Peptide Backbone Orientation and Dynamics in Spider Dragline Silk and Two-Photon Excitation in Nuclear Magnetic and Quadrupole Resonance

by

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Abstract

In the first part of the dissertation, spider dragline silk is studied by solid state NMR techniques. The dependence of NMR frequency on molecular orientation is exploited using the DECODER experiment to determine the orientation of the protein backbone within the silk fibre. Practical experimental considerations require that the silk fibres be wound about a cylindrical axis perpendicular to the external magnetic field, complicating the reconstruction of the underlying orientation distribution and necessitating the development of numerical techniques for this purpose. A two-component model of silk incorporating static b-sheets and polyglycine II helices adequately fits the NMR data and suggests that the b-sheets are well aligned along the silk axis (20 FWHM) while the helices are poorly aligned (68 FWHM). The effects of fibre strain, draw rate and hydration on orientation are measured. Measurements of the time-scale for peptide backbone motion indicate that when wet, a strain-dependent fraction of the poorly aligned component becomes mobile. This suggests a mechanism for the supercontraction of silk involving latent entropic springs that undergo a local strain-dependent phase transition, driving supercontraction.

In the second part of this dissertation a novel method is developed for exciting NMR and nuclear quadrupole resonance (NQR) by rf irradiation at multiple frequencies that sum to (or differ by) the resonance frequency. This is fundamentally different than traditional NMR experiments where irradiation is applied on-resonance. With excitation outside the detection bandwidth, two-photon excitation allows for detection of free induction signals during excitation, completely eliminating receiver dead-time. A theoretical approach to describing two-photon excitation is developed based on average Hamiltonian theory. An intuition for two-photon excitation is gained by analogy to the coherent absorption of multiple photons requiring conservation of total energy and momentum. It is shown that two-photon excitation efficiency improves when the two applied rf frequencies deviate from half-resonance. For two-photon NQR, it is shown that observable magnetization appears perpendicular to the excitation coil, requiring a second coil for detection, and that double quantum coherences are also generated. Several model systems and experimental geometries are used to demonstrate the peculiarities of two-photon excitation in NMR and NQR.
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<tr>
<td>NQR</td>
<td>nuclear quadrupole resonance</td>
</tr>
<tr>
<td>rf</td>
<td>radio frequency</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>DECODER</td>
<td>direction exchange with correlation for orientation distribution evaluation and reconstruction</td>
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<tr>
<td>AHT</td>
<td>average Hamiltonian theory</td>
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<tr>
<td>OD</td>
<td>orientation distribution</td>
</tr>
<tr>
<td>PAS</td>
<td>principle axis system</td>
</tr>
<tr>
<td>CSA</td>
<td>chemical shift anisotropy</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
</tr>
<tr>
<td>emf</td>
<td>electromotive force</td>
</tr>
<tr>
<td>BS</td>
<td>Bloch-Siegert</td>
</tr>
<tr>
<td>ħ</td>
<td>Plank’s constant</td>
</tr>
<tr>
<td>γ</td>
<td>gyromagnetic ratio</td>
</tr>
<tr>
<td>B</td>
<td>magnetic field strength</td>
</tr>
<tr>
<td>B₀</td>
<td>strength of the external magnetic field</td>
</tr>
<tr>
<td>B₁</td>
<td>strength of the rf field</td>
</tr>
<tr>
<td>B₂</td>
<td>strength of a second rf field</td>
</tr>
<tr>
<td>ω</td>
<td>angular frequency</td>
</tr>
<tr>
<td>ω₀</td>
<td>Larmor frequency = ( γB₀ )</td>
</tr>
<tr>
<td>ω₁</td>
<td>nutation frequency = ( γB₁ )</td>
</tr>
<tr>
<td>ωᵣf</td>
<td>frequency of applied rf field</td>
</tr>
<tr>
<td>ωₓ, ωᵧ, ωₗ</td>
<td>projection of the rf field onto an axis in the PAS of the interaction</td>
</tr>
<tr>
<td>ωₜd</td>
<td>strength of the dipole interaction</td>
</tr>
<tr>
<td>ω₀Q</td>
<td>nuclear quadrupole resonance frequency</td>
</tr>
<tr>
<td>( \hat{H} )</td>
<td>Hamiltonian</td>
</tr>
<tr>
<td>( \hat{H}_z )</td>
<td>Zeeman Hamiltonian</td>
</tr>
<tr>
<td>( \hat{H}_{rf} )</td>
<td>rf Hamiltonian</td>
</tr>
<tr>
<td>( \hat{H}_Q )</td>
<td>quadrupolar Hamiltonian</td>
</tr>
<tr>
<td>( \hat{H}_{DD} )</td>
<td>dipolar-dipole interaction</td>
</tr>
<tr>
<td>( \hat{H}_{CSA} )</td>
<td>chemical shift anisotropy Hamiltonian</td>
</tr>
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</table>
\( \hat{H}^{(0)}, \hat{H}^{(1)} \)  
zeroth and first order term in the Magnus expansion

\( \hat{H}_{\text{eff}} \)  
effective Hamiltonian obtained after a transformation into an interaction representation

\( \hat{H}_{\text{int}} \)  
Hamiltonian describing a generic interaction

\( \hat{U} \)  
unitary operator describing a transformation into an interaction representation

\( R \)  
second rank tensor

\( R_{\alpha\beta} \)  
Cartesian tensor components \( \alpha, \beta = x, y, z \)

\( R_{XX}, R_{YY}, R_{ZZ} \)  
principle tensor components in Cartesian representation

\( \hat{T}_{\alpha\beta} \)  
Cartesian tensor operator = \( \hat{T}_{\alpha} \hat{T}_{\beta} \)

\( \hat{T}_{lm} \)  
irreducible spherical tensor operator

\( \rho_{lm} \)  
irreducible spherical tensor components

\( R \)  
principle tensor components in irreducible spherical representation

\( \sigma_{\text{iso}} \)  
isotropic tensor value

\( \delta \)  
isotropic tensor value for the chemical shift interaction

\( \eta \)  
asymmetric tensor value

\( \sigma \)  
CSA tensor

\( \sigma_{XX}, \sigma_{YY}, \sigma_{ZZ} \)  
CSA tensor components

\( D_{m'm}^{i} \)  
Wigner rotation matrix elements

\( d_{m'm}^{i} \)  
azimuthal factors of the Wigner rotation matrix elements

\( \hat{\Omega} = (\alpha, \beta, \gamma) \)  
set of Euler rotations defined as successive rotations by \( \gamma \) about \( z \), \( \beta \) about \( y \), then \( \alpha \) about \( z \). Rotation are about space-fixed axes.

\( A(\hat{\Omega}) \)  
Euler rotation matrix

\( \hat{M} \)  
bulk magnetization vector

\( \hat{\mu} \)  
magnetic moment of a nuclear spin = \( \gamma \hat{I} \)

\( \langle \psi \rangle \)  
state vector describing the spin state

\( \hat{I}_{x}, \hat{I}_{y}, \hat{I}_{z} \)  
spin angular momentum operators

\( \hat{I}_{\pm} \)  
\( \hat{I}_{x} \pm \hat{I}_{y} \)

\( \hat{I}_{\pm \pm} \)  
\( \mp \hat{I}_{\pm} / \sqrt{2} \)

\( \hat{I} \)  
\( \hat{I}_{x}, \hat{I}_{y}, \hat{I}_{z} \)

\( \hat{S}_{i} \)  
spin angular momentum operator for a second nuclear species

\( \hat{f}_{\alpha}^{i-j} \)  
fictitious spin 1/2 operator connecting states \( i \) and \( j \) for \( \alpha = x, y, z \)

\( \hat{\rho} \)  
density matrix

\( \rho_{ij} \)  
elements of density matrix

\( k_{B}T \)  
temperature in units of energy

\( T \)  
Dyson time ordering operator
\text{Tr}\{\}

\begin{align*}
[A, B] & \quad \text{commutation operation} \\
\hat{A} & \quad \text{quantum mechanical operator} \\
A & \quad \text{matrix} \\
A^T & \quad \text{transpose of matrix} \\
A^\dagger & \quad \text{Hermitian conjugate} \\
\vec{A} & \quad \text{vector} \\
\vec{A}^T & \quad \text{transpose of vector} \\
\hat{x}, \hat{y}, \hat{z} & \quad \text{unit vectors} \\
r & \quad \text{internuclear distance} \\
S(\omega_1, \omega_2) & \quad \text{two-dimensional NMR spectrum} \\
 s(\omega_1, \omega_2, \Omega) & \quad \text{subspectra calculated for a given orientation of the PAS in the silk frame} \\
 s_{ij}(\omega_1, \omega_2, \Omega_{ij}) & \quad \text{set of subspectra calculated for discrete orientations, } \Omega_{ij}, \text{ of the PAS in the silk frame} \\
 P(\Theta, \Phi) & \quad \text{probability distribution for the PAS in the silk frame} \\
\Delta \alpha & \quad \text{width of Gaussian distribution describing orientation distribution of each secondary structure} \\
T_1 & \quad \text{longitudinal relaxation time constant} \\
T_2 & \quad \text{transverse relaxation time constant}
\end{align*}
Acknowledgements

Thanks first and foremost to Carl from whom I learned so much about so many things. As a mentor, role model and friend, you taught me to think critically, communicate clearly, and lead by example; lessons which, above all else, I will take away from my experience at UBC.

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Lastly, thanks to John Gosline and his group in the Zoology department for many useful discussions about all things related to spiders, and thanks to John for careful reading of the thesis and his valued input over the years.
For Colleen and Sophia
who provide inspiration
and perspective
for everything I do
Co-Authorship Statement

Chapters 5–9 have been previously published in peer reviewed journals as is indicated in the footnotes at the beginning of each of these chapters. These publications represent a collaborative effort with the present author’s Ph.D. supervisor, Dr. Carl A. Michal (C.A.M.), and is reflected in the fact that his name appears as a second author on each of the papers.

Regarding the respective contribution of the authors to each publication, it should be recognized that for the silk project (Chapters 5 and 6), the original idea for the wound fibre DECODER experiment, including the numerical approach for orientation reconstruction, was C.A.M.’s, while the majority of the experimental and computational work was performed by the present author. The idea for two-photon excitation of NQR (Chapters 7 and 9) is attributed to the present author and was inspired by a paper published by C.A.M. The theoretical description of two-photon excitation using average Hamiltonian theory and the prediction of two-color excitation (Chapter 8), are contributions made by the present author. All theoretical calculations and the majority of the experimental realizations of two-photon NMR and NQR were performed by the present author under the supervision of C.A.M. Finally, the manuscripts for the published works were written by the present author with significant input and editing by C.A.M.
Part I

Introduction
Chapter 1

Introductory Remarks

Nuclear magnetic resonance (NMR) was first demonstrated by the groups of Purcell and Bloch in 1946 at Harvard and Stanford respectively. The subsequent discovery that the NMR frequency depends on the local electron environment, which allows for spectroscopic resolution of various chemical groups, and the development of pulsed NMR techniques, multidimensional spectroscopy, and multiple quantum coherence techniques, has sparked an explosion in the field that has since yielded four Nobel prizes.¹

Nuclear spins experience a variety of interactions with neighbouring nuclei, local electrons, and externally applied magnetic fields. The dependence of these interactions on molecular parameters makes NMR an extraordinary technique that is used widely today in physics, chemistry, biochemistry and medicine in applications ranging from three-dimensional protein structure determination, to medical imaging, to measurement of molecular dynamics, to detection and identification of chemicals, to quantum computation. Now considered a mature field, NMR still receives much attention from researchers who use it as a tool to study a wide range of molecular systems, as well as from those striving to improve the sensitivity and spectral resolution of NMR experiments in order to study more and more complex systems.

In this dissertation two distinct studies are presented: one uses NMR to gain insight into the molecular structure of a very large protein; the other presents a fundamentally different way of performing NMR experiments.

Studies of protein structure represent a large subclass of NMR applications, with techniques being developed to study larger and larger proteins, especially those that are not soluble, are membrane-bound, do not crystallize well, or do not have a well defined structure. Often in such systems, the absence of rapid molecular tumbling leaves many inter-nuclear interactions intact resulting in a rich system from which much information can be gleaned. NMR studies of such systems fall under the general class of “solid state NMR”.

In the first part of this dissertation, solid state NMR techniques are applied to an especially large and complex protein system: spider silk. The size of the proteins that make up silk, and their inherent randomness makes impractical their study by other techniques. Chapter 3 introduces this amazing material whose combination of strength, extensibility and toughness has caught the attention of materials scientists. The following three chapters (Chapters 4–6) deal with measuring the orientation distribution of the protein backbone.

¹Purcell and Bloch shared the 1952 Nobel prize in physics, Richard R. Ernst received the 1991 prize in chemistry for development of pulsed NMR and multidimensional spectroscopy, Kurt Wüthrich won the 2002 prize in chemistry for techniques for determining the three dimensional structure of biological macromolecules, and Paul C. Lauterbur and Peter Mansfield shared the 2003 prize in medicine for the development of magnetic resonance imaging.
in silk. Experimental constraints stipulate that a large amount of silk be measured, and that the fibres be uniformly strained and hydrated. These requirements necessitate a sample geometry where the fibres are wrapped about a cylindrical support. Such a geometry complicates the reconstruction of the orientation distribution from NMR data, and in Chapter 4, models and numerical techniques are developed to perform such a reconstruction. This model-dependent reconstruction technique incorporates β-sheet and poly-glycine II helical secondary structures and assumes that their alignment along the fibre can be described by Gaussian probability distributions. In Chapters 5 and 6 results from solid-state NMR experiments on spider silk are presented from which the orientation and dynamics of silk’s protein backbone are reconstructed using the techniques developed in Chapter 4. Such experiments are performed as a function of fibre strain, draw rate, and hydration. The results suggest molecular mechanisms that are responsible for silk’s mechanical properties.

The second part of this dissertation concerns the way in which NMR experiments are performed. In traditional NMR experiments, high-power rf irradiation is applied at the resonance frequency to the sample. Afterwards, a weak free induction signal is detected at that same frequency. However, the sensitive receiver electronics must be decoupled from the system during the high-power irradiation. This results in a receiver dead-time after the rf has been turned off but before the receiver can be turned on during which the signal cannot be detected. In unfavorable circumstances, the dead-time may preclude detection of any NMR signal.

In Chapters 7–9, a fundamentally different way of exciting NMR is considered in which resonances are excited by multiple rf frequencies that sum to (or differ by) the resonance frequency; the simplest realization of this being excitation at half-resonance. We call this multi-photon excitation in analogy to the coherent absorption of multiple photons as is common in optics. Such excitation far from the resonance frequency allows for simultaneous excitation and detection in NMR, entirely eliminating receiver dead-time. Chapter 7 presents the first demonstration of two-photon excitation in nuclear quadrupole resonance (NQR), an experiment related to NMR where receiver dead-times are typically longer than in NMR. In Chapter 8, a general theoretical approach to multi-photon excitation is presented, and excitation of NMR at two frequencies that sum to the resonance frequency is demonstrated. In Chapter 9, two-photon excitation in NQR is described using the theory developed in the preceding chapter, and an investigation of the coherences generated by two-photon irradiation is presented.

Much of the scientific content presented in this dissertation has been previously published and versions of the manuscripts are presented in Chapters 5, 6, 7, 8 and 9. Each of these chapters is self-contained. To provide the reader with the necessary background to understand these works, this dissertation begins with some introductory material. Relevant concepts in nuclear magnetic resonance are first introduced (Chapter 2), followed by an introduction to spider silk (Chapter 3). An in-depth discussion of the methodology used for reconstruction of orientation distributions in wound fibres (Chapter 4) rounds out the introductory material. The manuscripts chapters (Chapters 5–9) follow, and the dissertation concludes (Chapter 10) with a summary of the findings presented in preceding chapters.
Chapter 2
An Introduction to Nuclear Magnetic Resonance

There exists an extensive body of literature that encompasses the many aspects of nuclear magnetic resonance (NMR). Background for much of what is presented in this dissertation can be found in most NMR texts, notably Refs [1-4] provide a good general background of NMR, while Refs [5, 6] are more pertinent to the work presented here.

This introductory chapter will review the quantum mechanical description of NMR with particular emphasis on those topics that will be relevant to the remainder of the dissertation. The chapter is organized into two main areas: 1) a description of the Hamiltonians defining the energy landscape that results from intrinsic physical properties of the sample (ie. the electronic and molecular configurations that are of ultimate interest to the NMR spectroscopist), and 2) the time-dependent evolution of the spin system under these Hamiltonians.

The organization of this introduction into these two categories also reflects the division of the background required for the two projects that make up this dissertation. The chapters on spider silk require introducing a complete description of the internal Hamiltonians from which molecular orientation information will be extracted. Because of the complex geometry of the silk samples, a formalism that facilitates rotations and frame transformations must be employed. For the chapters on two-photon excitation in NMR, the evolution of the system under a simple but time-dependent Hamiltonian must be described and is done so using Average Hamiltonian theory.

We begin with a few words outlining pulsed NMR and nuclear spins.

2.1 An Overview of Pulsed NMR

2.1.1 Nuclear Spin and Quantum Mechanics

NMR experiments measure the signal arising from nuclear spins in the sample that evolve under the influence of both an externally applied magnetic field, and other fields due to the local molecular environment.

Spin, or spin angular momentum as it is the properly termed, is an intrinsic property of fundamental particles such as quarks or electrons and of composites of these particles such as nuclei or atoms. Although purely quantum mechanical in nature, spin is classically analogous to orbital angular momentum. That is, it behaves like an orbiting charge, having a well defined magnetic dipole moment and angular momentum.
The nuclear spin moment is typically written as \( \vec{\mu} = \gamma \vec{I} \), where \( \gamma \) is the gyromagnetic ratio of the nucleus and \( \vec{I} \) is the quantum mechanical spin angular momentum operator. In a magnetic field, spin is typically quantized along the direction of the field, and for a sample containing an ensemble of spins this results in a net magnetization of the sample.

In typical NMR samples, the nuclear spin degrees of freedom are sufficiently weakly coupled to the translational and vibrational degrees of freedom that, to a first approximation, only the spin part of the wavefunction and the spin terms in the Hamiltonian need be considered.\(^1\) Furthermore, because of the relative insensitivity of NMR as a spectroscopic technique, typical NMR samples contain a large number of spins (on the order of Avogadro’s number). Although each individual member of such an ensemble is quantum mechanical in nature, the ensemble system can be described to a certain degree by classical analogies. Though the full power of NMR comes from its quantum mechanical nature, and the bulk of this introduction deals with the quantum mechanical description of NMR, it may be instructive to outline the basic principles of NMR classically.

### 2.1.2 A Classical Description of Pulsed NMR

A magnetic moment, \( \vec{\mu} \), in an external magnetic field, \( \vec{B} \), feels a torque such that its equation of motion is

\[
\frac{\partial \vec{\mu}}{\partial t} = \vec{\mu} \times (\gamma \vec{B}),
\]

where \( \gamma \) is the nuclear gyromagnetic ratio, an intrinsic property of a nucleus that describes the strength of the nuclear magnetic moment, and has units of Hz/Tesla. Since the torque is always along a direction perpendicular to both \( \vec{B} \) and \( \vec{\mu} \), the result is a precession of the magnetic moment about the external field, as shown in Fig. 2.1. The moment precesses at a frequency \( \omega_0 = \gamma |\vec{B}| \), known as the Larmor frequency. Thus, the gyromagnetic ratio can be defined as the ratio between the Larmor frequency and the applied field strength.

Such a time-varying magnetic moment will induce an AC current in a coil at the Larmor frequency. The principle behind detection of NMR signals relies on measuring this current at this specific frequency in a coil that surrounds the sample inside the magnetic field.

Unfortunately, a magnetic moment will not precess in such a way indefinitely. Microscopic fluctuations in the external field, and the effects of the magnetic moment in one part of the sample on another part (e.g. one nucleus on its neighbour) result in relaxation effects that 1) destroy the oscillating transverse magnetization (that is perpendicular to the external field) and 2) align the moment along the external field. The timescales for these two processes are governed by time constants called \( T_2 \) and \( T_1 \) respectively. The phenomenological equations that govern the evolution of the sample magnetization (i.e. the net magnetic moment of the sample) are

\[^1\text{Coupling to translational and vibrational degrees of freedom results in relaxation effects. Although relaxation is an important part of NMR [7], it will be ignored for the purposes of this dissertation.} \]
sample), in the presence of relaxation are the Bloch equations:

\[
\begin{align*}
\frac{\partial M_x}{\partial t} &= \omega_0 M_y - \frac{M_x}{T_2} \\
\frac{\partial M_y}{\partial t} &= -\omega_0 M_x - \frac{M_y}{T_2} \\
\frac{\partial M_z}{\partial t} &= -\frac{(M_z - M_0)}{T_1}.
\end{align*}
\]

Because of relaxation, magnetic moments tend to align along the magnetic field. Thus, in order to measure an NMR signal, one must first disturb the magnetization from its equilibrium orientation along the magnetic field before detecting the signal due to the precessing magnetization.

Excitation away from thermal equilibrium can be achieved by applying a weak magnetic field oscillating at the Larmor frequency along a direction perpendicular to the external field. This is done in practice by driving a large current at the Larmor frequency through the same coil that is later used for detection. Such excitation tips the magnetization away from thermal equilibrium. In typical modern NMR experiments where magnetic fields are of the order of 10 Tesla, the Larmor frequency is in the radiofrequency (rf) range. Thus, in this dissertation, the excitation is often referred to as rf irradiation, or just rf.
Chapter 2. An Introduction to Nuclear Magnetic Resonance

One can understand the effects of the oscillating magnetic field by going into a frame that is rotating at the Larmor frequency. In such a frame, the rf field appears constant along the x-direction. Furthermore, the precession due to the static field vanishes in this rotating frame, since the frame rotates with the precession. The net effect is that the magnetization precesses about the rf field at a frequency proportional to the strength of the field.

Once the magnetization is tipped away from thermal equilibrium, the high-power rf transmitter can be turned off, and the receiver turned on to detect the precessing magnetization (called a free induction decay, or FID). This simplest of pulsed NMR experiments is depicted in Fig. 2.2, and forms the conceptual basis of NMR.

Based on this classical description of NMR, it is possible to give a summary of the work presented in this dissertation. It turns out that the exact precession frequency depends on the orientation of molecules in the magnetic field, so in principle, for a distribution of measured frequencies, the distribution of orientations can be obtained. In the first part of the dissertation, this concept is applied to spider silk samples to determine the distribution of orientations of the peptide backbone within the fibre. In the second part of the dissertation, it is shown that applying a magnetic field oscillating at half the resonance frequency will also tip magnetization away from thermal equilibrium, but requires that the axis of the rf coil have a component perpendicular to and along the static field. The benefit of this type of excitation is that the excitation frequency is far from the detection frequency and it is possible to excite and detect simultaneously.

The remainder of this introductory chapter describes the quantum mechanical description of NMR and introduces the formalisms required to understand the details of the results obtained in subsequent chapters. Those readers not interested, or already proficient, in the technical details of NMR may skip ahead to Chapter 3 with no loss of continuity.

2.2 Internal and External Hamiltonians

2.2.1 The Zeeman Interaction

The dominant interaction in NMR is the Zeeman interaction that describes the coupling of a nuclear spin to a uniform external magnetic field, $\vec{B}_0 = B_0 \hat{z}$, oriented along a direction in the lab frame defined as the z-axis. The Zeeman Hamiltonian can be written as

$$\hat{H}_z = \hbar \mu \cdot \vec{B}_0 = \hbar \gamma B_0 \hat{I}_z,$$

where the operator $\hat{I}_z$ has eigenstates, $m$, that are the projection of the spin magnetic moment onto the z axis and can take on integer, or half-integer, values between $+I$ and $-I$.

---

2 This is the essence of the rotating frame transformation and will be revisited in more detail later.

3 In NQR, the quadrupole interaction is the dominant interaction. NQR will be considered later in the chapter but the formalism presented here is general enough to be easily transferable.
Figure 2.2: The simplest NMR experiment begins (A) with the sample magnetization (top row) in thermal equilibrium and directed along the external magnetic field. (B) The coil surrounding the sample (middle row) is connected to the rf transmitter which produces a current oscillating at the Larmor frequency and creates a time-dependent magnetic field in the coil, tipping the sample magnetization into the transverse plane. (C) Once the transmitter is turned off, the magnetization precesses in the transverse plane at the Larmor frequency and induces a current in the coil that can be detected in the receiver. (Bottom row) A schematic of the sequence of delays, transmitter pulses and subsequent free induction signal is typically depicted as such a “pulse sequence”.
The Applied rf Field

Closely related to the static Zeeman Hamiltonian is the rf Hamiltonian resulting from the oscillating transverse magnetic field present in the coil during rf irradiation. For an rf field of strength $B_1$, applied along the $x$-axis, the time-dependent rf Hamiltonian is

$$\hat{H}_{\text{rf}} = h\gamma B_1 \hat{I}_x \cos \omega_{\text{rf}} t.$$  \hspace{1cm} (2.6)

If the Zeeman interaction were the end of the story, NMR would not enjoy the widespread use that it does today. Nuclear spins feel a number of other, albeit weak, couplings to surrounding electrons (via chemical shift anisotropy, and quadrupole coupling) and to neighbouring nuclear spins (via dipole-dipole coupling and scalar coupling) that encode a variety of information such as internuclear distances, relative orientations, local electron configuration, etc.

2.2.2 Tensor Product Notation for the Hamiltonian of a Generic Interaction

Prior to elaborating on the interaction that nuclei experience, we introduce a tensor formalism for treating the Hamiltonian of a generic interaction. Later, describing specific interactions will only require specifying the form of the interaction tensor. Such a tensor formalism will be useful later in this dissertation as it simplifies rotations between frames of reference. This will be significant in the silk NMR experiments where Hamiltonians (specifically the CSA Hamiltonian) must be described in multiple frames of reference.

Consider a general interaction between spins $i$ and $j$, for which the Hamiltonian can be written as the tensor product:

$$\hat{H}_{\text{int}} = \vec{I} \cdot \vec{R} \cdot \vec{I}$$

$$= \sum_{\alpha, \beta} \hat{I}_\alpha^i R_{\alpha \beta} \hat{I}_\beta^j$$

$$= \sum_{\alpha, \beta} R_{\alpha \beta} \hat{T}_{\beta \alpha}$$ \hspace{1cm} (2.7) \hspace{1cm} (2.8) \hspace{1cm} (2.9)

where $\hat{I}_\alpha$ are the components of the spin operator, $\hat{T}_{\beta \alpha} \equiv \hat{I}_\alpha \hat{I}_\beta$ is a tensorial operator, and $R_{\alpha \beta}$ is a second rank Cartesian tensor that defines the interaction. The indices $\alpha, \beta = x, y, z$ for some choice of coordinate system.\(^4\) Since the dominant Zeeman interaction is tied to the lab frame and because ultimately only those parts of the interaction Hamiltonian\(^5\) that commute with $\hat{H}_z$ are considered, it is most convenient to work with spin operators in the

\(^4\)For chemical shift anisotropy $\vec{P}$ is replaced by $\vec{B}_0$ while for the quadrupolar interaction it is replaced by $\vec{P}$.

\(^5\)In this dissertation, we use the term "interaction Hamiltonian" to describe the Hamiltonian of some interaction, and do not refer to the Hamiltonian resulting from going into some interaction representation as is discussed in Section 2.3.4.
lab frame, where $\hat{H}_z$ is diagonal. Therefore to evaluate Eq. 2.9, the interaction tensor, $\mathbf{R}$, must also be written in the lab frame ($\mathbf{R}_{\text{Lab}}$).

However, the interaction tensor, which depends on physical parameters such as internuclear distance or local electronic configuration (and which is ultimately what one is interested in measuring), is most easily defined in its own principle axis system (PAS), that is generally different for each interaction. In its own PAS, the Cartesian interaction tensor is diagonal:

$$\mathbf{R}_{\text{PAS}} = \begin{pmatrix} R_{XX} & \ & \\ & R_{YY} & \\ & & R_{ZZ} \end{pmatrix}. \quad (2.10)$$

Oftentimes, it is convenient to define the quantities:

$$R = \frac{1}{3} \text{Tr}\{\mathbf{R}\},$$

$$\delta = R_{ZZ} - R,$$

$$\eta = \frac{R_{YY} - R_{XX}}{\delta},$$

where $R$ is the isotropic component of the tensor, $\delta$ is the anisotropy parameter, and $\eta$ is the asymmetry parameter. By convention, the $R_{ii}$ are defined such that $|R_{ZZ} - R| \geq |R_{YY} - R| \geq |R_{XX} - R|$, so that the anisotropy parameter is always positive, and the asymmetry parameter is between 0 and 1. In terms of these quantities, the interaction tensor can be rewritten as

$$\mathbf{R}_{\text{PAS}} = R\mathbf{I} - \delta \begin{pmatrix} \frac{1}{2}(1 + \eta) & & \\ & \frac{1}{2}(1 - \eta) & \\ & & -1 \end{pmatrix}. \quad (2.14)$$

To evaluate Eq. 2.9, the interaction tensor must be rotated into the lab frame. For each interaction there are a set of Euler angles, $\vec{\Omega}_{\text{PAS-Lab}} = (\alpha, \beta, \gamma)$, defined as the set of successive rotations that rotate the PAS into alignment with the lab frame. The convention for these rotations is such that the first rotation is about the lab z-axis by an angle $\gamma$, the second about the lab y-axis by an angle $\beta$, and last by an angle $\alpha$ about the lab z-axis,$^6$ as represented by the three rotation matrices:

$$\mathbf{A}(\vec{\Omega}) = \mathbf{A}_z(\alpha)\mathbf{A}_y(\beta)\mathbf{A}_z(\gamma)$$

$$= \begin{pmatrix} \cos \alpha & -\sin \alpha & 0 \\ -\sin \alpha & \cos \alpha & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} \cos \beta & 0 & \sin \beta \\ 0 & 1 & 0 \\ -\sin \beta & 0 & \cos \beta \end{pmatrix} \begin{pmatrix} \cos \gamma & \sin \gamma & 0 \\ -\sin \gamma & \cos \gamma & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad (2.15)$$

$^6$Note that the last rotation about the lab z-axis should never appear in the final expression for the Hamiltonian, since rotations about the static magnetic field should have no effect.
Chapter 2. An Introduction to Nuclear Magnetic Resonance

\[
\begin{pmatrix}
-c\gamma s\beta c\alpha - s\gamma s\alpha & s\gamma c\beta c\alpha + s\gamma s\alpha & s\beta c\alpha \\
-c\gamma c\beta s\alpha - s\gamma c\alpha & -s\gamma c\beta s\alpha + c\gamma c\alpha & -s\beta s\alpha \\
-c\gamma s\beta & -s\gamma s\beta & c\beta
\end{pmatrix}
\]  
\[\text{ (2.17)}\]

where the shorthand \( c \equiv \cos \) and \( s \equiv \sin \) has been used. It should be noted that the inverse rotation is given by the transpose of the above:

\[
A^{-1}(\Omega) = A_x(-\gamma)A_y(-\beta)A_z(-\alpha) = A^T(\Omega)
\]  
\[\text{ (2.18)}\]

In the lab frame, the interaction tensor can be written as

\[
R_{\text{Lab}} = A(\Omega_{\text{PAS-Lab}})R_{\text{PAS}}A^T(\Omega_{\text{PAS-Lab}})
\]  
\[\text{ (2.19)}\]

This expression can be inserted directly into Eq. 2.7 to evaluate the interaction Hamiltonian. However, such calculations become cumbersome with matrix multiplications that are littered with trigonometric functions, particularly when one must transform between several intermediate reference frames in going from the PAS to the lab frame (as is often the case as one typically must transform from the PAS to the molecular frame and, depending on the sample geometry, from the molecular frame to the sample frame as in Fig. 4.4). In such circumstances, another approach is more desirable.

### 2.2.3 Rotations with Irreducible Spherical Tensors

Rotations become simpler if, rather than using Cartesian tensors, one utilizes irreducible spherical tensors. One can regroup the Cartesian tensor components such that they transform predictably under rotation.\(^7\)

\[
\hat{\mathcal{H}}_{\text{int}} = C \sum_l \sum_{m=-l}^l (-1)^m R_{l,-m} \hat{T}_{lm}
\]  
\[\text{ (2.20)}\]

where \( \hat{T}_{lm} \) and \( R_{lm} \) are composed of linear combinations of \( T_{\alpha\beta} \) and \( R_{\alpha\beta} \) respectively. For a Cartesian interaction tensor that is symmetric (i.e. \( R_{\alpha\beta} = R_{\beta\alpha} \)), as is always the case in NMR,\(^8\) only the \( l=0 \) and \( 2 \) terms are required in the summation.

In the PAS of the interaction, only the \( m = 0, \pm 2 \) terms are non-zero. To distinguish the \( R_{lm} \) in this special frame, the tensor components are typically written as \( \rho_{lm} \) and can be expressed in terms of the variables \( R, \delta \) and \( \eta \) (which were defined in Eqs. 2.11–2.13):

\[
\rho_{00} = R,
\]  
\[\text{ (2.21)}\]

\(^7\)For example terms proportional to \( x^2 + y^2 + z^2 \) are invariant under rotation. See Ref. [8] for an introduction to irreducible spherical tensor operators and the 3-D rotation group, O3.

\(^8\)It is possible for the chemical shift tensor to have an antisymmetric component but it would not be observable by NMR techniques [5].
Chapter 2. An Introduction to Nuclear Magnetic Resonance

\[ \rho_{20} = \sqrt{\frac{3}{2}} \delta, \]  
\[ \rho_{2\pm 2} = \frac{1}{2} \eta \delta. \]  

(2.22)
(2.23)

Rotations for irreducible spherical tensors are defined simply. To rotate the interaction tensor into the lab frame by the set of rotations, \( \Omega_{\text{PAS-Lab}} = (\alpha, \beta, \gamma) \), one performs the following sum:

\[ R_{lm} = \sum_{m'=-l}^{l} \mathcal{D}_{m'm}^{l}(\Omega_{\text{PAS-Lab}}) \rho_{lm'}, \]  

(2.24)

where \( \mathcal{D}_{m'm}^{l}(\Omega) \) are elements of the Wigner rotation matrices defined to be

\[ \mathcal{D}_{m'm}^{l} = e^{im\alpha} d_{m'm}^{l}(\beta) e^{im'\gamma} \]  

(2.25)

with \( d_{00}^{l}(\beta) = 1 \) and \( d_{m'm}^{l}(\beta) \) and \( d_{m'm}^{l}(\beta) \) summarized in Tables 2.1 and 2.2.

Thus, the most useful form of the general interaction Hamiltonian is

\[ \mathcal{H}_{\text{int}} = C \sum_{l=0,2} \sum_{m=-l}^{l} \sum_{m'=-l}^{l} \rho_{lm'} \mathcal{D}_{m'm}^{l}(\Omega_{\text{PAS-Lab}}) \rho_{lm} \hat{T}_{lm} \]  

(2.26)

where the interaction is entirely defined by \( \rho_{lm} \) whereas \( \mathcal{D}_{m'm}^{l}(\Omega) \) and \( \hat{T}_{lm} \) are predefined.

