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Dipolar pathways in dipolar EPR spectroscopy†

Luis Fábregas-Ibáñez,^a Maxx H. Tessmer,^b Gunnar Jeschke^a and
Stefan Stoll^{a,b}

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Dipolar electron paramagnetic resonance (EPR) experiments such as double electron–electron resonance (DEER) measure distributions of nanometer-scale distances between unpaired electrons, which provide valuable information for structural characterization of proteins and other macromolecular systems. To determine these distributions from the experimental signal, it is critical to employ an accurate model of the signal. For dilute samples of doubly spin-labeled molecules, the signal is a product of an intramolecular and an intermolecular contribution. We present a general model based on dipolar pathways valid for dipolar EPR experiments with spin-1/2 labels. Our results show that the intramolecular contribution consists of a sum and the intermolecular contribution consists of a product over individual dipolar pathway contributions. We examine several commonly used dipolar EPR experiments in terms of dipolar pathways and show experimental results confirming the theoretical predictions. This multi-pathway model makes it possible to analyze a wide range of dipolar EPR experiments within a single theoretical framework.

1 Introduction

Dipolar electron paramagnetic resonance (EPR) experiments measure distributions of nanometer-scale distances between unpaired electrons in paramagnetic systems.^{1,2} Such distance distributions provide valuable information for the structural characterization of proteins and other macromolecular systems. Since dipolar EPR techniques do not require long-range order in a sample, such distance distributions provide unique information about structurally disordered or highly complex systems.

The most broadly applied type of dipolar EPR experiments is double electron–electron resonance (DEER). Following the principles of spin-echo double resonance (SEDOR)^{3,4} and since the introduction of the 3-pulse DEER experiment,¹ many variations and improvements have been developed, such as 4-pulse DEER,^{2,5,6} 5-pulse DEER,⁷ 7-pulse DEER,⁸ nDEER,⁹ light-induced DEER (LiDEER),¹⁰ laser-induced magnetic dipole (LaserIMD),¹¹ refocused out-of-phase DEER (ROOPH-DEER),¹² and reversed DEER.¹³ Related methods are dipolar double quantum coherence (DQC),^{14,15} relaxation-induced modulation enhancement (RIDME),^{16–18} and the single-frequency technique for refocusing (SIFTER).¹⁹ All these experiments use a sequence of microwave pulses to generate a spin echo and record the echo

amplitude as function of pulse timings. From the resulting modulated spin echo signal, the underlying spin–spin distance distribution is inferred.

The robust inference of the distance distribution from the recorded modulated spin echo trace is challenging, as the ill-posed nature of this process is very sensitive to noise and to errors in modeling the data.²⁰ It is essential to use an adequate theoretical model to describe the experimental data.

In this paper, we present a uniform theoretical model of DEER spectroscopy. The model is based on dipolar pathways.^{7,8,21–23} In general, a dipolar signal consists of a combination of oscillatory contributions from individual dipolar pathways. Existing analysis methodology limits models to a single dipolar pathway contribution, even though experimental signals can contain contributions from multiple dipolar pathways, as shown by Salikhov specifically for three-pulse and four-pulse DEER.^{22,23} Such signals cannot be correctly analyzed with a single-pathway model. In this work, we extend previous work and present a general multi-pathway approach that can be used to extract distance distributions from a broad range of DEER experiments, without the need of preprocessing to remove secondary contributions. This approach is particularly important for experiments such as 5-pulse DEER. The multi-pathway models are implemented in the open-source software DeerLab.²⁰

2 Theory

Fig. 1 depicts a general pulse sequence with N infinitely short microwave pulses with phases φ_n and flip angles ϑ_n . The time

^a ETH Zurich, Laboratory of Physical Chemistry, Vladimir-Prelog-Weg 2,
8093 Zurich, Switzerland

^b University of Washington, Department of Chemistry, Seattle, WA 98195, USA.
E-mail: stst@uw.edu

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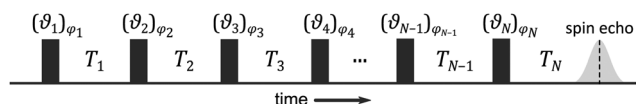


Fig. 1 General pulse sequence. The sequence consists of N pulses (black boxes) with flip angles θ_n and phase ϕ_n separated by the time intervals T_n . The spin echo after the last time interval T_N is detected.

intervals T_n separate the pulses and are chosen such that a spin echo is generated at a time T_N after the N -th pulse. The index $n = (1, 2, \dots, N)$ indicates a pulse or time interval. The sequence of timings and pulse parameters characterizes a specific dipolar EPR spectroscopy sequence.

By varying one or more intervals T_n simultaneously, the echo amplitude is modulated. In a sample with coupled electron spins, this modulation reports on the coupling between the spins. Writing the sequence of delays as a vector $\mathbf{T} = (T_1, T_2, \dots, T_N)$, the sequence timings for a one-dimensional experiment are written as²⁴

$$\mathbf{T}(t) = \mathbf{T}_0 + t\Delta \quad (1)$$

where $\mathbf{T}_0$ is the vector of initial delays and t is the time coordinate that is varied during the experiment. The unitless vector Δ specifies the incrementation scheme. Its elements indicate whether the interval T_n is incremented ($\Delta_n > 0$), decremented ($\Delta_n < 0$) or kept constant ($\Delta_n = 0$). If $\sum_n \Delta_n = 0$, then the overall length of the pulse sequence is independent of t , thus representing a constant-length experiment. We limit ourselves here to one-dimensional experiments where all elements of Δ are +1, 0 or −1.

The full theoretical description of the spin echo amplitude $V(t)$ as a function of t requires a full spin dynamics calculation that includes every dipole–dipole interaction between all spins in the sample. This is intractable. However, in highly diluted samples, where the doubly spin-labeled molecules or proteins in the sample are far from each other, the spin system can be approximately described in terms of just pair-wise interactions between spins. This yields the following product model as originally introduced by Milov *et al.*¹

$$V(t) = V_0 \cdot V_{\text{intra}}(t) \cdot V_{\text{inter}}(t) \quad (2)$$

Here, the prefactor V_0 is the echo amplitude in the absence of any dipolar interactions, and $V_{\text{intra}}(t)$ and $V_{\text{inter}}(t)$ are the echo modulations from intramolecular and intermolecular spin pairs, respectively. They are sometimes referred to as foreground and background. The intramolecular contribution depends on the intramolecular spin–spin distance distribution $P(r)$. The intermolecular contribution depends on the concentration of the spins and their spatial distribution.

In the rest of this section, we derive the expressions for $V_{\text{intra}}(t)$ and $V_{\text{inter}}(t)$ for an arbitrary pulse sequence. We limit all our theoretical treatments to $S = 1/2$ spins under weak dipolar coupling and in the high-temperature regime. We assume the absence of large g -factor anisotropies,^{25,26} multi-spin effects,^{27,28} exchange coupling,^{29,30} spectral diffusion,³¹

multi-spin multi-quantum coherences,³² dynamical decoupling effects,³³ and conformer-dependent relaxation rates.³⁴ If a sample or dipolar EPR experiment does not satisfy these assumptions, the model is not fully applicable.

2.1 Intramolecular contributions

We start by considering the echo contribution from an isolated pair of electron spins, A and B. The detailed derivation is given in Appendix A. The angular dipolar interaction frequency is

$$\omega_{\text{dip}} = \frac{\mu_0}{4\pi} \frac{g^2 \mu_B^2}{\hbar} \frac{1}{r^3} (1 - 3 \cos^2 \theta) = \frac{D}{r^3} (1 - 3 \cos^2 \theta) \quad (3)$$

where θ is the angle between the inter-spin vector and the applied magnetic field, r is the inter-spin distance and the constant D combines the g -value of the electron g (close to the value of the free electron, g_e), the Bohr magneton μ_B , the magnetic constant μ_0 and the reduced Planck constant $\hbar$. In addition to the dipole–dipole interaction, the two spins have resonance offsets Ω_A and Ω_B .

The state of an ensemble of spin pairs is described by its density operator.^{35,36} The density operator can be written as a linear combination with complex coefficients of 16 basis operators $\hat{B}_j$ that are products of single-spin Zeeman operators (e.g., $\hat{S}_{Az}\hat{S}_{B\beta}$, $\hat{S}_{Az}\hat{S}_{B+}$, $\hat{S}_{A+}\hat{S}_{B-}$, etc., abbreviated as $\alpha\beta$, $\alpha+$, $+-$, etc.).³⁷ These operators are called single-element operators. The density operator changes with time along the pulse sequence. Between pulses, some of the complex coefficients accumulate a phase, but all preserve their amplitude. In contrast, pulses transfer amplitude between different basis operators. A specific sequence of $N + 1$ basis operators along the pulse sequence constitutes the i -th single-element transfer pathway $\mathbf{B}_i$. Fig. 2 illustrates this. For an N -pulse sequence there are 16^{N+1} possible single-element transfer pathways, of which only those with non-zero amplitude at thermal equilibrium prior to the first pulse and ending in single-quantum coherence ($\alpha\pm$, $\beta\pm$, $\pm\alpha$, $\pm\beta$) after the last pulse are detectable as a free-induction decay (FID) or echo. In addition, only those lead to a stationary spin echo at time T_N after the last pulse whose resonance offset phase is refocused at that time, independent of t . As the total density is a sum of densities along these single-element transfer pathways, the total spin echo amplitude is a sum of the contributions over all detectable single-element transfer pathways leading to a stationary spin echo with amplitude

$$E(t) = \sum_i E_i(t) \quad (4)$$

where i is an index running over all single-element transfer pathways. The single-element transfer pathway echo amplitude is

$$E_i(t) = b_i e^{-i\phi_{\text{dip},i}(t)} \quad (5)$$

where b_i is the amplitude and $\phi_{\text{dip},i}(t)$ the net dipolar phase accumulated along the i -th single-element transfer pathway. The dipolar phase is the part of the phase angle accumulated by the spins after a certain time t that is proportional to the dipolar frequency ω_{dip} . Due to the requirement of echo refocusing, the resonance offset contributions to the overall echo phase are

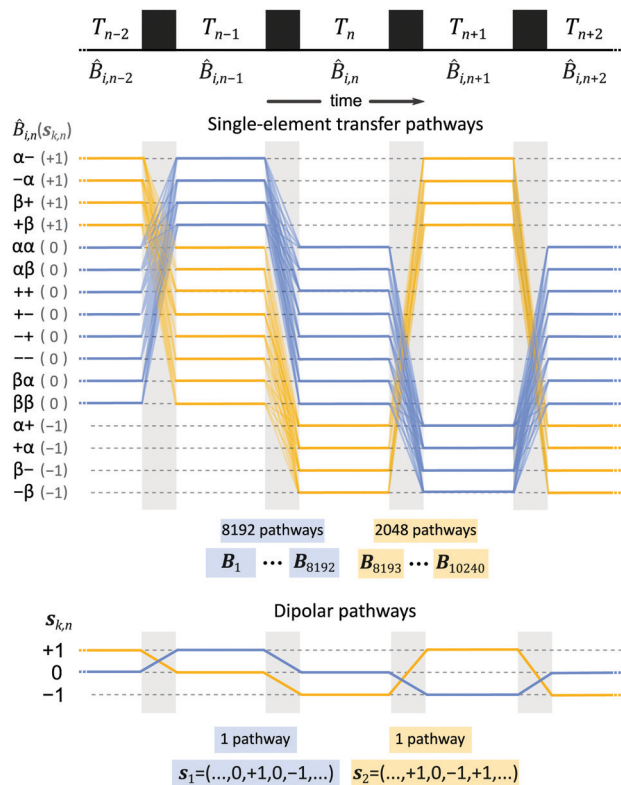


Fig. 2 Dipolar pathways as a condensed descriptor of dipolar EPR experiments. (Top) A subset of 10'240 single-element transfer pathways $\mathbf{B}_i$ is shown as colored lines connecting the basis operators $\hat{B}_{i,n}$ represented as dashed lines, for different time intervals T_n between the pulses. (Bottom) All the single-element transfer pathways shown can be collected according to their net dipolar phase accumulation trajectories into two dipolar pathways $\mathbf{s}_k$ shown as colored lines, with distinct progressions of $s_{k,n} = 0, \pm 1$. All single-element transfer pathways are colored according to their corresponding dipolar pathway.

independent of t upon detection (see Appendix A for details). This allows us to describe the echo modulation as a function of t solely in terms of the dipolar phase of the individual pathway. The total dipolar phase accumulated along a pathway i is the sum over the dipolar phases accumulated during all inter-pulse intervals n ,

$$\phi_{\text{dip},i}(t) = \sum_{n=1}^N \phi_{\text{dip},i,n}(t) \quad \phi_{\text{dip},i,n}(t) = \frac{1}{2} s_{i,n} \omega_{\text{dip}} T_n(t) \quad (6)$$

The quantity $s_{i,n}$ indicates whether the dipolar phase along pathway i increases (+1), remains constant (0), or decreases (−1) during a time interval $T_n(t)$.

