Dipolar recoupling NMR of biomolecular self-assemblies: determining inter- and intrastrand distances in fibrilized Alzheimer’s β-amyloid peptide

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Abstract

We demonstrate a new method for investigating the structure of self-associating biopolymers using dipolar recoupling NMR techniques. This approach was applied to the study of fibrillar β-amyloid (Aβ) peptides (the primary component of the plaques of Alzheimer’s disease) containing only a single isotopic spin label (13C), by employing the DRAWS (dipolar recoupling with a windowless sequence) technique to measure 13C-13C distances. The 'single-label' approach simplified analysis of DRAWS data, since only interstrand contacts are present, without the possibility of any intrastrand contacts. As previously reported T.L.S. Benzinger, D.M. Gregory, T.S. Burkoth, H. Miller-Auer, D.G. Lynn, R.E. Botto, S.C. Meredith, Proc. Natl. Acad. Sci. 95 (1998) 13407., contacts of approximately 5 Å were observed at all residues studied, consistent with an extended parallel β-sheet structure with each amino acid in exact register. Here, we propose that our strategy is completely generalizable, and provides a new approach for characterizing any iterative, self-associating biopolymer. Towards the end of generalizing and refining our approach, in this paper we evaluate several issues raised by our previous analyses. First, we consider the effects of double-quantum (DQ) transverse relaxation processes. Next, we discuss the effects of various multiple-spin geometries on modeling of DRAWS data. Several practical issues are also discussed: these include (1) the use of DQ filtering experiments, either to corroborate DRAWS data, or as a rapid screening assessment of the proper placement of isotopic spin labels; and (2) the comparison of solid samples prepared by either lyophilization or freezing. Finally, data obtained from the use of single labels is compared with that obtained in doubly 13C-labeled model compounds of known crystal structure. It is shown that such data are obtainable in far more complex peptide molecules. These data,
1. Introduction

NMR is rapidly becoming an established method for studying the 'solid-state' structures of biological molecules [1–4]. In their native state, many biomolecules are neither crystalline or soluble in solution. Some examples include membrane proteins [5–8], certain large enzymes [9,10] and forms of amyloid [11–14] and prion [15] proteins. Thus, solid state NMR techniques often are complementary to other methods because they extend the range of the physical state in which biomolecules can be investigated.

Modern solid-state NMR techniques that presently are being employed for measuring internuclear distances in biomolecules have evolved from earlier work on rotational resonance [16,17] and REDOR [18]. A commonality among all of these methods is their ability to restore dipolar couplings while preserving the spectral resolution afforded by implementation of the MAS experiment. Dipolar couplings are reintroduced in the experiment by using selective rotor-driven, r.f.-driven, or magnetization exchange techniques. The goal, then, has been to utilize these direct 'through-space' dipolar interactions as a means of accurately determining distances between individual spin pairs, which can be placed selectively within the molecule of interest. Accordingly, distances are measured to high precision from the static dipolar interaction between nuclear spins, because of the sensitivity of the coupling strength, which varies as the inverse cube of the internuclear separation.

The strategy most commonly employed in these experiments has been to introduce pairs of magnetically dilute spin-1/2 nuclei selectively, by isotopic enrichment. While this strategy has been successfully for measuring intramolecular distances between nuclei in a variety of systems, it also has some obvious disadvantages. A major disadvantage is that it is not always possible, when attempting the study of self-associating biomolecules, to unravel intermolecular effects in the presence of sometimes stronger intramolecular dipolar interactions. The resulting structural detail obtained has been limited to the tentative assignment of conformational structure only [12,13]. The synthesis of complex molecules containing labels at two specific sites for each distance measurement represents an additional difficulty.

In an effort to simplify investigations of biomolecular self-assemblies, we have devised a simple approach of using molecules that incorporate only a single isotopic label, $^{13}$C. This strategy is, in a sense, contrary to that of the general approach employing ‘multiple-spin’ labels. In our experiments, the only possible contacts between nuclei are intermolecular, facilitating analysis of the data in a relatively straightforward fashion.

However, measuring intermolecular distances between labeled sites in complex biomolecular assemblies presents its own set of challenges. For instance, selecting an appropriate site to label becomes paramount. One must be able to predict, a priori, the specific location of a site, which in the aggregated form brings the spin label in close proximity (i.e., < 6 Å) to the same site in an adjacent molecule. Even in the ideal case, the dipolar interaction for $^{13}$C-$^{13}$C spin pairs will be weak and difficult to detect. And finally, resolving the issue of whether one is detecting interactions between two spins in isolation or among multiple-spins is critical to measuring distances accurately.

We demonstrate how this approach can be used to elucidate the core structure of β-amyloid (Aβ) peptides, the main protein component of the plaques occurring in brains of patients with Alzheimer’s disease [31]. Alzheimer’s disease is the third leading cause of death in the elderly in the US [19]. The disease is characterized by the formation of extracellular, insoluble plaques in the brains of Alzheimer’s patients. β-Amyloid [20] is a family of peptides with the amino acid sequence, H$_2$N-DAEFRHDSGYEV-
HHQKLVFAEDVGSNKGEIGLMVGGV(VIAT)-CO₂H. Electron microscopy studies have shown that the β-amyloid plaques are comprised of long fibrils with a diameter of about 70–100 Å [21]. However, the molecular structure of Aβ in fibrils, as well as the mechanism for fibril formation is not well understood. This is in large part due to the difficulty in studying largely insoluble, non-crystalline forms of Aβ, which renders them unsuitable for the traditional high-resolution methods of protein structure determination such as solution-phase NMR or X-ray crystallography. We employed the DRAWS (dipolar recoupling with a windowless sequence) experiment [22] to measure 13C–13C distances to a precision of 0.2 Å, in Aβ peptides (Aβ₁₀–₃₅) labeled at carbonyl carbon atom of a single amino acid. From analysis of these data, we demonstrated that Aβ₁₀–₃₅ has a parallel β-sheet structure with amino acids in exact register.

In our analysis of DRAWS data, a number of critical issues arose, which we address in this paper. First, measurements of weak dipolar couplings are demonstrated on two model compounds, 13C-labeled succinic acid and adipic acid. DRAWS experiments are performed on several independent control samples, including Aβ₁₀–₃₅ peptide at natural 13C abundance, fibrilized and precipitated forms of labeled peptides, frozen suspensions of fibrils and isotopically diluted samples. In addition, intramolecular distances are obtained on Aβ₁₀–₃₅ isotopically labeled at two carbonyl carbons in contiguous amino acid residues, prior to and after fibrilization. The results document the conformational transition of the peptide from an α-helix in solution to a β-strand structure in the fibril. Also, new experimental details for measuring the double-quantum (DQ) relaxation rate are presented along with a theoretical description. The influence of both single-quantum (SQ) and DQ relaxation on the DRAWS simulations is discussed. Finally, the 13C-labeled peptides are investigated by DQ filtering and DRAWS experiments. Using numerical simulations that best fit the data, the spatial dependence of the nuclear dipolar interactions have been derived and subsequently are used to simulate the DRAWS decay curves. Results are presented using two-, three- and four-spin interaction models having different geometries. These data, taken together, refine the DRAWS method, and demonstrate its precision and utility in obtaining high resolution structural data in complex biomolecular aggregates such as Aβ.

