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Jerzy Haber Institute
of Catalysis and Surface Chemistry
Polish Academy of Sciences

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Kraków, 5 August 2023

Referee report for a PhD thesis submitted

by MSc Bartłomiej Surpeta

entitled „Molecular modeling of structure-dynamics-function relationships in proteins"

prepared under supervision of dr hab. Jan Brezovsky, prof. UAM

Introduction

This PhD thesis is a collection of four thematically coherent articles supplemented with: a list of publications, abstracts in Polish and in English, a list of used abbreviations, an introduction treating about structure-dynamics-function relationship in proteins, a presentation of the key results from the articles in an appropriate context, conclusions, a bibliography, and co-author statements about their contributions to the articles.

The introduction offers a thorough review of the development of our current understanding of the relationship between the structure and function of proteins and the role played by their dynamic nature. Here, landmark experimental and computational works are discussed to present their contribution to the field, as well as strong and weak sides of various research methods used to study protein structure and dynamics. The provided description of structure-dynamics-function relationships for enzymes, very neatly illustrated in Figure 1 (page 13), sets the stage for the presentation of the key results of the doctoral research, which are presented in 13 page long chapter entitled "Summary of the doctoral research".

The articles included in the thesis are indeed thematically coherent. Two of them (article 1 and 4) treat about the role of protein dynamics for enzymatic activity of native and rationally engineered quorum quenching hydrolases, article 2 is a review of computational approaches that include protein dynamics in protein design and engineering, whereas article 3 presents the TransportTools library useful for analysis of molecular

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dynamics trajectories. Thus, the protein dynamics is the common denominator for all four articles. Significantly, in three publications Mr. Surpeta is the first (shared) author, which together with the analysis of the co-author statements clearly shows that the computational study on quorum quenching hydrolases (article 1 and 4), as well as the review of computational methods for protein design or engineering (article 2) were done primarily by Mr. Surpeta.

The research work presented in the thesis is very timely and highly important. The studied N-terminal serine hydrolases are capable to cleave N-acyl-homoserine lactones, which serve as quorum sensing mediators in gram-negative bacteria. Thus, these enzymes have the potential to become new antibacterial agents to be used in medical and non-medical applications, and, thanks to their extracellular action, it is expected that they will not elicit new bacterial resistance mechanisms, as commonly observed for antibiotics. Mr. Surpeta and co-workers performed in-depth investigations of the catalytic mechanism of these enzymes, paying particular attention to dynamical factors governing enzymatic activity and specificity, and subsequently used thus gathered knowledge to perform rational redesign of *E. coli* penicillin G acylase to endow it with activity towards several N-acyl-homoserine lactones. The computational methodology used by Mr. Surpeta and co-workers for enzyme redesign has no precedence in the literature.

General comments

The first scientific article from the collection is dedicated to a comparative study of three enzymes: paPvdQ, ecPGA and aPGA. A range of different simulations were performed, including MD simulations for free forms of enzymes, molecular docking, MD simulations for enzyme-substrate complexes and steered QM/MM simulations for the first two steps of the catalytic reaction. In MD simulations for enzyme-substrate complexes, the Authors observed that the catalytically competent configuration was better maintained by paPvdQ than ecPGA or aPGA, which would suggest the former enzyme is more active towards the studied N-acyl-homoserine lactones. On the other hand, the PMF profiles obtained for C06-HSL (Figures 5 and 6, top) suggest that ecPGA should be more active towards this substrate than the reference paPvdQ, as the computed barriers for

ecPGA are not higher than those obtained for paPvdQ. Could the Author speculate about the reasons behind this apparent discrepancy?

In discussion of this issue, experimental data on enzymatic activity (k_{cat}, K_M) of the compared enzymes would be very valuable. Those for ecPGA and aPGA were determined by co-authors and reported in the article, however, those for paPvdQ I could not find. Are they not available in the literature? These data could also help in judging the application potential as quorum quenching enzymes of ecPGA and its rationally designed variants. Could the Author search for these data for paPvdQ (or for similar enzymes) and, if successful, present them (together with those for ecPGA and aPGA) during the public discussion of the thesis?

