Supporting Information

Insights into cerebral amyloid angiopathy type-1 and type-2 from comparisons of fibrillar assembly and stability of the Aβ40-Iowa and Aβ40-Dutch peptides

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Figure S1. Protofibrils of Aβ42-WT observed by TEM and AFM. (A,B) TEM images from Figure 3 showing the association of Aβ42-WT into protofibrils after 24 h at 25 °C and fibrils after 24 h at 37 °C. Protofibrils of both Aβ40-WT and Aβ42-WT are generally observed as short, worm-like aggregates of ~200 nm or less. Scale bar = 100 nm. (C-F) Single touch AFM images of Aβ42-WT after the monomeric peptide was warmed to room temperature and layered on the AFM grid (C), and after incubation for 2 h (D), 6 h (E) and 8 h (F) at 37 °C. The peptide rapidly begins to associate into low MW oligomers with heights of ~2 nm that stack to form high MW oligomers with heights of 4-5 nm (C) (reference 55). The low and high MW oligomers are attributed to the pentamer-hexamers and decamers-dodecamers that are characteristic of Aβ42.
These oligomers laterally associate to form protofibrils that coalesce to form fibrils, giving the “beads-on-a-string” appearance that is often observed by AFM and TEM. Time-resolved measurements of the transition of oligomers to protofibrils to fibrils typically reveal that straight fibrils of Aβ42 appear once the protofibrils reach a length of ~200 nm. The scale bars in (C), (D) and (F) were 100 nm and in (E) was 25 nm. Reproduced with permission from reference 48. Copyright 2015 American Chemical Society.
Figure S2. Transition of Aβ40-Iowa from small oligomers to protofibrils and fibrils by atomic force microscopy. (A,B) TEM images from Figure 3 showing the association of Aβ40-Iowa after 24 h at 25 °C and 37 °C. Scale bars = 50 nm. The structures formed at 25 °C have some features characteristic of the short (50-200 nm), worm-like protofibrils observed for Aβ40-WT and Aβ42-WT (see Supporting Figure 1), but elongate to much longer structures at 37 °C. (C-F) Single touch AFM images of the Aβ40-Iowa peptides after incubation of 10 min (C), 30 min (D), 60 min (E) and 24 h (F) at room temperature. The peptide rapidly begins to associate into oligomers and elongated structures after monomerization and warming to room temperature. (A). After 30 min, the AFM images reveal protofibrils resulting from the lateral association of oligomers. These are similar to the protofibrils observed for Aβ42-WT in Supporting Figure 1,
but generally are composed of low MW oligomers having heights of ~2 nm. The Aβ42-WT fibrils typically have heights of ~4-5 nm and are composed of two cross β-units. In contrast, the 2 nm height for the fibril in panel (F) corresponds to a single layer of Aβ, in agreement with the mass-per-unit length measurements of ~1 kDa/nm by Qiang et al. (2012) (reference 37). As a result, we refer to the elongated structures in panels (B) and (F) as single-layer fibrils rather than as protofibrils which are observed in panel (D). The scale bars for the AFM figures are 50 nm (B), 50 nm (C), 400 nm (D) and 25 nm.

