Fibril Structure

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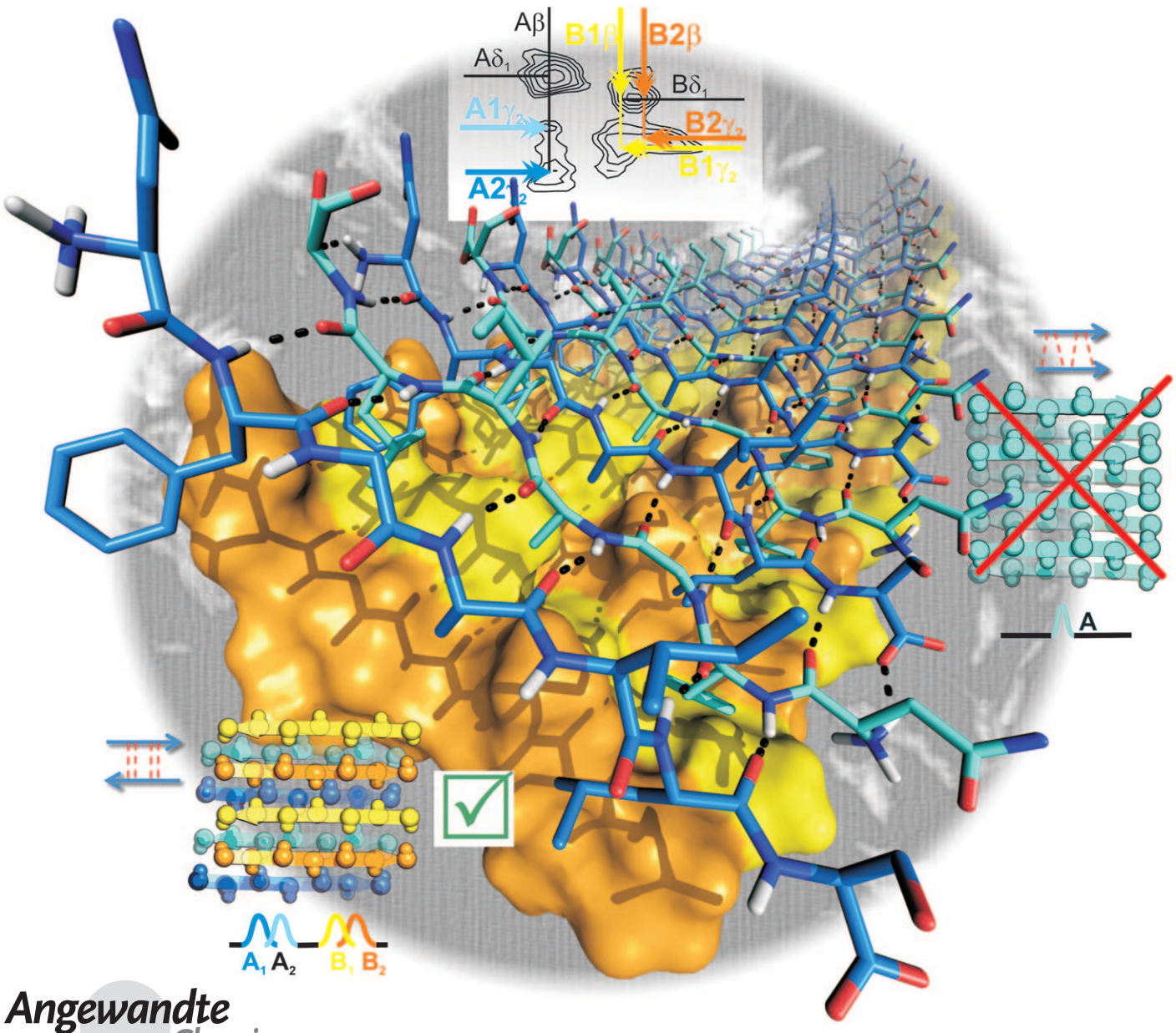
Unique Identification of Supramolecular Structures in Amyloid Fibrils by Solid-State NMR Spectroscopy**

