

Magn. Reson. Discuss., referee comment RC2  
<https://doi.org/10.5194/mr-2021-24-RC2>, 2021  
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## **Comment on mr-2021-24**

Alex Breeze (Referee)

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Referee comment on "Using delayed decoupling to attenuate residual signals in editing filters" by Kenneth A. Marincin et al., Magn. Reson. Discuss.,  
<https://doi.org/10.5194/mr-2021-24-RC2>, 2021

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This work describes an interesting enhancement to the toolkit of methods available for conducting isotope-filtered NMR studies of complexes containing labelled and unlabelled components (e.g. protein-ligand complexes). These studies are always challenging, not least because of the conflicting demands of obtaining effective isotope filtration across the range of JCH coupling constant values within <sup>13</sup>C-labelled proteins (which can only be met by concatenating filter elements tuned to >1 JCH value), and at the same time of keeping relaxation losses to a minimum by reducing the overall pulse sequence length. This is especially important for preserving proton signals of the bound ligand in potentially large and slowly-tumbling complexes and, coupled with the need also for water-suppression elements (e.g. WATERGATE) when working in H<sub>2</sub>O, presents a severe design challenge for effective isotope-filtered experiments.

Overall, I would rate this manuscript as a well-presented and valuable contribution to the field, because it introduces the neat trick of exploiting delayed decoupling to assist filtration efficiency by dephasing the antiphase components arising from the X-coupled protons. The authors are keen to emphasise that delayed decoupling is best deployed as an additional element, supplementing the traditional doubly-tuned filter block with an extra filter tuned to enhance the coverage of JCH values (e.g. to more effectively encompass the aromatic range). The key here is that this extra filter can be incorporated in a sequence that is shorter than would be possible by simply adding a third half-filter element, because the dephasing delay can be time-shared with the delay incurred by starting decoupling after beginning acquisition of the FID.

RC1 has already pointed out the omission of two relevant references on delayed decoupling (even though the Roessler et al. paper uses delayed decoupling to achieve a different goal), so I will not repeat these recommendations. But I do have a few minor points that I think the authors could consider addressing:

- Regarding the model system (PCP1:pantatheinate covalent adduct) it would be useful if they could confirm whether the adduct interacts with the protein (i.e. tumbles at the macromolecular rate), or whether it is mobile relative to the protein (the latter might present an easier case for isotope-filtering because of the reduced relaxation penalty incurred by extra filter delays). Also, what is the size of the complex (about 20kDa?)
- Although the authors point out the value of being able to combine the WATERGATE suppression block with the third (delayed-decoupling) tuned filter element, they are perhaps missing a trick in that earlier schemes as reviewed and described in (Breeze, 2000) already featured incorporation of WATERGATE into the second filter element. This approach already shortens the scheme relative to one in which the WATERGATE block is sequentially positioned after the double-tuned filter, so in that sense the comparison they present with their 'reference experiment' should reflect this fact (granted, their new scheme still has an advantage in filtering efficiency by introducing the third element with complementary J tuning – but it will suffer a slight sensitivity loss compared with the experiment with doubly-tuned filter incorporating WATERGATE into the second element).
- 2 is not as clear as it might be. It's unclear (needs to be stated) (i) that these are simulations (ii) what the solid grey and dotted lines are, and what the value of tau is in every case (i.e. ratio to J)
- Near the end of p5, it would be helpful to use consistent nomenclature to describe sinc function convolution (they use Sa function).
- Minor points to do with sample preparation: (i) why so much (presumably unlabelled) EDTA in a filtered experiment? (ii) Use of TCEP not advisable in phosphate buffer.