Institute of Chemistry SAS
Dúbravská cesta 5807/9, 845 38 Bratislava, Slovakia
Phone: +421 2 5941 0201 **Fax:** +421 2 5941 0222 **Email:** chemsekr@savba.sk **VAT ID:** SK2020894678

Review of doctoral thesis

Title: Molecular modeling of structure-dynamics-function relationships in proteins
Author: mgr inż. Bartłomiej Surpeta
Promotor: dr hab. Jan Brezovsky, prof. UAM

The formal page of the dissertation:

The thesis is presented as a collection of four 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 structure-dynamics-function relationship in proteins. In the first part this relationship is heavily studied using the molecular dynamics approach o selected enzymes. The two penicillin G acylases from *Escherichia coli* (ecPGA) and *Achromobacter* spp. (aPGA), and *Pseudomonas aeruginosa* acyl-homoserine lactone acylase (paPvdQ) have been chosen as a models for this study. Selected enzymes play a crucial role in the quorum sensing of the bacteria. The structure dynamics were studied employing molecular dynamics (MD) simulations of the free enzymes and their complexes with two signalling molecules of different lengths. The two step catalytic mechanism of the studied enzymes was followingly studied by the quantum mechanics/molecular mechanics (QM/MM) MD simulations. Obtained results pointed out on the differences between the enzymes in substrate binding and catalytic mechanism. Results were published in ACS Catalysis journal.

Within the second part of the thesis the comparison of the different approaches and methodologies used for the protein dynamics studies are compared and discussed in manner of dynamics-function relationship. Within the discussion clearly identifies the advantages and disadvantages of the discussed approaches. Authors also suggest the improvements of the procedures used in the *in silico* approaches for structure-dynamics studies, protein design and engineering. This part was published as the review article in International Journal of Molecular Sciences.

Third part of the presented results is dedicated to the developed computational tool named TransportTools. The tool is a specific library for high-throughput analyses of biomacromolecules structural dynamics responsible for the ligand transport and binding. The PhD candidate actively participate on the tool development especially to the user testing and debugging. Such tool is very useful tool for the computational chemists worldwide. The tool capabilities were published in the Bioinformatics journal.

In the last fourth part of the results the rational engineering of the penicillin G acylase binding pocket is discussed. The research is focused on biotechnologically optimized penicillin G acylase from Escherichia coli (ecPGA), which has quorum quenching activity on small signalling molecules. Within the research several different mutants of ecPGA were modelled and studied employing the computational chemistry tools. Several triple-point mutants were identified with slightly different substrate preference for the C8, C10, or C12 derivates of HSL (L-homoserine lactone) molecules. Designed triple-point mutants were prepared experimentally, their activity was examined and compared with the computational predictions. Observed experimental results were in good correlation with the computational predictions. The PhD applicant showed in this part that the used procedure is very robust and can predict the activity of the engineered enzymes. This work has been published on bioRxiv for now.

The PhD candidate published altogether six publication in highly impacted journals as *ACS Catalysis*, *Bioinformatics* or *Briefings in Bioinformatics*, where two of them are review articles. Four of the 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 protein engineering of the different enzymes.

Comments and questions:

1. The PGAs enzymes which were able to catalyse C12 derivatives of HSL are also able to handle HLS molecules with shortest acyl length? If so, what would be the shorter acyl length?
2. Would it be possible to design the "universal" PGA enzyme capable hydrolyse the derivatives with the acyl lengths from C6 to C12?
3. Is there any experimental validation of the PGA reaction mechanism?
4. What do you think how could influence the reaction mechanism model the change of the QM method from semi-empirical PM6-D to DFT?
5. During the catalytic reaction of the paPvdQ the His23β is included in the stabilization of the substrates where as in case ecPGA the Gln23β is included. What do you thing how could influence exchanging the His23β for Gln in paPvdQ and vice versa in ecPGA the enzyme activity?
6. The measured pH optima of the ecPGA and aPGA was 7 and 8, respectively. The zwitterionic state of the Ser1β is required for the catalytic reaction, however, according to tabulated values the pKa of the serine hydroxyl group is 13. Can you say, according to the performed computational experiments, what is helping to stabilize the zwitterionic state of the Ser1β?
7. In the "Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules" publication I miss the procedure of the "reactive stabilization score" calculation. Could you explain how this score is calculated?
8. In the Figure 4 of the same publication is table with calculated reactive stabilization score values, however colouring of the table do not correspond to the ranges presented in the figure description. Could you please explain this table?
9. What is the current state of the publication published in bioRxiv?

Chemický ústav Slovenskej akadémie vied, v. v. i.
Dúbravská cesta 5807/9, 845 38 Bratislava
Telefón: 02 5941 0201 Fax: 02 5941 0222 Email: chemsekr@savba.sk IČO: 00166618

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 protein engineering, but also have a methodological significance for the application of sophisticated theoretical methods, which is also indicated by the number of works published by the PhD candidate.

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. Due to the significant contribution to the field of *in silico* protein engineering I recommend the presented dissertation thesis of mgr inż. Bartłomiej Surpeta to the award of a distinction.

Based on the above facts, I recommend that the doctoral dissertation of mgr inż. Bartłomiej Surpeta entitled " Molecular modeling of structure-dynamics-function relationships in proteins " was accepted for defence and after its successful completion, the applicant was awarded a degree:

Philosophiae Doctor – PhD.

In Bratislava, 07. 08. 2023

Mgr. Stanislav Kozmon, Ph.D.