

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

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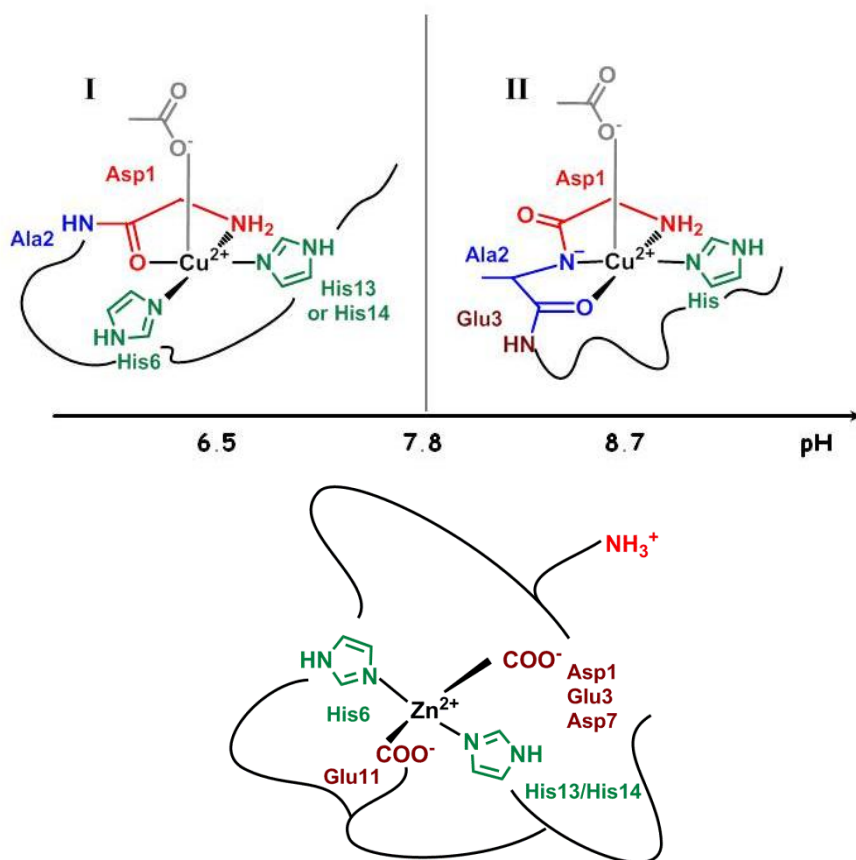
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## **SUPPORTING INFORMATION**

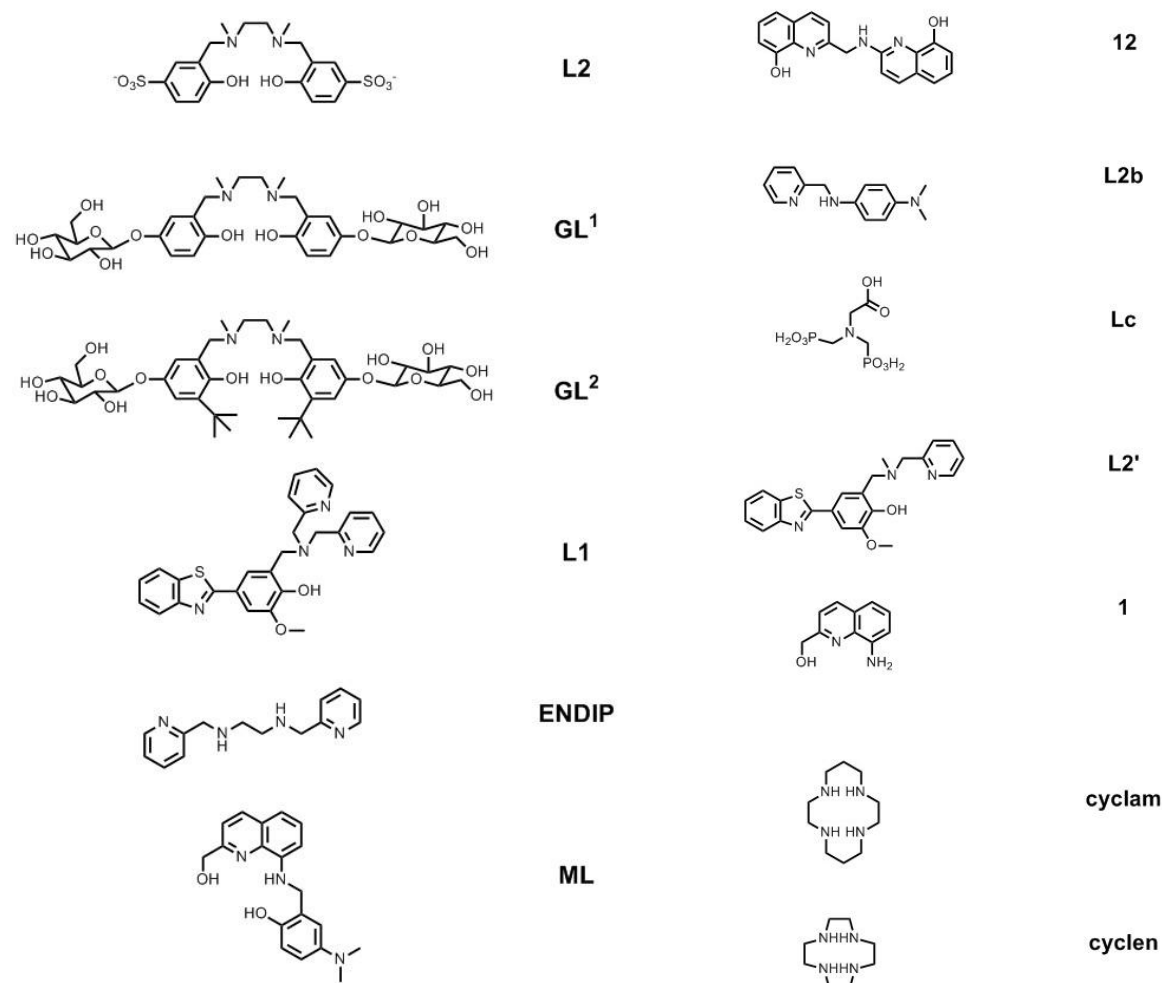
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## Cu(II) and Zn(II) coordination sites to A $\beta$



