# Supporting Information For: Copper Ligation to Soluble Oligomers of the English Mutant of the Amyloid-$\beta$ Peptide Yields a Linear Cu(I) Site That is Resistant to $O_2$ Oxidation.

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**Reagents and General Considerations.** Tetramethylethylenediamine (TEMED) and Thioflavin T were from Acros (NJ, USA). Sodium dodecyl sulfate (SDS, 99%) was from Sigma-Aldrich (MO, USA). Samples for XAS, GPC and EPR spectroscopy were prepared in NEM buffer containing 50 mM NEM/pH 7.4 with or without 50% glycerol (v/v). The trifluoroacetate salt of A$\beta$42H(6)R was prepared by solid-phase synthesis according to published procedures, and was treated with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Acros, NJ) according to literature protocols.(Stine 2003) After removal of HFIP, peptide was dissolved in sufficient dimethylsulfoxide (DMSO, Acros, NJ) to afford a 5 mM (22.7 mg/mL) solution. The A$\beta$42H(6)R DMSO stock solution was agitated gently at room temperature for 1 h prior to immediate use or storage at –20 °C. Reverse-phase HPLC and GPC were recorded on a Waters DeltaPrep 60. Fluorescence spectra were obtained on a Horiba Fluoromax 3 fluorometer. ATR-FTIR spectra were recorded on a Thermo-Nicolet 470 FTIR spectrometer with a SMART MIRacle Attenuated Total Reflectance (ATR) accessory (ZnSe crystal) as peptide films (2 $cm^{-1}$ resolution; average of 128 scans).

**Oligomer preparation.** Oligomers of A$\beta$42H(6)R were prepared according to the protocol outlined in Yu *et. al.*.(Yu 2009) A$\beta$42/A$\beta$42H6R was dissolved in HFP (total concentration of 6 mg/mL) and shaken at 37C for 1.5 hrs. HFP was allowed to evaporate. The dried A$\beta$ was dissolved in 132 $\mu$L DMSO, sonicated for 20 sec and shaken at room temperature for 10 min yielding a concentration of 5 mM A$\beta$ in DMSO. The globulomers were formed by adding 60 $\mu$L of A$\beta$/DMSO to 690 $\mu$L PBS buffer, pH 7.4, (20 mM $NaH_2PO_4$ and 140 mM NaCl) with 75 $\mu$L of 2% SDS (w/v) in water and incubated for 6 - 8 hr at 37C. The sample was then diluted with 2475 $\mu$L of Millipore water (at least 17.6 M$\Omega$ resistance) and incubated for 18 - 20 hr at 37 °C. This was then centrifuged at 3000 × G for 20 min and Centripreped with a 30 kDa cutoff centrifuge filter. The supernatant was dialyzed at 2 °C overnight in PBS/4 (PBS buffer, pH 7.4, diluted 4 times). The sample was then centrifuged at 10,000 × G for 10 min and stored on dry ice. The non-fibril oligomers (16/32 kDa) were formed by adding 11.9 $\mu$L of A$\beta$/DMSO to 393.1 uL of PBS buffer, pH 7.4, (20 mM $Na_2HPO_4$ and 140 mM NaCl) with 45 $\mu$L of 2 % SDS (w/v) in water and

incubated for 6 - 8 hr at 37 °C. This yielded the non-fibril oligomers with a concentration of 0.6 mg/mL. The sample was stored on dry ice.

**EPR Spectroscopy.** $Cu^{2+}$ containing A$\beta$(42)H(6)R oligomers were prepared as above and diluted with an equal volume of 50% buffer/glycerol to a final $Cu^{2+}$ concentration of 50 $\mu$M. Samples were clear prior to being stored in EPR tubes at -20 °C before collecting spectra. After thawing and incubating for 1 h at room temperature on the day spectra were collected no solid was observed in the tube and slowly frozen in liquid nitrogen yielding a clear glass.

