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¹H-detection and Dynamic Nuclear Polarization-Enhanced NMR of Aβ₁₋₄₂ Fibrils

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ABSTRACT: Several publications describing high-resolution structures of amyloid-β (Aβ) and other fibrils have demonstrated that magic-angle spinning (MAS) NMR spectroscopy is an ideal tool for studying amyloids at atomic resolution. Nonetheless, MAS NMR suffers from low sensitivity, requiring relatively large amounts of samples and extensive signal acquisition periods, which in turn limits the questions that can be addressed by atomic-level spectroscopic studies. Here, we show that these drawbacks are removed by utilizing two relatively recent additions to the repertoire of MAS NMR experiments – namely, ¹H detection and dynamic nuclear polarization (DNP). We show resolved and sensitive 2D and 3D correlations obtained on ¹³C, ¹⁵N-enriched and fully-protonated samples of M₀Aβ₁₋₄₂ fibrils by high-field ¹H detected NMR at 23.4 T and 18.8 T, and ¹³C detected DNP MAS NMR at 18.8 T. These spectra enable nearly complete resonance assignment of the core of M₀Aβ₁₋₄₂ (K16-A42) using sub-mg sample quantities, as well as the detection of numerous unambiguous inter-nuclear proximities defining both the structure of the core and the arrangement of the different monomers. An estimate of the sensitivity of the two approaches indicates that the DNP experiments are currently ~6.5 times more sensitive than ¹H detection. These results suggest that ¹H detection and DNP may be the spectroscopic approaches of choice for future studies of Aβ and other amyloid systems.

SIGNIFICANCE: Aβ is the subject of intense scrutiny because of its close association with Alzheimer's disease (AD), which currently afflicts about 50 M people worldwide. The results reported in this manuscript focus on the new possibilities provided by ultra-fast MAS ¹H-detection and fast-MAS dynamic nuclear polarization (DNP), which have ushered in a new era for NMR-based structural biology, but whose potential has not yet been demonstrated for the structural investigation of complex amyloid assemblies. This work demonstrates the expeditious structural analysis of amyloid fibrils without requiring preparation of large sample amounts, and sets the stage for future studies of unlabeled AD peptides derived from tissue samples available in limited quantities.

Competing interest statement: The authors declare no competing interests

Introduction

Amyloid fibrils are highly stable protein deposits found in β-sheet conformations and are notoriously recognized as disruptive agents to cellular function in over 40 human diseases.¹⁻² Alzheimer's disease (AD) is the most pervasive of all known plaque-related diseases and is associated with the presence of amyloid-β (Aβ) peptides in the extracellular space of the brain.³⁻⁶ As of 2021, there are ~6.2 M people in the US living with Alzheimer's dementia and ~50 M worldwide,⁷ and there is as of yet no cure available for AD. In order to address this epidemic, it is essential that we learn as much as possible about the formation and structure of Aβ plaques including the detailed features of their catalytic surface in order to design and develop appropriate treatments to limit the propagation of aggregates and the generation of toxic forms.

Aβ is derived from the C-terminal region of the amyloid precursor protein (APP), a membrane protein in neuronal cells, via proteolysis by β and γ-secretase.⁸⁻⁹ One of the principal challenges in rationalizing AD etiology is Aβ's diversity in peptide length, mutations, and post-translational modifications.¹⁰ Their low solubility renders solution nuclear magnetic resonance (NMR) ineffective, and high-resolution diffraction analyses have thus far been restricted to shorter peptides with all or most residues being ordered in the fibril core structure¹¹. CryoEM has made strides in resolution in fibril studies within the past decade¹²⁻¹⁸, but faces challenges studying with atomic level detail due to polymorphism and heterogeneity in the fibril macro-assemblies. Studying the individual and collective roles of amyloids at atomic resolution therefore requires alternative, high-resolution, high-throughput techniques for structural analysis. Magic angle spinning NMR (MAS-NMR) was introduced as a

technique with the potential to address these problems.¹⁹⁻²⁰ Recent technical advances²¹⁻²² and progress in sample preparation²³ have vastly improved the sensitivity and resolution of the spectra.²⁴ Accordingly, there are now publications describing high resolution structures of A β ²⁵⁻²⁹ and other amyloid³⁰⁻³⁷ fibrils based on distance and torsion angle constraints derived from MAS experiments.

