ELDOR-detected NMR: A general and robust method for electron-nuclear hyperfine spectroscopy?

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Keywords: EPR, NMR, ENDOR, ELDOR-detected NMR (EDNMR), nitroxide radical, hyperfine interaction

ABSTRACT

ELDOR-detected NMR (EDNMR) performed at higher magnetic fields is becoming an increasingly popular alternative to conventional ENDOR for the characterization of electron-nuclear hyperfine interactions owing to its enhanced sensitivity. However there are two key problems that limit its widespread adoption, with factors controlling: (i) lineshape distortions and; (ii) overall spectral resolution, still largely understood only at a qualitative level. Indeed highly anisotropic (dipolar) coupled species are particularly problematic in the EDNMR experiment. Nor is it clear as to whether line intensities measured in EDNMR can provide quantitative information. Here we describe how all these problems can be overcome for a nitroxide radical as model system. We introduce a simulation procedure/protocol for the simulation of EDNMR line-shapes collected over a range of high turning angle (HTA) pulse lengths. It is shown that spectral line-shapes can be robustly reproduced and that the intensities of spectral lines and the spin nutation behavior can be quantitatively assessed. This broadens the scope of the EDNMR experiment as a generally applicable, quantitative double resonance method.

GRAPHICAL ABSTRACT

1. INTRODUCTION

Electron-electron Double Resonance (ELDOR)-detected NMR (EDNMR) is growing in popularity as a means to characterize the hyperfine structure of complex chemical systems. First developed in the Schweiger laboratory [1], it uses selective microwave pulses which simultaneously pump EPR and NMR transitions of the spin manifold, so called spin forbidden transitions, where both the electron and nuclear spins change their projection direction ($\Delta M_S = \pm 1$, $\Delta M_I = \pm 1, \pm 2, \dots$). As compared to conventional Electron Nuclear Double Resonance (ENDOR) techniques it has a much higher sensitivity and does not exhibit nucleus dependent or pulse dependent spectral artifacts [2-5]. Such artifacts include: B_2 non-linearity, often preventing hetero-nuclear detection, nucleus dependent blind-spotting and additional harmonic frequencies and line-intensity modification due to hyperfine enhancement. It is also far more robust against fast T_1 relaxation allowing a greater range of chemical systems to be studied. The EDNMR experiment does however typically resolve broader spectral lines than the corresponding ENDOR experiment [3, 5]. In the text below we explain how this effect can however be overcome for many paramagnetic systems, including organic radicals and especially transition metal complexes.

Historically the wide spread adoption of EDNMR has been hampered by what is termed the central blind spot. Spin forbidden transitions which are sufficiently close in frequency to the allowed EPR transition are masked due to the simultaneous pumping of this allowed transition i.e. transitions within 5-10 MHz. Similarly, resonator bandwidth limitations lead to suppression of forbidden transitions which are far off resonance with the allowed EPR transition. Both of these problems are mitigated by performing the EDNMR experiment at higher magnetic fields as demonstrated by a number of recent studies [2-4, 6-21]. At such fields, many types weakly coupled nuclei, whose spectral position is chiefly governed by their nuclear Zeeman interaction (Larmor frequency), are shifted outside the central blind spot. Similarly, at the higher microwave frequencies used at higher magnetic fields, broadband resonance structures are more easily achieved without lowering the loaded quality factor (Q_L -value) of the cavity, i.e. without significant loss of sensitivity.

Questions though remain as to the general applicability of the EDNMR technique. As with Electron Spin Echo Envelope Modulation (ESEEM), EDNMR line intensities depend upon the probability of forbidden transitions which arise due to anisotropic (dipolar) hyperfine, nuclear quadrupole and/or zero-field interactions [22]. This has the practical consequence that the intensity of EDNMR transitions vanishes for orientations which coincide with the

canonical axes of these interaction tensors, leading to a distorted NMR spectrum as compared to ENDOR. For spin systems where several interactions exist this effect is less pronounced i.e. where both a hyperfine and quadrupole term ($I \geq 1$) contribute to the EDNMR line-intensity, as the principal axes for each interaction tensor typically do not coincide and thus the intensity is partially recovered in canonical orientation of any particular interaction tensor. But for the $I = 1/2$ nuclei, such as ^1H , ^{13}C , ^{15}N , ^{31}P , as only one hyperfine interaction is present, EDNMR line-shapes can be potentially distorted, see for example the ^1H -EDNMR spectrum of dipolar coupled protons in for the $\text{Mn}^{\text{II}}(\text{H}_2\text{O})_6$ complex [2].

Here we describe how this final limitation can be overcome, using the model nitroxide radical system. It is shown that by optimizing the HTA pulse length/amplitude, an ENDOR like line-shape can in most cases be achieved in the EDNMR experiment, regardless of the type of nucleus or nature of the hyperfine interaction i.e. isotropic vs. dipolar. We introduce a simulation procedure/protocol for the simulation of EDNMR line-shapes collected over a range of high turning angle (HTA) pulse lengths. Using this simulation protocol, both spectral line-shapes and the relative intensities can be interpreted, providing quantitative information on the magnitude and number of electron-nuclear contributions of the spin system of interest.

2. MATERIALS AND METHODS

2.1 Sample preparation. The perdeuterated 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolin-1-oxyl radical in its ^{14}N ($\text{R1-}^{14}\text{N-D}_{16}$) and ^{15}N ($\text{R1-}^{15}\text{N-D}_{16}$) form were synthesized as described previously. The solvents, methanol- D_3 (CD_3OH), toluene- D_8 and MTHF, were obtained from Fluka and Aldrich in their purest commercially available form and used without further purification. The nitroxide radical was directly dissolved in methanol- D_3 or toluene- D_8 /MTHF (90%/10% vol.). The 2-Methyltetrahydrofuran (MTHF) as cosolvent was used to improve the glass forming properties of toluene- D_8 and avoid the aggregation of nitroxide radicals due to intermolecular nitroxide-nitroxide hydrogen-bonding. The sample solution, containing 1 mM of nitroxide radical, was transferred into a quartz capillary (I.D. = 0.6 mm) for the W-band EPR measurements. A glass-type frozen solution was obtained by transferring the sample into the precooled EPR cryostat.

2.2 W-band pulse EPR measurements. High-field EPR experiments were performed using a modified W-band EPR spectrometer (Bruker Elexsys E680), equipped with a homebuilt ENDOR microwave cavity optimized for maximum RF performance at low RF frequencies ($< 100\text{MHz}$, optimum performance at 20 MHz) [4]. Electron spin echo-detected (ESE) field-swept spectra were measured using the Hahn echo pulse sequence:

$t_p - \tau - 2t_p - \tau$ -echo with $t_p = 50$ ns and $\tau = 200$ –500 ns. EDNMR measurements were performed using the pulse sequence: $t_{\text{HTA}} - T - t_p - \tau - 2t_p - \tau$ -echo. The high tuning angle (HTA) microwave pulse was applied at microwave frequency ν_1 . The detection Hahn echo pulse sequence at microwave frequency ν_0 matched to the cavity resonance was set 2 μ s post the HTA pulse to ensure the decay of the electron spin coherence (phase memory time of R1- ^{14}N -D $_{16}$ in toluene-D $_8$ /MTHF is about 4 μ s at 50 K). The pulse length for a detection $\pi/2$ pulse $t_p = 100$ ns and the pulse separation $\tau = 200$ ns were generally set. The echo was integrated 1400 ns around its maximum. The spectra were acquired continuously sweeping the HTA frequency ν_1 at fixed B_0 values. HTA parameters are given in the corresponding figure captions. The ^{14}N -, ^1H -ENDOR experiments were performed using a Davies-type pulse sequence: $t_{\text{inv}} - t_{\text{RF}} - T - t_p - \tau - 2t_p - \tau$ -echo with $t_{\text{inv}} = 200$ ns, $t_{\text{RF}} = 20$ μ s, $t_p = 32$ ns, $T = 3$ μ s and $\tau = 372$ ns for ^1H and $t_{\text{inv}} = 128$ ns, $t_{\text{RF}} = 20$ μ s, $t_p = 56$ ns, $T = 3$ μ s and $\tau = 372$ ns for ^{14}N . The D-ENDOR experiments were performed using a Mims-type pulse sequence: $t_p - \tau - t_p - t_{\text{RF}} - T - t_p - \tau$ -echo with $t_p = 32$ ns, $\tau = 400$ ns, $t_{\text{RF}} = 20$ μ s, $T = 3$ μ s.

3. THEORY

3.1 The spin Hamiltonian. Here we consider a single unpaired electron system ($S=1/2$), coupled to a single nuclear spin ($I \geq 1/2$). A basis set that describes the spin manifold can be built from the product of the eigenstates of the interacting spins:

$$\phi_n = \left| \frac{1}{2} \ M_S \right\rangle \otimes \left| I \ M_I \right\rangle \quad (1)$$

The number of basis vectors n is $(2S+1)(2I+1)$. M_S refers to the electronic magnetic sub-level ($\pm 1/2$), I refers to the nuclear spin state and M_I refers to the nuclear magnetic sub-level which takes the values $-I, 1-I, \dots, I-1, I$. The effective spin Hamiltonian that describes the spin manifold is:

$$\hat{H}_0 / h = \beta \cdot \vec{B}_0 \cdot \vec{g} \cdot \vec{S} - g_n \cdot \beta_n \cdot \vec{B}_0 \cdot \vec{I} + \vec{S} \cdot \vec{A} \cdot \vec{I} + \vec{I} \cdot \vec{P} \cdot \vec{I} \quad (2)$$

It contains: (i) an electronic Zeeman term; (ii) a nuclear Zeeman term; (iii) an electron-nuclear hyperfine term; and (iv) a nuclear quadrupole term. The incident microwave radiation is described by the B_1 field and its effect on the spin system is described by the spin Hamiltonian:

$$\hat{H}_1 / h = \beta \cdot \vec{B}_1 \cdot \vec{g} \cdot \vec{S} - g_n \cdot \beta_n \cdot \vec{B}_1 \cdot \vec{I} \quad (3)$$

And corresponding transition probability (I_{ik}) for the transitions between the i^{th} and k^{th} spin levels of the manifold:

$$I_{ik} = \left| \langle \psi_k | \hat{H}_1 | \psi_i \rangle \right|^2 \quad (4)$$

Where $\psi_k = \sum_n c_n \cdot \phi_n$ are the eigenstates of the spin Hamiltonian, Eq. (2), representing a linear combination of the original basis, Eq. (1).

