

Published in final edited form as:

J Neurochem. 2013 August ; 126(3): 305–311. doi:10.1111/jnc.12202.

Alzheimer's A β 42 and A β 40 peptides form interlaced amyloid fibrils

Lei Gu and Zhefeng Guo*

Department of Neurology, Brain Research Institute, Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA

Abstract

Deposition of A β in the brain is a pathological hallmark of Alzheimer's disease. There are two major isoforms of A β : the 42-residue A β 42 and the 40-residue A β 40. The only difference between A β 42 and A β 40 is that A β 42 has two extra residues at the C-terminus. The amyloid plaques in Alzheimer's brains consist of mostly A β 42 and some plaques contain only A β 42, even though A β 40 concentration is several-fold more than A β 42. Using electron paramagnetic resonance, we studied the formation of amyloid fibrils using a mixture of A β 42 and A β 40 *in vitro*. We show that A β 42 and A β 40 form mixed fibrils in an interlaced manner, although A β 40 is not as efficient as A β 42 in terms of being incorporated into A β 42 fibrils. Our results suggest that both A β 42 and A β 40 would be present in amyloid plaques if *in vivo* aggregation of A β were similar to the *in vitro* process. Therefore, there must be some mechanisms that lead to the preferential deposition of A β 42 at the extracellular space. Identifying such mechanisms may open new avenues for therapeutic interventions to treat Alzheimer's disease.

Keywords

Senile plaques; protein aggregation; electron paramagnetic resonance; spin labeling

Introduction

Aggregation of amyloid β (A β) plays a key role in the pathogenesis of Alzheimer's disease (AD). A β is a proteolytic product of amyloid precursor protein by β - and γ -secretases. The imprecise cleavage of γ -secretase at C-terminus of A β sequence results in two major A β isoforms: A β 42 (42 residues long) and A β 40 (40 residues long). The only difference between A β 42 and A β 40 is the two additional C-terminal residues on A β 42. The concentration of A β 40 in cerebral spinal fluid has been found to be several-fold more than that of A β 42. However, A β 42 is the major component of amyloid plaques in AD brains (Iwatsubo *et al.* 1995, Iwatsubo *et al.* 1994, Gravina *et al.* 1995, Miller *et al.* 1993, Mak *et al.* 1994), while A β 40 is detected only in a subset of plaques (Iwatsubo *et al.* 1994, Miller *et al.* 1993, Mak *et al.* 1994). These findings suggest that the A β 42 deposition precedes A β 40 deposition and the initial A β 42 aggregation does not involve A β 40.

The interplay between A β 42 and A β 40 has been generally considered to play a critical role in AD. Increased A β 42/A β 40 ratios appear to correlate with the early-onset familial AD cases caused by presenilin mutations (Kumar-Singh *et al.* 2006). Lowering A β 42/A β 40 ratios in transgenic mice decreases A β deposition (Kim *et al.* 2007). Higher neurotoxicity has been reported with samples of higher A β 42/A β 40 ratios (Kuperstein *et al.* 2010).

*To whom correspondence should be addressed: Zhefeng Guo, Department of Neurology, University of California, Los Angeles, 710 Westwood Plaza, Los Angeles, CA 90095. Phone: (310) 206-8773; zhefeng@ucla.edu.

Previous studies also suggest that A β 42 and A β 40 affect each other's aggregation rates and toxic activities (Snyder *et al.* 1994, Yoshiike *et al.* 2003, Yan & Wang 2007, Jan *et al.* 2008, Kuperstein *et al.* 2010, Pauwels *et al.* 2012). Furthermore, *in vitro* studies have shown that A β 42 and A β 40 form mixed aggregates (Frost *et al.* 2003).

Why is A β 42 the major, and sometimes only, component in amyloid plaques, when A β 40 is several-fold more abundant in the brain? One seemingly plausible explanation is that A β 42 is more aggregation prone than A β 40, and thus would be deposited before A β 40. However, this explanation assumes that A β 42 and A β 40 preferentially aggregate with their own species even if both isoforms are present. To this end, we use electron paramagnetic resonance (EPR) spectroscopy to investigate the interactions between A β 42 and A β 40 in amyloid fibrils at molecular level. Our results suggest that A β 42 and A β 40 form interlaced amyloid fibrils *in vitro*, suggesting some unrecognized mechanisms may contribute to the preferential deposition of A β 42 in AD brains.

Materials and Methods

Preparation of A β 42 peptides and spin labeling

The DNA constructs of GroES-ubiquitin-A β (Shahnawaz *et al.* 2007) and the deubiquitylating enzyme Usp2cc (Baker *et al.* 2005) were kindly provided by Dr. Il-Seon Park at Chosun University (South Korea) and Dr. Rohan T. Baker at Australian National University (Australia). The L17C mutation was introduced into A β 42 sequence using QuikChange kit (Agilent) and confirmed with DNA sequencing.

