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Porphyrins—valuable pigments of life

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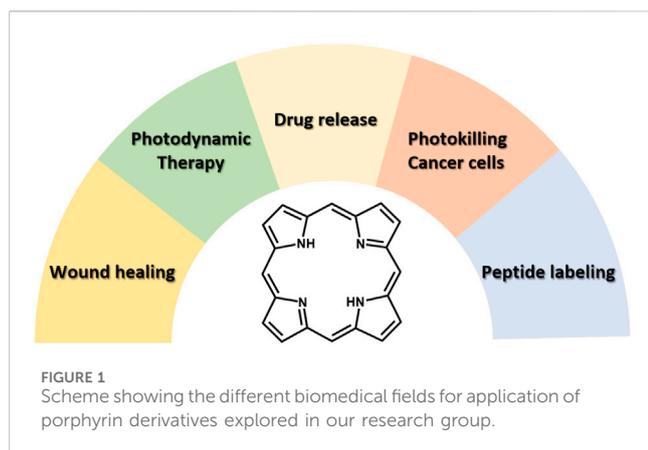
Porphyrin complexes are present in many natural systems and have significant biological roles, such as light harvesting, oxygen transport, and catalysis. Owing to their intrinsic aromatic structure, porphyrin derivatives exhibit characteristic photophysical and electrochemical properties. Porphyrins and porphyrin-based derivatives have been extensively utilized in biomedical applications during the last decade. Specifically, porphyrinoids have been tested as agents in antimicrobial and photodynamic therapy, as well as in imaging applications (e.g., diagnosis of cancer cells). This perspective article summarizes the recent developments in our group concerning the application of porphyrin derivatives in biomedical applications. The current challenges and future prospects concerning the exploitation of porphyrin-based materials in biomedical applications are also discussed.

1 Introduction

Porphyrinoids, frequently mentioned as "pigments of life" (Battersby, 2000), are tetrapyrrole derivatives with significant importance in natural occurring systems. Among the most well-known natural tetrapyrroles: i) heme is a porphyrin present in hemoglobin, being responsible for oxygen transport in blood, ii) chlorophyll (a chlorin) is the essential component in photosynthesis, by trapping sunlight, and iii) bacteriochlorophyll is a bacteriochlorin accountable for the photosynthesis in bacteria (Taniguchi and Lindsey, 2017). Ever since the seminal work of Battersby and co-workers regarding "pigments of life" (Battersby and McDonald, 1979; Battersby et al., 1980), porphyrin-based derivatives (and/or materials) were widely prepared for numerous applications due to their exceptional synthetic versatility, photo-, electro-, and thermal-stability, along with their unique photoelectrochemical features. The name of porphyrin derivatives originates from the Greek word "porphyra" which was used by ancient Greeks to describe the intense purple colour. From the structural viewpoint, porphyrin derivatives are composed of four pyrrolic units that are linked in a coplanar fashion by four methene bridges, resulting in a planar macrocyclic structure. It has an extended conjugated 18 π-electron system which is responsible for its aromatic nature, and its central cavity enables the accommodation of large number of metal cations.

FIGURE 1
Scheme showing the different biomedical fields for application of porphyrin derivatives explored in our research group.

On account of their substantial absorption in the visible region (400–450 nm Soret band, and 500–700 nm Q-bands) (Kim et al., 2008), as well as their ability to act as electron/energy donors/acceptors, porphyrins have been examined as artificial photosynthetic mimics (Bottari et al., 2012) and utilized in various solar cell technologies (Urbani et al., 2014; Zeng et al., 2020; Piradi et al., 2021; Molina et al., 2023). In addition, porphyrins (particularly metalloporphyrins), are considered as one of the most effective class of biomimetic catalysts (both in photo- and electro-catalysis), due to their significant catalytic activity in a great variety of chemical processes (Lin et al., 2021; Huang et al., 2022; Nikoloudakis et al., 2022; O'Neill et al., 2022; Domingo-Tafalla et al., 2023). Because of their tuneable optoelectronic properties, porphyrins are also used in molecular recognition and metal-ion sensing applications (Ogoshi and Mizutani, 1999; Ishizuka et al., 2022). Moreover, features of porphyrinoids such as biocompatibility, biological function mimicking, extended residence in cancer cells, are appealing properties for biomedical applications (Tian et al., 2023; Wang et al., 2023; Wu et al., 2023). For instance, photodynamic therapy (PDT) is a field dominated by the utilization of porphyrins (Mrinalini et al., 2021).

Researchers worldwide, have been utilizing porphyrinoinds for bio-imaging applications and drug delivery (Huang et al., 2015; Jenkins et al., 2016). This led to the development of porphyrin-based compounds as theragnostic agents in Magnetic Resonance Imaging (MRI), drug delivery and photodynamic cancer therapy (Stender et al., 2013; Cheng et al., 2014; Hammerer et al., 2014; Dong et al., 2017). Generally, porphyrins are used in the fields of disease treatment and *in vivo* diagnosis (Han et al., 2023). Regarding disease treatment, porphyrins can effectively treat inflammation and tumors since are able to produce reactive oxygen species (ROS) namely, singlet oxygen ($^1O_2$), hydroxyl and superoxide radicals, and hydrogen peroxide (Bustos et al., 2020; Wu et al., 2021). Apart from their exploitation in photodynamic therapy though, porphyrins can also have a photothermal therapy (PTT) effect against inflammation and tumors (Miao et al., 2021; Zhang et al., 2021). Concerning *in vivo* diagnosis, porphyrinoids can serve as organic ligands that coordinate paramagnetic ions (contrast agents for MRI) or as fluorescence chromophores, which both enable diagnosis in deep tissues (Egeblad and Werb, 2002; Zhang et al., 2007). Their intrinsic characteristics, such as: i) long resistance in tumors, ii) ability to mimic a great variety of biological processes,

iii) biocompatibility, and iv) minimal side effects are the most essential advantages that established them as potential agents in biomedical applications. Nevertheless, porphyrins are subjected to significant limitations, namely, nonspecific targeting, instability, self-quenching under physiological conditions. Researchers typically overcome these issues by incorporating porphyrins with carriers such as liposomes, inorganic particles, polymers, micelles, etc (Huang et al., 2017; Managa et al., 2018; Wang et al., 2018; Zhang et al., 2018).

Our research group, explored the features and behaviour of porphyrins in various biomedical applications (Figure 1). More specifically, a water-soluble porphyrin was encapsulated in a hydrogel as healing agent to treat skin defects in rats (Dontas et al., 2023). In another recent report, βCD-triphenylporphyrin conjugates found to specifically target and accumulate in lysosomes effectively acting as drug release agents (Panagiotakis et al., 2023). Similarly to the latter example, water insoluble tetraphenylporphyrins bearing a single carboxyalkyl side-arm were synthesized, showing remarkable photostability and a nearly 90% photokilling efficiency toward cancer cells (Panagiotakis et al., 2022). We have also examined the ability of porphyrin-peptide hybrids as labelling agents, in order to potentially be tested as theragnostic agents (Glymenaki et al., 2022). Finally, peptide nucleic acids (PNAs) were connected to photosensitizers (bodipy and porphyrin) to develop compounds for photodynamic therapy (PDT) (Chang et al., 2020).

2 Results and discussion

2.1 Wound healing

During the last decades, various approaches regarding the treatment and healing of skin defects have been explored (Takeo et al., 2015; Kamolz et al., 2022). The query though that frequently rises in clinical trials, is which treatment plan can be followed in each individual trauma. In many cases, a period of treatment with an effective agent is followed by the application of an alternative agent, since the wound healing process reaches a plateau (Seaman, 2002). Surgical procedures are arguably the most effective strategy to deal with widespread skin defects, namely, xenografts, autologous full- or split-thickness and allografts (Kamolz et al., 2022). However, apart from the aforementioned surgical techniques, hydrogels have also been extensively studied due to their unique features in healing skin defects (Loo et al., 2014). More specifically, hydrogels are biocompatible, uphold the desired hydration for the wound, are transparent which enables the observation of the healing process, provide gaseous permeability, and absorb the wound exudate (Amin and Abdel-Raheem, 2014; Li et al., 2021; Nuutila and Eriksson, 2021).

As a result, diverse hydrogels have been developed with the intention of creating a favourable environment for wound healing trials (Elangwe et al., 2022; Guamba et al., 2023). Self-assembling peptide hydrogels is one form of bioactive material tested in tissue regeneration and wound healing (Loo et al., 2014; Loo et al., 2015). This type of hydrogels is gaining considerable research attention as a result of their bioavailability and bioactivity. In addition, the encapsulation of a photosensitizer with antimicrobial properties
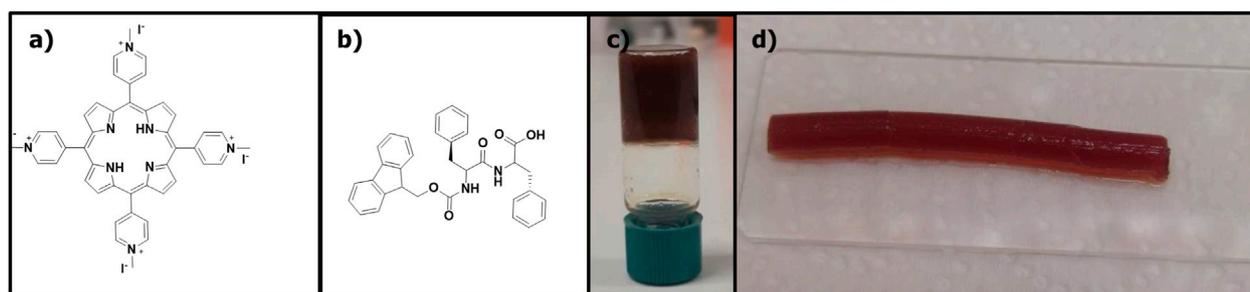
FIGURE 2
The molecular structures of: **(A)** (TMPyP^{4+})I$_4^-$, **(B)** Fmoc-FF, **(C)** hydrogel in a glass vial, **(D)** hydrogel upon syringe extrusion. Figure reproduced with permission from (Dontas et al., 2023). Copyright 2023, Springer Nature.

