Published in final edited form as:

Chembiochem. 2013 September 23; 14(14): . doi:10.1002/cbic.201300236.

Copper(II)-bis-Histidine Coordination Structure in Fibrillar Amyloid- β Peptide Fragment and Model Complexes Revealed by using Electron Spin Echo Envelope Modulation Spectroscopy

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Abstract

Truncated and mutated amyloid- β (A β) peptides are models for systematic study of the molecular origins of metal ion effects on A β aggregation rates, types of aggregate structures formed, and cytotoxicity, in homogeneous preparations. The 3-D geometry of bis-histidine imidazole coordination of Cu(II) in fibrils of the nonapeptide, acetyl-A β (13-21)H14A, is determined by using powder ¹⁴N electron spin echo envelope modulation (ESEEM) spectroscopy. The method of simulation of the anisotropic combination modulation is described, and benchmarked for a Cu(II)-bis-*cis*-imidazole complex of known structure. The revealed bis-*cis* coordination mode, and mutual orientation of the imidazole rings, for Cu(II) in Ac-A β (13-21)H14A fibrils are consistent with the proposed β -sheet structural model, and pair-wise peptide interaction with Cu(II), with alternating [–metal–vacancy–]_n pattern, along the N-terminal edge. Metal coordination does not significantly distort the intra- β -strand peptide interactions, which provides a rationale for the Cu(II)-acceleration of Ac-A β (13-21)H14A fibrillization, through stabilization of the associated state, and low-reorganization integration of β -strand peptide pair precursors.

Keywords

amyloid beta-peptides; copper; bioinorganic chemistry; EPR spectroscopy; ESEEM spectroscopy

Introduction

Alzheimer's disease (AD) afflicts over 25 million people, is among the top ten leading causes of death worldwide, and is the number one cause of dementia.^[1, 2] A morphological hallmark of AD is the accumulation of the amyloid- β protein [A β ; native peptides are $n=39-43$ residues in length, and denoted A β (1- n)] in plaques and neurofibrillary tangles in the human brain.^[3] The divalent metal ions, Zn(II) and Cu(II), have been found at elevated concentrations ($>10^{-4}$ M) in Alzheimer's plaques,^[4-8] and the association of A β with metal ions has been correlated with AD.^[4, 9, 10] Metal ions have also been shown to be correlated with amyloid aggregation in the neurodegenerative diseases, prion disease,^[11-13] and Parkinson's disease.^[14, 15] Electron spin echo envelope modulation (ESEEM)^[16-18] and hyperfine sublevel correlation (HYSCORE)^[19] spectroscopies have implicated the three

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histidine (H) residues, H6, H13, and H14 in the N-terminal domain of A β in Cu(II) binding, and elucidated the coordination structure, in soluble A β (1-16)^[20-25] and A β (1-28)^[26-28] peptides. Solid-state nuclear magnetic resonance (SS-NMR)^[8] and ESEEM^[29] spectroscopies have addressed the Cu(II) coordination structure in the fibrillar form of full-length A β (1-40). The ESEEM study identified bis-*cis*-H-imidazole coordination at two distinct sites, and led to a structural model, in which Cu(II)–H6/H13 and Cu(II)–H6/H14 sites alternate along the fibril axis, on opposite sides of the β -sheet fibril structure.^[29]

Truncated and mutated A β peptides allow systematic study of the molecular basis of the effects of metal ions on rates of A β aggregation, types of aggregate structures formed and cytotoxicity, in homogeneous preparations.^[30] Insights into metal ion control of amyloid structure are also valuable for manipulation of modified A β peptides, to form nanomaterial scaffolds, for templating ordered molecular arrays,^[31-33] or for metal deposition,^[34, 35] that create energy and charge transfer functionalities. The truncated and N-terminal-acetylated A β nonapeptide, Ac-HHQUALVFFA [Ac-A β (13-21)], contains the core hydrophobic segment, A β (17-21), that is necessary for fibrillization,^[31, 36, 37] and the metal binding dyad, H14–H15.^[38] The Ac-A β (13-21)H14A mutant nonapeptide forms homogeneous fibrils in the presence of Cu(II), and recapitulates the parallel, in-register β -sheet structure, that is found in fibrillar forms of A β (1-40)^[39-41] and A β (1-42).^[42] Cu(II)-Ac-A β (13-21)H14A also displays cytotoxicity comparable to full-length A β , whereas the apo-peptide is benign.^[30] Fibrillization of Ac-A β (13-21)H14A is accelerated by Cu(II). A structure model was proposed^[30] for the Cu(II)-Ac-A β (13-21)H14A fibril, in which Cu(II) is bis-coordinated by a histidine imidazole ligand from each of two peptides, in an arrangement that alternates along the fibril axis, as follows: (–peptide–Cu(II)–peptide–peptide–Cu(I)–peptide–)_n. In contrast to the interaction of Cu(II) with Ac-A β (13-21)H14A, interaction of Cu(II) with the peptide, A β (13-21)K16A, blocks the fibril formation characteristic of the apo-A β (13-21)K16A, and leads to formation of a dipeptide complex, which is not cytotoxic.^[30] The A β (13-21)K16A peptide displays Cu(II)-mono-H equatorial coordination, and Ac-A β (13-21)H14A shows Cu(II)-bis-H equatorial coordination.^[30] Thus, the Cu(II)-peptide coordination geometry is a critical determinant of the differences in peptide aggregation state, structure, and cytotoxicity.

We have developed an ESEEM spectroscopic approach for the determination of the mutual orientation of equatorial bis-imidazole ligands in Cu(II) complexes in disordered (powder) systems.^[29, 43] As depicted in Scheme 1, the method leads to specification of the bis-*cis* or bis-*trans* mode of coordination, and the mutual orientation of the two imidazole rings. The ¹⁴N ESEEM from the remote (non-coordinated) nitrogen of the H imidazole ligands is detected.^[44] The ESEEM experiment is performed at an external magnetic field value that corresponds to the $g_{\perp}$ position in the Cu(II) EPR lineshape, at the quasi-powder position^[45] of maximum transition intensity. This increases the detection sensitivity for the ESE at the low electron spin contents typical of A β protein samples. The analysis of the ¹⁴N ESEEM focuses on modulation from the combinations of the anisotropic, $\Delta m_{1,\beta} = \pm 2$ modulation, from the pair of coupled ¹⁴N. In the ESEEM spectrum, “combination lines” occur at sums of the fundamental ¹⁴N coupling frequencies. The ligand geometry information is extracted from the mutual orientation of the ¹⁴N dipolar superhyperfine (shf) tensors.^[29, 43] The large parameter space of the numerical simulation of the ESEEM is efficiently searched for the global minimum by the hybrid optimization algorithm in the ESEEM simulation software package, OPTESIM.^[43] OPTESIM also provides a statistical assessment (simultaneous confidence region) for each parameter.^[43] The method has been validated by application to the Cu(II)-bis-*trans*-imidazole model complex of known structure,^[43] Cu(II)-bis-histamine-bis-nitrate [Cu(II)(him)₂(NO₃)₂],^[46] and used to determine the Cu(II) coordination structure in fibrillar A β (1-40).^[29] Here, the method is benchmarked for Cu(II)-bis-*cis*-imidazole coordination, by using the complex of known structure, Cu(II)-bis-(acetate)-bis-(2-

methylimidazole) $[\text{Cu}(\text{II})(2\text{-MeIm})_2(\text{OAc})_2]$.⁵⁴ Application to $\text{Cu}(\text{II})$ coordination in $\text{A}\beta(13\text{-}21)\text{H14A}$ fibrils reveals a bis-*cis*-imidazole coordination and the mutual geometry of the imidazole rings. This quantifies features of the $\text{Cu}(\text{II})$ site structure $\text{A}\beta(13\text{-}21)\text{H14A}$ fibrils, and defines structural constraints for accurate modeling of the peptide configuration and fibril conformation. The refined model for of intra- β -sheet coordination of $\text{Cu}(\text{II})$ provides a rationale for $\text{Cu}(\text{II})$ -acceleration of fibrillization.

