Supplementary Information (SI) for Chemical Science.
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Supporting Information

Tau is a metal binding protein: Structural insights into copper and zinc coordination sites and their impact on amyloid aggregation.

Supporting information includes:

MATERIALS AND METHODS

Tau441 protein expression and purification. Protein Tau441 was expressed in *E. Coli* BL21-CodonPlus (DE3)-RIPL cells, using 0.5-1 L of Super Broth medium, enriched with ampicillin and chloramphenicol, at a final concentration of 50 µg/ml and 30 µg/ml, respectively. Cells were incubated at 37 °C, 220 rpm for 2h or 3h. Unlabeled protein expression was induced with 0.5mM IPTG at OD_{600} of 2.4-3.2. To obtain ^{15}N-labeled Tau441, cells were grown in LB medium until an OD_{600} of 0.6–0.8 was reached, then centrifuged at low speed and resuspended in minimal medium M9 supplemented with 1 g/L $^{15}NH_4Cl$, as the only nitrogen source, and D-glucose as carbon source; protein expression was induced with 0.5mM IPTG. After 3h of induction with IPTG, cells were harvested by centrifugation and cell pellets were resuspended in buffer A (50mM sodium phosphates, pH=6.2, 10 mM NaCl, 1 mM EDTA, 1mM DTT) supplemented with complete protease inhibitor cocktail (a tablet per liter of culture) and 0.1 mM of PMSF. The bacteria were lysed by 10 cycles of 30 s of sonication at 70% amplitude on ice and lysates were boiled for 10 min. Denatured proteins were removed by centrifugation with 25,000 g at 4 °C for 35 min. The clarified supernatant was recovered and DTT was added to a final concentration of 2 mM and incubated at 4 °C for 2h. The sample was filtered and loaded onto an equilibrated HiTrap™ SP HP (Cytiva) cation exchange chromatography column and the weakly bound proteins were washed out with buffer A. Tau441 protein was eluted with a linear gradient of 100% final concentration of buffer B (50mM sodium phosphates, pH=6.2, 500 mM NaCl, 1 mM EDTA, 2mM DTT). Purification was followed by SDS-PAGE, protein-enriched fractions were pooled and PMSF was added to a final concentration of 0.1 mM. The purified sample was concentrated by ultrafiltration (10 kDa Vivaspin, Cytiva) and buffer exchanged against 20mM MOPS, NaCl 50mM pH=7.4 prior to the experiments with metal ions. For the most concentrated samples the protein was dialyzed against MQ water and lyophilized prior to sample preparation.

Tau K18 protein expression and purification. Protein TauK18, encompassing the R1-R4 pseudo-repeats, was expressed and purified under the same conditions as Tau441, with some changes: protein expression was induced with 1mM IPTG; and cell pellets were resuspended in buffer A (20mM MOPS, pH=6.8, 10 mM NaCl, 1 mM EDTA, 1mM DTT) and 0.1 mM of PMSF. The sample was filtered and loaded onto an equilibrated HiTrap™ SP HP (Cytiva) cation exchange chromatography column and the weakly bound proteins were washed out with buffer A. TauK18 protein was eluted with a linear gradient of 100% final concentration of buffer B (20mM MOPS, pH=6.8, 500 mM NaCl, 1 mM EDTA, 2mM DTT). The purified sample was concentrated by ultrafiltration (3 kDa Vivaspin, Cytiva) and buffer exchanged against 20mM MOPS, NaCl 50mM pH=7.4 prior to the experiments with metal ions. For the most concentrated samples the protein was dialyzed against MQ water and lyophilized and stored at (-20°C) prior to sample preparation.

Tau peptide synthesis and purification. Peptides TauR2 (Ac-KCGSKDNIKHV-NH_2) and TauR3 (Ac-KCGSLGNIHHK-NH_2), that include the Cysteine and Histidine residues in R3 and R4 pseudo-repeats, were prepared by the solid-phase Fmoc method as described previously.[1] Briefly, Fmoc-protected amino acids were coupled using Oxyma/DIC on DMF. Remotion of the Fmoc-protecting group was achieved by the addition of 20% piperidine and 2% DBU in DMF, the peptides were acetylated at the amino terminus and amidated in the C-terminus. Peptides were cleaved by adding a mixture of 92.5% TFA, 2.5% TIS, 2.5% EDT, and 2.5% H2O. The peptides were purified by high-performance liquid chromatography (HPLC) using a semi-preparative column Pursuit C18 (10 µm, 250 × 10 mm) on a Waters HPLC system with a photodiode array (PDA) UV–vis detector. After

purification, the peptide was identified by mass spectrometry, lyophilized, and stored at −20 °C. Peptide purity (>98%) was measured by analytical HPLC.

Protein aggregation assays. Aggregation assays were performed with 25 μM Tau441 in 20 mM MOPS, 50 mM NaCl, pH 7.4 buffer (aggregation assay buffer). A total volume of 200μL of protein solution was pipetted in a well of 96 well plate (Nunc, microplate, 96 well Optical Bottom Plate, Polymer Base, black, Non-treated, Non-sterile, PS, item no-265301). Thioflavin T (ThT) was added at a final concentration of 25 μM to follow amyloid aggregation. Heparin was added to a final concentration of 6.25 μM to trigger aggregation. After 15 min of incubation at 37 °C, metal ions were added at different concentrations (0 to 10 equiv with respect to protein) in their sulphate salts. It should be noted that pre-incubation of Tau441 with metal ions, followed by addition of heparin, yields similar results; while controls performed without protein or with no heparin yielded no signs of aggregation. The aggregation assay was performed at 37 °C for 20h in a FLUOstar Omega Fluorescence reader (BMG Labtech) with double orbital shaking (shaking duration,1 min) at an interval of 5 min for 20 h. An excitation filter at a wavelength of 460 nm was used, and the emission wavelength was set to 485 nm (manual gain, 1000, number of flashes, 30).

SDS-PAGE Analysis. Protein samples at the endpoint of the turbidity assays were collected and an equivalent volume of Laemmli buffer (with or without 2-mercaptoethanol, BME) was added to each sample and boiled for 10 minutes. Each sample was loaded at room temperature into an SDS-PAGE gel (10%, 30:1 acrylamide/bis-acrylamide, 130 V, 75 min). After electrophoresis, the gel was stained with Coomassie blue, destained and rinsed with MQ water prior to scanning.

Transmission Electron Microscopy. The Tau441 amyloids aggregates samples were directly imaged by transmission electron microscopy (TEM) after the ThT assays. A 10 μL aliquot of the samples were adsorbed onto Formvar-coated copper grids for 2 min and stained by 2% uranyl acetate solution for 2 min. Samples were imaged with a JEOL 1400 EX transmission electron microscope.

