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Studies of Molecular Dynamics of Fmoc-Alanine-$d_3$ through Solid State Deuteron Nuclear Magnetic Resonance

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Studies of Molecular Dynamics of Fmoc-Alanine-d$_3$ Through Solid State Deuteron Nuclear Magnetic Resonance

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A Thesis presented to the Graduate Faculty of the College of William and Mary in Candidacy for the Degree of Master of Science

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The idea of devising and validating various multi-parameter models for intramolecular motion in solid, N-protected amino acid derivatives originates from the desire to quantify side chain molecular motion in proteins. It is well recognized that protein side chain motions play an important role in controlling protein structure and conformation, which directly determines protein function. This structure-function relationship necessitates the quantitative investigation of protein side chain dynamics.

In this thesis Fmoc-Alanine-d3 is chosen as the model system. Solid state deuteron quadrupole echo line shape analysis, measurements of Zeeman ($T_{1Z}$) and quadrupole order ($T_{1Q}$) relaxation and the associated anisotropies are combined with computer simulations to investigate the methyl group dynamics. $T_{1Z}$ and $T_{1Q}$ were measured using saturation recovery with quadrupole echo detection (SRQE) and Broadband Jeener Broekaert (BBJB) techniques respectively at 7.06 T between 230K and 330K. And at 17.6 T, $T_{1Z}$ was also measured using SRQE between 190K and 330K.

A smaller methyl deuteron quadrupole coupling constant was found for Fmoc-Alanine-d3 than for Alanine-d3, indicating the existence of an additional motional process in the Fmoc derivative. The Arrhenius activation energy calculated from the relaxation rates of the horns of the low field quadrupole echo spectra is $E_a = 13.6 \pm 0.8 \text{ kJ/mol}$. This indicates a less severe sterically crowded environment for the rotating CD$_3$ group than that found in crystalline Alanine-d3, for which $E_a = 20 \text{kJ/mol}$.

Motional spectral densities are derived from the best-fit simulated $R_{1Z}$ and $R_{1Q}$ profiles and compared to the experimental data. The $R_{1Z}$ and $R_{1Q}$ profiles exhibit unusual orientation dependence, which cannot be adequately reproduced by threefold methyl rotation alone. Therefore, more elaborate models are proposed that include motion of the C$_{3V}$ rotation axis of the methyl group. Stochastic Liouville formalism is applied to describe modulation of the threefold methyl rotational jumps by libration of the C$_{3V}$ axis. Approximate agreement with experimental data is found for two variants of this formalism, describing the libration either as two-site angular "jumps" or four-site "wobbling" in a cone respectively. However, the two-site jump model produces a significant non-vanishing asymmetry parameter, which is not observed in equilibrium line shapes, and therefore the more symmetric four-site wobble model is adopted. Finally, activation energies are calculated for the threefold methyl rotation and four-site wobbling based on the Arrhenius plots of the best-fit motional rates $k_3$ and $k_4$. 
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CHAPTER 1

Introduction

Nuclear magnetic resonance (NMR) arises from the interaction between the nuclear spin and the magnetic field, which was discovered in 1945 independently by Edward Purcell [1] and Felix Bloch [2]. NMR subsequently evolved into an extremely important tool for probing molecular structures and the physical, chemical, electronic properties of complex materials. NMR is advantageous over many other probing techniques in many aspects. Firstly, in NMR many nuclear spin interactions exist, every one of which features a huge amount of structural and dynamic information. Secondly, NMR is suitable for quantitative analysis and nondestructive to the sample. Lastly, NMR can be applied to different nuclei of specific interest. All these advantages have made NMR an ideal and powerful tool for investigating structures and molecular motions in solutions [3][9], solids [4-6] and partially ordered materials [7-8].

Nuclear spin interactions with their surroundings can be classified as isotropic and anisotropic. For anisotropic interactions, the NMR energy levels and transition
frequencies depend on the orientation of the spin-bearing material with respect to an externally applied magnetic field, while for isotropic interactions, they do not. In ordinary liquids, due to fast, isotropic molecular tumbling, anisotropic interactions that otherwise would cause significant line broadening are completely averaged to zero [9]. Thus the resonance signal in liquids is very sharp with high resolution. However, in solids the lack of the above molecular tumbling motions leads to the broadening of the spectra and the relatively lower signal-to-noise ratio and resolution. Fortunately, high-resolution solid-state NMR techniques [10] have been developed greatly and we now have an arsenal of mature techniques available to overcome the low sensitivity and resolution. Most importantly, the anisotropy of nuclear spin interactions, such as chemical shielding and quadrupole coupling, can now be utilized to reveal details of molecular structure, conformation and dynamics.

Local motions in biological macromolecules such as proteins are critical for their structures and thus functions. Also, they are important in understanding the microscopic basis of many human diseases and the mechanisms of drug actions [11]. NMR has emerged as a very informative tool for investigating biodynamics over the last twenty years [3][12-17]. Among all NMR techniques, deuteron NMR has proved to be the most informative for quantitative studies of solid-state protein dynamics [16][18-22]. Novel experimental techniques and motional models can be derived that may be applied to complex systems, after validation in simpler model systems. In this thesis Fmoc-alanine-d₃ is used to validate the composite motional model of the 3-fold methyl rotation combined with possible librational modes motion of the CD₃-C bond
axis. We chose this amino acid derivative for detailed investigation because its bulky protecting group, 9-fluorenymethoxycarbonyl (Fmoc), represents a bulky, relatively immobile group to which the amino acid is bound by a somewhat flexible linkage.

Quantitative investigation of methyl group dynamics has a long history [23-26]. It is well recognized that there exists fast rotational motion around the pseudo-threefold spinning axis, leading to an averaged small quadrupole coupling constant. Furthermore, there is a large variation in activation energies for methyl rotation, as monitored from the temperature dependence of spin lattice relaxation of the deuterated methyl group. The activation energy barrier for rotation of a specific methyl group is sensitive to its local environment and can be strongly affected by the degree of steric hindrance. For example, the activation energy in acetone $- d_6$ is $4.0 \pm 0.3 \text{ kJ/mol}$ [23] and only $2.6 \pm 0.5 \text{ kJ/mol}$ [24] in acetanilide $- d_3$. For $N - acetyl - DL - (y - d_6) - valine$, the barriers are much higher, 15.3 and 22.2 kJ/mol for the two methyl groups, due to the steric crowding experienced by two methyl groups bound to the same carbon atom [25]. Notably, $E_a = 20\text{ kJ/mol}$ for alanine $- d_3$ is larger than most other singly attached methyl groups [26]. This is probably a consequence of the tight packing found in the crystal structure. In general, such qualitative interpretation of the activation energy barrier ‘landscape’ is a useful means for visualizing the local intra- and inter- molecular environment.

Deuterons are spin-1 nuclei with a relatively small quadrupole moment ($Q = 2.8 \times 10^{-31} \text{ m}^2$). Deuteron NMR is simpler than NMR of other quadrupolar nuclei because the quadrupole interaction is sufficiently small to record well-defined spectra.
with minimum distortions of instrumental origin, and the line shape is sensitive to molecular motions with correlation times often found in molecular solids. In Chapter 2 the basic theory of deuteron NMR is presented. Various nuclear spin interactions that determine the solid state deuteron NMR spectra are introduced and analyzed, expressed in the form of irreducible spherical tensor operators. This includes the Zeeman interaction, chemical shielding, dipole-dipole coupling, and quadrupole – electric field gradient coupling. The physical mechanisms of these interactions and how they determine the NMR spectra are explained in detail. In the second part of Chapter 2, density matrix formalism is summarized for describing the evolution of spin ensembles under radio frequency pulses and relaxation due to perturbations caused by the relevant motional process. In the last part, Redfield theory (applicable in fast motion limit) and a more general stochastic Liouville formalism are explained as the theoretical foundation for interpreting the various relaxation phenomena.

In Chapter 3 the first part briefly introduces the general instrumental setup adopted in most solid state NMR experiments. In the second part, particular experimental methods used to record quadrupole echo (QE) spectra are reviewed. Experimental procedures for measuring deuteron spin lattice relaxation times of Zeeman order, \( T_{1Z} \), and quadrupolar order, \( T_{1Q} \), are explained. For \( T_{1Z} \) the pulse sequences include saturation recovery and inversion recovery pulse sequences. For \( T_{1Q} \), the broadband Jeener Broekaert (BBJB) pulse sequence is described. In the last part, issues about NMR signal processing are addressed.
Chapter 4 mainly focuses on the motional models and the simulation tool used to simulate the deuteron NMR spectra. Formalism for constructing motional models, calculating motional correlation functions and corresponding spectral densities are discussed in detail. Discrete jump models are extensively discussed. The NMR simulation software suite EXPRESS [27] developed by Professors Robert Vold and Gina Hoatson is briefly introduced. EXPRESS employs numerically efficient MATLAB algorithms for including effects of molecular motion in simulations of quadrupole echo, MAS and spikelet echo (QCPMG) line shape analysis, as well as measurements of Zeeman ($T_{1Z}$) and quadrupole order ($T_{1Q}$) relaxation and their anisotropies.

Chapter 5 is devoted to testing motional models for methyl group motion by comparing EXPRESS simulations with experimental data. As already mentioned above, Fmoc-alanine-$d_3$ is used as the model sample. Using our wide bore 17T superconducting magnet, QE spectra and deuteron spin lattice relaxation times of Zeeman order $T_{1Z}$ were measured as a function of sample temperature using the saturation recovery pulse sequence with QE detection. In addition, QE spectra and $T_{1Z}$ as well as $T_{1Q}$ were measured as a function of temperature in a different spectrometer, operating at 7T, using the saturation-recovery and BBJB pulse sequence, respectively.

The relaxation times exhibit prominent anisotropy, which validates the orientation dependence of the spectral densities. Spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ calculated from experimental measured spin lattice relaxation times $T_{1Z}$ and $T_{1Q}$
exhibit an unusual orientation dependence which cannot be adequately accounted for by three-fold methyl rotation alone. The Arrhenius plot of relaxation rate versus inverse temperature gives an averaged activation energy $E_a = 13.6 \pm 0.8 \text{kJ/mol}$. This activation energy for the threefold motion of the CD$_3$ group is smaller than that observed for crystalline Alanine-d$_3$, for which $E_a = 20 \text{kJ/mol}$ [26]. This indicates the less severe steric crowding of the –CD$_3$ group due to the existence of the bulky protecting Fmoc group. More importantly, activation energies calculated from temperature dependence of $J_1(\omega_0)$ and $J_2(2\omega_0)$ are uniform within the powder pattern horns but exhibit irregular distribution beyond the horns. This may indicate the existence of additional motions whose spectral densities have a different orientation dependence than that of threefold methyl jumps. EXPRESS simulations verify this assertion, and appear to rule out early suggestion [49] that associates the observed equilibrium quadrupole splitting to small deviations from tetrahedral bond geometry around carbon atoms.

Appendix A describes procedures for temperature calibration of the static probe using lead nitrate. Very accurate linear relations are known between each principal component of the lead chemical shielding tensor and sample temperature [50-52]. It has been proved that the observation of specific temperature-dependent chemical shift component of the $^{207}\text{Pb}$ resonance of lead nitrate has provided a convenient thermometer for both static [51] and MAS spectroscopy [50].
CHAPTER 2

Basic NMR Theory

This chapter will give an overview of nuclear magnetic resonance spectroscopy, including the physical mechanisms that determine what is observed, with a focus on how they affect solid-state deuteron NMR. The first section discusses the vector model and its applications in interpreting pulsed NMR experiments. The following section talks about nuclear spin interactions in deuteron NMR. The last section summarizes spin density matrix formalism, its applications in interpreting relaxation and the classical relaxation theories, with illustrations particularly relevant to deuteron relaxation.

2.1 Larmor Precession and Free Induction Decay

This section is concerned with the semi-classical vector model \[10\][28]. This model came with the birth of NMR but it is not old-fashioned. In reality, many pulsed NMR experiments can be understood more simply using the vector model instead of underlying quantum mechanical formalism.

In an NMR experiment, the sample has a statistically large number of nuclei (on the order of \(10^{20}\)). Associated with each nucleus there is a magnetic moment due to its nuclear spin. When there is no magnetic field, the nuclear spin magnetic moments
point in random directions at equilibrium. Applying a magnetic field $B_0$ results in a net magnetization $\mathbf{M}$ due to preferential alignment of individual nuclear magnetic moments $\mu_i$ along the direction, $Z$, of the applied magnetic field [10]:

$$M = \sum_i \mu_i = \gamma \hbar \sum_i I_i$$  \hspace{1cm} (2.1)

Where $\gamma$ is the gyromagnetic ratio and $\hbar$ is the Planck’s constant divided by $2\pi$.

If the bulk magnetization $\mathbf{M}$ is tilted away from the $Z$-axis (for example, by applying a radio frequency pulse), it will rotate about the magnetic field direction sweeping out a cone with a constant angle [Figure 2.1]:

$$\frac{dM}{dt} = \gamma \mathbf{M} \times \mathbf{B}_0$$  \hspace{1cm} (2.2)

$\mathbf{M}$ is said to precess about the field and this rotation is called Larmor precession. $\omega_0 = -\gamma B_0$ (in unit of rad/s) is called the larmor frequency.

