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Context Dependence of Protein Misfolding and Structural Strains in Neurodegenerative Diseases

Anil K. Mehta^{1,*}, Rebecca F. Rosen^{2,†}, W. Seth Childers^{1,‡}, John D. Gehman³, Lary C. Walker^{2,4,*}, and David G. Lynn^{1,*}

¹Departments of Chemistry and Biology, Alzheimer's Disease Research Center, Emory University, Atlanta, Georgia 30322, USA

²Yerkes National Primate Research Center, Center for Neurodegenerative Disease, Emory University, Atlanta, Georgia 30322, USA

³School of Chemistry, Bio21 Institute, University of Melbourne, Melbourne, Vic. 3010, Australia

⁴Department of Neurology, Emory University, Atlanta, Georgia 30322, USA

Abstract

Vast arrays of structural forms are accessible to simple amyloid peptides and environmental conditions can direct assembly into single phases. These insights are now being applied to the aggregation of the A β peptide of Alzheimer's disease (AD) and the identification of causative phases. We extend use of the imaging agent Pittsburgh compound B (PiB) to discriminate among A β phases and begin to define conditions of relevance to the disease state. And we specifically highlight the development of methods for defining the structures of these more complex phases.

Introduction

Protein misfolding events are now implicated in at least 50 disease states, and among these, the neurodegenerative maladies represent a personal and societal tragedy of immense and growing proportions. Despite progress in identifying these diseases, recent therapeutic strategies directed at controlling the production and aggregation of pathogenic proteins have all been disappointing.¹ However, several key observations common to these diseases have emerged, including (i) the demonstration of a remarkable diversity of assembled forms accessible to a given protein sequence, (ii) the documentation of individual forms that propagate through a conformational Darwinian selection process to give distinct strains, and (iii) the definition of nucleation and propagation events that are strongly influenced by the assembly environment. These proteinaceous aggregates then arise in a context dependent manner, and the selected forms, or strains, spread by an endogenous prion-like process of conformational templating.^{2–41}

The genetic and environmental risk factors associated with Alzheimer's disease (AD), probably the best known of these misfolding disorders, are largely associated with the metabolism, trafficking or aggregation of the 39–42 residue A β peptide.^{42–44} However, some individuals with substantial A β deposition in the brain show no clinical signs of AD.^{45,46} Similarly, nonhuman primates, which accumulate copious deposits of A β with age,⁴⁷ also fail to develop the key features of AD including neurofibrillary tangles, neuronal

*Corresponding authors: anil.mehta@emory.edu; lary.walker@emory.edu; david.lynn@emory.edu.

[†]Current address: American Institutes for Research, Washington, DC 20007

[‡]Current address: : Stanford School of Medicine, Department of Developmental Biology, Stanford, CA 94305

loss, and dementia.⁴⁸ Resolution of this apparent paradox may be rooted in proteins that aggregate and propagate into structurally and functionally distinct strains with only certain strains being critical for disease.^{8,47,49–61}

To explore the hypothesis that specific strains underlie the observed disease states, we have been developing methods to distinguish pathogenic from benign A β strains. Defining the architectural features of these strains⁵⁰ has required the development of new methods that address the structural challenges associated with these misfolded assemblies. Recognizing that defining the replicating environment will be critical for propagation of any selected assembly, we have further developed methods to propagate and characterize assemblies from more heterogeneous environments. Here we combine those methods in a coherent framework for defining the structural basis for AD and possibly other disease states.

Results and Discussion

Identifying AD-relevant strains

AD is diagnosed postmortem by the identification of two characteristic lesions in the brain: senile plaques and neurofibrillary tangles.^{43,62} Senile plaques contain extracellular deposits of micron long, unbranched fibers composed of A β , a ~4kDa cleavage product of the β -amyloid precursor protein (APP).^{44,63,64} Neurofibrillary tangles are intracellular polymers of the microtubule-associated tau protein.⁶⁵ Current evidence indicates that the tauopathy of AD is downstream of A β aggregation,^{42,43} and that the pathogenic process begins in the brain many years before the onset of dementia.⁶⁶ Furthermore, the type and distribution of A β deposits in the brain can vary considerably, both within and among AD brains, possibly reflecting different initiating events.⁵⁰ Hence, understanding the misfolding, aggregation, and propagation of A β phases will be an important step toward elucidating both the critical early stages of AD as well as the antecedents of degenerative changes in end-stage disease.