For rotations from the PAS to the lab frame by two (or more) successive rotations via an intermediate frame (or frames), the Wigner rotation matrices can be expressed as

\[ \mathcal{D}_{m'm}^{l}(\Omega_{\text{PAS-Lab}}) = \sum_{m''=-l}^{l} \mathcal{D}_{m'm''}^{l}(\Omega_{\text{PAS-Int}}) \mathcal{D}_{m''m}^{l}(\Omega_{\text{Int-Lab}}). \]  

(2.27)

The spin operator tensor, \( \hat{T}_{i\beta} \equiv \hat{T}_{i\alpha} \hat{T}_{\alpha\beta} \), describing the interaction between spins \( i \) and \( j \) (via dipole-dipole or scalar coupling) can also be expressed in spherical tensor notation:

\[ \hat{T}_{00} = \hat{I}_{0} \cdot \hat{I}_{0} \]  
(2.28)

\[ \hat{T}_{20} = \frac{1}{\sqrt{6}} \left( 3 \hat{I}_{0} \hat{I}_{0} - \hat{I}_{0} \cdot \hat{I}_{0} \right) \]  
(2.29)

\[ \hat{T}_{2\pm 1} = \frac{1}{\sqrt{2}} \left( \hat{I}_{\pm 1} \hat{I}_{0} + \hat{I}_{0} \hat{I}_{\pm 1} \right) \]  
(2.30)

\[ \hat{T}_{2\pm 2} = \hat{I}_{\pm 1} \hat{I}_{\pm 1}, \]  
(2.31)
Table 2.1: Elements of $d_{m'm}(\beta)$.

<table>
<thead>
<tr>
<th>$m'$</th>
<th>$m$</th>
<th>$\pm 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\frac{1}{\sqrt{2}} \sin \beta$</td>
<td>$\frac{1}{2} (1 \pm \cos \beta)$</td>
</tr>
<tr>
<td>0</td>
<td>$\cos \beta$</td>
<td>$\mp \frac{1}{\sqrt{2}} \sin \beta$</td>
</tr>
<tr>
<td>-1</td>
<td>$-\frac{1}{\sqrt{2}} \sin \beta$</td>
<td>$\frac{1}{2} (1 \pm \cos \beta)$</td>
</tr>
</tbody>
</table>

Table 2.2: Elements of $d_{m'm}(\beta)$.

<table>
<thead>
<tr>
<th>$m'$</th>
<th>$m$</th>
<th>$\pm 1$</th>
<th>$\pm 2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>$\sqrt{\frac{3}{8}} \sin^2 \beta$</td>
<td>$\frac{1 \pm \cos \beta}{2} \sin \beta$</td>
<td>$(\frac{1 \pm \cos \beta)^2}{4}$</td>
</tr>
<tr>
<td>1</td>
<td>$\sqrt{\frac{3}{8}} \sin 2\beta$</td>
<td>$\pm [\cos^2 \beta - \frac{1 \pm \cos \beta}{2}] \mp \frac{1 \pm \cos \beta}{2} \sin \beta$</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>$\frac{3 \cos^2 \beta - 1}{2}$</td>
<td>$\mp \sqrt{\frac{3}{8}} \sin 2\beta$</td>
<td>$\sqrt{\frac{3}{8}} \sin^2 \beta$</td>
</tr>
<tr>
<td>-1</td>
<td>$-\sqrt{\frac{3}{8}} \sin 2\beta$</td>
<td>$\mp [\cos^2 \beta - \frac{1 \pm \cos \beta}{2}] \mp \frac{1 \pm \cos \beta}{2} \sin \beta$</td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td>$\sqrt{\frac{3}{8}} \sin^2 \beta$</td>
<td>$-\frac{1 \pm \cos \beta}{2} \sin \beta$</td>
<td>$(\frac{1 \pm \cos \beta)^2}{4}$</td>
</tr>
</tbody>
</table>
where
\[ \hat{I}_{\pm 1} = \pm \frac{1}{\sqrt{2}} \hat{I}_\pm, \quad (2.32) \]
\[ \hat{I}_0 = \hat{I}_z, \quad (2.33) \]
and \( \hat{I}_\pm = \hat{I}_x \pm i\hat{I}_y \) are the raising and lowering operators. Such a definition is convenient because the rank 1 spherical tensors are trivially defined as \( \hat{T}_{10} = \hat{I}_z \) and \( \hat{T}_{1\pm 1} = \hat{I}_{\pm 1} \).

Although it may not appear so at first, this formalism proves computationally simpler than the Cartesian tensor formalism, particularly in the case of multiple frame transformations.

### 2.2.4 Secular Hamiltonians

In typical high-field NMR experiments where magnetic field strengths are on the order of several Tesla, the Zeeman interaction dominates all other interactions. The other interactions constitute perturbations on the Zeeman Hamiltonian.\(^9\) The interaction Hamiltonian can be broken down into two components: one that commutes with \( \hat{\mathcal{H}}_z \) (i.e. the secular part), and one that does not (i.e. the non-secular part). To first order in perturbation theory, the non-secular component of the interaction may be discarded. Thus, the secular part of the interaction Hamiltonian (that is henceforth referred to as the interaction Hamiltonian), has the following property:

\[ \left[ \sum_i \omega_i \hat{I}_z, \hat{\mathcal{H}}_{\text{int}} \right] = 0. \quad (2.34) \]

From Eqs. 2.28–2.31, it is clear that only \( \hat{T}_{00} \) and \( \hat{T}_{20} \) are secular while \( \hat{T}_{2\pm 1} \) and \( \hat{T}_{2\pm 2} \) are non-secular and can be neglected for our purposes.\(^10\) Furthermore, for heterogeneous interactions (such as dipole-dipole coupling between protons and carbon), only part of \( \hat{T}_{20} \) is secular, in which case only the part proportional to \( \hat{I}_z^2 \) is retained.

The generalized secular interaction Hamiltonian can therefore be written as

\[ \hat{\mathcal{H}} = C \left\{ \rho_{00} \hat{T}_{00} + \hat{T}_{20} \sum_{m'=-2}^{2} D_{m'0}^2 (\tilde{\alpha}_{\text{PAS-Lab}}) \rho_{2m'} \right\} \quad (2.35) \]

---

\(^9\)In NQR, the quadrupolar interaction dominates, but since the Zeeman and quadrupolar interaction commute for the case of \( \eta = 0 \), the Zeeman basis is still often used.

\(^10\)The \( \hat{T}_{2\pm 1} \) and \( \hat{T}_{2\pm 2} \) terms must be retained when calculating relaxation rates.
\[ = C \left\{ R \vec{I} \cdot \vec{P} + \frac{3}{2} \left( 3 \cos^2 \beta - 1 \right) + \frac{\eta}{2} \left( \sin^2 \beta \cos 2\gamma \right) \right\} \] (2.36)

The dot product \( \vec{I} \cdot \vec{P} = I_x P_x + I_y P_y + I_z P_z \) does not always commute with the Zeeman Hamiltonian, and often only the \( I_z P_z \) term remains after truncation. Eq. 2.36 is a useful expression if the Euler angles connecting the PAS to the lab frame are known. Otherwise, the more general Eq. 2.35 is used.

### 2.2.5 Other Interactions

Specializing now to specific interactions, we proceed by writing down the interaction Hamiltonian in the PAS of the interaction, and the secular interaction in the lab frame. We focus on the chemical shift anisotropy and quadrupolar interaction that are relevant to this dissertation, but also review the dipole-dipole interaction that is ubiquitous in solid state NMR.

#### 2.2.6 Chemical Shift Anisotropy

The external magnetic field induces currents in orbital electrons that shield the nucleus from the external field. Since the electronic orbitals are in general anisotropic, the shielding depends on molecular orientation in the external field, and the interaction can be described by the chemical shift tensor, \( \sigma \). The chemical shift anisotropy (CSA) interaction can be written as

\[ \hat{H}_{\text{CSA}} = \hbar \gamma \vec{I} \cdot \sigma \cdot \vec{B}_0. \] (2.37)

Using Eq. 2.35 and making the substitution \( \vec{I} \rightarrow \vec{B}_0 = (0, 0, B_0) \), the secular Hamiltonian can be written in its most general and useful form as

\[ \hat{H}_{\text{CSA}} = \hbar \gamma B_0 \hat{I}_z \left\{ \sigma_{\text{iso}} D_{00} + \delta \left( D_{00}^2 + \frac{\eta}{\sqrt{6}} [D_{20}^2 + D_{20}^2] \right) \right\} \] (2.38)

where \( C = \hbar \gamma \) and the \( \rho_{lm} \) were defined by Eq. 2.21–2.23. The isotropic tensor component, \( R \), has been written as \( \sigma_{\text{iso}} \), and is known as the isotropic chemical shift. The arguments of the \( D_{m'm}^l \) are \( \tilde{\Phi}_{\text{PAS-Lab}} \) and have been omitted for compactness. Using Eq. 2.27, the \( D_{m'm}^l \) may be rewritten for an arbitrary number of successive frame transformations.

It is worthwhile pointing out that Eq. 2.38 consists of the product of an orientation-dependent factor and the \( \hat{I}_z \) operator. The CSA Hamiltonian therefore describes a small orientation-dependent frequency shift with respect to the Zeeman interaction. In solids,
the NMR frequency therefore depends on molecular orientation. In liquids, rapid molecular tumbling averages away all terms except $D_{00}^0 = 1$, so only the isotropic shift remains. Since $\sigma_{\text{iso}}$ depends on electron environment, frequencies due to different chemical sites can be spectrally resolved. This forms the basis for liquid-state NMR.

It is useful to write down a few other less general formulations of Eq. 2.38. If $\tilde{\Omega}_{\text{PAS-Lab}} = (\alpha, \beta, \gamma)$ is known, then

$$\tilde{H}_{\text{CSA}} = \hbar \gamma B_0 \hat{I}_z \left\{ \sigma_{\text{iso}} + \delta \left( \frac{3 \cos^2 \beta - 1}{2} + \frac{\eta}{2} \sin^2 \beta \cos 2\gamma \right) \right\}. \tag{2.39}$$

Alternately, if one knows the projection of the PAS axes onto the lab z-axis (given by the direction cosines, $\cos \alpha_0$, $\cos \beta_0$, and $\cos \gamma_0$), then

$$\tilde{H}_{\text{CSA}} = \hbar \gamma B_0 \hat{I}_z (\sigma_{XX} \cos^2 \alpha_0 + \sigma_{YY} \cos^2 \beta_0 + \sigma_{ZZ} \cos^2 \gamma_0) \tag{2.40}$$

$$= \hbar \gamma B_2 \hat{I}_z (\sigma_{XX} \sin^2 \beta \cos^2 \alpha + \sigma_{YY} \sin^2 \beta \sin^2 \alpha + \sigma_{ZZ} \cos^2 \beta) \tag{2.41}$$

where $\sigma_{ij}$ are the principle components of the chemical shift tensor. This formulation is often useful if Cartesian rotations were performed to rotate the PAS into the Lab frame.

### 2.2.7 The Dipole-Dipole Interaction

The nuclear magnetic moment produces a local dipole field that is felt by its neighbors. Consider a pair of spins, $i$ and $j$, with one at the origin of some axis system, and another at $r = r\hat{r}$, where $r$ is the internuclear distance and $\hat{r}$ is the unit vector along the line intersecting both nuclei. The complete untruncated dipole-dipole interaction Hamiltonian is given by

$$\tilde{H}_{\text{DD}} = -\frac{\hbar \gamma_i \gamma_j}{r^3} \left[ 3(\hat{r} \cdot \tilde{I})(\tilde{I} \cdot \hat{r}) - \tilde{I} \cdot \tilde{I} \right] \tag{2.42}$$

In the PAS of the dipole interaction, where both nuclei sit along the $z$-axis, the dipolar Hamiltonian simplifies to

$$\tilde{H}_{\text{DD}}^{\text{PAS}} = -\frac{\hbar \gamma_i \gamma_j}{r^3} \left( 3\hat{I}_z \hat{I}_z - \tilde{I} \cdot \tilde{I} \right) \tag{2.43}$$

$$= C \rho_{20} \hat{T}_{20} \tag{2.44}$$

where the only non-zero member of the interaction tensor is $\rho_{20} = \sqrt{\frac{3}{2}} \frac{1}{3}$, and $C = -2\hbar \gamma_i \gamma_j$.

Transforming to the lab frame to obtain the equivalent of Eq. 2.42 written in spherical tensor notation

$$\tilde{H}_{\text{DD}} = C \rho_{20} \sum_m D_{0-m}^2 (\tilde{\Omega}_{\text{PAS-Lab}}) \hat{T}_{2m}. \tag{2.45}$$
Truncation of the non-secular part of the Hamiltonian depends on whether the interacting nuclei have the same (or close to the same) NMR frequency or not (i.e. whether the interaction is homonuclear or heteronuclear).

**Homonuclear Dipole-Dipole Coupling**

For homonuclear dipole-dipole coupling, only $\hat{T}_{20}$ commutes with the Zeeman Hamiltonian, $\hat{\mathcal{H}}_z = \omega_0 (I_z^1 + I_z^2)$, and Eq. 2.45 simplifies to

\[
\tilde{\mathcal{H}}_{DD} = C_{\rho_{20}} D_0^2 (\tilde{\mathcal{H}}_{\text{PAS-Lab}}) \hat{T}_{20} \\
= -\frac{\hbar \gamma^2}{r^3} \left( \frac{3 \cos^2 \beta - 1}{2} \right) \left( 3 I_z^1 I_z^2 - \vec{I}_z \cdot \vec{I}_z \right) \\
= \omega_{dd} \left( 2 I_z^1 I_z^2 - \frac{1}{2} (I_+^1 I_+^2 + I_-^1 I_-^2) \right).
\]

**Heteronuclear Dipole-Dipole Coupling**

For heteronuclear dipole-dipole coupling, only those terms in $\hat{T}_{20}$ proportional to $I_z^1 S_z^2$ commute with the Zeeman Hamiltonian, $\hat{\mathcal{H}}_z = \omega_I I_z + \omega_S S_z$, and Eq. 2.45 simplifies to

\[
\tilde{\mathcal{H}}_{DD} = C_{\rho_{20}} D_0^2 (\tilde{\mathcal{H}}_{\text{PAS-Lab}}) 2 I_z S_z \\
= -\frac{\hbar \gamma_I \gamma_S}{r^3} (3 \cos^2 \beta - 1) I_z S_z \\
= \omega_{dd} 2 I_z S_z.
\]

A useful relation for calculating the strength of dipole-dipole couplings is [6]:

\[
\omega_{dd} = -2\pi (122 \text{ kHz}) \frac{\gamma_I}{\gamma_{II}} \frac{\gamma_S}{\gamma_{II}} \frac{1}{(r \text{ [Å]}^3)} \frac{(3 \cos^2 \beta - 1)}{2}.
\]

### 2.2.8 The Quadrupolar Interaction

The interaction of the nuclear quadrupole moment, $eQ$, with an external electric field gradient due to its surrounding electrons is defined by the tensor $V$. In the PAS of the quadrupolar interaction, the interaction tensor is diagonal with elements given by the gradient of the electric field (i.e. the second derivative of the electric potential), $V_{ii} = \frac{\partial^2 V}{\partial x^2}$ for $i = x, y, z$.

By the Laplace equation ($\nabla^2 V = 0$), the tensor is traceless. Thus $\rho_{00} = 0$, $\rho_{20} = \sqrt{3/2} V_{ZZ} = \ldots$

---

111The angular momentum operator $\vec{S}$ is typically used to denote the second nucleus rather than keeping superscripts on $\vec{I}$ to distinguish the two spin species. $\omega_I$ and $\omega_S$ are the respective Larmor frequencies of the two spin species.
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\[ \sqrt{\frac{3}{2}} e eq, \text{ and } \rho_{2\pm 2} = \frac{1}{2} (V_{YY} - V_{XX}) = \frac{1}{2} \eta eq \text{ where } eq \equiv V_{ZZ}. \] The quadrupolar interaction is

\[ \hat{H}_Q = \frac{e^Q}{6I(2I-1)} \mathbf{I} \cdot \mathbf{V} \cdot \mathbf{I} \] (2.53)

\[ = \frac{e^Q}{6I(2I-1)} \hbar \sum_{m=-2}^{2} \sum_{m'=-2}^{2} (-1)^m \rho_{2,m'} \mathcal{D}_{m',-m}^2(\Omega) \hat{T}_{2m} \] (2.54)

\[ = \frac{e^2qQ}{6I(2I-1)} \hbar \times \sum_{m=-2}^{2} (-1)^m \left( \frac{3}{2} \mathcal{D}_{0,-m}^2 + \frac{\eta}{2} \left[ \mathcal{D}_{2,-m}^2 + \mathcal{D}_{2,-m}^2 \right] \right) \hat{T}_{2m} \] (2.55)

A useful form of Eq. 2.55, that is commonly used in high-field NMR, and represents the secular quadrupolar Hamiltonian in the lab frame, is:

\[ \hat{H}_Q = \frac{e^2qQ}{6I(2I-1)} \hbar \left( \sqrt{\frac{3}{2}} \mathcal{D}_{0,-m}^2 + \frac{\eta}{2} \left[ \mathcal{D}_{2,-m}^2 + \mathcal{D}_{2,-m}^2 \right] \right) \hat{T}_{20} \] (2.56)

\[ = \frac{e^2qQ}{4 \cdot 6I(2I-1)} \hbar \left( 3 \cos^2 \beta - 1 + \eta \sin^2 \beta \cos 2\gamma \right) \left( 3 \hat{T}_z^2 - \hat{I}^2 \right) \] (2.57)

were the \( \mathcal{D}_{m'm} \) have arguments \( \tilde{\Omega}_{PAS-Lab} \).

### 2.2.9 Nuclear Quadrupole Resonance

In Nuclear Quadrupole Resonance (NQR), the quadrupole Hamiltonian dominates, and the resonance frequency is determined by level splittings due to \( \hat{H}_Q \). In this case, it is useful to express the quadrupolar Hamiltonian in its own PAS, as can be done by setting the arguments of \( \mathcal{D}_{m'm} \) to zero in Eq. 2.55:

\[ \hat{H}_{Q}^{PAS} = \frac{e^2qQ}{2 \cdot 6I(2I-1)} \hbar \left( 3 \hat{T}_z^2 - \hat{I}^2 + \frac{\eta}{2} \left( \hat{T}_z^2 + \hat{I}_z^2 \right) \right). \] (2.58)

In many systems, the quadrupolar interaction is sufficiently large that experiments may be performed with no external magnetic field applied. In NQR, each nuclear spin is quantized along its own PAS, and only in the special case of a single crystal with one molecule per unit site (or multiple sites with mirror symmetry in the electric field gradient) does the sample have a unique quantization direction.

In NQR experiments, it is often more useful to think about things in the quadrupolar PAS. Thus, for an rf coil whose axis can be brought into the quadrupolar PAS by the set of rotations \( \tilde{\Omega}_{PAS-Lab} \), the rf Hamiltonian, for which \( \rho_{10} = \gamma B_1 \cos \omega_{rf} t \) and all other \( \rho_{lm} = 0 \),
can be written in the quadrupolar PAS as

\[ \hat{\mathcal{H}}^{\text{PAS}}_{\text{rf}} = \sum_{m=-1}^{1} (-1)^m \rho_{10} \mathcal{D}_{0-m}^{0} \hat{T}_{1m} \]

\[ = \gamma B_1 \cos \omega_{\text{rf}} t \left[ \mathcal{D}_{00}^{0} \hat{T}_{10} - \left( \mathcal{D}_{01}^{0} \hat{T}_{1-1} + \mathcal{D}_{0-1}^{0} \hat{T}_{11} \right) \right] \]

\[ = \gamma B_1 \cos \omega_{\text{rf}} t \left[ \cos \beta \hat{I}_z + \sin \beta \left( \hat{I}_x \cos \alpha + \hat{I}_y \sin \alpha \right) \right], \]

where the arguments of \( \mathcal{D}_{m'm} \) are \( \tilde{\Omega}_{\text{Coil-PAS}} \). For multiple crystal sites, each having a different \( \tilde{\Omega}_{\text{Coil-PAS}} \), the contribution to the signal from each must be summed.

### 2.3 The Time-Dependent State of the System

#### 2.3.1 The Density Matrix

In NMR, as in most experiments in quantum mechanics, the experimentally measurable quantity is the expectation value of certain operators. In NMR, this is typically \( \hat{I}_+ \) (see Section 2.5.2).

For an arbitrary time-dependent state \( |\psi(t)\rangle = \sum_i a_i(t) |i\rangle \), written in some basis, \( |i\rangle \), the expectation value of \( \hat{I}_+ \) is

\[ \langle \hat{I}_+ \rangle = \langle \psi | \hat{I}_+ | \psi \rangle \]

\[ = \sum_{i,j} a_i^\dagger a_j \langle i | \hat{I}_+ | j \rangle. \]

The coefficients \( a_i^\dagger a_j \) entirely define the time-dependent state of the system, while the factors \( \langle i | \hat{I}_+ | j \rangle \), quantities independent of the state, depends only on the choice of basis and can be calculated beforehand.

The density matrix operator,

\[ \hat{\rho}(t) \equiv |\psi(t)\rangle \langle \psi(t)| \]

\[ \equiv \sum_{ij} a_i^\dagger a_j \langle i | \langle j | \]

is defined as the matrix with elements

\[ \rho_{ij} = \langle i | \hat{\rho} | j \rangle \]

\[ = a_i^\dagger a_j. \]

and therefore entirely encodes the state of the system.
In terms of the density matrix, Eq. 2.63 can be written as the trace of the density matrix with $\hat{I}_+ = \hat{I}_x + i\hat{I}_y$

$$\langle \hat{I}_+ \rangle = \text{Tr}\{\hat{I}_+ \hat{\rho}\}.$$  \hfill (2.68)

In this definition of the density matrix, it has been assumed that the system was in a pure quantum mechanical state, but the same formalism holds for a mixed state (i.e. an ensemble in which each member has some probability of being in each of the pure states). NMR experiments deal with large ensembles of nuclei (on the order of Avogadro's number), where such a mixed state is realized.

In thermal equilibrium, the initial density matrix is given by

$$\hat{\rho}_{eq} = e^{-\hat{H}_z/k_B T} Z,$$  \hfill (2.69)

where $\hat{H}_z$ is the Zeeman Hamiltonian given by Eq. 2.5, $T$ is the temperature, $k_B$ is the Boltzmann constant, and $Z$ is the partition function necessary for proper normalization. In the high temperature limit, after Taylor expansion of the above relation and discarding the term proportional to unity, the equilibrium density matrix for a single nuclear spin species becomes

$$\hat{\rho}_{eq} \propto \left(\frac{-\hbar \gamma B_0}{2k_B T}\right) \hat{I}_z.$$  \hfill (2.70)

The prefactor is typically dropped for evolution of the system. It is important when considering the dependence of signal intensity on external ($B_0, T$), and internal ($\gamma$) parameters.

### 2.3.2 Time Evolution of the Density Matrix

The time evolution of the density matrix can be obtained by considering the derivative of $\hat{\rho}$ with respect to time

$$\frac{\partial \hat{\rho}(t)}{\partial t} = \frac{\partial}{\partial t} \left( |\psi(t)\rangle \langle \psi(t)| \right)$$  \hfill (2.71)

$$= \frac{\partial |\psi(t)\rangle}{\partial t} \langle \psi(t)| + |\psi(t)\rangle \frac{\partial \langle \psi(t)|}{\partial t},$$  \hfill (2.72)

into which can be substituted the time-dependent Schrödinger equation

$$i\hbar \frac{\partial}{\partial t} |\psi(t)\rangle = \hat{\mathcal{H}} |\psi(t)\rangle$$  \hfill (2.73)
to obtain the well known Liouville equation

$$\frac{\partial \hat{\rho}(t)}{\partial t} = i \frac{\hbar}{\hbar} \left[ \hat{\rho}(t), \hat{\mathcal{H}} \right] \quad (2.74)$$

where the square brackets indicate the commutation of $\hat{\mathcal{H}}$ with $\hat{\rho}$.

For an arbitrary Hamiltonian that may be time-dependent, the general solution to Eq. 2.74 is

$$\hat{\rho}(t) = T \exp \left( i \frac{\hbar}{\hbar} \int_0^t \hat{\mathcal{H}}(t') \, dt' \right) \hat{\rho}(0) T \exp \left( - i \frac{\hbar}{\hbar} \int_0^t \hat{\mathcal{H}}(t') \, dt' \right) \quad (2.75)$$

as can be easily seen by substituting back into Eq. 2.74. The Dyson time-ordering operator, $T$, ensures causality. Under certain circumstances, such as for time-independent Hamiltonians and certain time-dependent Hamiltonians, the above can be evaluated exactly, whereas for the more general case, approximate methods such as average Hamiltonian theory must be used. Both cases are reviewed below.

### 2.3.3 Evolution Under Time-Independent Hamiltonians

For a time-independent Hamiltonian, $\hat{\mathcal{H}}$, the solution of the Liouville equation is

$$\hat{\rho}(t) = \exp \left( i \frac{\hbar}{\hbar} \int \hat{\mathcal{H}} \, dt \right) \hat{\rho}(0) \exp \left( - i \frac{\hbar}{\hbar} \int \hat{\mathcal{H}} \, dt \right) \quad (2.76)$$

Practically speaking, such expressions can be evaluated by Taylor expansion of the exponential followed by matrix multiplication.

As an example, consider a spin-1/2 system in an initial state given by $\rho(0) = \hat{I}_z$ evolving under the Zeeman Hamiltonian, $\hat{\mathcal{H}} = \hbar \omega_0 \hat{I}_z$.\(^{12}\) Taylor expansion of Eq. 2.76 yields

$$\hat{\rho}(t) = \left( \sum_n \frac{(i\omega_0 t \hat{I}_z)^n}{n!} \right) \hat{I}_z \left( \sum_m \frac{(-i\omega_0 t \hat{I}_z)^m}{m!} \right) \quad (2.77)$$

$$= \left( \sum_{n \text{ even}} \frac{(i\omega_0 t/2)^n}{n!} \hat{I}_0 + \sum_{n \text{ odd}} \frac{(i\omega_0 t/2)^n}{n!} \hat{I}_z \right) \hat{I}_z$$

$$+ \left( \sum_{m \text{ even}} \frac{(i\omega_0 t/2)^m}{m!} \hat{I}_0 - \sum_{m \text{ odd}} \frac{(i\omega_0 t/2)^m}{m!} \hat{I}_z \right) \quad (2.78)$$

\(^{12}\)The solution of Eq. 2.76 for the case of an initial state $\rho(0) = \hat{I}_z$ is trivial since the density matrix commutes with the Hamiltonian and does not evolve. (i.e. $\rho(t) = \hat{I}_z$)
2.3.4 The Rotating Frame and the Interaction Representation

It is the evolution due to the perturbing interactions (whose strength depends on many physical parameters) that gives NMR its richness as an experimental technique. Thus it is useful to separate out the effects of the Zeeman Hamiltonian from that of other interactions. This is typically done by transforming into a frame of reference that is rotating at the Larmor frequency. This serves two purposes: to eliminate the effects of the Zeeman interaction (i.e. the rotations in Liouville space described in the previous section), and to make the time-dependent rf Hamiltonian (that typically oscillates at or near the Larmor frequency) time independent. This is a particularly useful example to work through as other variations of such frame transformations will be utilized in Chapters 7, 8, and 9.

Consider the time evolution according to the Liouville equation due to the Zeeman interaction, $\hat{H}_z = \omega_0 \hat{I}_z$, a sinusoidally varying rf field applied along the x-direction, $\hat{H}_{\text{rf}}(t) = 2\omega_1 \hat{I}_x \cos(\omega_0 t)$ and other miscellaneous internal and external interactions represented by the secular Hamiltonian $\hat{H}_{\text{int}}$:

$$\frac{\partial \hat{\rho}(t)}{\partial t} = i[\hat{\rho}(t), \omega_0 \hat{I}_z + 2\omega_1 \hat{I}_x \cos(\omega_0 t) + \hat{H}_{\text{int}}].$$

(2.82)

Multiplying on the left by $\hat{U} = e^{i\omega_0 t\hat{I}_z}$ and on the right by its complex conjugate, and

\[\hat{I}_x = \hat{I}_0 \cos \omega_0 t/2 + i \hat{I}_z \sin \omega_0 t/2\]

\[\hat{I}_y = \hat{I}_0 \cos \omega_0 t/2 - i \hat{I}_z \sin \omega_0 t/2\]

\[\hat{I}_z = \hat{I}_0 \cos \omega_0 t + \hat{I}_y \sin \omega_0 t\]

where we have used the fact that, for spin-$1/2$, $\hat{I}^{2n}_z = \frac{1}{2^n} \hat{I}_0$ and $\hat{I}^{2n+1}_z = \frac{1}{2^{n+1}} \hat{I}_z$. Though the above result was derived for spin-$1/2$, it applies to arbitrary spin.

It is oftentimes more intuitive to think of the density matrix as a vector (in what is known as Liouville space) rotating about the Hamiltonian (also a vector in Liouville space). This can be seen formally by decomposing the density matrix into a sum of spin operators:

$$\rho = c_0 \hat{I}_0 + c_x \hat{I}_x + c_y \hat{I}_y + c_z \hat{I}_z.\quad (2.81)$$

Neglecting the term containing the identity matrix, $\hat{I}_0$, the density matrix can be considered as the vector $(c_x, c_y, c_z)$ in Liouville space. Thus, for a single spin in Eq. 2.80, the time-dependent state represents a rotation in Liouville space about the z-axis at a frequency $\omega_0$.

This formulation is particularly convenient due to the commutation properties of spin operators $[\hat{I}_x, \hat{I}_y] = i \hat{I}_z$, and cyclic permutation of $x, y, z$. 

\[\hat{U} = e^{i\omega_0 t\hat{I}_z}\]
recognizing that
\[
\frac{\partial \hat{\rho}(t)}{\partial t} \hat{U}^\dagger = \frac{\partial \left( \hat{U} \hat{\rho}(t) \hat{U}^\dagger \right)}{\partial t} - \frac{\partial \hat{U} \hat{\rho}(t) \hat{U}^\dagger}{\partial t} - \hat{U} \frac{\partial \hat{\rho}(t)}{\partial t} \hat{U}^\dagger
\]
\[=
\frac{\partial \hat{\rho}_R(t)}{\partial t} + i[\hat{\rho}_R(t), \omega_0 \hat{I}_z] \tag{2.83}
\]
where \(\hat{\rho}_R(t) \equiv \hat{U} \hat{\rho}(t) \hat{U}^\dagger\) is the density matrix in the rotating frame, one obtains
\[
\frac{\partial \hat{\rho}_R(t)}{\partial t} = i[\hat{\rho}_R(t), 2\omega_1 \hat{I}_x \hat{U}^\dagger \cos \omega_0 t + \hat{\mathcal{H}}_{int}] \tag{2.84}
\]
\[= i[\hat{\rho}_R(t), 2\omega_1 (\hat{I}_x \cos \omega_0 t + \hat{I}_y \sin \omega_0 t) \cos \omega_0 t + \hat{\mathcal{H}}_{int}] \tag{2.85}
\]
\[= i[\hat{\rho}_R(t), \omega_1 \hat{I}_x + \hat{\mathcal{H}}_{int}] + i[\hat{\rho}_R(t), \omega_1 (\hat{I}_x \cos 2\omega_0 t + \hat{I}_y \sin 2\omega_0 t)] \tag{2.86}
\]
\[\approx i[\hat{\rho}_R(t), \omega_1 \hat{I}_x + \hat{\mathcal{H}}_{int}] \tag{2.87}
\]
In the second line, the result from Eq. 2.80 was used, and in the fourth line, the counter-rotating component of the rf was discarded since it varies quickly compared to the rate at which the system evolves.\(^{14}\) The resulting effective Hamiltonian has a time-independent contribution from the rf field, and no Zeeman term.

In an analogous way, one can take any time-independent interaction Hamiltonian, \(\hat{\mathcal{H}}_{int}\), and transform into a frame (known as an interaction representation) in which that Hamiltonian disappears by applying the unitary transformation \(\hat{U} = e^{i\hat{\mathcal{H}}_{int}t}\).

\[
\frac{\partial \hat{\rho}(t)}{\partial t} = i[\hat{\rho}(t), \hat{\mathcal{H}}_{int} + \hat{\mathcal{H}}_{other}] \tag{2.88}
\]
\[
\frac{\partial \hat{U} \hat{\rho}(t) \hat{U}^\dagger}{\partial t} = i[\hat{U} \hat{\rho}(t) \hat{U}^\dagger, (\hat{\mathcal{H}}_{int} - \frac{1}{i} \frac{\partial}{\partial t} \ln \hat{U}) + \hat{U} \hat{\mathcal{H}}_{other} \hat{U}^\dagger] \tag{2.89}
\]
\[
\frac{\partial \hat{U} \hat{\rho}(t) \hat{U}^\dagger}{\partial t} = i[\hat{U} \hat{\rho} \hat{U}^\dagger, \hat{U} \hat{\mathcal{H}}_{other} \hat{U}^\dagger]. \tag{2.90}
\]
This method, often referred to as the toggling frame transformation, is a useful way of taking into account the effects of certain terms in the Hamiltonian. The remaining Hamiltonian is referred to as the effective Hamiltonian in the interaction representation
\[
\hat{\mathcal{H}}_{eff} = \hat{U} \hat{\mathcal{H}}_{other} \hat{U}^\dagger. \tag{2.91}
\]
For other terms, such as those that are time-dependent, these approaches are not easily applied and a perturbative approach is required.

\(^{14}\)Later, the effect of this time-dependent term will be calculated.
2.3.5 Average Hamiltonian Theory

A time-dependent Hamiltonian can be written as piecewise time-independent over some small interval, $\tau_i$. This can always be done as long as the interval is chosen to be short enough. Thus, the density matrix can be evolved according to Eq. 2.76 for each time interval according to

$$\hat{\rho}(t) = T \left( \prod_i e^{i\hat{H}_i \tau_i} \right) \hat{\rho}(0) T \left( \prod_i e^{-i\hat{H}_i \tau_i} \right)$$

(2.92)

Here, the Dyson time-ordering operator, $T$, ensures that the product is applied to $\hat{\rho}(0)$ in the proper order to maintain causality. Using the Baker-Campbell-Hausdorff relation

$$e^{A} e^{B} = e^{\hat{A} + \hat{B} + \frac{1}{2}[\hat{A},\hat{B}]+\frac{1}{2}([\hat{A},[\hat{A},\hat{B}]]+[\hat{A},[\hat{A},\hat{B}]])+...}$$

(2.93)

to rewrite Eq. 2.92 as

$$\hat{\rho}(t) = e^{i(\hat{H}^{(0)}+\hat{H}^{(1)}+\hat{H}^{(2)}+... \hat{H}(t')) \hat{\rho}(0) e^{-i(\hat{H}^{(0)}+\hat{H}^{(1)}+\hat{H}^{(2)}+... \hat{H}(t))}.$$}

(2.94)

This is the well known Magnus expansion of the Hamiltonian, the first few terms of which, in the limit of $\tau_i \to 0$, are

$$\hat{H}^{(0)} = \frac{1}{t} \int_0^t \hat{\mathcal{H}}(t') dt'$$

(2.95)

$$\hat{H}^{(1)} = \frac{-i}{2t} \int_0^t \int_0^{t'} \frac{1}{t''} \frac{d}{dt''} [\hat{\mathcal{H}}(t'), \hat{\mathcal{H}}(t'')].$$

(2.96)

Restricting to the first term in the expansion, (i.e. the zeroth order term) one finds that the density matrix evolves under a Hamiltonian that is averaged over the time during which it is applied:

$$\hat{\rho}(t) = e^{i\hat{H}^{(0)}t} \hat{\rho}(0) e^{-i\hat{H}^{(0)}t}.$$}

(2.97)

However, when the first term disappears, the leading order effect is described by the second term. For an example of effects due to the first order Hamiltonian, see Section 2.4.4, on the Bloch-Siegert shift.

