Report on Ph.D. thesis
of
Vasilina Zayats, MSc.

"Structure and function of cation translocation systems: modeling and simulations"

The dissertation of Vasilina Zayats is a valuable contribution to the study of one of the most important biomolecular machineries namely cation translocation systems. The whole work represents very suitable combination of various computational methods used to shed light on interesting experimental findings.

The thesis document is organized in three main chapters. The first chapter - comprehensive and well-organized introduction - is related to description of structure and function of ion channels. In the second chapter a wide range of used methods (i.e. sequence alignment, homology modeling, molecular dynamics simulations, ligand docking etc.) is described. The third chapter is mainly focused on unpublished results (concerning the TRPA1 ion channel) supplemented by brief annotations of enclosed papers and manuscripts.

The main results of this dissertation are as follows. (i) Finding of severe steric overlap for NADH and benzoquinone substrates of the E. coli oxidoreductase WrbA (that has been implicated in oxidative defense), which was found using docking and QM/MM calculations using specialized method that include polarization of ligands to optimize (by redocking) the position of substrates in the binding pocket. This steric overlap is consistent with the ping-pong mechanism proposed for this protein. (ii) Further, a model of the N-terminal 17 ankyrin repeat structure of TRPA1, including the calcium-binding EF-hand was developed. In MD simulations the calcium-bound state was found to be rigid as compared to the calcium-free state, when the end-to-end distance can change by almost 50%. This increase in stiffness could affect the force acting on the gate of the TRPA1 channel. MD simulations of the transmembrane domain of TRPA1 showed that residue N855, which has been associated with familial episodic pain syndrome, forms a strong link between the S4-S5 connecting helix and S1, thereby creating a direct force link between the N-terminus and the gate. These findings were revised and further developed in the text of the thesis (i.e. homology models of the TRPA1 selectivity filter and transmembrane segment based on various templates etc.). (iii) Further, the hexameric C-terminal domain of arginine repressor of E. coli was studied by 100 ns molecular dynamics simulations in the presence and absence of the six L-arg ligands that bind at the trimer-trimer interface. MD simulations revealed that the binding of one L-arg results in a holoprotein-like conformational distribution. (iv) An extracellular Ca^{2+} binding site within plasma membrane Ca^{2+} channel Orai1 was located at the pore entrance. Efficiency of this Ca^{2+} sink was found to be fine-tuned by transient electrostatic interactions with the outer loop3 of Orai1. Notably, site directed mutagenesis of the Ca^{2+} sink structure attentuated Ca^{2+} permeation. (v) A refined structural model of the Sacharomyces cerevisiae Trk1 protein was developed based on homology modeling, molecular dynamics simulations and experimental evidence through functional analysis of mutants. The model and the experimental results showed that conserved glycine residues within the selectivity filter are not only important for protein function but also govern correct folding/membrane targeting.

The dissertation itself, as far as I can judge, is written in good and understandable English. Its graphical lay-out is appended with nice instructive pictures. The scientific results are of major interest and substantially contribute to the knowledge of the structural characteristics of cation translocation systems. It is evident without any doubts that MSc. Vasilina Zayats masters wide range of advanced modeling techniques. She knows to suitably apply them to get valuable results. In my opinion, the work certainly fulfils the requirements for Ph.D. thesis, and I recommend it for the defense.

Barvík

Prague 20th November 2014

RNDr. Ivan Barvík, PhD.
Institute of Physics
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Questions:

1) Various sequence alignments and homology models of the TRPA1 selectivity filter based on Kv1.2, NaK, CNG and NavAb template structures are presented on Fig. 11, p. 68. Compare them with analogous models based on TRPV1 templates (i.e. 3J5P, 3J5Q, 3J5R vs. 2AHY, 3K04).

2) Multimicrosecond all-atom molecular dynamics simulations produced using a special purpose ANTON hardware shed some light on the mechanism of ion channel voltage gating [1]. Further, Liao et al. used single-particle electron cryo-microscopy, with no help from any of the more established methods of structural biology, to solve the milestone structure of TRPV1 in open and close states [2]. Which of these studies provides more clues to understand TRPA1

gating?

3) It has been suggested [3-4] but challenged later [5], that activation of TRPA1 may depend on Ca^{2+} binding to an N-terminal EF hand motif. Intriguingly, the D469A mutant can still be activated by Ca^{2+} [5]. Did you test viability of this mutated EF hand using molecular dynamics simulations?

