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# **N<sub>4</sub>-Tetradentate chelators efficiently regulate copper homeostasis and prevent ROS production induced by copper-amyloid- $\beta_{1-16}$ , even in the presence of an excess of zinc.**

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## **Abstract**

The disruption of copper homeostasis and the oxidative stress induced by Cu-amyloids are crucial features of Alzheimer's disease pathology. The copper specific N<sub>4</sub>-tetradentate ligands TDMQ20 and **1** are able to fully inhibit *in vitro* the aerobic oxidation of ascorbate induced by Cu-A $\beta_{1-16}$ , even in the presence of 100 molar equivalents of Zn(II) with respect to Cu(II), while other ligands with N<sub>2</sub>O<sub>2</sub> or N<sub>3</sub>O<sub>2</sub> coordination spheres failed to do so. This essential result indicates that, in addition to metal selectivity, the coordination sphere of copper chelators should exhibit a N<sub>4</sub>-tetradentate motif to be able to reduce an oxidative stress in the zinc-rich physiological environment of brain. The N<sub>4</sub>-scaffolds of these two aminoquinoline-based ligands, TDMQ20 or **1**, suitable for a square-planar coordination of copper(II), allowed them to enhance both the selectivity for copper and also the ability to reduce the oxidative stress induced by copper-amyloid in a zinc-rich environment.

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## **Key-words**

Alzheimer's disease; Copper chelator; Copper homeostasis; Oxidative stress; Zinc.

Redistribution and restoration of the homeostasis of metal ions, especially copper, in the brain of patients with Alzheimer's disease (AD) is now considered as a valuable challenge in the chemotherapy of this neurodegenerative condition.<sup>1</sup> However, zinc element is abundant and strictly regulated in the brain to avoid depletion or excess.<sup>2</sup> In addition to zinc-protein complexes, large amounts of chelatable zinc are located in the cerebral cortex and the limbic region, notably in the hippocampal formation, and concentrated within synaptic vesicles of glutamatergic neurons where it acts as a modulator of synaptic transmission.<sup>2c</sup> Zinc concentration has been estimated to reach 300  $\mu\text{M}$  in the synaptic cleft system during strong stimulation.<sup>2,3</sup> The mean copper concentration in human frontal lobe and cerebellum has been reported to be in the range of 60-110  $\mu\text{M}$ .<sup>4</sup> The zinc/copper ratio can be as high as 10 to 100, in glutamatergic neurons.<sup>5a</sup> Therefore, any attempt to regulate copper homeostasis and to inhibit copper-induced oxidative stress in the brain, must take into account this zinc-rich environment.<sup>5b</sup>

Therefore, the ligands designed to regulate the homeostasis of redox metal ions in AD brains must be copper specific to avoid the perturbation of zinc. As an example of the importance of the possible copper/zinc competition, clioquinol has been discarded due to its neurotoxicity attributed to zinc chelation.<sup>6</sup> As a matter of fact, the metal selectivity of 8-hydroxyquinoline scaffold is very low.<sup>7</sup> To regulate copper homeostasis, chelators should be able to transfer copper from its pathological sink, mainly from the copper-amyloid complexes (Cu-A $\beta$ ), to glutathione which is a physiological provider of copper to copper enzymes,<sup>8</sup> even in the presence of zinc. In addition, a large excess of zinc should not prevent the ability of these copper chelators to inhibit the production of reactive oxygen species (ROS) generated by copper loaded amyloids. Moreover, such ligands should have logP values expected for membrane crossing, especially the blood brain barrier (BBB).

However, among the copper chelators which are currently under investigation, the selectivity for copper with respect to zinc is usually not (or poorly) documented,<sup>1a</sup> except for bis-8-aminoquinoline derivatives that are specific copper chelators.<sup>9</sup> In this view, some of us reported the capacity of a water-soluble negatively charged chelator named L2 (Figure 1) to remove copper from copper-loaded A $\beta$  in the presence of zinc in order to illustrate the importance of the Cu selectivity on ROS formation.<sup>5</sup> This N<sub>2</sub>O<sub>2</sub> chelator, based on a Schiff-base scaffold, was able to inhibit the reductive activation of dioxygen in the presence of 1

molar equivalent (mol equiv) of Zn(II) but failed to stop ROS production in the presence of 5 mol equiv of Zn(II) during the ascorbate consumption.<sup>5a</sup> In addition, as pointed out in the article, the ligand L2 is not able to cross the BBB ( $\log P = -3.7$ ).