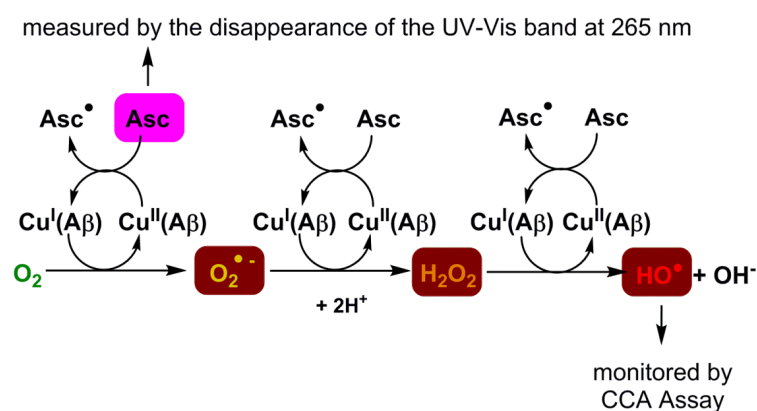
**Scheme S1.** Predominant coordination sites proposed for Cu(II) and Zn(II) to the A $\beta$  peptide near physiological pH.

## 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<sup>•</sup> 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 A $\beta$  or L to form the four possible L-Cu(II), L-Zn(II), A $\beta$ -Cu(II) and A $\beta$ -Zn(II) complexes.

In absence of Zn(II) the condition to predominantly remove Cu(II) from A $\beta$  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)	$\rightleftharpoons$	L-Cu(II)	$K_L^{Cu} = \frac{[L-Cu(II)]}{[L][Cu(II)]}$
$1 - \alpha_{Cu} - \alpha_{Zn}$		$1 - \alpha_{Cu} - \beta_{Cu}$		$\alpha_{Cu}$	
A $\beta$	+	Cu(II)	$\rightleftharpoons$	A $\beta$ -Cu(II)	$K_{A\beta}^{Cu} = \frac{[A\beta-Cu(II)]}{[A\beta][Cu(II)]}$
$1 - \beta_{Cu} - \beta_{Zn}$		$1 - \alpha_{Cu} - \beta_{Cu}$		$\beta_{Cu}$	
L	+	Zn(II)	$\rightleftharpoons$	L-Zn	$K_L^{Zn} = \frac{[L-Zn]}{[L][Zn]}$
$1 - \alpha_{Cu} - \alpha_{Zn}$		$1 - \alpha_{Zn} - \beta_{Zn}$		$\alpha_{Zn}$	
A $\beta$	+	Zn(II)	$\rightleftharpoons$	A $\beta$ -Zn	$K_{A\beta}^{Zn} = \frac{[A\beta-Zn]}{[A\beta][Zn]}$
$1 - \beta_{Cu} - \beta_{Zn}$		$1 - \alpha_{Zn} - \beta_{Zn}$		$\beta_{Zn}$	
<p>where <math>\alpha_{Cu}</math> corresponds to the proportions in L-Cu(II), <math>\beta_{Cu}</math> in A<math>\beta</math>-Cu(II), <math>\alpha_{Zn}</math> in L-Zn(II), and <math>\beta_{Zn}</math> in A<math>\beta</math>-Zn(II). We can then define <math>K_{Cu} = \frac{K_L^{Cu}}{K_{A\beta}^{Cu}}</math>; <math>K_{Zn} = \frac{K_L^{Zn}}{K_{A\beta}^{Zn}}</math> and <math>K = \frac{K_L^{Cu} K_{A\beta}^{Zn}}{K_L^{Zn} K_{A\beta}^{Cu}} = \frac{K_{Cu}}{K_{Zn}}</math>,</p>					
<p>where <math>K_M</math> (with M = Cu(II) or Zn) corresponds formally to the :</p>					
A $\beta$ -M	+	L	$\rightleftharpoons$	L-M + A $\beta$	
<p>and <math>\sigma</math> to the "exchange reaction"</p>					
A $\beta$ -Cu	+	L-Zn	$\rightleftharpoons$	L-Cu + A $\beta$ -Zn	
<p>It comes that <math>\alpha_{Cu} = \frac{\sqrt{K}}{1+\sqrt{K}}</math>.</p>					
<p>To have a Cu(II) removal from the A<math>\beta</math>, the condition is <math>\alpha_{Cu} \geq 0.5 \leftrightarrow K \geq 1</math>.</p>					

