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Studies of Molecular Dynamics of FMOC Amino Acids Using Solid State Deuteron Nuclear Magnetic Resonance Spectroscopy

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A Dissertation presented to the Graduate Faculty of the College of William and Mary in Candidacy for the Degree of Doctor of Philosophy

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August, 2014
This Dissertation is submitted in partial fulfillment of the requirements for the degree of

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Abstract

The purpose of devising and validating models for intramolecular motions for FMOC amino acids is to quantify side chain motion in proteins which plays an important role in understanding biological structure function relations of proteins.

In this thesis, spin lattice relaxation times ($T_1$) of FMOC amino acids were measured under both static and magic angle spinning (MAS) condition at variable temperatures. Lower activation energies of the relaxation times than the normal amino acids observed indicate a less sterically crowded environment for the rotation methyl group. A three-site jump model for the methyl group was developed to fit the $T_{1Z}$ and $T_{1Q}$ anisotropy under static condition. Under MAS, Multiple deuterated sites can be resolved and studied independently.

Finally, a temperature model for the spinning rotor was developed to account for the temperature gradient across the rotor. A comparison of using the single most probable temperature and the temperature distribution in the simulation of relaxation times concludes the difference between these two approaches is minimal.
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Acknowledgment

First I would like to express my greatest gratitude to my advisors, Professor Robert L. Vold and Professor Gina Hoatson for their patience, guidance and instructions over the years. I was overwhelmed with the academic integrity they insist on and the rigorous scientific methodology they impart. And that is the most valuable thing I have learned I believe I will benefit from it through all my life.

Much of the work in this thesis would not be possible without the cooperation of other members of the NMR lab, I would like to thank Jeremy Ellden and Chris Maher for helping me get familiar with the spectrometer and experiment procedures and providing some of the experiment data in this thesis. I also want to thank Rony Kalfarisi for some valuable discussions through my research.

I would like to thank my committee members, Professor Gunter Luepke and Professor Gregory Smith for taking the time to read and criticize my thesis. Last but not least, I would like to thank my parents for their unconditional support and understanding throughout the years. It allowed me to get where I am today.
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Chapter 1 Introduction

The importance of restricted molecular motion in understanding biological structure-function relations and their important role in the microscopic basis of many human diseases and mechanism of drug actions has long been recognized. For example, the side chain motions in proteins play an major role in protein stability, folding pathways and biological function.

Deuteron NMR (Nuclear Magnetic Resonance) has been proved to be a powerful tool to probe molecular structures and dynamics in various systems such as liquid crystals and biological molecules. Deuteron NMR line shapes and relaxation rates are usually dominated by a single particle mechanism which is the quadrupole interaction between the nuclear electric quadrupole moment and the electric field gradient. The deuteron has a relative small electric quadrupole moment \( Q = 2.8 \times 10^{-31} \text{ m}^2 \) which give rise to the quadrupole coupling constants in the range of 140 kHz-220 kHz in organic compounds. Available deuteron techniques such as line shape analysis and relaxation time measurements can cover a very broad range of time scales from picoseconds to milliseconds. All of these benefits make deuteron NMR an ideal tool for investigating protein side chain motions.

Solid state NMR offers several advantages over solution phase NMR, such as no constraints imposed by solubility, a typically much wider temperature range, and the
absence of hard to quantify contributions to spin relaxation from overall molecular tumbling. Although the lack of molecular tumbling leads to the broadening of the spectrum, and hence relatively low signal to noise ratio, development of high resolution solid NMR techniques combined with the use of ever higher static magnetic field have to a large extent overcome these difficulties. Moreover, the reward for the more complex nature of solid state NMR is that anisotropic interactions such as chemical shielding and quadrupole coupling, which are averaged to zero in solution, can now be utilized to reveal fine details of molecular structure and dynamics.

It is important that motional models intended for applications to complex systems, such as folded proteins, first to be validated by application to relatively simple model compounds. In this thesis, FMOC (9-fluorenylmethoxycarbonyl) amino acids are used to validate the motional models for rotating methyl groups in protein side chains. These amino acid derivatives were chosen because the N-protecting group, FMOC is a bulky, relatively immobile moiety to which the amino acid links, in rough analogy to the environment of an amino acid side chain linked to the backbone of a protein.

Quantitative studies of methyl group rotation have a long history\(^{7-9}\). It is well known that fast rotational motion of a C-D bond about an axis of 3-fold symmetry or higher leads to an averaged deuteron quadrupole coupling constant which is about one third of the value expected in absence of the rotational motion. Besides, there is a large variation in activation energy for the methyl rotation, which is often estimated from an Arrhenius plot of the relaxation time. The activation energy of methyl group
rotation is sensitive to its local environment, and is strongly affected by the degree of steric hindrance. For example, the activation energy for methyl group rotation in acetone-d$_6$ is only 4.0 kJ/mol$^{[8]}$, much smaller than the 20 kJ/mol$^{[9]}$ found for crystalline L-alanine-d$_3$.

Chapter 2 in this thesis will cover the basic principles in solid state NMR. The first part introduces the major nuclear spin interactions, including the Zeeman interaction, and perturbations arising from chemical shielding, dipolar coupling and quadrupole coupling. The second part presents a synopsis of Redfield relaxation theory$^{[11]}$ and a more general stochastic Liouville formulation suitable for describing restricted motion in solids$^{[43]}$.

Chapter 3 will focus on the experimental methods used in this thesis. This begins with an overview of the NMR instrumentation, followed by a description of various pulse sequences and experimental setups used to obtain quadrupole echo spectra and to measure spin relaxation times. After this the magic angle spinning technique is described and finally, data processing procedures are illustrated.

Static experiments on FMOC alanine will be discussed in Chapter 4. Experimental quadrupole spectra and both Zeeman ($T_{1Z}$) and quadrupolar ($T_{1Q}$) order relaxation time anisotropies are measured at several temperatures. Then a motional model is devised and the spectral densities are calculated. At last, the simulations are fit to the experimental data, the activation energy for the methyl rotation is calculated from the Arrhenius plot of simulated jump rates, and the result is interpreted in the context of literature values reported for similar molecules.

A second project, the study of FMOC-valine-d$_8$ under magic angle spinning
conditions, is discussed in Chapter 5. Per-deuterated valine-d₈ is of course much cheaper to buy and easier to synthesis than valine-d₃ with deuterons selectively introduced on the methyl group, but overlap between the chemically shifted powder patterns severely complicates the analysis of their spin-lattice relaxation behavior. A major goal of this study is to develop new procedures for analyzing relaxation rates of rotating samples that take advantage of the greatly enhanced spectral resolution available from high field MAS spectra. Experiments performed include measurements of MAS spectra and T₁z of different deuterated sites at variable temperatures. The overall spectrum as well as the spinning sidebands is interpreted, spectral densities are calculated for the spinning sample based on the discussion for the static case in chapter 4, and the activation energy for the jump rate of the methyl group is calculated and compared to values found for methyl groups in other FMOC-amino acid derivatives.

In the last chapter, a model for the temperature distribution in a spinning rotor is developed to account for effects of unavoidable frictional heating. Simulated lead nitrate spectra based on the assumed temperature model are fit to the experimental spectra obtained at various spin rates and temperatures (Appendix A discusses the details of the process of calibrating true sample temperature with lead nitrate spectra for both the static and MAS probes). Finally, a comparison of relaxation times calculated for methyl groups rotating at a single temperature is compared with simulations based on a full distribution of relaxation times calculated for the experimentally determined distribution of sample temperatures. The most important conclusion of this study is that when properly executed, the former (simpler)
procedure is entirely consistent with the latter if the thermal gradients are not too large.
Chapter 2 Basic principles of deuteron NMR

2.1 Nuclear spin interactions

The time independent Hamiltonian operator for spins in a static, uniform magnetic field is given by the following sum of interactions:\[10\]:

\[ \hat{H} = \hat{H}_z + \hat{H}_\sigma + \hat{H}_D + \hat{H}_Q \] (2-1)

The first term on the right is the Zeeman Hamiltonian, which is usually the dominant interaction in a nuclear spin system. The following three terms are chemical shielding, dipolar coupling and quadrupole coupling, respectively, and can be treated as perturbations of the Zeeman Hamiltonian. The following sections will discuss these interactions in detail, with particular reference to approximations relevant for deuterons.

2.1.1 Zeeman Hamiltonian

Zeeman Hamiltonian describes the interaction between the nuclear spin and the external static magnetic field. The Hamiltonian can be written as

\[ \hat{H}_z = -\hat{\mu} \cdot \mathbf{B}_0 \] (2-2)

Where \( \mu \) is the nuclear magnetic moment operator and \( \mathbf{B}_0 \) is the external magnetic field. This can be rewritten in terms of nuclear operators: assuming without loss of generality that the magnetic field is oriented along the laboratory-fixed z-axis, it can
be shown that

\[ \hat{H}_z = -\gamma \hbar \hat{I}_z B_0 \]  

(2.3)

In this expression, \( \gamma \) is the gyromagnetic ratio, \( \hbar \) is the reduced Planck's constant, \( 1.05 \times 10^{-34} \text{ J·s} \), and \( \hat{I}_z \) is the operator for total Z component of spin angular momentum.

Any possible state of the spin system can be expressed as a specific linear combination of the \((2l+1)\) eigenfunctions of \( \hat{I}_z \), denoted by \( |I,m\rangle \). Since \( \hat{H}_z \) is proportional to \( \hat{I}_z \), they have the same eigenfunctions. Thus,

\[ \hat{I}_z |I,m\rangle = m |I,m\rangle \]  

(2.4)

and

\[ \hat{H}_z |I,m\rangle = E_{i,m} |I,m\rangle \]  

(2.5)

from which it follows that

\[ E_{i,m} = -\gamma \hbar B_0 m \]  

(2.6)

For deuterons, the nuclear spin quantum number \( I \) is 1, so there are three possible eigenstates \( m = -1,0,1 \), with corresponding energies \( E = \gamma \hbar B_0, 0, -\gamma \hbar B_0 \).

In an ensemble of deuterons at thermal equilibrium with its surroundings, there is a Boltzmann distribution of nuclear spins among these three eigenstates. The population of each state is given by

\[ P_m = \frac{\exp(-E_m / kT)}{\sum_l \exp(-E_l / kT)} \]  

(2.7)

### 2.1.2 Chemical shielding Hamiltonian

The electrons surrounding a nucleus respond to the presence of an external magnetic...
field by producing a secondary field which, in general, is not parallel to the external field but is, to a high degree of approximation, proportional to its magnitude\(^1\). The response of nuclear spins to this induced magnetic field is described by the chemical shielding Hamiltonian:

\[
\hat{H}_{cs} = \hat{\mathbf{A}} \cdot \mathbf{\sigma} \cdot \mathbf{B}_0
\]  

(2-8)

Here, \(\mathbf{\sigma}\) is the chemical shielding tensor, which is a second-rank Cartesian tensor. The shielding tensor is real-valued but asymmetric, with nine independent elements. However, it can always be decomposed into the sum of a symmetric tensor with six independent elements, and a traceless, antisymmetric tensor with three independent elements\(^5\). In what follows, it is convenient to define a Cartesian axis frame in which the symmetric part is diagonal, called the principal axis system (PAS). It is conventional to define the quantities \(\sigma_{iso}, \Delta, \text{ and } \eta\)\(^5\), according to the definitions,

\[
\begin{align*}
\sigma_{iso} &= 1/3(\sigma_{xx}^{PAS} + \sigma_{yy}^{PAS} + \sigma_{zz}^{PAS}) \\
\Delta &= \sigma_{xx}^{PAS} - \sigma_{iso} \\
\eta &= \frac{\sigma_{xx}^{PAS} - \sigma_{yy}^{PAS}}{\Delta}
\end{align*}
\]  

(2-9)

As mentioned before, the chemical shielding Hamiltonian can treated as a first order perturbation to the Zeeman Hamiltonian. According to perturbation theory, only that part of the perturbing Hamiltonian that commutes with the Zeeman Hamiltonian has a significant effect on the energy levels of the spin states. The secular part of the chemical shielding Hamiltonian is given by\(^5\)

\[
H'(\theta, \varphi) = -\omega_0 I_z \{\sigma_{iso} + 1/2\Delta(3\cos^2 \theta - 1 + \eta \sin^2 \theta \cos 2\varphi)\}
\]  

(2-10)
Figure 2.1: Definition of polar angles $(\theta, \varphi)$. XYZ denotes laboratory fixed axes with Z along the external magnetic field and X along the rf coil axis (for a static NMR probe). PAS (Principal Axis System) denotes the molecule fixed axis system in which the symmetric part of the chemical shielding tensor is diagonal, with largest principal component along z. Only the Z-axis of this frame is shown. $\theta$ is the angle between the Z-axis in the lab-fixed frame and the Z-axis in the PAS frame, and is the angle between the projection of the molecule fixed Z-axis on the lab-fixed XY plane and the lab-fixed X-axis.

$(\theta, \varphi)$ are polar angles (Figure 2.1) that define the PAS in lab frame.

In NMR experiments, the chemical shift is not measured as an absolute value, instead, the observed resonance frequency is always measured with respect to a standard material for each nucleus, established by IUPAC convention\textsuperscript{[5]}. Thus the chemical shift is defined by
\[
\delta_{iso} = \frac{V - V_{ref}}{V_{ref}}
\]  

We can define isotropic chemical shifts $\delta_{iso}$, chemical shift anisotropies $\Delta_{cs}$ and asymmetry parameters $\eta_{cs}$ similar to those of the chemical shielding tensor itself:

\[
\delta_{iso} = 1/3(\delta_{11}^{PAS} + \delta_{22}^{PAS} + \delta_{33}^{PAS}) \\
\Delta_{cs} = \delta_{11}^{PAS} - \delta_{iso} \\
\eta_{cs} = \frac{\delta_{33}^{PAS} - \delta_{22}^{PAS}}{\Delta_{cs}}
\]  

Figure 2.2 shows the effect of an axially symmetric chemical shift tensor on the NMR spectrum of a polycrystalline sample of a molecular with axial symmetry. As discussed further in Section 2.2 below, this characteristic shape arises from a superposition of resonances from randomly oriented crystallites.

![Figure 2.2: NMR line shape of a polycrystalline powder sample with an axially symmetric chemical shielding tensor. Intensity at frequency $v_{//}$ arises from crystallites in which the PAS Z-axis is oriented along the lab-fixed Z-axis ($\theta = 0$), intensity at $v_{\perp}$ arise from crystallites in which the PAS Z-axis is oriented perpendicular to the lab-fixed Z-axis ($\theta = 90^\circ$), and $v_{iso}$ denotes the resonance frequency of the isotropic chemical shift that could be observed, for example, by fast magic angle spinning.](image)
2.1.3 Dipolar coupling Hamiltonian

The interaction between two nuclear magnetic dipole moments is called dipolar coupling. In solution, this interaction is averaged to zero by virtue of rapid, random molecular tumbling. However, in solid materials the dipolar coupling is not be averaged to zero and can be a major source of line broadening.

The interaction Hamiltonian for dipolar coupling between two spins is\(^\text{[5]}\)

\[
\hat{H}_D = -\frac{\mu_0}{4\pi} \gamma_1 \gamma_2 \delta \left( \frac{1}{r^3} - 3 \frac{(\mathbf{I} \cdot \mathbf{r})(\mathbf{S} \cdot \mathbf{r})}{r^5} \right)
\]  \hspace{1cm} (2-13)

We may also express the dipolar coupling Hamiltonian in tensor form

\[
\hat{H}_D = -2\mathbf{I} \cdot \mathbf{D} \cdot \mathbf{S}
\]  \hspace{1cm} (2-14)

Because we have to express spin Hamiltonians in many different frames related by Euler rotations, it is more convenient to write equation 2-14 in terms of second rank irreducible spherical tensors\(^\text{[12]}\)

\[
\hat{H}_D = \sum_{m=-2}^{2} (-1)^m \hat{A}^{(2,D)}_m T^{(2,D)}_m
\]  \hspace{1cm} (2-15)

The spin operators are given by

\[
\hat{A}^{(2,D)}_0 = \frac{1}{\sqrt{6}} (3\hat{I}_z \hat{S}_z - \mathbf{I} \cdot \mathbf{S})
\]

\[
\hat{A}^{(2,D)}_{\pm 1} = \frac{1}{2} (\hat{I}_z \hat{S}_\pm + \hat{I}_\pm \hat{S}_z)
\]  \hspace{1cm} (2-16)

\[
\hat{A}^{(2,D)}_{\pm 2} = \frac{1}{2} \hat{I}_\pm \hat{S}_\pm
\]

The lattice variables are defined as

\[
T^{(2,D)}_m(LAB) = \sum_{k=-2}^{2} D^{(2,D)}_{mk} (\Omega) T^{(2,D)}_k(PAS)
\]  \hspace{1cm} (2-17)
where $D_{mk}$ is Wigner rotation matrix, $\Omega_{lp}$ are the polar angles that rotate the Lab frame to PAS frame. The PAS values of the dipolar coupling tensor are

$$
T^{(2,D)}_0(PAS) = -\sqrt{3} \hbar \gamma_I \gamma_S r_{IS}^3
$$

$$
T^{(2,D)}_{\pm 1}(PAS) = T^{(2,D)}_{\pm 2}(PAS) = 0
$$

(2-18)

where $r_{IS}$ is the distance between spin $I$ and spin $S$.