To enhance the efficiency and drugability of copper chelators, we designed a new series of neutral N<sub>4</sub>-tetradentate copper ligands named TDMQ, based on an 8-aminoquinoline motif.<sup>10</sup> One of these ligands, TDMQ20 (Figure 1), is a specific ligand for copper compared to zinc. In the present work, we investigated the ability of TDMQ20 to inhibit the Cu-A $\beta$  activation of dioxygen in the presence of a large excess of zinc(II), up to 100 mol equiv. The extraction of copper from Cu-A $\beta$  by TDMQ20, as well as the release of copper from Cu-TDMQ20 in the presence of glutathione, were also evaluated in the presence of zinc, as these two discrete steps are required to put back copper into normal physiological circulation. The obtained data were compared with that of ligand **1**, a bis-8-aminoquinoline chelator previously reported to have a very high selectivity for Cu(II) with respect to Zn(II)  $\{\log [K_{\text{app}} \text{Cu-L} / K_{\text{app}} \text{Zn-L}] > 12\}$ , and to efficiently transfer copper from Cu-A $\beta$  to glutathione in the absence of zinc.<sup>9</sup> The 8-hydroxyquinoline derivative PBT2<sup>1c,11</sup> was used as a comparator. A detailed experimental section is provided as Supporting Information.

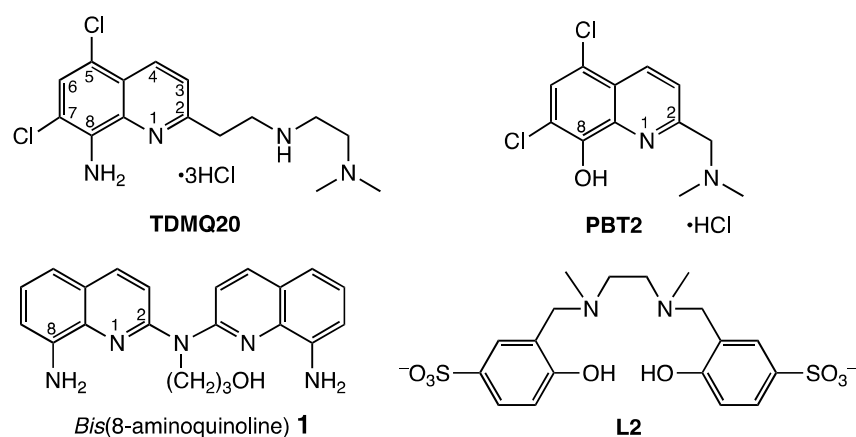


Figure 1. Structures of the N<sub>4</sub>-tetradentate ligands, mono-8-aminoquinoline TDMQ20 and bis(8-aminoquinoline) **1**, of the N<sub>2</sub>O<sub>2</sub> ligand L2, and of the bi/tridentate 8-hydroxyquinoline PBT2.

The ability of TDMQ20 to extract copper from Cu-A $\beta_{1-16}$  was evaluated as follows. The Cu(II) complex of A $\beta_{1-16}$  was prepared by incubation of A $\beta_{1-16}$  and Cu<sup>2+</sup> at 20  $\mu\text{M}$  (molar ratio = 1/1) in Hepes buffer pH 7.4. One or three mol equiv of Zn<sup>2+</sup> were added into the Cu<sup>II</sup>-

