Supporting Information For: One Octarepeate Expansion to the Human Prion Protein Alters Both the Zn^{2+} and Cu^{2+} Coordination Environments Within the Octarepeate Domain

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Metallopeptide Sample Preparation. All peptides were prepared as previously described by solid-phase peptide synthesis and purified by reverse-phase HPLC.(Walter 2006) Fragments that were prepared include the ORP (PHGGWGQ), 2-repeat, 3-repeat, 4-repeat and 5-repeat prion protein fragments ($\{PHGGWGQ\}_n$; n = 2 - 5). Peptide solutions were prepared using 50 mM *N*-ethylmorpholine buffer (pH = 7.4) with concentrations evaluated using the absorption of the W residue at 280 nm (ϵ_{280} = 5690 M^{-1} cm^{-1}). The peptides were then metallated using aliquots of concentrated $CuCl_2$ or $ZnCl_2$ solutions in water. For X-ray absorption studies these solutions were mixed with glycerol forming 1:1 metallopeptide-solution:glycerol samples. Final concentrations (in peptide) for X-ray absorption experiments ranged from 1.2 mM for the ORP samples to ∼0.40 mM for the 5-repeat samples.

Diethyl Pyrocarbonate (DEPC) Labeling Studies. Solutions of metallopeptides (125 μM) were prepared in 25 mM NEM buffer. To 300 μL of the metallopeptide solution 100 μL of a freshly prepared 0.5% v/v aqueous DECP solution was added. The reaction was vortexed for 1 min and was quenched with 100 μL of a 255 mM imidizole soution. The reaction was then analyzed by HPLC and LC-MS (Waters X-bridge C-18 column; 5 micron particle size; 4.5 × 150 mm) using a water:MeCN gradient (0.65% TFA) running from 10-90% over 50 min (1 mL/min flow-rate).

Peroxide Assays. Solutions containing 50 μM metallopeptide (in peptide) or $[Cu^{II}(imidizole)_4]$ $(ClO_4)_2$ (50 mM NEM buffer; pH 7.4) were aerated for 30 min. Ascorbate (300 μM) was then added to solution. Following 10 min. H_2O_2 production was quantified using a peroxide analysis kit (Biomedical Research Services; SUNY Buffalo; Cat # A-109). All analysis was baseline corrected using aerated 50 mM NEM buffer with the addition of 300 μM ascorbate, and normalized to the H_2O_2 produced from $[Cu^{II}(imidizole)_4](ClO_4)_2$. All experiments were repeated in triplicate.

Cu and Zn K–edge X–ray Absorption Data Collection and Measurement. The copper-containing metallopeptide solutions were placed in lucite sample holders and slowly frozen in liquid nitrogen to achieve a near optical glass. 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 or Zn foil (first inflection point assigned to 8980 eV for Cu and 9659 eV for Zn) 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). The primary hutch aperture was set to 0.8 mm and the hutch aperture was set to 1 $\times$ 5 mm. Cu k-edge 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 - 13.0 k). Zn k-edge data were obtained in 5.0 eV steps in the pre-edge region (9459 - 9654 eV), 0.5 eV steps in the edge region (9655 - 9704 eV), 2.0 eV steps in the near-edge region (9705 - 9957 eV), and 5.0 eV steps in the far-edge region (9958 eV - 13.0 k). The EXAFS spectra represent the averaged sum of 6 - 25 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 2010) For Zn single scatterer (SS) and multiple scatterer (MS) pathways the crystal structure of [Zn(bbi)] (Tian 2009) was used as a reference compound. Amplitude and phase functions for Zn SS and MS pathways were constructed as previously described(Shearer 2010) using the XAFS simulation package FEFF 8.20.(Rehr 2000) Each Zn SS pathways were based on eight simulated spectra while the imidazole MS pathway was based on 657 simulated spectra. Metric parameters for the imidizole MS-pathways are defined by three geometric parameters: the Cu/Zn-"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. All data refinements are based on a Fourier-filtered analysis (FT 2.0 – 12 k; BT 1 – 4 Å).

