

## Comment on mr-2022-3

Anonymous Referee #1

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Referee 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-RC1>, 2022

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The main result of this interesting paper on the use of pseudocontact shift data for characterizing protein structures is that multiple different paramagnetic tags attached to one site have similar information content as the same number of tags attached at different sites. This is of high practical relevance, as it simplifies experimental procedures and reduces the chance of undesired effects by paramagnetic tag introduction. Additional aspects, in particular regarding tag mobility, are treated, in general terms, in the informative Discussion. Tag flexibility remains a principal limiting factor.

The approach is both theoretical, by sampling generated tag orientations, and experimental, by measuring PCS values for four different tags attached at one site of ubiquitin. Both approaches have been well designed, executed, and described in the paper.

I propose publication after minor revision.

Minor points:

1. The statement in the Abstract that PCSs enable highly accurate structure determinations is somewhat overoptimistic. It is difficult to determine protein structures from PCS alone, in particular if the paramagnetic centers are in flexible tags.
2. Mathematical notion is somewhat non-standard. There is no summation index in Eq. 2.
3. The (finite) volume element in Eq. 3 is called  $dV$ , which is normally the symbol for an infinitesimal volume element. It would be better to call it  $\Delta V$  to make it clear that it is of finite size.
4. Using the gradient symbol for the scalar quantities defined in Eqs. 6 and 7 is an unusual notation.
5. It might be useful to point out that the term that is summed in Eq. 6 corresponds to  $|\cos(\tau_{ij})|$ , where  $\tau_{ij}$  is the angle between the vectors  $\mathbf{v}_i$  and  $\mathbf{v}_j$ . The quantity on the left hand side should carry an index  $j$ . Since the sum is over all  $i$  different from  $j$ , the normalization factor should be  $n - 1$  instead of  $n$ .

6. The same applies to Eq. 7, where the term that is summed corresponds to  $|\sin(\tau_{ij})| |v_i| |v_j|$ . In general, I find the localization space volume of Eq. 3 to be the quantity with the most straightforward interpretation.
7. In the caption of Fig. 2, it would be informative to report the  $\Delta V$  value that corresponds to the given parameters.