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Reply on RC1

Davy Sinnaeve et al.

Author 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-AC1, 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:

We thank Ranajeet Ghose for his kind comments.

- 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.

We agree with the referee that a comparison of chemical shifts would be facilitated by a side-by-side presentation of Table 1, but our attempts to reformat the table systematically led to a loss in readability. Instead, we now provide a supplementary figure 2 that displays the comparison of proton and carbon chemical shifts at positions delta and alpha between the two peptides. These positions are relevant to assess possible changes in the structure and/or dynamics of the polyproline peptide.

- I think on line 148 the authors mean $^{3}J_{F\text{-}\delta2}$ that shows a 5 Hz difference from the free amino acid.

We thank the referee for pointing out this mistake that has been corrected.

- 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.

Yes, that is correct. The first rank component is here effectively included in the relaxation analysis.

- Line 325 appears to have a typo – it should read "the higher affinity for MpSR relative to MpRS."

This has been corrected.

- 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.

The Kd values measured from the sole $^{1}H/^{15}N$ data for the two fluorinated peptides as well as for the equivalent non-fluorinated peptide are now provided in supplementary Table 1.

While we agree with the referee that ITC data could provide interesting additional insights on the binding mechanism (by comparing relative enthalpic and entropic contributions), we rather restricted the scope of this manuscript to the information provided by an extensive analysis of the fluorine signal of fluoroprolines to show the potential of such analysis. We agree that further analysis remains to be conducted to reveal some aspects of the recognition of polyproline motifs by SH3 domains that have been overlooked until now.