Electron Paramagnetic Resonances Spectroscopy. Sample preparation: Liophylized Tau441, TauK18, TauR2 and TauR3 were resuspended in 20mM MOPS and 50mM NaCl buffer pH=7.4 to reach a concentration of 0.2 mM for proteins or 0.5 mM for peptides. Samples were titrated with $CuSO_4$ and frozen in quartz tubes at liquid nitrogen temperature. Continuous-wave EPR spectra were acquired in an EMX Plus Bruker spectrometer using an X-band resonator (ER 4102ST) and a variable temperature nitrogen evaporation system (ER4131VT). The experimental parameters used were the following: a microwave frequency of ∼9.4 GHz, a temperature of ∼150 K, a modulation amplitude of 5 G, a microwave power of 100mW, and a modulation frequency of 100 kHz. Each EPR spectrum represents the mean of six or three scans. EPR simulations were performed using the Easy Spin (version 6.0.10) toolbox (on MATLAB R2023B).[2] A spin system of S = 1/2 of Cu with natural isotopic abundance was considered, coupled to nitrogen nuclei with natural isotopic abundance. All spectra were simulated considering an anisotropic broadening (g-strain).

Circular Dichroism Spectroscopy. The CD spectra were acquired using a Jasco J-815 CD spectropolarimeter at room temperature, using a quartz cell with a 10 mm path length. CD spectra were recorded from 240−280 to 800 nm, with a bandwidth of 5 nm, every 1 nm, using a scan speed of 200 nm/min and averaging two scans.

Nuclear Magnetic Resonance Spectroscopy. Sample Preparation: Tau441 lyophilized powder was resuspended in 20 mM MES, 50 mM NaCl Buffer pH=6.0, for copper titration, or 12 mM Phosphate buffer saline (PBS) pH=6.0, for zinc titration, at a final concentration of 100-120 μM. D_2O was added to 5% of the 200uL sample volume. The sample was finally placed into a 5 mm Shigemi NMR tube. The titration with metal ions was conducted added a stock solution of $CuCl_2$ or $ZnCl_2$. NMR experiments were performed on a Bruker 600 MHz or 700 MHz Avance III HD spectrometers

equipped with a cryogenically cooled proton-optimized $^{1}H[^{13}C/^{15}N]$ TCI probe. 2D ^{1}H-^{15}N HMQC spectra were acquired at 283 K. NMR spectra were processed with either Topspin (Bruker) and Sparky (University of California, San Francisco). Visualization and data analysis were carried out in Sparky. The previously reported signal assignment for Tau441 was used.[3] NMR signal intensity ratios (I/I_0) were determined for each residue by extracting the maximal signal height of the cross-peaks from the respective 2D ^{1}H-^{15}N NMR spectra.

X-ray Absorption Spectroscopy. XAS Sample Preparation: Tau441, TauK18, and TauR3 lyophilized powders were resuspended in 10mM MOPS and 50mM NaCl buffer pH=7.4 to a concentration of 0.8-1.0 mM. Metal ions were added from stock solutions of their sulphate salts at the indicated equivalents, and the samples were loaded in a sample holder and frozen in liquid Nitrogen. Cu K-edge XAS spectra were collected at beamline 7-3 at the SSRL synchrotron (Stanford, USA) using a LN_2 cooled Si(220) double-crystal monochromator. Spectra were collected in fluorescence mode by using a 30-element Ge solid state detector (Canberra). Sample was held in an Oxford Helium cryostat, cooled either by closed-cycle cooled He gas loop (10–100 K) or by open cycle liquid He dewar (4–200 K). Signal-to-noise ratios were improved by averaging spectra collected at different sample spots. XAS spectra were averaged (five to six scans per sample) after detector dead time and self-absorption correction and normalized to derive XANES spectra. The monochromator energy axis was calibrated using Cu foil as standard. The Zn K-edge X-ray absorption spectroscopy (XAS) spectra on Tau441-Zn^{2+}samples were collected at the SAMBA beamline (SOLEIL Synchrotron, France). All XAS acquisitions were performed at 20K using a He cryostat. Spectra were collected in fluorescence mode using a 13-pixel SDD detector (Mirion). Signal-to-noise ratios were improved by averaging several spectra collected on different sample spots. EXAFS spectra were extracted as described earlier.[4]

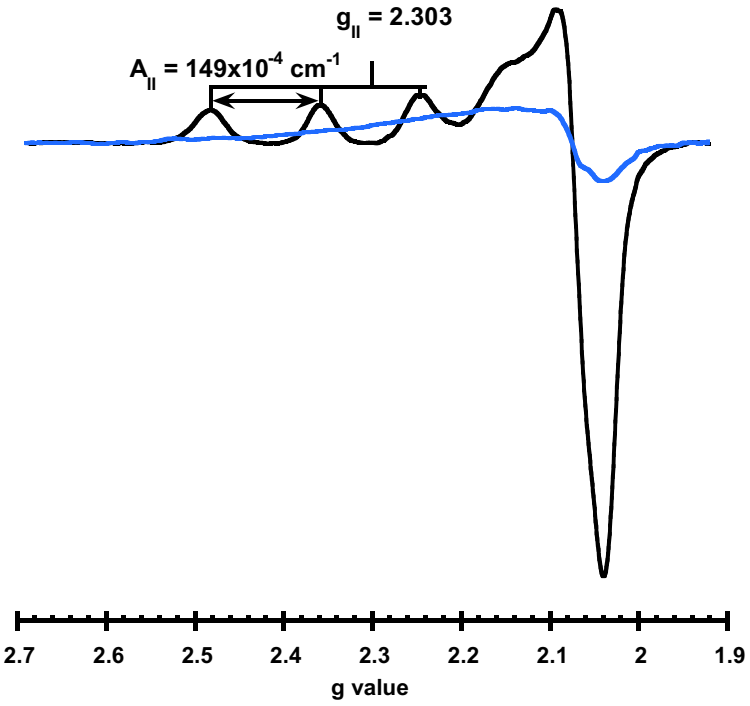


Figure S1. EPR spectra comparison of Tau441 with 1 eq of Cu^{2+} (black trace) and MOPS Buffer pH=7.4 with 1 eq of Cu^{2+} (blue trace).