Since there are 16 basis operators, but only 3 possible values for $s_{i,n}$, multiple single-element transfer pathways accumulate the same dipolar phase by the end of the pulse sequence. We can combine these pathways into a single dipolar pathway characterized by the sequence

$$\mathbf{s}_k = (s_{k,1}, s_{k,2}, \dots, s_{k,N}) \quad (7)$$

where k is an index running over all dipolar pathways. Fig. 2 illustrates this. Note that, while related, dipolar pathways are

not coherence transfer pathways. Coherence transfer pathways summarize the spin dynamics in terms of the spin coherence orders, whereas dipolar pathways are groupings in terms of the dipolar phase evolution. We can rewrite the dipolar phase in eqn (6) by factoring out ω_{dip} , yielding

$$\phi_{\text{dip},k}(t) = \omega_{\text{dip}} t_{\text{dip},k}(t) \quad (8)$$

where $t_{\text{dip},k}(t)$ is the effective dipolar evolution time (under ω_{dip}) for dipolar pathway $\mathbf{s}_k$,

$$t_{\text{dip},k}(t) = \frac{1}{2} \sum_{n=1}^N s_{k,n} T_n(t) = \frac{1}{2} \mathbf{s}_k \cdot \mathbf{T}(t) \quad (9)$$

The total echo amplitude $E(t)$ is the sum of the echo amplitudes $E_k(t)$ arising from the different dipolar pathways

$$E(t) = \sum_k E_k(t) = \sum_k \lambda_k e^{-i\phi_{\text{dip},k}(t)} = \sum_k \lambda_k e^{-i\omega_{\text{dip}} t_{\text{dip},k}(t)} \quad (10)$$

with the dipolar pathway amplitudes λ_k being the sum of b_i over all single-element transfer pathways that are part of the dipolar pathway k . They can be written as

$$\lambda_k = |\lambda_k| e^{i\Phi_k} \quad (11)$$

where $|\lambda_k|$ and Φ_k are the magnitude and phase of the dipolar pathways. For each dipolar pathway $\mathbf{s}_k$ there is another pathway $-\mathbf{s}_k$ with opposite phase. Since we already assumed echo refocusing of the resonance offset phase for all pathways, the magnitude of the complex echo signal can vary only due to dipolar dephasing. Thus, we can combine the overall magnitude of the pathway amplitudes $|\lambda_k|$ into V_0 in eqn (2) and impose the following normalization condition

$$\sum_k |\lambda_k| = 1 \quad (12)$$

meaning that any increase or decrease in a dipolar pathway's amplitude requires a compensating decrease or increase in amplitude of other dipolar pathways. Additionally, based on choice of pulse phases, different dipolar pathways can present in-phase and out-of-phase contributions of different intensities, and even negative contributions.

A dipolar pathway echo contribution $E_k(t)$ is modulated along t only if $t_{\text{dip},k}(t) \neq \text{const}$, otherwise its contribution will be constant. The spins along a dipolar pathway refocus at a time when the associated dipolar phase is zero ($\phi_{\text{dip},k}(t) = 0$), or, equivalently, when the effective dipolar evolution time is zero ($t_{\text{dip}}(t) = 0$). Thus, each modulated dipolar pathway has a characteristic refocusing time t_k given by

$$t_k = -\frac{\mathbf{s}_k \cdot \mathbf{T}_0}{\mathbf{s}_k \cdot \mathbf{A}} \quad (13)$$

where the dipolar phase of the pathway is zero and its contribution $E_k(t)$ to the total echo amplitude is at its largest.

We can combine the above equation with eqn (1) and (9) to give

$$t_{\text{dip},k} = \delta_k(t - t_k) \quad (14)$$

where the prefactor

$$\delta_k = \frac{1}{2} \mathbf{s}_k \cdot \mathbf{A} \quad (15)$$

characterizes how fast $t_{\text{dip},k}$ changes with the chosen experimental time coordinate t .

To obtain an expression for the intramolecular contribution $V_{\text{intra}}(t)$ in a disordered sample such as a frozen solution, we average the single-pair echo amplitude $E(t)$ of eqn (10) over the uniform orientational distribution of the spin pairs. If the inter-spin distance r is distributed, we additionally average over the distance distribution $P(r)$. As shown in Appendix B, in the high-temperature limit the contributions from the pathways $\mathbf{s}_k$ and $-\mathbf{s}_k$ can be collected into a pair of pathways $\mathbf{s}_p$ with amplitude $\lambda_p = 2\lambda_k$, same refocusing time $t_p = t_k$ and prefactor $\delta_p = |\delta_k|$. A subset M of the dipolar pathways exhibits modulations, while all other pathways contribute constant offsets. The intramolecular contribution reads

$$V_{\text{intra}}(t) = A_0 + \sum_{p \in M} \lambda_p \int_0^\infty dr P(r) K_0(|\delta_p|(t - t_p), r) \quad (16)$$

where A_0 is the total amplitude of the unmodulated pathways, M represents the set of modulated dipolar pathways, and K_0 is the single-pathway dipolar kernel, defined in eqn (47), that provides the form of the echo modulation function for a single distance r . eqn (16) shows that the intramolecular modulation is a sum over all the detectable dipolar pathways of unmodulated and modulated contributions. The modulated contributions have identical shapes and differ only in their refocusing times t_p and their amplitudes λ_p . Examples are shown in Section 3.

2.2 Intermolecular contributions

Next, we derive an expression for the last factor in eqn (2), which represents the echo attenuation due to dipole-dipole interactions between spins residing on different molecules. We assume a random uniform distribution of molecules in the sample^{1,38} and disregard any volume exclusion effects.³⁹ The details of the derivation are given in Appendix B.

To account for the intermolecular interactions, we must consider the full system with N_s dipolar coupled spins. As mentioned, in the case of high dilution we can describe the system in terms of pair-wise interactions between a single spin (A) on one molecule and all other spins residing on other molecules (B). The total echo arising from such a spin system is a product over the echo contributions from all A-B pairs

$$E_N(t) = \prod_b^{N_s} E(t, r_b, \theta_b) = \prod_b^{N_s} \sum_k \lambda_k e^{-i\phi_{\text{dip},k}(t, r_b, \theta_b)} \quad (17)$$

where the index b runs over all B spins, and r_b and θ_b are the coordinates of the b -th B spin relative to the A spin. Averaging this expression over all possible orientations and also over all possible configurations of B spins yields the total

intermolecular contribution to the signal

$$V_{\text{inter}}(t) = V_{\text{inter},0} \prod_{p \in M} \exp(-\kappa c_s \lambda_p |\delta_p|(t - t_p)) \quad (18)$$

Here, $\kappa \approx 5.065D$ is a constant (see Appendix D), c_s is the number concentration of spins in the sample, $V_{\text{inter},0}$ is a constant that collects all unmodulated dipolar pathway contributions, λ_p and t_p are again the amplitude and refocusing times of the p -th dipolar pathway pair, and p runs over all modulated dipolar pathways pairs $\mathbf{s}_k$ and $-\mathbf{s}_k$.

The most important feature of the intermolecular contribution in eqn (18) is that it is a product, and not a sum, of the contributions arising from the individual pathways, each with different refocusing times and different decay rates. Therefore, the intermolecular contribution exhibits kinks at the refocusing times of the different dipolar pathways. Examples are shown in Sections 3 and 4.

2.3 General dipolar EPR spectroscopy model

With the expressions for the intramolecular and intermolecular contributions to the dipolar signal $V(t)$ at hand, we can insert them back into eqn (2), giving

$$V(t) = V_0 \cdot \left(A_0 + \sum_{p \in M} \lambda_p \int_0^\infty dr P(r) K_0(|\delta_p|(t - t_p), r) \right) \cdot \prod_{p \in M} \exp(-\kappa c_s \lambda_p |\delta_p|(t - t_p)) \quad (19)$$

where we have included $V_{\text{inter},0}$ into V_0 . This shows that the spin-physical model of a dipolar signal consists of a combination of several modulated dipolar pathways of different amplitudes and refocusing times, with a constant offset contribution from all non-modulated pathways and with a single intermolecular contribution arising from a product over all modulated pathways. Importantly, this equation shows that all modulated intramolecular contributions in the dipolar signal contain the same amount of information about the underlying distance distribution, since they all dependent on $P(r)$ and their contributions differs only in time shift t_p and amplitude λ_p .

By utilizing $\int dr P(r) = 1$ and pulling out the integral over r , eqn (19) can be rewritten as

$$V(t) = \int_0^\infty dr P(r) K(t, r) \quad (20)$$

with the general dipolar kernel

$$K(t, r) = V_0 \cdot \left(A_0 + \sum_{p \in M} \lambda_p K_0(|\delta_p|(t - t_p), r) \right) V_{\text{inter}}(t) \quad (21)$$

This shows that within our set of assumptions the dipolar EPR signal $V(t)$ is a linear function of the distance distribution. This linearity can be exploited in maximum-likelihood and Bayesian analysis methods that aim at extracting $P(r)$ from $V(t)$.^{20,40,41}

3 Specific experiments

In this section, we analyze several specific dipolar EPR experiments in terms of dipolar pathways. Most pulse dipolar EPR sequences can be described by the model of eqn (19). Here, we focus on the 3-pulse, 4-pulse, and 5-pulse DEER experiments. We restrict the discussion to the high-temperature limit, where the contributions from the s_k and $-s_k$ dipolar pathways are equivalent and all their prefactors $|\delta_k| = 1$.

The dipolar pathway amplitudes λ_p can be estimated from pulse inversion probabilities and excitation overlaps. We will focus most of our discussion on typical experimental conditions as reported in the literature (rectangular and short microwave pulses, minimal or mild excitation overlap, *etc.*).

3.1 3-Pulse DEER

The simple 3-pulse DEER sequence features two different pairs of equivalent modulated dipolar pathways, s_1 and s_2 , refocusing at $t = 0$ and at $t = \tau$ respectively (see Fig. 3). These have been previously studied.^{21,23} Under common experimental conditions with selective pulses, the first and third pulses are probe pulses, and the second one is a pump pulse. Under these conditions, both dipolar pathways have significant amplitudes, with the amplitude of the s_1 pathway being considerably larger than that of the s_2 pathway.

