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# Copper-targeting approaches in Alzheimer's disease: how to improve the fallouts obtained from in vitro studies

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**Synopsis:** Since the discovery of Alzheimer's disease (AD) in 1906, scientists have made remarkable strides in understanding how AD affects the brain. Different therapeutic approaches have been proposed and yet there is no treatment that can cure AD or even halt its progression. In this viewpoint article, ligands targeting Cu(II) ion, regarded as a key player in the amyloid cascade linked to the etiology of AD, are under focus.

**Abstract:** According to the amyloid cascade hypothesis, metal ions, mainly Cu and Zn ions, bound to the amyloid- $\beta$  (A $\beta$ ) peptides are implicated in Alzheimer's disease (AD), a widespread neurodegenerative disease. They indeed impact the aggregation pathways of A $\beta$  and are involved in the catalytic generation of Reactive Oxygen Species (ROS) that participate to oxidative stress, while A $\beta$  aggregation and oxidative stress are regarded as two key events in AD etiology. Cu ions due to their redox ability have been considered as the main potential therapeutic targets in AD. A considerable number of ligands have been developed in order to modulate the toxicity associated to Cu in this context, *via* disruption of the A $\beta$ -Cu interaction. Among them, small synthetic ligands and small peptide scaffolds have been designed and studied for their ability to remove Cu from A $\beta$ . Some of those ligands are able to prevent Cu(A $\beta$ )-induced ROS production and can modify the aggregation pathways of A $\beta$  *in vitro* and *in cellulo*. Examples of such ligands are gathered in this viewpoint, as function of their structures and discussed with respect to their properties against Cu(A $\beta$ ) deleterious fallouts. Nevertheless, the beneficial activity of the most promising ligands detected *in vitro* and *in cellulo* have not been transposed to human yet. Some parameters that might explain this apparent contradiction and key concepts to consider for the design of "more" efficient ligands are thus reported and discussed. *En passant*, this viewpoint sheds light on the difficulties to compare the results from one study to another that hamper significant advances in the field.

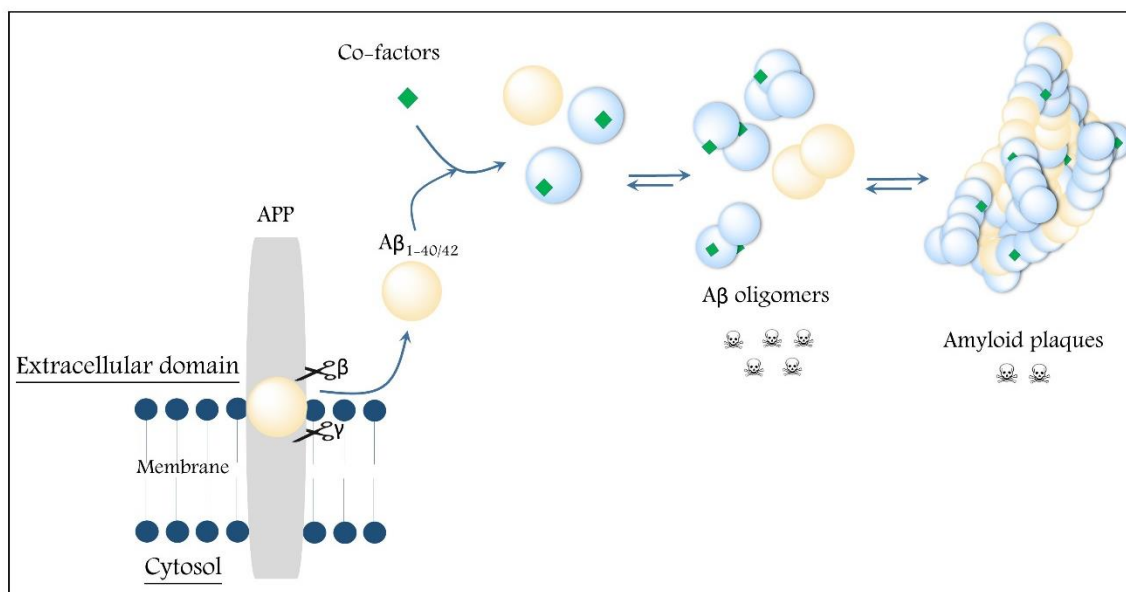
## 1. GENERAL CONSIDERATIONS

### 1. 1. Alzheimer's disease: a brief introduction

Dementias are frequent and serious neurological disorders that represent the main mental pathologies of the elderly. The dementia syndrome is characterized by a gradual deterioration of cognitive functions, especially memory, with a significant impact on the personality, activities of daily life, social functioning and patient autonomy. Alzheimer's disease (AD) is the most common form of dementia with a prevalence of around 47.5 million patients worldwide.<sup>1</sup> This number is expected to triple within the next 35 years and therefore it represents a major public health issue.<sup>1, 2</sup> Two morphological hallmarks are observed in AD,<sup>3-6</sup> both are abnormal accumulations of fibrils in different locations: (i) intracellular deposition of paired helical filaments of the hyperphosphorylated Tau protein in neurofibrillary tangles, which occurs in other neurodegenerative diseases such as Parkinson's disease as well; (ii) extracellular amyloid plaques (also known as senile plaques) made of amyloid- $\beta$  peptides ( $A\beta$ ) under aggregated structures. Hence targeting  $A\beta$  and Tau accumulation is crucial for diagnosis, treatment and prevention of AD.<sup>7, 8</sup> In the present article, we will focus on  $A\beta$  because of its interaction with metal ions that has been intensively studied in the last decades.<sup>5, 9, 10</sup> The  $A\beta$  peptides are produced from the amyloid precursor protein (APP) by the  $\beta$ - and  $\gamma$ -secretases (Figure 1).<sup>11-14</sup> Although several  $A\beta$  isoforms of various lengths are obtained,  $A\beta_{1-40}$  and  $A\beta_{1-42}$  are still regarded as the most abundant and their binding to metal ions the most investigated.<sup>5, 9, 10, 15, 16</sup> The mechanism of formation of the amyloid plaques and its role in the pathology of AD are controversial topics. However, the identification of  $A\beta$  in senile plaques and mutations in the APP, presenilin 1 and presenilin 2 genes leading to the accumulation of  $A\beta$  and early-onset familial dementia,<sup>11, 17, 18</sup> strengthened the proposition of the "amyloid cascade hypothesis" first formulated in 1992.<sup>19</sup> According to this hypothesis, the deposition of  $A\beta$  aggregates is the initial pathological trigger in the disease, which subsequently leads to the formation of neurofibrillary

Tau tangles, neuronal cell death and, later on, dementia.<sup>11, 17, 19, 20</sup> Several A $\beta$  aggregates have been identified that include dimers, oligomers, protofibrils, fibrils and amorphous aggregates.<sup>3, 21</sup> Their size varies from dimer to larger architectures of about ten nanometers in diameter while oligomers (*i.e.* small soluble assemblies made of a few peptides) are considered more toxic than high molecular weight aggregates.<sup>22, 23</sup> Note that here, for simplicity, high molecular weight assemblies (amorphous and/or fibrillar) are grouped under the term aggregates. The neurotoxicity of A $\beta$  aggregates is linked to various interconnected mechanisms such as: (i) oxidative stress,<sup>24, 25</sup> (ii) hyperphosphorylation of the Tau protein, leading to its inability to bind to microtubules, to the formation of neurofibrillary Tau tangles and further to the enhancement of the production of the A $\beta$  peptide,<sup>26, 27</sup> (iii) synaptic dysfunction due to the accumulation of soluble forms of the A $\beta$  peptides within the neurons,<sup>18, 25</sup> (iv) the insertion of the A $\beta$  peptides into cell membranes with the formation of ion channels inducing the dyshomeostasis of Ca<sup>2+</sup>.<sup>14, 22</sup> Besides these AD-specific features, considerable evidence indicates the presence of other hallmarks which are common for multiple neurodegenerative diseases; these include prominent activation of inflammatory and innate immune responses as well as excessive iron deposition and mitochondrial damage.<sup>28</sup>

The interconnection between the various dys- or malfunctions is an obstacle an unquestionable attribution of the original cause(s) of AD. Today, the accumulation of A $\beta$  peptide is no longer regarded as the only direct cause of the disease but rather as a key event, which is linked to a complex and branched cascade of several imbalances that favor the neuronal death. While APP is expressed ubiquitously, the amyloid deposits formed in AD are localized in very specific areas.<sup>4</sup> It could thus be hypothesized that, in addition to the accumulation of A $\beta$  (related to genetic causes or age), other factors may be involved in the formation of amyloid deposits.



**Figure 1.** Simplified view of the formation of amyloid plaques. Change in the color from yellow to blue corresponds to a change in the folding of the peptide upon metal ion binding. Co-factors = Cu and Zn,  $\beta$  and  $\gamma$  represent the  $\beta$ - and  $\gamma$ -secretases.

## 1. 2. Metal ions in AD

Among all the biological factors linked to the etiology of AD, metal ions held an essential place, since they are ill-regulated in AD brains.<sup>10, 29-32</sup> The total metal amount (Zn(II), Cu(I/II) and Fe(II) ions) detected in frozen brain tissues (lobes, amygdala, *hippocampus* ...) in Alzheimer's patients varies from healthy controls indicating of a global misregulation of metal ions in AD patient. Depending on the nature of the tissue the level are either up-regulated or down-regulated.<sup>33</sup> It has also been reported that the senile plaques are enriched in metal ions (about mM) in AD patients,<sup>34, 35</sup> and several *in vitro* experiments documented that metal ions can modulate the A $\beta$  aggregation (*vide infra*). The importance of those results with respect to the proposed impact of metal ions in the amyloid cascade would however deserve further investigations by other methods.<sup>36</sup> In addition, concentrations of the labile pool of Cu and Zn ions in the synaptic cleft can reach  $\mu$ M concentrations<sup>37-39</sup> and are thus available for coordination to A $\beta$ .

### 1. 2. a. Coordination of metal ions to A $\beta$

In vitro, Cu(I/II), Zn(II) and Fe(II) showed direct coordination to A $\beta$ ,<sup>9, 16, 40-44</sup> while Fe(II) bound to A $\beta$  does oxidize rapidly and finally converts into insoluble Fe(III)(OH)<sub>3</sub> making the *in vivo* interaction between Fe and A $\beta$  unlikely.<sup>45, 46</sup> The high affinity metal ion binding site of A $\beta$  lies between residues 1–16, a sequence regarded as the appropriate model to investigate A $\beta$  coordination ability in vitro.<sup>16</sup> Cu(II) binding sites to A $\beta$  peptides have been extensively studied and reviewed (see refs. <sup>40, 47</sup>). At neutral pH, A $\beta$  coordinates Cu in its +I and +II redox forms with very distinct environments,<sup>16</sup> with respective conditional affinity constant at pH 7.4 of 10<sup>10</sup> M<sup>-1</sup> and from 10<sup>7</sup> to 10<sup>10</sup> M<sup>-1</sup>.<sup>43</sup> Zn(II) ion is tetrahedrally bound to A $\beta$ ,<sup>9</sup> with a conditional affinity constant of about 10<sup>5</sup> -10<sup>6</sup> M<sup>-1</sup> for Zn(II).

### 1. 2. b. Metal ions - A $\beta$ and oxidative stress

It is now accepted that reactive oxygen species (ROS) such as O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub>, HO<sup>•</sup> over-generated under oxidative stress conditions play a key role in neurodegenerative diseases,<sup>24, 48</sup> while oxidative stress is one of the pathological hallmarks of AD although it remains uncertain whether this is a cause or a consequence of pathogenic processes occurring in the brain.<sup>49</sup> A relationship between ROS and A $\beta$  toxicity exists, mediated by redox active metal ions bound to A $\beta$  that produce ROS.<sup>24, 48, 50</sup> Indeed, in the presence of biological reductants such as ascorbate or glutathione, ROS can be generated by the redox cycling of Cu(I/II), which then contribute to neuronal oxidative damages. The involvement of Fe(A $\beta$ ) in ROS production is still unclear, thus, ROS production has been predominantly studied with Cu(A $\beta$ ). The *in vitro* techniques used to investigate ROS production rely on the UV-Vis monitoring of the consumption of ascorbate and on indirect methods for the detection of the ROS produced.<sup>51</sup> They mainly include the reduction of ferricytochrome C to ferrocycytochrome by O<sub>2</sub><sup>•-</sup> monitored by UV-Vis, the oxidation of Amplex Red<sup>®</sup> by H<sub>2</sub>O<sub>2</sub> leading to a fluorescent species, TBARS (Thiobarbituric acid reactive substances) assay relying on the reaction of 2-deoxyribose with

HO<sup>•</sup> and detection by UV-Vis of the resulting highly-colored dye, as well as the hydroxylation of the coumarin-3-carboxylic acid (CCA) by HO<sup>•</sup> leading to a fluorescent derivative (Figure 2.a).<sup>51</sup>

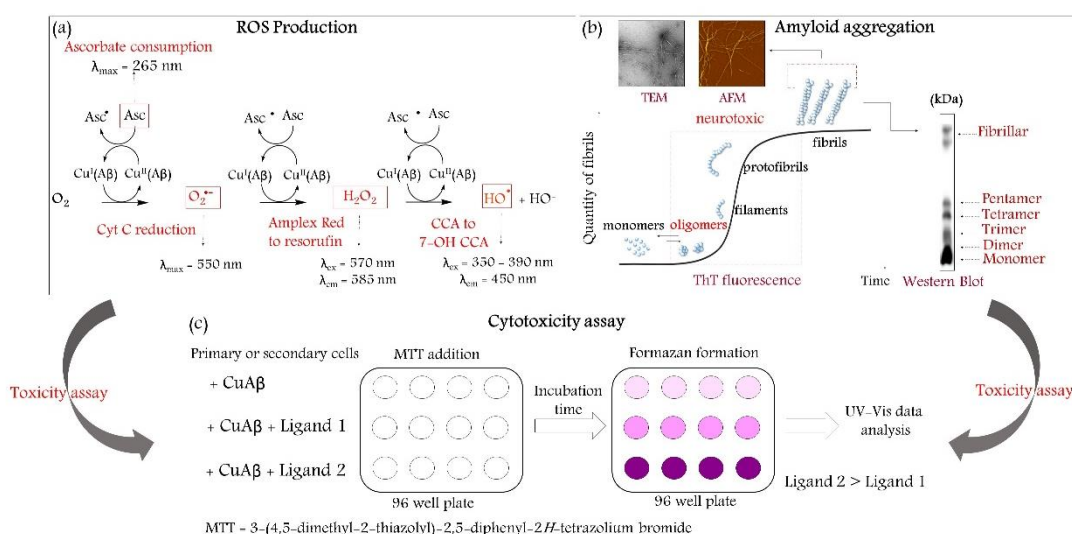
### **1. 2. c. A $\beta$ Aggregation**

The impact of Cu and Zn ions on aggregation pathways has been widely studied *in vitro* under different conditions including pH, temperature, concentration, nature of the metal ion, metal ion over peptide ratio, and clear effects were reported.<sup>10, 42, 52-54</sup> Metal ions can modify the thermodynamics and the kinetics of aggregation as well as the morphology of the final aggregates formed. The influence of Cu(II) on the A $\beta$  aggregation seems to be mainly dependent on its ratio and might lead to the production of highly cytotoxic oligomers.<sup>42</sup> As regarding Zn(II), once coordinated to A $\beta$  it has been mainly reported to promote a fast aggregation of species different from apo-fibrils.<sup>52</sup> Peptide aggregation can be monitored by different techniques. A widely used method to detect fibrils is the fluorescence enhancement of the Thioflavin-T (ThT) dye upon binding to the  $\beta$ -sheets present in amyloid structures.<sup>55, 56</sup> Experimentally, ThT fluorescence reports on the fibrillation kinetic that corresponds to a sigmoidal-like curve divided into three parts: the first one is a lag phase during which the nuclei are formed. Then, a second phase corresponds to the elongation of the nucleus into protofibrils and fibrils. Finally, the plateau of the sigmoid reflects a thermodynamic equilibrium between fibrils and soluble species (Figure 2.b). Other techniques have been used to monitor the formation of A $\beta$  aggregates<sup>22</sup> as turbidimetry (a very simple but weakly insightful method relying on the increase of absorbance near 400 nm), dynamic light scattering, gel electrophoresis/western blotting,<sup>57, 58</sup> or to identify their morphology by TEM (Transmission Electronic Microscopy) and AFM (Atomic Force Microscopy) microscopy (Figure 2.b).<sup>58, 59</sup>

### **1. 2. d. Toxicity studies on cells**



In addition to the aforementioned methods that measure ROS production and monitor A $\beta$  aggregation, the toxicity associated to these two events can be evaluated in cells. Samples of A $\beta$  and Cu(A $\beta$ ) have been measured towards their neurotoxicity while cell culture protocols are well documented in the literature.<sup>60</sup> Cellular models composed of primary neuronal cultures (cortical neuronal cells) or 2D neuron-like cell lines (*e.g.* PC12, SH-SY5Y and M17) are commonly used to study neuron cell death (induced by external stimuli) and to test the neuroprotective properties of specific compounds.<sup>61-63</sup> Recently, new cellular platforms have emerged *via* the development of induced pluripotent stem cells (iPSCs).<sup>64</sup> iPSCs offer the possibility to generate neuronal cultures from blood and fibroblasts. Cell viability has been measured using colorimetric tests, with the gold standard being the classical MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay relying on the reduction of the MTT to insoluble deep purple formazan dye (Figure 2.c).



