

Supporting Information

Experimental Section

Chemicals and methods. All solvents and commercially available reagents were purchased from Sigma-Aldrich, Fluka, Acros, or TCI, except A β ₁₋₁₆ that was purchased from Genecust. They were used without further purification. Cu(II)- and Zn(II) chloride or sulfate were used as source of metal ions. A β ₁₋₁₆ stock solution was prepared by dissolving the peptide in water, and the concentration was determined by UV-visible spectroscopy [$\epsilon_{276}(\text{Tyr10}) = 1410 \text{ M}^{-1} \text{ cm}^{-1}$].¹ UV-visible spectra were recorded from 200 to 600 nm, at 25 °C, on an Agilent 8453 spectrophotometer equipped with a magnetic stirrer, and a temperature controller. PBT2 was prepared as previously reported.² TDMQ20 and ligand **1** was prepared according to References 3 and 4, respectively. Except otherwise noted, the stock solutions were as follows: TDMQ20 (5 mM in H₂O), A β ₁₋₁₆ (3.37 mM in H₂O), CuCl₂ (3 mM in H₂O), ZnCl₂ (3 mM in H₂O), **1** (2.5 mM in DMSO), PBT2 (3 mM in MeOH), GSH (50 mM in H₂O), and sodium ascorbate (30 mM in H₂O). For each experiment, a fresh solution of glutathione or ascorbate was prepared.

Extraction of Cu(II) from A β ₁₋₁₆. The Cu^{II}/A β ₁₋₁₆ complex (molar ratio 1/1.1) was prepared by mixing adequate volumes of the metal salt (10 μL) and A β ₁₋₁₆ (8.9 μL) stock solutions, and incubated for 15 min at room temperature. The solution of ZnCl₂ (10 μL) was then added, and the mixture was incubated for a further 15 min. The solution of TDMQ20 (6 μL) was then added. The mixture was diluted in Hepes buffer 50 mM, pH 7.4, and the reaction was monitored by UV-visible spectroscopy. Final concentrations were [A β ₁₋₁₆] = [Cu²⁺] = [Zn²⁺] = [TDMQ20] = 20 μM .

Release of copper from Cu^{II}-TDMQ by glutathione. Under continuous stirring, adequate volumes of the stock solutions were added in the UV-visible cuvette containing Hepes buffer 50 mM, pH = 7.4, in the following order: TDMQ20 (6 μL), CuCl₂ (10 μL), and ZnCl₂ (10 μL). The UV-visible spectrum of the mixture was then recorded to evidence the chelation of Cu(II) by TDMQ20 ($\lambda_{\text{max}} = 243 \text{ nm}$ for Cu^{II}-TDMQ20, and ($\lambda_{\text{max}} = 259$ and 342 nm for TDMQ20 free ligand). 160 to 240 mol equiv of the GSH were then added in the cuvette, by aliquots of 20 equiv, and the spectrum was recorded after each addition of GSH. (Final concentrations

were : [TDMQ20] = [Cu²⁺] = [Zn²⁺] = 20 μM). The total variation of the volume in the cuvette was below 8% upon addition of 100 mol equiv of GSH.

Oxidation of ascorbate. Figure 4. The Cu^{II}-Aβ₁₋₁₆ complex was prepared by mixing adequate volumes of the copper chloride and Aβ₁₋₁₆ stock solutions (molar ratio 1/1.1). The mixture was incubated for 15 min at room temperature. The ZnCl₂ solution was then added, and the mixture was incubated for a further 15 min. A chelator (TDMQ20, **1**, or PBT2) was added, and the mixture was incubated for a further 15 min. The above mentioned mixtures (or CuCl₂, final concentration 10 μM, as a positive control) were then added to the UV cuvette containing sodium ascorbate in Hepes buffer 50 mM, pH 7.4, 25 °C, under continuous stirring. Monitoring of ascorbate absorbance ($\lambda_{\text{max}} = 265 \text{ nm}$, $\epsilon = 14500 \text{ M}^{-1} \times \text{cm}^{-1}$)⁵ was immediately started and continued over 30 min. The experiments without ascorbate were also performed in the same conditions and resulting absorbance was subtracted from measurements with ascorbate. Final concentrations in the cuvette were: [Cu] = 10 μM, [Aβ₁₋₁₆] = 11 μM, [Zn] = 30 μM, [TDMQ20] = [**1**] = 11 μM, [PBT2] = 22 μM, [ascorbate] = 100 μM, final volume = 1.5 mL, concentration of DMSO or MeOH, where present, was below 1 vol%.

