Performance of PELDOR, RIDME, SIFTER, and DQC in measuring distances in trityl based bi- and triradicals: exchange coupling, pseudosecular coupling and multi-spin effects

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Supporting Information

Content

1 Time traces prior to background correction	3
2 The effect of the pulse lengths and intensities on the echo intensity	5
3 Attenuation of the ELDOR channel	8
4 RIDME	9
5 Wavefunctions and energies in the different coupling regimes	11
6 Simulations of multi-spin effects using a geometrical model	22
7 References	29

1 Time traces prior to background correction



Figure S1. PELDOR (black) and SIFTER time trace (red) prior to background correction along with background fitting functions (dotted) on **1**.



Figure S2. PELDOR time traces for set-ups P_{2a} (black), P_{2b} (red), P_{2c} (green) and SIFTER time trace (blue) prior to background correction along with background fitting functions (dotted) on **2**. The SIFTER time trace was shifted by -0.1 along the Intensity axis for clarity.



Figure S3. PELDOR (black) and SIFTER time trace (red) prior to background correction along with background fitting functions (dotted) on **3**.

The DQC time traces did not require background correction and are therefore not included here.

2 The effect of the pulse lengths and intensities on the echo intensity

As mentioned in the main text, the echo intensity was found to be much lower for the classical $\pi/2 - \tau - \pi - \tau$ than for the $\pi/2 - \tau - \pi/2 - \tau$ echo sequence for a given duration of the $\pi/2$ pulse at a fixed MW power for compounds **1** – **3**. Figure S4 shows echo detected EPR spectra of **2** using the two aforementioned echo sequences. The spectra shown in Figure S4 have been recorded under identical conditions apart from the length of the second pulse (Table S1).



Figure S4. Echo detected EPR spectra of **2** at Q-band frequency using either the $\pi/2 - \tau - \pi - \tau$ (red graph) or the $\pi/2 - \tau - \pi - \tau$ (green graph) pulse sequences.

Table S1. Pulse length and interpulse separation used for the measurement shown in Figu	ure
S4. All other parameters as indicated in Table 1 in the main text.	

Color in Figure S1	1 st pulse ^a [ns]	2 nd pulse ^a [ns]	τ [ns]	Att ^c [dB]
Red	10	20	500	0
Green	10	10	500	0

^a Lengths of the two pulses in a two-pulse echo sequence. At the applied MW attenuation, a 10 ns pulse corresponds to a flipping angle of $\pi/2$. ^b Att = high power MW attenuation.

As can be seen in Figure S4, the red echo detected EPR spectrum recorded using the $\pi/2 - \tau - \pi - \tau$ sequence is clearly less intense than the green spectrum, which was recorded using two $\pi/2$ pulses. In the $\pi/2 - \tau - \pi - \tau$ sequence, the second MW pulse is twice as long as the first pulse. Therefore, one could suspect that the reduced excitation bandwidth of the pulse sequence is responsible for the reduced echo intensity. If that was true however, the signal stemming from the E' center (at ~1200.5 mT in Figure S4) should be affected in the same manner. Instead, the opposite is observed for this signal and the classical sequence (i.e. $\pi/2 - \tau - \pi - \tau$) yields better echo intensities than the $\pi/2 - \tau - \pi/2 - \tau$ sequence. Thus, normally the combination of $\pi/2$ and π pulses is expected to be superior. Here, the reason for the reduction of the echo intensity of compound **2** (and all other compounds investigated in this work) using the classical $\pi/2 - \tau - \pi - \tau$ echo sequence is the occurrence of

strong dipolar oscillations of the echo intensity in the polytrityl model compounds. This effect is so strong, that it overcompensates the gain in sensitivity when going from the usually inferior $\pi/2 - \tau - \pi/2 - \tau$ sequence to the $\pi/2 - \tau - \pi - \tau$ sequence. This is also apparent in the SIFTER and DQC time traces presented in the main text. where a combination of $\pi/2$ and π pulses is used to create coherences that evolve in two time intervals τ_1 (defocusing due to dipolar coupling) and τ_2 (refocusing of the dipolar couplings). The intensity of this echo is monitored as function of the two evolution periods. In the examples presented in the main text, the intensity peaks when the length of the first evolution period equals that of the second evolution period. Importantly, in these pulse sequences the coupled spins within a molecule have to be excited simultaneously to achieve de- and refocusing of the coherences due to dipolar interaction. In contrast, in a normal two pulse echo sequence, only one evolution period is present, in which the coherences defocus owed to the dipolar interaction. Therefore, the maximum echo intensity cannot be achieved in a twopulse echo experiment in polytrityl compounds at Q-band frequency, where the EPR spectrum is still narrow. Another way to demonstrate the effect is by monitoring the echo intensity of a two-pulse echo sequence as a function of the interpulse separation τ , i.e. by conducting a two-pulse ESEEM experiment. Figure S5 shows two-pulse ESEEM traces recorded at the maximum of the EPR spectrum using pulses with different flipping angles (Table S2).



