

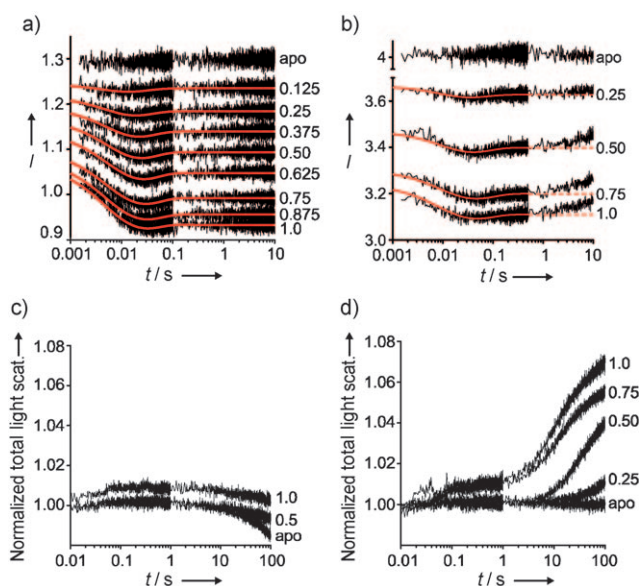
# Rapid Formation of a Preoligomeric Peptide–Metal–Peptide Complex Following Copper(II) Binding to Amyloid $\beta$ Peptides\*\*

Jeppe T. Pedersen, Kaare Teilum, Niels H. H. Heegaard, Jesper Østergaard, Hans-Werner Adolph, and Lars Hemmingsen\*

Copper(II)-induced extracellular aggregation of amyloid  $\beta$  peptides ( $A\beta$ ) is implicated in the pathogenesis of Alzheimer's disease (AD).<sup>[1]</sup> The exact role of the  $Cu^{II}$ – $A\beta$  interaction is not fully understood but it has been demonstrated that  $Cu$ – $A\beta$  oligomeric complexes may catalyze the formation of neurotoxic reactive oxygen species.<sup>[2]</sup>  $Cu^{II}$  also induces the rapid formation of insoluble  $A\beta$  oligomers that dissolve if  $Cu^{II}$  is removed.<sup>[3]</sup> Low-order oligomers both with and without  $Cu^{II}$  are key to the neurodegeneration associated with AD.<sup>[4]</sup> In the brain,  $A\beta$  are primarily found as 40-residue ( $A\beta_{1-40}$ ) and 42-residue ( $A\beta_{1-42}$ ) peptides. It is known that  $Cu^{II}$  binds to the N-terminal part of  $A\beta$  and the 16-residue fragment  $A\beta_{1-16}$  is well established as a nonfibrillating model for the  $Cu$ – $A\beta$  complex.  $A\beta_{1-16}$  coordinates  $Cu^{II}$  with the side chains of the amino acid residues D1, H6, H13, and H14 and presumably the N-terminal amino group;<sup>[5]</sup> two different pH-dependent coordination modes exist.<sup>[6]</sup> The importance of the elucidation of the mechanism of  $Cu^{II}$  binding to  $A\beta$  and its specific role in the aggregation process is emphasized by the fact that the  $Cu^{II}$  concentration is elevated in the amyloid plaques in brains from AD patients,<sup>[1a]</sup> and that  $Cu^{II}$  concentration transiently can reach micromolar concentrations in the extracellular space.<sup>[7]</sup> However, detailed knowledge of the dynamics of the initial events in  $Cu^{II}$  binding to  $A\beta$  and its relation to the formation of higher-order oligomers and aggregates is lacking. This information could be essential for the development of therapeutic strategies that could convert a toxic oligomerization pathway into a nontoxic pathway.

Herein we address the kinetic mechanism of  $Cu^{II}$  binding to both  $A\beta_{1-16}$  and  $A\beta_{1-40}$ , and copper(II)-induced  $A\beta_{1-40}$  oligomerization by a combination of stopped-flow spectroscopy (fluorescence spectroscopy and light scattering), NMR relaxation, and dynamic simulations. Global fitting and dynamic simulations resulted in a unifying model for the initial steps of  $Cu^{II}$  binding to  $A\beta$ , which includes a transient peptide–metal–peptide ( $A\beta$ – $Cu$ – $A\beta$ ) complex that does not participate in the subsequent aggregation.