**Figure 2.** Schematic representation of the most common techniques used to study Cu(A $\beta$ ) ROS production, A $\beta$  aggregation and their cytotoxicity. (a) evaluation of the catalytic production of ROS by Cu(A $\beta$ ) in presence of ascorbate and O<sub>2</sub>, and examples of detection techniques; (b) detection of A $\beta$  aggregation and examples of detection techniques; (c) evaluation of the Cu(A $\beta$ ) toxicity on cell culture and evaluation of the neuroprotective effect of drug candidates (ligands 1 and 2) using the MTT assay.

### 1. 3. Therapeutic approaches to fight AD

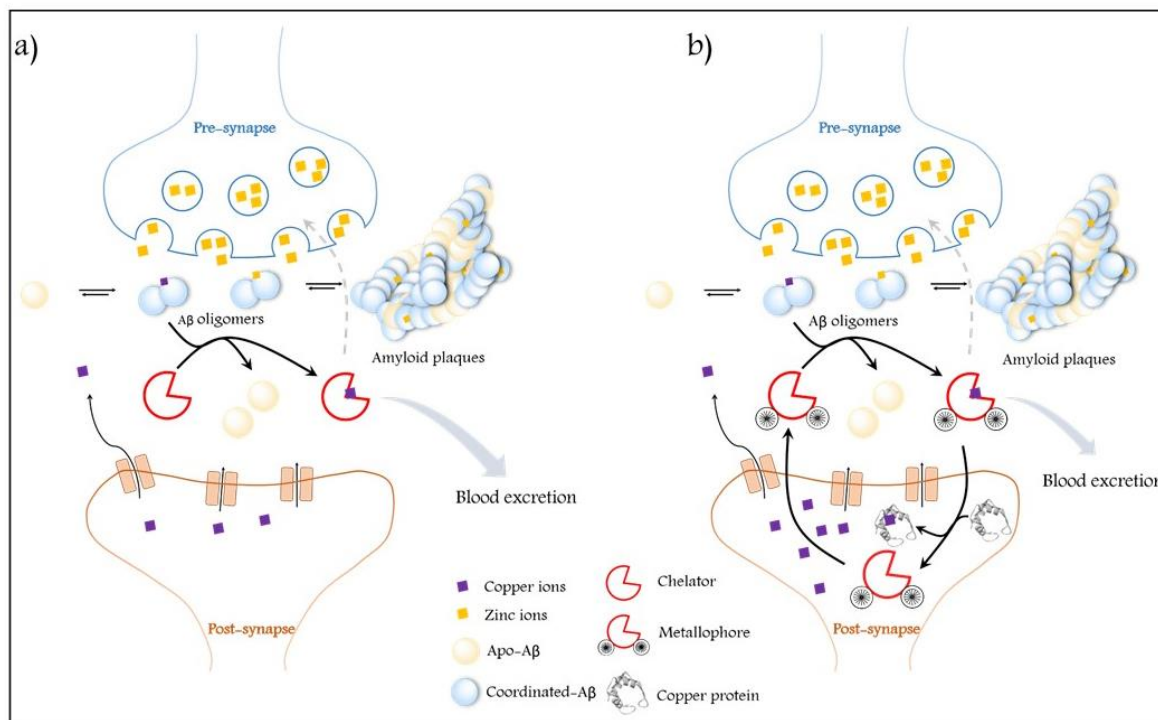
Two main therapeutic approaches are currently considered in the AD context depending on whether the symptoms or the causes of the disease are targeted.<sup>48, 65-67</sup> Disease-modifying approaches that aim at affecting the pathways responsible for neurodegeneration (*i.e.* the causes of the disease) include (i) reduction of the production of A $\beta$ , by the inhibition of the  $\beta$  and  $\gamma$  secretases, (ii) the increase of A $\beta$  elimination, including by the active or passive immunotherapy; the basis for these two first approaches being to counteract the deleterious accumulation of the A $\beta$  peptide, (iii) the targeting of metal ions in order to affect both aggregation and oxidative stress; this is the topic of the present viewpoint. The detection of a high level of metal ions in the brain areas affected by AD and the observation of the pathogenic consequences of the metal ions-A $\beta$  interactions (ROS, aggregation) were at the origin of therapeutic strategies aiming at preventing metal ions binding to A $\beta$ . Here, copper is under focus. Indeed, due to its redox ability, it participates in the production of ROS<sup>24</sup> and it modulates the aggregation of the A $\beta$  peptide possibly stabilizing oligomers,<sup>42</sup> regarded as the most toxic species of the aggregation process.<sup>22, 68</sup>

## 2. METAL TARGETING THERAPY

### 2. 1. Definitions: Chelator and Metallophore

Metal-targeting therapy refers to a medical treatment that reduce the toxic effects of metal ions by their removal or redistribution: (i) chelators have the intent of disrupting the deleterious interaction of metal ions with biomolecules thus inactivating them or helping their excretion (Figure 3.a) and (ii) metallophores that have the additional ability to transfer (across a membrane) the removed metal ions to biological compartment where they become beneficial.<sup>69,</sup>  
<sup>70</sup> In AD, it consists in redistributing the metal ions to regulate their homeostasis, reordering the metal ions traffic between extracellular and intracellular compartments (Figure 3.b).<sup>30, 43, 71-73</sup>

Here, for matter of simplicity, the term “ligands” is used for molecules capable of binding metal ions, with no precision on their releasing ability (*i.e.* a ligand can either be a chelator (binding only) or a metallophore (binding and redistribution)).



**Figure 3.** Schematic representation of the action of a chelator in the synaptic cleft (a) vs the action of a metallophore (b).

## 2. 2. Chemical and biological principles for in vivo metal-targeting

The essential properties of a ligand based on chemical and biomedical considerations have been progressively defined along with advances of research. The metabolic stability of the ligands and resulting complexes is a key parameter to fulfill : it has to be high enough to withstand the variety of physiological conditions, mainly pH. Other required properties may be combined as appropriate Absorption, Distribution, Metabolism, and Excretion (ADME) profile.<sup>74</sup>

Other thermodynamic properties have to be considered. The affinity of a ligand is the strength with which it binds the metal ions. The selectivity is the ability of the ligand to bind the targeted metal ion without strong interference from other metal ions. More precisely, the selectivity of a ligand for one metal ion versus another one is defined as the ratio of the affinity values of the

ligand for the two metal ions. As regard to the targeted Cu ions under focus here, the ligand has to compete with A $\beta$  (if we assume that the misplaced Cu is mainly bound to the A $\beta$  peptide). Thus the affinity of the ligand for Cu ions has to be higher than the one of A $\beta$  (about  $10^{10} \text{ M}^{-1}$  at pH 7.4 for A $\beta_{1-40/42}$  in the case of Cu(II)).<sup>75</sup> In addition, the ligand has to possess a Cu over Zn selectivity higher than the one of A $\beta$  (which equals about  $10^4$  in the case of Cu(II)) and because Zn is more concentrated than Cu in the synaptic cleft the intended selectivity should be largely higher than  $10^4$ .<sup>52</sup>

In addition to the conditions described above, for neurodegenerative diseases, the ability of the ligand to cross the blood–brain barrier (BBB) is a main factor to take into account.<sup>76-79</sup> Apart from respecting adapted Lipinski's rules for passive transport through the BBB,<sup>80, 81</sup> other approaches are currently developed mainly relying on active transport.<sup>82, 83</sup>

### **2. 3. Ligands of Cu ions**

Among the ligands developed in AD chelation therapy context (for a very recent review, see ref. <sup>48</sup>), some are monofunctional aiming at targeting metal ions and associated deleterious processes and others are multifunctional impacting several pathological features. In addition to the binding cavity, multifunctional ligands incorporate moieties to scavenge radical, to recognize A $\beta$  aggregates and to help BBB penetration. Proligands are ligands whose binding ability is obtained upon an appropriate stimulus.

In the following, monofunctional ligands are under focus. They have been grouped into three categories: (i) synthetic ligands; it regroups hydroxy/aminoquinoline (Figure 4, Table 1), tetraazamacrocycles, amino-phenol and amino-pyridines based ligands, phosphine and bis(thiosemicarbazonato) (Figure 5, Table 2) ; (ii) peptidic ligands (Figure 6, Table 3) and (iii) ligands possessing an A $\beta$ -targeting moiety in which the binding unit is embedded (Figure 7, Table 4). Their structures are shown in Figures 4 to 7, while their characteristics, and the studies carried out in AD context are described in the following paragraphs and their main properties

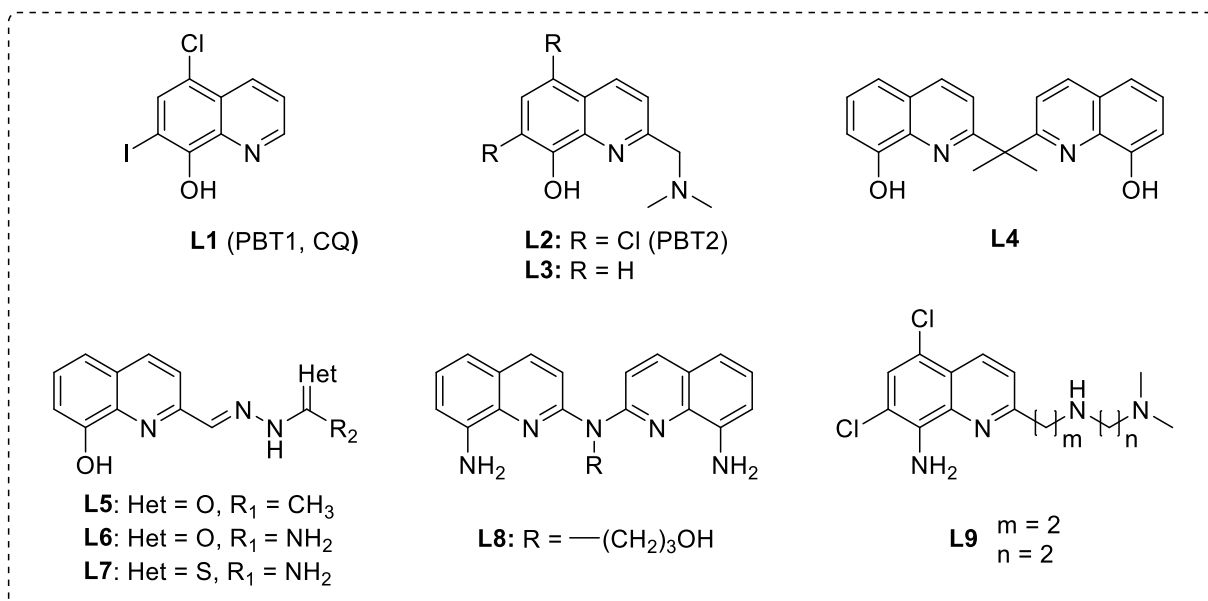
gathered in Tables 1 to 4. Note that, in Tables 1 to 4, the affinity of the ligand is reported as the  $pCu$  parameter that corresponds to the concentration of unbound Cu at a given pH (7.1 or 7.4) using the HySS program<sup>84</sup> when appropriate, and the results of the experiments carried out in the literature are reported as follows: ✓ means “beneficial effect”, ✗ means “no effect” and “n.r.” means “not reported” with several processes reviewed: (i) arrest of Cu-induced ROS production, (ii) ROS scavenging by the ligand itself, (iii) inhibition of Cu-induced aggregation, (iv) disassembly of Cu(A $\beta$ ) aggregates and (v) recovery of cell survival for cells confronted to toxicity associated to Cu(A $\beta$ ) aggregates. **Note that in the following text and Tables, unless otherwise specified, ROS production and aggregation under focus are Cu(II)-induced.**

“In the present viewpoint, beyond describing reported results on Cu(II) targeting strategies, our main aim is to gain deeper insights on the interaction between Cu / A $\beta$  / ligands to guide the design and investigations on next generations of ligands and that is why we examine and discuss several key parameters and criteria.

### **2. 3. a. Synthetic ligands**

#### **2. 3. a. 1. Hydroxy- and amino-quinolines ligands**

The quinoline motif is widely used in therapeutic chemistry. Quinoline derivatives substituted at the 8-position with a heteroatom (such as the 8-hydroxyquinoline or 8-aminoquinoline derivatives) can be used as ligands.<sup>85</sup> The hydroxyquinoline ligands and their amino-counterparts developed to target metal ions in AD are described in this first paragraph (Figure 4, Table 1).



**Figure 4.** Structures of hydroxy- and amino-quinolines ligands and derivatives.

### 2. 3. a. 1. 1. Hydroxyquinoline

Among all the hydroxyquinoline derivatives, the 8-HQ (8-Hydroxyquinoline) is the most interesting one due to its bioactivities and therapeutic potentials. It has been shown that the substitution with a chlorine group at the C5 or both C5 and C7 positions of 8-HQ increases the lipid solubility and the donor effect on the phenolic group that improves metal ions binding. **L1** (iodochlorhydroxyquin, Clioquinol (CQ), also named PBT1 for Prana BioTechnology 1) and **L2** (5,7-Dichloro-2-[(dimethylamino)methyl]quinolin-8-ol, PBT2) exert anti-neurodegenerative effects in transgenic model mice (Tg2576 and APP/PS1).<sup>85-88</sup> Cu(II)/Zn binding to **L1** was studied in solution,<sup>89, 90</sup> and in solid-state.<sup>91</sup> Effects of **L1** on metal-induced A $\beta$  aggregation,<sup>92-94</sup> and on its ability to cross the BBB<sup>95</sup> were reported. **L1** can partially dissolve amyloid plaques from human post-mortem brains<sup>96</sup> and induces a significant inhibition by almost 50% of A $\beta$  accumulation in APP Tg2576 transgenic mice.<sup>95</sup> A pilot phase II study of orally dosed **L1** reported that it improved the cognition and behavior of AD patients<sup>97</sup> but side effects such as mutagenicity and neurotoxicity were observed,<sup>98</sup> which led to the arrest of **L1** clinical development. A new bis-chloro 8-HQ derivative, the tridentate **L2** (PBT2) ligand was developed. It does not show severe side effects and has successfully completed phase IIa trials

in patients with early AD,<sup>99, 100</sup> but **L2** did not reach statistically significant reduction in amyloid plaque levels in the brains of patients with AD.<sup>85</sup> The proposed mechanism of action relies on a metallophore activity meaning that the compounds are able to lessen the level of Cu(II) bound to A $\beta$ , in combination with weakening of ROS production, re-solubilisation of amyloid plaques and promotion of metal ions uptake by cells.<sup>72, 101, 102</sup> While Cu(II) coordination to **L2** has been described,<sup>103</sup> the efficiency of Cu(II) removal from A $\beta$  has been mostly investigated in vitro for the **L3** analogue.<sup>104, 105</sup> It has been shown that **L3** can extract Cu(II) from A $\beta$  but also forms ternary species **L3**[Cu(II)]A $\beta$ , where A $\beta$  binds Cu via one of its His residues. This results in a decreased level of ROS production. However, one may consider that in vivo the A $\beta$  His will be replaced by imidazole-containing molecules and that **L3**[Cu(II)](Im) does produce ROS to a significant extent.<sup>104</sup>

### 2. 3. a. 1. 2. Hydroxyquinoline derivatives

Other structural modifications have been made to improve **L1-L3** efficiency. They include connection of two hydroxyquinoline ring moieties (**L4**) with various one-atom linkers leading to a tetradentate binding motif thus forming 1:1 (ligand:metal) complex (compared to the possible formation of 2:1 complex for **L1-L3**). As expected the bis-hydroxyquinoline derivatives has an higher affinity for Cu(II) and Zn(II) than the 8-HQ parent compounds but a moderate improvement in the ability to prevent A $\beta$  aggregation.<sup>106, 107</sup> Ligand **L4** is shown as an illustration of the wide series of bis-HQ ligands studied, since it recapitulates all the expected effects and since the crystallographic structures of Cu(II) and Zn(II) complexes have been reported as well.<sup>107</sup> Hydrazone, semi- and thiosemi-carbazones moieties have been incorporated into 8-HQ scaffold leading to **L5-L7**, respectively.<sup>108</sup> Their antioxidant capacity (*i.e.* the ability to scavenge ROS) are enhanced compared to the parent **L1** and **L2** ligands, a feature that is linked to the increased stabilization of the resulting phenoxyl radical due to the absence of halogenated substituent on the quinoline ring and the extended conjugation provided by the

substitution with the Schiff-base. Regarding aggregation, **L5** and **L6** are described as inhibitors of the formation of Cu(II)-induced oligomers, while **L6** has greater effect compared to **L5**, which may be attributed to its higher hydrophilicity and/or increased H-bonding interaction of the carboxamide function with the  $\beta$ -strand of the A $\beta$  peptides.<sup>108</sup>