Essentially, the precession of the magnetization vector is what we detect in an NMR experiment. More accurately, it is the precession of the transverse component of the magnetization that induces an oscillating, decaying signal in a coil mounted in the $xy$-plane as shown in Figure 2-1. This decaying signal is called the free induction decay (FID), and its Fourier transform gives the frequency domain NMR spectrum. In actual NMR experiments, pulses of alternating current at frequency $\omega_{rf}$ are delivered
to the same coil used to detect the FID. During such a pulse, when viewed in a frame rotating at the Larmor frequency, the magnetization experiences an *effective* field whose x-component is \( \omega_1 = \gamma B_1 \) (expressed in frequency units) and whose z-component is \( B_0 - \omega_{rf}/\gamma \). Since the magnetization in any frame precesses about the effective field, it will rotate essentially in the YZ plane if the amplitude of the rf pulse is much larger than the resonance offset \( B_0 - \omega_{rf}/\gamma \). In this case a pulse of duration \( t_{90} = 1/4\omega_1 \) will tip the magnetization from alignment along \( Z \) to alignment along the rotating \( y \)-axis, perpendicular to the applied field. This is called a 90° pulse.

Figure 2.1: Larmor precession and free induction decay (FID)
2.2 Nuclear Spin Interactions

Nuclear spin interactions can be divided into two categories: external and internal [9][10]. The former refer to the interactions between nuclear spins and the externally applied magnetic fields, such as static magnetic field $B_0$ and the radio frequency magnetic field, which can be controlled by the experimenter. The latter refer to interactions between nuclear spins and electric and magnetic fields produced by electrons and surrounding nuclei. Internal spin interactions include chemical shielding, dipolar coupling and quadrupole coupling, etc. Below the various interactions are discussed according to the order of dominance in deuteron NMR.

2.2.1 Zeeman Interaction

The Zeeman interaction refers to the interaction between the nuclear spin and the external static magnetic field. It can be expressed by the Hamiltonian [10]:

$$\hat{H}_z = -\mu \cdot B_0 = -\gamma h I \cdot B_0 = -\gamma h I_z B_0.$$  \hspace{1cm} (2.3)

The lab frame is defined with $z$ axis along the direction of the static magnetic field $B_0$, i.e. $B_0 = (0,0,B_0)$. $I_z$ is the $z$ component of the nuclear spin operator. Unlike the semi-classical vector model, here a rigorous quantum mechanical description is used. The possible states of the spin in the $B_0$ field are totally described by the eigenfunction
\[ \psi_{I,m} \] of the \( \hat{H} \) operator, written as \( |I, m> \) in the Dirac notation. The eigenvalues of \( \hat{H} \) are obtained through the Schrödinger equation:

\[ \hat{H} |I, m> = E_{I,m} |I, m> \]  \hspace{1cm} (2.4)

Here \( I \) is the nuclear spin quantum number, and \( m \) is the magnetic quantum number. \( m \) can take \( 2I + 1 \) values \(-I, -I+1, \ldots, I-1, I\). Substituting equation (2.3) into (2.4),

\[ \hat{H} |I, m> = -\gamma \hbar B_0 I_z |I, m> = -\gamma \hbar B_0 m |I, m> = E_{I,m} |I, m> . \]  \hspace{1cm} (2.5)

Thus, the zero-field degeneracy of the nuclear spin state is broken due to the effect of the magnetic field. The nuclear spin has \( 2I+1 \) discrete energy levels, which are called Zeeman energy levels.

\[ E_{I,m} = -\gamma \hbar B_0 m = \hbar \nu_0 m \]  \hspace{1cm} (2.6)

The simplest situation is when \( I = \frac{1}{2} \). Then \( m = \pm \frac{1}{2} \) and there are only two possible eigenstates corresponding to energy \( E_{\pm \frac{1}{2}} = \pm \frac{1}{2} \gamma \hbar B_0 = \pm \frac{1}{2} \hbar \nu_0 \). The energy level diagram is shown in Figure 2.2. At thermal equilibrium, the distribution of the nuclear spins among Zeeman states obey Boltzmann distribution (high temperature approximation),
\[
\frac{N_{\pm 1/2}}{N} = \frac{\exp(-E_{\pm 1/2})}{\exp(-E_{1/2} / k_B T) + \exp(-E_{-1/2} / k_B T)} \approx \frac{1}{2} \pm \frac{\gamma \hbar B_0}{k_B T}
\] (2.7)

\(T\) and \(k_B\) are Kelvin temperature and Boltzmann constant. Specifically, for nuclear spin with positive gyromagnetic ratio, the \(|\frac{1}{2}, +\frac{1}{2}\rangle\) state has lower energy and thus higher population. This implies the existence of a net, macroscopic longitudinal spin polarization, corresponding to a magnetization vector parallel to the external \(B_0\) magnetic field.

Electromagnetic radiation with appropriate frequency can stimulate the transitions between adjacent energy levels \((\Delta m = \pm 1)\). This frequency \(v_0\) is precisely equal to the Larmor frequency discussed previously.

Figure 2.2: Zeeman interaction for \(I = \frac{1}{2}\) with positive \(\gamma\).
For deuterons \( l = 1 \), there are three possible spin states \( |1, -1 \rangle, |1, 0 \rangle, |1, 1 \rangle \) corresponding to energies \( \nu_0, 0, -\nu_0 \) (in unit of rad/s), respectively.

### 2.2.2 Quadrupole Interactions

The electric quadrupole moment \((eQ)\) of quadrupole nucleus is nonzero and this reflects the deviation from spherical distribution of the nuclear electric charge. The quadrupole interaction refers to the interaction between this electric quadrupole moment and the electric field gradient \((EFG)\) at the site of the nucleus. Generally, the quadrupole interaction is the largest of the various anisotropic interactions that influences solid state NMR (although it is still usually much smaller than the Zeeman interaction). Specifically, solid state deuteron NMR is dominated by this single-particle interaction between the deuteron electric quadrupole moment and the EFG present at the nucleus. The deuteron has a relatively small electric quadrupole moment, which makes it easy to work with experimentally. In the principal axis system \((\text{PAS})\) the EFG can be expressed as a symmetric and traceless second-rank Cartesian tensor \( V \), with three diagonal elements \( V_{xx}, V_{yy}, V_{zz} \). By convention, the PAS axes are labeled such the \( |V_{zz}| > |V_{xx}| > |V_{yy}| \) and \( V_{xx} + V_{yy} + V_{zz} = 0 \).

Conventionally, the following parameters are defined [8]:

\[
eq q = V_{zz} \quad (2.8)
\]

\[
\eta = (V_{yy} - V_{xx})/V_{zz} \quad (2.9)
\]

\( eq \) is often called “the electric field gradient”. \( \eta \) is called quadrupolar asymmetry parameter and \( e \) is the magnitude of the electric charge.
For an isolated deuteron the quadrupole Hamiltonian can also be expressed in terms of irreducible spherical tensors [5][6]:

\[
H_Q = \sum_{m=-2}^{2} (-1)^m T_{-m}^{(2,Q)} R_m^{(2,Q)}
\]  

(2.10)

The spin operators \( T_{-m} \) are defined as

\[
T_0^{(2,Q)} = \frac{4}{\sqrt{6}} (3I_x^2 - I \cdot I)
\]  

(2.11a)

\[
T_{\pm 1}^{(2,Q)} = \pm \frac{1}{2} (I_x I_{\pm} + I_{\pm} I_z)
\]  

(2.11b)

\[
T_{\pm 2}^{(2,Q)} = \frac{1}{2} I_{\pm}^2
\]  

(2.11c)

The lattice variables are defined as

\[
R_m^{(2,Q)} (LAB) = \sum_{k=-2}^{2} D_{mk}^{(2)} (\Omega_{LP}) R_k^{(2,Q)} (PAS)
\]  

(2.12)

where

\[
R_0^{(2,Q)} (PAS) = \frac{\sqrt{5}}{4} C_Q
\]  

(2.13a)

\[
R_{\pm 1}^{(2,Q)} (PAS) = 0
\]  

(2.13b)

\[
R_{\pm 2}^{(2,Q)} (PAS) = \frac{1}{4} \eta C_Q
\]  

(2.13c)

\( C_Q = e^2 V_{zz} Q / \hbar \) is the quadrupole coupling constant expressed in rad s^{-1}. The PAS components of lattice variables are related to the Lab components through (2.12) by
introducing the Wigner rotation matrix $D^{(2)}_{mk}(\Omega_{LP})$[29], with Euler angle $\Omega_{LP} = (\alpha_{LP}, \beta_{LP}, \gamma_{LP})$.

By retaining the secular part of the quadrupole Hamiltonian, the Hamiltonian that determines the deuteron NMR spectrum is given by [6]

$$H^0 = H_z + H_Q = -\omega_0 I_z + \frac{1}{3} \omega_Q (3I_z^2 - I \cdot I)$$

(2.14)

The corresponding energy level diagram is shown in Figure 2.3.

Originally, there is a twofold degeneracy of single-quantum transitions between the Zeeman eigenstates at the Larmor frequency $v_0$. The quadrupole interaction breaks this degeneracy and leads to two transitions at frequencies $v_0 \pm v_Q$. The quadrupole splitting is calculated as

$$2v_Q = \frac{3}{4} \left( \frac{e^2 \nu_{zzQ}}{\hbar} \right) [(3 \cos^2 \beta_{LP} - 1) + \eta \sin^2 \beta_{LP} \cos 2\gamma_{LP}]$$

(2.15)

For single crystal in which the Z-axis of the PAS of the EFG tensor lies along $B_0$ direction, the spectrum includes two sharp lines. For powder sample in which there is an isotropic distribution of crystallite orientations, many such doublets will overlap and give the so-called powder pattern. Deuteron powder pattern line shape is sensitive to micro-to millisecond time scales of motions and thus is a conventional tool for probing molecular dynamics.
Quadrupole splitting: $2v_Q = (3/4)\left(\frac{e^2q_{zz}Q}{\hbar}\right)\left[ (3\cos^2\beta - 1) + \eta \sin^2 \beta \cos 2\psi \right]

Figure 2.3: Energy level diagram for deuteron in a magnetic field. In the lower left is a typical powder pattern. The lower right the definitions of polar angles are shown.
2.2.3 Chemical Shielding Interaction

Nuclei are surrounded by electrons and other nuclei. In external magnetic field, these electrons will revolve about the field and produce an induced magnetic field antiparallel to the applied field. Thus the actual magnetic field the nucleus feels is smaller than the external $B_0$ field. Or equivalently, the local induced magnetic field partly shields the nucleus. Because the electrons surrounding the nuclei reflect the chemical environment, this interaction between nuclear spin and the local magnetic field $B_{loc}$ is called chemical shielding interaction. Since the chemical shielding interaction is related to crystallite orientation, for polycrystalline powder sample this will lead to the broadening of the NMR signal.

In general, the secular part of chemical shielding Hamiltonian (the part that commutes with Zeeman Hamiltonian) is expressed as [6]

\[
H^0_\sigma = -\omega_0 l_z [\sigma_{iso} + \frac{1}{2} \delta (3\cos^2\beta - 1 + \eta \sin^2\beta \cos 2\gamma)]
\]  

(2.16)

Here the isotropic chemical shift is $\sigma_{iso} = \frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$. The asymmetric parameter of the chemical shielding tensor is $\eta = (\sigma_{xx} - \sigma_{yy})/\sigma_{zz}$. The chemical shift anisotropy is defined by $\Delta \sigma = \sigma_{zz} - \frac{1}{2}(\sigma_{xx} + \sigma_{yy}) = \frac{3}{2} \delta$. $(\beta, \gamma)$ are the polar angles defining the orientation of the $B_0$ field in the $(x^{PAF}, y^{PAF}, z^{PAF})$ axis frame, the principal axis frame of the shielding tensor.
Essentially, for deuteron the effect of chemical shift anisotropy is negligible compared to the anisotropy caused by quadrupole coupling.

2.2.4 Dipolar Coupling

Nuclear spins in a sample possess magnetic dipole moment and could interact with each other through space. This interaction is called dipolar coupling. The dipolar Hamiltonian (in angular frequency units) between spin I and S [10] is given by

\[ H_D = -2I \cdot D \cdot S \]  

(2.17)

Here \( D \) is the dipole-dipole coupling tensor. Its principal values include \(-d/2, -d/2, d\). And \( d \) is the so-called dipole-dipole coupling constant:

\[ d = \hbar \left( \frac{\mu_0}{4\pi} \right) \frac{1}{r^3} \gamma_I \gamma_S \]  

(2.18)

The dipolar coupling tensor describes how the coupling between two spins is related to the orientation of the \( I - S \) internuclear vector with respect to the applied field.

The average Hamiltonian theory calculation [10] gives

\[ H_D^{\text{hetero}} = -d(3\cos^2 \theta - 1)I_z S_z \]  

(2.19a)
Here $\beta$ is the polar angle specifying the orientation of the $I - S$ internuclear vector with respect to the $B_0$ field along the laboratory $z$-axis. So both homonuclear and heteronuclear coupling is proportional to $\frac{(3\cos^2\theta - 1)/2}{r^3}$, where $r$ is internuclear distance. For deuterons, the ratio of dipolar interaction to quadrupolar interaction is pretty small and thus negligible. Thus in practice it is very difficult to resolve deuteron-deuteron dipolar coupling in powder spectra, although they could contribute to the intrinsic homogeneous line width.

\[ H_{B_0}^{\text{homo}} = -d \cdot \frac{1}{2} (3\cos^2\theta - 1) [3I_z S_z - I \cdot S] \] (2.19b)
2.3 Relaxation

2.3.1 Phenomenological Interpretation

Generally speaking, “relaxation” refers to the returning to the equilibrium state of a physical system after a perturbation is applied. In NMR, relaxation is utilized widely for gaining insight into motional details. As pointed out in section 2.2.1, the spins will distribute unevenly among the Zeeman levels obeying Boltzmann distribution in an external magnetic field, giving rise to a net magnetization vector $M_0$ at equilibrium. Its magnitude is given by Curie’s Law [30]:

$$M_0 = \frac{NY^2h^2(I+1)}{3kT}B_0$$  \hspace{1cm} (2.20)

At equilibrium the magnetization aligns along $z$ and there is no transverse ($x$ or $y$) magnetization. So if transverse magnetization (by applying a 90° pulse) is created, relaxation will cause it to decay away to zero over time. The FID signal resulting from the magnetization precessing in the $xy$ plane will also decay in amplitude. The decaying of this $x$- and $y$- magnetization is defined as transverse relaxation or spin-spin relaxation.

