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Leszek Mateusz Mazur, Thomas J. Roland, Stephanie Leroy-Lhez,
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Efficient Singlet Oxygen Photogeneration by Zinc Porphyrin-Dimers Upon One- and Two-Photon Excitation.

Leszek M. Mazur[a], *Thomas Roland*[b], *Stephanie Leroy-Lhez*[c], *Vincent Sol*[c], *Marek Samoc*[a], *Ifor D.W. Samuel*[*b], *Katarzyna Matczyszyn*[*a]

[a] Advanced Materials Engineering and Modelling Group, Faculty of Chemistry, Wroclaw University of Science and Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland

[b] Organic Semiconductor Centre, SUPA, School of Physics and Astronomy, University of St Andrews, Fife, KY16 9SS, UK

[c] Laboratoire PEIRENE EA 7500, Faculté des Sciences et Techniques 123, Avenue Albert Thomas, Limoges cedex 87060, France

ABSTRACT

The development of photodynamic therapy at depth requires photosensitizers which have both sufficient quantum yield for singlet oxygen generation and strong two-photon absorption. Here we show that this can be achieved by conjugated linkage of zinc porphyrins to make dimers. We determined the quantum yield of generation of $^1O_2$, ϕ_Δ , by measuring emission at 1270 nm using a near infra-red streak camera and found it to increase from 15% for a single porphyrin unit to 27-47% for the dimers with a conjugated linker. Then, we measured the spectra of two-photon absorption cross section, σ_2, by a focus-tunable Z-scan method, which allows for nondestructive investigation of light-sensitive materials. We observed a strong enhancement of the two-photon absorption coefficient in the dimers, especially those with an alkyne linker. These results lead to an excellent figure of merit for two-photon production of singlet oxygen (expressed by the product $\sigma_2 \times \phi_\Delta$) in the porphyrin dimers, of around 3700 GM, which is very promising for applications involving treatment of deep tumors by photodynamic therapy.

1. INTRODUCTION

Photodynamic therapy (PDT) is a widely used cancer treatment, particularly for the treatment of superficial cancers such as many skin cancers[1]. PDT involves the use of light in combination with a photosensitizer to cause a photochemical reaction, leading to the generation of reactive oxygen species (ROS)[2], which then kill neighboring tumor cells. Whilst most often used to kill cancer cells, the reactive oxygen species can also be used to kill bacteria thereby providing a powerful alternative to antibiotics in the management of topical infections[3, 4]. For example, in addition to cancers, PDT has been used in the treatment of acne[5] (by killing Propionibacterium acnes), periodontology[6], treatment of viral lesions[7] and age-related macular degeneration[8].

In order to efficiently photogenerate singlet oxygen, a material should fulfill a few criteria. It needs to absorb excitation light and have a triplet energy level higher than 397 kJ/mol to allow for efficient energy transfer to ground state oxygen and hence the generation of singlet oxygen. Moreover, it should have high triplet quantum yield and long triplet state lifetime[2]. Despite these requirements, there are many materials capable of photogenerating singlet oxygen, they can be grouped into: (1) the organic dyes and aromatics[9]; (2) the porphyrins, chlorins, phthalocyanines, and related macrocycles[10]; (3) semiconductors[11]; and (4) metal nanoparticles[12, 13]. Among these photosensitizers, porphyrin derivatives attracted significant attention and some of them are already in clinical use.

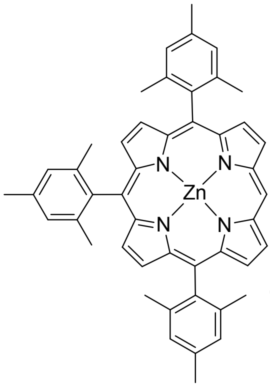
Photodynamic therapy has been widely used to kill tumor cells, which are located close to skin surface, due to the fact that the photosensitizers are excited with wavelengths which are strongly absorbed and scattered by the tissue. PDT could treat a much wider range of conditions if it could work at greater depth which could be achieved by using excitation wavelengths in the biological transparency windows of tissue at 650-950 nm and 1000-1250 nm[14]. As only few photosensitizers have a linear absorption band in this wavelength region[15], two-photon excitation in which two longer wavelength photons are simultaneously absorbed is a natural way to achieve the desired excitation energy with longer wavelength light that can penetrate further into tissue. The photosensitizers which aim to be used in two-photon excited PDT should be characterized by high quantum yield of singlet oxygen generation, ϕ_{Δ}, and large two-photon absorption cross-sections.

Typical porphyrin derivatives[16-18], even those accepted for clinical use like Photofrin®, Verteporfin® or Foscan® are indeed efficient singlet oxygen generators but suffer from low two-photon absorption cross-sections, on the order of 1 GM[19, 20]. In that context, there is a need to design and synthesize new molecules with excellent figure of merit for two-photon production of singlet oxygen (expressed by the product $\sigma_2 \times \phi_\Delta$). It is also relevant to consider this figure of merit when normalized by molecular weight i.e. $\sigma_2 \times \phi_\Delta / M$, marked as $(\sigma_2 \times \phi_\Delta)^*$ to facilitate comparison of large and small candidate photosensitizers. In the case of porphyrins, in order to maximize the parameter $\sigma_2 \times \phi_\Delta$, insertion of a metal ion leads to a trade-off as it causes reduction of the singlet oxygen quantum yield[21] and increase of the 2PA cross-section.

In the field of nonlinear optics, the general rules for molecular design to maximize 2PA cross-section exist in the literature and are supported by both theoretical and experimental work. It has been shown that centrosymmetric quadrupolar compounds have larger values of σ_2 than their dipolar counterparts[22]. In addition, introducing strong electron donating (D) or electron withdrawing (A) groups to yield the structures like D-A-D or A-D-A further improves 2PA properties[23]. Finally, the process of simultaneous absorption of two photons requires strong overlap of the π-orbitals over a large system, making it sensitive to molecular conformation changes, which may alter the electronic coupling.

The general structure-property rules can be applied to porphyrin based compounds. It has been shown that 2PA properties may be tuned by metal insertion, adding electron donating or withdrawing groups and expanding the π-conjugated system by creating dimers or higher order oligomers[17, 23, 24]. In the present work we use metal containing porphyrins and explore the effect of increasing electron delocalization by conjugated links between macrocycles. In particular, we explore the photogeneration of singlet oxygen and two-photon absorption in a series of zinc porphyrins, either being a monomer (compound **1**), or a dimer, linked by ethyne (**2**) or di-ethyne link (**3**) or by a single bond (**4**), as depicted in Scheme 1.