Recently, we found that high-affinity binding sites for the A β -imaging agent Pittsburgh compound B (PiB) (Fig 1A) are much more abundant on A β aggregates from human AD brains than from aged nonhuman primate brains⁴⁷, which deposit large amounts of A β in brain, but do not develop AD.^{48,67} *In vitro*, our studies and others show that the peptide conformation of A β aggregates is strongly determined by the conditions of aggregation,^{52,55,56,68–80} and thus PiB may serve as a critical probe for an AD-specific strain of A β and help define the conditions under which strains develop.

To test this assertion *in vitro*, we incubated 1mg/mL A β (1–40) peptides in 10mM phosphate buffer (pH 7.4) under conditions of constant agitation^{52,55} (Ag) and generated shorter and more twisted fibers compared to quiescent^{51,77,78} (Q) incubation (Fig 1B,C). Both fibers are composed of parallel in-register β -sheets, with the agitated fibers having a triangular cross-section with 3 peptides per unit-length⁵⁵, defined by the H-bonding repeat within the fiber, in contrast the quiescent fibers have two peptides per unit-length.^{81,82} A β (1–40) Ag vs. Q assemblies also show different affinity for [³H]PiB (Fig 1D); Ag fibers have almost three times as many binding sites as the Q fibers. A similar trend is observed for A β (1–42) fibers, with A β (1–42) Ag assemblies showing the highest [³H]PiB binding. These data suggest that very subtle environmental conditions can indeed nucleate and propagate structures that bind [³H]PiB with different affinities and set the stage for generating more disease-relevant structures.

Characterization of assemblies identified from *in vivo* PiB binding now provides a unique opportunity to define the structural features of β -amyloid strains that are specific for AD. [¹⁹F]PiB binds with similar affinity *in vivo*,^{83–85} opening the possibility to greatly increase sensitivity for specific ¹³C/¹⁵N – ¹⁹F distances to be defined with ¹³C/¹⁵N-enriched A β

peptide through $^{13}\text{C}/^{15}\text{N}\{^{19}\text{F}\}$ REDOR (Rotational-Echo Double-Resonance)^{86,87} solid-state NMR measurements. To optimize these experiments for eventual characterization of *in vivo* assemblies, we have developed the $^{13}\text{C} - ^{19}\text{F}$ distance measurement within amyloid assemblies of the central nucleating core of the A β peptide,^{88–90} A β (16–22), Ac- $^{16}\text{KLVFFA}^{22}\text{E-NH}_2$, using trifluoroacetic acid (TFA) association. A β (16–22) assembled phases are now the most thoroughly characterized amyloid structure (see ref⁶⁸ and references therein). $^{13}\text{C}\{^{15}\text{N}\}$ REDOR⁸⁶ solid-state NMR measurements of *N*-acetyl-[1- ^{13}C]LV18 [15N]A21 A β (16–22) peptides assembled at low pH resolves V18 ^{13}CO dipole-dipole coupling to two backbone A21 amide ^{15}N 's, one from the H-bonded residue (4.2 Å) and one from the non-H-bonded residue (5.4 Å) with an angle of 160° between the two ^{13}C - ^{15}N internuclear vectors.⁷⁵ These constraints place all of the peptides in anti-parallel, out-of-register β -sheets. Small-angle X-ray (SAXS) scattering of the *N*-acetyl A β (16–22) peptide assemblies fit to a hollow tube form factor with a 52 nm diameter and a 4.3 nm wall thickness.⁹¹ The ~4 nm wall thickness was confirmed by TEM of cross-sectioned A β (16–22) assemblies embedded in epoxy resin.⁹²