To date, all of the known NMR structures of amyloid fibrils were determined using constraints obtained from ¹³C/¹⁵N MAS spectra, which are inhomogeneously broadened and therefore feature well resolved lines at low spinning frequencies (<25 kHz).³⁸ However, resolution often remains insufficient for in-depth analysis, and the experiments require relatively large amounts of peptide and extensive signal acquisition periods. Two relatively recent additions to the repertoire of MAS NMR experiments – namely, ¹H detection and dynamic nuclear polarization (DNP) – promise to circumvent these issues by reducing signal acquisition times or, alternatively, the amount of protein required for the experiment³⁹. ¹H detection offers a factor of (γ_H/γ_S)^{3/2} gain in sensitivity, where S is usually a low γ -spin⁴⁰⁻⁴² such as ¹³C or ¹⁵N. In these two cases it is possible to achieve a factor of ~8 or ~32 gain in sensitivity, respectively. Importantly, ¹H detection also introduces an additional spectral dimension and therefore significantly increases the resolution. In parallel, DNP offers a general approach to enhancing sensitivity by factors of ~100, dramatically reducing signal acquisition times (by ~10⁴). It does so by exploiting the high spin polarization of unpaired electrons (of gyromagnetic ratio γ_e ~660 times larger than γ_H) of a paramagnetic polarizing agent to enhance sensitivity by a theoretical factor of γ_e/γ_H .⁴³⁻⁴⁶ Furthermore, DNP experiments are conducted at ~100 K, thereby increasing the Boltzmann polarization and sensitivity by another factor of ~3 over experiments conducted at ambient temperature.⁴⁷

While these arguments are well established for MAS NMR in many systems, it is less obvious that they are applicable to amyloid samples because spectra of amyloids are known to be broad for a variety of reasons, such as sample purity and polymorphism. Furthermore, ¹H-detected NMR at moderate MAS frequencies (approx. 20-60 kHz) needs to be coupled to different levels of deuteration to ensure high sensitivity and narrow linewidths.⁴² Accordingly, deuteration with partial reprotonation of the amide or H α sites has been implemented in pioneering studies on A β ₁₋₄₀ at 20 kHz MAS,⁴⁸ HET-s(218–289)⁴⁹ and D76N- β 2m at 60 kHz MAS.⁵⁰ In addition, selective protonation in A β ₁₋₄₀ fibril methyl groups at 18 kHz MAS has led to highly resolved ¹H-detected ¹³C correlations.⁵¹ In deuterated samples however, the amount of potentially available structural information is significantly reduced, which can impair high-resolution structure determinations. The advent of 0.7mm MAS rotors that achieve $\omega_r/2\pi > 110$ kHz attenuates ¹H-¹H dipole couplings and allows direct acquisition of multidimensional ¹H data without requiring deuteration.⁵² Furthermore, the spectra provide assignments and structural information. While a proof-of-concept application of this approach was demonstrated on fully protonated highly-regular prion fibrils,^{49,53-54} it is not clear whether this methodology is generally applicable and extendable to the detection of resolved inter- and intramolecular contacts in complex amyloid assemblies.

In parallel, our MAS DNP studies on M₀A β ₁₋₄₂^{28,32,55} report significant broadening of the NMR lines at cryogenic temperatures, which was attributed to distributions of conformations trapped at low temperature and is therefore inhomogeneous in origin. The loss of resolution associated with the MAS DNP methodology is a major obstacle for the detailed structural study of uniformly labeled amyloid samples. Concurrently, reports of well resolved spectra at high fields and spinning frequencies suggest that the broadening is homogeneous.⁵⁶⁻⁵⁸ The advent of DNP instrumentation operating at high magnetic fields (18.8 T) and faster MAS ($\omega_r/2\pi=40$ kHz) provides an approach to alleviate this limitation by attenuating homogeneous couplings.⁵⁹ However, this comes at the expense of the enhancement factor, potentially compromising the capacity to carry out expeditious multi-dimensional and multi-nuclear correlations. Moreover, NMR spectra of amyloid fibrils are known to suffer from additional debilitating broadening associated with their heterogeneous character (sample purity, polymorphism, etc.), which may mitigate the benefits of high magnetic fields.

In this work, we show that high resolution and sensitivity are possible for fibrils of M₀-A β ₁₋₄₂. Notably, we demonstrate rapid resonance assignment and site-resolved detection of numerous site-specific inter-nuclear proximities on sub-mg sample quantities via ¹H detected NMR at $\omega_r/2\pi \sim 110$ kHz and high field (23.4 T/ 1000 MHz for ¹H) at room temperature and ¹³C detected DNP MAS NMR at $\omega_r/2\pi=40$ kHz and high field (18.8 T/ 800 MHz for ¹H) at low temperature. While both ¹H detection and DNP afford increased sensitivity, we estimate, using approaches outlined by Ishii and Tycko⁴², that DNP, with our current $\epsilon=22$, yields a factor of ~6.5 higher sensitivity. These results therefore illuminate possible paths for the rapid structure elucidation of amyloid fibrils available in limited quantities.

