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Remodeling Cross- β Nanotube Surfaces with Peptide/Lipid Chimeras**

Rong Ni, W. Seth Childers, Kenneth I. Hardcastle, Anil K. Mehta,* and David G. Lynn*

Peptide/lipid chimeras, which are generally referred to as peptide amphiphiles, array specific peptides on the surface of ordered lipid assemblies.^[1] These materials are being explored as tissue scaffolds, in drug delivery, as antimicrobials, and for biomineralization applications.^[2–6] However, the increasing realization that simple peptides with high cross- β fold propensity can achieve long-range ordered arrays comparable to lipid amphiphiles has now allowed for the creation of an entirely new architectural framework. This framework not only offers precisely controlled positioning of charges and hydrophobic patches along a robust nanotube surface, but also the systematic adjustment of that spacing in a manner that to this point has been unrealized in self-assembling materials.

Peptide amphiphiles can achieve remarkable long-range order within a range of topological lipid phases.^[2–6] For example, the elongated worm-like micelles have been modeled as having alkane interiors with peptide surfaces,^[4] a morphology that reflects the same structural tension between head group and alkane that molds phospholipid assemblies.^[5] This structure suggests that various self-assembling peptide elements might be able to extend the standard amphiphile tensions into new frameworks, and focused our attention on the strong self-organizing potential of the cross- β fold.^[3,5,7,8] The nucleating core sequence of the amyloid forming A β -peptide associated with Alzheimer's disease, A β (16–22), K¹⁶LVFFAE²²-NH₂, which organizes into diverse cross- β assemblies,^[9–13] was acylated through conventional solid-phase Fmoc chemistry at the N-terminus with straight-chain fatty acids ranging in length from two to sixteen carbon atoms. The products were purified by RP-HPLC, assembled under a range of conditions, and the resulting assemblies scored by electron microscopy (EM).

Of the series assembled under acidic conditions, C₂ (*N*-acetyl) through C₄ (*N*-butyryl) assemblies were morpholog-

ically indistinguishable (Supporting Information, Figure S1), and assigned as hollow tubes based on the previously characterized *N*-acetyl-A β (16–22) assemblies.^[10] Intermediate acyl chain lengths, C₅ to C₁₀, assembled as ribbons, and longer lengths, C₁₄ and C₁₆, formed fibers (Supporting Information, Figure S1). Most notably however, the C₁₁ (*N*-undecanoyl), C₁₂ (*N*-lauroyl), and C₁₃ (*N*-tridecanoyl) A β (16–22) chimeras appeared as homogeneous tubes most similar to the *N*-acetyl assemblies, with the same distinct edges that arise from negative staining by uranyl acetate deposition inside and outside the tube cavity (Figure 1b).

Figure 1. a) Structure of *N*-lauroyl-A β (16–22); b) TEM, cryo-SEM (inset), and c) AFM micrographs of 1.2 mM *N*-lauroyl-A β (16–22) assembled in 40% acetonitrile/water with 0.1% trifluoroacetic acid for 1–2 weeks.

High-resolution cryo-SEM further confirmed the *N*-lauroyl-A β (16–22) assemblies as hollow nanotubes (Figure 1b, inset) and the diameters of (56 ± 8) nm were also remarkably similar to the well-characterized *N*-acetyl-A β (16–22) tubes.^[10] Powder X-ray diffraction analyses^[14] (Figure 2a) gave sharp and distinct reflections for the H-bonded β -strand repeat at 4.7 Å for all acyl chain lengths, but the lamination (β -sheet stacking) reflection varied systematically from 9.8 Å for *N*-acetyl-A β (16–22) tubes to 11.5 Å for *N*-lauroyl-A β (16–22) tubes (Figure 2b), to multiple laminate *d* spacings of 9.8 Å and ≥ 13 Å for the C₁₄ (*N*-myristoyl) and C₁₆ (*N*-palmitoyl) assemblies, respectively. This ability to systematically control the laminate spacing by simply changing the length of the acyl chain offers an as yet inaccessible materials design element and it focused our attention on the complete characterization of the homogeneous *N*-lauroyl-A β (16–22) tubes.