Given that the length of an extended seven-residue β -strand peptide is ~2.2 nm, one structural model has the tube wall defined by two peptides stacked end-to-end. If this arrangement was a peptide bilayer, the anti-parallel β -sheet registry would place the positively charged lysine side chain on both faces of each peptide leaflet, resulting in the burial of significant positive charge density at the leaflet interface (Fig 2). To determine whether TFA anions were involved in passivating this heavily charged interface, $^{13}\text{C}\{^{19}\text{F}\}$ REDOR methods were developed and used to establish that ^{13}C -enriched carbonyl carbons of the acetyl capping group at the N-terminus of *N*-acetyl A β (16–22) peptide, [1- ^{13}C]acetyl A β (16–22), were all within ~5 Å of an ^{19}F .⁹² Most importantly, when the peptide nanotubes were washed with sulfate, the $^{13}\text{C}\{^{19}\text{F}\}$ REDOR dephasing data plateaued at ~50%, demonstrating that only half of the acetyl groups are close to an ^{19}F .⁹² This result was consistent with a peptide bilayer where half of the lysines are solvent-exposed and half are buried within the leaflet interface (Fig 2). These findings demonstrate the potential of using ^{13}C - ^{19}F distance measurement with amyloid peptides to structurally localize small molecules such as TFA within the amyloid assembly. The high sensitivity offered by ^{13}C dephasing by ^{19}F now opens the possibility of defining the binding site of [^{19}F]PiB on complex amyloid assemblies.

Context Dependence of Assembly

As suggested above, A β aggregate strains are strongly dependent on the conditions of assembly *in vitro*,^{52,55,56,68–76} but little information exists on the nature of the assemblies that form *in vivo*. Since the A β peptide associated with Alzheimer's disease is generated by proteolytic secretase cleavage within the transmembrane region of APP,⁹³ the initial steps of assembly may well occur within the neuronal plasma membrane. Indeed, immunohistochemical analysis A β bound to ganglioside in intraneuronal organelles⁹⁴ and A β assembly in the presence of model lipids^{95–102} has been used to mimic assembly *in vivo*. We designed a simple initial test of the effect of this environment on A β assembly by assembling A β (1–42) in the presence of brain lipid extracts. TEM microscopy identified fibers and vesicles (Fig 3A). While characterization by IR and CD in these crude samples is challenging, β -sheet peptide conformation and registry can be specifically interrogated by measuring ^{13}C - ^{15}N and ^{13}C - ^{13}C backbone distances with solid-state NMR. [1- ^{13}C]L34 [15N]V36 A β (1–42) peptides were synthesized via solid-phase peptide chemistry and purified with reverse-phase HPLC to >99% purity. The Leu34 ^{13}CO chemical shift resonance at 172.8 ppm (Fig 3B) is consistent with protein β -strand chemical shifts.^{103,104} The *i* to *i*+2 intramolecular ^{13}C - ^{15}N distance measured with $^{13}\text{C}\{^{15}\text{N}\}$ REDOR⁸⁶ uniquely defines a $^{13}\text{C} - ^{15}\text{N}$ distance¹⁰⁵ of 4.1 Å, providing the initial assignment as parallel β -sheet

A β (1–42) peptide assemblies. A β (1–42) assembled *in vitro* has parallel in-register β -sheets,^{106–108} and this registry would place the Val34 carbonyl carbons 4.7 Å apart. That no ¹³C-¹³C dipolar coupling between [1-¹³C]Val34 carbons was observed in ¹³C Double Quantum Filtered DRAWS^{109,110} solid-state NMR experiments (data not shown) suggests that, in the presence of lipid extracts, A β (1–42) assembles as a different phase.

To develop the necessary methods for characterizing any mixed molecular assemblies forming in the presence of brain lipids, a series of simple peptide/lipid chimeras were prepared with A β (16–22) *N*-acylated with fatty acid chains containing from 2 (*N*-acetyl) to 16 (*N*-palmitoyl) carbon atoms.¹¹¹ Remarkably, all of these chimeric peptides assembled and displayed the characteristic H-bonded β -sheet reflection at 4.7 Å by X-ray diffraction (XRD).¹¹¹ The laminate XRD d-spacing systematically increased as the chain length increased from 9.8 Å for C₂ to 13.5 Å for C₁₆, with the two longest chains C₁₄ (*N*-myristoyl) and C₁₆ giving fibers with two laminate reflections consistent with assemblies that had distinctly different β -sheet stacking distances. C₁₁ (*N*-undecanoyl) to C₁₃ (*N*-tridecanoyl) assembled as tubes morphologically identical to those formed by the shorter chain (C₂ - C₄) chimeric peptides. This similarity between the morphology of the two distinctly different peptide amphiphiles (*N*-acetyl (C₂)- and *N*-lauroyl (C₁₂)-A β (16–22), Fig 4B & C) led us to investigate the structural differences between these assemblies, specifically the location of the acyl chain and the source of the altered laminate spacing of 9.8 Å for *N*-acetyl A β (16–22) to 11.5 Å for *N*-lauroyl A β (16–22) assembled peptides.¹¹¹

