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Comment on mr-2021-63

Marcellus Ubbink (Referee)

Referee comment on "Localising individual atoms of tryptophan side chains in the metallo-β-lactamase IMP-1 by pseudocontact shifts from paramagnetic lanthanoid tags at multiple sites" by Henry W. Orton et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-63-RC1, 2021

This manuscript describes how pseudocontact shifts generated with a lanthanoid tag attached to three different positions on a protein can be used to determine the position of a Trp indole ring in a protein. The position of the indole of Trp 28 in the metallo-beta-lactamase IMP-1 is a matter of debate because crystal structures give different conformations. The conformation was determined in the resting state and with an inhibitor bound and turned out to be very similar. The experimental work appears sounds and the analysis is supported by the data. The authors also discuss problems encountered, which is very useful. The results are highly relevant for a better understanding of this enzyme, so the work should be published.

Some points need addressing.

(1) The triangulation approach of using the intersections of three PCS isotherms has been reported before in other papers (e.g. J Biomol NMR 71, 27, 2018), so it is not clear why the current approach is not compared with published methods.

(2) In the discussion (l. 479 – 486), the point of not using different metals in the same tag but multiple orthogonal sites has been made by other studies, so references are required there.

(3) In line 246 it is mentioned that double peaks are observed for the Trp NHe groups. That could mean that the indoles are in different conformations in slow exchange. It is not discussed whether these could be the other conformations observed in the crystal structures. Could the PCS analysis be done for these minor peaks to exclude that possibility? In that case this work only yields the position of the major form, not the only form.

(4) The mass spectrometry shown in Fig. S2 and the yields mentioned give rise to questions. In line 204 the efficiency of 90% is mentioned. However, using the information in the caption of Fig. S2, a different result is suggested: The masses in the figure are about 9 Da lower than expected for 100% labelling (given the mentioned masses), which 25% of the expected 36 Da extra (in the caption it says +6 Da/Trp), so labelling efficiency would be 75%. However, after converting the indole to tryptophan, one deuteron is removed, so the expected mass increase is 4XD + 1 13C = 5 Da per Trp, not 6. That would result in a labelling of 90%, agreeing with the main text, but suggesting that the masses mentioned in the caption are too high. Please check.

(5) In section 2.2, mention the protein concentration(s) used for the NMR samples and indicate the tube type (3 mm, 5 mm, Shigemi), to know how much sample was used. Also mention the protein concentration in the captions of the NMR spectra figures in the supplementary material.

(6) l. 213, how were the assignments for the diamagnetic protein obtained? If from previous reports, give the reference and the BMRB entry.

(7) Some supplementary figures have the wrong numbers in the text:

line 165, Fig S1 > S8;

line 170, Fig. S2 > S9;

line 212, Fig. S2-S5, S3-S6 (?)

Tables S1 – S6 are not mentioned in the text.