Our answers are written in green, changes in the manuscript are in blue.

1. As I understand it, all analysis in terms of distance distributions in this work has been performed with the DEER kernel (S = 1/2 approximation), while you argue that the kernel should include effects due to the ZFS and provide software for computing such adapted kernels. You analyze in terms of distance distributions with the open-source software DeerLab. Why don't you directly compare analysis by Tikhonov regularization with the DEER kernel and your kernel? This would be particularly valuable for your experimental data.

   We performed the suggested analysis and added to results in the SI. They are discussed in the following paragraph which is added to the manuscript:

   To see how the additional decay of the ZFS affects the analysis of experimental LaserIMD traces, the recorded data were analyzed with Tikhonov regularization; and the results that are obtained with a LaserIMD kernel that includes the ZFS are compared with those obtained by a DEER kernel that ignores the ZFS (see S11 for a detailed overview of the results). The comparison of the obtained distance distributions shows that, even when the ZFS is ignored, the main distance peak is obtained correctly in all cases. For the measurements in Q-band, the entire distance distributions turn out to be virtually identical, regardless whether the ZFS is included in the analysis routine or not (see Fig. S13c-d). The situation is different in X-band. For TPP-AA_5-NO• in X-band, the strong additional decay is interpreted as an additional artifact peak at around 5.0 nm if the ZFS is ignored (see Fig. S13a). This peak disappears when the ZFS is considered. For TPP-AA_{10}-NO• in X-band, the analysis which ignores the ZFS also shows an additional peak around 7.0 nm. However, this artifact is not as pronounced as the one of TPP AA5 NO• and disappears in the validation. For the modulation depths and the background decay rates, there are notable differences when the ZFS is considered or not (see Table S5 and S6 in S11). In all cases, ignoring the ZFS leads to a reduced modulation depth. In Q-band, the modulation depth is reduced by a factor of ≈2/3 which means that the additional decay is completely assigned to the intermolecular background. In accordance with that, the background decay rates are larger when the ZFS is ignored. In X-band, these effects are not so pronounced. As the additional decay is partially fitted by introducing distance artifacts when ignoring the ZFS, the modulation depth is reduced only by a factor of 0.72 for TPP AA10-NO• and 0.84 for TPP-AA_5-NO•.
   These results show that ignoring the ZFS for the analysis of LaserIMD leads to artifacts in the obtained results. For TPP as transient spin label, the artifacts are not so prominent in Q-band. There, the additional decay mostly leads to a stronger background decay and reduced modulation depth and the distance distribution remains virtually unchanged. In X-band, however, artifact peaks in the distance distribution can occur if the ZFS is ignored.

   The following paragraph was added in the conclusion:

   The analysis of the experimental and simulated LaserIMD data with Tikhonov regularization showed that ignoring the ZFS compromises the obtained results. For transient triplet labels with a ZFS of ≈1 GHz like TPP, this is no so problematic in Q-band. There, only the obtained modulation depths and background decay rates are affected by ignoring the ZFS and the distance distribution remains unchanged. In X-band, however, ignoring the ZFS is more severe and can additionally lead to artifact peaks in the distance distributions.

A new section S11 was added in the SI:

S11 Analysis of the experimental LaserIMD data

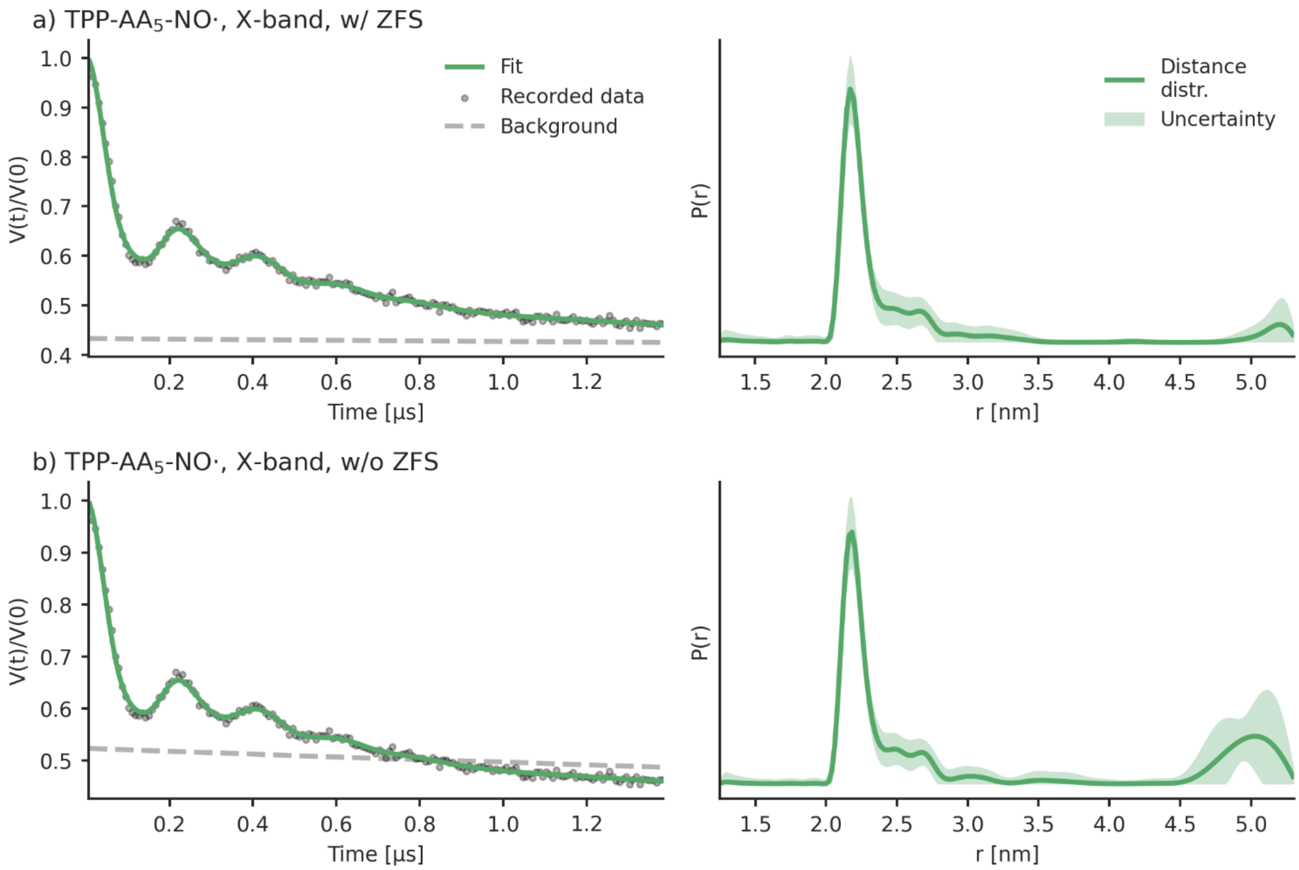

Figure S1: Experimental LaserIMD data of TPP-pAA$_5$-NO• recorded in X-band at 30 K in MeOD/D$_2$O (98/2 vol.%). a) Analyzed with a kernel that includes the ZFS and b) Analyzed with a kernel that ignores the ZFS. The raw data are depicted on the left side as grey dots with the fits as green line, the background fit is depicted as dashed grey line. The distance distributions obtained with Tikhonov regularization (Fábregas Ibáñez et al., 2020) is shown on the right side. The shaded areas correspond to the 95% confidence intervals that were obtained with bootstrapping.