Expression of GroES-ubiquitin-A β and Usp2cc proteins in *E. coli* and their purification were performed as previously described (Ngo & Guo 2011, Agopian & Guo 2012). Full-length A β 42 was cleaved from the fusion protein with Usp2cc in a buffer containing 19 mM phosphate, 3 M urea, 2 mM TCEP, pH 10.0. Usp2cc was added to the fusion protein at a Usp2cc:A β molar ratio of 1:100. The digestion reaction was allowed to proceed at 37°C for 15 min. The reaction mixture was then immediately filtered with 0.2- μ m filter (Whatman) and loaded on a 5-mL HisTrap column (GE Healthcare) equilibrated with PSU buffer (50 mM phosphate, 0.3 M NaCl, 8 M urea, pH 10.0). A β peptide was eluted with 25 mM imidazole. Purified A β was checked with SDS-PAGE, and no non-cleaved proteins were detected. Wild-type (WT) A β peptides were buffer exchanged to 30 mM ammonium acetate, pH 10.0, and then lyophilized.

For spin labeling of A β 42 L17C mutant, dithiothreitol was added to purified protein fraction to a final concentration of 10 mM and was allowed to incubate at room temperature for 20 min. Then the A β 42 sample was buffer exchanged to labeling buffer (20 mM MOPS, 7 M guanidine hydrochloride, 50 mM NaCl, pH 6.8) using a 5-mL HiTrap desalting column (GE Healthcare). The spin labeling reagent MTSSL (1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl methanethiosulfonate, Enzo Life Sciences) was added at 10-times molar excess and then incubated at room temperature for 1 h. The spin label is named R1. The spin-labeled A β 42 was further buffer exchanged to 30 mM ammonium acetate, pH 10.0. Spin-labeled A β 42 was lyophilized and stored at -80°C. MALDI-TOF mass spectrometry was performed to ensure that the mass of A β 42 is correct, and the extent of labeling is >95%.

Fibril growth

To mix spin-labeled A β with WT A β , lyophilized A β 42 L17R1 and WT A β were dissolved separately in 30 mM ammonium acetate, pH 10.0 and then mixed at molar ratios of 1:1 and 1:3 as described in the text. Then the mixture is lyophilized. For fibril formation, the mixture was suspended in 100% 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) at 1 mM and bath sonicated for 5 min. Then the sample was incubated at room temperature for 30 min. HFIP

was removed by evaporation overnight in the fume hood and then under vacuum for 1 h. Finally the A β sample was dissolved in PG buffer (20 mM CAPS, 7 M guanidine hydrochloride, pH 11) to 1 mM and then diluted 20 \times to HBS buffer (50 mM HEPES, 140 mM NaCl, pH 7.4) to a final concentration of 50 μ M. Then the A β solution was placed on a digital vortex mixer with a speed of 600 rpm at room temperature. Fibrils were collected by centrifugation at 14,000 g for 20 min after thioflavin T binding has plateaued (\sim 5–7 days). Soluble proteins were removed by washing the pellet with HBS buffer.

Transmission electron microscopy

The A β fibril sample (5 μ l) was placed on glow-discharged copper grids covered with 400 mesh formvar/carbon film (Ted Pella). The sample was negatively stained with 2% uranyl acetate. Samples were examined using a JEOL JEM-1200EX transmission electron microscope at 80 kV.

EPR spectroscopy

EPR measurements were performed at X-band frequency on a Bruker EMX spectrometer equipped with the ER 4102ST cavity. A modulation frequency of 100 kHz was used. Measurements were performed at 20 mW microwave power at room temperature. Modulation amplitude was optimized to individual spectrum (typically \sim 4 G). Approximately 20 μ L of fibril sample was loaded into glass capillaries (VibroCom) sealed at one end. EPR spectra in each figure panel were normalized to the same number of spins.

Spectral simulations

Spectral simulations were performed using the program MultiComponent of Dr. Christian Altenbach, which provides a LabVIEW (National Instruments) interface of the program NLSL developed by Freed and co-workers (Schneider & Freed 1989, Budil *et al.* 1996). A microscopic order macroscopic disorder model was used as previously described (Budil *et al.* 1996). A least-squares fit of the user-defined spectral parameters was performed using the Levenberg-Marquardt algorithm. For all fits, the values for the magnetic tensors A and g were fixed as $A_{xx} = 6.2$, $A_{yy} = 5.9$, $A_{zz} = 37.0$, and $g_{xx} = 2.0078$, $g_{yy} = 2.0058$, $g_{zz} = 2.0022$, which were determined previously for spin label R1 (Columbus *et al.* 2001). An anisotropic model for the motion of the spin label was assumed and was found to give better fits than isotropic models. For anisotropic simulations, diffusion tilt angles were fixed to $(\alpha, \beta, \gamma) = (0, 36^\circ, 0)$ for z -axis anisotropy as previously reported (Columbus *et al.* 2001). The diffusion tilt angles are the Euler angles relating the axes of the diffusion tensor and the magnetic tensor. The number of fitted parameters was kept at a minimum, which in this work includes the rotational diffusion constant (R), an order parameter (S), and Heisenberg exchange frequency (ω). We found that satisfactory fits were obtained with only these three parameters. Rotational correlation time (τ) was calculated using $\tau = 1/(6R)$. For two-components fitting of the 1:1 mixture of spin-labeled A β 42 and WT A β , the parameters (R , S , and ω) for the single-line component were fixed at the fitted parameters for the fully labeled A β 42 L17R1, and the parameters for the three-line component was allowed to vary.