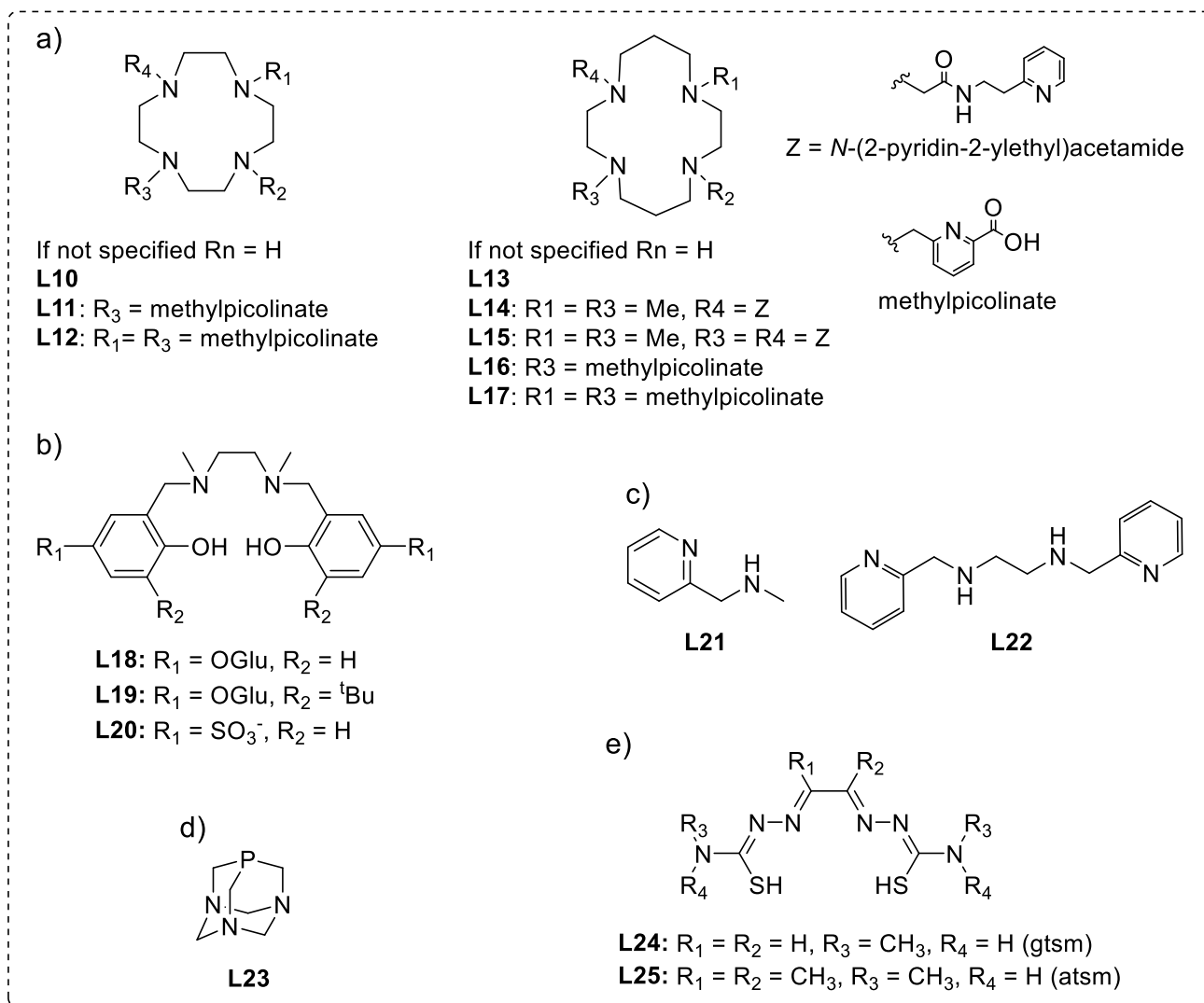
#### 2. 3. a. 1. 3. Aminoquinoline derivatives

In order to improve the Cu(II) affinity and the Cu(II) over Zn(II) selectivity, bis-aminoquinoline<sup>71, 109-112</sup> and aminoquinoline derivatives<sup>113, 114</sup> affording a 4N tetradentate binding unit have been recently developed. In particular, **L8** extracts Cu(II) from A $\beta$  peptide and can redistribute it to physiological ligands under reductive conditions. In addition, **L8** does inhibit ROS production.<sup>109-111</sup> Noticeably, after three weeks of treatment by oral route with a close analogue of **L8**, memory deficit mouse model induced by a single intracerebroventricular injection of A $\beta$  peptide showed a full recovery of deficit of episodic memory.<sup>112</sup> Later on, in order to improve BBB permeability, tetradentate monoquinoline ligands based on a 8-HQ moiety substituted at C2 by a more modular and low-weight bis-chelating side-chain were designed.<sup>113</sup> **L9** (obtained with n=2 and m=2, shown in Figure 4) is the best ligand of the series, as it has appropriate Cu(II) affinity, Cu(II) over Zn(II) selectivity and the ability to fully inhibit *in vitro* ROS production.<sup>114</sup>



**Table 1.** Hydroxy- and aminoquinolines reported from the literature as well as their impact on the ROS production and scavenging, the prevention of aggregation and aggregates disassembly and cell toxicity of the ligand. pCu =  $-\log[\text{Cu}]_{\text{unbound}}$ ,  $[\text{Cu}] = [\text{L}] = 10 \mu\text{M}$ , <sup>+</sup>: from conditional affinity value, <sup>□</sup>: from apparent affinity value, pH = 7.4.

	pCu	ROS		Aggregation			Refs.
		arrest of production	scavenging	prevention	disaggregation	cell toxicity	
Cu(A $\beta$ )	7.3-7.8 <sup>+</sup>	-	-	-	-	-	75, 115
L1	6.6	✓	✗	✓	✗	✗	85, 88, 89, 92, 93, 116, 117
L2	n.r.	n.r.	n.r.	✓	✓	✓	108, 118
L3	7.89 <sup>+</sup>	✓	✗	n.r.	n.r.	n.r.	104, 105
L4	10.45 <sup>□</sup>	✓	n.r.	✓	n.r.	n.r.	107
L5-L6	n.r.	n.r.	✓	✓	n.r.	n.r.	108
L7	n.r.	n.r.	✓	n.r.	n.r.	n.r.	108
L8	10.75 <sup>□</sup>	✓	n.r.	n.r.	n.r.	n.r.	109, 112
L9	10.75 <sup>+</sup>	✓	n.r.	n.r.	n.r.	n.r.	113, 114



**Figure 5.** Structures of selected ligands: tetraazamacrocycles (a), amino-phenol based ligands (b), amino-pyridine based ligands (c), phosphine (d) and bis(thiosemicarbazone) ligands (e).

### 2. 3. a. 2. Tetraazamacrocycles

Complexes of macrocyclic ligands are widely used in medicinal chemistry for imaging (MRI contrast agents, radiopharmaceuticals and luminescent probes). Indeed, the tetraazamacrocycle properties such as their high affinity for metal ions associated to kinetic inertness, their high selectivity for Cu(II) over Zn(II), their modularity (possible N- or C-functionalizations *via* the incorporation of pendant arms) and their low molecular weight prompted their use in medicine.<sup>119, 120</sup> In the AD context, the two tetraazamacrocycles, cyclen (**L10**) and cyclam (**L13**) have served as scaffolds for the construction of several substituted ligands (**L11-L12**, **L14-L17**, described later on, see Figure 5 and Table 2). **L10** and **L13** are able to modulate

aggregation thus improving neuronal cell survival but not to fully arrest ROS production.<sup>61</sup> Two cyclam derivatives displaying lipophilic pyridine groups in order to enhance their antioxidant activity and help them to cross the BBB (**L14** and **L15**) were reported.<sup>62</sup> They could modulate aggregation and lower H<sub>2</sub>O<sub>2</sub> production. In addition, **L14** and **L15** are able to attenuate Cu(II)(A $\beta$ ) induced toxicity *via* decreasing intracellular ROS on cells line. Although performed in absence of an external physiological reductant such as ascorbate as trigger of the ROS production reaction, this ability has been attributed to the interruption of the Cu(A $\beta$ ) redox cycle by **L14** and **L15**. **L15** is more efficient than **L14** in line with the antioxidant expected effect of additional pyridine rings. Other tetraazamacrocycles **L11-L12** and **L16-L17** functionalized with one or two methylpicolinate arms have been reported later on.<sup>121, 122</sup> All those ligands are able to prevent and stop ROS production and to restore formation of apo-like fibrils by removing the Cu(II). Importantly, the arrest of ROS was challenged during ROS production (and not only using pre-incubation of the ligand with the Cu(II)(A $\beta$ ) prior to the triggering of the ROS production reaction). In other words, the experiments were conducted in presence of both Cu(I) and Cu(II), which may best mirrors the physiological environment in the synaptic cleft. For the first time, it has been evidenced that the kinetics of Cu(II) removal from A $\beta$  by the ligand have to be considered since the unsubstituted **L10** and **L13** ligands were inefficient in stopping ROS production while the **L11-L12** and **L16-L17** were, due to the pendant arm(s) that foster(s) Cu(II) grasping. In addition, in contrast to the previous two studies, the aggregation was kinetically monitored by ThT fluorescence and the morphology of the apo and Cu-aggregates imaged by TEM, showing that in presence of the ligands apo-type fibrils are recovered.

### 2. 3. a. 3. Amino-phenol derivatives

**L18** and **L19** (H<sub>2</sub>GL<sub>1</sub>, H<sub>2</sub>GL<sub>2</sub>) are considered as multifunctional agents due to the phenolic moieties that can act as ROS scavengers, while the attached carbohydrate moieties enhance

solubility, minimize toxicity and improve BBB penetration (Figure 5, Table 2).<sup>123</sup> At neutral pH, **L18** and **L19** possess a moderate-to-high affinity for Cu(II) ( $10^{15} \text{ M}^{-1}$ ) while the affinity for Zn(II) ions is weak ( $10^6 \text{ M}^{-1}$ ) leading to an appropriate Cu(II) over Zn(II) selectivity. *In vitro* studies have shown efficiency to scavenge free radicals and to decrease the formation of A $\beta$  aggregates as measured by turbidimetry.<sup>124</sup> **L20** is an analogue to **L18** and **L19**, in which the phenolato groups are sulfonated to improve solubility and ease *in vitro* studies.<sup>125</sup> **L20** showed significant potential to arrest ROS production even in presence of Zn(II) and to restore the apo-like (in absence of Zn(II)) or Zn(II)-induced type aggregation of the A $\beta_{40}$  peptide.<sup>125, 126</sup>

#### 2. 3. a. 4. Amino-pyridine ligands

The characterizations of Cu(II) and Zn(II) complexes formation with the aminopyridine ligand **L21** show that it cannot compete with A $\beta$  for any of the metal ion. In order to form more stable complexes, **L22** a ligand that connects two **L21** moieties was used (Figure 5, Table 2).<sup>127</sup> It forms a stable 1:1 (ligand:metal) complex at neutral pH with both Cu(II) and Zn(II) through tetradentate coordination with affinity values ( $10^{15} \text{ M}^{-1}$  for Cu(II) and  $10^{10} \text{ M}^{-1}$  for Zn(II)) largely higher than those of A $\beta$ . The Zn(II)-aggregation studies displayed that **L22** is able to remove Zn(II) from A $\beta$  aggregates and thus to solubilize them as probed by Tyr10 fluorescence titration of the resulting soluble fraction.

#### 2. 3. a. 5. Phosphine derivatives

The ligands discussed above specifically target Cu(II). Knowing that Cu is engaged in A $\beta$  coordination in both redox forms, a Cu(I) chelator has been also investigated in AD. **L23** (triaz-7-phosphaadamantane, Figure 5, Table 2) is the first Cu(I) ligand able to retrieve both Cu(I) and Cu(II) from the A $\beta$  peptide. Indeed **L23** can reduce Cu(II) bound to A $\beta$  and then coordinates the resulting Cu(I) in oxidation-resistant complexes.<sup>128</sup> The same effect was also observed in presence of Zn(II), thus showing that PTA ligand has an appropriate Cu(I) versus Zn selectivity.<sup>129</sup>

### 2. 3. a. 6. Bis(thiosemicarbazonato) derivatives

Cu(II) and Zn(II) complexes of bis(thiosemicarbazonato) ligands (**L24-L25**, Figure 5) can be transported across the BBB, can cross neuron membranes and release metal ions. Thus they have been studied in neuron-like cells and AD animal models in order to evaluate the effects of intracellular increase of metal ions levels.<sup>130, 131</sup> Positive effects with regard to soluble A $\beta$  levels and restoration of cognitive performance in APP/PS1 transgenic AD model mice were reported for both metal ions while a fine-tuned redox mechanism was at play for the Cu(II) complexes: the intracellularly reduced Cu(II)(**L24**) species was efficient but not the Cu(II)(**L25**) analogue which is resistant to intracellular reduction and subsequent metal release.<sup>132</sup> The increased levels of intracellular bioavailable Cu(I) and Zn(II) initiated a signaling cascade involving activation of proteases that degraded A $\beta$ . Although there is no report about such investigations, the additional ability of **L24** to extract Cu(II) from A $\beta$  peptides and aggregates is anticipated. Hence similar metallophore effects as those reported for **L2** could be foreseen.<sup>72, 133, 134</sup>

**Table 2.** Other ligands reported from the literature as well as their impact on the ROS production and scavenging, the prevention of aggregation and aggregates disassembly and cell toxicity of the ligand. pCu =  $-\log[\text{Cu}]_{\text{unbound}}$ , [Cu] = [L] = 10  $\mu\text{M}$ , <sup>+</sup>: from conditional affinity value, <sup>□</sup>: from apparent affinity value, \* at pH = 7.1 (if not specified pH = 7.4).

