MAGNETIC RESONANCE
Discussions

Magn. Reson. Discuss., referee comment RC3
https://doi.org/10.5194/mr-2022-20-RC3, 2023
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# Comment on mr-2022-20

Anonymous Referee #2

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Referee comment on "The effect of the zero-field splitting in light-induced pulsed dipolar electron paramagnetic resonance (EPR) spectroscopy" by Andreas Scherer et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2022-20-RC3, 2023

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The manuscript by Scherer et al. provides a detailed discussion of the effect of the zero-field interaction in the triplet state on light-induced PDS traces obtained using the LaserIMD and the LiDEER experiments. The authors consider the effect of non-secular terms of the zero-field interaction and the dipole-dipole interaction Hamiltonians on the modulation frequencies and form factors for LaserIMD and LiDEER from a theoretical standpoint and illustrate how the modulated echo traces are affected by this for a series of different spin system and experimental parameters. They conclude that the effects on LiDEER traces will be negligible in all cases likely to be encountered experimentally, whereas LaserIMD traces contain an additional decaying contribution for spin pairs with triplets in the ms=0 state. The latter has a clear effect on the appearance of the trace, in particular for low magnetic fields (X-band), large ZFS parameters and short distances. The authors go on to show that experimental LaserIMD traces can be simulated accurately using a dipolar kernel obtained considering non-secular terms of the ZF and the dipole-dipole interaction.

This manuscript discusses a very important aspect that is relevant for the wider application of light-induced PDS for structure determination. After minor revision aimed at clarifying some points and at improving the connection to experiments and experimentally relevant cases based on the comments provided below, the manuscript will be a valuable contribution to the light-induced PDS literature.

1. In the abstract and conclusions of the current version of the manuscript, the authors are quite vague on the extent to which "the ZFS cannot be neglected" in the analysis of dipolar traces. It appears that in all cases that could reasonably be expected to be encountered, accurate distance distributions are obtained even with the standard S=1/2 kernel (except for clear artifacts at the end of the distance range accessible for the given length of the trace). This should be made clearer in the abstract and conclusions.

2. In the initial discussion of the different triplet labels that have been shown to be appropriate for light-induced PDS it would be useful to provide the corresponding D and E values, given the later discussion of spin labels with small or large ZFS D parameters and the corresponding effects on the dipolar traces. Since this manuscript was first made available online, an additional paper discussing the use of erythrosin B as a triplet spin label has been published and should be referenced as well (DOI: 10.3390/molecules27217526).

3. The authors should clarify in the main manuscript which non-secular terms of the Hamiltonian are included in their treatment and based on what arguments. The current statement "we did not consider all non-secular terms and pseudo-secular terms were also ignored" at the end of page 7 (and in the SI) is insufficient and not clear. I would also recommend clarifying in the main manuscript that non-secular terms are considered both for the zero-field interaction (S_T*D*S_T) and for the dipole-dipole interaction (S_T*T*S_D), since previous treatments of systems with ZFS have focused on the effect of the pseudo-secular term of the dipole-dipole interaction on PDS traces (DOI: 10.1063/1.4994084).
In general, the manuscript could be clearer regarding which interaction and which terms actually affect the traces rather than just referring to it generally as ZFS.

4. All of the simulations aimed at illustrating the effects of different spin system and experimental parameters on the dipolar trace have been performed for a single distance and show modulations that persist for a very long time. All of the systems encountered experimentally will be characterized by a distribution of distances. I believe it would be more instructive and more meaningful to show simulations performed for distance distributions in addition to or instead of the single-distance simulations as these would be closer to traces encountered experimentally and allow users to judge what effect they could expect in experimentally relevant situations. For example, it would be interesting to see how the V_mS=0 contribution would be affected by distance distributions with increasing widths. Also, would the effects illustrated in Figures 6, 8 and S8 even be visible in the presence of a distance distribution rather than a single distance?

5. In the initial discussion of LiDEER, transition selection is mentioned, but orientation selection is currently not. In the discussion of the LiDEER simulations and orientation selection, it would be useful to more clearly separate discussion of the effect of orientation selection on its own and the orientation selection effect on the additionally considered non-secular contributions. The sentence in lines 14-15 of page 20 will likely not be clear to readers not familiar with orientation selection in triplet states, I would consider including a plot to illustrate what is meant.

6. It would be very useful to compare distance distributions extracted from the experimental LaserIMD data using the standard S=1/2 kernel and the correct kernel including the effects of the non-secular terms in the Hamiltonian in Figure 10. It appears that the effect of the V_mS=0 contribution would mostly be compensated by the background contribution if the S=1/2 kernel is used and therefore not significantly affect the extracted distance distribution. Therefore, if only the distance distribution is of interest, it would appear that the standard analysis should be sufficient in almost all cases.

