2

Structural and Computational Studies of Interactions of Metals with Amyloid Beta

V. Chandana Epa
CSIRO Materials Science & Engineering,
Preventative Health Flagship and the CRC for Mental Health,
Parkville, Victoria,
Australia

1. Introduction

With rapidly ageing populations, dementia caused by neurodegenerative diseases has become a global socioeconomic issue. Globally, more than 24 million people were suffering from dementia in 2005 (C. Ferri et al.,2005). Alzheimer's disease (AD) is the most common cause of dementia. While characterized by the presence of Amyloid-beta (Aβ) peptide plaques in the brain (Masters and Beyreuther, 2006), a major source of the neurotoxicity in AD is now believed to be due to the action of soluble Aβ oligomers (Crouch et al., 2008). Aβ is generated from the amyloid precursor protein (APP) by the action of β- and γ- secretases, and yields peptides 39-43 amino acid residues long, with Aβ(1-40) and Aβ(1-42) the most common. Aβ(1-42) has the primary sequence $D_1AEFRH_6DSGY_{10}E_{11}VH_{13}H_{14}QK_{16}LVFFAEDVGSNK_{28}GAIIGLM_{35}VGGVVIA_{42}$). Structural characterization of the formation of the Aβ oligomers is currently the subject of intensive research. Among the possible mechanisms are those mediated by the interaction of Aβ with metal ions. This includes both redox-active metals such as Copper, and Iron, as well as redox-inactive metals such as Zinc. For example, interaction of Cu(II) (i.e. Cu^{2+}) with Aβ in the presence of reducing agents leads to the production of reactive oxygen species (ROS). This in turn can generate toxic soluble Aβ oligomers via the formation of di-tyrosine cross-linked Aβ dimers (Barnham et al., 2004). The Copper-chelating compound PBT2 has shown efficacy and safety in Phase IIa clinical trials (Adlard et al., 2008). More recently, Platinum and Ruthenium compounds have been synthesized and shown to ablate Aβ-mediated neurotoxicity. Clearly, interaction of metals with Aβ plays an important role in the aetiology of AD and is very relevant to the design of effective therapeutics. Knowledge of the atomic structure of metals bound to the Aβ peptide would greatly facilitate the design of such chemical entities and assist in the elucidation of the mechanisms of neurotoxicity.

This chapter will review important recent developments in determination of the structure of Aβ bound to transition metals (in different oxidation states) and organometallic compounds. While X-ray crystallography has so far been unsuccessful in determining the structure of any metal-Aβ complexes, methods such as NMR (nuclear magnetic resonance), EPR (electron paramagnetic resonance), and XAS (X-ray Absorption Spectroscopy) have been

used, with varying degrees of success, in the structural determinations of metal binding sites and interactions of Aβ. These experiments have been carried out on human and rat or murine Aβ, mutants of Aβ, and on constructs of different lengths, for example 1-16, 1-40, and 1-42. In a number of instances, these experiments have been supplemented by computational studies, usually *ab initio* quantum mechanical calculations. Computational simulations have also been instrumental in shedding light on how redox-active metals may initiate mechanisms of toxicity via binding to Aβ. This review will also discuss how recent computational studies have helped in the elucidation of metal interactions of Aβ and the interplay between theory/computation and experiment in furthering our understanding of the atomic structures of metal-Aβ complexes. The following sections of this chapter will consider, in turn, the interactions of amyloid beta with the transition metals copper, iron, and zinc. This will be followed by a section of the interaction of Aβ with organometallic compounds containing Pt, Ru, Rh, and Ir, before concluding the chapter.

2. Copper and amyloid beta

Copper interaction with amyloid-beta has been the subject of more experimental and computational investigations than any other metal. This is not only because plaques in AD brains are significantly enriched in copper, and Cu(II) binding facilitates aggregation of Aβ in vitro (Atwood et al., 1998), but also because as a very redox-active metal it plays a direct role in the generation of toxic reactive oxygen species (ROS).

Cu(II) concentration in the synaptic cleft can reach as much as 15 μM following post-synaptic release (Duce and Bush, 2010). Lovell et al. (1998) found that senile plaques in AD brains are enriched in copper almost five times compared with normal neuropils. AD brains also typically exhibit signs of oxidative stress such as enhanced levels of dityrosine species, 4-hydroxy nonenal, 8-hydroxy guanosine, protein carbonyl, and lipid peroxidation species (Sayre et al., 1997, Hensley et al., 1998). Cu(II) bound to Aβ can, in the presence of reducing agents such as ascorbate and glutathione, abundant in the brain, activate and reduce molecular O_2 to produce H_2O_2. During this redox cycle, Cu(II) bound to Aβ first gets reduced to Cu(I) by the reducing agent, and then gets oxidized back to Cu(II). This H_2O_2 produced can then lead to a cascade of ROS being generated through Fenton-like and Haber-Weiss chemistry (Smith et al., 2007). Attack by these very reactive free radicals on proteins, nucleic acids, and lipids would lead to the formation of the oxidatively modified products isolated from AD brain tissue. The amyloid beta peptide is itself modified by the ROS, leading for example, hydroxylation of the histidine side-chains and the oxidation of the methionine side-chain (Nadal et al. 2008). The most important modification of Aβ may be at the Tyrosine 10 position. The tyrosyl radicals produced may combine, leading to covalently cross-linked Aβ dimers. Barnham et al. (2004) showed that the Y10A mutant of Aβ was not toxic in neuronal cell assays. This was the case despite Y10A mutant producing H_2O_2 at half the rate of that by the wild-type peptide. Cappai and Barnham (2008) proposed that the covalently cross-linked Aβ oligomers produced by this Cu(II) catalyzed redox process is the genesis of Aβ-induced neurotoxicity. Hence, it is possible that the Cu(II)/Aβ redox chemistry initiates the generation of toxic soluble Aβ oligomers (which are toxic by mechanisms as of yet undetermined) rather than the neurodegeneration in AD being directly the result of the ROS generation.

Early reports (Huang et al., 1999a, 1999b) of Cu(II)/Aβ generation of H_2O_2 appeared to indicate that this process could occur in the absence of any external reducing agents, presumably via the involvement of Met 35 residue of the peptide itself. They also reported a rather high reduction potential (E^0) of +0.74-0.79 V versus the Normal Hydrogen Electrode (NHE) for the Cu(II)/Aβ system. However, later cyclic voltammetry experiments by Jiang et al. (2007) established this value to be +0.28 V. The latter also point out that the measured oxidation potential value for Met 35 makes it unlikely to act as a reductant in vitro. Likewise, the standard reduction potential values of 0.370 V, 0.372 V, and 0.384 V vs. NHE for dopamine, epinephrine, and norepinephrine, respectively, render them incapable of acting as external reducing agents in the generation of H_2O_2 by Cu(II)/Aβ. X-ray Absorption spectroscopy (XAS) studies performed on the Cu(II)/Aβ(1-16)/dopamine system by Streltsov and Varghese (2008) confirmed this when they did not observe the characteristic Cu(I) XANES (X-ray Absorption Near Edge Spectroscopy) spectra. On the other hand, the reduction potential for ascorbic acid, 0.051-0.058 V vs. NHE allows the oxidation of ascorbate by Cu(II)/Aβ to be thermodynamically favourable. Finally, Nadal et al. (2008) observed that the Cu(II)/Aβ/Ascorbate system generated the same amount of H_2O_2 as Cu(II) /Ascorbate (in the absence of Aβ) and inferred that Aβ acts as an antioxidant or free radical scavenger by quenching the hydroxyl ions produced by Cu(II)/Ascorbate. Their ^1H NMR spectra showed that the imidazoles of the histidine residues of Aβ had been oxidized to 2-oxo imidazoles, and also that Met 35 sulfur atom had been oxidized. It should be noted, however, that they did not investigate the kinetics of H_2O_2 production, i.e. did not compare the relative rates of H_2O_2 formation by Cu(II)/Ascorbate versus that of Cu(II)/Aβ/Ascorbate. This group also states that reduction of Cu(II) to Cu(I) by Aβ occurs in the absence of ascorbate using an bathocuproinedisulfonic acid assay. This is contrary to the conclusions drawn from the reduction potential measurements of Jiang et al. (2007) as discussed above. Moreover, the XAS experiments by Streltsov et al. (2008) did not show any evidence for the reduction of Cu(II) by Aβ alone in the absence of any addition of a reducing agent. The effect on the binding of Cu(II) and the neurotoxicity of such oxidative modifications of Aβ is also of some interest.

2.1 Cu(II) structural and modelling studies

With the critical role Cu(II) plays in the properties of Aβ when it is bound to the peptide, considerable effort has gone into the structural determination of the Cu(II) binding site on Aβ. While there have been a number of reports of widely varying values for the binding affinity of Cu(II) for Aβ, one of the more reliable was the study by Hatcher et al. (2008). Their isothermal calorimetry (ITC) experiments for Aβ(1-40) at 37 C gave values of $1.1x10^9$ M^{-1} and $2.4x10^9$ M^{-1} at pH 7.2 and pH 7.4, respectively. Under the same conditions Aβ(1-16) gave similar binding constants, indicating that the metal ion binding site is located in this N-terminal fragment. The stoichiometry between Cu(II) and Aβ was 1:1. This is likely the binding at the higher affinity site since there are also several findings in the literature of Aβ binding more than 1 mole equivalent of Cu(II). For example, Caine et al. (2007) found that their maltose binding protein (MBP) – Aβ(1-42) fusion protein bound Cu(II) with a stoichiometry of 1:2. There has been some speculation as to the location of the second, weaker affinity binding site of Cu(II), (which is most likely an intermolecular site), but the major effort, as discussed below, has been on the high affinity binding site.

