How Zn can impede Cu detoxification by chelating agents in Alzheimer's Disease: a proof-of-concept study

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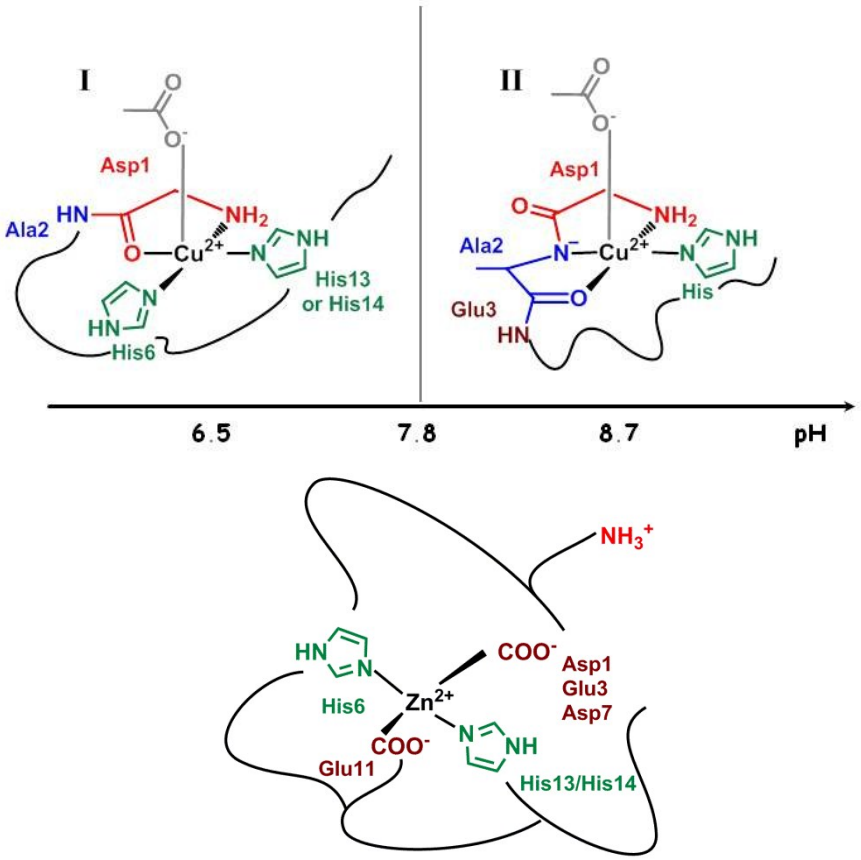
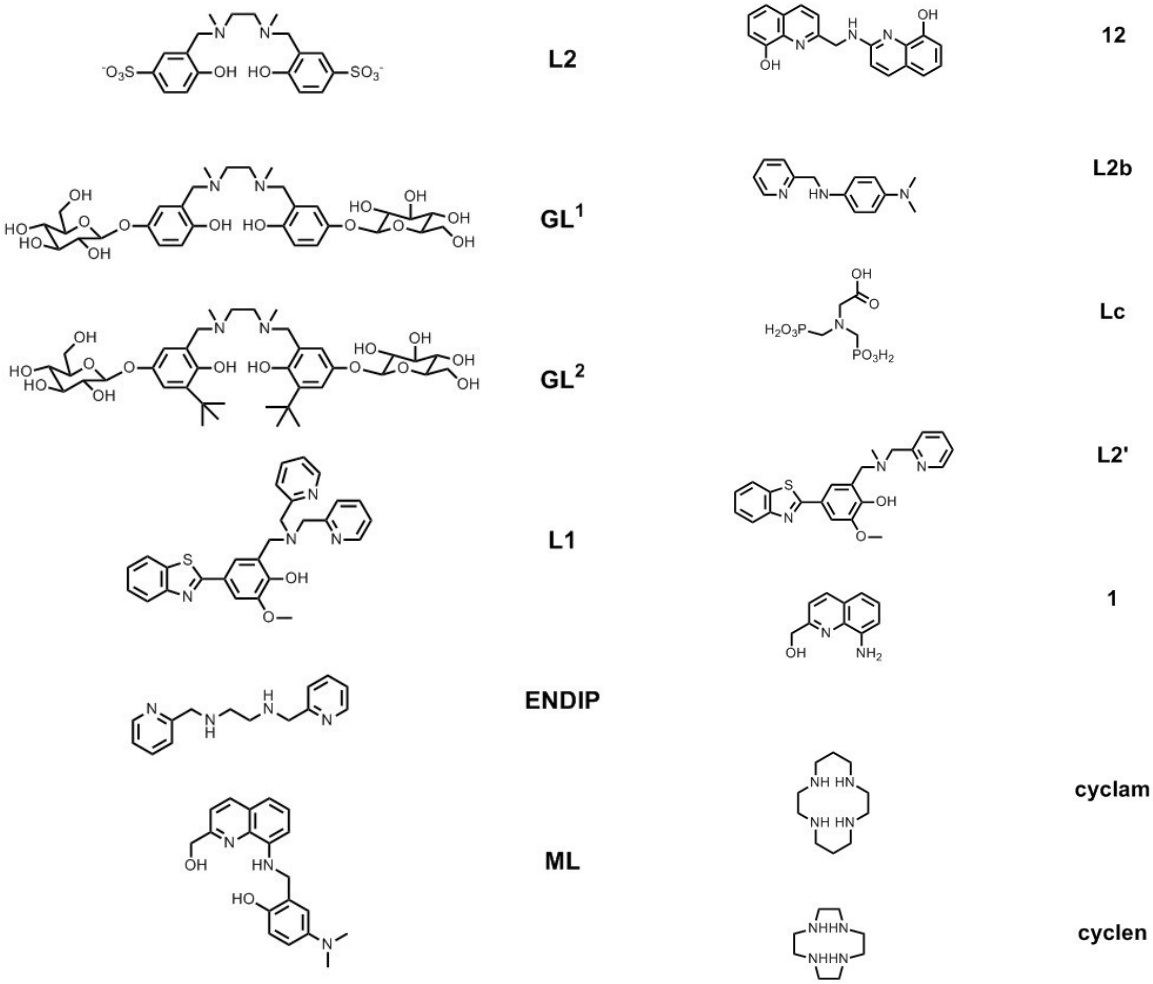
SUPPORTING INFORMATION

