Larkin University
College of Biomedical Sciences

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Stud. odd.- doktorske studium
Matematicko-fyzikální fakulta Univerzity Karlovy
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121 16 Praha 2

Oponentský posudek doktorské disertační práce

Vlastimil Zima: Molecular dynamics simulations of ion channel TRPA1

Dear committee:

In his PhD thesis Vlastimil Zima studied the structure/function relationship of one of the members of the transient receptor potential channel family, namely TRPA1. This human channel is involved in sensing pain and the candidate was using a computational approach including homology modeling and classical molecular dynamics (MD) simulations to interpret experimental data on an atomistic level. The used experimental data were mostly gained in collaborating labs specialized on electrophysiology and patch clamping and interested in understanding the molecular mechanism of those ion channels. I think there is no real need to emphasize the relevance of those research, as TRPA1 plays a central role in the pain response to endogenous inflammatory mediators and to a diverse array of volatile irritants and is an obvious prime target for drug design.

The PhD thesis consists of 88 pages (without bibliography and appendices). The research topic is introduced very briefly in one page of introduction, followed by chapters describing different voltage-gated ion channels including sodium, potassium and finally transient receptor potential channel (TRP) families with a special focus on TRPV1 and naturally TRPA1. This is followed by an overview over molecular dynamics simulations of lipid bilayers, membrane proteins, ion channels such as voltage-gated ion channels and in particular transient receptor potential channels in 20 pages. The consequent chapter then is devoted to the applied methodology giving details for classical molecular dynamics simulations, molecular dynamics flexible fitting, free energy perturbation and homology modeling. The author then presents and discusses his own results in chapter four of the thesis on more than 40 pages. The thesis concludes with a two page conclusions section. The thesis is written in good English with very minor errors and grammatical mistakes such as usage of prepositions and articles. The thesis is based on 7 scientific peer-reviewed publications presented in the appendix published in high impact factor journals such as Science Signaling with a current impact factor over 7 and

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Neuropharmacology with an impact factor still over 5 and other well established and highly recognized journals in the field as is the Journal of Biological Chemistry or the Journal of Physical Chemistry published by the American Chemical Society ACS.

The high level and relevance of the research conducted during his PhD is also demonstrated by the fact that the included papers were cited by September 16 already 35 times, which is a remarkable high number for a PhD student. Personally, I enjoyed reading the thesis as Vlastimil Zima's work is another nice example advertising the complementarity of experimental and computational approaches in the study of mechanistic aspects of ion channels. The thesis nicely shows and explains the different levels of simulation, including not only their relevance but also their shortcomings, and demonstrates the fast evolution/development in the field (due to technical progress and additional structural information, X-ray or cryo-EM) even in the few years of this PhD thesis, as the candidate started with comparatively simple modeling getting to free energy profiles and a level allowing rational drug design. Vlastimil Zima thus without any doubt does not only fulfill all formal requirements for a successful defense but has also proven the scientific relevance and recognition of his work within the international scientific community.

Finally, for the purpose of discussion, I have a few remarks/questions to the candidate:

1. I would be glad if the candidate could explain the use of the 4fs time step for their simulations with ACEMD (page 45 in the thesis). Please discuss this with respect to typical time steps in MD simulations and their physical reasoning.

2. On page 46 the candidate explains binding of a Ca^{2+} ion to the binding domain. How frequent or persistent is the occupancy of the ion in the pocket and how far it can move to be assumed unbound?

3. Does the candidate encounter non-ergodic conditions when simulating Ca^{2+} as the Ca^{2+} ion is double charged? Also I wonder whether water molecules in the first solvation shell around Ca^{2+} ions are influencing the occupancy of ion?

4. Calcium force field parametrization is a kind of tricky, and recent works of Jungwirth et al recommend corrections for the divalent calcium ions as most force field overestimate calcium-protein interactions. How dependent are the candidate results on the force field parameters used and did he "play" with different force fields or parametrization, corrections?

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5. On page 53 and 54 the author mentions the use of two Na^+ ions in the system and simulations have been performed for 30ns. I assume that the candidate considers this comparatively short simulations as sufficiently long to reveal the structural changes? Which criteria or parameters support this finding?

For all what is said above it is my pleasure to state that I can fully recommend Vlastimil Zima for being awarded the PhD degree.

(Český doplněk: Vlastimil Zima jásně prokázal tvůrčí schopností, prácé bez sebemenších pochybů splňuje požadavky kládené na disertační přáce)

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