A related minor remark; in publication 4, page 4 the Authors state: "Previously, we have shown that ecPGA possesses QQ activity towards short- to medium-long AHLs.[39]". As quorum sensing is a biological process, I would recommend not to equate quorum quenching activity with enzymatic activity towards AHLs, especially if the latter is rather modest.

Importantly, the computed PMF profiles clearly show that ecPGA is more active towards C06-HSL than C08-HSL, as was verified by experimentally determined k_{cat} and K_M values.

The knowledge on dynamical factors affecting ecPGA activity towards AHLs was subsequently used to rationally redesign the acyl-binding pocket, and the entrance to it, so that the ecPGA variants would display improved activity towards longer chain AHLs. For this purpose, the Authors (of publication 4) devised a novel in-silico protocol, which included, as a crucial step, extensive molecular dynamics simulations for enzyme-substrate complexes. Based on the MD results the ability of each tested variant to stabilize productive configuration of the complex was assessed, and this measure turned out to be a good prognostic for the experimental K_M value. I wonder if the observed correlation between K_M and stabilization of the productive configuration could be somehow rationalized on the basis of kinetic models of enzymatic reactions, e.g. Michaelis-Menten model? The three most promising variants were tested experimentally and they showed increased activity towards selected AHLs with longer chains, which is a genuine success.

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Minor typographical and stylistic errors noticed

Even though the thesis has been written very meticulously, a small number of minor mistakes / stylistic errors has been noticed and they are listed here.

- page 10: "HDX-MD" → „HDX-MS"
- page 11: „... lowering the barrier to reaction activation ..."
- page 14: „McCannon" → 'McCammon"
- page 16: „... although it lacks biotechnological potential because it is not used in large-scale processes." - by this argument none enzyme would ever have biotechnological potential, as sufficiently back in time it was not used in any process
- page 24: „... membrane-based fields such as industry, biotechnology, and wastewater treatment." → maybe „membrane-utilizing"
- page 25: „unpredictable polar groups"
- page 26: „synergistic agreement"
- page 29: „increasingly approaching"
- page 34, reference [78]: misspelled surneme of V. Stepanek
- article 1, page 6369: "... because Ala69β sampled catalytically suboptimal side-chain conformations ..." - shouldn't it be "Asn241β?
- article 4 page 7: "gradually decreasing the **distances** that restraint was applied in stage (iii) from 25 to 0 kcal/molA^2"
- article 4 page 10: "hot-spot resides" → "hot-spot residue"
- article 4, page 12 / Figure 4 page 13: "... specially the LSF mutant with seemingly similar properties but lacking the improvement for C10-HSL" – but on Figure 4 LSF/C10 box is green, which indicates improvement.

Conclusions

My above-listed comments to the thesis concern only very minor issues, mostly related to the way the results have been presented, and very few typographical errors, and they do not diminish my high assessment of the work presented in the thesis and the thesis itself. The thesis is very clearly written, the conclusions drawn from the results are sound and very well supported by the data. The results presented in the thesis have been published in international journals of high standard of peer review (3 published papers), which means they have already been positively assessed by scientific reviewers.

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The Author reviewed and used a range of state of the art computational methods, and contributed to the development of a new analysis tool, to perform an in-depth study of the reaction mechanism and dynamical features affecting enzymatic activity and specificity for highly interesting enzymes with antimicrobial potential. Moreover, in my view the Author proved with his thesis that he can formulate valid scientific questions and then, by adopting appropriate research methods and collaborating with other research groups, find answers to them.

Hence, I conclude that the thesis presented by Mr. Bartłomiej Surpeta meets all the requirements for doctoral dissertations included in the Act "Ustawa z dnia 20 lipca 2018 roku Prawo o szkolnictwie wyższym i nauce (Dz.U. z 2022 r. poz. 547)" and I submit the application to the Scientific Discipline Council, Biological Sciences, Adam Mickiewicz University Poznań for the admission of Mr. Bartłomiej Surpeta to further stages of the doctorate. Moreover, taking into account the high scientific value, quality and novelty of the research done by Mr. Surpeta, I propose that his doctoral dissertation be distinguished.

Prof. dr hab. Tomasz Borowski

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