Electronic Supplementary Material (ESI) for Metallomics
This journal is © The Royal Society of Chemistry 2014

An Integrated Study of the Affinity of the Aβ16 Peptide for Cu(I) and Cu(II): Implications for the Catalytic Production of Reactive Oxygen Species

Tessa R. Young, Angie Kirchner,‡ Anthony G. Wedd and Zhiguang Xiao*

School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria 3010, Australia.

Email: z.xiao@unmelb.edu.au

Fax: +61 3 9347 5180

‡ Internship trainee from Ludwig-Maximilian-University, Munich, Germany.

Supporting Information

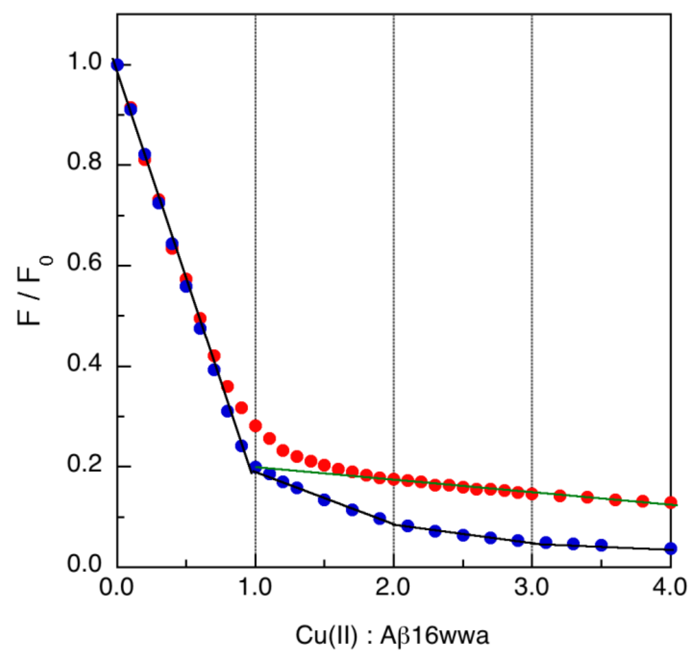

Figure S1. Fluorescence response at 360 nm upon titration of Cu^{2+} (10 μL, 400 μM) into a solution (2.0 mL) of Aβ16wwa (20 μM) in Mops buffer (50 mM, pH 7.4) is shown in blue. The effect of addition of BisTris (1.0 mM) is shown in red.

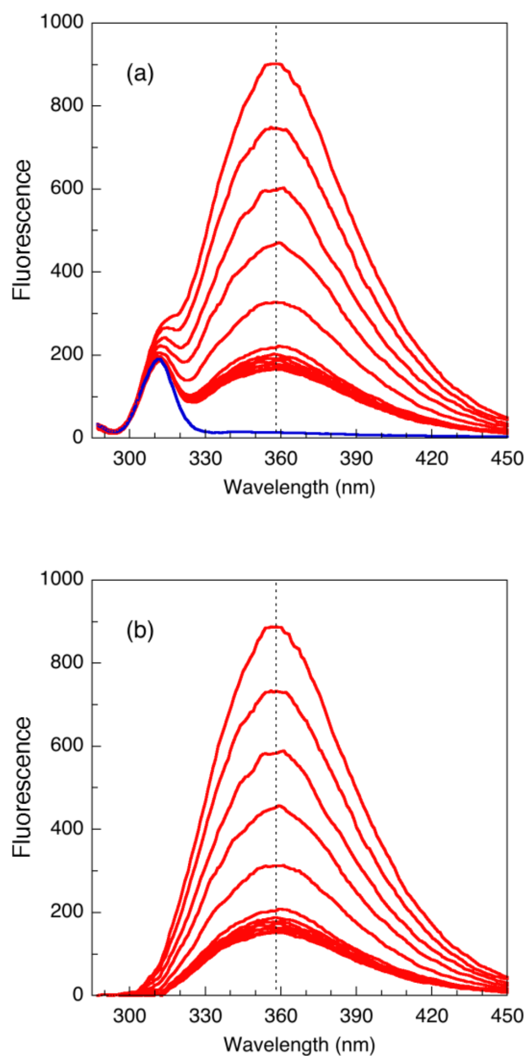

Figure S2. Quenching of fluorescence intensity of Aβ16wwa (0.20 μM) in Mops buffer (1.0 mM, pH 7.4) upon titration with Cu^{2+} (8.0 μM): (a) spectra without buffer background (blue trace) correction; (b) spectra with buffer background correction.