within them is feasible. One of the most appropriate dipeptide, that act as a hydrogelator is Fmoc (9-fluorenylmethoxy-carbonyl) protected phenylalanine-phenylalanine (Fmoc-FF). Fmoc-FF hydrogels are able to encapsulate and release small molecules in regulating manner promoting adhesion and proliferation (Mahler et al., 2006; Fichman and Gazit, 2014). In addition, hydrogels of Fmoc-FF are being formed with an excellent stability and are also studied because of their chemical simplicity and versatility (Arakawa et al., 2020; Martinez-Serrano et al., 2022). Porphyrinoids have been used as encapsulated photosensitizers in hydrogels, since are able to produce ROS and possess great photostability (Li et al., 2021; Ding et al., 2021; Martinez-Serrano et al., 2022). Their good biocompatibility, oxygen transfer, and molecular recognition outline porphyrnoids as ideal candidates for killing various microorganisms (McKenzie et al., 2019; Dias et al., 2020). Remarkably, in nature peptides and porphyrins work together to arrange molecules in an orderly fashion which is crucial for biological functions. For example, in the light-harvesting complex of Rhodopseudomonas acidophila's light-harvesting complex, the self-organization of porphyrinoids is being regulated by peptides (Frank and Cogdell, 2012).

Various porphyrin-based hydrogels have been investigated as antimicrobial agents in the last decades (Bonnett et al., 1993; Cabral et al., 2023; Thapa et al., 2023). Targeting the prevention of intraocular lens-associated infectious endophthalmitis, McCoy and co-workers developed hydrogels by combining anionic polymers of 2-(hydroxyethyl) methacrylate with the cationic tetrakis (4-N-methylpyridyl) porphyrin. The performed studies revealed high antimicrobial activity under intense light conditions due to $^1O_2$ generation (Parsons et al., 2009). The same year, McCarron and co-workers evaluated poly (vinyl alcohol)-borate complexes as drug delivery platforms in photodynamic antimicrobial chemotherapy of wound infections. A similar cationic porphyrin was utilized in this study as well, namely, the tetrakis (4-N-methylpyridyl) porphyrin tetra tosylate (Donnelly et al., 2009). The porphyrin-based hydrogels adopted the shape of the wound, however by preserving at the same time their structural integrity. Overall, the porphyrin-hydrogels were phototoxic toward methicillin-resistant *staphylococcus aureus*. In another report, Lovell et al. developed optically active polyamide hydrogels using tetrakis (4-carboxyphenyl) porphyrin (TCP) (Lovell et al., 2011). The hydrogels were stable, without any observable

degradation up to 2 months and also allowed a surgical resection *in vivo* using a fluorescence camera. The same TCP derivative, was also employed as a fluorescent tag in a biodegradable thermosensitive hydrogel, which demonstrated stable fluorescence for real time imaging (Lv et al., 2014).

In our recent report (Figure 2), we explored the treatment and healing of skin defects in rats using porphyrin-peptide hydrogels (Dontas et al., 2023). The developed hydrogels were transparent, in order to enable: i) the covering of the underlying tissue without adhesion, ii) the hydration during wound healing, and iii) the absorption of wound exudate. Two different families of rats were studied, namely, young and mature groups; interestingly, in both cases the application of the porphyrin-peptide hydrogel assisted in the healing process. Explicitly, concerning the mature animal group, the healing process was more pronounced due to the reduced healing rate of rats of advanced age. The porphyrinoid used in this work was a water-soluble 5,10,15,20-tetra-N-methyl-4-pyridyl) porphyrin tetra iodide ([TMPyP$_4^+$]I$_4^-$) which was synthesized according to our earlier work (Figure 2A) (Lazarides et al., 2014). For the successful development of the porphyrin-peptide hydrogel, a solvent-switch method was followed. In detail, ethanol was used as "good solvent" and water as the "bad solvent". The Fmoc-FF peptide (Figure 2B) was dissolved in ethanol and the porphyrin in water, resulting in a final porphyrin/peptide ratio of 1:30. As illustrated in Figures 2C, D, the type of the vessel (either a glass vial or a plastic syringe), resulted in two different morphologies. It is worth noting that the hydrogel formed in a plastic syringe (Figure 2D), was the most suitable because it permitted its direct application onto the defect of the rat.

Three different parameters were evaluated in the case of young rats: i) the diameter, ii) the perimeter, and iii) the total area. Overall, the porphyrin hydrogel outperformed both the control as well as the hydrogel experiment (formed by Fmoc-FF in ethanol/water) concerning the healing process of all defect parameters. Noteworthy, the hydrogel was also beneficial compared to the control experiment most likely due to the hydration of the skin defect; however, not at the extend of the porphyrin hydrogel. Similar to the experiment of the young rats, in the respective measurements using the mature rats, the porphyrin hydrogel outperformed both the hydrogel and the control experiment in terms of healing effect (for all three different defect parameters). More importantly, beneficial effect of the porphyrin hydrogel utilization was more

TABLE 1 Comparison of young and mature animals concerning the defect parameters (perimeter, diameter and area) for each experimental group (porphyrin-hydrogel, hydrogel, control).

Defect parameter	Porphyrin hydrogel		Hydrogel		Control	
	Young	Mature	Young	Mature	Young	Mature
Perimeter	71.06 ± 5.88	75.99 ± 10.65	72.73 ± 3.72	85.83 ± 4.80	87.03 ± 11.01	87.97 ± 6.00
Diameter	72.07 ± 6.48	78.72 ± 11.35	77.32 ± 4.56	82.51 ±10.99	93.47 ± 13.63	88.25 ± 6.88
Area	45.15 ± 9.54	56.52 ± 15.95	45.92 ± 7.39	69.16 ±10.30	69.62 ± 20.39	75.72 ± 11.21

visible concerning the statistical significance and the time of wound healing. It was evident, that all the different parameters illustrated a similar healing pattern in both young and mature rats. We have summarized the most important results in the following Table 1. We list herein, the comparison of porphyrin hydrogel, hydrogel and control experiment concerning the 3rd day of our measurements regarding the three skin defect parameters (perimeter, diameter and area) for both the mature and young rats.

2.2 Photodynamic therapy (PDT)

Photodynamic therapy (PDT) is a widely used oncological intervention which has approved by various countries, bringing hope in patients diagnosed with cancer each year (Siegel et al., 2022). Compared to conventional methods, such as surgery, chemotherapy and radiation treatment, PDT presents several advantages. In specific, PDT is non-invasive, nonradiative and with spatial accuracy of the operation, leading to minimal side effects, cumulative radiation dose and recurrence (Lucky et al., 2015). PDT is a type of phototherapy that combines light irradiation, photosensitizers, and oxygen to selectively destroy cancer cells (Brown et al., 2004; Castano et al., 2006; Li et al., 2019). The photosensitizer (PS) is excited with light in order to convert molecular oxygen into cytotoxic ROS, such as $^1O_2$ (Warszyńska et al., 2023). This procedure causes oxidative stress in cancer cells, which leads to cell death. PDT offers targeted treatment due to the specific activation of the PS by light, sparing normal tissues from unnecessary harm. Additionally, the targeted nature of PDT reduces side effects, establishing it as a more favourable choice compared to traditional cancer treatments. To that end, plenty research groups are exploring the utilization of PDT as a viable potential treatment for a variety of cancer types.
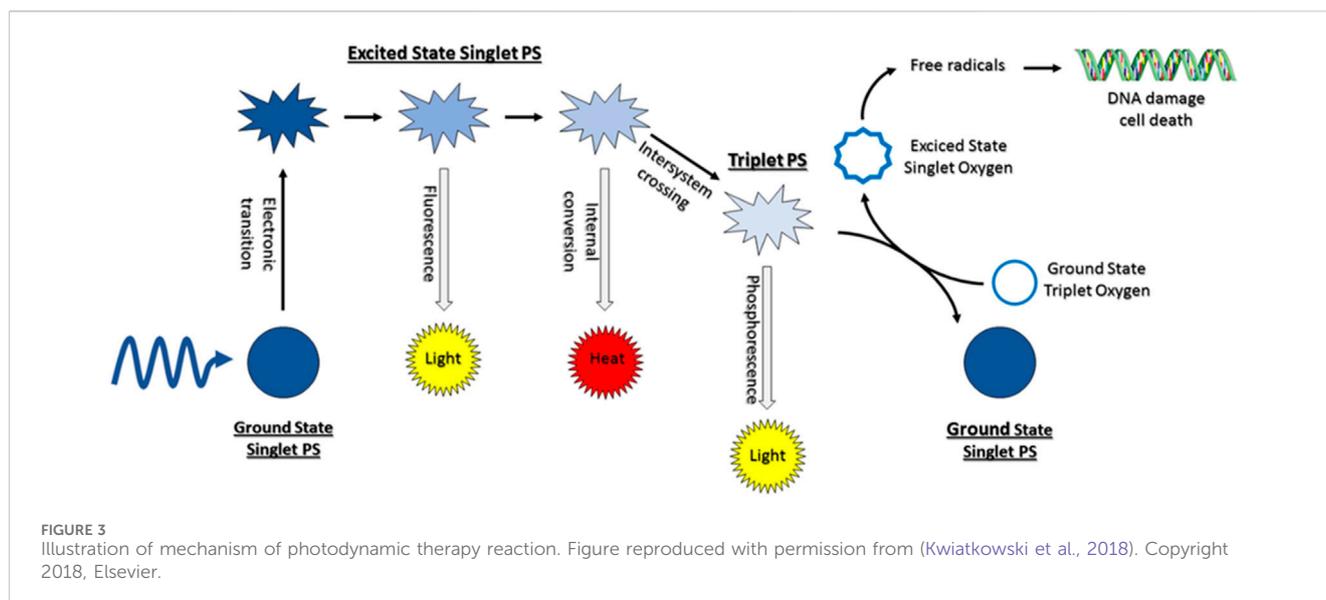
Even though PDT has been around for more than a century (Spikes, 1977; Szeimies et al., 2001), the vast interest in this kind of oncotherapy has grown as a result of recent progress in the fields of medicinal chemistry, cancer immunology, photochemistry, biochemistry, etc (Brown et al., 2004). Furthermore, PDT is employed in ophthalmology (age-related macular degeneration, AMD and treatment of localized infections) (Fine, 1999; Sułek et al., 2019), and dermatology (non-melanoma skin malignancies). The rising incidence of bacterial and fungal illnesses brought on by multidrug-resistant pathogens makes the latter use more crucial (Aroso et al., 2019). Many photosensitizers have been evaluated in PDT studies, both *in vitro* and *in vivo*; nonetheless, only a few of them demonstrated perfect abilities. As a result, contemporary research has been focussed on developing

