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Referee report

on the doctoral dissertation of Lizaveta Petrusevich entitled:

„Computer-aided design of two-photon-absorbing organic chromophores"

supervised by:

Dr. Robert Zaleśny

and Dr. Josep Maria Luis

the formal basis for the review was a letter from the Chairman of the Council of the Scientific Discipline Chemical Sciences of Wrocław University of Science and Technology, Robert Góra, Ph.D., D.Sc., informing me of Resolution No. 753/43/RDND10/2021-2024, which appointed me as a reviewer of the doctoral dissertation of Ms. Lizaveta Petrusevich.

The PhD thesis submitted for review concerns research on the design of fluorescent probes based on theoretical chemistry approaches. These probes could be used in bioimaging, and thus in diagnostics. The PhD student investigated the relationship between structure and two-photon absorption in selected groups of boron atom-containing compounds. Another important aspect of the research was the development of a fully theoretical protocol for the simulation of vibrationally-resolved absorption spectra for compounds containing a boron atom. Ms. Lizaveta Petrusevich also proposed a computational protocol on the basis of machine learning method (ML) for simulating the absorption spectrum, where inhomogeneous band broadening was taken into account. The topic of the carried out research is timely and very important, as it brings forth the deeper understanding of intramolecular interactions modifying the photophysical properties of the series of the studied compounds.

The doctoral dissertation of Ms. Lizaveta Petrusevich was written in English and

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contains 112 pages. At the beginning of the dissertation, the PhD student has included a list of used abbreviations in alphabetical order. The dissertation was divided into six chapters. The first chapter is the Introduction (15 pages), where Ms. Lizaveta Petrusevich presents a historical overview, examples of compounds applied in bioimaging, describes new fluorescent chromophores (e.g. BOPHY, BOPPY and BOAPH), and computational chemistry approaches used to study two-photon absorption phenomena. It is noteworthy that the Author critically evaluates the available theoretical chemistry approaches for electronic excited state simulations. In chapter two, the PhD student presents the objectives of the thesis (1 page), indicating the two most important:

1. Computer-aided design of improved fluorescent dyes (boron atom-containing) exhibiting one- and two-photon absorption, which may find application in bioimaging;

2. Development of nonempirical and computationally cheaper protocol for simulations of vibrationally-resolved spectra with improved accuracy.

In the third chapter, which contains seven pages and was divided into two subsections, the PhD student briefly presents the theoretical basis for the description of the electronic structure (two-photon absorption) as well as vibrational properties (in harmonic as well as anharmonic approximation). In this chapter it would also be useful to present a theoretical background of e.g. Density Functional Theory (DFT), the Time-Dependent Density Functional Theory (TD-DFT), the coupled cluster method (CC) and classical molecular dynamics (MD). In this way, the chapter would become more complete and clear, taking into account the fact, that the PhD student applied these methods in her research work. These methods constitute a foundation enabling further analysis of spectroscopic properties (2PA). In the fourth chapter, which contains 25 pages, the Author presents the results obtained from the electronic structure analysis of boron atom-containing dyes – analyzing four series of compounds: A, B, C and D (36 structures in total). These compounds differ structurally, which allows for the analysis of the influence of, e.g. cis/trans isomerism or substituent on the electronic structure of the investigated compounds. General conclusions were drawn, on the basis of which it was possible to derive the structure-property relationship, especially in the context of two-photon absorption. The Author begins this chapter by presenting the computational protocol applied in the research, which is common to the discussed series of compounds. On the basis of this protocol, it was

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possible to predict significant photophysical properties of the studied systems. Next, Ms. Lizaveta Petrusevich discussed the strategies used in the design of new boron atom-containing dyes. The PhD student presents the results of performed quantum-chemical simulations of the series of the studied compounds and draws conclusions regarding the electronic structure and, thus the spectroscopic properties important for the design of new fluorescent probes. She emphasizes the importance and difficulty of simulations, especially when the solvent effect should be included. She also pays attention to the importance of the conformation of the molecule, as seen e.g. in the A and C series of compounds. The D series consists of BOPHY derivatives, which are a new group of dyes discussed in the literature since 2014 and therefore knowledge of their photophysical features is still limited. It is worth underlining that the research carried out by Ms. Lizaveta Petrusevich on BOPHY derivatives, is very important for understanding the two-photon absorption and other photophysical properties of this group of compounds. Therefore, the results of the theoretical investigations presented in this chapter, have important impact making a significant contribution to the state of knowledge. A valuable part of this chapter is also the comparison of theoretically obtained results with available experimental data and their detailed discussion. The fifth chapter (32 pages), is devoted to the discussion of the theoretical results concerning the simulations of vibrationally-resolved absorption spectra. It was divided into two subsections. In the first one, the PhD student discusses the vibrationally-resolved absorption spectra obtained for the E series, compounds A1-A6, for compounds in the B series (but without compound B3) and the F series. Ms. Lizaveta Petrusevich writes on page 54 that the protocol was prepared for dyes containing the BF_2 group, but the B series of compounds does not contain such a moiety. The PhD student presents the results of a wide variety of benchmarks performed in order to propose an accurate and efficient protocol for spectra simulations in the harmonic approximation, as well as involving anharmonicity (F-series) - the computational protocol is schematically shown in Figure 27. It is worth to underline that Ms. Lizaveta Petrusevich discusses the advantages and disadvantages of the tested computational models, and illustrates observations in Figures. As in the previous chapter, the results obtained theoretically were compared with available experimental data. In the second subsection, the PhD student discusses the results of theoretical studies on inhomogeneous broadening on the basis of the series of compounds B (except

