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Magn. Reson. Discuss., referee comment RC1
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# Comment on mr-2021-19

Anonymous Referee #1

Referee comment on "Four-dimensional NOE-NOE spectroscopy of SARS-CoV-2 Main Protease to facilitate resonance assignment and structural analysis" by Angus J. Robertson et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-19-RC1, 2021

The very well written and well-illustrated manuscript by Robertson et al. from the Bax group entitled "Four-dimensional NOE-NOE spectroscopy of SARS-CoV-2 Main Protease to facilitateresonance assignment and structural analysis"describes two 4D [1H,1H]-NOESY NMR experiments and their application to a large protein system of 2×306 residues. The detailed precise description allows not only to reproduce the pulse sequences straight-forwardly, but also highlights the importance that are in the details such as the absence of phase distortion of the spectrum relevant for the application of NUS obtained by the introduction of two composite 1H 180° pulses, and the optimization of the phase cycle to 4 and the excitation of resonances only to the "amide side" of the water frequency to enable high resolution acquisition. Of high interest is the NOESY-NOESY-TROSY experiment reminiscent of the first 3D experiments (by the Kaptein group) because of its information content for assignment and distance restraint collection along with its inherent high signal to noise because it comprises a relaxation-based magnetisation transfer. This is well illustrated for the large system under study.

In short, the presented manuscript is recommended for publication. The only small suggestions perhaps are

(i) highlight the two composite pulses in the pulse sequence

(ii) indicate, that the 4D NOESY-NOESY-TROSY does not comprise NOEs from amide protons that are upfield of the water resonance.