The fitted values for ω , τ , and S are as follows. For A β 42 L17R1, $\omega = 160.4 \pm 1.4$ MHz, $\tau = 5.7 \pm 0.1$ ns, $S = 0.55 \pm 0.01$. For 1:1 spin dilution with A β 42 WT three-line component, $\omega = 70.9 \pm 3.5$ MHz, $\tau = 2.5 \pm 0.4$ ns, $S = 0.77 \pm 0.03$. For 1:3 spin dilution with A β 42 WT, $\omega = 68.2 \pm 0.6$ MHz, $\tau = 3.3 \pm 0.2$ ns, $S = 0.72 \pm 0.01$. For 1:1 spin dilution with A β 40 WT three-line component, $\omega = 70.9 \pm 4.9$ MHz, $\tau = 5. \pm 0.4$ ns, $S = 0.55 \pm 0.05$. For 1:3 spin dilution with A β 40 WT, $\omega = 64.8 \pm 0.8$ MHz, $\tau = 5.0 \pm 0.1$ ns, $S = 0.46 \pm 0.02$. The 1:1 dilution was repeated once with the following fitted parameters. For 1:1 spin dilution with A β 42 WT, $\omega = 72.5 \pm 3.1$ MHz, $\tau = 2.2 \pm 0.3$ ns, $S = 0.77 \pm 0.02$. For 1:1 spin dilution with A β 40 WT, $\omega = 71.4 \pm 5.4$ MHz, $\tau = 5.4 \pm 0.3$ ns, $S = 0.487 \pm 0.05$.

Statistical analysis

Data are presented as mean \pm SD. Statistical significance was defined at $p < 0.05$, using the Student's *t*-test.

Results and Discussion

Rationale for using EPR to study interactions between A β 42 and A β 40 in amyloid fibrils

Both A β 42 and A β 40 have been shown to form fibrils with parallel in-register β -sheet structures (Tycko 2006, Agopian & Guo 2012), in which the side chains from the same residue positions stack in ladder-like arrangement. When a spin label is introduced into the A β sequence, the stacking of spin labels lead to strong spin exchange interactions. Normally, a nitroxide spin label gives rise to an EPR spectrum with three spectral lines, but the strong exchange interaction causes the three lines to collapse into a single spectral line (Figure 1A). The single-line spectrum serves as a signature for the strong exchange interaction (Margittai & Langen 2008). The spin exchange interaction is related to inter-spin distance by an exponential function and quickly diminishes when inter-spin distances are beyond ~ 7 Å. Therefore, if the fibrils are formed by a mixture of spin-labeled and unlabeled A β , the EPR spectrum will lose the single-line feature (Figure 1B). The mixing experiment of spin-labeled and unlabeled samples is referred to as “spin dilution”.

Spin dilution provides a way to study the interactions between A β 42 and A β 40 at molecular level. When spin-labeled A β 42 is mixed with A β 42 WT, the EPR spectrum will change from a single-line to a three-line feature. When spin-labeled A β 42 is mixed with A β 40 WT, the EPR lineshape depends on whether A β 40 WT is incorporated into spin-labeled A β 42 fibrils. If A β 40 WT forms fibrils only with A β 40 WT, then no spin dilution effect will be seen and the EPR spectrum will be single-line. On the other hand, if A β 40 WT forms interlaced fibrils with A β 42, then we will observe similar effect of spin dilution as with A β 42 WT. With spin dilution, we can study the interactions of A β 42 and A β 40 at high spatial resolution. A simple co-aggregation without interlacing of A β 42 and A β 40 in the fibril would not generate any spin dilution effect.

Similar morphologies for spin-labeled A β 42 fibrils in the presence and absence of spin dilution

We introduced a spin label named R1 at position 17 of A β 42 sequence. The chemical structure of R1 is shown in Figure 2A. Previous studies show that A β 40 L17R1 gives rise to a single-line spectrum, suggesting strong spin exchange interactions at this position (Agopian & Guo 2012). Residue Leu-17 is located at the beginning of the central hydrophobic cluster, and previous studies suggest that Leu-17 is well ordered in A β 42 fibrils (Lührs *et al.* 2005, Olofsson *et al.* 2006). To study the effect of spin dilution with A β 42 and A β 40 WT proteins, we prepared five fibril samples under agitated condition: (1) A β 42 L17R1; (2) A β 42 L17R1 with A β 42 WT at 1:1 molar ratio; (3) A β 42 L17R1 with A β 42 WT at 1:3 molar ratio; (4) A β 42 L17R1 with A β 40 WT at 1:1 molar ratio; (5) A β 42 L17R1 with A β 40 WT at 1:3 molar ratio.