## Apparent affinity and selectivity values of the chelators

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

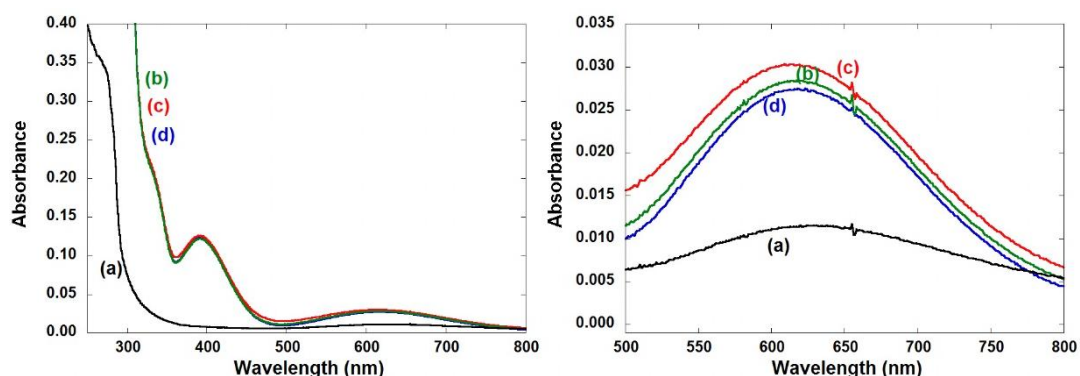
	A $\beta$ 16	L <sub>2</sub>	GL <sup>1</sup>	GL <sup>2</sup>	L1	ENDIP	ML	12	L2b	Lc	L2'	1	Cyclam <sup>[c]</sup>	Cylen <sup>[c]</sup>	
	$\log(K_L^{Cu})^{[a]}$	9.2	13.8	12.1	11.5	17.3	15.4	12.6	15.7	10.6	11.6	12.9	8.7	23.8	21.1
	$\log(K_L^{Zn})^{[a]}$	5.0	6.1	4.6	4.2	12.0	10.1	8.6	12.6	8.4	9.6	11.6	8.1	12.6	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 A $\beta$		✓	✓	✓	✓	✓	✓	✓	✗*	✓	✓	✗	✓	✓
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 A $\beta$		✓	✓	✓	✗*	✗*	✗	✗	✗	✗	✗	✗	✗	✓
	Ref.	1-3	2,4	5	5	6	7	8	9	10	11	6	8	12	12

<sup>[a]</sup> value at pH 7.4

<sup>[b]</sup> Apparent binding constant.

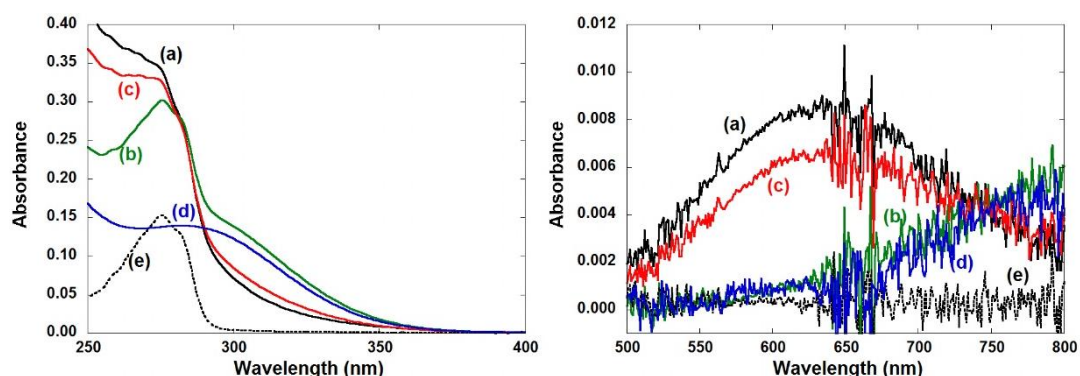
<sup>[c]</sup> The cyclen and cyclam ligands have the correct selectivity but do not stop the production of ROS.<sup>12</sup>