Results

EPR Spectroscopy of $\text{Cu}(\text{II})$ -imidazole model complexes and $\text{A}\beta$ peptides

Figure 1 shows the continuous-wave X-band powder EPR spectra of $\text{Cu}(\text{II})(\text{Dien})(2\text{-MeIm})$, $\text{Cu}(\text{II})(2\text{-MeIm})_2(\text{OAc})_2$, $\text{Cu}(\text{II})\text{-A}\beta(13\text{-}21)\text{K16A}$, and $\text{Cu}(\text{II})\text{-A}\beta(13\text{-}21)\text{H14A}$. The spectra are characteristic of a square-planar $\text{Cu}(\text{II})$ complex, and show resolved copper hyperfine splitting, which is centered at $g_{\parallel}$, from interaction of the unpaired electron spin with the $I=3/2$ copper nucleus.^[45, 47] The amplitudes of the powder EPR spectra are greatest around the $g_{\perp} \approx 2$ region. This corresponds to the position of the external magnetic field of 3030 Gauss that was used to achieve the maximum ESE amplitude in the ESEEM experiments. Each EPR spectrum in Figure 1 is consistent with a single spectral component. The EPR spectrum of $\text{Cu}(\text{II})\text{-A}\beta(13\text{-}21)\text{H14A}$ displays a general broadening of the $\text{Cu}(\text{II})$ hyperfine and $g_{\perp} \approx 2$ features, relative to the molecular $\text{Cu}(\text{II})(\text{Dien})(2\text{-MeIm})$, $\text{Cu}(\text{II})(2\text{-MeIm})_2(\text{OAc})_2$ and $\text{Cu}(\text{II})\text{-A}\beta(13\text{-}21)\text{K16A}$ complexes. This may arise from weak spin-spin interaction of $\text{Cu}(\text{II})$ in the fibrils. A comprehensive EPR simulation analysis of the $\text{Cu}(\text{II})$ model and $\text{A}\beta(13\text{-}21)$ spectra will be presented in a future publication.

ESEEM spectroscopy of mono- and bis-imidazole $\text{Cu}(\text{II})$ model complexes

Figure 2 shows the powder three-pulse ESEEM waveform and corresponding FT for the mono-imidazole complex, $\text{Cu}(\text{II})(\text{dien})(2\text{-MeIm})$. The ESEEM was collected at the maximum ESE amplitude in the EPR spectrum, and at a τ value of 310 ns. ESEEM at different τ and microwave frequency/magnetic field values has previously been reported for the $\text{Cu}(\text{II})(\text{dien})(2\text{-MeIm})$ complex.^[48] The ESEEM in Figure 2 exhibits a pattern of deep and sustained modulation in the time domain, and corresponding narrow lines in the frequency domain, that are characteristic of coupling to ^{14}N under the condition of exact cancellation, and specifically, to the remote nitrogen of imidazole.^[44, 49] The exact cancellation condition holds when the ^{14}N superhyperfine (shf) and ^{14}N nuclear Zeeman contributions approximately cancel for one electron spin manifold ($A/2 \approx \nu_{\text{N}}$, where A is the shf coupling constant and ν_{N} is the free nuclear frequency), which creates a near zero-field condition in one electron spin manifold (the $m_s = +1/2$, or α -, manifold, for isotropic shf coupling constant values, $A_{\text{iso}} > 0$). The energy level separations in this manifold are dominated by the nuclear quadrupole interaction (nqi). The ESEEM Fourier transform (FT) for $\text{Cu}(\text{II})(\text{dien})(2\text{-MeIm})$ in Figure 2B shows three narrow lines positioned at 0.55, 1.04, and 1.61 MHz, which correspond to the ν_0 , ν_- , and ν_+ nqi frequencies (expected relation, $\nu_0 + \nu_- = \nu_+$). The broad feature, centered at 4.2 MHz, corresponds to the $\Delta m_I = \pm 2$, or “double quantum” (ν_{dq}), splitting in the electron spin manifold in which the ^{14}N shf and nuclear Zeeman contributions are additive (here the $m_s = -1/2$, β -manifold). This feature is broadened by the dipolar shf coupling. The features corresponding to the $\Delta m_I = \pm 1$ splittings, which overlap the ν_0 , ν_- , and ν_+ features, are broadened by the dipolar anisotropy, and are generally not resolved in exact cancellation ESEEM. There are no features at frequencies higher than ν_{dq} , as anticipated for a mono-imidazole complex.

Figure 3 shows the powder three-pulse ESEEM waveform and corresponding FT for the bis-*cis*-imidazole complex of known structure,^[50] $\text{Cu}(\text{II})(2\text{-MeIm})_2(\text{OAc})_2$. The waveform and FT also show the characteristic exact cancellation pattern of coupling to the remote ^{14}N of

the imidazole ring.^[44, 49] The FT in Figure 3 shows the fundamental nqi ν_0 , ν_- , and β_+ lines at 0.61, 0.91, and 1.57 MHz, respectively, and ν_{dq} feature at 4.08 MHz. The three nqi and the ν_{dq} lines appear at slightly shifted frequencies (<10%), relative to those for the Cu(II)(dien)(2-MeIm) complex, which shows a subtle difference in the electronic structure around the remote ^{14}N in the two complexes, as expected from the difference in position of the alkyl substitution on the imidazole ring. The single set of narrow nqi fundamental features (line widths comparable to the corresponding features in Figure 2) indicates that the two remote ^{14}N nuclei in the complex have the same shf and nqi couplings, to within the resolution of the ESEEM experiment. In addition to the set of “fundamental” features observed for the mono-imidazole model, the Cu(II)-bisimidazole ESEEM shows “combination” lines that are positioned at sums of the fundamental frequencies. The combination lines are marked by arrows in Figure 3. These combination features arise from relatively low-probability, simultaneous nuclear spin-flip transitions among the two remote imidazole ^{14}N nuclear spin states. Prevalent features are observed at 2.20 ($\nu_0+\nu_+$), 2.49 ($\nu_-+\nu_+$), and 3.16 ($2\nu_+$) MHz. The weak feature from approximately 7.0 to 8.5 MHz represents the double quantum combination feature ($2\nu_{dq}$), which corresponds to the combination of the $\Delta m_I=\pm 2$ splittings in the non-cancellation electron spin manifold of each coupled remote ^{14}N . The feature, that is intermediate between the ν_{dq} and $2\nu_{dq}$ features, arises from combination of the $\Delta m_I=\pm 2$ and $\Delta m_I=\pm 1$ (denoted as monoquantum, “mq”) splittings in the non-cancellation electron spin manifold, and is denoted as $\nu_{mq}+\nu_{dq}$ in Figure 3.

