Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

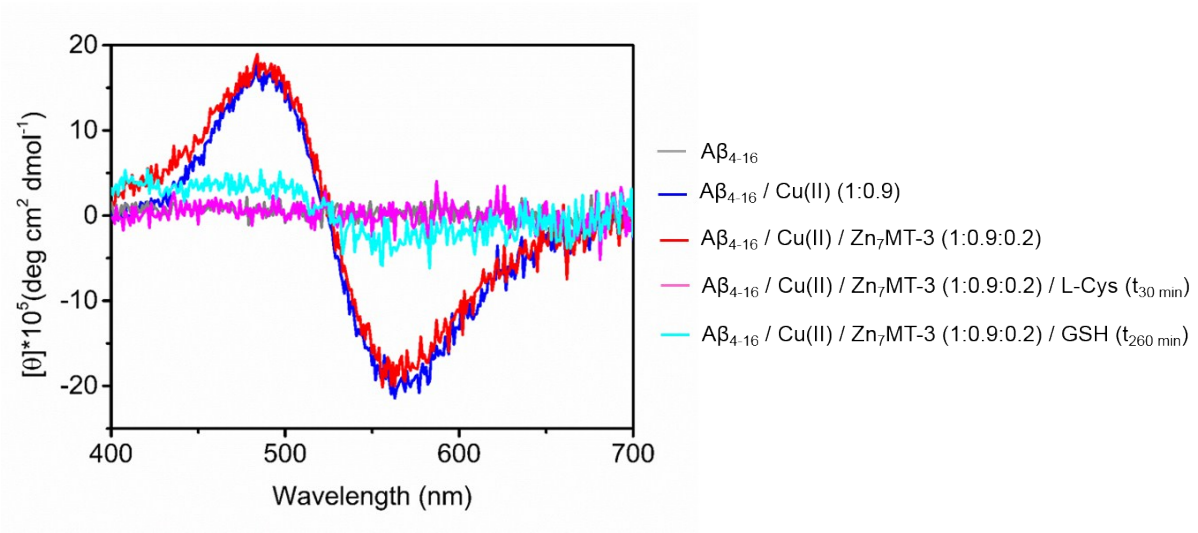


Fig S1. Circular Dichroism Spectroscopy (Vis-region, 400-700 nm) of Cu(II) release from $Cu^{II}(A\beta_{4-16})$ in the presence of Zn_7MT-3 with L-Cys or GSH. CD spectra of $A\beta_{4-16}$ (grey line); $A\beta_{4-16}$ / Cu(II) , 1:0.9 (blue line); $A\beta_{4-16}$ / Cu(II) / Zn_7MT-3, 1:0.9:0.2 (red line) recorded after 30 min from the reaction of $Cu^{II}(A\beta_{4-16})$ with Zn_7MT-3; $A\beta_{4-16}$ / Cu(II) / Zn_7MT-3/ L-Cys (magenta line) recorded after 30 min from L-Cys addiction to the mixture $A\beta_{4-16}$ / Cu(II) / Zn_7MT-3; $A\beta_{4-16}$ / Cu(II) / Zn_7MT-3/ GSH (cyane line) recorded after 260 min from GSH addiction to the mixture $A\beta_{4-16}$ / Cu(II) / Zn_7MT3. In this region both Zn_7MT-3 and $Cu(I)_4Zn(II)_4$MT-3 metallopeptides do not show any characteristic bands. As shown by UV-Vis spectroscopy in case of GSH the reaction was not complete after 260 min. Both the reaction with L-Cys and GSH were performed under the same experimental conditions. Reaction conditions (final concentrations for the reaction mixture $A\beta_{4-16}$ / Cu(II) / Zn_7MT3/ L-Cys or GSH): 500 µM $A\beta_{4-16,}$ 450 µM Cu(II), 100 µM Zn_7MT3, 3 mM L-Cys or GSH. The measurements were performed in phosphate buffer 50 mM, pH 7.4.

