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Comment on mr-2021-37

Anonymous Referee #2

Referee comment on "Exclusively heteronuclear NMR experiments for the investigation of intrinsically disordered proteins: focusing on proline residues" by Isabella C. Felli et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-37-RC2, 2021

In this work the authors present a simple to implement modification of the $^{13}C,^{15}N$ CON detection platform that is optimized to selectively record correlations from proline nitrogens with high resolution and short acquisition times. There is interest in this development because highly proline-enriched intrinsically disordered proteins (IDPs) remain challenging to study by NMR. Carbon direct-detect spectroscopy has arisen to meet this area of need and this work is a welcome step toward greater efficiency. Although this projet reports a minor step forward from the author's prior work (Murrali 2018 in the references) novel pulse programs are presented that were only conjectured in the discussion of the previous work and the contextualization of this method is more fully developed. Thus, this work is well aligned with the editorial scope of the journal and should be of use to the field. Minor suggestions for revision follow.

The authors and their past/present collaborators have always been careful to reserve the term "protonless" in reference to NMR experiments for those in which the coherence transfer pathway neither begins nor ends with the ^{1}H nucleus. Here, the authors present the strategy as being "protonless" and yet many of the pulse sequences utilize the proton-start strategy for higher sensitivity. Perhaps this is semantic, but a bit of clarification in the introduction may be helpful.

Table 1 is a very helpful guide for readers, but it did raise a question in my mind. Why are the carbon spectral widths set to 30 ppm for carbonyl (F2 in 2D and F3 in 3D)? This seems overly broad for carbonyl.

A critic might point out that these experiments are redundant for most systems; the regular CON already contains the proline resonances and coupling the CON-Pro to HSQC triple-resonance assignments offers completeness, but it probably could have been achieved with purely carbon-detected strategies anyway. However, this criticism misses the major point so carefully outlined by the authors in the discussion and conclusions: these experiments are excellent for exceptionally large or otherwise complex to deal with IDPs, filling a role analogous to methyl selective labeling and other strategies used for

large folded proteins. My question and comment therefore come down to the text beginning on line 236, which states "the initial count of cross-peaks in this spectrum reveals 42 out of the 45 expected correlations, highlighting the potential of this experimental stragegy for the investigation of IDRs/IDPs of increasing complexity" and the associated spectrum in Figure 4B. How much better is the resolution in the 2D CON^{Pro} compared to what would be achieved in the traditional 2D CON? How many peaks are resolvable with the new technology that are overlapped or ambiguous with the original CON? If it wouldn't be too distracting it seems that an overlay or some other visualization of the improvement on offer for a complex IDP like CBP-ID4 may be helpful for readers.

This is very minor, but on line 329 it seems "motives" should be replaced with "motifs".