### 2.1.4 Quadrupole coupling Hamiltonian

Nuclei with spin greater than 1/2 have electric quadrupole moments. These originate from the non-spherical distribution of charge within the nucleus. The charge distribution function can be expanded in a series of multipoles; the zeroth rank multipole is the total charge, the first rank term is the electric dipole moment and the second rank term is the electric quadrupole moment. For nuclei with spin $I = 1/2$, the second term of the multipole expansion is zero, so that only nuclei with spin $I$ greater than 1/2 process electric quadrupole moments. The nuclear quadrupole moment interacts with electric field gradients at the nucleus arising from nearby electrons, and thereby affects the spin energy levels.

The strength of this quadrupole interaction depends on both the nuclear quadrupole moment and the electric field gradient. It can be shown using the Wigner-Eckart theorem\[11\] that for a given nucleus, the quadrupole moment is a constant which does not depend on chemical environment.

The basic form of the quadrupole Hamiltonian is

$$
\hat{H}_Q = \frac{eQ}{2I(2I-1)\hbar} \hat{\mathbf{r}} \cdot \mathbf{\hat{r}}
$$

(2-19)
\( \hat{H}_Q = \frac{e^2 q Q}{4I(2I-1)\hbar} \left\{ 3\hat{I}_z^2 - \hat{I}_x^2 + \frac{1}{2} \eta_Q \left( \hat{I}_x^2 - \hat{I}_y^2 \right) \right\} \) (2-20)

In which \( eq \) is, by convention, the largest principal component of the electric field gradient tensor and \( \eta_Q \) is the asymmetry parameter, defined as follows,

\[
\begin{align*}
\text{\( eq = V_{zz}^{\text{PAS}} \)} \\
\text{\( \eta_Q = \frac{V_{xx}^{\text{PAS}} - V_{yy}^{\text{PAS}}}{V_{zz}^{\text{PAS}}} \)}
\end{align*}
\] (2-21)

Also, the quadrupole coupling constant is defined as \( C_Q = e^2 q Q / \hbar \).

To make it more convenient to express the quadrupole Hamiltonian in different frames, we can also write the Hamiltonian in terms of spherical tensors

\[
\hat{H}_Q = \sum_{m=-2}^{2} (-1)^m \hat{A}_m^{(2,Q)} T_m^{(2,Q)}
\] (2-22)

Where\(^{[12]}\)

\[
\begin{align*}
\hat{A}_0^{(2,Q)} &= \frac{4}{\sqrt{6}} (3\hat{I}_z^2 - \mathbf{I} \cdot \mathbf{I}) \\
\hat{A}_{\pm 1}^{(2,Q)} &= \pm \frac{1}{2} (\hat{I}_z \hat{I}_\pm + \hat{I}_\pm \hat{I}_z) \\
\hat{A}_{\pm 2}^{(2,Q)} &= \frac{1}{2} \hat{I}_\pm^2
\end{align*}
\] (2-23)

And

\[
T_m^{(2,Q)}(LAB) = \sum_{k=-2}^{2} D_{mk}^{(2)}(\Omega_{L'R}) T_k^{(2,Q)}(PAS)
\] (2-24)
As mentioned before, the quadrupole Hamiltonian is used as a perturbation of the Zeeman Hamiltonian. To first order, only the secular terms are included, and the resulting approximate Hamiltonian is

\[ H_Q = A_0^{(2)} T_0^{(2)} \]  

(2-26)

Only the PAS values of the electric gradient tensors are known, while \( T_0 \) is given in the lab frame. We therefore use a Wigner rotation matrix to relate the \( T_k \) (PAS values) to \( T_0 \)

\[ T_0^{(2)} \text{(Lab)} = \sum_k D_{0k}^{(2)*} (\Omega_{LP}) T_k^{(2)} \text{(PAS)} \]  

(2-27)

Substituting equation 2-26 with values from equations 2-23 and 2-25, yields the result

\[ \hat{H}_Q = \frac{e^2 qQ}{8I(2I-1)} (3 \hat{I}_z^2 - \hat{I}^2) \{3\cos^2 \theta - 1\} + \eta_0 \sin^2 \theta \cos 2\varphi \]  

(2-28)

Ignoring chemical shielding and dipolar coupling for the moment, for the moment, the total Hamiltonian is given by

\[ \hat{H} = -\gamma B_0 \hat{I}_z + \frac{e^2 qQ}{8I(2I-1)} (3 \hat{I}_z^2 - \hat{I}^2) \{3\cos^2 \theta - 1\} + \eta_0 \sin^2 \theta \cos 2\varphi \]  

(2-29)

For deuterons, \( I=1 \), so there are three energy levels as shown in equation 2-30 and Figure 2.3.
\[
E_{-1} = h\gamma B_0 + \frac{e^2qQ}{8} \left\{3\cos^2 \theta - 1\right\} + \eta_0 \sin^2 \theta \cos 2\varphi \\
E_0 = -\frac{e^2qQ}{4} \left\{3\cos^2 \theta - 1\right\} + \eta_0 \sin^2 \theta \cos 2\varphi \\
E_{+1} = -h\gamma B_0 + \frac{e^2qQ}{8} \left\{3\cos^2 \theta - 1\right\} + \eta_0 \sin^2 \theta \cos 2\varphi
\]  
(2-30)

There are two transition frequencies
\[
\omega_{-1,0} = \omega_0 + \omega_Q \\
\omega_{0,1} = \omega_0 - \omega_Q
\]  
(2-31)

Figure 2.3: Energy levels for a single deuteron in a constant magnetic field \(B_0\). In absence of quadrupole coupling (left), the Zeeman interaction produces equal spacing as shown between adjacent \(m\)-states in accord with equation 2-30 with \(Q = 0\). In this case only a single line will be observed, at frequency \(\omega_0 = \gamma B_0\). When \(H_Q \neq 0\) (right) the \(m = \pm 1\) states shift slightly upward by the same amount (to first order) and the middle \(m = 0\) state shifts downward by twice that amount. This lifts the degeneracy and the spectrum consists of two equally intense lines at frequencies \(\omega_0 \pm \omega_Q\), where \(\omega_Q\) is defined in equation 2-32.

where \(\omega_0\) is the Larmor frequency and
\[
\omega_Q = \frac{e^2qQ}{8} \left\{3\cos^2 \theta - 1\right\} + \eta_0 \sin^2 \theta \cos 2\varphi
\]  
(2-32)

So for a single crystal, a doublet will be observed and in powder sample, powder pattern (Figure 2.4) will be observed because of the orientation dependency of \(\omega_Q\).
The characteristic shape arises from a sum of doublet spectra over all crystallite orientations, weighted by the number of randomly oriented crystallites per unit solid.

![Figure 2.4: Origin of the deuteron powder pattern.](image)

Figure 2.4: Origin of the deuteron powder pattern. The NMR spectrum for one deuteron in a single crystal (left) consists of two peaks separated by $2\omega_0$, where $\omega_0$ is defined in equation 2-32 and $(\theta, \varphi)$ define the crystal orientation with respect to the external field. It follows that for a large, randomly oriented set of small crystallites, the spectrum consists of two partially overlapping signals, arising from a superposition of doublets with frequencies defined in equations 2-31 and 2-32, weighted by $\sin(\theta)$ to account for equal numbers of spins per unit solid angle. It follows from equations 2-31 and 2-32 that the outer "shoulders" of this powder pattern ($\theta = 90^\circ$), are separated by $3/2C_Q$, where $C_Q = e^2qzQ/h$ is the quadrupole coupling constant, while the "horns" ($\theta = 0^\circ$) are separated by exactly half that amount in the simple case of zero asymmetry parameter ($\eta_Q = 0$).

It is no accident that each component of the deuteron powder pattern resembles the chemical shift powder pattern for a nucleus of spin 1/2: according to equations 2-10 and 2-32, the transition frequencies exhibit precisely the same angular dependence. This is in fact quite general for all second rank perturbations of the Zeeman interaction.

### 2.1.5 Simplifications for deuterons

Energy levels and transition frequencies for the general case in which chemical shielding, dipolar coupling, and quadrupole coupling all produce comparable
contributions to the spin energy levels are quite complex, especially for dipolar coupling which extends over chains of coupled nuclei throughout the sample. One of the unique features of deuteron NMR spectroscopy is that the quadrupole coupling, while small enough so that second order effects are essentially negligible\cite{4}, are typically larger than either dipolar coupling or chemical shift anisotropy. Thus, it is often possible to record and quantitatively analyze experimental deuteron powder patterns that closely resemble the theoretical line shape shown in Figure 2.4. Moreover, magic angle spinning of a deuteron sample at experimentally achievable rotation rates can effectively remove all effects of chemical shift anisotropy and most residual dipolar coupling, so that chemically distinct sites can be experimentally resolved, at high magnetic fields, by virtue of their different isotropic chemical shifts without resorting to expensive and difficult procedures of selective deuteron isotopic labeling.

2.2 Relaxation theory

Once a system is in a non-equilibrium state, it has a tendency to return to the equilibrium state. This does not happen instantly, the general phenomenon of evolution back to equilibrium is called relaxation. In NMR, unlike most other forms of spectroscopy, spontaneous emission is far too slow to account for relaxation, which arises instead by virtue of transitions among the spin energy levels that are induced by randomly time-dependent, fluctuations of the interaction Hamiltonians described in section 2.1. These fluctuations arise through random rotational and librational motions of spin-bearing molecules, which introduce random time dependence into the angles that define molecule-fixed tensors (usually of second rank) with respect to
laboratory fixed axes. Relaxation studies are very important in NMR because not only because knowledge of the relaxation time scale is crucial for proper experimental design, but also because analysis of relaxation rates can provide unique, quantitative information about molecular motion on a wide range of time scales.

In this section, Redfield relaxation theory of spin relaxation is outlined and applied to the special case of deuterons. Also, the Stochastic Liouville Equation will be introduced to deal with the more general case slower molecular motion.

2.2.1 Density matrix

Spin relaxation is a macroscopically observable phenomenon, and methods of statistical mechanics are therefore necessary to relate the observable relaxation rates to the fluctuating Hamiltonian operators for individual spins. The density operator, or density matrix, is an elegant tool for developing such connections. For an ensemble of identical, isolated spin systems, each system can be in any one of the possible states \( \psi_i \) with probability \( p_i \), so the average expectation value of an observable quantity \( Q \) whose corresponding quantum operator is denoted by \( \hat{Q} \) is given by

\[
\overline{Q} = \sum_i p_i \langle \psi_i | \hat{Q} | \psi_i \rangle
\]  

(2-33)

The functions \( \psi_i \) form a complete basis set for any member of the ensemble, for example, \( | -1 \rangle, |0 \rangle, |1 \rangle \) form a complete set for a single spin with \( I = 1 \), so that an arbitrary state of the spin (not necessarily a stationary state of the corresponding Hamiltonian) can be written as

\[
\psi_i = \sum_k c_{ik} \phi_k
\]  

(2-34)
In which \( \{ \phi_i \} = | -1 \rangle, | 0 \rangle, | 1 \rangle \) is the basis set. From equations 2-33 and 2-34, it follows that

\[
Q = \sum_i \sum_{k,j} p_i^* c_{ik} c_{jl} \langle \phi_k | \hat{Q} | \phi_l \rangle
\]

(2-35)

Defining elements

\[
\rho_{kl} = \sum_i p_i^* c_{ik} c_{jl}
\]

(2-36)

So the expectation value of \( Q \) can be written as

\[
\overline{Q} = Tr(\hat{\rho} \hat{Q}) = \sum_k \sum_l \rho_{kl} Q_{jl}
\]

(2-37)

The diagonal elements of the density matrix represent the probability that a spin in the ensemble is in a particular state, that is to say the population of that state. Off-diagonal elements \( \rho_{jk} \) represent the fraction of spins in the ensemble that exist in a coherent superposition of basis states \( j \) and \( k \).

The state of thermal equilibrium is described by a density matrix whose elements, when represented in the basis of eigenstates of the spin Hamiltonian \( H \), are proportional to those of the exponential operator \( \exp(-iH/k_B T) \), where \( k_B \) is Boltzmann’s constant, and \( T \) is the temperature. However, it is important to realize that the trace relation in equation 2-37 is valid in any convenient basis.

2.2.2 Redfield relaxation theory

Redfield theory is a semi classical theory, in which spin systems are treated quantum mechanically and the environment of the spins, known as the lattice, is treated classically. The following section is a brief discussion of the basic principles.
The Hamiltonian in Redfield theory is divided into two parts: a time independent part $H_0$ and a time dependent perturbation $H_1(t)$. It is easy to show that the time evolution of the density operator is given in general by the Liouville-Von Neumann equation,

$$ \frac{d\hat{\rho}(t)}{dt} = i\left[\hat{\rho}(t), \hat{H}_0 + \hat{H}_1(t)\right] \tag{2-38} $$

To obtain explicit formula for the time evolution of the density matrix elements, it is convenient to transform equation 2-38 to the interaction representation, in which fast oscillations produced by $H_0$ are suppressed. Thus, we define

$$ \tilde{\rho}(t) = e^{i\hat{H}_0 t} \rho(t) e^{-i\hat{H}_0 t} \tag{2-39} $$

so that

$$ \tilde{\rho}_{\alpha\alpha}(t) = e^{(E_\alpha - E_\beta)t} \rho_{\alpha\alpha}(t) \tag{2-40} $$

And we also define a new $H_1$ in the interaction representation,

$$ \tilde{H}_1(t) = e^{i\hat{H}_0 t} \hat{H}_1(t) e^{-i\hat{H}_0 t} \tag{2-41} $$

From equations 2-38 ~ 2-40 it follows that

$$ \frac{d\tilde{\rho}(t)}{dt} = i\left[\tilde{\rho}(t), \tilde{H}_1(t)\right] \tag{2-42} $$

Equation 2-41 can be formally integrated from 0 to a short time $t$,

$$ \tilde{\rho}(t) = \tilde{\rho}(0) + i\int_0^t [\tilde{\rho}(t'), \tilde{H}_1(t')] dt' \tag{2-43} $$

equations 2-42 and 2-43 can be combined in iteration scheme (known to mathematicians as a Bernoulli series)\textsuperscript{13}. For the Hamiltonians appearing in equations (2-38) and (2-41), it is sufficient to truncate the series at second order:
\[ \tilde{\rho}(t) = \tilde{\rho}(0) + i \int_0^t \left[ \tilde{\rho}(0), \tilde{H}_1(t') \right] dt' \\
- i \int_0^t dt' \int_0^t \left[ \tilde{\rho}(0), \tilde{H}_1(t''), \tilde{H}_1(t') \right] dt'' \] (2-44)

Then, calculating the derivative and defining a time, it follows that

\[ \frac{d\tilde{\rho}(t)}{dt} = i \left[ \tilde{\rho}(0), \tilde{H}_1(t) \right] - \int_0^t \left[ \tilde{\rho}(0), \tilde{H}_1(t + \tau) \right] \tilde{H}_1(t) d\tau \] (2-45)

The first term on the right in equation 2-45 can be set to zero by replacing \( H_1(t) \) by \( H' = H_1(t) - \langle H_1(t) \rangle \), which fluctuates about average value zero; this implies that including the average \( \langle H_1(t) \rangle \) has been added to the time independent Hamiltonian. Then expanding the second term in equation 2-45 and transcribing this operator expressing in the basis of \( H_0 - \langle H_1(t) \rangle \) it can be shown\(^{[14]}\)

\[ \frac{d\tilde{\rho}_{aa'}}{dt} = - \sum_{\beta, \beta'} \int_0^t \left( \langle \alpha | \tilde{H}_1(t) | \beta \rangle \langle \beta | \tilde{H}_1(t + \tau) | \beta' \rangle \langle \beta' | \tilde{H}_1(t) | \alpha' \rangle \right) \\
- \langle \alpha | \tilde{H}_1(t + \tau) | \beta \rangle \langle \beta | \tilde{\rho}(0) | \beta' \rangle \langle \beta' | \tilde{H}_1(t) | \alpha' \rangle \\
- \langle \alpha | \tilde{H}_1(t) | \beta \rangle \langle \beta | \tilde{\rho}(0) | \beta' \rangle \langle \beta' | \tilde{H}_1(t + \tau) | \alpha' \rangle \\
+ \langle \alpha | \tilde{H}_1(t) | \beta \rangle \langle \beta | \tilde{H}_1(t + \tau) | \beta' \rangle \langle \beta' | \tilde{\rho}(0) | \alpha' \rangle \right) dt' \] (2-46)

Now we make a crucial approximation for validity of Redfield theory, the random motions of the spin environment are characterized by a correlation time \( \tau_c \) which is short enough that

\[ \omega_{SB} \tau_c << 1 \] (2-47)

where \( \omega_{SB} \) is the root mean square fluctuation of the perturbation \( H_1 \). We focus on the evolution of the density operator in the time scale \( t \)

\[ \tau_c << t << \omega_{SB}^{-1} \] (2-48)

Based on these assumptions, we can make three modifications to equation (2-45):
A. We can replace $\tilde{\rho}(0)$ with $\tilde{\rho}(t)$ because of the small variation of $\rho(t)$ on the timescale in equation 2-48.

