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Halle (Saale), 26. Februar 2024

The review of doctoral thesis - mgr inż. Maria BZÓWKA

Dear dr inż. Bartosz Kowalski,

I am writing to provide my evaluation report on the PhD thesis submitted by **mgr inż. Maria BZÓWKA** titled "*Analysis of Molecular Aspects of Proteins Regulation Considering Water Molecules as A Potential Mediator in Intermolecular Interactions.*" As per your request, I have thoroughly reviewed the thesis and have prepared a detailed assessment for your consideration.

Please find attached my comprehensive evaluation report for detailed insights into each of these aspects. Should you require any further clarification or additional information, please do not hesitate to contact me.

Thank you for entrusting me with the evaluation of this thesis. It has been a privilege to contribute to the academic assessment process at the Silesian University of Technology. I commend the author for their diligent work and look forward to seeing the continued development of their research.

Yours sincerely,



Dr. Mehdi Davari Dolatabadi

Reviewer Report for PhD Thesis Evaluation

Thesis Title: Analysis of Molecular Aspects of Proteins Regulation Considering Water Molecules as A Potential Mediator in Intermolecular Interactions

Candidate: mgr inż. Maria BZÓWKA

Institution: Silesian University of Technology

Date of Submission: Dec 2023

Summary of Evaluation:

The doctoral dissertation presents a comprehensive exploration of water molecule dynamics within biological systems, employing mainly AQUA-DUCT software and computational methodology. The thesis investigates the critical role of water molecules within proteins, focusing on three relevant areas: *drug design*, protein regulation and engineering, and *enzymatic reactions*.

- Regarding *drug design*, the author demonstrates the effectiveness of employing a combination of small-molecule tracking and local-distribution methodologies to elucidate variations in internal pocket dynamics within macromolecules. This approach facilitates the identification of potential binding sites for ligands across various targets, including SARS-CoV-2 main protease and human soluble epoxide hydrolase (hsEH). Additionally, the thesis explores the application of these techniques to evaluate the off-target risk for SARS-CoV-2 main protease and other proteases.
- In the field of *protein regulation and engineering*, the dissertation showcases how tracking water molecules during molecular dynamics simulations provides insights into tunnel networks and transportation phenomena inside proteins. Specifically, the analysis focuses on enzymes from the soluble epoxide hydrolase subfamily, establishing connections between enzyme structure, tunnel networks, and evolutionary patterns. Furthermore, a comparative study between small-molecule tracking and geometry-based approaches for tunnel detection and analysis is presented.
- In the context of *enzymatic reactions*, the thesis highlights the utility of combining small-molecule tracking and local-distribution methodologies to elucidate the diverse roles of water molecules throughout specific reaction cycles. A notable example includes the investigation of the proteolytic cleavage of the Z-loop in TLR8 by furin protease, where water molecules are involved in catalytic functions, stabilization of intermolecular interactions, and mediation processes such as proton transfer and dissociation.

In summary, the PhD thesis of Maria Bzówka presents original research results of significant importance for understanding the role of water in macromolecules. The findings contribute substantially to our understanding of water molecule dynamics in proteins, shedding light on the role of water as a crucial factor in protein structure, dynamics, and function. Moreover, the proposed strategy offers a promising avenue for advancing our comprehension of protein functions and presents promising approaches for drug design, protein engineering, and enzymatic reaction studies. The applied methodology demonstrates potential applicability to other challenging proteins. Additionally, the thesis is well-written, well-structured, and characterized by a comprehensive literature review and robust methodology. Maria Bzówka's numerous publications in peer-reviewed journals further underscore the impact and substantive contributions of her work to the field, warranting a PhD award without hesitation. These results have the potential to contribute to hypotheses concerning tunnel evolution in proteins, although further investigation into the generality of these hypotheses remains a topic for future study. **Overall, the dissertation significantly advances our understanding of water molecule dynamics in proteins, and I recommend unequivocally that the candidate be awarded the doctoral degree.**

Reviewer Report for PhD Thesis Evaluation

Here is my detailed evaluation report on PhD thesis:

1. Abstract and Introduction

The **abstract** of the thesis adheres to the established standards for a doctoral dissertation. It is well-structured, logically organized, and exhibits clarity, ensuring an accurate reflection of the entire work and its outcomes. The abstract presents the essence of the research, providing a clear overview that enables readers to grasp the scope and significance of the study.