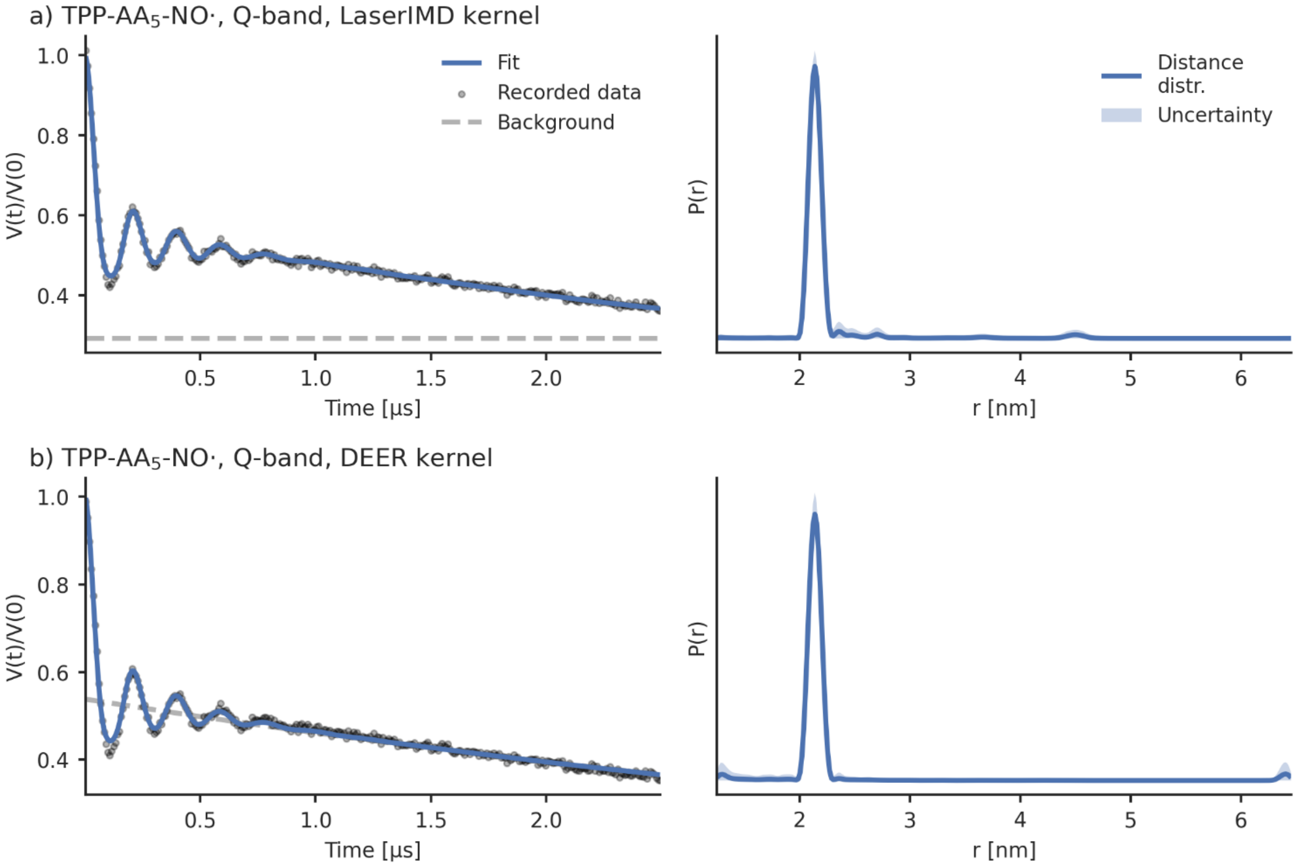

Figure S2: Experimental LaserIMD data of TPP-pAA$_5$-NO• recorded in Q-band at 30 K in MeOD/D$_2$O (98/2 vol.%). a) Analyzed with a kernel that includes the ZFS and b) Analyzed with a kernel that ignores the ZFS. The raw data are depicted on the left side as grey dots with the fits as blue line, the background fit is depicted as dashed grey line. The distance distributions obtained with Tikhonov regularization (Fábregas Ibáñez et al., 2020) is shown on the right side. The shaded areas correspond to the 95% confidence intervals that were obtained with bootstrapping.

