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INSTITUT NATIONAL DES SCIENCES APPLIQUÉES RENNES

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Rennes le 22/10/2022

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Design, optimisation, and characterisation of two- and multi-photon absorption photosensitizers for their prospective use in photodynamic therapy
Supervisor: Dr. Katarzyna Matczyszyn, Dr. Stéphanie Leroy-Lhez and *Co-supervisor:* Dr. Nicolas Villandier

***Report on the manuscript of Ms* Emma L. ROBBINS**

The manuscript presented by Ms Emma L. ROBBINS for the award of a PhD is a 137 pages document comprising an Introduction, four chapters and a Conclusion-Perspectives.

In the first chapter, entitled "**Porphyrin-loaded acetylated lignin nanoparticles**", three different porphyrins were encapsulated into acetylated lignin nanoparticles (**S1** (THPP), **S2** (ZnTHPP), and sample 3, **S3** (T(OAc)PP)). A control sample of empty acetylated lignin nanoparticles, was also examined (sample 4, **S4**). Surprisingly the zinc complex of sample 3, **S3** (T(OAc)PP) was missing in this series. Then, different experimental techniques were used to characterize these three porphyrin-loaded acetylated lignin nanoparticles: First the morphology was studied by atomic force microscopy (AFM), and then size separation was performed. To separate these nanoparticles, sucrose density gradient centrifugation (SDGC) was performed. The particles (**S1-S4**) were studied as dispersions in buffer solution, as well as size separated (**S1-0**, those from the 10% w/v sucrose fraction as **S1-10**, and those from the 20% w/v sucrose fraction as **S1-20**, etc.). Select fractions were examined to determine if the size of the particles had any effect on their photophysical properties. The Linear photophysical characterization was measured: (i) absorption spectra and (ii) One-photon excited (1PE) fluorescence, and (iii) Time-resolved 1PE fluorescence. Finally, non-linear optical (NLO) performances of aqueous dispersions of the porphyrin-loaded and empty nanoparticles were studied meaning: (i) the femtosecond laser-induced fluorescence spectra of all three porphyrin-loaded hybrids and the empty acetylated lignin nanoparticles were measured at several excitation wavelengths, (ii) Two-photon fluorescence microscopy

(2PFM) These results show that these nanoparticles are promising biomarkers able to emit in the NIR range, most significantly within the first biological optical window, as demonstrated particularly well with the single nanoparticle measurements. Therefore, in the first chapter, it is successfully demonstrated that porphyrins encapsulated in acetylated lignin nanoparticles are capable of emitting in the NIR range upon two-photon excitation. The candidate specifies that future work needs to be done regarding the singlet oxygen generation capabilities of the bulk dispersions, as well as the separated fractions, to determine their usefulness within 2PA-PDT, theranostic applications. In order to complete these results, additional experiments will need to be performed by the candidate: like studying the zinc complex of sample 3, **S3** (T(OAc)PP).

The second chapter is entitled "**Non-linear optical properties of Foscan® (*m*-THPC) and derivatives**". It begins with a quite detailed review of the literature concerning photosensitizer (PS) for photodynamic therapy (PDT) and for its use within two-photon absorption combined PDT (2PA-PDT) with a high 2PA cross-section value. Then three modified molecules named **PS1**, **PS3**, and **PS5** are studied and compared to the precursor 5, 10, 15, 20-tetra(*m*-hydroxyphenyl) chlorin. This last chlorin or ***m*-THPC**, nowadays registered as Foscan®, is approved for use as a PS for PDT for cancer treatment. Chemical modifications on the terminal groups of Foscan were done by Senge and Gomes-da-Silva; to optimize its use for 2PA applications by a better solubility in aqueous media (less aggregation), and higher 2PA, to improve treatment efficiency. These collaborators used esterification, and Sonogashira cross-coupling reactions to produce aldehyde and carboxylic acid derivatives of Foscan named **PS1**, **PS3**, and **PS5**. After rapidly describing these syntheses, their photophysical properties are reported. First two-photon excited fluorescence measurements (in DMF) were performed on the precursor ***m*-THPC** and modified chlorins (**PS1**, **PS3**, and **PS5**). The results showed that even simple modifications could lead to an improvement on the 2PA properties and this was confirmed by the determination of the 2PA cross-sections. In particular, **PS5** with its alkyne linkers between the core and the newly introduced carbonyl groups, indicated an increase at the 2PA maxima compared to the unmodified ***m*-THPC** core (from 26 to 69 GM). Additionally, the results of **PS3** demonstrating fluorescence in aqueous media upon two-photon excitation furthers the capabilities for this PS to be used not only in 2PA-PDT, but also potential as fluorescence imaging probes. Then power dependence measurements of emission intensities for ***m*-THPC**, **PS1**, **PS3**, and **PS5** were recorded with varying laser excitation power: These results indicate a quadratic power relation, thus, confirming a two-photon process is occurring.

