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**Developing high-field electron paramagnetic
resonance for studies of non-equilibrium dynamics**

A dissertation submitted in partial satisfaction
of the requirements for the degree

Doctor of Philosophy
in
Physics

by

Brad D. Price

Committee in charge:

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January 2025

Developing high-field electron paramagnetic resonance for studies of non-equilibrium
dynamics

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by

Brad D. Price

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Brad D. Price

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B. D. Price, A. Sojka, S. Maity, I. M. Chavez, M. Starck, M. Z. Wilson, S. Han, and M. S. Sherwin, “Field-domain rapid-scan EPR at 240 GHz for studies of protein functional dynamics at room temperature”, *Journal of Magnetic Resonance* **366**, 107744 (2024).

- B. D. Price, A. Sojka, N. I. Agladze, and M. S. Sherwin, “Compact module for complementary-channel THz pulse slicing”, *Applied Physics Letters* **124**, 021107 (2024).
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- S. Maity, B. D. Price, C. B. Wilson, A. Mukherjee, M. Starck, D. Parker, M. Z. Wilson, J. E. Lovett, S. Han, and M. S. Sherwin, “Triggered Functional Dynamics of AsLOV2 by Time-Resolved Electron Paramagnetic Resonance at High Magnetic Fields”, *Angewandte Chemie* **135**, e202212832 (2023).
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- B. D. Price, S. N. Lowry, I. D. Hartley, and M. Reid, “Subterahertz refractive flat-top beam shaping via 3D printed aspheric lens combination”, *Applied Optics* **59**, 5429–5436 (2020).

Patents

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Abstract

Developing high-field electron paramagnetic resonance for studies of non-equilibrium
dynamics

by

Brad D. Price

Life, and the world around it, is a collection of non-equilibrium dynamical systems: metabolism, protein function, gasoline engines, transistor operation, and even spin dynamics are examples of common non-equilibrium phenomena that govern day-to-day life. Therefore, a precise understanding of the world requires experimental techniques for probing these dynamics. Specifically, understanding biological life requires understanding the fundamental underpinnings of it: protein function and protein dynamics. Similarly, understanding the behavior of condensed matter systems requires understanding the dynamics of their spins when driven out of equilibrium.

Protein function is inherently non-equilibrium: energy is required to drive proteins through a set of conformational states that perform essential biological roles to enable life. There are a large collection of tools for studying and understanding the function of proteins, including cryo-electron microscopy, X-ray diffraction, infrared (IR) spectroscopy, Förster resonance energy transfer (FRET), and even magnetic resonance (MR). However, the majority of these techniques are limited to static measurements, meaning they are not capable of capturing dynamical protein function in a lifelike environment, and necessarily require flash-freezing or crystallization to achieve sufficient signal-to-noise. The others, that *are* capable of time-resolved, solution-state measurements (IR, FRET, MR), have their own limitations. IR spectroscopy, for example, is sensitive only to secondary (short-

range) structure of proteins; FRET is highly sensitive to tertiary (long-range) structure, but is sensitive only to mean distances and is unable to resolve distance distributions; and MR, in its conventional form, is a relatively slow technique that is capable of capturing distance distributions on minutes-to-hours timescales, which is significantly longer than the majority of dynamical protein responses.

Conversely, there are well-developed tools for studying non-equilibrium solid-state systems, including magnetic field-resolved heat capacity measurements, optical spectroscopy, neutron scattering, SQUID magnetometry, quantum sensing using optically-addressable spin centers, magneto-transport, and magnetic resonance. To study the *dynamic* non-equilibrium spins of spintronics and quantum magnetism, for example, a subset of these tools that are sensitive to non-linear dynamics are required, including pulsed optical spectroscopy or pulsed electron magnetic resonance spectroscopy.

This dissertation focuses on developing electron paramagnetic resonance (EPR), specifically at high fields and frequencies, to address fundamental questions pertaining to non-equilibrium biology, biophysics, and condensed matter physics. More specifically, the second chapter presents the development of a high-field EPR technique (time-resolved Gd-Gd EPR, called “TiGGER”) capable of “filming” protein dynamics in real-time and in solution state, a capability that is rare among current techniques. This technique was used to determine the mechanical refolding time of a light-activated phototropin from *Avena sativa* (AsLOV2). The technique is pushed further, toward resolving real-time spectroscopic changes using rapid-scan EPR, to resolve the spectral changes as a function of time during AsLOV2’s photocycle. Rapid-scan TiGGER is used to determine the effect of mutations on AsLOV2’s refolding, including measuring mutation-influenced refolding lifetimes, and was also used to measure the activation energy of the refolding process. Further, the time-and-field-resolved spectra were used to extract time-dependent

site-to-site distance distributions at room temperature in solution.

Though it is not required for solution-state protein dynamics studies, UCSB hosts a unique source for EPR spectroscopy: the free electron laser (FEL). This laser is capable of kilowatt power, microsecond long, narrowband pulses (500 kHz linewidth at 240 GHz) in the GHz-THz regime. Without a free electron laser (or gyrotron), it is notoriously difficult to generate such high powers in this range of the electromagnetic spectrum (known as the “terahertz gap”). The properties of the FEL make it particularly enticing as an electron magnetic resonance source, as it can provide access to a wide range of spin systems, including those with large zero field splittings due to its frequency agility, and short-lived excitations as a result of its exceptionally high power.

A pulsed FEL-EPR experiment aimed at determining the lifetime of low-lying magnetic excitations (called “spinons”) in CoNb_2O_6 is presented in the third chapter. CoNb_2O_6 is an experimental realization of the transverse field Ising chain, a common pedagogical tool for teaching many-body physics. It serves as an exciting playground for studying well-known, yet difficult-to-observe collective magnetic excitations. However, the current implementation of 240 GHz FEL-EPR was unable to unambiguously resolve these collective excitations or determine their lifetime, primarily due to the spectrometer’s limited sensitivity and tens-of-nanoseconds “dead-time” (*i.e.*, the amount of time required after a pulse sequence before a signal can be detected). These experiments motivated the development of the next generation of FEL-ESR spectroscopy (electron **spin** resonance rather than electron **paramagnetic** resonance, in order to include its application to non-paramagnetic spin systems), which required focused experimental redesign and development.

The necessary design modifications and upgrades, including those aimed at improving spectrometer dead-time, signal-to-noise, and the breadth of accessible experiments,

are discussed in the final sections of this dissertation. Key developments include: the design of a quasi-optical circuit that supports multi-pulse free electron laser experiments, pulsed low-power operation with arbitrary pulse sequences, or some combination of these two sources for novel pump-probe sequences; the implementation of novel pulse-slicer and phase-shifter modules; and the creation of a quasi-optical sample holder that greatly improves signal-to-noise and reduces dead-time. These advancements enable the next generation of high-power pulsed ESR and, hopefully, the precise characterization of excited state lifetimes of non-equilibrium dynamical spin systems.

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Chapter 1

Introduction

Electron paramagnetic resonance (EPR) is a spectroscopic technique that takes advantage of the Zeeman effect [1]. Pioneered in 1944 by Zavoisky [2], EPR leverages an applied magnetic field to probe local and long-range interactions between electrons and their environments. At the most fundamental level, an applied magnetic field splits the energies of spin quantum numbers of unpaired spins. For a free spin-1/2,

$$\hat{\mathcal{H}} = -\boldsymbol{\mu} \cdot \mathbf{B}, \quad (1.1)$$

where $\boldsymbol{\mu}$ represents the magnetic moment of the spin and $\mathbf{B}$ represents the applied magnetic field. The magnetic moment can be written $\boldsymbol{\mu} = \gamma \hat{\mathbf{S}}$, where γ is the gyromagnetic ratio of the spin and $\hat{\mathbf{S}}$ is the spin operator. For spin-1/2, the two energy eigenstates are spin-“up” and spin-“down” along the axis of the applied field, $\mathbf{B}$. Without loss of generality, if $\mathbf{B}$ is along $\hat{z}$, the spin is therefore quantized along the $\hat{z}$ axis, and

$$\hat{\mathcal{H}} = -\gamma \hat{S}_z B_z = -\frac{\gamma \hbar B_z}{2} \sigma_z, \quad (1.2)$$

where σ_z is the Pauli spin matrix and $\hbar$ is the reduced Planck constant. $\hat{H}$ has eigenvectors $|\Psi\rangle = (1, 0)^T, (0, 1)^T$ and eigenvalues $\lambda_{\pm} = \mp \gamma \hbar B_z / 2$, which are split linearly with

the strength of the applied field. Therefore, at some field value $B_z = B_0$, the splitting between the “up” and “down” energy levels is $E = \gamma\hbar B_0$. If the sample is irradiated by an electromagnetic wave of $\omega = E/\hbar$, a spin flip can be induced from the energetically favorable state to an excited one, permitting precise resolution of electronic spin energy levels, forming the basis of EPR spectroscopy.

Though EPR was pioneered 80 years ago, its development was delayed compared to its magnetic resonance cousin, nuclear magnetic resonance (NMR), which exploded in popularity since its advent in 1938 [3]. This can be explained by the relative difficulty to create the energy separation to excite a spin state: the gyromagnetic ratio of a proton is 267.5×10^6 rad/sT, and for an electron, it is -1.76×10^{11} rad/sT, which (ignoring the negative sign) is roughly 658 times larger. While NMR can be done with radio frequency (RF) coils at MHz frequencies, for the same field, EPR must be done with microwave sources in the GHz range. This GHz range bumps up against the “terahertz gap”, a region of the electromagnetic spectrum that is notoriously difficult to produce, as it sits between the easier-to-generate regimes of electronic and optical radiation [4]. Recent developments in superconducting field-swept magnets and GHz sources has enabled high field EPR and made it of great scientific interest [5], as its impressive sensitivity can inform a spectroscopist about an electron’s local environment and even longer-range (nanometer-scale) couplings within a sample. However, the “paramagnetic” part of EPR is crucial; unpaired electron spins must exist within a sample of interest, something that does not occur frequently in nature, especially in biological samples.

The rest of the Introduction discusses EPR fundamentals, including the “full” electron spin Hamiltonian, a simple block diagram of a typical high field EPR spectrometer, and high field EPR methodologies, including the two most common techniques, continuous wave (CW) and pulsed, as well as a burgeoning one, called rapid-scan. The

end of the chapter includes a brief discussion on advanced EPR techniques, including double-resonance and two-frequency techniques, such as DEER and ELDOR, as well as combining EPR and NMR for dynamic nuclear polarization (DNP).

Chapters 2 and 3 of this dissertation are made up of a collection of magnetic resonance research projects I have had the chance to contribute to over the past 5 years. Chapter 2 discusses time-resolved Gd-Gd EPR (TiGGER) as was first imagined by the Sherwin Group. TiGGER relies on the dipolar interaction between two gadolinium spins at high magnetic field to detect changes in intra-protein distances, with a long term goal of using this technique to track (and therefore, better understand) protein functional dynamics.

An extension of TiGGER to rapid-scan EPR is discussed in sec. 2.3, where the entire field-swept spectral lineshape is preserved, which provides a second dimension to the transient spectra of sec. 2.2. This technique enables long-time, phase-independent signal averaging which improves signal-to-noise while significantly reducing acquisition time. Further, the spectral lineshapes contain, in principle, all the information necessary to extract time-resolved spin-spin distance distributions, and preliminary results of lineshape fitting to do this extraction are presented.

Chapter 3 begins with an experiment on a transverse field Ising chain candidate, CoNb_2O_6 , using ITST's 240 GHz FEL-EPR spectrometer. FEL-EPR is powered by UCSB's free electron laser (FEL), which is capable of providing quasi-continuous wave beams at kilowatt powers in the "terahertz gap", a region of the electromagnetic spectrum that is notoriously difficult to access, especially at such high powers. The CoNb_2O_6 study attempted to probe the lifetime of short-lived fractionalized spin excitations (called "spinons") of the ordered 1D ferromagnet. However, it was not possible to directly measure the lifetime of spinons with pulsed FEL-EPR for a variety of reasons, including experimental limitations of possible pulse sequences, imprecise phase control of each pulse,

and a 70 ns experimental “dead-time”. Though a “spinon echo” could not be observed with the current capabilities of FEL-EPR, the experimental dead-time represented an upper bound on the spinons’ coherence time.

This experiment motivated many of the spectrometer upgrades that will (potentially) enable direct, echo-detected measurements of spinon lifetimes. Such upgrades include significant modification and re-design to the system’s

1. quasi-optics, enabling novel pump-probe experiments (sec. 3.4);
2. pulse slicer, enabling three-pulse FEL pulse sequences (sec. 3.5.3);
3. phase shifters, for signal-to-noise enhancement and coherence pathway selection (sec. 3.6.3);
4. sample holder, greatly improving signal-to-noise (sec. 3.7).

Each upgrade was individually designed and tested using existing 240 GHz EPR capabilities to verify their functionality. In combination, they permit three-pulse free electron laser experiments with arbitrary pulse length and phase control, with a reduction in dead-time and an improvement in signal-to-noise. Once fully implemented, the improvements may provide access to the dynamics of the relatively short-lived, low-lying excitations of ordered magnetic states in condensed matter systems such as CoNb_2O_6 .

In total, this dissertation presents a collection of projects from the past five years that span a broad range of disciplines from biochemistry and biophysics into engineering and condensed matter physics.

1.1 EPR Fundamentals

1.1.1 The electron spin Hamiltonian

For the purposes of EPR, the typical Hamiltonian of interest is as follows:

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_Z + \hat{\mathcal{H}}_{ZFS} + \hat{\mathcal{H}}_{dd} + \hat{\mathcal{H}}_{Ex} + \hat{\mathcal{H}}_N, \quad (1.3)$$

which, like all Hamiltonians used in physics, is a simplification of complicated physical phenomena, not designed to describe all interactions in perfect detail, but to provide a language and model that one can gain understanding from. This Hamiltonian is the *electron* spin Hamiltonian, meaning that it only includes terms that influence the energy levels of the electrons in a paramagnetic system. There are nuclear analogs for each term, but they rarely contribute any absorptions at EPR frequencies and are therefore omitted from this discussion. In order, the terms of $\hat{\mathcal{H}}$ are as follows: $\hat{\mathcal{H}}_Z$ is the Zeeman Hamiltonian, and the only one that applies for a free spin-1/2 in an applied magnetic field; $\hat{\mathcal{H}}_{ZFS}$ characterizes the “zero-field splitting” of a high spin ($S > 1/2$) system; $\hat{\mathcal{H}}_{dd}$ is the dipolar coupling term that represents the through-space interaction between two (or more) unpaired spins; $\hat{\mathcal{H}}_{ex}$ represents the exchange interaction between coupled spins—this requires their wavefunctions to be coupled and thus, only applies at very short distances; and $\hat{\mathcal{H}}_N$ represents the “hyperfine” interaction, a through-space coupling that is the nuclear analog of $\hat{\mathcal{H}}_{dd}$.

$\hat{\mathcal{H}}_Z$: Zeeman splitting

Zeeman splitting is the basis of eqn. (1.1), magnetic moments of opposite sign have energy levels of opposite sign when a magnetic field is applied.

$$\hat{\mathcal{H}} = -\hat{\boldsymbol{\mu}} \cdot \mathbf{B} \quad (1.4)$$

In the general case, for an electron, $\hat{\boldsymbol{\mu}} = -g\mu_B\hat{\mathbf{J}}$, where g is the g -factor for the electron (more on this later), μ_B is the Bohr magneton [6], and $\hat{\mathbf{J}}$ is the total angular momentum of the electron of interest. The free electron g , g_e is known to extreme accuracy [7],

$$g_e = 2.00231930436118(26), \quad (1.5)$$

but because electrons are never “free”, the electrons measured in EPR are parametrized by some g that is different from g_e . Further still, magnetic resonance adopts an effective g , g_{eff} , to treat local distortions in the applied magnetic field.

$$\hat{\mathcal{H}} = -\hat{\boldsymbol{\mu}} \cdot \mathbf{B}_{eff} \quad (1.6)$$

$$= -\hat{\boldsymbol{\mu}} \cdot (\mathbf{B}_0 + \mathbf{B}_{local}) \quad (1.7)$$

$$= -g\mu_B\hat{\mathbf{J}} \cdot (\mathbf{B}_0 + \mathbf{B}_{local}) \quad (1.8)$$

$$= -g_{eff}\mu_B\hat{\mathbf{J}} \cdot \mathbf{B}_0. \quad (1.9)$$

Here, $g_{eff} = g(1 - \sigma)$, where σ is small and analogous to the NMR chemical shift, σ_n . A diagram of the eigenvalues for such a system (assuming $\hat{\mathbf{J}} = \hat{\mathbf{S}}$) is shown in Fig. 1.1.

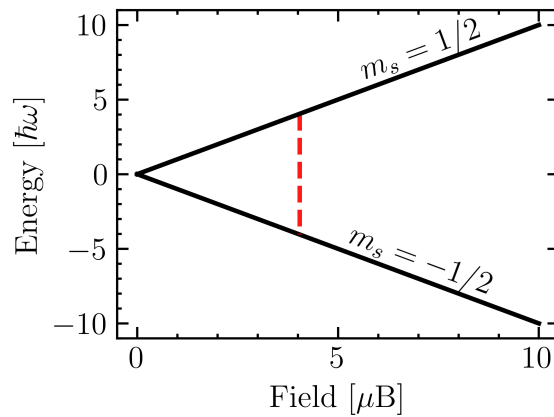


Figure 1.1: Cartoon demonstrating field-dependence of $\hat{\mathcal{H}}_Z$ eigenvalues (shown in black) for $S = 1/2$, $g = 2$ in an applied magnetic field (see eqn. (1.2)). Dashed red vertical lines represent allowed ($\Delta m_s = 1$) transitions at a microwave energy of $8\hbar\omega$.

Now, g is often not reported as an isotropic scalar, but as a rank-3 tensor. This is due to the quantum mechanical treatment of $\hat{\mathbf{J}}$. Because $\hat{\mathbf{J}}$, the total angular momentum, is made up of a sum of orbital, $\hat{\mathbf{L}}$, and spin, $\hat{\mathbf{S}}$, angular momenta, one must consider them individually, as well as their coupling, for a complete quantum mechanical treatment of the EPR Hamiltonian. Including the effect of orbital angular momentum, the Zeeman Hamiltonian expands to:

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_Z + \hat{\mathcal{H}}_{SO} = \mu_B \mathbf{B}_0 \cdot (\hat{\mathbf{L}} + g_e \hat{\mathbf{S}}) + \lambda \hat{\mathbf{L}} \cdot \hat{\mathbf{S}}, \quad (1.10)$$

where λ is a small parameter that is used for the perturbative expansion. Now, one can follow Weil and Bolton [6], Pryce [8], or Boča [9] for the complete treatment of second order perturbation theory in order λ ; only the result is presented here. For a given M_S to M'_S transition, the new, $\hat{\mathbf{L}}$ -affected matrix elements of the Hamiltonian are as follows:

$$\mathcal{H}_{M'_S, M_S} = \langle M'_S | \mu_B^2 \mathbf{B} \cdot \mathbf{A} \cdot \mathbf{B} + 2\lambda \mu_B \mathbf{B} \cdot \mathbf{A} \cdot \hat{\mathbf{S}} + \lambda^2 \hat{\mathbf{S}} \cdot \mathbf{A} \cdot \hat{\mathbf{S}} | M_S \rangle, \quad (1.11)$$

where $\mathbf{A}$ mixes the ground and n -th excited states, G and n , respectively.

$$\Lambda_{ij} = - \sum_{n \neq G} \frac{\langle G | \hat{\mathbf{L}}_i | n \rangle \langle n | \hat{\mathbf{L}}_j | G \rangle}{U_n^{(0)} - U_G^{(0)}}, \quad (1.12)$$

where the summation is over all non-ground states, n , $U^{(0)}$ are the zeroth order energies, and i, j denote a direction.

To simplify the interpretation of eqn. (1.11), proceed term-by-term. First, the term that scales with $\mathbf{B} \cdot \mathbf{A} \cdot \mathbf{B}$ does not depend on spin, and thus, increases the energy level of all eigenstates. Thus, EPR is not sensitive to it, since EPR can only detect the *differences* between energy levels; this term can then safely be ignored. Next, the term that scales as $\mathbf{B} \cdot \mathbf{A} \cdot \hat{\mathbf{S}}$ acts as a traditional Zeeman term with a $\mathbf{A}$ -matrix that allows for different coupling strengths along different directions. The third term, $\lambda^2 \hat{\mathbf{S}} \cdot \mathbf{A} \cdot \hat{\mathbf{S}}$ is a new spin

term that appears entirely different from the $\mathbf{B}$ -dependent terms that have been analyzed thus far. Reintroducing the original, non- $\hat{\mathbf{L}}$ -affected Zeeman term from eqn. (1.2) allows the Hamiltonian to be re-written with only relevant terms,

$$\hat{\mathcal{H}} = \mu_B \mathbf{B} \cdot \mathbf{g} \cdot \hat{\mathbf{S}} + \hat{\mathbf{S}} \cdot \mathbf{D} \cdot \hat{\mathbf{S}}, \quad (1.13)$$

where $\mathbf{g}$ is similar to the g -factor from eqns. (1.8) and (1.9), but it now allows for anisotropic couplings to the $\mathbf{B}$ -field, as it incorporates the effect of $\hat{\mathbf{L}}$,

$$\mathbf{g} = g_e \mathbf{1}_3 + 2\lambda \mathbf{\Lambda}. \quad (1.14)$$

Typically, especially at high fields (where the effects of anisotropic g become more pronounced due to the first term in eqn. (1.13) scaling with $\mathbf{B}$), g -values are reported as principal values of a rank-3 tensor rather than a scalar.

The second term in eqn. (1.13) modifies the splitting between spin sublevels, and is unimaginatively called “zero-field splitting”, which leads smoothly into the next term in eqn. (1.3), $\hat{\mathcal{H}}_{ZFS}$.

$\hat{\mathcal{H}}_{ZFS}$: Zero-field splitting

The zero-field splitting interaction, also known as the electronic quadrupolar interaction, may be written as

$$\hat{\mathcal{H}}_{ZFS} = \hat{\mathbf{S}} \cdot \mathbf{D} \cdot \hat{\mathbf{S}} = \hat{\mathbf{S}} \cdot \lambda^2 \mathbf{\Lambda} \cdot \hat{\mathbf{S}}. \quad (1.15)$$

As shown above, zero-field splitting manifests from the consideration of spin-orbit coupling. Kramer’s theorem requires that at zero field, half-integer spin systems must be doubly degenerate. This implies that the splitting can only be between different values of m_s , the individual $m_s^\pm$ energies cannot split [10]. Additionally, this implies that ZFS can only be observed for high-spin ($S > 1/2$) systems.

Typically, ZFS is described by two scalar parameters, D and E , that represent the axial and rhombic parts of $\mathbf{D}$. The complete expansion of eqn. (1.15) can be found in Appendix A.1—only the final result is quoted here:

$$\hat{\mathcal{H}}_{ZFS} = D \left\{ \hat{S}_z^2 - \frac{1}{3}S(S+1)\mathbf{1} \right\} + E \left(\hat{S}_x^2 - \hat{S}_y^2 \right). \quad (1.16)$$

Though this matrix is not traceless, because the values are obtained from EPR experiments, it is always reported as such. A non-zero trace modifies the energy of *all* spin eigenstates and thus does not contribute to the EPR spectrum. A simulation of the eigenvalues for a simple $S = 1$ system with nonzero ZFS is shown in Fig. 1.2.

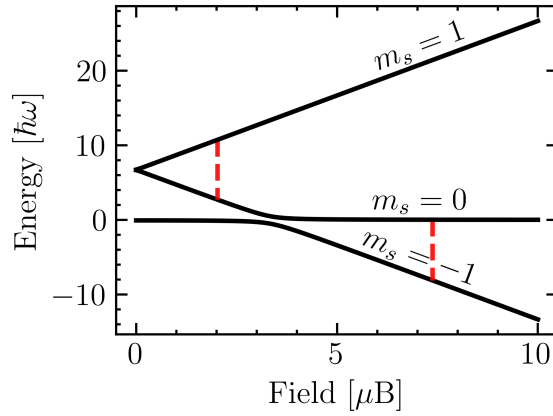


Figure 1.2: Cartoon demonstrating field-dependence of $\hat{\mathcal{H}}_Z + \hat{\mathcal{H}}_{ZFS}$ eigenvalues for $S = 1$, $g = 2$, $D = 10$, $E = 0.5$ (see eqn. (1.16)). Here, the ZFS tensor is assumed to be aligned with the lab frame ($D_z \parallel B_z$). Dashed red vertical lines represent allowed ($\Delta m_s = 1$) transitions at a microwave energy of $8\hbar\omega$; here, it is apparent that ZFS has split the two transitions that would otherwise be degenerate. There is an avoided crossing from sublevel mixing because $E \neq 0$.

Though the origin of ZFS is typically considered spin-orbit coupling, it can also result from electron-electron coupling [11], as the magnetic moment from other nearby electrons can induce a B_0 -independent splitting of coupled spin systems (see, for example, Chapter 14 of Levitt [12]). However, such splittings are distinguishable from the spin-orbit coupling based ZFS, and for the sake of this work, are called the electron-electron

dipolar interaction and given their own term in the Hamiltonian, $\hat{\mathcal{H}}_{dd}$.

$\hat{\mathcal{H}}_{dd}$: electron-electron dipolar interaction

$\hat{\mathcal{H}}_{dd}$ results from nearby electron spins (magnetic moments) imparting small magnetic fields on the electron spin of interest. This term is critically important to the work presented in Chapter 2, so it is described here in detail.

As a first step, write $\hat{\mathcal{H}}_{dd}$ using the well-known dipole-dipole interaction, where $\mathbf{r}$ represents the vector between the two spins (see Fig. 1.3):

$$\hat{\mathcal{H}}_{dd}(\mathbf{r}) = \frac{\mu_0}{4\pi} g^2 \mu_B^2 \left\{ \frac{\hat{\mathbf{S}}_1 \cdot \hat{\mathbf{S}}_2}{r^3} - \frac{3(\hat{\mathbf{S}}_1 \cdot \mathbf{r})(\hat{\mathbf{S}}_2 \cdot \mathbf{r})}{r^5} \right\}. \quad (1.17)$$

Since eqn. (1.17) is orientation dependent, this Hamiltonian is difficult to treat for randomly distributed molecules. In the case of a crystalline sample, where $\mathbf{r}$ is fixed between pairs (or groups, using a summation where required) of electrons, it is relatively simple; otherwise (and the case of the protein studies presented in Chapter 2), this anisotropic interaction must be simplified. Expanding and taking spatial averages over the electron ensemble (denoted by the angular brackets), $\hat{\mathcal{H}}_{dd}$ can be written:

$$\hat{\mathcal{H}}_{dd} = \hat{\mathbf{S}} \cdot \mathbf{T} \cdot \hat{\mathbf{S}} \quad (1.18)$$

$$= \left(\frac{\mu_0}{4\pi} g^2 \mu_B^2 \right) \hat{\mathbf{S}} \cdot \begin{pmatrix} \langle \frac{r^2-3x^2}{r^5} \rangle & \langle \frac{-3xy}{r^5} \rangle & \langle \frac{-3xz}{r^5} \rangle \\ \langle \frac{-3xy}{r^5} \rangle & \langle \frac{r^2-3y^2}{r^5} \rangle & \langle \frac{-3yz}{r^5} \rangle \\ \langle \frac{-3xz}{r^5} \rangle & \langle \frac{-3yz}{r^5} \rangle & \langle \frac{r^2-3z^2}{r^5} \rangle \end{pmatrix} \cdot \hat{\mathbf{S}} \quad (1.19)$$

where $\hat{\mathbf{S}}$ is the total spin, $\hat{\mathbf{S}}_1 + \hat{\mathbf{S}}_2$ [6]. There are certain geometrical situations where this can be simplified further (for example, $\theta = 0$ makes $x = y = 0$ so that the matrix is diagonal; see Fig. 1.3), but in general, this matrix representation does not help with conceptualization. Instead, it is instructive to follow the simplification from Abragam

[13]. By writing out the dot products for both spins, dependent on their polar and azimuthal angles, θ and ϕ , $\hat{\mathcal{H}}_{dd}$ becomes the “dipolar alphabet”.

$$\hat{\mathcal{H}}_{dd} = \omega_{dd} (A + B + C + D + E + F) \quad (1.20)$$

where

$$\omega_{dd} = \left(\frac{\mu_0}{4\pi} \frac{g^2 \mu_B^2}{r^3} \right) \quad (1.21)$$

$$A = (1 - 3 \cos^2 \theta) S_{1z} S_{2z} \quad (1.22)$$

$$B = -\frac{1}{4} (1 - 3 \cos^2 \theta) (S_{1+} S_{2-} + S_{1-} S_{2+}) \quad (1.23)$$

$$C = -\frac{3}{2} \sin \theta \cos \theta e^{-i\phi} (S_{1z} S_{2-} + S_{1-} S_{2z}) \quad (1.24)$$

$$D = C^\dagger \quad (1.25)$$

$$E = -\frac{3}{4} \sin^2 \theta e^{-2i\phi} S_{1+} S_{2+} \quad (1.26)$$

$$F = E^\dagger. \quad (1.27)$$

Here, the † operator represents a conjugate transpose. † does not modify the Pauli matrices—nor the spin operators ($S_i^\dagger = S_i$)—and it inverts the raising and lowering operators ($S_\pm^\dagger = \{\hat{S}_x \pm i\hat{S}_y\}^\dagger = \{\hat{S}_x \mp i\hat{S}_y\} = S_\mp$). For these purposes, the relevant terms in this expansion are A and B , the “secular” and “pseudosecular” terms, respectively. The B term is the “flip-flop” term, wherein energy and total angular momentum is conserved, but the spin quantum numbers of each individual spin is modified by ± 1 . By inspection, C , D , E , and F are not energy conserving as they induce spin flips with a net change in total spin quantum number (and therefore do not conserve energy when under an applied field). For this reason, they modify the dipolar-coupled energy levels infrequently and can therefore be neglected for most calculations.

Importantly, the coupling strength changes depending on whether spins 1 and 2 are distinguishable; one can look in Chapter 14 of Levitt [12] for the full derivation. In the

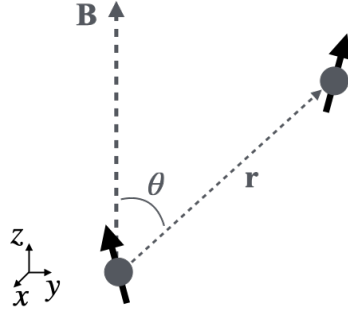


Figure 1.3: The vector between the two spins, $\mathbf{r}$ is set at the polar angle θ from the applied magnetic field, $\mathbf{B}$. The spins are precessing about the quantization direction (along $\mathbf{B}$), but may be “up” or “down”, which is fundamentally what causes the dipolar splitting.

case of “weak coupling” (*i.e.* $\omega_{dd} \ll \omega_{Z1} - \omega_{Z2}$), $\hat{\mathcal{H}}_{dd} = \omega_{dd} S_{z1} S_{z2}$, where ω_{dd} is the dipolar coupling term from eqn. (1.21) that has also absorbed the angle-dependent term in eqn. (1.22). ω_{Zi} are the Zeeman energies from eqn. (1.9). In such a system, the second term in the dipolar alphabet (B) does not contribute, because like C - F it is not energy-conserving, and the Hamiltonian simplifies to:

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_{Z1} + \hat{\mathcal{H}}_{Z2} + \hat{\mathcal{H}}_{dd} \quad (1.28)$$

$$= \omega_{Z1} S_{z1} + \omega_{Z2} S_{z2} + \omega_{dd} S_{z1} S_{z2}. \quad (1.29)$$

Here, it is clear that the individual Zeeman states are eigenstates of the coupled Hamiltonian. Working with the simplest case of two spin-1/2 particles, $|\alpha\rangle$ and $|\beta\rangle$, spin “up” and “down”, become product states, $|\alpha\alpha\rangle$, $|\alpha\beta\rangle$, $|\beta\alpha\rangle$, $|\beta\beta\rangle$ and the energy levels of each are:

$$\hat{\mathcal{H}}|\alpha\alpha\rangle = \frac{1}{2} \left(\omega_{Z1} + \omega_{Z2} + \frac{\omega_{dd}}{2} \right) |\alpha\alpha\rangle \quad (1.30)$$

$$\hat{\mathcal{H}}|\alpha\beta\rangle = \frac{1}{2} \left(\omega_{Z1} - \omega_{Z2} - \frac{\omega_{dd}}{2} \right) |\alpha\beta\rangle \quad (1.31)$$

$$\hat{\mathcal{H}}|\beta\alpha\rangle = \frac{1}{2} \left(-\omega_{Z1} + \omega_{Z2} - \frac{\omega_{dd}}{2} \right) |\beta\alpha\rangle \quad (1.32)$$

$$\hat{\mathcal{H}}|\beta\beta\rangle = \frac{1}{2} \left(-\omega_{Z1} - \omega_{Z2} + \frac{\omega_{dd}}{2} \right) |\beta\beta\rangle. \quad (1.33)$$

The allowed transitions therefore have energy splittings of $(\omega_{Z1} \pm \omega_{dd}/2)$, $(\omega_{Z2} \pm \omega_{dd}/2)$, and therefore the Zeeman transitions, ω_{Z1} and ω_{Z2} , each become two levels separated by ω_{dd} .

In the case of “strong coupling” or “magnetically equivalent spins”, the individual Zeeman states are no longer states of the coupled Hamiltonian. Here, B is no longer zero, and the flip-flop (off-diagonal) terms must be considered.

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_{Z1} + \hat{\mathcal{H}}_{Z2} + \hat{\mathcal{H}}_{dd} \quad (1.34)$$

$$= \omega_{Z1}S_{z1} + \omega_{Z2}S_{z2} + \omega_{dd}S_{z1}S_{z2} - \frac{1}{4}\omega_{dd}(S_{1+}S_{2-} + S_{1-}S_{2+}) \quad (1.35)$$

Working in the Zeeman basis, where an eigenvector represents the occupation of $\{|\alpha\alpha\rangle, |\alpha\beta\rangle, |\beta\alpha\rangle, |\beta\beta\rangle\}^T$, the Hamiltonian can be written as the following matrix:

$$\hat{\mathcal{H}} = \begin{pmatrix} \omega_Z + \omega_{dd}/4 & 0 & 0 & 0 \\ 0 & -\omega_{dd}/4 & -\omega_{dd}/4 & 0 \\ 0 & -\omega_{dd}/4 & -\omega_{dd}/4 & 0 \\ 0 & 0 & 0 & -\omega_Z + \omega_{dd}/4 \end{pmatrix}. \quad (1.36)$$

where $\omega_{Z1} = \omega_{Z2} = \omega_Z$ due to their magnetic equivalence. Two eigenstates of this Hamiltonian can be seen immediately, they are $|\alpha\alpha\rangle$ and $|\beta\beta\rangle$. The other two states are necessarily linear combinations of $|\alpha\beta\rangle$ and $|\beta\alpha\rangle$; $|\Psi_1\rangle = \frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle)$, $|\Psi_2\rangle = \frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle)$. Typically, these four coupled states are referred to as “singlet” and “triplet” states, where “singlet” refers to the one state with total spin zero ($S = 0$), $|\Psi_2\rangle$. The triplet refers to the other three that have nonzero total spin, $|\alpha\alpha\rangle, |\beta\beta\rangle, |\Psi_1\rangle$. The eigenvalues (energies) of these four states are: $\{0, -\omega_{dd}/2, -\omega_{dd}/2, -\omega_Z + \omega_{dd}/4\}$. The allowed ($\Delta S = 0, |\Delta m_s| = 1$) transitions are only between states in the triplet manifold, which gives the allowed transition energies of $\{\omega_Z + 3\omega_{dd}/4, \omega_Z - 3\omega_{dd}/4\}$. Therefore,

the single energy level at ω_Z is split into two levels with separation $3\omega_{dd}/2$, which is $3/2$ larger than the splitting in the weak coupling regime discussed above.

A similar treatment can be applied to higher spin systems (for example, $S = 7/2$): the biggest difference—apart from much larger matrices—is that the coupling with higher spin quantum numbers induces a larger splitting. As a result, high spin causes larger (and more observable) broadenings [14].

$\hat{\mathcal{H}}_{Ex}$: Exchange coupling

The exchange interaction is an additional consideration that is only relevant for strongly coupled spins. This coupling is typically weak and can only be observed when the spins are in very close proximity ($r < 1\text{nm}$), as it requires that the electron eigenstates are product states. This is common in crystalline samples, and less so in dilute organic materials, whether powder or solution. Exchange coupling is neglected for the dilute ($< 1\text{mM}$) solution samples in Chapter 2, for example.

The exchange interaction $\hat{\mathcal{H}}_{Ex}$, is typically expressed as

$$\hat{\mathcal{H}}_{Ex} = -J\hat{\mathbf{S}}_1 \cdot \hat{\mathbf{S}}_2, \quad (1.37)$$

where the sign of J determines the macroscopic behavior of the material: $J > 0$ returns the lowest energy with *aligned* spins, *i.e.* a ferromagnet; $J < 0$ returns the lowest energy with *anti-aligned* spins, *i.e.* an antiferromagnet. Note that this term depends on convention, and depending on the individual text, $-J$ can be written as $-J$, J , or even $-2J$.

Once again, begin by writing the Hamiltonian in matrix form, in the same product basis as eqn. (1.36) [15].

$$\hat{\mathcal{H}}_{Ex} = -J(S_{1x}S_{2x} + S_{1y}S_{2y} + S_{1z}S_{2z}) \quad (1.38)$$

$$= -J(S_{1z}S_{2z} + (S_{1+}S_{2-} + S_{1-}S_{2+})/2) \quad (1.39)$$

$$= \begin{pmatrix} -J/4 & 0 & 0 & 0 \\ 0 & J/4 & -J/2 & 0 \\ 0 & -J/2 & J/4 & 0 \\ 0 & 0 & 0 & -J/4 \end{pmatrix} \quad (1.40)$$

Diagonalizing this matrix returns the same eigenstates as eqn. (1.36), $\{|\alpha\alpha\rangle, |\beta\beta\rangle, \frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle), \frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle)\}$ with corresponding eigenvalues $\{-J/4, -J/4, -J/4, 3J/4\}$. These states are the same as the magnetically equivalent spins in the subsection above, which is intuitive—for “exchange” of the two spins to conserve energy, they of course must be equivalent.

$\hat{\mathcal{H}}_N$: Nuclear hyperfine interaction

The final term in the electron spin Hamiltonian results from coupling electron spins with nearby nuclei. The origin of the nuclear hyperfine interaction is dipole-dipole coupling between the nucleus $\hat{\mathbf{I}}$ and a nearby electron $\hat{\mathbf{S}}$, much in the same way as $\hat{\mathcal{H}}_{dd}$ [16]. It is most often written in a form analogous to eqn. (1.18):

$$\hat{\mathcal{H}}_N = \hat{\mathbf{I}} \cdot \mathbf{A} \cdot \hat{\mathbf{S}}. \quad (1.41)$$

This form is typically more informative than its electron-electron counterpart, as the electron orbitals are typically fixed to the frame of the nucleus. As a result, EPR can obtain the diagonal elements of the tensor $\mathbf{A}$ in its frame, and can report on the electron-nuclear coupling strength along different directions of the electrons orbital. However, there is an additional complication for electrons coupled to nuclei—there is nothing preventing the electron’s spatial wavefunction from overlapping that of the nucleus (*i.e.* the orbital radius, r , can be zero). In this case, the dipole-dipole coupling term diverges. Since this is

not physical, there must be a finite contribution to the hyperfine interaction when $r = 0$. This finite contribution was first derived by Fermi, and is named after him, called the “Fermi contact shift” [6, 16, 17]. This term makes up the *isotropic* part of $\mathbf{A}$, and is given by:

$$\hat{\mathcal{H}}_N^{iso} = \frac{2\mu_0}{3} g_e \mu_B g_N \mu_N \rho_0 \hat{\mathbf{S}} \cdot \hat{\mathbf{I}} \quad (1.42)$$

$$= a_{iso} \hat{\mathbf{S}} \cdot \hat{\mathbf{I}} \quad (1.43)$$

where g_N is the nuclear g -factor, μ_N is the nuclear magneton, and ρ_0 is the electron spin density at the nucleus ($r = 0$).

In the case of nitroxide spins (typically used for DEER and other site-directed spin labelling experiments of proteins), the splitting from $\mathbf{A}$ can be quite large ($A_{zz} \approx 100$ MHz [18]); on the other hand, with gadolinium spin labels (that are relevant in Chapter 2), $\mathbf{A}$ is typically taken to be negligible, as most isotopes of Gd nuclei have zero spin, and even those with nonzero spin have small couplings ($A_{zz} \approx 16$ MHz [14]).

1.1.2 Motional and exchange narrowing

Many EPR experiments are done in solution, and therefore, the paramagnetic sample of interest undergoes Brownian motion. In this case, an averaged coupling tensor is measured, rather than the true anisotropic one. The extent of the averaging depends on the rate of the motion relative to the timescale of the experiment—a helpful metric is $\omega_0 \tau_c$, where ω_0 is the relevant coupling, and τ_c is the motional correlation time within the sample. If $\omega_0 \tau_c \gg 2\pi$, the sample is not moving quickly enough to average the coupling, leaving the system in the “solid-state” (static) regime. In the solid-state regime, orientation-dependent interactions are fixed, and the resulting spectrum is a “powder pattern”, meaning it behaves as though the sample is not moving at all. If $\omega_0 \tau_c \ll 2\pi$,

the system is in the “fast-moving” regime, and completely averages over orientations, removing any orientation-dependent spectral features [12]; because all orientations are sampled within the timescale of the EPR experiment, an averaged coupling is returned. In the special case of angle-dependent dipolar couplings, this averaging goes to zero. Recall that

$$\hat{\mathcal{H}}_{dd} \propto 1 - 3 \cos^2 \theta; \quad (1.44)$$

assuming θ is a function of time, t , and that θ randomly samples all of its configuration space with equal likelihood over some time that is much shorter than the experimental timescale, T (*i.e.*, obeys “ergodicity”), averaging over time is equivalent to averaging over space (weighted by the likelihood of each individual orientation). Under these conditions [12],

$$\langle \hat{\mathcal{H}}_{dd} \rangle_T \propto \langle 1 - 3 \cos^2 \theta(t) \rangle_T \quad (1.45)$$

$$\propto \langle 1 - 3 \cos^2 \theta \rangle_r \quad (1.46)$$

$$\propto \int_0^\pi \int_0^{2\pi} \int_0^\infty (1 - 3 \cos^2 \theta) \rho(r) r \sin \theta dr d\phi d\theta \quad (1.47)$$

$$\propto \int_0^\pi (1 - 3 \cos^2 \theta) \sin \theta d\theta \quad (1.48)$$

$$= 0. \quad (1.49)$$

More complicated—and more difficult to treat—is the case where $\omega_0 \tau_c \sim 2\pi$. This is the “intermediate regime”, and results in partial averaging of orientationally-dependent couplings. In this case, the ergodic principle does not apply, as there is *some* auto-correlation of the sample’s orientation throughout the experimental timescale, T [19]. Therefore, simple approximations can no longer be made, and more advanced treatments must be used. For example, EasySpin, a popular EPR simulation software, treats this

regime by solving the stochastic Liouville equation while incorporating the effects of relaxation from motional correlation [20, 21]. τ_c varies widely depending on the individual system: there are contributions from sidechain kinetics, τ_{sc} ; backbone fluctuations, τ_{bb} ; and overall rotation of the molecular body (often a protein), τ_r [22]. In the case of nitroxide spin labels, which have been (and are being) used for monumental studies (see, *e.g.*, [23–28]), τ_{sc} and τ_{bb} are much shorter than τ_r , and thus, dominate the correlation time. The studies leverage the differing dynamics of sidechains and backbones to make EPR sensitive to electron spin mobility, and therefore extract dynamics-induced changes to site-specific secondary structure that manifest as transient changes to correlation time and therefore linewidth. In contrast, labels that are engineered to be rigid and isotropic (see, *e.g.* [29]) do not experience such rapid side-chain and backbone motion, and thus, can report directly on τ_r . Further, because τ_r is typically quite long for even small protein domains ($\tau_c \approx 1\text{-}10\text{ ns}$ for $m \approx 1\text{-}20\text{ kDa}$) [30], such spin labels are sensitive to weaker orientation-dependent couplings than nitroxide-based ones.

Though motion is the only line-narrowing mechanism discussed thus far, exchange coupling may also narrow spectroscopic splittings through a similar process [31]. Exchange between two magnetically equivalent particles causes rapid “motion” between sets of spatial coordinates, and thus, causes fast, random sampling of orientation-space, in the same way as discussed above for a short τ_c . Anderson and Weiss [31] provide a formula to estimate the lineshape in the rapidly exchanging regime, where $\omega_0 \ll \omega_e$:

$$I(\omega) = \frac{2}{\pi} \frac{(\omega_0^2/\omega_e)}{\omega^2 + (\omega_0^2/\omega_e)^2}. \quad (1.50)$$

Here, ω_0 is the intrinsic linewidth and ω_e is rate of the narrowing mechanism. This lineshape is a Lorentzian with linewidth $\Delta\omega = \omega_0^2/\omega_e$, which provides the ability to estimate the extent of averaging in the fast-exchange regime. Additionally, their treatment

was general enough that ω_e can be substituted for a more general ω_m to represent other motional fluctuations, including Brownian motion ($\omega_m = J/\hbar$ or $\omega_m = 2\pi/\tau_c$). This approximation is discussed further in sec. 2.3.5 in an attempt to estimate the extent of narrowing due to solution-state Brownian motion for an ensemble of spin-labeled protein domains.

1.1.3 The Bloch equations

Instead of approaching EPR as a quantum mechanics problem, one can approach it (semi-)classically by treating the net magnetization of a sample rather than the energy levels of individual spins. As noted by Bloch [32], the expectation value of a quantum mechanical observable will obey exactly the classical equations of motion. Since EPR is interested in the behavior of quantum spins, take the (expectation value of the) net magnetization per unit volume of the sample to be the observable of interest; call this observable $\mathbf{M}$. Then, take the static external field $\mathbf{B}_0$ to be along the $\hat{z}$ direction, such that

$$\mathbf{B} = B_0 \hat{z}. \quad (1.51)$$

The torque acting on the system is

$$\mathbf{T} = \mathbf{M} \times \mathbf{B}, \quad (1.52)$$

where $\times$ represents the cross product. From classical mechanics,

$$\frac{d\mathbf{A}}{dt} = \mathbf{T}, \quad (1.53)$$

where $\mathbf{A}$ is angular momentum. The gyromagnetic ratio relates the magnetization to the angular momentum such that

$$\frac{1}{\gamma} \frac{d\mathbf{M}}{dt} = \mathbf{M} \times \mathbf{B} \quad (1.54)$$

$$\frac{d\mathbf{M}}{dt} = \gamma [(M_y B_z - M_z B_y) \hat{x} + (M_z B_x - M_x B_z) \hat{y} + (M_x B_y - M_y B_x) \hat{z}] \quad (1.55)$$

$$= \gamma [(M_y B_0) \hat{x} + (-M_x B_0) \hat{y}]. \quad (1.56)$$

Which yield

$$\frac{dM_x}{dt} = \gamma M_y B_0 \quad (1.57)$$

$$\frac{dM_y}{dt} = -\gamma M_x B_0 \quad (1.58)$$

$$\frac{dM_z}{dt} = 0, \quad (1.59)$$

and may be solved by inspection, assuming $\mathbf{M}(t=0) = \mathbf{M}_0 = M_\perp \hat{x} + M_\parallel \hat{z}$.

$$M_x = M_\perp \cos \omega_L t \quad (1.60)$$

$$M_y = M_\perp \sin \omega_L t \quad (1.61)$$

$$M_z = M_\parallel \quad (1.62)$$

where $\omega_L = -\gamma B_0$. Now, this model must include phenomenological terms that account for relaxation back to equilibrium, both along the longitudinal ($\hat{z}$) and transverse ($\hat{x}, \hat{y}$) directions. These relaxations are assumed to be exponential, as the relaxation rate depends on the number of non-equilibrium spins. Therefore, the time-derivatives should scale with the inverse of these relaxation times:

$$\frac{dM_x}{dt} \propto -\frac{M_x}{T_2} \quad (1.63)$$

$$\frac{dM_y}{dt} \propto -\frac{M_y}{T_2} \quad (1.64)$$

$$\frac{dM_z}{dt} \propto -\frac{M_z - M_\parallel}{T_1}, \quad (1.65)$$

where T_1 and T_2 are intrinsic “relaxation times” that govern the out-of-equilibrium dynamics of the system. Incorporating these relaxation terms into eqn. (1.56) yields:

$$\frac{dM_x}{dt} = \gamma M_y B_0 - \frac{M_x}{T_2} \quad (1.66)$$

$$\frac{dM_y}{dt} = -\gamma M_x B_0 - \frac{M_y}{T_2} \quad (1.67)$$

$$\frac{dM_z}{dt} = -\frac{M_z - M_{\parallel}}{T_1} \quad (1.68)$$

Which can be expressed as a matrix equation,

$$\frac{d}{dt} \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} = \begin{pmatrix} -\frac{1}{T_2} & -\omega_L & 0 \\ \omega_L & -\frac{1}{T_2} & 0 \\ 0 & 0 & -\frac{1}{T_1} \end{pmatrix} \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ \frac{M_{\parallel}}{T_1} \end{pmatrix} \quad (1.69)$$

and has solutions [6]

$$M_x = M_{\perp} e^{-t/T_2} \cos \omega_L t \quad (1.70)$$

$$M_y = M_{\perp} e^{-t/T_2} \sin \omega_L t \quad (1.71)$$

$$M_z = M_{\parallel} (1 - e^{-t/T_1}). \quad (1.72)$$

These equations represent the magnetization of a sample in a static magnetic field that is applied along the $\hat{z}$ axis. They show that the magnetization precesses about this axis with frequency $\omega_L = \gamma B_0$, also known as the “Larmor frequency”. To make eqn. (1.69) more interesting (and relevant to EPR), the effect of resonant applied electromagnetic (EM) waves must be included, which can be modeled simply by adding a sinusoidal magnetic field along one of the two transverse directions. Taking a circularly polarized EM wave,

$$\mathbf{B}_1 = B_1 \cos \omega t \hat{x} + B_1 \sin \omega t \hat{y}, \quad (1.73)$$

and inserting this term into eqn. (1.54), the matrix equation becomes:

$$\frac{d}{dt} \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} = \begin{pmatrix} -\frac{1}{T_2} & -\omega_L & -\gamma B_1 \sin \omega t \\ \omega_L & -\frac{1}{T_2} & \gamma B_1 \cos \omega t \\ \gamma B_1 \sin \omega t & -\gamma B_1 \cos \omega t & -\frac{1}{T_1} \end{pmatrix} \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ \frac{M_{\parallel}}{T_1} \end{pmatrix}. \quad (1.74)$$

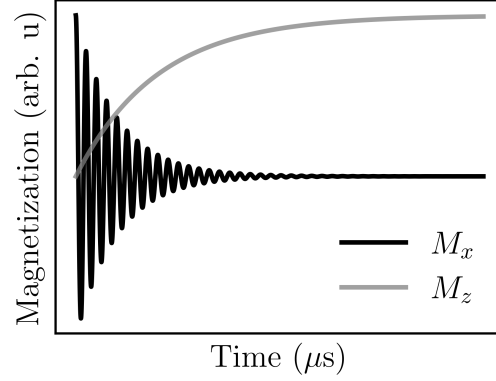


Figure 1.4: Simulation depicting the Bloch magnetization along the $\hat{x}$ (black) and $\hat{z}$ (gray) directions for a system with $\mathbf{B} = B \hat{z}$ and initial conditions $\mathbf{M} = M_{\perp} \hat{x}$. Here, it is obvious that M_x oscillates as a result of Larmor precession (decaying with T_2) and M_z relaxes to $M_{\parallel}$ (decaying with T_1).

To make this more informative, eqn. (1.74) can be transformed into the “rotating frame”. In this frame, the coordinate system rotates about the $\hat{z}$ axis at ω ; without loss of generality, take the phase of the frame to match the phase of the $\mathbf{B}_1$ ($\mathbf{B}_1 = B_1 \hat{x}$). Therefore, the effect of B_0 has been removed, as there is now no precession about the $\hat{z}$ axis, and the only magnetization precession now occurs about $\mathbf{B}_1$. If, for simplicity, the frequency of the rotating frame is assumed to match that of $\mathbf{B}_1$, the system is equivalent to what was presented in eqns. (1.60)-(1.62): magnetization under an applied static magnetic field. Call the new, rotating axes $\hat{x}'$, $\hat{y}'$, and $\hat{z}'$ (though $\hat{z}$ does not change under this transformation) and eqn. (1.55) becomes:

$$\frac{d\mathbf{M}'}{dt} = \gamma [(M'_z B_1) \hat{y}' + (-M'_y B_1) \hat{z}'], \quad (1.75)$$

which amounts to the magnetization precessing about the applied $\mathbf{B}_1$. Transforming back to the lab frame, the magnetization is then precessing about $\mathbf{B}_1$ and $\mathbf{B}_0$, meaning it is precessing *about* $\mathbf{B}_1$ while also rotating *with* it. It is also worth mentioning here that the applied EM wave does not need to be circularly polarized for this analysis to apply. A linearly polarized wave may be decomposed into two counter-propagating circular com-

ponents; only the propagation direction that rotates *with* the magnetization precession will induce a static field in the rotating frame (the counter-propagating component rotates at 2ω in the opposite direction and its effect is averaged to zero), and thus, the true EM polarization is irrelevant for this treatment.

Though it will not be worked through in detail here, one can refer to Weil and Bolton [6] for the steady-state solutions of the rotating frame system, if the frequency of the input wave is allowed to vary slightly from the Larmor frequency ($\omega \gg |\omega - \omega_L|$). The solutions, in the rotating frame, are:

$$M'_x = -M_{\parallel} \frac{\gamma B_1 (\omega_L - \omega) T_2^2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (1.76)$$

$$M'_y = M_{\parallel} \frac{\gamma B_1 T_2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (1.77)$$

$$M'_z = -M_{\parallel} \frac{1 + (\omega_L - \omega)^2 T_2^2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2}. \quad (1.78)$$

χ' , the dispersive lineshape, and χ'' , the absorptive lineshape, can be recovered by substituting for the static magnetization $M_{\parallel}$:

$$M_{\parallel} = \chi_0 B_0 / \kappa \mu_0 \quad (1.79)$$

where χ_0 is the static magnetic susceptibility, and $\chi_0 = \kappa \mu_0 N_V g^2 \mu_B^2 / 4k_b T$, where N_V is the spin density and T is the spin temperature. Now, define

$$\chi' = \frac{\kappa \mu_0 M'_x}{B_1} \quad (1.80)$$

$$\chi'' = -\frac{\kappa \mu_0 M'_y}{B_1}, \quad (1.81)$$

and substitute $M_{\parallel}$ to get expressions for dispersion and absorption:

$$\chi' = -\chi_0 \frac{\gamma B_0 (\omega_L - \omega) T_2^2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (1.82)$$

$$= \chi_0 \frac{\omega_L(\omega_L - \omega)T_2^2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (1.83)$$

$$\chi'' = -\chi_0 \frac{\gamma B_0 T_2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (1.84)$$

$$= \chi_0 \frac{\omega_L T_2}{1 + (\omega_L - \omega)^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2}. \quad (1.85)$$

Assuming the applied EM wave is weak (*i.e.* $\gamma^2 B_1^2 T_1 T_2 \ll 1$),

$$\chi' \approx \chi_0 \frac{\omega_L(\omega_L - \omega)T_2^2}{1 + (\omega_L - \omega)^2 T_2^2} \quad (1.86)$$

$$\approx \frac{-\omega_L^2 \Omega}{1/T_2^2 + \omega_L^2 \Omega^2} \quad (1.87)$$

$$\approx -\frac{\Omega}{1/\omega_L^2 T_2^2 + \Omega^2} \quad (1.88)$$

$$\chi'' \approx \chi_0 \frac{\omega_L T_2}{1 + (\omega_L - \omega)^2 T_2^2} \quad (1.89)$$

$$\approx \chi_0 \frac{\omega_L/T_2}{1/T_2^2 + \omega_L^2 \Omega^2} \quad (1.90)$$

$$\approx \chi_0 \frac{1/\omega_L T_2}{1/\omega_L^2 T_2^2 + \Omega^2}, \quad (1.91)$$

where $\Omega = (\omega - \omega_L)/\omega_L$, which can be recognized from Fig. 1.5 as a Lorentzian (χ'') and its Hilbert transform (χ'), with linewidth $\Gamma = 2/\omega_L T_2$ [33].

