
le Paige and co-authors presented a thorough discussion about packing nucleosome samples by sedimentation for ssNMR studies. It revealed that the nucleosomes in the ssNMR rotor are highly compacted, well hydrated and stable, meanwhile have sufficient disorder of nucleosome arrangement to allow proteins interacting with nucleosomes. This method allows studying the interactions between nucleosomes and binding factors, as demonstrated by the ssNMR study of a co-sedimented PHD2-nucleosome complex. The contribution is very helpful to practitioners interested in studying nucleosome interactions with NMR.

There are several minor comments.

- The authors added 2 mM Mg2+ in the nucleosome solutions prior to sedimentation. What is the reason to choose 2 mM Mg2+? Does it affect the sedimentation efficiency, final concentration or ssNMR data quality if adding less or adding more Mg2+? See also below on the effect of Mg2+ concentration on order as observed in SAXS X-ray diffraction spectra.
- When assessing the final concentration of nucleosomes packed in the ssNMR rotor, have the authors also tried quantifying this by other methods, such as quantifying the 13C or 1H signals using an external standard? It's not uncommon that considerable sample loss can happen during packing, clearing top for caps, closing caps etc, which would lead to overestimating the material quantity in the rotor by checking the supernatant.
- In the sedimented PHD2-nucleosome complex sample, 20:1 ratio was used. Does the free PHD2 remain in the supernatant or the sediment? If it is mostly sedimented, is it possible to prevent this by optimizing sedimentation time, or buffer or other conditions? It will be useful to include such information in the manuscript.
- In the introduction, line 60 "mainly via interactions mediated by the histone tails" is simplified, they interact by stacking in columns at various degree of order and the stacking for a condensed system is governed by not only close packing considerations,
but the contacts (stacking) between the histone octamer core surfaces are mediated in addition to tails, by charge-charge interactions by charged aa on the two surfaces as well as presence of divalent or higher charge salt counterions that screen electrostatic repulsion.

- In many places, the space between a value and the unit is missing, for example, Line106 - 10 mM; Line156, 10 bp; Line 148, 15 s; Line 117, 298 K; and many others.
- Line 76, “contacts” -> “contact”
- Line 120, ”monitored” -> "were monitored"
- How were the error bars in Figure 4 derived?

- Regarding SAXS data. "The sediments are devoid of pronounced long-range ordering of nucleosomes". Here they use 2 mM Mg2+. As the authors note later on, the long range order depends highly on the Mg2+ concentration used when precipitating the NCPs. As shown by Berezhnoy et al, the most pronounced order was achieved at 20 mM Mg2+, while there is no long range order at 2 mM.
- Line 265: “the sediments seem to primarily consist of heterogeneously packed nucleosomes with mean inter-particle distance of 78 nm.” Should not this read “7-8 nm”? Line 259 reads: “pronounced peak at q* ~0.08, corresponding to a characteristic distance of ~7-8 nm”.
- Line 348: “we estimate that the length scale of the regular structure in the sediment is ~1520 nm, corresponding to stacks of two to three nucleosomes, without a significant preference in relative orientation between the stacks”. How did the authors arrive at this conclusion? Is it based on FWH in SAXS spectra, if so details should be given? The peaks seem too broad and ill-defined for such an analysis.
- It would be good if the authors try to adhere to the recent guidelines for the description of SAXS results described in Acta Crystallogr. D Struct. Biol., 73(Pt 9), 710 (2017).
- Figure 3c (167 bp nucleosome SAXS solution data) lacks indication of the nucleosome concentration and details of calculation of the form factor profile (nucleosome structure used for calculation, program applied).
- For the condensed (precipitated) samples of the 167bp nucleosome, the authors obtained SAXS profiles that are different from data reported for condensed NCPs (nucleosome without linker DNA) and also from the recently published SAXS results for the 177bp nucleosome ( Soft Matter, 14, 9096 (2018); Sci. Rep., 11, 380 (2021)). It would be good to make some discussion and comparison with the indicated data. Is the difference due to the low Mg2+ concentration in the samples?