Historically, the minor contribution of the s_2 pathway has been termed “2 + 1 artifact” since the s_2 pathway is dominant in the 2 + 1 pulse sequence.⁴² However, from the perspective of

dipolar pathways, this is a signal component with the same information content as the s_1 pathway rather than an artifact. If a proper model is available, such contributions to dipolar signals arising from additional dipolar pathways can be included in the analysis and need not be considered artifacts.

Under typical experimental conditions, the intermolecular contribution from the s_1 pathway is dominant and results in a steady decay from the refocusing time at $t = 0$ while the minor s_2 pathway contribution shows a slower increase towards the refocusing time at $t = \tau$ (see Fig. 3). Still, since the s_1 pathway carries more amplitude, the overall modulation is a mono-exponential decay. If we use non-selective pulses, the overall intermolecular contribution would be a mono-exponential increase due to the dominating intermolecular contributions from the s_2 pathway.

3.2 4-Pulse DEER

The introduction of an additional pulse to form the 4-pulse DEER sequence results in a total of four different pairs of equivalent modulated dipolar pathways, as illustrated in Fig. 4. Their intramolecular contributions have also been discussed before.^{21,22} With the use of selective probe and pump pulses, the pathway s_1 refocusing at $t = \tau_1$ is commonly the dominant pathway, and experimental practice aims at maximizing its amplitude.

There are two other often non-negligible contributions: pathways s_2 and s_3 refocusing at $t = \tau_1 + \tau_2$ and $t = 0$, respectively (see Fig. 4). They have similar amplitudes, because along both

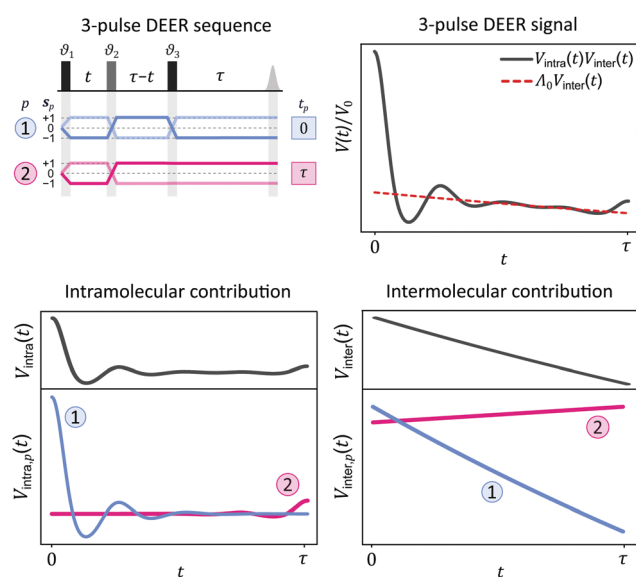


Fig. 3 Schematic illustration of dipolar pathways in 3-pulse DEER. (Top left) Pulse sequence and dipolar pathways. All detectable modulated dipolar pathway pairs s_p consisting of s_k (solid lines) and $-s_k$ (fainter lines) are shown along with their refocusing times t_p . (Top right) The full signal $V(t)$ is shown as a solid line and its unmodulated component as a dashed red line. (Bottom) Decomposition of the dipolar signal. The individual intramolecular (left) and intermolecular (right) contributions are shown as colored lines. The combined inter- and intramolecular contributions are shown as black lines on the top axes.

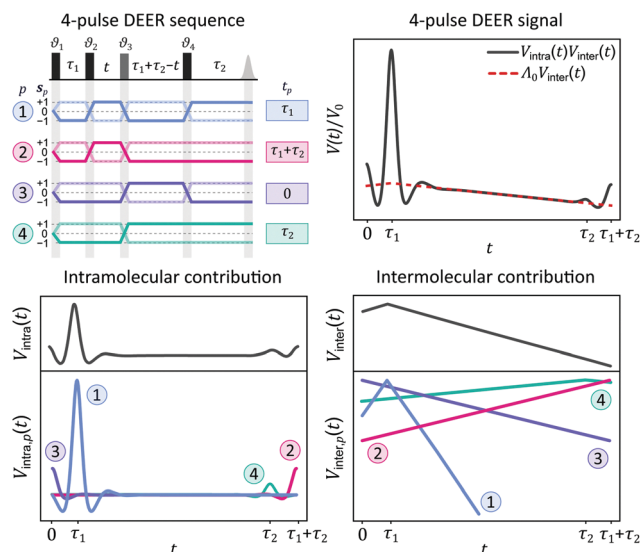


Fig. 4 Schematic illustration of dipolar pathways in 4-pulse DEER. (Top left) Pulse sequence and dipolar pathways. All detectable modulated dipolar pathway pairs s_p consisting of s_k (solid lines) and $-s_k$ (fainter lines) are shown along with their refocusing times t_p . The most commonly encountered pathways are highlighted in color. (Top right) The full signal $V(t)$ is shown as a solid line and its unmodulated component as a dashed red line. (Bottom) Decomposition of the dipolar signal. The individual intramolecular (left) and intermolecular (right) contributions are shown as colored lines. The combined inter- and intramolecular contributions are shown as black lines on the top axes.

pathways one of the probe pulses flips both spins. In 4-pulse DEER experiments these two contributions appear at the edges of the detectable signal since the time coordinate is typically limited to $\tau_1 \leq t < \tau_1 + \tau_2$, with a short stretch of $t < \tau_1$, to avoid pulses overlapping in time. The contribution s_2 refocusing at $t = \tau_1 + \tau_2$ has been discussed before. In analogy to its 3-pulse DEER counterpart, it has been referred to as a “2 + 1 artifact”,^{43,44} and many practitioners remove it partially by truncating the signal. The contribution s_3 refocusing at $t = 0$ refocuses outside the typical experimental range of t and its contribution overlaps with the refocusing time of the s_1 pathway. If the s_2 pathway amplitude is not negligible, it is essential to take the s_3 pathway into account as well in the data analysis, since it is not possible to remove it by cutting the trace without affecting the primary modulation.

The remaining modulated pathway s_4 , refocusing at $t = \tau_2$, has (mostly) negligible amplitude under standard 4-pulse DEER experimental conditions, since it is unlikely that both probe pulses will flip both spins. Nevertheless, this pathway can have non-negligible amplitude for substantial excitation band overlap between probe and pump pulses or non-conventional pulse excitation involving non-selective pulses. For example, the 4-pulse nDEER experiment⁹ (which uses two non-selective pulses) records the s_4 pathway as its main contribution.

Note that a general experimental strategy for suppressing contributions from secondary pathways is to strictly avoid excitation band overlap between probe and pump pulses, for example using Gaussian probe pulses.⁴⁴

When secondary pathways have non-negligible amplitude, not detecting a pathway's refocusing time or truncating the signal (a common practice) will not completely remove the contribution of that pathway from the data. This is clearly evident when looking at the intermolecular contributions to the 4-pulse DEER signal (see Fig. 4). While typically the intermolecular contribution is mostly dominated by the s_1 pathway, s_2 and s_3 also contribute over the full range of t , leading to an intermolecular contribution that is asymmetric about $t = 0$.

Essentially all 4-pulse DEER data in the literature have been analyzed utilizing a model containing the s_1 pathway alone, neglecting all other pathways. This approximation is valid if $\lambda_1 \gg \lambda_2, \lambda_3, \lambda_4$. In this case we can approximate $\lambda_2 = \lambda_3 = \lambda_4 \approx 0$. Using $\lambda = \lambda_1$ and $\Lambda_0 = 1 - \lambda$, we obtain the well-known single-pathway expression

$$V_{4p}(t) \approx V_0 \cdot \left(1 - \lambda + \lambda \int_0^\infty dr P(r) K(t', r) \right) \exp(-\lambda c_p \kappa |t'|) \quad (22)$$

where $t' = t - t_1$ shifts the time coordinate such that $t' = 0$ at the refocusing time of the s_1 pathway. The single-pathway approximation of eqn (22) is not applicable if there is excitation band overlap between probe and pump pulses. In that case, the secondary pathways need to be included in the analysis as well (see Section 4).

3.3 5-Pulse DEER

The 5-pulse DEER experiment introduces an additional pump pulse and can be realized in two different ways, as reverse 5-pulse DEER⁷ and forward 5-pulse DEER.⁴⁵ Here we focus on the former. Despite the specific dipolar pathways and refocusing times differing between the two experiments, the overall observations and discussions apply equally to both of them.

Fig. 5 shows that a 5-pulse DEER experiment has a total of eight different modulated dipolar pathway pairs. Under typically reported experimental conditions, the s_1 pathway refocusing at $t = \tau_3$ is the dominant contribution. The s_5 pathway contribution refocusing at $t = \tau_2$ is analogous to the s_1 contribution in the 4-pulse DEER signal. It usually has a considerable amplitude, since it is due to the second pump pulse failing to invert a spin that has been inverted by the first pump pulse. Both s_1 and s_5 were previously discussed in terms of dipolar pathways by Borbat *et al.*,⁷ and s_5 has also been regarded as an artifact.^{7,8,46,47} Experimental optimization has focused on minimizing its amplitude or shifting its refocusing time.⁴⁶ It is important to note that it is essentially impossible to completely avoid or suppress s_5 experimentally. For this reason, a single-pathway model is inaccurate, and a multi-pathway modeling approach is important.

For small excitation bandwidth overlap, pathways s_2 and s_3 can have non-negligible amplitude yet low in comparison to the s_1 and s_5 pathways. In analogy to the s_2 and s_3 pathways of the

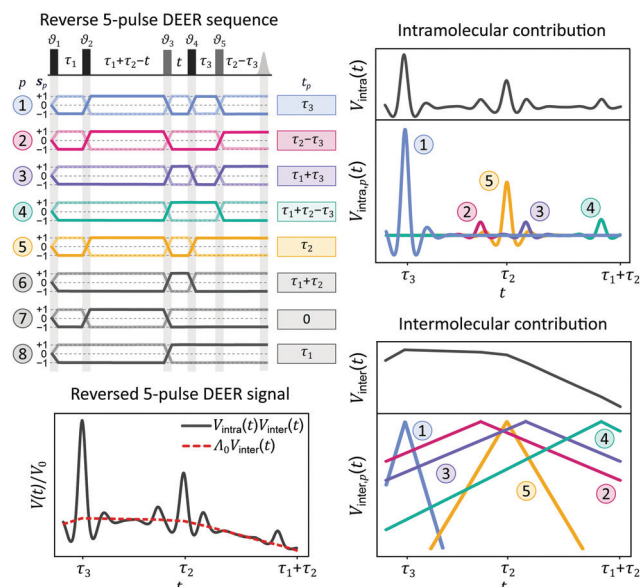


Fig. 5 Schematic illustration of dipolar pathways in 5-pulse DEER. (Left) Pulse sequence and dipolar pathways. All detectable modulated dipolar pathway pairs s_p consisting of s_k (solid lines) and $-s_k$ (fainter lines) are shown along with their refocusing times t_p . The most commonly encountered pathways are highlighted in color. (Right) Decomposition of the dipolar signal. The primary signal $V(t)$ is shown as a solid line and its constant unmodulated component as a dashed red line on the top panel. The individual intramolecular (top) and intermolecular (bottom) contributions from the most commonly encountered dipolar pathway are shown as colored lines in the two bottom panels. The combined inter- and intramolecular contributions are shown as black lines on the top axes.