Cu(II) and Zn(II) coordination sites to Aβ



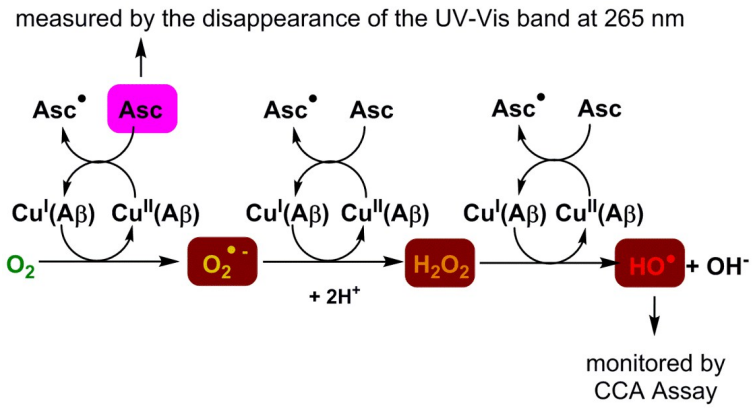
Scheme S1. Predominant coordination sites proposed for Cu(II) and Zn(II) to the Aβ peptide near physiological pH.

Ligands



Scheme S2. Ligands corresponding to Table 1 and Table S1.

ROS detection methods.



Scheme S3. Detection methods for ROS production based on the monitoring of ascorbate consumption by UV-Vis at 265 nm and HO° adduct formation with CCA observed at 450 nm by fluorescence.

Equations

The description of the situation (under thermodynamic control) within the synaptic cleft is represented by the equations and notations given in the box below, where Cu(II) and Zn(II) can both react with Aβ or L to form the four possible L-Cu(II), L-Zn(II), Aβ-Cu(II) and Aβ-Zn(II) complexes.

In absence of Zn(II) the condition to predominantly remove Cu(II) from Aβ is $K_{Cu} \gg 1$ while in presence of Zn(II), the condition is $K \gg 1$, using the following equations and corresponding reaction constants.

L	+	Cu(II)	⇌	L-Cu(II)	$K_L^{Cu} = \frac{[L-Cu(II)]}{[L][Cu(II)]}$
$1-\alpha_{Cu}-\alpha_{Zn}$		$1-\alpha_{Cu}-\beta_{Cu}$		α_{Cu}	
Aβ	+	Cu(II)	⇌	Aβ-Cu(II)	$K_{A\beta}^{Cu} = \frac{[A\beta-Cu(II)]}{[A\beta][Cu(II)]}$
$1-\beta_{Cu}-\beta_{Zn}$		$1-\alpha_{Cu}-\beta_{Cu}$		β_{Cu}	
L	+	Zn(II)	⇌	L-Zn	$K_L^{Zn} = \frac{[L-Zn]}{[L][Zn]}$
$1-\alpha_{Cu}-\alpha_{Zn}$		$1-\alpha_{Zn}-\beta_{Zn}$		α_{Zn}	
Aβ	+	Zn(II)	⇌	Aβ-Zn	$K_{A\beta}^{Zn} = \frac{[A\beta-Zn]}{[A\beta][Zn]}$
$1-\beta_{Cu}-\beta_{Zn}$		$1-\alpha_{Zn}-\beta_{Zn}$		β_{Zn}	

where α_{Cu} corresponds to the proportions in L-Cu(II), β_{Cu} in Aβ-Cu(II), α_{Zn} in L-Zn(II), and β_{Zn} in Aβ-Zn(II). We can then define $K_{Cu} = \frac{K_L^{Cu}}{K_{A\beta}^{Cu}}$; $K_{Zn} = \frac{K_L^{Zn}}{K_{A\beta}^{Zn}}$ and $K = \frac{K_L^{Cu}K_{A\beta}^{Zn}}{K_L^{Zn}K_{A\beta}^{Cu}} = \frac{K_{Cu}}{K_{Zn}}$,

where K_M (with M = Cu(II) or Zn) corresponds formally to the :

