

Magn. Reson. Discuss., author comment AC2
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Reply on RC2

Ulric B. le Paige et al.

Author comment on "Characterization of nucleosome sediments for protein interaction studies by solid-state NMR spectroscopy" by Ulric B. le Paige et al., Magn. Reson. Discuss., <https://doi.org/10.5194/mr-2021-21-AC2>, 2021

Dear prof. Schanda,

Many thanks for your insightful comments.

Thanks for pointing out the presence of some free bulk water which we had not addressed. There is indeed a minor component of free bulk water that likely evaporates over time (we did not use a rubber spacer and did not glue the caps to be able to retrieve the sample). Despite this water loss, the high quality of the spectra over time indicate that the nucleosome remains well-folded and hydrated, even if some hydration water is also lost (as we mention in the manuscript).

This indeed also implies that the higher centrifugal forces during MAS increase the packing densities beyond the values in Table 1. It is hard to say how much, especially since the sediment seems already close to the limiting concentration. Given the SAXS profiles of the retrieved samples there is no indication that MAS induces a crystal-like packing that would presumably give much stronger degree of order. We also realized that we made mistake in the calculation the nucleosome concentration in crystals. This should be ~ 3.2 mM, based on the unit cells dimensions and Matthew coefficient. The packing coefficient is the fraction of total volume occupied by the nucleosome, taken as a solid, completely filled particle. Thus, approximating the shape of nucleosome to a disk, 100% packing would equate to 4.2 mM concentration while the densest packing achievable would be 90% or 3.8 mM. Nucleosome crystals thus have a packing coefficient of $\sim 76\%$, substantially higher than the sediment. Because water can also be in void volumes and the enclosing surface envelope, the solvent content can still be 54%.

We will adapt the text accordingly to make these points clearer and also adapt the text to your other comments. The increased peak intensity in Fig 4e is likely due to the higher salt level in the sample, see Figure 4f.

On behalf of the co-authors,

Hugo