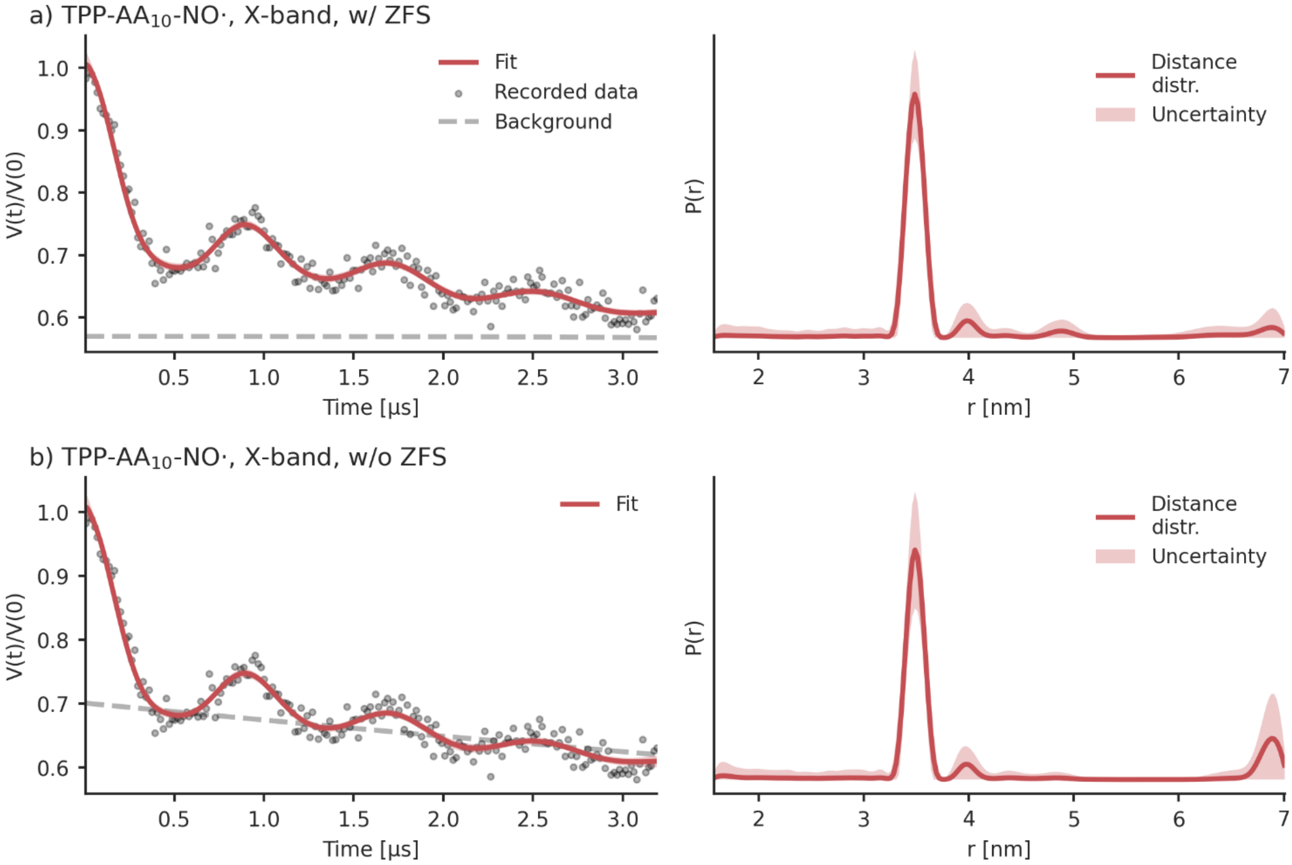

Figure S3: Experimental LaserIMD data of TPP-pAA$_{10}$-NO• recorded in X-band at 30 K in MeOD/D_2O (98/2 vol.%). a) Analyzed with a kernel that includes the ZFS and b) Analyzed with a kernel that ignores the ZFS. The raw data are depicted on the left side as grey dots with the fits as red line, the background fit is depicted as dashed grey line. The distance distributions obtained with Tikhonov regularization (Fábregas Ibáñez et al., 2020) is shown on the right side. The shaded areas correspond to the 95% confidence intervals that were obtained with bootstrapping.

