A catalytic antioxidant for limiting amyloid-beta peptide aggregation and reactive oxygen species generation†

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Alzheimer’s disease (AD) is a multifaceted disease that is characterized by increased oxidative stress, metal-ion dysregulation, and the formation of intracellular neurofibrillary tangles and extracellular amyloid-β (Aβ) aggregates. In this work we report the large affinity binding of the iron(II) 2,17-bis-sulfonato-5,10,15-tris(pentafluorophenyl)corrole complex FeL1 to the Aβ peptide (Kd ~ 10⁻⁷) and the ability of the bound FeL1 to act as a catalytic antioxidant in both the presence and absence of Cu(II) ions. Significant findings are that: (a) an Aβ histidine residue binds axially to FeL1; (b) that the resulting adduct is an efficient catalase; (c) this interaction restricts the formation of high molecular weight peptide aggregates. UV-Vis and electron paramagnetic resonance (EPR) studies show that although the binding of FeL1 does not influence the Aβ–Cu(II) interaction (Kd ~ 10⁻¹⁰), bound FeL1 still acts as an antioxidant thereby significantly limiting reactive oxygen species (ROS) generation from Aβ–Cu. Overall, FeL1 is shown to bind to the Aβ peptide, and modulate peptide aggregation. In addition, FeL1 forms a ternary species with Aβ–Cu(II) and impedes ROS generation, thus showing the promise of discrete metal complexes to limit the toxicity pathways of the Aβ peptide.

Introduction

Alzheimer’s disease (AD) is the most common form of dementia, representing between 50–75% of all cases. In 2017, an estimated 50 million people worldwide suffered from dementia, and this number is projected to grow sharply due to increased life expectancy. The lack of effective treatment strategies for AD, coupled with increased incidence, has stimulated extensive research efforts in this important field. Clinical diagnosis of AD is currently based on progressive loss of memory and impairment in cognition, with final diagnosis requiring post-mortem examination of the brain to determine the severity of two neuropathological hallmarks; amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs). It is still unclear whether Aβ-plaques, NFTs, or both, are a cause or an effect of the neurodegeneration in AD. NFTs are intracellular aggregates of oxidatively-modified and hyperphosphorylated microtubule-associated protein tau, while Aβ plaques are extracellular and contain the Aβ peptide as the major constituent. The Aβ peptide is a product of the amyloid precursor protein (APP), and a series of cleavage events by α-, β-, γ-secretases, afford the Aβ peptide as predominantly Aβ₄₀ or Aβ₄₂ (a 40- or 42-residue peptide). In addition, truncation at the N-terminus results in Aβ₃₈(p)-n, Aβ₄₄, and Aβ₁₁(p)-n (where p refers to pyroglutamate) peptides that are also significant components of amyloid deposits. Aβ can be found in three general forms in the brain: membrane associated, aggregated, and soluble. Most of Aβ is membrane-associated in healthy individuals, but in individuals with AD the aggregated and soluble fractions increase considerably. Early neuronal and pathological changes show indications of oxidative damage, indicating oxidative stress is involved in AD. The cause of oxidative stress in AD has been attributed to a number of factors, including impaired cellular energy metabolism and/or Fenton-type processes involving redox-active metal-ions (Fe, Cu), and metal-containing aggregates. Metal-ions, such as Zn, Cu and Fe, are essential for healthy organisms and brain function, and are tightly regulated under normal circumstances. However, a change in metal-ion homeostasis in the brain has been associated with protein aggregation, and the generation of reactive oxygen species (ROS) in neurodegenerative diseases such as AD. Metal-ions are present in increased concentrations in Aβ plaques in comparison to normal brain tissue, with concentrations of ca. 0.4 mM for Cu, 1 mM for Zn, and 0.9 mM for Fe. Metal-
ion binding can modify the aggregation pattern of Aβ, disrupt normal metalloenzyme activity, and facilitate the production of ROS.\textsuperscript{16b,14e,14c,35} Recent studies have shown that the Aβ–Cu(II) complex exhibits detrimental catalytic ROS generation, particularly so in the presence of cholesterol and vitamin C, and is able to reduce O\(_2\) generating the superoxide anion (O\(_2\)\(^{−}\)).