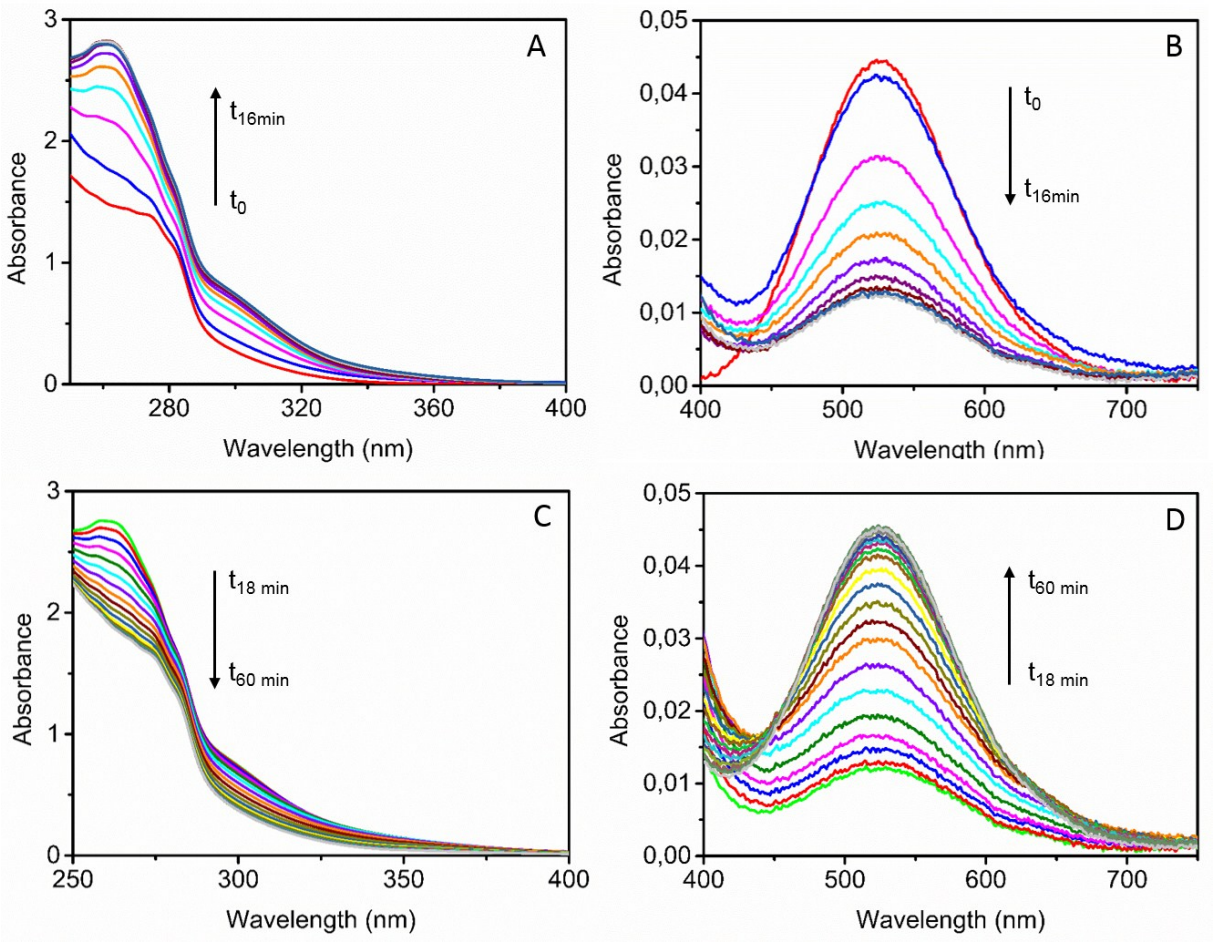


Fig S2. UV-Vis Spectroscopy (Uv-region, 250-400 nm; Vis-region, 400-750 nm) of Cu(II) release from $Cu^{II}(A\beta_{4-16})$ complex with L-Cys in the absence of Zn_7MT-3 in solution. A) UV region of the spectra for the first 16 min of the reaction after the addiction of L-Cys to $Cu^{II}(A\beta_{4-16})$ (spectra of $A\beta_{4-16}$ / Cu(II), 1:0.9, red line); B) corresponding Vis region of the spectra; C) UV region of the spectra from $t_{18\ min}$ to $t_{60\ min}$ min after the addiction after the addiction of Cys to $Cu^{II}(A\beta_{4-16})$ complex; D) corresponding Vis region of the spectra. Each spectra was recorded at 2 min intervals. Reaction conditions (final concentrations for the reaction mixture $A\beta_{4-16}$ / Cu(II) / Cys): 500 µM $A\beta_{4-16}$, 450 µM Cu(II), 3 mM L-Cys. The measurements were performed in phosphate buffer 50 mM, pH 7.4.