A $\beta_{1-16}$  solution. One mol equiv of TDMQ20 was then added, and the reaction was monitored by UV-visible spectroscopy. The spectrum of the resulting mixture containing A $\beta_{1-16}$ , Cu(II), Zn(II) and TDMQ20 was recorded (Figure 2, red trace, full line for 1 equiv of Zn, dashed line for 3 equiv of Zn). In both cases, the spectrum exhibited absorbance at 243 nm assigned to Cu-TDMQ20 (bright blue trace), but not at 259 and 342 nm, specific wavelengths of the TDMQ20 free ligand. In fact, the spectra of the mixture Cu<sup>II</sup>-A $\beta_{1-16}$  / TDMQ20 in the presence of Zn<sup>2+</sup> (red traces) were very similar to the arithmetic addition of the spectra of free A $\beta_{1-16}$  and Cu-TDMQ20 (black dashed trace). This result is the same as that obtained in a reaction carried out in the absence of zinc (Figure S1, Supporting Information). It therefore clearly indicates that 1 mol equiv of TDMQ20 was sufficient to fully demetallate the copper loaded amyloid, providing Cu-TDMQ20, in the presence of either 1 or 3 mol equiv of zinc with respect to copper. These data indicate that the presence of 3 mol equiv of zinc does not interfere with the demetalation of Cu<sup>II</sup>-A $\beta_{1-16}$  by one equivalent of TDMQ20.

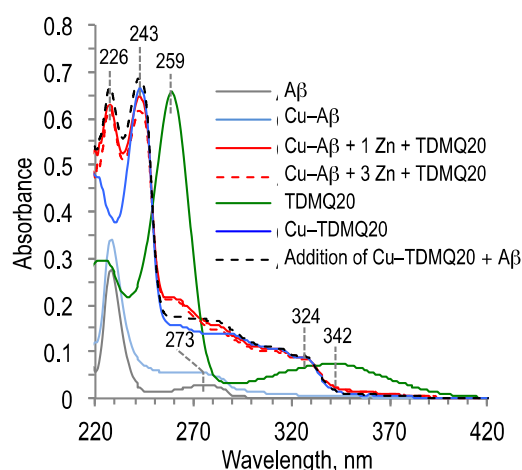


Figure 2. Extraction of copper from Cu<sup>II</sup>-A $\beta_{1-16}$  using 1 mol equiv of TDMQ20, in the presence of 1 or 3 mol equiv of Zn<sup>2+</sup> (full red trace, and dashed red trace, respectively) with respect to copper. For detailed experimental conditions, see Supporting Information.

The release of copper from Cu<sup>II</sup>-TDMQ20 in the presence of GSH was then monitored. As previously reported, when the Cu(II) complex of the tetradentate ligand **1** was treated by glutathione (GSH), GSH acts both as a reducing agent of Cu(II) and as a competitive ligand for the generated Cu(I).<sup>9c</sup> The resulting Cu(I)-glutathione complex is a physiological supplier of copper to the apo form of copper proteins.<sup>8</sup> However, the role of zinc in this process has

not been reported yet. Consequently, the influence of zinc on copper circulation in the presence of chelators remained to be established. We decided to monitor, by UV-visible spectrometry, the demetalation of Cu<sup>II</sup>-TDMQ20 in the presence of both glutathione and Zn<sup>2+</sup>, in Hepes buffer, pH 7.4. Firstly, CuCl<sub>2</sub> was incubated with TDMQ20 to generate Cu<sup>II</sup>-TDMQ20, and 1 mol equiv of ZnCl<sub>2</sub> was then added. The spectrum of the reaction mixture exhibited a maximal absorbance at 243 nm, typical of that expected for Cu<sup>II</sup>-TDMQ20 (Figure 3, red trace). Upon addition of glutathione (by aliquots of 20 mol equiv with respect to Cu-TDMQ), the demetalation of Cu-TDMQ was monitored by formation of the free ligand TDMQ20 detected at 259 and 342 nm (well-defined isosbestic points are detected at 279 and 332 nm; no isosbestic point can be detected below 250 nm, due to the own absorbance of glutathione and Cu(I)-glutathione at these wavelengths) (Figure 3). The plotting of the absorbance at 259 nm as a function of the number of glutathione equivalents (Figure 3, Insert) indicate that 80 equiv of glutathione were required to achieve full demetalation of Cu-TDMQ. Interestingly, a similar result was observed in the absence of ZnCl<sub>2</sub> (Figure S2, Supporting Information), indicating that the presence of zinc did not influence the demetalation of Cu-TDMQ20 by glutathione in these conditions.

