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Basic Experiments in ^2H static NMR for the Characterization of Protein Side-Chain Dynamics

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Abstract

The focus of this review is the basic methodology for applications of static deuteron NMR for studies of dynamics in the side chains of proteins. We review experimental approaches for the measurements of static line shapes and relaxation rates as well as signal enhancement strategies using the multiple echo acquisition scheme. Further, we describe computational strategies for modeling jump and diffusive motions underlying experimental data. Applications are chosen from studies of amyloid fibrils comprising the amyloid- β protein.

Keywords

static ^2H NMR; solid-state NMR; deuterium; motions; side-chains dynamics

1. General overview of the technique, advantages, and disadvantages

Static ^2H solid-state NMR has been known for decades as a sensitive tool for studies of the dynamics in systems ranging from polymers to peptides, nucleic acids, and proteins.(1–17)

Experiments are relatively easy to conduct over a broad temperature range and do not require microcrystals, assuming that the protein structure is maintained in the amorphous powders. In addition, the solid phase permits investigations of the effect of solvation. The major disadvantage/difficulty is the need for the selective-site labeling of the samples to obtain site-specific information (thus often requiring multiple samples) and inherent low sensitivity of the technique.

Deuterium has a spin of 1 and hence the main interaction governing deuteron NMR stems from the quadrupolar mechanism, i.e. interaction between the nuclear electric quadrupole moment and the electric field gradient (EFG). Thus, one gets a relatively simple system in terms of the spin degree of freedom. In most cases, the effect of the proton dipolar network is minor; thus, one can focus on the details of the molecular motions in which the nucleus is

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participating. Further, the magnitude of the electric quadrupole moment is relatively small compared with what is found for common half-integer quadrupolar nuclei and the ^{14}N nucleus with a spin of 1, leading to a relatively narrow spectrum that is easy to work with experimentally.

The goal of this review is to provide the methodological background for the most common ^2H static NMR techniques for the determination of the side-chain dynamics in proteins. It will cover the acquisition and analysis of line shapes and relaxation data and their interpretation using examples of motional modeling approaches.

2. Introduction to the theoretical basis of the effect

Details of the theoretical/computational basis have been described in numerous works over the past 50–60 years.(1, 2, 18–20) Here, brief overviews are presented for the line shape and relaxation experiments.

The quadrupolar Hamiltonian for a spin $I = 1$ system in a static magnetic field using the high field approximation and in the principal axis system of the EFG tensor is given by(2, 21)

$$H_q = \frac{e^2 q Q}{4I(2I-1)} \left[(3I_z^2 - I^2) + \frac{\eta}{2} (I_+^2 + I_-^2) \right]$$

in which eQ is the electric quadrupole moment of the nuclei and eq is the largest component of the EFG. The quadrupolar coupling constant is given by $C_q = \frac{e^2 q Q}{h}$ and $\eta \equiv \frac{q_{xx} - q_{yy}}{q_{zz}}$ represents the asymmetry of the tensor, defined to lie in the interval $0 \leq \eta < 1$ with $|q_{zz}| \geq |q_{xx}|, |q_{yy}|$.

For a given orientation of a crystallite, the time-domain response of the system $M(t) = M_+(t) + M_-(t)$ in a quadrupolar echo (QE) deuterium line shape experiment is governed by

$$\frac{dM_{\pm}}{dt} = A_{\pm} M_{\pm} \quad (1)$$

where $\pm$ corresponds to the $m=-1$ to $m=0$ and $m=0$ to $m=+1$ transitions of the deuteron spin. The response depends on the quadrupolar tensor orientation for each spin in the crystal-fixed frame and on the orientation of the crystallite in the laboratory frame. When the orientations of the deuteron EFG tensors in the crystal-fixed frame are specified by N “structural sites”, i.e. N sets of three Euler angles, and the deuteron dynamics are described by Markovian jumps among sites, M becomes a multidimensional vector with the components M_i ($i=1, \dots, N$) and A takes the form of an $N \times N$ matrix with the elements

$$A_{\pm}^{ij} = \pm i\omega_i \delta_{ij} + K_{ij}, \quad (2)$$

where i and j denote structural sites, ω_i is the orientation-dependent frequency of each site, δ is a Kronecker symbol, and K is a matrix of site-exchange rates (which does not depend on crystallite orientations). The matrix K satisfies the following two constraints,