novel photosensitizers (PSs) (Dąbrowski et al., 2016; Kwiatkowski et al., 2018). Chemical purity, tumor cell selectivity, physico-chemical stability, brief period of time between dose and maximum accumulation in tumor tissues, activation at longer wavelengths for deeper penetration in the biological tissue, and quick bodily clearance are all necessary features that an ideal PS should possess (Castano et al., 2005). Porphyrinoids such as porphycenes, porphyrins, phthalocyanines and chlorins are the four primary families of PSs. Interestingly, each class possess unique photochemical and photophysical characteristics which lead to wide-range methods of action and light activation (De Rosa and Bentley, 2000).

The mechanisms of light absorption and energy transfer are the two key components of PDT (Figure 3). The singlet ground state of PS is a low energy molecular orbital containing two electrons with opposing spins. One of these electrons is promoted to a higher energy orbital after absorption of a photon retaining its spin state. The additional energy of the PS in this transient state can be dissipated either by internal conversion as heat or emission of a photon, as fluorescence. The PS in the singlet excited state may also go through a process called intersystem crossing, in which the spin of the excited electron is inverted, converting the molecule to an excited triplet state with two electrons with parallel spin (Ochsner, 1996; Juzeniene and Moan, 2007). Then the PS from the triplet excited state returns to the singlet ground state by emission of light (phosphorescence) or undergoes two kinds of reactions with surrounding molecules.

First, it can react with a biological substrate, for instance a cell membrane or a molecule, by transferring an electron or a proton to generate a radical cation or anion, respectively. ROS might be created by these radicals after their reaction with molecular oxygen. As an alternative, the triplet PS can transfer its energy directly to molecular oxygen, resulting in the formation of excited singlet oxygen (Figure 3). The ratio between both routes, which can happen concurrently, is determined by the nature of the PS, as well as the substrate and oxygen concentrations (Castano et al., 2004). The major advantage of PDT lies to the fact that due to the high reactivity and brief half-life of singlet oxygen and hydroxyl radicals, only molecules that are close to the PS (i.e., regions of PS localization) are immediately impacted and eventually destroyed.

To efficiently produce ROS in PDT, a well-chosen PS is necessary. The ideal combination of optical and photophysical properties should be present, including long triplet state lifetimes, high triplet quantum yield, and efficient absorption (Dąbrowski et al., 2016). To guarantee proper photon penetration deep into tissues and avoid endogenous chromophores, it is preferred to utilize molecules that absorb in the red or even near-infrared range
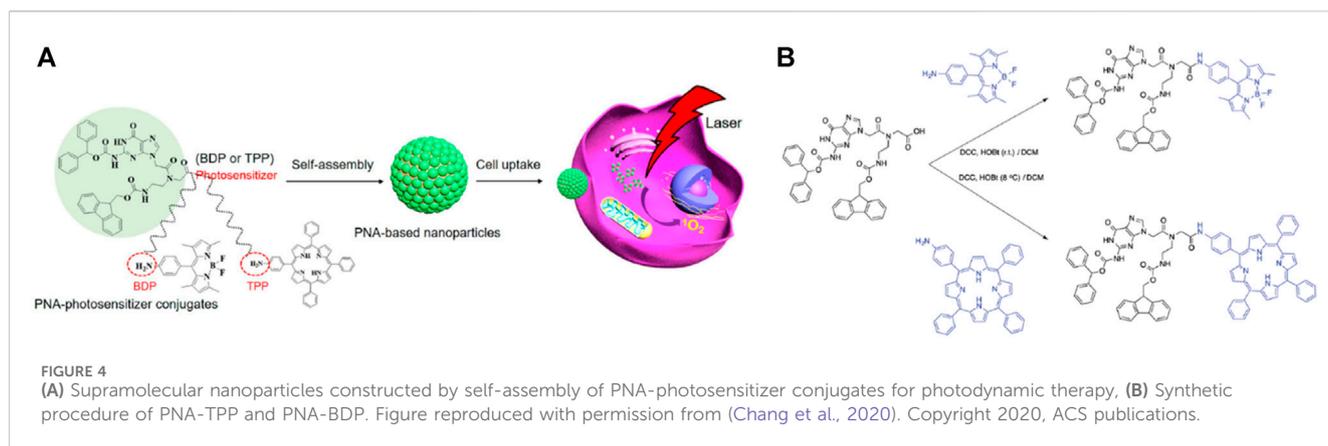
FIGURE 3
Illustration of mechanism of photodynamic therapy reaction. Figure reproduced with permission from (Kwiatkowski et al., 2018). Copyright 2018, Elsevier.

($\lambda \sim 630–850$ nm). To minimize the damage of healthy tissues, the PS should be biocompatible, non-toxic, and quickly removed from the body after photodynamic therapy avoiding long-term accumulation. This explains why the bulk of PSs used today are naturally occurring porphyrinoids, which may be found in a variety of animal and plant species. Because of their availability, low toxicity, and beneficial optical properties, porphyrinoids are frequently used in PDT. In addition, they can conveniently bind a wide range of metal ions. The first medication used in PDT was created by extracting hematoporphyrin (HP) from dried blood by eliminating iron, and was given the brand name Photofrin®. Albeit the first results indicated promising cancer treatment results, especially regarding esophageal and lung cancer, the medical world did not widely acknowledge it as a significant breakthrough. Other porphyrin-based PSs that were examined by the Food and Drug Administration (FDA) and applied in clinical trials are Visudyne and Foscan. PDT alone is typically insufficient to elicit a suitable immune response because cancer cells may produce additional promoting chemicals or immunosuppressive cytokines *via* non-immunogenic mechanisms. Thus, it is considered crucial to start with the most comprehensive approach when designing PDT PSs with the desired qualities and to appropriately combine various therapeutic approaches later on. One way to improve PDT effectiveness is the utilization of nanotechnology. Therefore, it is possible to engineer nanoparticles to deliver PSs to cancer cells improving selectivity and minimizing side effects. Additional development could be achieved, by enhancing photosensitizers' bioavailability and retention at the tumor site. Thus, many researchers designed novel nanomaterials with controllable structures *via* self-assembly approach (Charalambidis et al., 2011; Charalambidis et al., 2016; Karikis et al., 2016; Karikis et al., 2018; Li et al., 2018; Nikoloudakis et al., 2018; Xing et al., 2019).

Porphyrin chromophores present excellent biosafety and their absorption features are compatible with the biological window (650–900 nm), enabling deep tissue penetration. However, their low solubility in aqueous media significantly lowers singlet oxygen generation and limits their medical application.

Therefore, various approaches were employed to improve the solubility of the porphyrin-based PSs in water, such as, encapsulation of PS in appropriate carriers (Yang et al., 2019) and conjugation of PS with hydrophilic moieties (Feng et al., 2016). In another approach, van Hest's group prepared a peptide-porphyrin conjugate by coupling a pH-responsive dipeptide tryptophan-glycine (WG) to a hydrophobic porphyrin (P) core via amidation (Sun et al., 2020). This peptide-porphyrin hybrid (PWG) self-assembles into spherical nanoparticles under physiological conditions. However, when the nanoparticles were exposed to the acidic tumor microenvironment they transformed into nanofibers. The fibrillar nanostructures presented improved singlet oxygen generation ability and enabled high accumulation and long-term retention at tumor sites. In other report, Liang and co-workers prepared a porphyrin-oligopeptide conjugate (Ac-DEVDD-TPP) which demonstrated excellent solubility in water (Liu et al., 2023). Interestingly, after being taken up by oral cancer cells, Ac-DEVDD-TPP derivative was converted to D-TPP, which self-assembles into nanofibers around a mitochondrion. *In vivo* experiments demonstrated that upon laser irradiation, the porphyrin nanofibers induced efficient cell apoptosis and pyroptosis.