Results and Discussion

At physiological conditions (pH ~7-8), the structure of M₀-A β ₁₋₄₂ fibrils formed *in vitro* is a C₂ symmetric dimer, in which K16-A42 forms the fibril core while D1-H14 are disordered and likely solvent-exposed^{28-29,36}. Twelve residues of each monomer are buried in the hydrophobic core, while the fibril surface has a mixed hydrophobic, hydrophilic and charged character with the A42 C-terminal carboxyl group and K28 side chain forming a so-called a salt bridge. Recently, Wickramasinghe and coworkers identified a new form of A β ₁₋₄₂ using ¹H-detection on brain-seeded fibrils.⁶⁰ Likewise, a very different structure of A β ₁₋₄₂ was published by Gremer et al.¹⁵ However, these latter fibrils were grown at pH=2 in 30% CH₃CN, which likely accounts for the disparity. We can thus say that M₀-A β ₁₋₄₂ fibrils that have been formed at physiological conditions and without brain plaque seeding have a reproducible structure, and are therefore a well characterized system useful to develop novel techniques using MAS NMR.

¹H-detected A β spectra

Figure 1 shows 2D ¹H, ¹⁵N and ¹H, ¹³C fingerprints of fully protonated U-¹³C, ¹⁵N-labelled M₀-A β ₁₋₄₂ fibrils recorded at $\omega_r/2\pi=110$ kHz on a 1 GHz spectrometer. These spectra represent dipolar-driven correlations that reveal interactions between nuclei that have little or no dynamics⁶¹. Consequently, our existing ¹³C/¹⁵N assignments for M₀-A β ₁₋₄₂ fibrils⁶² led us to quickly identify many H ^{α} and H^N resonances from the fibril core without the need for further correlations.⁶³ These spectra feature both excellent resolution and sensitivity for all amide and aliphatic protons in the backbone and sidechains. Signals have linewidths of ~0.2-0.3 ppm, which are larger than those observed in similar conditions on microcrystalline proteins⁵² but nonetheless guarantee a good dispersion and limited overlap. Most importantly, the resonances feature very

long coherence lifetimes (eg bulk T_2 's of ~70 and 40 ms were measured for ^{15}N and $^{13}\text{C}_\alpha$, respectively). This allows the acquisition of two 3D experiments for sequential backbone assignment based on the detection of H^N protons, namely (H)CANH and (HCO)-CA-(CO)NH that correlate the intra- and inter-residual CA to the H^N -N pair,⁶³ as well as ^1H -detected (H)NCAHA and ^{13}C - ^{13}C TOCSY-HSQC correlations for side-chain resonance assignments⁵³. These spectra required only ~0.5 mg of sample and few days of data acquisition to yield the complete identification of backbone and side-chain signals (~92% completeness) within the fibril core, consistent with the existing $^{13}\text{C}/^{15}\text{N}$ assignments for $\text{M}_0\text{-A}\beta_{1-42}$ fibrils.⁶²

At the same time, $\omega_r/2\pi=110$ kHz MAS approaches the limit at which highly flexible regions appear in dipolar-based spectra. As a result, we observed that Gly37 and Ala42 exist in two distinct states, which are visible in the 2D CP-HSQC spectrum (in red in **Figure 1**). These instances of polymorphism were not detectable in our previous experiments at 20 kHz MAS, suggesting that they derive from backbone flexibility in the C-terminal region. Likewise, several more resonances appear that have no apparent correlation to neighboring nuclei, and likely arise from the N-terminal residues of the fibril or polymorphs of the fibril core.⁶⁴

Ultimately, ^1H detection has the potential to provide direct access to ^1H - ^1H distances for structure calculations, as shown in microcrystalline proteins⁶⁵, viral assemblies⁵², and membrane-embedded proteins.⁶⁶⁻⁶⁷ Here, we implemented RFDR mixing into 3D HN and HC dipolar correlation schemes ((H)NHH or (H)CHH sequences), with representative slices from these datasets in **Figure 2a**. Even with shorter mixing times, RFDR transfers polarization between protons that are further apart than typical 1- or 2-bond ^{13}C - ^{13}C distances that we typically achieve at moderate spinning frequencies. As a result, these spectra contain correlations between sequential or near-neighbor residues but also a number of unambiguous long-distance contacts up to 4-5 Å. These experiments can alternatively be performed as ^{13}C -edited variants, to increase the spectral resolution through the indirect evolution of two carbon dimensions in the 3D ^{13}C , ^{13}C -edited ^1H - ^1H RFDR experiment (**Figure 2b**). Although the additional pair of forward and backward H-C cross-polarization transfers causes a two-fold drop in sensitivity, it allowed identification of a large number of unambiguous contacts listed in the Supplementary Information.

Overall, while faster spinning would still be required to make clear and quantitative interpretation of these ^1H - ^1H distance measurements, these contacts map the aliphatic side chains within the hydrophobic “steric zipper”, some of which are illustrated in **Figure 2c**. One of the most important was to confirm the position of F19 and F20 on the inside of the β -strand as shown in **Figure 2c**. This conformation was observed in the ^{13}C - ^{13}C spectra but is unusual and an independent observation is important.