Transmission electron microscopy shows that A β 42 L17R1 forms short straight fibrils (Figure 2B). The fibrils have similar morphologies as previously studied A β 40 fibrils under agitated conditions (Petkova *et al.* 2005, Kodali *et al.* 2010, Agopian & Guo 2012). We chose agitated condition because solid-state NMR studies show that agitation leads to highly homogeneous fibrils for A β 40 (Bertini *et al.* 2011). Our previous study also reveals a more pronounced single-line feature for agitated A β 40 fibrils when compared to quiescent fibrils (Agopian & Guo 2012), suggesting better packing interactions in agitated fibrils. When spin labeled A β 42 is mixed with either A β 42 WT or A β 40 WT, the morphologies of the fibrils

remain unchanged, suggesting that similar fibril structures in different samples (Figure 2B). Our results are consistent with previous findings that mixtures of A β 42 and A β 40 at different ratios form similar fibrils as A β 42 or A β 40 alone (Pauwels *et al.* 2012).

Effect of spin dilution on EPR spectral lineshape of spin-labeled A β 42 fibrils

A β 42 L17R1 fibrils gave rise to a single-line spectrum, suggesting a parallel in-register β structure in A β 42 fibrils (Figure 3A, black traces). Spin dilution with A β 42 WT at 1:1 and 1:3 molar ratios led to an evident three-line feature. Similar effects were observed when spin dilution was performed with A β 40 WT. These results suggest that A β 40 WT can be incorporated into A β 42 fibrils. Meanwhile, the EPR lineshape for A β 42 WT-diluted fibrils is not identical to A β 40 WT-diluted fibrils, suggesting that A β 40 WT is not equivalent to A β 42 WT in terms of co-fibrillization with spin-labeled A β 42.

We performed spectral simulations to quantitatively analyze the population of single-line and three-line components in the spin-diluted spectra. The best non-linear least squares fits are shown in Figure 3A (red traces). The spectrum of fibrils formed by A β 42 L17R1 can be fitted with only a single-line component. On the other hand, when spin dilution is performed at the 1:3 molar ratio, the spectrum of spin-diluted fibrils, whether with A β 42 or A β 40 WT, can be fitted with only a three-line component, suggesting that A β 40 WT is capable of completely diluting out the spin-labeled A β 42. Distributions of different fibril populations as a result of different A β 42 and A β 40 mixing ratios are summarized in Figure 4.

When spin dilution is performed at 1:1 molar ratio, the EPR spectra contain both the single-line and three-line components (Figure 3A). A β 42 WT-diluted spectrum consists of 47% single-line and 53% three-line components, whereas A β 40 WT-diluted spectrum consists of 69% single-line and 31% three-line components (Figure 3B). Because the three-line component is a result of interlacing between unlabeled and labeled A β in the fibrils, the ratio of the three-line component populations in A β 40- versus A β 42-diluted samples can be used as an estimate for the relative efficiency of incorporating A β 40 into A β 42 fibrils. Using the data from Figure 3B, the efficiency of incorporating A β 40 into A β 42 fibrils, relative to incorporating A β 42 into A β 42 fibrils, is 58% (= 31% / 53%). In other words, when mixing A β 40 and A β 42 at 1:1 ratio, approximately 58% A β 40 proteins are forming interlaced fibrils with A β 42, and the remaining 42% forms A β 40-only fibrils.

Overall, the EPR studies in this work reveal interactions between A β 40 and A β 42 in amyloid fibrils at a resolution of <10 Å. The strong spin exchange interaction that gives rise to the single-line EPR spectrum requires spin labels to be within ~7 Å of each other, essentially the distance between adjacent β -strands in the fibril. The spin dilution experiments suggest that A β 40 WT is able to disrupt the spin exchange interactions in spin-labeled A β 42, suggesting A β 40 must be incorporated in between spin-labeled A β 42 molecules. Previous studies have suggested that A β 42 and A β 40, when mixed at different ratios, affect each other's aggregation behavior and the toxicities of the resulting aggregates (Jan *et al.* 2008, Yan & Wang 2007, Kuperstein *et al.* 2010, Pauwels *et al.* 2012). Sequestration of fluorescently-labeled A β 42 with unlabeled A β 40 (or vice versa) suggests that A β 42 and A β 40 form mixed aggregates (Frost *et al.* 2003), but the structural resolution for the mixed aggregates was low. A previous EPR study has shown that A β 42 can be incorporated into spin-labeled A β 40 fibrils (Török *et al.* 2002), but it was not clear whether A β 40 can be incorporated into A β 42 fibrils. Furthermore, Török *et al.* (2002) suggested that A β 42 and A β 40 co-mix equally well with spin-labeled A β 40. Our quantitative analysis suggests that A β 40 is less efficient in terms of being incorporated into A β 42 fibrils. Our results are consistent with previous surface plasmon resonance studies by Pauwels *et al.* (2012) suggesting that monomeric A β 42 and A β 40 interact with each other, albeit less strongly than with themselves.