Work Cited

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Table 1: Final Fits to the Cu K-edge EXAFS Data for Cu containing ORP, 2-repeat fragment and 3-repeat fragment

Shell	{Cu^{II}(ORP)}	Cu(II)2-repeat[a]	Cu(II)2-repeat[b]	Cu(II)3-repeat[a]
N/O				
n	3	3	3	2
r (Å)	1.928(3)	1.93(1)	1.93(1)	1.90(1)
σ^2 (Å^2)	0.005(2)	0.004(1)	0.004(1)	0.007(2)
imidizole #1				
n	1	1	1	1
r (Å)	1.98(2)	1.99(1)	1.986(3)	1.97(1)
σ^2 (Å^2)	0.003(1)	0.004(1)	0.003(2)	0.001(4)
ψ (°)	133(21)	138(11)	137(18)	125(3)
θ (°)	12(8)	8(4)	13(5)	10(6)
imidizole #2				
n	N/A	N/A	N/A	1
r (Å)				2.04(1)
σ^2 (Å^2)				0.006(4)
ψ (°)				138(11)
θ (°)				10(9)
E_o (eV)	8990.7	8990.6	8991.0	8990.4
GOF	0.42	0.59	0.53	0.78

a: one equivalent of Cu(II) per peptide; b: two equivalents of Cu(II) per peptide.

Table 2: Final Fits to the Cu and Zn K-edge EXAFS Data for 4-Repeat Prion Fragments

Shell	1 eq Cu(II)	4 eq. Cu(II)	1 eq Cu(II) and Zn(II)[a]	1 eq Cu(II) and Zn(II)[b]
N/O				
n	1	3	3	4
r (Å)	2.02(1)	1.946(4)	1.953(6)	2.086(4)
σ^2 (Å^2)	0.006(2)	0.0041(4)	0.0044(8)	0.0032(5)
imidizole #1				
n	2	1	1	1
r (Å)	1.94(1)	2.053(4)	2.011(3)	2.04(1)
σ^2 (Å^2)	0.0015(10)	0.004(2)	0.008(3)	0.007(2)
ψ (°)	133(4)	124(6)	125(17)	130(4)
θ (°)	9(7)	9(11)	7(2)	1(1)
imidizole #2				
n	1	N/A	N/A	1
r (Å)	2.08(2)			1.998(4)
σ^2 (Å^2)	0.006(2)			0.009(4)
ψ (°)	137(3)			141(4)
θ (°)	11(5)			3(1)
E_o (eV)	8992.8	8990.4	8990.5	9673.5
GOF	0.49	0.37	0.48	0.50

a: Cu K-edge; b: Zn K-edge.

Table 3: Final Fits to the Cu and Zn K-edge EXAFS Data for 5-Repeat Prion Fragments

Shell	1 eq Cu(II)	5 eq. Cu(II)	1 eq Cu(II) and Zn(II)[a]	1 eq Cu(II) and Zn(II)[b]
N/O				
n	2	3	2	2
r (Å)	2.01(1)	1.96(1)	1.92(1)	1.98(1)
σ^2 (Å^2)	0.009(2)	0.009(2)	0.001(1)	0.001(1)
N/O				
n	N/A	N/A	N/A	1
r (Å)				2.14(1)
σ^2 (Å^2)				0.006(2)
imidizole #1				
n	2	1	1	1
r (Å)	1.97(1)	1.999(12)	1.972(3)	1.94(1)
σ^2 (Å^2)	0.005(3)	0.003(10)	0.0014(7)	0.007(2)
ψ (°)	136(5)	129(15)	128(5)	124(8)
θ (°)	6(6)	9(1)	11(8)	1(1)
imidizole #2				
n	1	N/A	1	2
r (Å)	2.09(1)		2.033(5)	2.00(1)
σ^2 (Å^2)	0.001(5)		0.004(2)	0.005(5)
ψ (°)	124(3)		136(7)	131(2)
θ (°)	3(8)		14(6)	0(2)
E_o (eV)	8991.6	8990.8	8990.7	9672.2
GOF	0.86	0.64	0.85	0.52

a: Cu K-edge; b: Zn K-edge.