^{13}C DNP experiments

A full determination of fibril architecture additionally requires measuring inter-molecular interactions among different monomers, and the 3D spectra described above contain additionally cross peaks between pairs of residues at the interface (eg H K16 - ME M35 HA L17 - HG L34, or H L17 - ME M35). However, the repetitive structure of the assembly does not allow to assign the experimental cross-peak intensity as inter-molecular restraints in an unambiguous fashion. Current protocols to address this problem rely on mixed samples obtained from differently isotopically labeled species^{54, 68-69}. Such approaches have been successfully applied to describe inter-strand arrangements and inter-monomer contacts, but the necessary dilution of the labelled spins further reduces sensitivity and consequently requires even larger sample quantities. In these situations, MAS DNP at low temperature has a unique potential to complement ^1H -detected NMR studies at room temperature.

To this end, we combined high-field DNP (800 MHz/527 GHz) with MAS in a 1.3 mm rotor, which permits spinning frequencies up to 40 kHz. In addition, we used a suitably designed water-soluble polarizing agent, M-Tinypol⁷⁰, and a selectively labeled 1:1 mixture of 1,3- ^{13}C -glycerol/ $\text{U-}^{15}\text{N}$ - and 2- ^{13}C -glycerol, $\text{U-}^{15}\text{N}$ -enriched $\text{M}_0\text{A}\beta_{1-42}$. Here, the specific labeling scheme offers nearly the full range of labeled spins as that of a uniformly labeled sample but results in considerably sharper line widths due to the elimination of the J -couplings between neighboring ^{13}C nuclei.^{62, 71-73} The resulting enhancement factor ($\epsilon = 22$, **Figure 3a**) is the highest reported to date at this magnetic field on a biomolecular sample, and reduces a week's worth of experiment time to ~1/3 of an hour, enabling the rapid acquisition of 2D and 3D MAS NMR experiments with only ~1 mg of sample. Furthermore, this modest enhancement yields a sensitivity ~6.5 times larger than that available from ^1H detection at room temperature. Accordingly, improvements in polarizing agents will increase this number. Details of the calculation of this estimate are included in the SI.

Importantly, a 2D ^{13}C - ^{13}C CORD-RFDR spectrum (**Figure 3c**) features resolved cross-peaks from the fibril core with linewidths of ~0.6 ppm, on par with the best results reported for DNP^{58, 74-77} and equal to the linewidths we observed in spectra acquired at room temperature. In the carbonyl region, a few long-range contacts that reflect both inter- and intra-molecular contacts also appear in the carbonyl region (highlighted in red). For example, I32-CG1/V18-CO and G37-CA/V39-CO are consistent with the monomer structure, while L17-CB/M35-CO corresponds to the dimer interface. DNP enhanced ^{15}N - ^{13}C CO and ^{15}N - ^{13}C CA spectra (**Figure 3d**) also display very good resolution in the ^{15}N dimension, and feature all the signals from residues in the 15-22 range. Notably, the intensity is lower for residues 22-25, corresponding to a loop which is more dynamical at room temperature. This is consistent with a broader distribution of shifts corresponding to the freezing of multiple conformations. The spectra additionally reveal several peaks that were not present in the equivalent experiment at room-temperature.²⁴ These likely arise because cryogenic conditions required by DNP quench any dynamics that interfere with the ^1H decoupling that attenuates peak intensities.⁷⁸⁻⁷⁹ Concurrently, the microwave enhancement could potentially bring out minor polymorphs as well as the otherwise disordered or dynamic N-terminal region, opening new windows for the study of the extent of the fibrils' heterogeneity.

The excellent resolution and sensitivity suggest that resolved site-specific contacts could be monitored by combining these 2D datasets in 3D experiments. A pair of 3D NCACX and NCOCX spectra can be acquired by appending a ^{13}C - ^{13}C CORD-RFDR mixing to ^{15}N - ^{13}C CO and ^{15}N - ^{13}C CA modules. **Figure 4** shows for the fragment I31-V36 how the joint analysis of these spectra yields both sequential linking of the resonances and contacts between ^{13}C CO and ^{13}C CA/ ^{13}C CB across the interface of two stacked molecules. On the one hand, this demonstrates the possibility to perform an independent backbone sequential assignment of ^{13}C and ^{15}N resonances directly under cryogenic conditions, extending a strategy proposed by Sergeyev, et al.⁷⁷ Notably, joint analysis of the NCACX and NCOCX pair allows us to associate each ^{15}N frequency with

those of the ^{13}C and ^{13}C of both the same and the previous residue. This is illustrated in **Figure 4**, where residues I31-I32-G33 and L34-M35-V36 feature a clear sequential connectivity along the backbone. On the other hand, given the mixed labelling, we can easily highlight long-range contacts that reflect inter-molecular proximities. For example, among the strips displayed in **Figure 4**, we observe CA/CO correlations for G33, L34 and V36, which is consistent with the parallel-in-register architecture. The same strategy also allows us to detect unambiguous correlations such as e.g. L17-CB/M35-CO, corresponding to the dimer interface. Overall, these DNP experiments show that combining high field, fast spinning, up to date biradical polarizing agents, and specific labeling schemes produces a wealth of structural information in amyloid fibrils not available in other experiments. These data advantageously complement the capacity of the ^1H -detected experiments described earlier.