2.4 A Few Practical Tools in Solid State NMR

In this section, some practical tools of pulsed NMR are described, which are based on the concepts introduced in previous sections. The purpose is twofold: first, to make concrete some of the abstract concepts that have been introduced thus far, and second, because some of these tools will be used in experiments presented later in this dissertation. These examples
span a small subset of the tools currently in practice in modern NMR.

2.4.1 Echoes

Oftentimes, one wishes to suppress the evolution of the density matrix due to certain interactions (say $H_{\text{int}}$) over some period of time (say $\tau$). The general concept of echoes is that, by applying some set of rf pulses during $0 < t < \tau$, the average effect of the interaction will be zero at $\tau$. This is nothing more than restating the average Hamiltonian theory presented earlier, except that rather than a time-dependent Hamiltonian, we have a piecewise time-independent one consisting of $H_{\text{int}}$ and the rf pulses.

The specific pulse sequence used to achieve an echo depends on the interaction that one wishes to refocus. Echoes are best described by giving some common examples.

**Hahn Echo**

The simplest of echo sequences, the Hahn echo refocuses chemical shift anisotropy and heteronuclear dipole coupling (and heteronuclear scalar coupling in liquids) and consists of a 180° pulse at $t = \tau/2$. Thus, the propagator, $\hat{U}$, under which the density matrix evolves is given by

$$
\hat{U} = e^{i(\delta I_z + \omega_{dd} 2I_z S_z)\tau/2} e^{i\pi I_x} e^{i(\delta I_z + \omega_{dd} 2I_z S_z)\tau/2} e^{-i\pi I_x} e^{i\pi I_z}
$$

The net effect of the echo sequence is therefore a rotation of the $I$ spin by 180° about the $x$-axis. For an initial state, $\rho(0) = a\hat{I}_x + b\hat{I}_y + c\hat{I}_z$, the final state is $\rho(\tau) = a\hat{I}_x - b\hat{I}_y - c\hat{I}_z$ with no effect due to the chemical shift or heteronuclear dipole coupling.

**Solid (or Quadrupolar) Echo**

The solid echo serves to refocus Hamiltonians that are bilinear in the same spin species such as homonuclear dipole-dipole coupling and quadrupolar coupling. Here we will consider the former, but a similar calculation can be done for the latter.

The solid echo consists of a 90° pulse applied at $t = \tau/2$. The propagator for the duration of the echo sequence is:

$$
\hat{U} = e^{i(\omega_{dd} 3I_z^2 \hat{I}_z - \hat{I} \cdot \hat{P})\tau/2} e^{i\pi I_x} e^{i(\omega_{dd} 3I_z^2 \hat{I}_z - \hat{I} \cdot \hat{P})\tau/2} e^{-i\pi I_x} e^{i\pi I_z}
$$

The net effect of the echo sequence is therefore a rotation of the $I$ spin by 180° about the $x$-axis. For an initial state, $\rho(0) = a\hat{I}_x + b\hat{I}_y + c\hat{I}_z$, the final state is $\rho(\tau) = a\hat{I}_x - b\hat{I}_y - c\hat{I}_z$ with no effect due to the chemical shift or heteronuclear dipole coupling.
Multi-pulse Techniques for High-Resolution NMR in Solids

Other more complicated pulses schemes exist to fully refocus the dipolar coupling and other Hamiltonians. Such echo techniques are a simple application of a more general set of multi-pulse techniques widely used in NMR to recover high-resolution spectra from solids, as is often desirable to extract the effects of one Hamiltonian while eliminating the contribution from others, (for example to recover spectral chemical shift resolution while eliminating dipole-dipole interactions that would otherwise broaden the spectrum). The general approach involves the repeated application of echo techniques to refocus the effects of the unwanted coupling. By stroboscopically detecting only at the times when the echo has refocused, the resulting free induction signal arises from solely those terms in the Hamiltonian that were not refocused by the echo sequence. The first of such multi-pulse sequences was the WaHuHa sequence [9] but today dozens of such sequences exist. The interested reader is referred to Ref. [5] for a good review of these useful techniques as they are beyond the scope of this dissertation.

2.4.2 Heteronuclear CW Decoupling

In many solid state NMR spectra undesirable line-broadening effects often arise due to strong (compared to other interactions of interest) dipole-dipole couplings to neighbouring protons. The dipole-dipole Hamiltonian can be averaged to zero by applying a strong rf field at the Larmor frequency of one of the nuclear species.

To see this, consider the total Hamiltonian in the rotating frame:

$$\hat{H}_T = \gamma B_1 \hat{I}_x + 2\omega_{dd} \hat{I}_z \hat{S}_z$$

(2.107)

where the spin operator of the second nucleus has been written as $\hat{S}_z$. The effective Hamiltonian in an interaction representation given by $\hat{U} = \exp \left( i \gamma B_1 t \hat{I}_x \right)$ (using Eq. 2.91) is

$$\hat{H}_{\text{eff}} = 2\omega_{dd} \hat{U} \hat{I}_z \hat{S}_z \hat{U}^\dagger$$

(2.108)

$$= 2\omega_{dd} \left( \hat{I}_z \cos \omega_1 t - \hat{I}_y \sin \omega_1 t \right) \hat{S}_z$$

(2.109)

\[\text{\textsuperscript{15}}\text{Strong compared to the strength of the dipole interaction, } \omega_{dd}.\]
To zeroth order in Average Hamiltonian theory:

\[ H_{\text{eff}}^{(0)} = \frac{2\omega_{dd}\hat{S}_z}{t} \int_0^t \left( \hat{I}_z \cos \omega_1 t' - \hat{I}_y \sin \omega_1 t' \right) \partial t' \]

\[ = 0. \] (2.111)

Strictly speaking, the above is true only if \( t = n/\omega_1 \) for \( n = 0, 1, 2, \ldots \). However, for \( \omega_1 \gg \omega_{dd} \), where the evolution due to the rf field is fast compared to the evolution due to dipole-dipole coupling, the integral converges quickly and can, for convenience, be evaluated at \( t \to \infty \). This concept will be used later when considering the Magnus expansion for two-photon excitation.

### 2.4.3 Cross-Polarization

Polarization transfer between nuclei has many practical advantages in solid state NMR.\(^{16}\) Most solid state NMR experiments are performed on nuclei, such as \(^{13}\)C (spin 1/2), \(^{15}\)N (spin 1), or \(^3\)H (spin 1) which have small \( \gamma \) compared to protons (\(^{1}\)H, spin 1/2), and are often diluted in a sample by spin 0 isotopes. This keeps the strength of the homonuclear dipole couplings to surrounding nuclei relatively weak and is beneficial because strong couplings result in line broadening that is non-trivial to remove, especially in the case of homonuclear dipole-dipole coupling.

The small \( \gamma \) of these nuclei results in weak signal intensity due to weak initial polarization of the nuclei (see Eq. 2.70), as well as long relaxation times that require waiting long times (up to hours or days) between acquisitions making signal averaging impractical. For these reasons, it is desirable to transfer polarization from protons, which are very abundant in typical organic samples, have a relatively high gyromagnetic ratio hence a high thermal equilibrium polarization, and the short relaxation times (due to strong dipole coupling to neighbours).

The simplest polarization transfer scheme in solid state NMR is cross-polarization, in which simultaneous rf irradiation of both nuclei at specific intensities results in polarization transfer.

Consider a proton (that will be referred to as \( \hat{S} \)) in the vicinity of a low-gamma nucleus (\( \hat{I} \)) initially in thermal equilibrium:

\[ \hat{\rho}_{eq} = \omega_I \hat{I}_z + \omega_S \hat{S}_z \] (2.112)

where \( \omega_i = \gamma_i B_0 \). The state of the system following a \( \pi/2 \) pulse along x applied on-resonance to each spin, in an interaction representation obtained via the transformation

\(^{16}\)there are also many advantages in liquid state NMR, although the polarization transfer techniques are different than described here.
\[ \hat{U} = \exp\left(i(\omega_I \hat{I}_z + \omega_S \hat{S}_z)t\right) \] typically referred to as the double rotating frame, is

\[ \rho_0 = \omega_I \hat{I}_y + \omega_S \hat{S}_y. \tag{2.113} \]

If the phase of each rf channel is incremented by 90°, then the effective Hamiltonian is now along the direction of magnetization (in the rotating frame), and continued irradiation does not result in any further nutation. This is referred to as spin-locking. \(^\text{17}\) The effective Hamiltonian under spin locking, including the heteronuclear dipole-dipole coupling is:

\[ \hat{H}_{\text{eff}} = \omega_I \hat{I}_y + \omega_S \hat{S}_y + 2\omega_{dd} \hat{I}_z \hat{S}_z. \tag{2.114} \]

One can consider a second transformation to another interaction representation via \( \hat{U}_1 = \exp\left(i(\omega_I \hat{I}_y + \omega_S \hat{S}_y)t\right) \) in which

\[ \hat{H}'_{\text{eff}} = 2\omega_{dd} \hat{U}_1 \hat{I}_z \hat{S}_z \hat{U}_1^\dagger \]

\[ = 2\omega_{dd} \left( \hat{I}_z \cos \omega_I t + \hat{I}_x \sin \omega_I t \right) \times \left( \hat{S}_z \cos \omega_S t + \hat{S}_x \sin \omega_S t \right). \tag{2.116} \]

The zeroth order term in the Magnus expansion of the above Hamiltonian is

\[ \hat{H}^{(0)} = \omega_{dd} (\hat{I}_z \hat{S}_z + \hat{I}_x \hat{S}_x) \tag{2.117} \]

and is non-zero only if \( \omega_I = \omega_S \) (by orthogonality of trigonometric function with respect to integration). This requirement can be rewritten as:

\[ \frac{B_{I1}}{B_{S1}} = \frac{\gamma_S}{\gamma_I}, \tag{2.118} \]

the well-known Hartman-Hahn matching condition for cross-polarization. Qualitatively, what is happening, is that the strength of the spin-locking fields are such that the Zeeman-splitting is the same for both rf fields in their respective rotating frames. Therefore, mutual spin-flips (so-called "flip-flops") in which an \( S \) spin flips up while an \( I \) spin flips down (or vice versa) conserves the total energy. This can be seen further by rewriting Eq. 2.117 in terms of raising and lowering operators defined along the \( y \)-axis as \( \hat{I}_{\pm} = \hat{I}_x \pm i \hat{I}_z \):

\[ \hat{H}^{(0)} = \frac{\omega_{dd}}{2} (\hat{I}_+ \hat{S}_- + \hat{I}_- \hat{S}_+). \tag{2.119} \]

It can be shown [6] that evolution of the state in Eq. 2.113 under the above Hamiltonian

\(^{17}\)Note that since typically \( B_0 \gg B_1 \), the magnetization along the rf field is far from thermal equilibrium. It is commonly referred to as having a very low spin temperature.
yields

\[ \gamma I I_y \frac{\gamma_{dd}(I_x \hat{S}_x + I_y \hat{S}_y)}{2} \rightarrow \gamma I \left( I_y (1 + \cos \omega_{dd}t)/2 + \hat{S}_y (1 - \cos \omega_{dd}t)/2 \right. \]

\[ \left. + (I_z \hat{S}_y - I_y \hat{S}_z) \sin \omega_{dd}t \right) . \]  

(2.120)

In practice, there exist a range of \( \omega_{dd} \) (due to a range of internuclear distances and relative orientations) so that the oscillatory nature is not observed. The relevant result is that polarization is transferred from the highly polarized \( I \) spin to the weakly polarized \( S \) spin.

For polarization transfers from \(^1H\) to \(^13C\), for example, cross-polarization results in an increase in signal by up to a factor of four (\( \gamma_{1H} \approx 4 \gamma_{13C} \)). Furthermore, because protons relax much faster than \(^13C\) (100’s of milliseconds compared to 10’s of seconds), repetition of the experiment for signal averaging purposes is limited by the relaxation time of protons, resulting in an improvement in signal to noise per unit time by a factor of \( \sqrt{100} \approx 10 \).

### 2.4.4 The Bloch-Siegert Shift

In Section 2.3.4, a rotating frame transformation was demonstrated. The linear rf field was decomposed into two counter-rotating components and the transformation was made into a frame rotating with one of the components rendering it time-independent. The counter-rotating component (in Eq. 2.85) was thrown away, with a claim that its effects on the Hamiltonian were negligible. This counter rotating component does contribute a small correction in the form of a non-zero first order average Hamiltonian. The counter-rotating Hamiltonian is,

\[ \hat{H}_{CR} = \omega_1 \left( \hat{I}_x \cos 2\omega_0 t + \hat{I}_y \sin 2\omega_0 t \right) . \]  

(2.121)

It is obvious that the average of \( \hat{H}_{CR} \) is zero, hence the zeroth order term in the Magnus expansion (Eq. 2.95) vanishes. However, this is not so for the first order term (given by Eq. 2.96) that gives:

\[ \hat{H}^{(1)} = \frac{-i\omega_1^2}{2t} \int_0^t \partial t' \int_0^{t'} \partial t'' [\hat{I}_x \cos 2\omega_0 t' + \hat{I}_y \sin 2\omega_0 t', \hat{I}_x \cos 2\omega_0 t'' + \hat{I}_y \sin 2\omega_0 t''] \]

(2.122)

\[ = \frac{-\omega_1^2}{2t} \int_0^t \partial t' \int_0^{t'} \partial t'' \sin 2\omega_0 (t' - t'') \hat{I}_z \]

(2.123)

\[ = \frac{\omega_1^2}{4\omega_0 t} \int_0^t \partial t' (1 - \cos 2\omega_0 t') \hat{I}_z \]

(2.124)

\[ = \frac{\omega_1^2}{4\omega_0} \hat{I}_z . \]  

(2.125)
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In the last line, it has been assumed that the duration of the pulse is long compared to the inverse of the Larmor frequency \((\omega_0 t \gg 1)\), a valid assumption for most modern NMR experiments. This first order correction represents an offset to the Zeeman Hamiltonian and is present during rf irradiation. Compared to the strength of the Zeeman interaction, it is suppressed by a factor of \(\frac{1}{4} \left( \frac{\omega_1}{\omega_0} \right)^2\) that, for modern high-field NMR experiments where field strengths are on the order of \(\omega_0 = 2\pi(500 \text{ MHz})\) and rf field strengths are on the order of \(2\pi(100 \text{ kHz})\), yields a shift of 32 Hz or 0.06 ppm, a negligible amount.

For most NMR experiments, the Bloch-Siegert shift can be safely ignored. It is mentioned here because (1) the calculation is similar to the one performed for two-photon excitation (see Chapters 8 and 9), and (2) because in our observation of two-photon excitation with long and strong rf pulses, the Bloch-Siegert shift is observable.

2.5 Other Useful Concepts in NMR

2.5.1 Fictitious Spin-1/2 Operators

A useful concept in the theoretical description of NMR for spin>1/2 systems is that of fictitious spin-1/2 operators. [10] This formalism will be used Chapters 7–9 to describe certain Hamiltonians.

For a spin-1/2 system, any state can be expressed as a linear combination of the three spin-1/2 operators, \(\hat{I}_x\), \(\hat{I}_y\) and \(\hat{I}_z\) (as well as the identity operator, \(\hat{I}_0\)). Such a decomposition was done in section 2.3.3.) The coefficients naturally form a vector in a three-dimensional space known as Liouville space. Due to the commutation relations of spin-1/2 operators the evolution of the state often looks like a rotation of the state vector in Liouville space and corresponds well with a classical picture of magnetization precessing about an externally applied magnetic field. Unfortunately, for spin>1/2 (or for \(n\) coupled spin 1/2 nuclei), Liouville space has dimensionality \((2I + 1)^2\) (or \(2^n\)), and the vectorial representation is no longer useful.

It is often useful to decompose the rotations in the higher dimensional space into projections onto a three dimensional space (i.e. rotation about one dimension in a plane spanned by two other dimensions). This is the basic concept behind fictitious spin-1/2 operators. Some of their properties are reviewed below.

The fictitious spin 1/2 operators that connect states \(|r\rangle\) and \(|s\rangle\) can be written in matrix
representation as

\[
\hat{I}^{r-s}_{x} = \frac{1}{2} \begin{pmatrix}
\langle r | & \langle s | \\
\vdots & \vdots \\
0 & 1 & \vdots \\
\vdots & \vdots & \ddots & \ddots \\
1 & \vdots & \vdots & 0 \\
\vdots & \vdots & \vdots & \vdots \\
0 & \vdots & \vdots & -1 \\
\vdots & \vdots & \vdots & \vdots \\
\end{pmatrix}
\begin{pmatrix}
|r\rangle \\
|s\rangle \\
\end{pmatrix}
\]

\[
\hat{I}^{r-s}_{y} = \frac{1}{2} \begin{pmatrix}
\langle r | & \langle s | \\
\vdots & \vdots \\
0 & 1 & \vdots \\
\vdots & \vdots & \ddots & \ddots \\
1 & \vdots & \vdots & 0 \\
\vdots & \vdots & \vdots & \vdots \\
0 & \vdots & \vdots & -1 \\
\vdots & \vdots & \vdots & \vdots \\
\end{pmatrix}
\begin{pmatrix}
|r\rangle \\
|s\rangle \\
\end{pmatrix}
\]

All other entries in the matrices are zero.

For spin \( I \), there are \( 4I(2I + 1) \) such operators, whereas only \((2I + 1)^2\) basis states are required to span the space. It can be shown that the \( \hat{I}^{r-s}_{x} \) and \( \hat{I}^{r-s}_{y} \) operators are orthogonal (i.e. one can't construct any of these from a combination of the others). However, the \( \hat{I}^{r-s}_{z} \) and \( \hat{I}^{r-s}_{0} \) operators can be written as linear combinations of other \( \hat{I}^{r'}_{z'} \) and \( \hat{I}^{r'}_{0'} \) operators.\(^{18}\)

The operator basis has a set of commutation relations as are described below.

If the spin 1/2 operators address different transitions (i.e. \( r \neq t \neq u \) and \( s \neq t \neq u \)) then

\[
\left[ \hat{I}^{r-s}_{\alpha}, \hat{I}^{t-u}_{\beta} \right] = 0 \tag{2.126}
\]

for \( \alpha, \beta = x, y, z, 0 \).

If both operators address the same transition, then the commutation relations follow those for spin-1/2 operators, namely,

\[
\left[ \hat{I}^{r-s}_{i}, \hat{I}^{r-s}_{j} \right] = \epsilon_{ijk} \hat{I}^{r-s}_{k} \tag{2.127}
\]

\[
\left[ \hat{I}^{r-s}_{i}, \hat{I}^{r-s}_{0} \right] = 0 \tag{2.128}
\]

where \( i, j, k = x, y, z \).

The most non-trivial results are for when the operators address two transitions which have one state in common [2]. Then

\[
\left[ \hat{I}^{r-t}_{x}, \hat{I}^{s-t}_{x} \right] = \left[ \hat{I}^{r-t}_{y}, \hat{I}^{s-t}_{y} \right] = \frac{i}{2} \hat{I}^{r-s}_{y} \tag{2.129}
\]

\[
\left[ \hat{I}^{r-t}_{z}, \hat{I}^{s-t}_{z} \right] = \frac{i}{2} \hat{I}^{r-s}_{z} \tag{2.130}
\]

\(^{18}\)For example, \( \hat{I}^{1-2}_{z} = \frac{1}{2} \left( \hat{I}^{1-3}_{z} + \frac{1}{2} \hat{I}^{1-3}_{0} - (\hat{I}^{2-4}_{z} + \frac{1}{2} \hat{I}^{2-4}_{0}) \right) \)
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\[
\begin{align*}
[\hat{I}_z^{-t}, \hat{I}_z^{s-t}] &= 0, \\
[\hat{I}_x^{-t}, \hat{I}_y^{s-t}] &= \frac{i}{2} \hat{I}_x^{-s}, \\
[\hat{I}_x^{-t}, \hat{I}_z^{s-t}] &= -\frac{i}{2} \hat{I}_y^{-t}, \\
[\hat{I}_y^{-t}, \hat{I}_z^{s-t}] &= \frac{i}{2} \hat{I}_x^{-t}.
\end{align*}
\] (2.131)

Changing the order of indices results in sign changes according to

\[
\begin{align*}
\hat{I}_x^{s-r} &= \hat{I}_x^{-s}, \\
\hat{I}_y^{s-r} &= -\hat{I}_y^{-s}, \\
\hat{I}_z^{s-r} &= -\hat{I}_z^{-s}.
\end{align*}
\] (2.135) (2.136) (2.137)

Because of the non-triviality of these commutation relations, we strive to decompose operators into sets that do not share indices.

2.5.2 Measurement in NMR

NMR experiments detect the emf induced in a coil due to the precession of magnetization in the external field. The effects of the coil on the magnetization (radiation damping) and of the magnetization in one part of the sample on another (distant dipolar fields) are typically ignored. Formally, the signal induced in the coil can be written as:

\[
\text{emf} = \int \partial V \frac{\partial \vec{B}_1}{\partial t},
\] (2.138)

where \(\vec{B}_1\) is the field induced in the sample due to a current in the coil and the integral is over the volume of the coil, \(V\). This is just a statement of a reciprocity principle: the current induced in a coil due to a time-varying external magnetic field is the same as the current required to produce the same magnetic field.

In general, the magnetization will be some function that rotates about the external field \(\vec{M}(t) = M_x \hat{z} + (M_x \cos(\omega_0 t + \alpha) \hat{x} + M_y \sin(\omega_0 t + \alpha) \hat{y})\), the derivative of which is \(\frac{\partial \vec{M}(t)}{\partial t} = \omega_0 (-M_x \sin(\omega_0 t + \alpha) \hat{x} + M_y \cos(\omega_0 t + \alpha) \hat{y})\). It is generally assumed that the \(B_1\) field is homogeneous and points along the \(x\)-axis so that the emf is given by the time-dependence of the \(x\)-component of the magnetization:

\[
\begin{align*}
\text{emf} &\propto \langle M(t) \rangle \\
&\propto \langle \hat{I}_x(t) \rangle.
\end{align*}
\] (2.139) (2.140)
where $\langle \hat{I}_x \rangle$ is the expectation value of the $\hat{I}_x$ operator in the lab frame. Since the density matrix is almost always in the rotating frame associated with the unitary operator $\hat{U} = e^{i\omega_0 t \hat{I}_z}$, one can write:

$$
\langle \hat{I}_x \rangle = Tr \left\{ \hat{\rho}(t) \hat{I}_x \right\} \\
= Tr \left\{ \hat{U} \hat{\rho}(t) \hat{U}^\dagger \hat{I}_x \right\} \\
= Tr \left\{ \hat{\rho}'(t) \hat{U} \hat{I}_x \hat{U}^\dagger \right\} \\
= Tr \left\{ \hat{\rho}'(t) \hat{I}_x \right\} \cos \omega_0 t + Tr \left\{ \hat{\rho}'(t) \hat{I}_y \right\} \sin \omega_0 t. 
$$

where $\hat{\rho}' = \hat{U} \hat{\rho} \hat{U}^\dagger$ is the density matrix in the rotating frame. The trace operations represent the projection of this density matrix onto the x and y axis of the rotating frame. In practice, the receiver provides two output channels, one is the detected signal multiplied by $\cos \omega_0 t$, the other, multiplied by $\sin \omega_0 t$. Thus the signal in channel 1 is (applying a trigonometric identity):

$$
S_{ch1}(t) = Tr \left\{ \hat{\rho}'(t) \hat{I}_x \right\} (1 - \cos 2\omega_0 t) / 2 + Tr \left\{ \hat{\rho}'(t) \hat{I}_y \right\} (\sin 2\omega_0 t) / 2 \\
\approx Tr \left\{ \hat{\rho}'(t) \hat{I}_x \right\} / 2 
$$

where the second line is the signal remaining after a low-pass filter has been applied. Similarly, for the second channel the same signal is multiplied by $\sin \omega_0 t$ which, after after low-pass filtering yields:

$$
S_{ch2}(t) = Tr \left\{ \hat{\rho}'(t) \hat{I}_x \right\} (\sin 2\omega_0 t) / 2 + Tr \left\{ \hat{\rho}'(t) \hat{I}_y \right\} (1 - \cos 2\omega_0 t) / 2 \\
\approx Tr \left\{ \hat{\rho}'(t) \hat{I}_y \right\} / 2. 
$$

Now, if the signal from channel 2 is attributed to the imaginary part of the signal, the total signal, $S(t)$ is:

$$
S(t) = S_{ch1}(t) + iS_{ch2}(t) \\
= Tr \left\{ \hat{\rho}'(t)(\hat{I}_x + i\hat{I}_y) \right\} / 2 \\
= Tr \left\{ \hat{\rho}'(t) \hat{I}_+ \right\} / 2. 
$$

So this is what is meant when one says that the observable in NMR is $\langle \hat{I}_+ \rangle$ or that measurements are made in the rotating frame. In reality, the measurement is of the projection of the density matrix onto the x and y axes of the rotating frame.
Bibliography


Chapter 3

An Introduction to Spider Dragline Silk

3.1 Spider Silk as a Biomaterial

Biological materials have historically formed the building blocks from which humankind has made things. Optimized by millions of years of evolution for properties such as stiffness, toughness, extensibility, failure threshold, resistance to wear, fracture, environmental conditions, etc. and readily available in nature, materials such as wood, bone, leather and cotton are still used today despite advances in the development of man-made synthetic materials such as polymers, alloys, and ceramics. In the past century, much research has gone into the understanding and development of new synthetic materials with relatively simple molecular compositions (for example polymers such as nylon, polyethylene, polystyrene, and Kevlar synthesized by polymerization reactions with one or two precursors). More recently, there has been a resurgence of interest in biological materials as the source of inspiration for a new class of synthetic materials with the same complex structural hierarchy that is the hallmark of most biological materials. Materials derived from this type of approach are typically referred to as bio-inspired materials, biomimetic materials, or commonly just biomaterials. Curiously, silk is also being studied as a novel biomedical material [11].

One biomaterial that has recently attracted much attention is spider silk. With mechanical properties rivaling those of most man-made fibres, but synthesized under ambient conditions of temperature and pressure from environmentally benign materials (i.e. flying insects), development of silk-like analogs has in the past decade become an active area of research [12-15].

Spiders have the capacity to make up to seven distinct types of silk, each from a separate gland, that vary dramatically in mechanical properties and protein sequence [13, 16]. The function of these fibres ranges from the sticky capture spiral of the web made using very extensible fibres, to brittle fibres used to encase the egg sac, to adhesive plaques used to anchor strands to each other and to other structures. In particular, researchers have focused

---

1The term biomaterials is also used to describe materials that are biologically compatible with tissue and are used in medical application as medical sutures, tissue and bone scaffolding, implants, or for drug delivery. Curiously, silk is also being studied as a novel biomedical material [11].
their interest on the dragline silk, used for the radial spokes of the web, as well as for the lifeline used by the spider to rappel.

Although a number of spider draglines have been studied, this dissertation focuses on silk from the Golden Orb Weaver spider (Nephila clavipes), a spider large enough (~1-3 g) to provide the volume of silk (~100 mg) required for our NMR studies.\(^2\) The dragline silk of \(N.\) clavipes has been extensively studied and the amino acid sequence of its constituent proteins [17] was one of the first to be published. However, due to the inherent randomness of silk’s molecular constituents, the structure of silk has been elusive. Those regions where the protein backbone configuration is not well defined and where little long-range order exists have been particularly difficult to study by methods such as X-ray diffraction. It is these regions that play a major role in the mechanical properties of silk. Solid state NMR has been a useful tool in studying local molecular structure in silk [18–23], and we employ it in this dissertation to study the disordered regions in silk to better understand the molecular mechanisms responsible for silk’s mechanical properties.

This introduction to silk begins with a review of some of the mechanical properties of silk as further motivation for the study of this amazing fibre. This is followed with a hierarchical approach to describing silk, from peptide sequence to secondary structure to proposed models from supra-molecular structure, that will highlight those aspects of silk that are understood and those questions still unanswered. The chapter is concluded with a review of supercontraction, a unique property of silk whereby exposure to water causes extreme fibre shrinkage and swelling, which will be relevant later in this dissertation.

The introduction presented here is not meant to be an exhaustive survey of research into spider silk, but rather focuses on those studies relevant to the remainder of the dissertation. For a more in-depth review of spider silks, the interested reader is referred to Ref. [13] and references therein. Also, a series of snapshots in the evolution of the field can be obtained from compiled papers from International Symposia on Silk held in 1994, 1999 and 2003 [12, 24, 25].

### 3.2 Mechanical Properties of Dragline Silk

Dragline silk fibres have a combination of breaking strength, initial stiffness, toughness\(^3\) and extensibility [15] that is unsurpassed in man-made materials as can be seen from Table 3.1 where comparisons are made to other natural and man-made materials.

A typical stress-strain extension curve of dragline silk is shown in Fig. 3.1. The high initial stiffness is thought to be due to interchain van der Waals friction and hydrogen bonding along the peptide backbone. Once a sufficiently strong force is applied to overcome

---

2 Un Fortunately, studies of the dragline from the \textit{Nephila} genus are not done consistently on a single species. Many studies focus on the dragline from close relatives of \textit{N. clavipes}: \textit{N. edulis} and \textit{N. madagascariensis}. The dragline silks of these relatives are similar in tensile properties to \textit{N. clavipes}, and likely similar in peptide sequence and tertiary structure, though some differences in tensile properties are known to exist.

3 Toughness is defined as the energy required to break a fibre per unit volume and is obtained from the integral of the stress-strain curve.
Table 3.1: Typical values for mechanical properties of silk fibres and high-performance synthetic fibres. Adapted from Ref. [26].

<table>
<thead>
<tr>
<th>Fibre Type</th>
<th>Density (g/cm(^3))</th>
<th>Modulus (GPa)</th>
<th>Tensile Strength (GPa)</th>
<th>Breaking Strain (%)</th>
<th>Toughness (MJ/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. clavipes dragline</td>
<td>1.3</td>
<td>10</td>
<td>1.8</td>
<td>30</td>
<td>130</td>
</tr>
<tr>
<td>Silkworm silk</td>
<td>1.3</td>
<td>5</td>
<td>0.6</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>Nylon 6,6</td>
<td>1.1</td>
<td>5</td>
<td>0.9</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>Kevlar 49</td>
<td>1.4</td>
<td>130</td>
<td>3.6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>Steel</td>
<td>7.8</td>
<td>200</td>
<td>3.0</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 3.1: Typical stress strain curve for N. edulis dragline. The star depicts the breaking point. Adapted from Ref. [13]. Note that stress-strain curves vary depending on spinning conditions with breaking stress decreasing significantly for draw rate exceeding 10 cm/s and temperatures below 20°C [13].
these bonds, a yield region is observed where chain slippage results in a plastic deformation. Relaxation curves follow very different trajectories with stress decreasing dramatically as the fibre initially shortens, and returning to its original length with significantly less stress than during extension. This hysteresis indicates that a large fraction of the energy used to stretch silk is absorbed by the fibre and dissipated as heat, the hypothesis being that this property of silk prevents flying insects from being trampolined out of the web [28]. The most promising possible applications for synthetic silks such as bullet proof vests, parachute draglines, and seat belts take advantage of this property of silk.

3.3 What is Spider Silk?

Silk is a protein polymer.

3.3.1 What are Proteins?

All proteins are long molecular chains consisting of a string of subunits called amino acids that share a common backbone. Amino acids differ only in the nature of their side-chains, which is the molecular fragment that dangles from the side of the backbone. Side-chains vary in size and hydrophobicity and ultimately determine the three-dimensional structure of the protein. The sequence of amino acids that make up the protein is called the primary structure. Due to the rotational freedom of the backbone, peptide chains can and do fold into common structures that minimize the local free energy. These secondary structures often form helices (such as α-helices) or interstrand sheets (such as β-sheets). The secondary structures fold towards global free energy minima into the protein’s final three-dimensional tertiary structure which is the protein’s final functional form (though some proteins combine with other proteins to form quaternary structures.)

Primary structure can often be determined from sequencing DNA strands, whereas secondary and tertiary protein structure are typically probed by X-ray crystallography and NMR techniques. Predicting the folding of the primary sequence into the tertiary structure is a very difficult problem involving a complex free energy landscape, and is an active area of research. Folding of proteins is governed by electrostatic and other interactions between amino acids and with the molecules in which the protein is dissolved.

Unlike most proteins, silk fibres do not have a well defined tertiary structure.

3.3.2 Amino Acid Composition of Dragline Silk

Mass spectroscopy measurements of hydrolyzed dragline silk from the major ampullate gland of N. clavipes have revealed a predominance of glycine and alanine residues, with glutamine

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4There is little direct evidence to support these hypothesized molecular mechanisms though numerical models do reinforce them [27]. It is one of the goals of this dissertation to produce such evidence.

5The string of amino acids is often called a peptide, and makes up the protein.
Table 3.2: Amino acid composition of *N. clavipes* dragline silk (adapted from [29]) and abbreviations of amino acids.

<table>
<thead>
<tr>
<th>amino acid</th>
<th>abbreviation</th>
<th>% of silk</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycine</td>
<td>G</td>
<td>48.7</td>
</tr>
<tr>
<td>alanine</td>
<td>A</td>
<td>24.8</td>
</tr>
<tr>
<td>glutamine</td>
<td>Q</td>
<td>10.5</td>
</tr>
<tr>
<td>leucine</td>
<td>L</td>
<td>3.9</td>
</tr>
<tr>
<td>tyrosine</td>
<td>Y</td>
<td>2.6</td>
</tr>
<tr>
<td>serine</td>
<td>S</td>
<td>2.1</td>
</tr>
<tr>
<td>arginine</td>
<td>R</td>
<td>1.9</td>
</tr>
<tr>
<td>proline</td>
<td>P</td>
<td>1.9</td>
</tr>
<tr>
<td>asparagine</td>
<td>N</td>
<td>0.9</td>
</tr>
<tr>
<td>valine</td>
<td>V</td>
<td>0.7</td>
</tr>
<tr>
<td>histidine</td>
<td>H</td>
<td>0.4</td>
</tr>
<tr>
<td>threonine</td>
<td>T</td>
<td>0.4</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>F</td>
<td>0.2</td>
</tr>
<tr>
<td>lysine</td>
<td>K</td>
<td>0.2</td>
</tr>
<tr>
<td>isoleucine</td>
<td>I</td>
<td>0.1</td>
</tr>
<tr>
<td>cysteine</td>
<td>C</td>
<td>0.1</td>
</tr>
<tr>
<td>methionine</td>
<td>M</td>
<td>0.1</td>
</tr>
</tbody>
</table>

abundant to a lesser extent and trace amounts of other amino acids. The amino acid composition of silk is summarized in Table 3.2.

### 3.3.3 Primary Structure of Dragline Silk

To date, two genes (MaSpI and MaSpII) have been identified as coding for the proteins (Spidroin I and Spidroin II) found in dragline silk. Sequencing of cDNA has revealed partial amino acid sequences of each [17, 30]. However, the size and relative abundances of these proteins within silk has not been well established. Spidroin I, thought to be the more abundant protein, is estimated at 300 kDa [31] in size. This translates to approximately 5000 amino acids, extraordinarily large as far as proteins go. A partial sequence is reproduced in Fig. 3.2 from which can be identified two distinct repetitive motifs: 1) poly-alanine runs sandwiched by QAGAG and GAGGAQ on the N- and C-terminal ends respectively, and 2) glycine rich regions with repetitive motifs such as GGX and LGXQ.\(^6\)

\(^6\)An abbreviated formalism is used here for amino acids and is standard in biochemistry where, by convention, the leftmost residue is the N-terminus end. The relevant abbreviations are summarized in Table 3.2, while X is a placeholder for an arbitrary amino acid used to highlight the repetitive nature of the sequence.
Figure 3.2: Partial amino acid sequence of the spidroin I protein of *N. clavipes* dragline reproduced from Xu and Lewis [17]. Regions thought to be crystalline are italicized and are always surrounded on either side by bulky Glutamine (Q) residues. Dashes denote deletions inserted to highlight repetitive motifs. The partial sequence is to be read continuously from left (amino-terminal) to right, and top to bottom.
3.4 Evidence for the Molecular Structure of Dragline Silk

Unlike most proteins in nature, silk proteins have no well defined tertiary structure, though there is some local ordering in the form of secondary structures due to both inter- and intra-chain hydrogen bonding. The tertiary structure is governed by physical and chemical conditions in the spider's silk long narrow duct during fibre extrusion. Shear flow, dehydration, pH control and phosphorylation are thought to occur as the silk protein dope passes from the gland where it exists in a water soluble liquid-crystalline phase to where it exits the spinnerette as an insoluble drawn fibre [32–35].

