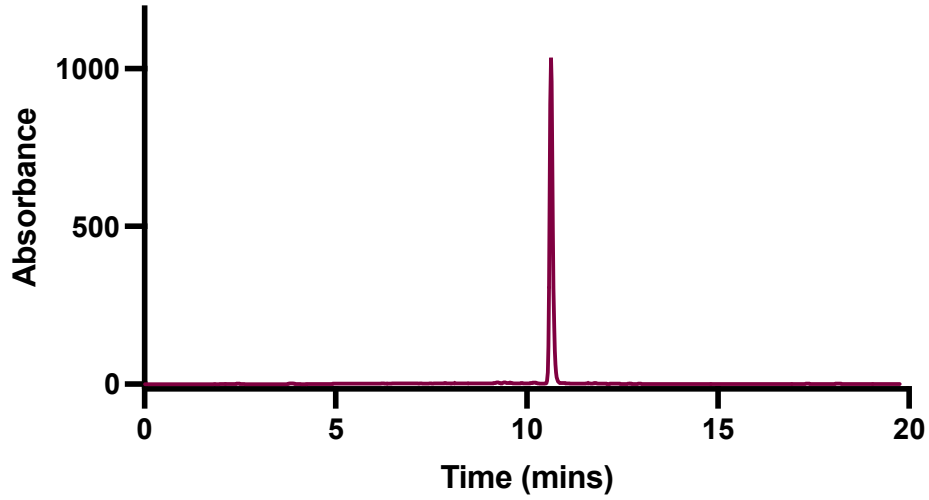
S 4 HPLC chromatogram of H_2atsm-Tz. Retention time = 11.65min, in MilliQ /acetonitrile mobile phase containing 0.1% TFA.

2.2 Cu(atsm-Tz)

H_2(atsm-Tz) (4.78 mg, 0.011 mmol) and Cu(II)OAc.H_2O (2.59 mg, 0.013 mmol) were dissolved in DMF and allowed to stir at room temperature for 1 hour, before the addition of H_2O. The precipitate was collected *via* centrifugation, and the solid was washed with H_2O, ethanol, and diethyl ether, then taken to dryness *in vacuo* yield a dark red solid. (4.62 mg, 0.009 mmol, 88%). R_t: 10.63 mins (system A). ESI MS[M+H^+]: 478.0529 calculated for $(C_{16}H_{18}CuN_{10}S_2)^+$: 478.0532.



S 5 Electrospray ionisation high resolution mass spectrum of Cu(atsm-Tz).

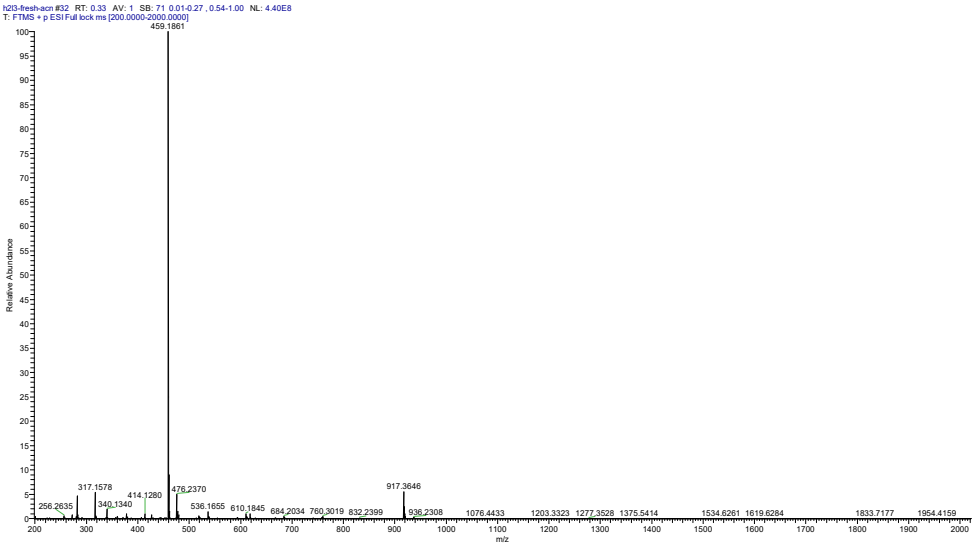


S 6 HPLC chromatogram of Cu(atsm-Tz). Retention time = 10.63 min, in MilliQ /acetonitrile mobile phase containing 0.1% TFA.

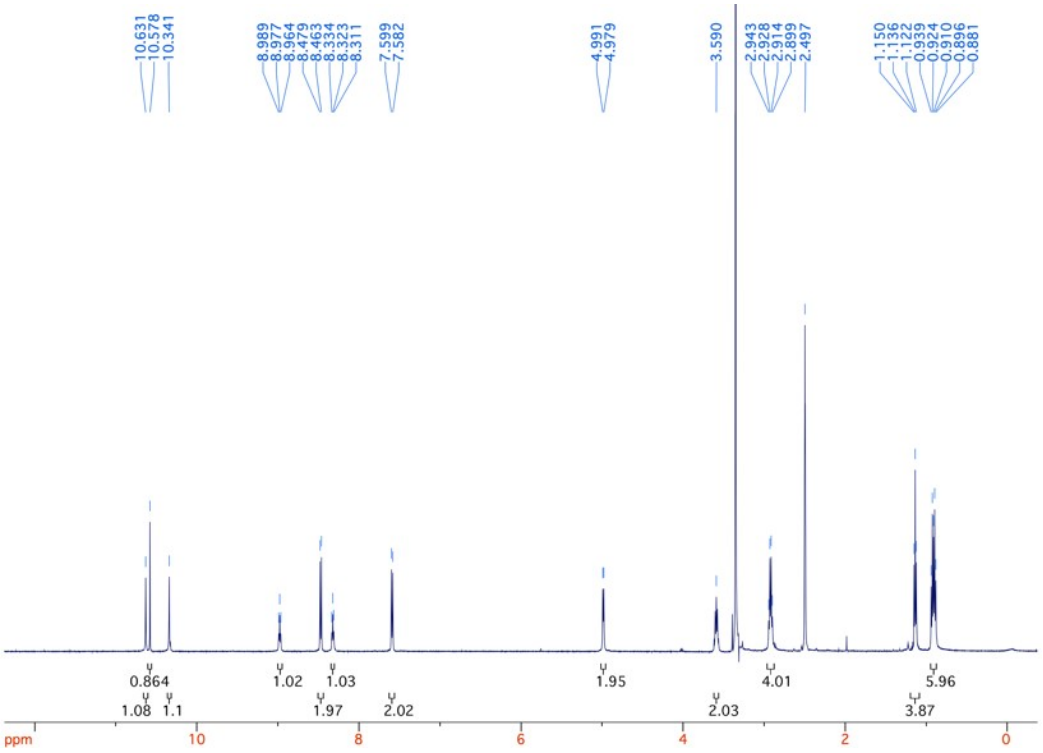
2.3 H_2dtse-Tz

Diethyl-4,4-dimethyl-4'-ethyl*bis*(thiosemicarbazone) (6.44 mg, 0.020 mmol), (4-(1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride (5.01 mg, 0.022 mmol) and DIPEA (3 µL, 0.023 mmol) were suspended in MeCN (1 mL) and irradiated with µW radiation for 5 minutes at 90°C with high absorbency. Upon cooling, the reaction mixture was taken to dryness under reduced pressure. The precipitate was purified *via* column chromatography (SiO_2, 10 - 40 % EtOAc in CH_2Cl_2) and combined fractions were taken to dryness *in vacuo* to yield a bright