Streltsov et al. (2008) used a combined extended X-ray Absorption Fine Structure – Density Functional Theory (EXAFS-DFT) approach in their study of the Cu(II) binding site in Aβ(1-16) where the experimental data was collected at 16.5 K with a metal:peptide ratio of 1:1 buffered to a pH of 7.4. As the initial data analysis indicated a first shell coordination number of 6, they computed with Density Functional Theory (DFT) (at B3LYP/LANL2DZ level) two different optimized geometries with 3N3O coordination: in each case the nitrogen coordination was via three histidine imidazoles while the oxygen coordination was with a glutamate carboxylate (bidentate) and a water molecule in one case, and with a tyrosine hydroxyl oxygen and two water molecules in the other case. Using these two models in the EXAFS spectra fitting and refinement they found that the fit was significantly better with the octahedral Cu(II)/Glu/3His/Wat geometry, where the three histidine N atoms (at distances of 1.9-2.1 Å from the Cu ion) and one of the Glu carboxylate O atoms (1.9 Å distant from the Cu) are in approximately square planar equatorial arrangement while the other carboxylate O atom (2.3 Å distant from the Cu) and the water O (2.0 Å distant from the Cu) are in an axial arrangement. Figure 1 shows the arrangement of Aβ residues at the Cu(II) binding site for a molecular mechanics (MM) model developed for Cu(II)/Aβ(1-16) using the EXAFS determined coordination distances as constraints. (No constraints were applied to the peptide termini.) In analogy with the NMR solution structure for Zn(II)/Aβ(1-16) by Zirah et al. (2006) (discussed in Section 4), the glutamate is taken as Glu 11. Streltsov et al. also found that their fit could be further improved by placing two more oxygen atoms (assumed as coming from the Asp 1 carboxylate) 4.4 Å distant from the Cu(II) and hydrogen-bonded to the axially placed water.

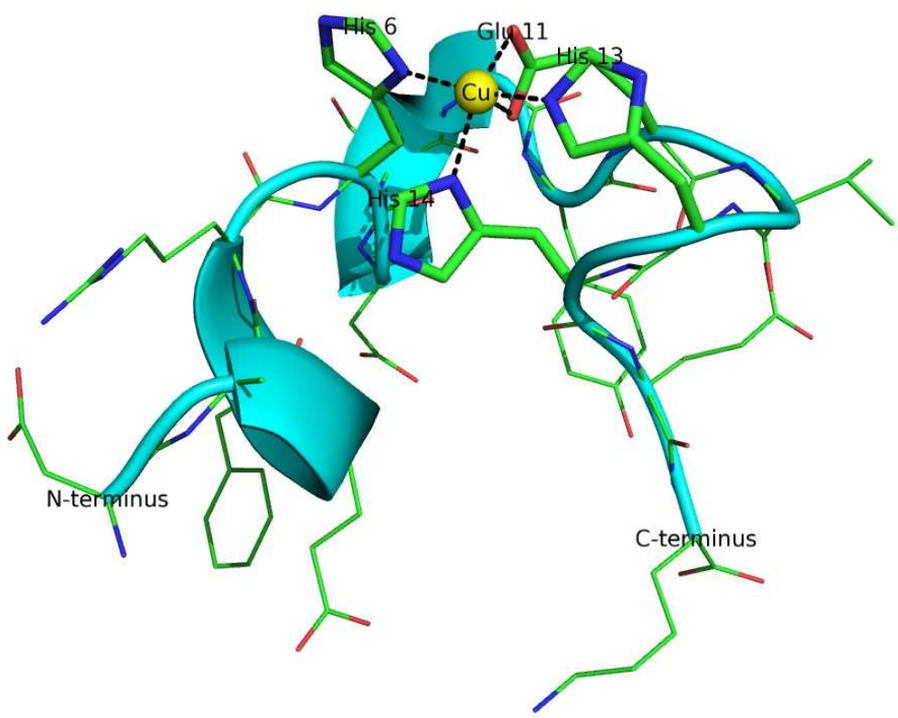


Fig. 1. Molecular model of Cu(II) bound to Aβ(1-16) from EXAFS-DFT studies of Streltsov et al. (2008).

Numerous experiments in the past have shown the importance of the histidine residues of Aβ to copper binding. Smith et al. (2006) reported the loss of Cu/Aβ-induced toxicity when the histidine imidazole δ or ε N atom is methylated. They reported the observation of a histidine –bridged Cu(II)/Aβ dimer by EPR spectroscopy at Cu:peptide ratios greater than 0.6:1. From the same laboratory, Smith et al. (2010) showed that the Aβ mutant H14A had no toxicity in primary neuronal cell cultures. Histidine-bridged Cu(II)/Aβ dimers were not seen in the EXAFS experiments of Streltsov et al.; however, as mentioned earlier this was at a Cu:peptide concentration ratio of 1:1. There is less evidence for the participation of Glu 11 in the Cu(II) binding. It is interesting to note that the x-ray crystal structure of quercetin 2,3-dioxygenase (Fusetti et al., 2002) contains a Cu(II) ion liganded by three histidines and a glutamate in a trigonal bipyramidal geometry. Furthermore, Hureau et al. (2011) very recently solved the x-ray crystal structure of Cu(II) bound to the peptide Gly-His-Lys, where Cu(II) displays a 3N1O coordination in the equatorial plane and a carboxylate O atom coordinating axially. Hence, the type of Cu(II) coordinating geometry proposed by Streltsov et al. has been seen in other contexts. However, the model would need to be validated by the results from EXAFS studies on Aβ mutants such as E11A.

EXAFS and X-ray crystallography result in structures that are mostly static. However, there is ample evidence that the binding of metal ions to Aβ is a dynamical process, and is exquisitely sensitive to the experimental conditions such as pH and type of buffer. Drew et al. (2009a, 2009b) in a series of experiments involving continuous wave electron paramagnetic spectroscopy (CW-EPR) and hyperfine sublevel correlation spectroscopy (HYSCORE) showed that Cu(II) binding to Aβ is pleomorphic in nature. Using a number of ^{15}N labelled and ^{13}C labelled Aβ(1-16) analogues, they found that the nature of the Cu(II) coordination shell was dependent on the pH. With these methodologies they conclude that at both pH 6.3 (“low pH”) and at pH 8.0 (“high pH”), the equatorial coordination of Cu(II) is 3N1O. At low pH, two binding modes predominate (“component Ia” and “component Ib”): the imidazole N of His 6 and N-terminal Asp 1 NH_2 and carbonyl O coordinate in both modes while the imidazole N of His 13 in one mode and the imidazole N of His 14 in the other mode constitute the fourth ligand. Drew et al. model (2009b) for the binding mode at high pH (“component II”) has the three histidine imidazole N atoms and the backbone carbonyl O atom of Ala 2 as the Cu(II) binding partners. They propose that this binding mode results in the polarization of the carbonyl C=O bond and facilitates the hydrolytic cleavage of the amide peptide bond between Ala 2 and Glu 3. This may be the possible source of Aβ(pyroglutamate 3-40 or 3-42) found in significant quantities in AD plaques. (Presumably glutaminyl cyclase in the brain cyclizes Glu 3 in the truncated N-terminal Aβ(3-40 or 3-42).) Moreover, ^{13}C, ^{15}N, and ^{17}O isotopic labelling provided no evidence of the involvement of the O atoms of the amino acid residues Glu 3, Asp 7, Glu 11 and Tyr 10. On the other hand, after performing both EPR and NMR experiments on isotopically labelled Aβ species, the Peter Faller group (Dorlet et al. 2009, Eury et al. 2011) proposed that the equatorial coordination of component II consists of one histidine imidazole N, N-terminal Asp 1 NH2, Ala 2 carbonyl O, and finally the deprotonated Asp1-Ala2 peptide backbone amide N atom. They also proposed that the carboxylate O atom of Asp 1 binds in an axial position. However, it should be noted that EPR measurements are less sensitive to axially coordinating ligands and the interpretation of data is not straightforward (Sarell et al., 2009, Faller and Hureau, 2009).

Binding of Cu(II) to rat or murine Aβ is also of some interest as rats do not display amyloid plaque deposits (Shivers et al., 1998). Compared to the human Aβ peptide, the rat or mouse sequence contains the mutations R5G, H13R, and Y10F. Again using isotopically labelled EPR and NMR studies, Eury et al. (2011) propose that the component II binding mode of Cu(II)/murine Aβ(1-16) is characterized by hexa-coordination, with the 3N1O equatorial binding via His 6 imidazole N, N-terminal Asp 1 NH2 and carbonyl O, and the (deprotonated) Gly 5-His 6 backbone amidyl N atom. The axial ligands proposed are the His 14 imidazole N atom and a carboxylate O atom from one of the acidic residues.

Recently, Streltsov et al. (2011) solved the x-ray crystal structure of Aβ(18-41) within the framework of the CDR3 loop of shark IgNAR (Ig New Antigen Receptor) single variable domain antibody. The Aβ(18-41) portion of the structure that is observed in the crystal structure is tetrameric. By constructing oligomeric models with these tetramer units (see Figure 2), they noticed that the neighbouring tetramers align Glu 22 and Asp 23 on the same face and speculate that these acidic side chains, along with contributions from solvent exposed backbone N and O atoms, may constitute the second, weaker affinity intermolecular Cu(II) binding site.

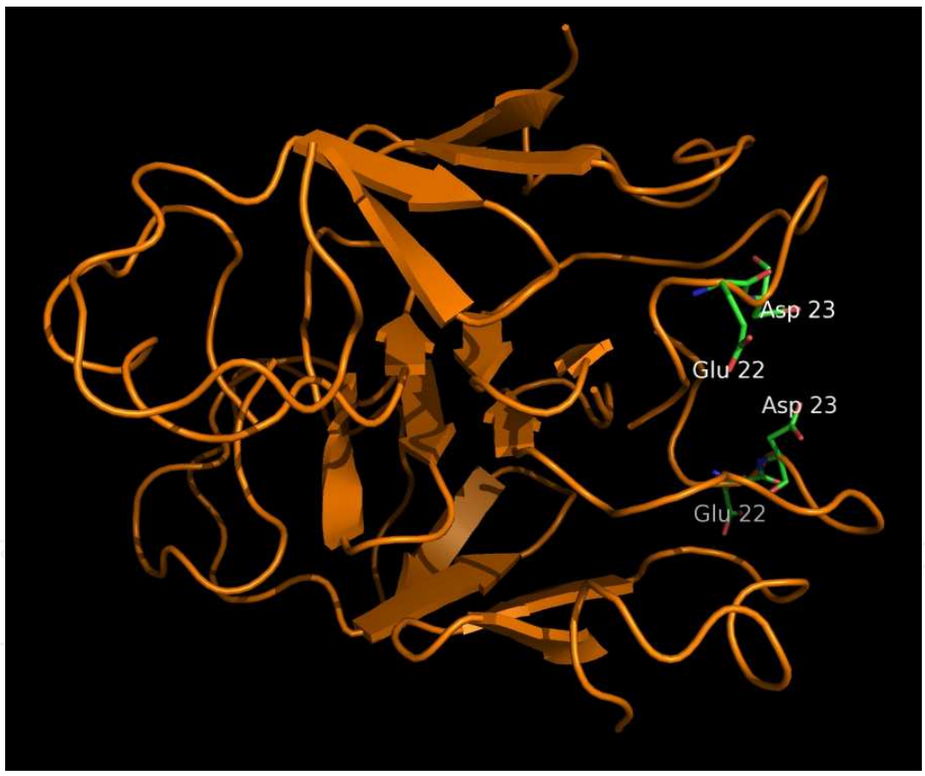


Fig. 2. Putative second binding site of Cu(II) from the Aβ(18-41) tetramer crystal structure of Streltsov et al. (2011)