Another significant drawback of porphyrin-based PSs is their strong inclination to aggregate, which leads to the electronic excited state's quenching and, therefore, reduces the quantum yield of singlet oxygen (Escudero et al., 2006; Helmich et al., 2010). Many approaches have been described to move past this difficulty, such as the use of metal-organic frameworks (MOFs) (Lu et al., 2014; Li et al., 2017), adding special groups onto porphyrin-based PSs (Haynes et al., 2017), and anchoring porphyrins onto nanoparticle drug carriers (Yu et al., 2016; Huang et al., 2017). To a certain extent, all of the aforementioned tactics may reduce aggregation-induced quenching of the PS and enhance singlet oxygen quantum yield. Nevertheless, hydrophobic porphyrinic PSs could be encapsulated into amphiphilic copolymers, and subsequently released properly. Zhang and co-workers reported an approach based on the preparation of novel

FIGURE 4
(A) Supramolecular nanoparticles constructed by self-assembly of PNA-photosensitizer conjugates for photodynamic therapy, **(B)** Synthetic procedure of PNA-TPP and PNA-BDP. Figure reproduced with permission from (Chang et al., 2020). Copyright 2020, ACS publications.

porphyrin-polymeric nanoparticles (Jin et al., 2018). In this report, pollyhedral oligomeric silsesquioxane (POSS) and cage-shaped Si8O12-based NPs (Cordes et al., 2010), were used as polymers with increased mechanical strength and thermal stability. Therefore, a porphyrin and POSS copolymer were synthesized from 4-vinylbenzyl-terminated tetraphenylporphyrin and maleimide isobutyl polyhedral oligomeric silsesquioxane, *via* alternating reversible addition-fragmentation chain transfer polymerization. It was found that the aggregation induced quenching of the material and the PDT efficacy of this new porphyrin/POSS co-polymer was increased.

Peptides are easily conjugated with other functional groups, including PSs, in many examples. The peptide-PS conjugates that are produced have the ability to operate like PS in addition to possess self-assembling capabilities. Consequently, phototherapy and catalysis can employ nanomaterials created by conjugate self-assembly (Li et al., 2015; Zhang et al., 2015; Carrión et al., 2017; Han et al., 2017). Additionally, DNA self-assembly relies on Watson-Crick base pairing, a specific interaction between base pairs. On the contrary, in the respective peptide self-assembly noncovalent interactions govern the process (Nikolova et al., 2011; Wu et al., 2017). A unique approach to create hybrid materials is to combine DNA nanoparticles with flexible and complicated peptide-based nanomaterials. The self-assembling properties of both peptides and DNA are combined in peptide nucleic acids (PNAs), synthetic structures having a peptide backbone and DNA bases (Berger et al., 2015). Because PNAs are very resistant to being broken down by nucleases or proteases, they are perfect building blocks for use in biomedical applications. The Watson-Crick base pairs, stacking interactions, and optical characteristics of PNAs were confirmed by Gazit and co-workers a few years ago (Berger et al., 2015). We recently developed two PNA-photosensitizer conjugates (PNA-BDP and PNA-TPP), in order to examine their catalytic potential and assembly behavior (Nikoloudakis et al., 2019). The assemblies that are produced have distinct architectures, suitable dimensions, and distinctive light-absorbing properties. In this work, Coutsolelos and co-workers explored their potency in antitumor phototherapy (Figure 4A) (Chang et al., 2020). The synthetic protocol for the preparation of both PNA-hybrids is illustrated in Figure 4B. The hybrid PNA-TPP was produced in high yield *via* an amide coupling between the TPP-NH$_2$ and the Fmoc-PNA-G-(Boc)-OH. The coupling reagents

for this reaction were dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt), resulting in the final product (PNA-TPP) upon stirring at low temperature (8°C) for 48 h (yield: 58%). A similar process was used to prepare PNA-BDP; however, in this case room temperature was needed for the successful amide coupling reaction.

Both PNA-BDP and PNA-TPP nanomaterials were prepared *via* self-assembly in aqueous solution through π-π stacking and hydrophobic interactions, forming materials with uniform spherical morphology as demonstrated in SEM images (Figure 5). The diameter of the spheres was measured 102 ± 34 nm via dynamic light scattering (DLS). UV-Vis absorption measurements of the nanomaterials showed broadening of the peaks compared to their molecular counterparts. It is worth noting that in the case of PNA-TPP, a red-shift was observed, which is indicative for formation of *J*-aggregates. Similarly, quenching of their fluorescence spectra revealed the self-aggregation of the composites. Moreover, since stability of nanodrugs is quite important DLS measurements resulted that the average size of the particles was stable in various concentrations. Additionally, in cell culture media, namely, phosphate buffered saline (PBS) the size of the nanoparticles was not significantly altered. Upon light irradiation, the absorption band of PNA-TPP was slightly decreased, while the band of PNA-BDP was significantly decreased. The ability of both materials to produce singlet oxygen was perceived with the use of a trapping reagent, 9,10-anthracenediyl-bis(methylene) dimalonic acid. *In vitro* experiments PNA-BDP and PNA-TPP nanoparticles were incubated with MCF-7 and 3T3 cells and in both cases the cells could uptake the nanocomposites efficiently. Then, PDT evaluation was performed by scanning at 488 nm excitation (4 mW). Both nanoparticles showed efficient cytotoxicity of cells in a certain selected area, while in the dark no cytotoxicity was observed (Figure 5).

To date many porphyrin-based PDT studies have been reported, where porphyrins are in conjunction with a variety of materials, including amphiphiles, polymers, supramolecular polymers, and 3D nanoparticles. Porphyrin-based materials can lead to an increased PDT efficiency and a high loading concentration. However, in order to fully utilize these types of materials, a number of difficulties must be resolved in the near future. First, in order to reach deeper tissue, porphyrin-based materials' emission and absorption must be shifted to longer wavelengths. Second, despite the variety of reported
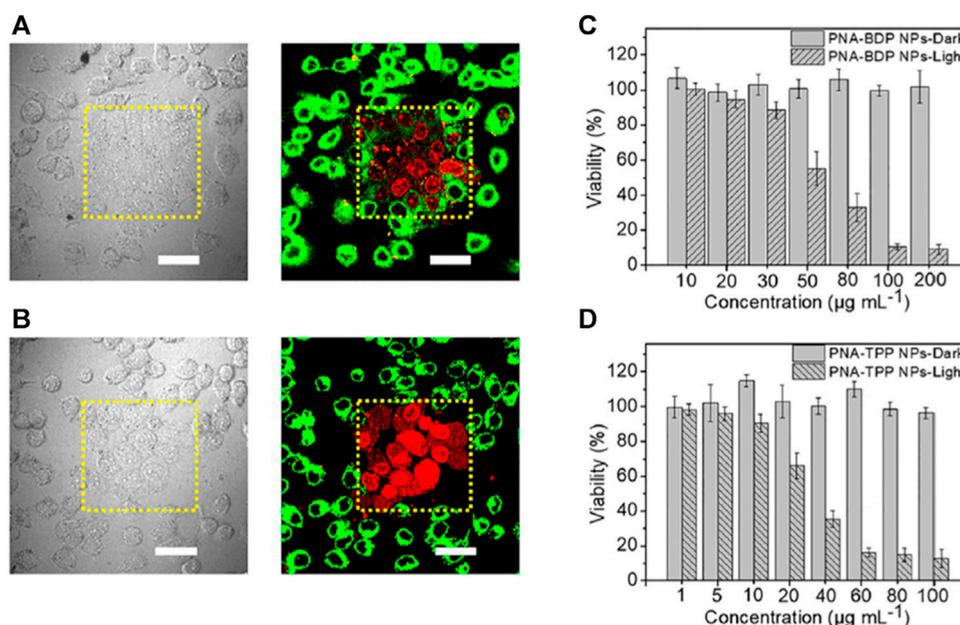
FIGURE 5
(A) Images from CLSM (right) and bright-field (left) demonstrating the PNA-BDP NPs' selective PDT action on MCF-7 cells. Dead cells are shown by the red signal from PI. **(B)** Bright-field (left) and CLSM (right) pictures demonstrating the PNA-TPP NPs' selective PDT action on MCF-7 cells; scale bars indicate 50 μm. The MCF-7 cells that were incubated with PNA-BDP NPs **(C)** and PNA-TPP NPs **(D)**, treated with or without light irradiation, were tested for viability. Scale bars indicate 50 μm. Standard deviation (n = 5) is shown by error bars. Figure reproduced with permission from (Chang et al., 2020). Copyright 2020, ACS publications.
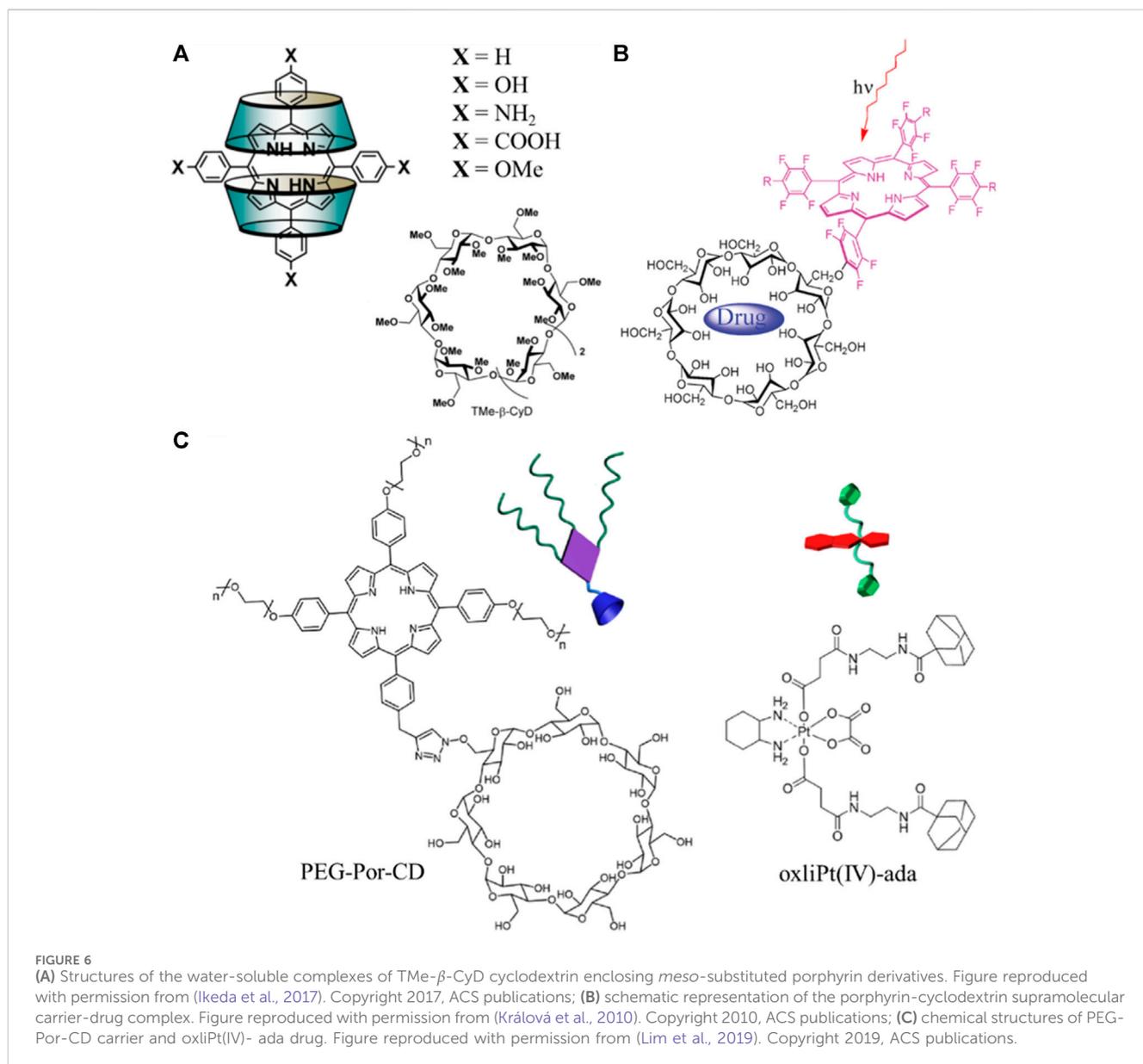
organic, inorganic, or hybrid porphyrin-based materials, it would be ideal to develop alternative therapies to rationally arrange the various functions against the particular tumor environment and get around PDT's inherent limitations. Thirdly, up to now, the majority of published research papers on porphyrin-based materials have mostly concentrated on fundamental and basic scientific study. Therefore, porphyrin-based materials activated by NIR light that have superior biocompatibility and biodegradability, high photocytotoxicity, outstanding distribution efficiency throughout the body, and efficient tumor targeting with minimum side effects are required for translational and clinical PDT practice. Consequently, further research and development are required to assure the effective use of these materials in clinical settings.