3.2 $S = 1/2$, $I = 1/2$ analytical solution. For the purposes of describing the EDNMR technique we briefly describe the two system, $S = 1/2$, $I = 1/2$ for which an analytical solution exists, for full description see chapter 1 in [22]. The spin Hamiltonian assuming an isotropic g-tensor and anisotropic hyperfine tensor re-expressed in the rotating frame is:

$$\hat{H} / h = \Omega_S \cdot S_Z + \nu_n \cdot I_Z + \vec{S} \cdot \mathbf{A} \cdot \vec{I} \quad (5)$$

Where $\Omega_S = \nu_e - \nu_{mw} = g_e \cdot \beta \cdot \Delta B_0 / h$ and $\nu_n = -g_n \cdot \beta_n \cdot B_0 / h$. The anisotropic hyperfine tensor is assumed to be axially symmetric having principal values $A_{\parallel}$ and $A_{\perp}$. There are six possible transitions within the four level model: two nuclear (NMR, $\Delta M_I = \pm 1$) transitions (α , β), two allowed EPR ($\Delta M_S = \pm 1$) transitions (a, d) and two forbidden EPR ($\Delta M_S = \pm 1$ and $\Delta M_I \pm 1$) transitions (b, c) with frequencies expressed in linear frequency units (see **Fig. 1**):

$$\begin{aligned} \text{Nuclear (NMR):} \quad \nu_{\alpha, \beta} &= \sqrt{\left(\nu_n \pm \frac{A}{2} \right)^2 + \frac{B^2}{4}} \\ \text{Allowed (EPR):} \quad \nu_{a, d} &= \Omega_S \pm (\nu_{\alpha} - \nu_{\beta}) \\ \text{Forbidden (ELDOR):} \quad \nu_{b, c} &= \Omega_S \pm (\nu_{\alpha} + \nu_{\beta}) \end{aligned} \quad (6)$$

where $A = A_{\parallel} \cos^2 \theta + A_{\perp} \sin^2 \theta$ and $B = (A_{\parallel} - A_{\perp}) \cdot \cos \theta \cdot \sin \theta$ where θ describes the polar angle between the $A_{\parallel}$ direction and B_0 . In this manuscript we will refer to the forbidden EPR transitions as ELDOR transition as these are the EPR transitions that are exploited in EDNMR experiment. The corresponding normalized transition probabilities for the allowed and spin forbidden transitions are:

$$I_a = I_d = \frac{\left| \nu_n^2 - \frac{1}{4} (\nu_{\alpha} - \nu_{\beta})^2 \right|}{\nu_{\alpha} \nu_{\beta}} \quad (7)$$

$$I_b = I_c = \frac{\left| \nu_n^2 - \frac{1}{4} (\nu_{\alpha} + \nu_{\beta})^2 \right|}{\nu_{\alpha} \nu_{\beta}} \quad (8)$$

The assignment of each transition as allowed or forbidden in Eqs. (6-8), is valid only for the weak coupling regime, $|A_{\parallel}|, |A_{\perp}| < 2 \cdot |\nu_n|$. In the strong coupling regime, $|A_{\parallel}|, |A_{\perp}| > 2 \cdot |\nu_n|$, the assignment of allowed and forbidden transitions has to be interchanged, i.e. $\nu_{a, d}$ –allowed and

$\nu_{b,c}$ –forbidden. For the case $|A_{||}| > 2 \cdot |\nu_n|$, $|A_{\perp}| < 2 \cdot |\nu_n|$ the change from the weak to the strong coupling limit takes place at a polar angle of

$$\cos 2\theta = \frac{8 \cdot \nu_n^2 - (A_{||}^2 + A_{\perp}^2)}{A_{||}^2 - A_{\perp}^2} \quad (9)$$

For the nitroxide radical at W-band the $^{14}\text{N}/^{15}\text{N}$ hyperfine interaction falls in the weak coupling regime for molecular orientations where $A_{\perp}$ is aligned with B_0 ($\theta = 90^\circ$ i.e. x-y plane) and in the strong coupling regime for orientations where $A_{||}$ is aligned with B_0 ($\theta = 0^\circ$ i.e. z axis), with $\theta = 79.5^\circ$ (for $A(^{15}\text{N}) = [-16; 16; 133]$ MHz and $B_0 = 3.34$ T) representing the crossing point.

3.3 The EDNMR experiment. The EDNMR experiment requires two discrete spin operations: a preparation (pump) microwave pulse and a subsequent detection (probe) pulse/sequence to read out the effect of the first pulse [1]. The probe sequence is performed at a fixed frequency, ν_0 , matched to the operating frequency of the microwave resonator whereas the pump pulse, ν_1 , is scanned about ν_0 . The resultant spectrum contains two basic features: (i) when the pump pulse frequency, ν_1 , matches ν_0 , the observed signal is diminished. This feature is termed the central hole and defines the zero frequency in the EDNMR experiment; and (ii) when the pump pulse frequency instead comes on resonance with another transition of the manifold the signal is also diminished, but to a lesser extent. These ‘side holes’ predominately represent the pumping of forbidden transitions of the manifold and match the frequencies of the NMR transitions of the spin manifold, relative to ν_0 . The pump pulse is termed the high-turning-angle (HTA) pulse as it overrotates the allowed EPR transitions of the spin manifold.

Fig. 1 Four energy-level scheme for the $S = 1/2$, $I = 1/2$ spin system. Both the strong coupling limit (a) and weak coupling limit (b) are shown. The blue, red and green lines show the EPR, NMR and forbidden (ELDOR) transitions of the spin manifold. The four eigenstates are labeled using a shorthand + and - (without $1/2$) in the diagram e.g. $|+,->$ represents $|+1/2, -1/2>$. The same color code is used for the corresponding stick spectrum. The top spectrum shows the relative energies of all four EPR/ELDOR transitions. The stick spectra below show the corresponding EDNMR spectrum representing from the pumping of two ELDOR transitions associated with each allowed EPR transition. In the strong coupling limit, the position of the EDNMR lines has the same sign relative to the EPR transition. In the weak coupling limit the EDNMR lines instead take the opposite sign. Under non-selective conditions, a symmetric EDNMR spectrum is observed regardless of the coupling regime. For selective detection RHS and LHS denote right hand side ($\Delta\nu = \nu_1 - \nu_0 > 0$) and left hand side ($\Delta\nu = \nu_1 - \nu_0 < 0$) of EDNMR spectrum, respectively. The stick diagram for nonselective detection is indicated by NS.

Figure 1 shows the allowed (EPR), forbidden (ELDOR) and NMR transitions of $S = 1/2$, $I = 1/2$ spin system in both the weak coupling and strong coupling limit. Depending on which transition of the spin manifold is on resonance i.e. falls within the microwave excitation bandwidth of the detection sequence at ν_0 , the resulting EDNMR spectrum changes. In the

instance where more than one transition fall within the excitation bandwidth, the resulting EDNMR pattern is the sum of each individual pattern. There are two contributions to the EDNMR pattern (**Fig. 2**): (i) from the pumping of forbidden (ELDOR) transitions associated with the allowed (EPR) transitions; and (ii) from the pumping of the allowed (EPR) transitions associated with the forbidden (ELDOR) transition. For $S = 1/2$, $I = 1/2$ and strong coupling limit there are twelve possible energy difference combinations of which eight contribute to the EDNMR spectrum.

$$\begin{array}{cccc}
\nu_a, I_a & \nu_b, I_b & \nu_c, I_c & \nu_d, I_d \\
\Delta_{ba} = \nu_b - \nu_a & \Delta_{ab} = \nu_a - \nu_b & \Delta_{ac} = \nu_a - \nu_c & \Delta_{bd} = \nu_b - \nu_d \\
= +\nu_\alpha & = -\nu_\alpha & = -\nu_\beta & = -\nu_\beta \\
\Delta_{ca} = \nu_c - \nu_a & \Delta_{db} = \nu_d - \nu_b & \Delta_{dc} = \nu_d - \nu_c & \Delta_{cd} = \nu_c - \nu_d \\
= +\nu_\beta & = +\nu_\beta & = +\nu_\alpha & = -\nu_\alpha
\end{array} \quad (10)$$

Note that the energy difference between transitions of the same type can be excluded i.e. a transition between two allowed transitions: $|\Delta_{ad}| = |\Delta_{da}| = |\nu_d - \nu_a| = |\nu_\alpha + \nu_\beta|$ or two spin forbidden transitions $|\Delta_{bc}| = |\Delta_{cb}| = |\nu_b - \nu_c| = |\nu_\alpha - \nu_\beta|$. This is because the pumping of a transition can only be detected if the transition has one level in common with the probe transition (see **Fig.1**). In practice, transitions at $|\Delta_{ad}|$ are observed, but these arise from the magnetization transfer (which can be caused by electron spin diffusion, nuclear relaxation, and slow rotational motions) from pumped allowed off-resonance EPR transitions [23] and not via an ELDOR effect. Finally it is noted: In the strong coupling limit, the frequencies of the two forbidden transitions associated with each allowed transition are either greater or smaller than that of the allowed transition, i.e. the EDNMR signals will appear at either $\Delta\nu = \nu_1 - \nu_0 > 0$ (right hand side, RHS) or $\Delta\nu = \nu_1 - \nu_0 < 0$ (left hand side, LHS). In contrast in the weak coupling limit, the forbidden transitions fall both below and above the allowed transition. Thus the asymmetry of the EDNMR pattern reveals information on the hyperfine coupling regime and the relative sign of the hyperfine coupling. However for asymmetry to be observed the hyperfine/quadrupole interaction needs to be larger than the EPR linewidth.

