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Review of the doctoral dissertation of M.Sc. Saruti Sirimatayanant

Entitled

Two-Photon Absorption in Isolated Retinal Protonated Schiff Base and Rhodopsin Studied by QM/MM methods

Review basis: Decision No 135/RDND10/2026 of the Discipline Council for Chemical Sciences at the Wrocław University of Science and Technology of appointing reviewers of the dissertation.

The doctoral dissertation entitled *“Two-Photon Absorption in Isolated Retinal Protonated Schiff Base and Rhodopsin Studied by QM/MM Methods”*, authored by M.Sc. Saruti Sirimatayanant, was prepared under the supervision of Prof. Tadeusz Andruniow, Ph.D., D.Sc. The research was conducted at the Institute of Advanced Materials of Wrocław University of Science and Technology. The topic of the dissertation falls within a research area that has been among the supervisor’s long-standing scientific interests.

Rhodopsin is a light-sensitive G protein-coupled receptor found in the rod cells of the retina, where it plays a crucial role in the visual process. Its key component is 11-cis-retinal, a vitamin A-derived aldehyde that serves as the chromophore and is covalently bound within the protein structure. Research into the molecular mechanisms underlying vision remains an important and highly relevant field of study. Consequently, the subject of this doctoral dissertation is both timely and scientifically significant.

In the dissertation, the author carried out a systematic investigation of the effects of structural modifications along the retinal polyene chain on its two-photon absorption properties. Particular attention was devoted to the influence of methylation and demethylation, that is, alterations in the methyl-group substitution pattern, on the chromophore’s geometry,

excitation energies, and other photophysical characteristics. Through these studies, the author sought to deepen our understanding of the structure-property relationships governing the behavior of retinal derivatives.

The dissertation is based on two research articles published in the prestigious journals: *The Journal of Chemical Physics* and *The Journal of Chemical Theory and Computation*. Thus, it satisfies the fundamental requirement concerning the scientific achievements forming the basis of a doctoral dissertation.

General description

The doctoral dissertation prepared by Saruti Sirimatayanant is a 148-page study organized into seven chapters, the last of which contains supplementary materials. The dissertation follows a classical structure. In the introductory chapter, spanning more than thirty pages, the author presents the historical development of research on rhodopsin and discusses the role of these photoactive proteins in optogenetics and medicine. The introduction also provides a detailed description of the chromophore, retinal, and explains the influence of the protein environment on its spectral properties, while outlining the physical principles underlying the two-photon absorption process.

The following chapter contains a concise presentation of the objectives of the study. The computational methodology adopted in the dissertation is described in Chapter 3. The subsequent two chapters summarize the results obtained by the author and constitute the most substantial part of the dissertation. Over the course of 65 pages, the author presents and discusses results obtained both in the gas phase (Chapter 4) and in the protein environment (Chapter 5).

The dissertation concludes with a concise summary and a presentation of the main findings and conclusions, provided in a four-page chapter. The main body of the thesis is preceded by Acknowledgements, a List of Abbreviations and Symbols used throughout the work. The dissertation is complemented by a bibliography and a list of the author's scientific publications and achievements. The bibliography of the doctoral dissertation is extensive and comprises 218 references, including 50 publications published within the last decade. This demonstrates that the research topic is very hot and that the doctoral candidate has engaged in addressing fundamental scientific problems.

The main achievements

The Author divided the study into two main stages: simulations of the isolated chromophore in the gas phase and simulations in the full protein environment. All computational models

employed in the dissertation were constructed to enable the achievement of the stated research objectives.

For the gas-phase investigations, the Author adopted a strategy of simplifying the chromophore structure (retinal). This approach is fully justified given the high computational cost of coupled-cluster methods. Three groups of structures, comprising a total of 16 computational models, were examined. The reduced PSB3 models (containing three conjugated double bonds in the polyene chain), m-PSB3 (the methylated variant of PSB3), PSB4 (containing four conjugated double bonds and one methyl group), and dm-PSB4 (the demethylated variant of PSB4) were used in the benchmark calculations. Models from the PSB5 family were employed to investigate the effect of demethylation/methylation and included singly, doubly, and triply methylated chromophore models. Full RPSB models were used to assess how the introduction of the ring moiety affects the spectra through complete π -electron delocalization. The protein-environment models were based on the crystal structure of bovine rhodopsin. The positions of crystallographic water molecules were optimized, and the protonation states of the protein were carefully verified. The entire system was then solvated in a box of water. This model served as the basis for the QM/MM calculations. For these calculations, the Author constructed a series of models, M1-M4, differing in the number of atoms included in the quantum region. The smallest of these models (M1) contained 77 atoms, whereas the largest (M4) comprised 148 or 152 atoms.

The sheer number of systems considered, together with the adopted computational methodology, clearly demonstrates that the Author undertook a highly demanding and computationally challenging research task.