There are numerous papers investigating either the two-photon absorption or singlet oxygen photogeneration properties of porphyrin based compounds, but not so many where the combination of these two effects is presented[25-27]. Our goal was to carry out interdisciplinary research covering the nonlinear optical properties and singlet oxygen photogeneration, thus providing strong support to the results and conclusions concerning the structure-property optimization. Singlet oxygen generation was studied using an infra-red streak camera. The two-photon absorption properties were investigated by the focus-tunable Z-scan method, which has the important advantage over alternative methods of being quick, thereby avoiding photodegradation which is a particular consideration in the measurement of materials intended to cause photochemistry.



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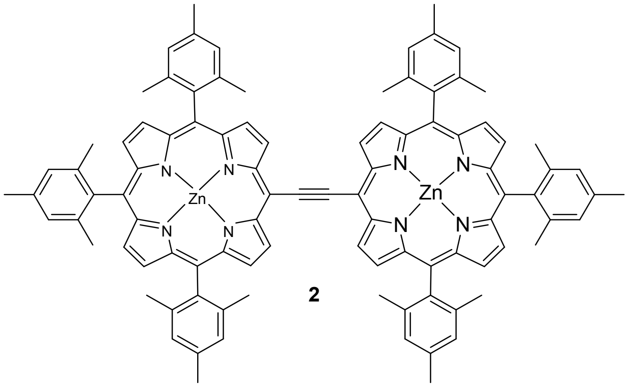

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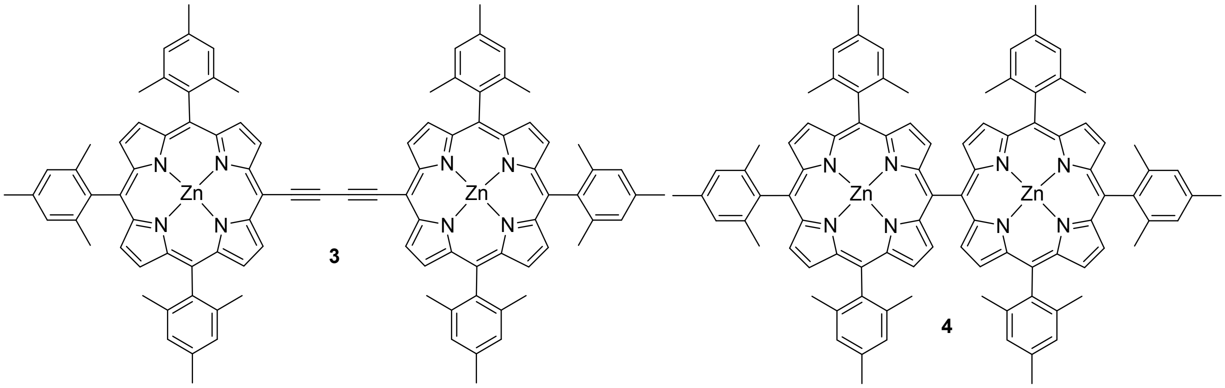

Scheme 1. Structures of investigated compounds.

2. EXPERIMENTAL SECTION

The molecules used in this work were synthesized and purified according to the protocols described in[28]. The molecules were dissolved in chloroform (Sigma-Aldrich) and quartz cuvettes were filled with the solutions for the photophysical measurements. UV-vis absorption spectra were measured using a Perkin Elmer Lambda 1050 UV/Vis/NIR spectrophotometer. The concentration of the solution was adjusted to reach optical densities suitable for the PL and emission from singlet oxygen experiments. Steady-state PL spectra were recorded using an automated spectrofluorometer (Fluorolog, Horiba Jobin-Yvon), with a 450 W Xenon lamp excitation source. The solution singlet oxygen quantum yield (SOQY) (ϕ_Δ)

values were determined using tetraphenylporphyrin (H_2TPP) from Fisher Scientific in chloroform solution as a standard reference because of its well-known SOQY of 55%[29]. The direct emission of singlet oxygen at 1270 nm was detected by a NIR streak camera from Hamamatsu Photonics. The camera was working in Photon Counting mode and the experiment was automatically stopped after 50 000 frames for each sample. In order to prevent the degradation, the samples solutions were placed in a rotating cell, placed in such a way that the exciting beam was shifted from the center. The beam from a Light Conversion PHAROS regenerative amplifier (output wavelength 1028 nm, pulse duration 290 fs, repetition rate 5 kHz) was directed to an optical parametric amplifier (OPA, a Light Conversion OPHEUS-F). The OPA generated 828 nm, which was further frequency doubled to obtain 414 nm used to excite the samples. The output pulse duration was less than 100 fs. The quantum yield of singlet oxygen generation may be determined using the modified method used by Tanielian and Heinrich[30] and using equation 1[21]:

$$\phi_{\Delta x} = \phi_{\Delta r} \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \frac{I(\lambda_r)}{I(\lambda_x)} \frac{D_x}{D_r} \frac{\tau_r}{\tau_s} \qquad (1)$$

where ϕ_Δ stands for the quantum yield of singlet oxygen generation, the subscripts *x* and *r* refer to the compound to be characterized and to the reference solutions, respectively. *λ* is the excitation wavelength, *A(λ)* is the absorbance, *I(λ)* is the relative intensity of the excitation light, *τ* is the lifetime of singlet oxygen emission in the sample and reference solvent, and *D* is the integrated area under the emission spectra.

As, the samples and reference solutions are excited at the same conditions and they are dissolved in chloroform, the above equation can be simplified to:

$$\phi_{\Delta x} = \phi_{\Delta r}\,\frac{A_r(\lambda_r)}{A_x(\lambda_x)}\frac{D_x}{D_r} \qquad (2)$$

The two-photon absorption cross-sections were determined by f-scan method described in[31]. The laser system used for this study was a Quantronix Integra Ti:Sapphire regenerative amplifier (output wavelength: 800 nm; pulse duration: 130 fs; repetition rate: 1 kHz) that pumped an optical parametric amplifier (OPA, a Quantronix Palitra) to provide tunable excitation. The sample solution was prepared by dissolving the corresponding chromophore in chloroform at concentration of 0.5% (w/w) and placed in a glass cell with 1 mm optical pathlength. The measurements were carried out in a relative manner, calibrating all the data against f-scans carried out on a fused silica plate and taking into account the nonlinear signals obtained from a cell containing pure solvent.

