Anales de la Asociación Química Argentina

División Jóvenes Profesionales

Mini-Review

SUPRAMOLECULAR PHOTOSENSITIZERS AS IMPROVED TOOLS FOR ANTICANCER AND ANTIMICROBIAL TREATMENTS

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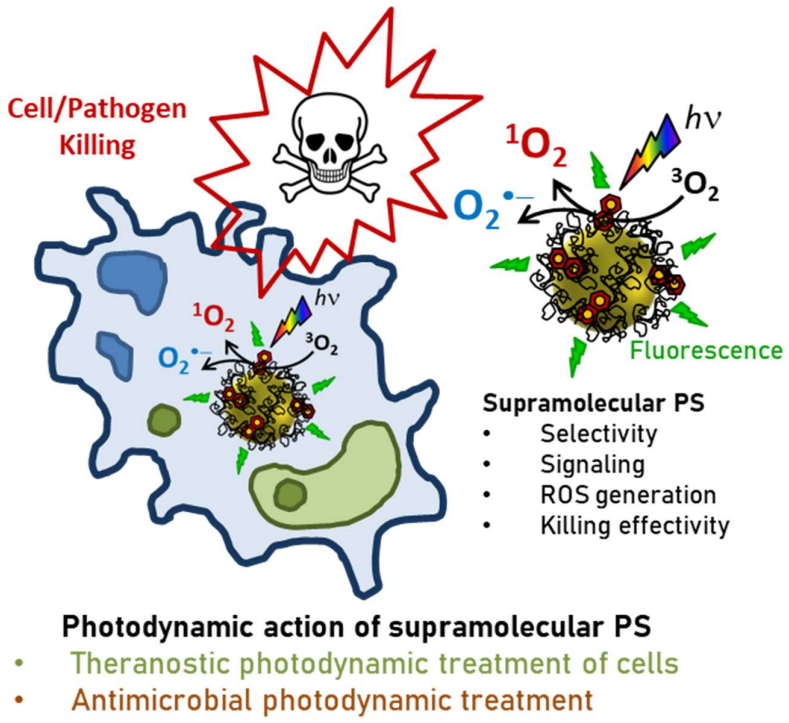
Graphical abstract



Resumen

La absorción de luz por una molécula llamada fotosensibilizador (PS) en presencia de oxígeno molecular desencadena la generación de especies reactivas de oxígeno (ROS), que en un medio biológico son capaces de degradar las moléculas objetivo. Este efecto se denomina acción fotodinámica (PDA) y es la raíz de la terapia fotodinámica (TFD), que en los últimos años se convirtió en una herramienta muy útil y versátil para inactivar tanto a las células dañinas como a los patógenos. Con el desarrollo de estructuras

supramoleculares funcionalizadas con propiedades fotosensibilizantes, hoy en día los horizontes de la TFD en aplicaciones antitumorales y antimicrobianas han mejorado y ampliado enormemente. En este artículo de revisión, discutimos los conceptos básicos de los procesos de fotosensibilización y TFD, revisando algunos de los avances más recientes en aplicaciones emergentes de fotosensibilizadores supramoleculares contra patógenos resistentes a múltiples fármacos, así como en tratamientos teranósticos del cáncer, donde se realiza con el mismo conjunto supramolecular tanto el diagnóstico como la terapia.

Abstract

Light absorption by a molecule called photosensitizer (PS) in the presence of molecular oxygen triggers the generation of reactive oxygen species (ROS), which can degrade target molecules in a biological milieu. This effect is called photodynamic action (PDA) and is the root of photodynamic therapy (PDT), which in recent years became a very useful and versatile tool for killing both harmful cells and pathogens. With the development of functionalized supramolecular structures with photosensitizing properties, nowadays the horizons of PDT in antitumoral and antimicrobial applications have been greatly improved and expanded. In this review article, we discussed the basics of the photosensitization and PDT processes, reviewing some of the most recent advances on emerging applications of supramolecular photosensitizers against multi-drug resistant pathogens as well as in cancer theranostic treatments, where both diagnostic and therapy is performed with the same supramolecular ensemble.

Palabras Clave: *fotosensibilización, terapia fotodinámica, fotosensibilizadores supramoleculares, antimicrobianos, anticancerígenos.*

Keywords: *photosensitization, photodynamic therapy, supramolecular photosensitizers, antimicrobial, anticancer.*

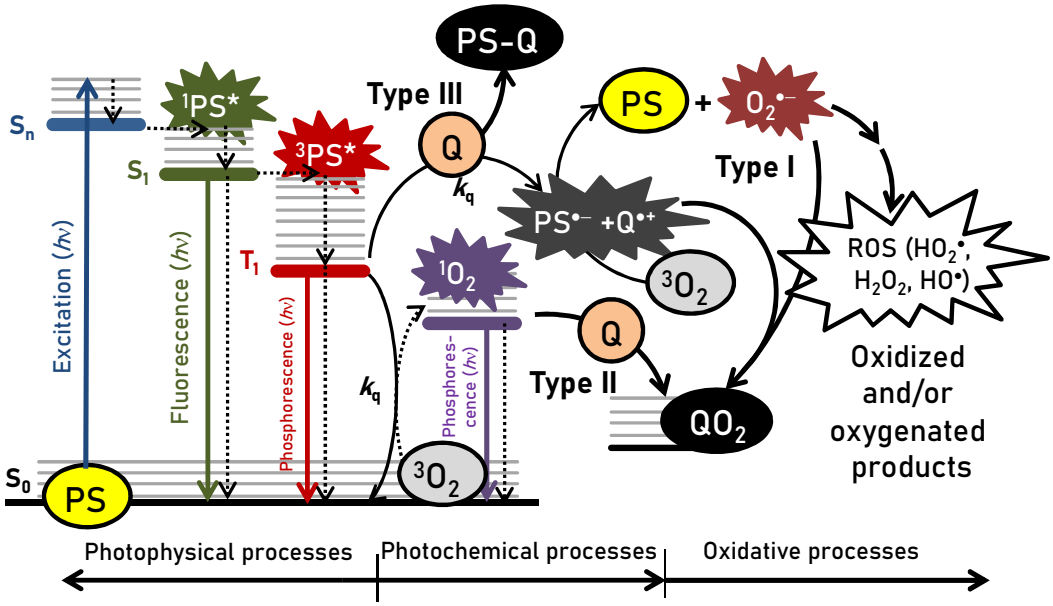
1. Basics of photosensitization

Solar light is a ubiquitous vector that promotes life on our planet, mainly by bacterial and plant photosynthesis. However, the photon flux incoming from the Sun also induces other photophysical and photochemical processes, such as visual and non-visual photoreception, photomovement, photoionization, photosensitization, photodegradation, environmental photochemistry, and photobiology, among others [1]. From all of them, from a biological point of view, photosensitization is particularly relevant due to their involvement from energy conversion to cell killing processes [2].

According to the "Gold Book" of the International Union of Pure and Applied Chemistry (IUPAC), the term photosensitization refers *to the process by which a photochemical or*

photophysical alteration occurs in one molecular entity as a result of initial absorption of radiation by another molecular entity called a photosensitizer (PS) [3]. Hence, a very vast number of natural and artificial photochemical and photobiological processes are easily initiated through photosensitized mechanisms by the absorption of lower energy photons of the visible or near-infrared region, *i.e.* above 400 nm, improving the utilization of the solar irradiation that impacts the Earth´s surface [1, 4].

Scheme 1 summarizes the photophysical and photochemical pathways involved during the photosensitization process of a PS molecule. The first step is the absorption of light by the ground state of the PS to finally produce the triplet excited state (^{3}PS*) by intersystem crossing from the lowest singlet excited state (^{1}PS*). Due to the spin-forbidden nature of the $S_0 \leftarrow T_1$ transition, the ^{3}PS* is a long-lived species (usually in a time-scale from hundreds of nanoseconds to the millisecond in fluid solutions) allowing a larger reactivity during its lifetime [5].



Scheme 1. Photophysical and photochemical pathways involved in photosensitized processes. See text for abbreviation meaning.

The primary photochemical reaction of ^{3}PS* with Q can produce radical/radical ion species by either hydrogen atom abstraction or electron-transfer reactions, respectively [6, 7]. Even if H-atom

transfer or electron-transfer reactions can take place in both directions, the ^{3}PS* acts most commonly as an oxidant. Thus, under aerobic conditions, the neutral or anion radicals PS$^{\bullet}$/PS$^{\bullet-}$ can react with molecular oxygen $^3O_2$ to generate anion superoxide ($O_2^{\bullet-}$) and the PS ground state molecule (*type I mechanism*) [6, 7]. In turn, $O_2^{\bullet-}$ can produce other reactive oxygen species (ROS) in subsequent secondary steps, such as hydroperoxyl radical ($HO_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), and hydrogen peroxide (H_2O_2), as well as other oxidants involving a substrate R, *e.g.* peroxyl radicals ($ROO^{\bullet}$) and alkoxyl radicals ($RO^{\bullet}$) [6-8].

Conversely, the diffusion-controlled quenching of ^{3}PS* by $^3O_2$ through an efficient down-hill energy-transfer process ($k_q^{O2} \approx 10^9$ $M^{-1}s^{-1}$) produces singlet molecular oxygen ($^1O_2$) and the ground state of the PS. $^1O_2$ is the lowest excited state of $^3O_2$, with an energy gap of 22.5 kcal mol^{-1}, showing in solution a lifetime ranging between hundreds of ns to several ms depending on the solvent nature, while in gas phase up to several seconds [9]. Since $^1O_2$ is an electrophilic species, it can react with electron-rich biomolecules such as guanine (but not with other nucleic acids), unsaturated lipids, and amino acid residues to form mainly endoperoxides from [4 + 2] cycloadditions, dioxetanes from [2 + 2] cycloadditions, hydroperoxides from *"ene"* reactions or phenol oxidations, and sulfoxides from sulfide derivatives [7,10,11]. The oxidation of target molecules by the photosensitized generation of $^1O_2$ is termed the *type II* mechanism [6,7].

