Reviewer 1

Thank you for your positive evaluation and constructive comments. Below we copied our evaluation in black and we present our response is in red.

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.

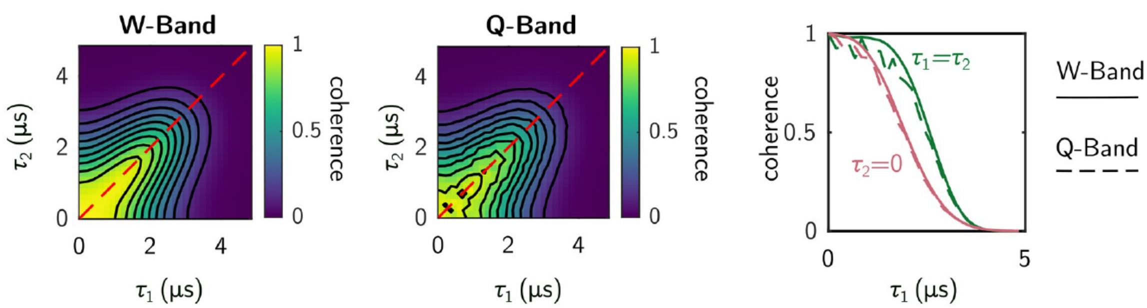
The experimental part of the paper and the choice of samples are a good compromise between freedom from other sources of echo decay and relevance to typical DEER measurements. So results at the longest times and for the highest deuteration may be limited by appearance of instantaneous diffusion, local 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.

We agree that the main purpose of the protein sample is to show that the results are relevant for biological 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 suppresses 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.

We agree with the reviewer that ESEEM is more significant at lower microwave frequencies. In our previous work (Canarie et al., J.Phys.Chem.Lett., 2019), we have already investigated the field dependence of the echo decay and have shown that the nuclear-spin-bath-driven decoherence is field independent, whereas the ESEEM modulation depth is field-dependent. The CCE simulations in the following figure illustrate this by way of a comparison between Q-band and W-band. The initial parts of the decay differ due to different ESEEM modulation depths, but the tails as well as the overall time scale of the decay remain unaffected. This will be added to the manuscript with a reference to the figure which will be as supplementary information as S2.



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?

Both the pulse widths and the turning angles are given in Table 1.

Does the strength of the perpendicular part of the microwave magnetic field vary across or along the samples?

Rabi nutation curves show several oscillation periods, from which we conclude that the B_1 inhomogeneity is small. We did not evaluate this quantitatively, as there is also an effect of the resonance offset. Also, in our simulations the observed decay time scale is independent of the pulse flip angles.

Were any checks made for instantaneous diffusion at the longest times?

The instantaneous-diffusion decay constant is 80 μs at 100 μM bulk concentration and a 25% flip probability. Its effect on the echo intensity for the time window where the echo is significant in our samples (5 – 10 μs) is therefore minor.

What was measured--peak point of echo, integral of echo, window between half height points of echo,...?

The echo was integrated over its full width at half maximum, as noted at the end of the "Spectroscopic measurements" paragraph in the Methods section.

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.

We agree with this assessment. These are the two major points in this work.

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

Thank you – fixed.

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).

Thank you for pointing them out, these are indeed important to refer to in the context of nuclear spin diffusion. We will add these references.