2.3 Photokilling cancer cells and drug release

As discussed in the previous paragraph, the key component in PDT, i.e., the PS, should possess the following features: i) ability to produce $^1O_2$ efficiently which is the cytotoxic agent responsible for the photokilling of tumor cells, ii) high absorbance in the long visible wavelengths (700–800 nm), iii) no toxicity in absence of light, iv) amphiphilic chemical structure as well as v) stability and solubility in injectable solvents (Ethirajan et al., 2011). Despite their great potential, typical porphyrins are insoluble in aqueous media; hence, demand a suitable molecular carrier which renders them water-soluble and assists their optimum delivery without changing their photosensitizing activity (Dąbrowski et al., 2016). Cyclodextrins (CDs) are promising candidates for this role since

they include guest molecules in their hollow structures producing inclusion complexes. In addition, cyclodextrins can host porphyrin molecules and improve their solubility in water, physicochemical stability and bioavailability as well as controlling their release (Tian et al., 2020). Several reports have been published dealing with porphyrin PSs combined with cyclodextrins towards PDT activity (Liu et al., 2016).

In a recent report, Ikeda et al. (2017) prepared water-soluble complexes of cyclodextrin enclosing porphyrin derivatives with different substituents in the *meso*-positions (Figure 6A) and investigated their activity towards PDT (Ikeda et al., 2017). In particular, aniline- and phenol-substituted porphyrins demonstrated high photodynamic activity which was even greater than photofrin; i.e., a common drug in clinical used as photosensitizer. Covalent attachment of cyclodextrin with porphyrin is another approach towards cancer therapy. The porphyrin-cyclodextrin conjugates (Figure 6B) prepared by Král and co-workers presented combined PDT activity with chemotherapy (Králová et al., 2010). The versatile supramolecular system combined efficient enclosing of the drug in the cavity of the cyclodextrin moiety as well as the photosensitizing properties of the porphyrin moiety while the entire complex was accumulating in the cancer tissue. The combination of PDT and chemotherapy was explored also in a more recent work, where Zhao and co-workers synthesized the porphyrin-cyclodextrin PEG-Por-CD nanocarriers presented in Figure 6C (Lim et al., 2019). These conjugates self-assembled with oxaliplatin via host-guest interactions and demonstrated enhanced cytotoxicity and apoptosis compared to the corresponding monotherapy. Besides covalent and Van-der Waals interactions, cyclodextrins can be combined with
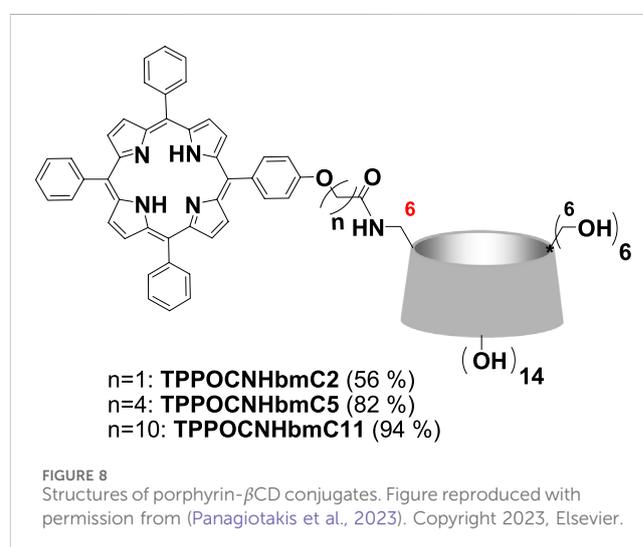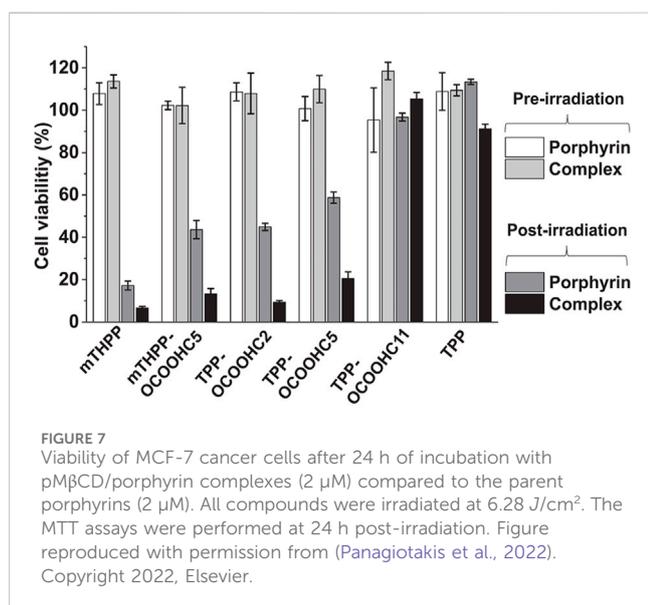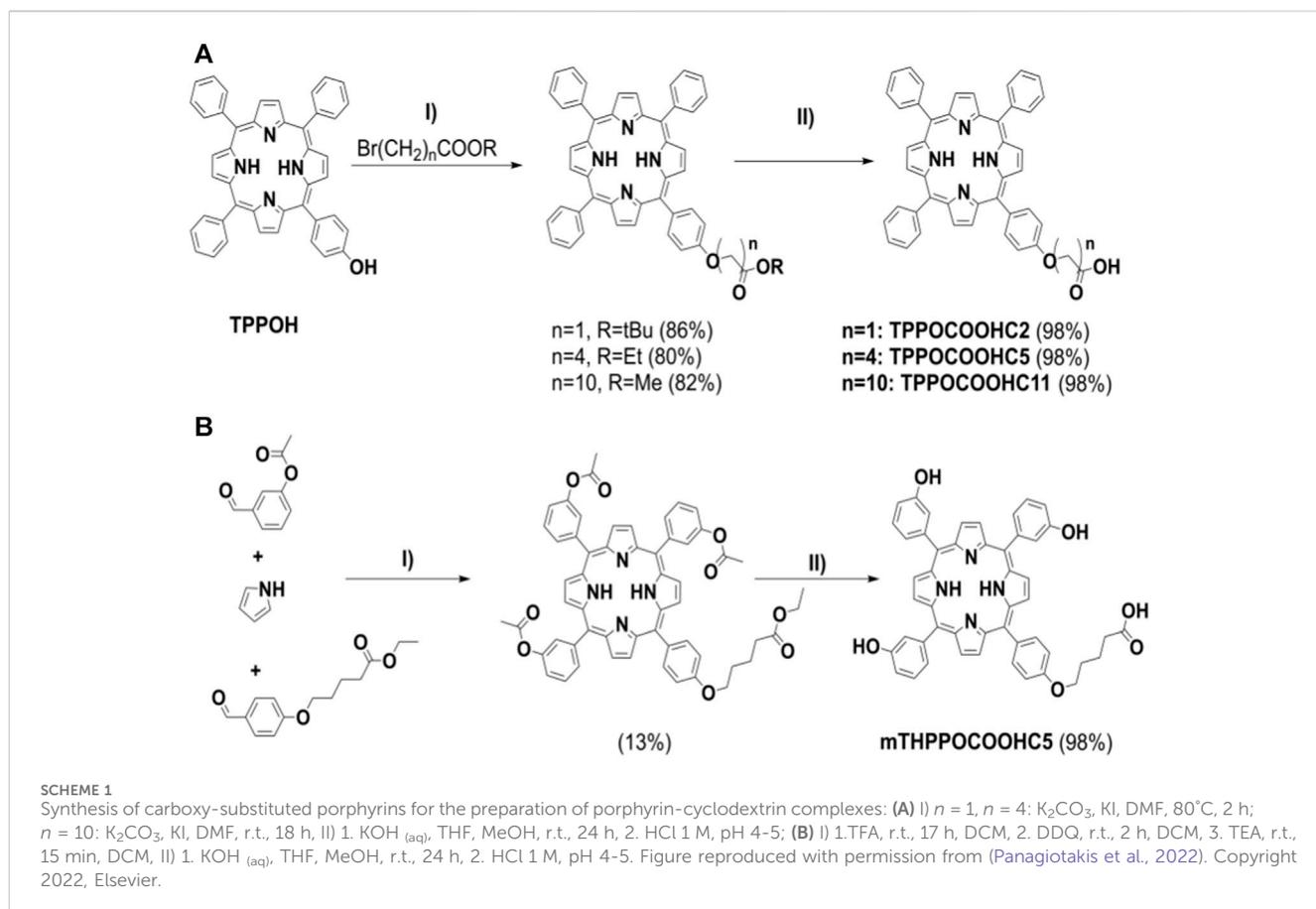
FIGURE 6
(A) Structures of the water-soluble complexes of TMe-β-CyD cyclodextrin enclosing *meso*-substituted porphyrin derivatives. Figure reproduced with permission from (Ikeda et al., 2017). Copyright 2017, ACS publications; **(B)** schematic representation of the porphyrin-cyclodextrin supramolecular carrier-drug complex. Figure reproduced with permission from (Králová et al., 2010). Copyright 2010, ACS publications; **(C)** chemical structures of PEG-Por-CD carrier and oxliPt(IV)- ada drug. Figure reproduced with permission from (Lim et al., 2019). Copyright 2019, ACS publications.