As a result of the possible role of metal-ion dysthromoglobin in AD, the development of multifunctional metal binding molecules as therapeutics has been actively explored.\textsuperscript{36} We, and others, have developed metal-binding agents with additional properties such as radical scavenging, peptide binding and aggregation inhibition, and acetylcholine esterase (AChE) activity.\textsuperscript{19} In addition, a number of groups have explored the use of discrete metal complexes for the diagnosis and treatment of AD.\textsuperscript{37} In terms of therapeutics, Pt,\textsuperscript{21} Ru,\textsuperscript{22} Ir,\textsuperscript{23} Co,\textsuperscript{10b,24} Re,\textsuperscript{25} Rh,\textsuperscript{26} Mn,\textsuperscript{27} and V\textsuperscript{38} complexes have been investigated for their ability to modify the aggregation of the Aβ peptide, and certain compounds have shown promising results in disease models. For example, an orally-available Pt(n) complex (Scheme 1) was shown to cross the blood–brain barrier (BBB), reduce plaque burden, and reduce Aβ peptide levels in an APP/PS1 mouse model.\textsuperscript{29} Therefore, a metal complex that can bind to the Aβ peptide, modulate aggregation, and reduce ROS production is a promising therapeutic for AD.

Corrole ligands are known to bind to metal ions, such as Al, Cu, Fe, Ga, and Au, and the corresponding metal complexes display outstandingly high hydrolytic stability.\textsuperscript{30} The iron(n) complex (FeL1, Scheme 1) displays excellent catalase activity,\textsuperscript{31} superoxide dismutase (SOD) activity,\textsuperscript{32} and catalytic activity for the decomposition of peroxynitrite (PN, ONOO\textsuperscript{−}).\textsuperscript{33} Additionally, FeL1 binds to and protects the cholesterol-carrying lipoproteins from oxidative stress; and oral administration of FeL1 to a mouse model of atherosclerosis leads to a decrease in atherosclerotic lesions.\textsuperscript{30c,33} We were thus interested to investigate the interaction of FeL1 with the Aβ peptide, and how this would modulate peptide aggregation and ROS generation. Strong inspiration came from reports by Dey et al., who have shown that heme binds to the Aβ peptide, that one of the three histidine residues (His\(5^\alpha\)) of Aβ is ligated to the heme’s iron, and that the heme-Aβ adduct induces ROS formation.\textsuperscript{17c,34} Furthermore, a study that focused on the uptake of iron complexes by macrophages, which are a major source of ROS, revealed that heme is cytotoxic while FeL1 is cytoprotective.\textsuperscript{35} Additionally, FeL1 was reported to have low cytotoxic activity while maintaining cell cycle distribution similar to untreated cancer cells.\textsuperscript{36}

We report the interaction of FeL1 with the Aβ peptide, how it affects peptide aggregation, and the radical scavenging ability of the FeL1–Aβ adduct, in both the presence and absence of Cu(II) ions.

**Results and discussion**

**Binding of Aβ His residues to FeL1**

The Fe(n) complex of the amphipolar 2,17-bis-sulfonato-5,10,15-tris(pentafluorophenyl)corrole (FeL1) has very strong affinity to human serum albumin (HSA) and lipoproteins, which is in part due to binding of histidine (His) residues to the metal ion.\textsuperscript{30e,37} The His ligation causes a shift in the Soret band of FeL1 from 390 to 410 nm, as well as the formation of a new band at 620 nm, and the intensity of the latter band is associated with the binding of either one or two axial His residues.\textsuperscript{30e,31e,37} There are three Aβ His residues (His\(5^\alpha\), His\(13^\alpha\), and His\(14^\alpha\)) and they play an important role in metal-ion binding (Scheme 2), with dissociation constants (K\textsubscript{d}) of \(\sim 10^{-16}\) M for Cu(II) and \(\sim 10^{-5}\) M for Zn(II).\textsuperscript{10b,14e,38} In addition, Aβ His residues have been reported to bind to discrete metal complexes such as heme,\textsuperscript{36a} Ru complexes,\textsuperscript{33e,36b,39} and Co complexes.\textsuperscript{10b,24} In addition to His binding, residues Asp\(\delta^\beta\), Tyr\(\alpha\), and Glu\(\alpha^\beta\) play a role in the coordination of Aβ to Cu(II) and Zn(II).\textsuperscript{40}