X-band CW EPR spectra were recorded on a Bruker EMX-plus spectrometer. Temperature was maintained by flowing nitrogen gas through liquid nitrogen and then into a nitrogen flow cryostat. Spectra were collected with the following experimental parameters: microwave frequency = 9.10 GHz, microwave power = 20 mW, modulation amplitude = 5.0 G, time constant = 40.96 ms, conversion time = 40.96 ms, gain = $1 \times 10^4$, scans = 100, T = 90 K). Spectra were decovolved using the package EasySpin 4.5.0, which runs under the MatLab software package.(Stoll, 2006)

**Cu K–edge X–ray Absorption Data Collection and Measurement.** The copper-containing oligomer solutions were injected into aluminum sample holders in between two windows made of Kapton tape (3M, cat. #1205; Minneapolis, MN) and quickly frozen in liquid nitrogen. Reduced samples were prepared by injecting 2 eq. of ascorbate to the oligomer solution under dinitrogen, injecting the samples in aluminium sample holders in between two windows made from Kapton tape, and quickly freezing the samples. Data were then collected at the National Synchrotron Light Source (Brookhaven National Laboratories; Upton, NY) on beamline X3b (ring operating conditions: 2.8 GeV; 200  305 mA). A focused Si(111) double monochrometer was used for energy selection along with a low-angle (4.5 mrad) Ni mirror for harmonic rejection. Energy calibrations were performed by recording a reference spectrum of Cu foil (first inflection point assigned to 8980.3 eV) simultaneously with the samples. All samples were maintained at 20 K throughout the data collection using a helium Displex cryostat. The spectra are reported as fluorescence data, which were recorded utilizing a 31-element Ge solid-state fluorescence detector (Canberra). Total count rates were maintained under 20 kHz per channel, and a deadtime correction of 3 $\mu$s was utilized (this had a negligible influence on the data). The primary hutch aperture height was set to 0.4 mm to obtain the maximum resolution (theoretical maximum is  0.9 eV), while the hutch aperture was set to 1 × 2 mm, and data were in 5.0 eV steps in the pre-edge region (8779  8958 eV), 0.5 eV steps in the edge region (8959  9023 eV), 2.0 eV steps in the near-edge region (9024  9278 eV), and 5.0 eV steps in the far-edge region (9279 eV  14.0 k). The spectra represent the averaged sum of 9 spectra. After every third scan the beam was moved to a different position on the sample to avoid potential radiation damage. All spectra were individually inspected prior to data averaging to insure that sample decomposition in the beam was not occurring.

Data analysis was performed as previously described using the XAS analysis package

EXAFS123.(Shearer 2007) The only deviation is that the number of scatterers in the individual shells were initially left as free parameters, and then restrained to the nearest whole number for analysis of the Cu(I) data. For the Cu(II) data we allowed for a full refinement of the number of scatterers. Single scatterer functions for Cu-N, Cu-O, and Cu-C interactions and multiple–scattering (MS) pathways for the full imidazole moiety were constructed as previously described. The MS-pathway is defined by three geometric parameters: the Cu-"N" distance (r(Im)), the in-plane angle ($\psi$), and the out–of–plane angle ($\theta$). In addition, a MS-pathway for the linear bis-His unit was constructed, which was defined with three geometric parameters: the average Cu-"N" distances (r(Im)), the N-Cu-N bond angle ($\psi$) and a twist angle ($\theta$) defining the orientation of the two rigs relative to each other. Individual spectra for 300 geometries were generated using FEFF *v.* 9.0 where the Cu-N bond-length was varied between 1.75 – 2.10 Å, the N-Cu-N bond angle was varied between 165 – 180° and the "twist" angle was varied between 0 – 90°. Phase and amplitude functions were then generated as previously described.

Although data were collected past 13 k, data refinements were only performed out to k = 12.0 $Å^{-1}$ due to noise at higher values of k. Best fits to the experimental data were determined by selecting the model that gave both chemically reasonable refinement parameters and the lowest value for the goodness of fit parameter:

$$\mathrm{GOF} = \mathrm{ave}[(\chi - \chi_{\mathrm{sim}})/\mathrm{esd}_{\mathrm{data}}](\mathrm{n_i}/(\mathrm{n_i} - \mathrm{n_p}))^{0.5} \quad (1)$$

where $n_i$ is the number of independent data points and $n_p$ is the number of parameters used in the data simulations. All data was modeled as FF-$k^3(\chi)$ (FT from 2.0 – 12.0 $Å^{-1}$; back-transformed from 1.0 – 4.0 Å). We note that the Cu(II) spectrum (although representing a mixture of two coordination environments) were not modeled as two distinct environments, as this could not be statistically justified by the GOF parameter. Instead the data were modeled using shells that accounted for fractional occupancies.