B. We neglect the possible correlation between $\rho(t)$ and $H_1(t)$ because of the separation of the timescale defined in equation 2-48.

C. We can extend the integral to infinity because of the short correlation time.

These assumptions lead to the operator equation

$$\frac{d\tilde{\rho}(t)}{dt} = \int_0^\infty \left[ \tilde{\rho}(t), \tilde{H}_1(t + \tau) \right] \tilde{H}_1(t) d\tau$$

(2-49)

Transcribing equation 2-49 in the basis of $H_0$, and after some tedious calculation, it can be shown that

$$\frac{d\tilde{\rho}_{\alpha\beta}}{dt} = \sum_{\alpha',\beta'} e^{i(\alpha - \alpha' - \beta + \beta')} R_{\alpha\beta,\alpha'\beta'} \tilde{\rho}_{\alpha'\beta'}(t)$$

(2-50)

the relaxation super matrix elements, $R_{\alpha\beta,\alpha'\beta'}$ are given by

$$R_{\alpha\beta,\alpha'\beta'} = \frac{1}{2} [ J_{\alpha\beta\alpha'\beta'} (\alpha - \beta') + J_{\alpha'\beta\beta'\alpha} (\alpha - \beta) ]$$

$$- \delta_{\alpha\beta} \sum_{\gamma} J_{\gamma\alpha\alpha'} (\gamma - \beta) - \delta_{\alpha'\beta'} \sum_{\gamma} J_{\gamma\beta\alpha'} (\gamma - \beta')$$

(2-51)

where

$$J_{\alpha\beta\alpha'\beta'} = 2 \int_0^\infty \langle \alpha | \hat{H}_1(t) | \alpha' \rangle \langle \beta' | \hat{H}_1(t + \tau) | \beta \rangle e^{-i\omega t} d\tau$$

(2-52)

is called the generalized spectral density. It should be noted that in equation 2-50, only terms for which $\alpha - \alpha' = \beta - \beta'$ need be kept because other terms will oscillate rapidly and hence average to zero. So equation 2-50 can be simplified to
\[
\frac{d\tilde{\rho}_{\alpha'\beta'}}{dt} = \sum_{\beta, \beta'} R_{\alpha\beta'\beta\beta'}(t)
\]

(2-53)

where the summation is now restricted to the terms that \(\alpha' = \beta = \beta'\).

2.2.3 Relaxation times \(T_{1z}\) and \(T_{1Q}\) for deuterons

For deuterons, \(\lambda = 1\) and the diagonal terms of equation 2-53 reduce to

\[
\dot{\rho}_{11} = R_{1111}[\rho_{11} - \rho_{11}(\infty)] + R_{1122}[\rho_{22} - \rho_{22}(\infty)] + R_{1133}[\rho_{33} - \rho_{33}(\infty)]
\]

\[
\dot{\rho}_{22} = R_{2211}[\rho_{11} - \rho_{11}(\infty)] + R_{2222}[\rho_{22} - \rho_{22}(\infty)] + R_{2233}[\rho_{33} - \rho_{33}(\infty)]
\]

\[
\dot{\rho}_{33} = R_{3311}[\rho_{11} - \rho_{11}(\infty)] + R_{3322}[\rho_{22} - \rho_{22}(\infty)] + R_{3333}[\rho_{33} - \rho_{33}(\infty)]
\]

(2-54)

Note that these expressions include the additional approximation that the relaxation super-operator acts not on \(\rho\), but rather on \(\rho - \rho(\infty)\) so that \(\rho(t)\) relaxes to thermal equilibrium as time goes to infinity.

From equation 2-52, the generalized spectral densities involve two factors: matrix elements of tensor spin operators \(A_{m}\) which are the same for all systems in the ensemble, and ensemble averages over the random lattice variables, correlated at two different times. Thus, we can write

\[
J_{\alpha\alpha'\beta'\beta'}(M\omega_0) = \sum_{M} \langle \alpha | A_{M} | - \alpha \rangle \langle \beta | A_{M} | - \beta \rangle (T_{0}^{(\Phi)})^2 J_{M}(M\omega_0)
\]

(2-55)

where

\[
J_{M}(M\omega_0) = (T_{0}^{(\Phi)})^{-2} \int_{0}^{\infty} T_{M}^{(l)}(t) T_{M}^{(l')\dagger}(t + \tau)e^{-i\omega_0\tau} d\tau
\]

(2-56)

With equations 2-23 and 2-25, the spectral densities can be calculated as
Zeeman order is defined as the ensemble average of $I_z$

$$\text{Tr}(\rho I_z) = \rho_{11} - \rho_{33}$$  \hspace{1cm} (2-58)

Quadrupole order is defined by the ensemble average of $Q_z = (3I_z^2 - I \cdot I) / 3$

$$\text{Tr}(\rho Q_z) = \rho_{11} - \rho_{22} + \rho_{33}$$  \hspace{1cm} (2-59)

From the spectral densities defined by equation 2-57, the relaxation times of Zeeman order and quadrupole order are\[^{[4]}\]

$$R_{1Z} = \frac{1}{T_{1Z}} = \frac{3\pi^2 C_q^2}{2} [J_1(\omega_b) + 4J_2(2\omega_b)]$$

$$R_{1Q} = \frac{1}{T_{1Q}} = \frac{9\pi^2 C_q^2}{2} J_1(\omega_b)$$  \hspace{1cm} (2-60)

$R_{1Z}$ and $R_{1Q}$ can be measured in experiments and $J_1$ and $J_2$ can thus be evaluated independently and compared results obtained by Fourier transforms of model-dependent time correlation functions.

### 2.2.4 Stochastic Liouville equation for deuterons

Redfield theory is limited according to equations 2-47 and 2-48 to motions with short correlation times. The more complicated Stochastic Liouville Equation (SLE)\[^{[15]}\]
discussed below is valid over wider motional time scales.

In a frame rotating at Larmor frequency, the evolution of the spin density operator for an ensemble of deuterons with identical quadrupole Hamiltonians $H_Q$ can be written

$$\frac{d\hat{\rho}(t)}{dt} = i\left[\hat{\rho}(t), \langle \hat{H}_Q \rangle \right] + i\left[\hat{\rho}(t), \hat{H}_1(t) \right]$$

(2-61)

Where $H_1(t) = H_Q - \langle H_Q \rangle$. The first term on the right of equation 2-61 gives rise to coherent precession but not relaxation, which arises only from the second term.

We can rewrite equation 2-61 in matrix form and rearrange the matrix elements so that

$$\rho = \begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix}$$

(2-62)

where

$$P = \begin{pmatrix} \rho_{11} - \rho_{11}(\infty) \\ \rho_{22} - \rho_{22}(\infty) \\ \rho_{33} - \rho_{33}(\infty) \end{pmatrix}, \quad M_+ = \begin{pmatrix} \rho_{12} \\ \rho_{23} \\ \rho_{13} \end{pmatrix}, \quad M_- = \begin{pmatrix} \rho_{21} \\ \rho_{32} \\ \rho_{31} \end{pmatrix}.$$ 

Then equation 2-61 becomes

$$\frac{d}{dt} \begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix} = \begin{pmatrix} R_1 & 0 & 0 \\ 0 & i\omega - R_2 & 0 \\ 0 & 0 & -i\omega - R_2 \end{pmatrix} \begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix}$$

(2-63)

Where

$$\omega = \begin{pmatrix} \omega_0 & 0 & 0 \\ 0 & \omega_0 & 0 \\ 0 & 0 & 2\omega_0 \end{pmatrix}, \quad R_1 = \begin{pmatrix} R_{1111} & R_{1122} & R_{1133} \\ R_{2211} & R_{2222} & R_{2233} \\ R_{3311} & R_{3322} & R_{3333} \end{pmatrix}, \quad R_2 = \begin{pmatrix} R_{1212} & R_{1223} & 0 \\ R_{2312} & R_{2323} & 0 \\ 0 & 0 & R_{3313} \end{pmatrix}$$

In highly constrained environments such as molecule crystals, motions can be modeled by instantaneous jumps among different orientational "sites". These
Markovian jumps can be included in the time evolution of the spin density matrix, which now must be extended to separate 3x3 matrices, one for each site. Thus for N different sites, if $k_{ij}$ represents the jump rate between sites $i$ and $j$. The dimension of equation 2-63 will become $9N^4$.

$$\frac{d}{dt} \begin{pmatrix} \rho^{(1)} \\ \rho^{(2)} \\ \vdots \\ \rho^{(N)} \end{pmatrix} = \begin{pmatrix} i\omega_1 + R_1 + k_{11} & k_{12} & \cdots & k_{1N} \\ k_{21} & i\omega_2 + R_2 + k_{22} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ k_{N1} & k_{N2} & \cdots & i\omega_N + R_N + k_{NN} \end{pmatrix}$$ (2-64)

The principle of detailed balance is observed,

$$k_{ii} = \sum_{i \neq j}^N k_{ji}$$ (2-65)

Solving the Stochastic Liouville Equation is very complicated and time consuming, the program EXPRESS\textsuperscript{16} developed by Dr. R. L. Vold deals with this task nicely. In the following chapters, EXPRESS will be utilized to model and solve the deuteron relaxation problem.
Chapter 3 Experimental methods

3.1 Equipment overview

Most of the experiments performed in this thesis were performed on a Bruker WB 750 magnet, which is operating consistently at 17.6T (proton resonance frequency at 750MHz) with a 89mm clear bore inside the room temperature shim coil assembly, and controlled by a Bruker AVANCE spectrometer system. The magnetic field is generated by a superconducting coil. To maintain such a high magnetic field, the superconducting coil is immersed in a liquid helium dewar which is pumped on by a vacuum pump all the time to keep the coil temperature below 4.2K, the boiling point of helium. The helium dewar is surrounded by a liquid nitrogen dewar, for better isolation, there is a vacuum chamber between the helium and nitrogen dewar. Besides the main static magnet, there are some lower power room temperature shim coils that can be used to eliminate or reduce the inhomogeneity of the magnetic field around the sample.

There are two probes used in the 17.6 T field, a 2.5 mm MAS probe and a 5 mm static probe. All experiments can be performed as a function of temperature, which is varied by a stream of dry nitrogen gas heated under control of a digital PID controller supplied by Bruker Biospin, Inc.. Temperature calibrations were performed using lead nitrate and D$_2$O with details in Appendix A.

The probes which hold the sample are inserted into the bore of the magnet from the
bottom of the spectrometer, a stack which matched the probe is then inserted into the bore from the top to maintain proper gas flow and to protect the magnet bore and shim coil assembly from exposure to temperature extremes. Higher pressure nitrogen supply lines, used to control air bearing and spin rate drive by means of a Bruker spin rate controller for MAS experiments, are is routed through the top stack. A single solenoid coil in the probe is used both to provide the rf pulses and collect the signal generated by the nuclear spins.

In the static probe, the coil is oriented perpendicular to the static field and can be changed depending on the range of the Larmor frequency of the observed nucleus. The induced NMR signal passes through a frequency filter to reduce noise and is finally amplified and digitized so that it can processed by the computer. Before a new experiment, the probe needs to be tuned by adjusting built-in capacitors and observing the wobble curve (power reflected back from the coil as a function of the frequency of a low level, CW signal). By adjusting the tuning capacitor, the position of minimum reflected power can be set Figure 3.1 to the desired Larmor frequency and by adjusting the matching capacitor, the reflected power at the tuning point can be minimized (so that the impedance of the tuned probe matches the transmitter output impedance).

For the MAS probe, the spinning of the rotor is controlled by both the driving and bearing gas, the bearing gas is blown towards the rotor from the side of the stator to stabilize it and the drive gas is blown along the fluted top cap of the rotor to spin it. Part of the bottom cap edge of the rotor is marked for an optical sensor to monitor the spin rate so that the drive and bearing gas can be adjusted to reach the target spin rate.
An illustration of the bearing and driving gas can be found in Chapter 6, Figure 6.1 where a model of frictional heating is developed.

Other experiments in this chapter are performed on an OXFORD 7.06T (300MHz) Bruker AVANCE spectrometer, whose circuitry differs from that of the high field spectrometer mostly in the use of intermediate frequencies designed to generate properly heterodyned frequencies corresponding to the approximately 2.5 times lower Larmor frequencies. These experiments are mainly used for comparison with results from the 17.6T spectrometer.

![Figure 3.1: An optimized probe tuning "wobble" curve for the static 5mm probe. This is a display of reflected power (vertical axis) against the frequency of a low-level signal applied continuously from the transmitter. The position of the sharp dip defines the tuning point (adjusted here to the deuteron Larmor frequency 115.17 MHz for a 17.6 T magnetic field) and when the probe tuning and matching capacitors are properly adjusted for minimum reflected power, the probe is accurately matched to the 50 ohm output impedance of the transmitter. This ensures maximum B1 magnetic field applied to the sample during an rf pulse.](image-url)
3.2 Pulse sequences

In addition to the static magnetic field, a radiofrequency (rf) oscillating magnetic field is applied along a direction perpendicular to the static field. This field, obtained from a gated transmitter amplifier signal, generates pulses whose duration, amplitude, and relative phase (defined in reference an arbitrary, user selectable zero) can be used to manipulate the evolution of the density matrix to realize different experiment goals. In this section, several common pulse sequences used in this thesis are briefly described.

3.2.1 Zero-go pulse sequence

The ZG (zero-go) sequence is the most basic and one of the most commonly used pulse sequences (Figure 3.2(A)). It consists of a recycle delay used to ensure an equilibrium state before the pulse, a single $90^\circ_x$ pulse which can rotate the magnetization from the Z-axis to the y-axis of a frame rotating about the lab-fixed Z-axis at the carrier frequency, a short delay to allow the probe and receiver amplifiers to recover from pulse overload, and a user-selectable interval for accumulating the free induction decay (FID) signal detected by the receiver.

