Dear Editor

Here is our response to the reviewers. Their comments were copied (black fonts), and our response, along with a description of changes made in the manuscript (blue fonts). We also made minor changes in the manuscript according to the comments of Alberto Collauto. These, along with the changes made in response to the reviewers, are highlighted in a copy of the revised manuscript.

Reviewer 1 – Gunnar Jeschke

While understanding of decoherence of electron spins in nitroxide spin labels improved substantially during the past few years, data for Gd(III) spin labels were relatively scarce, in particular at high frequencies (95 GHz) where these labeles perform particularly well. The current manuscript addresses this gap in a systematic way and presents very interesting results. Experiments and data analysis are state-of-the-art, data quality is high, and the presentation is clear. I have little to criticize. The following points should be addressed in minor revision.

Thank you !

1. The manuscript would profit from a Table that provides an overview of Tm (or relaxation rate 1/Tm) for the various samples.

   Making such a table for the various samples (labels and proteins), concentrations and temperatures and fields would be an endless table which will be hard to follow. We added a table to the SI for conditions that are usually used in DEER, namely 10K and 50 μM (or 25 μM), measured at the central transition.

Table S3 : Overview of the T_m and β values of the samples studied in this work measured by Hahn echo at the CT and 10 K.

Sample	Conc. (μM)	T_m (μs)		β	
Gd-PyMTA	50	16.53 ± 0.77		1.21 ± 0.07	
Gd-PyMTA-$d_{8,}$	50	19.59 ± 0.31		1.14 ± 0.05	
Gd-PyMTA-d_{12}	50	18.41 ± 0.84		1.16 ± 0.08	
Gd-TPMTA[a]	50	9.96 ± 0.57		0.92 ± 0.03	
Gd-TPMTA-d_8[a]	50	10.09 ± 1.33		0.94 ± 0.07	
Gd-TPMTA-d_{12}[a]	50	9.75 ± 0.42		0.90 ± 0.04	
		^{1}H protein	^{2}H protein	^{1}H protein	^{2}H protein
Ubi-Gd-PyMTA	25	8.1 ± 0.04	9.73 ± 0.05	1.43 ± 0.01	1.38 ± 0.01

Ubi-Gd-PyMTA-d_8	25	8.25 ± 0.04	9.1 ± 0.05	1.33 ± 0.01	1.27 ± 0.01
Ubi-PyMTA-d_{12}	25	8.18 ± 0.03	9.1 ± 0.07	1.35 ± 0.01	0.99 ± 0.01
Ubi-Gd-TPMTA[a]	50	5.4 ± 0.6	5.2 ± 0.3	1.19 ± 0.04	0.99 ± 0.01
Ubi-Gd-TPMTA-d_8[a]	50	6.2 ± 0.6	6.6 ± 0.4	1.02 ± 0.05	1.00 ± 0.02
Ubi-Gd-TPMTA-d_{12}[a]	50	5.6 ± 0.3	9.1 ± 0.05	1.02 ± 0.02	1.27 ± 0.01
Ub-Gd-DO3A	50	8.98 ± 0.04	8.09 ± 0.04	1.05 ± 0.01	0.912 ± 0.004
Ub-Gd-DO3A-d_8	50	8.77 ± 0.04	9.34± 0.05	1.014 ± 0.004	0.916 ± 0.004

[a]Average of measurements carried out at fields 1,2, and 3.

2. In the Conclusion (point 3), the authors discuss residual nuclear spin diffusion as a contribution to 1/Tm for C -> 0 and focus this discussion on only the label protons. A potential contribution from residual protons in the deuterated matrix should be mentioned.

   We changed to "At the limit of [C]→0, the contributions to T_m (0) can be residual NSD of the protons on the pyridine rings with hyperfine couplings below 0.4 MHz or residual protons in the deuterated matrix, tZFS, and direct T_1."

3. In principle, simulation tools exist for predicting the contribution of the label protons to 1/Tm (at least for the Hahn echo/CP1 case). While such predictions may be beyond the scope of the current manucsript, I encourage the authors to address this issue in the future, also relating this to point 2 (residual matrix protons).

   We added in point 3 of the conclusions : "In principle, it would possible to predict the contribution of the above mentioned weakly coupled protons and residual solvent protons to the Hahn echo decay using the analytical pair product approximation which allows for computationally efficient simulations and provides a good prediction.(Canarie et al., 2020; Jeschke, 2023). This, however, is beyond the scope of this manuscript."

4. Given the importance of Gd(III) longitudinal relaxation as a contrubtion to 1/Tm, it would be helpful to include a paragraph with a few references to previous work on T1 of Gd(III).