Binding of  $Cu^{II}$  to  $A\beta_{1-16}$  induces a large change in the environment of Y10 in  $A\beta_{1-16}$  that allows characterization of the binding kinetics by stopped-flow fluorescence spectroscopy.  $A\beta_{1-16}$  (40  $\mu$ M) was mixed with solutions of  $Cu^{II}$  with varying concentrations in HEPES buffer (0–40  $\mu$ M), thus resulting in biphasic time traces (Figure 1 a). The fast phase (< 30 ms) that exhibits a large decrease in signal intensity shows observed rates from approximately 35 to  $1.7 \times 10^2$  s<sup>−1</sup>



**Figure 1.** Tyrosine fluorescence of a)  $A\beta_{1-16}$ , b)  $A\beta_{1-40}$ , and normalized light scattering of c)  $A\beta_{1-16}$  and d)  $A\beta_{1-40}$  after rapid mixing with  $Cu^{II}$  in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) + 100 mM NaCl buffer solution, pH 7.4 at 37 °C. The peptide concentration was kept constant at 40  $\mu$ M and the  $Cu^{II}$  concentration was increased stepwise to a molar  $Cu^{II}/A\beta$  ratio of 1 as indicated. Fluorescence data were globally fit (red traces) to a two-step model using KinTek Explorer. Broken red traces (b) show extrapolated fit for  $A\beta_{1-40}$  beyond 500 ms to visualize the second phase of the fluorescence increase. Four equivalents of glycine were present in the  $Cu^{II}$  solution (the light-scattering intensity does not directly reflect the aggregation number, see the Supporting Information).

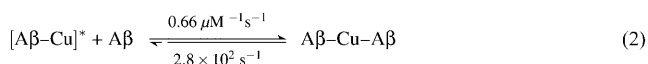
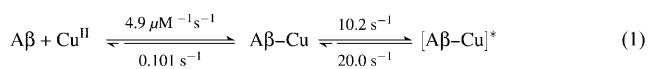
[\*] Prof. H.-W. Adolph, Prof. L. Hemmingsen  
Department of Basic Sciences and Environment  
Faculty of Life Sciences, University of Copenhagen  
Thorvaldsensvej 40, 1871 Frederiksberg (Denmark)  
Fax: (+45) 3533-2398  
E-mail: lthe@life.ku.dk

J. T. Pedersen, Prof. J. Østergaard  
Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen (Denmark)  
Prof. K. Teilum  
Structural Biology and NMR Laboratory, Department of Biology  
University of Copenhagen (Denmark)  
MD DSc N. H. H. Heegaard  
Department of Clinical Biochemistry and Immunology  
Statens Serum Institut, Copenhagen (Denmark)

[\*\*] This work was supported by Statens Serum Institut and the Drug Research Academy at the Faculty of Pharmaceutical Sciences, The Villum Kann Rasmussen Foundation, and The Danish Research Council for Independent Research | Natural Sciences.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ange.201006335>.

depending on the  $\text{Cu}^{\text{II}}$  concentration. The slow phase (ca. 30–100 ms) exhibits a small increase in signal intensity and has an observed rate of approximately  $30 \text{ s}^{-1}$ , which is independent of  $\text{Cu}^{\text{II}}$ . As two well-separated reaction phases characterize the binding kinetics of  $\text{Cu}^{\text{II}}$  to  $\text{A}\beta_{1-16}$ , a mechanism must include at least three states. This prerequisite is consistent with the result that at least two conformations of the  $\text{Cu}^{\text{II}}\text{-A}\beta$  are in a dynamic equilibrium.<sup>[6f]</sup> We used the numerical program KinTek Explorer<sup>[8]</sup> for stopped-flow data analysis to globally fit all reaction traces to a two-step mechanism [Scheme 1, Equation (1)]. The first step in this model is the

