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A review of the doctoral Thesis by Saruti Sirimatayanant, *Two-Photon Absorption in Isolated Retinal Protonated Schiff Base and Rhodopsin Studied by QM/MM methods.*

The assessed dissertation was written under the supervision of Prof. Dr. Hab. Tadeusz Andruniów at the Institute of Advanced Materials of Wrocław University of Science and Technology. The doctoral Thesis presents theoretical research results on two-photon absorption in isolated retinal protonated Schiff base rhodopsin using coupled-cluster methods, TD-DFT assessment, de/methylation studies, and configurationally sampled QM/MM computational methods, establishing consistent relationships between molecular environment, excitation energies, dipole-based properties, and two-photon response.

The subject of the dissertation is highly topical, and the research is significant and desirable, both from the cognitive side and potential applications, e.g., near-IR two-photon activation of opsins for optogenetics and non-invasive imaging.

Saruti Sirimatayanant's doctoral Thesis was written in English and contains over 140 pages. The literature is extensive and well-matched to the issues presented. Much of the work has already been published in two papers: J. Chem. Phys., vol. 158, no. 9, p. 094106, 2023, and J. Chem. Theory Comput., vol. 20, no. 18, pp. 8118–8126, 2024, with Saruti Sirimatayanant as a first author.

The work begins with an unusually thorough, literature-driven introduction which situates retinal/rhodopsin in photobiology and optogenetics, reviews the physical basis of 2PA and its advantages over 1PA, and critically surveys the methodological state of the art: benchmark studies of CC2/CCSD/CC3 coupled cluster methods and TD-DFT functionals for 1PA/2PA, embedding schemes (ME/EE/PE/FPE), and prior QM/MM studies. It also identifies the specific gaps: no systematic coupled-cluster hierarchy benchmark for 2PA on rPSB, no systematic study of de/methylation on 11-



cis rPSB 2PA, and no systematic QM-region-size convergence study of rhodopsin 2PA. This gap analysis directly motivates the main objectives of the Thesis, presented in the second chapter. Chapter 3 is a self-contained theory chapter covering most of the methods used in the Thesis, including coupled-cluster and DFT, and, in detail, multiscale QM/MM and system partitioning methods. It contains a careful derivation of 1PA/2PA within response theory: linear, quadratic, and cubic response; the sum-over-states (SOS) formalism; the two-state model (2SM); and the deterministic conversion of the microscopic δ^{2PB} transition strength to the macroscopic σ^{2PB} cross-section (GM) with an explicit, clearly-stated line-shape convention.

The next two chapters present the main results obtained during the doctoral dissertation research. Chapter 4: gas-phase computational benchmarking and 2PA tuning, and Chapter 5: the Rhodopsin protein (QM/MM). The results presented have already been published in two articles. The presentation is comprehensive, clear, honest about its limitations, extremely careful about method validation, and internally consistent in its conclusions, which are summarized in the last chapter.

From a technical and linguistic standpoint, and, most importantly, on merit, the work is written at an excellent level, although the language is sometimes highly specialized.

At this point, I would like to make a few minor editorial and substantive comments:

- Unfortunately, there is no Abstract in the Thesis, and the two-page "Summary and Conclusions" (Chapter 6) does not substitute for it.
- Also, the title of subsection 3.1, "Schrödinger's Time-Independent Equation and the Hartree-Fock method," is slightly confusing because it also contains a description of correlated methods, including FCI.
- The electron density is correctly introduced as $\rho(r)$ but is then repeatedly and inconsistently written (pages 47-49) as the Latin letter p — including inside display equations (e.g., "a functional of $p^0(r)$ ")
- The sentence "...2nd and 3rd order variants of coupled cluster (CCSD, CCSD(dt), CC3)..." contains several confused statements:
 - There is an incorrect characterization of the coupled-cluster hierarchy (repeated also in the Introduction, §1.4.3, p. 30, p. 46), especially in the context of mixing the excitation rank of the cluster operator and perturbative order. ("2nd and 3rd order variants of coupled cluster (CCSD, CCSD(dt), CC3)") Could the Author explain the meaning of the "order" in this context (CC2, CC3)?
 - CCSD(dt) is not a real method name – what is the proper method name in this context?

- Could the Author define and characterize CC approximations used in the CCS, CCSD, CCSDT, CCSD(T), CC2, and CC3, focusing on the excitation rank, iterative and non-iterative corrections, and "orders"

The above comments are minor and do not affect my overall positive opinion of the Thesis.

In summary, Saruti Sirimatayanant proposed an original solution to a scientific problem. The Thesis establishes, for the first time, a validated and internally consistent computational protocol for predicting and rationally tuning two-photon absorption in retinal-based chromophores and in the full rhodopsin protein environment, and identifies the specific electronic descriptors and modeling decisions that govern 2PA rather than 1PA. The Thesis meets, and in several respects exceeds, the standard expected of a doctoral dissertation in computational chemistry. It addresses a nontrivial, timely problem (two-photon activation of rhodopsins for optogenetics/imaging), combines rigorous method validation with a genuine scientific result (2PA is tunable by methylation and predictable by the two-state model), and delivers a practical, caveated protocol for QM/MM 2PA studies of a full membrane protein.

Saruti Sirimatayanant has demonstrated excellent general knowledge and proficiency with advanced theoretical tools, including computer programs, as well as thorough analysis and elaboration of the results. It proves the doctoral student's ability to perform scientific work effectively.

In conclusion, I am pleased to state that the Saruti Sirimatayanant doctoral dissertation is a well-structured, methodologically rigorous, and coherently argued thesis combining gas-phase benchmark work with a polarizable QM/MM study of a complete protein. It clearly demonstrates the Author's high scientific competence in the chemical sciences. The quantity and quality of the results obtained, all already published in reputable journals, fully meet the substantive and formal requirements for doctoral dissertations. Therefore, I recommend that Saruti Sirimatayanant be admitted to the next steps in awarding the doctoral degree.

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Ireneusz Grabowski