

## Comment on mr-2022-22

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Referee comment on "Facilitating the structural characterisation of non-canonical amino acids in biomolecular NMR" by Sarah Kuschert et al., Magn. Reson. Discuss., <https://doi.org/10.5194/mr-2022-22-RC3>, 2023

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The manuscript of Sarah Kuschert et al. describes an extension of a program (ATB) that facilitates the modelling of non-canonical amino-acids using commonly used structure determination programs such as CYANA or CNS. This contribution is particularly useful for the community since the use of modified peptides in pharmacopeia receives a growing interest. The manuscript presents applications on various compounds including stapled peptides which is of particular interest. One important aspect of this work is the description of an automated atom naming procedure that complies with the IUPAC standards. Such tool represents an important and valuable technical contribution for researchers working in structural biology. However, in its present form, the manuscript focus mainly on applications with CYANA program while the authors state (and I tend to believe it) that the approach is general and applicable to CNS or XPLOR-NIH. The manuscript could be greatly improved if the authors provide some examples of this eucumenism with structure calculations performed by CNS or XPLOR-NIH. Beside, I have several specific points that should be addressed to improve the overall clarity:

- Figure 2 shows the template for the description of ncAAs. What happens if the nitrogen atom of the peptide bond is not bound to an hydrogen but to a carbon such as in methylated AA or di-amino butyric acid found in some bacterial siderophores or a modified proline ?

- The statement in the first result paragraph is rather odd:  
" In general the recalculated structures are very similar to those previously calculated ...."  
I disagree with the statement that it is beyond the scope of the work to compare in detail the results of both procedure. The demonstration that the automated approach delivers the same results as the manual one should be provided in a quantitative way and the origin of possible "subtle" differences should be carefully analysed and addressed. The results of structure calculations should comply with accepted standards showing the tables with structural statistics.

- As already mentioned, comparative structural calculations should be provided also for CNS or XPLOR-NIH. It would be very helpful to have the example of a topology entry for a modified amino-acid in one of the routinely used force field of CNS.

- In section 3.3, the authors present a practical application on a stapled peptide. Details that are provided should be displaced in the method section rather. As for other examples, a table recapitulating structural statistics should be provided. It would also be interesting to detail how the cis-trans isomery of the double bond is defined from the input structure.

- It would be very interesting to provide an example where fluorinated amino-acids are incorporated in a peptide or a protein.