Electronic Supplementary Material (ESI) for Metallomics
This journal is © The Royal Society of Chemistry 2014

Quantification of Cu(II) affinity to Aβ16wwa probe via competition with NTA ligands

NTA reacts with Cu(II) to yield 1:1 complex only with $K_A = 10^{10.7}$ at pH 7.4,[1] and thus the eqs 7-9 for Gly are simplified to eqs S1-S3 for NTA:

$$[Cu^{II}(NTA)] + P \rightleftharpoons Cu^{II}\text{-}P + NTA \quad (S1)$$

$$[Cu(II)]_{tot} = [Cu^{II}(NTA)] + [Cu^{II}\text{-}P] \quad (S2)$$

$$[Cu_{aq}^{2+}] = \frac{[Cu(II)]_{tot} - [Cu^{II}\text{-}P]}{K_A[NTA]} \quad (S3)$$

where [NTA] in eq S3 refers to free NTA concentration in equilibrium eq S1. Other details of experiments and data processing are similar to those for Gly ligand and are detailed in ref [2]. The results are given in Figure S3.

1. R. M. C. Dawson, D. C. Elliot, W. H. Elliot and K. M. Jones, *Data for Biochemical Research*; Clarendon Press: Oxford, UK, 1986.
2. Z. Xiao and A. G. Wedd, The challenges of determining metal-protein affinities, *Nat. Prod. Rep.*, 2010, **27**, 768-89.

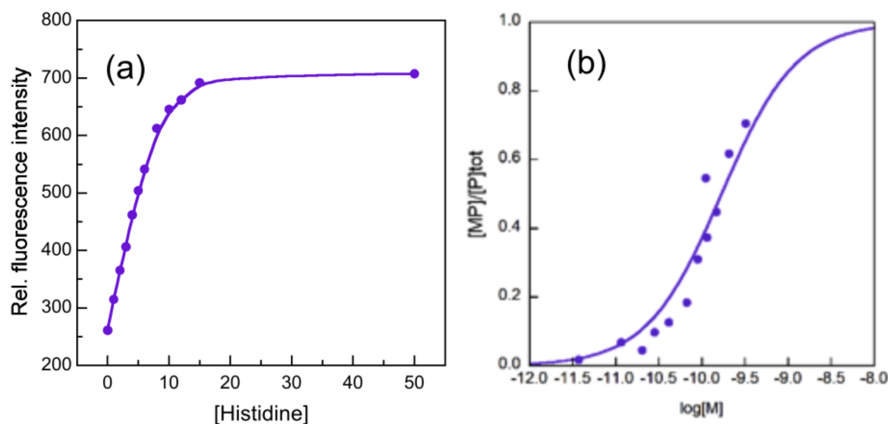

Figure S3. Determination of Cu(II) affinity of Aβ16wwa in Mops buffer (10 mM, pH 7.4) by competition with Cu(II) ligand NTA: (a) recovery of fluorescence intensity for $Cu^{II}_{0.8}$-Aβ16wwa (2.0 μM) with increasing concentration of NTA; (b) plot of corresponding $[Cu^{II}\text{-}P]/[P]_{tot}$ versus $\log[Cu_{aq}^{2+}]$ and fitting of the data to eq 10 estimate $K_D^{II} = 10^{-9.8}$ for Cu^{II}-Aβ16wwa.

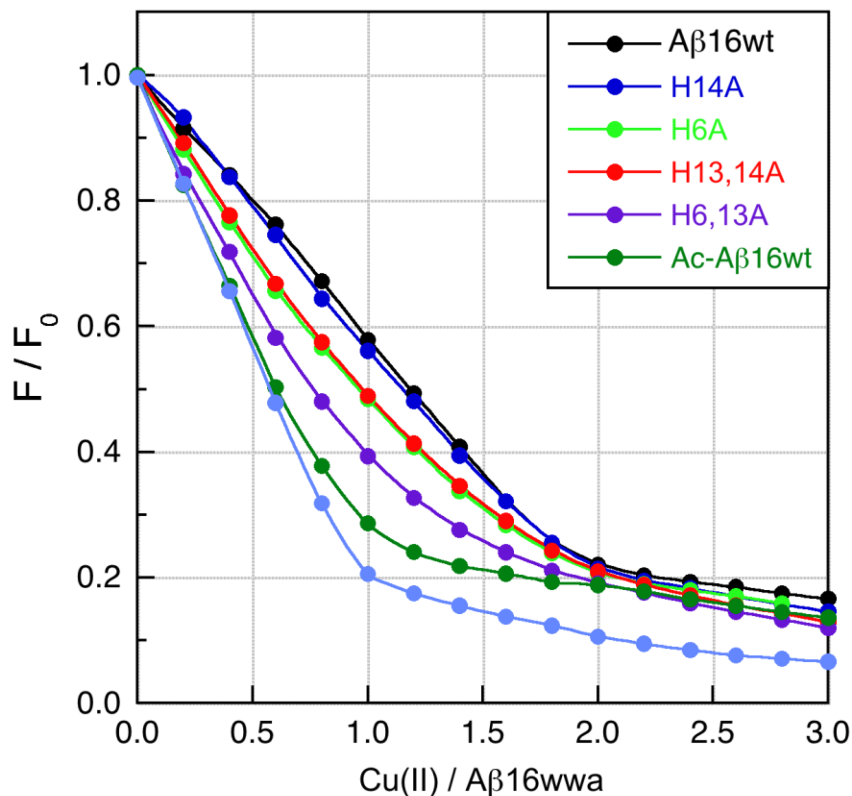

Figure S4. Determination of Cu^{II} dissociation constants (K_D^{II}) for Aβ16 peptides using Aβ16wwa as fluorescence probe at pH 7.4. The experiments were conducted by titration of Cu^{2+} (10 μL, 80 μM) into a solution (2.0 mL) containing either Aβ16wwa alone (2.0 μM; pale blue circles) or both Aβ16wwa and the target peptide (each 2.0 μM) in Mops buffer (10 mM, pH 7.4). Each target peptide is identified in the inset. The complete set of K_D^{II} values derived is summarised in Table 3.