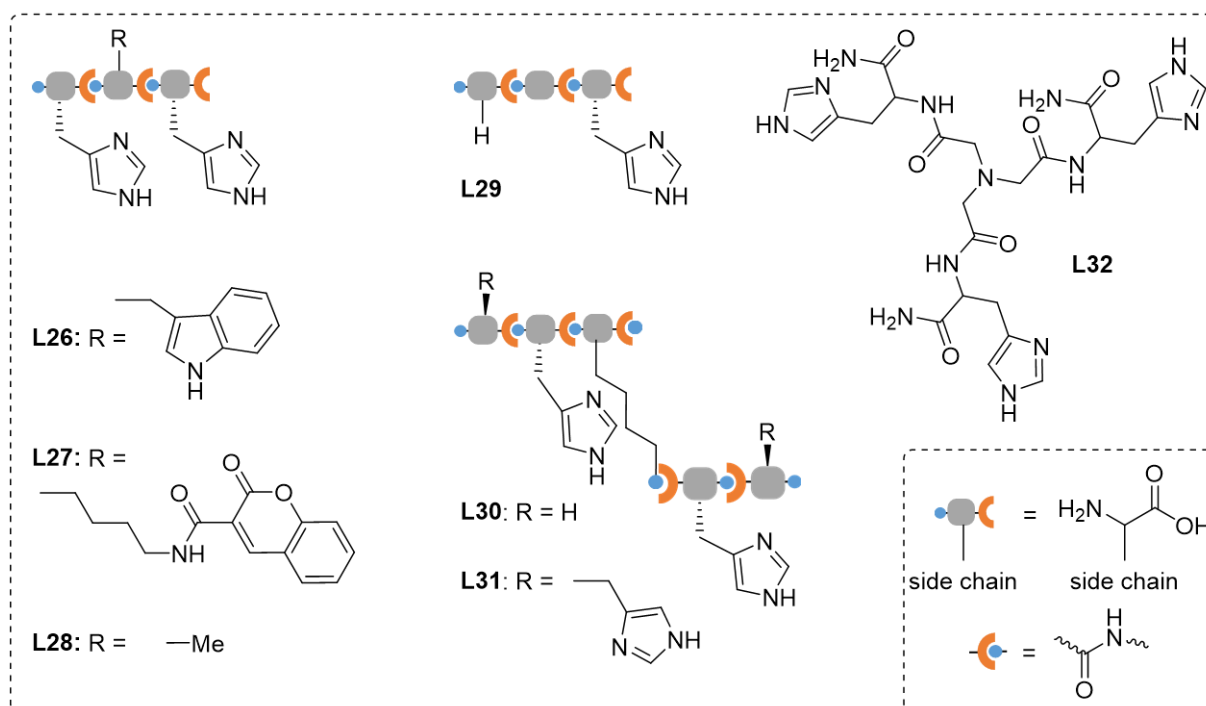
	pCu	ROS		Aggregation			Refs.
		arrest of production	scavenging	prevention	disaggregation	cell toxicity	
Cu(A $\beta$ )	7.3-7.8 <sup>+</sup>	-	-	-	-	-	75, 115
L10	11.5 <sup>*+</sup>	✗	n.r.	✓	~	✓	61, 121, 122
L11	11.8 <sup>*+</sup>	✓	n.r.	✓	n.r.	n.r.	121, 122
L12	11.2 <sup>*+</sup>	~	n.r.	✓	n.r.	n.r.	121, 122
L13	12.7 <sup>*+</sup>	✗	n.r.	✓	~	✓	61, 121, 122
L14	n.r.	~	n.r.	✓	✓ (2eq L /Cu)	✓	62
L15	n.r.	~	n.r.	✓	✓	✓	62
L16	11.5 <sup>*+</sup>	✓	n.r.	✓	n.r.	n.r.	121, 122
L17	10.0 <sup>*+</sup>	✓	n.r.	✓	n.r.	n.r.	121, 122

L18	10.8 <sup>+</sup>	n.r.	✓	✓	n.r.	n.r.	124
L19	11.4 <sup>+</sup>	n.r.	✓	✓	n.r.	n.r.	124
L20	9.6 <sup>+</sup>	✓	n.r.	~	n.r.	n.r.	125, 126
L21	6.6 <sup>+</sup>	n.r.	✗	✗	✗	n.r.	127
L22	10.8 <sup>+</sup>	n.r.	n.r.	✓	✗	n.r.	127
L23	5.4 <sup>+,a</sup>	~	n.r.	✓	n.r.	n.r.	128

<sup>a</sup>. relative to Cu(I)

### 2. 3. b. Peptidic ligands

The use of biocompatible peptides and proteins as metal ligands to capture Cu in AD pathology has been explored.<sup>48</sup> Peptides have many advantages: they are suitable for physiological conditions (appropriate water-solubility for instance), they can be easily and routinely synthesized with commercially available building blocks, reagents and apparatus by trained researchers and they can be functionalized. In addition, simple strategy like the use of the D-analogues, cyclisation, substitution or branched peptides can be applied to lessen recognition and further degradation of the peptide by peptidases *in vivo*.<sup>135</sup> Last, they have been extensively studied for their ability to bind Cu(II) ion and to a lesser extent Zn(II) ion.<sup>136</sup> In the following, the use of peptidic ligands with ATCUN-like motif, His-containing peptides and peptidomimetic ligands are described in the context of Cu targeting in AD (Figure 6, Table 3).



**Figure 6.** Structures of selected peptidic ligands.

### 2. 3. b. 1. ATCUN-like motif

The ATCUN (Amino-terminal Cu and Ni binding) motif is characterized by the  $\text{H}_2\text{N-Xxx-Zzz-His}$  sequence, with Xxx and Zzz being any amino acid residues except proline. It has a high affinity for Cu(II) which is higher by about three orders of magnitude than the one of A $\beta$ . Inside such a peptide sequence, the Cu(II) is coordinated by four nitrogen atoms: the N-terminal amine, the first two amides and the nitrogen atom of imidazole.<sup>137</sup> Some reports indicate that Cu(II) in an ATCUN motif is stable and cannot be reduced to Cu(I).<sup>137, 138</sup> Overall these beneficial features make ATCUN peptides good candidates as ligands in the AD context. Gamez and co-workers described the preparation of tripeptides (His-Zzz-His, **L26-L28**) ligands.<sup>139</sup> Unexpectedly, the binding constants reported for these three peptides are lower than those of other ATCUN motifs by three to four orders of magnitude.<sup>137, 138</sup> Anyway, the removal of Cu(II) from A $\beta$  was directly probed by competition between the **L27** and A $\beta$  as well as indirectly since the addition of **L26-L28** at the beginning of aggregation process restores the A $\beta$  apo-aggregation behaviour. **L26-L28** were able to moderately prevent ROS production

while based on the complete Cu(II) removal from A $\beta$  by **L26-L28** and the anticipated redox inertness of the resulting Cu(II)-ATCUN complexes, a total preclusion of the ROS production would be expected. Another tripeptide **L29** (Gly-Gly-His) was reported to prevent ROS production in superstoichiometric ratio **L29**:Cu(II) (4:1) and accordingly restore viability of PC12 cells confronted to ROS production.<sup>63</sup> Given the intrinsic affinity, formation of stoichiometric Cu(II) complexes and redox properties of such ATCUN motif, it is quite surprising that 4 equivalents (and not only one) of **L29** were required to observe the arrest of ROS production.

#### 2. 3. b. 2. His-containing peptides and peptide-like ligands

The preparation and activity against A $\beta$  aggregation of two histidine-rich branched peptides with one lysine as a linking unit, **L30** and **L31** have been reported.<sup>140</sup> The strategy based on branched peptides is interesting due to the resistance of these structures to proteolytic cleavage. Cu(II) and Zn coordination of the branched peptide have been investigated at neutral pH and notably, any peptide can chelate two metal ions. **L31** shows a slightly better ability than **L30** to bind Cu(II) from A $\beta$  in line with its higher Cu(II) affinity value (Table 3) but neither **L30** nor **L31** could effectively compete with A $\beta$  for Zn(II) *in vitro* affording an appropriate Cu(II) over Zn selectivity. Later on, **L32** was designed based on the assumption that the main Cu redox state in the synaptic cleft is not clearly defined. **L32** was inspired by the amino acid side chains responsible for Cu binding in A $\beta$  to be able to coordinate both Cu(II) and Cu(I) ions. **L32** displays His coordination moieties that have been introduced on the nitrilotriacetic acid platform and it has moderate binding affinity constants for both Cu(I) and Cu(II) but slightly higher than those of A $\beta$ . As a proof of concept, **L32** was able to target both redox states of Cu, prevent and stop ROS production.<sup>141</sup> The beneficial effect observed was due to a more sluggish electron transfer between the Cu(I) and Cu(II) for the Cu(**L32**) complex compared to Cu(A $\beta$ ),<sup>142, 143</sup> due to a more constrained geometry of **L32**.

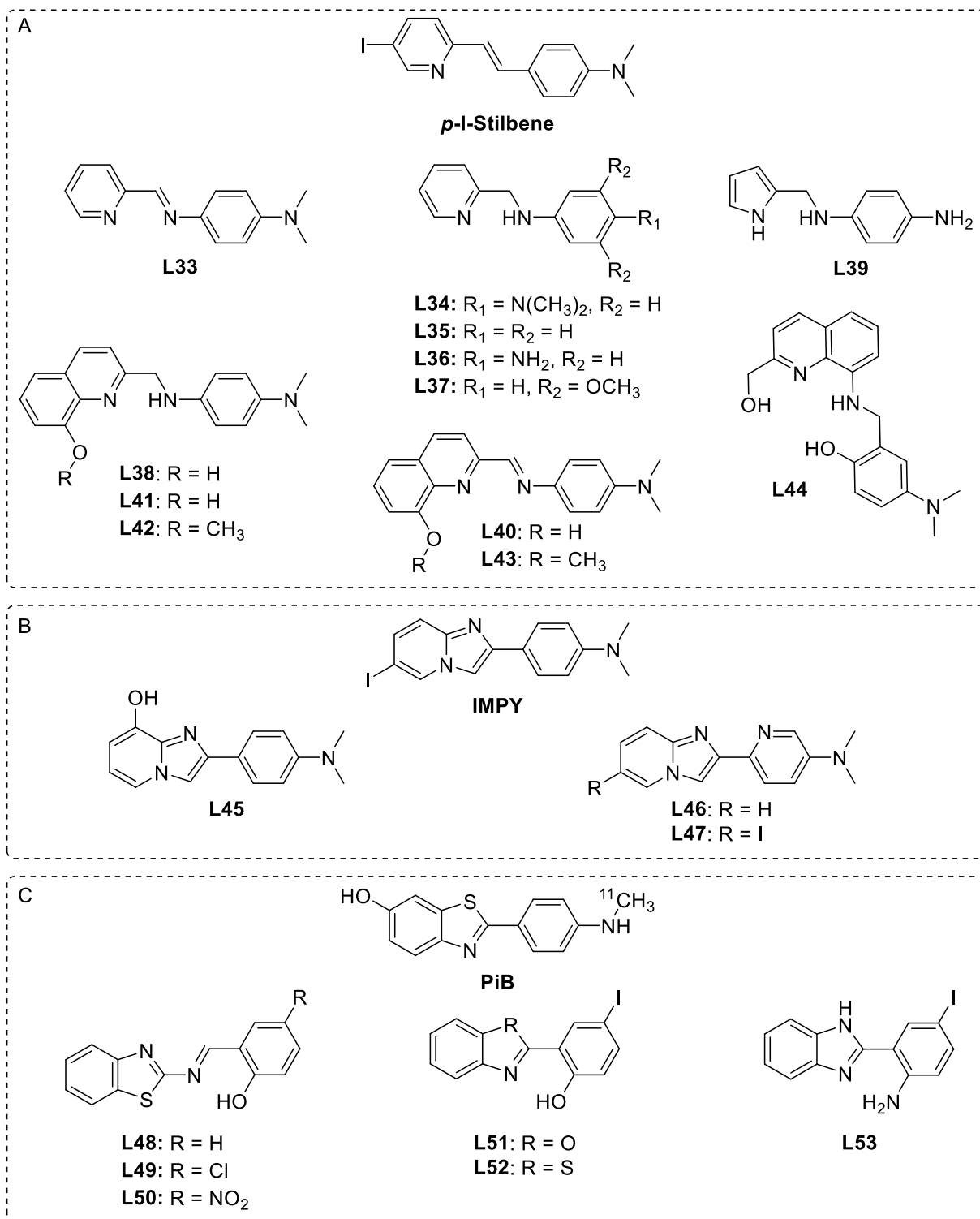


**Table 3.** Peptidic ligands reported from the literature as well as their impact on the ROS production and scavenging, the prevention of aggregation and aggregates disassembly and cell toxicity of the ligand. pCu =  $-\log[\text{Cu}]_{\text{unbound}}$ ,  $[\text{Cu}] = [\text{L}] = 10\mu\text{M}$ , <sup>+</sup>: from conditional affinity value, <sup>□</sup>: from apparent affinity value, pH = 7.4. # here not due to aggregation but ROS-induced toxicity (measurement performed in presence of ascorbate).

	pCu	ROS		Aggregation			Refs.
		arrest of production	scavenging	prevention	disaggregation	cell toxicity	
Cu(A $\beta$ )	7.3-7.8 <sup>+</sup>	-	-	-	-	-	75, 115
L26	7.2 <sup>+</sup>						139
L27	6.7 <sup>+</sup>	✓	n.r.	✓	n.r.	n.r.	
L28	n.r.						
L29	n.r.	✓ 4 eq	n.r.	✗	n.r.	✓# 4 eq	63
L30	12.7 <sup>+</sup>						140
L31	14.1 <sup>+</sup>	n.r.	n.r.	✓	n.r.	n.r.	
L32	8.8 <sup>+</sup>	✓	n.r.	n.r.	n.r.	n.r.	141

### 2. 3. c. « 2 in 1 » integrated bifunctional ligands

Many bi-functional and/or multifunctional molecules have been extensively studied in last decade and recently reviewed in several articles, the reader can refer to (refs. <sup>48, 123, 144, 145</sup>). Hence in this paragraph, we will only recapitulate the main bifunctional ligands that possess an appropriate binding moiety buried inside a scaffold aiming at targeting A $\beta$  aggregates (Figure 7, Table 4). For example, imaging agents of A $\beta$  plaques<sup>55, 146-148</sup> such as the *p*-I-stilbene ((*E*)-4-Iodo-4'-dimethylamino-stilbene), the IMPY (2-(4'-dimethylaminophenyl)-6-iodoimidazo[1,2-a]pyridine) and the PiB (Pittsburgh compound B, 2-(4'-[<sup>11</sup>C]methylaminophenyl)-6-hydroxybenzothiazole) have been used as host scaffolds for the design of new compounds of this family of ligands. Those molecules are of interest because they could target and thus remove specifically the Cu bound to A $\beta$  and have a molecular weight low enough to fulfil the adapted Lipinsky's rules. This paragraph will focus on the integration of the binding moiety in A $\beta$ -targeting scaffolds thus keeping the size of molecules appropriate for further therapeutic/theranostic purpose.



**Figure 7.** Structures of selected ligands in which the binding unit is integrated in A $\beta$  recognition moiety. A) Stilbene-like, B) IMPY-like, and C) PiB-like moieties.

### 2. 3. c. 1. Stilbene-like

In a pioneering work, two nitrogen atoms have been introduced to the *p*-I-stilbene to generate **L33**.<sup>88</sup> **L33** can bind Cu(II) and Zn(II) ions (with modest selectivity for Cu(II) over other metal ions in ethanol),<sup>149</sup> is able to reduce A $\beta$  aggregation, to disassemble preformed A $\beta$  aggregates and it lessens the H<sub>2</sub>O<sub>2</sub> production by 70 %. Despite these interesting abilities, further biological applications have been hindered by its poor stability in water due to the imine moiety ( $t_{1/2} < 5$  min when metalated with Cu(II)). Thus, it may be anticipated that the results observed on aggregation and ROS production are linked to the activity of products of hydrolysis rather than on the ligand itself. To solve this issue, the water stable amine derivative **L34** has been prepared and studied.<sup>150, 151</sup> **L34** can form a mixture of 1:1 and 1:2 metal-to-ligand complexes at neutral pH. The affinity constant of **L34** for Cu(II) and Zn(II) are weaker than the those of A $\beta$  (Table 4). Similarly to the parent imine containing compound, **L34** is able to limit the H<sub>2</sub>O<sub>2</sub> production (reduction by 84 %) as determined by the HRP (HorseRadish Peroxidase)/Amplex Red<sup>®</sup> assay, to regulate Cu(II)- or Zn(II)-induced A $\beta$  aggregation and disassemble preformed aggregates. *In cellulo* and *in vivo* treatment with **L34** (on N2a cells and 5xFAD mice, respectively) showed both improvement in cell viability (in presence of exogenous added Cu and A $\beta_{1-40-42}$ ) and noticeable cognitive improvements compared to the control mice according to the Morris water maze (MWM) test.<sup>152</sup> More detailed structure-activity-relationship investigations have shown that the dimethylamino group is crucial for the recognition of A $\beta$  aggregates and could serve as an anchor. Indeed, the analogue **L35**, lacking the dimethylamino group, shows a modest regulation of the A $\beta$  aggregation and disassembly compared to the parent **L34**.<sup>150</sup> Furthermore, changing the dimethylamino to amino (**L36**) or donating methoxy (3,5-dimethoxy, for **L37**) functionalities on the phenyl ring results in a partial (**L36**) or complete (**L37**) loss of regulating effect on A $\beta$  aggregation when [A $\beta$ ] = [Cu(II)] = 25  $\mu$ M.<sup>153</sup> Modification of the pyridine moieties into a quinolone (**L38**) or a pyrrole (**L39**) moieties causes intrinsic problems of

solubility (**L39**) and toxicity on M17 cells in presence of Cu(II) ions (**L38**) and thus, such modifications have not been further explored.<sup>153</sup> The *N*-dimethylaniline moiety inspired from the *p*-I-stilbene has also been conjugated to hydroxyquinoline, a motif found in the PBT2 molecule (**L2**), by imine (**L40**) or amine bond (**L41**) and to the low-binding methoxyquinoline counterparts (**L42**, **L43**).<sup>118</sup> In contrast to **L33**, the water stability of the imine containing ligands has not been reported. These four molecules have limited effect on the H<sub>2</sub>O<sub>2</sub> production as evaluated on a HRP/Amplex-Red<sup>®</sup> assay, but exhibit effective inhibition of A $\beta$  aggregation and also show significant abilities to disassemble A $\beta$  aggregates as shown by ThT assay and TEM. Last, a stilbene derivative integrating a tetradentate [N<sub>2</sub>O<sub>2</sub>] binding moiety has been reported (**L44**).<sup>154</sup> It gathers structural elements for interaction with A $\beta$ , improved metal coordination, control of ROS generation, antioxidant activity, water solubility, and BBB permeability. This ligand can form more stable 1:1 metal-to-ligand stoichiometry complexes (the conditional affinity constants for **L44** with Cu(II) or Zn(II) being in the 10<sup>12</sup>M<sup>-1</sup> and 10<sup>9</sup>M<sup>-1</sup> range, respectively, at pH 7.4) than the bidentate ligands described above and has an higher Cu(II) affinity than A $\beta$  (Table 4). Moreover, the geometry of the scaffold is not suitable for Cu(I) coordination that prefers tetrahedral geometry and thus **L44** keeps the Cu ions under the +II state thus preventing redox cycling and ROS production. **L44** is a key example of designed molecule able to control multiple parameter involved in AD pathway, including metal-induced A $\beta$  aggregation, toxicity induced by A $\beta$  and metal-A $\beta$  on N2aAPPswe neuroblastoma cells, ROS generation, and free radical reactions. The excellent results obtained with **L44** were attributed to its ability to directly interact with monomeric and oligomeric A $\beta$ , confirmed by the detection of **L44** in the ternary complex (A $\beta$ )[Cu(II)]**L44** (and (A $\beta$ )[Zn(II)]**L44**) by ion mobility-mass spectrometry and to its ability to remove metal ions from metal ions-A $\beta$  complexes generating apo-A $\beta$  species.