Aβ-M	+	L	⇌	L-M + Aβ

and σ to the "exchange reaction"

Aβ-Cu	+	L-Zn	⇌	L-Cu + Aβ-Zn

It comes that $\alpha_{Cu} = \frac{\sqrt{K}}{1+\sqrt{K}}$.

To have a Cu(II) removal from the Aβ, the condition is $\alpha_{Cu} \geq 0.5 \leftrightarrow K \geq 1$.

Apparent affinity and selectivity values of the chelators

Table S1. Apparent affinity values at pH 7.1 for Cu and Zn, for Aβ and representative ligands and corresponding K_{Cu} and K values. Only 1:1 metal:ligand complexes are considered.

		Aβ16	L_2	GL[1]	GL[2]	L1	ENDIP	ML	12	L2b	Lc	L2'	1	Cyclam[c]	Cyclen[c]
	$log(K_L^{Cu})_{[a]}$	9.2	13.8	12.1	11.5	17.3	15.4	12.6	15.7	10.6	11.6	12.9	8.7	23.8	21.1
	$log(K_L^{Zn})_{[a]}$	5.0	6.1	4.6	4.2	12.0	10.1	8.6	12.6	8.4	9.6	11.6	8.1	12.6	12.7
	$log(S_L)$	4.2	7.7	7.5	7.3	5.3	5.3	4.0	3.1	2.2	2.0	1.3	0.6	11.1	8.4
Without Zn	$log(K_{Cu})$		4.6	2.9	2.3	8.1	6.2	3.4	6.5	1.4	2.4	2.7	-0.5	14.6	11.9
	Complete Cu removal from Aβ		⚑	⚑	⚑	⚑	⚑	⚑	⚑	⚐*	⚑	⚑	⚐	⚑	⚑
With Zn	$log(K)$		3.5	3.3	3.1	1.1	1.1	0.2	-1.1	-2.0	-2.2	-2.9	-3.6	6.9	4.2
	Complete Cu removal from Aβ		⚑	⚑	⚑	⚐*	⚐*	⚐	⚐	⚐	⚐	⚐	⚐	⚑	⚑
	Ref.	1-3	2, 4	5	5	6	7	8	9	10	11	6	8	12	12

[a] value at pH 7.4

[b] Apparent binding constant.

[c] The cyclen and cyclam ligands have the correct selectivity but do not stop the production of ROS.[12]

UV-Vis, EPR and XANES monitoring of Cu(II) removal form Aβ in presence of Zn(II)

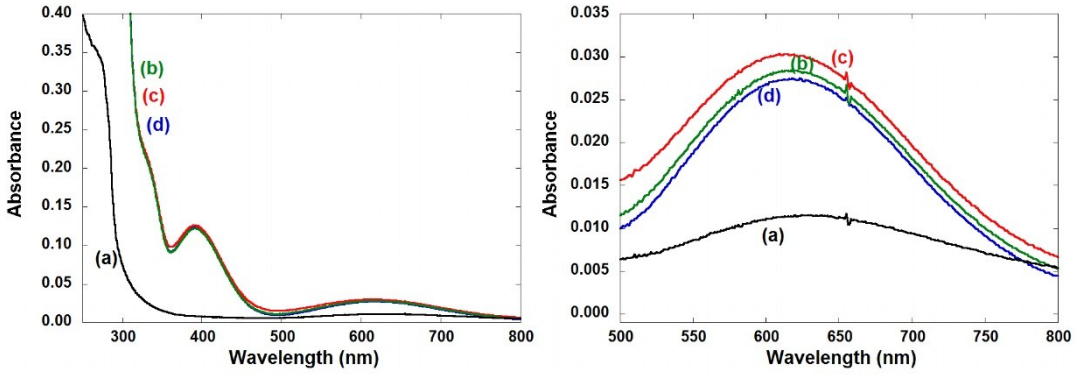


Figure S1. UV-Vis absorption spectra of Cu(Aβ16) (black, a) ; Cu(Aβ16) + L_2 (green, b) ; Cu(Aβ16) + Zn(L_2) (red, c) ; Cu(L_2) (blue, d). [Aβ16] = [L_2] = 0.1 mM, [M] = 0.1 mM, [hepes] = 0.1 M, pH 7.1, T = 25 °C, ℓ=1 cm.

Regardless the presence of Zn, the removal of Cu(II) from Aβ by L_2 is total as can be seen by comparison between curves (b, c and d), in line with the data described previously EPR and XANES.