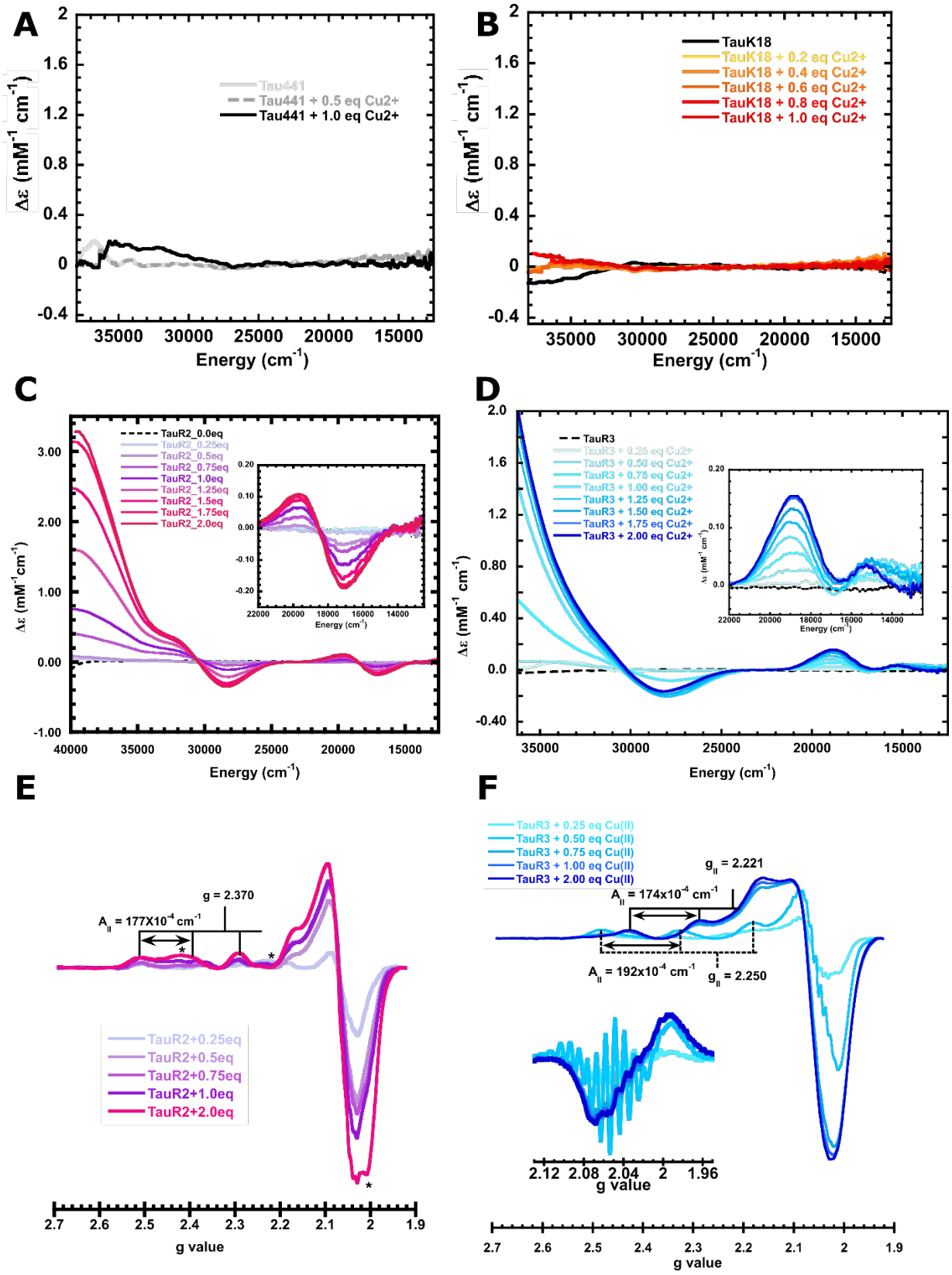


Figure S2. Titration of Tau441 and different fragments with Cu^{2+} as followed by CD and EPR. (A) CD spectra for Tau441 with 0, 0.5 and 1.0 equiv of Cu^{2+}. (B) Titration of TauK18 with 0-1.0 equiv of Cu^{2+}, as followed by CD. Titrations of TauR2 (C) and TauR3 (D) peptides with 0-2.0 equiv of Cu^{2+}, as followed by CD. Titrations of TauR2 (E) and TauR3 (F) peptides with 0-2.0 equiv of Cu^{2+}, as followed by EPR. The CD signals associated

with Cu^{2+} binding to both, R2 and R3 fragments saturate at around 2 equiv of metal ion. For the R2 fragment, EPR spectra reveal the formation of one main species with $g_{\|} = 2.370$ and $A_{\|} = 177\text{x}10^{-4}$ cm^{-1}, while signals associated with a second species appears at higher equiv of copper (> 1 equiv) (asterisks in panel E), but it is not possible to extract EPR parameters. On the other hand, for the R3 fragment, the EPR spectra at low equiv of metal ion (0.25 and 0.5 equiv) reveals the formation of a complex rich in nitrogen-based ligation and with $g_{\|} = 2.250$ and $A_{\|} = 192\text{x}10^{-4}$ cm^{-1}, that is no longer observed at higher equiv of copper (>0.5 equiv). This behavior suggests the formation of a Cu:R3 peptide fragment with a 1:2 stochiometry at <0.5 equiv, that later yields a 1:1 complex at >0. 5 equiv. with $g_{\|} = 2.370$ and $A_{\|} = 177\text{x}10^{-4}$ cm^{-1}; the latter is the main species at 1 and 2 equiv of metal ion, since no significant changes in the EPR spectrum are observed. Hence, for the purpose of comparison to the Cu^{2+} binding site in Tau441, the CD and EPR spectra at 1 equiv of metal ion were used.