2. Methods

2.1. Synthesis, purification and analysis of β-amyloid peptides

1-13C-L-lysine, 1-13C-L-glutamine, 1-13C-L-leucine and 1-13C-L-valine were obtained from Cambridge Isotope Laboratories. Protection of the 13C-labeled amino acids was performed by Midwest Biotech, Aβ₁₀–₃₅ peptides were synthesized using an Fmoc amide resin (ABI) and standard Fmoc chemistry on an ABI model 431A peptide synthesizer.

For cleavage of the peptide from the resin, a solution containing 10 ml neat trifluoroacetic acid (TFA), 0.25 ml EDT, 0.5 ml thioanisole, and 0.5 ml ddH₂O was prepared and added to the peptide–resin on ice. The reaction was then allowed to proceed at room temperature, with stirring, for 1–1/2 to 2 h. The beads were then separated from the peptide by filtration and the beads and glassware were washed with 30 ml DCM. The peptide solution was reduced to 2 ml under reduced pressure, then precipitated by 100 ml ice cold ether. The peptide was then purified by 6–12 ether extractions, after which the purity was assessed by analytical HPLC (at 70°C) and the identity confirmed by mass spectrometry. Peptides determined to be 96–99% pure by this method were stored as the ether precipitate at −20°C. For some peptides, further purification by preparatory HPLC was required. Preparatory HPLC was performed on a ZORBAX 300-SB C4 column at 70°C under isocratic conditions (27% ACN, 0.1% TFA).

Fibrils were formed by solubilizing Aβ₁₀–₃₅ at 0.2 mM (by mass) in water at pH 3.0, and then slowly adjusting the pH to 7.4 or 5.6 by dropwise addition of dilute sodium hydroxide. The samples were then stirred at room temperature for 3 days under nitrogen. Fibril formation was monitored by the disappearance of the monomeric peptide peak on analytical reverse phase HPLC, and for the appearance of fibrils by electron microscopy. Once fibril formation was complete, the peptides were lyophilized and stored at −20°C.
For experiments performed at $-80^\circ$C, instead of lyophilization, the fibrils were reduced in volume to 400 $\mu$L using 3000 molecular weight cutoff Microcon and Centriprep Centrifugal Concentrators (Amicon) and stored at 4$^\circ$C.

Malonic acid-1,3-13C, succinic acid-1,4-13C and adipic acid-1,6-13C were purchased from Isotec. Both samples were diluted in parent compounds at the natural 13C abundance to 20%, 5% and 1%, respectively, by co-recrystallization. Methyl-13C,d3 alcohol-d was purchased from Aldrich.

2.2. Solid state NMR experiments and analysis

DRAWS data were acquired on a Bruker Advance DSX-200 spectrometer operating at a 13C Larmor frequency of 50.3 MHz. A Bruker 7 mm MAS probe was used to perform all experiments. Sample spinning was maintained at 4525 ± 2 Hz. The 13C magnetization was prepared initially by employing the ramped-amplitude cross-polarization (RAMP-CP) sequence [23,24]. The 13C r.f. power was 50 kHz during the cross-polarization period, then reduced to 38.5 kHz ($t_{\text{rf}} = 6.5$ ms) during the DRAWS period. The 1H r.f. power was varied between 30 kHz and 70 kHz during the RAMP-CP period. The proton power was increased to 115 kHz during the DRAWS period and decreased to 80 kHz during acquisition. The Bruker four-phase modulator was used in all experiments.

The DRAWS experiment has been described previously; the basic pulse sequence is shown in Fig. 1. DRAWS is based on an earlier experiment called DRAMA [25]. With DRAMA the dipolar interaction is reintroduced in a MAS experiment with the application of two 90° pulses per rotor period. In addition to the 90° pulses, the DRAWS sequence incorporates a train of 360° pulses which give better compensation for chemical-shift anisotropy and offset effects. In this work, a four-rotor period supercycle RRRR was used in the DRAWS experiments (see Fig. 1b).

A 13C π-pulse was centered one rotor period after the conclusion of the DRAWS pulse sequence and a Hahn echo was detected one rotor period later.

The pulse sequence used for DQ filtering [26] and measurement of DQ relaxation rate is shown in Fig. 1c. A standard DQ phase cycle was used in the experiment to minimize signal that is not in a DQ state at the beginning of the first the $\tau$ period [27].

For phase cycling purposes, the composite 90°$X$–90°$Y$–90°$X$ pulse is phase cycled the same as the preparation period of the pulse sequence. Proton decoupling remains on during the $\tau$ periods at the same level as during the DRAWS pulses.

We took several steps to insure that our results were reproducible. Kel-F spacers were placed on the top and bottom of samples contained within the 7-mm MAS rotors; this greatly reduced effects arising from $B_z$ inhomogeneities. In this way, the available sample volume was reduced by approximately one half. We also found it necessary to restrict each sample to the identical volume element in the rotor. KBr was mixed with smaller samples so that the entire available volume was utilized. Amyloid samples were mildly hydroscopic; thus, to insure dryness, rotors were placed in a desiccator under vacuum after they were packed. All samples were homogeneously mixed with a small amount of hexamethylbenzene as a control.
The $^{13}$C r.f. power levels were initially set using a sample of methyl-$^{13}$C,d$_4$ alcohol-d. A standard tune-up procedure was used to minimize pulse errors [28]. We found that the effects of phase transients could be reduced by placing 29 $\Omega$ of resistance just before the $^{13}$C channel of the probe, and thus, slightly reducing the $Q$ of the probe circuit. Phase transients were further reduced by adjusting the r.f. cable length between the preamplifier and probe.

The DRAWS experiment is sensitive to $^{13}$C r.f. power level. Thus, the carbon power level was optimized for each sample. This was done by performing the DRAWS experiment with a fixed mixing period while varying the r.f. power produced by the $^{13}$C amplifier. If the $^{13}$C r.f. B$_1$ field is not set to the proper value producing the greatest signal determines the optimal $^{13}$C r.f. B$_1$ field. We found that we needed to vary the $^{13}$C power level only very slightly from sample to sample, as long as the samples were kept dry. Before starting an experiment we retuned the probe after at least 30 min of pulsing.