*Jakob T. Nielsen, Morten Bjerring, Martin D. Jeppesen, Ronnie O. Pedersen, Jan M. Pedersen, Kim L. Hein, Thomas Vosegaard, Troels Skrydstrup, Daniel E. Otzen, and Niels C. Nielsen**



In recent years, considerable progress has been made in using solid-state NMR spectroscopy to determine atomic-resolution structures of amyloid fibrils[1–4] associated with serious disorders such as Alzheimer's and Parkinson's diseases, prion diseases, and type 2 diabetes. Although the architecture of fibrils is considered to be a continuous stack of β-sheet ladders, termed a cross-β structure,[5] there may be significant variations in the supramolecular organization of the peptides within the fibrils. Detailed insight may shed light on the assembly process and inspire design of fibril-binding compounds and inhibitors.

Like its liquid-state analogue, solid-state NMR spectroscopy is based on local structure information and does not automatically provide long-range structure-symmetry information as known from X-ray crystallography. As a consequence, large amounts of data on several differently labeled samples have typically been required to obtain sufficient long-range information. Herein, we demonstrate that it is possible to identify the supramolecular conformation of fibrils directly from symmetry-induced resonance patterns in 2D solid-state NMR spectra for a single ^{13}C,^{15}N-labeled sample. Our target is the hIAPP20–29 (SNNFGAILSS) decapeptide from the human islet amyloid polypeptide (hIAPP), which is believed to form the fibrillation core domain of fibrils in the pancreas of type 2 diabetes patients.[6,7]

To appreciate the NMR signatures of fibril symmetry, it is necessary to understand the structural determinants of fibrils. Fibrils are formed by peptides in extended conformations (β strands) into ladders (Figure 1a, top) through parallel or antiparallel bridges (hydrogen bonds), which through steric effects further combine into stacked ladders, called a zipper[8] (Figure 1a, bottom). The stacking of the two ladders may occur with different orientation of the strands defining, in total, eight classes of steric zippers[8] with different numbers of nonequivalent copies of the peptides, as illustrated for the NFGAILS fragment of hIAPP (Figure 1b). Such zippers can, in combination with a few long-range constraints, be uniquely distinguished using solid-state NMR spectroscopy since structurally nonequivalent peptides give rise to distinct resonance sets.

Addressing the fibrillation core domain hIAPP20–29 of hIAPP, AFM and X-ray fiber diffraction (Figure 2a,b) provided typical signatures of fibril structures. Solid-state NMR spectra (Figure 2c, and S2 and S3 in the Supporting Information) of the decapeptide, with the central six residues (NFGAIL) uniformly labeled with ^{13}C and ^{15}N, displayed sufficient resolution for peaks to be assigned to specific atoms in the peptides (see the Supporting Information). Secondary chemical shift analysis[9] revealed β-strand conformations for all residues, as typical for fibrils. Two sets of resonances were detected (A and B in Figure 2c) with equal intensities and markedly different chemical shifts. Splitting of some of the Ile5 A and B side-chain resonances revealed the presence of two further pseudoequivalent chains, yielding a total of four different resonances sets, A_1, A_2, B_1, and B_2, with A_1/A_2 and B_1/B_2 denoting pseudoequivalent sets of peptides. In addition, we detected two sets of resonances with lower intensities, differing from the A and B sets only in the Ala4, Ile5, and Leu6 resonances, which we interpreted as a minor form fibril population (Figure 2c, Table S1 in the Supporting Information). No unambiguous contacts between major and minor forms were detected.

Detection of four sets of resonances revealed that the supramolecular organization of the major population of hIAPP20–29 fibrils belongs to one of the four classes with antiparallel ladders. Among these the "homo zipper" is unlikely since it would have four pseudoequivalent chains and therefore four very similar resonance sets. For further discrimination of the supramolecular organization we exploited the distinct long-range contacts between copies of the peptide. Among ten long-range contacts obtained from solid-state NMR DARR (dipolar-assisted rotational resonance)[10] spectra (see the Supporting Information, Figures S3 and S5, Table S1) we detected contacts between Leu6ACδ and Ile5BCα and between Phe2ACγ and Gly3BCα with A (A_1/A_2) and B (B_1/B_2) denoting two different resonance sets. In combination with the symmetry information, the SNNFGAILSS fibrils could thus be unambiguously assigned to the antiparallel ladder "hetero zipper" class. The possibility of the multiple resonance sets corresponding to different structural polymorphs can rigorously be excluded based on the contacts between chains A and B (see the Supporting Information).