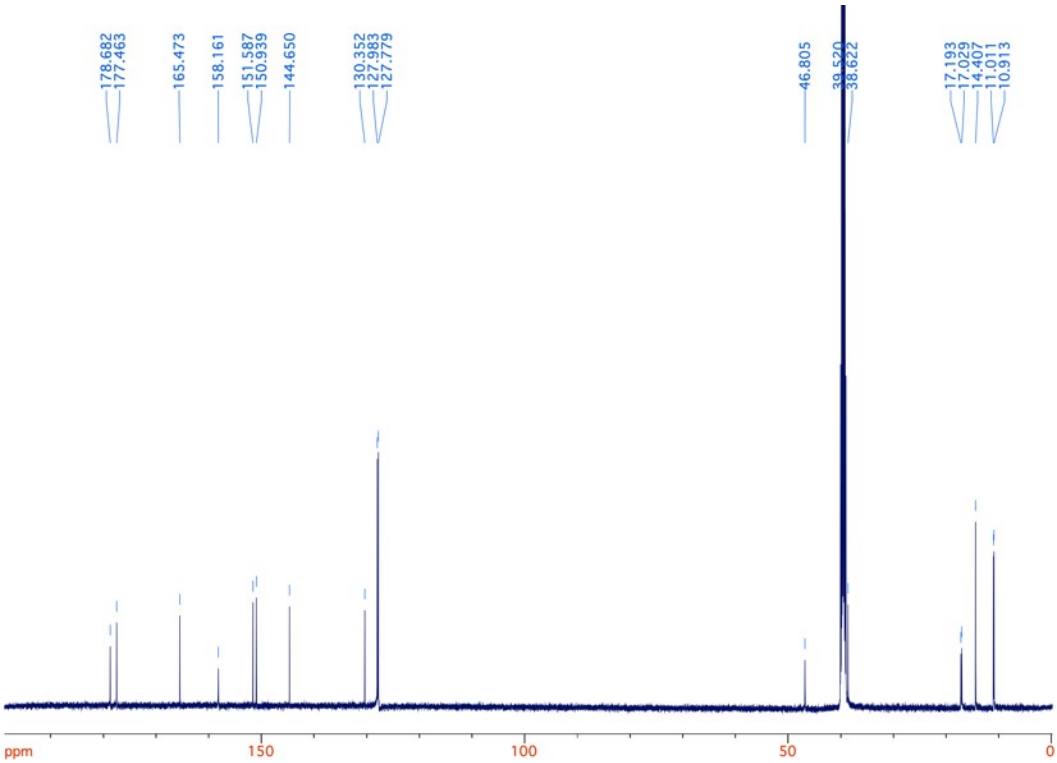
pink/purple solid. (6.08 mg, 0.013 mmol, 67 %). R_t: 13.15 min (system A). ESI MS[M+H$^+$]: 459.1861 calculated for $(C_{19}H_{27}N_{10}S_2)^+$: 459.18. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.64 (s, 1H), 10.58 (s, 1H), 10.35 (s, 1H), 8.98 (t, *J* = 6.2 Hz, 1H), 8.48 (d, *J* = 8.3 Hz, 2H), 8.33 (t, *J* = 5.8 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 2H), 4.99 (d, *J* = 6.1 Hz, 2H), 3.63 – 3.57 (m, 2H), 2.93 (q, *J* = 7.3 Hz, 4H), 1.14 (t, *J* = 7.1 Hz, 3H), 0.92 (dt, *J* = 14.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 179.1, 177.9, 165.9, 158.6, 152.0, 151.3, 145.1, 130.8, 128.4, 128.2, 47.2, 39.0, 17.6, 17.4, 14.8, 11.4, 11.3.



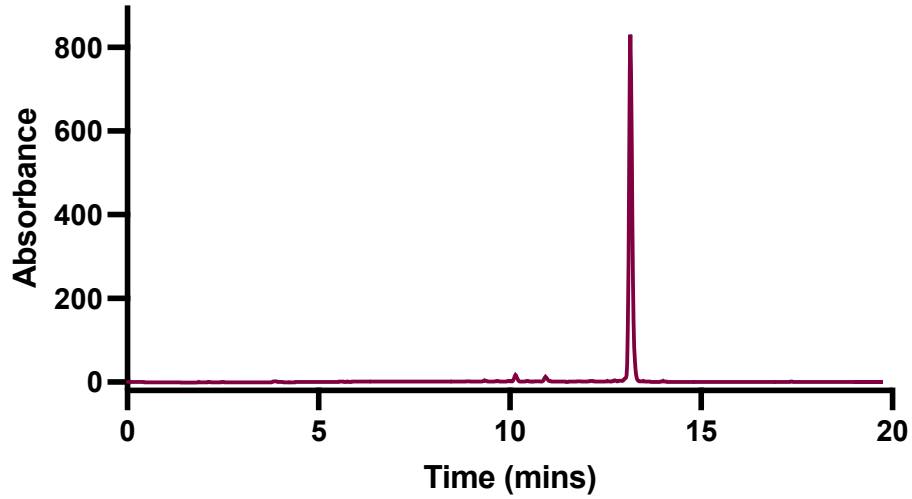
S 7 Electrospray ionisation high resolution mass spectrum of H_2dtse-Tz



S 8 ^{1}H NMR spectrum of H_2atsm-Tz. 500 MHz, d_6-DMSO



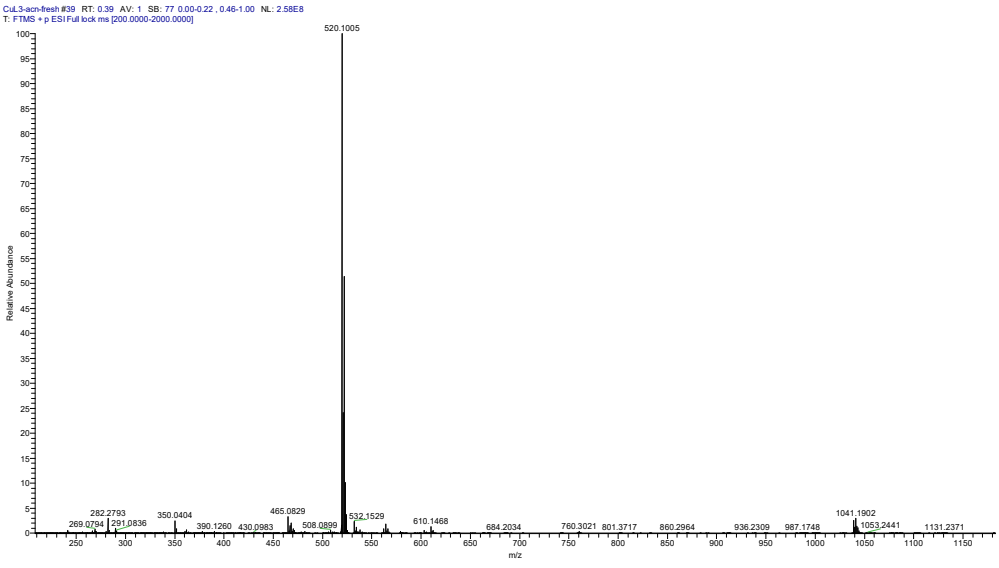
S 9 ^{13}C NMR spectrum of H_2atsm-Tz. 126 MHz, d_6-DMSO