After tilted away from the initial $B_0$ direction, the $z$-magnetization will try to return to its equilibrium position. This is the so-called longitudinal relaxation or spin lattice relaxation. Essentially, the evolution obeys the following monoexponential relation:
\[ M(T) - M(\infty) = [M(0) - M(\infty)] \exp \left( -\frac{T}{T_{1z}} \right) \] (2.21)

Also, spin-lattice relaxation could be equivalently understood as the return to equilibrium population distribution.

### 2.3.2 Density Matrix Formalism

The simple, single-particle Hamiltonian of deuteron makes density matrix a very tractable means of interpreting the effects of radio frequency pulses and relaxation phenomena [10][29]. Based on quantum mechanics, the state of a spin system could be described by a superposition state:

\[
\Phi = \sum_{\psi} \sqrt{p_{\psi}} \psi
\] (2.22)

\(p_{\psi}\) is the probability that an individual spin system is in state \(\psi\). Then the expectation value of a quantity \(A\) is calculated from

\[
< A > = \langle \Phi | \hat{A} | \Phi \rangle = \sum_{\psi} p_{\psi} \langle \psi | \hat{A} | \psi \rangle
\] (2.23)

And each state \(\psi\) could be further expanded in a complete basis set composed of the eigenfunctions of the Zeeman Hamiltonian.

\[
\psi = \sum_{i} c_{\psi i} \phi_i
\] (2.24)
Substituting (2.24) into (2.23):

\[ < A > = \sum_{\psi} p_\psi \sum_{i,j} c_{\psi i}^* c_{\psi j} < \phi_i | A | \phi_j > = Tr(A \rho) \]  \hspace{1cm} (2.25)

Where the matrix element \( A_{ij} = < \phi_i | A | \phi_j > \). And the \textit{density matrix} element is defined as

\[ \rho_{ji} = \sum_{\psi} p_\psi c_{\psi j} c_{\psi i}^* = c_{\psi j} c_{\psi i}^* \]  \hspace{1cm} (2.26)

The bar over means the \textit{ensemble average}. Particularly, the diagonal elements of the density matrix represent the \textit{populations} of the corresponding basis functions. And the nonvanishing off-diagonal elements \( \rho_{ij} \) represents that there is a correlation between basis functions \( \phi_i \) and \( \phi_j \) or equivalently, there is a coherence between \( \phi_i \) and \( \phi_j \) in the superposition state \( \Phi \).

The density matrix for a deuteron spin ensemble is:

\[
\begin{bmatrix}
\rho_{11} & \rho_{12} & \rho_{13} \\
\rho_{21} & \rho_{22} & \rho_{23} \\
\rho_{31} & \rho_{32} & \rho_{33}
\end{bmatrix}
= \begin{bmatrix}
\bar{c}_1 c_1^* & \bar{c}_1 c_2^* & \bar{c}_1 c_3^* \\
\bar{c}_2 c_1^* & \bar{c}_2 c_2^* & \bar{c}_2 c_3^* \\
\bar{c}_3 c_1^* & \bar{c}_3 c_2^* & \bar{c}_3 c_3^*
\end{bmatrix}
\]  \hspace{1cm} (2.27)
Zeeman and quadrupole order could then be constructed from the eigenstate populations as follows:

\[ M_z = \langle I_z \rangle = \rho_{11} - \rho_{33} \]  
(2.28)

\[ Q_z = \langle Q_z \rangle = \rho_{11} - 2\rho_{22} + \rho_{33} \]  
(2.29)

Zeeman order is also called *Zeeman polarization*. Quadrupole order is coined as *spin alignment*.

### 2.3.3 Redfield Theory

In fast motion limit (motional rate \( k > \omega_0 \)), the Redfield motional narrowing theory [32] comes into play. The evolution of the density matrix elements obeys the following equation:

\[ \frac{\partial \rho_{ij}(t)}{\partial t} = i\omega_{ij} \rho_{ij} + \sum_{kl} R_{ijkl} [\rho_{kl}(t) - \rho_{kl}(\infty)] \]  
(2.30)

Here \( \omega_{ij} \) is the transition frequency between eigenstates \(|i\rangle\) and \(|j\rangle\). \( R_{ijkl} \) is element of the Redfield relaxation supermatrix. Detailed calculations of the deuteron Zeeman order and quadrupolar order relaxation times expressed in the spectral
densities of motions are given elsewhere [5][33]. Here only the final results are listed below:

\[
\frac{1}{T_{1z}} = \frac{3\pi^2}{2} \left( \frac{e^2 q Q}{\hbar} \right)^2 J_1(\omega_0) + 4J_2(2\omega_0)) \quad (2.31)
\]

\[
\frac{1}{T_{1Q}} = \frac{9\pi^2}{2} \left( \frac{e^2 q Q}{\hbar} \right)^2 J_1(\omega_0) \quad (2.32)
\]

Thus the relaxation time \( T_{1z} \) and \( T_{1Q} \) are related to the spectral densities of the motions at the single quantum and double quantum transition frequencies. And spectral density is defined as the Fourier transformation of the motional correlation function. Essentially, the decay of quadrupole order (i.e. spin alignment) can be used to probe slow and ultraslow motions and the recovery of Zeeman order can be used to detect fast motions. To summarize, relaxation time measurements of \( T_{1z} \) and \( T_{1Q} \) could be combined together to determine the orientation dependent spectral densities, which enables the probing of fast motions feasible.

**2.3.4 Stochastic Liouville Equation**

For slow molecular motions, the fast motion limit is not valid and the Redfield theory cannot be applied for the calculation of relaxation times any longer. The Stochastic Liouville equation [34][35] gives a complete description because it does not depend on the scale of the motional correlation time:
\[ \dot{\rho}(t) = L(\rho(t) - \rho_{\text{eq}}) \]  \hfill (2.33)

\( L \) is the time-independent Liouville operator and it is composed of two parts: 
\( L = i\Omega + R \). \( \Omega \) is composed of the Zeeman level transition frequencies. \( R \) is derived from the Redfield relaxation supermatrix elements. Specifically, applying this formalism to deuteron spin relaxation and arranging elements of \( \rho \) as a column vector [5],

\[
\rho = \begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix}
\]  \hfill (2.34)

\( P \) is the population vector

\[
P = \begin{pmatrix} \rho_{11} - \rho_{11\text{eq}} \\ \rho_{22} - \rho_{22\text{eq}} \\ \rho_{33} - \rho_{33\text{eq}} \end{pmatrix}
\]  \hfill (2.35)

The \textit{coherence} elements of density matrix are enclosed as

\[
M_+ = \begin{pmatrix} \rho_{12} \\ \rho_{23} \\ \rho_{13} \end{pmatrix}, \\
M_- = \begin{pmatrix} \rho_{21} \\ \rho_{32} \\ \rho_{31} \end{pmatrix}
\]  \hfill (2.36)
Then the Stochastic Liouville equation can now have the form:

$$\frac{d}{dt}\begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix} = \begin{pmatrix} W & i\omega Z & -i\omega Z \\ i\omega Z & \omega - R_2 & 0 \\ -i\omega Z & 0 & -\omega - R_2 \end{pmatrix} \begin{pmatrix} P \\ M_+ \\ M_- \end{pmatrix} \quad (2.37)$$

Here

$$Z = \frac{1}{\sqrt{2}}\begin{pmatrix} 1 & 0 & 0 \\ -1 & 1 & 0 \\ 0 & -1 & 0 \end{pmatrix} \quad (2.38)$$

$\omega_1$ is the rf field frequency. $\omega$ is a diagonal matrix composed of the single quantum and double quantum transition frequencies. $W$ is composed of transition probabilities and $R_2$ is the Redfield submatrix including the transverse relaxation rates.

$$W = \begin{pmatrix} R_{1111} & R_{1212} & R_{1313} \\ R_{2112} & R_{2222} & R_{2323} \\ R_{3131} & R_{3232} & R_{3333} \end{pmatrix} \quad (2.39)$$
CHAPTER 3

Experimental Procedures

This chapter is concerned with the experimental aspect of solid state NMR. Firstly, basic solid state NMR facility is introduced. Secondly, the pulse sequences used to record quadrupole echo and measure the spin lattice relaxation times of Zeeman and quadrupole orders are briefly overviewed. Finally, the problems needed to pay attention to in NMR signal processing are discussed.

3.1 Deuteron Solid State NMR Spectroscopy

First part of the experiment is done using a 17.6 T WB 750 (proton resonance frequency 750MHz) Bruker spectrometer with a 89mm clear bore inside the room temperature shim coil assembly. It is equipped with the AVANCE I electronics with three rf channels. A two-channel (H-X) Broadband static probe with replaceable coils operating in the temperature range from 190K to 330K is used to measure the deuteron quadrupole echo line shapes and the Zeeman order spin lattice relaxation time $T_{1\pi}$.

A 7.05T spectrometer (Oxford 89mm bore magnet) driven by AVANCE 3000 electronics and a two-channel (H-X) 4mm MAS probe are used to record the deuteron
quadrupole echo and spin alignment echo line shapes. Besides, spin lattice relaxation times of Zeeman order $T_{1z}$ and quadrupole order $T_{1Q}$ are measured using this spectrometer.

All experiments were carried out at various temperatures, using a stream of cold nitrogen gas and a heater controlled by a Bruker variable temperature control unit to stabilize within the error of 0.1K. The temperature calibration of the static sample was done using lead nitrate.

A brief outline of the solid state NMR spectrometer is given in Figure 3.1. A superconducting magnet generates the magnetic field used to induce the NMR transitions. The superconducting coil is immersed into liquid helium to maintain its temperature below the critical point. To reduce the evaporation of liquid helium, the heat flow from the NMR lab to the magnet core needs to be minimized. Thus outside the liquid helium tank there is a liquid nitrogen bath. And a vacuum chamber is created between the liquid nitrogen tank and the outside to gain more thermal insulation.

A set of current carrying coils (i.e. shimming coils) is mounted into the lower end of the magnet. It produces additional magnetic field in the region of the sample that will either enhance or impair the static magnetic field. Its aim is to offset any existing inhomogeneous field and maximize field homogeneity. Shimming the magnet well is a prerequisite for gaining good resolution and signal-to-noise ratio. Unfortunately, shimming is a time-consuming process especially for inexperienced spectrometer operators and thus personal experience is the best way to master shimming.
The probe is inserted into the magnet bore, holding the sample. It has special designed rf coils built inside to transmit radio frequency pulses to excite the sample and receive the emitted NMR signal. *Preamplifiers* are applied before the transmittance of rf excitation and the reception of the NMR signal. The amplified response is then received by the receiver and digitized through a digitizer. And the digitized FID is sent to the computer for further processing, i.e. Fourier transforming. Modern pulsed NMR is performed exclusively in the Fourier transform mode. In addition, before finally starting to acquire the data, *tuning* and *matching* procedure need to be done on the probe. Because the sensitivity of the probe varies with the frequency of the transmitted signal and at a certain optimum frequency the probe is most sensitive. Specifically, tuning and matching are accomplished by adjusting the capacitors built into the probe circuitry. In the actual experiment, the Wobble Curve is used for the tuning and matching.
Figure 3.1: Schematic diagram of solid state NMR spectrometer
3.2 Pulse Sequences

3.2.1 “zg” Pulse Sequence

The most common pulse sequence in FT-NMR experiment is the simple pulse-acquire experiment [30] given in Figure 3.2.

In actual NMR experiment, this pulse-acquire sequence will be repeated many times so as to improve the signal-to-noise ratio (S/N), which is proportional to the square root of the number of scans (NS). The radio frequency pulse is irradiated on the sample to excite the nuclei and during the acquisition time the nuclei re-radiate to induce the NMR signal in the form of an exponentially decaying sine wave, which is coined as free induction decay. In BRUKER NMR instrument, the radio frequency pulse has a characteristic frequency-spectrometer frequency SFO1, which is placed in
the center of the spectrum. In reality, no pulse with a single frequency is applied. Due to the existence of various anisotropic interactions, the NMR spectrum is spread into a range of frequencies. Thus to obtain a complete excitation, a pulse with a wide range of frequency components is used. Short pulse with a high power level corresponds to a wide frequency distribution. Long pulse with lower power level possesses much less frequency components.

In order to gain the best result, the pulse needs to be a 90° pulse, which means that the flip angle of the pulse is 90°. The flip angle is calculated from

\[ \theta = \frac{360 \gamma B_1 t_p}{2 \pi} \]  

(3.1)

Here \( \gamma \) is the gyromagnetic ratio, \( B_1 \) is the radio frequency field, \( t_p \) is the time of the pulse (i.e. the pulse width \( p \)). The approximate excitation width is estimated by

\[ EW = \frac{1}{4 \cdot p(90^\circ pulse)} \]  

(3.2)

For example, for a 2\( \mu \)s, the field width is \( \frac{1}{4 \cdot 0.000002} = 125kHZ \). And the quadrupole coupling constant is around 45-55 \( kHZ \) for methyl group in organic compound. So this pulse width is enough to cover the typical range of deuteron resonance in our experiment.
Other important parameters include time domain data size (TD), i.e. the number of points in the FID, acquisition time (AQ) and the spectral width (SW). Generally speaking, AQ should be reasonably large so that enough information is obtained. However, unnecessary long AQ will introduce more noise and thus reduce the signal-to-noise ratio. Furthermore, the spectral width should be set as large as possible at first. Because sometimes the signal may fall out of the spectral window if it is too narrow and thus cannot be detected. In reality, the spectral width, the number of points, and the acquisition time are interrelated through the following equations:

\[
AQ = \frac{TD}{2SW}
\]  

(3.3)

\[
Resolution = \frac{1}{AQ} = \frac{2SW}{TD}
\]  

(3.4)

Here ‘Resolution’ stands for the digital resolution of the spectrum, which is in units of Hz/point. It should be less than a half of the full width at half maximum of the peak (FWHM).

