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Interactive comment on "High sensitivity Gd^{3+}-Gd^{3+} EPR distance measurements that eliminate artefacts seen at short distances" *by* Hassane El Mkami et al.

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The authors propose a very interesting application of a high-bandwidth, high-power W-band setup to measure undistorted Gd(III)-Gd(III) DEER traces even for short distances, condition under which the mixing of the $|+\frac{1}{2},-\frac{1}{2}>$ and $|-\frac{1}{2},+\frac{1}{2}>$ states caused by the pseudosecular terms of the dipolar Hamiltonian results, under normal measuring conditions, in dampening of the dipolar modulation. A very interesting conclusion is proposed suggesting the use of the already available Gd(III)-based tags with low zero-field splitting even for short distances, provided that both the pump pulse and the detection sequence completely avoid the excitation of the central transition.

The manuscript is well written, and definitely suitable for publication on Magnetic Resonance; the conclusions are substantial and nicely supported by the presented data and analyses. However, there are some points that I would like to be addressed by the authors.

1. The style of the references is not homogeneous: in some cases the full DOI hyperlink is reported, whereas in other ones only the DOI number is displayed; some references make use of journal abbreviations, whereas in other ones the full journal title is mentioned. Besides, the absence of spacing and/or indentation makes it really hard to find a specific item. Moreover, references having the same first author are not always listed chronologically. I advise to follow thoroughly the author guidelines.

2. As far as I could see, no specific literature for Gd(III) labelling of DNAs has been cited although reference has been made in the text to this application (line 49).

3. I found the nomenclature proposed in Table 1 rather unclear; for example, why is a 10 ns-long pump pulse set to the maximum of the central transition once identified as P1 and once identified as P3 (6.0 nm Gd ruler)? I would find easier for the reader to have the relevant experimental conditions reported for each experiment (pulse length and frequency offset) in the figure caption or as inset, and, to improve the readability of the manuscript, I would consider moving the sensitivity considerations reported in Table 1 to the supporting material.

4. Is the (rather lengthy) discussion about the echo decay traces relevant for the purpose of this paper? After all, the measurements were performed on the maximum of the central transition, whereas the DEER detection sequence was always placed at spectral positions where the largest contribution to the echo comes from other transitions. A possible solution could be to move this section to the supporting material.

5. A high sensitivity of the experimental setup is claimed. However, a rather large sample amount (around 75 μL of a 40 μM solution, hence 3 nmol) was used compared to the typical ones used for conventional W-band or Q-band spectroscopy (around 5 μL

of a 40 μM solution, hence 0.2 nmol; 15 times smaller!), or even X-band spectroscopy (around 20 μL of a 40 μM solution, hence 0.8 nmol). An extension of the proposed approach to applications where the limiting factor is the sample amount, such as investigations inside cells or on systems that are challenging to express and/or label, is therefore in my opinion still not straightforward.

6. Throughout the main paper and the SI plots belonging to the same figure have different sizes and are not always aligned (see for example Figures 3 a/b, 5, S3, S4, S5).

7. Table 1: the shot repetition time should be given in time units; what is reported is the shot repetition rate.

8. Table 1: why was the shot repetition rate decreased from 3 kHz to 1 kHz for some of the measurements on the 2.1 nm Gd ruler (see Table 1)? Are measurements available to justify this choice?

9. Table 1: what was the used value of τ1 for the DEER experiments?

10. Were the DEER measurements performed with or without a phase cycling of the π/2 pulse? If without, which precautions were taken so as to have no constant offset of the DEER traces?

11. In Figure S3a the intermolecular contribution for the experimental condition P3O3 has been modelled as an increasing function, a clearly unphysical assumption (as also stated by the authors). The analysis of these experimental data has to be repeated by taking an exponential decaying function. Furthermore, the primary data are displayed only for t >= 0; is this the way in which the data were recorded? If so, why? If not, it would be advisable to plot the whole data, in such a way that the maxima of the recorded traces are visible.

12. Figures 5d and 7b: what do the black arrows highlight?

13. Table S1: which distribution of E values was taken to fit the experimental data

shown in Figure 2? Were the simulations perform assuming a monomodal distribution of D values around +D or a bimodal distribution of D values around $\pm$D? (I am not able to deduce this information from lines 197-199 of the main text).

14. Table S2: is the time corresponding to a decay of the echo signal to 10% of its initial value given as τ or 2τ? In which units is this value reported?

15. Captions of the Tables S2/S3: what is x? Was the dead time 2τ0 taken into account for the fit of the echo decay curves? (This is relevant as the traces were fitted with a non-exponential function).

16. Table S3: a biexponential behavior of the inversion recovery curves has been reported. Were other kind of experiments attempted aimed at minimizing the role of spectral diffusion? Besides, a T1 value resulting from a monoexponential fit of the experimental traces has been reported but no comparison between the biexponential and monoexponential fits is shown in Figure S2.

17. Figure S2: because of the poor resolution I can hardly see the experimental data points.

18. Figure S2a: what was the minimum used value of τ? This can't be deduced from the figure, where the first point of the decay trace has been set at 2τ = 0.

19. Figure S2b: the inversion recovery curves have not been collected till a plateau corresponding to the full recovery of the echo signal has been observed. This may result in severe uncertainties in the estimation of the longitudinal relaxation rate by fitting of the experimental data (Table S3).

20. Caption of Table S2 and lines 312-313 of the main text: why the fit of the echo decay curves has been described as a "sum of two stretched exponential functions" although for one of the components the exponent has been fixed to 1?

21. Figure S3: given the amount of free space on the page, I would consider useful to quickly recap, maybe in the form of a table, the relevant settings corresponding to the

different traces.

22. Figures S4, S5, S6: in my opinion, a reminder to the legend of Figure 2 for what concerns the color code used in the simulation of the EDFS-EPR spectra would be useful.

23. In my opinion, it would be useful to add the frequency response of the EIKA, which dominates the bandwidth of the system, to the plots in the supporting materials showing the excitation profiles of the pump and detection pulses. This would make immediately clear to the reader where the pulses have been positioned within the bandwidth of the transmission chain.

24. Figures 3, 5, 7, S4, S5, S6: how was the excitation profile of the detection sequence calculated?

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