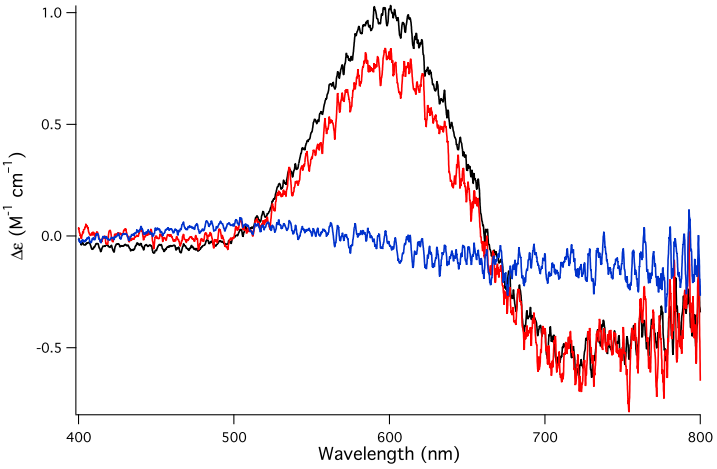

Figure 1: CD spectra of ∼0.5 mM solutions of {Cu^{II}ORP} (black), 4-repeat fragment with 1 eq. of Cu^{2+} added (blue) and the 4-repeat fragment with 1 eq. of Cu^{2+} and 1 eq. of Zn^{2+} added (red). All spectra were acquired in 25 mM NEM buffer, pH 7.4.

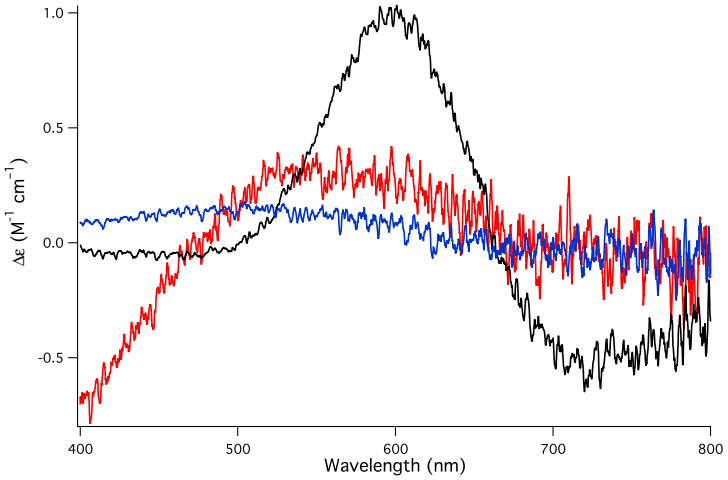

Figure 2: CD spectra of ∼0.3 mM solutions of {Cu^{II}ORP} (black; ∼0.5 mM from figure above), 5-repeat fragment with 1 eq. of Cu^{2+} added (blue) and the 5-repeat fragment with 1 eq. of Cu^{2+} and 1 eq. of Zn^{2+} added (red). All spectra were acquired in 25 mM NEM buffer, pH 7.4.

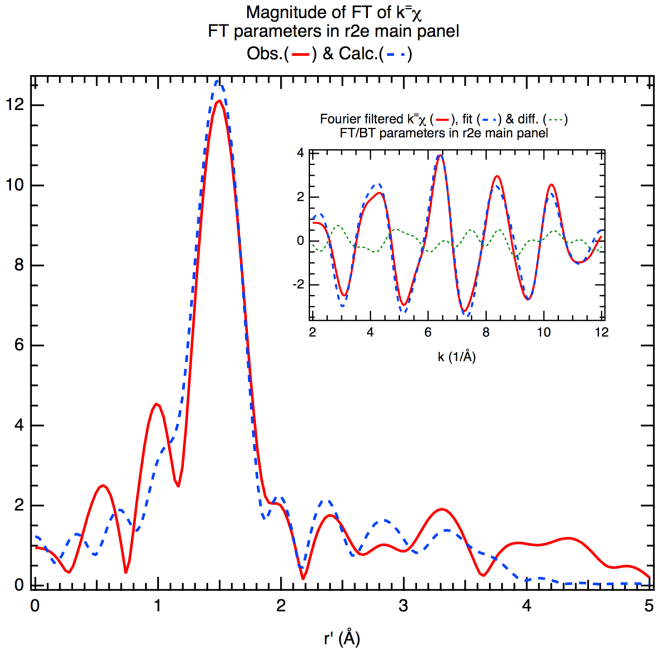

Figure 3: FT and FF (inset) $k^3(chi)$ of {Cu^{II}ORP}. The solid red line represents the data while the dashed blue line represents the best fit. FT from k = 2 - 12$Å^{-1}$; BT from 1 - 4 Å.