Figure S5. Two-pulse ESEEM time traces of **2** at Q-band frequency using either the $\pi/2 - \tau - \pi - \tau$ (red and blue graphs) or the $\pi/2 - \tau - \pi/2 - \tau$ (green graph) pulse sequences. The modulation period is determined by the dipolar coupling frequency of about 1.2 MHz.

Table S2. Pulse length and interpulse separation used for the measurement shown in Figure
S5. All other parameters as indicated in Table 1 in the main text.

Color in Figure S2	1 st pulse ^a [ns]	2 nd pulse ^a [ns]	τ ^ь [ns]	Att ^c [dB]
Red	10	20	300	0
Green	10	10	300	0
Blue	10	20	300	6

^a Lengths of the two pulses in a two-pulse echo sequence. At an MW attenuation of 0 dB, a 10 ns pulse corresponds to a flipping angle of $\pi/2$. ^b Initial τ value. ^c Att = high power MW attenuation.

As can be seen in Figure S5, using the classical combination of $\pi/2$ and π pulses (red graph) leads to the lowest overall, initial echo intensity and the strongest dipolar modulations. If the length of the second pulse is reduced by 50%, the overall echo intensity rises whereas the dipolar modulations diminish (green graph). The higher echo intensity is a consequence of the diminishing dipolar modulations, as the probability to flip several spins in one molecule simultaneously is strongly reduced if the second pulse is too short by 50%. A similar effect on the overall echo intensity and the modulation depth is observed if the pulse lengths are left unchanged but the attenuation is increased (blue graph). This has the same effect as shortening of both pulses would have. With an MW attenuation of 6 dB, the pulse lengths correspond to flipping angles of approximately 45 and 90°. Despite these low flipping angles, the overall echo intensity in the blue graph is higher than in the red graph. Again, this is caused by the diminishing of the dipolar modulation overcompensates the reduced echo intensity caused by the attenuation of the MW power.

3 Attenuation of the ELDOR channel

To quantify the effect of attenuating the ELDOR channel, transient nutation experiments have been performed using the ELDOR channel for the first pulse of the sequence. The position of the first minimum gives an estimate for the length required to achieve a flipping angle of π . These experiments were conducted without any further attenuation of the MW power. Using our hardware, we found an irregular but consistent behavior of the ELDOR channel with respect to the MW attenuation. Thus, for low attenuation values hardly any change of the flipping angles were observed, and only at 16 dB a doubling of the required pulse length was observed. This corresponds to a reduction of 50% for the flipping angle for a given pulse length. From 16 dB on, normal behavior of the ELDOR channel was observed, i.e. reduction of the flipping angle by ~50% when increasing the attenuation by 6 dB. Figure S6. and Table S3 summarize the results of the transient nutation experiments.



Figure S6. Transient nutation experiments are various attenuations of the ELDOR channel. The minimum length of the ELDOR pulse amounts to 2 ns, where the graphs start.

Table S3. Pulse length and interpulse separation used for the measurement shown in Figur	е
S6. All other parameters as indicated in Table 1 in the main text.	

ELDOR Attenuation [dB]	Approx. position of minimum ^a [ns]	Reduction of flipping angle ^b
0	14	0%
6	16	~0%
16	30	50%
22	58	75%
28	118	87.5%

^a The resolution of the x axis amounts to 2 ns, limiting the accuracy of the value for low attenuation. At high attenuations, the minima get very broad, which also limits the accuracy of value. ^b For a given pulse length relative to 0 dB ELDOR attenuation.

4 RIDME

Figure S7 shows a RIDME time trace measured on **1** with a T value of 1 ms as an example of an unsuccessful measurement. Importantly, the chosen T value is on the same order as the spin lattice relaxation time for the trityl spin centers used herein, therefore fulfilling one of the experimental prerequisites to observe modulations in the RIDME experiment. In the case at hand no modulation is observed. Furthermore, the quick decay of the signal intensities suggests that the method would be inferior to the other methods, even if modulations would be visible.



Figure S7. RIDME time trace obtained on 1.

Noteworthy, it was possible in one case to obtain a modulated RIDME time trace for **3**, using pure dichloromethane as solvent (i.e. a microcrystalline frozen solvent). This could be caused by the increased heterogeneity around the spin centers, leading to different relaxation behaviors for spins on different trityl groups. However, using non-glassy solvent led to a strongly decreased overall sensitivity in all experiments and also stronger damping in the time traces of PELDOR, SIFTER, and DQC (data not shown). Furthermore, the modulated part at the end of the time trace shown in Figure S8 is noted. These modulations have the same period as those observed at the beginning of the time trace and appear to be caused by the dipolar interaction between the electrons. Thus, there appears to be an alternative coherence pathway in the five-pulse RIDME sequence which leads to the formation of a modulated time trace, possibly somewhat similar to the mechanism of the other single frequency sequences SIFTER and DQC.



Figure S8. RIDME time trace obtained on 3 in pure DCM.