4-pulse DEER experiment, they occur due to one of the probe pulse inverting both spins. These have also been rationalized in the past,⁴⁶ but regarded as artifacts. If the same configuration is used for both probe pulses, both pathways will present the same amplitudes. Similarly, pathway s_4 can have non-negligible amplitude if the excitation overlap of the pulses is considerable. It is analogous to the s_4 pathway of the 4-pulse DEER experiment, since it arises from both probe pulses inverting both spins. A 5-pulse nDEER experiment would record pathway s_4 as its main contribution. The dipolar pathways s_6 to s_8 might contribute to the dipolar signal, although their amplitudes are typically negligible in comparison to the other pathways. However, when using non-selective pulses, these can present larger amplitudes and even become dominant.

A very interesting feature of the 5-pulse DEER experiment is the typical form of its intermolecular contribution (see Fig. 5, bottom right) for low excitation overlap. It is asymmetric around $t = \tau_3$ and (typically) exhibits kinks not only at $t = \tau_3$, but also at $t = \tau_2$. This is due to the relatively large amplitudes of the s_1 and s_5 pathways. Therefore, the overall intermolecular contribution has typically three time intervals with different decay rates.

4 Experimental demonstrations

To validate the multi-pathway model and to demonstrate its utility, we performed a series of DEER experiments on several different samples using a Bruker ELEXSYS spectrometer operating at Q-band frequencies at 50 K (see ESI,[†] for details). For each experiment, we estimated the pulse inversion efficiency profiles and their overlap from simulations using the experimental parameters. Note that we use the indicated excitation overlap only for illustration and not in data analysis. In several

datasets, the data exhibits spikes at certain times caused by moving pump spin echoes which cannot be removed by phase-cycling with an incoherent pump source.⁴⁵ To remove their influence, we omitted those points from the analysis whose out-of-phase values after phase correction exceeded multiple standard deviations of the estimated noise.

The data were modeled according to the theoretical models presented above. In the 4-pulse DEER model we accounted for all four modulated pathways shown in Fig. 4 (s_1 to s_4) and in the 5-pulse DEER model we included pathways s_1 to s_5 shown in Fig. 5. We simultaneously fitted the dipolar-pathway amplitudes, the distance distribution and the spin concentration to the data. We also allowed the pathways refocusing times to deviate within ± 100 ns from their theoretically predicted values (based on the experimental timings) to account for the finite length of the pulses. The distance distribution and the other model parameters were fitted *via* separable non-linear least-squares (SNLLS) using a Tikhonov penalty optimized *via* the Akaike information criterion (AIC).²⁰ All data analysis was performed in DeerLab v0.14.0 with Python 3.8–3.9. The analysis scripts and their outputs are available in the ESI.[†]

4.1 4-Pulse DEER

We performed multiple 4-pulse DEER measurements on a 50 μM solution of maltose-binding protein (MBP, see ESI,[†] for details). The experiments were conducted using rectangular probe and pump pulses with different pulse length and frequency separation (see ESI,[†] for details) resulting in excitation overlap of approximately 10% to 70%. The datasets are shown in Fig. 6. We fitted a non-parametric distance distribution and the spin concentration globally to all the datasets, while fitting all other parameters locally.

Additionally, we performed two 4-pulse DEER measurements on a 90 μM solution of a rigid oligo(*p*-phenyleneethynylene)

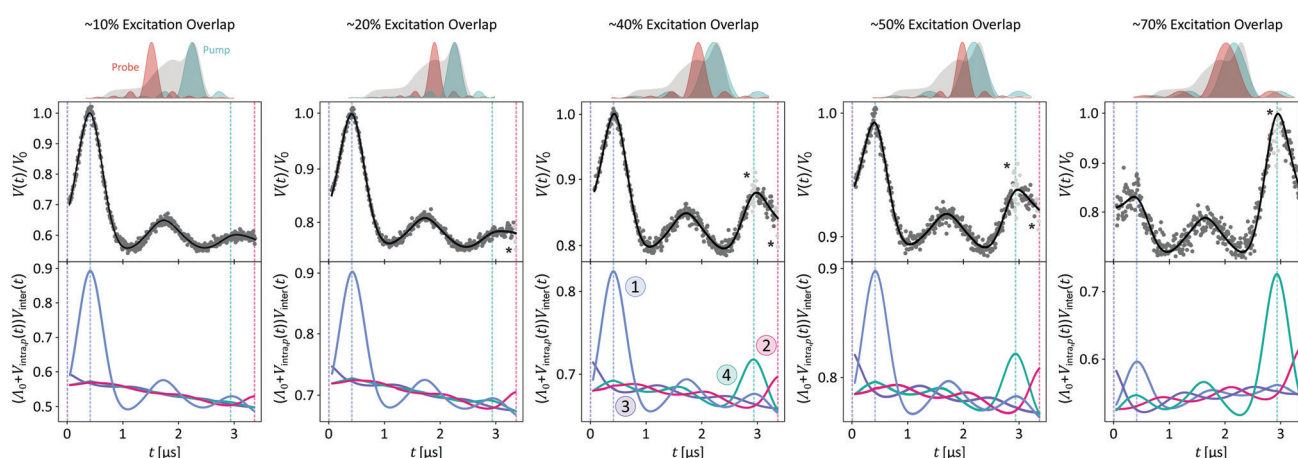


Fig. 6 Global analysis with DeerLab of experimental 4-pulse DEER signals obtained from a 50 μM solution of maltose-binding protein (MBP) using different configurations with varying pump–probe overlap (see ESI,[†] for details) with $\tau_1 = 0.4$ s and $\tau_2 = 3.0$ s. The experimental data are shown as grey dots. The experimental data corresponding to moving echoes are shown as fainter dots marked with a dark asterisk and were omitted from the analysis. The data was fitted to the full 4-pulse DEER multi-pathway model, with a global non-parametric distance distribution. The resulting fit to the data is shown as a black line. The fitted contributions to each signal are displayed for each fit as colored lines using the same notation as in Fig. 4. The simulated inversion efficiency profiles of the probe (red) and pump (green) pulses are superimposed on the nitroxide spectrum (grey) are shown on top for each dataset. The fitted distance distribution is shown in the ESI.[†]

(oligoPPE) bi-radical in deuterated *o*-terphenyl (dOTP)⁴⁸ as an example for DEER signals with long-lasting oscillations. We performed the experiments with two configurations of the rectangular probe pulse (see ESI,[†] for details) resulting in approximately 10% and 45% excitation overlap. The datasets are shown in Fig. 7. The distance distribution arising from the flexibility of such a short and rigid oligomer is well described by a worm-like chain parametric model,⁴⁹ here with a chain length of about 4 nm. The distribution was fitted globally to all datasets while keeping all other parameters local. To account for the orientational correlation of the two radicals in such a rigid biradical, we included orientation selection in this analysis and modeled the orientation weights as a four-point cubic spline. Note that the presence of orientation selection does not affect the multi-pathway analysis as it only alters the shape shared by all pathway contributions.

The data and fitted models shown in Fig. 6 and 7 show that our theoretical model accurately describes the experimental data. For very small pump-probe excitation overlap, the s_1 pathway solely dominates the dipolar signal. However, larger pump-probe excitation overlap results in multiple dipolar pathways having non-negligible amplitude. Particularly, in our data not only the well-known s_2 pathway (*i.e.* the “2 + 1” contribution) is present, but also the s_3 and the s_4 pathways. While the s_2 and s_4 pathways influence the signal mainly at

later times, the s_3 contribution strongly overlaps with the s_1 pathway signal at very early times. In case of moderate overlap, s_4 pathway's amplitude becomes significant and even exceeds that of the s_2 and s_3 pathways for severe overlap. Our experiment with the largest overlap closely resembles a 4-pulse nDEER experiment. In this situation, the s_4 pathway becomes the main contribution and the s_1 pathway the minor contribution, as predicted. These data show that interpretation of 4-pulse DEER data in terms of just the s_1 and s_2 pathways is also limited, and that pathways s_3 and s_4 must also be taken into account if there is pulse excitation overlap. Truncating the signal at later times to remove possible contributions from the s_2 pathway is incomplete as it fails to remove the contributions from the s_3 or s_4 pathways. When the sequence timings are such that the dipolar modulation extends over the entire measured time interval (as in the case of Fig. 6), the contributions from all pathways overlap over most of the signal, making their separation or removal impossible.

4.2 5-Pulse DEER

We conducted a set of 5-pulse DEER experiments on the same MBP sample. We used the same pulses as in the 4-pulse DEER experiments (see ESI,[†] for details) resulting again in excitation overlaps in the range of approximately 10% to 70%. All the datasets are shown in Fig. 8. To provide further illustration, we performed a multi-pathway analysis on the experimental 5-pulse DEER data acquired from an A7R1/W22R1 mutant of the membrane-inserting peptide WALP23 and previously published by Breitgoff *et al.*⁴⁶ (see ESI,[†] for details) as an example for a case with a relatively short distance that is moderately distributed. The two datasets are shown in Fig. 9. They were recorded with different timings and different configurations of the rectangular probe pulses and broad-band hyper-secant pump pulses, resulting in approximately 4% and 10% overlap of the pulse excitation profiles.

The experimental data and the model fits in Fig. 8 and 9 show that our theoretical model also provides an accurate description of the experimental 5-pulse DEER data. As expected, even for very low excitation overlap (as in Fig. 9), the s_5 pathway presents significant amplitude, while increased overlap results in substantial contributions of additional pathways, such as s_2 and s_3 . Moderate overlap results in significant amplitude of these pathways in addition of pathway s_4 . In analogy to the 4-pulse DEER case, as expected, for the most severe overlap cases the s_4 pathway becomes the main contribution with s_1 becoming a minor contribution to the signal. This analysis shows that the multi-pathway model approach reduces the need for removing (experimentally or *via* processing) any secondary contributions. In cases where several pathway contributions overlap (such as s_2 , s_3 , and s_5 in Fig. 8), their separation and removal are no longer possible. These results bear significant relevance for the experimental application of 5-pulse DEER as well. A multi-pathway analysis provides the means to analyze 5-pulse DEER data acquired with rectangular pulses (as in Fig. 8), without the need of shaped pulses. Still, the use of shaped pulses (as in Fig. 9) significantly reduces

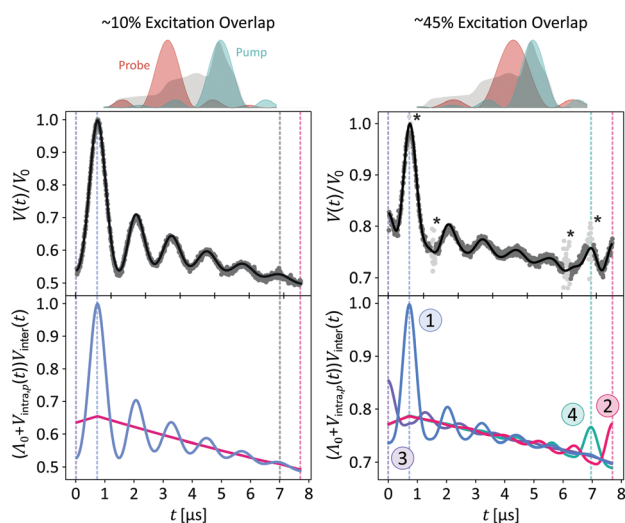


Fig. 7 Global analysis with DeerLab of experimental 4-pulse DEER signals obtained from a 80 μ M oligoPPE bi-radical solution using different configurations with varying pump-probe overlap (see ESI,[†] for details). with $\tau_1 = 0.75$ s and $\tau_2 = 7.5$ s. The experimental data are shown as grey dots. The experimental data corresponding to moving echoes are shown as fainter dots marked with a dark asterisk and were omitted from the analysis. The data was fitted to the full 4-pulse DEER multi-pathway model, with a global worm-like chain distance distribution. For the orientational distribution, a phenomenological model based on a cubic spline was used. The resulting fit to the data is shown as a black line. The fitted contributions to each signal are displayed for each fit as colored lines using the same notation as in Fig. 4. The inversion efficiency profiles of the probe (red) and pump (green) pulses are superimposed on the nitroxide spectrum (grey) are shown on top for each dataset. The fitted distance distribution is shown in the ESI.[†]