$$K_{ii} = - \sum_{j=i, j \neq i}^N K_{ji}, \text{ and the microscopic reversibility condition given by } K_{ij}p_j^{eq} = K_{ji}p_i^{eq},$$

where p_i^{eq} is the equilibrium population of site i and K_{ij} is the rate of jumps from site j to site i . The orientation dependence of the frequencies ω_i can be expressed by

$$\omega_i = C_q \sum_{a=-2}^2 \left(D_{0,a}^{(2)}(\Omega_{PC,i}) + \frac{\eta}{\sqrt{6}} (D_{2,a}^{(2)}(\Omega_{PC,i}) + D_{-2,a}^{(2)}(\Omega_{PC,i})) \right) D_{a,0}^{(2)}(\Omega_{CL}) \quad (3)$$

where $D^{(2)}$ are the second-order Wigner rotation matrices defining the transformations from the principal axis frame (PAS) of the quadrupolar tensor to the crystal-fixed frame with the relative orientations given by the Euler angles $\Omega_{PC,i}$ and from the crystal-fixed frame to the laboratory frame with the relative orientations given by the Euler angles Ω_{CL} . In a polycrystalline sample, the line shape is the average over all possible orientations:

$$I(\omega) = \int d\Omega_{CL} \text{Re} \int dt \sum_i (M_{+,i}(t) + M_{-,i}(t)) e^{-i\omega t} \quad (4)$$

The Zeeman T_{1Z} and quadrupolar order T_{1Q} relaxation rates are given by the following expressions in the Redfield approximation:(2, 22)

$$\frac{1}{T_{1Z}} = \frac{3\pi^2}{2} C_q^2 (J_1(\omega_0) + 4J_2(2\omega_0)) \quad (5a)$$

$$\frac{1}{T_{1Q}} = \frac{9\pi^2}{2} C_q^2 J_1(\omega_0) \quad (5b)$$

where ω_0 is the Larmor frequency and J_1 and J_2 are the spectral density functions. J_1 and J_2 are dependent on the time scales and types of the underlying motional processes as well as on the crystallite orientations. The dependence on the crystallite orientation is unique for each motional model and thus the anisotropies in the relaxation rates can be useful in discriminating among several motional models. The spectral density functions can be

obtained analytically for several simple models of motion.(19) However, motional models with multiple modes usually require computer simulations.

In some systems, the relaxation decays $M(t)$ are non-exponential due to the presence of conformers with different underlying motional parameters. Approaches have to be developed to interpret such decays. One useful phenomenological function for characterizing non-exponentiality is given by the so-called “stretch-exponential” of the form(23, 24)

$$M(t) - M(\infty) = (M(0) - M(\infty)) \exp(-(t/T_1^{\text{eff}})^\beta), \quad (6)$$

where $M(t)$ is the signal intensity, T_1^{eff} is the effective relaxation time, and β is the parameter that reflects the degree of non-exponentiality, $0 < \beta \leq 1$. β less than 1 corresponds to non-exponential behavior.

3. Sample preparation of the peptide and protein samples

Sample preparation is usually guided by the necessity to introduce site-specific labels to obtain residue-specific information with this technique. The most common route is solid-state peptide synthesis, with modern capabilities to about 120 residues in length,(25) and protein expression with media enhancement strategies to label selective residue types.(26) For hydration dependence studies, water is often reintroduced by either vapor diffusion or pipetting the desired amount directly into lyophilized powder. The latter approach permits the use of deuterium-depleted water to avoid the NMR signal arising from HOD. For protein sizes between 30 to 70 amino acids, it is usually necessary to obtain 10 to 25 mg of material for a satisfactory signal. Thus, containers of at least 4 mm in cross-section/diameter are usually required. Other site-specific techniques such as magic-angle spinning (MAS) NMR are necessary to confirm the integrity of the sample structure under these conditions.

4. Static solid-state NMR probes

Optimal probe/console performance optimization for these types of measurements boils down to two major factors: power handling to withstand the $2 \mu\text{s}$ 90° pulses necessary to cover the full-width powder pattern and the minimization of the acoustical ring-down to 20–30 μs beyond which signal loss due to transverse relaxation becomes pronounced (an example is shown in Figure 1). If a wide temperature range is desired for the measurements, as is often the case for detailed dynamics information, the set-up has to be optimized for evacuating cold/hot air out of the magnet not to risk the damage to the magnet. Currently, commercial options for satisfactory static probes for protein ^2H NMR measurements are relatively limited, with the best performance provided by Phoenix NMR and Doty Scientific in regard to the parameters discussed above. Very interesting options were created with low E capabilities (Gorkov and coworkers),(27) and cryogenic capabilities to perform experiments down to liquid He temperatures (Lipton and coworkers).(28)