### 2. 3. c. 2. IMPY-like

Incorporation of chelating atoms (N, O) into IMPY scaffold, an A $\beta$  aggregates imaging probe led to the synthesis of **L45**, **L46** and **L47** which are able to control inhibition of Cu(II) and Zn(II)-triggered A $\beta$  aggregation and disassemble preformed aggregates but to a lesser extent than bifunctional *p*-I-stilbene-based molecules. Based on control experiments on apo-A $\beta$  aggregation, it seems that **L46** and **L47** have a preferential reactivity toward metal-induced over metal-free A $\beta$  aggregation pathways. More detail investigations on **L45** indicated its ability to reduce H<sub>2</sub>O<sub>2</sub> production (70 %) but pointed out its intrinsic toxicity for SK-NBE(2)-M17 cells.<sup>88, 155</sup>

### 2. 3. c. 3. PiB-like

Inspired by PiB and its water-soluble counterpart ThT, new bifunctional molecules that contain both amyloid binding and metal chelating properties were developed. **L48-L50** can extract Cu(II) from A $\beta$  and form dimer (1:2 copper to ligand) complexes at a working concentration of 20  $\mu$ M.<sup>156</sup> **L48-L50** showed reduction of the amount of aggregated A $\beta$  when added at the beginning of the aggregation and disaggregation of preformed A $\beta$  aggregates. In addition, **L48-L50** could moderately reduce H<sub>2</sub>O<sub>2</sub> produced by Cu(A $\beta$ ) *in vitro* and in PC12 cells (25%, 27%, and 41%, respectively) and restore cells viability. On the same principle, **L51-L53** are suitable ligands for at least partially sequestering Cu(II) and Zn(II) metal ions present in A $\beta$  peptide and thus are able to prevent A $\beta$  aggregation as shown by turbidity assays (Table 4).<sup>117</sup>

### 2. 3. c. 4. Other bifunctional ligands

While the interaction of stilbene, IMPY and PiB derivatives with metal ions have been thoroughly characterized, other bifunctional molecules incorporating metal binding moieties into resveratrol-, chalcone- and DPP (Diphenylpropynone)-based structures have also been studied in the context of AD (although to a lesser extent) and have been recently reviewed in ref.<sup>48</sup>.

**Table 4.** « 2 in 1 » integrated bifunctional ligands reported from the literature as well as their impact on the ROS production and scavenging, the prevention of aggregation and aggregates disassembly and cell toxicity of the ligand.  $pCu = -\log[Cu]_{unbound}$ ,  $[Cu] = [L] = 10\mu M^+$ : from conditional affinity value,  $^{\square}$ : from apparent affinity value,  $pH = 7.4$ .

	pCu	ROS		Aggregation			Refs.
		arrest of production	scavenging	prevention	disaggregation	cell toxicity	
Cu(A $\beta$ )	7.3-7.8 <sup>+</sup>	-	-	-	-	-	75, 115
L33	n.r.	✓	n.r.	✓	✓	✓	88
L34	7.4 <sup>+</sup>	✓	n.r.	✓	✓	✓	150, 152
L35	n.r.	✓	n.r.	~	~	✗	150
L36	n.r.	~	✓	✓	✓	✓	153
L37		~	~	✗	~	✓	
L38		~	✓	✓	~	n.r.	
L39		n.r.	✓	~	~	n.r.	
L40	n.r.	~	n.r.	✓	✓	n.r.	164
L41		✗					
L42		~					
L43		✗					
L44	8.5 <sup>+</sup>	✓	✓	✓	✓	✓	154
L45	n.r.	✓	n.r.	~	✓	✗	88
L46	n.r.	n.r.	n.r.	~	~	n.r.	155
L47							
L48	n.r.	~	n.r.	✓	✓	✓	156
L49							
L50							
L51	7.9 <sup>+</sup>	n.r.	n.r.	~	n.r.	n.r.	117
L52	6.9 <sup>+</sup>						
L53	5.9 <sup>+</sup>						

### 3. DISCUSSION

#### 3. 1. How to improve fallouts from in vitro experiments

##### 3. 1. a. Introducing remarks

The fundamental causes of AD are still not yet fully understood. Nevertheless the current ever-increasing knowledge of the disease has led to the identification of several promising therapeutic targets. Metal ions are one of those,<sup>43, 48</sup> thus metal-targeting approaches have been developed with a special emphasis for copper ions that is redox active and thus may contribute to the oxidative stress encountered in AD<sup>24</sup> in addition to its involvement in modulation of the

amyloid-beta aggregation.<sup>42</sup> The field knows an increase of interest since more than a decade encouraged by the clinical outcomes achieved by **L1** and **L2** in AD animal models. This in turn has driven medicinal inorganic scientists to produce a growing range of new ligands. This review has described such ligands. Depending on their structures, they have been separated in four groups (Figures 4-7), while bifunctional molecules only include those where the binding cavity is embedded in A $\beta$ -recognition motif. An attempt to sort them in term of efficiency against some of the key deleterious events linked to the etiology of AD (*i.e.* Cu-induced ROS production and Cu-altered A $\beta$  aggregation) is shown in Tables 1-4.

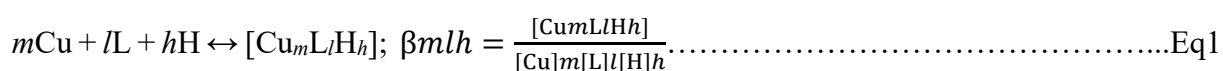
### **3. 1. b. Variability of the *in vitro* assays**

The main observation that clearly appears when trying to review the ligands designed against metal-related toxicity in AD, is the wide diversity of the *in vitro* methods used to test them against Cu-induced ROS production and Cu-altered A $\beta$  aggregation (see § 1.2.b and c). Regarding the aggregation process, its stochastic nature makes it very difficult to reproduce from one group to another one. This mainly depends on the quality of the starting monomeric A $\beta$  peptides and of the monomerisation protocol. Hence the aggregation itself and the formed aggregates do differ even without addition of external molecules.<sup>42</sup> For instance, it was reported that most of the A $\beta$ <sub>40</sub> remains soluble after incubation at 37 °C for two days<sup>61</sup> or does aggregate, with very similar experimental conditions.<sup>121, 122</sup> Hence the study of the effect of the ligands is dependent on the aggregation experiment itself making comparison between different studies/groups difficult or even irrelevant.

When performed, the *in cellulo* experiments are using different cell lines with different culture medium. In addition, except for a few reports,<sup>63</sup> Cu(II) and A $\beta$  are introduced in the cellular media but with neither control on the aggregation state nor in conditions of ROS production (*i.e.* without addition of an external reductant).

### 3. 1. c. Variability in the determination of ligand thermodynamic characterizations

The Cu(II) (and Zn(II)) affinity values of the ligands are not always reported, although it is a primordial criterion for metal-targeting approach, as the ligand has to compete with  $A\beta$ . Those values, when reported, are mainly determined using potentiometric titrations, competitions with a ligand of very well-known affinity (such as ethylenediaminetetraacetic acid) monitored by an appropriate spectroscopy (UV-Vis, EPR) or direct titrations monitored spectroscopically. In the former case, the affinity can be recalculated at any pH values while in the second and third one, the affinity value is determined at the pH where the competition or titration experiments are performed. Potentiometric titrations lead to the determination of conditional affinity (*i.e.* the affinity at a given pH with no competing molecules) while titrations lead to apparent affinity (*i.e.* the affinity at a given pH in presence of buffer). Here, the difficulty in comparing the data reported arises from inappropriate terminology, as often the apparent affinity is used instead of the conditional affinity.<sup>157</sup> Also several kinds of values related to the affinity are given, including the formation constants ( $\beta_{mlh}$ , see Eq. 1), the affinity itself and the  $pCu = -\log [Cu]_{free}$ , as used here.



A convenient way to compare the affinity is the use of the  $pCu$  value that corresponds to the amount of unbound metal ion at given pH, metal ion concentration and metal to ligand ratio. The  $pCu$  has the main advantage to be calculated for complexes of all metal ion to ligand stoichiometries while the affinity values correspond only to the formation of 1:1 species. Hence, for instance,  $pCu$  makes possible the comparison between ligands forming either 1:1 only or 1:1 and 1:2 (metal:ligand) complexes. It is worth noting that for the later type of ligands when the concentration decreases, 1:1 species are favored compared to 1:2 (metal:ligand) complexes.



### 3. 1. d. Variability of experimental conditions

Furthermore, the way to perform the experiments are also different from one report to another. Some primordial experimental conditions like pH, temperature, buffer, reaction time and solvent differ and the results obtained are therefore barely comparable. For instance, the effect of the ligand on the aggregation can be monitored on minutes or days. Another illustration lies in the ROS production assay where the ratio between the Cu and the evaluated ligand can vary from 1 to several equivalents. It is also important to maintain reaction conditions as close as the ones in biological environment in particular regarding the aqueous medium. However, as water solubility is a major issue for intended ligands, this condition is not always fulfilled.

### 3. 1. e. Guidelines for improvement

To be able to draw valuable comparisons between ligands, there is thus an urgent need of going through more standardized procedures, experimental conditions and even (analytical) language, this would ideally include purification and monomerization process for A $\beta$  peptide samples for further aggregation experiments. It is worth mentioning that *in vitro* investigations may also be complemented by measurements on 2D neuron-like cells (*e.g.* obtained from PC12, SH-SY5Y and M17) regarding toxicity of the ligands and Cu(A $\beta$ ) (*via* formation of aggregates and/or ROS production) and viability recovery in the additional presence of ligands. For the inorganic chemist community, studies on differentiated 2D neuron cells would be a first and accessible way to challenge straightforwardly the ligand effect in an environment mimicking the biological medium, thus more appropriate than the test tube. However, a step forward would be the use of more accurate models as 3D human neural cells as they are more physiologically relevant and have successfully recapitulated the AD pathogenic cascades (neurons generating extracellular aggregation of A $\beta$  and tauopathy driven by the A $\beta$  accumulation).<sup>64</sup> While ultimately, better would be transgenic animal models as they allow behavioral tests, 2D cell lines are well known, more easily accessible, less difficult to handle and cheaper than the use of living animals.

### 3. 2. Toward better ligands ?

The important properties of ligands aiming at targeting Cu(II) in the context of AD are shown in the graphical abstract and can be summarized as followed : Cu(I) and Cu(II) chelation, redox inertness, BBB permeability, good affinity, selectivity, kinetic and stability properties and low toxicity.

#### 3. 2. a. General criteria

In spite of the variability of investigations reported in the literature, the designed ligand generally fulfill some basic criteria that are briefly reminded below. The designed molecules has to be soluble and stable in biological medium (buffered water at neutral pH). The toxicity of the ligands on cell lines should be tested and weak. The ligand should keep its binding ability including in presence of oxidative stress (*i.e.* should not be rapidly degraded by oxidative damages). AD occurs in the brain separated from blood circulation by the BBB, which the ligand must cross. In addition, *in vitro*, the ligands should be able to retrieve Cu from A $\beta$ , prevent and stop ROS production, prevent the formation of A $\beta$  toxic aggregates and further to restore viability of cells confronted to Cu(A $\beta$ )-induced toxicity.

#### 3. 2. b. Thermodynamic parameters

The affinity of the intended ligands should be high enough to compete with A $\beta$  for Cu(II) binding but not too high to withdraw Cu from essential metalloproteins.<sup>30, 43, 44, 48, 71, 144</sup> One rapid way to probe the ability to remove Cu from Cu(A $\beta$ ) is to add the ligand to Cu(A $\beta$ ) and monitor the exchange by appropriate spectroscopy (EPR, UV-Vis..). This method is straightforward for tetradentate ligands making 1:1 species only but could be much more complicated for ligand with lower denticity leading to 1:2 (metal:ligand) species as well. If the ability to remove Cu(II) removal from A $\beta$  is kept in presence of Zn, this will also allow determining whether the selectivity of the ligand is appropriate. Indeed, the importance of taking Zn(II) into account when designing Cu(II)-targeting ligands has been demonstrated.<sup>52, 71,</sup>

<sup>113, 114, 126</sup> The ligand has to display a high Cu(II) over Zn(II) selectivity, *i.e.* higher than those of A $\beta$  which equals about 10<sup>4</sup> at neutral pH. This value is quite high (as matter of comparison the selectivity of ethylenediaminetetraacetic acid is 10<sup>2</sup>) and thus this criteria is difficult to fulfill. The lack of Cu(II) selectivity over Zn(II) may be an explanation for the difference of efficiency observed *in vitro*, *in cellulo* (most of studies were performed with Cu or Zn only not a mix of the two metal ions) and *in vivo*, in case of **L1** and **L2** ligands. In the synaptic cleft, ligands with insufficient selectivity will bind the most abundant Zn(II) and be saturated by them, precluding any activity against Cu(II)-related events. The ability of a ligand to remove Cu(II) from A $\beta$  in presence of Zn(II) or the evaluation of its selectivity, has been reported only in a few cases.<sup>113, 114, 126</sup>

### **3. 2. c. “chelators versus metallophores”**

The fate of the Cu removed from A $\beta$  is a controversial issue. Among the large number of ligands that have been proposed as therapeutics for AD, a handful of them has been clinically trialed. Based on *in vivo* results, metallophores such as **L1** (CQ) and **L2** (PBT2) have shown the most satisfying results.<sup>72, 158</sup> In addition, alterations of metal ions levels in AD patients involve both a dyshomeostasis and anomalous interactions with proteins rather than a large overload of loosely-bound metal ions.<sup>30, 31, 159</sup> Thus, the current development of metal-targeting strategies in AD is directed toward favoring brain metal redistribution rather than decreasing metal ions levels.<sup>31, 32, 72, 160</sup> It should be noted that the metallophore ability has only been tested for a few of the reported ligands, namely **L1**, **L2**, **L24** and **L25**.<sup>30, 72</sup>

### **3. 2. d. Bi- and tridentate ligand versus tetradentate ones**

The ligands described here can be gathered into three categories depending on their denticity. Bidentate ligands such as CQ or bi-functional ligands integrating an A $\beta$  recognition moiety **L33-L53**, can form 1:2 (metal:ligand) complex, but this will depend on the metal concentration and metal to ligand ratio, the biologically relevant value of this latter parameter being hard to

predict. The *in vitro* study of such ligands is made difficult by the possibility to form different complexes. The affinity of such ligands is quite weak and it may be anticipated that, rather than removing the Cu(II) ion from A $\beta$ , they form A $\beta$ [Cu(II)]L ternary species in physiological conditions. Such ability has been shown for the tridentate ligand **L3** that leaves free one binding position in the Cu(II) equatorial plane. This position can be occupied by imidazole group of His from the A $\beta$  sequence, for instance.<sup>104, 105</sup> This led to another kind of metal-targeting approach where the metal could stay bound to A $\beta$  but its properties are modulated by the presence of the ligand as for instance **L44**.<sup>154</sup> With respect to tetradentate ligands, their *in vitro* study are eased since they make 1:1 species only. In addition, when rigid enough, they prevent the redox cycling to Cu(I), thus being able to redox silence Cu(II) extracted from A $\beta$ .<sup>43</sup>

### **3. 2. e. Cu(II) versus Cu(I)**

Among all the ligands, only few have been designed to bind Cu(I),<sup>128, 141</sup> investigations being mainly focused on Cu(II)-targeting ligands. However the redox state of Cu in the synaptic cleft is not clearly identified, since  $\mu$ M levels of the reductant Asc are present.<sup>161, 162</sup> Thus the question of the most appropriate redox state of Cu to target is still open. One pragmatic way to solve this issue is to design ligands able to bind both redox states.<sup>128</sup> But, then, a particular attention has to be to the redox activity of the resulting Cu-complexes, so that the Cu-complexes don't produce ROS on their own.