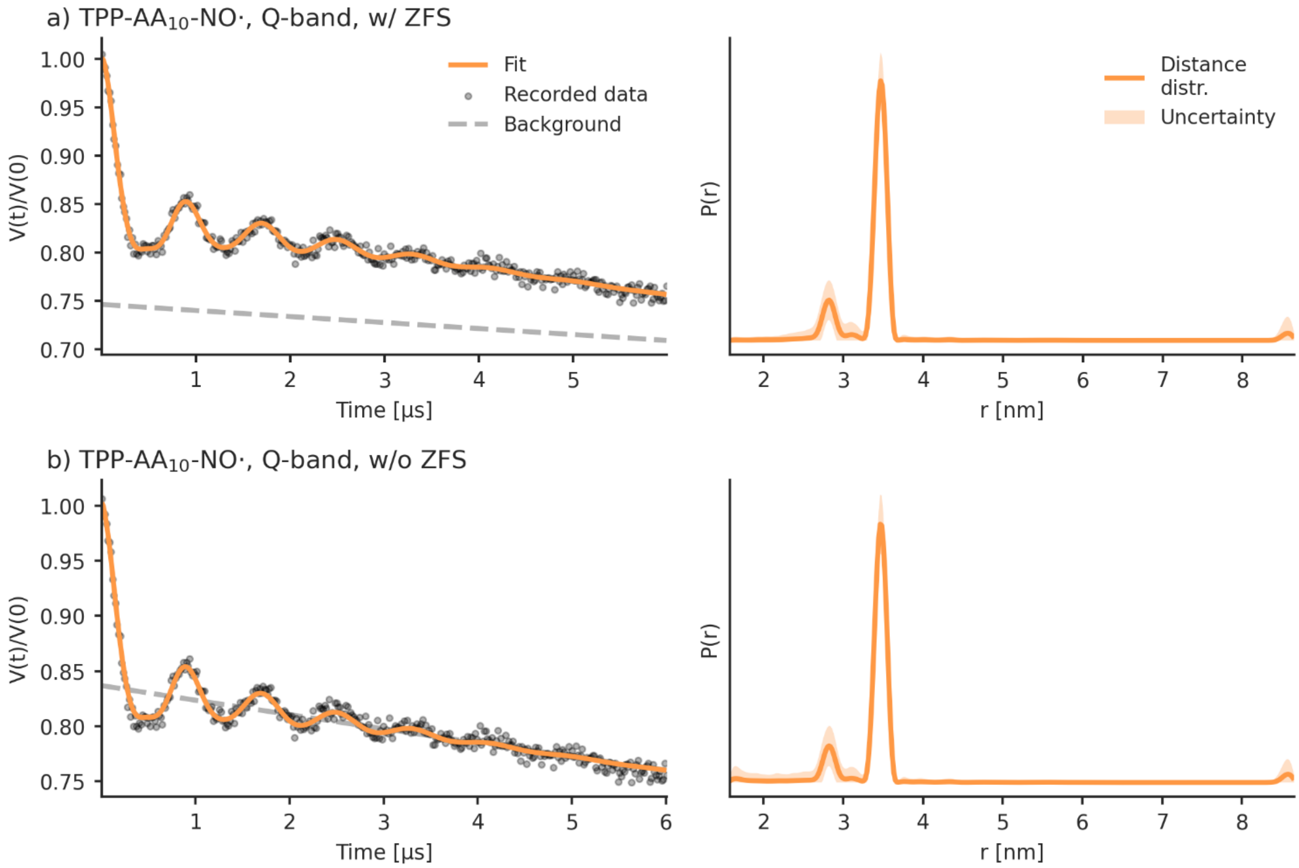

Figure S4: Experimental LaserIMD data of TPP-pAA$_{10}$-NO• recorded in Q-band at 30 K in MeOD/D_2O (98/2 vol.%). a) Analyzed with a kernel that includes the ZFS and b) Analyzed with a kernel that ignores the ZFS. The raw data are depicted on the left side as grey dots with the fits as orange line, the background fit is depicted as dashed grey line. The distance distributions obtained with Tikhonov regularization **(Fábregas Ibáñez et al., 2020)** is shown on the right side. The shaded areas correspond to the 95% confidence intervals that were obtained with bootstrapping.