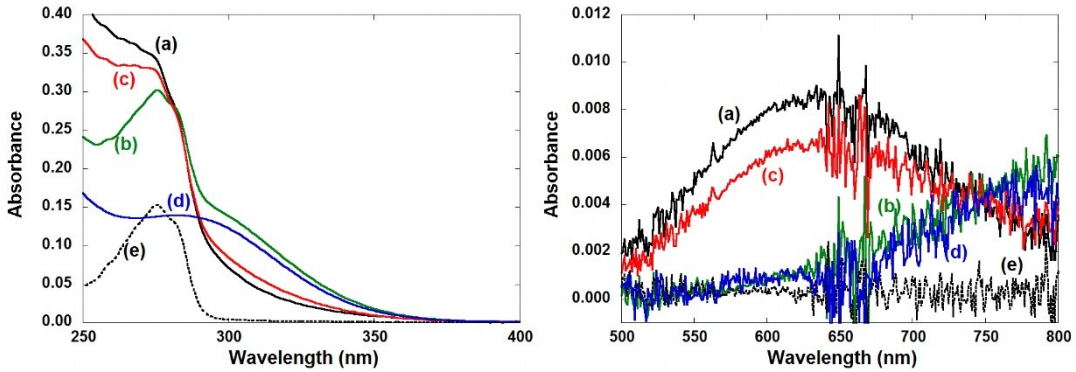


Figure S2. UV-Vis absorption spectra Cu(Aβ16) (black, a); Cu(Aβ16) + L_c (green, b); Cu(Aβ16) + Zn(L_c) (red, c); Cu(L_c) (blue, d) ; (Aβ16) (e, dotted black line). [Aβ16] = [L_c] = 0.1 mM, [M] = 0.1 mM, [hepes] = 0.1 M, pH 7.1, T = 25 °C, ℓ=1 cm.

In absence of Zn, the removal of Cu(II) from Aβ by L_c is almost total as can be seen by comparison between curves (b, d and e). Indeed, curve (b) corresponds to the addition of curves (d) and (e), in line with the presence of Cu(II) bound to L_c and free Aβ in the solution. In presence of Zn, the removal of Cu(II) from Aβ by L_c is hampered and the ratio of Cu(II) bound to L_C is approx. 25% in line with the data described previously EPR and XANES.

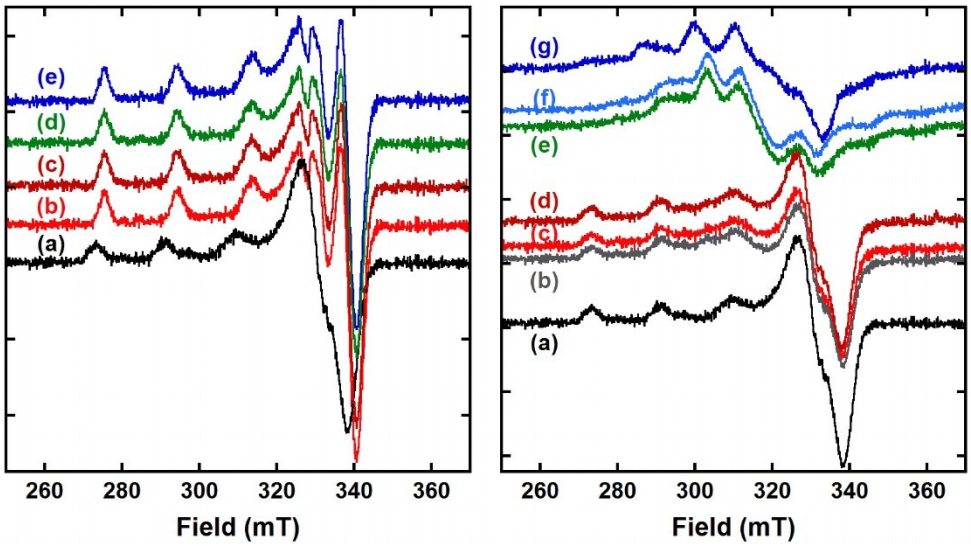


Figure S3. Left panel: EPR signatures of Cu(Aβ16) (a) ; Cu(Aβ16) + Zn(L_2) (b) ; Cu,Zn(Aβ16) + L_2 (c) ; Cu(Aβ16) + L_2 (d) ; Cu(L_2) (e). Right panel: EPR signatures of Cu(Aβ16) (a) ; calculated spectrum according to 0.3 spectrum (d) + 0.7 spectrum (a) (b) ; Cu(Aβ16) + Zn(L_C) (c) ; Cu,Zn(Aβ16) + L_C (d) ; Cu(L_C) + Aβ16 (e) ; Cu(L_C) + 1 equiv. of imidazole (f) ; Cu(L_C) (g). [Cu(II)] = 0.18 mM in 50 mM hepes buffer at pH 7.1. ν = 9.5 GHz, amplitude modulation = 0.5 mT, microwave power = 20 mW. T = 110 K.