Implications for AD pathogenesis

Specific interactions between A β 42 and A β 40 as revealed by EPR provide insight on how A β 42 to A β 40 ratio plays an important role in AD. Our results suggest that coexistence of A β 42 and A β 40 in the extracellular space may generate aggregates containing three populations: A β 42 alone, A β 40 alone, and A β 42/A β 40 mix. Transgenic mice with over expression of A β 40 alone do not develop amyloid pathology or form insoluble aggregates (McGowan *et al.* 2005), suggesting that the aggregation of A β 40 is too slow to account for amyloid deposition in AD. The mixture of A β 42 and A β 40 also aggregates slowly compared to A β 42 alone (Snyder *et al.* 1994, Yoshiike *et al.* 2003, Yan & Wang 2007, Jan *et al.* 2008, Kuperstein *et al.* 2010, Pauwels *et al.* 2012). Therefore, it is likely that the A β 42-alone population is responsible for amyloid pathology in AD. The spin dilution experiments in this work show that the amount of A β 42-alone population is determined by the A β 42 to A β 40 ratio. At A β 42:A β 40 ratio of 1:3, most of A β 42 is in the A β 42/A β 40 mixture, while at A β 42:A β 40 ratio of 1:1, ~69% of A β 42 exists in A β 42-alone population (Figure 3). As a result, increases in A β 42/A β 40 ratio lead to increased A β 42-alone population, which causes AD pathology.

The finding that A β 42 and A β 40 form interlaced amyloid fibrils raises an intriguing question regarding *in vivo* A β aggregation. The amyloid plaques in AD brains contain mostly, sometimes only, A β 42 (Iwatsubo *et al.* 1995, Iwatsubo *et al.* 1994, Gravina *et al.* 1995, Miller *et al.* 1993, Mak *et al.* 1994), even though the A β 40 concentration in cerebrospinal fluid is several folds more than A β 42. What is the explanation for this phenomenon when A β 40 can be incorporated into A β 42 fibrils *in vitro*? Two potential mechanisms may contribute to the preferential deposition of A β 42 in amyloid plaques. First, the local concentration of A β 42 at plaque deposition sites may be significantly higher than currently assumed. A β concentration in the extracellular space of human brain is difficult to analyze. An intracerebral microdialysis study of brain interstitial fluid A β in patients with acute brain injuries shows high A β 40/A β 42 ratios (~14) for the pooled sample of 5 patients (Brody *et al.* 2008). However, microdialysis studies can only detect A β peptides in solution. Preferential binding of A β 42 to extracellular matrix or cell membrane may lead to a high local A β 42 concentration. Second, the aggregation of A β 42 *in vivo* may be assisted by some other proteins or cell components such as lipids, which may preferentially promote the aggregation of A β 42. Future investigations to achieve a better understanding of the discrepancy between *in vitro* and *in vivo* observations may shed new light on the pathogenesis of AD.

Acknowledgments

We thank Dr. Christian Altenbach and Dr. Wayne Hubbell for providing EPR analysis programs. This work was supported by the Alzheimer's Association (Grant NIRG-09-133555) and American Health Assistance Foundation (Grant A2010362). We acknowledge the use of instruments at the Electron Imaging Center for NanoMachines supported by NIH (1S10RR23057 to ZHZ) at the California NanoSystems Institute, UCLA. The authors declare no conflict of interest.

References

- Agopian A, Guo Z. Structural origin of polymorphism for Alzheimer's amyloid- β fibrils. *Biochem J.* 2012; 447:43–50. [PubMed: 22823461]
- Baker RT, Catanzariti AM, Karunasekara Y, Soboleva TA, Sharwood R, Whitney S, Board PG. Using deubiquitylating enzymes as research tools. *Methods Enzymol.* 2005; 398:540–554. [PubMed: 16275357]
- Bertini I, Gonnelli L, Luchinat C, Mao J, Nesi A. A new structural model of A β 40 fibrils. *J Am Chem Soc.* 2011; 133:16013–16022. [PubMed: 21882806]