Electron diffraction of oriented *N*-acetyl and *N*-lauroyl A β (16–22) tubes both displayed two cross- β diffraction patterns from the top and bottom tube walls with the H-bonds of the *N*-lauroyl A β (16–22) β -sheets tilted 11° from the tube long axis, half the value of the *N*-acetyl tubes.¹¹¹ The peptide nanotube architectures were intriguingly similar;¹¹¹ both tubes had a wall thickness of ~4 nm as measured by AFM. Isotope-edited IR of [1-¹³C]F19 *N*-acetyl and *N*-lauroyl A β (16–22) had identical ¹²C and ¹³C band splittings, indicating that both peptides form anti-parallel out-of-register β -sheets. Negatively charged 5nm Au colloidal nanoparticles bound to the surfaces of both assemblies with similar density and distribution, indicating that the terminal lysine side chain amines were protonated and exposed on the tube surface. ¹³C{¹⁹F} REDOR measurements of *N*-[1-¹³C]acetyl and *N*-[1-¹³C]lauroyl A β (16–22) peptides assembled in the presence of TFA and slowly bundled in the presence of sulfate or NADH, respectively, to remove the solvent-exposed TFA ions had similar REDOR dephasing plateaus, consistent with TFA anions buried within a peptide bilayer.¹¹¹

Initial molecular modeling of the *N*-lauroyl peptides arranged into a single anti-parallel out-of-register β -sheet suggested that the lauroyl chain was bent over the peptide side chains. To localize the alkyl chain, distances from both ends of the lauroyl chain with ¹³C enrichment of either the lauroyl methyl or carbonyl carbon were measured to the peptide backbone enriched with ¹⁵N. ¹³C{¹⁵N}REDOR measurements of *N*-[1-¹³C]lauroyl [¹⁵N]L17 A β (16–22) peptides assembled into nanotubes fit to a ¹³C-¹⁵N distance of 3.7 Å indicating that, at the attachment point to the peptide, all of the lauroyl chains were bent as shown in Fig. 4A. Further, ¹³C{¹⁵N}REDOR measurements from the terminal methyl of the lauroyl chain to [¹⁵N]L17 and [¹⁵N]V18 (Fig 4A) placed the methyl group closer to V18 than L17, and molecular modeling placed the end of the chain close to the middle of the peptide with the lauroyl chain snorkeling between two stacked anti-parallel out-of-register β -sheets. The commensurate increase in the distance between the β -sheets as observed in the XRD pattern, from 9.8 Å to 11.5 Å in this case, can now be used as a diagnostic signature for the incorporation of small molecules between the peptide bilayer leaflets and within the β -sheet laminate (Fig 4B & C). This demonstrated structural plasticity of the cross- β architecture greatly expands the range of structures available in the cellular milieu where amyloid proteins assemble, and provides the methods necessary to define their structures.

Conclusion

In AD, the nucleation and aggregation of A β is an early and obligatory pathogenic event^{1,42–44,62} that begins decades before symptoms appear.^{66,112} This timing, together with the context-dependent nature of assembly within neuronal tissues,⁵⁰ conspires to present one of the most daunting structural problems facing peptide science. Here we have demonstrated the development of methods for defining small molecule binding to complex amyloid arrays that exploit the greater sensitivity of the ¹³C{¹⁹F}REDOR experiments. These methods can now be directly applied to the differential binding of the A β -imaging agent PiB to A β aggregates from human AD brains.⁴⁷ Structural characterization will, however, require the preparation of sufficient amounts of the peptide assemblies for characterization. Maybe not surprisingly, our initial data suggest that A β (1–42) assemblies have different β -sheet registries in the presence of lipid brain extract. The full characterization of peptide/lipid chimera assemblies has identified unique areas of plasticity within the β -sheet laminates, and while this discovery does not directly explain the apparent registry shift in A β (1–42), it does provide perspective for how assembling within the complex milieu of brain tissues may impact the final structure. This sets the stage for the structural interrogation of amyloid strains similar to *in vivo* assemblies and the definition of their ultimate relationship to disease. Such methods may finally provide a route forward for the elucidation of the critical features that underlie AD and possibly other amyloid assemblies in a growing class of devastating diseases.