Electron diffraction^[13,15] (Figure 2c,d) and simulations^[16] (Supporting Information, Figure S2) from the top and bottom walls of oriented tubes^[10,17] defined a distinct tilt angle of the

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Figure 2. Powder and oriented electron diffraction of *N*-acyl-Aβ(16-22) peptide assemblies. a) X-ray powder diffraction of *N*-lauroyl-Aβ(16-22) (red) and *N*-acetyl-Aβ(16-22) tubes (black), indicating that each has a 4.7 Å H-bonding *d* spacing but differ in lamination *d* spacing (9.8 Å vs. 11.5 Å). b) Lamination spacing as a function of increasing *N*-terminal acyl chain length. c, d) Oriented electron diffraction of c) *N*-lauroyl-Aβ(16-22) tubes and d) *N*-acetyl-Aβ(16-22) tubes. The tube long axis is oriented vertically with respect to image. Each tube has two cross-β patterns, shown as solid blue and dashed red lines, owing to diffraction from the top and bottom tube walls.^[10,17]

spiraling ribbon along the tube axis. The *N*-lauroyl-Aβ(16-22) tube tilt angle was 11°, which is less than half the 27° angle of the *N*-acetyl-Aβ(16-22) tubes.^[10,17–18] This angle further implicates the laminate groove as the structural constraint most significantly impacted by addition of the *N*-lauroyl chain.

Indeed, the overall peptide architectures of the *N*-lauroyl and *N*-acetyl nanotubes were remarkably similar. Both tubes gave a wall thickness of 4 nm by atomic force microscopy (AFM) measurements (Figures 1c; Supporting Information, Figures S3 and S4), which is consistent with TEM and SEM analyses. Negatively charged gold colloidal particles bound to both assemblies with indistinguishable particle distribution and density (Supporting Information, Figure S5),^[10,19] thus suggesting that the terminal lysine residues were protonated and equally accessible on each tube surface. Additionally, the amide I IR stretching modes at 1628 cm^{−1} (Supporting Information, Figure S6) and the common shoulder at 1693 cm^{−1} were observed for both tubes and are diagnostic of antiparallel strand arrangements.^[20] Assemblies prepared with *N*-acetyl-[1-¹³C]F19 Aβ(16-22) and *N*-lauroyl-[1-¹³C]F19 Aβ(16-22) gave identical ¹²C and ¹³C band assignments at 1639 cm^{−1} and 1597 cm^{−1} (Figure 3),^[12,21] uniquely aligning the Phe19 carbonyl groups and positioning the strands in a one-residue-out-of-register antiparallel configuration (Figure 3b).^[10] Finally, when *N*-[1-¹³C]lauroyl-Aβ(16-22) peptides assembled in the presence of 0.1 % trifluoroacetic acid (TFA) were slowly bundled with Na₂SO₄/NADH and evaluated by

Figure 3. a) FTIR spectra of [1-¹³C]Phe19 *N*-lauroyl-Aβ(16-22) tubes (red), *N*-acetyl Aβ(16-22) tubes (black), and *N*-acetyl Aβ(16-22) fibers (blue). The vertical dashed lines highlight the ¹²C and ¹³C stretches at 1639 cm^{−1} and 1597 cm^{−1} respectively, consistent with b) antiparallel one-residue out-of-register β-sheets.^[10] *N*-Acetyl-Aβ(16-22) fibers assembled at neutral pH served as a control for antiparallel in-register β-sheets.^[10] Yellow circles denote [1-¹³C]Phe19 carbonyl carbon atoms and green circles indicate the position of acyl chain.

solid-state NMR, ¹³C{¹⁹F}REDOR,^[22] at least 50 % of the ¹³C signal was dephased by fluorine (Supporting Information, Figure S7), which is consistent with similar TFA access to the C₂ and C₁₂ bilayer surfaces.^[23]

These structural constraints placed the C₁₂ acyl chain between the laminated β-sheets, but further constraints were required to position the acyl chain relative to the peptide backbone. We first attempted to localize the carbonyl end of the acyl chain by preparing and assembling *N*-[1-¹³C]lauroyl-[¹⁵N]L17 Aβ(16-22) for solid-state NMR analyses. The unbiased fit using a Boltzmann maximum-entropy approach (BS-REDOR)^[24] of the ¹³C{¹⁵N}REDOR dephasing data as isolated ¹³C-¹⁵N spin pairs (Figure 4c,d) indicated that 92 % of *N*-lauroyl carbonyl carbon atoms were 3.8 Å from the leucine amide backbone nitrogen atoms. A molecular mechanics conformational search of the assembly without distance constraints showed that the acyl chain could bend over the β-sheet sidechains and give a distance of (3.7 ± 0.3) Å from the lauroyl carbonyl carbon to the Leu17 nitrogen (Supporting Information, Figure S8). For reference, a fully extended chain (Figure 4a), analogous with the peptide amphiphile model, would have a lauroyl ¹³CO to [¹⁵N]Leu17 distance of 4.5 Å.