5 Wavefunctions and energies in the different coupling regimes

5.1 Isotropic interactions

The isotropic case is of importance for the treatment of the exchange interaction observed in the room temperature cw EPR spectra and was discussed previously.¹

5.2 Assessment of the $\Delta \omega$ values in frozen solution

As discussed in the main text and above, the ratio $|J-D/2|/|\Delta\omega|$ is of large importance. To evaluate this ratio, it is necessary to assess the accessible values of $\Delta \omega$ in a given molecule and experiment. For the investigation of the exchange coupling constants in liquid solution, molecules of **3** with one ¹³C nucleus have been considered. The very small linewidth of the main signal and of the satellite lines in liquid solution (< 1 MHz) was clearly lower than the hyperfine coupling constants of all satellite lines. Hence, $\Delta \omega$ for the two electron spins on the two trityl groups was entirely determined by the hyperfine coupling constant in this situation. Therefore, it was possible to neglect residual contributions to the frequency difference caused by the EPR linewidth (for example stemming from coupling to ¹H nuclei within the diamagnetic backbone of **1**, for example) when considering the spectra obtained from liquid samples. The echo detected EPR spectrum of 3 (and the other trityls measured in this work) in frozen solution at Q-band frequency however has a larger linewidth, which is of the same order as the other EPR parameters. Furthermore, in SIFTER and DQC the main line is used for observation and pumping simultaneously. Thus, in these experiments the $\Delta \omega$ of the two spins within a molecule **3** is entirely caused by anisotropic broadening due to hyperfine coupling to nearby protons (or deuterons from the solvent) and g anisotropy. To analyze $\Delta \omega$ in **3** in frozen solution, Figure S9 is considered.



Figure S9. a) Echo detected EPR spectrum of **3** at Q band frequency and Gaussian fit to the main signal. The arrow indicates the lower and upper limit of the integration window used to obtain the plot shown in b), the dotted red lines include 98% of the signal's intensity. b) Integral of the Gaussian fitting function. The *x* axis has been converted from magnetic field scale to a frequency scale and shifted to start at 0 MHz.

Figure S9a) shows the echo detected EPR spectrum of **3** along with a Gaussian function which has been used to fit the main part of the EPR signal. The agreement between the Gaussian fitting function and the main part of the signal is quite good. This suggests that the main signal can be treated as isotropic, despite being recorded in frozen solution. In an isotropic signal, the resonance fields of the two electron spins in **3** are uncorrelated. For a molecule of **3** without ¹³C nuclei, the most extreme case would be encountered if electron A had its resonance field at the low field edge of the main signal while electron B had its resonance field at the high field edge of the signal. By integrating the signal it is possible to assess the probability for either of the two spins to have a given resonance field. To avoid complications stemming from noise and the satellite lines, the Gaussian function is integrated instead of the actual signal. The result of the integration is shown in Figure S9b) and demonstrates that 98 % of all molecules resonate within a frequency window of 16 MHz. If this window is subdivided into 16 intervals one can calculate the probabilities of either spin to resonate at a frequency range of 1 MHz (Table S4).

Freqency range [MHz]	Probability [%]	Freqency range [MHz]	Probability [%]
11 – 10	1.3	19 – 18	11.3
12 – 11	2.1	20 – 19	10.4
13 – 12	3.3	21 – 20	8.7
14 – 13	5	22 – 21	7
15 – 14	7	23 – 22	5
16 – 15	8.7	24 – 23	3.3
17 – 16	10.3	25 – 24	2.1
18 – 17	11.3	26 – 25	1.3

Table S4. Probability of either spin to resonate within a certain frequency window as defined in the x axis of Figure S9b).

With these values it is possible to calculate the probability to find a certain frequency difference for any spin pair. Taking the definition for the occurrence of strong coupling given in the main text (i.e. $|J-D/2|/\Delta \omega \ge 10$), J = 37.5 MHz, and D/2 = 2.4 MHz one arrives at |J-D/2| = 35.1 MHz (the relative sign of J and D is discussed below. This means, that all spin pairs with a frequency offset of 3.5 MHz or lower are expected to be strongly coupled. Using the tabulated values one can calculate that 51.7 % of all spin pairs in 3 have a frequency offset of 3 MHz or less, while 63.2 % of all spins have a frequency offset of 4 MHz. Thus, taking the average of these two values one can estimate that ~57.5 % of all molecules will be in the strong coupling regime. Since the contribution from the satellites to the signal is not considered in this calculation, this value is probably an overestimation and should be taken as an upper bound. If one assumes that only the strongly coupled carbon atoms (i.e. the 18 ortho and para ¹³C nuclei) resonate outside the main signal one can estimate a lower bound by multiplying the obtained value with 0.82 to arrive at an estimate of 47.1%. Thus, between 47.1 and 57.5 % off all spin pairs in 3 are expected to be in the strong coupling regime in SIFTER and DQC. An experimental assessment on the fraction of molecules which are coupled strongly can be obtained by integrating the distance distribution derived from these experiments (Figure S10). Since the distance distribution has a rather complicated shape it is difficult to justify an exact integration window. Here, the lower bound is defined as 1.90 nm, which is the average distance of the intense peak minus 0.035 nm which is about 1.5 times the half width at half height. The distances in between the dashed line correspond to distances caused by molecules in the strong coupling regime. Molecules in the intermediate coupling regime are responsible for the broad shoulder towards higher distances. Here, the upper bound for the integral is taken to be ~2.24 nm as indicated in Figure S10a). Figure S10b) shows the integral of the distance distributions. Importantly, the Figure shows that 54% of all molecules are experimentally observed to be in the strong coupling regime in agreement with the estimate given above. As will be discussed below, the second value indicated in Figure S10b) is also in agreement with theoretical predictions based on the arguments outlined above.