1. Morten O. Jensen, Vishawanath Jogini, David W. Borhani, Abba E. Leffler, Ron O. Dror, David E. Shaw
Mechanism of Voltage Gating in Potassium Channels
Science 336 (2012) 1216533

2. Erhu Cao, Maofu Liao, Yifan Cheng & David Julius
TRPV1 structures in distinct conformations reveal activation mechanisms
Nature 504 (2013) 113-118

3. S. Zurborg, B. Yurgionas, J. A. Jira, O. Caspani, P. A.
Heppenstall direct activation of the ion channel TRPA1 by Ca(2+)
Nat. Neorsci. 10 (2007) 277-279

4. J. F. Doerner, G. Gisselmann, H. Hatt, Ch. Wetzel
Transient receptor potential chanel A1 is directly gated by calcium ions
J. Biol. Chem. 282 (2007) 13180-13189

5. B. Nilius, J. Prenen, G. Owsianik
Irritating Channels: the case of TRPA1
J. Physiol 589 (2011) 1543-1549

Reviewer´s report

Structure and function of cation translocation systems: Modeling and simulations

Vasilina Zayats, MSc

The thesis of the Ph D candidate is focused on investigations of the molecular structure of cation translocation systems, using various representative membrane proteins specialized on the translocation of mono- and divalent cations. The combined approach of bioinformatics and molecular dynamics simulation has been used for the theoretical explanation of mechanisms in different groups of cation translocation systems.

The first membrane protein studied was the transient receptor potential ankyrin 1 receptor channel TRPA1. It is a polymodal excitatory ion channel found in sensory neurons of different organisms. Since its discovery as an uncharacterized transmembrane protein , TRPA1 has become one of the most intensively studied ion channels. Its function has been linked to regulation of heat and cold perception, mechanosensitivity, hearing, inflammation, pain, chemoreception, and other processes. Some of these proposed functions remain controversial, while others have gathered considerable experimental support. The polymodal ion channel TRPA1 is activated by various stimuli, including electrophilic chemicals, oxygen, temperature, and mechanical force, yet the molecular mechanism of TRPA1 gating remains obscure.

The first aim of the thesis was focused on the structural organization of the TRPA1 N-terminus which contains 17 ankyrin repeats and one EF- hand loop, and the transmembrane region. In TRPA1 channel, the sequence identity between the N-terminal domain with a template structure (based on crystal stucture of potassium Kv channels) is at lower border 25% where homology modeling is possible, manual interference is needed, including additional sequence analysis to identify proper secondary structure elements. The transmembrane domains of TRPA1 indicate a similar fold and quartenary structure arrangement like in potassium voltage gated channels.

The second goal of the thesis was to build the model of human Orai1 channel and to identify potential binding sites for cholesterol. This protein belongs to the store-operated calcium entry proteins SOCE and associates with TRPA1.Using molecular dynamics simulations based on the template Drosophila Orai 1 structure allowed to get a reasonable human Orai1 structure with a good atomistic duality. This model was used for investigation of the structure-function relationship in collaboration with the group of dr. C. Romanin from Linz.

Third topic of the thesis was aimed at the structure of Trk1 potassium –selective translocation system from Saccharomyces cerevisiae.Trk1 protein is distantly related to prokaryotic Trk and plant HKT proteins. Using combined theoretical and experimental approaches, a three – dimensional model of Trk1 was obtained with a good correlation with experimental results from the group of dr. J. Ludwig. It also characterized a distinct role of glycine amino acid residues in the selectivity filter of Trk1 and examined the structure-function role of conserved salt bridge between the first pore domain and the last transmembrane helix of the protein.

This main part of the thesis has been complemented with kinetic data on bacterial enzyme WrbA that is implicated in oxidative defense .Steady- state kinetics results shown that WrbA conforms to a ping pong mechanism.

The thesis contains substantial amount of work , is well written and organized in ordinary way with introduction part, results and discussion chapters to highlight main findings and finishes with a short conclusion. The number of references is sufficient. There are attached

five papers, two already published and the candidate is the first author on one of them, the rest is submitted.
I have the following questions for discussion:

