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Referee comment (Frueh)

Dominique Frueh (Referee)

Referee comment on "Rapid measurement of heteronuclear transverse relaxation rates using non-uniformly sampled $R_{1\rho}$ accordion experiments" by Sven Wernersson et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-46-RC1, 2021

Hello Sven, Mikael et al.

Here is my review. I hope all is clear and happy to resolve ambiguities if any. Note that I won't have access to the site until June 1st though.

Best,

Dominique Frueh

General comments and significance:

This is an excellent work, in which the authors demonstrate successful measurement and analysis of transverse relaxation rates collected through non-uniformly sampled accordion methods. This manuscript is a significant contribution as the new method can dramatically reduce acquisition times and hence provides access to studies that would otherwise be

prohibited by experimental limitations, e.g low sample concentrations. The author's analysis convinces that the precision and accuracy of the rates obtained through NUS-accordion and the analytical methods employed (relying on DSURE) are comparable to those of rates obtained through conventional methods when sampling factors of 50% or above are used.

The manuscript is very well written, suitable for Magnetic Resonance, and I only have very minor cosmetic or pedagogical recommendations, with a few points to discuss.

In short, I am looking very forward to trying those experiments.

specific comments and detailed critique:

You performed a really exhaustive comparison and the introduction, in my opinion, does not reflect the extent of the work presented, which results in a little confusion when reading the experimental method where one is left to wonder why so many different types of experiment were collected. The introduction could specify that the rates obtained through NUS-accordion-R1rho are compared against uniform acquisition of the same experiments as well as conventional non-accordion experiments for both R1rho, using two implementations, and CPMG. Also, hint at why CT-CPMG were collected.

Speaking of CT-CPMG, why describe the experiment and make use of it if you are not showing the data? There is no need for a full RD analysis here; showing the profile of L219 would be enough. It seems to me that 'data not shown' goes against the philosophy of Mag Res.

One challenge when presenting such work is that when providing a rigorous demonstration of new improvements, users may be deterred from trying an experiment or protocol that is in fact more simple than it looks. Currently, because NUS was sampled from a full set for comparison, it may appear that the full set is needed to generate the optimized schedule. This would be an obstacle to popularize the method as a user would certainly not want to collect a full set before using NUS. As you briefly mentioned in a previous publication (JCP 2019), it is possible to do an optimization using a regular HSQC and an estimate of relaxation rates. Please include a few sentences to describe this alternative in the method section and briefly mention how it works whilst mentioning the names of the scripts you provide and what variables to use in these scripts to perform the task (see the note about data availability).

I was a little surprised to see larger error bars in rates obtained through F-ref when compared to F-Reverse as the signal-to-noise in the ref experiment should be higher.

Was there a reason to use 132 complex points for the accordion-adiabatic experiment but 128 points for all others? It may be wise to remind the reader of this distinction when appropriate.

Figure 4(b,e) are currently not informative as the impact of the offset is masked by that of the number of signals analyzed. Would it be possible to only select a subset of slices with the same number of residues involved? Or maybe re-color the points according to the number of residues co-analyzed? Depending on the outcome, you may move the current (b,e) to the appendix.

Reproducibility and data analysis:

In the method section, sample preparation. Please describe the final protein concentration and NMR buffer and comment on sample stability. Was the same sample used for all measurements? How was the integrity of the sample monitored for all data that are compared (e.g. comparing Watergates or HN traces of HSQC)?

Please describe how the errors on the rates were estimated for all classes of experiments (Monte Carlo? If so, how many repeats?)

Accessibility:

Magnetic Resonance requires deposition of pulse sequences, experimental parameters and codes for analysis in repositories that generate a DOI. We ended up using Zenodo because others used it before us, although we did not find any recommendation as to what site to use.