Conclusions

The possibility of directly recording and assigning resolved resonances throughout the backbone and side-chains is key for obtaining a large set of contacts in amyloid fibrils. Here we have shown that this information can be accessed using only sub-mg sample amounts and without the need for deuteration from high-field ^1H -detected NMR with 110 kHz MAS and from high-field MAS DNP. Our results demonstrate the significant benefits of these methodologies in the context of amyloid studies and suggest the potential to perform high-throughput analyses of different amyloid constructs without requiring preparation of large quantities of protein, or even to study unlabeled peptides derived from tissue samples available in limited quantities.

Additionally, the high spectral resolution provided by high spinning frequencies and high signal enhancements reported here lay the ground-work for implementing ^1H -detection and DNP in a combined fashion for amyloid research in the near future. The ultimate purpose of our exploration is to determine whether combining ^1H -detection and high field DNP may be feasible for studying fibrils. Together with the $\epsilon \sim 22$ enhancement from M-TinyPol, ^1H -detected DNP at low temperature may bring a factor of several thousand-fold increase in sensitivity, which will likely improve with further development of hetero-biradical polarizing agents. Indeed, ^1H -detected DNP experiments have recently been accomplished on histidine at 65 kHz MAS and 100K at high field⁸⁰. Extending this work to amyloid fibrils at natural abundance is thus a challenge that is within reach, and these new possibilities will enhance our understanding of molecular events underlying Alzheimer's disease and improve the perspectives for therapeutic invention.

Materials and Methods

Sample preparation

The biosynthetic preparation of U- $^{13}\text{C}/^{15}\text{N}$ MoA β_{1-42} fibrils sample was accomplished by expression of the MoA β_{1-42} peptide in *E. coli* in M9 minimal medium supplemented with ^{15}N -ammonium chloride and $^{13}\text{C}_6$ -glucose, and purification of the peptide from inclusion bodies using ion exchange and size exclusion chromatography, followed by a second size exclusion step to isolate pure monomer prior to spontaneous fibril formation under quiescent condition. This procedure was used previously^{28,36,81} and is adapted from Walsh, et.al⁸². The 1,3/2- ^{13}C glycerol/ ^{15}N mixture used in the DNP experiments was prepared by expressing the peptide in *E. coli* in two different M9 media, one containing 1,3- $^{13}\text{C}_2$ glycerol, and the other with 2- ^{13}C glycerol. The peptides were purified independently but then combined as monomers after the final size exclusion step, using the absorbance of the collected monomer peak to determine the concentration of each sample before preparing a 1:1 mixture of the two ^{13}C -labeled variants. Fibrils were formed by incubating the mixture under quiescent conditions, at pH = 8.

^1H detection experiments

For the ^1H -detected experiments, U- ^{13}C , ^{15}N -MoA β_{1-42} labeled fibrils were resuspended in water and directly centrifuged at 165,000 $\times g$ for 15 h at 12°C into the NMR rotor using a device provided by Giotto Biotech, similar to those described in literature.⁸³⁻⁸⁴

All spectra were acquired at the static field of 23.5 T (1000 GHz ^1H) with a three-channel (HCN) 0.7 mm Bruker MAS probe and at the static field of 18.8 T (800 MHz ^1H) with a four-channel (HCND) 0.7 mm Bruker MAS probe. The sample was spun at 111.111 kHz, and we maintained the temperature using a Bruker cooling unit (BCU III) with a regulated dry N_2 gas directed at the rotor. The temperature detected by the sensor (260 K) at the point where bearing, drive and VT gases are mixed, corresponds to roughly 285 \pm 5 K inside the 0.7 mm rotor. On both probes, high-power pulse durations were 2.5, 3.5 and 4.5 μs for ^1H , ^{13}C and ^{15}N , respectively. ^1H , ^{13}C and ^{15}N chemical shift data is available in the BMRB.

The pulse sequences follow, with no modifications, those described in Barbet-Massin, et al.⁶³, Andreas, et al.⁵² and Stanek, et al.⁵³. For the dipolar-based ^{15}N , ^1H and ^{13}C , ^1H CP-HSQC experiments, ^1H - ^{15}N and ^1H - ^{13}C CP transfers were optimized around nutation frequencies of $5/4 \omega_r$ and $1/4 \omega_r$, respectively for proton and ^{15}N (or ^{13}C), with a 10 % linear ramp applied on the ^1H channel. For 3D CANH and (H)(CO)CA(CO)NH⁸⁵, ^{13}C - ^{15}N CP was performed with a 10% tangent ramp applied on the ^{15}N frequency at $2/5 \omega_r$, while the ^{13}C nutation frequency was kept around $3/5 \omega_r$. The H(C)CH and (H)CCH TOCSY sequences were implemented with composite ^{13}C pulses applied with a low nutation frequency of $1/4$ the MAS frequency (~ 27.5 kHz).