## UV-Vis, EPR and XANES monitoring of Cu(II) removal from A $\beta$ in presence of Zn(II)



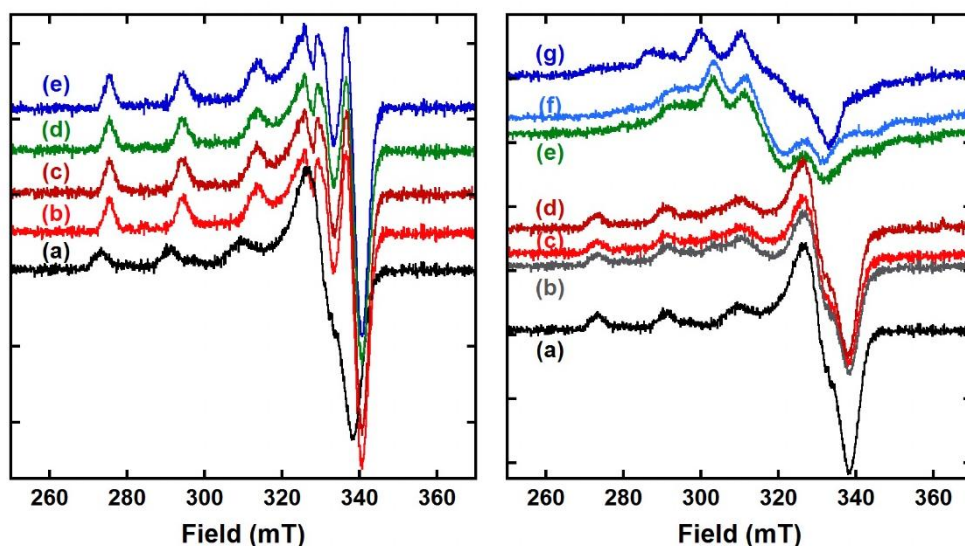
**Figure S1.** UV-Vis absorption spectra of Cu(A $\beta$ 16) (black, a) ; Cu(A $\beta$ 16) + L<sub>2</sub> (green, b) ; Cu(A $\beta$ 16) + Zn(L<sub>2</sub>) (red, c) ; Cu(L<sub>2</sub>) (blue, d). [A $\beta$ 16] = [L<sub>2</sub>] = 0.1 mM, [M] = 0.1 mM, [hepes] = 0.1 M, pH 7.1, T = 25 °C,  $\ell$ =1 cm.

Regardless the presence of Zn, the removal of Cu(II) from A $\beta$  by L<sub>2</sub> 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(A $\beta$ 16) (black, a); Cu(A $\beta$ 16) + L<sub>c</sub> (green, b); Cu(A $\beta$ 16) + Zn(L<sub>c</sub>) (red, c); Cu(L<sub>c</sub>) (blue, d) ; (A $\beta$ 16) (e, dotted black line). [A $\beta$ 16] = [L<sub>c</sub>] = 0.1 mM, [M] = 0.1 mM, [hepes] = 0.1 M, pH 7.1, T = 25 °C,  $\ell$ =1 cm.