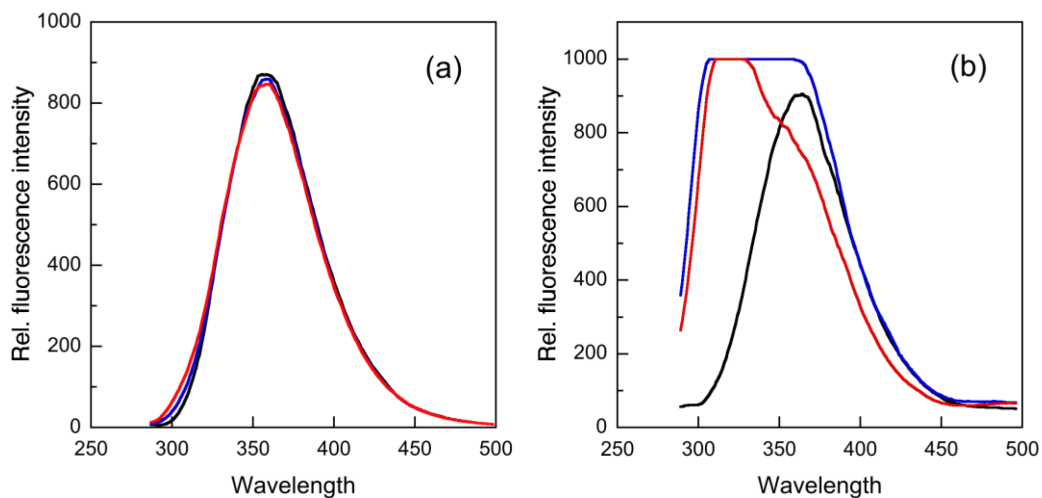

Figure S5. (a) fluorescence spectra in Mops buffer (10 mM, pH 7.4) of Aβ16wwa (2.0 μM; black trace); plus Aβ16-wt (1.0 eq, blue trace; 2.0 eq, red trace); (b) fluorescence spectra of Aβ16wwa (1.0 μM; black trace); plus 20 eq of Aβ16-wt (20 μM, blue trace); further addition of 0.8 eq of Cu^{II} (0.8 μM, red trace).

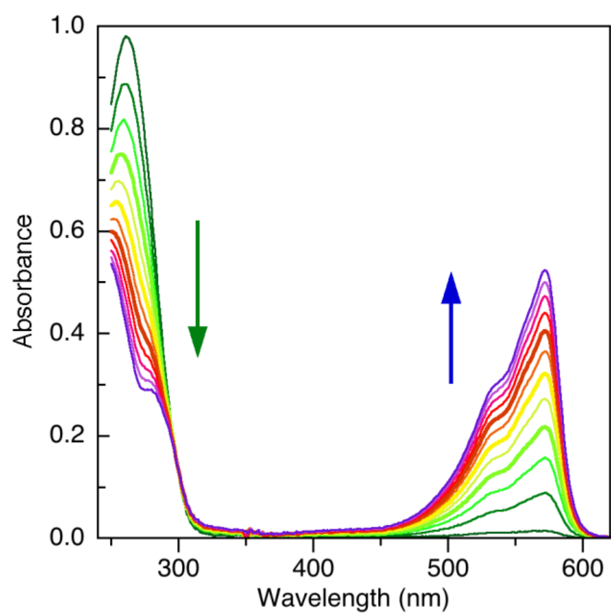

Figure S6. UV-Vis monitoring of catalytic aerobic oxidation of Asc (via absorbance decay at 265 nm) and production of H_2O_2 (via absorbance increasing at 571 nm for resorufin formation). See Figure 8 legend for the experimental conditions.

Table S1. LogK_D^I determined for two controlled CopK proteins within the pH range 5.5 - 7.9 using $[Cu^I(Fs)_2]^{3-}$ as probe [a]

Buffer	pH	Log($K_D^{I\prime}$)	
		CopK-wt	CopK-H70F
Mes	5.5		−10.94
	5.6	−11.07	
	6.3		−10.73
	6.3	−11.00	
Mops	6.9	−11.02	
	7.4	−10.88	
	7.5		−10.65
	7.7	−10.88	
	7.9		−10.78

[a] The probe $[Cu^I(Fs)_2]^{3-}$ compositions: $[Cu]_{tot}$ = 30 μM; $[Fs]_{tot}$ = 280 μM.