Prior to investigating the interaction(s) of FeL1 with the Aβ peptide by UV-Vis spectroscopy, its binding to 1-methylimidazole (1-MeIM) was examined as to determine the spectral features and binding affinity associated with exogenous imidazole as the axial ligand. Gradual addition of up to 150 equiv. of 1-MeIM led to a shift in the near UV (Soret) band, a decrease in the band at 533 nm, and the formation of a new band at 620 nm (Fig. 1). The spectral changes matched those for histidine binding (Fig. S1†),\textsuperscript{30e} however in both cases a large excess of ligand is required (150 and 700 equiv. respectively) to observe spectral endpoints. A variable pH UV-Vis titration (Fig. S2†), at a concentration ratio of 1 : 2 FeL1 : 1-MeIM, together with subsequent data fitting using Hypspec and HySS,\textsuperscript{41,45} provides binding constants of log M(1-MeIM) = 5.81 ± 0.01 (where M = FeL1) and a much smaller log M(1-MeIM)\textsubscript{2} = 2.57 ± 0.02. Our results are in accord with the higher stability of 5-coordinate mono-axial ligated Fe(n) corroles in comparison to 6-coordinate bis-axial ligated Fe(n) corroles,\textsuperscript{42} which is opposite to that

![Scheme 1](image1.png)

**Scheme 1** Structures of Pt(n) complex, and Fe(n) corrole (FeL1) complex.

![Scheme 2](image2.png)

**Scheme 2** Representation of Component I (l\_\_l, l\_\_l, l\_\_l) and Component II, the two major pH-dependent Aβ–Cu(II) binding modes (modified from Borghesani et al., 2018).\textsuperscript{46}
reported for Fe(III) porphyrins.\textsuperscript{41} The main reason for this difference is that upon bis-axial ligation Fe(III) porphyrins gain more crystal field stabilization energy (CFSE) as they transform from high spin (HS) to low spin (LS), while Fe(III) corroles only transform from intermediate spin (IS) to LS.\textsuperscript{42,43,44}

The studies with 1-MeIm provided critical information for examining the interaction of FeL1 with the full length Aβ₁₋₁₆ and two truncated peptides: Aβ₁₋₁₆ that contains the metal binding N-terminus (His\textsuperscript{6}, His\textsuperscript{13}, His\textsuperscript{14}), and Aβ₁₇₋₄₀ with the hydrophobic portion of the peptide lacking any His. Addition of Aβ₁₇₋₄₀ to FeL1 did not induce any significant spectral changes, while even a single equivalent of either Aβ₁₋₁₆ or Aβ₁₋₁₆ led to a red shift and intensity-increase of the Soret band, accompanied by the appearance of a λ\textsubscript{max} = 620 nm band (Fig. 2). While this experiment clearly proves the importance of His–Fe binding, the comparison of Fig. 2 and 1 exposes major differences. Importantly, the binding of the protein-provided histidine must be much stronger than that of 1-MeIm as full spectra changes are achieved with 1 vs. >100 equivalents, respectively.