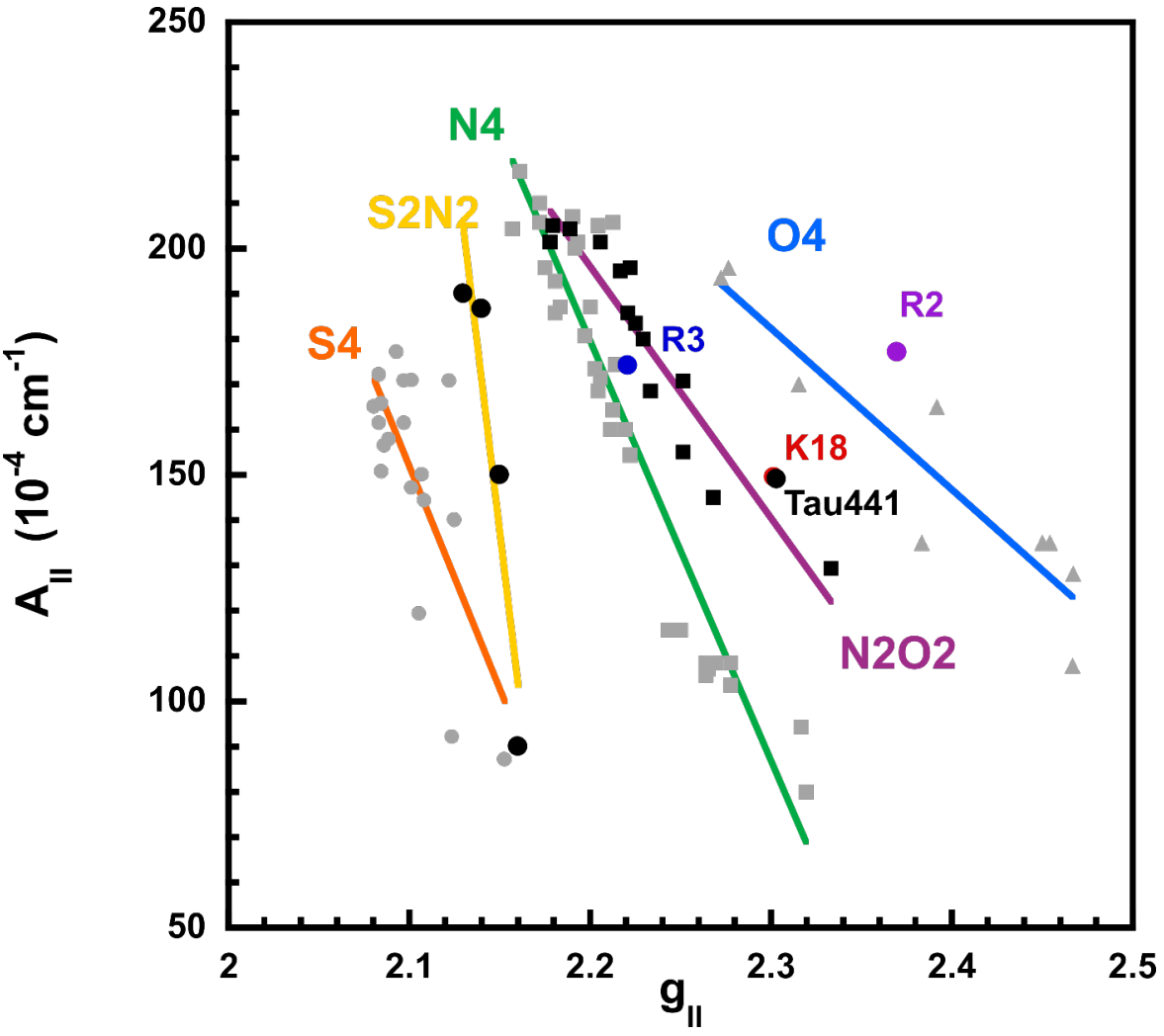


Figure S3. Peisach-Blumberg diagram of the EPR parameters of the different fragments of Tau441 with 1 equivalent of Cu^{2+}.

Table S1. Parameters for EXAFS fits for the Cu^{2+}-Tau441 complex with different coordination spheres (2N2O, 2N1O1S, 2N2S, 3N1O and 3N1S).

2N2O				**2N1S1O**				**2N2S**			
Path	***N[a]***	***R (Å)***	***σ² (Å²)***	***Path***	***N[a]***	***R (Å)***	***σ² (Å²)***	***Path***	***N[a]***	***R (Å)***	***σ² (Å²)***
Cu-N	2	1.93(3)	0.004(2)	Cu-N	2	1.89(9)	0.006(6)	Cu-N	2	1.84(8)	0.014(6)
Cu-O	2	2.02(4)	0.003(2)	Cu-S	1	2.16(5)	0.008(7)	Cu-S	2	2.11(2)	0.006(1)
				Cu-O	1	1.99(9)	0.002(2)				
S_0^2=0.9, **E_0**=-3.87 eV , **R_{factor}[b]**=5.07% **BVS**=1.97				**S_0^2**=0.9, **E_0**=-10.29 eV , **R_{factor}[b]**=6.99% **BVS**=2.51				**S_0^2**=0.9, **E_0**=-18.14 eV , **R_{factor}[b]**=10.65% **BVS**=3.24			

3N1O				**3N1S**			
Path	***N[a]***	***R (Å)***	***σ² (Å²)***	***Path***	***N[a]***	***R (Å)***	***σ² (Å²)***
Cu-N	3	2.02(1)	0.004(20)	Cu-N	3	1.96(2)	0.008(20)
Cu-O	1	2.99(3)	0.004(10)	Cu-S	1	2.02(1)	0.006(2)
S_0^2=0.9, **E_0**=-4.07 eV , **R_{factor}[b]**=5.71% **BVS**=1.94				**S_0^2**=0.9, **E_0**=-9.55 eV , **R_{factor}[b]**=9.58% **BVS**=2.42			

a. Coordination numbers have been fixed. b. Filtered for the FT between 1-2 Å.

Reduction of the Cu^{2+}-Tau441 complex. Addition of 20 equiv of ascorbic acid (AA) to the Cu^{2+}-Tau441 complex (at 0.74 mM concentration) did not achieve any reduction, as assessed by EPR (data not shown). When the protein was pre-reduced with dithiothreitol (DTT) and a small excess (2 mM, 2.7 equiv) remained in the protein solution, EPR spin quantitation indicated the presence of 21% residual Cu^{2+}, i.e. 79% of reduction to Cu^{+} (Fig. S4A). Addition of ascorbic acid (AA) 50 mM (68 equiv) to this solution yielded full reduction of the site, as EPR spin quantitation indicated 2% residual Cu^{2+}, i.e. 98% of reduction to Cu^{+} (Fig. S4A). Hence, full reduction of the Cu^{2+}-Tau441 complex is only achieved using both, DTT and AA, as reducing agents. XANES spectra for the reduced samples display the typical feature at 8982 eV associated with the electric dipole-allowed 1s → 4p transition for Cu^{+} ions (inset Fig. S4B). The intensity of the signal at 8982 eV is consistent with the extent of reduction of the site, as determined by EPR of Cu^{+} ions species (Fig. S4A); namely, the intensity of the 8982 eV signal for the sample with 79% reduction (2.7 equiv of DTT; purple trace) is ~ 80% of that for the fully reduced sample (2.7 equiv DTT with 68 equiv AA; cyan trace). A fourth sample was prepared by adding 80 equiv of AA to the Cu^{2+}-Tau441 complex at 0.26 mM concentration; no EPR data was collected for this sample, however, it is clear from the intensity of the feature at 8982 eV that reduction was partial (~ 30%) and that DTT is absolutely needed to achieve full reduction of the site. Moreover, the EXAFS spectra clearly show distinct differences between the samples, depending on the extent of reduction (Fig. S4C&D). The sample with 79% reduction (2.7 equiv DTT) displays very similar features to that of the fully reduced sample (2.7 equiv DTT and 68 equiv AA), while the small differences can be explained by the presence of residual Cu^{2+} species in the sample. EXAFS analysis and fitting for the Cu^{+}-Tau441 complex was performed with the data collected on the fully reduced sample (2.7 equiv DTT and 68 equiv AA, cyan trace in Fig. S4). It is important to note that the XANES spectrum of the fully reduced complex is quite different from that reported for the Cu^{+}-DTT complex (5).