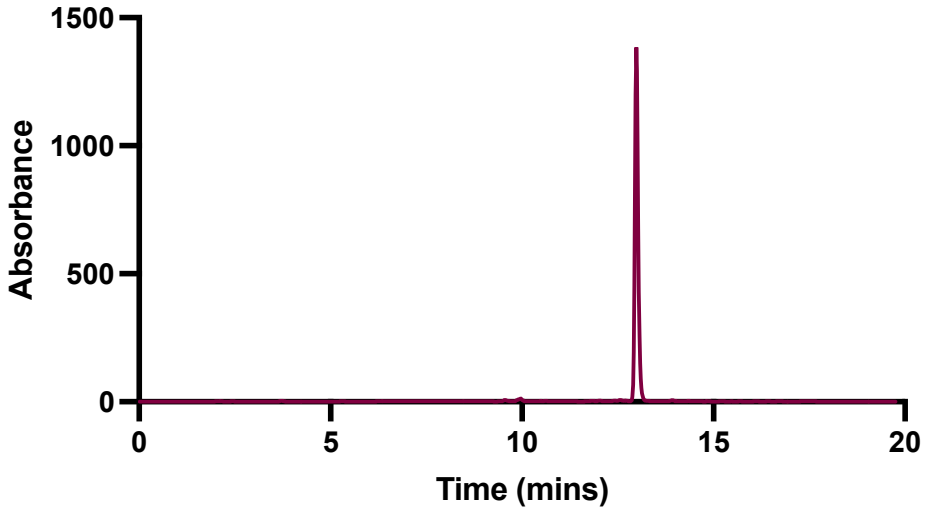
S 10 HPLC chromatogram of H_2dtse-Tz. Retention time = 13.15 min, in MilliQ /acetonitrile mobile phase containing 0.1% TFA

2.4 Cu(dtseTz)

H_2dtseTz (15.67 mg, 0.034 mmol) and Cu(II)OAc.H_2O (8.37 mg, 0.042 mmol) were suspended in MeOH and allowed to stir at room temp overnight. The suspension was centrifuged and washed with H_2O and ethanol then taken to dryness *in vacuo* to yield a dark red/purple solid. The residue was purified *via* column chromatography to yield a red solid. (15.89 mg, 0.030 mmol, 88%). R_t: 12.98 min (system A). ESI MS[M+H^+]: 520.1005 calculated for $(C_{19}H_{24}CuN_{10}S_2)^+$: 520.1001



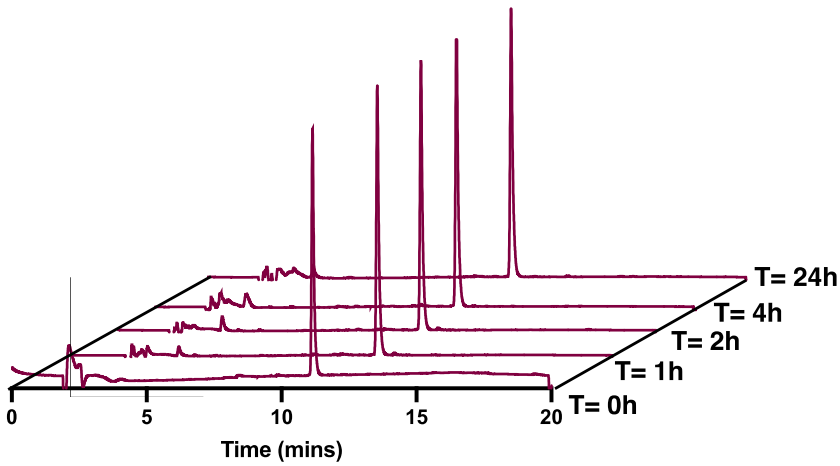
S 11 Electrospray ionisation high resolution mass spectrum of Cu(dtse-Tz)



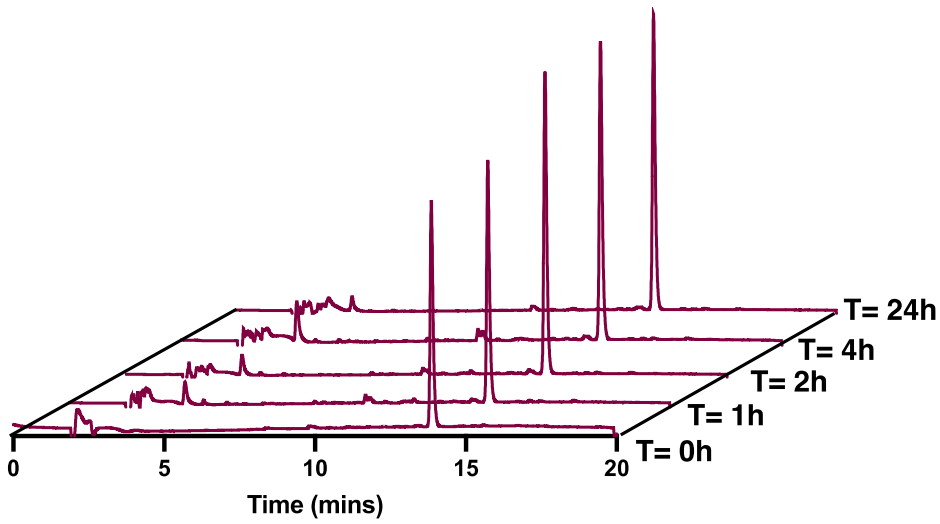
S 12 HPLC chromatogram of Cu(dtse-Tz). Retention time = 12.98 min, in MilliQ /acetonitrile mobile phase containing 0.1% TFA

2.5 Stability study with Glutathione (GSH)

A solution of Cu(atsm-Tz) and Cu(dtse-Tz) (10 µL, final concentration 0.2 mM) was added to glutathione (GSH, 40 µL, 16 mM) and shaken at 37 °C (20% DMSO total volume). Aliquots were taken at 1, 2, 4 and 24 hours and diluted then analysed via RP-HPLC and compared to the unchallenged complexes. Both tetrazine complexes are stable >90% with respect to GSH.



S 13 HPLC chromatogram of Cu(atsm-Tz) and GSH in MilliQ /acetonitrile mobile phase containing 0.1% TFA



S 14 HPLC chromatogram of Cu(dtse-Tz) and GSH in MilliQ /acetonitrile mobile phase containing 0.1% TFA

2.6 Crystallographic Data

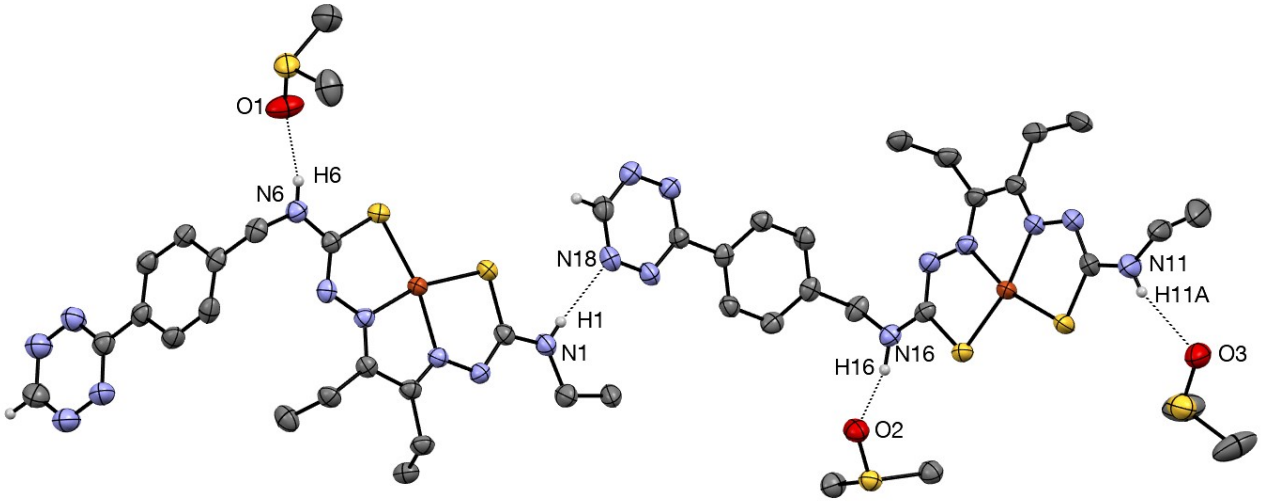
Cu(dtse-Tz) CCDC 2205695
[Cu(H_2atsm-Tz)(ClO_4)$_2$ CCDC 2205696