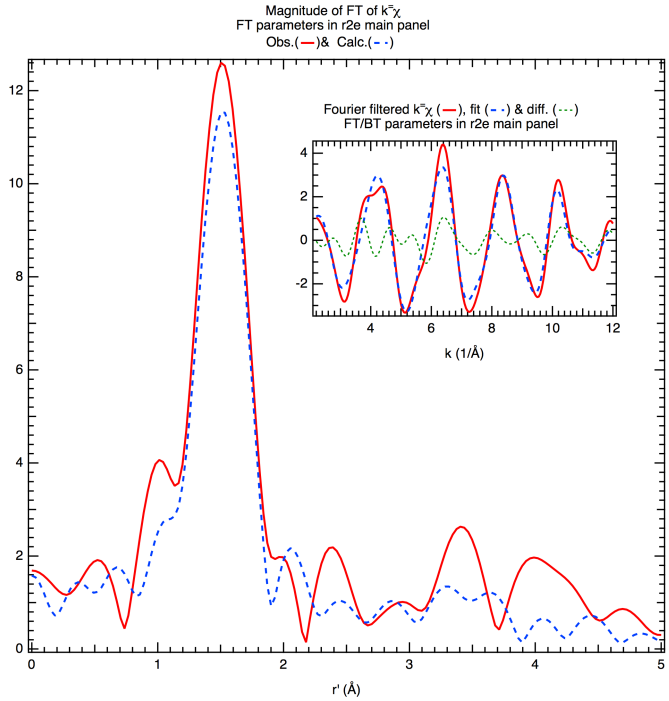

Figure 4: FT and FF (inset) $k^3(chi)$ of the 2-repeat fragment with one eq of Cu(II) added. The solid red line represents the data while the dashed blue line represents the best fit. FT from k = 2 - 12Å^{-1}; BT from 1 - 4 Å.

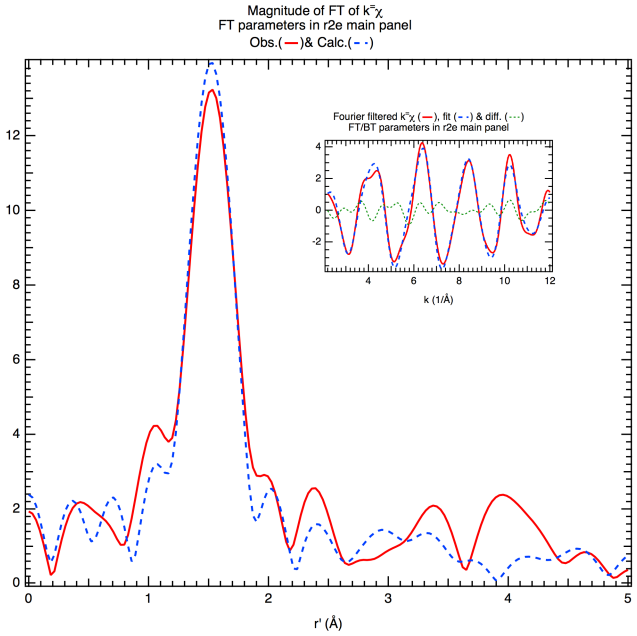

Figure 5: FT and FF (inset) $k^3(chi)$ of the 2-repeat fragment with two eq of Cu(II) added. The solid red line represents the data while the dashed blue line represents the best fit. FT from k = 2 - 12Å^{-1}; BT from 1 - 4 Å.

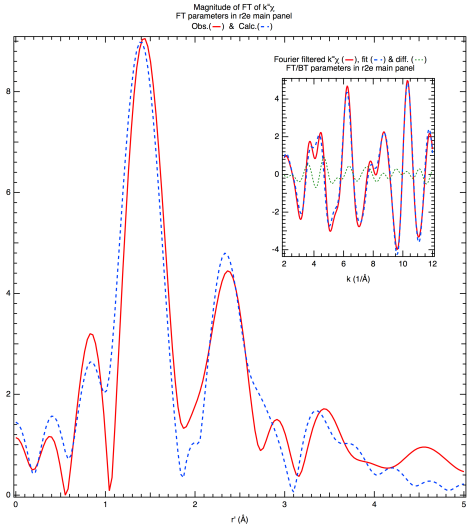

Figure 6: FT and FF (inset) $k^3(chi)$ of the 3-repeat fragment with three eq of Cu(II) added. The solid red line represents the data while the dashed blue line represents the best fit. FT from k = 2 - 12Å^{-1}; BT from 1 - 4 Å.