5. Experimental determination of line shapes

The QE pulse sequence is the most common way to obtain the line shapes: $[90^\circ_x - \tau - 90^\circ_y - \tau]$. Several phase cycling schemes have been suggested.(22, 29, 30). The 90° pulse has to be of sufficient power to cover the full spectral band and is usually used at around 2.0–2.5 μ s. Composite pulses and other techniques are sometimes used to overcome this power limitation.(31, 32) The second τ delay is usually set up for time smaller than necessary for the echo to get refocused, so that one can easily locate the maximum of the FID and left shift the spectrum to its exact position. The minimum delay time possible is given by the acoustical ring-down, and in the best performing probes can be as small as 15–20 μ s. Long echo times significantly diminish overall intensity due to transverse relaxation (see the example in Figure 1).

Further, due to the possibility of anisotropy in transverse relaxation, the resulting line shapes can have a different form as a function of τ . The line shapes are most sensitive to motions when the time scale is of the order of the quadrupolar coupling constant. Examples of the effects of motions on line shapes are shown in the simulated spectra in Figure 2. As will be elaborated in the Modeling section, to obtain detailed dynamics information, one has to select the most likely motional model and then parametrize it. The line shape experiment can provide insights into the dependence of the dynamics on the presence of the solvent, and examples are shown in Fig. 3

6. QCPMG-static analog of magic-angle spinning

The inherent low sensitivity of the technique calls for methods of signal enhancements. One of the most powerful approaches is the multi-echo acquisition scheme (Figure 4), also referred to as Quadrupolar Carr-Purcell-Meiboom-Gill(QCPMG), which consists of several loops of QE cycles $[\tau_3 - 90^\circ - \tau_4]$. It breaks the powder pattern into a series of spikelets and serves as a static analog of the MAS enhancement. An important distinction is that while MAS acts on the spatial part of the Hamiltonian, QCPMG affects the spin part. The distance between the spikelets in the QCPMG-detected spectrum is inversely related to the interpulse delay τ_3 in the QE sequence.

The scheme is only applicable if a) the equipment can handle the required duty cycle with multiple 1 kW pulses; b) transverse relaxation is slow compared with the length of the power train; and c) heating the sample can be minimized with a relatively small number of echo trains. For hydrated protein powders, 10–15 echo pulses are empirically found to be a reasonable compromise between the desirable signal enhancement and other undesirable effects. Examples of the FID and spectra collected on the amyloid- β (A β) fibril samples with the use of the QCPMG scheme are shown in Figs. 5 and 6, respectively.

7. Experimental determination of the relaxation rates: Zeeman and Quadrupolar order

7.1 Zeeman order, T_{1Z}

Inversion or saturation recovery can be coupled to either the QE or multiple echo acquisition schemes. Relaxation anisotropy is known to provide important constraints for the determination of the motional mechanisms.(2) An example is shown in Figure 7A below for the side-chain of L69 in villin headpiece subdomain protein.(38) First, the existence of observable anisotropy supports the model of 3-site jumps about the methyl spinning axis, as opposed to rotation diffusion, in agreement with earlier works.(4) However, the magnitude of the anisotropy is smaller than that predicted for 3-site jumps alone (shown by dotted line). This reduction reflects the presence of a slower additional motional mode for the methyl axis, in particular restricted rotational diffusion and rotameric jumps that will be discussed in detail in the modeling section.

Unlike MAS analog, QCPMG does *not* refocus relaxation anisotropy,(39) which can thus be studied under static conditions. An example is shown for hexa-methyl-benzene(HMB): relaxation anisotropy profiles are compared between the QE and QCPMG detection schemes with two different values of the inter-pulse delay τ of the echo train (Fig. 4).(39) The general qualitative pattern of anisotropy is similar in all three schemes, however, QCPMG detection does not lead to a simple averaging of T_1 relaxation times in the nearest frequency regions. Rather, a more complex pattern is observed that is likely affected by the differential amount of transverse relaxation in the QCPMG detection as compared to QE detection. Additionally, there is an effect of the feed-through signal, which is particularly strong for more rigid compounds such as HMB.(39)

In the example below, the T_{1Z} measurements coupled to QCPMG detection (Figs. 6 and 8) enabled the characterization of the dynamics of a phenylalanine side chain buried in the core of amyloid- β fibrils across a wide temperature range.(34) From the change in the slope, it is evident that more than one motional mechanism is present.

