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Comment on mr-2021-13

Anonymous Referee #3

Referee comment on "Competing transfer pathways in direct and indirect dynamic nuclear polarization magic anglespinning nuclear magnetic resonance experiments on HIV-1 capsid assemblies: implications for sensitivity and resolution" by Ivan V. Sergeyev et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-13-RC3, 2021

The authors report highly intriguing DNP MAS NMR spectra of tubular assemblies made up by the HIV-1 capsid protein. They study the effects of biradical concentration and of different excitation schemes (CP, direct and SCREAM). In line with work by the group of Corzilius, different DNP polarization transfer pathways are made active, indirect, direct and SCREAM DNP, all relying on the cross-effect. For this study the authors use mainly Amupol in three different concentrations (4.3, 22.8, and 28.2 mM). Radical concentration was determined by EPR measurements using a bench top devise. On the whole, the study is very interesting and the following remarks are meant to be part of a final ironing process. Its particular value is in the display of spectra obtained with a small amount of radical (4.3 mM). The authors demonstrate nicely the effects of radical concentration on spectral quality and enhancement, as well as the discriminating effect of cross polarization versus direct polarization using one of the already classic DNP test systems. The paper is certainly worthwhile publishing, and I enjoy reading it; however, it can do with a lot of clarifications to improve readability.

- The authors claim that 13.8 watts of MW were applied at the sample. Where was that measured, and how was that measured? This should be included in the experimental. Was it measured in front of the sample or in the sample? What exactly means ‘at’ in this case?
- The spectra shown in Fig. 1 were all recorded with a recycle delay of 10 s. The authors should briefly state why they choose to do the experiment in this way and not using appropriately chosen multiples of T1 which is very different for the samples, and which would lead to optimal signal-to-noise-ratios for the fast relaxing ones. More importantly, they should discuss the implications of choosing the same delay for all samples appropriately. Enhancements are given in the top row of Fig. 1a, and I wonder whether there is a typo somewhere. Since so different radical concentrations are used I would expect different depolarization effects, by the way. One sentence discussing their possible contributions and in general the change with radical concentration would be good.

- The authors should also indicate the scaling factors between the displays of spectra in the top and bottom rows in Fig. 1. They cannot be the same, otherwise the statement that in all three cases E=76 is likely not correct.
- It should always clearly be stated which pulse sequences were used; names are not enough. Fig. 3 is good to have, but where is the SCREAM sequence, where are the CORD sequences (yes, they are easy), which sequence is used for which spectra in Fig. 1, 2 and 4, etc. For the outside reader it is very difficult to understand what TB, TB+ and TB- refers to, there is nothing like this in the pulse sequences of Fig. 3 and there is also no description in the experimental. Furthermore, the letters in the pulse sequences of Fig. 3 are far too small, especially those for the DANTE delay. It requires quite some magnification to see that it is Tr. Elderly persons who need to print the manuscript will not see anything.
- Fig. 4 reports CP-CORD spectra whereas this name does not appear in Fig. 3 nor in the experimental part of the paper. There is no reference where I would expect it. Again, its needs to be stated to which pulse sequence Fig. 4 correlates to, and what the numbers mean above the spectra. By the way, they are very misleading, they could be mistaken for the CORD mixing time by an unexperienced reader. Better write RD= and define somewhere in the paper or in the legend.
- It is very instructive to see spectra recorded with 4.3 mM Amupol only, it is an important point of the paper, but it is always tricky to compare contour plots. To me it is obvious that spectra are different yet comparing one or two selected cross sections would be probably wise.
- In the conclusion section it is said that 4.3 mM Amupol yielded the highest DNP signal enhancements. In stark contrast, Fig. 1 announces exactly the same enhancement for all three concentrations. Furthermore, the statement needs probably some seasoning, since there was no T1-optimized relaxation delay employed, and the S/N would be better with appropriate choices for the samples with higher Amupol concentrations. On the other hand, the better resolution in the spectra for the sample 4.3 mM Amupol does its job, too. Resolution is good, yes. To me it looks like similar T1 effects lead to the appearance of narrow signals in those direct polarization experiments recorded with longer relaxation delays. Maybe this should also be discussed appropriately.