Oponentský posudek doktorské disertační práce

Tomáš Kučera: Impact of the glycine-rich loop on the function of processing peptidases of the mitochondrial type

In his PhD thesis Tomáš Kučera makes an attempt to understand the evolution of the glycine-rich loop of mitochondrial processing peptidases and MPP-like peptidases and to elucidate its physiological role. The PLOS one paper that Tomáš Kučera authored as a first author and which I therefore consider the core of his PhD work represents a theoretical study on the glycine-rich loop of MPP, finally concluding that this unstructured secondary structure element acts as a balanced structural element, holding both peptidase subunits in a partially closed conformation. The research has been conducted with great care and the paper is technically sound without any flaws in the experiments. Although the paper itself is a theoretical paper, the interpretation takes into account the available wealth of biochemical experimental data, including the data presented in the three papers co-authored by the candidate, and tries to match the gained knowledge with available experimental data and the conclusions are drawn appropriately. The four papers published by the candidate gained to date altogether 36 citations, which is remarkable for a PhD thesis and demonstrates the actuality of the research and its international recognition.

The thesis itself is written in a classical way as a full thesis and not as a collection of papers. The thesis is very nicely structured, and the English is appropriate with only minor mistakes, clear and easy to read. It is visible that the author invested a lot of time and care in writing up his research and did care even about small details that are often overseen. 40 pages of literature review is an extensive introduction into the topic, nevertheless I miss an introduction into the theoretical background of the major methods used, as the "Materials and Methods" section only gives the technical details. However, given the huge amount of experimental and computational techniques this might be a nearly impossible task without blowing-up the thesis significantly. On the other side, the remarkable broadness demonstrates the broad theoretical and practical knowledge of the candidate and the reader is left with the feeling that the candidate knows what he is writing about.

Questions for the defense that should be addressed by the candidate:

1. The thesis describes in detail a huge variety of experimental and theoretical methods ranging from molecular biology via crystallization over SAXS to targeted MD. I can hardly imagine that all of the mentioned methods are mastered by the candidate with the same perfection. I would be glad if the candidate could describe a bit to which degree he manages the various techniques with his "own hands" and where he sees his main strength.

2. I find the targeted MD that uses the linear minimization of the RMSD a very smart alternative to the classical steered MD that uses a pulling force on the moving target. I would be very happy if the candidate could describe a bit his experience with that method, its pitfalls and if possible its advantages/disadvantages over classical steered-MD

2. The homology model of HPP is based on the very weak homology to MPP. Especially the alpha-subunit shows a sequence identity in the "twilight" zone (17%). As far as I am aware the SWISS-MODEL server has a default cut-off at 30% identity, and as it used classical fragment-based modeling probably results below that...
border have to be taken with great caution. Did you try alternative methods as for example restraint-based modeling (Modeller) that should give better results for low identities or loop modeling to improve the surface exposed loops that are not stable in the MD refinement?

3. The HPP results are yet to be published. Is there a paper in the pipeline that includes all the structural work, experimental as well as computational?

3. The MD simulations were run on a server in the Otyepka group in Olomouc. How many cores were you able to employ for a typical 100ns simulation of your system and how long did it take to calculate 100ns (days)?

4. One paper is mentioned as a review ready for submission. Has it been submitted already and where?

5. The author mentions a book chapter in the Handbook of Proteolytic Enzymes published by Elsevier. Generally, a book chapter is mostly by invitation from the editor only and is regarded as a prestigious publication one should be proud of. Certainly, it should not be hidden under the title "publications without IF". How many pages does the chapter comprise?

Finally, it is my pleasure to state that Tomáš Kučera until now conducted internationally recognized high quality science, clearly manifested in the citations to his work. The well written thesis tells a very exciting story and the publications that back up the thesis show, without leaving any doubts, that the applicant fulfills all criteria for being awarded a PhD degree. Therefore I can fully recommend Tomáš Kučera for being awarded the PhD degree.

(Český doplněk: Tomáš Kučera jasně prokázal tvůrcí schopnosti, práce bez sebemenších pochyb splňuje požadavky kláděné na disertační práce v oboru biochemie)

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