2.2 Cu(I) structural and modelling studies

During the production of H_2O_2 by Copper/Abeta in the presence of a reducing agent, the oxidation state of copper continually cycles between the +2 and +1 states. Hence, the

structure of Cu(I) bound to Aβ is also of interest. XAS and EPR experiments by Shearer and Szalai (2008) on Cu(II)/Aβ(1-16) reduced with ascorbate showed the disappearance of the characteristic near-edge (XANES) spectral peaks of Cu(II) and the appearance of the peaks characteristic of Cu(I). The EXAFS data could be best fit with a linear imidazole-Cu(I)-imidazole geometry with Cu-N distance of 1.9 Å. They hypothesize that these imidazoles belong to His 13 and His 14. Large basis set DFT calculations (B2-PLYP hybrid functional of Grimme, Ahlrichs' def2-aug-TZVP basis for Cu and ligating N atoms, Ahlrichs' TZVP basis set for other atoms) resulted in an optimized geometry with parameters similar to those measured by EXAFS. Figure 3 depicts their DFT-optimized geometry. In contrast, the XAS and NMR studies done by Hureau et al. (2009) indicate pleotropy in Cu(I) binding to Aβ , showing that all three histidines contribute in a dynamical process. They propose a model where Cu(I) moves between binding to a histidine dyad (His 13 and His 14) and binding to a histidine triad (all three His). An alternative model would be an equilibrium between three histidine dyads: (His 13, His 14), (His 13, His 6), and (His 6, His 14).

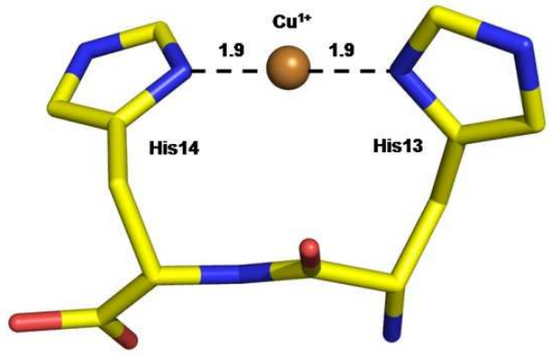


Fig. 3. Structural model of His 13 – Cu(I) – His 14 from DFT studies of Shearer & Szalai (2008), Reprinted from http://www.publish.csiro.au/nid/51/paper/CH09454.htm.

2.3 Cu(II)/Cu(I) ROS chemistry modeling

As mentioned above, quantum mechanical (QM) and molecular mechanical (MM) calculations have played important roles in the structural elucidation of Cu binding to Aβ. Structural models of DFT optimized geometries were an integral part of the EXAFS high-affinity Cu(II) binding site determination by Streltsov et al. (2008).

Computational chemistry, in particular *ab initio* QM calculations also have a major role to play in the elucidation of the mechanisms of ROS chemistry that occur as the result of Cu(II) binding to the N-terminus of Aβ. The laboratory of Rauk has carried out a number of *ab initio* computational studies of Cu binding to Aβ and the resultant H_2O_2 production (Raffa et al., 2005; Raffa et al., 2007; Hewitt and Rauk, 2009). Hewitt and Rauk (2009) examined the mechanism of H_2O_2 generation by Cu(II)/Aβ model system in the presence of an unspecified external reducing agent. The model system that they selected was two imidazoles linked by a peptide backbone, representing the His 13 – His 14 fragment of Aβ. The geometry optimizations were done with DFT calculations at B3LYP/6-31+G(d) level while enthalpy calculations were done with single point energy calculations at B3LYP/6-311+(2df, 2p) level. The reaction pathway or redox cycle computed in this study is depicted

schematically in Figure 4. In the first step, the most stable (according to their calculations) Cu(II) species is reduced to the most stable Cu(I) species. The former species has 2N2O coordination, with the N ligands being His 13 and His 14 imidazole N atoms while the O ligands are the backbone carbonyl O and a water molecule. The most stable Cu(I) species has a linear geometry, with the Cu(I) coordinated by His 13 and His 14 imidazole N atoms.

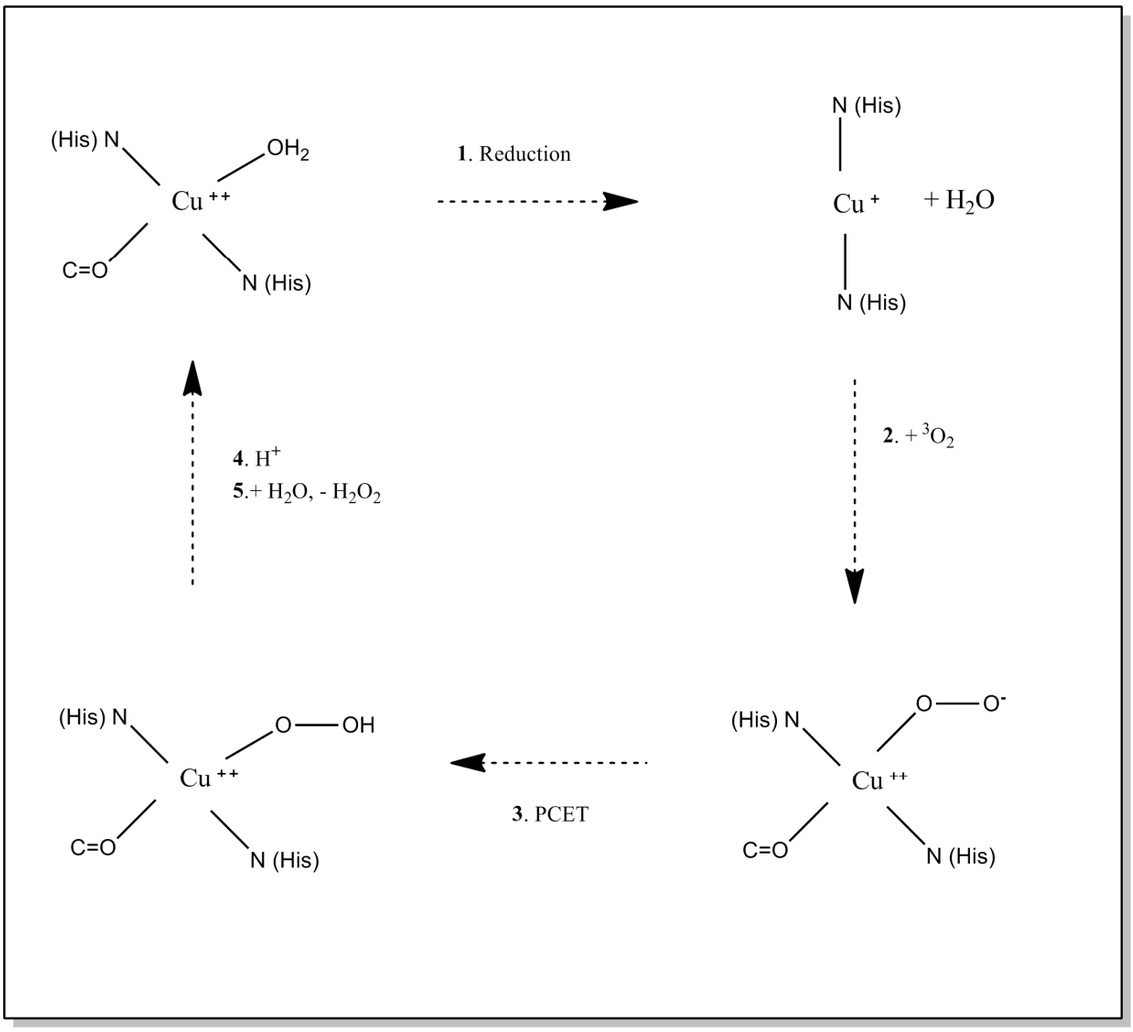


Fig. 4. Simplified reaction scheme of Hewitt and Rauk for the generation of H_2O_2 by Cu(II)/Aβ. Adapted with permission from Hewitt and Rauk (2009). Copyright (2009) American Chemical Society.

In the next step, this Cu(I) species forms a loose adduct with molecular O_2 (in its triplet spin state). This species then takes part in a proton coupled electron transfer (PCET) reaction due to the participation by some external reducing agent (such as ascorbate or glutathione). In the final step, protonation followed by associative substitution by a water molecule leads to the production of H_2O_2 and the regeneration of the original Cu(II) species. Among their findings is that the generation of superoxide to be energetically unfavourable, consistent with the non-observation of superoxide by Huang et al. (1999b) during the Cu(II)/Aβ

generation of H_2O_2. However, their starting structural model for Cu(II)/Aβ, i.e. 2N2O coordination, is at variance with most experimental studies on the Cu(II) binding site as discussed in the previous section. Hewitt and Rauk state that at the level of theory that they employed, axial ligands to Cu(II) dissociate in water. They computed a reduction potential of 0.52 V (vs. the NHE) for the first step. This is significantly higher than the value of 0.28 V measured by Jiang et al. (2007) for Cu(II)/Cu(I) couple when bound to Aβ.

A few years previous to the work of Hewitt and Rauk, Barnham et al. (2004) also used *ab initio* DFT calculations to propose a reaction mechanism for the production of H_2O_2 by Cu(II)/Aβ in the presence of (excess of) ascorbate. Their computation (at the B3LYP/LANL2DZ level) starts the redox cycle with Cu(II) coordinated by three histidines and tyrosine. Cu(II) is reduced to Cu(I) by ascorbate in a PCET step, dissociating the tyrosine. Molecular O_2 coordinates the Cu(I) and oxidizes it back to Cu(II). Hydrogen atom transfer from the tyrosine and simultaneous abstraction of a proton from the medium leads to the formation of H_2O_2 and a tyrosyl radical. Tyrosyl radicals from Aβ molecules close to each other can then lead to the formation of experimentally observed dityrosine-linked Aβ dimer. The presence of transient radicals was shown by the use of the radical trapping agent 2-methyl-2-nitrosopropane. Such radicals were absent in the case of the Aβ mutant Y10A, which was also not toxic in neuronal cell assays. On the other hand, subsequent structural work on the Cu(II) binding site (discussed above) have now ruled out the participation of tyrosine 10 in the binding of Cu(II). Furthermore, Barnham et al. (2004) also reported that the Y10A mutant still produced H_2O_2, albeit at half the rate of wild-type Aβ. Obviously, other mechanisms, not involving binding of Tyr 10 to Cu(II), can lead to the generation of H_2O_2 by Cu(II)/Aβ in the presence of ascorbate.