Note that the rapid conversion of Aβ40-Iowa protofibrils to single layer fibrils in the AFM studies is in agreement with the SEC experiments showing a rapid conversion to large structures that do not enter the SEC column (Figure 3) and the HSQC experiments (Figure 4) where the Aβ40-Iowa “aggregates” do not revert back to monomer or small oligomers upon temperature cycling between the 3 h and 24 h time points at 25 °C. Reprinted in part with permission from reference 39. Copyright 2021 Multidisciplinary Digital Publishing Institute Journals.
Figure S3. Transition of Aβ40-WT and Aβ40-Iowa oligomers to fibrils by FTIR and TEM. (A,B) FTIR from Figures 4 and 5 that have been overlaid to highlight the transition from oligomers to fibrils. In these spectra, mature fibrils are characterized by a decrease in the amide I frequency to 1626-1630 cm\(^{-1}\) along with an increase in the amide I intensity. The amide II frequency generally increases to ~1545-1550 cm\(^{-1}\) in the transition from oligomers to fibrils. For Aβ40-Iowa, the small shift in the amide II vibration is likely associated with its anti-parallel character. (C,D) TEM images of Aβ40-WT and Aβ40-Iowa after two weeks of incubation at 37 °C. The Aβ40 fibrils are composed of at least two protofilaments, which have both twisted and flat morphologies. The Aβ40-Iowa fibrils contain the curvilinear fibrils having a single protofilament as observed after 24 h of incubation at 37 °C, but also twisted fibrils that appear to form via association of protofilaments. FTIR spectra of these fibrils containing the \(^{13}\)C-LAGG labeling scheme (Figure 5D) exhibit the characteristic anti-parallel splitting of the amide I vibration and also exhibit a strong \(^{13}\)C – \(^{13}\)C cross peak in DARR NMR spectra between 2-\(^{13}\)C.
Ala30 and 1-^{13}C-Val36, which is expected for the single-layer anti-parallel fibrils described by Qiang et al. (2012) (reference 37) (see Figure S6).
**Figure S4. Antiparallel β-sheet structure as monitored by FTIR.** Illustration of the differences exhibited in FTIR spectra of two types of β-sheet structure using model compounds that have previously been characterized as having anti-parallel or parallel β-strands. (A) The seven-residue GNNQQNY peptide, a fragment of the yeast prion protein Sup35p, forms fibrils with parallel, in-register β-strands. The parallel, in-register structure in this peptide was established by solid-state NMR measurements and is likely driven by hydrogen bonding interactions between the side chain amide groups of Asn and Gln. Isotope labeling does not shift the major symmetric 1629 cm\(^{-1}\) band but results in a weak isotope shifted resonance at 1596 cm\(^{-1}\). The broad intensity at \(\sim\)1650 cm\(^{-1}\) is attributed to the side chain Asn and Gln amide vibrations. (B) The seven residue KLVFFAE peptide forms fibrils with anti-parallel β-sheet structure. This peptide corresponds to residues 16-22 of Aβ40. The anti-parallel structure, which was also established by solid state NMR spectroscopy, is likely driven by the complementary electrostatic interactions of the N- and C-termini of the peptide when the β-strands associate in anti-parallel arrangement. The amide I region is shown of Aβ16-22 (red) containing 1-\(^{13}\)C labeled Leu2 (corresponding to Leu17 in the Aβ40 sequence) and unlabeled Aβ16-22 (black). The 1626 cm\(^{-1}\)
resonance in the spectrum of the unlabeled peptide is characteristic of β-sheet secondary structure. The splitting into two intense bands at 1602 and 1630 cm$^{-1}$ is associated with anti-parallel β-sheet. a.u. = arbitrary units.

Molecular structures of the parallel and anti-parallel β-strands are shown below the FTIR spectra to illustrate that the hydrogen bonding arrangement is different in the two geometries. This difference in hydrogen-bonding gives rise to the differences in the amide I vibration, which corresponds largely to the C=O stretching vibration.

The differences in the vibrational spectra for anti-parallel and parallel β-strands within β-sheet secondary structure observed for these two model peptides are the same as those found by vibrational calculations on anti-parallel and parallel β-sheets. Namely, for anti-parallel structure the major component of the amide I normal mode splits into two equally intense bands (one with a higher frequency and one with a lower frequency). In contrast, for parallel structure the major β-sheet peak does not change frequency or intensity, but a very weak isotope shifted band is observed at a lower frequency than the intense low-frequency component of the amide I band observed in the spectra associated with anti-parallel structure. The differences arise in how the internal coordinates couple within the amide I normal mode. This coupling is dependent on the geometry. Reprinted in part with permission from reference 42. Copyright 2016 Springer-Nature.
Figure S5. Protofibril and fibril formation in Aβ42-WT. TEM of Aβ42-WT after 2-weeks of incubation at 25 °C (A) and 37 °C (B). Both curvilinear protofibrils of 50-100 nm in length and straight fibrils of >300 nm are observed after 2-weeks at 25 °C. In contrast, protofibrils are absent after 2-weeks of incubation at 37° C and long twisted fibrils are observed. The FTIR spectrum in Figure 4A of unlabeled Aβ42-WT incubated at 25 °C exhibits an amide I vibration of 1630 cm\(^{-1}\) and an amide II vibration of 1543 cm\(^{-1}\). The LAGG \(^{13}\)C-labeled FTIR spectrum, however, exhibits a defined splitting of the amide I band characteristic into components at 1609 and 1643 cm\(^{-1}\). The frequency and relative intensities of these bands suggest the Aβ peptide has a largely anti-parallel conformation, which likely arises from the protofibrils in the sample. Scale bars = 50 nm.
Figure S6. Solid-state NMR spectroscopy of Aβ40-Dutch and Aβ40-Iowa fibrils.