Last but perhaps most importantly, the recycle delay \(D_1\) is very noteworthy. After the radio frequency pulse, the equilibrium magnetization is tilted away from equilibrium and thus it will relax back through a process called ‘spin lattice relaxation’. Or quantum mechanically speaking, the pulse breaks the equilibrium distribution of the nuclear spins among Zeeman levels. Spin lattice relaxation time \(T_1\) is the time it takes for around 63% of magnetization to return to the equilibrium z-axis. Normally, at least \(1.5T_1\) is needed between every scan to ensure that the system relaxes completely [31]. Otherwise, the sample will be saturated, because more and
more energy will be accumulated in the sample and cannot be released. Actually, 1.5$T_1$ gives optimum S/N per unit acquisition time, at the expense of line shape distortion if there is significant $T_1$ anisotropy. You need wait at least 5$T_1$ between scans for full recovery before recording an "infinity" spectrum. The beauty of saturation recovery is that you do not have to wait at all between scans for shorter tau-values, since the starting point is one of complete saturation. Thus comes at the expense of a 2-fold loss of dynamic range (signal recovers from zero to $M(\text{inf})$ rather than from $-M(\text{inf})$ to $M(\text{inf})$ as in inversion recovery).

3.2.2 Quadrupole Echo

Due to the isotropic distribution of crystallite orientations in a solid, polycrystalline sample, there is a corresponding distribution of quadrupolar doublet splittings and the resulting NMR spectrum, which is the sum of independent spectra from each crystallite, is therefore, very broad. Such spectra are sensitive to motions over a wide timescale. Typically in organic compounds, the deuterium quadrupole coupling constant lies in the range of 140-220 kHz, which makes the powder lineshape sensitive to motions with a rate between $10^4 \text{s}^{-1}$ and $10^7 \text{s}^{-1}$.

Broad deuterium powder lineshapes correspond to rapidly decaying FIDs. Due to unavoidable receiver overload from residual pick-up of the observation pulses, the FID signal cannot be measured immediately after excitation, which leads to significant signal loss and line distortions [10]. In order to overcome this receiver dead time problem, the quadrupole echo technique [36,37] has long been used. The quadrupole echo (QE) or solid echo pulse sequence, $90_\theta^\alpha - \tau - 90_\theta^\alpha - \tau - \text{acquire}$,
is applicable for lines broadened by quadrupole coupling. The classic quadrupole echo pulse sequence is shown in Figure 3.3. In the lower half of Figure 3.3, the behavior of transverse magnetization is also illustrated [10].

Figure 3.3: The quadrupole echo pulse sequence. The behavior of the transverse magnetization is shown in the lower half. After the first $90_x$ pulse, the magnetization components will fan out and dephase during the first $\tau$ period. The second $90_y$ pulse rotates the magnetization component $180^\circ$ about the pulse axis and after a further $\tau$ period the magnetization components refocus. Then the FID could be recorded from the true echo maximum. The receiver dead time problem is thus overcome by delaying the start of acquisition to the top of the echo.
After the first $90^\circ$, the equilibrium magnetization is tilted to align in the $x-y$ plane. Under the effects of various anisotropic interactions, the transverse magnetization components would fan out ('dephase') during the first $\tau$ period. The second $90^\circ$ pulse rotates the magnetization components around the pulse axis so that they refocus after $\tau$ time. After that the FID signal is recorded. As long as $\tau$ is appropriately selected to cover the receiver dead time, the FID can then be acquired completely.

In practice, special attention needs to be paid when setting up the quadrupole echo experiment. Firstly, the second $\tau$ period is usually set shorter than the first one so that the true echo maximum can be clearly identified. Actually, the FID should be recorded right from the echo maximum. Briefly speaking, the pulse spacing should be adjusted to give a point exactly at the top of the echo so that an integer number of left shift yields spectra that need no first-order (linear) phase correction. It is also useful to set the receiver phase so that little or no zero order phase correction is needed. The real and imaginary parts of the FID signal are recorded in two channels, and adjusting the receiver phase so that the signal is confined to the real channel alone makes it easier to determine the echo maximum.

Last but not least, an important source of distortion needs to be considered to evaluate the QE lineshape correctly [6]. If orientation-dependent transverse relaxation during the time between the first pulse and the top of the echo is not negligible,
contributions of fast-relaxing crystallites to the quadrupole echo spectrum will be selectively reduced, and this results in characteristic line shape “distortions” that can, if properly simulated, actually provide significant additional information about the rates and trajectories of restricted rational motions.

3.2.3 Saturation Recovery with Quadrupole Echo (QE) Detection

Saturation recovery pulse sequence with quadrupole echo detection, $90^\circ - T - 90^\circ_x - \tau - 90^\circ_y$ [30], is adopted to measure the Zeeman order spin lattice relaxation time $T_{1z}$. It is illustrated in Figure 3.4. The first $90^\circ$ pulse flips the equilibrium magnetization to align within the $x - y$ plane. $T$ is a variable relaxation delay. After a certain $T$, the longitudinal magnetization will partially relax back to the $z$-axis. Following the variable delay is a series of quadrupole echo pulse sequence, which aims at overcoming the receiver dead time and detecting the partially relaxed magnetization. In practice, around ten relaxation delays $T$ that span at least one full decade of recovery are randomly set and a series of partially relaxed QE spectra are recorded. In order to avoid the over saturation of the sample, a recycle delay at least $5T_{1z}$ is left out between every scan.
After obtaining the partially relaxed spectra, $T_{1z}$ is acquired by fitting to the following phenomenological equation:

$$M(T) - M(\infty) = [M(0) - M(\infty)] \exp \left( -T / T_{1z} \right)$$  \hspace{1cm} (3.5)$$

$M(T)$ is the sum intensity of two symmetrically selected points on the quadrupole echo spectra at time $T$, $M(\infty)$ is its equilibrium value, and $M(0)$ is the intensity immediately after the first saturation pulse. In essence, perfect saturation pulse should give $M(0) = 0$. 

Figure 3.4: Saturation recovery with solid echo detection pulse sequence
3.2.4 Broadband Jeener-Broekaert (BBJB) Pulse Sequence

In order to gain complete excitation and create quadrupole order over a full width powder pattern, the broadband Jeener-Broekaert pulse sequence (BBJB) [38-40] is used instead of the frequency selective conventional Jeener-Broekaert sequence, which is illustrated in Figure 3.5. Quadrupole order $< Q_z > = \rho_{11} - 2\rho_{22} + \rho_{33}$ correspond to the components of the quadrupole doublet pointing parallel and antiparallel to the magnetic field. Thus it is also called spin alignment.

A series of broadband composite excitation sequence is used to excite the quadrupole order. $D_1$ is the recycle delay, $5\tau$ is the total pulse spacing and $T$ is the variable relaxation delay. $D_3$ is the pre-echo pulse delay and $D_4$ is the post-echo pulse delay. An echo pulse is applied after the relaxation delay $T$ to move the resulting FID out of the receiver dead time. This BBJB scheme has been proven to be frequency independent and give a uniform excitation of the quadrupole order, which also provide more accurate and reliable results.

In practice, the last echo pulse is set up separately to make it a variable echo pulse. With proper phases that were set to give undistorted QE line shapes for the saturation recovery measurement of $T_{1z}$, the BBJB time domain signals will have a zero crossing at exactly the same point as the echo maximum for $T_{1z}$ signals. Thus the same number of left shifts could be used to get undistorted quadrupole order spectra.
In principle, the relaxation of the quadrupole order obey the following relation:

\[ Q_z(T) = \exp(-R_{1Q}T)Q_z(0) \]  

(3.6)

Where \( R_{1Q} \) is the relaxation rate of the quadrupolar order. \( Q_z(T) \) is the difference of the intensities corresponding to two symmetrically selected points in the partially relaxed quadrupole spectra at time \( T \).

Figure 3.5: Broadband Jeener-Broekaert (BBJB) pulse sequence with QE detection. This sequence could achieve uniform excitation of the quadrupole order and allow for variable echo detection. The width of the echo pulse could be adjusted so that an echo maximum is achieved.
3.3 Practical Signal Processing Issues [41]

The relevant signal processing including $T_{1z}$ and $T_{1Q}$ calculation was done using a home-built NMR data processing software written by Professor Vold. Four times zero filling is applied to the experiment FID before subsequent Fourier transform. As stated in Section 3.1, the digital resolution is $\frac{1}{AQ}$. Increasing the acquisition time might work to gain better resolution. However, the trade-off of increasing too much is the decrease of the signal-to-noise ratio. Because the FID has a finite lifetime determined by the longitudinal relaxation time $T_1$. Simply increasing the acquisition time would increase the total experiment time and acquire unwanted noise. Fortunately, zero filling, (i.e. padding the FID by zero intensity points) provides a means of improving the digital resolution while at the meantime keeping the signal-to-noise ratio.

The emitted radio frequency signal in NMR spectroscopy is very weak and thus it suffers from low signal-to-noise ratio (S/N). S/N could be improved by increasing the number of scans (NS) as stated in section 3.1. Another very important approach is apodization, which means applying a weighting function (or window function) to the FID. The most used weighting function for NMR spectra is the exponential window multiplication function, which multiplies each data point $i$ with factor $\exp\left(-\frac{(i-1)LB\pi}{2SW}\right)$. Here LB is the line-broadening factor and SW is the spectral width. In practice, a 2000 Hz line broadening is applied to the QE and spin alignment echo spectra to filter out the noise.
Furthermore, additional zero order and first order phase corrections \((phasing)\) are applied so that the spectra display purely absorptive peaks. The zero-order phase correction is frequency independent and can be replaced by properly adjusted receiver phase that makes the FID signal for a quadrupole echo experiment appear exclusively in the real channel. The first-order phase correction is frequency dependent. In practical experiments, the pulse spacing in the QE sequence can be adjusted to give a spectrum with one point exactly at the echo maximum. In this case, an integer number of left shifts applied prior to Fourier transformation obviates the need for post acquisition, first-order phase correction.
CHAPTER 4

Motional Models: Correlation Functions

and Spectral Densities

In typical molecular crystals, molecular motions are not isotropic due to the highly
constrained environment. In many circumstances the molecules are confined to
potential wells separated by high activation energy barriers. Thus the motion is better
described by a random walk along a spatially restricted and well-defined angular
trajectory [5][42,43]. Basically, the motional models of this situation are divided into
two categories. Firstly, the motion could proceed as sudden (Markovian) jumps
among a number of discrete orientational sites. Alternatively, the motion may proceed
by continuous, small step diffusive motion. For the actual simulations in this thesis,
the computationally more efficient discrete jump formalism is adopted.

4.1 Construction of Motional Models [43]

In a frame rotating at the deuteron Larmor frequency, the single-particle deuteron
Hamiltonian is time dependent due to the modulation of the random molecular
motions. After constructing an averaged Hamiltonian, the Hamiltonian is expressed
by [5]
\[ H_Q = H_Q + H(t) - \dot{H}_Q = \dot{H}_Q + H'(t) \] (4.1)

\( H'(t) \) is responsible for relaxation and it fluctuates about zero. Recall the definition of \( H_Q \) in the form of irreducible spherical tensors in Section 2.2.2, we have

\[ H'(t) = \sum_{m=-2}^{2} (-1)^m T_{m}^{(2,Q)} [R_m^{(2,Q)}(t) - < R_m^{(2,Q)} >] \] (4.2)

The spin operator \( T_{2m}^2 \) and the lattice variable \( R_m^2 \) are both defined within the experiment laboratory frame with \( z - \text{axis} \) parallel to the external magnetic field.

Dropping out the superscript \( Q \) the rotational molecular motions could be enclosed into the Wigner rotation matrix elements, which transform the quadrupole coupling tensor from its principal axis frame \( (p) \) to the lab frame \( (l) \) through the Euler angles \( \Omega_{lp}(t) = (\alpha_{lp}, \beta_{lp}, \gamma_{lp}) \):

\[ R_m^{2,l}(t) = \sum_{n=-2}^{n=2} D_{mn}^{2*} [\Omega_{lp}(t)] R_n^{2,p} \] (4.3)

\( T_{n}^{2,p} \) is the component of the EFG coupling tensor in its principal axis system.

More detailed molecular motions could be modeled by dividing the transformation from the lab to the PAS frame into two or three steps as needed: from the PAS frame
(p) to the molecule-fixed system (m); from the molecular frame to the crystal-fixed frame (x); finally from the crystal frame to the lab frame (f):

$$R_{m}^{2,j}(t) = \sum_{lmn} D_{ml}^{2}(\Omega_{ls}) D_{hl}^{2}(\Omega_{sm}(t)) D_{kn}^{2}(\Omega_{np}) R_{n}^{2,p}$$  \hspace{1cm} (4.4)

In practical simulations the Wigner rotations can be done easily using the home-built NMR simulation suite written by Professor Robert L. Vold [27]. Generally, this involves defining a sequence of coordinates axes (“frames”), whose relative orientations constitute a rotational “trajectory” from the principal axis frame to the crystal-fixed reference frame. Different motional models correspond to different angular trajectories and jump rates among the orientational “sites” defined in each frame.