3.4 EDNMR line-intensities. As in the corresponding ENDOR experiment, the transition intensities of EDNMR lines are governed by the transition probability connecting the eigenstates which define the transition. This intrinsic intensity however can be modulated by the choice of the HTA pulse, leading to the enhancement and suppression of different

EDNMR lines relative to each other. As a consequence, meaningful line-intensity information is only obtained by measuring the power dependence of EDNMR lines, within the approximation that the excitation bandwidth remains constant.

Fig. 2 ELDOR stick spectrum for the $S = 1/2$, $I = 1/2$ spin system in the strong coupling limit. All four components of the EDNMR spectrum are shown i.e. the pump excitation for the transitions a to d centered about their respective probe frequency a to d. The * represents the transition within the spin manifold not seen in the EDNMR experiment (see text).

The effective flip angle of the HTA pulse connecting the ELDOR active levels are defined in terms of the transition probability connecting the two states (see Eq. (4)):

$$\begin{aligned}\beta_a = \beta_d &= \omega_1 \cdot t_{HTA} \cdot \sqrt{I_a} = \beta_0 \cdot \sqrt{I_a} \\ \beta_b = \beta_c &= \omega_1 \cdot t_{HTA} \cdot \sqrt{I_b} = \beta_0 \cdot \sqrt{I_b}\end{aligned}\tag{11}$$

Where $\omega_1 = g_e \cdot \beta \cdot B_0 / \hbar$ and β_0 represents the nominal flip angle. The product $\omega_1 \cdot t_{HTA}$ determines the efficiency of the HTA pulse. As described above, (see **Fig. 2**) each EDNMR line is made up of two components under conditions of nonselective detection [1]. For example, the EDNMR peak corresponding to the positive NMR frequency α , is derived from pumping transition b, as detected by a (Δ_{ba} , **Fig. 2**), and pumping d as detected by c (Δ_{dc}). These two contributions to the EDNMR line intensity (W) correspond to the product of the

transition probability of the probe transition multiplied by the transition intensity of the pump frequency [1], i.e.

$$\begin{aligned} W_{EDNMR} &= 1 - I_a \cdot \cos(\beta_b) - I_c \cdot \cos(\beta_d) \\ &= 1 - I_a \cdot \cos(\beta_b) - I_b \cdot \cos(\beta_a) \end{aligned} \quad (12)$$

A similar equation can be deduced for the intensity of the central blind spot.

$$W_{Center} = 1 - I_a \cdot \cos(\beta_a) - I_b \cdot \cos(\beta_b) \quad (13)$$

A more general approach to model any paramagnetic system would be to treat the system using the density operator formalism [10, 24, 25], which would accommodate relaxation phenomena and spin/spectral diffusion, necessary for understanding the dynamic nuclear polarization transfer mechanisms. While this is the most rigorous approach, it is our opinion that it is not necessary for the simulation of EDNMR spectra. Importantly when using the density operator formalism, the spin Hamiltonian needs to be evaluated over all frequency offsets. This can become computationally expensive in the case of complex spin systems, especially those containing multiple nuclear spins, which give rise to combination transitions.

As we will show below, EDNMR experiments should be optimized to minimize spin and spectral diffusion effects to yield high resolution quantitative data. We also note that we do not consider it necessary to explicitly include the detection sequence, as the mode of detection does not influence the resultant EDNMR spectrum, other than the resolution.

A simpler approach for the calculation of EDNMR transition intensities for any arbitrary spin system is to model the effect of the HTA pulse using the Bloch equations [26], expressed in the rotating frame. The equations of motion for each transition (k) of the spin manifold, with transition probability I_k are:

$$\begin{aligned} \frac{dM_{k,x}}{dt} &= -\frac{M_{k,x}}{T_m} \\ \frac{dM_{k,y}}{dt} &= -\frac{M_{k,y}}{T_m} - \omega_1 \cdot \sqrt{I_k} \cdot M_{k,z} \\ \frac{dM_{k,z}}{dt} &= \omega_1 \cdot \sqrt{I_k} \cdot M_{k,y} \end{aligned} \quad (14)$$

Here we exclude the static magnetic field and only consider the effect of the HTA pulse and the loss of the coherence primarily due to T_2 relaxation. However, for the simulation of experimental spectra T_2 is replaced by the phase memory time, T_m , which also accounts for

spectral diffusion processes. Re-expressing the set of ordinary differential equations as a matrix yields:

$$\frac{d}{dt}\mathbf{M}_k(t) = \begin{bmatrix} -\frac{1}{T_m} & 0 & 0 \\ 0 & -\frac{1}{T_m} & -\omega_1 \cdot \sqrt{I_k} \\ 0 & \omega_1 \cdot \sqrt{I_k} & 0 \end{bmatrix} \cdot \begin{bmatrix} M_{k,x}(t) \\ M_{k,y}(t) \\ M_{k,z}(t) \end{bmatrix} = \mathbf{T}_k \cdot \begin{bmatrix} M_{k,x}(t) \\ M_{k,y}(t) \\ M_{k,z}(t) \end{bmatrix} \quad (15)$$

Taking the matrix exponent of $\mathbf{T}$ allows the $\mathbf{M}$ vector to be solved for an arbitrary time evolution i.e. length of the HTA pulse. Note at $t = 0$ (thermal equilibrium) the M vector is aligned along z.

$$\mathbf{M}_k(t_{HTA}) = e^{\mathbf{T}_k t_{HTA}} \cdot \mathbf{M}_k(t_0) = e^{\mathbf{T}_k t_{HTA}} \cdot \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} \quad (16)$$

From this the corresponding EDNMR transition intensity for the k^{th} contribution to the EDNMR pattern can be calculated:

$$W_k = \frac{1 - M_{k,z}(t_{HTA})}{2} \quad (17)$$

Note that we are only interested in the z-component of the magnetization vector. The x/y components do not contribute to the EDNMR spectrum, dephasing during the delay between the HTA and the start of the detection sequence. The denominator of 2 normalizes the effect of the HTA pulse to be a maximum of 1, i.e. the M_z magnetization runs from +1 (no inversion) to -1 (complete inversion). The saturation condition occurs for $M_z = 0$.

Thus Eq. (12) can then be re-expressed in the general form which includes the effect of T_2 relaxation:

$$W_{EDNMR} = I_a \cdot W_b + I_b \cdot W_a \quad (18)$$

In practice not all ‘probe’ transitions contribute equally to the observed echo. To account for this each contribution to the intensity function is weighted by a Gaussian function which describes the frequency offset between the probe frequency and the operating frequency of the resonator.

$$W_{EDNMR} = G(\omega_a - \omega_{mw}, \sigma) \cdot I_a \cdot W_b + G(\omega_b - \omega_{mw}, \sigma) \cdot I_b \cdot W_a \quad (19)$$

where σ is the excitation bandwidth. An analogous approach is used to implement orientation selectivity in ENDOR. We note however that this procedure does not completely capture the selectivity of the EDNMR experiment, which is discussed in **section 4.1.3**.

3.5 General simulation procedure. The general procedure for calculation of the EDNMR spectrum is as follows:

- 1) Solve eigenvectors and eigenfunctions of the spin Hamiltonian
- 2) Calculate all possible energy differences of the spin ladder
- 3) Calculate the transition intensities using the Bloch equations for the pumped transition

When implementing this procedure we make no distinction as to the ‘identity’ of the transition, i.e. as allowed or forbidden. We simply apply the same procedure for all combinations of pump and probe pulse and as a final step add all contributions together. In this way we ensure that we capture all contributions to each EDNMR line, with the correct transition weighting. This then allows simulation of any arbitrary spin system such as the nitroxide radical for which the $^{14}\text{N}/^{15}\text{N}$ hyperfine interaction falls in the weak or strong coupling regime depending on magnetic field position measured leading to reassignment of the transition identities, see Eq. (9). Similarly the procedure allows calculation of combination transitions involving more than one nucleus.

General formulae for the spectra position and intensity of each contribution to the EDNMR spectrum can be expressed in terms of three indices (i, k, l) defined as follows. The frequency difference (Δ_{ikl}) in linear units, between the pump transition between the k^{th} and l^{th} magnetic sublevels, associated with the probe transition between the i^{th} and l^{th} sublevels and corresponding transition intensity (ζ_{ikl}) are:

$$\begin{aligned} \Delta_{ikl} &= (E_k - E_l) - (E_k - E_i) = \nu_{lk} - \nu_{ik} \\ \zeta_{ikl} &= G(\nu_{ik} - \nu_{mw}, \sigma) \cdot I_{ik} \cdot W_{lk} \end{aligned} \quad (20)$$

where $I_{ik} = \left| \langle \psi_k | \hat{H}_1 | \psi_i \rangle \right|^2$. W_{lk} is calculated using Eqs. (15-17) with the flip angle for the transition of $\beta_{lk} = \omega_1 \cdot \beta_0 \cdot \sqrt{I_{lk}}$.