So, *in vitro*, TDMQ20 is able to abstract copper from Cu-Aβ<sub>1-16</sub> and then to transfer it to glutathione, indicating that this N<sub>4</sub>-tetradentate ligand has the capacity for regulation of copper homeostasis in the presence of zinc.

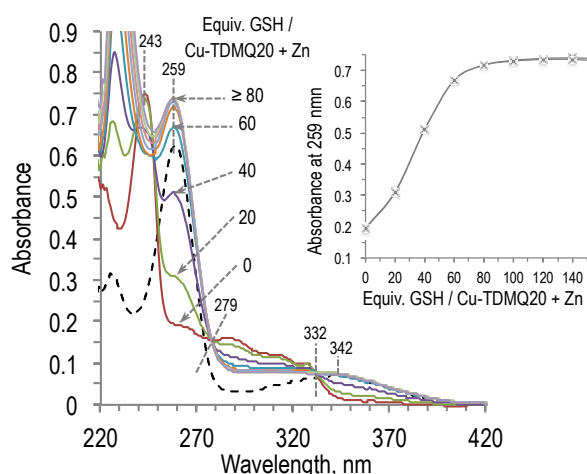


Figure 3. Extraction of copper from Cu<sup>II</sup>-TDMQ20 by glutathione (GSH) in the presence of 1 mol equiv of Zn<sup>2+</sup>. The spectrum of the free ligand TDMQ20 (dashed black line) is given for comparison. Insert: Variation of the absorbance at 259 nm as a function of the number of GSH mol equiv with respect to Cu-TDMQ / Zn<sup>2+</sup>. For detailed experimental conditions, see Supporting Information.

The *in vitro* ROS production mediated by Cu-A $\beta$ <sub>1-16</sub> in the presence or in the absence of any additive (namely zinc or a putative chelator) can be evaluated based on the kinetics of ascorbate oxidation, easily monitored by UV-visible at 265 nm.<sup>9c,12,13</sup> We investigated the influence of TDMQ20 (and ligands **1** and PBT2 as comparators, structures in Figure 1) on the oxidation of ascorbate induced by Cu-A $\beta$ <sub>1-16</sub> in the presence of Zn<sup>2+</sup>. Firstly, a Zn/Cu molar ratio equal to 3 was investigated since senile plaques contain roughly 3 times more Zn than Cu (25 and 69  $\mu$ g/g for Cu and Zn, respectively).<sup>14</sup> The results are showed in Figure 4. Trace (a) shows the ascorbate autoxidation, without any additive. In the presence of CuCl<sub>2</sub>, ascorbate was fully oxidized in only 5 min (trace b). Cu-A $\beta$ <sub>1-16</sub> induced *ca.* 40% of ascorbate oxidation in 5 min, and full oxidation occurred in *ca.* 25 min (trace c). In the presence of 3 mol equiv of Zn<sup>2+</sup>, TDMQ20 or ligand **1** drastically inhibited the ascorbate oxidation induced by Cu-A $\beta$ <sub>1-16</sub>, the oxidation of ascorbate in 30 min being only 15-16% in both cases (traces d and e, respectively), very close to the ascorbate autoxidation (8%, trace a).