3. RESULTS AND DISCUSSION

Steady-state electronic absorption.

The absorption and photoluminescence spectra of the investigated compounds were measured in dilute $CHCl_3$ solutions with 1×10^{-6} mol/L concentration. Maximum absorption wavelengths and maximum emission wavelengths are listed in Table 1. The absorption spectra are displayed in Figure 1. For the monomeric porphyrin, compound **1**, (Figure 1a), two absorption bands are clearly visible. The Soret band is located at 414 nm and so called Q-band at 543 nm. For the dimers, the Q-bands are intensified they show bathochromic shift compared to molecule **1**. Moreover, both Soret and Q-bands are much broader with additional peaks visible in the absorption spectra for compounds **2-4**. It is worth to mention that the stronger Q-band absorption is responsible for enhanced 2PA properties in the NIR. Fluorescence occurs from the Q-band, whichever band is excited. The fluorescence bands of compounds **2** and **3** show a bathochromic shift with respect to compound **1** (Figure 1a-c). The bathochromic shift of the absorption and fluorescence bands can be explained by the decreased HOMO-LUMO gap due to π-delocalization between the neighboring porphyrin units[24]. Polarized absorption spectroscopy revealed that for the dimers linked with ethyne and di-ethyne bonds, the lowest energy transitions were polarized along the molecular axis (*x*) and can be called $Q_x(0\text{-}0)$[17]. They are followed by vibronic satellites. In the Soret region, there is a group of *x*- and *y*-polarized transitions. Among them, the lowest energy and relatively narrow transitions is parallel to $S_1 \leftarrow S_0$, which can be assigned as $B_x(0\text{-}0)$ transition. There are also other B_x and B_y transitions which span a wide region to the blue from the $B_x(0\text{-}0)$ transition. In the case of compound **4** the molecule is no longer planar due to steric hindrance, and so the dimerization does not lead to increased electron delocalization. There is an almost equally intense split in the Soret absorption and Q-band emission of **4** (Figure 1d), which can be explained by the excitonic coupling between the two parallel strong dipole transitions of each porphyrin ring[28, 32]. The magnitude of the absorption band split is equal to 2002 cm^{-1} and the emission band split is equal to 854 cm^{-1}.

The crystallographic structure analysis performed by another group yielded the torsional angle of the porphyrin dimer linked by a single bond to be 69.1(2)°[33]. The torsional angle of the dimers linked by ethyne and di-ethyne bonds were determined by DFT calculations to be zero, which means that the molecules are planar[34], which enhances both 2PA and singlet oxygen photogeneration due to faster intersystem crossing (S_1–T_1), compared to twisted conformers[35].

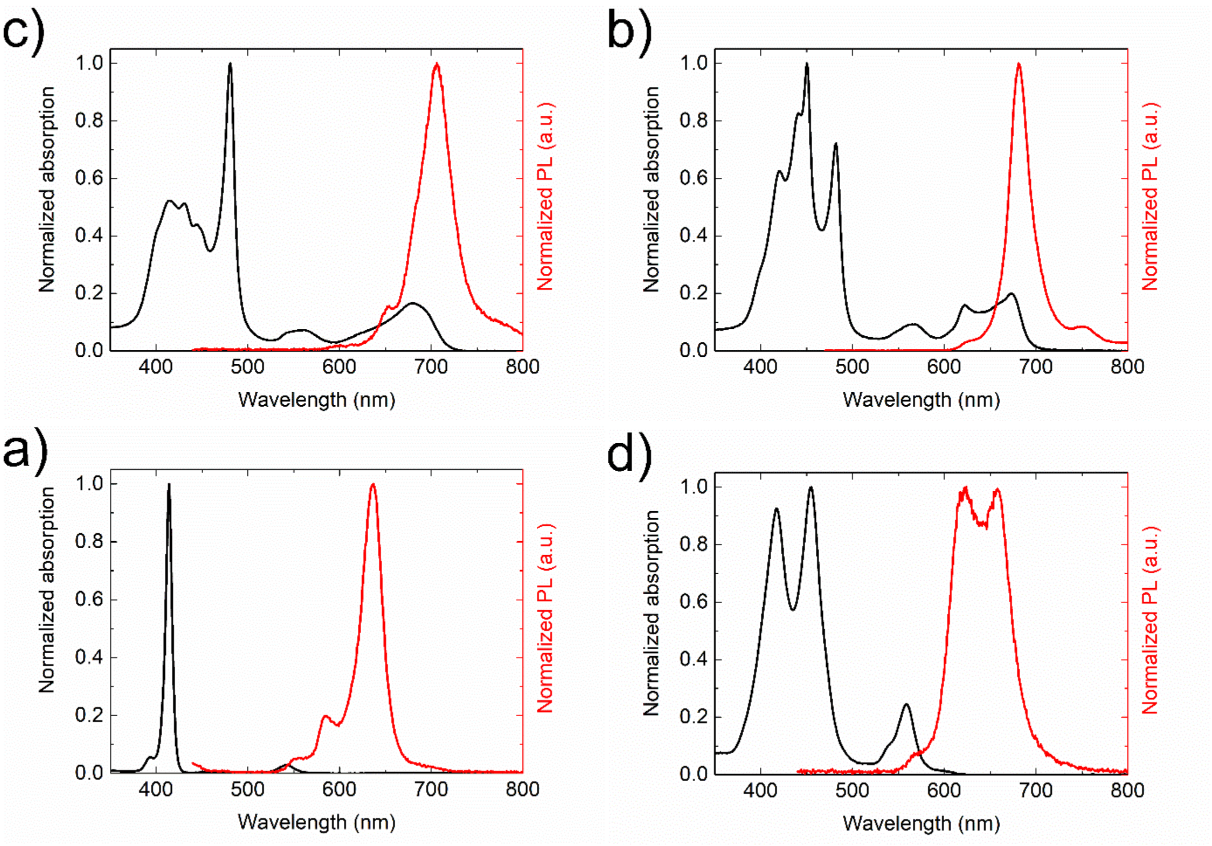

Figure 1. Normalized absorption (black curves) and steady-state fluorescence spectra (red curves) of investigated porphyrins. Figures a) to d) correspond to molecules **1** to **4**, respectively.

Singlet oxygen detection.