Similarly, the magnitude of the probe transition associated with the pumped transition described by Eq. (21), which contributes the central blind spot centered at $\Delta_{ikl} = 0$ can be calculated in the exactly the same way:

$$\zeta_{ik} = q \cdot \sum_k \sum_i G(\nu_{ik} - \nu_{mw}, \sigma) \cdot I_{ik} \quad (21)$$

The W_{lk} term is absent as time evolution of the detection is not included. This is because the simulation assumes that the saturation condition is always met for the probe transition. The parameter q represents the number of transitions which contribute to the central blind spot. In the case of selective excitation $q = 1$. In the case of non-selective excitation it takes a positive integer value e.g. for the system represented in **Fig. 2**, $q = 2$. Thus, for the theoretical maximum EDNMR response under selective conditions it is 1, normalized to the central blind spot. In contrast, under non-selective conditions the maximum response is less than 1. As an example, for the $S = 1/2$, $I = 1/2$ spin system the maximum response is 0.5, i.e. the blind spot is twice the size of the EDNMR signal as it is distributed over both sides (positive and negative) of the EDNMR spectrum.

3.6 Measuring the B_1 field strength: the central blind spot. As the formulae above include the B_1 field strength, this parameter needs to be accurately measured to simulate EDNMR data. The excitation function of the rectangular microwave pulse of length t_p and amplitude ω_1 is given by [27]:

$$f = \left(\frac{\omega_1}{\omega_{eff}} \right)^2 \cdot \sin^2(\omega_{eff} \cdot t_p / 2) \quad (22)$$

where $\omega_{eff}^2 = \Delta\omega^2 + \omega_1^2$, and $\Delta\omega = 2\pi \cdot (\nu_l - \nu_0)$ is the frequency difference between the ELDOR and detection mw frequencies and ω_1 is the microwave field strength. In the case of long HTA pulses, $\omega_{eff} \cdot t_{HTA} \gg 1$, the lineshape of the central blind spot should approximate a Lorentzian function [3].

$$C(\Delta\nu) = \left(\frac{\omega_1}{\omega_{eff}} \right)^2 = \frac{\omega_1^2}{\Delta\omega^2 + \omega_1^2} \quad (23)$$

with FWHM (full width at half maximum) of $2 \cdot \omega_1$, or $\Delta\nu_{1/2} = \omega_1/\pi$ in linear frequency units. Physically what happens is that the oscillations along the $\Delta\omega$ dimension become so dense that it is safe to neglect them due to spectral diffusion during the pulse. This result is important

because the lineshape of the central hole is independent of the HTA pulse length and thus the width of the central blind spot can be used to measure ω_1 (B_1 field strength).

3.7 Correction to the B_1 field to account for the bandwidth of the resonator. Finally, we note that Eq. 15 assumes that the effective flip angle for pumping a spin forbidden transition is constant. This is not the case, as the resonator in which the EDNMR experiment is performed has a finite bandwidth. In practice this means that as the pump frequency, ν_1 , shifts away from the resonator frequency ν_0 the HTA pulse must be increased in amplitude to compensate for the effective decrease in the B_1 field, an effect similar to B_2 field non-linearity as seen in pulse ENDOR. Importantly, in EDNMR this instrumental line-intensity modification is predictable and can be taken into account in the simulation package described above. The relative amount of microwave power entering the cavity at frequency ν_1 is equal to the square of the B_1 field. In case that there is no frequency dependence of the excitation microwave power, this power is determined by the frequency dependence of the cavity reflection coefficient $|\Gamma|$. For a critically coupled cavity this relative power is given by

$$\frac{P(\nu_1)}{P(\nu_0)} = \left(\frac{\omega_1(\nu_1)}{\omega_1(\nu_0)} \right)^2 = 1 - |\Gamma|^2 = \left[1 + \left(2 \cdot Q_L \cdot \frac{\nu_1}{\nu_0} \right)^2 \right]^{-1} \quad (24)$$

This factor is then used to scale ω_1 which appears in Eq. (15).

$$\omega_1(\Delta_{ikl}) = \omega_1(\nu_0) \cdot \left[1 + \left(2 \cdot Q_L \cdot \frac{\Delta_{ikl}}{\nu_0} \right)^2 \right]^{-1/2} \quad (25)$$

with Δ_{ikl} defined by Eq. (21), ν_0 the resonator frequency and Q_L the loaded quality factor of the resonator. The effect of this correction is best seen for broad transitions, which fall both within and outside the resonator bandwidth. Lines further from the resonator frequency are suppressed, falling off following a Lorentzian profile, see Eq. (23). The Q_L -factor bandwidth of the resonator can be experimentally determined by performing a microwave nutation experiment. This is achieved by measuring the inversion pulse length of the allowed EPR transition as a function of the operating frequency around the resonator frequency. Note that the frequency of the transient nutation, ω_n , for an $S = 1/2$ system is equal to the B_1 field amplitude. For our W-band system a loaded quality factor of $Q_L = 750$ was determined corresponding to a resonator bandwidth of 130 MHz [3].

4. RESULTS AND DISCUSSION

4.1 Optimizing the EDNMR experiment. Before attempting the simulation of EDNMR spectra we discuss important features of the EDNMR experiment which can lead to line intensity modification, line shape artifacts and line broadening. Arguable EDNMR is more susceptible to signal manipulation as compared to conventional techniques such as ESEEM and ENDOR, from incorrect experimental settings, leading to spectra that cannot be readily interpreted. We use the nitroxide radical as a model system to explain these key features.

The frozen solution EPR spectrum of the nitroxide radical is defined by two parameters: the molecular g-tensor and the hyperfine tensor of the nitroxide ^{14}N , which carries significant spin density. Both are resolved at W-band (94 GHz) microwave frequency used in this study. The molecular g and ^{14}N hyperfine tensor are collinear [3], for further discussion see **section 4.2.2**.

Fig. 3. (a) Energy-level scheme showing the EPR, ELDOR and NMR transitions of the deuterated nitroxide complex associated with the high field edge of the W-band EPR spectrum. The eigenstates are described using the shorthand $+, 0$ and $-$. The first entry represents the two M_S states $\pm 1/2$ and are labeled $+$, or $-$. The second and third entries correspond to the M_I states $(-1, 0, 1)$ of the ^{14}N and ^2H nuclei and are labeled $+$, 0 or $-$ i.e. the $|+++\rangle$ state correspond to an electronic spin of $+1/2$, and ^{14}N and ^2H nuclear spin projection of $+1$ and $+1$. The left most six level scheme shows the ^{14}N levels that define the edge EPR pattern. Two single quantum (SQ, b, c) and double quantum (DQ, d, e) ELDOR transitions are associated with this EPR transition (a), which all appear on the RHS of the EDNMR spectrum ($\nu_1 > \nu_0$). The inclusion of a third spin (^2H) results in a twelve level scheme (center). As the ^2H hyperfine coupling is small, all three EPR transitions within this sub-manifold are excited, however only the ELDOR and NMR transitions associated with the $|-, +, -\rangle \rightarrow |+, +, -\rangle$ EPR transition are shown for clarity of presentation. This results in two additional SQ ^2H (g, f) and DQ (h, i) ELDOR transitions that appear on both sides of the EDNMR spectrum. In the instance where the ^{14}N and ^2H nucleus couple together two further pairs of ELDOR transitions are expected (j, k and l, m). Importantly they do not show a one-to-one correspondence with the allowed NMR transitions of the manifold unlike the SQ or DQ ELDOR transition described above.

(b) Experimental EDNMR recordings for R1- ^{14}N -D $_{16}$ nitroxide in frozen toluene-D $_8$ solution acquired at the high field edge of the W-band EPR spectrum (indicated in Fig. 4a) at 50 K using $t_{\text{HTA}} = 1 \mu\text{s}$ (green trace) and $64 \mu\text{s}$ (blue trace) with $\omega_1/2\pi = 5.6 \text{ MHz}$. The shape of subtracted blind spot is shown by the gray dashed line. The stars mark the natural abundance ^{13}C -EDNMR signals.

The EDNMR measurement performed at the high-field edge of the W-band EPR spectrum (g_{zz} , $M_I = -1$ spectral region, see **Fig. 5a**) selectively probes a single EPR transition of the spin manifold: the $|-1/2, -1\rangle \rightarrow |+1/2, -1\rangle$ transition. Associated with this EPR transition are two single quantum (SQ, $\Delta M_S = \pm 1$, $\Delta M_I = \pm 1$) and two double quantum (DQ, $\Delta M_S = \pm 1$, $\Delta M_I = \pm 2$) ^{14}N ELDOR transitions, all of which appear at higher energy to the allowed EPR transition and thus contribute to only one side of the resultant EDNMR spectrum (RHS, $\Delta \nu = \nu_1 - \nu_0 > 0$), see **Fig. 3a**. In addition to the ^{14}N lines, SQ and DQ transitions from weakly coupled deuterium (D) nuclei are also observed (**Fig. 3**). Unlike the ^{14}N transition, the D-ELDOR transitions appear on either side of the allowed EPR transition, centered at the D nuclear Larmor frequency (SQ) and twice the Larmor frequency (DQ), i.e. on both the left and right hand side of the EDNMR spectrum ($\nu_n(\text{D}) = 21.8 \text{ MHz}$, $B_0 = 3.34 \text{ T}$). Finally, a third class of signals is observed. These transitions represent double nuclear double quantum (DNQ, $\Delta M_S = \pm 1$, $\Delta M_I(^{14}\text{N}) = \pm 1$, $\Delta M_I(\text{D}) = \pm 1$) transitions in which both the ^{14}N and D nuclear spins change their projection. These low probability transitions present are also seen in ESEEM and are analogous to two photon excitations in optical spectroscopy. They appear as satellites about the SQ transition of the first nucleus, separated by twice the Larmor frequency and the hyperfine interaction of the second nucleus. SQ transitions are also observed for weakly coupled ^{13}C (natural abundance) nuclei centered at the Larmor frequency of $\nu_n(^{13}\text{C}) = 35.8 \text{ MHz}$. The relative intensities of the SQ, DQ and DNQ transitions are dependent on the HTA pulse efficiency. A higher HTA efficiency enhances the low probability DQ and DNQ signals, **Fig. 3b** and see **section 4.2**.

