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Review of the PhD Thesis of M. Sc. Sylwia Czach

Title: "Computational Modeling of Structural Changes in Selected Optically Regulated Proteins and Ligands"

1. Character and Subject Matter of the Thesis

The doctoral dissertation of M. Sc. Sylwia Czach concerns the use of computational methods to study light-induced structural changes in proteins and the interaction of photoactive ligands with receptors. The topic lies at the border of computational biophysics, photobiology, structural bioinformatics, and molecular pharmacology.

The dissertation was prepared at Nicolaus Copernicus University in Toruń, at the Department of Biophysics, Institute of Physics, Faculty of Physics, Astronomy and Informatics. The main supervisor of the thesis is prof. dr hab. Wiesław Nowak, and the auxiliary supervisor is dr inż. Jakub Rydzewski.

The thesis focuses on three main systems. The first is bacteriophytochrome, used to study signal transfer after photoisomerization of biliverdin. The second is Photoactive Yellow Protein, used as a model system for machine learning analysis of protein dynamics. The third is the P2X7 receptor, where the Candidate explores whether photoactive ligands could be used for light-controlled regulation of a medically relevant receptor.

The dissertation has a publication-based character and is built around papers and manuscripts. It contains a general introduction, three research chapters, a summary, and a bibliography. Chapter 2 is based on a publication in *The Journal of Physical Chemistry B* from 2022. Chapter 3 is based on a manuscript in preparation. Chapter 4 is based on a publication in *Cellular Signalling* from 2023. The publication list

also includes a first-author paper in *Physical Biology* from 2025, which is not included as a separate thesis chapter.

The subject of the thesis is important and timely. It combines basic questions about how photoactive proteins work with more applied questions related to photopharmacology and drug design. The range of methods is broad and includes molecular dynamics, enhanced sampling, machine learning, homology modeling, and molecular docking.

The biological systems studied in the thesis are diverse: bacteriophytochrome, Photoactive Yellow Protein, and the human P2X7 receptor. This broad scope is justified by the common theme of optical regulation and structural response, although the links between the chapters could have been stated more explicitly.

2. Structure, Language, and Clarity

The thesis is written in English and has a clear general structure. The introduction gives a broad overview of photoactive proteins and computational methods used to study them. A particularly strong aspect of the dissertation is the quality of the graphical presentation. The figures are clear, visually attractive, and professionally prepared. They support the text very well, especially in the sections showing protein structures, photocycles, free-energy landscapes, and structural comparisons.

The descriptions of computational methods are detailed and show that the Candidate understands the theoretical background of the techniques used in the thesis, especially molecular dynamics, enhanced sampling, and machine-learning methods.

The language is generally understandable and appropriate for a scientific dissertation. Minor grammatical and typographical errors are present, but they do not affect the overall clarity of the thesis.

Since the dissertation is partly based on multi-author publications, it is important to clearly define the Candidate's own contribution. Two of the three main research chapters are based on papers in which the Candidate is the third author. This is acceptable, because computational biophysics is often collaborative. However, the thesis should make it clear which simulations, analyses, and interpretations were performed by the Candidate herself and which were part of the broader team effort.

3. Scientific Evaluation

The thesis presents a broad set of computational studies. It shows that the Candidate has experience with several important methods used in computational biophysics and structural modeling.

3.1. Bacteriophytochrome - Chapter 2

Chapter 2 presents an advanced computational study of the bacteriophytochrome–biliverdin complex. The use of enhanced sampling to explore free-energy landscapes and metastable states is well justified, and the analysis of possible signal-transfer routes from the chromophore region to distant parts of the protein is interesting.

This chapter is methodologically advanced. However, the Candidate is the third author of the underlying publication. According to the contribution statement, her role included literature review, analysis and discussion of results, and writing. It is not clearly stated whether she personally set up and performed the enhanced sampling simulations. Therefore, her exact technical contribution should be clarified during the defense.

I would also like to comment on one central claim. The thesis abstract states that “previously unknown metastable states were revealed, and two signal transduction pathways from the chromophore-binding pocket to the remote protein domains were identified.” The first part of this statement is generally supported by Chapter 2, which provides evidence for multiple metastable states of the Pr and Pfr conformers and for dark thermal reversion involving heterogeneous Pfr chromophore conformations. However, the second part should be formulated more cautiously. The two signal-transduction routes discussed in the chapter are described in the thesis itself as routes previously found by X-ray structures and NMR studies. Thus, the computational work appears to provide atomistic and thermodynamic support for these pathways, rather than identifying them de novo.

3.2. Photoactive Yellow Protein - Chapter 3

Chapter 3 is, in my opinion, the strongest part of the thesis in terms of the Candidate’s independent work. It applies the Spectral Map method to Photoactive Yellow Protein, using a modern machine-learning approach to construct slow collective variables from molecular dynamics data.

The Candidate is the first author of the related manuscript in preparation. Her declared contribution includes force-field parameterization, molecular dynamics simulations, analysis, visualization, and writing. This shows broad and direct involvement in the project.

