Dr. habil. Janusz M. Dąbrowski, Assoc. Prof.
Faculty of Chemistry, Jagiellonian University
Gronostajowa 2, 30-387 Kraków
✉ jdabrows@chemia.uj.edu.pl
☎ (+48)126862464, 602348234

Kraków, 02.11.2022

Review report on the PhD thesis of Ms. Emma L. Robbins entitled *"Design, optimisation, and characterisation of two- and multi-photon absorption photosensitizers for their prospective use in photodynamic therapy"*

Multidrug resistance (MDR) is one of the main causes of limited treatment efficacy for both cancer and bacterial infections. With the emergence of new, difficult-to-treat strains resistant to most antibiotics, we are rapidly approaching the end of the antibiotic era. Increasing bacterial resistance therefore justifies an intensive search for alternative therapeutics, including methods to combat infections caused by multidrug-resistant microorganisms. The current state of knowledge of both bacterial infections and cancer allows us to conclude that medical strategies that operate through diverse molecular mechanisms, targeting the destruction of cancer and bacteria cells are required to achieve full therapeutic success. Such approaches include photodynamic therapy of cancer (PDT) and photodynamic inactivation of microorganisms (PDI), discussed in the reviewed dissertation. The active agents in both strategies are reactive oxygen species (ROS), which are basically products of either photoinduced electron or energy transfer from the excited triplet state of photosensitizer molecules to the molecular oxygen in its ground state. The ability to generate ROS in a controlled manner depends largely on the properties of the photosensitizers used. Extensive research is undertaken to search for new compounds that absorb light in the visible and/or near-infrared range, with enhanced selectivity against bacterial cells and/or in the tumor microenvironment, high efficacy in the generating both singlet oxygen and free radicals, as well as high therapeutic efficacy.

This important and timely research topic was addressed by Emma L. Robbins, M.Sc., during the preparation of her PhD project. The dissertation was prepared in the collaboration between the Advanced Materials Engineering and Modelling group at the Wrocław University of Science and Technology in Poland, and the Peirene Laboratory at the University of Limoges in France. The work was done as part of POLYTHEA Joint Doctorate programme, financially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant. It should be noted at this point that the PhD Candidate was very fortunate to participate in this

JAGIELLONIAN UNIVERSITY
IN KRAKÓW

Dr. habil. Janusz M. Dąbrowski, Assoc. Prof.
Faculty of Chemistry, Jagiellonian University
Gronostajowa 2, 30-387 Kraków
✉ jdabrows@chemia.uj.edu.pl
☎ (+48)126862464, 602348234

project, which provided access to several high-tech laboratories, participation in training schools, as well as courses and scientific meetings with top specialists in the field of PDT.

In a broader context, the topics addressed in the dissertation contribute to a better understanding of the chemistry of porphyrins and their derivatives - chemical compounds that are crucial for a number of biochemical processes occurring in living organisms. Future implementation of these findings may contribute to solving several medical and environmental problems. Basic research in the chemistry of porphyrins and their derivatives has a growing potential for application in catalysis, photovoltaics, optoelectronics, nanotechnology, and, of course, in biology and medicine. This interdisciplinary approach leads to an understanding of the physicochemical properties of the porphyrins studied herein and the nanomaterials derived from them.

The reviewed thesis is 138 pages long, written in English, and its layout although non-standard is not objectionable. It consists of 4 main chapters preceded by a list of abbreviations and an introduction. The dissertation ends with a general summary of the work and perspectives, a bibliography, and a list of publications coauthored by PhD Candidate. The main concept of this dissertation involves the design, synthesis, optimization, and characterization of selected photophysical and photochemical properties of a series of porphyrin-based photosensitizers.

In a brief introduction, providing a review of the scientific literature on the research topic, the author presented basic information on photodynamic therapy. Firstly, the photochemical mechanisms of PDT leading to the generation of reactive oxygen species are discussed. Secondly, the definition and importance of 'phototherapeutic window' is provided, followed by a brief discussion of the significance of two-photon PDT. Finally, the properties of porphyrins are described, as well as their possible modifications, both within the tetrapyrrole ring (reduction of pyrroles, introduction of desired substituents and/or metal ions) and through the use of appropriate formulations to improve their features enabling more effective use in PDT.

After reading this section, several comments and questions immediately arise:

1. Could the predominance of one of the photochemical mechanisms described herein (Type I versus Type II) result in a correspondingly more favorable anticancer and antimicrobial effect? As I did not find Prof. Michael Hamblin's work in the cited literature, I am also curious if you are familiar with his proposed Type III Mechanism.

JAGIELLONIAN UNIVERSITY
IN KRAKÓW

Dr. habil. Janusz M. Dąbrowski, Assoc. Prof.
Faculty of Chemistry, Jagiellonian University
Gronostajowa 2, 30-387 Kraków
✉ jdabrows@chemia.uj.edu.pl
☎ (+48)126862464, 602348234

2. Is the phototherapeutic window range indeed 600- 900 nm? Is the energy of 1.3 kJ/mol (900 nm) sufficient for the photochemical reactions discussed?
3. What are the differences in the criteria for selecting photosensitizers for photodynamic therapy of cancer versus photodynamic inactivation of microorganisms? During the defense, I would like to hear about both the chemical properties of the photosensitizers and the protocols used in both therapeutic strategies.

Despite the concerns presented, this chapter fulfills its role, as it enables the reader of the thesis to understand the essence of the research undertaken by the PhD student and provides an adequate introduction to the complex topics.