It is by now quite apparent that Cu binding to Aβ is a pleotropic, dynamical phenomenon, although at a given set of conditions such as pH and buffer a particular species may predominate over others. Molecular dynamics (MD) calculations would be the preferred computational tool to investigate such dynamical processes. Classical MD employing empirical force fields cannot deal with breaking and formation of bonds, so techniques such as CPMD and QM/MM-MD are advantageous in this regard. Furlan et al. (2010) have used *ab initio* (or Car-Parrinello) molecular dynamics (CPMD) calculations to investigate the Cu(I) binding to Aβ. Starting from a number of different Cu(I)/Aβ geometries (employing either two histidine or three histidine topologies), the simulations of Furlan et al. showed that a linear His 13 – Cu(I) – His 14 arrangement was the most stable, although certain interactions between the peptide and the metal ion may lead to the approach and binding of His 6. It must be noted that because of the highly compute-intensive nature of CPMD, their simulation was quite short at 1 ps. It is known that the linear imidazole-Cu(I)-imidazole geometry is particularly stable (Le Clainche et al., 2000), and it is interesting to speculate whether a transient formation of a triply coordinated Cu(I) species might be necessary for its reactivity towards molecular dioxygen.

Concurrent with the studies described above on elucidating the nature of Cu interaction with Aβ and the effects on toxicity, there have been work designing novel chemical entities to ablate the neurotoxicity by interfering with the Cu binding to Aβ. As mentioned in the introduction, successful results of phase IIa clinical trials of a copper ligand PBT2 have been announced (Adlard et al., 2008; Lannfelt et al., 2008). This compound is the second-

generation version of another copper ligand, Clioquinol (Figure 5). These two compounds were shown to be capable of significantly reducing the amount of Aβ aggregation, H_2O_2 generation, and dityrosine-linked Aβ production. They significantly improved the level of cognition in AD patients. Adlard et al. (2008) hypothesized that these compounds act more as ionophores, i.e. removing the copper bound to Aβ and transporting it inside the neuronal cells, leading to upregulation of matrix metalloproteases and subsequently to the degradation of Aβ.

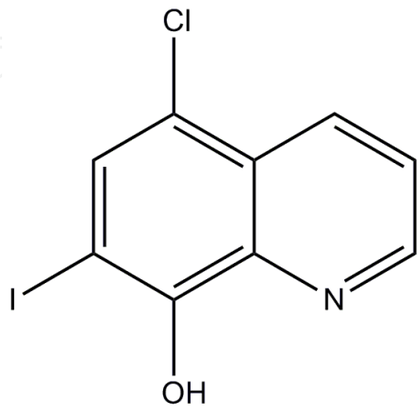


Fig. 5. Chemical structure of Clioquinol

3. Iron and amyloid beta

Iron, like copper, is a redox-active metal and plays a critical role in the human body, being an essential component of haemoglobin and a number of enzymes. After the liver, the organ with the richest concentration of iron is the brain, normally containing in the order of 60 mg of non-haeme iron in the adult brain (Duce & Bush, 2010). However, being redox-active, it is also capable of participating in Fenton and Haber-Weiss type reactions and generating hydroxyl radicals, superoxide, and other reactive oxygen species, and is a potential source of oxidative stress in the brain. The Fe(III)/Fe(II) system has a standard reduction potential of 0.77 V, i.e. greater than that of the Cu(II)/Cu(I) system. Maintaining strict homeostasis of the iron levels and the oxidation state it is in is essential for maintaining the health of the body. This occurs in the healthy body with the activities of ferroxidases such as ceruloplasmin, iron transport proteins like transferrin, and iron strorage proteins such as ferritin. Recently, Duce et al. (2010) proposed that the amyloid precursor protein, APP, also has ferroxidase activity, converting Fe(II) to Fe(III). They found this function was located in the E2 domain (residues 365-495) of APP, associated with the motif REXXE, and to be inhibited by Zn(II). With normal ageing, the iron content in the brain has been found to increase. Iron also has been found to be concentrated in senile plaques of AD brains. Lovell et al. (1998) found AD neuropils to contain more than twice the amount of iron found in control neuropils.

Despite this importance of iron, there has not been much published work on the interaction of iron with amyloid beta. Liu et al. (2010) have proposed that iron promoted the toxicity of Aβ by actually delaying the formation of well-ordered aggregates of Aβ such as the fibrils found in AD. The easy oxidation of Fe(II) to Fe(III) and the hydrolysis and precipitation of

Fe(III) at physiological pH are some of the reasons hindering experimental studies. To get around the latter problem, Jiang et al. (2009) used the complex between Fe(III) and nitrilotriacetic acid (NTA) in their work on the interaction with Aβ and redox properties. They found that Fe(III)-NTA bound to Aβ extremely strongly, with a measured dissociation constant of $6.3x10^{-21}$ M. In comparison, that for Fe(II)-NTA was $5.0x10^{-12}$ M. Furthermore, using cyclic voltametry they determined that the redox potential for Fe(II)-NTA to be 0.23 V when complexed to Aβ.

Just as in the case of Cu(II), there have been contradictory reports on the amino acid residues of Aβ involved in coordinating Fe. An early Raman spectroscopic study by Miura et al. (2001) concluded that while Tyr 10 was central to the binding of Fe(III), the three histidines in the N-terminal Aβ region were not involved. On the other hand, Nakamura et al. (2007) in their study of the redox activity of Cu and Fe in association with Aβ, conclude that, just like for Cu, the three histidines are necessary of the binding of Fe. Most recently, the group of Faller and Hureau used NMR, including ^{1}H, ^{13}C, and 2D studies in an attempt to determine the coordination shell of Fe(II) in binding to Aβ (Bousejra-El Garah et al., 2011). Analyzing the line broadening in the NMR spectra induced by Fe(II) binding to Aβ(1-16), Aβ(1-28), and Aβ(1-4) peptides, they concluded that firstly, neither Tyr 10 nor Met 35 had any role in Fe(II) coordination. Secondly, they identified Asp 1, Glu 3, His 6, His 13, and His 14 as the residues involved in binding Fe(II). Assuming hexa-coordination of Fe(II), they propose a 3N3O first coordination shell for Fe(II). i.e. The equatorial ligands are the imidazoles of His 6 and His 13 or His 14 as well as the N-terminus of Asp1 and the backbone carbonyl oxygen from Asp1 or His 6. The axial ligands consist of the carboxylate oxygens of Asp 1 and Glu 3. This is schematically depicted in Figure 6. More precise determination of the 3D structure of the binding site awaits future work. No EXAFS studies on Fe binding to Aβ have been reported yet. Notably, Bousejra-El Garah et al. did not find any pH dependence for the Fe(II) binding to Aβ near physiological pH.

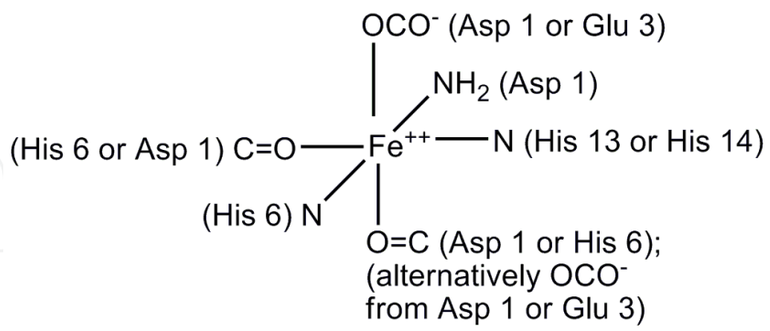


Fig. 6. Model for Fe(II)/Aβ coordination by Bousejra-El Garah et al. (2011). Adapted with permission from Bousejrah-El Garah et al. (2011). Copyright (2011) American Chemical Society.

Also very recent is an *ab initio* study by Rauk and co-workers on the structures and stabilities of Fe(II) and Fe(III) complexes with Aβ fragments (Ali-Torres et al., 2011). Their calculations consisted of single point energies calculated at MP2 level with a large 6-311+G(2df,2p) basis set at geometries optimized with the DFT functional B3LYP with a

small 6-31G(d) basis set. Solvent effects were included with an IEFPCM (polarisable continuum model with the integral equation formalism variant). They determined that the most stable complexes containing His-His (i.e. His 13 – His 14) and phenolate (derived from Tyr 10) are the two penta-coordinated species [Fe(II)(*O*-HisHis)(PhO^-)(H_2O)]$^+$ and [Fe(III)(*N*-HisHis)(PhO^-)(H_2O)]$^+$. These structures are shown in Figure 7. They concluded that the simultaneous coordination of Tyr10 and His 13 - His 14 to Fe(II) and to Fe(III) is thermodynamically favourable. However, as we saw from the NMR results by Bousejra-ElGarah et al., it is unlikely that Aβ utilizes Tyr 10 in coordination to Fe(II). Ali-Torres et al. computing the standard reduction potentials, determined that this coordination by Tyr and His-His reduces Fe(III)/Fe(II) reduction by about 0.5 V (compared to aqueous Fe(III)/Fe(II)) to 0.20 V. We note that this value is quite close to the value of 0.23 V experimentally determined by Jiang et al. (2009) for the Fe(III)-NTA system complexed to Aβ.

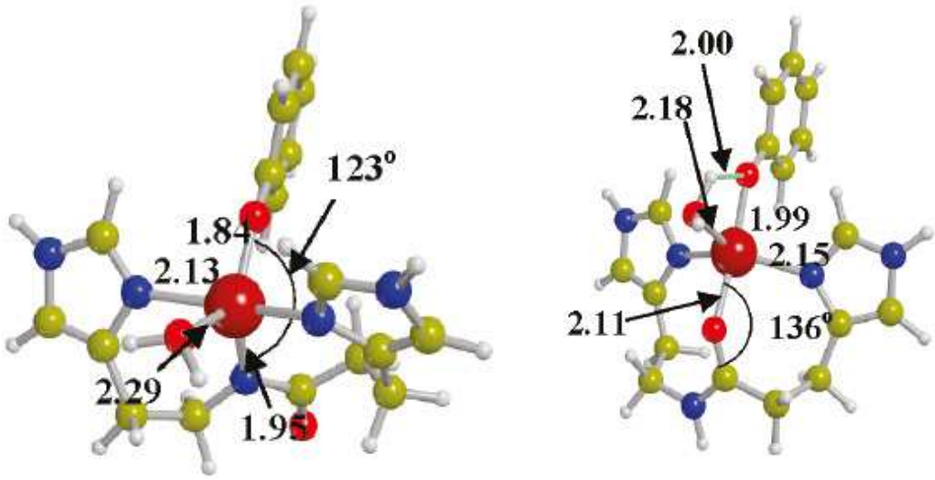


Fig. 7. The most stable Fe(II) (left) and Fe(III) (right) structural models by Ali Torres et al. (2011). Reprinted with permission from Ali Torres et al. (2011). Copyright (2011) American Chemical Society.