Methods

Peptide Synthesis and Purification

Peptides were synthesized using a Liberty CEM Microwave Automated Peptide Synthesizer (NC, USA) and a Fmoc-L-Val-PEG-PS or Fmoc-L-Ala-PEG-PS Resin (AnaSpec, CA, USA) for A β (1–40) and A β (1–42) (¹DAEF ⁵RHDSG ¹⁰YEVHH ¹⁵QKLVF ²⁰FAEDV ²⁵GSNKG ³⁰AIIGL ³⁵MVGGV ⁴⁰VIA) synthesis, respectively, to give the free acid carboxy terminus upon cleavage from resin. Fmoc-PEG-PS Resin was swollen using dimethylformamide for 15 minutes. Microwave-assisted Fmoc deprotection was completed using 20% piperidine and 0.1M N-Hydroxybenzotriazole (HOBt) in dimethylformamide at 45–55°C for 180 sec, followed by 3X dimethylformamide flushes. Each Fmoc-amino acid coupling step was performed using 0.1M Fmoc protected amino acid, and activated with 0.1 M 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and 0.2M N,N-Diisopropylethylamine (DIEA) in DMF. Coupling temperatures using microwave activation were maintained between 75 and 82°C for 300 sec except for histidine and arginine residues. Histidine was double-coupled for 600 sec at 50°C and arginine was coupled at room temp for 900 sec and 300 sec between 75 and 82°C. After each coupling step, resin was rinsed with three aliquots of dimethylformamide. Isotope-enriched peptides were synthesized by the Peptide Technology Facility of the Bio21 Molecular Science and Biotechnology Institute peptide synthesis facility (Melbourne, Australia).

Resin was filtered and washed with dichloromethane and allowed to air dry. Peptides were cleaved from the resin using trifluoroacetic acid/thioanisole/1,2-ethanedithiol/anisole (90:5:3 2, v/v/v/v) at room temperature for 3 hrs. The cleaved peptide-TFA solution was filtered, and precipitated by drop-wise addition to cold (–20°C) diethyl ether. Precipitated product was centrifuged at 3000 rpm for 10 min, and the pellet was further washed 3 times with cold diethyl ether. Dried peptides were dissolved in minimal volume of 40% acetonitrile + 0.1% trifluoroacetic acid and 8M Guanidinium chloride (Aldrich) and purified by RP-HPLC using a C18-reverse phase column with an acetonitrile-water +0.1%TFA gradient. In each case, the peptide purity was evaluated by HPLC. Molecular weight was confirmed by electro

spray ionization (ESI) mass spectrometry. Acetonitrile was removed with rotary evaporation and peptides were lyophilized to dryness.

Peptide Assembly

For microscopy, A β (1–40) and A β (1–42) lyophilized powder, synthesized as described above, was dissolved in minimal hexafluoroisopropanol (HFIP, Aldrich, MO) in glass vials for 2hrs, then dried under a steady stream of argon gas overnight to form a clear film. Peptides were assembled in 10mM phosphate buffer, pH 7.4, with 0.02% Na azide in 15 or 50 mL Falcon tubes. Agitated samples were placed on an orbital shaker (BellCo Glass Inc., Vineland NJ) with the shaker table maintained at 40 revolutions per minute. Agitated and quiescent samples were assembled for at least two weeks. For PiB binding assays, HFIP-treated lyophilized A(1–40) and A(1–42) (purchased from Anaspec) were brought up to 5mM stock concentration with anhydrous DMSO. Peptides were brought up to 100 μ M in sterile 0.01M PBS in 1.5mL polypropylene tubes and samples were spun at 14,000g for 10min at room temperature. Clear supernatants were transferred to clean tubes and incubated at 37°C for 25 days at rest or shaking at 450 rpm on an orbital shaker.