The singlet oxygen quantum yield, ϕ_{Δ}, was determined by recording direct emission from singlet oxygen centered at 1270 nm by a NIR streak camera. The transition from first excited singlet state $^1\Delta_g$ to ground state being triplet, $^3\Sigma_g^-$, is spin forbidden[2]. This is the reason for the emission lifetime on the order of μs, which strongly depends on the solvent[21].

The signals were integrated from 8 to 50 μs to obtain the spectra of $^1O_2$ emission. The spectra obtained for sample **4** and the reference are shown in Figure 2. Finally, the spectra were integrated to obtain the *D* factors necessary to calculate the quantum yield of singlet oxygen photogeneration. The values of ϕ_{Δ} of all investigated compounds are summarized in Table 1. The least efficient is the monomeric unit (**1**). The dimerization leads to enhanced properties of singlet oxygen photogeneration, regardless the type of the link. One can notice that the value of ϕ_Δ for compound **4**, which is not conjugated due to steric hindrance, is approximately doubled, compared to **1**. On the other hand, compounds **2** and **3**, which have large π-delocalization and are close to planar, are characterized with much larger ϕ_Δ being equal to 35 and 47 %. These findings are in good agreement with other works on porphyrins dimers, e.g. with porphyrin units linked by butadiyne bond linked to TIPS-protected ethynyl groups[36]. The determined ϕ_Δ of such porphyrin dimer in DCM was reported to be 37 %, which means that the structural design of compounds **2** and **3** are more efficient in terms of singlet oxygen production.

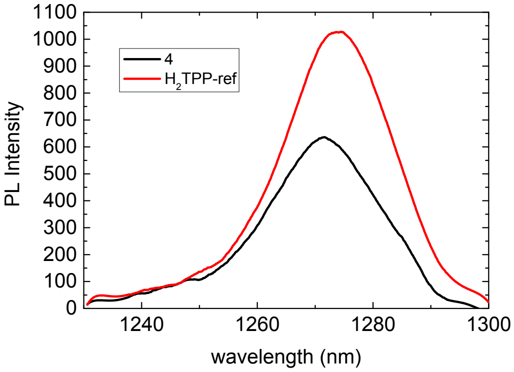

Figure 2. Singlet oxygen emission spectra of **4** and H$_2$TPP, obtained by integrating the streak camera images from 8 to 50 μs.

Table 1. Steady-state photophysical properties of the investigated compounds (**1-4**) and their singlet oxygen quantum yields. The positions of the most intensive bands are given in bold.

porphyrins	λ_{abs} (nm)	λ_{em} (nm)	ϕ_Δ (%)

	Soret band	Q-band		
1	393 **414**	**542**	554 585 **637** 700	15
2	420 441 **450** 482	566 622 **673**	627 **681** 751	35
3	414 431 444 **481**	562 **681**	599 654 **706** 785	47
4	417 **455**	538 **558** 600	567 **624** **658**	27

Two-photon absorption cross-section spectra.

The spectra of two-photon absorption cross-section may be obtained by many methods, two-photon excited fluorescence (TPEF)[37] and fs Z-scan[38-41] being the most common nowadays. They strongly differ from each other, as in TPEF one detects fluorescence spectrum at various wavelengths and compares the integrated PL with a standard reference with known PLQY and σ_2. In Z-scan one measures the changes of sample transmission as a function of the sample position in Z direction and fit the data recorded on so called Open Aperture curve as described in the literature[38, 41] to determine the value of two-photon absorption cross-section at the specific wavelength. In order to obtain reliable data by these two aforementioned techniques, the samples have to be perfectly photostable.

Upon photoexcitation with one- and two-photons the singlet oxygen which is generated by the investigated porphyrins causes their degradation. It is important to mention that each photosensitizer molecule can typically produce 10^3-10^5 molecules of $^1O_2$ before being degraded through photobleaching by $^1O_2$ or by some other process[2]. Thus, it is crucial to minimize the time of irradiation especially with high power laser used to study the nonlinear effects. Hence we use the so-called “f-scan” technique which is a modification of the standard Z-scan method, to determine the spectra of two-photon absorption cross-section. In the f-scan technique, instead of translating the sample, the focal point is shifted by an electrically controlled lens[31]. Combined with improved data acquisition technique, this method reduces the time necessary for a single scan from a few minutes to several seconds, thus limiting the possibility of photodegradation. While the vast majority of published papers described only the singlet oxygen photogeneration process, or two-photon absorption properties of the studied photosensitizers, in this work both are treated with equal attention and detail, allowing for more reliable conclusions.

The spectra of two-photon absorption cross-sections of investigated porphyrins obtained by f-scan technique are shown in Figure 3. They are overlaid with linear absorption spectra, shown as red curves, plotted *vs*. twice the wavelength. For all porphyrins, both the monomer (**1**) and dimers (**2-4**) the maximum of two-photon absorption cross-sections is within first biological window which exists at wavelengths between 650 nm and 950 nm[14].