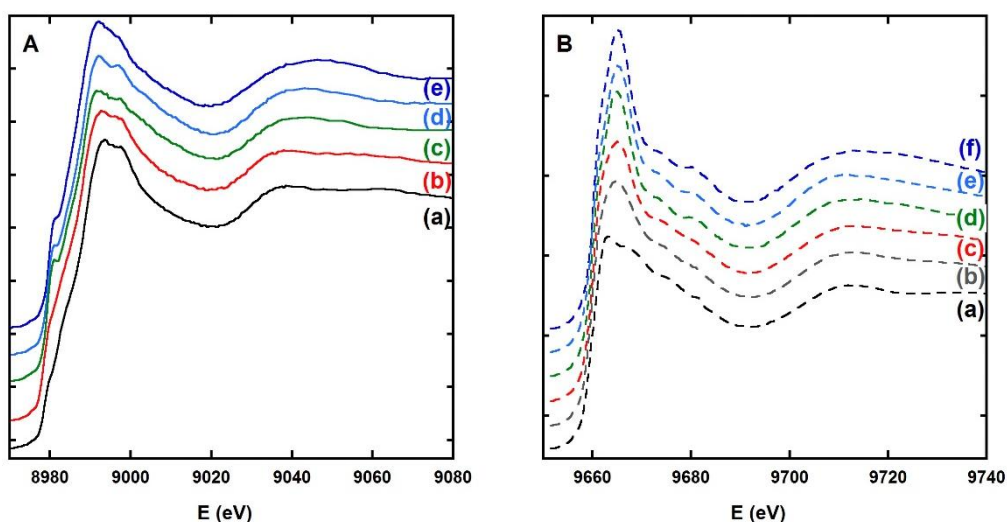
In absence of Zn, the removal of Cu(II) from A $\beta$  by L<sub>c</sub> is almost total as can be seen by comparison between curves (b, d and e). Indeed, curve (b) corresponds to the addition of curves (d) and (e), in line with the presence of Cu(II) bound to L<sub>c</sub> and free A $\beta$  in the solution. In presence of Zn, the removal of Cu(II) from A $\beta$  by L<sub>c</sub> is hampered and the ratio of Cu(II) bound to L<sub>c</sub> is approx. 25% in line with the data described previously EPR and XANES.



**Figure S3.** Left panel: EPR signatures of Cu(A $\beta$ 16) (a) ; Cu(A $\beta$ 16) + Zn(L<sub>2</sub>) (b) ; Cu,Zn(A $\beta$ 16) + L<sub>2</sub> (c) ; Cu(A $\beta$ 16) + L<sub>2</sub> (d) ; Cu(L<sub>2</sub>) (e). Right panel: EPR signatures of Cu(A $\beta$ 16) (a) ; calculated spectrum according to 0.3 spectrum (d) + 0.7 spectrum (a) (b) ; Cu(A $\beta$ 16) + Zn(L<sub>C</sub>) (c) ; Cu,Zn(A $\beta$ 16) + L<sub>C</sub> (d) ; Cu(L<sub>C</sub>) + A $\beta$ 16 (e) ; Cu(L<sub>C</sub>) + 1 equiv. of imidazole (f) ; Cu(L<sub>C</sub>) (g). [Cu(II)] = 0.18 mM in 50 mM hepes buffer at pH 7.1.  $\nu$  = 9.5 GHz, amplitude modulation = 0.5 mT, microwave power = 20 mW. T = 110 K.

Cu(II) extraction from the A $\beta$  peptide is observed with L<sub>2</sub> in all the conditions tested, regardless of the absence or presence of Zn.

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



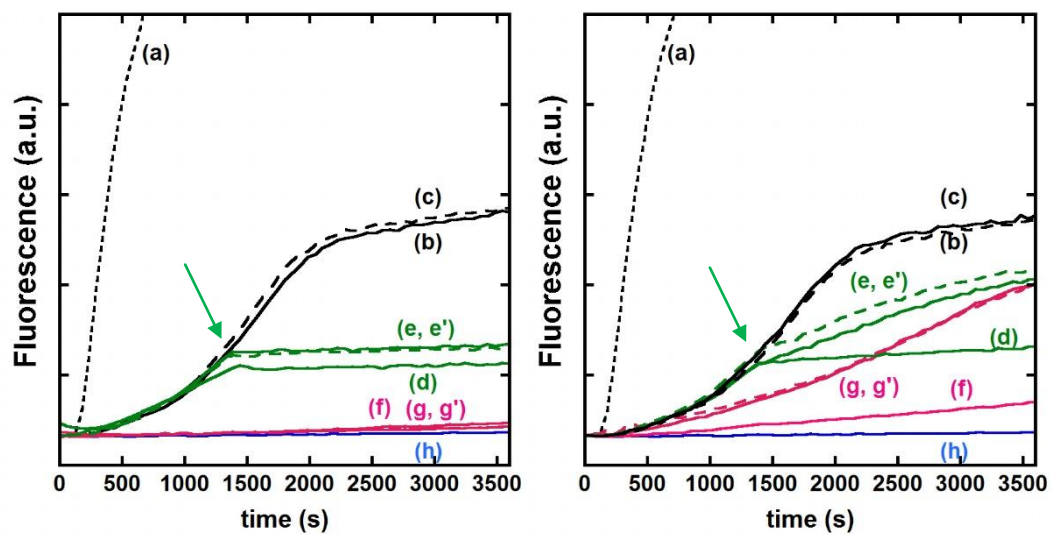
**Figure S4.** Panel A: Normalized Cu K-edge X-ray absorption near edge structure (XANES) spectra of Cu(A $\beta$ 16) (a) ; Cu(A $\beta$ 16) + Zn(L<sub>C</sub>) (b) ; Cu(L<sub>C</sub>) + A $\beta$ 16 (c) ; Cu(L<sub>C</sub>) + 1 equiv. of imidazole (d) ; Cu(L<sub>C</sub>) (e). Panel B: Normalized Zn K-edge XANES spectra of Zn(A $\beta$ 16) (a) ; calculation spectrum according to 0.4 spectrum (a) + 0.6 spectrum (d)) (b) ; Cu(A $\beta$ 16) + Zn(L<sub>C</sub>) (c) ; Zn(L<sub>C</sub>) + A $\beta$ 16 (d) ; Zn(L<sub>C</sub>) + 1 equiv. of imidazole (e) ; Zn(L<sub>C</sub>) (f). [A $\beta$ ] = [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<sub>C</sub> 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).

