

Supporting information

Impact of N-Truncated A β Peptides on Cu- and Cu(A β)-Generated ROS: Cu(I) Matters!

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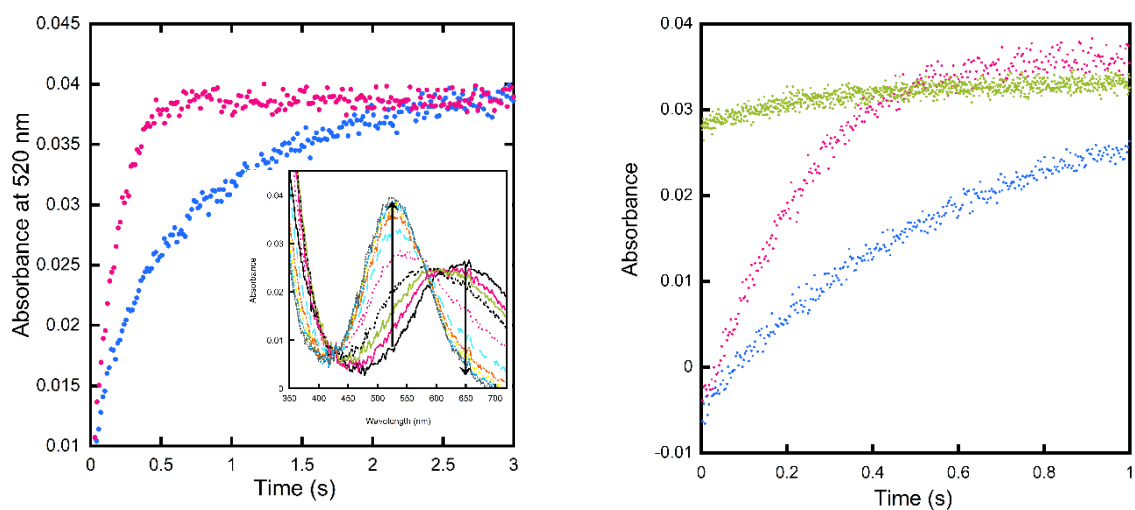


Figure S1 : Stopped-flow Kinetic traces of Cu coordination by the peptides A β_{4-16} (blue), A β_{11-16} (pink) and A β_{1-16} (green). *Left*: Representative stopped-flow traces showing UV-vis changes of the absorbance at 520 nm (λ_{max} of the Cu^{II}(A $\beta_{4/11-16}$) upon mixing Cu and A β_{4-16} (blue) and A β_{11-16} (pink) reflecting the formation of the complexes Cu^{II}(A β_{4-16}) and Cu^{II}(A β_{11-16}). The inset shows the typical evolution of the UV-vis, the arrows indicates the disappearing of the “free” copper and the appearance of the Cu^{II}(A β_{4-16}) complex, only the UV-vis spectra recorded at 0.006, 0.051, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3 s are shown for clarity. *Right*: Representative stopped-flow traces showing UV-vis changes of the absorbance at 630 nm corrected at 440 nm upon mixing Cu and A β_{1-16} (green), data recorded for A β_{4-16} (blue), A β_{11-16} (pink) are shown for comparison. The formation of the Cu^{II}(A β_{1-16}) is too fast to be measured with our system < 10 ms. Experiments were carried out in HEPES (100 mM, pH 7.4). Final Cu concentration was 416.5 μM and final peptide concentration was 500 μM .