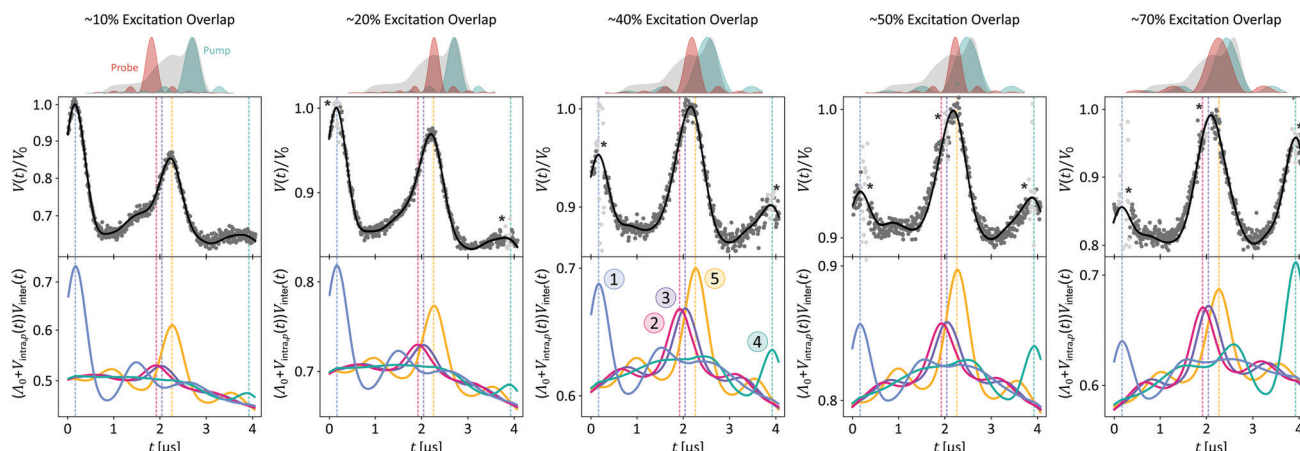


Fig. 8 Global analysis with DeerLab of experimental 5-pulse DEER signals obtained from a 50 μM solution of maltose-binding protein (MBP) using different configurations with varying pump–probe overlap (see ESI,[†] for details) with $\tau_1 = 1.8$ s, $\tau_2 = 2.3$ s, and $\tau_3 = 0.2$ s. The experimental data are shown as grey dots. The experimental data corresponding to moving echoes are shown as fainter dots marked with a dark asterisk and were omitted from the analysis. The data was fit with a 5-pulse DEER multi-pathway model consisting of the first five dipolar pathways, with a global non-parametric distance distribution. The resulting fit to the data is shown as a black line. The fitted contributions to each signal are displayed for each fit as colored lines using the same notation as in Fig. 4. The simulated inversion efficiency profiles of the probe (red) and pump (green) pulses are superimposed on the nitroxide spectrum (grey) are shown on top for each dataset. The fitted distance distribution is shown in the ESI.[†]

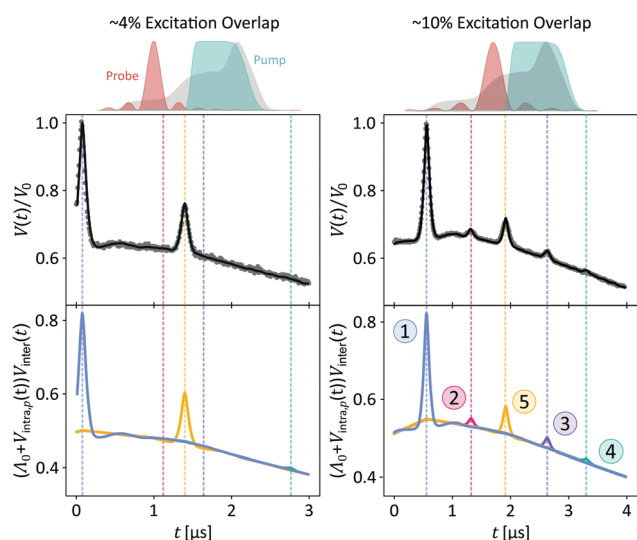


Fig. 9 Global analysis with DeerLab of experimental 5-pulse DEER signals from a A7R1/W22R1 mutant of the membrane-inserting peptide WALP23, acquired and published by Breitgoff *et al.*,⁴⁶ using different configurations with varying pump–probe overlap (see ESI,[†] for details). The left panel correspond to a 5-pulse DEER experiment with $\tau_1 = \tau_2 = 1.5$ s and $\tau_3 = 0.2$ s, and the right panel to $\tau_1 = \tau_2 = 2.0$ s and $\tau_3 = 0.5$ s. The experimental data are shown as grey dots. The data was fit with a 5-pulse DEER multi-pathway model consisting of the first five dipolar pathways, with a non-parametric distance distribution. The resulting fit to the data is shown as a black line. The fitted contributions to each signal are displayed for each fit as colored lines using the same notation as in Fig. 5. The insets show the fitted dipolar pathway amplitudes λ_k as colored bars. The simulated inversion efficiency profiles of the probe (red) and pump (green) pulses are superimposed on the nitroxide spectrum (grey) are shown on top for each dataset. The fitted distance distributions are shown in the ESI.[†]

the complexity of the data analysis *via* a strong reduction of the different pathway contributions to the signals. However, even

when using shaped pump pulses, multi-pathway analysis of 5-pulse DEER data may be required in order to properly account for the intermolecular contribution and for any remaining intramolecular contributions from pathways other than s_5 .⁵⁰

The model fits of the data in Fig. 9 also show the aforementioned shape of the intermolecular contribution, which exhibits kinks particularly at the refocusing times of the s_1 and s_4 pathways.

To more directly validate the theoretical predictions regarding the form of the intermolecular contribution in the 5-pulse DEER signal, we performed another series of experiments on a sample of dilute mono-radicals. We performed the measurements on a 400 μM solution of TEMPOL in a 1:1 v/v water/glycerol solvent mixture at 50 K, using a home-built AWG spectrometer operating at Q-band frequencies (see ESI,[†] for details).⁵¹ To observe changes in the relative amplitudes of the pathway contributions, we employed different pulse configurations with deteriorating inversion efficiency (see ESI,[†] for details). To illustrate this, we estimated the average spectral inversion efficiency from simulations. We modeled the data using the multi-pathway intermolecular model including pathways s_1 to s_5 of the 5-pulse DEER model. To ensure a stable fit, the spin concentration was fixed at 400 μM during the analysis.

The experimental datasets and the model fits are shown in Fig. 10. Across the different measurements, the relative amplitudes of the two dipolar pathways vary, changing both the shape and overall amplitude of the intermolecular signal. The intermolecular contributions seem to be well described mainly by the s_1 and s_5 pathways. As the inversion efficiency of the pulses deteriorates, s_5 becomes the main contribution since the probability of the second pump pulse not inverting the spins increases. In some cases, due to the overlap of the pulses, we can see some minor contributions from pathways s_2 , s_3 , and s_4 . As expected, due to the relatively large amplitudes of the s_1 and

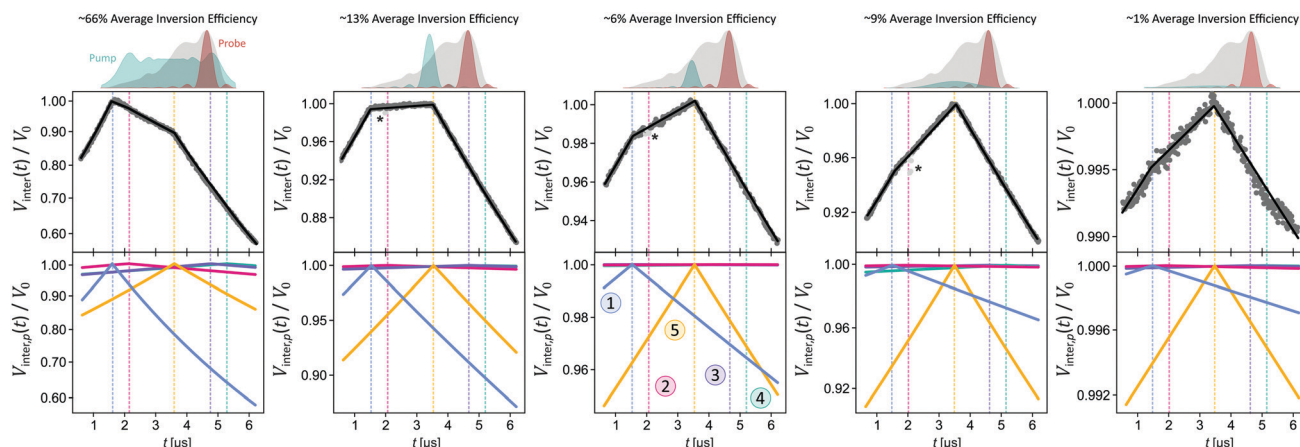


Fig. 10 Global analysis with DeerLab of experimental 5-pulse DEER signals obtained from a 400 μM TEMPOL solution using different pulse configurations (see ESI,[†] for details). The experimental data are shown as grey dots. The experimental data corresponding to moving echoes are shown as fainter dots marked with a dark asterisk and were omitted from the analysis. The data was fit with a 5-pulse DEER multi-pathway model consisting of the first five dipolar pathways. The resulting fit to the data is shown as a black line. The fitted contributions to each signal are displayed for each fit as colored lines using the same notation as in Fig. 4. The simulated inversion efficiency profiles of the probe (red) and pump (green) pulses are superimposed on the nitroxide spectrum (grey) are shown on top for each dataset. The fitted distance distribution is shown in the ESI.[†]

s_5 pathways, the theoretically predicted kinks at the refocusing times of the individual pathways are clearly visible in the data.

Overall, the experiments and the model fits show that the multi-pathway model provides an accurate and general description of the dipolar signal.

5 Conclusions

While dipolar EPR spectroscopy has grown into a colorful collection of experimental techniques, their interpretation has been focused on a simplified model of the 3-pulse DEER experiment with a single modulated dipolar pathway. Any observed features deviating from this model have been deemed artifacts. Here, we have introduced a more general multi-pathway model, with each pathway leading to a separate contribution to the overall signal. The model accurately describes pulse dipolar EPR experiments (within the assumptions listed in this work). We have illustrated this by examining several multi-pulse DEER experiments within this theoretical framework and validating them against experimental evidence. The model correctly describes the secondary pathways previously reported in the literature such as the s_2 pathway (referred to as “2 + 1” “artifact”) in 4-pulse DEER or the s_5 pathway (referred to as “overlap artifact”) in 5-pulse DEER. Additionally, in combination with our experimental evidence our model has shown the practical significance of other dipolar pathways, such as the s_3 and s_4 pathways in 4-pulse DEER. Furthermore, this model has allowed the quantitative derivation an explicit expression for the intermolecular contribution of dipolar EPR experiments beyond the single-pathway approximation.