Upon addiction of L-Cys to the preformed $Cu^{II}(A\beta_{4-16})$ complex, Cu(II) is released from $A\beta_{4-16}$ with concomitant formation of Cu(I)-Cys complex. In line with the instability of Cu(I)-Cys complex, after 20 min the oxidation of Cu(I) to Cu(II) allows the complete reformation of $Cu^{II}(A\beta_{4-16})$ complex (C, D).

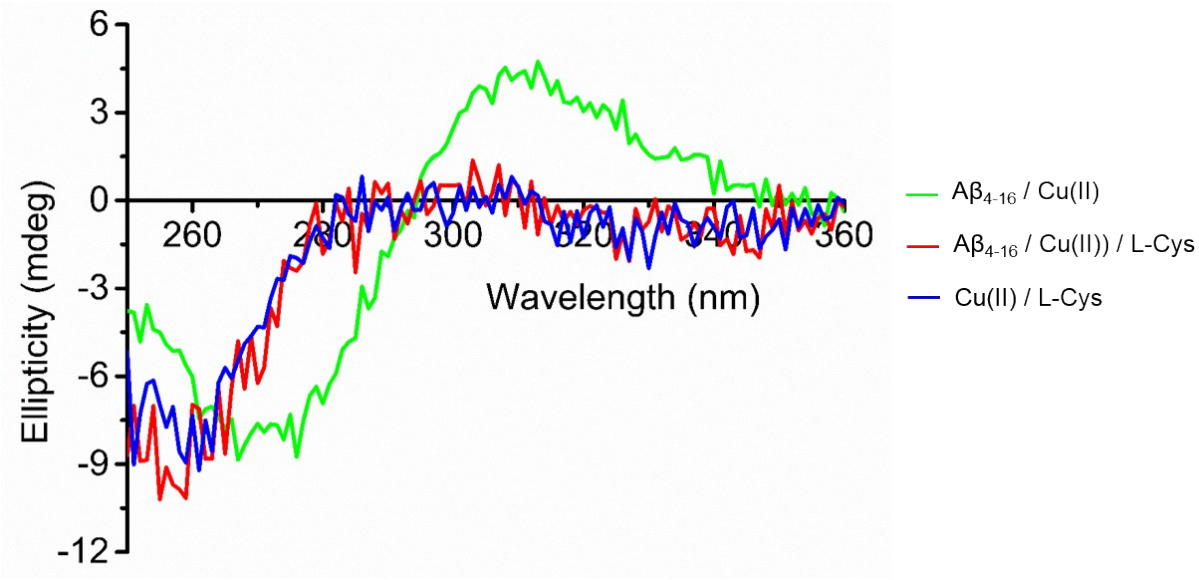


Fig S3. Circular Dichroism Spectroscopy (Uv-region, 250-360 nm) of Cu(II) release from $Cu^{II}(A\beta_{4-16})$ complex with L-Cys in the absence of Zn_7MT-3 in solution. CD spectra of $A\beta_{4-16}$/ Cu(II), 1:0.9 (light green line); CD spectra of Cu(I)-Cys / cystine recorded after 18 min from L-Cys addition to $Cu^{II}(A\beta_{4-16})$ complex ($A\beta_{4-16}$ / Cu^{II} / L-Cys ,red line); control CD spectra of Cu(I)-Cys /cystine obtained from the addiction of free Cu(II) to L-Cys solution (Cu(II) / L-Cys, blue line). The measurements were performed in phosphate buffer 50 mM, pH 7.4. The final concentration of each single component in solution is 100 µM $A\beta_{4-16,}$ 90 µM Cu(II), 20 µM Zn_7MT3, 3 mM L-Cys.