**Molecular Dynamics Simulations**. Conventional, all-atom molecular dynamics simulations were performed using a linear array starting structure that facilitates efficient aggregation into oligomeric conformations. The Amber 12 Molecular Dynamics software package was utilized(Case, 2012) with the ff03.r1 force field, using the Langevin thermostat (ntt = 3; collision frequency = 5.0), SHAKE algorithm (ntc = 2), and the Generalized Born solvent model. NVidia GPU CUDA Version 12.1 was utilized for these simulations and previously tested and found to significantly reduce calculation run time while maintaining consistency with CPU data, and was used with mixed single/double/fixed point precision [SPFP] for these calculations.(Goetz, 2012; Grand 2012) Minimization was performed (0.5 fs step time; 500 steps each of steepest descent gradient and conjugate gradient algorithms), followed by heating from 0 K to 50 K above physiologic temperature (so as to escape local potential energy minima) and relaxation back to 310 K for the equilibration phase (350 ns; 2 fs step time).

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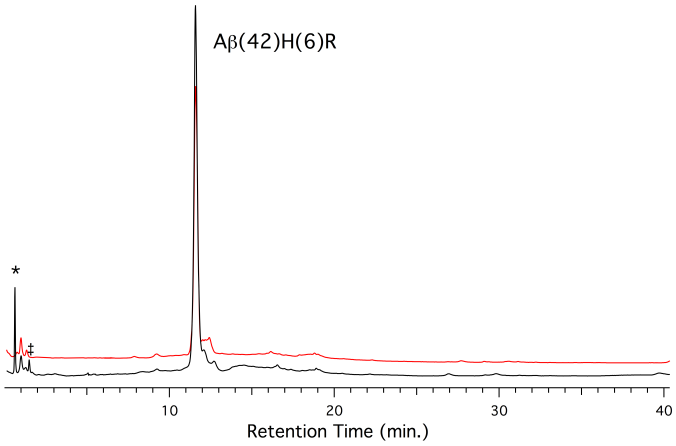


Figure 1: HPLC traces of purified A$\beta$(42)H(6)R (red) and cooper-coordinated A$\beta$(42)H(6)R after reduction and subsequent 48 hour air-exposure (black). The symbol * is the void volume and ‡ is ascorbate. HPLC conditions: C-4 column (15 micron particle size; 250 $\times$ 4.6 mm); flow-rate = 1.0 mL/min.; solvent A = 0.05% $NH_4HCO_3$(aq.), solvent B = 80:20 $CH_3CN$:0.05% $NH_4HCO_3$(aq.); gradient: 85:15 A:B to 40:60 A:B over 35 min and then held at 40:60 A:B for 10 min.

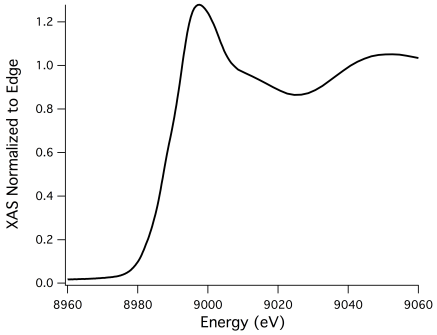


Figure 2: XANES region of the Cu K-edge X-ray absorption spectrum of $\{Cu^{II}(A\beta(42)H(6)R)\}$ oligomers.