In the former case, the observed spectral changes occur immediately upon mixing, with no further spectral changes apparent after monitoring for 1 h, and addition of excess Aβ₁₋₁₆ (up to 16 equivalents, Fig. S3\textsuperscript{†}) did not induce further spectral changes. The last result is also highly relevant to the other spectral difference: while the 533 nm band disappears in the presence of a large excess of 1-MeIm (Fig. 1), the bands at 533 and 620 nm remain of essentially equal intensity starting from a 1 : 1 (Fig. 2) to a 1 : 16 (Fig. S3\textsuperscript{†}) ratio of FeL1 : Aβ₁₋₁₆. Taken together, the results show that FeL1 and Aβ form a 1 : 1 adduct that relies on only one of the His residues in Aβ. The other two His residues are either too far away to approach the metal center and/or are unable to bind due to steric interference. Previous reports agree with our findings in that axial ligand binding to Fe(m) corroles shows that the 5-coordinate species is stabilized in comparison to the 6-coordinate bis-axial ligated species.\textsuperscript{42,43,44}

The stability of the 1 : 1 FeL1 : Aβ₁₋₁₆ adduct was determined via a variable pH titration (Fig. 3), which together with subsequent data fitting using Hypspec and HySS,\textsuperscript{34,45} provided binding constants of log M(Aβ₁₋₁₆) = 11.90 ± 0.01, and
log M(Aβ1-16)(H) = 4.90 ± 0.02 (where M = FeL1, and (H) indicates a mono-protonated peptide species). This experiment demonstrates the much higher affinity of FeL1 for the Aβ peptide in comparison to 1-MeIm (see below). At higher pH values (≥9.5) a metal hydroxy species is evident (modelled as log M(Aβ1-16)(OH), presumably due to deprotonation of a bound H2O ligand). As indicated from the speciation diagram (Fig. 3), the interaction of FeL1 with the Aβ peptide coincides with His deprotonation (reported pKₐ values of 5.72, 6.5, and 6.95). Further analysis of the speciation diagram of FeL1 with Aβ1-16 provides the binding affinity at physiological pH. The concentration of free FeL1 present in solution at a given pH, referred to as pH [p(FeL1)) = -log([FeL1]unchelated)], is a direct estimate of metal–ligand affinity when all species in solution are considered. The calculated value for p(FeL1) is 6.6 ([FeL1] = [Aβ1-16] = 30 μM), which affords a Kₐ value of ~10⁻⁷ M. This value shows that the affinity of Aβ for FeL1 is lower than that of Cu(II), but larger than for Zn(II).

To gain more insight into the binding event, both ¹H NMR and ESI-MS studies were performed. The MS spectrum of a 1 : 1 FeL1 : Aβ1-16 adduct showed multiple m/z peaks consistent with FeL1 binding to Aβ1-16, with the most intense adduct peak corresponding to [FeL1–Aβ1-16]²⁺ (Fig. S5†). FeL1 has been reported to bind to human serum albumin (HSA), and in addition the Aβ peptide shows a specific interaction with HSA. Based on these reports we investigated the binding of FeL1 to Aβ in the presence of HSA, and under these conditions observed the [FeL1–Aβ1-16]³⁺ adduct (Fig. S6†). The ¹H NMR of Aβ1-16 was recorded in the presence of 0.10 and 0.25 equivalents of the paramagnetic FeL1. Initially, the signals from the three histidines and the tyrosines were quite sharp and well resolved (Fig. 4, bottom trace). Addition of FeL1 induced broadening of all signals attributed to the histidines (7.95, 7.05, 7.00 ppm), while those of Tyr10 (7.10 and 6.82 ppm) were not affected (Fig. 4, mid and top traces). Overall, the data are consistent with binding of an Aβ His residue to FeL1, and there is likely no preference for any of the available peptide His residues (His⁶, His¹³, His¹⁴).