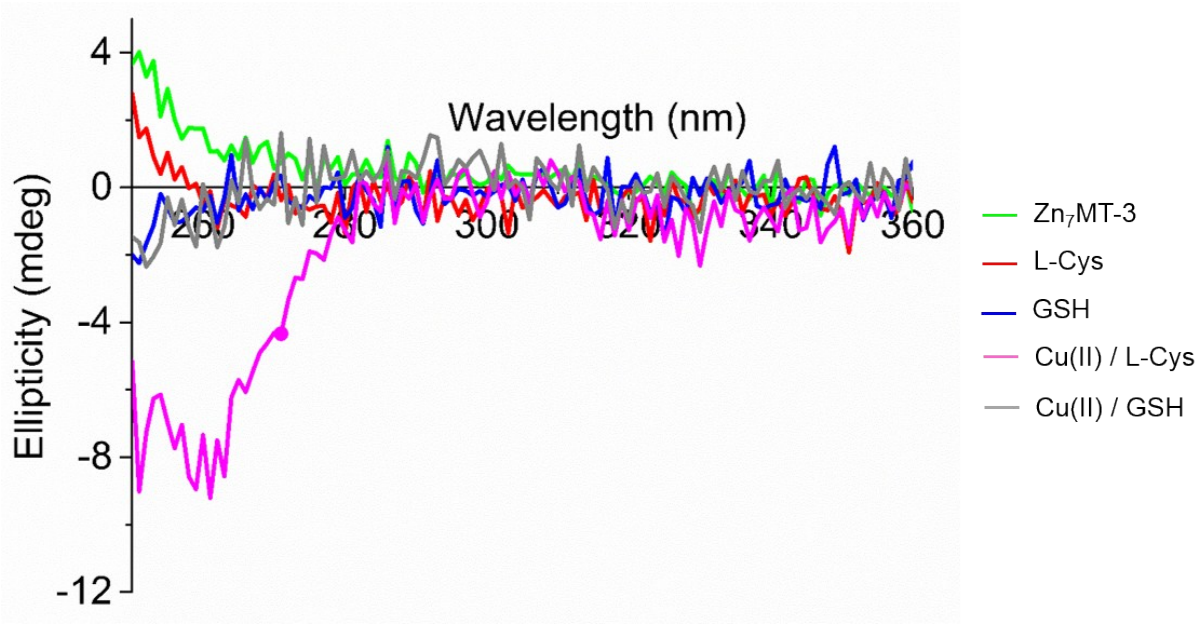
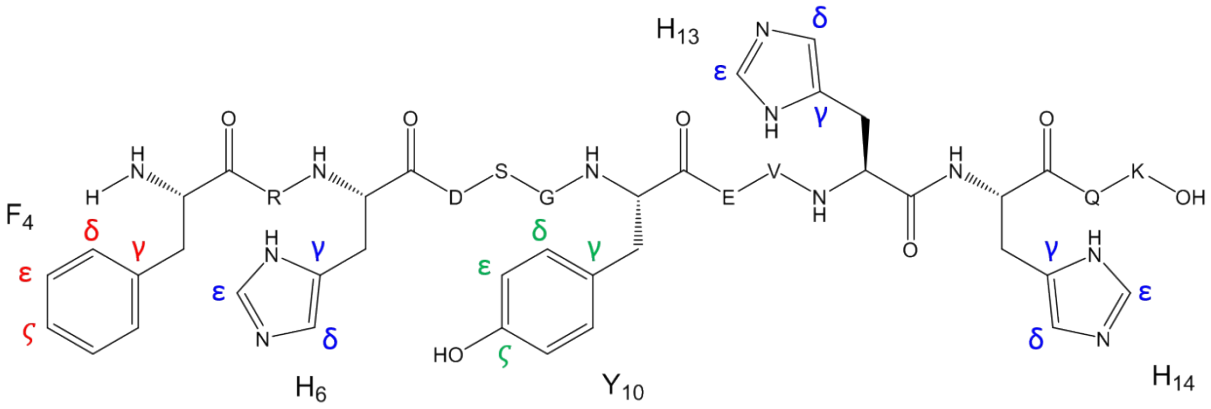