Table 1 Crystallographic Table for Cu(H_2atsm-Tz)(ClO_4)$_2$ and Cu(dtse-Tz)

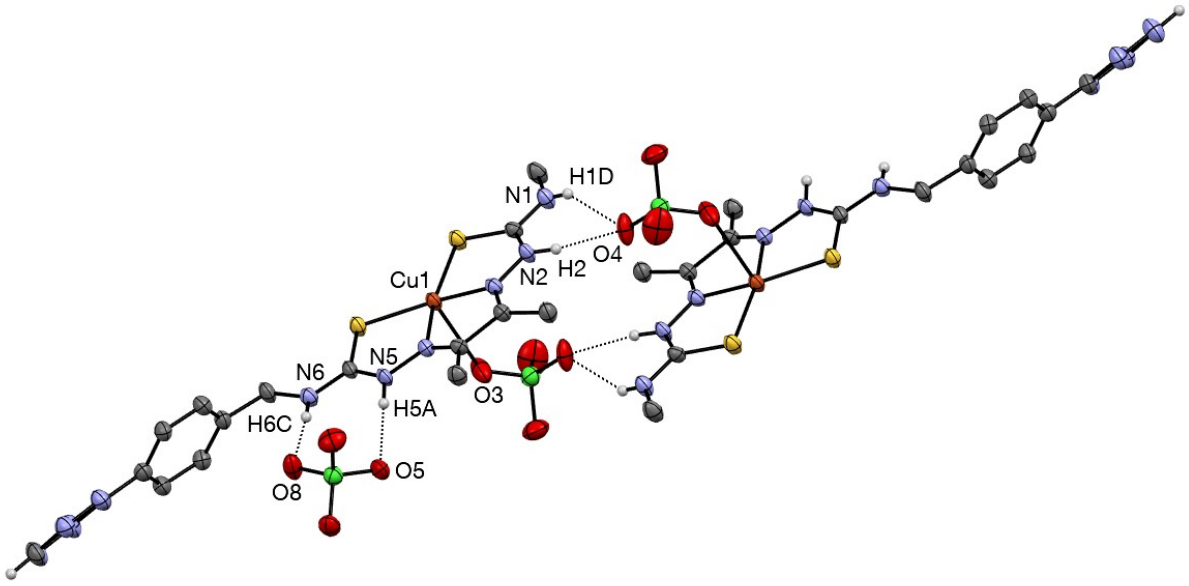
	[Cu(H_2atsm-Tz)(ClO_4)$_2$	**Cu(dtse-Tz)**
Empirical Formula	$C_{16}H_{20}Cl_2CuN_{10}O_8S_2$	$C_{19}H_{24}CuN_{10}S_2$
Formula Weight	678.98	519.0923
Crystal System	Triclinic	Monoclinic
Space Group	P $\bar{1}$	P $2_{1/n}$
***a*/Å**	8.0247(2)	17.7445(7)
***b*/Å**	8.5035(3)	8.2723(3)
***c*/Å**	18.5600(6)	37.4544 (13)
α/(°)	78.558(3)	90
β/(°)	87.910(3)	94.522(3)
γ/(°)	89.697(3)	90
***V*/Å^3**	1240.50(7)	5785.5(4)
Z	2	10
Temperature/K	100.0(2)	130.01(10)
λ/Å	1.54184	1.54184
Refinement method	Full-matrix least-squares on F2	
R-Factor/%	4.4	5.93

The structure of (Cudtse-Tz)$_2$.(DMSO)$_3$ is characterised by N-H…N hydrogen bonding between the two independent molecules of the ligand in addition to N-H…O hydrogen bonds to the three DMSO solvate molecules. The dihedral angles between the tetrazene rings and the phenyl rings are 2.7(3)° and 12.8(2)° for the two molecules respectively. The structure of CuH_2atsm-TZ.(ClO_4)$_2$ is characterised by two sets of N-H…O hydrogen bonds between the

two thiourea moieties and the two ClO_4^- anions. One set involves the 'free' ClO_4^- anion while the second set involves the coordinated ClO_4^- anion resulting in a centrosymmetric dimer. The dihedral angle between the tetrazene ring and the phenyl ring is 26.6(2)°.

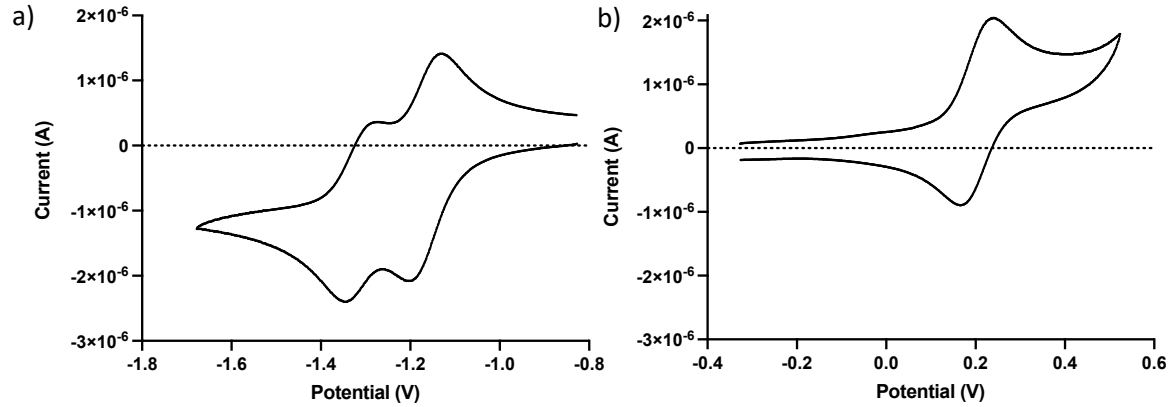


S 15 Thermal ellipsoid plot (50% ellipsoids) of the asymmetric unit of CuDTSE-Tz showing the hydrogen bonding interaction between the two complexes and with the three molecules of the DMSO solvate: N1-H…N18 (N1…N18 3.000(5) Å, N1-H…N18 173(5)°); N6-H…O1 (N6…O1 2.792(5) Å, N6-H…O1 164(5)°); N16-H…O2 (N16…O2 2.824(5) Å, N16-H…O2 157(5)°); N11-H…O3 (N11…O3 2.986(5) Å, N11-H…O3 153(6)°).

