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Comment on mr-2021-54

Ranajeet Ghose (Referee)

Referee comment on "Fluorine NMR study of proline-rich sequences using fluoroprolines" by Davy Sinnaeve et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-54-RC1, 2021

This is a very interesting paper that looks at the structural and dynamical aspects of the introduction of fluorine at the 4-position for a proline residue in a stereospecific fashion. Some of the results are quite unexpected and that suggests a level of caution that should be employed while introducing 4R- or 4S- fluoroprolines to quantitively probe protein interactions involving proline-rich segments. Conversely, conformational biases can be introduced to probe specific aspects of protein-protein interactions. Overall, the paper is well-written and well referenced; the analyses are robust and complete. This paper should be interest to the readership of Magnetic Resonance Discussions. I have a few minor suggestions and queries listed below:

- Table 1 lists the shifts for MpRS and MpSR peptides separately making comparison a bit cumbersome. I suggest that a two-column format that lists the corresponding shifts side by side be used.
- I think on line 148 the authors mean $^{3}J_{F\text{-}\delta2}$ that shows a 5 Hz difference from the free amino acid.
- For Table 3, by the anti-symmetric component of the shift tensor, I assume that the authors mean the rank-1 component. Best to clarify that since this is generally neglected in most relaxation analyses.
- Line 325 appears to have a typo – it should read “the higher affinity for MpSR relative to MpRS.”
- For the K_d calculations, while I agree that a combined analysis of fluorine and $^{1}H/^{15}N$ data is the most robust way to proceed, given that the affinity of the non-fluorinated peptide was determined using $^{1}H/^{15}N$ data only, it is worth also reporting just that analysis for the fluorinated peptides for completeness. If possible, I would also suggest a bulk measurement using ITC perhaps, given the somewhat strange behavior of the RS peptide. Though I admit that similar non-canonical binding models may complicate the ITC analysis.