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Review of doctoral thesis

Title: Insights into Molecular Mechanism behind Rare Transport Processes in Enzymes with Buried Active Sites
Author: mgr Nishita Mandal
Promotor: dr hab. Jan Brezovsky, prof. UAM

The formal page of the dissertation:

The thesis is structured as a collection of three published scientific articles, each interconnected thematically. It begins with a succinct introduction to the studied field, followed by a summary of the doctoral research. This well-written introduction effectively sets the stage, aiding readers in grasping the research's motivation. The summary showcases the key findings from the PhD study in a clear and comprehensive manner, allowing readers to easily understand the work accomplished. Both the text and graphics in the submitted dissertation meet the required standards. The scope aligns with the current field norms, and the work's components are organized in a clear and logical manner. I have just one comment to the formal page of dissertation thesis. I miss some page numbering at the Publications part of the thesis. Writing of the comments referencing to this part is then slightly complicated without the page numbers.

The content page of the dissertation:

Presented dissertation thesis is focused on the study of the dynamics of the tunnels in enzymes used to transport of molecules from the environment to the buried catalytic site. The tunnels can modulate the 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 conventional and enhanced sampling molecular dynamics simulations techniques have been used within the study. The Gaussian accelerated or coarse-grained molecular dynamics were used for the conformations sampling acceleration. The majority of the computational studies have been done on the haloalkane dehalogenase LinB and its two mutants within all computational experiment as a model system. Moreover, nine selected enzymes have been used for coarse grain simulations.

The initial section focuses on the development of a computational tool named TransportTools. This specialized library facilitates high-throughput analyses of biomacromolecules' structural dynamics, particularly in understanding how enzymes utilize tunnels to water or substrate transport by analysing trajectories from molecular dynamic simulations. The PhD candidate was actively involved in the tool's development, specifically in user testing, data generation, performance evaluation, and summarizing use case where the LinB haloalkane dehalogenase was used as a model system. This tool is invaluable to computational chemists globally. Its capabilities were published in the *Bioinformatics journal*.

Within the second part, the efficiency of using the GaMD method to explore the tunnel networks in model system haloalkane dehalogenase LinB has been studied. The GaMD performance was compared to conventional molecular dynamics of the studied enzyme. Altogether 150μs of the simulation data has been produced and analysed. The CAVER and TransportTool programs have been used to analyse the possible tunnels in the obtained trajectories. Analyses

show that the GaMD simulation has correctly identified all tunnels known from conventional MD and moreover sampling of the higher energy conformations reveal the new one transient side tunnel unidentified before. Lately the CaverDock tool has been used to study the possible transport rate of the selected molecules in the primary p1, auxiliary p3 and new side tunnel. The CaverDock tool can calculate interaction energy profile, which can identify energy barriers or resistance points. Using the tool, the applicant has been able to identify the differences in energetics between particular tunnels and more over also t in the mutated homologue tunnels. The observed results suggest that the newly observed side tunnel has similar energetic profile as auxiliary p3 tunnel. At the final the calculated trajectories have been analysed using the Principal Component Analysis. The comparison of the results between the conventional MD and GaMD shows that GaMD was able to sample the distinct conformational states of LinB. The analysis identified the movement of the short alpha helical structure which was responsible for opening and closing the side tunnel. The study presented in this part was published in *Journal of Chemical Information and Modeling*.

The last third part is focused to the study whether the Corse Grain molecular dynamics methods can be effective and accurate enough for study of tunnel network in enzymes. The applicant has used several coarse grain approaches and compare it with the all atom molecular dynamics. Namely the Martini 3.0 with Elastic Network or with GoMartini3 modification models, and SIRAH model have been employed. The haloalkane dehalogenase LinB has been chosen as a model also in this case. Moreover, other nine selected enzymes have been studied from three different Enzyme Classes where three enzymes from each group were selected. The production molecular dynamics trajectories have overall length of 300μs. Produced trajectories has been analysed by analogical procedure as in previous studies employing the CAVER and TransportTools software. In comparison with all atom molecular dynamics simulations, the presented results of the study show that all tested coarse grain models were able to properly identify all main tunnels in selected enzymes. Analyses also show that coarse grain molecular dynamics simulations can effectively differentiate between permanent and transient tunnels and accurately reflect the structural characteristics of the respective tunnel ensembles. This study is described in 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 four publications where three of them are used in the dissertation thesis and one publication not directly connected to thesis topic. Three publications were already published in highly impacted journals as *Bioinformatics*, *Journal of Chemical Information and Modeling* or *Computational Biology and Chemistry* and the latest one has been published on bioRxiv for now. 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 procedures eligible for the studying of the tunnel dynamics and its identification in the enzymes.

Comments and questions:

1. On the page 23 you state “The residue L176 is mutated for LinB-Closed and LinB-Open mutant, forming H-bond with D146, making it more difficult for ST opening in mutants”, however you do not mention what mutation is present instead of L176. Could you please specify the mutation? Or on other hand, I would speculate that you used incorrect amino acid numbering compared to the rest of the text.

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2. On the page 26 you state "…, I developed in-house restraint protocol to keep my CG model stable", however you do not mention what protocol. I haven't found any description of the new protocol in the text of the manuscript either. I'm wondering whether you somehow modify the protocol in the used programs, or it means that you just tuned some protocol parameters. Could you please clarify this?
3. I'm wondering how the specific restrain parameters described on page 26 or in publication 3 in the "System setup and Coarse-Grained MD simulation using Martini." paragraph for the coarse grain simulations were chosen. Did you run short simulations to test the stability of the system with different parameters?
4. On page 28 you state "I observed many additional transient tunnels captured through CG method, that have little or no comparable counterparts from cMD, observed likely due to sampling enhancement". Do you really think that the "sampling enhancement" is the only reason for such behaviour?
5. I assume that the plots in Figure 5B in publication 2 represent the average of the energy costs of successful transport events. I'm wondering whether the same analysis can be used to plot the energy cost of the transport according to the tunnel length to identify the area of the tunnel with highest energy cost.
6. What is the current state of the publication published in bioRxiv?

Overall assessment:

In the final evaluation of the submitted dissertation, I commend the extensive array of computer experiments conducted by the PhD candidate. The results obtained are undeniably beneficial, not only for the specific study of molecule flow in tunnels but also for their methodological significance in applying advanced theoretical methods.

In conclusion, the dissertation's goals were fully achieved, and the PhD candidate has demonstrated the capability to conduct independent scientific research.

Based on the above facts and in accordance with the requirements specified in Article 187 paragraphs 1-2 and Article 190 paragraph 3 of the Law of July 20, 2018, on Higher Education and Science (Journal of Laws 2024 item 1572), I recommend that the doctoral dissertation of mgr Nishita Mandal entitled " Insights into Molecular Mechanism behind Rare Transport Processes in 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, 17. 01. 2025

Mgr. Stanislav Kozmon, Ph.D.