### **3. 2. f. Ligands with A $\beta$ recognition moiety**

Multifunctional ligands aiming at retrieving Cu(II) and targeting A $\beta$  aggregates have been the subject of intense investigations in the last years.<sup>48, 123, 144, 145</sup> However, one has also to take into account that disassembling A $\beta$  aggregates or preventing aggregation could lead to the formation of lower molecular weight and more toxic species than fibrils. For instance, the ability of such ThT-based ligands to inhibit A $\beta$  fibril formation and promote fibril disaggregation has been shown to increase the cellular toxicity. This was likely due to the formation of neurotoxic

soluble A $\beta$ <sub>42</sub> oligomers.<sup>163</sup> Hence, the strategy consisting to generate molecules able to interact, inhibit and disassemble A $\beta$  fibrils may not be the optimal one until a complete understanding of the neurotoxic role of the various A $\beta$  aggregates and of the mechanisms underlying A $\beta$  aggregation is reached.

### 3. 2. g. Concluding remarks

The lack of strong positive results obtained with the metal-targeting approach in general and the failure of the clinical trials of **L1** and **L2** may cast some doubts about the relevance of using ligands, especially copper ones, against AD and even, more essentially, on the role of metal ions in AD. Prior to this reasonable question, one may wonder whether medicinal inorganic chemists learn as much as they can from *in vitro* studies. A more systematic approach leading to comparable results and studies from one group to another could help learning more from *in vitro* researches. Also going deeper in mechanisms at play between the various actors (A $\beta$ , ligand, Cu, Zn and other competing biomolecules) is required to improve fallouts from *in vitro* data. In such a context, testing ligands in biological medium on neuron-like cells appears as a good balance between the insights that can be obtained and the complexity of the methodology. We may also wonder whether the absence of strong beneficial effects of the first ligands tested in clinical trials could be linked to their inability to fulfill all required criteria, such as those emphasized in the previous paragraphs.

### 3. 2. h. Further improvements

New insights into, for instance, the interactions between A $\beta$ –metal ion–L involved when bidentate or tridentate ligands are used<sup>104</sup> or the kinetic aspects of Cu(II) removal,<sup>121, 122, 164</sup> may be valuable. Future improvements also include or better mathematical model of the synaptic cleft,<sup>165</sup> and taking into account the other isoforms of A $\beta$ .<sup>104, 158</sup> One crucial point is to perform the investigations in better mimicking media (no organic solvent), more realistic cellular models, such as 3D ones and/or primary neurons in cultures.<sup>64</sup>

## **CONCLUDING REMARKS**

The present viewpoint article has gathered, described and discussed most of the ligands targeting Cu(II) reported in the last years in the context of AD and of the amyloid and metal hypotheses. A key point for inorganic chemists working in the field will be to deliver directly comparable data obtained with standardized methodologies on their ligands' properties that can be useful to discriminate between the ligands prior to in vivo assays.

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## REFERENCES

1. 2017 Alzheimer's disease facts and figures. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* **2017**, *13* (4), 325-373.
2. Wimo, A.; Jönsson, L.; Bond, J.; Prince, M.; Winblad, B., The worldwide economic impact of dementia 2010. *Alzheimer's & Dementia* **2013**, *9* (1), 1-11.e3.
3. Hamley, I. W., The Amyloid Beta Peptide: A Chemist's Perspective. Role in Alzheimer's and Fibrillization. *Chem. Rev.* **2012**, *112* (10), 5147-5192.
4. Jakob-Roetne, R.; Jacobsen, H., Alzheimer's Disease: From Pathology to Therapeutic Approaches. *Angew. Chem. Int. Ed.* **2009**, *48* (17), 3030-3059.
5. Savelieff, M. G.; Lee, S.; Liu, Y.; Lim, M. H., Untangling Amyloid- $\beta$ , Tau, and Metals in Alzheimer's Disease. *ACS Chem. Biol.* **2013**, *8* (5), 856-865.
6. Ross, C. A.; Poirier, M. A., Protein aggregation and neurodegenerative disease. *Nat. Med.* **2004**, *10*, S10.
7. Polancon, J. C.; Li, C.; Bodea, L. G.; Martinez-Marmol, R.; Meunier, F. A.; Götz, J., Amyloid- $\beta$  and tau complexity - towards improved biomarkers and targeted therapies. *Nat. Rev. Neurol.* **2018**, *14* (1), 22-39.
8. Li, C.; Götz, J., Tau-based therapies in neurodegeneration: opportunities and challenges. *Nat. Rev. Drug Discov.* **2017**, *12*, 863-883.
9. Atrian-Blasco, E.; Gonzalez, P.; Santoro, A.; Alies, B.; Faller, P.; Hureau, C., Cu and Zn coordination to amyloids: a chemistry of pathological importance ? *Coord. Chem. Rev.* **2018**, *371*, 38-55.
10. Cristóvão, J. S.; Santos, R.; Gomes, C. M., Metals and Neuronal Metal Binding Proteins Implicated in Alzheimer's Disease. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 9812178.
11. Holtzman, D. M.; Morris, J. C.; Goate, A. M., Alzheimer's disease: the challenge of the second century. *Sci. Transl. Med.* **2011**, *3* (77), 77sr1.
12. Roher, A. E.; Kokjohn, T. A.; Clarke, S. G.; Sierks, M. R.; Maarouf, C. L.; Serrano, G. E.; Sabbagh, M. S.; Beach, T. G., APP/A $\beta$  structural diversity and Alzheimer's disease pathogenesis. *Neurochem. Int.* **2017**, *110*, 1-13.
13. Norstrom, E., Metabolic Processing of the Amyloid Precursor Protein — New Pieces of the Alzheimer's Puzzle. *Discovery Medicine* **2017**, *23* (127), 269-276.
14. del C. Cárdenas-Aguayo, M.; del C. Silva-Lucero, M.; Cortes-Ortiz, M. J.-R., B. ; Gómez-Virgilio, L.; Ramírez-Rodríguez, G.; Vera- Arroyo, E.; Fiorentino-Pérez, R.; García, U.; Luna-Muñoz, J.; Meraz-Ríos, M. A., Physiological Role of Amyloid Beta in Neural Cells: The Cellular Trophic Activity, Neurochemistry. In *Neurochemistry*, Heinbockel, T., Ed. IntechOpen: 2014.
15. Kepp, K. P., Bioinorganic chemistry of Alzheimer's disease. *Chem. Rev.* **2012**, *112* (10), 5193-5239.
16. Hureau, C., Coordination of redox active metal ions to the APP and to the amyloid- $\beta$  peptides involved in AD. Part 1: an overview. *Coord. Chem. Rev.* **2012**, *256* (19-20), 2164-2174.
17. Tanzi, R. E.; Bertram, L., Twenty Years of the Alzheimer's Disease Amyloid Hypothesis: A Genetic Perspective. *Cell* **2005**, *120* (4), 545-555.
18. Selkoe, D. J.; Hardy, J., The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO molecular medicine* **2016**, *8* (6), 595-608.
19. Hardy, J. A.; Higgins, G. A., Alzheimer's disease: the amyloid cascade hypothesis. *Science* **1992**, *256*, 184-185.
20. Hardy, J., The discovery of Alzheimer-causing mutations in the APP gene and the formulation of the "amyloid cascade hypothesis". *The FEBS Journal* **2017**, *284* (7), 1040-1044.

21. Schnabel, J., Amyloid: Little proteins, big clues. *Nature* **2011**, *475*, S12-S14.
22. Lee, S. J. C.; Nam, E.; Lee, H. J.; Savelieff, M. G.; Lim, M. H., Towards an understanding of amyloid- $\beta$  oligomers: characterization, toxicity mechanisms, and inhibitors. *Chem. Soc. Rev.* **2017**, *46* (2), 310-323.
23. Walsh, D. M.; Selkoe, D. J., A beta oligomers - a decade of discovery. *J. Neurochem.* **2007**, *101* (5), 1172-1184.
24. Cheignon, C.; Tomas, M.; Bonnefont-Rousselot, D.; Faller, P.; Hureau, C.; Collin, F., Oxidative stress and the amyloid beta peptide in Alzheimer's Disease. *Redox Biology* **2018**, *14*, 450-464.
25. Tönnies, E.; Trushina, E., Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. *J Alzheimers Dis.* **2017**, *57* (4), 1105-1121.
26. Bloom, G. S., Amyloid- $\beta$  and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis. *JAMA Neurology* **2014**, *71* (4), 505-508.
27. Nisbet, R. M.; Polanco, J.-C.; Ittner, L. M.; Götz, J., Tau aggregation and its interplay with amyloid- $\beta$ . *Acta Neuropathol. (Berl)*. **2015**, *129* (2), 207-220.
28. Urrutia, P.; Mena, N.; Nunez, M., The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. *Frontiers in Pharmacology* **2014**, *5* (38).
29. Ayton, S.; Lei, P.; Bush, A. I., Metallostasis in Alzheimer's disease. *Free Radic. Biol. Med.* **2013**, *62*, 76-89.
30. Barnham, K. J.; Bush, A. I., Biological metals and metal-targeting compounds in major neurodegenerative diseases. *Chem. Soc. Rev.* **2014**, *43*, 6727-6749.
31. Faller, P.; Hureau, C., "A bioinorganic view of Alzheimer's disease: When misplaced metal ions (re)direct the electrons to the wrong target? ". *Chem. Eur. J.* **2012**, *18* (50), 15910-15920.
32. Budimir, A., Metal ions, Alzheimer's disease and chelation therapy. *Acta Pharm.* **2011**, *61* (1), 1-14.
33. Trapani, G.; Satriano, C.; La Mendola, D., Peptides and their Metal Complexes in Neurodegenerative Diseases: from Structural Studies to Nanomedicine Prospects. *Curr. Med. Chem.* **2018**, *25* (6), 715-747.
34. Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R., Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.* **1998**, *158* (1), 47-52.
35. Miller, L. M.; Wang, Q.; Telivala, T. P.; Smith, R. J.; Lanzirotti, A.; Miklossy, J., Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with  $\beta$ -amyloid deposits in Alzheimer's disease. *J. Struct. Biol.* **2006**, *155* (1), 30-37.
36. Schrag, M.; Mueller, C.; Oyoyo, U.; Smith, M. A.; Kirsch, W. M., Iron, zinc and copper in the Alzheimer's disease brain: A quantitative meta-analysis. Some insight on the influence of citation bias on scientific opinion. *Prog. Neurobiol.* **2011**, *94* (3), 296-306.
37. Kardos, J.; Kovács, I.; Hajós, F.; Kálmán, M.; Simonyi, M., Nerve endings from rat brain tissue release copper upon depolarization. A possible role in regulating neuronal excitability. *Neurosci. Lett.* **1989**, *103* (2), 139-144.
38. Hartter, D. E.; Barnea, A., Evidence for release of copper in the brain : depolarization-induced release of newly-taken <sup>67</sup>Copper. *Synapse* **1988**, *2*, 412-415.
39. Frederickson, C. J., Neurobiology of zinc and zinc-containing neurons. *Int. Rev. Neurobiol.* **1989**, *31*, 145-238.
40. Drew, S. C.; Barnham, K. J., The Heterogeneous Nature of Cu(2+) Interactions with Alzheimer's Amyloid- $\beta$  Peptide. *Acc. Chem. Res.* **2011**, *44* (11), 1146-1155.



41. Migliorini, C.; Porciatti, E.; Luczkowski, M.; Valensin, D., Structural characterization of Cu<sup>2+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup> binding sites of model peptides associated with neurodegenerative diseases. *Coord. Chem. Rev.* **2012**, *256* (1-2), 352-368.
42. Rana, M.; Sharma, A. K., Cu and Zn interactions with A $\beta$  peptides: consequence of coordination on aggregation and formation of neurotoxic soluble A $\beta$  oligomers. *Metallomics* **2019**, *11*, 64-84.
43. Hureau, C., Metal Ions and Complexes in Alzheimer's Disease: From Fundamental to Therapeutic Perspectives. In *Encyclopedia of Inorganic and Bioinorganic Chemistry*, Scott, R. A., Ed. 2019.
44. Faller, P.; Hureau, C., Bioinorganic chemistry of Cu and Zn ions coordinated to A $\beta$  peptide. *Dalton Trans.* **2009**, 1080-1094.
45. Valensin, D.; Migliorini, C.; Valensin, G.; Gaggelli, E.; La Penna, G.; Kozlowski, H.; Gabbiani, C.; Messori, L., Exploring the Reactions of  $\beta$ -Amyloid (A $\beta$ ) Peptide 1–28 with Al<sup>III</sup> and Fe<sup>III</sup> Ions. *Inorg. Chem.* **2011**, *50* (15), 6865-6867.
46. Bousejra-El-Garah, F.; Bijani, C.; Coppel, Y.; Faller, P.; Hureau, C., Iron(II) Binding to Amyloid- $\beta$ , the Alzheimer's Peptide. *Inorg. Chem.* **2011**, *50* (18), 9024-9030.
47. Hureau, C.; Dorlet, P., Coordination of redox active metal ions to the APP protein and to the amyloid- $\beta$  peptides involved in Alzheimer disease. Part 2: How Cu(II) binding sites depend on changes in the A $\beta$  sequences. *Coord. Chem. Rev.* **2012**, *256* (19-20), 2175-2187.
48. Savelieff, M. G.; Nam, G.; Kang, J.; Lee, H. J.; Lee, M.; Lim, M. H., Development of Multifunctional Molecules as Potential Therapeutic Candidates for Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis in the Last Decade. *Chem. Rev.* **2019**, *119* (2), 1221-1322.
49. Andersen, J. K., Oxidative stress in neurodegeneration: cause or consequence? *Nat. Rev. Neurosci.* **2004**, *10*, S18.
50. Miroslav, P., Alzheimer's Disease and Oxidative Stress: A Review. *Curr. Med. Chem.* **2014**, *21* (3), 356-364.
51. Chassaing, S.; Collin, F.; Dorlet, P.; Gout, J.; Hureau, C.; Faller, P., Copper and heme-mediated A $\beta$  toxicity: redox chemistry, A $\beta$  oxidations and anti-ROS compounds. *Curr. Top. Med. Chem.* **2012**, *12* (22), 2573-2595.
52. Atrian-Blasco, E.; Conte-Daban, A.; Hureau, C., Mutual interference of Cu and Zn ions in Alzheimer's disease: perspectives at the molecular level. *Dalton Trans.* **2017**, *46*, 12750-12759.
53. Faller, P.; Hureau, C.; Berthoumieu, O., Role of Metal Ions in the Self-assembly of the Alzheimer's Amyloid- $\beta$  Peptide. *Inorg. Chem.* **2013**, *52*, 12193-12206.
54. Viles, J. H., Metal ions and amyloid formation in neurodegenerative diseases. *Coord. Chem. Rev.* **2012**, *256* (19-20), 2271-2284.
55. Noël, S.; Cadet, S.; Gras, E.; Hureau, C., The benzazole scaffold: a SWAT to combat Alzheimer's Disease. *Chem. Soc. Rev.* **2013**, *42*, 7747-7762.
56. Blancas-Mejia, L. M.; Weber, B.; Buchner, J.; Ramirez-Alvarado, M.; Naiki, H.; Otzen, D., ThT 101: a primer on the use of thioflavin T to investigate amyloid formation AU - Gade Malmos, Kirsten. *Amyloid* **2017**, *24* (1), 1-16.
57. Pryor, N. E.; Moss, M. A.; Hestekin, C. N., Unraveling the early events of amyloid- $\beta$  protein (A $\beta$ ) aggregation: techniques for the determination of A $\beta$  aggregate size. *Int. J. Mol. Sci.* **2012**, *13* (3), 3038-3072.
58. Stine, W. B.; Jungbauer, L.; Yu, C.; LaDu, M. J., Preparing synthetic A $\beta$  in different aggregation states. In *Alzheimer's Disease and Frontotemporal Dementia. Methods in Molecular Biology (Methods and Protocols)*, Roberson, E., Ed. Humana Press, Totowa, NJ: 2011; Vol. 670, pp 13-32.