Figure S10. a) Experimental distance distribution of **3** obtained from SIFTER and DQC. The left dashed line indicates the start of the integration window, the arrow indicates the end of the integration window. **b)** Integral of the distance distribution with the upper and lower bounds as indicated in **a**).

5.3 Anisotropic and isotropic interactions

The discussion of the different coupling regimes including anisotropic coupling proceeds along the same lines as discussed above for the isotropic case.¹ The most important difference is that the discussion in this chapter focuses on those molecules of **3** which do *not* contain any ¹³C atom. Therefore, only the spin functions of the two electrons have to be considered. First, the spin Hamiltonian matrix is inspected. Taking the biradical wavefunctions

$ 1\rangle = \alpha_1 \alpha_2\rangle$	(S-1)
$ 2> = \alpha_1\beta_2>$	(S-2)
$ 3\rangle = \beta_1 \alpha_2\rangle$	

$$|4\rangle = |\beta_1\beta_2\rangle \tag{S-4}$$

and the spin Hamilton operator given by eq. 1 in the main text, one arrives at the spin Hamiltonian matrix for a spin system consisting of two electron spins and no nuclear

spins. To simplify the equations, the factor $(1-3(\cos\theta)^2)$ is dropped from the equations. This means that only molecules in which the interspin vector is aligned perpendicular to the external field B_0 are taken into account.ⁱ In frequency units, the spin Hamiltonian matrix is given as

$$\bar{H} = \begin{pmatrix} \omega + \frac{J+D}{4} & \frac{2J-D}{4} & \\ & \frac{\Delta\omega}{2} - \frac{J+D}{4} & \frac{2J-D}{4} & \\ & \frac{2J-D}{4} & -\frac{\Delta\omega}{2} - \frac{J+D}{4} & \\ & & -\omega + \frac{J+D}{4} \end{pmatrix}$$
(S-5)

very similar to the matrix obtained in the isotropic case. The derivations made here are general and apply for any spin pair with different resonance frequencies regardless of the origin of the frequency difference $\Delta \omega/2$. For **3**, $|\Delta \omega|$ may have any value lower or equal to the linewidth of the EPR spectrum (see section 3.2) and may stem from hyperfine coupling of the nuclei to nearby protons or the small *g*-anisotropy. As for the isotropic case, the block diagonal form of the matrix \overline{H} reveals that the functions $|1\rangle$ and $|4\rangle$ do not mix with any other function, i.e. are eigenfunctions of the spin Hamiltonian operator in all coupling regimes. The eigenvalues of these functions can be read off directly from the matrix. Therefore, the remaining discussion focusses on the functions $|2\rangle$ and $|3\rangle$, for which the submatrix

$$H_{freq,|2>,|3>} = \begin{pmatrix} \frac{\Delta\omega}{2} - \frac{J+D}{4} & \frac{2J-D}{4} \\ \frac{2J-D}{4} & -\frac{\Delta\omega}{2} - \frac{J+D}{4} \end{pmatrix}$$
(S-6)

has to be considered to obtain the eigenvalues and eigenfunctions. For the eigenvalues, the determinant

$$\begin{vmatrix} \frac{\Delta\omega}{2} - \frac{J+D}{4} - E & \frac{2J-D}{4} \\ \frac{2J-D}{4} & -\frac{\Delta\omega}{2} - \frac{J+D}{4} - E \end{vmatrix} = 0$$
(S-7)

or

$$E^{2} + \frac{J+D}{2}E - \frac{3}{16}J^{2} + \frac{6}{16}JD - \frac{\Delta\omega^{2}}{4} = 0$$
 (S-8)

has to be solved, yielding eigenvalues of

$$E_{2',3'} = -\frac{J+D}{4} \pm \frac{1}{2} \sqrt{\left(J - \frac{D}{2}\right)^2 + \Delta\omega^2}$$
(S-9)

ⁱ These molecules contribute the highest weight to the ensemble of molecules. The omitted factor can be reintroiduced at later stages to consider coupling frequencies at arbitrary orientations.

where the subscripts 2'and 3' refer to the eigenfunctions |2'> and |3'> and the plus sign before the square root is valid for |2'>. The simplest cases are the weak and strong coupling regimes. In the weak coupling regime $|\Delta \omega| \gg |J - \frac{D}{2}|$ holds and the eigenvalues amount to

$$E_{2'} = -\frac{J+D}{4} + \frac{1}{2}\Delta\omega$$
 (S-10)
$$E_{3'} = -\frac{J+D}{4} - \frac{1}{2}\Delta\omega$$
 (S-11)