FeL1 binding to Aβ in the presence of Cu(II)

UV-Vis analysis. The affinity between Cu(II) and Aβ is very large (Kₐ of ~10⁻¹⁰ M) and the inner coordination sphere of Cu(II) in the adduct is usually composed of the N-terminal amine, one carboxyl group and two histidines (Scheme 2, Component I, major species at pH = 7.4). Although the affinity of FeL1 to Aβ was found to be quite large (Kₐ ~ 10⁻⁷ M) it is still 3 orders of magnitude lower than that of Cu(II), and thus is not expected to compete for the Cu(II) binding site. Considering, however, that the Aβ–Cu(II) adduct still has one His residue not involved in Cu binding, concurrent binding of Cu(II) and FeL1 to Aβ is possible and of large potential interest. This aspect was addressed by combining FeL1 with a preformed Aβ–Cu(II) complex and also vice versa, by adding Cu(II) to a FeL1/peptide mixture. Identical results were obtained in both cases (Fig. 5 and S4†) and the corresponding UV-Vis spectra clearly revealed the earlier outlined spectral features associated with axial His binding. This shows that FeL1 binds to the peptide even in the presence of Cu(II) and also provides another independent indication that the inner coordination sphere of FeL1 has only one axial histidine ligand. The formation of the ternary adduct (1 : 1 : 1 FeL1 : Aβ1-16 : Cu adduct) was further confirmed by ESI-MS, with m/z peaks corresponding to [FeL1–Aβ–Cu(II)]⁺ and sodium adducts (Fig. S5†).

EPR characterization. In order to better understand the binding of both Cu(II) and FeL1 to the Aβ peptide, we analyzed the electron paramagnetic resonance (EPR) spectra of Aβ1-16–Cu(II), FeL1–Aβ1-16, and finally FeL1–Aβ1-16–Cu(II). The EPR data for Aβ1-16–Cu(II) are in agreement with previous report; and simulation of the EPR spectra indicate the existence of both Component I and Component II therein (Scheme 2 and Fig. S7†). The simulation parameters are detailed in Table 1, and an approximate intensity ratio of 0.6 : 0.4 for Component I : Component II at pH 7.4 is in agreement with the measured pKₐ value of 7.8 ± 0.5 (Scheme 2) via the Henderson–Hasselbalch equation.29,30

![Fig. 4](https://example.com/fig4.png)

**Fig. 4** Changes in the ¹H NMR spectra of Aβ1-16 in the presence of FeL1. Shown are spectra obtained at 210 μM Aβ1-16, in PBS buffer prepared in D₂O pH 7.4 at 25 °C (red), with addition of 0.10 (green) and 0.25 equivalents (blue) of FeL1. *His⁶, His¹³, and His¹⁴. **Tyr10.

![Fig. 5](https://example.com/fig5.png)

**Fig. 5** UV-Vis spectra of binding of FeL1 (30 μM, black) in PBS buffer (0.01 M, pH 7.4) to 1 equiv. of Aβ1-16 with (blue) or without (green) Cu(II) (0.9 equiv.).
**FeL1** in buffer displays a weak intermediate-spin Fe(III) signal at \( g_{\perp} = 3.9 \) and \( g_{\parallel} = 2.0 \),\(^{16,34,55}\) whereas **FeL1–Aβ\(_{1-16}\)** shows, in addition to this signal, also a rhombic spin system consistent with low-spin Fe(III) \( S = \frac{1}{2} \) species (Table 1 and Fig. S8†). The latter is similar to the spectrum for **FeL1** in the presence of 20 equiv. of 1-MeIm (Fig. S8†) and to reported EPR data for other 6-coordinate low-spin Fe(III) corroles (with CN- and pyridine as axial ligands).\(^{54}\) These data are consistent with contributions from both mono- and bis-axial ligated **FeL1** in the EPR experiment, likely due to the increased ligand affinity upon freezing the solutions for EPR analysis. Increased bis-axial ligation to **FeL1** is observed for both 1-MeIm and Aβ\(_{1-16}\) in solution at lower temperatures (\(10^\circ\)C) by UV-Vis (Fig. S10), and freezing a 5-coordinate (OEC)Fe(III)(py) corrole (OEC = trianion of 2,3,7,8,12,13,17,18-octaethylcorrole) in pyridine results in a similar spectral pattern with both intermediate and low spin signals.\(^{56}\) Due to the distinct temperature-dependence of signal intensity for the EPR spectra of the Fe species it is not possible to accurately determine their ratios from these experiments.\(^{55}\)