Though an absolute tertiary structure may not exist, it is expected that there exist regular structural components in the form of β-sheets and helices that represent preferred secondary structures and that these structures are assembled in a way as to give silk its unique mechanical properties.

3.4.1 The Multi-Component Nature of Silk

It is generally accepted that spider silk is a semicrystalline polymer with small crystalline regions embedded in an “amorphous” matrix [36]. The crystalline regions were initially thought to consist of the glycine-rich motifs [37, 38], but it was later found that they were in fact due to the poly-alanine regions [39], while the glycine-rich regions formed the amorphous matrix. This was subsequently confirmed by NMR [19].

The multi-component nature of silk’s secondary structure has been confirmed by several studies. Wide angle X-ray diffraction [40] studies found 10-15% crystallinity with crystallites in the form of anti-parallel β-sheets that were $2 \times 5 \times 7$ nm in size well aligned along the fibre (15.7° full width at half maximum), as well as an oriented amorphous phase (30% with 30° FWHM), and an isotropic phase (55%). Solid-state NMR measurements of relaxation times in alanine C$_\beta$ [19, 20] suggested alanines exist in two crystalline phases and fits to wideline deuterium NMR spectra [20] required a well oriented (27% with a 5° FWHM) and poorly oriented (67% with a 75° FWHM) crystalline component.

Less well characterized is the amorphous component of silk. Proton-driven spin diffusion NMR experiments [21] support the crystalline nature of alanine and suggest a highly extended 3_1 helical conformation of the glycine-rich regions (such helices are also called polyglycine II helices). More accurately, the data is qualitatively in agreement with neighbouring glycines in an extended conformation. However, there was no evidence that long-range extended helices exist.

In an elegant set of experiments by van Beek et al. [23], more accurate torsion angles between adjacent glycine residues or between adjacent alanine residues were obtained using

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The term amorphous refers to the lack of any long-range structural order. There is evidence of orientational order along the fibre (which this dissertation endeavors to characterize) and possibly some local short-range structural order.
Chapter 3. An Introduction to Spider Dragline Silk

the DOQSY (DOuble Quantum correlation Spectroscopy) NMR experiment. The results indicated that the alanine local conformation is consistent with extended anti-parallel $\beta$-sheet model, while the glycines exist in both the extended $\beta$-sheet and $3_1$ helical conformations. Based on this same data, the random coil hypothesis which involves a Boltzmann-weighted distribution of torsion angles (based on assumptions of energetically favorable torsion angles [41]) was rejected, as was the notion of the amorphous matrix consisting of highly mobile entropic springs [41]. In this same study a DECODER (see Chapter 4) experiment was performed on oriented silk fibres, with results consistent with 70% of alanines in $\beta$-sheets well aligned along the fibre and 61% of glycines poorly aligned along the fibre with conformations not inconsistent with $3_1$ helices. However quantitative descriptions of the widths of the distributions, as will be determined in the course of this dissertation by similar experimental techniques, were not readily obtained from this analysis.

A cartoon of a recently proposed model of silk which assimilates the data presented in this chapter is reproduced from Ref. [23] in Fig. 3.3.

3.4.2 Fibre Stretching and Molecular Orientation

Due to the difficulty in uniformly straining multiple fibres, little information has been collected on protein motion and orientation as a function of fibre strain.

In the X-ray diffraction study referred to above [40], an increased degree of orientation was observed in the crystalline and oriented amorphous component as the fibre was stretched, consistent with affine deformation. NMR data showed no change in alignment of alanine regions as a function of fibre strain [20].

3.5 Supercontraction

In 1977, Work [42] was the first to report supercontraction in major ampullate silk fibres. He observed that, when exposed to water, dragline silk from the araneus genus of spider contracted by up to 45% and swelled to more than twice their original diameter. Supercontraction was not observed in minor ampullate silk from the same species of spider. X-ray diffraction measurements showed that the crystallite unit cell rotates due to macro distortion during supercontraction, but is otherwise unchanged [43].

Gosline et al. [36] found that silk's initial modulus falls by 3 orders of magnitude after supercontraction, with resulting stress-strain curves that were rubber-like (i.e. linear over a large extension range). They surmised that supercontraction was due to mobility in the protein backbone of the amorphous regions and was thought to be a result of the hydration-sensitivity of polar amino acids [28]. Bonthrone et al. [44], using NMR spectroscopy, showed that the elastic nature of the capture spiral, which also has rubber-like stress-strain curves, was due to water-induced mobility. NMR spin diffusion measurements [45] in supercontracted dragline silk found increased motion in the peptide backbone that could be frozen out at low temperature. These results however did not show any conclusive evidence for local structural changes in supercontracted silk.
Figure 3.3: Structural model of silk as proposed by van Beek et al. (reproduced from Ref. [23]). (A) The structure consists of $\beta$-sheet regions containing alanine (straight lines) interconnected with $3_1$ helices (a.k.a. polyglycine II helices) (curled lines). Ordered regions rich in poly-alanine runs are crystallites (boxes). (B and C) Conformation of $3_1$ helices showing 3-fold symmetry and extended nature of a polypeptide with torsion angles (-60,135). The poorly understood hierarchical structure of silk, which includes a skin, core, and potentially other fibrular structures, is depicted by the black and white cylinders at the bottom of the figure.
In a CPMAS NMR experiment on supercontracted and dry silk, Yang et al. [46] observed peptide backbone dynamics on a timescale faster than 100 $\mu$s in all amino acids except alanine, which showed no fast motion. Furthermore, analysis of wideline $^{13}$C spectra of 1-$^{13}$C-glycine-labeled silk revealed that 40% of glycine residues undergo limited motion when wet, while the remainder undergo motion on a timescale faster than 50 $\mu$s. The immobilized glycine residues were associated with the amino acid sites physically closest to the polyalanine runs.

It had originally been suggested that supercontraction could be a means to maintain tautness in the web, preventing it from sagging in the morning dew [36]. This theory was later questioned [47] because measurements suggested that the force generated by a single supercontracting fibre was transient. With the humidity increasing monotonically with time, the supercontracting force reached a maximum after 1 minute (generating a maximum force of 4.5 mN). By 4 minutes, the supercontracting force had vanished. More recent measurements [48] contradict this result, by demonstrating a continuous supercontraction stress of 0.06 GPa at 100% humidity persistent for many hours, supporting the original claim. Another independent study published similar results concurrently [49].

Recent interest in supercontraction has been sparked by results by Perez-Rigueiro et al. [50] who show that by allowing silk to supercontract to predetermined lengths and subsequently allowing it to dry at those lengths, the mechanical properties could be tailored from very stiff as in the original fibres to very soft and extensible (up to 100% breaking strain). All fibres demonstrated the same initial stiffness before showing very different yield regions which depended on their degree of supercontraction.
Bibliography


Chapter 4

Reconstructing Orientation Distributions from DECODER NMR Spectra for an Axially Symmetric Fibre Wound About a Cylindrical Support using Multi-Component Models

4.1 Introduction

One may often be interested in the orientation of molecules within a sample. Spectroscopic techniques such as X-ray diffraction and NMR can, in principle, provide such information as long as there is some orientational order in the sample. Samples which are macroscopically ordered with a unique director axis (such as single crystals) facilitate these measurements because the sample frame of reference has a well defined orientation in the lab frame. Conversely, completely disordered samples such as powders can never provide any information about the orientation of molecules in the sample frame. For samples that are ordered, but whose frame does not have a unique orientation in the lab frame, some information regarding molecular orientation in the sample frame can be extracted, though this is in general more difficult.

Such is the case for the experiments on silk presented in this dissertation. Silk fibres, which have cylindrical symmetry about the fibre axis, are wound about a cylindrical support. This is done in order to maximize the amount of silk which can fit into the NMR coil because the experiments presented here are signal-limited. Furthermore, it is difficult to maintain uniform strain on large fibre bundles, and even more difficult to uniformly vary the strain in all fibres, as can be done with a cylindrical support with a variable diameter. Lastly, uniformly hydrating silk samples is easily done in such a configuration by covering the cylindrical sample with a water-filled test tube.

For these reasons, the wound geometry is preferred over other geometries. The techniques used to recover the underlying molecular orientation distribution (OD) from a sample with such a geometry make up the content of this chapter. We begin with a few words on the spectroscopic techniques used to recover orientation information in solid organic samples.
4.1.1 Introduction to Wideline NMR Spectra

In liquids, rapid molecular tumbling averages dipolar, quadrupolar and chemical shift interactions resulting in sharp well resolved NMR lines that lack any orientational information. In contrast, for solid samples these same interactions result in spectra that are broadened and unresolved. These wideline spectra carry information about internuclear distances and molecular orientation in the external field, and thus, in principle carry a great deal of information about the sample. The extraction of underlying molecular parameters from a wideline spectrum is referred to as an inverse problem, and is in general non-trivial.

One method to simplify wideline spectra is to dilutely isotopically label a sample with a spin 1/2 nucleus (such as $^{13}$C) at a single molecular site. In such a case the quadrupolar and homonuclear dipole interactions can be ignored, and it can be assumed that the observed broadening comes purely from chemical shift anisotropy (CSA) and can be described by a single CSA tensor.\(^1\)

As discussed in section 2.2.6, the CSA interaction results in an orientation-dependent frequency shift given by:

$$\omega(\Omega) = \omega_0 \left\{ \sigma_{\text{iso}} + \delta \left( \frac{3 \cos^2 \beta - 1}{2} + \frac{\eta}{2} \sin^2 \beta \cos 2\gamma \right) \right\} . \quad (4.1)$$

where $\sigma_{\text{iso}}$, $\delta$, and $\eta$ describe principle values of the CSA tensor while $\beta$ and $\gamma$ are the polar and azimuthal angles describing the orientation of the CSA in the lab frame (or conversely, the orientation of the magnetic field in the CSA PAS).

For a powder sample where all orientations are equally likely, a characteristic powder spectrum is obtained whose shape is dictated by the principle values of the chemical shift tensor. Figure 4.1 shows a powder pattern for silk labeled with $^{13}$C at the carbonyl carbon of glycine. The CSA-broadened spectrum is spectrally resolved from several other unresolved peaks due to aliphatic carbons. For samples that are ordered (non-isotropic), wideline spectra deviate from the powder pattern shape and carry information regarding the OD of molecular sites within the sample. However, reconstructing the underlying OD of molecules within the sample is non-trivial [51–53], especially for complex sample geometries.

4.1.2 The Problem of Orientation Reconstruction from CSA-broadened Spectra and the DECODER Experiment

Consider a single molecule with a given orientation in an external field. From Eq. 4.1, the chemical shift frequency can be readily obtained. However, the inverse is not true. Given the NMR frequency, one cannot infer the orientation. In fact, there is a continuous set of orientations that yield the same frequency (see Fig. 4.2). If one were to rotate the

\(^1\)If multiple molecular sites were labeled, overlapping CSA-broadened spectra would result and would be more difficult to separate. Additional broadening comes from heteronuclear dipole coupling to neighbouring protons, though this can be mostly decoupled as described in section 2.4.2.
Figure 4.1: CSA-broadened powder pattern of silk labeled with $^{13}C$ at the carbonyl carbon site of glycine. Fit includes broadening parameters. The right hand peak is due overlapping spectral lines from natural abundance aliphatic carbons. The chemical shift anisotropy of each aliphatic carbon is negligible compared to its linewidth.
Figure 4.2: There is no unique mapping of the CSA frequency to molecular orientation though this degeneracy may be broken by rotating the sample. In the sample frame of reference, when the external magnetic field is directed along $B_{01}$, a measured NMR frequency $\omega_1$ may correspond to a set of possible orientations as depicted by the solid circle. Rotation of the sample about some axis moves the magnetic field to $B_{02}$ and the measured NMR frequency corresponding to a new set of possible orientations. The true orientation of the sample in the lab frame is given by the intersection of the two circles. Due to inversion symmetry of the CSA interaction, there is a similar pair of circles on the backside of the sphere. (Adapted from Ref. [52].)

For a sample in the magnetic field, a new set of possible orientations would arise given the new chemical shift frequency. It turns out that there is a unique orientation for which the NMR frequency would have one value at one orientation and another value at the second orientation. This is the principle behind the two-dimensional NMR experiment dubbed DECODER (direction exchange with correlation for orientation distribution evaluation and reconstruction): a macroscopic rotation of the sample by 90° about an axis perpendicular to the external field during the mixing time of a two-dimensional NMR experiment gives a direct mapping of frequency to the orientation of the molecule in the external field. The DECODER pulse sequence is given in Fig. 4.3.

For a sample with some distribution of molecular orientations, reconstruction of the underlying OD from a DECODER spectrum is relatively straightforward [52, 54]. However, the reconstruction yields the OD in the lab frame, which, except for samples macroscopically

\[^2\]There are actually 4 orientations that satisfy this condition but they reflect the symmetry of the CSA interaction and cannot be extracted by NMR techniques.
Chapter 4. Reconstructing Orientation Distributions from DECODER

Figure 4.3: DECODER pulse sequence. Cross-polarization to protons for sensitivity enhancement results in transverse polarization and is followed by an evolution under the CSA interaction during time $t_1$. The magnetization is stored along the $z$-axis by a $\pi/2$ pulse that marks the start of the mixing time during which the sample is rotated about the rf coil axis by $90^\circ$ and the transverse magnetization is destroyed by $T_2$ processes. A second $\pi/2$ pulse tips the magnetization into the transverse plane where it evolves under the CSA interaction in its new orientation. A short echo is used to collect undistorted wideline spectra.

aligned along the external field, does not correspond to the OD in the sample frame. Such is the case in our silk samples which are uniaxial and wound around an axis perpendicular to the external field.

To reconstruct the underlying OD in the silk frame, numerical simulations of DECODER spectra are generated for discrete orientations of the PAS in the silk frame (which will be referred to as subspectra), and linear combination of these subspectra are fit to the experimental spectrum. Rather than fitting each of the subspectra independently (resulting in a large number of fitting parameters), a physical model of silk is proposed that assumes secondary structures that are aligned along the silk fibre with a Gaussian probability distribution.

The following sections develop the approach used for generating simulated DECODER subspectra for samples with uniaxial geometry that are wrapped about the lab $x$-axis. The molecular model for silk is subsequently developed and is used to fit the generated subspectra to the experimental DECODER spectrum.
4.2 Theory and Methods: An Approach to Orientation Reconstruction from DECODER Spectra

4.2.1 Relevant Frames of Reference

Consider three sets of Cartesian coordinates corresponding to 1) the PAS of the CSA, 2) the silk fibre frame, and 3) the lab frame as depicted in Fig. 4.4 where the sets of Euler angles $\Omega$, $\Omega'$, and $\Omega^0$ describe the orientation of the PAS in the silk frame, the silk frame in the lab frame and the PAS in the lab frame respectively.

From Eq. 2.38, the chemical shift frequency can be written using Wigner rotation matrices in terms of $\Omega^0$:

$$\omega(\Omega^0) = \omega_0 \left\{ \sigma_{\text{iso}} + \delta \left( D^2_{00}(\Omega^0) + \frac{\eta}{\sqrt{6}} [D^2_{20}(\Omega^0) + D^2_{-20}(\Omega^0)] \right) \right\}, \quad (4.2)$$

or by using the properties of Wigner rotation matrices (Eq. 2.27), in terms of $\Omega$ and $\Omega'$:

$$\omega(\Omega, \Omega') = \omega_0 \left\{ \sigma_{\text{iso}} + \delta \sum_{m'=-2}^{2} \left( D^2_{m'}(\Omega) + \frac{\eta}{\sqrt{6}} [D^2_{2m'}(\Omega) + D^2_{-2m'}(\Omega)] \right) D^2_{m'0}(\Omega') \right\}. \quad (4.3)$$

In a similar fashion, after rotation of the sample about the rf coil axis by 90° (i.e. $\Delta\Omega' = (0, 90^\circ, 0)$), the chemical shift may be written as $\omega(\Omega, \Omega' + \Delta\Omega')$.

4.2.2 Closed-Form Expression for DECODER Spectra

The DECODER spectrum can be written as

$$S(\omega_1, \omega_2) = c \int_0^{4\pi} \partial \Omega P(\Omega) s(\omega_1, \omega_2, \Omega) \quad (4.4)$$

where $c$ is a constant which gives the overall spectral intensity, and the integration is over all possible orientations of the PAS in the silk frame. $P(\Omega)$ is the probability of finding a given orientation (i.e. the OD), while

$$s(\omega_1, \omega_2, \Omega) = \int_0^{\pi} \partial \Theta' \int_0^{2\pi} \partial \Phi' \delta(\omega_1 - \omega(\Omega, \Omega')) \delta(\omega_2 - \omega(\Omega, \Omega' + \Delta\Omega')) \quad (4.5)$$

is the kernel representing the DECODER spectrum that would be obtained for a specified orientation of the PAS in the silk frame (i.e. for a fixed $\Omega$). The integrals in Eq. 4.5 reflect
Figure 4.4: The relevant reference frames for the DECODER experiment are connected by Euler rotations. The PAS system of the carbonyl carbon lies with $\sigma_{22}$ colinear with the carbonyl bond and $\sigma_{33}$ out of the peptide plane [55]. The z-axis of the silk frame is colinear with the silk fibre axis while the z-axis of the lab frame is colinear with the $B_0$ field. By our convention, successive rotations of $\Phi$, $\Theta$, and $\Psi$ are performed about the space-fixed $z$, $y$ and $z$ axis respectively.)
Chapter 4. Reconstructing Orientation Distributions from DECODER

the sample geometry. In Eq. 4.5, \( \delta(x) \) is the well known Dirac delta function.

The goal is to invert Eq. 4.4 to obtain the OD, \( P(\Omega) \). However, Eqs. 4.4 and 4.5 cannot be evaluated analytically, and numerical integration techniques must be employed.

4.2.3 Numerical Simulations of DECODER Spectra

The integration over \( \Omega \) in Eq. 4.4 was done by Riemann sums:

\[
S(\omega_1, \omega_2) = c \sum_{i,j} \Delta P(\Theta_i, \Phi_j) s_{ij}(\omega_1, \omega_2, \Omega_{ij})
\]

(4.6)

where

\[
\Delta P(\Theta_i, \Phi_j) = P(\Theta_i, \Phi_j) \sin \Theta_i \Delta \Theta_i \Delta \Phi_j
\]

(4.7)

is the probability of finding the PAS in the area on the unit sphere between \( \Theta_i \) and \( \Theta_i + \Delta \Theta_i \) and between \( \Phi_j \) and \( \Phi_j + \Delta \Phi_j \), and

\[
s_{ij}(\omega_1, \omega_2, \Omega_{ij}) = \sum_{\Theta', \Phi'} \delta(\omega_1 - \omega(\Theta_i, \Phi_j, \Theta', \Phi'))
\]

\[
\times \delta(\omega_2 - \omega(\Theta_i, \Phi_j, \Theta' + \Delta \Theta', \Phi')) \Delta \Theta' \Delta \Phi'
\]

(4.8)

are DECODER subspectra calculated for each discrete orientation, where \( \delta(x) \) is defined to be 1 for \( x = 0 \) and 0 otherwise.

Subspectra were calculated according to Eq. 4.8 with a resolution of \( \Delta \Theta = \Delta \Phi = 5^\circ \) using CSA tensor values of (242, 178, 96) ppm [56]. A subset of these subspectra are shown in Fig. 4.5.

As a test, a sample is considered for which the OD is known, namely a powder where \( P(\Theta, \Phi) = 1/(4\pi) \). An experimental DECODER spectrum of glycyl-glycine powder is shown in Fig. 4.6a, with a simulated spectrum that assumes \( P(\Theta, \Phi) = 1/(4\pi) \) shown in Fig. 4.6b.

Cross-correlation of Subspectra

In order to gain any information about the OD, the individual subspectra must differ from each other significantly. In order to get a qualitative feel for the difference between subspectra, cross-correlations were performed between each of the subspectra with all the other subspectra. The cross-correlation between subspectra, \( s_{ij} \) and \( s_{kl} \) is defined as:

\[
C_{ij}^{kl} = \frac{s_{ij} \cdot s_{kl}}{\sqrt{s_{ij} \cdot s_{ij} s_{kl} \cdot s_{kl}}}
\]

(4.9)

\(^3\)It is worthwhile to pause here and to note, that had the second integral in Eq. 4.5 been over \( \partial(\cos \Theta') \) rather than over \( \partial \Theta' \) this would have represented an isotropic sample geometry from which no orientational information could be gleaned. Thus, our sample geometry has indeed made it difficult to extract orientational information.
Figure 4.5: Subspectra for discrete $(\Theta, \Phi)$ calculated using Eq. 4.8.
Figure 4.6: a) DECODER spectrum for natural abundance glycyl-glyine powder. b) Simulated spectrum generated using Eq. 4.6 with $P(\Theta, \Phi)$ constant. Units on both axes are ppm.
Figure 4.7: Cross-correlation of each subspectrum with other subspectra. Each pixel in the image represents the cross-correlation of the spectrum representing \((\Theta, \Phi)\) in the supergrid with the corresponding spectrum representing \((\Theta, \Phi)\) in the subgrid. Ideally, each subgrid would contain a single point of amplitude 1. Because of the spherical geometry, all spectra with \(\Theta = 0\) are identical.
where the dot product between subspectra refers to the product of each pixel in the subspectrum summed over all pixels. The cross-correlation function has a value of 1 when $ij = jk$, and goes to zero as spectral overlap decreases.

Figure 4.7 depicts the cross-correlation function between subspectra. From this and from visual inspection of Fig. 4.5, it is evident that except near $\Theta = 0$, subspectra begin to differ significantly from each other when $\Theta$ and $\Phi$ change by $\pm 10^\circ$.

Eq. 4.6 cannot be inverted to yield $P(\Theta_i, \Phi_j)$ directly from the DECODER spectrum. The problem of direct inversion is ill-posed due to non-orthogonality of subspectra. A solution is to fit the components of $P(\Theta_i, \Phi_j)$ to the data. However, with the grid resolution which was used, this would result in 361 free parameters.

Thus, a model of silk is introduced that is parameterized by a small number of physically meaningful variables $\bar{a}$ and generates a probability distribution $P(\Theta_i, \Phi_j, \bar{a})$. The inversion problem is thereby recast from large $(\Theta_i, \Phi_j)$ coordinate space into a much smaller $\bar{a}$ coordinate space.

### 4.2.4 A Two-Component Model of Silk

Based on studies that suggest that dragline silk contains extended antiparallel $\beta$-sheets [57, 58] and more recent results suggesting the presence of highly extended polyglycine II helices [56, 59, 60], silk is modeled with two-components consisting of $\beta$-sheets and polyglycine II helices (see Fig. 4.8). The OD is therefore separated into two independent components

$$P(\alpha) = \epsilon P_\beta(\Theta, \Phi) + (1 - \epsilon) P_{\Pi}(\Theta, \Phi)$$

where $\epsilon$ is the fraction of the signal originating from the $\beta$-sheet component.

Studies have shown that in many drawn semi-crystalline polymers including silk, the crystallites are aligned along the fibre axis with a Gaussian OD [58, 61, 62]. This assumption is made for each of the components so that

$$P_i(\Theta, \Phi) = A \exp \left( -\frac{\sin^2 \alpha}{2\Delta \alpha^2} \right)$$

where $\alpha$, the angle between the secondary structure axis and the fibre axis, is a function of $\Theta$ and $\Phi$, as well as $\theta_i$ and $\phi_i$ that are the rotations that bring the PAS into the secondary structure frame ($i = \beta$ for $\beta$-sheets, and $i = II$ for helices). For the $\beta$-sheet, $\theta_\beta = 75^\circ$ and $\phi_\beta = 5^\circ$, while for the polyglycine II helices, $\theta_{\Pi} = 55^\circ$ and $\phi_{\Pi} = 10^\circ$. $\Delta \alpha$ is a measure of the width of the distribution, and $A$ is a normalization factor such that the total probability over the entire sphere is unity.

---

4Both of these secondary structures represent highly extended peptide conformations. With this two-component model, a rigorous assignment of secondary structure is not meant to be implied.
Figure 4.8: Rotations connecting the molecular (PAS) frame to the fibre frame via intermediate frames representing secondary structures. Each secondary structure is assumed to be aligned preferentially along the silk axis with a Gaussian probability distribution of width $\Delta\alpha$. 
The change of variables from $P(\alpha)$ to $P(\Theta, \Phi)$ is facilitated by the fact that

$$\sin \alpha = \frac{1}{\sqrt{2}} \left( D^1_{10}(0, \alpha, 0) - D^1_{-10}(0, \alpha, 0) \right). \quad (4.12)$$

Using Eq. 2.27, and the fact that the Euler rotation $(0, \alpha, 0)$ can be alternately written as the successive rotations $(-\phi_i, -\theta_i, -\gamma)$ and $(0, \Theta, \Phi)$ (see Fig. 4.8), we get

$$\sin \alpha = \frac{1}{\sqrt{2}} \sum_{m=-1}^{1} \left( D^1_{1m}(-\phi_i, -\theta_i, -\gamma) - D^1_{-1m}(-\phi_i, -\theta_i, -\gamma) \right) D^1_{m0}(0, \Theta, \Phi)$$

$$= (-\sin \theta_i \cos \Theta + \cos \theta_i \sin \Theta \cos(\phi_i - \Phi)) \cos \gamma$$

$$- \sin \Theta \sin(\phi_i - \Phi) \sin \gamma. \quad (4.13)$$

This expression can be inserted directly into Eq. 4.11 to yield the desired $P(\Theta, \Phi)$. Numerical integration over $\gamma$ ensures rotational symmetry about the secondary structure axis.

Thus, we have arrived at a model of silk that has only 4 parameters: the width of each of the Gaussian distributions, $\Delta \alpha_{ii}$ and $\Delta \alpha_{gg}$, the relative intensity of each, $\epsilon$, and the overall intensity of the spectrum.\textsuperscript{5} The remaining step is to fit the experimental DECODER data to the model.

### 4.2.5 Fitting

The Port Library [63] non-linear least squares fitting algorithm was used to fit the simulated spectra to the data using the fitting parameters described above.

Finite pulse width amplitude corrections [52] were performed on the simulated spectra in both dimensions, and a background subtraction was performed using a DECODER spectrum from unlabeled silk. The background spectrum was properly scaled and shifted along the frequency axis according to the relative size and position of the alpha carbon peaks from the labeled and unlabeled spectra. It was found that for spectra obtained from samples with good $1^{-13}$C labeling, the background subtraction was unnecessary, as it did not affect the results significantly.

The fitting algorithm was used to minimize the square of the residuals between the experimental spectrum $S_{\text{Expt}}$ and a spectrum $S_{\text{Model}}(\vec{a})$ generated from Eqs. 4.6, 4.10, and 4.14. The algorithm searches for a local minimum of

$$\chi^2 = \sum_{\omega_1, \omega_2} (S_{\text{Expt}}(\omega_1, \omega_2) - S_{\text{Model}}(\omega_1, \omega_2, \vec{a}))^2. \quad (4.15)$$

\textsuperscript{5}In practice, two extra parameter are included for Lorentzian and Gaussian broadening of the simulated spectra to correspond to the broadening due to homo- and heterogeneous relaxation. Another parameter is included to allow an arbitrary frequency shift of the data along the diagonal, $\omega_1 = \omega_2$. 
Such parameter space searches as done by the fitting algorithm become increasingly less robust with larger number of fitting parameters (i.e. searching through a higher-dimensional space). Reduction of the problem to a small number of parameters as has been outlined in this chapter results in a more robust algorithm, as is evidenced by the fact that the fitting algorithm converges to the same minima for a large range of initial starting parameters.

4.3 Conclusions

This chapter has presented a methodology for the difficult problem of reconstructing the molecular OD from a fibre that was wound onto a cylindrical support from DECODER NMR data. A model of silk was introduced, consisting of β-sheets and polyglycine II helices aligned along the fibre with a Gaussian distribution, which will facilitate the fitting of simulated spectra to silk DECODER data.

In the following chapters, this technique will be applied to determine the OD in silk, and to observe changes in orientation as a function of fibre strain, draw rate, and hydration.
Bibliography


Part II

Manuscripts: Spider Silk
Chapter 5

Orientation of Spider Silk’s Peptide Backbone Under Varying Strain and Draw Rate

5.1 Introduction

The dragline silk of the golden orb weaver spider, Nephila clavipes, is widely regarded as a high performance fibre with a combination of strength and extensibility that has been difficult to achieve in man-made materials [64] especially under benign processing conditions. These unique physical properties are attributed to the secondary and tertiary structure of the silk protein [65] in the fibrous state. An understanding of this structure-function relationship will complement ongoing efforts of biomimeticists to spin fibres [66, 67] based on recombinant silk proteins [68] in commercial quantities, having mechanical properties matching those of fibres produced in nature. The size of the silk protein, its insolubility in the fibrous state, and its inherent inhomogeneity precludes it from conventional solution nuclear magnetic resonance (NMR) or X-ray crystallographic protein structure determination, and other techniques must be employed. Because of its sensitivity to structure and dynamics, solid-state NMR is a powerful tool for the study of polymer fibres [69], and several groups have applied various solid-state NMR techniques to silks [70–72].

Based on solid-state NMR [70, 72–75], fibre X-ray diffraction [76, 77], and amino acid sequences from cDNA sequencing [65, 78], it is known that dragline silk is a semicrystalline polymer comprised of rigid alanine-rich β-sheet crystallites connected by a network of soft glycine-rich chains, with the latter believed responsible for silk’s extensibility. Computer simulations suggest that such a model accounts well for silk’s mechanical properties [79]. X-ray diffraction [77] and solid state NMR [70] studies have shown that the crystalline regions exhibit a high degree of orientational order and that these regions reorient under tensile deformation [77]. The structure of the glycine-rich regions has been described as amorphous [77], random coil [64, 80] and most recently as a highly extended poly-glycine II (or 3i) helix [72, 75, 81]. and although the orientation distribution (OD) of these structures is expected to be affected by macroscopic fibre deformation, such changes have not been observed.

A version of this chapter has been previously published as:  
In this work, we use a new variation of DECODER (Direction Exchange with Correlation for Orientation Distribution Evaluation and Reconstruction) NMR [82] to reconstruct the OD of glycine's carbonyl groups within the silk fibre under varying strain and fibre draw rate. Our implementation of DECODER NMR is novel due to the sample geometry which complicates the reconstruction but provides a number of practical benefits.

5.1.1 DECODER NMR

Due to the anisotropy of the local electronic environment, the NMR chemical shift of a nucleus depends on molecular orientation in an external magnetic field [83] according to:

$$\omega = \omega_0 \left( \delta_{iso} + \delta \left[ \left( \frac{3}{2} \cos^2 \Theta - \frac{1}{2} \right) + \eta \sin^2 \Theta \cos 2\Phi \right] \right),$$  \hspace{1cm} (5.1)

where $\delta_{iso}$, $\delta$ and $\eta$ describe the chemical shift anisotropy (CSA) tensor which is attached to the molecular frame, and $(\Theta, \Phi)$ specify the orientation of the magnetic field in this frame. Thus, the NMR spectrum contains information about the OD of molecular fragments within the sample. However, the problem of reconstructing an OD from a spectrum is ill-posed because each NMR frequency does not correspond to a unique orientation. This degeneracy can be largely lifted by a two-dimensional (2D) NMR experiment known as DECODER, which correlates the NMR frequency at two sample orientations using a macroscopic rotation of the sample during the mixing time. Each point in the 2D spectrum corresponds to one of eight orientations, which are not further differentiable due to the symmetry of electronic shielding.

Here we introduce a new implementation of DECODER which incorporates a novel geometry with uniaxial fibres wound around a cylindrical support rod (Fig. 5.1). Such a geometry complicates the reconstruction of the underlying OD by breaking the direct correspondence between points in the 2D spectrum and orientation in the fibre. Instead, each molecular orientation in the fibre corresponds to a distinct spectral signature as shown in Fig. 5.2, and we employ simulation and fitting techniques to reconstruct the underlying OD. This sample geometry offers several practical advantages over an oriented fibre geometry by allowing for: 1) a large amount of sample to fit inside the measurement volume, thus increasing the signal to noise ratio and opening the door for measurement of low abundance amino acids and unlabeled fibre samples, 2) uniform variation of the fibre strain attained by changing in the diameter of the PTFE rod, 3) smooth and constant silking rates during sample collection, and, 4) the possibility of performing measurement of hydrated samples with the PTFE rod fitted into a water-filled NMR tube. Such practical benefits are difficult to achieve in an oriented sample for the large fibre bundles required to obtain adequate signal to noise from multidimensional NMR experiments.
Chapter 5. Orientation of Spider Silk's Peptide Backbone

5.2 Experimental Methods

5.2.1 Silk Samples

Selectively labeled silk from 8-10 adult female N. clavipes spiders collected in central Florida was harvested by forced silking [84] during five sessions over the course of two weeks to yield ~100 mg samples. Spiders were fed Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich, Steinheim, Germany) enriched with 5% w/v 1-\(^{13}\)C glycine (Cambridge Isotope Laboratories, Andover, MA) and supplemented with 2.5% w/v unlabeled alanine to approximately reflect the amino acid composition of major ampullate silk. Silk was drawn at 2 cm/s and 4 cm/s, within the range typically found in nature, and at rates that have recently been shown to yield silk fibres with appreciably different crystallite ordering in N. edulis dragline [85]. Silk was drawn onto specially designed PTFE rods which could collapse to ~90% of their original diameter when a polychlorotrifluoroethylene (PCTFE) insert was removed, releasing some of the inherent fibre drawing strain (Fig. 5.1). We measured a drawing strain of ~10% for the 2 cm/s drawing rate. Thus, the decrease in the sample rod circumference represents an appreciable change in fibre strain.

5.2.2 NMR Spectroscopy

DECODER spectra were collected on a homebuilt NMR spectrometer [86] based upon an 8.4 Tesla magnet (Oxford Instruments, Oxford, UK) providing a \(^{13}\)C resonance frequency of 91.480 MHz. Hartmann-Hahn cross-polarization from protons was performed with 50 kHz rf fields and 1.5 ms contact times. TPPM [87] proton decoupling at 50 kHz was used.
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during both evolution periods.  

A 90° sample flip about the rf coil axis was performed by a spectrometer controlled stepper motor during the 0.1 second mixing time. Fifty complex points were obtained in the indirect dimension with an 8 µs dwell time. FID's were zero-filled to achieve the same digital resolution in both dimensions and were apodized before Fourier transformation with an exponential corresponding to a 50 Hz full width at half maximum (FWHM). The sample’s starting orientation was cycled in 1.8° increments while signal averaging to ensure cylindrical symmetry about the rotation axis.