Finally, it should be mentioned that some Fe(III) chelating compounds have been tested as possible therapeutics for AD. Two such compounds are desferrioxamine and deferiprone (Figure 8). While they are used as iron chelators to treat iron overload conditions, their efficacy as AD therapeutics is doubtful, and they also bind other metal ions such as Cu(II) and Zn(II).

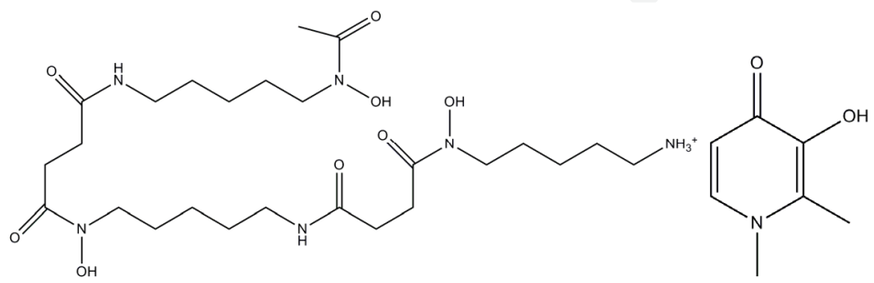


Fig. 8. Chemical structures of desferrioxamine (left) and deferiprone (right)

4. Zinc and amyloid beta

Zinc, like Copper, is known to play a major role in Alzheimer's Disease pathogenesis. However, unlike copper (and iron), zinc, normally found only in the Zn(II) state, is not redox-active. While Zn is an essential component in a number of enzymes, its role is probably mostly structural rather than reactive chemically. The brain is a rich source of Zn(II). During neuronal activity, the concentration of Zn(II) released into the synaptic cleft can be as high as 1mM (Duce & Bush, 2010). The zinc transporter ZnT3 activity is key during this process. With age, hippocampal Zn as well as ZnT3 levels have been found to decrease (Adlard, 2010). Zn(II) binding to Aβ facilitates the amyloid aggregation. AD brain plaques are highly enriched in zinc, reaching millimolar concentrations (Lovell, 1998). The metal ionophores, Clioquinol and PBT2, discussed earlier, show moderate binding of Zn(II), and Adlard et al. (2008) observed the dissolution of Zn-induced Aβ(1-42) aggregates upon treatment with these compounds. Faller and Hureau (2009) concluded that Zn(II) had a binding affinity to Aβ of 1-20 μM, which is weaker than that shown by Cu(II). However, this value is highly dependent on the assay conditions and probably on the aggregation state of Aβ.

The structure of the Zn(II) binding site in Aβ has been the subject of a number of studies. While most experiments appear to agree that the triad of histidines, His 6, His 13, and His 14 coordinate the Zn(II) ion, there is some disagreement on the identity of the fourth coordinating amino acid residue. In 2006, Zirah et al. (2006) determined the solution structure of Zn(II) bound to Aβ(1-16) in aqueous solution at pH 6.5 using ^{1}H and ^{13}C 1- and 2-dimensional NMR experiments. Here, Zn(II) displays a flattened tetrahedral geometry with the N^{δ} atoms of His 6 and His 14, and the N^{ε} atom of His 13 at the 'base' and the carboxylate O atoms of the bidentate Glu 11 at the apex. Notably, the two O atoms of Glu 11 are equidistant from the Zn at 2.1 Å. His 6 and His 13 are also 2.1 Å distant from the metal while His 14 is at 2.3 Å. It is noteworthy that the peptide used in these studies was N-acetylated at the N-terminus, thus hindering any potential involvement of Asp 1 in the Zn coordination. The geometry of the Zn(II) binding site is depicted below in Figure 9 (PDB id: 1ZE9).

When the N-terminal of Aβ is not acetylated, NMR studies point to the involvement of Asp 1 in the coordination of Zinc. For example, using high resolution NMR ^{1}H-^{13}C and ^{1}H-^{15}N heterocorrelation experiments on Aβ(1-40), Danielsson et al. (2007) propose that the three histidines and most likely the N-terminal NH2 of Asp 1 coordinate Zn(II). However, they do not rule out the possibility of the carboxylate oxygen atom of Asp 1 as a Zn(II) ligand. On the other hand, Minicozzi et al. (2008) observed that at pH 7, the EXAFS spectrum of Aβ(5-23) looked very similar to those of Aβ(1-16), Aβ(1-28), and Aβ(1-40). They determine that the best fit to the data is with 4 histidines and a oxygen atom, and hence conclude that what they observe is best explained by Zn(II) cross-linking 2 Aβ chains, each chain contributing 2 histidines to the first coordination shell. Finally, comparative ^{1}H NMR studies on human and rat Aβ(1-28) in water/sodium dodecyl sulphate (SDS) micelles were carried out at pH 7.5 by Gaggelli et al. (2008). The chemical shift variations and line broadening led them to a penta-coordinated structural model for Zn(II)/Aβ(1-28) where the Zn(II) is liganded by Asp 1 (N-terminal NH2 group), His 6, His 13, His 14, and the carboxylate oxygen atom of Glu 11. The last ligand was strongly evidenced by the down field ^{1}H chemical shifts for Glu 11 upon

the addition of Zn(II). Notably, large downfield shifts were also observed for Tyr 10, which were ascribed to conformational changes brought about by Zn(II) binding rather than direct involvement of Tyrosine as a ligand. They also determined the Zn(II) coordination shell when bound to the rat Aβ(1-28). The rat Aβ differs from the human peptide in three important mutations: Arg 5 → Gly, Tyr 10 → Phe, and His 13 → Arg. Their structural model for Zn(II) with rat Aβ(1-28) has the metal ion tetrahedrally coordinated with the ligands Asp 1 NH_2 group, His 6 and His 14 imidazoles, and Glu 11 carboxylate. Thus there is very little change in the mode of binding in going from the human to rat sequence, resulting only in the loss of His 13 as a ligand.

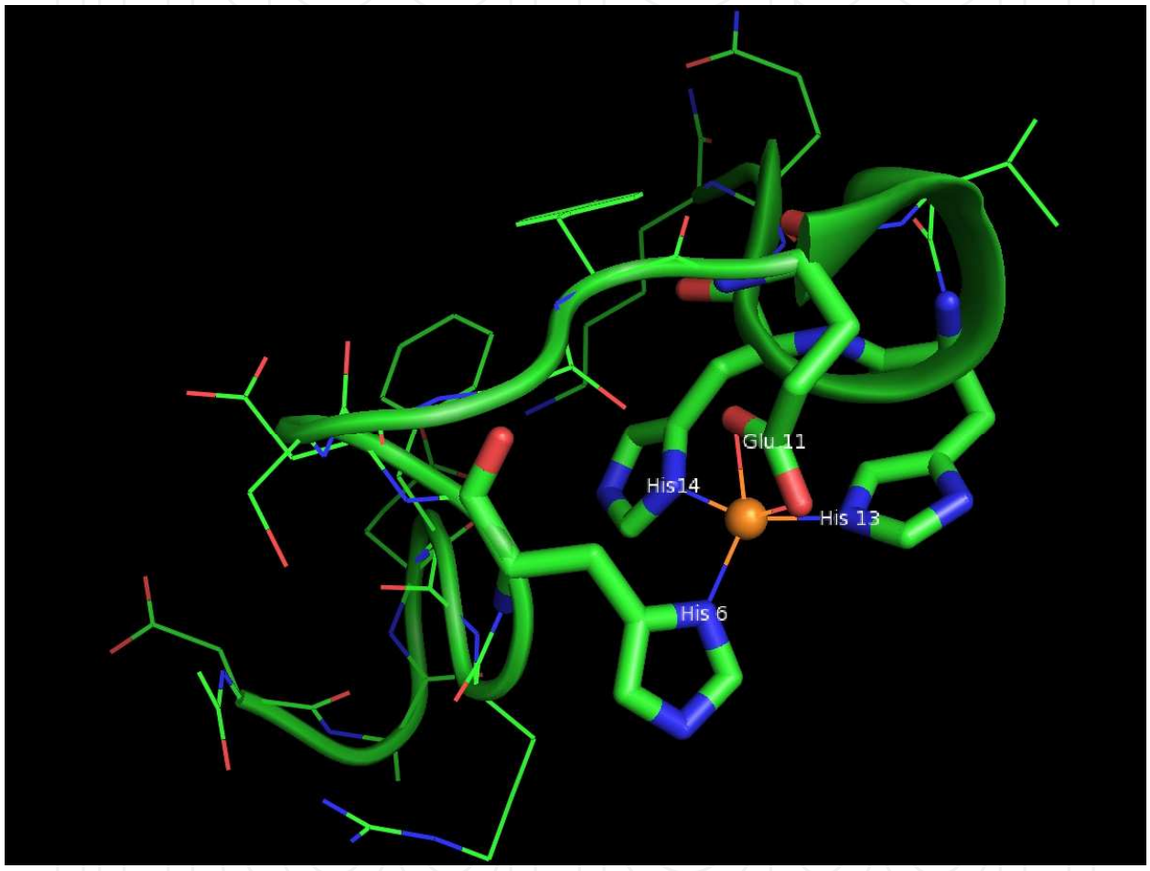


Fig. 9. Zn(II)/Aβ(1-16) NMR solution structure of Zirah et al. (2006).