In the concluding section of the abstract, I recommend adding a paragraph that discusses the general impact of the research and any advancements made in the field because of the findings presented in the thesis. This paragraph could also reflect on any valuable lessons learned throughout the research process. By highlighting the broader implications of the study and reflecting on its significance in advancing the domain, the thesis will provide a more comprehensive and insightful abstract.

In the **Introduction** section, the author lays the groundwork for the research problem, presenting the motivations and objectives and provides its relevance to the field. The introduction is well-structured, guiding the reader through the conceptual landscape and establishing a solid foundation for the investigation. Particularly noteworthy is the inclusion of philosophical underpinnings and the creation of the origin and evolution of the research idea, which enriches the narrative and contextualizes the study within a broader intellectual framework.

However, a potential area for enhancement lies in the discussion of the role of water in protein folding and its implications for protein stability. While the introduction sets the stage for the research, a deeper exploration of the interplay between water molecules and protein structure, as well as the underlying thermodynamic principles, governing protein stability, and related time scales for the protein function and various aspects of hydration simulations (hydration layer and bulk water) would provide valuable context and deepen the reader's understanding of the research problem.

2. Literature Review

The Literature Review section provides a detailed exploration of relevant theoretical frameworks, methodologies, and prior research, offering readers a solid foundation for understanding the context of the study. Overall, the section demonstrates a good understanding of the existing literature in the field.

Nonetheless, upon careful examination, there are areas where the literature review could be enhanced to provide a more comprehensive understanding of the role of water in biological systems. Specifically, there is an opportunity to go deeper into key factors related to modeling water's role in biological processes. Addressing these aspects would provide valuable insights for the readers. I recommend augmenting the literature review with a brief overview of state-of-the-art water models. This could include a comparative analysis of the structural and dynamic properties of commonly used models, as well as an assessment of the quality and limitations of different water models. Additionally, discussing the recent advances in polarizable and flexible water models, along with the associated drawbacks and challenges in their development, would further enrich the discussion and provide readers with an understanding of the topic. These suggestions are minor in nature and can be easily addressed. Integrating these additional elements into the literature review would not only enhance the depth and breadth of the discussion but also contribute to the overall rigor of the presented work.

3. Research Methodology (Information about the conditions of the computational experiments)

Reviewer Report for PhD Thesis Evaluation

The employed Research Methodology demonstrates a rigorous approach in addressing the research objectives, includes thorough discussions of data collection procedures, sampling techniques, and analysis methods (described in the original published works). The author has provided a clear outline of the steps undertaken to achieve the stated research aims.

The inclusion of detailed discussions on statistical analysis of potential numerical errors would further enhance the methodological rigor and ensure transparency and reproducibility in the research process. While the chosen research methodology is robust, addressing this aspect would provide valuable insights into the potential limitations and uncertainties associated with the data analysis process. It is presumed that such discussions may be included in the related publications; however, incorporating a brief overview in the thesis itself would strengthen the coherence and completeness.

4. Results and Discussions

The aim of the work (introduced on p. 28) has been to study the impact of water on proteins (developing and employing methods in AQUA-DUCT software) in different topics including computational drug design-related studies for different molecular targets. The thesis showed that AQUA-DUCT methods can be successfully applied in several different areas as demonstrated by the variety of the subjects addressed. The results and discussions are presented in different sections. The application of AQUA-DUCT software in areas including structural and functional analyses of macromolecules, the evolution of tunnels, and computational drug design-related studies has been successfully demonstrated.