Anyway, herein the theoretical definitions and calculations of the motional correlation functions as well as spectral densities are given for illustration following the formalism put forth by Torchia and Szabo [43] based only on the two-step transformation. Recall that the spin lattice relaxation times of the Zeeman and quadrupole order could be expressed by the spectral densities corresponding to the single quantum and double quantum spin flips $J_1(\omega_0)$ and $J_2(2\omega_0)$. The spectral density functions are defined as the Fourier transformation of the correlation functions:

$$J_{m}(m\omega_0) = \int_{0}^{\infty} C_{m}(t)e^{-im\omega_0 t} dt$$  \hspace{1cm} (4.5)
The autocorrelation function $C_m(t)$ is given by

\[ C_m(t) = \langle R_m^{2,(t)}(0) R_m^{2,(t)}(t) \rangle / \langle R_0^{2,(o)} \rangle^2 \quad (4.6) \]

Where $\langle \rangle$ stands for ensemble average. Adopting the two-step transformation: from lab frame ($l$) to the crystal frame ($x$) through $\Omega_{xl}$; from the crystal frame to the principal axis frame ($p$) through $\Omega_{px}$, the correlation function is rewritten as

\[ C_m(t) = (R_0^{2,(p)})^{-2} \sum_{n, n', \alpha, \alpha'} R_n^{2,(p)} R_{n'}^{2,(p)} D_{nn'}^{2*}(\Omega_{p}(t)) D_{\alpha \alpha'}^{2} (\Omega_{p}) \]

\[ \times \langle D^2_{\alpha \alpha} (\Omega_{px}(0)) D^2_{n n'} (\Omega_{px}(t)) \rangle \quad (4.7) \]

For carbon-bonded deuteron it is reasonable to presume that the quadrupole coupling tensor is axially symmetric ($\eta = 0$). Then only the tensor components having $n = n' = 0$ are retained to simplify the correlation function as

\[ C_m(t) = \sum_{\alpha \alpha'} d^{2}_{m\alpha} (\theta) d^{2*}_{m\alpha} (\theta) e^{i(\alpha - \alpha')} C_{\alpha \alpha'} (t) \quad (4.8) \]

The correlation function in the crystal-fixed frame is given by
\[ C_{aa'}(t) = \langle D_{0u}^{zz}(0,0,0) D_{0u}^{zz}(0,0,0) \rangle \]  \hspace{1cm} (4.9)

\((\theta, \phi)\) are the polar angles that define the orientation of the external magnetic field \(B_0\) in the crystal-fixed frame. Likewise \((\Theta, \Phi)\) are the polar angles that specify the orientation of the principal \(z-axis\) in the crystal frame. Their definitions are illustrated in Figure 4.1.

![Figure 4.1](image.png)

Figure 4.1: Definitions of the polar angles \((\theta, \phi)\) and \((\Theta, \Phi)\)

\(C_{aa'}(t)\) describes the reorientation of the principal \(z-axis\) \((Z_p)\) of the quadrupole coupling tensor. Usually \(Z_p\) aligns along the direction of the C-D bond. The specific calculation of \(C_{aa'}\) depends on the motional model and is deferred to the next section.
4.2 Discrete Jumps Model

The motional models used for simulation in this thesis were put forth by Torchia and Szabo [43] and further developed by Vold and Vold [5]. Due to the fact for steric reasons, molecules in molecular solids typically exist in deep potential wells separated by high barriers to rotation, the motions that do occur are often best described by discrete (Markovian) jumps among a small number of discrete orientational “sites”.

The N-site jump model. Denoting the direction of $Z_p$ (i.e. the symmetry axis of the coupling tensor) by a unit vector $z_p$, then $z_p$ is assumed to jump among N discrete orientations in the crystal frame. The orientation of site $i$ is specified by Euler angles $\Omega_i = (\Theta_i, \Phi_i)$. Defining the rate of jump from site $j$ to site $i$ as $k_{ij} = k_{i\rightarrow j}$, the principle of microscopic reversibility requires that

$$p_j k_{ij} = p_i k_{ji}$$  \hspace{1cm} (4.10)

Where $p_j$ stands for the equilibrium population of site $j$.

Following the scheme in Torchia and Szabo and using the standard matrix method, the correlation function $C_{aa'}(t)$ is given by

$$C_{aa'}(t) = \sum_{j=1}^{N} \sum_{i=1}^{N} \sum_{m=2}^{N} (p_i p_j)^{1/2} U_{m} U_{m} e^{\lambda_j t} D_{0a} (\Omega_i) D_{0a} (\Omega_j)$$  \hspace{1cm} (4.11)
Where \( \Omega_i = (\Theta_i, \Phi_i) \) and \( \Omega_j = (\Theta_j, \Phi_j) \) are the Euler angles specifying the orientation of site \( i \) and site \( j \) in the crystal frame respectively, and \( \mathbf{U} = \{\mathbf{U}_{jn}\} \) is the orthogonal matrix that diagonalizes the real symmetric matrix \( \mathbf{K} \), whose eigenvalues are \( \lambda_n \). \( \mathbf{K} \) is constructed from the original matrix of site-to-site jump rates by the symmetrizing transformation

\[
K_{ij} = (k_{ij} k_{ji})^{1/2} = \left(p_j\right)^{-1/2}k_{ji}(p_i)^{1/2}
\] (4.12)

A very important and widely used class of models consists of precessional jumps such that the polar angle \( \Theta \) is constant and only \( \Phi \) is time dependent. In this case [43] the correlation function reduces to

\[
C_{aa'}(t) = d_{\Theta\Theta}^2(\Theta)d_{\phi\phi'}^2(\Theta)\Gamma_{aa'}(t)
\] (4.13)

\( d_{\Theta\Theta}^2(\Theta) \) is the reduced Wigner rotation matrix. Specifically, the \textit{N-Equivalent sites with nearest neighbor jumps} model is used in our simulations. Suppose that \( z_p \) could execute nearest neighbor jumps among \( N \) sites on an arc with \( \Theta_j = \Theta \) and \( \Phi_j = \frac{2\pi j}{N} \), \( j = 0, 1 \ldots N - 1 \). The equilibrium site population for every site is the same. The nearest jump between site \( j \) and \( j+1 \) proceeds at constant rate \( k \). Then it can be shown [43] that
\[ \Gamma_{aa'}(t) = e^{-4ktsin^2(\pi a/N)} \quad a = a' \mod N \]

= 0 otherwise \hspace{1cm} (4.14)

In this research, we mainly focus on modeling the motion of a methyl group attached to a rigid carbon. A three-site jump model with correlation time \( \tau = 1/3k \) is plausible. For this model

\[ \Gamma_{aa'}(t) = 1 \quad a = a' = 0 \]

\[ = e^{-t/\tau} \quad a = a' = \pm 1, \pm 2, \]

\[ a = 1, a' = -2 \]

\[ a = -1, a' = 2 \]

\[ a = 2, a' = -1 \]

\[ a = -2, a' = 1 \] \hspace{1cm} (4.15)

It follows from Eqs. 4.14 and 4.15 that the correlation functions, and hence ultimately the spin relaxation rates, will be different for crystallites that are oriented differently with respect to the external magnetic field. Moreover, assuming axial symmetry of the EFG tensor, the spectral line frequency depends only on powder angle \( \theta \). Thus, relaxation rates observed for different points on the powder line shape are expected to exhibit a characteristic anisotropy. Since the deuteron spin lattice relaxation times depend on both \( \theta \) and \( \phi \) due to the existence of the nondiagonal terms in the correlation function, the magnetization from a given point on the line shape may not evolve in time as a single exponential function, but this effects turns out to be small.
and it is possible to interpret the observed recovery by averaging the theoretical expressions over all values of $\phi$.

Correlation functions and spectral densities for more complicated models are given in the literature and will not be detailed here. Notably, additional librational motion of the axis about which the methyl group rotates can also be described by discrete jumps. By decomposing the Wigner rotation from the lab frame to the principal axis frame into successive transformations, the correlation functions for the methyl group symmetry axis motions can be constructed following the above scheme. It is of special importance to keep in mind that this additional motion will lead to further averaging of the deuteron quadrupole coupling constant and may produce unusual relaxation behavior which cannot be accounted for by the simple three-site jump model. Detailed discussions are deferred into the next chapter.

### 4.3 Practical Simulations

The practical simulations are performed using the NMR simulation software suite EXPRESS [27] developed by Professors Robert L. Vold and Gina Hoatson. With this software, we avoid the tedious and error-prone calculation of analytic expressions for the correlation functions and their Fourier transforms, i.e. the spectral densities. EXPRESS stands for “Exchange Program for Relaxing Spin Systems” and features a Graphical User Interface for simulating effects of jump type motion on various pulsed NMR experiments. The spectrometer parameters (including the nucleus to be simulated, a menu of possible pulse sequences, the Larmor frequency, spectral
acquisition window, etc.) and parameters of the motional model (including the site populations, frame-specific jump rates and site-to-site connectivities) are all input through the interface. EXPRESS then constructs an equivalent one-frame rate matrix from the user-specified set of intermediate jump frames and performs summations of Wigner rotation matrix elements like those shown in Eq. 4.7 internally. The correlation functions and spectral densities are internally computed for a user-specified (optimally chosen) set of crystallite orientations and the total signal for a particular pulse experiment is obtained by summing over the FID computed for each crystallite.
CHAPTER 5

A Study of Molecular Dynamics in

*Fmoc – Alanine – d$_3$*

5.1 Overview

The idea of devising and validating various multi-parameter motional models in N-protected amino acid derivatives originates from the desire to quantify molecular motions of protein side chains. It has been well recognized that the motion of protein side chains and their interaction with the protein backbone and other side chains play an important role in the protein structure and conformation, which in turn relates to biological function. This structure-dynamics-function relationship necessitates the quantitative investigation of protein side chain dynamics [11-17]. Many research results have demonstrated that the NMR line shape and anisotropic relaxation times of a selectively introduced –CD$_3$ group are able to provide unambiguous, quantitative information about the relevant
motional time scales [44-46], but obtaining more detailed method that allows one to experimentally distinguish among different models for composite motions is more challenging.

N-protected amino acid derivatives are widely used in the synthesis of synthetic polypeptides and many such derivatives are commercially available with specifically deuterated methyl groups. Amino acid derivatives with the bulky protecting group 9-fluorenylmethoxycarbonyl, (Fmoc) or t-butoxycarbonyl (Boc), are of special interest in this thesis. The bulky protecting group interferes with crystal packing and thus produces a less sterically hindered environment for solid-state side-chain motion in comparison to that of the crystalline amino acid itself, [24-26]. Furthermore, these model systems still retain some protein-like features grouping that the methyl-bearing side chain is attached through a peptide link to a larger, less mobile moiety, in a manner which can result in restricted side chain motions of similar amplitude to those hypothesized for real proteins. These motions in real protein side chains are forbiddingly complicated and the most informative experiments are plagued by very low signal to noise ratio due to high molecular weight and limited sample quantities. Thus, the N-protected amino acid derivatives provide a welcome opportunity test of the relevant models on systems that are complex enough to be relevant yet simple enough to be tractable.

In this thesis Fmoc-Alanine-d$_3$ is chosen for detailed study. The structure of Fmoc-Alanine-d$_3$ is illustrated in Figure 5.1. This is perhaps the simplest useful model system since the “side chain” consists of a single methyl group. Attached
to the central carbon are a hydrogen atom, a carboxylic group, one deuterated methyl group (-CD₃) and an amino group with one of its hydrogen replaced by the bulky 9-fluorenylmethoxycarbonyl (Fmoc) functional group.

Figure 5.1: Structure of Fmoc-Alanine-d₃. The deuterated methyl group we are interested in modeling is labeled by blue color and it is attached to the central carbon
5.2 Experimental Methods

Sample Preparation and Experiment Setup

QE line shape and SRQE relaxation experiments were carried out in first at 17.6 T (deuteron Larmor frequency 115.157 MHz), using a WB750 Bruker spectrometer equipped with AVANCE I electronics and a two-channel (H-X) Broadband DVT static (non-spinning) probe operating between 190 K and 330K. Subsequently, QE line shape, SRQE and broad band Jeener-Broekaert (BBJB) relaxation experiments were performed using a 7.06T (46 MHz deuteron Larmor frequency) spectrometer (Oxford 89mm bore magnet) driven by Bruker AVANCE I electronics and a two-channel (H-X) 4mm MAS probe operating between 230K and 330K with a Bruker DVT control unit for controlling the temperature. The powdered Fmoc-Ala-OH-3, 3, 3-d$_3$ purchased from Sigma-Aldrich and used without purification, was packed in 3.2mm and 4mm rotors for measurements at high and low field respectively. The 3.2mm rotor was wrapped by Parafilm to fit into the 5mm coil in the static probe.

The line shape experiments were carried out at 17.6T by applying a quadrupole echo pulse sequence with a delays 50 $\mu$s between the 90° pulses and 18 $\mu$s prior to data acquisition to ensure that a true echo maximum is observable. The duration of the 90° pulse was 2.1 $\mu$s and the transmitter power level was -5dB. For the $T_{1z}$ measurement by SQRE, 1024 scans were used for each of 10 relaxation delays in order to achieve adequate signal-to-noise ratio.
At 7.06 T, the quadrupole echo experiments were performed with a 40μs interpulse delay and a 15μs delay prior to acquisition. These acquisition delays were adjusted so that several points could be observed prior to the echo maximum, and fine tuned such that the point at the echo maximum was flanked symmetrically by points on either side. Also, the carrier frequency was adjusted to the exact midpoint of the powder pattern and receiver phase was adjusted so that the FID signal focuses mostly in the real channel. The 90° pulse length was 2.2μs at power level 0dB. The number of scans was 2048 and the spectral window was 200kHz. T_{1z} measurements were performed using the SQRE sequence. Pre-scan delay is 4.5μs. Particularly, the recycle delay between successive scans is set at 150ms to make sure that the system returns to thermal equilibrium before every scan starts.