To establish a full structure, we complemented this information with ambiguous distance restraints[11] to overcome overlap in the 2D DARR spectra, in particular between nonequivalent resonance forms of the same nucleus which could not be removed by traditional approaches with selectively labeled or spin-diluted samples.[2,4] In total, 75 distance constraints for the NFGAIL residues were applied with ambiguities at three different levels (see the Supporting Information), enabling establishment of a structure for the hIAPP20–29 fibrils. 500 structures were calculated and the root-mean-square deviation (RMSD) for the ten lowest-energy structures (Figure 3a) is 0.52 Å for backbone and 1.16 Å for all heavy atoms (see the Supporting Information, Table S3). The fibril structure belonging to the antiparallel hetero zipper class shows hydrophobic interactions between side-chains of Phe2 and Leu6 within the same ladder and Phe2 and Ile5 of the opposite ladder, and it displays a slight twist around the fibril axis which is reproducible within the ensemble (Figure 3, and S4 and S7 in the Supporting Information). A fibril model structure of hIAPP was recently

[*] Dr. J. T. Nielsen, Dr. M. Bjerring, M. D. Jeppesen, R. O. Pedersen, Dr. J. M. Pedersen, K. L. Hein, Dr. T. Vosegaard, Prof. T. Skrydstrup, Prof. D. E. Otzen, Prof. N. C. Nielsen
Center for Insoluble Protein Structures (inSPIN)
Interdisciplinary Nanoscience Center (iNANO)
University of Aarhus, 8000 Aarhus C (Denmark)
Fax: (+45) 8619-6199
E-mail: ncn@inano.dk

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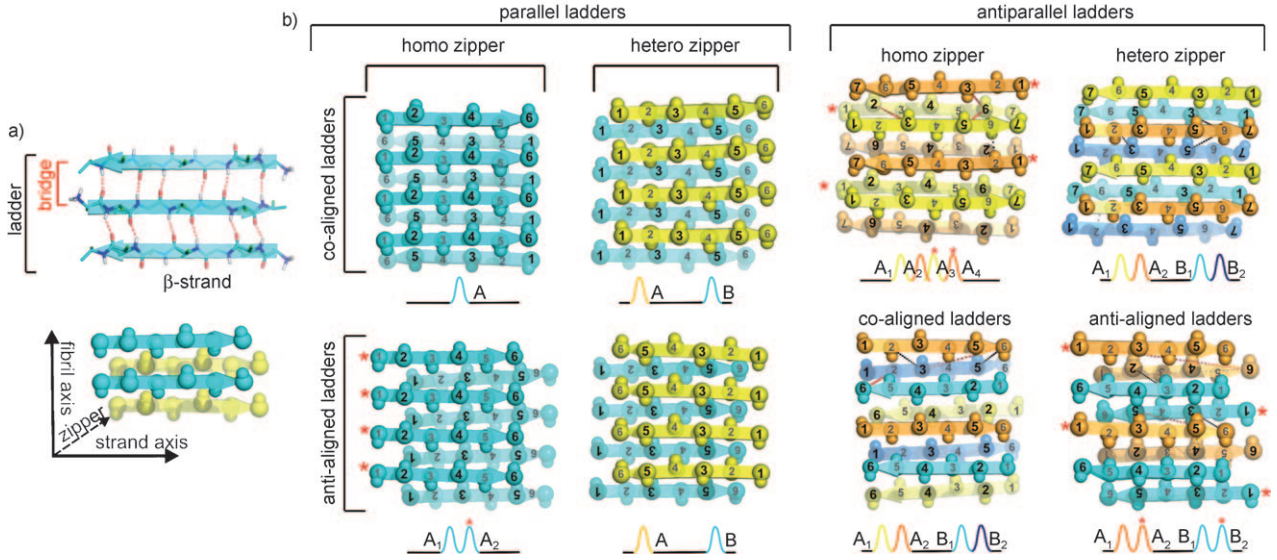

