T_1 Relaxation and Inhomogeneous Magnetization Transfer in Brain: Physics and Applications

by

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Abstract

A major goal of the Magnetic Resonance Imaging (MRI) community is quantifying myelin in white matter. MRI contrast depends on tissue microstructure, so quantitative models require detailed understanding of Nuclear Magnetic Resonance (NMR) physics in white matter's complex, heterogeneous environment. In this thesis, we study the underlying physics behind two different ¹H contrast mechanisms in white and grey matter tissue: T_1 relaxation and the recently developed inhomogeneous Magnetization Transfer (ihMT).

Using *ex-vivo* white and grey matter samples of bovine brain, we performed a comprehensive solid-state NMR study of T_1 relaxation under six diverse initial conditions. For the first time, we used lineshape fitting to quantify the non-aqueous magnetization during relaxation. A four pool model describes our data well, matching with earlier studies. We also show examples of how the observed T_1 relaxation behaviour depends upon the initial conditions.

ihMT's sensitivity to lipid bilayers, like those in myelin, was originally thought to rely upon hole-burning in the supposedly inhomogeneously-broadened lipid lineshape. Our work shows that this is incorrect and that ihMT only requires the presence of dipolar couplings, not a specific kind of line broadening. We developed a simple explanation of ihMT using a spin-1 system. Using solid-state NMR, we then performed measurements of ihMT and T_{1D} (dipolar order relaxation time) on four samples: a multilamellar lipid system (Prolipid-161), wood, hair, and bovine tendon. ihMT was observed in all samples, even those with homogeneous broadening (wood and hair). Moreover, we saw no evidence of hole-burning.

Lastly, we present results from ihMT experiments with CPMG acquisition on the bovine brain samples. We show that myelin water has a higher ihMT signal than water outside the myelin. It was determined that this was due to the unique thermal motion in myelin lipids. In doing so, we developed a useful metric for determining the relative contributions from magnetization transfer and dipolar coupling to ihMT. Also, we applied a qualitative four pool model with dipolar reservoirs. Together, our results are consistent with myelin lipids having a T_{1D} which is appreciably longer than the T_{1D} of non-myelin lipids, despite recent measurements to the contrary.

Lay Summary

In diseases like Multiple Sclerosis (MS), a material in the brain called "myelin" is damaged. If nerve cells are like wires, then myelin is like their insulation: when myelin is damaged, nerve signals can't travel properly. My research focuses on methods for measuring myelin using an MRI scanner. This is important for more accurate diagnoses and for deeper study of diseases such as MS.

An MRI scanner is like an X-ray machine that's really good at taking pictures of brain tissue. Instead of X-rays, the pictures taken by MRI scanners are made using magnets and radio waves. How to distinguish myelin's unique radio waves, and thereby be able to quickly and accurately measure myelin using an MRI scanner, is the topic of my thesis.

Preface

The experiments performed in Chapter 5 were suggested by my two co-supervisors, Carl Michal and Alex MacKay. The pulse sequences were written by Carl Michal and modified by me. I built the sample holders, modified the spectrometer, and carried out the experiments. I wrote the data analysis and simulation scripts with guidance from Alex and Carl.

Chapter 6 of this thesis is based on the published paper:

AP Manning, KL Chang, AL MacKay, CA Michal, Journal of Magnetic Resonance **274**, 125–136 (2017) https://doi.org/10.1016/j.jmr.2016.11.013

Portions of Section 4.4.3 were also taken from this paper. Carl Michal conceived of the initial experiments. Kimberley Chang made the Prolipid-161 sample, wrote the pulse programs, and performed these experiments, under guidance from Carl and me. Carl and I developed the spin-1 model together. I developed the Provotorov model (building off of previously published work) and wrote the code to perform the simulations. Apart from Prolipid-161, I obtained all the other samples and ran the experiments on them. The majority of the manuscript was written by me based on Kimberley's undergraduate thesis. Alex and Carl also helped with the manuscript preparation.

The ihMT-CPMG experiments in Chapter 7 were originally envisioned by Carl. They were carried out by Patricia Angkiriwang and me. She wrote the final pulse program under guidance from Carl and I. This was based on an earlier version of the pulse program written by Esther Lin. Patricia also performed the preliminary analysis of the data for her undergraduate thesis. I performed the majority of the analysis and developed the four pool model with dipolar couplings independently.

I developed the circuit analogies in Appendix C and the derivation of the Provotorov Equations in Appendix A independently.

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List of Symbols and Acronyms

- β_D Inverse spin temperature of the Dipolar reservoir, page 30
- β_Z Inverse spin temperature of the Zeeman reservoir, page 30
- Δ Offset from resonance frequency, page 33
- η Weighting factor for MW residuals where TI < 37 ms, page 83
- γ nuclear gyromagnetic ratio, page 5
- \hat{H}_D Secular dipolar coupling Hamiltonian, page 24
- \hat{H}_Z Zeeman Hamiltonian, page 5
- \mathbf{B}_0 NMR/MRI main magnetic field, page 5
- \mathbf{B}_1 NMR/MRI oscillating, transverse magnetic field applied with coils., page 9
- \mathbf{B}_{eff} The total effective field in a rotating frame., page 9
- \mathbf{M}_0 Equilibrium magnetization, page 7
- Ω Fractional offset relative to ω_D , page 33
- ω_0 Larmor frequency, page 5
- ω_1 RF (B_1) field amplitude in rad/s, page 10
- ω_D Residual (RMS) dipolar coupling strength, page 25
- ρ Density matrix, page 18
- ρ_0 Equilibrium density matrix, page 20
- B_L The local field, page 27

m Magnetization in reduced units, page 63

 $M(\infty)$ Pool size, page 63

 $M_{\rm aq}$ The total aqueous magnetization (MW+IEW in the four pool model), page 71

 $M_{\rm non-aq}\,$ The total non-aqueous magnetization (M+NM in the four pool model), page 71

$$S_+$$
 ihMT experiment with prepulse at an offset $+\Delta$, page 56

- S_{-} ihMT experiment with prepulse at an offset $-\Delta$, page 56
- S_0 ihMT experiment with no prepulse, page 56
- S_{dual} ihMT experiment with prepulse at offsets $\pm \Delta$ simultaneously, page 56
- T_1 Longitudinal relaxation time / spin-lattice relaxation time, page 11
- T_2 Transverse relaxation time / spin-spin relaxation time, page 11
- T_2^* Observed T_2 relaxation time ($T_2^* < T_2$ because of field inhomogeneities), page 37
- T_{1D} Dipolar order (magnetization) spin-lattice relaxation time, page 32
- T_1^* The four pool model effective T_1 relaxation times, page 61
- T_{cr} Cross-relaxation time for adjacent pools, page 63
- TI Cross-relaxation delay in T_1 relaxation experiments, page 65
- W Saturation rate, page 33

ADRF/ARRF Adiabatic Demagnetization/Remagnetization in the Rotating Frame, page 31

- BPP Bloembergen, Purcell, and Pound saturation/relaxation theory, page 27
- BW bulk water (isolated) pool, page 72
- CNS Central Nervous System, page 41
- CPMG Carr-Purcell-Meiboom-Gill acquisition, page 37
- CSF Cerebrospinal Fluid, page 2
- E/R Exchange/relaxation factor for four pool model eigenvectors, page 84
- FID Free Induction Decay, page 14

- GS Goldman-Shen NMR experiment, page 67
- IEW Intra/Extra-cellular Water, page 58
- ihMT inhomogeneous Magnetization Transfer, page 56
- ih
MTR ih
MT Ratio, page 57
- IR Inversion-Recovery experiment, page 36
- M four pool model Myelin: the non-aqueous protons in the myelin sheath, page 62
- MAS Magic Angle Spinning NMR experiments, page 61
- MRI Magnetic Resonance Imaging, page 1
- MS Multiple Sclerosis, page 1
- MT Magnetization Transfer, page 54
- MTR Magnetization Transfer Ratio, page 54
- MW Myelin Water, page 44
- MWF Myelin Water Fraction, page 59
- NM four pool model pool "Non-Myelin": the non-aqueous protons outside the myelin sheath, page 62
- NMR Nuclear Magnetic Resonance, page 1
- NNLS Non-Negative Least Squares, page 59
- PNS Peripheral Nervous System, page 41
- qMT quantitative Magnetization Transfer, page 56
- rf magnetic fields at radio frequencies, page 7
- SNR Signal to Noise Ratio, page 6
- TR Tilted Rotating frame, page 30

Acknowledgements

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Finally, Asha, thank you for your love and encouragement. I'm a better person because of your patience—and, through much practice, a better cook.

Dedication

This work is dedicated to the memory of Evan MacRae

Chapter 1

Introduction

1.1 MRI

Magnetic Resonance Imaging (MRI) uses the physical phenomenon of Nuclear Magnetic Resonance (NMR) to create images. Nowadays, MRI is a ubiquitous medical imaging technology, given its excellent performance in soft tissue and its absence of ionizing radiation. It is especially useful for brain and spinal cord imaging and is the only modality suitable for diagnosing Multiple Sclerosis (MS) [1].

Ultimately, the contrast in MRI scans depends only on the NMR properties of the nucleus under study within distinct microstructural environments. In the majority of clinical and research contexts, the scanner detects the NMR signal from aqueous protons (¹H nuclei). That said, this signal's properties are dictated largely by interactions between aqueous and non-aqueous protons. Hence, these aqueous/non-aqueous interactions can allow indirect imaging of the non-aqueous protons.

Using different pulse sequences, an MRI scanner produces images which emphasize the variation within one or more of the NMR properties of different tissue. For example, two common ways of distinguishing between white and grey matter is by their T_1 relaxation times and aqueous proton concentrations. When a single aqueous T_1 time is assumed, white and grey matter have values of ~0.7 s and ~1.2 s respectively at a field strength of 1.5 T [3]. And grey matter has a higher water content than white matter, so the two are distinguishable on an image showing only differences in aqueous proton density.

To see these concepts in practice, Fig. 1.1 shows four images from different kinds of ¹H MRI scans of the same MS patient with obvious lesions. The image in panel A is weighted by (that is, its contrast reflects) the T_2 relaxation times. Aqueous proton density is emphasized



Figure 1.1: MR images of a 30 year old patient with MS. Different types of MRI techniques were used to generate contrast in different ways. Lesions (areas with myelin damage) can be seen in all of the images. (A) A T_2 weighted image. Short T_2 s appear dark. (B) A proton density weighted image. High density appears light. (C) A fluid-attenuated inversion recovery image, which attenuates signals from CSF. (D) T_1 weighted image after injection of a gadolinium contrast agent. Short T_1 s appear dark. Reproduced from ref. [2] [Journal of Neurology, Neurosurgery & Psychiatry, Trip & Miller, Volume 76, iii11–iii18], © 2005, with permission from BMJ Publishing Group Ltd.

by the pulse sequence used to acquire the image in panel B. The image in panel C was acquired after inverting the magnetization in the aqueous protons and waiting for a delay before acquisition. This reduces the signal from the cerebrospinal fluid (CSF), isolating the signal from aqueous protons within the tissue. Finally, the patient has had a contrast agent injected prior to acquiring the image in panel D, which is T_1 -weighted. The contrast agent selectively reduces the T_1 relaxation time of specific tissues.

This thesis will cover the physics behind these images extensively except for contrast agents. These are administered intravenously or orally and selectively change the relaxation times in specific tissues, compartments, or organs. Currently, contrast agents are used in about 25% of all MRI exams [4,5]. Compounds with high specificity to certain tissues (such as myelin [6]) or pathologies (such as amyloid plaques [7]) have been developed. However, because of safety and regulatory considerations, their use is often limited to animal models [8]. In this thesis,

we are concerned with ways of improving the specificity of MRI without the use of contrast agents, so they won't be discussed further.

1.2 Motivation: quantitative MRI of myelin

In clinical practice, non-quantitative MRI scans are used most of the time; apart from observing the presence and morphology of lesions, the end result rarely contains precise measures of the tissue microstructure properties. Quantitative MRI is a highly desirable goal for both clinicians and scientists, however: quantifying microscopic disease pathologies would lead to better diagnosis and management, more advanced research into disease mechanisms, and more precise metrics for judging treatment effectiveness. For these reasons, there is now a push by the MRI research community towards developing and implementing quantitative techniques.

Quantitative MRI sequences and models seek to develop biomarkers for specific tissue components or morphologies. This requires a fundamental understanding of the physical origins of the NMR signal and its properties. In this thesis, we are concerned with the development of biomarkers for myelin. Myelin is a substance surrounding axons and is essential for proper nervous signal transmission. It is a major component of white matter tissue.

The research in this thesis covers two approaches to myelin quantification: T_1 relaxation and inhomogeneous Magnetization Transfer (ihMT). We try to understand both of these on a fundamental, physical level. Currently, measurements of T_1 relaxation in white matter disagree. The values obtained by different groups show unexplained variation and even the number of T_1 components present is unclear. Our work emphasizes that there are indeed multiple components, but they cannot be cleanly associated with specific compartments. ihMT is a new technique which allows one to calculate a simple ratio whose value may be a biomarker for myelin. In this thesis we argue that the original hypothesis explaining it—inhomogeneous broadening of the non-aqueous lineshape—is incorrect. More generally, we explore its fundamental physics, from its origin in the non-aqueous protons to its manifestation in separate compartments of aqueous protons.

Our tool to study white and grey matter is solid-state NMR spectroscopy, allowing straightforward observation of both the aqueous and non-aqueous proton signals. The samples we use are biological materials or phantoms of tissue. In particular, the grey and white matter we investigate is from *ex-vivo* bovine brain. Because we are concerned with fundamental NMR properties, there is no imaging performed in this thesis, but we expect that the work here will be useful in guiding future quantitative MRI development.

1.3 Outline

The next three chapters are background material. In Chapter 2 we give an overview of the classical and quantum physics of NMR. Topics which are relevant to the rest of the thesis are emphasized, including the dipolar interaction and saturation theories. After this, Chapter 3 is a short introduction to the structure and function of myelin. Diseases of the myelin are briefly covered, focusing on multiple sclerosis (MS). Moving to Chapter 4, we give some necessary information about the relaxation and spectral properties of NMR in white and grey matter. The spectral lineshape of the non-aqueous ¹H nuclei—the super-Lorentzian—is introduced. Then, we explore some of the controversy surrounding T_1 relaxation in white matter. We show how Magnetization Transfer (MT) and the MT ratio (MTR) is a useful consequence of aqueous/non-aqueous magnetization exchange. T_2 relaxation, which reveals distinct aqueous compartments in white matter, is the final topic.

With the background material out of the way, Chapter 5 is the first chapter with original work: an exhaustive study of T_1 relaxation in bovine white and grey matter. There, the four pool model is introduced and is used to analyze the results.

Chapter 6 discusses a suite of experiments investigating the fundamental physics of ihMT. We performed ihMT experiments and measured dipolar order relaxation in a multilamellar lipid system (a phantom for myelin), hair, wood, and bovine tendon. Based on ihMT's connection with dipolar couplings, we also introduce a spin-1 model of ihMT. Our results suggest that ihMT does not rely on inhomogeneous broadening.

Our last results are in Chapter 7, where we unite concepts from the previous two chapters. We carried out ihMT experiments with Carr-Purcell-Meiboom-Gill (CPMG) acquisition in the same bovine white and grey matter. This allowed observation of the separate ihMT signals from the myelin water and intra/extra-cellular water. We apply the four pool model, now modified with the addition of dipolar reservoirs, to qualitatively model our results.

Finally, in Chapter 8 we review the results and suggest future experiments.

The appendices contain additional calculations which are not integral to the main thesis. These include a derivation of the Provotorov equations, an outline of how to calculate the correction factor for exchange during CPMG acquisition, circuit analogies of the four pool model and ihMT, and modeling of pulse-train ihMT prepulses.

Chapter 2

NMR theory

2.1 The Zeeman interaction and its implications

Nuclei with unpaired protons or neutrons have nonzero nuclear spins. This leads to a nuclear magnetic moment, $\boldsymbol{\mu} = \gamma(\hbar \mathbf{I})$, where γ is the gyromagnetic ratio and \mathbf{I} the nuclear spin. (We will use the convention of unitless spin operators, hence the explicit factor of \hbar .) Nuclei like this interact with magnetic fields via the Zeeman interaction. The Zeeman Hamiltonian is¹

$$\hbar \hat{H}_Z = -\boldsymbol{\mu} \cdot \mathbf{B}_0.$$

The units of \hat{H}_Z are rad/s and \mathbf{B}_0 is the main spectrometer field, which is taken to be in the z direction: $\mathbf{B}_0 = B_0 \hat{z}$. Hence, the Zeeman Hamiltonian (in rad/s) is

$$\hat{H}_Z = -\gamma B_0 \hat{I}_z \tag{2.1}$$
$$= \omega_0 \hat{I}_z,$$

where $\omega_0 = -\gamma B_0$ is the Larmor frequency of precession. This identification anticipates the connection to the classical theory of precession.

With an expression for the Zeeman energy, it is illuminating to calculate the thermal polarization in a typical NMR spectrometer or MRI scanner B_0 field. Consider protons², which, as spin- $\frac{1}{2}$ particles, have $I = \frac{1}{2}$ and $m = \pm \frac{1}{2}$. The energies of these two eigenstates are

¹In this chapter we will cite references only when necessary, since the content here parallels most introductory textbooks. Sources which the author relied on for this content were Duer [9], Slichter [10], Schmidt-Rohr & Spiess [11], and a useful report by Goldman [12].

²When NMR and MRI physicists say "protons", they are always referring to a ¹H nucleus. By the same token, "spins" almost always refer to nuclear spins.

 $\left\langle \pm \frac{1}{2} \left| \hat{H}_Z \right| \pm \frac{1}{2} \right\rangle = \pm \frac{1}{2} \omega_0$ and the energy difference is $\Delta E = \hbar \omega_0$. If $N_+ + N_-$ is the total number of spins in a sample, with N_{\pm} representing the number in the $|\pm \frac{1}{2}\rangle$ state, then the relative polarization is

$$\begin{split} \frac{N_+ - N_-}{N_+ + N_-} &\approx \frac{1}{2} \left(1 - \frac{N_-}{N_+} \right) \\ &= \frac{1}{2} \left(1 - \exp(-\Delta E/k_B T) \right) \\ &\approx \frac{\Delta E}{2k_B T}, \end{split}$$

where k_B is Boltzmann's constant and T is the temperature in Kelvin. In a 100 MHz (2.3 T) field³ at 300 K, this is only 8 ppm for protons—a very small polarization indeed! We can compare this to the typical polarizations seen in Electron Spin Resonance (ESR). For protons, $\gamma/2\pi = 42.577$ MHz/T, and for an unpaired electron, $\gamma_e/2\pi = 28.025$ GHz/T. The thermal polarization of a sample with unpaired electrons is about $\gamma_e/\gamma \approx 658 \times$ higher.

The relative weakness of the nuclear Zeeman effect dictates the experimental constraints and features of NMR and MRI. Without $B_0 \gtrsim 0.1$ T, performing NMR and MRI experiments is difficult.⁴ For MRI, the range of nuclei which can be imaged in clinically reasonable times is limited to ¹H and a few others (eg. ²³Na and ³¹P). All of these are abundant enough in the human body to be imaged directly. However, protons are the most common nuclei to image due to their superior signal to noise ratio (SNR) in biological samples. There are three reasons for this. First, with tissue being ~70% water, they are ubiquitous in biochemical systems. Second, NMR SNR is approximately proportional to ω_0^2 [15, 16], so it makes sense to use nuclei with high Larmor frequencies.⁵ ¹H also leads in this category, for only the extremely rare ³H has a higher γ . Finally, naturally occurring Hydrogen is isotopically pure, with ~99.98% of all H atoms containing ¹H nuclei [19].

On a more fundamental level, the weak nuclear Zeeman effect sets NMR apart from many other forms of spectroscopy because of its unique method of relaxation. Unlike optical spectroscopy, excited states in NMR do not relax via stimulated and/or spontaneous emission (see Hoult [20] and references therein). Rather, relaxation is driven by environmental fluc-

³The B_0 and B_1 field strengths of NMR spectrometers is typically stated in the Larmor frequency (in Hz) of protons in that field. Conversely, in MRI these are usually given in Tesla.

⁴But not impossible. In fact, NMR and MRI has been performed in the Earth's magnetic field (*eg.* see references [13,14]). Although weak, the Earth's magnetic field is extremely homogeneous. However, Earth's Field NMR and MRI are of limited practical utility, and typically can't observe any nuclei except ¹H.

⁵In MRI, the SNR is more complicated. It is very sensitive to the sample geometry and composition. Also, because repeated scans are required to cover all of k-space, it doesn't make sense to speak of the SNR for a single acquisition. Furthermore, the image is typically made from the magnitude of the NMR signal, and this has a Rician noise profile. See Macovski [17] and Ocali & Atalar [18] for more details.

tuations, *i.e.* coupling to the lattice. Therefore, the relaxation processes in NMR and MRI can be a useful window into the microscopic structure and motion of a sample. We will have much more to say on this in the following chapters.

Because of the nuclear Zeeman effect's low energy, NMR frequencies are in the 1 MHz – 1 GHz range, corresponding to radiation wavelengths of 300–0.3 m. How then are MRI scanners capable of sub-millimeter resolution? Even though precessing spins emit radio waves in the far-field limit, NMR and MRI operate in the near-field limit (*i.e.* the distance to the emitters is small or comparable to the wavelength) [15, 20, 21]. In this regime, the emission from an ensemble of precessing spins manifests as an oscillating magnetic field which, unlike electromagnetic radiation, does not impose limits on resolution based on wavelength [20–22]. Correspondingly, the spins are manipulated with magnetic fields oscillating at radio frequencies, and, in MRI, the spatial resolution is instead limited by the strength of the magnetic field gradients [23]. Obviously, this is the reason for the "M" in NMR! In the literature, the transmitted and detected magnetic fields are commonly referred to as "rf".

Magnetization is the macroscopic result of the Zeeman interaction. The equilibrium magnetization in a sample inside a field \mathbf{B}_0 is

$$\mathbf{M}_{\mathbf{0}} = \chi_0 \mathbf{B}_{\mathbf{0}},\tag{2.2}$$

where χ_0 is the static magnetic susceptibility.

2.2 Classical treatment of NMR

2.2.1 Precession and the rotating frame

The classical picture of NMR is a straightforward way to introduce basic NMR dynamics and experiments. The equations of motion for a magnetization \mathbf{M} in a field \mathbf{B} are

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times (\gamma \mathbf{B}),\tag{2.3}$$

which describes the precession of **M** around **B** at a frequency $\omega = -\gamma B$.

Excluding relaxation, this compact equation contains all the classical dynamics of NMR. It applies whether \mathbf{M} and/or \mathbf{B} are constant or varying. Nonetheless, it isn't terribly straightforward to use in practice. One of the problems with Eq. 2.3 is that it describes the dynamics

of precession in the laboratory frame. Most calculations in NMR are simplified by working in the rotating frame, a coordinate system which rotates around the z axis.

Slichter [10] has a nice derivation of how vectors are transformed between the lab and rotating frames. Consider an arbitrary vector function $\mathbf{F}(t) = \sum_{i=x,y,z} F_i \hat{i}$, where the \hat{i} unit vectors are aligned with the lab frame. Now, assume that this coordinate frame rotates with an angular velocity $\boldsymbol{\omega}$. In this case, the lab frame time rate of change for \hat{i} is

$$rac{d\hat{i}}{dt} = oldsymbol{\omega} imes \hat{i}.$$

The time derivative of $\mathbf{F}(t)$ in the lab frame is now more complicated, but gives a useful result:

$$\frac{d\mathbf{F}(t)}{dt}\Big|_{lab} = \sum_{i=x,y,z} \left(\frac{dF_i}{dt}\hat{i} + F_i\frac{d\hat{i}}{dt}\right) \\
= \sum_{i=x,y,z} \frac{dF_i}{dt}\hat{i} + \sum_{i=x,y,z} F_i\frac{d\hat{i}}{dt} \\
= \sum_{i=x,y,z} \frac{dF_i}{dt}\hat{i} + \boldsymbol{\omega} \times \mathbf{F}(t) \\
= \left.\frac{d\mathbf{F}(t)}{dt}\Big|_{rot} + \boldsymbol{\omega} \times \mathbf{F}(t),$$
(2.4)

where $\frac{d\mathbf{F}(t)}{dt}\Big|_{rot}$ is the time rate of change in the rotating frame. If we apply this to **M**, then from Eq. 2.3 we have

$$\begin{aligned} \left. \frac{d\mathbf{M}}{dt} \right|_{lab} = \mathbf{M} \times (\gamma \mathbf{B}) \\ = \left. \frac{d\mathbf{M}}{dt} \right|_{rot} - \mathbf{M} \times \boldsymbol{\omega} \end{aligned}$$
$$\Rightarrow \left. \frac{d\mathbf{M}}{dt} \right|_{rot} = \mathbf{M} \times (\gamma \mathbf{B} + \boldsymbol{\omega}). \end{aligned}$$

And so, in the rotating frame Eq. 2.3 still applies, provided that we replace \mathbf{B}_0 with an effective field

$$\mathbf{B}_{\rm eff} = \mathbf{B} + \frac{\boldsymbol{\omega}}{\gamma}.\tag{2.5}$$

When only B_0 is present, by convention the precession axis is taken to be \hat{z} . Hence, in the

 \hat{z} direction we have

$$B_{\text{eff}} = B_0 + \frac{\omega}{\gamma}$$

$$\Rightarrow \gamma B_{\text{eff}} = \gamma B_0 + \omega$$

$$\omega_{\text{eff}} = \omega_0 - \omega.$$
(2.6)

Where $\omega_{\text{eff}} = -\gamma B_{\text{eff}}$ is the effective precession frequency in the frame rotating at ω . If $\omega = \omega_0$, then ω_{eff} and B_{eff} are both zero: the effect of the main spectrometer field \mathbf{B}_0 has been completely removed.

NMR uses a B_1 field to manipulate the magnetization. The B_1 field is applied in the transverse plane and oscillates at ω :

$$\mathbf{B}_{1}(t) = B_{1}\cos(\omega t + \phi)\hat{x} + B_{1}\sin(\omega t + \phi)\hat{y}, \qquad (2.7)$$

where ϕ is some arbitrary phase factor. We make no assumptions about ω : it could be on or off resonance. It's easiest to analyze the problem in a frame rotating at ω . The time derivative is then

$$\frac{d\mathbf{B}_{1}(t)}{dt}\Big|_{rot} = \left.\frac{d\mathbf{B}_{1}(t)}{dt}\right|_{lab} - (\omega\hat{z}) \times \mathbf{B}_{1}(t)$$
$$= B_{1}(\omega - \omega)\sin(\omega t + \phi)\hat{x} + B_{1}(\omega - \omega)\cos(\omega t + \phi)\hat{y},$$
$$= 0.$$

In this frame it appears as a constant magnetic field perpendicular to \hat{z} , around which **M** rotates according to Eq. 2.3. This rotation around **B**₁ is called nutation. Combining this result for B_1 with the result for B_0 in Eq. 2.6, the total effective field in the frame rotating at the frequency of the rf pulse is

$$\mathbf{B}_{\text{eff}} = \left(B_0 + \frac{\omega}{\gamma}\right)\hat{z} + B_1\hat{x},\tag{2.8}$$

where we have chosen the phase of the rf pulse ϕ such that B_1 is along \hat{x} . Another way to write this is

$$\boldsymbol{\omega}_{\text{eff}} = (\omega_0 - \omega)\hat{z} + \omega_1\hat{x} \tag{2.9}$$

with

$$\omega_1 = |\gamma| B_1 \tag{2.10}$$



Figure 2.1: Dynamics in a frame rotating at the B_1 frequency ω . Field strengths are indicated in units of rad/s (see Eqs. 2.8 and 2.9). (A) The effective field, $\boldsymbol{\omega}_{\text{eff}}$, composed of the effective main field $\omega_0 - \omega$ and the amplitude of the rf field, ω_1 . (B) The magnetization **M** precesses around the effective field. After Fig. 2.4 in Slichter [10].

as the amplitude of the rf pulse in rad/s—which, by convention, is always positive. The dynamics of **M** in this frame are shown in Fig. 2.1. If the rf pulse is on resonance ($\omega = \omega_0$), the effect of B_0 is completely removed.

In the preceding discussion, we have glossed over the issue of the sign of γ and the direction of **M**'s precession and nutation. Most NMR-active nuclei, including ¹H and ¹³C, have $\gamma > 0$. Referring to Eq. 2.3 and applying the right-hand rule, this implies a clockwise precession of **M** about **B**, which is a negative angular frequency. Indeed, if $\mathbf{B} = \mathbf{B}_0$, then the definition $\omega_0 = -\gamma B_0$ gives a negative Larmor frequency, as required. If $\gamma < 0$ (eg. ¹⁵N), then the precession of **M** is in the counter-clockwise direction and $\omega_0 > 0$. Regarding nutation in the B_1 field, the definition of ω_1 (Eq. 2.10) means it will always be positive—independent of the sign of γ —implying a counter-clockwise nutation of **M** about the effective field in the rotating frame. Experimentally, we could ensure $\omega_1 > 0$ by appropriately selecting ϕ , the B_1 rf pulse phase.

In practice, it is rarely necessary to keep track of the correct precession and nutation directions in the analysis of single-nuclei experiments, so long as one is consistent. For a deeper discussion, the reader is referred to Levitt's papers on the subject [24, 25]. For the sake of clarity, we will assume counter-clockwise nutation of \mathbf{M} about the B_1 field for the remainder of this thesis.

2.2.2 The Bloch equations

Before getting too far into the discussion of how rf pulses (the B_1 field) affect the spins, the Bloch equations should be introduced. These phenomenological equations incorporate relaxation into the dynamics described by Eq. 2.3. The Bloch equations are approximate and do not accurately describe the dynamics of NMR in all situations—only in systems of isolated spin- $\frac{1}{2}$ nuclei in low-viscosity solutions are they exact. Still, they provide an excellent framework for intuitive understanding. And, even where they aren't rigorously accurate, they can often be modified to model the situation anyway.

The Bloch equations in the lab frame are

$$\frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1} + \gamma (\mathbf{M} \times \mathbf{B})_z$$

$$\frac{dM_{x,y}}{dt} = \gamma (\mathbf{M} \times \mathbf{B})_{x,y} - \frac{M_{x,y}}{T_2}.$$
(2.11)

 T_1 is the spin-lattice or longitudinal relaxation time, T_2 is the spin-spin or transverse relaxation time, and $T_2 \leq T_1$. T_1 is a result of the spin-lattice coupling that returns the magnetization to thermal equilibrium $\mathbf{M}_0 = M_0 \hat{z}$ via

$$M_{z}(t) = M_{0} \left(1 - \left(1 - \frac{M_{z}(0)}{M_{0}} \right) \exp\left(-\frac{t}{T_{1}} \right) \right).$$
(2.12)

There can be no transverse components of magnetization in equilibrium: M_x and M_y must decay. They do so exponentially with time constant T_2 via

$$M_{x,y}(t) = M_{x,y}(0) \exp\left(-\frac{t}{T_2}\right).$$
 (2.13)

Dephasing of the precessing spins causes T_2 relaxation. In practice, we often refer to spinlattice and spin-spin relaxation as T_1 and T_2 relaxation, even in cases where there is not a single, well-defined value for either.

Now, consider the Bloch equations for a sample in an NMR spectrometer under the influence of rf pulses on resonance. In this situation, $\mathbf{B}_{lab} = \mathbf{B}_0 + \mathbf{B}_1(t)$, where $\mathbf{B}_1(t)$ is rotating in the transverse plane at ω_0 (Eq. 2.7). Following the last section, in the rotating frame this becomes $\mathbf{B}_{rot} = B_1 \hat{x}$, where we have chosen the *x*-axis to be the direction of $\mathbf{B}_1(0)$. Correspondingly, $\mathbf{M} \times \mathbf{B}_{rot} = B_1(M_z \hat{y} - M_y \hat{z})$, so the Bloch equations in the rotating frame are

$$\frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1} - \omega_1 M_y$$

$$\frac{dM_y}{dt} = \omega_1 M_z - \frac{M_y}{T_2}$$

$$\frac{dM_x}{dt} = 0.$$
(2.14)



Figure 2.2: The relative orientation of the sample, \mathbf{B}_0 , and $\mathbf{B}_1(t)$.

2.2.3 A simple NMR experiment and the Lorentzian lineshape

The relative orientation of \mathbf{B}_0 and $\mathbf{B}_1(t)$ to the sample and the transceiver coil is shown in Fig. 2.2. The solenoidal coil style in this figure was used to complete all NMR experiments in this thesis, though many other coil styles exist.

In thermal equilibrium, the sample has a magnetization $\mathbf{M}_0 = M_0 \hat{z}$. In a simple NMR experiment, a B_1 pulse is applied to tilt \mathbf{M} away from the z-axis. Experimentally, this is achieved by applying an oscillating voltage V(t) across the coil resonance circuit,

$$V(t) \sim \exp(-i(\omega t + \phi))$$

where ϕ is phase under the experimenter's control. This causes an oscillating, linearlypolarized B_1 field,

$$\mathbf{B}_1(t) = 2B_1 \cos(\omega t + \phi')\hat{x},$$

where $2B_1$ is an amplitude under the control of the experimenter. Regarding the phase factors, ϕ and ϕ' vary only by an additive constant for a given sample and experimental set-up. Therefore, the relative phases of multiple B_1 pulses (either in the same experiment or in repeated experiments) can be carefully controlled. In other words, the rf pulses used in NMR and MRI pulse sequences are coherent.

The oscillating B_1 field along the x-axis may be mathematically decomposed into two

counter-rotating fields in the x-y plane:

$$\mathbf{B}_{1}(t) = 2B_{1}\cos(\omega t + \phi')\hat{x}$$
$$= B_{1}\left(\cos(\omega t + \phi')\hat{x} + \sin(\omega t + \phi')\hat{y}\right) + B_{1}\left(\cos(\omega t + \phi')\hat{x} - \sin(\omega t + \phi')\hat{y}\right). \quad (2.15)$$

Under most circumstances, the field rotating opposite to the precession direction of **M** has negligible effect on the spins' dynamics, and can be safely ignored in the analysis of most experiments. As such, with a linearly-polarized B_1 field, half of the rf power is wasted. In NMR, this rarely poses a large enough problem to address, since doing so requires nonstandard coil designs and resonance circuits. In these configurations, changing the sample is often difficult. On the other hand, in MRI the wasted power is absorbed by the patient, posing limitations on B_1 strength and duration. In modern MRI scanners, this is addressed by using quadrature coils [26, 27]. In the most simple configuration, two perpendicular linearly-polarized B_1 coils are used. When transmitting, the sinusoidal current in the two coils has a relative phase shift of 90°, producing circular B_1 polarization. Due to the large bore of MRI scanners, coils with geometries designed for imaging specific regions can be placed directly on the patient.

Returning to the simple experiment, when B_1 is turned on with an amplitude $\omega_1 \gg T_1^{-1}$ and $\omega_1 \gg T_2^{-1}$, relaxation effects may be temporarily ignored. If the total duration of the B_1 pulse is τ , then in the rotating frame

$$\mathbf{M}(\tau) = M_0 \cos(\omega_1 \tau) \hat{z} - M_0 \sin(\omega_1 \tau) \hat{y}.$$

Let's consider the case of $\omega_1 \tau = \frac{\pi}{2}$, which is a "90-degree" pulse. Immediately following this pulse, $\mathbf{M}(\tau) = -M_0 \hat{y}$, which is a 90° rotation from equilibrium. With components in the transverse plane, \mathbf{M} precesses around \hat{z} at ω_0 .

The precessing magnetization induces a voltage in the coil, which is now used as a receiver. The voltage is converted into a complex signal, S(t). Up to a constant, this is given by

$$S(t) = M_{transverse}(t) \exp\left(i(\omega_0 - \omega_{ref})t\right)$$

$$= \sqrt{M_x(t)^2 + M_y(t)^2} \exp\left(i(\omega_0 - \omega_{ref})t\right),$$
(2.16)

where $M_{transverse}(t)$ is the magnitude of the magnetization in the transverse (x-y) plane precessing at ω_0 and ω_{ref} is a reference frequency corresponding to the center of the spectrum. Taking into account T_2 relaxation, the signal after the 90° pulse is

$$S(t) = M_0 \exp(-t/T_2) \exp(i(\omega_0 - \omega_{ref})t).$$
(2.17)

The 90° pulse gives the maximum signal intensity. If a 45° pulse was used instead, $M_{transverse}(0)$ and S(0) would both be reduced by $\frac{1}{\sqrt{2}}$.

The NMR signal acquired with a single pulse is called the Free Induction Decay (FID). The Fourier transform of the FID gives the NMR spectrum, which in this case is [28]

$$S(\omega) = \mathcal{F}\left\{S(t)u(t)e^{i\phi_0}\right\}$$

= $e^{i\phi_0}\left[\frac{T_2}{1+(\omega-\Delta\omega)^2 T_2^2} - i\frac{(\omega-\Delta\omega)T_2^2}{1+(\omega-\Delta\omega)^2 T_2^2}\right],$ (2.18)

where $\Delta \omega = \omega_0 - \omega_{ref}$. The first and second terms in the brackets represent the absorption and dispersion parts of the Lorentzian lineshape. Two factors have been explicitly inserted prior to the Fourier transform: the zeroth-order phase factor, $e^{i\phi_0}$, and the unit step or Heaviside function u(t), given by

$$u(t) = \begin{cases} 0 & t < 0 \\ 1 & t > 0. \end{cases}$$

The first is necessary to complete the Fourier transform properly since S(t < 0) = 0. The second arises from the NMR receiver chain. In NMR, spectra are represented using the absorption lineshapes, so phase correcting $S(\omega)$ (multiplying by $e^{-i\phi_0}$ for a zeroth order correction) is necessary to isolate the pure absorption part. Exponential decay of the FID from the T_2 time corresponds to a Lorentzian lineshape in the frequency domain. Evidently, the Bloch equations naturally lead to a Lorentzian lineshape.

2.3 Quantum mechanical treatment of NMR

2.3.1 NMR in Hilbert space

Having seen the classical approach, the next step is some basic calculations using state vectors evolving under the Schrödinger equation. This "Hilbert Space" approach is actually not used very often, since the ensemble average of $\sim 10^{23}$ magnetic moments in a sample lends itself

to classical calculations (preceding sections) or density matrices (following section). Still, it a useful bridge into the quantum mechanics of NMR.

The Schrödinger equation in the lab frame is

$$i\frac{\partial}{\partial t}\left|\psi\right\rangle = \hat{H}\left|\psi\right\rangle \tag{2.19}$$

where \hat{H} has units of rad/s. For the time being we shall only deal with cases where the Hamiltonian is constant, leading to the formal solution

$$|\psi(t)\rangle = \exp(-i\hat{H}t) |\psi(0)\rangle. \qquad (2.20)$$

The operator $\exp(-i\hat{H}t)$ is called the propagator. Two important properties of the propagator are i) $\hat{A} \exp(\hat{B}) = \exp(\hat{B})\hat{A}$ only if $[\hat{A}, \hat{B}] = 0$; and ii) if $|\alpha\rangle$ is an eigenstate of \hat{A} , then $\exp(\hat{A})|\alpha\rangle = \exp(\alpha)|\alpha\rangle$.

Let's consider the expectation values of two different states for a spin-1/2 nucleus under $\hat{H} = \omega_0 \hat{I}_z$ (no B_1 field). The first is the eigenstate $\left|\frac{1}{2}\right\rangle$. Unsurprisingly,

$$\begin{aligned} \langle I_z \rangle &= \langle \frac{1}{2} | \exp(i\omega_0 \hat{I}_z t) \hat{I}_z \exp(-i\omega_0 \hat{I}_z t) | \frac{1}{2} \rangle \\ &= \langle \frac{1}{2} | \hat{I}_z | \frac{1}{2} \rangle \\ &= \frac{1}{2} \end{aligned}$$

In the same way we can also show that $\langle \hat{I}_x \rangle = \langle \hat{I}_y \rangle = 0$ for this state. For a more interesting example, if the spin state is now $|x; +\frac{1}{2}\rangle = \frac{1}{\sqrt{2}} \left(|\frac{1}{2}\rangle + |-\frac{1}{2}\rangle \right)$, then $\langle I_z \rangle$ is

$$\begin{aligned} \langle I_z \rangle &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \exp(i\omega_0 \hat{I}_z t) \hat{I}_z \exp(-i\omega_0 \hat{I}_z t) \left(|\frac{1}{2}\rangle + |-\frac{1}{2}\rangle \right) \\ &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \left(\frac{1}{2} |\frac{1}{2}\rangle - \frac{1}{2} | -\frac{1}{2}\rangle \right) \\ &= 0. \end{aligned}$$

And $\langle \hat{I}_x \rangle$ is

$$\begin{split} \langle \hat{I}_x \rangle &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \exp(i\omega_0 \hat{I}_z t) \hat{I}_x \exp(-i\omega_0 \hat{I}_z t) \left(|\frac{1}{2} \rangle + |-\frac{1}{2} \rangle \right) \\ &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \exp(i\omega_0 \hat{I}_z t) \hat{I}_x \left(\exp(-i\frac{\omega_0}{2} t) |\frac{1}{2} \rangle + \exp(i\frac{\omega_0}{2} t) |-\frac{1}{2} \rangle \right) \\ &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \exp(i\omega_0 \hat{I}_z t) \left(\frac{1}{2} \exp(-i\frac{\omega_0}{2} t) |-\frac{1}{2} \rangle + \frac{1}{2} \exp(i\frac{\omega_0}{2} t) |\frac{1}{2} \rangle \right) \\ &= \frac{1}{2} \left(\langle \frac{1}{2} | + \langle -\frac{1}{2} | \right) \left(\frac{1}{2} \exp(-i\omega_0 t) |-\frac{1}{2} \rangle + \frac{1}{2} \exp(i\omega_0 t) |\frac{1}{2} \rangle \right) \\ &= \frac{1}{2} \cos(\omega_0 t). \end{split}$$

Similar calculations also show that $\langle \hat{I}_y \rangle = \frac{1}{2} \sin(\omega_0 t)$. In the lab frame, $\langle \hat{I}_y \rangle \hat{x} + \langle \hat{I}_x \rangle \hat{y}$ represents the precession of the spin's magnetic moment around \mathbf{B}_0 at the Larmor frequency. One isolated spin's expectation values behaves like the macroscopic magnetization.

Now let's apply a B_1 pulse rotating around \hat{z} at a frequency ω with an amplitude ω_1 . In the lab frame,

$$\hat{H} = \omega_0 \hat{I}_z + \omega_1 \cos(\omega t) \hat{I}_x + \omega_1 \sin(\omega t) \hat{I}_y$$

$$= \omega_0 \hat{I}_z + \omega_1 \exp(-i\omega \hat{I}_z t) \hat{I}_x \exp(i\omega \hat{I}_z t).$$
(2.21)

On the second line we have applied a useful property of the angular momentum operators,

$$\exp(-i\phi\hat{I}_l)\hat{I}_m \exp(i\phi\hat{I}_l) = \hat{I}_m \cos\phi + \hat{I}_n \sin\phi$$
for cyclic permutations of $l, m, n = \{x, y, z\},$

$$(2.22)$$

which arises from their well-known commutation relations, $[\hat{I}_l, \hat{I}_m] = i\hat{I}_n$ (and cyclic permutations thereof). In other words, $\exp(i\phi\hat{I}_l)$ is a generator of rotations around axis l. We will use this property extensively when working with density matrices under rf pulses.