- Brody DL, Magnoni S, Schwetye KE, Spinner ML, Esparza TJ, Stocchetti N, Zipfel GJ, Holtzman DM. Amyloid-beta dynamics correlate with neurological status in the injured human brain. *Science*. 2008; 321:1221–1224. [PubMed: 18755980]
- Budil DE, Lee S, Saxena S, Freed JH. Nonlinear-least-squares analysis of slow-motion EPR spectra in one and two dimensions using a modified Levenberg-Marquardt algorithm. *J Magn Reson, Ser A*. 1996; 120:155–189.
- Columbus L, Kálai T, Jeko J, Hideg K, Hubbell WL. Molecular motion of spin labeled side chains in α -helices: analysis by variation of side chain structure. *Biochemistry*. 2001; 40:3828–3846. [PubMed: 11300763]
- Frost D, Gorman PM, Yip CM, Chakrabarty A. Co-incorporation of A β 40 and A β 42 to form mixed pre-fibrillar aggregates. *Eur J Biochem*. 2003; 270:654–663. [PubMed: 12581205]
- Gravina SA, Ho LB, Eckman CB, Long KE, Otvos L, Younkin LH, Suzuki N, Younkin SG. Amyloid β protein (A β) in Alzheimer's disease brain: Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A β 40 or A β 42(43). *J Biol Chem*. 1995; 270:7013–7016. [PubMed: 7706234]
- Iwatsubo T, Mann DMA, Odaka A, Suzuki N, Ihara Y. Amyloid β protein (A β) deposition: A β 42(43) precedes A β 40 in Down syndrome. *Ann Neurol*. 1995; 37:294–299. [PubMed: 7695229]
- Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A β 42(43) and A β 40 in senile plaques with end-specific A β monoclonals: Evidence that an initially deposited species is A β 42(43). *Neuron*. 1994; 13:45–53. [PubMed: 8043280]
- Jan A, Gokce O, Luthi-Carter R, Lashuel HA. The ratio of monomeric to aggregated forms of Abeta40 and Abeta42 is an important determinant of amyloid-beta aggregation, fibrillogenesis, and toxicity. *J Biol Chem*. 2008; 283:28176–28189. [PubMed: 18694930]
- Kim J, Onstead L, Randle S, Price R, Smithson L, Zwizinski C, Dickson DW, Golde T, McGowan E. Abeta40 inhibits amyloid deposition in vivo. *J Neurosci*. 2007; 27:627–633. [PubMed: 17234594]
- Kodali R, Williams AD, Chemuru S, Wetzel R. A β (1-40) forms five distinct amyloid structures whose β -sheet contents and fibril stabilities are correlated. *J Mol Biol*. 2010; 401:503–517. [PubMed: 20600131]
- Kumar-Singh S, Theuns J, Van Broeck B, et al. Mean age-of-onset of familial Alzheimer disease caused by presenilin mutations correlates with both increased A β 42 and decreased A β 40. *Hum Mutat*. 2006; 27:686–695. [PubMed: 16752394]
- Kuperstein I, Broersen K, Benilova I, et al. Neurotoxicity of Alzheimer's disease Abeta peptides is induced by small changes in the A β 42 to A β 40 ratio. *EMBO J*. 2010; 29:3408–3420. [PubMed: 20818335]
- Lührs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Doeli H, Schubert D, Riek R. 3D structure of Alzheimer's amyloid- β (1-42) fibrils. *Proc Natl Acad Sci USA*. 2005; 102:17342–17347. [PubMed: 16293696]
- Mak K, Yang FS, Vinters HV, Frautschy SA, Cole GM. Polyclonals to beta-amyloid(1-42) identify most plaque and vascular deposits in Alzheimer cortex, but not striatum. *Brain Res*. 1994; 667:138–142. [PubMed: 7895077]
- Margittai M, Langen R. Fibrils with parallel in-register structure constitute a major class of amyloid fibrils: molecular insights from electron paramagnetic resonance spectroscopy. *Q Rev Biophys*. 2008; 41:265–297. [PubMed: 19079806]
- McGowan E, Pickford F, Kim J, et al. Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron*. 2005; 47:191–199. [PubMed: 16039562]
- Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, Iqbal K. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. *Arch Biochem Biophys*. 1993; 301:41–52. [PubMed: 8442665]
- Ngo S, Guo Z. Key residues for the oligomerization of A β 42 protein in Alzheimer's disease. *Biochem Biophys Res Commun*. 2011; 414:512–516. [PubMed: 21986527]
- Olofsson A, Sauer-Eriksson AE, Ohman A. The solvent protection of Alzheimer amyloid- β (1-42) fibrils as determined by solution NMR spectroscopy. *J Biol Chem*. 2006; 281:477–483. [PubMed: 16215229]