porphyrins via electrostatic interactions towards $^1O_2$ generation (Zagami et al., 2023).

In 2022, our research group contributed in this field through the preparation of porphyrin-cyclodextrin complexes towards PDT. In detail, we prepared and fully characterized unsymmetrical, mildly amphiphilic porphyrin derivatives, in addition to their complexes with pMβCD in water, and investigated their intracellular delivery, phototoxicity and dark toxicity against MCF-7 breast cancer cells (Panagiotakis et al., 2022). Scheme 1 illustrates the synthetic procedures for the preparation of the porphyrin derivatives bearing different peripheral substituents and a carboxylic side-chain of different length. In detail TPPOCOOHC2, TPPOCOOHC5, TPPOCOOHC11 and the *meta*-trihydroxy derivative mTHPPOCOOHC5, were synthesized in high yields starting from the corresponding ester followed by basic hydrolysis. The ester precursor porphyrins were prepared via nucleophilic substitution reactions between TPPOH and the

appropriate ω-bromoalkyl ester (Brulé et al., 2004) (Scheme 1A). The *meta*-substituted ester precursor was synthesized from the suitable ester-protected aldehydes and pyrrole (Scheme 1B) using trifluoroacetic acid (TFA) according to Lindsey's method (Lindsey et al., 1987). The symmetrical compounds TPP and mTHPP were also synthesized by the Adler and Longo method for comparison purposes (Adler et al., 1964). Analysis of the 1D and 2D NMR spectra proved that the stoichiometry of the complexes of pMβCD with the unsymmetrical amphiphilic porphyrins is 2:1, while there are variations in the complexation modes and in the number of supramolecular isomers.

Photogeneration of singlet oxygen was monitored using the water-soluble $^1O_2$ sensor 9,10-anthracenediylbis(methylene) dimalonic acid (ABDA) and clearly verified that the rates of $^1O_2$ production were larger in case of the porphyrin-CD complexes than for the free porphyrins, especially for TPPOCOOHC5, while no difference was detected for mTHPPOCOOHC5. After establishing

SCHEME 1
Synthesis of carboxy-substituted porphyrins for the preparation of porphyrin-cyclodextrin complexes: **(A)** I) $n = 1$, $n = 4$: K_2CO_3, KI, DMF, 80°C, 2 h; $n = 10$: K_2CO_3, KI, DMF, r.t., 18 h, II) 1. KOH (aq), THF, MeOH, r.t., 24 h, 2. HCl 1 M, pH 4-5; **(B)** I) 1.TFA, r.t., 17 h, DCM, 2. DDQ, r.t., 2 h, DCM, 3. TEA, r.t., 15 min, DCM, II) 1. KOH (aq), THF, MeOH, r.t., 24 h, 2. HCl 1 M, pH 4-5. Figure reproduced with permission from (Panagiotakis et al., 2022). Copyright 2022, Elsevier.

FIGURE 7
Viability of MCF-7 cancer cells after 24 h of incubation with pMβCD/porphyrin complexes (2 μM) compared to the parent porphyrins (2 μM). All compounds were irradiated at 6.28 J/cm^2. The MTT assays were performed at 24 h post-irradiation. Figure reproduced with permission from (Panagiotakis et al., 2022). Copyright 2022, Elsevier.

FIGURE 8
Structures of porphyrin-βCD conjugates. Figure reproduced with permission from (Panagiotakis et al., 2023). Copyright 2023, Elsevier.

the excellent stability of the pMβCD/porphyrin complexes under prolonged light irradiation in the presence of cell culture proteins for 24 h, the authors studied their cellular uptake by MCF-7 breast cancer cells using confocal microscopy. It was proven that pMβCD promotes enhanced cell uptake as well as endoplasmic reticulum (ER) distribution and high phototoxicity, while toxicity without light

remains low. In Figure 7, the photodynamic activity against MCF-7 cells of the pMβCD/porphyrin complexes compared to the parent porphyrins is illustrated. The most effective monocarboxyporphyrins may be suggested as parts of more advanced photo and chemotherapy platforms, and their function can be facilitated by pMβCD.

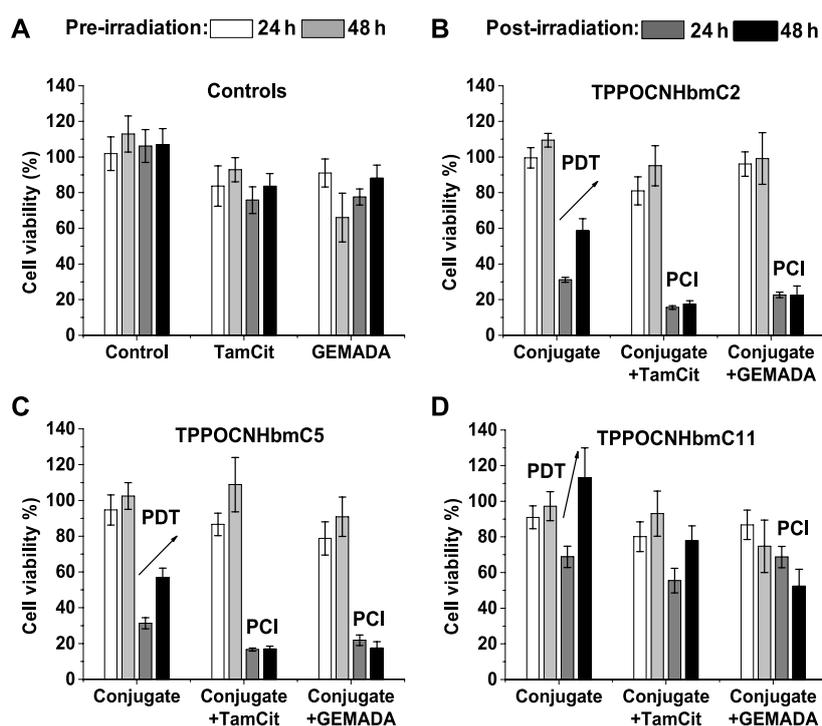In addition, our research group recently developed combinational PDT and chemotherapy systems based on

FIGURE 9
Toxicity against MCF-7 cells after 24 h of incubation with porphyrin-βCD NPs (10 µM) with or without anticancer drugs (7.5 µM), without (white and light grey bars) or with irradiation at 4.18 J/cm², λ = 640 nm (dark grey and black bars). The MTT assays were performed at 24 h (dark grey bars) or 48 h (black bars) post-irradiation: **(A)** Control experiments with cells alone (control), TamCit or GEMADA; **(B)** TPPOCNHbmC2; **(C)** TPPOCNHbmC5; **(D)** TPPOCNHbmC11, alone and loaded with the drugs Figure reproduced with permission from (Panagiotakis et al., 2023). Copyright 2023, Elsevier.

cyclodextrins and porphyrins (Panagiotakis et al., 2023). The aim was to develop photoactive nanoparticle targeting cancer cell lysosomes for drug delivery and light-controlled release. In detail they covalently connected the best performing porphyrins of their previous work with mono-amino-substituted cyclodextrin and obtained new amphiphilic porphyrin-βCD conjugates. The successful synthesis of the porphyrin-βCD conjugates (Figure 8) was achieved via amide linkage between the mono(6-amino-6-deoxy)-βCD (Petter et al., 1990) and TPPOCOOHCx (Scheme 1) utilizing 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) (Kunishima et al., 1999) coupling reagent. Noteworthy the employment of common coupling reagents and conditions such as DCC and HOBt, or hexafluorophosphate azabenzotriazolyl-tetramethyluronium (HATU) or thionyl chloride (SOCl$_2$) lead only to the recovery of the starting materials. Absorption and emission spectroscopy studies revealed that the length of the aliphatic chain linkers between the porphyrin and βCD moieties porphyrin-βCD molecules does not alter the photophysical properties both in DMF and in aqueous media. The conjugates self-assembled into nanoparticles of approximately ~60 nm as demonstrated by scanning and transmission electron microscopy as well as DLS experiments. Importantly, the self-assembled nanoparticles (NPs) were photoactive and selectively targeted the lysosomes of MCF-7 cells.