In the second part of this chapter, the transfer effect from DMF to aqueous solution was studied because the intense red fluorescence of the PS shows great potential for these compounds to be used as 2PE fluorescence imaging agents. As a result, the fact that there is significantly strong red 2PE fluorescence occurring in physiological conditions demonstrates the potential of **PS3** in biomedical applications. The candidate concludes that these results, show that these PSs are promising for the development of two-photon induced PDT.

The third chapter is entitled "**Synthesis and characterisation of functionalised porphyrin for prospective use in PDT/PACT**". This chapter, the most important (48 pages), is different from the others because it shows the candidate's ability to propose synthesis strategies and to carry them out with more or less success. The target is a π-conjugated extended porphyrin to develop its 2PA properties for their prospective use within PDT. As an introduction, the retrosynthetic pathway of the synthetic strategy is presented. Then, the synthesis of porphyrinic synthons is described; these porphyrin cores need to bear either iodine (**ER-4**) or acetylene (**ER-5**) followed by the synthesis of the novel 2-(4-bromophenyl)-5-iodopyrimidine (**ER-6-I**). The synthesis of the porphyrin core, as well as of the pyrimidine, have been optimized for efficiency and yield. Finally, the coupling of porphyrin **A3** and the para-substituted pyrimidine arm is described showing unsuccessful strategy of the Sonogashira coupling of the acetylated porphyrin **ER-5.** Unfortunately, due to lack of time, the candidate could not complete this last key step to obtain the target molecule. Characterization of intermediates (NMR, UV-Vis, Mass Spectrometry, and 2PA properties) is also well detailed in this chapter. However, the uncoupled porphyrin structures were measured and characterised for their 2PA properties via Z-scan measurements.

The fourth chapter is entitled "**Two-photon absorption properties of expanded macrocycles: naphthiporphyrins**". In this last chapter another way to enhance porphyrin 2PA properties, *via* expansion of the macrocycle core, is tried. The chapter begins with a detailed review of the literature concerning **expanded macrocycles**. The goal is to study photophysical properties of heteroporphyrins, namely carbaporphyrinoids, with their potential application within PDT. The candidate proposes to measure the 2PA properties of three 28-hetero-2,7-naphthiporphyrins where heterocycle D differs with the presence of sulfur (**1-S**), selenium (**1-Se**), and tellurium (**1-Te**) within the cavity of the macrocycle. The obtained results for **1-S**, **1-Se**, and **1-Te** show a moderate improvement upon well-known PS that are currently used for PDT. The one- and two-photon absorption properties of three naphthiporphyrins were measured using UV-Visible absorption and the Z-scan technique, respectively. The moderate 2PAcross-section values recorded at 1400 nm is the longest wavelength that these types of compounds have been measured at before.

The thesis ends with a concise "**Conclusion and perspectives**" which provides a concise and helpful summary of the work. Overall, this is a complex and detailed manuscript. The Thesis is agreeable and nicely balanced; detail is provided where necessary. The text, is homogenous and attractive to read, and the presentation overall is of a high standard.

To conclude, the Thesis provides in-depth physicochemical and optical studies of the compounds. The Thesis seems likely to be very interesting to PDT specialists. In summary, the manuscript nicely fulfils the requirements for a candidature for the title of "Doctor", so I recommend Ms Emma L. ROBBINS for it, and look forward to her oral presentation in defence of her work.

Fait à Rennes le 27/10/2022

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