- Pauwels K, Williams TL, Morris KL, et al. The structural basis for increased toxicity of pathological A β 42:A β 40 ratios in Alzheimer's disease. *J Biol Chem*. 2012; 287:5650–5660. [PubMed: 22157754]
- Petkova AT, Leapman RD, Guo ZH, Yau WM, Mattson MP, Tycko R. Self-propagating, molecular-level polymorphism in Alzheimer's β -amyloid fibrils. *Science*. 2005; 307:262–265. [PubMed: 15653506]
- Schneider, DJ.; Freed, JH. Calculating slow motional magnetic resonance spectra. In: Berliner, L.J.; Reuben, J., editors. *Spin Labeling: Theory and Applications*. Plenum Press; New York: 1989. p. 1p. 76
- Shahnawaz M, Thapa A, Park IS. Stable activity of a deubiquitylating enzyme (Usp2-cc) in the presence of high concentrations of urea and its application to purify aggregation-prone peptides. *Biochem Biophys Res Commun*. 2007; 359:801–805. [PubMed: 17560941]
- Snyder SW, Ladror US, Wade WS, Wang GT, Barrett LW, Matayoshi ED, Huffaker HJ, Krafft GA, Holzman TF. Amyloid-beta aggregation: Selective inhibition of aggregation in mixtures of amyloid with different chain lengths. *Biophys J*. 1994; 67:1216–1228. [PubMed: 7811936]
- Török M, Milton S, Kaye R, Wu P, McIntire T, Glabe CG, Langen R. Structural and dynamic features of Alzheimer's A β peptide in amyloid fibrils studied by site-directed spin labeling. *J Biol Chem*. 2002; 277:40810–40815. [PubMed: 12181315]
- Tycko R. Molecular structure of amyloid fibrils: insights from solid-state NMR. *Q Rev Biophys*. 2006; 39:1–55. [PubMed: 16772049]
- Yan Y, Wang C. A β 40 protects non-toxic A β 42 monomer from aggregation. *J Mol Biol*. 2007; 369:909–916. [PubMed: 17481654]
- Yoshiike Y, Chui DH, Akagi T, Tanaka N, Takashima A. Specific compositions of amyloid-beta peptides as the determinant of toxic beta-aggregation. *J Biol Chem*. 2003; 278:23648–23655. [PubMed: 12716908]

Abbreviations

Aβ	amyloid β
AD	Alzheimer's disease
EPR	electron paramagnetic resonance
WT	wild-type

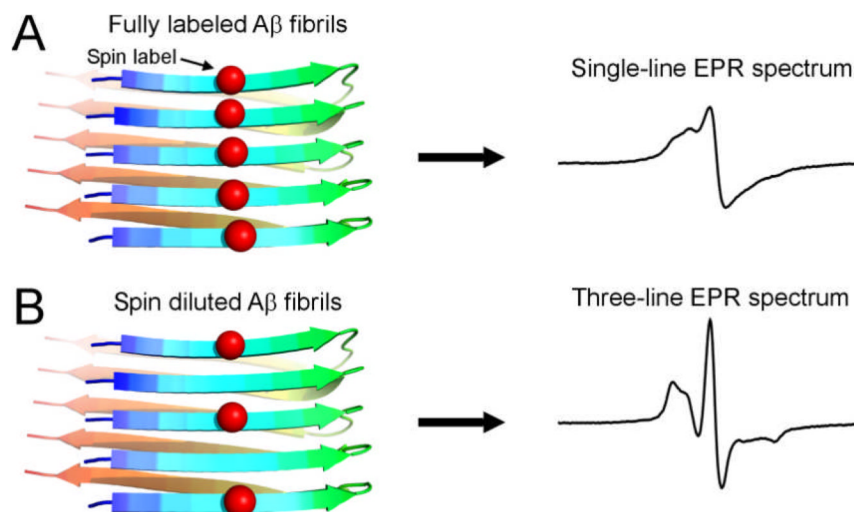


Figure 1. Effect of spin dilution on the EPR lineshape of spin-labeled fibrils

Red balls represent spin labels. (A) In a parallel in-register β -sheet structure of amyloid fibrils, the spin label side chains pack closely against each other and lead to strong spin exchange interactions between spin labels. As a result, the EPR spectrum shows a characteristic single-line feature. (B) When the fibrils are formed by spin-labeled A β and unlabeled A β (i.e. spin dilution), the inter-spin label spacing is more than ~ 7 Å, and thus spin exchange interactions are weak, leading to the normal three-line spectrum.

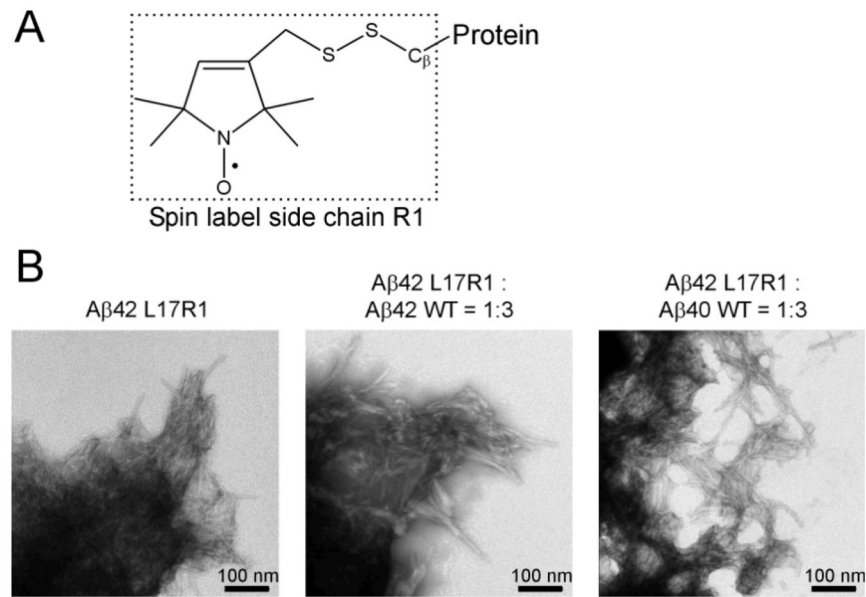


Figure 2. Characterization of spin labeled Aβ42 fibrils

(A) Chemical structure of spin label R1 used in this work. (B) Transmission electron microscopy images show similar morphologies for Aβ42 L17R1 and its mixture with either Aβ40 or Aβ42 wild type peptides.