EPR lineshapes are often made up of many Lorentzians, split by differences in individual spin's local environments, which combine to form a collective Gaussian, called “inhomogenously broadened”. If a lineshape is solely Lorentzian, it is “homogenously broadened”, meaning it is lifetime-broadened as a result of quantum mechanical uncertainty in the coherence lifetime ($\Gamma \propto T_2^{-1}$, as seen in eqn. (1.91)).

1.1.4 A simple EPR spectrometer

Low and high-field EPR spectrometers operate with similar principles, though the exact techniques and hardware required tend to differ significantly. A basic EPR spectrometer

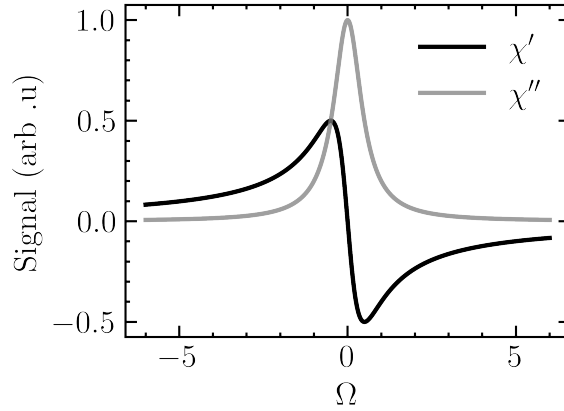


Figure 1.5: Simulated lineshapes of χ' (black) and χ'' (gray) in the weak-excitation regime, where $\gamma^2 B_1^2 T_1 T_2 \ll 1$. For this simulation, $\Gamma = 1$.

block diagram is shown in Fig. 1.6. Here, an electronic clock signal is converted to an EM wave by a microwave source. This EM wave is directed to the magnet by the microwave bridge, where it is directed onto the sample. Depending on the magnet design, the EM wave may be coupled into a waveguide that guides the light to-and-from a sample sitting within the magnet, at center of the static magnetic field. Microwaves that are reflected at the sample space are directed toward the detector by a circulator, where the signal is then mixed with the original clock signal to produce an intermediate frequency (IF) signal (AC or DC). This signal is processed and digitized. For CWEPR in particular (see sec. 1.2), the digitization is done by a lock-in amplifier that is phase-locked to the modulation coil. The coil slightly perturbs the applied field B_0 at a well-defined frequency, which allows the lock-in amplifier to attenuate noise by selecting only the EPR signals that are modulated at the same frequency. In the case of pulsed EPR (see sec. 1.3), no modulation coil is used and the signal is directly digitized in the time domain by a digitizer [34]. For high-field (and therefore high-frequency) EPR, there are a few specifics that are different from low-field EPR and therefore worth discussing in greater detail:

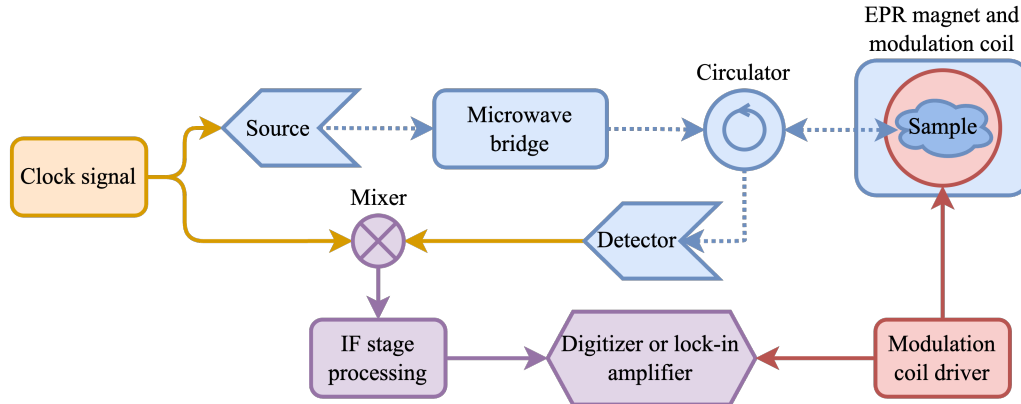


Figure 1.6: A simple diagram of a basic CW EPR spectrometer. A clock signal is converted to EM waves by a microwave source, which propagates along a microwave bridge to the sample that is housed within a magnet. Reflected microwaves are directed toward the detector by a circulator, where they are then mixed with the original clock signal to return an intermediate frequency. This signal is processed and digitized by a digitizer or lock-in amplifier.

1. The microwave bridge is necessarily quasi-optical.
2. Detection is done in “induction mode”.
3. The source frequency is (usually) fixed and therefore the magnet must be field-swept.
4. The IF stage operates at a nonzero frequency (usually called “heterodyne” detection).
5. Single-mode cavity resonators are no longer practical.

These topics are addressed separately below, as they all play an important role in the experiments discussed throughout this dissertation.

1. Quasi-optical bridges

At low frequencies, circulators, attenuators, isolators, phase shifters, etc., are implemented using transmission lines. At higher frequencies (more than a few tens of GHz), these components do not exist, nor do cables to carry signals between them. As a result, rather than transmission lines, optics that are similar in size to the wavelength of the beam (*e.g.* a 10 cm diameter mirror for a 1 cm wavelength), called “quasi-optics” are required. In such a system, the EM wave is treated as a Gaussian beam, and it is repeatedly imaged using pairs of off-axis elliptical or parabolic mirrors in order to transfer it from the source, through the circulators and isolators, and into the magnet (see Fig. 1.7) [5, 35]. In a quasi-optical system, the isolators are a single optical component—wiregrid polarizers. Wiregrid polarizers contain thin, conductive wires along one polarization direction—in that direction, the wires are conductive, and thereby act as antennas that reflect EM waves. In the perpendicular direction, there is no wire for electrons to travel along, and thus, the wires are not conductive—the polarizer is transparent. Therefore, a polarizer is used to filter one polarization from an arbitrarily polarized (or unpolarized) beam, “isolating” it from the others. A circulator is more complicated (and requires an isolator as part of its makeup); it requires that light sent one direction through a quasi-optical circuit is not transmitted along the same beam path when propagating the opposite direction. Since ray optics obey reciprocity—meaning rays traveling in the both directions must follow the same path—a component that violates this symmetry must be incorporated [36]. This is done with a Faraday rotator, which is made up of a ferrite plate with a static magnetic field that imparts a polarization rotation into an incoming beam. These plates rotate the polarization the same direction (relative to the lab frame), regardless of beam propagation direction, making them capable of violating reciprocity.

Typically, a Faraday rotation will rotate the polarization by $\Delta\theta = \pi/4$, such that light that propagates through it twice will be polarized exactly perpendicular to the input polarization ($2\Delta\theta = \pi/2$) [37, 38]. These rotators have been commercialized and can be purchased from off-the-shelf manufacturers like Thomas Keating [39], for instance. To take advantage of the Faraday rotation, the circulator includes a wiregrid polarizer isolator that is oriented with its surface normal at 45° degrees to the beam propagation axis. The beam is initially transmitted through this polarizer and is rotated $\pi/4$ by a Faraday rotator. Upon reflection at an end mirror (potentially in the sample space), it is sent back to the Faraday rotator where it is rotated another 45° and then completely *reflected* by the polarizer and directed *out* of the original optical path into a new one that is perpendicular to the original. A circulator is used for induction mode detection as well (as shown in Figs. 1.6 and 1.8), which is discussed in detail in the section below.

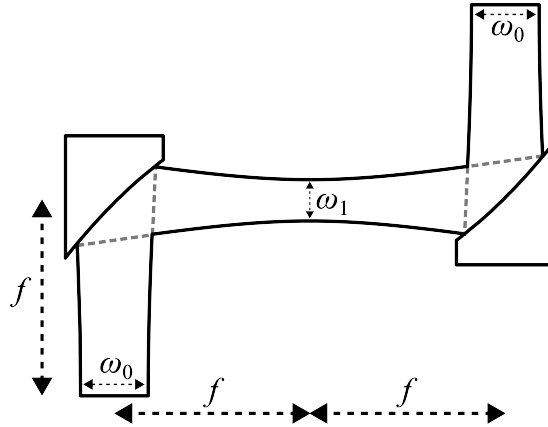


Figure 1.7: Cartoon depicting Gaussian beam imaging using a pair of off-axis parabolic mirrors. The input beam waist (ω_0) is set a distance f , the focal length of the mirror, away from the mirror surface. The mirrors are separated by $2f$, such that the focus of the mirrors (ω_1) is equidistant between them. The output mirror inverts the focusing by the first mirror and returns the image of the input beam waist ω_0 with very little losses.

The EM wave is coupled into the magnet using a corrugated waveguide. At high frequency, corrugated cylindrical waveguides minimize power loss while accepting and

emitting fundamental Gaussian beam profiles [40], making them beneficial for use with the rest of the quasi-optics that also operate with fundamental Gaussians. This dissertation does not review Gaussian beam optics or quasi-optical systems, the reader is referred to the text by Goldsmith et al. [41] for details.

2. Induction mode detection schemes

Because samples are small and quasi-optics are imperfect, it is beneficial to use “induction mode” detection at high field. This means that a Faraday rotator-wiregrid combination is used to isolate EPR signals from background stray EM waves. As discussed in sec. 1.1.3, if linearly polarized light is sent into the sample space, only one component—circularly polarized that is *co-rotating* with the Larmor precession—can be absorbed. As a result, all counter-rotating polarization is reflected and *slightly less* co-rotating is. This amounts to elliptical (no longer linear) total polarization, and thus, there is now some component of linear polarization that is orthogonal to the initial input polarization. Placing a wiregrid and Faraday rotator above the sample space selects the initial polarization and provides the isolation of the orthogonal polarization upon reflection, which is what is meant by “induction mode” (see Fig. 1.8). This architecture allows for detection of very tiny signals from large backgrounds by almost completely isolating (30-50 dB [42]) the output from the input, as ideal operation would only return a signal output when on resonance [43].

3. Single-frequency sources

High-field EPR requires frequencies > 100 GHz. This frequency range is right on the edge of the famous “terahertz gap”, a region of the electromagnetic spectrum (0.3-10 THz) that is notoriously difficult to produce, as it lies between the ranges of well-known techniques

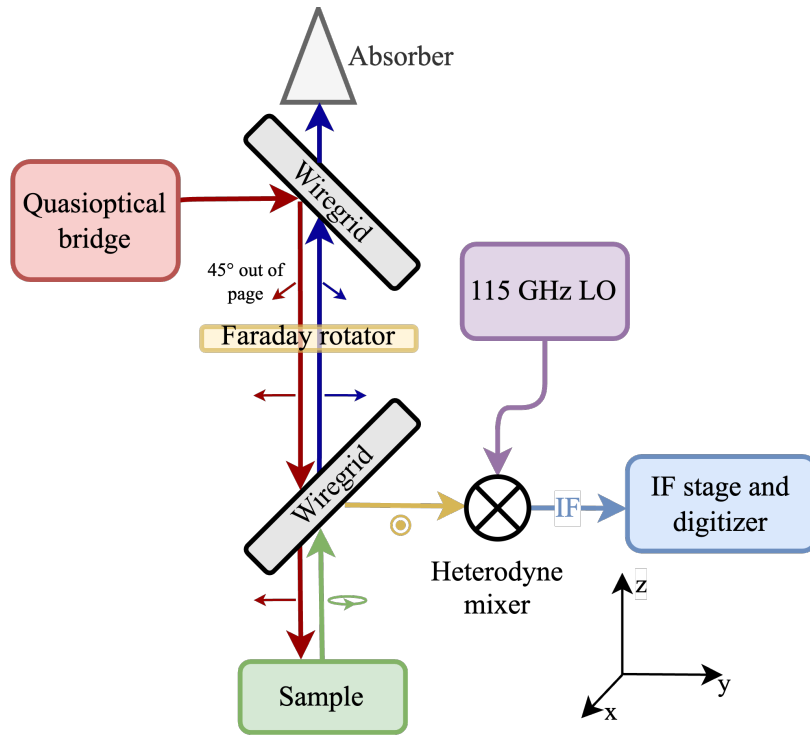


Figure 1.8: Cartoon of induction mode detection using a Faraday rotator and wiregrid polarizer. The input beam (red) is polarized out of the page at 45° . This polarization is reflected by a wiregrid polarizer oriented at 45° . It is then rotated 45° by the Faraday rotator so that it passes completely through a 90° wiregrid polarizer. This polarization interacts with the sample and returns slightly elliptical polarization (green) which is then filtered by the polarizer such that only out-of-page polarization (yellow) makes it to the detector when sample absorption occurs. The Faraday rotator rotates the in-plane polarization (blue) again, to 45° out of the page and therefore orthogonal to the input polarization, where it is transmitted by the first wiregrid polarizer into an absorber.

of electronic devices (lower frequencies, *e.g.* radio) and optics (higher frequencies, *e.g.* visible lasers) [4]. Recent advances in EM wave generation at the low end of this gap has enabled continuous wave emission with milliwatt powers [44–48]. However, such sources are typically single frequency or only offer a small range of frequency-sweeping capabilities (*e.g.*, a few GHz), which limits their potential for EPR. On the opposite end of the spectrum is the highly-specialized, high-power (kW or more) sources that can be (and have been) used for EPR: gyrotrons and free electron lasers [49, 50]. However, gyrotrons are designed for a single frequency (or its harmonics [51]), and while free electron lasers can operate at multiple frequencies, each pulse is narrow-band and pulse repetition rate is restrictively low (~ 1 Hz), requiring a very long acquisition time for time-averaging or multi-frequency experiments [52, 53]. Additionally, the quasi-optical components discussed in sec. 1.1.4 (namely the Faraday rotators and waveguides) operate ideally in narrow frequency ranges, and thus, even if the sources *could* sweep a sufficiently wide frequency range, the same quasi-optical components would not operate (well) across that range. Therefore, in order to record entire high-field EPR spectra, because the frequency of the source cannot be swept over a broad range, the magnet must be capable of field sweeps. Contrast this to the MHz frequencies required for NMR, for example, where quasi-optics are not required, and arbitrary wave generators can produce broadband excitations that are more than sufficient to sweep NMR resonances of interest [54].

4. Heterodyne detection architectures

The majority of modern high-field, high-frequency EPR spectrometers operate with “heterodyne” or “superheterodyne” detection architectures [34]. This means that the detector signal (RF) is mixed with a local oscillator (LO) that has a frequency offset from the RF. This mixing returns a sinusoidal intermediate frequency (IF). In *superheterodyne*

architectures, this IF is processed and mixed again (or rectified), this time to DC for output [55]. In contrast, “homodyne” mixing implies that $f_{LO} = f_{RF}$ such that $f_{IF} = 0$. The benefit of a nonzero f_{IF} is that DC noise is suppressed, and there are a vast selection of 1 MHz-10 GHz electronic components capable of low-noise amplification, mixing, multiplying, and filtering with extremely narrow bands thanks to the radio and microwave communications industries. Therefore, if the signal needs a significant amount of amplification or filtering, for example, it is simpler to implement at an IF than DC.

5. Nonresonant sample holders

At high EPR frequencies, the wavelengths become quite small (at 100 GHz, $\lambda = 3$ mm). As a result, it is impractical to design single-mode resonant cavities for the purpose of enhancing the B_1 field strength at the sample space. Many high-frequency spectrometers simply do not use a resonator, and instead directly place the sample onto a flat mirror at the output port of the waveguide. This has the trade-off of not amplifying B_1 , but the benefit of necessitating that B_1 is essentially maximized on the sample (boundary conditions of EM waves require a B_1 antinode at the metal-air interface [56]). Additionally, though single-mode cavities are difficult (impossible) to use with such small sample volumes (~ 1 mm³), there are clever techniques for multimode cavities that allow B_1 amplification at these wavelengths; for example, photonic bandgap resonators [57], Fabry-Pérot-type resonators [43, 58], and even non-resonant dielectric stacks [59].

1.2 Continuous wave EPR

Continuous wave EPR (CWEPR) implies that the sample is constantly irradiated with EM waves and the magnetic field is swept to record an absorption spectrum. Typically,

this is what is reported in the literature; though it is usually shown as a “derivative” or “first harmonic” spectrum due to lock-in amplification. CWEPR relies on the ability of lock-in amplifiers to greatly reduce noise amplitude and thereby amplify signal. They operate by modulating the signal and selecting signal that is exclusively at the modulated frequency. For a signal S , the modulated signal S' , is given by

$$S' = S \cos \omega_{mod} t. \quad (1.92)$$

To detect the signal of interest, the lock-in amplifier mixes S' with $\cos \omega_{mix}$ and $\sin \omega_{mix}$ to get a lock-in phase dependent signal.

$$S_{LI} = S' e^{i(\omega_{mix} t + \phi)} \quad (1.93)$$

$$= S \cos \omega_{mod} t [\cos (\omega_{mix} t + \phi) + i \sin (\omega_{mix} t + \phi)] \quad (1.94)$$

$$= S [\cos (\omega_{mod} t) \cos (\omega_{mix} t + \phi) + i \cos (\omega_{mod} t) \sin (\omega_{mix} t + \phi)] \quad (1.95)$$

$$= \frac{S}{2} [\cos ([\omega_{mod} + \omega_{mix}] t + \phi) + \cos ([\omega_{mod} - \omega_{mix}] t - \phi)] \quad (1.96)$$

$$+ i \frac{S}{2} [\sin ([\omega_{mod} + \omega_{mix}] t + \phi) - \sin ([\omega_{mod} - \omega_{mix}] t - \phi)] \quad (1.97)$$

Then, since the lock-in is producing ω_{mod} , it can set the mix frequency $\omega_{mix} = \omega_{mod}$. Therefore, one mixed frequency is DC and the other is at $2\omega_{mod}$ (and DC noise is at $\omega_{noise} = -\omega_{mix}$). Low-pass filtering returns the signal of interest:

$$S_{LI}^{LP} = \frac{S}{2} [\cos \phi + i \sin \phi] \quad (1.98)$$

Now, this signal can be phased to contain the entire EPR signal in one channel (multiply by $e^{-i\phi}$) or both channels can be summed in quadrature to acquire the magnitude ($|S_{LI}^{LP}| = \frac{S}{2}$). Because the lock-in selects the modulated signal amplitude as a function of modulation field, the output spectrum is the effect of one upon the other. Therefore,

the CWEPR signal due to lock in modulation S_{CW} is:

$$S_{CW} \propto \frac{\Delta S}{\Delta B_{mod}} \quad (1.99)$$

where Δ represents the magnitude of the modulation. Assuming the Δ is small, this is the definition of the discrete derivative (see Fig. 1.11). Since it is a *discrete* derivative, an EPR spectroscopist must take caution to not “overmodulate”, which means the modulation field strength is too large to obtain a lineshape that accurately represents a derivative: if ΔB is too large, the “derivative” ends up as the average slope over the range ΔB , not an approximation of the instantaneous slope. However, this effect can be useful: the modulation field-induced broadening can be used to calibrate the field strength of the modulation coil as a function of input current. As shown in Fig. 1.9, increasing coil input current overmodulates and distorts the lineshape. The peak-to-peak distance in units of field (ΔB_{pp} , often called the CWEPR “linewidth”) increases linearly with input current. The slope of the linewidth vs. current relationship provides the calibration curve for a given coil, in units of mT/mA, as is demonstrated by Fig. 1.9.

Even though the fit shown in Fig. 1.9 is assumed to be linear, that is only the case if $\Delta B_{mod} \gg \Delta B_{pp}$ —there is a more complex equation to fit the full current dependence that can be found in [60, 61], for example. For completeness, it is written here:

$$\Delta B_{pp}^{obs} = \Delta B_{pp} \left\{ \left(\frac{\Delta B_{mod}}{\Delta B_{pp}} \right)^2 + 5 - 2 \left[4 + \left(\frac{\Delta B_{mod}}{\Delta B_{pp}} \right)^2 \right]^{1/2} \right\}^{1/2}. \quad (1.100)$$

Of course, for the case $\Delta B_{mod} \gg \Delta B_{pp}$, this can be simplified to

$$\Delta B_{pp}^{obs} \approx \Delta B_{pp} \left\{ \left(\frac{\Delta B_{mod}}{\Delta B_{pp}} \right)^2 - 2 \left(\frac{\Delta B_{mod}}{\Delta B_{pp}} \right) \right\}^{1/2} \quad (1.101)$$

$$\Delta B_{pp}^{obs} \approx \Delta B_{mod}, \quad (1.102)$$

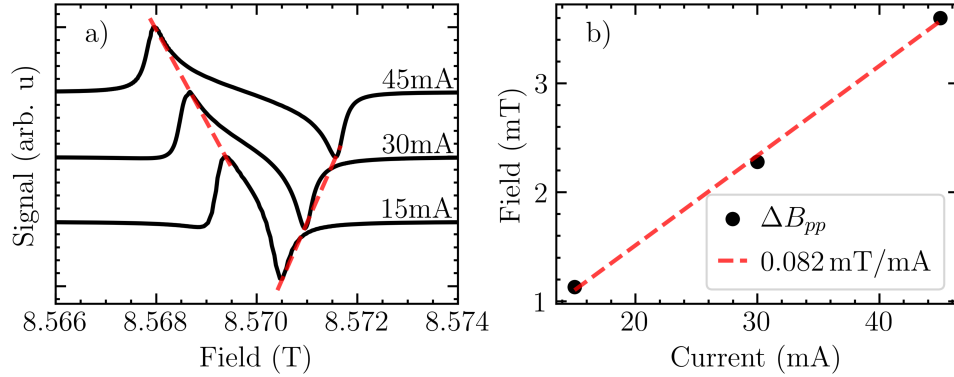


Figure 1.9: Effect of overmodulation on the linewidth of a very narrow (~ 0.2 mT intrinsic linewidth) crystal of LiPC. (a) Derivative-mode lineshapes of LiPC with three difference input currents (from bottom to top, 15 mA, 30 mA, 45 mA). Here, even the lowest current setting has distorted the intrinsically Lorentzian lineshape. (b) Raw linewidths (black) and a linear fit (dashed red) that returns the modulation field strength as a function of input current. In this case, the fit returns $m = 0.082 \pm 0.007$ mT/mA where the error represents a 95% confidence interval to the fitted parameter.

as expected given the quality of the linear fit shown in Fig. 1.9.

It is worth mentioning here that the effect of modulation (and even overmodulation) may be simulated by simple convolution of the EPR spectrum with a Bessel function of the first kind (see *e.g.*, [62]). Since convolution is equivalent to multiplication in Fourier space,

$$X(Z) = \mathcal{F}\{x(k)\} \quad (1.103)$$

$$x_{mod} = Ai^n \mathcal{F}^{-1} \left\{ X(Z) J_n \left(\frac{\Delta B_{mod} Z}{2} \right) \right\}, \quad (1.104)$$

where A is a scaling constant; i is the imaginary unit; $x(k)$ is the discrete version of the EPR spectrum, $S(B)$; $X(K)$ is the Fourier space spectrum; J_n is the n -th order Bessel function of the first kind (which returns the n -th order harmonic); and $\mathcal{F}$ is the discrete Fourier transform. If a given spectrum is overmodulated, this principle can be applied to deconvolve the true derivative spectrum from the overmodulated one.

CWEPR has many benefits, including its extreme sensitivity as a result of induction mode and lock-in detection, as well as the ability to sweep arbitrarily broad resonances (limited only by the magnet’s sweep range) [63]. However, it does have some drawbacks, including (1) the effects of saturation (discussed below) and (2) the inability to obtain relaxation lifetimes of the spins within a sample; for single-molecule magnet qubit candidates, for example, these metrics are critical. Pulsed and rapid-scan EPR provide access to them.

Saturation effects

EPR signals result due to a difference in population between the ground and excited state(s). When a spin in the ground state is excited, it absorbs a photon. When a spin in the excited state decays, it emits a photon of the same wavelength. As a result, if the two populations are exactly equivalent, there will be no net absorption, and thus, no EPR signal will be recorded. However, thanks to the applied static field, these two populations are not equal, and their ratios are governed by a Boltzmann distribution:

$$\frac{n_{\downarrow}}{n_{\uparrow}} = \exp\left(\frac{\Delta E}{k_B T}\right) = \exp\left(\frac{g\mu_B B_0}{k_B T}\right). \quad (1.105)$$

Though only spin-1/2 has been considered here, high spin is similar and the lowest spin quantum numbers are the most populated (see, *e.g.* Wilson [59]). At low frequencies and fields (*e.g.* X-band, 9.6 GHz, 343 mT), $n_{\downarrow}/n_{\uparrow} \approx 1.002$, meaning only 0.2% more spins are down than up. At high field (*e.g.* 240 GHz, 8.6 T), this ratio rises to $n_{\downarrow}/n_{\uparrow} \approx 1.04$ (4% more down than up). It is often said that high-field EPR has higher sensitivity than low-field EPR—this stems from the pronounced difference in equilibrium populations. The sensitivity can be improved further by reducing the sample temperature. At 240 GHz, the “Zeeman temperature”, where $g\mu_B B_0 = k_B T$ is 11.5 K. Below 11.5 K, the population

difference grows quickly and thus, cryogenic experiments typically benefit greatly from operating as cold as possible (*e.g.* at liquid helium temperatures, 4.2 K). The relative populations can also be used to calculate the net magnetization of the paramagnetic centers under an applied static field. The magnetic moment of a sample is given by the sum of the density-weighted magnetic moments of each spin, $M = N\langle\mu\rangle$. The magnetic moment per unit volume, $\langle\mu\rangle$, is therefore:

$$\langle\mu\rangle = n_{\downarrow}\left(\frac{g\mu_B}{2}\right) + n_{\uparrow}\left(-\frac{g\mu_B}{2}\right) \quad (1.106)$$

$$= \frac{g\mu_B}{2} \left(\frac{e^{g\mu_B B/2k_B T} - e^{-g\mu_B B/2k_B T}}{e^{g\mu_B B/2k_B T} + e^{-g\mu_B B/2k_B T}} \right) \quad (1.107)$$

$$= \frac{g\mu_B}{2} \tanh\left(\frac{g\mu_B B}{2k_B T}\right). \quad (1.108)$$

The total magnetic moment M is then (see Fig. 1.10) [64]

$$M = \frac{N g \mu_B}{2} \tanh\left(\frac{g \mu_B B}{2 k_B T}\right). \quad (1.109)$$

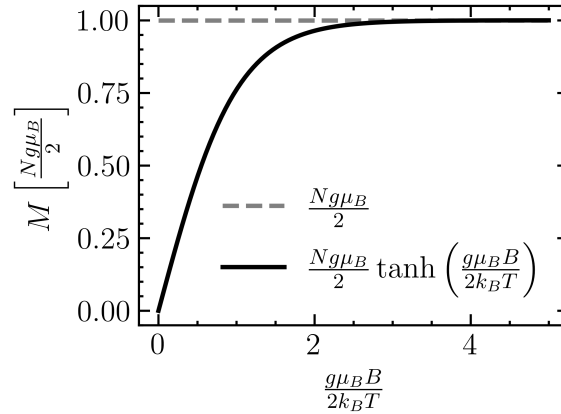


Figure 1.10: Simulation of the net magnetic moment of a spin-1/2 paramagnetic sample in a magnetic field. The magnetization approaches complete polarization ($M = N\mu_B$) quickly, reaching near saturation around $g\mu_B B/2k_B T \approx 3$.

When the sample is irradiated on resonance, transitions are induced from down-to-up via absorptive excitation and up-to-down via radiative decay. This is governed by

equilibrium kinetics:

$$\frac{dn_{\downarrow}}{dt} = -kn_{\downarrow} + k'n_{\uparrow} \quad (1.110)$$

$$\frac{dn_{\uparrow}}{dt} = -k'n_{\uparrow} + kn_{\downarrow} \quad (1.111)$$

where k and k' are rate coefficients for each transition. The solutions are [65]:

$$n_{\downarrow} = \frac{k' + ke^{-(k+k')t}}{k' + k} n_{\downarrow}(0) \quad (1.112)$$

$$n_{\uparrow} = 1 - n_{\downarrow} = 1 - \frac{k' + ke^{-(k+k')t}}{k' + k} n_{\downarrow}(0) \quad (1.113)$$

Where k , the rate of down-to-up transitions, is necessarily dependent on source power ($k = k(P)$, where higher power induces more transitions). Note that k' must be proportional to T_1^{-1} , as T_1 is the timescale with which the spins relax back to ground state from the excited state. As long as $k(P) \ll k'$, there is no saturation; however, if $k \sim k'$, a new equilibrium is established and the amount of absorption that can be observed by EPR is reduced [63]; in fact, if $k' = k$, no absorption is detected at all (and the “spin temperature”, which relates the population distribution to an intrinsic temperature, is infinite).

Saturation can be conceptualized in another way using eqn. (1.85): if the source power is large, B_1 in eqn. (1.85) is also large, and $\gamma^2 B_1^2 T_1 T_2 \sim 1$, which reduces the peak height and smears out the Lorentzian lineshape, as is typical for saturated EPR spectra.

1.3 Pulsed EPR

Pulsed EPR is the most common of the three techniques at low field. Microwave technologies have made high-power, frequency-tunable sources ubiquitous, and thus, even field-swept EPR is typically done in pulsed mode. As mentioned above, the relaxation

lifetimes, T_1 and T_2 are often of particular interest; a long T_2 a critical property of qubit candidates, for example. T_2 is usually defined as the spin-spin coherence time, which is the timescale with which a qubit can coherently store quantum information.

Pulsed EPR allows experiments that sweep not only magnetic field, but also pulse timings, allowing for complicated sequences that provide information in two dimensions, field (or frequency) and time. Two simple experiments are discussed here: (1) one-pulse free induction decays and (2) two-pulse Hahn echoes.

1. One-pulse free induction decays

A single, high power pulse can rotate the Bloch magnetization of a sample [66] about an axis perpendicular to the applied static magnetic field B_0 . In the rotating frame, where $B_0 = 0$, a powerful pulse introduces a static magnetic field along some direction in the plane perpendicular to B_0 (and parallel to $M_\perp$ from eqn. (1.60)). This rotation angle comes from the precession about the pulse direction, and thus, is given by $\theta = \omega_{nut}t$, where ω is called the “nutaton” frequency. This frequency is really a Larmor precession frequency, but is called ω_{nut} to distinguish it from the precession about B_0 .

$$\omega_{nut} = \gamma B_1 \tag{1.114}$$

Back in the lab frame, if a pulse is applied that rotates the Bloch magnetization 90° , the magnetization is now in the $M_\perp$ plane, and the spins emit dipolar radiation that is circularly polarized in the direction of the B_0 Larmor precession—output circular polarization is partially reflected by the induction mode isolators, and thus, results in an EPR signal called a “free induction decay”, or FID (since the source is off after a pulse, all power detected is dipolar radiation). This dipolar radiation is dampened with lifetime T_m , the “phase memory time”, which includes T_2 relaxation but also includes coupled-

spin effects like spin diffusion; as all the spins dephase, the sum of their dipolar radiations destructively interfere. If T_m is long ($T_m \approx T_2 \approx T_1$), the signal is still dampened on the timescale of T_m , but this dampening results from the magnetization along $M_\perp$ relaxing back to M_z . Since M_z produces no dipolar radiation in the transverse direction, it does not modify the input polarization and thus is not detected in an EPR experiment.

2. Two-pulse Hahn echoes

Hahn echo experiments leverage the spin precession generated by FIDs. After an initial $\pi/2$ pulse, the spins precess, dephase as a result of local fields inducing small variations to each spin's ω_{nut} , and decohere through T_m relaxation. Next, after a delay τ , a π pulse is applied that inverts the direction of Bloch magnetization (and therefore the precession direction). Since each spins precession frequency is stable (ignoring stochastic T_m processes), the spins will precess *back* to their initial state τ time after the $\pi/2$ pulse, and emit coherent dipole radiation—called an “echo”. However, the longer the time delay τ between the $\pi/2$ and π pulses, the more T_2 and T_m relaxation that can occur, and thus, the lower the resulting echo amplitude. Two-pulse Hahn echo experiments will often be used to measure the T_m of a spin system. In such an experiment, the amplitude of the echo is recorded as a function of τ . This profile is then fit to a decaying exponential $A = A_0 \exp(-2\tau/T_m)$, which returns T_m [66]. Though T_m is not a direct measurement of T_2 , it is a useful experimental analog that requires less complicated pulse sequences than those that recover relaxation times closer to the true T_2 (such as the CPMG sequence, for example [67]).

Pulsed EPR benefits immensely from high power sources. The B_1 of an applied pulse scales as the square root of pulse power, $B_1 \propto \sqrt{P}$. This means that rotations can be imparted with shorter pulses, and thus, shorter relaxation times can be probed. Since

powerful sources are well-developed at low frequency, so is pulsed EPR. Conversely, at high frequency, high-power pulsed EPR is quite difficult. There are a few sources capable of high powers at high frequencies (*e.g.* gyrotrons and FELs [68, 69]), but each come with difficulties, one of which is that it is very difficult to create flexible pulse sequences. A lot of the technology used to create high-frequency pulses at low powers either (1) has a low damage threshold, (2) does not provide sufficient contrast ratios to have well-defined “off” states with zero spin excitation, or (3) is not fast enough to create pulses with sharp rising and falling edges [70]. THz modulation techniques, and a potential solution to these problems, are discussed in greater detail in sec. 3.5.3.

1.4 Rapid-scan EPR

The final of the three “main” EPR techniques is rapid-scan EPR. It is a much newer technique than the other two, though something akin to rapid-scan NMR was observed at least as early as 1948 [71]. Rapid-scan EPR relies on rapidly sweeping either the source frequency or magnetic field to record thousands of spectra per second. The data is directly digitized and averaged, providing similar time-weighted signal-to-noise as CWEPR, but recorded much more quickly, which is helpful in situations where temporal resolution is important. Additionally, because the field is swept rapidly, the frequency/field is only on resonance for a very short period of time during each sweep cycle. As a result, few spins are excited during a sweep, and they are provided ample time to relax before a new sweep is started (in most cases). This means that it is quite difficult to saturate a spin system with rapid-scan EPR, and thus, much higher source powers may be used. As a result of these higher powers, the time-weighted signal-to-noise ratio (SNR) of rapid-scan can be *much* higher than CWEPR and therefore, similar SNRs can be recorded in significantly

less time, improving the experimental throughput of the spectrometer [72, 73].

Rapid-scan EPR is recognized by its characteristic “wiggles” when the scan rate dB/dt is greater than $1/\gamma T_2^2$ [74]. These wiggles result from continued coherent precession of out-of-equilibrium spins, even after the applied field (frequency) has swept through the resonance. This Larmor precession, as the magnetic field is changing, emits dipolar radiation that has a variable frequency offset from the excitation EM wave, and thus, causes a beat frequency (the wiggles) to be observed at the detector [71, 75].

Because the rapid-scan lineshape has a strong functional form dependence on T_2 , it is often the case that T_2 may be extracted from rapid-scan studies. As a result, one can obtain both the field-swept lineshape and the spin-spin relaxation time in a single rapid-scan experiment. Though it has not yet been shown experimentally, it may be possible to also extract T_1 , assuming the power of the excitation source is quite high, since T_1 also appears in the rapid-scan Bloch equations (through discussions with M. Tseytlin in 2023 this is potentially possible with $\sim$ kW source power) [74]. Rapid-scan EPR is a major consideration of sec. 2.3 and will be discussed there in detail.

1.5 Double electron-electron resonance and other advanced EPR techniques

An extremely common modern application of EPR is pulsed electron double resonance (PELDOR), *aka* double electron-electron resonance (DEER). DEER is a technique that is used to measure distances between pairs of paramagnetic electron spins, often attached at two sites to biological samples of interest. It relies on multifrequency pulsed EPR to determine the coupling strength between the pairs (or clusters) of spins. For efficient

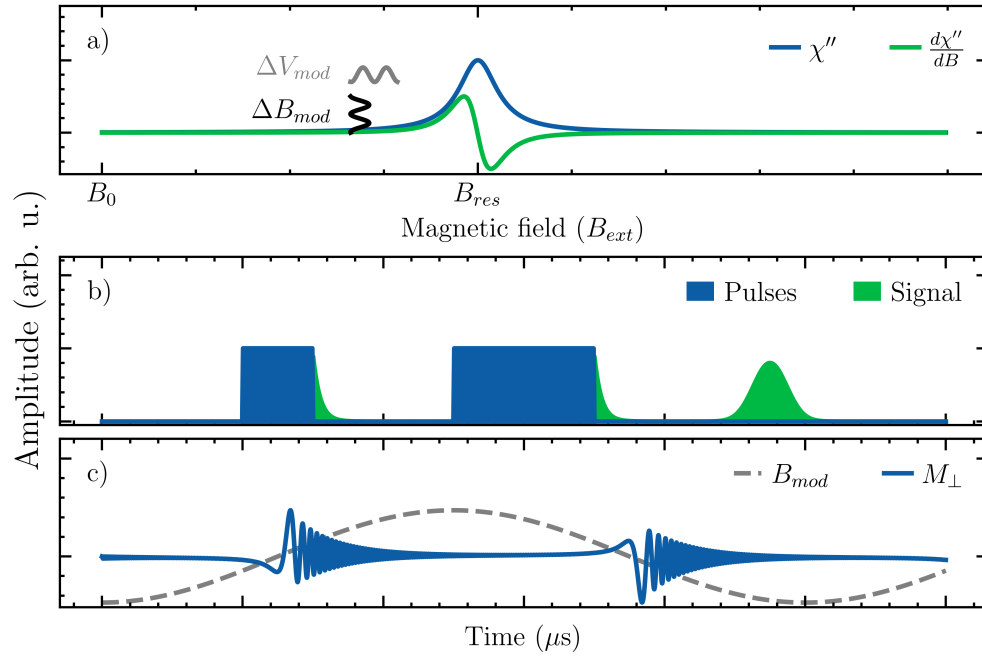


Figure 1.11: Simulations of the three most common EPR techniques. (a) CW EPR, where the raw absorptive signal (χ'' , blue) is shown with the derivative signal that is actually recorded (green). (b) Pulsed EPR depicting a Hahn echo sequence that consists of a $\pi/2$ excitation pulse and a π refocusing pulse (blue) as well as each pulse's FID and the resultant refocused echo (green). (c) Transverse magnetization ($M_{\perp}$, blue) as a function of time and modulation field (gray dashed line). Wiggles are observed because the peak sweep rate $\frac{dB_{mod}}{dt}$ is larger than $1/\gamma T_2^2$ [74].

DEER, spectrally distinct spins are required—meaning one spin of the pair can be excited while the other is not, and vice versa. A standard PELDOR experiment consists of a Hahn echo sequence at one frequency (ν_A), a delay τ , and a third refocusing π pulse at the same frequency. The result of this sequence is an echo with amplitude that depends on the length of τ . Now, if an additional π pulse at a different frequency (ν_B) is inserted into the τ delay window, the Bloch magnetization of B-spins are flipped, which modifies the static magnetic field B_{eff} felt by the A-spins, assuming the A and B spins are through-space coupled through the dipolar interaction (see sec. 1.1.1 and Fig. 1.12). This modification of the A-spin resonance by a B-spin causes ν_A to no longer be on resonance, and thus, reduces the amplitude of the A-spin echo. The strength of this effect depends only on the distance between the two spins, r_{AB} . Recovering the distance distribution $P(r_{AB})$ from the echo response as a function of t is an ill-posed problem, but has been done using Tikhonov regularization and machine learning-based techniques to great success [76, 77].

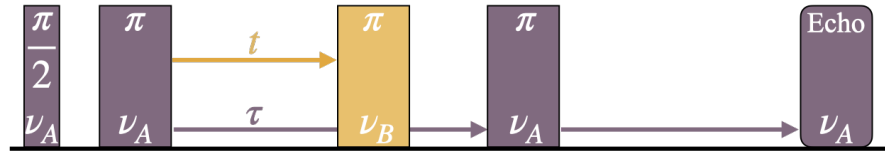


Figure 1.12: Four-pulse DEER sequence. A-spins undergo a Hahn echo ($\pi/2 \dots \pi$) sequence, then a dipolar mixing time τ , before a final refocusing π pulse that refocuses and generates the final echo. B-spins undergo a π -pulse during the τ window such that the inversion of the B-spins magnetization has an effect on the ability to refocus the A-spins, assuming they are dipolar-coupled.

There are a wide variety of pulsed, multifrequency EPR techniques that are similar to DEER but probe different couplings. Electron-nuclear double resonance spectroscopy (ENDOR), for example, probes the effect of nuclei coupled to electron spins; Mims ENDOR is very similar to DEER, but rather than a short π pulse at ν_B , it has a long, RF excitation pulse at the resonance frequency of a nucleus of interest [78]. The num-

ber of possible pulse sequences, experiments, and acronyms are vast: DEER, ENDOR, RIDME [57], ESEEM [79], etc. The list goes on. For an incomplete reference to many of these techniques, the reader is directed to a wonderfully thorough online textbook about magnetic resonance, *eMagRes* [80].

The last topic discussed in this section is dynamic nuclear polarization (DNP) due to its immense promise and applications (especially for protein science) at high fields. Initially conceptualized by Overhauser [81] in 1953, DNP relies on the hyperfine coupling-mediated cross-relaxation between electrons and nuclei to transfer the extreme polarizability of electrons at high field to the much less polarizable nuclei (due to their vastly different magnetic moments $\gamma_e/\gamma_n \approx 658$). To achieve such a result, the electron spins must be saturated via high-power CW (or repeatedly pulsed) EM waves that correspond to the electrons' resonance frequency at high field. With an optimized DNP medium, the electron polarization may be transferred to the nuclei, and thus, increase their polarizability, enhancing the signal strengths (and thereby also the SNR) of NMR experiments by well over $100\times$ [82]. Today, DNP development is an extremely rich field that includes research on polarizing agents; developing pulsed, high-power EPR sources; and studying DNP mechanisms, including the eponymous "Overhauser effect", solid effect, and cross effect. Rather than reviewing these topics here, the curious reader is directed to one of the many reviews on the topic (*e.g.* [82–92]). The enhanced spectral resolution provided by DNP allows for more precise studies of structural biology using NMR. Though recent work has highlighted the potential of solution-state DNP [93], to maximally benefit from DNP for structural biology studies, experiments must be performed at cryogenic temperatures to (1) permit magic-angle spinning that improves sensitivity by reducing broadenings (2) quench thermal fluctuations to reduce structural noise (3) maximize the polarizability of the electrons within the sample.

Chapter 2

High-field EPR for triggered functional dynamics of proteins

The impressive sensitivity of EPR, as discussed in Chapter 1, makes it an attractive technique for structural biology studies, much like the much more established NMR. To its benefit, EPR requires a smaller sample volume than NMR, and has much higher signal-to-noise at room temperature. This chapter discusses the pursuit of EPR for room-temperature studies of protein dynamics.

2.1 Introduction

2.1.1 The need for a time-resolved measurement of tertiary structure

Proteins are fundamental building blocks of life. Understanding their functions is key to understanding biological processes, and the collective effort to decode these functions

has culminated in more than 230,000 structures being logged into the Protein Data Bank [94]. The thoroughness with which static structures have been mapped leads one to begin considering a functional “movie”—observing multiple biologically relevant amino acid sites move in real time—which can be combined to create a 3D rendition of site-specific motion. However, most state-of-the-art structural biology tools require the protein to be immobilized (cryo-electron microscopy, solid-state NMR, or double electron-electron resonance—DEER) or mechanically inhibited (X-ray crystallography), which may cause a significant amount of information to be lost (*e.g.*, time-dependence, environmental effects, pH effects) [95, 96]. By rapidly freezing an ensemble of proteins after triggering a conformational change, several structural biology tools have been used to elucidate the triggered functional dynamics of proteins [97–99]. However, it has been shown that freeze-quenching does change the structure of proteins (see, *e.g.*, [100]) and depends quite strongly on the amount of time required for freezing. Because a sample cannot be frozen instantaneously, it is not possible to capture the true solution-state configuration, though microsecond freeze times should be very close. To avoid these complications, ideally, a “movie” would be “filmed” in real-time, by *in vitro* tracking of the positions of residues at (or near) physiological temperatures in the solution state.

Pioneering work of Steinhoff and Hubbell demonstrated that continuous-wave electron paramagnetic resonance at X-band (9.5 GHz, 0.35 T) can be used to report on site-specific structural changes in proteins upon activation under physiological conditions [101–103]. Such experiments made use of nitroxide-based labels with anisotropic g and hyperfine tensors that provide extensive knowledge about the local environment in which a spin label resides. Specifically, the label tumbling rate reveals the rigidity and the local spatial constraints of the spin labeled residue. When triggered mechanical activation alters the local rigidity and environment, the CW-EPR lineshape can resolve these changes to re-

port on protein conformation. Unfortunately, the sensitivity of nitroxide-based CWEPR lineshapes to the local environments of the nitroxide spins makes it challenging to extract distance information from the small contribution of dipolar broadening to the spectra of proteins doubly labeled with nitroxide probes [26, 104]. Pulsed electron dipolar spectroscopies such as DEER solve this problem by isolating the dipolar interaction from other factors, and hence are widely used for measuring distances between protein residues [105–109], but, with a few exceptions (*e.g.*, [110]), are always performed at cryogenic temperatures.

In summary, the tools available for studies of tertiary (the 3D shape of the protein) are wonderfully well-developed at cryogenic temperatures (and therefore in the solid state); in contrast, few tools are capable of these studies at room temperature, in the solution state. As a result, there is a significant need for an EPR-based technique that leverages the impressive spectral sensitivity of high-field EPR to perform such studies. The first attempt at such a technique is presented below.

Nitroxide-based spin labels

Nitroxide-based spin labels are used ubiquitously throughout the EPR literature for biological studies of protein (secondary) structure (specifically, the label is often MTSSL, sometimes called “R1” in the literature) [18, 25, 28, 103, 108, 109, 111–119]. These labels are attached to protein sites of interest using site-directed spin labelled (SDSL) and provide information on site-specific dynamics. The ligand design of these probes is an entire scientific field on its own, but in most cases, the linker that allows the N-O molecule to be attached to a protein is not rigid. It allows for bending and rotation, both of which are on the timescale of typical EPR experiments and modify the resulting lineshape. Further, because the unpaired electron is shared between the nitrogen and

oxygen atoms, its orbit is anisotropic about the N nucleus with nonzero spin ($S_I^N = 1$). Therefore, orientational information is captured within the EPR spectrum. Such labels are well-equipped for biological studies (and others that require long acquisitions) due to their chemical stability despite their radical nature [120], and their relatively small size that affects protein motion very little [116]. The majority of DEER experiments are done using nitroxides with these benefits in mind, and numerous solution-state CWEPR experiments have also been performed to understand site-specific dynamics (*i.e.* site mobility) of proteins [101, 121–123]. However, because nitroxide lineshapes are anisotropic due to g -anisotropy and nuclear hyperfine interactions (see *e.g.* Fig. 2.1)—and their rapid linker motion reduces relatively weak dipolar couplings—dipole-dipole distance measurements beyond 2 nm are not possible with CWEPR in the solution state [114]. The radius of a protein may be roughly estimated by $r \approx 0.66 \text{ nm/kDa}^{1/3}(M)^{1/3}$ [30]; to make studies of solution-state distance dynamics possible, a protein must be smaller than $\sim 25 \text{ kDa}$ —without even considering the additional distance added by the labels—meaning that the majority of proteins too large for such studies. DEER is able to work around this effect, as the experiment is designed to be sensitive to only dipolar couplings, extending it to 6 nm or more, but solution-state CWEPR studies cannot access distances nearly that far [124].

2.1.2 Gadolinium at high field

In contrast, gadolinium (III) spin labels at high field have been shown to be sensitive to dipolar couplings up to 4 nm at 8.6 T [14]. For high-field EPR applications measuring biological distances, Gd(III) has multiple benefits over nitroxide-based labels:

1. Gd nuclei have effectively zero hyperfine coupling to their valence electrons.

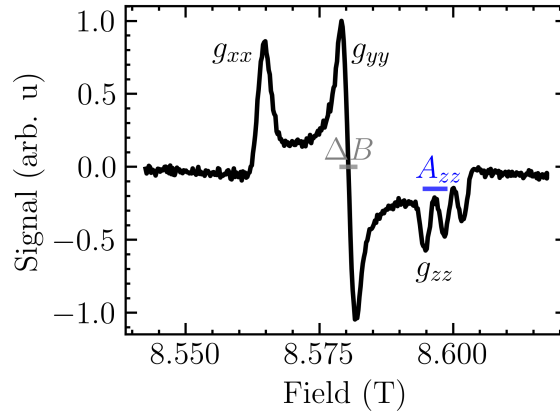


Figure 2.1: Nitroxide-based TEMPO (black) radical suspended in solid polystyrene at 0.5% w/w% at 160 K. Central g_{yy} transition $\Delta B = 2.5$ mT peak-to-peak (gray horizontal line). A_{zz} (blue horizontal line) is the largest component of the hyperfine tensor, measured to be roughly 3.8 mT (106 MHz). Spectrum recorded by JS.

2. Gd spin label ligands can be designed to be very isotropic.
3. Gd(III) is an f -block, high spin ($S = 7/2$) system.
4. Broadening as a result of its zero field splitting is *reduced* at high field.

1. Gd(III) hyperfine interaction

The majority of stable ${}^{64}\text{Gd}$ isotopes have an even number of neutrons and thus, have no spin (${}^{154}\text{Gd}$, ${}^{156}\text{Gd}$, ${}^{158}\text{Gd}$, ${}^{160}\text{Gd}$). The two stable isotopes that do not have nonzero spin are ${}^{155}\text{Gd}$ and ${}^{157}\text{Gd}$, but their relative abundance is small $\sim 30\%$ and their hyperfine coupling strength is weak (~ 16 MHz). Therefore, the hyperfine broadening of Gd(III) is typically unresolved in experiments, and only contributes a small amount to the intrinsic linewidth [14, 125].

2. Gd(III) isotropic lineshape

The lineshape of Gd(III) spin labels can be engineered to be particularly isotropic (see Fig. 2.2 for an example). With these labels, the electron spin is localized about the Gd nucleus and not the ligand, which contributes to the small hyperfine interaction and narrows the label-label distance distributions recovered from DEER (or CWEPR) [126]. Further, the linkers required for SDSL can be quite short so that the position of the ligand is well-defined relative to the protein (*e.g.* only three bonds, [29], see Fig. 2.3).

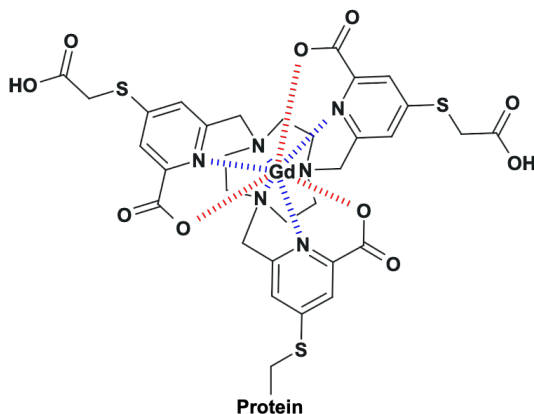


Figure 2.2: Gd-sTPATCN spin label. The spin label is engineered to be highly isotropic with a short, rigid linker that reduces the available rotamers when attached to a site of interest [29]. Figure made with Marvin (version 24.3.139) [127].

4. Gd(III) high spin

The high spin ($S = 7/2$) of Gd(III) is advantageous for SDSL and biological studies. Typically, such studies have limited sensitivity as a result of spin concentration. High-spin labels, however, can improve the sensitivity of EPR experiments by increasing the transition probability (and therefore amount of absorbed microwaves) between spin sublevels. An EPR transition from a lower spin sublevel to a higher spin sublevel is proportional to $\langle m' | \hat{S}_+ | m \rangle$, where $\hat{S}_+$, the raising operator discussed in sec. A.2, is provided by a

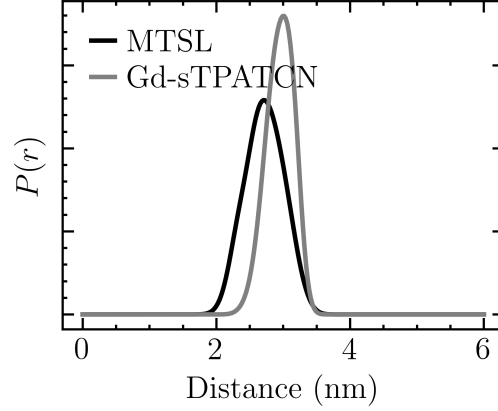


Figure 2.3: **chiLife** [128] simulations of MTSL (a well-known nitroxide-based label) and Gd-sTPATCN (a Gd(III)-based label, [29]) spin labels attached to a protein domain (AsLOV2, PDB 2V1A, [129]). The rigid, short linked of Gd-sTPATCN provides a narrower distance distribution than MSTL. Both simulations used off-rotamer sampling and 5000 samples [130].

transverse magnetic field ($B_1 \propto \sigma_{x,y}$).

$$\langle m' | \hat{S}_+ | m \rangle = \delta_{m',m+1} \hbar \sqrt{(S-m)(S+m+1)} \quad (2.1)$$

When $S = 1/2$, $\langle m' | \hat{S}_+ | m \rangle = \langle 1/2 | \hat{S}_+ | -1/2 \rangle = \hbar$; when $S = 7/2$, the matrix element for the central transition is $\langle m' | \hat{S}_+ | m \rangle = \langle 1/2 | \hat{S}_+ | -1/2 \rangle = 4\hbar$. According to Fermi's golden rule, the transition probability is proportional to this matrix element [131]. Therefore, the EPR signal from the central transition of spin-7/2 electrons is four times stronger than the central (only) transition of spin-1/2 electrons.

Additionally, the spin-spin dipolar coupling of high-spin labels is stronger than spin-1/2 and thereby provides access to longer distances. In a high-spin ensemble, the majority of the spins coupled to a probe spin will have $|m_s| \neq 1/2$; at high temperatures, all spin sublevels will be equally populated, and at low temperatures, the lowest spin sublevels (with $|m_s| > 1/2$) will be most populated. Therefore, the average spin of the ensemble is $\overline{|m_s|} > 1/2$, and according to eqn. (1.22), the dipole-dipole coupling is stronger than two coupled spin-1/2s and provides sensitivity to longer distances.

4. Gd(III) zero-field splitting

A critical feature of Gd(III) lineshapes, especially for SDSL distance measurements, is a significant strain of zero field splitting values [132, 133]. This strain (spread of observed values) causes a broad distribution of energy levels observed in the spectral lineshape. However, it is a feature of the ZFS Hamiltonian that the central transition energy does not depend on the ZFS parameters, to first order in perturbation theory. All other transitions *do* depend on this term, and thus, the strain impacts a “smearing” of their transitions into a very broad background. To determine the effects of ZFS on the central transition, it is necessary to carry out perturbation theory to second order. In this case, the central transition energy splitting scales as D^2/B_0 , where D is the axial ZFS parameter (see Appendix C of [134] for the second-order treatment). Therefore, as the field is increased, the linewidth *narrows*. This effect was confirmed up to around 250 GHz, where the line begins broadening once again due to competing g -anisotropy that is unresolved at lower frequencies [135]. This feature alone makes Gd(III)—at high fields, in particular—enticing for SDSL studies of protein structure. The extremely narrow central transition at high fields (~ 5 G at 8.6 T) allows for coupling sensitivity to much longer-range dipolar couplings than the 2 nm that can be accessed with nitroxides [14].