The minimal Zn(II) binding fragment of Aβ has been the subject of QM/MM (hybrid quantum mechanics / molecular mechanics) simulations and ITC (isothermal calorimetry) experiments (Tsvetkov et al., 2010). The QM/MM calculations (performed combining the Car-Parinello molecular dynamics code CPMD with the MM package GROMACS) used the NMR solution structure of Zirah et al. (2006) as the initial geometry and included the metal ion and the side-chains of His6, His 13, His 14 and Glu 11 in the QM region. These calculations (run for a maximum of 8 ps) on Zn(II) complexed to Aβ(1-16) and Aβ(6-14) showed stable structures when Zn(II) was liganded by the three histidines and Glu 11. ITC on different sized Aβ fragments as well as H6R and E11A mutants of Aβ(1-16) at pH 7.3 all indicated that (6-14)

contained the minimal binding region of Aβ. They proposed Zn(II) is first coordinated by $E^{11}VH^{13}H^{14}$, followed by His 6 binding. *Ab initio* molecular dynamics was also the computational methodology of choice used by Furlan and La Penna (2009) to investigate the stability of Zn(II) binding by different Aβ(1-16) residues (this time using an implementation of CPMD in the Quantum Espresso package). Simulations *in vacuo* at 300 K of 1-2 ps length supported the structural model of coordination by the three histidines and Glu 11. On the other hand, involvement of Asp1 in the binding resulted in the expulsion of a histidine from the coordination shell. Finally, Tyr 10 liganding the metal ion in competition with Glu 11 or Asp1 appeared to be possible only when the tyrosine is first deprotonated. It should also be noted that in these theoretical models discussed above, Glu 11 displays monodentate, not bidentate binding to Zn(II). Miller et al. (2010) performed replica exchange molecular dynamics simulations with Zn(II)/Aβ models found that the zinc ions can simultaneously coordinate Aβ both intramolecularly as well as intermolecularly, and hence facilitate Aβ aggregation. Their simulations, commenced from structural models constructed using the Zirah et al. Zn(II)/Aβ(1-16) structure discussed above and the Aβ(17-42) fibril structure of Luhrs et al. (2005), ran for 20 ns. They observed that the Zn(II) coordination decreased the solvation energy of Zn(II)/Aβ oligomers, again facilitating the amyloid aggregation.

Finally, it is also interesting that the high resolution NMR studies by Danielsson et al. (2007) also appeared to suggest the presence of a second, weaker, Zn(II) binding site in the central region of Aβ, involving residues 23, 24, 26, and 28. The putative second Cu(II) binding site mentioned in section 2.1, suggested by the p3-IgNAR tetrameric crystal structure of Strelsov et al. (2011) has the potential also to be a second Zn(II) binding site. Confirmation of this as a second Zn(II) binding site awaits future experiments.

5. Platinum, Ruthenium, Rhodium, and Iridium metals and amyloid beta

While the major body of work of on the interactions of metals with Amyloid beta (Aβ) has been of those metals with biological significance and found in the brain, i.e. Cu, Zn, and Fe, more recently there has been interest in the study of the interaction of Aβ with organometallic compounds involving transition metals Platinum (Pt), Ruthenium (Ru), Rhodium (Rh), and Iridium (Ir). While there is no evidence that the interaction of these metals with Aβ is of any biological significance, and indeed that such interactions occur at all in the human brain, they do have significance as prototypes or templates for potential therapeutics in order to ablate the neurotoxicity induced by Aβ.

The first study in this area was that by Barnham et al. (2008) on the effects of binding of Aβ to Cisplatin and Cisplatin-derived compounds. The Pt compounds involved in this study were cisplatin, Pt(1,10-phenanthroline)Cl_2, Pt(4,7-diphenyl-[1,10]-phenanthroline)Cl_2 and Pt(4,7-diphenyl-[1,10]-phenanthroline disulfonate)Cl_2, (i.e. compounds 1, 2, 3, and 4 in this study), and are depicted in Figure 10. The impetus behind this work was the knowledge that the N-terminal residues, and in particular the histidines at positions 6-, 13-, and 14- were important in the toxicity induced by Aβ(Atwood et al., 2000), and that aromatic compounds appeared to bind to Aβ and, at the least, affect its fibril forming tendency. In particular, 1,10-phenanthroline compounds (in the absence of any metals) appear to have a weak affinity to the N-terminal residues Phe 4, Tyr 10, and Phe 19 (Yao et al., 2004). The three histidines, His 6, His 13, and His 14, of course, are included in this segment of Aβ.

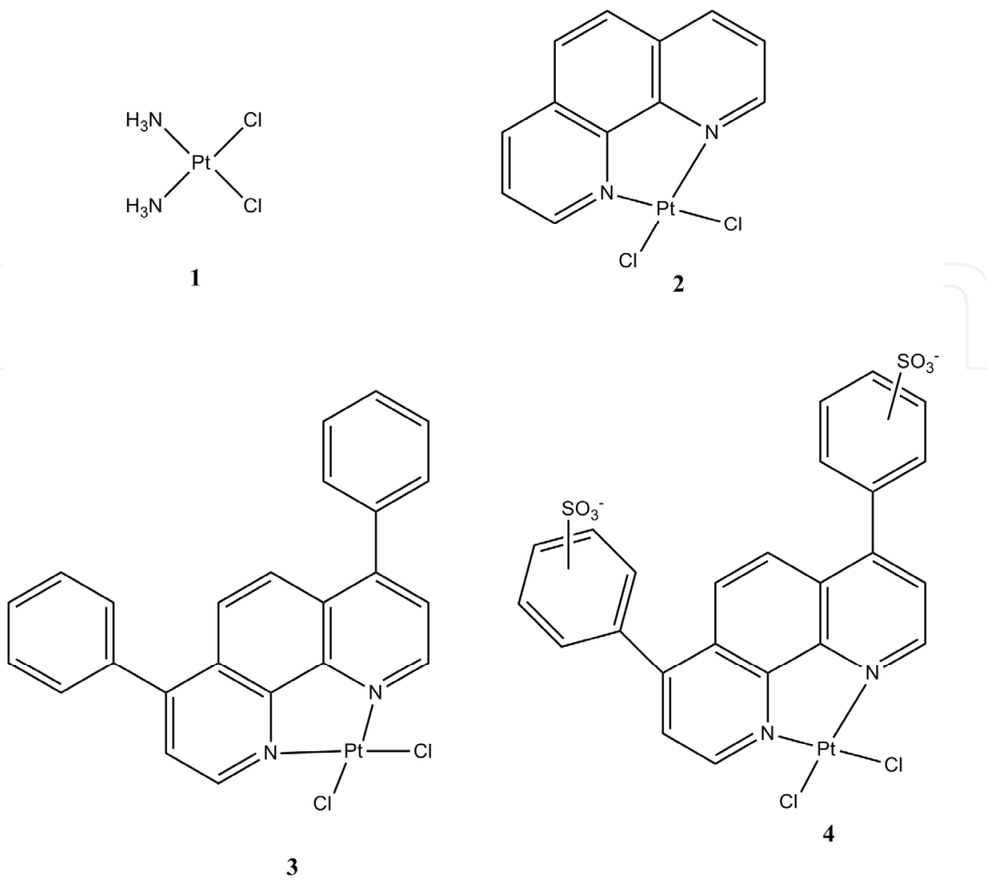


Fig. 10. Platinum compounds used for binding to Aβ by Burnham et al. (2008)

Barnham et al. found that while all four compounds bound to Aβ(1-40), only compounds 2, 3, and 4, and not 1, (i.e. cisplatin), had significant effects on the behaviour of the peptide. Compounds 2, 3, 4 inhibited the aggregation of Aβ(1-40), with EM showing the absence of fibrils. They significantly inhibited the Cu(II) induced generation of H_2O_2, with the measured IC_{50} values in the nanomolar range. This is comparable to the inhibition of ROS generation by clioquinol as discussed earlier. Furthermore, these compounds significantly inhibited the Aβ induced neurotoxicity, increasing the cell viability of primary mouse cortical neurons in cell cultures. Finally, they rescued the Aβ induced inhibition of long-term potentiation (LTP) in mouse hippocampal slices. How do these compounds have such beneficial effects on Aβ biological properties while cisplatin does not? The ^1H NMR experiments of Barnham et al. appear to show that while compounds 2-4 bind the N-terminal histidines of Aβ, cisplatin binds the C-terminal Met 35. At a first glance, this appears to explain why compounds 2-4 and not cisplatin inhibits H_2O_2 generation. (And incidentally, is further confirmation that Met 35 is not involved in the ROS generating activity of Aβ.) However, determination of all the coordinating residues of Aβ has not been done, and more extensive and definitive structural studies on the complexes of Aβ with these Pt compounds are necessary. Such studies, both experimental as well as computational, are now underway at our laboratory (CSIRO). These would, hopefully, provide complete 3-dimensional structural models of the Aβ-Pt compound complexes. Such

structures would not only explain the differences in the chemical properties of the different compound complexes, but also be templates or leads for the development of effective therapeutics for AD. Among the qualities effective neuroprotective agents should have are good blood-brain barrier crossing properties. It should be noted that cisplatin has been used for some time as a cytotoxic drug in anti-cancer therapy. However, Barnham et al. did not notice toxicity to cells of these Pt compounds at the concentrations that were used in their experiments, i.e. 5 µM and 10 µM.

Following this work, Valensin et al. (2009) published their study of the interaction of Aβ(1-28) with the Ruthenium compound *fac*-[$Ru(CO)_3Cl_2$-N^1-1,3-thiazole] (where Ru has a +2 charge). Given the fact that Ru compounds are in general far less cytotoxic than Pt compounds, and the fact that Ru compounds prefer coordination to imidazole nitrogen atoms, they strongly suggest Ru compounds such as theirs be considered as potential therapeutics for Alzheimer's Disease. The ^{1}H NMR experiments they carried out appear to indicate that the chlorines in the compound are substituted by the histidine imidazoles of the Aβ peptide. In addition to the histidines, Tyr 10 also appears to be implicated. Supporting their conclusions, rat Aβ(1-28) (which differs in the mutations R5G, Y10F, and H13R) was significantly less affected by the Ru compound. Once again, definitive structural determinations remain to be done. The circular dichroism experiment carried out by Valensin showed that the peptide underwent conformational changes upon binding to the Ru compound. However, they did not perform any peptide aggregation, ROS generation, cell viability, or LTP studies with their compound.

More recently, Man et al. (2011) have investigated the use of Group 9 transition metal Rhodium (Rh(III)) and Iridium (Ir(III)) compounds to inhibit Aβ aggregation via these metals' coordination to the N-terminal histidines. Their Rh and Ir solvato-complexes contain 2-phenyl pyridine, benzoquinoline, or phenyl quinoline ligands. Presumably, the labile solvent (H_2O) molecules are displaced by the histidine imidazoles upon binding to Aβ. Man et al. found that the Rh(III) compound with phenyl pyridine as ligand was the most effective in the inhibition of fibril formation by Aβ(1-40).