59. Drolle, E.; Hane, F.; Lee, B.; Leonenko, Z., Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *Drug Metab. Rev.* **2014**, *46* (2), 207-223.
60. Gordon, J.; Amini, S.; White, M. K., General overview of neuronal cell culture. In *Neuronal Cell Culture. Methods in Molecular Biology (Methods and Protocols)*, Amini, S.; White, M., Eds. Humana Press, Totowa, NJ: 2013; Vol. 1078, pp 1-8.
61. Chen, T.; Wang, X.; He, Y.; Zhang, C.; Wu, Z.; Liao, K.; Wang, J.; Zijian, G., Effects of Cyclen and Cyclam on Zinc(II)- and Copper(II)-Induced Amyloid  $\beta$ -Peptide Aggregation and Neurotoxicity. *Inorg. Chem.* **2009**, *48* (13), 5801-5809.
62. Yang, Y.; Chen, T.; Zhu, S.; Gu, X.; Jia, X.; Lu, Y.; Zhu, L., Two macrocyclic polyamines as modulators of metal-mediated A $\beta$ 40 aggregation. *Integr. Biol.* **2015**, *6*, 655-662.
63. Hu, X.; Zhang, Q.; Wang, W.; Yuan, Z.; Zhu, X.; Chen, B.; Chen, X., Tripeptide GGH as the Inhibitor of Copper-Amyloid- $\beta$ -Mediated Redox Reaction and Toxicity. *ACS Chem. Neurosci.* **2016**, *7* (9), 1255-1263.
64. Choi, S. H.; Kim, Y. H.; Quinti, L.; Tanzi, R. E.; Kim, D. Y., 3D culture models of Alzheimer's disease: a road map to a "cure-in-a-dish". *Mol. Neurodegener.* **2016**, *11* (1), 75.
65. Kulshreshtha, A.; Piplani, P., Current pharmacotherapy and putative disease-modifying therapy for Alzheimer's disease. *Neurol. Sci.* **2016**, *37* (9), 1403-1435.
66. Daniela Galimberti, E. S., Treatment of Alzheimers Disease: Symptomatic and Disease-Modifying Approaches. *Current Aging Science* **2010**, *3* (1), 46-56.
67. Bachurin, S. O.; Bovina, E. V.; Ustyugov, A. A., Drugs in Clinical Trials for Alzheimer's Disease: The Major Trends. *Med. Res. Rev.* **2017**, *37* (5), 1186-1225.
68. P, C.; Sani, M. A.; Ding, F.; Kakinen, A.; Javed, I.; Separovic, F.; Davis, T. P.; Mezzenga, R., Implications of peptide assemblies in amyloid diseases. *Chem. Soc. Rev.* **2017**, *46* (21), 6492-6531.
69. Giampietro, R.; Spinelli, F.; Contino, M.; Colabufo, N. A., The Pivotal Role of Copper in Neurodegeneration: A New Strategy for the Therapy of Neurodegenerative Disorders. *Mol. Pharm.* **2018**, *15* (3), 808-820.
70. Rivera-Mancía, S.; Pérez-Neri, I.; Ríos, C.; Tristán-López, L.; Rivera-Espinosa, L.; Montes, S., The transition metals copper and iron in neurodegenerative diseases. *Chem. Biol. Interact.* **2010**, *186* (2), 184-199.
71. Robert, A.; Liu, Y.; Nguyen, M.; Meunier, B., Regulation of copper and iron homeostasis by metal chelators: a possible chemotherapy for Alzheimer's disease. *Acc. Chem. Res.* **2015**, *48* (5), 1332-1339.
72. Crouch, P. J.; Barnham, K. J., Therapeutic redistribution of metal ions to treat Alzheimer's disease. *Acc. Chem. Res.* **2012**, *45* (9), 1604-1611.
73. Adlard, P. A.; Bush, A. I., Metals and Alzheimer's Disease: How Far Have We Come in the Clinic? *J Alzheimers Dis.* **2018**, *62* (3), 1369-1379.
74. Fan, J.; de Lannoy, I. A. M., Pharmacokinetics. *Biochem. Pharmacol.* **2014**, *87* (1), 93-120.
75. Alies, B.; Renaglia, E.; Rozga, M.; Bal, W.; Faller, P.; Hureau, C., Cu(II) affinity for the Alzheimer's Peptide: Tyrosine fluorescence studies revisited. *Anal. Chem.* **2013**, *85* (3), 1501-1508.
76. Pardridge, W. M., Alzheimer's disease drug development and the problem of the blood-brain barrier. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **2009**, *5* (5), 427-432.
77. Paul, C. T., Selecting Good 'Drug-Like' Properties to Optimize Small Molecule Blood-Brain Barrier Penetration. *Curr. Med. Chem.* **2016**, *23* (14), 1392-1407.

78. Banks, W. A., From blood–brain barrier to blood–brain interface: new opportunities for CNS drug delivery. *Nat. Rev. Drug Discov.* **2016**, *15*, 275.
79. Rosenberg, G. A., Blood-Brain Barrier Permeability in Aging and Alzheimer's Disease. *The journal of prevention of Alzheimer's disease* **2014**, *1* (3), 138-139.
80. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug. Deliv. Rev.* **2001**, *46* (1), 3-26.
81. Walters, W. P., Going further than Lipinski's rule in drug design. *Exp. Opin. Drug. Discov.* **2012**, *7* (2), 99-107.
82. Tsou, Y.-H.; Zhang, X.-Q.; Zhu, H.; Syed, S.; Xu, X., Drug Delivery to the Brain across the Blood–Brain Barrier Using Nanomaterials. *Small* **2017**, *13* (43), 1701921.
83. Pardridge, W. M., Drug transport across the blood–brain barrier. *J. Cereb. Blood Flow Metab.* **2012**, *32* (11), 1959-1972.
84. Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A., Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coord. Chem. Rev.* **1999**, *184* (1), 311-318.
85. Oliveri, V.; Vecchio, G., 8-Hydroxyquinolines in medicinal chemistry: A structural perspective. *Eur. J. Med. Chem.* **2016**, *120*, 252-274.
86. Bareggi, S. R.; Cornelli, U., Clioquinol: Review of its Mechanisms of Action and Clinical Uses in Neurodegenerative Disorders. *CNS Neuroscience & Therapeutics* **2012**, *18* (1), 41-46.
87. Valeria, L.; Danilo, M.; Giuseppe Di, N.; Giuseppe, P., Repurposing of Copper(II)-chelating Drugs for the Treatment of Neurodegenerative Diseases. *Curr. Med. Chem.* **2018**, *25* (4), 525-539.
88. Hindo, S. S.; Mancino, A. M.; Braymer, J. J.; Liu, Y.; Vivekanandan, S.; Ramamoorthy, A.; Lim, M. H., Small Molecule Modulators of Copper-Induced A $\beta$  Aggregation. *J. Am. Chem. Soc.* **2009**, *131* (46), 16663-16665.
89. Budimir, A.; Humbert, N.; Elhabiri, M.; Osinska, I.; Biruš, M.; Albrecht-Gary, A.-M., Hydroxyquinoline based binders: Promising ligands for chelatotherapy? *J. Inorg. Biochem.* **2011**, *105* (3), 490-496.
90. Pushie, M. J.; Nienaber, K. H.; Summers, K. L.; Cotelesage, J. J. H.; Ponomarenko, O.; Nichol, H. K.; Pickering, I. J.; George, G. N., The solution structure of the copper clioquinol complex. *J. Inorg. Biochem.* **2014**, *133*, 50-56.
91. Di Vaira, M.; Bazzicalupi, C.; Orioli, P.; Messori, L.; Bruni, B.; Zatta, P., Clioquinol, a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(II) and copper(II) complexes. *Inorg. Chem.* **2004**, *43* (13), 3795-3797.
92. Raman, B.; Ban, T.; Yamaguchi, K.-i.; Sakai, M.; Kawai, T.; Naiki, H.; Goto, Y., Metal Ion-dependent Effects of Clioquinol on the Fibril Growth of an Amyloid  $\beta$  Peptide. *J. Biol. Chem.* **2005**, *280* (16), 16157-16162.
93. Mancino, A. M.; Hindo, S. S.; Kochi, A.; Lim, M. H., Effects of Clioquinol on Metal-Triggered Amyloid- $\beta$  Aggregation Revisited. *Inorg. Chem.* **2009**, *48* (20), 9596-9598.
94. Bolognin, S.; Zatta, P.; Drago, D.; Parnigotto, P. P.; Ricchelli, F.; Tognon, G., Mutual Stimulation of Beta-Amyloid Fibrillogenesis by Clioquinol and Divalent Metals. *Neuromol. Med.* **2008**, *10* (4), 322-332.
95. Cherny, R. A.; Atwood, C. S.; Xilinas, M. E.; Gray, D. N.; Jones, W. D.; McLean, C. A.; Barnham, K. J.; Volitakis, I.; Fraser, F. W.; Kim, Y.; Huang, X.; Goldstein, L. E.; Moir, R. D.; Lim, J. T.; Beyreuther, K.; Zheng, H.; Tanzi, R. E.; Masters, C. L.; Bush, A. I., Treatment

with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* **2001**, *30* (3), 665-676.

96. Cherny, R. A.; Legg, J. T.; McLean, C. A.; Fairlie, D. P.; Huang, X.; Atwood, C. S.; Beyreuther, K.; Tanzi, R. E.; Masters, C. L.; Bush, A. I., Aqueous dissolution of Alzheimer's disease Abeta amyloid deposits by biometal depletion. *J. Biol. Chem.* **1999**, *274* (33), 23223-23228.

97. Ritchie, C. W.; Bush, A. I.; Mackinnon, A.; et al., Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting A $\beta$  amyloid deposition and toxicity in alzheimer disease: A pilot phase 2 clinical trial. *Arch. Neurol.* **2003**, *60* (12), 1685-1691.

98. Mao, X.; Schimmer, A. D., The toxicology of Clioquinol. *Toxicol. Lett.* **2008**, *182* (1), 1-6.

99. Lannfelt, L.; Blennow, K.; Zetterberg, H.; Batsman, S.; Ames, D.; Harrison, J.; Masters, C. L.; Targum, S.; Bush, A. I.; Murdoch, R.; Wilson, J.; Ritchie, C. W., Safety, efficacy, and biomarker findings of PBT2 in targeting A $\beta$  as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *The Lancet Neurology* **2008**, *7* (9), 779-786.

100. Faux, N. G.; Ritchie, C. W.; Gunn, A.; Rembach, A.; Tsatsanis, A.; Bedo, J.; Harrison, J.; Lannfelt, L.; Blennow, K.; Zetterberg, H.; Ingelsson, M.; Masters, C. L.; Tanzi, R. E.; Cummings, J. L.; Herd, C. M.; Bush, A. I., PBT2 rapidly improves cognition in Alzheimer's Disease: additional phase II analyses. *J Alzheimers Dis.* **2010**, *20* (2), 509-516.

101. Crouch, P. J.; Savva, M. S.; Hung, L. W.; Donnelly, P. S.; Mot, A. I.; Parker, S. J.; Greenough, M. A.; Volitakis, I.; Adlard, P. A.; Cherny, R. A.; Masters, C. L.; Bush, A. I.; Barnham, K. J.; White, A. R., The Alzheimer's therapeutic PBT2 promotes amyloid- $\beta$  degradation and GSK3 phosphorylation via a metal chaperone activity. *J. Neurochem.* **2011**, *119* (1), 220-230.

102. Adlard, P. A.; Cherny, R. A.; Finkelstein, D. I.; Gautier, E.; Robb, E.; Cortes, M.; Volitakis, I.; Liu, X.; Smith, J. P.; Perez, K.; Laughton, K.; Li, Q.-X.; Charman, S. A.; Nicolazzo, J. A.; Wilkins, S.; Deleva, K.; Lynch, T.; Kok, G.; Ritchie, C. W.; Tanzi, R. E.; Cappai, R.; Masters, C. L.; Barnham, K. J.; Bush, A. I., Rapid Restoration of Cognition in Alzheimer's Transgenic Mice with 8-Hydroxy Quinoline Analogs Is Associated with Decreased Interstitial A $\beta$ . *Neuron* **2008**, *59* (1), 43-55.

103. Nguyen, M.; Vendier, L.; Stigliani, J.-L.; Meunier, B.; Robert, A., Structures of the Copper and Zinc Complexes of PBT2, a Chelating Agent Evaluated as Potential Drug for Neurodegenerative Diseases. *Eur. J. Inorg. Chem.* **2017**, *2017* (3), 600-608.

104. Mital, M.; Zawisza, I. A.; Wiloch, M. Z.; Wawrzyniak, U. E.; Kenche, V.; Wróblewski, W.; Bal, W.; Drew, S. C., Copper Exchange and Redox Activity of a Prototypical 8-Hydroxyquinoline: Implications for Therapeutic Chelation. *Inorg. Chem.* **2016**, *55* (15), 7317-7319.

105. Kenche, V. B.; Zawisza, I. A.; Masters, C. L.; Bal, W.; Barnham, K. J.; Drew, S. C., Mixed ligand Cu $^{2+}$  complexes of a model therapeutic with Alzheimer's amyloid- $\beta$  peptide and monoamine neurotransmitters. *Inorg. Chem.* **2013**, *52* (8), 4303-4318.

106. Deraeve, C.; Pitié, M.; Mazarguil, H.; Meunier, B., Bis-8-hydroxyquinoline ligands as potential anti-Alzheimer agents. *New J. Chem.* **2007**, *31* (2), 193-195.

107. Deraeve, C.; Boldron, C.; Maraval, A.; Mazarguil, H.; Gornitzka, H.; Vendier, L.; Pitié, M.; Meunier, B., Preparation and study of new poly-8-hydroxyquinoline chelators for an anti-Alzheimer strategy. *Chem. Eur. J.* **2008**, *14* (2), 682-696.

108. Gomes, L. M. F.; Vieira, R. P.; Jones, M. R.; Wang, M. C. P.; Dyrager, C.; Souza-Fagundes, E. M.; Da Silva, J. G.; Storr, T.; Beraldo, H., 8-Hydroxyquinoline Schiff-base

- compounds as antioxidants and modulators of copper-mediated A $\beta$  peptide aggregation. *J. Inorg. Biochem.* **2014**, *139*, 106-116.
109. Nguyen, M.; Robert, A.; Sournia-Saquet, A.; Vendier, L.; Meunier, B., Characterization of New Specific Copper Chelators as Potential Drugs for the Treatment of Alzheimer's Disease. *Chem. Eur. J.* **2014**, *20* (22), 6771-6785.
110. Nguyen, M.; Rechignat, L.; Robert, A.; Meunier, B., The Necessity of Having a Tetradentate Ligand to Extract Copper(II) Ions from Amyloids. *ChemistryOpen* **2015**, *4* (1), 27-31.
111. Nguyen, M.; Bijani, C.; Martins, N.; Meunier, B.; Robert, A., Transfer of Copper from an Amyloid to a Natural Copper-Carrier Peptide with a Specific Mediating Ligand. *Chem. Eur. J.* **2015**, *21* (47), 17085-17090.
112. Ceccom, J.; Coslédan, F.; Halley, H.; Francès, B.; Lassalle, J. M.; Meunier, B., Copper Chelator Induced Efficient Episodic Memory Recovery in a Non-Transgenic Alzheimer's Mouse Model. *PLoS One* **2012**, *7* (8), e43105, 1-7.
113. Zhang, W.; Huang, D.; Huang, M.; Huang, J.; Wang, D.; Liu, X.; Nguyen, M.; Vendier, L.; Mazères, S.; Robert, A.; Liu, Y.; Meunier, B., Preparation of Tetradentate Copper Chelators as Potential Anti-Alzheimer Agents. *ChemMedChem* **2018**, *13* (7), 684-704.
114. Zhang, W.; Liu, Y.; Hureau, C.; Robert, A.; Meunier, B., N<sub>4</sub>-Tetradentate Chelators Efficiently Regulate Copper Homeostasis and Prevent ROS Production Induced by Copper-Amyloid- $\beta_{1-16}$ . *Chem. Eur. J.* **2018**, *24* (31), 7825-7829.
115. Kowalik-Jankowska, T.; Ruta, M.; Wisniewska, K.; Lankiewicz, L., Coordination abilities of the 1-16 and 1-28 fragments of  $\beta$ -amyloid peptide towards copper(II) ions: a combined potentiometric and spectroscopic study. *J. Inorg. Biochem.* **2003**, *95*, 270-282.
116. Martínez, A.; Alcendor, R.; Rahman, T.; Podgorny, M.; Sanogo, I.; McCurdy, R., Ionophoric polyphenols selectively bind Cu<sup>2+</sup>, display potent antioxidant and anti-amyloidogenic properties, and are non-toxic toward *Tetrahymena thermophila*. *Bioorg. Med. Chem.* **2016**, *24* (16), 3657-3670.
117. Rodríguez-Rodríguez, C.; Sánchez de Groot, N.; Rimola, A.; Álvarez-Larena, Á.; Lloveras, V.; Vidal-Gancedo, J.; Ventura, S.; Vendrell, J.; Sodupe, M.; González-Duarte, P., Design, Selection, and Characterization of Thioflavin-Based Intercalation Compounds with Metal Chelating Properties for Application in Alzheimer's Disease. *J. Am. Chem. Soc.* **2009**, *131* (4), 1436-1451.
118. Fu, C. L.; Hsu, L. S.; Liao, Y. F.; Hu, M. K., New Hydroxyquinoline-Based Derivatives as Potent Modulators of Amyloid-beta Aggregations. *Arch. Pharm. (Weinheim)*. **2016**, *349* (5), 327-341.
119. Liang, X.; Sadler, P. J., Cyclam complexes and their applications in medicine. *Chem. Soc. Rev.* **2004**, *33* (4), 246-266.
120. Mewis, R. E.; Archibald, S. J., Biomedical applications of macrocyclic ligand complexes. *Coord. Chem. Rev.* **2010**, *254* (15), 1686-1712.
121. Conte-Daban, A.; Beyler, M.; Tripier, R.; Hureau, C., Kinetic is crucial when targeting copper ions to fight Alzheimer's disease: an illustration with azamacrocyclic ligands. *Chem. Eur. J.* **2018**, *24* (33), 8447-8452.
122. Conte-Daban, A.; Beyler, M.; Tripier, R.; Hureau, C., Corrigendum: Kinetics Are Crucial When Targeting Copper Ions to Fight Alzheimer's Disease: An Illustration with Azamacrocyclic Ligands. *Chem. Eur. J.* **2018**, *24* (49), 13058-13058.
123. Rodríguez-Rodríguez, C.; Telpoukhovskaia, M.; Orvig, C., The art of building multifunctional metal-binding agents from basic molecular scaffolds for the potential application in neurodegenerative diseases *Coord. Chem. Rev.* **2012**, *256* (19-20), 2308-2332.