ESEEM spectroscopy of Cu(II)-A β (13-21) mutant complexes

Figure 4 shows the powder three-pulse ESEEM waveform ($\tau=310$ ns) and the corresponding FT for the mono-imidazole complex,^[30] Cu(II)-A β (13-21)K16A. ESEEM at a different τ value (156 ns) has been reported for this complex.^[30] The ESEEM in Figure 4 is characteristic of coupling to the remote nitrogen of imidazole under the condition of exact cancellation.^[44, 49] As expected for the mono-imidazole equatorial coordination in Cu(II)-A β (13-21)K16A,^[30] only the fundamental ESEEM features are observed.

Figure 5 shows the powder three-pulse ESEEM waveform and corresponding FT for the bisimidazole complex, Cu(II)-A β (13-21)H14A. ESEEM at a different τ value (156 ns) has been reported for this complex.^[30] In addition to the fundamental features, which show unresolved ν_0 and ν_- lines, weak combination lines are observed, which correspond to the ($\nu_0/\nu_-+\nu_+$) and ($2\nu_+$) frequencies, as expected for the previously assigned^[30] equatorial bis-histidine imidazole coordination. As shown in Figure 5, the Cu(II)-A β (13-21)H14A ESEEM, collected at $\tau=310$ ns, also shows the $\nu_{mq}+\nu_{dq}$ and $2\nu_{dq}$ features. The relatively narrow, single-peak line shape of the $2\nu_{dq}$ feature, which has a maximum at approximately 7.8 MHz, is similar to the $2\nu_{dq}$ line shape for the Cu(II)(2-MeIm)₂(OAc)₂ equatorial bis-*cis*-imidazole complex, and distinct from the broader, twin-peak $2\nu_{dq}$ line shape of the Cu(II)(him)₂(NO₃)₂ equatorial bis-*trans*-imidazole complex.^[43]

Simulation of the ^{14}N ESEEM from the different Cu(II) complexes

Simulations of the ESEEM in Figures 2-5 are shown as overlaid dotted curves. The best-fit simulation parameters, and the simultaneous confidence regions at the 99% confidence level, are presented in Table 1. The principal values of the shf tensor, the nuclear quadrupole coupling constant (e^2qQ/h) and the electric field gradient (efg) asymmetry parameter (η) for Cu(II)(Dien)(2-MeIm) are comparable to values previously reported.^[48] The A_{iso} values for Cu(II)(Dien)(2-MeIm), Cu(II)(2-MeIm)₂(OAc)₂, Cu(II)-A β (13-21)H14A lie in the range of 1.7-1.9 MHz, and the principal values of the dipolar shf tensors for the complexes all approximate the $[A\ 0.2A\ -A]$ form that is common among 2- and 4-alkyl-substituted imidazole ligands to Cu(II).^[51-53] Therefore, the electronic structure of the imidazole ring

and the coupling of the unpaired spin density with the remote ^{14}N , are relatively constant across the four complexes. The narrow confidence regions for the shf and nqi parameters in Table 1 show a high relative certainty for the best-fit values, and indicate that the parameters are strong determinants of the experimental ESEEM (a wide confidence region indicates that the parameter is poorly constrained). The excellent quality of the simulations and narrow confidence regions for the Cu(II)-bis-imidazole complexes provide further support for the assumption of comparable shf and nqi parameters for the two remote ^{14}N in each complex.

Discussion

Mutual Orientation of Imidazole Ligands in the Cu(II)(2-Melm)₂(OAc)₂ Complex

Figure 6 shows the physical model of the imidazole remote ^{14}N shf principal axis system (PAS) orientations in the Cu(II)(2-Melm)₂(OAc)₂ complex, which is constructed by using the Euler angles in Table 1. A stereo plot of the physical model is shown in Figure S1 (Supporting Information). The shf PAS (black), that is rotated through the Euler angles, $[\alpha_A \beta_A \gamma_A]$, displays the simultaneous confidence intervals at the 99% confidence level. In order to assess the match of the physical model and X-ray crystallographic structure, we superposed the shf PAS onto the atomic coordinates of the X-ray structure,^[50] by using the shf PAS–molecular PAS relation derived from the single crystal ESEEM studies.^[51, 52] The calculated physical model, which is based on the X-ray crystallographic coordinates, is shown in Figure 7A, and the structure of the complex, as determined by X-ray crystallography,^[50] is shown in Figure 7B. The calculated model displays reasonable agreement with the ESEEM-derived physical model in Figure 6. In particular, the *x*-axes in each model are approximately perpendicular, which is characteristic of the bis-*cis* coordination mode. The deviations from agreement of the ESEEM-derived and calculated structures, beyond the confidence regions of the angular parameters, are attributed to differences between the crystal and glassy solution structures of the complexes. Differences of the shf PAS orientations between the crystal and frozen glassy solution structures are expected for the Cu(II)(2-Melm)₂(OAc)₂ complex, because rotation of each imidazole ring about the Cu(II)-ligand axis is possible. A difference in the imidazole rotamer states for the complexes in the crystal and frozen glassy solution is supported by the ability to rotate the two shf PAS in the physical model about the *x*-axes, which coincide with the proximal-N-Cu(II) bond direction, to achieve a very good match with the shf PAS determined for the X-ray crystallographic structure (Figure S2, Supporting Information).

Assessment of the ESEEM Method of bis-Imidazole Coordination Geometry Determination in Cu(II) Complexes

We previously reported the physical model for the bis-*trans*-imidazole complex,^[43] Cu(II)(him)₂(NO₃)₂, and found excellent agreement of this model with the X-ray crystallographic structure.^[46] The superposition of the shf PAS for Cu(II)(him)₂(NO₃)₂ onto the atomic coordinates of the X-ray structure for Cu(II)(him)₂(NO₃)₂^[46] (Figure S3, Supporting Information), by using the shf PAS–molecular PAS relation derived from the single crystal ESEEM studies,^[51, 52] further quantifies the agreement with the reported physical model.^[43] In the case of the bis-*trans*-imidazole complex, the deviations between the two models are within the angular range of the confidence intervals for the Euler angles, at the 99% confidence level. This is expected, because rotation of the imidazole rings about the Cu(II)-ligand axis is constrained by the bidentate equatorial histamine coordination in the Cu(II)(him)₂ complex.

The reasonable agreement, within the limits expected for crystal versus glassy frozen solvent conditions, of the powder ESEEM-derived physical models for the mutual orientation of the imidazole remote ^{14}N PAS with the mutual orientation calculated from the combined single

crystal ESEEM and X-ray crystallographic results, demonstrates that the powder ^{14}N ESEEM experiment and numerical simulation approach, based on analysis of the $\nu_{\text{mq}} + \nu_{\text{dq}}$ and $2\nu_{\text{dq}}$ line shapes, is capable of accurately specifying the mutual orientation, to within the confidence intervals of the angular parameters. In contrast, in the general case of a Cu(II)-bis-imidazole complex of unknown structure and unknown relation of the shf PAS to the imidazole molecular PAS, the *cis*- or *trans*- coordination mode cannot be uniquely determined. The finding of a rotation matrix with no eigenvalue of -1 does, however, introduce the constraint that the mode is not *trans*. For example, if the two shf PAS of the two imidazoles in a bis-*trans* configuration are related by a rotational matrix $\mathbf{R}$, then there must exist a vector $\mathbf{v}$, corresponding to the bonding direction of the proximal ^{14}N and Cu(II), such that, when rotated by $\mathbf{R}$, becomes $-\mathbf{v}$. The relationship, $\mathbf{R}\mathbf{v} = -\mathbf{v}$, requires $\mathbf{R}$ to have an eigenvalue of -1 , corresponding to a 180° rotation about some reference axis. However, an eigenvalue of -1 is not sufficient for the assignment of the bis-*trans* configuration. Information about the direction of the proximal ^{14}N -Cu(II) bond relative to the shf PAS is required.