Overall, heterogeneity of the conformational ensemble was observed from the magnetization decay curves (Fig. 8) fitted to the stretched exponential function of Eq. (6).

7.2 Quadrupolar order T_{1Q}

Due to their dependence on different spectral density terms, T_{1Q} measurements can be more sensitive to somewhat different transition frequencies, as can be seen from Eq. (5). Thus, in combination with the T_{1Z} measurement, they permit very precise model refinement. T_{1Q} measurements tend to be more demanding experiments than T_{1Z} due to intensity losses in the excitation period. One of the common pulse sequences for the measurement of the quadrupolar order is the broad-band Jeener-Broekaert variant: $[90^\circ_x - 2\tau_e - 67.5^\circ_y - 2\tau_e - 45^\circ_y - \tau - 45^\circ_y - \text{acq}]$.(40, 41) To avoid substantial signal losses, the excitation delay τ_e often needs to be limited to 5 μs in hydrated protein systems. The acquisition scheme can be either the QE or, if signal enhancement is desired, QCPMG.(39) T_{1Q} analysis permitted

for detection of the differences in the dynamics of various polymorphs of the amyloid- β fibrils (Fig. 9).(37)

8. Motional modeling based on experimental data

In this section, we provide specific examples on how to model several modes on the basis of ^2H static line shape and relaxation data. One of the most powerful tools for such data analysis is the EXPRESS program developed by Vold and co-workers.(33) Its predecessor MXET1 can also be found in the literature.(18) EXPRESS permits us to model a variety of NMR measurements including the QE, QCPMG, and MAS detection schemes as well as Zeeman and Quadrupolar order relaxation. Importantly, one can model motions comprising multiple motional modes which is especially relevant to complex protein systems.

8.1 Line shapes: Modeling rotameric jumps in methyl groups

Besides specifying the obvious parameters such as those involved in the experimental QE set-up, one has to input the values of C_q , value of tensor asymmetry, number of conformers, geometry of conformers, populations of conformers, and number of molecular orientations in the lab frame for the simulations of the powder state (tiles). It is almost always assumed that the principal axis system of the quadrupolar tensor for each deuteron is aligned along the position of the C—D bond.

When certain motions occur in the fast regime with respect to C_q (i.e., their rate is much faster than the value of C_q), the resulting spectrum is essentially a static one with the parameters of the averaged quadrupolar tensor. In general, this leads to a reduced effective C_q value, resulting in spectral compression, but can also lead either to a reduced or to an increased asymmetry parameter (an effect known as motional asymmetry). For example, when methyl rotations or three-site exchange between deuterons in the CD_3 group are in the fast limit, their only effect on C_q is to reduce it by a factor of three, assuming the tetrahedral geometry of methyl carbon.(2) Typical values of non-averaged C_q are between 160 and 180 kHz.(1, 2, 20, 44) Deviations from the ideal tetrahedral geometry were observed in some cases.(45)

The typical number of powder pattern tiles for the QE line shape experiment is between 500 and 5,000 and as always is governed by the compromise between spectral quality and simulation time.

8.1.1 Valine side chain—Rotameric jumps in this case occur around the χ_1 angle with three rotamers (Figs. 10 and 11), usually assumed to correspond to g^+ , g^- , and t orientations. To set up these simulations in EXPRESS, one has to specify the Euler angles Ω_{PC} representing this geometry: $\{(0, 109.5^\circ, 0^\circ), (0, 109.5^\circ, 120^\circ), \text{ and } (0, 109.5^\circ, 240^\circ)\}$. Further, one has to specify the relative population of the rotamers. For example, if there is one major and two minor conformers with equal populations, we would specify x:1:1.

8.1.2 Leucine side chain—In leucine side chains with the methyl-d3 labeling pattern, the situation is more complicated due to possible rotameric interconversions between both χ_2 and χ_1 angles, in principle resulting in nine possible conformers.(46) Out of these, only four

are magnetically inequivalent (Fig. 11).(13, 47) Thus, it is possible to approximate all rotamers with effective jumps of the methyl axis using only four directions, pointing toward the corners of the tetrahedron. In this situation, the Euler angles for the motions of $C^\gamma-C^\delta$ axis become: $\{(0, 109.5^\circ, 0^\circ), (0, 109.5^\circ, 120^\circ), (0, 109.5^\circ, 240^\circ), \text{ and } (0, 0^\circ, 0^\circ)\}$. A two-conformer model has also been employed for modeling leucine rotameric jumps.(13, 48)