The lab frame Hamiltonian in Eq. 2.21 is unsuitable to solve with the propagator in Eq. 2.20 because of its time dependence. As in the classical case, a transformation into the rotating frame simplifies the problem. We do this by moving the time-dependent part of the Hamiltonian into the state kets. This technique can be applied to any Hamiltonian in the form $\hat{H}(t) = \hat{H}_0 + \hat{V}(t)$ and moves the problem into what is known as the *interaction representation*. Consider an arbitrary state ket in the lab and rotating frame, indicated by $|\psi\rangle$ and $|\psi'\rangle$ respectively. They are related by

$$\begin{aligned} |\psi'\rangle &= \exp(i\omega t \hat{I}_z) |\psi\rangle \\ \Rightarrow |\psi\rangle &= \exp(-i\omega t \hat{I}_z) |\psi'\rangle \end{aligned}$$

The procedure to find the dynamics of the transformed state is similar to the one above for the transformation of a classical vector into the rotating frame (Eq. 2.4). The time derivative is

$$\frac{\partial}{\partial t}|\psi\rangle = -i\omega\hat{I}_z \exp(-i\omega t\hat{I}_z)|\psi'\rangle + \exp(-i\omega t\hat{I}_z)\frac{\partial}{\partial t}|\psi'\rangle,$$

which we equate to $\hat{H}|\psi\rangle$ (via Schrödinger's equation, Eq. 2.19). After some algebra, we find Schrödinger's equation in the rotating frame,

$$i\frac{\partial}{\partial t}|\psi'\rangle = \left((\omega_0 - \omega)\hat{I}_z + \omega_1\hat{I}_x\right)|\psi'\rangle.$$

$$i\frac{\partial}{\partial t}|\psi'\rangle = \hat{H}'|\psi'\rangle.$$
(2.23)

The effective Hamiltonian in the rotating frame, \hat{H}' , is now static. The B_1 field is constant along the *x*-axis and the effective B_0 field is

$$B_{\text{eff}} = B_0 + \frac{\omega}{\gamma},$$

as in the classical case (Eq. 2.5).

Now, we apply this rotating frame representation to calculate the effect of a B_1 pulse of duration τ . If we start with a $|\frac{1}{2}\rangle$ state, the expectation values change in time:

$$\begin{aligned} \langle \hat{I}_z \rangle &= \langle \frac{1}{2} |\exp(i\hat{H}'\tau)\hat{I}_x \exp(-i\hat{H}'\tau)|\frac{1}{2} \rangle \\ &= \langle \frac{1}{2} |\exp(i\left((\omega_0 - \omega)\hat{I}_z + \omega_1\hat{I}_x\right)\tau)\hat{I}_x \exp(-i\left((\omega_0 - \omega)\hat{I}_z + \omega_1\hat{I}_x\right)\tau)|\frac{1}{2} \rangle \end{aligned}$$

This is tedious to calculate when $\omega \neq \omega_0$, since $e^{\hat{A}+\hat{B}} = e^{\hat{A}}e^{\hat{B}}$ only if $[\hat{A}, \hat{B}] = 0$, which isn't the case for \hat{I}_z and \hat{I}_x . One possible approach would be to use the matrix form of \hat{H}' and diagonalize it. This would be written as $H' = PDP^{-1}$, where D is a diagonal matrix with entries $\lambda_1, \lambda_2, \ldots, \lambda_n$, the eigenvalues of H'. Then, the properties of the matrix exponential are such that

$$\exp(-iH'\tau) = \exp(-iPDP^{-1}\tau)$$
$$= P\exp(-iD\tau)P^{-1}$$
$$= P\begin{bmatrix} e^{-i\lambda_{1}\tau} & \cdots & 0\\ \vdots & \ddots & \vdots\\ 0 & \cdots & e^{-i\lambda_{n}\tau} \end{bmatrix} P^{-1}.$$

Another approach is to use a different frame transformation, such as the double-rotated
frame or the tilted frame, which we will meet in Section 2.5.4 below. In this example, we will proceed assuming the B_1 pulse is on resonance.

With $\omega = \omega_0$, $\langle \hat{I}_z \rangle$ becomes

$$\begin{aligned} \langle \hat{I}_z \rangle &= \langle \frac{1}{2} |\exp(i\omega_1 \hat{I}_x t) \hat{I}_z \exp(-i\omega_1 \hat{I}_x t) |\frac{1}{2} \rangle \\ &= \langle \frac{1}{2} |\cos(\omega_1 t) \hat{I}_z - \sin(\omega_1 t) \hat{I}_y |\frac{1}{2} \rangle \\ &= \frac{1}{2} \cos(\omega_1 t), \end{aligned}$$

where we used the spin operators as generators of rotation (Eq. 2.22). By the same token,

$$\langle \hat{I}_y \rangle = \frac{1}{2} \sin(\omega_1 t)$$

 B_1 rf pulses allow transitions between eigenstates of \hat{I}_x , \hat{I}_y , and \hat{I}_z . After a 90° pulse ($\omega_1 t = \frac{\pi}{2}$), the system has moved from an eigenstate of \hat{I}_z to one of \hat{I}_y , corresponding classically to nutation of the magnetization around the \mathbf{B}_1 field by 90°.

2.3.2 NMR in Liouville space: density matrices

Density matrices are used extensively in NMR since they provide a concise way of dealing with ensembles of many spins. We will briefly review their properties and motivation, following Lynden-Bell [22]. Consider a spin with a wavefunction $|\Psi\rangle$ written in terms of the eigenstates $|\psi_n\rangle$:

$$|\Psi\rangle = c_1|\psi_1\rangle + c_2|\psi_2\rangle + \dots + c_n|\psi_3\rangle.$$

The density matrix ρ has elements given by

$$\rho_{ij} = c_i c_j^*,$$

which are the elements of the Cartesian product $|\Psi\rangle \langle \Psi|$ of the wavefunctions. One of the density matrix's strengths is that expectation value calculations are straightforward. For an

arbitrary operator \hat{Q} with a matrix representation Q in the $\{|\psi_n\rangle\}$ basis,

$$\begin{split} \langle \hat{Q} \rangle &= \langle \Psi | \hat{Q} | \Psi \rangle \\ &= \sum_{i,j} c_i^* c_j \langle \psi_i | \hat{Q} | \psi_j \rangle \\ &= \sum_{i,j} \rho_{ji} Q_{ij} \\ &= \operatorname{Tr} \{ \rho Q \} = \operatorname{Tr} \{ Q \rho \}. \end{split}$$

Moreover, it can be shown that the trace is independent of the basis set for ρ and Q, allowing one to choose the simplest representation for the calculation at hand.

The density matrix formalism provides a method of dealing with ensembles of spins. Consider a system of N spin- $\frac{1}{2}$ nuclei which are spatially localized and therefore distinguishable. To a good approximation, this is the case for protons in a sample of liquid water. The system could be described by the set of wavefunctions for each spin, $\{|\psi_1\rangle, |\psi_2\rangle, \ldots, |\psi_N\rangle\}$. These would have forms like

$$\begin{aligned} |\psi_1\rangle &= \left|\frac{1}{2}\right\rangle \\ |\psi_2\rangle &= -\left|-\frac{1}{2}\right\rangle \\ |\psi_3\rangle &= \frac{1}{\sqrt{2}}\left|\frac{1}{2}\right\rangle + \frac{1}{\sqrt{2}}\left|-\frac{1}{2}\right\rangle \\ &\dots \text{ etc.}, \end{aligned}$$

for example. Keeping track of these wavefunctions is impossible in a sample with ~10²⁰ water molecules. Moreover, it isn't useful, since in NMR we can only ever measure expectation values from all spins at once. In other words, we don't care about each individual wavefunction; it is the ensemble average which is the useful quantity. Finding this is simplified by the density matrix. Formally, the density matrix for this system is given by $\rho = \frac{1}{N} (|\psi_1\rangle \langle \psi_1| + |\psi_2\rangle \langle \psi_2| + \cdots + |\psi_N\rangle \langle \psi_N|)$, which is a 2x2 matrix—a significant reduction in the number of terms to keep track of! And since the equilibrium density matrix is known (see below), there is never any need to deal with the wavefunctions corresponding to individual spins.

The time evolution of the density matrix under a Hamiltonian \hat{H} (with units of rad/s) is given by the Liouville-Von Neumann equation,

$$\frac{\partial \rho}{\partial t} = -i[\hat{H}, \rho]. \tag{2.24}$$

When \hat{H} is constant, the formal solution is

$$\rho(t) = \exp(-i\hat{H}t)\rho(0)\exp(i\hat{H}t), \qquad (2.25)$$

where $\exp(-i\hat{H}t)$ is the propagator we met before in the formal solution to Schrödinger's equation (Eq. 2.20).

Density matrices exist in Liouville space, where they are the analogue of Hilbert space state vectors. In Liouville space the trace is the analogue of the inner product and "superoperators" act on state density matrices via commutators. While a comprehensive overview of the Liouville formalism would be useful for some topics in NMR, it isn't necessary for this thesis.

2.3.3 A simple NMR experiment using density matrices

We'll calculate again the evolution of a simple NMR experiment consisting of a 90° pulse followed by signal acquisition, this time using density matrices. Our model system now is a collection of isolated protons coupled loosely to the lattice. Together, they form a canonical ensemble. At equilibrium in the spectrometer field B_0 , the density matrix is

$$\rho_{0} = \frac{\exp(-\hbar\hat{H}_{Z}/k_{B}T)}{\operatorname{Tr}\left\{\exp(-\hbar\hat{H}_{Z}/k_{B}T)\right\}} \\
\approx \frac{\mathbf{1} - \hbar\hat{H}_{Z}/k_{B}T}{\operatorname{Tr}\left\{\mathbf{1}\right\}} \tag{2.26}$$

$$= \frac{\mathbf{1}}{N} - \frac{\hbar\hat{H}_{Z}}{Nk_{B}T} \\
= \frac{\mathbf{1}}{N} - \frac{\hbar\omega_{0}}{Nk_{B}T}\hat{I}_{z}.$$

with **1** as the unit operator and N as the number of spins. A density matrix of $\frac{1}{N}$ **1** describes an ensemble of spins oriented completely randomly, which isn't measurable. Rather, it is the deviation from the random orientation which is detectable, so the constant term is dropped. Also, the constant prefactor in front of \hat{I}_z is usually set to 1 since it doesn't typically change over the course of an experiment. We are left with the simple result,

$$\rho_o = \hat{I}_z. \tag{2.27}$$

The equilibrium magnetization is proportional to $\langle \hat{I}_z \rangle$. If the spins have $I = \frac{1}{2}$,

$$M_0 \propto \langle \hat{I}_z \rangle$$

= Tr { $\rho_0 \hat{I}_z$ }
= Tr { $\hat{I}_z \hat{I}_z$ }
= $\frac{1}{2}$.

Up to now, the calculations have been valid for the rotating or lab frame, since only \hat{I}_z operators have been involved. From now on, we will assume that ρ is in the rotating frame.

With the system in equilibrium, we now apply a pulse of the B_1 field at frequency ω . The first step in determining the evolution of ρ is to calculate the propagator of the Hamiltonian in the rotating frame (Eq. 2.23):

$$\exp(-i\hat{H}'t) = \exp(-i\left((\omega_0 - \omega)\hat{I}_z + \omega_1\hat{I}_x\right)\tau).$$

Again, we'll assume that $\omega = \omega_0$. Hence,

$$\rho(\tau) = \exp(-i\omega_1\tau \hat{I}_x)\rho(0)\exp(i\omega_1\tau \hat{I}_x)$$
$$= \exp(-i\omega_1\tau \hat{I}_x)\hat{I}_z\exp(i\omega_1\tau \hat{I}_x)$$
$$= \cos(\omega_1\tau)\hat{I}_z - \sin(\omega_1\tau)\hat{I}_y,$$

where we have used the rotation relations for the spin operators given in Eq. 2.22. We are considering a 90° pulse, so $\omega_1 \tau = \frac{\pi}{2}$ and now

$$\rho = -\hat{I}_y.$$

This corresponds to magnetization precessing in the transverse plane. The signal measured by the spectrometer after quadrature detection, S(t), is easily found using density matrices. In Section 2.2.3, we found S(t) classically from the precessing magnetization. The signal from the FID detected in the coil is always mixed with a reference frequency, ω_{ref} . So, we first need to determine the evolution of the density matrix in a frame rotating at $\omega_0 - \omega_{ref}$ relative to the stationary lab frame. Since $\rho = -\hat{I}_z$ is in a frame rotating at ω_0 , the new frame has a relative rotation of $-(\omega_0 - \omega_{ref})$. The density matrix in this new frame, ρ' , is:

$$\rho' = \exp\left(i\left(\omega_0 - \omega_{ref}\right)t\hat{I}_z\right)\rho\exp\left(-i\left(\omega_0 - \omega_{ref}\right)t\hat{I}_z\right)$$
$$= \exp\left(i\left(\omega_0 - \omega_{ref}\right)t\hat{I}_z\right)(-\hat{I}_y)\exp\left(-i\left(\omega_0 - \omega_{ref}\right)t\hat{I}_z\right)$$
$$= -\cos((\omega_0 - \omega_{ref})\tau)\hat{I}_y - \sin((\omega_0 - \omega_{ref})\tau)\hat{I}_x.$$

In the rotating frame, S(t) is given by [9, 11]

$$S(t) \propto \operatorname{Tr}\left\{\hat{I}_{+}\rho'\right\},$$

where $\hat{I}_{+} = \hat{I}_{x} + i\hat{I}_{y}$. Up to a constant we have

$$S(t) = \operatorname{Tr} \left\{ \left(-\cos((\omega_0 - \omega_{ref})\tau)\hat{I}_y - \sin((\omega_0 - \omega_{ref})\tau)\hat{I}_x \right) \left(\hat{I}_x + i\hat{I}_y \right) \right\}$$

$$= -\sin((\omega_0 - \omega_{ref})\tau)\operatorname{Tr} \left\{ \hat{I}_x \hat{I}_x \right\} - i\cos((\omega_0 - \omega_{ref})\tau)\operatorname{Tr} \left\{ \hat{I}_y \hat{I}_y \right\}$$

$$= -\frac{1}{2}\sin((\omega_0 - \omega_{ref})\tau) - i\frac{1}{2}\cos((\omega_0 - \omega_{ref})\tau)$$

$$= -i\frac{1}{2}\left(\cos((\omega_0 - \omega_{ref})\tau) + i\sin((\omega_0 - \omega_{ref})\tau)\right)$$

$$= -i\frac{1}{2}\exp(i(\omega_0 - \omega_{ref})\tau).$$

Ignoring the exponential decay from T_2 relaxation, this is the same signal we calculated classically (Eq. 2.17). The presence of the phase factor -i here is irrelevant and arises only from how we have defined our pulse phases. We are free to rotate the coordinate system within the rotating frame around \hat{z} without changing the physics.

2.4 Other spin interactions

2.4.1 Chemical shielding and quadrupolar interactions

So far we have not explicitly considered any interactions apart from the nuclear Zeeman effect. This thesis focuses mostly on the effects of the dipolar interaction, a topic we will soon cover in detail. First, however, we briefly discuss two other ways a nuclear spin may interact with its environment.

Chemical shielding is the interaction of the electrons surrounding a nucleus in a field \mathbf{B}_0 . This results in a slightly higher or lower field at the nucleus, changing the Larmor frequency in a measurable way. Because of this effect, the same nucleus in a molecule often has site-specific spectral frequencies. In rapidly-tumbling molecules in solution, an isotropic part of the chemical shielding interaction remains. This is one of the main reasons NMR spectroscopy is so useful in analytical chemistry.

Nuclei with $I > \frac{1}{2}$ (~74% of all NMR-active nuclei [9]) experience quadrupolar couplings. This is an interaction of the nucleus with electric field gradients. Quadrupolar coupling is usually quite strong relative to other interactions. Because its interaction strength depends on its orientation relative to \mathbf{B}_0 , it can be a useful tool for studying the structure and motion of certain molecules, like liquid crystals. To first order, the quadrupolar interaction has no isotropic part, so it is averaged away in small molecules in solution.

2.4.2 The dipolar Hamiltonian for two nuclei

Dipolar coupling⁶ is the interaction of one nuclear spin's magnetic moment with one or more magnetic moments from neighbouring nuclear spins. Imagine a system with two spins, I and S, separated by a vector **r**. Their dipolar Hamiltonian (in rad/s) is

$$\hat{H}_{D,tot} = \left(\frac{\mu_0}{4\pi}\right) \gamma_I \gamma_S \hbar \left(\frac{\hat{\mathbf{I}} \cdot \hat{\mathbf{S}}}{r^3} - 3\frac{(\hat{\mathbf{I}} \cdot \mathbf{r})(\hat{\mathbf{S}} \cdot \mathbf{r})}{r^3}\right)$$
$$= -d \left[A + B + C + D + E + F\right]$$

in units of rad/s. Here, $\gamma_{I,S}$ are the gyromagnetic ratios, $\hat{\mathbf{I}} = \hat{I}_x \hat{x} + \hat{I}_y \hat{y} + \hat{I}_z \hat{z}$ (and similarly for $\hat{\mathbf{S}}$), and

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

$$A = \hat{I}_z \hat{S}_z (3\cos^2\theta - 1)$$

$$B = -\frac{1}{4} \left(\hat{I}_+ \hat{S}_- + \hat{I}_- \hat{S}_+\right) (3\cos^2\theta - 1)$$

$$C = \frac{3}{2} \left(\hat{I}_z \hat{S}_+ + \hat{I}_+ \hat{S}_z\right) \sin\theta \cos\theta \exp(-i\phi)$$

$$D = \frac{3}{2} \left(\hat{I}_z \hat{S}_- + \hat{I}_- \hat{S}_z\right) \sin\theta \cos\theta \exp(+i\phi)$$

$$E = \frac{3}{4} \left(\hat{I}_+ \hat{S}_+\right) \sin^2\theta \exp(-2i\phi)$$

$$F = \frac{3}{4} \left(\hat{I}_- \hat{S}_-\right) \sin^2\theta \exp(+2i\phi),$$
(2.28)

where $\hat{I}_{\pm} = \hat{I}_x \pm i \hat{I}_y$. In these terms θ and ϕ are the polar and azimuthal angles and \hat{z} is the direction of \mathbf{B}_0 . The angles refer to the orientation of the inter-nuclear vector with respect

⁶In this thesis we are dealing exclusively with *magnetic* dipoles and dipolar coupling.

to \mathbf{B}_0 . Proton-proton dipolar couplings in organic solids have a maximum strength of 20–30 kHz. This is small compared to the Zeeman interaction (~100 MHz), so $\hat{H}_{D,tot}$ may be treated as a perturbation. In light of this, the dipolar Hamiltonian simplifies immensely [10]. Terms C, D, E, F are off-diagonal—they connect non-degenerate states—so their contributions to the spectrum are small (although they are responsible for relaxation). Conversely, terms A and B are diagonal. In any Hamiltonian, keeping terms like A and B is called the secular approximation. The secular, homonuclear ($\gamma_I = \gamma_S$) dipolar Hamiltonian is

$$\hat{H}_D = -d(3\cos^2\theta - 1)\left(\hat{I}_z\hat{S}_z - \frac{1}{4}\left(\hat{I}_+\hat{S}_- + \hat{I}_-\hat{S}_+\right)\right) = -d(3\cos^2\theta - 1)\left(\hat{I}_z\hat{S}_z - \frac{1}{2}(\hat{I}_x\hat{S}_x + \hat{I}_y\hat{S}_y)\right) = -\frac{d}{2}(3\cos^2\theta - 1)\left(3\hat{I}_z\hat{S}_z - \hat{\mathbf{I}}\cdot\hat{\mathbf{S}}\right).$$
(2.29)

We will refer to the secular approximation as the dipolar Hamiltonian from now on, using the three forms given in Eq. 2.29. As usual, the Hamiltonian has been written in units of rad/s. $\hat{I}_+\hat{S}_- + \hat{I}_-\hat{S}_+$ is often called the "flip-flop" term since it swaps the z-component of the spins in a spin pair. The heteronuclear Hamiltonian ($\gamma_I \neq \gamma_S$) doesn't have these terms—the states it connects are non-degenerate—and so retains only $\hat{I}_z\hat{S}_z$.

The Zeeman Hamiltonian for these two spins, $\hat{H}_Z \sim (\hat{I}_z + \hat{S}_z)$, commutes with \hat{H}_D : $[\hat{I}_z + \hat{S}_z, \hat{I}_z \hat{S}_z] = 0$ trivially, and by using the identity $2\hat{\mathbf{I}} \cdot \hat{\mathbf{S}} = (\hat{\mathbf{I}} + \hat{\mathbf{S}})^2 - \hat{I}^2 - \hat{S}^2$, we can see that $[\hat{I}_z + \hat{S}_z, \hat{\mathbf{I}} \cdot \hat{\mathbf{S}}] = 0$ as well (this is only true under the assumption that \hat{I}_z and \hat{S}_z have identical prefactors). This implies that \hat{H}_D is the same in the lab frame and in any frame rotating around \hat{z} . Also, it means that \hat{H}_D and \hat{H}_Z have simultaneous eigenstates.

As an example application, consider the special case of two identical dipolar-coupled spin- $\frac{1}{2}$ nuclei, such as protons in a methylene group. For this system,

$$\hat{H}_D = -\frac{d}{2} (3\cos^2\theta - 1) \left(3\hat{I}_z^2 - \hat{I}^2\right).$$

Since the spins are identical, we can only measure the total spin, not the spin of any one nucleus. The total spin eigenstates are separable into the singlet state,

$$|00\rangle = \frac{1}{\sqrt{2}} \left(|\uparrow\downarrow\rangle - |\uparrow\downarrow\rangle\right),$$

and the triplet states,

$$\begin{split} |11\rangle &= |\uparrow\uparrow\rangle\\ |10\rangle &= \frac{1}{\sqrt{2}}\left(|\uparrow\downarrow\rangle + |\uparrow\downarrow\rangle\right)\\ |1-1\rangle &= |\downarrow\downarrow\rangle, \end{split}$$

where the arrows indicate the spin of the two protons and $|Is\rangle$ indicates the total spin state has quantum numbers I and s. The singlet state is of no interest here since no transitions are possible and it is therefore unobservable. Using only the triplet state, we have $\hat{I}^2 = I(I+1)\mathbf{1} = 2\mathbf{1}$, where $\mathbf{1}$ is the unit operator. And so, in the B_0 field the total Hamiltonian is

$$\hat{H}_{Z} + \hat{H}_{D} = \omega_{0}\hat{I}_{z} - \frac{1}{3}\omega_{D}(\theta) \left(3\hat{I}_{z}^{2} - 2\mathbf{1}\right)$$
(2.30)

with
$$\frac{1}{3}\omega_D(\theta) = \frac{d}{2}(3\cos^2\theta - 1).$$
 (2.31)

This causes transitions at frequencies $\omega_0 \pm \omega_D(\theta)$ (Fig. 2.3A and B). The splitting $2\omega_D(\theta)$ depends on the relative orientation of the two nuclei (as above, θ is the angle the inter-nuclear vector makes with **B**₀). At the "magic angle" $\omega_D(\theta_{MA}) = 0$. This angle is

$$\theta_{MA} = \cos^{-1} \left(1/\sqrt{3} \right)$$

$$\approx 54.7^{\circ} \tag{2.32}$$

We will use this Hamiltonian again in Chapter 6 to describe ihMT.

2.4.3 Dipolar line broadening in many-spin systems

The two-nucleus dipolar Hamiltonian above (Eq. 2.29) is simple enough to solve exactly. However, in naturally-occuring organic solids or soft matter, proton-proton dipolar couplings are rarely limited to two spins. These systems have a many-spin dipolar Hamiltonian,

$$\hat{H}_D = -\sum_{i \neq j} d_{ij} (3\cos^2 \theta_{ij} - 1) \left(\hat{I}_{z,i} \hat{I}_{z,j} - \frac{1}{4} \left(\hat{I}_{+,i} \hat{I}_{-,j} + \hat{I}_{-,i} \hat{I}_{+,j} \right) \right).$$

This still has simultaneous eigenstates with the Zeeman Hamiltonian for the system,

$$\hat{H}_Z = \sum_i \omega_{0,i} \hat{I}_{z,i},$$



Figure 2.3: The effects of dipolar broadening in two identical spins and in many spins. (A) The spectrum of an isolated nucleus, with slight broadening from T_2 relaxation. (B) When two of these spins are coupled via the dipolar interaction, the spectrum is a doublet, where $\omega_D(\theta)$ is given by Eq. 2.30. (C) When many spins are coupled together, individual lines cannot be distinguished and the spectrum is broad. The spectral intensities are not to scale.

but we have no easy way of finding what they are. As more spins are added to the system, the Zeeman energy levels are smeared out into a continuum. As a result, the spectrum is broadened, as illustrated in Fig. 2.3C. In organic solids, ¹H spectral broadening from the dipolar interaction can be up to ~ 50 kHz.

Experimentally, it is found that the spectral broadening from dipolar couplings in many systems is approximately Gaussian. This cannot easily be derived directly from the Hamiltonian. However, it can be motivated using other models, like assuming a randomly-fluctuating field [29]. Also, we can approximate the lineshape of a system under the influence of the many-spin dipolar Hamiltonian without explicitly knowing the energy eigenstates. This technique is called the Van Vleck expansion [10, 30]. This expands the lineshape in terms of its moments (*e.g.* the second moment of the Gaussian is the variance, σ^2). Using this technique, many systems, such as cubic lattices, are only non-Gaussian in negligible higher order moments [30].

2.5 Saturation

2.5.1 The problem statement and the local field

Imagine performing an NMR experiment on a dipolar-coupled system of many spins. When rf is applied via the B_1 field, the lab frame Hamiltonian is composed of the Zeeman, dipolar, and rf parts:

$$\hat{H}_{lab} = \hat{H}_{Z,lab} + \hat{H}_D + \omega_1 \cos(\omega t) \hat{I}_{x,lab}.$$

We can pose what may seem to be a simple question: what is the evolution of the density matrix ρ under this Hamiltonian? In fact, this is a difficult problem to solve because $\hat{I}_{x,lab}$ does not commute with $\hat{H}_{Z,lab}$ or \hat{H}_D , so only in simple systems are there exact solutions. In practice we must use approximations, each valid in a different regime. (Note that we are assuming \hat{H}_D is the secular dipolar Hamiltonian, which is the same in the lab and rotating frames.)

In order to determine the regime, we need a relative measure of the energies of each interaction. The Zeeman and rf terms have the energy scales $\hbar\omega_0$ and $\hbar\omega_1$ respectively. The dipolar interaction energy is less straightforward. What we need for the dipolar Hamiltonian is a measure for the typical field strengths at one spin due to its neighbouring spin. When there is only once spin species, this is given by [31]

$$\omega_D = \sqrt{\langle \hat{H}_D^2 \rangle} = \sqrt{\frac{1}{3} \langle \Delta B^2 \rangle}.$$
 (2.33)

Here, $\langle \Delta B^2 \rangle$ is the second moment of the absorption lineshape, and we call ω_D the *local field* strength or RMS average dipolar interaction strength. It has units rad/s. In the literature, it is often called the local field B_L , defined as

$$B_L = \omega_D / \gamma. \tag{2.34}$$

Note that ω_D applies to one coupled network of spins, not to all spins of a specific species.

With an energy scale at hand for all parts of the total Hamiltonian, we can now explore some fundamental theories which apply in various regimes. The following is the program for the remainder of Section 2.5. After describing the trivial case of pulsed rf, we will introduce the work of Bloembergen, Purcell, and Pound (BPP), which uses perturbation theory to model saturation under extremely small rf irradiation (we will make explicit what "extremely small" means later). However, BPP theory fails to explain much of the behaviour seen in solids. This was the motivating factor for development of the next topic, Redfield theory, which introduces the concept of spin temperature. Redfield theory applies under strong rf irradiation. Provotorov Theory, the last topic discussed, extends the concept of spin temperature to explain the saturation of solids under weak rf irradiation. For a more detailed picture of how these theories fit together, the reader is referred to the introduction of Janzen's paper [32].

What is meant by saturation? If the system starts in thermal equilibrium, then a saturation rf pulse slowly equalizes the populations of the states, reducing the magnetization, $\langle \hat{I}_z \rangle$.

Depending on the specific regime, $\langle \hat{I}_z \rangle \to 0$ (the sample is completely saturated) or $\langle \hat{I}_z \rangle \to const$. Note that saturation also implies randomly distributed phases among the spins. A 90° pulse may tip the magnetization into the transverse plane, causing $\langle \hat{I}_z \rangle = 0$, but the phases of the spins are coherent, so this is not saturation.

On a historical note, many of the theories describing saturation were developed in the early days of NMR. At that time, *continuous-wave* NMR spectroscopy was used. In this technique, one applies a constant oscillating B_1 field to the sample as the B_0 field is increased, allowing for measurements of the absorption and dispersion of the sample. Obviously, quantifying the effects (*eg.* saturation narrowing) on the spectrum from the continuous B_1 field was important. Nowadays, Fourier transform spectroscopy is used, where the B_0 field is constant and the spectrum is determined from the FID after a series of intense B_1 pulses. However, saturation theories are useful in MRI, where certain techniques we discuss in the next chapter use long, low-power B_1 pulses to generate contrast.

2.5.2 Pulsed rf

The simplest regime: $\omega_1 \gg \omega_D$, $\omega_0 \gg \omega_1$, and B_1 duration τ is much less than the lifetime of the FID. Hence, we can safely ignore the effects of dipolar couplings during the pulse. Working in the rotating frame allows us to directly calculate solutions, as exemplified in Section 2.3.3. Saturation would occur when $\omega_1 \leq \omega_D$ and $\tau \gtrsim T_2$.

2.5.3 BPP theory

Bloembergen, Purcell, and Pound were the first to treat the rf pulse as a perturbation to the main Hamiltonian, consisting of the Zeeman and dipolar terms [33]. Their calculation used two coupled spin-1/2 nuclei under a weak rf field. However, the BPP saturation theory is only useful in the regime $\omega_1^2 T_1 T_2 \ll 1$. Higher rf powers will cause population equilibration, which changes the wavefunction of the system, invalidating the perturbation theory approach [34]. While BPP saturation theory predicts the right behaviour in liquids, in solids it deviates from observations at long times.

2.5.4 Spin temperature and Redfield theory

Redfield theory is applicable to strong rf pulses, where $\omega_1^2 T_1 T_2 \gg 1$ [10, 12, 30]. It uses the concept of spin temperature. This emerges because nuclear spins form a canonical ensemble,

describable by a Boltzmann distribution with a well-defined temperature. This is true under most circumstances in solids, where there is weak coupling to the lattice and spin-spin couplings redistribute the populations of the energy levels in a time $T_2 \ll T_1$.

Let us first consider the system at equilibrium with no rf applied. The total Hamiltonian consists of the Zeeman and dipolar parts,

$$\hat{H} = \hat{H}_Z + \hat{H}_D.$$

Then, following the derivation of Eq. 2.27, the density matrix is

$$\rho = -\beta \hat{H}.$$

= $-\beta \hat{H}_Z - \beta \hat{H}_D$ (2.35)

where β is the inverse lattice temperature. The spin temperature in each reservoir is the same, but the order (or magnetization/polarization) is not. To see this, we write

$$\rho = -\beta\omega_0 \left[\hat{I}_z \right] - \beta\omega_D \left[\frac{\hat{H}_D}{\omega_D} \right].$$

The operators in $[\cdots]$ are written this way to make them both unitless. The order p in each reservoir is

$$p_D = \left\langle \frac{\hat{H}_D}{\omega_D} \right\rangle = -\beta \omega_D$$

$$p_Z = \left\langle \hat{I}_z \right\rangle = -\beta \omega_0.$$
(2.36)

Because $\omega_D \ll \omega_0$, $p_D \ll p_Z$. Since the energy of the system is bounded, the order may be negative or positive in general.

The partition function is

$$Z = \operatorname{Tr} \{ \exp(-\beta \hat{H}) \}$$

= $\operatorname{Tr} \left\{ \mathbf{1} - \beta \hat{H} + \frac{1}{2} \beta^2 \hat{H}^2 + \cdots \right\}$
 $\approx (2I+1)^N + \frac{1}{2} \beta^2 \operatorname{Tr} \left\{ \hat{H}^2 \right\}.$

.

We have made use of the fact that $\text{Tr}\{\hat{H}\} = 0$ since $\text{Tr}\{\hat{H}_Z\} = \text{Tr}\{\hat{H}_D\} = 0$. $\text{Tr}\{\mathbf{1}\} = (2I+1)^N$ is the dimensionality of a system with N particles of spin I. We may now calculate

expectation values. The magnetization is [10, 12]

$$\mathbf{M} = \frac{\operatorname{Tr}\left\{\hat{\mathbf{M}}\exp(-\beta\hat{H})\right\}}{Z}$$
$$\approx \frac{\operatorname{Tr}\left\{\hat{\mathbf{M}}(\mathbf{1}-\beta\hat{H})\right\}}{(2I+1)^{N}}$$
(2.37)

$$= C\beta \mathbf{B},\tag{2.38}$$

where $\hat{\mathbf{M}} = (\hat{I}_x \hat{x} + \hat{I}_y \hat{y} + \hat{I}_z \hat{z})$ and *C* is the Curie constant. This result is Curie's law. It says that the local field has no effect on the magnetization, which is either parallel or anti-parallel to **B**. In the same way we can find the entropy of the system, *s*, a quantity we will make use of later. This is [10, 12]

$$s = s_Z + s_D \tag{2.39}$$

$$= -\frac{1}{2}\beta^2 \operatorname{Tr}\left\{\hat{H}_Z^2\right\} - \frac{1}{2}\beta^2 \operatorname{Tr}\left\{\hat{H}_D^2\right\} + const$$
(2.40)

$$= \left(-\frac{1}{2}C\beta^{2}B_{0}^{2}\right) + \left(-\frac{1}{2}C\beta^{2}B_{L}^{2}\right) + const.$$
 (2.41)

Now, imagine suddenly turning on an rf field $\omega \gg \omega_D$, so $\mathbf{B}_{\text{eff}} = (B_0 - \omega/\gamma)\hat{z} + (\omega_1/\gamma)\hat{x}$ (Eq. 2.5). According to Curie's law (Eq. 2.38), **M** must eventually point along \mathbf{B}_{eff} . How long does this take? When the rf field is turned on, **M** precesses around \mathbf{B}_{eff} until its "transverse" components (the components perpendicular to \mathbf{B}_{eff}) dephase. This happens in a time $\sim T_2$ ($\sim 1/\omega_D$). Only the projection of **M** along \mathbf{B}_{eff} is retained.

Say B_1 is rapidly turned on with an amplitude and frequency such that \mathbf{B}_{eff} is 45° to \mathbf{B}_0 (realized when $\omega = \omega_0 - \omega_1$). If the equilibrium magnetization in the lab frame was $\mathbf{M}_0 = M_0 \hat{z}$, then in a time $\sim T_2$ after the rf is turned on it will be parallel to \mathbf{B}_{eff} and have a magnitude of $M_0/\sqrt{2}$. The tilted rotating frame (henceforth the TR frame) is a rotating frame where \hat{z} is along \mathbf{B}_{eff} . The rapid application of B_1 means that once $\mathbf{M}_0 || \mathbf{B}_{\text{eff}}$, the system is in quasi-equilibrium: both the Zeeman and dipolar reservoirs in this tilted-rotating frame are describable by different inverse spin temperatures β'_Z and β'_D , but neither is in equilibrium with the lattice.

The new Zeeman inverse spin temperature β'_Z in the tilted frame may be found from the

Curie law in Eq. 2.38:

$$\frac{\beta_Z'}{\beta_Z} = \frac{B_0}{B_{\text{eff}}} \frac{\sqrt{2}M_0}{M_0}$$
$$= \sqrt{2} \frac{B_0}{B_{\text{eff}}} \gg 1.$$
(2.42)

Thus, $\beta'_Z \gg \beta_Z$ and the Zeeman reservoir has a significantly colder spin temperature in the TR frame than in the lab frame. We shall not attempt a similar calculation for the dipolar order—there is no analogue of the simple Curie law and the dipolar Hamiltonian in the tilted frame, \hat{H}'_D , is complicated.

We may also calculate the new entropy:

$$s' = s'_{Z} + s'_{D}$$

= $\left(-\frac{1}{2}CB^{2}_{\text{eff}}\beta'^{2}_{Z}\right) + \left(-\frac{1}{2}CB'^{2}_{L}\beta'^{2}_{D}\right) + const,$ (2.43)

where primes indicate the TR frame.

In the above discussion, the rapid application of the B_1 field with $\omega_1 \gg \omega_D$ led to a quasiequilibrium state after time $\sim T_2$. In the TR frame, the Zeeman reservoir has energy spacings of γB_{eff} . In the dipolar reservoir, the energy spacings are γB_L . When $\omega_1 \gg \omega_D$, $\gamma B_{\text{eff}} \gg \gamma B_L$ and "heat" (magnetization) cannot flow between the two reservoirs. Such transitions would be energy non-conserving. Yet when $\omega_1 \sim \omega_D$ these transitions can take place: the reservoirs are coupled together, and their spin temperatures equilibrate on timescales of $\sim 1/\omega_D$.

As for equilibrium with the lattice, in the TR frame this has timescales of $\sim T_{1\rho}$ for the Zeeman reservoir (the spin-lattice relaxation time in the rotating frame, ~0.1–1 s) and T_{1D} for the dipolar reservoir (the dipolar relaxation time, ~0.1–10 ms for the samples considered in Chapter 6). In the following discussion, we assume the rf duration is much shorter than $T_{1\rho}$ or T_{1D} , so we may ignore their effects while the rf is on.

2.5.5 ADRF/ARRF: An application of Redfield theory

We will now give an example of an experiment which can be quantified using Redfield theory. This is called Adiabatic Demagnetization/Remagnetization in the Rotating Frame (ADRF/ARRF) [10,30]. We will use this experiment in Chapter 6 to measure the dipolar relaxation time, T_{1D} .

From an equilibrium state with a spin temperature of β , a 90°_{y} on-resonance pulse rotates

the magnetization: $\mathbf{M} = M_0 \hat{x}$. Immediately after (taken to be t = 0), the rf is phaseshifted to the rotating frame's *x*-axis. At this point, the Zeeman reservoir is already in quasi-equilibrium since $\mathbf{M}_0 || \mathbf{B}_{\text{eff}}$. An analysis like the one leading to Eq. 2.42 shows that the Zeeman reservoir's inverse spin temperature at t = 0 is $\beta_Z = \frac{B_0}{B_1} \beta$.

The amplitude of the rf pulse, which starts off at $\omega_1 \gg \omega_D$, is now ramped down adiabatically to zero, leaving a final inverse spin temperature β '. The entropy is constant, so we can use the expression in Eq. 2.43. Dropping the primes for convenience, we equate the entropy at the start and end of the ADRF ramp (state 1 and 2 respectively):

$$s_{1} = s_{2}$$

$$s_{Z,1} + s_{D,1} = s_{Z,2} + s_{D,2}$$

$$\left(-\frac{1}{2}CB_{1}^{2}\beta_{Z,1}^{2}\right) + \left(-\frac{1}{2}CB_{L}^{2}\beta_{D,1}^{2}\right) = \left(-\frac{1}{2}CB_{\text{eff},2}^{2}\beta_{Z,2}^{2}\right) + \left(-\frac{1}{2}CB_{L}^{2}\beta_{D,2}^{2}\right)$$

$$B_{1}^{2}\left(\frac{B_{0}}{B_{1}}\beta\right)^{2} \approx B_{L,2}^{2}\beta_{D,2}^{2}$$

$$\omega_{0}^{2}\beta^{2} = \omega_{D}^{2}\beta_{D,2}^{2}$$

$$\rightarrow \beta_{D,2} = \beta\frac{\omega_{0}}{\omega_{D}}.$$

where we have used the fact that $\omega_1 \gg \omega_D$ and that $B_{\text{eff},2} = 0$ in the third line. At this point, $\mathbf{B}_{\text{eff}} = 0$ hence $\mathbf{M} = 0$, meaning that there is no magnetization in the Zeeman reservoir. Instead, it has been moved to the dipolar reservoir, which now has an inverse temperature of $\beta \frac{\omega_0}{\omega_D}$ and a polarization (Eq. 2.36) of $-\beta \omega_0$. Note that because we are on resonance, ω_D is the same as in the lab frame. During demagnetization, the Zeeman and dipolar reservoirs remain uncoupled until $\omega_1 \sim \omega_D$.

Since we reached this using an adiabatic process, reversing it will transfer observable magnetization back to the Zeeman reservoir. This is the ARRF part of the sequence. While in the dipolar reservoir, the dipolar order decays with spin-lattice relaxation time T_{1D} . Therefore, the ADRF/ARRF sequence can be used to measure T_{1D} .

2.5.6 Provotorov theory

Provotorov Theory deals with the case where ω_1 is weak so that we can't assume the Zeeman and dipolar reservoirs have the same spin temperature [10, 30, 34, 35]. Experimentally, this is usually the case in solids when $\omega_1 \ll \omega_D$. It was first introduced by Provotorov [36] but the canonical reference is Goldman's book [30].

In Appendix A we derive the Provotorov equations. Here, we simply state the results and

put them into a useful form for later. We will use the form of the equations introduced by Lee *et al.* [37, 38]. The density matrix in a rotating frame is

$$\rho = -(\omega_0 - \omega)\beta_Z \hat{I}_z - \beta_D \hat{H}_D$$

= $-2\pi\Delta\beta_Z \hat{H}_Z - \beta_D \hat{H}_D$
= $-2\pi\Delta\beta_Z \hat{I}_z - \omega_D\beta_D \left(\frac{\hat{H}_D}{\omega_D}\right).$ (2.44)

In the above, $2\pi\Delta = \omega_0 - \omega$, where ω is the frequency of the rf (not yet applied). Δ is the offset from the center of the spectrum and is stated in Hz. We can now express ρ as a vector with $\{I_z, \hat{H}_D/\omega_D\}$ as the basis

$$\boldsymbol{\rho} = \begin{pmatrix} -(2\pi\Delta)\beta_Z \\ -\omega_D\beta_D \end{pmatrix}.$$
(2.45)

The components of ρ are the magnetizations or orders in each reservoir

$$\langle I_z \rangle = (\boldsymbol{\rho})_1 = -(2\pi\Delta)\beta_Z$$

$$\left\langle \frac{\hat{H}_D}{\omega_D} \right\rangle = (\boldsymbol{\rho})_2 = -\omega_D\beta_D.$$
 (2.46)

When weak rf is applied with amplitude $\omega_1 \ll \omega_D$, the Provotorov equations are

$$\frac{d\boldsymbol{\rho}_{\pm}}{dt} = W \begin{pmatrix} -1 - \frac{1}{WT_1} & \Omega \\ \Omega & -\Omega^2 - \frac{1}{WT_{1D}} \end{pmatrix} \boldsymbol{\rho}_{\pm} + \begin{pmatrix} \frac{\langle I_z \rangle_0}{T_1} \\ 0 \end{pmatrix}, \qquad (2.47)$$

with

$$W = \pi \omega_1^2 g(\Delta) \tag{2.48}$$

$$\Omega = \frac{2\pi\Delta}{\omega_D}.\tag{2.49}$$

Here, $g(2\pi\Delta)$ the symmetric, normalized lineshape (in units of s). In Appendix A we also show how rf applied at offsets $\pm\Delta$ simultaneously decouples the Zeeman and dipolar reservoirs, leading to [30]

$$\frac{d\boldsymbol{\rho}_{\text{dual}}}{dt} = W \begin{pmatrix} -1 - \frac{1}{WT_1} & 0\\ 0 & -\Omega^2 - \frac{1}{WT_{1D}} \end{pmatrix} \boldsymbol{\rho}_{\text{dual}} + \begin{pmatrix} \frac{\langle I_z \rangle_0}{T_1}\\ 0 \end{pmatrix}.$$
(2.50)

Eq. 2.47 and 2.50 are at the heart of ihMT, discussed in Chapter 6.