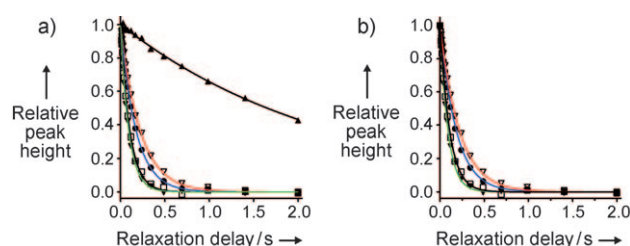


**Scheme 1.** Proposed two-step (1) and three-step (1 + 2) mechanism for initial binding of  $\text{Cu}^{\text{II}}$  to  $\text{A}\beta_{1-16}$ . Rate constants are determined from stopped-flow data, NMR relaxation decay, and simulation.  $[\text{Cu-A}\beta]^*$  indicates an alternative conformation of the complex. See also the Supporting Information.

bimolecular binding of  $\text{Cu}^{\text{II}}$  to free  $\text{A}\beta_{1-16}$  in consistency with the observed increase of the rate with an increase in  $\text{Cu}^{\text{II}}$  concentration; the second step is an intramolecular rearrangement of the initial  $\text{Cu-A}\beta$  complex to an alternative conformation  $[\text{Cu-A}\beta]^*$  that accounts for approximately 33% of the  $\text{Cu-A}\beta$  complexes at equilibrium (see the Supporting Information). The fluorescence of  $[\text{Cu-A}\beta]^*$  is less quenched than that of  $\text{Cu-A}\beta$ , and may correspond to the coordination mode favored by high pH values, which has been found to account for approximately 20–25% of the  $\text{Cu-A}\beta$  complexes at pH 7.4.<sup>[6e]</sup>

The model satisfactorily describes the stopped-flow kinetic data up to one equivalent of  $\text{Cu}^{\text{II}}$  (Figure 1a and Table S1 in the Supporting Information). Supporting the model, we find that the affinity ( $K_d = (14 \pm 1) \text{ nM}$ ) of  $\text{A}\beta_{1-16}$  for  $\text{Cu}^{\text{II}}$  derived from the kinetic parameters agrees with values reported by others<sup>[9]</sup> (see the Supporting Information). At superstoichiometric concentrations of  $\text{Cu}^{\text{II}}$ ,  $\text{A}\beta_{1-16}$  is known to bind a second  $\text{Cu}^{\text{II}}$  ion albeit 100 times weaker than the first  $\text{Cu}^{\text{II}}$  ion;<sup>[10]</sup> this event is not detected in our experiments.

To complement the stopped-flow kinetics, the  $\text{Cu}^{\text{II}}$  binding kinetics were measured by  $^1\text{H}$  NMR spectroscopy (Figure S3) taking advantage of the paramagnetic relaxation enhancement (PRE) induced by  $\text{Cu}^{\text{II}}$ . The PRE of the longitudinal relaxation of  $^1\text{H}$  spins in  $\text{A}\beta_{1-16}$ ,  $R_{1,\text{PRE}}$ , was quantified by measuring  $R_1$  on samples of  $\text{A}\beta_{1-16}$  in the absence and presence of  $\text{Cu}^{\text{II}}$  ions at  $\text{A}\beta_{1-16}$  concentrations of 150 and 300  $\mu\text{M}$  (Figure 2a). The observed  $R_{1,\text{PRE}}$  for  $\text{H}^{\delta 2}$  and  $\text{H}^{\epsilon 1}$  in H6, H13, and H14 is simply the apparent rate constant,  $k_{\text{app}}$ , by which metal-free  $\text{A}\beta_{1-16}$  coordinates the  $\text{Cu}^{\text{II}}$  ion (see the Supporting Information). Owing to the strong PRE, Cu-loaded  $\text{A}\beta$  is invisible in the spectra. Thus the time courses shown in Figure 2a reflect the rate by which  $\text{A}\beta$  molecules that are Cu-free at time zero experience the transient binding



**Figure 2.** Longitudinal relaxation decays of  $\text{H6 H}^{\delta 2}$  in  $\text{A}\beta_{1-16}$  at varying concentrations of peptide and  $\text{Cu}^{\text{II}}$  a) measured by  $^1\text{H}$  NMR and b) simulated. (▲) 300  $\mu\text{M}$   $\text{A}\beta_{1-16}$ , (▽) 300  $\mu\text{M}$   $\text{A}\beta_{1-16}$  + 15  $\mu\text{M}$   $\text{Cu}^{\text{II}}$ , (●) 150  $\mu\text{M}$   $\text{A}\beta_{1-16}$  + 15  $\mu\text{M}$   $\text{Cu}^{\text{II}}$ , (□) 300  $\mu\text{M}$  peptide + 30  $\mu\text{M}$   $\text{Cu}^{\text{II}}$ , and (▼) 150  $\mu\text{M}$   $\text{A}\beta_{1-16}$  + 30  $\mu\text{M}$   $\text{Cu}^{\text{II}}$ . All samples were measured in 10%  $\text{D}_2\text{O}$ , pH 7.4 at 37°C. Solid lines are a) fits of monoexponential decays to the experimental data points or b) simulated traces with  $k_3 = 0.66 \mu\text{M}^{-1} \text{ s}^{-1}$  and  $k_{-3} = 276 \text{ s}^{-1}$ , see Scheme 1, Equation (2).