On the other hand, the ^{3}PS* can also react with Q to form a stable product (P) [8,12]. This oxygen-independent process is sometimes denominated as a *type III* reaction, but strictly it cannot be considered as a photosensitized reaction since the PS molecule is depleted [7, 13].

Hence, both *type I* and *type II* photosensitization mechanisms drive to the final formation of highly reactive and harmful ROS species that react with biological molecular targets (*e.g.* DNA, lipids, proteins) inducing damage of subcellular organelles, which in turn can lead to tissue injury, inflammation, and finally cell killing [14-17]. Usually, *type II* mechanism prevails and the main ROS generated is $^1O_2$, albeit the *type I* pathway can eventually result in the generation of

the highly toxic hydroxyl radical (HO•) from the secondary transformation of H_2O_2 by Fenton reaction with iron (II) [18].

Thus, the term "photodynamic action" (PDA) refers exclusively to oxygenation reactions of organic substrates that only occurs under the illumination of the PS added to the reaction mixture, but not in darkness, and that the PS is not consumed during these reactions [18]. Hence, any cell-killing process produced by the combined use of light, photosensitizer, and $^3O_2$ is denominated photodynamic therapy (PDT), while the use of the term "oxygen-independent photodynamic action" to describe the *type III* reaction is inaccurate [7].

2. Molecular and supramolecular PS

Molecular photosensitization is a field of high current interest in several scientific and technological areas. Figure 1A shows the exponential growth of the number of published articles since 1985 reported by the Scopus® database obtained by using "molecular photosensitizer" as the search term. Hundreds of organic and inorganic molecules have been used as PS in organic synthesis, energy conversion, clinical and environmental applications among others [19-21].

Many natural and artificial PS have been tested in PDT, mainly those with high efficiency of $^1O_2$ generation (type II mechanism) [22, 23], albeit *type I* oxidation processes are also relevant in the modification of nucleic acids [7, 24, 25] and proteins [26-30].

Typical organic PS include compound groups like tetrapyrroles (*e.g.* porphyrins, chlorins, phthalocyanines); organic dyes (*e.g.* xanthenic and phenothiazines derivatives such as rose bengal (RB) and methylene blue (MB), respectively, boron-dipyrromethenes BODIPYs, cyanines, and coumarins); quinones (anthraquinones and hypericins), isoalloxazines derivatives or flavins (lumiflavin, riboflavin, flavin mononucleotide), phenalenones, biological molecules (proteins, chlorophylls, verdins, vitamins, etc.) [1, 2, 14, 17, 19, 22, 23, 31, 32] . Chart 1 shows some of these structures, but most of them can be found in the preceding citations.

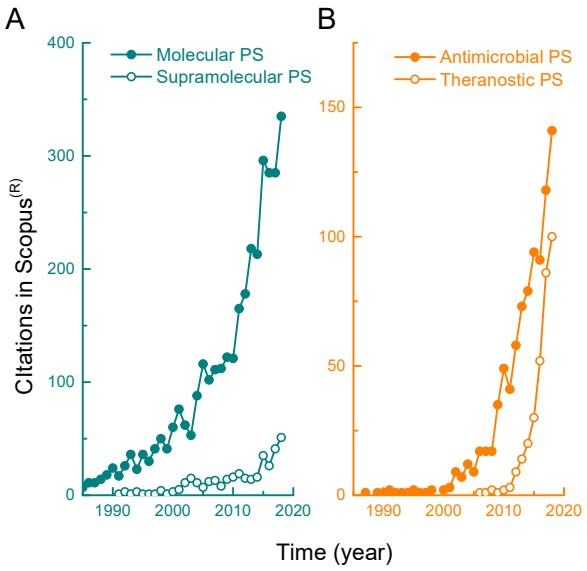


Figure 1. Number of the photosensitizer (PS)-related papers published since 1985 obtained from Scopus® database by searching in the title, abstract, and keyword sections the following terms: (A) "molecular photosensitizer" and ''supramolecular photosensitizer''; and (B) ''antimicrobial photosensitizer'' and "theranostic photosensitizer".

An ideal molecular PS for photodynamic applications is expected to fulfill several photophysical and photochemical features, such as a high molar absorptivity coefficient, mainly in the visible and near-infrared (NIR) light spectrum, efficient intersystem crossing leading to high excited triplet quantum yields ($\Phi_T \geq 0.2$), with larger triplet-state energy than that of $^1O_2$ (*i.e.* > 22.5 kcal mol^{-1}) and long lifetime (~μs) to increase the probability of interaction with triplet molecular oxygen for the subsequent efficient ROS generation [15, 31, 33]. Figure 2 shows the absorptivity spectra of some typical organic molecular PS covering the visible region together with their singlet oxygen quantum yield average values in fluid solutions (Φ_Δ). Furthermore, PS molecules are expected to be photo-stable and no cytotoxic in the absence of light, together with the ability to interact with cell targets [15, 33]. Nevertheless, all these requirements are very difficult to be satisfied by a simple molecule, and hence new strategies are required to obtain PS systems able to satisfy multiple functions [34-42].

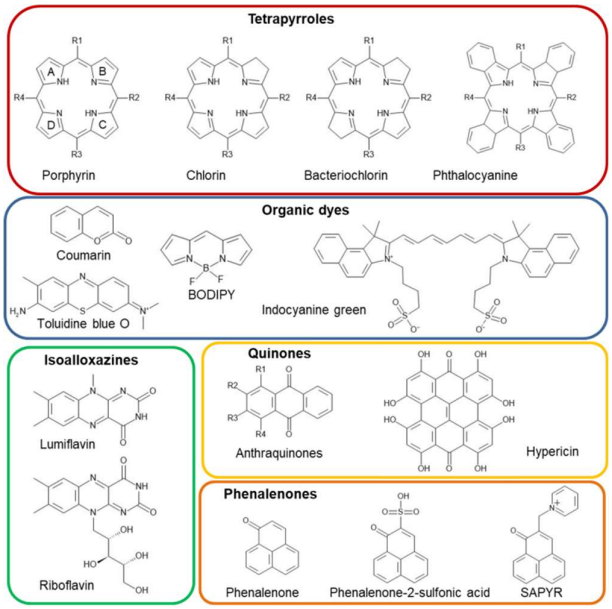


Chart 1. Molecular structures of some selected organic photosensitizers.

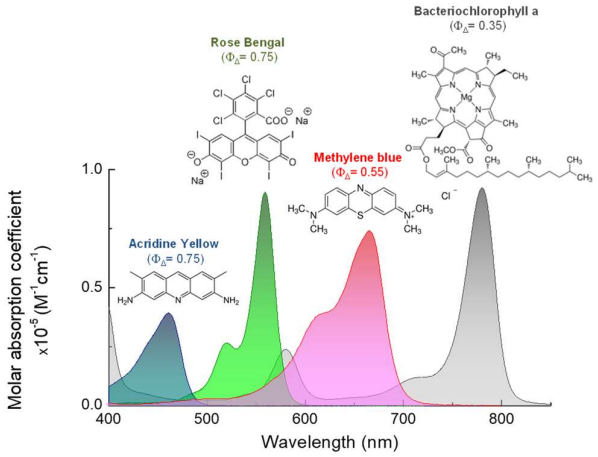


Figure 2: Structure and absorptivity spectra and singlet oxygen quantum yield (Φ_Δ) values of typical molecular PS covering the visible region of the electromagnetic spectrum.

Regarding this, ***supramolecular chemistry*** can afford practical solutions for the development and design of PS systems with multiple desired functionalities. The concept behind supramolecularity is to obtain molecular systems of higher complexity by the association of two or more chemical species held together due to intermolecular forces [43, 44]. The association driving forces include van der Waals, electrostatic, hydrogen bonding, hydrophobic interactions, etc.,

some of which are often cooperatively working in one supramolecular complex [43, 44]. Even more, in many cases, the supramolecular complex owns new advantageous properties missing in the individual components [44]. Nature is an inspiration source of supramolecular examples, for instance, the building of three-dimensional structures of proteins, DNA, and phospholipid membranes, among others [45, 46]. The development of facile approaches to fabricate submicron-structured assemblies allows to obtain supramolecular structures with high specificity, selective targeting and/or signaling, generation or scavenging of ROS (pro- or antioxidant function, respectively), sensitive to external stimuli (pH, ionic strength, light, temperature, etc.) [45-47].

A new article search in Scopus® using the term "supramolecular photosensitizer" leads to a fewer number of articles than the former search for "molecular photosensitizer" in the same period, but with almost twice-faster growth in the last decade (Figure 1A). In particular, photosensitizing processes using supramolecular devices are being intensively explored, especially for medical and energy conversion applications [38, 48, 49].

Usually, the bottom-up strategy is selected for the building up of the supramolecular PS, using a large diversity of nanomaterials (*e.g.* metal nanoparticles, fullerenes, carbon nanotubes, semiconductors, nanocellulose, etc) [35, 37, 40, 50-54], biomolecules as proteins [26, 55-58], and self-assembled nano/micro-systems (micelles, vesicles, multilayers, microcapsules, polymers, peptides, etc.) [24, 53, 59-63]. Chart 2 shows a pictorial representation of some representative supramolecular PS systems.