Cu(II) extraction from the Aβ peptide is observed with L_2 in all the conditions tested, regardless of the absence or presence of Zn.

In the case of L_C, the situation is a little bit more complex, due to the formation of a ternary species between Cu(II), L_C and the Aβ peptide. Indeed, at this high concentration, the peptide can complete the Cu sphere in the Cu(L_C) complex, very likely via the coordination of the imidazole ring from one of the three His, as recently observed for Cu(II), Aβ and alpha-synuclein.[13, 14] This is further confirmed by the high similarity between the EPR signatures of the Cu(L_C) species in presence of Aβ or of one equiv. of imidazole. Anyway, in presence of Zn, the removal of Cu(II) from Aβ is hampered and the ratio of Cu(II) bound to L_C is only approx. 30% as expected based on the relative Cu over Zn selectivity of Aβ ($10^{4.2}$) and L_C (10^2) (compare spectra (b) and (c) and see Table S1).

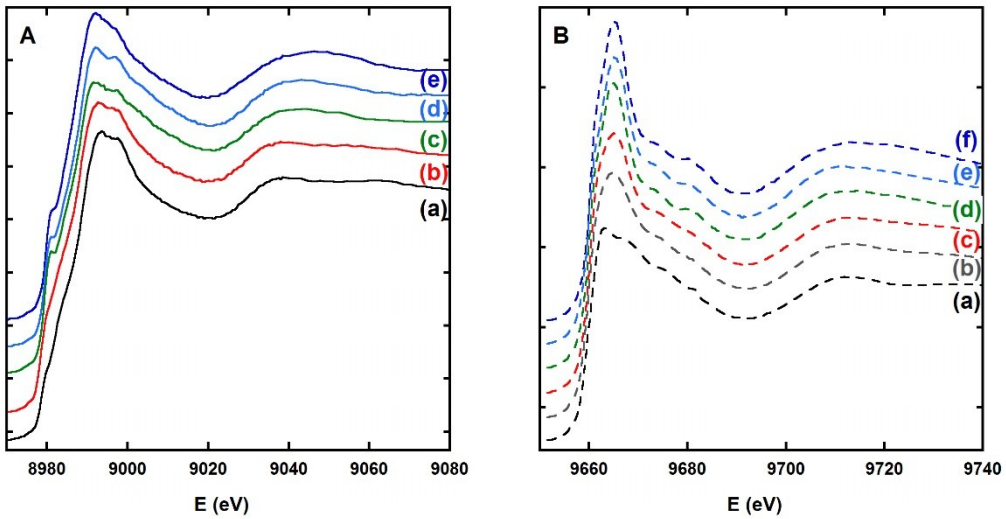


Figure S4. Panel A: Normalized Cu K-edge X-ray absorption near edge structure (XANES) spectra of Cu(Aβ16) (a) ; Cu(Aβ16) + Zn(L_C) (b) ; Cu(L_C) + Aβ16 (c) ; Cu(L_C) + 1 equiv. of imidazole (d) ; Cu(L_C) (e). Panel B: Normalized Zn K-edge XANES spectra of Zn(Aβ16) (a) ; calculation spectrum according to 0.4 spectrum (a) + 0.6 spectrum (d)) (b) ; Cu(Aβ16) + Zn(L_C) (c) ; Zn(L_C) + Aβ16 (d) ; Zn(L_C) + 1 equiv. of imidazole (e) ; Zn(L_C) (f). [Aβ] = [L] = 1 mM, [M] = 1 mM, [hepes] = 0.1 M, pH 7.1, T = 20 K.

The evaluation of the distribution of Cu(II) between the peptide and the L_C ligand in the exchange experiment by XANES is difficult due to the high similarity between the various signatures (and to a lesser extent by the presence of Cu(II) photoreduction). EPR is more appropriate (see next paragraph).

In contrast, the evaluation of the distribution of Zn(II) between the peptide and the L_C ligand in the exchange experiment by XANES at the Zn K-edge is possible. This leads to approx. 60% of Zn bound to the L_C (compare spectra (b) and (c)) in line with the proportion of Cu(II) bound to the ligand found by EPR (see next paragraph).