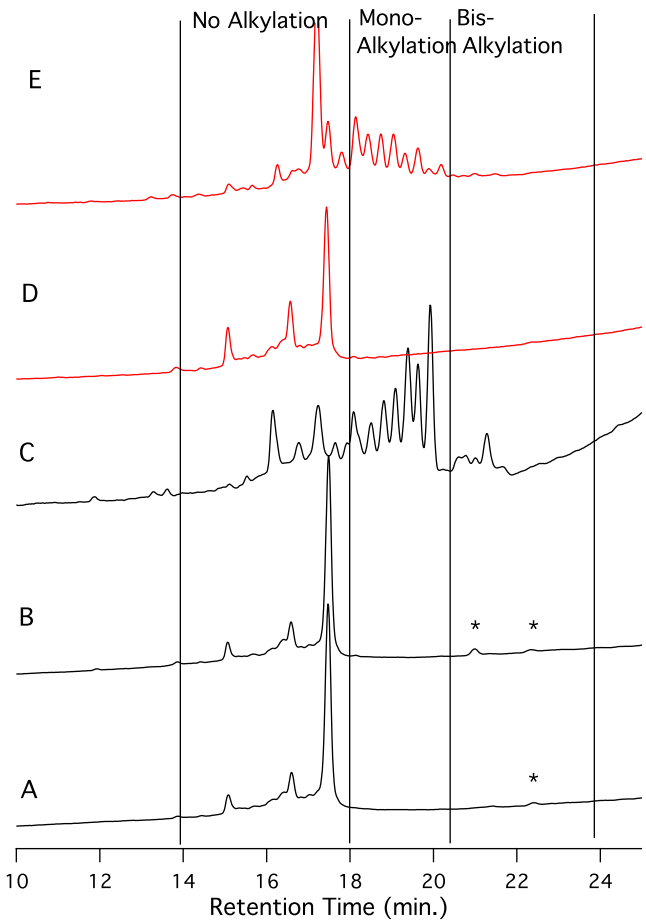

Figure 7: HPLC traces of DECP labeling experiments performed on the 4-repeat PrP fragment. * represents unidentified impurities. Trace A: With 4 equivalents of Cu(II); Trace B: With 4 equivalents of Cu(II) added with DECP treatment; Trace C: With 1 equivalent of Cu(II) added with DEPC treatment; Trace D: With 1 equivalent of Cu(II) and Zn(II) added; Trace E: With 1 equivalent of Cu(II) and Zn(II) added with DEPC treatment.