8.1.3 Methionine side chain—Methionine is expected to have the need for the most sophisticated models due to the length of its side chain with three torsional angles (Fig. 10). In this case, it is often necessary to make an approximation rather than model complete rotameric behavior. For example, rotameric jumps of M35 side chains in the $A\beta_{1-40}$ fibrils (corresponding to the spectra depicted in Fig. 3 were modeled with four conformers in which the $S-C^\epsilon$ axis points toward the corners of the tetrahedron, analogous to the case of leucine above.(35)

8.2 Line shapes: Modeling ring flips in an aromatic side chain

We will consider an example of phenylalanine, for which the rings flips correspond to rotations around the χ_2 angle and do not involve the ζ (para) deuteron.(34, 49) Thus, only the D^δ and D^ϵ deuterons participate in the jumps. The D^ζ deuteron can be excluded from the simulation when its contribution is filtered by recycle delay, which is too short for these rigid nuclei. Under the assumption of the ideal ring geometry, the contributions from the D^δ and D^ϵ deuterons are identical.

Several sets of tensor parameters have been reported for crystalline phenylalanine amino acid. For example, Gall *et al.*(7) and Kinsey *et al.*(50) reported a C_q of 180 kHz and $\eta = 0.05$ based on line shape data at 100 K and 298 K, respectively. Alternatively, Hiyama *et al.*(3) reported $= 171 C_q$ kHz and $\eta < 0.02$ in conjunction with deviations from the ideal ring geometry based on the spectral data in the range of 293–421 K, and a C_q of 180 kHz and $\eta = 0$ based on 80–100 K QCPMG-detected spectra in the villin headpiece subdomain protein(49). In several cases, slight deviations from the ideal ring geometry were observed, (3, 34, 49) which may render slight differences between the D^δ and D^ϵ deuterons.

The following examples demonstrate the values of the Euler angles Ω_{PC} for the D^δ deuteron, in which θ is the angle between the $C-D^\delta$ bond and phenyl axis and $\eta = 0$: $\{(0, \theta, 0^\circ), (0, \theta, 180^\circ)\}$

8.3 Line shapes: Modeling fluctuations inside rotameric wells

Diffusive small-angle fluctuations within potential wells can be approximated by jump processes.(2, 19, 51, 52) The example below demonstrates the simulations of restricted asymmetric motions inside rotameric wells for the side chain of leucine. The $C^\gamma-C^\delta$ axis moves on the arc of a cone with an apex angle of 141° as defined by the tetrahedral geometry (Fig. 12).

We assume that the $C^\gamma-C^\delta$ axis has a preferred direction in the crystal-fixed frame corresponding to $\varphi = 0$, where φ is the azimuthal angle in a spherical coordinate system associated with the C^γ atom and the $C^\beta-C^\gamma$ bond lies on the polar axis. The arc itself is represented by several sites with values of φ incremented in $\varphi = 5^\circ$ steps. For example, a

30° arc is given by seven sites with $\varphi = -15^\circ$ to $\varphi = 15^\circ$. The effective diffusion coefficient D is given by $D = \varphi^2 k$, where $k = K_{i,i+1}^{arc} = K_{i+1,i}^{arc}$ is the jump rate between two neighboring sites in the arc frame. Only nearest-neighbor jumps are allowed. A similar approach was used by Meints *et al.*(51) to describe local motions in solid deoxyribonucleic acids. The Euler angles for this mode can be written as $\{(0, 109.5^\circ, \varphi_i)\}$.

These asymmetric motions such as motions along the arc lead to the effective asymmetry of the tensor.(38) When the motions inside the rotameric well occur in the fast motional regime with respect to C_q , one does not need to do the full simulation but can rather specify the value of the effective asymmetry parameter. Such an approach was employed for the M35 side chain in amyloid fibrils.(35) The motions inside the rotameric wells are not constrained to methyl-bearing side chains, but can also be evident in aromatic side chains.(49)

8.4 Line shapes: Simultaneous multiple modes

Line shapes often cannot be described with only one motional modes. Two examples are shown below, the first one combining rotameric jumps and fluctuations inside rotameric wells for a leucine side chain, and the second one combining pi-flips and small-angle fluctuations of phenyl axis for a phenylalanine side chain.