To constrain the remainder of the acyl chain, both *N*-[12-¹³C]lauroyl-[¹⁵N]V18 Aβ(16-22) and *N*-[12-¹³C]lauroyl-[¹⁵N]L17 Aβ(16-22) chimeras were prepared and assembled separately. The lauroyl ω-¹³CH₃ ¹³C{¹⁵N}REDOR dephasing in the ¹⁵N Val18 assemblies identified two distances (4.8 Å and 6 Å; Figure 5d). ¹³C dephasing in the [¹⁵N]Leu17 assemblies resolved as a single distance of 6.4 Å (Figure 5e); however at this distance, assuming that each ¹³C is coupled to only a single ¹⁵N, about 20 % of the ω-CH₃ carbon atoms have dipolar couplings that are too weak to measure, even with 120 ms REDOR evolution times. The distance measurements on both ω-¹³CH₃-enriched samples were consistent with the lauroyl chain methyl group being closer to the valine18

Figure 4. NMR ^{13}C – ^{15}N distance measurements to localize acyl chain within cross- β laminate. a) Peptide amphiphile model with a fully extended and b) bent lauroyl chain. The peptide pleated backbone is shown as black lines, the position of the leucine nitrogen is shown as blue spheres, the lauroyl chain is drawn as a red line, and the lauroyl chain CO as red spheres. c) $^{13}\text{C}\{^{15}\text{N}\}$ REDOR dephasing with calculated dephasing curve for distances from d) BS-REDOR fit, consistent with a ^{13}CO to ^{15}N Leu distance centered at 3.7 Å.

Figure 5. NMR ^{13}C – ^{15}N distance measurements to distinguish between a) extended and b) bent lauroyl chain with CH_3 laying over peptide within cross- β laminate. The leucine (black) and valine (blue) nitrogen atoms and lauroyl methyl (red) are drawn as spheres. c) $^{13}\text{C}\{^{15}\text{N}\}$ REDOR measurement of N -[$^{12-13}\text{C}$]lauroyl-[^{15}N]L17 A β (16-22) (blue) and N -[$^{12-13}\text{C}$]lauroyl-[^{15}N]V18 A β (16-22) (black) peptide nanotubes. BS-REDOR fits (solid lines) with $^{13}\text{CH}_3$ to ^{15}N Val distances centered at 4.8 Å (30%) and 6 Å (55%; blue), shown in (d), and with $^{13}\text{CH}_3$ to ^{15}N Leu distances centered at 6.5 Å (80%; black), shown in (e).

nitrogen than the leucine17 nitrogen, and maps of the possible solutions for these NMR constraints place the ω - $^{13}\text{CH}_3$ well within the cross- β core (Figure 6a). Constraining both ends of

Figure 6. a) Allowable positions of the terminal $^{13}\text{CH}_3$ carbons for N -lauroyl-K¹⁶ LVFFAE²²-NH₂ that match the ^{13}C – ^{15}N distances of 6.4 Å to ^{15}N Leu and 4.8 Å to ^{15}N Val (red spheres) or 6.4 Å to ^{15}N Leu and 6 Å to ^{15}N Val (green spheres). Blue spheres highlight leucine and valine nitrogen atoms. b) Model of lauroyl chain (red) packing within the β -strand. Lysine residue is colored blue; the peptide backbone and side chains are gray. c) Orientation and packing of N -lauroyl-KLVFFAE peptides within tube. Expansion shows three laminated β -sheets and a peptide–peptide bilayer, consistent with the structural data. d) Comparison of solvent-exposed surface patterns highlighting the lipophilic potential^[26] for N -lauroyl and N -acetyl KLVFFAE tubes. The most hydrophobic (lipophilic) regions are colored brown and the least blue.

the acyl chain restricts the chain to a well-specified position within the laminated β -sheets (Figure 6b,c). The resulting model for the N -lauroyl-KLVFFAE peptide–amphiphile assembly increases both the distance between adjacent β -sheets (Figure 6c) and the hydrophobicity of the solvent exposed tube surfaces (Figure 6d) relative to the N -acetyl capped peptide.