References:
In the introduction, at several points very recent papers are cited for relatively general aspects that are better discussed in older papers or reviews or that are in some cases even not actually discussed in the cited paper. Examples are on p.3, line 21, p.3 line 24, p.4 line 3, p.4 line 5, p.20 line 11.
On page 2, line 4, the correct reference to cite would be the original paper proposing a triplet state spin label (DOI:10.1021/ja502615n).
When Tikhonov regularization is first mentioned on page 4, the Bowman (DOI:10.1007/BF03166560) and Jeschke papers (e.g. DOI 10.1007/BF03166574) first discussing its use for the extraction of distance distributions should also be cited.
On p.6, line 12, a book or review would be a more appropriate reference.
When ReLaserIMD is mentioned on page 12, the original paper proposing it should be cited DOI: 10.1002/cphc.201900139.
In the discussion of the LiDEER experiment and orientation selection, the references exploring the effects of orientation selection in light-induced PDS should be cited.

Additional minor comments:
- The zero-field splitting is a manifestation of the effect of the zero-field interaction on the energy levels, the authors should consider referring to it as the "zero-field interaction" or just "zero-field splitting" rather than "zero-field splitting interaction" in the title and throughout the manuscript.
- The initial discussion of the light-induced PDS experiments on page 2 goes into some detail, but currently does not explicitly mention the reason for using the nitroxide as the observer spin in LaserIMD but as pump spin in LiDEER. In an introduction intended for an audience not familiar with light-induced PDS, this would be useful information to include.
- In the discussion of orientations and powder averaging, the authors should clarify whether they are considering a fixed orientation of the dipolar vector with respect to the triplet molecular frame or not and why.
- The authors should consider including the simulation script for LiDEER used for the time-domain simulations with Spinach in the SI.
- On page 13, line 14, the authors state that the frequency shift "seems to be averaged out after integration". Would it be possible to expand on this or explain why?
- On page 13, the authors state that modulation depths larger than 66.6% could be reached as the "modulation depth is increased by the ZFS". Given that the additional contribution from ms=0 appears to be a decay rather than a modulation, can it really be considered an increase in modulation depth?
- The effects on the distance distributions in Figure 7 are hard to make out in the current plots.
- The authors consider two different sets of spin system parameters for their simulations, with a D parameter of ca. 1 GHz, which is assigned to TPP, and with a D parameter of 3.5 GHz, for which they only vaguely state that "such high values are possible for some labels". I would recommend specifying which label(s) they mean.
- When the software tool for the calculation of LaserIMD kernels is mentioned, it would be useful to also mention this can be used in conjunction with DEERLab.
- In Figure S8, the authors should specify more clearly and indicate where the observer pulse was placed with respect to the triplet spectrum.

Typos and language:
p.2, line 11: on the other side -> on the other hand
p.3, line 1: clarify what is meant by contributions
p.3, line 2: spliting -> splitting
p.5, line 24: do not dependent on -> do not depend on

p.6, line 11: of the zero-field eigenstates (remove "of the ZFS")
p.9, eq. 25-27: some of the Euler angles have a subscript t and some a subscript T, they should all be T
p.9, line 14: insight in these expression -> insight into this expression
p.11, line 5: microwave pulses who -> microwave pulses which
p.11, line 16 and p.20, line 3: Euler angels -> Euler angles
p.12, line 17: pi - pump -> pi_pump
p.13, line 3, p.14, line 8, captions of Fig.4-5,7, p.17, line 8, p.23, line 15: omega is used for angular frequency units elsewhere, here it is used as a frequency in GHz
p.13, line 6: the abbreviation TPP is used without specifying what it stands for
p.13, line 15: for this terms -> for these terms
p.13, line 19: with and a modulation -> with a modulation
p.14, line 13: triplet states spin being -> triplet states being
p.15, lines 2-3: This fits to -> This agrees with
p.15, line 4: feels a stronger effect of -> is more strongly affected by
p.15, lines 8-9: rephrase
p.15, line 9 and p.21, line 11: dependency -> dependence
p.17, line 11: in principle by influenced -> in principle be influenced
p.17, line 17: extend -> extent
p.18, Figure 7: consider using the same y-axis limits for X- and Q-band data to more clearly show differences
p.19, line 12: correspond closer to -> are closer to
p.19, line 14: rephrase
p.19, line 18: can thus an option -> can thus be an option?
p.21, lines 21-22: rephrase
p.22, line 1: The is -> This is
p.25, line 9-10: rephrase end of sentence
SI, eq. S5: w_S -> w_D
SI, page 5: rephrase sentence after eq. S31