| Component | **FeL1** | Aβ–Cu(II)–FeL1 | Aβ–Cu(II)–FeL1 | Aβ–Cu(II)–FeL1 | Aβ–Cu(II)–FeL1 |
|-----------|---------|----------------|----------------|----------------|----------------|
| \(g_{\parallel}\) | 2.26    | 2.22           | 2.22           | 2.22           | 2.22           |
| \(g_{\perp}\) | 2.05    | 2.05           | 2.05           | 2.05           | 2.05           |
| \(A_{1}^{Cu}\) | 186     | 170            | 170            | 170            | 170            |
| \(\chi^{b}\) | 0.6     | 0.4            | 0.4            | 0.4            | 0.4            |

\(\alpha\) See experimental section for details. \(\beta\) Component relative abundance.

**Table 1** X-band EPR simulation parameters

Influence of **FeL1** on Aβ aggregation

Gel electrophoresis and western blotting, in combination with Transmission Electron Microscopy (TEM), were used to investigate if binding of **FeL1** to the Aβ peptide would alter the size distribution of Aβ species and the morphology of the resulting aggregates. The longer Aβ\(_{1-42}\) peptide was employed for this study, as it is most aggregation prone and neurotoxic.\(^{136,56}\) Incubation with low concentrations of **FeL1** (0.1 to 1 equiv., for 24 h) significantly affected the aggregation pattern (Fig. 7A). While the Aβ\(_{1-42}\) peptide forms mostly high molecular weight aggregates (lane 1) in the absence of **FeL1**, consistent with previous reports,\(^{19,24,57}\) **FeL1** exhibits a concentration-dependent effect on the aggregation pattern (lanes 2–5). Only low molecular weight species were observed after 24 h with one equiv. of **FeL1** (lane 5). The influence of **FeL1** on Aβ\(_{1-42}\) aggregation was further confirmed by TEM (Fig. 7B–D). The TEM image for peptide alone shows long fibrils and large aggregate size, matching previous reports.\(^{24,57}\) However, as the
concentration of FeL1 is increased, a reduction in aggregate size is observed, with only small aggregates present with 1 equiv. of FeL1 (Fig. 7D). We also investigated the effect of the free ligand L1 on Aβ1-42 peptide aggregation. Under the same experimental conditions, L1 also displays a concentration-dependent effect on aggregation (Fig. S11†), however aggregate species are observed over a broad molecular weight range. We hypothesize that L1 alters the aggregation pattern via hydrophobic interactions with the Aβ peptide,11,37,58 while the covalent interaction of FeL1 with Aβ His residues results in the preferential formation of low molecular weight species (Fig. 7).

Catalytic antioxidant activity

FeL1 has been previously reported to exhibit exceptional antioxidant activity for the disproportionation of H2O2,30 and catalytic activity for the decomposition of peroxynitrite (PN, ONOO−).32 In addition, the antioxidant activity of FeL1 is maintained, and even enhanced, when bound to albumin, lipoproteins, or imidazole since this minimizes formation of the less catalytically-active μ-oxo iron(IV) dimer.30,32 This work highlighted that the FeL1–Aβ species could act as a potent antioxidant, and possibly minimize ROS generation from Aβ–Cu([n] when both FeL1 and Cu([n] are bound to the peptide simultaneously.

Catalase activity. The catalase activity of FeL1 has been demonstrated to exceed that of any other synthetic mimic of the enzyme,10,11,17,59 and its activity increases in the presence of excess imidazole. In order to determine the influence of FeL1 binding to Aβ1-16 on its catalase activity, an “Amplex Red”/H2O2 catalase assay was performed. This assay relies on competing with the very fast color producing reaction by adding a complex that catalytically decomposes H2O2. A catalase standard curve (Fig. S12A†) was prepared and different concentrations of FeL1 in the presence and absence of Aβ1-16 (Fig. S12B†) were tested to determine their activity. Both FeL1 and the 1 : 1 FeL1–Aβ1-16 adduct displayed good catalase activity, with the latter being superior. This shows that His binding of the Aβ peptide to FeL1 results in an enhancement of catalase activity at all concentrations studied (1–5 μM).