In the case of a known relation of the shf PAS to the imidazole molecular PAS, the coordination mode can be uniquely determined, in addition to the mutual orientation of the imidazole molecular PAS. Therefore, with the inclusion of the shf PAS-molecular PAS correlation from the single crystal ESEEM studies,^[51, 52] the bis-*cis*- or bis-*trans*-imidazole coordination mode of the complex can be determined.

Mutual Orientation of Imidazole Ligands in the Cu(II)-Ac-A β (13-21)H14A Peptide

Figure 9 depicts the physical model of the relative orientation of the shf PAS for Cu(II)-Ac-A β (13-21)H14A, which is based on the Euler angles presented in Table 1. The *x*-axes in the model are approximately perpendicular, as shown by the confidence region of 75° to 110° . Perpendicular *x*-axes are characteristic of the bis-*cis*-imidazole coordination mode, as demonstrated for the Cu(II)(2-Melm)₂(OAc)₂ complex. Therefore, we conclude that the Cu(II) in fibrils of Ac-A β (13-21)H14A is coordinated in the equatorial plane by two histidine imidazoles in the *cis* geometry. The ability to simulate the results by using a discrete model of Cu(II)-remote ^{14}N coupling (single set of shf, nqi, and orientational simulation parameters), indicates a single coordination geometry for Cu(II) with the Ac-A β (13-21)H14A peptide in the fibril. This is consistent with the homogeneous fibril morphology on the mesoscale, which is shown by electron microscopy studies.^[30]

Cu(II)-bis-*cis*-His coordination in the context of the Ac-A β (13-21)H14A fibril

In the structural model for fibrils of Ac-A β (13-21)H14A, individual peptides, in a β -strand configuration, combine to form a parallel, in-register β -sheet structure, that extends along the inter-strand, hydrogen-bonding direction, to define the long axis of the fibril.^[30] Multiple β -sheets stack, or laminate, in a direction perpendicular to the long axis.^[30] The inter- β -strand, backbone-to-backbone spacing within each sheet is $4.7 \text{ \AA}$, and the inter-sheet separation is approximately $10 \text{ \AA}$.^[30, 54]

Figure 9 depicts a structural model for the local Cu(II) coordination site in the context of the global Ac-A β (13-21)H14A fibril structural model.^[30] The molecular modeling program, MacroModel (version 9.9; Schrödinger, LLC, New York, NY), was used to construct the model, by incorporating the following constraints: (a) intra-sheet, bis-*cis*-imidazole coordination by H13 on adjacent peptides, with hydrogen-bonded peptides separated by $4.7 \text{ \AA}$,^[30] (b) mutual orientation of the two imidazole rings from the physical model (Figure 8), (c) angle between the proximal ^{14}N -Cu(II) bonds of 93° , which corresponds to the center of the confidence region (Figure 8), (d) a distance between the proximal (directly coordinated) imidazole ^{14}N and Cu(II) of $2.0 \text{ \AA}$ [characteristic of the ^{14}N -metal distance in Cu(II)-

imidazole complexes, including the bis-*trans*- (2.03 Å) and bis-*cis* (1.97, 1.98 Å) imidazole model Cu(II) complexes.^[46, 50], (e) direct coordination by imidazole ring Nε atoms, (f) fixed coordinates for all peptide atoms, other than atoms of H13 and the acetyl capping group, and (g) adherence to local steric constraints, by using the MMFF94^[55] force field. As depicted in Figure 9, the bis-*cis* coordination mode is accommodated by the 4.7 Å intra-strand distance. The bis-*cis* mode is obligatory for Cu(II) binding, relative to the bis-*trans* mode, because *trans*-coordination significantly distorts the N-terminal region, and thus, destabilizes the intra-β-strand interactions distal to the Cu(II) site.

The bis-*cis*-imidazole coordination mode partially resolves the structure of the intra-sheet coordination of Cu(II) at the N-terminal region of the fibrils, and is consistent with the structural model proposed for Ac-Aβ(13-21)H14A fibrils.^[30] Figure 10 shows a representation of the global Ac-Aβ(13-21)H14A fibril model, that incorporates the local Cu(II) site geometry. The pair-wise interaction of the peptides with Cu(II), which creates an alternating [–metal–vacancy–]_n pattern with an approximately 9.4 Å periodicity along the N-terminal region of the β-sheet, is accommodated by the global β-sheet structure. Thus, we propose that Cu(II)-induced acceleration of Ac-Aβ(13-21)H14A fibril formation arises from the following two factors: (a) The β-sheet structure is stabilized by Cu(II) coordination, which reduces the monomer dissociation rate. (b) Cu(II) coordination aligns and stabilizes β-strand peptide pair precursors, that integrate into the fibril, with a minimum reorganization energy, thus increasing the effective peptide association rate.

Experimental Section

Synthesis of Model Complexes and Peptides and EPR Sample Preparation

All chemicals used in the syntheses were obtained from commercial sources (Sigma-Aldrich or Fisher), and used without further purification.

Cu(II)(Dien)(2-Melm)—The Cu(II)(Dien)(2-MeIm) complex was prepared as described by McCracken et al.^[48] Briefly, copper(II) diethylenetriamine acetate (2.6 mg), copper(II) acetate (5.0 mg), and 2-methylimidazole (41.0 mg) in water (5 ml) were mixed, and ethylene glycol was added to form a 1:1 ethylene glycol/water solution. The pH was adjusted to 8.0. The solution was frozen to a glass in a 4 mm outer diameter quartz EPR tube, and stored in liquid nitrogen (77 K).

Cu(II)(Him)₂(NO₃)₂—The [Cu(II)(Him)₂(NO₃)₂] complex was prepared by following the procedure described by Zhang et al.^[46] Briefly, histamine dichloride (25.95 mg) and sodium hydroxide (0.28 ml of 1 M) in water (2.72 ml), were added to ethanol (3.93 ml) containing copper(II) nitrate 3-hydrate (0.07 ml of 1 M). The mixture was then added to ethylene glycol (7 ml), placed in a 4 mm outer diameter quartz EPR tube, frozen to a glass and stored in liquid nitrogen.

Cu(II)(2-Melm)₂(OAc)₂—The [Cu(II)(2-MeIm)₂(OAc)₂] complex was prepared as described by Abuhijleh et al.^[50] Cu(II) acetate (0.5 g) and 2-methylimidazole (1.0 g) and were dissolved in chloroform (10 ml) and methanol (2.5 ml). The mixture was stirred for 30 min at 50 °C. The mixture was filtered, and ether (15 ml) was added to the filtrate. The filtrate was stirred again for 10 min, followed by the addition of ether (5 ml). The solution was filtered under reduced pressure and washed with ether and chloroform. The solid was air dried, and recrystallized from methanol/ether. The crystals were dissolved in 1:4 methanol/chloroform in a 4 mm outer diameter quartz EPR tube, then frozen to a glass and stored in liquid nitrogen.

A β (13-21) peptides—The A β (13-21)H14A peptide was synthesized, as described.^[30] The peptide was dissolved in buffer containing 25 mM HEPES and 10 mM NaCl (pH 7.2). After incubating the sample for two weeks, fibrils were produced, as verified by electron microscopy.^[30] The Ac-A β (13-21)H14/Cu(II) ratio was 2:1. The peptide A β (13-21)K16A was synthesized as described.^[30] The peptide was dissolved in buffer containing 25 mM MES (pH 5.6) and 10 mM NaCl. The A β (13-21)K16A/Cu(II) ratio was 1:1. Samples for EPR and ESEEM were mixed with an equal volume of ethylene glycol, transferred to a 4 mm outer diameter quartz EPR tube, then frozen to a glass and stored in liquid nitrogen.