Figure 1B also shows the evolution of reported articles on photosensitization oriented to antimicrobial [64-67], and cancer theranostic applications [68-70]. The term ***theranostics*** implies the combination of **therapy** + diag**nostic** functions by a single composite and is a new field of medicine allowing specific targeted diagnostic and efficient therapy [71]. Hence, supramolecularity became a very helpful tool for the design of photosensitizing systems with multiple functions and/or properties.

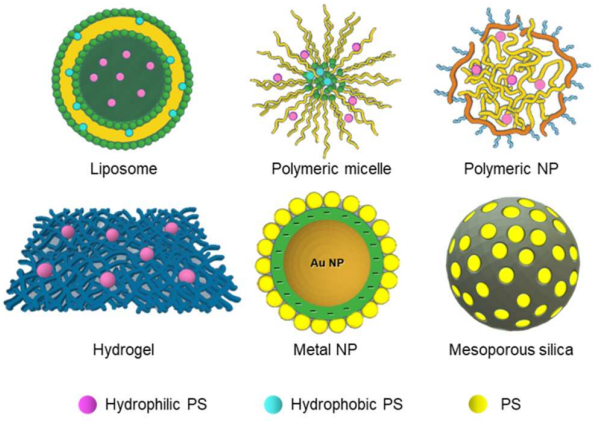


Chart 2. Schematic representation of some supramolecular PS systems

Both applied research areas have blasted within the last two decades, probably associated with two world-wide concerns: i) the increase of microbial resistance to the common antibiotics used to treat infections caused by bacteria, fungi, viruses, and parasites, that has become one of the leading causes of morbidity and mortality [72-74], and ii) the need of painless and less invasive cancer treatments [75]. For the latter, the design of supramolecular PS became a very interesting challenge oriented to obtain theranostic agents with multiple functions such as specific targeting to the neoplastic cells, tracking through the body tissues by exploiting the intrinsic luminescence/photoacoustic/magnetic responses, and with efficient PDT in the treated cells [76-78]. This application field shows a promising horizon for PDT as illustrated by the increase of reported articles since 2010 (Figure 1B).

3. Antitumoral vs. antimicrobial photodynamic treatments

Despite the PDA was discovered more than a hundred years ago by Oscar Raab [79], who found that *Paramecium* spp. protozoans were killed after staining with acridine orange and subsequent exposure to bright light, it was not until a quarter of a century ago that photodynamic therapy (PDT) was clinically approved for the treatment of a small number of selected tumors [15, 33]. Nowadays, its application has been tremendously expanded to include health specialties such as

cardiology, urology, immunology, ophthalmology, dentistry, dermatology, and cosmetics [15, 33, 80, 81]. Hence, the PDT term was initially coined to describe the minimally invasive therapeutic modality used for the selective treatment of a variety of neoplastic and non-neoplastic diseases [15, 33]. In PDT, the photo-generated cytotoxic species induce the killing of the target cells by different death mechanisms, *e.g.* necrosis, apoptosis, and autophagy [82].

Most effective anti-cancer PS molecules are relatively lipophilic compounds, with little or no overall positive or negative charges, so they can rapidly diffuse towards subcellular membrane structures such as mitochondria and endoplasmic reticulum (ER) of tumor cells. Nevertheless, for more polar PS, slower incorporation by endocytosis can be expected [15].

The successful utilization of an anticancer PS depends on several factors: a) good light-absorbing properties in the "phototherapeutic window" located between 650 and 1300 nm, where the absorption and scattering of light by tissues is minimal; b) large capability of $^1O_2$ generation close to the target biomolecules or organelles in the treated cells or tissues, considering the short lifetime (ns to μs) of this species; c) selective up-take of the PS into neoplastic cells to minimize non-desired toxic effects on healthy cells; and d) photobleaching capability (*i.e.* light-mediated destruction of the PS) since some new studies suggest that this phenomenon can avoid undesired over-treatment effects [14, 17, 36, 54, 83].

Most PS used in PDT show absorption bands in the far-red and NIR spectral regions for deeper tissue penetration [17, 31, 33, 81, 83]. Cyclic tetrapyrrolic structures as porphyrins and their analogs, chlorins, bacteriochlorins, phthalocyanines, etc. absorb light in the "phototherapeutic window" where tissue components such as hemoglobin and water are poor light absorbers [31].

The development of high power NIR lasers has overcome the poor or no light absorption by PS in the phototherapeutic window, *i.e.* PS with absorbing properties at the UVA and blue-light edges, through a two-photon excitation process allowing deeper penetration in the tissue, enhancement of the three-dimensional space selectivity, and less PS photobleaching, improving

the application of PDT in precise cancer treatment [84-86]. Furthermore, two-photon imaging was successfully integrated with PDT to diagnose diseases, to guide and monitor the treatment, and to assess the success of therapy (*i.e.* theranostic action) [84-86].

Another application field of PDT was prompted by the increasing challenge of antimicrobial resistance [72-74]. The so-called ‘‘ESKAPE’’-pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* strains) are one of the main current threats to public health for they have become resistant to almost all kinds of available antibiotic treatments [73]. As for antifungal drugs, the development of resistance against all drug classes has been reported in *Candida* and *Aspergillus* species such as *Candida glabrata*, *Candida auris*, and *Aspergillus fumigatus* [87]. Nowadays, with the recent COVID-19 outbreak, more scientific research efforts on the efficacy of photosensitized and PDT methods to combat the SARS-CoV-2 virus is needed, but until now, few reported articles about PDT methods applied to treat COVID-19 can be found [88].

Moreover, several microorganisms, such as bacteria, fungi, green algae, cyanobacteria, and lichen, possess the ability to grow in biofilm ecosystems rather than as planktonic single-species cultures, where they are usually incorporated in a matrix of extracellular polymeric substances (EPS) auto-produced by the microorganisms. Biofilm communities have developed resistance mechanisms that act synergistically, making them even less susceptible to antibiotic treatments [89, 90]. These mechanisms include, but are not limited to, slow penetration of antimicrobial agents through the biofilm matrix and interaction with its components, reduced growth rates, the formation of a sub-population of “persister cells”, the quorum-sensing systems, and the inter-species propagation of antibiotic resistance through horizontal gene transfer [74, 89, 91]. For the above mentioned, and considering that 80% of human infections are caused by biofilms [92], the search for treatments that allow the eradication of biofilms has become a key issue.

In this context, antibacterial photodynamic therapy (APDT) was outlined as a suitable alternative antimicrobial treatment to deal with the antibiotic-resistance issue [16, 35, 72, 74, 80, 93]. One advantage

of APDT over conventional antibiotic treatments is its unspecific killing mechanism since it involves the generation of ROS that can react with a wide variety of molecular components in the pathogen, with the additional potential that the PS can behave as a broad-spectrum antimicrobial agent. Thus, the possibility that microorganisms generate resistances through random mutations becomes extremely unlikely.

In general terms, PSs for antimicrobial applications do not need to absorb light in the "phototherapeutic window" as in the case of anti-cancer PSs, since the superficial nature of the microbial infections and contaminations does not require a deep light penetration, being also very efficient the PS molecules absorbing blue-light [22, 23, 67, 93]. Besides, antimicrobial PS are expected to be photo-stable, mainly in the case of immobilized PS intended to be reutilized [20, 34, 41, 51], lack dark toxicity and to be active within an appropriate concentration range, among other parameters involved in the photodynamic inactivation of pathogens to avoid harming eukaryotic mammalian cells [80]. Additional structural features of antimicrobial PS should be considered regarding their ability to interact with microbial targets. Molecular photosensitizers generally consist of planar π-conjugated aromatic structures, a prerequisite for light absorption in the visible and NIR regions. However, for this reason, these types of molecules tend to be hydrophobic and prone to aggregation, and hence self-quenching processes of the excited states of the PS constrains their applicability on APDT in aqueous media [20, 34, 41].

Furthermore, for the same PS, APDT efficiency can be different among microbial agents, mainly due to their variable cell surface structures. A good antimicrobial PS must photo-induce the reduction of the colony-forming unit (CFU) by at least 3 log units. Positively charged PSs have a broader action spectrum, being able to inactivate both Gram (+) and Gram (−) bacteria, as well as pathogenic yeasts [16, 64, 80, 94]. Hence, Gram (+) bacteria and yeasts are the most susceptible to APDT, as they are also affected by neutral and negatively charged PS molecules. Moreover, the porosity of Gram (+) outer wall, located outside the cytoplasmic membrane, allows the passage of complex nutrients with molecular weights between 30–60 KDa, so they are as well permeable

to supramolecular PSs within this size range [95]. In contrast, Gram (–) bacteria have an additional asymmetric and highly organized outer membrane to which porins, lipopolysaccharide, and lipoprotein constituents bestow a high negative charge density. This extra barrier constrains cell permeability, letting only small hydrophilic molecules (up to ~700 Da) pass through [96].

For the above mentioned, the impartment of positive charges in a PS proved to be a successful strategy to improve antimicrobial photodynamic activities of all kind of PS families (phenothiazidium dyes, porphyrins, phthalocyanines, flavins, among others) [16, 97]. An illustrative example is represented by a water-soluble phenalen-1-one derivative, *i.e.* (2-((4-pyridinyl)methyl)-1H-phenalen-1-one chloride) termed SAPYR, Chart 1 [98]. This cationic phenalenone derivative showed a similar $^1O_2$ quantum yield close to one, as the anionic derivative PNS [1H-Phenalen-1- one-2-sulfonic acid] [99]. However, PNS did not produce APDT action because of its negative charge, while the positively charged pyridinium-methyl moiety in SAPYR facilitated its incorporation in bacteria, allowing a successful APDT for inactivation of a polybacteria biofilm in a single treatment, with efficacy of ≥ 99.99% [98].