Aβ–Cu([n] ROS production. Under biologically relevant reducing conditions, which are commonly mimicked by reducing agents such as ascorbate (Asc), Aβ–Cu([n] species are known to produce an array of ROS composed of superoxide anion radical, hydrogen peroxide, and hydroxyl radical.34,57 There are many protocols for investigating the multiple steps that lead to these damaging species (Scheme 3), of which the first one is the oxidation of Asc by Cu([n]). This process was followed by monitoring the time course for disappearance of the Asc absorption band at 265 nm (Fig. 8) in the presence of CuCl2 and FeL1 only, their binary adducts with Aβ1-16, and the ternary adduct formed by combining Aβ1-16 with Cu([n] and FeL1. Consistent with previous reports,34 the high rate of Asc consumption in the presence of Cu([n]) is diminished when bound to Aβ1-16 and consistent with expectations, both FeL1 and FeL1–Aβ did not promote Asc oxidation. The most revealing result is that the ternary FeL1–Aβ1-16–Cu([n]) complex displayed only slightly enhanced Asc oxidation in comparison to Aβ1-16–Cu([n]) only. Overall, and in accordance with the EPR results that show that FeL1 does not alter the Cu([n]) binding site, this assay suggests that the presence of FeL1 does not significantly affect the reduction of Aβ–Cu([n]) by Asc.

Cu–Aβ species can transform O2 to H2O2 through a series of steps, which can be detected via its reaction with Amplex Red, which forms the intensely colored resorufin (Scheme 3).60 Following this process by monitoring the formation of resorufin (Fig. 9A) revealed that: (a) Cu([n]) alone induces the fastest rate of formation of H2O2; (b) the binding of Cu([n]) to Aβ slows down the process, as reported previously,60 and (c) the ternary FeL1–Aβ1-16–Cu([n]) species shows a reduced rate of H2O2 formation.

Scheme 3 Reactive oxygen species generated by Aβ–Cu([n]) in the presence of ascorbate and the possible assays to detect them, modified from C. Cheignon et al. 2018.58a
A 1 : 1 adduct with Aβ via axial binding of one His residue with a moderate binding affinity ($K_d$ of $\sim 10^{-7}$ M), which is weaker in comparison to Cu binding to Aβ ($K_d$ of $\sim 10^{-10}$ M) but still stronger than Zn(II) binding ($K_d$ of $\sim 10^{-5}$ M). It is interesting to note that FeL1 has a much higher affinity for Aβ peptide His residues than 1-MeIm or free His, suggesting significant non-covalent interactions between FeL1 and the Aβ peptide. These results are in agreement with the specific binding of FeL1 to HDL2 proteins in comparison to other serum constituents, due to the amphipolar character of FeL1. Indeed, L1 was also shown to influence Aβ peptide aggregation likely due to hydrophobic interactions, albeit to a significantly lower extent. In a similar manner, non-covalent π–π stacking interactions, in addition to covalent binding, have been shown to dictate the association of Pt[n](phenanthroline) complexes with the Aβ peptide.  

We have also shown herein that FeL1 binds to the Aβ peptide concurrently with Cu(n). Our EPR data suggests no significant change in the Cu-binding site with FeL1 His coordination. This is further corroborated by the ascorbate oxidation assay, which displays only minor changes to the rate of ascorbate oxidation for Aβ–Cu(n) and the ternary species FeL1–Aβ–Cu(n). However, the bound FeL1 acts as an efficient catalase, and decomposes free form and when bound to the Aβ peptide (Fig. S12B†). In addition to catalase activity, FeL1 displays exceptional antioxidant activity for the dismutation of $\mathrm{O}_2^\cdot$ and thus the complex may also quench superoxide formed as shown in Scheme 3. Detection of this reactivity using a cytochrome c assay$^{46,17a}$ was challenging due to interference of FeL1 absorption bands.

