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Atomic structure and hierarchical assembly of a cross- β amyloid fibril

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The cross- β amyloid form of peptides and proteins represents an archetypal and widely accessible structure consisting of ordered arrays of β -sheet filaments. These complex aggregates have remarkable chemical and physical properties, and the conversion of normally soluble functional forms of proteins into amyloid structures is linked to many debilitating human diseases, including several common forms of age-related dementia. Despite their importance, however, cross- β amyloid fibrils have proved to be recalcitrant to detailed structural analysis. By combining structural constraints from a series of experimental techniques spanning five orders of magnitude in length scale—including magic angle spinning nuclear magnetic resonance spectroscopy, X-ray fiber diffraction, cryoelectron microscopy, scanning transmission electron microscopy, and atomic force microscopy—we report the atomic-resolution (0.5 Å) structures of three amyloid polymorphs formed by an 11-residue peptide. These structures reveal the details of the packing interactions by which the constituent β -strands are assembled hierarchically into protofilaments, filaments, and mature fibrils.

It is well established that a wide variety of peptides or proteins without any evident sequence similarity can self-assemble into amyloid fibrils (1, 2). These structures have many common characteristics, typically being 100–200 Å in diameter and containing a universal “cross- β ” core structure composed of arrays of β -sheets running parallel to the long axis of the fibrils (3). These fibrillar states are highly ordered, with persistence lengths of the order of microns (4) and mechanical properties comparable to those of steel and dragline silk, and much greater than those typical of biological filaments such as actin and microtubules (5). Amyloid fibrils can also possess very high kinetic and thermodynamic stabilities, often exceeding those of the functional folded states of proteins (6), as well as a greater resistance to degradation by chemical or biological means (7). Several functional forms of proteins that exploit these properties have been observed in biological systems (8). More generally, however, the conversion of normally soluble functional proteins into the amyloid state is associated with many debilitating human disorders, ranging from Alzheimer’s disease to type II diabetes (1, 9). Our understanding of the nature of this type of filamentous aggregate has greatly improved in recent years (3, 10–19), particularly through the structural determination of their elementary β -strand building blocks (20) and the characterization of their assembly into cross- β steric zippers (21, 22). However, a thorough understanding of the hierarchical assembly of these individual structural elements into fully-formed fibrils, which display polymorphism but possess a range of generic features (23), has so far been limited by the absence of a complete atomic-resolution cross- β amyloid structures (2).

We report here the simultaneous determination of the atomic-resolution structures of a cross- β amyloid fibril and two polymorphic variants, formed by an 11-residue fragment of the protein transthyretin, TTR(105–115) (20). These fibrils have the classic amyloid morphology, being 100–200 Å in diameter and

typically 1–3 μ m in length (*SI Appendix, Fig. S1*). We have achieved this objective by bringing together a set of complementary biophysical techniques to provide atomic structures of these complex aggregates. Specifically, we have combined interatomic structural restraints from magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy with high-resolution electron density maps from cryoelectron microscopy (cryo-EM), together with data from X-ray fiber diffraction, scanning transmission electron microscopy (STEM), and atomic force microscopy (AFM) measurements. Our results reveal the molecular basis of the stability and polymorphism of these amyloid fibrils by defining at high resolution the variety of structural elements in their hierarchical self-assembly.

Results

Assembly of Individual Molecules into β -Sheet Arrays. A large number of intramolecular distance and torsion angle restraints (76 total) have been measured previously using MAS NMR methods, resulting in the high-resolution structure of the individual TTR(105–115) molecules in amyloid fibrils (20). To extend these studies to probe higher-order elements in the amyloid assemblies by identifying site-specific intermolecular restraints, we prepared eight different samples, each having a single isotopically labeled carbonyl atom per TTR(105–115) molecule at one of the residues I107 to P113 and S115. Double quantum dipolar recoupling experiments (24) on these singly labeled samples provided eight high-precision (<0.2 Å), intrasheet ¹³CO–¹³CO distance restraints (*SI Appendix, Table S1 and Fig. S24*). The restraints span the entire backbone of the extended β -strand conformation adopted by TTR(105–115) peptides in fibrils (*SI Appendix, Table S1 and Fig. S24*) and unequivocally define a parallel, in-register arrangement of neighboring strands within the β -sheets.

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The authors declare no conflict of interest.

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Data deposition: The structures of the TTR(105–115) amyloid fibrils have been deposited in the Protein Data Bank, www.pdb.org [PDB ID code 2m5n (Protofilament), 2m5k (Doublet), 2m5m (Triplet), and 3zpk (Quadruplet)] and the Electron Microscopy Data Bank [EMDB accession no. EMD-5590 (Doublet), EMD-2323 (Triplet), and EMD-2324 (Quadruplet)].

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Although knowledge of the intrasheet registry drastically reduces the number of permutations for the peptide molecules within the structure of the protofilaments, the number of possible intersheet arrangements is still very large (22). To address the challenge of defining this level of structural detail, we prepared two separate TTR(105–115) samples labeled uniformly with ^{13}C and ^{15}N isotopes at (underscored) residues YTIAALLSPYS and YTIAALLSPYS to characterize selectively the intermolecular packing of adjacent β -sheets in the fibrils. Internuclear distance restraints from Z-filtered transferred echo double resonance (ZF-TEDOR) (25) and rotational resonance in the tilted frame width (R2TRW) (26) experiments on centrally labeled YTIAALLSPYS samples (*SI Appendix*, Table S2 and Fig. S2B) strongly suggest that the β -strands in a given β -sheet are antiparallel to those in the adjacent β -sheet.