[³H]PiB binding

In a 96-well polypropylene plate, 10 or 20 μ L of A β assemblies (diluted in 5% ethanol/PBS) were added to each of duplicate wells. 100 or 200 μ L of 1 nM [³H]PiB (SA = 82 Ci/mmol, custom synthesis, GE Healthcare, UK) diluted in 5% ethanol/PBS were quickly added to each well. Samples were incubated at ambient temperature for 3.5 h, without shaking, transferred to a 96-well Multiscreen HTS Hi Flow FB filter plate, and filtered with a vacuum manifold (Millipore Corporation, Bedford, MA, USA). Filters were rapidly washed with 3 $\times$ 200 μ L of PBS at room temperature and dried on the manifold, after which 50 μ L of MicroScint 20 scintillation fluid (PerkinElmer, Waltham, MA, USA) were added to each well. After 24 h incubation on an orbital shaker, [³H]PiB binding was quantified in a TopCount scintillation counter (PerkinElmer).

A β and Lipid Preparation

Multilamellar vesicles (MLVs) for NMR experiments were prepared by individually dissolving 3.5 mg of [1-¹³C]L34[¹⁵N]V36 A β 42 to 2 mg/mL in HFIP and 17.25 mg of Brain Total Lipid Extract (Avanti Polar Lipids, Alabaster, AL) in chloroform and methanol (9 : 1), then combining to evaporate to a film under vacuum. The film was placed under high vacuum overnight to remove any residual solvent before being suspended in 21 μ L 50 mM sodium phosphate buffer (pH 7.2) and 2.6 mL 18 M Ω water and homogenized with three freeze–thaw cycles between liquid N₂ and a 40°C water bath. The sample was then lyophilized and packed into the NMR rotor under positive pressure argon gas.

Solid-state NMR

NMR sample (20 mg) was packed into a 4 mm solid-state NMR rotor and centered using boron nitride spacers. NMR spectra were collected with a Bruker (Billerica, MA) Avance 600 spectrometer using a 4mm HCN BioSolids magic-angle spinning (MAS) probe. MAS frequency was actively controlled at 10,000 $\pm$ 2 Hz with cooling and spinning air exit temperature maintained below –1 °C to ensure that MAS and radio-frequency (RF) heating did not denature the samples. ¹³C (150.8 MHz) CP-MAS spectra before and after REDOR experiments confirmed that the samples did not change during the experiment. The pulse sequence for ¹³C{¹⁵N} rotational-echo double-resonance (REDOR)⁸⁶ consists of two parts, an S sequence with both ¹³C and ¹⁵N pulses, and the S₀ sequence, which is identical but does not contain any ¹⁵N dephasing pulses. Applying pulses to the dephasing ¹⁵N spins interferes with the averaging due to magic-angle spinning, and reintroduces the dipolar

coupling that is observed in the REDOR S spectrum, where the signal decays according to both T_2 (spin-spin relaxation) and the heteronuclear ^{13}C - ^{15}N dipolar coupling. Maximum dephasing occurs when the spacing between π -pulses is equal to $\frac{1}{2}$ of the rotor cycle. The sequence without any ^{15}N dephasing π -pulses gives the REDOR full-echo or S_0 spectra, where the magnetization decays according to only T_2 . The difference between the REDOR S and S_0 signal (ΔS) is directly proportional to the dipolar coupling, hence the distance between the two spins.

xy8 $^{13}\text{C}\{^{15}\text{N}\}$ -REDOR pulse sequence¹¹³ with the dephasing ^{15}N pulses centered at $\text{Tr}/2$ and refocusing pulses centered at Tr with EXORCYCLE phase cycling¹¹⁴ of the final Hahn-echo $4\text{ }\mu\text{s}$ ^{13}C (150.8 MHz) π pulse was used. To compensate for pulse imperfections, both the $4\text{ }\mu\text{s}$ ^{13}C and $8\text{ }\mu\text{s}$ ^{15}N REDOR rotor synchronized dephasing π pulses were xy8 phase-cycled⁸⁶. The initial ^1H 90° pulse was $1.9\text{ }\mu\text{s}$, ^1H CP RF fields were ramped from 50 to 70 kHz and the ^{13}C CP RF field was kept constant at 50 kHz. 128 kHz Spinal64¹¹⁵ ^1H (600.3 MHz) decoupling was applied during REDOR evolution and acquisition. SPINAL64 pulse widths and ^1H decoupling resonance frequency were optimized by comparing the peak heights of the CH and CH_2 resonances of fumaric acid monoethyl ester.