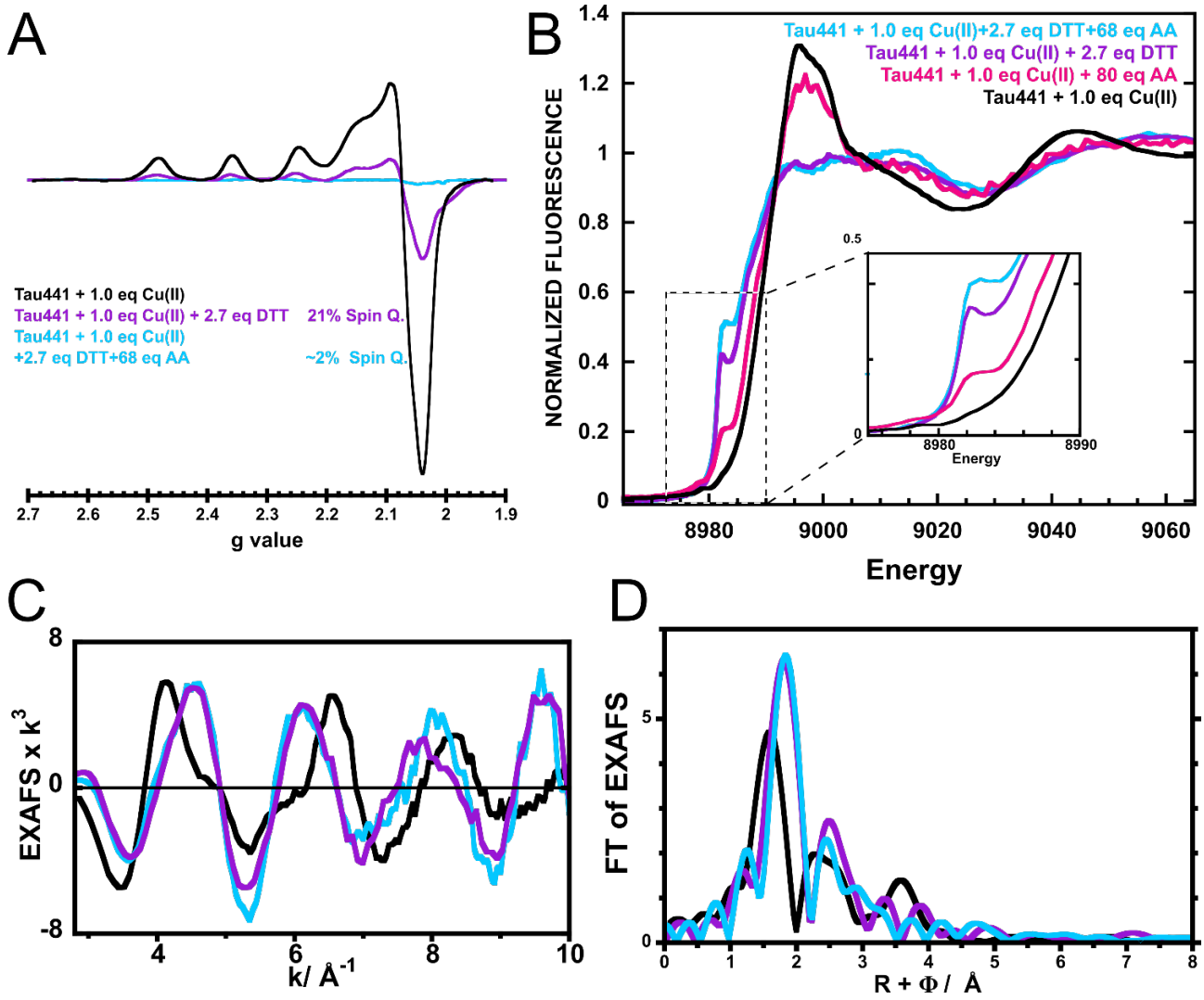


Figure S4. (A) EPR spectra for the Cu^{2+}-Tau441 complex (black trace) at 0.74 mM concentration, and in the presence of reducing agents: with dithiothreitol (DTT) 2mM (2.7 equiv) (purple trace), and with both, DTT 2 mM and ascorbic acid (AA) 50mM (68 equiv) (cyan trace). Spin quantitation yields 21% of residual Cu^{2+} for the sample with DTT 2mM (i.e. 79% reduction), and 2% (practically full reduction) for the sample with DTT and AA. A fourth sample was prepared with the Cu^{2+}-Tau441 complex at 0.29 mM concentration, without DTT and with 80 equiv of AA (magenta trace), although no EPR spin quantitation was performed, XANES spectra shows the presence of Cu^{+} ions (approximately 30%). Cu-K edge XANES **(B),** EXAFS **(C)** and FT**(D)** for the Cu^{2+}-Tau complex (black trace) and the reduced complexes: with 2.7 equiv of DTT (purple trace), with 80 equiv of AA (magenta trace), and with both, DTT (2.7 equiv) and AA (68 equiv) (cyan trace). Inset in (B) shows the feature associated with the electric dipole-allowed 1s → 4p transition for Cu^{+} ions.

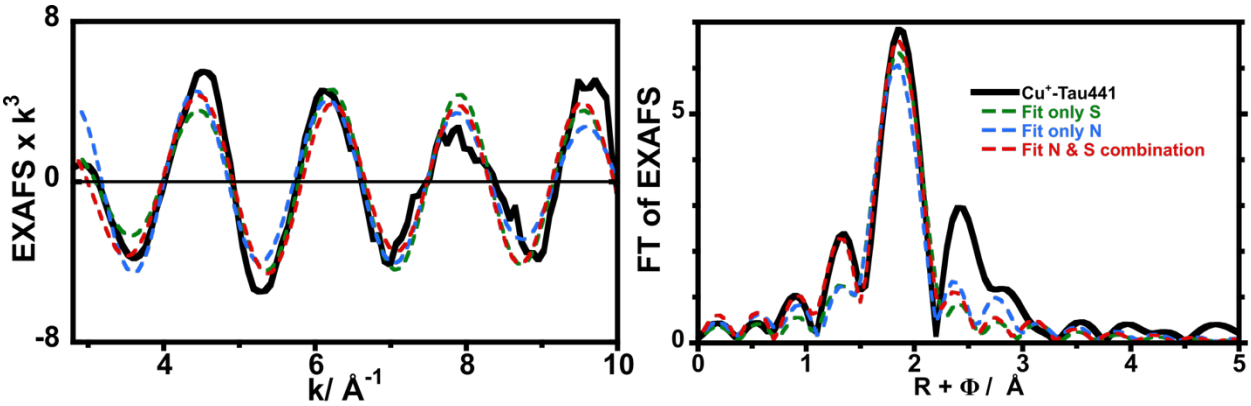


Figure S5. EXAFS and FT spectra of the Cu$^+$-Tau441 complex (black trace) and the different EXAFS fits that consider only first coordination sphere interactions, namely: using only S-based ligands (green dotted line), with only N-based ligands (blue dotted line) and with a combination of N- and S- based ligands (red dotted line), using the parameters listed in Table S2. The best result was achieved with a combination of N and S ligands.