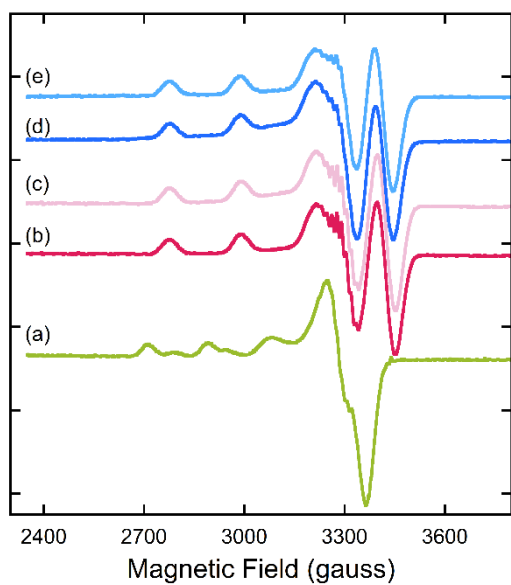


Figure S2 : X-band EPR spectra of (a) $\text{Cu}^{\text{II}}(\text{A}\beta_{1-16})$, (b) $\text{Cu}^{\text{II}}(\text{A}\beta_{11-16})$, (c) competition experiments between $\text{A}\beta_{1-16}$ and $\text{A}\beta_{11-16}$, $\text{Cu}(\text{II}) + \text{A}\beta_{1-16} + \text{A}\beta_{4-16}$, (d) $\text{Cu}^{\text{II}}(\text{A}\beta_{4-16})$, (e) competition experiments between $\text{A}\beta_{1-16}$ and $\text{A}\beta_{4-16}$, $\text{Cu}(\text{II}) + \text{A}\beta_{1-16} + \text{A}\beta_{11-16}$. Experimental conditions $[\text{A}\beta_{1/4/11-16}] = 200 \mu\text{M}$, $[\text{Cu}^{\text{II}}] = 180 \mu\text{M}$, $[\text{HEPES}] = 50 \text{ mM}$, $\text{pH } 7.4$. 10% of glycerol was used as cryoprotectant. $T = 110 \text{ K}$. The EPR parameters are listed in the Table 1 in the main text.

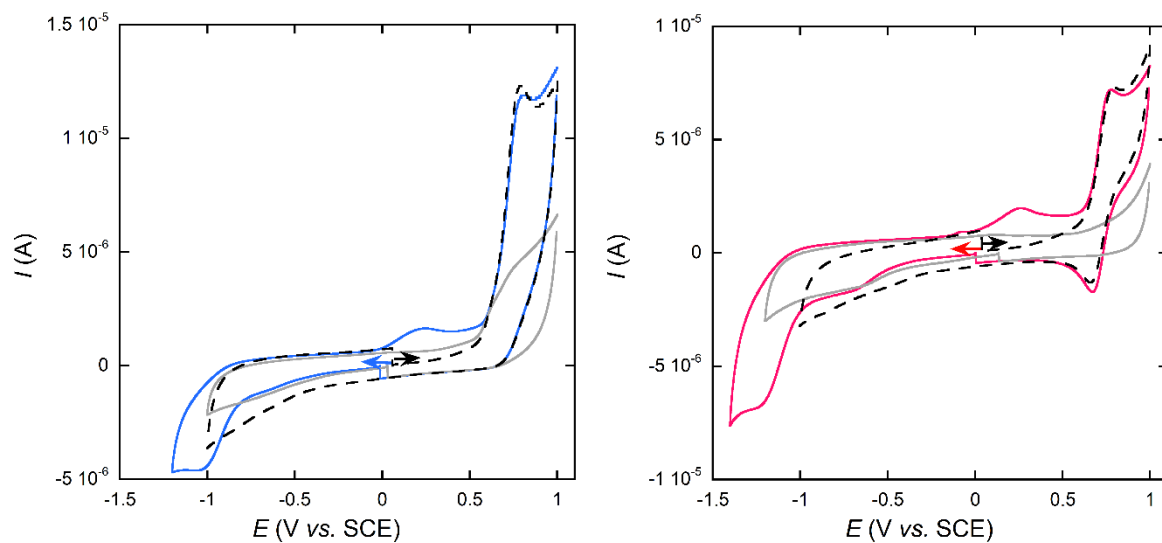


Figure S3 : Cyclic voltammogram (CV) of $\text{Cu}^{\text{II}}(\text{A}\beta_{11-16})$ (pink line) and $\text{Cu}^{\text{II}}(\text{A}\beta_{4-16})$ (blue line). The dotted black lines are CV measured in oxidation showing no reduction process at 0.24 V vs SCE. The grey line are the CV of the peptide alone (without $\text{Cu}(\text{II})$) $[\text{A}\beta_{4/11-16}] = 0.2 \text{ mM}$, $[\text{Cu}(\text{II})] = 0.19 \text{ mM}$ in [phosphate buffer] = 50 mM at pH 7.4 under Ar. Scan rate = $100 \text{ mV}\cdot\text{s}^{-1}$; WE = Glassy carbon, Ref = SCE, CE = Pt wire. First scan are shown starting from the open circuit potential.

