Supporting Information For: Cu K–Edge X–ray Absorption Spectroscopy Reveals Differential Copper Coordination Within Amyloid-β Oligomers Compared to Amyloid–β Monomers

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Reagents. Protein molecular weight marker was from Invitrogen (CA,USA). Acrylamide and electrophoresis grade ammonium persulfate were from FisherBiotech (NJ, USA). Tetramethylethylenediamine (TEMED) and Thioflavin T were from Acros (NJ, USA). Sodium dodecyl sulfate (SDS, 99%) for electrophoresis was from Sigma-Aldrich (MO, USA). The buffer for Thioflavin T fluorescence experiments is 50 mM glycine-NaOH (pH 8.5) and was prepared as previously described.(Karr 2008; LeVine 1999) Samples for gel electrophoresis and EPR spectroscopy were prepared in phosphate buffer containing 20 mM NaP_i, 140 mM NaCl, pH 7.2 with or without 50% glycerol (v/v). The trifluoroacetate salt of Aβ42 was purchased from AnaSpec (San Jose, CA) 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 Aβ42 DMSO stock solution was agitated gently at room temperature for 1 h prior to immediate use or storage at –20 °C.

Preglobumomer preparation. Preglobulomers of Aβ42 were prepared according to the protocol outlined in Yu *et. al.*, except that our buffer pH was 7.2 instead of 7.4.(Yu 2009) The preglobulomers were prepared by combining 11.9 μL of a 5 mM Aβ42 DMSO stock, 45 μL of 2% SDS (w/v), 393.1 μL of buffer with or without 50% glycerol, and water to generate a sample with 0.6 mg Aβ42/mL in 20 mM NaP_i, 140 mM NaCl, pH 7.2. This sample was divided to make three types of samples: an untreated sample, a sample to which Cu^{2+} was added prior to incubation, and a third sample to which Cu^{2+} was added following incubation. The final [Cu^{2+}] was 120 μM in both of the Cu^{2+}-containing samples. Samples were incubated for 6-8 h at 37 °C.

SDS-PAGE Denaturing PAGE was performed as previously described by Barghorn et al.(Barghorn 2005) The gel was run at 200 V for about 45 min at room temperature. A solution of coomassie blue was added to the gel. The gel was then microwaved on high for 20 s at 700W (IEC 60705 rating standard for the microwave output). The stained gel was

incubated for 30 min before destaining with a mixture of ethanol, acetic acid, and water overnight without agitation.

EPR Spectroscopy. Each preglobulomer sample was prepared by combining 5 μL of 5 mM Aβ42 in DMSO, 12.5 μL of 2% SDS in water and 230 μL buffer. The Cu^{2+} before sample, referred to as Cu^{2+} + Aβ42 preglobulomer, was prepared by adding 2.3 μL of a 10.56 mM Cu^{2+} stock in water to the Aβ42/SDS/buffer solution prior to incubation for 6.5 h at 37 °C. The Cu^{2+} after sample referred to as Aβ42 preglobulomers + Cu^{2+} - was prepared by adding 2.3 μL of 10.56 mM Cu^{2+} stock in water after the 6.5 h incubation at 37 °C. The Cu^{2+} stock was as previously described.(Karr 2004) Samples were clear prior to being stored in EPR tubes at -20 °C for 6 days before collecting spectra. After thawing and incubating for 7.5 h at room temperature on the day spectra were collected, a flocculent solid was observed in both tubes. The samples were mixed by inversion three times and then spectra were collected. The Aβ42 preglobulomer + Cu^{2+} sample has less flocculent solid than the Cu^{2+} + Aβ42 preglobulomer sample does. The relative amount of flocculent solid initially present in each sample was not recorded for comparison. .

X-band CW EPR spectra were recorded on a Bruker EMX 6/1 spectrometer equipped with a microwave frequency meter and an Oxford Instruments ESR900 liquid He cryostat system. All spectra were collected with the following experimental parameters: microwave frequency = 9.38 GHz, microwave power = 0.5 mW, modulation amplitude = 10 G, time constant = 40.96 ms, conversion time = 40.96 ms, gain = 5×10^4 (four scans) or 1×10^4 (eight scans), temperature 20 K. Spectra of calibration standards were collected every run and a calibration curve to determine the Cu^{2+} concentration was generated.

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 13-element Ge solid-state fluorescence detector (Canberra). Total count rates were maintained under 20 kHz per channel, and a deadtime correction of 3 μs was utilized (this had a negligible influence on the data). For XANES spectra 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 obtained in 10 eV steps in the pre-edge region (8779 8958 eV), 0.3 eV steps in the edge region (8959 9023 eV), and 2.0 eV steps in the near-edge region. For EXAFS spectra the primary hutch aperture was set to 0.8 mm, the hutch aperture was set to 1 × 8 mm, and data were obtained 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 EXAFS spectra represent the averaged sum of 20 spectra, while the XANES spectra represent the averaged sum of 7 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. 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 (ψ), and the out–of–plane angle (θ). Although data were collected to 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:

$$\text{GOF} = \text{ave}[(\chi - \chi_{\text{sim}})/\text{esd}_{\text{data}}](\text{n}_\text{i}/(\text{n}_\text{i} - \text{n}_\text{p}))^{0.5} \quad (1)$$

where n_i is the number of independent data points and n_p is the number of parameters used in the data simulations.