2.6 Relaxation in homogeneous systems

2.6.1 What drives relaxation?

Environmental fluctuations in magnetic fields felt by the nuclei are responsible for spin-spin (T_2) and spin-lattice (T_1, T_{1D}) relaxation. Generally, molecular motion causes these fluctuations. Consider a proton on a tumbling molecule in a B_0 field. Depending on the molecular orientation, there may be different electron screening around the nucleus. Also, there will inevitably be fluctuating fields from the magnetic moments of other protons and nuclei. Time-independent couplings also cause precessing nuclei to dephase, so these contribute to T_2 as well. Finally, the fluctuations of paramagnetic centers also play a role.

In MRI, it isn't typically necessary to perform exhaustive calculations of the quantum origins of relaxation. In fact, given the complexity of most biochemical environments, like lipid bilayers, this would be impossible without molecular dynamics simulations (*e.g.* reference [39]). Instead, it is usually adequate to either measure the relaxation rate, predict its magnitude from the fundamental physics, or predict its value based on knowledge of similar systems. For example, we can measure the T_1 and T_2 times of aqueous protons in white and grey matter. We can predict that the non-aqueous protons will have a $T_2 \sim 10-100 \ \mu$ s due to their slow tumbling (see next section). This short T_2 time can be confirmed experimentally using NMR spectroscopy and computationally using molecular dynamics simulations. Similarly, in Chapter 7 there is an extensive discussion on predicting the relative T_{1D} times in different types of white matter lipids.

We shall now present some general results from BPP relaxation theory (the same theory that describes saturation in the limit $\omega_1^2 T_1 T_2 \ll 1$). This provides a suitable framework for understanding the quantum origin of relaxation in tissue.

2.6.2 BPP relaxation theory

Here we provide a flavour of BPP theory without going into the details. Relaxation is the redistribution of populations in the density matrix. So for an arbitrary matrix element $\rho_{nm} = \langle n | \rho | m \rangle$, how does this change in time? It can be shown (*e.g.* see Slichter [10]) that

$$\frac{d}{dt}\rho_{nm} = \frac{1}{\hbar^2} J_{mn}(\omega_n - \omega_k)$$

where $J_{mn}(\omega)$ is the is the spectral density and $\omega_n - \omega_k$ is the energy difference between $|m\rangle$ and $|n\rangle$. The spectral density is a measure of which frequency components are present in the random fluctuations felt by a nucleus. Intuitively this makes sense: when the random fluctuations are on resonance for a transition, the populations of the corresponding levels will change. The spectral density is given by the Fourier transform of the correlation function $G_{mn}(\tau)$:

$$J_{mn}(\omega) = \mathcal{F}\left\{G_{mn}(\tau)\right\}$$

and

$$G_{mn}(\tau) = \overline{\langle m | \hat{H}_r(t-\tau) | n \rangle \langle n | \hat{H}_r(t) | m \rangle}.$$

Here, \hat{H}_r is some Hamiltonian that is responsible for the fluctuations, such as the dipolar Hamiltonian. The overbar indicates an ensemble average. We often make the assumption

$$G_{mn}(\tau) \sim \exp(-\tau/\tau_c),$$

where τ_c is called the correlation time. This is very nearly exact for small molecules like water. With this assumption, $J_{mn}(\omega)$ has a Lorentzian profile around $\omega = 0$ with width $\sim \tau_c$. The above is the starting point for deriving expressions for the relaxation times. By considering the case of two rapidly-tumbling dipolar-coupled spins (*e.g.* protons in a water molecule), it can be shown that [33, 40, 41]

$$\frac{1}{T_1} = C \left(\frac{\tau_c}{1 + (\omega_o \tau_c)^2} + \frac{4\tau_c}{1 + (2\omega_0 \tau_c)^2} \right)
\frac{1}{T_2} = C \left(\frac{3}{2} \tau_c + \frac{(5/2)\tau_c}{1 + (\omega_0 \tau_c)^2} + \frac{\tau_c}{1 + (2\omega_0 \tau_c)^2} \right).$$
(2.51)

Where C is a constant and ω_0 the Larmor frequency. Fig. 2.4 plots these expressions under various conditions, we see that they give the right qualitative behaviour. In solids, $T_2 \ll T_1$ and in liquids $T_1 \sim T_2$. In tissues, the molecules are often restricted by compartment walls, so $T_2 < T_1$.

The same sort of approach has also been applied to T_{1D} for a dipolar-coupled proton pair [43]. Using a more general relaxation theory (Redfield theory [10]), the value is found to be [43–45]

$$\frac{1}{T_{1D}} = \frac{27}{8} \gamma^4 \hbar J^1(\omega_D).$$
(2.52)



Figure 2.4: T_1 and T_2 as functions of correlation time τ_c . Modified from reference [42] with permission from Hans Reich. Figure is based on work in reference [33].



Figure 2.5: The inversion-recovery sequence used to measure T_1 .

Here, $J^{1}(\omega)$ is the spectral density of the spherical tensor function $F^{(1)}$, and

$$F^{(1)} = \frac{\sin\theta\cos\theta\exp(-i\phi)}{r^3},$$

where **r** is the vector connecting the two nuclei. Eq. 2.52 says that slow fluctuations around the frequency of the local field strength ω_D drive T_{1D} relaxation.

2.7 Some experimental methods

2.7.1 T_1 measurement with inversion-recovery

 T_1 is commonly measured in NMR studies by using an inversion-recovery (IR) experiment. Its pulse sequence is shown in Fig. 2.5. In equilibrium the magnetization is $M_0\hat{z}$. If a 180° B_1 rf pulse is applied, now the magnetization is $-M_0\hat{z}$. According to Eq. 2.12, the system will now return to equilibrium via

$$M_z(t) = M_0 (1 - 2 \exp(-t/T_1)).$$

 $M_z(t)$ can be observed by a 90° pulse and the T_1 time extracted. The IR experiment is considered the "gold standard" for T_1 measurements. But in MRI scanners, it requires prohibitively long scan times [46–48]. Instead, Look-Locker methods (where a 180° pulse is followed by a train of low flip-angle pulses) or variable flip angle methods (where a sequence of images are acquired with varying flip angles) are used [48].

2.7.2 T_2 measurements: the spin echo and CPMG acquisition

From the Bloch equations, it seems that the envelope of the FID will decay with $\exp(-t/T_2)$. We assumed this in our example above (Eq. 2.17). In reality, we often find that the FID for aqueous protons decays with $\exp(-t/T_2^*)$, where $T_2^* < T_2$. T_2^* takes into account inhomogeneities in the B_0 field which are static on timescales of T_2 :

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2,inhomo}}$$

 $T_{2,inhomo}$ is the contribution from inhomogeneities in the static field. These are caused by varying magnetic susceptibilities throughout the sample, limitations on shimming, and paramagnetic impurities. However, there is a simple method to reverse the effects of $T_{2,inhomo}$ and measure the true T_2 time. To explain what this is, we first need to introduce the concept of the spin echo.

Fig. 2.6 gives a pictorial description of the spin echo, which is a sequence consisting of a 90° pulse, then a delay τ , then another 180°. Following the 90° pulse, the spins will precess at slightly different frequencies due to static field inhomogeneities. Correspondingly, they lose phase coherence and the net magnetization decays with a time constant T_2^* . However, after the 180° pulse, the spin with the lowest precession frequency now has the most advanced phase, and vice versa. Therefore, at a time 2τ , we are only left with the effects of T_2 relaxation.

The Carr-Purcell Meiboom-Gill (CPMG) acquisition is a series of repeated spin echoes. Its pulse sequence is shown in Fig. 2.7. The amplitude of each echo, plotted as a function of time, decays with time constant T_2 . The experiments in Chapters 5 and 7 use this acquisition method.

In MRI, B_1 and B_0 inhomogeneities mean that the 180° refocusing pulses are imperfect,



Figure 2.6: The spin echo in the rotating frame. Because of static field inhomogeneities, each spin has a slightly different speed of precession. As they lose phase coherence, the net magnetization is reduced. After evolving for a time τ , a 180° pulse flips the phases: now the slow spin is in front and the fast spin is behind. At 2τ , an echo is observed when the spins refocus. While the effects of $T_{2,inhomo}$ can be removed, T_2 relaxation from field fluctuations is unavoidable.



Figure 2.7: The CPMG acquisition sequence. A few points around the echo are acquired on each echo. The 90° phase shift between the 90_x and 180_y pulses prevents small errors from imperfect 180° pulses from accumulating.

leaving some magnetization in the longitudinal direction after each pulse. Ultimately, this produces stimulated echoes, which arise after two or more pulses, and causes errors when fitting the CPMG decay curve. The effect of these stimulated echoes can be corrected post-acquisition and is necessary when analyzing MRI CPMG curves [49]. In NMR, because the sample is significantly smaller and the pulse widths shorter, the B_1 and B_0 inhomogeneities are relatively insignificant and this correction is not usually necessary.

2.7.3 Determining FID deadtime

After an intense B_1 pulse, there is a period where the resonant circuit in the probe rings down. Following an observation pulse, this prevents immediate detection of the FID, forcing the experimenter to wait before turning on the receiver. This delay, t_d , is known as the receiver deadtime and is typically a few μ s.

The deadtime must be accounted for when different frequency components of the signal dephase or decay appreciably during the deadtime. For example, the FID of tissue contains a signal from non-aqueous protons that decays in 10–100 μ s, whereas the signal from the aqueous protons can last up to about 1 s. If one desires to precisely model the non-aqueous signal, a sizable portion of it may be missing due to the deadtime. It is important, therefore, to know the true t = 0 point, otherwise the modeling could over or under-estimate the non-aqueous signal's amplitude. If only the aqueous protons are of interest, then the deadtime is comparatively insignificant and the start of the acquired FID is taken to be the t = 0 point.

With some equipment, the receiver clock is easily synchronized with the pulse sequence such that the true t = 0 time is known. However, signal propagation delays depend on filters in the receiver chain, which may vary between experiment setups. For this reason, it is often easier to measure t_d . For the work in this thesis, the following measurement technique was used.

Let f(t) be the envelope of an FID that has a resonance with a lineshape g at a frequency ω_0 in a field B_0 . The spectrum $S(\omega)$ with zero deadtime is

$$S(\omega) = \mathcal{F} \{ f(t) \exp(-i\omega_0 t) \}$$
$$= \exp(-i\phi_0)g(\omega) * \delta(\omega - \omega_0)$$
$$= \exp(-i\phi_0)g(\omega_0)$$

Here, ϕ_0 is the the zeroth-order phase correction chosen to make a pure absorption spectral line at ω_0 : $S(\omega) \exp(i\phi_0) = g(\omega_0)$. Its value is determined by timing in the spectrometer receiver chain and can be measured by visually phasing the spectrum.

To find t_d , imagine two spectra are acquired on the same sample in slightly stronger and weaker B_0 fields, $B_0 + \Delta B$ and $B_0 - \Delta B$, where ΔB is a small offset achieved through the z_0 shim. Including the deadtime, their FIDs are $f(t - t_d) \exp(-i\omega_{0,\pm}t)$, leading to spectra

$$S(\omega_{\pm}) = \exp(-i\phi_0) \exp(-i\omega t_d)g(\omega) * \delta(\omega - \omega_{0,\pm})$$
$$= \exp(-i(\omega_{0,\pm}t_d + \phi_0))g(\omega_{0,\pm})$$
$$= \exp(-i(\theta_{\pm} + \phi_0))g(\omega_{0,\pm}),$$

where $\theta_{\pm} = \omega_{0,\pm} t_d$ Now, $\theta_{\pm} + \phi_0$ is the phase correction term, where ϕ_0 is approximately constant over the small changes in frequency at hand. Finally, the difference $(\theta_+ + \phi_0) - (\theta_- + \phi_0) = t_d(\omega_{0,+} - \omega_{0,-})$ leads to

$$t_d = \frac{\frac{1}{360}(\theta_+ - \theta_-)}{f_{0,+} - f_{0,-}} \tag{2.53}$$

where θ_{\pm} is in degrees and $f_{0,\pm} = \omega_{0,\pm}/2\pi$ is in Hz.

In practice, the procedure is as follows:

- 1. Set the spectrometer frequency to be at the center of the spectrum.
- 2. Adjust the z_0 shim to shift the spectrum about +10 kHz off of the spectrometer frequency and acquire. Phase the spectrum using the zero-order correction and record the value (θ_+). Also record the frequency of the spectrum's central line ($f_{0,+}$).
- 3. Adjust the z_0 shim the other way to shift the spectrum about -10 kHz off of the spectrometer frequency and acquire. Record the corresponding values for θ_{-} and $f_{0,-}$.
- 4. Use Eq. 2.53 to calculate t_d .

Note that defining $\Delta \theta = \theta_+ - \theta_-$ and $\Delta f = f_{0,-} - f_{0,+}$, Eq. 2.53 implies $\Delta \theta \propto \Delta f$, which is a first-order phase correction (*i.e.* the phase correction is a linear function of frequency). So finding t_d is the same as finding the first-order phase correction. In a spectrum with multiple, well-defined lines over a reasonably broad frequency range, this correction can be done by eye from a single spectrum.

Chapter 3

White matter, grey matter, and myelin

3.1 Introduction

Improving the sensitivity and specificity of MRI to myelin is the ultimate goal of this thesis. This chapter's aim is to briefly explore the biology and physics of myelin. We first introduce the nervous system and the role played by myelin in neuron signal propagation. Myelin's unique structure, which is the source of its MR properties, is discussed in detail. We also discuss Multiple Sclerosis (MS), a disease in which MRI plays a central role in diagnosis and study.

3.2 The nervous system

The nervous system in vertebrates is separated into the Peripheral Nervous System (PNS) and the Central Nervous System (CNS). Neurons in the PNS relay sensory information from external and internal sources. They also relay signals to control muscles and other organs. The CNS receives and processes information from the PNS and coordinates responses.

The CNS, comprising the spinal cord and the brain, receives a great deal of study with MRI. However, the function of the CNS, from a cognitive to a genetic level, does not much concern us here. Instead, our focus is on the CNS microstructure, since this determines the properties of the NMR signals within an MR image.

The CNS tissue is separated into grey matter and white matter. White matter tissue contains myelinated axons, glial cells, and capillaries. Its pale white appearance is from the high lipid



Figure 3.1: A cartoon of a neuron. Image modified from the original created by "Quasar Jarosz" on English Wikipedia [50] with permission under the CC-BY-SA.

content in the myelin sheaths. White matter connects different parts of the grey matter tissue, which is composed of neuronal dendrites and cell bodies, glial cells, and capillaries. Its relatively low myelin content is responsible for its darker appearance. In the brain, grey matter is mostly found on the surface whereas white matter is mostly found underneath in the bulk tissue. In the spinal cord, the opposite is true.

On a microscopic level, the vertebrate nervous system is composed of neurons and glial (support) cells. Fig. 3.1 shows an idealized neuron, the fundamental unit of the nervous system. Signals, called action potentials, are received on dendrites. Outgoing signals propagate down the axon. At the axon terminals, neurotransmitters are released into the synapse, reaching the next neuron's dendrite.

3.3 Myelin

3.3.1 Myelin structure

Myelin forms a multi-layered sheath around nerve cell axons (also called compact myelin). The myelinated regions are called internodes and alternate periodically with short, unmyelinated regions called the Nodes of Ranvier. These nodes are typically about 1–2 μ m long and are spaced at intervals about 100× the axon diameter [52,53]. Myelin is a plasma membrane extension of a specialized glial cell. In the PNS, Schwann cells form the myelin with one cell per sheath. In the CNS, the myelin sheaths are extensions of oligodendrocyte cells, and one oligodendrocyte can form up to about 30 nodes [53]. At the end of the internode, each layer of the cytoplasmic membrane is attached to an invagination in the axon called the perinodal loop. While these nodes occur at regular intervals, the relative total length of unmyelinated axon is quite short at <0.5% of the surface length [54].



Figure 3.2: The physical and chemical composition of myelin. The proteins are myelin basic protein (MBP), proteolipid protein (PLP), cyclic nucleotide phosphodiesterase (CNP), and myelin-associated glycoprotein (MAG). Note that the extracellular water and cytoplasm water indicated on this diagram together form the myelin water pool which will covered in detail in Section 4.5. The intra/extra-cellular water we also introduce in that section is found areas outside the myelin sheath, such as the axon shown here. Reprinted by permission from Springer Neurotherapeutics, Laule *et al.* [51], © 2007.



Figure 3.3: Electron micrographs of human brain white matter with increasing levels of magnification in panels A–C. In (C) the multi-layered structured of the myelin sheath is obvious, showing the alternating major dense and intraperiod lines. Between the myelin's bilayers a pool of water called the Myelin Water (MW) is trapped. Figure modified from Liu & Schumann [59] with permission under CC-BY-4.0.

Fig. 3.2 shows a diagram of the myelin sheath's physical and chemical composition. At each internode, the myelin sheath wraps around the axon like a toilet paper roll, forming a system of alternating lipid bilayers, where the bilayers are the plasma membrane of the oligodendrocyte. Cytoplasmic fluid is contained between apposed internal surfaces of the membrane, and extra-cellular fluid is contained between apposed external surfaces. These are called the major dense lines and intra-period lines respectively, due to their alternating appearance on electron micrographs (Fig. 3.3). Both the extra-cellular fluid in the intraperiod line and the cytoplasmic fluid in the major dense line share a unique NMR relaxation property: their T_2 time (~10 ms) is measurably shorter than the T_2 time (~50 ms) of water elsewhere in the tissue [55–58]. Because of this collective behaviour, fluids in both the intraperiod lines and the major dense lines are known as Myelin Water (MW) [51].

The composition of myelin (Table 3.1) is what enables its remarkable structure, where the membrane surfaces in compact myelin are "zippered" together [60]. Its lipid content is unusually high, comprising around 70% of the dry weight [53, 61, 62], with the remaining weight from proteins. This is in contrast to typical biomembranes, which have much higher protein to lipid ratios, typically somewhere between 1:1 and 4:1 [61]. Also, a high proportion of myelin lipids are saturated (94%) and/or have very long hydrocarbon chains (20% have more than 18 carbons). This is significant compared to the grey matter average, where only 80% of lipids are saturated and just 1% have chains longer than 18 carbons [61]. The reduced membrane fluidity from the tight packing of the saturated chains is offset by myelin's high (~30%) cholesterol content, which increases fluidity [63]. The structure of the major lipid

| | Myelin | White matter | Grey matter |
|---|--------|--------------|-------------|
| Proteins | 30% | 39% | 55% |
| myelin basic protein (MBP) | 30% | - | - |
| proteolipid protein (PLP) | 50% | - | - |
| cyclic nucleotide phosphodiesterase (CNP) | 4% | - | - |
| myelin-associated glycoprotein (MAG) | 1% | - | - |
| Lipids | 70% | 55% | 33% |
| cholesterol | 28% | 28% | 22% |
| phospholipids | 43% | 46% | 70% |
| glycosphingolipids | 28% | 26% | 7% |

Table 3.1: The composition of human myelin, white matter, and grey matter. Bold numbers are percent weight in wet tissue, all others are percent dry weight of total lipid or total protein content. Myelin protein values are from Laule *et al.* [51], white and grey matter values are from Norton & Cammer [62].



Figure 3.4: The structure of the major lipid types in myelin. After van der Knaap [64].

types seen in myelin is shown in Fig. 3.4.

Myelin's major proteins are myelin basic protein (MBP) and proteolipid protein (PLP). MBP helps to stabilize the membrane structure by neutralizing the charge on the phospholipid head group. PLP is often referred to as a "spacer" and has domains in both the intraperiod and major dense lines. It maintains a constant spacing between the plasma membranes [64]. Other less-abundant proteins include cyclic nucleotide phosphodiesterase (CNP, an enzyme) and myelin-associated glycoprotein (MAG, which plays a role in cellular recognition and intra-cellular interactions) [51, 64].

The values for myelin composition in Table 3.1 are averages across a number of individuals and structures and variation from these values is expected. Variation is also seen on the microscopic level. For example, the corpus callosum (which enables communication between the cerebral hemispheres) has some fibre tracts with myelin sheaths on only 30% of the axons, despite being highly myelinated in general. [65]. There may also be significant differences in myelination between individuals, especially those of different age. Children are born with relatively few CNS structures fully myelinated and myelination isn't completed until early adulthood [64].

3.3.2 Myelin function

Neurons have resting potentials around -70 mV. The net negative charge inside the cytosol is established by K^+ and Na^+ ion pumps. At rest, the Na^+ concentration is higher outside but K^+ is higher inside. Signal transmission is a temporal and spatial change in this membrane potential. It happens in two main ways: by graded potentials and by action potentials. We shall describe graded potentials first, then action potentials, and then finally bring the two concepts together by discussing the role of myelin.

Graded potentials are changes in membrane potential which are variable in size, are additive, and decay spatially from a source. These can be observed in unmyelinated axons with voltage clamp experiments and naturally occur at the postsynaptic dendrites in response to neurotransmission. There, neurotransmitters activate ion channels (distinct from the Na⁺ and K⁺ channels already mentioned), causing membrane depolarization. The depolarization is localized at the postsynaptic dendrite and causes a decaying membrane potential—the graded potential—away from the synapse. This potential spreads via the attraction and repulsions of ions inside the cytosol [66]. The length constant of this decay is a function of the axial resistance and the membrane resistance. In a cylindrical axon the axial resistance is [67, 68]

$$r_a = \frac{\rho_c}{\pi a^2}$$

where ρ_c is the resistivity of the cytosol and *a* the axon radius, so r_a has units of Ω/m . The membrane resistance, r_m , is a function of the specific resistance of an area of plasma membrane, R_m :

$$r_m = \frac{R_m}{2\pi a}$$

 R_m has units of Ωm^2 , so r_m has units of Ω m. Graded potentials spread away from the source, decaying exponentially in strength with a length constant λ given by [67,68]

$$\lambda = \sqrt{\frac{r_m}{r_a}} = \sqrt{\frac{R_m}{\rho_c} \frac{a}{2}}.$$
(3.1)

Hence, increasing the membrane resistance and the axon diameter increases the length constant, allowing graded potentials to spread over longer distances. The duration of the graded potential is given by the time constant τ via [67, 68]

$$\tau = r_m c_m$$

where c_m is the membrane capacitance in F/m. An estimation of the velocity of the graded potential down the nerve cell is then [67]

$$v \approx \frac{\lambda}{\tau}$$

$$= \frac{1}{\sqrt{r_m r_a c_m}}$$

$$\propto \sqrt{\frac{a^3}{\rho R_m c_m^2}}$$

$$= \sqrt{\frac{a}{\rho R_m C_m^2}}$$
(3.2)

where C_m is the capacitance per unit area of the membrane. The constants which were dropped are given explicitly in Tasaki's work [69].

Action potentials are the second type of signals which are transmitted in neurons, where they occur in the axons. In contrast to graded potentials, action potentials are capable of propagating over long distances without decaying in strength. They are caused by voltage-gated Na⁺ and K⁺ ion channels in the axon membrane. When an action potential approaches, voltage-gated Na⁺ ion channels open, allowing external Na⁺ ions to diffuse down their concentration gradient into the axon. This causes a positive voltage across the membrane, eventually triggering the K⁺ ion channels. The K⁺ ions then start to move out of the cytosol across the membrane, causing a negative membrane potential. Finally, there is a small delay when the section of axon is unable to transmit any signals. During this *refractory period*, K⁺ and Na⁺ ion pumps re-establish the resting potential. (The number of ions that move across the membrane is actually quite small; for K⁺, this is less than <0.03% of the total number of ions within the axon [60].)

Both graded potentials and action potentials can propagate in unmyelinated axons. Of the two, graded potentials have a much faster signal velocity, relying on short-range charge reorganization within the cytosol instead of diffusion through ion channels, as for action potentials. However, action potentials are able to propagate over long distances without diminishing in strength. Nature's ingenious solution to these problems is the myelin sheath, which allows the best of both worlds.

In a myelinated axon, action potentials occur only at the nodes of Ranvier. These cause a

graded potential, which then spreads underneath the internode at greater speed and over a longer distance compared to what it could in an unmyelinated axon. To see why the speed is greater, consider that myelin increases the membrane resistance, R_m . Referring to Eq. 3.1, this increases the length constant λ . Myelin also increases the membrane capacitance C_m . With a myelin thickness of b, $R_m \propto b$ and $C_m \propto b^{-1}$, if the approximation of a parallel plate capacitor is used. Substituting these into Eq. 3.2 gives [67]

$$v \propto \sqrt{ab}$$

The myelin thickness and axon diameter are key parameters in determining the conduction velocity.

When the graded potential reaches the next node of Ranvier, it triggers an action potential, and the process repeats. Because of the refractory period, the graded potential cannot cause an action potential at the previous node. Thus, the signal appears to "hop" from node to node, hence its name: *saltatory* conduction, from the latin word for hopping, *saltare*. Myelin increases the speed of action potential transmission $10-100 \times$ compared to an unmyelinated axon and also requires less energy, since ion pumping is required only at the nodes. [51]. Also, saltatory conduction ensures signals can travel in one direction and do not diminish in strength.

3.3.3 Multiple Sclerosis

Changes in myelin have been associated with many cognitive disorders, including Alzheimers [70] and schizophrenia [71]. Even mild trauma can affect CNS myelin [72]. There are also diseases which directly damage the myelin, known as demyelinating diseases. Of these, Multiple Sclerosis (MS) is perhaps the most well-known. MS is of particular concern in Canada, which has one of the highest rates of in the world [73]. The cause of the disease is unknown, and while it is likely autoimmune in nature, vitamin D deficiency (prevalence is higher is northern countries), viral (*e.g.* Epstein-Barr), and genetic factors have all been implicated [74–76]. Onset of MS is typically in early adulthood and ultimately leads to decreased motor and cognitive abilities, although progression is generally slow—on average, patients live 30 years after diagnosis [76]. There is no cure, but symptomatic treatments exist.

In MS, localized areas of demyelination occur, which can be followed by axonal loss or remyelination (in earlier stages of the disease). These significant changes to the tissue microstructure are often (although not always) visible using MRI. For this reason, MS diagnostic criteria relies upon localization of MRI-visible lesions in time and space [77].

Chapter 4

Relaxation and spectra in brain: properties and applications

4.1 Introduction

MR imaging of white and grey matter tissue makes use of their relaxation and spectral properties, which is the concern of this chapter. We first emphasize the theory behind the NMR signal from non-aqueous protons, which is measured directly in Chapter 5's experiments. At the heart of this is the super-Lorentzian spectral lineshape. Then, the section on T_1 relaxation highlights its inconsistent experimental results. We offer an explanation for these inconsistencies later in our work in Chapter 5. A section on the physics and uses of magnetization transfer follows which includes a detailed overview of inhomogeneous magnetization transfer (ihMT). This will be useful for Chapters 6 and 7, where we explore the fundamental physics of ihMT. Finally, we end this chapter with an overview of T_2 relaxation, which can be used to separate signals from water inside and outside the myelin sheath.

4.2 Spectral properties of white and grey matter

The molecules in tissues can be broadly separated into two types: non-aqueous and aqueous. The non-aqueous molecules are restricted in their motion in some way, whether by their size (such as large proteins) or by their environment (such as molecules in lipid membranes).¹

¹We will use the terms "aqueous" and "non-aqueous" throughout this work. However, the nomenclature in the literature is inconsistent. Some of the synonyms encountered are: aqueous protons = free protons, water protons, unbound protons

non-aqueous protons = macromolecular protons, tissue-associated protons, bound protons, semi-solid protons



Figure 4.1: Dipolar couplings in lipid molecules and lipid bilayers. (A) A generic lipid acyl chain. There is strong ¹H intra-methylene coupling. Weaker inter-methylene couplings lead to spectral broadening. (B) In a lipid bilayer, rapid spinning averages away inter-molecular dipolar couplings. The residual strength of the dipolar couplings is now a function of bilayer orientation.

The aqueous protons are mostly on water molecules, which have very similar ¹H spectral properties to unconfined water. Tissue water in a homogeneous B_0 field also has an FID that decays exponentially, leading to a Lorentzian lineshape with a precisely defined T_2 (Eq. 2.18).

There is significantly more to say regarding the ¹H spectral properties of non-aqueous molecules. Their motion is restricted, so the dipolar interaction is not averaged to zero as it is for aqueous molecules. Lipid-rich tissues like white matter have a super-Lorentzian² non-aqueous lineshape [79–82]. Fundamentally, this is caused by strong intra-methylene dipolar couplings in the lipid acyl chains (Fig. 4.1A). At physiological temperature, lipid bilayers in brain are in the fluid lamellar (L α) phase [83,84], which has three kinds of motions that average the proton-proton dipolar couplings in the acyl chains. First, lateral diffusion averages away the effects of intra-molecular dipolar couplings on the spectrum (although these can still produce relaxation) [85, 86]. Second, rapid spinning of the lipid molecules about their long axes creates a $P_2(\cos \theta)$ dependence on the average strength of the dipolar couplings [87], where P_2 is the second Legendre polynomial and θ is the angle between **B**₀ and the bilayer normal, as shown in Fig. 4.1B. Third, the lipid tails fluctuate via transgauche isomerisation, with motion increasing towards the tail ends in the middle of the bilayer [87, 88].

Collectively, these motions average the inter-methylene proton couplings more than the intra-

 $^{^{2}}$ The Super-lorentzian is named because its ratio of the width of the line at half max to the width at the inflection points is greater than a Lorentzian lineshape [78]. It is not actually a special case of a Lorentzian line.



Figure 4.2: Dipolar couplings in acyl chains causes the super-Lorentzian lineshape. (A) The ¹H NMR spectrum of lamellar-phase potassium palmitate - $(\beta - \omega)$ - d₂₉ in D₂O. In these deteurated molecules, only the α -methylene group retains its protons. The top trace is the signal from all protons, which is a sum of the spectrum just from the α -CH₂ methylenes (middle trace) plus contamination from the protons in the headgroup and in water (bottom trace). The middle trace is the angular average of two Gaussian-broadened doublets with a splitting determined by the dipolar coupling $\sim \frac{1}{2}(3\cos^2\theta - 1)$. This is also called a Pake pattern. Because the acyl chain is deuterated, inter-methlyene dipolar couplings are minimized. (B) A super-Lorentzian lineshape with $\sigma_0=20$ kHz and $\sigma_{\min}=40$ Hz and example contributions from specific angles. One would expect undeteurated potassium palmitate to have such a lineshape since it has significant inter-methylene coupling. Hence, its spectra from bilayers at specific orientations would be well described as single Gaussians rather than the doublets seen in the deuterated case in panel A. Panel A is modified slightly to improve readability from ref. [85] [Chemistry and Physics of Lipids, Volume 20, Higgs & MacKay, Determination of the complete order parameter tensor for a lipid methylene group from ¹Hand ²H-NMR spin labels, 105–114], © 1977, with permission from Elsevier.

methylene couplings. (Methyl groups can also produce super-Lorentzians [89], but their contribution is small compared to the large number of methylenes.) Consequently, the lipid tail spin system could well be called a system of strongly-coupled spin- $\frac{1}{2}$ pairs weakly coupled together [88,90]. Such systems nominally have spectra of superimposed Gaussian-broadened doublets [87,91]. Indeed, in a sample of lamellar-phase potassium palmitate - $(\beta - \omega)$ - d_{29} in D₂O where the protons have been retained only in the α -methylene group, the spectrum (Fig. 4.2A, from ref. [85]) shows this doublet behaviour clearly. In this sample, the intermethylene interaction responsible for more significant Gaussian broadening is not present. In naturally-ocurring lipids in the lamellar phase, however, the ¹H spectra are substantially broadened by these inter-methylene interactions. Therefore, the most common approach to modeling the super-Lorentzian is as a spectrum of superimposed Gaussians with widths modulated by $P_2(\cos \theta)$ [78, 79, 81, 87, 91, 92]. The Gaussian standard deviation for a bilayer whose normal makes an angle θ to **B**₀ is

$$\sigma(\theta, \sigma_0, \sigma_{\min}) = \sqrt{\frac{1}{4} (3\cos^2\theta - 1)^2 \sigma_0^2 + \sigma_{\min}^2}.$$
(4.1)

Here, $3\sigma_0/2$ is the maximum linewidth at $\theta = 0^\circ$. Bilayer fluctuations and field inhomogeneities are responsible for the minimum linewidth σ_{\min} of molecules oriented at the magic angle $\theta_{MA} \approx 54.7^\circ$ [81]. When all bilayer orientations are possible, integrating over them (powder averaging) gives the super-Lorentzian FID and spectral lineshape: [79–81,89,93,94]

$$S(t;\sigma_0,\sigma_{\min}) = \int_0^{\pi/2} d\theta \sin\theta \exp\left(-\frac{1}{2}\sigma(\theta,\sigma_0,\sigma_{\min})^2 t^2\right)$$
(4.2)

$$s(\omega; \sigma_0, \sigma_{\min}) = \int_0^{\pi/2} d\theta \sin \theta g \left[\omega, \sigma(\theta, \sigma_0, \sigma_{\min})\right]$$
$$= \int_0^{\pi/2} \frac{d\theta \sin \theta}{\sigma(\theta, \sigma_0, \sigma_{\min})} \exp\left(-\frac{\omega^2}{2\sigma(\theta, \sigma_0, \sigma_{\min})^2}\right).$$
(4.3)

Here, g is the Gaussian lineshape at one orientation. An example of the super-Lorentzian along with g from different orientations is displayed Fig. 4.2B. Note that because of the nerve fibre tracts in white matter, its *in vivo* lineshape in certain regions may more accurately be described as a partially-averaged super-Lorentzian [81]. As we will discuss in the next chapter, the *ex vivo* samples used in this thesis do not retain fibre tract orientation and isotropic powder averaging (*i.e.* a super-Lorentzian lineshape as described by Eq. 4.3) is seen.

The super-Lorentzian is typically discussed in the context of lipids, but it also arises from
other macromolecules (like proteins) which undergo similar averaging. As an example, super-Lorentzian lineshapes have been observed from suspensions of whole cells [80], and have successfully modeled quantitative Magnetization Transfer (qMT, Section 4.4.2) in muscle [82] and hydrated durum wheat and gluten [95]. In light of these varied applications, it seems that a super-Lorentzian may arise in systems where there are strongly-coupled proton groups (*e.g.* methylene or methyl groups) undergoing rotational thermal averaging, creating angulardependent dipolar couplings.

We will return to the super-Lorentzian in Chapter 5 when it is used to fit FID data from white matter.

4.3 T_1 relaxation

4.3.1 A common, simple model

 T_1 relaxation is a key source of contrast in brain and spinal cord imaging, but a comprehensive understanding of its physics has been elusive. One reason for this is simplicity—the goal of clinical MRI is to obtain adequate contrast in the shortest amount of time possible. Complicated models that involve many parameters are less useful in a clinical context than simple empirical or semi-empirical ones. Indeed, the assumption of a single T_1 in aqueous protons is suitable for understanding and developing many forms of MRI contrast.

The simplest approach to T_1 in tissue is the solvation layer model [96, 97]. Briefly, this assumes three distinct populations of protons in tissue: free water, a solvation layer surrounding non-aqueous molecules, and non-aqueous protons. The assumption of fast exchange leads to the well-known empirical relation,

$$\frac{1}{T_1} \propto \frac{1}{\text{WC}} + const, \tag{4.4}$$

where T_1 is the single value measured for aqueous protons, and WC is the water content (the weight fraction of water) in the tissue. This model suffices in many cases.

4.3.2 The controversy of quantitative T_1 measurements

For quantitative imaging, the specifics of T_1 relaxation become important. And in brain and spinal cord, the details of the observed aqueous T_1 relaxation unfortunately remain unclear.

For example, when a single T_1 component is assumed, the reported values in white matter tissue at 3 T vary from 690–1735 ms depending on the site and technique used [48,98].

Many recent MRI studies have measured both a short (~100 ms) and a long (~1 s) T_1 component [99–103]. These have been associated with two separate aqueous pools and some have suggested that myelin water is responsible for the faster T_1 component [101, 103]. However, still more studies suggest that axonal water (water inside myelinated axons) may have its own unique intrinsic T_1 time and should be accounted for explicitly [104–106]. Finally, confounding effects from T_1 's sensitivity to iron content may also play a significant role (see MacKay *et al.* [3] and references therein).

Our work in Chapter 5 is an attempt to show how some of these disagreements arise and in what situations simple models are suitable. We emphasize there that cleanly associating any one T_1 component with a specific aqueous compartment is generally not possible. This naturally arises from multiple-compartment models, such as the two pool model explained below and the four pool model used in Chapter 5.

4.4 Magnetization transfer

4.4.1 The magnetization transfer ratio

In tissue, magnetization can exchange between the aqueous and non-aqueous protons. Under physiological conditions, this happens primarily via proton exchange between water and the hydroxyl protons in lipid headgroups on macroscopic timescales of 0.1-1 s [83,107,108]. Microscopically, the residence time of protons in a macromolecular group is often as low as 10^{-11} s [107]. If the magnetization in the non-aqueous pool is reduced, magnetization exchange causes a subsequent reduction in the aqueous pool's magnetization. We can realize such a situation by making use of the ¹H spectral properties of tissue, exemplified in Fig. 4.3A. Magnetization Transfer (MT) is depicted in panel B. Low-amplitude rf irradiation (a prepulse) is applied far off resonance from the water peak prior to acquisition. This saturates the non-aqueous protons, reducing their magnetization. Magnetization exchange then leads to a net magnetization reduction in both the non-aqueous and aqueous pools.

To easily see the effects of MT in MRI, we need to compare our image with (S) and without (S_0) the weak, off-resonance MT pulse. The Magnetization Transfer Ratio (MTR) is [109]

$$MTR = \frac{S_0 - S}{S_0}.$$



Figure 4.3: How magnetization transfer works. (A) A cartoon of the ¹H NMR spectrum in tissue, consisting of a broad line from the non-aqueous protons and a narrow line from the aqueous protons. (B) Magnetization transfer. Weak rf irradiation at an offset Δ from the water peak reduces the non-aqueous magnetization. Because of magnetization exchange, the aqueous magnetization decreases as well.

Because MT originates in the non-aqueous pool, MTR can reveal changes in tissue structure. For example, in CNS tissue, MTR is lower in MS lesions and in areas with inflammation [110]. Also, MTR is sensitive to changes that may be invisible to other techniques. For example, non-lesion white matter in MS patients (the normal-appearing white matter) was observed to have a measurably different MTR than white matter in healthy controls [110,111].

4.4.2 qMT and the two pool model

MT is often modeled using a two pool model, also called the binary spin bath model (Fig. 4.4) [35, 112, 113]. Under rf irradiation ω_{rf} at an offset Δ from resonance, the coupled Bloch equations are [113, 114]:

$$\frac{dM_{x,1}(t)}{dt} = -\frac{M_{x,1}(t)}{T_{2,1}} - 2\pi\Delta M_{y,1}(t) - \omega_1 \sin(2\pi\Delta t)M_{z,1}
\frac{dM_{y,1}(t)}{dt} = -\frac{M_{y,1}(t)}{T_{2,1}} + 2\pi\Delta M_{x,1}(t) + \omega_1 \cos(2\pi\Delta t)M_{z,1}
\frac{dM_{z,1}(t)}{dt} = \frac{M_1(\infty) - M_{z,1}(t)}{T_{1,1}} - k_{12}M_{z,1}(t) + k_{21}M_{z,2}(t) + \omega_{rf} \sin(2\pi\Delta t)M_{x,1}(t) - \omega_1 \cos(2\pi\Delta t)M_{y,1}
\frac{dM_{z,2}(t)}{dt} = \frac{M_2(\infty) - M_{z,2}(t)}{T_{1,2}} - k_{21}M_{z,2}(t) + k_{12}M_{z,1}(t) - WM_{z,2}.$$
(4.5)

Here, k_{ij} is the exchange rate for magnetization from pool *i* to pool *j* and the $M(\infty)$ terms are the pool sizes. Because of the short T_2 in the non-aqueous pool, it may be assumed that its transverse magnetization is zero at all times. The saturation rate *W* is the same as for



Figure 4.4: The two pool model of tissue.

Provotorov theory (introduced in Section 2.5.6),

$$W = \pi \omega_1^2 g(2\pi\Delta),$$

where $g(2\pi\Delta)$ is the lineshape for the non-aqueous pool. Note that these are modified Bloch equations, because $g(2\pi\Delta)$ is usually assumed to be Gaussian or super-Lorentzian, not Lorentzian. This is not rigorous, but works well enough in practice [35, 79, 112–114].

Using these equations, "quantitative MT" (qMT) imaging can be performed by fitting different MT experiments to the model. Typically, one fixes $T_{1,2} = 1$ s, assumes a functional form for $g(2\pi\Delta)$, and measures $T_{2,1}$ and $T_{1,1}$ in separate experiments. Then, the remaining parameters are fit to repeated MT experiments at different prepulse offsets.

Because of the need for multiple values of Δ , qMT MRI takes a relatively long time to acquire in practice, about 20–30 minutes [115]. Moreover, with only one aqueous pool, qMT is sensitive to any changes in the tissue microstructure, not only those associated with myelin [109, 115, 116]. Hence, while qMT can detect changes in the myelin, it is not specific to them [117].

4.4.3 Inhomogeneous magnetization transfer

Recently, a modification of MT, called inhomogeneous Magnetization Transfer (ihMT), has been developed which appears to be selective for lipid bilayers, such as those found in myelin [84, 110, 118–131]. ihMT was originally thought to arise from inhomogeneous broadening of the non-aqueous lineshape, a hypothesis we explore at length in Chapters 6 and 7. Our results indicate that ihMT's specificity arises from dipolar couplings alone (a connection also made by others), so the "inhomogeneous" name is perhaps unfortunate. In this section, we shall describe the technique and some applications of ihMT, leaving the details of the physics for later chapters.

In ihMT experiments, a series of NMR spectra or MRI images are acquired: first, one with

a prepulse at a single offset frequency $+\Delta$ (spectrum S_+); next, one with an offset $-\Delta$ (spectrum S_-); then, one with the same power split between both $+\Delta$ and $-\Delta$ (spectrum S_{dual}); and finally, one with no prepulse (spectrum S_0). Experiments have shown that many non-aqueous non-lipids (such as heat-denatured ovalbumin, agarose, and gelatin [84, 118]) result in an equal attenuation of the aqueous signals in S_+ , S_- , and S_{dual} . However, materials containing a substantial proportion of lipid bilayers (such as brain and spinal cord WM, hair conditioner, Prolipid-161, and DPPC:Cholesterol phospholipids) show larger attenuation in S_{dual} [84, 118]. A quantitative measure of this difference, the ihMT ratio, has been defined as [118]

$$ihMTR = \frac{S_+ + S_- - 2S_{dual}}{S_0}.$$
(4.6)

The sum $S_+ + S_-$ provides a first-order correction for MT asymmetry. In this thesis, we use a definition that also includes a 2 in the denominator:

$$ihMTR = \frac{S_+ + S_- - 2S_{dual}}{2S_0}.$$
(4.7)

This is to maintain consistency with our publication on ihMT [131].

