The authors present a detailed NMR investigation of two SH3 class II binding peptides, containing each a (4R)- and (4S)-fluoroproline but at different location. They first present the NMR assignment of the peptides, and exploit a high resolution NOESY-HSQC spectrum to assign all proline resonances.

In this aspect, I am suprised why they only give a single value for the H_δ protons, knowing that the two H_δ's can be distinguished, and actually give infomation on the flexibility of the residues (see for example Ahuja et al. JMB 2016). I also wonder whether the larger dispersion of the Pro C_δ carbons in the MpRS peptide compared to that of the MpSR peptide (Figure 2) has a meaning ? When I compare the C_δ spread of the two prolines flanking the 4R-FP in the MpRS peptide (ΔC_δ(3-5) = -0.3ppm) with the same value in the MpSR peptide (ΔC_δ(7-9) = +0.27ppm), I again wonder whether the chemical shift contains structural information.

Not only the ring pucker but also the backbone conformation of the proline is influenced by the fluorine incorporation, with the (4S)-FP favoring the cis conformation. Here, I am somewhat confused. If the fluorine spectrum of the (4R)-FP4 in MpRS shows a major and minor peak in a 1 :3 ratio, what do they represent ? A major trans form and a minor cis form of this floroproline? But these should then also show up in the 1H-13C HSQC spectra, and the cis form should be characterized by a H_α-H_α cross peak ? And is the situation different for the (4R)-FP8 in the MpSR peptide ? What about the (4S)-FP in both peptides ? Are they in the cis conformation ? Elucidating these points seems important for the interested reader.

In order to characterize the movements of the FP rings, they turn to relaxation measurements. These are not easy to interpret, knowing the multiple dipolar terms and the important csa contribution. With the help of Spinach simulations, they obtain reasonable estimates for the different rates as a function of correlation time. The experimental data are then presented in a Table form, but I would suggest the authors indicate them by lines on the theoretical curves to allow easier interpretation by the reader. The heteronuclear NOE values indicate the surprising finding that the position in the peptide rather than being a (4R)- or (4S)-proline dictates the dynamic behaviour ? This is puzzling, and so is the large exchange contribution to the R2 rates. The delay between pulses is 400µs, so this implies movements on the millisecond time scale ? Finally, for the MpRS peptide, do both lines for the (4R)-FP have similar relaxation parameters ?

They finally look into the binding to the SH3 domain by a titration experiment, and measure both a protein 1H-15N spectrum and a direct 19F spectrum. Both peptides interact, but with a threefold different affinity and apparently different mode. The amplitude of the 19F CSPs in the MpSR peptide are different from those in the MpRS peptide, even for the residue in position 4 that should not interact (lines 114-115) ? I would have expected the red spectrum in Figure 6C to be identical to the ones of the free peptides (Figure 4), is there a referencing issue ? Finally, can the authors distinguish the major and minor 19F signals in the interacting spectra, or is line broadening too important ?

In conclusion, this is a thorough study of the influence of a fluorinated proline in a peptide motif, that should lay the basis for further use of this residue in advanced protein studies.

Minor remarks

Line 126 strong H_δ(i) to H_α(i-1) NOE

Line 549 Acknowledgments