4.1.1 EDNMR linewidths. It is important to recognize that line-shape distortion easily arises in the EDNMR experiment. The observed linewidth is dependent on the experimental settings used. Of particular importance is the detection sequence used and to a lesser extent the pump pulse. The linewidth in the EDNMR experiment is determined by the decay of EDNMR responses, i.e. by the phase memory time T_m . The measured linewidth is solely determined by the EDNMR signal integration window (t_{int}). The empirical linewidth dependence for a rectangular HTA pulse using a Hahn echo detection sequence is [3]:

$$t_{\text{int}} = 1.4/\nu_R \quad (26)$$

where ν_R is the desired resolution. Thus an integration window of $1.4 \mu\text{s}$ yields a resolution of 1 MHz in the EDNMR experiment. An integration window of this magnitude is already

sufficient to measure the real linewidth of the SQ ^{14}N transitions, with the linewidth measured using EDNMR matching that measured by Davies ENDOR, **Fig. 4b**. Interestingly we note that the ^{14}N SQ spectral linewidth is approximately half the linewidth of the corresponding DQ spectral lines (**Fig. 3b**). The linewidth for the corresponding ^{15}N nitroxide increases by a factor 1.5 (the ratio of the $^{15}\text{N}/^{14}\text{N}$ g-values) suggesting that hyperfine strain defines the linewidth parameter in this instance [3].

What happens in case where the linewidth of a nuclear transition is intrinsically very narrow? This situation arises for weakly coupled D nuclei in the nitroxide system. In **Fig. 4c** we show a 2D contour plot of the entire integration window extending out $\pm 1\ \mu\text{s}$ across the D peak centered at 21.8 MHz. The EDNMR signal is then plotted in **Fig. 4d** as a function of the integration width. It is observed that the intensity (peak amplitude) is invariant of the choice of the integration window normalized to the central blind spot, demonstrating that while the detection sequence can modify the lineshape it does not influence line-intensities. It therefore does not need to be explicitly accounted for in spectral simulations. A maximum linewidth of $\approx 5\ \text{MHz}$ for the D line can be achieved using a narrow integration window, approximately equal to the bandwidth of the π pulse of the detection sequence. This decreases to $\approx 1\ \text{MHz}$ upon integration of the entire window ($\pm 1\ \mu\text{s}$). This is approximately the same width seen in the corresponding Mims-ENDOR spectrum, albeit with the loss of detailed structure, of the order of 100 kHz. Note that in the Mims-ENDOR data, hyperfine contrast acts to suppress the matrix line of the deuterons, which still strongly contribute to the EDNMR spectrum, partially explaining the change in the overall signal profile. More importantly, this dataset shows that lineshape artifacts can arise due to the choice of the integration window, similar to that seen in ESEEM. They come about from both a FID-type EDNMR response, see **Fig 4c**, and possibly from a free induction decay (FID) signal caused by the π -pulse of the detection sequence, see **Fig. 4c** signals at negative integration window time, leading to side structure on the EDNMR signal. Such lineshape artifacts should be suppressed by using two-step phase cycling. Nevertheless, care needs to be taken when analyzing details of the EDNMR lineshape of intrinsically narrow spectral features measured by echo integration. We recommend measuring EDNMR spectra using at least two integration settings to ensure all spectral features are reproduced.

The length of the HTA pulse can also lead to artificial line broadening. However this circumstance can always be avoided by using an HTA pulse of optimized efficiency (length/amplitude). There are two reasons why this comes about:

- (i) Magnetization transfer for long HTA pulse lengths [23]. This is observed in our model system, **Fig. 3b**. Both the ^{14}N and D SQ transitions are broadened by approximately a factor of two when measured under conditions optimal for visualizing the DQ transitions, i.e. a long HTA pulse. We note that the width of the central blind spot (allowed EPR transition) also broadens due to this effect.
- (ii) Unresolved DNQ transitions of low- γ nuclei. Especially at low magnetic fields DNQ transitions can be superimposed and, thus, broaden, the higher frequency EDNMR lines.

Thus EDNMR correctly performed can often contain the same linewidth information content as ENDOR, with the exception of very narrow lines (<500 kHz). But care must be taken to avoid introducing linewidth artifacts and to correctly measure all spectral features multiple instrument setting may be required.

Fig. 4. Integration window and spectral resolution. **(a)** W-band echo detected EPR spectrum of the R1- ^{14}N -D₁₆ nitroxide radical in frozen toluene-D₈/MTHF solution at 50 K. **(b)** Comparison of ^{14}N -EDNMR and Davies-ENDOR line shapes. Integration window in EDNMR was set to 1.4 μs . Gray dotted lines show the best fit simulation of experimental recordings to Gaussian line. **(c)** Time-frequency dependence of EDNMR intensities recorded for the D-line centered at $v_n(\text{D})=21.8$ MHz using $t_{\text{HTA}} = 8$ μs , $\omega_1/2\pi=1.3$ MHz. The EDNMR intensities were calculated subtracting echo recordings from the echo trace at 26 MHz **(d)** Comparison of D-EDNMR and Mims-ENDOR line shapes. EDNMR spectra were calculated from data in (c) using the integration windows indicated.

4.1.2 EDNMR line intensities as a function of the HTA pulse length. The dependence of the EDNMR line intensities on the HTA pulse is best observed by performing a microwave nutation experiment, **insert in Fig. 5d**. In this experiment the intensity of the EDNMR

transition is monitored as a function of HTA pulse length. In case of selective excitation, for instance the spectral position marked in the top panel of **Fig. 5a**, a damped oscillation is observed which can be empirically described by the following function:

$$S_{\text{nut}} = 1 - K \cdot \left[1 - \cos(2 \cdot \pi \cdot \nu_{\text{nut}} \cdot t_{\text{HTA}}) \cdot e^{-t_{\text{HTA}}/\tau} \right] \quad (27)$$

In which K represents the modulation depth (maximum EDNMR intensity) and ν_{nut} the frequency of oscillation. It is these two parameters (K, ν), which themselves are a function of the amplitude of the HTA pulse, that the simulation procedure outlined in **section 3** needs to be able to reproduce. The remaining parameters are the experimental settings: t_{HTA} , the HTA pulse length and τ , the signal decay time.

Fig. 5. Microwave nutation experiment. **(a)** W-band echo detected EPR spectrum of R1- ^{14}N -D $_{16}$ nitroxide radical in frozen toluene-D $_8$ /MTHF solution at 50 K. **(b)** MW nutation traces recorded at different settings of mw power. They are calculated from the echo intensities, $\text{Signal}(\nu_{\text{ON}}) - \text{Signal}(\nu_{\text{OFF}})$, acquired at ON and OFF resonance frequencies, marked in (c). The experimental settings: $t_d = 10 \mu\text{s}$, $t(\pi/2) = 100 \text{ ns}$, $\tau = 2 \mu\text{s}$, $t_{\text{int}} = 1.4 \mu\text{s}$. Dashed lines show the best fit of experimental data to Eq. (26). **(c)** Experimental (blue line) and calculated (red line) EDNMR spectra for magnetic field position marked in (a). **(d)** Calculated MW nutation traces for ω_1 evaluated from the central blind spot width indicated. The parameters are $T_m = 4 \mu\text{s}$, $Q_L = 700$. The insert shows the principal scheme of the nutation experiment. The same color code is used for the traces in panels (b) and (d).

Experimental microwave nutation curves are shown in **Fig. 5b**. For a HTA pulse amplitude of $\omega_1/2\pi = 7.5 \text{ MHz}$ a maximum depth parameter $K = 0.46$ is achieved as obtained from simulation of the experimental trace by Eq. (27). Simulations of the microwave nutation curve shown in **Fig. 5d**, nominally match the frequency and damping of the signal. As expected the oscillation frequency is linear with the amplitude of the HTA pulse (see **section 3.4**). At higher HTA pulse amplitudes the oscillation damping is faster compared to the T_m time for

both experimental and calculated curves approaching, however, T_m at low HTA amplitudes. The faster damping seen at higher HTA pulse amplitudes results from the intensity distribution of the number of ELDOR transitions excited by the HTA pulse at the magnetic field position of detection.

An important deviation between experiment and simulation is however observed. The modulation depth (K) parameter is only reproduced by the simulation package at high HTA amplitudes. The simulation predicts that the depth parameter should approximately be the same in the case where the HTA π -pulse length is comparable or shorter than T_m . This is not seen in the experiment, where instead the depth parameter rapidly falls off as the amplitude of the HTA pulse decreases. This effect is due to incomplete excitation of the spectral envelope which makes up the ELDOR transition. Thus, only under conditions where the HTA excitation bandwidth matches the EPR linewidth including unresolved hyperfine couplings is the depth parameter reproduced by the simulation. If this condition is met, the depth parameter, relative to the central blind spot, can be considered quantitative (see **Fig. 5c**). In order to account for this effect, the simulation package should include independent excitation bandwidths for both detection sequence (important only for bandwidth larger than the inhomogeneous EPR linewidth) and HTA pulse. This is ongoing work in our laboratory.