To obtain further information on this crucial issue, we carried out additional ZF-TEDOR experiments using terminally labeled YTIAALLSPYS samples and observed three clearly resolved cross-peaks (Fig. 1A and *SI Appendix*, Fig. S3). The mixing-time dependence of the cross-peak intensities yielded three highly precise (± 0.3 Å) internuclear distances (*SI Appendix*, Table S2). This precision establishes an antiparallel intersheet stacking of β -sheets because not only do the contacts arise between residues at opposite ends of the TTR(105–115) molecule, but also the measured distances are much too short to occur either intramolecularly or within a parallel, in-register β -sheet. These measurements were further complemented by proton-driven ^{13}C spin diffusion (PDS) (27) experiments (Fig. 1B and *SI Appendix*, Fig. S4) on the YTIAALLSPYS sample; cross-peaks from these spectra provided a total of 16 ^{13}C - ^{13}C intersheet restraints (*SI Appendix*, Table S2 and Fig. S2B). The complete set of 23 intersheet distance restraints (*SI Appendix*, Table S2 and Fig. S2B) reveals the specific manner in which adjacent β -sheets within the fibril are stacked in an

antiparallel arrangement, thereby solving the first crucial aspect of the assembly of these multimolecular species.

The quantitative intersheet constraints between L111 and A108 and A109 (Fig. 1C, blue dashed lines), between A108 and S112 and P113 (Fig. 1C, red dashed lines), and between I107 and S112 (Fig. 1C, red dashed lines) are consistent only with a structure in which the even-numbered side chains, TALS Y (Fig. 1C, violet sticks), are packed against odd-numbered side chains, YIALPS (Fig. 1C, orange sticks), thus yielding an even–odd–even–odd interface between the sheets (Fig. 1C). This arrangement means that the side chains of all residues in the sequence appear both on the internal (dry) and external (wet) faces of each protofilament, and so in principle two sets of chemical shifts could be expected for each site. Indeed, some side chains do exhibit clear peak doubling, for example, I107C δ , C γ 1 and C γ 2 and S112C α and C β as illustrated in *SI Appendix*, Fig. S5. Additionally, Y105 displays two conformations for the ^{15}N H $_3^+$ and $^{13}\text{C}\alpha$ (*SI Appendix*, Fig. S5). Because some side chains are more prone to dispersion than others, the chemical shift differences between the two different conformations for other cross-peaks are not resolved but contribute to the inhomogeneous broadening of the cross-peaks. For example, the linewidths of A108C β , A109C β , L110C δ , and L111C δ are ~ 1.0 – 1.2 ppm. Similar doubling of some peaks has also been detected for polyglutamine (poly-Q) fibrils, although in this case this phenomenon is likely to arise from an antiparallel β -sheet structure, which is also a packing geometry leading to residues alternately pointing into (buried) and out (solvent exposed) of the fibril (28, 29). The intensities of the peaks that exhibit doubling in *SI Appendix*, Fig. S5 are not 1:1, unlike the peaks in poly-Q spectra (28, 29), because here, the solvent exposed side chains are more likely to be dynamic than those buried in the dry interface between the β -sheets.

A summary of the restraints used in the calculation of the protofilament structure is given in *SI Appendix*, Table S1 (intra-sheet; *SI Appendix*, Fig. S2A) and *SI Appendix*, Table S2 (inter-sheet; *SI Appendix*, Fig. S2B). The resulting set of structures, which were calculated with an average of 10 restraints per residue, has a backbone heavy atom root mean square deviation to the mean of the 20 lowest energy conformers in the NMR ensemble of 0.4 Å for the backbone and 0.7 Å for all heavy atoms (*SI Appendix*, Fig. S6). There are no residues with torsion angles in disallowed regions of the Ramachandran plot (*SI Appendix*, Table S3). Complete details concerning the structure calculations and statistics are provided in *SI Appendix*, *SI Materials and Methods* and Table S3.

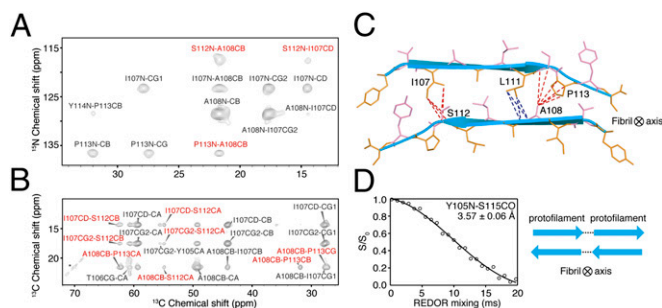


Fig. 1. MAS NMR experiments used to probe the intermolecular arrangement of TTR(105–115) fibrils. (A and B) Shown are 2D ^{13}C - ^{15}N ZF-TEDOR ($\tau_{\text{mix}} = 10.24$ ms) (A) and 2D ^{13}C - ^{13}C PDS ($\tau_{\text{mix}} = 200$ ms) (B) MAS NMR spectra of TTR(105–115) U- ^{15}N , ^{13}C labeled as YTIAALLSPYS and recorded at $\omega_{\text{OH}}/2\pi = 750$ MHz and $\omega_r/2\pi = 12.5$ kHz and $\omega_{\text{OH}}/2\pi = 900$ MHz and $\omega_r/2\pi = 11$ kHz, respectively, and $T = 280$ K. The spectra were used to constrain the intermolecular distances between the β -sheets within the TTR(105–115) protofilament. In the two spectra, intermolecular and intramolecular cross-peaks are labeled in red and black, respectively, and all cross-peaks have been assigned. (C) A cross-section of the TTR(105–115) protofilament depicting the sheet-to-sheet interface and the 11 distance constraints labeled in the spectra of A and B (red lines). The blue lines denote four additional distances between L111- $^{13}\text{C}\delta$ 1 and - $^{13}\text{C}\delta$ 2 and A109- ^{15}N and A108- $^{13}\text{C}\alpha$ detected in additional spectra of a sample labeled as YTIAALLSPYS. The complete set of 23 intersheet distance restraints is shown in *SI Appendix*, Fig. S2B. Odd- and even-numbered side chains are shown as orange and violet sticks, respectively, with secondary structure shown in a cyan ribbon representation. (D) ^{13}C - ^{15}N REDOR data obtained from a TTR(105–115) sample labeled as Y105- ^{15}N , S115- $^{13}\text{C}\text{O}_2\text{H}$ (YTIAALLSPYS) and designed to detect interprotofilament contacts in the fibril. The intermolecular ^{13}C - ^{15}N interaction between the terminal ^{15}N and ^{13}C atoms corresponds to a distance of 3.57 ± 0.06 Å, consistent with a head-to-tail protofilament arrangement as illustrated in *Right*. The curve was fitted by using the program SPINEVOLUTION (*SI Appendix*, *SI Materials and Methods*).