Each point of the DRAWS decay curve was obtained by integrating over the centerband peaks in the NMR spectra. Sideband peak intensities were found to be negligible in this work and thus were ignored. The integrated intensities were normalized to the intensity obtained from a CP/MAS spectrum with no DRAWS applied. Each DRAWS experimental point was repeated five times. The points reported are the average, and the error bars represent one standard deviation.

Given the inherent resolution of solid-state NMR spectra, virtually all carbonyl chemical shifts for $\beta$-sheet structures are superimposable at $\delta = 171$ ppm. It was necessary, therefore, to subtract the natural abundance signal derived from other amino acid residues. A similar situation occurs when the $^{13}$C-labeled dicarboxylic acids are diluted in parent compounds containing $^{13}$C at natural abundance. For $S_T$ = total experimentally observed signal, one obtains:

$$S_T = aS_L + bS_U$$  

where $S_L$ = normalized signal attributable to the labeled carbonyl carbon(s), and $S_U$ = normalized background signal due to $^{13}$C at natural abundance from the 24 or 25 other carbonyl carbon atoms, where the sum of the coefficients, $a$ and $b$, is unity. This treatment assumes that the $^{13}$C abundance at the labeled position is 100%, and 1.1% for each of the other 24 or 25 amino acids in the peptide. Thus:

$$S_T = aS_L + (1 - a)S_U$$  

where $a = 0.78$ for mono $^{13}$C-labeled $\beta$-amyloid peptides, $a = 0.88$ for di $^{13}$C-labeled peptides, $a = 0.77$ for the succinic acid-1,4-$^{13}$C$_2$ sample, and $a = 0.48$ for the adipic acid-1,6-$^{13}$C$_2$ sample.

Simulated data were created by performing precise numerical calculations of the nuclear spin dynamics under the full time-dependent Hamiltonian [22]. The input parameters to the numerical calculation program included the chemical shift tensor (CSA) elements for the spin-1/2 nuclei, the dipolar coupling strengths, Euler angles which rotate the CSA tensors from the molecular frame to their respective principle axis systems, an initial density matrix $\rho(0)$, an observable $\hat{\theta}$, and any relevant relaxation parameters. In this work the Euler angles were set to zero, as it was determined that they had a negligible effect on the simulated curves. The CSA parameters were taken from the data of Ye et al. [29]. The initial density matrix and the observable are given by:

$$\rho(0) = \hat{\theta} = I_{1Y} + I_{2Y}$$ (two spins)

$$\rho(0) = \hat{\theta} = I_{1Y} + I_{2Y} + I_{3Y}$$ (three spins).

$$\rho(0) = \hat{\theta} = I_{1Y} + I_{2Y} + I_{3Y} + I_{4Y}$$ (four spins).  

This is propagated in the usual fashion using the truncated high field, rotating frame MAS Hamiltonian that describes the nuclear magnetic moments. The calculated ‘signal’ is found by taking the trace:

$$\left< \hat{\theta}(t) \right> = \text{tr} \left[ \rho(t) \hat{\theta} \right]$$

$$\rho(t) = U^{-1}(t) \rho(0) U(t)$$  

$$U_r = \prod_{m=1}^{n} \exp(iH(m \cdot \Delta t))$$ and $n = t/\Delta t$.

The time increment was typically 2–3 $\mu$s. Relaxation effects were modeled by multiplying the SQ
density matrix elements by an exponential factor at the end of each time increment. Data from unlabeled samples were used to determine the appropriate SQ relaxation time constant. The program also performs a powder average of 2000–4000 randomly selected crystallite orientations.

3. Theory

Theoretical descriptions of the DRAWS and DQ filtering experiments have been presented previously [22,26]. To accurately measure weak dipolar coupling interactions between $^{13}$C nuclei, particularly for the case of protonated carbons, it is often necessary to include effects from $T_2^{SQ}$ on the calculated DRAWS curves. Here we describe an experiment created to measure the DQ relaxation rate and present a theoretical description of it. The idea is to measure $T_2^{DQ}$ using a pulse method analogous to the Hahn spin–echo experiment, which is typically used to measure $T_2^{SQ}$ relaxation [30]. First, coherence is produced in a DQ-DRAWS experiment (see Fig. 1c) and allowed to evolve for a time $\tau$. The DQ coherence is subsequently refocused using a composite 90°-90°-90° pulse. This serves the same function as the 180°-pulse in a Hahn spin–echo experiment, as will be described below. DQ coherence is allowed to evolve for a second time period $\tau$ and then converted back to SQ coherence for detection. Signal intensity is plotted as a function of time $2\tau$, and the decay rate is fit to a single exponential yielding $T_2^{SQ}$.

The effect of a 180°-pulse on SQ coherences is to leave magnetization of one phase unchanged while inverting the sign of the other phase. For instance, a 180° pulse leaves the $I_y$ spin state unaffected while it changes $I_x$ to $-I_x$. In order to refocus DQ coherences, it is necessary to create the same effect on the DQ spin states $I_x^{DQ}$ and $I_y^{DQ}$, Let the effect of the three-pulse sequence, 90°-90°-90°, be described by the operator $Q$. Then $Q(I_x^{DQ}) = I_x^{DQ}$ and $Q(I_y^{DQ}) = -I_y^{DQ}$.

To describe this more explicitly, let

$$U_x = \exp \left( \frac{i\pi}{2} (I_x + S_x) \right)$$

and

$$U_y = \exp \left( \frac{i\pi}{2} (I_y + S_y) \right)$$

Then, using the product operator basis of Sorensen et al. [32],

$$Q(I_x^{DQ}) = Q(I_x S_y + I_y S_x) = U_x U_y U_y (I_x S_y + I_y S_x) U_y^{-1} U_y^{-1} U_x^{-1} U_x$$

Similarly,

$$Q(I_y^{DQ}) = Q(I_x S_x - I_y S_y) = U_x U_y U_x (I_x S_x - I_y S_y) U_y^{-1} U_y^{-1} U_x^{-1} U_x$$

Thus, the composite pulse produces the desired effect, refocusing DQ coherences in a manner analogous to a Hahn spin–echo for SQ coherences.

4. Results

4.1. General strategy

In our previous studies [32], we employed the DRAWS pulse sequence to measure internuclear distances in $^{13}$C-labeled β-amyloid peptides, mostly in lyophilized samples. Here, we also compare results from various samples prepared under different conditions; these include samples at natural $^{13}$C abundance, those prepared by ether precipitation from TFA, and samples which were prepared to form fibrils at pH 5.6 and 7.4. Also, several fibrillized samples were never lyophilized, but instead were measured directly as a frozen solution. In this way, we were able to verify whether the peptide structure was altered by the lyophilization procedure.