In 3D (H)NHH, (H)CHH and (H)C(HH)CH spectra, ^1H - ^1H RFDR recoupling⁸⁶⁻⁸⁸ was applied after the first back-CP at a ^1H RF frequency of 100 kHz, yielding ^1H - ^1H contacts resolved using the shift of ^{15}N or aliphatic ^{13}C .

In all experiments, low power WALTZ-16 decoupling of 10 kHz was employed for heteronuclear decoupling, while ^{13}C decoupling during acquisition was performed with DIPSI-2 irradiation of $\gamma\text{B}_1/2\pi=20$ kHz. The MISSISSIPPI scheme⁸⁹ without the homospoil gradient was used for 100 ms in order to suppress the water signal.

Spectra were apodized in each dimension with 50-degree shifted squared sine-bells ('qsine 3' in Bruker Topspin), and zero-filled to at least twice the number of points in the indirect dimensions. Where linewidths are reported, no apodization was applied for the reported frequency. Acquisition parameters specific for each spectrum can be found in Table S1.

Experimental parameters for DNP

For the DNP MAS experiments, we resuspended $M_{\alpha}\beta_{1-42}$ fibrils prepared from a mixture of 1,3- ^{13}C -glycerol and 2- ^{13}C -glycerol labeled samples in 25 μL of DNP juice (d_8 -glycerol/ $\text{D}_2\text{O}/\text{H}_2\text{O}$ 60/30/10) containing 10 mM of M-TinyPOL. The suspension was left in the refrigerator overnight and packed into a 1.3 mm ZrO_2 rotor the following morning by centrifugation for 3 h at 120,000 g and 4° C.

DNP MAS NMR spectra were recorded on a Bruker Avance III wide bore spectrometer, operating at 18.8 T, and equipped with a triple resonance H/X/Y 1.3 mm low-temperature MAS probe. The sample was irradiated with high-power microwaves at a frequency of 527 GHz generated by a gyrotron that was operating continuously during the DNP experiments (stability of better than $\pm 1\%$). A microwave power of 22 W was used (measured at the bottom of the probe), with a field position we adjusted for optimal cross-effect. The MAS rate was 40 kHz. Sample temperature, estimated from a sensor placed inside the stator that measures the temperature of gas flows around the rotor, was about 115 K (VTU was 95.0 K, bearing 94.5 K, drive 127.8 K). High-power pulse durations were 2.5, 3 and 6 μs for ^1H , ^{13}C and ^{15}N , respectively. For all experiments, ^1H - ^{15}N and ^1H - ^{13}C CP transfers were optimized around nutation frequencies of 100 kHz for proton and 60 kHz for ^{15}N or ^{13}C , with a 50 % linear ramp applied on the ^1H channel. CP transfers between ^{13}C and ^{15}N were performed with a 10% tangent ramp applied on the ^{15}N frequency at 15 kHz while the ^{13}C nutation frequency was kept around 25 kHz. The ^{13}C - ^{13}C CORD-RFDR experiment was recorded as described by Hou, et al ⁹⁰, with a mixing time of 104 ms. In the NCOCX and NCACX experiments, we employed the CORD-RFDR mixing scheme for an identical duration.

Spectra were apodized in each dimension with 50-degree shifted squared sine-bells ('qsine 3' in Bruker Topspin), and zero-filled to at least twice the number of points in the indirect dimensions. Details about the used acquisition and processing parameters are given in Table S2 below.

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Figure Captions

Figure 1. High-resolution ^1H -detected spectra recorded on fully protonated $\text{U-}^{13}\text{C},^{15}\text{N}$ -labeled $\text{M}_0\text{-A}\beta_{1-42}$ at 111.111 kHz MAS. Above: 2D ^{13}C - ^1H CP-HSQC; Below: 2D ^{15}N - ^1H CP-HSQC.

Figure 2. Representative restraints from the (a) 3D (H)NHH, (H)CHH and (b) (H)C(HH)CH spectra that show long-range ^1H - ^1H contacts corresponding to the intra-molecular structure of $\text{M}_0\text{-A}\beta_{1-42}$, highlighted in red. Asterisks mark diagonal peaks. (a) The NHH and CHH experiments establish contacts between F20H and I32 γ_{12} and A30a, between A30 α and F20 ζ F19 β_3 and I42 δ_1 , and from F19 α to I32 $\gamma_2/\gamma_{12}/\delta_1$. (b) The CCH experiments establish contacts between G33 α and V36 β and V36 γ_1 and between I41 β and G29 α (c) Display of intramolecular contacts found in the spectra shown in Figure 3 on the lowest energy NMR structure of $\text{M}_0\text{A}\beta_{1-42}$ (PDB ID 5kk3²⁸). The contacts illustrated in the figure are listed in the caption for Figure 3.

