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Comment on mr-2021-58

Anonymous Referee #1

Referee comment on "Rapid assessment of Watson–Crick to Hoogsteen exchange in unlabeled DNA duplexes using high-power SELOPE imino ^{1}H CEST" by Bei Liu et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-58-RC1, 2021

In the manuscript by Liu et al, the authors evaluate if imino ^{1}H experiments CEST can be carried out on unlabeled nucleic acid sample to detect conformational exchange. Although ^{13}C and ^{15}N relaxation dispersion experiments are routinely used to study conformational exchange process like Watson-Crick to Hoogsteen basepair transitions in nucleic acids they require expensive samples enriched in ^{15}N and/or ^{13}C that are also very laborious to prepare and measurements are often restricted to a few judiciously chosen constructs. Hence to study exchange in a large number of sequences to identify sequence dependent conformational dynamics it will be useful to have an NMR experiment that can be used to study exchange in unlabeled sample. The authors show that despite the presence of 1H-1H NOE effects (relatively) 'artifact free' CEST profiles can be obtained by using selective imino excitation and short CEST delays (< 100ms) in unlabeled nucleic acid samples that are significantly cheaper and easier to produce opening up the possibility of studying conformational exchange in several DNA and RNA sequences. I have only a few minor comments.

- Here conclusions regarding the Watson-Crick to Hoogsteen basepair transition are being drawn based on a single imino Δω value. Is it safe to do this, as breaking of the hydrogen bond will result in Δω value of ~-1.5 ppm similar to the Δω values being observed here. There should a discussion on how robust this conclusion is and if other measurements like pH dependence of the population etc are required to confirm this.
- Figure S9: Please specify what is being plotted on the Y axis.
- Figure S5: Increase the range of Δω values for T9-H3.
- In materials and methods please specify the number of scans and the d1 used to record the 1H CEST data.
- In figure 1b, it might be safe to destroy all the magnetization after the acquisition, to avoid any accidental offset dependence of the starting 1H magnetization.
- In the legend to figure 1b specify the 1H carrier is position at various points in the experiment.
- In the legend to figure 1b specify the range (in ppm) that is being excited by the Eburp pulse.

- Line 346: "However, since no NOE dips were observable for non-imino protons within 2.8 Å (Fig. 3a), a sizeable cross-relaxation contribution from neighboring imino protons is unlikely considering they are separated by a longer internuclear distance of ~3.7- 3.9 Å (Fig. 3a)" This is a bit confusing: In figure 3b, there are NOE contributions in the 0.1s CEST profiles of G2-H1 due to A3-H2 (3.9 Å) when the selective pulse is turned off. This suggests that NOE effects due to T22-H3 (3.9 Å) will be there in 0.1s CEST profile with selective excitation so long as all the iminos are excited by the Eburp pulse. Artefacts might have been reduced because the T22 imino proton exchanges with water or because the artefacts are very close to the G2-H1 dip. However one may get around the problem by exciting just the Guanine nucleotides with the Eburp or by exciting just G2-H1 and not T22-H3 with the Eburp.
- While it is clear that selective imino excitation coupled with short exchange delays (<0.1s) results in imino 1H CEST profiles that are largely free of NOE induced artefacts due to non imino protons, they can still contain artefacts due to imino protons. Hence the authors should include a few guidelines on safely interpreting the 1H CEST data. When can we get Δω values, when can we get exchange parameters etc? When do we have to discard the CEST profiles entirely? While the manuscript contains the guidelines in various places summarizing them in a single paragraph will be useful.