An example of ihMTR in various phantoms is shown in Fig 4.5. MTR shows similar signals from the two samples with non-aqueous protons (heat-denatured ovalbumin in row 1 and hair conditioner in row 2). Hair conditioner is a phantom for myelin, containing a high concentration of lipid bilayers. For this reason, ihMTR's selectivity to hair conditioner is of substantial interest.

Since its introduction, work on ihMT has rapidly advanced. Various improvements to the technique have been made, such as enabling measurements of T_{1D} [127] and generating T_{1D} -dependent contrast [127,132,133]. Moreover, by modifying the concentration of power during the prepulse, the ihMT signal can be boosted considerably [123, 134].

Comparison between ihMT and other techniques *in vivo* have shown promise of myelin sensitivity. Ercan *et al.* performed ihMT, MTR, diffusion tensor imaging, and myelin water fraction imaging (MWF imaging; see Section 4.5 below) in different white matter tracts [121]. Those authors found a strong correlation between ihMTR and MWF, the latter which is known to be a biomarker for myelin. MTR was only weakly correlated with MWF. In a study by Geeraert *et al.*, they found strong correlation between the myelin volume fraction and ihMTR [129]. Finally, recent work by Prevost *et al.* used mice that were genetically modified to produce green fluorescent protein in their myelin [135]. They observed a linear correlation between the fluorescence intensity and the ihMTR values, albeit with a considerable offset.

ihMT also holds considerable promise for rapid adoption in clinical settings given the sim-



Figure 4.5: ihMTR compared to in different phantoms. Row 1 contains heat-denatured ovalbumin, row 2 contains hair conditioner, rows 3–10 are aqueous solutions with varying T_2 times. (A) The measured T_2 times of the phantoms. (B) The MTR shows similar signals from two samples (hair conditioner and ovalbumin) with a non-aqueous proton pool. (C) The ihMTR is selective to the sample with lipid bilayers (hair conditioner). Figure reproduced from Varma *et al.* [118], © 2015, with permission from John Wiley & Sons, Inc.

plicity of calculating ihMTR. Like MTR, ihMTR is just a ratio and isn't dependent on model fitting. Two studies have shown its potential for use in patients. Rasoanandrianina *et al.* used ihMT to image spinal cord in Amyotrophic Lateral Sclerosis (ALS) patients [128]. That work found correlation between ihMTR and the clinical disability scores. In a similar study, ihMTR in lesions and in normal-appearing white matter of MS patients had a stronger correlation than MTR with their clinical disability scores. [110].

One of the possible confounding factors with ihMT is its orientation dependence, which has been shown *in-vivo* in brain [121, 129] and *ex-vivo* in sections of spinal cord [136]. This is likely because ihMT is sensitive to the strength of dipolar couplings, and dipolar couplings in lipid bilayers are orientation-dependent.

4.5 T_2 relaxation

4.5.1 Myelin water and intra/extra-cellular water

In contrast to T_1 relaxation, there is a consensus on T_2 relaxation in white matter [3,51,55, 58,137–139]. T_2 distributions calculated from CPMG decay curves show distinct peaks. MW typically has $T_2 \sim 10$ ms, and the water in intra/extra-cellular compartments (the IE water or IEW) has T_2 values in the range 40–90 ms [3,51,57,139]. Often, a third peak at ~1 s from

CSF is also present. It is somewhat remarkable that the IEW appears as one pool given its complex environment. However, glial cells are about 80% water and their membranes have a high concentration of aquaporin, allowing the free diffusion of water molecules on the timescale of one CPMG echo [54].

Regularized non-negative least squares (NNLS) is used to obtain the T_2 distribution from CPMG decay curves. Let A be a matrix whose elements are

$$A_{ij} = \exp(-t_i/T_{2,j}).$$

Then, the T_2 distribution $f(T_2)$ is found from the CPMG echo amplitudes $y(t_i)$ by solving

$$\arg\min_{f} ||Af - y||_{2}^{2} + \lambda^{2} ||f||_{2}^{2} \quad \text{subject to } f > 0.$$

In this expression, λ is the regularization parameter chosen so the regularized NNLS fit chi-squared is 1%–2% larger than in the non-regularized fit [58]. Regularization prevents over-fitting by forcing f to be smooth. Examples of these types of distributions are shown in the next chapter. Once $f(T_2)$ has been calculated, a useful quantity called the myelin water fraction (MWF) can be found. If $A_{\rm MW}$, $A_{\rm IEW}$, and $A_{\rm other}$ are the areas of the peaks corresponding to the MW, IEW, and other water, then

$$MWF = \frac{A_{MW}}{A_{MW} + A_{IEW} + A_{other}}.$$

The MWF has been validated as a marker for myelin using histology [140] and has found extensive use in research. However, as a myelin-sensitive MRI technique, it does have some drawbacks. Acquisition can be lengthy and processing can be technically demanding. Also, MWF imaging has difficulty distinguishing between intact myelin and myelin debris. Finally, MW/IEW and MW/non-aqueous exchange can introduce errors. For in-depth discussion of the applications and challenges of MWF imaging, the reader is referred to the reviews by MacKay & Laule (and references therein) [55, 141].

4.5.2 CPMG exchange correction

Magnetization exchange between the MW and IEW pools can lead to slight shifts in the measured T_2 and pool sizes. Exchange during CPMG acquisition will lead to an underestimation of the T_2 times and the MW size, and slight overestimation of the IEW size [142]. Previous work by Bjarnason *et al.* in bovine brain showed that these corrections were approximately 5–10% of the MW size [142]. Kalantari *et al.* used a slightly different procedure to estimate corrections in human brain. They fit the four pool model (discussed in the next chapter) to relaxation data with and without MW/IEW exchange, finding corrections up to 15% of the MW size [143]. With this approach, the corrections were sensitive to the initial conditions.

In this thesis, we will use the correction method introduced by Bjarnason *et al.*, which uses a two-pool model of exchange between the MW and IEW to calculate the correction factors for both the pool sizes and the T_2 times. How to do so was introduced by Bjarnason *et al.* in ref [142]. In Appendix B, their derivation is repeated using slightly different notation. Fig. 4.6 gives some examples of this correction on the T_2 times and the MW pool size. In that plot, T_{cr} is a measure of the MW/IEW exchange time, where lower values mean faster exchange. With a typical T_{cr} time of about 1 s, corrections are usually <20%. With this approach, the correction factor is independent of the initial conditions.



Figure 4.6: Examples of the CPMG exchange correction to pool sizes and T_2 under different conditions. T_{cr} (defined in Section 5.2) is a measure of the MW/IEW exchange time, where lower values mean faster exchange. (A) T_2/\tilde{T}_2 (corrected T_2 / observed T_2) for MW and IEW for different observed MWFs and $T_{cr,MW/IEW}$. The tilde indicates the observed value prior to having the correction applied. (B) The actual MWFs under the same conditions. Here we are assuming MW and IEW are the only aqueous pools and have fixed $\tilde{T}_{2,MW} = 6$ ms and $\tilde{T}_{2,IEW} = 55$ ms. These values were typical of our measurements in bovine brain (Chapter 5).

Chapter 5

Aqueous and non-aqueous T_1 relaxation in brain under six different initial conditions

5.1 Introduction

Ever since Edzes and Samulski's pioneering work on cross-relaxation in hydrated tendon [144], it has been known that in general, the intrinsic spin-lattice relaxation time of aqueous protons in tissue is not directly observable. Instead, the observed aqueous T_1 s (called T_1^* s in this work) convolve intrinsic spin-lattice relaxation with cross-relaxation to the non-aqueous protons and pools of other aqueous protons.

Some recent studies have emphasized the fact that only T_1^* s are directly observable [100, 102, 145, 146]. They have also highlighted how a system's relaxation depends on the initial conditions [102, 142, 145–147]. This may help explain the disagreement in the number of T_1 components present in white matter as highlighted in Section 4.3.2. As was noted, reproducibility is difficult in T_1 measurements, where pulse sequence, field strength, and site seem to be confounding variables [48, 98, 147].

One significant limitation to studying T_1 relaxation with MRI scanners is their difficulty in observing the rapidly-decaying signal from the non-aqueous protons. Yet this is straightforward with NMR spectrometers, and there has been some work on solid-state NMR spectroscopy in white matter. For example, some groups have looked at magic angle spinning (MAS) spectra of white matter [148, 149], while others have investigated the short T_2^* times [89,148,150]. Wilhelm *et al.*'s recent publication on high-resolution spectra of extracted myelin lipids and rat thoracic spinal cord in D_2O clearly showed their super-Lorentzian lineshapes [89]. However, the author is only aware of one previous paper by Bjarnason *et al.* [142] that followed the aqueous and non-aqueous magnetization during T_1 relaxation.

This chapter discusses T_1 relaxation in bovine white matter brain tissue under six different initial conditions. We analyzed the data in the context of a four pool model, where the two aqueous pools are MW and IEW, and the two non-aqueous pools are in myelin and nonmyelin. As in Bjarnason's work, an NMR spectrometer was used to acquire both FIDs and CPMGs, allowing the magnetization in different pools (MW, IEW, and total non-aqueous) to be found. The data and analysis here are significant improvements on this previous study, however. Because of improved sample holders and new equipment, we were able to acquire more FID data points and perform lineshape fitting instead of relying on moment analysis. This new approach gives deeper insights into the physico-chemical composition of the non-aqueous signal fraction. Furthermore, our CPMG analysis technique allowed for modeling of negative amplitude components, whereas Bjarnason *et al.* required subtraction of two complimentary experiments to ensure a positive signal. Also, their four pool model parameters were estimated from relaxation analysis on the data, whereas we fit the data directly. Finally, we also present FID experiments on grey matter for the first time.

Our analysis is performed with an eigenvector solution to the four pool model, introduced in Barta *et al.*'s recent work [145]. We emphasize how the eigenvector coefficients provide rich information about the effects the initial conditions have on relaxation. Lastly, we discuss how our results may be used guide pulse sequence design.

5.2 Theory: the four pool model

In white matter, there are at least two distinct pools of aqueous protons, separable by their T_2 times: the myelin water (MW) and the intra/extra-cellular water (IEW). Also, as discussed in Section 4.3.2, measurements of T_1 using MRI typically reveal two additional time constants, one short (~100 ms) and one long (~1 s). With the assumption that these four time constants are distinct, then in a simple model with first-order exchange between pools, four pools are required. The Four Pool Model [142,143,145,151–153] assumes that the MW and IEW pools exchange with each other via self-diffusion. Physiological considerations also require that each aqueous pool also exchanges with an adjacent non-aqueous pool. For MW, this is the non-aqueous myelin (pool M), composed of lipids and proteins in the myelin bilayers. IEW exchanges with the non-aqueous non-myelin (pool NM), which is mostly lipids and proteins in the glial cell membranes. The aqueous/non-aqueous exchange is mediated primarily by proton exchange (see Section 4.4).

Fig. 5.1 is the schematic of the four pool model. The dynamics of the longitudinal magnetization in the four pools are described by a system of coupled differential equations:

$$\frac{dM_{\rm M}}{dt} = -\frac{M_{\rm M} - M_{\rm M}(\infty)}{T_{1,\rm M}} - k_{\rm M,\rm MW}M_{\rm M} + k_{\rm MW,\rm M}M_{\rm MW}$$

$$\frac{dM_{\rm MW}}{dt} = -\frac{M_{\rm MW} - M_{\rm MW}(\infty)}{T_{1,\rm MW}} + k_{\rm M,\rm MW}M_{\rm M} - k_{\rm MW,\rm M}M_{\rm MW} - k_{\rm MW,\rm MW}M_{\rm MW} - k_{\rm MW}M_{\rm M} - k_{\rm$$

Here, $M_i(\infty)$ and $T_{1,i}$ are the size and intrinsic spin-lattice relaxation time of pool *i*, respectively; and k_{ij} is the magnetization exchange rate between pools *i* and *j*. k_{ij} and k_{ji} are related through

$$k_{ij}M_i(\infty) = k_{ji}M_j(\infty) \tag{5.2}$$

and

$$\frac{1}{T_{cr,ij}} = \frac{1}{k_{ij}} + \frac{1}{k_{ji}}.$$
(5.3)

 $T_{cr,ij}$ is the cross-relaxation time between pools *i* and *j*, which depends on the pool size through the k_{ij} s.

It is convenient to work in reduced magnetization units m, which are defined for pool i as [144]

$$m_i = -\frac{M_i - M_i(\infty)}{2M_i(\infty)}.$$
(5.4)

In these units, m = 0 corresponds to equilibrium magnetization, m = 1 is inverted magnetization, and m = 1/2 is zero magnetization (in the longitudinal direction). This transforms Eq. 5.1 into a homogeneous system of differential equations,

$$\frac{d\mathbf{m}}{dt} = R\mathbf{m},\tag{5.5}$$

where R is

$$R = \begin{pmatrix} -\frac{1}{T_{1,M}} - k_{M,MW} & +k_{M,MW} \\ +k_{MW,M} & -\frac{1}{T_{1,MW}} - k_{MW,M} - k_{MW,IEW} \\ 0 & +k_{IEW,MW} \\ 0 & 0 \\ -\frac{1}{T_{1,M}} - k_{M,MW} & +k_{M,MW} \\ +k_{MW,M} & -\frac{1}{T_{1,MW}} - k_{MW,M} - k_{MW,IEW} \\ 0 & +k_{IEW,MW} \\ 0 & 0 \end{pmatrix}$$
(5.6)

and \mathbf{m} is a vector of the pools' reduced magnetizations. The solution to Eq. 5.5 is [154]

$$\mathbf{m}(t) = \sum_{p=1}^{4} c_p \mathbf{v}_p \exp(\lambda_p t)$$
$$= \sum_{p=1}^{4} c_p \mathbf{v}_p \exp(-t/T_{1,p}^*), \tag{5.7}$$

where \mathbf{v}_p and λ_p are the p^{th} eigenvector and eigenvalue of R, c_p is a coefficient that depends on the initial conditions, and

$$T_{1,p}^* = -1/\lambda_p \tag{5.8}$$

is the p^{th} apparent T_1 relaxation time. $T_{1,p}^*$ is associated with eigenvector p, not with just one specific pool.¹

Finally, in regular magnetization units, the solution for pool i is

$$M_{i}(t) = M_{i}(\infty) \left(1 - 2\sum_{p=1}^{4} c_{p} v_{p,i} \exp(\lambda_{p} t) \right),$$
(5.9)

where $v_{p,i}$ is the *i*th component of the *p*th eigenvector.

In Appendix C, we show how there is an analogous electronic circuit for the four pool model.

¹A few words on notation: despite sharing the "*" superscript with T_2^* , T_1^* has nothing to do with B_0 homogeneity. Both are apparent relaxation times, but T_1^* is intrinsic to the sample microstructure and reflects the inability to distinguish between changes in longitudinal magnetization due to exchange and due to spin-lattice relaxation. On the other hand, T_2^* is a function of sample geometry and shimming and can be distinguished from T_2 by means of a CPMG acquisition. Also, our definition of "apparent T_1 relaxation time" is different than Rioux *et al.*'s [147]. They define T_1^* (same symbol) to be the single T_1 time one would measure if mono-exponential behaviour is assumed in a system relaxing with multiple exponentials. In our work, we take it to mean the time constants of those multiple exponentials.



Figure 5.1: The Four Pool model.

5.3 Methods

5.3.1 Sample preparation

A chilled, unfrozen bovine brain was ordered from Innovative Research (Novi, MI, USA) and received about 30 hours after harvesting. The age of the cow at the time of slaughter was less than 30 months old. Tissue samples were immediately excised and stored at 5 °C until use. All experiments were completed within 72 hours of receiving the brain.

Four tissue samples were extracted: 11.6 mg and 36.7 mg of white matter from two different locations in the splenium of the corpus callosum (samples WM-sp1 and WM-sp2), 52.2 mg of frontal white matter (sample WM-fr), and 28.6 mg of basal ganglia grey matter (sample GM-bg). Each sample was sandwiched between two cylindrical spacers made from protonfree Kel-F (the flourinated polymer PCTFE) inside a 3.5 mm NMR tube. This improved B_0 homogeneity over previous experiments which did not use spacers [145]. Proton-free o-rings on the spacers minimized water loss. During white matter sample preparation, the tissue was folded several times, ensuring that the nerve fiber tracts were oriented isotropically.

5.3.2 NMR experiments

The pulse sequences, shown in Fig. 5.2, consisted of three parts. First, the preparation pulses put the longitudinal magnetization into a non-equilibrium state. Second, the longitudinal magnetization relaxed (via intrinsic spin-lattice relaxation and exchange between pools) during the variable cross-relaxation delay, TI. Finally, an FID or CPMG echo train was acquired. The preparation pulses are the only variation between the sequences. Fig. 5.3 shows how the preparation pulses of each experiment gives the four pools unique initial magnetization values. The size and direction of the arrows qualitatively represent the magnitude and direction of the longitudinal magnetization.

The hard inversion-recovery (IR-hard, Fig. 5.2A) and soft inversion-recovery (IR-soft, Fig.

(A) Pulse sequence scheme



Figure 5.2: The NMR pulse sequences. All sequences have the general form shown in (A) consisting of three periods: preparation, cross relaxation, and acquisition. The hard inversionrecovery sequence (IR-hard) shown in (B) uses a broadband inversion pulse, inverting the aqueous and non-aqueous magnetization. The soft inversion-recovery sequence (IR-soft) in (C) uses a narrowband inversion pulse (1.1 kHz bandwidth), completely inverting only the aqueous magnetization. (D) shows the Goldman-Shen sequences, consisting of a spin-echo T_2 filter of 1 or 50 ms followed by a broadband pulse that either rotates the magnetization in the +z ("up") or -z ("down") direction.



Figure 5.3: The initial conditions on the four pools immediately after the preparation period in the various pulse sequences. The arrows' sizes and directions represent the longitudinal magnetization's direction and magnitude. Ideally, no two experiments have similar initial conditions, ensuring that the behaviour during cross-relaxation depends on a wide range of the four pool model parameters.

5.2B) experiments use different types of inversion pulses. The "hard" inversion pulse is a short (~6 μ s) rectangular pulse whose broadband excitation profile completely inverts the magnetization in all the pools. The "soft" inversion pulse is a 3 ms three-lobe sinc pulse that has a narrow (1.1 kHz) excitation bandwidth. This pulse is designed to invert the magnetization of the aqueous protons (due to their narrow linewidth) while only marginally decreasing the non-aqueous protons' magnetization. This is similar to the soft pulses typically used in MRI sequences.

Goldman-Shen (GS) experiments separate proton populations with distinct T_2 times [155, 156]. Our implementation (Fig. 5.2C) uses a spin-echo as a " T_2 filter" followed by a pulse that puts the magnetization back in the +z ("up", parallel to \mathbf{B}_0) or -z ("down", antiparallel to \mathbf{B}_0) direction. The spin echo in the middle of the T_2 filter is necessary due to the short T_2^* time, which is ~10 ms for the aqueous signals from all samples. The echo or filter time, τ_f , is either 1 ms or 50 ms. $\tau_f = 1$ ms separates the non-aqueous and aqueous magnetization due to the latter's short, ~10–500 μ s decay time. $\tau_f = 50$ ms separates the myelin water ($T_2\sim 6$ ms) and the IE water ($T_2\sim 60$ ms). The two τ_f times, combined with the two directions possible for the magnetization after the spin echo, give a total of four Goldman-Shen experiments: GS-1ms-up/down and GS-50ms-up/down.

Experiments were performed using a Bruker solenoidal probe (HP WB73ASOL10) in a 200 MHz (4.7 T) magnet with a home-built NMR spectrometer. The temperature was regulated at 37 °C. This setup allows acquisition of both FID and CPMG signals. During the FID,

131072 points were acquired with a dwell time of 1 μ s (10⁶ samples/sec).

The CPMG acquisition collected 300 echoes spaced 2 ms apart. 100 data points spaced 10 μ s apart were collected around the center of each echo and averaged. For all samples, the 90° pulse width was within the range of 3.1–3.3 μ s (a B_1 amplitude of 18–19 mT). The recycle delay was 7 s and 8 acquisitions were averaged in all experiments. During the cross-relaxation delay, 23 TI times were used, arrayed logarithmically from 0.77 ms to 10 s. With TI = 10 s, the system had fully recovered to equilibrium.

5.3.3 Analysis



Figure 5.4: (A) The analysis flowchart for fitting and combining the FID and CPMG data and (B) for fitting the four pool model parameters. BW is an isolated bulk water pool, discussed below.

The analysis pipeline, drawn in Fig. 5.4, broadly consists of two parts. In part A, the FID and CPMG data are fitted and then combined to extract the signals of different separable pools as functions of TI. Ultimately, separate signals from MW, IEW, and the sum of all non-aqueous protons are extracted. We remind the reader that in the context of the four pool model introduced in Section 5.2, the non-aqueous protons are composed of separate pools of non-aqueous myelin (pool M) and non-aqueous non-myelin (pool NM). In part B, these separate signals (MW, IEW, and M+NM) are fit to the four pool model. These steps will be covered in detail below.

Except when stated otherwise, for each parameter p_i its errors σ_i^+ and σ_i^- were determined in the following way. Using a least squares minimizer (regardless of the minimizer used to find p_i), 50 repeated fits were performed with synthetic Gaussian noise whose standard deviation was equal to the standard deviation of the best fit residuals. The initial guess for the i^{th} parameter in these fits was chosen randomly from $[0.8p_i, 1.2p_i]$ each time. Then, σ_i^{\pm} were determined from the average positive and negative deviation from p_i over the repeated fits.

All analysis was performed in Python with the Scipy/Numpy library [157] and with the LMFIT library [158].

5.3.3.1 FID fitting

The FID signals were modeled using one or more super-Lorentzians for the broad, nonaqueous component and a combination of Voigtians, Lorentzians, and Gaussians for the aqueous components (yellow boxes on the flow chart in Fig. 5.4A). In a perfectly-shimmed B_0 field, the aqueous lineshapes are nominally Lorentzian. However, one Lorentzian could not adequately model the aqueous component in our samples due to field inhomogeneities. In such cases, the Voigtian lineshape, which is a Gaussian-broadened Lorentzian, is often appropriate [159, 160]. In the time domain, the functions of the Lorentzian, Gaussian, and Voigtian positioned at f_0 are

$$g_{\text{Lorentzian}}(w, f_0; t) = \exp(-\pi w t) \exp\left(-i(2\pi f_0)t\right)$$
(5.10)

$$g_{\text{Gaussian}}(\sigma, f_0; t) = \exp\left(-(2\pi\sigma)^2 t^2\right) \exp\left(-i(2\pi f_0)t\right)$$
(5.11)

$$g_{\text{Voigtian}}(\sigma, f_0, w; t) = \exp(-\pi w t) \exp\left(-(2\pi\sigma)^2 t^2\right) \exp\left(-i(2\pi f_0)t\right).$$
(5.12)

We use widths to characterize all the lines. For the Lorentzian, w is the FWHM, and $T_2^* = (\pi w)^{-1}$. For the Gaussian, the width σ is the standard deviation. The Voigtian's widths correspond to its Gaussian and Lorentzian parts. In the frequency domain, the Voigtian is

a convolution of Gaussian and Lorentzian lines, which is a complicated function [160]. For completeness, the super-Lorentzian function is repeated from Eq. 4.2:

$$g_{\rm SL}(\sigma,\sigma_0,f_0;t) = \int_0^{\pi/2} d\theta \sin\theta \exp\left(-\frac{1}{2}\sigma(\theta,\sigma_0,\sigma_{\rm min})^2 t^2\right)$$
$$= \int_0^{\pi/2} d\theta \sin\theta \exp\left(-\frac{1}{2}(\frac{1}{4}(3\cos^2\theta - 1)^2\sigma_0^2 + \sigma_{\rm min}^2)t^2\right)$$

where $3\sigma_0/2$ is the maximum linewidth at $\theta = 0^\circ$ and σ_{\min} is the minimum linewidth at the magic angle.

Fitting was performed directly on both the real and imaginary parts of the phased timedomain data; there are two reasons why this was done instead of fitting in the frequency domain. First, the non-aqueous component in the frequency domain is low amplitude and spread out over multiple data points. Second, because of probe ring-down, the FIDs do not start immediately after the end of the 90° acquisition pulse. With our equipment, this deadtime had to be determined by calculating the first-order phase correction, the procedure for which is outlined in Section 2.7.3. Without knowledge of this, the amplitude of the rapidly-decaying non-aqueous signals may be over- or under-estimated by fitting. With the time of the first FID datapoint known, the data were phased to achieve a zero imaginary component at t = 0 when extrapolated backwards. Accounting for the deadtime in the frequency domain is not straightforward.

Fits to all FIDs give the total aqueous and total non-aqueous magnetizations at each TI:

$$M_{\rm aq}(TI) = [M_{\rm MW}(TI) + M_{\rm IEW}(TI) + M_{\rm BW}(TI)]$$
(5.13)

$$M_{\rm non-aq}(TI) = [M_{\rm NM}(TI) + M_{\rm M}(TI)].$$
(5.14)

The terms in [...] cannot be individually determined by FID data alone. But, by fitting the CPMG, one can determine the relative sizes of the isolated MW, IEW, and BW terms. Note that this is not so for the NM and M terms: they cannot be separated by any of the experiments in this work.

5.3.3.2 CPMG fitting

Multi-exponential regularized NNLS distributions, introduced in Section 4.5, are the usual way of fitting CPMG data when one is interested in separating the MW and IEW [51,55,57, 58]. However, this approach can't account for signals where different components may have opposite signs, as may occur in our experiments. Moreover, in regularized NNLS, separate peaks in the T_2 spectrum often coalesce when dealing with low-amplitude signals. In light of these issues, we used a sparse exponential distribution instead to fit the CPMG data (blue boxes in flowchart A). With this method, there is one exponential for the low-amplitude MW peak, two exponentials for the IEW peak (separated by 10 ms), and a final exponential for the low-amplitude ~200 ms peak.

Two comments in anticipation of the results are necessary here. First, we found the use of two exponentials instead of one for the IEW signal gave superior fits to the CPMG. This was especially true when the longitudinal magnetization was in a non-equilibrium state. Second, we have identified this last ~200 ms peak as bulk water (BW). This will be justified later.

With this sparse exponential distribution, we can measure the magnetization, positive or negative, of each aqueous component as a function of TI. The signal fraction p(TI) of each aqueous pool is

$$\tilde{p}_{MW}(TI) = \frac{A_{MW}(TI)}{\tilde{A}_{MW}(\infty) + \tilde{A}_{IEW}(\infty) + A_{BW}(\infty)}$$

$$\tilde{p}_{IEW}(TI) = \frac{\tilde{A}_{IEW}(TI)}{\tilde{A}_{MW}(\infty) + \tilde{A}_{IEW}(\infty) + A_{BW}(\infty)}$$

$$p_{BW}(TI) = \frac{A_{BW}(TI)}{\tilde{A}_{MW}(\infty) + \tilde{A}_{IEW}(\infty) + A_{BW}(\infty)},$$
(5.15)

where $A_i(TI)$ is the total intensity of the signal from pool *i* and $A_i(\infty)$ is the amplitude of the pool's exponential fits to the CPMG at TI = 10 s. The tilde on the MW and IEW terms indicates observed values—as covered in Section 4.5.2 and Appendix B, MW/IEW exchange during the CPMG acquisition means the actual pool amplitudes and T_2 times cannot be directly measured. The actual values are determined later in the analysis. In contrast, the BW pool is relatively isolated from exchange, so its actual value can be directly observed (indicated by the absence of a tilde).

5.3.3.3 Combining CPMG and FID fits

Steps in combining the CPMG and FID fits are shown in grey in part A of the analysis pipeline in Fig. 5.4. Using Eqs. 5.15 and 5.17 together gives the magnetization in each aqueous pool,

$$\widetilde{M}_{\rm MW}(TI) = \widetilde{p}_{\rm MW}(TI)M_{\rm aq}(\infty)$$

$$\widetilde{M}_{\rm IEW}(TI) = \widetilde{p}_{\rm IEW}(TI)M_{\rm aq}(\infty)$$

$$M_{\rm BW}(TI) = p_{\rm BW}(TI)M_{\rm aq}(\infty).$$
(5.16)

 $\widetilde{M}_{\text{MW}}(TI)$, $\widetilde{M}_{\text{IEW}}(TI)$, and, from Eq. 5.14, $M_{\text{non-aq}}(TI)$ are the inputs to the four pool model fitting routines. For each of the six experiments, we take the initial conditions in pool *i* from extrapolation back to TI = 0. Similarly, at the longest TI time the magnetization will be close to equilibrium, giving a measure of the pool sizes:

$$M_i(\infty) \approx M_i(10 \text{ s}). \tag{5.17}$$

The median value from $M_i(10 \text{ s})$ in each of the six experiments is used. $M_{BW}(TI)$ is fit separately to the standard equation for T_1 relaxation,

$$M_{BW}(TI) = M_{BW}(\infty) \left(1 - f \exp(-TI/T_{1,BW})\right), \qquad (5.18)$$

where f is the inversion efficiency, which depends on the initial magnetization (f = 2 for complete inversion) and $T_{1,BW}$ is the single T_1 time of the BW pool. When fitting, f is allowed to vary in each experiment while $M_{BW}(\infty)$ and $T_{1,BW}$ vary as global values.

5.3.3.4 Four pool model analysis

In Fig. 5.4's flowchart B, the input data to the fitting routine is in green boxes and calculations to modify those data are in red. One such modification is the CPMG exchange correction, applied at each iteration to find the "actual" magnetization in MW and IEW (assuming the trial parameters are correct in that iteration). This is done first by correcting the pool size (finding $M_{\rm MW}(\infty)$ and $M_{\rm IEW}(\infty)$ as described in Section 4.5.2). Then, each data point in these pools is multiplied by $\widetilde{M}_{MW}(\infty)/M_{\rm MW}(\infty)$ or $\widetilde{M}_{IEW}(\infty)/M_{\rm IEW}(\infty)$ to propagate the correction across all TI times.

Another modification is splitting the total non-aqueous data, $M_{\text{non-aq}}(TI)$ into its approximated constituent contributions from $M_{\text{M}}(TI)$, and $M_{\text{NM}}(TI)$. To do so, we define a parameter $\alpha_M = M_{\text{M}}(\infty)/(M_{\text{NM}}(\infty) + M_{\text{M}}(\infty))$ such that

$$M_{\rm M}(TI) = \alpha_{\rm M} M_{\rm non-aq}(TI)$$
$$M_{\rm NM}(TI) = (1 - \alpha_{\rm M}) M_{\rm non-aq}(TI).$$

This also gives values for the initial conditions in these pools, $M_{\rm M}(0)$ and $M_{\rm NM}(0)$. Following previous work [142, 143, 145, 152], we set $\alpha_M = 0.5$, which assumes equal proton amounts in both non-aqueous pools.

Fig. 5.4B's white boxes are the core steps to simulate the magnetization evolution. We used Scipy's implementation of the Differential Evolution algorithm, which is a global minimizer [161]. On each iteration, the algorithm first introduces new four pool model test parameters (4 T_{1} s and 3 T_{cr} s). It also could vary the pool sizes by $\pm 5\%$ (not shown on the flowchart). The penalty function is found using the model's estimation for the MW, IEW, and M+NM data. Once the global minimum is found, the fitting terminates. The penalty function is nominally the sum of the residuals squared, and we use this calculation with one slight adjustment—as we explain later, some of the MW early-time data data points are weighted slightly higher.

5.4 Results

5.4.1 Spectra and FIDs

The ¹H equilibrium spectrum of white matter (Fig. 5.5A) has a narrow, intense line from the aqueous protons sitting on a low-amplitude, broad ~10–15 kHz line from the non-aqueous protons. This non-aqueous lineshape was fit well by a super-Lorentzian, characterized by broad wings and a sharp central peak. The grey matter spectrum (Fig. 5.5B) is similar, but with a much smaller non-aqueous amplitude. In both the grey and white matter spectra, some additional structure is also visible, likely from metabolites, lipid headgroups, and/or methyl groups [80,89,148,149]. The B_0 shimming was imperfect due to sample geometry, so interpreting small spectral details is difficult.

While the fitting was ultimately performed in the time domain, we will discuss the frequencydomain spectra in detail since some aspects are easier to interpret. Fig 5.6A and B shows two examples of fitting WM-sp2's spectrum at a short and long time after a soft inversion pulse, and Table 5.1 lists all of the lineshape functions used for fitting each sample's spectra. While numerous functions are involved, the end result is simply an overall amplitude for the aqueous and non-aqueous signals. Through trial and error, we found that the aqueous line in each sample was fit well by one main Lorentzian (for WM-fr, WM-sp2, and GM-bg) or Voigtian (for WM-sp1). The need for the Voigtian indicates the B_0 field was less homogeneous in WM-sp1, likely due to its small size (11.6 mg of tissue compared to WM-sp2's 36.7 mg). Lower-intensity lines were also necessary to supplement this main aqueous line. These helped to isolate the wings of the main water peak, which due to its intensity, had a width of about 2 kHz near the baseline. In the case of IR-soft (Fig 5.6A), the central portion of the aqueous peak was inverted by the 1.1 kHz-wide inversion pulse, so the extremities of the wings were largely untouched. Even though the maximum amplitude of these wings was <1% of the aqueous line, we found they must be accounted for in order to distinguish their signal from



Figure 5.5: The equilibrium NMR spectra from WM-fr and GM-bg. The narrow peak is from aqueous protons: IEW, MW (in white matter), and BW. The broad super-Lorentzian is from non-aqueous protons: NM and, in white matter, M. "SL" indicates the peak of the super-Lorentzian, clearly visible on the white matter spectra in (A) but not in the grey matter spectrum in (B), where the non-aqueous pool is smaller and composed of relatively fewer lipids.



Figure 5.6: Fits to the equilibrium and non-equilibrium spectra for sample WM-sp2. (A) When the center of the aqueous peak is inverted, multiple low-amplitude lines are necessary to fit the non-inverted wings of the aqueous signal. (B) In equilibrium, the aqueous peak in this sample is largely accounted for by a Lorentzian, although a small, negative correction is required for better definition near the baseline. In both cases, two super-Lorentzians (one broad, one narrow) at the same frequency account for the non-aqueous magnetization. (C) When the second, narrower super-Lorentzian is not present, the fit to the same data as (B) is comparatively worse, as indicated by the arrows.

the super-Lorentzians from the non-aqueous protons. As seen in the example in Fig 5.6A, a series of Gaussians modeled these wings. These Gaussians were fixed at zero amplitudes at all TI times after the entire aqueous line relaxed enough to have a positive amplitude.

Two super-Lorentzians, one broad and one relatively narrow, with both constrained to be at the same frequency, were required in all white matter samples to fit the non-aqueous signal. Fig 5.6B and C demonstrates why two were needed by comparing the same equilibrium spectrum fit with two and one super-Lorentzians. Arrows in plot C indicate where the single super-Lorentzian's fit was inferior. Moreover, when single super-Lorentzian fits were attempted on non-equilibrium spectra like the one in plot A, the results were often unphysical estimations for the non-aqueous magnetization (*i.e.* $M_{\text{non-aq}}(TI) > M_{\text{non-aq}}(\infty)$). Specifically, using only one broad super-Lorentzian alone did not adequately fit the intensity near the center. The additional ~2 kHz super-Lorentzian ensured the central singularity fit well at all TI times. This extra intensity in the second, narrower super-Lorentzian may be from other, distinct proton groups. For example, in Wilhelm *et al.*'s high-resolution spectral fits of purified myelin [89], they included lower-intensity super-Lorentzians from Choline CH₂s and acyl CH₃s at approximately the same frequency as the broad, intense methylene super-Lorentzian. Whether this is the reason the spectra here required an additional, narrow super-Lorentzian is difficult to judge. Our B_0 shimming was comparatively worse than in the Wilhelm study (their sample was a solution of myelin extract, allowing a highresolution probe to be used), so the lines from these other protons, if present, could not be definitively resolved. Another potential explanation for the necessity of including the narrower super-Lorentzian is inadequate numerical integration of the broader super-Lorentzian. From Eq. 4.3, one can see that around the magic angle, the super-Lorentzian's constituent Gaussians rapidly decrease in width, which has has a nearly $P_2(\cos \theta)$ dependence. However, doubling the resolution from the 400 evenly-spaced steps in θ used here to 800 did not change the results appreciably. Beyond this, the fitting routine takes too long, though a more efficient discretization scheme could be used.

Since FID fitting was performed mainly through trial and error, there was a risk of overfitting. Yet, for our purposes, any over-fitting introduced negligible error, since we were only interested in the sum of all aqueous and non-aqueous lineshape function amplitudes. With the non-aqueous lineshape functions consisting solely of super-Lorentzians, the aqueous/nonaqueous contributions were easily distinguishable. On the other hand, if the goal was to distinguish contributions from specific metabolites, then the inclusion of multiple lineshape functions must be carefully mapped to each species (which would probably be impossible with the B_0 field homogeneity seen here). As it stands, we added additional lineshape functions until plots of the total aqueous and total non-aqueous amplitudes as a function of TI were seen to vary negligibly (not shown).

For all white matter samples, the spectral frequency of the super-Lorentzians were fixed at the position of the visible central peak of the non-aqueous spectrum. This peak was not visible in GM-bg so the position was approximated from the white matter samples. Correspondingly, without the obvious central peak, it wasn't necessary to include the more narrow super-Lorentzian when fitting the data as in the white matter samples. The central peaks were found to be about 3 ppm upfield from the water line. This is consistent with the signal originating mostly from acyl methylene groups [87, 89, 94, 162]. The minimum width was set to $\sigma_{\min}=40$ Hz, which is close to the narrowest width of the aqueous line.

Examples of time domain (FID) data are given in Fig. 5.7, along with the total aqueous and total non-aqueous fits (the sum of the functions in Table 5.1). Here, the broad, non-aqueous super-Lorentzians correspond to signals which have mostly decayed by ~100 to 500 μ s. This means that the number of FID data points with non-aqueous signals was relatively small. Consequently, we found it essential to have appropriate lineshape functions for the beginning of the FID. Also given in this table are the approximate T_2^* times, when the aqueous part of the FID decayed to about 1/e of its initial amplitude. These times are much shorter than

| Sample | Non-aqueous equilibrium lines | Aqueous equilibrium lines | Aqueous |
|--------|--|--|--------------|
| | | | T_2^* (ms) |
| WM-fr | $54 \cdot SL(\sigma=16.2 \text{ kHz}, \sigma_{\min}=40 \text{ Hz}, f_0=-673 \text{ Hz})$ 19 \cdot SL(\sigma=2.4 \km Hz, \sigma_{\min}=40 \text{ Hz}, f_0=-673 \text{ Hz}) | 158 · $L(w=44$ Hz, $f_0=0$ Hz) | 10.3 |
| WM-sp1 | $\begin{array}{l} 27 \cdot SL(\sigma {=} 14.9 \text{ kHz}, \ \sigma_{\min} {=} 40 \text{ Hz}, \ f_0 {=} {-} 679 \text{ Hz}) \\ 15 \cdot SL(\sigma {=} 4.5 \text{ kHz}, \ \sigma_{\min} {=} 40 \text{ Hz}, \ f_0 {=} {-} 679 \text{ Hz}) \end{array}$ | $\begin{array}{l} 161 \cdot V(\sigma_l{=}41 \ {\rm Hz}, \ \sigma_g{=}33 \ {\rm Hz}, \ f_0{=}4 \ {\rm Hz}) \\ 5 \cdot G(\sigma{=}11 \ {\rm Hz}, \ f_0{=}7 \ {\rm Hz}) \end{array}$ | 8.6 |
| WM-sp2 | $\begin{array}{l} 29 \cdot SL(\sigma = 13.6 \text{ kHz}, \ \sigma_{\min} = 40 \text{ Hz}, \ f_0 = -679 \text{ Hz}) \\ 1 \cdot SL(\sigma = 549 \text{ Hz}, \ \sigma_{\min} = 40 \text{ Hz}, \ f_0 = -679 \text{ Hz}) \end{array}$ | $ \begin{array}{l} -5 \cdot V(\sigma_l = 224 \; {\rm Hz}, \; \sigma_g = 176 \; {\rm Hz}, \; f_0 = 35 \; {\rm Hz}) \\ 5 \cdot L(w = 9 \; {\rm Hz}, \; f_0 = 3 \; {\rm Hz}) \\ 126 \cdot L(w = 45 \; {\rm Hz}, \; f_0 = 0 \; {\rm Hz}) \end{array} $ | 6.6 |
| GM-bg | $12 \cdot SL(\sigma = 18.8 \text{ kHz}, \sigma_{\min} = 40 \text{ Hz}, f_0 = -679 \text{ Hz})$ | $\begin{array}{l} -35 \cdot V(\sigma_l{=}118 \; {\rm Hz}, \; \sigma_g{=}795 \; {\rm Hz}, \; f_0{=}39 \; {\rm Hz}) \\ 33 \cdot G(\sigma{=}1.0 \; {\rm kHz}, \; f_0{=}21 \; {\rm Hz}) \\ 99 \cdot L(w{=}96 \; {\rm Hz}, \; f_0{=}{-}3 \; {\rm Hz}) \\ 13 \cdot L(w{=}25 \; {\rm Hz}, \; f_0{=}10 \; {\rm Hz}) \end{array}$ | 6.3 |

Table 5.1: The functional forms of the equilibrium FID fits (TI = 10 s) for all samples and their aqueous T_2^* s. The functions' amplitudes are adjusted at every TI and then summed together to determine the total aqueous and total non-aqueous signal. All lineshape functions are normalized to 1. The amplitudes are in relative units. SL=super-Lorentzian function (Eq. 4.2; $3\sigma/2$ is the standard deviation of the widest component Gaussian, σ_{\min} is standard deviation the narrowest component Gaussian), G=Gaussian function (Eq. 5.11; σ is standard deviation), L=Lorentzian function (Eq. 5.10; $w = (\pi T_2)^{-1}$ is FWHM) V=Voigtian function (Eq 5.12; σ and w correspond to its Gaussian and Lorentzian widths). The widths vary from sample to sample to account for differences in B_0 homogeneity. The centers of the nonaqueous lines were fixed at the center of the super-Lorentzian, visible in the white matter samples. In GM-bg, the super-Lorentzian center not visible on the spectrum and so was approximated from the white matter samples. The aqueous T_2^*s are approximately the time it took for the aqueous portion of the FIDs to decay by 1/e.



Figure 5.7: Examples of fits to FIDs in an IR-Soft experiment on WM-sp1 at different TI times. (A) The FID shortly after the soft inversion pulse. Here, the non-aqueous signal's magnetization is positive, whereas the aqueous magnetization is inverted. (B) The same TI after a hard inversion pulse. The non-aqueous magnetization is now inverted as well. The equilibrium FID in (C) shows the long-lasting signal from the aqueous protons as well as the short-lived signal from the non-aqueous protons.

the IEW T_2 (~60 ms) because of B_0 inhomogeneity.