Our synthetic labeling scheme included placing 1-$^{13}$C-carbonyl labels in amino acids of the truncated β-amyloid sequence $\text{AB}_{10-35}$; $^1\text{YEVHHQKLVFEADVGSNKGAIGL}$. The rationale for the use of the truncated peptide as a model for the full length peptide is given in detail elsewhere. Briefly, the truncated peptide is competent to form fibrils [33–35]; contains the central core region in which mutant
perturb fibrillogenesis [36–41]; is neurotoxic [42], and yet is more tractable than the full length peptide, allowing for homogeneous fibril preparations. The initial region for placement of $^{13}$C labels was guided by the observation that the enzyme tissue transglutaminase specifically crosslinks Gln$_{15}$ and Lys$_{16}$ of Aβ, but never involves Lys$_{28}$ [43,44], suggesting that Gln$_{15}$ and Lys$_{16}$ are proximate in an aggregated form of Aβ. Studies of peptides labeled along the entire sequence of Aβ$_{10–35}$ have been synthesized, and these studies will be reported elsewhere.

The rationale of incorporating a single label into peptides was that any dipolar interaction detected by DRAWS had to come from an intermolecular association of the peptide. In contrast, in a doubly labeled peptide there is the potential for both intra- and intermolecular interactions, making analysis of the DRAWS data inherently more complex and ambiguous. We did, however, also study one intramolecular distance in Aβ$_{10–35}$ using a sample with labels in contiguous amino acids, i.e., both Val$_{18}$ and Phe$_{19}$, from which the conformational structure of the peptide could be confirmed.

Despite the relative ease of analyzing DRAWS data obtained from singly labeled peptides, a number of potential complications remained to be investigated. As outlined above, these included issues of transverse relaxation processes, numbers and geometries of spins used in simulations of the DRAWS data, the obtaining of corroborating data from DQ filtering, and techniques of sample preparation. We also address the study of small, doubly labeled compounds of known crystal structure, and the extension of such studies, for corroboration of DRAWS data, to peptides doubly labeled in contiguous amino acids. Each of these issues is addressed below.

4.2. Doubly labeled model compounds

Before DRAWS distance measurements are presented for the amyloid samples, results obtained from several control experiments on model compounds are described. Two di-$^{13}$C-labeled dicarboxylic acid samples of known structure were investigated to assess the accuracy of DRAWS for measuring long (4–6 Å) $^{13}$C–$^{13}$C distances, see Fig. 2. Both samples were diluted with unlabeled material to minimize intermolecular effects. In the case of succinic acid-1,4-$^{13}$C$_2$, which was diluted to 5% in succinic acid, 17% of the signal of the carboxyl peak was due to $^{13}$C at natural abundance. Because most of these nuclei do not experience any dipolar coupling, the raw data points appear well above the simulated curve for a 3.8 Å distance. However, once signal from these nuclei are taken into account, the compensated data fit well to the simulated curve, as seen in Fig. 2. The DRAWS distance obtained agrees well with the distance of 3.8 Å measured by X-ray diffraction [45].

Fig. 2 also shows data for adipic acid-1,6-$^{13}$C$_2$. Because of the longer $^{13}$C–$^{13}$C distances involved, the sample had to be diluted to 1% with unlabeled material. Even for this case, where approximately one-half of the signal was derived from natural abundance $^{13}$C, a reasonable DRAWS curve was obtained. The DRAWS distance of 6.0 ± 0.5 Å was also in reasonable agreement with the distance obtained by X-ray diffraction, 6.3 Å [46]. This distance clearly demonstrates the upper limit measurable by DRAWS for a $^{13}$C–$^{13}$C spin pair.

4.3. Intramolecular distances in peptides doubly labeled in contiguous amino acids

Intramolecular distances were obtained via DRAWS on amyloid samples which were isotopi-
cally labeled at the carbonyl carbons of amino acids Val₁₈ and Phe₁₉. Samples were prepared under three different conditions: the first was prepared as an ether precipitate from TFA, and two remaining samples were fibrilized from aqueous solution at pH 5.6 and 7.4. DRAWS curves for the ether precipitate and fibril sample prepared at pH 5.6 are presented in Fig. 3. The distance obtained in DRAWS experiments between the contiguous amino acid labels depended on sample preparation. Simulations of the data clearly represent two distinctly different distances: 3.0 ± 0.1 Å for the ether precipitate and 3.5 ± 0.1 Å for the fibrils.

In order to correlate carbonyl–carbonyl distances with the secondary structure of the peptide, molecular modeling was performed for both β-strand and α-helical Aβ peptide conformations with the aid of X-ray diffraction data obtained for peptide structures in the Brookhaven Protein Data Bank. For an α-helix structure, carbonyl–carbonyl distances were found in the range of 2.9–3.1 Å, which compares well to the distance of the ether precipitate. Correspondingly, carbonyl–carbonyl distances determined for the β-strand conformation were in the range of 3.3–3.5 Å, in agreement with the measured DRAWS distance for the amyloid fibrils.

Further evidence in support of a conformational transition of Aβ from an α-helix in solution to a β-strand structure in fibrils comes from a comparison of ¹³C chemical shifts. Empirical shift relationships established by Saito [47] have shown that carbonyl carbon shifts of valine in α-helix and β-strand structures are 174.9 and 171.5 ppm, respectively. The corresponding shifts for phenylalanine appear at 175.2 and 169.0 ppm. A similar trend is observed for valine and phenylalanine resonances of the ether precipitate and fibrilized form of 1-¹³C-Val₁₈,1-¹³C-Phe₁₉-Aβ(10–35). Broad resonances of the labeled carbonyl carbons in the amyloid sample are observed at 175 ppm for the ether precipitate and 170 ppm for fibrils prepared at pH 7.4, and are consistent with a transition from an α-helix to a β-strand structure.

DRAWS data for fibrils prepared at pH 7.4 are similar to the data obtained at pH 5.6 with one exception, the dipolar oscillations observed at the longer DRAWS cycle times seem to be less pronounced. DRAWS curves shown in Fig. 4a, however, indicate that the carbonyl–carbonyl distance between Val₁₈–Phe₁₉ in these fibrils is compatible with a β-strand structure, 3.4 ± 0.1 Å. The source of the dampened oscillations observed for the DRAWS curve of this sample is not readily apparent. Explanations include possible averaging effects resulting from greater disorder in this region of the peptide, or overdamping effects as a result of DQ relaxation processes or additional weak internuclear interactions. Insights into these possibilities are discussed in greater detail below.