These data highlight a remarkable plasticity of the cross- β structural element as revealed in this unique and unexpected capacity of the cross- β fold to accommodate acyl chains within the interior of the β -sheet laminate. Although the structure models suggest that the C_{12} acyl chain does not

occupy the complete laminate surface, it is positioned to fill a well-defined cavity around these side chains. The shorter C₅ to C₁₀ chain may only partially occupy this laminate cavity and therefore not assemble as a single thermodynamic assembly, whereas the longer C₁₄ to C₁₆ alkanes may assemble into multiple structures. Isotope-edited IR of [1-¹³C]Phe19-enriched peptides (Supporting Information, Figure S9) indicate that the β -sheet registry for chain lengths from C₂ to C₁₃ are identical with antiparallel out-of-register β -sheets, but that C₁₄ and C₁₆ A β (16-22) form parallel β -sheets. The parallel β -sheet registry would allow for peptide amphiphile conformations, as suggested in worm-like micelle and nanobelt models, where acyl chains are sequestered from solvent and charged head groups are exposed to solvent;^[4-6,8,25] however, further solid-state NMR analysis is needed to localize the C₁₄ and C₁₆ acyl chains with respect to the peptide backbone.

A key difference between the *N*-lauroyl-A β (16-22) peptide amphiphile model (Figure 5c) and typical worm-like micelles is the antiparallel β -sheet registry and burial of half of the positively charged lysine sidechains, which are passivated by negatively charged TFA anions (Supporting Information, Figure S7) as previously observed^[23] in the *N*-acetyl A β (16-22) bilayer structure. Remarkably however, the antiparallel β -sheet registry and the ability of these simple cross- β assemblies to expand in an accordion-like fashion (Figure 1b) to accommodate acyl chains between the stacks of β -sheets greatly extends the molecular geometries available to cross- β assemblies and introduces a radically new phase for the peptide amphiphiles that must now be considered in the structural models for all known assemblies.^[2-4] The ability to systematically alter the spacing on these solvent accessible surfaces, ones already known to bind small molecules^[18] and metal arrays,^[27] by simply incorporating a saturated carbon chain within the cross- β laminate core will provide entirely new strategies for the creation of well-ordered mesoscale assemblies.^[5,25,28] It has also not escaped our attention that other amyloid and prion proteins could well sequester lipids as they assemble, particularly those generated within biological membranes, providing further insight into the physiological data that implicates more complex aggregates as critical for disease symptoms.^[29]

Experimental Section

Peptides were synthesized by standard solid-phase Fmoc (9-fluorenylmethoxycarbonyl) chemistry.^[10] N-termini of *N*-acyl-A β (16-22) were capped with standard coupling of the corresponding acyl acid with resin overnight. The ¹³C{¹⁵N} REDOR pulse sequence^[22] consists of an *S* sequence with both ¹³C and ¹⁵N π pulses, and the *S*₀ sequence, which does not contain any ¹⁵N pulses. The difference between the REDOR *S* and *S*₀ signal (ΔS) is directly proportional to the dipolar coupling, and thus the distance between the two spins.

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Supporting Information

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Supporting information

Peptide synthesis and purification: The peptides *N*-X-A β (16-22) (X=acetyl to palmitoyl) were synthesized through standard Fmoc/HBTU chemistry with Fmoc Rink-amide polystyrene resin (Anaspec, Inc.) on a Rainin Symphony Quartet peptide synthesizer. The first 5 residues (VFFAE) were single coupled for 2 hours and the last two residues (Lys16 and Leu17) double coupled for 4 hours. The N-terminus of *N*-acyl-A β (16-22) peptides were capped manually by coupling the corresponding fatty acid with resin overnight with HBTU/NMM activation. The dried resin was mixed with cleavage cocktail (90 vol% TFA, 5 vol% thioanisole, 3 vol% ethanedithiol and 2 vol% anisole) over 1.5 hours at room temperature to cleave the peptide from the resin. Cleavage solution was added drop-wise to cold diethylether 4 times to extract the crude peptide. The peptide was purified by reverse-phase HPLC (Waters Delta 600) using a Waters Atlantis C-18 preparative column (19 x 250mm) and employed a linear gradient at 10 mL/min starting at 20% acetonitrile and ending with 95% acetonitrile over 75min. Water and acetonitrile mobile phases each included 0.1% TFA. Crude purity was typically 80-90%. After removing acetonitrile under vacuum, peptide fractions were frozen and lyophilized to yield a peptide powder that was stored at 4°C. Product purity of greater than 99% by peak area was confirmed by reverse phase HPLC using a Water Atlantis C-18 (4.6 x 250mm) analytical column, with identical gradient as for preparative HPLC, but with a flow rate of 1mL/min. Product mass was confirmed by MALDI-TOF (Voyager-DETM STR Biospectrometry Workstation) with α -Cyano-4-hydroxycinnamic acid (CHCA) matrix. For isotope-enriched peptides, the same procedure was applied except the isotope-enriched amino acid (Cambridge Isotopes, Andover, MA) was manually coupled for 4 hours.