Atomic Structure of the Cross- β Protofilaments. The results presented above define a parallel, in-register β -sheet geometry within the sheets of the TTR(105–115) fibrils (Fig. 2A and *Movie S1*), indicating a full complement of nine backbone–backbone hydrogen bonds (Fig. 2A) along the entire length of the molecule—i.e., involving all residues but the proline at position 113. The fact that this optimized interbackbone hydrogen-bonding network adopts this particular arrangement indicates that the intrinsically more favorable hydrogen-bonding pattern found in antiparallel β -sheets is offset by the juxtaposition of side chains with identical hydrophobic and hydrophilic character in the ordered self-assembly of these amphiphilic molecules. The in-register alignment of matching residues generates very tight packing (shape complementarity = 0.76; Fig. 2A), also maximizing favorable hydrophobic and van der Waals side-chain contacts along the long axis of the fibril (14); in addition, the structures may gain further stabilization through π - π stacking of the tyrosine aromatic rings (10) at the termini of the TTR(105–115) molecule (Fig. 2A and B). The low degree of left-handed twist ($\sim 1^\circ$ between successive β -strands; Fig. 2B and *SI Appendix*, *SI Supporting Equation*) favored by the constituent parallel β -sheets of the fibrils is similar to the nearly flat β -sheets proposed in models of SH3 (11) and insulin (12) fibrils and limits lateral growth in the sheet–sheet direction (Fig. 2B and C), thus discriminating individual fibrils from 3D amyloid-like microcrystals (21, 22).

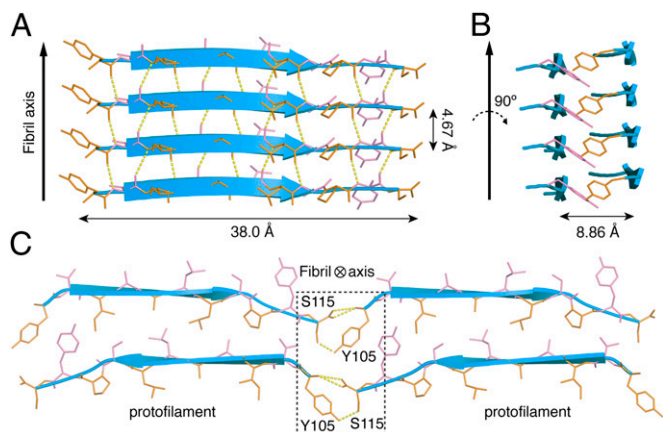


Fig. 2. Atomic-resolution structure of the TTR(105–115) protofilament determined by MAS NMR. The structures (calculated with CNSolve; see *SI Appendix, SI Materials and Methods*) have a rmsd of ~ 0.4 Å for the backbone and 0.7 Å for all atoms. Odd- and even-numbered side chains are shown as orange and violet sticks, respectively, with secondary structure shown in a cyan ribbon representation. (A) The β -sheet viewed perpendicularly to the fibril axis illustrating the parallel in-register β -strands and the hydrogen bonds defining the β -sheet (yellow lines). The conformation was determined from eight $^{13}\text{C}=\text{O}-^{13}\text{C}=\text{O}$ double quantum distance measurements and one $^{13}\text{C}=\text{O}-^{13}\text{C}=\text{O}$ REDOR distance measurement (*SI Appendix, Table S1*). (B) Cross-sectional view of the two-sheet protofilament along the peptide chain direction. There is a sheet-sheet offset of approximately one-fifth the separation of hydrogen-bonded β -strands (i.e., $0.2 \times 4.67 \text{ \AA} = 0.93 \text{ \AA}$) shown clearly by the interdigitation of the Y105 (orange sticks) and Y114 (violet sticks) side chains. (C) Protofilament-protofilament interface viewed looking down the long axis of the fibril showing the head-to-tail packing arrangement. Interprotofilament hydrogen bonds between the terminal C=O and N-H groups and between the Y105 OH atoms and the S115 O_γ atoms are depicted as yellow dashes.

The structure shows that in-register, parallel β -sheets stack antiparallel to one another via a C_2 axis parallel to the intersheet direction (Fig. 2C and *Movie S1*) to create a two-sheet protofilament as suggested in previous models of cross- β fibrils (11, 12). There is also an average sheet-sheet shift of 0.9 ± 0.6 Å, which is approximately one-fifth the separation of hydrogen-bonded β -strands (Fig. 2B). This staggered arrangement results in the burial of even-numbered side chains, TALSYS (Fig. 2B, violet sticks), on one sheet between two sets of odd-numbered side chains, YIALPS (Fig. 2B, orange sticks), on the opposite face and vice versa, in a highly specific manner similar to the “parallel, face-to-back, up-down” class of steric zipper (22). The self-complementary interdigitation of the side chains of two β -sheets (shape complementarity = 0.79; Fig. 2B and C) creates a tightly packed, hydrophobic core composed primarily of the contiguous hydrophobic stretch IAALL. The ~ 1 -Å sheet-sheet shift, however, leads to incompletely paired β -strands at the fibril ends (13) (Fig. 2B and *Movie S1*) with exposed hydrophobic residues and unsatisfied hydrogen bonds. The staggering of the cross-sheet stack therefore breaks the symmetry of the growing ends of the fibril, resulting in a single binding site for the addition of monomers (30). The offset of the sheets is also likely to make the fibrils more resistant to bending and stretching forces and thus less prone to fracture, which can play key roles in the kinetics of self-templated aggregation (31).