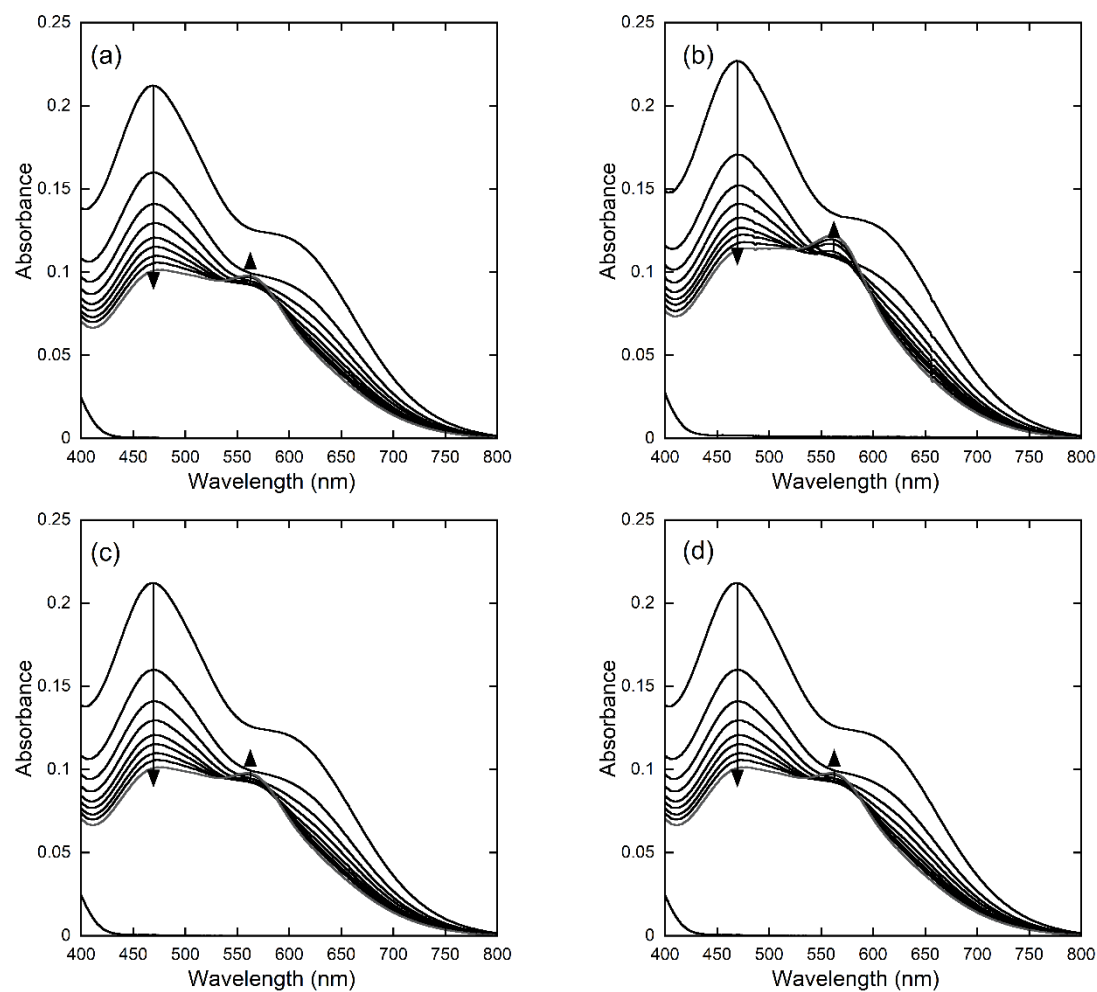


Figure S4 : UV-vis spectra of successive additions of $A\beta_{4-16}$ and $A\beta_{11-16}$ (ca. 100 nM per addition) into a solution of $[Cu^I(Fz)_2]^{3-}$ (55 μM) in HEPES (100 mM, pH 7.4). First spectrum correspond to $[A\beta_{4/11-16}] = 0 \mu M$. The solid arrow indicates the decrease of the 470 nm absorption band characteristic of the $[Cu^I(Fz)_2]^{3-}$ complex. The dashed arrow indicates the increase of the 562 nm absorption band characteristic of the $[Fe^{II}(Fz)_3]^{4+}$ complex, its formation is due to the presence of very small amount of Fe in the peptide batches. Each experiment has been done in triplicate. As a control the affinity constants of the $Cu^I(A\beta_{11-16})$ species has been measure the same day and gave consistent data with published results.