In all, these four effects have made Gd(III)-based labels particularly useful for DEER-based distance measurements of SDSL proteins [117, 119, 136–141]—the extension of those techniques to room temperature and solution state is a natural step for the field.

2.2 CW TiGGER of AsLOV2[†]

A blue light-activated phototropin domain from *Avena sativa* (AsLOV2) was selected to demonstrate the technique. This domain is of interest to the optogenetic community because of its functional application as a light-activated micro-machine [143–147].

2.2.1 Results

For the purposes of a CW time-resolved Gd-Gd EPR experiment at high field (dubbed “TiGGER”), the following residues of AsLOV2 were chosen: one on the J α -helix (near the C-terminus, site 537) that is expected to unfold and move significantly upon light activation, and one on the A α -helix (near the N-terminus, site 406) that was expected to remain relatively still [129, 148]. The two sites were mutated to cysteines (mutations T406C and E537C) and subsequently spin-labeled with MTSL and Gd-sTPATCN. Numerical simulations on Multiscale Modeling of Macromolecules software (version 2018.2) show that according to the protein’s crystal structure, nitroxide-based MTSL labels attached to residues 406 and 537 would be roughly 2.6 nm apart; this distance in the solution state is not known for certain, but is expected to remain similar (± 0.4 nm) [149, 150]. As shown in Fig. 2.3, a pair of Gd-sTPATCN labels at 406 and 537 are expected to be slightly further apart (around 3 nm). Upon light activation in the solution state, it was expected that sites 406 and 537 move apart and that TiGGER would be sensitive to that motion [148, 151].

The photoresponse of spin labeled AsLOV2 was first established using UV-Vis spectroscopy. As shown in Fig. 2.4b, the spectrum changed most dramatically at 447 nm

[†]The text and many of the figures in secs. 2.1-2.2 are adapted from [142]. Reprinted with permission according to Wiley’s Rights & Permissions guidelines.

while under blue (450 nm) light illumination due to changes in the local environment of AsLOV2's flavin mononucleotide (FMN) chromophore. Time-dependent UV-Vis measurements (Fig. 2.4c) clearly show that the protein is photoswitching and relaxing as a result of this illumination, and that site directed mutagenesis and spin labeling (SDSL) at sites 406 and 537 does not affect the photoresponse of AsLOV2.

To confirm that the protein mechanically actuates, and that the actuation is correlated to its photoresponse, EPR spectra of MTSL-labeled AsLOV2 were recorded at X-band. The X-band spectra showed changes between the light on and off states, which corresponded to a change in MTSL's local environment (see Fig. 2.4d). Time-resolved X-band EPR experiments showed mobility relaxation times ($65\text{ s} < \tau < 81\text{ s}$) that were similar to optical absorption relaxation times as obtained from UV-Vis spectra ($\tau = 65.00 \pm 0.03\text{ s}$), confirming that the protein is responding mechanically to light activation (Fig. 2.4e). There was no distinguishable difference between singly and doubly MTSL-labeled samples, which implied that the changes seen in the CWEPR spectral amplitude under light activation were not due to changes in the inter-spin label couplings, but instead to changes in the local environment of the individual MTSL spin labels. Such changes were expected, as the J α - and A α -helices unfolding could readily alter the tumbling rate, anisotropy, and, potentially, tertiary contact of the MTSL spin label. This observation illustrates the need for spin labels sensitive to intra-protein distance-dependent dipolar coupling.

It is important for TiGGER that the sample contains a minimal fraction of singly labeled proteins. To this end, an already spin-labeled preparation of double mutants was incubated with biotin-maleimide and filtered with a streptavidin-agarose resin, which removed any proteins with free cysteines (*i.e.*, imperfectly labeled, see Fig. 2.5a for a cartoon of this process). After filtering, all samples had a protein concentration of

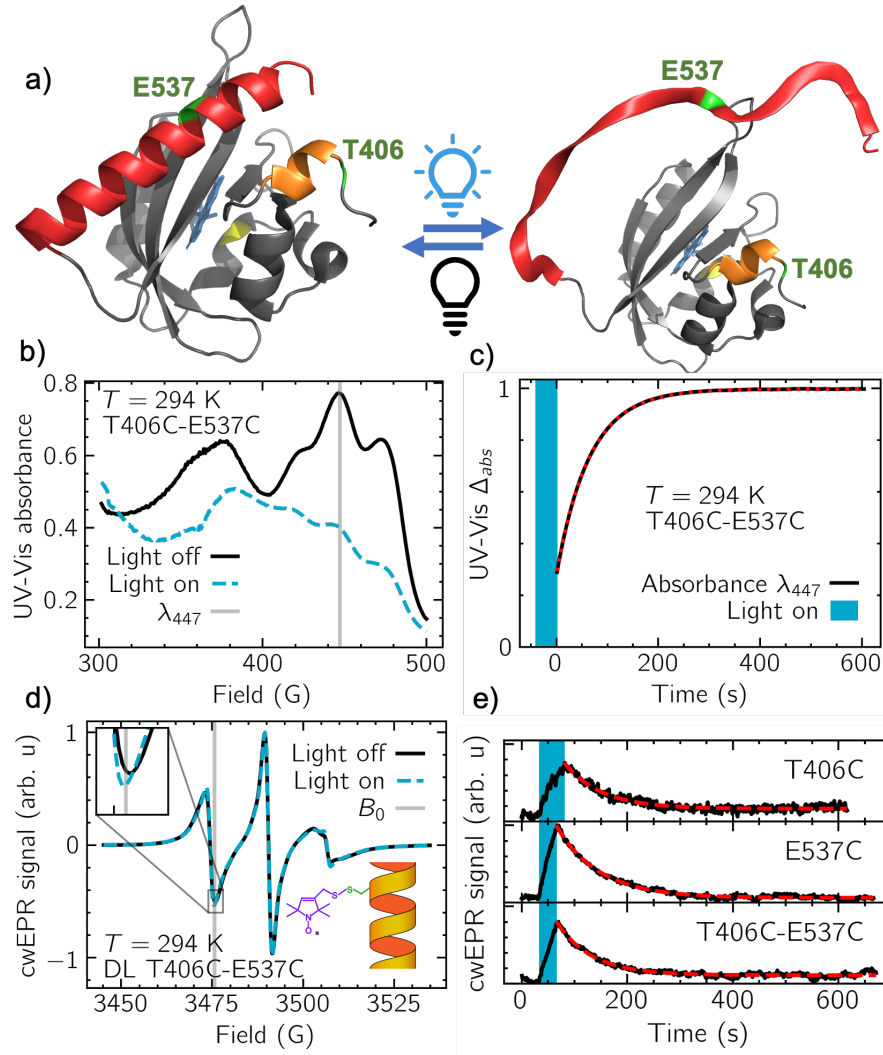


Figure 2.4: Photoresponse of AsLOV2. (a) PYMOL(v2.5.2, [152])-generated structure demonstrating AsLOV2 structural change that occurs after 450 nm illumination [129, 153]. Residues 406 and 537 are marked green on the A α (N-terminus, orange) and J α -helices (C-terminus, red), respectively. (b) UV-Vis absorption spectra of AsLOV2 T406C-E537C with (dashed blue line) and without (solid black line) blue light activation. The vertical gray line indicates the wavelength at which the lifetime of the protein's FMN chromophore was measured. (c) The lifetime of the chromophore's excited state after activation was measured with UV-Vis absorbance at 447 nm ($\tau = 65.00 \pm 0.03$ s). (d) X-band CW-EPR spectra of AsLOV2 MTSL-labeled at sites 406 and 537. (e) Transient X-band EPR demonstrating similar results for singly and doubly MTSL-labeled AsLOV2. Exponential fits returned $\tau_{T406C} = 70.1 \pm 1.3$ s, $\tau_{E537C} = 80.5 \pm 0.6$ s, $\tau_{T406C-E537C} = 66.2 \pm 0.8$ s. Transient measurements were performed near 3475 G (± 1 G), at the field where each spectrum changed the most.

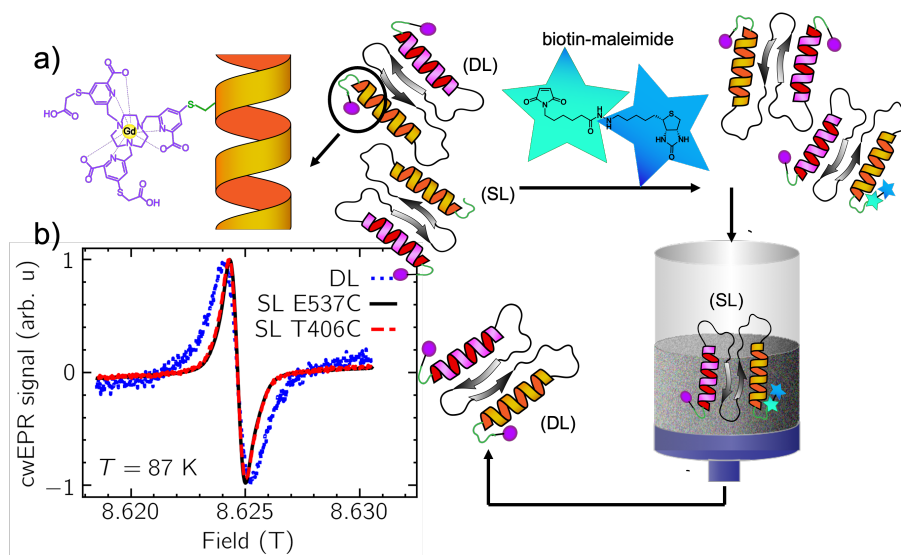


Figure 2.5: Site-directed spin labeling of AsLOV2 with Gd-sTPATCN. (a) Enrichment of DL AsLOV2. The reaction products from the spin-labeling are mixed with biotin maleimide. Singly labeled AsLOV2 with free cysteine is trapped in the column with streptavidin agarose and the fully labeled DL T406C-E537C gets through the column. (b) Comparison of 240 GHz CW-EPR lineshapes of AsLOV2 singly and doubly labeled with Gd-sTPATCN. Lineshapes are normalized to highlight dipolar broadening. The experiment was performed at 87 K to eliminate effects of motional averaging. The dotted blue line, solid black line, and dashed red line correspond to AsLOV2 samples labeled at residues 406-537, 537, and 406, respectively.

$\sim 1.5 \text{ mM}$. The sample volume for high field EPR experiments was $1.2 \mu\text{L}$, meaning roughly 1×10^5 protein domains (2×10^5 spins) were present within the sample.

To ensure that EPR was sensitive to the dipolar broadening of doubly labeled (DL) AsLOV2, 240 GHz CW-EPR experiments were first performed at 87 K such that tumbling was frozen in order to eliminate motional narrowing. Dipolar broadening was clearly observed, as is shown in Fig. 2.5b: the doubly Gd-labeled AsLOV2 EPR line has a larger peak-to-peak linewidth, as well as broader wings, than that of AsLOV2 singly labeled (SL) at sites 406 or 537. The lineshapes of the two singly labeled proteins are essentially identical, which emphasizes the insensitivity of this spin label to its local environment.

The principal results of this paper are shown in Fig. 2.6. Fig. 2.6a and 2.6b show that, at room temperature, the CWEPR spectra of singly labeled proteins at either site 406 or 537 do not change upon light activation; while the protein does activate (see Fig. 2.4c and 2.4d), the Gd-sTPATCN label is not sensitive to the associated changes. Doubly-labeled AsLOV2 samples (Fig. 2.4d), by comparison, show a significant lineshape narrowing upon light activation at room temperature. This change is attributed to a reduction in dipolar broadening between spin labels at sites 406 and 537, as these sites moved apart when the J α - and A α -helices unfolded. The results from high field with Gd spin labels are in contrast to the X-band EPR experiment presented in Fig. 2.4d and 2.4e: the lineshape change observed at X-band did not depend on whether the sample was singly or doubly labeled. This implies that TiGGER was able to directly observe a change in dipolar coupling—and therefore protein motion—after activation by an external stimulus in solution state. TiGGER of doubly Gd-sTPATCN labeled AsLOV2 yielded a time constant for relaxation to equilibrium similar to those from X-band EPR and UV-Vis spectroscopy ($\tau_{\text{TiGGER}} = 51.9 \pm 0.3 \text{ s}$, $65 \text{ s} < \tau_{\text{X-band}} < 81 \text{ s}$, $\tau_{\text{UV-Vis}} = 65.00 \pm 0.03 \text{ s}$). This observation confirms that mechanical relaxation is correlated to that of the local environment of MTSL and the triplet state of the FMN.

The internal cysteine residue C450 is intimately coupled to the chromophore. Previous studies have shown that mutation of this conserved residue leads to complete inhibition of the photocycle and suppression of the associated secondary structural changes [154, 155]. As a negative control for TiGGER, experiments were performed on DL AsLOV2 with the internal cysteine mutated to alanine (C450A). No illumination-dependent effects were observed under static or transient EPR conditions (static shown Fig. 2.7; transient shown in orange in Fig. 2.6d). UV-Vis experiments on C450A DL AsLOV2 corroborated this result and confirmed that laser illumination initiated no spectroscopic changes, consistent

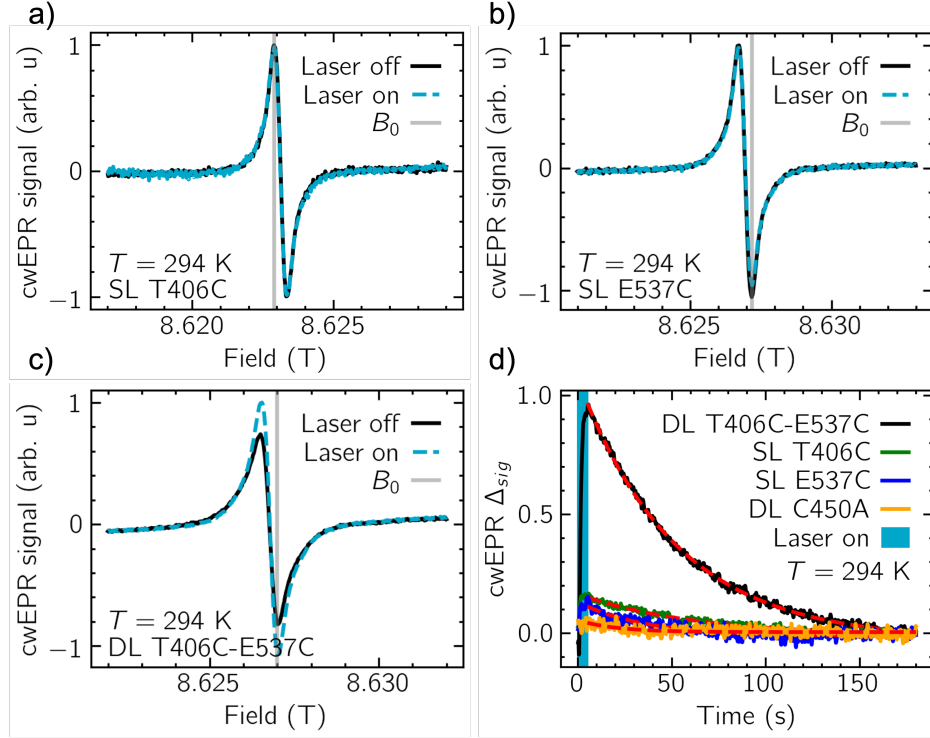


Figure 2.6: Effect of laser illumination on CWEPR spectra of Gd-labeled AsLOV2. SL CWEPR spectra of AsLOV2 residues (a) 406 and (b) 537 demonstrating that the spectrum with the laser off is unchanged when the laser is turned on. (c) DL CWEPR spectra of AsLOV2 demonstrating that the spectrum with the laser off is narrowed when the laser is turned on. Laser off spectra are shown in solid black and laser on spectra are shown in dashed blue. B_0 , where maximum time-dependent change occurred, is shown on all three plots by a vertical gray line. Note that B_0 is not the same for all three samples; the field value with maximum change was chosen for each time-dependent experiment. (d) CWEPR time-dependent signal change of SL and DL Gd-AsLOV2 due to laser illumination. Overlaid exponential fits returned time constants of $\tau_{T406C-E537C} = 51.9 \pm 0.3$ s, $\tau_{T406C} = 62.5 \pm 1.8$ s, $\tau_{E537C} = 34.6 \pm 1.9$ s, and $\tau_{T406C-E537C} C450A = 20.8 \pm 3.4$ s. All plots are normalized to the magnitude of DL T406C-E537C signal change.

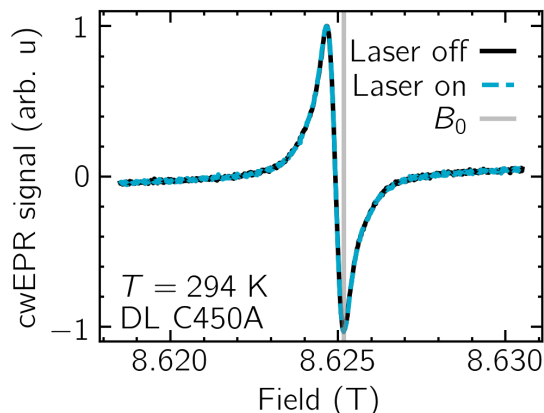


Figure 2.7: CW-EPR spectra of DL AsLOV2 with an additional C450A mutation. After the mutation of the central cysteine to a nonpolar amino acid (in this case, alanine), the light off (dashed black) and light on (solid blue) lineshapes overlapped exactly, implying that the light-activated motion had been suppressed.

with previously published results. It is therefore unambiguous that the line narrowing seen by TiGGER in DL AsLOV2 is due to a light-activated inter-label distance increase caused by photoactivation.

Additional control experiments were done with SL samples. It was expected that such samples would not show any lineshape or time-dependent change. However, as can be seen from Fig. 2.6d, both T406C and E537C SL showed small time-dependent relaxations after light activation. Initially, this effect was attributed to nonspecific double-labeling, potentially of the central cysteine (C450) in the protein's core. There was evidence to this effect, as CW-EPR measurements of AsLOV2 proteins that went through the labeling procedure without either cysteine mutation (T406C or E537C) still showed a small EPR resonance that was attributed to unintentional labeling of the central cysteine (see Fig. 2.8), though the low SNR implied the likelihood occurrence of this labeling was quite small. However, as will be shown in sec. 2.3, this change is actually in the direction *opposite* what would be expected for lineshape narrowing of T406C or E537C with an unintentionally-labeled second site. As is mentioned later, this effect may be caused by

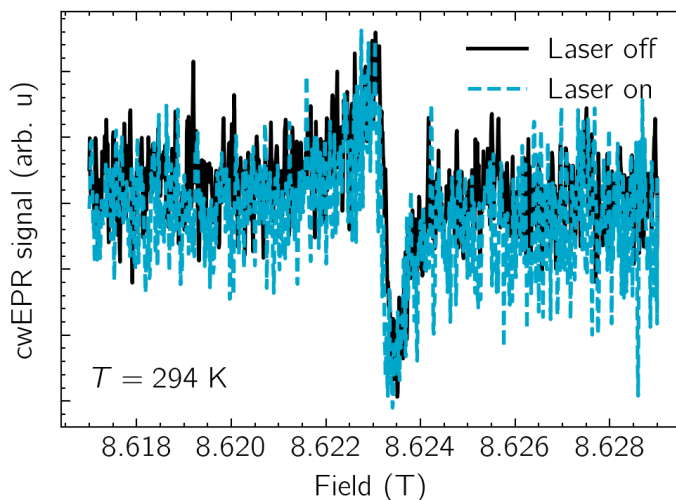


Figure 2.8: Unintentional labeling of central cysteine. CW-EPR spectrum of wild type AsLOV2 that shows a distinct Gd-sTPATCN resonance. This resonance was attributed to labeling of the native central cysteine. The signal-to-noise of the spectrum is very low, which implies that the relative occurrence of this labeling is small.

light-activated AsLOV2 dimerization in solution; recent literature has reported evidence to this effect [156].

Next, a topic of current interest in the AsLOV2 literature is the conserved glutamine residue (Q513) [96, 148, 151, 155, 157–159]. The Q513 site is located on the I β -sheet, interacts with the J α -helix, and is in the immediate proximity of the chromophore binding site. It is suspected that, upon light activation, this residue switches its hydrogen bonding pattern with the chromophore and plays a key role in the transmission of stress to the J α -helix, which causes it to unfold [157, 160]. Mutation to an uncharged, non-polar amino acid (*e.g.*, alanine), is known to slow down photoactivation and relaxation of AsLOV2. TiGGER is well-equipped to determine whether a Q513A mutation also alters the long-range structural changes as manifested by the J α -helix unfolding. In order to elucidate the consequence of Q513A mutation in the refolding of the J α -helix, experiments were performed on a Q513A-mutated variant of DL AsLOV2 with Gd-sTPATCN spin

labels attached to the same sites (T406C and E537C) as before. TiGGER and transient UV-Vis absorption spectroscopy were first used to confirm that the Q513A mutation does not inhibit photoswitching of DL AsLOV2, but does slow the photoactivity of the chromophore by a factor of approximately 2 ($\tau_{\text{Q513A DL}} = 135.4 \pm 0.3$ s, see Fig. 2.9b for comparison to DL AsLOV2, $\tau_{\text{DL}} = 61.91 \pm 0.07$ s).

Further, as a proxy to measuring distance changes between sites 406 and 537, CWEPR showed that the Q513A mutation caused similar, though appreciably smaller, dipolar narrowing to that of DL T406C-E537C upon 450 nm illumination (*i.e.* less effective movement). There was also a slowing of the TiGGER relaxation time after activation (approx. $3\times$, $\tau_{\text{Q513A DL}} = 174.5 \pm 0.5$ s, see Fig. 2.9b) that was comparable to what was observed by UV-Vis kinetics. This result is consistent with earlier studies from other groups on AsLOV2 using circular dichroism spectroscopy, where light-activated changes were observed in molar ellipticity per residue, $[\theta]_{\text{MRW}}$, at 208 nm and 220 nm in the mutant Q513L AsLOV2 [96, 148, 157]. The results presented here demonstrate that the Q513 residue plays an important role in modulating the structural changes of J α -helix upon blue light illumination but is not indispensable for motion, as the refolding of the J α -helix is slowed but still apparent.

2.2.2 Discussion

Upon light activation, the FMN chromophore is excited to a triplet state and reacts with the nearby cysteine residue, C450. It forms a covalent bond between the C(4a) atom of the chromophore and the sulfur atom of C450 [162]. This cysteinyl adduct formation is accompanied by destabilization of the J α -helix in the C-terminus of the protein. The recovery rates of the covalent adduct to the dark state vary widely across LOV domains,

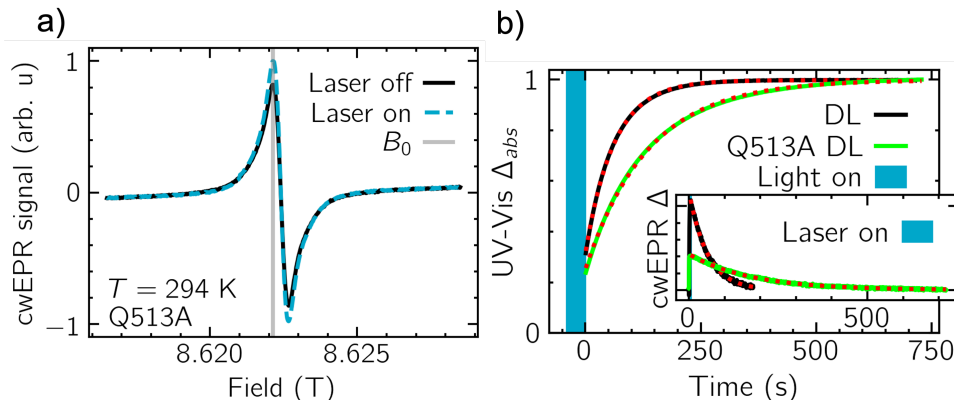


Figure 2.9: The Q513A mutation slows the light-activated chromophore and mechanical photocycle kinetics as detected by TiGGER. (a) CW-EPR spectra of Q513A DL T406C-E537C AsLOV2 showing light-activated change between dark (solid black line) and lit (dashed blue line) states. (b) Time-resolved UV-Vis demonstrates a slowing of the chromophore photocycle in AsLOV2 after illumination. Black and green solid lines represent T406C-E537C at λ_{447} and Q513A DL T406C-E537C at λ_{442} (both $\sim 30 \mu\text{M}$), respectively. Both time-resolved experiments were performed at the wavelength of each spectrum's maximum absorption; as has been reported in refs. [157, 160, 161], a Q513A mutation modifies the FMN's electronic environment and its UV-Vis absorption spectrum changes as a result. Exponential fits returned time constants of $\tau_{\text{DL}} = 61.91 \pm 0.07 \text{ s}$ and $\tau_{\text{Q513A DL}} = 135.4 \pm 0.3 \text{ s}$. Δ_{abs} are presented as fractions of the peak signal to report on the relative change induced. (Inset) TiGGER at B_0 with and without Q513A mutation demonstrating that Q513A slows refolding. Exponential fits returned $\tau_{\text{DL}} = 51.9 \pm 0.3 \text{ s}$ and $\tau_{\text{Q513A DL}} = 174.5 \pm 0.5 \text{ s}$.

on a timescale of seconds to days [148, 163–165]. This widely varying photocycle is interesting; it is believed that the adduct decay is base-catalyzed, limited by a proton transfer step [166]. The presence of a glutamine residue, Q513, within hydrogen-bonding distance with the chromophore, seems to be the only LOV residue in proximity to the chromophore that is capable of catalyzing this deprotonation process [157]. The UV-Vis experiment presented here, on AsLOV2 Q513A variant, in agreement with previous studies by others, show that mutation to the glutamine residue slows the photocycle, but does not prohibit it [96, 148, 160]. Studies in the literature have suggested that the preservation of the photocycle activity of the Q513A variant may be owed to effects

of hydration of the LOV domain, where water molecules can directly act as a base catalyst by entering the chromophore binding pocket and hydrogen-bonding with the FMN chromophore [96, 167–170].

Indeed, TiGGER demonstrated that the chromophore and mechanical photocycles are still intact, even in the absence of the glutamine residue in the Q513A variant, albeit $2\text{--}3\times$ slower (see Fig. 2.9). The rate of mechanical refolding, as measured by TiGGER, is slowed more than that the chromophore photocycle. This may imply that an unknown rate-limiting step is caused by the Q513A mutation, partially decoupling the chromophore photocycle from the mechanical response. The Q513 site that is known to be important for chromophore base catalysis is also important for driving mechanical movement between the N and C termini. However, these results imply that the stress transmission through glutamine at residue 513 is not the only mechanism capable of driving $J\alpha$ unfolding, consistent with discussions in the literature [96, 148, 157].

The observation that the $J\alpha$ -helix unfolds-and-refolds in the wild type and the Q513A variant highlights the complementarity of TiGGER with other structural biology tools. Recent high-resolution X-ray crystal structures of the Q513L variant in Dietler et al. [96] show that related structural changes (sub-Å displacements) are induced by light in the Q513L and the wild type, though it has long been known that when crystallized, the $J\alpha$ -helix remains folded upon light activation [153]. In contrast, because TiGGER is performed in solution state, the $J\alpha$ -helix is free to unfold-and-refold and readily does so. These seemingly contrasting results imply that the magnitude of some biophysical protein dynamics cannot be readily seen by X-ray crystallography.

Further still, solution-state NMR studies on Q513L AsLOV2 conducted by others suggest that, upon illumination, the majority of the Q513L AsLOV2 population remains in the dark state. However, chemical shift changes are sensitive to local electronic envi-

ronments and do not report directly on inter-residue distance changes [157]. TiGGER, on the other hand, is not sensitive to changes in FMN electron configurations (SL samples showed almost no light-activated change) and can directly inform on the effect of mutations on both unfolding yield and refolding rate of the J α -helix. As was expected, the chromophore excitation efficiency (as measured by relative amplitude of the UV-Vis response) was not affected by the Q513A mutation, but the amplitude of the TiGGER response was. If a larger fraction of Q513A proteins remained in the dark state after light activation as compared to the wild type, as reported by Nash et al. [157], it would explain the reduced TiGGER amplitude observed in that mutant. However, an alternative explanation is a reduction in the *magnitude* of motion; the qualitative evidence presented here is not sufficient to definitively separate the two and presents an exciting direction for future studies.

To the author's knowledge, besides TiGGER, the only method capable of tracking dynamical distance changes between a pair of specific residues on a protein in solution state is Förster resonance energy transfer (FRET). FRET relies on a distance-dependent non-radiative energy transfer from donor to acceptor fluorophores that have been introduced site-specifically into the macromolecule of interest [171]. Recent advances have made it possible for FRET to be applied to single molecules [172–174], but the application of FRET to explore dynamics in a light sensitive protein can be complicated due to the need for additional correction factors to resolve the spectral crosstalk between the light sensitive protein and donor-acceptor pairs [175].

Extracting distances

The contrast between lit and dark states will be enhanced by maximizing the fraction of proteins that are loaded with a chromophore, and the effects of rotational averag-

ing may be mitigated by incorporating proteins into stabilizing agents (*e.g.*, hydrogels). Enabling the extraction of time-dependent distance distributions from TiGGER will require recording the entire field-swept EPR spectrum as a function of time rather than the CWEPR signal at a fixed magnetic field; rapid-scan EPR offers an attractive path towards this goal. Additionally, methods that have been developed to extract distance distributions from nitroxide lineshapes will need to be extended to Gd(III) spin labels at high fields in order to quantify the extent of light-activated motion in future experiments (*e.g.*, [176, 177]).

2.2.3 Conclusion and next steps

This section demonstrated the first step to “filming” a protein in action using TiGGER. Site-specific labeling allowed for tracking changes in distances between a pair of residues in an ensemble of room temperature proteins in solution state. TiGGER was able to help confirm a mutation-induced slowing of the mechanical photocycle, which highlights the importance of the time-resolved, solution state technique to the optogenetic community. Additional experimental development and an application of TiGGER to a range of protein residues will enable three-dimensional, time-dependent mapping of protein mechanical action, and may play an important role in improving design of optogenetic actuators and fluorescent reporters.

The technique presented here should be applicable to studying conformational changes triggered by other factors, such as ligand binding by rapid mixing, voltage actuation, or temperature jumps. In the future, TiGGER may be used to fill in the gaps of stories of protein triggered functional dynamics that are partially told by time-resolved X-ray crystallography and IR spectroscopy, FRET, freeze-quench cryo-EM, solid-state NMR,

and DEER, among others.

Future work for TiGGER on photoresponsive proteins will focus on enhancing the contrast between dark and lit states, mitigating the effects of rotational averaging, and making TiGGER a more quantitative method that can report on the time evolution of distance distributions between labeled residues.

2.3 Extending TiGGER with rapid-scan EPR[†]

2.3.1 High-field rapid-scan EPR

The results presented in sec. 2.2 represented a large step forward in experimental sensitivity to solution-state protein dynamics, but there were certainly areas for potential improvement. Specifically, the experiment lacked quantitative information—though TiGGER can tell *if* a protein is moving, it is impossible to say confidently *how much*. Additionally, the long-term goal of the TiGGER project was to access information similar to what is provided by DEER: inter-site distance *distributions*, except this time, in real-time and the solution state. To achieve this goal, a different experimental technique was required, one that did not require repeated acquisitions for sufficient signal averaging, and one that could provide more detailed spin-spin coupling information to allow for reliable distance extraction. Luckily, a technique with these abilities is undergoing active development: rapid-scan EPR, with some modifications, is capable of providing the time-and-field-resolved spectra required.

Rapid-scan electron paramagnetic resonance (RSEPR) is a technique that has received increased attention in recent decades [72, 179–188]. It can provide substantial

[†]The text and many of the figures in sec. 2.3 are adapted from [178], reproduced with permission according to Elsevier’s Copyright Policies.

benefits compared to conventional CWEPR, including improved signal-to-noise (SNR) ratio for equivalent acquisition times as well as the ability to record entire field-swept spectra with high temporal resolution [189–194]. In RSEPR, either the source frequency or the applied magnetic field is rapidly swept to record a spectrum. Frequency sweeps can be extremely quick using an arbitrary waveform generator (AWG) or voltage-controlled oscillator (up to 10^7 T/s [195, 196]) and do not generate eddy currents in metallic elements. Field sweeps, in contrast, are much slower (10^3 T/s) and do generate eddy currents [197], but do not suffer from frequency-dependent standing wave modes in the quasi-optics, as the source frequency is fixed [198]. It is also comparatively cost-effective and simpler to implement field-domain rapid-scanning in a fixed-frequency CWEPR spectrometer because a variable-frequency source and receiver are not required.

To date, RSEPR has been primarily utilized at low field (*i.e.* “X-band”, 9.6 GHz and 342 mT), where commercial options are prevalent. However, there are benefits to performing EPR at high magnetic fields and frequencies (> 3.6 T and > 100 GHz): larger fields improve spectral resolution, increase sensitivity through increased electron spin polarization, and can even *decrease* the width of an EPR line in the case of $-1/2 \rightarrow 1/2$ transitions of paramagnetic species with non-integer spin $> 1/2$, if the primary broadening mechanism is zero-field splitting. In particular, as reported in [14, 29, 135] the linewidth of the $-1/2 \rightarrow 1/2$ transition in Gd-sTPATCN, a highly-symmetric gadolinium spin label with spin $S = 7/2$, has been shown to decrease to less than 10 G at 8.6 T (240 GHz) in sufficiently dilute samples, due to its small zero-field splitting and otherwise symmetric, isotropic EPR spectrum. Hence, dipolar broadening of Gd spin labels at 8.6 T is visible if other Gd spins are sufficiently close (< 4 nm separation), making their linewidth useful for probing distances between residues in spin-labeled proteins (a cartoon demonstration of this concept is shown in Fig. 2.10) [14].

Combining the benefits of high-field and rapid-scan EPR in the frequency domain is still rare [188, 195, 199], but has been demonstrated up to 330 GHz [74]. Rapid-scan was first demonstrated at high field in the frequency domain [195]. Resonator-free, high-field spectrometers—such as the one used throughout this dissertation—do not limit the range of frequencies that may be used [197], and thus can provide access to broad spectra when the frequency is swept. Still, frequency-domain rapid scanning is complicated to implement given the baseline instability caused by standing waves.

In contrast to frequency domain, implementing field-domain modulation is less complicated, but many EPR resonance lines are too broad for a 100 G field-domain modulation coil to achieve meaningful results. However, the $-1/2 \rightarrow 1/2$ transition in Gd-sTPATCN is sufficiently narrow to sweep through with a 100 G modulation coil, thereby enabling field-domain RSEPR of protein samples of interest at high magnetic field; to the author’s knowledge, Price et al. [178], recapped here, is the first demonstration of field-domain RSEPR above X-band.

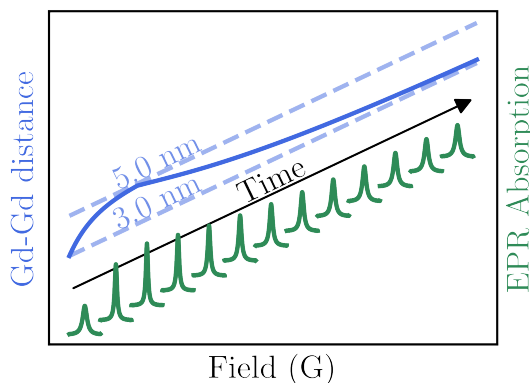


Figure 2.10: Cartoon of RS TiGGER concept. As the spin-spin distance (blue) of a doubly labeled protein changes, RS TiGGER captures field-swept lineshapes as a function of time (green), combining the spectral information of CWEPR with the time resolution of the CW TiGGER experiments of sec. 2.2.

For this experiment, a function generator and modulation coil are required in order

to sweep through an entire EPR transition of interest in a matter of microseconds. This implementation also requires a digitizer with a sampling rate of order 500 MSa/s (see below) and real-time onboard averaging that can continuously average rapid scans as they are acquired (thousands of points, updating at tens of kHz), in order to operate at an achievable data throughput and minimize post-processing. To enable field-domain rapid-scan in the homebuilt EPR spectrometer housed at UCSB's Institute for Terahertz Science and Technology (ITST), hardware and software upgrades were required. One such upgrade was implementation of the recently reported quasi-optical sample holder that greatly improves SNR by reducing the intensity of the baseline relative to the signal (discussed in detail in sec. 3.7) [194]. The sample holder also makes it possible to finely position the sample at a B_1 maximum to amplify the resulting EPR response. It does not contain metallic components within the coil and all metallic components are quite far from the coil center, eliminating the background effects of eddy currents in rapid-scan experiments. The nearest metallic element is a copper waveguide that is approximately 1.25 mm from the top of the coil along its field axis (7.25 mm from the center of the coil) and the cylindrical cryostat that houses the probe is 6 cm in diameter, which is quite far away from the 2.5 cm outer diameter of the coil (and 3 cm from its center) [194]. The second upgrade was the installation and programming of a field-programmable gate array (FPGA)-enabled digitizer for rapid data acquisition and real-time averaging. In total, the work presented here represents a large leap in field-domain RSEPR from 9.4 GHz to 240 GHz.

2.3.2 Development of ITST's RSEPR spectrometer

All experiments were performed using the homebuilt EPR spectrometer at ITST. The spectrometer operates in field domain with a 12.5 T field-swept magnet (Oxford Instruments, Inc., UK), 240 GHz source (~ 30 mW output power, Virginia Diodes, Inc., USA), and Schottky-diode based subharmonically-mixed heterodyne receiver (WR4.3SHM with single-sideband noise temperature approx. 1400 K and 8 dB conversion loss, Virginia Diodes, Inc., USA) that returns the EPR signal at an intermediate frequency (IF) of 10 GHz. All rapid-scan experiments presented here made use of the quasi-optical sample holder presented in Sojka et al. [194] (and sec. 3.7) to maximize the signal-to-noise ratio, which in turn enhances the temporal resolution of kinetic processes of interest. Spectrometer details that do not pertain specifically to rapid-scan operation can be found elsewhere (*e.g.*, [50, 134]).

Hardware

Field-domain RSEPR is achieved by driving a modulation coil with a sinusoidal AC current (100 mA RMS; produced by 8111A function generator, Hewlett-Packard, USA) to sweep rapidly through the resonance (modulation field is ~ 100 G at 25 kHz, depending on the inductance and number of turns of the individual coil, as well as the capacitance of an in-series capacitor, which is usually set to 10 nF). Therefore, the peak sweep rate, at the center of a sinusoidal sweep, is approximately 600 T/s. The modulation coil strength at a given frequency may be improved by reducing the AC impedance of the coil of choice or amplifying the driver current. This can be done with Litz wire [200] or an amplifier circuit like those reported in refs. [201–203]. Because this coil is approximately $5\times$ smaller in each dimension than coils used for X-band RSEPR, it can be driven to

similar field strengths with much lower currents. This reduces spurious microphonic effects as well as associated eddy current effects and parasitic signals to negligible levels [204]. The function generator that drives the modulation coil is also used to trigger data acquisition at the digitizer (16-bit PXIe-5673, National Instruments™, USA) so that field sweeps start and stop at repeatable field positions. Only the low-to-high field sweeps are recorded so that the digitizer has time to arm and trigger before each low-to-high sweep.

After detection and subharmonic mixing, the IF signal that contains the EPR spectrum is variably attenuated to control input power, amplified, then IQ-mixed with an in-phase and quadrature 10.10 GHz local oscillator (LO) signal (MLSL-0911, Micro Lambda Wireless, Inc., USA) that is phase-locked to the subharmonic mixer LO. This mixing results in a 100 MHz carrier frequency (see IF stage block diagram in Fig. 2.11) that is directly digitized and subsequently added in quadrature by a field-programmable gate array (Xilinx KU040 FPGA, Advanced Micro Devices, Inc., USA) onboard an AC-coupled 500 MS/s, 225 MHz input bandwidth digitizer.

Software

Because a significant amount of co-polar leakage (*i.e.*, reflected source power parallel to the input polarization that is not a result of induction mode EPR absorption) is detected, the signals that are digitized contain a large DC offset. Somewhat surprisingly, due to this offset, it is possible to extract both the dispersion, χ' , and absorption, χ'' , by only recording the magnitude of the input IF signal ($\sqrt{I^2 + Q^2}$). To demonstrate, assume one channel contains χ' and some DC offset, a , and the other contains χ'' and some DC offset, b , such that the digitized signal, S , may be written as (up to a scaling factor):

$$S \propto (a + \chi') + i(b + \chi''), \quad (2.2)$$

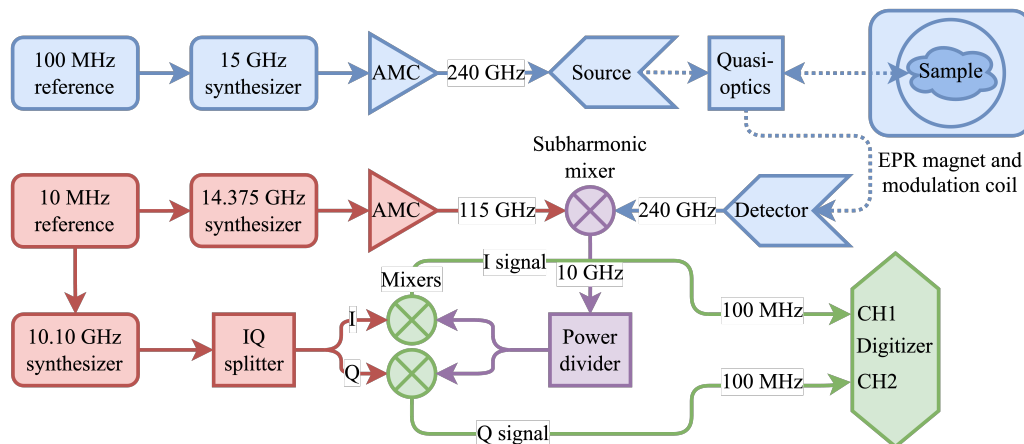


Figure 2.11: Simplified block diagram of the rapid-scan EPR spectrometer. Signal acquired by the digitizer is sampled every 2 ns and time and field-domain traces are summed at the modulation frequency of ~ 25 kHz. An amplifier-multiplier chain (AMC) that appears in the source chain (blue, top) multiplies the 15 GHz LO frequency $16\times$. The source power emitted is ~ 30 mW at 240 GHz. An AMC in the detector chain (red, middle) multiplies the 14.375 GHz LO frequency $8\times$. The resulting 240 GHz and 115 GHz signals are subharmonically mixed (WR4.3SHM, Virginia Diodes, Inc., USA) to 10 GHz for amplification, filtering, and further mixing with 0° and 90° 10.10 GHz reference signals, which produces two orthogonal signals at a 100 MHz carrier frequency. The 100 MHz modulated signals (green, bottom right) are then digitized by an FPGA-enabled digitizer. The 100 MHz and 10 MHz reference sources do not need to be phase-locked because of the phase-insensitive magnitude averaging technique used. Solid lines represent SMA cables carrying electronic signals and the dotted lines represent electromagnetic waves that propagate through quasi-optical components and in-and-out of the EPR magnet where the sample is located. RF amplifiers and variable attenuators are omitted here for simplicity.

such that

$$|S| \propto \sqrt{I^2 + Q^2} = \sqrt{(\chi' + a)^2 + (\chi'' + b)^2}. \quad (2.3)$$

Then, since the co-polar leakage is large compared to the EPR signal, the DC offset in each channel is large compared to the susceptibility ($a \gg \chi'$ and $b \gg \chi''$). Applying the binomial approximation twice returns:

$$|S| \propto \sqrt{a^2 + b^2} + \frac{a\chi' + b\chi''}{\sqrt{a^2 + b^2}}. \quad (2.4)$$

Then, subtracting the Hilbert transform, $\mathcal{H}$, of $|S|$ provides an analytic signal (which is denoted $\widetilde{|S|}$) that can be deconvolved using the algorithm in Tseytlin [205].

$$\widetilde{|S|} = |S| - i\mathcal{H}\{|S|\} \propto \sqrt{a^2 + b^2} + \frac{a\chi' + b\chi''}{\sqrt{a^2 + b^2}} - i \left(\frac{-a\chi'' + b\chi'}{\sqrt{a^2 + b^2}} \right). \quad (2.5)$$

Simplifying and applying an overall phase correction of $\phi = \pi/2 - \tan^{-1}(a/b)$ leaves

$$\widetilde{|S|}e^{i\phi} \propto (a + ib) + \chi' + i\chi'' \propto S, \quad (2.6)$$

which is the original signal, S , up to a scaling factor, that contains separable components of both χ' and χ'' , the rapid-scan signals of interest. For a full derivation, see Appendix B.1.

It is possible to obtain χ' and χ'' by phasing each I and Q signal separately before averaging. In this case, the 100 MHz carrier frequency must remain very stable throughout the experiment so that each sine wave may be precisely aligned. The final result of separately phasing each I and Q signal is the same as the one described above, but implementing the necessary frequency stability is challenging and real-time phasing is much slower computationally; therefore, to maximize the FPGA's ability to perform computations in real time, magnitudes were continuously averaged. Importantly, averaging magnitudes eliminates any dependence on phase drift between the signal and

reference chains and thereby enables phase-independent signal averaging. For this reason, the 10 MHz and 100 MHz reference sources shown in Fig. 2.11 are not required to be phase-locked to one another, and the resulting spectra are not sensitive to phase drifts due to the IF stage temperature changing, for example.

The magnitude signals are continuously summed a user-specified number of times in the FPGA and transferred to the control PC for further processing (see block diagram in Fig. 2.12). The control PC computes the root-mean-square time-dependent signal, digitally filters high frequency noise (LabVIEW 21.0 Butterworth low-pass filter with $f_{\text{cutoff}} = 25$ MHz), and writes the signal to an experiment file. Currently, the averaging is limited to ≥ 250 scans (~ 10 ms with 25 kHz field modulation) due to limitations with memory onboard the digitizer and data throughput rates to the control PC.

Experiment files are processed using Python3 [206]: beginning with Eqn. (2.7), the Hilbert transform is computed from the raw magnitude of I and Q , $r(t) = \sqrt{I^2 + Q^2}$, to return the complex analytic signal of the acquired component,

$$\tilde{r}_a(t) = r(t) - i * \text{Im} \{ \mathbf{H} \{ r(t) \} \}, \quad (2.7)$$

where $\mathbf{H}$ is `scipy.signal.hilbert` [207]. As discussed above, this process returns a complex signal that contains both $\chi'(t)$ and $\chi''(t)$ with some DC offset and an unknown phase. Next, a GUI (`onefileRapidscanGUI.py`, [206]) is used to implement the deconvolution algorithm for sinusoidal scans developed by Tseytlin [205]. The combination of these programs returns χ^* , the complex magnetic susceptibility χ with some phase shift. To obtain the field-swept absorptive and dispersive lineshapes, the spectra must be phased. There are multiple ways to apply the phasing, but a typical method requires minimizing (nulling) any negative values in χ'' , the absorptive signal of interest.

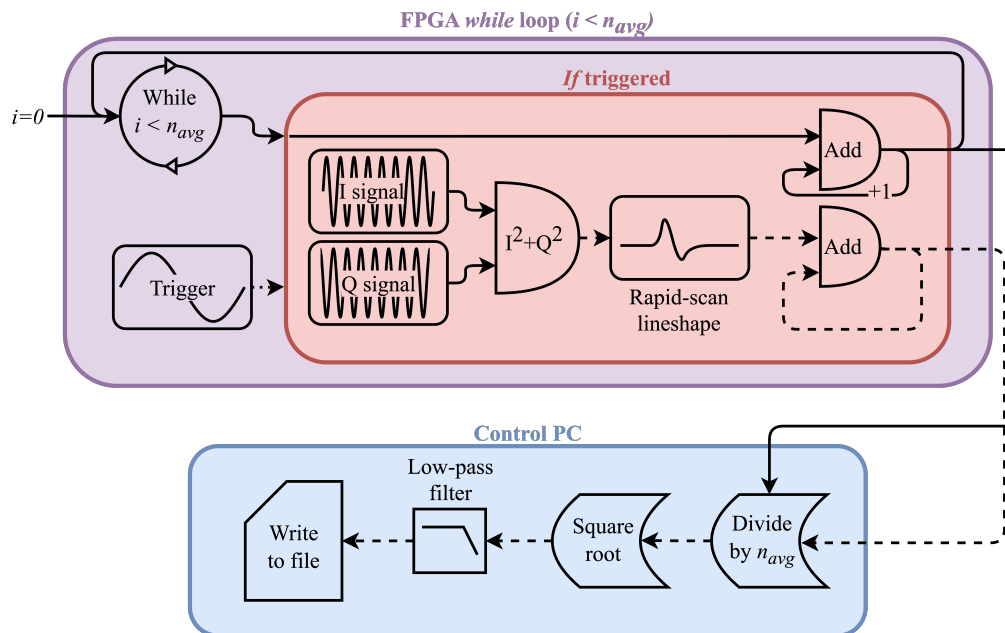


Figure 2.12: Digital signal processing block diagram. The AC current that drives the rapid-scan field serves as a data acquisition trigger (~ 25 kHz), which is looping inside a *while* loop (purple). The trigger activates an acquisition cycle in an *if* loop (red) that proceeds until it executes a user-specified number of times. Inside the *if* loop, the I and Q signals (100 MHz carrier frequency) are added in quadrature, and then continuously averaged by summing. After the *while* loop has run n times, the loop exits, the data is sent to a control PC for additional processing (blue), and the *while* loop is restarted. On the control PC, the root-mean-square signal is calculated and then low-pass filtered at 25 MHz, which removes high frequency noise and harmonics of the carrier frequency while preserving any features that may appear in the rapid-scan spectrum. The data is then written to a file. Division-by- n and square-rooting take place on the Control PC to avoid having to modify data types onboard the FPGA. Additionally, both division and square-rooting require *for* loops, which take more than a single clock cycle and add complexity without appreciably improving processing speed.

Symbolically,

$$\chi^*(B) = \mathbf{D} \{ \tilde{r}_a(t) \} \quad (2.8)$$

$$e^{i\phi}(\chi' + i\chi'') = \mathbf{D} \{ \tilde{r}_a \}, \text{ where } \chi^* = e^{i\phi} \chi = e^{i\phi}(\chi' + i\chi'') \quad (2.9)$$

$$\chi'' = \mathbf{Im} \{ e^{-i\phi} \mathbf{D} \{ \tilde{r}_a \} \} \quad (2.10)$$

where $\chi^*(B)$ is the complex magnetic susceptibility χ as a function of field, B , with unknown phase ϕ ; $\mathbf{D}$ is the deconvolution algorithm from Tseytlin [205]; and χ' and χ'' are the slow-scan dispersive and absorptive signals, respectively.

2.3.3 Results of rapid-scan TiGGER

In order to extract quantitative, time-resolved changes of AsLOV2 residue-residue distances under physiological conditions, it is necessary to record the entire EPR lineshape of interest at every time step in solution state, which requires RS TiGGER. This section reports time-dependent measurement of EPR lineshapes by RS TiGGER before, during, and after light activation. From these lineshapes, an activation energy of 15.5 ± 1.5 kcal/mol is extracted for AsLOV2 refolding using a temperature-dependent Arrhenius plot of refolding rates that agrees well with the NMR-determined values presented in Harper et al. [208].

Gd-sTPATCN

Field-domain RSEPR relies on the ability to sweep the entire resonance using the modulation coil, without sweeping the main field. ITST's CWEPR modulation coils typically reach field strengths of ~ 100 G peak-to-peak, and thus, can only be used for RS on lines narrower than 25 G, as the post-processing Hilbert transform requires that the

scan extends far past the wings of the resonance. At 8.6 T, Gd-sTPATCN has a full-width at half-maximum linewidth of less than 10 G, even when attached to a protein [29, 142], due to its very small zero-field splitting (ZFS) and significant ZFS strain (at 10 K, $D = 485$ MHz [132]), which makes it a great candidate for TiGGER. To first order, ZFS does not alter the spin $-1/2 \rightarrow 1/2$ transition, but to second order, broadening scales as $\Delta\omega \propto \frac{1}{B_0}$, where B_0 is the strength of the applied magnetic field [134, 136, 209]. Therefore, in a typical field-swept EPR spectrum, only the narrow central transition is observed: the others are spread into a very broad background as a result of this ZFS strain. Further, it has been shown that 240 GHz is close to the optimal frequency for Gd-sTPATCN, as it produces nearly the narrowest linewidth of all accessible EPR frequencies; this is a result of the competing effects of narrowing due to ZFS and increased broadening due to unresolved g -anisotropy [135].

Spectrometer sensitivity

For a 1 mM solution of Gd-sTPATCN spin label in D_2O , the SNR for 250 scans—using a conservative low-pass filter cutoff of 25 MHz—was found to be $\overline{\text{SNR}} = 60 \pm 10$ over 1000 experimental runs.

SNR of rapid-scan experiments was calculated from the raw rapid-scan data by dividing the difference between the mean of the eight largest and eight smallest data points by the standard deviation of the final 5% of the baseline, similar to the calculation in Möser et al. [210]. Symbolically,

$$\text{SNR} = \frac{\langle \{x_1, \dots, x_8\} \rangle - \langle \{x_{N-8}, \dots, x_N\} \rangle}{\sigma(\{x(t = 0.95 * t_f), \dots, x(t = t_f)\})}, \quad (2.11)$$

where the integer subscripts denote sorted rank from maximum (1) to minimum (N). The baseline is calculated in the last 5% of time points, from $0.95 * t_f$ to t_f ; for a typical

23.6 kHz RS experiment and 500 MHz digitizer sampling rate, this is 529 points. These values were then averaged over N field sweeps to produce $\overline{\text{SNR}} \pm 2\sigma$, where σ represents the standard deviation.

The SNR of CWEPR was quantified in the same way, using the difference between the largest and smallest 8 points and the last 5% of the x -axis field points. This calculation yielded $\text{SNR}_{cw} = 7850$ for the lock-in amplifier-detected dataset that is plotted in Fig. 2.13.

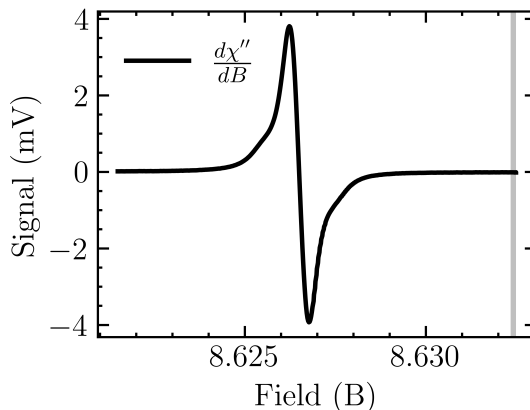


Figure 2.13: CWEPR spectrum of 1 mM Gd-sTPATCHN in D₂O (blue). SNR was calculated using the difference between the average of the largest and smallest 8 points, divided by the RMSE of points within the baseline, highlighted by the gray vertical bar. This calculation returned $\text{SNR} = 7850$.