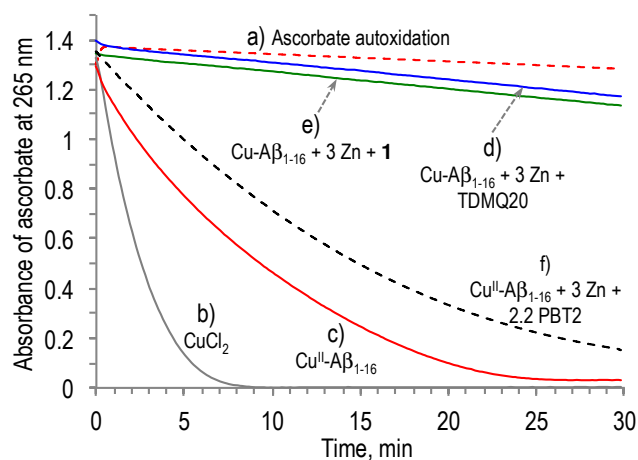


Figure 4. UV-visible (265 nm) kinetic spectra of ascorbate oxidation under air, in the presence of a) no additive (ascorbate autoxidation), b) CuCl<sub>2</sub>, c) CuCl<sub>2</sub>/A $\beta$ <sub>1-16</sub>, 1/1.1, d) CuCl<sub>2</sub>/A $\beta$ <sub>1-16</sub>/ZnCl<sub>2</sub>/TDMQ20 = 1/1.1/3/1.1, e) CuCl<sub>2</sub>/A $\beta$ <sub>1-16</sub>/ZnCl<sub>2</sub>/**1** = 1/1.1/3/1.1, f) CuCl<sub>2</sub>/A $\beta$ <sub>1-16</sub>/ZnCl<sub>2</sub>/PBT2 = 1/1.1/3/2.2. For detailed experimental conditions, see Supporting Information.

We also performed similar experiments with PBT2, a bi- or tridentate ligand based on 8-hydroxyquinoline scaffold, that has been developed as a potential AD drug.<sup>1c,15</sup> Since the Cu-PBT2 complex has potentially a metal/ligand stoichiometry = 1/2,<sup>16</sup> we therefore used 2.2 mol equiv of PBT2 with respect to Cu-A $\beta$ <sub>1-16</sub>, to avoid the presence of free Cu<sup>2+</sup> ions in the

solution. With 3 mol equiv of Zn with respect to copper, 89% of ascorbate oxidation was observed in 30 min (Figure 4, trace f), close to the 80% value reported in the absence of zinc<sup>9c</sup>, indicating that PBT2 did not inhibit ascorbate oxidation under these reaction conditions.

Since both TMDQ20 and compound **1** efficiently inhibited the ascorbate oxidation induced by Cu-A $\beta$ <sub>1-16</sub> in the presence of 3 mol equiv of Zn<sup>2+</sup>, we decided to investigate the same reaction with a very large excess of zinc, 100 mol equiv of zinc with respect to copper, a Zn/Cu ratio that has been proposed to be biologically relevant.<sup>5</sup> In these conditions, Cu-A $\beta$ <sub>1-16</sub> induced 48% of ascorbate oxidation in 5 min, and 86% in 10 min (Figure 5, trace a). This is a moderate increase of ROS production compared to the result obtained in the absence of zinc (trace b, 39% and 63% in 5 min and 10 min, respectively). A previous report indicated that a *single* zinc equivalent had no effect on Cu-A $\beta$  induced ROS production, even though the coordination of Zn<sup>2+</sup> impacts the Cu(II) and Cu(I) binding sites of A $\beta$ , and may lead to the formation of a hetero-bimetallic Cu-Zn-A $\beta$  complex, without release of Cu.<sup>17</sup>

Our result indicates that a 100-fold excess of Zn<sup>2+</sup> resulted in the release of a fraction of Cu<sup>2+</sup> from Cu<sup>II</sup>-A $\beta$ <sub>1-16</sub>, which is consistent with a higher ascorbate oxidation. So, the presence of a local huge excess of zinc with respect to Cu-A $\beta$  may significantly exacerbate the redox toxicity of Cu-A $\beta$  *in vivo*.

In order to evidence the fast action of such N<sub>4</sub>-tetradendate ligands on the kinetics of the ascorbate oxidation induced by CuCl<sub>2</sub>/A $\beta$ <sub>1-16</sub>/ZnCl<sub>2</sub> (1/1/100 molar ratio), we added one of these copper chelators, TDMQ20 or **1** (1 mol equiv), after 6.6 min of reaction (Figure 5, traces d and e, respectively). In both cases, the oxidation of ascorbate was immediately and completely stopped, indicating that stoichiometric amounts of TDMQ20 and **1** were able to fully inhibit the ROS production induced by the mixture Cu(II)/A $\beta$ <sub>1-16</sub>/Zn(II) = 1/1/100. The slopes of traces d and e after 6.6 min ( $\Delta\text{Abs}_{265} = 0.002 / \text{min}$ ) were not significantly different to that of ascorbate autoxidation (Figure 4, trace a,  $\Delta\text{Abs}_{265} = 0.003 / \text{min}$ ).