Fig S4. CD spectra (Uv-region, 250-360 nm) of Zn_7MT-3 (light green spectra), L-Cys (red spectra), GSH (blue spectra), Cu(II) / L-Cys mixture (magenta line), Cu(II) / GSH mixture (grey line). The final concentration of each single component in solution is 20 µM Zn_7MT-3, 3 mM Cys, 3 mM GSH, 90 µM Cu(II). The measurements were performed in phosphate buffer 50 mM, pH 7.4.



Scheme S1. Amyloid Beta (4-16) peptide sequence (FRHDSGYEVHHQK) with the aromatic amino-acid residues nomenclature.

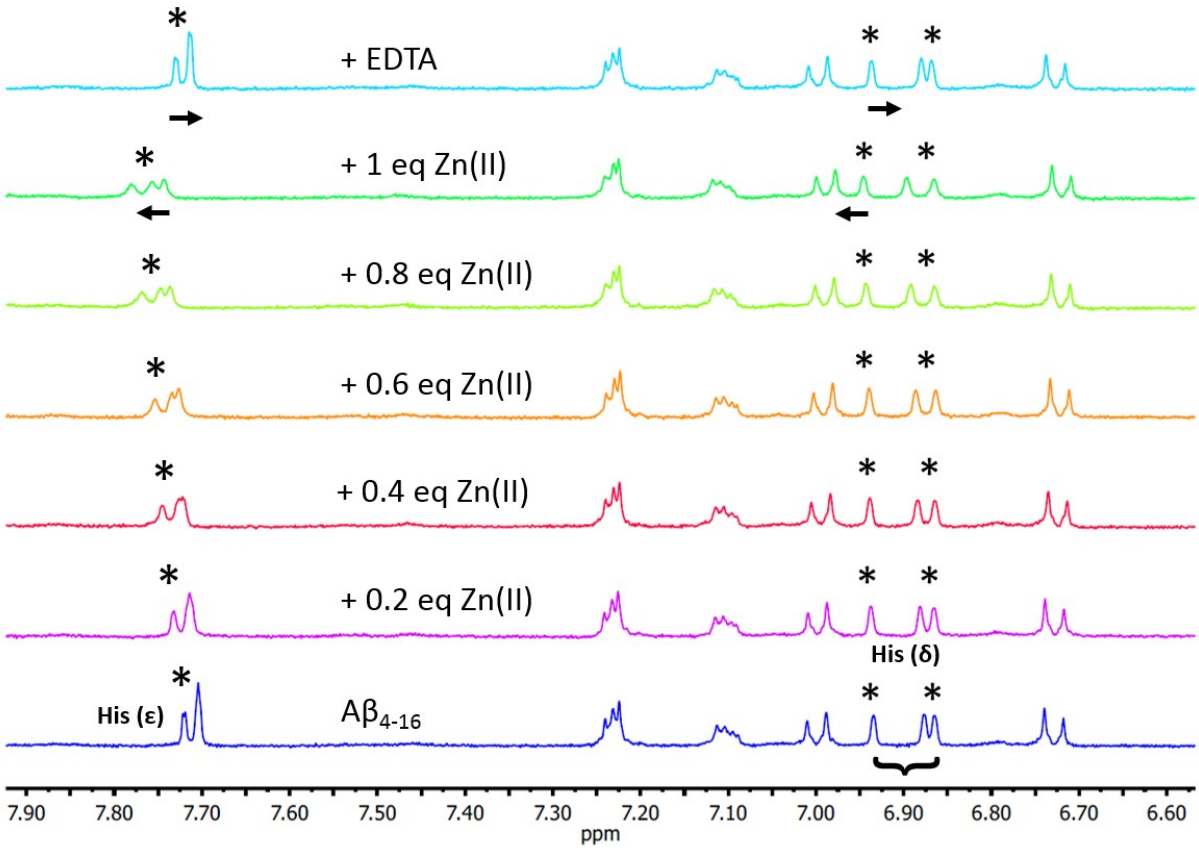


Fig S5. ^{1}H-NMR of the effect of Zn(II) binding to $A\beta_{4\text{-}16}$ peptide. ^{1}H-NMR spectra were obtained from 10%D_2O/90%H_2O solution in 50 mM phosphate buffer, pH 7.4. A concentrated Zn(II) solution (36 mM) was added to 300 µM $A\beta_{4\text{-}16}$ to obtain a ratio $A\beta_{4\text{-}16}$/Zn(II) (from the bottom): 1/0 (blue spectrum), 1/0.2 (purple spectrum), 1/0.4 (red spectrum), 1/0.6 (orange spectrum), 1/0.8 (light green spectrum), 1/1 (green spectrum). To the 1/1 complex $Zn^{II}(A\beta_{4\text{-}16})$ a concentrated stock solution of EDTA (50 mM, at pH 7.4) was added (final EDTA concentration 600 µM) to recover the signature of the peptide alone and confirm the Zn-related shift.

Experimental Section

The peptide Aβ4-16 (AA sequence: FRHDSGYEVHHQK-NH2) was synthesized according to Fmoc strategy and purified by HPLC as described before (M. Mital, N. E. Wezynfeld, T. Frączyk, M. Z. Wiloch, U. E. Wawrzyniak, A. Bonna, C. Tumpach, C. L. Haigh, K. J. Barnham, W. Bal, S. C. Drew, Resistance of Cu(Aβ4–16) to Copper Capture by Metallothionein-3 Supports a Function for the Aβ4–42 Peptide as a Synaptic Cu^{II} Scavenger, *Angew. Chem. Int. Ed.* **2015**, 54, 10460).

