



doc. Ing. Petr Kolenko, Ph.D.
Břehová 7
115 19 Praha 1
kolenpe1@cvut.cz
+420 770 130 060
kmlinux.fjfi.cvut.cz/~kolenpe1/

Opponent Review of Doctoral Thesis

The doctoral thesis of Stefan Djukic "Structure-assisted design of inhibitors targeting medically relevant enzymes" describes the author's contribution to the development of new inhibitors of purine nucleoside phosphorylase and cyclin-dependent kinase 2. Both proteins represent a valuable target for the improvement of current medical therapies.

The thesis is written as a collection of four papers already published in high-profile international journals. The text starts with 25 pages of introduction to the problematics. The overall knowledge of both protein targets is described sufficiently to understand the motivation of the thesis. The Aims of the thesis are clearly defined.

Section Results contains reprints of four manuscripts. Each manuscript is introduced by subsection Motivation for the study and another subsection about the inhibitors. Here, the author nicely describes the rationale behind all studies and explains its importance. Another subsection Structural studies usually describe the main author's contribution to the manuscript. Apparently, they played a pivotal role in the research. The next subsection Conclusions summarizes the most important outcome of the research. In all sections mentioned above, the author is original and provides a reasonable amount of well-written text for evaluation of the quality of a writer. However, the subsection "My contribution" is usually very limited and does not report the involvement of the author in writing the text. This had to be supplied by the "Supervisor's declaration".

The last original contributions of the author are sections Discussion and Conclusion (four pages). The author discusses the observation from his perspective – the perspective of a structural biologist. The crystal structure analysis is put together with results from other studies. The text manifests an overall understanding of a complex research project, but also the importance of the results. Moreover, the aims of the thesis were successfully achieved.

The section References reports a reasonable amount of original sources.

All reported manuscripts document the ability of the author to work in collaboration with a big multidisciplinary team of scientists. His contribution is essential for understanding the complex and unique features of analyzed proteins.

The weakest point of the thesis is the authorship of the reported manuscripts. Stefan Djukic shares the first authorship only in one of the papers. He also does not report his contribution to text

writing in the description of individual papers in the thesis. Nevertheless, this deficiency is compensated by the quality of here presented text.

Minor comments:

- 1) The author uses the abbreviation PNP right from the beginning, already in the Abstract. The reader does not know what it stands for.
- 2) A list (or number) of the entries in the PDB database (*e.g.* page 10) does not say much about its content. Are there multiple conformations? Ligands? Other complexes?
- 3) Page 10: β -sheet, but alpha-helices?
- 4) Page 11: Unclear, what is subunit? Is it chain, domain?
- 5) The thesis contains a few typographic mistakes, *e.g.* alphaC-helix, missing dots, too many spaces, Protein d(D)ata b(B)ank.
- 6) What is a single digit IC50 value towards CDK2 (page 113)?

Questions:

- 1) Page 12, last paragraph: Statement “Phe200 and Tyr188 in both targets have identical orientation and do not form direct contacts with substrate” is in contradiction to Figure 4 C and D on page 13.
- 2) What is the structural rationale for no inhibition of PfPNP by inhibitors of hPNP and MtPNP? Can something be deduced from crystal structures, models, or sequence alignment?

- 3) *Purely technical question: Some of the Rmerge or Rmeas values of here reported structures are out of expectation (around 30%, or even 60% on page 151). Is there some evident reason for that?*

My questions are more or less supplementary questions. Minor comments are also marginal points. Overall, the thesis is written well. I am confident that the author will be able to provide satisfactory answers. Therefore, the thesis can be considered as a foundation for awarding a doctoral degree – Ph.D.

10.10.2023 - doc. Ing. Petr Kolenko, Ph.D.