The pulse flip angle, which needs to be optimized before the experiment, is defined as

$$\theta_{rf} = \omega_1 \tau_1 = \gamma B_1 \tau_1 \quad (3-1)$$

$B_1$ is the pulse field, for a given power level, pulse length $\tau_1$ is adjusted to make $\theta_{rf} = \pi/2$. To optimize the $90^\circ_x$ pulse, a sequence of zg experiments with different pulse
Figure 3.1: Pulse sequences used for observing solid state line shapes. A) The basic zero-go (ZG) or one-pulse sequence, in which signal following a single pulse is accumulated as often as needed to achieve adequate signal to noise ratio. This averaged signal is converted to a frequency domain spectrum by subsequent Fourier transformation. B) The two-pulse quadrupole echo sequence, used to accumulate fast decays (and hence broad lines) without loss of signal and line shape distortion arising from pulse overload immediately following the intense rf pulses. C) Behavior of transverse magnetizations during the quadrupole echo sequences, illustrating the formation of a quadrupole echo as described in the text.
length are performed on liquid D$_2$O, and the pulse length with the strongest FID signal is identified as the optimal $\tau_1$.

The delay $D_1$ before the pulse is used to prepare an equilibrium $z$-axis magnetization, $D_1$ needs to be selected in order that the $z$-axis magnetization is fully recovered before the pulse and the total experiment time is minimized. $D_1$ should therefore be set to $3\sim 5$ times $T_{1z}$ so that the $z$ magnetization can return to more than 95% of the infinity value\textsuperscript{[19]}. For liquid D$_2$O $D_1$ is relatively long (1-2 seconds is sufficient) but for solid samples, where deuteron $T_{1z}$ can vary from as short as a few milliseconds to over an hour for very rigid materials, a common source of apparent instrument failure is operator impatience with the necessity of long $D_1$ values.

In practice, instrumental imperfections such as DC offsets in the digitizers and audio amplifiers, imperfect pulse amplitudes and phase transients at the start and end of nominally square pulses and, for deuterons, the use of oscillating magnetic fields that are not strong enough to fully suppress effects of the quadrupole Hamiltonian during the pulse can lead to pernicious spectral artifacts. An extensive literature\textsuperscript{[19],[22]} is devoted to implementing phase cycled pulse sequences designed to minimize these artifacts. In a phase cycled pulse sequence, the total number of acquisitions is an integral multiple of a small number (typically between 2 and 64) of individual sub-sequences that may include variation of the receiver reference phase and re-routing of digitized signals between real and imaginary channels of the phase detector, as well as relative phases of different pulses in a multi-pulse sequence. A properly designed phase cycle will result in cancellation of undesired coherence transfer artifacts, while preserving coherent addition of the desired signal from scan to
scan. For the zg experiment, the most common phase cycle sequence is listed in (Table 3.1):

<table>
<thead>
<tr>
<th>90°x</th>
<th>X -X -X X Y -Y -Y Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACQ</td>
<td>X -X -X X Y -Y -Y Y</td>
</tr>
</tbody>
</table>

It can be shown\cite{22} that this "Cyclops" phase cycle removes artifacts due to audio amplifier imbalance between the real and imaginary channels and also DC offsets arising from the digitizers.

3.2.2 Quadrupole echo experiment

In order to achieve uniform excitation of deuteron whose full width powder pattern is on the order of 300 kHz, a high pulse power is needed to create a short 90° pulse, but in a typical 5 mm static probe, it takes about 15-20 μs for a rf coil to recover after the high power pulse which is the dead time for the coil. We cannot record the FID immediately after the pulse, so the information in the beginning of the FID signal is lost. Fortunately, the quadruple echo pulse sequence\cite{20,21} (Figure 3.2(B)) has been developed to overcome this problem.

The idea of the pulse sequence is to add another 90° pulse with π/2 phase shift after the first pulse with interval τ₁. The detection starts at time τ₂ after the second pulse and τ₂ = τ₁, the second pulse will make an echo and refocus to a maximal after τ₂.

An illustration of the evolution of transverse magnetization in a quadrupole experiment is shown in Figure 3.2(C). The transverse component of the net spin will
start to dephase after the first pulse, we can view this as two magnetizations precessing with different directions, the second pulse will reverse the direction of the precessing magnetization, so after the same interval, the two magnetizations will refocus to form a maximal of the signal.

There are two potentially serious source of spectral distortions in the quadrupole echo sequence\textsuperscript{[5]}, the first one arises from the assumption of a \( \delta \)-function rf pulses. While the phase cycle described below can suppress phase distortions arising from pulses of finite power and duration, it does not suppress effects of quadrupole-induced precession during the pulses, which result in loss of signal in the outer "shoulders" of the powder pattern\textsuperscript{[45]}. Fortunately, the quadrupole coupling for rapidly rotating methyl deuterons is sufficiently small that this distortion is not large, so it is not necessary to resort to elaborate, exact simulations\textsuperscript{[45]}.

The other source of spectral distortion arises from different chemical shifts of magnetically non-equivalent deuterons. The refocusing pulse (which must be applied exactly on resonance) cannot simultaneously refocus the different phase lost from different chemical shift Hamiltonian. This distortion can be avoided, if necessary, by inserting 180° pulses in the sequence at appropriate times.

The phase cycle sequence for the basic quadrupole echo is listed in Table 3.2.

| 90°\(_x\) | X -Y -X Y X -Y -X Y |
| 90°\(_y\) | -Y -X Y X Y X -Y -X |
| ACQ | -Y -X Y X -Y -X Y X |
3.2.3 Saturation recovery with quadrupole detection

A saturation-recovery pulse sequence\cite{22} can be used to measure Zeeman order spin-lattice relaxation times, $T_{1Z}$, of anisotropically relaxing deuteron powder patterns. The pulse sequence (Figure 3.3(A)) first applies a 90° pulse to the equilibrium sample to rotate the longitudinal magnetization to transverse magnetization, and after a variable delay to let allow z-magnetization to build up, a quadrupole echo pulse sequence is appended to sample the recovered z-magnetization. The evolution of the longitudinal magnetization at each point on the powder pattern is given by

$$M_z(t) = M_\infty (1 - e^{-t/T_{1Z}})$$

(3-2)

$M_\infty$ is the longitudinal magnetization at equilibrium, where now $T_{1Z}$ is a function of the crystallite orientations and hence the position on the powder pattern where the Fourier transformed signal is sampled. A typical recovery curve is shown in Figure 3.3(C). The pulse sequence needs to be optimized so that after the first 90° pulse, the longitudinal magnetization should be close to zero, in practice, to account for imperfect initial saturation, we add one more parameter, the initial z-magnetization $M(0)$, to equation (3-2)

$$M_z(t) = M(0) + [M_\infty - M_z(0)](1 - e^{-t/T_{1Z}})$$

(3-3)

With this three parameter fit we obtain far more accurate $T_{1Z}$ values.

The various delays need to be chosen carefully to yield $T_{1Z}$ values of good quality. We first get an estimate of the $T_{1Z}$ value and select the delay series to distribute evenly along the recovery curve, about 10 different delays spanning at least the first decade.
of recovery is good enough. During the somewhat lengthy experiments, it is better to randomize the order of the delays to minimize the cumulative effects of slow changes in pulse amplitudes and other instrumental conditions.

A good phase cycle for saturation recovery consists of 32 steps, only the first 8 steps is shown in Table 3.3. The initial pulse phase need to be increased by $\pi/2$ every 8 steps to get the remaining sub-sequences. It can be shown that this phase cycle prevents double quantum coherence generated by the first two pulses from back transfer by the third pulse to one quantum coherence, which would otherwise lead to oscillatory recovery for shorter recovery delays.

Table 3.3 Phase cycle for saturation recovery with QE detection

<table>
<thead>
<tr>
<th></th>
<th>X X X X X X X X X</th>
</tr>
</thead>
<tbody>
<tr>
<td>$90_x$</td>
<td>X -Y -X Y X -Y -X Y</td>
</tr>
<tr>
<td>$90_y$</td>
<td>-Y -X Y X Y X -Y -X</td>
</tr>
<tr>
<td>ACQ</td>
<td>-Y -X Y X -Y -X Y X</td>
</tr>
</tbody>
</table>

Inversion recovery (Figure 3.3(B)) is another widely used pulse sequence to measure $T_{1Z}$ relaxation times. The only difference is that the initial $90^o$ saturating pulse is replaced by a $180^o$ pulse. The evolution of the transverse magnetization is given by

$$M(t) = M_\infty (1 - 2e^{-t/T_{1z}})$$

(3-4)

And the recovery curve is shown in (Figure 3.3(D)). The phase cycling steps are the same with saturation recovery pulse sequence.
Figure 3.3: Pulse sequences used to measure Zeeman relaxation times $T_{1}\text{Z}$. A) Saturation recovery (SRQE), in which a short train of $90^\circ$ pulses is used to establish an initial state of complete saturation, followed by a variable delay period $t$ evolution to $Z$-magnetization towards equilibrium, and a quadrupole echo pulse pair to monitor the recovery at each point on the line shape. B) Inversion recovery (IRQE), in which the initial state (population) is accomplished with a single $180^\circ$ pulse. The rest of this sequence is the same as for saturation recovery. In principle, the IRQE experiment gives superior signal to noise ratio compared with SRQE because as shown in C) and D) the dynamic range of recovery is twice as great (from $-M_{eq}$ to $+M_{eq}$) for IRQE. However, since the initial state for SRQE is zero, while that for IRQE involves the equilibrium magnetization, SRQE can be repeated without waiting for full recovery and is therefore far more efficient when many scans must be repeated to achieve adequate sensitivity.
3.2.4 Conventional and broadband Jeener-Broekaert pulse sequence

The Jeener-Broekaert\textsuperscript{[23, 24]} pulse sequence is widely used in the measurement of the deuteron quadrupole order spin-lattice relaxation time $T_{1Q}$. The spectral density $J_1(\omega)$ can be determined directly by measuring $T_{1Q}$. The conventional JB sequence is shown in Figure 3.4(A), which includes an initial 90° pulse, an excitation time $\tau$, a 45° pulse, a relaxation delay list and another 45° pulse for detection. After the initial 90° pulse, the longitudinal magnetization will be rotated to the horizontal plane, after the first 45° pulse, both quadrupole order and double quantum coherence are created, during the delay time, the spin system start going back to equilibrium, normally the double quantum terms will be suppressed by phase cycling (Table 3.4). The final 45° pulse will convert both the Zeeman and quadrupole order to observable single quantum coherence.

The conventional JB pulse sequence is frequency selective. In single crystal or mono domain liquid crystals, to maximize the creation of the quadrupole order, the excitation time $\tau$ can be adjusted so that

$$\omega_0 \tau = (n + \frac{1}{2})\pi$$

(3-5)

Table 3.4 phase cycling sequence for JB experiment

\begin{center}
\begin{tabular}{|c|c|}
\hline
Angle & Pulse Sequence \\
\hline
90° & X X Y Y -X -X -Y -Y \\
\hline
45° & -Y -Y X X Y Y -X -X \\
\hline
45° & X -Y -X Y X Y -X Y \\
\hline
ACQ & X -Y -X Y X -Y -X Y \\
\hline
\end{tabular}
\end{center}
Figure 3.4: JB and BBJB pulse sequences used to measure relaxation times $T_{1Q}$ of quadrupole order. (A) Conventional Jeener-Broekaert (JB) sequence, where a single 45° pulse is applied a non-selective 90° pulse, with delay time $\tau$ to optimize quadrupole order for one specific quadrupole doublet splitting. (B) Broadband excitation of quadrupole order is achieved by replacing the 45° excitation pulse with three pulses as shown. In both sequences, quadrupole order is allowed to decay towards zero (equilibrium) for time $T$, and monitored either by a single 45° pulse or better (as in BBJB) by an echo sequence similar to the quadrupole echo sequence but with modified phases chosen as shown to suppress residual Zeeman order. (C) Exact solution of the density matrix equations in absence of relaxation, equations 2-38 – 2-42, showing how the extra pulses of the BBJB sequence (dotted line) improves the uniformity of the excitation profile.
For powdered samples, it is impossible to create uniform excitation because of the continuous distribution of quadrupole splitting instead of quadrupole doublet in single crystals. Broadband Jeener-Broekaert (BBJB) pulse sequence was developed to overcome this problem[25]. The BBJB sequence provides a more uniform excitation on a much wider range than the conventional JB sequence (Figure 3.4 (C)) so that $T_{1Q}$ anisotropy of a powdered sample can be measured accurately.

Figure 3.4 (B) is the BBJB sequence with echo detection. The first four pulses create quadrupole order uniformly across the spectrum. During the variable delay time, quadrupole order decays exponentially, then the last two pulses convert the remaining quadrupolar coherence to observable single quantum coherence and refocus the FID to avoid the dead time signal loss.

### 3.3 Magic angle spinning

Magic angle spinning[26] is widely used in solid state NMR. It can greatly improve the resolution and signal to noise ratio by "concentrating" spectral intensity spread across the powder pattern into a limited set of narrow, well resolved spinning sidebands whose total integrated intensity is the same as that of the powder pattern. The effects of chemical shift anisotropy, dipolar coupling are usually not observed in solution NMR experiments because of the fast tumbling of the molecule in solution. The time scale of the motion of the molecule is much shorter than that of these interactions so that the orientation dependence of the relevant interaction tensor(s) is averaged to zero. Unlike the rapid random molecular tumbling in a liquid, magic angle spinning produces coherent time dependence of the orientation dependent parts of the spin Hamiltonians.
Figure 3.5: Illustration of the Magic Angle Spinning (MAS) experiment. The angle between the rotor axis and the magnetic field, $\theta_R$, is set to the "magic" value of $54.7^\circ$. For reasons described in the text, this yields spectra that consist of a center band flanked by side bands at multiples of the spin rate, with line widths largely free of broadening due to different crystallite orientations.

Figure 3.5 illustrates the experiment setup of a MAS experiment. The sample is packed in a rotor that is to spin about an axis oriented at the magic angle ($\theta_R=54.7^\circ$) with respect to the external static magnetic field. The orientation dependence (for all second rank tensor interactions) includes terms proportional to $3\cos^2\theta-1$, where for each crystallite in a polycrystalline sample, $\theta$ is the angle between the PAS $z$-axis and the external magnetic field. When the sample is spun fast enough compared with the anisotropy of the interaction, the orientation dependence of the interaction tensors such as chemical shift anisotropy can be averaged to zero: it follows from the transformation rules for second rank tensors that

$$\left<3\cos^2\theta-1\right>=\frac{1}{2}(3\cos^2\theta_R-1)(3\cos^2\beta-1)=0 \quad (3-6)$$
Here, the angular brackets indicate an average over one complete rotor cycle and for each crystallite, $\beta$ is the angle between the principal z-axis of the relevant interaction tensor, fixed in the crystallite, and the z-axis of the spinning rotor as shown in Figure 3.5. Additional time dependent terms in the spin Hamiltonian, corresponding to rotational averages of spherical harmonics with $m \neq 0$, do not average to zero and lead to modulation sidebands at multiples of the spin rate whose intensity decreases with increasing spin rate$^{[27]}$.

The top of the center band and the sideband envelope trace out the discrete powder pattern whose line shape can provide accurate values tensor components as well as motional rate on the time scale of the spin rate.

3.4 Basic signal processing procedure

In general, a free induction decay (FID) signal will be recorded after some particular pulse sequence; both real and imaginary components will be retained as separate arrays in computer memory. The FID is initially recorded in the time domain. In order to analyze the data in the frequency domain, a discrete Fourier transform of the complex-valued FID is performed on the FID signal, according to the definition$^{[28]}$

$$f_n = \frac{1}{\sqrt{N}} \sum_{k=0}^{N-1} d_k e^{-2\pi i k n / N} \quad (3-7)$$

where $d_k$ is the k-th time domain point and $f_n$ is the n-th frequency domain point. Before Fourier transformation, it is convenient to perform a left shift that drops the first few points (which may be subject to pulse overload) and perhaps to replace the entire $d_k$ array with spline-interpolated points that place the top of a quadrupole echo precisely at this first point of the array (fractional left shift). Also prior to Fourier
transformation, it is customary to apply a decaying, multiplicative function (apodization) designed to reduce or eliminate high frequency noise without producing excessive additional line broadening. For example, by using an exponential decay, the beginning part of the FID where the signal is large compared to the noise is more weighted so that the overall S/N is improved. But the improvement of the S/N is incurs the cost of the broadening of the peaks, so there is a trade off between sensitivity and resolution when selecting the appropriate apodization method. Exponential and Gaussian apodization are two widely used methods. The exponential apodization function is given by

\[ a_k = e^{-\pi W k \Delta t} \]  

(3-8)

Where \( W \) is the broadening constant in Hertz. This function preserves the Lorentzian nature of lines, a Lorentzian line with width \( L \) will have width \( L+W \) after exponential apodization. The Gaussian apodization is similar to exponential apodization but with \( a_k \) given by

\[ a_k = e^{-W (k \Delta t)^2} \]  

(3-9)

The exponential decay for Gaussian depends on the square of the time so it decays faster than the exponential apodization. Real line shapes are often intermediate between Lorentzian and Gaussian and we need to experiment to find the most pleasing apodization function. More fundamentally, Gaussian apodization is often a good approximate model for small, unresolved dipolar coupling while exponential apodization (Lorentzian line broadening) is occasionally a useful model for residual transverse relaxation due to a variety of processes not otherwise accounted for.