T_{1Q} measurements were done using the BBJB sequence with variable echo detection. Note that the 90° pulse length for echo detection in this experiment was optimized at 2μs, slightly shorter than the value used for SQRE measurements. 150ms recycle delay is still enough and 5μs pulse spacing was used to give a good excitation of the quadrupole order across the line shape. 10 and 8 relaxation delays were used for T_{1z} and T_{1Q} measurements, respectively.
Data Processing

Data processing was done using the home-built NMR data processing software NMRLV developed by Professor Vold. For 17.6T, time domain data were left shifted and apodized with 500Hz exponential line broadening to filter out high frequency noise. Meanwhile, the 1024 points was padded with zeros to 4096 points prior to Fourier transformation to improve the apparent resolution.

For relaxation experiments at 7.6T, 2000 exponential apodization was needed to filter the noisier signals generated at lower magnetic field, 2048-point FIDs were zero-filled to 4096 points before Fourier transformation. The pulse spacings of the quadrupole echo detection part of the pulse sequence in T_{1z} experiments were carefully adjusted so that an integer number of left shifts could place the true echo maximum at zero time, and thus no first-order phase correction was needed. Then in the BBJB experiments the same pulse spacings were used, and the same number of left shifts thus yielded the expected zero crossing of the BBJB echo [47] at the proper position, even though this point could not be accurately set by direct observation since the signal is zero at the time of the echo.

Finally, in order to obtain the relaxation times the signal intensities at selected frequencies, i.e., points on the line shape, were fitted as a function of relaxation delay to mono-exponential recovery functions for T_{1z} (Equation 3.5) and mono-exponential decay functions for T_{1Q} (Equation 3.6). Signal intensities for T_{1Z} were determined as the sum of the intensities for two symmetric points selected on the quadrupole echo
line shape, while for $T_{1O}$, signal amplitude was taken as the difference between the two symmetrically chosen line shape points.

**Temperature Control and Calibration**

The Bruker variable temperature control unit (VTU) allows automatic control of sample temperature. Low temperatures were achieved by flowing the cold nitrogen gas past the probe coil. A heater works together with the cold gas to produce the desired sample temperature. After setting the controller to a new temperature, acquisition was deferred for 20 to 30 minutes to allow the system to reach steady state with constant temperature and minimal thermal gradients across the sample. Because the temperature sensor cannot be located inside the sample tube, the set-point temperature is not the true sample temperature. Temperature calibration of the static probe was done using an internal standard of solid lead nitrate [50-52], as described in Appendix A.

**5.3 Line Shape and Relaxation Data Summary**

**Line Shape Analysis**

At 7.06 T, quadrupole echo spectra were recorded between 230K and 330K. The spectra recorded at various temperatures are shown in Figure 5.2. Along with each spectrum, the actual temperature and the quadrupole coupling constant measured from the separation between horn positions are shown, respectively. For each temperature the horn positions and the calculated $C_Q$ are listed in Table 5.1.
Table 5.1: Quadrupole coupling constant at various temperatures.

<table>
<thead>
<tr>
<th>Set-point T (K)</th>
<th>True T (K)</th>
<th>left horn (KHz)</th>
<th>right horn (KHz)</th>
<th>Cq(KHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>330</td>
<td>346.1</td>
<td>-18.65</td>
<td>17.8</td>
<td>48.6</td>
</tr>
<tr>
<td>320</td>
<td>333.9</td>
<td>-18.67</td>
<td>18.05</td>
<td>48.9</td>
</tr>
<tr>
<td>310</td>
<td>321.2</td>
<td>-18.66</td>
<td>18.11</td>
<td>49</td>
</tr>
<tr>
<td>300</td>
<td>310.6</td>
<td>-18.73</td>
<td>18.12</td>
<td>49.1</td>
</tr>
<tr>
<td>290</td>
<td>297.8</td>
<td>-18.56</td>
<td>18.55</td>
<td>49.5</td>
</tr>
<tr>
<td>280</td>
<td>286.4</td>
<td>-18.72</td>
<td>18.4</td>
<td>49.6</td>
</tr>
<tr>
<td>270</td>
<td>274.3</td>
<td>-18.72</td>
<td>18.53</td>
<td>49.7</td>
</tr>
<tr>
<td>260</td>
<td>264.2</td>
<td>-18.77</td>
<td>18.57</td>
<td>49.8</td>
</tr>
<tr>
<td>250</td>
<td>253.4</td>
<td>-18.8</td>
<td>18.67</td>
<td>50</td>
</tr>
<tr>
<td>240</td>
<td>240.1</td>
<td>-19.2</td>
<td>18.4</td>
<td>50.1</td>
</tr>
<tr>
<td>230</td>
<td>228.7</td>
<td>-19.2</td>
<td>18.43</td>
<td>50.2</td>
</tr>
</tbody>
</table>

According to Ref [26], quadrupole coupling constant (Cq) for the methyl group in Alanine-d$_3$ is 55.0 KHz and the asymmetry parameter is zero within experiment error. The small Cq is due to the fast rotational motion of the methyl group about its threefold spinning axis [25,26], and the zero asymmetry parameter is the result of the threefold symmetry. The quadrupole coupling constants we obtain for Fmoc-alanine-d$_3$ are all smaller than those for Alanine-d$_3$, and they exhibit a small (~3%) decrease with increasing temperature. This suggests that the methyl spinning axis experiences progressively larger amplitude libration as temperature increases.
Figure 5.2: Experimental quadrupole echo spectra measured at 7.06 T spectrometer and various temperatures. Temperatures and corresponding quadrupole coupling constant are specified for each spectrum.
Relaxation Data

The spin lattice relaxation time of the Zeeman order $T_{\text{iz}}$ was measured using the SRQE sequence. Typical partially relaxed spectra of Zeeman order are shown as a function of relaxation delay in Figure 5.3. Relaxation times across the line shape were obtained by fitting the signal intensities as a function of the relaxation delay according to Equation (3.5):

$$M(T) - M(\infty) = [M(0) - M(\infty)] \exp \left(-\frac{T}{T_{\text{iz}}^*}\right)$$

(3.5)

Here $M(T)$ stands for the signal intensity, which was taken as the sum of the intensities of two points symmetrically placed about the center of the line shape (corresponding to total $z$-component of magnetization, which is expected to recover as a single exponential). $M(\infty)$ represents the intensity at thermal equilibrium (i.e., for infinite relaxation delay) and $M(0)$ should be zero for saturation recovery. In practice, accurate relaxation times were obtained only if these parameters, as well as $T_{\text{iz}}$ itself, are incorporated in a 3-parameter nonlinear least squares fit. Representative semi-log plots of $(M(\infty)-M(\tau))/M(\infty)$ vs. relaxation delay, $\tau$, are shown in Figure 5.4 for three points on the line shape corresponding to the shoulders, horns and center of the spectrum. The overall $R_{\text{iz}}$ profile is shown in Figure 5.5. Recall that the characteristic powder pattern is due to the isotropic distribution of crystallite orientations in the polycrystalline powder, so that the $R_{\text{iz}}$ profile reflects the orientation dependence of the relaxation rates and their underlying spectral densities.
Figure 5.3: Partially relaxed spectra of Zeeman order as a function of the relaxation delay. A quadrupole echo is applied after a 90° saturation pulse.
Figure 5.4: Semi-logarithmic plots of recovery of Zeeman order measured at 310K and 7.05T (46MHz) as a function of relaxation delay $\tau$. ■: shoulder, $T_1=22.1\pm3.8\text{ms}$; •: horn, $T_1=36.6\pm1.6\text{ms}$; inverse triangles: center, $T_1=32.3\pm2.1\text{ms}$. 
Figure 5.5: \( R_{1z} \) anisotropy profile of Fmoc-Alanine-\( d_3 \) at 310K and 46 MHz Larmor frequency.
**Relaxation of quadrupole order:** $T_{1Q}$ was measured using the BBJB sequence. Experimental partially relaxed spectra at 310K were given in Figure 5.6. The pulse phases were cycled during the experiment to cancel Zeeman order while preserving quadrupole order, which decays to zero at thermal equilibrium. Relaxation times were obtained by fitting signal intensities to this decay according to Eq. (3.6):

$$Q_Z(T) = \exp(-R_{1Q}T)Q_Z(0)$$

(3.6)

Here signal intensity $Q_Z(T)$ is the *difference* between two intensities selected symmetrically on opposite sides of the line shape. Specifically, the decay for the horn is shown in Figure 5.7. The corresponding relaxation time $T_{1Q}=50.1\pm1.3$ ms is significantly longer than $T_{1Z}$. The $R_{1Q}$ profile, shown in Figure 5.8, exhibits prominent anisotropy very different from that found for $R_{1Z}$. Recall in the last section that position on the line shape corresponds to different crystallite orientations with respect to the external field. Analysis of this orientation dependence provides information about both the rates and the trajectories of restricted orientational motion.
Figure 5.6: Partially relaxed spectra of quadrupole order as a function of the relaxation delay measured at 46 MHz and 310K.
Figure 5.7: Semi-logarithmic plot of experimental decay curve of quadrupole order as a function of relaxation delay $\tau$. Blue circles stand for experimental data corresponding to the line shape horns and the dashed line is the best linear fit to a single exponential decay. $T_{1Q}=50.1\pm1.3$ ms.
Figure 5.8: $R_{1Q}$ anisotropy profile of Fmoc-Alanine-d$_3$ at 310K and 46 MHz Larmor frequency.
Spectral Densities $J_1(\omega_0), J_2(2\omega_0)$

In Table 5.2, spin lattice relaxation times of Zeeman and quadrupole order $T_{1Z}, T_{1Q}$ are listed along with the spectral densities $J_1(\omega_0), J_2(2\omega_0)$ for Fmoc-alanine-d$_3$ in 7.06 T magnetic field at 310K temperature. As outlined in Chapter 2, $R_{1Z}$ and $R_{1Q}$ can be expressed in terms of the motional spectral densities at the Larmor frequency and twice the Larmor frequency. Thus, both $J_1(\omega_0)$ and $J_2(2\omega_0)$ can be determined [48] as a function of crystallite orientation by simple inversion of Eqs. (2.31) and (2.32):

$$R_{1Z} = \frac{1}{T_{1Z}} = \frac{3\pi^2}{2} C_q \, \omega_0^2 \,[J_1(\omega_0) + 4J_2(2\omega_0)] \quad (2.31)$$

$$R_{1Q} = \frac{1}{T_{1Q}} = \frac{9\pi^2}{2} C_q \, \omega_0^2 \, J_1(\omega_0) \quad (2.32)$$

In Figure 5.9 both spectral densities are plotted with respect to the quadrupole frequencies and a quadrupole echo spectrum is given as a reference for the chosen points. Both $J_1(\omega_0)$ and $J_2(2\omega_0)$ exhibit orientation dependence, which depends characteristically on the details of the motional trajectories and rates. Thus by comparing the simulated orientation dependence of $J_1(\omega_0)$ and $J_2(2\omega_0)$ with experimental anisotropy profiles, the motional model can be validated.
Table 5.2: Experimental relaxation rates $R_{1Z}$ and $R_{1Q}$ and the motional spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$, of Fmoc-Alanine-d$_3$ in 7.06 T magnetic field at 310K.

