

Magn. Reson. Discuss., author comment AC3  
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## Reply on RC2

Christopher A. Waudby and John Christodoulou

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Author 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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We are grateful to the reviewer for their careful reading of our manuscript and appreciate their positive and helpful feedback. We provide a point-by-point response to these points below, to be incorporated into a revised manuscript.

*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).*

Yes, this is an excellent idea, and we will be reporting on such applications in a forthcoming manuscript.

*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.*

The primary aim of calculating dipolar interactions with protons was to demonstrate the absence of intra-methyl relaxation pathways, but we agree that contributions from external deuterons will be an important contribution to the total relaxation rate. For completeness, as the reviewer suggests, we will include a calculation of these contributions in the revised manuscript.

*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?*

This is a great question. In our analyses we have used the individually measured values. However, it is informative to make an order-of-magnitude estimate of the contribution of CSA to the observed rates. Considering  $^{13}C$  spins alone, for 'typical' values  $\Delta\delta C = 1.5$  ppm,  $\sigma C = 30$  ppm,  $pB = 0.1$ ,  $k_{ex} = 5000$  s $^{-1}$ ,  $S2\tau_c = 50$  ns, the relative contribution of CSA vs Rex is ca. 10%. Variations in the CSA between methyls are of the order of 20%,

resulting in a net effect on the order of 2%, likely well within experimental uncertainties (although for faster exchange in larger molecules the CSA contribution may become more significant). This suggests that it will be sufficient for most users to use average values (and indeed the variation in CSA can be incorporated into calculated uncertainties), which will help to accelerate applications of this analysis. We're grateful to the reviewer for prompting this analysis, which we will incorporate into the revised manuscript.

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

No – given the additional information provided by the CPMG data, we have fitted the data using a more complete model of chemical exchange (Eq. 11) which interpolates between fast and slow exchange limits. We will add a note to the figure legend in the revised manuscript to clarify this point.

*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:*

- *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 <sup>13</sup>C relaxation:*

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

We thank the reviewer for highlighting these interesting papers – we'll be very pleased to include them in the revised manuscript.