5.4.2 CPMG multi-exponential fitting

Fig. 5.8 shows the difference between the regularized NNLS and sparse exponential distributions of WM-fr. The distributions of all white matter samples were similar. In GM-bg, no MW contribution was detected, even in the sparse exponential distribution. Possible reasons for this are explored in the discussion. Another difference was that GM-bg's BW T_2 time was closer to 300 ms (instead of 200 ms as for the white matter samples). In the sparse distribution, two exponentials were necessary to fully account for the IEW's potential for high positive and negative signals across the whole range of experiments. The sparse distributions were fairly consistent with Barta *et al.'s* work in bovine brain [145], although their BW T_2 was much higher (650 ms) than ours. This was likely due to the confined volume of our sample, where the BW could only form a thin film between the sample and the walls of the NMR tubes and plugs. This restriction would have reduced the T_2 . Barta *et al.* used a small piece of tissue inside an NMR tube without any spacers, so the BW pool was less restricted.



Figure 5.8: The regularized NNLS distribution compared to the sparse exponential distribution. The distributions were calculated from WM-fr's equilibrium CPMG. The sparse distribution allowed for negative amplitudes. Two exponentials were used for the IEW peak, which has the highest intensity.

5.4.3 White matter four pool and bulk water fitting

Using the four pool model, $M_{\rm MW}(TI)$, $M_{\rm IEW}(TI)$, and $M_{\rm non-aq}(TI)$ (= $M_{\rm M}(TI) + M_{\rm NM}(TI)$) data series were fit for the three white matter samples. Given its lack of a measurable MW pool, sample GM-bg was fit to a two pool model, discussed later. These results are presented in Fig. 5.9, which also includes the BW fits using Eq. 5.18. The MW and IEW data in this plot have been corrected for MW/IEW exchange during the CPMG and so represent the actual magnetization in these pools. In the case of MW, we found data points near $M_{\rm MW}(TI) \approx 0$ tended to be unreliable and were omitted (indicated by "×" markers). The sparse exponential distribution could not adequately detect the MW when its absolute intensity is very small, leading to values which tended to correlate (erroneously) with the IEW signal. Because the omitted data includes the start of the GS-50ms-up/down experiments, in these cases $M_{\rm MW}(0)$ was fixed at 0. The MW plots also show the observed magnetization, calculated by performing the CPMG exchange correction in reverse. The same correction was applied to the IEW data but the difference is not visible on the graph.



Figure 5.9: Four pool model fits to WM-fr. All MW and IEW data with opaque markers have been corrected for exchange during the CPMG and are the actual magnetizations in those pools. For MW, the observed magnetization, $\widetilde{M}_{\rm MW}(TI)$, is plotted (translucent markers) along with its fit (dashed line). These are not plotted for IEW because the difference between its observed and actual magnetization is very slight. "×" markers indicate data which were omitted (data points where $M_{\rm MW}(TI) \approx 0$, as described in the text). The BW was fit separately using Eq. 5.18; for this pool, each experiment was well characterized using a unique initial magnetization and a single, global T_1 time. This is evidence that the BW pool can be considered isolated from the the other four pools.

(A) White matter four pool model fit parameters

(B) Fit eigenvectors and eigenvalues

| Parameter | Units | WM-fr | WM-sp1 | WM-sp2 | | <u>WM-fr</u> | | | |
|--|-------------|---------------------|---------------------|-----------------------|--------|------------------|----------------------|----------------|--------------------------------|
| T _{cr,M/MW} | s | 0.13 (+0.01/-0.01) | 0.16 (+0.01/-0.02) | 0.154 (+0.007/-0.016) | | 22.9 (+1.1/-0.8) | | | |
| T _{cr,MW/IEW} | | 0.82 (+0.05/-0.04) | 0.84 (+0.07/-0.07) | 0.73 (+0.05/-0.07) | T1* | ms | 74 (+3/-4) ms | 130 (+4/-4) ms | 1.268 (+0.004/-0.005) s |
| T _{cr,IEW/NM} | | 0.67 (+0.04/-0.03) | 0.86 (+0.16/-0.06) | 0.86 (+0.19/-0.05) | м | 6.16 | 2.19 | -11.71 | 5.25 |
| T _{1,M} | | 0.23 (+0.01/-0.01) | 0.19 (+0.05/-0.01) | 0.149 (+0.066/-0.004) | MW | -8.11 | 0.32 | -5.09 | 4.05 |
| T _{1,NM} | | 0.63 (+0.06/-0.06) | 1.0 (+0.5/-0.2) | 2.5 ^b | IEW | 2.67 | -14.97 | 7.65 | 51.55 |
| T _{1,IEW} ^a | S | 3.0 (+0.2/-0.1) | 2.4 (+0.2/-0.1) | 2.24 (+0.10/-0.08) | NM | -0.15 | 14.52 | 3.32 | 8.29 |
| T _{1,MW} ^a | | 3.0 (+0.2/-0.1) | 2.4 (+0.2/-0.1) | 2.24 (+0.10/-0.08) | Size | 17.09 | 32 | 27.78 | 69.15 |
| M _M (∞) | | 14.92 (+0.06/-0.07) | 9.26 (+0.07/-0.07) | 9.11 (+0.04/-0.04) | E/R | 0.03 | 0.06 | 0.21 | 1 |
| M _{NM} (∞) | | 14.92 (+0.06/-0.07) | 9.26 (+0.07/-0.07) | 9.11 (+0.04/-0.04) | | | | M-sn1 | |
| M _{IEW} (∞) | rel. ° | 85.9 (+0.1/-0.2) | 91.65 (+0.14/-0.10) | 84.2 (+0.2/-0.2) | T1* | 22 (+1/-2) ms | 67 (+5/-4) ms | 102 (+6/-5) ms | 1.453 (+0.006/-0.005) s |
| <i>M</i> _{MW} (∞) | | 8.92 (+0.08/-0.10) | 5.46 (+0.03/-0.02) | 6.92 (+0.08/-0.04) | м | 2.94 | 1.25 | -8.01 | 3.01 |
| $M_{\rm non-aq}(\infty) = M_{\rm NM}(\infty) + M_{\rm M}(\infty)$ | | 29.8 (+0.1/-0.1) | 18.5 (+0.1/-0.1) | 18.22 (+0.09/-0.08) | MM | -5.17 | 0.01 | -2.55 | 2.59 |
| T _{2,MW} | | 8.6 | 7.9 | 8.3 | IEW | 2.66 | -9.6 | 4.92 | 53.71 |
| T _{2,IEW} | ms | 63 | 66 | 66 | NM | -0.11 | 9.13 | 1.62 | 5.29 |
| $\tilde{T}_{2,MW}/T_{2,MW}$ | | 0.9 | 0.86 | 0.87 | Size | 10.88 | 19.98 | 17.1 | 64.6 |
| $\tilde{T}_{2,\text{IEW}} / T_{2,\text{IEW}}$ | | 0.93 | 0.93 | 0.92 | E/R | 0.03 | 0.04 | 0.24 | 1 |
| $\widetilde{M}_{MW}(\infty) / M_{MW}(\infty)$ | - | 0.78 | 0.7 | 0.73 | | | 1 | 1 | 1 |
| $\widetilde{M}_{-}(\infty) / M_{-}(\infty)$ | | 1 | 1.00 | 1 | | | <u>w</u> | <u>M-sp2</u> | |
| (In the second s | rel C | 5 40 (10 02/ 0.02) | 2,802 (10,02(0,02) | 0.00 (10.04/ 0.04) | T1* | 24 (+1/-2) ms | 72 (+3/-3) ms | 91 (+6/-2) ms | 1.376 (+0.005/-0.004) s |
| /// _{BW} (∞) | Tel | 5.19 (+0.03/-0.03) | 2.092 (+0.02/-0.02) | 0.00 (+0.04/-0.04) | м | 3.99 | 2.73 | -7.22 | 2.74 |
| T _{1,BW} | s | 2.20 (+0.02/-0.02) | 1.60 (+0.02/-0.02) | 2.19 (+0.02/-0.02) | N/1\A/ | -6.21 | 0.78 | -3.45 | 3.17 |
| T _{2,BW} | ms | 211 (±5%) | 211 (±5%) | 184 (±5%) | | 2.96 | 10.91 | 2 10 | 40.1 |
| MW residual weight, η | - | 8 | 10 | 5 | IEW | 2.50 | -10.81 | 3.19 | 43.1 |
| a) Parameters were cons | trained to | be the same value | | | NM | -0.13 | 8.55 | 3.16 | 5.46 |
| b) Parameters are at the | imit of all | owed range | | | Size | 13.3 | 22.87 | 17.02 | 60.47 |
|) Relative pool size units are scaled so that aqueous pools add to 100 | | | | | E/R | 0.05 | 0.05 | 0.25 | 1 |

Table 5.2: The results of the four pool model fits on all white matter samples. (A) The fit parameters. Errors on the T_{2} s are estimated to be 5%, which is close to the FWHM of the peaks on the regularized NNLS distributions (Fig. 5.8). Errors on other parameters are the standard deviations of repeated fits with noise, as described in the Methods section. (B) The eigenvectors and eigenvalues derived from the fit parameters. To show the amount of magnetization flow represented each eigenvector, the components listed are $v'_i = v_i M_i(\infty)$. The size $(\sum_{i=1}^4 |v'_i|)$ is a measure of how much magnetization flow is associated with each eigenvector. Eigenvectors with larger sizes generally have more easily observable T_1^* values. E/R is the exchange/relaxation factor from Eq. 5.21 (E/R=0 means the T_1^* corresponds to pure exchange, whereas E/R=1 indicates pure spin-lattice relaxation).

The data show that the expected initial conditions in the six experiments (Fig. 5.3) were achieved, leading to unique relaxation behaviour in each case. The fits from the model described the magnetization in each pool well, with the largest relative deviation occurring in MW during the GS-50ms-up experiment around $TI \sim 100$ ms. However, this large relative error was consistent with MW being the smallest pool. In fact, when the standard penalty function was used in the fitting routine (the sum of residuals squared), the solver was relatively insensitive to the MW pool due to its comparatively lower amplitude. This led to the solver omitting a short, ~30 ms T_1^* component, present most obviously in MW. To ensure this component was accounted for, the fitting results shown here have had the residuals from the start (TI < 37 ms) of the MW data up-weighted by a factor η when calculating the fitting penalty function:

$$MW \text{ Residuals} = \begin{cases} M_{MW,data}(TI) - M_{MW,fit}(TI) & TI > 37 \text{ ms} \\ \eta \left(M_{MW,data}(TI) - M_{MW,fit}(TI) \right) & TI < 37 \text{ ms.} \end{cases}$$
(5.19)

We will deal with choosing the value of η and the consequences of this choice in the next section.

Turning now to the best fit parameters listed in Table 5.2A, the size of the MW pool, $M_{\rm MW}(\infty)$, is immediately understandable. Because the pool sizes in the table have been normalized so that $M_{\rm MW}(\infty) + M_{\rm IEW}(\infty) + M_{\rm BW}(\infty) = 100$, this is equal to the myelin water fraction (MWF). WM-fr had the highest $M_{\rm MW}(\infty)$, likely due to the presence of more myelin. This was reflected in WM-fr's total non-aqueous pool size, $M_{\rm non-aq}(TI)$, which was larger than in the other samples. In all cases $T_{1,\rm MW}$ and $T_{1,\rm IEW}$ were constrained to have the same value, which could vary. Without this constraint, $T_{1,\rm MW}$ had large (~1 s) variability.

The ratios of the observed to true values for IEW and MW pool sizes and T_2 s are also given in the table. The largest correction was for $\widetilde{M}_{MW}(\infty)$ of WM-sp2, where the true pool size, $M_{MW}(\infty)$, was about 40% larger. In other words, the observed value only underestimated the pool size by less than 2% of the total aqueous signal (the size of $M_{MW}(\infty)$ in WM-sp2 is 6.9% of the total aqueous signal). The effect on the IEW pool size was negligible.

The T_1^* s and eigenvectors in Table 5.2B offer a simple way to consider the meaning of all of the fitted parameters at once. In the table, the values listed are scaled eigenvectors \mathbf{v}' whose components are

$$v_i' = M_i(\infty)v_i. \tag{5.20}$$

 v'_i can be interpreted as amount of physical magnetization entering (positive values) or leaving (negative values) the pool, keeping in mind that only the relative sign differences in the eigenvector components matter—multiplying and eigenvector by -1 does not change the physics. Each eigenvector describes a varying amount of magnetization exchanging or relaxing, so it is convenient to define their "size" as $\sum_{i=1}^{4} |v'_i|$. Generally, the larger the size, the easier it is to observe the T_1^* associated with this eigenvector—although this ultimately depends on the initial conditions. Another related metric is the relaxation/exchange factor [145],

$$E/R = \frac{\left|\sum_{i=1}^{4} v_{i}'\right|}{\sum_{i=1}^{4} |v_{i}'|},$$
(5.21)

where E/R=0 indicates the corresponding T_1^* arises from inter-pool exchange only, and E/R=1 indicates the T_1^* is from pure spin-lattice relaxation. All the white matter samples' eigenvectors had a similar structure and matched well with those found in a previous study in bovine brain [145]. Looking at WM-fr, the most rapid relaxation time was $T_1^* = 23$ ms from an eigenvector that primarily described M/MW exchange. This matches with the theory that MW's short T_2 time is caused by exchange with the myelin lipids [57,145]. There was also some MW/IEW exchange on this timescale as well, which was more significant with the WMsp1/sp2 samples. This is consistent with their smaller non-aqueous pool sizes. A smaller myelin sheath would be more permeable, increasing the MW/IEW exchange. The next eigenvector ($T_1^*\approx 60-80$ ms) was almost pure IEW/NM exchange. The last two eigenvectors can be associated with MW/IEW or (M+MW)/(IEW+NM) exchange ($T_1^*\approx 90-130$ ms) and spin-lattice relaxation ($T_1^* > 1$ s). Because this last one had the largest size and the longest T_1^* , it is therefore the easiest to observe using MRI. T_1 measurements of brain often only report this relaxation time.

Fig. 5.9 and Table 5.2 also show the results of fitting the BW data to Eq. 5.18, the single inversion-recovery equation. BW was well-described using a single, global T_1 and $M_{\rm BW}(\infty)$ values, but with unique initial magnetization for each experiment. The majority of its relaxation was single-exponential, with only small fluctuations indicating some exchange with other pools. In light of this, treating BW as an isolated pool outside of the four pool model seems justified.

5.4.4 White matter fitting variations

As mentioned in the last section, when all MW, IEW, and M+NM residuals were treated without weighting in the fitting penalty function, the four pool model tended to ignore the ~30 ms T_1^* component. Its associated eigenvector in Table 5.2 shows this component represents M/MW exchange. Fig. 5.10 summarizes this issue by plotting the residuals from fits to the first 10 points of the MW magnetization on a linear scale. The GS-50ms-up/down



Figure 5.10: Residuals from four pool model fits to short-TI MW data in sample WM-fr. In the weighted fit, the residuals for the 10 MW data points across all experiments where TI < 37 ms were multiplied by η (defined in Eq. 5.19), emphasizing the importance of these points. This forced the rapid, 20–30 ms decay most visible in the MW pool to be accounted for. This is particularly clear on this graph's linear scale in the case of IR-Soft, where the weighted fit was superior. The GS-50ms-up/down experiments were not included since their initial MW data points were excluded from the four pool model fit.

| | | χ^2_{tot} | |
|---|-------|----------------|--------|
| | WM-fr | WM-sp1 | WM-sp2 |
| three pool | 6.83 | 7.61 | 3.67 |
| four pool, $\eta = 1$ (unweighted) | 2.98 | 4.16 | 2.27 |
| four pool, $\eta > 1$ (MW start weighted) | 5.86 | 6.79 | 3.05 |

Table 5.3: Comparison of total chi-squares for three pool, weighted four pool, and unweighted four pool model in white matter. The three pool model consisted only of MW, IEW, and one non-aqueous pool. The unweighted four pool model treated the residuals of all data points the same. η , the weighting factor, is given in Table 5.2. Larger values force recognition of the $T_1^* \sim 30$ ms time. Weighting was removed prior to calculation of χ^2_{tot} (see Eq. 5.24). The values of η when $\eta > 1$ are listed for each sample in Table 5.2.



Figure 5.11: The effect of varying the weighting of the start of the MW residuals (η , Eq. 5.19) for sample WM-fr. (A) Chi-square from each data series across all experiments (Eq. 5.23) and the total chi-square (Eq. 5.24). (B) The change in the four T_1^* times as η is increased. Only at larger values were the times from MW/M exchange and IEW/NM distinguishable. The longest T_1^* time, associated with spin-lattice relaxation, is minimally influenced by η . For this sample, the optimal factor was $\eta=8$, determined by the start of the plateau in most of the chi-square and T_1^* values.



Figure 5.12: A comparison of three different models applied to WM-sp2: two four pool models (one weighted, one not) and one unweighted three-pool model (consisting only of MW, IEW, and one combined non-aqueous pool). The chi-square plotted, $\chi^2(x_s, x_e)$, is the sum over all data points for each data series x_s and experiment x_e (Eq. 5.22). Overall, the four pool models provided a better fit, particularly in the IEW pool. However, weighting was necessary in order to fit the MW pool properly.

MW data was not plotted since it was excluded from the fits (as explained above).

The four pool model fits shown were performed in two different ways. The first fit method treated all data points in the residuals with the same weight ($\eta = 1$, where η was defined in Eq. 5.19). With no weighting, the rapid decay seen most clearly at the beginning of the IR-soft and GS-1ms-up/down experiments was ignored. This was also visible in IR-soft non-aqueous magnetization (not shown). In the second method, the residuals from the first ten MW data points (TI < 37 ms) in each experiment were up-weighted by the factor, η defined in Eq. 5.19. As evident by the weighted fits, $\eta > 1$ forced recognition of this short time constant. However, forcing the fit to more closely match the beginning of the MW curve came at the price of a worse overall fit.

Fig. 5.11 motivates the ultimate choices of η , which are reported for each sample in Table 5.2. The total chi-square for fits to the MW, IEW, and total non-aqueous (M+NM) data are shown in panel A, and T_1^* times are in panel B. Both are plotted as functions of η . There is an inflection point in the plotted quantities around a similar value of η . For WM-fr, this was around $\eta = 8$. Note that without a large η , the T_1^* times associated with MW/M exchange and IEW/NM exchange were unacceptably close (the corresponding eigenvalues were also
equal).

Table 5.3 gives the total chi-square values, χ^2_{tot} , for different fitting methods, including weighted and unweighted four pool models.² The chi-square $\chi^2(x_s, x_e)$ for data series $x_s \in \{MW, IEW, M+NM\}$ and experiment $x_e \in \{IR-Hard, IR-Soft, GS-50ms-up, GS-50ms-down, GS-1ms-up, GS-1ms-down\}$ is

$$\chi^{2}(x_{s}, x_{e}) = \sum_{i=1}^{N_{TI}} \left(\text{data}(x_{s}, x_{e}; TI_{i}) - \text{fit}(x_{s}, x_{e}; TI_{i}) \right)^{2},$$
(5.22)

where N_{TI} is the number of TI times in that series (which depends on x_s and x_e since data points were removed in certain series). From this, the total chi-square for a particular series x_s across all experiments is

$$\chi^2(x_s) = \sum_{\substack{x_e \in \\ \text{experiments}}} \chi^2(x_s, x_e), \tag{5.23}$$

and the total chi-square for all series and experiments has the form

$$\chi^2_{tot} = \sum_{\substack{x_s \in \\ \text{series}}} \chi^2(x_s).$$
(5.24)

Importantly, these values are calculated independent of the value of η .

In the three white matter samples, χ^2_{tot} was ~1.2–1.8× worse in the weighted fits than in the unweighted fit. To investigate why, the chi-square values for each experiment and data series, $\chi^2(x_s, x_e)$, are shown in Fig. 5.12. Unsurprisingly the weighted four pool model fit gave superior MW modeling, particularly for the IR-soft experiment where the ~30 ms T_1^* component is most obvious. However, the trade-offs were worse IEW and total non-aqueous fits.

To confirm the four pool model is necessary to model white matter, we also tried fitting the same data to an unweighted ($\eta = 1$) three pool model. In the three pool model, the two non-aqueous pools in the four pool model (M and NM) are combined into a general non-aqueous pool. Exchange happens between MW/IEW, IEW/non-aqueous, and MW/non-aqueous. This was motivated by the fact that we were unable to observe the M and NM magnetization separately. When this model was used, the result was a worse overall chi-square than either of the four pool fitting methods (Table 5.3). Looking at the chi-square

²The χ^2 as defined here is actually the Residual Sum of Squares (RSS). If the errors on each data point were independently and identically distributed with a variance σ^2 (which is not the case for our data), then RSS/σ^2 has a chi-square distribution.

| (A) Grey matter two | o pool model | fit parameter |
|---------------------|--------------|---------------|
|---------------------|--------------|---------------|

| Parameter | Units | GM-bg | |
|---------------------------|----------------------|----------------------------|--|
| T _{cr,non-aq/aq} | s 1.42 (+0.12/-0.11) | | |
| T _{1,non-aq} | | 0.23 (+0.02/-0.01) | |
| T _{1,aq} | 3 | 3.63 (+0.25/-0.18) | |
| M _{non-aq} (∞) | rol | 8.26 (+0.12/-0.04) | |
| M _{aq} (∞) | 101. | 98.52 (+0.20/-0.16) | |
| T _{2,IEW} | s | 1.482 (+0.016/-0.014) | |
| M _{BW} (∞) | rel. | rel. 1.480 (+0.016/-0.011) | |
| T _{1,BW} | s | 1.823 (+0.036/-0.026) | |
| T _{2 BW} | ms | 288 (±5%) | |

| <i>T</i> ₁ * | 73 (+1/-2) ms | 2.000 (+0.003/-0.008) s |
|-------------------------|----------------------|-------------------------|
| Aqueous | -5.83 | 80.5 |
| Non-Aq | 8.25 | 4.7 |
| Size | 13.08 | 85.1 |
| E/R | 0.16 | 1 |

Table 5.4: The fitted two pool model parameters (A), and eigenvectors and T_1^* s (B) for the grey matter sample GM-bg. Values listed in the eigenvectors are $v_i M_i(\infty)$. Error values are described in the caption of Fig. 5.2.

values of the individual experiments, the three pool model performed better in MW than the unweighted four pool model. This may hint that the assumption of equal non-aqueous protons in the M and NM pools was incorrect. Yet, the three pool fit was significantly worse in both the IEW and the total non-aqueous than either of the four pool models.

5.4.5 Grey matter two pool fitting

With no detectable MW, the GM-bg sample was fit to a two pool model representing aqueous and non-aqueous protons. The fit results are summarized in Fig. 5.13 and Table 5.4. The non-aqueous data are comparatively noisier than in the white matter samples, due to GMbg's smaller non-aqueous pool size (8% of the total aqueous pool size compared to about 18% in WM-sp1/sp2 and 30% in WM-fr). The IR-soft non-aqueous data was especially noisy; as in the white matter samples, this was the most difficult experiment for FID fitting because of its complicated lineshape. This was exacerbated by the relatively low non-aqueous signal in the grey matter. Looking at the eigenvectors and T_1^* times, the rapid, 73 ms T_1^* is associated with aqueous/non-aqueous exchange, whereas the longer 2.0 s time is associated with spin-lattice relaxation.



Figure 5.13: Two pool fits to grey matter sample GM-bg. Since there was no detectable MW in this sample, the two pool model was more appropriate. Bulk water was fit separately using Eq. 5.18.

5.5 Discussion

5.5.1 Comparison with other studies

Overall, the values measured in the four pool model fits were reasonable and in-line with previous *ex-vivo* and *in-vivo* studies. Comparing the size of the MW pool (*i.e.* the MWF) in white matter, we find it is on average lower than in previous human and bovine studies (see Minty *et al.* and references therein [163]). For example, our white matter values were about 2–10% smaller than those from two previous studies in bovine brain [142, 145]. That said, our data was internally consistent: the fitted $M_{\rm MW}(\infty)$ in WM-sp1 and WM-sp2 were similar (within 2 units of amplitude, where 100 units comprises the entire aqueous magnetization). This is expected given that they are from the same brain and same area. Differences in the sample preparation and between individual cows (such as their age) likely account for the discrepancies between our work and the other studies.

Between the WM-sp1/sp2 and WM-fr samples, WM-fr had a $\sim 2 \times$ larger non-aqueous fraction. Confirmation of this in the literature is mixed: one quantitative MT (qMT) *in-vivo* human study also showed a 10-15% higher non-aqueous fraction in frontal white matter than in splenium [116, 164], although a different study found little difference [165]. Still, because we are able to directly compare the aqueous and non-aqueous FIDs, our accuracy in this regard is likely better than in previous work, with one caveat: with the *ex-vivo* sample used here, there is the potential for water loss.

The intrinsic T_1 s of the aqueous and non-aqueous pools are also of interest. In all our samples (acquired at 4.7 T and 37 °C), $T_{1,M} \approx 200$ ms, which is similar to both the value of 171 ± 22 ms measured in human brain *in-vivo* at 1.5 T [166]; and the values of 250 ms at 3 T and 500 ms at 7 T for human brain *in-vivo* [102]. Both of these studies used a two-pool model, so their values would include protons in what we consider the NM pool as well. However, our value is shorter than another study's 0.5–1 s measured in bovine optic nerve at 20 °C and 1.5 T [151]. Evidently, the T_1 of the non-aqueous pool is temperature and field dependent. The former is primarily a result of correlation time changes from increased or decreased thermal motion away from physiological temperatures [83,84,167]. The field-dependence, discussed in Section 2.6, arises from T_1 relaxation's sensitivity to fluctuations at ω_0 and $2\omega_0$ (ω_0 is the Larmor frequency) [3, 10, 102, 168]. In contrast, the intrinsic aqueous protons already experience significant averaging, so their T_{1s} ($T_{1,MW}$ and $T_{1,IEW}$) don't depend as strongly on the field [102]. Our data suggest a large, >1 s T_1 for both MW and IEW. However, the values obtained from the data here may be imprecise.

Another important quantity to compare with previous work is the MW/IEW "exchange

time". Using the T_1^* associated with the MW/IEW exchange eigenvector, our samples are in the range of about 90–130 ms. These are perhaps slightly faster than with previous reported values of similar quantities, including ~200 ms (for human white matter *in-vivo* [169]), 127 ms (for human white matter *in-vivo* [170]) ~220 ms (bovine splenium *ex-vivo* [145]), and ~140 ms (bovine white matter *ex-vivo* [142]). Still, this confirms previous conclusions that MW/IEW exchange has only a slight effect on the MWF measurements. Indeed, the error in the MW amplitude due to exchange during the CPMG, $(M_{\rm MW}(\infty) - \widetilde{M}_{\rm MW}(\infty))/\widetilde{M}_{\rm MW}(\infty)$, is about 20% for WM-fr and for WM-sp1/sp2.

Turning now to the FID results, there are relatively few studies which have directly observed the non-aqueous proton FIDs in CNS tissues (e.g. references [89, 142, 148]). In fact, most measurements of the super-Lorentzian lineshape in white and grey matter have been presented in qMT studies [79, 81, 93, 94, 116, 171, 172]. In those experiments, the non-aqueous protons are indirectly observed via their influence on the aqueous protons' magnetization. Grey matter is less frequently studied this way, given its smaller non-aqueous pool [171]. Still, qMT studies have generally shown that the effective T_2 —the T_2 that is measured if one assumes a Lorentzian lineshape—for the non-aqueous signals is similar in both white and grey matter (see Sled [171] and references therein). Although assigning an effective T_2 to the distinctly non-Lorentzian non-aqueous NMR signal is crude, it is consistent with our results: The wide super-Lorentzians in our white (13.5–16.2 kHz) and grey matter (18.8 kHz) samples have similar spectral widths. Techniques to perform *in-vivo* observations of the non-aqueous signal also support this: in T_2^* measurements in ovine (sheep) brain, Fan *et al.* found white and grey matter values of 209±9 μ s and 258±4 μ s respectively [173].

The similarity between the non-aqueous lineshapes in white and grey matter may hint at the inability of FID fitting to distinguish between M and NM protons (those non-aqueous protons within the myelin bilayers and those outside it). If NM had a very different lineshape, this would dominate in grey matter where there is little myelin. However, this doesn't appear to be the case. There is an analogous observation in the aqueous protons: nothing in our results suggests MW and IEW have different lineshapes, a possibility previously raised [169]. While the broad linewidths from field inhomogeneities may have been a limitation in this regard, the same result was present in a high-resolution study of rat thoracic spinal cord [89]. That said, fitting the non-aqueous component is intrinsically difficult compared to the aqueous component; in the frequency domain its highest amplitude is $\sim 50 \times$ lower than the aqueous peak (Fig. 5.5), and in the time domain its longest signal lasts for $\sim 1/300$ as long as the aqueous FID.

The lack of a MW pool in the GM-bg sample is surprising. Indeed, grey matter has signif-

icantly less myelin, yet *in-vivo* human MWF measurements typically show non-zero values (*eg.* Laule *et al.* [140]). It may be that the little myelin present in our GM-bg was damaged during sample preparation and loading. Due to its higher water content, grey matter tissue is significantly more delicate than white matter tissue. Still, with only one grey matter sample, it is hard to draw any definitive conclusions, and more measurements would be required. This is especially true given that GM-bg is sub-cortical grey matter; hence, future work should also include samples of cortical grey matter as well.

5.5.2 Imaging applications

It is unlikely the four pool model could ever be completely characterized *in-vivo*, given the number of parameters involved. Moreover, acquiring the FIDs of both the aqueous and non-aqueous protons is probably unfeasible on clinical MRI systems due to the short, intense, broadband pulses required. Instead, this work may prove useful in experimental design and analysis, particularly when there is an interest in the MW pool signal.

The total aqueous signal, $M_{\rm MW}(TI) + M_{\rm IEW}(TI)$, is approximately what is measured in an imaging experiment (BW is excluded since it is external to our samples). As an example, we can analyze this quantity in detail for IR-soft and IR-hard. Following standard procedure in IR experiments, each recovery curve is fit to a multiple-component inversion-recovery equation,

$$M_{\rm MW}(TI) + M_{\rm IEW}(TI) = \sum_{i=1}^{n} a_i (1 - f_i \exp(-TI/\tau_i)).$$
(5.25)

Here a_i is the amplitude of the component with an apparent relaxation time of τ_i and f_i its inversion efficiency. We use the generic notation a_i instead of $M_i(\infty)$ to emphasize that each component is most likely associated with multiple pools—and using this type of analysis alone, we can't know which pools these are, only what their combined magnetization is. Fig. 5.14A shows how a single component fit is clearly inadequate in the soft-IR experiment: it requires two components ($\tau_1 = 1.4$ s and $\tau_2 = 63$ ms), whereas in plot B the IR-hard fits well with just one ($\tau_1 = 1.4$ s). These relaxation times are comfortably similar to two of the T_1^* s (71 ms and 1.27 s) from the four pool model fitting (Table 5.2B). Taken together, the results strongly suggest that broadband inversion and selective inversion will generally result in measurably different T_1 relaxation values. Note that broadband inversion is possible in clinical MRI using adiabatic pulses [174].

This behaviour—where the value and number of components measured depends on the initial conditions—has been discussed by some recent publications [102, 142, 145–147]. Both two and four pool models can explain this result. However, the eigenvector formalism that we



Figure 5.14: One and two-component fits to IR-soft and IR-hard for sample WM-fr. These were fit to Eq. 5.25. The IR-soft curve in (A) appears to be bi-exponential ($\tau_1 = 1.4$ s and $\tau_2 = 63$ ms), whereas the IR-hard curve in (B) is mono-exponential ($\tau_1 = 1.4$ s).

used (following Barta *et al.* [145]) can give a particularly clear depiction of how the initial conditions affect T_1 relaxation. This is made explicit by Fig. 5.15 where the eigenvector coefficients are plotted. Plot A are these coefficients (the c_j s in Eq. 5.7), showing which T_1^* components are excited by the preparation pulses. Plot B shows $c_p\left(\sum_{i=1}^4 |v'_{pi}|\right)$, where each coefficient is multiplied by the sum of the absolute values of the eigenvector components. This roughly corresponds to the magnitude of the perturbation from equilibrium corresponding to that eigenvector.

Turning again to the contrast between IR-hard and IR-soft, the coefficient plots make a few things clear. First, the most prominent appearance of the shortest T_1^* time (23 ms) is in the IR-soft experiment; in IR-hard, it is negligible. In fact, IR-hard primarily excited the $T_1^* = 1.3$ s eigenvector, consistent with Fig. 5.14. The IR-soft behaviour is remarkably different, despite having almost exactly the same total aqueous signal amplitude. Here, the soft inversion pulse excited all components except $T_1^* = 130$ ms. The nonzero signal strengths (plot B) from the other eigenvectors hints they should all be observable. Indeed, even in the bi-exponential T_1^* fit of IR-soft data above (Fig. 5.14A), there appears to be a small component in the <100 ms range, likely corresponding to the 23 ms T_1^* . Still, it is not very well-defined, since the lowest two T_1^* s were separated only by a few tens of ms (23 ms vs. 74 ms). The difficulty separating these components was one of the reasons for up-weighting the start of MW residuals during the four pool fitting.

This type of eigenvector analysis may be useful when interpreting the T_1^* components measured in a particular experiment. Importantly, it can be applied to two or three pool models as well, since—as we discuss next—the four pool model may not always be necessary.



Figure 5.15: The eigenvector excitation and aqueous pool magnetizations of the initial condition for sample WM-fr. (A) The eigenvector coefficients c_j s, showing which T_1^* components are excited by the different preparation pulses. (B) The same coefficients weighted by the scaled eigenvector sizes. This is approximately a measure of the expected signal size from each component. In IR-hard and IR-soft, there are distinctly different sets of eigenvectors which will relax. This is despite the aqueous magnetization being essentially the same, which is shown in (C).

5.5.3 Is the four pool model necessary to understand T_1 relaxation?

The comparison of the MW weighted and unweighted four pool model fits with the three pool model fits (Table 5.3 and Fig. 5.12) makes it clear that while the four pool model's fits were superior, none of the three performed best in all pools simultaneously. What's more, different models performed better in different experiments. The fact that we had to up-weight the start of the MW data in order to fit the ~30 ms T_1^* component indicates that the experiments did not equally expose all time constants. In Barta *et al.*'s recent work, they fit only the MW and IEW signals from IR-soft and IR-hard experiments similar to the ones performed here. Yet, their fit picked up the ~30 ms T_1^* component without any need to weight the MW residuals as we have done [145]. This is an indication that our inclusion of four more experiments washed out the importance of the ~30 ms component's appearance in the IR-soft experiment.

To summarize, the four pool model provides the most comprehensive description of relaxation in all the protons at once, and future refinements will probably help improve its accuracy. However, this information may not always be required. For example, the commonly-used two pool model (Section 4.4.2) ignores the distinction between MW and IEW on the one hand, and M and NM on the other. This is reasonable in many applications given the MW's small size compared to the IEW. The eigenvector analysis just discussed could be a useful tool in determining which model is adequate for quantitative and/or qualitative modeling.

5.5.4 Limitations

While the present study has shown the applicability of the four pool model to white matter, there were a number of limitations. Perhaps the most significant of these was the assumption of equivalent M and NM pool sizes. Myelination varies in different brain structures, and presumably this results in different proportions of M and NM pool sizes [65]. At present, there is no clear way to separate the FIDs from these two non-aqueous pools, although some ideas are given in the last chapter. When this proportion was allowed to vary, it was poorly constrained by the data from these experiments and tended to put all the protons in one of M or NM and none in the other. Histology may yield better estimation in specific tissues, and one recent study applied this to quantify the myelin volume fraction in mice [117].

On the experimental front, the choice of initial conditions could be improved in similar future studies. The need for additional weighting in order to fit the short ~30 ms T_1^* component

means that most of the experiments used here did not sufficiently excite this corresponding eigenvector. Eigenvector analysis (the plots in Fig. 5.15) may be a useful tool in choosing groups of experiments in the future. Combinations with MT and ihMT (see the next two chapters) could also be explored. Because we can directly measure the magnetization after the preparation pulses, these can be arbitrarily complicated without concern about modeling them. Some suggestions for future experiments are given in the last chapter.

Regarding the samples, experimental constraints meant that we were limited in the number of samples we could study. With only three white matter samples, one grey matter sample, and all of them coming from the same brain, more work will be needed before the results here can be conclusively generalized. Sample preparation could also be improved, perhaps by soaking the tissue in D_2O to reduce the intensity of the aqueous peak. This approach was successfully used by Willhelm *et al.* in spinal cord and by Fan *et al.* in ovine brain [89, 173].

Chapter 6

Is "inhomogeneous" MT mis-named?

6.1 Introduction

Inhomogeneous Magnetization Transfer (ihMT) seems to show enhanced contrast in materials containing lipid bilayers, such as myelin.¹ We introduced the ihMT technique in Section 4.4.3, and in the work below we investigate its physical origins in detail. The original ihMT paper by Varma *et al.* suggested that it relied upon the non-aqueous spectrum of lipids being inhomogeneously-broadened [118]. They claimed it would be possible to "burn a hole" in such a spectrum, thereby causing sensitivity to the prepulse frequency [118,120,175]. Fig. 6.1 is reproduced from their paper and its caption outlines this hypothesis, which was the origin of ihMT's name.

Portis was the first to define homogeneous and inhomogeneous broadening of magnetic resonance spectra (the publication focused on electron spin resonance, but the same physics applies to NMR) [176]. That paper defined a homogeneously-broadened spectrum as one which spreads any absorbed energy equally throughout the spin system. In other words, an rf saturation pulse will attenuate the magnetization in a homogeneously-broadened spectrum equally. Additionally, Portis called spectra consisting of overlapping narrow lines from isochromats inhomogeneously broadened. In such a spectrum, a low power rf pulse will saturate a localized frequency range only, corresponding to the spins whose resonance condition is met. Thus, one will "burn a hole" in the spectrum.

Maricq and Waugh introduced a slightly different definition of homogeneous and inhomogeneous in their paper on magic angle spinning (MAS) [177]. If a spectrum is broadened

¹This chapter is modified from the following publication:

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Figure 6.1: The original explanation of ihMT, which suggested that the prepulses were burning holes in the non-aqueous spectra of lipids. This would necessarily require the spectrum to be inhomogeneously-broadened under the definition of Portis (see text). Under these conditions, the S_- (A) and S_+ (B) prepulses would burn a hole at single offsets, whereas the S_{dual} prepulse (C) would burn a hole at both offsets. The difference (D) would lead to an observable ihMT signal. Figure modified slightly for readability from Varma *et al.* [118], © 2015, with permission from John Wiley & Sons, Inc.

by a Hamiltonian \hat{H} (such as the many-spin dipolar Hamiltonian) and $[\hat{H}(t_1), \hat{H}(t_2)] = 0$ at times $t_1 \neq t_2$, then by their definition the spectrum is inhomogeneously-broadened. During MAS experiments, one would see spinning sidebands as a result. These are NMR signals that appear in the spectrum separated by the NMR spinning frequency. Conversely, if $[\hat{H}(t_1), \hat{H}(t_2)] \neq 0$, then under this definition it is homogeneous and no spinning sidebands are seen.

Portis's definition relies only on the spectrum, whereas Maricq and Waugh's definition arises from the properties of the Hamiltonian. Hence, the nomenclature in the literature is inconsistent. Schmidt-Rohr and Spiess discuss this point in Section 3.13.4 of their book [11]. In fact, in certain situations, an inhomogeneous Hamiltonian may give rise to a homogeneouslybroadened spectrum [11, 177]!

To connect this to the lipid systems of interest to this thesis, we discussed in Section 4.2 how dipolar couplings within methylene groups on lipid acyl chains lead to their super-Lorentzian ¹H lineshape. The rapid translational diffusion and spinning of the lipid molecules results in an effective homonuclear dipolar Hamiltonian that commutes with itself at all times while spinning [178]. High-resolution MAS spectra of lamellar lipids can be obtained, where spinning sidebands are evident [178,179]. Therefore, in these systems the Hamiltonian is inhomogeneous by the Maricq and Waugh definition. However, as we explore below, the overlapping nature of the orientation-dependent subspectra in the super-Lorentzian lineshape characteristic of lipids does not permit asymmetric hole burning—so the spectra are *not* inhomogeneous in the Portis sense.

In any case, in addition to the explanation based on inhomogeneous broadening, more recent work by Varma *et al.* used Provotorov Theory to describe the fundamental physics of ihMT [30, 119]. When rf irradiation is applied at one offset, magnetization is able to flow between the Zeeman and dipolar reservoirs, but when rf is applied at both offsets simultaneously, the coupling between the reservoirs is severed. In this framework, the dipolar relaxation time T_{1D} is a key parameter in determining the ihMT signal magnitude. Notably, the application of Provotorov Theory does not require any assumptions about the type of spectral broadening, only that there be dipolar couplings present.

To summarize, to date there are two possible explanations for ihMT: one based on holeburning, which assumes an inhomogeneously-broadened spectrum in the Portis sense for lipid membranes' methylenes; and one based on Provotorov theory, which makes no assumptions on the type of broadening. In this chapter, we test both explanations by focusing on the NMR behavior of the non-aqueous protons. In the Theory section, we describe the fundamental physics of ihMT rigorously, first through a minimal model of an isolated methylene group using density matrices, and then for any system using Provotorov Theory. In doing so we characterize the fundamental timescales of the non-aqueous protons required for a non-zero ihMTR. Our experimental results show that the model lipid system, Prolipid-161 (PL161), does not exhibit hole-burning and that its non-aqueous spectrum behaves like that of a weakly-coupled ensemble of strongly-coupled spin pairs. Moreover, our results make clear that some samples with *homogeneously*-broadened lineshapes do exhibit ihMT, refuting the explanation based upon inhomogeneous broadening. Taken together, we show that ihMT arises simply from the dipolar interaction, not from a specific broadening mechanism.

6.2 Theory

The physics of both MT and ihMT may be considered as two separate processes: 1) the irradiation of non-aqueous protons, and 2) magnetization exchange between the non-aqueous and aqueous protons. A complete model of ihMT using Provotorov Theory that considers both processes (by including aqueous and non-aqueous protons) has already been published by Varma *et al.* [119], and we do not seek to replicate it here. What sets ihMT apart from MT is only a change in prepulse irradiation (comparing single vs dual-sided irradiation). Therefore, fundamental understanding why ihMT appears more selective to lipids requires modeling the behavior of the non-aqueous protons only, which we do in the models below.

Both of our models describe calculation of a "non-aqueous ihMTR" in analogy to the "aqueous ihMTR" typically used in ihMT. Experimentally, since NMR spectroscopy can detect both the non-aqueous and aqueous parts of the proton spectrum, the two ihMTRs are found by integrating the corresponding parts of the spectrum. In a sample that exhibits MT signals, a non-aqueous ihMT (where the non-aqueous ihMTR $\neq 0$) will cause an aqueous ihMT because of magnetization exchange.

Our first model of ihMT is based on the simplest system in which ihMT can occur: a spin-1 system. Our second model uses Provotorov theory to describe ihMT. It predicts ihMT can arise in spectra with either inhomogeneous or homogeneous broadening.

6.2.1 ihMT model 1: a simple spin-1 system

6.2.1.1 Selective and non-selective pulses in a spin-1 system

We now consider the behavior of a simple spin-1 system, which is motivated by the behaviour of coupled protons in the methylene groups in lipid acyl chains (Section 4.2). We will

use this to both semi-quantitatively model the non-aqueous protons in the lipids and as a straightforward means of understanding the general mechanism of ihMT.