EPR Spectroscopy

X-band continuous-wave EPR spectroscopy was performed by using a Bruker ELEXSYS E500 EPR spectrometer with an ER 4123SHQE X-band cavity resonator and a Bruker ER 4131VT liquid nitrogen flow temperature control system. EPR spectra of the Cu(II) model and Cu(II)-A β complexes were acquired at temperatures of 120 and 140 K, respectively.

ESEEM Spectroscopy: ¹⁴N ESEEM of Cu(II)-imidazole complexes

ESEEM sensitively detects the coupling of the unpaired electron spin ($S=1/2$) on Cu(II) with the “remote” (not directly coordinated) nitrogen nucleus (¹⁴N; nuclear spin, $I=1$) of equatorial imidazole ligands.^[44] The electron-nuclear coupling condition that is characteristic of the Cu(II)-remote ¹⁴N interaction, known as “exact cancellation”,^[44, 49] leads to the presence of modulation components in the ESEEM that correspond to the remote ¹⁴N shf and ¹⁴N nqi energy level splittings. The shf principal tensor components, [$A_x A_y A_z$], and nqi parameters (nuclear quadrupole coupling constant and electric field gradient asymmetry parameter) and the relative orientation of the shf and nqi principal axis systems (PAS), are obtained from numerical simulations of the ¹⁴N ESEEM. ¹⁴N ESEEM has previously been used to determine the shf and nqi parameters for the imidazole remote ¹⁴N in Cu(II) sites in copper proteins,^[48, 56] and in Cu(II) model complexes in frozen solution,^[48, 56, 57] in single crystals,^[51-53, 58, 59] and in a single crystal/powder comparison study.^[60]

ESEEM Spectroscopy: Experimental

ESEEM data were collected at 6 K on a home-built pulsed-EPR spectrometer by using the three-pulse ($\pi/2$ - τ - $\pi/2$ - T - $\pi/2$ - τ -echo) microwave pulse sequence with pulse-swapping.^[61] The pulse-swapping sequence reduces the effective dead time to the intrinsic spectrometer dead time, which is the time required for the ring-down of the microwave pulse energy in the resonator to the level of the ESE signals, at the particular spins/Gauss values for the Cu(II) samples used in these studies (160–200 ns). The τ value was selected from a scan of

the τ values, corresponding to the relation, $\tau = \frac{n}{\nu_{1H}}$, where $n=1, 2, 3, \dots$, ν_{1H} is the free precession frequency of the ¹H nucleus at the prevailing external magnetic field (3030 Gauss). These τ values correspond to τ -suppression^[62] of the free ¹H contribution to the ESEEM. The $2\nu_{dq}$ feature achieved the largest amplitude at τ values of 310 ns and 620 ns, but was near to, or within, the noise amplitude at the other τ values. The τ value of 310 ns was selected for analysis, because the shorter time period of the phase memory decay in the pulse-swapping sequence afforded the best signal-to-noise ratio, relative to $\tau=622$ ns. ESEEM was cosine Fourier transformed to generate ESEEM frequency spectra. The deadtime modulation was reconstructed by using the simulated ESEEM. All data processing was performed by using laboratory-written MATLAB (Mathworks, Natick, MA) code running on personal computers.

ESEEM Simulations

Numerical simulations of the ESEEM were performed by using the OPTESIM ESEEM simulation and analysis software suite.^[43] The stationary-state spin Hamiltonian for treatment of the interaction of the electron spin on Cu(II) with the remote ¹⁴N nuclear spin is formulated with a shf coupling term, a nuclear Zeeman term, and a nqi term, as follows:^[63]

$$\mathbf{H} = h \vec{S} \cdot \mathbf{A} \cdot \vec{I} - g_N \beta_N \vec{B}_0 \cdot \vec{I} - \vec{I} \cdot \mathbf{Q} \cdot \vec{I}' \quad (1)$$

where $\beta_N \vec{S}$, and $\vec{I}$ are the nuclear magneton, electron spin operator, and nuclear spin operator, respectively, g_N is the nuclear g -value, $\mathbf{A}$ is the shf coupling tensor, and $\mathbf{Q}$ is the nqi tensor.^[64] The I and I' indicate that the eigenfunctions of $\mathbf{A}$ and $\mathbf{Q}$ are, in general, different. The shf tensor has the principal components, $\mathbf{A} = [A_{xx} A_{yy} A_{zz}]$, and is composed of an isotropic part, $A_{iso} = \frac{1}{3} \sum_i A_{ii}$, and a dipolar part, $\mathbf{A} - A_{iso}$. The nqi tensor, $\mathbf{Q}$, is defined by the nuclear quadrupole coupling constant, $e^2 q Q / h$, and the electric field gradient (efg) asymmetry parameter, η .^[65] In its PAS, the traceless nqi tensor, $\mathbf{Q} = [Q_{xx} Q_{yy} Q_{zz}]$, is related to $e^2 q Q / h$ and η by the following expressions:

$$Q_{zz} = \frac{e^2 q Q}{2I(2I-1)h} \quad (1)$$

$$\eta = \frac{(Q_{xx} - Q_{yy})}{Q_{zz}} \quad (3)$$

where $|Q_{xx}|$, $|Q_{yy}|$, $|Q_{zz}|$. In OPTESIM, the orientation between the nqi tensor PAS and the shf tensor PAS is defined by the Euler angles, $[\alpha_Q \beta_Q \gamma_Q]$. In systems with more than one coupled nucleus ($N > 1$), the orientation of the shf PAS of each nucleus, relative to an arbitrary reference frame, is defined by the Euler angles, $[\alpha_A \beta_A \gamma_A]$. For the two remote N couplings in the Cu(II)-bis-imidazole complexes studied here, $[\alpha_A \beta_A \gamma_A]$ are chosen to define the mutual orientation of the two shf PAS.

A coupled electron–single ¹⁴N system is therefore parameterized by using the following eight adjustable parameters: A_{xx} , A_{yy} , A_{zz} , α_Q , β_Q , γ_Q , $e^2 q Q / h$, η . In the Cu(II)-bis-imidazole complexes, these eight parameters are assumed to be common among the two imidazole ¹⁴N. The bis-imidazole complexes have α_A , β_A , and γ_A as additional adjustable parameters. The method of calculation of the simultaneous confidence region for each adjustable parameters, at a specific confidence level (here, 99%), has been described.^[43]

The ESEEM calculation in OPTESIM incorporates the full density matrix diagonalization from the Mims treatment of ESEEM,^[63, 66] for evaluation of the transition frequencies and intensities, but uses a computationally efficient numerical procedure^[18, 67, 68] for constructing the three-pulse modulation. For the case of a powder distribution, which is well approximated by the nominally octahedral Cu(II)-bisimidazole complexes near the $g_{\perp}$ position of the EPR spectrum,^[45] a spherical average is calculated for the ESEEM. For more than one coupled nucleus ($N > 1$), the computed spherical average in OPTESIM is performed by using a physical model which includes information about the particular arrangement of coupled nuclei relative to the paramagnet. This geometry is defined by the set of Euler angles, $[\alpha_A \beta_A \gamma_A]$, for each nucleus, which together specify the mutual orientation of the different shf PAS. Therefore, the ESEEM is calculated and combined at each powder orientation. A physical model has been used previously to simulate ¹⁴N ESEEM in a metal complex.^[69] The three-pulse envelope modulation for the $N=2$ electron-nuclear couplings is

combined separately for the α - and β - electron spin manifolds by using the following product rule:^[70]

$$E_{\text{total}}(\tau, T) = \prod_i^N E_{\alpha,i}(\tau, T) + \prod_i^N E_{\beta,i}(\tau, T) \quad (4)$$