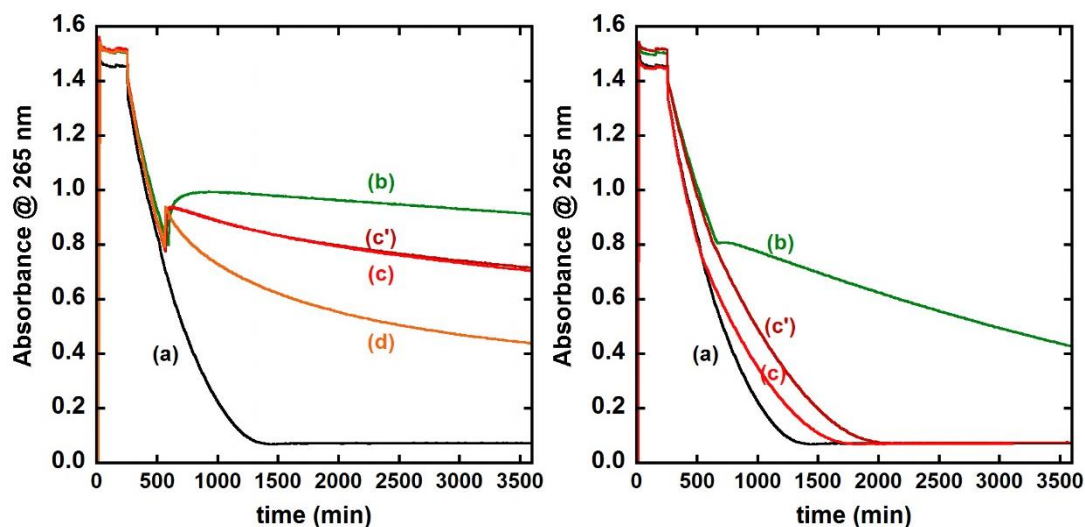
In contrast, the evaluation of the distribution of Zn(II) between the peptide and the L<sub>C</sub> ligand in the exchange experiment by XANES at the Zn K-edge is possible. This leads to approx. 60% of Zn bound to the L<sub>C</sub> (compare spectra (b) and (c)) in line with the proportion of Cu(II) bound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