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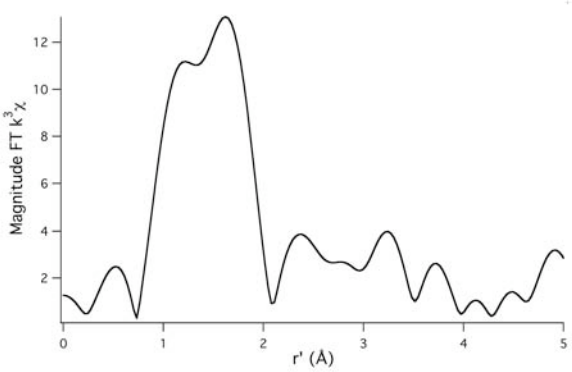

Figure 1: Magnitude FT k^3 EXAFS spectrum for Cu^{1+} loaded Aβ(42) oligomers. A reasonable solution to the EXAFS could not be found.

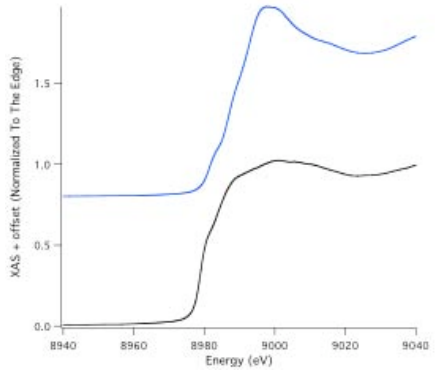

Figure 2: Edge spectra of re–oxidized (top) and re–reduced (bottom) copper–loaded Aβ(42) oligomers.

Table 1: Alternate Fits to the Cu^{II} Loaded Aβ(42) Oligomer EXAFS Data

Fit	N/O Shell	Imidazole Shell #1	Imidazole Shell #2
1			
n	4	N/A	N/A
r	1.984(8) Å		
σ^2	0.0052(12) Å^2		
GOF	1.17		
2			
n	2	1	1
r	1.93(X) Å	1.94(3) Å	2.11(2) Å
σ^2	0.006(2) Å^2	–0.001(3) Å^2	0.008(2) Å^2
θ	N/A	60(3)°	20(2)°
ψ	N/A	46(2)°	44(4)°
GOF	0.87		
3			
n	2	2	N/A
r	2.03(2) Å	1.99(2) Å	
σ^2	0.010(3) Å^2	0.002(1) Å^2	
θ	N/A	20(2)°	
ψ	N/A	54(1)°	
GOF	0.94		
4			
n	1	3	N/A
r	1.989(13) Å	1.97(2) Å	
σ^2	0.004(2) Å^2	0.012(5) Å^2	
θ	N/A	20(1)°	
ψ	N/A	49(4)°	
GOF	1.12		

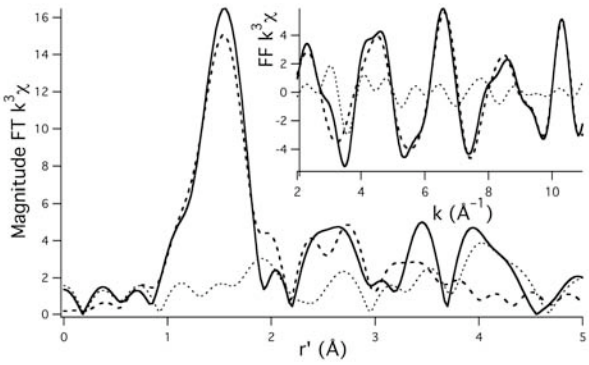

Figure 3: Magnitude FT k^3 EXAFS spectrum for reoxidized Cu^{2+} loaded Aβ(42) oligomers. The solid spectrum is the experimental data, the thick dashed spectrum is the best fit to the experimental data, and the thin dotted line is the difference spectrum. The inset depicts the FF k^3 EXAFS spectrum (FT from 2.0 to 12.0 $Å^{-1}$; backtransformed from 1.0 to 4.0 Å). Best fits to the data: N–shell (n = 1.0; r = 1.948(11)Å; σ^2 = 0.003(1)$Å^2$); 1st imidizole–shell (n = 2.0; r = 1.97(2)Å; σ^2 = 0.006(2)$Å^2$; θ = 5(4)°; ψ = 41(11)°); 2nd imidizole–shell (n = 1.0; r = 2.03(2)Å; σ^2 = 0.002(2)$Å^2$; θ = 31(6)°; ψ = 44(7)°); E_o = 8985.7 eV; GOF = 0.82.