In accordance with expectations for a biradical system, in which the pseudosecular parts of the spin Hamiltonian operator can be omitted. Therefore, the eigenfunctions |2'> and |3'> are identical with the biradical basis set functions |2> and |3>, respectively. For distance measurements in such a biradical system the energy differences of the transitions $|1\rangle \rightarrow |2\rangle$ and $|4\rangle \rightarrow |3\rangle$ monitored in PELDOR are of interest

$$E_{1} - E_{2'} = \omega - \frac{\Delta \omega}{2} + \frac{J + D}{2}$$
(S-12)
$$E_{3'} - E_{4} = \omega - \frac{\Delta \omega}{2} - \frac{J + D}{2}$$
(S-13)

The coherent superposition of these two transitions yields the modulation frequency in PELDOR for the weak coupling case as

$$v_{mod,weak} = J + D \tag{S-14}$$

in accordance with eq. 5 in the main text. In the strong coupling regime $|\Delta \omega| \ll |J - \frac{D}{2}|$ holds and the eigenvalues amount to

$$E_{2'} = +\frac{J}{4} - \frac{D}{2}$$
(S-15)
$$E_{3'} = -\frac{3J}{4}$$
(S-16)

As for the isotropic case, |2'> corresponds to a triplet state function whereas |3'> is a singlet state function in the strong coupling regime.

$$|2'_{strong} > = \frac{1}{\sqrt{2}} (|2 > + |3 >)$$

$$|3'_{strong} > = \frac{1}{\sqrt{2}} (|2 > - |3 >)$$
(S-17)
(S-18)

As before, transitions are only allowed between the triplet state functions in this regime, i.e. the transitions $|1>\rightarrow|2'>$ and $|4>\rightarrow|2'>$. The superposition of the coherences from these transitions yield the modulation frequency

$$v_{mod,strong} = \frac{3D}{2}$$
(S-19)

in accordance with eq. 8 in the main text. In the intermediate coupling regime the general eq. S-9 has to be used. Before this is discussed, the wavefunctions |2'> and |3'> have to be considered in more detail. As in the isotropic case, the wavefunctions |2'> and |3'> are admixtures of the biradical wavefunctions with coefficients c_2 and c_3 . These coefficients can be obtained by solving the secular equations

$$(\frac{\Delta\omega}{2} - \frac{J+D}{4} - E) \cdot c_1 + (\frac{2J-D}{4}) \cdot c_2 = 0$$

$$(\frac{2J-D}{4}) \cdot c_1 + (-\frac{\Delta\omega}{2} - \frac{J+D}{4} - E) \cdot c_2 = 0$$
(S-20)

and by taking into account the normalization condition

$$c_1^2 + c_2^2 = 1 \tag{S-21}$$

This yields

$$|2^{'}\rangle = c_{1}|2\rangle + c_{2}|3\rangle$$
 (S-22)
 $|3^{'}\rangle = c_{2}|2\rangle - c_{1}|3\rangle$ (S-23)

And

$$c_{1} = \sqrt{\frac{(J - \frac{D}{2})^{2}}{(J - \frac{D}{2})^{2} + (\left(\sqrt{(J - \frac{D}{2})^{2} + \Delta\omega^{2}} \mp \Delta\omega\right))^{2}}}$$
(S-

24)

$$c_{2} = \sqrt{\frac{\left(\left(\sqrt{\left(J - \frac{D}{2}\right)^{2} + \Delta\omega^{2}} \mp \Delta\omega\right)\right)^{2}}{\left(J - \frac{D}{2}\right)^{2} + \left(\left(\sqrt{\left(J - \frac{D}{2}\right)^{2} + \Delta\omega^{2}} \mp \Delta\omega\right)\right)^{2}}}$$
(S-

25)

where the upper sign corresponds to $|2'\rangle$ and the lower sign to $|3'\rangle$. As apparent from eqs. S-24 and S-25, the coefficients are complex functions of the coupling constants *J* and *D* as well as the frequency difference $\Delta \omega$. Figure S11 shows the coefficients c_1 and c_2 as a function of $|(J+D/2)/\Delta \omega|$.



Figure S11. Coefficients C_1 and C_2 for $|2\rangle$ as a function of $|J-D/2|/\Delta\omega$.