5.2.3 Spectral Reconstruction

DECODER spectra of wound uniaxial fibres were simulated for discrete orientations of the chemical shift tensor in the fibre with resolution of 5° in θ and φ (see Fig. 5.2). These angles describe the orientation of the CSA in the fibre and are defined here to be successive rotations of the CSA tensor about the fibre-fixed z and y axes with the CSA initially oriented with the largest component along x and the smallest component along z (the fibre axis). The glycine carbonyl CSA was assumed to lie with the most shielded component perpendicular to the peptide plane and the intermediate component tilted by 5° away from the carbonyl bond [88]. CSA tensor values of (242, 178, 96) ppm [72] were used in the simulations. A linear superposition of the simulated subspectra was used to reconstruct the experimental spectrum with the resulting weight of each subspectrum indicating the relative number of residues that have the corresponding orientation.

Due to intrinsic broadening of the experimental spectra, the non-orthogonality of the simulated subspectra, and our discrete sampling of orientation space in the simulations, our reconstruction technique is limited in the degree to which the underlying OD can resolved. For this reason, we choose a model-dependent approach for reconstructing the experimental spectrum where the molecular OD in the silk is described by a small number of free parameters in a physically relevant model, from which weights for individual subspectra can be calculated. Because glycines reside in both the crystalline and soft interconnecting domains, we model our data with a two-component distribution, assuming that the two environments have varying degrees of alignment along the fibre. We introduce a pair of intermediate structures, shown in Fig. 5.3, and assume that the OD of each structure is described by a Gaussian,

\[
P(\alpha) = A \exp \left( -\frac{\sin^2(\alpha)}{2\Delta\alpha^2} \right),
\]

parameterized by a width Δα, in radians. The model assumes that all possible orientations of the intermediate structures rotated about the structure axis (by an angle γ, as in Fig. 5.3) are equally likely. By defining the set of rotations which connect the molecular and

\[\text{1Compared to cw decoupling at the same rf power, TPPM is a more efficient means of proton decoupling. It involves alternating the phase of the cw proton channel between two values. The period of this phase alternation and the phase difference must be calibrated for each experiment.}\]
Figure 5.2: Simulated DECODER spectra of wrapped fibre samples for discrete orientations of the carbonyl carbon in the fibre for a sample rotation of 90°.
intermediate frame, we write $\alpha$ in terms of $\theta$ and $\phi$ and thus the Gaussian probability, $P(\alpha)$ as $P(\theta, \phi)$, yielding the appropriate weighting of each subspectrum.

Based on recent results [72, 75, 81], we chose to model the two component nature of our data in terms of extended anti-parallel $\beta$-sheets ($\theta_\beta=75^\circ$, $\phi_\beta=5^\circ$) and poly-glycine II helices ($\theta_{II}=55^\circ$, $\phi_{II}=10^\circ$), where the angles given here describe the orientation of the CSA tensor in the fibre frame when the secondary structures are aligned with their long axes along the fibre (Fig. 5.3). We henceforth refer to the two respective components in the model as “crystalline” and “helical” but do not, as will be discussed, mean to imply a rigorous assignment of secondary structure.

We implemented other more complicated two-component models which also reproduced our data well, including one model containing random coils, and another model with the angles that define the intermediate structures left as fitting parameters. However, the complexity of these models (i.e. the number of fitting parameters) resulted in many sets of parameters yielding fits of similar quality (i.e. a shallow minimum in the multi-dimensional $\chi^2$-space), making an interpretation of changes as a function of fibre strain and draw rate difficult. The simplicity of the model described here, containing very few free parameters, facilitates interpretation of our results under varying conditions of strain and draw rate.
Table 5.1: Best fit parameters from model and calculated order parameters.

<table>
<thead>
<tr>
<th></th>
<th>β-sheet</th>
<th></th>
<th>helix</th>
<th></th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%  Δα  f</td>
<td>%  Δα  f</td>
<td>%  Δα  f</td>
<td>%  Δα  f</td>
<td></td>
</tr>
<tr>
<td>2 cm/s As drawn</td>
<td>47  8.5° 0.93</td>
<td>53  27° 0.33</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxed</td>
<td>44  8.6° 0.93</td>
<td>56  24° 0.42</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 cm/s As drawn</td>
<td>47  8.5° 0.93</td>
<td>53  23° 0.45</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxed</td>
<td>32  9.2° 0.92</td>
<td>68  29° 0.29</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2.4 Fitting

Simulations were fit to experimental spectra with a non-linear least squares fitting algorithm from the PORT Mathematical Subroutine Library (AT&T Bell Labs, Murray Hill, NJ) [89]. The relative contribution of signal from each intermediate structure and the width of each Gaussian distribution were left as fitting parameters as were spectral broadening parameters and frequency offsets.

5.3 Results

The carbonyl region of the $^{13}$C DECODER spectrum of $L^{13}$C glycine labeled silk drawn at 2 cm/s under residual draw strain is shown in Fig. 5.4a. No background subtraction was deemed necessary because, based on electrospray mass spectroscopy of a portion of the sample following hydrolysis in 12M HCL for 24 hours at 100°C, we estimate that 84% of the carbonyl signal intensity arises from glycine residues.

Spectra similar to Fig. 5.4a were collected for silk drawn at 4 cm/s and under drawing tension and with tension relaxed. Spectra obtained under varying conditions showed qualitative differences and were appreciably different from spectra obtained on unoriented samples [90].

Figs. 5.4b, and 5.4c depict the best fit spectrum, and the residuals respectively. The OD resulting from the fit to the data, shown in Fig. 5.4d, reveals that 47% of glycines are well aligned along the fibre (Δα = 8.5°) in the crystalline components while 53% are poorly aligned (Δα = 27°) in the helical component. Table 5.1 summarizes the fitting results for the various glycine-labeled samples. Although we have not undertaken a detailed error analysis, we find the goodness of fit to the data changes on a scale of about ± 2% for the % contribution and ±1° for the distribution widths. These uncertainties do not reflect significant correlations amongst the parameters, whereby changes in the relative populations of the components may be offset by a compensating change in a distribution width. The order parameter $f = (3 \cos^2 \alpha - 1) / 2$ is calculated for comparison with previously published data.
Figure 5.4: a) Carbonyl peak from the $^{13}$C DECODER spectrum of isotopically labeled silk with aliphatic carbon peak due to natural abundance $^{13}$C shown in the inset, b) best fit spectrum, c) residuals, and d) orientation distribution, $P(\alpha)$, from fit with an 8.5° and 27° Gaussian width for the $\beta$-sheet and poly-glycine II helix respectively.
5.4 Discussion

5.4.1 Orientation Distribution

DECODER experiments were recently reported [72] for 1-13C glycine and alanine labeled dragline silk from N. edulis spiders in an oriented fibre geometry. Although a quantitative analysis of orientation was not performed in that study, those data appeared to be consistent with two components of roughly equal intensity, one well aligned, the other poorly aligned, in qualitative agreement with our results.

The degree of alignment of our model’s crystalline component corresponds to a FWHM of 20° (where FWHM = 2 arcsin(1.18Δα)), while that of the helical component is about 68°. These results compare to a 16° FWHM crystalline component (12%), a 30° FWHM oriented amorphous component (34%) and an isotropic component (54%) reported by Grubb et al. [77] from X-ray diffraction measurements. We also compare to the results of Simmons et al. [70] who report a 40% well-aligned crystalline component with a 5° FWHM and a 60% poorly aligned crystalline component with a 75° FWHM, based on 2H NMR measurements of alanines.

The sequence of amino acids generally accepted as forming the core of the crystalline domains (see Table 5.2) is the alanine-rich sequence bounded by glutamine residues. Because glycines are found near the ends of these domains, where deviations from perfect crystallinity, or “fraying,” may be expected, it is not surprising that the glycines show a greater distribution width than did the alanines. It has also been suggested [81] that a staggering of poly-alanine sequences in the β-sheets would create an interphase domain between the crystalline and amorphous domains that would contain glycine and glutamine residues which, due to the differing size and shape from alanines, would break the crystal symmetry and result in a broader OD than that of the bulk crystallites.

We thus make a connection between our crystalline component and the combination of Grubb’s crystalline and oriented amorphous components and between our model’s helical component and Grubb’s isotropic component. To account for the 47% of glycines within our crystalline component, we must include all of the glycines within the crystalline sequence as well as the majority of glycines within three residues of the terminating glutamines.

Such an assignment appears to be at odds with the Simmons et al. results, because based on the Simmons model, in which linker regions are unoriented, we would expect to find much less orientation in the glycines than we do. One potential explanation for this discrepancy is a different spectroscopic weighting of the components due to a difference in cross polarization efficiency or a difference in 13C or 1H relaxation. We have ruled out this possibility by observing single exponential behavior in carbonyl carbon (T1=50 s and T2=0.8 ms) and proton relaxation times (T1=0.7 s) and observed overall cross-polarization efficiencies similar to those in glycine powder, leading to the conclusion that more glycines are well aligned along the fibre than expected from the Simmons model.

The results from the relaxed fibres drawn at 4 cm/s show a reduced (32%) contribution from the aligned component, suggesting that fibre tension and drawing conditions affect the relative contribution of these poorly and highly aligned components to the makeup of silk.
5.4.2 Tension and Fibre Drawing Rate

Our data suggest that the dependence of OD on tension is a function of fibre drawing rate. The sample drawn at 2 cm/s actually showed a slight decrease in alignment of the helical component when the draw tension was relaxed. The overall order parameter however remained nearly constant, as the increase in alignment of the poorly aligned helical component was offset by an increase in the population of that component. As discussed below, our data, especially for the poorly aligned component, are insensitive to the details of the secondary structure and so we do not interpret this change in population to indicate a shift from sheets to helices. Rather we claim the fitting results for this sample reflect subtle changes in the OD.

In contrast, the sample drawn at 4 cm/s exhibited a significant broadening of the distribution (i.e. a decrease in the total order parameter) when tension was released. From the fits, it is clear that the decreased degree of alignment along the fibre is due to an increase in width of the distribution for the helices, as well as an increased contribution from the helical component to the total signal intensity. Again we do not mean to suggest that our data provide evidence for sheet structures becoming helical when the tension is relaxed. The fits are not sensitive enough to local structure to warrant such an interpretation.

We attribute the changes in OD observed in the faster drawn fibres to the presence of metastable conformations that exist under tension and arise from expedited fibre processing in the duct. The release of tension allows for molecular rearrangement into a lower energy conformation. The slower drawing rate may allow time for this stable conformation to be reached in the duct during processing.

Alternately, the fact that we observed greater changes in OD in the fibres drawn more quickly can be attributed to the dependence of inherent drawing strain on draw rate. Fibres drawn faster may be closer to their breaking strain whereas fibres drawn slower would be nearer a fully relaxed state. Our results would imply that more chain reordering occurs at high values of strain.

Comparison of the OD in the two samples as drawn seems to indicate that drawing rate affects the distribution of the helical component much more than the crystalline component.

The crystalline regions is defined here as the amino acid sequence between the glutamine residues that contains polyalanine runs. Fractions are based on the peptide sequence of Xu and Lewis [78] containing 656 residues, a representative sample of which we reproduce here with crystalline region in bold:

...AGRGGLGQGAGGAAAAAGGAGQGGGYGLGSQG...
An increased drawing rate yields fibres with helical components that are better aligned along the fibre, as would be expected from alignment due to elongational and shear flow [91, 92]. These results are consistent with recent X-ray diffraction measurements of orientation in the ordered regions of as-drawn silk [85], which found an increase in order with increasing draw rate, with appreciable changes in order parameter occurring between 1 and 40 mm/s draw rates. Our results suggest that these data should be interpreted as an increased alignment of the amorphous region, as opposed to more perfect alignment of the already well aligned crystalline regions.

### 5.4.3 Secondary Structures

We have chosen to simulate our spectra with a two component model consisting of β-sheets and poly-glycine II helices. A more complete model might incorporate more components or other secondary structures. We find, however, that our results are fairly insensitive to the exact nature of the intermediate structures, and cannot justify the inclusion of additional fitting parameters to describe a third component. This insensitivity is due to the similarity of \((\theta_B, \phi_B)\) and \((\theta_{II}, \phi_{II})\), reflecting the fact that the protein backbones in both structures are well aligned along the structure axis. What sensitivity there would be to secondary structure is further obscured by a broad distribution in \(\alpha\), especially for the component we have labeled “helices”. We think it unlikely that long regular 3I-helical structures exist in silk. Rather, we believe that there exist highly extended chains, that, while not having a well defined long-range structure, may retain some local short-range order, for example between adjacent glycine residues, with dihedral angles resembling a 3I-helix. In this article, wherever we have referred to “helices” we have alluded to such extended structures. Thus, our DECODER data appears to be consistent with β-sheets and poly-glycine II helices but is not conclusive evidence for their existence. Despite this caveat, it is clear that our data are not well described by a single component, but require at least two overlapping components to be adequately simulated.

### 5.5 Conclusions

We have shown that DECODER NMR in a wound fibre geometry is sensitive to molecular orientation within the fibre and that this method presents some practical benefits over oriented sample DECODER by allowing for uniform changes in strain, constant drawing rates, sample hydration and increased signal to noise rendering low abundance amino acids and unlabeled samples accessible to such techniques.

We have been able, for the first time, to directly visualize the changes in orientation of the soft parts of the silk as functions of strain and draw rate. Our results indicate that the orientation of the helical component increases with increased draw rate and that the OD appears to change appreciably when the drawing tension on the fibre is released in a sample drawn at 4 cm/s but not in one drawn at 2 cm/s.
Bibliography


Chapter 6

Dynamics of Spider Silk’s Peptide Backbone During Supercontraction

6.1 Introduction

Spider silks are protein fibers whose mechanical properties range from strong and stiff to soft and elastic [93] depending on species, silk gland, and environmental conditions. Silks have become the subjects of intensive study [94, 95], in part due to advances in recombinant DNA technology [96], which promises to allow large-scale production of silk-inspired fibers having properties customized by amino acid sequence [97, 98] and spinning conditions [99]. Realization of this promise depends on a thorough understanding of the microscopic structural basis of the fibers’ macroscopic properties [100].

Many polymer fibers, including dragline silks, demonstrate a phenomenon known as supercontraction [101], where an absorbed solvent induces large-scale molecular motions in a transition from a glassy to a rubbery state, causing a macroscopic reduction in the length of the fiber and a concomitant swelling in diameter. Supercontraction in synthetic polymers is usually observed only at high temperature or in harsh solvents, whereas spider silks supercontract in water at room temperature [101, 102]. It has been suggested that in nature, supercontraction induced by morning condensation maintains tension in spider webs [101]. Based on measurements of diminishing supercontraction stress as a function of time, this suggestion was later challenged [103], but is supported by more recent measurements [104].

Because of its sensitivity to dynamics, solid-state NMR has been used to probe supercontracted silk [105–107]. Other NMR experiments have probed local structure [108, 109] and bulk orientation [109, 110] in dry silk. However, due to the challenges of uniformly varying strain in large oriented fiber bundles, molecular orientation and dynamics in partially supercontracted samples have not previously been studied.

We employ a two-dimensional (2D) solid-state NMR technique known as DECODER (Direction Exchange with Correlation for Orientation Distribution Evaluation and Reconstruction) [111] that, for oriented samples, facilitates a full reconstruction of the orientation distribution of static molecular subunits within a polymer by correlating the chemical shift frequencies of nuclear spins at two macroscopic sample orientations. Our sample geometry, fibers wrapped around a collapsible plastic support whose axis lies parallel to the NMR coil

A version of this chapter has been previously published as:
axis, complicates the orientation distribution reconstruction, but allows control of the sample strain and environmental conditions [110].

In addition to orientation, the DECODER experiment is sensitive to molecular motions on a number of timescales (see Fig. 6.1). Motions with correlation times, $\tau_c$, less than $1/(10 \text{ Hz})$ cause a loss of correlation during the mixing time of the experiment (100 ms) and result in a 2D spectrum that is simply the product of the one dimensional spectrum with itself. Motions faster than ~ 20 kHz average the $^{13}\text{C}$ chemical shift anisotropy, reducing the wide-line spectrum into a sharp peak at the isotropic chemical shift, and also average the $^1\text{H}$-$^{13}\text{C}$ dipolar couplings resulting, for cross polarization experiments like ours, in a loss of signal intensity. Still faster motions manifest themselves in enhanced spin relaxation, to which this experiment is relatively insensitive because the mixing and evolution times are much shorter than the fastest $T_1$ in any of our silk samples.

In this work, we restrain silk fiber bundles before hydration, allowing them to supercontract to varying predetermined lengths, and measure protein backbone orientation and dynamics as a function of degree of supercontraction. In such a way, we obtain snapshots of silk in the process of supercontraction.

6.2 Experimental Methods

Silk Samples. The NMR experiments were carried out on $1^{-^{13}\text{C}}$ glycine-labeled silk samples under three different conditions of restraint: the first was used as collected, and was under considerable draw tension, estimated at 50% of the fibers' breaking strength, corresponding to a strain of about 10%. The second sample was allowed to contract 10% in length, largely relieving the draw tension. Finally, the first sample was cut off the collection spool and rolled into an isotropic bundle and so was not restrained.

Dragline silk from adult female *Nephila clavipes* spiders fed a $1^{-^{13}\text{C}}$ glycine enriched diet was spooled at 2 cm/s onto polytetrafluoroethylene (PTFE) rods that had been hollowed, slit on one side, and expanded with polychlorotrifluoroethylene (PCTFE) cylindrical inserts that could be later removed to relax the drawing tension [110]. NMR spectra of restrained samples were obtained with the silk on the collecting rod and hydration was achieved by placing a water-filled NMR tube over the end of the sample and sealing with PTFE tape. Unrestrained spectra were collected from silk that had been removed from the rod and packed unoriented inside a hollowed PCTFE cylinder. Hydration was achieved by filling the sample holder with water before sealing.

NMR spectroscopy. $^{13}\text{C}$ DECODER NMR spectra were collected at 91.480 MHz on a homebuilt NMR spectrometer [112] with a homebuilt doubly tuned wideline probe equipped to rotate the sample about the solenoidal rf coil axis via a spectrometer-controlled stepper motor. Spectra were collected with 5 $\mu$s 90° carbon pulses, a cross-polarization contact time of 1.5 ms, CW proton decoupling at 50 kHz during both evolution periods, and a 90° rotation
Figure 6.1: Molecular motions on multiple timescales can be differentiated based on spectral characteristics in the DECODER experiment. Simulated powder spectra are shown in the different motional regimes.
of the sample about the rf coil axis during the 0.1 s mixing time. FID’s were zero-filled to achieve the same spectral resolution in both dimensions and apodized with a 50 Hz full width at half maximum (FWHM) exponential before Fourier transformation.

**Spectral Reconstruction.** The unrestrained carbonyl spectra were decomposed into contributions from static \(1/\tau_c<10\) Hz and mobile \(1/\tau_c>10\) kHz components by fitting a linear superposition of a simulated carbonyl powder spectrum with chemical shift tensor values of [242,178,96] ppm [109], and a sharp (1.9 kHz FWHM) peak at the isotropic chemical shift (172 ppm). The restrained spectra were reconstructed by a linear superposition of subspectra simulated for a discrete set of molecular orientations of the CSA tensor in the silk fiber using the PORT Mathematical Subroutine Library’s (AT&T Bell Labs, Murray Hill, NJ) adaptive non-linear least squares fitting algorithm [113]. A two-component model, details of which are described elsewhere [110], was used to decompose the broad carbonyl spectrum into contributions from a well aligned and poorly aligned component in addition to a mobile component identical to that used above.

**Mass Spectroscopy.** Electrospray mass spectroscopy was performed on a portion of the sample that had been hydrolyzed in HCL for 24 hours at 100°. The isotopic analysis indicated that approximately 84% of the signal intensity of the dry sample arises from glycines, with the remainder coming largely from alanines. Thus, no background subtraction was deemed necessary.

### 6.3 Results

The \(^{13}\text{C}\) DECODER NMR spectra of the silk as-drawn and unrestrained are shown in Figs. 6.2A and 6.2E respectively. The dramatic difference between the as-drawn spectrum and the isotropic powder pattern of the unrestrained spectrum reflects the high degree of orientational order in the silk fibers. The fit shown for the as-drawn spectrum (Fig. 6.2B) is based upon a model [110] incorporating two static components, each of which has an orientation distribution described by a Gaussian centered along the fiber axis.

The fit reveals that one component is well aligned, the other poorly aligned, their distributions having respective FWHM of about 20° and 70° with respect to the fiber axis. The unrestrained spectrum is fit (Fig. 6.2F) solely to an isotropic static powder pattern. It is clear from these spectra, along with one-dimensional \(^{13}\text{C}\) NMR spectra acquired without cross polarization that there is little molecular motion at time scales faster than \(\sim10\) Hz.

Spectra for these same samples when hydrated are shown in Figs. 6.2C and 6.2G. Upon hydration, a sharp peak appears at the isotropic chemical shift frequency, and the overall spectral intensity decreases. These changes are indicative of large amplitude molecular reorientations on timescales faster than \(\sim20\) kHz occurring within some regions of the silk, as the chemical shift anisotropy is well averaged in the sharp peak, and the loss of signal intensity reflects inefficient cross polarization of the mobile component. In addition to the
Figure 6.2: Carbonyl region of $^{13}$C DECODER spectra of 1-$^{13}$C glycine labeled dragline silk. The sample in (A) and (C) was restrained with drawing tension, unrestrained in (E) and (G). (B), (D), (F) and (G) are simulations described in the text.

The DECODER data in Fig. 6.2 completely lack the spectral characteristics of slow motions on the time scale of $10^{-10^4}$ Hz (see Fig. 6.1). This is especially dramatic in the hydrated samples which exhibit both static ($1/\tau_c<10$ Hz) and fast moving ($1/\tau_c>10^4$ Hz) components but lack an intermediate dynamical regime. The absence of slow motions is also evident from the lack of off-diagonal intensity in a DECODER experiment performed without sample rotation. This is strong evidence that supercontraction is the result of a hydration-induced phase transition between glassy and rubbery states mediated by the disruption of interchain hydrogen bonds by waters of hydration.

The relative abundance of the static and fast components, as determined by the intensities of the multicomponent fits to the data, are summarized in Fig. 6.3A, and show a marked increase in the fraction of the sample that becomes mobile as the fiber strain decreases. The degree of orientational order in hydrated silk observed here is in qualitative agreement with X-ray diffraction [116] and birefringence [101] measurements made as a function of strain.

The results of the two-component fits to the restrained samples' spectra indicate that the glycines that become mobile are almost exclusively those that were poorly aligned before hydration. Upon hydration, the remainder of the poorly aligned static fraction is fit by a Gaussian so broad as to be completely isotropic, while the well aligned component retains a FWHM of about 20°. The apparent increase in the population of the well aligned component...
Figure 6.3: Dependence of orientation, dynamics, and mechanical properties on hydration and strain. (A) Integrated intensity of NMR signals of dry and wet silk fibers under varying strain. The shading of the unrestrained sample reflects the fact that no information on the relative abundance of well and poorly aligned components is obtained. These data suggest a reinterpretation of recently measured stress-strain curves, shown schematically in (B), based on Refs. [98, 114] and [115] (see text). The dotted curves represent fibers treated by allowing them to partially or fully supercontract before drying.
of the as-drawn sample, from 45 to 51%, upon hydration likely reflects residual alignment of the poorly aligned component, which is now split amongst the isotropic and well aligned components.

Based on the spidroin-1 amino acid sequence [107], roughly 37% of glycines reside inside or within a few amino acids of the alanine-rich crystalline regions [106], and are known from previous solid-state NMR [106, 117] and X-ray diffraction measurements [116] to remain immobilized upon hydration. The remaining glycines are distributed throughout the inter-crystal linkers. The 44% of glycines that we find remain static in the fully supercontracted sample corresponds well with the fraction expected to reside inside or immediately adjacent to the crystalline regions, in agreement with the fraction found by Yang et al. [106] based on the analysis of one-dimensional NMR spectra of fully supercontracted 1-13C glycine labeled silk.

Based on these orientational and dynamic data, we make a connection between the linker regions, which become mobile during supercontraction, with the poorly aligned component, and the crystalline regions, which are unaffected by hydration, with the well aligned component. These are not rigorous assignments of the fit components, because as mentioned above, there is likely some (strain dependent) contribution from each of the structural regions to each of the fit components, due to the limitations of the two component model in describing the true orientation distribution.

6.4 Discussion

Our results illuminate the molecular origin of a large body of mechanical and structural measurements (eg. Refs. [94, 95] and references therein) for which we propose a model mechanism based on the existence of preformed extended structures in the linker regions of as-drawn silk. The interchain hydrogen bonds stabilizing these structures are broken by fiber stress and hydration; the former leading to elastic then plastic fiber deformation and the latter leading to local phase transitions to a rubbery state in the process of supercontraction. We outline this model in detail below.

In dry, as-drawn dragline, the linker regions largely assume highly extended conformations [110] (Fig. 6.4A). The amino acid sequence and fiber spinning process encourage the formation of some local structure, possibly extended poly-glycine II helices [108, 109], as well as compact turns [106, 118]. On hydration, hydrogen bonds stabilizing these structures are broken and the chains collapse into mobile entropic springs, with the fraction of the linker regions able to participate governed by the restrained length of the fiber (Figs. 6.4B and 6.4C). Based on our results above, the separation between mobile and static components is a sharp one, and is understood in terms of local phase transitions. Existing heterogeneity provides nucleation sites so that regions with low enough local stress cooperatively hydrate and collapse, increasing the stress on neighboring regions, reinforcing their quiescence, as depicted in Fig. 6.4B. The collapse results in a broadening of the orientation distribution of the uncollapsed linkers as the fiber shrinks.

Straining the supercontracted dragline results in a very low initial modulus, as the soft
Figure 6.4: Cartoon depicting the proposed mechanism of fiber supercontraction. (A) Static linkers (extended green chains) in as-drawn dragline silk connect crystalline regions (gold pleated blocks) and are stabilized by interchain hydrogen bonds (red dotted lines). (B) As the hydrated fiber is allowed to contract, an abrupt transition to a highly mobile phase (blurred blue chains) occurs in some regions, increasing strain and enforcing quiescence in neighboring regions. (C) For a fiber allowed to fully supercontract, more regions become mobile. An actual fiber has a diameter several hundred times that of the region depicted here.
entropic springs extend (region i in Fig. 6.3B). On reaching the extension limits of the springs, chain reorientation and rearrangement dominate the response and the modulus increases.

If allowed to dry while contracted, the entropic spring regions lose their mobility as waters of hydration are replaced with intra- and inter-chain hydrogen bonds, forming “latent entropic springs”, static structures that may regain some local structure [105], but remain compact. When these fibers are strained, the initial modulus is similar to that of as-drawn dragline (ii of Fig. 6.3B), which is well accounted for by detailed numerical models [119] that include the rigid crystalline regions in addition to the soft linkers and allow processes such as chain slippage and breakage. As the stress becomes great enough to break hydrogen bonds, there is a pronounced plateau in the stress-strain curve (iii of Fig. 6.3B) as the latent springs elongate.

In the as-drawn dragline, a small, spinning condition dependent fraction of the linker regions exist as latent entropic springs, accounting for the plateau observed in stress-strain curves (iv in Fig. 6.3B). The hysteresis observed in dry fibers [115] is due to the fact that once stretched out, the latent entropic springs contract much more reluctantly than when hydrated, but remain entropically driven. In the absence of water, the contraction is unlikely to complete, as hydrogen bonds, some transient, may reform to stabilize intermediate configurations. Differences between successive load-unload cycles may also be due to the rupturing of the initial extended structures found in as-drawn fibers.

Our proposed model succeeds in explaining the mechanical properties of dragline silk including recent tensile measurements on silk fibers having undergone controlled supercontraction during which the length of a fiber is fixed while it is wetted and subsequently dried [114] (dotted lines in Fig. 6.3B). Such post-draw fiber processing holds the promise of tailored fiber stiffness. Furthermore, our model explains the tensile measurements of wet silk by Work [120]. These measurements showed that the fibre stress during supercontraction was significantly less than the stress when the wet silk was subsequently extended back to its original length. According to our model, once fully supercontracted, there are more regions acting as entropic springs compared to the number in partially supercontracted silk. Our model also explains the X-ray diffraction results of Grubb and Jelinski [116], who differentiated between crystalline, aligned amorphous, and isotropic components in supercontracted fibers.

Such a picture of supercontraction is consistent with previous measurements [98] including NMR experiments probing chain dynamics of various amino acids in supercontracted silk [106]. Our experiments complete this picture by elucidating the role of fibre strain in the process of supercontraction. The major new feature in our interpretation is the incorporation of abrupt strain-dependent transitions between static and mobile phases arising from cooperative reinforcement in local structure by inter- and intra-chain hydrogen bonding.

In contrast to as-drawn dragline silk, viscid silk, found in the capture spiral of the spider web, functions in a microscopically hydrated environment [121] and has mechanical properties similar to supercontracted dragline, suggesting that similar mechanisms are active in these silks. The abundance of proline in the linker regions of viscid silk [122] prevents the formation of stable extended chain structures, which, in addition to a hygroscopic glycoprotein
coating [121], allows viscid silk to maintain its entropic spring character in lower humidity. It has recently been suggested [123] that highly pitched coils act as “nanosprings” in viscid silk. We see no evidence in our data of the existence of such nanosprings in dragline. Rather our results suggest that latent and mobile entropic springs are responsible for the elastic character of silks.
Bibliography


Part III

Manuscripts: Two-Photon NMR
Chapter 7

Two-Photon Excitation in Nuclear Quadrupole Resonance

7.1 Introduction

Pulsed NQR was first demonstrated by Bloom and Norberg [124], who observed the $^{35}$Cl resonance in sodium chlorate (NaClO$_3$) both in zero external magnetic field ($B_0 = 0$) and with a small $B_0$ using single pulse and spin-echo excitation. Since then, NQR has become a powerful method for the study of structure and dynamics in solids [125, 126]. Because NQR frequencies are specific to chemical structure, NQR has also become a diagnostic tool to test for the presence of specific compounds, and has found particular application to the detection of $^{14}$N rich contraband substances such as TNT [127], RDX [127-130], and cocaine [129, 130].

Based on the work of Grechishkin [131] and related multidimensional experiments by Liao et al. [132, 133], Sauer and coworkers [134] recently demonstrated a new approach for separating the excitation and detection frequencies for some NQR experiments in order to reduce receiver recovery times associated with the high-Q probe circuits and low resonance frequencies that have limited the detection of many important quadrupolar species. They simultaneously excited a 3-level system at two of the transition frequencies and detected a signal at the frequency of the third transition (the sum or difference of the two excitation frequencies).

In a recent communication introducing two-photon nuclear magnetic resonance (NMR) noise spectroscopy [135], it was demonstrated that radio frequency (rf) excitation at half the Larmor frequency could be used to excite NMR signals, allowing for simultaneous detection on-resonance. The potential application of two-photon excitation to NQR was suggested because it promises to completely eliminate the receiver dead-time. We distinguish two-photon excitation, which directly excites transitions between quantum levels having spin quantum number difference $\Delta m = \pm 1$, from the much more common suite of techniques comprising multiple-quantum NMR, which has come to describe the probing of coherences between levels having $\Delta m \neq \pm 1$ [136], most commonly employing only on-resonance or nearly on-resonance rf.

In this letter, we investigate the application of two-photon excitation to NQR spectroscopy, and demonstrate theoretically and experimentally that two-photon NQR is pos-

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A version of this chapter has been previously published as:
sible, but, in the case of axially symmetric quadrupole interactions introduces a dramatic change in the way the experiment must be performed. Specifically, two-photon excitation results in NQR signals that cannot be detected in the excitation coil, but require a receive coil, ideally perpendicular to both the excitation coil and the quadrupole symmetry axis, for observation. The two-photon NQR experiment described here differs fundamentally from the two-frequency excitation method [134] because our excitation does not correspond to a transition frequency, rather it employs a process involving the simultaneous absorption of two photons to achieve transitions between adjacent levels.

7.2 Theory

We begin with the Hamiltonian for an axially symmetric ($\eta = 0$) quadrupole interaction in the presence of a linearly oscillating rf field:

$$\hat{H} = \hat{H}_Q + \hat{H}_{rf}$$

$$= \hbar \omega_Q \left( 3\hat{I}_z^2 - \hat{I}^2 \right) + \hbar \omega_1 \cos(\omega_{rf}t) \left( \hat{I}_z \cos \alpha + \hat{I}_x \sin \alpha \right),$$

where $\omega_Q = e^2 qQ/(4I(2I-1))$ is the strength of the quadrupole interaction, $\omega_1 = \gamma B_1$ is the strength of the rf field, $\alpha$ is the angle between the rf field and the quadrupole symmetry axis, and $\omega_{rf}$ is the frequency of the rf field. Because the quadrupolar and Zeeman Hamiltonians commute, for $\eta = 0$, the familiar Zeeman basis can be used with the nuclear magnetic moment quantized along the symmetry axis of the quadrupolar interaction.

Transforming into an interaction representation with a unitary operator given by $\hat{U} = \hat{U}_1 \hat{U}_2 = \exp \left( iA(t)\hat{I}_z \right) \exp \left( iB(t)(3\hat{I}_z^2 - \hat{I}^2) \right)$ yields an effective Hamiltonian

$$\hat{H}_{\text{eff}} = \hbar \left( \omega_Q - \frac{\partial B(t)}{\partial t} \right) \left( 3\hat{I}_z^2 - \hat{I}^2 \right) + \hbar \omega_1 \cos(\omega_{rf}t) \cos \alpha - \frac{\partial A(t)}{\partial t} \right) \hat{I}_z$$

$$+ \hbar \omega_1 \cos(\omega_{rf}t) \sin \alpha \hat{U}^{-1} \hat{I}_x \hat{U}. \quad (7.2)$$

Choosing $A(t) = \frac{\omega_1}{\omega_{rf}} \sin(\omega_{rf}t) \cos \alpha$ and $B(t) = \omega_Q t$ eliminates the longitudinal terms leaving

$$\hat{H}_{\text{eff}} = \hbar \omega_1 \cos(\omega_{rf}t) \sin \alpha \hat{U}^{-1} \hat{I}_x \hat{U}. \quad (7.3)$$

Because $\hat{I}_x$ commutes with $(3\hat{I}_z^2 - \hat{I}^2)$, the two parts of the unitary operator $\hat{U}$ can be handled independently. Transforming under $\hat{U}_1$ results in

$$\hat{U}_1^{-1} \hat{I}_x \hat{U}_1 = \hat{I}_x \cos A(t) + \hat{I}_y \sin A(t). \quad (7.4)$$

The second transformation under $\hat{U}_2$ is facilitated by expanding $\hat{I}_x$ in terms of fictitious
spin-1/2 operators [137]. Because $\hat{I}_x$ is a single quantum operator, it is sufficient to write

$$\hat{I}_x = \sum_{m=-I}^{I-1} a_m \hat{I}_x^{m-m+1}. \quad (7.5)$$

The fictitious spin-1/2 operator $\hat{I}_x^{m-m+1}$ addresses only the transition between the $m$ and $m+1$ eigenstates and will herein be written $\hat{I}_x^m$. The coefficients $a_m$ are given by the matrix elements of the $\hat{I}_x$ operator, namely $a_m = \sqrt{I(I+1) - m(m+1)}$. The unitary transformation of $\hat{I}_x$ under $\hat{U}_2$ yields

$$\hat{U}_2^{-1} \hat{I}_x \hat{U}_2 = \sum_{m=-I}^{I-1} a_m \left[ \hat{I}_x^m \cos (\omega_Q^m t) + i \hat{I}_y^m \sin (\omega_Q^m t) \right], \quad (7.6)$$

where $\omega_Q^m = 3\omega_Q(2m+1)$ is the frequency corresponding to the $m \rightarrow (m+1)$ transition. We note that $\omega_Q^m = -\omega_Q^{-(m+1)}$, so the two degenerate transitions correspond to fictitious spin-1/2 magnetizations that precess with the same frequency but in opposite directions. An analogous expression can be written for a transformation of the $\hat{I}_y$ operator.

