

Magn. Reson. Discuss., referee comment RC2  
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## **Comment on mr-2021-21**

Paul Schanda (Referee)

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Referee 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-RC2>, 2021

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This is a very interesting article that examines the properties of sedimented samples of nucleosome particles, obtained by ultracentrifugation into ssNMR rotors. The properties of the sample are evaluated by ssNMR and SAXS, and in addition to nucleosome samples, also a complex with a weakly binding protein (PHD2) is studied.

While many groups have been using sedimentation, often without reporting the details of the sample, this study reports their properties, and this will certainly be useful for others that use similar procedures.

The study is rigorously done and I recommend publishing it, after considering the following points.

Page 5, line 138: "weighting factor of the  $^{15}\text{N}$  chemical shift differences (in ppm) of 6.51". Actually, the weighting factor is  $1/6.51=0.154$ . This is in the range of commonly used value, reviewed here: Williamson, M.P. (2013). Using chemical shift perturbation to characterise ligand binding. *Prog. Nucl. Magn. Reson. Spectrosc.* 73, 1–16.

The methods indicate that the rotors were closed, but no rubber spacer was used, nor was the cap glued into our rotor. In our hands, the samples can lose water, often excessively, without rubber spacer or gluing. This observation has also been reported in one case: Asami, S., Szekely, K., Schanda, P., Meier, B.H., and Reif, B. (2012). Optimal degree of protonation for  $^1\text{H}$  detection of aliphatic sites in randomly deuterated proteins as a function of the MAS frequency. *J. Biomol. NMR* 54, 155–168; see Figure 11.

The spectra of the  $^1\text{H}$  water signal change in shape (Fig 2a); there are clearly several components in the first measurement, which disappear after some time. This could be related to loss of bulk water, as described here: Böckmann, A., Gardiennet, C., Verel, R., Hunkeler, A., Loquet, A., Pintacuda, G., Emsley, L., Meier, B.H., and Lesage, A. (2009). Characterization of different water pools in solid-state NMR protein samples. *J. Biomol. NMR* 45, 319–327.

My interpretation is that:

- There is bulk water, probably in the center of the rotor, in the beginning – this actually means that the calculated packing density is underestimated, see below.
- The bulk water is lost
- The hydration water is largely (but not totally) retained.

Can the authors comment on this? Have the caps been glued? Probably not, which may explain the losses. For other samples, such water loss can have dramatic consequences on the sample, as we reported for the case of SH3 crystals (reference above, Asami et al).

I am not convinced that the following statement (page 9) is totally true: “Taken together these data demonstrate the nucleosomes in the sediment remain well-folded and hydrated through the measurements without evidence for direct nucleosome-nucleosome contacts” The spectra of day 1 and day 34 are clearly different, and a change in hydration is likely.

On page 7, the authors state that the packing density (61%) is lower than in crystals.

They state that in crystals the "packing coefficients of ~67% and a solvent content of ~54% ". I did not find out what is meant by "packing coefficient". I assumed that it is the volume occupied by the protein relative to the total volume. But if it was so, then the solvent content should be the complement to the packing coefficient, i.e. 33% in this case. Could the authors clarify this?

As eluded to above, it is likely that the packing density in the rotor during MAS is higher than after ultracentrifugation into the rotor. In typical samples, at least crystalline ones, we always find that bulk water accumulates in the center of the rotor. The water 1H peak in the beginning of the measurement shows an additional component (on the right), which most likely is bulk water, located in the center of the rotor. Thus, I believe that the actual packing density of the species that is detected by NMR is higher than the authors' estimate.

Figure 4e: it is surprising that some peaks (res. 20-25) have much higher intensity in the complex (INEPT based). This suggests that these residues become more mobile. Can the authors comment on this?