ROS production assays

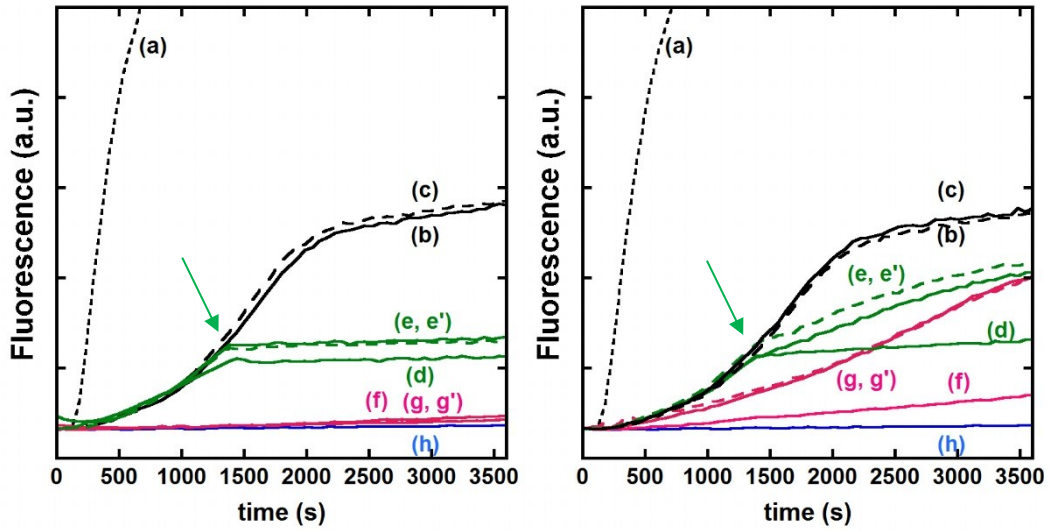


Figure S5. Cu(II) induced 7-OH-CCA formation. Cu(II) (a), Cu(Aβ16) (b), Cu,Zn(Aβ16) (c), Cu(Aβ16) + L added @ t = 20 min (d) ; Cu(Aβ16) + Zn(L) added @ t = 20 min (e) ; Cu,Zn(Aβ16) + L added @ t = 20 min (e', dotted line) ; Cu(Aβ16) + L (f) ; Cu(Aβ16) + Zn(L) (g) ; Cu,Zn(Aβ16) + L (g', dotted line) ; control experiment (without Cu) (h). Ascorbate is added to trigger the reaction. Left panel L = L_2 ; Right panel : L = L_C. [Cu(II)] = 10 µM, 1.2 equiv of Aβ, L and Zn. λ_{em} = 452 nm, [Asc] = 0.5 mM, [PO_4] = 50 mM, pH 7.1, [CCA] = 0.5 mM, T = 25°C.

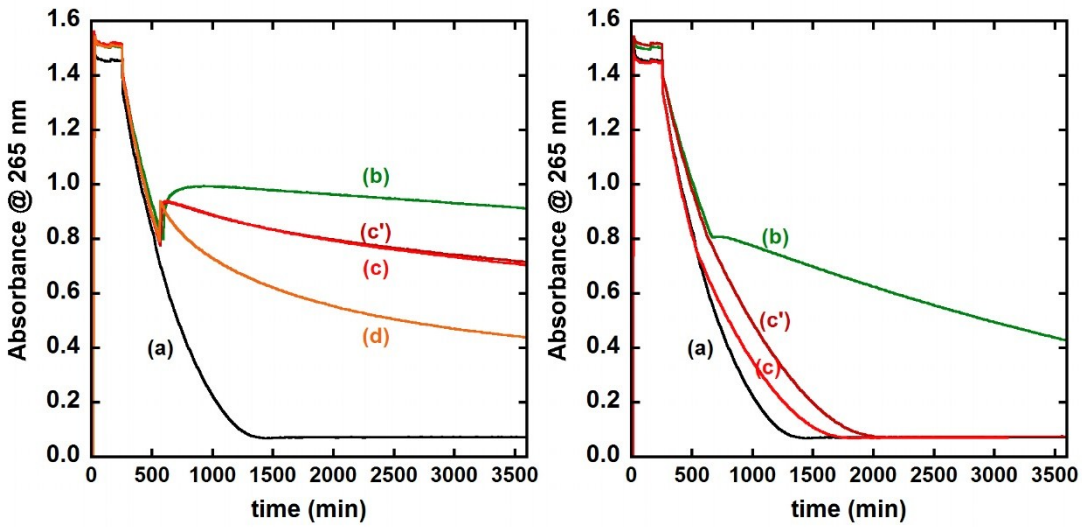


Figure S6. Cu(II) induced ascorbate consumption. Cu(Aβ16) (a), Cu(Aβ16) + L added @ t = 590 s (b) ; Cu(Aβ16) +Zn(L) added @ t = 560 s (c) ; Cu,Zn(Aβ16) + L added @ t = 570 s (c') ; Cu(Aβ16) + 5eq. Zn + L added @ t = 560 s (d). Left panel L = L_2 ; Right panel : L = L_C. [Cu(II)] = 10 µM, 1.2 equiv of Aβ, L and Zn. [Asc] = 0.1 mM, [hepes] = 100 mM, pH 7.1, T = 25°C.