REDOR data points are the integrated sum of center- and sideband peaks. Error bars were calculated using the noise of each spectrum as the maximum peak height deviation. To normalize for the decay due to T_2 , individual REDOR curves are plotted as $\Delta S/S_0$. The ideal scaling factor was determined with $^{13}\text{C}\{^{15}\text{N}\}$ REDOR spectra of $[1\text{-}^{13}\text{C}, ^{15}\text{N}]$ alanine diluted 10:1 with natural abundance alanine. This dilution ratio does not create a perfectly isolated spin-pair due to the probability of having an enriched $^{13}\text{C}/^{15}\text{N}$ alanine as a next nearest neighbor.¹⁰⁵ Correcting for this, the probe REDOR scaling factor was determined to be 93%.

Transmission Electron Microscopy Imaging

After addition to TEM grids, the amyloid peptide assemblies were allowed to adsorb for 1 min before excess peptide solution was wicked away with filter paper. Freshly prepared 2-wt % uranyl acetate solutions were added to TEM grids and incubated for 1–2 minutes before wicking and then placed in a vacuum desiccator overnight. TEM micrographs were recorded with a Philips 410 TEM at magnifications ranging from $7100\times$ to $69\,000\times$ with a Tungsten filament at an accelerating voltage of 80 kV. Negatives were scanned at 2000 dpi resolution on an Agfa DuoScan flatbed scanner (Agfa Corp., Ridgely Park, NJ). All staining solutions were prepared fresh and filtered prior to staining to minimize distinguishable artifacts.

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Figure 1.

(A) Pittsburgh Compound B (PiB), X=H and [^{19}F] PiB (X=F). Transmission electron microscopy (TEM) micrographs of A β (1–40) assembled (B) quiescently, or (C) with constant agitation. (D) [^3H]PiB binding to A β fibers assembled under quiescent (Q) or agitated (Ag) conditions.

Figure 2.

N-acetyl A β (16–22) peptide bilayer of anti-parallel β -sheets places lysine residues (colored blue) at both ends of each leaflet with TFA ions trapped within the peptide bilayer interface. Expansion shows 6 laminated β -sheets, viewed down the H-bonding axis, with alanine – valine cross-strand pairing within each β -sheet. Peptide is drawn as sticks with lysines colored blue and the remaining amino acids colored grey or white to highlight the two leaflets. TFA anions are shown as spheres with green fluorines, gray carbons and red oxygens.

Figure 3.

A β (1–42) assembled in the presence of lipid brain extract. (A) TEM micrographs of the co-assembly after 7 days, showing fibers (arrowhead) and vesicles (arrow with tail). $^{13}\text{C}\{^{15}\text{N}\}$ REDOR (B) full-echo (S_0) spectrum and (C) dephasing for $[1-^{13}\text{C}]\text{L34}[^{15}\text{N}]\text{V36}$ A β 42 assembled in lipid brain extracts. The i to $i+2$ $^{13}\text{CO}-^{15}\text{N}$ idealized distance for the α -helix is 3.3 Å, for an anti-parallel β -sheet is 4.4 Å, and for a parallel β -sheet is 4 Å.

Figure 4.

Molecular model of *N*-lauroyl Aβ(16–22) showing (A) positions of ¹³CH₃, [¹⁵N] Leu17 and Val18 and with distances consistent with ¹³C{¹⁵N}REDOR measurements places the ω-CH₃ of the lauroyl chain closer to the middle of the peptide than the ends. High-resolution SEM and resulting models for (B) *N*-acetyl and (C) *N*-lauroyl Aβ(16–22) highlight the position of the lauroyl chain with the increased laminate distance.