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 will present the following table for conditions that are usually used in DEER, namely 10K and 50 μM (or 25 μM), measured at the central transition.

Table : Overview of the T_m values of the samples studied in this work measured by Hahn at the CT and 10 K. For Gd-TMTPA the values measured at fields 1,2,and 3 are averaged.

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	50	9.96 ± 0.57		0.92 ± 0.03	
Gd-TPMTA-$d_{8,}$	50	10.09 ± 1.33		0.94 ± 0.07	
Gd-TPMTA-d_{12}	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	50	5.4 ± 0.6	5.2 ± 0.3	1.19 ± 0.04	0.99 ± 0.01
Ubi-Gd-TPMTA-$d_{8,}$	50	6.2 ± 0.6	6.6 ± 0.4	1.02 ± 0.05	1.00 ± 0.02
Ubi-Gd-TPMTA-d_{12}	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

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 will change 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 will add 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 will add 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, will be fixed.

   References:

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