Transmission Electron Microscopy (TEM): A TEM copper grid with a 200 mesh carbon supported (Electron Microscopy Sciences, Hatfield, PA) was covered with 10 μ L of a diluted nanotube sample (0.05 mM to 0.1 mM) for 1 minute before removing excess solution with filter paper. A 10 μ L staining solution (2% uranyl acetate, Sigma-Aldrich) was added and incubated for 2 minutes. After wicking away excess solvent, sample grids were dried under vacuum overnight. A Hitachi H-7500 transmission electron microscope was used to image the samples at 75 kV. There is variation in sample density from square to square on a given EM grid even when the same volume and concentration are added to the EM grids. Imaged regions were chosen based on optimizing for sample density and to reflect all the species observed on the entire grid. Within these areas, the surface coverage of non-tube species is less than 1 % of observed tube assemblies on the grid.

Atomic Force Microscopy (AFM): 20 μ L of assembled peptide solutions were diluted to desired concentration and placed on a cleaned silicon chip (4" diameter diced silicon wafer (TedPella, Inc, Redding, CA) cleaned by sonication in methanol for 20min) for one minute at room temperature. Excess solution was removed with filter paper and the chip was rinsed with distilled H₂O. Tapping mode analysis on a JEOL JSPM-4210 scanning probe microscope employed ultra-sharp non-contact silicon cantilevers (MikroMasch, Wilsonville, OR) with typical frequencies between 240 and 350 KHz. Images were collected under dry conditions.

Electron Diffraction: 15 μ L of assembled nanotubes (0.2 mM to 0.5 mM) was incubated on a TEM grid for 1min, followed by slowly wicking excess solution in one direction to align tubes on grids. These micrographs were recorded on Philips 4500 transmission electron microscope in diffraction mode. *d*-spacing, where $d = \lambda L / R$, was calculated, where *R* is half the distance (mm) between two opposite arcs, λ is the electron wavelength (75 kV), and *L* is the camera length (distance in mm between specimen and photographic film), calibrated using an aluminum polycrystalline standard (Electron Microscopy Sciences, Hartfield, PA).

X-ray powder diffraction

Assembled peptide nanotubes were bundled with sulfate or nicotinamide adenine dinucleotide (NADH) with the ratio of peptide to sulfate 1:10 or peptide to NADH 1:4. The white precipitate was collected by centrifugation. The pellet was frozen and lyophilized to yield dry powder for X-ray diffraction. The powder spectra were obtained using a Bruker D8 Discover diffractometer, equipped with a multiposition X,Y,Z stage, a cobalt X-ray tube with Goebel mirror and a Vantec-1 solid state detector. The sample was placed in a zero-background holder on the stage and the spectrum obtained using Bragg-Brentano geometry. The scan step was repeated several times to maximize the diffracted intensity and minimize noise.

Cryo-Etch High-Resolution SEM: 2 μ L nanotube solution (0.2mM) on gold plunchet was plunge-frozen in liquid ethane.^{1,2} Frozen sample was transferred and mounted in a precooled (-180°C) Gatan 500 CT cryostage, followed by fracturing of the sample surface with a pre-chilled blade. The cryostage was transferred to a Denton DV-602 Cr coater, where the sample temperature was gradually increased to -105°C over 70min and held at that temperature to sublime ice for 10 min at 0.3 μ Torr. The etched sample was quickly cooled to -170 °C. The Cr coater was flushed with 5mTorr nitrogen for 5min to remove air. Then the sample was sputter-coated with 4.5nm Cr at a rate of 0.3 Å/s and a current of 50 mA under 2 mTorr. After the coated sample was transferred to a DS-130F scanning electron microscope, the cryostage was heated slowly to -120°C and equilibrated for 30 min before imaging.