<table>
<thead>
<tr>
<th>$v_Q(KHz)$</th>
<th>$T_{1Z}(ms)$</th>
<th>$T_{1Q}(ms)$</th>
<th>$J_1(\omega_0)(ps)$</th>
<th>$J_2(2\omega_0)(ps)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.75</td>
<td>24.25±1.1</td>
<td>57.22±6.8</td>
<td>163.08±19.5</td>
<td>247.87±15.3</td>
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<td>29.285</td>
<td>26.52±1.5</td>
<td>65.4±7.6</td>
<td>142.69±16.7</td>
<td>228.21±11.1</td>
</tr>
<tr>
<td>26.843</td>
<td>26.64±1</td>
<td>62.8±6.6</td>
<td>142.50±15.6</td>
<td>225.57±6.53</td>
</tr>
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<td>24.35</td>
<td>29.95±1.1</td>
<td>58.7±5.17</td>
<td>158.92±13.3</td>
<td>193.93±5.5</td>
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<td>21.912</td>
<td>31.76±0.9</td>
<td>55.98±2.5</td>
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<td>178.69±4.4</td>
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<td>20.276</td>
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<td>51.73±1.3</td>
<td>180.39±4.6</td>
<td>145.61±5.13</td>
</tr>
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<td>18.225</td>
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<td>50.8±1.03</td>
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<td>145.44±3.2</td>
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<td>15.369</td>
<td>35.47±1.5</td>
<td>58.7±1.72</td>
<td>158.72±4.6</td>
<td>157.61±7.2</td>
</tr>
<tr>
<td>13.318</td>
<td>34.70±1.4</td>
<td>63.2±2.4</td>
<td>147.47±5.5</td>
<td>164.81±6.9</td>
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<td>10.437</td>
<td>33.55±1.5</td>
<td>70.37±3.4</td>
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<td>175.45±8.1</td>
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<td>4.285</td>
<td>31.56±1.3</td>
<td>79.44±9.4</td>
<td>117.4±13.99</td>
<td>192.39±6.2</td>
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<td>-3.894</td>
<td>32.22±1.3</td>
<td>81.2±10.5</td>
<td>114.9±14.93</td>
<td>188.49±5.3</td>
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<td>71.98±3.6</td>
<td>129.64±6.53</td>
<td>177.36±8.86</td>
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<td>35.21±1.3</td>
<td>62.0±1.92</td>
<td>150.42±4.66</td>
<td>161.17±6.53</td>
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<td>-16.199</td>
<td>35.85±1.4</td>
<td>56.9±1.29</td>
<td>163.80±3.7</td>
<td>154.28±6.76</td>
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<td>-18.665</td>
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<td>185.98±3.7</td>
<td>144.17±3.96</td>
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<td>-21.13</td>
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</tr>
<tr>
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<td>57.72±4.6</td>
<td>161.68±13</td>
<td>199.52±10.7</td>
</tr>
<tr>
<td>-27.673</td>
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<td>66.43±5.7</td>
<td>140.46±12.1</td>
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<td>59.62±5.6</td>
<td>156.53±14.9</td>
<td>245.06±11.66</td>
</tr>
</tbody>
</table>
Figure 5.9: Motional spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ as a function of spectral frequencies corresponding to points selected across the quadrupole echo line shape. Anisotropy (orientation dependence) of $J_1(\omega_0)$ and $J_2(2\omega_0)$ can be used to validate the motional models.
**Temperature Dependence of $R_{1\text{Z}}$ – Activation Energy**

In fast motion limit ($\omega_0 \tau_c \ll 1$), for the deuteron relaxation in Fmoc-Alanine-d$_3$, the following relation exists [43]:

$$R_{1\text{Z}} \propto C_q^2 \tau_c \propto C_q^2 k \quad (5.1)$$

Where $\tau_c$ is the motional correlation time assuming that the methyl group executes threefold rotation and $k = 1/3 \tau_c$ is the corresponding motional rate. Quantitative basis of the relationship between the rate of motion and the activation energy is given by the Arrhenius equation:

$$k = k_0 e^{-E_a/RT} \quad (5.2)$$

Here R is the universal gas constant and T is the temperature. $E_a$ is the activation energy. $E_a$ can be considered as the potential energy barrier separating two potential wells. For the discrete jump type model, $E_a$ is the least energy it takes for the methyl group jumping from one orientation site to the nearest neighbor site. Based on Equation (5.1) and (5.2) we have

$$R_{1\text{Z}} = R_{1\text{Z}}^0 e^{E_a/RT} \quad (5.3)$$
In Figure 5.10, the semi-logarithmic plots of $R_{12}$ versus 1000/T for three points selected on the QE line shape (one point in the center region, one point on the shoulder and one point on the horn) are shown. The activation energy corresponding to every point is listed in the legend. Specifically,

\[ E_a(\text{shoulder}) = 13.5 \pm 2.0 \text{ kJ/mol} \]
\[ E_a(\text{center}) = 13.9 \pm 1.5 \text{ kJ/mol} \]
\[ E_a(\text{horn}) = 13.6 \pm 0.8 \text{ kJ/mol} \]

It is obvious that the error of the calculated $E_a$ is much smaller for the horn than that for the shoulder and center due to the relatively poor signal-to-noise ratio in the shoulder and center part of the spectrum. Within experiment error, the three activation energies are about the same. Thus, the activation energy for methyl group motion in Fmoc-Alanine-d$_3$ is significantly lower than that in Alanine-d$_3$, for which $E_a=20\text{kJ/mol}$ [26]. According to Ref. [26], average distance between methyl groups in the crystal lattice of Alanine-d$_3$ is less than the sum of their van der Waals radii. The lower activation energy we find for methyl rotation in Fmoc-Alanine-d$_3$ suggests a less sterically crowded environment presumably due to the competition between aromatic stacking forces associated with the bulky Fmoc group and amino acid hydrogen bonding interactions, such that alanine methyl groups in adjacent molecules can rotate more freely. Unfortunately, this competition between aromatic stacking and hydrogen bonding precludes the possibility of growing single crystals suitable for high resolution X-ray experiments.
log(R_{1z})

- shoulder, $E_a = 13.5 \pm 2.0$ kJ/mol
- horn, $E_a = 13.6 \pm 0.8$ kJ/mol
- center, $E_a = 13.9 \pm 1.5$ kJ/mol

--- shoulder, linear fitting
--- horn, linear fitting
--- center, linear fitting

Figure 5.10: Arrhenius plots of $R_{1z}$ versus $1000/T$ for three points selected on the shoulder, center and horn parts of quadrupole echo line shape with the activation energy specified respectively.
5.4 Simulation and Motional Models

This section is concerned with the simulation and derivation of the motional models. The NMR simulation software suite EXPRESS (EXchange Program for RElaxing Spin Systems) developed by Professors Robert L. Vold and Gina Hoatson [27] was used to simulate quadrupole echo line shapes and the relaxation anisotropy profiles corresponding to various motional models. The first part gives a brief overview of the Stochastic Liouville formalism used in EXPRESS. The second part evaluates the validity of the proposed motional models through comparisons with experimental line shapes and anisotropy profiles, measured as a function of temperature at both 46 MHz (low field) and 115 MHz (High field).

5.4.1 Basic Theory

EXPRESS simulates NMR line shapes and relaxation times in which the dominant spin interactions are modulated by multi-site discrete jump dynamics. This is done by solving the stochastic Liouville equation for the reduced spin density matrix. In particular, let \( \rho_j = \rho(\Omega_j, t) \) be the reduced density matrix for spins in a molecular fragment described by Enler angles \( \Omega_j = (\alpha_j, \beta_j, \gamma_j) \) with respect to the molecule-fixed reference frame. Then its evolution is described by jumps between \( N \) discrete orientational sites [27]:

\[
\frac{d\rho_i}{dt} = i[\rho_j, H_j(t)] + \sum_{k=1}^{N} \frac{p(j,k)\rho_j p(j,k) - \rho_j}{\tau_{jk}}
\]  

(5.3)
Here, the interchanging of the site j and k is achieved by the permutation operator $P(j, k)$ and $k_{jk} = 1/\tau_{jk}$ is the jump rate from site k to site j. $H_j(t)$ is the Hamiltonian related to orientational site j.

In practice, only a subset of density matrix elements corresponding to single quantum coherence (i.e. transverse magnetization) is picked out. Let $m^{(j)} = [\rho_{lm}^{(j)}, \rho_{kn}^{(j)} ... ]$ stand for the single quantum coherences in site j, Equation (5.3) is reduced to:

$$\frac{d}{dt} \begin{bmatrix} m^{(1)} \\ \vdots \\ m^{(N)} \end{bmatrix} = i \begin{bmatrix} \omega^{(1)} & 0 & 0 & 0 \\ 0 & \omega^{(2)} & 0 & 0 \\ 0 & 0 & \ldots & 0 \\ 0 & 0 & 0 & \omega^{(N)} \end{bmatrix} \begin{bmatrix} m^{(1)} \\ \vdots \\ m^{(N)} \end{bmatrix}$$

$$+ \begin{bmatrix} k_{11}I & k_{12}I & \ldots & k_{1N}I \\ k_{21}I & k_{22}I & \ldots & k_{2N}I \\ \vdots & \vdots & \ldots & \vdots \\ 0 & k_{N2}I & \ldots & k_{NN}I \end{bmatrix} \begin{bmatrix} m^{(1)} \\ \vdots \\ m^{(N)} \end{bmatrix}$$

(5.4)

Here $I$ is a $N \times N$ identity matrix. $\omega^{(i)}$ are diagonal matrices with elements being the single quantum precession frequencies of site i in the rotating frame. Express provides a graphical user interface for inputting geometric parameters and motional rates of the model. Multi-axis jump processes are specified in terms of successive Wigner rotations. For example, for deuteron the first rotations is from the principal
axis frame of the quadrupole coupling tensor to an intermediate jump frame, followed by a second rotation from the intermediate frame to the crystallite frame. For polycrystalline powder sample, the time domain response is obtained by integration over a set of crystallite orientations using an optimized tiling algorithm.

5.4.2 Motional Models

Model 1: Three-Site Hops of the Methyl Group about the $C_{3v}$ Axis

It has long been recognized that the methyl groups execute fast threefold rotational jumps [25-26][49]. As stated in previous sections, $f_1(\omega_0)$ and $f_2(2\omega_0)$ are dependent on time scales and geometry of underlying motional processes as well as on crystallite orientations. For each motional model the crystallite orientation dependence is unique. Thus the anisotropy in the relaxation rates (or equivalently, spectral densities) could be utilized to distinguish among the motional models. In the last section, it is pointed out that the apparently lower average quadrupole coupling constant for methyl deuteron in Fmoc-Alanine-d$_3$ might suggest the existence of additional motional mechanism. Here we will see that three-site hops of the methyl group alone cannot fully reproduce the experimental $R_{1Z}$ and $R_{1Q}$ anisotropy profile. Therefore, an additional motional mechanism (for example, small amplitude libration of the $C_{3v}$ symmetry axis) should be incorporated.

In Figure 5.11(A), a brief chemical structure of Fmoc-Alanine-d$_3$ is given. Figure 5.11(B) illustrates the fast three-site jump of the methyl group about its $C_{3v}$ axis.
Figure 5.11: (A) Brief structure of Fmoc-Alanine-d$_3$; (B) Fast three-site hops of the methyl group about the C$_\alpha$-C$_\beta$ symmetry axis.
The best-fitting simulated $R_{1Z}$ and $R_{1Q}$ profiles are plotted together with the experimental profiles in Figure 5.12. And the site angles jump rates and other parameters are listed in Table 5.3.

$R_{1Z}, R_{1Q}$ (1/s)

![Graph showing $R_{1Z}$ and $R_{1Q}$ profiles with experimental data compared to simulated profile.](image)

$k3 = 8.6e+9$ Hz

Figure 5.12: $R_{1Z}$ and $R_{1Q}$ anisotropy profiles at 310K and 7.06 T for Fmoc-Alanine-d$_3$. Experimental data (circles) are compared to the simulated profile (solid line) based on the three-site hop model.
Table 5.3: Site angles, jump rates and other parameters for the three-site hop model.

<table>
<thead>
<tr>
<th>One-frame parameters</th>
<th>From PAS to Crystal frame</th>
<th>One-frame rate matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jump rate $k_3 = 8.6 \times 10^9 s^{-1}$</td>
<td>$\alpha$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>74</td>
</tr>
</tbody>
</table>

Other parameters:
- $C_Q = 155$ kHz, $\eta=0.09$ for all sites;
- $\tau_d = 1,2,3,5,10,20,30,50,100,500$ ms
- Powder increments: 1597 zcw;
- Echo pulse spacing: 40$\mu$s; 512 fid points; no apodization

In Figure 5.12, red circles and lines represent $R_{1Z}$ experiment and simulated profiles; Blue circles and lines represent $R_{1Q}$ experiment and simulated profiles, respectively. For the proposed three site hops model, simulated $R_{1Z}$ profile is in agreement with the experiment profile. For $R_{1Q}$, the part of simulated profile corresponding to the quadrupole horn matches the experiment very well. While for the center and the shoulder region, the simulation does not accurately reproduce the experiment profile. For the center region, this disagreement is within expectation; the center of the spectrum corresponds to zero quadrupole order \[6\]. Thus, despite the term “broadband, uniform excitation” associated with the BBJB pulse sequence, the excitation profile has a dip in the middle of the spectrum and this is not modeled by EXPRESS. This may explain why the center of the experiment $R_{1Q}$ profile is significantly lower than the simulation. However, the noteworthy difference for the shoulder of an apparent trend of rising around 30 kHz frequency, is also not captured by the simulation no matter what geometric parameters or jump rates are used. Thus the three-site hops model cannot fully account for the anisotropy profiles. We
conclude that the unusual curvature of the $R_{1Q}$ anisotropy profile indicates the existence of an additional motional mechanism. In the next two sections, librational motion of the methyl group $C_{3v}$ axis is incorporated in our simulation.

**Model 2: Threefold Methyl Rotation + Two Site Jumps of Methyl Spinning Axis**

In this model the methyl group executes rapid rotation and the rotation axis also jumps at a rate $k_2 = 1.5 \times 10^7 \text{s}^{-1}$ between two orientations, $(\alpha_2, \beta_2, \gamma_2) = (0,0,0)$ and $(0,40,0)$. As above methyl rotation was modeled as threefold jump but at rate $k_3 = 2.85 \times 10^9 \text{s}^{-1}$. The Euler angle $\beta_1$ with respect to the methyl spinning axis was 74°. All sites populations were assumed to be equal and $C_Q = 155 \text{kHz}$ and $\eta = 0$ were used. Parameters for this two-mode motional model are chosen to match the experiment measured $C_Q$. A schematic representation of the motional model is shown in Figure 5.13. The corresponding experiment was done at 310K.

Fast rotation of the methyl group leads to an averaged quadrupole coupling tensor $< C_Q > = \frac{1}{2} (3\cos^2 \beta - 1)C_Q = 59.8 \text{kHz}$ with zero asymmetry parameter, where the intrinsic quadrupole coupling constant in absence of motion is assumed to be 155 kHz [27], with principal EFG tensor z-axis aligned along the threefold $C_{3v}$ symmetry axis. Recall that in the previous line shape analysis, it is mentioned that the experiment measured $C_Q$ for Fmoc-Alanine-d$_3$ is much lower than Alanine-d$_3$ and this might be from the further averaging caused by the libration of the methyl spinning axis. Here in this model, the two sites jumps of the $C_{3v}$ axis could produce slightly further
reduction in the average $C_Q$ ($< C_Q \geq 49.4 \text{ kHz}$) and a non-vanishing asymmetry parameter ($\eta = 0.2$) whose magnitude is determined by the amplitude of the libration. Nevertheless, the experimental quadrupole line shape demonstrates the existence of an almost zero asymmetry parameter in contradiction with the large $\eta$ from the simulation. This is more clearly shown in Figure 5.14, where the simulated QE does not match the experiment line shape. Thus this model is not suitable and a more valid model is needed.