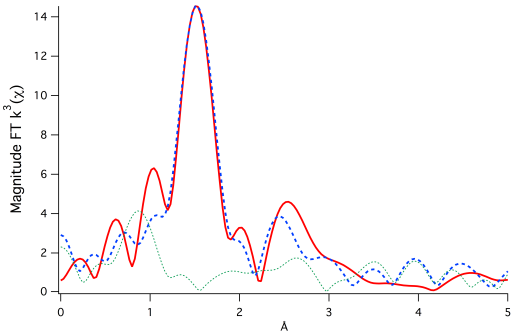


Figure 3: Magnitude FT $k^3(\chi)$ of $\{Cu^{II}(A\beta(42)H(6)R)\}$ oligomers (FT from 2.0 – 12.0 k). The red spectrum represents the experimental data, the blue dashed spectrum is the simulated data and the dashed green line is the difference spectrum. Fit parameters: N/O shell: n = 2.443(17), r = 1.963(4) Å, $\sigma^2$ = 0.0044(7) Å$^2$; Im shell 1: n = 1.07(8), r = 2.02(4), $\sigma^2$ = 0.008(3) Å$^2$, $\phi$ = 17(5)°, $\theta$ = 137(2)°; Im shell 2: n = 0.68(2), r = 1.922(3), $\sigma^2$ = 0.004(2) Å$^2$, $\phi$ = 2(5)°, $\theta$ = 122(6)°; $E_o$ = 8987.1(2) eV. GOF = 0.93.

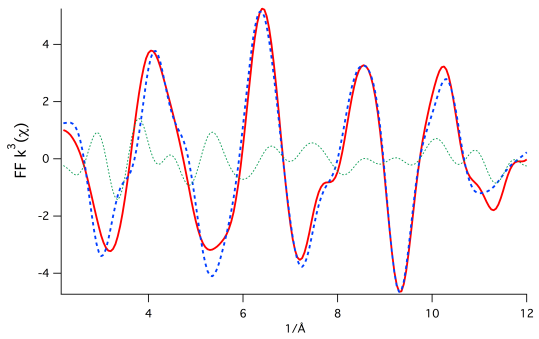


Figure 4: FF $k^3(\chi)$ of $\{Cu^{II}(A\beta(42)H(6)R)\}$ oligomers (FT from 2.0 – 12.0 k; back transformed 1.0 – 4.0 Å). The red spectrum represents the experimental data, the blue dashed spectrum is the simulated data and the dashed green line is the difference spectrum. Fit parameters: N/O shell: n = 2.443(17), r = 1.963(4) Å, $\sigma^2$ = 0.0044(7) Å$^2$; Im shell 1: n = 1.07(8), r = 2.02(4), $\sigma^2$ = 0.008(3) Å$^2$, $\phi$ = 17(5)°, $\theta$ = 137(2)°; Im shell 2: n = 0.68(2), r = 1.922(3), $\sigma^2$ = 0.004(2) Å$^2$, $\phi$ = 2(5)°, $\theta$ = 122(6)°; $E_o$ = 8987.1(2) eV. GOF = 0.93.

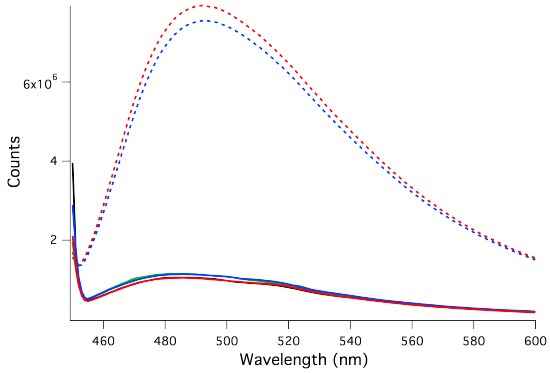


Figure 5: Thioflavin T binding assay. (440 nm excitation; 10 nm emission slit size; 5 nm excitation slit size; 1 nm step size with a 1 sec. integration time per step). All fluorescence measurements were taken using 50 $\mu$M ThT in PBS buffer, pH 7.3. Concentrations of all A$\beta$ peptides were 0.6 mg/mL. The red spectra are for samples of A$\beta$(42)H(6)R, the blue are for samples of A$\beta$(42), the green is A$\beta$(16), and the black are a blank. Solid are for the oligomers that were used in this study (non-fibril oligomers). Dashed line spectra represent solution of soluble oligomers under fibril forming conditions (pre-fibrils), which were not used in this study. Dotted line spectra are for controls (blank and A$\beta$(16)).