4. Antitumoral supramolecular PS

Most of anti-cancer PDT difficulties that could not be resolved by a single molecular PS have been overcome by combining concepts of supramolecular chemistry, nanotechnology, and photophysics to obtain new supramolecular PS systems with dual or multiple functions, *e.g.* imaging, specific recognition and attaching to cancer cells, drug/PS transport and controlled delivery, ROS generation, etc. [37, 54, 68, 69, 77, 78, 100-105].

The role of supramolecular and nanocomposites structures to build multifunctional theranostic PSs using metal nanoparticles, carbon nanotubes, carbon quantum dots, fullerenes, titanium dioxide, polymeric dendrimers, vesicles, microbubbles, graphene, mice, silica nanoparticles, nanogels, etc., have been extensively described in several recent reviews [17, 34, 37, 38, 68, 83,100,105-107].

Among the diagnostic techniques used are the optical microscopy (OM), photoacoustic image (PAI), computed tomography (CT), positron emission tomography (PET), single-photon computed tomography (SPCT), magnetic resonance imaging (MRI), ultrasound (US), etc. [68, 77, 78, 84, 103, 108-110]. Some of the most recent examples of theranostic supramolecular PS are discussed in the following paragraphs.

Chlorin e6 (Ce6) was loaded onto superparamagnetic iron oxide (SPION) nanoparticles via an oil-in-water emulsion [77]. The Ce6-SCs nanocomposite of 92 nm of size showed high solubility and excellent stability under physiological conditions. The enhanced permeability and retention achieved by this supramolecular formulation led to its selective accumulation within tumors, as observed by magnetic resonance (MR) and fluorescence dual-mode imaging following intravenous injection of the nanocomposite in a murine tumor model. After Ce6-SCs administration, PDT was performed through the excitation of Ce6 by a 665 nm laser. A high singlet oxygen generation was observed leading to a significant delay of tumor growth in mice.

Wu et al. [102] tested a theranostic nanocomposite prepared through the electrostatic interaction between a cationic tetraplatinated porphyrin complex (PtPor) and negatively charged carbon quantum dots (CQDs) for PDT against human cervical carcinoma cells (HeLa). The CQDs@PtPor nanocomposite integrates both optical properties of CQDs and the anticancer PDA of porphyrins. The PDT efficiency of CQDs@PtPor was stronger than that of the organic molecular PtPor, mainly by the enhanced production of $^1O_2$ induced by the presence of CQDs.

Li et al. [60] have recently revised peptide-modulated self-assembly strategies for supramolecular nanotheranostics. Three major assembly schemes were discussed: (1) self-assembly of peptide-photosensitizers, (2) self-assembly of peptide-anticancer drugs, and (3) multicomponent cooperative (PS-anticancer drugs) self-assembly. In the case of supramolecular PS systems, the desired strategy is the precise releases of the PS at tumoral cells as induced by some tumor microenvironment stimulus (*e.g.* pH < 6.0, heightened glutathione (GSH) level, overexpressed

enzymes, and biomarkers) to achieve controlled photosensitizer activation at tumor sites rather than normal tissues.

Non-covalent assembled nanoparticles made with the conjugated polymer poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) and the amphiphilic copolymer stabilizer (PS-PEG-COOH) doped with hydrophobic platinum octaethylporphyrin (PtOEP) as PS were tested as theranostic PDT agent in glioblastoma (T98G), colorectal adenocarcinoma (SW480) and macrophage (RAW 264.7) cell lines.[62] The non-covalent assembled nanoparticles showed $\Phi_\Delta = 0.24$ in water, as confirmed by monitoring the NIR phosphorescence emission of $^1O_2$, and the PDT treatment was effective in the three types of cells [62].

Self-assembled micelles and liposomes were formerly used as nano-spherical carriers of molecular PS [83, 107, 111, 112]. Micelles can carry hydrophobic PS in their hydrocarbon interior [111], while liposomes can encapsulate either hydrophilic drugs within the aqueous regions or lipophilic molecules within the lipid bilayers [107]. Liposomes were used as delivery systems for 5-aminolevulinic acid (5-ALA), a pro-drug for the biosynthesis of the potent endogenous photosensitizer protoporphyrin IX (PpIX) in neoplastic cells, and the synthetic lipophilic temoporfin (mTHPC), for PDT of superficial skin lesions [107]. Liposomes bearing PS enhance penetration and accumulation of the PS in the skin compared with free molecules, leading to higher PDT efficacy and enable topical applications for deep and hyperkeratotic skin lesions. Moreover, PDT-driven by liposomes reduced the absorption of the PS into systemic circulation compared with the free drug, minimizing the risk of generalized photosensitivity.

Recently, Xu et al. [108] showed a study of various PS such as benzoporphyrin monoacid ring A (BPD), tetraphenylporphinesulfonate (TPPS4), and sodium 4-[2-[(1*E*,3*E*,5*E*,7*Z*)-7-[1,1-dimethyl-3-(4-sulfonatobutyl)benzo[e]indol-2-ylidene]hepta-1,3,5-trienyl]-1,1-dimethylbenzo[e]indol-3-ium-3-yl]butane-1-sulfonate (ICG) targeted with polyethyleneglycol (PEG) attached to 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) nanoliposomes (50-100 nm). *In vitro* studies showed that BDP encapsulated in PEGylated liposomes (LBDP) had an

increase in the efficiency of PDT and greater destruction of blood vessels in tumor tissues compared to free BPD. The *in vivo* localization of the supramolecular PS in tumor-bearing mice was performed by both fluorescence imaging (FLI) and photoacoustic imaging (PAI) dual-model. This multifunctional theranostic agent has exhibited its potential for clinical translatability since liposomal encapsulation of PS is the most popular clinically accepted nanosized drug delivery strategy.

The use of semiconductor-based PS like titanium dioxide (TiO_2) for PDT applications is strongly restricted by the low penetration of UV light into the tissues. Despite TiO_2 shows minimal dark cytotoxicity, it needs UVA light for ROS generation (*e.g.* the stronger oxidant $HO^{\bullet}$). However, this limitation can be overcome by using NIR two-photon excitation through upconversion energy processes using lanthanides [113]. Within this framework, Hou et al.[84] presented a novel NIR light-activated photosensitizer for PDT based on TiO_2-coated upconversion nanoparticle (UCNP) core-shell nanocomposites (UCNPs@TiO2 NCs), using a $NaYF4:Yb^{3+},Tm^{3+}@NaGdF4:Yb^{3+}$ core/shell. The UCNPs can efficiently convert NIR light to UV emission, corresponding to the TiO_2 shells absorption region. The nanocomposite is able to generate intracellular ROS under NIR irradiation, decreasing the mitochondrial membrane potential to release cytochrome c into the cytosol and then activating caspase 3 to induce cancer cell apoptosis. Thus, the combination of penetrating NIR radiation with efficient light-triggered ROS generation is a powerful weapon for cancer cell destruction.

Mesoporous silica nanoparticles (MSNs) functionalized with amine groups were used as a support to build up a layer-by-layer (LbL) supramolecular structure for tumor theranostic application. Multilayer coated MSNs (MCMSN) were obtained by alternative sequential adsorption of 1) hyaluronic acid (HA) with β-cyclodextrin (CD) and 2) 5, 10, 15, 20 - tetrakis (4-sulfonatophenyl) - porphyrin (TPPS4) onto the nanoparticles [69]. Then, tirapazamine (TPZ) was introduced due to its cytotoxicity toward tumoral cells, obtaining the nanoplatform (TPZ@MCMSN). Finally, the integrated TPZ@MCMSN-Gd^{3+} theranostic agent was formed by

a chelation reaction with gadolinium-III (Gd3+). TPZ@MCMSN-Gd^{3+} was tested in SCC-7 (Squamous cell carcinoma), MCF-7 (human breast adenocarcinoma), and COS7 (African green monkey kidney fibroblast) cell lines. The developed TPZ@MCMSN-Gd^{3+} supramolecule showed several advantages, such as dual-model imaging (NIR fluorescence and MR) guidance, effective tumor targeting, and efficient tumor growth inhibition by enhanced ROS production.

The correlation between the photobiological and photophysical properties of the organometallic compounds of Ru(II) with the extent of the π conjugation of the cyclometalation ligand was investigated [103]. Three of the organometallics derived from non-π-expansive cyclometalating ligands presented dark cytotoxic to cancer cells, which was not appreciable amplified by light exposure. On the other hand, the Ru(II) organometallic system derived from a π-expansive cyclometalating ligand, such 4,9,16-triazadibenzo[a,c]napthacene (pbpn), was completely non-toxic to cells in the dark, but photo-toxicity to cancer cells increased with a moderate light treatment. In addition to excellent its photocytotoxicity, this compound displayed intense green intracellular fluorescence.

Zhang et al. [104] reported that nanoscale supramolecular network formed by co-assembly of the amphiphilic amino acid 9-fluorenylmethyloxycarbonyl-L-leucine (Fmoc-L-L) and ionic manganese (Mn^{2+}) to encapsulate Ce6. The obtained bio-metal-organic nanoparticles exhibit a high Ce6 loading capability, inherent good biocompatibility, robust stability and smart disassembly in response to glutathione (GSH). The cooperative assembly of the multiple components is synchronously dynamic in nature and enables enhanced PDT to damage tumor cells and tissue by efficiently delivering of Ce6 via the competitive coordination of GSH with Mn^{2+}. Real-time in vivo evaluation of the antitumor effect was done by MRI through the long-term intracellular biochelation of Mn^{2+}.

