

Comment on mr-2022-3

Henry W. Orton et al.

Author comment on "Localising nuclear spins by pseudocontact shifts from a single tagging site" by Henry W. Orton et al., Magn. Reson. Discuss.,
<https://doi.org/10.5194/mr-2022-3-AC2>, 2022

Daniel Häussinger commented:

1) "The results indicate that the number of tags is much more important than the number of tagging sites. ... I don't see that the claim of the abstract is supported by the data."

Response: Looking at the RMSD volume associated with the 95th percentile in Figure 2B, it is quite generally smaller for $n+1$ tags (regardless of the number of tagging sites!) than n tagging sites.

2) "The distribution of the magnitude observed PCSs for a given tag / protein conjugate is not a Gauss distribution, but rather characterized by a few large, some intermediate and many small experimental PCSs. I, therefore, wonder whether the RMSD is really the most suitable criterion for the assessment, or if the normalised q-factor would be more suitable."

Response: If a nuclear spin is located near the PCS isosurface, where the PCS is zero (i.e. the sign of the PCS changes), the conventional Q-factor calculation would involve division by a small number although this isosurface defines the location of the spin with particularly high precision. By calculating the RMSD in ppm, it can be compared directly with the uncertainty of PCS measurements (which also is in ppm).

3) "In figure 6 it seems, as if the ubiquitin structure is for all panels A-D depicted in an identical orientation. If this is the case, it should be mentioned in the caption – if not – then figure 6 should be modified accordingly."

Response: All panels of Figure 6 indeed show the protein in the same orientation.

4) "It is not entirely clear to me, if experimental noise on PCS has been considered in the simulation. It seems likely that noise would affect the one site / multiple tags scenario differently as the multiple sites / one tag, because the S/N ratio of PCS decreases with

increasing SoI-metal distance and the coverage by several sites may compensate this to a certain extent.”

Response: The simulations are based on random tensor orientations. As tensors were not fitted using PCSs, the tensor orientations did not vary in response to PCS uncertainties. Furthermore, the simulations were for tensors that all have their origin at the same distance from the site of interest. If the tensor origins are at different distances from the site of interest (which is much more likely in the case of multiple than single tagging sites), smaller PCSs from more remote tensors will indeed be associated with larger relative errors. Importantly, however, the absolute uncertainty remains unchanged as far as it results from measuring the PCS as the difference between two chemical shifts. This uncertainty is well captured by the PCS RMSD (in ppm) we used to define the boundary of the localisation space.