The content of the dissertation provided in Chapters I-IV is consistent with the titles. The dissertation was implemented according to a well-thought-out scheme, which enabled the research hypothesis to be verified and some interesting conclusions to be drawn. The experimental data obtained by the PhD Candidate were critically discussed in the context of the international literature and the direct experience of the scientific teams in which the work was carried out. Chapter I deals with the encapsulation of three different porphyrins (THPP, ZnTHPP,T(OAc)PP), using acetylated lignin. Obtained in this way nanomaterials were further studied using several techniques such as two-photon excited fluorescence measurements, two-photon microscopy, and atomic force microscopy. It is rather disappointing that the effect of encapsulation on the thermal and photochemical stability of the studied porphyrins, and especially on the generation of ROS, was not investigated. On the other hand, it can be concluded from preliminary studies that the described nanoparticles can serve as optical sensors capable of emission in the NIR. Chapter II is concerned with the optimization of clinically used, chlorin-type photosensitizer called temoporfin (Foscan®). The structure of temoporfin was modified and the two-photon absorption properties were determined. The results described here show a significant increase in the two-photon absorption cross-section values for the modified photosensitizer compared to Foscan® and confirm its feasibility in two-photon PDT. Chapter III involves the design, synthesis, and characterization of a new porphyrin derivative. Moreover, the optical properties of the obtained compound in the linear and nonlinear regimes were investigated demonstrating modest enhancement of two-photon absorption cross section. Chapter IV focuses on the analysis of the effect of selected modifications that led to the development of so called naphthiporphyrins on their linear and non-linear optical properties. The described photosensitizers

JAGIELLONIAN UNIVERSITY
IN KRAKÓW

Dr. habil. Janusz M. Dąbrowski, Assoc. Prof.
Faculty of Chemistry, Jagiellonian University
Gronostajowa 2, 30-387 Kraków
✉ jdabrows@chemia.uj.edu.pl
☎ (+48)126862464, 602348234

are characterized by an average two-photon absorption up to 1400 nm. The obtained results provide a solid ground of new knowledge and have broad cognitive significance, fulfilling the research gap, especially in the field of optical properties of porphyrins with possible application in PDT, photodiagnosis or theranostics.

As in any such study, there are some inaccuracies and flaws, which, out of my obligation as a reviewer, I list below:

1. Page 12: there is: ***1PE** – One-Photon Excited* and should be: ***1PE** – One-Photon Excitation*.
2. The Jabłoński diagram presented in Fig. 1 is too simplified, resulting in a laconic elucidation of the key photophysical processes and photochemical reactions that determine the effectiveness of PDT.
3. On page 22 it is written that the main drawback of 2PA-PDT is the need of expensive laser light sources. In my opinion, the cost of lasers is becoming less of a concern, but it overlooks another important limitation that makes two-photon PDT rather impossible to be used to treat all types of tumors.
4. On page 23 it is written *"Common metals used within PDT applications are zinc and ruthenium"*. Please provide other (perhaps even more important) metal ions present in the structures of compounds used in PDT and discuss their impact on the photochemical properties and efficiency of PDT.

It is noteworthy that the results of the research included in the dissertation were published in the form of four articles in good and very good specialized scientific journals such *RSC Advances*, *Journal of Porphyrins and Phthalocyanines*, *ChemPhotoChem* and *Molecules*. I have no doubt that the experimental data already published are of great scientific value.

Among the most important achievements obtained by Emma Robbins, M.Sc. within the framework of the reviewed dissertation, I would include:

1. Demonstration that studied porphyrins encapsulated in acetylated lignin nanoparticles are capable to emit NIR photons upon two-photon excitation.
2. Demonstration that appropriate modification (introduction of aldehyde and carboxylic acid groups) of the commercially available temoporfin led to the enhanced two-photon absorption properties.

JAGIELLONIAN UNIVERSITY
IN KRAKÓW

Dr. habil. Janusz M. Dąbrowski, Assoc. Prof.
Faculty of Chemistry, Jagiellonian University
Gronostajowa 2, 30-387 Kraków
✉ jdabrows@chemia.uj.edu.pl
☎ (+48)126862464, 602348234

3. Synthesis and characterization (^{1}H-NMR, UV-Visible absorption, and mass spectrometry) of a π-conjugated extended porphyrin for future application either in PDT or PDI.
4. Demonstration that studied naphthiporphyrins absorb NIR photons at much longer wavelengths compared to commercial porphyrin-based photosensitizers. This is a significant achievement because photons from these range are not harmful and penetrate much deeper into the tissue.

I believe that the reviewed dissertation is well conceived and prepared, contains scientific novelty and originality. The ability to solve complex scientific problems in the field of interdisciplinary topics deserves mention. The PhD candidate has proven her knowledge of numerous research techniques. The work from the linguistic and editorial aspects is also well prepared. The critical remarks I have made are an invitation for discussion during the defense, rather than pointing out substantive mistakes affecting the final very good evaluation of the work. In addition, some of the questions I asked may be seen as a suggestion to undertake further research to gain a deeper understanding of the processes described and to clarify the photochemical and biological mechanisms. Considering the above, I conclude that the reviewed work fully meets the conditions specified in Article.187 of the Law of July 20, 2018. Law on Higher Education and Science (i.e. Journal of Laws of 2022, item 574, as amended).

I therefore request the Council of the Scientific Discipline Chemical Sciences of the Wrocław University of Science and Technology to continue the procedure for awarding the degree of Doctor of Chemical Sciences and to admit Emma Robbins, M.Sc. to the public defense.

Dąbrowski Janusz

Dr hab. Janusz M. Dąbrowski, prof. UJ