As a side note, it is expected that the accuracy of simulations in this context to be highly sensitive to the choice of water model used. The author should generally provide commentary on the extent to which the selection of a water model or molecular dynamics simulation time may alter the behavior of complex networks of transport tunnels, which are critical for the function of many enzymes with buried active sites. This additional discussion would enhance the methodological rigor of the study.

As a minor comment, in the list of publications included in the doctoral thesis (page 5), it would be beneficial to specify the contributions made by the author to each publication. This information could be incorporated on page 5 of the thesis, providing clarity regarding the author's role and contributions to the research presented in the publications.

6. Conclusion

The Conclusion section of the thesis serves as a summary of the key findings and contributions, effectively summarizing the essence of the research and its alignment with the stated objectives. The author presents the main outcomes of the study, providing readers with a clear understanding of the significance and implications of the research findings.

Furthermore, the conclusion provides closure to the study, offering a narrative that brings the research journey to a satisfying conclusion. The inclusion of recommendations for future research or practical applications is particularly commendable, as it underscores the potential avenues for further exploration and underscores the practical relevance of the study.

7. Writing Style and Presentation

The writing style of the thesis is characterized by clarity, coherence, and an engaging narrative. The author effectively communicates complex ideas and arguments, demonstrating clear language and an ability to convey information in a clear and accessible manner. Particularly noteworthy is the engaging storytelling employed in the introduction, which serves to captivate the reader's interest and set the stage for the research journey (e.g. different theories for evolution).

Reviewer Report for PhD Thesis Evaluation

The overall appearance of the thesis is appropriate, with no obvious grammatical or spelling errors. The text is written in a coherent and proper style, maintaining a consistent tone and flow throughout. Citations are accurately presented, ensuring the integrity of the scholarly discourse. The author demonstrates a command of the relevant literature and other sources, effectively integrating existing knowledge into the research narrative. The work is well-presented, with tables and diagrams used to enhance understanding and illustrate key concepts. These visual aids complement the written text and contribute to the overall clarity and effectiveness of the presentation. However, there is room for improvement in the references of concerned literature, which could be expanded to cover a broader spectrum of relevant sources to enrich the context (see comment above for the introduction).

8. Overall Evaluation

The dissertation represents an original contribution to the field of protein modeling, showcasing the candidate's clear and well-defined position within the research domain. The researcher demonstrates a high level of competence in the subject matter, evidenced by the rigorous methodology employed and the depth of analysis presented throughout the thesis. The language and style of the dissertation are fitting for scientific research work.

Moreover, the candidate's track record of publication in prestigious journals within the field underscores the significance and impact of their research findings. These publications serve as a testament to the scholarly merit and relevance of the dissertation, further validating the candidate's expertise and contributions to the field of protein modeling and design.

Considering the originality, rigor, and scholarly impact demonstrated in the dissertation, coupled with the candidate's evident proficiency in the subject matter, I am confident in affirming that the thesis meets the standards required for the award of a PhD degree.

9. Summary and Recommendation

The thesis demonstrates a clear and coherent presentation of original research findings, supported by a rigorous methodology and a comprehensive literature review. The thesis fulfills all the criteria and standards expected of a doctoral dissertation, showcasing originality, rigor, and scholarly excellence. Maria BZÓWKA's work significantly advances the understanding of water molecule dynamics in proteins, making substantial contributions to the field of biomolecular science. Moreover, Maria BZÓWKA's track record of publications in reputable journals further confirms the significance and impact of her research contributions. Based on the comprehensive evaluation conducted, I recommend the PhD thesis of Maria BZÓWKA for acceptance.

10. Declaration

I declare that this review is my original work and is based solely on my evaluation of the PhD thesis titled "Analysis of Molecular Aspects of Proteins Regulation Considering Water Molecules as a Potential Mediator in Intermolecular Interactions". I have no conflicts of interest to disclose regarding this review.

Reviewer Name: Dr. Mehdi Davari Dolatabadi

26.02.2024