A pet-3d (Novagen) plasmid encoding for human MT-3 sequence was used for recombinant protein expression. Zn_7MT-3 was expressed in Escherichia coli strain BL21(DE3)pLys and purified as previously described. (Faller, P., Hasler, D. W., Zerbe, O., Klauser, S., Winge, D. R. and Vašák, M., Evidence for a dynamic structure of human neuronal growth inhibitory factor and for major rearrangements of its metal-thiolate clusters., Biochemistry. **1999**, 38, 10158)

Stock solution of the $A\beta_{4-16}$ peptide was prepared by dissolving the powder in to Milli-Q water (resulting pH = 2). Concentration of $A\beta_{4-16}$ peptide was determined by UV-Visible Spectroscopy from free Tyr^{10} absorption with $\Delta\varepsilon(^{\lambda}_{276-296})$ = 1410 M^{-1} at pH 2. Peptide concentration was confirmed by titration of Cu(II) in phosphate buffer 50 mM pH 7.4, monitoring by Uv-Visible Spectroscopy the formation of the characteristic d-d band for the 1:1 $Cu^{II}(A\beta_{4-16})$ complex at 525 nm. The titration was carried out adding a $CuCl_2$ solution of known concentration to $A\beta_{4-16}$.

Stock solution of Zn_7MT-3 was prepared by dissolving the powder in Milli-Q water. In order to remove DTT (previously added to preserve the thiol groups from oxidation), a SEC (size exclusion chromatography) column was run, employing a PD mini trap G10 column, equilibrated with phosphate buffer 10 mM, pH 7.4. Concentration of Zn_7MT-3 metallopeptide was then determined by UV-Visible Spectroscopy from the metal induced shoulder at 230 nm (ε = 80000 $M^{-1}cm^{-1}$) in phosphate buffer 50mM, pH 7.4. The absorbance of Zn_7MT-3 at 230 nm has been corrected for that of APO-MT-3 in the same region. APO-MT-3 species was obtained at pH 2.