The application of this method to PYP is well justified, as this protein is a classic model system for photoinduced structural changes. The integration of machine learning provides significant methodological novelty.

The main limitation is that the manuscript is still in preparation and has not yet passed external peer review. The results also appear partly preliminary, especially because reversible transitions could not be simulated. It would be useful to explain whether this was due to insufficient sampling, force-field limitations, the Spectral Map method, or the intrinsic complexity of the PYP photocycle.

Despite these limitations, this chapter gives the strongest evidence that the Candidate can independently carry out a computational project.

3.3. P2X7 Receptor and Photoactive Ligands - Chapter 4

Chapter 4 moves toward structural bioinformatics, molecular docking, and photopharmacology. The biological motivation is interesting, because the P2X7 receptor is relevant to glioma biology, and light-controlled ligands could in principle offer spatial and temporal control of receptor activity.

The chapter has a broad screening character. The Candidate docks several groups of compounds, which is an understandable design, as it builds a hierarchy of comparison: from native and reference ligands to candidate photoswitchable molecules.

However, the logic of this multi-step screening could have been presented more clearly. At times, the large number of docked compounds makes the chapter difficult to follow, and the discussion becomes mostly score-based. It is important to emphasize that docking scores are useful for preliminary ranking, but they do not have sufficient physical accuracy to be interpreted as direct measures of biological activity, especially for a complex, gated ion channel.

The main limitation of this chapter is that docking is a static method. It does not fully account for receptor flexibility, the membrane environment, or the complex dynamics of the P2X7 trimer. For this reason, I interpret Chapter 4 as a promising exploratory screening study rather than as a final demonstration of optical control. The chapter would be significantly stronger if the most promising hits had been followed by molecular dynamics simulations to assess the stability of cis and trans binding modes or to explore possible allosteric effects on receptor opening.

3.4. General Assessment of the Candidate's Contribution

The publication record is solid and shows that the Candidate worked in several computational projects. The strongest evidence of independent work comes from the first-author PYP manuscript and the first-author *Physical Biology* paper listed in the dissertation.

The *Physical Biology* paper is not included as a research chapter, which is understandable because it does not concern optical regulation. However, it is relevant to the overall assessment of the Candidate's computational skills, as it involves molecular docking, molecular dynamics simulations, and analysis of substrate-binding dynamics. Thus, it supports the view that the Candidate has practical experience in computational studies of protein–ligand systems.

Taken together, the listed publications and the material presented in the dissertation provide sufficient evidence of the Candidate's scientific development and practical computational skills. Although part of the publication portfolio is still at the manuscript stage and has not yet undergone external peer review, the presented material is sufficient to assess the Candidate's contribution positively.

4. Remarks and Questions for Defense

I would like to ask the Candidate the following questions:

1. In Chapter 2, concerning bacteriophytochrome, you are the third author of the underlying publication, and your contribution statement mentions analysis and discussion of the results but does not explicitly mention performing the VES simulations. Could you precisely define your role in this project? Did you set up and run the enhanced sampling simulations, or did you analyze trajectories generated by others?
2. In Chapter 3, concerning PYP, you mention that the findings are preliminary and that simulating reversible transitions was not possible. What were the specific bottlenecks? Was it a limitation of the Spectral Map method, force-field accuracy, or sampling time? How would you overcome this in future work?
3. In Chapter 3, you selected 45 mechanistic descriptors (pairwise distances) for the Spectral Map input, stating they explain most of the variance. Could you clarify the exact workflow for this selection? Why was the number fixed at exactly 45, and how sensitive are the resulting collective variables to this choice?

4. In Chapter 4, you compared your homology model of P2X7 with AlphaFold2 predictions, noting significant inconsistencies in the intracellular region. Given the rapid development of AI-based structure prediction methods, including AlphaFold 3, how would you assess the reliability of your docking results, which targeted the extracellular pocket, in light of these structural uncertainties?
5. The thesis title emphasizes “structural changes”. However, Chapter 4 relies mainly on static docking. How might the flexibility of the P2X7 receptor, not accounted for in static docking, influence the binding of photo-switchable ligands, particularly in their different isomeric states, cis and trans?

5. Final Conclusion

The doctoral dissertation of M. Sc. Sylwia Czach presents a broad and relevant application of computational methods to biological systems connected with optical regulation. It combines studies of native photoactive proteins with an exploratory photopharmacological approach to receptor regulation, giving the thesis both fundamental and applied value.

The Candidate demonstrates knowledge of modern computational biophysics and practical experience with molecular dynamics, enhanced sampling, machine learning-based analysis, homology modeling, and molecular docking. The dissertation and the publication record together document her scientific development in molecular modeling of protein–ligand systems and light-regulated biological processes.

In my opinion, the thesis contains original scientific material and shows that the Candidate has acquired the knowledge, methodological competence, and research maturity expected at the doctoral level.

In light of the above, I state that the presented PhD thesis **meets the requirements for a doctoral degree** as specified in the Act on Higher Education and Science. **I recommend its acceptance and admission of M. Sc. Sylwia Czach to the public defense.**

Electronically signed by

Sebastian Kmiecik; Uniwersytet Warszawski

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