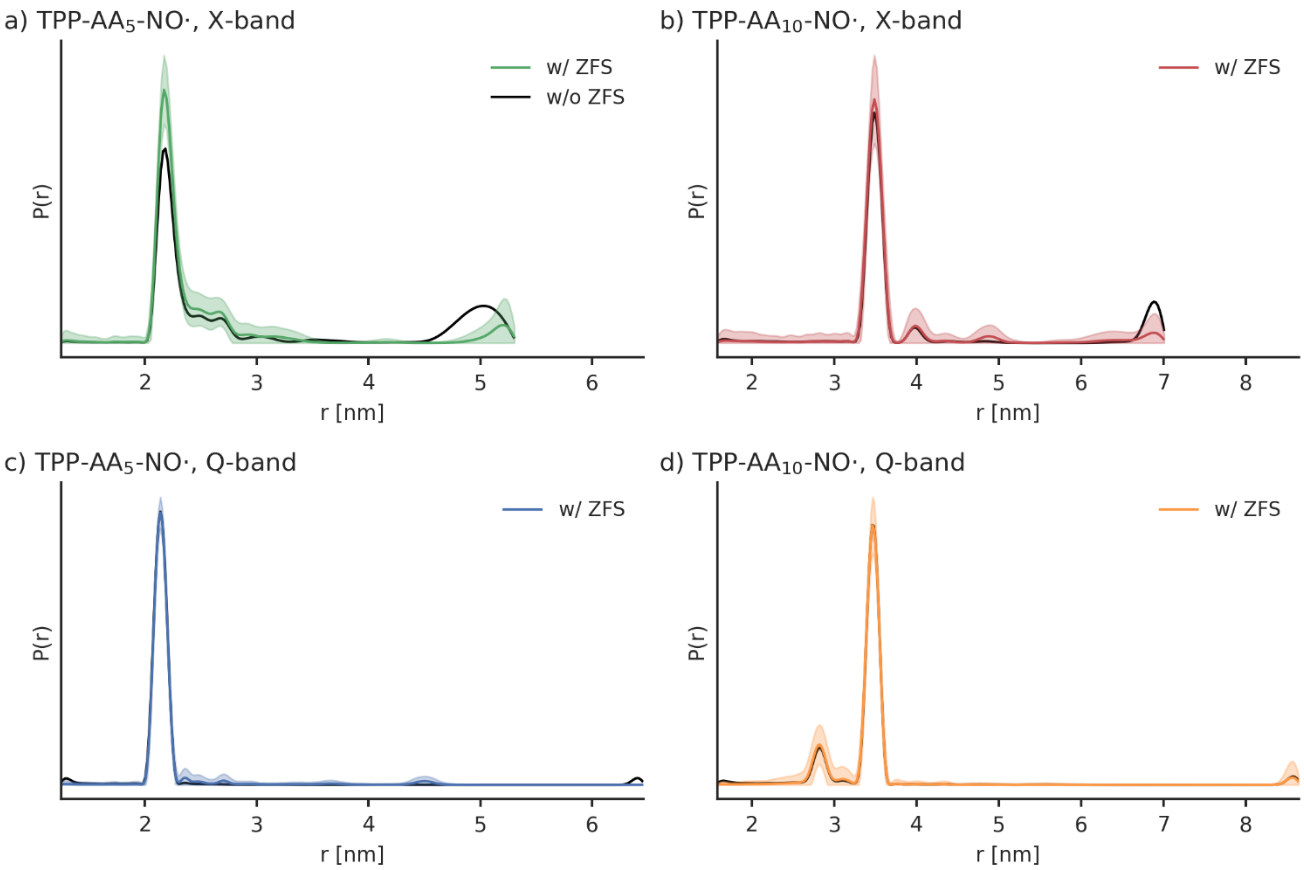

Figure S5: A comparison of the distance distributions that were obtained by analyzing the experimental LaserIMD data with a kernel that includes the ZFS (coloured lines) and with a kernel that ignores the FS (black lines). a) TPP-pAA$_5$-NO• in X-band. b) TPP-pAA$_5$-NO• in Q band. c) TPP-pAA$_{10}$-NO• in X-band. a) TPP-pAA$_{10}$-NO• in Q-band.

Table S1: Background decay rates and modulation depths as obtained by the analysis of the LaserIMD data of TPP-pAA$_5$-NO•.

	X-band		Q-band	
	w/ ZFS	w/o ZFS	w/ ZFS	w/o ZFS
Background decay rate [μs^{-1}]	0.0 (0.0, 0.2)	0.1 (0.0, 0.3)	0.000 (0.003, 0.000)	0.33 (0.27, 0.36)
Modulation depth [%]	57 (52, 59)	48 (43, 52)	71 (70, 72)	46 (45, 49)

Table S6: Background decay rates and modulation depths as obtained by the analysis of the LaserIMD data of TPP-pAA$_{10}$-NO•.