Oxidation of ascorbate. Figure 5. The stock solution of ZnCl₂ was 200 mM in H₂O. The Cu^{II}-Aβ₁₋₁₆ complex was prepared by mixing adequate volumes of the copper chloride and Aβ₁₋₁₆ stock solutions (molar ratio 1/1.1). The mixture was incubated for 15 min at room temperature. The ZnCl₂ solution was then added, and the mixture was incubated for a further 15 min. The above-mentioned mixture was then added to the UV cuvette containing sodium ascorbate in Hepes buffer 50 mM, pH 7.4, 25 °C, under continuous stirring. Monitoring of ascorbate absorbance ($\lambda_{\text{max}} = 265 \text{ nm}$, $\epsilon = 14500 \text{ M}^{-1} \times \text{cm}^{-1}$) was immediately started and continued over 30 min (traces a-c). For traces d and e, TDMQ20 or **1**, respectively, was added after 6.6 min of reaction, and the monitoring of absorbance was continued until 30 min of total reaction duration. Final concentrations in the cuvette were: [Cu] = 10 μM, [Aβ₁₋₁₆] = 11 μM, [Zn] = 1000 μM, [TDMQ20] = [**1**] = 11 μM, [ascorbate] = 100 μM, final volume = 1.5 mL, concentration of DMSO or MeOH, where present, was below 1 vol%. The own absorbances at 265 nm of [Cu-Aβ₁₋₁₆ + Zn], [Cu-Aβ₁₋₁₆], CuCl₂, [Cu-Aβ₁₋₁₆ + Zn + TDMQ20], [Cu-Aβ₁₋₁₆ + Zn + **1**] were subtracted from crude data, to provide traces a, b, c, d, and e, respectively.

References

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Figures

Figure S1. Extraction of copper from Cu^{II}-A β ₁₋₁₆ using 1 mol equiv of TDMQ20, in the absence of Zn²⁺ monitored by UV-visible spectrophotometry. Full red trace: Cu-A β ₁₋₁₆ + TDMQ20, bright blue trace: Cu-TDMQ20. Spectra of A β ₁₋₁₆ (grey trace), Cu-A β ₁₋₁₆ (light blue trace), and TDMQ20 (green trace) are given for comparison. The dashed black spectrum results from the mathematical addition of spectra of A β ₁₋₁₆ and Cu-TDMQ20.

Figure S2. Extraction of copper from Cu^{II}-TDMQ20 by glutathione (GSH), in the absence of Zn²⁺, monitored by UV-visible spectrophotometry. The spectrum of the free ligand TDMQ20 (dashed black line) is given for comparison. Insert: Variation of the absorbance at 259 nm as a function of the number of GSH mol equiv with respect to Cu-TDMQ.

Figure S1

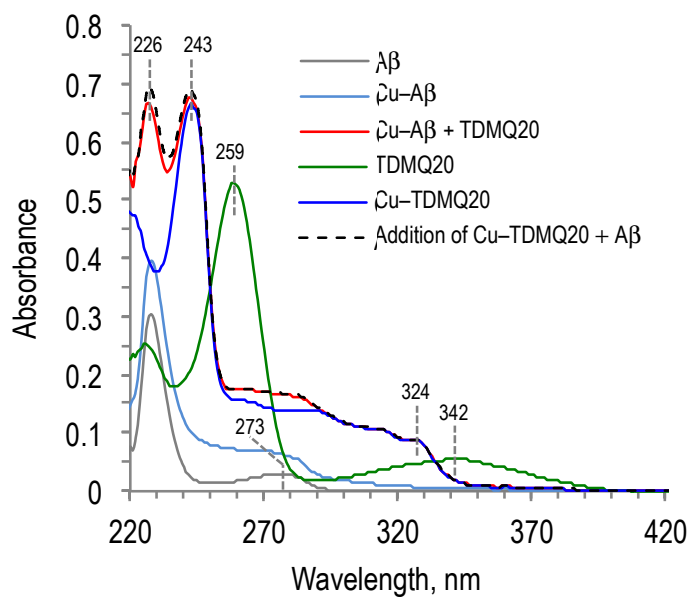


Figure S2