An interesting combination of covalent and supramolecular chemistry for efficient antitumoral activity was reported by Ren et. al. [101] with the development of a nanosized supramolecular system formed by hyaluronic acid (HA) as carrier polymer with Ce6 as PS

covalently linked by an adipic dihydrazine bridge, and the anticancer drug doxorubicin (DOX) bound by non-covalent interactions. Despite the nanocomposite HA-Ce6 (DOX) could specifically bind to A549 cells through the CD44-HA receptor, it showed no PDA due to Ce6 molecular crowding. The study of cellular uptake and distribution of HA-Ce6 (DOX) by confocal fluorescence microscopy showed that, under acidic conditions and enzymatic stimulation, both Ce6 and DOX drugs were quickly released inside A549 cells, improving the therapeutic effect for individual photodynamic or chemotherapy with free Ce6 or DOX, respectively. Thus, the pH-responsive nanocomposite resulted in a potent anticancer theranostic agent [101].

5. Antimicrobial supramolecular PS

The increasing antibiotic resistance developed by the microorganisms [72-74, 89], together with some significant drawbacks of molecular PS for the application of APDT in clinical treatments, like their lack of selectivity and photobleaching, accelerated the application of supramolecular PS in APDT as a new alternative for the local treatment of infections as well as for disinfection of different surfaces and materials [20, 35, 39, 41, 65, 92, 114-117].

As well as for cancer PDT applications, self-assembled polymeric micelles and liposomes have been successfully used as nanocarriers for the delivery of PSs molecules into microbial cells, as they can prevent PS aggregation and preserves its photophysical properties [111, 112, 114].

Poly (β-amino ester)s with pH buffering capacities were recently employed for the encapsulation of Ce6 in charge-conversion nanoparticles, resulting in an efficient targeting and photodynamic inactivation of pathogenic bacteria in a weakly acidic urinary tract infection environment [118]. Surface charge switching on the nanoparticles conferred them an enhanced recognition towards Gram (+) and Gram (–) bacteria (*S. aureus* and *E. coli*, respectively). Moreover, the nanoparticles yielded efficient ROS photo-production leading to significant in vitro antibacterial effect, with increased minimum inhibitory concentration (MIC) values when compared to free Ce6, but with

low systemic cytotoxicity [118]. Ce6 grafted onto α-cyclodextrin (α-CD) was also used for selective targeting and photodynamic inactivation of *P. aeruginosa* and methicilin-resistant *Staphylococcus aureus* (MRSA) biofilms towards the formulation of bacteria-targeted PS delivery polymeric micelles. Selectivity was achieved by the covalent bounding of antimicrobial peptide Magainin I to PEG, then exploiting α-CD/PEG supramolecular assembly by host-guest complexation [119].

An amphiphilic calix[4]arene was utilized for the formation of micellar-like nanocontainers ca. 40 nm in diameter to encapsulate hydrophilic and hydrophobic phthalocyanine and porphyrin derivatives, respectively [120]. In aqueous solutions, the hydrophilic phthalocyanine showed self-aggregation with consequent changes on its spectral properties precluding its use as PS, while the hydrophobic porphyrin derivative was insoluble and photochemically inactive under these conditions. However, in the presence of the amphiphilic calix[4]arene micellar-like nanocontainers, both PS@calixarene nanoassemblies showed high quantum yields of $^1O_2$ photogeneration and remarkable visible light-induced inactivation of *S. aureus and P. aeruginosa*, representative Gram (+) and Gram (–) bacteria, respectively [120].

Metallic nanoparticles (MNP), *e.g.* Au, Ag, and Pt, have been extensively used for the conjugation, coating or loading of PSs for their application in APDT [114]. As MNP have antimicrobial activities themselves [121], they can act synergistically with the PS through PDA for the inactivation of microbial pathogens. Gold NP (AuNPs) obtained from green-synthesis using *Aloe vera* leaf extracts and conjugated with the phenothiazidium dyes MB and toluidine blue O (TBO) as PSs showed photodynamic inactivation activity when tested against both planktonic and biofilm *C. albicans* populations [122]. Moreover, in vivo experiments in mice demonstrated the ability of AuNP@MB and AuNP@TBO conjugates to suppress superficial skin as well as oral *C. albicans* infection, suggesting their potential application on APDT of cutaneous and nosocomial infections [122]. Other formulations comprising non-covalent conjugates of MB and

AuNPs also showed inhibitory effect over MRSA biofilms and *S. aureus* isolates from impetigo lesions, when irradiated with a red laser (≈660 nm) [123, 124].

A nanocomposite formed by conjugating the phenothiazidium dye TBO with AgNPs (AgNP@TBO) was tested for the photo-sensitized inactivation of *Streptococcus mutans* [125]. The resulting AgNP@TBO composites showed a higher photo-toxicity against *S. mutans* biofilm than the isolated TBO, as it was evidenced by an increased uptake of propidium iodide and by the leakage of cellular constituents. Fluorescence spectroscopic studies were conducted to confirm that photo-toxicity corresponded to a type I mechanism, with the generation of $HO^{\bullet}$ as the main ROS [125].

A large variety of supramolecular formulations involved the association of PSs with synthetic or naturally occurring polymers to yield diverse structured photo-active materials, such as polymeric nanoparticles, hydrogels, antimicrobial coatings, or surfaces [41, 51, 65, 66, 114, 126, 127].

Chen et al. [50] proposed a double-advantageous strategy for biofilm elimination based on degradable polymers. On one hand, high local concentrations of an organic PS are achieved by its binding to a supramolecular photodynamic polymer through host-guest interactions. Besides, after light-induced inactivation of the microorganism, the PS can be detached by competition with cucurbit[7]uril, favoring polymer degradation, and thus hindering the progression of drug resistance [50].

Manjón and co-workers employed porous silicone as a support for the immobilization of two different Ru(II) complexes (RDP^{2+} and RDB^{2+}) by hydrophobic interactions [128]. The resulting PS-doped porous silicon materials (RDP/pSil and RDB/pSil) yielded increased $^1O_2$ lifetimes compared to those of PSs in water, and were effective for waterborne *Enterococcus faecalis* photodynamic inactivation using a lab-scale solar simulator or a solar photoreactor as illumination sources. After sunlight irradiation, reloading of RDP/pSil with free RDP^{2+} resulted in even higher photodynamic efficiencies than the unused material, which was explained in terms

of the aggregation of the silicone-supported photosensitizer on pSil surface, as evidenced by photochemical characterization [128]. On a later work of the same group, RDP/pSil was compared with silicon-supported pristine C60-fullerene or its derivative 1-(4-methyl)-piperazinylfullerene (MPF), for their photodynamic water solar disinfection abilities [129]. C60/pSil showed poor photo-induced antibacterial activity due to fullerene aggregation, which results in a negatively charged surface. However, the positively charged MPF fullerene derivative was not effective either, because the C60 structural modification introduced led to lower $^1O_2$ generation efficiency [129].

Mesoporous silica nanoparticles (MSNP) functionalized with either amino- or mannose- moieties were reported as MB delivery systems for APDT [130]. Loading of MB into the MSNPs was driven by electrostatic interactions, and two populations of the dye were differentiated according to its location on the outer surface of the MSNPs or on the walls of the inner mesopores, as suggested by time-resolved spectroscopic studies. Both MSNPs showed photo-inactivation activities similar to free MB when tested against *E. coli* and *P. aeruginosa* [130].

A sort of polymer-based structure that has been widely explored for the immobilization of PSs is represented by hydrogels, which consist of three-dimensional networks made of cross-linked water-soluble polymers [114]. Some shared characteristics of these platforms are their high porosity, biocompatibility, biodegradability, and flexible shape [114]. For example, an anti-infective intraocular lens were developed by immobilization of a cationic porphyrin on the surface of hydrogels made of acrylate co-polymers. The incorporation of the porphyrin resulted in a great reduction of bacterial adhesion on the material [131].

The development of antimicrobial surfaces involving PSs loaded on polymeric matrices was exploited as a healthcare infection prevention strategy. Cahan et al. reported the physical immobilization of RB, TBO, or MB by scattering a mixed powder of poly(vinylidene fluoride) (PVDF) and PSs onto a polyethylene sheet, and further pressing with a heating press device [41]. This procedure yielded hydrophobic surfaces with antibacterial photodynamic properties as demonstrated by the reduction of *E. coli* and *S. aureus* CFUs by >4 logs when illuminated with

a fluorescent lamp during 6-24 h [41]. More recently, a photodynamic spray coating was formulated by taking advantage of the host-guest interaction between β-cyclodextrin and MB, which prevented MB aggregation and resulted in a material with high $^1O_2$ photogeneration efficiency with low PS density [132].

An antimicrobial coating with combined virucidal and bactericidal activities was obtained by the immobilization of C70 fullerene and AgNPs into polystyrene-block-poly- 4-vinylpyridine (PS-P4VP) templates, yielding photo-active thin films [133]. This nanocomposite presents two distinct nanoscale functional domains as C70 is preferentially integrated into PS block domains (due to speculated π–π interactions), whereas AgNPs are formed in situ in P4VP domains. The significant amounts of $^1O_2$ generated by visible-light activation of C70, together with the Ag^+ release properties, allowed a synergistic inactivation of both *E. coli* and PR772 bacteriophage [133].