4.1.4 General recipe for high-quality EDNMR data. The two proceeding sections outline the most important considerations for the collection of high-quality EDNMR data. The important physical parameters of the system are: the position of the EDNMR line, namely where the line falls within the bandwidth of the resonator; the width of the EDNMR line, which constrains the detection sequence and the phase-memory time T_m . It is these parameters that then determine the choice of the instrumental settings, namely:

- (i) amplitude of the HTA pulse (ω_1)
- (ii) length of the HTA pulse (t_{HTA})
- (iii) spin echo integration window (t_{int})
- (iv) interpulse distance in the detection pulse sequence (τ)
- (v) pulse length in the detection pulse sequence (t_p).

As every spin system is different and we cannot offer an ideal choice of experimental settings. We do however suggest the following procedure as a guide for optimizing the EDNMR experiment:

1. All measurements should begin by measuring the relaxation properties of the spin system, throughout the spectrum. The most important parameter here is the T_m time which can be estimated by measuring the decay of the spin echo as a function of the interpulse separation. These properties then dictate the choice of the HTA pulse length and interpulse distance.
2. The resonator bandwidth should be measured. This can be achieved by measuring a series of microwave nutation curves in which the operating frequency is incremented around the resonator frequency.
3. It is important to know the inhomogeneous linewidth of the EPR spectrum. The excitation bandwidth of the detection should match this value or be smaller. No gain in information content can be obtained by the bandwidth exceeding this value. For larger excitation bandwidths the possibility of resolution loss should be taken into account.
4. Ideally the excitation bandwidth of the HTA pulse should be set to excite all EDNMR transitions at the magnetic field position of detection, for optimal signal intensities. This is achieved by optimizing the amplitude of the HTA pulse. Remember that the excitation width of the HTA pulse is proportional to the width of the central blind spot.
5. Care needs to be taken to ensure that the length of the HTA pulse is long enough to avoid deviation of the central blind spot shape from that of a Lorentzian, see Eq. (22). Long HTA pulses can be used to minimize the central blind spot thus making small couplings observable.
6. The HTA pulse should however be as short as possible to avoid magnetization transfer artifacts. This may lead to the situation in which the optimal EDNMR signal intensity is sacrificed in order to measure the undistorted EDNMR lineshape. This situation could potentially arise for signals that lie close to the central line spot, i.e. signals from weakly coupled low γ -nuclei.
7. It is noted though that a broad integration window requires a correspondingly large interpulse separation (τ) for the echo detection pulse sequence. In case where the phase-memory time, T_m , is short this can lead to a significant reduction in sensitivity. Under such circumstances FID detection may be preferable to echo detection. Also note that if in this case the linewidth information is not crucial, detection of the second

half of the echo (using a constrained Hahn echo sequence) also allows high S/N data collection.

4.2 Simulation of EDNMR spectra. In the next section we report simulations of EDNMR spectra collected for the nitroxide system. The spectra we report meet the criteria listed in section 4.1.4, namely that the pumped transition is completely excited. As such the spectra and their corresponding simulations are quantitative. We examine two circumstances: the non-selective EDNMR spectrum arising from a dipolar coupled ^1H and the partially selective 2D-EDNMR spectrum arising from the ^{15}N and ^{14}N nucleus of the nitroxide radical.

4.2.1 ^1H -EDNMR for the dipolar coupled proton. The nitroxide radical has a number of internal proton signals in addition to proton signals that arise from coupling to the solvent. In the EDNMR experiment, all of these species can potentially be observed. To limit the complexity of the system, the perdeuterated nitroxide (3-hydroxymethyl-2,2,5,5-tetramethylpyrrolin-1-oxyl) was examined in deuterated methanol (CD_3OH). This system should contain only one proton signal, arising from a dipolar coupled proton due to the H-bonding interaction of the alcohol group of the methanol with the oxyl of the nitroxide. There is however still a proton matrix component due to the higher proton molarity of non-bound methanol molecules. The dipolar coupled proton species has been characterized by W-band Davies ENDOR. As its ENDOR signal is dependent on the orientation of the nitroxide-methanol complex relative to B_0 , the ENDOR spectrum was measured across the entire EPR spectrum to completely describe the species. This is represented in **Fig. 6b** as a 2D surface where the x and y axes represent the magnetic field and radio frequency respectively, and the colored contour lines the intensity of the ENDOR signal. Averaging across the entire EPR spectrum, yields a Pake-pattern with $A_{\parallel}$ and $A_{\perp}$ of about 12.5 MHz and -5 MHz respectively. The surface shows the relative orientations of the hyperfine and g-tensor components with $A_{\parallel}$ falling in the g_{xx}/g_{yy} plane and $A_{\perp}$ aligned along g_{zz} .

In ESEEM as in EDNMR, line intensities depend upon the probability of forbidden transitions which arise due to anisotropic (dipolar) hyperfine, nuclear quadrupole and/or zero-field interactions. This has the practical consequence that the intensity of ESEEM transitions vanishes for orientations which coincide with the canonical axes of these interaction tensors, leading to a distorted NMR spectrum as compared to ENDOR. The view held in the literature is that the same should be true in EDNMR [25]. However, this is not always the case. In the small HTA efficiency limit, the same regime the ESEEM experiment is performed, the EDNMR lineshape will indeed resemble the ESEEM spectrum. If however instead a higher

HTA efficiency pulse is used, weaker components of the EDNMR spectral profile can be enhanced such that in the limit of a highly efficient HTA pulse the EDNMR spectrum should approach the appearance of the ENDOR spectrum. This is demonstrated in **Fig. 6a** using the non-selective excitation case in which the whole nitroxide EPR spectrum is excited. With increasing HTA pulse amplitudes the simulated EDNMR profile changes: using a weak HTA efficiency the profile is the same as that seen in ESEEM (blue) with the spectral singularities lost, whereas using a highly efficiency HTA pulse the profile is almost identical to that seen in ENDOR.

The predicted dependence of the EDNMR profile on the HTA efficiency can be readily demonstrated for the H-bonded nitroxide-methanol complex. Using a long HTA pulse the characteristic 'butterfly' like 2D surface as seen by Davies ENDOR can also be observed using EDNMR. Unfortunately the 2D-EDNMR spectrum contains distortions at the proton Larmor frequency. These come about due to incomplete integration of the matrix proton line. Such matrix peaks are amplified under the instrumental conditions necessary to visualize the Pake pattern in EDNMR. This would seem to be a general problem, not encountered in Davies ENDOR, where matrix signals are suppressed [22].

The orientationally resolved EDNMR simulation is shown in **Fig. 6c**. It can readily be observed that the simulation matches the intensity profile of the experimental spectrum. It shows a better agreement with the experimental ENDOR spectrum, owing to the integration artifacts described above. Most importantly the simulation accurately reproduces the modulation depth parameter. The intensity of the signal profile outside the matrix envelope is exactly reproduced in the simulation (0.03 of the primary echo), demonstrating that EDNMR has the potential to quantify the number of proton interactions, in this instance the number of H-bonds the complex contains. This coupled with the key advantage of EDNMR, its significantly higher detection sensitivity, demonstrates that EDNMR may be the method of choice for characterizing a number of chemical systems. In the example shown in **Fig. 6** the field-frequency EDNMR spectrum was acquired in one hour while the ENDOR spectrum required 8 hours.

Fig. 6 (a) The W-band ^1H -EDNMR spectra calculated for $t_{\text{HTA}} = 1 \mu\text{s}$ (blue trace); $5 \mu\text{s}$ (green trace) and $25 \mu\text{s}$ (black trace) using $t_{\text{HTA}} = 25 \mu\text{s}$, $\omega_1/2\pi = 9.5 \text{ MHz}$, $T_m = 8 \mu\text{s}$, $Q_L = 700$ and the proton hyperfine tensor $A(^1\text{H}) = [12.4; -5.5; -4.6] \text{ MHz}$. The gray trace shows the ^1H -ENDOR spectrum calculated using the salt routine (Easyspin). The black and gray traces are shifted up by 0.02 for clarity.

(b) Frequency-field dependence of the experimental W-band proton Davies-type ENDOR of $\text{R1-}^{14}\text{N-D}_{16}$ in frozen CD_3OH solution at 50 K. The contour lines are shown as isohypses from 0.05 to 1 of the maximum ENDOR intensity due to the bonded proton. The pulse settings of the Davies-type ENDOR experiment are $t_{\pi}(\text{pump}) = 200 \text{ ns}$, $t_{\text{RF}} = 20 \mu\text{s}$, $t_{\pi/2}(\text{det.}) = 32 \text{ ns}$, averages per point = 256, pulse repetition rate = 200 Hz. Total acquisition time was 8 hours.

(c) Frequency-field dependence of the calculated W-band EDNMR spectrum using $t_{\text{HTA}} = 25 \mu\text{s}$, $\omega_1/2\pi = 9.5 \text{ MHz}$, $T_m = 8 \mu\text{s}$, $Q_L = 700$. The proton hyperfine tensor $A(^1\text{H}) = [12.4; -5.5; -4.6] \text{ MHz}$ is rotated 43° in the xy-plane of the nitroxide g-tensor. The direction of $A_x(^1\text{H})$ coincides with the direction of the nitroxide g_{zz} . On top, the calculated W-band EPR spectrum is shown to refer to the spectral positions.