Fourier Transform Infrared (FTIR): Spectra were collected on a Nicolet MAGNA-IR 560 Spectrometer with 4 cm^{-1} resolution and generally 100 scans were averaged. For sample preparation, the mature nanotubes were bundled with NADH with the molar ratio of peptide to NADH 1:4. The white precipitate was pelleted at 16,100Xg for 5 minutes to remove un-assembled material, and lyophilized to yield dry powder. The dried powder was mixed well with grounded KBr (at a ratio of 1:10 (w/w)) and pressed into a transparent disk for IR analysis.

Circular Dichroism spectroscopy: Jasco-810 CD spectropolarimeter was used to record CD spectra of 20 μ L sample in 0.1 mm path length cell at room temperature. Spectra are the average of three scans between 260nm to 190nm with a step size of 0.2 nm and a speed of 100 nm/s.

Molecular Modeling. Conformational searches were performed with the MMFFs force-field within MacroModel³ (Schrodinger, Inc. Portland, OR) with mixed torsional/low mode sampling. Three peptides with an anti-parallel out-of-register conformation were constructed, including a protonated glutamic acid side chain. Only the middle peptide had the *N*-lauroyl acyl chain. All conformers within 19 kJ/mol of the lowest energy structure were retained. Multiple conformational searches were performed with different starting orientations for the acyl chain. Lowest and highest energy structures from the resulting conformational searches were used as starting points for new conformational searches. This resulted in two families of conformers, with the acyl chain being on either β -sheet face. In these searches, all atoms were frozen except for the acyl chain and lysine residues and the glutamic acid or leucine side-chain atoms or the valine side-chain atoms closest to the acyl chain. No distance restraints were used in the conformational search

NMR: Mature nanotubes were bundled with sulfate or NADH with the ratio of peptide to sulfate 1:10 or peptide to NADH 1:4. Previously, sulfate bundling of nanotubes has been shown to protect assemblies from freezing and lyophilization.⁴ The resulting white precipitate was pelleted by centrifugation, frozen and lyophilized to yield dry powder. Each NMR sample (25-50 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 MAS and radio-frequency (RF) heating did not denature the samples. ^{13}C (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 $^{13}\text{C}\{^{15}\text{N}\}$ rotational-echo double-resonance (REDOR)⁵ consists of two parts, an S sequence with both ^{13}C and ^{15}N pulses, and the S_0 sequence which is identical but does not contain any ^{15}N dephasing pulses. Applying pulses to the dephasing ^{15}N spins interferes with the averaging due to magic-angle spinning and reintroduces the dipolar coupling which 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 and ^{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 sequence⁶ with the dephasing ^{15}N pulses centered at $\text{Tr}/2$ and refocusing pulses centered at Tr with EXORCYCLE phase cycling⁷ of the final Hahn-echo $4\ \mu\text{s}$ ^{13}C (150.8 MHz) π pulse was used. To compensate for pulse imperfections both the $4\ \mu\text{s}$ ^{13}C and $8\ \mu\text{s}$ ^{15}N REDOR rotor synchronized dephasing π pulses were xy8 phase cycled⁵. The initial ^1H 90° pulse was $1.9\ \mu\text{s}$, ^1H CP RF fields were ramped from 50 to 70 kHz and the ^{13}C CP RF field was kept constant at 50kHz. 128 kHz Spinal64⁸ ^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.

Long REDOR evolution times required to measure the weak dipolar couplings between the lauroyl $^{13}\text{CH}_3$ carbons and the peptide backbone ^{15}N 's are susceptible to RF homogeneity.^{7,9} RF homogeneity will lead to a lower REDOR dephasing plateau than predicted with stronger dipolar couplings. Therefore, to determine proper level for ^{13}C 180° pulses the power level was arrayed in REDOR S_0 pulse sequence at long REDOR evolution times (>50 ms typically corresponding to ~ 500 $4\ \mu\text{s}$ ^{13}C π pulses) and choosing the power level that corresponded to the maximum signal intensity (Fig S10). Similarly, ^{15}N 180° pulses were determined by arraying the ^{15}N power level using the REDOR S experiment at REDOR evolution times corresponding to a $\Delta S/S_0$ between 0.3 and 0.5 (Fig. S11). Comparison of optimized vs. non-optimized pulses on the REDOR dephasing are shown in Fig. S12.

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 $\Delta S/S_0$. A ^{13}C -CP-Echo spectrum was run before and after each REDOR collection to ensure that the sample had not degraded. The ideal scaling factor was determined with $^{13}\text{C}\{^{15}\text{N}\}$ REDOR spectra of [1- ^{13}C , ^{15}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.¹⁰ Correcting for this, the probe REDOR scaling factor was determined to be 93%.