Finally, still before Fourier transformation, we may add a series of zeros to the end of
the data (zero-filling), that is equivalent to smoothing the frequency domain spectrum by interpolating points, we can (optionally) adjust the overall phase of the frequency domain spectrum by judicious linear combinations of the real and imaginary components of the time domain signal, and we divide the first point of the shifted, apodized time domain signal by 2 because the discrete Fourier transform starts discontinuously from a non-zero value. This eliminates an annoying DC offset from the frequency domain spectrum.

We rarely get a pure absorption line shape by the manipulations described above: since shifting the origin in the time domain introduces a proportional linear phase shift in the frequency domain, it is often necessary to "undo" the effects of such a shift by applying a linear phase shift after the Fourier transformation. In practice, we first select a "pivot point" $p$ which is phased by only by a constant (zero order) phase correction and then apply this plus a linear correction is applied to all the other frequency domain points. Thus the overall phase correction is given by

$$f'_n = e^{i(\phi_0 + (n-j)\phi_1/N)} f_n$$

(3.10)

Where $\phi_0$ is the zero order phase correction and $\phi_1$ is the first order phase correction.

All of these processing procedures are included in a MATLAB program NMRLV developed in the Vold/Hoatson laboratory as a more flexible (and cheaper) alternative to powerful data processing software available from Bruker Biospin, Inc.
Chapter 4  Study of FMOC-Alanine-d3 under static conditions

4.1 Overview of the sample and experiments

The notion of designing and validating motional models for FMOC-alanine-d3 and similar materials was inspired by experimental measurements and associated simulations of solid state NMR spectra of deuteron labeled side chains in the hydrophobic core of chicken villin headpiece protein HP36[18, 37]. It is widely recognized that constraints on protein side chain motion imposed by the folded polypeptide backbone, as well as by interactions with other side chains, play a major role in protein structures, folding pathways and biological function[2, 18]. Recent research results[18] for HP-36 suggest that line shape and relaxation time studies of selectively labeled methyl groups in protein side chains can be used to quantify the side chain spectrum of protein side chain molecular motion in highly constrained environments.

However, since quantitative motional parameters such as jump rates and activation energies are necessarily dependent on the details of the heuristic models used to fit the data, it is prudent to validate the models by applying them to simpler systems which nevertheless mimic the more complex environment of a folded protein side chain.

FMOC-alanine-d3 was chosen as the model sample in this chapter. Alanine is the
simplest naturally occurring amino acid that has a "side chain" consisting of a simple methyl group. By attaching the bulky protecting group 9-fluorenylmethoxycarbonyl (FMOC) to the amide nitrogen, the resulting crystalline substance can be regarded as consisting of a hydrogen bonded chain, whose configurations in the crystal are constrained by packing constraints associated with the aromatic ring plane of the FMOC moiety (see Figure 4.1). Presumably, competition between these stacking interactions and the hydrogen bond interactions is responsible for the fact that there are apparently no reports in the literature of single crystal X-ray structures for any of the FMOC-amino acid derivatives, despite their widespread use in automated solid state peptide synthesis\cite{46}. It should be possible to account very accurately for the motion of the \(-\text{CD}_3\) "side chain" in FMOC-alanine-d3 simply by random jumps among about the three equilibrium orientations of a methyl C-D bond, and to quantitatively investigate possible complications that could arise, for example, by slow, restricted libration of the RC-\(\text{CD}_3\) bond that attaches the methyl group to the rest of the molecule.

Polycrystalline FMOC-L-alanine-d3 used in our experiments was purchased from Sigma-Aldrich and used without purification. Experiments were performed on both of the 17.6 T and 7.06 T spectrometers. T\(_{1Z}\) experiments were carried out with a quadrupole echo detection pulse. The delay between the two 90\(^{\circ}\) pulses (calibrated using liquid D\(_2\)O) was 60 \(\mu\)s and the 90\(^{\circ}\) pulse was 2.1 \(\mu\)s at -3 dB power for the 17.6 T spectrometer and 2.2 \(\mu\)s at 0 dB power for the 7.06T spectrometer. Data acquisition was initiated before the echo maximum, and the starting time of acquisition was adjusted to place a data point exactly at the echo maximum. The number of scans was
set to 1024 for the high field measurements and 2048 for the low field measurements. The spectral window was set to 200 kHz. In order to provide enough time for the spins to return equilibrium, the recycle delay was 250 millisecond, which is at least 3 times longer than the longest $T_{1Z}$. $T_{1Z}$ data was measured using the BJB pulse sequence with quadrupole echo detection. The excitation time $\tau$ was 5 $\mu$s, which was found to provide optimally flat excitation over the methyl deuteron spectrum. The number of scans was 2048 for both the high and low field spectrometers and the recycle delay was set to 400 millisecond. The temperature range for the high field measurements is from 180 K to 340 K, and for the low field measurements it is 230
K-330 K. Temperature calibration was done by using measured $^{207}$Pb static line shapes of lead nitrate as described in Appendix A to determine a linear relation between controller set point temperature and the true temperature inside the sample tube.

Relaxation times were obtained by fitting the signal intensity as a function of the variable relaxation delay to mono-exponential saturation recovery curves (for $T_{1Z}$) and mono-exponential decays (for $T_{1Q}$).

4.2 Qualitative discussion of high field results

4.2.1 Line shape analysis

The temperature dependence of quadrupole echo line shapes is shown in Figure 4.2. The quadrupole coupling constant ($C_Q$) is determined by measuring the horn splitting of the spectrum and the results are shown in Table 4.1.

For deuterons in a rigid C-D bond, expected value of the quadrupole coupling constant is on the order of 160-170 kHz, with zero asymmetry parameter$^{47}$. Thus the fact that $C_Q$ of the methyl deuterons in L-alanine-$d_3$ is only 55 kHz (with zero asymmetry parameter) at room temperature$^9$ is entirely consistent with a low temperature (rigid lattice value) of 167 kHz$^9$, reduced by the factor

$$\frac{1}{2} (3\cos^2 \beta - 1) = 1/3$$

where $\beta$ is the tetrahedral angle made by each C-D bond with the
Table 4.1 Quadrupole coupling constant at different temperatures.

<table>
<thead>
<tr>
<th>T_{se}/K</th>
<th>T_{true}/K</th>
<th>Left hom (kHz)</th>
<th>Right hom (kHz)</th>
<th>C(_{Q}) (kHz)</th>
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<td>176.80</td>
<td>19.51</td>
<td>-18.77</td>
<td>51.04</td>
</tr>
</tbody>
</table>

C-C methyl spinning axis. At first glance, the slightly smaller values observed for FMOC-alanine-d\(_3\) suggest a deviation from the tetrahedral symmetry of the methyl group due to the effect of the bulky FMOC group. However, this explanation requires relatively large distortions from tetrahedral geometry (C-C-D bond angle between 111° and 111.7° depending on temperature) and more importantly, fails to account for the observation of a small but finite asymmetry parameter as discussed below in section 4.3.2.
Figure 4.2: Experimental temperature dependent quadrupole echo spectra of FMOC-alanine -d$_3$. The vertical dotted lines emphasize the very small decrease of hom splitting with increasing temperature.
4.2.2 Relaxation data anisotropy profile

The spin lattice relaxation times of Zeeman order were measured by saturation recovery with quadrupole echo detection after a variable recovery delay. Figure 4.3 shows partially relaxed spectra recorded at 280.2 K. Relaxation times were obtained by fitting the intensity of a user selected set of points distributed over the line shape using NMRLV software. The software automatically constructs recovery curves for the sum magnetization (i.e., user-selected pairs of points placed symmetrically about the center of the powder pattern) and fits the results as a function of the variable delay t, to a single exponential:

\[ M_z(t) = M(0) + [M_\infty - M_z(0)](1 - e^{-t/T_z}) \]  \hspace{1cm} (4-1)

In order to minimize the effect of imperfect initial saturation, the starting point of the signal was a parameter included in the fit instead of being set to zero. We note in passing that equation 4-1 is not applicable to individual points on the line shape, which relax as the sum of two different exponentials. Figure 4.4 is a semi log plot of normalized recovery curve of three representative points which were selected from the center, horn and shoulder of the quadrupole echo line shape at 280.2 K. The overall R\textsubscript{1z} anisotropy profile is shown in Figure 4.5. Anisotropy (orientation dependence) of R\textsubscript{1z} occurs because the observed spectrum is the sum of independent doublet spectra, whose peak separation as well as relaxation times depend on the specific crystallite orientation with respect to the static magnetic field \cite{30}. The mono-exponential fitting procedure used by NMRLV ignores non-exponentiality introduced by overlap of doublet components with different relaxation times, and
Figure 4.3 Partially relaxed spectra of Zeeman order for FMOC-alanine-d₃ at 280.2K as a function of relaxation delay. NMRLV software can be used to automatically extract values for the sum of symmetrically chosen points with respect to the spectrum centers, whose recovery curves yield values for the Zeeman relaxation time $T_{1Z}$ at each point on the line shape. It is evident from these data that $T_{1Z}$ is anisotropic (i.e., dependent on crystallite orientation as specified by position on the line shape): the shoulders clearly recover more quickly than the horns. Typical results of this procedure are shown in Figure 4.4
Figure 4.4: Semilog plot of normalized magnetization recovery of Zeeman order as a function of relaxation delay at 280.2 K. For the center point, $T_{1Z} = 29.2 \pm 0.3$ ms, for the horn point, $T_{1Z} = 33.3 \pm 0.2$ ms, and for the shoulder point, $T_{1Z} = 21.2 \pm 1.3$ ms. Uncertainties reported here are 95% confidence levels (two standard deviations) returned from NMRLV software, which uses the base line noise in the spectrum to estimate the uncertainty of each point on the recovery curve and minimizes chi-squared for a three parameter fit in which $M(0)$, $M(\infty)$ and $R_{1Z} = 1/T_{1Z}$ are fit to equation 4-1.
Figure 4.5: Zeeman relaxation rate $R_{1z} = 1/T_{1z}$ anisotropy profile for FMOC-alanine-d$_3$ at $T=280.2$K. The relaxation is slower (smaller rate, longer relaxation time) at the horns and faster at the shoulders and center.
Figure 4.6: Partially relaxed spectra of quadrupole order for FMOC-alanine-d3 at 280.2K as a function of relaxation delay. NMRLV software can automatically extract the difference between signal intensities of points on opposite sides of the spectrum center, which is proportional to quadrupole order remaining at time t, and fit the results to mono-exponential functions to determine the orientation dependence of $R_{1Q} = 1/T_{1Q}$. A typical example of this procedure is shown in Figure 4.7.
Figure 4.7: Semi log plot of normalized magnetization recovery of quadrupole order as a function of relaxation delay. For the horn position, $T_{1Q} = 22.7 \pm 0.3$ ms. Note that for long delays, there appears to be deviation from single exponential decay. However, this occurs only after the residual signal has decayed to ~ 1-2% of its initial value, and may simply be a consequence of a small but systematic mis-estimate of the spectrum base line from which signal intensities are measured.
Figure 4.8: Quadrupole order relaxation rate $R_{1Q}$ profile for FMOC-alanine-d$_3$ at $T = 280.2$K. In contrast to $R_{1z}$ (Figure 4.5) the relaxation rate is faster at the horns and slower at the shoulders.
tends to provide an effective average relaxation rate $<1/T_{1Z}>$ that corresponds to the initial slope of the recovery curves, averaged over the (very) limited set of crystallite orientations that contribute to each line shape point. For this reason, it is important when analyzing the relaxation behavior to simulate complete, partially relaxed line shapes for precisely the same set of relaxation delays used in the experiments, and then fit them using the same algorithms used to extract relaxation rate from the experimental data.

Quadrupole order relaxation times were measured using the BBJB sequence with quadrupole echo detection. A set of partially relaxed spectra at 280.2 K is shown in Figure 4.6. The relaxation time $T_{1Q}$ was obtained by fitting equation 4-2,

$$m(t) = m(0)e^{-t/T_{1Q}}$$

(4-2)

where $m(t)$ is the difference between the two magnetization intensities selected from pairs symmetrically placed points about the center of the spectrum. $m(0)$ was treated as a variable parameter in the fit to minimize errors associated with non ideal initial excitation. A representative recovery curve (for the horn position) is shown in Figure 4.7 and the overall $R_{1Q}$ profile is shown in Figure 4.8. The $R_{1Q}$ profile shows an orientation dependence different from that for $R_{1Z}$.

As shown in Section 2.2.3, The relaxation rates are related to motional spectral densities spectral according to the equations[4]

$$R_{1Z} = \frac{1}{T_{1Z}} = \frac{3\pi^2 C_q^2}{2} [J_1(\omega_0) + 4J_2(2\omega_0)]$$

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

(4-3)
Figure 4.9: Experimental spectral densities for FMOC-alanine-d$_3$ at 280.2 K. These orientation-dependent spectral densities are obtained from the relaxation data in Figure 4.5 by procedures described in the text. Vertical error bars represent 95% confidence levels (two standard deviations) returned by the nonlinear least squares fitting procedure.
where now, for each of the randomly oriented crystallites in the polycrystalline powder sample, $J_1$ and $J_2$ are functions of the Euler angles that define the crystallite orientation with respect to the external field. By inverting equation 4.3 for each point of the line shape, we obtain the spectral densities shown in Figure 4.9 as a function of position in the powder pattern. This procedure requires an estimate of the quadrupole coupling constant $C_Q$, averaged only over fast motions not specifically incorporated in expressions for the motional spectral densities. For this purpose we assume a fixed value, $C_Q = 160.1$ kHz, an assumption that is rationalized in Section 4.3.3. The spectral densities, and especially their orientation dependence, are more sensitive to the details of molecular motion than are simple measurements of $R_{1Z}$ alone, because their intrinsic orientation dependence tends to partially cancel from the sum $J_1 + 4J_2$.

4.2.3 Temperature dependence of relaxation rates

It shows the temperature dependence of both $R_{1Z}$ and $R_{1Q}$ for FMOC-alanine-d$_3$ in Figure 4.10. Both relaxation rates reveal a maximum as a function of temperature, which generally implies the existence in the experimentally accessible temperature range of motions whose rates match the Larmor frequency and/or twice the Larmor frequency. The position of the maximum is at higher temperatures for $R_{1Z}$ than for $R_{1Q}$ (i.e., to the left in Figure 4.10) because according to equation 2-60, the spectral density $J_2$, which appears only in $R_{1Z}$, is evaluated at twice the Larmor frequency and motional rates generally increase with increasing temperature.

For the fast motion temperature range, the activation energies can be obtained by linear fits to the semi log plots. Thus for the powder pattern horns, we obtain
Figure 4.2 Arrhenius plot for $R_{1z}$ and $R_{1q}$ at temperature range from 180K-340K. The temperature of maximum relaxation rate is different for $R_{1z}$ and $R_{1q}$ for reasons discussed in the text.
Ea = 11.7 ± 0.2 kJ/mol from the R12 values, in agreement with 11.7 ± 0.2 kJ/mol found from the R10 values.