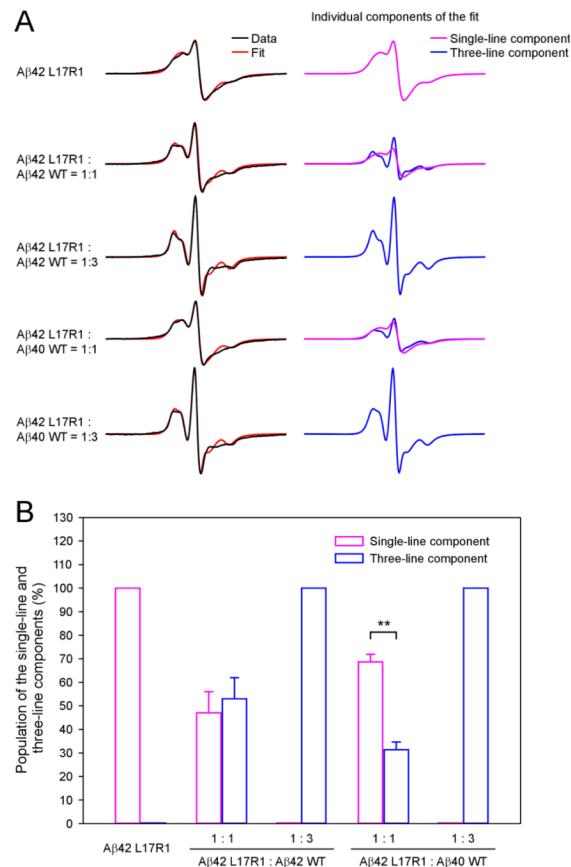


Figure 3. EPR analysis of spin-labeled Aβ42 fibrils

(A) EPR spectra of fully labeled Aβ42 fibrils and spin-diluted with either Aβ42 or Aβ40 wild-type proteins. The experimental spectra are shown in black and the best fits from spectral simulations are shown in red. Individual spectral components are shown in magenta and blue. (B) A bar graph showing the population of the single-line and three-line components from spectral simulations in the absence and presence of spin dilutions. For the 1:1 spin dilutions, results are expressed as mean ± SD (** $p < 0.01$, Student's t -test).

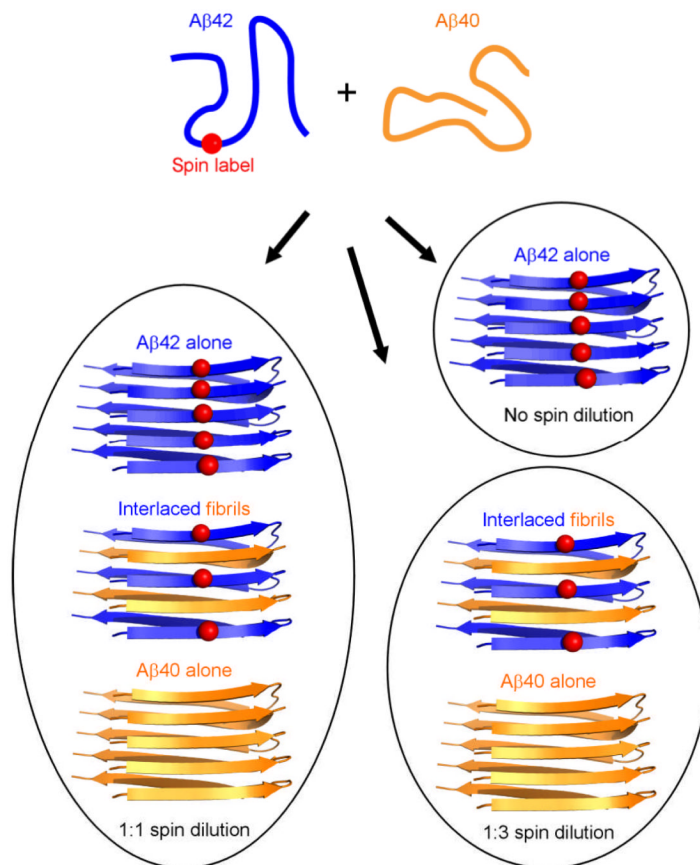


Figure 4. A schematic drawing shows distributions of different fibril populations resulting from spin dilution of spin-labeled Aβ42 with unlabeled Aβ40

With 1:3 dilution, two fibril populations are present: interlaced fibrils and Aβ40 fibrils. With 1:1 dilution, all three fibril populations (labeled Aβ42, interlaced, and unlabeled Aβ40) are present.