Taking $\omega_1/\omega_{rf} \ll 1$, we keep from Eq. 7.4 only terms of leading order in $A(t)$. The effective Hamiltonian, for any axially symmetric quadrupolar interaction in zero magnetic field under rf irradiation, becomes

$$\hat{H}_{\text{eff}} = \frac{1}{2} \hbar \omega_1 \sin \alpha \sum_{\ell=\pm 1} \sum_{m=-I}^{I-1} a_m \left[ \hat{I}_x^m \cos \left( (\omega_Q^m + \ell \omega_{rf})t \right) + \hat{I}_y^m \sin \left( (\omega_Q^m + \ell \omega_{rf})t \right) \right] + \frac{\omega_1}{2 \omega_{rf}} \cos \alpha \left\{ \hat{I}_y^m \sin \left( (\omega_Q^m + 2\ell \omega_{rf})t \right) + \ell \hat{I}_x^m \cos \left( (\omega_Q^m + 2\ell \omega_{rf})t \right) \right\}. \quad (7.7)$$

If $\omega_{rf}$ is in the vicinity of one of the transition frequencies (i.e. $\omega_{rf} \approx \omega_Q^n/n$ for integer $n > 2$), higher order terms in $A(t)$ that have been dropped from Eq. 7.7 will be significant. This Hamiltonian has stationary terms when $\omega_{rf} = |\omega_Q^n|$ (on-resonance excitation) and when $\omega_{rf} = |\omega_Q^n|/2$ (two-photon excitation).

For $\omega_{rf} = |\omega_Q^n|$, the time averaged Hamiltonian is

$$\hat{H}^{(0)} = \frac{a_m}{2} \hbar \omega_1 \sin \alpha \left( \hat{I}_x^m + \hat{I}_x^{-(m+1)} \right). \quad (7.8)$$

Thus irradiation at a frequency corresponding to the $\pm m \rightarrow \pm (m+1)$ transition excites only off-diagonal terms in the density matrix connecting the $m \rightarrow m+1$ and $-m \rightarrow -(m+1)$ transitions. We may then treat the two degenerate transitions as independent spin-1/2 systems, an approach used by Bloom and Herzog for their description of an axially symmetric spin-3/2 system [138], and generalized by Goldman [139] and Odin [140] for $\eta \neq 0$. Fig. 7.1(a-c) illustrates the evolution of the density matrix in Liouville space during and after an on-
Figure 7.1: A degenerate pair of transitions of an axially symmetric quadrupole interaction, selectively excited by an applied rf field, is analogous to two independent spin-1/2 systems. The cartoon depicts density matrix elements corresponding to these two transitions as vectors in Liouville space, following them as they evolve during and just after an rf pulse produced by a coil whose axis lies in the \(x-z\) plane. (a) Before the pulse, the system is in thermal equilibrium, \(\rho_{\text{eq}} \propto \hat{I}_z^{1/2} - \hat{I}_z^{-3/2}\). During the pulse, the fictitious spin-1/2 systems nutate about the \(x\) axis in the interaction frame: in the same direction for the on-resonance case (b) and in opposite directions for the two-photon case (d) according to Eqs. 7.8 and 7.9 respectively. Following the rf pulse, the two systems precess in opposite directions about the quadrupole symmetry axis, and refocus to produce observable signals along the \(x\) axis in the on-resonance case (c) or along the \(y\) axis for the two-photon case (e).
Chapter 7. Two-Photon Excitation in Nuclear Quadrupole Resonance

resonance rf pulse. The observable signal occurs along the $x$ axis and no signal would be detected by a coil whose axis lay in the $y$-$z$ plane.

For rf applied at exactly half the frequency corresponding to the $\pm m \rightarrow \pm (m + 1)$ transition (i.e. $\omega_{rf} = |\omega_Q^m|/2$), the time averaged Hamiltonian is

$$\hat{H}^{(0)} = \frac{\alpha_m}{2} \left( \frac{\omega_1}{\omega_Q^m} \right) \hbar \omega_1 \sin \alpha \cos \alpha \left( \hat{I}_x^m + \hat{I}_x^{-(m+1)} \right).$$

(7.9)

This result differs from the on-resonance case (Eq. 7.8) in several ways. First, the pre-factor includes the ratio of the rf field strength to the quadrupolar coupling strength. Second, the geometric factor $\sin \alpha \cos \alpha$ vanishes when the rf is applied either along or perpendicular to the static interaction. Both of these results are analogous to features obtained in two-photon NMR [135]. Third, the sign of the fictitious spin-1/2 operator, $\hat{I}_x$, differs for the $m \rightarrow (m+1)$ and the $-m \rightarrow -(m+1)$ transitions. Thus magnetizations corresponding to the two fictitious spin-1/2 systems now nutate about the applied field in opposite directions, in contrast to the on-resonance case. The two counter-rotating components add constructively along the $y$ axis, perpendicular to the coil, but exactly cancel along the $x$ axis (Fig. 7.1(a,d,e)), necessitating a second coil for detection.

A weak static magnetic field may be used to shift the frequencies so that $\omega_Q^m = -\omega_Q^{-(m+1)}$, allowing a signal to be detected in the excitation coil. This Zeeman splitting would result in the detection of an anti-phase doublet, in contrast to the on-resonance case where a splitting of the transitions would result in an in-phase doublet.

For integer spin nuclei at sites lacking axial symmetry, the degeneracy of the $m \rightarrow m + 1$ and $-m \rightarrow -(m + 1)$ transitions is lifted [141], and in this case the two transitions may be excited independently.

For half-integer spin nuclei with $I \geq 5/2$ the case can arise where the two-photon excitation frequency for one transition is the same as the single-photon frequency for another transition. This could result in an effective Hamiltonian with both on-resonance and two-photon terms, presenting the possibility of exciting multiple transitions with a single frequency rf pulse.

7.3 Results and Discussion

We present preliminary results from two-photon NQR experiments performed on a single crystal of NaClO$_3$ in a weak magnetic field with excitation and detection on a single coil.

Spectra acquired using on-resonance and two-photon excitation are shown in Fig. 7.2 as a function of magnetic field strength. The two-photon signal appears as an anti-phase doublet, as predicted, while the on-resonance excitation always produces an in-phase signal. This allows us to exclude second harmonic from the power amplifier as the excitation source of the signals shown in Fig. 7.2b, as such an excitation source would produce a conventional on-resonance excitation. Furthermore, as the strength of the static field decreases, the intensity
Figure 7.2: $^{35}$Cl spectra, acquired at 29.041 MHz, of single-crystal NaClO₃ in an external magnetic field of varying strength obtained after (a) on-resonance and (b) two-photon excitation. The insets show the integrated signal intensity. In (b), the spectra were multiplied by $\omega/|\omega|$ before integration. All spectra were acquired on a Unity Inova NMR spectrometer (Varian Inc, Palo Alto, CA) using a home-built single-coil double-resonant probe and home-built 15 MHz low-pass and 30 MHz bandpass external filters. The magnetic field is the stray field of an actively shielded 9.4 T NMR magnet (Oxford Instruments, Oxford, UK), measured with a model 4048 Gaussmeter (F.W. Bell, Orlando, FL). The spectra and integrals have been scaled by the number of repetitions (32 for each spectrum in (a), and 25000, 1120, 1304, 1276 from top to bottom in (b)) and to compensate approximately for the difference in tip angle (produced by 50 $\mu$s rf pulses with field strengths of $\sim$10 kHz in (a) and $\sim$90 kHz in (b)).
of the two-photon peaks decrease, as shown in the inset, while for the on-resonance excitation it remains approximately constant. At zero field, we expect to see no two-photon NQR signal when exciting and detecting in a single coil.

The reduced signal to noise ratio of the spectra in Fig. 7.2b is a result of a small tip angle (\( \sim 2^\circ \)) due to the suppression of the Hamiltonian by a factor \( \omega_1/\omega_Q \) compared to on-resonance excitation. This is a general feature of two-photon excitation [135]. However, the decrease in nutation rate compared to on-resonance excitation may be less severe in NQR than in NMR because the quadrupolar frequency is typically much less than the Larmor frequency. For quadrupolar couplings of 10 MHz, and typical rf fields of 100 kHz, the two-photon Hamiltonian is still reduced by a factor of \( \sim 10^2 \) from the on-resonance case. As a result, significant increases in rf power or very long pulses are required to significantly tip the magnetization. Stochastic excitation [142] may be employed to overcome this limitation as was demonstrated [135] for NMR. The application of stochastic excitation to on-resonance NQR suffers from a limitation on the size of the spectral window due to receiver dead-times.

A complication of two-photon excitation for axially symmetric quadrupole interactions results from sample symmetry considerations. The signal from a given crystal site will appear along a direction perpendicular to both the excitation coil axis and the symmetry axis of the quadrupole interaction. For our NaClO\(_3\) crystal, it may be shown by summing the signals produced from the four crystal sites that in the absence of a static magnetic field, the signals add destructively. Thus no observable two-photon signal can be produced. This same consideration leads to the conclusion that two-photon excitation of an observable signal from a powder sample having \( \eta = 0 \) will not be possible in zero field. The introduction of a magnetic field breaks the sample symmetry. Because only the secular component of the Zeeman term (i.e. the component parallel to the quadrupolar symmetry axis) is retained in a perturbation treatment, each crystal site in the sample will, in general, experience a different Zeeman splitting. This is reflected by the broadening of the peaks in Fig. 7.2 with increasing field strength.

Like the two-frequency excitation method [134], our two-photon experiment offers the advantage over on-resonance excitation of eliminating magnetoacoustic and piezoelectric ringing artifacts [143]. Such ringing can be a serious problem for contraband detection if the acquisition and detection frequencies coincide. Both techniques have the potential to reduce or eliminate receiver deadtime arising from probe ring-down, because neither employs on-resonance excitation. One related difference between the techniques is that nonlinearities in the dielectric response of probe construction materials and circuit components can mix the two high-power rf excitation signals of the two frequency method to produce on-resonance rf, but as long as such responses are symmetric, on-resonance rf will not be produced in the two-photon technique.

The two photon technique offers the advantages that it is not restricted to \( \eta \neq 0 \) and hardware requirements are somewhat simplified as irradiation is required at only one frequency.

The two-photon method suffers the disadvantages of increased excitation power requirements, and, for systems with \( \eta = 0 \) a second coil or static magnetic field is required. A
circularly polarized rf excitation [143] provides another option for breaking the symmetry to produce observable signals. The excitation power requirements will likely prohibit the use of the method for large volume coils, as required for contraband detection, but are feasible for laboratory scale experiments.

Three or more photon excitation is also possible. As mentioned, the higher order terms in \( A(t) \) responsible for these multi-photon processes have been discarded from Eq. 7.7. The efficiency of such excitation decreases by an additional factor of \( \omega_1/\omega_Q^m \) for each additional photon, severely decreasing the effective nutation frequency. Additionally, the broadband rf power amplifiers often used for NMR and NQR tend to produce large quantities of odd harmonics, that if not adequately filtered, would mask any multi-photon excitation. Even with sufficient filtering or use of narrow-band amplifiers, non-linearities in the dielectric response of probe components can generate odd harmonics of the excitation rf at unacceptably high levels, making such higher-order excitation experimentally difficult.

7.4 Conclusions and Future Work

We have theoretically and experimentally demonstrated a new technique for exciting NQR signals without the use of an on-resonance rf field. This two-photon excitation scheme offers several advantages over other methods, most notably the potential to completely eliminate the receiver dead-time. The method does suffer from the requirement that for axially symmetric quadrupole interactions, a weak static magnetic field is required to observe signals from poly-crystalline samples or single crystals of sufficient symmetry.

A more comprehensive theoretical and experimental treatment of two-photon NQR, including detailed discussion of the cases \( \eta \neq 0 \), and \( B_0 \neq 0 \) is currently underway, and will be published elsewhere, along with an implementation of stochastic excitation with two-photon excitation.
Bibliography


Chapter 8

Two-Photon Two-Color Nuclear Magnetic Resonance

8.1 Introduction

Pulsed NMR has traditionally been performed in two distinct stages [144]: first, nuclear spins are excited by some sequence of radio-frequency (rf) pulses, and second, NMR signals are detected as a free induction decay. With the introduction of stroboscopic detection techniques such as CPMG [145] and WaHuHa [146], the two stages may be interleaved but still require the receiver to be turned off during excitation. In practice, probe ringdown and transients from receiver gates impose a receiver deadtime [147] that can, in unfavorable cases, last longer than the free induction signal, but more commonly results in distortions to the desired spectra. Recently, it was demonstrated that receiver deadtime can be entirely eliminated by exciting nuclear spins with an rf field at half the transition frequency, allowing NMR signals to be acquired during excitation [148]. This excitation was denoted "two-photon" in analogy to the simultaneous absorption of two optical photons.

While nonlinear processes form the basis for a wide variety of important optical techniques and devices, similar effects in NMR, for example two-photon excitation and the Bloch-Siegert [149] shift, are either little-known or viewed as artifacts, and consequently form the basis for few if any common experimental techniques. Even so, multi-photon excitation of magnetic resonance transitions has been known for many years [150, 151], and encompasses a rich variety of processes. These processes may be divided into two broad types, the first involving the absorption of two or more near resonance photons to directly excite transitions between nuclear spin levels with spin quantum number difference \( \Delta m \neq 1 \), as observed in early cw NMR experiments [152], while the second type involves the excitation of transitions between levels having \( \Delta m = \pm 1 \) by a multi-photon process. Of this second type, cases with multiple rf fields near the ordinary resonance frequency have been considered, both in cw [153] and in pulsed NMR [154], while excitation at a submultiple of the resonance frequency has been discussed by Abragam [155] and Shirley [156].

We distinguish multi-photon excitation from the much more commonly-used suite of techniques comprising multiple-quantum NMR [157], where single-photon processes are used to indirectly excite and detect coherences between nuclear spin levels with \( |\Delta m| > 1 \).

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Our laboratory has recently demonstrated two-photon excitation of NMR [148] and NQR [158] transitions, and shown that such two-photon excitation may be effectively combined [148] with noise spectroscopy (stochastic excitation) [159, 160]. A principle drawback of two-photon excitation is the relatively low nutation rates produced, requiring either very large applied rf fields or long pulses to produce appreciable tip angles.

In this work, we consider two-photon excitation theoretically using average Hamiltonian theory (AHT) [161] and generalize to include excitation with two rf fields at different frequencies. We identify two-photon excitation with the first order term in the Magnus expansion, and, in solving the case for a single spin, show that the excitation occurs if the frequencies of the two applied rf fields sum to or differ by the transition frequency. Significantly, the rate of nutation increases as the two frequencies deviate from half resonance. We demonstrate this so-called two-color excitation on \( ^1\text{H} \) nuclear spins in water excited at 30 and 370 MHz with detection at 400 MHz, and close with a discussion of some practical considerations for two-color excitation along with potential opportunities for non-linear phenomena in NMR.

8.2 Theory

8.2.1 A General Approach to Two-Photon Excitation from First Order Average Hamiltonian Theory

The Hamiltonian of a nuclear spin under rf irradiation can be written

\[
\hat{\mathcal{H}}(t) = \hat{\mathcal{H}}_{\text{static}} + \gamma \vec{B}_1 \cdot \vec{I} \cos \omega_{\text{rf}} t,
\]

where \( \hat{\mathcal{H}}_{\text{static}} \) is due to some static interaction such as the Zeeman interaction, \( \gamma \) is the gyromagnetic ratio of the nucleus of interest, \( \vec{B}_1 \) specifies the amplitude and direction of the applied rf field, \( \omega_{\text{rf}} \) is the angular frequency of the rf, and \( \vec{I} \) is the spin's angular momentum operator.

Diagonalizing the static Hamiltonian with a change of basis represented by the matrix \( \mathbf{A} \) (yielding the eigenvalues \( \omega^i \)) and transforming into an interaction representation via \( \hat{U} = \exp(-i(\mathbf{A}^{-1}\hat{\mathcal{H}}_{\text{static}}\mathbf{A})t) \) removes the effect of the static interaction leaving

\[
\hat{\mathcal{H}}_{\text{eff}}(t) = \hat{U}^{\dagger} \mathbf{A}^{-1} \left( \gamma \vec{B}_1 \cdot \vec{I} \right) \mathbf{A} \hat{U} \cos \omega_{\text{rf}} t.
\]

The element in the \( i^{th} \) row and \( j^{th} \) column of \( \hat{\mathcal{H}}_{\text{eff}} \) has the following time dependence:

\[
\left( \hat{\mathcal{H}}_{\text{eff}}(t) \right)_{ij} = \left( \hat{\mathcal{H}}_{\text{eff}}(0) \right)_{ij} e^{i(\omega^i - \omega^j)t} \cos \omega_{\text{rf}} t.
\]

For \( i = j \), the only time dependence is in the cosine, while for \( i \neq j \), there is a time dependence corresponding to the transition frequency between the \( i^{th} \) and \( j^{th} \) eigenstates. Thus
the effective Hamiltonian can be decomposed into terms according to their time dependences:

$$\hat{H}_{\text{eff}}(t) = \left( \hat{H}^0 + \sum_n \hat{H}^n e^{i\omega_n t} \right) \cos \omega_{\text{rf}} t,$$

where we sum over the transitions, labeled by the index $n$, having frequencies $\omega_n$.

From AHT, the leading order effect of a time-dependent Hamiltonian is given by the first non-zero term in the Magnus expansion, where the zeroth and first order terms are given by [162]

$$\hat{H}^{(0)} = \frac{1}{t} \int_0^t dt' \hat{H}_{\text{eff}}(t'),$$

$$\hat{H}^{(1)} = -\frac{i}{2t} \int_0^t dt' \int_0^{t'} dt'' [\hat{H}_{\text{eff}}(t'), \hat{H}_{\text{eff}}(t'')].$$

Although AHT is more commonly applied to systems evolving under cyclic Hamiltonians [161], it is valid here as long as all time-dependent terms in the integrand vary rapidly compared to the time-scale of spin evolution in the interaction frame. The integrals of Eqs. 8.5 and 8.6 then display brief transients, but rapidly converge to steady-state values. For convenience we evaluate the integrals in the limit $t \to \infty$.

From the orthogonality of trigonometric functions under integration over time, it is clear from Eqs. 8.4 and 8.5 that the zeroth order term will vanish unless the rf is applied on-resonance for one of the transition frequencies ($\omega_{\text{rf1}} = \omega_n$), in which case it becomes $\hat{H}^{(0)} = \hat{H}^n/2$.

For two-photon excitation of the $n$th transition at half the transition frequency, $\omega_{\text{rf}} = \omega_n/2$, the zeroth order Hamiltonian will always vanish. However, there will be a first order contribution that survives due to cross terms between $\hat{H}^0$ and $\hat{H}^n$ (i.e. integrands of the form $e^{i\omega_n t'} \cos \omega_{\text{rf1}} t' \cos \omega_{\text{rf2}} t''$). It is exactly these cross terms that result in two-photon excitation for $\omega_{\text{rf}} = \omega_n/2$.

It is straightforward to extend the above argument to two-photon excitation with two rf fields whose frequencies sum to the resonant frequency ($\omega_{\text{rf1}} + \omega_{\text{rf2}} = \omega_n$). Cross terms inside the integral of Eq. 8.6 of the form $e^{i\omega_n t'} \cos \omega_{\text{rf1}} t' \cos \omega_{\text{rf2}} t''$ that do not vanish after integration lead to two-photon, two-color excitation. Higher order terms of the Magnus expansion will lead in a similar fashion to three and greater photon excitation.

### 8.2.2 Explicit Calculation for Two-Color excitation in NMR

We consider an isolated spin in an external magnetic field of strength $B_0 = \omega_0/\gamma$ in the presence of two colinear rf fields, as would be the case in a doubly tuned rf coil, applied at an angle $\alpha$ to the external field. We define the $z$-axis to lie along the static field and choose the $x$-axis so that the rf fields lie in the $x$-$z$ plane. With the two rf fields having respective strengths $B_1$ and $B_2$, phases $\phi_1$ and $\phi_2$, and frequencies $\omega_{\text{rf1}} = (\omega_0 - \delta)/2$ and
\( \omega_{r2} = (\omega_0 + \delta)/2 \), so that \( \omega_{r1} + \omega_{r2} = \omega_0 \), the Hamiltonian may be written

\[
\hat{H}(t) = \omega_0 \hat{I}_z + \gamma B_1 \left( \hat{I}_x \sin \alpha + \hat{I}_z \cos \alpha \right) \cos(\omega_{r1}t + \phi_1) \\
+ \gamma B_2 \left( \hat{I}_x \sin \alpha + \hat{I}_z \cos \alpha \right) \cos(\omega_{r2}t + \phi_2).
\] (8.7)

Transforming into an interaction frame with \( \hat{U} = e^{i\omega_0 t \hat{I}_z} \), the effective Hamiltonian becomes

\[
\hat{H}_{\text{eff}}(t) = \gamma B_1 \left[ (\hat{I}_x \cos \omega_0 t + \hat{I}_y \sin \omega_0 t) \sin \alpha + \hat{I}_z \cos \alpha \right] \cos(\omega_{r1}t + \phi_1) \\
+ \gamma B_2 \left[ (\hat{I}_x \cos \omega_0 t + \hat{I}_y \sin \omega_0 t) \sin \alpha + \hat{I}_z \cos \alpha \right] \cos(\omega_{r2}t + \phi_2).
\] (8.8)

Using the commutation relations of spin operators and the double integrals over trigonometric functions summarized in Table 8.1, the two-color excitation Hamiltonian may be calculated from Eq. 8.6 yielding

\[
\hat{H}^{(1)} = \omega_{\text{nut}} \left( \hat{I}_x \cos(\phi_1 + \phi_2) - \hat{I}_y \sin(\phi_1 + \phi_2) \right) + (\Delta \omega_1^{BS} + \Delta \omega_2^{BS}) \hat{I}_z
\] (8.9)

where

\[
\omega_{\text{nut}} = \left( \frac{\gamma B_1 \sin \alpha}{2 \omega_1} \right) \frac{1}{2} \gamma B_2 \cos \alpha + \left( \frac{\gamma B_2 \sin \alpha}{2 \omega_2} \right) \frac{1}{2} \gamma B_1 \cos \alpha \\
= \frac{\gamma B_1 B_2 \sin 2\alpha}{2 B_0 \left( 1 - \left( \frac{\delta}{\omega_0} \right)^2 \right)}
\] (8.10)

and

\[
\Delta \omega_i^{BS} = \frac{-\gamma B_i^2 \sin^2 \alpha}{4 B_0 \left( 1 - \left( \frac{\omega_{\text{nut}}}{\omega_0} \right)^2 \right)}.
\] (8.11)

The first term in Eq. 8.9 results in nutation at an angular frequency \( \omega_{\text{nut}} \), while the second is responsible for a frequency shift, known as the Bloch-Siegert (BS) shift [149], and is the sum of two shifts, one due to each of the rf fields. As a consequence of the presence of both \( B_1 \) and \( B_2 \) in Eq. 8.10, the nutation occurs only if both rf fields are present, while each of the shifts of Eq. 8.11 persists if only its respective rf field is applied. The \( \alpha \) dependence of Eq. 8.10 reveals that two-photon excitation occurs only if the coil has components both parallel and perpendicular to \( B_0 \). This requirement reflects the fact that two-photon transitions between levels with \( |\Delta m| = 1 \) require one transverse (\( \sigma^+ \) or \( \sigma^- \)) and one longitudinal (\( \pi \)) photon in order to conserve angular momentum [163]. The first line in equation 8.10 is written to emphasize the absorption of two photons. The factors in brackets are due to \( \sigma^\pm \) photons while those outside the brackets are due to \( \pi \) photons. The first term corresponds to absorption of a \( \sigma^\pm \) photon at \( \omega_1 \) and a \( \pi \) photon at \( \omega_2 \), while in the second term the frequencies of the
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\[ CQs(u_{ril} + \phi_1) \cos \omega_0 t'' \sin \omega_0 t'' \]
\[ \cos \omega_0 t' \] \[ \sin \omega_0 t' \] \[ 1 \]
\[ \cos (\omega_{rt1} t' + \phi_1) \]
\[ \cos (\omega_{rt2} t' + \phi_2) \]
\[ \cos (\omega_{rt1} t' + \phi_1) \cos \omega_0 t'' \] \[ \cos \omega_0 t'' \]
\[ \sin \omega_0 t'' \]
\[ 1 \]
\[ \cos (\omega_{rt2} t' + \phi_2) \cos \omega_0 t'' \] \[ \cos \omega_0 t'' \]
\[ \sin \omega_0 t'' \]
\[ 1 \]

Table 8.1: Integrals for Eq. 8.6 for non-integer \( \delta/\omega_0 \). Each entry in the table is the integral \( \frac{x}{2t} \int_0^t dt' \int_0^{t'} dt'' \) over an integrand that is the product of the entries in the first two columns and entries in the first two rows of the table. Only one half of the table is presented here, the other half is obtained by exchanging \( \omega_{rt1} \) and \( \omega_{rt2} \), and \( \phi_1 \) and \( \phi_2 \).

Table 8.2: Integrals \( \frac{x}{2t} \int_0^t dt' \int_0^{t'} dt'' \) for \( \delta = 0 \), i.e. \( \omega_{rt1} = \omega_{rt2} = \omega_0/2 = \omega_r \), interpreted in a similar fashion to Table 8.1.
two photons are reversed. For an experimental setup in which one rf field is applied along $z$, while the other is applied along $x$, only one of the two terms would be present.

In what follows, we take $\omega_0$ to be the receiver reference frequency. In the presence of a resonance offset, where $\omega_0$ is not exactly the nuclear resonance frequency, the offset adds in a straightforward fashion to the BS shifts of Eq. 8.9.

Eq. 8.10 reveals that the nutation frequency increases as $\delta$, the difference between the two excitation frequencies, increases, as is depicted in Fig. 8.1. In fact the nutation rate appears to diverge at $\delta = \omega_0$. Our AHT treatment breaks down when terms in the integrand of Eq. 8.6 vary slowly compared to the spin evolution time. This is certainly the case in the vicinity of $\delta = \omega_0$, and in fact near $\delta/\omega_0 = m$ for any integer $m$. The region of $\delta$ around $m\omega_0$ in which Eq. 8.9 can not be applied is given roughly by the larger of the rf amplitudes multiplied by $\gamma$. Except for the cases of $m = 1$ and 3 where on-resonance excitation (by $\omega_{rf1}$ and $\omega_{rf2}$ respectively) dominates, our approach is again valid exactly at $\delta/\omega_0 = m$, but the results of the integration of Eq. 8.6 in Table 8.1 no longer apply, and the integrals must be reevaluated for these special cases.
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If both rf fields are applied at $\omega_0/2$ ($\delta = 0$), the cross terms surviving integration are displayed in Table 8.2, yielding

$$\mathcal{H}^{(1)} = \frac{\gamma \sin 2\alpha}{4B_0} \left( (B_1^2 \cos 2\phi_1 + B_2^2 \cos 2\phi_2 + 2B_1B_2 \cos(\phi_1 + \phi_2)) \hat{I}_x ight.$$

$$- (B_1^2 \sin 2\phi_1 + B_2^2 \sin 2\phi_2 + 2B_1B_2 \sin(\phi_1 + \phi_2)) \hat{I}_y \bigg)$$

$$- \frac{\gamma \sin^2 \alpha}{3B_0} \left( B_1^2 + B_2^2 + 2B_1B_2 \cos(\phi_1 - \phi_2) \right) \hat{I}_z. \quad (8.12)$$

Depending on the relative phases and strengths of the two rf fields, the nutation rate varies between 0 and twice that given by Eq. 8.10. In practice, for irradiation at $\omega_0/2$, typically only one rf field is applied, reducing the nutation rate by a factor of 4. In this case we recover the previously derived result [148] for two-photon excitation:

$$\mathcal{H}^{(1)} = \frac{\gamma B_0^2 \sin 2\alpha}{4B_0} (\hat{I}_x \cos 2\phi - \hat{I}_y \sin 2\phi) - 2B_0^2 \sin^2 \alpha \hat{I}_z. \quad (8.13)$$

For $\delta > \omega_0$, $\omega_{rf2}$ becomes negative, and resonance corresponds to the difference frequency, $\omega_{rf1} - |\omega_{rf2}|$. For $1 < \delta/\omega_0 < 3$, one of the excitation frequencies is above resonance and the other below. For the special condition $\delta/\omega_0 = 2$ ($\omega_{rf1} = -\omega_0/2$ and $\omega_{rf2} = 3\omega_0/2$) both two-color and half-frequency excitation will occur. Our present approach remains valid, but requires a recalculation of the integrals in Table 8.1. Beyond $\delta/\omega_0 > 3$, the absolute values of both excitation frequencies are above resonance.

8.3 Experimental Setup

Two-color excited $^1$H spectra were acquired from a sample of H$_2$O in a sealed 4 mm magic-angle-spinning (MAS) rotor. Spectra were obtained on a Varian $^{UNITTY}$ INOVA 400 spectrometer with a Varian Chemagnetics HFX MAS probe tuned to 30, 370 and 400 MHz. This MAS probe was chosen not only for its triple-resonance tuning, but also for the oblique angle the rf coil makes with the external field, providing $\alpha = 54.7^\circ$. Spectra were acquired, without MAS, on the 400 MHz channel with irradiation on the 30 and 370 MHz channels. The lower excitation frequency, $\omega_{rf1}/2\pi$ was fixed at 30.00 MHz, while the exact value of $\omega_{rf2}/2\pi$ (369.74 MHz) was set to the difference between the desired receiver reference (399.74 MHz) and $\omega_{rf1}/2\pi$. A 400 MHz selective band-pass filter was used on the detection channel to minimize 30 and 370 MHz rf feedthrough to the receiver.

All spectra were obtained with excitation powers of 600 and 300 W on the 30 and 370 MHz channels respectively unless otherwise noted. Each spectrum was obtained with a single scan, although phase coherence between transmitter and receiver channels did allow for signal averaging.

Calibrations of the off-resonance field strengths were performed on $^{14}$N and $^{19}$F using
acetonitrile and poly(tetrafluoroethylene) samples with the probe re-tuned to 28.88 and 376.08 MHz.

Adiabatic passage was performed with an rf frequency sweep on the 30 MHz channel, implemented as an accelerating phase shift to obtain an 8 kHz sweep width centered about the proton resonance line. Phase steps were taken every 20 µs during the 50 ms detection time with acquisition of a complex point every 20 µs.

8.4 Results

Fig. 8.2A shows the time-domain signal acquired during nutation due to two-color excitation. The trace begins when the receiver and one of the rf channels are turned on. Nutation begins and a non-zero signal is observed when the second rf channel is turned on 120 ps later. An identical plot is obtained if the order in which the rf channels are turned on is reversed.

To obtain an accurate measure of \( \omega_{\text{nut}} \), the rf frequencies were selected to sum to the ordinary resonance frequency plus the BS shift \( \omega_{r1} + \omega_{r2} = \omega_0 + \Delta \omega_1^{\text{BS}} + \Delta \omega_2^{\text{BS}} \) so that only the \( \hat{I}_x \) term in Eq. 8.9 is responsible for the observed nutation.

The contributions to the BS shift of the two rf fields may be measured by allowing the magnetization to evolve with no rf fields present (Fig. 8.2B), with only the 370 MHz rf present (Fig. 8.2C), or with only the 30 MHz rf present (Fig. 8.2D). We find shifts of \( \Delta \omega_1^{\text{BS}}/2\pi = 1220 \text{ Hz} \) and \( \Delta \omega_2^{\text{BS}}/2\pi = 150 \text{ Hz} \) for the 30 and 370 MHz channels respectively. From these values, using Eq. 8.11, we calculate respective rf field strengths of \( \gamma B_1/2\pi = 1710 \text{ kHz} \) and \( \gamma B_2/2\pi = 228 \text{ kHz} \) (expressed in units of proton frequency), and, using Eq. 8.10, a nutation frequency of 1.65 kHz which agrees well with the 1.71 kHz that may be measured directly from Fig. 8.2A.

The rf field strengths calculated from the BS shifts also compare well with the directly measured values of 1630 kHz and 190 kHz obtained from calibrations on \( ^{14}\text{N} \) and \( ^{19}\text{F} \). The expected nutation frequency from the calibrations is 1.33 kHz. The differences between these and the directly measured values can be attributed to differences in probe and amplifier performance at the different frequencies used for the experiments and calibrations.

Potential mixing of the 30 MHz and 370 MHz rf’s by non-linearities in passive probe components was investigated by coupling the probe’s 400 MHz channel directly into an oscilloscope during continuous irradiation on both the 30 and 370 MHz channels. No detectable 400 MHz rf was observed, whereas the level of on-resonance rf excitation required to achieve a comparable nutation frequency was more than three orders of magnitude (in voltage) greater than the detection limit using the oscilloscope. This result, in concert with the fact that we were able to detect an NMR signal during excitation without a DC offset as would be expected from any rf mixing, leads us to believe that the signal we observe is due entirely to two-photon excitation.

Fig. 8.3 depicts a series of two-photon-excited water spectra that display a linear increase in amplitude with increasing rf field strength for each channel separately (Figs. 8.3A and 8.3B) as expected from Eq. 8.9. When the rf field strengths are ramped simultaneously, the amplitude of the observed signal increases quadratically (Fig. 8.3C). These data highlight
Figure 8.2: Two-color excitation of $^1$H spins in H$_2$O. (A) Direct observation of nutation. The transmitter frequencies were chosen to be exactly on resonance, including the BS shifts, during irradiation. (B) Excitation followed by free precession. (C) and (D) Excitation followed by precession with continued irradiation at 30 MHz (C) or 370 MHz (D). The grey bars indicate the times during which the rf channels were turned on. The panels on the right are projections of the signals in the complex plane. The precession frequencies displayed following excitation in (B), (C), and (D) are due to the BS shift terms of Eq. 8.11.
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Figure 8.3: $^1$H spectra of $\text{H}_2\text{O}$ obtained following two-color excitation with a fixed length (10 $\mu$s) pulse and an increasing rf field strength on (A) the 370 MHz channel and (B) the 30 MHz channel and (C) both channels simultaneously. The strongest pulses correspond to a tip angle of 0.3°. The dashed lines are linear (A) and (B) or quadratic (C) guides to the eye.

The evolution of the nuclear magnetization during an adiabatic fast passage is displayed in Fig. 8.4A. One rf channel remains purely sinusoidal while the other is swept through the two-color resonance condition. Typically, adiabatic fast passage experiments are visualized in a frame rotating with the excitation field, in which case magnetization follows the effective field from the positive to negative $z$-axis in the $x$-$z$ plane [164]. Here, we detect in a frame rotating at constant frequency (on-resonance) so that we observe a signal whose frequency decreases as resonance is approached, becoming zero on resonance and changing sign after resonance is passed. Due to the BS shift, the center of the observed peak does not fall in the center of the sweep and we observe the peak move towards the center as the rf power is decreased. In Fig. 8.4B, by reducing the rf field strength, we have entered the linear fast passage regime [164] in which some of the magnetization is left in the transverse plane.
Figure 8.4: Observation of frequency sweeps through resonance. The data, depicted here in a frame rotating at a constant 400 MHz, are plotted to show the transverse magnetization in a plane perpendicular to the time axis. The magnitude of the transverse magnetization is shown above each data set. In (A), the excitation powers were 600 and 300 W on the 30 and 370 MHz channels while in (B) the power at 30 MHz was reduced to 10 W.
resulting in an asymmetric peak consisting of the swept peak plus a transient decay. By stopping midway through an adiabatic fast passage, or by optimizing the effective field strength and sweep rate for a linear fast passage (known as a chirped pulse [165]), one can, in principle, achieve \( \pi/2 \) pulses with very small rf field strengths.