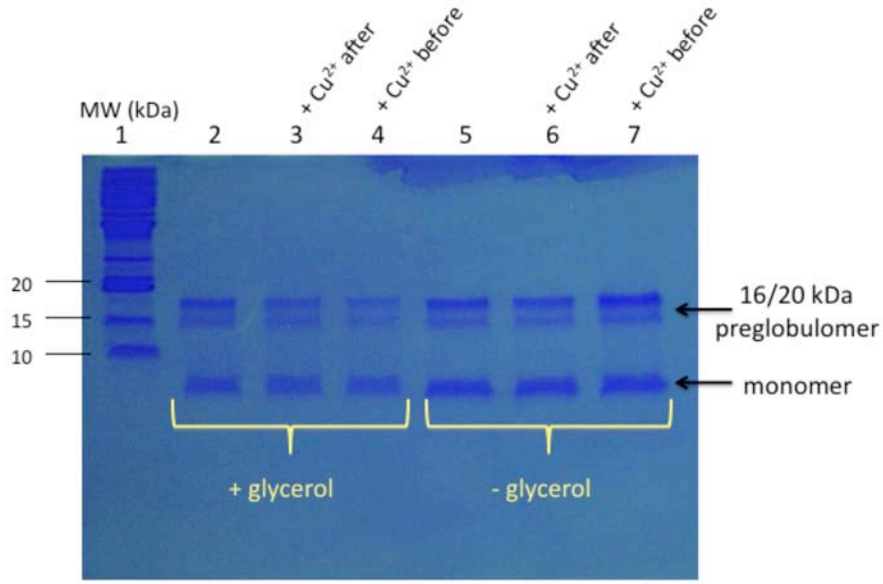

Figure 4: SDS–PAGE of Aβ(42) oligomers. Lane 1: standard protein ladder. Lanes 2 & 5: no Cu^{2+}; lanes 3 & 6: Cu^{2+} added after 6-8 h incubation at 37 °C; lanes 4 & 7: Cu^{2+} added before incubation for 6-8 h at 37 °C.

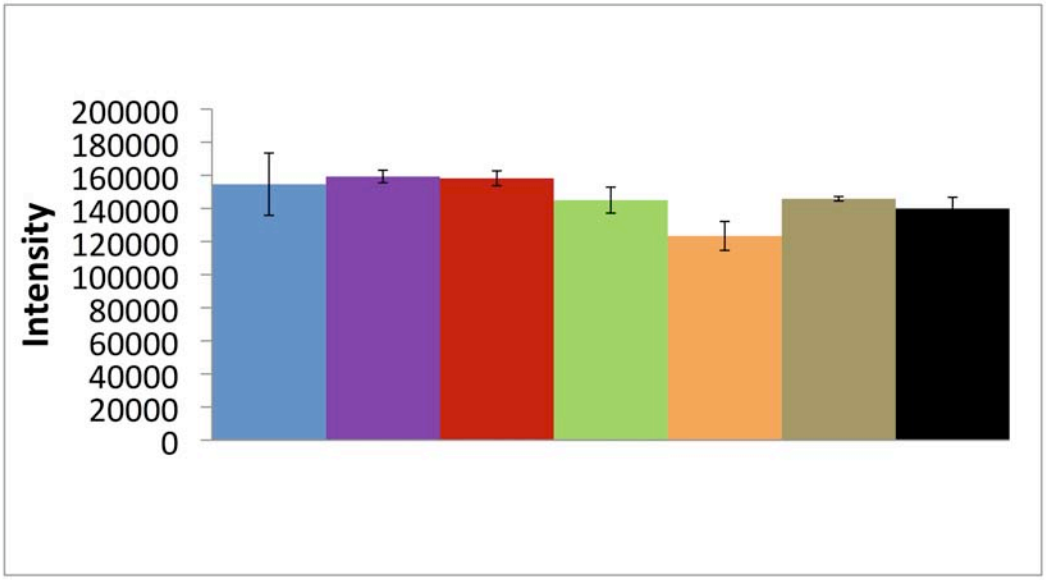

Figure 5: Thioflavin T dye-binding assays on SDS-PAGE samples from Figure 4. From left to right, columns 1 – 3 are for samples prepared in the presence of glycerol; columns 4– 6 are for samples prepared without glycerol. Columns 1 & 4, no Cu^{2+}; columns 2 & 5: Cu^{2+} added after 6 – 8 h 37 °C incubation; columns 3 & 6, Cu^{2+} added prior to 6 – 8 h 37 °C incubation. Column 7 (black) is the control (buffer with ThT dye).