In general, this multi-pathway model describes the anatomy of any conceivable dipolar EPR experiment. Different experiments may differ in the shape of the oscillations due to additional effects (e.g. orientation selection, as shown above), but not in their

refocusing times and amplitudes. They will still consist of a sum of intramolecular contributions and a product of intermolecular contributions arising from dipolar pathways. In the future, the multi-pathway model can be expanded to account for the shapes of, e.g., SIFTER signals (by introducing dynamical decoupling effects³³), RIDME signals (by accounting for overtones⁵² and nuclear-driven spectral diffusion³¹), or DQC intermolecular contributions (by including multi-spin high-order multi-quantum coherences³²).

When applying these models to analyze experimental data, it is rarely necessary to include all possible dipolar pathways in the analysis. As seen in the previous section, many modulated pathways will have negligible contributions under common experimental conditions. Often, a reduced subset of dipolar pathways is sufficient for an accurate description of the data in such cases. For 4-pulse DEER, a single pathway is sufficient if the amplitudes of the 2 + 1 pathway (s_2), the s_3 pathway, and the s_4 pathway can be kept close to zero by avoiding pump–probe excitation overlap. If there is overlap, then both s_2 and s_3 (and potentially s_4) will have significant amplitude and must be included in the analysis, as shown experimentally above. For 5-pulse DEER, it is generally necessary to include both s_1 and s_5 in the analysis. Depending on the extent of pump–probe excitation overlap, additional pathways will also have significant amplitude and must be included in the analysis. In general, one has to exercise care to make sure any simplified model is applicable, *i.e.* that contributions from neglected pathways have indeed negligible amplitude in the experimental signal. The incentive to simplify the model comes from the two additional fit parameters for each additional pathway (refocusing time and amplitude). Larger models are harder to fit robustly and, in certain cases, multiple datasets may be required to robustly identify and separate pathway contributions. Alternatively, refocusing times t_p of individual pathways can be shifted,⁴⁶ which provides additional data that should

stabilize model fitting. Global fitting, as implemented in DeerLab,²⁰ provides a general approach for this.

The general multi-pathway model presented here is implemented in the DeerLab software package, and examples of its use to fit data can be found in the software documentation.²⁰ It provides the means to fit multi-pathway models to experimental data without the need for removing secondary pathway contributions during data preprocessing. This leads to more accurate estimates of the underlying distance distributions if secondary pathway contributions are present. The ability to analyze data with more than one contributing dipolar pathway also opens up new ways of thinking about the optimization of experiments. Many established procedures for routine dipolar EPR experiments are based on finding a delicate balance between maximal spectral coverage of probe and pump pulses to maximize the sensitivity and minimal excitation overlap to minimize any additional pathway contributions. Being able to handle the latter within the model allows us to focus on the former. Describing experiments in terms of dipolar pathways can also be helpful for the design of new dipolar EPR experiments.

Conflicts of interest

There are no conflicts to declare.

Appendix

A Spin density operator description

The high-field spin Hamiltonian of an isolated spin-1/2 pair A–B in angular-frequency units reads

$$\hat{\mathcal{H}}/\hbar = \omega_A \hat{S}_{A,z} + \omega_B \hat{S}_{B,z} + \omega_{\text{dip}} \hat{S}_{A,z} \hat{S}_{B,z} \quad (23)$$

where $\hat{S}_{i,z}$ are spin operators and z labels the direction of the applied magnetic field. The first two terms describe the Larmor frequencies ω_A and ω_B of the A and B spin packets, respectively. The Larmor frequencies on electron Zeeman and hyperfine contributions to the resonance frequency. *i.e.*, ω_A and ω_B depend on magnetic quantum numbers of hyperfine-coupled nuclei. In the high-field approximation, the electron Zeeman contribution dominates. The last term represents the dipolar interaction between the two spin, with the dipolar frequency

$$\omega_{\text{dip}} = \frac{\mu_0}{4\pi} \frac{g^2 \mu_B^2}{\hbar} \frac{1}{r^3} (1 - 3 \cos^2 \theta) = \frac{D}{r^3} (1 - 3 \cos^2 \theta) \quad (24)$$

where θ is the angle between the inter-spin vector and the external magnetic field, r is the inter-spin distance and D is a constant that combines the g -value of the electron g (close to the value of the free electron, g_e), the Bohr magneton μ_B , and the reduced Planck constant $\hbar$. Note that we have neglected any exchange interaction contributions, which is a valid approximation for distances beyond 1.5 nm.

The spin Hamiltonian can be further simplified by transformation from the laboratory frame to a rotating frame with

reference frequency ω_{ref} . With these changes, the spin Hamiltonian reads

$$\hat{\mathcal{H}}/\hbar = \Omega_A \hat{S}_{A,z} + \Omega_B \hat{S}_{B,z} + \omega_{\text{dip}} \hat{S}_{A,z} \hat{S}_{B,z} \quad (25)$$

where $\Omega_i = \omega_i - \omega_{\text{ref}}$ are the resonance offsets of the A and B spins, respectively.

The state of the coupled two-spin system is fully described by its spin density operator $\hat{\rho}$. It can be represented in a basis of 16 orthogonal basis operators $\hat{B}_j$,³⁵

$$\hat{\rho} = \sum_j b_j \hat{B}_j \quad (26)$$

One possible set of $\hat{B}_j$ is the set of single-element operators, which consists of all products of the α , β , raising (+), and lowering (−) operators of the two spins. The operators describe polarization, single-quantum coherence (SQC), zero-quantum coherence (ZQC), and double-quantum coherence (DQC) states. This is visualized in Fig. 11.

At the beginning of the pulse sequence, the system is assumed to be at thermal equilibrium, with the associated density operator

$$\hat{\rho}_{\text{eq}} = \frac{e^{-\hat{\mathcal{H}}/(k_B \mathcal{T})}}{\text{tr}\{e^{-\hat{\mathcal{H}}/(k_B \mathcal{T})}\}} \approx \hat{1} - \frac{\hat{\mathcal{H}}}{k_B \mathcal{T}} \quad (27)$$

where k_B is the Boltzmann constant and $\mathcal{T}$ is the temperature. This operator contains only polarization components.

A pulse acting on the spin system induces transfers of amplitude from any of the 16 terms in the basis expansion of $\hat{\rho}$ to any other. Each transfer has an associated transfer amplitude a , which is a function of the pulse parameters such as frequency, flip angle and phase. For an N -pulse sequence, a sequence of $N + 1$ single-element basis operators

$$\mathbf{B}_i = (\hat{B}_{i,0}, \hat{B}_{i,1}, \hat{B}_{i,2}, \dots, \hat{B}_{i,N}) \quad (28)$$

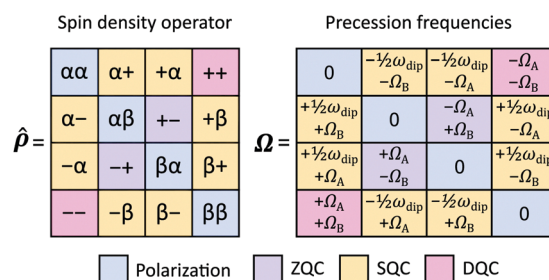


Fig. 11 Matrix representation of the spin density operator $\hat{\rho}$ and precession frequencies Ω . (Left) The 16 operators $\hat{B}_j$ in the single-element Zeeman product basis for a coupled spin-pair are shown. $\alpha\alpha$ indicates $\hat{S}_{A,z} \hat{S}_{B,z}$ etc. (Right) The precession frequencies of the individual components during the free evolution time between pulses are shown. The matrix elements are color-coded according to the type of spin system state: polarization (blue), zero-quantum coherence (ZQC, purple), single-quantum coherence (SQC, yellow), or double-quantum coherence (DQC, red).

constitutes the i -th single-element transfer pathway, with overall amplitude

$$b_i = b_{i,0} \prod_{n=1}^N a_{i,n} \quad (29)$$

where $b_{i,0}$ is the coefficient of the $\hat{B}_{i,0}$ operator in the expansion of $\hat{\rho}_{\text{eq}}$. During the time intervals T_n the density component $\hat{B}_{i,n}$ accumulates a phase

$$\phi_{i,n}(t) = \Omega_{i,n} T_n(t) \quad (30)$$

where $\Omega_{i,n}$ is the frequency with which the density component evolves, as shown in (Fig. 11). Polarization components of the density ($\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$) do not evolve ($\Omega_{i,n} = 0$), but coherence components evolve with different frequencies.

Assuming pulses are short enough that evolution during the pulses can be neglected, the accumulated phase at the end of the pulse sequence along the i -th single-element transfer pathway is

$$\phi_i(t) = \sum_{n=1}^N \phi_{i,n}(t) = \sum_{n=1}^N \Omega_{i,n} T_n(t) \quad (31)$$

We can separate this phase into the contributions from the two resonance offset terms and the dipolar interaction,

$$\phi_i(t) = \phi_{A,i}(t, \Omega_A) + \phi_{B,i}(t, \Omega_B) + \phi_{\text{dip},i}(t, \omega_{\text{dip}}) \quad (32)$$

For dipolar EPR spectroscopy, the quantity of interest is the dipolar phase $\phi_{\text{dip},i}(t)$.

Among all 16^{N+1} possible single-element pathways in an N -pulse sequence, there are $4 \times 16^{N-1} \times 8$ pathways that start in polarization (thermal equilibrium) and end in detectable SQC. Among these, only those are observable along which the spins refocus and generate an echo at a time T_N after the last pulse. A necessary condition for this is that at time T_N the net phase ϕ_i from eqn (32) is independent of Ω_A and Ω_B . Both Ω_A and Ω_B have broad distributions (100 MHz or more) and therefore dominate the rapid dephasing away from the refocusing time. In contrast, ω_{dip} has a much narrower distribution (a few MHz or less). Therefore, dephasing due to the dipolar frequency distribution is much slower, and we consider its effect as a dipolar modulation of the echo. Among the echo-generating pathways, we retain only those that form a stationary echo, *i.e.* whose refocusing time is T_N after the last pulse, independent of t . All other echoes move as t is changed and cause spikes in the detected signal. Here, we neglect all moving echos. Experimentally, they are removed *via* appropriate phase cycling.⁴⁵

Summing over all single-element transfer pathways that yield a stationary echo at T_N after the last pulse, the overall echo amplitude is

$$E(t) = \sum_{i \in \mathcal{R}} E_i(t) = \sum_{i \in \mathcal{R}} b_i e^{-i\phi_{\text{dip},i}(t)} \quad (33)$$

where E_i is the echo from single-element transfer pathway i . Since both the A and B spins refocus at T_N to form an echo, the pathway phase consists of just the dipolar phase, and the echo contributions will be modulated by the dipolar frequency.