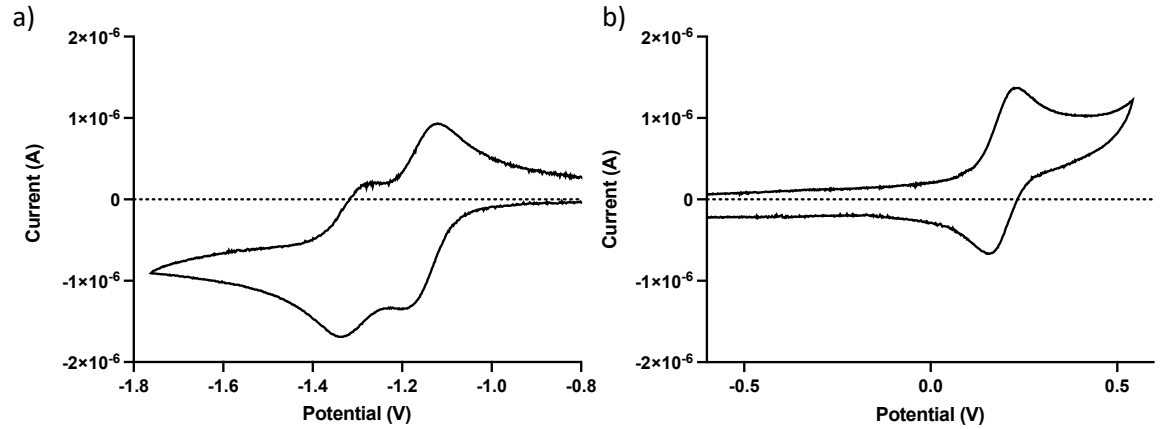


S 16 Thermal ellipsoid plot (50% ellipsoids) showing centrosymmetric CuH2ATSMTz dimers bridged by N-H…O hydrogen bonds: N1-H…O4 (N1…O4 2.955(5) Å, N1-H…O4 143(5)°); N2-H…O4 (N2…O4 2.731(5) Å, N2-H…O4 157(4)°); N5-H…O5 (N5...O5 2.863(4) Å, N5-H...O5 172(4)°); N6-H...O8: (N6…O8 2.878(4) Å, N6-H...O8 174(4)°); O3-Cu1 2.504(3) Å

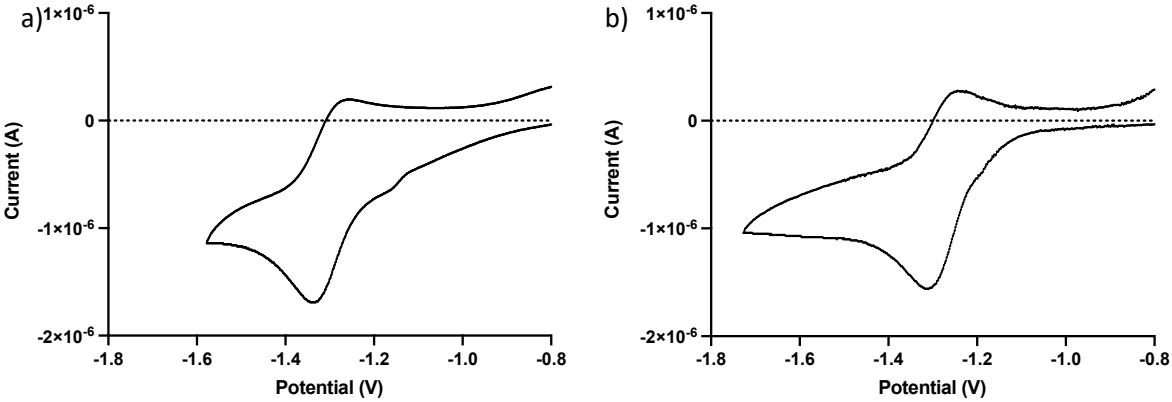
2.7 Cyclic Voltammetry



S 17 Cyclic voltammetry of Cu(atsm-Tz) (1mM) in DMF (versus Ag/Ag^+), referenced to Fc/Fc+ couple E°' = 0V) with $(Bu)_4NPF_6$ supporting electrolyte 0.1M.



S 18 Cyclic voltammetry of Cu(dtse-Tz) (1mM) in DMF (versus Ag/Ag^+), referenced to Fc/Fc+ couple E°' = 0V) with $(Bu)_4NPF_6$ supporting electrolyte 0.1M.

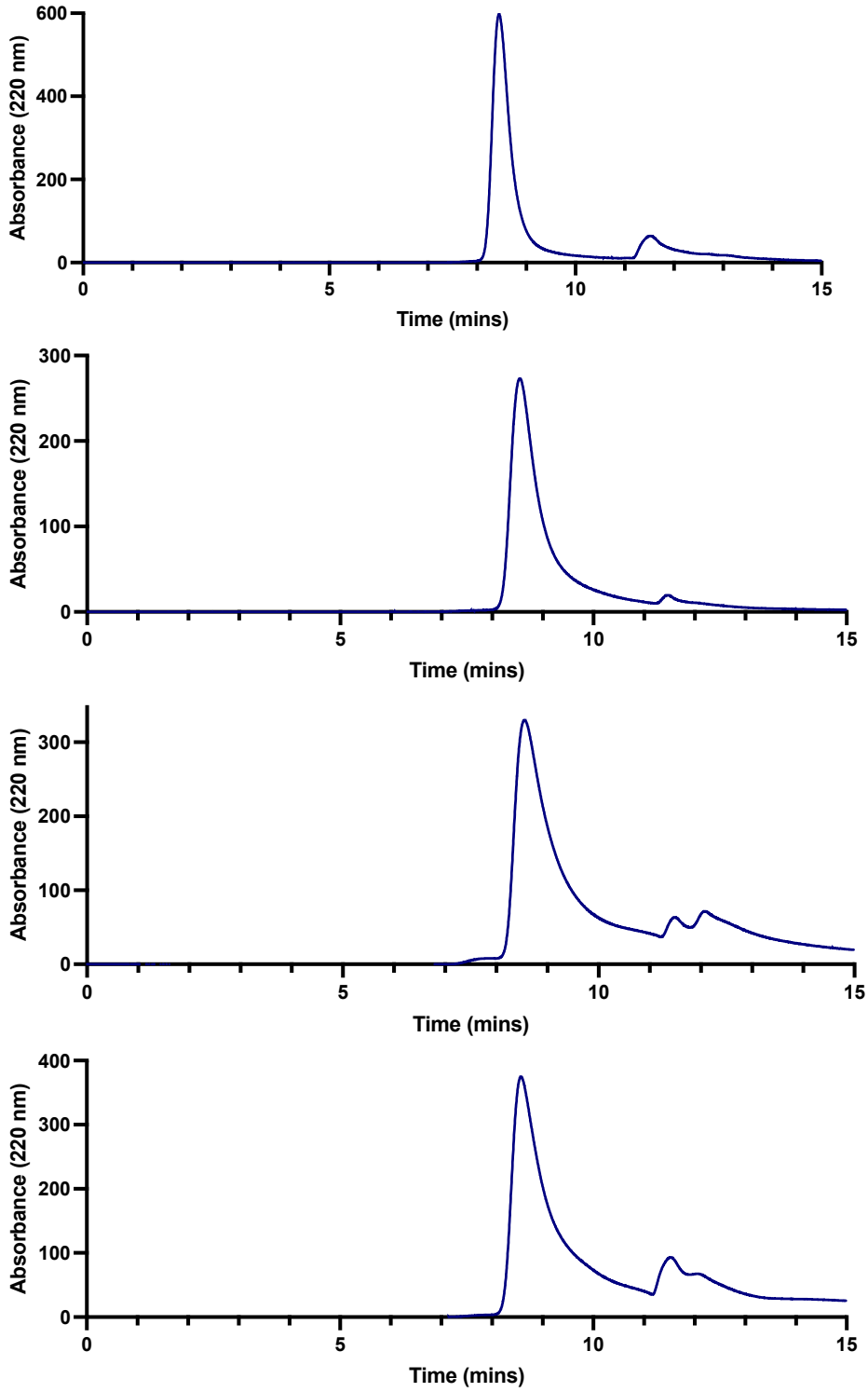


S 19 Cyclic voltammetry of (a) H_2atsm-Tz and (b) H_2dtse-Tz (1mM) in DMF (versus Ag/Ag^+), referenced to Fc/Fc+ couple E°' = 0V) with $(Bu)_4NPF_6$ supporting electrolyte 0.1M.