The automated optimization of the simulations used a genetic algorithm, followed by the Nelder-Mead simplex method. The optimization was performed on the experimental ESEEM wave forms, only. The genetic algorithm had a starting population of 300. The genetic algorithm converged after approximately 100 generations, and the best individual was selected as the starting point for the simplex method. Simulations were run in parallel on a collection of personal computers, which were tasked with subsets of the powder orientation sampling vectors, under the Java-RMI based distributed computational framework that is included in OPTESIM.^[43]

Relation between shf tensor PAS and molecular PAS of imidazole

Single crystal ESEEM studies of the Cu(II)-mono-imidazole complex in Cu(II)-doped crystals of L-histidine hydrochloride monohydrate^[51] and in Cu(II)-doped bis-(L-histinato)cadmium dihydrate,^[52] determined, in addition to the shf and nqi parameters, the relation of the imidazole remote ^{14}N shf and nqi tensors to the respective crystal axes.^[51, 52] This allowed the ^{14}N shf PAS and nqi PAS to be related to the molecular frame of the imidazole. The A_z component is tilted by 8° relative to the imidazole ring plan normal. The A_x axis lies in the ring plane and makes an angle of 13° with the imidazole $\text{N}_\delta\text{-N}_\epsilon$ axis. In these systems, the ^{14}N dipolar shf tensor, which represents the through-space interaction between the unpaired electron distribution and the nucleus, has a rhombic form.^[51] The orientation of the dipolar shf tensor in the molecular frame of the imidazole ring was accounted for by using a multipoint electron spin density model.^[51, 53] Density functional theory (DFT) calculations found a positive sign for the isotropic hyperfine coupling ($A_{\text{iso}} > 0$) for the remote ^{14}N in the two imidazole ligands in Cu(II)-bis-histamine in frozen solution.^[71] Collectively, the results show that the remote ^{14}N dipolar shf tensor in different Cu(II) complexes with 2- and 4-alkyl substituted imidazoles has the approximate rhombic form, $[A \ 0.2A \ -A]$,^[51, 52, 56] to within 20% of the component values, which implies a comparable unpaired electron spin density distribution over the atoms of the imidazole rings, and an associated common angular relation of the dipolar shf PAS to the imidazole molecular PAS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported, in part, by an Emory University Arts and Sciences Partnership Seed Funding Program grant. The authors acknowledge the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DEER15377 for peptide synthesis and analyses. Research reported in this publication was supported, in part, by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number R01 DK054514. The purchase of the Bruker E500 EPR spectrometer was funded by National Institutes of Health, National Center for Research Resources grant RR17767, and by Emory University.

References

1. Mebane-Sims I, A. s. Association. *Alzheimers & Dementia*. 2009; 5:234–270.

2. Selkoe DJ. *Nature Med.* 2011; 17:1060–1065. [PubMed: 21900936]
3. Selkoe DJ. *Science.* 2002; 298:789–791. [PubMed: 12399581]
4. Mantyh PW, Ghilardi JR, Rogers S, Demaster E, Allen CJ, Stimson ER, Maggio JE, *Neurochem J.* 1993; 61:1171–1174.
5. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. *J. Neurol. Sci.* 1998; 158:47–52. [PubMed: 9667777]
6. Bush AI. *Trends Neurosci.* 2003; 26:207–214. [PubMed: 12689772]
7. Miller LM, Wang Q, Telivala TP, Smith RJ, Lanzirotti A, Miklossy J. *J. Struct. Biol.* 2006; 155:30–37. [PubMed: 16325427]
8. Parthasarathy S, Long F, Miller Y, Xiao Y, McElheny D, Thurber K, Ma B, Nussinov R, Ishii Y. *J. Am. Chem. Soc.* 2011; 133:3390–3400. [PubMed: 21341665]
9. Bush AI, Pettingell WH, Multhaup G, Paradis MD, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL, Tanzi RE. *Science.* 1994; 265:1464–1467. [PubMed: 8073293]
10. Allsop D, Mayes J, Moore S, Masad A, Tabner BJ. *Biochem. Soc. Trans.* 2008; 36:1293–1298. [PubMed: 19021543]
11. Brown DR, Hafiz F, Glasssmith LL, Wong BS, Jones IM, Clive C, Haswell SJ. *Embo J.* 2000; 19:1180–1186. [PubMed: 10716918]
12. Chattopadhyay M, Walter ED, Newell DJ, Jackson PJ, Aronoff-Spencer E, Peisach J, Gerfen GJ, Bennett B, Antholine WE, Millhauser GL. *J. Am. Chem. Soc.* 2005; 127:12647–12656. [PubMed: 16144413]
13. Riihimäki ES, Martinez JM, Kloo L. *Phys. Chem. Chem. Phys.* 2008; 10:2488–2495. [PubMed: 18446248]
14. Rasia RM, Bertoncini CW, Marsh D, Hoyer W, Cherny D, Zweckstetter M, Griesinger C, Jovin TM, Fernandez CO. *Proc. Natl. Acad. Sci. USA.* 2005; 102:4294–4299. [PubMed: 15767574]
15. Binolfi A, Lamberto GR, Duran R, Quintanar L, Bertoncini CW, Souza JM, Cervenansky C, Zweckstetter M, Griesinger C, Fernandez CO. *J. Am. Chem. Soc.* 2008; 130:11801–11812. [PubMed: 18693689]
16. Kevan, L.; Bowman, MK. *Modern pulsed and continuous-wave electron spin resonance.* Wiley; New York: 1990.
17. Schweiger A. *Angew. Chem., Int. Edit.* 1991; 30:265–292.
18. Dikanov, SA.; Tsvetkov, YD. *Electron spin echo envelope modulation (ESEEM) spectroscopy.* CRC Press; Boca Raton: 1992.
19. Höfer P, Grupp A, Nebenführ G, Mehring M. *Chem. Phys. Lett.* 1986; 132:279–283.
20. Shin BK, Saxena S. *Biochemistry.* 2008; 47:9117–9123. [PubMed: 18690709]
21. Drew SC, Noble CJ, Masters CL, Hanson GR, Barnham KJ. *J. Am. Chem. Soc.* 2009; 131:1195–1207. [PubMed: 19119811]
22. Dorlet P, Gambarelli S, Faller P, Hureau C. *Angew. Chem. Int. Edit.* 2009; 48:9273–9276. S9273/9271-S9273/9214.
23. Alies B, Eury H, Bijani C, Rechinat L, Faller P, Hureau C. *Inorg. Chem.* 2011; 50:11192–11201. [PubMed: 21980910]
24. Drew SC, Barnham KJ. *Acc. Chem. Res.* 2011; 44:1146–1155. [PubMed: 21714485]
25. Shin, B.-k.; Saxena, S. *J. Phys. Chem. A.* 2011; 115:9590–9602. [PubMed: 21491887]
26. Syme CD, Nadal RC, Rigby SEJ, Viles JH. *J. Biol. Chem.* 2004; 279:18169–18177. [PubMed: 14978032]
27. Karr JW, Akintoye H, Kaupp LJ, Szalai VA. *Biochemistry.* 2005; 44:5478–5487. [PubMed: 15807541]
28. Sarell CJ, Syme CD, Rigby SEJ, Viles JH. *Biochemistry.* 2009; 48:4388–4402. [PubMed: 19338344]
29. Gunderson WA, Hernandez-Guzman J, Karr JW, Sun L, Szalai VA, Warncke K. *J. Am. Chem. Soc.* 2012; 134:18330–18337. [PubMed: 23043377]