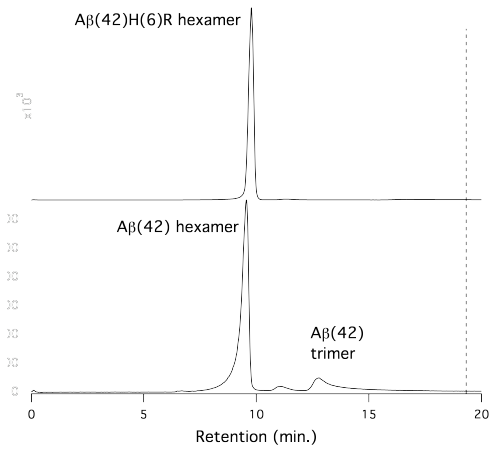


Figure 6: Gel permeation chromatography performed on A$\beta$(42)H(6)R soluble non-fibril oligomers used in this study and A$\beta$(42) soluble non-fibril oligomers. Note the lack of monomeric A$\beta$ in either sample (the calculated monomer retention time is indicated by the vertical dashed line).

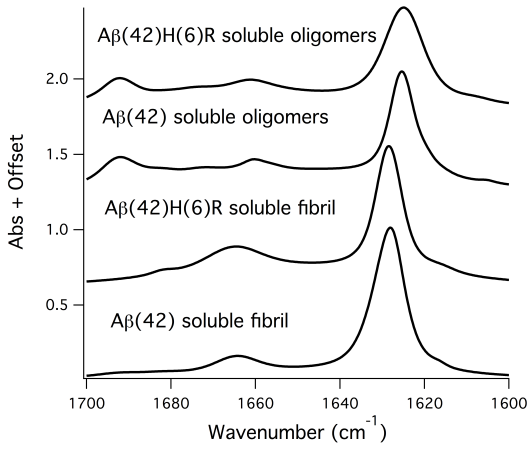


Figure 7: ATR-FTIR spectra of the amide I area for A$\beta$(42)H(6)R and A$\beta$(42) soluble non-fibril oligomers and soluble fibrils. The band at 1696 cm$^{-1}$ is characteristic of non-fibril oligomers.

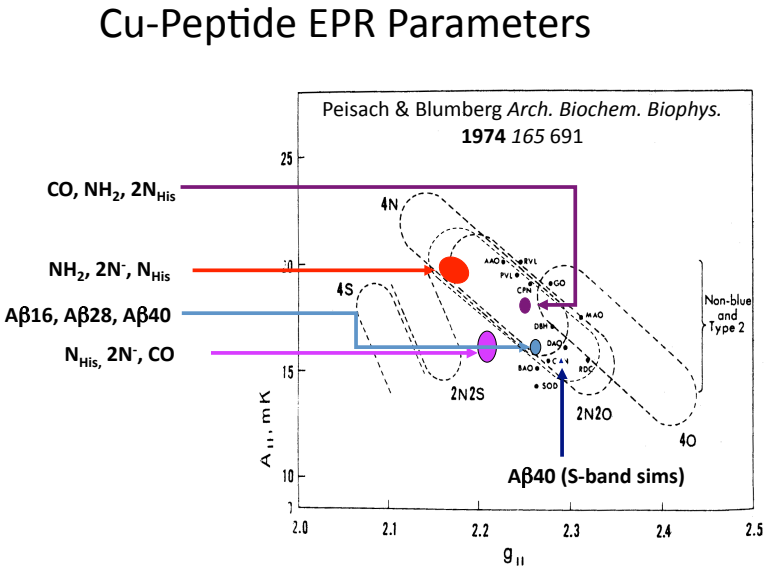


Figure 8: EPR truth tables (*Arch. Biochem. Biophys.* **1974**, *165*, 691) with highlighted regions for wild-type Cu(II)A$\beta$, Cu(II)ATCUN binding motif and Cu(II)A$\beta$16H(6)R. This table was kindly provided by Dr. V. Szalai and modified slightly.