2.8 Conjugation of TCO-PEG$_4$-NHS to aducanumab and 6E10 antibodies

Aducanumab was expressed in CHO cells and purified by Protein A affinity and size exclusion chromatography in PBS (pH 7.4). A solution of TCO-PEG$_4$-NHS ester (7 μL of 5 mg/mL stock solution in DMSO, 10 equivalents) was added to a solution of Aducanumab (5mg/ mL) in PBS (1x, pH 7.4) and the reaction was incubated in the dark at room temperature for 1 hour. Tris-HCl buffer (25 μL, 3M, pH 8.0) was added to quench the reaction and excess reagents were removed and buffer exchanged (HEPES, 0.1M, pH7.4) *via* spin filtration (50 KDa MW cut off). Purified anti-β-amyloid, 1-16 antibody (clone 6E10, purchased from Biolegend, United States) (30 μg, 1mg/ mL in PBS 1x) was conjugated under the same conditions (10-fold excess of TCO-PEG$_4$-NHS, 2.5 μL of 1mg/mL anhydrous DMSO.

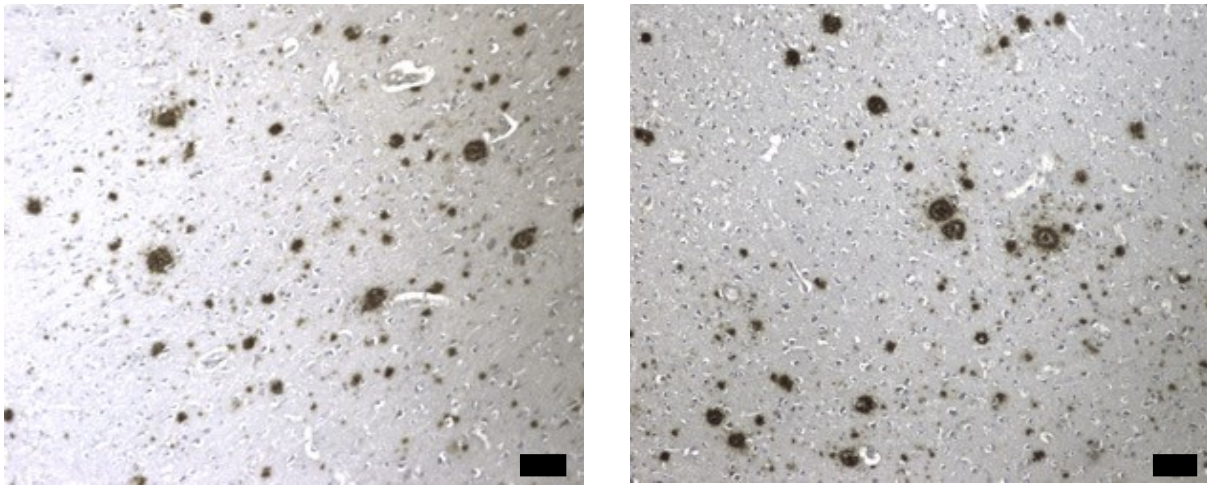
2.9 Size Exclusion High Performance Liquid Chromatography of aducanumab and aducanumab conjugates



S 20 Size exclusion HPLC chromatograms for (a) unmodified Aducanumab, and (b) Aducanumab-TCO, (c) Aducanumab-TCO + Cu(atsm-Tz) click and (d) Aducanumab-TCO + Cu(dtse-Tz) click. Eluent: 1x PBS pH 7.4, flow rate: 0.35 mL/min, UV detection: 220 nm.

2.10 Immunohistochemistry

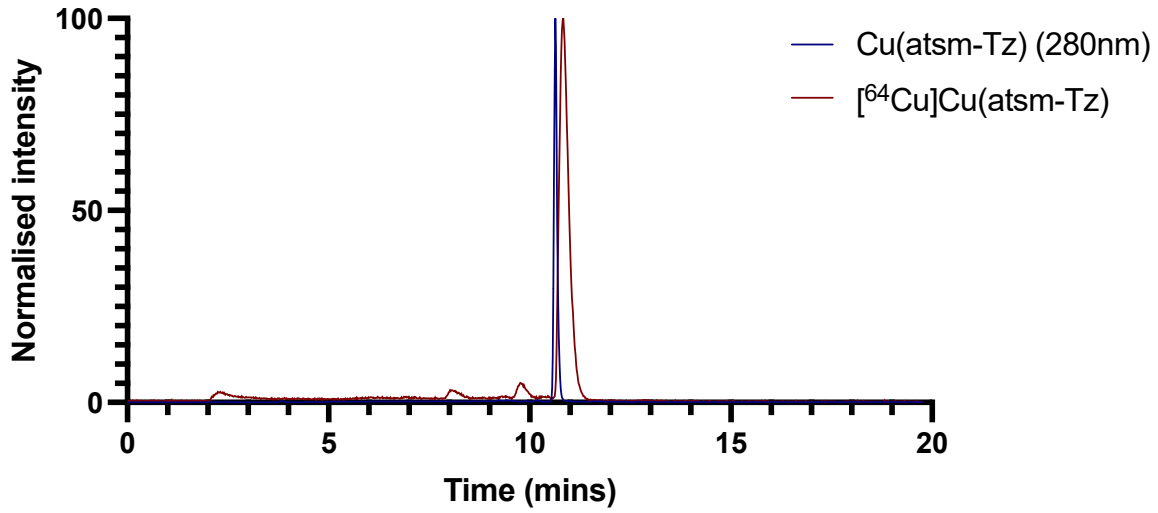
Brain tissues isolated from the frontal cortex of AD and healthy control subjects were fixed in 10% formalin/PBS and embedded in paraffin. Serial sections (7 μm) were deparaffinized and treated with 80% formic acid (5 min) and endogenous peroxidase activity was blocked utilizing 3% hydrogen peroxide. The section was then treated with blocking buffer (20% fetal calf serum, 50 mM Tris-HCl, 175 mM NaCl pH 7.4) before incubation with primary antibody to Aβ (6E10 1:200 or 6E10-TCO; 1:400) for one hour at room temperature. Visualization of antibody reactivity was achieved using the LSABTM+ kit (DAKO) and hydrogen-peroxidase-diaminobenzidine (H_2O_2-DAB) to visualise the Aβ deposits and cover slipped with DAKO fluorescent mount. Bright field images were visualized using a Leica DMIL LED microscope.