Because the SNR of rapid-scan is dependent on modulation coil frequency, number of averages, as well as digitizer and low-pass filter bandwidths, a quantification of sensitivity that can be easily compared to reported CWEPR sensitivities is difficult. In lieu of this, the absolute sensitivity of the spectrometer was determined in CW mode, and the SNR of CW and RS were compared under similar conditions, which scales the same was absolute sensitivity and provides a fair comparison. For CW, following the calculation in Nir-Arad

et al. [211], the sensitivity, E , is:

$$E_{CW} = \frac{N}{\text{SNR} * n * \Delta H_{pp} * \sqrt{\Delta f}}, \quad (2.12)$$

where N is the number of spins in the sample (for 1.3 μL capillary of 1 mM Gd-sTPATCHN, this is 7.8×10^{14} spins), SNR is the signal-to-noise ratio, n is the number of EPR lines (1), ΔH_{pp} is the peak-to-peak linewidth (5 G), and Δf is the detection bandwidth (for a lock-in time constant of $\tau = 100$ ms with a 24 dB/oct roll-off, the equivalent noise bandwidth is $\Delta f = 5/64\tau = 0.78$ Hz [212]). Therefore, E_{CW} is:

$$E_{CW} = \frac{7.8 \times 10^{14} \text{ spins}}{8 \times 10^3 * 1 * 5 \text{ G} * \sqrt{0.78 \text{ Hz}}} = 2.2 \times 10^{10} \text{ spins/G}\sqrt{\text{Hz}}, \quad (2.13)$$

which is comparable to the room temperature, continuous wave sensitivities of other high-field, resonator-free spectrometers [198, 211, 213]. To compare rapid-scan to CW, it is simplest to divide the SNR of CW and RS by the square roots of their acquisition times, similar to what was done in Mitchell et al. [73]. In both cases, the SNR was carefully maximized by reducing high frequency noise as much as possible without distorting the line: for CW, this meant acquiring a point every 200 ms, twice the lock-in time constant; for rapid-scan, this meant using 2.5 MHz as the cut-off frequency for the digital low-pass filter, as any lower cut-off began to distort the lineshape (see Fig. 2.14). The SNR of CW was 8×10^3 and was acquired by sweeping over 11 mT with a 0.1 mT/s sweep rate, meaning the experiment took $t_{CW}^{acq} = 110$ s. The SNR of 2.5×10^4 averaged rapid-scan sweeps filtered at 2.5 MHz had a mean SNR of $1.7 \pm 0.6 \times 10^3$ over 9 experimental runs, acquired at 23.6 kHz, each with a total acquisition time, $t_{RS}^{acq} = 1.06$ s. Therefore,

$$\text{SNR}_{CW} / \sqrt{t_{CW}^{acq}} = 760 \sqrt{\text{Hz}}, \quad (2.14)$$

$$\text{SNR}_{RS} / \sqrt{t_{RS}^{acq}} = 1650 \sqrt{\text{Hz}}, \quad (2.15)$$

implying that while using UCSB's homebuilt EPR spectrometer, rapid-scan with a digital filter of 2.5 MHz can provide better SNR (and therefore sensitivity) with a total acquisition time similar to CW. This approximately $2\times$ improvement may be due to the elimination of phase noise between the signal and reference chains as a result of the magnitude averaging scheme used. As discussed by Mitchell and collaborators [72, 73, 191], if the sample can be saturated with continuous wave excitation, even larger SNR benefits may be obtained by using rapid-scan. In rapid-scan mode, higher source power may be used without saturating, which provides a much larger signal and thus higher SNR. However, Gd-sTPATCN at high field and room temperature cannot be saturated with the approximately 0.1 G B_1 field strength that is available at the sample position.

Rapid-scan also allows signal averaging for as long as is necessary, which is a marked improvement over CW at high field, where repeated signal averaging is difficult and electronic phase drift often negates its benefits. As shown in Figs. 2.15 and 2.16, longer acquisition times (more averages) improved SNR by the expected value of $\sqrt{N_{avg}}$, where N_{avg} is the number of summed signals. There are likely to be diminishing returns at some N_{avg} , when the amplitude of high frequency noise has been reduced below the resolution of the digitizer, but up to at least 2.5 million scans (100 s), that limit had not been reached.

It is possible to use Eqn. (2.15) to estimate the SNR at the lower limit of time resolution for a 1 mM Gd-sTPATCN sample. Assuming a 23.6 kHz modulation frequency, a single scan would have:

$$\text{SNR}_{RS} = 1650 \sqrt{\text{Hz}} * \sqrt{(23.6 \text{ kHz})^{-1}} = 10.7, \quad (2.16)$$

which would still be sufficient for the post-processing and linewidth fitting that is presented below.

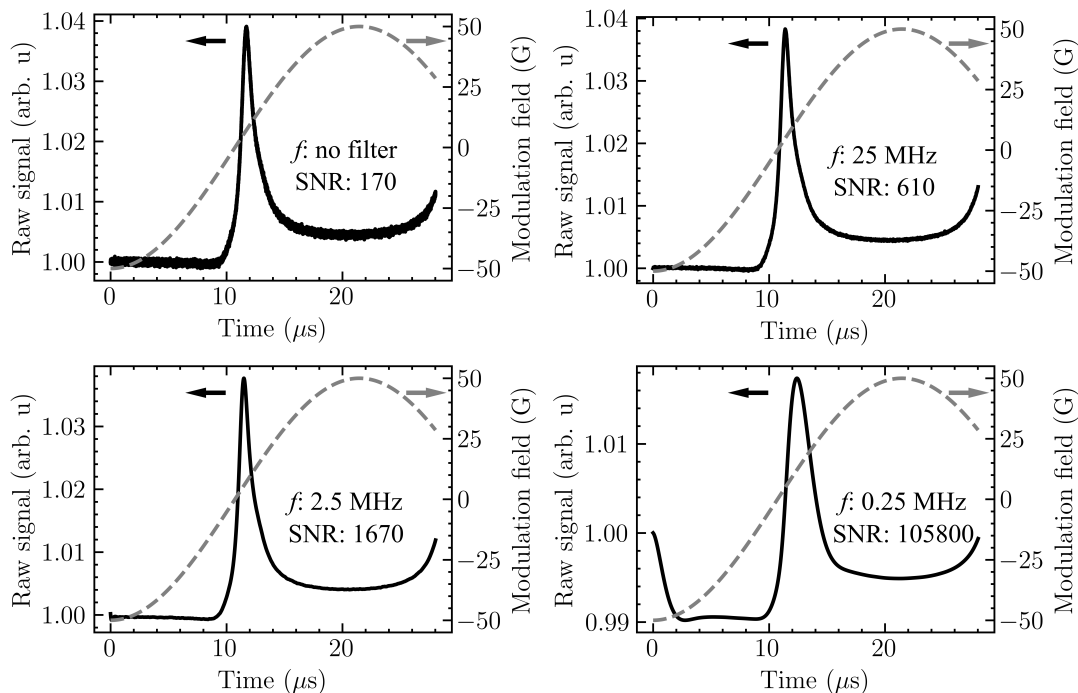


Figure 2.14: RSEPR lineshapes at various low-pass filter cutoff frequencies. Raw rapid-scan spectra of 1 mM Gd-sTPATCN in D_2O ; no filter, 25 MHz low-pass filter, 2.5 MHz low-pass filter, and 0.25 MHz low-pass filter (left-to-right, top-to-bottom). Filter frequency and SNR of each dataset are shown within each plot window. The lowest cutoff frequency, 0.25 MHz, is not acceptable, as it distorts the lineshape due to the signal frequency overlapping with the cutoff. Filtering is done in LabVIEW 21.0 using a built-in Butterworth filter function [214]. Raw rapid-scan data is shown in solid blue, modulation field is shown in dashed gray. Colored arrows point toward each line's respective y -axis.

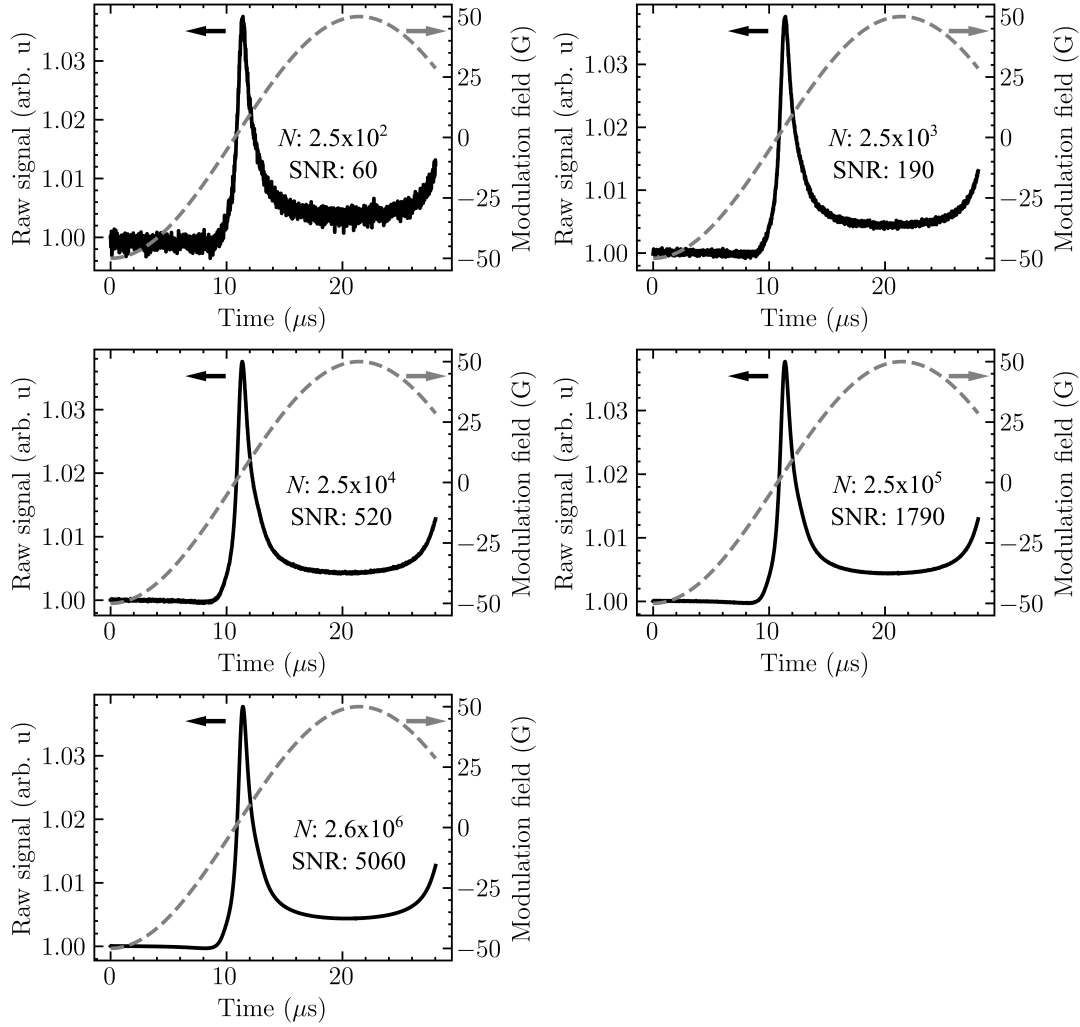


Figure 2.15: Raw rapid-scan spectra of 1 mM Gd-sTPATCHN in D₂O; 2.5×10^2 , 2.5×10^3 , 2.5×10^4 , 2.5×10^5 , and 2.5×10^6 scans at 23.6 kHz (left-to-right, top-to-bottom). The number of averages, N , and SNR of each dataset is shown within plot window. SNR is proportional to $\sqrt{N}$, at least up to 2.5×10^6 scans. Digital low-pass filter cut-off was set to 25 MHz. Raw rapid-scan data is shown in solid blue, modulation field is shown in dashed gray. Colored arrows point toward each line's respective y-axis.

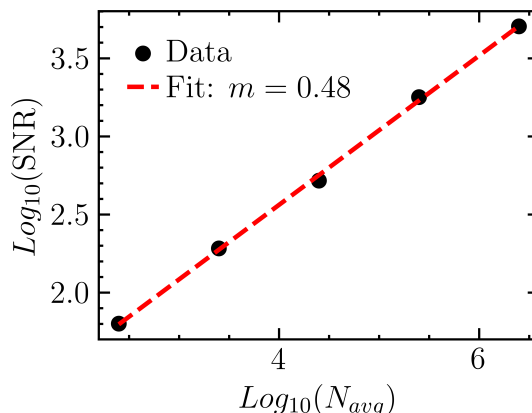


Figure 2.16: Log-log plot of signal-to-noise (black dots) as a function of the number of rapid-scan acquisitions (*i.e.*, acquisition time). Up to at least 2.5 million scans, the relationship is linear, and the slope is $m = 0.48 \pm 0.2$ (fit shown by a dashed red line, uncertainty represents a 95% confidence interval for the fit), meaning SNR improves as expected, scaling as $\sqrt{N_{\text{avg}}}$ [215]. Sample was 1 mM Gd-sTPATCN in D₂O, experiment was performed at 10 °C.

Rapid-scan TiGGER of Gd-sTPATCN-labeled AsLOV2

While the CW TiGGER spectra reported in sec. 2.2 and Maity et al. [142] return information on the timescale and *existence* of inter-residue motion, time-dependent field-swept spectra are required for quantitative analysis of time-resolved distances and distance distributions. Additionally, a two-dimensional time-field map makes data interpretation more obvious and robust than one-dimensional information at a single field position, where artifacts can obscure a quantity of interest. For example, if light activation slightly changes the peak field position of a CW spectrum, the resulting one-dimensional transient would be indistinguishable from the peak signal decreasing due to linewidth broadening. With a RS field sweep, the spectroscopic quantities of interest can be extracted more reliably, as artifacts such as small field-dependent peak shifts can be handled in post-processing.

RSEPR is able to resolve the entire Gd-sTPATCN AsLOV2 central transition and

continuously average as few as 250 scans per frame (totaling 10 ms), making it well-suited for the task of tracking AsLOV2's inter-residue movement. The shortest time resolution for continuous averaging is currently 10 ms due to a bottleneck of data transfer throughput and limited memory onboard the digitizer. However, the AsLOV2 spectra shown in Fig. 2.17a were averaged 25,000 times to achieve roughly 1 frame per second, which is sufficient for the timescale of the kinetics of interest (in these measurements, this timescale is ~ 100 s) while maximizing the signal-to-noise ratio and minimizing the amount of data that needs to be handled in post-processing. All RS TiGGER experiments presented here were captured in a single photocycle because averaging over multiple photocycles was not required to achieve a sufficient signal-to-noise ratio. As a result, the experiments presented here yield much more information than those in Maity et al. [142] while requiring only 10% of the total acquisition time.

The AsLOV2 experiments presented in this manuscript were done in the presence of excess flavin mononucleotides (FMN), a chromophore that appears naturally in the protein's central binding pocket. FMN is responsible for absorbing a 450 nm photon and initiating AsLOV2's light-activated conformational changes. Preliminary results showed a correlation between dark-state linewidth and temperature that implied the protein seemed to "condense" with increasing temperature. However, loading excess FMN ($100\times$ the protein concentration) eliminated this effect: it is suspected that the central FMN exchanged more readily between the center of the protein and the surrounding environment when it had more thermal energy. In the case of the FMN-surplus samples, the high concentration of external FMN ensured that an equilibrium was established with a higher fraction of loaded proteins. Therefore, to avoid a reduction in TiGGER sensitivity due to proteins lacking an FMN chromophore, all reported AsLOV2 RS TiGGER experiments were done with excess FMN at approximately 100 mM. All other spin labelling,

purification, enrichment, and sample buffer details match what were reported in Maity et al. [142].

To recover the refolding rate, the lineshape of each time-dependent RS TiGGER scan can be fitted to a Lorentzian,

$$\mathcal{L}(B) = \frac{A}{\pi} \frac{\frac{1}{2}\Delta B}{(B - B_0)^2 + (\frac{1}{2}\Delta B)^2}, \quad (2.17)$$

where A is the fit amplitude, B is the magnetic field, B_0 is the resonance position, and ΔB is the full-width at half-maximum in units of field. The result of these fits for data recorded at 18 °C are shown in Fig. 2.17d. The 18 °C relaxation time (54.9 ± 1.4 s) agreed well with the CW experiment performed at 21 °C (51.9 ± 0.3 s) in sec. 2.2.

The RS experiment was repeated at a variety of temperatures from 6 °C to 33 °C. The relaxation times decrease with increasing temperature, as expected for a thermally-activated process, per the Arrhenius equation [216, 217]. The equation states that the relaxation rate constant, k , should behave according to

$$k = A \exp(-E_A/RT), \quad (2.18)$$

where A is a scaling factor, E_A is the activation energy for a conformation change, R is the ideal gas constant, and T is temperature. Rearranging,

$$\ln(k) = \ln(\tau^{-1}) = -\frac{E_A}{R} \frac{1}{T} + \ln(A), \quad (2.19)$$

which can be used to fit the natural log of the refolding rate to a linear equation in inverse temperature to extract an activation energy for refolding, E_A . The results of fitting the data to eqn. (2.19) are shown in Fig. 2.18. The extracted activation energy of 15.5 ± 1.5 kcal/mol is in good agreement with the value of 15.1 kcal/mol reported by Harper et al. [208], which was recorded using time-resolved NMR.

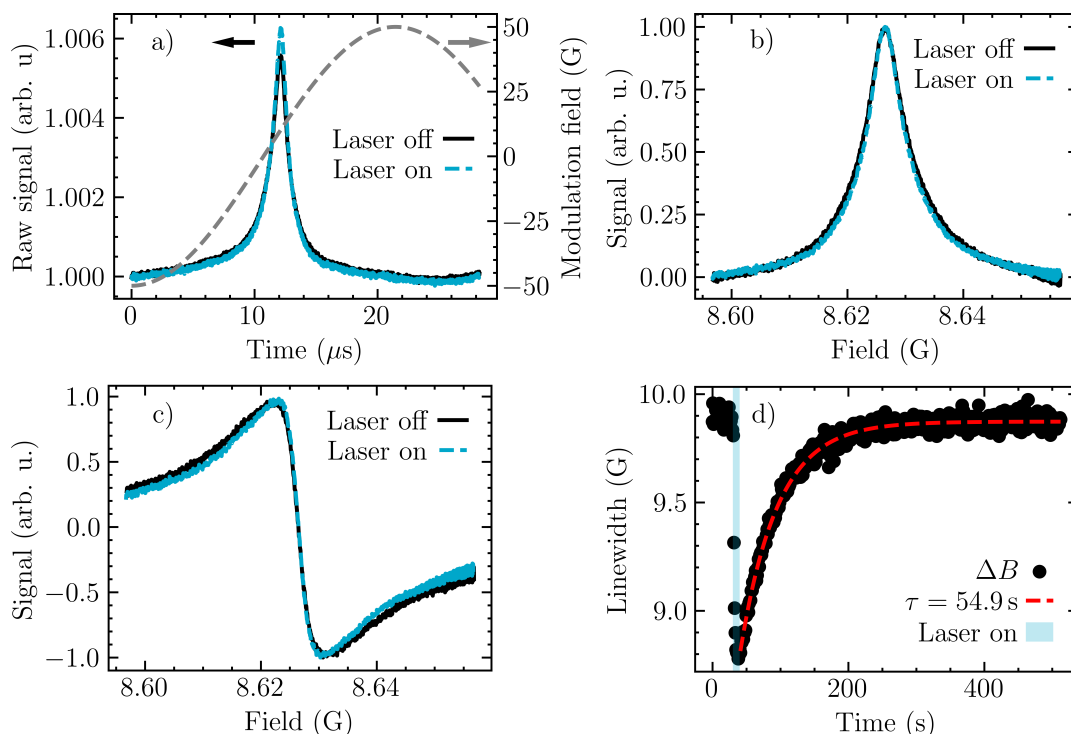


Figure 2.17: Rapid-scan TiGGER of AsLOV2 doubly labeled with Gd-sTPATCN. (a) Raw rapid-scan data of 2.5×10^4 averages (black, at $t = 0$ s with the laser off, and dashed blue at $t = 40$ s, after the laser has been on for 10 s) and modulation field during the rapid-scan sweeps (dashed gray). Colored arrows point toward each line's respective y-axis. (b) Deconvolved rapid-scan absorptive data as a function of field and light activation (a video animation of this plot can be found in the Supplementary Material of [178]). Though only 2 frames are shown, roughly 500 were captured during the 500 s experimental run. When the protein was illuminated with 450 nm light, the linewidth narrowed, and then subsequently relaxed back toward the initial spectrum. The lineshapes are normalized to their individual peak intensities to highlight the light-induced narrowing. (c) Deconvolved rapid-scan dispersive data as a function of field and light activation. (d) Linewidth of doubly labeled AsLOV2 absorptive spectra as a function of time. Linewidths (black points) were extracted from Lorentzian fits of each rapid-scan frame captured during a TiGGER experiment. Fit of an exponential decay (dashed red) returned 54.9 ± 1.4 s, where the uncertainty represents a 95% confidence interval for the time constant returned by the fit. Samples were doubly labeled AsLOV2 at ~ 1 mM in buffer; the buffer included 100 mM surplus FMN. The experiment shown was performed at 18 °C. Samples were loaded into a 100 μ m-thick, 2-by-5 mm (approx. 1 μ L sample volume) borosilicate glass capillary (VitroCom, Mountain Lakes, NJ) to maximize the ratio between surface area and sample thickness and thereby reduce 240 GHz attenuation [59].

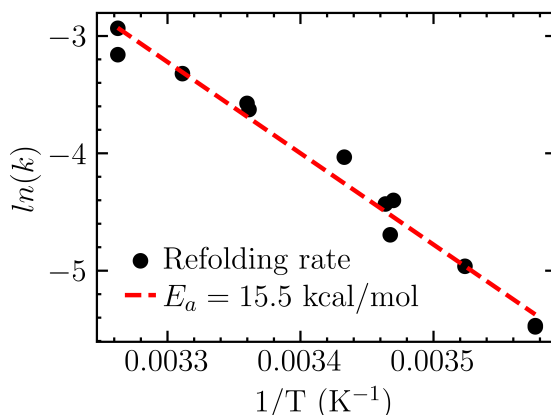


Figure 2.18: Arrhenius plot of the natural logarithm of refolding rate (k ; units of s^{-1}) of doubly labeled AsLOV2 versus inverse temperature. A linear fit (dashed red line) to the raw data (solid black dots) returned an activation energy $E_A = 15.5 \pm 1.5$ kcal/mol.

As a control experiment, similar to what was done in Fig. 2.6d, RS TiGGER was done on single label T406C mutants. Fig. 2.6d showed a small, light-activated, transient peak-height change with the single label mutants. At the time, this was attributed to unintentional labelling of other sites, resulting in a small fraction of proteins being double labeled, and thus, exhibiting a small amount of lineshape narrowing due to light-induced unfolding. The expectation was that similar RS TiGGER experiments would confirm this explanation or even show no light-induced change if the affinity for unintentional labeling was reduced. However, as can be seen in Fig. 2.19, this is clearly not the case. The linewidth is initially narrower than the DL due to lack of dipolar broadening, but actually *broadens* upon light activation, though the timescale matches quite closely with the DL *narrowing* observed in Fig. 2.17. The relative broadening in this case is roughly 25% of the amplitude of the DL narrowing, and cannot be explained by unintentional labeling, as it was assumed that no two residues should get closer together when the protein is light activated and becomes disordered.

A possible explanation for this effect is an unfolding-induced reduction in rotational

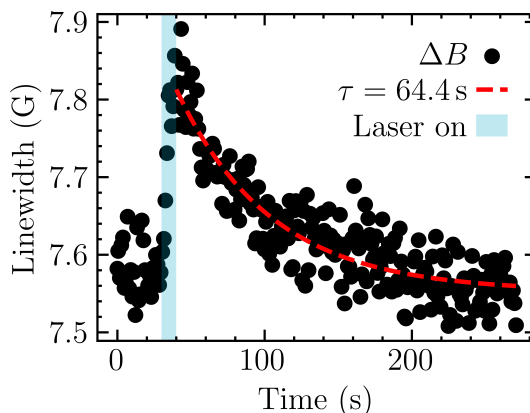


Figure 2.19: Linewidth of singly labeled AsLOV2 absorptive spectra as a function of time. Linewidths (black points) were extracted from Lorentzian fits of each rapid-scan frame captured during a TiGGER experiment. Fit of an exponential decay (dashed red) returned 64.4 ± 11 s, where the uncertainty represents a 95% confidence interval for the time constant returned by the fit. Protein concentration was $\sim 800 \mu\text{M}$ in buffer. The experiment shown was performed at 20°C . Other experimental conditions matched that of Fig. 2.17.

correlation time. Previous studies have shown that at frequencies above roughly 250 GHz, the linewidth of Gd-sTPATCN starts to broaden; this broadening is attributed to g -anisotropy that is unresolved at lower fields [135]. It was hypothesized that after unfolding, the moment of inertia of the protein was larger than its folded conformation; this increased moment of inertia would slow tumbling and reduce τ_c , reducing the amount of motional averaging occurring, and thus, would return a broader line.

While a changing rotational correlation time may explain part of the unexpected broadening, there is a more likely explanation: very recent literature has reported the light-activated dimerization of AsLOV2 [156]. It is well known that some LOV domains dimerize upon light activation [218–222], but no evidence had been shown of such dimerization with AsLOV2 until recently. In light of these results, it was deemed likely that the unexpected broadening shown here was a result of dimerization, highlighting the power of TiGGER to observe functional dynamics of proteins in solution and the relative

simplicity in interpreting its results.

2.3.4 Discussion and conclusion

Field-modulated rapid-scan TiGGER was employed to record EPR lineshapes as a function of time during the photocycle of AsLOV2, which lasts hundreds of seconds, with a temporal resolution of approximately one second. The lineshape contains much more information than a similar CW TiGGER experiment, which samples the EPR spectrum at a fixed field as a function of time. The extra information yielded by RS includes entire lineshapes of Gd(III)'s central transition that are robust to electronic phase and power drift. Such lineshapes are required to extract electronic dipolar couplings. Though current data throughput limits the time resolution to ~ 10 ms, corresponding to averaging ≥ 250 scans at 25 kHz, there is no fundamental reason that the temporal resolution cannot be decreased to well-below 1 ms, or even down to the time required for a single field-swept scan. As discussed above (see Eqn. (2.16)), the SNR for a single scan would still be greater than 10. The data throughput can, in principle, be increased with improved digital hardware and software to enable time resolution into this single-scan regime. Further, the time required for a single-scan may be reduced by increasing the modulation frequency (potentially up to 1 MHz). With these hardware changes together with modest improvements in signal-to-noise ratio, for example by implementing a low- Q resonator, RS TiGGER should be able to resolve much faster dynamics (*e.g.*, the unfolding of AsLOV2, which has been reported to occur on 10-100 μ s time scales [151]).

Eqn. (2.15) may be used to estimate the SNR at 1 MHz while including the effect of a resonator:

$$\text{SNR}(t^{acq} = 1 \mu\text{s}) = 1650\sqrt{\text{Hz}}\sqrt{1 \mu\text{s}} * \eta Q = 1.65 * \eta Q, \quad (2.20)$$

where η is the filling factor and Q is the quality factor of the resonator [223, 224]. For a high field-and-frequency system, a practical resonator candidate would be Fabry-Pérot [225, 226] or open Fabry-Pérot [43, 227, 228] type, with Q -factors reported up to 10,000. However, such high- Q EPR resonators have only been demonstrated at 94 GHz. More attainable Q -factors at 240 GHz, due to the smaller wavelengths (1.25 mm), is $Q \sim 100$, as reported in [40, 111, 229, 230] in an open Fabry-Pérot configuration. Though η depends on resonator and sample relative sizes, the value of $\eta = 0.05$ from Burghaus et al. [231] may be used as an estimate. Therefore, ηQ would improve SNR by approximately 5, up to $\text{SNR} \approx 8$, which is mainly limited by a low filling factor. Photonic bandgap resonators, by comparison, can achieve higher filling factors with comparable Q -factors at high frequency, with reported $Q \approx 450$ at 94 GHz [57]. Since the resonator structure can be scaled to smaller wavelengths quite easily, by simply reducing the thickness of dielectric layers, similar Q -factors should be achievable at 240 GHz. Conservatively assuming a photonic bandgap resonator has double the filling factor of Fabry-Pérot type ($\eta \approx 0.1$), a 240 GHz photonic bandgap resonator with $Q \approx 450$ would still increase the $1 \mu\text{s}$ SNR by a factor of 45, up to $\text{SNR} \approx 72$.

The field-swept lineshapes recorded by RS TiGGER contain, in principle, all the information required to extract inter-spin-label distance distributions as a function of time, rather than only qualitative changes as a result of deviation from the initial coupling strength. Quantitative distance distributions, obtained by fitting entire EPR lineshapes, can report on the entire ensemble of conformations rather than the mean of the population, which is especially important for intrinsically disordered regions of proteins. Further, using RS TiGGER to extract distance distributions *and* activation energies of kinetic processes simultaneously may allow for spectroscopic identification of conformational intermediates in multistep unfolding or refolding processes, for example. For

guidance on how extract distance distributions from EPR spectra, one can look to the extensive literature on the topic between nitroxide and gadolinium-based spin labels, including CW dipolar broadening [14, 23, 134, 232–235], DEER and other pulsed techniques [27, 107, 112, 136, 138, 236], and even R^{-6} dipolar relaxation effects in rapidly tumbling liquids [25].

This section has demonstrated the hardware and software upgrades required to convert an existing CWEPR spectrometer that enable high-field rapid-scan EPR. RS preserves the central transition’s entire time-dependent lineshape as well as all of its underlying information, and can record an entire EPR spectrum in tens of microseconds, making it valuable for observing time-dependent kinetics that manifest as changes in the spectra. The SNR provided by RS is shown to be approximately twice that of CWEPR, after normalizing by the square root of acquisition time. The current lower limit on temporal resolution is approximately 10 ms, corresponding to 250 scans, which returned an average $\overline{\text{SNR}} = 60 \pm 10$ (when low-pass filtered at 25 MHz) for a 1 mM solution of Gd-sTPATCN labels in D_2O . Additionally, using the magnitude-averaging technique discussed, RSEPR enables averaging for long times (above 2.5 million scans) while eliminating the typical susceptibility to electronic phase drift. The applicability of time-resolved, high-field RSEPR to the study of time-resolved protein conformational changes was demonstrated by “filming” the EPR spectrum as a function of time during a single photocycle, without the need for averaging over multiple experimental runs. In the future, the field-swept lineshape may be used to determine time-dependent distance distributions between spin labels in solution state. Further, time-resolved, high-field RSEPR may prove useful for “filming” other kinetic processes, potentially using different samples or spin labels, that can be observed through time-dependent lineshape changes of narrow-linewidth spectra.

Next steps

Although addressing the challenges of extracting distance distributions from RS TiGGER lineshapes deserves its own chapter (and maybe entire dissertation), two of them are briefly stated here, along with approaches to solving them. First, protein tumbling in solution reduces the dipolar broadening via motional narrowing [237, 238]. Therefore, it is necessary to either (1) mitigate tumbling by slowing it down, for example using a viscogen like ficoll [28] or trehalose [239] which may not inhibit protein conformational changes, or (2) devise a method to quantify the incomplete motional averaging, possibly by direct spectral simulation of an intermediate motional regime using a tool such as EasySpin [240, 241] with a rotational correlation time deduced from NMR [242, 243] that is augmented by molecular dynamics simulation [244]. Second, while the distribution of zero-field splittings for Gd-sTPATCN has been measured at cryogenic temperatures [132], it may be different at room temperature, so further experimental or theoretical estimates, or models that do not require ZFS as an input (*i.e.*, Pake convolution) are needed.

The payoff for successfully addressing these challenges is significant, as no other experimental methods are well-equipped to measure time-resolved inter-residue distance distributions of proteins after photoactivation in solution state. DEER [124], cryo-EM [245], and X-ray crystallography [246, 247] all provide high-resolution residue-residue distances, but they cannot be done in solution state. Förster resonance energy transfer (FRET) can be performed at room temperature, in solution, and even on single molecules. [173, 248]. However, it is difficult to use FRET to extract more than a mean distance [175, 249]. Combining FRET with freeze-quenching followed by DEER [100] or cryo-EM [250, 251] will be helpful for benchmarking and validating approaches to extracting distance distributions from RS TiGGER.

2.3.5 Preliminary distance extraction

In order for any least-squares fitting technique to be effective, a subset of free parameters must be chosen that allow for sufficient fitting and distance extraction, without allowing a model to overfit the dataset with extraneous free parameters. A solution of doubly-labeled proteins has many possible fit parameters: these include the entire distance distribution (which is a many-parameter function that may be a combination of Gaussian distributions, but it also may not), the ratio of doubly labeled to singly labeled proteins, the rate of their tumbling motion, the unfolding and folding relaxation rates, the fraction of proteins that do not activate (always in the dark, folded state), the fraction of proteins that always unfolded, and the rest of the Hamiltonian parameters (ZFS, exchange, hyperfine splitting, intrinsic anisotropy from the apparatus, etc.). To assist with this complication, two techniques were utilized: (1) simplification of the distance distribution used for fitting using results by principal component analysis (2) Pake convolutional fitting that allowed the fitting scheme to omit the details of the Hamiltonian parameters not associated with dipolar coupling.

1. Principal component analysis

Principal component analysis was used to decode the number of primary distance configurations within a given solution-state sample. The leading hypothesis for the refolding kinetics is that they happen quite rapidly on an individual-protein scale, and the exponential recovery is a result of many proteins undergoing “spontaneous” relaxation with some intrinsic lifetime that is related to the likelihood of this relaxation [208]. This implies that most proteins will be in some stable or pseudo-stable conformational state, and the number of these states may be detectable by analyzing the “principal” compo-

nents of the spectrum, using principal component analysis (PCA) [252]. PCA is often used with multi-variate biological assays to pick the variables (or combinations of them) that introduce the largest change in the output. As such, it was hypothesized that PCA could provide an eigenvector that is related to the changing relative populations of n pseudo-stable configurations.

The PCA presented here used a singular value decomposition (SVD)-based technique (SVDcleaned.py using `numpy.linalg.svd`, [206]). SVD can provide the information necessary to extract the number of spectrally distinguishable species in a combined spectrum, which is exactly the quantity of interest in this case; the number of spectrally distinguishable species is equivalent to the number of accessible protein configurations—assuming all other spin labels conditions are unchanged—and therefore can be used to significantly reduce the parameter space for least-squares fitting [253]. As written in Henry and Hofrichter [253], if the data matrix can be represented as A_{ij} , where i is the spectral position (for RS TiGGER, this is field point) and j is the time point, the combination of spectra is given by:

$$A_{ij} = \mathbf{A}(B_i, t_j) = \sum_{n=1}^{n_s} f_n(B_i) c_n(t_j), \quad (2.21)$$

where f_n is the distinguishable spectral shape and c_n is the time-dependent weight of each spectrum. Of course, the time-dependent weight of a folded-versus-unfolded configuration would be of interest in a TiGGER experiment, so too would be the f_n s, if they could be extracted using PCA. This is not necessarily the case, as PCA can only pick the axes of which the dataset varies the *most*, these axes are not necessarily physical. However, if the experiment of interest has only a few independent variables, it is conceivable that these f_n s may be recognized after decomposition.

For SVD-PCA, the data acquired from RS TiGGER was placed into a 2D data matrix,

with field positions along the vertical dimension and time points along the horizontal. There is some jitter present due to imperfect trigger timing (limited by the digitizer’s time resolution) and current noise in the modulation coil, so the data was processed to center each spectrum at the same field position. After centering, the 2D data matrix is shown in Fig. 2.20.

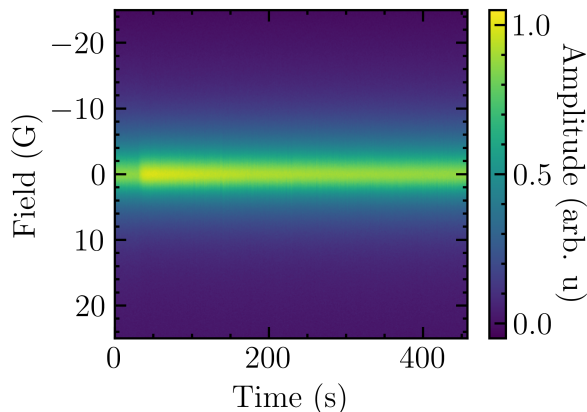


Figure 2.20: 2D matrix generated from a single RS TiGGER experiment of doubly labeled AsLOV2. The laser was turned on at $t = 30$ s and turned off at $t = 45$ s. Data is centered so that each time point has its peak maximum at the same field position. Data is normalized so that the maximum value in the dataset is equal to unity. Large values (close to 1) are yellow, small values (close to 0) are blue. Experiment was performed at 10.0°C .

This matrix, $\mathbf{X}$, was decomposed according to

$$\mathbf{X} = \mathbf{U}\mathbf{\Sigma}\mathbf{V}^T, \quad (2.22)$$

where $\mathbf{X}$ is the raw data (shown in Fig. 2.20), $\mathbf{U}$ is the left-singular matrix, which has column vectors that make up the field-dependent orthogonal basis, and $\mathbf{V}$ is the right-singular matrix, which is made up of column vectors that set the time-dependent weight of each left-singular column.

To determine the number of “principal components”, which can be somewhat subjective, a Scree plot may be used [254]. A Scree plot shows the PCA eigenvalues σ_n (the

diagonal elements of Σ), which allows for determination of the “significant” components. There are a few ways to choose these components, including taking all eigenvalues σ_n that are larger than unity, or finding the inflection point using the second derivative, though there is no one “correct” technique and the validity of each technique varies depending on the individual dataset. For the purpose of this dissertation, the significant components were defined such that $\sigma_n/\sigma_{n+1} > 1.5$. A log-linear Scree plot for the experiment in Fig. 2.20 is shown in Fig. 2.21.

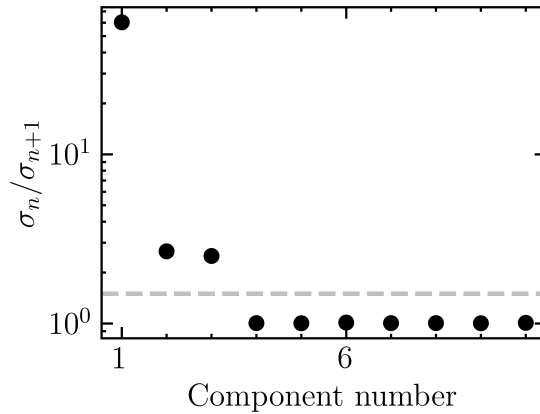


Figure 2.21: Scree plot from RS TiGGER experiment of doubly labeled AsLOV2. Principal components are chosen such that the eigenvalues σ_n of each component (black dots) are $1.5\times$ larger than the next eigenvalue. Experiment was performed at 10.0°C .

PCA of this dataset with $n = 3$ components is shown in Fig. 2.22. Note that the data was *not* mean-centered, as is often done before PCA, as interpretation of the results was less obvious after mean-centering.

As was hypothesized, the second component, $\mathbf{u}_2$ appears to be made up of the difference of two Lorentzians. These can be interpreted as the components of the spectra that are changing in time; the fit returned two of them, as one might expect if there were two conformational states within the protein sample. Further, the time-varying weight of this component changes sign when the laser is turned on. This means that the posi-

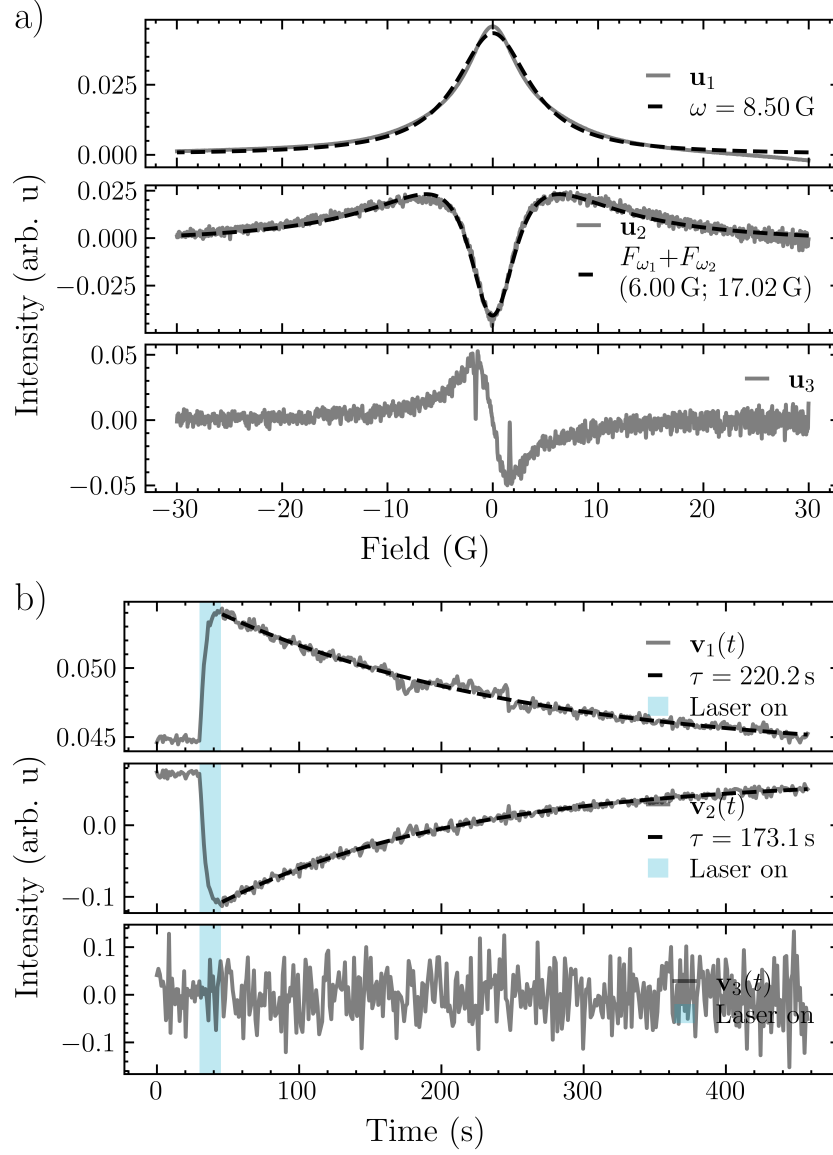


Figure 2.22: SVD-based PCA from RS TiGGER experiment of doubly labeled AsLOV2. (a) Column vectors $\mathbf{u}_i$ of $\mathbf{U}$. $\mathbf{u}_1$ and $\mathbf{u}_2$ are fitted to Lorentzians. $\mathbf{u}_1$ is fitted to a single Lorentzian with $\Gamma = 8.50 \pm 0.05$ G. $\mathbf{u}_2$ is fitted to two Lorentzians with widths $\Gamma_1 = 6.00 \pm 0.10$ G and $\Gamma_2 = 17.02 \pm 0.39$ G. The ratio of the two Lorentzians' integrals is $1.03 \pm 0.04 : 1.92 \pm 0.03$. (b) Column vectors $\mathbf{v}_i$ of $\mathbf{V}$. $\mathbf{v}_1$ and $\mathbf{v}_2$ are fitted to exponential decays. The fits return $\tau_1 = 220.2 \pm 12.9$ s and $\tau_1 = 173.1 \pm 6.3$ s, respectively. The uncertainties represent 95% confidence intervals for the parameters returned by the fit.

tive (broad) Lorentzian component of $\mathbf{u}_2$ is initially contributing to the EPR lineshape before the laser illumination, and this contribution is replaced by the negative (narrow) contribution during it. Therefore, a subpopulation of individual proteins go from their dark state (nearby, broader line) configurations to their lit state (further apart, narrower line) configurations.

The first component is related to the mean of the data, and is made up of only a single Lorentzian-like spectrum. The amplitude of this spectrum changes with time, as seen in 2.22b, but the sign does not; this component may be related to the amplitude of the spectrum, for example, though it does not seem to be relevant to the spin-spin distances. The third component has a peculiar shape that is difficult to interpret. This shape is reproducible across a range of similar experiments, and seems to be a real component of the TiGGER lineshape. However, since it displays no time dependence, its interpretation is outside the scope of this dissertation.

The separability of the two populations within $\mathbf{u}_2$ provide constraints for the convolutional fitting discussed above. As there seems to be only *two* main components (and therefore distances) switching back-and-forth with light activation, the fit can be constrained to use only two distance distributions.

2. Pake convolutional fitting

By global fitting a combination of R^{-3} static dipolar couplings via convolution with a linear combination of Pake patterns [76], extraction of distance distributions should be possible. At room temperature, however, this is a complicated and ill-posed problem—it involves Fourier deconvolution of a noisy spectrum *and* motional narrowing through tumbling ($\tau_c \approx 10$ ns, $2\pi/\omega_{dd} \approx 100$ ns).

Direct simulation of dipolar-broadened lineshapes of proteins tumbling in solution

may provide simpler access to distance distributions, but requires both estimating values for Gd-sTPATCN ZFS at room temperature and is prohibitively slow, which is further complicated by the fact that the inter-residue distances require a calculation over a distribution rather than a single value. Additionally, no other well-established technique is capable of providing room-temperature distance distributions between residues as a function of time after light activation, which complicates the validation any distribution extraction routine developed here. DEER, cryo-EM, and X-ray crystallography all provide high-resolution structures, but because they cannot be done in solution state, their findings may not necessarily return the same distance distributions as provided by TiGGER. Active development of freeze-quench cryo-EM has improved the stability of its rapid freezing and has made it potentially capable of capturing the solution-state structure of AsLOV2 [255]; experiments to this effect constitute future work for the project. Förster resonance energy transfer (FRET) may also prove useful for validation, as it can be performed at room temperature on single molecules and therefore may be able to provide a useful guideline for the ensemble-based TiGGER measurements [173, 248]. However, difficulties exist with FRET as well: orthogonally labeling a donor and acceptor is nontrivial, and it is very difficult to use FRET to extract more than a mean distance (*i.e.*, a distance distribution) [175, 249].

Forward-convolving Pake patterns with intrinsic lineshapes can mitigate some of these issues. These intrinsic (*i.e.*, not broadened by dipolar coupling) lineshapes contain all the information about the spin Hamiltonian, without needing to know $\mathbf{A}$, D , E , etc. Convolution introduces the effect of dipolar broadening such that the final output, assuming the simulated Pake pattern is representative of reality, replicates the experimental dipolar-broadened lineshape. For this purpose, a library of Pake patterns was generated with EasySpin chili (version 6.0.4 [20]) at a range of Gd-sTPATCN-accessible distances

($r = 2\text{-}7\text{ nm}$).

The electron-electron coupling tensor was calculated using

$$\hat{\mathcal{H}}_{dd}(r) = \hbar\omega_{dd}(r)\hat{\mathbf{S}}_1 \cdot \begin{pmatrix} 1 & & \\ & 1 & \\ & & -2 \end{pmatrix} \cdot \hat{\mathbf{S}}_2, \quad (2.23)$$

where ω_{dd} is given by:

$$\omega_{dd}(r) = \frac{\mu_0}{4\pi} \frac{(\mu_B g)^2}{\hbar} \frac{1}{r^3} \quad (2.24)$$

(see [256] for details). The simulation incorporated the dipolar coupling between one spin-1/2 ($g = 1.992$) and one spin-7/2 ($g = 2$) to most closely match the experimental conditions, as only the $|-1/2\rangle \rightarrow |1/2\rangle$ transition is observed with an RS TiGGER experiment (at $g = 1.992$ field). The two spins were given slightly different isotropic g -values in order to simulate the weak-coupling regime, where $\omega_{dd} \ll |\omega_1 - \omega_2|$. The weak-coupling approximation is presumed valid because most of the spins that the “probe” $m_s = -1/2$ spins interact with have a much different resonant field due to Gd-sTPATCN’s ZFS strain; at room temperature, the majority of nearby spins are not spin-1/2, and thus exist in the roughly 100 mT ZFS-strain-induced background [29, 132]. The correlation time of the Brownian motion of the spin system was taken to be $\tau_c = 13.8\text{ ns}$, as estimated using the equations from Cavanagh et al. [30] for a 17.3 kDa protein domain and two 0.9 kDa spin labels in an H₂O-based buffer at 10 °C [29]. Of course, the fit is highly dependent on the Pake patterns used, which themselves depend strongly on the correlation time chosen. Autocorrelating molecular dynamics simulations using a second order Legendre polynomial (see [244, 257]) returns a correlation time of 2.2 ns. Pake convolving EasySpin simulations that use $\tau_c = 2.2\text{ ns}$ returns much shorter distances than $\tau_c = 13.8\text{ ns}$, as expected, because Pake patterns get much narrower with faster tumbling (see Fig. 2.23).

However, since the MD simulations did not account for the viscosity of the buffer solution nor that the experimental temperature was different from room temperature, it is likely that the MD estimate of τ_c is below the true value. Additionally, MD is traditionally not used for analyses of overall tumbling, and the standard water models are not optimized to perfectly replicate water dynamics on those distance scales as a result. See, for example Daragan and Mayo [258]—the τ_c for a protein of AsLOV2’s size (144 residues) would be expected to be around 10 ns at 30 °C. For this reason, 13.8 ns is chosen as the “correct” τ_c at 10 °C for the following treatment. Since 2 ns and 13.8 ns do not agree—and are almost an order of magnitude apart—the results presented in this section are not yet reliably quantitative; they are presented here as a first step toward time-resolved distance extraction—significant future work will be required before the results can be considered accurate.

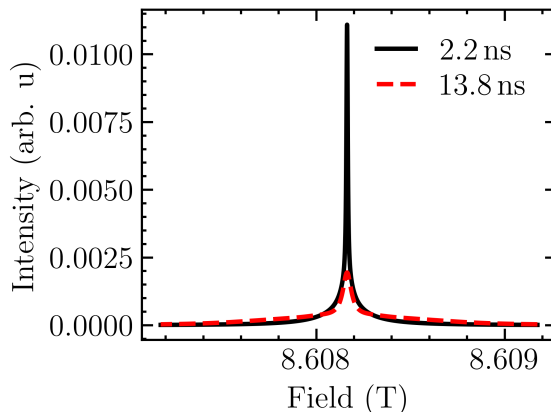


Figure 2.23: *chili*-simulated Pake patterns for Gd-sTPATCN separated by 2 nm at 2.2 ns and 13.8 ns. The 2.2 ns pattern is narrower with less shoulder as a result of motional narrowing (see section 1.1.2), which requires the fitted distance distributions to be much closer together than when fitting with the 13.8 ns Pake pattern. Both lineshapes were normalized by their integral.

To roughly confirm that 13.8 ns is the correct rotational correlation time, the estimate from Anderson and Weiss [31] may be used (see eqn. (1.50)), assuming the frequency

of motion is much larger than the width of the coupling. An estimate of the dipolar coupling strength is provided by eqn. (1.21). For $r = 3 \text{ nm}$, $\omega_{dd} = 2\pi * 1.9 \text{ MHz}$ for two spin-1/2s. In the case of high spin at high temperature, the spin manifold is almost equally populated across $-7/2 \dots 7/2$ and the average m_s of a nearby spin is $\overline{m_s} = 2$, four times that of two spin-1/2s, which makes the coupling frequency $\omega_{dd} = 2\pi * 7.6 \text{ MHz}$. Compared to $\omega_m = 2\pi/13.8 \text{ ns}$, ω_{dd} is only roughly ten times smaller and the condition $\omega_0 \ll \omega_m$ is not fulfilled. Regardless, the motional narrowing may be roughly estimated using eqn. (1.50). A singly labeled T406C sample in buffer was taken to be the “intrinsic” linewidth, and a doubly labeled T406C-E537C sample in a buffer-Ficoll-70 mixture at roughly 30% w/w% was used as a “stationary” linewidth. Ficoll-70 is well-known to reduce overall rotation when added at 20-40% w/w% in solution [28, 259–262]. ω_0 , as a result of dipolar broadening, was approximated by taking the difference between the stationary and the intrinsic linewidths. The experiments are shown in Figs. 2.24a and 2.24c. The difference between the two is roughly 2.4 G. In field units, 2.4 G is 6.7 MHz. Following eqn. (1.50),

$$\Delta\omega = \omega_0^2/\omega_m \quad (2.25)$$

$$= (2\pi * 6.7 \text{ MHz})^2/(2\pi/13.8 \text{ ns}) \quad (2.26)$$

$$= 2\pi * 0.6 \text{ MHz} \quad (2.27)$$

which, in field units, corresponds to a broadening of $\Delta B = 0.2 \text{ G}$. This is quite a bit smaller than the observed value of roughly 1.6 G, based on experiments comparing Fig. 2.24a and 2.24b. The discrepancy is likely a result of three things: (1) the formula from Anderson and Weiss [31] is meant as an estimate, (2) the tumbling was in the intermediate, not the rapid motion regime, as the estimate was designed for, and (3) the protein was not completely stationary in Ficoll-70, so some tumbling still occurred.

Wenner and Bloomfield [263] report that Ficoll-70 at 20% w/w% increases the viscosity by roughly a factor of 10; following Cavanagh et al. [30], this should correspond to a factor of 10 slowdown in tumbling. This slowdown would only increase τ_c to roughly 100 ns, which is still in an intermediate motional regime where some averaging would be expected. Further studies, potentially using NMR [242, 264], are required to precisely measure the correlation time; for now, fitting is performed with $\tau_c = 13.8$ ns, which is expected to be roughly correct.

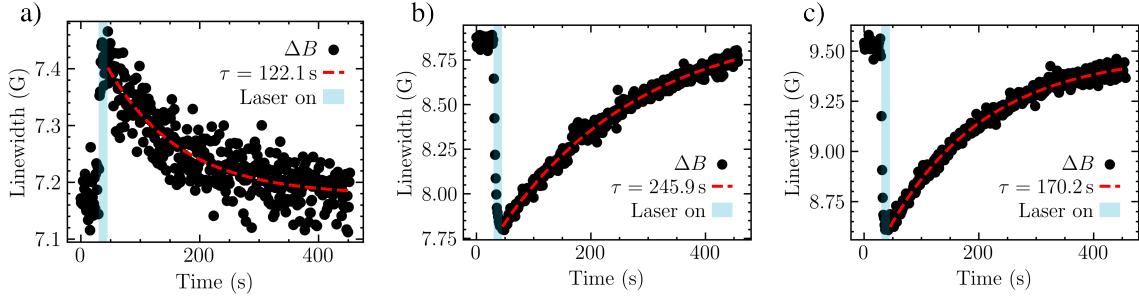


Figure 2.24: RS TiGGER measurements to record τ_c -dependent dark-state linewidth. (a) 800 μ M T406C SL AsLOV2 in buffer at 9.8 $^{\circ}$ C. Dark state linewidth is roughly 7.2 G. (b) 600 μ M DL AsLOV2 in buffer at 10.0 $^{\circ}$ C. Dark state linewidth is roughly 8.8 G. (c) 600 μ M DL AsLOV2 in 25% w/w% Ficoll-70 in buffer at 10.2 $^{\circ}$ C. Dark state linewidth is roughly 9.6 G.

The Pake convolution works as follows: if the intrinsic (unbroadened) spin label lineshape is $S_I(B)$, where S_I contains all the spectral information about $\hat{\mathcal{H}}_Z$, $\hat{\mathcal{H}}_N$, $\hat{\mathcal{H}}_{ZFS}$, etc. The Pake pattern, $P(B)$ contains *only* the dipolar coupling, and can be modeled using a piecewise function [134, 265], or simulated using EasySpin, which is able to incorporate the effects of tumbling in solution using the function `chili` [20]. It then follows that the broadened lineshape, $S_B(B)$ can be computed by convolving the two lineshapes, such that

$$S_B(B) = \int_{-\infty}^{\infty} P(\beta) S_I(B - \beta) d\beta. \quad (2.28)$$

The most complete way to perform this calculation is to allow the Pake pattern to be made up of a *distribution* of spin-spin distances, $P_r(B)$, which more closely simulates how they exist in nature:

$$P_r(B) = \int_0^\infty P(B, r) \rho(r) dr, \quad (2.29)$$

where $\rho(r)$ is the distance distribution of interest. To extract $\rho(r)$, global fitting of the data matrix in equation 2.20 is performed using the `lmfit` Python package [266] and allows for variation of the following:

1. The centers of the two distance distributions.
2. Their relative weights, requiring their functional form to be a time-dependent exponential.
3. The decay constant of the exponential.
4. Peak-centering to account for trigger jitter and modulation coil current noise.
5. An overall amplitude to scale the fit.