4.3 Dynamic simulations

4.3.1 Model correlation functions and spectral densities of motion

The dominant spin relaxation mechanism for deuterons is the quadrupole interaction between the electric quadrupole moment and the electric field gradient tensor, whose Hamiltonian in spherical tensor notation form is given by

\[ H = -\omega_0 I_z + \sum_m (-1)^m A^{(2, Q)} T^{(2, Q)}(t) \] (4-4)

Here, the spherical irreducible tensor spin operators are given by

\[ \hat{A}^{(2, Q)}_0 = \frac{4}{\sqrt{6}} (3I_z^2 - I \cdot I) \]
\[ \hat{A}^{(2, Q)}_{\pm 1} = \frac{1}{2} (I_z \hat{I}_\pm + \hat{I}_\pm I_z) \] (4-5)
\[ \hat{A}^{(2, Q)}_{\pm 2} = \frac{1}{2} I_{\pm}^2 \]

while the time independent components of the (diagonal) quadrupole coupling tensor are defined in a molecule-fixed principal axis system by the relations

\[ T^{(1, Q)}_0(PAS) = \frac{\sqrt{6}}{4} (e^2 q_2 Q / h) \]
\[ T^{(1, Q)}_{\pm 1}(PAS) = 0 \]
\[ T^{(1, Q)}_{\pm 2}(PAS) = \frac{1}{4} \eta (e^2 q_2 Q / h) \] (4-6)

In the frame which is rotating at Larmor frequency of the deuteron, H0 is still time dependent because of the random molecular motion, so it is necessary to construct an
average Hamiltonian \( H'(t) \) that fluctuates randomly about average value zero:

\[
H(t) = \bar{H}_Q + H(t) - \bar{H}_Q = \bar{H}_Q + H'(t)
\]  

(4-7)

where \( H'(t) \) is given by

\[
H'(t) = \sum_m (-1)^m A_m^{(2, Q)} [T_m^{(2, Q)}(t) - \langle T_m^{(2, Q)} \rangle]
\]

(4-8)

and is responsible for relaxation.

The spin operators in equation 4.5 are valid in any coordinate system, but must be expressed with respect to laboratory fixed axes with \( z \)-axis parallel to the external magnetic field. Then to make use of equation 4.4 it is necessary to transform the quadrupole coupling tensor components from molecule-fixed principal axes to the laboratory fixed frame, using randomly time dependent Wigner rotation matrix elements:

\[
T_m^{(2, l)}(t) = \sum_{n=-2}^{2} D_{mn}^{(2)*} \Omega_{lp}(t) T_n^{(2, p)}
\]

(4-9)

In practice, this transformation is further decomposed into several steps to reflect the nature of the motion. For example, the quadrupole coupling tensor for each orientational "site" can first be transformed from its PAS frame to a molecular fixed frame and then to a common crystal fixed frame whose time independent orientation with respect to laboratory fixed axes involves the angles used to construct powder average quantities. The general form of this series of transformations can be written as

\[
T_m^{(2, j)} = \sum_{i, k, j, \ldots} D_{mi}^{(2)*} (\Omega_{mi}) D_{ik}^{(2)*} (\Omega_{ik}) \ldots D_{jn}^{(2)*} (\Omega_{jn}) T_n^{(2, p)}
\]

(4-10)

The spectral density of motion in the lab frame is defined as the Fourier transform of
the correlation function

\[ J_{MM}^r(M \omega_0) = \delta_{MM}^r \int_0^\infty C_{MM}^r(\tau) e^{-iM \omega_0 \tau} d\tau \] (4-11)

Where the correlation function is defined as

\[ C_{MM}^r(\tau) = \frac{1}{(T_0^p)^2} \left\langle \left[ T_M^i(0) - \overline{T_M^i} \right] \left[ T_M^i(\tau) - \overline{T_M^i} \right] \right\rangle \] (4-12)

The denominator \((T_0^p)^2\) ensures that the spectral densities are independent of the strength of the quadrupole interaction. This can important if the values determined by NMR are to be compared with results obtained by other experimental techniques such as inelastic neutron scattering.

Here, we focus on spectral densities and correlation functions defined in the crystal-fixed frame because the transformation from lab to crystal fixed axes describes their orientation dependence in polycrystalline powder sample.

\[ J_{LL}^r(M \omega_0) = \int_0^\infty C_{LL}^r(\tau) e^{-iM \omega_0 \tau} d\tau \] (4-13)

The correlation function is given in very general form by

\[ C_{LL}^r(\tau) = \sum_{KK} \left\langle \left[ D_{LK}^i(\Omega_{sm}(0)) - \overline{D_{LK}^i(\Omega_{sm})} \right] \langle D_{LK}^i(\Omega_{sm}(\tau)) - \overline{D_{LK}^i(\Omega_{sm})} \rangle \right\rangle \] (4-14)

where the form of \(C_{LL}^r\) depends on specific motional models.

For the deuterated methyl group in FMOC-alanine, the motional model applied in this thesis is simply a set of Markovian jumps among three equilibrium orientations of one C-D bond in a rotating methyl group. This discrete jump model originates from the fact that molecules in solids typically exist in deep potential wells separated by high energy barriers, so the Markovian jumps among some discrete sites can well describe this kind of motion. In its simplest form, this traditional "3-fold jump" model assumes
exact $C_{3v}$ symmetry for the rotational motion, so that the EFG tensors for each of the three orientation sites must be identical. However, it is important to recognize that this need not be the case in any crystalline environment with symmetry lower than $C_{3v}$ and indeed, this loss of symmetry has been shown previously\textsuperscript{[48,49]} to be responsible for different, site-dependent N-D deuteron quadrupole coupling constants in L-alanine-d$_3$.

For the general N sites jump, defining $P(j, t; i, 0)$ is the probability of finding a spin in site j at time t when the spin is at site i at time 0, and $k_{ij}$ is the rate of jump from j to i, the correlation function in the crystal fixed frame can be written\textsuperscript{[16]} as

$$C_{LL'}(t) = \sum_{KK'} \frac{T_{KK'}^{(2,T)}}{(T_0^{(2,T)})^2} \sum_{i=1}^N \sum_{j=1}^N p_{ij} P(j, t; i, 0) D_{LK}^{(2)}(\Omega_j(0)) D_{L'K'}^{(2)}(\Omega_j'(t)) \quad (4-15)$$

And the population evolution at each site is be described by a set of differential equations

$$\dot{p}_i = \sum_{j=1}^N k_{ij} p_j - p_i \sum_{j=1, j \neq i}^N k_{ji} \quad (4-16)$$

These equations can be solved using standard matrix methods to give the result

$$C_{LL'}(t) = \sum_{KK'} \frac{T_{KK'}^{(2,T)}}{(T_0^{(2,T)})^2} \sum_{i=1}^N \sum_{j=1}^N \sum_{m,n=1}^N (p_{ij} p_{nm} e^{ij})^{1/2} U_{jm} U_{in} e^{ij} D_{LK}^{(2)}(\Omega_j(0)) D_{L'K'}^{(2)}(\Omega_j'(t)) \quad (4-17)$$

A widely used jump model consists of sites in which only the azimuthal angle varies with time (Figure 4.11a). In this case, the correlation function can be simplified to

$$C_{LL'}(t) = d_{L0}^{(2)}(\theta) d_{L'0}^{(2)}(\theta) \Gamma_{LL'}(t) \quad (4-18)$$

where $d_{L0}^{(2)}$ is the reduced Wigner rotation matrix and
\[ \Gamma_{LL'}(t) = e^{-4k \sin^2 (L \pi / N)} \quad L = L' \mod N \]
\[ \Gamma_{LL'}(t) = 0 \quad otherwise \]

For the methyl group, specializing to three sites mutually "connected" by jumps at rate \( k \), (so the correlation time \( \tau = \frac{1}{3k} \)) it can be shown that\[^{32} \]

\[ \Gamma_{LL'}(t) = 1 \quad L = L' = 0 \]
\[ = e^{-t/\tau} \quad L = L' = \pm 1, \pm 2 \]
\[ L = 1, L' = -2 \]
\[ L = -1, L' = 2 \]
\[ L = 2, L' = -1 \]
\[ L = -2, L' = 1 \]

Spectral densities can be obtained by Fourier transform of the correlation functions defined in equation 4-20. It is important to note that the tempting approximation that cross terms \( \Gamma_{LL'}(t) \) with \( L \neq L' \) are zero but this is not the case: while for a methyl group rigidly attached to an isotropically tumbling macromolecule, the contribution of these non-diagonal terms will cancel\[^{32} \] but in solids the presence of the non-diagonal terms will cause nonexponentially decaying correlation functions. The resulting spin lattice relaxation times will depend on two powder angles even for axially symmetric EFG tensors, so that exact analytic expressions become unmanageably cumbersome.

**4.3.2 Simulation results**

Practical dynamic simulations in this thesis are all performed with the software EXPRESS\[^{16} \], which stands for "Exchange Program for Relaxing Spin Systems", 

\[ \text{66} \]
developed by Professors Vold and Hoatson. This software is MATLAB based and features a graphical user interface for simulating various motional models on different NMR experiments. By using EXPRESS, we can avoid the tedious calculation of analytic expressions for correlation functions and spectral densities and it is very convenient to construct very general multi-site motional models. EXPRESS also includes routines that process the simulated time domain free induction decay signals and analyze the resulting partially relaxed spectra to yield relaxation time anisotropies suitable for direct comparison with the experiments.

The motional model used here consists of three-site jumps of the methyl group about the C-C bond axis that joins the methyl group to the rest of the molecule (Figure 4.11(A)). Since the line shape we get from experiment exhibits a small but non-vanishing asymmetry parameter, we hypothesize unequal populations and quadrupole coupling constants for the three sites. The polar angles that transform from principal axes to crystal-fixed axes are held constant in the simulations, but the fractional populations and quadrupole coupling constants of each site were adjusted for best fit to the equilibrium quadrupole echo powder spectrum at each temperature. Then, the jump rate $k_3$ was adjusted to yield the minimum sum of mean square deviations between experimental and calculated $R_{1Z}$ and $R_{1Q}$ values for 50-100 points distributed across the experimentally measured anisotropy profiles. In order to reduce the number of variable parameters to an acceptable number, the static values $C_Q$
Figure 4.3: Comparison of experimental and best-fit simulations of quadrupole echo line shapes for fast three-site jump model simulations of the methyl group in FMOC-alanine-ds. (A) Cartoon showing the three equilibrium orientations of the C-D bonds. The angle between the $C_3\nu$ axis and CD bond is assumed to be 70.52°. (B) Best fit fully relaxed (equilibrium) line shape. The solid blue line represents experimental data and the red dotted line is the simulation. For this simulation, $C_Q = 52.8$ kHz and $\eta_Q = 0.07$. 
for the three sites were fixed at (157, 159, 159) kHz at all temperatures and the relative weight of the first site was adjusted to reproduce the best equilibrium powder line shape at each temperature. Specific parameters used to simulate the relaxation rate anisotropies at 280.2 K are shown in Table 4.2. These are probably not unique; it is likely that a small change in the angle \( \beta \) between the C-D bond axis and the methyl spinning axis is indistinguishable from a compensating change in the assumed \( C_Q \) values. However, best simultaneous fits to equilibrium line shapes and relaxation rate anisotropies are noticeably worse for models based on equal site populations and for models which attempt to reproduce the observed asymmetry parameters by means of restricted wobbling of the methyl spinning axis (details not shown).

<table>
<thead>
<tr>
<th>site</th>
<th>( \alpha )</th>
<th>( \beta )</th>
<th>( \gamma )</th>
<th>weight</th>
<th>( C_Q ) (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>70.52</td>
<td>0</td>
<td>1.07</td>
<td>157</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>70.52</td>
<td>120</td>
<td>1</td>
<td>159</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>70.52</td>
<td>240</td>
<td>1</td>
<td>159</td>
</tr>
</tbody>
</table>

The number of powder increments is 987 and it takes less than one minute to run one simulation. The best fit motionally induced asymmetry parameter observed at 280.2 K was fixed at 0.07 due to averaging over the unequal weights and different \( C_Q \) values for the three sites listed in Table 4.2. For the simulated the line shape shown in Figure 4.11, 500Hz exponential apodization was applied to the simulated line shape to account heuristically for motionally averaged, unresolved residual deuteron dipolar couplings. Figure 4.12 shows the best fit simulations of both \( R_{1Z} \) and \( R_{1Q} \) anisotropy.
profile, both calculated for the same value of the three-site jump rate $k_3$, 2.9x10$^8$ s$^{-1}$.
This rate was optimized by simultaneously minimizing the sum of the squared residuals for both $R_{1Z}$ and $R_{1Q}$ data points.

The simulated $R_{1Z}$ profile matches the experiment values essentially exactly with experimental error. However the simulated values for $R_{1Q}$ in the shoulder region of the powder pattern are seen to be uniformly larger than the observed values. This discrepancy could be due to transfer of unequally stimulated initial quadrupole order among adjacent molecules via spin diffusion; an effect which is not captured by the EXPRESS simulation algorithms. Based on the best fit simulations of $R_{1Z}$ and $R_{1Q}$, the best fit spectral densities are calculated and shown in Figure 4.13. The results show that the anomalous relaxation behavior of $R_{1Q}$ is confined to one-quantum spin flips and is not observed for the 2-quantum spin flips that govern $J_2$.

Figure 4.14 shows the best fits of the temperature dependence of $R_{1Z}$ and $R_{1Q}$ at the horn position. At each temperature, the weights of the three sites are slightly adjusted to match the horn experimental splitting. These simulations are based on a single, unique value of $k_3$ at each temperature, and the excellent simultaneous fit to both experimental relaxation rates provides strong support for the adequacy of the motional model. The $k_3$ values used in the simulation are shown in the Arrhenius plot of Figure 4-15, from which the activation energy for $k_3$ is found to be 12.5 ± 0.2 kJ/mol.
Figure 4.12: Simulations of relaxation rate anisotropy profiles of FMOC-alanine-d$_3$ at 280.2K. For both R$_{1Z}$ (A) and R$_{1Q}$ (B), blue points represent experimental relaxation rates and the red dashed line represent simulations. Vertical error bars represent 95% confidence levels (two standard deviations) returned by the nonlinear least squares fitting procedure.
Figure 4.13: Simulation of anisotropic spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ of FMOC alanine-d$_3$ at 280.2K. The blue dots with error bars denote experimental values of $J_2(2\omega_0)$ and blue crosses with error bars denote experimental values of $J_1(\omega_0)$. Red dashed lines are simulations of the respective anisotropy profiles. The values shown here were obtained by inverting equation 2-60 with $C_0$ chosen to be 158 kHz, which is close to the static quadrupole coupling constant (as opposed to the motionally averaged value).
Figure 4.14: $R_{1Z}$ and $R_{1Q}$ of FMOC alanine-$d_3$ at the horn position for temperatures between 176.7K and 341.8K. The blue circles with error bars denote experimental data and red crosses denote best fit EXPRESS-based simulations. Vertical error bars denote 95% confidence levels of the measured relaxation rates.
Figure 4.15: Arrhenius plot for the three-site jump rate $k_3$ of FMOC alanine-d$_3$. The temperatures range from 176.7 K to 341.8K. The slope of the red straight line corresponds to activation energy $E_a = 12.5 \pm 0.2$ kJ/mol, where the error limit represents one standard deviation.
Chapter 5  Study of FMOC-valine-d$_8$ under MAS conditions

5.1 Overview of sample and experiments

FMOC-L-valine is an N-protected amino acid derivative with the bulky protecting group 9-fluorenylmethoxycarbonyl (FMOC) attached to the amino nitrogen. The polycrystalline powder material deuterated at sites shown in Figure 5.1 (A) was purchased from Sigma-Aldrich and used without purification. The sample was packed in a 2.5 mm rotor and T$_{1Z}$ measurements were carried out with magic angle spinning at spin rate 10 kHz using the 17.6 T spectrometer. The 90° pulse width was 3.5 ms with power level -3 dB, and data acquisition was timed so that the maximum of the first rotational echo fell precisely on a data point. The recycle delay was 15 s and the spectral window was 400 kHz. 128 scans were accumulated to obtain sufficient signal to noise ratio. The delay list for saturation recovery experiments included 22 values, constructed to distribute over the first two decades of the recovery curve. The magic angle was set to achieve the narrowest possible sideband line widths (~70 Hz at 298 K) for a deuterated sample of Urea-sebacic-acid-d$_4$, spinning at 10 kHz. Variable temperature experiments were performed between set points 250 K~340 K(true temperature range, 252.5 K to 347.9 K) in 10 K increments. Temperature was calibrated by lead nitrate and palmitic acid as described in Appendix A. A quadrupole
Figure 5.1: Molecular structure of FMOC-L-valine-ds. The deuteron labels are shown in blue in Figure 5.1 (A), but from the non-spinning quadrupole echo powder pattern (B) it is possible only to distinguish signals from methyl and methylene deuterons. One way to obtain signals from each labeled position is to synthesize separate materials with labels in each position; a cheaper and easier alternative is to employ magic angle spinning as shown in Figures 5.2 and 5.3.
echo experiment at 284.3 K was also performed on a sample of the same material, packed in 5 mm non-rotating sample tube, yielding the line shape shown in Figure 5.1 (B). The outermost features at ~ ±60 kHz correspond to horns of the powder pattern expected for more or less rigid –CD deuterons (Cq ~160 kHz) and the more intense, much narrower powder pattern in the center of the spectrum is assigned to –CD3 methyl deuterons undergoing rapid 3-fold rotation. However, the extensive of these powder patterns precludes resolution of signals from individual –CD and CD3 groups.