Overall Architecture of the Mature Fibrils. Because fibrils are typically composed of between two and six protofilaments (12), a vast number of interprotofilament packing arrangements are possible, for example, as observed in A β fibrils (16, 17, 19, 32). Although variations in intrasheet and intersheet stacking within protofilaments give rise to molecular-level polymorphism (15), interprotofilament arrangements represent an ultrastructural polymorphism (33) that can

result in vast numbers of fibrils with distinct morphologies, where the nature of the interactions are unlikely to affect significantly the chemical shifts of any of the residues (12). Knowledge of the atomic structure of the protofilaments from the MAS NMR analysis, however, provides clues as to the possible higher-order packing interfaces. We therefore have combined single-particle and helical cryo-EM approaches (11, 16) to determine the overall structure of the complete fibrils formed by TTR(105–115). The fibrils are observed to be twisted ribbons with regular cross-over distances ranging from 850 to 1,000 Å (Fig. 3A–C, and *SI Appendix, Fig. S1* and *Table S4*). Three prominent fibril types were identified (Fig. 3A–C) with distinct widths between crossovers (84 Å, Fig. 3A and D; 121 Å, Fig. 3B and E; and 154 Å, Fig. 3C and F). All fibrils have a width at crossover of ~ 37 Å and thus a nearly rectangular cross-section (Fig. 3D–F). The widths between crossovers correspond to integer multiples of the peptide chain length (~ 38 Å $\times 2, 3,$ or 4) and indicate the presence of multiple protofilaments (11, 19).

The internal packing of the protofilaments is revealed by examination of cross-sections through the 3D fibril reconstructions. Although the three fibril morphologies differ in the dimensions of the long side of their cross-sections (Fig. 3D–F), they are remarkably similar in other respects. In particular, they are all composed of two elongated, high-density regions separated by a well-defined region of low density (~ 13 Å wide at 1σ above the mean density) that extends to the slightly open cross-sectional ends (Fig. 3D–F). The high-density regions are ~ 12 Å in width (at 1σ above the mean density) and correspond to the paired β -sheets of individual protofilaments (11, 12, 16, 19) arranged in a linear fashion. The electron density maps all display twofold symmetry (Fig. 3D–F), which was not imposed during image processing, about the long axis of the fibril. This axial symmetry relates the paired β -sheet density regions to each other so that all of the fibrils described here are left-handed, double-layered helical ribbons with a hollow core (Fig. 3A–F).

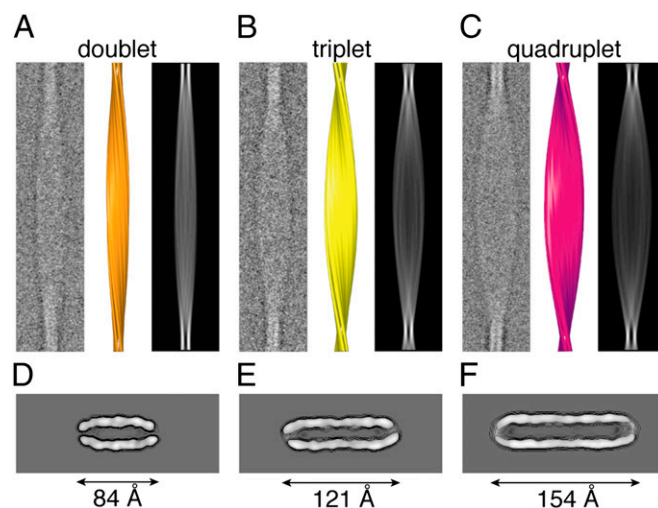


Fig. 3. Representative cryo-EM images of averaged fibrils (class averages), surface representations of reconstructions, 2D projections, and contoured density cross-sections of the three types of fibril formed by TTR(105–115). (A) Doublet class average (Left), 3D reconstruction (Center; orange), and 2D projection of the fibril reconstruction (Right). (B) Triplet class average (Left), 3D reconstruction (Center; yellow), and 2D projection of the fibril reconstruction (Right). (C) Quadruplet class average (Left), 3D reconstruction (Center; purple), and 2D projection of the fibril reconstruction (Right). (D–F) Contoured density cross-sections of the doublet (D), triplet (E), and quadruplet (F) fibrils. Contours represent density levels of multiples of 0.5σ above the average density of the background (outermost contour). Measurements of dimensions were determined at 1.0σ above the mean density.

Because the cryo-EM maps strongly suggest that the fibrils formed by TTR(105–115) are composed of integer multiples of linearly connected protofilaments, we sought to identify the interprotofilament packing interface. Low-angle X-ray experiments on TTR(105–115) fibrils (34) indicate an equatorial repeat of $37 \pm 2 \text{ \AA}$, which closely matches the length of the TTR(105–115) peptide in an extended β -strand conformation (38 \AA ; Fig. 2A); this observation suggests that the protofilaments are stacked in a periodic manner in the peptide chain direction. We therefore isotopically labeled the termini of the peptide (^{15}N -Y105 and ^{13}C -S115) and performed rotational-echo double-resonance (REDOR) (35) experiments to probe for backbone-to-backbone contacts. The observation of a strong dipolar coupling between S115- ^{13}C O $_2$ - ^{15}N H $_3^+$ -Y105, corresponding to an interprotofilament distance of $3.57 \pm 0.06 \text{ \AA}$, indicates the presence of head-to-tail contacts between adjacent protofilaments (Fig. 1D). This result is crucial in determining the relative orientations of individual protofilaments within the overall fibril topology (Fig. 1D).

The head-to-tail, interprotofilament packing interface (Fig. 2C) is very closely matched (shape complementarity = 0.83) and appears to be stabilized by dipole-dipole interactions between the N and C termini of peptide chains in neighboring protofilaments (SI Appendix, Fig. S7) and by four hydrogen bonds between the terminal C=O and N-H groups (Fig. 2C) and two tyrosine (OH...O) hydrogen bonds (Fig. 2C).