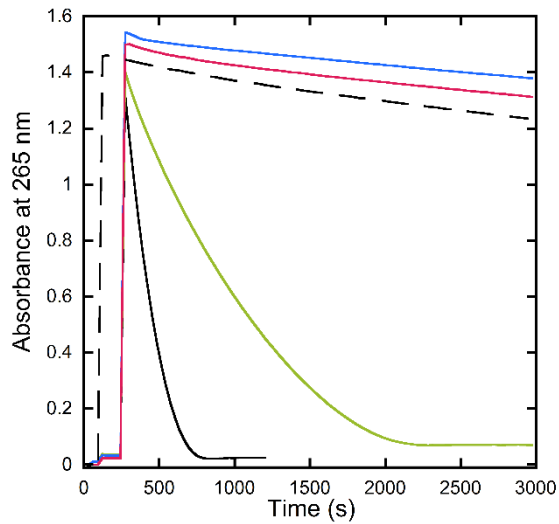


Figure S5: Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Asc alone (dotted black curve), Cu(II) + Asc (black curve), Cu(II) + A β_{1-16} + Asc (green curve), Cu(II) + A β_{11-16} + Asc (pink curve), Cu(II) + A β_{4-16} + Asc (blue curve). [A $\beta_{1/4/11-16}$] = 12 μ M, [Cu(II)] = 10 μ M, [Asc] = 100 μ M, [HEPES] = 100 mM, pH 7.4.

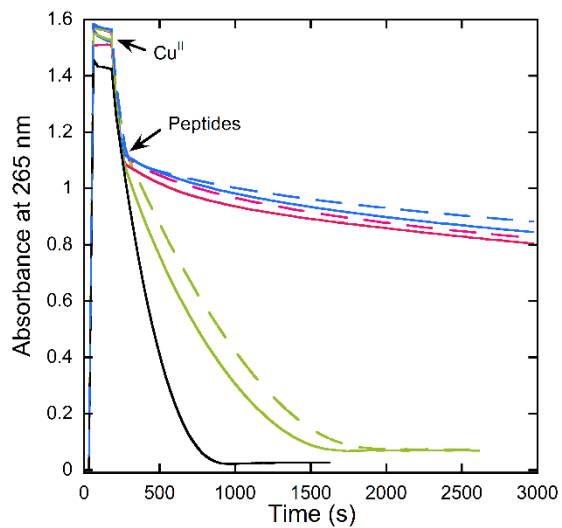


Figure S6 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Asc + Cu(II) + A β_{1-16} (dotted green curve), Asc + Cu(II) + A β_{11-16} (dotted pink curve), Asc + Cu(II) + A β_{4-16} (dotted blue curve). [A $\beta_{1/4/11-16}$] = 20 μ M, [Cu(II)] = 10 μ M, [Asc] = 100 μ M, [HEPES] = 100 mM, pH 7.4. The other curves are the curves of found in figure 3 in the main text for comparison.

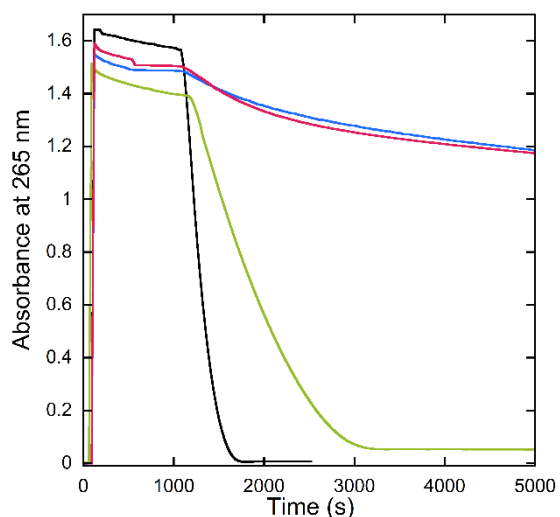


Figure S7 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Cu(II) + Asc (black curve), Cu(II) + Asc + A β_{1-16} (green curve), Cu(II) + Asc + A β_{1-16} (pink curve), Cu(II) + Asc + A β_{4-16} (blue curve). [A $\beta_{1/4/11-16}$] = 12 μ M, [Cu(II)] = 10 μ M, [Asc] = 100 μ M, [HEPES] = 100 mM, pH 7.4. All the solutions were deoxygenated by bubbling argon and were added to a sealed UV-vis cuvette under a little overpressure of argon in order to keep Cu under its +I oxidation state, generated by the reduction of the Cu(II) initially present by ascorbate. The cuvette was open at 1120 s and air bubble inside.