of  $\text{Cu}^{\text{II}}$ . In all the NMR experiments the  $\text{Cu}^{\text{II}}/\text{A}\beta$  molar ratio is less than or equal to 0.1. Nonetheless, the decay of the NMR signal reflects that all  $\text{A}\beta$  molecules in the sample have experienced  $\text{Cu}^{\text{II}}$  coordination to H6. Thus, either the  $\text{Cu}^{\text{II}}$  is rapidly bound and dissociated or the  $\text{Cu-A}\beta$  and the  $[\text{Cu-A}\beta]^*$  complexes bind to another peptide in a metal-ion-bridged species.

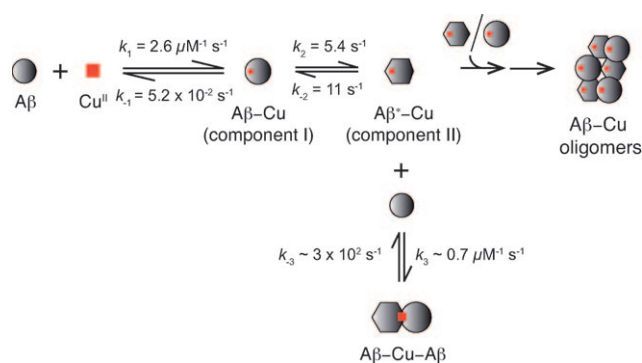
Monoexponential  $R_1$  decays were observed for the peak from  $\text{H6 H}^{\delta 2}$  (Figure 2a) and the two peaks from  $\text{H13} + \text{H14 H}^{\delta 2}$  and  $\text{H13} + \text{H14 H}^{\epsilon 1}$ . The  $R_{1,\text{PRE}}$  determined from the three peaks gave average apparent rate constants,  $k_{\text{app}}$ , of  $(10.7 \pm 0.5) \text{ s}^{-1}$ ,  $(9.6 \pm 0.5) \text{ s}^{-1}$ ,  $(5.9 \pm 0.8) \text{ s}^{-1}$ , and  $(4.7 \pm 0.7) \text{ s}^{-1}$  for total concentrations  $c_{\text{A}\beta_{1-16}}/c_{\text{Cu}}$  of 150  $\mu\text{M}/30 \mu\text{M}$ , 300  $\mu\text{M}/30 \mu\text{M}$ , 150  $\mu\text{M}/15 \mu\text{M}$ , and 300  $\mu\text{M}/15 \mu\text{M}$ , respectively. Thus, the observed rate depends on the total concentration of both  $\text{A}\beta_{1-16}$  and  $\text{Cu}^{\text{II}}$ . This behavior is inconsistent with the two-step mechanism [Equation (1) in Scheme 1] that was initially found to be sufficient to describe the stopped-flow data. In that model, the rates observed in the NMR experiments would depend only on the concentration of free  $\text{Cu}^{\text{II}}$ , thus the expected  $K_d$  in the low or subnanomolar range<sup>[9]</sup> is determined by the ratio  $c_{\text{Cu}}/(c_{\text{A}\beta_{1-16}} - c_{\text{Cu}})$ . Hence the overall rate-limiting step in Equation (1) would be the dissociation of  $\text{Cu}^{\text{II}}$  from  $\text{Cu-A}\beta$  ( $k_{-1}$ ). Direct exchange of  $\text{Cu}^{\text{II}}$  between the  $\text{Cu-A}\beta$  complex and the pool of free  $\text{Cu}^{\text{II}}$  would also infer that the off-rate for the complex is in the order of 50–90  $\text{s}^{-1}$ . However,  $k_{-1}$  derived from the stopped-flow data is two to three orders of magnitude too slow to explain the NMR experiments (see the Supporting Information). Instead we propose that the exchange of  $\text{Cu}^{\text{II}}$  proceeds by a metal-ion-bridged ternary complex ( $\text{A}\beta\text{-Cu-A}\beta$ ) as seen for other proteins<sup>[11]</sup> and also suggested for  $\text{A}\beta$ .<sup>[12]</sup> This requires an extension of the binding model with Equation (2) in Scheme 1. In this model, we assumed for simplicity that  $[\text{Cu-A}\beta]^*$  is the precursor for the ternary complex. However, from our current data we cannot determine if  $[\text{Cu-A}\beta]^*$ ,  $\text{Cu-A}\beta$ , or both are able to interact with free  $\text{A}\beta$  molecules. Rate constants for the proposed minimum three-step model were obtained by combining numerical fitting of the stopped-flow data with NMR relaxation data and simulations (see the Supporting Information). The  $\text{A}\beta\text{-Cu-A}\beta$  complex could not be detected in the