The fit minimizes the sum-of-squares error between the experimental and fitted 2D data matrices. The fitted 2D matrix is generated by first producing a set of linearly combined Pake patterns that incorporate a simulated distance distribution, which itself is made up of two normalized Gaussians using the following functional form:

$$\rho(r, t) = \frac{A}{\sqrt{2\pi}} \left[\frac{(1 - \alpha(t))}{\sigma_1} \exp\left(-\frac{(r - R_2)^2}{2\sigma_1^2}\right) + \frac{\alpha(t)}{\sigma_2} \exp\left(-\frac{(r - R_2)^2}{2\sigma_2^2}\right) \right] \quad (2.30)$$

with

$$\alpha(t) = \alpha_0 \left\{ H(t - 30 \text{ s}) H(45 \text{ s} - t) \exp\left(-\frac{t - 30 \text{ s}}{\tau_1}\right) + H(t - 45 \text{ s}) \exp\left(-\frac{t - 45 \text{ s}}{\tau_2}\right) \right\}, \quad (2.31)$$

where r is between 2-7 nm; A , R_i , α_0 , and τ are free parameters for the fit; and $H(t)$ is the Heaviside step function. During the experiment, the laser was on from 30 s to 45 s, represented by the first two Heaviside steps in (2.31). The Pake patterns are arranged in an $n_r \times n_B$ matrix, where n_r is the number of simulations along the distance axis, and n_B is the number of field points in each EasySpin simulation (for this example, the number of field points was 2048). Each summed Pake pattern is

$$P_r(B) = \mathbf{P}_{n_B \times n_r}(B, r) \boldsymbol{\rho}_{n_r \times 1}(r, t), \quad (2.32)$$

and the 2D fit matrix, $\mathbf{M}^{fit}$, is therefore given by

$$M_{ij}^{fit} = \int_{-\infty}^{\infty} \mathbf{P}_{n_B \times n_r}(\beta, r) \boldsymbol{\rho}_{n_r \times 1}(r, t_j) S_I(B_i - \beta) d\beta, \quad (2.33)$$

for discrete field and time steps B_i and t_j .

The astute reader will note that the widths of the distance distributions are not free parameters for the fit. This was done to minimize the number of free parameters while still recovering physically meaningful results. The widths of the distributions were fixed at $\sigma = 0.34$ nm and $\sigma = 0.6$ nm for the near and far distances, respectively. These values were estimated from previous DEER experiments (see Fig. 2.25) and the `chilife` DEER simulations (see Fig. 2.3). Though it is likely the DEER distances in a frozen state do not perfectly match the true distances in the solution state, fixing this parameter constrains the fit while still allowing the main parameters of interest (mean distances) to vary.

The global fit of the raw DL data shown in Fig. 2.20, fitted using the `lmfit` package's Nelder-Mead method [267] with T406C SL data as S_I , is shown in Fig. 2.26. It can be seen that the fit agrees with the raw data quite well and exhibits the same time-dependent narrowing and re-broadening. The residuals from the fits are shown in Fig. 2.27; the deviation from the raw data is small (almost entirely $< 5\%$ of the signal amplitudes).

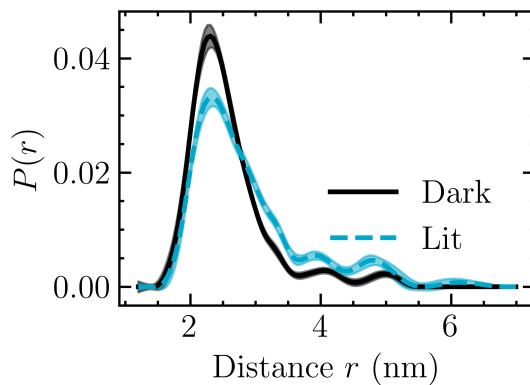


Figure 2.25: AsLOV2 DEER in dark and lit state frozen at 30 K. In the dark state, the first distribution has a width of approximately 0.8 nm at full-width half-max, which corresponds to roughly to $\sigma = 0.34$ nm, as $\text{FWHM} = 2.355\sigma$ for Gaussian distributions. The second distribution is complicated and is made up of two peaks. This is most likely an artifact of the regularization process, not the true distribution. Therefore, for the purposes of the fitting performed here, the two peaks were assumed to be a single distribution with a FWHM of roughly 1.5 nm, corresponding to a Gaussian width of $\sigma = 0.6$ nm.

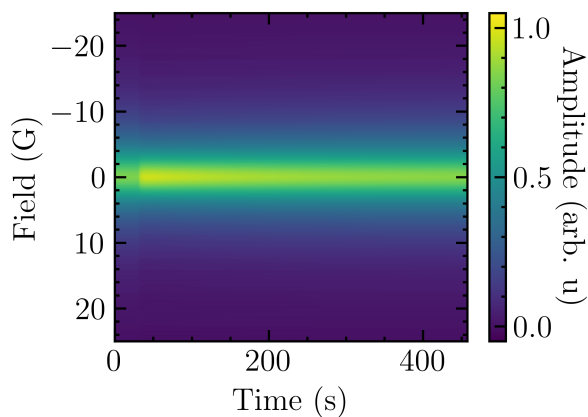


Figure 2.26: 2D globally-fitted matrix of RS TiGGER experiment in Fig. 2.20. The laser activation was simulated with two Heaviside step functions, $H(t-30\text{ s})H(45\text{ s}-t)$. Large values (close to 1) are yellow, small values (close to 0) are blue.

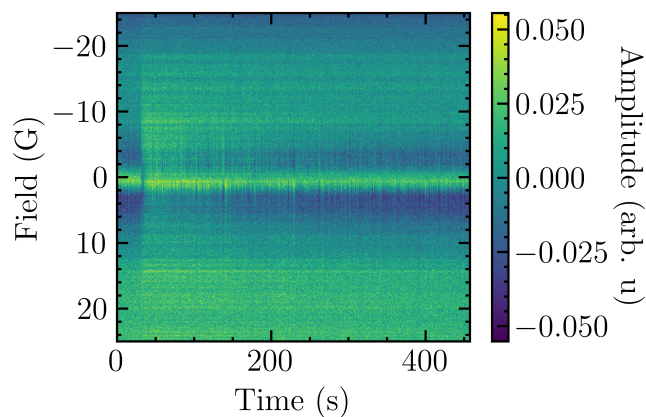


Figure 2.27: Residuals of global fit for 2D matrix of RS TiGGER experiment. Result is difference between the data in Figs. 2.20 and 2.26. The residual was $\pm 5\%$ or less of the total signal, and its relatively constant shape is due to the experimental lineshape being slightly narrower than a true Lorentzian.

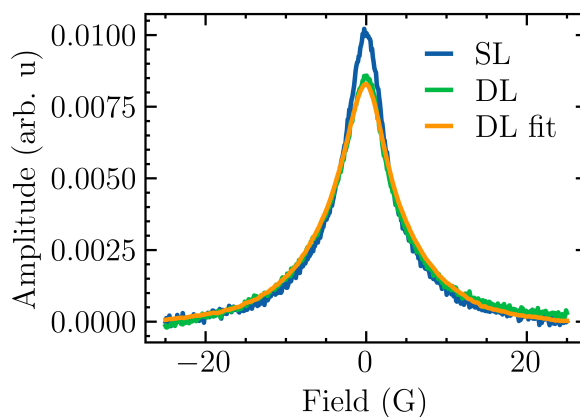


Figure 2.28: 1D slice of Pake convolution-based fitted spectra at $t = 0$, before the laser is turned on. The singly labeled (SL, blue) plot is normalized to its integral and the doubly labeled (DL, green) and DL fit (orange) plots are normalized to the integral of DL. The slice shows that the fit is very close to the observed DL lineshape. SL is the intrinsic lineshape used for the fitting routine ($S_I(B)$ in eqn. (2.28)).

The fits shown in Figs. 2.26 and 2.28 are quite promising. The fitting procedure is reliable and returns similar results across a handful of experiments at similar temperatures, meaning the extracted distance distribution, $\rho(r)$, may be correct. The distance distribution corresponding to the fit in Fig. 2.26 is shown in Fig. 2.29, and the relative fraction of unfolded proteins during the photocycle (as returned by the fit) is shown in Fig. 2.30. Because the function in eqn. (2.30) does not allow for singly labeled or dark-state-unfolded proteins, and it was able to fit the data quite well, it may be assumed that the ensemble was effectively (near 100%) doubly labeled. Further, the dark-state distance distribution was centered at 3.2 nm, quite close to the expected value of 3.0 nm from the **chiLife** simulations that used the crystal structure of AsLOV2 from Halavaty and Moffat [153] (see Fig. 2.3).

Though this represents significant progress, it remains possible that the results returned by this fitting are incorrect. As discussed earlier, there currently no datasets that can be used to validate TiGGER with certainty: **chiLife** uses the crystal structure, which may be different from solution state, and DEER is frozen, which again, may alter the distance distributions. Cryo-EM measurements of a freeze-quenched ensemble will provide apt comparison in order to validate, though work is ongoing in this regard. It seems possible (even intuitive) that proteins that are free to tumble in solution would be less compact than when crystallized or frozen, and thus would return slightly longer distances with TiGGER than with techniques that require immobilization, but more evidence is required before such a claim can be made. Establishing a “correct” answer to compare TiGGER to remains open at the time of this writing, and progress toward one would be of significant importance to the structural biology and optogenetic communities.

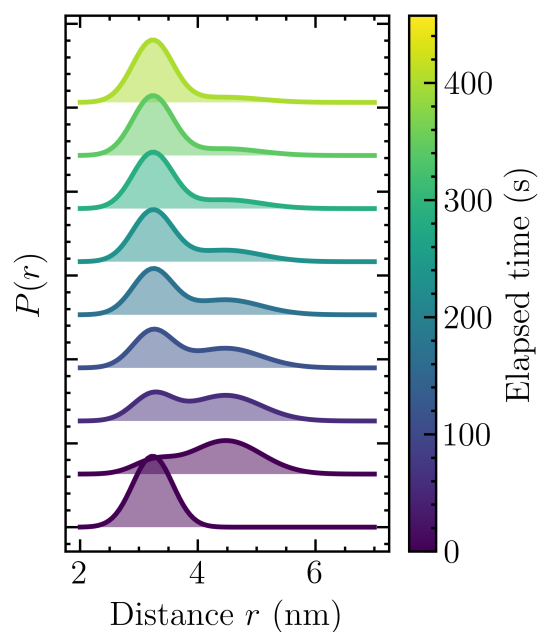


Figure 2.29: Time-resolved distance distributions from an RS TiGGER experiment. It can be seen that before the laser is “turned on” at $t = 45$ s, the distribution is a single Gaussian and centered at roughly 3.2 nm. After the laser is turned on, a second distribution appears around 4.5 nm, representing the narrowed lineshape due to the protein moving. The width of the distributions were fixed to $\sigma = 0.34$ nm and $\sigma = 0.6$ nm, respectively. There are 9 distributions shown over a total experimental time of roughly 456 s, corresponding to 57 s of elapsed time between each distribution from bottom to top. Each distribution is normalized to 1.

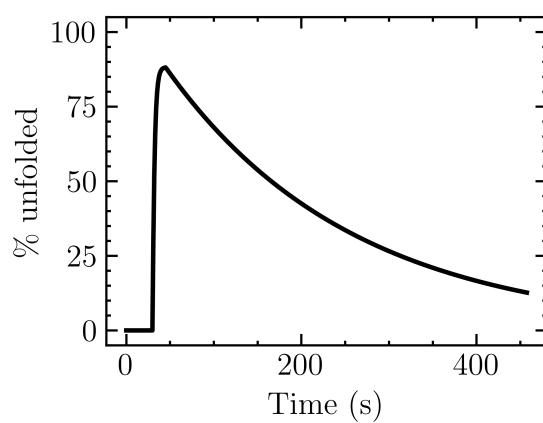


Figure 2.30: Fraction of “unfolded” proteins (*i.e.*, long-distance conformers) as a function of time after light activation, as returned by the Pake convolution fitting process. According to the fit, roughly 90% of the proteins unfolded and moved apart in the lit state. The time constants of the unfolding and refolding exponentials were 2.2 s and 213 s, respectively.

Chapter 3

FEL-powered EPR and ESR for studies of spin dynamics at high magnetic fields

3.1 Introduction

High-field ESR is an area of active research, as it can provide a significant boost to spectral resolution when compared to low field; the spectral resolution increases due to the increased splitting of the anisotropic field-dependent Zeeman terms in the spin Hamiltonian. Further, the high frequency sources that are required for high field EPR can provide access to large ZFS (at low fields) terms that are otherwise inaccessible with low frequency (*e.g.* 9.5 GHz X-band) sources [5]. However, high frequency systems require hardware considerations that are different from their low-frequency counterparts. Some of these considerations include quasi-optical bridge designs, non-resonant sample holders, and induction mode detection architectures [268] (see sec. 1.1.4).

Most high frequency sources suffer from very low pulsed power (milliwatts), which reduces the efficiency of pulsed EPR in particular. ITST is home to a rare instrument that can produce kilowatt powers in the sub-THz regime: a free electron laser (FEL). Utilizing the free electron laser as an EPR source permits probing short-lived excited states due to many orders of magnitude more power than state-of-the-art solid-state mm-wave sources (roughly 6 kW vs. mW) [134]. However, configuring the FEL for EPR is challenging. As an example of the difficulties involved with using the FEL for EPR, simply creating more than one pulse with precise temporal control of on-and-off timings from a quasi-continuous wave FEL pulse requires dozens of optical components, two optical tables, and two high-power solid-state pulsed laser sources. Detection poses another challenge, as the kW-level powers from the FEL is enough to damage the sensitive Schottky-diode based subharmonic mixers that are often used for mm-wave detection. An in-depth discussion of some of these challenges and solutions to them, among others, are presented in this chapter.

Considering the difficulties of using a free electron laser (FEL) or other high power, quasi-continuous wave source (*e.g.*, gyrotrons) for EPR, the potential benefits must be great. The most significant benefit is greater access to excitations with lifetimes too short to be probed with low-power (milliwatt) sources. Typically, pulsed EPR is assumed to be performed with “hard pulses”, where the sample’s spin dynamics are treated as negligible during the pulse. However, for fast-relaxing samples, this is not possible with the low-power sources that are prevalent at high frequencies (> 100 GHz). For example, the low-power source at UCSB (30 mW VDI-0027, Virginia Diodes, Inc.) has a π -pulse length of roughly $1 \mu\text{s}$. For dense or strongly coupled spin systems, this is much longer than relevant spin excitation lifetimes (usually hundreds of ns, though can be even shorter, see *e.g.* [269–272] and sec. 3.2), and thus, low-power pulse sequences are unable

to resolve these dynamics. Further still, at high frequencies, as has been mentioned previously, the B_1 field strength can not be amplified by resonant cavities because the wavelengths ($\lambda \approx 1$ mm or less) are too small for practical operation of such resonators [268]; this means that to approach the higher sensitivities that, in theory, pulsed EPR at high frequencies can provide, the excitation power must be large enough to ensure similarly “hard” pulses when compared to resonator-enhanced low-frequency EPR [273]. The FELs at ITST are capable of kW-power, μ s-long, quasi-continuous wave pulses across a range of frequencies from 0.1 to a few THz. These properties make it ideal for EPR operation across a wide frequency range (100-500 GHz or 3-18 T) with powers that are otherwise inaccessible [134]. UCSB’s FEL and FEL-EPR operation has been described in great detail elsewhere (see [50, 53, 59, 69, 134, 274–277]) and will not be reviewed here, except where comparison is relevant.

Sec. 3.2 discusses using 240 GHz FEL-EPR to probe spin dynamics in CoNb_2O_6 , a condensed matter system that well-replicates transverse field Ising chain (TFIC) dynamics as a result of strong ferromagnetic exchange interaction along one crystal direction and very weak antiferromagnetic exchange in the other two. These studies were able to place an upper bound on the coherence time of the low-lying excitations of this system. However, because of the relatively long dead-time (70 ns), and limited pulse sequences and phase cycling capabilities, the precise lifetime of these excitations could not be determined. It is possible that upgrades, specifically engineered to mitigate these problems, will provide future access to short-lived excitations in systems like CoNb_2O_6 that host exotic magnetism.

The later sections of this chapter are dedicated to these upgrades and the construction of an FEL-powered electron **spin** resonance (FEL-ESR) spectrometer, including the design and construction of a novel frequency-agile pulse-slicer that enables more compli-

cated (T_1 sensitive) pulse sequences; a frequency-agile, continuous phase-shifter module; and a frequency-agile quasi-optical sample holder that greatly reduces signal background while enhancing EPR response through mechanical adjustment to greatly improve spectrometer SNR.

3.2 FEL-EPR studies of fractionalized excitations

This section represents a dramatic change in focus from those preceding it—rather than dilute paramagnetic samples in a biological system, attention is turned to dense, many-body interacting spin systems in condensed matter crystals. This section also serves as additional motivation the following sections, as it highlights the shortcomings of the current 240 GHz FEL-EPR spectrometer and the potential of the resulting novel design improvements that eliminate those shortcomings. The primary topic of interest is an experimental realization of the “transverse field Ising chain”, a condensed matter system that is well-known to obey the physics of the analytically solvable Ising chain [278]. Though the Ising chain is typically thought of as a pedagogical tool, the applicability of the Ising Hamiltonian to TFIC systems allows for direct comparison between analytically solvable theory and realizable experiments [279]. Further, the excitations of such systems are “fractionalized”, meaning they host partial conventional degrees of freedom. This will be discussed in more detail, but is of immense current interest due to their existence in topological condensed matter systems such as some quantum spin liquids. UCSB’s FEL-powered EPR and novel ESR, and the very short excitation lifetimes they are sensitive to because of their high power, are uniquely equipped to probe “spinon echoes” and measure, for the first time, the lifetime of the fractionalized excitations hosted within quantum magnets.

3.2.1 The transverse field Ising chain

The “transverse field Ising chain” is a common pedagogical problem. It considers the effect of a 1D spin chain with strong nearest neighbor coupling and neglects all other couplings. In the simplest case,

$$\hat{\mathcal{H}}_I = -J \sum_{\langle ij \rangle} \sigma_i^z \sigma_j^z \quad (3.1)$$

where J is the exchange coupling, i, j represents an integer location along the chain, $\langle ij \rangle$ is a sum over nearest neighbors, and σ^z is the usual Pauli matrix in the z direction,

$$\sigma^z = \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}. \quad (3.2)$$

Because this Hamiltonian is diagonal in the two-spin product basis, the eigenstates are obvious—they are the product version of the spin “up” and “down” eigenstates discussed in section 1.1.1. J may be positive or negative: when $J > 0$, the ground state is a ferromagnetically aligned spin chain; when $J < 0$, the ground state is antiferromagnetic. These states can be represented by:

$$|\psi_{FM}^G\rangle = |\dots \uparrow\uparrow \dots\rangle \quad (3.3)$$

$$|\psi_{AM}^G\rangle = |\dots \uparrow\downarrow\uparrow\downarrow \dots\rangle \quad (3.4)$$

where ψ_{FM}^G represents the ground state of the ferromagnetic system and ψ_{AM}^G represents the ground state of the antiferromagnetic system, which are represented pictorially in Fig. 3.1. Since the Hamiltonian is $\mathbb{Z}_2$ (spin-flip) symmetric, the ferromagnetically aligned ground state must be degenerate, with an equivalent spin “down” ordered state.

Of course, this is not particularly interesting on its own. The model becomes less trivial when a magnetic field is applied along a transverse axis. For the sake of the

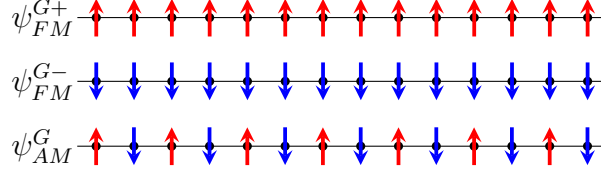


Figure 3.1: Ground states of the exchange-coupled 1D Ising chain. The ferromagnetic chain ($J > 0$) has two degenerate ground states because the Hamiltonian is $\mathbb{Z}_2$ symmetric, but the states individually are not (there is spontaneous symmetry breaking within this system, but this is not relevant to the fractionalized excitation discussion presented in this chapter). The antiferromagnetic ($J < 0$) chain has only one ground state, as the state is already $\mathbb{Z}_2$ symmetric.

discussion here, a unitless field g will be applied along $\hat{x}$, such that $g = g_x/g_c$ for some critical field g_c . In the other chapters of this dissertation, the applied field B_0 is taken to be along $\hat{z}$, since the applied field strength is typically much stronger than other couplings. However, here, this assumption is no longer true, as B_0 is often weaker than the spin-spin coupling such that the eigenstates are no longer just up and down in the applied field.

$$\hat{\mathcal{H}}_I = -J \sum_{\langle ij \rangle} \sigma_i^z \sigma_j^z - Jg \sum_i \sigma_i^x \quad (3.5)$$

Now, if $g \ll 1$, g can be treated as a perturbation to the Hamiltonian in eqn. (3.1) and the applied field introduces off-diagonal elements that induce spin flips; note that turning on an applied field cannot flip all spins simultaneously, this would constitute a nonlocal operation. Therefore, each spin must be flipped individually, such that one σ_i^x is applied at a time [280]:

$$-g\sigma_i^x |\psi_{FM}\rangle = -g |\dots \uparrow \downarrow_i \uparrow \dots\rangle. \quad (3.6)$$

Which, for a very weak field, increases the exchange-coupled energy by $2J$ since there are now two pairs of spins that are anti-aligned (the flipped spin and its left nearest-neighbor, and the flipped spin and its right nearest-neighbor). To first order in g , the

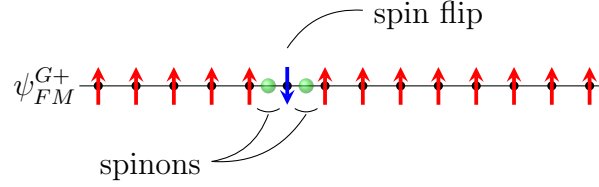


Figure 3.2: Spinon creation in ferromagnetic Ising 1D ground state. After a single spin flip, two quasiparticles called “spinons” are created that each carry one-half a conventional spin degree of freedom, as the spin flip causes $\Delta S = 1$ and each quasiparticle carries an equal share

energy required to generate a spin flip (*i.e.* the “gap” to create one spinon) in this regime is $\Delta = 2J(1 - g)$, where $g = g_x/g_c$; therefore, 2Δ is required to flip a single spin.

The full Hamiltonian, including the $\hat{x}$ field contribution, can be diagonalized using the Jordan-Wigner transformation; this is not done here, but can be found in the Supplemental Material of Wan and Armitage [272], for example. It is immediately apparent that if a second spin is flipped, the energy level is unchanged *as long as the spins are nearest neighbors* because the Hamiltonian is only concerned with nearest neighbor coupling, and the number of anti-aligned nearest neighbors has not changed. In this way, only the number of *domains* matters, and all states with the same number of magnetic domains have the same energy. It is therefore energetically permissible for the excitation to propagate along the chain, flipping spins as it does so. To simplify the language, such excitations are often described with the number of “kinks”, which refers to the number of domain walls, *i.e.* separations between magnetically aligned groups. In 1D, the number of kinks is the same as the number of distinct domains, N_D , minus one.

$$N_{kink} = N_D - 1 \quad (3.7)$$

These “kinks” are often referred to as “spinons”, quasiparticles that carry spin angular momentum and may even carry linear momentum $\mathbf{k}$, if a photon deposits more energy

than is required for the initial spin flip (see Figs. 3.2 and 3.3). The dispersion of one spinon within a pair is well-known to be [281, 282]:

$$\epsilon_k = 2J\sqrt{1 + g^2 - 2g \cos k} \quad (3.8)$$

with linear momentum k . Note that taking $k = 0$ and approximating small g to first order gives the field-dependent spinon gap $\Delta \approx 2J(1 - g)$ as reported above. For completeness, once entering the paramagnetic phase where $g > 1$, the excitation gap is quite similar, $\Delta = 2J|1 - g|$, except that a single spin flip “quasiparticle” is allowed in the paramagnetic regime, and 2Δ is no longer required [282].

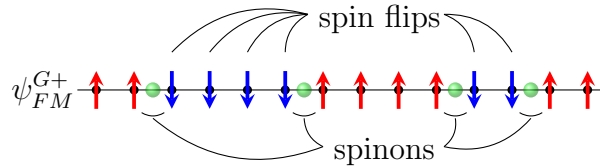


Figure 3.3: Spinon propagation in ferromagnetic Ising 1D ground state. There are 5 magnetic domains and therefore 4 kinks. Because the applied field is in a direction perpendicular to the ferromagnetic alignment, systems with 1 and n spin flips are degenerate, as long as the number of flipped *domains* does not change. These kinks (spinons) may propagate with linear momentum $\mathbf{k}$ if $E_s > 2J(1 - g)$.

This system can host spinons at a given frequency as long as the applied field g is strong enough to shrink the gap to less than the excitation frequency, but weak enough to not alter the ferromagnetically aligned states. If $g \gg 1$ (*i.e.* $g_x \gg g_c$), the field overwhelms the exchange coupling and the system becomes a paramagnet in the applied field; of course, there must be a quantum phase transition between these two phases at some critical field g_c . According to Sachdev [281] and Cabrera et al. [283], a mean-field treatment and the Jordan-Wigner treatment provide slightly different critical fields, with transitions at $g_x = g_c$ and $g_x = g_c/2$, respectively, though the precise value is not

important for the experiments or discussion presented here. A stronger g -field that is still lower than the critical g_c can more effectively drive spin-flips; at high temperatures or exactly at $g = g_c$, the system enters a quantum critical phase where neither the ferromagnetic (FM) nor paramagnetic (PM) phases are stable (see Fig. 3.4).

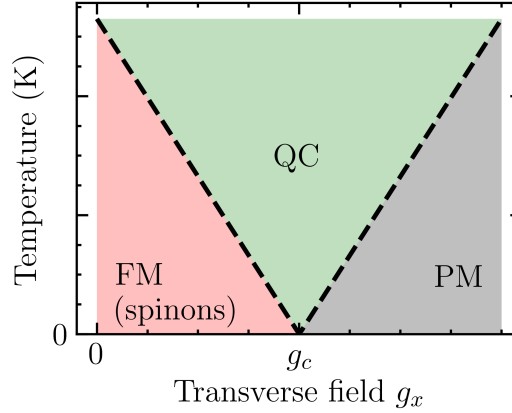


Figure 3.4: Phase diagram for the transverse field Ising chain [278]. Below g_c and at low temperatures, the ferromagnetic (FM) phase that hosts spinons as its low-lying excitation dominates. At higher temperatures and fields (crossover $T \sim \Delta \propto g_x$, [282]), the system becomes quantum critical (QC) and is not stable in either of its two magnetic regimes. Above $g_x = g_c$ with sufficiently low temperatures, the system becomes a quantum paramagnet, where excitations are simple Zeeman spin flips in the direction opposite the applied field.

Though the quantum phase transition is in itself interesting, this system also has correspondence to the Kitaev lattice model of a p-wave superconductor in 1D [284, 285] and spinons are even well-known to be the low-lying excitations of quantum spin liquids [286–289]. Further still, their very existence is topologically protected—since they are created with a $\Delta S = 1$ excitation, and each carry $S = 1/2$, they are their own anti-“particle” and may only be destroyed via collision. Their parity is therefore cannot be changed, meaning that a spin chain *must* host an even number of spinons [280]. As a result, a spinon may collide with another spinon and annihilate ($\Delta N_s = -2$), or it may decay into 3 spinons all with less linear momentum than the initial one ($\Delta N_s = 2$), but

there are no allowed decay pathways with $\Delta N_s = \pm 1$. This topological protection allows spinons to live for particularly long times after creation.

When operating in the low-transverse-field regime that allows for spinon creation, a broad range of energies can excite them. Because the spinons may carry linear momentum (total linear momentum is conserved if two spinons propagate with $\mathbf{k}$ and $-\mathbf{k}$), any energy that is larger than the gap is sufficient; this is very different from paramagnetic EPR, where absorption peaks are necessarily sharp. The spinons carry any additional energy as kinetic energy. As a result, the characteristic signature of the existence of spinons in magnetic systems is a broad absorption *continuum*, where spectral features cannot be resolved [280].

Spectral features, however, can arise as a result of *domain wall confinement* in such systems, if a longitudinal field is included. With this treatment, there is an additional Zeeman energy requirement to flipping a spin. This can be treated theoretically by modeling the spin's perspective with the following Hamiltonian [290]:

$$\hat{\mathcal{H}} = -J \sum_{\langle ij \rangle} \sigma_i^z \sigma_j^z - \sum_i \{h_x \sigma_i^x + h_z \sigma_i^z\} \quad (3.9)$$

or with the following Schrödinger equation from the perspective of the two-spin center-of-mass from Coldea et al. [278]:

$$\frac{-\hbar^2}{\mu} \frac{d^2 \psi}{dx^2} + \lambda |x| \psi = (m - 2m_0) \psi, \quad (3.10)$$

where μ is the center of mass, x is the position coordinate, m is the mass (energy) of the spinons, m_0 is their rest mass (takes $2m_0$ of energy to create a pair), λ is the linear confining potential, and ψ is the two-spinon state. Under confinement, spinons may still propagate, but the propagation requires energy. Such a confining field may manifest as a result of intra-chain magnetic ordering below some critical temperature T_c . This

confinement also creates a set of well-resolved bound states that are discussed in detail in the literature (see *e.g.* [278, 279, 290, 291]).

3.3 Cobalt niobate (CoNb_2O_6)

A recent realization of a condensed matter system that experimentally approximates a TFIC is crystalline cobalt niobate (CoNb_2O_6) [278, 280]. Though it is not a *perfect* realization of the TFIC because the spins are slightly canted (31°) relative to the strong exchange coupling easy axis (in the c -direction) and the “linear” chain is a zig-zag [279, 280, 290], the physics replicates what would be expected from a TFIC: it has ferromagnetically-ordered 1D chains and an overall antiferromagnetic ordering at low temperature (individual chains anti-align relative to neighbors), a transverse field along the b -direction destroys the ferromagnetic ordering and induces a phase transition to a paramagnet at $g_c \approx 5.3 \text{ T}$ ($J \approx 17.5 \text{ K}$ or 1.5 meV , [282, 292]), and bound states as a result of a confining longitudinal field have been observed [280].

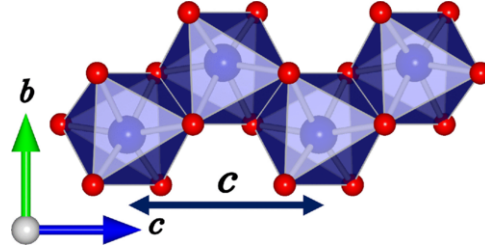


Figure 3.5: TFIC realization in CoNb_2O_6 . Magnetic Co^{2+} ions are shown in blue with octahedral O^{2-} coordination ions shown in red. Figure reproduced from [290] with permission according to Phys. Rev. B Creative Commons licensing policies.

3.3.1 240 GHz EPR measurements

UCSB’s Free Electron Laser-powered EPR spectrometer is uniquely equipped to probe the fractionalized excitations hosted in CoNb_2O_6 . To recap the main benefits of 240 GHz FEL-EPR: it is capable of operating at temperatures slightly above CoNb_2O_6 ’s ordering temperature (operating T between 310 K and 2.2 K; CoNb_2O_6 aligns antiferromagnetically below 3 K [279, 292–296]), sweeping through the critical field (B_0 between 0 T and 12.5 T), has quasi-continuous wave kW-level power with a narrow bandwidth, can produce up to 2 pulses for echo measurement, and has a dead time of roughly 70 ns.

Within this section, T_1 is considered the spinon population lifetime (*i.e.* how long the low-lying excited states are able to exist in the TFIC before decaying). This lifetime is best-measured by saturation or inversion recovery three-pulse experiments that are sensitive to the polarizability of the population: if all the spins have relaxed back to their original polarization, by definition, no spinons remain in the chain. T_2 on the other hand, is defined to be the spinon coherence time. A physical interpretation of this lifetime is less obvious, but T_2 can be measured more easily (with a two-pulse Hahn echo, for example). It is expected that for spinons in the TFIC, T_1 is much longer than T_2 ; this is because population decay is topologically protected by parity, which means there is no mechanism outside pairwise annihilation that allows for T_1 relaxation. There is no similar argument for coherence time, and thus, T_2 is not impacted by this topological protection. The current capabilities of 240 GHz FEL-EPR are limited to two pulses, and as a result, only experiments sensitive to the shorter T_2 were possible (to date).

CW EPR

The field at which 240 GHz is sufficient to excite spinons is not exactly known, but can be estimated from the field-dependent THz absorption spectroscopy presented in Fig. 3 of Morris et al. [280]. According to their data, at 240 GHz, the spinon continuum should begin at roughly 2.5 T, and exist up to $g = g_c$ (5.3 T). The dispersion of TFIC spinons may even be simulated by assuming the TFIC model applies, and choosing the magnitude of J to best match their results. As discussed in their paper, in reality, CoNb_2O_6 spin physics is best-represented by a “twisted Kitaev chain” Hamiltonian rather than the simpler TFIC model. However, the TFIC model should be quantitatively correct near the critical point, as the two models belong to the same universality class [278, 280]. Simulations of the field-dependent TFIC dispersion (with $J = 0.5$ meV) are shown in Fig. 3.6. As shown, at roughly $0.2g_c$, there is no overlap between 0.5 meV (120 GHz) and the dispersion (meaning no absorption). Above roughly $0.5g_c$, the dispersion crosses 0.5 meV (absorption begins), and at $g = g_c$, the system becomes critical and the gap goes to zero. Note that FEL-EPR at ITST is performed at 240 GHz (1 meV) and the energy plotted in Fig. 3.6 is half of that. This is because one photon may induce one spin flip, and a single spin flip creates two spinons—meaning half the energy goes to each spinon in order to conserve total linear momentum.

To confirm that the system behaved as expected magnetically, CWEPR was performed at 4.2 K in a flow cryostat (the crystal sample used for these experiments, as well as those in [280], is shown in Fig. 3.7). For these experiments, the B_0 applied field was parallel to the crystal’s b -direction and the microwave applied field B_1 was parallel to the a -direction (known as “Faraday geometry”, [297]). Though the static and the spin-flip inducing (energy depositing) fields were perpendicular, both were also perpendicular to

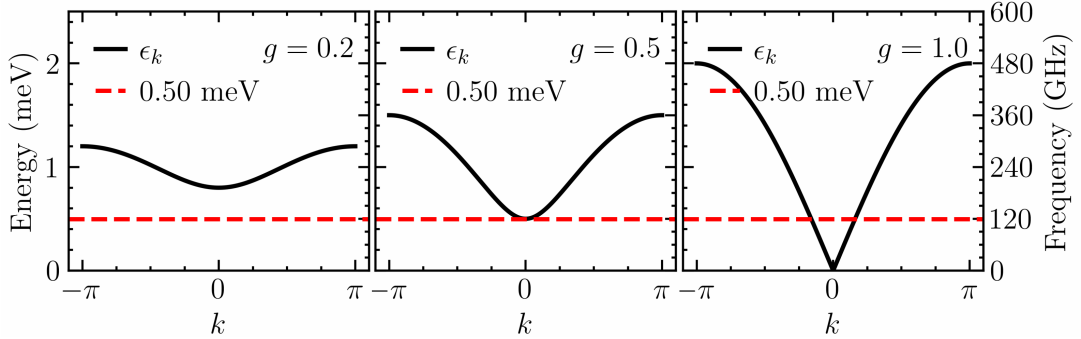


Figure 3.6: Cartoon of field-dependent dispersion in CoNb_2O_6 . At low field ($g = 0.2$, there is no overlap between the energies accessible with 240 GHz EPR (dashed red) and the spinon dispersion (solid black). Between $0.5 \leq g \leq 1$, there is overlap that allows pairs of spinons to be created with a roughly 1 meV photon at 240 GHz. At $g = 1$ ($g_x = g_c$), the gap goes to zero at $k = 0$ and the system becomes quantum critical.) For this figure, J was chosen to be 0.5 meV, as it best-matched the observed field-dependence of the spinon continuum [280].

the c -axis (the main direction of ferromagnetic alignment), as is necessary for the TFIC model. The small amount of spin canting ($\pm 31^\circ$, alternating back-and-forth, [280, 292]) toward the a -direction imposed *some* longitudinal field, which was acceptable; because the axial alignment was imperfect, and the crystal was flat along a plane perpendicular to the b -direction, it was not possible in any configuration to completely eliminate longitudinal fields. In the future, a precise, rotatable sample holder or rapid sample-exchange mechanism would allow for iterative measurements to perfectly align the c -direction orthogonal to the applied B_1 field. Further, aligning B_1 parallel to B_0 in the so-called “Voigt geometry” more closely resembles the TFIC model, but introduces engineering challenges; work is ongoing to enable these experiments.

The results of the experiment described are shown in Fig. 3.8. At roughly 2.3 T, a sharp absorption can clearly be seen that corresponds to the opening of the spinon continuum at 240 GHz. Above that field, there is a broad absorption that agrees nicely with a propagating spinon interpretation. At roughly $B_0 = 5.3$ T, there appears to be

a very small change in slope of the absorption, which may correspond to the critical field that separates the ferromagnetic (spinon) phase from the paramagnetic phase. At roughly 8.8 T, there is a broad absorption peak that likely corresponds to the paramagnetic absorption of a single spin-flip quasiparticle; this agrees qualitatively with the data presented in Fig. 3b of [280], though the temperature is slightly higher (and above the antiferromagnetic ordering transition) here (4.2 K vs. 2.5 K in [280]). Above 9 T, the spectrum becomes difficult to interpret—it is likely that there was a large torque on the dense paramagnetic spins in the crystalline sample and this torque caused the sample to move in the holder, creating the seemingly chaotic fluctuations.

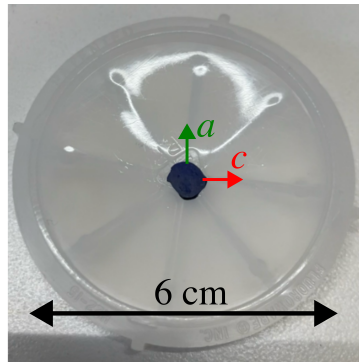


Figure 3.7: Photo of CoNb_2O_6 crystal. The crystal's a and c -axes are depicted by the green and red arrows, respectively. The b direction is coming out of the plane of the page, toward the reader. The black scale bar shows 6 cm.

The broad absorption due to the spinon continuum has also been seen with neutron scattering and THz spectroscopy, and has been taken as indirect evidence of the existence of spinons. These techniques provide no tractable information about the linewidth or lifetime of the excitations. Echo-based measurements provide an avenue to such information, particularly 2D (multi-frequency) ones. Two dimensions allow for unambiguous determination of the makeup of the continuum and can resolve the linewidths of each spinon pair's absorption spectrum. Both two-dimensional coherence spectroscopy [298,

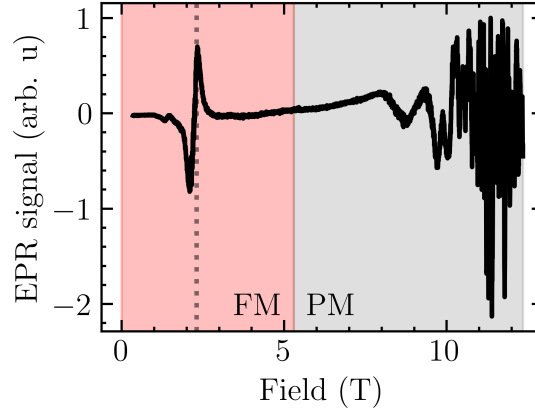


Figure 3.8: CW EPR of CoNb_2O_6 crystal at 240 GHz and 4.2 K. $\vec{B}_0$ was parallel to the b -direction (out of the plane of Fig. 3.7) and the microwave $\vec{B}_1$ was parallel to the crystal's a -direction (Faraday geometry). At low fields (below roughly 2.3 T), there is no absorption because the domain wall dispersion has not yet crossed below 0.5 meV. At roughly 2.3 T, a relatively sharp absorption appears when the field is strong enough to create spinons. Spinons are created with higher and higher kinetic energies up to roughly 5.3 T, where a small kink *may be* present in the absorption, and the system shifts from ferromagnetic (FM, shown red) to paramagnetic (PM, shown gray). A broad absorption can be seen at roughly 8.8 T that may be a result of Zeeman spin flips in the paramagnetic regime (this agrees roughly with the data presented in Fig. 3b of [280]). Above those fields, the CW absorption becomes very difficult to interpret. At such high fields, it is likely that the dense paramagnet had significant torque on it, which caused it to move within the sample holder and may explain the extreme signal fluctuations at the high-field side of the spectrum.

299] and pulsed electron spin resonance are capable of generating such echoes. A theoretical model for a TFIC spinon echo was proposed by Wan and Armitage [272]: the third-order term of magnetic susceptibility includes a “spinon echo” term that is identical to a photon echo in optical spectroscopy—the next step is to observe this echo experimentally.

Pulsed FEL-EPR

It is hypothesized that the $\Delta N_s = 2$ ($1 \rightarrow 3$ spinon) decay mechanism becomes possible above roughly 4.5 T [300]. Here, it would be expected that the spinon coherence time (and potentially, also the spinon lifetime) would shrink dramatically; this would be indisputable evidence of their existence and would provide additional experimental evidence that the TFIC model is applicable to CoNb_2O_6 . To this end, field-swept Hahn echo measurements were performed to determine the coherence time of spinons at various relevant fields (2.3 T, 2.3-4.5 T, 4.5 T, 4.5-5.3 T, > 5.3 T). Though it has not been unambiguously determined at this stage whether T_1 , the spinon population lifetime, or T_2 , the spinon coherence time, is more sensitive to field strength-dependent effects, initial experiments were aimed at observing a spinon echo with 240 GHz FEL-EPR, as echo-detected techniques can provide robust, low dead-time measurements of both T_1 and T_2 .

Two-pulse Hahn echo experiments with four-step phase cycling were performed at 4.2 K and a variety of magnetic fields in the same orientation as in Fig. 3.8. An example experiment at 4 T is shown in Fig. 3.9: the experiment was repeated many times at different fields and pulse timings, with similar results for all. No echo could be observed with the 240 GHz FEL-EPR spectrometer. Fig. 3.9 shows the leaked power of the FEL to the left of the pulses as a result of imperfect coupling and switching. The first (roughly $\pi/2$) pulse P_1 , the second (roughly π) pulse P_2 , and the activation switch

timing (shown with dashed vertical gray line) can be seen clearly in the data. After phase cycling (Fig. 3.9, bottom), some small modulations near the expected echo time were apparent. However, varying the τ delay between the pulses did not change the timing of the modulations in the expected way and thus, the modulations were determined to be FEL ringdown noise (see Appendix sec. C.1.2 for a comparison to an obvious echo signal from P1 centers in diamond).

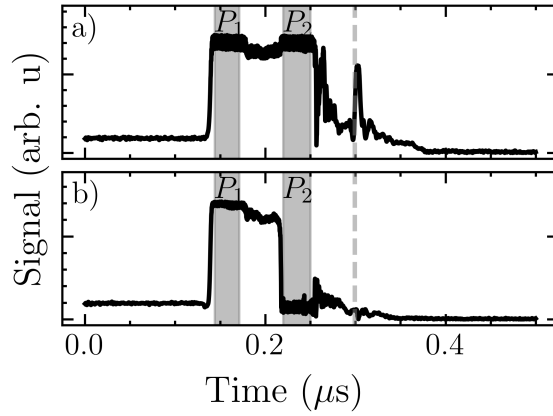


Figure 3.9: Pulsed FEL-EPR of CoNb_2O_6 crystal with 75 ns τ at 240 GHz, 4 T, and 4.2 K. (a) Raw FEL-EPR data of Hahn echo sequence with 75 ns delay between pulses. There is a large spike where the detector is activated (shown by the dashed vertical gray line, for details, see *e.g.* Edwards et al. [275]); this is due to FEL standing waves that cause ringdown. To reduce the effects of these standing waves, two-step phase cycling was performed (b). Here, the coherence pathway corresponding to the first pulse and an echo is selected. There are small modulations at the timing an echo would be expected, but repeated experiments while varying the P_1 – P_2 delay, τ , did not change the position of the small modulations—they remained near the timing of detector activation and were likely a result of ringdown from leaked power before the pulses and the non-cycled first pulse (see Appendix C.1.1). To compare to a strong, obvious 240 GHz FEL-EPR echo signal, echo signals of P1 in diamond are shown in section C.1.2; for more figures of obvious FEL echoes, the reader is referred to Fig. 4c of [276].

Because the 240 GHz FEL-EPR spectrometer has a dead-time of roughly 70 ns and the pulses could not be perfectly phase cycled as a result of the phase plate technique discussed in section 3.6.2, shorter τ times could not be used. As a result, the experiments

were not able to confirm the existence of spinon echoes with pulsed FEL-EPR.

This experiment *was* able to place an upper bound on the spin-spin coherence time of spinons in CoNb_2O_6 at 70 ns, which contrasted with the simple TFIC model described here, that predicts virtually no decay mechanism except pairwise annihilation. It is likely that crystalline defects, inter-chain exchange, and spin-lattice couplings cause decoherence that reduce the coherence time (T_2^*) of the quasiparticles, though T_1 may still be quite long. The combined FEL-RS experiments described in sec. 3.4.3 have the potential to provide direct access to the potentially long-lived T_1 even though echo-detected pulsed experiments are not currently possible.

3.3.2 Future work

The promise of confirming the existence of spinons, as well as measuring their lifetimes, is an exciting one. In fact, it motivated much of the following work: the considerations and upgrades included in the novel FEL-ESR design were inspired by the experiments discussed here. Though not exhaustive, these upgrades include:

1. Enabling three-pulse FEL measurements using the novel pulse slicer, including an inversion recovery pulse sequence that is capable of measuring the T_1 of short-lived excitations (sec. 3.5.3).
2. Improving the angular precision and available phases of FEL-ESR phase cycling with the continuously-variable phase shifter to greatly reduce FID and ringdown effects and thereby improve SNR (sec. 3.6.3).
3. Reducing experimental dead-time with the quasi-optical sample holder's > 20 dB improvement to induction mode isolation (sec. 3.7).

4. Improving SNR with the quasi-optical sample holder by adjusting sample position to an antinode of applied B_1 field (sec. 3.7).
5. Implementing a “dry” magnet to enable closed-cycle cryogenic measurements so that experiments may be performed without the limitation of liquid helium supply.
6. Implementing an arbitrary waveform generator to produce complex, low-power pulse sequences with control of individual pulse frequency and phase that enables multifrequency experiments and low-power phase cycling.
7. Enabling sample rotation to perfectly align the canted spins orthogonal to the B_1 field direction in the Faraday geometry.
8. Enabling Voigt geometry measurements that align B_1 and B_0 so that spin-flip perturbations are parallel to the applied static field.

Expected to begin experiments in early 2025, the novel FEL-ESR spectrometer will be able to operate from 0-16 T and ~ 2 -300 K at 170-420 GHz, with excitation powers that cannot be accessed with any other high frequency ESR source. With the upgrades discussed in the following sections, a second attempt at determining the lifetime of spinons in CoNb_2O_6 (and exotic magnetism in other condensed matter systems) is within sight.

3.4 FEL-ESR quasi-optical design

To motivate the construction of a novel FEL-ESR spectrometer that can operate between 170-500 GHz and 0-16 T, it is worth reviewing the capabilities of spectrometers that operate in similar regimes; the operating fields and frequencies of some modern high-field EPR spectrometers are highlighted in Fig. 3.10. The novel FEL-ESR spectrometer has

been designed to operate within the entire gray region of Fig. 3.10, providing kW-power pulses across a broad range of frequencies and fields to probe $g = 2$ organic samples, single-molecule magnets, antiferromagnetic spintronics, and highly entangled spins, such as the system discussed in sec. 3.2. There are a few systems with comparably high frequencies: for example, the newest Bruker ELEXSYS commercial system operates at 263 GHz [301]; the dual DNP/EPR system in Tel Aviv operates at 388 GHz and 14 T [211]; and FRaSCAN in Brno, CZ, can operate from 82-1100 GHz and 0-16 T. However, these three systems are all limited to (at most) hundreds of milliwatts of power, which is still many orders of magnitude lower than the FEL. HiPER, a kW-power 94 GHz spectrometer built by Graham Smith's group [302] can access comparable powers to ITST's FEL, but like the current 240 GHz FEL-EPR system at ITST, is not frequency-agile and is limited to the lower frequency of 94 GHz. In total, there is a clear need for high-frequency, high-power, frequency-agile FEL-ESR to access the rich physics that exists at such frequencies, some of which includes spintronics, low-lying excitations of magnetically ordered spin systems, and single-molecule magnet qubit candidates.

The novel FEL-ESR spectrometer was designed to operate in three distinct modes: low-power (LP) source mode, FEL mode, and combined mode. The 240 GHz FEL-EPR spectrometer currently housed at ITST is able to operate in LP and FEL mode, but is not capable of a combined mode. The next-generation quasi-optics that permit switching between these three modes were designed to minimize the number of components that must be adjusted or switched in (or out) of the system to make it as user-friendly as possible.

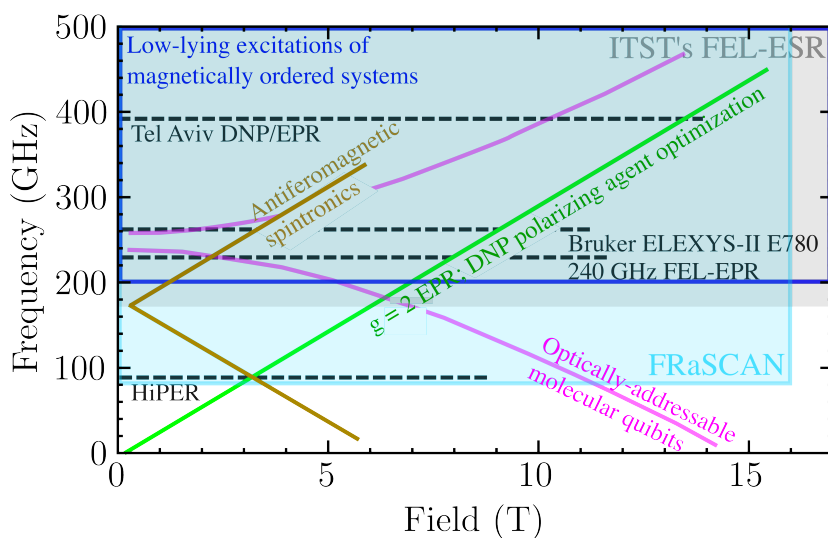


Figure 3.10: Review of some high-field, high-frequency EPR systems compared to the expected operating regime of FEL-ESR. Bruker ELEXSYS [301], Tel Aviv DNP/EPR [211], and FRaSCAN [198] all operate at high frequency (> 100 GHz), but low power (< 500 mW on the high end). HiPER [302] is capable of kW-power, but is limited to a single frequency that is much lower than the frequency-agile capabilities of ITST's FEL. ITST's current 240 GHz FEL-EPR is capable of kW-power pulsed EPR, but is limited to a single frequency, which restricts its potential to only certain (optimal) samples. The field dependence of some relevant experimental regimes are shown as guides to compare to the operating regimes of modern high-field spectrometers.

3.4.1 Design for low-power ESR

The low-power ESR design is much like the 240 GHz LP system discussed in Edwards [134], Wilson [59], or even those discussed in Schnegg [5]. A schematic of LP source operation is shown in Fig. 3.11. The LP source emits linearly polarized electromagnetic radiation at 170-500 GHz, which is then reflected by adjustable wire grid polarizers that are aligned to transmit the maximum amount of LP source power to the sample. The light is transmitted through the first wire grid, its polarization is rotated by the Faraday rotator (contributing 3 dB insertion loss), and it is then transmitted through the second wire grid. Upon interaction with the sample and reflection by an end mirror, the EM radiation is sent back toward the quasi-optics, where it is filtered by an induction mode Faraday-rotator-based isolator that selects only the polarization orthogonal to the input polarization. After this isolation, the ESR signal is sent to the cross-polar detector and the background light is sent to the co-polar detector, which can be used to monitor source power and phase. If lower source power is desired (*i.e.* if the EPR signal is saturated), it can be controlled by adjusting the angle of the wire grid polarizers in the LP source (pink) path. Regardless of the angle of the adjustable wire grids, the fixed wire grids that make up the Faraday rotator isolator will select only the polarization that works with the isolator and receiver, any cross-polarized light will be dumped to the left of the LP source path.

3.4.2 Design for FEL-powered ESR

In the FEL operating mode, the LP source is used for injection locking [274]. Injection locking requires a very small amount of power (roughly nanowatts) and can pre-excite a single cavity mode which promotes FEL lasing at that mode; this returns a quasi-

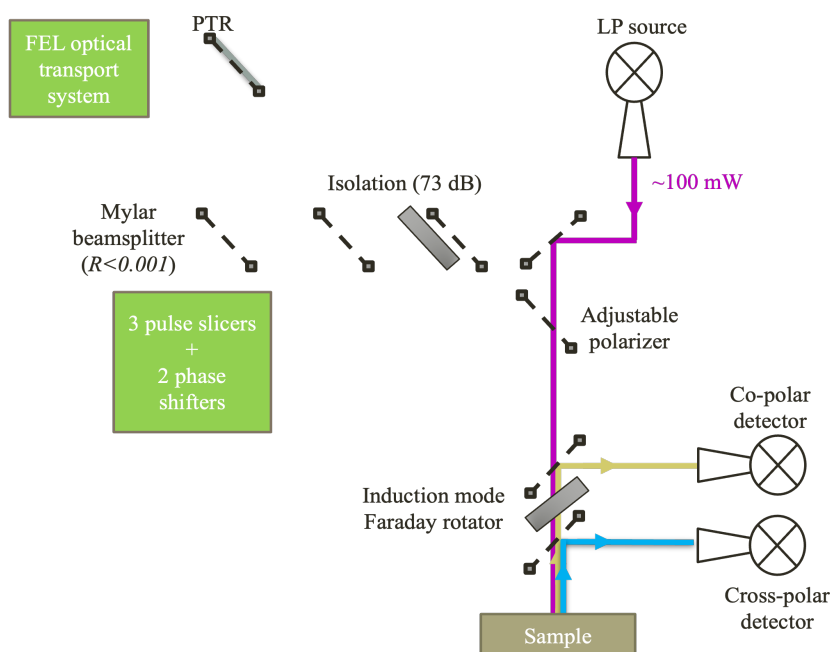


Figure 3.11: Low-power operating mode of novel FEL-ESR. Light is emitted by the LP source at ~ 100 mW and sent along the pink path. It is directed toward the magnet by wire grid polarizers that can be used to adjust the power for an experiment. After interaction with the sample, a Faraday-rotator-based isolator selects only the polarization that results from ESR absorption and directs it toward the cross-polar detector. Background light is directed toward the co-polar detector for measurements of beam power and phase. In this setup, the FEL quasi-optics (left side of the figure) are not used. Off-axis parabolic mirrors used for beam imaging and propagation are omitted from this figure for simplicity.

continuous wave FEL beam with a linewidth that is roughly 2 ppm of the operating frequency (500 kHz at 240 GHz as reported in [274]). To achieve this, the first adjustable wire grid polarizer in the pink path is rotated 90° from the LP operating mode such that all power is transmitted. The LP light is there sent through a Faraday-rotator based isolator and reflected by a Mylar film beamsplitter with a very low reflectance ($R < 0.001$); these isolators sum to roughly 73 dB of isolation and exist to protect the LP source's waveguide amplifiers from the kW power levels of the FEL.

After injection locking and FEL lasing, FEL radiation is directed via the optical transport system (OTS) to the ESR bridge. Because the FEL has multiple undulators that produce different polarizations (linear undulators produce linear polarization and helical undulators produce circular polarization), a polarization transformer is required. A reflective transformer of this type (polarization transforming reflector, PTR) is used to transform the FEL output polarization to arbitrary polarization [303]. For the case of FEL-ESR, this polarization is linearly polarized out of the plane of the page. The Mylar film transmits the majority ($T > 0.999$) of the FEL power to the pulse slicers and phase shifters, where individual ESR pulses are created. These pulses are then directed to the next adjustable polarizer, which is oriented 90° to its orientation in LP mode. The operation after this polarizer is identical to that of LP mode (with the same 3 dB Faraday rotator loss), except that a protection switch must be installed that only allows light to reach the cross-polar detector **after** the FEL pulse sequences to protect the Schottky diode within the detector's subharmonic mixer. This is achieved by inserting an additional "pulse slicer" module (see sec. 3.5.3) in which the additive channel directs light to the detector.