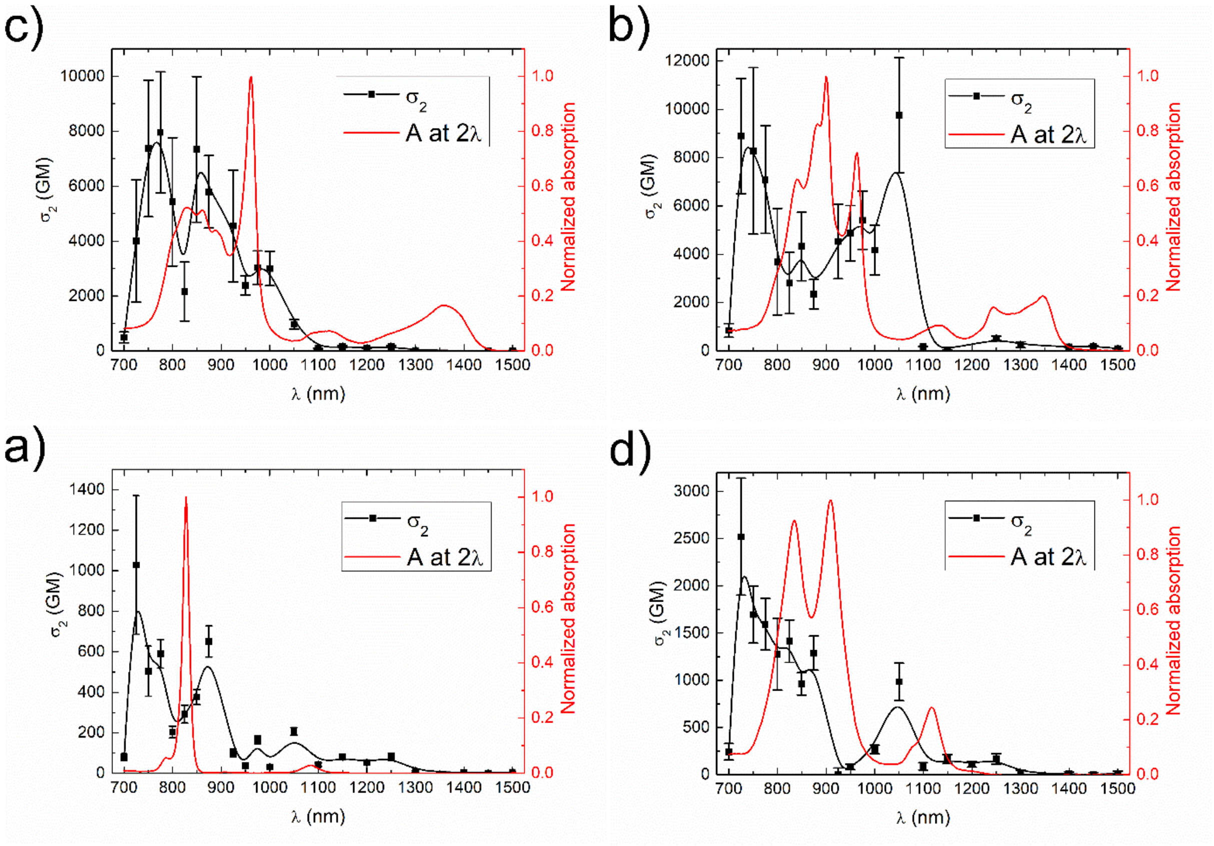

Figure 3. Spectra of two-photon absorption cross-sections of the investigated porphyrins (**1-4**). The spectra were overlaid with linear absorption (red curves) plotted vs. twice the wavelength. Figures a) to d) correspond to compounds **1** to **4**.

In case of the monomer **1**, the maximum value of σ_2 is around 1000 GM at 725 nm, as shown in Figure 3. a). One can see that the band of 2PA is split into two, even though the linear absorption shows only one peak at 414 nm. It is caused by different selection rules for one- and two-photon absorption, as described in the literature[22]. It is worth noticing that the high energy 2PA band at 725 nm is observed for all the compounds and has the highest value of σ_2 among other 2PA bands. It means that it is favorable for the molecules to be excited to higher vibronic state upon 2PA excitation, compared to 1PA process. Despite the fact that linear absorption of **4** is split into two equally intense bands, the 2PA spectrum is different. One can see that the band at 725 nm is much more intensive that that at 875 nm. The maximum value of σ_2, measured at 725 nm is approximately doubled compared to those of compound **1** and is equal to 2500 GM. In the studies of two-photon absorption cross-sections various normalization factors are used, among which the normalization to molar mass is the most popular. If the maximum σ_2 is divided by molar mass, a similar value is obtained, which means that such structural change does not impose neither cooperation nor deleterious effects on the multiphoton absorption properties of the investigated compounds **1** and **4**. In case of compounds **2** and **3**, the spectra of two-photon absorption cross-section show more complex structures, similarly to their 1PA spectra. For compound **2** (Figure 3. b), the higher energy 2PA band is broader than for compounds **1** and **4**, but its position remains the same, the wavelength of maximum σ_2 is equal to 725 nm. However, the low energy band, which is located at 875 nm for other investigated compounds, is bathochromically shifted to 1050 nm. Similarly to compound **1**, there is a dip in the two-photon absorption cross-section spectra at twice the wavelength of maximum 1PA band. The 2PA spectrum of compound **3** is presented in Figure 3. c). The higher energy band is bathochromically shifted by 50 nm to 775 nm and the value of σ_2 is around 8000 GM being slightly lower than for compound **2**. For both compounds **2** and

3, the maximum value of two-photon absorption cross-section is enhanced few times, while keeping the molar mass similar to compound **4**. The results prove that a simple modification of the linker, from single bond (**4**) to ethyne (**2**) and di-ethyne (**3**) links between the zinc porphyrins cores cause cooperation effect, enhancing the σ_2* figure of merit. The values of maximum two-photon absorption cross-section and the figure of merit are listed in Table 2. The dimers of zinc porphyrins, especially connected by ethyne and di-ethyne link, are much more effective two-photon photosensitizers than tetraphenyl porphyrin, or other porphyrin derivatives that are commercially used for PDT.

Table 2. Two-photon absorption cross-section, merit factors and two-photon excited oxygen sensitization properties of the investigated porphyrins (**1-4**).

	λ_{2PA}^{max} (nm)	σ_2^{max} (GM)	$\sigma_2^{max}*$ (GM·mol/g)	$\sigma_2^{max}\cdot\phi_\Delta$ (GM)	$\sigma_2^{max}\cdot\phi_\Delta*$ (GM·mol/g)	Two-photon excited oxygen sensitization enhancement factor[c]
1	725	1030	1.4	155	0.21	21
2	725	8900	6.1	3115	2.15	433
3	775	7950	5.4	3737	2.53	519
4	725	2500	1.7	675	0.45	94
H_2TPP	790	12[a]	0.02	7.2[b]	0.01	1

[a] taken from reference 42

[b] taken from reference [27]

[c] Enhancement factor: $\sigma_2^{max}\cdot\phi_\Delta$ of the compound normalized to that of H_2TPP.

It was previously shown e.g. by TPEF measurements[17], that dimerization of porphyrin units leads to enhanced 2PA cross-sections and enhanced two-photon production of singlet oxygen. The σ_2 determined for the porphyrin dimer linked by ethyne and di-ethyne bonds were on the order of 8200 and 5500 GM, which is similar to the values found in the current work for similar porphyrin derivatives. The cooperative effect was explained by a combination of several factors, including strong enhancement of the lowest one-photon Q-transition, better resonance conditions in the three-level system and dramatic enhancement of the excited-state singlet-singlet transition due to linear elongation of the π-conjugated system. Insertion of side groups, being either electron acceptors (A) or donors (D) influence the σ_2 in a way that two-photon absorption cross-section is the lowest for neutral porphyrins, higher for the porphyrins extended with A and the highest for compounds with D groups[43].