### 4.4. Relaxation effects

To accurately measure longer distances (4–6 Å) in the DRAWS experiment, it is necessary to separate effects of the dipolar interaction, which are
Fig. 4. DRAWS data from 1-13C-Val,1-13C-Phe-Ab fibrilized at different pH levels. The data has been separated into two figures to make the presentation clearer. However, the meaning to the symbols is the same in both (a) and (b). The 1-13C-Val,1-13C-Phe-Ab sample was fibrilized at two different pH levels: pH 5.6 circles and pH 7.4 squares. The solid line represents simulated data for a 3.5 Å distance without DQ relaxation effects. The dashed lines represent simulated data for three different distances and including a DQ relaxation parameter of $R_{DQ}$. The three different distances are 3.5 Å (long dashes), 3.3 Å (short dashes), and 3.0 Å (alternating short and long dashes).

manifest in an accelerated rate of decay of the NMR signal with increasing DRAWS mixing time, from the effects of other relaxation mechanisms. In particular, effects arising from SQ and DQ transverse relaxation processes become important to the analysis of the data. DRAWS produces very little zero-quantum (ZQ) magnetization [48], and thus, ZQ relaxation can be safely ignored. While SQ transverse relaxation ($R_{SQ} = 1/T_{SQ}$) can be measured easily in a control experiment on an unlabeled sample, earlier work established that it is necessary to include a $R_{DQ}$ parameter in the calculated fits when carbons have directly bonded protons. However, it has been shown that $R_{DQ}$ processes seemingly are unimportant for unprotonated carbons [48]. The need to include $R_{DQ}$ for protonated carbons is probably related to relaxation effects from incomplete proton decoupling.

In an attempt to quantify these effects more rigorously, we have developed a new pulse method for measuring $T_{SQ}^{DQ}$ directly. The experiment is based on a combination of the DQ-DRAWS and Hahn spin–echo pulse sequences [30]. Results from $T_{SQ}^{DQ}$ and $T_{DQ}^{DQ}$ measurements are summarized in Table 1. Values of $T_{SQ}^{DQ}$ and $T_{DQ}^{DQ}$ are found to vary widely for different samples when they are measured in the absence of any r.f. field. However, in the presence of DRAWS irradiation, the $T_{SQ}^{DQ}$ values become quite different from SQ transverse relaxation measured in the Hahn spin–echo experiment. This suggests that r.f. irradiation is in some way modifying the relaxation pathways that are available to the spin system. If this is correct, it seems likely that $T_{DQ}^{DQ}$ will also be modified by DRAWS irradiation, although its influence on $T_{DQ}^{DQ}$ cannot be tested experimentally.

As an alternate approach, we have attempted to predict the influence of $T_{DQ}^{DQ}$ on DRAWS decay curves from simulation studies. The dipolar coupling and $T_{DQ}^{DQ}$ have different effects on the DRAWS curve, and hence, it is possible to fit both parameters independently using the same curve. The dipolar coupling, $D$, determines the initial slope of the curve and the frequency of the dipolar oscillations. DQ relaxation dampens the dipolar oscillations if $R_{DQ} < D$ or, if $R_{DQ} > D$, the effect is to raise the asymptotic value of the curve above zero, i.e., $R_{DQ}$ has the effect of an overdamped oscillator. Both effects can be observed in a powder averaged DRAWS curve since the effective value of $D$ will depend on crystallite orientation.

DRAWS data and simulation curves for 1-13C-Val,1-13C-Phe-Ab fibrilized at pH 5.6 are shown in Fig. 4b. The best fit to the data occurs when $R_{DQ}^{DQ}$ is set to zero (solid line). Simulations for several different distances, in which the $R_{DQ}^{DQ}$ parameter was set to 185 s$^{-1}$ (the value determined experimentally in the absence of DRAWS), are also shown for comparison (dashed lines). The effect of $R_{DQ}^{DQ}$ is to dampen the oscillations and raise the curves above the zero intensity line. Thus, it is impossible to adequately fit the experimental data to any reasonable 1-13C–13C distance, using the experimentally determined value of $R_{DQ}^{DQ}$ in the absence of 13C r.f.
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_2^{SQ}$ without r.f. (ms)</th>
<th>$T_2^{SQ}$ with DRAWS r.f. (ms)</th>
<th>$T_2^{DQ}$ without r.f. (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malonic acid-1,3-$^{13}$C$_2$</td>
<td>70$^a$</td>
<td>20$^b$</td>
<td>46$^c$</td>
</tr>
<tr>
<td>Succinic acid-1,4-$^{13}$C$_2$</td>
<td>144$^a$</td>
<td>20$^b$</td>
<td>45$^a$</td>
</tr>
<tr>
<td>Adipic acid-1,6-$^{13}$C$_2$</td>
<td>220$^a$</td>
<td>20$^b$</td>
<td>49$^a$</td>
</tr>
<tr>
<td>Hexamethylbenzene (HMB) (ring carbons)</td>
<td>580$^a$</td>
<td>19</td>
<td>NC$^c$</td>
</tr>
<tr>
<td>1-$^{13}$C-Val$_{18}$-Aβ (ether)</td>
<td>10.0$^d$</td>
<td>17$^e$</td>
<td>NC$^e$</td>
</tr>
<tr>
<td>1-$^{13}$C-Val$_{18}$-Aβ (pH 5.6)</td>
<td>9.6$^d$</td>
<td>17$^e$</td>
<td>NC$^e$</td>
</tr>
<tr>
<td>1-$^{13}$C-Val$_{18}$-Aβ (pH 7.4)</td>
<td>8.8</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>1-$^{13}$C-Leu$_{17}$-Aβ (pH 5.6)</td>
<td>8.5</td>
<td>17$^e$</td>
<td>NC$^e$</td>
</tr>
<tr>
<td>1-$^{13}$C-Leu$_{17}$-Aβ (pH 7.4)</td>
<td>8.9</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>1-$^{13}$C-Glu$_{15}$-Aβ (pH 7.4)</td>
<td>9.4</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>1-$^{13}$C-Val$<em>{18}$-Phe$</em>{19}$-Aβ (pH 5.6)</td>
<td>8.6</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Unlabeled Aβ</td>
<td>9.5</td>
<td>17</td>
<td>NC$^e$</td>
</tr>
</tbody>
</table>

$^a$Measurement error in dicarboxylic acid and HMB samples is ±2.0 ms for $T_2^{SQ}$ (with the exception of adipic acid were the error is ±10 due to poor signal to noise). The error is ±1.0 ms for $T_2^{SQ}$ with DRAWS r.f. applied. The accuracy for the $T_2^{SQ}$ measured in the absence of r.f. is difficult to assess with these samples. In order to avoid probe arcing, no delay greater than 40 ms was attempted in these experiments as high power proton decoupling was applied during spin evolution. Thus, values much greater than 40 ms are most likely underestimates of the true value due to a lack of data points at longer evolution times.

$^b$These numbers were measured with an unlabeled sample.