Intermolecular $^{13}$C – $^{13}$C distances that are characteristic of parallel, in-register or anti-parallel fibril structures can be measured using solid-state NMR spectroscopy. These studies make use of two Aβ peptides incorporating specific $^{13}$C labels. The $^{13}$C labels are chosen such the intermolecular distance separating the $^{13}$C sites on neighboring peptides within an Aβ fibril is less than ~6 Å, the approximate distance limit for the DARR NMR method. The first peptide contains 2-$^{13}$C Ala30 and 1-$^{13}$C Val36. The single layer anti-parallel fibril structure that has been determined using solid-state NMR shows that the intermolecular distance between these $^{13}$C sites is ~4.5 Å. Conversely, these $^{13}$C labels are >10 Å apart in structures of parallel, in-register fibrils. The second peptide contains 3-$^{13}$C Ala30. In parallel, in-register fibrils, Ala30 on one peptide packs against Ala30 on the neighboring peptide resulting in close 2-$^{13}$C Ala30 to 3-$^{13}$C Ala30
distances (~4-5 Å) when the two peptides are mixed in equimolar concentrations. As such, mixing both peptides in one sample will result in cross peaks for anti-parallel fibrils between 2-\(^{13}\)C Ala30 and 1-\(^{13}\)C Val36, and cross peaks for parallel, in-register fibrils between 1-\(^{13}\)C Ala30 and 3-\(^{13}\)C Ala30.

(A,B) **Two-dimensional \(^{13}\)C DARR NMR spectra of A\(\beta\)40-Dutch and A\(\beta\)40-Iowa fibrils after two weeks of incubation at 37 °C.** The regions containing cross peaks corresponding to parallel and anti-parallel fibril structures are shown. The parallel cross peaks form between 2-\(^{13}\)C Ala30 and 3-\(^{13}\)C Ala30 labels on neighboring peptides (blue boxes), while the anti-parallel cross peaks form between 2-\(^{13}\)C Ala30 and 1-\(^{13}\)C Val36 labels on neighboring peptides (red box). Other than the intense diagonal resonances, peaks that appear in each of the 2D NMR spectra that are not labeled correspond to spinning sidebands or aliased spinning side bands. Rows taken from the 2D NMR spectra are shown for the cross peaks (above the diagonal resonance) diagnostic of anti-parallel structure (left) and parallel, in-register structure (right). For each peptide, the intensity of the cross peaks on both sides of the diagonal resonance were integrated (peak volumes) and the total intensity for the regions containing the parallel and anti-parallel cross peaks was normalized to 1.

(C,D) **TEM micrographs of A\(\beta\)40-Dutch and A\(\beta\)40-Iowa.** Representative TEM micrographs are shown of A\(\beta\) fibrils after two weeks of incubation at 37 °C. A small aliquot was taken from the solutions used for the NMR measurements above. Scale bars = 100 nm.
Figure S7. Comparison of FTIR spectra of Aβ40-WT, Aβ40-Dutch, Aβ40-Iowa and Aβ42-WT. (A) Overlay of FTIR spectra at t = 0 h of the four Aβ peptides with LAGG $^{13}$C labeling. (B, C) Overlay of FTIR spectra at t = 3 h of the four Aβ peptides without $^{13}$C labeling obtained at 25 °C (B) and 37 °C (C). (D) Overlay of FTIR spectra at t = 2 weeks of the four Aβ peptides with LAGG $^{13}$C labeling.