5.2 Experiment results and analysis

Partially relaxed FID signals obtained from the spinning sample were processed using NMRLV. Appropriate left shift and phase correction parameters were established to get an absorption line shape for the fully relaxed (equilibrium) spectrum (see Figure 5.2(A)) and the same left shift and phase corrections were automatically applied by NMRLV routines to the partial relaxed FID signals before calculating the relaxation times. A typical MAS spectrum for FMOC-L-valine-ds, obtained at 284.2K is shown in Figures 5.2 and 5.3. In the center band spectrum, Figure 5.2 (B), there are four distinct peaks which arise from magnetically nonequivalent deuterons with different site-specific chemical shifts. Peaks A and B can be identified unambiguously with the two different -CD deuterons, while peaks C and D may be identified on the basis of their greater intensity and shift to higher field as arising from the two different CD3 groups. The sideband intensities of the two methyl peaks (Figure 5.3) start to decrease at the third sideband at ±30 kHz because the motionally averaged quadrupole coupling constant for methyl groups is no larger than about 55 kHz. Only the methylene deuterons, whose quadrupole coupling tensors are not averaged by rapid
large angle motions, contribute to sidebands at observed at higher multiples of the 10 kHz spin rate. These differences in $C_Q$ also contribute to the relative intensities of the center band peaks: the signal from a deuteron with a larger $C_Q$ will distribute over more side bands so its center band intensity will be smaller.

The chemical shift between Peaks A ($-C_\alpha$-D methylene deuterons) and B($-C_\beta$-D methylene deuterons) is significantly larger than that observed for analogous $-C$-$H$ peaks in solution state NMR spectra of similar materials, which also reveal only one peak assignable to the methyl groups. These differences between the solid and solution spectra arise because the more restricted environment in the solid material suppresses internal rotation around the $C_\alpha$-$C_\beta$ bond as well as large angle motions of the FMOC moiety. Unfortunately, this limits the utility of solution state NMR spectra for unambiguous assignment of methylene peaks A and B in the solid state spectra, as well as methyl peaks C and D, to specific functional groups.

Compared with the low signal to noise and limited resolution of the overlapping powder patterns shown in Figure 5.1 (B), the spectacular improvements obtained using magic angle spinning offer an opportunity to measure site-specific relaxation times of individual deuterons without recourse to expensive and difficult procedures required to achieve selective isotropic labeling.

Figure 5.4 shows the relaxation rates measured at 284.3 K for each member of the spinning side band manifold for all four peaks. Only peak B is shows significant relaxation time anisotropy in the sense that sidebands of different order have different relaxation rates. The anisotropy is unusual, all the sidebands for a given C-D moiety are expected to exhibit the same relaxation rate since the sample rotation, which is
fast on the timescale set by the spin lattice relaxation rate produces exactly overlapping contributions to each sideband from sets of crystallite orientations that relax at different rates, leading to an effective orientation independent average over one rotor cycle\textsuperscript{30}.

The relaxation rates for sidebands of Peak B that fall outside the manifold of methyl group signals (peaks C and D) are significantly larger than those found for the center band and first two side bands, which fall inside the manifold of the methyl group peaks. This cannot be due simply to spectral overlap of peaks with different relaxation rates, because no such differences are found for the peak A methylene resonance. From the side band spectra shown in Figure 5.3, it is evident that peak methylene peak B, but not A, overlaps with methyl peak C. This enhances the possibility of dipole-dipole induced cross-relaxation between peaks B and C, which would tend to equalize their relaxation rates. But in the 5\textsuperscript{th} and 6\textsuperscript{th} sidebands from which methyl contributions are absent, such cross relaxation is suppressed and the relaxation intrinsic relaxation rate for peak B can be accurately measured. The lower relaxation rate found for peak A most likely arises from smaller libration angles than are possible for peak B deuterons, suggesting that peak A most likely arises from the C\textsubscript{\alpha}-D deuterons.
Figure 5.2: 115 MHz deuteron MAS spectrum of FMOC-L-valine-d$_8$ at 284.3K. (A) full spectrum showing side bands at multiples of the 10 kHz spin rate. (B) Expanded center band region showing well resolved signals for deuterons assigned in the text to four specific sites as shown.
Figure 5.3: Sidebands of the FMOC-valine-d$_8$ MAS spectrum. According to the static powder pattern shown in Figure 5.1, the motional narrowed methyl deuteron signal is confined to frequencies within about ±45 kHz of the spectrum center. For this reason, methyl deuteron signals are absent from sidebands of order five and higher. Thus from these outer sidebands, it is possible to study the relaxation behavior of methylene signals without interference from the more intense methyl signals.
Figure 5.4: Relaxation rates for each spinning side band of FMOC-valine-d₈. The sample temperature is 284.3K and spin rate is 10kHz. Relaxation time anisotropy is suppressed by magic angle spinning; the unexpected, enhanced relaxation of the center band and first two sidebands of methylene peak B is probably due to rotor-assisted spin diffusion with nearby methyl deuterons as described in the text.
Figure 5.5: Arrhenius plot for center band spin lattice relaxation of all four peaks of FMOC-valine-d₅. The temperature range is 252.5 K-347.9 K. Vertical error bars represent one standard deviation confidence limits for the measured rates. The activation energies for peak methyl peaks C and D are 11.4 ± 0.6 kJ/mol and 12.6 ± 0.6 kJ/mol, respectively. For methylene peaks A and B, the values 1.0 ± 0.4 kJ/mol and 2.4 ± 0.3 kJ/mol obtained from the dashed lines are smaller than or comparable to the thermal energy k_BT over the temperature range, implying that for these deuterons, the dominant motion process is most likely small angle C-D bond libration.
Figure 5.5 shows Arrhenius plots for center band relaxation of each peaks over the temperature range between 252.5K and 347.8K. The relaxation rates are almost temperature independent for methylene peaks A and B, consistent with small angle librational motion. The relaxation rates for the two methyl groups increase as expected with decreasing temperature; activation energies calculated by fitting the two curves are 11.4 ± 0.6 kJ/mol and 12.6 ± 0.6kJ/mol. In the following section, simulations for methyl group relaxation under MAS conditions are described in details.

5.3 Relaxation time calculation under MAS

To calculate the laboratory frame spectral densities of a spinning sample, the rotor fixed coordinate system needs to be added as an intermediate in the string of transformations from PAS to LAB fixed frames: this transformation is then summarized as[4]

\[ \begin{align*}
\text{PAS} & \xrightarrow{\Omega_{\text{rot}}} \text{MOL} \xrightarrow{\Omega_{\text{cr}}(t)} \text{CRY}
\xrightarrow{\Omega_{\text{rot}}(t)} \text{ROT} \xrightarrow{\Omega_{\text{lab}}(t)} \text{LAB}
\end{align*} \]

The transformation from crystal fixed to rotor fixed frames is time independent while the transformation from rotor to LAB fixed frame is coherently time dependent because of the spinning rotor.

The quadrupole coupling tensor components in the lab-fixed frame are related to those in the PAS frame by an impressive sum of no less than 625 Wigner rotation matrix elements[35]

\[ T_{nm}^{(2,3)}(t) = \sum_{rl, ..., pm} D_{ri}^{(2)*} (\Omega_{rl}(t)) D_{cr}^{(2)*} (\Omega_{cr}) D_{mc}^{(2)*} (\Omega_{mc}(t)) D_{pm}^{(2)*} (\Omega_{pm}) T_{p}^{(2,3)} \]  

(5-1)

It follows that the spectral density can be written in the form
\[ J_{MM}(M\omega) = \delta_{MM} \int_0^{\infty} C_{MM}(\tau)e^{-iM\omega t} d\tau \] (5-2)

Where \(C_{MM}(\tau)\) is the correlation function,

\[ C_{MM}(\tau) = \frac{1}{(T_0^p)^2} \left( [T_M'(0) - T_M'(\tau)] - [T_M'(\tau) - T_M'(0)] \right) \] (5-3)

Time independent Wigner matrix terms can be removed from the integral, so the spectral densities reduce to

\[ J_M(M\omega) = \sum_{c,r} \sum_{\Omega_{cR}} D^{(2)}_{c'c}\left( \Omega_{cR} \right) D^{(2)*}_{r'r'}\left( \Omega_{cR} \right) D^{(2)}_{pm}\left( \Omega_{PM} \right) D^{(2)*}_{pm'}\left( \Omega_{PM} \right) \int_0^{\infty} \langle D^{(2)}_{c'c'}(\Omega_{RL}(0)) D^{(2)*}_{r'r'}(\Omega_{RL}(\tau)) D^{(2)}_{m'm'}(\Omega_{MC}(0)) D^{(2)*}_{m'm'}(\Omega_{MC}(\tau)) \rangle e^{-iM\omega t} d\tau \] (5-4)

The time scale set by the rotor period is typically \(\sim 10^{-4}\) s, while the correlation time for fast methyl group is \(10^{-8} - 10^{-9}\) s. Therefore, the rotor can be considered stationary when calculating the integral for the \(\Omega_{MC}\) transformations (which have already been discussed in Chapter 4). The relaxation time for methyl groups is on the order of 100 ms. Thus, the rotor has undergone thousands of rotations before the deuteron has fully relaxed. It follows that the rotor-averaged relaxation time can be calculated by averaging the spectral densities over a rotor cycle. The \(\Omega_{RL}\) transformation is determined by the angle between the rotor axis and the applied magnetic field (i.e. the magic angle \(\beta_{RL}\)) and it follows that the integral over a rotor cycle for the \(\Omega_{RL}\) transformation is \(38\)

\[ \langle D^{(2)}_{c'c'}(\Omega_{RL}(0)) D^{(2)*}_{r'r'}(\Omega_{RL}(\tau)) \rangle = \left| D^{(2)}_{c'c'}(\beta_{RL}) \right|^2 \delta_{c'r'} \] (5-5)
Figure 5.6: Arrhenius plot for 3-fold methyl jumps (peak D) in FMOC-valine-d₈, determined as described in the text and in Appendix B by matching MAS simulations with measured center band relaxation rates over the temperature range from 252.5K to 347.8K.

Based on the three site jump model discussed in the previous chapter, spectral densities calculated were averaged over a rotor cycle to fit the experimental methyl relaxation rates for FMOC-valine-d₈. The Arrhenius plot for the best fit three site jump rate is shown in Figure 5.6, from which the activation energy for $k₃$ is found to be $13.7 \pm 0.6$ kJ/mol.
Chapter 6 Temperature distributions in a 2.5mm rotor

When we perform a temperature calibration with lead nitrate, the peak position in the spectrum is chosen to determine the true temperature of the sample and this single temperature is used to calculate the relaxation times. This procedure assumes a constant temperature throughout the sample and ignores any thermal gradients. However, since relaxation times are usually dependent on temperature, the experimental relaxation times are actually the average of contribution from the different parts of the sample with different temperatures. For static experiments, this effect is minimal because the temperature gradients across the sample rendered negligible by careful design and thermal insulation of the sample coil, but in MAS experiments, because of the frictional heating and high flow rates of spinner drive and bearing gas flow, temperature gradients across the rotor is significantly larger. For example, when a 2.5 mm rotor spins at 30 kHz and the temperature controller set point is 309 K, experiments and simulations described below indicate that the true temperature in the rotor varies between 329 K to 340 K. In order to account for thermal gradients in the simulation of the relaxation times, we have developed a model to estimate the temperature distribution in the rotor by analysis of $^{207}$Pb MAS NMR line shapes.
6.1 The model of temperature distribution in rotor

A schematic illustration of the rotor housed in its mounting block (the stator) is shown in Figure 6.1. Room temperature nitrogen gas (bearing gas) is blown towards the top and bottom part of the rotor and a second stream of nitrogen gas (driving gas) is directed towards fins at the top cap of the rotor to spin the sample. The variable temperature gas enters the stator through a 5 mm hole and is directed tangentially towards the middle of the rotor. The VT gas exits from the other side of the stator, where it is mixes with some of the driving and bearing gas.

In order to obtain the temperature distribution in the rotor, we need to solve a 3 dimensional heat equation

\[
\frac{dT}{dt} = K \left( \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right)
\]

Here \( T \) is the temperature at point \((x,y,z)\), \( K \) is the thermal conductivity, \( \mu \) is the specific heat capacity and \( \rho \) is density of the material in the rotor. Fortunately, we only need to consider the steady state solutions, which can conveniently be obtained by rewriting equation 6.1 cylindrical coordinates and solving the time independent equation

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2 T}{\partial \theta^2} + \frac{\partial^2 T}{\partial z^2} = 0
\]

The rotor has cylindrical symmetry when spinning, so the temperature does not depend on the angle \( \theta \), and equation 6-2 then simplifies to a 2 dimensional problem,

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) + \frac{\partial^2 T}{\partial z^2} = 0
\]

In our model, boundary conditions consists for solving equation 6-3 consist of
Figure 6.1: Schematic diagram of a MAS rotor in the stator. Side (left) and top (right) views indicate where heat is transferred intentionally to and from the sample by a combination of ambient temperature spinner drive and bearing gas flows and a temperature controlled gas stream used to adjust the sample temperature. As described in the text, Dirichlet boundary conditions for solving the heat equation are specified by five parameters, consisting of the temperature at the rim top, middle, and bottom, and the center top and bottom of the sample.

quadratic functions fixed by five adjustable parameters: temperatures at the top, middle and bottom edges of the rotor, and temperature at the top and bottom center as shown in Figure 6.1. Then an appropriate mesh size is chosen and the temperature distribution in the rotor can obtained using MATLAB PDE toolbox. In order to compare match these simulations to experimental data, the computed temperature distributions were used to construct lead nitrate MAS spectra line shapes. The contribution from each mesh point to the spectrum can be viewed as a Lorentzian peak with a fixed width, whose frequency relative to an arbitrary reference is determined by the temperature dependent chemical shift for the specified mesh point.
temperature. The well established empirical relation between chemical shift and the temperature is given by\(^{[51]}\)

\[
\delta(\delta_{cs}(T) = 0.753 \times (T - T_{ref}) + \delta_{ref})
\]

Here, \(\delta_{ref}\) is the chemical shift of lead nitrate at \(T_{ref}\). Because the temperature is defined in a 2D plane, the points closer to its center correspond to fewer points in the 3D sample, so the Lorentzian spectra need to be weighted by the distance from the mesh point to the center of the rotor when added together to get the final simulated line shapes.

6.2 Experiment results and simulation

A powdered sample of lead nitrate was packed in a 2.5 mm rotor and ZG MAS spectra were collected using the 17.6 T spectrometer. Lead nitrate spectra were obtained 249.2 K, 279.2 K and 309.2 K. These are controller set point temperatures, fixed by the sensor which in our probe is located downstream from the sample. At each set point temperature, spectra were obtained for different spin rates varying from 5 kHz to 30 kHz in 5 kHz increments. A typical 90° pulse with a pulse length of 2.8 μs and power level of 4 dB was used and the recycle delay was set to be 10 s. Experimental lead nitrate spectra are shown in Figures 6.4, 6.5 and 6.6 together with best fit simulated line shapes. It was found as expected\(^{[50]}\) that with the increasing spinning speed, the deviation between the true and set temperatures increases quadratically with increasing spin rate (Figure 6.2). This deviation can be as high as 30 K when the spinning speed reaches 30 kHz, which emphasizes the need for accurate temperature calibration in MAS experiments. The increasing width of the
Figure 6.2: Deviation of the most probable sample temperature from the set point temperature at different spinning speeds. The most probable sample temperature for each set point was determined from the chemical shift of the highest point in $^{207}$Pb lead nitrate MAS spectra as described in Appendix 1 and shown in Figures 6.4 – 6.6.

center band line shapes with increasing spin rate demonstrates a corresponding increase in thermal gradients.