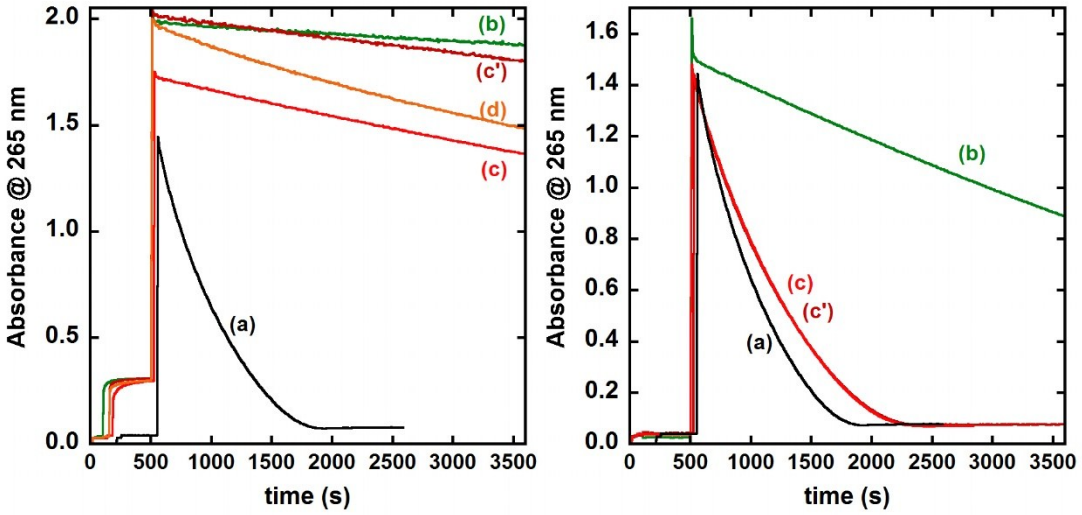


Figure S7. Cu(II) induced ascorbate consumption. Cu(Aβ16) (a), Cu(Aβ16) + L (b) ; Cu(Aβ16) +Zn(L) (c) ; Cu,Zn(Aβ16) + L (c') ; Cu(Aβ16) + 5eq. Zn + L (d). Ascorbate is added to trigger the reaction. Left panel L = L_2 ; Right panel : L = L_C. [Cu(II)] = 10 µM, 1.2 equiv of Aβ, L and Zn. [Asc] = 0.1 mM, [hepes] = 100 mM, pH 7.1, T = 25°C. Ascorbate is added at t ≈ 500 s.

Aggregation assay

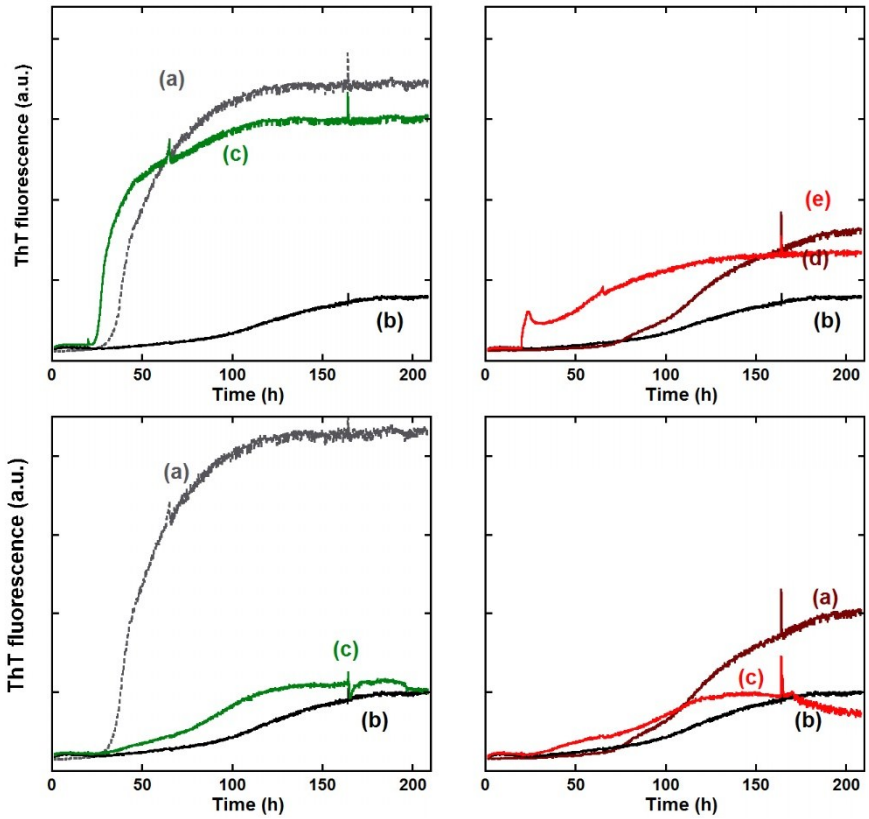


Figure S8. Kinetic measurement of amyloid fibrils formation using ThT Fluorescence. Left panel: (a) Aβ; (b) Cu(Aβ) and (c) Cu(Aβ) + L_2 added @ t = 20 h (top) and 162 h (bottom) ; Right panel: (a) Zn(Aβ); (b) Cu(Aβ) and (c) Cu(Aβ) + Zn(L_2) added @ t= 20 h (top) and 162 h (bottom). [Aβ] = [L_2] = [L_2-Zn] = 20 μM, [Cu] = [Zn] = 18 μM, [phosphate buffer] = 50mM, [ThT] = 10 μM, pH 7.1, T = 37°C.