The Hamiltonian of a dipolar-coupled spin- $\frac{1}{2}$ pair or a single spin-1 particle was given in Eq. 2.30. Repeating it here:

$$\hat{H} = \hat{H}_Z + \hat{H}_D = -\omega_0 \hat{I}_z + \frac{\omega_D}{3} (3\hat{I}_z^2 - 2\mathbf{1}).$$
(6.1)

The first and second terms are the Zeeman and dipolar Hamiltonians, ω_0 is the Larmor frequency, and ω_D is the dipolar interaction strength (where $\omega_D \ll \omega_0$). In thermal equilibrium the density matrix is

$$\rho_0 = M_0 \operatorname{diag}(1, 0, -1) = M_0 \hat{I}_z. \tag{6.2}$$

The spectrum of this system, $g(\omega)$, following a broadband on-resonance rf pulse is

$$g(\omega) \propto \delta(\omega_0 + \omega_D) + \delta(\omega_0 - \omega_D). \tag{6.3}$$

This is a doublet centered at ω_0 with a splitting of $2\omega_D$.

Now, a non-selective pulse is applied at ω_0 with an amplitude $\omega_1 \gg \omega_D$ such that both transitions are affected. If the pulse is applied on the *y*-axis in the rotating frame, then via Eq. 2.22,

$$\rho = M_0 \cos(\omega_1 \tau) \hat{I}_z - \sin(\omega_1 \tau) \hat{I}_x,$$

where τ is the duration of the non-selective pulse. In this spin-1 system, this is exactly the case of the dual ihMT prepulse, so we may write

$$M_{\text{dual}} = \langle \hat{I}_z \rangle$$

= $M_0 \cos(\omega_1 \tau).$ (6.4)

Alternatively, we may also use a selective pulse with $\omega_1 \ll \omega_D$ such that it is on resonance for only one transition. If the pulse frequency is $\omega_0 + \omega_D$, then we can analyze just the $\{1, 0\}$ subspace of ρ [9, 180]. In this subspace, there is one transition, so it is identical to a spin- $\frac{1}{2}$ particle. In equilibrium, the subspace is

$$\rho_{0,1/2} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}$$
$$= \frac{M_0}{2} \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} + \frac{M_0}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}$$
$$= \frac{M_0}{2} \mathbf{1}_{1/2} + M_0 \hat{I}_{z,1/2},$$

where we have used spin- $\frac{1}{2}$ operators. The same subspace in the rf Hamiltonian is

$$\hat{H}_{rf} = \begin{pmatrix} 0 & -i\omega_1 \\ i\omega_1 & 0 \end{pmatrix}$$
$$= 2\omega_1 \hat{I}_{y,1/2}.$$

Outside of this subspace, \hat{H}_{rf} is zero, since the pulse is selective to the one transition. Again, using Eq. 2.22, we find that the density matrix evolves under this Hamiltonian to

$$\rho_{1/2} = \frac{M_0}{2} \mathbf{1}_{1/2} + M_0 \cos(2\omega_1 \tau) \hat{I}_{z,1/2} - M_0 \sin(2\omega_1 \tau) \hat{I}_{x,1/2}.$$

The nutation frequency is twice as fast than in the dual case. This is a well-known effect and is seen in the nutation of the central transition in quadrupolar couplings as well [9,180]. Now, substituting this subspace back into the complete spin-1 density matrix, we find for a selective pulse on a single transition

$$M_{\rm single} = M_0 \left(\frac{3}{4} + \frac{1}{4} \cos(2\omega_1 \tau) \right).$$
 (6.5)

Selectively irradiating the other transition at $\omega_0 - \omega_D$ yields the same result.

6.2.1.2 Application to ihMT

We now show how the spin-1 system is sensitive to the frequency of selective pulses explicitly through one simplified example. The selective pulses are used to saturate or invert one or both transition populations. This has a corresponding impact on the spectral line amplitudes. Consider a selective pulse applied at $\omega_0 + \omega_D$, with an amplitude ω_1 and pulse length τ calibrated to invert the transition (a π pulse), yielding

$$\rho_{+} = M_0 \operatorname{diag}(0, 1, -1) = \frac{M_0}{2} I_z - \frac{M_0}{2} (3I_z^2 - 2\mathbf{1}), \tag{6.6}$$

in which the subscript + on ρ_+ indicates a pulse applied at a positive frequency offset. The $(3I_z^2 - 2\mathbf{1})$ term indicates the presence of dipolar order. Similarly, applying the selective π pulse at $\omega_0 - \omega_D$ to invert the other transition yields

$$\rho_{-} = M_0 \operatorname{diag}(0, 1, -1) = \frac{M_0}{2} I_z + \frac{M_0}{2} (3I_z^2 - 2\mathbf{1}), \tag{6.7}$$

In either case, the coefficient of the \hat{I}_z term is reduced from the thermal equilibrium value of M_0 to $M_0/2$. Next, we consider applying the same pulse power to both transitions simultaneously. We know from Eqs. 6.4 and 6.5 that if $2\omega_1\tau = \pi$ in the selective case, the flip angle in the dual case will be $\pi/2$ (half as much). Since we don't care about the off-diagonal elements, we will call this a saturation pulse. Thus,

$$\rho_{\rm dual} = M_0 \,{\rm diag}(0,0,0),\tag{6.8}$$

where now clearly there is no magnetization or dipolar order.

The difference from equilibrium for the single prepulse cases are $M_0 - M_0/2 = M_0/2$, whereas in the dual case it is $M_0 - 0 = M_0$. Therefore in his example, *irradiating both transitions* simultaneously provides twice as much difference from the equilibrium value of $\langle I_z \rangle$ for the same rf power. Calculating the non-aqueous ihMTR for this experiment, we have

$$ihMTR = \frac{\langle I_z \rangle_+ + \langle I_z \rangle_- - 2 \langle I_z \rangle_{dual}}{2 \langle I_z \rangle_0}$$
$$= \frac{M_0/2 + M_0/2 - 2(0)}{2M_0}$$
$$= 1/2$$
(6.9)

Using Eqs. 6.4 and 6.5, we can determine this generally for any prepulse power, showing that

the spin-1 system always has a nonzero ihMTR:

$$ihMTR = \frac{\langle I_z \rangle_+ + \langle I_z \rangle_- - 2 \langle I_z \rangle_{dual}}{2 \langle I_z \rangle_0}$$
$$= \frac{2M_0 \left(\frac{3}{4} + \frac{1}{4}\cos(2\omega_1\tau)\right) - 2M_0\cos(\omega_1\tau)}{2M_0}$$
$$= \frac{3 + \cos(2\omega_1\tau) - 4\cos(\omega_1\tau)}{4} > 0.$$
(6.10)

In the absence of spin-lattice relaxation, the limit $\tau \to \infty$ averages the cosine terms to zero and ihMTR approaches 3/4.

If our spin-1 system here represents a typical methylene group in a lipid bilayer, then magnetization exchange would take place with aqueous protons on the timescale of 10–100 ms. This would decrease the aqueous magnetization by an amount proportional to the non-aqueous $\langle I_z \rangle$, thereby causing a non-zero aqueous ihMTR. This shows that a non-zero *aqueous* ihMT signal is expected from a system consisting of water in contact with strongly-coupled spin pairs. Moreover, it shows that ihMT does not arise from a specific type of spectral broadening, but from dipolar couplings alone.

6.2.1.3 Spectral asymmetry from dipolar order

The population differences in the density matrices of Eqns. 6.6, 6.7, and 6.8, suggest that inverting (or saturating) the transition of one of the lines in the doublet spectrum should result in an enhancement in the amplitude of the other line. Perhaps surprisingly, a nonselective $\frac{\pi}{2}$ (or any $(2n+1)\frac{\pi}{2}$) pulse applied to this system produces a spectrum with both peaks of the doublet having identical amplitudes. However, if the flip angle is $\neq n\frac{\pi}{2}$, an intensity difference between the two lines is observed. This is a manifestation of the "flipangle effect" [181, 182].

The diagonal of the density matrix following an arbitrary prepulse can be written as

$$\rho = \operatorname{diag}(\rho_{11}, \rho_{22}, \rho_{33}) = aI_z + \frac{b}{3}(3I_z^2 - 2\mathbf{1}) + c\mathbf{1}, \tag{6.11}$$

where $a = \frac{1}{2}(\rho_{11} - \rho_{33})$, $b = \frac{1}{2}(\rho_{11} + \rho_{33}) - \rho_{22}$, and $c = \frac{1}{3}(\rho_{11} + \rho_{22} + \rho_{33})$. The off-diagonal components are set to zero, which may be accomplished experimentally by appropriate phase cycling. Next, we calculate the effect of a hard observe pulse of flip angle α along the +x axis using product operators (Eq. 2.22). Keeping only the observable terms, we find

$$\rho = -a\sin\alpha I_y - b\cos\alpha\sin\alpha (I_z I_y + I_y I_z). \tag{6.12}$$

The I_y term is from the initial Zeeman magnetization (given by a), and the $I_z I_y + I_y I_z$ term is antiphase magnetization from the initial dipolar order (given by b). Evolving this expression under the Hamiltonian in Eq. 6.1 gives the amplitudes of the two transitions:

$$A_{\pm} = a \sin \alpha \pm b \cos \alpha \sin \alpha, \tag{6.13}$$

where A_{\pm} are the amplitudes of the transitions at $\pm \omega_D$. It is clear that when the flip angle is $\frac{\pi}{2}$, A_+ and A_- are identical $(A_{\pm}(\frac{\pi}{2}) = a = \frac{1}{2}(\rho_{11} - \rho_{33}))$, and both lines have identical amplitudes regardless of the amount of dipolar order. However, for flip angles $\neq n\frac{\pi}{2}$, the amplitudes of the lines will differ by $2b \cos \alpha \sin \alpha$. For small flip angles, where $\cos \alpha \approx 1$, $A_{\pm} = (a \pm b) \sin \alpha$, so that $A_+ = (\rho_{11} - \rho_{22}) \sin \alpha$ and $A_- = (\rho_{22} - \rho_{33}) \sin \alpha$ as expected. For all flip angles, the amplitudes of the two lines are correlated. The amplitude of one cannot be reduced independently of the other. This is in contrast to hole burning, where the amplitudes of the overlapping lines that make up the spectrum can be changed independently.

Experiments like the ones considered here, demonstrating the interplay between dipolar coupled spectral lines, have been carried out experimentally on the ensemble of dipolar coupled ¹H spin pairs in oriented 5CB (which has a spectrum of two lines) by Lee *et al.* [37]. When one of the spectral lines of the dipolar coupled pairs was irradiated, the intensities behave as predicted by the model described above. Nakashima *et al.* have also shown similar effects in the behavior of spin-3/2 systems in ²³Na NMR of NaNO₃ crystals [182].

Spin-1 behavior is not immediately obvious from the proton NMR spectra of lamellar lipids, where the chain-position dependent coupling strength, along with residual inter-molecular and inter-methylene couplings broaden the doublets to the point where the lipid lineshape is well described as a superposition of Gaussian *singlets* having widths and intensities modulated by $P_2(\cos \theta)$ [80,87,91]. This fact is explored more deeply in Section 4.2.

6.2.2 ihMT model 2: a homogeneously-broadened system using Provotorov theory

6.2.2.1 The Provotorov equations for continuous-wave ihMT prepulses

While the spin-1 theory just described forms a minimal model demonstrating the origin of the ihMT effect, a general approach for all systems is based on Provotorov Theory. This describes the evolution of dipolar-coupled spins under weak rf irradiation (*i.e.* $2\pi\nu_1 \ll \omega_D$), such as ihMT prepulses [30, 183, 184]. We introduced Provotorov Theory in Section 2.5.6 and derive it in Appendix A. Previous ihMT studies have used two varieties of prepulses. The "Continuous-Wave" (CW) type applies a single rectangular prepulse, which is sine-modulated in the case of the S_{dual} experiment [118]. The "pulse-train" type uses prepulses consisting of a train of shaped pulses (typically Hann or Gaussian) [84,119,120]. We analyze the behavior of a coupled spin system under both varieties. Because our experiments use CW prepulses, their analysis is presented below. Appendix D contains a similar treatment of pulse-train prepulses, which are common in imaging applications. The derivation below follows the approach by Lee *et al.* [37,38].

We briefly review the Provotorov equations. The density matrix for a dipolar-coupled system in a rotating frame at angular frequency $\omega_0 + 2\pi\Delta$ is [30, 38]

$$\rho = \mathbf{1} - (2\pi\Delta)\beta_Z I_z - \omega_D \beta_D \left(\frac{\hat{H}_D}{\omega_D}\right).$$
(6.14)

Or, as a vector with $\{I_z, \frac{\hat{H}_D}{\omega_D}\}$ as the basis (the **1** term is dropped):

$$\boldsymbol{\rho} = \begin{pmatrix} -(2\pi\Delta)\beta_Z \\ -\omega_D\beta_D \end{pmatrix}.$$
(6.15)

Here, $\beta_{Z,D}$ are the inverse spin temperatures for the Zeeman and dipolar reservoirs, and the vector basis is $\{\hat{I}_z, \frac{\hat{H}_D}{\omega_D}\}$, with \hat{H}_D as the dipolar Hamiltonian. When weak rf is applied at a single offset Δ , the Provotorov equations including spin-lattice relaxation are [30]

$$\frac{d\boldsymbol{\rho}_{\pm}}{dt} = W \begin{pmatrix} -1 - \frac{1}{WT_1} & \Omega \\ \Omega & -\Omega^2 - \frac{1}{WT_{1D}} \end{pmatrix} \boldsymbol{\rho}_{\pm} + \begin{pmatrix} \frac{\langle I_z \rangle_0}{T_1} \\ 0 \end{pmatrix}, \qquad (6.16)$$

with

$$W = \pi (2\pi\nu_1)^2 g(2\pi\Delta)$$
 (6.17)

$$\Omega = \frac{2\pi\Delta}{\omega_D}.\tag{6.18}$$

Here, ν_1 is the prepulse amplitude (in Hz), and $g(2\pi\Delta)$ the symmetric, normalized lineshape. ω_D is the RMS average dipolar interaction strength (the residual dipolar couplings).

Eq. 6.16 describes the evolution during the S_+ or S_- prepulse, the only difference between the two being the sign of Ω and consequently the sign of dipolar magnetization. However, qualitatively different behavior occurs during the S_{dual} prepulse, where rf irradiation with amplitude $\nu_1/\sqrt{2}$ is applied to dual offsets $\pm \Delta$ simultaneously. This causes the Zeeman and dipolar reservoirs to decouple, leading to [30]

$$\frac{d\boldsymbol{\rho}_{\text{dual}}}{dt} = W \left(\begin{array}{cc} -1 - \frac{1}{WT_1} & 0\\ 0 & -\Omega^2 - \frac{1}{WT_{1D}} \end{array} \right) \boldsymbol{\rho}_{\text{dual}} + \left(\begin{array}{c} \frac{\langle I_z \rangle_0}{T_1}\\ 0 \end{array} \right).$$
(6.19)

Applying standard differential equation techniques (e.g. see reference [154]) to solve Eqs. 6.16 and 6.19 under a prepulse of duration τ yields

$$\boldsymbol{\rho}(t) = c_1 \mathbf{v}_1 e^{\lambda_1 W \tau} + c_2 \mathbf{v}_2 e^{\lambda_2 W \tau} + \mathbf{v}_0 \tag{6.20}$$

where

$$\lambda_{1,2} = -\frac{1}{2} \left[\frac{1}{WT_1} + \frac{1}{WT_{1D}} + 1 + \Omega^2 \pm \sqrt{\left(\frac{1}{WT_1} - \frac{1}{WT_{1D}} + 1 - \Omega^2\right)^2 + 4\Omega^2} \right]$$
(6.21)

$$\mathbf{v}_{0} = \frac{\langle I_{z} \rangle_{0}}{WT_{1}(\lambda_{1} - \lambda_{2})} \left(\begin{array}{c} \frac{\lambda_{2} + \Omega^{2} + (WT_{1D})^{-1}}{\lambda_{2}} - \frac{\lambda_{1} + \Omega^{2} + (WT_{1D})^{-1}}{\lambda_{1}} \\ \frac{\Omega}{\lambda_{2}} - \frac{\Omega}{\lambda_{1}} \end{array} \right).$$
(6.22)

Lee *et al.* also give the steady-state solution vector \mathbf{v}_0 explicitly, assuming the system starts from thermal equilibrium [37]. The non-aqueous ihMTR as a function of prepulse duration τ is

$$ihMTR(\tau) = \frac{\langle I_z \rangle_+(\tau) + \langle I_z \rangle_-(\tau) - 2 \langle I_z \rangle_{dual}(\tau)}{2 \langle I_z \rangle_0}.$$
 (6.23)

In Appendix C, we present an analogous electronic circuit of the above equations.

6.2.2.2 Model details

We now consider the behavior of the non-aqueous protons under the three different types of prepulses, assuming the system starts at equilibrium, $\rho(0) = \langle I_z \rangle_0 I_z$. In the S_+ and $S_$ cases, $\langle I_z \rangle$ decays bi-exponentially since the eigenvalues $W\lambda_1$ and $W\lambda_2$ are always negative. This is in contrast to the mono-exponential decay behavior in the S_{dual} case.

Under the S_{dual} prepulse, the two reservoirs are decoupled. In the Zeeman reservoir, relaxation and saturation are responsible for a loss of magnetization at the rate $(W + T_1^{-1})\langle I_z \rangle$. The solution to Eq. 6.19 is

$$\langle I_z \rangle_{\text{dual}}(\tau) = \langle I_z \rangle_0 \frac{1 + W T_1 e^{-(W + T_1^{-1})\tau}}{1 + W T_1},$$

$$\langle \frac{\hat{H}_D}{\omega_D} \rangle(\tau) = 0.$$
 (6.24)

The magnetization in the Zeeman reservoir decays exponentially toward the steady-state value $(1 + WT_1)^{-1}$ at a rate that is independent of T_{1D} , because no magnetization enters the dipolar reservoir.

During the S_+ prepulse (or with $\Omega \to -\Omega$, the S_- prepulse), the dipolar reservoir must also be considered, where magnetization saturates and relaxes at a rate $(W\Omega^2 + T_{1D}^{-1})\langle \frac{\hat{H}_D}{\omega_D} \rangle$. Magnetization flows into the dipolar reservoir from the Zeeman reservoir at a rate $W\Omega\langle I_z \rangle$. If $T_{1D}W \ll 1$, rapid relaxation means very little magnetization is left to flow back to the Zeeman reservoir. The net result is that $\langle I_z \rangle_+(\tau)$ (and $\langle I_z \rangle_-(\tau)$) behaves similarly to $\langle I_z \rangle_{\text{dual}}(\tau)$ and ihMT is negligible. Alternatively, if $T_{1D}W \gtrsim 1$, then non-negligible magnetization flows back into the Zeeman reservoir at a rate $W\Omega\langle \frac{\hat{H}_D}{\omega_D} \rangle$. This leads to a significant deviation in behavior of the Zeeman magnetization from the S_{dual} case, so ihMT is measurable.

From this, we see that WT_{1D} is a key parameter in controlling ihMT. If W is held constant, then, as others have pointed out, ihMT generates T_{1D} -dependent contrast in MRI [84, 119, 133]. However, since W is, within limits, under control of the experimenter (by controlling ν_1), it may afford detection of ihMT in systems with short T_{1D} .

Fig. 6.2 explores the dependence of non-aqueous ihMTR on prepulse duration, WT_{1D} and offset frequency. Fig. 6.2A shows the ihMTR dependence on prepulse length, τ . Generally, there is a peak followed by a falloff to a lower steady-state value. At short times ($\tau \sim 1$ ms), the bi-exponential decay of $\langle I_z \rangle_+(\tau)$ (and $\langle I_z \rangle_-(\tau)$) and the mono-exponential decay of $\langle I_z \rangle_{dual}$ are similar, so ihMTR is small. At times $\tau \sim 10$ –100 ms, the difference between the two behaviors is at a maximum, leading to a maximum ihMTR. At longer times, the difference decreases as the system achieves steady state, yielding constant ihMTR values. Also shown in this plot is that ihMTR increases with WT_{1D} .

Fig. 6.2B isolates the WT_{1D} dependence more explicitly for a prepulse duration of $\tau = 500$ ms. This plot shows similar behavior in ihMTR as in Fig. 6.2A, where ihMTR is plotted as a function of τ . Analysis of Eqs. 6.21 and 6.24 shows that only $W\tau$ appears, not τ alone. Therefore, holding τ and T_{1D} constant and varying W produces the same behaviour as holding T_{1D} constant and varying $W\tau$. This plot also shows the highly-sensitive T_{1D} -dependence, which is approximately linear unless $T_1 \sim T_{1D}$.

Fig. 6.2C shows the dependence on the offset frequency of the prepulse. The general shape of these curves is similar to plots of aqueous ihMTR vs. $|\Delta|$ in previous studies [84,118–120]. At short prepulse lengths, the maximum ihMTR occurs near the resonance condition in the local field ($\Omega = 1$). However, at long prepulse lengths, the maximum shifts to higher offset frequencies where saturation effects are suppressed.

This model considers a system of isolated, non-aqueous protons only. However, including an



Figure 6.2: Simulation of non-aqueous ihMT in an isolated spin system using CW prepulses (Eq. 6.23). No coupling to aqueous protons is included. (A) The dependence of ihMTR on prepulse length. If couplings to aqueous protons were included, the long-time behavior would deviate from what is shown here. (B) The effect of WT_{1D} and T_{1D} . The T_{1D} -dependence is approximately linear and for $WT_{1D} \ll 1$, ihMT is unobservable. (C) The dependence on offset for different prepulse lengths. The resonance condition in the local field is $\Omega = 1$. Parameters unless otherwise indicated: $T_1 = 1$ s, $\Omega = 1$, $\nu_1 = 400$ Hz, with $g(2\pi\Delta)$ as a Gaussian with standard deviation $\omega_D = 10$ kHz.

exchangeable aqueous proton pool would not change the qualitative behavior significantly, except in the case of ihMTR as a function of prepulse length (Fig. 6.2A). When ihMT is performed in the presence of an aqueous proton pool, a longer prepulse produces a greater change in the aqueous magnetization due to the slow exchange. In this case, there will not be a maximum in the aqueous ihMTR at a prepulse length of 10–100 ms.

Appendix D contains simulations of the pulse-train model (Fig. D.2) similar to those shown for the CW model. The term $W_{\text{eff}}T_{1D}$ is shown to play the role of WT_{1D} , where the effective $W(W_{\text{eff}})$ is scaled by the duty cycle.

6.2.2.3 Spectral asymmetry from dipolar order

As in the spin-1 model, dipolar order in large spin systems gives rise to spectral asymmetry. Starting with the rotating-frame density matrix in Eq. 6.14, a hard pulse of flip angle α is applied along y. After a time t, the components of magnetization are [30]

$$\langle I_x \rangle(t) \propto -(\beta_Z (2\pi\Delta) \sin\alpha) f(t) \langle I_y \rangle(t) \propto (\beta_D \sin\alpha \cos\alpha) \frac{df(t)}{dt}.$$
(6.25)

Here, f(t) is the envelope of the FID. The resonance frequency at the center of the spectrum is ω_0 , so the spectrum (up to a constant) is

$$A(\omega) = \left[-\beta_Z(2\pi\Delta)\sin\alpha - (\omega - \omega_0)\beta_D\sin\alpha\cos\alpha\right]g(\omega - \omega_0).$$
(6.26)

Here, $g(\omega - \omega_0)$ is the Fourier transform of f(t) and describes a symmetric spectrum centered at ω_0 . The factor of $\omega - \omega_0$ in the second bracketed term causes spectral asymmetry, which is only visible when $\alpha \neq n\pi/2$.

We can use this equation to re-derive the spin-1 model amplitudes. Substituting the spin-1 spectrum (Eq. 6.3) into the expression for $A(\omega)$, we calculate

$$A_{\pm} \propto \int_{0}^{\pm \infty} A(\omega) d\omega$$

= $-\beta_{Z} (2\pi\Delta) \sin \alpha \mp \beta_{D} \omega_{0} \sin \alpha \cos \alpha,$ (6.27)

which has the same form as Eq. 6.13, showing that it is applicable to any dipolar-coupled lineshape.



Figure 6.3: Pulse sequences used in this work. The dipolar order creation sequence uses a Gaussian prepulse. The two ihMT sequences were used to measure ihMTR. During their prepulses the rf power was the same. The ADRF/ARRF sequence was used to measure T_{1D} .

6.3 Methods

PL161 samples were prepared by melting ca. 50 mg of prolipid-161 (Ashland Specialty Ingredients, DE, USA) at 80-90° C in distilled water and/or deuterated water (Cambridge Isotope Laboratories, Inc. MA, USA). Samples of PL161/D₂O (10%/90% w/w), PL161/D₂O/H₂O (10%/88%/2% w/w), and PL161/H₂O (10%/90% w/w) were made. PL161 forms a lamellar liquid crystal with MT properties similar to those of myelin [118,185–187]. A 63 mg sample of curly, black human hair was obtained 10–20 cm from the scalp. This was thoroughly washed in water and soap then air-dried prior to measurement. A 61 mg sample of Douglas Fir (*Pseudotsuga menziesi*) sapwood was obtained from a branch with a ~3 cm diameter and was air-dried for 2 weeks prior to measurement. A 57 mg sample of Western Red Cedar (*Thuja plicata*) sapwood was obtained from a branch with a ~1 cm diameter and dried the same way. Beef (*Bos taurus*) tendon was obtained frozen from a local butcher. A 70 mg sample was extracted from the tendon sheath, patted dry, then sealed inside an NMR tube. Experiments on tendon were completed within 48 hours of thawing.

The four styles of pulse sequences used are shown in Fig. 6.3. The dipolar order creation sequence was used for spectral asymmetry experiments. It features a short, intense Gaussian prepulse three standard deviations wide (typically, $\tau = 1-3 \text{ ms}$, $\nu_1 = 2.5 \text{ kHz}$, $\Delta = 8 \text{ kHz}$, δ variable). For ihMT-related experiments, the S_+ and S_- spectra were produced by a rectangular prepulse at offsets $+\Delta$ and $-\Delta$ respectively, whereas the S_{dual} spectra were produced with a rectangular prepulse modulated by $\sin(2\pi\Delta)$, thereby irradiating $\pm\Delta$ simultaneously. The S_0 spectra had no prepulse. When observing the differences in the non-aqueous portions of S_0 , S_+ , S_- , and S_{dual} spectra, typically $\tau = 2-50 \text{ ms}$ and $\nu_1 = 10 \text{ kHz}$. When measuring aqueous ihMTR, typically $\tau = 500 \text{ ms}$ and $\nu_1 = 460 \text{ kHz}$. In both cases, $\delta \leq 0.5 \text{ ms}$. Lastly, the Adiabatic Demagnetization/Remagnetization in the Rotating Frame (ADRF/ARRF) sequence was used for measuring the dipolar order decay constant T_{1D} , discussed in Section 2.5.5. It had a ramp time $\tau = 1 \text{ ms}$ and a variable relaxation delay δ . In all experiments, the observe pulse flip angle α was either 33° (pulse width 2 μ s) or 90° (pulse width 5.5 μ s).

All spectra were acquired at $21\pm1^{\circ}$ C using 64 acquisitions, a 10 s recycle delay, and were processed with 500 Hz of Gaussian line broadening. $\Delta = 0$ kHz is approximately the center of the non-aqueous lineshape. Experiments were carried out using a horizontal solenoid coil probe on a 200 MHz home-built NMR spectrometer incorporating a digital receiver, based on an Oxford Instruments 4.7 T, 89 mm bore superconducting magnet [188]. Curve fitting was performed with SciPy's least squares package [157]. Errors given on fitted parameters are one standard deviation found with the bootstrap method using 1000 permutations.

6.4 Results

6.4.1 PL161 spectral asymmetry from dipolar order

¹H NMR spectra of a PL161/D₂O sample showing the effects of an off-resonance prepulse for 33° and 90° observe pulses are shown in Fig. 6.4. The spectra consist of a super-Lorentzian arising from the non-aqueous lipid protons and a residual HDO line. Because $\tau = 1$ ms and $\delta = 0.5$ ms, magnetization transfer to the aqueous protons is insignificant. Therefore, plotting $S_+ - S_0$ removes the HDO line and highlights differences between the non-aqueous S_+ and S_0 . Spectra acquired with an observe flip angle of $\alpha = 33^\circ$ (Figs. 6.4A and 6.4C) are asymmetric: the prepulses with $\Delta = 8$ kHz attenuate the spectrum near +8 kHz and enhance it near -8 kHz, indicating the presence of dipolar order. When $\alpha = 90^\circ$ (Figs. 6.4B and 6.4D), the prepulse appears to attenuate the entire non-aqueous spectrum *uniformly*. In Fig. 6.4, a single prepulse at positive offset was used. In work by Swanson *et al.* [84], plots of $S_+ - S_-$ for PL161 also show asymmetry from dipolar order, but remove the effects of Zeeman order, obscuring any potential hole-burning. Plotting $S_+ - S_0$ as done here shows both the effects of both Zeeman order and dipolar order on the spectrum.

These experiments show no evidence of hole-burning in the non-aqueous parts of the PL161 spectrum as would be expected based on the explanation of the ihMT effect in Ref [118]. The behavior is consistent with the presence of strong dipolar couplings within the lipid methylenes. Two discrete spectral peaks are not observed for the non-aqueous component due to the orientation and chain-position dependence of the dipolar coupling strengths and residual dipolar couplings to neighboring methylene groups.

 S_0 , S_+ , S_- , and S_{dual} for PL161/D₂O using CW ihMT sequences are plotted together in Fig. 6.5. The purpose of these experiments is to highlight the response of the non-aqueous



Figure 6.4: Effect of the observation flip angle α on the PL161 non-aqueous proton spectrum. For S_+ , a Gaussian prepulse ($\tau = 1 \text{ ms}$, $\nu_1 = 2.5 \text{ kHz}$, $\Delta = 8 \text{ kHz}$, $\delta = 0.5 \text{ ms}$) was applied with $\alpha = 33^{\circ}$ (A) or $\alpha = 90^{\circ}$ (B). (C) and (D) are difference spectra which highlight the asymmetry caused by dipolar order observed in $\alpha = 33^{\circ}$ case.

protons to the various ihMT prepulses. Again, enhancement of the non-irradiated side of the spectrum can be seen in S_+ and S_- . In contrast, the non-aqueous component of the S_{dual} spectrum is symmetric and strongly suppressed.

We can approximate the degree of Zeeman and dipolar magnetizations in these four spectra. Integrals of the positive frequency $(I_{>})$ and negative frequency $(I_{<})$ sides of the non-aqueous components of a spectrum S(f) are calculated by

$$I_{>} = \sum_{f_{i}=+3}^{f_{i}=+80 \text{ kHz}} S(f_{i}),$$

$$I_{<} = \sum_{f_{i}=-80 \text{ kHz}}^{f_{i}=-3 \text{ kHz}} S(f_{i}).$$
(6.28)

Following the description of the spectral lineshape in the presence of dipolar order (Eq. 6.26), the sum and difference are approximate measures of the Zeeman and dipolar magnetizations, respectively:

$$\langle I_z \rangle \approx I_> + I_< \tag{6.29}$$

$$\left| \left\langle \frac{\hat{H}_D}{\omega_D} \right\rangle \right| \approx |I_> - I_<|. \tag{6.30}$$

Fig. 6.6 compiles these results for our samples, allowing comparison of the different behaviors. The spectra used in the calculations are shown in Figs. 6.5 and 6.7. The prepulse length τ varies and was chosen to give the maximum dipolar order in the S_+ and S_- spectra for each



Figure 6.5: Manifestation of dipolar order in ihMT. Spectral asymmetry from dipolar order is present in S_+ and S_- but not S_0 or S_{dual} . Sequence parameters are listed in the caption of Fig. 6.6.

sample. Also, the recovery time δ was decreased for samples with smaller T_{1D} values. In contrast to the short duration used here, in ihMT-MRI experiments reported to date, $\tau + \delta$ is typically ~ 1 s [84, 118–120]. However, a prepulse this long would push the non-aqueous protons toward steady-state conditions, decreasing the visible differences in their portion of the spectra, observation of which is our goal for these experiments.

In all of these samples, we see that non-aqueous ihMT occurs since $\langle I_z \rangle_{\text{dual}} < \langle I_z \rangle_{\pm}$. In PL161/D₂O, these results are consistent with the behavior of the spin-1 view of the lipid spin system. The non-aqueous Zeeman magnetization $\langle I_z \rangle$ in the S_+ and S_- cases are 0.71 and 0.70, respectively, which is a reduction of about 0.3 from the S_0 case where $\langle I_z \rangle = 1$. In the S_{dual} case the reduction is about twice as much, *i.e.* $\langle I_z \rangle = 0.34 \approx 1 - 2 \times 0.3$. This two-fold reduction in the case of S_{dual} is consistent with the predictions of the spin-1 model above.

6.4.2 Flip-angle dependence of spectral asymmetry

Fig. 6.8A shows the observe pulse flip-angle (α) dependence of the non-aqueous spectrum of PL161/D₂O. $I_{>}$ and $I_{<}$ of $S_{+} - S_{0}$ as functions of α are fit to the A_{+} and A_{-} line intensities of the spin-1 model (Eqs. 6.13 and 6.27). In order to account for B_{1} inhomogeneity effects, A_{+} and A_{-} were multiplied by $\exp(-\pi \,\delta B_{1} \,\alpha/2\pi B_{1})$, where δB_{1} is the full-width at half-max of a Lorentzian distribution of B_{1} field strengths.

The best fit was found with a Zeeman order of $a = 6.4 \pm 0.7$, a dipolar order of $b = 4.4 \pm 0.6$,



Figure 6.6: Summary of the positive/negative frequency integrals $I_>/I_<$, approximate dipolar magnetization $\left|\left\langle\frac{\hat{H}_D}{\omega_D}\right\rangle\right|$, and Zeeman magnetization $\langle I_z \rangle$ for the non-aqueous proton portions of the S_+ , S_- , and S_{dual} spectra from Figs. 6.5 and 6.7. $I_<$ and $I_>$ are normalized to the S_0 values. The ihMT sequences were used with $\nu_1 = 1$ kHz, $|\Delta| = 10$ kHz, and $\alpha = 33^{\circ}$. τ was chosen to maximize the amount of dipolar order, and δ decreased for samples with a shorter T_{1D} . PL161/D₂O: $\tau = 50$ ms, $\delta = 0.5$ ms, Douglas fir: $\tau = 50$ ms, $\delta = 0.1$ ms, hair: $\tau = 2$ ms, $\delta = 0.1$ ms, tendon: $\tau = 2$ ms, $\delta = 0.01$ ms.



Figure 6.7: Beef tendon, human hair, and Western Red Cedar sapwood spectra following CW ihMT prepulses. These are used to calculate the Zeeman and dipolar magnetizations shown in the chart of Fig. 6.6, which also lists the pulse sequence parameters. Spectral asymmetry from dipolar order is evident in all three samples. The hair and tendon spectra had more line broadening applied due to their lower signal-to-noise ratio.



Figure 6.8: Flip-angle dependence of spectral asymmetry and saturation method measurement of T_{1D} in PL161. (A) The $I_{>}$ and $I_{<}$ integrals of $S_{+} - S_{0}$ as functions of α closely follow Eq. 6.13. A single Gaussian prepulse was used with $\tau = 3 \text{ ms}$, $\nu_{1} = 2.5 \text{ kHz}$, $\Delta = +10 \text{ kHz}$, and $\delta = 0.5 \text{ ms}$. (B) Saturation method data, showing T_{1} and T_{1D} relaxation in PL161 from the recovery of the $S_{+} - S_{0}$ difference spectrum. $I_{>}$ and $I_{<}$ are fit to Eq. 6.32. A Gaussian prepulse with $\tau = 1 \text{ ms}$, $\nu_{1} = 2.5 \text{ kHz}$, $\Delta = +8 \text{ kHz}$, and $\alpha = 33^{\circ}$ was used. In both plots, deviations apparent in nearby data points provide estimates of the measurement error.



Figure 6.9: An ADRF/ARRF spectrum of $PL161/D_2O$. There are no contribution from aqueous protons, since their dipolar couplings are averaged away. This also applies for lipids oriented near the magic angle, hence the dip at 0 kHz.

and a B_1 inhomogeneity of $\gamma \delta B_1/2\pi = 11 \pm 1$ kHz (about 20% of ν_1).

6.4.3 PL161 dipolar order relaxation

Measurements of the dipolar order relaxation time T_{1D} in PL161 were made with two different methods. The ADRF/ARRF sequence converts Zeeman magnetization to dipolar order and allows it to relax for time δ before reconverting to an observable signal. An example of an ADRF/ARRF spectrum of PL161/D₂O is shown in Fig. 6.9. In comparison to the S_0 spectrum, the residual HDO peak is absent and the peak of the super-Lorentzian is replaced with a dip. The peak of the super-Lorentzian corresponds to lipids in bilayers whose normal points along the magic angle. When aligned with the magic angle, the intra-methylene residual dipolar coupling strength is averaged to near zero. The signal from these lipids and from the water are eliminated by the pulse sequence phase cycle.

Measurements of T_{1D} made with the ADRF/ARRF sequence were not well fit with single exponential decays, likely due to the distribution of bilayer orientations and chain positions. Stretched exponentials of the form

$$\langle I_z \rangle(t) = C \exp(-(t/T_{1D})^s),$$
 (6.31)

did adequately describe the data. Best fit parameters are given in Table 6.1. Our value of ~60 ms for PL161/D₂O is similar, but somewhat greater than, the 48.8 ± 2.5 ms measured by Swanson *et al.* [84]. The discrepancy is probably due to their slightly higher sample temperature (25°C) and their measurement technique. They used a Jeener-Broekaert sequence, which weights T_{1D} distributions differently than ADRF/ARRF sequences [189].

We have also measured T_{1D} for PL161 in 88%/2% D₂O/H₂O and 90% H₂O. Increasing the



Figure 6.10: Inversion-recovery T_1 measurements in PL161/D₂O. The aqueous proton intensity is integrated in a 1 kHz-wide window around the aqueous peak. Integrals of the entire spectrum and the aqueous protons only were fit to a stretched exponential of the form $\langle I_z \rangle(t) = C [1 - \gamma \exp(-(\delta/T_1)^s)]$. The fit values are as follows. Entire spectrum: $T_1 = 1.245 \pm 0.007$ s, $\gamma = 1.831 \pm 0.004$, $s = 0.818 \pm 0.005$. Aqueous peak only: $T_1 = 1.594 \pm 0.005$ s, $\gamma = 1.961 \pm 0.003$, with s set to 1. Here, γ is the inversion efficiency, δ is the recovery time, and C is a constant.

aqueous proton concentration decreases the T_{1D} , from 61 ms in D₂O to 23 ms in H₂O. This suggests that both spin diffusion from within the bilayer to the surface and exchange with aqueous protons at the surface, which destroys the dipolar order, are important contributors to the rate.

Another way to measure the T_{1D} is by using the "saturation method" [190–192]. A weak, offresonance pulse first creates dipolar order. Then, the sample's T_{1D} is extracted by observing the decay of spectral asymmetry (assuming $\alpha \neq \frac{\pi}{2}$) as a function of δ . We have performed this experiment on PL161/D₂O. In the difference spectrum $S_+ - S_0$, the non-aqueous integrals $I_>$ and $I_<$ relax toward zero with time constant T_{1D} . The sum $I_> + I_<$ decays toward zero with time constant T_1 . The data were fit simultaneously to

$$I_{<} = C_{1} \exp(-\delta/T_{1}) + C_{2} \exp(-\delta/T_{1D})$$

$$I_{>} = C_{1} \exp(-\delta/T_{1}) - C_{2} \exp(-\delta/T_{1D}),$$
(6.32)

where $C_{1,2}$ are constants. The results are shown in Fig. 6.8B. The best fit is $T_{1D} = 58 \pm 4$ ms and $T_1 = 390 \pm 60$ ms. This T_{1D} value agrees with the value found from the ADRF/ARRF sequence, but the T_1 value disagrees with measurements made with an inversion-recovery (IR) sequence. These data, which are shown in Fig. 6.10, were also fit to a stretched-exponential, yielding $T_1 = 1.245 \pm 0.008$ s. However, the IR non-aqueous signal is likely contaminated by the residual HDO, causing the apparent T_1 to shift toward the longer aqueous T_1 . This is supported by a fit to the aqueous peak intensities only, which was found to be longer (about 1.6 s).

| Sample | $T_{1D} \ (ms)$ | s | Measurement Technique |
|--|-------------------|-----------------|-----------------------|
| PL161/D ₂ O $(10\%/90\% \text{ w/w})$ | 61 ± 1 | 0.74 ± 0.01 | ADRF/ARRF |
| | 58 ± 4 | 1 (fixed) | saturation method |
| PL161/D ₂ O/H ₂ O (10%/88%/2% w/w) | 49.8 ± 0.8 | 0.70 ± 0.01 | ADRF/ARRF |
| PL161/H ₂ O (10%/90% w/w) | 23 ± 1 | 0.66 ± 0.02 | ADRF/ARRF |
| Douglas fir | 2.6 ± 0.1 | 0.84 ± 0.05 | ADRF/ARRF |
| Human hair | 1.58 ± 0.03 | 0.84 ± 0.02 | ADRF/ARRF |
| Beef tendon | 0.634 ± 0.010 | 1 (fixed) | saturation method |

Table 6.1: T_{1D} values for our samples. T_{1D} values greater than about 1 ms could be measured using the ADRF/ARRF technique. The saturation method had to be used to measure the short tendon T_{1D} . s is the stretched exponential parameter defined by Eq. 6.31. In the case of Beef Tendon, a single exponential fit the data well, hence s was fixed at 1.

6.4.4 Dipolar order of homogeneously-broadened spin systems

Here, we show explicitly that homogeneously-broadened spin systems can behave similarly to the PL161 spin system discussed so far, in agreement with the predictions from Provotorov Theory. Measurements were made of three biological materials with homogeneouslybroadened non-aqueous spectra: Douglas fir sapwood, human hair, and beef tendon. Fig. 6.6 displays measures of their Zeeman and dipolar magnetizations following the ihMT prepulses, as well as their T_{1D} s. In contrast to PL161, these samples are not well-described by the spin-1 model, due the large size of their coupled spin systems.

The Douglas fir S_0 , S_+ , S_- , and S_{dual} spectra are shown in Fig. 6.5. As in PL161, the S_+ and S_- spectra display spectral asymmetry from dipolar order, as predicted by Eq. 6.26. The S_0 lineshape is approximately Gaussian, due to the large, rigid spin system of wood constituents including cellulose, xylan, and lignin [193]. Of these, cellulose makes up about half the mass of wood. In Douglas fir, these exist as crystalline microfibrils with diameters of about 12–20 nm, interspersed with amorphous regions every 100–200 nm [194,195]. From the aqueous and non-aqueous zero-time intercepts of the FID, the moisture content of the Douglas Fir sample was found to be about 10% [196]. At this level, most of the remaining water is in the cell wall, where it hydrates the cellulose microfibrils [196]. Experiments on a sample of Western Red Cedar sapwood (Fig. 6.7) gave similar behavior as the Douglas Fir.