Stock solution of Cu(II) (100 mM) was prepared in Milli-Q water from $CuCl_2 \cdot 2H_2O$. Its concentration has been verified by Uv-Vis absorption spectroscopy from Cu(II) d-d band at 780 nm (ε = 12 $M^{-1}cm^{-1}$). Stock solution of Zinc (100 mM) was prepared in Milli-Q water from $ZnSO_4 \cdot 7H_2O$. Stock solution of EDTA (100mM) was prepared in Milli-Q water, increasing the pH up to 8 by addiction of a 5 M solution of NaOH. Stock solution of phosphate buffer (500 mM, pH 7.4) was prepared by mixing potassium dihydrogen phosphate 99 % (KH_2PO_4) with potassium hydrogen phosphate 98 % (K_2HPO_4) in Milli-Q water, adjusting the pH with a 5M solution of NaOH.

Stock solutions of L-Cys, and GSH were freshly prepared before each experiment by dissolving their powders in to a 72.4 mM solution of HCl in Milli-Q water.

Uv-Vis measurements were performed on a Cary 5000 and on a Cary 60 spectrophotometer at room temperature (between 20-25 °C). The spectra are express in Absorbance. A Jasco Circular Dichroism (model 810) Spectropolarimeter was used to record CD spectra at room

temperature (20-25 °C) with a scanning speed of 50 nm/min. The spectra are express as ellipticity (mdeg). Both for UV-Vis and CD measurements 1 cm quartz cuvettes have been used.

The release of copper from $Cu^{II}(A\beta_{4-16})$ complex in the presence of Zn_7MT-3 and L-Cys and the concomitant formation of $Cu(I)_4Zn(II)_4$MT-3 was investigated by UV-Visible and Circular Dichroism Spectroscopy, over the spectral range 250-800 nm. First $Cu^{II}(A\beta_{4-16})$ complex was generated adding 0.9 eq of $CuCl_2$ solution to $A\beta_{4-16}$ (ratio $Cu^{II}/A\beta_{4-16}$, 1/0.2). Then Zn_7MT-3 stock solution was mixed to $Cu^{II}(A\beta_{4-16})$, and the reaction monitored over a period of 30 min through the d-d band of $Cu^{II}(A\beta_{4-16})$ over a period of 30 min. After that, L-Cys or GSH stock solution were added in to the mixture $Cu^{II}(A\beta_{4-16})$ / Zn_7MT-3 (ratio 1:0.2) and the release of copper monitored over a period of 60 min in case of Cys or 260 min in case of GSH. Kinetic of copper release from $Cu^{II}(A\beta_{4-16})$ was obtained plotting the UV-Vis absorbance at 525 nm (maximum of the $Cu^{II}(A\beta_{4-16})$ d-d band) versus time of collection of each spectra. The final concentration of $A\beta_{4-16,}$ Cu(II), Zn_7MT-3, L-Cys or GSH in the reaction mixture are respectively 500 uM, 450 uM, 100 uM , 3mM (reaction followed in the Vis-region), 100 µM, 90 µM, 20 µM, 3mM (reaction followed in the UV-region). All the reactions have been performed in phosphate buffer 50 mM, pH 7.4.

The measurements have been performed at least 3 times at different days with different solutions. No significant differences were observed and hence representative measurements are shown in the Figures. We did also analogue experiments with the peptide $A\beta_{4-7}$ (that has the same Cu(II) binding site as $A\beta_{4-16}$), and the results were confirmed with similar kinetics.

^{1}H-NMR experiments were realized on a 400 NMR Bruker Spectrometer, in 10%D_2O/90%H_2O solutions at 298K, using water gate technique for water suppression. $A\beta_{4-16}$ peptide sample was freshly prepared in 10%D_2O/90%H_2O in phosphate buffer 50 mM (final concentration 300 µM); Cu(II), Zn_7MT-3 and L-Cys were then respectively added into the NMR tube with a final concentration of 270 µM, 60 µM, 3mM. In order to confirm the Zn(II) effect upon binding to the $A\beta_{4-16}$, a freshly prepared solution of the peptide (300 µM) was titrated increasing the concentration of Zn (up to 300 uM).