(A)

(B)

Figure 5.13: Schematic representation of the motional model: 3-site hops of methyl group in combination with 2-site wagging of the methyl spinning axis.
Figure 5.14: Simulated QE spectrum based on model 2 (red, dashed line) in contrast with the experiment spectrum (blue, solid line). Simulated spectrum indicates an obviously non-vanishing asymmetry parameter ($\eta = 0.2$).
Model 3: Threefold Methyl Rotation + Four Sites Wobbling In A Cone of the Methyl Spinning Axis

This model retains three-site jumps of the methyl group mentioned in the previous models, and the libration of the C$_{3v}$ axis is incorporated as jumps among four sites that define wobbling in a narrow cone as illustrated in Figure 5.15. Relevant model parameters are listed in Table 5.4. In efforts to match the simulation to low field experiments at 310K, the 3-fold jump rate, $k_3$, and cone wobble rate, $k_4$, were varied over wide ranges. The cone angle, 15°, was chosen to match the observed quadrupole coupling constant at 310K.

Table 5.4: Site angles, jump rates and other parameters for model 3.

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<thead>
<tr>
<th>Two-frame parameters</th>
<th>Rate matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frame 1(PAS) $k_3 = 5.4 \times 10^{10}s^{-1}$</td>
<td>$\begin{bmatrix} -2k_3 &amp; k_3 &amp; k_3 \ k_3 &amp; -2k_3 &amp; k_3 \ k_3 &amp; k_3 &amp; -2k_3 \end{bmatrix}$</td>
</tr>
<tr>
<td>$\alpha$ $\beta$ $\gamma$</td>
<td></td>
</tr>
<tr>
<td>1 0 74 0</td>
<td></td>
</tr>
<tr>
<td>2 0 74 120</td>
<td></td>
</tr>
<tr>
<td>3 0 74 240</td>
<td></td>
</tr>
<tr>
<td>Frame 2 $k_4 = 2.85 \times 10^{6}s^{-1}$</td>
<td>$\begin{bmatrix} -3k_4 &amp; k_4 &amp; k_4 &amp; k_4 \ k_4 &amp; -3k_4 &amp; k_4 &amp; k_4 \ k_4 &amp; k_4 &amp; -3k_4 &amp; k_4 \ k_4 &amp; k_4 &amp; k_4 &amp; -3k_4 \end{bmatrix}$</td>
</tr>
<tr>
<td>1 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2 0 15 0</td>
<td></td>
</tr>
<tr>
<td>3 0 15 120</td>
<td></td>
</tr>
<tr>
<td>4 0 15 240</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.15: Schematic representation of Model 3. The first mode (A) corresponds to fast three-site hops of the methyl group; the second mode (B) corresponds to four-site wobbling in a cone of the $C^\alpha - C^\beta$ axis.
The asymmetry parameter $\eta = 0$ and averaged quadrupole coupling constant $C_Q = 49.4 kHz$. Model 3 reproduces the experimental QE line shape very nicely, as shown in Figure 5.16. Jump rates $k_3 = 5.45 \times 10^{10}$ and $k_4 = 2.85 \times 10^6$ are chosen to achieve a compromise fit with both the experimental $R_{1Z}$ and $R_{1Q}$ profile. The simulated $R_{1Z}$ and $R_{1Q}$ profiles are plot with the experimental profiles in Figure 5.17. It is apparent that Model 3 can account for both the $R_{1Z}$ and $R_{1Q}$ anisotropy profiles better than models 1 or 2, but there is still significant disagreement in the central region of the spectrum.

![Figure 5.16: Simulated QE spectrum based on model 2 (red, dashed line) in contrast with the experiment spectrum (blue, solid line). The slight difference may be due to the bad shimming, different phasing or apodization.](image-url)

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Figure 5.17: Simulated and experimental $R_{1Z}$ and $R_{1Q}$ anisotropy profiles based on the Model 3. $k_3 = 5.45 \times 10^{10}$ and $k_4 = 2.85 \times 10^6$. 
Based on Equations (2.31) and Equation (2.32), the simulated spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ could be derived from the simulated relaxation rates $R_{1Z}$ and $R_{1Q}$, which is shown in Figure 5.18. The good agreement between simulated and experimental spectral densities further validates Model 3.

$J_1(\omega_0)$, $J_2(2\omega_0)$, (ps)

Figure 5.18: Simulated and experimental spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$. Experiment was at 310$K$ and 7.06$T$. 

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Given the success of Model 3 in reproducing the low field data at 310K, we were encouraged to fit the low field data at other temperature. For every other temperature at which the experiment was done, $k_3$ and $k_4$ were adjusted to achieve the best compromise fit, with results summarized in Table 5.5. Arrhenius plots of $k_3$ and $k_4$ (given in Figure 5.19) then give activation energies for the methyl rotation and the symmetry axis libration, respectively.

\[
E_a(\text{methyl rotation}) = 16.2 \text{kJ/mol}
\]

\[
E_a(\text{methyl spinning axis libration}) = 2.1 \text{kJ/mol}
\]

Table 5.5: Best fit simulated jump rates $k_3$ and $k_4$ as a function of sample temperature.

<table>
<thead>
<tr>
<th>set-point T(K)</th>
<th>True T(K)</th>
<th>1000/T</th>
<th>$k_3$(1/s)</th>
<th>$k_4$(1/s)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>228.7</td>
<td>4.4</td>
<td>5.98E+09</td>
<td>2.08E+06</td>
</tr>
<tr>
<td>240</td>
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<td>4.2</td>
<td>8.80E+09</td>
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</tr>
<tr>
<td>250</td>
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<td>1.16E+10</td>
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</tr>
<tr>
<td>260</td>
<td>264.2</td>
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<td>1.66E+10</td>
<td>2.53E+06</td>
</tr>
<tr>
<td>270</td>
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<td>3.6</td>
<td>2.30E+10</td>
<td>2.60E+06</td>
</tr>
<tr>
<td>280</td>
<td>286.4</td>
<td>3.5</td>
<td>3.00E+10</td>
<td>2.70E+06</td>
</tr>
<tr>
<td>290</td>
<td>297.8</td>
<td>3.4</td>
<td>4.15E+10</td>
<td>2.78E+06</td>
</tr>
<tr>
<td>300</td>
<td>310.6</td>
<td>3.2</td>
<td>5.45E+10</td>
<td>2.85E+06</td>
</tr>
<tr>
<td>310</td>
<td>321.2</td>
<td>3.1</td>
<td>6.85E+10</td>
<td>2.90E+06</td>
</tr>
<tr>
<td>320</td>
<td>334</td>
<td>3</td>
<td>8.25E+10</td>
<td>2.94E+06</td>
</tr>
<tr>
<td>330</td>
<td>346.1</td>
<td>2.9</td>
<td>1.00E+11</td>
<td>2.98E+06</td>
</tr>
</tbody>
</table>
Figure 5.19: Arrhenius plots. (A) $\log(k_3)$ vs $1000/T$; (B) $\log(k_4)$ vs $1000/T$
5.5 Simulation of High Field Results

In order to further validate the model proposed in the last section, we tried to model the experimental QE line shape and R_{12} anisotropy in the high magnetic field. Take the 302 K experiment as an illustration. We continue to use the same geometric parameters. The jump rates $k_3 = 4 \times 10^{10} \text{s}^{-1}$ and $k_4 = 1 \times 10^7 \text{s}^{-1}$ gives a best fitting. The simulated line shape and R_{12} profile is plot in contrast to the experiment results in Figure 5.20 and Figure 5.21, respectively.

Figure 5.20: Simulated and Experimental QE line shape at high field and 302 K.
$R_{1Z}$ (1/s)

$k_3 = 4e+10$ 1/s
$k_4 = 1e+7$ 1/s

Figure 5.21: Simulated and experimental $R_{1Z}$ anisotropy profile in high magnetic field at 301 K. The best fit value for $k_3$ is close to that determined from low field data at the same temperature, but the best fit value for $k_4$ from high field data is about 3 times greater than that found from low field data.

Fitting the temperature dependence of high field relaxation rates would be risky, since unfortunately the requisite experimental values of $R_{1Q}$ are not yet available. We note in passing that the best fit parameters determined from low field experiments may require significant refinement to account for the frequency dependence of both spectral densities.
5.6 Conclusion

A smaller methyl deuteron quadrupole coupling constant found for Fmoc-Alanine-d$_3$, indicates the existence of an additional motional process in the Fmoc derivative. The Arrhenius activation energy calculated from the relaxation rates of the horns of the low field quadrupole echo spectra is $E_a(\text{horn}) = 13.6 \pm 0.8$ kJ/mol. This indicates a less severe sterically crowding environment for the CD$_3$ group than that in crystalline Alanine-d$_3$, for which $E_a = 20$kJ/mol [26]. We infer that the less sterically hindered environment is due to the bulky Fmoc group, which prevents the tight packing of the molecules like that in Alanine-d$_3$.

The $T_{1Z}$ and $T_{1Q}$ profiles exhibit unusual orientation dependence, which cannot be adequately reproduced by threefold methyl rotation alone. Therefore, additional libration of the C$_{3V}$ axis was incorporated in more elaborate motional models. Stochastic Liouville formalism was applied to describe the modulation of the threefold methyl rotational jumps by libration of the C$_{3V}$ axis. Agreement in the anisotropy profiles is found for two variants of this formalism, describing the libration as two-site “jumps” and four-site “wobbling” in a cone respectively. However, the former two-site “jumps” model produces a significantly non-vanishing asymmetry parameter and cannot reproduce the experiment QE line shape. Thus the four-site “wobbling” in a cone formalism was adopted. Motional spectral densities derived from the best-fit simulated $R_{1Z}$ and $R_{1Q}$ profiles match the experimental spectral densities well, which further proves the validity of the model. Finally, activation energies for the threefold methyl rotation and four-site wobbling in a cone
based on the Arrhenius plots of the motional rates $k_3$ and $k_4$ are $E_a(\text{methyl rotation}) = 16.2 \text{kJ/mol}$ and $E_a(\text{methyl spinning axis libration}) = 2.1 \text{kJ/mol}$, respectively. The very weak temperature dependence for the four-site wobbling motion suggests that this motion may not actually involve jumps over a barrier: the value determined for “$E_a$” is actually less than the average thermal energy at the experimentally realized temperature.
APPENDIX A

Temperature Calibration of the Static Probe Using Lead Nitrate

In practical experiment, the temperature sensor is placed in the vicinity of the sample tube. Thus the displayed temperature is actually not the true temperature inside the sample and temperature calibration is indispensable for determining the true sample temperature. The temperature-dependent isotropic shifts of the $^{207}$Pb resonance of lead nitrate has provided a thermometer for calibrating MAS probe [50]. For non-spinning sample, the temperature could be determined from the temperature variation of the lead nitrate peak shift [51,52].

Lead nitrate has very accurately established linear relations between each principal component of the shielding tensor and temperature of the spins in the sample:

$$
\sigma_\perp = a_\perp \cdot T_{\text{sample}} + b_\perp \quad (A.1)
$$

$$
\sigma_\parallel = a_\parallel \cdot T_{\text{sample}} + b_\parallel \quad (A.2)
$$

If the temperature is uniform across the sample, the non-spinning spectrum will be a simple chemical shift anisotropy powder pattern with two singularities since the shift tensor is axially symmetric. Line shape deviations from a simple, axially symmetric
powder pattern thus provide information about variations (gradients) in temperature across the sample.

In this thesis, the real temperature was estimated from measured lead nitrate "horn" frequencies $\delta_{11}$ using the following linear relation

$$\delta_{11}(T_{\text{real}}) = 0.666T_{\text{real}} + \delta_0$$  \hspace{1cm} (A.3)

The value of the intercept $\delta_0$ depends on the frequency to which measured horn positions are refereed and is relatively not important. For 7.06 T and 17.6 T spectrometers $\delta_0$ was independently determined by measuring $\delta_{11}(T_{\text{real}})$ of lead nitrate at one fixed point, $T_{\text{real}} = 277K$. This was determined in each probe by noting the set point at which D$_2$O freezes, monitored by static deuteron NMR. Denoting $\delta_{11}(T_{\text{real}})$ at the temperature $T^*$ at which D$_2$O freezes by $\delta_{\text{peak}}^*$, then other temperatures relative to that temperature could be determined by

$$T - T^* = (1.50 \pm 0.01 \ K/\text{ppm})\{\delta_{\text{peak}} - \delta_{\text{peak}}^*\}$$  \hspace{1cm} (A.4)

Then there also exists a linear relation between the set points and the real temperatures. $T_{\text{real}}$ versus $T_{\text{set}}$ for the static probe at 17.6 and 7.06 T spectrometers are given in Figure A.1.

At 7.06 T:  \hspace{0.5cm} $T_{\text{real}} = 1.166\times T_{\text{set}} - 39.448$  \hspace{1cm} (A.5)

At 17.6 T:  \hspace{0.5cm} $T_{\text{real}} = 1.006\times T_{\text{set}} + 0.071$  \hspace{1cm} (A.6)
Figure A.1: Temperature calibration (A) 7.06 T; (B) 17.6 T
Bibliography


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