Studies on the reduced benchmark models PSB3 and PSB4 (protonated retinal Schiff bases with a shortened polyene chain and lacking the β -ionone ring) enabled the Author to formulate a number of key conclusions regarding the accuracy of Coupled Cluster (CC) methods and time-dependent density functional theory (TD-DFT) in the description of nonlinear optical properties. The most important findings derived from these model systems concern vertical excitation energies, one-photon optical properties, two-photon absorption intensities, the influence of molecular size on the performance of selected methods relative to the reference approach, and the performance of various TD-DFT functionals in comparison with the reference method. These conclusions are highly significant for researchers involved in modeling the optical properties of complex systems, where the use of various approximations is often unavoidable. The Author subsequently relied on these benchmark investigations when studying the chromophore in its protein environment. In this sense, the benchmark studies proved highly useful and successfully fulfilled their intended purpose. The benchmark analysis demonstrated that nonlinear optical properties are extremely sensitive to the choice of density functional. Pure functionals (without exact Hartree-Fock exchange) and hybrids containing only a small fraction of exact exchange drastically underestimated signal intensities and failed to provide an accurate description of charge-transfer states. The Author showed that CAM-B3LYP and BHandHLYP were the most reliable functionals for describing two-photon excitations. The latter was subsequently employed in the QM/MM calculations

performed for the chromophore embedded in the protein environment. Particularly noteworthy are the conclusions drawn from the QM/MM calculations.

The Author emphasized the necessity of conformational sampling and ensemble averaging over molecular configurations. The use of a single, static molecular structure for the calculation of nonlinear optical properties is insufficient and may lead to misleading results. Averaging spectroscopic properties over a representative ensemble of snapshots extracted from molecular dynamics (MD) trajectories is absolutely essential for the reliable interpretation of spectroscopic data.

The Author demonstrated that the small M1 model is sufficient for the description of the S_1 state. The lowest excited state (S_1) of retinal exhibits remarkable stability and is virtually converged already at the level of the smallest QM model (M1). At the same time, the Author demonstrated the dramatic influence of the embedding scheme on the nonlinear optical spectrum, particularly on the two-photon absorption (2PA) response. While the excitation energy of the S_1 state remains relatively stable regardless of the MM environment representation, the choice of embedding scheme has a profound impact on the calculated two-photon absorption intensity. Also noteworthy is the role of the β -ionone ring, whose inclusion leads to an approximately order-of-magnitude enhancement of the 2PA signal, as convincingly demonstrated in the dissertation.

In summary, the Author of the doctoral dissertation obtained a number of interesting and valuable results concerning both methodological aspects and more general physicochemical features of the phenomenon under investigation. These findings constitute a meaningful contribution to the understanding and computational modeling of nonlinear optical properties in retinal-based chromophoric systems.

Remarks and questions

During my review of the doctoral dissertation, I noted a number of observations, comments, and questions that I would like to address to the Doctoral Candidate. Some of these remarks are of a technical nature. Nevertheless, the quality of a dissertation is determined not only by its scientific content but also by the manner in which it is written and presented. A clear, well-structured, and carefully written dissertation facilitates the communication of scientific ideas and enhances the overall value of the work. Moreover, dissertations of this quality may serve as useful reference materials and educational resources for future members of a research group.

- The notation employed throughout the dissertation lacks consistency. In particular, the variables introduced in the displayed equations differ from those defined in the text. In such a way two distinct sets of symbols have been used for the same quantities.

- The dissertation is overburdened with abbreviations. The list of abbreviations contains nearly 100 entries. Sentences containing too many abbreviations are not reader-friendly and make the text more difficult to follow.
- The work presented by the PhD student is based on coupled-cluster (CC) theory and time-dependent density functional theory (TD-DFT). The introduction to these methods should be more comprehensive. At a minimum, the reader should be able to appreciate the differences between CC2, CCSD, and CC3. The cluster operator should also be formally defined. A proper connection to the Hartree–Fock reference orbitals would help the reader better understand the formalism.
- Three formalisms are mentioned in the context of two-photon absorption. The author should explain why quadratic response theory was chosen as the primary computational approach. Are there any alternative methods available in the literature for treating two-photon absorption?
- The Author should provide a more detailed description of the molecular dynamics (MD) simulations, focusing on their physical foundations rather than limiting the discussion to the computational details of specific software packages.
- Based on the calculations performed and the knowledge acquired during this study, can the Author venture a comment on why retinal, out of the vast pool of possible chromophores, was selected by nature through natural selection?
- A vast amount of data was generated during the PhD research. Have the resulting data especially trajectories been deposited in any specialized repositories used by the scientific community to ensure data availability and reproducibility of the results?

Final Remarks

My comments are primarily of a technical nature, and some of them stem from my scientific curiosity. However, they in no way diminish the scientific value of the doctoral dissertation under review. Having carefully examined the thesis, I have no doubt that it constitutes an original and significant scientific contribution within the discipline of chemical sciences.

The subject addressed in the dissertation is both challenging and inherently multidisciplinary, and it is likely to remain of considerable interest to the scientific community for many years to come. Through the work of Saruti Sirimatayanant, our understanding of rhodopsin and the retinal chromophore has been enriched by a number of novel and insightful findings. The author demonstrates that the theoretical description of two-photon absorption is considerably more demanding than that of one-photon absorption, owing to its pronounced sensitivity to both the size of the computational model and the choice of electronic-structure method. To address these challenges, the author conducted a comprehensive and technically demanding comparative analysis. The results obtained are not only relevant to the chemistry

of retinal-containing molecular systems but also have broader implications for the understanding of two-photon absorption processes in complex molecular environments.

In conclusion, the doctoral dissertation fully satisfies all requirements set forth in the Act of 20 July 2018, *Law on Higher Education and Science* (Poland). **I therefore recommend that the dissertation of Saruti Sirimatayanant be admitted to the subsequent stages of the doctoral procedure. Furthermore, provided that all formal requirements have been met, I consider the dissertation worthy of being awarded with distinction.**



Jacek Korchowiec