Atomic-Level Structures of the Fibril Polymorphs. The reconstructed cross-sectional density maps are fully consistent with the NMR-derived protofilament structure discussed above (SI Appendix, Fig. S8) and can accommodate perfectly four, six, or eight of the two-sheet protofilaments interconnected in a head-to-tail fashion (Fig. 4A–C). Side-chain details are only present in exceptionally high-resolution electron density maps (3.3–4 \AA) (36), which is not the case here because the resolution of the three maps is $\sim 11 \text{ \AA}$ (SI Appendix, Table S4 and Fig. S9). As a result of the slightly curved cross-section of the protofilaments (Fig. 2C), the presence of a twofold symmetry axis (Fig. 3D–F), and the nanometer resolution (SI Appendix, Table S4) of the 3D reconstructions, there is a common structural arrangement that gives the best fit to the set of electron density maps (up to 7% less atoms outside the electron density maps at 1.0σ threshold) and on average more than seven times more interfacial contact area (with no steric clashes; Fig. 4D–F) than other alternative structures. This structural arrangement involves a pair of oppositely directed, linear stretches of two (Fig. 4A and SI Appendix, Fig. S10A and Table S3), three (Fig. 4B and SI Appendix, Fig. S10B and Table S3) and four (Fig. 4C and SI Appendix, Fig. S10C and Table S3) protofilaments with the even-numbered residues (TALSY) lining the outer surface of the fibrils and the odd-numbered residues (YIALPS) buried in the hollow core (Fig. 4D–F). Therefore, different numbers of otherwise identical protofilaments (12) are able to self-assemble into a generic structural arrangement.

To obtain an independent estimate of the number of peptides contained in the fibril cross-sections, we performed STEM mass-per-length (MPL) measurements on an ensemble of fibrils (Fig. 5A). The distribution can be described by three Gaussian curves peaking at 9.7 ± 1.2 , 12.8 ± 1.2 , and 15.9 ± 1.2 peptides per 4.67- \AA layer of the different types of fibril. These MPL measurements are in good agreement with the cryo-EM fibril reconstructions (Fig. 4A–C), which have cross-sections with twofold symmetry (Fig. 3D–F) able to accommodate 8 (Fig. 4A), 12 (Fig. 4B), or 16 (Fig. 4C) peptides—i.e., 4, 6, or 8 two-sheet protofilaments—respectively. As an additional validation, we simulated the X-ray diffraction pattern of the fibril cross-sections and found that the resulting peaks closely match the primary and secondary reflections in the experimental diffraction pattern (34) (Fig. 5B).

The cryo-EM maps of the three fibrils are characterized by a nearly constant $\sim 16\text{-\AA}$ backbone-to-backbone low-density region between the high-density regions of paired β -sheets (Figs. 3D–F and 4A–C). Such a separation suggests that this interface may contain structured water involved in a hydrogen-bonded network with the exposed side chains (Fig. 4D–F) and water molecules. The dimensions of the cavity (Figs. 3D–F and 4A–C) can accommodate the two sets of exposed β -sheet side chains ($\sim 9 \text{ \AA}$), leaving a $2 \times 3.4 \text{ \AA}$ (the diameter of a water molecule) $\sim 7 \text{ \AA}$ layer for water molecules to occupy (Fig. 4D–F). Moreover, the cavity dimension matches very closely the size of the wet interfaces in amyloid-like microcrystals in which crystal contacts result in a bilayer of water molecules that can be observed crystallographically (21). Molecular dynamics simulations (SI Appendix, SI Materials and Methods) suggest that a single layer of water molecules becomes bound to each of the slightly hydrophilic protofilament surfaces (SI Appendix, Fig. S11).

Extensive AFM imaging, which has a much higher contrast than single cryo-EM images, of TTR(105–115) samples (Fig. 5C) revealed the presence of a low population ($\sim 3\%$ of the total fibril dataset) of singlet fibrils, or “filaments,” with a uniform height of $38.7 \pm 4.4 \text{ \AA}$ (Fig. 5C). The dimensions of this species match perfectly to the predicted height profile of a pair of protofilaments separated by the constant $\sim 16 \text{ \AA}$ (backbone to backbone) water cavity (overall dimensions $38 \times 43 \text{ \AA}$; SI Appendix, Fig. S12). We also saw no evidence of the number of protofilaments per fibril varying along the length of a single TTR(105–115) fibril (37). Rather, it is clear from the cryo-EM images (Fig. 3) and 3D reconstructions (Fig. 4), AFM images, and STEM MPL measurements of TTR(105–115) fibrils (Fig. 5) that there are three main subsets of the fibril population—namely, the doublet, triplet, and quadruplet fibrils (Fig. 6 and SI Appendix, Figs. S13–S15).

To determine the complete structures of the amyloid fibrils (Fig. 6, SI Appendix, Figs. S13–S15, and Movies S2, S3, and S4), a helical symmetry was imposed on to the NMR/cryo-EM atomic cross-sections to generate longer fibril segments (Fig. 6, SI Appendix, Figs. S13–S15, and Movies S2, S3, and S4). The fit of four,

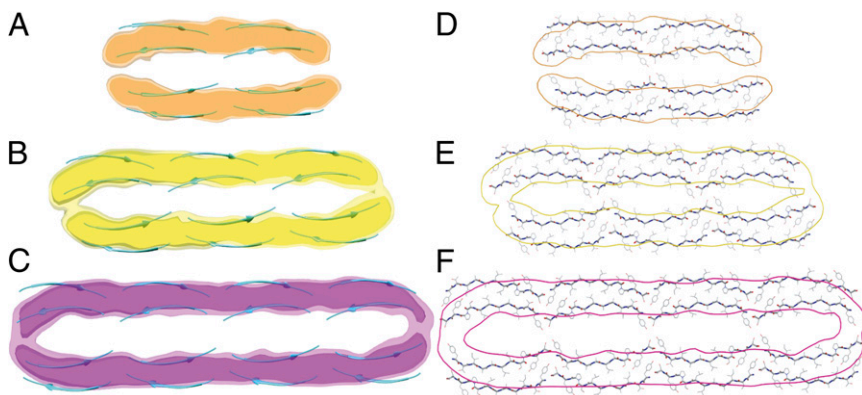


Fig. 4. Atomic-resolution cross-sections of the three types of TTR(105–115) amyloid fibrils determined by combining MAS NMR and cryo-EM. Reconstructed cross-section density maps are shown as two electron density isosurfaces (1.0σ and 2.2σ above the mean density) with the secondary structure of the constituent NMR-derived protofilaments shown in a cyan ribbon representation. (A–C) The doublet (A), triplet (B), and quadruplet (C) fibril cross-sections can accommodate pairs of two, three, and four interconnected protofilaments, respectively. (D–F) All-atom representation of the doublet (D), triplet (E), and quadruplet (F) cross-sections with cryo-EM envelopes superimposed. Cryo-EM envelopes are shown as orange, yellow, and purple contours at 1.0σ above the mean density for doublet, triplet, and quadruplet fibrils, respectively. For a discussion on the resolution of the cryo-EM maps, see SI Appendix, Table S4.