Noteworthy, below $|J+D/2|/\Delta\omega = 0.1$ and above $|J+D/2|/\Delta\omega = 10$ the coefficients only change marginally and are already almost identical to the coefficients needed to reproduce the functions of the weak and strong coupling regimes, respectively. In the intermediate coupling regime, the coefficients and the energies of the spin states depend in a complex manner on J, D, and $\Delta \omega$ and change rapidly as a function of $|J+D/2|/\Delta\omega$. Therefore, it is not possible to give simple instructions on how to read off J and D from the frequency spectrum in the intermediate coupling regime. However, using eqs. S-5 and S-9, it is possible to calculate the modulation frequency obtained from the superposition of coherences stemming from two different transitions. Since $\Delta \omega$ amounts to a maximum value of only ~15 MHz (and is even lower than 10 MHz) for more than 94 % of the molecules without ¹³C atom, see section 5.2), it can be assumed that even the molecules in the intermediate coupling regime are still close to the strong coupling regime. This is also in agreement with the coefficients shown in Figure S11. Consequently, the dominating transitions that can be observed are still $|1>\rightarrow|2>$ and $|4>\rightarrow|2>$ as in the strong coupling case. The superposition of these transitions leads to

$$|v_{mod,int}| = |J + D - \sqrt{\left(J - \frac{D}{2}\right)^2 + \Delta\omega^2}|$$
 (S-26)

Eq. S-26 requires input-values for *J*, *D*, and $\Delta \omega$. From the cw measurements it was possible to obtain an approximate value of |J| = 20 - 38 MHz (details concerning the

magnitude of the exchange coupling constant J are discussed in a previous paper¹) $\frac{3}{3}$

whereas ²D could be determined from the singularities in the Pake pattern obtained from SIFTER and DQC as ~7.2 MHz. Figure S12 plots $|v_{mod,int}|$ according to eq. S-26 for both antiferromagnetic and ferromagnetic coupling against $|J+D/2|/\Delta\omega$. Noteworthy, the results depend strongly upon whether or not *J* and *D* have the same sign. If *J* and *D* have the same sign (i.e. antiferromagnetic coupling), the expected modulation frequencies are lower than the frequency obtained in the strong coupling regime while the frequencies are higher if the signs are different. Since all measurements yield modulation frequencies of 7.2 MHz (strong coupling) or below, the first scenario is plausible. Furthermore, this scenario also agrees with expectations based on the *p*-substituition pattern of molecule **3**, which leads to antiferromagnetic coupling.^{2,3} The plot of eq. S-26 in Figure S12 can be compared to the experimental data, with regimes of interest regarding $|J+D/2|/\Delta\omega$ for SIFTER and DQC as well as for PELDOR indicated in the Figure.

For SIFTER and DQC, the plot shows that 94% of all molecules without ¹³C atom of **3** (i.e. ~77% of all molecules of **3**, if the contribution of molecules with one ¹³C atom in the strongly hyperfine coupling ortho- or para-positions to the molecular ensemble (amounting to about 18 %) is taken into account) for J = 38 MHz are expected to have a modulation frequency of 6.3 MHz or higher, corresponding to an apparent distance of 2.023 nm or lower. Assuming the lower bound of J = 20 MHz, this value reduces to ~62%. Both values agree reasonably with Figure S10, which shows that 72% of all molecules yield apparent distances corresponding to such modulation frequencies.

For PELDOR, the frequency offset of the pulses amounts to 15 MHz. Therefore, J- $D/2|/\Delta \omega = 1.33 - 2.66$ on average for PELDOR. At $|J-D/2|/\Delta \omega = 2.66$, the expected dipolar frequency at θ = 90° amounts to ~4.6 MHz, in good agreement with the value of 4.5 MHz observed experimentally. In addition, Figure S13 shows that the modulation depends strongly on the angle θ in the intermediate regime, in contrast to the strong or weak coupling regime, where the dipolar coupling frequency roughly changes at values of θ close to 90°. This is shown in Figure S13 for a series of different frequency offsets $\Delta \omega$, assuming J = 37.5 MHz (the graph can also be used to estimate the behavior for J = 20 MHz, cf. Figure caption of Figure S13). Importantly, the frequencies decrease rapidly with decreasing θ and reach zero at around 80°. Such angles have high statistical weights whereas contributions from low angles θ decay rapidly.⁴ This explains the large contribution of low frequencies to the frequency spectrum observed in the PELDOR experiment and the lack of singularities. Now, the behavior for the lower bound of the exchange coupling with J= 20 cm⁻¹ is discussed, for which $|J-D/2|/\Delta \omega$ = 1.33 holds. Here, the expected dipolar frequency at θ = 90° is close to zero, but the angular dependence leads to a rapid increase in the absolute value of modulation frequencies and values close to 4 MHz are expected for θ around 80°. Thus, the frequencies observed in the PELDOR

experiment on **3** do not conflict with the frequencies observed experimentally for the range of coupling constants determined earlier.¹



Figure S12. Expected modulation frequencies for J = 37.5 MHz and D/2 = 2.4 MHz (black) as well as for J = -37.5 MHz and D/2 = 2.4 MHz (red) plotted against $|J-D/2|/\Delta\omega$. The dashed horizontal line indicates the value of *D*.



Figure S13. Dipolar coupling as a function of the angle θ for J = 37.5 MHz and various frequency offsets. The red double arrow indicates the predominantly observed frequencies in the PELDOR experiment, coinciding with those orientations which have a high statistical

weight. An offset of 25 MHz for J = 37.5 MHz produces approximately the same angle dependence as J = 20 MHz for an offset of 15 Mhz, since $|J-D/2|/\Delta \omega \approx 1.5$ in both cases.