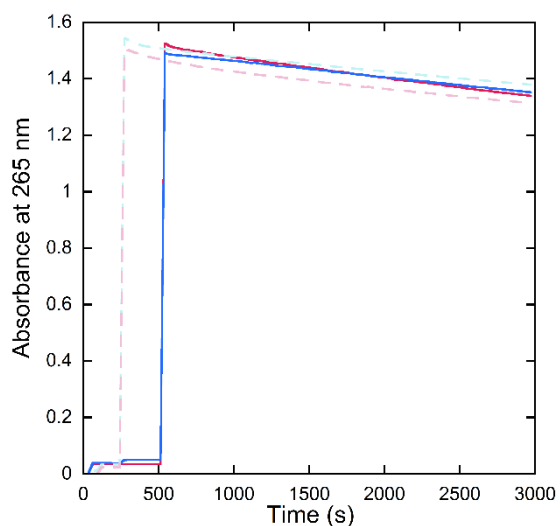


Figure S8 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Cu(II) + A β_{11-16} + Asc (dashed pink curve), Cu(II) + A β_{4-16} + Asc (dashed blue curve), Cu(II) + equimolar mixture of A β_{11-16} and A β_{1-16} + Asc (pink curve), Cu(II) + equimolar mixture of A β_{4-16} and A β_{1-16} + Asc (blue curve). [A $\beta_{1/4/11-16}$] = 12 μ M, [Cu(II)] = 10 μ M, [Asc] = 100 μ M, [HEPES] = 100 mM, pH 7.4. The dotted lines are the data presented in Figure S6 (Cu(II) + peptides), for comparison purpose.

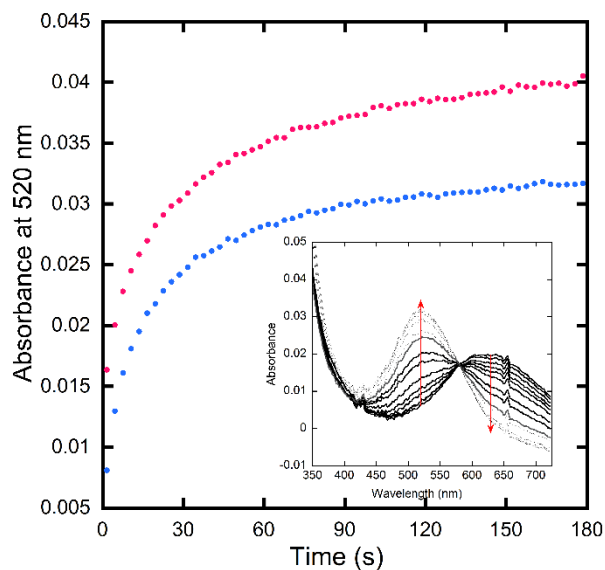


Figure S9 : Stopped-flow Kinetic traces of Cu coordination by the peptides A β_{4-16} (blue) and A β_{11-16} (pink). Representative stopped-flow traces showing UV-vis changes of the absorbance at 520 nm upon mixing Cu and A β_{4-16} (blue) and A β_{11-16} (pink) reflecting the formation of the complexes Cu^{II}(A β_{4-16}) and Cu^{II}(A β_{11-16}). Experiments were carried out in HEPES (100 mM, pH 7.4). Final Cu concentration was 500 μ M and final peptide concentration was 500 μ M.

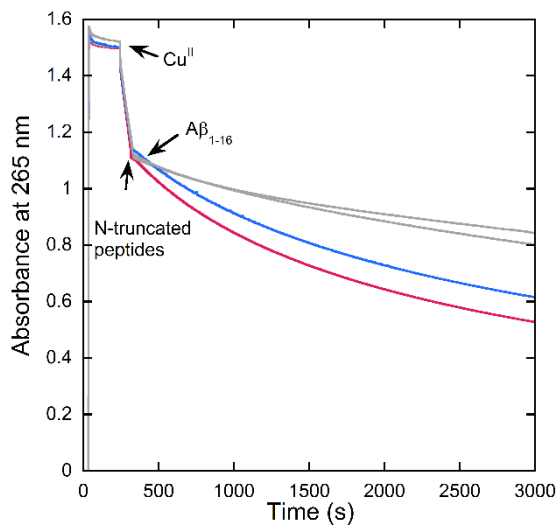


Figure S10 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Asc + Cu(II) + A β_{11-16} + A β_{1-16} (pink curve), Asc + Cu(II) + A β_{4-16} + A β_{1-16} (blue curve). The arrows indicate the order and time of the different addition into the UV-vis cuvette. [A $\beta_{1/4/11-16}$] = 12 μ M, [Cu(II)] = 10 μ M, [Asc] = 100 μ M, [HEPES] = 100 mM, pH 7.4. The grey lines are data equivalent to the one presented Figure 3 (Cu(II/I) + peptides), for comparison purpose.