Table S2. Parameters for the EXAFS fits for the Cu$^+$-Tau441 complex considering only first coordination sphere interactions. Nitrogen- and sulfur-based ligands were probed.

1st Sphere

	Fit with only S			**Fit with only N**			**Fit with S & N**		
Path	***N***	***R (Å)***	***σ² (Å²)***	***N***	***R (Å)***	***σ² (Å²)***	***N***	***R (Å)***	***σ² (Å²)***
Cu-S	2.1(3)	2.246(8)	0.004(1)	-	-	-	2.2(1)	2.225(8)	0.004
Cu-N	-	-	-	3.4(2)	2.145(8)	0.003(2)	0.9(2)	1.85(2)	0.004
	***S_0^2*=0.95, *e0*=-1.75 eV, *Rfactor*[a]=18.9% *BVS*=0.648**			***S_0^2*=0.95, *e0*=11.27 eV, *Rfactor*[a]=21.6% *BVS*=0.803**			***S_0^2*=0.95, *e0*=-5.15 eV, *Rfactor*[a]=11.3% *BVS*=1.189**		

a. R-factors are filtered for the FT between 1-2.2 Å.

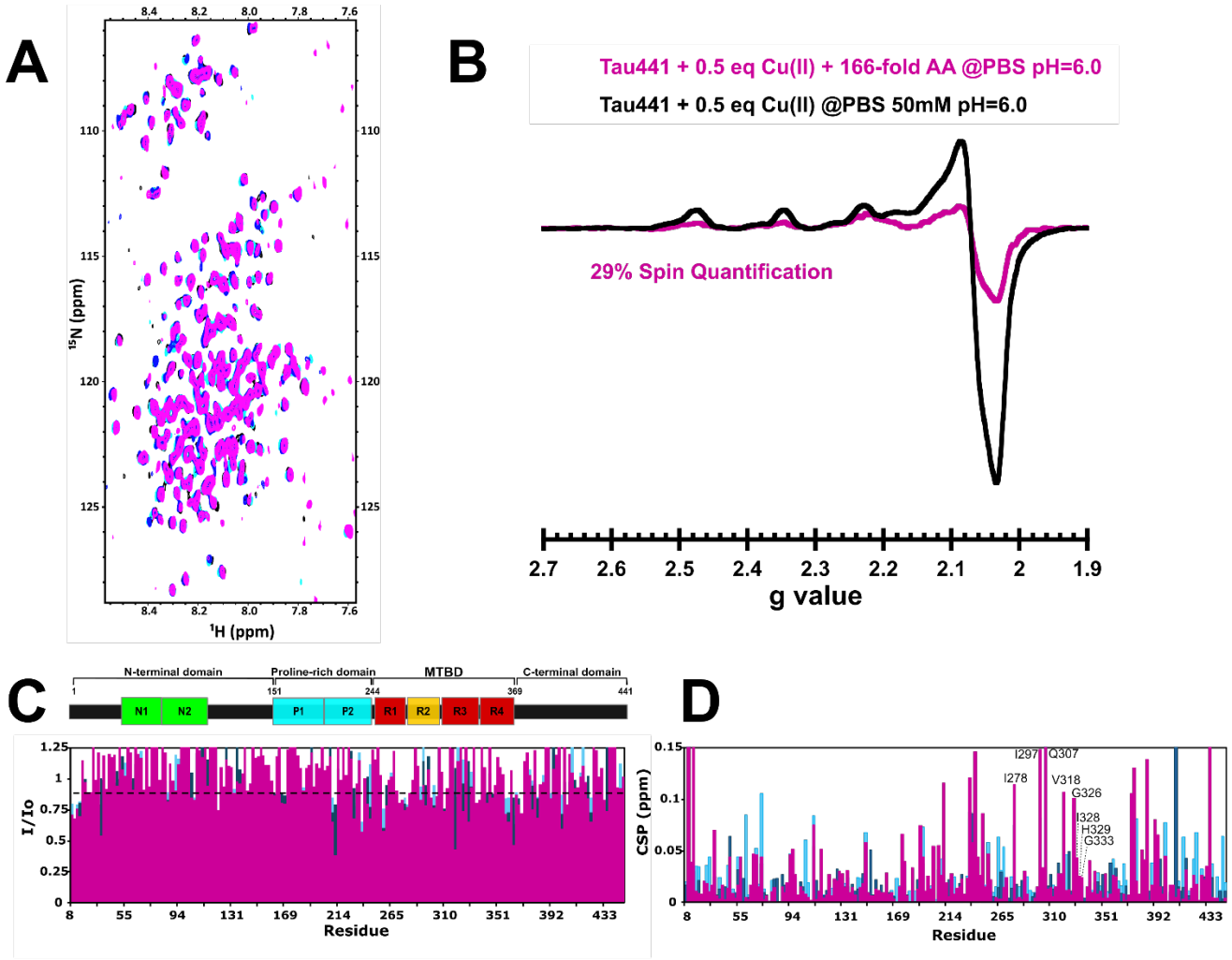
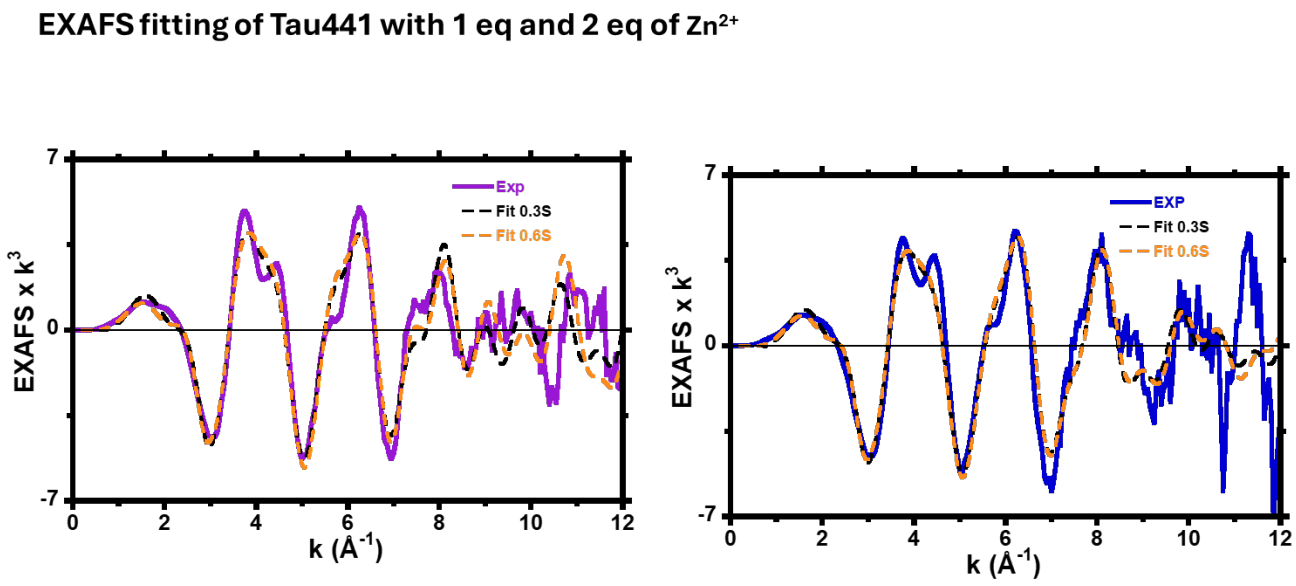