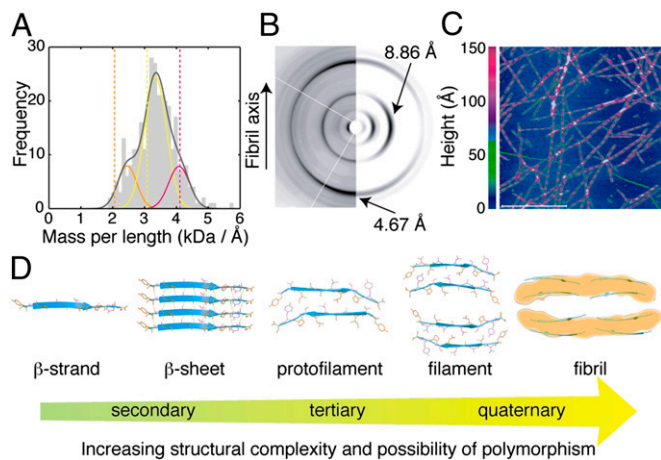


Fig. 5. Five diverse biophysical techniques were integrated to determine unambiguously the structures of each of the motifs that make up the TTR(105–115) amyloid fibrils. Spanning five orders of magnitude, the overlapping length scales of MAS NMR (0.1–10 Å), X-ray diffraction (3–100 Å), cryo-EM (8–1,000 Å), AFM (30–1,000 Å), and STEM (80–1,000 Å) enabled us to derive self-consistent, high-precision structural restraints on the secondary (β -strand and -sheet; distance restraints of <6 Å), tertiary (protofilament; distance restraints of 4.5–37 Å), and quaternary structure (filament and fibril; distance restraints of 16–1,000 Å) of the TTR(105–115) amyloid fibrils. (A) Histogram of STEM MPL measurements of TTR(105–115) fibrils, which reveals three populations of fibrils, with a best fit (gray solid line) being the sum of three Gaussian curves with values of 2.5 ± 0.3 kDa/Å (orange solid line), 3.3 ± 0.3 kDa/Å (yellow solid line), and 4.1 ± 0.3 kDa/Å (purple solid line). The orange, yellow, and purple dashed lines refer to the number of TTR(105–115) peptides per 4.67-Å repeat in the doublet (8 peptides), triplet (12 peptides), and quadruplet (16 peptides) fibrils, respectively. (B) Comparison of the high-resolution experimental X-ray diffraction pattern from TTR(105–115) fibrils (34) (Left) and the simulated X-ray diffraction pattern for TTR(105–115) fibrils (Right). The fibril axis is vertical, with the incident beam directed orthogonally to this axis. The meridional reflection at 4.67 Å and the equatorial reflection at 8.86 Å are characteristic of cross- β structure. (C) High-resolution AFM image of fibrils (pink and purple) and filaments (green) formed by TTR(105–115). (Scale bar, 1 μ m.) Fibrils (pink and purple) have heights ranging from 70 to 160 Å and pitches of 950 ± 100 Å. The filament has an average height of 38.7 ± 4.4 Å. (D) Hierarchy of atomic-resolution motifs involved in the self-assembly of the amyloid fibrils and their polymorphism.

six, and eight protofilament structures winding around a hollow core to the doublet, triplet, and quadruplet fibril densities is excellent with correlation coefficients of 0.85, 0.88, and 0.86, respectively (Fig. 6, *SI Appendix*, Figs. S13–S15, and *Movies S2, S3, and S4*). These structures have a rmsd of the backbone and all heavy atoms in the ensemble of the 20 lowest energy conformers of 0.4, 0.6, 0.6 Å and 0.6, 0.7, 0.7 Å, respectively (*SI Appendix*, Table S3 and Fig. S10). None of the structures has any residue with torsion angles in disallowed regions of the Ramachandran plot (*SI Appendix*, Table S3).

These results indicate that, in analogy to microtubules (38), interprotofilament interactions are highly conserved across different types of filamentous structures. However, because the termini of the β -sheets are involved in the higher-order assembly, the lateral association of two or more protofilaments occludes the hydrophilic surfaces so that the proportion of exposed hydrophobic surface area increases steeply with increasing fibril diameter. Because the fibrils have a large fraction of exposed hydrophobic residues (up to 23% more than typical globular proteins), this free energy penalty may be offset by the stabilization arising from the highly optimal packing that allows extensive backbone hydrogen bonding (39). The specific head-to-tail electrostatic interactions between protofilaments that we have identified (Fig. 2C and *SI Appendix*, Fig. S7) indicate how these elements can self-associate under one set of chemical conditions into three highly populated and distinct ultrastructural

polymorphs, with varying widths and pitches that are propagated over many microns (4) (Fig. 6, *SI Appendix*, Figs. S13–S15, and *Movies S2, S3, and S4*).