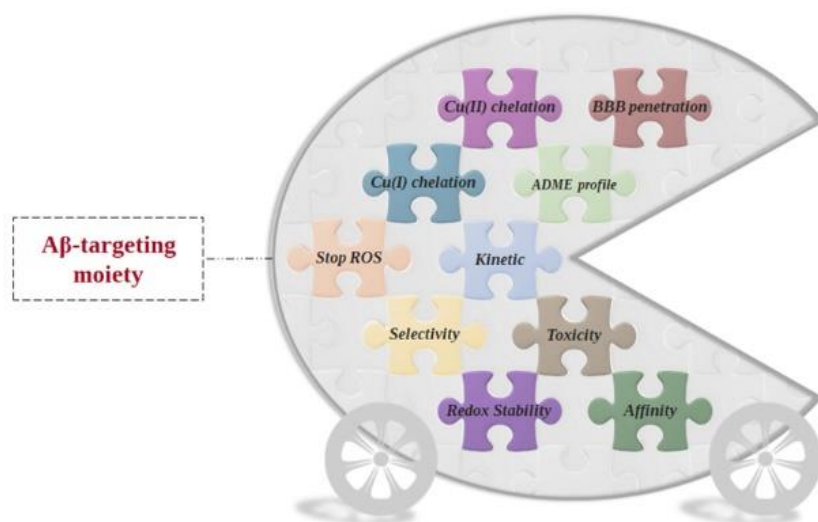
124. Storr, T.; Merkel, M.; Song-Zhao, G. X.; Scott, L. E.; Green, D. E.; Bowen, M. L.; Thompson, K. H.; Patrick, B. O.; Schugar, H. J.; Orvig, C., Synthesis, characterization, and metal coordinating ability of multifunctional carbohydrate-containing compounds for Alzheimer's therapy. *J. Am. Chem. Soc.* **2007**, *129* (23), 7453-7463.
125. Noël, S.; Perez, F.; Ladeira, S.; Sayen, S.; Guillon, E.; Gras, E.; Hureau, C., A new water-soluble Cu(II) chelator that retrieves Cu from Cu(A $\beta$ ) species and stops associated ROS production. *J. Inorg. Biochem.* **2012**, *117*, 322-325.
126. Conte-Daban, A.; Day, A.; Faller, P.; Hureau, C., How Zn can impede Cu detoxification by chelating agents in Alzheimer's disease: a proof-of-concept study. *Dalton Trans.* **2016**, *45* (39), 15671-15678.
127. Lakatos, A.; Zsigo, v.; Hollender, D.; Nagy, N. V.; Fülöp, I.; Simon, D.; Bozso, Z.; Kiss, T., Two pyridine derivatives as potential Cu(II) and Zn(II) chelators in therapy for Alzheimer's disease. *Dalton Trans.* **2010**, *39*, 1302-1315.
128. Atrian-Blasco, E.; Cerrada, E.; Conte-Daban, A.; Testemale, D.; Faller, P.; Laguna, M.; Hureau, C., Copper(I) targeting in the Alzheimer's disease context: a first example using the biocompatible PTA ligand. *Metallomics* **2015**, *7*, 1229-1232.
129. Atrian-Blasco, E.; Cerrada, E.; Faller, P.; Laguna, M.; Hureau, C., Role of PTA in the prevention of Cu(Amyloid- $\beta$ ) induced ROS formation and Amyloid- $\beta$  oligomerisation in the presence of Zn. *Metallomics* **2019**, doi: 10.1039/c9mt00011a.
130. Donnelly, P. S.; Caragounis, A.; Du, T.; Laughton, K. M.; Volitakis, I.; Cherny, R. A.; Sharples, R. A.; Hill, A. F.; Li, Q. X.; Masters, C. L.; Barnham, K. J.; White, A. R., Selective intracellular release of copper and zinc ions from bis(thiosemicarbazone) complexes reduces levels of Alzheimer disease amyloid-beta peptide. *J. Biol. Chem.* **2008**, *282* (8), 4568-4577.
131. Crouch, P. J.; Hung, L. W.; Adlard, P. A.; Cortes, M.; Lal, V.; Filiz, G.; Perez, K. A.; Nurjono, M.; Caragounis, A.; Du, T.; Laughton, K.; Volitakis, I.; Bush, A. I.; Li, Q.-X.; Masters, C. L.; Cappai, R.; Cherny, R. A.; Donnelly, P. S.; White, A. R.; Barnham, K. J., Increasing Cu bioavailability inhibits A $\beta$  oligomers and tau phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (2), 381-386.
132. Xiao, Z.; Donnelly, P. S.; Zimmermann, M.; Wedd, A. G., Transfer of Copper between Bis(thiosemicarbazone) Ligands and Intracellular Copper-Binding Proteins. Insights into Mechanisms of Copper Uptake and Hypoxia Selectivity. *Inorg. Chem.* **2008**, *47* (10), 4338-4347.
133. Bica, L.; Crouch, P. J.; Cappai, R.; White, A. R., Metallo-complex activation of neuroprotective signalling pathways as a therapeutic treatment for Alzheimer's disease. *Mol. Biosyst.* **2009**, *5* (2), 134-142.
134. Kenche, V. B.; Barnham, K. J., Alzheimer's disease & metals: therapeutic opportunities. *Br. J. Pharmacol.* **2011**, *163* (2), 211-219.
135. Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D., The Future of Peptide-based Drugs. *Chem. Biol. Drug. Des.* **2013**, *81* (1), 136-147.
136. Sóvágó, I.; Kállay, C.; Várnagy, K., Peptides as complexing agents. Factors influencing the structure and thermodynamic stability of peptide complexes. *Coord. Chem. Rev.* **2012**, *256* (19-20), 2225-2233.
137. Gonzalez, P.; Bossak, K.; Stefaniak, E.; Hureau, C.; Raibaut, L.; Bal, W.; Faller, P., N-terminal Cu Binding Motifs Xxx-Zzz-His (ATCUN) and Xxx-His and their derivatives: Chemistry, Biology and Medicinal Applications. *Chem. Eur. J.* **2018**, *24* (32), 8029-8041.
138. Borghesani, V.; Alies, B.; Hureau, C., Cu(II) binding to various forms of amyloid- $\beta$  peptides. Are they friends or foes? *Eur. J. Inorg. Chem.* **2018**, 7-15.

139. Caballero, A. B.; Terol-Ordaz, L.; Espargaró, A.; Vázquez, G.; Nicolás, E.; Sabaté, R.; Gamez, P., Histidine-Rich Oligopeptides To Lessen Copper-Mediated Amyloid- $\beta$  Toxicity. *Chem. Eur. J.* **2015**, *22* (21), 7268-7280.
140. Lakatos, A.; Gyurcsik, B.; Nagy, N. V.; Csendes, Z.; Weber, E.; Fulop, L.; Kiss, T., Histidine-rich branched peptides as Cu(II) and Zn(II) chelators with potential therapeutic application in Alzheimer's disease. *Dalton Trans.* **2012**, *41* (6), 1713-1726.
141. Conte-Daban, A.; Boff, B.; Candido Matias, A.; Montes Aparicio, C. N.; Gateau, C.; Lebrun, C.; Cerchiaro, G.; Kieffer, I.; Sayen, S.; Guillon, E.; Hureau, C.; Delangle, P., A trishistidine pseudopeptide with ability to remove both Cu(I) and Cu(II) from the Alzheimer's peptide and to stop the associated ROS formation. *Chem. Eur. J.* **2017**, *23*, 17078-17088.
142. Balland, V.; Hureau, C.; Savéant, J.-M., Electrochemical and Homogeneous Electron Transfers to the Alzheimer Amyloid- $\beta$  Copper Complex Follow a Preorganization Mechanism *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (48), 17113-17118.
143. Cheignon, C.; Jones, M.; Atrian-Blasco, E.; Kieffer, I.; Faller, P.; Collin, F.; Hureau, C., Identification of key structural features of the elusive Cu–Ab complex that generates ROS in Alzheimer's disease. *Chem. Sci.* **2017**, *8*, 5107-5118.
144. Santos, M. A.; Chand, K.; Chaves, S., Recent progress in multifunctional metal chelators as potential drugs for Alzheimer's disease. *Coord. Chem. Rev.* **2016**, *327-328*, 287-303.
145. Savelieff, M. G.; DeToma, A. S.; Derrick, J. S.; Lim, M. H., The Ongoing Search for Small Molecules to Study Metal-Associated Amyloid- $\beta$  Species in Alzheimer's Disease. *Acc. Chem. Res.* **2014**, *47* (8), 2475-2482.
146. Nordberg, A.; Rinne, J. O.; Kadir, A.; Långström, B., The use of PET in Alzheimer disease. *Nat. Rev. Neurol.* **2010**, *6*, 78-87.
147. Koo, J.; Byun, Y., Current status of PET-imaging probes of  $\beta$ -amyloid plaques. *Arch. Pharm. Res.* **2013**, *36* (10), 1178-1184.
148. Angelina, C.; Pierpaolo, A.; Federico, C.; Laura, C., Radiotracers for Amyloid Imaging in Neurodegenerative Disease: State-of-the-Art and Novel Concepts. *Curr. Med. Chem.* **2018**, *25* (26), 3131-3140.
149. Braymer, J. J.; Choi, J.-S.; DeToma, A. S.; Wang, C.; Nam, K.; Kampf, J. W.; Ramamoorthy, A.; Lim, M. H., Development of Bifunctional Stilbene Derivatives for Targeting and Modulating Metal-Amyloid- $\beta$  Species. *Inorg. Chem.* **2011**, *50* (21), 10724-10734.
150. Choi, J.-S.; Braymer, J. J.; Nanga, R. P. R.; Ramamoorthy, A.; Lim, M. H., Design of small molecules that target metal-A $\beta$  species and regulate metal-induced A $\beta$  aggregation and neurotoxicity. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (51), 21990-21995.
151. Lee, H. J.; Korshavn, K. J.; Nam, Y.; Kang, J.; Paul, T. J.; Kerr, R. A.; Youn, I. S.; Ozbil, M.; Kim, K. S.; Ruotolo, B. T.; Prabhakar, R.; Ramamoorthy, A.; Lim, M. H., Structural and Mechanistic Insights into Development of Chemical Tools to Control Individual and Inter-Related Pathological Features in Alzheimer's Disease. *Chem. Eur. J.* **2017**, *23* (11), 2706-2715.
152. Beck, M. W.; Oh, S. B.; Kerr, R. A.; Lee, H. J.; Kim, S. H.; Kim, S.; Jang, M.; Ruotolo, B. T.; Lee, J.-Y.; Lim, M. H., A rationally designed small molecule for identifying an in vivo link between metal–amyloid- $\beta$  complexes and the pathogenesis of Alzheimer's disease. *Chem. Sci.* **2015**, *6* (3), 1879-1886.
153. Beck, M. W.; Derrick, J. S.; Kerr, R. A.; Oh, S. B.; Cho, W. J.; Lee, S. J. C.; Ji, Y.; Han, J.; Tehrani, Z. A.; Suh, N.; Kim, S.; Larsen, S. D.; Kim, K. S.; Lee, J.-Y.; Ruotolo, B. T.; Lim, M. H., Structure-mechanism-based engineering of chemical regulators targeting distinct pathological factors in Alzheimer's disease. *Nat. Commun.* **2016**, *7*, 13115-13127.
154. Lee, S.; Zheng, X.; Krishnamoorthy, J.; Savelieff, M. G.; Park, H. M.; Brender, J. R.; Kim, J. H.; Derrick, J. S.; Kochi, A.; Lee, H. J.; Kim, C.; Ramamoorthy, A.; Bowers, M. T.;

- Lim, M. H., Rational Design of a Structural Framework with Potential Use to Develop Chemical Reagents That Target and Modulate Multiple Facets of Alzheimer's Disease. *J. Am. Chem. Soc.* **2014**, *136* (1), 299-310.
155. Choi, J.-S.; Braymer, J. J.; Park, S. K.; Mustafa, S.; Chae, J.; Lim, M. H., Synthesis and characterization of IMPY derivatives that regulate metal-induced amyloid- $\beta$  aggregation. *Metallomics* **2011**, *3* (3), 284-291.
156. Geng, J.; Li, M.; Wu, L.; Ren, J.; Qu, X., Liberation of Copper from Amyloid Plaques: Making a Risk Factor Useful for Alzheimer's Disease Treatment. *J. Med. Chem.* **2012**, *55*, 9146-9155.
157. Zawisza, I.; Rozga, M.; Bal, W., Affinity of peptides (A $\beta$ , APP,  $\alpha$ -synuclein, PrP) for metal ions (Cu, Zn). *Coord. Chem. Rev.* **2012**, *256* (19-20), 2297-2307.
158. Drew, S. C., The Case for Abandoning Therapeutic Chelation of Copper Ions in Alzheimer's Disease. *Front. Neurosci.* **2017**, *11*, 317.
159. Faller, P., Copper in Alzheimer disease: too much, too little, or misplaced? *Free Radic. Biol. Med.* **2012**, *52* (4), 747-748.
160. Hegde, M. L.; Bharathi, P.; Suram, A.; Venugopal, C.; Jagannathan, R.; Poddar, P.; Srinivas, P.; Sambamurti, K.; Rao, K. J.; Scancar, J.; Messori, L.; Zecca, L.; Zatta, P., Challenges Associated with Metal Chelation Therapy in Alzheimer's Disease. *J Alzheimers Dis.* **2009**, *17* (3), 457-468.
161. Harrison, F. E.; May, J. M., Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radic. Biol. Med.* **2009**, *46* (6), 719-730.
162. Rice, M. E., Ascorbate regulation and its neuroprotective role in the brain. *Trends Neurosci.* **2000**, *23* (5), 209-216.
163. Sharma, A. K.; Pavlova, S. T.; Kim, J.; Finkelstein, D.; Hawco, N. J.; Rath, N. P.; Kim, J.; Mirica, L. M., Bifunctional compounds for controlling metal-mediated aggregation of the a $\beta$ 42 peptide. *J. Am. Chem. Soc.* **2012**, *134* (15), 6625-6636.
164. Branch, T.; Girvan, P.; Barahona, M.; Ying, L., Introduction of a Fluorescent Probe to Amyloid- $\beta$  to Reveal Kinetic Insights into Its Interactions with Copper(II). *Angew. Chem. Int. Ed.* **2015**, *54* (4), 1227-1230.
165. Branch, T.; Barahona, M.; Dodson, C. A.; Ying, L., Kinetic Analysis Reveals the Identity of A $\beta$ -Metal Complex Responsible for the Initial Aggregation of A $\beta$  in the Synapse. *ACS Chem. Neurosci.* **2017**, *8* (9), 1970-1979.



**TOC.** Schematic representation of the essential properties that a ligand should possess to be efficient at targeting Cu(II) in the AD context.





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Christelle Hureau has a background in physical inorganic chemistry with a focus on EPR and electrochemistry. She is interested in chemistry in link with the biological world. In 2015, she became group leader of the “Alzheimer and amyloids” team hosted by the Laboratoire de Chimie de Coordination in Toulouse where she investigates structural, dynamic and kinetic aspects of metal ions coordination to the amyloid- $\beta$  peptide and new chelating concepts to fight against Alzheimer’s disease.