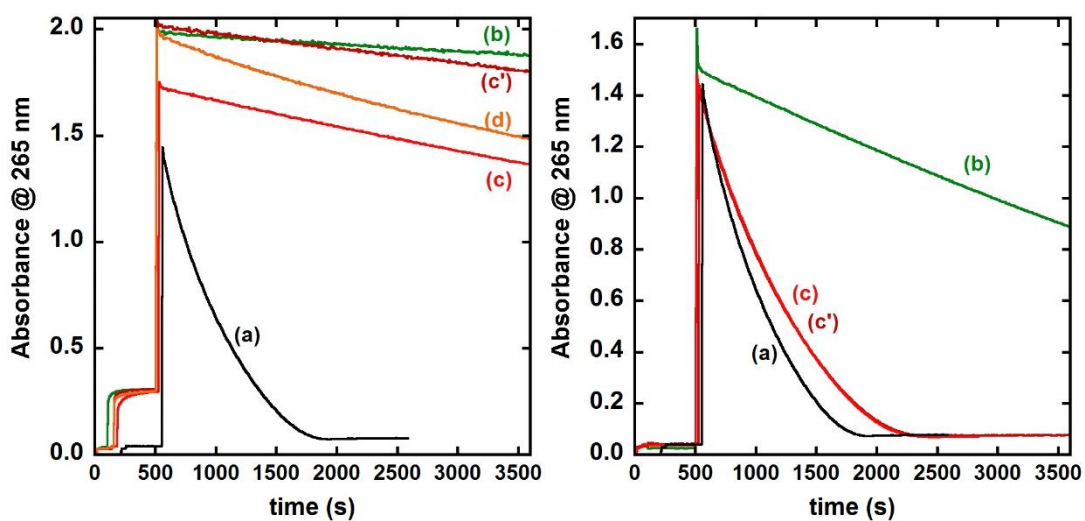
## ROS production assays



**Figure S5.** Cu(II) induced 7-OH-CCA formation. Cu(II) (a), Cu(A $\beta$ 16) (b), Cu,Zn(A $\beta$ 16) (c), Cu(A $\beta$ 16) + L added @ t = 20 min (d); Cu(A $\beta$ 16) + Zn(L) added @ t = 20 min (e); Cu,Zn(A $\beta$ 16) + L added @ t = 20 min (e', dotted line); Cu(A $\beta$ 16) + L (f); Cu(A $\beta$ 16) + Zn(L) (g); Cu,Zn(A $\beta$ 16) + L (g', dotted line); control experiment (without Cu) (h). Ascorbate is added to trigger the reaction. Left panel L = L<sub>2</sub>; Right panel : L = L<sub>C</sub>. [Cu(II)] = 10  $\mu$ M, 1.2 equiv of A $\beta$ , L and Zn.  $\lambda_{em}$  = 452 nm, [Asc] = 0.5 mM, [PO<sub>4</sub>] = 50 mM, pH 7.1, [CCA] = 0.5 mM, T = 25°C.

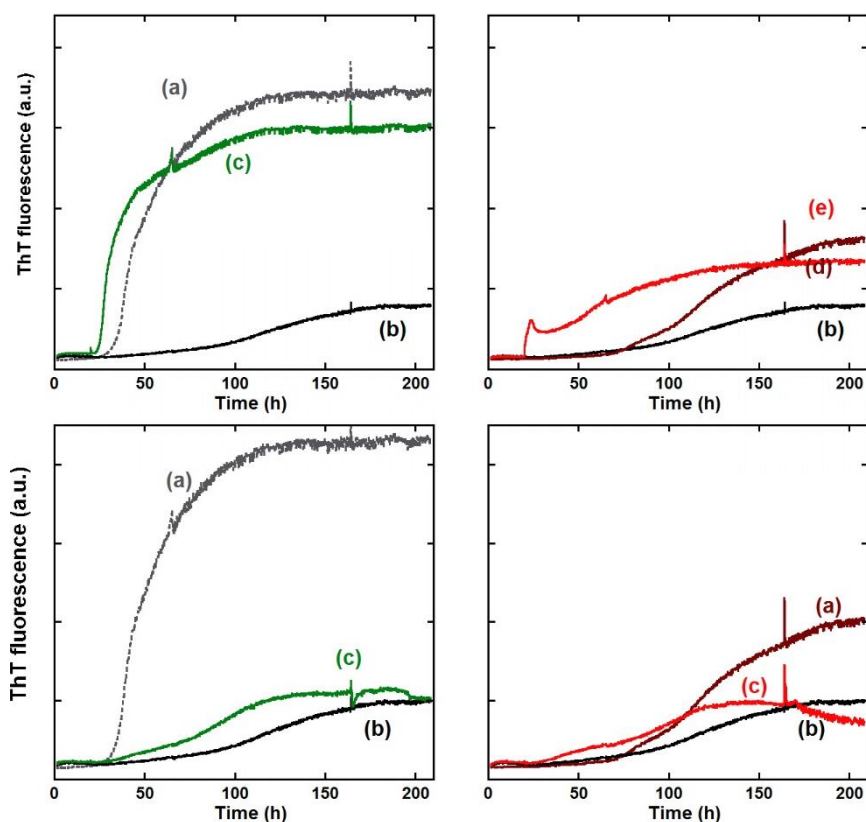


**Figure S6.** Cu(II) induced ascorbate consumption. Cu(A $\beta$ 16) (a), Cu(A $\beta$ 16) + L added @ t = 590 s (b) ; Cu(A $\beta$ 16) +Zn(L) added @ t = 560 s (c) ; Cu,Zn(A $\beta$ 16) + L added @ t = 570 s (c') ; Cu(A $\beta$ 16) + 5eq. Zn + L added @ t = 560 s (d). Left panel L = L<sub>2</sub> ; Right panel : L = L<sub>C</sub>. [Cu(II)] = 10  $\mu$ M, 1.2 equiv of A $\beta$ , L and Zn. [Asc] = 0.1 mM, [hepes] = 100 mM, pH 7.1, T = 25°C.