Biopolymer-based nanoparticles, mainly chitosan and cellulose, have shown to be a very advantageous PSs delivery agent in APDT due to their biocompatibility, stable formulation, versatility and generally easy preparation [134]. Chitosan can be isolated from the chitin exoskeleton of crustacea and is structurally composed of poly(D-glucosamine) chain. Due to its protonated amino groups, chitosan has an intrinsic antimicrobial activity that can be enhanced by covalent or non-covalent association with different bioactive compounds, among which are PS molecules for APDT applications. Recently, Castro and co-workers reported the ability of porphyrinic-chitosan films to inhibit *Listeria innocua* attachment and prevent subsequent biofilm formation [127].

Several works have reported the application of PS-chitosan hydrogels on topic APDT for wound infections, alone or in association with other components, such as hydroxypropylmethylcellulose, to improve the mechanical properties of the gels. Chitosan hydrogels were combined with PSs like MB or TBO for APDT against both planktonic and biofilm-forming pathogens [134, 135].

Supramolecular structures involving cellulose association with PSs have been explored in multiple configurations for APDT applications. For example, photo-active antimicrobial cellulosic fabrics were obtained by embedding the polymeric material with porphyrins, which resulted to be immobilized both by electrostatic and covalent interactions, as confirmed by diffuse reflectance (UV-Vis) spectroscopy [126]. Immobilization of cationic zinc phthalocyanine (ZnPc) derivatives on the negatively-charged surface of sulfated cellulose nanocrystals (CNC) by electrostatic interactions, yielded hybrid structures with photodynamic inactivation properties that outcome those observed for the free ZnPc [51]. Both PS concentrations and light doses applied for the photodynamic inactivation of *S. aureus* and *E. coli* were one to two orders of magnitude lower than those reported in the literature for similar but covalently linked porphyrin-based systems [136, 137]. Interestingly, the ZnPc@CNC composites in aqueous solution did not photogenerate $^1O_2$, suggesting that the observed efficient APDT relies on the transfer of ZnPc from the CNC surface to the microorganism, standing out the advantage of a non-covalent supramolecular strategy [51].

Carbon-based nanomaterials such as graphene, carbon nanotubes, fullerenes, carbon dots, and mesoporous carbon nanoparticles have been used for APDT applications either as PSs carriers or as nanomaterials with inherent photosensitizing properties. Akbari et al. [138] demonstrated the enhanced PDA of indocyanine green (ICG) conjugated with graphene oxide (GO), if compared with ICG alone [138]. The conjugate ICG@GO, in which ICG was loaded on the surface of GO by strong π–π stacking and hydrophobic interactions, not only showed a 1.3 more effective APDT activity against *E. faecalis* biofilms but also presented some advantages for endodontic applications, such as its cost-effectiveness and the much lower dye concentrations needed, with concomitantly reduced toxicity. A later work from the same group reported that nanocomposites of carnosine-GO decorated with hydroxyapatite had a better ICG loading capacity and longer stability than the conjugates prepared with GO alone [139].

Multi-walled carbon nanotubes (MWCNT) have been also utilized as delivery vehicles of toluidine blue (TB) for the efficient photo-inactivation of *P. aeruginosa* and *S. aureus* both in planktonic cultures and biofilms [140]. The nanocomposite MWCNT@TB allowed the gradual releases of the dye and led to a greater APDT efficiency when compared to free TB [140].

6. Conclusions

In this review, we have presented several supramolecular strategies addressed to overcome some of the limitations of molecular photosensitizers (PS) for photodynamic therapy (PDT) and antimicrobial photodynamic therapy (APDT) applications.

Typically, those supramolecular strategies included nanostructured PS self-assemblies, PS co-assemblies with antibiotics or anticancer drugs, and PS non-covalently and covalently attached with polyelectrolytes, biopolymers, and nanoparticles together with other additional materials. The main goal is to regulate ROS generation for specific and efficient anticancer or antimicrobial therapies, with a focus on the increment of ROS generation at tumor cells or tissue lesions, and also taking advantage of known anomalies in tumoral cells and tissues to triggering ROS generation with the light stimulus.

As mentioned through the review, the use of nano/micro-sized supramolecular systems for emerging photodynamic applications against multi-drug resistant pathogens and in the theranostic application in tumors became a powerful tool, since supramolecular PS systems can be designed with very high selectivity to target either tumor or pathogen cells, and with poor or null toxicity for normal cells. Also, in the field of theranostic treatments, a large variety of optical and non-optical monitoring signals can be obtained, allowing a large variety of tracking non-invasive methods. Nevertheless, most supramolecular PS systems are still in the initial development stages and their safety in animals has not been fully evaluated. Therefore, before clinical applications, the safety issues of supramolecular PS should be fully addressed.

Advanced or interested readers can find extra information in recent comprehensive reviews on both PDT and APDT application fields [16, 17, 20, 31, 32, 37-40, 54, 65, 66, 68, 92, 93, 105, 197, 114].

Acknowledgments:

C.C.V. and F.T. thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for postdoctoral fellowships at INBIONATEC. C. D. B. is member of the Research Career of CONICET. Authors also thank Universidad Nacional de Santiago del Estero (Grant# CICyT-23A215), CONICET (Grant# PUE-2018-0035), and ANPCyT-FONCyT (Grant# PICT-2015-0828) for financial supports.

References

(1) K. C. Smith, Ed. , The Science of Photobiology, Plenum Press, New York and London, **1989**.
(2) L. O. Björn, Ed. , Photobiology: The Science of Light and Life, Springer, New York, **2015**.
(3) A. D. McNaught, A. Wilkinson, IUPAC. Compendium of Chemical Terminology, Blackwell Scientific Publications, Oxford, **1997**.
(4) Lars Olof Björn, Ed. , Photobiology. The Science of Light and Life, Springer, New York, **2015**.
(5) J. D. Coyle, Introduction to Organic Photochemistry, John Wiley & Sons Ltd, London, UK, **1986**.
(6) C. S. Foote, Photochem. Photobiol. **1991**, 54, 659–659.
(7) M. S. Baptista, J. Cadet, P. Di Mascio, A. A. Ghogare, A. Greer, M. R. Hamblin, C. Lorente, S. C. Nunez, M. S. Ribeiro, A. H. Thomas, et al., Photochem. Photobiol. **2017**, 93, 912–919.
(8) G. Laustriat, Biochimie **1986**, 68, 771–778.
(9) C. Schweitzer, R. Schmidt, Chem. Rev. **2003**, 103, 1685–1757.
(10) A. Greer, Acc. Chem. Res. **2006**, 39, 797–804.
(11) P. Di Mascio, G. R. Martinez, S. Miyamoto, G. E. Ronsein, M. H. G. Medeiros, J. Cadet, Chem. Rev. **2019**, 119, 2043–2086.
(12) R. Mang, H. Stege, J. Krutmann, Contact Dermatitis **2011**, 97–104.
(13) A. D. McNaught, A. Wilkinson, IUPAC. Compendium of Chemical Terminology, Blackwell Scientific Publications, Oxford, **1997**.
(14) E. J. Dennis, G. J. Dolmans, D. Fukumara, K. J. Rakesh, Nat. Rev. Cancer **2003**, 3, 375–380.
(15) L. Benov, Med. Princ. Pract. **2015**, 24, 14–28.
(16) M. Tim, J. Photochem. Photobiol. B Biol. **2015**, 150, 2–10.
(17) H. Abrahamse, M. R. Hamblin, Biochem. J. **2016**, 473, 347–364.
(18) A. A. Krasnovsky Jr., in Photodyn. Ther. Cell. Lev. (Ed.: A.B. Uzdensky), Research Signpost, **2007**, 17–62.
(19) M. C. DeRosa, R. J. Crutchley, Coord. Chem. Rev. **2002**, 233–234, 351–371.
(20) C. Spagnul, L. C. Turner, R. W. Boyle, J. Photochem. Photobiol. B Biol. **2015**, 150, 11–30.
(21) B. Quintero, M. A. Miranda, Ars Pharm. **2000**, 41, 27–46.
(22) R. W. Redmond, J. N. Gamlin, Photochem. Photobiol. **1999**, 70, 391–475.
(23) F. Wilkinson, W. P. Helman, A. B. Ross, J. Phys. Chem. Ref. Data **1993**, 22, 113–262.
(24) M. P. Serrano, C. Lorente, C. D. Borsarelli, A. H. Thomas, ChemPhysChem **2015**, 16, 2244–2252.
(25) M. P. Serrano, C. D. Borsarelli, A. H. Thomas, Photochem. Photobiol. **2013**, 89, 1456–1462.
(26) E. Alarcón, A. M. Edwards, A. Aspée, C. D. Borsarelli, E. A. Lissi, Photochem. Photobiol. Sci. **2009**, 8, 933–943.
(27) C. L. Hawkins, M. J. Davies, Biochim. Biophys. Acta **2001**, 1504, 196–219.
(28) M. J. Davies, Biochem. J. **2016**, 473, 805–825.
(29) M. Mariotti, C. Lopez-Alarcon, M. Rykaer, P. Hägglund, M. J. Davies, F. Leinisch, Free Radic. Biol. Med. **2017**, 112, 240–252.
(30) E. Alarcón, A. M. Edwards, A. M. Garcia, M. Muñoz, A. Aspée, C. D. Borsarelli, E. A. Lissi, Photochem. Photobiol. Sci. **2009**, 8, 255–263.
(31) I. Yoon, J. Z. Li, Y. K. Shim, Clin. Endosc. **2013**, 46, 7–23.
(32) R. Yin, M. Hamblin, Curr. Med. Chem. **2015**, 22, 2159–2185.
(33) P. Agostinis, K. Berg, K. Cengel, CA Cancer J Clin **2011**, 61, 250–281.
(34) J. Mosinger, K. Lang, P. Kubát, Top. Curr. Chem. **2016**, 370, 135–168.
(35) K. Liu, Y. Liu, Y. Yao, H. Yuan, S. Wang, Z. Wang, X. Zhang, Angew. Chemie Int. Ed. **2013**, 52, 8285–8289.