After establishing the above desired properties, we proceeded with the inclusion of anticancer drugs in the NPs. Two different drugs were investigated, the citrate salt of Tamoxifen (TamCit) and the N-adamantyl derivative of Gemcitabine (GEMADA). PBS solutions

of the pre-assembled NPs were incubated with TamCit, GEM and a model guest 1-adamantylcarboxylic acid (ADACOOH) and according to DLS experiments the diameter of the nanoparticles was significantly increased. Confocal laser scanning microscopy was utilized once again for live imaging of human breast adenocarcinoma MCF-7 cells incubated with the conjugate NPs. Either empty or with the drug, the NPs present no toxicity in the dark for 48 h; thus, light irradiation induces the release of the drugs. After irradiation at 640 nm with a LED array lamp, the cells incubated with drug-loaded NPs displayed a major decrease of viability (>85%) for 48 h. The results showed strong synergy between the photodynamic and chemotherapeutic effects and therefore, photochemical internalization (PCI) is suggested to be the drug release mechanism. The above results are illustrated in Figure 9 which describes the viability of MCF-7 cells after 24 h of incubation with the NPs, with or without the investigated anticancer drugs. The authors suggest that the prepared porphyrin-βCD NPs, should be considered, along with other nano-assemblies (Xue et al., 2019), as efficient vehicles for the photo delivery of small drugs.

2.4 Peptide labelling

Visualizing the trafficking and localization of proteins in living systems is the key to understand protein functions in their natural environment. Thus, labelling and tracking of biomolecules (peptides, antibodies, and proteins) inside alive cells provides a pathway for the determination of their interactions, mobility, dynamics, and functions (Schneider and Hackenberger, 2017). Several labelling methods such as

radioactive tracers, photochromic compounds, electrochemical sensors, colorimetric biosensors, isotope markers, photo switchable biomaterials and fluorescent labels are available for this purpose (Ong and Mann, 2006; Chudakov et al., 2010; Rocha-Santos, 2014; Velikyan, 2014; Abdollahi et al., 2019; Rai and Ferreira, 2021). Among them, optical imaging by fluorescence microscopy is the most widely used and versatile visualization strategy in research and clinical practice mainly due to its non-destructive features and high sensitivity. Additionally, it fulfils the requirements of low concentration of fluorescent material and small measurement volume (Dean and Palmer, 2014). Recently, fluorescence microscopy and imaging have gained significant attention, owing to the growing availability of dyes, fluorescent proteins and probes alongside with an increasing number of fluorescent imaging methods.

Optical visualization by fluorescence microscopy requires the site-specific labelling of a protein of interest (POI) with a fluorescent derivative. The connection of the fluorophore with the desired biomolecule can be achieved biologically or chemically (Lotze et al., 2016). Peptide-based recognition tags provide an attractive strategy for protein labelling. In the tag-based method, the POI is genetically fused with an oligo-peptide that binds site-specifically to a desired synthetic fluorophore (Lai et al., 2015). The attachment of the fluorophore with the tag through metallochelate coupling is one of the most widely used strategies and has been employed in biotechnology and molecular biology by several research groups (Connell and Donnelly, 2018). It is based on the capability of nitrilotriacetic (NTA) complexes with transition metals (Zn^{2+}, Ni^{2+}, Co^{2+}, or Cu^{2+}) to coordinate specific amino acid residues, mostly oligo-histidine sequences, such as the hexa-histidine tag (His_6-tag) (Leonhardt et al., 2023). The His_6-tag is an oligo-histidine sequence in which six histidine residues have the ability to interact noncovalently and reversibly with a metallic complex of NTA. The main advantage of this approach is the high specificity, and additionally the tag can be expressed either at the N-termini or C-termini during the expression of the protein (You and Piehler, 2014).

Porphyrins and metallo-porphyrins are considered as promising labelling tools (Ethirajan et al., 2011; Rabiee et al., 2020; Sandland et al., 2021; Jin et al., 2023). Additionally, porphyrin derivatives were employed in PDT of tumours and as labels for the detection of cancer cells (Tian et al., 2020; Chen et al., 2021; Park et al., 2021; Shi et al., 2021). Dmitriev et al. (2016) reported the first example where porphyrin complexes bearing NTA moieties were applied in the labelling of various biomolecules that possess the His_6-tag. More specifically, they succeeded to label through metallochelate coupling several peptides and proteins with phosphorescent Pt-porphyrin derivatives bearing NTA group. Their studies revealed that all synthesized metallochelate complexes display phosphorescence and sensitivity to O_2, which is comparable with other (poly)peptide-based probes.
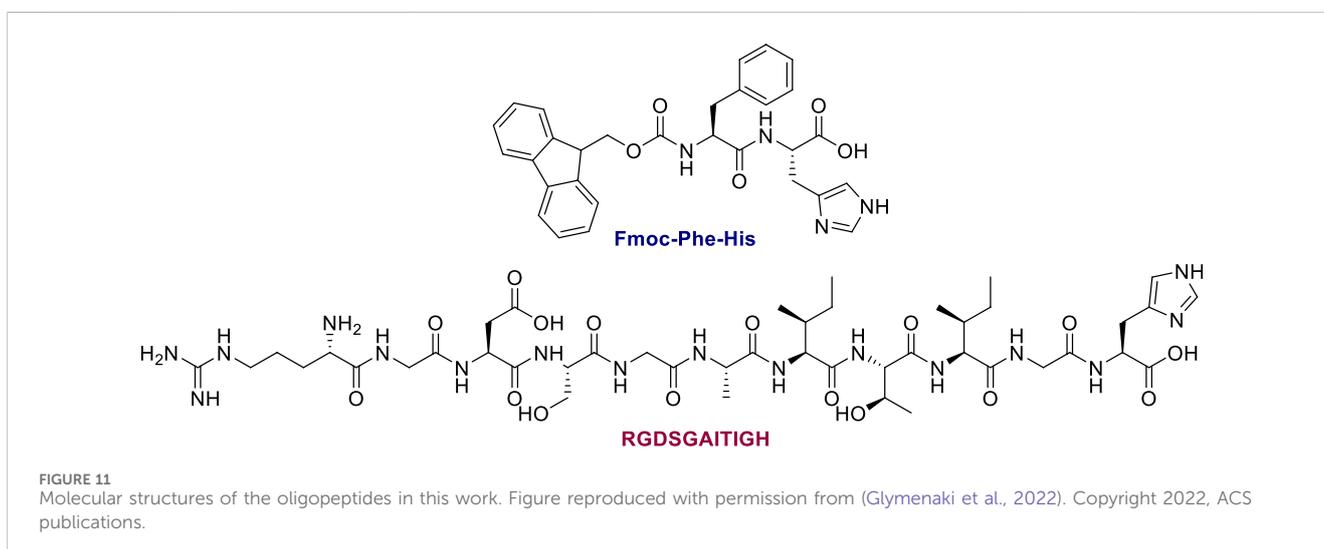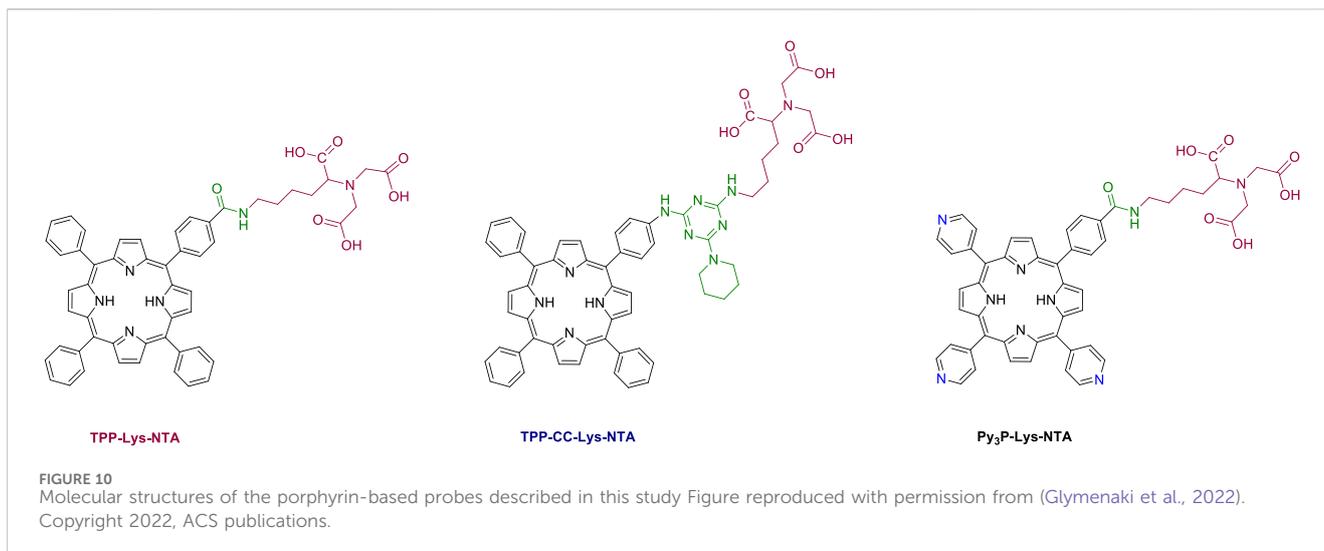
Coutsolelos and co-workers reported the synthesis and characterization of three porphyrin-based fluorescent probes (Figure 10), bearing modified lysine-NTA groups and could be applied in protein labelling through metallochelate coupling chemistry (Glymenaki et al., 2022). All hybrids were based on a free base porphyrin macrocycle which was covalently connected with an appropriately NTA-substituted ligand. In the first two probes (TPP-Lys-NTA and TPP-CC-Lys-NTA) the same phenyl-substituted porphyrin ring was employed, with the only difference being the group linking the two moieties. In TPP-Lys-NTA, the linkage was performed through an amide bond, while in TPP-CC-Lys-NTA, a triazine ring was employed as linker in order to increase the distance between the porphyrin and the NTA ligand. In the

third dyad (Py_3P-Lys-NTA) three pyridyl groups were introduced to the porphyrin periphery, targeting to increase the hydrophilicity of the probe; a desirable feature for labelling water-soluble peptides. The connection of the porphyrin with the NTA ligand was achieved through an amide bond using standard coupling reagents.