Finally, the expected modulation frequencies depend heavily on the exact frequency offset of the spin pairs used for observation, which also contributes to broadening of the frequency spectrum and the distance distribution (this statement is also corroborated by our data, that shows vastly different time traces for $\langle \Delta \omega \rangle = 15$ MHz in PELDOR as compared to $\langle \Delta \omega \rangle = 0$ for SIFTER and DQC). The heavy dependence of the frequencies on $\Delta \omega$ and on θ explain the very strong damping of the PELDOR time traces obtained on molecule **3** and the resulting, apparently strongly broadened distance distributions.

6 Simulations of multi-spin effects using a geometrical model

In this section, some of the choices concerning parameters for the simulation of multispin effects (MSEs) are discussed. In addition, the effect of including angular correlations between the three spin centers is demonstrated in section 4.1.

6.1 Correlated angles versus uncorrelated angles

As discussed in connection to Figure 2 in the main text, the angles θ_{ij} between the magnetic field and the vectors joining spins *i* and *j* in a triangular molecule with spin centers i,j = A, B, or C are correlated. Thus, if spin A is observed, choosing the angles θ_{AB} and ϕ_{AB} suffices to define the angles $\theta_{AC and} \theta_{BC}$ for a given conformation and geometry of the molecule incorporating the spin centers A, B, and C (see also Figure 2 in the main text). This means, the three-spin correlations T_3 of an observed spin A can be calculated using eq. S-27:

$$T_{3} = \iint \cos(\omega_{AB}(\theta_{AB}) \cdot \Delta t) \cdot \cos(\omega_{AC}(\theta_{AB}, \phi_{AB}) \cdot \Delta t) d\theta_{AB} d\phi_{AB}$$
(S-27)

Note, that the coupling frequency ω_{AC} of the spin pair AC for a given interspin distance depends on the angle θ_{AC} which in turn depends on θ_{AB} and ϕ_{AB} . If no angular correlations were present, the three-spin correlation would instead be calculated using eq. S-28:

$$T_{3} = \iint \cos(\omega_{AB}(\theta_{AB}) \cdot \Delta t) \cdot \cos(\omega_{AC}(\theta_{AC}) \cdot \Delta t) d\theta_{AB} d\theta_{AC}$$
(S-28)

Since the angles would then not be correlated, the integration of the two angles can be performed individually, yielding eq. S-29:

$$T_{3} = \int \cos\left(\omega_{AB}(\theta_{AB}) \cdot \Delta t\right) d\theta_{AB} \cdot \int \cos\left(\omega_{AC}(\theta_{AC}) \cdot \Delta t\right) d\theta_{AC} = P_{3}^{AB} \cdot P_{3}^{AC}$$
(S-29)

This equation shows, that for uncorrelated angles the three spin correlation of spin A are just the product of the two-spin form-factors in the three spin system ABC P_{3}^{AB} and P_{3}^{AC} . Equations S-27 and S-29 are clearly different and thus the obtained form-factors are in general different as well for the two different equations. Figure S14 shows the form-factors obtained using the two different equations. Noteworthy, while the shape of the experimental time trace of compound **2** (black full line in Figure S14) is reproduced reasonably if equation S-27 is used (dotted black line in Figure S14), the coincidence of simulation and experiment is rather poor if equation S-29 is used (dotted red line in Figure S14) despite using identical molecular geometries for the

simulation in both cases, the only difference being whether or not angular correlation is taken into account.



Figure S14. Simulated form factor for 2 with and without angular correlation.

Noteworthy, DeerAnalysis is able to suppress MSEs in the simulated time traces only if eq. S-29 is used. Thus, taking the red dotted line as input and using the ghost suppression feature yields a form factor corresponding to the pair contribution, i.e. complete suppression of MSEs, which is not the case if the black dotted line is taken as input (Figure S15). In this second case, only partial suppression is achieved (this helps identifying MSEs).



Figure S15. Simulated time traces shown in Figure S14 after treatment in DeerAnalysis with the ghost suppression feature enabled. To use DeerAnalysis, a constant value of 0.07 was added to the form factors shown in Figure S15.

The inability to fully suppress MSEs with a simple approach as implemented in DeerAnalysis is in line with observations made previously by Valera *et al.*⁵ and underlines the importance of either experimentally suppressing MSEs or simulating the time traces using a more sophisticated model as done herein.

6.2 The bending angle of the molecular triangle

As explained in the main text, the different conformations 2 have been obtained by treating the molecule as an object with bendable arms. Obviously, one needs to define a range of allowed angles for this bending motion. Empirically, this could be done by scanning a large variety of ranges and observing the resulting effect on the simulated time traces. However, since the script in its current form needs approximately three hours to run on a normal personal computer, this approach was unpractical here. Instead, a small, representative variety of angular ranges was tested. The results of these tests are summarized in Figure S16, which shows simulations (dashed lines) using bending angles of ±16.5° as already shown in the main text(black), ±14° (red), ±19° (green), ±9° (blue), and ±24° (cyan). As can immediately be seen, using bending angles which are far too small (±9°) or too large (±24°) produce time traces which do not compare favorably to the experimental data. Contrastingly, the red and green simulated time traces are of similar quality as the black simulated time trace. There, the range of angles is only slightly lower or larger than for the black time trace, respectively. If the deviation of the simulations from experiment is quantified by means of the root-mean-square deviation, it is recognized that a range of $\pm 16.5^{\circ}$ is indeed the best of the tried ranges (Table S5). Nevertheless, it is clear that the apparent precision of that value is not to be taken too seriously, and an error of about ±3° within the used geometrical model is estimated.