(36) S. Wang, R. Gao, F. Zhou, M. Selke, J. Mater. Chem. **2004**, 487–493.
(37) G. M. F. Calixto, J. Bernegossi, L. M. De Freitas, C. R. Fontana, M. Chorilli, A. M. Grumezescu, Molecules **2016**, 21, 1–18.
(38) E. Paszko, C. Ehrhardt, M. O. Senge, D. P. Kelleher, J. V. Reynolds, Photodiagnosis Photodyn. Ther. **2011**, 8, 14–29.
(39) S. Perni, P. Prokopovich, J. Pratten, I. P. Parkin, M. Wilson, Photochem. Photobiol. Sci. **2011**, 10, 712-720
(40) X. Li, S. Lee, J. Yoon, Chem. Soc. Rev. **2018**, 47, 1174–1188.
(41) R. Cahan, R. Schwartz, Y. Langzam, Y. Nitzan, Photochem. Photobiol. **2011**, 87, 1379–86.
(42) S. Sansaloni-Pastor, J. Bouilloux, N. Lange, Pharmaceuticals **2019**, 12, 148.
(43) P. A. Gale, Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. **2000**, 358, 431–453.
(44) K. Ariga, in Biomater. Nanoarchitectonics (Ed.: M. Ebara), Elsevier Inc., Oxford, **2016**, 25–40.
(45) B. Bhushan, Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 2009, 367, 1445–1486.
(46) M. Sarikaya, C. Tamerler, A. K. Jen, K. Schulten, F. Baneyx, Nat. Mater. **2003**, 2, 577–585.
(47) E. Dujardin, S. Mann, Adv. Mater. **2002**, 14, 709–716.
(48) H. Ahmad, S. K. Kamarudin, L. J. Minggu, M. Kassim, Renew. Sustain. Energy Rev. **2015**, 43, 599–610.
(49) A. P. Nikalje, Med. Chem. (Los. Angeles). **2015**, 5, 81–89.
(50) L. Chen, Y. Yang, P. Zhang, S. Wang, J. F. Xu, X. Zhang, ACS Appl. Bio Mater. **2019**, 2, 2920–2926.
(51) E. Anaya-Plaza, E. van de Winckel, J. Mikkilä, J. M. Malho, O. Ikkala, O. Gulías, R. Bresolí-Obach, M. Agut, S. Nonell, T. Torres, et al., Chem. Eur. J. **2017**, 23, 4320–4326.
(52) M. R. Hamblin, Photochem. Photobiol. Sci. **2018**, 17, 1515–1533.
(53) X. Li, S. Yu, D. Lee, G. Kim, B. Lee, Y. Cho, B. Y. Zheng, M. R. Ke, J. D. Huang, K. T. Nam, et al., ACS Nano **2018**, 12, 681–688.
(54) C. K. Lim, J. Heo, S. Shin, K. Jeong, Y. H. Seo, W. D. Jang, C. R. Park, S. Y. Park, S. Kim, I. C. Kwon, Cancer Lett. **2013**, 334, 176–187.
(55) E. Alarcón, A. M. A. M. Edwards, A. M. A. M. Garcia, M. Muñoz, A. Aspée, C. D. C. D. Borsarelli, E. A. E. A. Lissi, Photochem. Photobiol. Sci. **2009**, 8, 255–263.
(56) M. R. Hamblin, E. L. Newman, J. Photochem. Photobiol. B Biol. **1994**, 26, 147–157.
(57) S. Monti, I. Manet, Chem. Soc. Rev. **2014**, 43, 4051–4067.
(58) M. B. E. Turbay, V. Rey, N. M. Argañaraz, F. E. Morán Vieyra, A. Aspée, E. A. Lissi, C. D. Borsarelli, J. Photochem. Photobiol. B Biol. **2014**, 141, 275–282.
(59) C. Li, F. Lin, W. Sun, F. G. Wu, H. Yang, R. Lv, Y. X. Zhu, H. R. Jia, C. Wang, G. Gao, et al., ACS Appl. Mater. Interfaces **2018**, 10, 16715–16722.
(60) S. Li, Q. Zou, R. Xing, T. Govindaraju, R. Fakhrullin, X. Yan, Theranostics **2019**, 9, 3249–3261.
(61) W. Q. Lim, G. Yang, S. Z. F. Phua, H. Chen, Y. Zhao, ACS Appl. Mater. Interfaces **2019**, 11, 16391–16401.
(62) L. E. Ibarra, G. V. Porcal, L. P. Macor, R. A. Ponzio, R. M. Spada, C. Lorente, C. A. Chesta, V. A. Rivarola, R. E. Palacios, Nanomedicine **2018**, 13, 605–624.
(63) R. M. Spada, L. P. Macor, L. I. Hernández, R. A. Ponzio, L. E. Ibarra, C. Lorente, C. A. Chesta, R. E. Palacios, Dye. Pigment. **2018**, 149, 212–223.
(64) F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, E. Hellwig, A. Al-Ahmad, T. Maisch, Crit. Rev. Microbiol. **2018**, 44, 571–589.
(65) M. Q. Mesquita, C. J. Dias, M. G. P. M. S. Neves, A. Almeida, M. A. F. Faustino, Molecules **2018**, 23, 2424.
(66) R. A. Craig, C. P. McCoy, S. P. Gorman, D. S. Jones, Expert Opin. Drug Deliv. **2015**, 12, 85–101.
(67) M. Tim, J. Photochem. Photobiol. B Biol. **2015**, 150, 2–10.
(68) P. Rai, S. Mallidi, X. Zheng, R. Rahmanzadeh, Y. Mir, S. Elrington, A. Khurshid, T. Hasan, Adv. Drug Deliv. Rev. **2010**, 62, 1094–1124.
(69) W. H. Chen, G. F. Luo, W. X. Qiu, Q. Lei, L. H. Liu, S. B. Wang, X. Z. Zhang, Biomaterials **2017**, 117, 54–65.
(70) G. Yu, S. Yu, M. L. Saha, J. Zhou, T. R. Cook, B. C. Yung, J. Chen, Z. Mao, F. Zhang, Z. Zhou, et al., Nat. Commun. **2018**, 9, 4335.
(71) S. S. Kelkar, T. M. Reineke, Bioconjug. Chem. **2011**, 22, 1879–1903.
(72) R. K. Fukuda, Antimicrobial Resistance: Global Report on Surveillance, World Health Organization, Geneva, **2014**.
(73) S. Santajit, N. Indrawattana, Biomed Res. Int. **2016**, 2455067.
(74) N. Venkatesan, G. Perumal, M. Doble, Future Microbiol. **2015**, 10, 1743–1750.
(75) K. G. Andersen, H. Kehlet, J. Pain **2011**, 12, 725–746.
(76) Y. Qin, L. J. Chen, F. Dong, S. T. Jiang, G. Q. Yin, X. Li, Y. Tian, H. B. Yang, J. Am. Chem. Soc. **2019**, 141, 8943–8950.
(77) A. Amirshaghaghi, L. Yan, J. Miller, Y. Daniel, J. M. Stein, T. M. Busch, Z. Cheng, A. Tsourkas, Sci. Rep. **2019**, 9, 1–9.