$^c$NC indicates no contact observed, i.e., the DRAWS curves for the labeled and unlabeled samples decayed at the same rate. No dipolar coupling was detected, thus $T_2^{DQ}$ could not be measured.

$^d$Measurement error in amyloid samples is ±1.0 ms.

$^e$This assumes no contact in this sample.

Similar trends are apparent in simulations of DRAWS data for succinic and adipic acid as well.

Remarkably, $T_2^{SQ}$ relaxation times of carbonyl carbons measured in the presence of DRAWS irradiation are very similar for all samples investigated, $T_2^{SQ} \approx 19$ ms (see Table 1). Similar values have been obtained previously for carbonyl (unprotonated) carbons in other systems [48]. Furthermore, DRAWS $T_2^{SQ}$ values are similar regardless of the magnitude of $T_2^{SQ}$ relaxation times measured in the Hahn spin–echo experiment. It seems reasonable to assume that $T_2^{SQ}$’s of carbonyl carbons will also follow a similar trend in the presence of DRAWS r.f. irradiation. Moreover, $T_2^{DQ}$ values measured for the amyloid samples tend to be similar. On the basis of the foregoing arguments and given the behavior of $R_2^{DQ}$ on the outcome of the DRAWS simulations on doubly labeled samples, we have neglected the effects of DQ relaxation in our analyses.

Thus, measuring $T_2^{DQ}$ for use in DRAWS simulations turns out not to be as straightforward as we had hoped. Nevertheless, simulating the effects of DQ relaxation lends insight into the dampened oscillations observed for the Aβ fibrils prepared at pH 7.4. Clearly, incorporating DQ relaxation effects in the simulations does not adequately model the behavior seen, making structural ordering effects in the Val$_{18}$–Phe$_{19}$ region of the peptide the most plausible explanation for the observed results.

Parenthetically, we remark that the arguments presented in the Theory section of this paper show that the composite 90°–90°–90° pulse will refocus ZQ coherence as well. Therefore, it may be possible to measure $T_2^{ZQ}$ using a similar experiment. This might have applications to rotational resonance experiments, which are affected by $T_2^{ZQ}$ but have no r.f. applied during the mixing period.

4.5. Intermolecular distance measurements—simulations using multiple-spin geometries

The primary goal of this work concerns accurately measuring weak dipolar couplings in self-associated forms of $^{13}$C-labeled amyloid samples. Estimation of these distances is based on performing dipolar recoupling DRAWS experiments, in which the decay of signal intensity of a $^{13}$C-labeled sample is compared to the decay rate of a control sample at natural $^{13}$C abundance. The problem is that the difference in the two decay rates is inherently small, owing to the
weak nature of the intermolecular interactions involved. Thus, it is essential that highly reproducible results can be achieved in our studies.

Fig. 5 presents an example of the experimental reproducibility attained for various preparations of $^{13}$C-Val$_{18}$-$\alpha$-A$_{10-35}$. This peptide was prepared as the ether precipitate from TFA, and fibrilized at pH 5.6 and 7.4. It was later confirmed by other techniques that only the preparation at pH 7.4 produced fibrils. Results for unlabeled $\alpha$-A$_{10-35}$ preparations are included, and are comparable to labeled samples obtained as the ether precipitate, fibrils prepared at pH 5.6, and fibrils prepared at pH 7.4 but isotopically diluted to 10% (not shown). Each experiment was performed on a different day. Different DRAWS curves for the same sample preparation are identical to within an experimental error of ±0.2 Å.

A second important aspect of this work is to accurately model the DRAWS data; simulations using a typical ‘two-spin’ interaction model did not fit the experimental data well. Fig. 6 presents the results of DRAWS distance measurements on $^{13}$C-Val$_{18}$-$\alpha$-A$_{10-35}$ fibrilized at pH 7.4. Calculated DRAWS curves were then performed using ‘two-spin’ and ‘three-spin’ models and are presented in Fig. 6a and b, respectively. It clearly is seen that the ‘three-spin’ model with a distance of 5.0 ± 0.2 Å provides a much better fit to the experimental data. Simulations for three other fibrilized samples, $^{13}$C-labeled Gln$_{15}$, Lys$_{16}$, and Leu$_{17}$, give similar results. In all cases, the data fit a ‘three-spin’ model better; distances in the range of 4.9–5.1 ± 0.2 Å are obtained (see Table 2).

Thus far, spin simulations were performed using a model in which three spins are placed in a linear arrangement, and for which the dipolar coupling strength between the two spins at the ends is the same as the coupling from the middle spin to either outer spin. Thus, each spin is coupled to two others, all of the coupling strengths are identical, and the dipolar vectors are all parallel. This geometry, which we define as an ‘infinite-loop’ model, is simple to calculate and is intended to model a propagating structure where an infinite number of spins are in a line. For instance, the proposed model of amyloid fibrils has the peptides arranged as extended β-sheets.
However, performing density matrix calculations on such a large number of spins turns out to be impractical. Instead, we chose to study the influence of different geometries on the simulations involving three or four interacting spins. Fig. 7a shows simulations comparing two different geometries: the three-spin ‘infinite loop’ model given above, and a similar four-spin ‘infinite loop’ model with each spin placed in a linear arrangement. The results for the two geometries are almost identical at the initial decay rates, i.e., prior to the onset of the dipolar oscillations seen at the shorter distances.

In the case of simulations for a 5 Å distance, both models give the same result for DRAWS mixing times approaching 20 ms. The foregoing modeling implies that, under the mixing times employed in our experiments, all 5 Å simulation models in which every spin interacts with two others of equal magnitude and the dipolar vectors are all parallel will be indistinguishable.

In addition, we simulated additional multiple-spin systems in using a restricted linear arrangement in which there is no coupling between the two spins at the ends. These were compared to the results obtained for the three-spin ‘infinite-loop’ model. The results of simulations on three- and four-spin systems having a restricted linear geometry are shown in Fig. 7b and c, respectively. Higher order spin simulations were not attempted because of computational time constraints. The two models clearly become more divergent at shorter interaction distances. However, the 5 Å simulation curves for restricted linear vs. ‘infinite-loop’ geometries are fairly similar and appear to become convergent as the number of spins applied to the restricted linear model increases.

4.6. Obtaining of corroborating data or rapid screening using DQ-filtering

In addition to DRAWS distance measurements, we performed experiments using a DQ filtering pulse sequence (vide supra). While the latter experiment does not provide additional information, it has been useful both for corroboration of the DRAWS distance measurements and for rapid screening of samples. Inspection of the data, which are summarized in Table 2, reveals that measured DQ efficiencies correlate roughly with the distances measured in the DRAWS experiment.