Figure S1. TEM of A β (16-22), K¹⁶LVFFAE²²-NH₂, peptides with various *N*-acyl chain lengths, assembled at pH2 in 40% acetonitrile. Morphologies consistent with nanotubes are boxed in red. Cryo-SEM are shown as insets for *N*-acetyl-A β (16-22) and *N*-lauroyl-A β (16-22) assemblies.

Figure S2. Oriented electron diffraction of *N*-acyl-A β (16-22) peptide assemblies. (c) *N*-Lauroyl-A β (16-22) tubes, (b) *N*-acetyl-A β (16-22) tubes and (c,d) simulations. Panels a and b are identical to Fig 2 c,d in the main text and are shown here for comparison to the calculated diffraction patterns. Tube long-axis is oriented vertically with respect to image. The two sets of cross- β patterns for the tubes are indicated with solid blue and dashed red lines (see main text). Unit-cell parameters used in Clearer¹¹ simulations (e) $a=4.7\text{\AA}$, $b=11.5\text{\AA}$, $c=6.9\text{\AA}$, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma=88^\circ$, fiber axis (1,0.194,0) and (F) $a=4.7\text{\AA}$, $b=9.9\text{\AA}$, $c=44\text{\AA}$, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma=90^\circ$, fiber axis (1,0.466,0).

Figure S3. Example AFM micrographs of (Left) *N*-acetyl-Aβ(16-22), CH₃CO-K¹⁶LVFFAE²²-NH₂, and (Right) *N*-lauroyl-Aβ(16-22), CH₃(CH₂)₁₀CO-K¹⁶LVFFAE²²-NH₂, peptides assembled into nanotubes. AFM heights are average of 10 separate height measurements.

Figure S4. Peptide orientation with respect to tube axis. Oriented electron diffraction indicates that the β -sheet H-bonds run parallel to the blue lines on the tube surface and the peptide long-axis makes up the tube wall. The length of *N*-lauroyl-A β (16-22) anti-parallel out-of-register β -sheets with fully extended *N*-lauroyl chains is shown in the right panel. The orientation shown here would have the solvent exposed tube surface be composed of hydrophobic chains.

C-2 KLVFFAE

C-12 KLVFFAE

Figure S5. TEM images of gold colloid bound nanotubes. **(A)** *N*-Acetyl-A β (16-22) tubes; **(B)** *N*-lauroyl -A β (16-22) tubes. The tubes were prepared in 40% acetonitrile/water with 0.1% TFA for 2 to 3 weeks. The aliquot of tubes were mixed with negatively-charged gold colloid with molar ratio of peptide to gold 1:10. After 1 hour incubation, the purple precipitate was pelleted at 16,100 xg for 5 minutes and resuspended in fresh water for TEM sample preparation and imaging.

Figure S6. FT-IR of *N*-acetyl-Aβ(16-22) tubes (black) and *N*-lauroyl-Aβ(16-22) (red) tubes.

Figure S7. $^{13}\text{C}\{^{19}\text{F}\}\text{REDOR}$ dephasing of N -[1- ^{13}C]acetyl- $\text{A}\beta(16-22)$ (orange)¹² and N -[1- ^{13}C]lauroyl- $\text{A}\beta(16-22)$ peptides assembled in 0.1% TFA and 40% acetonitrile. Tubes were slowly bundled with $\text{Na}_2\text{SO}_4/\text{NADH}$. The REDOR dephasing plateau for the N -[1- ^{13}C]Acetyl- $\text{A}\beta(16-22)$ nanotubes indicates that, as shown previously,¹² only $\frac{1}{2}$ of the acetyl ^{13}CO are close to an ^{19}F . This was attributed to the TFA being the counter-ion for lysines within the bilayer, but being replaced by sulfate during bundling for the solvent exposed lysines. The plateau for the N -[1- ^{13}C]lauroyl- $\text{A}\beta(16-22)$ tubes is slightly higher than for the N -[1- ^{13}C]acetyl- $\text{A}\beta(16-22)$ tubes. This is still consistent with TFA buried within the N -lauroyl- $\text{A}\beta(16-22)$ tubes peptide bilayer. The slightly higher plateau for the N -[1- ^{13}C]lauroyl- $\text{A}\beta(16-22)$ $^{13}\text{C}\{^{19}\text{F}\}$ dephasing could be due to incomplete removal of TFA from the solvent exposed lysine sidechains due to the addition of NADH as the bundling agent for N -lauroyl- $\text{A}\beta(16-22)$ tubes or the presence of a peptide-peptide-peptide trilayer. However, the oriented electron diffraction placement of the long-axis of the peptide chain perpendicular to the electron beam, hence the wall being made up by the length of the peptide, and the AFM measurement of the wall thickness (Fig S3) rules out the possibility of a peptide-peptide-peptide trilayer.

