

Magn. Reson. Discuss., referee comment RC2
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Comment on mr-2021-60

Anonymous Referee #2

Referee comment on "Analysis of conformational exchange processes using methyl-TROSY-based Hahn echo measurements of quadruple-quantum relaxation" by Christopher A. Waudby and John Christodoulou, Magn. Reson. Discuss.,
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This paper describes an important development of a novel Hahn echo experiment for methyl quadruple quantum coherences. The theory shows that the Hahn echo relaxation rate constant is very sensitive to exchange and breaks the problem of symmetry that prevents absolute sign determination of chemical shift differences in other Hahn echo experiments. Pulse sequence for the experiment is well-described and validated (and shows excellent sensitivity). The experimental work is very thorough and includes careful assessment of the contributions of chemical shift anisotropy to the field-dependent relaxation rates. Finally, the paper shows how joint analysis of the Hahn echo and CPMG measurements leads to more complete characterization of the exchange process. I expect this new experiment to be widely adopted in the set of powerful NMR experiments using the unique properties of methyl groups to characterize dynamics processes.

It may be that the authors are already at work on extensions to this work, but it seems feasible to modify the sequence for dispersion type measurements, using for example the heteronuclear double resonance relaxation approaches of Bodenhausen and coworkers, because dephasing of longitudinal $1H$ operators is not an issue (as in DQ/ZQ TROSY approaches).

Minor:

Should table 1 include contributions for relaxation from dipole interactions with remote deuterons? The dipole interaction is weaker, but in a deuterated background, there are many deuterons.

In the joint analysis (Fig. 6 for example), were individual CSA values used for each spin or

average values for residue type? Does it matter within the experimental uncertainties shown? Do other users need to remeasure the CSA's for each protein or are average values enough?

In Fig. 6C and 6D, should the x-axis be magnetic field-squared?

The authors have done an excellent job of referencing a now extensive literature on chemical exchange in biological molecules. A couple of other references might be of interest:

Multiple quantum relaxation outside the fast-exchange limit:

C. Wang and A. G. Palmer, Differential multiple quantum relaxation caused by chemical exchange outside the fast exchange limit, *J. Biomol. NMR* 24, 263-268 (2002)

Joint Hahn echo/CPMG analysis of ^{13}C relaxation:

N. E. O'Connell, et al., Partially folded equilibrium intermediate of the villin headpiece HP67 defined by ^{13}C relaxation dispersion, *J. Biomol. NMR* 45, 85-98 (2009).