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Review of the Ph.D. Thesis entitled "Two-Photon Absorption in Isolated Retinal Protonated Schiff Base and Rhodopsin Studied by QM/MM methods"

The Ph.D. Thesis I received to review has been realized by Mr. Saruti Sirimatayanant and prepared under the supervision of Professor Tadeusz Andruniów in the Institute of Advanced Materials of Wrocław University of Science and Technology. The dissertation consists of seven chapters followed by the list of references. Chapter VII, the last one, entitled as "Supporting information" is of more technical character and contains detailed results of calculations, collected in ten tables.

The dissertation begins with the lengthy introduction continued with the presentation of the research objectives shown in a concise way in chapter II. In chapter III the author discusses theoretical methods used in this work. He provides a sketchy characteristic of both the wave function based approaches (including a coupled cluster (CC) and a perturbation theory) as well as those based on the density functional theory (DFT). A more detailed analysis has been given to the multiscale approach engaging both quantum and molecular mechanics (QM/MM), presented in the last section of the chapter. Chapters IV and V provide results of calculations: the former focuses on both the CC and DFT results for the retinal models while the latter is devoted to the analysis of properties of the full rhodopsin protein. A summary of the results and conclusions are provided in chapter VI. The dissertation, written in correct English, is 148 pages long.

The results presented in the dissertation are a subject of two published papers authored by the Ph.D. candidate and his supervisor. They appeared

in the renowned journals: the first one in *Journal of Chemical Physics* and the other in *Journal of Chemical Theory and Computation*.

As it is indicated in the title of the Thesis the relevant research is focused on the studies of the selected protonated Schiff bases (PSB), derived from the well known rhodopsin chromophore and built around the retinal chain. The retinal protonated Schiff base (rPSB) is relatively large molecule containing over 20 heavy (C and N) atoms. Its molecular formula is $C_{20}H_{28}N$. The structure and properties of rhodopsin and its chromophore were a subject of many papers, the large part of them were devoted to the computational studies. What makes the current research different from the others is a focus on the theoretical description and calculations of spectroscopic parameters connected with the two-photon absorption (2PA) process.

It is commonly accepted strategy in the studies of rhodopsin and its chromophore that in order to make calculations more feasible one replaces the original retinal fragment by its models containing smaller number of carbon atoms. Thus, the real molecules on which a majority of the research is focused are two prototypes of the original chromophore containing three (PSB3, molecular formula C_5H_8N) or four (PSB4, molecular formula $C_7H_{10}N$) conjugated double bonds with the β -ionone ring and methyl substituents eliminated.

One of the objectives of the reviewed research reported in section 4.1 is an analysis of the performance of three selected coupled cluster schemes: CC2, CCSD and CC3. The excited state properties were obtained via linear or quadratic response theory. The CC calculations were carried out for two basis sets: one belonging to the Dunning system and known under the acronym aug-cc-pVDZ (23 functions per heavy atom) and the other, originating from Pople’s nomenclature, known as 6-31+G* (14 f.p.a). The results, obtained for the PSB3 and PSB4 and their methylated derivatives and shown in Table 1, indicate a quite satisfactory performance of the CC2 method which in many cases provides the results closer to the CC3 (considered here as a reference) than the CCSD model. This is important conclusion since the CC2 is a relatively fast computational scheme (scaling N^5), in particular when combined with the efficient treatment of four-index integrals via the resolution of identity (RI) trick.

An essential quantity in the 2PA spectroscopy is a two-photon transition strength δ^{2PA} and two-photon absorption cross-section σ^{2PA} . Both of them are the prime target in the current research. In the mentioned table the quoted results refer to the lowest excited singlet state S_1 . In the next table,

Table 2, the calculations were extended to six lowest lying singlet excited states of the PSB3 molecule.