Figure S16. Simulated form factors with different ranges of bending angle (dotted lines) and comparison to experimental data (full lines). The form factors of the three-spin system **2** are shifted by 0.4.

Table S5. RMSD values in	n arbitrary ur	nits of the	simulations	and the	e experimental	data	for
different angular ranges in	the geometric	cal model					

Angular range	RMSD (two spins)	RMSD (three spins) ^a
9.0	0.08985	0.02591
14.0	0.05760	0.01963
16.5	0.05717	0.01968
19.0	0.06384	0.03011
24.0	0.08141	0.03156

^a The RMSD for the three-spin form factor is dominated by the error in the steep fall at the beginning. Therefore, the RMSD for a range of bending angles of $\pm 9^{\circ}$ is lower than the RMSD for an angular range of $\pm 19^{\circ}$, despite the coincidence of the latter function qualitatively being better than that of the former function.

6.3 The weighting of the different conformers

Finally, the weighting of the different conformations within the molecular ensemble is discussed. To that end, Figure S17 is considered.



Figure S17. a) Recapitulation of the geometrical model presented in Figure 2 in the main text. b) Conformer obtained by moving one arm about a certain angle. The dashed thin arrow is at the position of the "unmoved" arm. c) Conformer obtained by moving arms about the same angle as in b) but in opposite directions. d) Conformer obtained by moving arms about the same angle as in b) but in the same direction.

Figure S17a) is similar to Figure 2a) in the main text and recapitulates the generation of conformers when the molecule is treated as triangular object with the interspin distance r_{ij} and the angles α_k . Parts b), c), and d) of Figure S17 show examples for conformers obtained by moving either one or two arms by a certain angle either clock- or counterclockwise. Now, for the simulations shown in the main text, each of that conformer would have the same probability/weighting in the simulation. This decision was made, as the simulations should only show that it was possible to simulate the time traces of **2** using a simple geometrical model if angular correlation is taken into account. Thus, not implementing any kind of weighting means reducing the number of parameters and focus entirely on the geometric relation between the spin centers A, B, and C. Introducing certain weighting would also mean using a more physical model to generate an ensemble of conformers. Implementing such a physical model is beyond the scope of the presented work, as this would require extended theoretical studies on the potential energy surface in the conformational space of 2. Two examples are discussed and the corresponding simulations are shown. First, one could argue that bending of the molecular arms comes at a certain energetic cost und should therefore reduce the probability of finding that conformation. Thus, the conformation shown in b) would be less likely than the undistorted conformation shown in a) and the "doubly" deformed conformation in c) would be even less likely, because two arms are now bent. The conformation in d) on the other hand has the same bending of the two arms as in c), just with the direction of one bending changed. Thus, since angle α_2 is not affected by the overall deformation, this conformer is possibly more likely than the one shown in c) but less likely than the one shown in b). While it is of course possible to find a useful weighting relation taking this behavior into account based on gualitative arguments, it is now obvious that this would require a complex weighting function and therefore introduce many further parameters. In the second example, the deformed conformations are favored over the undeformed conformer. Obviously, finding an appropriate weighting function poses the same problem as in the case before. Here, the movement of the individual arms is weighted using a Gaussian distribution to obtain simulations for the two discussed scenarios. For just one of the two arms, the functions are shown in Figure S18.



Figure S18. Weighting function for bending one of the two arms out of the position shown in Figure S17a) in dependence of the bending angle. Black line: No weighting, i.e. as used in the simulations shown above and in the main text. Red Line: Gaussian weighting, favoring undistorted conformations. Blue line: Gaussian weighting favoring the distorted conformers.

The resulting weighting functions in the two-dimensional coordinate space are shown in Figure S19.



Figure S19. Weighting function for the movement of both arms favoring the undistorted conformation (**a**) or the distorted conformations (**b**).

Using the functions shown in Figures S18 and S19 to weight the different conformations yields the simulations shown in Figure S20.



Figure S20. Simulated form factors (dotted lines) with different with different weighting of the conformers and comparison to the experimental data (full lines). The form factors of the three-spin system **2** are shifted by 0.4, the color code is identical to the one used in Figure S18.

Figure S20 shows that using the weighting depicted in Figure S19a) simulations of similar quality to those obtained using uniform weighting of all conformers are

obtained, whereas favoring the conformers in which the arms are displaced from their positions leads to clearly worse agreement between simulation and experiment. This shows that implementing a physical model holds potential to improve the simulations. However, as already stated above, the effort necessary to find a justified model merits a study fully devoted to that subject and is therefore outside the scope of the present work.

7 References

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