Discussion

An atomic-resolution structure of a cross- β amyloid fibril and its constituent self-assembling subunits has proved elusive to structural biology. Such a structure is of great interest, because the thermodynamic and kinetic properties of amyloid fibrils, as well as their frequently adverse effects on cellular homeostasis leading to disease, are linked to the details of their morphological features (40). In this study, we have resolved each of the structural elements in a cross- β amyloid fibril to atomic resolution, thus revealing the structural basis for the hierarchical organization of these aggregates. The architecture of the fully assembled amyloid fibrils is not strongly dependent on sequence because the constituent β -strands within the core of the fibril are linked by a vast array of interbackbone hydrogen bonds (Fig. 2A and *Movie S1*), which are main-chain interactions common to all polypeptides (23). This generic character is evident in the marked similarity in appearance (17), dimensions (12), X-ray diffraction patterns (3), material properties (5), kinetics (31), thermodynamic stabilities (6), and dye-binding properties (41) of fibrils formed by peptides and proteins varying widely in sequence and native conformation.

The nature of the side-chains does, however, modulate the details of the fibrillar scaffold by determining which regions of the sequence self-associate to form the intermolecular packing arrangement within, and between, the constituent β -sheets of the fibril core (1, 2). Although side chains influence which protofilament structures are adopted under a given set of experimental conditions, ultrastructural polymorphism is also a common feature of amyloid fibrils (1, 2). This variability at the intra- and interprotofilament levels provides an explanation of different fibril polymorphs characteristic of distinct clinical subphenotypes, in analogy to the strains of prions (42).

The ability to understand the hierarchical organization of cross- β amyloid fibrils (Fig. 5D) reveals how this structure

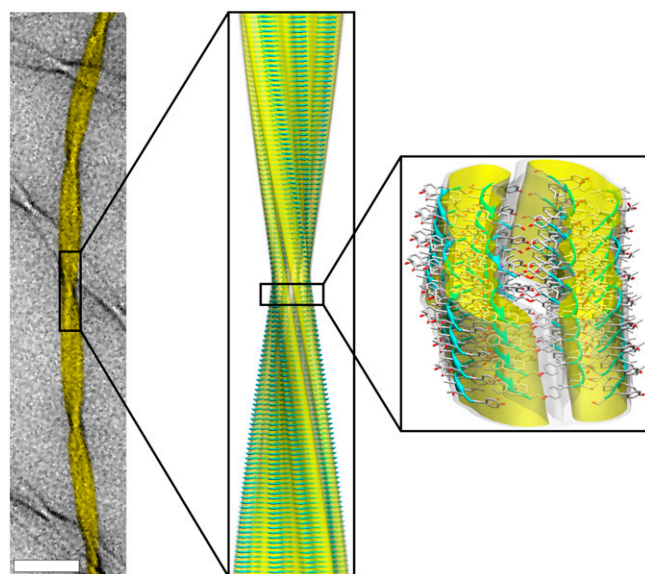


Fig. 6. Close-up view of the MAS NMR atomic-resolution structure of the triplet fibril fitted into the cryo-EM reconstruction (Center). The background image of the fibril (Left) was taken using TEM. (Scale bar, 50 nm.) The fibril surfaces (Right) are shown at 1.0σ (white) and 2.2σ (yellow) above the mean density, respectively, and the constituent β -sheets are shown in a ribbon representation; oxygen, carbon, and nitrogen atoms are shown in red, gray, and blue, respectively.

represents a template that many amino acid sequences can adopt (23). Thus, although in longer sequences additional residues will undoubtedly affect the overall assembly of the fibrils and the nature of interactions between protofilaments (11), the basic structural elements (Fig. 5D) remain closely similar (12, 17, 43). In this respect, understanding the formation and molecular pathology of the cross- β fibrils formed by other peptides and proteins involves the study of variations on a theme common to most polypeptide chains (23). Indeed, both polar and non-polar (22), and even homopolymeric (44) sequences can adopt the cross- β form of polypeptide states. By contrast, the β -helical structure of HET-s(218–289) fibrils (18) which represents a functional yeast prion, is stabilized by specific interactions selected through evolution and therefore not accessible to most amino acid sequences. The structures presented here (Fig. 6, *SI Appendix*, Figs. S13–S15, and *Movies S2, S3, and S4*) have instead the widely accessible cross- β geometry commonly associated with misfolding disorders such as Alzheimer's disease and type II diabetes (1, 2).

Conclusions

We have determined the atomic-resolution structure of a cross- β amyloid fibril and two of its associated polymorphic variants. Our approach has enabled us to dissect each distinct level of the structural hierarchy of the fibrils at atomic resolution and to

identify the packing interactions that drive the self-assembly and ultimately stabilize these persistent filamentous aggregates. The results that we have presented illustrate the increasing potential for amyloid polymorphism with the ascending hierarchy of fibrillar structures, a phenomenon that we expect to be common for many other peptides and proteins.

Methods

Amyloid fibrils were prepared by dissolving TTR(105–115) in a 10% (vol/vol) acetonitrile/water solution (adjusted to pH 2 with HCl) at a concentration of 15 mg/mL. The samples were incubated for 2 d at 37 °C followed by incubation for 14 d at room temperature (20). The samples were routinely characterized by TEM, and great care was taken to ensure that the morphologies of the fibrils studied by NMR were identical to those examined by cryo-EM and the other techniques (X-ray diffraction, AFM, and STEM).

Full methods are available as *SI Appendix, SI Materials and Methods*.