We added the following to page 5:
The T_1 values of Gd(III) complexes in solution are relatively short and therefore expected it to affect the Gd(III) phase relaxation. For example, Gd(III) ruler with a PyMTA chelate with distances of 3.4 nm has at W-band T_1 values in the range of 80-11 μs at the temperature range of 6-30 K respectively, (Seal et al., 2022), (Razzaghi et al., 2014). For the same type of ruler with distances of 2.1 and 6 nm T_1 of ~30 μs was reported at 10 K (Mocanu et al., 2025). The reported T_1 values of the spin label BrPsPy-DO3A-Gd(III) in the temperature range of 6-40 K are 132-9 μs (Seal et al., 2022). At Q-band the T_1 values are longer than at W-band; for the complexes of the [Gd^{III}(NO3Pic)] family, which have a small ZFS with D~500 MHz T_1 in the range of 190-200 μs was reported (Ossadnik et al., 2023).

5. Reference (Pannier et al., 2011) points to a 10th anniversary reprint of the original paper [(Pannier et al., 2000, https://doi.org/10.1006/jmre.1999.1944)]. It might be more appropriate to cite the original paper

   Oops, sorry about this, was fixed.

   References:

Canarie, E. R., Jahn, S. M., and Stoll, S.: Quantitative Structure-Based Prediction of Electron Spin Decoherence in Organic Radicals, J. Phys. Chem. Lett., 11, 3396-3400, 10.1021/acs.jpclett.0c00768, 2020.

Jeschke, G.: Nuclear pair electron spin echo envelope modulation, Journal of Magnetic Resonance Open, 14-15, 100094, https://doi.org/10.1016/j.jmro.2023.100094, 2023.

Mocanu, E. M., Ben-Ishay, Y., Topping, L., Fisher, S. R., Hunter, R. I., Su, X.-C., Butler, S. J., Smith, G. M., Goldfarb, D., and Lovett, J. E.: Robustness and Sensitivity of Gd(III)–Gd(III) Double Electron–Electron Resonance (DEER) Measurements: Comparative Study of High-Frequency EPR Spectrometer Designs and Spin Label Variants, Appl. Magn. Reson., 56, 591-611, 10.1007/s00723-024-01741-0, 2025.

Ossadnik, D., Kuzin, S., Qi, M., Yulikov, M., and Godt, A.: A GdIII-Based Spin Label at the Limits for Linewidth Reduction through Zero-Field Splitting Optimization, Inorganic Chemistry, 62, 408-432, 10.1021/acs.inorgchem.2c03531, 2023.

Razzaghi, S., Qi, M., Nalepa, A. I., Godt, A., Jeschke, G., Savitsky, A., and Yulikov, M.: RIDME Spectroscopy with Gd(III) Centers, J. Phys. Chem. Lett., 5, 3970-3975, 10.1021/jz502129t, 2014.

Seal, M., Feintuch, A., and Goldfarb, D.: The effect of spin-lattice relaxation on DEER background decay, J. Magn. Reson., 345, 107327, https://doi.org/10.1016/j.jmr.2022.107327, 2022.

Reviewer 2 - Giuseppe Sicoli

The manuscript represents an interesting example of understanding to design and design for understanding related to the impact of the deuteration within the context of the relaxation processes. The message of the manuscript is clear and exhaustively delivered; however, for fulfilling the main 'take-home' message of the manuscript, some minor points can be revised:

Thank you.

- For the comparison of the values of $1/T_{m,f}$ and b_f for different samples and temperatures (figure 7 C, F; please notice into the text at page 20 such figure has been referred as '5'), the authors refer to Figure 10 (page 22) for describing the contribution of the fast component. Besides the *fairly constant behaviour* for the PyMTA, it would be interesting to provide further elements to the discussion on the behaviour of TPMTA, exhibiting a completely different behaviour.

  Thank you; we changed 5 to 7.

  As for the different behavior of TPMTA, we added in p. 21 the following :

  The relative contribution of the two components is fairly constant in the temperature range tested for Gd-PyMTA, whereas for Gd-TPMTA the contribution of the fast component is constant for 1.6-4 K and thereafter, a significant increase with increasing temperature is observed in the range of 6-15 K **(Fig. 10)**. This trend seems to correlate with the relative intensity of the central transition (**Fig. 2**). Currently we do not have an explanation for this behavior.

- The general approach proposed does not mention the effect of the pH, which may have an impact into the affinity of the two main ligands described; such an effect on the relaxation is probably beyond the scope of the manuscript, but it can be worth to mention also that tuneable parameter (i.e., pH).

  We usually prepare the spin label at a pH where all carboxylates are deprotonated and able to coordinate the Gd(III), changing pH may lead to variation in the number of ligands and the ZFS, and will complicate things. We therefore think that this should be a completely different study.

- The assignment of dominating mechanism assigned for the two populations (*slow* and *fast*), as summarized on page 23 (lines 8-11) can eventually be reinforced by citing known structures where the T_1 and tZFS are distinctively contributing to the relaxation paths. It may support the effect of the deuteration for 'small' molecules and validate the less pronounced effect on labelled proteins.

  Unfortunately, as far as we know, there are no such studies, except the study of Raitsimring. It would be nice to have a correlation of T_1 with the ZFS, but currently, there is not enough data (measured at the same frequency and temperature) to support such a correlation.

- Please notice that the authors refer to Figure 2D (page 10) but the capital letter on the figure 2 (page 11) is missing. A-B-C-D on the four panel must be revised.

  This was fixed.