(78) Z. Liu, W. Shi, G. Hong, W. Chen, B. Song, X. Peng, X. Xiong, F. Song, J. Control. Release **2019**, 310, 1–10.
(79) O. Raab, Zeitung Biol . **1900**, 39, 524–526.
(80) G. B. Kharkwal, S. K. Sharma, Y. Y. Huang, T. Dai, M. R. Hamblin, Lasers Surg. Med. **2011**, 43, 755–767.
(81) R. Mang, H. Stege, J. Krutmann, in Contact Dermatitis (Ed.: Springer), **2011**, 97–104.
(82) J. Soriano, I. Mora-Espí, M. E. Alea-Reyes, L. Pérez-García, L. Barrios, E. Ibáñez, C. Nogués, Sci. Rep. **2017**, 7, 1–13.
(83) S. A. Sibani, P. A. McCarron, A. D. Woolfson, R. F. Donnelly, Expert Opin. Drug Deliv. **2008**, 5, 1241–1254.
(84) Z. Hou, Y. Zhang, K. Deng, Y. Chen, X. Li, X. Deng, Z. Cheng, H. Lian, C. Li, J. Lin, ACS Nano **2015**, 9, 2584–2599.
(85) Y. Q. Huang, L. J. Sun, R. Zhang, J. Hu, X. F. Liu, R. C. Jiang, Q. L. Fan, L. H. Wang, W. Huang, ACS Appl. Bio Mater. 2019, 2, 2421–2434.
(86) Z. Zheng, T. Zhang, H. Liu, Y. Chen, R. T. K. Kwok, C. Ma, P. Zhang, H. H. Y. Sung, I. D. Williams, J. W. Y. Lam, et al., ACS Nano **2018**, 12, 8145–8159.
(87) D. S. Perlin, R. Rautemaa-Richardson, A. Alastruey-Izquierdo, Lancet Infect. Dis. **2017**, 17, e383–e392.
(88) M. Eickmann, U. Gravemann, W. Handke, F. Tolksdorf, S. Reichenberg, T. H. Müller, A. Seltsam, Vox Sang. **2020**, 146–151.
(89) C. W. Hall, T. F. Mah, FEMS Microbiol. Rev. **2017**, 41, 276–301.
(90) P. S. Stewart, J. William Costerton, Lancet **2001**, 358, 135–138.
(91) O. Ciofu, E. Rojo-Molinero, M. D. Macià, A. Oliver, APMIS **2017**, 125, 304–319.
(92) X. Hu, Y. Y. Huang, Y. Wang, X. Wang, M. R. Hamblin, Front. Microbiol. **2018**, 9, 1–24.
(93) J. Ghorbani, D. Rahban, S. Aghamiri, A. Teymouri, A. Bahador, Laser Ther. **2018**, 27, 293–302.
(94) T. Dai, B. B. Fuchs, J. J. Coleman, R. A. Prates, C. Astrakas, T. G. St. Denis, M. S. Ribeiro, E. Mylonakis, M. R. Hamblin, G. P. Tegos, Front. Microbiol. **2012**, 3, 1–16.
(95) G. Jori, M. Camerin, M. Soncin, L. Guidolin, O. Coppellotti, Antimicrobial Photodynamic Therapy: Basic Principles, **2011**.
(96) L. Leive, Ann. N. Y. Acad. Sci. **1974**, 235, 109–129.
(97) A. Segalla, C. D. Borsarelli, S. E. Braslavsky, J. D. Spikes, G. Roncucci, D. Dei, G. Chiti, G. Jori, E. Reddi, Photochem. Photobiol. Sci. **2002**, 1, 641–648.
(98) F. Cieplik, A. Späth, J. Regensburger, A. Gollmer, L. Tabenski, K. A. Hiller, W. Bäumler, T. Maisch, G. Schmalz, Free Radic. Biol. Med. **2013**, 65, 477–487.
(99) S. Nonell, M. Gonzalez, F. R. Trull, Afinidad **1993**, 50, 445–450.
(100) J. Xie, S. Lee, X. Chen, Adv. Drug Deliv. Rev. **2010**, 62, 1064–1079.
(101) Q. Ren, Z. Liang, X. Jiang, P. Gong, L. Zhou, Z. Sun, J. Xiang, Z. Xu, X. Peng, S. Li, et al., Int. J. Biol. Macromol. **2019**, 130, 845–852.
(102) F. Wu, L. Yue, H. Su, K. Wang, L. Yang, X. Zhu, Nanoscale Res. Lett. **2018**, 13, 357.
(103) T. Sainuddin, J. McCain, M. Pinto, H. Yin, J. Gibson, M. Hetu, S. A. McFarland, Inorg. Chem. **2016**, 55, 83–95.
(104) H. Zhang, K. Liu, S. Li, X. Xin, S. Yuan, G. Ma, X. Yan, ACS Nano **2018**, 12, 8266–8276.
(105) O. J. Fakayode, N. Tsolekile, S. P. Songca, O. S. Oluwafemi, Biophys. Rev. **2018**, 10, 49–67.
(106) N. Fomina, J. Sankaranarayanan, A. Almutairi, Adv. Drug Deliv. Rev. **2012**, 64, 1005–1020.
(107) N. Dragicevic-Curic, A. Fahr, Expert Opin. Drug Deliv. **2012**, 9, 1015–1032.
(108) H. Xu, Y. Liu, J. Qu, Z. Yuan, J. Innov. Opt. Health Sci. **2019**, 12, 1–9.
(109) S. Jenni, A. Sour, Inorganics **2019**, 7, 1–13.
(110) S. M. Janib, A. S. Moses, J. A. MacKay, Adv. Drug Deliv. Rev. **2010**, 62, 1052–1063.
(111) C. F. Van Nostrum, Adv. Drug Deliv. Rev. **2004**, 56, 9–16.
(112) H. Cabral, K. Miyata, K. Osada, K. Kataoka, Chem. Rev. **2018**, 118, 6844–6892.
(113) M. Nyk, R. Kumar, T. Y. Ohulchanskyy, E. J. Bergey, P. N. Prasad, Nano Lett. **2008**, 8, 3834–3838.
(114) Y. Feng, L. Liu, J. Zhang, H. Aslan, M. Dong, J. Mater. Chem. B **2017**, 5, 8631–8652.
(115) N. Kashef, Y.-Y. Huang, M. R. Hamblin, Nanophotonics **2017**, 176, 139–148.
(116) H. S. Kim, E. J. Cha, H. J. Kang, J. H. Park, J. Lee, H. D. Park, Environ. Res. **2019**, 172, 34–42.
(117) R. Khurana, A. S. Kakatkar, S. Chatterjee, N. Barooah, A. Kunwar, A. C. Bhasikuttan, J. Mohanty, Front. Chem. **2019**, 7, 452.
(118) S. Liu, S. Qiao, L. Li, G. Qi, Y. Lin, Z. Qiao, H. Wang, C. Shao, Nanotechnology **2015**, 26, 495602.
(119) Y. Gao, J. Wang, D. Hu, Y. Deng, T. Chen, Q. Jin, J. Ji, Macromol. Rapid Commun. **2019**, 40, 1800763.
(120) I. Di Bari, A. Fraix, R. Picciotto, A. R. Blanco, S. Petralia, S. Conoci, G. Granata, G. M. L. Consoli, S. Sortino, RSC Adv. **2016**, 6, 105573–105577.
(121) M. J. Hajipour, K. M. Fromm, A. Akbar Ashkarran, D. Jimenez de Aberasturi, I. R. de Larramendi, T. Rojo, V. Serpooshan, W. J. Parak, M. Mahmoudi, Trends Biotechnol. **2012**, 30, 499–511.
(122) M. A. Sherwani, S. Tufail, A. A. Khan, M. Owais, PLoS One **2015**, 10, 1–20.

(123) E. Darabpour, N. Kashef, S. M. Amini, S. Kharrazi, G. E. Djavid, J. Drug Deliv. Sci. Technol. **2017**, 37, 134–140.
(124) A. A. Tawfik, J. Alsharnoubi, M. Morsy, Photodiagnosis Photodyn. Ther. **2015**, 12, 215–220.
(125) L. Misba, S. Kulshrestha, A. U. Khan, Biofouling **2016**, 32, 313–328.
(126) R. Rahimi, F. Fayyaz, M. Rassa, Mater. Sci. Eng. C **2016**, 59, 661–668.
(127) K. A. D. F. Castro, N. M. M. Moura, A. Fernandes, M. A. F. Faustino, M. M. Q. Simões, J. A. S. Cavaleiro, S. Nakagaki, A. Almeida, Â. Cunha, A. J. D. Silvestre, et al., Dye. Pigment. **2017**, 137, 265–276.
(128) F. Manjón, M. Santana-Magaña, D. García-Fresnadillo, G. Orellana, Photochem. Photobiol. Sci. **2010**, 9, 838–845.
(129) F. Manjón, M. Santana-Magaña, D. García-Fresnadillo, G. Orellana, Photochem. Photobiol. Sci. **2014**, 13, 397–406.
(130) O. Planas, R. Bresolí-Obach, J. Nos, T. Gallavardin, R. Ruiz-González, M. Agut, S. Nonell, Molecules **2015**, 20, 6284–6298.
(131) C. P. McCoy, R. A. Craig, S. M. McGlinchey, L. Carson, D. S. Jones, S. P. Gorman, Biomaterials **2012**, 33, 7952–7958.
(132) T. Yao, J. Wang, Y. Xue, W. Yu, Q. Gao, L. Ferreira, K.-F. Ren, J. Ji, J. Mater. Chem. B **2019**, 7, 5089–5095.
(133) K. J. Moor, C. O. Osuji, J. H. Kim, ACS Appl. Mater. Interfaces **2016**, 8, 33583–33591.
(134) M. L. Frade, S. R. de Annunzio, G. M. F. Calixto, F. D. Victorelli, M. Chorilli, C. R. Fontana, Molecules **2018**, 23, 473.
(135) C.-P. Chen, C.-M. Hsieh, T. Tsai, J.-C. Yang, C.-T. Chen, Int. J. Mol. Sci. **2015**, 16, 20859–20872.
(136) E. Feese, H. Sadeghifar, H. S. Gracz, D. S. Argyropoulos, R. A. Ghiladi, Biomacromolecules **2011**, 12, 3528–3539.
(137) B. L. Carpenter, E. Feese, H. Sadeghifar, D. S. Argyropoulos, R. A. Ghiladi, Photochem. Photobiol. **2012**, 88, 527–536.
(138) T. Akbari, M. Pourhajibagher, F. Hosseini, N. Chiniforush, E. Gholibegloo, M. Khoobi, S. Shahabi, A. Bahador, Photodiagnosis Photodyn. Ther. **2017**, 20, 148–153.
(139) E. Gholibegloo, A. Karbasi, M. Pourhajibagher, N. Chiniforush, A. Ramazani, T. Akbari, A. Bahador, M. Khoobi, J. Photochem. Photobiol. B Biol. **2018**, 181, 14–22.
(140) V. T. Anju, P. Paramanantham, S. L. Sruthil, A. Sharan, A. Syed, N. A. Bahkali, M. H. Alsaedi, K. K., S. Busi, Photodiagnosis Photodyn. Ther. **2019**, 27, 305–316.

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