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- Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75:333–366.
- Eisenberg D, Jucker M (2012) The amyloid state of proteins in human diseases. *Cell* 148(6):1188–1203.
- Sunde M, et al. (1997) Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol* 273(3):729–739.
- Knowles TPJ, Smith JF, Craig A, Dobson CM, Welland ME (2006) Spatial persistence of angular correlations in amyloid fibrils. *Phys Rev Lett* 96(23):238301.
- Knowles TP, et al. (2007) Role of intermolecular forces in defining material properties of protein nanofibrils. *Science* 318(5858):1900–1903.
- Baldwin AJ, et al. (2011) Metastability of native proteins and the phenomenon of amyloid formation. *J Am Chem Soc* 133(36):14160–14163.
- Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* 475(7356):324–332.
- Fowler DM, Koulou AV, Balch WE, Kelly JW (2007) Functional amyloid—from bacteria to humans. *Trends Biochem Sci* 32(5):217–224.
- Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8(2):101–112.
- Gazit E (2002) A possible role for pi-stacking in the self-assembly of amyloid fibrils. *FASEB J* 16(1):77–83.
- Jiménez JL, et al. (1999) Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. *EMBO J* 18(4):815–821.
- Jiménez JL, et al. (2002) The protofilament structure of insulin amyloid fibrils. *Proc Natl Acad Sci USA* 99(14):9196–9201.
- Lührs T, et al. (2005) 3D structure of Alzheimer's amyloid-beta(1–42) fibrils. *Proc Natl Acad Sci USA* 102(48):17342–17347.
- Petkova AT, et al. (2002) A structural model for Alzheimer's beta-amyloid fibrils based on experimental constraints from solid state NMR. *Proc Natl Acad Sci USA* 99(26):16742–16747.
- Petkova AT, et al. (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* 307(5707):262–265.
- Sachse C, Fändrich M, Grigorieff N (2008) Paired beta-sheet structure of an Abeta(1–40) amyloid fibril revealed by electron microscopy. *Proc Natl Acad Sci USA* 105(21):7462–7466.
- Schmidt M, et al. (2009) Comparison of Alzheimer Abeta(1–40) and Abeta(1–42) amyloid fibrils reveals similar protofilament structures. *Proc Natl Acad Sci USA* 106(47):19813–19818.
- Wasmer C, et al. (2008) Amyloid fibrils of the HET-s(218–289) prion form a beta solenoid with a triangular hydrophobic core. *Science* 319(5869):1523–1526.
- Zhang R, et al. (2009) Interprotofilament interactions between Alzheimer's Abeta(1–42) peptides in amyloid fibrils revealed by cryoEM. *Proc Natl Acad Sci USA* 106(12):4653–4658.
- Jaroniec CP, et al. (2004) High-resolution molecular structure of a peptide in an amyloid fibril determined by magic angle spinning NMR spectroscopy. *Proc Natl Acad Sci USA* 101(3):711–716.
- Nelson R, et al. (2005) Structure of the cross-beta spine of amyloid-like fibrils. *Nature* 435(7043):773–778.
- Sawaya MR, et al. (2007) Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature* 447(7143):453–457.
- Dobson CM (1999) Protein misfolding, evolution and disease. *Trends Biochem Sci* 24(9):329–332.
- Caporini MA, et al. (2010) Accurate determination of interstrand distances and alignment in amyloid fibrils by magic angle spinning NMR. *J Phys Chem B* 114(42):13555–13561.
- Jaroniec CP, Filip C, Griffin RG (2002) 3D TEDOR NMR experiments for the simultaneous measurement of multiple carbon-nitrogen distances in uniformly (13)C,(15)N-labeled solids. *J Am Chem Soc* 124(36):10728–10742.
- Ladizhansky V, Griffin RG (2004) Band-selective carbonyl to aliphatic side chain 13C-13C distance measurements in U-13C,15N-labeled solid peptides by magic angle spinning NMR. *J Am Chem Soc* 126(3):948–958.
- Szeverenyi NM, Sullivan MJ, Maciel GE (1982) Observation of spin exchange by two-dimensional fourier-transform C-13 cross polarization-magic-angle spinning. *J Magn Reson* 47:462–475.
- Sivanandam VN, et al. (2011) The aggregation-enhancing huntingtin N-terminus is helical in amyloid fibrils. *J Am Chem Soc* 133(12):4558–4566.
- Schneider R, et al. (2011) Structural characterization of polyglutamine fibrils by solid-state NMR spectroscopy. *J Mol Biol* 412(1):121–136.
- Collins SR, Douglass A, Vale RD, Weissman JS (2004) Mechanism of prion propagation: Amyloid growth occurs by monomer addition. *PLoS Biol* 2(10):e321.
- Knowles TPJ, et al. (2009) An analytical solution to the kinetics of breakable filament assembly. *Science* 326(5959):1533–1537.
- Goldsbury CS, et al. (2000) Studies on the in vitro assembly of a beta 1–40: Implications for the search for a beta fibril formation inhibitors. *J Struct Biol* 130(2–3):217–231.
- Goldsbury CS, et al. (1997) Polymorphic fibrillar assembly of human amylin. *J Struct Biol* 119(1):17–27.
- Gras SL, et al. (2008) Functionalised amyloid fibrils for roles in cell adhesion. *Bio-materials* 29(11):1553–1562.
- Gullion T, Schaefer J (1989) Rotational-echo double-resonance NMR. *J Magn Reson* 81:196–200.
- Orlova EV, Saibil HR (2011) Structural analysis of macromolecular assemblies by electron microscopy. *Chem Rev* 111(12):7710–7748.
- Komatsu H, Feingold-Link E, Sharp KA, Rastogi T, Axelsen PH (2010) Intrinsic linear heterogeneity of amyloid β protein fibrils revealed by higher resolution mass-per-length determinations. *J Biol Chem* 285(53):41843–41851.
- Sui HX, Downing KH (2010) Structural basis of interprotofilament interaction and lateral deformation of microtubules. *Structure* 18(8):1022–1031.
- Fitzpatrick AW, Knowles TPJ, Waudby CA, Vendruscolo M, Dobson CM (2011) Inversion of the balance between hydrophobic and hydrogen bonding interactions in protein folding and aggregation. *PLoS Comp Biol* 7(10):e1002169.
- Vendruscolo M, Knowles TPJ, Dobson CM (2011) Protein solubility and protein homeostasis: A generic view of protein misfolding disorders. *Cold Spring Harb Perspect Biol* 3(12):3.
- Krebs MRH, Bromley EHC, Donald AM (2005) The binding of thioflavin-T to amyloid fibrils: Localisation and implications. *J Struct Biol* 149(1):30–37.
- Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. *Science* 318(5852):930–936.
- Bayro MJ, et al. (2011) Intermolecular structure determination of amyloid fibrils with magic-angle spinning and dynamic nuclear polarization NMR. *J Am Chem Soc* 133(35):13967–13974.
- Fändrich M, Dobson CM (2002) The behaviour of polyamino acids reveals an inverse side chain effect in amyloid structure formation. *EMBO J* 21(21):5682–5690.