	X-band		Q-band	
	w/ ZFS	w/o ZFS	w/ ZFS	w/o ZFS
Background decay rate [μs^{-1}]	0.00 (0.00, 0.05)	0.13 (0.01, 0.28)	0.03 (0.01, 0.04)	0.09 (0.07, 0.11)
Modulation depth [%]	43 (40, 45)	31 (25, 36)	26 (25, 27)	17 (16, 18)

2. Except perhaps for the case of LiDEER performed at non-canonical orientations in X band, effects of ZFS on the extracted distance distribution are so minor that they are likely overwhelmed by other uncertainties in application work. If you agree with this assessment, you should clearly state this in the Conclusion.

   The following sentence was added on p. 26, l. 15:
   "For experimental LiDEER data which are recorded under such conditions the effect of the ZFS is negligible and a standard DEER kernel that does not consider the ZFS can be employed for data analysis."

3. I think that the experimental data is underused. Even if you perform only simulations with your own kernel (instead of using it in Tikhonov regularization), you should make an effort to assess the influence that ZFS has on the background decay rate and modulation depth for these examples.

   We think that this is also covered by the answer to point one.

4. Your referencing does not follow established rules. If you provide a reference for a statement, it should be either the first paper where this was found or a review/book chapter. If the statement can be considered as textbook knowledge, no reference is needed. In several cases you rather appear to cite the papers where you first encountered the same statement. For example, you cite me for textbook knowledge (distance dependence of the dipolar coupling) and for work by Salikhov, Tsvetkov, and Milov (p. 4, l. 11, citation (Jeschke, 2016) for the term "background", if this really needs a citation). There are many more instances, also affecting others. In a very general Introduction as you write it, the absence of citations to the pioneering work from the Novosibirsk lab is problematic.

   We updated the referencing to fit common usage. The references to textbook knowledge were deleted and other citations were corrected to the original work. We also added the work from Novosibirsk.

5. In the Introduction, you come close to considering orientation selection, but you never mention it. You should do so, as neglect of orientation selection is a feature of your treatment.

   On page 6, line 9 the following sentence was added:
   "In absence of orientation selection, the orientation of the dipolar vector and the transient triplet label are not correlated and the integration over the corresponding Euler angles can be done independently."

   On page 9, line 9, the following sentence was added:
   "Still assuming no orientation selection, this gives the following integrals."

6. "Please note that we did not consider all non-secular terms and pseudo-secular terms were also ignored." It is not clear to me, which terms you consider as pseudo-secular and how you selected the terms that you included. Section S1 of the Supplementary Information does not help. Common usage is that terms that you consider on top of the secular terms are pseudo-secular and terms that you drop are (considered as) non-secular.

   We rephrased the corresponding paragraph starting from p.7, l. 12:
   Even though in the secular approximation the ZFS has no effect in LaserIMD, it cannot be taken for granted that the non-secular terms can be ignored because the ZFS of some transient triplet labels can be quite large (Williams et al., 2020). Here, we additionally consider the terms $\hat{S}_{\mathrm{T},z}\hat{S}_{\mathrm{T},+} + \hat{S}_{\mathrm{T},+}\hat{S}_{\mathrm{T},z}$ and $\hat{S}_{\mathrm{T},-}\hat{S}_{\mathrm{T},z} + \hat{S}_{\mathrm{T},-}\hat{S}_{\mathrm{T},z}$ from the ZFS interaction and the terms $\hat{S}_{\mathrm{D},z}\hat{S}_{\mathrm{T},+}$ and $\hat{S}_{\mathrm{D},z}\hat{S}_{\mathrm{T},-}$ from the dipolar coupling. They connect the adjacent triplet states $|+1\rangle$ and $|0\rangle$ and $|0\rangle$ and $|-1\rangle$ of the triplet manifold and shift their energy in second order (Hagston and Holmes, 1980). This is illustrated in Fig. 2. The details of this calculation are described in S1. For this calculation, the remaining ZFS terms $\hat{S}^2_{\mathrm{T},+}$ and $\hat{S}^2_{\mathrm{T},-}$ were ignored. They connect the triplet states $|+1\rangle$ and $|-1\rangle$, which have a larger energy difference than adjacent states. Therefore, the second order energy shift of $\hat{S}^2_{\mathrm{T},+}$ and $\hat{S}^2_{\mathrm{T},-}$ is weaker than those of the considered terms. The terms $\hat{S}_{\mathrm{D},+}\hat{S}_{\mathrm{T},+}$, $\hat{S}_{\mathrm{D},-}\hat{S}_{\mathrm{T},+}$, $\hat{S}_{\mathrm{D},+}\hat{S}_{\mathrm{T},-}$, $\hat{S}_{\mathrm{D},-}\hat{S}_{\mathrm{T},-}$, $\hat{S}_{\mathrm{D},+}\hat{S}_{\mathrm{T},z}$ and $\hat{S}_{\mathrm{D},-}\hat{S}_{\mathrm{T},z}$ of the dipolar coupling were also ignored. They connect spin states of different manifolds of the doublet spin and the corresponding energies cannot be significantly shifted by the comparably weak dipolar coupling. It is shown in S2 that at magnetic field strengths that are relevant for experimental conditions the included non-secular terms from Eq. (S3) are sufficient and no further distortions are to be expected by the left-out ones.