30. Dong J, Canfield JM, Mehta AK, Shokes JE, Tian B, Childers WS, Simmons JA, Mao Z, Scott RA, Warncke K, Lynn DG. *Proc. Natl. Acad. Sci. USA.* 2007; 104:13313–13318. [PubMed: 17686982]
31. Lu K, Jacob J, Thiyagarajan P, Conticello VP, Lynn DG. *J. Am. Chem. Soc.* 2003; 125:6391–6393. [PubMed: 12785778]
32. Liang Y, Guo P, Pingali SV, Pabitt S, Thiyagarajan P, Berland KM, Lynn DG. *Chem. Commun.* 2008:6522–6524.
33. Knowles TPJ, Buehler MJ. *Nature Nanotech.* 2011; 6:469–479.
34. Scheibel T, Parthasarathy R, Sawicki G, Lin X-M, Jaeger H, Lindquist SL. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:4527–4532. [PubMed: 12672964]
35. Carny O, Shalev DE, Gazit E. *Nano Lett.* 2006; 6:1594–1597. [PubMed: 16895341]
36. Hilbich C, Kisterswoike B, Reed J, Masters CL, Beyreuther K. *J. Mol. Biol.* 1992; 228:460–473. [PubMed: 1453457]
37. Gordon DJ, Balbach JJ, Tycko R, Meredith SC. *Biophys. J.* 2004; 86:428–434. [PubMed: 14695285]
38. Dong JJ, Shokes JE, Scott RA, Lynn DG. *J. Am. Chem. Soc.* 2006; 128:3540–3542. [PubMed: 16536526]
39. Petkova AT, Yau W-M, Tycko R. *Biochemistry.* 2006; 45:498–512. [PubMed: 16401079]
40. Paravastu AK, Leapman RD, Yau W-M, Tycko R. *Proc. Natl. Acad. Sci. USA.* 2008; 105:18349–18354. [PubMed: 19015532]
41. Bertini I, Gonnelli L, Luchinat C, Mao J, Nesi A. *J. Am. Chem. Soc.* 2011; 133:16013–16022. [PubMed: 21882806]
42. Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JJ, Van Nostrand WE, Smith SO. *Nature Struct. Mol. Biol.* 2010; 17:561–567. [PubMed: 20383142]
43. Sun L, Hernandez-Guzman J, Warncke K. *J. Magn. Reson.* 2009; 200:21–28. [PubMed: 19553148]
44. Mims WB, Peisach J. *J. Chem. Phys.* 1978; 69:4921–4930.
45. Weltner, W. *Magnetic atoms and molecules.* Scientific and Academic Editions; New York: 1983.
46. Zhang CG, Duan CY, Hu Q, Yan DY. *J. Chem. Crystallogr.* 1999; 29:1153–1155.
47. Pasenkiewicz-Gierula M, Subczynski WK, Antholine WE. *J. Phys. Chem. B.* 1997; 101:5596–5606.
48. Mccracken J, Pember S, Benkovic SJ, Villafranca JJ, Miller RJ, Peisach J. *J. Am. Chem. Soc.* 1988; 110:1069–1074.
49. Flanagan HL, Singel DJ. *J. Chem. Phys.* 1987; 87:5606–5616.
50. Abuhijleh AL, Woods C, Ahmed IY. *Inorg. Chim. Acta.* 1991; 190:11–17.
51. Colaneri MJ, Peisach J. *J. Am. Chem. Soc.* 1992; 114:5335–5341.
52. Colaneri MJ, Peisach J. *J. Am. Chem. Soc.* 1995; 117:6308–6315.
53. Colaneri MJ, Potenza JA, Schugar HJ, Peisach J. *J. Am. Chem. Soc.* 1990; 112:9451–9458.
54. Burkoth TS, Benzinger TLS, Urban V, Morgan DM, Gregory DM, Thiyagarajan P, Botto RE, Meredith SC, Lynn DG. *J. Am. Chem. Soc.* 2000; 122:7883–7889.
55. Halgren TA. *J. Comput. Chem.* 1999; 20:720–729.
56. Jiang F, Mccracken J, Peisach J. *J. Am. Chem. Soc.* 1990; 112:9035–9044.
57. Ames WM, Larsen SC. *Phys. Chem. Chem. Phys.* 2009; 11:8266–8274. [PubMed: 19756283]
58. Colaneri MJ, Peisach J. *J. Magn. Reson. Ser. A.* 1993; 102:360–363.
59. Colaneri MJ, Vitali J, Peisach J. *Biochemistry.* 2000; 39:584–591. [PubMed: 10642183]
60. Shane JJ, Vanderheijden PAAW, Reijerse EJ, Deboer E. *Appl. Magn. Reson.* 1994; 6:427–454.
61. Fauth J-M, Schweiger A, Braunschweiler L, Forrer J, Ernst RR. *J. Magn. Reson.* 1986; 66:74–85.
62. Peisach J, Mims WB, Davis JL. *J. Biol. Chem.* 1979; 254:2379–2389.
63. Mims WB. *Phys. Rev. B.* 1972; 5:2409–2419.
64. Wertz, JE.; Bolton, JR. *Electron spin resonance; elementary theory and practical applications.* McGraw-Hill; New York: 1972.
65. Lucken, EAC. *Nuclear quadrupole coupling constants.* Academic P.; London, New York: 1969.

66. Mims WB. Phys. Rev. B. 1972; 6:3543–3545.
67. Schweiger, A.; Jeschke, G. Principles of pulse electron paramagnetic resonance. Oxford University Press; Oxford, UK ; New York: 2001.
68. Stoll S, Schweiger A. J. Magn. Reson. 2003; 163:248–256. [PubMed: 12914840]
69. Reijerse EJ, Tyryshkin AM, Dikanov SA. J. Magn. Reson. 1998; 131:295–309. [PubMed: 9571105]
70. Dikanov SA, Shubin AA, Parmon VN. J. Magn. Reson. 1981; 42:474–487.
71. Baute D, Arieli D, Neese F, Zimmermann H, Weckhuysen BM, Goldfarb D. J. Am. Chem. Soc. 2004; 126:11733–11745. [PubMed: 15366921]

Figure 1.

X-band continuous-wave EPR spectra of Cu(II)-imidazole model and A β (13-21) complexes. A) [Cu(II)(Dien)(2-MeIm)]. B) [Cu(II)(2-MeIm)₂(OAc)₂]. C) [Cu(II)-A β (13-21)K16A]. D) [Cu(II)-A β (13-21)H14A]. The vertical dashed line shows the position of the magnetic field that corresponds to the ESEEM collection, scaled for the different microwave frequencies used in the CW-EPR and ESEEM experiments. *Conditions:* microwave frequency, 9.3411 GHz; microwave power, 2 mW; microwave attenuation, 20 dB; magnetic field modulation 1.5 G; modulation frequency, 100 kHz; field sweep rate, 1.5 G s⁻¹; time constant, 2.56 ms; temperature, 120 K for model complexes and 165 K for A β (13-21) peptides; average of 12 sweeps, minus average of 12 baseline spectra.

Figure 2.