(d) Frequency-field dependence of the experimental W-band EDNMR. The contour lines are shown as isohypses from 0.05 to 1 of the maximum EDNMR intensity due to the bonded proton. The settings of the EDNMR experiment are $t_{\text{HTA}} = 25 \mu\text{s}$, $\omega_1/2\pi = 9.5 \text{ MHz}$, $t_{\pi/2}(\text{det.}) = 100 \text{ ns}$, the interpulse time in the detection sequence = $1.6 \mu\text{s}$, echo integration window = $2 \mu\text{s}$, averages per point = 50, pulse repetition rate 300 Hz. Total acquisition time was 1 hour.

4.2.2 ^{15}N -EDNMR simulations for ^{15}N -labelled nitroxide. As described above the molecular g and N hyperfine tensors are collinear. The unique tensor axis (z), describes the unpaired spin associated with the p-orbitals of the conjugated ring. It is characterized by a g-value approximately that of the free electron value and a large ^{14}N hyperfine coupling, in the

strong coupling limit, $|A_{zz}|/2 > |\nu_n(N)|$. The other two g-tensor principal components appear at higher values with ^{15}N hyperfine couplings in the weak coupling limit, $|A_{xx,yy}|/2 < |\nu_n(N)|$. As a consequence, the corresponding ENDOR/EDNMR spectrum of the nitroxide radical is highly dependent on the position at which the double resonance experiment is performed.

The complex field dependence of the nitrogen signal can be demonstrated using a 2D contour plot representation. The EDNMR spectrum has both contributions appearing at positive and negative frequency differences, $\Delta\nu$. Because the experiment is selective the pattern is asymmetric (on both edges). The EDNMR spectrum clearly resolves one peak on the LHS and one on the RHS on the low field edge, indicating the weak coupling regime whereas both peaks appear on the RHS on the high field edge, indicating the strong coupling regime, see **Fig. 1b**. This asymmetry allows the sign of the hyperfine coupling (relative to the sign of g_n) to be determined by inspection of the data, a feat that cannot be achieved with ESEEM or ENDOR, without recourse to TRIPLE type techniques [28, 29].

The intensity of spin forbidden transitions changes dramatically across the EPR spectrum because the nitrogen coupling switches between the weak and strong coupling regime. On the edges where the coupling regime is well defined, I_f is much smaller than I_a whereas in the center of the spectrum where the couple regime switches, I_f and I_a are equal, i.e. at the cancellation condition. As a consequence the 2D-EDNMR spectrum is strongly influenced by the choice of the HTA length and amplitude. For a fixed HTA pulse length, using a low amplitude HTA pulse the ^{15}N signals in the center of the spectrum are amplified whereas using a high amplitude HTA pulse, the edges of the pattern are amplified. Indeed, for measuring the absolute edges of the pattern low HTA pulses are required to enhance near-canonical orientations, as shown above for the dipolar proton example. This system thus represents a good system to test the robustness of our simulation package under a variety of different HTA conditions.

Simulations of the ^{15}N -EDNMR are shown in **Fig. 7**. While the ^{15}N coupling is variable it is nevertheless large enough to be resolved throughout the spectrum. As such the EDNMR spectra are in the selective regime, or at least semi-selective regime. Absolute transition intensities should be reproduced by the simulation package on the low and high field edge of the spectrum where a single EPR transition contributes. In the center, where overlapping contributions are seen only relative transition intensities can at this point be calculated. However as the absolute intensity is known on the edges this is of no consequence.

Fig. 7. (a) Frequency-field dependence of the experimental W-band EDNMR of R1- ^{15}N -D $_{16}$ in frozen toluene-D $_8$ /MTHF solution at 50 K. The color code of ^{15}N EDNMR intensity is shown on the left. Experimental settings: $t_{\text{HTA}} = 3.1 \mu\text{s}$, $\omega_1/2\pi = 0.6 \text{ MHz}$, $t_{\pi/2}(\text{det.}) = 100\text{ns}$, $t_{\text{int}} = 1.4 \mu\text{s}$. (b) Frequency-field dependence of the calculated W-band EDNMR spectrum using experimental parameters from (a) and $T_m = 4 \mu\text{s}$, $Q_L = 500$. The nitroxide radical paramagnetic parameters: $g = [2.00906; 2.0062; 2.0023]$ and $A(^{15}\text{N}) = -[16; 16; 133] \text{ MHz}$. (c) Frequency-field dependence of the experimental W-band EDNMR recorded using $\omega_1/2\pi = 1.6 \text{ MHz}$. The color code for isohypses is adjusted according to the EDNMR intensities of the calculated spectrum shown in (d). Experimental and calculated EPR spectra are shown on the top to refer to the spectral positions. Insert demonstrates the large intensity difference of EDNMR spectra from (b, red line) and (d, blue line) at the magnetic field position indicated by arrow in upper panel.

In the simulation of spectrum a, in which the HTA is optimized (length/amplitude) to measure the ^{15}N signal in the center of the EPR spectrum, reproduces all features of the experimental data quantitatively, with the exception of the deuterium matrix line at $\Delta\nu = \pm 21.8 \text{ MHz}$. The simulated depth parameter matches experiment on both edges of the spectrum and the intensity and structure of the overlapping ridges throughout the spectrum are the same.

As expected, the ^{15}N signal falls off on the high field edge approaching the canonical orientation (see **Fig. 7a**). To better visualize this feature, a HTA pulse of the same length but of higher amplitude was used to record the EDNMR spectrum (**Fig. 7c**). This enhances the

ridges extending to higher fields. The enhancement of these ridges could be quantitatively reproduced by the simulation routine (compare **Fig. 7b** and **d**. Also see the insert **Fig. 7d**, red trace vs. blue trace). The simulation did not, however, accurately reproduce the center of the profile. Under the experimental conditions used, magnetization transfer is strongly enhanced, which both amplifies and broadens features in the center of the spectrum. Thus, under these conditions quantitative information cannot be recovered. We note, however, that the structure of the spectrum is still reproduced by the simulation routine.

4.2.2 ^{14}N -EDNMR simulations for ^{14}N -nitroxide. Experimental data and corresponding simulations were also collected for the ^{14}N natural abundance system. **Figure 8a** shows an EDNMR spectrum using the same pulse length and amplitude as for the ^{15}N system described above, optimized to the ^{14}N signal in the center of the EPR spectrum i.e. a low amplitude HTA pulse. The simulation, **Fig. 8b**, reproduces all spectral features and the depth parameter on both the low and high field edge. There is a small discrepancy in the central region concerning relative intensities. This may indicate the small difference of the nitroxide spin Hamiltonian parameters used in the calculation from experimental used, specifically the orientation of the quadrupole tensor to the ^{14}N hyperfine and g-tensor may not be exactly correct. This may also explain small differences in the EPR lineshapes in these regions. In addition to the SQ transitions, the simulation also reproduces the experimental signals due to ^{14}N -DQ transitions which appear in the x-y spectral region at the positions with highest forbidden transition intensity, **Fig. 8ab**.

Fig. 8. (a) Frequency-field dependence of the experimental W-band EDNMR of R1- ^{14}N -D $_{16}$ in frozen toluene-D $_8$ /MTHF solution at 50 K. The contour lines are shown as isohypses, the maximum ^{14}N EDNMR intensity coded on the left. Experimental settings: $t_{\text{HTA}} = 3.1 \mu\text{s}$, $\omega_1/2\pi = 0.6 \text{ MHz}$, $t_{\pi/2} = 100\text{ns}$, $t_{\text{int}} = 1.4 \mu\text{s}$. (b) Frequency-field dependence of the calculated W-band EDNMR spectrum using experimental parameters from (a) and $T_m = 4 \mu\text{s}$, $Q_L = 500$. The nitroxide radical magnetic parameters are: $g = [2.00906; 2.0062; 2.0023]$ and $A(^{14}\text{N}) = [11.5; 11.5; 95] \text{ MHz}$. (c) Frequency-field dependence of the experimental W-band recorded using $t_{\text{HTA}} = 64 \mu\text{s}$ and $\omega_1/2\pi = 2.9 \text{ MHz}$. (d) Frequency-field dependence of the calculated W-band EDNMR spectrum using experimental settings from (c). The EDNMR spectra in (c) and (d) are shown in different intensity scales due to the large spectral diffusion contributions to experimental spectrum. Experimental and calculated EPR spectra are shown on the top to refer to the spectral positions.

Figure 8d shows a simulation of the 2D-EDNMR surface using HTA settings to enhance the EDNMR signal at canonical orientations and to better observe DQ transitions ($M_I = \pm 1$) for the ^{14}N nucleus. The corresponding experimental EDNMR spectrum is shown in **Fig. 8c**. Good agreement between the experimental data and simulation is only observed in the high field spectral region. The remainder of the spectral envelope disagrees substantially. Note that the 2D spectrum and simulation are depicted on different intensity scales. This is mainly because of the spectral contributions due to magnetization transfer processes which operate on the time scale of EDNMR pulse sequence of $80 \mu\text{s}$ (see section 3.3). For this system these

effects are best seen at g_{zz} , ($M_I = 0$ spectral position) at which no ^{14}N -DQ transitions are expected (3357mT, LHS **Fig. 8c**). At this position an additional peak at $A_{zz}=95$ MHz is observed which comes about from the direct pumping at g_{zz} , $M_I = -1$ transition i.e. it represents a second probe/zero frequency (see also **Fig. 9**). There are also EDNMR lines associated with this second probe frequency, namely D peaks which are located either side of A_{zz} i.e. $A_{zz} \pm \nu_h(\text{D})$, see **Fig. 8c**. Therefore in these spectral regions we do not consider experimental line intensities to be trustworthy under these conditions and thus we make no comment as to the reliability of our simulation package to predict relative or absolute line intensities under these conditions. Our conclusion, unsurprisingly, is that very long and high amplitude HTA pulses should usually be avoided when collecting EDNMR spectra.