stopped-flow experiments where the peptide concentration is 3.8–7.5 times lower than in the NMR experiments (see Supporting Information). The combined analysis of stopped-flow and NMR data thus demonstrates that the first higher-order species of A $\beta$ <sub>1–16</sub> to form in the presence of Cu<sup>II</sup> is a transient dimer that coordinates one equivalent of Cu<sup>II</sup>.

To determine if our model also accounts for the initial events in binding of Cu<sup>II</sup> to A $\beta$ <sub>1–40</sub>, a series of stopped-flow experiments were performed with A $\beta$ <sub>1–40</sub>. Two subsecond phases similar to those observed in A $\beta$ <sub>1–16</sub> were detected upon Cu<sup>II</sup> binding (Figure 1 b). The kinetics of the two initial phases could be described by the same model used for the binding to A $\beta$ <sub>1–16</sub> and with almost identical rate constants and affinities (see the Supporting Information). Thus the initial binding mechanism of Cu<sup>II</sup> to A $\beta$ <sub>1–40</sub> is most likely very similar to that in A $\beta$ <sub>1–16</sub>. Our model also explains previous findings of line broadening induced by substoichiometric amounts of Cu<sup>II</sup> in NMR spectra of A $\beta$ <sub>1–40</sub>.<sup>[13]</sup> Such line broadening infers that Cu<sup>II</sup> exchanges faster between different A $\beta$ <sub>1–40</sub> molecules than explained by a kinetic model including only Equation (1) in Scheme 1.

On timescales longer than 1 s, A $\beta$ <sub>1–16</sub> and A $\beta$ <sub>1–40</sub> behave differently. For A $\beta$ <sub>1–40</sub>, a third phase in the fluorescence signal is observed, which is absent for A $\beta$ <sub>1–16</sub> (Figure 1 a and b). This late phase parallels what is observed by stopped-flow Rayleigh scattering of the A $\beta$ –Cu<sup>II</sup> binding process. In this experiment, where the formation of higher-order species is probed,<sup>[14]</sup> the light scattering of A $\beta$ <sub>1–16</sub> did not change significantly upon mixing with Cu<sup>II</sup> (Figure 1 c), thus supporting that the dominant species is the monomer under the experimental conditions employed. In contrast, a pronounced increase in light scattering with a rate that is dependent on the Cu<sup>II</sup> concentration is observed for timescales longer than 1 s for A $\beta$ <sub>1–40</sub> (Figure 1 d). The observation that the rate of oligomerization increases with the concentration of Cu<sup>II</sup> and thus the concentration of Cu–A $\beta$  and [Cu–A $\beta$ ]\* suggests that one or both of these A $\beta$  species are direct precursors of the observed oligomers as previously proposed.<sup>[15]</sup> The light-scattering signal for apo-A $\beta$ <sub>1–40</sub> remains constant throughout the experiment (Figure 1 d), hence indicating that spontaneous oligomerization does not take place on the seconds-to-minutes time scale. This behavior is consistent with previous observations.<sup>[16]</sup> However, we cannot conclude any further on the initial steps of the oligomerization pathway in the absence of Cu<sup>II</sup>.