Figure S6. (A) Overlay of ^{1}H-^{15}N HMQC NMR spectra of Tau441 60 μM with no metal ions (black), and with 0.05 (cyan), 0.1 (dark blue) and 0.5 (magenta) equiv of Cu^{2+} in the presence of 5 mM ascorbic acid (AA). (B) EPR spectra comparison of Tau441 60 μM with 0.5 equivalent of Cu^{2+} (black trace) and with 166-fold excess of AA (5mM). Spin quantitation indicates that the reduced sample contains 29% residual Cu^{2+} species, i.e. 71% of reduction was achieved. (C) Normalized I/Io profiles of the ^{1}H-^{15}N HMQC NMR signals of assigned residues for Tau441 at different equivalents of copper: 0.05 equiv (cyan bars), 0.1 equiv (dark blue bars) and 0.5 equiv (magenta bars). (D) Chemical shift perturbations (CSP) for the HMQC signals of assigned residues of Tau441 with different equivalents of copper. Residues with major CSP in the region R2 and R3 are indicated.

A



B

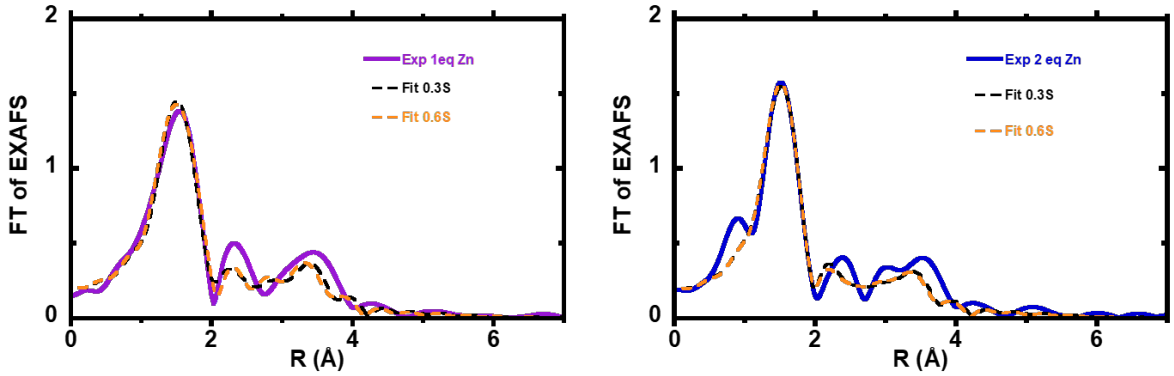


Figure S7. EXAFS fitting (**A**) and FT **(B)** of the Tau441 with 1 equivalent of Zn^{2+}. Experimental spectra are shown in magenta and the fit in black dotted line.

Table S3. Parameters of the best EXAFS fitting of the Tau441 with 1 and 2 equivalent of Zn^{2+}.

TAU441 + 1 eq Zn^{2+}

	2N2O			**2N 1.7O 0.3S**			**2N 1.4O 0.6S**			**2N1O1S**		
Path	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***
Zn-S	0	-	-	0.3	2.28(5)	0.00154	0.6	2.27(3)	0.0015(4)	1	2.26(3)	0.005(3)
Zn-N	2	1.9708	0.00154	2	1.95(1)	0.00154	2	1.94(2)	0.00154	2	1.94(2)	0.00154
Zn-O	2	2.1339	0.00154	1.7	2.12(3)	0.00154	1.4	2.12(3)	0.0154	1	2.12(5)	0.00154
N-N	2	4.2006	0.0007	2	4.17(3)	0.0045	2	4.14(4)	0.00035	2	4.11(4)	0.00066
C-N	2	4.2111	0.0007	2	4.18(3)	0.0045	2	4.15(4)	0.00035	2	4.13(4)	0.00066
	S_0^2=1.3, **e0=**3.40 eV , **Rfactor=**5.21% **BVS=1.47**			**S_0^2=**1.3, **e0=**1.78 eV , **Rfactor=**4.75% **BVS=1.94**			**S_0^2=1.3, e0=**0.81 eV , **Rfactor=**5.20% **BVS=2.39**			**S_0^2=**0.9, **e0=**0.59 eV , **Rfactor=**6.38% **BVS=2.93**		

TAU441 + 2 eq Zn^{2+}

	2N2O			**2N 1.7O 0.3S**			**2N 1.4O 0.6S**			**2N1O1S**		
Path	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***	***N***[a]	***R (Å)***	***σ² (Å²)***
Zn-S	0	-	-	0.3	2.24(5)	0.003(9)	0.6	2.25(3)	0.007(8)	1	2.25(4)	0.0097(67)
Zn-N	2	1.96(1)	0.00166	2	1.95(2)	0.00166	2	1.95(2)	0.00166	2	1.95(2)	0.00166
Zn-O	2	2.11(2)	0.00166	1.7	2.11(3)	0.00166	1.4	2.11(3)	0.00166	1	2.10(5)	0.00166
N-N	2	4.19(3)	0.002	2	4.18(4)	0.00261	2	4.16(4)	0.00265	2	4.14(5)	0.00272
C-N	2	4.20(3)	0.002	2	4.19(4)	0.00261	2	4.17(4)	0.00265	2	4.15(5)	0.00272
	S_0^2=1.3, **e0=**3.28 eV , **Rfactor=**4.73% **BVS=1.52**			**S_0^2=**1.3, **e0=**2.28 eV , **Rfactor=**4.55% **BVS=1.90**			**S_0^2=**1.3, **e0=1.25** eV , **Rfactor=**5.02% **BVS=2.31**			**S_0^2=**1.3, **e0=**-0.01 eV , **Rfactor=**5.76% **BVS=2.85**		

a. Coordination numbers have been fixed.