Another aspect of the calculations, done for the PSB3 and PSB4 models, is related to the performance of various DFT functionals in comparison with the considered CC schemes, realized within the framework of TD-DFT approach. The results, obtained for the first excited state S_1 of the PSB3 example, are presented in Table 3. They indicate comparatively satisfactory performance of CAM-B3LYP and BHandHLYP functionals with respect to the calculated energy of the S_1 state. The same conclusions can be derived from the histograms given in Fig. 8.

A final issue discussed in the context of the calculations focused on the retinal chromophore refers to the effect of the methylation of original 11-cis rPSB with truncated β -ionone ring. The latter chromophore, containing five conjugated double bonds, can be named as PSB5, i.e. the next element in the hierarchy: PSB3 and PSB4.

The results presented in section 4.2 refer to the derivatives of the rPSB with various number of the methyl substituents (1, 2 or 3) placed in different positions in rPSB chain (shown in Fig. 11). To estimate the impact of the methyl substituent on the 2PA properties the author has carried out the calculations for the retinal model containing one, two or three methyl substituents. In the latter two cases the studied molecule exhibits two stable global minima: the planar and twisted conformation, and the calculations were done for both cases.

Two-photon absorption properties are presented for the ground and first (Table 7) and higher (Table 8) excited states. The present calculations are limited to the RI-CC2 scheme and aug-cc-pVDZ basis set. The conclusions reached on the basis of this level of theory indicate that the methyl group in C13 position has the largest impact on the nonlinear response.

A challenging issue has been addressed in chapter V, where the rhodopsin-chromophore system has been studied with the QM/MM modeling. Within the latter approach we have to identify the QM region (treated with quantum mechanics) and MM one (based on the force field and Newtonian equations). As it was concluded by the author the sensible results could be obtained by applying ensemble averaging the QM results. This has been done on the basis of molecular dynamics simulation (done with GROMACS suite) to identify the configurations evolving within the given time intervals, necessary in the process of the ensemble averaging. The calculations done in this study refer to two QM regions M1 and M2. The former consists of 77 atoms and includes

the retinal chromophore, lysine amino acid (Lys296) and several atoms of the adjacent molecules. The M2 model expands the QM region to 122 atoms including an additional amino acid (glutamine), a number of water molecules and selected atoms from the neighbouring amino acids. The M2 model made it possible to estimate the impact of the QM-region size on the calculated photochemical properties. The author concludes that the expansion of the QM region from M1 to M2 has rather moderate effect on the structure of the low-lying excited states.

In summing up I would focus on two aspects of the reviewed work.

The first one is a significance of the accomplished research. Although the rhodopsin protein has been studied in numerous projects any effort contributing to the better understanding of its properties and behaviour is very welcome. The current research provides a very thorough study of the rhodopsin and its chromophore with its several models of reduced size and using the collection of computational methods. Particularly important is the analysis of the behaviour of the retinal chromophore in the protein environment reported in chapter V of the manuscript.

The second aspect concerns the competences and proficiency of a Ph.D. candidate, Mr. Saruti Sirimatayanant, M.Sc. I am strongly convinced that the research conducted by him deserves recognition. All the principal objectives of the research has been achieved. The investigations carried out were really demanding. They required, on-one-hand, an adequate knowledge of the wave function based methods and a full command of the DFT schemes with plethora of various DFT functionals on the other. The performed calculations are by no means trivial and their successful realization demonstrates expertise of the Ph.D. candidate in using quantum-chemical tools.

The editorial aspect of the dissertation does not raise any objections, the manuscript has been prepared with the due care and I did not encounter any meaningful errors.

In conclusion, I declare that the doctoral dissertation of Mr. Saruti Sirimatayanant, M.Sc., meets both the requirements typically set for doctoral dissertations and the currently applicable statutory requirements, so he fulfills all formal and substantive requirements and therefore I strongly support his application for the Doctor of Philosophy degree. Thus, I request the Council for the Discipline of Chemical Sciences of the Wrocław University of Science and Technology to admit Mr. Saruti Sirimatayanant, M.Sc., to the further stages of the doctoral process.