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Opponent Review of Doctoral Thesis

The doctoral thesis of Shivam Shukla "*Structural studies of metal-dependent hydrolases: Histone Deacetylase 6 and Glutamate Carboxypeptidase II*" describes structural biology approaches to understand the two above-mentioned metalloenzymes. Both enzymes belong to hot topics in the field and their investigation has the potential to significantly improve the overall quality of life.

The thesis consists of twenty pages of Introduction, ten pages of Materials and Methods, about twenty pages of Results, and four pages of Discussion and Conclusions. The author used more than a hundred literature sources. There are also about ten pages of supplementary information in the Appendix. The thesis is further supplied with two publications and a statement about the author's contribution to a manuscript to be submitted.

The Introduction contains three subsections. It provides information on both proteins of interest and also practical insight into the application of various structural biology methods that were used in the research. The introduction to both proteins is written sufficiently to understand the motivation of the research. However, the description of methods is naive in several places and gives only a limited insight into the methods. For example, section 1.3.2 lacks a description of the validation parameters, no physical background is described for any of the methods.

Section Materials and Methods contains a standard description of used methods and concisely provides sufficient information that would be necessary to reproduce here described results. This section documents the large extent of laboratory work that has been done by the author. He used a large repertoire of biophysical and biochemical methods to support all statements discussed in manuscripts and in the thesis.

Sections Results and Discussions are very concise and brief. Frequently, expressions like "*in vitro*" are not written in italics. The author frequently uses the words "we", "our group", and this raises the issue of how much help was given by the collaborators and what was the extent of the author's contribution. Such words should be avoided in the text.

Section Conclusions provides the overall summary of the thesis. When compared to Aims, the objectives of the thesis were fulfilled.

Section References reports literature used in this thesis. I have discovered several inconsistencies, typos, and one invalid citation. The amount of references is adequate for a standard doctoral thesis.

Reading the thesis brings the expectation that the manuscript (or its draft) mentioned in section 9.3 will be published in the thesis in the form of an appendix. However, this is not the case. The author should briefly present the stage of the manuscript during the defense and highlight the contribution to the manuscript including text writing.

Questions:

- 1) HDAC6 is a multi-domain protein that adopts multiple conformations in solution. Six conformations were selected to demonstrate its flexibility. Is there a possibility to select two border cases with remaining conformations potentially classified as “morphing snapshots” between these extremes?
- 2) Can you explain the statement on page 44 that the inhibitor is hydrolyzed by the wild-type enzyme? Can you exclude the influence of E424M mutation on your interpretation? I recommend the creation of a figure of the active site with an overlap of wild-type and mutant variant with inhibitor.

Overall, the thesis is written in a standard way, but the extent is rather limited. However, it brings new information for a better understanding of HDAC6 complexity and improvement of biotechnologies based on inhibition of GCPII. After correct answers to this review, the thesis can be considered as a foundation for awarding a doctoral degree – Ph.D.

9.10.2023 - doc. Ing. Petr Kolenko, Ph.D.