Our current minimal model of the initial steps in Cu<sup>II</sup> binding to A $\beta$ <sub>1–40</sub> is summarized in Figure 3. It should be emphasized that although the identified A $\beta$ –Cu–A $\beta$  complex is the multimeric A $\beta$  species that kinetically is most directly accessible from the monomeric state, it is unlikely to be a direct precursor for higher-order oligomers or aggregates. As increasing Cu<sup>II</sup> concentrations above 0.5 equivalents inevitably lead to lowered concentrations of the A $\beta$ –Cu–A $\beta$  complex, a reduced rate of oligomerization of A $\beta$  would be expected if this complex was a precursor—in contrast to what is observed. The model provides an explanation for the observation that low concentrations of Cu<sup>II</sup> destabilize A $\beta$ <sub>1–40</sub> oligomers and inhibit fibrillation.<sup>[17]</sup> As the A $\beta$ –Cu–A $\beta$  complex apparently does not oligomerize or aggregate, this



**Figure 3.** Schematic minimal mechanism for initial binding of Cu<sup>II</sup> to A $\beta$  followed by the copper(II)-induced A $\beta$  oligomerization based on this study.  $k_1$ ,  $k_{-1}$ ,  $k_2$ , and  $k_{-2}$  are determined from fitting the A $\beta$ <sub>1–40</sub> stopped-flow data while  $k_3$  and  $k_{-3}$  are derived from A $\beta$ <sub>1–16</sub> NMR relaxation and simulation. Components I and II refer to the complexes formed at low and high pH values, respectively.<sup>[6a,b]</sup>

may suggest selective stabilization of this complex as a strategy to avoid A $\beta$  oligomerization and aggregation.

Received: October 8, 2010

Revised: December 28, 2010

Published online: February 17, 2011

**Keywords:** Alzheimer's disease · amyloid  $\beta$  peptides · copper · kinetics · peptides

- [1] a) M. A. Lovell, J. D. Robertson, W. J. Teesdale, J. L. Campbell, W. R. Markesbery, *J. Neurol. Sci.* **1998**, *158*, 47–52; b) L. M. Miller, Q. Wang, T. P. Telivala, R. J. Smith, A. Lanzirrotti, J. Miklossy, *J. Struct. Biol.* **2006**, *155*, 30–37.
- [2] a) G. F. da Silva, L. J. Ming, *Angew. Chem.* **2007**, *119*, 3401–3405; *Angew. Chem. Int. Ed.* **2007**, *46*, 3337–3341; b) X. Huang, M. P. Cuajungco, C. S. Atwood, M. A. Hartshorn, J. D. Tyndall, G. R. Hanson, K. C. Stokes, M. Leopold, G. Multhaup, L. E. Goldstein, R. C. Scarpa, A. J. Saunders, J. Lim, R. D. Moir, C. Glabe, E. F. Bowden, C. L. Masters, D. P. Fairlie, R. E. Tanzi, A. I. Bush, *J. Biol. Chem.* **1999**, *274*, 37111–37116; c) X. D. Huang, C. S. Atwood, M. A. Hartshorn, G. Multhaup, L. E. Goldstein, R. C. Scarpa, M. P. Cuajungco, D. N. Gray, J. Lim, R. D. Moir, R. E. Tanzi, A. I. Bush, *Biochemistry* **1999**, *38*, 7609–7616.
- [3] A. Olofsson, M. Lindhagen-Persson, M. Vestling, A. E. Sauer-Eriksson, A. Ohman, *FEBS J.* **2009**, *276*, 4051–4060.
- [4] a) D. P. Smith, G. D. Ciccotosto, D. J. Tew, M. T. Foderio-Tavoletti, T. Johanssen, C. L. Masters, K. J. Barnham, R. Cappai, *Biochemistry* **2007**, *46*, 2881–2891; b) D. P. Smith, D. G. Smith, C. C. Curtain, J. F. Boas, J. R. Pilbrow, G. D. Ciccotosto, T. L. Lau, D. J. Tew, K. Perez, J. D. Wade, A. I. Bush, S. C. Drew, F. Separovic, C. L. Masters, R. Cappai, K. J. Barnham, *J. Biol. Chem.* **2006**, *281*, 15145–15154; c) Y. Yoshiike, K. Tanemura, O. Murayama, T. Akagi, M. Murayama, S. Sato, X. Y. Sun, N. Tanaka, A. Takashima, *J. Biol. Chem.* **2001**, *276*, 32293–32299; d) G. M. Shankar, S. Li, T. H. Mehta, A. Garcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, M. A. Farrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. L. Sabatini, D. J. Selkoe, *Nat. Med.* **2008**, *14*, 837–842.
- [5] a) C. D. Syme, R. C. Nadal, S. E. J. Rigby, J. H. Viles, *J. Biol. Chem.* **2004**, *279*, 18169–18177; b) C. J. Sarell, C. D. Syme, S. E. Rigby, J. H. Viles, *Biochemistry* **2009**, *48*, 4388–4402.