At low temperature ($T_{\text{set}} = 249.2$ K), there are two peaks and the stronger feature appears on the low temperature end of the spectrum. As expected, the intensity of the high temperature peak becomes stronger as the spinning speed increases. At intermediate temperatures ($T_{\text{set}} = 279.2$ K), the double peaks does not appear until the spinning speed reaches 20kHz, and the most probable population shifts from the low temperature to the high temperature end. At high temperatures ($T_{\text{set}} = 309.2$ K), only a single peak appears and the most probable population stays at the high temperature
end. The following tables show the best fit parameters used in the simulations

Table 6.1 Simulation parameters for $T_{set} = 249.2\, K$

<table>
<thead>
<tr>
<th>SR/kHz</th>
<th>$T_{me}/K$</th>
<th>$T_{bc}/K$</th>
<th>$T_{tc}/K$</th>
<th>$T_{te}/K$</th>
<th>$T_{be}/K$</th>
</tr>
</thead>
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<td>279</td>
<td>279</td>
<td>282.3</td>
<td>281</td>
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Table 6.2 Simulation parameters for $T_{set} = 279.2\, K$

<table>
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<th>SR/kHz</th>
<th>$T_{me}/K$</th>
<th>$T_{bc}/K$</th>
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<th>$T_{te}/K$</th>
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Table 6.3 Simulation parameters for $T_{set} = 309.2\, K$

<table>
<thead>
<tr>
<th>SR/kHz</th>
<th>$T_{me}/K$</th>
<th>$T_{bc}/K$</th>
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<th>$T_{te}/K$</th>
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Typical mesh plots for the temperature profile in the rotor are shown in Figure 6.3. Comparing these plots with the lead nitrate spectra, it is evident that the most probable population comes from the middle part of the rotor. When the temperature of
Figure 6.3: Simulated temperature profiles. (A) Set point temperature 249.2 K and spin rate 30 kHz. (B) Set point temperature 279.2 K and spin rate 10 kHz. (C) Set point temperature 279.2 K and spin rate 30 kHz. (D) Set temperature 309.2 K and spin rate 30 kHz.
Figure 6.4: Lead nitrate spectra (solid lines) and simulations (dotted lines) for set point temperature 249.2 K. The bottom x-axis is the chemical shift in ppm and the top x-axis is the corresponding temperature determined by procedures described in Appendix A.
Figure 6.5: Lead nitrate spectra (solid lines) and simulations (dotted lines) for set point temperature 279.2 K. The bottom x-axis is the chemical shift in ppm and the top x-axis is the corresponding temperature determined by procedures described in Appendix A.
Figure 6.6: Lead nitrate spectra (solid lines) and simulations (dotted lines) for set point temperature 309.2 K. The bottom x-axis is the chemical shift in ppm and the top x-axis is the corresponding temperature determined by procedures described in Appendix A.
the middle part of the rotor is lower, the highest peak in the spectrum appears at the low temperature end of the spectrum and vice versa. This is reasonable because the cold VT gas blows directly to the middle the rotor.

6.3 Calculation of methyl relaxation times in presence of thermal gradients

For MAS experiments, the temperature gradient across the sample can be as high as 10K when the spinning rate reaches 30 kHz. The jump rate for three site methyl hopping is given by the Arrhenius equation,

\[ k = k_0 \exp\left(-\frac{E_a}{RT}\right) \]  \hspace{1cm} (6.5)

where for methyl motion in FMOC-alanine-d\textsubscript{3}, the activation energy is 12.5 kJ/mol with the intercept \( k_0 \) to be \( 6.24 \times 10^{11} \) s\textsuperscript{-1}. Thus, for set point temperature 309.2 K and spin rate 30 kHz, the temperature distribution computed at 1000 increments, using the temperature model developed in the preceding section and then the average jump rate is calculated to be \( 7.14 \times 10^9 \) s\textsuperscript{-1} and the corresponding relaxation time is 65.1 ms. The most probable jump rate calculated by fitting a weighted superposition of EXPRESS generated MAS line shapes is \( 6.98 \times 10^9 \) s\textsuperscript{-1} and the relaxation time is 63.7 ms.

Figure 6.7 shows a comparison of the spin lattice recovery curves for both cases. The difference between the two methods of calculating the relaxation time is less than 3%, which is well below the error in measuring relaxation time. It can be concluded that it is acceptable to use the highest peak a lead nitrate spectrum to determine a single sample temperature, which can be used in the calculation of the relaxation times even in presence of thermal gradients generated in high spinning rate MAS experiments. However, we emphasize that this conclusion pertains only to the particular probes and
sample conditions we have simulated, and generalization to other experimental conditions should be treated with caution.

**Figure 6.7:** Simulated recovery curves for deuterons relaxing by thermally activated threefold jumps in a rotating sample (spin rate 30 kHz) in presence of steady state temperature gradients. The solid line was obtained by procedures described in Appendix 2 using the most probable jump rate for the value of \( k_3 \). The dotted line, which more closely resembles reality, is based on using a weighted average jump rate resulting from the (simulated) temperature distribution across the sample.
Conclusions

Solid state deuteron NMR line shape and relaxation time analysis presented in this thesis demonstrate their scope and power for quantitative characterization of molecular motions in FMOC amino acid derivatives. An motional model based on Markovian sudden jumps among three unequally populated rotational sites is enough to describe the hindered rotation of methyl groups in FMOC-alanine-d$_3$. The observed lower methyl activation energy than L-alanine-d$_3$ suggests a less sterically crowded environment so that the methyl group can rotate more freely. Comparison with the analysis reported recently by Vugmeyster et al. [18, 36, 37] for FMOC-valine as well as specific valine and iso-leucine residues in the small protein HP36, demonstrates that the simple three-site jump model used in the present work must be supplemented by explicit descriptions additional motional process before it can be applied to methyl groups attached to flexible chains.

The present study of FMOC-valine-d$_8$, shows that the increased resolution obtained by high field MAS permits measurement and interpretation of site specific deuteron spin lattice relaxation rates without recourse to expensive, difficult to synthesize selectively labeled materials.

Finally, the thermal diffusion model of temperature gradients induced by fast, gas-driven sample spinning developed here provides an encouragingly positive answer to nagging questions the potential importance of systematic errors arising
from the widespread practice of neglecting them altogether. From the comparison of
two different methods for calculating methyl relaxation rates in presence of thermal
gradients, it can be concluded that MAS-induced thermal gradients, at least those no
larger than about 10-12 K across the spinning sample, do not introduce significant
systematic error in the interpretation of temperature dependent spin lattice relaxation
times of properly calibrated samples.
Appendix A  Temperature calibration using lead nitrate.

When studying the temperature dependence of NMR spectra, it is necessary to determine the true temperature of the sample. This is a non-trivial exercise, since as discussed in Chapter 3, the temperature sensor is necessarily placed outside the sample tube and the temperature reported by the sensor is not the actual temperature of the sample. This difference is especially important in magic angle spinning experiments, where the difference between the true temperature and the sensor temperature can be very large due to frictional heating\textsuperscript{[50, 52]}. For example, in a 2.5 mm rotor spinning at 30 kHz, the difference between controller set point and true sample temperature can be as large as 30 K\textsuperscript{[42]}. Lead nitrate is a good candidate for calibrating the sample temperature, because there are well established strong, precisely linear relations between temperature and each component of the $^{207}$Pb chemical shift tensor\textsuperscript{[40-43]}. It follows that the isotropic chemical shift of $^{207}$Pb in lead nitrate, is also a linear function of temperature, which careful measurements\textsuperscript{[40]} have shown is given by the relation

$$\delta_{\text{iso}}(T) = 0.75\times(ppm/K) \times (T - T_{nfs}) + \delta_{nfs} \quad (A-1)$$

$\delta_{\text{iso}}(T)$ is readily measured from the center band frequency of a $^{207}$Pb MAS spectrum, while for a static $^{207}$Pb chemical shift powder pattern, it is easier to measure instead
the chemical shift of the highest point in the spectrum, for which the chemical shift is given by\[^{41}\],

\[ \delta_{\text{peak}} = 0.666 (\text{ppm} / K) \times (T - T_{\text{ref}}) + \delta_{\text{ref}} \]  (A-2)

The temperature calibration process consists of measuring the chemical shift for several different set point temperatures, using equation A-1 (for MAS experiments) or A-2 (for non-rotating samples) to compute the corresponding true sample temperature, and fitting the results to determine the slope \( a \) and intercept \( b \) for the linear relation

\[ T_{\text{true}} = a T_{\text{set}} + b \].

However, it is important to realize that since chemical shifts are reported relative to a standardized but arbitrary reference, the above relations establish only the slope of the temperature calibration: At least one fixed point, i.e., a measurement of the chemical shift with \( T_{\text{set}} \) adjusted very precisely to give an independently known true sample temperature, is needed to determine \( b \), the intercept of the calibration line. For our static probe, the D\(_2\)O melting point (3.8°C) was used as the fixed point and for the MAS probe, the melting point of a deuterated palmitic acid sample (62.5°C) was used. Temperature calibrations were repeated periodically, especially after disassembly, repair, and reassembly of the probe.

During the calibration process, the lead nitrate sample was maintained at identical conditions as the deuteron experiments to be performed, including the range of set point temperatures, the nitrogen VT gas flow rate, and spinner drive and bearing pressures for the MAS probe. Before each measurement, the sample was allowed to equilibrate for at least 15 minutes.
Figure A.1: Temperature calibration curves for static and rotating samples. For each calibration the horizontal axis is the set point temperature and the vertical axis is the "true" sample temperature, estimated from the $^{207}$Pb chemical shift of lead nitrate in 5 mm (A) a static probe and (B) 2.5 mm MAS probe with spin rate 10 kHz. These curves are specified to the probes and sample conditions described in this thesis and cannot be used to estimate the true sample temperature under different conditions.
A typical calibration result for each probe is shown in Figure A-1 and the fitting parameters is shown in Table A-1.

Table A.1 Temperature calibration parameters. $T_{true} = aT_{set} + b$.

<table>
<thead>
<tr>
<th>Probe</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mm static</td>
<td>1.03 ± 0.01</td>
<td>-9.33 ± 0.15</td>
</tr>
<tr>
<td>2.5mm MAS (10kHz)</td>
<td>1.06 ± 0.01</td>
<td>-12.55 ± 0.24</td>
</tr>
</tbody>
</table>

It is important to realize that these results are specific to these particular probes and also to the details of the gas flow supply lines and the degree of thermal isolation between the probe and its surroundings, they should not be used for other probe/spectrometer configurations. We note in passing that for the 7.06T spectrometer equipped with a MAS probe operated in non-spinning mode to record deuteron powder patterns\(^{31}\), the temperature calibration parameters were found to be significantly larger: a = 1.17 and b = -39.45. This may arise from poorer thermal isolation and unexpected gas flow patterns when the low field MAS probe is operated with spinner supply lines connected but turned off.
Appendix B  Calculating spin lattice relaxation times for a spinning sample

To calculate the relaxation time of a methyl group in a spinning rotor, lattice variables in the relevant spin Hamiltonian need to be transformed from the PAS to the LAB frame, including an intermediate frame between Crystal and LAB fixed frames. However, for the case of a single motional process treated here, it is not necessary to distinguish between molecule and crystal fixed axes so the three step transformation defined in Figure B.1 is sufficient.

\[
\Omega_{\text{pc}}(\alpha(t), \beta(t), \gamma(t)) \text{ is determined by the three site jump motion and is thus time dependent; } \Omega_{\text{cr}}(\omega_R t, \beta_R, 0) \text{ is also time dependent because the spinning of the rotor.}
\]

In the rotor fixed frame, the polar angles of all the crystallites distribute randomly on a sphere just as they do under static conditions and the spectral densities (or } R_{\text{cr}}(\theta, \varphi) \text{)
for each crystallites can be calculated by EXPRESS\textsuperscript{[16]} (in T1Zsim.m) for a general model or more simply by Torchia & Szabo's formula\textsuperscript{[32]} for three-site jumps. It should be noted that because of the separation of time scales between rotor motion (~$10^4$ s$^{-1}$) and spin relaxation rates (>10$^2$ s$^{-1}$) of methyl group, relaxation rates can be averaged over a rotor cycle. The following section shows how to calculate the average relaxation rates using Monte Carlo methods.

First, define $u$ and $v$ to be random variables on $[0,1]$, then

$$
\begin{align*}
\theta &= \arccos(2u - 1) \\
\varphi &= 2\pi v
\end{align*}
$$

will give uniform distributions of polar angles on a sphere\textsuperscript{[54]}.

\textbf{Figure B.2:} A thousand random points uniformly distributed on a sphere.
Next, generate $N \sim 1000$ such randomly oriented crystallites and track their the orientation over a rotor period (in 100 steps), using the following rotation matrix.

Given a unit vector, the matrix for a rotation by an angle of $\theta$ about an unit axis $(u_x, u_y, u_z)$ is given by $^{[53]}$

$$R = \begin{bmatrix} \cos \theta + u_z^2(1 - \cos \theta) & u_x u_z(1 - \cos \theta) - u_y \sin \theta & u_y u_z(1 - \cos \theta) + u_x \sin \theta \\ u_x u_z(1 - \cos \theta) + u_y \sin \theta & \cos \theta + u_x^2(1 - \cos \theta) & u_z u_x(1 - \cos \theta) - u_y \sin \theta \\ u_y u_z(1 - \cos \theta) - u_x \sin \theta & u_z u_y(1 - \cos \theta) + u_x \sin \theta & \cos \theta + u_y^2(1 - \cos \theta) \end{bmatrix} \quad (B-2)$$

The Cartesian coordinates after the rotation is given by

$$\begin{bmatrix} x' \\ y' \\ z' \end{bmatrix} = R \begin{bmatrix} x \\ y \\ z \end{bmatrix} \quad (B-3)$$

The orientation of each crystallite changes over the rotor period thus the relaxation rate for each crystallite will differ, so an average $R_{1Z}$ for each crystallite can be calculated. Finally, a second average of the 1000 crystallites will yield $R_{1Z}$ for the rotating sample. It should be noted that this procedure of averaging relaxation rates over a rotor cycle is not strictly correct; one should compute the total magnetization (including spinning sidebands) at each value of the relaxation delay for each crystallite, average that quantity over a rotor cycle, and finally compute an effective relaxation time by fitting the resulting multi-exponential decay. However, the initial slopes of these curves will be determined exactly by the average relaxation rate described above, and will be the same for the center band and all spinning side bands.

The results of the calculated $R_{1Z}$ will show a small variation for repeated calculations because of the randomly generated orientations of the crystallites. Trial calculations (details not shown) indicate that this difference is much less than 1%, for 1000
crystallite orientations; more precise results could be achieved if desired by increasing the number of crystallites.
## Appendix C  Summary of activation energies

<table>
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<tr>
<th>Sample</th>
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<tr>
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<tr>
<td>Chicken villin HP 36&lt;sup&gt;[18]&lt;/sup&gt;</td>
<td>10.7–12.4</td>
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<td>FMOC-alanine-d$<em>3$ (from R$</em>{1Z}$ Arrhenius plot)</td>
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<tr>
<td>FMOC-alanine-d$<em>3$ (from R$</em>{1Q}$ Arrhenius plot)</td>
<td>11.7 ± 0.2</td>
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<tr>
<td>FMOC-alanine-d$_3$ ($k_3$)</td>
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<td>FMOC-valine-d$<em>8$ (peak C) (measured from R$</em>{1Z}$) (SR=10kHz)</td>
<td>11.4 ± 0.6</td>
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<td>FMOC-valine-d$_8$ (peak D) $k_3$ (simulation) (SR=10kHz)</td>
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<td>Methyl group (Most probable $k_3$) (SR=30kHz)</td>
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