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compound B3) and F. She also presents the proposed computational protocol applied in the investigations, which was prepared based on machine learning methods. However, it does not explain precisely, how the spectra of the compounds were obtained, in particular, there is no broader description of how the structures obtained from MD simulations were selected. In addition, the description of the MD protocol used in the simulations (Chapter 4, page 29) does not allow to assess whether the non-covalent interactions of the dye with the solvent molecules, which may affect the reorganization of the solvation sphere, have been taken into account. The sixth chapter contains concluding remarks of the presented theoretically obtained results (2 pages). Subsequently, the doctoral dissertation included a Bibliography, which contains 292 references. The PhD student primarily cites scientific articles, occasionally books, and computer programs that were used during the research. The reference 253, is a citation for a publication where the PhD student is one of the co-authors. At this point, the question arises why in the Bibliography, the Author does not cite other publications in which she is a co-author and which are related to the doctoral dissertation ? The next parts of the dissertation contain: the list of schemes (11), figures (41), and tables (10). The last part of the doctoral dissertation is the list of publications co-authored by Ms. Lizaveta Petrusevich, which were published during the PhD study. At the time of writing the review, it came to my attention that the PhD student in the list of publications (included at the end of the dissertation) wrote Petrusevich, L., while in publications and citation 253 (Bibliography) there is written Petrusevich, E.F. However, this issue has already been clarified. It should also be mentioned that in ORCID the PhD student has both versions of her name transcription.

Concluding, in my opinion as the most important results obtained by Ms. Lizaveta Petrusevich could be considered:

1. indication that the introduction of an additional nitrogen atom into an electronacceptor group containing a boron atom leads to an increase in the strength of the two-photon absorption, a shift in excitation energy and an improvement in the photostability of the studied compounds;
2. observation that derivatives containing two nitrogen atoms in a heterocyclic ring exhibit a similar two-photon transition strength, but the fluorescence quantum yield of these dyes is highly dependent on the position of the heteroatom in the ring;

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3. investigation of two-photon absorption in BOPHY derivatives for the first time. The Author noticed that substituents exhibiting weaker electrondonor properties in quadrupole structures exhibit a higher two-photon transition strength, compared to stronger electrondonor substituents in dipolar structures;
4. development of a nonempirical computational protocol to simulate vibrationally-resolved absorption spectra for boron atom-containing fluorescent dyes;
5. development of a computational protocol based on the machine learning method taking into account inhomogeneous broadening in the simulated spectra;
6. identification of 11 of the 36 studied compounds as promising probe candidates for two-photon fluorescence microscopy.

I would like to emphasize that Ms. Lizaveta Petrusevich has achieved the intended research objectives. The Author has correctly chosen the theoretical approaches. The obtained research results have been carefully interpreted and appropriate conclusions have been drawn. The critical assessment of the obtained results by the PhD student is valuable. Therefore, on the basis of the submitted doctoral dissertation, I can conclude that Ms. Lizaveta Petrusevich has general theoretical knowledge and the ability to carry out independent research.

In my opinion, the doctoral dissertation of Ms. Lizaveta Petrusevich presented for review is an original solution to a scientific problem that can be defined as the computer-aided design of organic chromophores containing a boron atom and exhibiting two-photon absorption.

However, in the doctoral dissertation, Ms. Lizaveta Petrusevich did not avoid the so-called editing errors (I will mention only some of them), e.g. on page 8 there should be Scheme 2, instead of Figure 2. On page 21 it should be iv instead of vi. In some drawings, the y-axis designation is missing. In the Bibliography, references 44 and 50, one can notice the use of different abbreviations of the title of the same journal. References 93, 130, 195, 247, 257 are missing page numbers, while references 190, 223 are missing the author names. In reference 219, the Author gave the full title of the journal instead of an abbreviation. There is also a lack of citations of Hohenberg, P.; Kohn, W. "Inhomogeneous electron gas". Phys. Rev. 1964, 136, B864–B871 and Kohn, W.; Sham, L.J. "Self-consistent equations including exchange and correlation effects". Phys. Rev. 1965, 140, A1133–A1138. However, I would like to emphasize

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that the cited "faults" do not have a major impact on the value of the reviewed doctoral dissertation.

It is worth noting that Ms. Lizaveta Petrusevich is a co-author of **13 scientific publications**, which were published during her education at the Doctoral School of the Wrocław University of Science and Technology.

The papers were published in international journals, including e.g. *Journal of Physical Chemistry Letters*, *Journal of Materials Chemistry C*, *Journal of Physical Chemistry A*, and *Journal of Chemical Theory and Computation*.

In conclusion of the doctoral dissertation review, I would like to point out that Ms. Lizaveta Petrusevich has presented interesting theoretical results in her PhD thesis, which make an important contribution to the design and search for new probes based on boron atom-containing dyes. In addition, she proposes a computational protocol for vibrationally-resolved absorption spectra prediction, which is more accurate and computationally advantageous.

Therefore, the doctoral dissertation of Ms. Lizaveta Petrusevich **meets the conditions set out in Art. 187(1-2) of the Act of 20 July 2018 - Law on Higher Education and Science (i.e. Journal of Laws of 2023, item 742, with later amendments)**, and I request the Council of the Scientific Discipline Chemical Sciences of Wrocław University of Science and Technology to grant Ms. Lizaveta Petrusevich permission to proceed to the further stages of the PhD degree conferment.

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