When L_2 or Zn(L_2) addition is performed before the elongation phase, a trend similar to the one observed with an addition at t_0 is observed. The abrupt step in trace (c) of the top, right panel coincides with the addition of the Zn(L_2). In contrast, when L_2 or Zn(L_2) addition is performed later on, i.e. on the plateau phase, no modification of the ThT fluorescence is observed on the time scale of the experiment (two days), although Cu(II) is readily removed from the fibrils (Figure S9). This indicates that the transformation of Cu-induced oligomers, into fibrils is extremely slow in line with aggregation energetic profile.[15, 16]

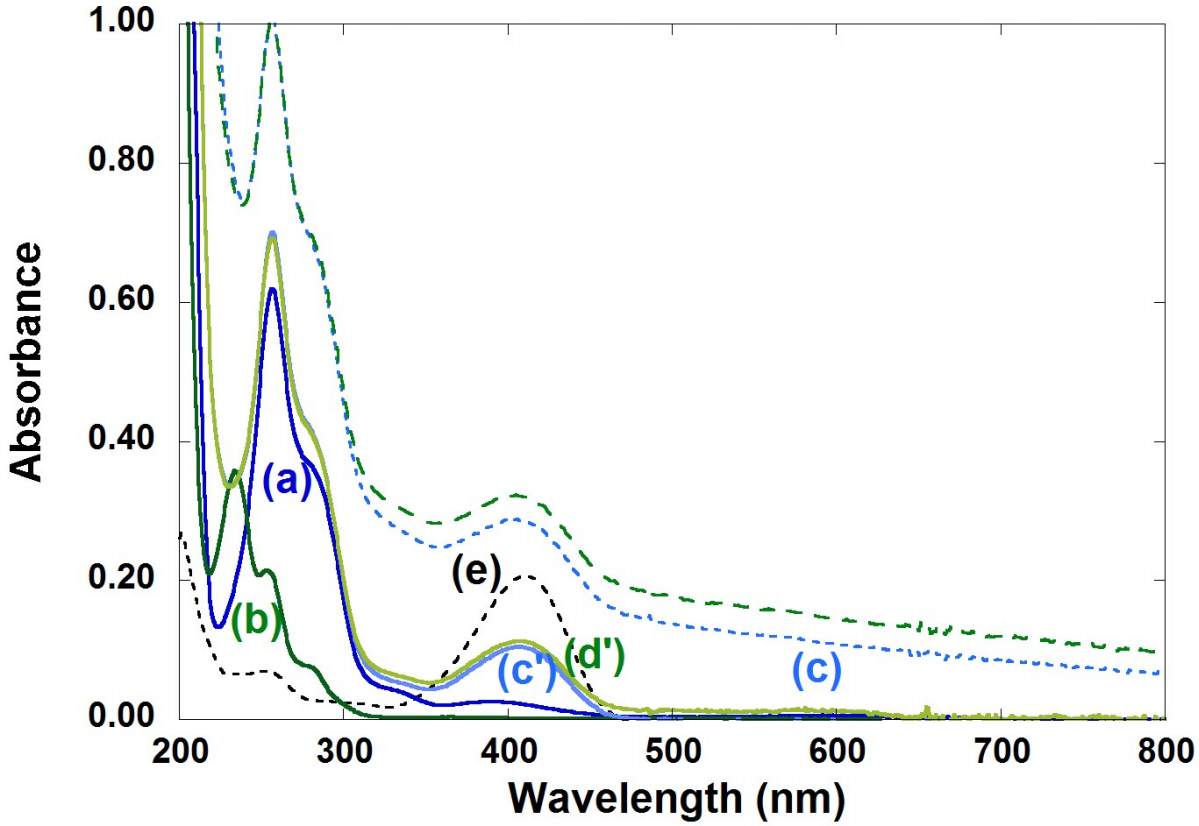


Figure S9. UV-Vis absorption spectra of $Cu(L_2)$ (blue, a) ; $Zn(L_2)$ (green, b) ; Cu(Aβ) after aggregation and addition of L_2 @ t = 162 h (dotted light blue line, c) ; in the supernatant of Cu(Aβ) after aggregation and addition of L_2 @ t = 162 h (plain light blue line, c') ; Cu(Aβ) after aggregation and addition of $Zn(L_2)$ @ t = 162 h (dotted light green line, d) ; in the supernatant of Cu(Aβ) after aggregation and addition of $Zn(L_2)$ @ t = 162 h (plain light green line, d') and free ThT (dotted black line, e); $[L_2] = [A\beta] = 20$ µM, [M] = 18 µM, [phosphate buffer] = 0.05 M, pH 7.1, [ThT] = 10 µM, T = 25 °C, ℓ=1 cm.

References.

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