B Averaging of the intramolecular contribution

In a disordered sample such as a frozen solution, the direction of the inter-spin vector is distributed and has an associated orientation distribution $P(\theta, \varphi)$, where θ and φ are the spherical coordinates describing the orientation of the applied magnetic field relative to the molecule. The average of the spin echo from eqn (10) over this distribution is

$$\langle E \rangle_{\varphi, \theta} = \int_0^{2\pi} d\varphi \int_0^\pi d\theta \sin \theta P(\theta, \varphi) E(t, r) \quad (34)$$

Under certain experimental conditions, different orientations of the inter-spin vector are excited by the pulses with different efficiency. This effect is commonly known as orientation selection and described by a non-uniform distribution $P(\theta, \varphi)$.⁵³ In its absence, the direction of the inter-spin vector is uniformly distributed, *i.e.* $P(\theta, \varphi) = 1/4\pi$, and evaluates to

$$\langle E \rangle_{\varphi, \theta} = \sum_k \lambda_k \mathcal{K}_0(t_{\text{dip},k}(t), r) \quad (35)$$

where $\mathcal{K}_0$ is the complex-valued single-pathway dipolar kernel given by the integral

$$\mathcal{K}_0(t, r) = \int_0^{\pi/2} d\theta \sin \theta \exp\left(-i \frac{Dt}{r^3} (1 - 3 \cos^2 \theta)\right) \quad (36)$$

An expression for $\mathcal{K}_0$ in terms of Fresnel integral functions is derived in Appendix C and given in eqn (57).

Next, if the intramolecular spin-spin distance r is distributed, the intramolecular contribution must be averaged over the associated distance distribution $P(r)$, yielding

$$V_{\text{intra}}(t) = \langle E \rangle_{\varphi, \theta, r} = \int_0^\infty dr P(r) \langle E \rangle_{\varphi, \theta} \quad (37)$$

$$= \sum_k \lambda_k \int_0^\infty dr P(r) \mathcal{K}_0(t_{\text{dip},k}(t), r) \quad (38)$$

$$= \sum_k \lambda_k V_{\text{intra},k}(t_{\text{dip},k}(t)) \quad (39)$$

Now, from the whole set of dipolar pathways, there is typically a subset M of modulated pathways, while all others contribute a constant offset to $V_{\text{intra}}(t)$ for being independent of t . We can rewrite $V_{\text{intra}}(t)$ by separating the two subsets

$$V_{\text{intra}}(t) = \sum_{k \notin M} \lambda_k V_{\text{intra},k}\left(\frac{1}{2} \mathbf{s}_k \cdot \mathbf{T}_0\right) + \sum_{k \in M} \lambda_k V_{\text{intra},k}\left(\frac{1}{2} \mathbf{s}_k \cdot \mathbf{T}_0 + \frac{1}{2} t \mathbf{s}_k \cdot \mathbf{D}\right) \quad (40)$$

where we can introduce the definition of the dipolar pathway refocusing time t_k from eqn (13) and of the factor δ_k from eqn (15). This can be rewritten as

$$V_{\text{intra}}(t) = A_0 + \sum_{k \in M} \lambda_k V_{\text{intra},k}(\delta_k(t - t_k)) \quad (41)$$

where

$$A_0 = \sum_{k \notin M} \lambda_k \int_0^\infty dr P(r) \mathcal{K}_0(t_{\text{dip},k}(t), r) \quad (42)$$

collects all unmodulated contributions. In principle, A_0 can adopt negative values. Additionally, since the integral in eqn (42) can only adopt values of magnitude ≤ 1 , the following inequality must hold

$$|A_0| \leq \left| \sum_{k \notin M} \lambda_k \right| \quad (43)$$

We can now rewrite eqn (39) with separated contributions

$$V_{\text{intra}}(t) = A_0 + \sum_{k \in M} \lambda_k \int_0^\infty dr P(r) \mathcal{K}_0(\delta_k(t - t_k), r) \quad (44)$$

Due to the complex-valued nature of $\mathcal{K}_0$, the intramolecular contribution will have both detectable in-phase and out-of-phase components. The out-of-phase component is significant only at high fields and low temperatures where the spin polarization is large.^{54,55} Note that the in-phase component of $\mathcal{K}_0$ is an even function while the out-of-phase component is an odd function. Therefore, for two pathways $\mathbf{s}_k$ and $\mathbf{s}_j = -\mathbf{s}_k$ with the same refocusing times $t_k = t_j$ and $\delta_k = -\delta_j$ we can see that

$$\begin{aligned} & \lambda_k V_{\text{intra},k}(\delta_k(t - t_k)) + \lambda_j V_{\text{intra},j}(\delta_j(t - t_j)) \\ &= (\lambda_k + \lambda_j) \text{Re}\{V_{\text{intra},k/j}(|\delta_{k/j}|(t - t_{k/j}))\} \\ &+ i(\lambda_k - \lambda_j) \text{Im}\{V_{\text{intra},k/j}(|\delta_{k/j}|(t - t_{k/j}))\} \end{aligned} \quad (45)$$

In the high-temperature limit, which is typically encountered experimentally, there is such a small fraction of polarized spins that the initial states for the pathways $\mathbf{s}_j$ and $\mathbf{s}_k$ are approximately equally populated. As a consequence, the magnitudes of these pathways must be approximately equal in addition to having opposite phase. Therefore, in this limit we can approximate $\lambda_k \approx \lambda_j^*$ and we can see in the expression above that the out-of-phase components cancel leaving purely in-phase contributions. In this limit the contributions from the $\mathbf{s}_k$ and $\mathbf{s}_j$ pathways are equivalent, and we can combine them into a dipolar pathway pair $\mathbf{s}_p$ with amplitude $\lambda_p = 2\lambda_k$ and prefactor $\delta_p = |\delta_k|$. Therefore, we can simplify the expression above to

$$V_{\text{intra}}(t) = A_0 + \sum_{p \in M} \lambda_p \int_0^\infty dr P(r) K_0(|\delta_p|(t - t_p), r) \quad (46)$$

where $K_0(t, r) = \text{Re}(\mathcal{K}_0)$ is given by

$$\begin{aligned} K_0(t, r) &= \sqrt{\frac{\pi r^3}{6Dt}} \left[\cos\left(\frac{Dt}{r^3}\right) F_C\left(\sqrt{\frac{6Dt}{\pi r^3}}\right) \right. \\ &\quad \left. + \sin\left(\frac{Dt}{r^3}\right) F_S\left(\sqrt{\frac{6Dt}{\pi r^3}}\right) \right] \end{aligned} \quad (47)$$

C Single-pathway dipolar kernel

In this section we solve the integral of the complex-valued single-pathway dipolar kernel from eqn (36). We introduce the substitution $z = \cos \theta$ which leads to an integral in the range $[-1, 1]$. However, since the integrand is an even function, we can restrict the integration to $[0, 1]$ and adding a factor of 2. Using the abbreviation $\zeta = Dt/r^3$ yields

$$\mathcal{K}_0(t, r) = \int_0^1 dz e^{-i\zeta(1-3z^2)} = e^{-i\zeta} \int_0^1 dz e^{-3i\zeta z^2} \quad (48)$$

The additional substitution

$$x = z\sqrt{-3i\zeta} = z\frac{i-1}{\sqrt{2}}\sqrt{3\zeta} \quad dx = dz\frac{i-1}{\sqrt{2}}\sqrt{3\zeta} \quad (49)$$

leads to

$$\mathcal{K}_0(t, r) = \frac{e^{-i\zeta}}{\sqrt{3\zeta}} \frac{\sqrt{2}}{i-1} \int_0^{\frac{i-1}{\sqrt{2}}\sqrt{3\zeta}} dx e^{-x^2} \quad (50)$$

In the above expression we can identify the definition of the error function $\text{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-x^2} dx$ and substitute it to give

$$\mathcal{K}_0(t, r) = \frac{e^{-i\zeta}}{\sqrt{3\zeta}} \sqrt{\frac{\pi}{2}} \frac{1}{i-1} \text{erf}\left(\frac{i-1}{\sqrt{2}}\sqrt{3\zeta}\right) \quad (51)$$

This is the general expression. We can rewrite it using Fresnel integrals utilizing the connection between the Fresnel integrals and the error function⁵⁶

$$F_C(x) + iF_S(x) = \frac{i+1}{2} \text{erf}\left(\frac{\sqrt{\pi}(1-i)}{2}x\right) \quad (52)$$

We first rewrite

$$\mathcal{K}_0(t, r) = -e^{-i\zeta} \sqrt{\frac{\pi}{6\zeta}} \sqrt{\frac{\pi}{2}} \frac{i+1}{2} \text{erf}\left(\frac{1-i}{\sqrt{2}}[-\sqrt{3\zeta}]\right) \quad (53)$$

$$= -e^{-i\zeta} \sqrt{\frac{\pi}{6\zeta}} \frac{i+1}{2} \text{erf}\left(\frac{\sqrt{\pi}(1-i)}{2}\left[-\sqrt{\frac{6\zeta}{\pi}}\right]\right) \quad (54)$$

and then substitute

$$\mathcal{K}_0(t, r) = -e^{-i\zeta} \sqrt{\frac{\pi}{6\zeta}} \left[F_C\left(-\sqrt{\frac{6\zeta}{\pi}}\right) + iF_S\left(-\sqrt{\frac{6\zeta}{\pi}}\right) \right] \quad (55)$$

$$= e^{-i\zeta} \sqrt{\frac{\pi}{6\zeta}} \left[F_C\left(\sqrt{\frac{6\zeta}{\pi}}\right) + iF_S\left(\sqrt{\frac{6\zeta}{\pi}}\right) \right] \quad (56)$$

where in the last line we have exploited the odd parity of the Fresnel integrals, *i.e.* $F_{C/S}(-x) = -F_{C/S}(x)$. Introducing back the definition of ζ we obtain

$$\mathcal{K}_0(t, r) = e^{-i\frac{Dt}{r^3}} \sqrt{\frac{\pi r^3}{6Dt}} \left[F_C\left(\sqrt{\frac{6Dt}{\pi r^3}}\right) + iF_S\left(\sqrt{\frac{6Dt}{\pi r^3}}\right) \right] \quad (57)$$

which is the final expression for the complex-valued single-pathway dipolar kernel.

D Intermolecular contribution in a homogeneous medium

In this section, we derive the product form of the intermolecular contribution. We utilize the approach by Sweger *et al.*⁵⁵ As described in the main text, in dilute (low-density) situations, we can describe an N_s -spin system in terms of its pair-wise interactions. The echo arising from a certain configuration ($\mathbf{r} = (r_1, \dots, r_b, \dots, r_{N_s})$ and $\mathbf{q} = (\theta_1, \dots, \theta_b, \dots, \theta_{N_s})$) of the N_s spins reads

$$E_N(t) = \prod_b^{N_s} E_b(t, r_b, \theta_b) = \prod_b^{N_s} \sum_k \lambda_k e^{-i\phi_{\text{dip},k}(t, r_b, \theta_b)} \quad (58)$$

We must average this expression over the whole configuration space S_R . As a configuration space, we take a Euclidean sphere of radius R and volume $V_R = 4\pi R^3/3$ with N_s different spins centered about one of the spins. The configuration S_R inside of the sphere is given by the set $\{\mathbf{r}, \mathbf{q}\}$. The configurational average reads

$$\langle E_N(t) \rangle_{S_R} = \left\langle \prod_b^{N_s} E_b(t, r_b, \theta_b) \right\rangle_{S_R} \quad (59)$$

If we can assume the spin locations for a configuration $\{\mathbf{r}, \mathbf{q}\}$ to be uncorrelated we can swap the product and the average in the above expression, yielding

$$\langle E_N(t) \rangle_{S_R} = \prod_b^{N_s} \langle E_b(t, r_b, \theta_b) \rangle_{S_R} = \langle E(t, r, \theta) \rangle_{S_R}^{N_s} \quad (60)$$