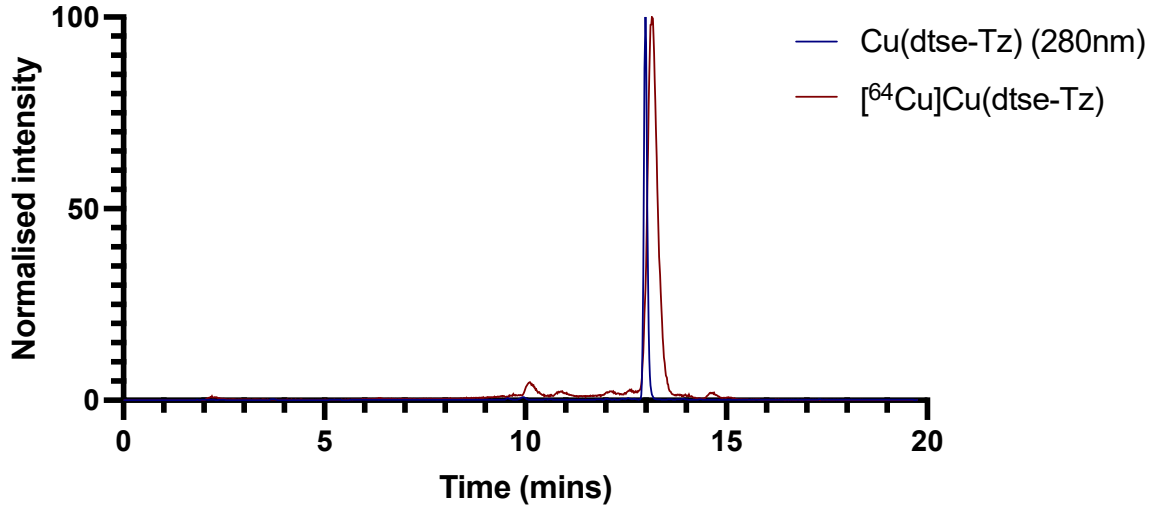
S 21 Section of human brain tissue from AD affected subject immuno-stained with 6E10 antibody (1:200 dilution); This staining shows that there is retention of antigen binding site and comparable binding between the undofied 6E10 antibody and the 6E10-TCO conjugate. black scale bar = 100 μm.

2.11 Radiochemistry

The copper-64 complexes were prepared by diluting [^{64}Cu]$CuCl_2$ (aq) (0.05M HCl, 20 μL, 50 MBq) with aqueous sodium acetate (0.1 M, pH 5.5 180 μL) to pH 5 or by diluting [^{64}Cu]Cu^{2+} (aq) (H_2O, pH 6, 20 μL, 35 MBq) with aqueous sodium acetate (1M, 2 μL), then H_2atsm-Tz or H_2dtse-Tz in DMSO was added (1mg/ mL, 5 μL) at room temperature for 30 minutes. An aliquot of the reaction solution (1 MBq) was taken for analysis by reverse phase radio-HPLC.



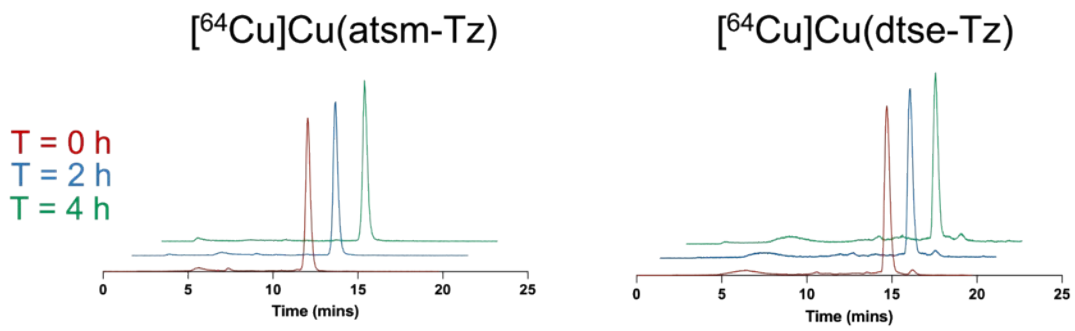
S 22 [HPLC of [^{64}Cu]Cu(atsm-Tz) (scintillation detector, red) Cu(atsm-Tz) (λ = 280 nm, blue). Retention time = 10.8 min and 10.6 min respectively



S 23 Radio HPLC of [^{64}Cu]Cu(dtse-Tz) (scintillation detector, red) Cu(dtse-Tz) (λ = 280 nm, blue). Retention time = 13.1 min and 13.0 min respectively.

2.12 Stability studies with cysteine and histidine

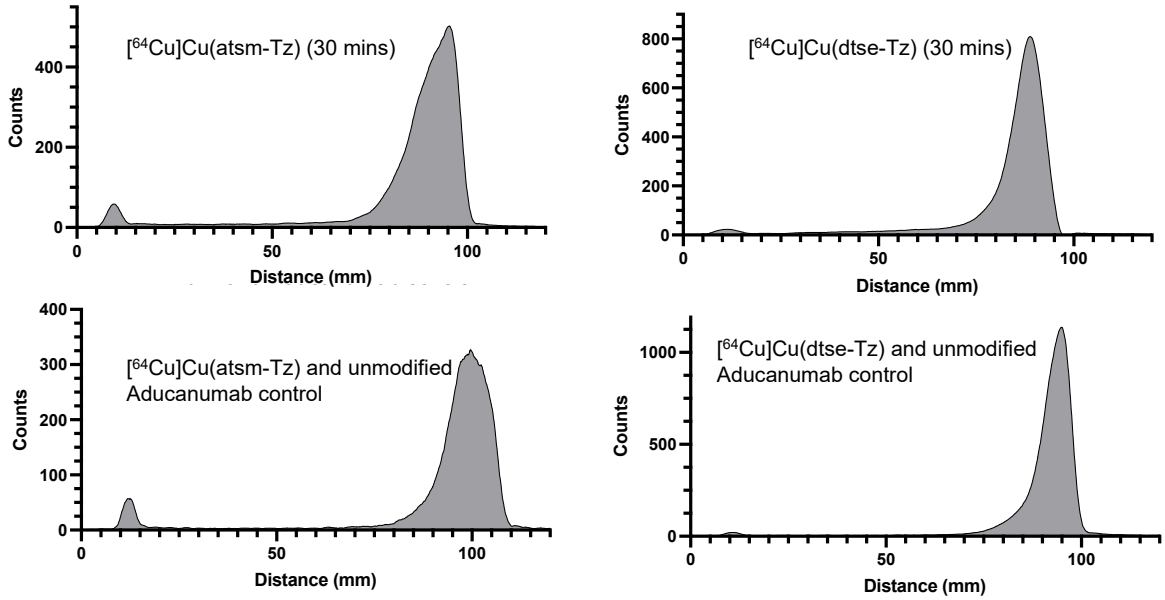
A solution of Cysteine and Histidine (5 mM) was added to a solution of both [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) (4 MBq in 50 uL) and incubated at 37°C. Aliquots of the mixture (12.5 µL) were injected into the HPLC at 0, 2 and 4 hours incubation time.