To model both the rotameric mode and the fluctuations along the restricted arc for the $C^\gamma-C^\delta$ axis of leucine, a two-frame nested model has to be set-up in EXPRESS.(38) The first frame corresponds to the arc motion and its Euler angle represents the rotations from the PAS system to the orientation associated with the single rotamer; the second rotameric frame transforms from the first frame to the crystal-fixed frame. For an arc length of 35° , the set of Euler angles become arc motion frame $\{(0, 109.5^\circ, \varphi_i)\}$ with the polar angle φ_i taking values between -17.5° and 17.5° in steps of 5° ; and rotameric frame $\{(0, 0, 0), (180^\circ, 109.5^\circ, 120^\circ), (300^\circ, 109.5^\circ, 240^\circ), \text{ and } (300^\circ, 109.5^\circ, 0)\}$.

The choice of angles for the rotameric frame was made such that the orientations of the $C^\gamma-C^\delta$ axis corresponding to the center of the arc still point to the four corners of the tetrahedron. When the π -flips and small-angle fluctuations around the χ_2 angle are considered, it is neither necessary nor convenient to set up simulations as two frames. Rather, it is easier to set up a single frame where the Euler angles correspond to all the values of the angles included in the model.(49) At the same time, this set-up requires the careful selection of the jump rates between different sites, realized through the “custom” jump rate matrix in EXPRESS.

In the example below, a so-called strong collision limit is shown for the small-angle fluctuations, such that they correspond to jumps between four sites, schematically represented in Fig. 13. The large-angle flips occur between sites 1–3 and 1–4 with an equal probability; this also holds for the 2–3 and 2–4 pairs. The small-angle jumps with the amplitude α occur between sites 1–2 and 3–4. The change in the χ_2 angle for transitions 1–3 and 2–4 is exactly 180° due to the symmetry of the ring. The corresponding Euler angles for the four sites are $\{(0, \theta, 0^\circ), (0, \theta, \alpha), \text{ and } (0, \theta, 180^\circ + \alpha)\}$.

The rate constants of the transitions between the four positions are specified in the transition rate matrix:

$$\begin{pmatrix} -k^{small} - k^{large} & k^{small} & k^{large}/2 & k^{large}/2 \\ k^{small} & -k^{small} - k^{large} & k^{large}/2 & k^{large}/2 \\ k^{large}/2 & k^{large}/2 & -k^{small} - k^{large} & k^{small} \\ k^{large}/2 & k^{large}/2 & k^{small} & -k^{small} - k^{large} \end{pmatrix}, \quad (7)$$

where k^{small} is the small-angle jump rate and $k^{large}/2$ is the rate for large-angle jumps. For example, if small-angle jumps have the amplitude of 5° , then $k^{large}/2$ corresponds to the rate of jumps from 0° to either 175° or 180° . The diagonal elements are chosen to satisfy the transition rate matrix conditions.

In addition to explicit motional modeling, several phenomenological approaches can be found in the literature for the interpretation of the line shape data. They range from the determination of the effective order parameter from the narrowing of the tensor compared with the rigid case under the assumption of axially symmetric motions,(15, 44) to the sophisticated slowly relaxing local structure approach.(53, 54)

8.5 Relaxation: Example of methyl jumps

Relaxation modeling will be first demonstrated for the case of a methyl-bearing side chain with the tree-site methyl jumps representing the dominant relaxation. EXPRESS has built-in blocks for the T_{1Z} and T_{1Q} relaxation experiments with either the QE or the QCPMG detection schemes. Assuming the tetrahedral methyl geometry, the Euler angles for the individual C-D δ bonds are $\{(0, 109.5^\circ, 0^\circ), (0, 109.5^\circ, 120^\circ), \text{ and } (0, 109.5^\circ, 240^\circ)\}$.

8.6 Relaxation: Creation of libraries and global fits at all temperatures

For cases in which one needs to model the distribution of rates due to the presence of non-exponential relaxation in the experimental data, EXPRESS can be used to create libraries of relaxation times corresponding to different rate constant values. Importantly, the intensities of the NMR signal can vary dependent on the rate and such libraries contain this information. When averaging is done assuming a certain functional form of the distribution, the intensities obtained for the individual values of the rate constants act as additional weighing factors.