Figure S8. Family of structures within 19kJ/mol of lowest energy structure of Macromodel conformational search using the MMFFs force field, for *N*-lauroyl-A β (16-22) peptides. No distance restraints were used, yet all structures had a lauroyl chain carbonyl carbon distance of $3.7\text{\AA} \pm 0.3\text{\AA}$ to the leucine nitrogen. Three anti-parallel out-of-register peptides consistent with the isotope-edited IR (Fig 3 – main text) were used in the search. The *N*-lauroyl group was attached to only the middle chain to approximate an infinitely long β -sheet. All atoms except for the lysine attached to the lauroyl chain and the lauroyl chain were frozen. This 3 peptide configuration does not test for interactions between chains within the same sheet (which would be spaced $9.4\text{\AA}$ apart) or between the lauroyl chain atoms and sidechain atoms to adjacent β -sheets (which would be spaced by $11.5\text{\AA}$).

Figure S9. Isotope-edited IR spectra of $[1-^{13}\text{C}]$ F19 enriched *N*-acylated A β (16-22) peptides assembled at acidic pH. Acyl group and number of carbons are indicated at the right side of each trace. An IR-stretch at 1693cm^{-1} is consistent with anti-parallel β -sheets. The ^{12}C and ^{13}C bands at 1638cm^{-1} and 1597cm^{-1} respectively indicate anti-parallel out-of-register β -sheets. The absence of an IR stretching frequency at 1693cm^{-1} for C-14 and C-16 A β (16-22) peptides suggests a parallel β -sheet registry, however, the observed ^{13}C splitting in parallel β -sheets should correspond to an IR stretch at $\sim 1605\text{cm}^{-1}$.^{13,14} The observed weaker splitting for the ^{13}C band suggests that the Phe19 carbonyl carbons are further apart than in an in-register β -sheet, however the precise registry will require further solid-state NMR characterization.

Figure S10. Normalized $^{13}\text{C}\{^{15}\text{N}\}$ REDOR S_0 intensity as a function of ^{13}C π -pulse power level. In each experiment, the ^{13}C pulse width was fixed to $4\mu\text{s}$ and rotor-synchronized pulses were applied every rotor period for a total of 16-Tr (black), 288-Tr (red), 544-Tr (green) and 1056-Tr. The vertical dashed line indicates the power-level that was measured with the simple CP-Flip Back – Dipolar Dephase – Read pulse sequence to optimize a $4\mu\text{s}$ 180° pulse. Using the power level determined from the flip-back sequence for the ^{13}C π -pulse results in incomplete refocusing as the number of ^{13}C π -pulses are increased. A narrow range of power-levels give maximum signal (hence maximum refocusing) for 1056 refocusing pulses. In the above example, a ^{13}C power level corresponding to the Bruker pulse programming parameter of -2.4dB was chosen.

Figure S11. Normalized $^{13}\text{C}\{^{15}\text{N}\}$ REDOR S intensity as a function of ^{15}N dephasing π -pulse power level. In each experiment, the ^{15}N pulse width was fixed to 8 μs and rotor-synchronized pulses were applied every rotor period at Tr/2 for a total of 544-Tr (black) and 1056-Tr (red). The dephasing pulse power-level is much less sensitive than the ^{13}C refocusing pulse widths shown in Fig. S10. In this example a power level of -3.8db was chosen for the $^{13}\text{C}\{^{15}\text{N}\}$ REDOR experiment.

Figure S12. $^{13}\text{C}\{^{15}\text{N}\}$ REDOR dephasing showing the effect of optimized pulses shown in Figs S10,S11 (red) vs. pulses and power levels measured with the CP - Flip Back – dipolar dephase – Read pulse sequence (black). At short REDOR evolution times <50ms the REDOR dephasing is less sensitive to power-level/pulse width mismatch, than at longer REDOR evolution times.

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