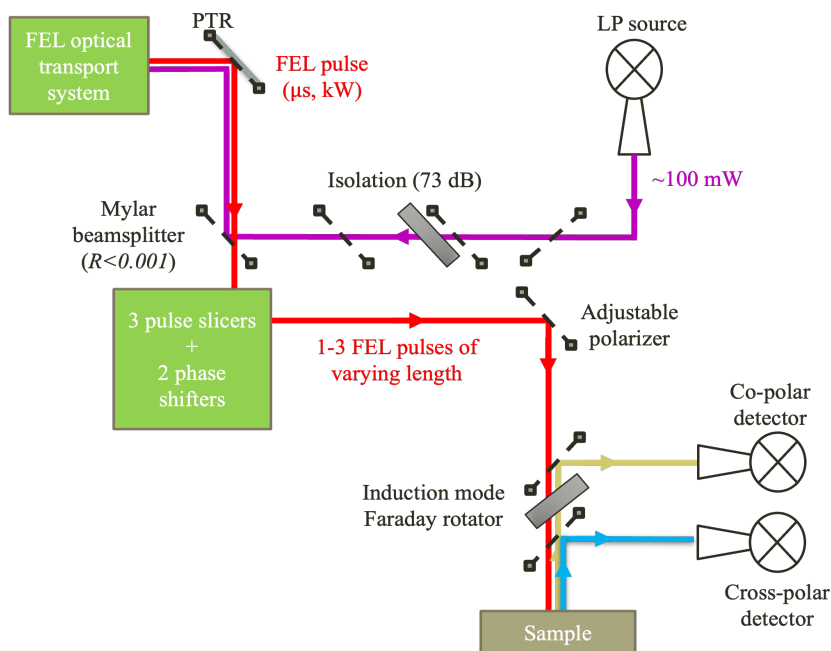


Figure 3.12: FEL operating mode of novel FEL-ESR. LP source is used to injection lock the FEL. After lasing, FEL radiation is directed to the ESR bridge by the OTS, where its polarization is altered by the PTR, and it is sliced and phase shifted. It is then directed to the sample by an adjustable wire grid polarizer that is rotated 90° to its orientation in LP mode. The rest of the quasi-optics are the same as in LP operation. A pulse slicer-based protection switch (not shown) is used to ensure FEL radiation does not destroy the subharmonic mixer. Off-axis parabolic mirrors used for beam imaging and propagation are omitted from this figure for simplicity.

3.4.3 Design for combined FEL and low-power source ESR

New to this design is the combined FEL-LP mode. This mode was designed with pump-probe experiments in mind, such that a dense spin system could be pumped with the FEL, and after the FEL pulse, the LP source could be used to probe the transient excitations. Because the LP source is driven by an AWG, the FEL injection lock (and therefore FEL pump) frequency does not need to be the same as the LP source probe frequency. This enables multifrequency experiments such as DEER or spectral hole-burning (see below section on “FEL-powered rapid-scan ESR” for further discussion on spectral hole-burning). The optics do not differ much from the other two modes, the important feature is that the first LP adjustable polarizer reflects most ($\sim 90\%$) of the light that hits it, and the second adjustable polarizer is rotated 45° from the vertical orientation. Through careful, slow manipulation of the FEL and LP polarizations (not shown in these figures), the LP polarization can be completely transmitted by this adjustable wire grid, and the FEL can be completely reflected. When these two polarizations are incident on the first (fixed) wire grid of the induction mode assembly, half of the LP and FEL power will be transmitted (3 dB additional conversion loss for both) to the same polarization, allowing all the other induction mode optics to behave the same as separate LP and FEL modes. An equivalent treatment (that simplifies things conceptually) would simply consist of rotating both induction mode polarizers by 45° . However, because the detector waveguides are sensitive to input polarization, it is simpler in practice to preserve the induction mode polarization scheme and adjust the polarizations before the induction mode detection assembly.

The total power losses through each path in the three operating modes are shown in Table 3.1. These values are not precise, as additional treatment of fine details (Brewster’s

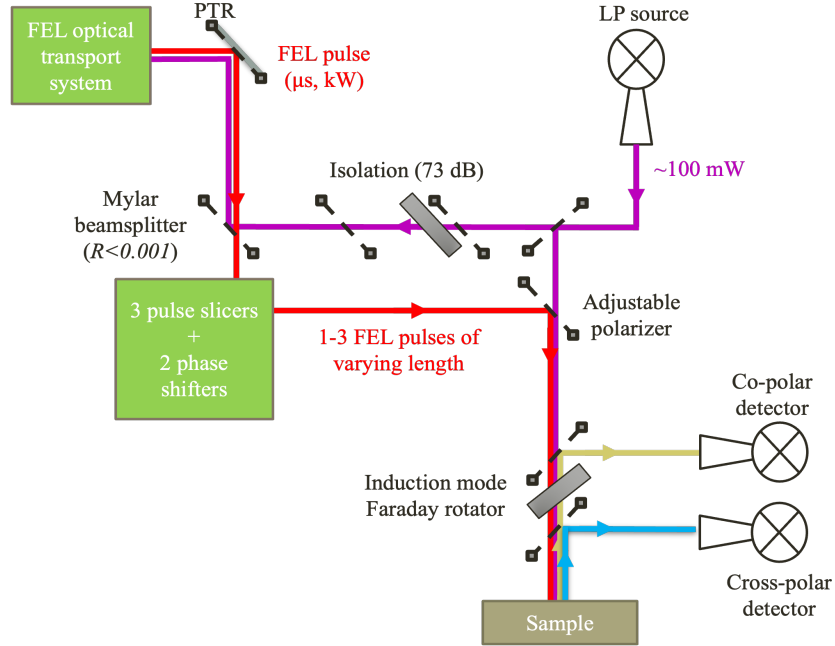


Figure 3.13: FEL-LP operating mode of novel FEL-ESR. In this mode, the first wire grid polarizer is rotated to split the LP beam. The amount of power in each direction can be chosen based on use-case, but for this treatment it is assumed to be a roughly a 95-5 beamsplitter. 5% of the power from the LP source is used to injection lock the FEL (assuming significant additional ~ 30 dB power loss from the Mylar); at the time of writing, 5% of the LP source has not been shown to sufficiently injection lock the FEL, but it is expected to be more than sufficient. After lasing, FEL radiation is directed to the ESR bridge by the OTS, where it is sliced and phase shifted. It is then directed to the sample by an adjustable wire grid polarizer that is rotated to reflect all the FEL polarization and transmit all the LP polarization: the two sources are polarized orthogonal to each other through this wiregrid. In this case, though all the optics are not shown, the LP and FEL polarization has been rotated 45° degrees with low losses through gradual changes (though they are orthogonal to each other). This allows the first polarizer of the induction mode assembly to equally reduce the power of both the FEL and LP sources to 50% of their initial power while combining them to the same polarization through transmission. In this way, half the power of both sources is lost (3 dB), and another half is lost at the Faraday rotator (3 dB). A pulse slicer-based protection switch (not shown) is used to ensure FEL radiation does not destroy the subharmonic mixer. Off-axis parabolic mirrors used for beam imaging and propagation are omitted from this figure for simplicity.

angle input to pulse slicers, multiple wire grid polarizers can more efficiently rotate polarization, ohmic loss, etc.) can provide higher-order corrections. The simplest treatment is provided here for clarity; after the system is installed, these fine details are more simply (and accurately) treated by measurement rather than calculation.

Table 3.1: Power loss through FEL-ESR quasiotics. Adjustable wire grid polarizers allow optimization for minimizing losses in each mode. These values will change slightly when including the Brewster's angle input of the pulse slicer, but the additional complications are omitted here.

Mode	LP loss	FEL loss
LP	3 dB	—
FEL	—	3 dB
FEL-LP	6 dB	6 dB

FEL-powered rapid-scan ESR

The rapid-scan Bloch equations have a B_1 and T_1 dependence along the $\hat{z}$ axis. In a frame that rotates with B_1 [205],

$$\frac{dm_x}{dt} = -\frac{m_x}{T_2} + \gamma B_0 m_y \quad (3.11)$$

$$\frac{dm_y}{dt} = -\frac{m_y}{T_2} - \gamma B_0 m_x + \gamma B_1 m_z \quad (3.12)$$

$$\frac{dm_z}{dt} = \frac{M_0}{T_1} - \frac{m_z}{T_1} + \gamma B_1 m_y, \quad (3.13)$$

where γ is the gyromagnetic ratio, m_x and m_y are magnetization along directions transverse to the applied static magnetic field B_0 , m_z is magnetization parallel to B_0 , and M_0 is the equilibrium magnetization, assumed to be parallel to B_0 . As can be seen, these coupled differential equations all explicitly or implicitly depend on B_1 and T_1 : for

this reason, it should be possible to observe T_1 dependent RS lineshapes and may make dead-time free T_1 measurements possible with high-power RS-ESR, assuming the RS scan rate is fast enough. Because the FEL cannot be frequency-swept on RS timescales, such experiments would rely on sweeping the magnetic field, as in a RS TiGGER experiment. However, current scan rates are limited to tens of kHz, making the minimum time resolution roughly $10\ \mu\text{s}$. This is much longer than the current 240 GHz FEL-EPR dead-time and pulse length (μs), and thus, would need to be reduced greatly to provide a benefit over inversion-recovery three-pulse sequences using FEL-ESR. Increasing the scan rate should be possible, and in case the spin system could not produce (1) an FID with two-pulse EPR experiments, or (2) an echo with three-pulse FEL-ESR experiments due to strong coupling or homogeneously broadened lines, FEL-RS may provide an avenue to extract T_1 when other techniques cannot. An FEL-RS experiment would be quite simple: RS lineshapes would be recorded only *during* an FEL pulse and the experiment would mimic LP-RS in all other ways.

Another potential combined-mode experiment is FEL-powered spectral hole-burning. Using the “combined” ESR quasioptics shown in Fig. 3.13, the LP source would injection lock the FEL at one frequency (f_1) for “pumping” the spin system away from equilibrium, and then the arbitrary wave generator that drives the LP source would switch frequency, and probe the spin system using a different frequency (f_2). This could be used for multifrequency experiments like DEER, but could also be used for spectral hole-burning (SHB). For SHB, a long FEL pulse would be used to saturate the spin system at a given frequency (with a nonzero, roughly MHz bandwidth), and the LP source would be used to probe the saturation recovery *across* those frequencies using LP RS-ESR after the FEL pulse (see Fig. 3.14). The decay profile of the number of nonequilibrium spins may be fit to extract T_1 —to account for spectral diffusion, the *integral* of the spectrum is the

correct order parameter to track the T_1 return to equilibrium. A cartoon of the data recovered during such an experiment is shown in Fig. 3.15.

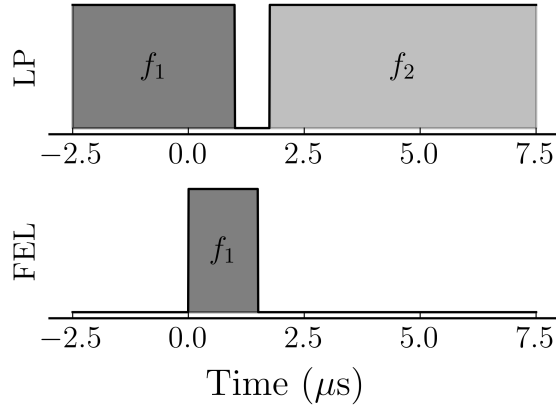


Figure 3.14: FEL-LP pulse timing diagram. The LP source (top) is initially used to injection lock the FEL at f_1 . After the FEL (bottom) begins lasing at $0\ \mu\text{s}$, the LP source is switched off. After the FEL pulse, the LP source is turned on again at a potentially new frequency f_2 . For rapid-scan spectral hole burning recovery experiments, if the field is modulated, f_2 does not need to be different from f_1 (though of course it can be); if the frequency is modulated, f_2 will be swept rapidly through f_1 .

Figs. 3.11-3.13 leave space in the quasi-optical design for a black-box of “3 pulse slicers” and “2 phase shifters” that are compact and frequency agile, compared to the large, single-frequency designs used for previous FEL-EPR setups. The design, construction, and operation of these components are discussed in detail below, in secs. 3.5.3 and 3.6.3.

3.5 FEL pulse slicing[†]

[†]The text and many of the figures in sec. 3.5 are reproduced from B. D. Price, A. Sojka, N. I. Agladze, and M. S. Sherwin, “Compact module for complementary-channel THz pulse slicing,” *Appl. Phys. Lett.* **124**, 021107 (2024), with the permission of AIP Publishing [194].

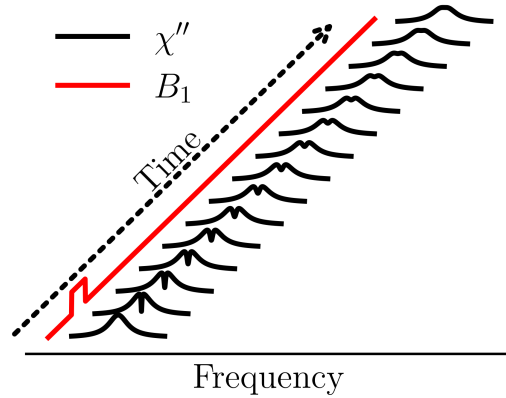


Figure 3.15: Cartoon of spectral hole-burning experiment. After the FEL pulse (red), the LP source is used for RS-ESR to sweep the entire lineshape as a function of time (black) to observe the recovery of the saturation “hole” in the lineshape. The recovery of this hole can be used to extract T_1 . Time after the FEL pulse is increasing diagonally up-and-right to simplify visualization.

3.5.1 Introduction

Electron spin resonance (ESR) is not the only application for pulsed, high power THz radiation. High power, narrowband terahertz (0.1–10 THz) waves are crucial for heating plasmas in fusion reactors [304, 305], wireless communications with ultra-high data rates [306–308], radar with mm-range spatial resolution [309, 310], electron beam accelerators [311, 312], and coherent manipulation of quantum states [313, 314]. For all of these applications, complex THz waveforms are desirable. Traveling-wave-tube and gyrotron amplifiers are under development to create such waveforms, but scaling them to high frequencies is challenging. Laser-generated narrowband waveforms may be used to produce high-power THz pulses, but the output bandwidth is still larger than 1 GHz [312], and therefore not suitable for EPR. Complex, narrowband THz waveforms can be, in principle, generated by modulating nearly-monochromatic quasi-continuous wave sources. Examples of sources with kW power and sub-MHz bandwidths include free electron lasers (FEL) and gyrotron oscillators [49, 68, 69, 315, 316].

The leading technique for modulating such waveforms uses laser-driven semiconductor switches (LDSS), where the optical excitation of a semiconducting wafer creates a high-carrier-density surface from what is otherwise a transparent material: reflectances of up to 90% have been reported for silicon [274, 317] and gallium arsenide [318–320] substrates for switching powers up to nearly 1 MW [321]. Electronic modulation of such high power THz sources is also possible, but is complicated by the low damage threshold of such modulators [322].

In recent decades, ESR at frequencies above 95 GHz (3.5 T for $g \approx 2$) has grown in popularity because spectral resolution is greater, new phenomena can be accessed, and the increased polarizability of electrons can be transferred to nuclei to enhance the signal strength of high field NMR spectroscopy through dynamic nuclear polarization (DNP) [5, 85, 323–326]. Pulsed ESR, in particular, offers the opportunity to obtain spin-lattice and spin-spin relaxation times, as well as resolve small spin-spin couplings [66]. It is crucial for an ESR pulse modulator to have sufficiently low power transmission in the “off” state to avoid undesirable induced magnetization. Since the flip angle, α , is proportional to $B_1 \propto \sqrt{P_{mw}}$ and the duration of the applied field [66], this means that the time integral of the leaked microwave magnetic field in the “off”-state must be much less than that of the pulses:

$$\sqrt{P_l}t_l \ll \sqrt{P_p}t_p \quad (3.14)$$

where P is power, t is time, and the indices l and p represent “leaked power” and “pulse power”, respectively. In addition, it is of course desirable to have maximal transmission in the “on” state in order to maximize the flip-angles that can be achieved with a given pulse.

A typical pulsed ESR experiment requires more than one pulse and the ability to

control the relative phase in a sequence of pulses [276]. An LDSS apparatus that is capable of slicing up to two pulses from the output of an FEL, with the ability to control relative phases, has enabled FEL-powered EPR experiments, including free induction decay, Hahn echo, and spin-lattice relaxation time measurements in select systems [50, 276]. However, pulse sequences of *more* than two pulses are typically desirable for pulsed ESR spectroscopy, and it is challenging to scale the existing pulse slicer design to more than two pulses due to spatial requirements. Furthermore, all pulse slicers reported to date have dumped the beam energy that is outside the pulse timings into an absorber to minimize standing waves and spectrometer ringdown. This dumped “complementary” beam is thus wasted in existing pulse slicer designs [50, 321, 327].

3.5.2 ITST’s current pulse slicer

The existing pulse slicing configuration as used for 240 GHz FEL-EPR at the Institute for Terahertz Science and Technology is described in sec. 5.2.4 of Edwards [134]. The configuration was designed to ensure that all switches were at Brewster’s angle and in the same plane as the rest of the quasioptics. This Brewster’s angle setup has close to zero “off”-state transmission (< -60 dB) and is capable of producing one ($< 1 \mu\text{s}$) or two sharp pulses (one of 1 ns to 20 ns, the other of 1 ns to 40 ns) from a $1 \mu\text{s}$ FEL pulse [50]. Such a design has a very large footprint to generate only two pulses: one full optical table ($1.2 \times 2.4 \text{ m}^2$) is required for optical components and another is required for the high-power ($\sim 100 \text{ mJ}$) Nd:YAG lasers and physical delay stages used for rise-and-fall timing manipulation. The large footprint and many optical components required (both visible and THz) also make the design prone to misalignment and imperfect coupling, which reduces the sharpness of the generated pulses as well as the peak ESR pulse power that

can be achieved. Further, the large footprint requires the use of high-density polyethylene THz lenses in order to preserve the input beam radius; however, such lenses also introduce partially reflective, normal-incidence surfaces that cause standing waves and increase spectrometer dead-time through ringdown. To reduce the effects of ringdown, the setup requires that the complementary quasi-CW beam must be dumped into a THz absorber.

3.5.3 Novel pulse slicing module

To mitigate the issues described above, a modular pulse slicing design was invented that can be stacked in sequence to slice an arbitrary number of pulses from a THz quasi-continuous wave (CW) Gaussian beam. The module is frequency agile, and cheaper than the current design by an order of magnitude. It is also more robust to optical misalignment and approximately 30 times smaller than previous designs [50]. The modular design provides two outputs: the “additive” channel that contains the desired pulses, and—unique to this design—its complementary “subtractive” channel, that contains the complement of the sliced pulses. The subtractive channel—which has been dumped in previous pulse slicers—can be used as an input to a subsequent module to slice additional pulses, or concurrently measure beam parameters such as power and phase.

The design incorporates parabolic mirrors ($f = 50.8$ mm, 35-644, Edmund Optics, NJ, USA) that focus the beam onto a silicon wafer and subsequently image the input beam to the output (see Fig. 3.16). This design has two qualities that assist in minimizing “off”-state reflection by the silicon wafer, which is crucial for pulsed ESR: incoming THz is directed toward the silicon switches at Brewster’s angle ($n_{Si} = 3.4175$, $\theta_B = 73.69^\circ$, Dai et al. [328]), and the modular assembly also allows silicon wafers of optimal thickness—such that front and back surface reflections at the silicon wafer destructively interfere—to

be inserted by a stepper motor when the FEL frequency is changed.

Fig. 3.16 demonstrates the operation of a pulse slicing assembly, made up of two pulse slicing modules, to generate one pulse. The input THz beam (pink) is reflected by a parabolic mirror and subsequently passes through a silicon wafer (dark green) into the subtractive channel output. Upon activation of this wafer using Laser-1, the input beam is reflected by the front surface of the wafer and directed into the additive channel (blue), while the subtractive channel is turned off. Each channel is collimated using its own parabolic mirror, producing two beams that match the original input beam's size and profile. These beams are used as inputs for the second module and the entire process is repeated. When Laser-2 activates the second wafer, the beam in the additive channel is directed into the subtractive channel (now black), which turns off the additive channel and leaves a sharp pulse behind (see Fig. 3.16, bottom). Additional pulses can be generated using the output of the subtractive channel as the input for subsequent modules.

Matrix formulation

It is informative to formulate the operation of a module as a transfer matrix so that successive modules can be simulated by simply raising the matrix to the n -th power. Taking an input vector $\vec{E}_i$ that contains the relative field strengths in the subtractive and additive channels, an individual pulse slicer module can be represented as a 2-by-2 matrix,

$$\mathbf{E}_f = \mathbf{A}\mathbf{E}_i \quad (3.15)$$

$$\begin{pmatrix} E_f^S \\ E_f^A \end{pmatrix} = \begin{pmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{pmatrix} \begin{pmatrix} E_i^S \\ E_i^A \end{pmatrix} \quad (3.16)$$

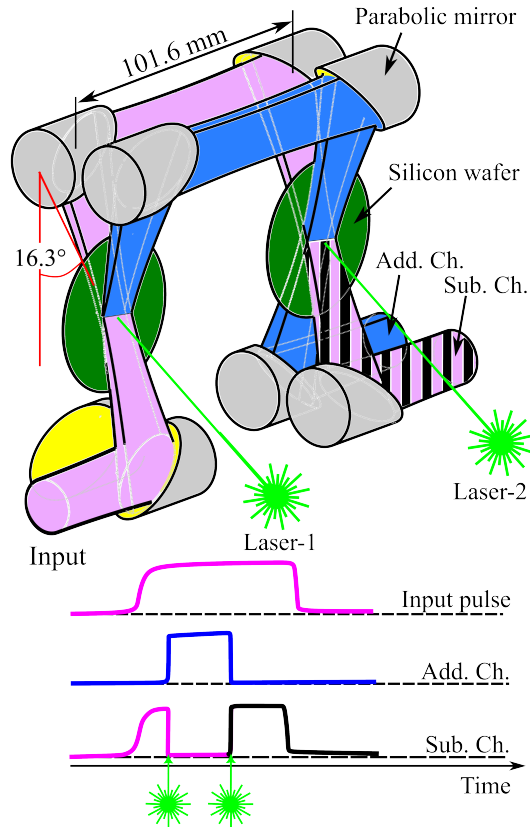


Figure 3.16: Quasioptics incorporated in one modular pulse slicer assembly. Parabolic mirrors focus collimated incoming (sub-)THz waves (pink) onto a 50.8 mm diameter silicon wafer at Brewster's angle. Depending on switch state, the THz waves are either transmitted (pink) or reflected (blue) at the first switch. Similarly, pulses are either transmitted (pink and blue) or reflected (black) by the second switch. As shown in the timing diagram (bottom), during slicing, pink is output first, then blue, and finally black. A pulse (~ 3.8 mm beam diameter) from a 532 nm diode-pumped laser incident on the silicon wafer (in this figure, illumination is from the right-hand side of both wafers) promotes electrons into the conduction band and switches silicon from transmissive-to-reflective to THz for the duration of the lifetime of the carriers. Eight parabolic mirrors and two silicon wafers (as shown) constitute one pulse slicing assembly.

where subscripts f and i denote final and initial, and superscripts S and A denote the subtractive and additive channel, respectively. For an individual laser (and module) timing, t_i , each matrix element A_{ij} can be represented with a simple expression,

$$A_{11} = A_{22} = p * (1 - H(t - t_i)) \quad (3.17)$$

$$A_{12} = A_{21} = r(t - t_i) * H(t - t_i) \quad (3.18)$$

where p is the transmission coefficient of an unactivated silicon wafer, r is the reflection coefficient of the silicon wafer as a function of time after laser excitation t_i , and $H(t)$ is the Heaviside step function. Since $\mathbf{A}$ only represents a single module, one pulse can be sliced by two modules ($E_f^1 = \mathbf{A}^2 E_i$) and n pulses can be sliced by $2n$ modules ($E_f^n = \mathbf{A}^{2n} E_i$).

A simulation with 100% of the input power into the subtractive channel, taking $n = 3$, $p = 1$, $r = r_0 e^{-(t-t_i)/\tau}$, $r_0 = \sqrt{0.7}$, $\tau = 6 \mu\text{s}$ for laser timings of 50, 150, 300, 500, 750, and 1050 ns is shown in Fig. 3.17 ($r_0 = \sqrt{0.7}$ was chosen because the peak “on”-state power obtained during experiments was approximately 70%).

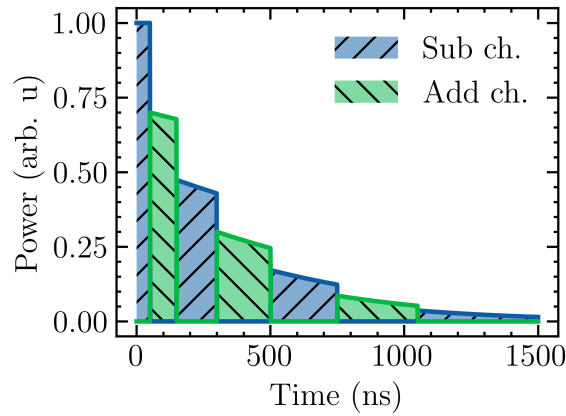


Figure 3.17: Transfer matrix simulation of six pulse slicing modules. Taking 100% of the input power to be in the subtractive channel, the output power of the subtractive (blue) and additive (green) channels is shown as a function of time. For this simulation, $p = 1$, $r = r_0 e^{-(t-t_i)/\tau}$, $r_0 = \sqrt{0.7}$, $\tau = 6 \mu\text{s}$, and laser timings are 50, 150, 300, 500, 750, and 1050 ns. The plotted result is power (E_f^2), normalized to 1 at $t = 0$.

Tuning thickness for destructive interference

Precisely tuning the wafer thickness for specific frequencies leads to destructive interference between front- and back-surface reflections from the wafer, and thereby minimizes undesirable reflection of rays that are not exactly at Brewster's angle or those that are cross-polarized. Doty et al. [329] showed that this destructive thin-film interference can result in an additional 30 dB suppression of “off”-state reflections.

Therefore, in order to achieve optimal performance across a wide range of frequencies, the silicon wafers need to be replaceable. The design incorporates an automated solution for this process through a silicon exchange mechanism (see Fig. 3.18) that facilitates switching between two pairs of wafers of different thicknesses. Positioned between two modules, this mechanism not only facilitates wafer replacement but also ensures effective microwave coupling between pairs of parabolic mirrors via an adjustable set screw; each wafer's position can be finely adjusted to ensure its precise alignment within the microwave beam waist.

“Off”-state reflection can be caused by cross-polarization as a result of reflection by the curved surface of the parabolic mirrors and the divergence of the Gaussian beam near its waist, which causes the edges of the beam to have incident angles that are different from its center [330]. Following Murphy [35], an estimate for the fraction of p -polarized power that is converted to s -polarized through cross polarization is given by:

$$\eta_{cp} = \frac{\omega_m^2}{4f^2} \tan^2 \theta_i \quad (3.19)$$

$$= \frac{\omega_0^2 \left[1 + \left(\frac{z}{z_r} \right)^2 \right]}{4f^2} \tan^2 \theta_i \quad (3.20)$$

$$= \frac{\omega_0^2 \left[1 + \left(\frac{z}{\pi \omega_0^2 / \lambda} \right)^2 \right]}{4f^2} \tan^2 \theta_i \quad (3.21)$$

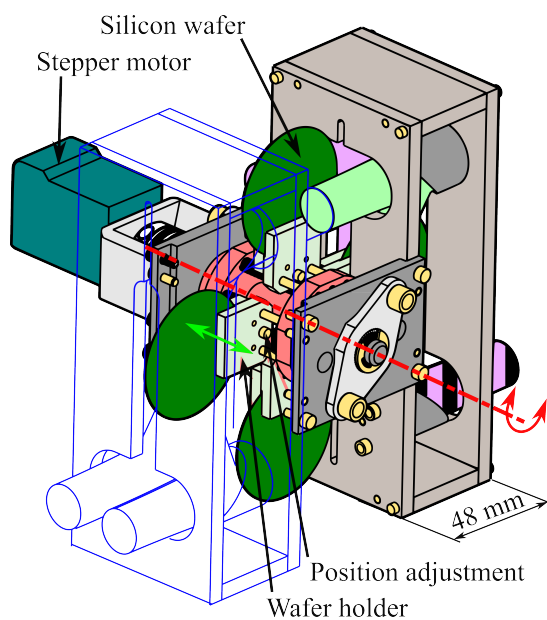


Figure 3.18: Pulse slicer silicon exchange mechanism. Two slicer boxes are interconnected and aligned using the exchange mounting brackets. The brackets also support and align a stepper motor and shaft (TSM17Q-1RG, Applied Motion Products, Inc., CA, USA). This mechanism accommodates up to four wafers: two within the boxes and two positioned between them. Additionally, spring-loaded wafer holders in each box allow for precise fine-tuning of wafer positions via a set screw.

$$= \frac{(5.79 \text{ mm})^2 \left[1 + \left(\frac{50.8 \text{ mm}}{\pi(5.79 \text{ mm})^2/0.937 \text{ mm}} \right)^2 \right]}{4(50.8 \text{ mm})^2} \tan^2(\pi/4) \quad (3.22)$$

$$\eta_{cp} = 0.004, \quad (3.23)$$

which means that 0.4% of the Gaussian beam power incident on the semiconducting switch will be partially reflected (beam parameters: $\omega_0 = 5.79 \text{ mm}$, $\lambda = 0.937 \text{ mm}$). Here, ω_m is the beam radius at the mirror surface, f is the mirror focal length, and θ_i is the angle of incidence. In eqn. (3.20), ω_m is substituted for using the equation for Gaussian beam propagation along z from an input beam radius, ω_0 . z_r represents the beam's Rayleigh range, which is substituted for in eqn. (3.21) with π , ω_0 , and λ , the input wavelength [331]. Though only a fraction of the 0.4% cross-polarized light will be reflected and cause “off”-state leakage, any leakage at all reduces experimental sensitivity and is therefore worth minimizing.

Off-axis reflection by a parabolic mirror also causes a distortion that shifts the center of the Gaussian beam relative to its propagation axis. However, since the 532 nm excitation pulse can be positioned to maximize overlap with the THz beam, this shift can be compensated for at the wafer surface. In the modular configuration presented here (and in Fig. 1.7), the second parabolic mirror of each pair corrects for the distortion and reduces it to negligible levels at the output, as is discussed in Murphy [35].

A pulse slicing assembly is illustrated in Fig. 3.20; the largest components are two diode-pumped 532 nm lasers (VRN20-50-G, Lumibird Photonics, FR) that are used to drive the silicon switches. 532 nm lasers were chosen for optical excitation due to the maturity of their technology: diode-pumped lasers with small form factors are able to produce significant power at this wavelength and their photon energies are sufficiently high (larger than the bandgap of Si, $\lambda_{ex} < hc/E_{g, Si} = hc/1.13 \text{ eV} = 1.1 \text{ } \mu\text{m}$, [332]). These

lasers, in conjunction with the modules' housing and optics for laser beam expansion, are securely mounted onto a base plate. Both 532 nm laser beams are reflected and expanded by a 9.5 mm focal length concave spherical mirror (CM127-010-G01, Thorlabs, NJ, USA): beam expansion ensures the precise overlap of THz and laser beam profiles on the silicon wafer's surface. Individual modules (highlighted in red in Fig. 3.20) were constructed from CNC-milled (CNC track precision $2.5\text{ }\mu\text{m}$) aluminum housing that was designed to fix all quasi-optical components in place and minimize positioning errors (see constructed test assembly in Fig. 3.19).

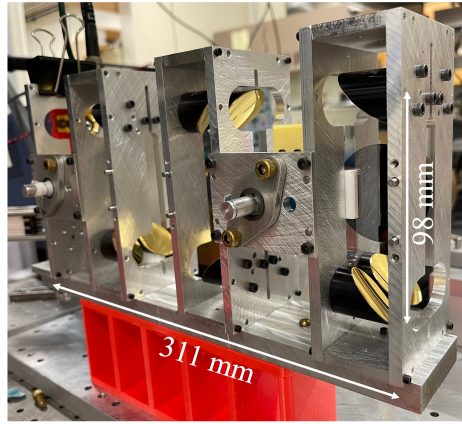


Figure 3.19: CNC-machined pulse-slicing box. Readily available parabolic mirrors (35-644, Edmund Optics, NJ, USA) focus collimated incoming mm waves onto a 50.8 mm diameter silicon wafer at Brewster's angle. All metallic components were machined from high-precision 6.35 mm-thick plates (3511T115, McMaster-Carr, IL, USA). The parts were designed to be symmetric so that only 3 distinct parts were required for the design, which simplified the manufacturing process. Plastic components (the wafer holder and carousel) were 3D-printed for rapid prototyping and flexible, scratch-free mounting of the Si wafers (for 320 GHz, the wafer thickness used was $279 \pm 25\text{ }\mu\text{m}$; wafers provided by Virginia Semiconductor, VA, USA). The wafer-holder carousel features springs an adjustable set screw that positions the wafer to ensure the beam is nominally at Brewster's angle and intra-module alignment is optimized.

Each pulse slicer assembly (consisting of two modules) is designed to generate a single pulse. Because of its small footprint, additional assemblies could be stacked to generate

more than two pulses (outlined in Fig. 3.20), and a protective enclosure could easily be employed to increase eye safety and reduce scattered laser light.

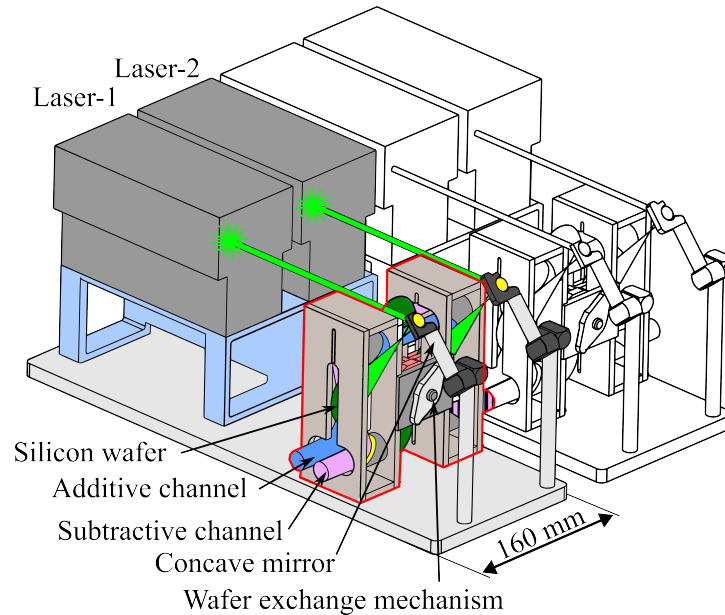


Figure 3.20: Modular pulse slicing assembly. The arrangement of mirrors, silicon wafers, and THz Gaussian beams (as depicted in Fig. 3.16) is integrated within two pulse slicer modules, which are highlighted by the red outlines. The overall assembly involves two 25 mJ lasers that both emit a 9 ns 532 nm pulse, ~ 3.8 mm in diameter, that is expanded beyond the focal length (9.5 mm) of a spherical concave mirror to fully cover the face of the silicon wafer (and incident THz Gaussian beam) inside each module. The pulse slicer module precisely aligns four parabolic mirrors with a silicon wafer at Brewster’s angle using slip- and press-fit pairs of dowel pin holes. The assembly’s stackable nature is visually depicted in a black and white illustration—the configuration shown would be used to make two sharp pulses from a high power quasi-CW beam.

Novel pulse slicer results

The capabilities of complementary-channel modular pulse slicer design were demonstrated using a 320 GHz source-and-detector test setup (VDI-S027 source, Virginia Diodes, Inc., VA, USA; AC-coupled quasi-optical detector, 3DL 12C LS2500 A2, ACST gmbH, DE) and two $279 \pm 25 \mu\text{m}$ float-zone, high-resistivity, $\langle 111 \rangle$ undoped silicon

wafers (Virginia Semiconductor, VA, USA). The wafer thickness was optimized such that front and back surface reflections at 320 GHz destructively interfered when oriented at Brewster’s angle, using

$$T = \frac{m\lambda}{2 \left(\sqrt{n_{\text{Si}}^2 - n_{\text{air}}^2 \sin^2 \theta_i} \right)} \quad (3.24)$$

from Doty et al. [329] after substitution using Snell’s law, where T is the wafer thickness and m is an integer that represents the interference fringe order. The AC-coupled detector, with an amplifier bandwidth of 0.008-4 GHz, converted 320 GHz power into a voltage. Pulse slicing experiments were done in either “CW” mode, where a pulse was directly sliced from a low-power CW beam, or “stepped” (convolution) mode, where a 20 ns pulse was stepped through the slicing timing. In both modes, the 320 GHz power was approximately 100 μW .

Fig. 3.21 demonstrates sliced pulses with durations between 1 ns and 50 ns generated by two lasers and two wafers. The rise and fall times of the pulses are currently limited by the 9 ns pulse length of the laser used (depicted in gray in Fig. 3.21). Shorter pulses with faster rise and fall times could be achieved by activating the silicon switch with a picosecond or even femtosecond laser [333, 334]. Recorded pulse profiles are distorted due to the AC-coupling of the detector, but the detector’s fast response was able to capture the slopes of the rising and falling edges.

To avoid the limitations of the AC-coupled quasi-optical detector, the transfer function of the modular pulse slicer was also measured by stepping the timing of a 20 ns, 320 GHz close-to-square probe pulse with respect to the timing of laser pulses 1 and 2. This method returns a convolution of the transfer function and probe pulse with rising and falling edges less steep than a pulse sliced from a CW beam (simulation of this convolution effect is shown in Fig. 3.22). The additive and subtractive outputs of

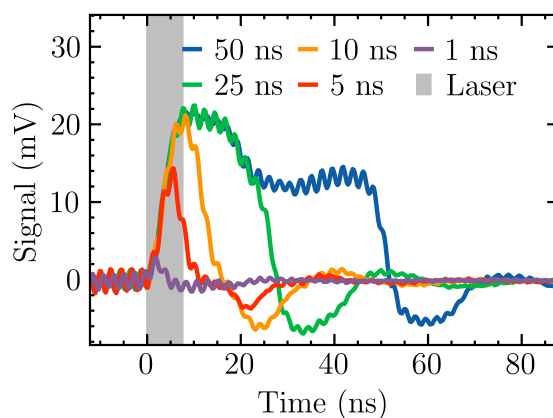


Figure 3.21: Pulses sliced from CW 320 GHz Gaussian beam. The delays between on and off switch timings are shown in the legend. Peak power remains consistent down to approximately 10 ns pulses, below which it is reduced. Below 10 ns on-and-off delay, the pulse length of the laser (~ 9 ns [335], shown by the gray bar) does not allow for the full 25 mJ to be deposited onto the wafer before the pulse is switched off. The deviations from square temporal profiles are due to the AC coupling of the detector. The fine structure (with fringe period of 2.2 ns) is attributed to electromagnetic interference that manifested when the 532 nm laser is fired. Experiments were repeated at 20 Hz. Exponential continuous averaging was used, with an effective time constant of 1.55 seconds, corresponding to 31 oscilloscope traces at 20 Hz (see Appendix D.1 for further explanation of exponential averaging).

complementary-channel pulse slicing are shown in Fig. 3.23, which highlights the temporal flexibility of the modular design. Fig. 3.23a shows 25 ns and 100 ns pulses with peak reflectances around 70%. Fig. 3.23b shows the power returned by the complementary subtractive channel; the power in this channel can be used to create additional pulses by stacking modules. The maximum power of each subsequent pulse n is given by $P_n = P_0 R^{2n-1}$, where R is the peak reflectance of the wafer.

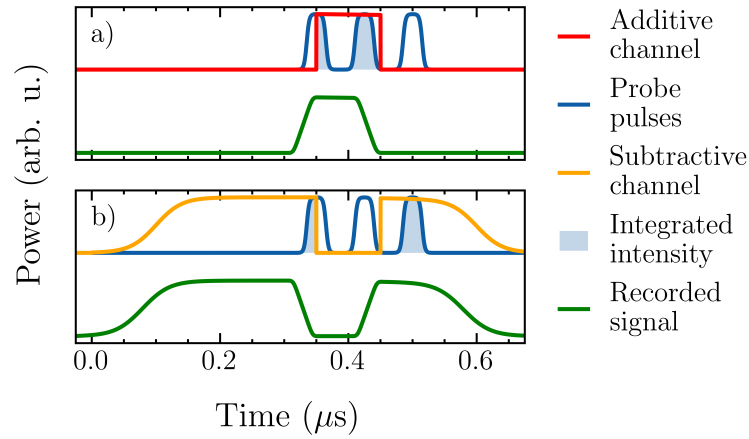


Figure 3.22: Effect of 320 GHz pulse convolution with slicing profile. (a) Convolution (green) of pulses (blue) with additive slicing profile (red). (b) Convolution (green) of pulses (blue) with subtractive slicing profile (orange). As seen here, convolution reduces the sharpness of the slicing profile and can also reduce the modulation depth for short slicing times.

Single-laser reflectance

The modular pulse slicer relies on the long recombination time of photo-excited electrons and holes in silicon. As shown in Fig. 3.24, for the wafers used, the reflectance remains substantial for 10 μs , and over the sub- μs duration of a typical FEL-EPR pulse sequence ($< 1 \mu\text{s}$, shown in red in Fig. 3.24), decays by less than 10%. The specific shape of the decay depends on the charge carriers' surface recombination, Auger lifetime, diffusion, and the Shockley-Read-Hall recombination due to impurities [321, 336]. Recombination

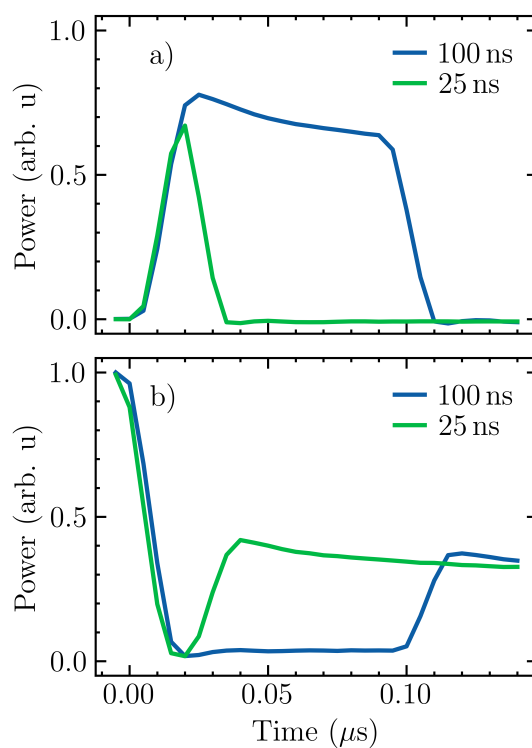


Figure 3.23: Convolutional sliced pulses of 25 and 100 ns. Pulses shorter than 25 ns, while possible, suffer significant distortion due to convolution with the 20 ns probe pulse. Exponential continuous averaging was used, with an effective time constant of 1.55 seconds, corresponding to 31 oscilloscope traces at 20 Hz. (a) Additive channel that makes up the sequence of pulses. Power is normalized to the transmitted power with both lasers off. (b) Subtractive channel that subsequent pulses are sliced from. Power is normalized to the transmitted power with both lasers off. Each data point represents the integrated power of a 20 ns THz pulse that was swept through the laser timing. The recorded data is a convolution of the slice timing with the probe pulse and results in a less sharp recorded profile than that shown in Fig. 3.21.

due to surfaces and impurities may be mitigated in the future by adapting methods and materials developed for silicon photovoltaics [337].

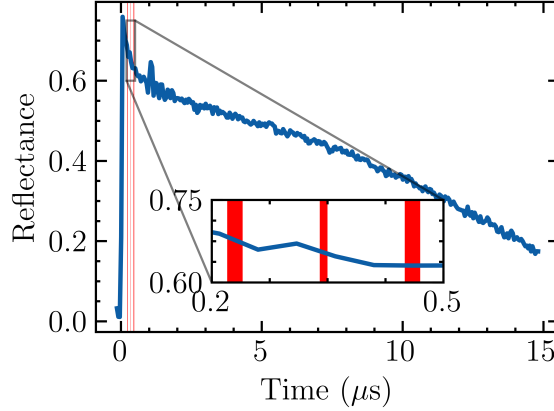


Figure 3.24: Reflectance of a single silicon wafer after excitation by a 25 mJ, 532 nm laser pulse. Power in the additive channel was measured by sweeping a 20 ns THz pulse in time across the laser pulse timing and integrating the total power of the THz pulse. Reflectance (solid blue) was calculated by dividing the power measured in the additive channel by the power in the subtractive channel when the switch was not activated. Experiments were repeated at 20 Hz. Exponential continuous averaging was used, with an effective time constant of 1.55 seconds, corresponding to 31 oscilloscope traces at 20 Hz. (Inset) Reflectance between 0.2-0.5 μs overlaid with a typical echo-detected inversion recovery FEL-EPR pulse sequence (pulse timings shown with red vertical bars).

The results demonstrate the feasibility of a frequency-agile quasi-optical pulse slicing module for slicing short (> 1 ns) pulses from a CW or quasi-CW THz source. The modular design vastly reduces the required space compared to the most sophisticated previous design and can slice pulses with a $0.048 \times 0.072 \text{ m}^2$ footprint rather than a large fraction of a $1.2 \times 2.4 \text{ m}^2$ optical table [50]. The modules can also be stacked together to slice as many pulses as necessary, which will enable three-pulse echo-detected inversion recovery ESR experiments, for example. The timings of the pulse edges are set entirely electronically and do not require a physical delay stage that would cap the maximum pulse length. Additionally, this modular design requires no custom optics and can be

almost entirely constructed with three CNC-milled parts.

The insertion loss of the subtractive channel of the pulse slicer was measured to be 1.2 dB at 320 GHz, which was obtained by comparing the power into and out of the slicing assembly test setup that consisted of three pulse slicing modules. This loss can be attributed to five main factors:

1. *Small off-axis parabolic mirror distortions*

Parabolic mirror distortions, resulting from off-axis reflection of a Gaussian beam, convert small amounts of power from the fundamental mode to higher-order modes, resulting in small power losses from the fundamental mode. A pair of mirrors in the reported pulse slicer configuration should reduce this distortion to negligible amounts. An upper bound on losses due to mode conversion is thus the loss from one mirror without compensation, which are be estimated (following Murphy [35] and Finn et al. [338]) to be

$$\eta_d = \frac{\omega_m^2}{8f^2} \tan^2 \theta_i \quad (3.25)$$

$$\eta_d = \frac{1}{2} \eta_{cp} = 0.002 \quad (3.26)$$

at 320 GHz. This implies a maximum loss of 0.0085 dB by each mirror pair, totaling 0.026 dB through the three module test setup.

2. *Spatial filtering of the THz beam by the pairs of off-axis parabolic mirrors*

The pulse slicer was designed for slicing a TEM₀₀ beam; higher order modes that can appear in an input beam due to imperfections in quasioptics are larger than the fundamental TEM₀₀ mode and are thereby filtered by the pairs of parabolic mirrors and contribute to the measured insertion loss [331].

3. *Ohmic losses at the metallic surfaces of the mirrors*

The estimated ohmic loss on a metallic mirror surface was around 0.2% [339–341]; the loss as a result of seven mirrors in series totals approximately 0.06 dB. The seven mirrors consisted of the six parabolic mirrors incorporated in three modules, and one flat mirror in place of a third silicon wafer that was used to facilitate switching between detected output channel.

4. *Losses at the semiconducting wafer face due to cross-polarization*

The losses as a result of divergence of the beam at the focus and slight cross-polarization are assumed to be negligible in this setup because the wafer thickness was tuned such that reflections from the front and back surfaces destructively interfered. However, an upper bound of the cross-polarization losses can be estimated by assuming that all s -polarized THz radiation incident on the wafer is lost through reflection, similar to the treatment for distortion losses. As calculated by eqn. (3.23), cross-polarization creates a fractional loss of $\eta_d = 0.004$ of input p -polarized beam power at each semiconducting wafer interface, leading to a total loss of 0.034 dB through one assembly [35, 331].

5. *Misalignment of optical components or imperfections in mirror manufacturing*

Under the above assumptions 1-4, the total loss through the test bench setup is approximately 0.12 dB, which is much smaller than the observed loss. The rest of the insertion loss (approx. 1.1 dB) can be attributed to alignment and machining imperfections. The finite thickness of the semiconducting wafer necessarily contributes some misalignment in (at least) one of the two channels that cannot be accounted for, as a variety of wafer thicknesses are required for operation across

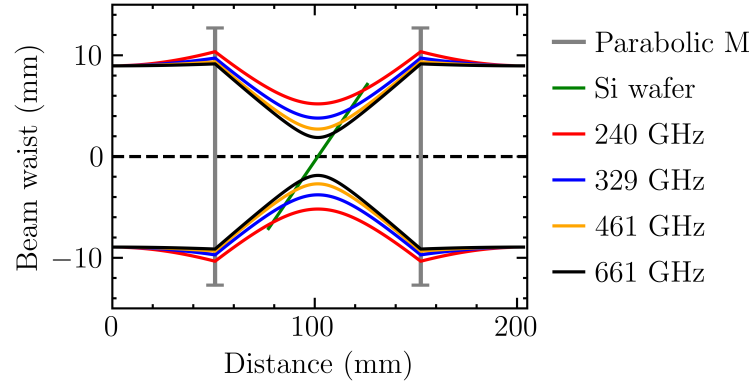


Figure 3.25: Simulation of the Gaussian beam profile through a pulse slicer module at a selection of frequencies commonly used for dynamic nuclear polarization (DNP). As shown, frequencies of 240 GHz and above will propagate through the quasi-optics with few losses. Lower frequencies, due to additional divergence, will partially fall outside the diameter of the silicon wafer. A larger wafer would be required to use this design at frequencies < 240 GHz. Vertical gray lines represent the position and input diameter of the parabolic mirrors, the angled green line represents the silicon wafer, and the red, blue, yellow, and black lines represent a circle that encloses 99% of the Gaussian beam energy at 240 GHz, as well as DNP frequencies 329 GHz, 461 GHz, and 661 GHz respectively [342].

the desired 170-500 GHz band. Though insertion losses of this magnitude are not ideal, 1.2 dB is acceptable when working with kW-power THz beams.

The insertion loss of the additive channel is dominated by the peak reflectance of the semiconducting wafer, with additional losses from imperfect quasi-optical coupling, spatial filtering, and ohmic losses, comparable to those of the subtractive channel. Fig. 3.25 depicts a simulation of a diameter that encloses 99% of the encircled energy for a selection of DNP frequencies and demonstrates that frequencies higher than 240 GHz will propagate without issue. Below 240 GHz, additional power will be lost as the circle enclosing 99% of the Gaussian beam energy falls outside the diameter of the silicon wafer. Below 150 GHz, the circle enclosing 99% encircled beam energy also falls outside the input diameter of the parabolic mirrors.

3.6 FEL phase cycling

Phase cycling is a common NMR technique that allows for selection of individual coherence orders within a pulsed magnetic resonance experiment [343]. Echoes from different sets of pulses will return (in general) different coherence orders; if one is clever about the phase of each pulse, individual echoes or free induction decays (FIDs) may be selected, which removes background signals and can improve signal-to-noise. In the case of the FEL, where background signals are large, “off-state” switching cannot be perfect, and the source repetition rate is quite low, phase cycling is critical to obtain high signal-to-noise.

3.6.1 Coherence order and coherence transfer pathways

Though they are abstract, “coherence orders” or “coherence pathways”, simplify the discussion of phase cycling techniques, so they are briefly reviewed here. Pulsed magnetic resonance is concerned with the coherences of spin states, and the “coherence pathways” involving transitions between them. As discussed by Bain [343], a coherence level is a good quantum number, as it cannot change without external influence via an RF (in the case of NMR) or mm-wave (in the case of EPR) pulse. Defining coherence order transitions for individual pulse types and coherence order pathways for pulse sequences allows a spectroscopist to design pulse sequences with the desired phase-cancellation effects. This is a rich field of study in NMR, and sequences can get extremely complicated with NMR’s long relaxation times and ubiquitous use of arbitrary waveform generators (*e.g.* SPINAL64 64-pulse sequence [344]).

For a spin-1/2 in a magnetic field, there are precisely two available coherences that are denoted ± 1 . These correspond to precession in the clockwise and counterclockwise directions about the applied static magnetic field B_0 . The critical feature of coherence

orders is that a coherence of order m will have its phase shifted by $m\phi$ if a pulse is applied with phase ϕ ; essentially, the coherence order determines the scaling factor applied to the pulse-dependent phase of a given pathway.

The coherence order of other pulses can easily be calculated using the definition provided above. More formally, for a quantum mechanical operator of order p , the coherence order is determined by how the operator transforms by a rotation about $\hat{z}$ [345].

$$\hat{\rho} \xrightarrow{\mathcal{R}_z(\phi)} \hat{\rho} \exp \{-ip\phi\}. \quad (3.27)$$

Here, p is necessarily an integer. To calculate the coherence order generated by a spin flip ($\hat{S}_+$), for example, perform the rotation using the well-known quantum mechanical rotation operator on some inner product $\langle f | \hat{S}_+ | i \rangle$:

$$\langle f | \mathcal{R}_z^\dagger(\phi) \hat{S}_+ \mathcal{R}_z(\phi) | i \rangle, \quad (3.28)$$

which means the operator of interest is

$$\hat{S}_+^{\phi_z} = \mathcal{R}_z^\dagger(\phi) \hat{S}_+ \mathcal{R}_z(\phi). \quad (3.29)$$

Therefore,

$$\hat{S}_+^{\phi_z} = \exp \left(\frac{i\phi \hat{S}_z}{\hbar} \right) \hat{S}_+ \exp \left(\frac{-i\phi \hat{S}_z}{\hbar} \right) \quad (3.30)$$

$$= \hat{S}_+ \exp(-i\phi), \quad (3.31)$$

such that the coherence order of a $\pi/2$ pulse, as provided by the raising operator $\hat{S}_+$, is $p = 1$ according to eqn. (3.27). A similar treatment gives the coherence order of the lowering operator, $\hat{S}_-$ as $p = -1$. $\hat{S}_z$ has a coherence order $p = 0$, as $\hat{S}_z$ commutes with $\mathcal{R}_z(\phi)$, such that $\hat{S}_z^\phi = \hat{S}_z$. See Appendix A.2.1 for a complete walkthrough of these

calculations; a different technique that provides the same result can be found in Appendix A.11.3 of Levitt [12]. Product operators, for example $2\hat{S}_{1+}\hat{S}_{2+}$, return $p = 0, \pm 2$ via similar treatment. A product operator is required for $|p| > 1$, as a single spin-1/2 can only have coherence order of 1.

For a spin eigenstate in a magnetic field applied along $\hat{z}$, applying a pulse along (*i.e.*, rotation about) the x -axis generates the following coherence orders [345]:

$$\hat{S}_z^{\phi_x} = \exp\left(\frac{-i\phi\hat{S}_x}{\hbar}\right) \hat{S}_z \exp\left(\frac{i\phi\hat{S}_x}{\hbar}\right) \quad (3.32)$$

$$= \cos \phi \hat{S}_z - \sin \phi \hat{S}_y \quad (3.33)$$

$$= \cos \phi \hat{S}_z - \sin \phi \frac{1}{2i} (\hat{S}_+ - \hat{S}_-), \quad (3.34)$$

which generates equal amounts of $p = \pm 1$ (from $\hat{S}_+$ and $\hat{S}_-$), scaled by the flip angle of the pulse, ϕ . It is clear from eqn. (3.34) that a $\pi/2$ pulse generates coherences of $p = \pm 1$.