For later use, we expand this expression as follows

$$\langle E_N(t) \rangle_{S_R} = \langle 1 + E(t, r, \theta) - 1 \rangle_{S_R}^{N_s} \quad (61)$$

$$= \left\langle 1 + \sum_k \lambda_k e^{-i\phi_{\text{dip},k}(t, r, \theta)} - \sum_k |\lambda_k| \right\rangle_{S_R}^{N_s} \quad (62)$$

$$= \left\langle 1 + \sum_k |\lambda_k| \left(e^{-i(\phi_{\text{dip},k}(t, r, \theta) + \Phi_k)} - 1 \right) \right\rangle_{S_R}^{N_s} \quad (63)$$

$$= \left(1 + \sum_k |\lambda_k| \left\langle e^{-i(\phi_{\text{dip},k}(t, r, \theta) + \Phi_k)} - 1 \right\rangle_{S_R} \right)^{N_s} \quad (64)$$

where we have used the normalization condition $\sum_k |\lambda_k| = 1$ in eqn (12) and the complex definition of the pathway amplitude in eqn (11). The configuration-averaged term is given by

$$\begin{aligned} \left\langle e^{-i(\phi_{\text{dip},k}(t, r, \theta) + \Phi_k)} - 1 \right\rangle_{S_R} &= \frac{1}{V_R} \int_{S_R} d\mathbf{r} d\theta \left(e^{-i(\phi_{\text{dip},k}(t, r, \theta) + \Phi_k)} - 1 \right) \\ &= \frac{1}{V_R} C_{k,R} \end{aligned} \quad (65)$$

We can rewrite the previous expression as

$$\langle E_N(t) \rangle_{S_R} = \left(1 + \sum_k |\lambda_k| \frac{C_{k,R}}{V_R} \right)^{N_s} \quad (66)$$

$$= \left(1 + \sum_k |\lambda_k| \frac{C_{k,R}}{V_R} \right)^{c_s V_R} \quad (67)$$

$$= \left[\left(1 + \sum_k |\lambda_k| \frac{C_{k,R}}{V_R} \right)^{V_R} \right]^{c_s} \quad (68)$$

where we have introduced the concentration of spins $c_s = N_s/V_R$. At this point we have an expression for the average intermolecular echo contribution over a sphere of finite size R . In order to account for every possible intermolecular interaction in a system we must consider the limit $R \rightarrow \infty$ (and thus $V_R \rightarrow \infty$) for the previous expression which yields the intermolecular contribution to the primary DEER signal

$$V_{\text{inter}}(t) = \lim_{R \rightarrow \infty} E_{\text{tot}} S_R = \left[\exp \left(\sum_k |\lambda_k| C_k \right) \right]^{c_s} \quad (69)$$

$$= \exp \left(c_s \sum_k |\lambda_k| C_k \right) \quad (70)$$

where we have used

$$\lim_{n \rightarrow \infty} \left(1 + \frac{x}{n} \right)^n = \exp(x) \quad (71)$$

The configuration integral $C_{k,R}$ can now be evaluated in the limit $R \rightarrow \infty$. A detailed solution of this integral is provided in Appendix E. The configuration integral C_k at the limit $R \rightarrow \infty$ reads

$$C_k = \lim_{R \rightarrow \infty} C_{k,R} = -e^{i\Phi_k} (\kappa |\mathbf{s}_k \cdot \mathbf{T}(t)| + i\zeta (\mathbf{s}_k \cdot \mathbf{T}(t))) \quad (72)$$

where κ and ζ are numerical constants introduced for the sake of compactness (see eqn (92) and (93)). Inserting this expression back into the expression for $V_{\text{inter}}(t)$ yields

$$V_{\text{inter}}(t) = \exp \left(-c \sum_k |\lambda_k| e^{i\Phi_k} (\kappa |\mathbf{s}_k \cdot \mathbf{T}(t)| + i\zeta (\mathbf{s}_k \cdot \mathbf{T}(t))) \right) \quad (73)$$

$$= \exp \left(-\kappa c_s \sum_k \lambda_k |\mathbf{s}_k \cdot \mathbf{T}(t)| \right) \exp \left(-i\zeta c_s \sum_k \lambda_k (\mathbf{s}_k \cdot \mathbf{T}(t)) \right) \quad (74)$$

As in the previous section we can separate the pathways into modulated and unmodulated ones.

$$V_{\text{inter}}(t) = \exp\left(-\kappa c_s \left[\sum_{k \notin M} \lambda_k |\mathbf{s}_k \cdot \mathbf{T}_0| + \sum_{k \in M} \lambda_k |\delta_k(t - t_k)| \right]\right) \exp\left(-i\zeta c_s \left[\sum_{k \notin M} \lambda_k (\mathbf{s}_k \cdot \mathbf{T}_0) + \sum_{k \in M} \lambda_k \delta_k(t - t_k) \right]\right) \quad (75)$$

which can be rewritten as

$$V_{\text{inter}}(t) = \exp\left(-c_s \sum_{k \notin M} \lambda_k (\kappa |\mathbf{s}_k \cdot \mathbf{T}_0| + i\zeta \mathbf{s}_k \cdot \mathbf{T}_0)\right) \exp\left(-\kappa c_s \sum_{k \in M} \lambda_k |\delta_k(t - t_k)|\right) \exp\left(-i\zeta c_s \sum_{k \in M} \lambda_k \delta_k(t - t_k)\right) \quad (76)$$

which is equivalent to

$$V_{\text{inter}}(t) = \prod_{k \notin M} \exp(-c_s \lambda_k (\kappa |\mathbf{s}_k \cdot \mathbf{T}_0| + i\zeta \mathbf{s}_k \cdot \mathbf{T}_0)) \prod_{k \in M} \exp(-\kappa c_s \lambda_k |\delta_k(t - t_k)|) \exp(-i\zeta c_s \lambda_k \delta_k(t - t_k)) \quad (77)$$

We can identify the first term as a constant scaling and phasing factor $V_{\text{inter},0}$ and rewrite the above expression as

$$V_{\text{inter}}(t) = V_{\text{inter},0} \prod_{k \in M} \exp(-\kappa c_s \lambda_k |\delta_k(t - t_k)|) \quad (78)$$

$$\prod_{k \in M} \exp(-i\zeta c_s \lambda_k \delta_k(t - t_k)) \quad (79)$$

The multi-pathway dipolar intermolecular contribution is the *product* of the individual contributions arising from the individual pathways, each with different refocusing times and different decay rates $\kappa c_s \lambda_k$. This is in contrast to the intramolecular contribution, which is a *sum* over all individual pathway signals. The underlying reason is the exponential form of the intermolecular contributions, since a sum in an exponent converts into a product of the functions.

The first product in eqn (79) collects the decay of the intermolecular contribution to the dipolar signal. The second product in eqn (79) represents a time-dependent phase-shift, that is particularly prominent under large spin polarization at low temperatures.⁵⁵ Therefore, in the high-temperature limit this phase-shift averages out and can be safely neglected. In this limit the contributions of the $\mathbf{s}_k$ and $-\mathbf{s}_k$ pathways are again equivalent, and we can combine them into a paired pathway $\mathbf{s}_p$ leading to a simpler expression for the

intermolecular contribution

$$V_{\text{inter}}(t) = V_{\text{inter},0} \prod_{p \in M} \exp(-\kappa c_s \lambda_p |\delta_k(t - t_p)|) \quad (80)$$

E Integral over all space

Here, we solve the integral $C_{k,R}$ of eqn (65) at the limit $R \rightarrow \infty$, following the approach by Sweger *et al.*⁵⁵ $C_{k,R}$ is best determined in spherical coordinates

$$C_k = \lim_{R \rightarrow \infty} C_{k,R} \quad (81)$$

$$= \int_0^{2\pi} d\varphi \int_0^\pi d\theta \sin \theta \int_0^\infty dr r^2 \left[e^{-i(\phi_{\text{dip},k}(t,r,\theta) + \Phi_k)} - 1 \right] \quad (82)$$

which can be rewritten as

$$C_k = 2\pi \int_0^\pi d\theta \sin \theta \int_0^\infty dr r^2 \left[e^{-i(q_k(1-3\cos^2\theta)/r^3 + \Phi_k)} - 1 \right] \quad (83)$$

where $q_k = D \mathbf{s}_k \cdot \mathbf{T}(t)$. We introduce again the substitution $z = \cos \theta$ which leads to an integral in the range $[-1,1]$. However since the integrand is an even function, we can restrict the integration to $[0,1]$ and adding a factor of 2, as follows

$$C_k = 4\pi \int_0^1 dz \int_0^\infty dr r^2 \left[e^{-i(q_k(1-3z^2)/r^3 + \Phi_k)} - 1 \right] \quad (84)$$

Expanding the complex exponential integral in terms of trigonometric functions gives

$$C_k = 4\pi \int_0^1 dz \int_0^\infty dr r^2 \left(e^{i\Phi_k} \cos(q_k(1-3z^2)/r^3) - 1 \right) - i4\pi e^{i\Phi_k} \int_0^1 dz \int_0^\infty dr r^2 \sin(q_k(1-3z^2)/r^3) \quad (85)$$

Both inner integrals are improper integrals not defined for $r = 0$. The first inner integral can be solved according to

$$\lim_{\varepsilon \rightarrow 0} \int_\varepsilon^\infty dr r^2 \left[e^{i\Phi_k} \cos\left(\frac{b}{r^3}\right) - 1 \right] = -\frac{\pi}{6} e^{i\Phi_k} |b| \quad (86)$$

and the second double integral can be solved according to

$$\lim_{\varepsilon \rightarrow 0} \int_0^1 dz \int_\varepsilon^\infty dr r^2 \sin\left(\frac{q_k}{r^3}\right) = \left[\frac{2}{9} - \frac{4}{9\sqrt{3}} \text{acoth}(\sqrt{3}) \right] q_k \quad (87)$$

leading to the expression

$$C_k = -\frac{2\pi^2}{3} e^{i\Phi_k} \int_0^1 dz |q_k(1-3z^2)| - i e^{i\Phi_k} \left[\frac{8\pi}{9} - \frac{16\pi}{9\sqrt{3}} \text{acoth}(\sqrt{3}) \right] q_k \quad (88)$$

$$= -e^{i\Phi_k} \left(\frac{2\pi^2}{3} D |\mathbf{s}_k \cdot \mathbf{T}(t)| \int_0^1 dz |(1-3z^2)| + i \left[\frac{8\pi}{9} - \frac{16\pi}{9\sqrt{3}} \text{acoth}(\sqrt{3}) \right] D \mathbf{s}_k \cdot \mathbf{T}(t) \right) \quad (89)$$

where we have used the fact $|D| = D$. The remaining integral can be evaluated analytically to obtain the result

$$C_k = -e^{i\Phi_k} \left(\frac{8\pi^2}{9\sqrt{3}} D |\mathbf{s}_k \cdot \mathbf{T}(t)| - i \left[\frac{8\pi}{9} - \frac{16\pi}{9\sqrt{3}} \operatorname{acoth}(\sqrt{3}) \right] D \mathbf{s}_k \cdot \mathbf{T}(t) \right) \quad (90)$$

$$= -e^{i\Phi_k} (\kappa |\mathbf{s}_k \cdot \mathbf{T}(t)| + i\zeta \mathbf{s}_k \cdot \mathbf{T}(t)) \quad (91)$$

where

$$\kappa = \frac{8\pi^2}{9\sqrt{3}} D \approx 5.065D \quad (92)$$

and

$$\zeta = \left[\frac{8\pi}{9} - \frac{16\pi}{9\sqrt{3}} \operatorname{acoth}(\sqrt{3}) \right] D = \frac{8\pi^2}{9\sqrt{3}} \frac{\sqrt{3} + \ln(2 - \sqrt{3})}{\pi} D \approx 0.669D \quad (93)$$

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