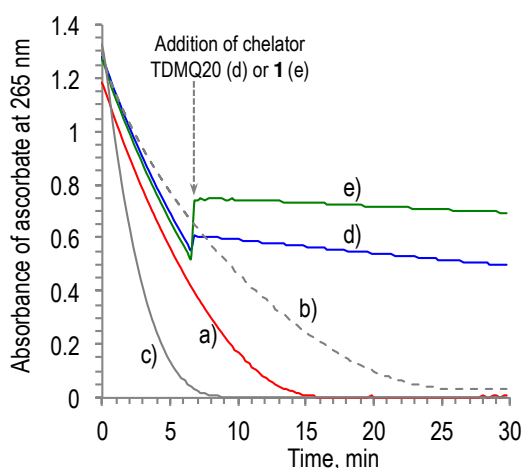


Figure 5. UV-visible (265 nm) kinetic spectra of ascorbate oxidation under air, in the presence of  $\text{CuCl}_2/\text{A}\beta_{1-16}/\text{ZnCl}_2 = 1/1/100$ , then addition of L (1 mol equiv) at 6.6 min (arrow): L = no ligand (trace a), L = TDMQ20 (trace d), L = **1** (trace e). The ascorbate oxidation in the presence of  $\text{CuCl}_2/\text{A}\beta_{1-16}$ , (trace b), or  $\text{CuCl}_2$  alone (trace c) are given for comparison. The increase of absorbance upon addition of TDMQ20 (d) or **1** (e) at 6.6 min, is due to the own absorbance of  $\text{Cu}^{\text{II}}$ -TDMQ20, or  $\text{Cu}^{\text{II}}$ -**1** at 265 nm. For detailed experimental conditions, see Supporting Information.

These tetradentate ligands TDMQ20 and **1**, that specifically chelate Cu(II) in a  $\text{N}_4$ -square planar fashion, are able to immediately and completely stop the *in vitro* ongoing aerobic oxidation of ascorbate induced by  $\text{Cu-A}\beta_{1-16}$ , even in the presence of 100 mol equiv of Zn(II) with respect to copper ions, while the  $\text{N}_2\text{O}_2$ -tetradentate ligand L2, able to chelate copper with metal/ligand stoichiometry = 1/1, was efficient only in the presence of a stoichiometric amount of zinc.<sup>5</sup> PBT2, whose copper complex has a  $\text{N}_3\text{O}_2$  coordination sphere, and a metal/ligand stoichiometry = 1/2,<sup>16</sup> failed to inhibit oxygen reduction either in the presence or in the absence of zinc.

These  $\text{N}_4$ -tetradentate ligands TDMQ20 and **1** have a high selectivity for Cu(II) with respect to Zn(II)  $\{\log [K_{\text{app}} \text{Cu-L} / K_{\text{app}} \text{Zn-L}] > 12\}$ ,<sup>9a,10</sup> much higher than that of  $\text{A}\beta$   $\{\log [K_{\text{app}} \text{Cu-A}\beta / K_{\text{app}} \text{Zn-A}\beta] = 4-5\}$ ,<sup>5b</sup> and thus fulfill the criteria for biologically pertinent copper chelators mentioned in the introduction. The obtained results indicated that both ligands TDMQ20 and **1** have the potential to reduce or inhibit *in vivo* ROS production induced by  $\text{Cu-A}\beta$ , even in a very rich zinc physiological environment.

In conclusion, such N<sub>4</sub>-tetradentate copper-specific ligands, like TDMQ20 and **1**, are suitable for a square-planar scaffold with Cu(II) and have the ability to largely stabilize Cu(II) over Cu(I). This structural feature makes them efficient inhibitors of the *in vitro* ROS production induced by Cu-A $\beta$ , including in a zinc-rich physiological environment. Finally, the calculated logP values are 2.5 and 3.5, for TDMQ20 and **1**,<sup>18</sup> respectively, in the optimal range expected for crossing the blood-brain barrier.<sup>19</sup> The design of copper ligands able to counteract metal deregulation and deleterious consequences in AD brain requires a good understanding of their coordination properties. Pharmacological studies on TDMQ20 are in progress.