Now, consider a π pulse applied to an eigenstate of $\hat{S}_z$. According to eqn. (3.34), $\phi = \pi$ returns no change in coherence. However, if a π pulse is applied to a nonzero coherence, the sign of the coherence is flipped [345]. Consider, for example, $\hat{S}_+$ and a π pulse along $\hat{x}$:

$$\hat{S}_+^{\phi_x} = \exp\left(\frac{-i\phi\hat{S}_x}{\hbar}\right) \hat{S}_+ \exp\left(\frac{i\phi\hat{S}_x}{\hbar}\right) \quad (3.35)$$

$$= \hat{S}_x + i \cos \phi \hat{S}_y + i \sin \phi \hat{S}_z \quad (3.36)$$

$$= \hat{S}_x - i \hat{S}_y \text{ for } \phi = \pi \quad (3.37)$$

$$= \hat{S}_-, \quad (3.38)$$

which is also worked through in detail in Appendix A.2.1. In general, multi-pulse sequences of coupled spins (*e.g.* $\hat{S}_{x1}\hat{S}_{x2}$) will produce equal amounts of $p = \pm 2$, regardless of if the coherence order from the first pulse is $p = +1$ or $p = -1$; this is to say that

$\Delta p \neq 1$ necessarily, only that after a $\pi/2$ pulse, $||p_f| - |p_i|| = 1$.

These properties of coherence order are fundamental to the concept of phase cycling. When chosen carefully, coherence orders and transfer pathways allow a spectroscopist to choose the phases of an experiment to extract only the coherence orders of interest. In the case of 240 GHz FEL-EPR, this is typically a two-pulse echo. In the future, this technique will likely be expanded to three-pulse echoes in, for example, inversion recovery T_1 experiments.

To illustrate the idea of a coherence pathway, consider a two-pulse Hahn echo experiment. The system begins in an eigenstate of B_0 , either “up” or “down” in the magnetic field, which can be represented as an eigenstate of $\hat{S}_z$. It has been shown that applying a $\pi/2$ pulse changes the magnitude of the coherence order $|p|$ by 1 and that a π pulse flips the sign of an existing coherence order. It has also been demonstrated (see eqn. (3.34)) that a pulse applied to an $\hat{S}_z$ eigenstate generates equal amounts of available coherences. Though it is not demonstrated here, this is true in general: a $\pi/2$ pulse creates both $\pm\Delta p$.

A magnetic resonance experiment observes only $p = \pm 1$, as a single circularly-polarized photon can only change the spin of the system by ± 1 . Since the spins are precessing either clockwise or counterclockwise, the experiment is really only sensitive to one of either $p = +1$ or $p = -1$. Usually, by convention, the “detectable” coherence is $p = -1$. Therefore, after the full pulse sequence, an EPR experiment is only sensitive to the coherence transfer pathways that go from $p = 0 \rightarrow -1$ [346].

First, suppose that the Hahn echo sequence is ideal, such that the first pulse is a perfect $\pi/2$ pulse, and the second is a perfect π pulse. Though relaxation effects can alter these coherence orders between pulses, they are ignored for this treatment. There

are only two possible coherence transfer pathways in this case:

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} -1 \quad (3.39)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} +1. \quad (3.40)$$

These pathways are shown graphically in Fig. 3.26.

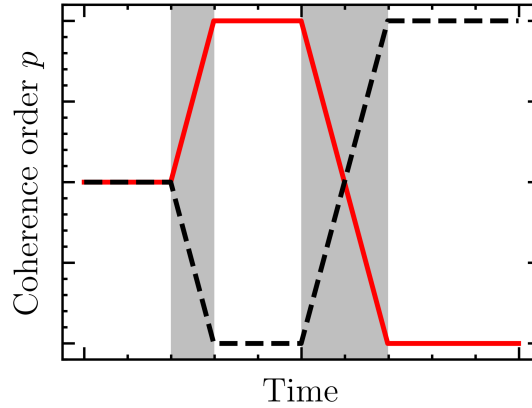


Figure 3.26: Coherence transfer pathways for an ideal Hahn echo. As can be seen here, the coherence order is initially 0, a $\pi/2$ pulse (first gray rectangle) converts it to $p = \pm 1$, and a π pulse (second gray rectangle) flips the order of both pathways. The only coherence that is detectable with ESR is $p = -1$, meaning only the solid red pathway can be detected. The dashed black pathway can be ignored.

Of course, no magnetic resonance experiment is ideal. The bandwidths of pulses are finite, the lengths are never exactly $\pi/2$ and π , relaxation effects can eliminate coherence orders between pulses, and each pulse does not necessarily excite all the spins in a sample. Therefore, each pulse can be either a $\pi/2$ or a π pulse, or some combination of both. This creates detectable signals (called FIDs) from imperfect “ π ” pulses that should be cycled away. Incorporating imperfect pulses makes the possible coherence transfer pathways much more complex:

$$0 \xrightarrow{\pi/2} 0 \xrightarrow{\pi} 0 \quad (3.41)$$

$$0 \xrightarrow{\pi/2} 0 \xrightarrow{\pi} +1 \quad (3.42)$$

$$0 \xrightarrow{\pi/2} 0 \xrightarrow{\pi} -1 \quad (3.43)$$

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} +1 \quad (3.44)$$

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} -1 \quad (3.45)$$

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} 0 \quad (3.46)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} +1 \quad (3.47)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} -1 \quad (3.48)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} 0 \quad (3.49)$$

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} +2 \quad (3.50)$$

$$0 \xrightarrow{\pi/2} +1 \xrightarrow{\pi} -2 \quad (3.51)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} +2 \quad (3.52)$$

$$0 \xrightarrow{\pi/2} -1 \xrightarrow{\pi} -2 \quad (3.53)$$

$$(3.54)$$

Again, this is shown graphically in Fig. 3.27

The task of phase cycling is then to separate the coherence transfer pathway of interest from the other detected ones. In the case of the Hahn echo, assuming the coherent echo is the signal of interest, this means selecting the red coherence pathway at the top of Fig. 3.27 from the two red coherence pathways at the middle and bottom (all have $p = -1$). The three coherences that will be detected by ESR are shown in Fig. 3.28. With typical NMR phase cycling, the pulses' and receiver's phase can be arbitrarily shifted to perform phase cycling. When using the FEL as a source, the receiver is not phase-locked to its pulses, and thus, the receiver phase (relative to the pulses) cannot be

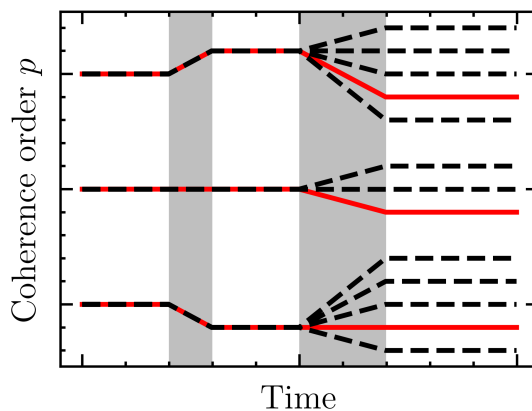


Figure 3.27: Coherence transfer pathways for an imperfect Hahn echo. The coherence order is initially 0; a “ $\pi/2$ ” pulse (first gray rectangle) transfers it to 0 or ± 1 ; a “ π ” pulse (second gray rectangle) then either flips the order, acts as a $\pi/2$ pulse, or does not act at all. The only coherence that is detectable with ESR is $p = -1$, meaning only the solid red pathways can be detected. The dashed black pathways can be ignored. The 13 pathways are vertically offset by $5\times$ their coherence order after the first pulse so that they are all visible.

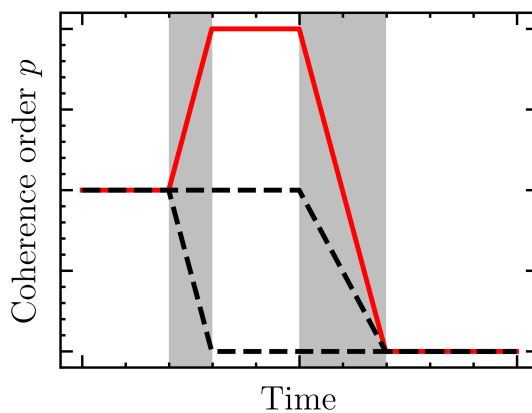


Figure 3.28: Detected coherence transfer pathways for the imperfect Hahn echo shown in Fig. 3.27. Phase cycling to improve the echo signal of a Hahn echo experiment amounts to selecting the solid red (echo) pathway, Σ_1 , from the dashed black (FID) pathways, Σ_2 and Σ_3 .

easily adjusted. However, this can be amended in post-processing—the effect of adjusting the “receiver phase”, ϕ_R , can be added by simply adjusting the phase of the entire coherent time-domain signal. To phase cycle and select the red coherence pathway in Fig. 3.28, first perform a reference experiment, where all phases are taken to be zero ($\phi_{\pi/2} = \phi_{\pi} = \phi_R = 0$). Now, cycle the phase of only the second pulse. If $\phi_{\pi} = \pi$, the 3 coherence pathways will all return signals of different phases. The desired pathway will therefore add a phase of $(-2)\pi$ to the resulting signal and the other two will add $(-1)\pi$ and 0, respectively. Since -2π is equivalent to 0, and -1π flips the sign of the resulting signal, adding the $\phi_{\pi} = 0$ and $\phi_{\pi} = \pi$ signals will cancel the FID from the second pulse and leave the echo (desired) signal and the first-pulse FID, which is usually small enough that it can be ignored. Since there was only two phases used in the cycle and 2π is equivalent to 0, it is not possible to separate coherence transfer pathways that are separated by $|\Delta p| = 2$.

Higher order phase cycles can allow higher order filtering. For example, if a phase cycle is chosen with $\phi_{\pi} = \frac{2\pi}{4}m, m \in [0, 3]$, coherence pathways that are not separated by integer multiples of 4 can be filtered from each other. The phases of the pulses and output signals from a four-pulse pulse sequence are shown in Table 3.2. The receiver phase is adjusted into $[0, 2\pi]$ to match the phase of the desired coherence pathway (Σ_1). Including this phase when detecting the other two pathways makes their phases sum to zero, as shown in Table 3.3. If all of these signals are summed, the contributions from Σ_2 and Σ_3 sum to zero, while the contribution from Σ_1 sums coherently, thus amplifying the desired signal and suppressing the others. This is the basis of phase cycling. Of course, for a three pulse experiment, a similar procedure can be constructed; it gets exponentially more complicated, as the phase of the pulses must be cycled *independently*, which requires $4 \times$ the number of experiments per pulse—for a three-pulse experiment, this would be 16

Table 3.2: Phase cycling for a Hahn echo. The phase of the $\pi/2$ pulse is $\phi_{\pi/2}$, the phase of the π pulse is ϕ_{π} , the total phase from the Hahn echo pathway is ϕ_{Σ_1} , from the second pulse FID pathway is ϕ_{Σ_2} , and from the first pulse FID pathway is ϕ_{Σ_3} . The phase of the receiver phase (*i.e.*, post-processing phase) is ϕ_R .

Sequence	$\phi_{\pi/2}$	ϕ_{π}	ϕ_{Σ_1}	ϕ_{Σ_2}	ϕ_{Σ_3}	ϕ_R
1	0	0	0	0	0	0
2	0	$\frac{\pi}{2}$	$-\pi$	$-\frac{\pi}{2}$	0	π
3	0	π	-2π	$-\pi$	0	0
4	0	$\frac{3\pi}{2}$	-3π	$-\frac{3\pi}{2}$	0	π

sequences (four-pulse would be 64 sequences, etc.).

3.6.2 ITST's current phase shifters

The phase shifters used at ITST for FEL-ESR are phase-plates custom-machined from HDPE that adjust the path length of one of the FEL pulses after slicing by the slicing optics. The technique has been reported in detail by Wilson et al. [276]. The HDPE plates function well, but have a few drawbacks:

1. They are difficult to machine to the precision required ($3.5\ \mu\text{m}$ thickness precision is required for 1° phase precision at 240 GHz).
2. Two plates are required, which doubles machining errors.
3. They must be at Brewster's angle to the FEL beam to minimize stray reflections.
4. They must be manually swapped in-and-out during an experiment which lengthens the duration of all experiments.

Table 3.3: Phased coherence pathways for a Hahn echo. After compensating for receiver phase, the phase of the $\pi/2$ pulse is $\phi_{\pi/2}$, the phase of the π pulse is ϕ_{π} , the total phase from the Hahn echo pathway is ϕ_{Σ_1} , from the second pulse FID pathway is ϕ_{Σ_2} , and from the first pulse FID pathway is ϕ_{Σ_3} . For this table, all phases are converted into the range $[0, 2\pi)$.

Sequence	$\phi_{\pi/2}$	ϕ_{π}	ϕ_{Σ_1}	ϕ_{Σ_2}	ϕ_{Σ_3}	ϕ_R
1	0	0	0	0	0	0
2	π	$\frac{3\pi}{2}$	0	$\frac{\pi}{2}$	π	0
3	0	π	0	π	0	0
4	π	$\frac{\pi}{2}$	0	$\frac{3\pi}{2}$	π	0

5. Because of the manual swapping, the experiment cannot be completely automated; an operator must be in the room during long acquisition cycles.
6. Only $\frac{2\pi}{4}m$ plates were machined, so only $\frac{2\pi}{4}m$ phase cycles are possible.
7. Most critically, the plates are only the correct thickness for a single frequency; phase-cycled ESR experiments are restricted to 240 GHz with this design.

A fully automated, continuously variable system mitigates all of these issues and can access more precise phases without the need for manual operation across a range of FEL frequencies. A device for this application was designed and tested for implementation into the novel FEL-ESR system.

3.6.3 Novel phase shifting module

The novel phase-shifter was designed to be continuously adjustable and broadband so that it could work across the full range of FEL-ESR frequencies. It was also designed to

have one stationary path and one adjustable path that aligned with the outputs of the pulse slicer module, such that phase shifters could be placed between modules to allow for precise phase control of all the pulses of a given FEL-ESR experiment. A first design incorporated two parabolic mirrors in the mirrored telescoping configuration (similar to Fig. 1.7, but the handedness of one of the mirrors was inverted) on a movement stage that could extend the path of one with respect to the other. However, as shown in Murphy [35], this configuration introduces modal and cross-polarization distortions that will increase the conversion loss of the system. To avoid these losses, the design was modified to incorporate two 45° off-axis parabolic mirrors and a folding mirror between them: in this way, they are configured similar to the initial design, but optically equivalent to a periscope-like configuration that eliminates distortions (see Figs. 3.29 and 3.30) [338].

In this system, the optical path length is designed to image the input beam at the output. To achieve this, the paths a and b sum to the length of the path c , which is equal to the effective focal length of the parabolic mirror. A micrometer sets the path length by adjusting the position of the two parabolic mirrors and one folding mirror. This configuration ensures that the focus lies exactly on the flat folding mirror at all times.

The phase shifter was automated with a stepper motor and tested using a vector network analyzer (VNA) by undergraduate Casey Bernd. As shown in Fig. 3.31, the phase shift as a function of stepper motor step was very repeatable. The experiments were repeated five times: the mean and standard deviation of the five experiments is shown in orange. A small (repeated) deviation from linear appeared between 2000 and 3000 steps. It is likely that the guide rails for the movement plate were slightly misaligned. This misalignment may have caused tensile strain and sticking in the system; once the plate had moved past the misalignment, the tension was released, which may have caused

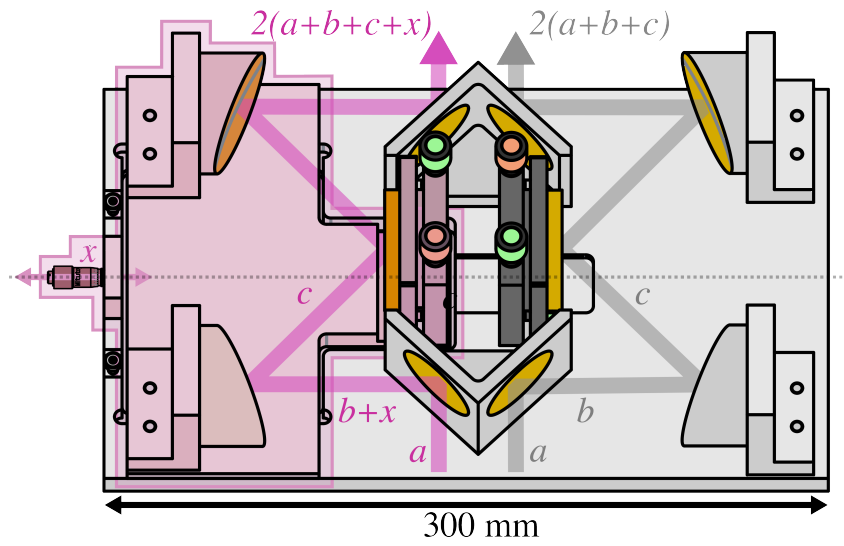


Figure 3.29: Schematic of mirror-based continuous phase shifter module. Each path couples directly to-and-from pulse slicer modules. The parabolic mirrors are 45° off-axis parabolic mirrors with a 50.8 mm input diameter and 80 mm effective focal length. The system is designed to image the input beam at the output; the focus of both mirrors lies directly on the flat folding mirror. The folding mirror is mounted on adjustable mirror mount for precise control of the positioning of the output beam. A micrometer is used to adjust the plate position, x . The total phase shift of the pink path relative to the gray path is $\phi = \frac{2\pi}{\lambda} 2x$. The red and green knobs adjust the vertical and horizontal alignment of the output beam, respectively.

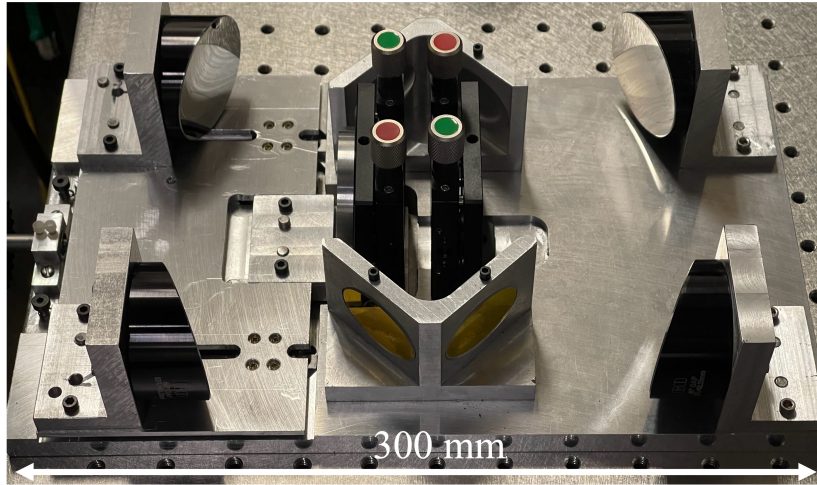


Figure 3.30: Image of mirror-based continuous phase shifter module. Parabolic mirrors are Edmund Optics, part no. 35-553. The springs that are attached to the moving plate maintain constant contact between the moving plate and the micrometer and function to eliminate backlash when reversing movement direction. Frame components were custom-machined from aluminum in UCSB’s student machine shop, the mirrors, guide rails, etc., were purchased from external vendors.

the nonlinear behavior as the system returned to linearity at around 3000 steps. If this “sticking” can not be mitigated, the stepper motor controller program may be set up as a feedback control system: in this case, a user would enter a desired phase, and the control software would iteratively adjust the plate position until the FEL phase matched the desired setting.

Combining three pulse slicer modules with two phase shifter modules allows for precise control of the timing of all three pulses, as well as individual control of the relative phase of the second and third pulse with respect to the first. Because phase is relative, and the first pulse phase can always be taken as $\phi = 0$ (by post-processing or adjusting receiver phase), only two phase shifters are necessary to fully define the phase of the entire pulse sequence.

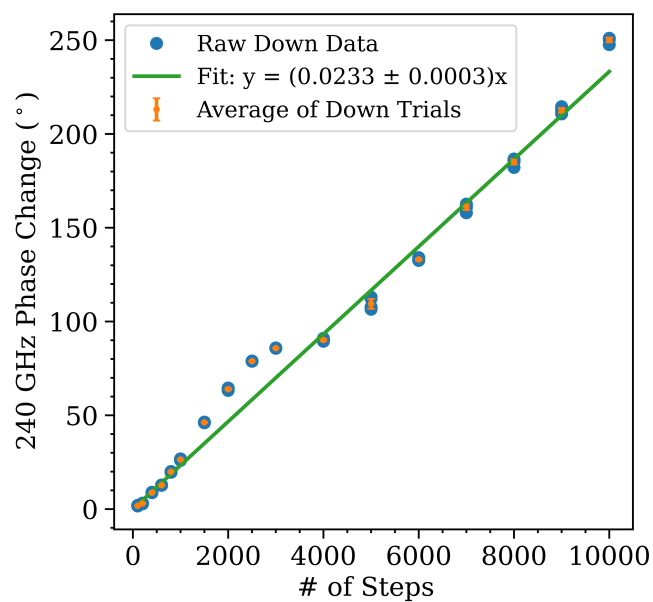


Figure 3.31: VNA testing of phase shifter module. Phase as a function of stepper motor step is shown in blue. The average of 5 experimental runs is shown in orange, along with error bars representing the standard deviation. A fit for the data is shown in green, which returned $23.3 \pm 0.3 \text{ m}^\circ$ per step. The stage was moved in the same direction for all experiments (“down”, reducing the path length). Data acquired and plotted by Casey Bernd, reproduced with permission.

3.7 Quasi-optical sample holder[†]

To achieve sufficient sensitivity without a cavity, high-field (HF) EPR spectrometers typically operate in induction mode; this means that a wire grid polarizer above the sample ideally only reflects radiation into the detector that is perpendicular to the input polarization (discussed earlier, in section 1.1.4). The perpendicular radiation (called “cross-polar”) is made of information about the sample, whereas the incident polarization (“co-polar”) is made up of almost entirely background. Therefore, maximizing the cross-polar-to-co-polar contrast also means maximizing the spectrometer’s sensitivity. While typically capable of isolating the cross-polar from the co-polar with 30 dB of contrast [198, 303], induction mode architectures still impart an undesirable background to EPR experiments, as the excitation power is typically much larger than the orthogonal induction-mode signal; as a result, improving induction-mode isolation for high-field and high-frequency experiments presents a potential avenue for improving HFEPR resolution and is a topic of interest for many researchers [193, 268, 302, 342, 347–349].

This section details a frequency-independent high-field sample holder that enables an important improvement in SNR for HFEPR spectrometers that is easy to design, prototype, and implement. The quasi-optical sample holder (QSH) presented here, designed to operate in all three modes (CW, pulsed, and RS), was fitted to attach to the EPR probe used in EPR spectrometer at ITST. Minor adjustments to the 3D printed model would allow for simple incorporation into other probe designs. The QSH was entirely 3D printed for rapid prototyping, except for the 12.7 mm diameter parabolic mirror (focal

[†]The text and many of the figures in sec. 3.7 are adapted from [194]: A. Sojka, B. D. Price, and M. S. Sherwin, “Order-of-magnitude SNR improvement for high-field EPR spectrometers via 3D printed quasi-optical sample holders,” *Sci. Adv.* **9**, eadi7412 (2023). Reprinted with permission from AAAS.

length of 25.4 mm), which was purchased from Thorlabs Inc., and the rooftop mirror, which was end-milled from a 7 mm diameter aluminum rod (see fig. S1). The QSH enhanced the SNR of all three EPR modes. For CWEPR, the enhanced SNR can provide equal sensitivity with fewer spins. For pulsed EPR, it reduces undesirable co-polar power enough that direct measurements of cross-polar power can immediately return a field-swept EPR spectrum without requiring an engineered pulse sequence. For rapid-scan EPR, the SNR enhancement enables time-resolved kinetic studies with tunable time resolution (currently down to 10 ms).

3.7.1 Design

This section discusses two types of sample holders: the “simple” sample holder (SSH) that has been used for many years at ITST, and the novel QSH. A part similar to the SSH can be found in almost any HFEPR lab and is, therefore, a useful comparison to the QSH. The SSH typically consists of two parts that are machined from plastic: one is permanently attached to the corrugated waveguide and supports the modulation coil, and the other is a detachable insert on which the sample sits. By varying the insert’s length, the sample can be placed close to the corrugated waveguide output at the top of the modulation coil (Fig. 3.32 right, “SSH-T”) or in the center of the modulation coil (Fig. 3.32 right, “SSH-M”). In both SSH configurations, the sample sits directly on a planar mirror that reflects incident radiation back into a corrugated waveguide. For pulsed EPR, the SSH-T position is used to minimize microwave loss due to divergence of the propagating Gaussian beam. For RS-EPR and CW-EPR, the SSH-M position is used to obtain the largest and maximally homogeneous modulation field at the sample space.

The QSH was designed with a broad range of applications and user-friendliness in mind. The frequency-independent design of the QSH uses focusing quasi-optics to ensure that the beam waist is kept within the sample holder's specified optical path, reducing microwave beam clipping due to the waveguide aperture. The quasi-optical design does not require a waveguide or end mirror within its modulation coil, which eliminates eddy currents inside the coil, and results in an improvement of field modulation amplitude and homogeneity. The quasi-optics consist of a 25.4 mm focal length off-axis parabolic mirror (MPD019-M03, Thorlabs Inc., USA) mounted along the axis of the corrugated waveguide and a rooftop mirror machined using an end mill. A simulation of quasi-optical fundamental mode Gaussian beam propagation is shown in Fig. 3.33 [41]. These simulations show the beam waist compared to the quasi-optical elements and highlight that clipping loss is reduced due to the focusing quasi-optics.

The QSH was designed for 3D printing and room temperature operation and works well in these conditions. With a different printing substrate (e.g., nylon-based filament), liquid nitrogen temperatures should be easily achievable [350]. If higher precision or durability is required, then a QSH could be made using subtractive machining after a suitable design has been prototyped via 3D printing.

The prototype and gears were 3D printed from polylactic acid by an Original Prusa i3 MK3S+ (Prusa Research, CZ). A Helmholtz modulation coil was used (32 AWG copper wire) to get direct access to the sample space from outside the coil. Sample space access is necessary for fiber optical excitation in experiments similar to those presented in [142] and may also allow for fast sample loading in future designs. The modulation coil field strength was calibrated using a reference sample of LiPc (see Fig. 3.34), as discussed in section 1.2 and eqn. (1.102).

To motivate the design of the QSH, it is instructive to first review the design of the

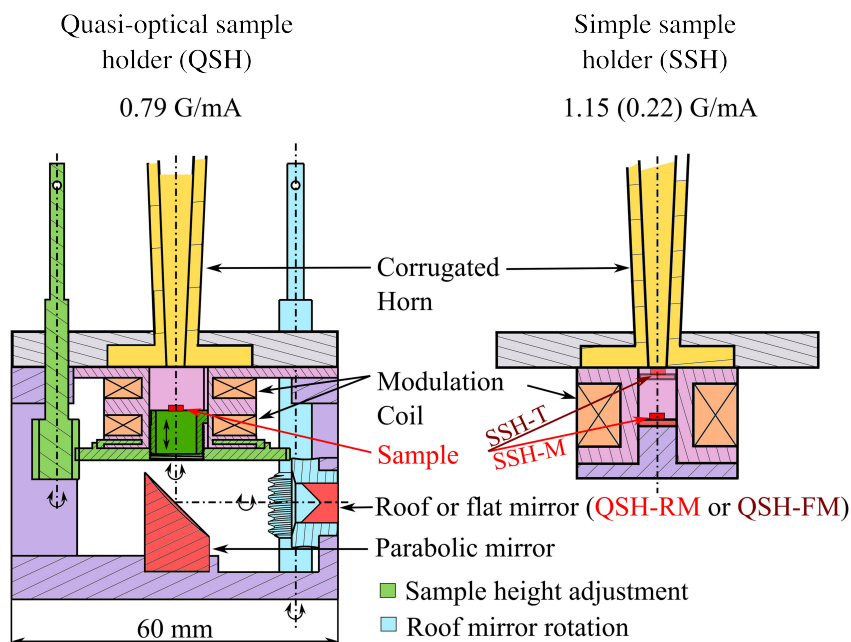


Figure 3.32: Schematic comparison of novel QSH and SSH. The SSH has been used at ITST previously with two sample positions: the middle of the coil to maximize modulation amplitude (SSH-M) and the top of the coil to minimize losses due to divergence of the 240 GHz Gaussian beam (SSH-T). The QSH uses one of two end mirrors: a rooftop mirror (RM) or a flat mirror (FM) and uses two mechanical adjustment knobs: one to optimize sample position along the optical axis (green) and one to rotate the rooftop mirror to optimize cross-polar isolation (blue). The sample is positioned using a spur gear assembly with a 5:1 gear ratio. The rooftop mirror is rotated by a worm gear assembly with a 30:1 gear ratio.

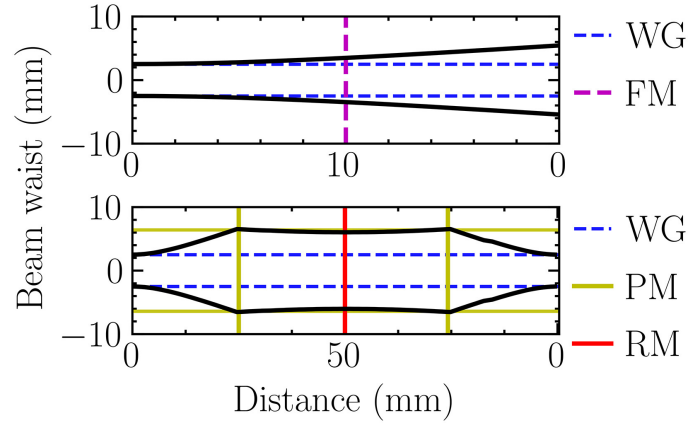


Figure 3.33: Simulation of Gaussian beam propagation through QSH and SSH. The black lines denote a circle that encloses 99% of the energy in the beam [41]. The thin dotted blue lines represent the diameter of the input waveguide. The beams are reflected by a mirror at the middle of each propagation path. (Top) In the SSH-M, after reflection from a flat mirror at $x = 10$ mm (FM; dashed purple line), the beam expands to a size larger than waveguide (WG; dashed blue line) that results in microwave losses. (Bottom) In the QSH, the beam expands after leaving the end of the waveguide (dashed blue lines) and is focused by parabolic mirror of 12.7 mm diameter (PM; solid yellow lines) onto the rooftop mirror at $x = 51.8$ mm (RM; solid red line) and then back the waveguide along the same path. Because the sample dimensions are small compared to the beam, the small shift of beam waist position at the output of the corrugated taper as well as any higher order Gaussian beam modes that are produced as a result of parabolic mirror aberrations, may be safely neglected [35, 41].

Table 3.4: Sample holder field strength coefficients as returned by linear fits to the data in Fig. 3.34.

SH	Coil coefficient
SSH-T	0.22 G/mA
SSH-M	1.15 G/mA
QSH	0.79 G/mA

ITST 240-GHz EPR spectrometer, which is typical of quasi-optical HFEPR spectrometers. A wire grid polarizer at the end of the quasi-optical bridge reflects the polarized 240-GHz radiation into a 1.2 m gold-plated corrugated waveguide that is coupled to the sample space. On resonance, the radiation that is reflected by the sample acquires a small, orthogonally polarized component (cross-polar) that is the EPR signal of interest. This signal is reflected by a wire grid at the end of the bridge, received by a WR3.4 Schottky diode-based subharmonic mixer (Virginia Diodes Inc.), and subsequently mixed down to 10 GHz (see Fig. 2.11). The 10 GHz signal must be limited to no more than -35 dBm to avoid saturating intermediate frequency (IF) stage mixers and distorting the resulting EPR signal. Ideally, all the co-polar radiation is directed back toward the source and does not reach the receiver. However, without the rooftop mirror, enough co-polar radiation reaches the receiver that both the signal and co-polar leakage must be attenuated by a variable attenuator. This attenuation degrades the SNR, especially if the noise from subsequent IF amplifiers is greater than the noise of the attenuated IF signal. Although it depends largely on the absorptive properties, size, and thickness of a given sample, most SSH experiments require the attenuator to be set between 15 dB and 25 dB.

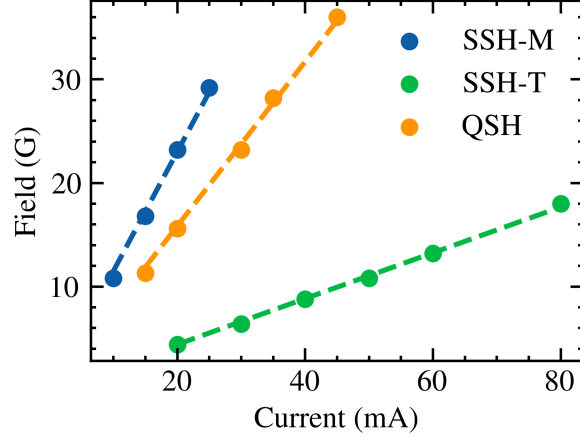


Figure 3.34: Peak-to-peak distance of LiPc spectrum as a function of drive current. SSH-M (blue) has a much larger modulation field than SSH-T (green) as a result of position within the coil. QSH (red) is most similar to SSH-M as the sample is located in the center of the Helmholtz coil. Points are fit by linear regression; slopes are used as calibration coefficients and are presented in Table 3.4.

The benefits of decreasing the co-polar intensity to the cross-polar mixer positively influences the noise figure of the detection scheme; this result is demonstrated in Figure 3.35. Figure 3.35 shows the intensity of noise recorded as a function of the receiver's variable attenuation. These measurements show that above 20 dB, the recorded noise is dominated by low-noise amplifier on the IF stage. However, with the rooftop mirror tuned to minimize co-polar intensity, the attenuation can be reduced and the noise produced by the subharmonic mixer (which scales with the signal intensity) begins to dominate. In this regime, the signal-to-noise ratio will be higher, as the mixer noise is no longer dominated by the fixed noise power added by the LNA.

The Jones matrix for a rooftop mirror is

$$\mathbf{J}(\theta) = \begin{pmatrix} \cos 2\theta & \sin 2\theta \\ -\sin 2\theta & \cos 2\theta \end{pmatrix} \quad (3.55)$$

where θ is the angle between the incident polarization and the axis of the rooftop mirror [351]. This matrix demonstrates the 2θ polarization rotation due to an angular offset

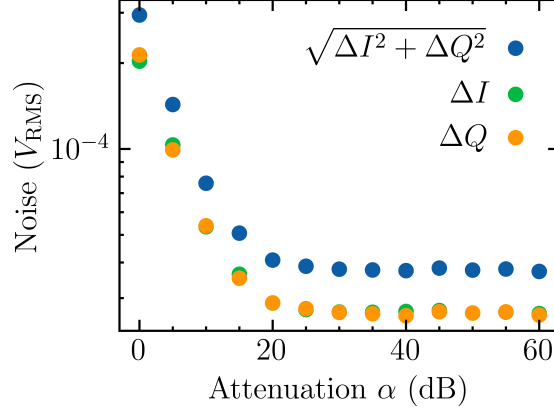


Figure 3.35: RMS noise of IF stage in ITST’s EPR spectrometer as a function of attenuator value α . Green and orange represent the noise in I and Q channels of the phase-sensitive mixer, respectively. Blue represents their magnitude, added in quadrature. Two regimes are visible: above 20 dB, the noise is dominated by the LNA and approximately constant, and below approximately 20 dB, the subharmonic mixer noise, which increases exponentially with reducing attenuation, begins to dominate. Note that signal intensity also scales exponentially with decreasing attenuation. Noise was defined as the standard deviation of detected time-domain data when the microwave source was completely blocked.

from the $\hat{y}$ -axis, α , as shown in the xy basis:

$$\mathbf{J}(\theta)\vec{E} = \begin{pmatrix} \cos 2\theta & \sin 2\theta \\ -\sin 2\theta & \cos 2\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} \quad (3.56)$$

$$= \mathbf{J}(-\alpha/2) \begin{pmatrix} \sin \alpha \\ \cos \alpha \end{pmatrix} \quad (3.57)$$

$$= \begin{pmatrix} 0 \\ 1 \end{pmatrix} \quad (3.58)$$

which has the effect of rotating all co-polar radiation into the direction that is discarded by the $\hat{y}$ -axis induction mode wiregrid. This property allows for adjustment of the output polarization to minimize background noise by ideally coupling to the induction mode isolation, and therefore minimizing α in order to maximize the induction mode SNR.

If a sample is thin compared to the wavelength, then the EPR signal can be further improved by placing it at an antinode of the microwave's magnetic field (B_1). Thus, two independent adjustments are necessary: one to rotate the rooftop mirror to minimize co-polar leakage and one to translate the sample to a maximum of B_1 .

Roof mirror rotation and sample translation are implemented with two mechanical transmission systems (see Fig. 3.36). The first system rotates the rooftop mirror about an axis perpendicular to the magnetic field; as reported by previous work, subdegree rotation of a rooftop end mirror can result in approximately 30 dB of additional cross-polar isolation [302]. To achieve subdegree precision, a 30:1 worm gear assembly was used. The worm gear pinion is connected to a lead that ends outside the magnet and can be rotated by hand or a connected stepper motor. When using the rooftop end mirror, the cross-polar isolation is greatly improved, and much less attenuation was required (even as low as $\alpha = 0$ dB) while still limiting power to the IF stage to -35 dBm.

The second transmission system positions the sample at a maximum of B_1 using a 5:1 spur gear assembly rotating about an axis parallel to the magnetic field. The sample rests on 12.7- μ m-thick Mylar film that is glued to a slot-guided, threaded shaft. Rotation of the spur gear is translated into linear motion of the M10 $\times$ 0.5 threaded shaft along the applied microwave axis. Translation along the microwave axis is not necessary in the case of the SSH, as the boundary conditions of EM waves ensures that an electric field node (and, hence, a magnetic field antinode) appears on the surface of the mirror [352].

3.7.2 Results

The QSH was tested with three different samples and EPR modes (see Table 1): a solution of 100 μ M gadolinium chloride (GdCl_3) in D_2O by CWEPR as a proxy for the

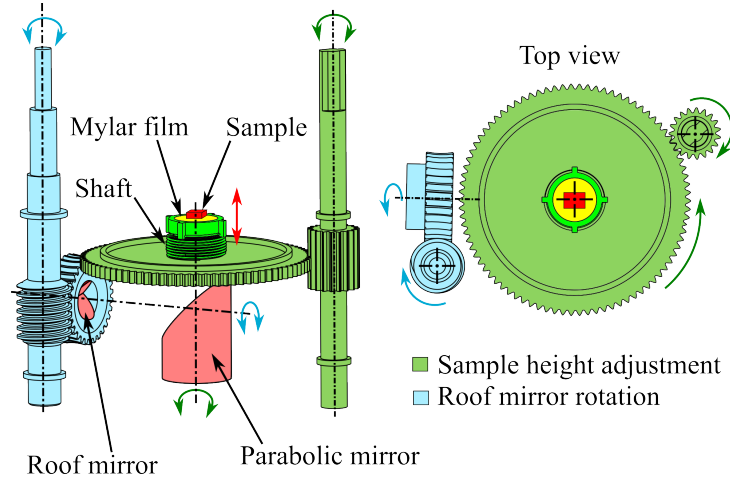


Figure 3.36: Mechanical motion of the QSH as controlled by rotating mechanical feedthroughs. The green feedthrough couples two spur gears with a 5:1 gear ratio to rotate a M10×0.5 threaded shaft that allows the sample to move up-and-down. The blue feedthrough couples a worm gear to a spur gear (30:1 ratio) to control the angle of the rooftop mirror.

Gd-sTPATCN spin labels discussed in Chapter 2, a diamond crystal with nitrogen substitutional defects (P1 centers) by pulsed EPR as a proxy for solid-state spin qubit candidates, and a single crystal of lithium phthalocyanine (LiPc) by rapid-scan EPR as a narrow-line radical that is sensitive to the local oxygen concentration [353]. The measurements were compared with those done with the SSH, in both the SSH-M and SSH-T positions. The QSH experiments were performed with a flat end mirror (QSH-FM) and rooftop end mirror (QSH-RM) for comparison. The optimization procedure for each experiment using the QSH-RM is described in detail in Appendix E.

CWEPR was performed on 100 μM GdCl_3 dissolved in D_2O . The GdCl_3 solution was inserted into a 100- μm -thick, 2 mm-by-5 mm borosilicate glass capillary (VetroCom, Mountain Lakes, NJ, USA) to maximize the ratio of surface area to thickness and sealed with wax [59]. The modulated signal was amplified using a lock-in detector (SR830, Stanford Research Systems) and is shown in Fig. 5. The SNR was best in the case

of QSH-RM (SNR = 292) because of the combined abilities to minimize the co-polar baseline while also maximizing the spin response by optimizing the sample position. Next best was SSH-T (SNR = 53), due to the near-zero microwave losses as a result of the mirror being placed directly at the end of the waveguide. SSH-M had the lowest SNR (SNR = 14), which is due to microwave losses as a result of divergence of the Gaussian beam. QSH-FM (SNR = 37) had an SNR greater than that of SSH-M because it was able to reduce divergence loss with focusing quasi-optics but, as expected, still had more divergence loss and scattering than SSH-T, which had no free-space propagation.

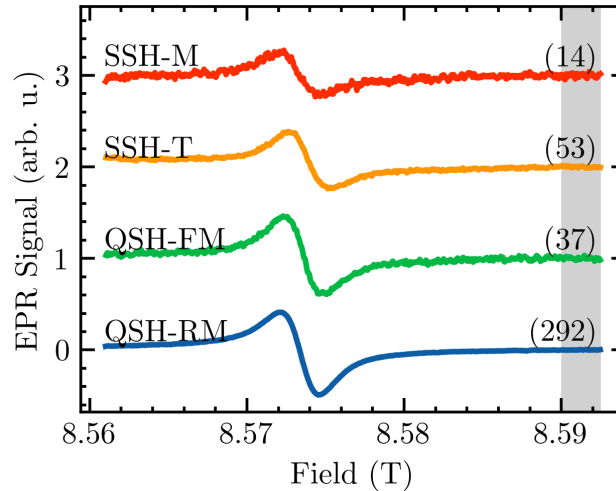


Figure 3.37: Improvement of CWEPR SNR using QSH. EPR signal of $100\ \mu\text{M}$ GdCl_3 as a function of field, as detected by a lock-in amplifier (100 ms lock-in time constant). The applied magnetic field was modulated at the resonant frequency of the modulation coil and an accompanying 10 nF capacitor ($B_{\text{mod}} = 14.8\ \text{G}$ at 33 kHz in the case of the SSH and $B_{\text{mod}} = 7.9\ \text{G}$ at 23 kHz in the case of the QSH). The SNR was best in the case of the QSH-RM (blue) because of the ability to reduce co-polar noise, as well as to position the thin GdCl_3 -filled capillary at a maximum of the irradiated B_1 field. The baseline was removed by subtracting the mean of points within the vertical gray column. SNR (in parentheses) was calculated by dividing peak baseline-corrected signal by the SD of baseline-corrected points within the vertical gray column.

Pulsed EPR was performed at 240 GHz on P1 nitrogen centers in a diamond crystal using a 30 mW CW source Virginia Diodes, Inc. that was pulsed using a fast PIN

(p-type, intrinsic, n-type) diode switch (SWM-0JV-1DT-2ATT, American Microwave Corporation, USA). A Hahn echo pulse sequence, consisting of a 600 ns excitation pulse, 1.2 μ s delay time, and 800 ns refocusing pulse, was used to create a spin echo. The pulse sequence was repeated at 1 kHz and averaged over 512 repetitions per field position. Integrated echo intensity was recorded as a function of field and plotted in Fig. 3.38. The results from each sample holder were quite similar. The SSH-M performed the worst (SNR = 178) because its long optical path length and beam divergence cause clipping at the waveguide aperture (see Fig. 3.33). The SSH-T did not suffer from this clipping and, therefore, performed second best (SNR = 472). It was expected that the QSH-FM (SNR = 219) and QSH-RM (SNR = 524) would perform similarly, as the co-polar background cannot obscure the cross-polar signal after the pulse sequence has occurred. However, optimizing the roof mirror angle for the maximum Hahn echo did improve SNR and in total, the QSH-RM performed the best overall.

Rapid-scan EPR was performed on a needle of LiPc at 240 GHz in three sample holders (SSH-M, QSH-FM, and QSH-RM). Rapid-scan SSH-T experiments were not performed because the modulation field strength (approximately 16 G tip-to-trough; see Fig. 3.34) was insufficient to entirely sweep the LiPc resonance. The rooftop mirror increased the cross-polar isolation by ~ 20 dB. As shown in Table 3.5 and Fig. 3.39, the SNR of the QSH represented an improvement over the SSH. In the QSH-FM, the SNR improved by approximately $3\times$ over the SSH-M ($278 \rightarrow 788$). After replacing the end mirror with a rooftop mirror (QSH-RM), the SNR was improved by an additional $2\times$ ($788 \rightarrow 1620$), for a total improvement of $6\times$ ($278 \rightarrow 1620$).

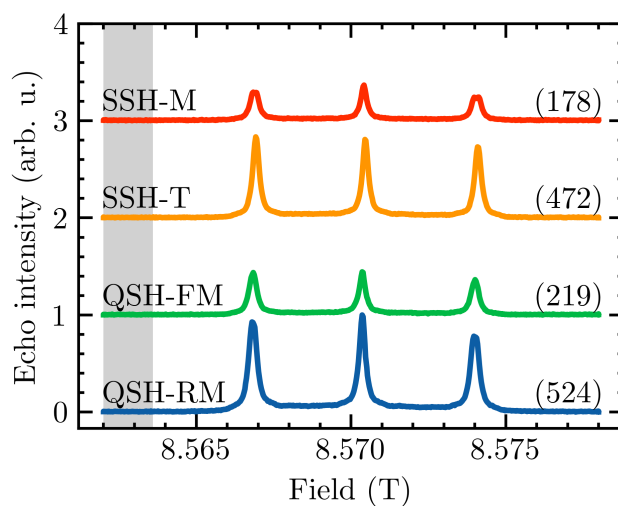


Figure 3.38: Improvement of pulsed EPR SNR using QSH. Integrated field-swept echo magnitudes of P1 centers in diamond, plotted as a function of field for each sample holder configuration (1 kHz repetition rate with 512 averages at each field position). SSH-M (red) performed the worst (SNR = 178). SSH-T (orange) performed as the second best; the optical path length was minimal and, therefore, so were losses due to propagation (SNR = 472). QSH-FM (green) performed second worst (SNR = 219). QSH-RM (blue) performed the best as a result of focusing optics and the ability to maximize desirable cross-polar signal (SNR = 524). SNR (in parentheses) was calculated by dividing peak signal by the SD of points within the vertical gray column.

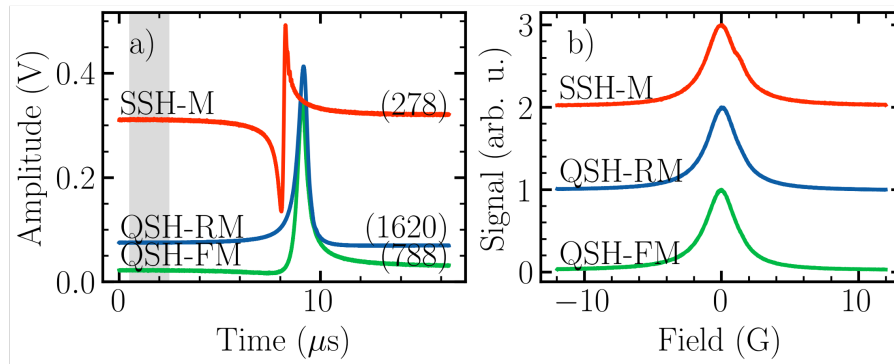


Figure 3.39: Improvement of RSEPR SNR using QSH. (a) Time-resolved rapid-scan EPR spectra of LiPc with 10,000 averages. SSH-M is shown in red ($B_{mod} = 144$ G at 33 kHz, variable IF attenuator setting $\alpha_{var} = 25.5$ dB, SNR = 278); QSH-FM is shown in green, and sample position was adjusted using the optical axis positioning thread to maximize signal amplitude ($B_{mod} = 75$ G at 23 kHz, $\alpha_{var} = 20.5$ dB, SNR = 788); QSH-RM is shown in blue, sample position was adjusted using the optical axis positioning thread to maximize signal amplitude, and the rooftop mirror was adjusted to minimize co-polar baseline ($B_{mod} = 75$ G at 23 kHz, $\alpha_{var} = 5.75$ dB, SNR = 1620). SNR (in parentheses) was calculated by dividing peak amplitude by the root-mean-square deviation of points within the vertical gray bar. (b) Deconvolved LiPc spectra after application of the driving-function Fourier deconvolution algorithm found in Tseytlin [205].

Table 3.5: SNR and insertion loss of ITST’s sample holders. For CW, GdCl_3 in D_2O was used as a sample; for pulsed EPR, P1 (nitrogen) centers in diamond; for RS, a LiPc crystal grain. RS could not be done with SSH-T because the modulation field strength at that sample position is not large enough to sweep the entire (very narrow, roughly 1 G) LiPc lineshape.

Sample holder	CW (GdCl_3)	Pulsed (P1 dia.)	RS (LiPc)	Insertion loss
SSH-T	53	472	–	2.67 ± 0.4 dB
SSH-M	14	178	278	4.18 ± 0.2 dB
QSH-FM	37	219	788	1.84 ± 0.6 dB
QSH-RM	292	524	1620	1.53 ± 0.4 dB

3.7.3 Discussion and conclusion

This section demonstrated a rapidly prototyped, cost-efficient 3D printed sample holder that is capable of greatly improving the SNR of CW and RSEPR by reducing co-polar leakage and optimizing B_1 field strength at the sample position. This sample holder represents an easy-to-implement solution for improving the SNR of a variety of homebuilt EPR spectrometer designs through custom-fit modifications of the .stp files included in the Supplementary Materials of [194]. 3D-printed components enable simple incorporation into a variety of induction mode HFEPR spectrometers and provide a method to improve SNR while still allowing further customization for possible additional functionality. The three main benefits of the design can be summarized as follows.

1. *Better SNR and sensitivity improvement*

The QSH showed SNR improvements for all three EPR modes: CW, RS, and pulsed (see Table 3.5). In the case of CW and RSEPR, reducing the baseline is critical for improving the SNR. In this case, the rooftop mirror provides a substantial

benefit. It can improve co-polar isolation by $\gtrsim 20$ dB and SNR by around $6\times$, when compared to the best possible configuration using the SSH. In addition, the QSH benefited CW and RSEPR by allowing the sample to be placed in the middle of the modulation coil where the best possible performance could be achieved. Note that the results presented here were established using an imperfect rooftop mirror end-milled from an aluminum rod that may perform even better with additional polishing or electron-beam deposition of a low-loss metal.

2. *Reduction of co-polar intensity during pulsed EPR measurements*

For pulsed EPR, the signal is usually detected after the excitation is turned off, which makes co-polar leakage a smaller concern than in the other two modes. Therefore, in this mode, the sample is typically placed as close as possible to the waveguide to limit losses due to Gaussian beam propagation. Such losses cannot be avoided in the QSH-FM/RM, but they can be partially mitigated by the parabolic focusing mirrors. Nevertheless, even for the Hahn echo experiment presented here, the QSH-RM was able to improve SNR by $\sim 11\%$. To achieve this improvement, the polarization of the echo was slightly rotated to better couple with the induction mode quasi-optics, and the sample was positioned at a magnetic field (B_1) antinode.

For high-power pulsed HFEPR measurements, in which the microwave pulse is generated by a gyrotron or free-electron laser, improved cross-polar isolation should greatly reduce the time between the end of the pulse sequence and the time at which detection of an echo or free-induction decay begins (often called the “dead time”). As an illustration, Fig. 3.40 shows experiments in which a pulsed EPR signal from P1 centers in diamond is recorded *during* a 240 GHz microwave pulse—a so-called

“zero-dead time” experiment [302]. With the SSH, which has no roof mirror, the co-polar signal dominates, resulting in a large baseline, and the features associated with P1 centers show up as a decrease in the signal. When using the QSH with the roof mirror properly adjusted, the baseline associated with co-polar leakage is reduced enough that the cross-polar EPR signal dominates and sample absorption is directly observed as an *increase* in detected pulse power, returning the expected EPR spectrum.

3. *Enables real-time rapid-scan EPR of transient phenomena*

The QSH improved co-polar isolation by a factor of $\gtrsim 20$ dB and SNR by approximately $6\times$, corresponding to a $36\times$ reduction in signal acquisition time for comparable SNR. For rapid-scan experiments, a reduction in requisite acquisition time increases the time resolution, enabling capture of more rapid transient phenomena. This sample holder enabled the experiments throughout sec. 2.3 as a result of its marked improvement in signal-to-noise.

3.7.4 Future work

Future work for the QSH-RM includes incorporating a fast-loading sample design and identifying and optimizing a 3D printing substrate that is capable of handling liquid nitrogen temperatures for cryogenic experiments [350, 354]. Furthermore, the QSH may provide an avenue to greatly reduce dead time of pulsed EPR using high-power microwave sources such as ITST’s free electron laser [50]. Although experiments performed here were done without a cavity, the QSH-RM sample holder should be compatible with a Fabry-Pérot type cavity, which would further enhance SNR and time resolution [355]: work is ongoing on these fronts.

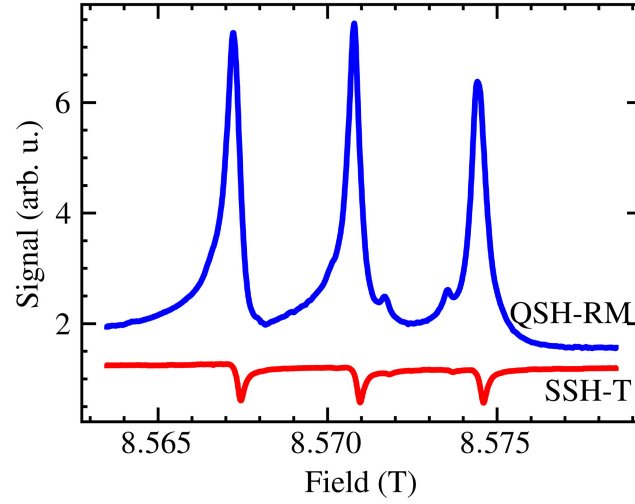


Figure 3.40: Dead-time free measurement of P1 centers in diamond using QSH shown via integrated intensity of a long ($1\ \mu\text{s}$) microwave pulse during a resonant field sweep. In the case of the SSH-T experiment (red), the resonant signal is a result of a decrease of the detected co-polar power due to substantial leakage. After optimization, the QSH-RM experiment signal (blue) is primarily cross-polar and thus results in a signal *increase* while on resonance.

There are two techniques currently being developed to further improve the SNR that the QSH can provide: (1) implementation of a piezo-electric rotator in place of the manual rotation knob to optimize rooftop mirror angle and (2) implementation of stepper- or piezo-controlled adjustment of the rooftop mirror position that can modify the length of the beam path thereby change the positions of B_1 antinodes, equivalent to moving the sample. To this end, preliminary design work has been done and is previewed in Fig. 3.41.

It will be essential to incorporate the SNR-improving, dead-time-reducing techniques presented here to the novel FEL-ESR system. These improvements will provide access to smaller signals and allow them to be more easily distinguished from background noise.