Even though the vast majority of molecules targeted to be used in 2PA PDT are based on the porphyrin structure, there are some alternative approaches with reasonable figures of merit. One example is a macromolecular photosensitizer, which was obtained via the covalent anchoring of several molecular PS based on a dibromobenzene (DBB) core onto a water-soluble polymer chain based on poly(N-acryloylmorpholine). It resulted in large 2PA cross-section up to 2600 GM at 740 nm and was proved to be an efficient candidate to cause melanoma cells death upon photo irradiation[44].

4. CONCLUSIONS

In this work, we investigated two-photon absorption and singlet oxygen photogeneration properties in a series of zinc porphyrins, either in a monomer or dimer form. The dimers were connected by single bond, or by ethyne, or di-ethyne link. We observed strong enhancement of the obtained quantum yields for two-photon excited singlet oxygen generation (expressed by the product $\sigma_2^{max} \times \phi_\Delta$) in the porphyrin dimer connected by di-ethyne link, around 3700 GM, which is around 500 times more efficient than tetraphenyl porphyrin. It can be seen that in case of zinc porphyrin derivatives, structure optimization towards high two-photon absorption cross-section simultaneously enhances the properties of singlet oxygen photogeneration. Thus, in order to obtain a material capable of efficient production of singlet oxygen upon two-photon excitation, one should increase the conjugation length, so that π-delocalization is maximized, while maintaining planar structure, as shown for compounds **2** and **3**. The results are very promising for applications involving treatment of deep tumors by photodynamic therapy.

AUTHOR INFORMATION

Corresponding Author

katarzyna.matczyszyn@pwr.edu.pl;
idws@st-andrews.ac.uk

ORCID

Leszek M. Mazur: 0000-0002-4562-0743

Vincent Sol: 0000-0003-0175-0156

Marek Samoc: 0000-0002-5404-2455

Ifor D.W. Samuel: 0000-0001-7821-7208

Katarzyna Matczyszyn: 0000-0001-8578-8340

Conflict of interest statement

There are no conflicts to declare.

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REFERENCES:

1. Brown, S. B.; Brown, E. A.; Walker, I., The Present and Future Role of Photodynamic Therapy in Cancer Treatment. *Lancet Oncol.* **2004**, *5*, 497-508.
2. DeRosa, M. C.; Crutchley, R. J., Photosensitized Singlet Oxygen and Its Applications. *Coord. Chem. Rev.* **2002**, *233–234*, 351-371.
3. Yin, R.; Agrawal, T.; Khan, U.; Gupta, G. K.; Rai, V.; Huang, Y.-Y.; Hamblin, M. R., Antimicrobial Photodynamic Inactivation in Nanomedicine: Small Light Strides against Bad Bugs. *Nanomedicine* **2015**, *10*, 2379-2404.
4. Tardivo, J. P.; Adami, F.; Correa, J. A.; Pinhal, M. A. S.; Baptista, M. S., A Clinical Trial Testing the Efficacy of PDT in Preventing Amputation in Diabetic Patients. *Photodiagn. Photodyn. Ther.* **2014**, *11*, 342-350.
5. Bissonnette, R., Treatment of Acne with Photodynamic Therapy. *G. Ital. Dermatol. Venereol.* **2011**, *146*, 445-456.
6. Kumar, V.; Sinha, J.; Verma, N.; Nayan, K.; Saimbi, C.; Tripathi, A., Scope of Photodynamic Therapy in Periodontics. *Indian J. Dent. Res.* **2015**, *26*, 439-442.
7. Kharkwal, G. B.; Sharma, S. K.; Huang, Y.-Y.; Dai, T.; Hamblin, M. R., Photodynamic Therapy for Infections: Clinical Applications. *Lasers Surg. Med.* **2011**, *43*, 755-767.
8. Boscia, F.; Furino, C.; Sborgia, L.; Reibaldi, M.; Sborgia, C., Photodynamic Therapy for Retinal Angiomatous Proliferations and Pigment Epithelium Detachment. *Am. J. Ophthalmol.* **2004**, *138*, 1077-1079.
9. Redmond, R. W.; Gamlin, J. N., A Compilation of Singlet Oxygen Yields from Biologically Relevant Molecules. *Photochem. Photobiol.* **1999**, *70*, 391-475.
10. Murtinho, D.; Pineiro, M.; Pereira, M. M.; Rocha Gonsalves, A. M. d. A.; Arnaut, L. G.; Miguel, M. d. G.; Burrows, H. D., Novel Porphyrins and a Chlorin as Efficient Singlet Oxygen Photosensitizers for Photooxidation of Naphthols or Phenols to Quinones. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2441-2447.
11. Yamamoto, Y.; Imai, N.; Mashima, R.; Konaka, R.; Inoue, M.; Dunlap, W. C., Singlet Oxygen from Irradiated Titanium Dioxide and Zinc Oxide. *Methods Enzymol* **2000**, *319*, 29-37.
12. Chen, C.-W.; Hsu, C.-Y.; Lai, S.-M.; Syu, W.-J.; Wang, T.-Y.; Lai, P.-S., Metal Nanobullets for Multidrug Resistant Bacteria and Biofilms. *Adv. Drug Del. Rev.* **2014**, *78*, 88-104.
13. Huang, C.-C.; Liu, T.-M., Controlled Au–Polymer Nanostructures for Multiphoton Imaging,

Prodrug Delivery, and Chemo–Photothermal Therapy Platforms. *ACS Appl. Mater. Interfaces* **2015**, *7*, 25259-25269.

14. Smith, A. M.; Mancini, M. C.; Nie, S., Bioimaging: Second Window for in vivo Imaging. *Nat. Nanotechnol.* **2009**, *4*, 710-711.

15. Habermeyer, B.; Guilard, R., Some Activities of Porphychem Illustrated by the Applications of Porphyrinoids in PDT, PIT and PDI. *Photochem. Photobiol. Sci.* **2018**, *17*, 1675-1690.

16. Goyan, R. L.; Cramb, D. T., Near-Infrared Two-Photon Excitation of Protoporphyrin IX: Photodynamics and Photoproduct Generation. *Photochem. Photobiol.* **2000**, *72*, 821-827.

17. Drobizhev, M.; Stepanenko, Y.; Dzenis, Y.; Karotki, A.; Rebane, A.; Taylor, P. N.; Anderson, H. L., Extremely Strong near-IR Two-Photon Absorption in Conjugated Porphyrin Dimers: Quantitative Description with Three-Essential-States Model. *J. Phys. Chem. B* **2005**, *109*, 7223-7236.