In all cases, DQ efficiencies were calculated for a 13C-labeled sample compared to that of a internal standard, hexamethylbenzene (HMB), which served as a negative control. The DQ efficiencies for all unlabeled compounds studied, including HMB, typically fell in the range of 3.0 to 4.5%; values of DQ efficiency had an estimated precision of ±2%. The non-zero departure from ideality found for the unlabeled control samples is most likely due to pulse
imperfections in the DQ filtering experiment, and/or to the presence of residual $^{13}\text{C}^{-13}\text{C}$ interactions at natural abundance. In this work, DQ efficiencies greater than 7%, which is the result obtained for adipic acid and represents the largest distance that can be measured by DRAWS, are considered to be significant and are taken to be indicative of a contact.

Fig. 8 shows CP/MAS and DQ-DRAWS spectra for two $^{1-13}\text{C}\text{-Val}_{10}\text{-A}{}_{\beta (10-35)}$ samples, one prepared as fibrils and another isolated as an ether precipitate. The ether precipitate shows no evidence of a $^{13}\text{C}^{-13}\text{C}$ coupling; the DQ efficiency measured is 3.8%. On the other hand, the result for the fibrillized amyloid sample demonstrates a DQ efficiency of 13.2%, indicating an intermolecular dipolar contact is present in this sample. On the basis of DQ efficiencies obtained for the dicarboxylic acid samples, we estimate distances in the range of 4–6 Å for this sample. Similar results have been obtained for all $^{13}\text{C}$-labeled amyloid samples that were fibrillized at pH 7.4.

4.7. Sample preparation conditions

Contrasted DRAWS results have been obtained on lyophilized amyloid preparations that were fibrillized under different pH conditions. Lyophilized peptides fibrillized at pH 7.4 always showed a positive intermolecular contact, whereas those fibrillized at pH 5.6 never exhibited a dipolar coupling interaction. This trend was observed in both the DQ filtering and DRAWS experiments.

Fig. 8 shows solid-state $^{13}\text{C}$ NMR spectra of $^{1-13}\text{C}\text{-Val}_{10}\text{-A}{}_{\beta (10-35)}$ admixed with a small amount of hexamethylbenzene (HMB). HMB resonances appear at 132 and 16 ppm. The broad resonance at 170 ppm in spectra at the left results from fibrils and the corresponding resonance at 175 ppm in the spectra at the right results from ether precipitate. Smaller resonances are background signals of peptides at natural abundance or spinning sidebands. The spectra above were acquired using a standard CP/MAS pulse sequence, while spectra below are results from the DQ-DRAWS filtering experiment.
In an attempt to understand the difference between samples fibrillized at the two pH conditions, we performed DRAWS experiments directly on frozen solutions of the fibrillized peptides. Four samples were studied in this manner: $1^{13}$C-Val$_{18}$, $1^{13}$C-Phe$_{19}$-$\alpha$B$_{10-35}$, $1^{13}$C-Gin$_{15}$-$\alpha$B$_{10-35}$, $1^{13}$C-Lys$_{16}$-$\alpha$B$_{10-35}$ and an unlabeled peptide sample. Samples were fibrillized at pH 5.6 or 7.4 and flash frozen immediately before performing the DRAWS measurements, which were carried out at a temperature of $-80^\circ$C.

Results of the measurements on frozen samples are summarized in Fig. 9. The quantities of peptides studied as frozen solutions were far less than the amounts of lyophilized powders, resulting in spectra with lower signal-to-noise, hence larger scatter in the

![Graph](image)

**Fig. 9.** DRAWS curves from experiments performed on frozen amyloid samples at $-80^\circ$C. Circles represent $1^{13}$C-Val$_{18}$, $1^{13}$C-Phe$_{19}$-$\alpha$B$_{10-35}$ fibrilized at pH 5.6. Triangles represent unlabeled $\alpha$-amyloid. Three experiments performed on $1^{13}$C-Lys$_{16}$-$\alpha$B$_{10-35}$ are represented by the squares. Closed squares represent a sample prepared at pH 5.6 and run immediately after flash freezing. Squares with crosses represent the same sample run after one freeze–thaw cycle. Open squares represent a sample prepared at pH 7.4 and run immediately after flash freezing. Simulated curves are represented by the lines as follows: control curve is the dotted line; large dashes, 5.5 Å; solid line 5.0 Å; large dashes, 4.5 Å; and small dashed is 3.4 Å.

DRAWS data. Nevertheless, the DRAWS curve obtained for frozen $1^{13}$C-Val$_{18}$, $1^{13}$C-Phe$_{19}$-$\alpha$B$_{10-35}$ was nearly identical to the one obtained for the lyophilized powder at room temperature. More importantly, intermolecular contacts were detected for the frozen $1^{13}$C-Lys$_{16}$ and $1^{13}$C-Gin$_{15}$ samples fibrillized at both pH’s. Allowing samples to go through a freeze–thaw cycle prior to the DRAWS analysis, however, resulted in the lose of intermolecular contacts for both samples fibrillized at pH 5.6.

5. Discussion

In this paper, we outline a general methodology for obtaining accurate interatomic distances in biomolecular self-assemblies. We demonstrate the goodness of DRAWS data for measuring long interatomic distances ($\leq 6$ Å) to support our previous work [31], where we have used the DRAWS method to show that $\alpha$B peptide in the form of solid fibrils has a parallel $\beta$-sheet structure, with the amino acids in exact register. The DRAWS method allowed this conclusion to be drawn with a degree of certainty whereby interatomic distances can be measured to a resolution of $\pm 0.2$ Å. The analysis of DRAWS data was greatly simplified by the inclusion of only a single $^{13}$C label at the carbonyl atom of one amino acid per peptide strand. In this way, contacts at a distance of $\leq 6$ Å are detected between peptide chains, but no such contacts are possible within a chain.

The precision of the experiments was determined from repeated experiments on the same sample (see Fig. 5). The accuracy of the measurements depends, however, on the correctness of the assumptions used in our simulations for the analysis of the DRAWS data. These assumptions include the following: (1) spin–spin relaxation processes can affect the interpretation of DRAWS data, but as we have shown above, the only major contributor to
such relaxation can be modeled as a single exponential decay. In other data, we have modeled different geometries of multiple interacting spins in an iterative ‘infinite-loop’ pattern, and have compared these to restricted linear arrays of three and four spins. For the case of the Aβ(10–35) peptide, a significantly closer fit of the data was obtained using an infinite arrangement of three spins, rather than a two-spin model. Such improved fit re-confirmed the conclusion that the structure of these peptides was an extended, propagating array of peptides in a parallel β-sheet.

If the second assumption is not correct and DQ relaxation does affect the DRAWS experiment, then including $T_{2}^{DQ}$ in the analysis would make the internuclear distances appear to be shorter. Hence, the $5.0 \pm 0.2$ Å measurement must simply be treated as an upper limit. Molecular modeling places the lower bound at 4.7 Å. Thus, the distance would still be constrained to the rather narrow range, 4.7–5.2 Å.