The spectra of Human hair (Fig. 6.7) also revealed the presence of dipolar order and a similar ratio of aqueous to non-aqueous intensities. In hair, crystalline α -keratin filaments about 8 nm in diameter are embedded in an amorphous hydrophilic keratin matrix [197,198]. The water signal originates from this matrix, where the water hydrates the keratin filament exteriors.



Figure 6.11: T_{1D} of beef tendon as measured by the saturation method. The data are fit to a mono-exponential decay (Eq. 6.31 with s fixed at 1), yielding $T_{1D} = 634 \pm 10 \ \mu$ s. For S_+ , a Gaussian prepulse was used with a width $\tau = 3\sigma = 0.5$ ms and $\nu_1 = 7$ kHz. The observe pulse flip angle was 33°.

The behavior of tendon (Fig. 6.7) is similar, although the short T_{1D} hinders the creation and observation of dipolar order under these experimental conditions. The non-aqueous spectrum is visually similar to the PL161 super-Lorentzian. Tendon is highly-ordered, consisting of triple helices of collagen, which are organized into fibrils that run parallel to the tendon [199]. As large, relatively immobile molecules, collagen proteins have substantial dipolar couplings. At the same time, mobile water molecules permeate the entire fibril and are partially ordered by its structure [200].

The T_{1D} values from the three non-lipid samples (Table 6.1) reflect the microstructure of the samples. As with lipid bilayers, dipolar order in the non-aqueous protons of hair, wood, and tendon is destroyed at the aqueous/non-aqueous proton interface due to proton exchange. When there is no exchange, the dipolar order can evolve for much longer: crystalline cellulose prepared in D₂O has $T_{1D} \sim 50$ ms, for example [201].

The T_{1D} of tendon was too short to be measured by the ADRF/ARRF sequence. Instead, the saturation method was used. As shown in Fig. 6.11, this gave a value of $T_{1D} = 634 \pm 10$ μ s. This is comparable to the value of $230 \pm 20 \ \mu$ s measured by Swanson *et al.* for chicken hyaline cartilage at 25° using a Jeener-Broekaert sequence [84]. The unique samples probably account for the difference between these values. Tendon is mostly type I collagen (about 86% of the dry weight), whereas hyaline cartilage is mostly type II collagen (about 60% of the dry weight) [202–204].

The saturation method was used to measure the T_{1D} of beef tendon. Fig. 6.11 shows the decay of the spectral integrals of $S_+ - S_0$ and the fit to Eq. 6.31 with s was fixed at 1, describing a mono-exponential decay. In contrast to PL161, in tendon $T_1 \gg T_{1D}$, so T_1 relaxation can be ignored during this analysis. A single exponential fit this data well so it



Figure 6.12: Aqueous and non-aqueous ihMTRs as functions of offset frequency. CW ihMT sequences were used with $\tau = 500$ ms, $\nu_1 = 460$ Hz, and $\delta = 0.5$ ms. Samples with homogeneously broadened spectra show non-zero ihMT, in contradiction to the hypothesis that ihMT occurs due to inhomogeneous broadening.

was not necessary to introduce the stretched exponential (*i.e.* s was fixed at 1).

6.4.5 ihMT in lipids and homogeneously-broadened systems

Fig. 6.12 shows the ihMTR for hair, Douglas fir sapwood, PL161, and tendon as functions of offset frequency $|\Delta|$. We have calculated two ihMTRs: one using aqueous proton intensities (integrated in a 1 kHz window around the aqueous peak), and one using non-aqueous proton intensities (= $I_{<} + I_{>}$, where $I_{<}$ and $I_{>}$ are defined in Eq. 6.32). The general shape these curves follow is consistent with previous studies and follows our model of non-aqueous ihMTR using CW prepulses (Fig. 6.2C). At low offset frequencies, the ihMTR becomes unreliable, due to discretization of the $\sin(2\pi\Delta t)$ modulation in the S_{dual} prepulse, the direct saturation of the aqueous protons, and smaller differences between the S_{+}/S_{-} and S_{dual} spectra.

Another observation is that the $\sim 3 \times$ larger ihMTR seen in the non-aqueous protons of



Figure 6.13: ihMTR vs. prepulse power (which is $\propto WT_{1D}$) in the samples with the longest and shortest T_{1D} values. These curves are qualitatively similar to those in our model of CW ihMTR (Fig. 6.2B). A CW ihMT sequence was used with $\tau = 500$ ms, $\delta = 0.5$ ms, and with ν_1 calibrated by nutation of the water peak. $|\Delta|$ is 11 kHZ in PL161 and 19 kHz in tendon, which are close to the values that maximize the ihMTR in Fig. 6.12.

 $PL161/D_2O$ than in the aqueous protons is due to diffusion-limited proton exchange to the aqueous pool. Even the slightly higher concentration of aqueous protons in the sample with 2% H₂O causes more similar non-aqueous and aqueous ihMTRs. This provides confirmation that ihMT occurs due to the behavior of the non-aqueous protons' dipolar reservoir only, and that it is observable via the aqueous protons because of proton exchange. In samples with abundant protons, under long prepulses the aqueous and non-aqueous ihMTRs will be very similar.

These results are in agreement with Varma *et al.*'s claim that ihMTR is highly sensitive to T_{1D} [119]. However, the differences in T_{1D} alone are not enough to account for the differences in ihMTR between the samples. For example, despite having a T_{1D} that is 20–40 times larger than Douglas Fir or hair, PL161 has a maximum ihMTR only 4–5 times greater. This is likely due to differences in W contributing to WT_{1D} . Different values of W arise due to the different lineshapes $g(2\pi\Delta)$ and local field strengths ω_D amongst the samples. Testing the model's predicted sensitivity to WT_{1D} would involve quantifying these values accurately, and was not attempted here.

We have not attempted to fit the results of Fig. 6.12 to the models described above, as our models do not include the effects of exchange with the aqueous proton pools. Varma *et al.* did quantitative fits of a Provotorov-theory based model to experimental data and found excellent agreement [119]. Our goal here is not to reproduce that work, but instead to consider simplified models that provide greater understanding of the fundamental physics underlying the ihMT effect.
We have also measured ihMTR vs. prepulse power (ν_1^2) for the samples with the highest (PL161/D₂O) and lowest (beef tendon) T_{1D} s. These results are shown in Fig. 6.13. The offset frequencies were set to the values that maximized the non-aqueous ihMTR in Fig. 6.12, these are 19 kHz for tendon and 11 kHz for PL161. As shown in Eq. 6.17, $W \propto \nu_1^2$. Furthermore, T_{1D} is constant, so these are effectively plots of ihMTR vs. WT_{1D} . These results follow the behaviour predicted by our model of ihMT in Fig. 6.2B: at low values of WT_{1D} ihMTR increases linearly and at high values it saturates.

6.5 Discussion

Our results have clearly shown that ihMT originates in the non-aqueous protons but is observed in the aqueous protons following magnetization exchange. Moreover, ihMT does not arise because of inhomogeneous broadening but instead through dipolar couplings, and does in fact occur in homogeneously-broadened spin systems. Finally, a hole cannot be burned in the PL161 proton spectrum. Hole-burning is not a part of the mechanism responsible for the ihMT signals observed here.

These experimental findings are consistent with our theoretical models of the non-aqueous spin systems under ihMT prepulses. Spin-1 theory provides a minimal model allowing an intuitive understanding of the origin of the effect. The Provotorov theory-based approach allowed the identification of the timescale ratio WT_{1D} for CW ihMT, and $W_{\text{eff}}T_{1D}$ and τ_2/T_{1D} for pulse-train ihMT (see Appendix D), as key parameters controlling whether ihMT occurs. Moreover, the spin-1 model results can be derived from the Provotorov theory model, showing their generality beyond spin-1 systems. Neither of our models require assumptions about the type of spectral broadening (homogeneous vs. inhomogeneous) present. All they require is the presence of the dipolar interaction.

We are now in a position to definitively answer the question, "When will a material have an ihMT response?" The short answer to this for CW ihMT sequences is that WT_{1D} for the non-aqueous protons must be "large enough". A quantitative cutoff can be estimated by estimating WT_{1D} for tendon and PL161, which respectively have the lowest and highest ihMTR among our samples. We assume a Gaussian lineshape with a standard deviation ω_D equal to the offset that maximizes the ihMTR in Fig. 6.12 (about 11 kHz for PL161 and 19 kHz for tendon). Then, using $|\Delta| = \omega_D$ and $\nu_1 = 460$ Hz, we find $WT_{1D} \approx 5.6$ for PL161 and $WT_{1D} \approx 0.04$ for tendon. From this, we may estimate a rule of thumb that ihMT will not be easily observable unless $WT_{1D} > 0.01$.

Experimentally, for a given W, ihMT depends sensitively upon a material's T_{1D} . This value

reflects structure of the microstructure and its motion. Dipolar order relaxes from motions that occur on a timescale ~ ω_D^{-1} , and is also destroyed by proton exchange. In most tissues, motions from exchange with water are probably the primary driver of T_{1D} [201, 205]. In proteins, reorientation of methyl groups also play a role, and in lipids the residual interand intra-molecular dipolar couplings weakly contribute as well [205, 206]. If a bottleneck exists for spin diffusion from a reservoir to these sites of relaxation, then T_{1D} can be large enough for ihMT to occur. This is exemplified using simple spin diffusion models of PL161 and cellulose crystallites.

Lamellar lipids tend to have very slow spin diffusion along the lipid tails due to the weak inter-methylene coupling. In contrast, dipolar order relaxation proceeds rapidly once magnetization reaches the headgroup because of proton exchange. Taken together, this means spin diffusion along the lipid tails in PL161 is the rate-limiting process for dipolar order relaxation. As shown in Table 6.1, T_{1D} of PL161 seems sensitive to aqueous proton concentration only when the aqueous proton concentration is small. T_{1D} decreases by ~20% when the sample is changed slightly from pure 90% D₂O (10% PL161) to a 88%/2% D₂O/H₂O mixture. Yet, the PL161 sample in neat H₂O shows a further decrease of only 50% in T_{1D} . Aqueous protons destroy non-aqueous dipolar order at the headgroups via proton exchange, but after a critical concentration of aqueous protons is reached, this is not the rate-limiting process, rather spin diffusion inside the lipid bilayer is.

A simple one dimensional model of spin-diffusion in an infinite plane can be solved analytically (e.g. Eq. 4.16 in Crank [207]). With parameters chosen to represent a single lipid bilayer ($D \approx 0.016 \text{ nm}^2/\text{ms}$ [208] and layer thickness $l \approx 3 \text{ nm}$ and $D \approx 0.016 \text{ nm}^2/\text{ms}$ [208], corresponding to a 15–18 carbon chain), initial conditions of uniform dipolar order within the plane, and boundary conditions where the dipolar order is destroyed at the plane surfaces, the decay of dipolar order predicted fits well to a stretched exponential (Eq. 6.31), with $T_{1D} \approx 45 \text{ ms}$ and s = 0.8. This T_{1D} value is within a factor of 2 from the measured value of 23 ms.

A similar analysis can be performed for wood cellulose. We assume that H₂O permeates the cellulose microfibrils and relaxes dipolar order at the surface of each cellulose crystallite. These crystallites have a diameter of about 5 nm and lengths 4–10× this [209]. The crystallites can be modelled as infinitely long cylinders with radius $a \approx 2.5$ nm. In solid organic polymers like these, the spin diffusion coefficient is ~ 1 nm²/ms [11]. With these parameters, and similar initial conditions and boundary conditions as before, the solution to the diffusion equation in this geometry (*e.g.* Eq. 5.18 in Crank [207]) is again well described as a stretched exponential, with $T_{1D} = 1.05$ ms, s = 0.98. Here T_{1D} is within a factor of three from the measured value of 2.6 ms.

The agreement between these models and our results show that in systems with magnetization exchange to abundant aqueous protons, T_{1D} is largely dependent on the spin diffusion rate. It also suggests that even if spin diffusion is relatively fast, T_{1D} may be long enough for ihMT to be observable if the physical size of the reservoir is large enough, as in the case of cellulose crystallites in wood and keratin crystallites in hair.

The observation of non-zero ihMT in tendon suggests that this technique may not be as myelin-specific as previously thought. In brain, lipid membranes in myelin and glial cells are likely the only structures with an ihMT response. Such experiments however may prove to be useful in imaging other areas of the body, Further work to rigorously identify tissues producing non-zero ihMT is required.

Taken together, this work suggests that thinking of ihMT as resulting from a type of spectral line broadening is misleading. While ihMT may occur in inhomogeneously-broadened systems, it occurs in homogeneously-broadened ones as well. The presence of a dipolar term in the Hamiltonian of the non-aqueous protons is enough to ensure the presence of a dipolar reservoir, and if WT_{1D} is large enough, then ihMT will be visible from the non-aqueous proton intensities. If magnetization exchanges with aqueous protons, then ihMT will be visible from their intensity too. Others have already shown the applicability of Provotorov theory, and our results have confirmed that ihMT is driven by the dipolar interaction alone and that inhomogeneous broadening is not involved. For this reason, we suggest changing the name ihMT to dipolar magnetization transfer (dMT) to better reflect the underlying mechanism.

Chapter 7

Pool-specific ihMT in white matter

7.1 Introduction

There is little doubt of ihMT's sensitivity to T_{1D} . Indeed, studies performed on phantoms [84, 125, 131] and *in-vivo* [127] show how ihMT may be considered a T_{1D} -weighted imaging modality. It is also clear that ihMT is sensitive to myelinated tissues such as white matter. Moreover, myelin bilayers are known to be unique, possessing on average fewer proteins, more long-chained lipids, and a higher proportion of saturated lipids than in other biomembranes [51, 61, 63, 64, 84]. Accordingly, the prevailing theory for ihMT's sensitivity to myelin is that myelin's unique lipid bilayers possess a long T_{1D} .

Recent measurements of T_{1D} in white and grey matter are inconsistent with this understanding of ihMT. Using the sequence developed by Varma *et al.* to measure T_{1D} *in-vivo* with ihMT prepulses [127], multiple studies have shown remarkably similar T_{1D} values in white and grey matter. However, still other measurements by Swanson *et al.* give very different T_{1D} values in these two tissues. The state of T_{1D} measurements is summarized in Table 7.1. Differences in the samples (fixed vs. *in-vivo*) may account for the large variation. Also, each technique for measuring T_{1D} is biased towards certain values.

These discrepancies show the need for closer examination of the ihMT signal from myelin. To this end, the present study combines ihMT with CPMG acquisition in order to measure the distinct signals from myelin water (MW) and intra/extra-cellular water (IEW). This is possible through multi-exponential fitting of the CPMG decay. Since MW is nominally the first aqueous pool in which the ihMT signal from myelin arises, observing its ihMT signal directly may highlight the differences between the non-aqueous protons inside and outside the myelin. To qualitatively model the results, we apply the four pool model of white matter

| Study | Sample | Technique | White | Grey matter | Muscle |
|-------------------|----------------------|----------------|--------------------|-----------------------|---------------|
| | | | matter T_{1D} | T_{1D} (ms) | T_{1D} (ms) |
| | | | (ms) | | |
| Prevost <i>et</i> | Mouse | T_{1D} -ihMT | 6.1 ± 0.8 (IC) | $5.6{\pm}1.2$ | $2.2{\pm}0.6$ |
| al. [125] | in-vivo | | | (cGM) | |
| Swanson et | Formalin- | Jeener- | 11.1 ± 1.8 | $4.06{\pm}1.20$ | - |
| al. [84] | fixed bovine | Broekaert | (SC) | (SC) | |
| | spinal cord | | | | |
| | in D_2O | | | | |
| Varma et | Human | T_{1D} -ihMT | $6.2{\pm}0.4$ | $5.9{\pm}1.2$ | - |
| al. [127] | brain <i>in-vivo</i> | | (avg) | (avg) | |
| Carlvalho et | Mouse brain | T_{1D} -ihMT | 4.9 ± 1.0 (IC) | $4.8 {\pm} 0.7$ | $1.9{\pm}0.3$ |
| al. [210] | in- $vivo$ | | | (cGM) | |
| | Rat spinal | | $6.6 \pm 0.5 (SC)$ | $6.6 \pm 0.9 \; (SC)$ | - |
| | cord ex-vivo | | | | |

Table 7.1: Measurements from the literature of white and grey matter T_{1D} s. IC = internal capsules, cGM = cortical grey matter, SC = spinal cord. T_{1D} -ihMT refers to the sequence developed by Varma *et al.* for measuring T_{1D} using ihMT prepulses [127].

from Chapter 5. The model is modified to include dipolar reservoirs in each non-aqueous pool.

7.2 Theory

7.2.1 The four pool model with dipolar reservoirs

The four pool model, used extensively in Chapter 5, models longitudinal relaxation in white matter tissue. To model ihMT as well, a four pool model with dipolar reservoirs is required (Fig. 7.1). The dipolar couplings in the non-aqueous pools must now be taken into account. The protons in these pools are on large molecules like lipids, which either tumble slowly or are restricted in some way. As a result, the proton-proton dipolar interactions are incompletely averaged. This causes broad non-aqueous NMR lineshapes, such as the super-Lorentzian seen extensively in previous chapters. What is relevant here is that the residual dipolar couplings also forms a thermodynamic reservoir. This reservoir can store dipolar magnetization (also called dipolar order) [10, 30].

Provotorov Theory, derived in Section 2.5.6 and applied in the previous chapter, describes the coupling of Zeeman and dipolar magnetization. We remind the reader of its key equations. In an isolated system of dipolar-coupled protons, the evolution of a vector $\boldsymbol{\rho} = [M_D, M]^{\mathrm{T}}$



Figure 7.1: The four pool model with dipolar reservoirs. Pools 1 to 4 can hold Zeeman (longitudinal) magnetization. The dipolar reservoirs hold dipolar magnetization.

(where M_D and M are the dipolar and Zeeman magnetizations respectively) under weak rf irradiation is described by

$$\frac{d\boldsymbol{\rho}}{dt} = \begin{pmatrix} -W - \frac{1}{T_1} & W\Omega \\ W\Omega & -W\Omega^2 - \frac{1}{T_{1D}} \end{pmatrix} \boldsymbol{\rho}_{\pm} + \begin{pmatrix} \frac{\langle I_z \rangle_0}{T_1} \\ 0 \end{pmatrix}.$$
(7.1)

 T_1 and T_{1D} are the Zeeman and dipolar spin-lattice relaxation times and

$$\Omega = 2\pi\Delta/\omega_D$$

where Δ is the offset frequency of the rf and ω_D is the RMS dipolar interaction strength. Finally,

$$W = \pi (2\pi B_1)^2 g(\Delta), \tag{7.2}$$

which is a function of the prepulse RMS amplitude B_1 and the lineshape of the non-aqueous protons g evaluated at the offset. The S_{dual} prepulse causes the off-diagonal elements of the matrix above to vanish.

Combining this with the four pool model is straightforward. This combined model builds off of two pool models with a dipolar reservoir for modeling MT [35,93], and Varma *et al.*'s introduction of this two pool model for simulating ihMT [119]. In the last chapter, we used Provotorov Theory in a single non-aqueous proton system as a model for discussing ihMT physics. In the combined model below, the dipolar reservoirs remain coupled to non-aqueous pools only, which in turn can exchange with aqueous pools. The combined model is described by a coupled system of homogeneous differential equations,

$$\frac{d\mathbf{M}}{dt} = R\mathbf{M}.\tag{7.3}$$

M is a vector of Zeeman (M) and dipolar (M_D) magnetizations

$$\mathbf{M}(t) = [M_{D,M}(t), \ M_{M}(t), \ M_{MW}(t), \ M_{IEW}(t), \ M_{NM}(t), \ M_{D,NM}(t), \ 1]^{\mathrm{T}}.$$
 (7.4)

Starting from thermal equilibrium, the initial condition vector is

$$\mathbf{M}(0) = [0, \ M_{\rm M}(\infty), \ M_{\rm MW}(\infty), \ M_{\rm IEW}(\infty), \ M_{\rm NM}(\infty), \ 0, \ 1]^{\rm T}.$$
(7.5)

The last component of these two vectors is a constant equal to 1 whose purpose is to convert the inhomogeneous differential equations in Eqs. 2.47 and 5.1 to homogeneous equations. This approach is possible because the inhomogeneous terms (the $M_i(\infty)/T_{1,i}$ s) are constant. This last dimension in $\mathbf{M}(t)$ has no physical interpretation and can be ignored. When the four pool model is used without a dipolar reservoir, substituting reduced magnetization units, $m_i = -\frac{M_i - M_i(\infty)}{2M_i(\infty)}$, also makes the system homogeneous [144, 145]; this was our approach in Chapter 5. That is not possible here because $M(\infty)$ in the dipolar reservoirs is close to zero [30].

The matrix R containing the dynamics is given by



where

$$\Phi = \begin{cases} 1, & \text{during } S_+ \text{ and } S_- \text{ prepulse} \\ 0, & \text{during } S_{\text{dual }} \text{ prepulse.} \end{cases}$$
(7.7)

In experiment S_0 there is no prepulse and $W_{\rm M} = W_{\rm NM} = 0$.

One significant limitation of this combined model is that it assumes single values for physical parameters. For the four pool model alone, its excellent fit to the relaxation experiments in Chapter 5 shows little need to model distributions for pool sizes, T_{cr} times, and T_1 times. This is likely because rapid diffusion of magnetization in aqueous compartments averages out local variations. However, the same simplification is not necessarily expected with regards to the dipolar reservoir parameters.

We showed that the non-aqueous lineshape from our white matter samples were super-Lorentzian in Chapter 5; in the notation here, that is the function $g_M(\Delta) + g_{NM}(\Delta)$. The super-Lorentzian itself is an integral of orientation-dependent lineshapes, primarily from the acyl chains in lipid bilayers. Therefore, the use of single values for g_M and g_{NM} (via Eq. 7.2) implicitly assumes the presence of angular averaging in the lipid bilayers, occurring much faster than magnetization transfer to the aqueous pool. Orientation dependence of ω_D and T_{1D} (see Eq. 2.52) means there is a distribution of these parameters as well. Nonetheless, accounting for distributions is beyond the scope of this work. Rather, the purpose of this simple model is to lead to straightforward, qualitative conclusions.

7.2.2 The grey matter analogue

In addition to modeling the MTR and ihMTR data for a white matter sample, the four pool model with dipolar reservoirs can be used to model a "grey matter analogue". From a structural perspective, grey matter is very roughly like white matter with the myelin removed. In both cases, the components (glial cells, unmyelinated axons, blood vessels, *etc.*) are similar, though there are some obvious structural differences: the presence of neuron cell bodies in actual grey matter, for example. Still, in a thought experiment where the myelin in white matter is removed and replaced with water, this reduces the quantity of non-aqueous protons by about half, nicely matching with the fact that there are roughly half as many non-aqueous protons in grey matter compared to white matter (when measured as a fraction of total proton number) [165,211]. And so, to simulate ihMTR in the white matter sample's grey matter analogue, we set $T_{cr,IEW/MW} \rightarrow \infty$ and record the signal from the IEW pool only. This grey matter analogue is indeed a crude model, but it has been used before in biochemical assays. Norton & Cammer note that despite being an oversimplification, this model does yield accurate results when quantifying lipids in grey and white matter [62]. Its use here is justified given the qualitative nature of our analysis.

7.3 Methods and materials

7.3.1 Sample preparation

The same four samples (bovine white matter samples WM-fr, WM-sp1, WM-sp2, and grey matter sample GM-bg) were used here as in the T_1 relaxation experiments in Chapter 5. See Section 5.3.1 for details of the sample preparation.

7.3.2 NMR experiments

The pulse sequences used are shown in Fig. 7.2. This style of ihMT experiment, where the prepulse is continuous-wave rf, requires three experiments with different prepulses plus a reference experiment for a total of four experiments. Experiments S_+ and S_- use prepulses at offsets $+\Delta$ and $-\Delta$ relative to the center of the lipid super-Lorentzian. Experiment S_{dual} uses a prepulse which is sine-modulated at Δ to irradiate $\pm \Delta$ simultaneously. The maximum prepulse amplitude in S_{dual} is $\sqrt{2}B_1$, but in S_+ and S_- it is B_1 . This ensures that the prepulse RMS power is the same in all three cases. In the reference experiment S_0 , $B_1 = 0$ during the prepulse.

For sample WM-sp1, 20 ihMT experiments were acquired, varying the prepulse duration, τ , and the relaxation delay, δ . In the first 14 experiments, τ was increased from 10 to 1000 ms (with δ held at 0.01 ms), and in the last 7 experiments, δ was increased from 0.01 to 500 ms (with τ held at 1000 ms). In order to complete all experiments on all samples within 72 hours, for the remaining samples only 12 experiments were acquired, where τ was increased from 10 to 1000 ms in the first 7 (holding δ constant at 0.01 ms), and δ was increased from 0.01 to 500 ms in the last 6 (holding τ constant at 1000 ms). More data was acquired at low values of τ and δ in order to capture any short-time behaviour.

 $|\Delta|$ was fixed at 7 kHz and B_1 was either 141 or 283 Hz (3.32 or 6.64 μ T). In a previous study by Varma *et al.*, similar parameters gave a large ihMT in human WM [119]. This is also close to the optimal parameters (Δ =7–9 kHz and an RMS B_1 of 4–5.5 μ T) suggested



Figure 7.2: The four ihMT NMR experiments with CPMG acquisition. S_{dual} uses a sinemodulated prepulse to irradiate offsets at $\pm \Delta$ simultaneously. In experiment S_0 there is no prepulse.

by Mchinda *et al.*, although their *in-vivo* study used a pulse-train prepulse instead of cosinemodulation [123]. Our CPMG acquisition used broadband rectangular pulses with a typical 90° pulse duration of 3.1–3.3 μ s (a B_1 amplitude of 18–19 mT). The CPMG train used 300 echoes with 2 ms spacing. 8 or 4 transients were acquired with a recycle delay of 7 s.

Like the experiments on these samples reported on earlier, the data for this study was collected using a Bruker solenoidal probe (HP WB73ASOL10) in a 200 MHz (4.7 T) magnet with a home-built NMR spectrometer. The temperature was regulated at 37 °C.

7.3.3 CPMG fitting

As in the T_1 relaxation experiments in Chapter 5, the CPMG curves were analyzed using sparse exponential distributions. Section 5.3.3.2 outlined this approach, where the CPMG signal was modeled as a sum of four exponentials: one corresponding to the MW peak (~6 ms), two corresponding to the IEW peak (constrained to be 10 ms apart, centered near 60 ms), and a last one for a small ~200 ms component. Previously, we identified this longest component as an external bulk water (BW) pool, based off of its mono-exponential T_1 relaxation behaviour. Our magnetization transfer results below confirms this association. The amplitudes of these exponentials give the relative amount of magnetization in each pool. From the four experiments, the standard Magnetization Transfer Ratio (MTR) can be calculated separately for single and dual-sided ihMT prepulses. For each pool, the MTR is

$$MTR_{single} = \frac{2A_0 - A_+ - A_-}{2A_0}$$
(7.8)

$$MTR_{dual} = \frac{A_0 - A_{dual}}{A_0},$$
(7.9)

and the ihMTR calculated through

$$ihMTR = MTR_{dual} - MTR_{single}$$
$$= \frac{A_{-} + A_{+} - 2A_{dual}}{2A_{0}}.$$
(7.10)

As in the last chapter, we caution the reader that this definition of ihMTR has a two in the denominator, which most imaging studies now omit. We keep it here for internal consistency within the thesis. When we later quote results from other papers, we will convert the values appropriately.

Because the quantities of interest in this chapter are ratios, we can ignore the effects of MW/IEW exchange during the CPMG in this chapter. MW/IEW exchange causes the the measured amplitudes (and T_2 s) of the MW and IEW pools to deviate slightly from their true values (see Section 4.5.2). In Chapter 5, we calculated a multiplicative correction factor for the pool amplitudes which was applied to each of the observed A_0 , A_{dual} , A_+ , and A_- terms at all times. However, this cancels out in MTR and ihMTR.

Fitting was performed using the least squares solver in the SciPy package [157]. Errors on T_2 component amplitudes are the standard deviations of 50 repeated fittings to the CPMG with synthetic Gaussian noise. The noise standard deviation was equal to the standard deviation of the best fit residuals.

7.3.4 Four pool model fitting

When the four pool model with dipolar reservoirs is fit to the data from the white matter samples, the following constraints are imposed on physiological grounds:

$$T_{1D,\text{NM}} \le T_{1D,\text{M}}$$
$$\omega_{D,\text{NM}} \le \omega_{D,\text{M}}.$$
(7.11)

These are justified by the properties of the myelin lipid bilayers, which Chapter 3 mentioned are unique in three ways. When compared to other membranes, myelin has, on average, i)

longer lipid acyl chains, ii) a higher degree of saturated lipids, and iii) a smaller protein:lipid ratio [51, 61, 63, 64, 84]. Taken together, compact myelin is more closely-packed and rigid than other biomembranes [60], meaning the membrane fluctuation amplitudes which drive T_{1D} relaxation are expected to be smaller [84, 210], whereas ω_D is similar or slightly higher. This last point is supported by the similar linewidths seen in our measurements of the nonaqueous spectra of WM-fr, WM-sp1, WM-sp2, and GM-bg (Table 5.1).

Fitting used SciPy's implementation of the Differential Evolution algorithm [161] via the LMFIT package [158]. The four pool parameters from the results in Chapter 5 were used, so only the dipolar reservoir parameters need to be fit. These include T_{1D} , ω_D , and $g(\Delta)$ in both non-aqueous pools, which is six parameters in total.

7.4 Results



Figure 7.3: Comparison of regularized NNLS distributions in WM-fr after the four ihMT prepulse conditions. The equilibrium distribution (experiment S_0 , no prepulse) shows the three peaks from distinct populations of aqueous protons. The integrated intensity is displayed beside each peak. Immediately after a prepulse ($B_1 = 283$ Hz, $\tau = 215$ ms, $\delta = 0.01$ ms), the S_{dual} experiment showed a significantly larger reduction of the MW and IEW peaks compared to the S_+ or S_- experiments. The BW pool appears to increase due to regularized NNLS fitting artifacts.

To illustrate how the different prepulses uniquely affect each aqueous pool, Fig. 7.3 shows the regularized NNLS distributions in WM-fr for all four experiments under a 215 ms prepulse. Each peak is labeled with its integrated intensity. The MW and IEW peaks decreased more when a dual-sided prepulse was used (S_{dual}) than in the case of single-sided prepulses $(S_{+}$ and $S_{-})$. Conversely, the BW pool appeared to increase in intensity. However, this is an

artifact of the NNLS fitting. As the total signal decreases, so too does the SNR. Smaller SNR values are known to introduce errors into the regularized NNLS distribution [212]. However, the more robust sparse exponential fits below showed only a decreasing BW amplitude for the white matter samples.

The MTRs (Eqs. 7.8 and 7.9) are plotted in Fig. 7.4 for the white matter samples (MW, IEW, and BW pools) and for GM-bg (IEW and BW pools). The corresponding ihMTRs (Eq. 7.10) are given in Fig. 7.5. We first focus on the white matter MTR and ihMTR, leaving the GM-bg results for later.

The ihMTR and MTR values are plotted as functions of increasing prepulse duration τ and recovery time δ . The results in all white matter samples are visually similar. The MT response from MW was the largest, followed closely by IEW. This matches with the results found in previous MT-CPMG experiments [169]. The BW MT response was significantly lower, confirming that this pool is relatively isolated. Across all pools, $B_1 = 283$ Hz caused a higher MT response. There are striking differences in MW and IEW MTRs during the relaxation period, when $\delta > 0$. These can be explained by comparing the size of their corresponding non-aqueous pools (given by the four pool fits in Table 5.2). MW relaxes quickly, since it is ~0.5× the size of pool M and these two pools are in close contact. Conversely, the IEW pool size is about 5–7× the NM pool, so the magnetization exchange rate between the two is much smaller. For example, when $B_1 = 141$ Hz, the IEW MTR appears to plateau until about $\delta = 100$ ms, whereas MW's MTR starts decreasing immediately.

Focusing now on the ihMTR plots (Fig. 7.5), given the relatively small difference between MTR_{dual} and MTR_{single}, these ihMTR data were much noisier than the MTR data. All white matter samples showed gradual growth in MW and IEW ihMTR as τ increases when $B_1 = 141$ Hz. However, when $B_1 = 283$ Hz, the MW ihMTR peaks and then decays. Previous *in vivo* studies [125] and modeling [119, 131] that looked at the total aqueous response have also shown similar behaviour. The same feature would be expected in the IEW pool at higher B_1 values. This occurs because the MW magnetization became saturated more rapidly than the IEW magnetization. When this saturation happened, the effect of the single and dual prepulses became similar, and ihMTR decreased.

We also plot ihMTR for the combined MW and IEW pools in white matter, which is approximately what would be observed for the total aqueous signal *in-vivo* (assuming TE, the time between the MRI excitation pulse and acquisition, was short compared to the MW T_2). Excluding the BW pool is justified due to its isolation. This MW+IEW ihMTR closely followed the IEW ihMTR since that pool was 90–95% of the aqueous signal.

Concerning the grey matter, the results indicate a relatively poor fit. Firstly, the MTR from



Figure 7.4: MTR for all samples after single and dual prepulse irradiation as a function of prepulse duration τ and recovery time δ . The consistently lower MTR in the BW pools confirms its relative isolation. In general, MW and IEW showed a higher MTR_{dual} than MTR_{single}, indicating ihMT occurs in these pools. Error bars are plotted but are smaller than the data points for most series. In GM-bg, no MW signal was observed and the BW MTR was constrained to be positive. This was necessary due to the large fitting error on the BW pool. Note that in the interests of completing all experiments on the samples promptly, fewer τ and δ times were acquired on WM-fr, WM-sp2, and GM-bg compared to WM-sp1.

| | WM-fr | WM-sp1 | WM-sp2 |
|---|-------|--------|--------|
| $T_{1D,\mathrm{M}} (\mathrm{ms})$ | 7.4 | 30* | 8.9 |
| $\omega_{D,\mathrm{M}}/2\pi \;(\mathrm{kHz})$ | 4.1 | 8.4 | 6.0 |
| $g_{ m M}/10^{-6}({ m s})$ | 3.4 | 5.0 | 12 |
| $T_{1D,\rm NM}~(\rm ms)$ | 3.8 | 7.5 | 4.0 |
| $\omega_{D,\rm NM}/2\pi~(\rm kHz)$ | 3.8 | 7.4 | 5.3 |
| $g_{\rm NM}/10^{-6}({ m s})$ | 3.0 | 9.6 | 7.5 |

Table 7.2: The dipolar reservoir fit parameters. In these simulations, the four pool model parameters in Table 5.2 were used. Errors on parameters were not included since this model is qualitative only, as evident from the fits in Fig. 7.6. $T_{1D,M}$ for WM-sp1 was at the allowed limit for that value (30 ms). Parameter constraints are listed in Eq. 7.11.

GM-bg's BW pool (Fig. 7.4D) had to be artificially constrained to be positive, otherwise unphysical negative values appeared. This fitting difficulty is reflected in the large error bars on GM-bg's BW MTR data, which are about $\pm 2\%$; in the white matter samples, these are <0.5%. It is unsurprising that the lowest ihMTR signal did come from GM-bg's IEW pool, which had a maximum signal about half that of the white matter samples. This is expected, given the low quantity of myelin in grey matter. Yet, the negative ihMTR values, particularly in the B_1 =141 Hz data, are not expected—these are clearly unphysical. We will delay an exploration of possible causes for this until the Discussion.

The plots in Fig. 7.6 show the four pool model fits to the data, using the technique described in the methods. It's immediately clear that the model describes the MTR data well, but only qualitatively describes the ihMTR. Still, the general trends are captured. For instance, the model matches how the MW ihMTR rose rapidly compared to the IEW ihMTR. Also, in the case of $B_1 = 283$ Hz, it simulates the maximum in the MW ihMTR at short τ . Where the model falls short is the magnitude of the IEW ihMTR. This is the case even though we make no assumptions about the functional form of g_M and g_{NM} or the relationship between these factors and the respective $\omega_D s$. No fitting was performed on the GM-bg sample—its negative ihMTR values at small τ are unphysical.

Table 7.2 gives the dipolar reservoir fit parameters (the four pool parameters were fit in Chapter 5 and are listed in Table 5.2). The qualitative nature of this model means the precision of these values is low. Indeed, two pool model fits to similar data tended to have large variations in the parameters [119]. Even so, the relative magnitudes are illuminating. For example, these parameters are consistent with the view that M has a significantly longer T_{1D} than NM. Also, the $g_{\rm M}$ and $g_{\rm NM}$ values are similar, which is expected if these two pools have similar lineshape widths.



Figure 7.5: The ihMT response for all samples under the two different prepulse amplitudes. The maximum MW ihMTR was always higher than IEW's. The total aqueous ihMTR (MW+IEW) was very close to the IEW ihMTR, since that pool contains the majority of the aqueous protons. Error bars are not drawn for clarity on the line for MW+IEW, but they are approximately the same size as IEW's error bars. GM-bg sample had no measurable MW pool so no combined ihMTR is plotted. The negative ihMTR values in that sample are non-physical. In all cases, the solid lines are plotted as guides to the eye.



Figure 7.6: The fits of the four pool model with dipolar reservoirs in all white matter data. The fits to the MTR data are shown in plots (A,C,E) and the fits to the ihMTR data are shown in plots (B,D,F). There is significantly higher error in the ihMTR fits due to ihMT's sensitivity to subtle differences between MTR_{dual} and MTR_{single} . The model fails to capture the details, but is qualitatively correct. The simulation used parameters from Tables 5.2 and 7.2.



Figure 7.7: ihMT in white matter samples and in their grey matter analogues. The four pool simulations are the same fits shown in Fig. 7.6. The grey matter analogues are a simulation of the same sample with the myelin removed. The maximum ihMTR in both cases is indicated.

Using these parameters, we also simulated the grey matter analogue. This is plotted in Fig. 7.7. If we imagine the white matter sample's myelin being removed, the behaviour in the dashed lines is expected, which shows lower ihMTRs in every case.

7.5 Discussion

The work here has two main portions: ihMT-CPMG measurements, which allow the ihMT in MW and IEW to be observed separately; and qualitative simulations using a four pool model with dipolar reservoirs. While the precise values of ihMTR depend on sequence, sample, and parameters used, in general the total ihMTR (MW+IEW) we measured matches well with

previous *in-vivo* ihMT studies. For example, in a previous study also using cosine-modulated S_{dual} prepulses, doubling the RMS prepulse power also roughly doubled the ihMT signal [118]. The same was seen with pulse-train prepulses [120]. Moreover, the grey matter sample had a maximum ihMTR that was roughly half the maximum ihMTR in the white matter samples, which was also seen previously [118, 120].

The measurements of ihMT in MW are one novel aspect of the work here. Its higher MT response reflects the close contact between this pool and the myelin lipids. Tracing the origin of the higher ihMT response is less clear. On one hand, we expect the myelin lipids to have more ihMT compared to non-myelin non-aqueous protons since their lipid bilayers are unique: as mentioned previously, they are more rigid, more compact, and—although measurements disagree on this—are thought to have a long T_{1D} [84,119,131]. On the other hand, MW's MTR is larger (especially at $\delta < 500$ ms, see Fig. 7.4), which, all things being equal, would allow a higher ihMTR to be realized. The essential question is the following: is MW's ihMTR higher than IEW's because of i) a difference in the dipolar reservoirs of the non-aqueous pools to which they are coupled, or ii) the higher MT in MW because of its more intimate contact with its non-aqueous pool?

In an attempt to answer this, we define the ratio

$$\frac{\text{ihMTR}}{\overline{\text{MTR}}} = \frac{\text{MTR}_{\text{dual}} - \text{MTR}_{\text{single}}}{\frac{1}{2} (\text{MTR}_{\text{dual}} + \text{MTR}_{\text{single}})},$$
(7.12)

which will be large when ihMTR is due mainly to the behaviour of the dipolar reservoir and small when it is mostly caused by MT. This ratio doesn't contain much information on its own, but it is illuminating to compare its values for different pools like MW and IEW, as is plotted in Fig. 7.8 for the duration of the prepulses. Because of MW/IEW exchange effects, 50 ms $<\delta<$ 200 ms is the easiest period to interpret. (The eigenvectors from the four pool model fits show that MW/IEW exchange in these samples operates on a timescale of 100-150 ms, see Table 5.2.) While the MW data were noisy, a general trend emerges across the samples and prepulse amplitudes: $\frac{ihMTR}{MTR}$ is higher in MW than in IEW. In short, this is good evidence that MW's higher ihMT is at least partially due to a distinction between the dipolar reservoir in myelin non-aqueous protons and the dipolar reservoir in non-myelin non-aqueous protons.

The four pool model with dipolar reservoirs, though qualitative, provides a description of ihMT that is also consistent with the dipolar reservoir in myelin being unique. In the model, this uniqueness manifests as $T_{1D,M} > T_{1D,NM}$, which has indeed been the accepted theory for the sensitivity of ihMT to tissues with a high abundance of myelin [119]. However, as



Figure 7.8: The relative contribution of MT to ihMT during the prepulse. The ratio plotted is defined in Eq. 7.12. Higher values indicate ihMTR is from dipolar reservoir properties, not from MT behaviour. After about 200 ms, MW/IEW exchange effects start to become significant, making interpretation difficult. Values of $\delta < 50$ ms are not plotted because in that regime there is little distinction between MTR_{single} and MTR_{dual}.

mentioned, there have been conflicting measurements of T_{1D} in white and grey matter, the latter of which has similar structural and biochemical properties as the NM pool [62]. Some studies report different values [84], and others have measured similar values [127,210]. Taken together with our work, there is obviously a clear need for a careful measurement of T_{1D} in white and grey matter. We explore how this could be accomplished in Chapter 8.

Future studies like the work here could be improved in a number of ways. Firstly, we only varied two parameters: the prepulse duration, δ , and the prepulse peak amplitude, B_1 . Varying the offset frequency, Δ , may allow the lineshapes of the M and NM pool to be determined. More generally, the SNR could be improved upon with more signal averaging. This is particularly important for the small MW pool. FID acquisitions may also be useful in constraining model fitting by providing information about the non-aqueous amplitudes.

Any continuation of this work should also ensure multiple grey matter samples are studied, for the negative ihMTR values at low prepulse times in the single grey matter sample (GMbg) are difficult to interpret. If real, it would suggest that response to single-sided prepulses is completely different in grey matter than in white matter. It is not an artifact of the analysis: in the cases where GM-bg's ihMTR is negative, the CPMG decay curves (not shown) do have a slightly larger amplitude in S_{dual} than in any other experiments. One possibility is the presence of paramagnetic ions (*eg.* iron in blood), which could introduce spectral asymmetries. Still, such an effect should be mitigated by the inclusion of both S_+ and S_- in the ihMTR calculation. Besides, the S_+ and S_- experiments show similar attenuation. In any case, it is a small enough effect to largely ignore for the purposes of this work. And notably, none of the other experiments discussed in this chapter or in Chapter 5 show similar behaviour, so there is little concern of a systematic error.