6. Conclusion

Diverse experimental techniques have been utilized over the past few years in elucidating the nature of the interactions of transition metal ions with the amyloid beta peptide. Much of it has concerned copper, in both its oxidation states, due to its redox activity and biological relevance. It is clear that the interactions are complex, and very sensitive to ambient conditions. This makes it quite challenging to discern the interactions that are occurring in the brain. As of yet there is no x-ray crystallographic structure of a metal ion complexed with Aβ. The pleotropic nature of the binding as well as the lack of definite (secondary) structure in the N-terminal region of Aβ makes obtaining such a crystal structure an arduous task. On the other hand, crystal structures of metal ions with Aβ as suitable fusion protein constructs or within stable protein scaffolds or ternary complexes with Aβ and antibody fragments are worthy goals. Also of great interest would be the structural determination of complexes of any of the organometallic compounds with Aβ. Such structures could provide templates for the design of therapeutics and diagnostics for AD. Computational studies, particularly when done in conjunction with experiments, also

have contributed considerably to our current understanding of metal interaction with Aβ. *Ab initio* MD and hybrid QM/MM-MD methodologies hold great promise in providing insight into the dynamical processes and the ROS chemistry resulting from the metal-peptide interactions. In performing these simulations carrying them out in biologically relevant time scales will be a challenge that will need to be met. It will also be of use to tie in the calculations as close as possible to the experiments, for example commencing simulations from reliable and relevant experimental structural models. Finally, we note that two fundamental questions in the study of the aetiology of AD are yet to be answered satisfactorily: what is the toxic species and what is the mechanism of toxicity.

7. References

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., (2008). Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Aβ, *Neuron*, 59, pp. 43-55.

Adlard, P.A., Parncutt, J.M., Finkelstein, D.I., & Bush, A.I. (2010). Cognitive Loss in Zinc Transporter-3 Knock-Out Mice: A Phenocopy for the Synaptic and Memory Deficits of Alzheimer's Disease? *Journal of Neuroscience*, 30, pp. 1631-1636.

Ali-Torres, J., Rodriguez-Santiago, L., Sodupe, M. & Rauk, A. (2011). Structures and Stabilities of $Fe^{2+/3+}$ Complexes Relevant to Alzheimer's Disease: An Ab Initio Study, *J. Phys. Chem. A*, on-line, dx.doi.org/10.1021/jp2026626

Atwood, C.S., Moir, R.D., Huang X., Scarpa, R.C., Bacarra, N.M.E., Romano, D.M., Hartshorn, M.E., Tanzi, R.E., & Bush, A.I. (1998). Dramatic Aggregation of Alzheimer Aβ by Cu(II) Is Induced by Conditions Representing Physiological Acidosis, *Journal of Biological Chemistry*, 273, pp. 12817-12826.

Atwood, C.S., Scarpa, R.C., Huang, X., Moir, R.D., Jones, W.D., Fairlie, P., Tanzi, R.E., & Bush, A.I. (2000). Characterization of Copper Interactions with Alzheimer Amyloid β Peptides: Identification of an Attomolar-Affinity Copper Binding Site on Amyloid β 1–42, *Journal of Neurochemistry*, 75, pp. 1219-1233.

Barnham, K.J, Haeffner, F, Ciccotosto, G.D., Curtain, C.C., Tew, D Mavros, C., Beyreuther, K., Carrington, D., Masters, C.L., Cherny, R.A., Cappai, R.,& Bush, A.I., (2004). Tyrosine gated electron transfer is key to the toxic mechanism of Alzheimer's disease β-amyloid, *FASEB Journal*, 18, pp. 1427-1429.

Barnham, K.J., Kenche, V.B., Ciccotosto, G.D., Smith, D.P., Tew, D.J., Liu, X., Perez, K., Cranston, G.A., Johanssen, T.J., Volitakis, Bush, A.I., Masters, C.L., White, A.R., Smith, J.P., Cherny, R.A., & Cappai, R. (2008). Platinum-based inhibitors of amyloid-β as therapeutic agents for Alzheimer's disease, *Proc. Nat. Acad. Sci. USA,* 105, pp. 6813-6818.

Bousejra-ElGarah, F., Bijani, C., Coppel, Y. Faller, P. & Hureau, C. (2011). Iron(II) Binding to Amyloid-β, the Alzheimer's Peptide, *Inorganic Chemistry*, 50, pp. 9024-9030.

Cappai, R., & Barnham, K.J. (2008). Delineating the Mechanism of Alzheimer's Disease Aβ Peptide Neurotoxicity, *Neurochemical Research*., 33, pp. 526-532.

Crouch, P.J., Harding, S.M., White, A.R., Camakaris, J., Bush, A.I., & Masters, C.L., (2008). Mechanisms of Aβ-mediated neurodegeneration in Alzheimer's disease, *International Journal of Biochemstry & Cell Biology,* 40, pp. 181-198.

Danielsson, J., Pierattelli, R., Banci, L.& Graslund, A. (2007). High-resolution NMR studies of the zinc-binding site of the Alzheimer's amyloid β-peptide, *FEBS Journal*, 274, pp. 46-59.

Dorlet, P., Gambarelli, S., Faller, P. & Hureau, C., (2009). Pulse EPR Spectroscopy Reveals the Coordination Sphere of Copper(II) Ions in the 1–16 Amyloid-β Peptide: A Key Role of the First Two N-Terminus Residues, *Angew. Chem. Int. Ed*. 48, pp. 9273 – 9276

Drew, S. C., Noble, C. J., Masters, C.L., Hanson,G. R.& Barnham, K. J. (2009). Pleomorphic Copper Coordination by Alzheimer's Disease Amyloid-β Peptide,. *J. Am. Chem. Soc.* , 131, pp. 1195–1207.

Drew, S.C., Masters, C.L., & Barnham, K.J. (2009), Alanine-2 Carbonyl is an Oxygen Ligand in Cu^{2+} Coordination of Alzheimer's Disease Amyloid-β Peptide - Relevance to N-Terminally Truncated Forms, *J. Am. Chem. Soc*., 131, pp. 8760–8761.

Duce, J.A. & Bush A.I., (2010). Biological metals and Alzheimer's Disease: implications for therapeutics and diagnosis, *Progress in Neurobiology*, 92, pp. 1-18.

Duce, J.A., Tsatsanis, A., Cater, M.A., James, S.A., Robb, E., Wikhe, K., Leong, S.L., Perez, K., Johanssen, T., Greenough, M.A., Cho, H.-H., Galatis, D., Moir, R.D., Masters, C.L., McLean, C., Tanzi, R.E., Cappai, R., Barnham, K.J, Ciccotosto, G.D., Rogers, J.T., & Bush, A.I., (2010), Iron export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's Disease, *Cell*, 142, pp. 857-867.

Eury, H., Bijani, C., Faller, P. & Hureau, C. (2011). Copper(II) Coordination to Amyloid β: Murine versus Human Peptide, *Angew. Chem. Int. Ed*., 50, pp. 901-905.

Faller, P. & Hureau, C. (2009). Bioinorganic chemistry of copper and zinc ions coordinated to amyloid-β peptide, *Dalton Transactions*, 2009, pp. 1080-1094.

Ferri, C., Prince, M., Brayne, C., Brodaty, H., Fratigliani, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., Menzes, P.R., Rimmer, E. & Scazufca, M., (2005). Global Prevalence of Dementia: A Delphi consensus study, *Lancet*, 366, pp. 2112-2117.

Furlan, S, Hureau, C. Faller, P., & La Penna, G. (2010). Modeling the Cu+ Binding in the 1-16 Region of the Amyloid-β Peptide Involved in Alzheimer's Disease, *Journal of Physical Chemistry B*, 114, pp. 15119-15133.

Furlan, S. & La Penna, G. (2009). Modeling of the Zn^{2+} binding in the 1–16 region of the amyloid β peptide involved in Alzheimer's disease, *Physical Chemistry Chemical Physics*, 11, pp. 6468-6481.

Fusetti, F., Schroeter, K.H., Steiner, R.A., van Noort, P.I., Pijning, T., Rozeboom, H.J., Kalk, K.H., Egmond, M.R. & Dijkstra, B.W. (2002). Crystal Structure of the Copper-Containing Quercetin 2,3-Dioxygenase from Aspergillus japonicas, *Structure*, 10, pp. 259-268.

Gaggelli,E., Janicka-Klos, A., Jankowska, E., Kozlowski, H., Migliorini, C., Molteni, E., Valensin, D., Valensin, G., & Wieczerzak, E. (2008). NMR Studies of the Zn^{2+} Interactions with Rat and Human β-Amyloid (1-28) Peptides in Water-Micelle Environment, *Journal of Physical Chemistry B*, 112, pp. 100-109.

Hensley, K., Maidt, M.L. Yu, Z. Sang, H., Markesbery, W.R.,& Floyd, R.A. (1998). Electrochemical Analysis of Protein Nitrotyrosine and Dityrosine in the Alzheimer Brain Indicates Region-Specific Accumulation, *Journal of Neuroscience*, 18, pp. 8126–8132.

Hewitt, N. & Rauk, A. (2009). Mechanism of Hydrogen Peroxide Production by Copper-Bound Amyloid Beta Peptide: A Theoretical Study, *Journal of Physical Chemistry B*, 113, pp. 1202-1209.

Huang, X., Cuajungco, M.P., Atwood, C.S., Hartshorn, M.A., Tyndall, J.D.A., Hanson, G.R., Stokes, K.C., Leopold, M., Multhaup, G., Goldstein, L.E., Scarpa, R.C., Saunders, A.J., Lim, J., Moir, R.D., Glabe, C., Bowden, E.F., Masters, C.L., Fairlie, D.P., Tanzi,R.E. & Bush, A.I., (1999b). Cu(II) Potentiation of Alzheimer Aβ neurotoxicity correlation with cell-free hydrogen peroxide production and metal reduction, *Journal of Biological Chemistry*, 274, pp. 37111-37116.

Huang, X., Atwood, C.S., Hartshorn, M.A., Multhaup, G., Goldstein, L.E., Scarpa, R.C., Cuajungco, M.P., Gray, D.N., Lim, J., Moir, R.D., Tanzi, R.E.&, Bush, A.I. (1999a). The Aβ Peptide of Alzheimer's Disease Directly Produces Hydrogen Peroxide through Metal Ion Reduction, *Biochemistry*, 38, pp. 7609-7616.

Hureau , C., Eury, H., Guillot, R., Bijani, C., Sayen, S., Solari, P.-L., Guillon, E., Faller, P.,& Dorlet, P. (2011). X-ray and Solution Structures of Cu^{II}GHK and Cu^{II}DAHK Complexes: Influence on Their Redox Properties, *Chemistry A European Journal*, 17, pp. 10151-10160.

