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reply to RC2

Bei Liu et al.

Author comment on "Rapid assessment of Watson–Crick to Hoogsteen exchange in unlabeled DNA duplexes using high-power SELOPE imino ^1H CEST" by Bei Liu et al., Magn. Reson. Discuss., <https://doi.org/10.5194/mr-2021-58-AC5>, 2021

The article entitled « Rapid measurement of Watson-Crick to Hoogsteen exchange in unlabeled DNA duplexes using high-power SELOPE amino ^1H CEST » submitted by Liu et al. is an important contribution to the study of Watson-Crick - Hoogsteen exchange occurring in DNA duplexes. The main achievement of the study is the application of a recently found new pulse sequence SELOPE (Schlagniweit et al., 2018) by the group of K. Petzold to DNA duplexes and WC-HG equilibrium. The work is also of a methodological nature with a systematic study of the possible artifacts related to the ^1H - ^1H cross-correlation in the study of systems in equilibrium exchange. There is really a huge wealth of data that are very convincing and that support the main message of the work that is the interest of the application of SELOPE sequence to unlabelled DNA duplexes permitting to obtain large quantity of data about HG-WC equilibrium at a lower cost and with improved efficiency, additionally the method permits to characterize HG-WC equilibrium with a lower HG population and faster kinetics that it was possible using the previously used R1p Relaxation dispersion $^{13}\text{C}/^{15}\text{N}$ methodology. The method, because it permit to obtain rather easily a lot of data on many DNA base pairs, is well adapted to the study of WC-HG equilibria whose the dependence from sequence is quite complex.

We have just some comments about specific points

Legend of Figure 1 the delay d_1 is not explicitly defined

We thank the reviewer for this suggestion and have specified the d_1 in the figure legend

1b: " $\tau = \frac{1}{2} d_1 = 0.7 \text{ s}$ "

To be clearer , we suggest to make mention earlier in the text that the minor shoulder stated in l 297-298 is indicated with the black line ES in the figure 3B

We have modified the text on page 20 to incorporate this good suggestion:

"On the other hand, a minor shoulder was observed in the ^1H CEST profile of T5-H3 (Fig. 3b, the $\Delta\omega$ is highlighted by dashed red lines in the profile and labeled "ES")."

There is no comments on the large variations observed in r_{χ^2} in figure 4 (and also Fig S2, S3) by example why so large variations between U9H3 (498-476) and U5H3 (7.2-6.9) with or without exchange while the experimental data shown with the fit appear similar, additionally for proton T9-H3 or G10H1 while a very significant reduction in reduced r_{χ^2} is

observed when considering or not the existence of WC-HG exchange, justifying clearly the existence of exchange processes for these protons, the reduced $r\chi^2$ for the correct model (with exchange) remain rather elevated considering what is expected generally (Rangadurai et al. Prog. In Nuclear Magn .Res., 2019). If this results from a peculiar definition of the reduced chideux, the error bars or any other reason is not clear and needs some explanations.

We thank the reviewer for pointing this out. The different values for different ^1H CEST profiles is most likely due to differences in the quality of the NMR data and differences in uncertainty as well as poor estimation of the real experimental uncertainty. Large variations in were also observed for $^{13}\text{C}/^{15}\text{N}$ CEST profiles reported previously (Shi et al., 2019; Liu et al., 2020; Zhao et al., 2014). In general, the values for $R_{1\rho}$ profiles (estimated using Monte Carlo simulations) (Rangadurai et al. Prog. In Nuclear Magn .Res., 2019) are smaller than those of CEST likely due to better estimation of the experimental uncertainties. In the case for U9-H3 and U5-H3 ^1H CEST profiles, the errors for U9-H3 are 0.00239 and 0.00045 for spin lock power 250 Hz and 500 Hz respectively, while these two errors for U5-H3 are 0.00240 and 0.00845 respectively. The ~ 18 -fold differences in the error of 500 Hz data likely contributes to the large difference in . We have added a statement in the method section on page 49 to explain the variations in

“Note that the variations in values for different ^1H CEST profiles in Fig. 4 and Fig. S2-4 are most likely due to differences in the quality of the NMR data, variations of errors and poor estimation of the real experimental uncertainty.”

Lines 374 not clear -6ppm is repeated

We thank the reviewer for pointing this out. We meant the range of offset is from -6 ppm to 6ppm. To avoid confusion, we have modified the text on page 27:

“we restricted the offset to -6 ppm to 6 ppm when analyzing and fitting the ^1H CEST profiles.”