18. Nowak-Król, A.; Wilson, C. J.; Drobizhev, M.; Kondratuk, D. V.; Rebane, A.; Anderson, H. L.; Gryko, D. T., Amplified Two-Photon Absorption in Trans-A2B2-Porphyrins Bearing Nitrophenylethynyl Substituents. *ChemPhysChem* **2012**, *13*, 3966-3972.

19. Balaz, M.; Collins, H. A.; Dahlstedt, E.; Anderson, H. L., Synthesis of Hydrophilic Conjugated Porphyrin Dimers for One-Photon and Two-Photon Photodynamic Therapy at NIR Wavelengths. *Org. Biomol. Chem.* **2009**, *7*, 874-888.

20. Karotki, A.; Khurana, M.; Lepock, J. R.; Wilson, B. C., Simultaneous Two-Photon Excitation of Photofrin in Relation to Photodynamic Therapy. *Photochem. Photobiol.* **2006**, *82*, 443-452.

21. Mathai, S.; Smith, T. A.; Ghiggino, K. P., Singlet Oxygen Quantum Yields of Potential Porphyrin-Based Photosensitisers for Photodynamic Therapy. *Photochem. Photobiol. Sci.* **2007**, *6*, 995-1002.

22. Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L., Two-Photon Absorption and the Design of Two-Photon Dyes. *Angew. Chem. Int. Ed.* **2009**, *48*, 3244-3266.

23. Wilkinson, J. D.; Wicks, G.; Nowak-Krol, A.; Lukasiewicz, L. G.; Wilson, C. J.; Drobizhev, M.; Rebane, A.; Gryko, D. T.; Anderson, H. L., Two-Photon Absorption in Butadiyne-Linked Porphyrin Dimers: Torsional and Substituent Effects. *J. Mater. Chem. C* **2014**, *2*, 6802-6809.

24. Kim, K. S.; Lim, J. M.; Osuka, A.; Kim, D., Various Strategies for Highly-Efficient Two-Photon Absorption in Porphyrin Arrays. *J. Photochem. Photobiol., C* **2008**, *9*, 13-28.

25. Yao, D.; Hugues, V.; Blanchard-Desce, M.; Mongin, O.; Paul-Roth, C. O.; Paul, F., Dendritic Molecular Assemblies for Singlet Oxygen Generation: Meso-Tetraphenylporphyrin-Based Biphotonic Sensitizers with Remarkable Luminescence. *New J. Chem.* **2015**, *39*, 7730-7733.

26. McIlroy, S. P.; Cló, E.; Nikolajsen, L.; Frederiksen, P. K.; Nielsen, C. B.; Mikkelsen, K. V.; Gothelf, K. V.; Ogilby, P. R., Two-Photon Photosensitized Production of Singlet Oxygen: Sensitizers with Phenylene−Ethynylene-Based Chromophores. *J. Org. Chem.* **2005**, *70*, 1134-1146.

27. Yao, D.; Zhang, X.; Triadon, A.; Richy, N.; Mongin, O.; Blanchard-Desce, M.; Paul, F.; Paul-Roth, C. O., New Conjugated Meso-Tetrafluorenylporphyrin-Cored Derivatives as Fluorescent Two-Photon Photosensitizers for Singlet Oxygen Generation. *Chem. - Eur. J.* **2017**, *23*, 2635-2647.

28. Jiblaoui, A.; Baudequin, C.; Chaleix, V.; Ducourthial, G.; Louradour, F.; Ramondenc, Y.; Sol, V.; Leroy-Lhez, S., An Easy One-Pot Desilylation/Copper-Free Sonogashira Cross-Coupling Reaction Assisted by Tetra-Butylammonium Fluoride (TBAF): Synthesis of Highly π-Conjugated Porphyrins. *Tetrahedron* **2013**, *69*, 5098-5103.

29. Poon, C. T.; Zhao, S. S.; Wong, W. K.; Kwong, D. W. J., Synthesis, Excitation Energy Transfer and Singlet Oxygen Photogeneration of Covalently Linked N-Confused Porphyrin-Porphyrin and Zn(II) Porphyrin Dyads. *Tetrahedron Lett.* **2010**, *51*, 664-668.

30. Tanielian, C.; Heinrich, G., Effect of Aggregation on the Hematoporphyrin-Sensitized Production of Singlet Molecular Oxygen. *Photochem. Photobiol.* **1995**, *61*, 131-135.

31. Kolkowski, R.; Samoc, M., Modified Z - Scan Technique Using Focus-Tunable Lens. *J. Opt.* **2014**, *16*, 125202.

32. L. Anderson, H., Building Molecular Wires from the Colours of Life: Conjugated

Porphyrin Oligomers. *Chem. Commun.* **1999**, 2323-2330.

33. Senge Mathias, O.; Pintea, M.; Ryan Aoife, A., Synthesis and Crystal Structure of a Meso-Meso Directly Linked Bisporphyrin. *Z. Naturforsch., B: Chem. Sci.* **2011**, *66*, 553-558.

34. Peeks, M. D.; Neuhaus, P.; Anderson, H. L., Experimental and Computational Evaluation of the Barrier to Torsional Rotation in a Butadiyne-Linked Porphyrin Dimer. *Phys. Chem. Chem. Phys.* **2016**, *18*, 5264-5274.

35. Kuimova, M. K.; Balaz, M.; Anderson, H. L.; Ogilby, P. R., Intramolecular Rotation in a Porphyrin Dimer Controls Singlet Oxygen Production. *J. Am. Chem. Soc.* **2009**, *131*, 7948-7949.

36. Schmitt, J.; Heitz, V.; Jenni, S.; Sour, A.; Bolze, F.; Ventura, B., π-Extended Porphyrin Dimers as Efficient near-Infrared Emitters and Two-Photon Absorbers. *Supramol. Chem.* **2017**, *29*, 769-775.

37. Deiana, M.; Mettra, B.; Mazur, L. M.; Andraud, C.; Samoc, M.; Monnereau, C.; Matczyszyn, K., Two-Photon Macromolecular Probe Based on a Quadrupolar Anthracenyl Scaffold for Sensitive Recognition of Serum Proteins under Simulated Physiological Conditions. *ACS Omega* **2017**, *2*, 5715-5725.