Relaxation rates at different temperatures do not usually constitute unrelated parameters that can be fitted individually. They depend on the underlying mechanism. Typically, motionally activated Arrhenius-type behavior is chosen for the temperature dependence of the rate constants with the distribution arising from the distribution of activation energies. In this situation, the best course of action is to perform a global fit of the model to the data collected at all temperatures.(52, 55) For example, when the experimental behavior is fitted to the stretched exponential function of Eq. (6), one obtains the values of T_1^{eff} and β at all temperatures. Within the chosen motional model, one can calculate the predicted values of

T_1^{eff} and β using the library of relaxation rates and intensities described above. Then, the predicted values of T_1^{eff} and β compared with the experimental values and best fit parameters are obtained through χ^2 minimization.

8.7 Relaxation: Multimodal systems

When more than one relaxation mechanism is present, modeling is performed similar to what has been described for the simulation of the line shapes. An interesting situation can occur when the dominant motional mechanism that causes relaxation is different at different temperature intervals. For example, in the model of the π -flips and small-angle fluctuations described above (Fig. 13), the π -flips are likely to dominate relaxation at high temperatures, while at low temperature their effectiveness is greatly reduced and small-angle fluctuations dominate relaxation. Such a situation has been found for the hydrophobic core F19 in the A β fibrils (Fig. 8).(34) In terms of the modeling procedure, one now has to take into account motional processes.

Because relaxation in this model depends on two jump rates, both of which can change in the intervals spanning many orders of magnitude, it becomes impractical to construct libraries of relaxation rates and intensities covering all possible combinations of such rates. An approach more tailored to the specifics of the experimental results provides a better way. In particular, if two dominant relaxation regimes are well established at the ends of the experimental temperature range, the parameters governing the two relaxation mechanisms can be approximately determined in the relevant limits assuming Arrhenius behavior. They are then fine-tuned by a χ^2 minimization procedure, each step of which requires the simulation of the full-model relaxation process.

9. Concluding remarks

^2H solid-state static NMR techniques have great potential to complement and expand the information obtained by modern powerful MAS-based approaches for the elucidation of protein dynamics (9, 56–58), especially for amorphous systems. The rapid development of solid-state probes technology coupled with increasing field strength will open up new avenues for investigations of low-sensitivity biological systems such as the site-specific labeled disordered proteins imbedded in membranes and protein-nucleic acid complexes. Motional parameters obtained by computational modeling or phenomenological approaches can be used as constraints in molecular dynamics simulations for the elucidation of the thermodynamic and kinetic picture governing dynamics in the solid state.

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Highlights

- We discuss experimental static deuteron NMR techniques and computational approaches
- Application are chosen from studies of amyloid fibrils
- The focus is placed on basic methodology such as line shape and relaxation data
- We discuss computational modeling approaches for data interpretation

Figure 1.

Dependence of QE line shapes on the interpulse delay τ . The spectra were normalized per the number of scans acquired. Data collected on the F19-ring- d_5 side chains of hydrated amyloid fibrils (3-fold symmetric polymorph) consisting of the A β protein in the 3-fold symmetric polymorph. The A β protein in the 3-fold symmetric polymorph, at 17.6 T and 25°C.

Figure 2.

Line shape simulations, performed using EXPRESS software.(33) A–C methyl groups. A) Pake pattern narrowed by the three-site jump motions with the effective quadrupolar constant of $C_q = 53.3$ kHz. No methyl axis motions. B) Methyl axis motion along a restricted arc corresponding to the diffusion of the methyl axis occurring in the fast limit with respect to C_q . The arc length is 60° . C) Methyl axis undergoes rotameric motions, simulated by four magnetically distinct conformers with the weight of the major conformer twice that of each of the minor conformers; the jump rate is $2.5 \times 10^5 \text{ s}^{-1}$. D–F Ortho- and meta-deuterons of phenylalanine rings. D) Static Pake pattern with $C_q = 180$ kHz and an asymmetry parameter of 0.05. E) π -flip motion in the intermediate regime with a flip rate of $2.5 \times 10^6 \text{ s}^{-1}$. F) Fast π -flips with a flip rate of $1 \times 10^8 \text{ s}^{-1}$. Adapted from (17), license number 4226710808630

Figure 3.

Examples of the QE line shape spectra corresponding to selected side-chain sites in fibrils comprised of the A β protein in dry and hydrated (200% by weight) states in the 3-fold symmetric polymorph.(34, 35) Labeling pattern: F19-ring-d5. M35-methyl-d3. QE delay was 31 μ s. Collected at 17.6 T and 37°C.

Figure 4.