8.5 Discussion

8.5.1 Advantages and Disadvantages of Two-Color Excitation

While retaining the novel features resulting from the separation of excitation and detection frequencies that were introduced with half-frequency excitation, two-color excitation offers several further advantages. The most prominent of these is an improved excitation strength that increases as the difference between the two rf frequencies increases (Fig. 8.1). At the frequencies used here \( (\delta/\omega_0 = 0.85) \), Eq. 8.10 gives a 3.5-fold improvement in nutation frequency compared to excitation at \( \omega_0/2 \) assuming the same total power applied to the probe, neglecting variations in probe performance as a function of frequency. Further substantial improvements are possible with a modest increase in \( \delta \). Along with the limits to the validity of Eq. 8.9 set by the proximity of \( \delta \) to \( m\omega_0 \) compared to the rf amplitudes discussed above, the practical difficulties associated with applying rf close to resonance while maintaining acceptable isolation between transmission and reception channels may ultimately limit the range of accessible \( \delta \).

A second advantage of two-color excitation over excitation at \( \omega_0/2 \) is the fact that the fidelity requirements of the rf fields are greatly reduced. Harmonic output of rf power amplifiers will not generally contribute to any excitation nor interfere with detection, except for unfortunate choices of \( \delta \) (for example where \( \delta/\omega_0 = 2/3 \) so that \( \omega_{rft} = \omega_0/3 \) that are easily avoided. This greatly relaxes the need for high power rf band-pass filters on the excitation channels. Provided that any non-linearities in the electrical response of probe components are symmetric with respect to the sign of the voltage, such non-linearities will not mix the two excitation frequencies to produce \( \omega_0 \) in the absence of a DC offset.

The two-color technique can be performed on most commercial spectrometers with no special hardware or software modification aside from retuning of an MAS probe. It does however, require three rf synthesizers operating from the same reference clock.

Two-color excitation suffers from the disadvantage of requiring a triply-tuned probe in order to observe signals from a single nuclear species. Our implementation also employed two rf transmitters and power amplifiers; however, it may be possible in some cases to amplify the two rf excitation signals in the same power amplifier and couple them to the probe on a single port.

8.5.2 Optimizations

In order to achieve the maximum \( \omega_{\text{nut}} \) possible, it is clear from Eq. 8.10 that \( B_1 \) and \( B_2 \) should be as large as possible, and \( \delta \) should be as close to \( \omega_0 \) as possible. The restriction
that the rf amplitudes remain small compared to |δ − ω₀| (as δ approaches ω₀) for Eq. 8.9 to apply, implies a trade-off: as B₁ and B₂ are increased, the required separation between δ and ω₀ increases, attenuating the boost to ωₙₜᵤₓ provided by the denominator of Eq. 8.10. At some point as B₁ and B₂ are increased, the limits they place on δ actually cause a decrease in ωₙₜᵤₓ. This limit is not reached, however, until the rf fields are a sizable fraction of B₀.

A more likely limitation on the field strengths may be placed by the probe’s power handling capability, and we consider the optimal distribution of the total rf power (Pₜ) between the two rf channels. The rf field produced in an NMR coil by an rf pulse having power Pᵢ is

\[ Bᵢ = k \sqrt{Pᵢ/R(ωᵢ)} \]

where k is a constant of proportionality fixed for a given probe and R(ω) is the resistance of the coil, generally a complicated function of frequency [166]. Substituting this relation into Eq. 8.10 we find

\[ ωₙₜᵤₓ \propto kPₜ \sqrt{\frac{r₁(1-r₁)}{R(ωᵢ₁)R(ωᵢ₂)}} \]  

(8.14)

where Pₜ = P₁ + P₂ is the total rf power and r₁ = Pᵢ / Pₜ is the fraction applied at ωᵢ₁. The optimal choice of r₁ is clearly given by 1/2, independent of the choice of frequencies and the functional form of R(ω), and corresponds to equal power applied to the two channels. In our experiments with a value of r₁ = 0.67, we achieved 94% of the optimal efficiency, based on limiting the input power to the probe at 900 W.

### 8.5.3 Opportunities for Non-Linear Processes in NMR

Despite the increase in ωₙₜᵤₓ afforded by two-color excitation over half-frequency excitation, the nutation frequencies produced remain small compared to those required for many potential applications. Ongoing developments in the use of microcoils, with which significantly stronger rf fields may be produced [167–170], may provide opportunities for practical applications for two-photon excitation and other non-linear effects in NMR.

Any case where minimizing the receiver dead-time is important would be a clear candidate for two-photon excitation. An example is wide-line NMR, especially in the presence of molecular motion, where echo techniques to refocus rapidly decaying NMR signals are ineffective. Clearly, for broadband excitation, an adequate effective field strength is a key requirement.

The potentially important application of simultaneous homonuclear decoupling and detection [148] that is currently being pursued using microcoil probes elsewhere [167] may also benefit from the boost in nutation frequency provided by two-color excitation.

Two-color excitation also offers the opportunity for more exotic, though potentially less practical manipulation and detection of spin systems. The excitation condition, ωᵢ₁ + ωᵢ₂ = ω₀, leaves one degree of freedom and allows the selection of one of the frequencies to correspond to another nuclear resonance frequency, enabling the simultaneous excitation of two nuclear species with a triply tuned probe. This may be useful for example to decouple one nucleus while simultaneously exciting another with a two-photon process. In a similar
arrangement, with careful adjustment of rf amplitudes, one could achieve a Hartmann-Hahn match between two nuclear species, with one species spin-locked directly (at $\omega_{rf1}$ or $\omega_{rf2}$) while the other is spin-locked with a two-photon field (at $\omega_{rf1} + \omega_{rf2}$).

A final proposal is motivated by a close inspection of the nutation signal in Fig. 8.2A, which reveals a decay that can not be described by a single exponential but rather has a quickly and slowly decaying component. We attribute the quickly decaying component to inhomogeneity of the rf fields, with the slowly decaying part due to a region of high homogeneity in the center of the coil. Rf inhomogeneity is magnified compared to on-resonance excitation due $\omega_{\text{mut}}$'s quadratic dependence on field strength. In principle, if the field inhomogeneity was known or deliberately non-uniform, the BS shift, under irradiation with one or more off-resonance rf fields, could be used for spatial localization without the use of a separate gradient coil. Such an implementation may have a niche for certain microimaging applications.

### 8.6 Conclusions

We have shown that two-photon excitation is a consequence of first order terms in the Magnus expansion and occurs whenever two applied rf frequencies sum to or differ by $\omega_0$. Excitation away from half the transition frequency, referred to as two-color excitation, presents significant improvements in the excitation strength as well as other practical benefits. We have demonstrated that two-color excitation is robust and amenable to current NMR spectrometer hardware and software with minimal modification. Ongoing developments of microcoil probe technology may provide opportunities for a variety of practical applications based on two-photon excitation and other non-linear effects.
Bibliography


Bibliography


Chapter 9

Crossed-Coil Detection of Two-Photon Excited Nuclear Quadrupole Resonance

9.1 Introduction

Multi-photon excitation is a general consequence of higher order perturbation theory and is well understood, typically in the context of atomic electrons coupled to a radiation field as is the case in many optics experiments [171]. In early cw optics [171], electron paramagnetic resonance (EPR) [172, 173] and nuclear magnetic resonance (NMR) [174, 175] experiments, multi-photon excitation was described by absorption cross-sections or transition probabilities calculated using time-dependent perturbation theory. Where phase sensitive excitation and detection is important, as in pulsed EPR and NMR as well as more recent coherent optics experiments [176], coherent descriptions of multi-photon excitation are necessary. Coherent multi-photon excitation has been described in a number of different formalisms: vectorially by Bloch equations [177, 178], by rotating frame transformations [179-181], by second quantization [182, 183], or by Floquet theory [184-187]. Recently, we presented a new approach [188] based on average Hamiltonian theory [189].

Practical applications of multi-photon excitation abound in optics [190] and in EPR [187] where various combinations of linearly and circularly polarized microwave and radio-frequency irradiations have been used to produce two-, three-, and four-photon excitation. Due to the inherent insensitivity of NMR and nuclear quadrupole resonance (NQR) experiments, multi-photon processes have not been widely exploited to date. Pulsed multi-photon excitation of NMR has been demonstrated experimentally [185, 186, 191] but has largely been limited to the absorption of an odd number of photons, or to the excitation of unobservable $|\Delta m| > 1$ coherences. Only recently have we demonstrated practical two-photon excitation of directly observable single-quantum coherences by applying an rf field with components both parallel and perpendicular to the static magnetic field [180, 188].

A general result of monochromatic $n$-photon excitation of a resonance line with a transition frequency $\omega_0$ is that the nutation rate is proportional to $(\gamma B_1)^n/\omega_0^{n-1}$, where $\gamma$ is the gyromagnetic ratio of the nucleus and $B_1$ is the strength of the applied rf field. The nutation

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A version of this chapter has been previously published as:

rate is thus significantly suppressed compared to on-resonance \((n = 1)\) excitation, but the suppression is less severe when \(\gamma\) is reasonably large and the resonance frequency is low due to a weak static external field (NMR) or quadrupolar coupling (NQR).

The production of observable coherences by far off-resonant irradiation has a number of distinct advantages over on-resonant irradiation including the complete elimination of receiver dead-time, allowing simultaneous excitation and detection [180, 188]. This benefit of two-photon excitation is of greater importance at low frequencies where long receiver dead times due to probe ringing may outlast the free induction signal. For this reason, the application of two-photon excitation to nuclear quadrupole resonance has been proposed [181].

The work of Sauer et al. [192, 193] in the case of a 3-level NQR system (a spin-1 nucleus with \(\eta \neq 0\)) achieved a similar goal, where excitation at two of the transition frequencies allowed detection at the third. That work differs from our two-photon approach as it involves generating a single quantum coherence between two levels via an intermediate energy level. In our approach no intermediate levels are required and the irradiation frequency does not correspond to any transition.

In this paper, we treat two-photon excitation of nuclear quadrupole resonance, first for an axially symmetric spin-3/2 nucleus, with average Hamiltonian theory as described in Ref. [188]. Results of the theory applied to the more general but less intuitive case without axial symmetry are then described. The current treatment builds on our previous demonstration of two-photon NQR [181] by providing a more complete description of the two-photon excitation Hamiltonian as well as a quantitative comparison of experiment with theory for the important \(\eta = 0\) case. The current formalism reproduces the single-quantum excitation terms of the effective Hamiltonian found with the earlier rotating frame approach, and further provides expressions for the Bloch-Siegert shift and for terms that directly generate double-quantum coherences. For the axially symmetric case, we experimentally confirm the angular dependence of the two-photon single-quantum excitation, demonstrate that observable signal arises perpendicular to the excitation axis, and directly observe the double-quantum coherences generated by the newly predicted terms by application of a weak static external magnetic field.

\section{9.2 Theory}

In the principle axis system (PAS) of the quadrupole interaction, the Hamiltonian of a single spin-3/2 nucleus in an external electric field gradient and a linear rf magnetic field is:

\begin{equation}
\hat{H} = \hat{H}_Q + \hat{H}_{\text{rf}} \\
= \frac{\omega_Q}{6} \left[ 3\hat{I}_z^2 - \hat{I}_z^2 + \eta(\hat{I}_x^2 - \hat{I}_y^2) \right] \\
+ \left( \omega_x \hat{I}_x + \omega_y \hat{I}_y + \omega_z \hat{I}_z \right) \cos \omega_{\text{rf}} t, \tag{9.1}
\end{equation}

\begin{equation}
\frac{\hat{H}}{\hbar} = \frac{3\omega_Q}{6} \left[ 3\hat{I}_z^2 - \hat{I}_z^2 + \eta(\hat{I}_x^2 - \hat{I}_y^2) \right] \\
+ \left( \omega_x \hat{I}_x + \omega_y \hat{I}_y + \omega_z \hat{I}_z \right) \cos \omega_{\text{rf}} t, \tag{9.2}
\end{equation}
where \( \omega_Q = e^2 qQ/2 \) is the strength of the quadrupolar interaction, \( \eta \) is the asymmetry parameter of the electric field gradient, and \( \omega_x = \gamma \left( \vec{B}_1 \cdot \hat{x} \right) \), \( \omega_y = \gamma \left( \vec{B}_1 \cdot \hat{y} \right) \) and \( \omega_z = \gamma \left( \vec{B}_1 \cdot \hat{z} \right) \) are the projections of the rf field onto the \( x \), \( y \) and \( z \) axes of the PAS respectively.

Following the prescription for calculating the two-photon excitation Hamiltonian as set out in Ref. [188], we transform Eq. 9.2 into an interaction representation using the unitary operator \( U = e^{i\mathcal{H}_Q t} \) such that the static quadrupolar Hamiltonian is removed.

### 9.2.1 Two-Photon Excitation of NQR for a Spin-3/2 Nucleus with \( \eta = 0 \)

Specializing now to the axially symmetric case where the interaction frame transformation is straightforward, and choosing the \( x \) axis so that the rf lies in the \( x-z \) plane (making \( \omega_y = 0 \)), we find an effective Hamiltonian given by

\[
\begin{align*}
\mathcal{H}_{\text{eff}} &= \left[ \omega_x \hat{I}_x + \omega_z \hat{I}_z \right] \cos \omega_{\text{rf}} t \\
&= \left[ \sqrt{3} \omega_x \left( \cos \omega_Q t \left( \hat{I}_{1x}^{1-2} + \hat{I}_x^{3-4} \right) \right) + \sin \omega_Q t \left( \hat{I}_{1y}^{1-2} - \hat{I}_y^{3-4} \right) \right] \\
&\quad + 2 \omega_x \hat{I}_{x}^{2-3} + \omega_z \hat{I}_z \right] \cos \omega_{\text{rf}} t,
\end{align*}
\]

where Eq. 9.4 has been written in terms of fictitious spin-1/2 operators [194]. In the axially symmetric case, the Zeeman states, quantized along the \( z \) axis, are eigenstates of the system, and the superscripts \( 1 \rightarrow 4 \) correspond to the states \( |3/2\rangle, |1/2\rangle, |1/2\rangle \) and \( |3/2\rangle \) respectively.

The time-averaged effects resulting from this time-dependent Hamiltonian can be described by average Hamiltonian theory via the Magnus expansion [195], the first two terms of which are

\[
\begin{align*}
\mathcal{H}^{(0)} &= \frac{1}{t} \int_0^t dt' \mathcal{H}_{\text{eff}}(t'), \\
\mathcal{H}^{(1)} &= \frac{-i}{2t} \int_0^t dt' \int_0^{t'} dt'' \left[ \mathcal{H}_{\text{eff}}(t'), \mathcal{H}_{\text{eff}}(t'') \right].
\end{align*}
\]

For irradiation at half-resonance (\( \omega_{\text{rf}} = \omega_Q/2 \)), the first non-zero term in the Magnus expansion is the first order term, which, calculated for convenience in the limit \( t \to \infty \), yields the time-independent two-photon excitation Hamiltonian:

\[
\mathcal{H}^{(1)} = \omega_{BS}^{0Q} \left( 3 \hat{I}_x^2 - \hat{I}_y^2 \right) / 6 \\
+ \omega_{\text{nut}}^{1Q} \left( \hat{I}_{1x}^{1-2} - \hat{I}_{x}^{3-4} \right) \\
+ \omega_{\text{nut}}^{2Q} \left( \hat{I}_{x}^{1-3} + \hat{I}_x^{2-4} \right),
\]

(9.7)
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Figure 9.1: The single- and double-quantum terms in the two-photon Hamiltonian represent absorption of one longitudinal (I) and one transverse (O or O) photon or two transverse photons resulting in a $\Delta m = \pm 1$ and $\Delta m = \pm 2$ transitions respectively. The latter does not result in observable signal in a zero field NQR experiment. By contrast, on-resonance excitation with a single photon only couples the $\Delta m = \pm 1$ states.

where

\[
\omega_{BS}^0 = -\frac{\omega_x^2}{\omega_Q} = -\frac{(\gamma B_1)^2}{2} \sin^2 \alpha, \tag{9.8}
\]

\[
\omega_{\text{nut}}^1 = \frac{\sqrt{3} \omega_x \omega_z}{2 \omega_Q} = \frac{\sqrt{3} (\gamma B_1)^2}{2} \cos \alpha \sin \alpha, \tag{9.9}
\]

\[
\omega_{\text{nut}}^2 = -\frac{\sqrt{3} \omega_x^2}{2 \omega_Q} = -\frac{\sqrt{3} (\gamma B_1)^2}{2} \sin^2 \alpha, \tag{9.10}
\]

and $\alpha$ is the angle between the quadrupolar symmetry axis and the rf coil. The Hamiltonian in Eq. 9.7 has been arranged to group together the zero-, single- and double-quantum terms. The last two are responsible for nutation into single- and double-quantum coherences whereas the first term, proportional to the quadrupolar interaction Hamiltonian, is a resonance offset analogous to the Bloch-Siegert shift in NMR [196]. This Bloch-Siegert shift is typically small (approximately 100 Hz for the experiments presented here) and is simply a resonance offset that is effective only when the rf field is present.

The single-quantum term in Eq. 9.7 results in $\Delta m = \pm 1$ transitions between the $|\pm 3/2\rangle$ and $|\pm 1/2\rangle$ states that are due to the absorption of one transverse and one longitudinal photon (see Fig. 9.1). The resulting single-quantum coherence leads to directly observable signal. We use the terms longitudinal and transverse to describe photons according to the orientation of the corresponding rf field component with respect to the $z$ axis in the PAS. These photons carry zero or one unit of angular momentum and correspond to the zero ($\hat{I}_z$) or single ($\hat{I}_x$ and $\hat{I}_y$) quantum operators of Eq. 9.2.

The double-quantum term in Eq. 9.7 that addresses the $|\pm 3/2\rangle$ and $|\mp 1/2\rangle$ transitions (with $\Delta m = \pm 2$ due to absorption of two transverse photons) does not lead to a state that is
observable under evolution due solely to the quadrupolar interaction. This double-quantum coherence does contribute to the observed signal if a static magnetic field is present during detection.

### 9.2.2 Observable Free Induction Decay for $\eta = 0$

The evolution of the system under two-photon irradiation is complicated by the presence of the double-quantum terms of Eq. 9.7, as they interfere with the simple nutation produced by the single-quantum terms. In the absence of a static magnetic field, the free induction signal, $\langle \vec{I} \rangle$, along an arbitrary direction in the PAS of the quadrupolar interaction after a half-resonant pulse of duration $\tau$ is given by:

\[
\langle \vec{I}(t > \tau) \rangle = \text{Tr} \left\{ \hat{\rho}(t) \hat{I} \right\} = \text{Tr} \left\{ e^{i\vec{\mathcal{H}}_Q \tau} \hat{\rho}(0) e^{-i\vec{\mathcal{H}}_Q \tau} \hat{U} \hat{U}^\dagger \right\} . \tag{9.11}
\]

Assuming that the initial density matrix is $\hat{\rho}(0) \propto \mathcal{H}_Q$, the above evolution yields:

\[
\langle \vec{I} \rangle \propto \cos \alpha \sin \left( \frac{\omega_{1Q} \tau}{\cos \alpha} \right) \cos(\omega_Q t) \hat{y}, \tag{9.12}
\]

revealing that observable signal appears only along the $y$ axis, which is perpendicular to both the excitation axis and the symmetry axis of the quadrupolar interaction. The interference of the double-quantum term accelerates $\omega_{1Q}$ by a factor of $1/\cos \alpha$ while reducing the amplitude of the observed signal by $\cos \alpha$. In the limit of short pulses ($\omega_{\text{nut}} \tau \ll 1$), and assuming detection along the $Z$ axis in the lab frame, the amplitude of the detected signal, $S$, is:

\[
S \propto \omega_{\text{nut}} \tau (\hat{y} \cdot \hat{Z}) = \frac{\sqrt{3} \omega_x \omega_z}{2 \omega_Q} \tau (\hat{y} \cdot \hat{Z}), \tag{9.13}
\]

where the interference of the double-quantum terms is no longer apparent.

### 9.2.3 The Case of $\eta \neq 0$

The calculation of the two-photon excitation Hamiltonian for a spin-$3/2$ system with non-zero asymmetry parameter is similar to the $\eta = 0$ case and is reproduced in full in Appendix A.
The result is:
\[
\mathcal{H}^{(1)} = \Omega_{BS}^{QQ} \left[ 3\hat{j}_x^2 - \hat{i} + \eta(\hat{j}_x^2 - \hat{j}_y^2) \right] / 6 \\
+ \Omega_{xz}^{1Q} (\hat{j}_x^{1-2} - \hat{i}^{1-4}) \\
+ \Omega_{yz}^{1Q} (\hat{j}_y^{1-2} - \hat{i}^{1-4}) \\
+ \Omega_{xy}^{1Q} (\hat{j}_y^{1-3} + \hat{i}_y^{2-4}) \\
+ \Omega_{xz}^{2Q} (\hat{j}_x^{1-3} + \hat{i}_x^{2-4}),
\] (9.14)

where
\[
\Omega_{BS}^{QQ} = \frac{(4\kappa^2 + 3)(\omega_x^2 + \omega_y^2) + \frac{\sqrt{5}}{2}\kappa(3\kappa^2 + 1)(\omega_x^2 - \omega_y^2) - 2\kappa^2\omega_z^2}{3\xi^3\omega_Q'},
\] (9.15)
\[
\Omega_{xz}^{1Q} = \frac{-\omega_x\omega_z(3\kappa + \sqrt{3})}{2\xi\omega_Q'},
\] (9.16)
\[
\Omega_{yz}^{1Q} = \frac{\omega_y\omega_z(3\kappa - \sqrt{3})}{2\xi\omega_Q'},
\] (9.17)
\[
\Omega_{xy}^{1Q} = \frac{\sqrt{3}\omega_z\omega_y}{\xi\omega_Q'},
\] (9.18)
\[
\Omega_{xz}^{2Q} = \frac{-\kappa(\omega_x^2 + \omega_y^2) - \frac{\sqrt{3}}{2}(\kappa^2 - 1)(\omega_x^2 - \omega_y^2) + 2\kappa\omega_z^2}{\xi\omega_Q'}
\] (9.19)

and \( \kappa = \eta/\sqrt{3}, \xi = \sqrt{1 + \kappa^2}, \) and \( \omega_Q' = \xi\omega_Q. \) The resulting excitation Hamiltonian is qualitatively similar to the \( \eta = 0 \) case in that it consists of a Bloch-Siegert shift and single- and double-quantum excitation terms. Eq. 9.14 reduces to Eq. 9.7 for the symmetric case of \( \eta = 0 \) if the \( x \) axis is chosen so that the rf again lies in the \( x-z \) plane.

If \( \eta \neq 0, \) the Zeeman states are no longer eigenstates of the system and the excitation Hamiltonian terms we have labeled as single- and double-quantum do not produce states that are properly described as single- or double-quantum coherences. Furthermore, the evolution of the density matrix under such irradiation is non-trivial. An intuition for the evolution of the system may be obtained by considering short pulses (\( \mathcal{H}^{(1)} \tau \ll 1 \)) where each term in Eq. 9.14 can be considered independently. The result (presented in full in Appendix A) is that observable signal is generated by the second, third or fourth terms of Eq. 9.14 when the rf has components along any two of the quadrupolar axes, with the signal appearing along the third axis. However, the vector sum of the signals generated by each of the three terms points in a direction perpendicular to the excitation axis (see Eq. A.8). Therefore, even in the \( \eta \neq 0 \) case, a second coil is required for detection. As expected, the first term in Eq. 9.14 (the Bloch-Siegert shift term), which has been grouped in such a way as to be proportional to the quadrupolar interaction, generates no observable signal. Also, the fifth term generates no observable signal as it is the equivalent of a double-quantum excitation.
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Further intuition into the $\eta \neq 0$ case can be gained from the $\eta = 0$ case where we discussed the concept of excitation through absorption of longitudinal and transverse photons. Because, in the $\eta \neq 0$ case, none of the quadrupolar axes is preferred, excitation along any one of the axes should be possible, requiring the absorption of two photons: one transverse and one longitudinal with respect to that axis to generate observable coherences, or two transverse photons to generate an unobservable coherence. This description is born out in the geometric factors in Eqs. 9.15-9.19.

In our treatment, the linear rf field applied along an arbitrary direction in the quadrupolar PAS can be decomposed into a set of transverse and longitudinal fields capable of supplying both transverse and longitudinal photons along any, depending on crystal orientation, of the quadrupolar axes. In other experimental configurations, where independent transverse and longitudinal fields may be applied in arbitrary directions, only certain terms in Eq. 9.14 may survive.

### 9.3 Methods

A 300 mg potassium chlorate (KClO$_3$) single crystal (of approximate dimensions $3 \times 8.25 \times 9.5$ mm) was grown by slow evaporation of a saturated solution. Potassium chlorate forms a monoclinic crystal with two molecules per unit cell in the space group $P2_1/m$ [197]. The symmetry axis of the quadrupolar interaction is perpendicular to the plane defined by the three oxygens [198], which form a nearly tetrahedral arrangement with the chlorine atom. The two molecular sites are related by an inversion center and therefore are indistinguishable in our magnetic resonance experiments. The symmetry axis of the quadrupolar interaction makes an angle of $\beta = 57^\circ$ with the crystal $c$ axis, which lies perpendicular to the one large crystal face.

All NQR spectra were acquired on a home-built spectrometer [199] in a home-built wide-line probe with a 9.6 mm (inner diameter) solenoid coil resonant at 14 MHz inside a larger saddle coil resonant at 28 MHz. Thus, for two-photon excitation, the excitation and detection axes were orthogonal, whereas for on-resonance excitation, the saddle coil was used for both excitation and detection. Those two-photon spectra obtained with a single excitation and detection coil were acquired with the probe circuit rebuilt so that the solenoid coil was doubly resonant at 14 and 28 MHz.

Unless otherwise stated, two-photon spectra were collected after 100 $\mu$s of rf irradiation, while on-resonance excitation spectra were obtained after a $\pi/2$ pulse of 13 $\mu$s, with approximately 600 W of rf power in both cases. In order to determine the proper first order phase correction for the two-photon excited spectra, on-resonance excited spectra were also collected with weak 100 $\mu$s pulses. A home-built high-power 15 MHz lowpass filter, which attenuated on-resonance rf by 80 dB compared to half-frequency rf, was used to minimize any on-resonance excitation by second harmonic generated in the amplifier. Any 28 MHz rf that may have been generated was below the detection level of a digital oscilloscope (<0.1 mW) when coupled directly to the probe's 28 MHz channel during a half-resonance rf pulse. To cancel the earth's magnetic field, or to generate an external field, the probe was placed inside
Figure 9.2: Schematic of crystal orientation in the lab frame, with the $Z$ axis defined to be along the on-resonance coil axis and the $Y$ axis along the two-photon rf coil. The symmetry axis of the quadrupolar interaction, $z$, makes an angle of $\beta = 57^\circ$ with the lab $Z$ axis. $\alpha$ is defined to be the angle between $z$ and the $Y$ axis and depends on the rotation of the crystal about the $Z$ axis by an angle $\theta$ defined to be zero when $z$ is in the $Y-Z$ plane.

A 20 cm diameter Helmholtz coil that was powered by a computer-controlled power supply (Circuit-Test Electronics, Burnaby, Canada). The field was characterized by a hand-held Hall effect Gaussmeter (F.W. Bell Inc., Orlando FL).

Rotation plots were obtained with a smaller (170 mg) KClO$_3$ single crystal mounted onto a poly(tetrafluoroethylene) (PTFE) holder inside the rf coil with the crystal $c$ axis colinear with the on-resonance coil axis (see Fig. 9.2). Sample rotation about the $c$ axis was achieved by a spectrometer controlled stepper motor that was coupled to the PTFE holder via a plastic gear and chain set. Each spectrum is the average of 1024 scans taken one per second.

The temperature sensitivity of the quadrupole coupling constant (approximately $-5.7$ kHz/K near room temperature [200]) was significant. Temperature fluctuations of less than 0.1 degrees would result in frequency shifts greater than the spectral linewidth. High-power rf irradiation during two-photon excitation resulted in transient temperature fluctuations at the beginning of an acquisition, requiring several hundred scans be discarded before signal averaging was commenced.
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Figure 9.3: $^{35}\text{Cl}$ NQR spectra of KClO$_3$. (a) Spectrum acquired following an on-resonance excitation pulse with excitation and detection in the same coil. (b) and (c) Spectra acquired following a pulse at half the resonance frequency with the rf applied along (b) $\alpha = 53.6^\circ (\theta = 45^\circ)$ and (c) $\alpha = 90^\circ (\theta = 90^\circ)$. Detection was along an axis perpendicular to the direction of excitation as described in the text.

9.4 Results and Discussion

9.4.1 Single-Quantum Coherences and Rotation Plots

The $^{35}\text{Cl}$ NQR spectrum of potassium chlorate excited on-resonance is shown in Fig. 9.3a. A two-photon spectrum, shown in Fig. 9.3b, was obtained with detection along an axis perpendicular to the excitation axis, following an excitation pulse applied at half the NQR frequency.

The relative integrated intensities of the peaks in Figs. 9.3a and 9.3b indicate a tip angle of 2.64$^\circ$ for the 100 $\mu$s two-photon pulse, or a nutation frequency of $\omega_{\text{n}}^{1Q} = 2\pi (73 \text{ Hz})$. The strength of the 14 MHz rf field, calibrated by measuring the nutation rate of $^{14}\text{N}$ spins in a saturated solution of glycine on an MSL200 (Bruker) NMR spectrometer ($\omega_0 = 14.451 \text{ MHz}$), was measured to be $B_1 = 23.0 \text{ mT}$. According to Eq. 9.9, such a $B_1$ would result in a two-photon nutation frequency of $\omega_{\text{n}}^{1Q} = 2\pi (87 \text{ Hz})$, in good agreement with the measured value.
Figure 9.3c shows the two-photon excited spectrum of KCIO₃ with the crystal rotated such that the excitation rf is oriented along an axis perpendicular to the quadrupole axis (α = 90°, θ = 90°). As predicted from Eqs. 9.7 and (9.9), no excitation was observed with the excitation coil in this orientation. Similarly, for θ = 0° (data not shown), when the symmetry axis was in the plane defined by the excitation and detection coils, no signal was observed because the free induction signal appears along a direction perpendicular to the detection coil. Based on the angular dependence of ω_{n1}' during a complete rotation about Z (θ = 0 → 2π), there should be four crystal orientations at which no signal can be observed. In fact, for the coil geometry used in these experiments the expected signal, as given by Eq. 9.13, is

\[ S \propto \frac{(\gamma B_1)^2 \tau}{\omega_Q} \sin^2 \beta \sin 2\theta \sqrt{1 - \sin^2 \beta \cos^2 \theta}. \]  

Indeed for two-photon excitation (Fig. 9.4b), we observe the two-photon excited intensity...
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Figure 9.5: Energy level diagram for an axially symmetric quadrupolar interaction of a spin-3/2 nucleus perturbed to first order by a weak external magnetic field, $B_0$, oriented at an angle $\Theta$ to the symmetry axis of the quadrupolar interaction. The $|\pm 1/2\rangle$ states are mixed such that $|+\rangle = \cos \delta |1/2\rangle + \sin \delta |-1/2\rangle$ and $|-\rangle = -\sin \delta |1/2\rangle + \cos \delta |-1/2\rangle$, where $\tan \delta = \sqrt{(f-1)/(f+1)}$ and $f = 1 + (I + 1/2)^2 \tan^2 \Theta$. The transitions $|\pm 3/2\rangle \rightarrow |\pm\rangle$ and $|\pm 3/2\rangle \rightarrow |\mp\rangle$ yield the well known $\alpha$ and $\beta$ lines with splittings $\Delta \omega_{\alpha} = (3 - f) \cos \Theta$ and $\Delta \omega_{\beta} = (3 + f) \cos \Theta$ and relative intensities $\cos \delta$ and $\sin \delta$ [201].

undergo two full periods through one rotation of the crystal as predicted. By contrast, the on-resonance excited signal intensity is expected to be independent of the rotation angle as is observed in Fig. 9.4a.

9.4.2 Excitation and Detection of Double-Quantum Coherences

The double-quantum term in Eq. 9.7 generates coherences that are unobservable in a pure quadrupole resonance experiment where $\Delta m \neq \pm 1$ transitions are forbidden. To demonstrate that these coherences do exist, a weak static magnetic field was applied.

A degenerate perturbation theory treatment [201] shows that the static field mixes the $|\pm 1/2\rangle$ states (see Fig. 9.5) so that as long as the magnetic field is not colinear with the quadrupolar symmetry axis, the Zeeman states are no longer eigenstates of the system, and any double-quantum coherences of the form $a |\pm 3/2\rangle + b |\mp 1/2\rangle$ generated by two-photon excitation are observable. Level splittings due to the magnetic field result in a set of two doublets, with the inner doublet (known as the $\alpha$ lines) due to $|\pm 3/2\rangle$ to $|\mp\rangle$ transitions and the outer doublet (the $\beta$ lines) due to $|\pm 3/2\rangle$ to $|\pm\rangle$ transitions.

In general, if both the single- and double-quantum terms were to contribute to excitation, in the limit of short pulses, the resulting spectrum would be an admixture of the spectra that would be obtained from each term. However, from Eqs. 9.9 and (9.10) the angular dependence is different for the two terms with the single-quantum term requiring projections of the excitation field both parallel and perpendicular to the $z$ axis of the quadrupolar PAS.
Figure 9.6: $^{35}$Cl NQR spectra of KClO$_3$ with excitation rf perpendicular to the symmetry axis of the quadrupolar interaction. Top spectra are in zero external magnetic field, while an external magnetic field of 8 Gauss was applied to bottom spectra to render double-quantum coherences observable. (a) On-resonance excitation, (b) two-photon excitation with crossed-coil detection, and (c) two-photon excitation detected along the excitation axis. A Helmholtz coil was used to cancel the earth's magnetic field in (a) and (b) but not in (c), resulting in a small 'antiphase' peak in the upper panel of (c). The asymmetric lineshape of some of the multi-line spectra is due to small imperfections in the crystal.

whereas the double-quantum term requires only a perpendicular projection. Thus, by setting $\alpha = 90^\circ$, only the double-quantum term will contribute to excitation and in the absence of a static magnetic field no signal should be observed.$^1$

$^{35}$Cl NQR spectra of KClO$_3$ with the excitation rf perpendicular to the symmetry axis of the quadrupolar interaction are shown in Fig. 9.6. For on-resonance excitation, as depicted in Fig. 9.6a, the single peak splits into 4 lines in the presence of an external magnetic field. As expected, for two-photon excitation with crossed-coil detection, no signal is observed in the absence of an external field whereas a symmetric set of lines appears when a field is applied obliquely to the quadrupolar symmetry axis (Fig. 9.6b). When repeated with excitation and

---

$^1$In the present analysis, it has been assumed that the magnetic field is weak ($\gamma B_0 \tau \ll 1$), so that the static field may be neglected during excitation, acting only during the subsequent evolution time. A more careful analysis must be performed if this condition is not fulfilled or if a more quantitative agreement between experiment and theory is necessary. In such circumstances, numerical methods such as a stepwise integration of the Bloch equations may be more practical.
detection in a single doubly tuned rf coil (Fig. 9.6c), a set of lines appears when an external field is applied but this time is antisymmetric with respect to zero frequency. The symmetries of the spectra confirm that the signal does indeed appear along an axis perpendicular to the excitation axis. The fact that in Fig. 9.6b the two doublets have opposite sign is a reflection of the excitation geometry which should result in no observable signal as the magnetic field is turned off and the lines collapse together.

