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Mike Bowman (Referee)

Referee comment on "The decay of the refocused Hahn echo in DEER experiments" by Thorsten Bahrenberg et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-10-RC1, 2021

The manuscript "The decay of the refocused Hahn echo in DEER experiments" is a significant advance toward understanding nuclear spin diffusion and its role in limiting most types of pulse EPR spectroscopic experiments. Nuclear spin diffusion and other mechanisms contributing to spectral diffusion and decay of signals limit sensitivity and the length of time for which a signal can be measured. Efforts to model it have been made since the 1960's, but required oversimplification of the model to such an extent that in many cases echo decay would be impossible or results were qualitative.

However, this paper uses computational power and modeling techniques that are now available to treat the spin system and spin-spin interactions without oversimplification and to construct realistic molecular models of the distribution of nuclei in the sample. The result is an impressive quantitative agreement with experimental measurements in three different systems relevant to many DEER experiments. This provides some insights and guidance on how to optimize samples and measurements. However the model applied here also has some relevance to other pulse EPR measurements such as: ESEEM, ENDOR (both Mims and Davies), and HYSCORE, to name a few. This paper has relevance and impact for other forms of pulse EPR.

The experimental part of the paper and the choice of samples are a good compromise between freedom from other sources of echo decay and relevance to typical DEER measurements. So results at the longest times and for the highest deuteration may be limited by appearance of instantaneous diffusion, local modes, molecular motion, and methyl group rotation. But within those boundaries, the calculations and experiments seem in good agreement.

Measurements were also made of a Gd-labelled protein. There are many grounds for criticizing the use of this particular sample. It certainly cannot be used to validate the modeling and calculations. However, it provides an important indication that the results, that are validated in better defined model systems, do have relevance to 'real' samples.

Although it is not really mentioned in the paper, one of the important aspects of the experimental measurements is that they are made at W-band. This almost completely supresses any ESEEM from protons and deuterons both because of its tiny amplitude at high magnetic field and because of the difficulty in exciting it with microwave pulses broader than the nuclear Zeeman period. Labs operating at lower microwave frequencies will be affected by ESEEM but the computations as described here also would include ESEEM. The point is that ESEEM becomes relevant at lower frequencies and may modify the results obtained here for W-band, but that point lies beyond the scope of this paper in
establishing the modeling and calculations.

However, the paper does not disclose some very important and relevant experimental details needed for readers to evaluate the experimental results. What are the approximate pulse widths and turning angles of the microwave pulses in the measurements? Does the strength of the perpendicular part of the microwave magnetic field vary across or along the samples? Were any checks made for instantaneous diffusion at the longest times? What was measured--peak point of echo, integral of echo, window between half height points of echo,...?

Although it is possible to find many things that could have been added to this paper, they do not seem to reach the importance of two major results: 1) a framework for quantitatively modeling the effect of nuclear spin diffusion on pulse EPR measurements; 2) confirming the importance of pairs and triples of nuclei in nuclear spin diffusion-driven electron spin echo decay.

I did find a couple of typos that need correcting: line 279 - "couplings IS neglected"; and line 351 - "socalled".

The chapter by Ian Brown should be supplemented by the chapter (W. B. Mims, in Electron Paramagnetic Resonance, ed. S. Geschwind, Plenum, New York, 1972, pp. 263-352.) and by the book on spin echoes by Salikhov, Semenov and Tsvetkov (or perhaps the chapter by Salikhov and Tsvetkov in Kevan and Schwartz, I think it covers nuclear spin diffusion).