Figure 1. The eight classes of steric zippers with their respective NMR signatures: a) β Strands are arranged by hydrogen bonding (bridge) into a ladder, which can be parallel or antiparallel. Two ladders combine into a steric zipper (spheres indicate Cα and Hα). b) The eight classes are defined by different arrangement of β strands within ladders through identical/different faces (homo/hetero) and aligned in the same (co-) or opposite (anti-) direction along the fibril axis. Different arrangements may lead to different chemical-shift patterns, as represented by the NMR spectrum below each structure with labeling of the resonance sets. Parallel ladders incorporate one or two distinct peptides, whereas antiparallel ladders incorporate four distinct peptides. Peptides orienting side-chains of odd and even residues into the zipper are colored cyan/marine and yellow/orange, respectively. The chemical shifts depend on whether the side-chain points into or away from the zipper, leading to large shift differences for odd/even combinations, relative to even/even or odd/odd combinations. The latter combinations may lead to equivalent or pseudoequivalent peptides, where pseudoequivalence may arise either for peptides with buried side-chains close to the Hα protons of the two nearest peptides within the ladder (Hα-sandwiched; colored orange or marine blue; Figure S6 in the Supporting Information) or for two equivalent peptides screwed along an axis parallel to the strand axis. Red asterisks indicate peptides with the N-terminal nearest the edge of the zipper. Contacts between residues corresponding to signals in ^{13}C–^{13}C DARR[10] spectra for SNNFGAILSS fibrils are shown as dashed lines when consistent (black) or inconsistent (red) with the class.