The last and most damaging step in Scheme 3 occurs via the formation of the hydroxyl radical (·OH) from the reaction of Cu(i) with H$_2$O$_2$ (Scheme 3), which may be detected by the reaction of 3-coumarin carboxylic acid (3-CCA) with ·OH to form the fluorescent 7-hydroxy-3-coumarin-carboxylic acid (7-OH-3-CCA).$^{62}$ Consistent with previous reports,$^{12e}$ ·OH production is quite fast and significant for Aβ–Cu(n) albeit much less than for non-His-coordinated Cu(n) (Fig. 9B). The addition of FeL1, to form the ternary FeL1–Aβ1–16–Cu(n) species, resulted in a further 6-fold reduction in the amount of 7-OH-3-CCA. In principle, this may reflect either the lower availability of H$_2$O$_2$ due to the catalase-like activity or the direct quenching of ·OH by FeL1, or a combination of both. Another possible interpretation is that Cu in the ternary complex is less reactive, which is unlikely considering the minimal interaction of FeL1 with Aβ1–16–Cu(n) binding motif. In any case, the almost complete elimination of hydroxyl radical formation demonstrates that the potent antioxidant activity of FeL1 is maintained when bound to the Aβ peptide.

**Summary**

This study underlines the ability of FeL1 to target both Aβ peptide aggregation and ROS formation, two factors influencing AD progression. FeL1 and the free corrole ligand L1 influence Aβ aggregation differently; FeL1 stabilizes low molecular weight species while L1 stabilizes aggregates over a broad MW range. FeL1 forms a 1 : 1 adduct with Aβ via axial binding of one His residue with a moderate binding affinity ($K_d$ of $\sim 10^{-7}$ M), which is weaker in comparison to Cu binding to Aβ ($K_d$ of $\sim 10^{-10}$ M) but still stronger than Zn(II) binding ($K_d$ of $\sim 10^{-5}$ M). It is interesting to note that FeL1 has a much higher affinity for Aβ peptide His residues than 1-MeIm or free His, suggesting significant non-covalent interactions between FeL1 and the Aβ peptide. These results are in agreement with the specific binding of FeL1 to HDL2 proteins in comparison to other serum constituents, due to the amphipolar character of FeL1. Indeed, L1 was also shown to influence Aβ peptide aggregation likely due to hydrophobic interactions, albeit to a significantly lower extent. In a similar manner, non-covalent π–π stacking interactions, in addition to covalent binding, have been shown to dictate the association of Pt[n](phenanthroline) complexes with the Aβ peptide.  

We have also shown herein that FeL1 binds to the Aβ peptide concurrently with Cu(n). Our EPR data suggests no significant change in the Cu-binding site with FeL1 His coordination. This is further corroborated by the ascorbate oxidation assay, which displays only minor changes to the rate of ascorbate oxidation for Aβ–Cu(n) and the ternary species FeL1–Aβ–Cu(n). However, the bound FeL1 acts as an efficient catalase, and decomposes...
a significant fraction of the H$_2$O$_2$ generated by Aβ–Cu(n) (Fig. 9A). In the presence of FeL1 we also observe a decrease in the formation of ·OH (Fig. 9B), consistent with the result from the amplex red assay. Our results show that amphiplor FeL1
binds specifically to the Aβ peptide via a His residue in a 1:1 stoichiometry, and this interaction modulates the peptide aggregation pathway. In addition, the peptide-bound FeL1 maintains its exceptional antioxidant activity, limiting ROS formation from Aβ–Cu(n). Overall, our results highlight the promising multifunctional character of FeL1 to limit Aβ peptide aggregation and the formation of damaging ROS, two hallmarks of AD.

Conflicts of interest
There are no conflicts to declare.

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