Putting these problems to one side, as stated above EDNMR spectra measured in the high field edge region are not strongly contaminated by magnetization transfer processes and thus can be reproduced using our simulation package. A comparison of experimental and simulated 1D EDNMR spectra collected on the high field edge are shown in **Fig. 9**. As expected using a low efficiency HTA pulse only the SQ ^{14}N transitions are observed (bottom green trace). The corresponding simulation quantitatively matches experiment, both in terms of line intensities and positions. Using a longer, high efficiency HTA pulse amplifies DQ ^{14}N transitions (top blue trace, mark in Fig. 9) which appear at higher frequencies. Interestingly, while the absolute intensity of the SQ transitions scaled to the central blind spot is correctly reproduced in both regimes (low vs. high efficiency HTA pulse) DQ transition intensities are systematically over-estimated, in this case by 50-100% depending on the exact field position. This same behavior is observed for any EDNMR transition for which there is insufficient microwave power to saturate the transition. The calculated intensities for low probability transitions should be viewed as the theoretical maximum that could be achieved in the absence of magnetization transfer processes. This observation again highlights the importance of performing the EDNMR experiment in the short HTA pulse regime and the need to develop higher power sources at high field (W-band) to better characterize these types of transitions.

In the simulation shown in Fig. 9 we also explicitly include both a single ^2H and ^{14}N nucleus, allowing us to rationalize the positions of all DNQ combination peaks (red dots). We note that the relative intensities of the DNQ peaks to either the SQ D peak (peak g) or the SQ ^{14}N (peaks c and b) do not exactly match. This is because the experimental D line represents a number of coupled intra- and intermolecular deuteriums – with the intensity of this transition

scaling with the number of D interactions. This is in contrast to the DNQ transitions scale with the ^{14}N SQ lines. This property can potentially be exploited. For instance, in systems in which the number of interacting molecules is unknown e.g. in solvation science, analyzing the intensity of the DNQ transitions would provide an estimate of the average hyperfine interaction between the electron spin and the solvent molecules of the 1st coordination sphere, with subsequent fitting of the SQ D peak using this information, constraining the number of solvent interactions. Note that DQ D peak (h) is absent for the same reason as given above i.e. both the SQ and DQ D peaks are underestimated as only 1 deuterium is included in the simulation.

Fig. 9. (lower traces) Experimental EDNMR recordings (LHS) for R1- ^{14}N -D₁₆ nitroxide in frozen toluene-D₈/MTHF solution acquired at the high field edge of the W-band EPR spectrum at 50 K using $t_{\text{HTA}} = 1 \mu\text{s}$ (green trace) and $64 \mu\text{s}$ (blue trace) with $\omega_1/2\pi = 5.6 \text{ MHz}$. **(upper traces)** Calculated EDNMR spectra using the settings given in Fig. 8 but including additional D nuclei with hyperfine tensor $A(\text{D}) = [-0.7 \ -0.7 \ 1.4] \text{ MHz}$. The red cycles mark the field positions of ^{14}N -D DNQ lines. The stick diagram of EDNMR transitions is shown in the upper panel, see also Fig. 3.

5 CONCLUSIONS AND OUTLOOK

The experiments and the simulations shown above demonstrate that EDNMR is a quantitative double resonance spectroscopy that in most circumstances contains the same information as established hyperfine spectroscopy techniques. An ENDOR like line-shape can in most cases be achieved in the EDNMR experiment, regardless of the type of nucleus or nature of the

hyperfine interaction i.e. isotropic vs. dipolar. Furthermore the dependence of line intensities on the HTA pulse length/amplitude provides additional information that can be used to quantify the number of electron-nuclear contributions of the spin system of interest.

As yet, we have only implemented the simulation procedure for the $S = 1/2$, $I \geq 1/2$ spin system (see appendix). In principle the procedure outlined can easily be extended to any arbitrary spin system, as we demonstrate in section 4.1.1. A robust simulation code for any spin system forms ongoing work of our laboratory. Similarly, we are extending the current package to account for both multinuclear transitions and the excitation bandwidth selectivity of the HTA pulse, when calculating transition intensities.

Acknowledgements

Dr. Yuri Kutin is gratefully acknowledged for his critical reading of the manuscript. The authors own work, cited in the manuscript was financially supported the BMBF (MANGAN), the Cluster of Excellence RESOLV (EXC 1069) funded by the Deutsche Forschungsgemeinschaft and the Max Planck Society. N.C. acknowledges the support of the Australian Research Council (FT140100834).

APPENDIX – Simulation Code

The simulation routine uses Easyspin and MATLAB functions. The first step is to solve the spin Hamiltonian for a given magnetic field (B_0), and orientation (θ, ϕ), constructed using the ‘Sham’ function:

$$\mathbf{H}(B_0, \theta, \phi) \psi(B_0, \theta, \phi) = \mathbf{E}(B_0, \theta, \phi) \psi(B_0, \theta, \phi) \quad \text{Eq. A1}$$

This is solved using the MATLAB “eig” function, yielding a diagonal matrix ($\mathbf{Z}$) of energies and a matrix $\mathbf{W}$ whose columns represent the weights $c_1, \dots, c_n$ describing the wavefunctions of the system in the original basis. Using these two matrices all possible energy differences ($\Delta\mathbf{E}$) are calculated as follows:

$$\Delta\mathbf{E} = \mathbf{Z} \cdot \mathbf{T}_L - \mathbf{T}_L \cdot \mathbf{Z} = \begin{bmatrix} 0 & E_{1,2} & E_{1,3} & \dots & E_{1,n-1} & E_{1,n} \\ E_{2,1} & 0 & E_{2,3} & \dots & E_{2,n-1} & E_{2,n} \\ E_{3,1} & E_{3,2} & 0 & \dots & E_{3,n-1} & E_{3,n} \\ \dots & \dots & \dots & \dots & \dots & \dots \\ E_{n-1,1} & E_{n-1,2} & & \dots & 0 & E_{n-1,n} \\ E_{n,1} & E_{n,2} & E_{n,3} & \dots & E_{n,n-1} & 0 \end{bmatrix} \quad \text{Eq. A2}$$

Where $\mathbf{T}_L$ is a lower triangular matrix of the same dimensions as $\mathbf{Z}$ with zeros along the diagonal. A corresponding matrix of transition probabilities $\mathbf{P}$ can be generated for each energy difference $E_{n,m}$, i.e. transition between the n th and m th state by applying the transition operators S_X and S_Y averaged over the third euler angle:

$$\mathbf{P} = |\mathbf{W}' \cdot \mathbf{T}_X \cdot \mathbf{W}|^{\circ 2} + |\mathbf{W}' \cdot \mathbf{T}_Y \cdot \mathbf{W}|^{\circ 2} \quad \text{Eq. A3}$$

Where the $\circ 2$ superscript denotes the Hadamard or entrywise product, i.e.

$$(\mathbf{A} \circ \mathbf{B})_{i,j} = (\mathbf{A})_{i,j} (\mathbf{B})_{i,j} \quad \text{Eq. A4}$$

Such that each term in the sum resembles:

$$|\mathbf{W}' \cdot \mathbf{T}_X \cdot \mathbf{W}|^{\circ 2} = \begin{bmatrix} 0 & \langle \psi_1 | \mathbf{T}_X | \psi_2 \rangle^2 & \dots & \langle \psi_1 | \mathbf{T}_X | \psi_{n-1} \rangle^2 & \langle \psi_1 | \mathbf{T}_X | \psi_n \rangle^2 \\ \langle \psi_2 | \mathbf{T}_X | \psi_1 \rangle^2 & 0 & \dots & \langle \psi_2 | \mathbf{T}_X | \psi_{n-1} \rangle^2 & \langle \psi_2 | \mathbf{T}_X | \psi_n \rangle^2 \\ \dots & \dots & \dots & \dots & \dots \\ \langle \psi_{n-1} | \mathbf{T}_X | \psi_1 \rangle^2 & \langle \psi_{n-1} | \mathbf{T}_X | \psi_2 \rangle^2 & \dots & 0 & \langle \psi_{n-1} | \mathbf{T}_X | \psi_n \rangle^2 \\ \langle \psi_n | \mathbf{T}_X | \psi_1 \rangle^2 & \langle \psi_n | \mathbf{T}_X | \psi_2 \rangle^2 & \dots & \langle \psi_n | \mathbf{T}_X | \psi_{n-1} \rangle^2 & 0 \end{bmatrix}$$

The upper left and lower right quadrants of $\Delta \mathbf{E}$ and $\mathbf{P}$ represent the NMR transitions and corresponding transition intensities of the spin manifold. The relative magnitude of the NMR transitions are used to correctly assign the sign of the pump transition with respect to its probe transition. From this two arrays are generated that define the position and intensity of each transition. The arrays **Position** and **Intensity** take the form:

$$\mathbf{Position, Intensity} = \begin{bmatrix} probe(1) & pump(1,1) & \dots & pump(m,1) \\ probe(2) & pump(1,2) & \dots & pump(m,2) \\ \dots & \dots & \dots & \dots \\ probe(n) & pump(1,n) & \dots & pump(m,n) \end{bmatrix} \quad \text{Eq. A6}$$

Where n is the number of possible pump transitions and m is the number of transitions associated with each pump transition. In the case of a $I = 1/2$ nucleus, $m = 1$. In the case of an $I > 1/2$ nucleus, $m = 2I$.

These entries can then be used in Eqs. (20, 21) to simulate the EDNMR spectrum. The full simulation routine is posted on the Easyspin forum.

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