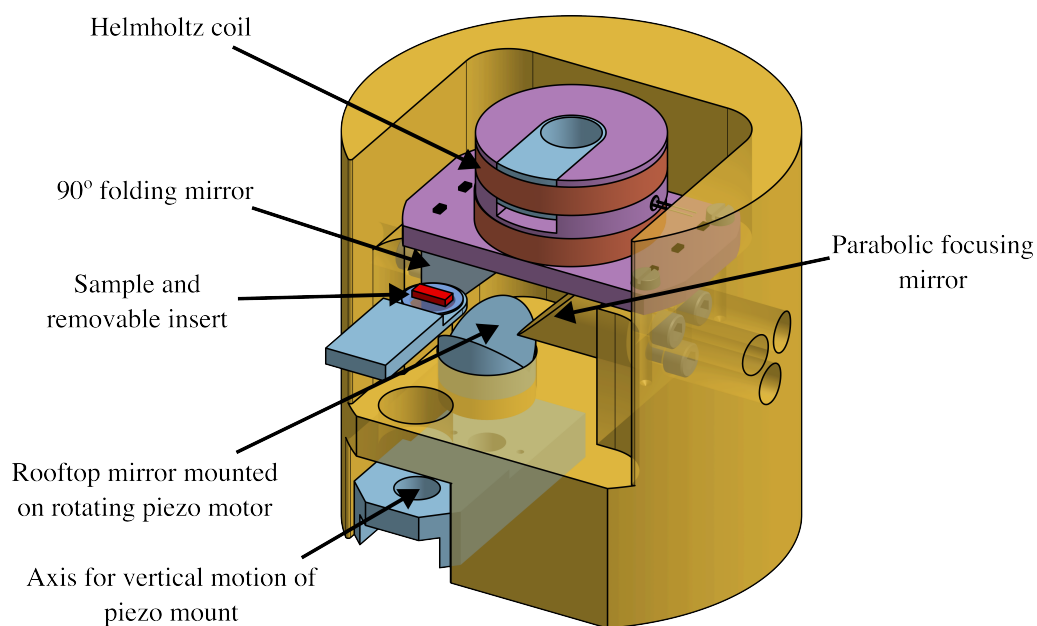


Figure 3.41: Conceptual design of modified QSH. This design incorporates rotating and linear piezos to adjust the angle and position of the rooftop mirror, which enables all the benefits of the first design without the need for mechanical feedthroughs or manual control. Further, it is intended to be simple to machine such that it can be made from a plastic material that has favorable cryogenic properties (PEEK, G10, etc.).

Chapter 4

Conclusion

This dissertation spans a broad range of magnetic resonance-based concepts, with a particular focus on high field-and-frequency EPR spectroscopy, and the experimental benefits and challenges therein. A particular focus is the development of high field EPR for applications in biology, biophysics, and condensed matter physics of driven, non-equilibrium dynamics. Namely, the discussion includes the use of high-field EPR for transient measurements of protein dynamics in a novel technique called “TiGGER”. This technique is expanded and improved by the first demonstration of field-domain rapid-scan EPR at high field, which provides more information than comparable transient measurements, including the entire spectral lineshape of interest, with which additional dynamical information can be elucidated. The discussion then turns to free electron laser studies of CoNb_2O_6 , an experimental realization of the transverse field Ising chain. Such studies were not able to resolve the low-lying excitations of the systems, likely due to the limited spectrometer sensitivity and 70 ns dead-time. These limitations motivated significant redesign and experimental development to increase the resolution and reduce the dead-time in order to enable studies of short-lived dynamical spin systems.

TiGGER, time-resolved Gd-Gd EPR, was shown to be capable of “filming” the folding dynamics of a protein in Chapter 2. Using site-directed spin labeling, high-field EPR is sensitive to the distances between two gadolinium spin labels. A well-studied protein, AsLOV2, has ordered domains that become disordered upon light activation. The distance sensitivity of high-field EPR was able to clearly resolve this structural change: two protein residues moved further apart when the protein was light-activated, which changed (narrowed) the spin labels’ EPR lineshape. Using TiGGER, the role of a glutamine residue at site 513 was partially elucidated—while this polar residue is *important* for signal transduction after light activation, it is not *critical*. Mutating this glutamine to alanine significantly slowed the protein re-folding, but did not inhibit it. Additional studies probing how the signal transduction remains possible are ongoing—there are some hypotheses that suggest that polar water molecules within the proteins hydrodynamic radius can take the place of the polar Q513 and transduce the signal in its place. An obvious drawback of TiGGER, in the form discussed here, is that it is only capable of telling that two protein residues moved, not by how much.

The natural next step for TiGGER is to combine the impressive spectral resolution of EPR with the time resolution of transient measurements. A technique capable of this already exists, and is called rapid-scan EPR. Rapid-scan relies on modulating the applied magnetic field at kHz rates, digitizing the entire lineshape during the modulation, and averaging the lineshapes in real-time to acquire time-dependent, signal averaged EPR spectra. With rapid-scan, TiGGER would be possible with both field and time-resolution. Further, since it is well-known how coupling two spins modifies their lineshape as a function of their relative through-space distance, in theory, the distance distribution of the pairs of spins in an ensemble may be back-calculated as a function of time. Currently, there is no technique capable of such a measurement in real-time and the solution state

with millisecond-to-second time resolution. To enable such a technique, rapid-scan EPR had to be demonstrated in the field domain at high field (> 0.3 T) for the first time. The spectrometer was upgraded to enable rapid-scan, which included implementation of a novel sample holder with greatly improved signal-to-noise, a field programmable gate array (FPGA)-based digitizer that could be custom-programmed in hardware, as well as modification of the EPR IF stage. Inclusion of the FPGA meant that triggering, data acquisition, and custom signal processing could all happen onboard, in real time. The result of these modifications enabled rapid-scan TiGGER, and in turn, resolved the time-dependent lineshape broadening and narrowing that results from the protein's dynamics. Further, preliminary work toward back-calculation of time-resolved distances shows promise toward a reliable, real time “movie” that can resolve the site-site distances within a solution state protein ensemble, which is not possible with any other technique.

ITST hosts one of the few THz FELs in the world, and the only EPR spectrometer based off of one. As a result, it is capable of unique experiments, including excitation lifetime measurements (T_1 and T_2) of systems with very short-lived excitations (currently, as short as ~ 100 ns). The second half of this dissertation focuses on FEL-powered electron magnetic resonance and begins with discussion on CoNb_2O_6 , an experimental realization of the transverse field Ising chain. FEL-powered electron magnetic resonance is well-equipped to study the low-lying excitations of this system, which are spin-1/2 quasiparticles called “spinons”. However, to date, no unambiguous experimental observation of spinons has been shown (*e.g.*, a spinon echo), due in part to limiting FEL-EPR spectrometer properties: the experiments presented here were able to place an upper bound on the spin-spin coherence time in CoNb_2O_6 at 70 ns, but could not access any shorter times due to the spectrometers 70 ns dead-time. There is reason to believe that a careful application of a modernized version of FEL-EPR would be able to resolve a

spinon echo, which motivated the design of a novel FEL-ESR spectrometer that should be capable of such measurements. If FEL-ESR is, in fact, able to characterize spinons in 1D, similar measurements may be possible on other systems that are known to host exotic magnetism, such as quantum spin liquid candidates.

The second part of Chapter 3 presents the hardware developments necessary for FEL-ESR that could enable such measurements. The spectrometer development was done in parts, including design and realization of a novel pulse-slicing module that allows for pulse sequences of more than two pulses (with flexible pulse lengths of 1-1000 ns), a novel phase-shifting module with continuous control of pulse phases (with millidegree precision), and a sample holder that reduces spectrometer dead-time by a large amount and may one day enable sub-ns dead-times (and improved signal-to-noise by more than $6\times$ in continuous wave and rapid-scan modes). The finished spectrometer will also include multi-source (FEL and low power) source operation, a dry magnet, and rapid sample exchange, which will greatly improve usability and experimental throughput.

In total, it is obvious that high-field (> 100 GHz) EPR is a work in progress. Improvements are incremental and development is slow, as only a few groups are actively addressing the experimental challenges. However, as demonstrated by the overwhelming success of nuclear magnetic resonance, the potential benefits are great. Through collaborations between scientific disciples and the desire to push science forward, high field EP(S)R has a promising future.

Appendix A

Additional quantum mechanical formalism

A.1 Zero-field splitting parameters

Choosing a coordinate system to be along the principal axis of $\mathbf{D}$, $\hat{\mathcal{H}}_{ZFS}$ may be expanded to:

$$\hat{\mathcal{H}}_{ZFS} = \hat{\mathbf{S}} \cdot \mathbf{D} \cdot \hat{\mathbf{S}} \quad (\text{A.1})$$

$$= D_x \hat{S}_x^2 + D_y \hat{S}_y^2 + D_z \hat{S}_z^2 \quad (\text{A.2})$$

$$= \frac{D_x}{2} \left(\hat{S}_x^2 + \hat{S}_y^2 + \hat{S}_z^2 + \hat{S}_x^2 - \hat{S}_y^2 - \hat{S}_z^2 \right) \quad (\text{A.3})$$

$$+ \frac{D_y}{2} \left(\hat{S}_x^2 + \hat{S}_y^2 + \hat{S}_z^2 - \hat{S}_x^2 + \hat{S}_y^2 - \hat{S}_z^2 \right) \quad (\text{A.4})$$

$$+ D_z \left(\hat{S}_z^2 \right) \quad (\text{A.5})$$

Then, since $\hat{S}_x^2 + \hat{S}_y^2 + \hat{S}_z^2 = S(S+1)\mathbf{1}$,

$$\hat{\mathcal{H}}_{ZFS} = D_x \left(\frac{S(S+1)}{2} \mathbf{1} + \frac{\hat{S}_x^2 - \hat{S}_y^2}{2} - \frac{\hat{S}_z^2}{2} \right) \quad (\text{A.6})$$

$$+ D_y \left(\frac{S(S+1)}{2} \mathbf{1} - \frac{\hat{S}_x^2 - \hat{S}_y^2}{2} - \frac{\hat{S}_z^2}{2} \right) \quad (\text{A.7})$$

$$+ D_z \left(\hat{S}_z^2 - \frac{S(S+1)}{3} \mathbf{1} + \frac{S(S+1)}{3} \mathbf{1} \right) \quad (\text{A.8})$$

$$= \frac{D_x + D_y + D_z}{3} S(S+1) \mathbf{1} \quad (\text{A.9})$$

$$+ \frac{2D_z - D_y - D_x}{2} \left(\hat{S}_z^2 - \frac{1}{3} S(S+1) \mathbf{1} \right) \quad (\text{A.10})$$

$$+ \frac{D_x - D_y}{2} \left(\hat{S}_x^2 - \hat{S}_y^2 \right) \quad (\text{A.11})$$

$$= C + D \left\{ \hat{S}_z^2 - \frac{1}{3} S(S+1) \mathbf{1} \right\} + E \left(\hat{S}_x^2 - \hat{S}_y^2 \right), \quad (\text{A.12})$$

where D and E are the commonly referred to ZFS parameters. C can be dropped for EPR arguments, as it modifies the energy of all spin states and thus has no relevance when looking at level transitions.

A.2 Spin operator formalism

This section will briefly review the spin operator formalism. First, it must be taken for granted that fermions (*e.g.*, electrons) are spin-1/2 particles (*i.e.* their total spin must be $n/2$, where n is an integer) and that spin is quantized. The spin-1/2 state basis may be spanned by eigenvectors (called “spinors”) that represent the occupation of a spin sublevel, m_s [131].

In the simplest case, $S = 1/2$, the two eigenstates are:

$$|\Psi_1\rangle = |\uparrow\rangle = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad (\text{A.13})$$

$$|\Psi_2\rangle = |\downarrow\rangle = \begin{pmatrix} 0 \\ 1 \end{pmatrix}, \quad (\text{A.14})$$

where Ψ_1 represents “spin up” ($m_s = 1/2$) and Ψ_2 represents “spin down” ($m_s = -1/2$).

Now, if a measurement is made along $\hat{z}$, the $\hat{S}_z$ operator must return the expected spin up and down eigenvalues (in units of angular momentum).

$$\hat{S}_z |S, m_s\rangle = \hbar m_s |s, m_s\rangle \quad (\text{A.15})$$

$$\hat{S}_z |\uparrow\rangle = \frac{\hbar}{2} |\uparrow\rangle \quad (\text{A.16})$$

$$\hat{S}_z |\downarrow\rangle = -\frac{\hbar}{2} |\downarrow\rangle \quad (\text{A.17})$$

Here, s is the total angular momentum and m_s is the angular momentum of an individual sublevel. By inspection, $\hat{S}_z$ must therefore be:

$$\hat{S}_z = \frac{\hbar}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} = \frac{\hbar}{2} \sigma_z. \quad (\text{A.18})$$

Where σ_z is the Pauli matrix in the z direction. Without working through their derivation, the spin operators in the other orthogonal directions are also Pauli matrices,

$$\hat{S}_x = \frac{\hbar}{2} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} = \frac{\hbar}{2} \sigma_x, \quad (\text{A.19})$$

$$\hat{S}_y = \frac{\hbar}{2} \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix} = \frac{\hbar}{2} \sigma_y. \quad (\text{A.20})$$

Now, in the same way as orbital angular momentum, $\hat{\mathbf{L}}$, there exist ladder operators that raise and lower the spin sublevel of a given state.

$$\hat{S}_{\pm} = \hat{S}_x \pm i\hat{S}_y \quad (\text{A.21})$$

Further, spin angular momentum must obey the general angular momentum operations.

The commutation relation for general angular momentum operators are as follows:

$$[J_i, J_j] = i\hbar\epsilon_{ijk}J_k \quad (\text{A.22})$$

where ϵ_{ijk} is the Levi-Cevita symbol that is only nonzero if i, j, k are all different. And, without working through the derivation, the matrix elements of these operators are:

$$J_{\pm} |j, m\rangle = \hbar \sqrt{(j \mp m)(j \pm m + 1)} |j, m \pm 1\rangle. \quad (\text{A.23})$$

Here, it can be seen that these operators return zero if the raising operator is applied to the highest accessible sublevel ($j = m$) or the lowering operator is applied to the lowest accessible sublevel ($j = -m$); this is intuitive, there cannot—by definition—be a state outside the range of accessible states.

Spin operators of higher S exist, and can be derived by building up $\hat{S}_x$ and $\hat{S}_y$ from the known behavior of $\hat{S}_z$ and $\hat{S}_{\pm}$, or they can simply be looked up in a table such as [356]. For example, $\hat{S}_x$ for $S = 5/2$ is:

$$\hat{S}_x = \frac{\hbar}{2} \begin{pmatrix} 0 & \sqrt{5} & 0 & 0 & 0 & 0 \\ \sqrt{5} & 0 & \sqrt{8} & 0 & 0 & 0 \\ 0 & \sqrt{8} & 0 & \sqrt{9} & 0 & 0 \\ 0 & 0 & \sqrt{9} & 0 & \sqrt{8} & 0 \\ 0 & 0 & 0 & \sqrt{8} & 0 & \sqrt{5} \\ 0 & 0 & 0 & 0 & \sqrt{5} & 0 \end{pmatrix}. \quad (\text{A.24})$$

A.2.1 Rotation of spin operators

The rotated version of $\hat{S}_+$, $\hat{S}_+^{\phi_z}$, is given by $\mathcal{R}_z(\phi) \hat{S}_+ \mathcal{R}_z^{-1}(\phi) = \mathcal{R}_z(\phi) \hat{S}_+ \mathcal{R}_z^{\dagger}(\phi)$.

$$\hat{S}_+^{\phi_z} = \exp\left(\frac{-i\phi \hat{S}_z}{\hbar}\right) \hat{S}_+ \exp\left(\frac{i\phi \hat{S}_z}{\hbar}\right) \quad (\text{A.25})$$

Expand the exponentials and substitute in $\hat{S}_+ = \hat{S}_x + i\hat{S}_y$ and $\hat{S}_i = \frac{\hbar}{2}\sigma_i$ to get:

$$\hat{S}_+^{\phi_z} = \left[\sum_n \frac{1}{n!} \left(\frac{-i\phi \sigma_z}{2} \right)^n \right] \frac{\hbar}{2} (\sigma_x + i\sigma_y) \left[\sum_n \frac{1}{n!} \left(\frac{i\phi \sigma_z}{2} \right)^n \right] \quad (\text{A.26})$$

$$= \frac{\hbar}{2} \left[\sum_n^{\infty} \frac{1}{(2n)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n} + \frac{1}{(2n+1)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n+1} \right] (\sigma_x + i\sigma_y) R \quad (\text{A.27})$$

where

$$R = \sum_n^{\infty} \frac{1}{n!} \left(\frac{i\phi\sigma_z}{2} \right)^n. \quad (\text{A.28})$$

Proceed by expanding term-by-term:

$$\sum_n^{\infty} \frac{1}{(2n)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n} \sigma_x = \sum_n^{\infty} \frac{(-1)^n}{(2n)!} \left(\frac{\phi}{2} \right)^{2n} \sigma_z^{2n} \sigma_x \quad (\text{A.29})$$

$$= \sum_n^{\infty} \frac{(-1)^n}{(2n)!} \left(\frac{\phi}{2} \right)^{2n} \mathbf{I}_2 \sigma_x \quad (\text{A.30})$$

$$= \cos \frac{\phi}{2} \sigma_x. \quad (\text{A.31})$$

Similarly,

$$\sum_n^{\infty} \frac{1}{(2n)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n} i\sigma_y = \sum_n^{\infty} \frac{(-1)^n}{(2n)!} \left(\frac{\phi}{2} \right)^{2n} \sigma_z^{2n} \sigma_y \quad (\text{A.32})$$

$$= i \cos \frac{\phi}{2} \sigma_y. \quad (\text{A.33})$$

For the second term in the left sum,

$$\sum_n^{\infty} \frac{1}{(2n+1)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n+1} \sigma_x = \sum_n^{\infty} \frac{-i(-1)^n}{(2n+1)!} \left(\frac{\phi}{2} \right)^{2n+1} \sigma_z^{2n+1} \sigma_x \quad (\text{A.34})$$

$$= -i \sum_n^{\infty} \frac{(-1)^n}{(2n+1)!} \left(\frac{\phi}{2} \right)^{2n+1} \sigma_z \sigma_x \quad (\text{A.35})$$

$$= -i \sin \frac{\phi}{2} (i\sigma_y) \quad (\text{A.36})$$

$$= \sin \frac{\phi}{2} \sigma_y \quad (\text{A.37})$$

$$\sum_n^{\infty} \frac{1}{(2n+1)!} \left(\frac{-i\phi\sigma_z}{2} \right)^{2n+1} i\sigma_y = \sum_n^{\infty} \frac{-i(-1)^n}{(2n+1)!} \left(\frac{\phi}{2} \right)^{2n+1} \sigma_z^{2n+1} i\sigma_y \quad (\text{A.38})$$

$$= \sin \frac{\phi}{2} \sigma_z \sigma_y \quad (\text{A.39})$$

$$= -i \sin \frac{\phi}{2} \sigma_x. \quad (\text{A.40})$$

Then, treat the right side sum:

$$R = \sum_n \frac{1}{n!} \left(\frac{i\phi\sigma_z}{2} \right)^n \quad (\text{A.41})$$

$$= \sum_n \frac{1}{(2n)!} \left(\frac{i\phi\sigma_z}{2} \right)^{2n} + \frac{1}{(2n+1)!} \left(\frac{i\phi\sigma_z}{2} \right)^{2n+1} \quad (\text{A.42})$$

$$= \sum_n \frac{(-1)^n}{(2n)!} \left(\frac{\phi\sigma_z}{2} \right)^{2n} + \frac{i(-1)^n}{(2n+1)!} \left(\frac{\phi\sigma_z}{2} \right)^{2n+1} \quad (\text{A.43})$$

$$= \sum_n \frac{(-1)^n}{(2n)!} \left(\frac{\phi}{2} \right)^{2n} \mathbf{I}_2 + \frac{i(-1)^n}{(2n+1)!} \left(\frac{\phi}{2} \right)^{2n+1} \sigma_z \quad (\text{A.44})$$

$$= \cos \frac{\phi}{2} \mathbf{I}_2 + i \sin \frac{\phi}{2} \sigma_z. \quad (\text{A.45})$$

Combine all the terms to get:

$$\hat{S}_+^{\phi_z} = \frac{\hbar}{2} \left(\cos \frac{\phi}{2} \sigma_x + i \cos \frac{\phi}{2} \sigma_y + \sin \frac{\phi}{2} \sigma_y - i \sin \frac{\phi}{2} \sigma_x \right) \left(\cos \frac{\phi}{2} \mathbf{I}_2 + i \sin \frac{\phi}{2} \sigma_z \right) \quad (\text{A.46})$$

$$= \frac{\hbar}{2} \left[\cos^2 \frac{\phi}{2} \sigma_x + i \cos \frac{\phi}{2} \sin \frac{\phi}{2} \sigma_x \sigma_z + i \cos^2 \frac{\phi}{2} \sigma_y - \cos \frac{\phi}{2} \sin \frac{\phi}{2} \sigma_y \sigma_z \right. \\ \left. + \sin \frac{\phi}{2} \cos \frac{\phi}{2} \sigma_y + i \sin^2 \frac{\phi}{2} \sigma_y \sigma_z - i \sin \frac{\phi}{2} \cos \frac{\phi}{2} \sigma_x + \sin^2 \frac{\phi}{2} \sigma_x \sigma_z \right] \quad (\text{A.47})$$

$$= \frac{\hbar}{2} \left[\cos^2 \frac{\phi}{2} \sigma_x + i \cos \frac{\phi}{2} \sin \frac{\phi}{2} (-i\sigma_y) + i \cos^2 \frac{\phi}{2} \sigma_y - \cos \frac{\phi}{2} \sin \frac{\phi}{2} (i\sigma_x) \right. \\ \left. + \sin \frac{\phi}{2} \cos \frac{\phi}{2} \sigma_y + i \sin^2 \frac{\phi}{2} (i\sigma_x) - i \sin \frac{\phi}{2} \cos \frac{\phi}{2} \sigma_x + \sin^2 \frac{\phi}{2} (-i\sigma_y) \right] \quad (\text{A.48})$$

$$= \frac{\hbar}{2} \left[\left(\cos^2 \frac{\phi}{2} - \sin^2 \frac{\phi}{2} \right) (\sigma_x + i\sigma_y) + \cos \frac{\phi}{2} \sin \frac{\phi}{2} (2\sigma_y - 2i\sigma_x) \right] \quad (\text{A.49})$$

$$= \frac{\hbar}{2} [\cos \phi (\sigma_x + i\sigma_y) - i \sin \phi (\sigma_x + i\sigma_y)] \quad (\text{A.50})$$

$$= \exp(-i\phi) (\hat{S}_x + i\hat{S}_y) \quad (\text{A.51})$$

$$\hat{S}_+^{\phi_z} = \exp(-i\phi) \hat{S}_+. \quad (\text{A.52})$$

Which implies that the coherence order, p , of $\hat{S}_+$ is $p = 1$. A similar treatment returns $p = -1$ for $\hat{S}_-$. Importantly, $\hat{S}_z$ has a coherence order $p = 0$, as $\hat{S}_z$ commutes with $\mathcal{R}_z(\phi)$, such that $\hat{S}_z^\phi = \hat{S}_z$.

For a π pulse is applied to a nonzero coherence, the sign of the coherence is flipped. As an example, take $\hat{S}_+$ and a π pulse along $\hat{x}$:

$$\hat{S}_+^{\phi_x} = \exp\left(\frac{-i\phi\hat{S}_x}{\hbar}\right) \hat{S}_+ \exp\left(\frac{i\phi\hat{S}_x}{\hbar}\right) \quad (\text{A.53})$$

$$= \exp\left(\frac{-i\phi\hat{S}_x}{\hbar}\right) \hat{S}_x \exp\left(\frac{i\phi\hat{S}_x}{\hbar}\right) + \exp\left(\frac{-i\phi\hat{S}_x}{\hbar}\right) i\hat{S}_y \exp\left(\frac{i\phi\hat{S}_x}{\hbar}\right) \quad (\text{A.54})$$

$$= \hat{S}_x + \frac{i\hbar}{2} \exp\left(\frac{-i\phi\sigma_x}{2}\right) \sigma_y \exp\left(\frac{i\phi\sigma_x}{2}\right) \quad (\text{A.55})$$

$$= \hat{S}_x + \frac{i\hbar}{2} \left[\sum_n \frac{1}{(2n)!} \left(\frac{-i\phi\sigma_x}{2}\right)^{2n} + \frac{1}{(2n+1)!} \left(\frac{-i\phi\sigma_x}{2}\right)^{2n+1} \right] \sigma_y \exp\left(\frac{i\phi\sigma_x}{2}\right) \quad (\text{A.56})$$

$$= \hat{S}_x + \frac{i\hbar}{2} \left[\cos \frac{\phi}{2} \mathbf{I}_2 - i \sin \frac{\phi}{2} \sigma_x \right] \sigma_y \left[\cos \frac{\phi}{2} \mathbf{I}_2 + i \sin \frac{\phi}{2} \sigma_x \right] \quad (\text{A.57})$$

$$= \hat{S}_x + \frac{i\hbar}{2} \left[\cos^2 \frac{\phi}{2} \sigma_y + i \cos \frac{\phi}{2} \sin \frac{\phi}{2} (-i\sigma_z) + \cos \frac{\phi}{2} \sin \frac{\phi}{2} \sigma_z - \sin^2 \frac{\phi}{2} \sigma_y \right] \quad (\text{A.58})$$

$$(\text{A.59})$$

$$= \hat{S}_x + i \cos \phi \hat{S}_y + i \sin \phi \hat{S}_z \quad (\text{A.60})$$

$$= \hat{S}_x - i \hat{S}_y \text{ for } \phi = \pi \quad (\text{A.61})$$

$$\hat{S}_+^{\phi_x} = \hat{S}_-. \quad (\text{A.62})$$

Appendix B

RS TiGGER software

B.1 Recovering $\chi'(B)$ and $\chi''(B)$

Because the 10 GHz IF EPR signal in Fig. 2.11 is mixed with a 10.10 GHz LO, both 100 MHz channels contain in-phase and quadrature components. The FPGA onboard the RS digitizer records the magnitude of the two channels, *i.e.*, $\sqrt{I^2 + Q^2}$ and retains no information about phase. However, it can be recovered.

Up to a scaling constant, the digitized signal S can be written as:

$$S(t) \propto \chi'(t) + i\chi''(t) + C = \chi'(t) + i\chi''(t) + (a + ib), \quad (\text{B.1})$$

for a rapid-scan signal with a DC offset, $C = a + ib$, allowed to be different for each channel. As a magnitude and phase,

$$S \propto \sqrt{(\chi' + a)^2 + (\chi'' + b)^2} e^{i \tan^{-1} \left(\frac{\chi'' + b}{\chi' + a} \right)} e^{i2\pi\omega t}, \quad (\text{B.2})$$

where $\omega/2\pi = 10$ GHz, the IF stage frequency. Then, mix S with a 10.10 GHz LO of unknown phase, θ :

$$S \times LO \propto \sqrt{(\chi' + a)^2 + (\chi'' + b)^2} e^{i \tan^{-1} \left(\frac{\chi'' + b}{\chi' + a} \right)} e^{i2\pi\omega t} \times \rho e^{i(2\pi\omega + 100 \text{ MHz})t} e^{i\theta}. \quad (\text{B.3})$$

Now, take only the magnitude, as is done by the FPGA, to get

$$|S \times LO| \propto r\rho, \quad (\text{B.4})$$

where $r = \sqrt{(\chi' + a)^2 + (\chi'' + b)^2}$. Even when using the quasi-optical sample holder described in [194], the rapid-scan signal is less than 1% of the digitized background signal, and usually much less (see *e.g.*, the y-axis of Fig. 2.15). Therefore, because $a \gg \chi'$ and $b \gg \chi''$, the binomial approximation can be applied to both terms:

$$|S| \propto r = \sqrt{a^2(1 + \chi'/a)^2 + b^2(1 + \chi''/b)^2} \quad (\text{B.5})$$

$$\approx \sqrt{a^2 + 2a\chi' + b^2 + 2b\chi''} \quad (\text{B.6})$$

$$r \approx \sqrt{a^2 + b^2} \sqrt{1 + \frac{2a\chi' + 2b\chi''}{a^2 + b^2}}. \quad (\text{B.7})$$

Again, use the binomial approximation:

$$r \approx \sqrt{a^2 + b^2} \left\{ 1 + \frac{1}{2} \frac{2a\chi' + 2b\chi''}{a^2 + b^2} \right\} \quad (\text{B.8})$$

$$|S| \propto r \approx \sqrt{a^2 + b^2} + \frac{a\chi' + b\chi''}{\sqrt{a^2 + b^2}}. \quad (\text{B.9})$$

Now, in the Python3 processing code, the Hilbert transform is used to return the imaginary component of this real signal; subtracting this imaginary component from the real component provides an analytic signal, denoted by $|\widetilde{S}|$. It is important to subtract in order to return a final result of $\chi' + i\chi''$, rather than $\chi' - i\chi''$.

$$|\widetilde{S}| = |S| - i\mathcal{H}\{|S|\} \quad (\text{B.10})$$

$$|\widetilde{S}| \approx |S| - ic\mathcal{H}\left\{\sqrt{a^2 + b^2} + \frac{a\chi' + b\chi''}{\sqrt{a^2 + b^2}}\right\}, \quad (\text{B.11})$$

where c is a scaling constant. Using two properties of the Hilbert transform, namely that $\mathcal{H}$ is linear and $\mathcal{H}\{c\} = 0$ for some constant c , it can be simplified:

$$|\widetilde{S}|' \approx |S| + 0 - i\frac{a\mathcal{H}\{\chi'\} + b\mathcal{H}\{\chi''\}}{\sqrt{a^2 + b^2}}, \quad (\text{B.12})$$

where $|\widetilde{S}'|$ has absorbed the scaling constant. Then, since $\mathcal{H}\{\chi'\} = -\chi''$ and $\mathcal{H}\{\chi''\} = \chi'$,

$$|\widetilde{S}'| \approx |S| - i \frac{-a\chi'' + b\chi'}{\sqrt{a^2 + b^2}} \quad (\text{B.13})$$

$$\approx \left(\sqrt{a^2 + b^2} + \frac{a\chi' + b\chi''}{\sqrt{a^2 + b^2}} \right) - i \frac{-a\chi'' + b\chi'}{\sqrt{a^2 + b^2}} \quad (\text{B.14})$$

$$\approx \sqrt{a^2 + b^2} + \frac{a - ib}{\sqrt{a^2 + b^2}} \chi' + \frac{b + ia}{\sqrt{a^2 + b^2}} \chi'' \quad (\text{B.15})$$

$$\approx \sqrt{a^2 + b^2} + e^{i \tan^{-1}(-b/a)} \chi' + e^{i \tan^{-1}(a/b)} \chi'' \quad (\text{B.16})$$

$$|\widetilde{S}'| \approx \sqrt{a^2 + b^2} + e^{-i \tan^{-1}(b/a)} \chi' + e^{i \tan^{-1}(a/b)} \chi''. \quad (\text{B.17})$$

Since $\tan^{-1}(b/a)$ and $\tan^{-1}(a/b)$ are two angles of the same right triangle, they sum to $\pi/2$. Let $\theta = \tan^{-1}(a/b)$:

$$|\widetilde{S}'| \approx \sqrt{a^2 + b^2} + e^{-i(\pi/2-\theta)} \chi' + e^{i\theta} \chi''. \quad (\text{B.18})$$

Applying an overall correction phase, $\phi = \pi/2 - \theta$:

$$|\widetilde{S}'| e^{i(\pi/2-\theta)} \approx \sqrt{a^2 + b^2} e^{i(\pi/2-\theta)} + e^{i(\pi/2-\theta)} e^{-i(\pi/2-\theta)} \chi' + e^{i(\pi/2-\theta)} e^{i\theta} \chi'' \quad (\text{B.19})$$

$$\approx e^{i(\pi/2-\theta)} \sqrt{a^2 + b^2} + \chi' + e^{i\pi/2} \chi'' \quad (\text{B.20})$$

$$\approx e^{i\pi/2} e^{-i \tan^{-1}(a/b)} \sqrt{a^2 + b^2} + \chi' + i\chi'' \quad (\text{B.21})$$

$$\approx e^{i\pi/2} (b - ia) + \chi' + i\chi'' \quad (\text{B.22})$$

$$\approx (a + ib) + \chi' + i\chi'' \quad (\text{B.23})$$

$$\therefore |\widetilde{S}'| e^{i\phi} \propto S. \quad (\text{B.24})$$

Therefore, after taking the magnitude of the complex-valued IQ rapid-scan signal, Hilbert transforming, and applying a correction phase, the true magnetic susceptibility, χ , is recovered, up to a scaling constant and DC offset. This result requires that the DC offset be much larger than the time-dependent rapid-scan signal, which has been true for all experiments conducted at ITST to date.

From here, a standard deconvolution algorithm (see Tseytlin [205]) can be applied to convert $|\widetilde{S(t)}|'e^{i\phi}$ to slow-scan absorption and dispersion lineshapes of the modulated magnetic field, B .

B.2 Programming LabVIEW FPGA

LabVIEW FPGA is not fundamentally different from programming hardware control using LabVIEW and GPIB or VISA. However, there are some points to make note of and tips that may make the experience easier.

- Begin by navigating to LabVIEW **Help** and selecting **Find Examples**
- From here, search for the hardware to be programmed; in sec. 2.3, this was a PXIe-5763 FlexRIO digitizer
- Select an example LabVIEW project (.lvproj file) with a similar purpose to the proposed application
- Within the .lvproj, open the .vi that is hosted on the hardware; this should be under “My Computer/<hardware folder>/<device>/file_i.vi”
- There should be multiple, concurrent loops inside the program file (a minimum of two): (1) acquisition and (2) data handling, processing, and transfer
- The acquisition loop should be run at the digitizer clock cycle (for PXIe-5763, this is 80 MHz); it is best to use the provided code from the example .vi with minimal changes, as there are the FPGA API .vis that have a lot of the hardware interfacing packed inside them; some examples are:

- AI channels (returns signals from the 4 analog channels)
 - Convert FXP to Integer (converts fixed point number to integer)
 - Analog edge trigger
 - Start trigger
 - Streaming v1 FPGA (continuous acquisition of the requested number of samples after a trigger event)
 - Channel arbiter (combines the channel and data into a single memory element that can be stored in the FPGA RAM)
 - DRAM FIFO (dynamic RAM first-in first-out element that stores data without loss)
- The processing loop is application dependent. For rapid-scan, the following was necessary:
 - Read from DRAM FIFO (receive data packed by acquisition loop)
 - DRAM to channel arbiter (unpack the data and read out which channel it was acquired from)
 - If data is from channel 1 or 2, pack data into a new FIFO that stores the data temporarily
 - If channel 3 (trigger channel), both data 1 and 2 have already been acquired; read from the channel 1 and 2 FIFOs, square and sum their elements, and pass to a new FIFO that stores *all* data for a given time-sweep; pack channel 3 data into a FIFO that stores its data

- Repeat this loop for as many sets of data go into a given time-sweep; for example, for an acquisition of 10k points, this loop would repeat 2.5k times; 4 points are acquired per channel sample of the digitizer
 - After the 10k points, read the first four points of the time-sweep FIFO, add (sum) the incoming data, and pack the data back into the FIFO
 - Repeat for the full 10k points until the number of “averages” has been achieved
 - * By operating like this, no fixed-sized arrays are needed and the frequency of the modulation coil can be changed without needing to modify the size of any data structures (which isn’t allowed at run time); the size of the “array” is simply set by the number of times data is packed into the time-sweep FIFO before any is read
 - Send the averaged time-sweeps to a streaming FIFO that can be read out by the control PC; this FIFO can be found in the .lvproj and can be read by a .vi on the control PC
- There is an included “bit shift” option that adjusts the bitness of the integers by n to the right; this throws away some precision, but is useful if the signal is large or there are a lot of acquisitions during the continuous averaging and the summed value becomes larger than the max size of a given datatype (for RS TiGGER, the datatype was 16-bit integer)
 - Attempt to use code that runs very quickly in hardware; this is not always possible, but it is faster and simpler; if slow calculations must be done (*e.g.* trig functions, division, etc.), pay careful attention to the number of cycles for each calculation and ensure that a sufficient number are provided before re-running the calculation

- By right-clicking on the FPGA target in the .lvprog, the execution mode can be changed to “Simulation”; this will run the code as though it is onboard the FPGA but does not require compilation, making it much faster to test; note that simulation mode does not apply the same restrictions that are present in hardware, meaning that even though something runs in simulation does not necessarily mean it will run in production
- As a result of the above point, iterate step-by-step: write a loop, test in simulation mode, then compile and test onboard to make sure all functions and datatypes are permitted and the code runs as expected on the hardware

B.3 Processing data for a RS TiGGER experiment

To run the RS TiGGER processing code hosted at <https://github.com/bradley/rapidscan-epr>, follow the directions in the README.md. They are reproduced here for completeness:

Begin with `pip install -r requirements.txt` to install necessary python packages.

For a typical RS TiGGER experiment:

1. Data is acquired with LabVIEW .vis on the control PC; each experiment should be given its own folder as the data processing produces a lot of additional files that are sorted into the parent folder; it is good in the file names to at least include the modulation coil current and frequency since they are needed later; usual naming:
`./<experiment>/<temp>/<current>mA_<freq>kHz.dat`
2. Run `onfileRapidscanGUI.py` on the computer with the data file (run with

- Python, open GUI using web browser of choice)
3. Open file in the GUI using the full pathname (app will strip ' or " from each side of the string if they are present)
 4. Adjust experimental settings to match experiment and coil
 5. Auto-phase signal (if this doesn't work, try manual phasing to match what is expected for given EPR spectrum)
 6. **Batch deconvolve** to apply same deconvolution process to all time points in the file (this will also generate a `times.txt` file that uses the number of averages from the TiGGER file name to calculate the amount of time that has passed as the experiment progressed)
 7. Run `onfileAnimateSlowscan.py` in your command line using `python onfileAnimateSlowscan.py [filename: string] [B field offset: float (default 0)] [auto-phase: boolean (default False)]`
 - This will make all the TiGGER output files for the given experimental file name (it actually reads the `.feather` filename that is generated from `onfileRapidscanGUI.py`, but you can pass it the name of the original file)
 - The `B field offset` allows you to shift the center of the output plots so that the resonances are centered even if the RS modulation coil phase or timing is off
 - `<auto-phase>` set to `True` does its best to phase each time point to a pure absorptive signal which is useful if the temperature of the sample drifts during the experiment

- If `<auto-phase>` is off (`False` or not set), the phase that as applied using `onfileRapidscanGUI.py` is applied to all time points
8. The results are all in the folder of your original data file!

Appendix C

Additional experiments

C.1 240 GHz pulsed FEL-EPR measurements

C.1.1 CoNb_2O_6 at 4 T and 4.2 K

240 GHz FEL-EPR of CoNb_2O_6 was repeated many times at 2 T, 3 T, 4 T, and 5 T, and no obvious echo could be seen. The experiments at 4 T are shown here (Figs. C.2-C.4); no difference was observed at the other fields.

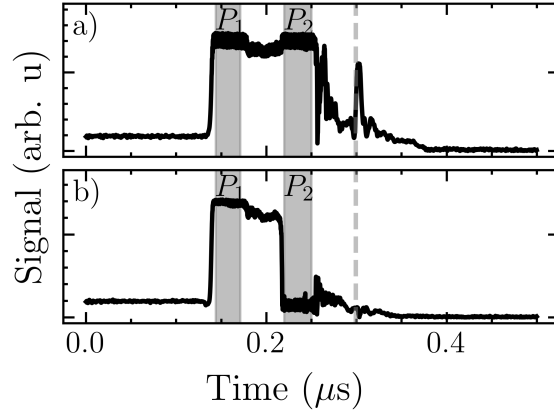


Figure C.1: Pulsed FEL-EPR of CoNb_2O_6 crystal with $\tau = 75$ ns at 240 GHz, 4 T, and 4.2 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line.

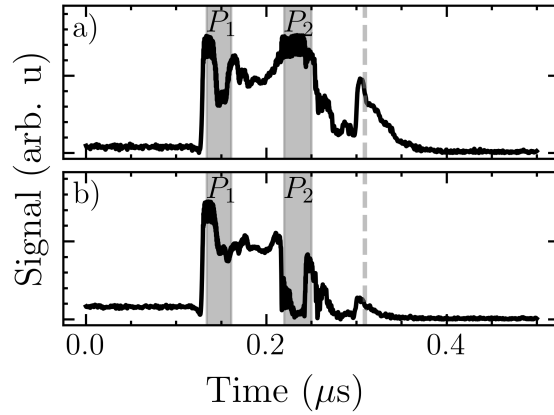


Figure C.2: Pulsed FEL-EPR of CoNb_2O_6 crystal with $\tau = 85$ ns at 240 GHz, 4 T, and 4.2 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line.

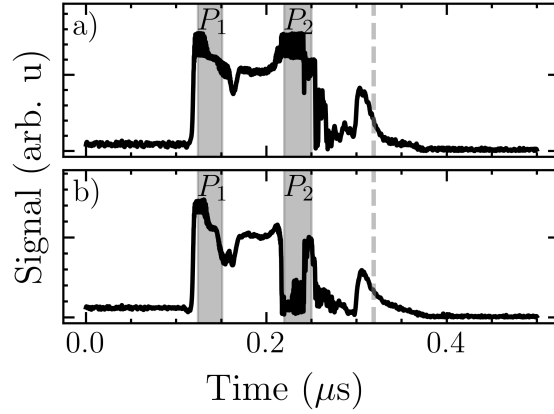


Figure C.3: Pulsed FEL-EPR of CoNb_2O_6 crystal with $\tau = 95$ ns at 240 GHz, 4 T, and 4.2 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line.

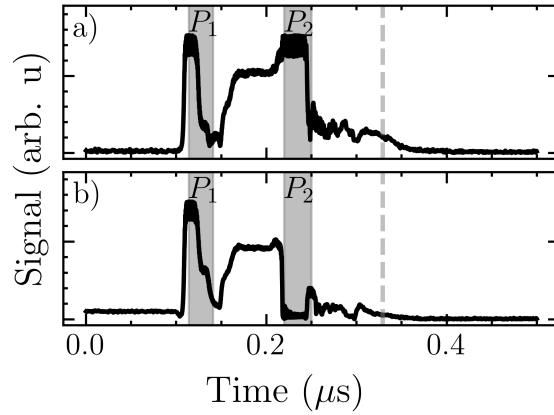


Figure C.4: Pulsed FEL-EPR of CoNb_2O_6 crystal with $\tau = 105$ ns at 240 GHz, 4 T, and 4.2 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line.

C.1.2 P1 centers in diamond at 8.6 T and 290 K

For comparison, 240 GHz FEL-EPR experiments on diamond are shown here (Figs. C.5 and C.6). $g = 2$ for this sample, putting the resonance at 8.6 T. As can be seen, the phase-cycled echo moves as expected when τ is varied.

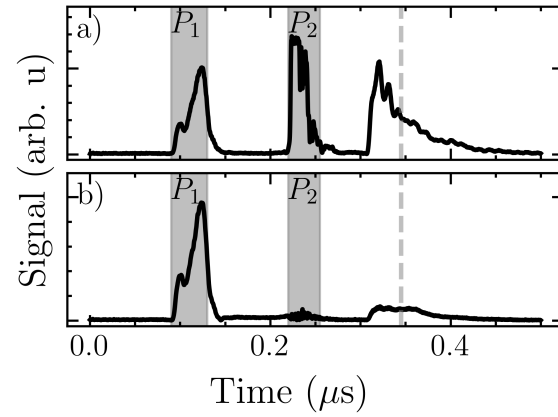


Figure C.5: Pulsed FEL-EPR of P1 center in diamond with $\tau = 125$ ns at 240 GHz, 8.6 T, and 293 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line, echo appears right afterward.

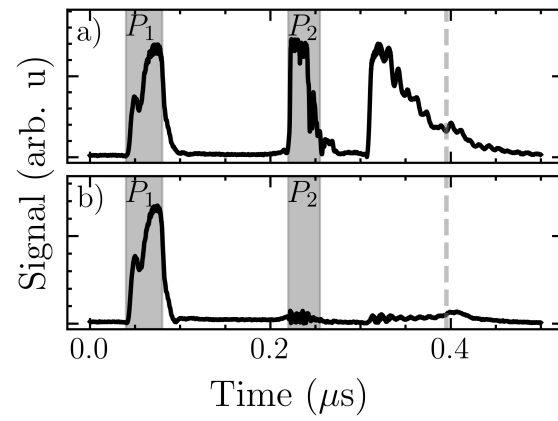


Figure C.6: Pulsed FEL-EPR of P1 center in diamond with $\tau = 175$ ns at 240 GHz, 8.6 T, and 293 K. (a) Raw time-domain data. (b) Two-phase phase-cycled data, phased to select echo coherence. Pulse 1 and 2 timings are highlighted by gray rectangles, expected echo location is marked by dashed vertical gray line, echo appears right afterward.

Appendix D

Lab hardware

D.1 Oscilloscope signal averaging

The oscilloscope (WavePro950, LeCroy Corp., NY, USA) was used in continuous averaging mode to eliminate any noise fluctuations in the pulse slicer's temporal response. This means that the output signal was

$$S(t) = \sum_{n=0}^{\infty} S_n(t) e^{-n/N} \quad (\text{D.1})$$

where S_n is the n th time trace for a given experiment and N is the user-determined continuous averaging value [357]. The experiment was automated to step the pulse timings without user intervention, and thus, each averaged signal would include some contribution from the delays preceding it. Therefore, when triggering at 20 Hz, if the desired experimental delay is ~ 10 s, contributions after ~ 10 s are required to be negligible:

$$S_n(t) e^{-10 \text{ s} * 20 \text{ Hz} / N} \approx 0. \quad (\text{D.2})$$

More specifically, requiring all contributions from more than 10 s ago to make up approximately 1/1000th of the signal,

$$\sum_{n > n_{10\text{ s}}}^{\infty} e^{-n/N} \approx \int_{10\text{ s}}^{\infty} e^{-t*20\text{ Hz}/N} dt \approx 10^{-3}, \quad (\text{D.3})$$

$$\therefore \frac{N}{20} e^{-200/N} \approx 10^{-3}. \quad (\text{D.4})$$

Numerically solving this equation gives $N < 27.7$. The options available on the oscilloscope were $N = 2^k - 1$; $k \in \mathbb{Z}$, so $N = 31$ was used, which amounts to

$$\frac{31}{20} e^{-200/31} = 2.4 \times 10^{-3} \quad (\text{D.5})$$

of the total signal, which was sufficiently low for the experiments performed.

D.2 Cryomech helium reliquifier

Due to the prohibitive cost of running a helium flow cryostat to maintain the superconducting coils in the Oxford 12.5 T field-swept magnet (roughly \$25/L, totaling nearly \$4000/month), in October 2024, a helium reliquifier was installed that makes the superconducting system (almost) closed cycle. The recovery is well above 95%.

D.2.1 Start-up

Before inserting the fill needle into the magnet, ensure that dry helium is flowing through the system (see below on dry helium over-pressurizing). This is to prevent air freezing on the condenser inside the cold head and blocking flow. If there is frozen air/water on the cold head, the pulse tube temperature will read below 4.2 K when the system is operating. If there is only helium in the system, the temperature will pulse right around

4.2 K as helium is being liquified. To insert the needle, lower the winch on the back of the gantry and guide the needle into the fill port. This is a two-person job. If the needle needs to be removed, it is best to reduce the operating pressure of the system down to 0.1 or 0.25 psig before doing so to reduce the amount of pressurized gaseous helium that escapes (see below about HRC-110).

D.2.2 Ice in the pulse tube

In case air or water get in the system and freezes in the pulse tube:

1. Turn off the compressor.
2. Remove the needle with the winch.
3. Allow the system to warm overnight.
4. Lower it back in while over-pressurizing He (ideally on both sides of the connection).

D.2.3 Frozen vacuum jacket

If liquifier jacket starts condensing air, the vacuum jacket has been compromised. It probably just needs to be pumped out again. Cryomech provides a special part for this (see photo below). This part connects to the opening on the bottom edge of the jacket and adapts to a KF flange. This part is tightened down by a hex nut and o-ring. Begin by pumping down the line connecting to this adapter. Once the lines have been evacuated, turn the thumbscrew into the valve on the jacket. This grabs the sealing plug. Then, pull out the plug by tugging on the thumbscrew. Wedge something in so that the thumbscrew cannot be pulled back in by the vacuum. You are now pumping the jacket. Allow the rough to go down to 5×10^{-2} Torr and then activate the turbo. Let this run overnight. If

the vacuum gets down to 10^{-6} Torr on the ion gauge, you are good to go. Now, push the thumbscrew back in. Once it is in, break the vacuum on the outside (ensure the turbo and rough pump are off!). This will ensure the valve stays in place as a result of the external pressure. Now, unscrew the thumbscrew until you are sure it is all the way out. Unscrew a lot more. It is now safe to unscrew the hex nut holding the adapter in place and the adapter can be removed.



Figure D.1: Specialized Cryomech vacuum jacket adapter. Bronze nut tightens O-ring onto liquifier vacuum jacket port. Thumbscrew (black) is used to turn screw threads into the valve plug, pulling this thumbscrew out removes the plug and allows the system to be pumped down. It may be helpful to wedge something between the thumbscrew and port during pumping to ensure the plug stays removed.

D.2.4 Heater and inverter operation

Helium gas boils off from the magnet jacket, flows through the vapor return line, is liquified, and falls back into the jacket due to gravity. To ensure that the whole system remains under positive pressure (so that no air can leak in), there is a heater inside the cold head that is controlled by the HRC-110 heater controller. This heater can be used to set the pressure set point for the whole system (currently 0.5 psig). If the boil-off rate of the cryostat is not high enough to maintain positive pressure, the heater ensures

the system does not go below atmospheric pressure. There is also a needle valve on the helium vapor return line that controls the rate that gas makes it to the cold head; it should be opened exactly 2 full turns.

The heater should almost never activate. There is an inverter in the compressor that reduces the compressor power to minimize the cooling power while maintaining a stable positive pressure. The inverter PID is set to 1 psig. Therefore, the system should remain stable at 1 psig. However, sometimes the PID fails and the inverter gets stuck at the maximum frequency (70 Hz) which forces the heater into always-on operation. This is not ideal and will cause the system to wear down more quickly. If this happens and the inverter PID stops functioning, it can be reset with a hard re-boot of the compressor. Turn off the pulse tube with the “Off” button on the compressor and wait for it to spin down. Then, shut the compressor off by flipping the breaker switch on its front panel. Flip the breaker back on, wait for the system to boot, and re-start compression by pushing “On”. The inverter frequency should stabilize around 40 Hz if the PID is now functioning properly.

D.2.5 Starting the system

The system can be started with either the front panel control on the compressor (in the FEL vault, follow the 2” diameter metal hoses) or the software front panel on the EPR computer. The computer communicates with the compressor via ethernet through the ITST network.

After starting the system, you should hear the compressor motor turn on and the pulse tube begin pulsing (repetitive chirps, roughly 1 per second). The temperature should begin dropping. It will stabilize at 4.2 K. If this does not happen, get help or

consult the operating manual for the Cryomech system (manual located on Sherwin shared drive under /Manuals/).

D.2.6 Monitoring

The helium level, system pressure, pulse tube temperature, and heater output can be monitored at magnet-log.onrender.com. The code for the operating the site is here (<https://github.com/brad-ley/data-reader>) in case it ever goes down or Render becomes a paid service. It is written to be run in a Docker container on Render and should be portable to other services/machines if needed. The code was written hastily, it could be significantly sped up and improved with a refactor.

D.2.7 HRC-110 pressure controller

The HRC-110 pressure controller sets the pressure setpoint of the cryo-cooler pulse tube (and therefore also the superconducting jacket of the magnet). The set pressure can be controlled via the HRC-110 front panel. Operation and recovery is very stable at 0.50 psig with PID values of $P = 1$, $I = 0.1$, $D = 40$.

D.2.8 Slow filling

Since recovery is imperfect, it is still necessary to inject helium into the system roughly once per month. A cylinder of UHP300 He from Airgas is sufficient. Connect the cylinder to the He gas line in the lab. Open all valves except the ones directly out of the cylinder and directly into the magnet. Using the KF flange manifold on the wall near the magnet, pump down the lines to ~ 0.1 Torr. This is not great vacuum, but good enough for this purpose. The idea is to ensure there is very little air inside the lines when helium starts

flowing into the magnet system to reduce the risk of air rocks forming in the cryostat. Once it has been pumped down, close the pump valve and open the cylinder. The regulator pressure should be roughly 3-5 psig. Allow this to sit for a few minutes as the overpressure of helium should force remaining air molecules out through the leaks. Open the valve directly into the magnet. The pressure of the magnet should rise to roughly 2.5 psi (which is controlled by the small regulator near the magnet itself) and the heater should turn off. The magnet is now filling, as that warm gas is being liquified by the liquifier. Allow this to fill as long as necessary. Before the cylinder is empty, close the valves so that air does not enter the system.

D.2.9 Stable operation

Under stable operating conditions, the magnet loses very little helium. There is a regular, roughly 1 day periodicity to the liquid helium percentage (LHe %) that can be read out from the Oxford ILM200 cryogen level monitor. A five-day period is shown in Fig. D.2. Because the sensor is resistive, the level is read every 1 hour to minimize the amount of heat that must be dumped into the helium bath.

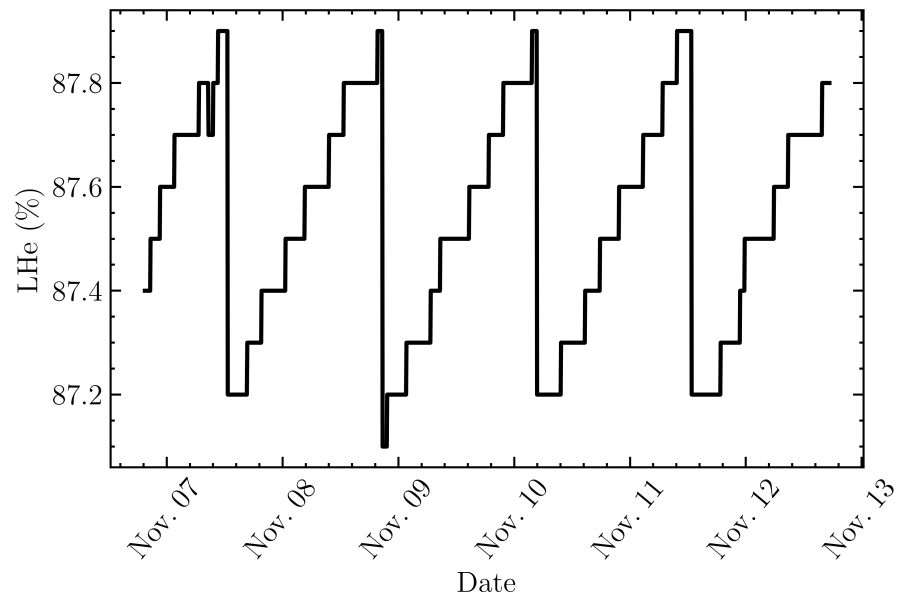


Figure D.2: LHe % under normal operation (with the inverter frequency at 70 Hz and the heater enabled at roughly 0.5 W) in Nov. 2024. There is a roughly one day periodicity in the level reading and very little (none over this timescale) drift downward under normal operation. Recovery is near 100%.

Appendix E

Quasi-optical sample holder operation

E.1 Optimization of QSH for EPR experiments

For RS and CW:

1. Sweep the magnetic field off resonance.
2. Roughly adjust power received at cross-polar detector until power is decreasing steadily with rotation.
3. Reduce rotation speed and monitor cross-polar power received at detector.
4. Find rooftop mirror position that corresponds to the least amount of cross-polar power received into detector.
5. Slightly adjust sample position; monitor cross-polar power to see if any large changes occur as stressed or bent joints and gears can quickly pop into a new

position.

6. Check that a very small adjustment of the rooftop mirror angle does not greatly change cross-polar power (if yes, begin again at step 2)
7. Sweep magnetic field to resonance.
8. Adjust sample position to maximize on-resonance signal relative to baseline.
9. Check once again that a very small rooftop mirror angle adjustment does not impart a large change on cross-polar power received (if yes, begin again at step 2).
10. If total power received at cross-polar detector is larger than what was expected for a given sample, try a 90° or 180° rooftop mirror angle from the current position and begin again at step 2.

For pulsed EPR:

1. Sweep the magnetic field to resonance.
2. Adjust rooftop mirror to maximize dead time free signal (*i.e.* Hahn echo intensity).
3. Adjust sample position to maximize dead time free signal.
4. Check that a very small rooftop mirror angle adjustment does not impart a large change in cross-polar power received (if it does, begin again at step 1).
5. If total power received at cross-polar detector is larger than what was expected for a given sample, try a 90° or 180° rooftop mirror angle from the current position and begin again at step 2.

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