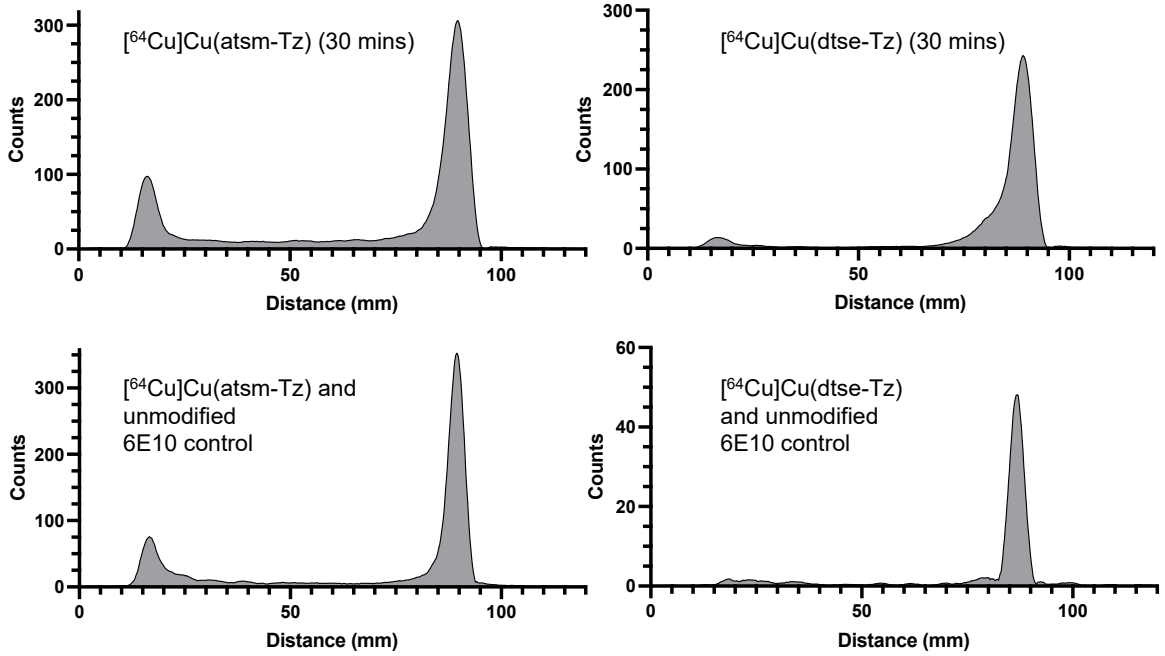
S 24 HPLC chromatograms of [^{64}Cu]Cu(atsm-Tz) (left) and [^{64}Cu]Cu(dtse-Tz) (right) at 0, 2 and 4 hours incubation time with an excess of cysteine and histidine.

2.13 In vitro click reaction with aducanumab-TCO and 6E10-TCO

The copper-64 complexes were prepared by diluting [^{64}Cu]$CuCl_2$ (aq) (0.05M HCl, 20 µL, 50 MBq) with aqueous sodium acetate (0.1 M, pH 5.5 180 µL) to pH 5 or by diluting [^{64}Cu]Cu^{2+} (aq) (H_2O, pH 6, 20 µL, 35 MBq) with aqueous sodium acetate (2 µL, 1M). H_2atsm-Tz or H_2dtse-Tz in DMSO was added (1mg/ mL, 0.5 ug per 10 MBq of [^{64}Cu]Cu^{2+}) and the reaction was left at room temperature for 30 minutes. Once TLC had confirmed >95% RCY (iTLC stationary phase, ethanol mobile phase, free [^{64}Cu]Cu^{2+} Rf = 0 and complex Rf = 1), the copper complexes were added directly into a solution of either aducanumab and aducanumab-TCO with equal molar amounts compared to H_2atsm-Tz and H_2dtse-Tz , or a slight excess of ligands for 6E10 or 6E10-TCO in NaOAc buffer (pH 5.5, 0.1, total volume 100 –125 µL, 6 –16 µM concentration before analysis via TLC under identical conditions.



S 25 radio-TLC of [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) incubation with unmodified aducanumab control



S 26 Radio TLC of ^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) incubation with unmodified 6E10 control

2.14 Biodistribution of [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) in Balb/c mice

This study was performed in strict accordance with the National Health and Medical Research Council (2013) Australian code for the care and use of animals for scientific purposes, 8th edition. Canberra: National Health and Medical Research Council and was approved by the Monash Animal Research Platform (MARP) Animal Ethics Committee of Monash University (Victoria, Australia). To characterise the biodistribution properties of [^{64}Cu]Cu(atsm-Tz) and

[^{64}Cu]Cu(dtse-Tz) and their capacity to cross the blood brain barrier, healthy wild type mice (balb/c) received intravenous injections (0.6 – 3.1 MBq in 200 μL) of [^{64}Cu]Cu(atsm-Tz)(*n*= 5 for 2 m.p.i and n=6 at 60 m.p.i)and [^{64}Cu]Cu(dtse-Tz), groups of mice (*n*= 6 per time point) were sacrificed by cervical dislocation and biodistribution was assessed at 2 and 60 minutes post injection. Organs [liver, spleen, kidney, muscle, skin, bone (femur), lungs, heart, stomach, brain, small intestines, large intestines, and tail) were collected immediately. All samples were counted in a Multi-Wiper™ HE γ-radiation well-counter (2008 Laboratory Technologies, Inc.). The tissue distribution data were calculated as the mean ± SD percent injected activity per gram tissue (%IA/g) for each construct per time point.

	[^{64}Cu]Cu(atsm-Tz) (2 mins) n= 5	[^{64}Cu]Cu(atsm-Tz) (60 mins) n=6	[^{64}Cu]Cu(dtse-Tz) (2 mins) n=6	[^{64}Cu]Cu(dtse-Tz) (60 mins) n=6
Blood	7.27 ± 2.51	1.51 ±0.15	4.29 ± 0.68	0.85 ± 0.05
Urine	1.44 ± 1.04	2.40 ± 0.40	0.06 ± 0.02	0.41 ± 0.10
Bladder	1.91 ± 0.36	1.80 ± 0.14	1.66 ± 0.36	0.92 ± 0.06
Heart	13.18 ± 4.20	1.83 ± 0.13	7.76 ± 0.91	1.57 ± 0.09
Brain	2.48 ± 0.49	0.79 ± 0.08	1.69 ± 0.28	0.41 ± 0.03
Liver	23.93 ±5.71	19.76 ± 1.22	21.99 ± 0.90	34.48 ± 3.73
Kidney	8.07 ± 0.43	4.84 ± 0.35	7.57 ± 0.77	2.86 ± 0.12
Spleen	4.48 ± 1.05	9.40 ±1.48	11.15 ± 1.86	19.74 ± 3.00
Stomach	3.19 ± 0.54	3.54 ± 0.68	2.20 ± 0.20	1.59 ± 0.12
Small Intestine	3.19 ± 0.31	9.85 ± 0.55	2.70 ± 0.32	5.81 ± 0.39
Large Intestine	1.10 ± 0.12	1.42 ± 0.09	0.86 ± 0.12	0.70 ± 0.05
Lungs	18.12 ± 0.14	3.41 ± 0.77	12.79 ± 2.06	12.41± 2.01
Muscle	1.05 ± 0.14	0.84 ± 0.25	0.92 ± 0.12	0.67 ± 0.03
Bone	1.56 ± 0.11	0.95 ± 0.06	2.09 ± 0.42	0.72 ± 0.02
Tail	11.14 ± 2.34	5.79 ± 2.69	3.10 ± 0.56	1.26 ± 0.10

Table 2 Biodistribution data for [^{64}Cu]Cu(atsm-Tz), and [^{64}Cu]Cu(dtse-Tz)

2.15 PET/CT of [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) in Balb/c mice

For mouse imaging studies, [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) complexes were made with a final activity of approximately 3.3 MBq/injection or 6MBq/ injection respectively. Doses were prepared with a final concentration of DMSO (10%), ethanol (8%) in sodium Acetate buffer (0.1M).. Wild type (WT) mice (n=6) underwent microPET/computed tomography (CT) imaging on an Inveon PET-CT (Siemens). Mice were anesthetized with isoflurane, catheterized and placed in the PET-CT and PET listmode data was collected for for 1 minute before injection of [^{64}Cu]Cu(atsm-Tz) and [^{64}Cu]Cu(dtse-Tz) via the tail vein. Following the 30 min PET acquisition, mice received a CT scan for co-localisation of the PET signal. The 30-minute list-mode data was histogrammed into 1x60s, 15x1s, 15x15s and 25x 60s for kinetic analysis. Image analysis was carried out using PMODv4.2 software (Bruker).