1. A key issue for computational modeling is the quality of the initial input structure. In your work you used in several cases homology models based either on the same protein from a different organism (Orai) or based on more distantly related ion channels or transporters (TrpA1, TrK). In crystallography the quality of a structure is indicated by its resolution between 1-3.5A for all atom models. Could you comment on the quality of your initial models for the three cases in your thesis and relate their probability to a resolution similar as for crystal structures?
2. The molecular dynamics analysis of the Orai1 channel is based on the recently described hexameric structure for a modified Drosophila Orai. In contrast, several reports in the literature have previously concluded that the structure of the functional CRAC channel is a tetrameric assembly of Orai1 subunits. Could you comment on if this dispute is considered as solved in the community or if this still considered an open issue?
3. In TrK1 four glycines are predited by your model to form the narrowest part of the selectivity filter. The functional tests of the Trk1 mutant G959A demonstrated that the G to A mutants in the selectivity filter had growth defects at low external [K+], while the fusion protein Trk1G959A]/GFP was folded ant targeted correctly. Can you computationally predict what would be the effect of the point mutation in structural or dynamical terms? Does the alanine just block the pore or do you observe a change in dynamics or even a distortion of the filter?
4. In your thesis you write that your model based on NavAb structure was surprisingly close to the TrpV1 crystal structure that came out later, and most conclusions that you draw can be still considered as correct. The main difference you describe for the menthol-sensitive residues S873 and T874, which are not easily accessible from the cytosol in the TRPV1 based model of the channel. Do you think that the TRPV1 based model might be incorrect in that region (while the older model was right), or that it is rather a different conformation of the channel that would allow the interaction with menthol?

The candidate has proven to be capable of conducting independent research using molecular dynamics simulations. Therefore, I recommend the submitted thesis for further procedures in the process of granting a PhD degree to the candidate.

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November 18th 2014

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Prof Tomas Polivka
Faculty of Science
University of South Bohemia in Ceske Budejovice

17th November 2014

Reviewer's report on PhD thesis "Structure and function of cation translocation systems: modelling and simulation" by Vasilina Zayats

Dear Prof Polivka

The thesis by Ms Zayats uses computer simulation to provide new insight into the relationship between structure and function for the translocation of ions by membrane bound ion channels.

The thesis provides a sound background to the biological relevance of the systems studied during the PhD. In the "Materials and Methods" section, a brief introduction to the main computational tools of homology modelling, molecular dynamics and ligand docking is provided, as is a brief summary of the quantum mechanical methods commonly employed in computational chemistry. In the "Results" chapter, the use of homology modelling to provide a structure for sections of TRPA1 and atomistic MD simulations were performed to test the results for the N-terminal domain. The use of docking calculations to understand the structural basis for the regulation of the human Orai1 ion channel was described, and reported that cholesterol is most likely to be bound to transmembrane helices TM2 and TM3. The binding of Ca^{2+} ions to the channel entrance was also studied. Due to its low homology with other systems, a combination of homology modelling, secondary structure and experimental restraints were used to predict the structure of the Trk1 protein. Finally, the thesis briefly introduces modelling applications to non-membrane systems, such as the binding of co-factors to the proteins WrbA and ArgG.

There are, however, aspects of the work that if discussed in more detail would improve the thesis. Most importantly, the thesis does not contain any discussion of the limitations of the modelling methods used, such as potential problems with homology models, or the limitations associated with the short MD trajectories that can be obtained in atomistic simulations. Moreover, none of the issues associated with the difficulties of modelling divalent counterions with current common forcefields, such as Ca^{2+}, have been described.

The PhD thesis clearly represents a large body of computational work, much of which has been successful. The work described in the thesis has provided invaluable insight into experimental data and has assisted in the design of future laboratory work. The thesis contains five papers that have

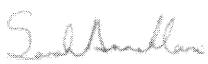
either already been published, or have been submitted for publication, which demonstrates that the candidate has certainly fulfilled the requirements for the award of a PhD.

I would like to pose the following questions to the candidate in order to increase my understanding of the work presented:

1) In the work described by Zayats et al, J Mol Model 2013, the change in the mechanical properties of the ankyrin repeats on calcium binding is described. PCA of the simulations is also presented in Fig 3 of the paper. Could the candidate please discuss whether the global motions extracted by the PCA are able to explain the mechanism of amplification of small chemical changes by the ankyrin repeat structure, and how this is modulated by calcium?
2) In Fig 7, the fitting of the model structure into a cryoEM map is described. How exactly was this fitting performed, and how well does the model agree with the experimental data?
3) On pg 92 of the thesis, docking calculations of cholesterol into the binding pocket of hOrai1 are described. What approximations are inherent in such docking calculations? How reliable are the results, and how might these be tested either experimentally or computationally?
4) Computing is playing an increasingly important role in biomedicine, as the candidate describes in the Preface to the thesis on pg 14. Based on her experience of using computing in the biomolecular sciences, and her understanding of the limitations and advantages of the methodologies employed in the thesis, I invite the candidate to speculate the most important contributions that computational methods may make in the next 5 or 10 years. What developments are necessary to make the field of computational biology a fully quantitative discipline?

I am very much looking forward to discussing the research of the candidate in more detail at the defense.

Yours sincerely,



Sarah Harris

Dr Sarah A Harris
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