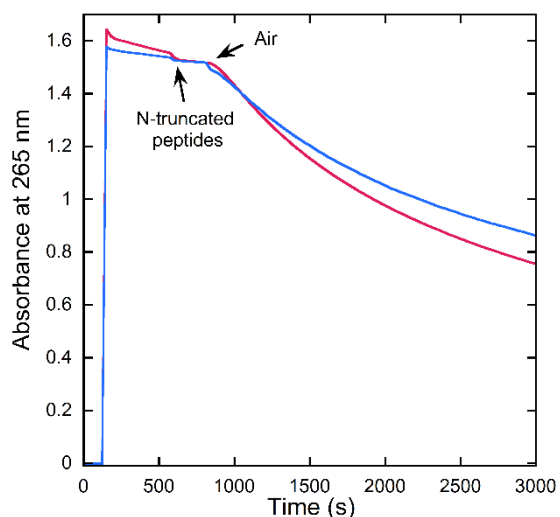


Figure S11 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. $\text{Cu}^{\text{I}}(\text{A}\beta_{1-16}) + \text{A}\beta_{4-16}$ (blue curve) and $\text{Cu}^{\text{I}}(\text{A}\beta_{1-16}) + \text{A}\beta_{11-16}$ (pink curve). All the solutions were deoxygenated by bubbling argon and were added to a sealed UV-vis cuvette under a little overpressure of argon in order to keep Cu under its +I oxidation state. The $\text{Cu}^{\text{I}}(\text{A}\beta_{1-16})$ complex was generated *is situ* from the reduction of $\text{Cu}^{\text{II}}(\text{A}\beta_{1-16})$ by ascorbate, the N-truncated peptides were added at 600 s and the UV-vis cuvette was open to air at 950 s, air was then bubbled into the cuvette. $[\text{A}\beta_{1/4/11-16}] = 12 \mu\text{M}$, $[\text{Cu}(\text{II})] = 10 \mu\text{M}$, $[\text{Asc}] = 100 \mu\text{M}$, $[\text{HEPES}] = 100 \text{mM}$, pH 7.4.

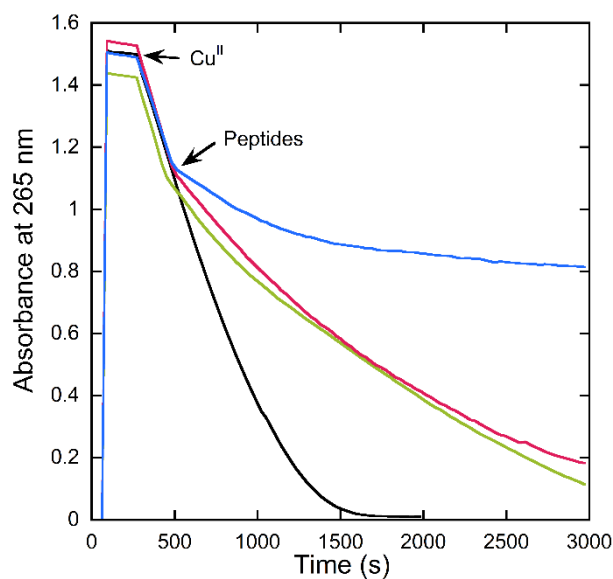


Figure S12 : Kinetics of ascorbate consumption, followed by UV-visible spectroscopy at 265 nm. Asc + Cu(II) (black curve), Asc + Cu(II) + $\text{A}\beta_{1-16}$ (green curve), Asc + Cu(II) + $\text{A}\beta_{11-16}$ (pink curve), Asc + Cu(II) + $\text{A}\beta_{4-16}$ (blue curve). $[\text{A}\beta_{1/4/11-16}] = 12 \mu\text{M}$, $[\text{Cu}(\text{II})] = 10 \mu\text{M}$, $[\text{Asc}] = 500 \mu\text{M}$, $[\text{HEPES}] = 100 \text{mM}$, pH 7.4, the experiments were realized in a 2 mm path length UV-vis cuvette. The arrows indicate the time of additions.