Initially, absorption and emission spectroscopy studies were performed to explore if the three probes were able to coordinate with Ni^{2+} ions. These experiments demonstrated that the NTA ligand can bind Ni^{2+} and the characteristic absorption features of the dyes are preserved after the coordination. Noteworthy, emission studies revealed that the emission of these probes is not quenched after the coordination with Ni^{2+} ions. This observed feature is stimulating, since the majority of the reported fluorophores a significant emission quenching is observed upon the connection with the transition metal. This is the most common drawback of many fluorescent probes, since quenching of the emission decreases the detection limit of the probe after its connection with the desired POI (Dmitriev et al., 2016). Additionally, as a proof of concept, we studied two oligo-peptides (Fmoc-FH and RGDSGAITIGH, Figure 11) to examine whether the porphyrin-NTA dyads could be used as fluorescent probes and coordinate with them through the NTA ligand. Interestingly, these two peptides were able to self-assemble and form various nanostructures. Moreover, one histidine residue is present in their backbone; thus, they can be regarded as His_6-tag models. Self-assembly based on oligo-peptides provides a novel approach for tumour nanotheranostics that possess functionality, biosafety, controllability and programmability, enabling the transition of supramolecular nanotheranostics from lab to bedside (Li et al., 2019).

Field-emission scanning electron microscopy (FESEM) experiments were performed to study the capability of porphyrin-NTA dyads to coordinate with histidine bearing peptides in the presence of Ni^{2+} ions. TPP-Lys-NTA hybrid self-assembles and forms flake-shaped nanostructures (Figure 12A). However, in the presence of Ni^{2+} ions the morphology of the porphyrin self-assembling nanostructures was altered, resulting in spherical architectures (Figure 12B). On the other hand, the Fmoc-FH peptide self-assembles forming a network of fibrils (Figure 12D). Noteworthy, in the case where both Fmoc-FH and TPP-Lys-NTA were present the two derivatives self-assembled independently and formed a fibrillar network covered with spherical nanostructures (Figure 12E). Finally, the sample which contained Fmoc-FH with TPP-Lys-NTA-Ni^{2+} gave rise to the formation of more well-defined nanostructures with spherical shape and increased diameter (Figure 12C). Similar results were observed for the other two probes as well. The differences that were observed before and after the addition of the oligo-peptides provide a strong indication for the successful metallochelate coupling. Solid state absorption and emission experiments also supported the connection of the probes with the oligopeptides through metallochelate coupling.

Moving one step forward, the authors examined the capability of the porphyrin-peptide hybrids to penetrate and accumulate in mammalian HeLa cancer cells, studying their potential application as anticancer agents. Confocal microscopy studies revealed that the porphyrin-NTA hybrids alone and their corresponding complexes with the oligopeptides can enter the HeLa cancer cells and accumulate into the cytoplasm. Thus, peptides with histidine residues in their backbone are especially suitable for intracellular deliver of labelling fluorophores or different type of molecules. The pKa of histidine's imidazole group is around 6 and in can be protonated in the intracellular environment, especially in the

FIGURE 10
Molecular structures of the porphyrin-based probes described in this study Figure reproduced with permission from (Glymenaki et al., 2022). Copyright 2022, ACS publications.

FIGURE 11
Molecular structures of the oligopeptides in this work. Figure reproduced with permission from (Glymenaki et al., 2022). Copyright 2022, ACS publications.

acidic conditions within endosomes. This can result in endosomal lysis and release of the fluorophore or another type of molecule inside the cytoplasm.

3 Conclusion and outlook

Overall, in this perspective article we illustrated the development of properly functionalized porphyrinoids as agents in several biomedical applications, namely, wound healing, PDT, photokilling cancer cells, drug release, and peptide labelling. The wound healing results using porphyrin-hydrogels in young and mature rats based on our work, illustrated that further studies would be of great interest. More precisely, as future directions we suggest the utilization of metallated porphyrinoids with higher $^1O_2$ production rates, as well as diverse peptides which can still form transparent hydrogels though. These two approaches could potentially improve the stability and the efficiency of hydrogels targeting defect parameters similar to our study. We have also

introduced a method for producing PDT nanodrugs by mixing PNAs with suitably functionalized photosensitizers. We were able to develop nanoparticles with distinct structures through the self-assembly of PNA-BDP and PNA-TPP hybrids, which were effectively internalized by cells and exhibited stability in biological fluids. In the absence of irradiation negligible cytotoxicity was observed; however, by utilizing laser irradiation selective cytotoxicity was detected rendering these self-assembled NPs as effective nanoagents. Moving one step forward, additional PNA derivatives connected with appropriate decorated porphyrin-based PSs should be developed, targeting to further improve both the identification of tumour cells as well as their treatment. Overall, this approach could advance the preclinical assessment and the clinical use of bioinspired biomaterials via the incorporation of such novel supramolecular therapeutic nanoagents. In addition, we have utilized cyclodextrin moieties in an effort to develop water-soluble porphyrin complexes with PDT properties. This strategy was proved very efficient; thus, other chromophores with alike photophysical features such as corroles, phthalocyanines and
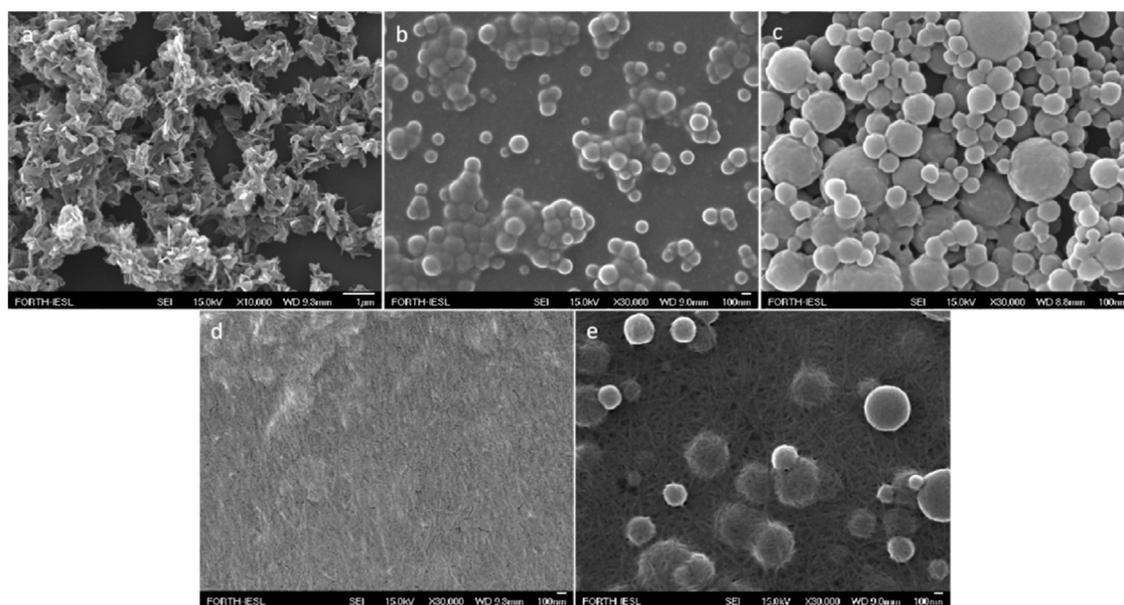
FIGURE 12
FESEM picture of: **(A)** TPP-Lys-NTA, **(B)** TPP-Lys-NTA and Ni^{2+}, **(C)** Fmoc-FH and TPP-Lys-NTA with Ni^{2+}, **(D)** Fmoc-FH, and **(E)** Fmoc-FH and TPP-Lys-NTA. All samples were prepared in HFIP:HEPES 50 mM pH 7.4 (3:7, v/v) solvent mixture. Figure reproduced with permission from (Glymenaki et al., 2022). Copyright 2022, ACS publications.

BODIPY dyes could also be used as photosensitizers in PDT studies. The porphyrin-cyclodextrin conjugates that we developed, targeting cancer cells for light-controlled drug release, demonstrated a facile way to develop photoactive nanoparticles; expanding the field of combinational PDT and chemotherapy into different small molecule chemotherapeutics. Regarding the protein labelling applications, we covalently connected appropriately modified NTA ligands with porphyrins in an effort to form fluorophores. One of the possible future direction in this field could be the preparation of similar hybrids by taking advantage of the metallochelate coupling chemistry, and eventually penetrate into cancer cells. Porphyrinoids have merited a distinct spot in biomedicine, owing to: i) their broad and intense absorption, ii) their facile structural modification, and iii) their ability to chelate various metals. As a consequence, porphyrin-based nanohybrids have attractive photoredox, photochemical, and photophysical properties and are considered as ideal theragnostic agents with vast potentials. However, in order to find a practical application, we should improve their stability against demetallation, biocompatibility, selectivity, off-target phototoxicity, $^1O_2$ quantum yield, and absorption in the visible-NIR region. Undoubtedly, the successful preparation of porphyrin macrocycles with the above-mentioned characteristics will result in useful theragnostic agents in multiple disease areas.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

VN: Conceptualization, Writing–original draft, Writing–review and editing. EN: Writing–original draft, Writing–review and editing. KL: Investigation, Writing–original draft, Writing–review and editing. GC: Investigation, Writing–original draft, Writing–review and editing. AC: Investigation, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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