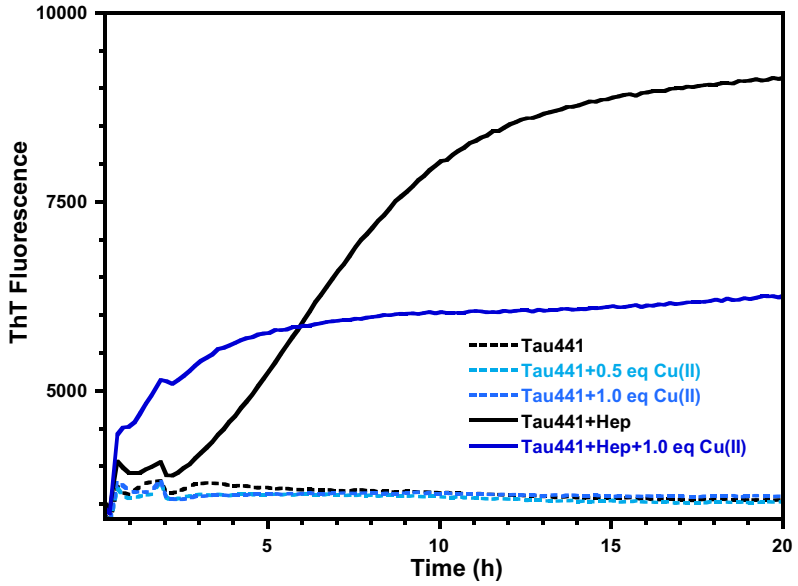
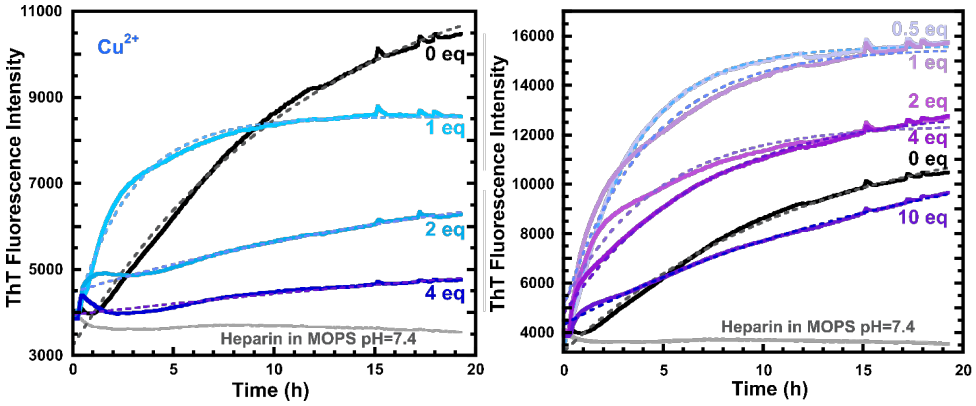


Figure S8. Aggregation assay of Tau441 protein (25 μM) in aggregation buffer, with 0.5 eq or 1 eq Cu^{2+} in the presence or absence of heparin (6.25 μM), as followed by ThT fluorescence at 37 °C.



Exponential growth equation y=m1+m2*exp(-m3*x)			
Cu^{2+} equiv	**Exponential rate m3 (h^{-1})**	**Zn^{2+} equiv**	**Exponential rate m3 (h^{-1})**
0	**0.0823**	**0.5**	0.3095
1	0.3538	**1**	0.2385
2	0.0458	**2**	0.2161
4	0.0209	**4**	0.1464
		10	0.05364

Figure S9. Fitting Tau protein (25 μM) aggregation curves with Cu^{2+} or Zn^{2+} in the presence of heparin (6.25 μM) at 37°C. The aggregation kinetics were fitted to an exponential growth curve and the calculated constants are reported in the Table.

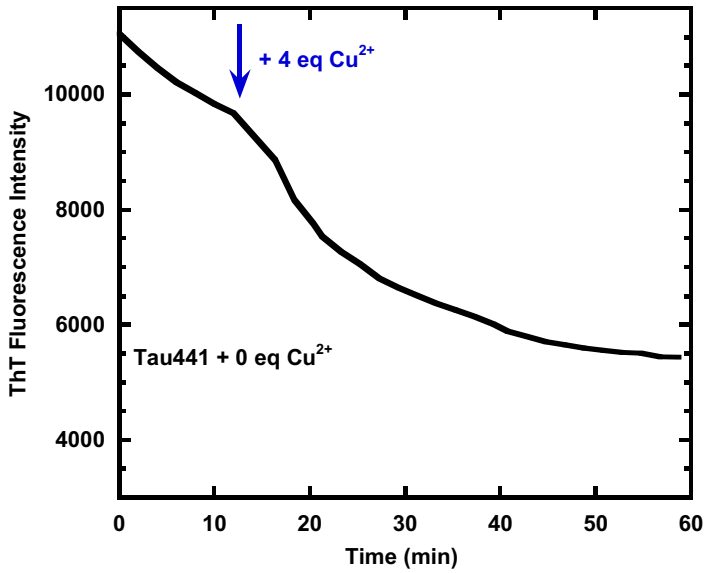
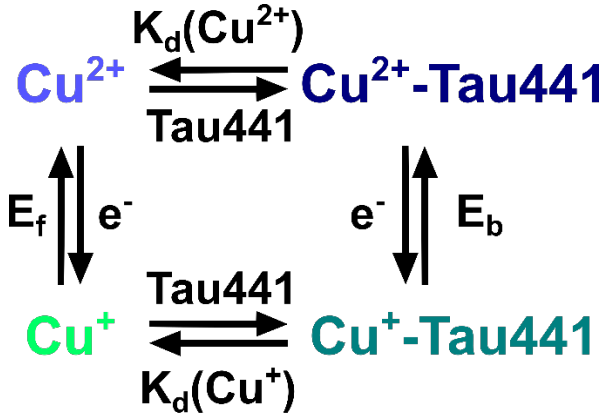


Figure S10. ThT Fluorescence intensity of Tau441 (25 μM) fibrils grown in the absence of metal ions and after the addition of 4 eq of Cu^{2+} ions (blue arrow). ThT fluorescence of the fibrils is quenched significantly.

Scheme S1. Thermodynamic cycle scheme for reduction of copper-Tau complexes.

A thermodynamic cycle scheme can be constructed, relating the reduction potential of the Cu-Tau441 complex to that of free Cu and the relative binding affinities of Tau441for Cu^{2+} and Cu^{+} ions:



$$E_b - E_f = 0.059 \log\left(\frac{K_d\,(Cu^{2+})}{K_d\,(Cu^{+})}\right)$$

where E_f is the midpoint reduction potential for free Cu ions, known to be E_f =0.158 Vs. NHE at physiological pH; E_b is the reduction potential for the Cu-Tau441 species; K_d (Cu^{+}) corresponds to the dissociation constant of the Cu^{+}-Tau441 complex; and K_d (Cu^{2+}) corresponds to the dissociation constant of the Cu^{2+}-Tau441 complex, which is reported to be K_d=0.5 mM (6). Since $E_{b(Cu\text{-}Tau441)}$ is estimated to be < +0.052 V, a lower limit can be set for K_d (Cu^{+}), i.e. K_d (Cu^{+}) >32 mM, using the equation above.

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