38. Van Stryland, E. W.; Sheik-Bahae, M., Z-Scan Measurements of Optical Nonlinearities. In *Characterization Techniques and Tabulations for Organic Nonlinear Materials*, Kuzyk, M. G.; Dirk, C., Eds. Marcel Dekker: 1998; pp 655-692.

39. Gao, B.; Mazur, L. M.; Morshedi, M.; Barlow, A.; Wang, H.; Quintana, C.; Zhang, C.; Samoc, M.; Cifuentes, M. P.; Humphrey, M. G., Exceptionally Large Two- and Three-Photon Absorption Cross-Sections by OPV Organometalation. *Chem. Commun.* **2016**, *52*, 8301-8304.

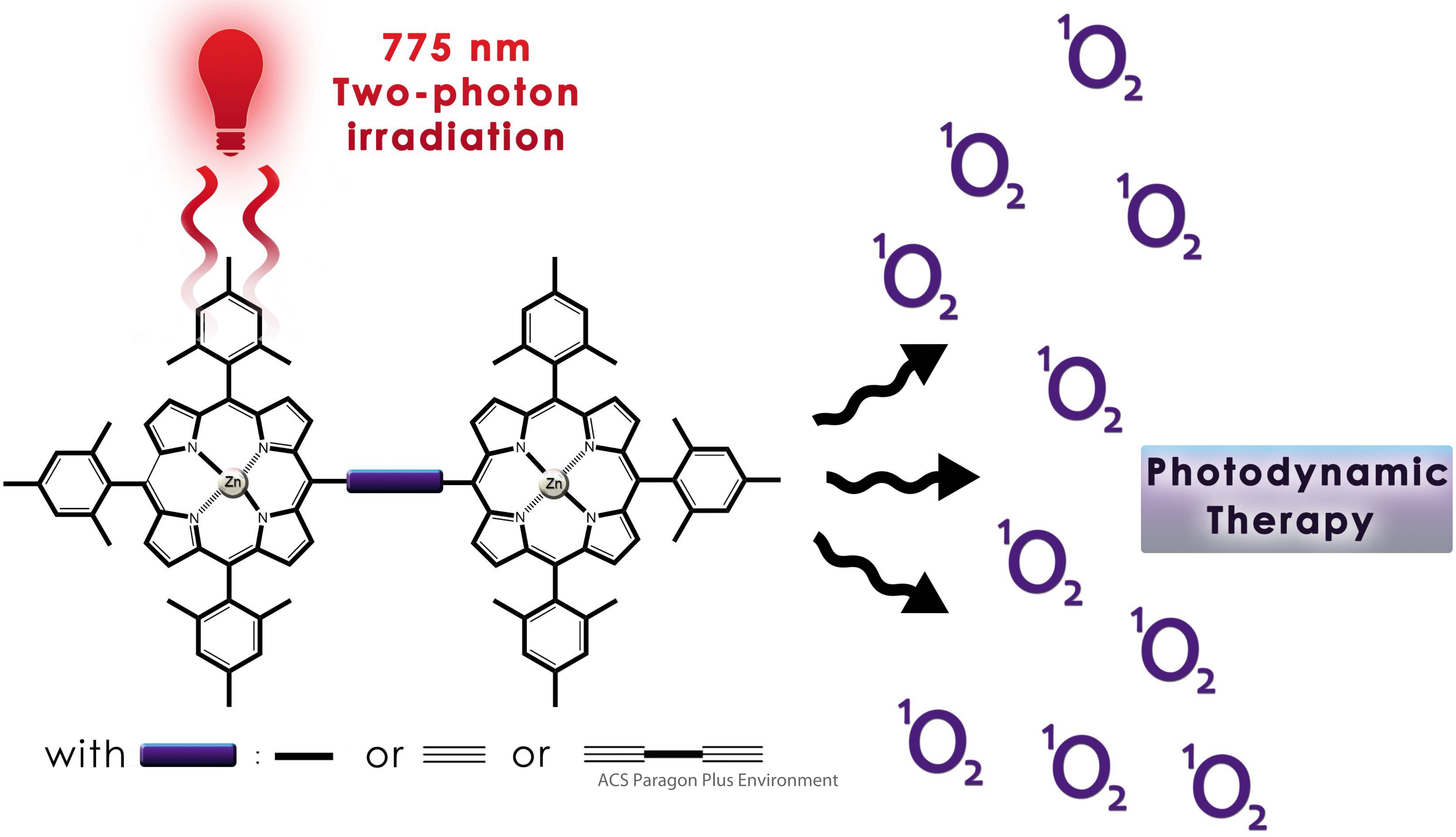
40. Lin, T.-C.; Chien, W.; Mazur, L. M.; Liu, Y.-Y.; Jakubowski, K.; Matczyszyn, K.; Samoc, M.; Amini, R. W., Two- and Three-Photon Absorption Properties of Fan-Shaped Dendrons Derived from 2,3,8-Trifunctionalized Indenoquinoxaline Units: Synthesis and Characterization. *J. Mater. Chem. C* **2017**, *5*, 8219-8232.

41. Sheik-Bahae, M.; Said, A. A.; Wei, T. H.; Hagan, D. J.; Stryland, E. W. V., Sensitive Measurement of Optical Nonlinearities Using a Single Beam. *IEEE J. Quantum Electron.* **1990**, *26*, 760-769.

42. Makarov, N. S.; Drobizhev, M.; Rebane, A., Two-Photon Absorption Standards in the 550–1600 nm Excitation Wavelength Range. *Opt. Express* **2008**, *16*, 4029-4047.

43. Koszelewski, D.; Nowak-Krol, A.; Drobizhev, M.; Wilson, C. J.; Haley, J. E.; Cooper, T. M.; Romiszewski, J.; Gorecka, E.; Anderson, H. L.; Rebane, A.; Gryko, D. T., Synthesis and Linear and Nonlinear Optical Properties of Low-Melting π-Extended Porphyrins. *J. Mater. Chem. C* **2013**, *1*, 2044-2053.

44. Cepraga, C.; Marotte, S.; Ben Daoud, E.; Favier, A.; Lanoë, P.-H.; Monnereau, C.; Baldeck, P.; Andraud, C.; Marvel, J.; Charreyre, M.-T.; Leverrier, Y., Two-Photon Photosensitizer–Polymer Conjugates for Combined Cancer Cell Death Induction and Two-Photon Fluorescence Imaging: Structure/Photodynamic Therapy Efficiency Relationship. *Biomacromolecules* **2017**, *18*, 4022-4033.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