Figure 3: (a) DNP enhanced ^{13}C spectra of 1,3- $^{13}\text{C}_2$ / 2- ^{13}C / ^{15}N labeled $\text{M}_0\text{A}\beta_{1-42}$ illustrating and enhancement of $\varepsilon=22$ using (b) bis-nitroxide polarizing agent M-TinyPol (c) DNP-enhanced 13C-13C CORD-RFDR spectra of 1,3- $^{13}\text{C}_2$ / 2- ^{13}C / ^{15}N labeled $\text{M}_0\text{A}\beta_{1-42}$. The resolution in the spectrum is comparable to that obtained at ambient temperatures, ~ 0.6 ppm as indicated for I31C β -C α . In red we show the long-range contacts that correspond to the intra-molecular monomer structure of the fibril. (d) DNP-enhanced $^{15}\text{N}^{13}\text{CO}$ and $^{15}\text{N}^{13}\text{CA}$ (right) spectra acquired using M-TinyPol as the polarizing agent. $\omega_r/2\pi=40$ kHz and $T=100$ K

Figure 4. Selected cross-sections from DNP-enhanced NCOCX (blue) and NCACX (red) spectra of 1,3- $^{13}\text{C}_2$ / 2- ^{13}C / ^{15}N labeled $\text{M}_0\text{-A}\beta_{1-42}$. Green circles indicate signals which reflect the inter-dimer contacts and the parallel-in-register arrangement of the fibrils. Asterisks mark diagonal peaks.