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### References

1. a) M. Kawahara, M. Kato-Negishi, K. Tanaka, *Metallomics*, **2017**, *9*, 619-633; b) A. Robert, Y. Liu, M. Nguyen, B. Meunier, *Acc. Chem. Res.* **2015**, *48*, 1332-1339; c) K. J. Barnham, A. I. Bush, *Chem. Soc. Rev.* **2014**, *43*, 6727-6749; d) M. A. Telpoukhovskaia, C. Orvig, *Chem. Soc. Rev.* **2013**, *42*, 1836-1846; e) P. Faller, C. Hureau, *Chem. Eur. J.* **2012**, *18*, 15910-15920; f) L. R. Perez, K. J. Franz, *Dalton Trans.* **2010**, *39*, 2177-2187;
2. a) A. Takeda, H. Fujii, T. Minamino, H. Tamano, *J. Neurosci. Res.* **2014**, *92*, 819-824; b) M. S. Shetty, M. Sharma, S. Sajikumar, *Aging Cell*, **2017**, *16*, 136-148 and references cited; c) E. P. Huang, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13386-13387.
3. S. L. Sensi, P. Paoletti, J-Y. Koh, E. Aizenman, A. I. Bush, M. Hershfinkel, *J Neurosci.* **2011** *31*, 16076-16085.
4. E. D. Gaier, B. A. Eipper, R. E. Mains, *J. Neurosci. Res.* **2013**, *91*, 2-19.
5. a) A. Conte-Daban, A. Day, P. Faller, C. Hureau, *Dalton Trans.* **2016**, *45*, 15671-15678.  
b) E. Atrián-Blasco, A. Conte-Daban, C. Hureau, *Dalton Trans.* **2017**, *46*, 12750-12759.

6. a) J. L. Arbiser, S.-K. Kraeft, R. van Leeuwen, S. J. Hurwitz, M. Selig, G. R. Dickersin, A. Flint, H. R. Byers, L. B. Chen, *Mol. Med.* **1998**, *4*, 665–670; b) D. A. Andersson, C. Gentry, S. Moss, S. Bevan, *Proc. Natl. Acad. Sci. USA* **2009**, *109*, 8374–8379.
7. a) J. P. Phillips, *Chem. Rev.* **1956**, *56*, 271–297; b) E. Ferrada, V. Arancibia, B. Loeb, E. Norambuena, C. Olea-Azar, J. P. Huidobro-Toro, *Neurotoxicology*, **2007**, *28*, 445–449.
8. a) G. Musci, S. Di Marco, G. C. Bellenchi, L. Calabrese, *J. Biol. Chem.* **1996**, *271*, 1972–1978; b) M. R. Ciriolo, A. Desideri, M. Paci, G. Rotilio, *J. Biol. Chem.* **1990**, *265*, 11030–11034; c) J. H. Freedman, M. R. Ciriolo, J. Peisach, *J. Biol. Chem.* **1989**, *264*, 5598–5605.
9. a) M. Nguyen, A. Robert, A. Sournia-Saquet, L. Vendier, B. Meunier, *Chem. Eur. J.* **2014**, *20*, 6771–6785; b) M. Nguyen, L. Rechinat, A. Robert, B. Meunier, *ChemistryOpen* **2015**, *4*, 27–31; c) M. Nguyen, C. Bijani, N. Martins, B. Meunier, A. Robert, *Chem. Eur. J.* **2015**, *21*, 17085–17090.
10. a) Y. Liu, X. Liu, D. Huang, M. Huang, D. Wang, M. Nguyen, A. Robert, B. Meunier, Chinese patent 201610369550.X, May 27, 2016; b) W. Zhang, D. Huang, M. Huang, J. Huang, D. Wang, X. Liu, M. Nguyen, L. Vendier, S. Mazères, A. Robert, Y. Liu, B. Meunier, *ChemMedChem* **2018**, *16*, 684–704.
11. K. J. Barnham, E. C. L. Gautier, G. B. Kok, G. Krippner, US Patent 2006/0089380 A1, Apr. 27, 2006.
12. G. R. Buettner, B. A. Jurkiewicz, *Free Rad. Biol. Med.* **1993**, *14*, 49–55.
13. E. Atrián-Blasco, E. Cerrada, A. Conte-Daban, D. Testemale, P. Faller, M. Laguna, C. Hureau, *Metallomics* **2015**, *7*, 1229–1232.
14. M. A. Lovell, J. D. Robertson, W. J. Teesdale, J. L. Campbell, W. R. Markesbery, *J. Neurol. Sci.* **1998**, *158*, 47–52.
15. N. G. Faux, C. W. Ritchie, A. Gunn, A. Rembach, A. Tsatsanis, J. Bedo, J. Harrison, L. Lannfelt, K. Blennow, H. Zetterberg, M. Ingelsson, C. L. Masters, R.E. Tanzi, J. L. Cummings, C. M. Herd, A. I. Bush, *J. Alz. Dis.* **2010**, *20*, 509–516.
16. M. Nguyen, L. Vendier, J.-L. Stigliani, B. Meunier, A. Robert, *Eur. J. Inorg. Chem.* **2017**, 600–608.
17. B. Aliès, I. Sasaki, O. Proux, S. Sayen, E. Guillon, P. Faller, C. Hureau, *Chem. Commun.* **2013**, *49*, 1214–1216.