Experimental powder three-pulse ESEEM and Fourier transform for [Cu(II)(Dien)(2-MeIm)], and overlaid simulation (dashed curve). In the ESEEM panel, the vertical scale bar corresponds to 25% of the constant echo amplitude at $\tau+T=12\ \mu\text{s}$. *Experimental conditions:* sample temperature, 6 K; microwave frequency, 8.772 GHz; magnetic field strength, 3030 Gauss; initial τ value -190 ns; τ increment, 20 ns; $\pi/2$ pulse width, 20 ns; pulse repetition rate, 120 Hz; 64 pulses per repetition; average of 2 accumulations.

Figure 3.

Experimental powder three-pulse ESEEM and Fourier transform for $[\text{Cu(II)}(2\text{-MeIm})_2(\text{OAc})_2]$, and the overlaid simulation (dashed curve). In the ESEEM panel, the vertical scale bar corresponds to 25% of the constant echo amplitude at $\tau+T=12\ \mu\text{s}$. In the Fourier transform panel, arrows indicate combination lines that correspond to the labeled fundamental lines. Simulation parameters are presented in Table 1. *Experimental conditions:* sample temperature, 6 K; microwave frequency, 8.772 GHz; magnetic field strength, 3030 Gauss; τ value, 310 ns; initial $\tau+T$ value, $-190\ \text{ns}$; τ increment, 20 ns; $\pi/2$ pulse width, 20 ns; pulse repetition rate, 120 Hz; 64 pulses per repetition; one accumulation.

Figure 4.

Experimental powder three-pulse ESEEM and Fourier transform for [Cu(II)-A β (13-21)K16A], and overlaid simulation (dashed curve). In the ESEEM panel, the vertical scale bar corresponds to 25% of the constant echo amplitude at $\tau + T = 12 \mu\text{s}$. *Experimental conditions:* sample temperature 6 K; microwave frequency 8.831 GHz, magnetic field strength, 3050 Gauss; initial τ value -190 ns; τ increment, 20 ns; $\pi/2$ pulse width, 20 ns; pulse repetition rate, 120 Hz; 64 pulses per repetition; average of 2 accumulations.

Figure 5.

Experimental powder three-pulse ESEEM and Fourier transform for Cu(II)-Ac-A β (13-21)H14A, and the overlaid simulation (dashed curve). In the ESEEM panel, the vertical scale bar corresponds to 25% of the constant echo amplitude at $\tau + T = 12 \mu\text{s}$. In the Fourier transform panel, arrows indicate combination lines that correspond to the labelled fundamental lines. Simulation parameters are presented in Table 1. *Experimental conditions:* sample temperature, 6 K; microwave frequency, 8.831 GHz; magnetic field strength, 3050 Gauss; τ value, 310 ns; initial $\tau + T$ value, -190 ns ; τ increment, 20 ns; $\pi/2$ pulse width, 20 ns; pulse repetition rate, 120 Hz; 64 pulses per repetition; 48 accumulation.

Figure 6. Physical model of the mutual orientation of the two remote ^{14}N shf PAS in $[\text{Cu}(\text{II})(2\text{-MeIm})_2(\text{OAc})_2]$, derived from the powder ^{14}N ESEEM. The hatched surface represents the simultaneous confidence interval at the 99% level of confidence. *Axis color code: x (blue), y (green), z (red).*

Figure 7. Calculated physical model and molecular structure of the $\text{Cu(II)(2-MeIm)}_2(\text{OAc})_2$ complex. A) Calculated mutual orientation of the imidazole remote ^{14}N shf PAS in the $\text{Cu(II)(2-MeIm)}_2(\text{OAc})_2$ complex, based on the X-ray crystallographic structure,^[50] and the relation of the shf PAS to the imidazole molecular PAS.^[51, 52] Axis color code: x (blue), y (green), z (red). B) Molecular model, based on the X-ray crystallographic structure.^[50]

Figure 8.

Physical model of the mutual orientation of the two remote ^{14}N shf PAS in [Cu(II)-Ac-A β (13-21)H14A], derived from the powder ^{14}N ESEEM. The hatched surface represents the simultaneous confidence interval at the 99% level of confidence. *Axis color code: x (blue), y (green), z (red)*

Figure 9.

Molecular structural model for the local Cu(II) coordination site in the context of the global Ac-A β (13-21)H14A fibril structural model. The local interaction of Cu(II) with two β -strands from the β -sheet is shown. A) View along β -strand axis. Fibril axis is along horizontal. B) View along fibril axis. The view is rotated by 90°, relative to panel A, as shown. C) View of side of β -sheet. Fibril axis is along horizontal. The view is rotated by 90°, relative to panel A, as shown. *Atom color code:* Orange, Cu(II); red, oxygen; blue, nitrogen; grey, carbon; white, hydrogen.

Figure 10.

Representation of the molecular structure model for the Cu(II)-Ac-A β (13-21)H14A amyloid fiber. The Cu(II) and H13 positions correspond to the local site geometry, displayed in Fig 9A-C. Only peptide backbone and H sidechains are shown, for clarity. Distances correspond to the hydrogen-bonded, intra-sheet peptide spacing (4.7 Å), intra-sheet spacing of Cu(II) sites (9.4 Å), and β -sheet lamination distance (10 Å). *Atom color code:* Orange, Cu(II); red, oxygen; blue, nitrogen; grey, carbon; white, hydrogen.

Scheme 1.

Depiction of the equatorial bis-*cis* and bis-*trans*-imidazole coordination of Cu(II) and rotational degrees of freedom of the imidazole group. A) Cu(II)-bis-*trans*-imidazole. B) Cu(II)-*cis*-imidazole.

Table 1

ESEEM simulation parameters for the Cu(II)-imidazole model and Ap(13-21) complexes. The best-fit values and the simultaneous confidence region at the 99% confidence level are shown for each adjustable parameter. For each complex, the best-fit values for each simulation parameter are shown in bold font (left column), and the upper and lower limits of the simultaneous confidence regions for the parameters are given (right column).

Sample	Cu(II)(Dien)(2-MeIm)		Cu(II)(2-MeIm) ₂ (OAc) ₂		Cu(II)-Aβ(13-21)K16A		Cu(II)-Ac-Aβ(13-21)H14A	
A _x (MHz)	2.41	2.44 2.38	2.43	2.44 2.41	2.12	2.17 2.08	2.25	2.29 2.20
A _y (MHz)	1.94	1.98 1.88	1.77	1.85 1.70	1.80	1.88 1.73	1.82	1.86 1.77
A _z (MHz)	1.42	1.47 1.38	1.40	1.42 1.38	0.90	0.95 0.86	1.25	1.33 1.16
<i>e</i> ² <i>qQ</i> / <i>h</i> (MHz)	1.71	1.72 1.70	1.64	1.64 1.63	1.51	1.52 1.50	1.52	1.53 1.51
<i>η</i>	0.64	0.65 0.63	0.72	0.72 0.71	0.71	0.74 0.70	0.82	0.83 0.81
<i>a</i> _Q (°)	64	75 54	20	24 15	228	246 217	360	357 355
<i>β</i> _Q (°)	22	27 16	18	24 15	9	10 7	67	70 65
<i>γ</i> _Q (°)	20	32 9	359	3 353	291	306 285	291	297 288
<i>a</i> _Q (°)	–	–	25	30 14	–	–	1.0	6 351
<i>β</i> _Q (°)	–	–	132	143 112	–	–	113	122 105
<i>γ</i> _Q (°)	–	–	312	320 302	–	–	299	305 292