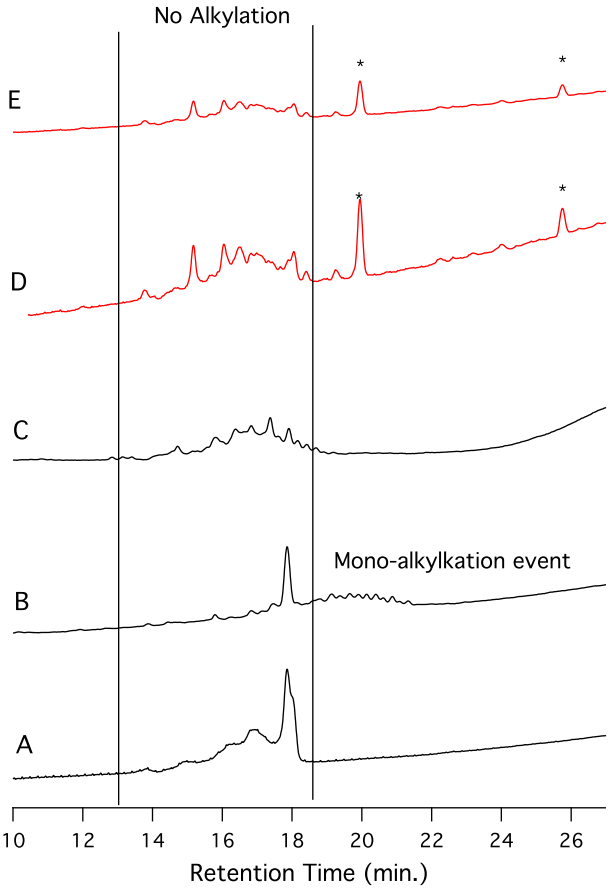

Figure 8: HPLC traces of DECP labeling experiments performed on the 5-repeat PrP fragment. * represents unidentified impurities. Trace A: With 5 equivalents of Cu(II); Trace B: With 1 equivalents of Cu(II) added with DEPC treatment; Trace C: With 5 equivalent of Cu(II) added with DECP treatment; Trace D: With 1 equivalent of Cu(II) and Zn(II) added with DEPC treatment; Trace E: With 1 equivalent of Cu(II) and Zn(II) added.

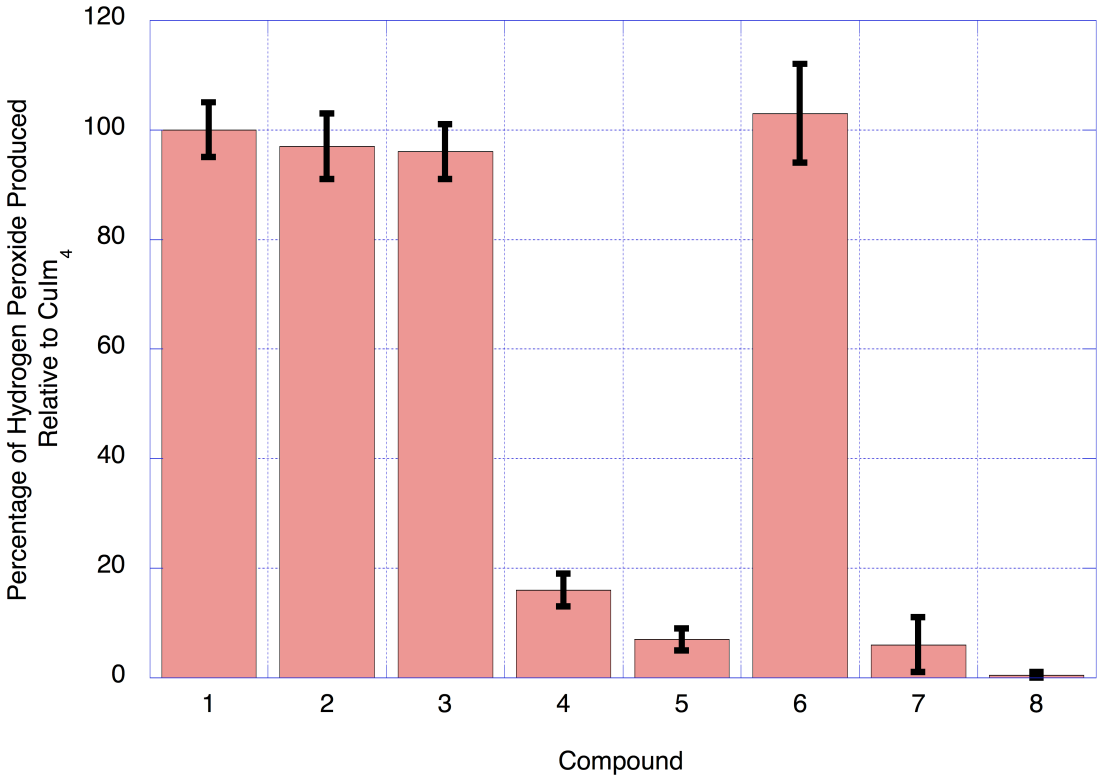

Figure 9: H_2O_2 production studies. Column 1: $[Cu^{II}Im_4]^{2+}$; Column 2: 5-repeat with one equivalent of Cu(II); Column 3: 4-repeat with one equivalent of Cu(II); Column 4: 5-repeat with five equivalents of Cu(II); Column 5: 4-repeat with four equivalent of Cu(II); Column 6: 5-repeat with one equivalent of Cu(II) and Zn(II); Column 7: 4-repeat with one equivalent of Cu(II) and Zn(II); Column 8 $\{Cu^{II}(ORP)\}$.