The following sentences starting from p.7, l.13 were deleted:
~~In Fig. 2 it is shown, how the energy levels E_(m_D, m_T)^(non-sec) get shifted when additional non-secular terms of the ZFS and dipolar coupling are considered. This shift was calculated by including the non-secular terms from Eq. (S3) in S1 and diagonalizing the Hamiltonian with a second-order perturbation approach (Hagston and Holmes, 1980). This is described in detail in S1. Please note that we did not consider all non-secular terms and pseudo-secular terms were also ignored.~~

We also deleted these sentences from S1, p.3, l.4-7 to avoid repetition:
~~Here, $\hat{S}_{T,+}$ and $\hat{S}_{D,+}$ are the raising ($\hat{S}_+ = \hat{S}_x + i\hat{S}_y$) and $\hat{S}_{D,-}$ and $\hat{S}_{T,-}$ are the lowering ($\hat{S}_- = \hat{S}_x - i\hat{S}_y$) operators. Note that this non-secular part does not include all non-secular terms and all pseudo secular terms are also ignored here. As is shown in S2 these remaining terms have only a negligible effect at the magnetic fields strengths that are relevant in most experiments, and this is why they can be left out.~~

7. In powder averaging, an equidistant grid over cos β_{dip} would have been more efficient (all grid points would have had the same weight). I do not suggest that you repeat the work. This is just advice for future work.

   Thank you very much for the advice.

8. p. 5, l. 10: “The dipolar coupling tensor is axial”. This presumes the point-dipole approximation, which might be questionable for a TPP triplet at the shorter distance of 2.2 nm. In any case you should mention that your treatment uses the point-dipole approximation.

   We added a half sentence to clarify this point:
   “In the point-dipole approximation, the dipolar coupling tensor $\boldsymbol{T}$ is axial with the eigenvalues $\boldsymbol{T}_x = \boldsymbol{T}_y = -\boldsymbol{\omega}_{\mathbf{dip}}$ and $\boldsymbol{T}_z = 2\boldsymbol{\omega}_{\mathbf{dip}}$.”

9. 8. p. 12, l. 13: "In X-band the resonator was critically coupled to a Q-value of ≈ 900-2000 for higher sensitivity". Did you check this? A higher Q improves detection sensitivity, but reduces excitation bandwidth. Common wisdom is that, as long as you have sufficient microwave power, you should overcouple. What is different in your case?

   Bieber et al. found that LaserIMD performs better in critically coupled than in overcoupled resonators (Bieber et al., 2018). However, this was done on a different spectrometer in Q-band and we did not check it again in X-band. Therefore, we deleted the last part:
   "In X-band the resonator was critically coupled to a Q-value of ≈ 900-2000 ~~for higher sensitivity~~."

10. p. 13, l. 8: “effects of the background were ignored”: You probably want to say that background decay was ignored.

    This was changed to:
    “no background was added”

11. Typos

    The typos were corrected.