It should be noted, however, that the parallel model for β-amyloid does not depend on the level of accuracy claimed in the DRAWS experiments. As we reported in other work [31], although the first set of distances obtained for Gln$_{15}$ and Lys$_{16}$ were compatible with either a parallel or antiparallel structure, the propagation of 5 Å interstrand contacts between labeled carbons of Leu$_{14}$ or Val$_{16}$ along the polypeptide chain made the antiparallel conformation impossible, since in the antiparallel conformation, the distances between like residues would diverge from the point of contact between Gln$_{15}$ and Lys$_{16}$ in adjacent chains (compare Fig. 10a and b). Even without obtaining the additional, high resolution DRAWS data, however, it would have been possible to distinguish between parallel and antiparallel structures from the DQ filtering results, which by themselves imply $^{13}$C-$^{13}$C distances of $5 \pm 1$ Å in fibrils. Thus, even with these looser distance constraints, the only reasonable model of the structure of the peptide is a parallel arrangement. Accordingly, the simpler DQ-DRAWS experiments can serve either to corroborate the higher resolution DRAWS experiments, or even more so, can serve as a check of the appropriateness of initial synthetic placement of labeled amino acids.

We also examined the effects of sample preparation on the presence of contacts in Aβ. Solid samples of the peptide were prepared by either lyophilizing peptides at different pH, or only flash freezing samples without lyophilization. While both lyophilized and frozen solutions of fibrils prepared from singly $^{13}$C labeled peptides at pH 7.4 exhibit intermolecular contacts, only frozen solutions of those prepared at pH 5.6 show contacts, consistent with a strongly pH dependent transition noted by us and others. Two other inferences can be drawn, from the data on the doubly labeled Aβ(10–35) fibrils prepared at pH 5.6 (see Fig. 9a). First, manifestation of dipolar oscillations in the DRAWS data suggest a well-ordered local structure of the fibrilized peptide. Structural disorder in the fibrils would lead to a distributional average of these distances in the peptide, and to a dampening of the oscillations in the DRAWS curve. Comparing the experimental and simulated data shows no evidence of this dampening, however. Second, effects from DQ relaxation appear to be negligible. While DQ relaxation also are seen to dampen DRAWS oscillations in the simulations, data are best fit with $R_{2}^{DQ} = 0$, Fig. 4.
The observation that effects from $T_2^{DQ}$ relaxation can be neglected in simulations of the DRAWS data on amyloids and other model systems is surprising and deserves further comment. For the case where relaxation at the labeled carbonyls on different molecules is uncorrelated, one would expect $T_2^{DQ}$ to be at least half of $T_2^{DQ}$. Accordingly, it is seen that average $T_2^{DQ}$ times measured for amyloid samples in the absence of any carbon r.f. are considerably shorter than $T_2^{DQ}$ (av. ~9 ms), on the order of 5.5 ms (see Table 1). It could be argued that $T_2^{DQ}$ with r.f. should be even shorter, owing to interferences resulting in less efficient proton decoupling. However, simulations of DRAWS data clearly establish that $T_2^{DQ}$ and $T_2^{DQ}$ for amyloid samples generally appear longer in the presence of DRAWS. One possible interpretation is that relaxation processes are more akin to $T_1^{DQ}$ than $T_2^{DQ}$ in the DRAWS experiment, in which a 'windowless' sequence of $^{13}$C irradiation is applied in the rotating frame and about which the carbons spins are free to precess. $T_1^{DQ}$ is typically much larger than $T_2^{DQ}$ in the slow motion regime for a rigid solid, and the presence of strong DRAWS dipolar oscillations observed for the doubly labeled peptide certainly support a certain degree of rigidity for this portion of the molecule.

Further insight into this issue comes from recent experiments on the doubly labeled peptide, $\text{1-13C-Gly}_{25}$, $\text{1-^{13}C-Ser}_{26}$-$\text{Aβ}_{(10-35)}$. DRAWS data on the fibrilized peptide exhibit remarkably dampened oscillations, and data simulations can best be modeled only by incorporating a parameter of $T_2^{DQ}$ = 10 ms, in addition to a 0.25 Å dispersion of distances into the calculations. Along with slightly longer distances observed for the singly labeled peptides (for Gly$_{25}$, 5.7 Å; for Ser$_{26}$, 5.6 Å) over the other segments of Aβ, the simulations suggest that while these residues retain the parallel β-sheet structure, with residues in register, this particular segment of residues has greater mobility than is observed for other parts of the chain. We presently are performing low temperature studies on the doubly labeled peptide to see if we can 'freeze out' motions in this segment of the peptide, with the hope of following changes in the DRAWS curves with temperature.

DRAWS results for fibrils prepared at pH 7.4, while yielding the same intramolecular carbonyl–carbonyl distance, show somewhat dampened oscillations compared to fibrils prepared at pH 5.6. While this could be caused by structural disorder of the peptide resulting in a distribution of distances, a more likely explanation is the existence of additional, weaker $^{13}$C–$^{13}$C couplings. Simulations with three- and four-spin models show that, when both a strong a weaker couplings are present, the strong coupling dominates the experimental results. The effect of the weaker couplings is to dampen oscillations in the DRAWS curve. This interpretation is consistent with a propagating β-sheet structure of the peptide in fibrils prepared at pH 7.4, resulting in the presence of additional weaker intermolecular contacts that are absent in fibrils at pH 5.6.

Presently, we are performing additional experiments with Aβ containing labels on other residues. The question of whether interstrand contacts span the entire length of the peptide chain has been addressed by preparing four additional peptide fibrils, each containing a single $\text{1-^{13}C}$ amino acid residue in place of Val$_{12}$, Phe$_{20}$, Val$_{24}$ and Lue$_{34}$. A summary of the distances measured in fibrilized samples of Aβ$_{(10-35)}$ is shown in Fig. 11. The level of molecular detail from these experiments is now, for the first time, sufficient to allow us to develop a tertiary structure of the fibrilized peptide and to rationally design agents to control fibril formation. Work also is progressing with neutron and X-ray scattering techniques to investigate long-range order of Aβ aggregates.

In conclusion, we have examined a plaque-competent peptide of Alzheimer’s disease under fibrillogenesis conditions and have determined the molecular arrangement of self-association. The secondary structure of Aβ fibrils consists of an extended parallel β-sheet with each amino acid in exact register. The data presented in this paper refine this approach, and demonstrate a new strategy of general applicability to any iterative self-associating biopolymer.

In general, any biopolymer is amenable to this approach, provided that the following are known. First, the overall secondary structure must be approximated by low resolution techniques such as CD or
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