- [6] a) P. Dorlet, S. Gambarelli, P. Faller, C. Hureau, *Angew. Chem.* **2009**, *121*, 9437–9440; *Angew. Chem. Int. Ed.* **2009**, *48*, 9273–9276; b) S. C. Drew, C. J. Noble, C. L. Masters, G. R. Hanson, K. J. Barnham, *J. Am. Chem. Soc.* **2009**, *131*, 1195–1207; c) S. C. Drew, C. L. Masters, K. J. Barnham, *J. Am. Chem. Soc.* **2009**, *131*, 8760–8761; d) C. Hureau, Y. Coppel, P. Dorlet, P. L. Solari, S. Sayen, E. Guillon, L. Sabater, P. Faller, *Angew. Chem.* **2009**, *121*, 9686–9689; *Angew. Chem. Int. Ed.* **2009**, *48*, 9522–9525; e) J. W. Karr, V. A. Szalai, *J. Am. Chem. Soc.* **2007**, *129*, 3796–3797; f) T. Kowalik-Jankowska, M. Ruta, K. Wisniewska, L. Lankiewicz, *J. Inorg. Biochem.* **2003**, *95*, 270–282.
- [7] a) J. Kardos, I. Kovács, F. Hajós, M. Kálmán, M. Simonyi, *Neurosci. Lett.* **1989**, *103*, 139–144; b) M. L. Schlieff, A. M. Craig, J. D. Gitlin, *J. Neurosci.* **2005**, *25*, 239–246.
- [8] K. A. Johnson, Z. B. Simpson, T. Blom, *Anal. Biochem.* **2009**, *387*, 20–29.
- [9] P. Faller, C. Hureau, *Dalton Trans.* **2009**, 1080–1094.
- [10] L. Guilloreau, L. Damian, Y. Coppel, H. Mazarguil, M. Winterhalter, P. Faller, *J. Biol. Inorg. Chem.* **2006**, *11*, 1024–1038.
- [11] U. Heinz, L. Hemmingsen, M. Kiefer, H. W. Adolph, *Chem. Eur. J.* **2009**, *15*, 7350–7358.
- [12] a) C. S. Atwood, R. C. Scarpa, X. Huang, R. D. Moir, W. D. Jones, D. P. Fairlie, R. E. Tanzi, A. I. Bush, *J. Neurochem.* **2008**, *75*, 1219–1233; b) M. Rózga, A. M. Protas, A. Jablonowska, M. Dadlez, W. Bal, *Chem. Commun.* **2009**, 1374–1376.
- [13] C. C. Curtain, F. Ali, I. Volitakis, R. A. Cherny, R. S. Norton, K. Beyreuther, C. J. Barrow, C. L. Masters, A. I. Bush, K. J. Barnham, *J. Biol. Chem.* **2001**, *276*, 20466–20473.
- [14] D. Noy, I. Solomonov, O. Sinkevich, T. Arad, K. Kjaer, I. Sagi, *J. Am. Chem. Soc.* **2008**, *130*, 1376–1383.
- [15] C. Talmard, L. Guilloreau, Y. Coppel, H. Mazarguil, P. Faller, *ChemBioChem* **2007**, *8*, 163–165.
- [16] C. S. Atwood, R. D. Moir, X. D. Huang, R. C. Scarpa, N. M. E. Bacarra, D. M. Romano, M. K. Hartshorn, R. E. Tanzi, A. I. Bush, *J. Biol. Chem.* **1998**, *273*, 12817–12826.
- [17] K. Garai, P. Sengupta, B. Sahoo, S. Maiti, *Biochem. Biophys. Res. Commun.* **2006**, *345*, 210–215.