Alternatively, it would be possible to observe the double-quantum coherences in the absence of an external magnetic field with a two-pulse experiment in which two-photon excitation was followed by an on-resonance pulse with phase cycling or field gradients applied to select out double-quantum contributions to the signal. In fact this has been done previously by Gold and Hahn [177] for the case of a spin-3/2 nucleus in a strong magnetic field where double-quantum coherence between the [3/2] and [−1/2] states was excited by irradiation at half the transition frequency, which did not correspond to on-resonance excitation involving the intermediate [1/2] state because of the quadrupole interaction. Zur and Vega [186] similarly demonstrated double-quantum coherence in a spin-1 nucleus. In both instances, rf was applied along an axis perpendicular to the external magnetic field so that only $\Delta m = \pm 2$ transitions were allowed by absorption of two transverse photons.

Excitation of $|\Delta m| > 1$ coherences by absorption of multiple transverse photons is interesting from the point of view of exciting higher order multiple-quantum coherences. Such excitation is not limited to single spins or to quadrupolar systems and has previously been achieved in dipolar coupled spin systems [202]. However since the multi-photon excitation efficiency decreases exponentially with the number of photons involved, other techniques such as multiple pulse sequences [203] have proven more efficient and are used widely today. Furthermore, because multiple-quantum coherences are not directly observable in a standard NMR experiment, the advantages of off-resonance excitation, namely detection during excitation, do not extend to any such coherences that may be excited. It is the practical advantages of detection outside the excitation bandwidth that have fueled our recent interest in multi-photon excitation in NMR [180, 188] and NQR [181], and it is in this respect that we expect two-photon excitation to find practical application.

The generality of the technique in NQR will be limited by the fact that, in the experiment which we present here, the two-photon excited NQR signal arising from a powder sample averages to zero as a consequence of the angular dependence of Eq. 9.13. Furthermore, the weak nutation rates achieved in our experiments result in a very limited excitation bandwidth if 90° pulses are required. This latter difficulty can be overcome by combining two-photon NQR with noise spectroscopy [204, 205], where small tip angle pulses provide a broad excitation bandwidth even with low nutation frequencies. In addition, application of two-photon NQR to samples with smaller quadrupole couplings (or larger $\gamma$) will improve the nutation rate, as will improvements in rf field strengths made possible by developments with microcoils, being developed in our lab and elsewhere [206]. Further improvements may also be achieved with two-color excitation using two independent rf fields whose frequencies sum to or differ by the resonance frequency [188].
Chapter 9. Crossed-Coil Detection of Two-Photon Excited NQR

9.5 Conclusions

We have applied an average Hamiltonian treatment to two-photon excitation of NQR in a spin-3/2 system, predicting that excitation at half the normal resonance frequency produces single-quantum coherences (via absorption of one longitudinal and one transverse photon), double-quantum coherences (via absorption of two transverse photons), and a resonance offset analogous to the Bloch-Siegert shift in NMR. We have demonstrated two-photon excitation in a single crystal of potassium chlorate, and have observed the single- and double-quantum coherences directly. We verified the angular dependence of the nutation rate on rf coil orientation and demonstrated that signal appears along an axis perpendicular to the rf field.
Bibliography


Part IV

Conclusions
Chapter 10

Concluding Remarks

In this conclusion, the major findings of the dissertation are summarized, and future directions are suggested. The two parts of the dissertation are considered separately.

10.1 Spider Dragline Silk

It was found in Chapter 5 that the alignment of glycine residues in the peptide backbone along the axis of the silk fibre can be adequately modeled with two components: the first, a $\beta$-sheet structure that was found to be well aligned along the fibre (with a FWHM of 20°); the second a polyglycine II helix found to be poorly aligned (with a FWHM of 68°). The results indicate that a significantly larger fraction of residues are in the well aligned component than would have been expected from previous models. Glycines outside of the previously proposed crystalline regions must be included in the well aligned fraction suggesting that the previously drawn distinction between crystalline regions being composed of poly-alanine runs with glycines making up the surrounding matrix may have been overly simplistic. The results also suggest that the change in orientation with fibre strain depends on the draw rate. Those fibres drawn faster show a higher degree of initial alignment and changed significantly when the fibres are allowed to relax from their draw-induced stress, while those fibres drawn slowly do not show such a significant change in orientation.

In Chapter 6, it was shown that the poorly aligned component of silk undergoes rapid motion when fibres are exposed to water, while the well aligned component does not. The fraction of the poorly aligned component that participates in the fast motion increases as the fibre supercontracts. The absence of intermediate motional regimes suggests a glass-rubber phase transition in these regions. These results imply a molecular model for silk's supercontraction that involves prestrained chains stabilized by hydrogen bonding that collapse entropically when exposed to water. Permeation of water into an increasing fraction of the poorly oriented regions drives the process of supercontraction. These findings support previous hypotheses proposing that supercontraction is a result of water-induced mobility in the amorphous region [207].

The insensitivity to water of a surprising number of glycine residues implies that the crystalline regions may be more extensive than originally expected. The degree of alignment observed in the well aligned component was less than had previously been found for alanines suggesting that glycine residues surrounding the alanines are more disordered, but, like alanines, are impermeable to water. This suggests that there exists an intermediate region between the poly-alanine crystals and the amorphous matrix, whose role in the mechanical
properties of silk is, as yet, not understood.

Upon supercontraction and subsequent drying, it is expected that the collapsed regions would take on a low-energy secondary structure, perhaps helical. The change in local torsion angles should be significant, though initial attempts to observe such changes as reported in Ref. [208] as well as preliminary experiments by our group have not shown dramatic changes. A more careful study may reveal well defined secondary structures in such supercontracted and dried silk. Such findings would have implications for recent results in which tensile properties of silk were altered by supercontraction to predetermined lengths followed by drying of the fibres [209].

In conclusion, NMR probes of the structure of silk continue to provide new clues into silk’s amazing mechanical properties. Future directions in the field may focus on comparative studies of structure and dynamics between native silk and synthetic silk fibres obtained by expression of silk-like proteins sequences and proper processing. Current state-of-the-art synthetic silk-like fibres still lack the mechanical properties of native silk. An understanding of the molecular structure of these synthetic fibres may aid in future attempts to produce fibres with mechanical properties rivaling those of silk.

### 10.2 Two-Photon Excitation

In Chapter 7, two-photon excitation of nuclear quadrupole resonance (NQR) was demonstrated for the first time with rf irradiation at half the normal resonance frequency. A simple theory involving a transformation into an interaction representation predicted the single quantum excitation Hamiltonian for a nucleus with arbitrary spin. The theory also predicted observable magnetization appearing along an axis perpendicular to the excitation field. Experiments on $^{35}$Cl (spin-3/2) in a single crystal of NaClO$_3$ were consistent with these predictions though an external magnetic field was required to render the signal observable in the excitation coil.

In Chapter 8 a general theoretical description of two-photon excitation in NMR was presented. Using average Hamiltonian theory, two-photon excitation was worked out for the case of excitation by two rf fields whose frequencies summed to, or differed by, the normal resonance frequency. Such excitation was called two-color excitation. It was shown that the excitation efficiency increased as the two excitation frequencies deviated from half-resonance. A demonstration of two-color excitation of NMR for a spin-1/2 nucleus was performed, and the practical advantages over half-resonance excitation were discussed.

In Chapter 9, the new theoretical approach was used to provide a more complete description of two-photon excitation in NQR for a spin-3/2 nucleus, predicting a Bloch-Siegert shift and double quantum excitation. A probe with a crossed detection coil was used to observe two-photon excited signal in $^{35}$Cl in a single crystal of KClO$_3$. The free induction signal appeared along a direction perpendicular to the excitation field. Rotation plots confirmed the theoretical prediction of signal amplitude dependence on coil orientation. Double quantum coherences were observed directly by the application of a weak magnetic field.
In addition to the theoretical framework for two-photon excitation provided by Chapters 7–9, an intuitive picture of two-photon excitation was also presented. The concept of conservation of energy and angular momentum in the process of coherent absorption of multiple photons provides an intuition for the results that are obtained from a rigorous theoretical calculation. This concept of absorption of multiple photons extends to other areas of NMR.

Several limitations of two-photon excitation exist and must be addressed if two-photon excitation is to find widespread application. Foremost is the inherently weak nutation frequency associated with weak two-photon excitation efficiency, resulting in narrow excitation bandwidths. We suggest combining two-photon excitation with noise spectroscopy, which alleviates this limitation, and simultaneously alleviates some technical limitations of noise spectroscopy. As another potential method of increasing excitation bandwidth, we propose the use of two-color excitation very far from half-resonance, and the use of very strong $B_1$ fields as can be attained using microcoils. Realistically, however, two-photon excitation may be limited to applications where transition frequencies are low while nuclear $\gamma$ are high such as in NQR and low-field NMR. Long receiver deadtimes due to probe ringing are often problematic at these low frequencies.

Practical applications of two-photon excitation to NQR are also limited by the fact that in powders, two-photon NQR, as we have presented it here, yields no observable signal due to cancellation of signal by integration over all crystal orientations. Novel coil geometries and rf fields (such as circularly polarized rf) may potentially solve this problem.

The intuition for two-photon excitation provided by this dissertation and by the early work on two-photon excitation in NMR [210–213] may spark other applications of this technique. In particular such a picture provides a means of predicting the effects of novel excitation/detection coil geometries without tedious calculations.

We hope that in the future two-photon excitation finds a niche in the world of nuclear magnetic resonance as it has in optics and electron spin resonance.
Bibliography


Part V

Appendices
Appendix A

Two-Photon Excitation of NQR in a Spin-3/2 Nucleus with $\eta \neq 0$

For the case of a non-zero asymmetry parameter ($\eta \neq 0$), the quadrupolar Hamiltonian of a single spin-3/2 nucleus in an electric field gradient, written in the Zeeman basis is

$$\hat{\mathcal{H}}_Q = \frac{\omega_Q}{2} \begin{pmatrix} 1 & 0 & \kappa & 0 \\ 0 & -1 & 0 & \kappa \\ \kappa & 0 & -1 & 0 \\ 0 & \kappa & 0 & 1 \end{pmatrix}.$$  \hspace{1cm} (A.1)

The quadrupolar Hamiltonian can be diagonalized using the matrix

$$A = \frac{1}{2\xi(\xi - 1)} \begin{pmatrix} \kappa & 0 & \xi - 1 & 0 \\ 0 & \kappa & 0 & 1 - \xi \\ 1 - \xi & 0 & \kappa & 0 \\ 0 & \xi - 1 & 0 & \kappa \end{pmatrix}$$  \hspace{1cm} (A.2)

becoming

$$\hat{\mathcal{H}}'_Q = A^{-1} \hat{\mathcal{H}}_Q A$$

$$= \frac{\omega'_Q}{2} \begin{pmatrix} 1 & -1 \\ -1 & 1 \end{pmatrix}.$$  \hspace{1cm} (A.3)

Thus, for $\eta \neq 0$, the asymmetry parameter increases the level splitting by a factor $\xi$. The spin operators in this new basis are

$$\hat{I}'_x = c_{0x} \hat{I}^{1-2}_x + c_{1x} \hat{I}^{3-4}_x + c_{2x} \hat{I}^{1-4}_x + c_{3x} \hat{I}^{2-3}_x$$

$$\hat{I}'_y = c_{0y} \hat{I}^{1-2}_y + c_{1y} \hat{I}^{3-4}_y + c_{2y} \hat{I}^{1-4}_y + c_{3y} \hat{I}^{2-3}_y$$

$$\hat{I}'_z = c_{0z} \hat{I}^{2-4}_z + c_{1z} \hat{I}^{1-3}_z + c_{2z} \hat{I}^{1-4}_z + c_{3z} \hat{I}^{2-3}_z$$  \hspace{1cm} (A.4) \hspace{1cm} (A.5) \hspace{1cm} (A.6)

where Table A.1 summarizes the coefficients used above. The expansion is unique except for
Appendix A. Two-Photon Excitation of NQR in a Spin-3/2 Nucleus with \( \eta \neq 0 \)

Table A.1: Coefficients used in expansion of spin operators to fictitious spin-1/2 basis.

<table>
<thead>
<tr>
<th>( i )</th>
<th>( c_{0i} )</th>
<th>( c_{1i} )</th>
<th>( c_{2i} )</th>
<th>( c_{3i} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( x )</td>
<td>( \sqrt{3+\xi} )</td>
<td>( \sqrt{3+\xi} )</td>
<td>( \sqrt{3\xi-1+\xi} )</td>
<td>( -\sqrt{3\xi+1+\xi} )</td>
</tr>
<tr>
<td>( y )</td>
<td>( \sqrt{3-\xi} )</td>
<td>( \sqrt{3-\xi} )</td>
<td>( \sqrt{3\xi+1-\xi} )</td>
<td>( \sqrt{3\xi+1+\xi} )</td>
</tr>
<tr>
<td>( z )</td>
<td>( \frac{2\xi}{\xi} )</td>
<td>( \frac{2\xi}{\xi} )</td>
<td>( \frac{1}{\xi} (\xi+2) )</td>
<td>( -\frac{1}{\xi} (\xi-2) )</td>
</tr>
</tbody>
</table>

terms in \( \hat{I}_z^{r-s} \) which can be expanded in terms of linear combinations of \( \hat{I}_y^{r-s'} \) and \( \hat{I}_z^{r-s''} \).

From here, we proceed as in the case of \( \eta = 0 \) by choosing an interaction representation, \( \hat{U} = e^{i\hat{H}_Q t} \), that removes the diagonal quadrupolar Hamiltonian. By taking advantage of the commutation relations of fictitious spin-1/2 operators, we write the effective Hamiltonian in this interaction representation as

\[
\hat{H}_{\text{eff}}'(t) = \begin{pmatrix}
\omega_x \\
\omega_y \\
\omega_z
\end{pmatrix} \left( \begin{array}{c}
\hat{I}_x^{3/4} \cos \omega_Q t - \hat{I}_y^{3/4} \sin \omega_Q t \\
\hat{I}_y^{3/4} \cos \omega_Q t + \hat{I}_x^{3/4} \sin \omega_Q t \\
c_0 \hat{I}_x^{1/2} \cos \omega_Q t - c_3 \hat{I}_x^{1/2} \sin \omega_Q t \\
c_0 \hat{I}_x^{1/2} \cos \omega_Q t + c_3 \hat{I}_x^{1/2} \sin \omega_Q t \\
\hat{I}_x^{1/2} \cos \omega_Q t + c_3 \hat{I}_x^{1/2} \sin \omega_Q t \\
\hat{I}_x^{1/2} \cos \omega_Q t - c_3 \hat{I}_x^{1/2} \sin \omega_Q t
\end{array} \right)
+ \cos \omega_{\text{rf}} t. \quad (A.7)
\]

Calculation of first order terms in the Magnus expansion requires keeping track of \( 18^2 = 324 \) terms, each of which contains a commutation and a temporal integral over trigonometric functions. Fortunately, the latter factor comes in 9 varieties, 5 of which vanish as summarized in Table 8.2 in Chapter 8. The non-vanishing first order terms in the Magnus expansion for \( \omega_{\text{rf}} = \omega_Q/2 \) (expressed in the original Zeeman basis) are given by Eq. 9.14.

Although evolution under the full two-photon excitation Hamiltonian is non-trivial, some insight can be gained by considering only short pulses \( (\hat{H}_Q^{(1)})_T \ll 1 \) so that each term in Eq. 9.14 contributes independently to the signal. According to Eq. 9.11, using the Hamiltonian of Eq. 9.14, the free induction signal in the quadrupolar PAS is:

\[
\langle \hat{I} \rangle \propto \left( \begin{array}{c}
- \omega_y \omega_z (\eta^2 + 2\eta - 3) \\
\omega_z \omega_x (\eta^2 - 2\eta - 3) \\
\omega_x \omega_y 4\eta
\end{array} \right) \frac{\tau \cos \omega_Q t}{2(1 + \frac{\eta^2}{3})\omega_Q}. \quad (A.8)
\]
The $x$-, $y$-, and $z$-components are due to the third, second, and fourth terms in Eq. 9.14 respectively, whereas the first and fifth terms produce no observable signal. As in the $\eta = 0$ case, this signal appears perpendicular to the excitation coil axis, shown by the vanishing of the dot product of Eq A.8 with the unit vector defining the excitation coil axis $((\omega_x, \omega_y, \omega_z)/(\gamma B_1))$. 
Appendix B

Two-Color Excitation of NQR in a Spin-3/2 Nucleus with \( \eta = 0 \) using Two Excitation Coils.

We consider the case of two-color excitation of NQR in an \( \eta = 0 \) spin-3/2 system with the two rf fields produced by two coils of arbitrary orientation in the quadrupolar PAS.

The total Hamiltonian during a pulse on both coils is

\[
\hat{H}_T = \hat{H}_Q + \hat{H}_{rf1} + \hat{H}_{rf2}
\]
\[
= \frac{\omega Q}{6} (3\hat{I}_z^2 - \hat{I}_z^2)
\]
\[
+ \cos \omega_{rf1} t \left( \omega_{\perp} \hat{I}_x + \omega_{\parallel} \hat{I}_z \right) + \cos \omega_{rf2} t \left( \omega_x \hat{I}_x + \omega_y \hat{I}_y + \omega_z \hat{I}_z \right), \quad (B.2)
\]

where the \( x \)-axis has been chosen so that one of the rf coils lies in the \( x-z \) plane. Transforming into an interaction representation via the unitary operator \( \hat{U} = \exp(\frac{i}{2} \omega_Q t (\hat{I}_z^2 - \frac{1}{4} \hat{I}_z^4)) \), yields the effective Hamiltonian

\[
\hat{H}_{eff} = \cos \omega_{rf1} t \left( \cos \omega_Q t \left[ \sqrt{3} \omega_{\perp} \left( \hat{I}_x^0 - \hat{I}_x^3 \right) \right] 
+ \sin \omega_Q t \left[ \sqrt{3} \omega_{\parallel} \left( \hat{I}_y^0 - \hat{I}_y^3 \right) \right] + \left[ 2 \omega_{\perp} \hat{I}_x^2 + \omega_{\parallel} \hat{I}_z \right] \right)
\]
\[
+ \cos \omega_{rf2} t \left( \cos \omega_Q t \left[ \sqrt{3} \omega_x \left( \hat{I}_x^0 - \hat{I}_x^3 \right) + \sqrt{3} \omega_y \left( \hat{I}_y^0 - \hat{I}_y^3 \right) \right] 
+ \sin \omega_Q t \left[ \sqrt{3} \omega_x \left( \hat{I}_y^0 - \hat{I}_y^3 \right) - \sqrt{3} \omega_y \left( \hat{I}_x^0 - \hat{I}_x^3 \right) \right] 
+ \left[ 2 \omega_x \hat{I}_x^2 + 2 \omega_y \hat{I}_y^2 + \omega_z \hat{I}_z \right] \right). \quad (B.3)
\]

Applying first order average Hamiltonian theory and using the temporal integrals in Table 8.1, we find the following time-independent two-color excitation Hamiltonian:

\[
\hat{H}^{(1)} = \omega_{BS} \left( \hat{I}_z^1 - \hat{I}_z^3 \right)
\]
\[
+ \omega_x \left( \hat{I}_x^1 - \hat{I}_x^3 \right) + \omega_y \left( \hat{I}_y^1 - \hat{I}_y^3 \right)
\]
\[
+ \omega_z \left( \hat{I}_z^1 + \hat{I}_z^3 \right). \quad (B.4)
\]
Appendix B. Two-Color Excitation of NQR in a Spin-3/2 Nucleus with \( \eta = 0 \)

where

\[
\omega_{BS}^{0Q} = -\frac{3}{4\omega_Q} \left[ \frac{\omega_1^2}{1 - \left( \frac{\omega_1}{\omega_Q} \right)^2} + \frac{\omega_2^2 + \omega_y^2}{1 - \left( \frac{\omega_y}{\omega_Q} \right)^2} \right], \tag{B.5}
\]

\[
\omega_x^{1Q} = \frac{\sqrt{3}(\omega_1^{x} + \omega_{||}^{x})}{2\omega'_Q}, \tag{B.6}
\]

\[
\omega_y^{1Q} = \frac{\sqrt{3}\omega_{||}^{y}}{2\omega'_Q}, \tag{B.7}
\]

\[
\omega_x^{2Q} = -\frac{\sqrt{3}\omega_1^{x}}{\omega'_Q}, \tag{B.8}
\]

\[
\omega_y^{2Q} = \frac{\sqrt{3}\omega_1^{y}}{\omega'_Q}, \tag{B.9}
\]

with \( \omega'_Q = \omega_Q \left( 1 - \left( \frac{\delta}{\omega_Q} \right)^2 \right), \) and \( \delta = \omega_{r2} - \omega_{r1} \) the difference between excitation frequencies.

The observable signal is given by:

\[
\langle \hat{I} \rangle = Tr \left\{ \hat{U}^\dagger e^{i\hat{H}(1)t} \rho(0) e^{-i\hat{H}(1)t} \hat{U} \right\}, \tag{B.10}
\]

where \( \rho(0) = (\hat{I}_z^{1-4}) \propto \hat{H}_Q. \) The resulting observable signal during the pulse, expressed as a vector in the quadrupolar PAS is:

\[
\langle \hat{I} \rangle = \cos \omega_Q t \frac{\sqrt{3} \sin \omega_T t}{\omega_T} (\omega_x^{1Q}, \omega_y^{1Q}, 0), \tag{B.11}
\]

where \( \omega_T = \sqrt{(\omega_x^{1Q})^2 + (\omega_y^{1Q})^2 + (\omega_x^{2Q})^2 + (\omega_y^{2Q})^2}. \)

For short pulses, the Eq. B.11 can be Taylor expanded resulting in a signal of

\[
\langle \hat{I} \rangle = \sqrt{3} t (\omega_y^{1Q}, \omega_x^{1Q}, 0). \tag{B.12}
\]

Consider now the case where the two excitation coils are perpendicular (along \( \vec{B}_1 \) and \( \vec{B}_2 \) respectively), and a third detection coil (along \( \vec{B}_{det} \)) is perpendicular to both of the excitation coils. In the quadrupolar PAS, the orientation of the three coils can be written in terms of the polar and azimuthal angles, \( \theta \) and \( \psi \) (note that rotation about the symmetry axis should
Appendix B. Two-Color Excitation of NQR in a Spin-3/2 Nucleus with \( \eta = 0 \)

have no effect):

\[
\begin{align*}
\vec{B}_1 &= B_1(\sin \theta, 0, \cos \theta) = (\omega_\perp, 0, \omega_\parallel)/\gamma \\
\vec{B}_2 &= B_2(\cos \theta \sin \psi, \cos \psi, \sin \theta \sin \psi) = (\omega_x, \omega_y, \omega_z)/\gamma \\
\vec{B}_{\text{det}} &= (\cos \theta \cos \psi, \sin \psi, -\sin \theta \cos \psi).
\end{align*}
\]

The signal received in the detection coil for a single orientation of this set of coils in the quadrupolar PAS is:

\[
S(\theta, \psi) = \left\langle \vec{I} \right\rangle \cdot \vec{B}_{\text{det}}
\]

\[
= \frac{-3\gamma B_1 B_2 t}{2\omega_Q'} \left[ \cos 2\theta \sin^2 \psi + \cos^2 \theta \cos^2 \psi \right].
\]

Unfortunately, the powder average vanishes:

\[
S_T = \int_0^{2\pi} \partial \psi \int_0^\pi \partial \theta \sin \theta S(\theta, \psi) = 0.
\]

In fact, the orientation of the coil does not matter. The total observable signal disappears upon powder averaging:

\[
\int_0^{2\pi} \partial \psi \int_0^\pi \partial \theta \sin \theta \left\langle \vec{I} \right\rangle = 0.
\]

Higher order terms in the Taylor expansion of Eq. B.11 don’t produce signal either. Thus, unfortunately, two-color excitation in two separate excitation coils does not break the symmetry which prevents measurement of two-photon excited NQR in powders containing spin-3/2 nuclei.
Appendix C

Two-Photon Excitation of NQR in a Spin-1 Nucleus with \( \eta \neq 0 \)

Another potentially important application of two-photon excitation of NQR is in a spin-1 system (e.g. \(^{14}\text{N}\)). Such a case is worked out here using the theory described in Chapter 8. The result is qualitatively similar to the spin-3/2 case as presented in Chapter 9, expect that for \( \eta \neq 0 \), excitation and detection can be achieved in a single coil as long as it has components along all three principle axes of the quadrupolar PAS.

For a pure quadrupolar interaction in the presence of an external rf field, the total Hamiltonian in the principle axis system (PAS) of the local electric field gradient of a single nuclear site is

\[
\hat{H}_T = \hat{H}_Q + \hat{H}_{rf}
\]

\[
= \omega_Q (3I_x^2 - 2 + \eta(I_x^2 - I_y^2))
+ (\omega_xI_x + \omega_yI_y + \omega_zI_z) \cos \omega_{rf}t,
\]

where \( \omega_Q = e^2qQ/(12I(2I-1)\hbar) \) is the strength of the quadrupolar interaction. For spin-1, the quadrupolar Hamiltonian can be written in the Zeeman basis as

\[
\hat{H}_Q = \omega_Q \begin{pmatrix} 1 & 0 & \eta \\ 0 & -2 & 0 \\ \eta & 0 & 1 \end{pmatrix}.
\]

Diagonalization of the Hamiltonian with

\[
A_{\pm1} = \frac{1}{\sqrt{2}} \begin{pmatrix} 1 & 0 & \mp1 \\ 0 & \sqrt{2} & 0 \\ \pm1 & 0 & 1 \end{pmatrix}
\]

yields

\[
\hat{H}'_Q = A^{-1}\hat{H}_Q A
\]

\[
= \omega_Q \begin{pmatrix} (1+\eta) & 0 & 0 \\ 0 & -2 & 0 \\ 0 & 0 & (1-\eta) \end{pmatrix}
\]

\[
= \omega_Q (2\eta I_z^1 - 3I_0^1 - 2),
\]
The energy level diagram for the quadrupolar Hamiltonian is depicted in Fig. C.1.

In the diagonalized basis, the spin operators can be rewritten as

\[
I'_x = A^{-1}I_xA = \hat{I}^{1-2}_x, \\
I'_y = A^{-1}I_yA = \hat{I}^{2-3}_y, \\
I'_z = A^{-1}I_zA = -\hat{I}^{1-3}_z, 
\]

and the total Hamiltonian can be written

\[
\hat{H}' = \omega_Q \left(2\eta \hat{I}^{1-3}_x + 3 \hat{I}^{1-3}_0 - 2\right) \\
+ \left(\omega_x \hat{I}^{1-2}_x + \omega_y \hat{I}^{2-3}_y - \omega_z \hat{I}^{1-3}_z\right) \cos \omega_{rt}t
\]

Transforming into an interaction representation with the unitary operator

\[
\hat{U} = e^{i\omega_Q(2nI_z^{1-3} + 3l_0^{1-3} + 2)t},
\]

the effective Hamiltonian becomes

\[
\hat{H}'_{\text{eff}}(t) = \left(\omega_x \hat{U}^\dagger \hat{I}^{1-2}_x \hat{U} + \omega_y \hat{U}^\dagger \hat{I}^{2-3}_y \hat{U} - \omega_z \hat{U}^\dagger \hat{I}^{1-3}_z \hat{U}\right) \cos \omega_{rt}t.
\]

Using the commutation relations of fictitious spin 1/2 operators, we obtain

\[
\hat{U}^\dagger \hat{I}^{1-2}_x \hat{U} = \hat{I}^{1-2}_x \cos \omega^+t + \hat{I}^{1-2}_y \sin \omega^+t, \\
\hat{U}^\dagger \hat{I}^{2-3}_y \hat{U} = \hat{I}^{2-3}_y \cos \omega^-t + \hat{I}^{2-3}_x \sin \omega^-t, \\
\hat{U}^\dagger \hat{I}^{1-3}_z \hat{U} = \hat{I}^{1-3}_z \cos \omega^0t + \hat{I}^{1-3}_y \sin \omega^0t.
\]
Appendix C. Two-Photon Excitation of NQR in a Spin-1 Nucleus with $\eta \neq 0$

From Eq. C.8 and C.9 and from the orthogonality of trigonometric functions (with an inner product defined as the integral over time), it is clear that the zeroth order terms in the Magnus expansion will be non-zero only if $\omega_{\text{tf}} = \omega^0$, $\omega^+$ or $\omega^-$:

$$\mathcal{H}^{(0)} = \frac{1}{2} \begin{cases} -\omega_z \hat{f}_z^{-1} & \omega_{\text{tf}} = \omega^0 \\ \omega_y \hat{f}_y^{-2} & \omega_{\text{tf}} = \omega^- \\ \omega_x \hat{f}_x^{-1} & \omega_{\text{tf}} = \omega^+ \end{cases} \quad (C.12)$$

This is just on-resonance excitation of nuclear quadrupole resonance as has been described elsewhere.

For two photon excitation, we must look to first order terms in the Magnus expansion. Since there are 6 terms in the effective Hamiltonian in Eq. C.8, we will be required to keep track of 36 terms in the first order expansion. The resulting first order term in the Magnus expansion for such two photon excitation reduces to:

$$\mathcal{H}^{(1)} = \begin{cases} \Omega_{xy}^0 \hat{f}_y^{-1} + (\Omega_{zy}^0 \hat{f}_z^{-1} + \Omega_{yx}^0 \hat{f}_x^{-1} + \Omega_{xy}^0 \hat{f}_z^{-1}) & \omega_{\text{tf}} = \omega_{\text{tf}}^0 \\ \Omega_{zx}^{-2} + (\Omega_{yx}^{-2} + \Omega_{yy}^{-2} + \Omega_{zx}^{-2}) & \omega_{\text{tf}} = \omega_0^- \\ \Omega_{yz}^{+1} + (\Omega_{yz}^{+1} + \Omega_{yy}^{+1} + \Omega_{zx}^{+1}) & \omega_{\text{tf}} = \omega_0^+ \end{cases} \quad (C.13)$$

where the following have been defined:

$$\begin{align*}
\Omega_{xy}^0 &= \frac{-\omega_x \omega_y}{4(\omega^0 + \omega^-)} \\
\Omega_{zx}^0 &= \frac{-\omega_x^2}{3\omega^0} \\
\Omega_{yy}^0 &= \frac{(3-\eta)\omega_0^2}{6(2\omega^0 - \omega^-)} \\
\Omega_{zx}^{-2} &= \frac{-3\omega_0^2}{2(3-\eta)(\omega^0 + \omega^-)} \\
\Omega_{yz}^{+1} &= \frac{-2\omega_0^2}{3(5-\eta)(\omega^0 - \omega^-)} \\
\Omega_{yy}^{+1} &= \frac{(3-\eta)\omega_0^2}{6(2\omega^0 + \omega^+)} \\
\Omega_{zx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{xy}^{+1} &= \frac{2\omega_0^2}{3(5+\eta)(\omega^0 - \omega^0)} \\
\Omega_{yx}^{+1} &= \frac{(\omega^0 - \omega^0 - \omega^0)}{3(5-\eta)(\omega^0 + \omega^0)} \\
\Omega_{xx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{yy}^{+1} &= \frac{(3-\eta)\omega_0^2}{6(2\omega^0 + \omega^0)} \\
\Omega_{zx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{xy}^{+1} &= \frac{2\omega_0^2}{3(5+\eta)(\omega^0 - \omega^0)} \\
\Omega_{yx}^{+1} &= \frac{(\omega^0 - \omega^0 - \omega^0)}{3(5-\eta)(\omega^0 + \omega^0)} \\
\Omega_{xx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{yy}^{+1} &= \frac{(3-\eta)\omega_0^2}{6(2\omega^0 + \omega^0)} \\
\Omega_{zx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{xy}^{+1} &= \frac{2\omega_0^2}{3(5+\eta)(\omega^0 - \omega^0)} \\
\Omega_{yx}^{+1} &= \frac{(\omega^0 - \omega^0 - \omega^0)}{3(5-\eta)(\omega^0 + \omega^0)} \\
\Omega_{xx}^{+1} &= \frac{-3\omega_0^2}{3(9-\eta)(\omega^0 - \omega^0 - \omega^0)} \\
\Omega_{yy}^{+1} &= \frac{(3-\eta)\omega_0^2}{6(2\omega^0 + \omega^0)} \end{align*}$$

Note that the terms in brackets in Eq. C.13 commute with the equilibrium density matrix, $\rho_{\text{eq}}$, (i.e. they are diagonal) and the first term is required to obtain observable signal. Thus, to achieve two-photon excitation at $\omega_0^0$, the rf must have components along the $x$- and $y$-axes; for excitation at $\omega_0^-$, the rf must by along $x$ and $z$; and excitation at $\omega_0^+$ occurs if there component of the rf along $y$ and $z$. This is a common feature of two photon excitation as described in Chapter 9. However, unlike the spin-3/2 case considered earlier where a non-zero $\eta$ did not split the degeneracy of the $|\pm m\rangle$ states, the various transitions can be addressed independently by choosing the appropriate excitation frequency.

Considering only short pulses where each term contributes independently to the evolution of the initial state of the system, it can be shown that the free induction signal detected on-resonance (i.e. at the frequency of the transition that is being irradiated) is:

$$\mathcal{G} = \begin{cases} \frac{(2\eta)\omega_x \omega_y}{16(\omega^0 + \omega^-)} T \hat{z} & \omega_{\text{tf}} = \frac{\omega^0}{2} \\
\frac{(3-\eta)\omega_x \omega_z}{16(\omega^0 + \omega^-)} T \hat{y} & \omega_{\text{tf}} = \frac{\omega^-}{2} \\
\frac{(3+\eta)\omega_x \omega_z}{16(\omega^0 - \omega^0)} T \hat{x} & \omega_{\text{tf}} = \frac{\omega^+}{2}. \end{cases} \quad (C.15)$$
Note that the signal appears along a direction perpendicular to the two directions along which rf must be applied to excite any given transition. (For example, to excite the $\omega_0/2$ transition, rf is required along both $x$ and $y$ and signal appears along $z$.) required to excite each transition.

Unlike the spin-3/2 case, each of the principle planes in the quadrupolar (PAS) (i.e. the planes described by pairs of principle axes) correspond to a unique transition. Irradiation of a given transition produces signal along a unique direction, and unlike the spin-3/2 case, a single coil can be used for both excitation and detection, as long as it has components along three of the principle axes. This is true as long as $\eta$ is large enough that the transitions may be excited individually.

Unfortunately, no observable signal can be produced by two-photon excitation of a powder at half-resonance. This can be seen immediately from Eq. C.15 where the strength of the signal is proportional to terms like $\omega_x \omega_y$. One can imagine a crystal orientation where $\omega_y$ has a value that is exactly negative of that for another crystal orientation while $\omega_x$ are the same for both crystal orientations. The signal from these two orientations would appear along exactly opposite directions along the $z$-axis, producing no observable signal.

For the special case of an asymmetry parameter close to 1, the denominator in equation C.15 becomes very small for irradiation at $\omega^+/2$. In this case nutation rates will be significantly enhanced. However, the present analysis breaks down at $\eta = 1$ where other terms in the first order Magnus expansion begin to matter.