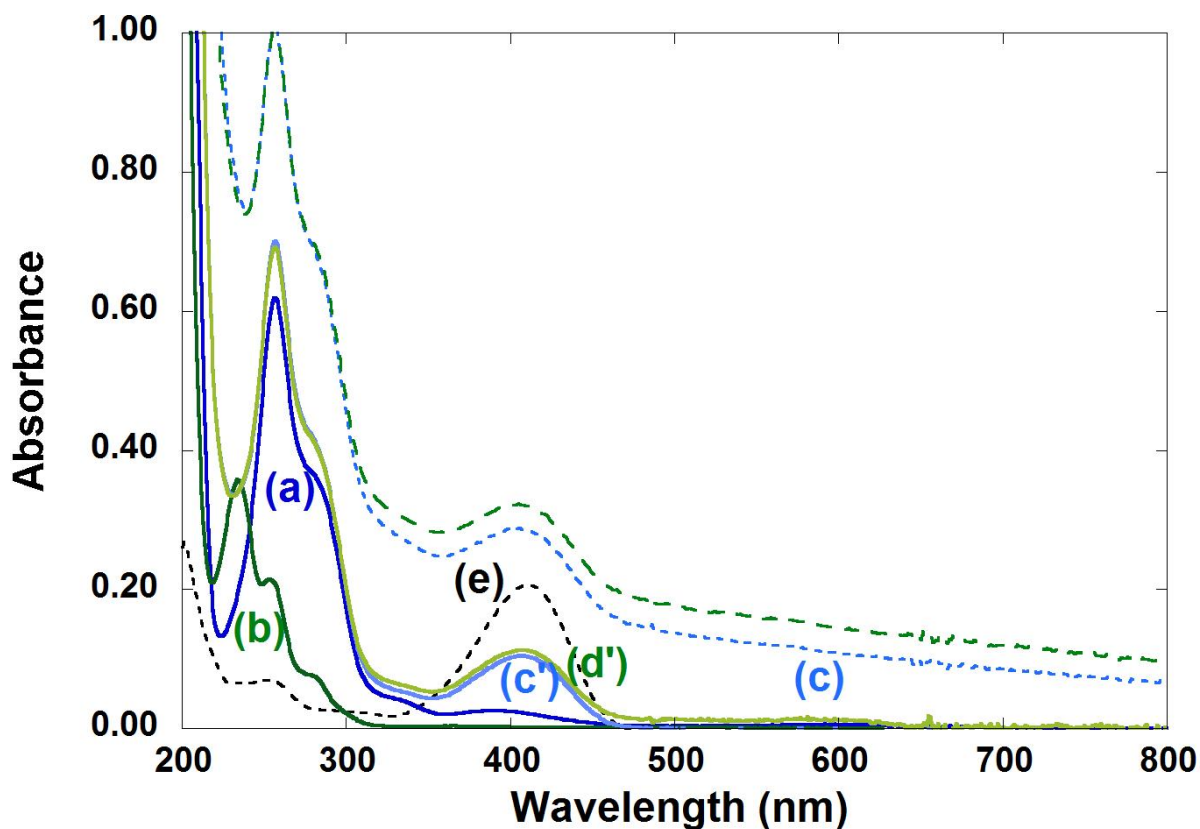
**Figure S7.** Cu(II) induced ascorbate consumption. Cu(A $\beta$ 16) (a), Cu(A $\beta$ 16) + L (b) ; Cu(A $\beta$ 16) +Zn(L) (c) ; Cu,Zn(A $\beta$ 16) + L (c') ; Cu(A $\beta$ 16) + 5eq. Zn + L (d). Ascorbate is added to trigger the reaction. Left panel L = L<sub>2</sub> ; Right panel : L = L<sub>C</sub>. [Cu(II)] = 10  $\mu$ M, 1.2 equiv of A $\beta$ , L and Zn. [Asc] = 0.1 mM, [hepes] = 100 mM, pH 7.1, T = 25°C. Ascorbate is added at t  $\approx$  500 s.

## Aggregation assay



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

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.<sup>15, 16</sup>



**Figure S9.** UV-Vis absorption spectra of Cu(L<sub>2</sub>) (blue, a) ; Zn(L<sub>2</sub>) (green, b) ; Cu(Aβ) after aggregation and addition of L<sub>2</sub> @ t = 162 h (dotted light blue line, c) ; in the supernatant of Cu(Aβ) after aggregation and addition of L<sub>2</sub> @ t = 162 h (plain light blue line, c') ; Cu(Aβ) after aggregation and addition of Zn(L<sub>2</sub>) @ t = 162 h (dotted light green line, d) ; in the supernatant of Cu(Aβ) after aggregation and addition of Zn(L<sub>2</sub>) @ t = 162 h (plain light green line, d') and free ThT (dotted black line, e); [L<sub>2</sub>] = [Aβ] = 20 μM, [M] = 18 μM, [phosphate buffer] = 0.05 M, pH 7.1, [ThT] = 10 μM, T = 25 °C, ℓ=1 cm.

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