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Review of doctoral thesis

Title: Role of Transient Tunnels in Function of Enzymes with Buried Active Sites
Author: mgr inż. Aravind Selvaram Thirunavukarasu
Promotor: dr hab. Jan Brezovsky, prof. UAM

The formal page of the dissertation:

The thesis is presented as a collection of three published and thematically related scientific articles. The short introduction in the studied field and summary of the doctoral research precedes the attached publications. The introduction is well written and helps reader to understand the motivation of the presented research. Summary of the doctoral research contains the most important findings of the research done during the PhD study. The summary is written clearly and reader can observe clear image of the accomplished work. Text and graphics of the submitted dissertation are prepared at the required level. The scope of the work is adequate to current standards in the field. The individual parts of the written work are clearly and logically structured. I do not have any comments on the formal page of dissertation thesis.

The content page of the dissertation:

Presented dissertation thesis is focused on the study of the dynamics and functionality of the water molecules transferring tunnels. The tunnels can modulate the water molecules flow into the buried active site of the enzyme what can have a serious implication on the enzyme function and also to its reaction kinetics. The molecular dynamics simulations and adaptive sampling techniques have been used within the study. Different hydrolytic enzymes with buried active sites, such as epoxide hydrolase or haloalkane dehalogenase, have been used as a model enzymes.

The first part is dedicated to the development of the computational tool named TransportTools. The tool is a specific library for high-throughput analyses of biomacromolecules structural dynamics responsible among others for the utilization of water by the enzyme by analysing the trajectory obtained from the molecular dynamics simulation. The PhD candidate actively participate on the tool development especially to the user testing, data generation, performance evaluation and use case summarization. Such tool is very useful tool for the computational chemists worldwide. The tool capabilities were published in the Bioinformatics journal.

Within the second part, the previously developed tools are used for characterization of the tunnels in the haloalkane dehalogenase (DhaA) from *Rhodococcus rhodochrous*. This enzyme is known to have a three main water transferring tunnels where one of the main tunnels is split to three side tunnels. The enzyme was subjected to the comprehensive molecular dynamics simulations. All obtained trajectories were analysed employing the CAVER and AQUA-DUCT tools dedicated for the possible tunnels clustering and water flow over the tunnels. Finally, all the analyses were combined in the TransportTools. Using this approach, the candidate has been able to identify two new water molecules transporting tunnels which were not known. The candidate has also been able to quantify the water molecules flow within the tunnels and define the tunnels

responsible for the hydrolytic activity. The obtained results have been published as a part of the second presented publication which has been published on bioRxiv for now.

The last third part is focused to the study of the different water models impact on the tunnel analyses. It is generally known that different molecular mechanics water models have different physiochemical properties. These differences influence the macroscopic water properties extracted from the simulation trajectories. The candidate has used three different commonly used water models and studied how these models influence the water flow over the tunnels. The OPC, TIP3P and TIP4P-Ew were models has been employed. Previously studied haloalkane dehalogenase (DhaA) from *Rhodococcus rhodochrous* has been used as a test system. All identified tunnels in DhaA have been grouped in to five Tunnel Conformational Groups (TCGs) according their bottleneck radii. These five TCGs have been solvated with different water models and subjected to molecular dynamics simulations. Obtained trajectories has been analysed employing the same procedure as in the previous studies. The results showed clear differences in the water flow through the studied TCGs and imply that the differences in water flow are strongly influenced by the choice of the water model. The obtained results have been published as a part of the third presented publication where the candidate is the first author and which has been published on bioRxiv for now.

The PhD candidate published altogether three publications where one was already published in highly impacted journal *Bioinformatics* and the other two have been published on bioRxiv for now. All three publications are a part of the presented dissertation thesis. I really appreciate the amount of the computational experiments done and the amount of the data which had to be analysed. All used computational methodologies are high quality and PhD candidate was able to develop very efficient computational procedure eligible for the studying of the water molecules flow in the tunnels in enzymes.

Comments and questions:

1. On the page 18 you mention that you used the dihedral angles as a used-defined metrics for the constructing Markov State Models. I'm wondering why you decided for the dihedral angles and what another metric can be used.
2. In the last paragraph on the page 20 you name three amino acid residues which are part of the helices α4 and α5, however, you do not mention what amino acid residues are they. Could you please specify these amino acid residues?
3. In the Publication 2 on page 6 you mention that you run 201 ns long equilibration with positional restrains. It is unusually long restrained equilibration. I'm wondering what was the reason for a such long restrained equilibration?
4. For the network tunnel calculations in the CAVER you set the probe radius to 0.7 Å instead of commonly used value of 1.4 Å. I'm wondering why you chose such value and whether you did some test how this value can influence your results.
5. In the Publication 2 you conclude that that the water molecule can pass extremely narrow, subangstrom, bottlenecks in the tunnels. What do you think how this observation is influenced by the flexibility or dynamics of the amino acid chains?
6. Within the Publication 3 I did not catch the procedure of the Tunnel Conformational Groups (TCGs) creation. Could you please explain the TCGs creation in more detailed way?

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7. I'm wondering whether it is possible to estimate the energetics of the water flow within the tunnels based on the performed calculations in the Publication 3.
8. What is the current state of the publication published in bioRxiv?

Overall assessment:

In the final evaluation of the submitted dissertation, I appreciate the huge amount of implemented computer experiments that the PhD candidate conducted. The obtained results are an indisputable benefit not only in the specific area of studying of the water molecules flow in the tunnels, but also have a methodological significance for the application of sophisticated theoretical methods.

In conclusion, I conclude that the set goals of the dissertation were fully met and the PhD candidate demonstrated the ability to do independent scientific work.

Based on the above facts, I recommend that the doctoral dissertation of mgr inż. Aravind Selvaram Thirunavukarasu entitled "Role of Transient Tunnels in Function of Enzymes with Buried Active Sites" was accepted for defence and after its successful completion, the candidate was awarded a degree:

Philosophiae Doctor – PhD.

In Bratislava, 07. 01. 2024

Mgr. Stanislav Kozmon, Ph.D.