Chapter 8

Conclusions and future work

8.1 Conclusions

This thesis looked at the fundamental physics of T_1 relaxation and ihMT in brain. T_1 relaxation is a key contrast mechanism which is highly-dependent on myelin, but quantitative studies have so far disagreed on the value, number, and source of T_1 components in white matter. ihMT is a new technique that is sensitive to materials rich in lipid bilayers, like myelin. However, the hypothesis that it requires inhomogeneous spectral broadening is unproven. Also, recent studies with conflicting T_{1D} measurements in white and grey matter have questioned how it is selective to myelin lipids in particular. Together, these factors provided the motivation for this work. More broadly, the research here is part of a larger effort to improve quantitative MRI of myelin.

In Chapter 5 we reported on a suite of solid-state NMR spectroscopy experiments on ex-vivo bovine grey and white matter brain tissue. We separately observed T_1 relaxation of the MW, IEW, and total non-aqueous protons from six unique initial conditions. For the first time, we performed non-equilibrium lineshape analysis on the non-aqueous signal from these samples. These data were fit to a four pool model, and the fit parameters in general matched well with the literature values. Our results also confirmed that MW/IEW exchange only causes minor errors in the accuracy of MWF measurements. We also explored why different initial conditions lead to different relaxation behaviour, showing this explicitly for hard and soft inversion-recovery experiments. In doing so we exemplified how eigenvector analysis could be a useful tool for predicting the relaxation behaviour under different pulse sequences.

Chapter 6 encompassed a close look at the physics of ihMT. We introduced a simple spin-1 model, showing how ihMT arises from the dipolar interaction. Then, an analysis of the

Provotorov Theory model of ihMT showed how WT_{1D} was a key parameter in determining its strength. Our experiments showed how the off-resonance pulses created dipolar order in PL161, tendon, wood, hair—the last two having homogeneously-broadened non-aqueous spectra. None of the samples showed evidence of hole burning, but all exhibited ihMT. T_{1D} measurements were carried out using ADRF/ARRF and saturation-recovery experiments, showing the validity of WT_{1D} as a rough measure of the intensity of ihMT.

The last study in Chapter 7 combined aspects of the four pool model and ihMT. ihMT experiments with CPMG acquisition were used to observe MW and IEW separately in the same bovine brain samples from Chapter 5. A higher ihMT signal from MW was observed. To separate out the relative contribution from MT and ihMT in this larger signal, the ratio IHMTR/MTR was compared for MW and IEW. This new metric showed evidence that the comparatively higher MW ihMT is due to a distinction between the myelin and non-myelin lipids. A qualitative four pool model with dipolar reservoirs suggested that this was due to a difference in T_{1D} s, which matches with earlier explanations. Together, this suggests that recent observations of similar T_{1D} s in grey and white matter need to be carefully examined.

8.2 Future work

The work performed in this thesis has shed new light on T_1 relaxation and ihMT in brain. Inevitably, certain areas of this research could be refined, and it has also raised new questions. Below, we offer some suggestions on how research in this area could proceed.

8.2.1 The non-aqueous lineshape and the effect of soft pulses

When fitting the non-aqueous lineshapes in Chapter 5, we used super-Lorentzians, which are superpositions of Gaussians with orientation-dependent widths. When a low-amplitude, off-resonance pulse is applied, do these Gaussians get saturated individually, or does the entire lineshape get saturated as a whole? Typically, the latter behaviour is assumed when super-Lorentzians are used in qMT studies (*e.g.* see references [79,81,93,171]), but the former seems correct on a fundamental level. We were unable to study this in the *ex-vivo* samples because of the intense aqueous line. Varma *et al.* considered this in one of their models for ihMT [119], but they did not directly observe the non-aqueous protons and could not make any firm conclusions on this point.

The ideal experiment might be to simply use the $PL161/D_2O$ from Chapter 6 with a saturation pulse of variable length and offset followed by a 90° pulse and FID acquisition. In order to remove confounding effects from dipolar order [93], one could saturate at an offset $\pm \Delta$ simultaneous (decoupling the Zeeman and dipolar reservoirs), as in an S_{dual} ihMT prepulse. Experiments on *ex-vivo* brain samples could also be performed. Soaking the sample in D₂O to reduce the intensity of the water line may help to isolate the non-aqueous signal. This would be similar to Wilhelm *et al.*'s recent high-resolution spectra of rat spinal cord in D₂O [89].

It may be worthwhile attempting these experiments in a high-resolution probe. This would require longer 90° pulse lengths, therefore causing more truncation at the start of the FID. Also, adiabatic pulses may be required to completely excite the broad, ~ 20 kHz non-aqueous line. However, these may be worthwhile trade-offs if the resolution is significantly better.

8.2.2 Improved quantification of T_1 relaxation

The six different experiments in Chapter 5 were chosen to establish a diverse set of initial magnetization within the distinct pools of protons in the brain tissue sample. Future studies could build upon this by using other experiments to better separate the two non-aqueous pools (M and NM). We used the assumption of an equal number of protons in M and NM, but this should be a well-constrained free parameter, or if possible, a measured value.

One experiment which may help reveal this is saturation of the non-aqueous protons during the cross-relaxation period [100, 213, 214]. Low-amplitude, continuous rf would be applied at offsets $\pm \Delta$, saturating the non-aqueous protons (the dual offsets are to prevent dipolar order creation). If the rf power was high enough, it may be possible to saturate the nonaqueous protons completely while leaving the aqueous protons relatively unaffected. This would turn the non-aqueous pools into magnetization sinks. The relaxation dynamics would be extremely different, and may help reveal differences in the M and NM pools. It may also more obviously show the contribution from M/MW exchange, which had to be manually emphasized when fitting our data in Chapter 5.

Better separation of the relaxation associated with MW/M exchange $(T_1^* \approx 30 \text{ ms})$ and IEW/NM exchange $(T_1^* \approx 70 \text{ ms})$ is also desirable. One way to do this may be with a double inversion-recovery sequence [215]. In this sequence, one inverts the magnetization and then waits until the MW magnetization passes through zero (~30 ms). Another inversion pulse brings the magnetization into the +z direction. Then, during the cross-relaxation period, it may be easier to view the M/MW exchange behaviour. The inversion pulses could be hard or soft.

Complimentary data could also be collected using two different B_0 field strengths. We

discussed how the intrinsic spin-lattice relaxation time (T_1 for each pool) is a function of the B_0 field strength in Section 2.6. However, all other four pool model parameters—the T_{cr} and $M(\infty)$ values—will remain constant. One could run the same experiments on the same sample in two different spectrometers with different B_0 fields. Then, when fitting the model, one could impose all parameters except the T_1 times to be identical in the two experiment sets. Sample aging, shimming, and variation in T_2 times may be confounding factors with this approach.

8.2.3 T_{1D} measurements in brain

In the introduction to Chapter 7, it was mentioned that recent measurements of grey and white matter T_{1D} s are inconclusive. Some research has suggested that grey and white matter T_{1D} s are the same, whereas other papers have measured very different (~10 ms) values. The outcome of these measurements seems to depend on the technique and sample used. Still, the sensitivity of ihMT to myelin is thought to rely on it having a uniquely-long T_{1D} time. Our results seemed to confirm this, although we did not measure T_{1D} directly—something that future research should focus on.

Measuring T_{1D} is difficult because most techniques are biased towards certain values. For example, the ADRF/ARRF sequences used in Chapter 6 could not measure T_{1D} s less than ~1 ms. It would likely be necessary (and illuminating) to perform the measurements with multiple techniques on both white and grey matter. These could include the saturation method, the ADRF/ARRF sequence, the Jeener-Broekaert sequence, and the ihMT sequence. Regarding the analysis of this type of data, regularized NNLS may help identify distributions in T_{1D} .

Because the presence of the intense water signal makes measurement difficult in tissue samples, a sample soaked in D_2O may prove useful here too. Even though D_2O will likely affect T_{1D} due to the reduced chemical exchange rate, it may still show a relative difference between white and grey matter.

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Appendix A

Derivation of the Provotorov equations

Provotorov first published the theory of saturation under weak rf fields for long times in 1962 [A1]. However, the canonical derivation is in Goldman's book [A2]. We now derive the equations in another way, hinted at in Section 6.7 of Slichter's book [A3]. This follows Schumacher's work on the thermodynamics of coupled generic reservoirs in spin systems [A4]. Our derivation is by no means rigorous, but provides a simple sketch of where the Provotorov Equations come from.

Consider a spin system governed by a total time-independent Hamiltonian

$$\hat{H}_T = \hat{H}_1 + \hat{H}_2$$

where \hat{H}_1 and \hat{H}_2 contain terms from the Zeeman and secular many-spin dipolar interactions. This Hamiltonian does not include terms leading to spin-lattice relaxation. Also, we assume $[\hat{H}_1, \hat{H}_T] = [\hat{H}_2, \hat{H}_T] = 0$, so both \hat{H}_1 and \hat{H}_2 are constants of motion. These terms may be thought of as forming separate thermodynamic reservoirs, each with a unique spin temperature, θ_1 and θ_2 [A3]. Therefore, the high-temperature density matrix is

$$\rho \approx -\frac{1}{\theta_1}\hat{H}_1 - \frac{1}{\theta_2}\hat{H}_2$$

Section 2.5.4 shows how a situation where $\theta_1 \neq \theta_2$ could be realized by working in the rotating frame, where θ_1 and θ_2 correspond to the Zeeman and dipolar reservoirs. For now we don't specify what frame we are working in or what \hat{H}_1 and \hat{H}_2 are.

Our ultimate goal is find $\frac{d}{dt}(\frac{1}{\theta_{1,2}})$. Using the fact that time rate of change for the energy in

a reservoir is $\frac{dE}{dt} = \frac{dE}{d\theta} \frac{d\theta}{dt}$, for any reservoir [A4]

$$\frac{d}{dt}\left(\frac{1}{\theta}\right) = \frac{dE/dt}{-\theta^2 dE/d\theta}.$$
(A.1)

The derivative in the denominator is relatively straightforward. For a generic reservoir with a Hamiltonian \hat{H} and spin temperature θ

$$E = \frac{\operatorname{Tr}\left\{\hat{H}\exp(-\hat{H}/k\theta)\right\}}{\operatorname{Tr}\left\{\exp(-\hat{H}/k\theta)\right\}}$$
$$= \frac{\operatorname{Tr}\left\{\hat{H}(1-\hat{H}/k\theta+\hat{H}^2/(k\theta)^2+\cdots)\right\}}{\operatorname{Tr}\left\{1-\hat{H}/k\theta+\hat{H}^2/(k\theta)^2+\cdots\right\}}$$
$$\approx \frac{-1}{k\theta}\frac{\operatorname{Tr}\left\{\hat{H}^2\right\}}{\operatorname{Tr}\left\{1\right\}}$$
$$= \frac{-1}{kN\theta}\operatorname{Tr}\left\{\hat{H}^2\right\}.$$
(A.2)

Where N is the dimensionality of the Hamiltonian. This uses the high-temperature approximation and the assumption that $\text{Tr}\{\hat{H}\} = 0$, which is true for dipolar and Zeeman Hamiltonians. And so,

$$\frac{dE}{d\theta} = \frac{1}{kN\theta} \operatorname{Tr}\left\{\hat{H}^2\right\}.$$
(A.3)

Finding $dE/d\theta$ is more involved. Assume there are M energy levels in a reservoir (because of the possibility of degeneracy, $M \leq N$). Then,

$$\frac{dE}{d\theta} = \sum_{n=1}^{M} \frac{dp_n}{dt} E_n,\tag{A.4}$$

where p_n is the population of the n^{th} level and E_n is its energy. We use the standard approach to calculate changes in populations: a first-order rate equation [A3,A4]:

$$\frac{dp_n}{dt} = \sum_{m \neq n} \left(W_{m \to n} p_m - W_{n \to m} p_n \right) + \sum_{m \neq n, r \neq s} \left(V_{(m \to n), (s \to r)} p_m q_s - V_{(n \to m), (r \to s)} p_n q_r \right).$$
(A.5)

This is also known as the "master equation". Here, p and q indicate populations of specific levels in reservoirs one and two, and $W_{m\to n}$ is rate of transitions from level m to n in reservoir one. $V_{(m\to n),(s\to r)}$ is the rate of a simultaneous transition in reservoir one from m to n and in reservoir two from s to r.¹

We assume that the reservoirs are coupled via energy-conserving interactions, such as the flip-flop transitions from dipolar coupling. Therefore, $V_{(m \to n),(s \to r)} = V_{(n \to m),(r \to s)}$ and the energy difference in both reservoirs is the same: $\Delta E_{nm} = \Delta E_{rs}$, where *n* and *s* are the higher-energy states. Then,

$$\frac{p_n}{p_m} = \exp\left(-\frac{\Delta E_{mn}}{k\theta_1}\right) \approx 1 - \frac{\Delta E_{mn}}{k\theta_1}$$

$$\frac{q_s}{q_r} = \exp\left(-\frac{\Delta E_{mn}}{k\theta_2}\right) \approx 1 - \frac{\Delta E_{mn}}{k\theta_2}.$$
(A.6)

If we substitute this high-temperature expansion into the second term of Eq. A.5, we get

$$\sum_{m \neq n, r \neq s} V_{(m \to n), (s \to r)} p_m q_r \frac{\Delta E_{mn}}{k} \left(\frac{1}{\theta_1} - \frac{1}{\theta_2} \right)$$

Eq. A.4 tells us that we need to know $\frac{dp_n}{dt}E_n$. Calculating this for the second term in Eq. A.5 yields:

$$\frac{dp_n}{dt}E_n\Big|_{\text{term2}} = \sum_{m \neq n, r \neq s} E_n V_{(m \to n), (s \to r)} p_m q_r \frac{\Delta E_{mn}}{k} \left(\frac{1}{\theta_1} - \frac{1}{\theta_2}\right)$$

$$= \sum_{m \neq n, r \neq s} V_{(m \to n), (s \to r)} p_m q_r \frac{\Delta E_{mn}^2}{2k} \left(\frac{1}{\theta_1} - \frac{1}{\theta_2}\right)$$

$$= \sum_{m \neq n, r \neq s} V_{(m \to n), (s \to r)} \frac{\Delta E_{mn}^2}{2kM_1M_2} \left(\frac{1}{\theta_1} - \frac{1}{\theta_2}\right).$$
(A.7)

Where $M_{1,2}$ specifies the number of energy levels in reservoirs one and two. On line two, we used the fact that $\sum_{m \neq n} E_n \Delta E_{mn} = -\sum_{m \neq n} E_m \Delta E_{mn}$. On line three, we expanded p_m as

$$p_m = \frac{\exp(-E_m/k\theta_1)}{\sum_{j=1}^{M_1} \exp(-E_j/k\theta_1)}$$
$$\approx \frac{1}{\sum_{j=1}^{M_1} 1}$$
$$= \frac{1}{M_1}.$$

These are standard maneuvers when dealing with master equations for NMR relaxation (see

¹There is an error in Schumacher's paper on the last line in equation 4. The term $W'_{nr,ms}p_nq_s$ should read $W'_{nr,ms}p_nq_r$. This term relies upon flip-flop transitions from $n \to m$ in reservoir 1 and $r \to s$ in reservoir 2. The same term is written correctly later in that paper in equation 6.

Section 5.2 in Slichter [A3]).

We can substitute Eqs. A.3, A.5, and A.7 into Eq. A.1, giving [A3]

$$\frac{d}{dt}\left(\frac{1}{\theta_1}\right) = -R_1\left(\frac{1}{\theta_1} - \frac{1}{\theta_{1,L}}\right) - R_{12}\left(\frac{1}{\theta_1} - \frac{1}{\theta_2}\right)$$

$$\frac{d}{dt}\left(\frac{1}{\theta_2}\right) = -R_2\left(\frac{1}{\theta_2} - \frac{1}{\theta_{2,L}}\right) - R_{21}\left(\frac{1}{\theta_2} - \frac{1}{\theta_1}\right)$$
(A.8)

where

$$R_{12} = \frac{\sum_{m \neq n, r \neq s} V_{(m \to n), (s \to r)} \Delta E_{mn}^2}{2 \operatorname{Tr} \{\hat{H}_1^2\} M_1},$$

$$R_{12} = \frac{\sum_{m \neq n, r \neq s} V_{(m \to n), (s \to r)} \Delta E_{mn}^2}{2 \operatorname{Tr} \{\hat{H}_2^2\} M_2},$$

$$R_1 = \frac{\sum_{n \neq m} W_{m \to n} \Delta E_{mn}^2}{2 \operatorname{Tr} \{\hat{H}_1^2\}},$$

$$R_2 = \frac{\sum_{n \neq m} W_{m \to n} \Delta E_{mn}^2}{2 \operatorname{Tr} \{\hat{H}_2^2\}}.$$
(A.9)

We have manually inserted the term $1/\theta_{1,L}$ in the equation above, forcing the system to relax to the lattice temperature. The justification for this is discussed in Slichter [A3] and Slichter & Hebel [A5].

The above treatment has been quite general, but now we shall assume explicit forms for the Hamiltonians. Let

$$\hat{H}_1 = \Delta \sum_j \hat{I}_{z,j}$$

which is a many-spin Zeeman Hamiltonian in a frame rotating at ω , and $\Delta = \omega_0 - \omega$. Also,

$$\hat{H}_2 = \hat{H}_D,$$

a many-spin dipolar coupling Hamiltonian. With this, we can immediately identify [A3–A5]

$$R_1 = \frac{1}{T_1}$$
 and $R_2 = \frac{1}{T_{1D}}$.

Next, R_{12} is tackled. This is a constant, so we may find it from comparison to BPP saturation

theory, which is correct for short times [A3]:

$$R_{12} = W$$
$$= \pi \omega_1^2 g(\Delta),$$

which is defined in Eq. 2.48. We find the last rate

$$R_{21} = R_{12} \frac{\operatorname{Tr}\{\hat{H}_1^2\}M_1}{\operatorname{Tr}\{\hat{H}_2^2\}M_2}$$
$$= W \frac{\operatorname{Tr}\left\{\left(\Delta \sum_j \hat{I}_{z,j}\right)^2\right\}}{\operatorname{Tr}\left\{\hat{H}_D^2\right\}}$$
$$= W \frac{\Delta^2}{\omega_D^2},$$

making use of the fact that $M_1 = M_2$. Here, ω_D is the local field strength, discussed in Section 2.5.

Finally, we have the Provotorov Equations (using "Z" for Zeeman and "D" for dipolar):

$$\frac{d}{dt}\left(\frac{1}{\theta_Z}\right) = -\frac{1}{T_1}\left(\frac{1}{\theta_Z} - \frac{1}{\theta_{L,1}}\right) - W\left(\frac{1}{\theta_Z} - \frac{1}{\theta_D}\right)$$

$$\frac{d}{dt}\left(\frac{1}{\theta_D}\right) = -\frac{1}{T_{1D}}\left(\frac{1}{\theta_D}\right) - W\frac{\Delta^2}{\omega_D^2}\left(\frac{1}{\theta_D} - \frac{1}{\theta_Z}\right).$$
(A.10)

The dipolar reservoir lattice temperature $\theta_{L,2}$ is extremely hot, so that term is dropped. We can put these into a more useful form. The density matrix for this system is

$$\rho = -\frac{\Delta}{\theta_Z} \sum_j \hat{I}_{z,j} - \frac{\omega_D}{\theta_D} \left(\frac{\hat{H}_D}{\omega_D}\right),$$

so we define the Zeeman and dipolar polarizations as (see Section 2.5.6)

$$p_Z = \langle \hat{I}_z \rangle = -\frac{\Delta}{\theta_Z}$$
$$p_D = \left\langle \frac{\hat{H}_D}{\omega_D} \right\rangle = -\frac{\omega_D}{\theta_D}.$$

Substituting these into Eq. A.10 yields the form of the Provotorov equations introduced in Section 2.5.6.

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Appendix B

CPMG exchange correction

B.1 Introduction

Exchange between myelin water (MW) and intra/extra-cellular water (IEW) occurs during CPMG acquisition. This can cause erroneous observed values of their pool sizes and T_2 times. The percent error depends on the exchange rate, but in bovine brain it is typically 10–20% (see Chapter 5 and ref [B1]). This can be corrected for, however; how to do so is the focus of this appendix. This correction was described by Bjarnason *et al.* in ref [B1] and by Bjarnason in ref [B2]. It has been repeated here in a slightly different form for clarity.

In the following, we indicate observed values (found from CPMG acquisition with no correction) by a tilde, and set MW as pool 1 and IEW as pool 2. Our goal is to develop two algorithms:

- 1. Take the actual T_2 times $(T_{2,1} \text{ and } T_{2,2})$ and actual sizes $(M_{0,1} \text{ and } M_{0,2})$ as inputs. Return the observed values $(\tilde{T}_{2,1}, \tilde{T}_{2,2}, \tilde{M}_{0,1}, \tilde{M}_{0,2})$ as outputs. Note that we use this notation for the pool sizes instead of $M(\infty)$ as in the rest of this thesis since $M \to 0$ as $t \to \infty$ in the CPMG.
- 2. Takes the observed values $(\tilde{T}_{2,1}, \tilde{T}_{2,2}, \tilde{M}_{0,1}, \tilde{M}_{0,2})$ as inputs. Return the actual values $(T_{2,1}, T_{2,2}, M_{0,1}, M_{0,2})$ as outputs.

B.2 Equations from a two pool model

To develop these algorithms, the strategy will be to first derive equations linking the actual and observed values, then determine how to convert between the two. Using a vector $\mathbf{M}(t)$ to represent the actual magnetization,

$$\mathbf{M}(t) = \begin{bmatrix} M_1(t) \\ M_2(t) \end{bmatrix},\tag{B.1}$$

the time evolution for these two pools is represented by

$$\frac{d}{dt}\mathbf{M}(t) = \begin{pmatrix} -k_{12} - R_1 & k_{21} \\ k_{12} & -k_{21} - R_2 \end{pmatrix} \mathbf{M}(t)$$

$$= A\mathbf{M}(t)$$
(B.2)

where $R_i = 1/T_{2,i}$ and $R_1 > R_2$ (since pool 1 is MW). As Bjarnason pointed out, we do not have to include the non-aqueous pools; their short T_2 times means they act like transverse magnetization sinks. They return no magnetization to the aqueous pool, so their effects can be incorporated into R_1 and R_2 .

We can simplify matters by noting that total magnetization remains constant before and after the correction, $\sim \sim \sim$

$$M_{tot} = \widetilde{M}_{0,1} + \widetilde{M}_{0,2} \\ = M_{0,2} + M_{0,2}.$$
(B.3)

Using

 $k_{12}M_{0,1} = k_{21}M_{0,2}$

and

$$T_{cr} = k_{12}^{-1} + k_{21}^{-1},$$

we write

$$k_{12} = \frac{M_{tot}}{M_{0,1}T_{cr}}$$
$$k_{21} = \frac{M_{tot}}{M_{0,2}T_{cr}} = \frac{M_{tot}}{(M_{tot} - M_{0,1})T_{cr}}.$$

To solve Eq. B.2, in which the matrix A contains the dynamics, we use the well-known equations for the eigenvalues λ_{\pm} and eigenvectors \mathbf{v}_{\pm} of a 2x2 matrix:

$$\lambda_{\pm} = \frac{T}{2} \pm \sqrt{\frac{T^2}{4} - D} \tag{B.4}$$

$$\mathbf{v}_{\pm} = \begin{pmatrix} \lambda_{\pm} - A_{2,2} \\ A_{2,1} \end{pmatrix}$$
$$= \begin{pmatrix} \lambda_{\pm} + k_{21} + R_2 \\ k_{12} \end{pmatrix}$$
(B.5)

where

$$T = Tr(A)$$

= $A_{1,1} + A_{2,2}$
= $-k_{12} - k_{21} - R_1 - R_2$

and

$$D = \text{Det}(A)$$

= $A_{1,1}A_{2,2} - A_{1,2}A_{2,1}$
= $(k_{12} + R_1)(k_{21} + R_2) - k_{12} - k_{21}$
= $k_{12}R_2 + k_{21}R_1 + R_1R_2$.

After some simplification,

$$\lambda_{\pm} = -\frac{1}{2}(k_{12} + k_{21} + R_1 + R_2) \pm \frac{1}{2}\sqrt{(R_1 - R_2 + k_{12} - k_{21})^2 + 4k_{12}k_{21}}.$$
 (B.6)

Assuming $\tilde{T}_{2,1} < \tilde{T}_{2,2}$, the eigenvalues are related to the observed T_2 times via

$$\widetilde{T}_{2,1} = -\frac{1}{\lambda_{-}}$$

 $\widetilde{T}_{2,2} = -\frac{1}{\lambda_{+}},$
(B.7)

since $|\lambda_-| > |\lambda_+| \text{The formal solution to Eq. B.2}$ is

$$\mathbf{M}(t) = c_{+}\mathbf{v}_{+}e^{\lambda_{+}t} + c_{-}\mathbf{v}_{-}e^{\lambda_{-}t}$$

$$\begin{bmatrix} M_{1}(t) \\ M_{2}(t) \end{bmatrix} = \begin{bmatrix} c_{+}v_{+,1}e^{\lambda_{+}t} + c_{-}v_{-,1}e^{\lambda_{-}t} \\ c_{+}v_{+,2}e^{\lambda_{+}t} + c_{-}v_{-,2}e^{\lambda_{-}t} \end{bmatrix}.$$
(B.8)

Here, $v_{+,1}$ is the first component of the \mathbf{v}_+ eigenvector (and similarly for the other terms), and c_{\pm} are constants defined below. Connecting this equation to the observed pool sizes, the total CPMG signal is

$$M_1(t) + M_2(t) = \{c_+v_{+,1} + c_+v_{+,2}\} e^{\lambda_+ t} + \{c_-v_{-,1} + c_-v_{-,2}\} e^{\lambda_- t},$$

where the prefactors in {} are the observed pool amplitudes:

$$\widetilde{M}_{0,1} = c_+ v_{+,1} + c_+ v_{+,2} \tag{B.9}$$

$$\widetilde{M}_{0,2} = c_{-}v_{-,1} + c_{-}v_{-,2} = M_{tot} - \widetilde{M}_{0,1}.$$
(B.10)

If the actual values are known, the c_{\pm} constants are determined by the t = 0 initial condition:

$$\mathbf{M}(0) = \begin{bmatrix} M_1(\infty) \\ M_2(\infty) \end{bmatrix} = \begin{bmatrix} M_{0,1} \\ M_{tot} - M_{0,1} \end{bmatrix} = c_+ \mathbf{v}_+ + c_- \mathbf{v}_-$$
$$= V \begin{bmatrix} c_+ \\ c_- \end{bmatrix},$$
(B.11)

where V is a matrix whose columns are \mathbf{v}_+ and \mathbf{v}_- . This leads to

$$c_{+} = \frac{1}{\text{Det}(V)} \left(V_{2,2}M_{0,1} + V_{1,2}(M_{tot} - M_{0,1}) \right)$$

$$c_{-} = \frac{1}{\text{Det}(V)} \left(-V_{2,1}M_{0,1} + V_{1,1}(M_{tot} - M_{0,1}) \right).$$
(B.12)

With that, we have derived the necessary equations. Now we will see how they are applied.

B.3 Algorithm 1: Actual to observed values

Given the actual T_2 times $(T_{2,1} \text{ and } T_{2,2})$ and pool sizes $(M_{0,1} \text{ and } M_{0,2})$, along with a T_{cr} time, Eqs. B.6 and B.7 give the observed T_2 times $\tilde{T}_{2,1}$ and $\tilde{T}_{2,2}$. The observed pool sizes, $\tilde{M}_{0,1}$ and $\tilde{M}_{0,2}$, are found using Eqs. B.9 and B.10.

B.4 Algorithm 2: Observed to actual values

Going the other way (finding the actual T_2 values and pool sizes from the observed values) is less straightforward. The observed values $(\tilde{T}_{2,1}, \tilde{T}_{2,2}, \tilde{M}_{0,1}, \text{ and } \tilde{M}_{0,2})$ are typically taken from a CPMG acquisition when the system starts in equilibrium. The first step is to find the R_1 and R_2 values (the inverse of the actual T_2 times). Using Eq. B.6,

$$\lambda_{+} + \lambda_{-} = -(k_{12} + k_{21} + R_1 + R_2)$$

$$\implies R_1 = -(k_{12} + k_{21} + R_2 + \lambda_{+} + \lambda_{-})$$
(B.13)

and

$$(\lambda_{+} - \lambda_{-})^{2} = (k_{12} - k_{21} + R_{1} - R_{2})^{2} + 4k_{12}k_{21}$$

$$\implies R_{2} = -\frac{1}{2}\sqrt{(\lambda_{+} - \lambda_{-})^{2} - 4k_{12}k_{21}} - k_{21} - \frac{1}{2}(\lambda_{+} + \lambda_{-}).$$
(B.14)

Note the choice of the negative root: $\sqrt{(k_{12} - k_{21} + R_1 - R_2)^2} = -(k_{12} - k_{21} + R_1 - R_2)$. This ensures that when $T_{cr} \to \infty$ (causing $k_{12} \to 0, k_{21} \to 0, \lambda_+ \to -R_2$, and $\lambda_- \to -R_1$), then the RHS of Eq. B.14 correctly becomes $-\frac{1}{2}((-R_2) - (-R_1)) - \frac{1}{2}(-R_2) - \frac{1}{2}(-R_1) = R_2$.

Now, T_{cr} and $M_{0,1}$ are the only remaining unknown parameters ($M_{0,2}$ is found via Eq. B.3). In the case of fitting the four pool model (Chapter 5) T_{cr} is a fit parameter. At each step in the solver iteration, T_{cr} will have some trial value, so $M_{0,1}$ is found by solving the transcendental equation given in Eq. B.9. We found that a bracketed root finder like SciPy's implementation of the quadratic Brent algorithm [B3] worked well.

[B1] Bjarnason, T., Vavasour, I., Chia, C. & MacKay, A. Characterization of the NMR behavior of white matter in bovine brain. *Magnetic Resonance in Medicine* 54, 1072–1081 (2005). URL https://doi.org/10.1002/mrm.20680

[B2] Bjarnason, T. The Effect of Cross Relaxation on the NMR Behaviour of Bovine White Matter. UBC Master's thesis (2005). URL http://hdl.handle.net/2429/16429

[B3] Brent, R. P. Algorithms for Minimization Without Derivatives. Englewood Cliffs, NJ: Prentice-Hall, (1973). Ch. 3-4.

Appendix C

Circuit analogies in NMR relaxation

C.1 Introduction

Certain relaxation problems have one-to-one correspondence with electric circuits. These amusing circuit analogies offer no new physics, but they do provide an interesting way of looking at certain problems which may be more intuitive. Circuit analogies have been used before by Bloch to describe the Nuclear Overhauser Effect [C1]. Here, we apply them to the four pool model and Provotorov equations.



Figure C.1: The equivalent circuit of the four pool model.

C.2 Four pool model

The four pool model equations are

$$\begin{aligned} \frac{dM_{\rm M}}{dt} &= -\frac{M_{\rm M} - M_{\rm M}(\infty)}{T_{1,\rm M}} - k_{\rm M,\rm MW}M_{\rm M} + k_{\rm MW,\rm M}M_{\rm MW} \\ \frac{dM_{\rm MW}}{dt} &= -\frac{M_{\rm MW} - M_{\rm MW}(\infty)}{T_{1,\rm MW}} + k_{\rm M,\rm MW}M_{\rm M} - k_{\rm MW,\rm M}M_{\rm MW} - k_{\rm MW,\rm M}M_{\rm MW} - k_{\rm MW,\rm MW}M_{\rm HW} \\ \frac{dM_{\rm IEW}}{dt} &= -\frac{M_{\rm IEW} - M_{\rm IEW}(\infty)}{T_{1,\rm IEW}} + k_{\rm MW,\rm IEW}M_{\rm MW} - k_{\rm IEW,\rm MW}M_{\rm IEW} - k_{\rm IEW,\rm MW}M_{\rm IEW} - k_{\rm IEW,\rm MW}M_{\rm IEW} + k_{\rm NM,\rm IEW}M_{\rm MM} \\ \frac{dM_{\rm NM}}{dt} &= -\frac{M_{\rm NM} - M_{\rm NM}(\infty)}{T_{1,\rm NM}} + k_{\rm IEW,\rm NM}M_{\rm IEW} - k_{\rm IEW}M_{\rm NM}. \end{aligned}$$

The circuit in Fig. C.1 is described by equivalent equations. Consider the node where C_1 , R_1 , and R_{12} meet. The current flowing into the node from the capacitor is dQ_1/dt , where Q_1 is the charge on the capacitor. Kirchoff's node rule gives

$$\begin{aligned} \frac{dQ_1}{dt} &= \frac{V - Q_1/C_1}{R_1} + \frac{Q_2/C_2 - Q_1/C_1}{R_{12}} \\ &= \frac{-(Q_1 - C_1V)}{R_1C_1} - \frac{Q_1}{C_1R_{12}} + \frac{Q_2}{C_2C_{12}} \\ &= -(C_1R_{12})^{-1}Q_1 - \frac{(Q_1 - C_1V)}{R_1C_1} + (C_2R_{12})^{-1}Q_2 \end{aligned}$$

Comparing this to the expression for $dM_{\rm M}/dt$ above, we make the connections

$$Q_1 = M_{\rm M}$$

 $(C_1 R_{12})^{-1} = k_{\rm M,MW}$
 $(C_2 R_{12})^{-1} = k_{\rm MW,M}$
 $M_{\rm M}(\infty) = C_1 V$
 $T_{1,{\rm M}} = R_1 C_1.$

Similar expressions will apply for the other pools.

In this analogy, magnetization is charge, and our goal is to quantify all of the component values. We do this by putting different initial charges on the capacitors, and then observing how it returns to equilibrium.



Figure C.2: The equivalent circuit of the Provotorov equations with $\Omega = 1$.

C.3 Provotorov equations

The Provotorov equations also lend themselves to an analogous circuit, shown in Fig. C.2A and B. In this case, only with $\Omega = 1$ does the circuit provide an easy analogy. The Provotorov Equations (Eq. 2.47) for the Zeeman and dipolar magnetizations M_Z and M_D are then

$$\frac{dM_Z}{dt} = -\frac{(M_Z - M_Z(\infty))}{T_1} - WM_Z + WM_D$$

$$\frac{dM_D}{dt} = -\frac{M_D}{T_{1D}} - WM_D + WM_Z.$$
(C.1)

Panel A shows the case for single-sided irradiation. Here, we have for the charge across each capacitor (following the analysis from the previous section)

$$\frac{dQ_Z}{dt} = -\frac{(Q_Z - VC_Z)}{C_Z R_Z} + (C_Z R_{DZ})^{-1} Q_D - (C_Z R_Z)^{-1} Q_Z$$

$$\frac{dQ_D}{dt} = -\frac{Q_D}{C_D R_D} - (C_D R_{DZ})^{-1} Q_D + (C_Z R_Z)^{-1} Q_Z.$$
(C.2)

The D and Z subscripts refer to dipolar and Zeeman reservoirs respectively. Comparing Eqs. C.1 and C.2, we can make the connections:

$$VC_Z = M_Z(\infty)$$
$$Q_{Z,D} = M_{Z,D}$$
$$C_Z R_Z = T_1$$
$$C_D R_D = T_{1D}$$
$$(C_D R_{DZ})^{-1} = (C_Z R_{DZ})^{-1} = W.$$

In ihMT, this would be the case for a single off-resonance prepulse (an S_+ or S_- prepulse). In panel B, the equivalent circuit behaves like the Zeeman and dipolar reservoir for an S_{dual} prepulse: they are uncoupled.

This analogy makes it clear why WT_{1D} is a key parameter for ihMT. This is

$$WT_{1D} = C_D R_D (C_D R_{DZ})^{-1}$$
$$= R_D / R_{DZ}$$

If $R_{DZ} \to \infty$ (W = 0), there is obviously no ihMT. If $R_D \approx 0$ ($T_{1D} \approx 0$), then the discharge of C_Z behaves the same in both cases, and there is also no ihMT. In the intermediate case, if $R_D \sim R_{DZ}$, then the discharge of C_Z will behave differently in both cases, and there is ihMT.

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Appendix D

Model of ihMT using pulse-train prepulses

The pulse-train variety of ihMT experiments use prepulses consisting of trains of shaped pulses. Typically, in the S_+ or S_- experiments, these shaped pulses are all at the offset $+\Delta$ or $-\Delta$, respectively. In the S_{dual} experiment, these shaped pulses alternate between $+\Delta$ and $-\Delta$. In the following, we show a simple model of the behavior of a many-spin system under these prepulses. As with the model of CW prepulses presented above, we ignore magnetization transfer to aqueous protons.

Fig. D.1 shows our simple model of pulse-train prepulses. We make the assumption of rectangular pulses so that while the RF is on the Provotorov equations in Eqs. 6.16 and 6.19 apply. As discussed earlier in Chapter 7, we can add an extra dimension to eliminate the inhomogeneous term from T_1 relaxation, leading to

$$\frac{d\boldsymbol{\rho}_{\pm}}{dt} = W \begin{pmatrix} -1 - \frac{1}{WT_1} & \Omega & \frac{\langle I_z \rangle_0}{T_1} \\ \Omega & -\Omega^2 - \frac{1}{WT_{1D}} & 0 \\ 0 & 0 & 0 \end{pmatrix} \boldsymbol{\rho}_{\pm}$$

$$= A \boldsymbol{\rho}_{\pm}.$$
(D.1)

where A is the coefficient matrix. The solution to this equation for an arbitrary initial condition vector $\boldsymbol{\rho}(0) = (\rho_1, \rho_2, 1)$ and an irradiation length τ_1 is [154]

$$\rho_{+}(t) = P_{+}\rho_{+}(0)$$

$$P_{+} = F(\tau_{1})F^{-1}(0),$$
(D.2)



Figure D.1: A model of ihMT prepulses of the pulse-train variety. With the assumption of square pulses, the sequence can be modeled as a product of matrices. An actual ihMT experiment consists of many cycles ($n \sim 100-1000$).

where the matrix $F(\tau_1)$ has columns composed of

$$\mathbf{F}(\tau_1) = \begin{bmatrix} \mathbf{v}_1 e^{\lambda_1 \tau_1} & \mathbf{v}_2 e^{\lambda_2 \tau_1} & \mathbf{v}_3 e^{\lambda_3 \tau_1} \end{bmatrix}.$$
 (D.3)

Here, $\mathbf{v}_{1,2,3}$ are the eigenvectors of A and $\lambda_{1,2,3}$ are the eigenvalues, given by Eq. 6.21. The matrix for irradiation at $-\Delta$ is $P_{-} = P_{+}(\Delta \rightarrow -\Delta)$. During the delay τ_{2} between pulses, a matrix R describes the dipolar relaxation:

$$\mathbf{R} = \begin{pmatrix} -\frac{1}{T_1} & 0 & \frac{\langle I_z \rangle_0}{T_1} \\ 0 & -\frac{1}{T_{1D}} & 0 \\ 0 & 0 & 0 \end{pmatrix}.$$
 (D.4)

As shown in Fig. D.1, we can now represent a pulse-train prepulse sequence of n cycles by products of matrices. The Zeeman magnetization at the end of the pulse train is

$$\langle I_z \rangle_+(n) = ((\mathbf{P}_+ \mathbf{R} \, \mathbf{P}_+ \mathbf{R} \, \boldsymbol{\rho_0})^n)_1$$

$$\langle I_z \rangle_-(n) = ((\mathbf{P}_- \mathbf{R} \, \mathbf{P}_- \mathbf{R} \, \boldsymbol{\rho_0})^n)_1$$

$$\langle I_z \rangle_{\text{dual},1}(n) = ((\mathbf{P}_+ \mathbf{R} \, \mathbf{P}_- \mathbf{R} \, \boldsymbol{\rho_0})^n)_1$$

$$\langle I_z \rangle_{\text{dual},2}(n) = ((\mathbf{P}_- \mathbf{R} \, \mathbf{P}_+ \mathbf{R} \, \boldsymbol{\rho_0})^n)_1,$$

(D.5)

where the subscript 1 indicates the value of the first (Zeeman) component of the vector. Finally,

Non-aqueous ihMTR =
$$\frac{\langle I_z \rangle_+ + \langle I_z \rangle_- - \langle I_z \rangle_{\text{dual},1} - \langle I_z \rangle_{\text{dual},2}}{2 \langle I_z \rangle_0}.$$
 (D.6)

Although we do not consider this here, some recent studies with pulse-train prepulses also

used dual irradiation during τ_1 to measure and increase sensitivity to T_{1D} [127, 133]. This could be included through the equation

$$\frac{d\boldsymbol{\rho}_{\text{dual}}}{dt} = W \begin{pmatrix} -1 - \frac{1}{WT_1} & 0 & \frac{\langle I_z \rangle_0}{T_1} \\ 0 & -\Omega^2 - \frac{1}{WT_{1D}} & 0 \\ 0 & 0 & 0 \end{pmatrix} \boldsymbol{\rho}_{\text{dual}}.$$
 (D.7)

This approach can also be used to model shaped pulses by discretizing them as rectangular pulses. Also, coupling to the aqueous protons could be included as well. Here we only consider the effect of rectangular pulses on isolated non-aqueous protons..

Fig. D.2 shows the results of simulations using this pulse-train model. Unless otherwise stated, the following parameters were used: $\Delta = \omega_D/2\pi = 10$ kHz, $T_1 = 1$ s, $\tau_1 = \tau_2 = 3$ ms, n = 41, and $g(2\pi\Delta)$ as a Gaussian with standard deviation of ω_D . Fig. D.2A replicates Fig. 6.2A. The plot is discretized because the prepulse time increases in steps of $2(\tau_1 + \tau_2)$, which is the time for one cycle. Because there is a delay of time τ_2 between pulses in the pulse train, the effective transition rate W_{eff} is

$$W_{\text{eff}} = W \frac{\tau_1}{\tau_1 + \tau_2}.\tag{D.8}$$

The relative rates term for the pulse-train model is $W_{\text{eff}}T_{1D}$, and for the equivalent value of WT_{1D} in the CW model, the ihMT behavior is nearly identical.

Fig. D.2B replicates Fig. 6.2B using the pulse-train model. Again, very similar behavior is seen. This plot also shows the importance of inter-pulse period τ_2 . The non-aqueous ihMTR will have a multiplicative term of $\exp(-\tau_2/T_{1D})^n$, so the ihMTR is suppressed unless $\tau_2 \gtrsim T_{1D}$.

Finally, Fig. D.2C plots the offset frequency dependence, as in Fig. 6.2C for the CW model. A similar trend is seen, albeit with a smaller effect, at shorter overall pulse lengths. This is likely because the effective irradiation time is actually 25/2=12.5 ms, which is too short to generate a large ihMT effect.



Figure D.2: Simulation of non-aqueous ihMT in non-aqueous spin system using pulse-train prepulses. Coupling to aqueous protons has not be included. (A) shows the dependence on prepulse duration, which is discretized to fit in an integer number of cycles. This shows similar behavior as in the CW model (see Fig. 6.2A). (B) shows the dependence on the effective relative rates term $W_{\rm eff}T_{1D}$. Again, the behavior is very similar to the CW model in Fig. 6.2B. Finally, (C) shows the offset-dependence, similar to Fig. 6.2C. Because the duty cycle is only 50%, when $\tau = 25$ ms, the ihMT repsonse is significantly smaller than in the CW case. Unless otherwise indicated, in all plots $\Delta = \omega_D/2\pi = 10$ kHz, $T_1 = 1$ s, $\tau_1 = \tau_2 = 3$ ms, n = 41, and $g(2\pi\Delta)$ as a Gaussian with standard deviation of ω_D .