18. Calculated with ChemDraw Prime v. 16.0.0.82.
19. C. Hansch, A. J. Leo, Substituent constant for correlation analysis in chemistry and biology, New York: Wiley (1979).

## Captions for Figures

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- Figure 2. Extraction of copper from  $Cu^{II}$ - $A\beta_{1-16}$  using 1 mol equiv of TDMQ20, in the presence of 1 or 3 mol equiv of  $Zn^{2+}$  (full red trace, and dashed red trace, respectively) with respect to copper monitored by UV-visible spectrophotometry.
- Figure 3. Extraction of copper from  $Cu^{II}$ -TDMQ20 by glutathione (GSH), in the presence of 1 mol equiv of  $Zn^{2+}$ , 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  $Cu$ -TDMQ20 /  $Zn^{2+}$ .
- Figure 4. UV-visible (265 nm) kinetic spectra of ascorbate oxidation under air, in the presence of a) no additive (ascorbate autoxidation), b)  $CuCl_2$ , c)  $CuCl_2/A\beta_{1-16}$ , 1/1.1, d)  $CuCl_2/A\beta_{1-16}/ZnCl_2/TDMQ20 = 1/1.1/3/1.1$ , e)  $CuCl_2/A\beta_{1-16}/ZnCl_2/1 = 1/1.1/3/1.1$ , f)  $CuCl_2/A\beta_{1-16}/ZnCl_2/PBT2 = 1/1.1/3/2.2$ .
- Figure 5. UV-visible (265 nm) kinetic spectra of ascorbate oxidation under air, in the presence of  $CuCl_2/A\beta_{1-16}/ZnCl_2 = 1/1/100$ , then addition of L (1 mol equiv) at 6.6 min (arrow): L = no ligand (trace a), L = TDMQ20 (trace d), L = **1** (trace e). The ascorbate oxidation in the presence of  $CuCl_2/A\beta_{1-16}$ , (trace b)  $CuCl_2$  (trace c) are given for comparison. The increase of absorbance upon addition of TDMQ20 (d) or **1** (e) at 6.6 min, is due to the own absorbance of  $Cu^{II}$ -TDMQ20, or  $Cu^{II}$ -**1** at 265 nm.

## Scheme and text for Table of Contents



N<sub>4</sub>-tetradentate copper-specific ligands generate Cu(II) square planar complexes and stabilize Cu(II) but not Cu(I). This feature makes them able to regulate copper homeostasis and to efficiently inhibit the *in vitro* oxidative stress induced by Cu-A $\beta$ , including in a zinc-rich physiological environment like Alzheimer's disease brain. The design of ligands able to counteract copper deregulation and deleterious effects in AD brain must take into account their coordination modes.