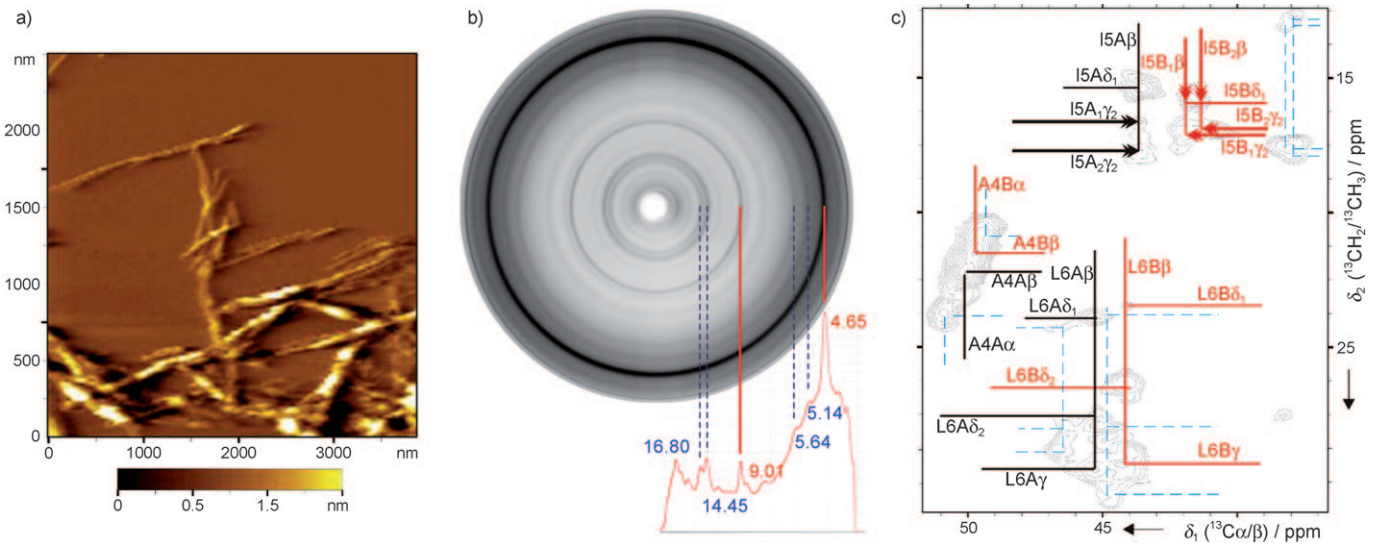

Figure 2. Characteristics of hIAPP20–29 fibrils: a) AFM image in derivative mode (see the Supporting Information) revealing typical amyloid fibril structures. b) X-ray fiber diffraction pattern and a radial average highlighting the 4.65 Å distance between bridging strands and the 9.01 Å distance between ladders. c) Part of a 40 ms 2D DARR[10] solid-state NMR spectrum of fibrils with six central residues, N1, F2, G3, A4, I5, and L6, uniformly labeled with ^{13}C and ^{15}N (more spectra in Figure S2 and S3 in the Supporting Information). Resonance positions are labeled ($A/A_1/A_2$ and $B/B_1/B_2$ are chain identifiers; see Figure 1) and indicated by lines. Minor forms and pseudoequivalent forms are shown with dashed and heavy lines, respectively. Peak groups that are split as a result of pseudoequivalence are marked with arrows.

derived where NFGAIL was part of a non-β-strand turn.[12] The difference could be due to different sample preparations. Interactions in the hetero zipper class could favor addition of a third ladder, but this addition can be excluded by symmetry,

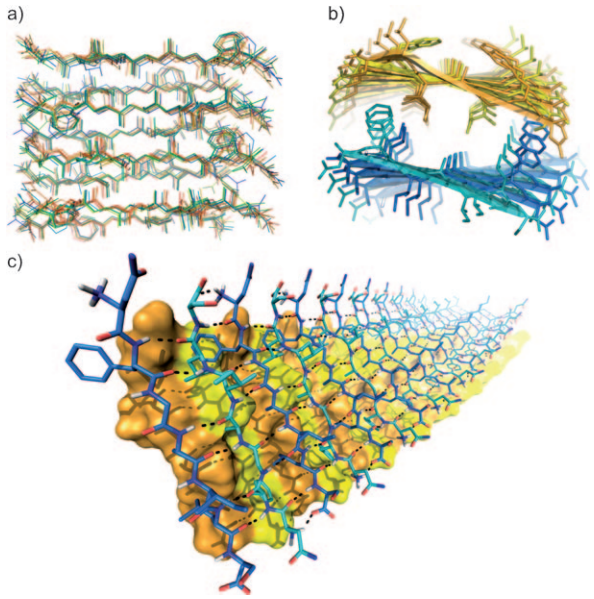

Figure 3. The supramolecular structure of the NFGAILS section of the hIAPP20–29 fibrils. a) Superimposed ensemble of the ten lowest-energy structures. b) View down the fibril axis. The two ladders are shown in blue/cyan and yellow/orange c) One ladder shown as surface and the adjacent ladder as sticks, with hydrogen bonds between strands indicated by black dashed lines.

since it would give rise to three different resonance sets of equal intensity.

In conclusion, exploiting the combination of symmetry information and ambiguous distance constraints (with an average of 12.5 restraints per labeled residue) from standard 2D NMR spectra, it was possible to determine the supramolecular structure of the hAIPP20–29 fibrils with high resolution. We envisage that the exploitation of spectral symmetry signatures will become an integral part of future structure calculations of multimeric systems of insoluble proteins such as amyloid fibrils. It is expected that the method may find interesting applications in the study of equilibria between different structural forms using simple 1D spectra of fibrils with a single label.

After submission of this work, a paper by Middleton and co-workers[13] presented a structural model of hIAPP20–29 consistent with solid-state NMR spectroscopy and fiber diffraction constraints. The authors discuss the presence of parallel and antiparallel polymorphs depending on sample preparation, and focus major attention to the parallel arrangement. As described above, our data is not consistent with mixed polymorphs and we present a full high-resolution structure of antiparallel fibrils.

Experimental Section

All NMR spectra are recorded with a Bruker Avance II 700 MHz spectrometer using a standard 4 mm triple resonance Bruker probe at 12 kHz MAS and 263 K. The structure was calculated using Xplor-NIH. For more details on the NMR experiments and on the biophysical studies, we refer to the Supporting Information.

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