Timing scheme for the static QCPMG experiment for deuterium nuclei. Part A corresponds to a quadrupolar echo experiment with τ_2 adjusted such that the acquisition starts at an echo maximum. Part B is the repeating unit with the $\pi/2$ refocusing pulses bracketed by delays τ_3 , τ_4 followed by an acquisition period τ_a . Part C is an additional acquisition period ensuring the full decay of the FID. The phases ϕ_1 – ϕ_3 are cycled to select the coherence transfer pathway $p = 0 \rightarrow \pm 1 \rightarrow \mp 1$ etc. Reprinted with permission from (36). Copyright (1997) American Chemical Society.

Figure 5.

An example of the free induction decay resulting from the QCPMG detection scheme. Collected on M35 side chains (methyl- d_3) of hydrated fibrils consisting of the $A\beta_{1-40}$ protein.(37) 15 echos were collected and 3072 scans. Breakthrough pulses were removed in the processing. Collected at 17.6 T and 37°C. The FID demonstrates a typical loss of signal in a hydrated protein powder sample.

Figure 6.

Examples of QCPMG spectra corresponding to selected side-chain sites in the fibrils comprised of the A β protein.(34, 37) Labeling patterns: F19-ring-d₅ and M35-methyl-d₃. Interpulse delay in the QCPMG cycle was 52 μ s and the number of QCPMG cycles was 15. Collected at 17.6 T.

Figure 7.

A) T_{1Z} relaxation anisotropy profiles at 10°C and 17.6 T for L69-d3 side-chain in villin headpiece subdomain. Experimental data (circles) are compared to the simulated profile according to the single 3-site jumps mode (dotted line), and a multimode model involving 3-site jumps, restricted diffusion of methyl axes, and rotameric jumps (solid line). B) T_{1Z} relaxation anisotropy profile for HMB at 25 °C and 17.6 T,(39) measured by QE detection scheme (black line), QCPMG detection scheme with inter-pulse delay τ of 302 μ s (blue circles) and 102 μ s (red squares).

Figure 8.

Examples of the T_{1Z} time measurements shown for the F19 hydrophobic core side chain in the amyloid fibrils consisting of the A β protein in the 3-fold symmetric polymorph; the data correspond to the 60 kHz spikelets of the QCPMG spectra. A) Values of T_1^{eff} and β obtained from the fits of magnetization decay curves to the stretched exponential function of Eq. (6). Compared are the hydrated (red) and dry (black) states of the fibrils. Arrows demonstrate the temperature of the dynamical transition temperature and solid lines are the fits to the model elaborated in (34). B) Saturation recovery magnetization build-up curve at 220 K for the hydrated state demonstrating the quality of the fit to the single exponent (dashed line) and the stretched exponential function (solid line). Data collected at 17.6 T.

Figure 9.

Plots of T_{1Z}^{eff} and T_{1Q} vs. $1000/T$ at a 17.6 T magnetic field strength for the M35 site in the amyloid fibrils comprised of the amyloid- β (1-40) protein in the 2-fold and 3-fold symmetric polymorphs. The measurements employed the QCPMG signal-enhancement scheme. B) Structural representation of the 2-fold and 3-fold symmetric polymorphs of the amyloid- β fibrils, view down the fibril axis.(42, 43) The M35 side chain pointing into the water-accessible cavity is shown in red. Reprinted with permission from (37), Copyright (2016) Elsevier, License 4226720132799.

Figure 10. Dihedral angles involved in the rotameric interconversion of valine, leucine, and methionine side chains. Common commercially available labeling patterns of methyl groups are shown in red. Taken from (17), license number 4226710808630.

Figure 11.

Schematic representation of rotamers in leucine and valine side chains. In leucine, rotameric jumps corresponding to hops between four non-equivalent positions of the leucine side chain out of the nine possible configurations. In valine, the interconversions are among the g^+ , g^- , and t conformers.

Figure 12. Restricted diffusion on an arc for the methyl axes of the leucine side chain, approximated by small nearest neighbor jumps of the C^γ - C^δ axis.

Figure 13.

A) The phenylalanine side chain with the deuterium-labeling pattern marked in orange. B) Motional model for the fluctuations around the χ_2 dihedral angle. The diagram displays sites' connectivities according to the four-site strong collision model, illustrated for one of the C^δ-D bonds. The large-angle flips occur between sites 1-3 and 1-4 with an equal probability; this also holds for the 2-3 and 2-4 pairs. The small-angle jumps with the amplitude α occur between sites 1-2 and 3-4. Adapted with permission from (49). Copyright (2015) American Chemical Society