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Figure 1

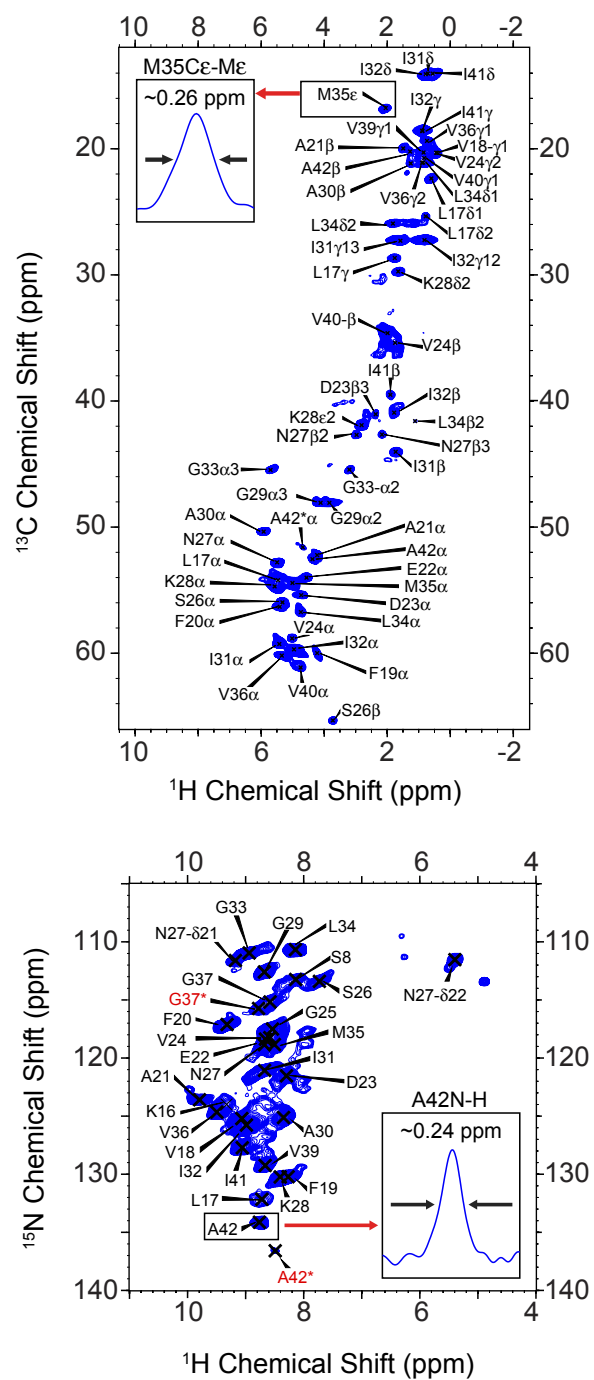


Figure 2

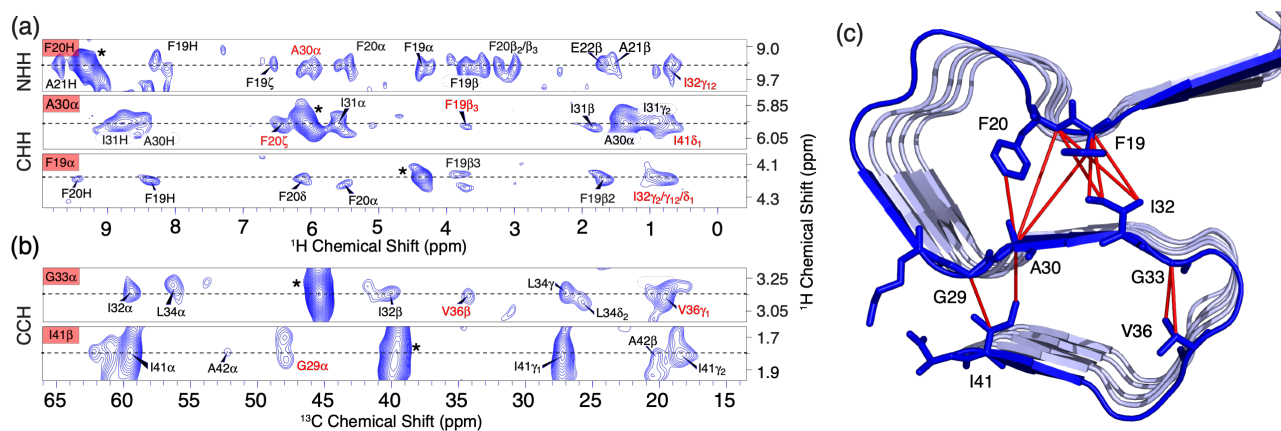


Figure 3

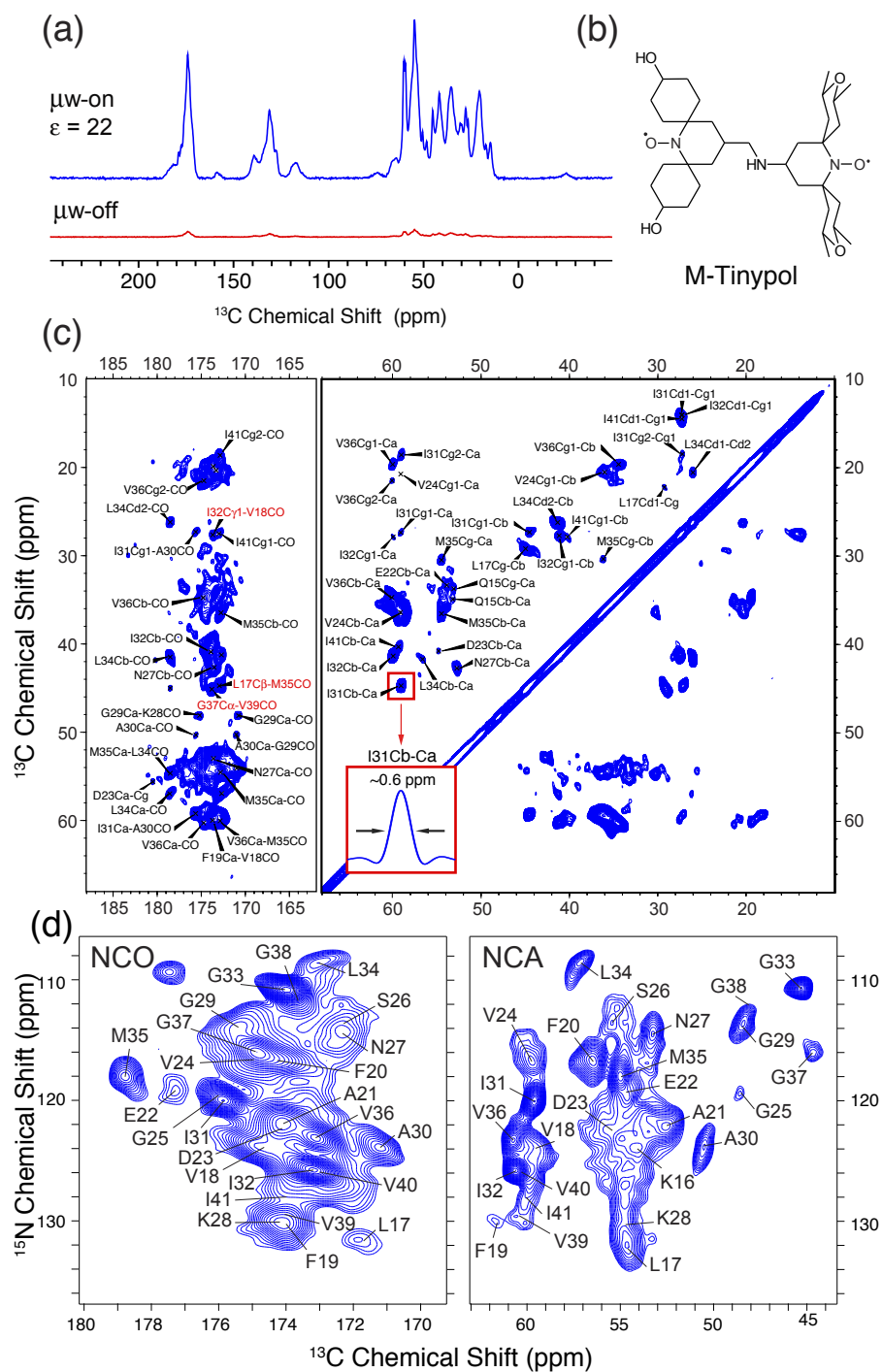


Figure 4

