

The manuscript by H. Orton et al. describes the localisation of nuclear spins in a “site of interest”, (SoI) by pseudocontact shift NMR. Specifically, the question is addressed, whether several tagging sites with one tag or one tagging site using several structurally different tags yield more precise results. A Monte Carlo based simulation as well as a case study on Ubiquitin are provided. Surprisingly, both methods deliver comparable quality of localisation, which is a very important finding, as it is considerably easier and quicker to perform experimentally the latter approach, provided sufficient tags are at hand.

This contribution is highly original and scientifically sound, the documentation of the results in the SI and in the zenodo repository is exemplary. Literature coverage is complete and in most cases the hypotheses are backed by the data. I recommend to accept this excellent and relevant contribution after minor revisions of the following points.

1) The phrasing of the abstract: “The results indicate that the number of tags is much more important than the number of tagging sites.” is evidently contrasting the wording in the conclusion: “In the case of a single selected site of interest, such as the conformation of a loop region, the binding site of a ligand or a protein–protein interface, our results indicate that PCSs generated with a single tagging site and four different tags most likely yield structural information that is nearly as good as that obtained with a single tag deployed at four different sites.” I don’t see that the claim of the abstract is supported by the data, whereas I completely agree with the statement in the conclusion.

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.

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.

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.