Hureau, C., Balland, V.,Coppel, Y. Solari , P.L., Fonda, E. & Faller, P. (2009). Importance of dynamical processes in the coordination chemistry and redox conversion of copper amyloid-β complexes, *Journal of Biological Inorganic Chemstry*, 14, pp. 995–1000.

Jiang, D., Li, X., Williams, R., Patel, S., Men, L., Wang, Y. & Zhou, F. (2009). Ternary Complexes of Iron, Amyloid-β, and Nitrilotriacetic Acid: Binding Affinities, Redox Properties, and Relevance to Iron-Induced Oxidative Stress in Alzheimer's Disease, *Biochemistry,* 48, pp. 7939–7947 7939.

Jiang, D., Men, L., Wang, J., Zhang, Y., Chickenyen, S., Wang, Y. & Zhou, F. (2007). Redox Reactions of Copper Complexes Formed with Different β-Amyloid Peptides and Their Neuropathological Relevance, *Biochemistry*, 46, pp. 9270-9282.

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. (2008). Safety, effi cacy, and biomarker fi ndings of PBT2 in targeting A β as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial, *Lancet Neurology*, 7, pp. 779-786.

Le Clainche, L., Giorgi, M. & Reinaud O. (2000). Synthesis and Characterization of a Novel Calix[4]arene-Based Two-Coordinate Copper(I) Complex That Is Unusually Resistant to Dioxygen, *European Journal of Inorganic Chemistry*, 2000, pp. 1931-1933.

Liu, B., Molone, A., Meehan, S., Morris, K., Thomas, S.E., Serpell, L.C., Hider, R., Marciniak, S.J., Lomas, D.A & Crowther, D.C. (2010). Iron Promotes the Toxicity of Amyloid β Peptide by Impeding Its Ordered Aggregation, *Journal of Biological Chemistry*, 286, pp. 4248-4256.

Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L., Markesbery, W.R. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques, *Journal of the Neurological Sciences*, 158, pp. 47–52.

Luhrs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Doebeli, H., Schubert, D., & Riek, R. (2005). 3D structure of Alzheimer's amyloid-beta(1-42) fibrils, *Proc. Natl. Acad. Sci. USA*, 102, pp. 17342-17347.

Man, B. Y.-W., Chan, H.-M., Leung,C.-H., Chan, D.S.-H., Bai, L.-P., Jiang, Z.-H., Li, H.-W. & Ma, D.-L. (2011). Group 9 metal-based inhibitors of β-amyloid (1–40) fibrillation as potential therapeutic agents for Alzheimer's disease, *Chemical Science,* 2, pp. 917-921.

Masters, C.L. & Beyreuther, K., (2006). Alzheimer's centennial legacy: prospects for rational therapeutic intervention targeting the Aβ amyloid pathway, *Brain*, 129, pp. 2823-2839.

Minicozzi, V., Stellato, F., Comai, M., Dalla Serra, M., Potrich, C., Meyer-Klaucke, W., & Morante, S. (2008). Identifying the Minimal Copper- and Zinc-binding Site Sequence in Amyloid-β Peptides*, Journal of Biological Chemistry*, 283, pp. 10784-10792.

Miura, T., Suzuki, K. & Takeuchi, H. (2001). Binding of iron(III) to the single tyrosine residue of an amyloid-β peptide probed by Raman spectroscopy, *Journal of Molecular Structure*, 598, pp. 79-84.

Nadal, R.C., Rigby, S.E.J. & Viles, J. H. (2008). Amyloid β-Cu^{2+} Complexes in Both Monomeric and Fibrillar Forms Do Not Generate H_2O_2 Catalytically but Quench Hydroxyl Radicals, *Biochemistry*, 47, pp. 11653-11664.

Nakamura, M., Shishido, N., Nunomura, A., Smith, M.A., Perry, G., Hayashi, Y., Nakayama, K. &. Hayashi, T. (2007). Three Histidine Residues of Amyloid-β Peptide Control the Redox Activity of Copper and Iron, *Biochemistry*, 46, pp. 12737-12743

Raffa, D.F., Go´mez-Balderas, R., Brunelle, P. Rickard , G.A. & Rauk, A. (2005). Ab initio model studies of copper binding to peptides containing a His–His sequence: relevance to the β-amyloid peptide of Alzheimer's disease, *Journal of Biological Inorganic Chemistry*, 10, pp. 887-902.

Raffa, D.F., Rickard , G.A. & Rauk, A. (2007). Ab initio modelling of the structure and redox behaviour of copper(I) bound to a His–His model peptide: relevance to the β-amyloid peptide of Alzheimer's disease*, Journal of Biological Inorganic Chemistry,* 12, pp. 147-164.

Sarell, C.J., Syme, C.D., Rigby, S.E.J. & Viles, J.H. (2009). Copper(II) Binding to Amyloid-β Fibrils of Alzheimer's Disease Reveals a Picomolar Affinity: Stoichiometry and Coordination Geometry Are Independent of Aβ Oligomeric Form, *Biochemistry*, 48, pp. 4388- 4402.

Sayre, L.M., Zelasko, D.A., Harris, P.L.R., Perry, G., Salomon, R.G. & Smith, M.A. (1997). 4-Hydroxy Nonenal-derived Advanced Lipid Peroxidation End Products Are Increased in Alzheimer's Disease, *Journal of Neurochemistry*, 68, pp. 2092-2097.

Shearer, J. & Szalai, V.A. (2008). The Amyloid-β Peptide of Alzheimer's Disease Binds Cu^{I} in a Linear Bis-His Coordination Environment: Insight into a Possible Neuroprotective Mechanism for the Amyloid-β Peptide, *J. Am. Chem. Soc.*, 130, pp. 17826-17835.

Shivers, B.D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K. & Seeburg, P.H. (1988). Alzheimer's disease amyloidogenic glycoprotein expression pattern in rat brain suggests a role in cell contact, *EMBO Journal*, 7, pp. 1365- 1370.

Smith D.G., Ciccotosto, G.D., Tew, D.J., Perez, K., Curtain, C.C., Boas, J.F., Masters, C.L., Cappai, R., Barnham K.J. (2010). Histidine 14 modulates membrane binding and neurotoxicity of the Alxzheimer's Disease amyloid-beta peptide, *Journal of Alzheimer's Disease*, 19, pp. 1387-1400.

Smith, D.G, Cappai, R.& Barnham, K.J., (2007). The redox chemistry of the Alzheimer's disease amyloid β peptide, *Biochimica et Biophysica Acta*, 1768, pp. 1976–1990.

Smith, D.P, Smith, D.G., Curtain, C.C., Boas, J.F., Pilbrow, J.R., Ciccotosto, G.D., Lau, T.-L., Tew, D. J., Perez, K., Wade, J.D., Bush, A.I., Drew, S.C., Separovic, F., Masters, C.L., Cappai, R.,& Barnham, K.J. (2006). Copper-mediated Amyloid-_ Toxicity Is Associated with an Intermolecular Histidine Bridge, *Journal of Biological Chemistry*, 281, pp. 15145-15154.

Streltsov, V.A. & Varghese, J.N. (2008). Substrate mediated reduction of copper-amyloid-β complex in Alzheimer's disease, *Chemical Communications*, 2008, pp. 3169-3171.

Streltsov, V.A., Titmuss, S.J., Epa, V.C., Barnham, K.J., Masters, C.L. & Varghese, J.N. (2008). The Structure of the Amyloid-β Peptide High-Affinity Copper II Binding Site in Alzheimer's Disease, *Biophysical Journal*, 95, pp. 3447-3456.

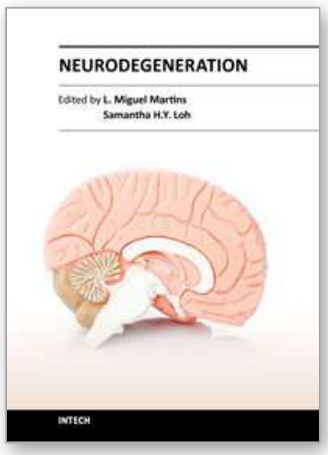
Streltsov, V.A., Varghese, J.N., Masters, C.L., & Nuttall, S.N. (2011). Crystal Structure of the Amyloid-β p3 Fragment Provides a Model for Oligomer Formation in Alzheimer's Disease, *Journal of Neuroscience*, 31, pp. 1419 –1426.

Tsvetkov, P.O., Kulikova, A.A., Golovin, A.V., Tkachev, Y.V., Archakov, A.I., Kozin, S.A., & Makarov, A.A. (2010). Minimal Zn^{2+} Binding Site of Amyloid-β, *Biophysical Journal*, 99, pp. L84–L86.

Valensin,D., Anzini, P., Gaggelli,E., Gaggelli,N., Tamasi, G., Cini, R., Gabbiani, C., Michelucci, E., Messori, L., Kozlowski, H., & Valensin, G. (2010). fac-$\{Ru(CO)3\}^{2+}$ Selectively Targets the Histidine Residues of the β-Amyloid Peptide 1-28. Implications for New Alzheimer's Disease Treatments Based on Ruthenium Complexes, *Inorganic Chemistry*, 49, pp. 4720–4722.

Yao S, Cherny RA, Bush AI, Masters CL, & Barnham K.J. (2004). Characterizing bathocuproine self-association and subsequent binding to Alzheimer's disease amyloid beta peptide by NMR,. *Journal of Peptide Science*, 10, 210–217.

Zirah, S., Kozin, S.A., Mazur, A.K., Blond, A., Cheminant, M., Se´galas-Milazzo, I., Debey, P. & Rebuffat, S., (2006). Structural Changes of Region 1–16 of the Alzheimer Disease Amyloid β-Peptide upon Zinc Binding and in Vitro Aging, *Journal of Biological Chemistry*, 281, pp. 2151–2161.



Neurodegeneration

Edited by Dr. L. Miguel Martins

ISBN 978-953-51-0502-2
Hard cover, 362 pages
Publisher InTech
Published online 11, April, 2012
Published in print edition April, 2012

Currently, the human population is on a collision course for a social and economic burden. As a consequence of changing demographics and an increase in human individuals over the age of 60, age-related neurodegenerative disorders are likely to become more prevalent. It is therefore essential to increase our understanding of such neurodegenerative disorders in order to be more pro-active in managing these diseases processes. The focus of this book is to provide a snapshot of recent advancements in the understanding of basic biological processes that modulate the onset and progression of neurodegenerative processes. This is tackled at the molecular, cellular and whole organism level. We hope that some of the recent discoveries outlined in this book will help to better define the basic biological mechanisms behind neurodegenerative processes and, in the long term, help in the development of novel therapeutic approaches.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen