Smart molecular designs and applications of activatable organic photosensitizers

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Review article

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Smart molecular designs and applications of activatable organic photosensitizers

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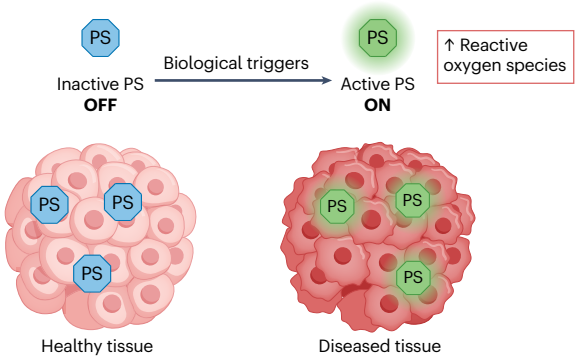
Abstract

Photodynamic therapy (PDT) — which combines light, oxygen and photosensitizers (PS) to generate reactive oxygen species — has emerged as an effective approach for targeted ablation of pathogenic cells with reduced risk of inducing resistance. Some organic PS are now being applied for PDT in the clinic or undergoing evaluation in clinical trials. A limitation of the first-generation organic PS was their potential off-target toxicity. This shortcoming prompted the design of constructs that can be activated by the presence of specific biomolecules — from small biomolecules to large enzymes — in the target cells. Here, we review advances in the design and synthesis of activatable organic PS and their contribution to PDT in the past decade. Important areas of research include novel synthetic methodologies to engineer smart PS with tuneable singlet oxygen generation, their integration into larger constructs such as bioconjugates, and finally, representative examples of their translational potential as antimicrobial and anticancer therapies.



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Key points

- Activatable organic photosensitizers (PS) are designed for targeted photodynamic therapy (PDT), reducing off-target toxicity by activating only at disease sites.
- Chemical activation strategies include pH-responsive, redox-mediated, and enzyme-activatable PS, enhancing selectivity and efficacy in anticancer and antibacterial treatments.
- Near-infrared (NIR) PS improve tissue penetration and targeting for clinical translatability, with modifications such as extended conjugation and heavy atom inclusion to increase the excitation wavelengths.
- Successful biomedical applications demonstrate the potential of activatable PS in treating cancers and infectious diseases, with ongoing challenges in clinical adaptation.
- Future directions focus on optimizing PS formulations for better biodistribution, developing NIR PS for deeper tissue penetration, and exploring sonodynamic activation.

Introduction

Since the early 1990s, photodynamic therapy (PDT) has been used to safely remove pathogenic or malignant cells during the treatment of infectious diseases, skin conditions and cancer[1,2]. PDT makes use of the ability of photosensitizers (PS) to produce toxic reactive oxygen species (ROS) and cause cell death in areas where ultraviolet (UV) light or visible light is irradiated. Numerous chemical constructs have been reported as PDT agents, and they can range in their structures from nanoparticles and metal–organic frameworks to small organic molecules[3–7]. The smaller size, versatility and modularity of the latter have favoured their diversification for biological targeting and translation to clinical studies.

In addition to producing high levels of ROS upon light illumination, an important requirement of PDT agents is to induce toxicity only in target cells without causing major damage to neighbouring healthy tissues. Most conventional PDT agents (for example, photofrin$^{(R)}$ and talaporfin) lack targeting groups to facilitate accumulation in target sites, which can result in side effects such as off-target cytotoxicity or skin photosensitivity. Additional desired features in targeted PS include excitation in the near-infrared (NIR) region of the electromagnetic spectrum to maximize tissue penetration and clinical translatability.

One approach to enhance the accumulation of PS in targeted tissues is by conjugating them to biomolecules (for example, peptides, proteins and antibodies) able to bind specific cell-surface markers[8–11]. This strategy is effective at disease stages wherein proteomic signatures are distinct, but it can have limitations at earlier stages of pathological processes. Moreover, the size difference between the PS and large biomolecules can limit the therapeutic efficacy of the constructs owing to inadequate PS–protein ratios and heterogeneous infiltration into targeted tissues[12]. Alternatively, synthetic chemists have described smart molecular designs — similar to those reported for fluorescent probes[13–17] — wherein the reactivity (and, therefore, phototoxicity) of activatable PS is modulated chemically or biologically to improve cell selectivity. Activatable fluorophores are widely used in bioimaging[18,19], super-resolution microscopy[20] and fluorescence-guided surgery[21], among others. By using similar designs, the activatable PS counterparts can harness unique features of the tumour microenvironment or the cell envelope of bacterial cells to cause preferential damage in targeted cells, thereby reducing bystander toxicity and improving overall efficacy.

This article reviews recent advances (in the past 5–10 years) in the chemical synthesis and biological applications of activatable organic PS. Specifically, we cover (1) chemical strategies to fine-tune the photodynamic activity of small-molecule PS from different chromophores, (2) the main mechanisms behind the differential behaviour of organic PS in their inactive and active states, (3) biological triggers able to induce efficient and selective photodynamic responses to tumour and bacterial metabolism and (4) successful examples of biomedical PDT studies and a short discussion of the future challenges to enhance the translatability of activatable PS to clinical applications.

Biomarker-triggered PS

The first examples of organic PS for anticancer PDT were built from porphyrin structures, which are in a permanently active state (that is, they are able to generate singlet oxygen upon illumination) and display limited ability to distinguish between normal and pathogenic cells. To overcome their potential off-target toxicity, subsequent generations of activatable PS have been engineered to induce phototoxicity only at target sites, even when they are administered systemically[22]. In the context of anticancer PDT, chemists have designed bespoke PS that exploit physiological and/or molecular signatures of cancerous tissues to cause cell death upon activation by defined biomarkers (Fig. 1a). Among these, triggers such as pH, redox mediators or enzymatic activity have been reported for the activation of organic PS[23,24].

pH-activatable PS

Tumour tissues exhibit overall lower pH values than average healthy tissues owing to the anaerobic glycolysis under hypoxic conditions[25,26]. Most pH-activatable PS make use of quenching mechanisms reported in optical probe development, such as photoinduced electron transfer (PeT) and intramolecular charge transfer (ICT)[27]. For instance, PS can be chemically linked to electron-donating groups that are protonated at low pH values, thus inhibiting PeT or ICT and restoring the capacity of the PS to generate ROS (Fig. 1b). Such groups can be incorporated into the molecular structures of different PS (for example, porphyrins, xanthenes, cyanines or boron dipyrromethenes (BODIPY))[28–32], such as the works of Ju and colleagues on the rational design of pH-responsive azaBODIPY PS[31,33]. Two bromophenyl moieties were incorporated to enhance singlet oxygen generation, whereas diethylaminophenyl groups conferred pH-specific activation. To facilitate accumulation in cancer cells expressing $\alpha_v\beta_3$ integrins, this activatable PS was encapsulated in a nanomicelle derivatized with cyclic RGD peptides.

Redox-mediated PS

Other molecular signatures, such as the high levels of glutathione (GSH) or cysteine (Cys) found inside cancer cells, have also been used to activate PS. GSH is an intracellular reducing agent responsible for counteracting oxidative stress, and it is abundant in cancer cells as a result of their highly active metabolism[34]. Previous studies in drug delivery have demonstrated that GSH-mediated cleavage of disulfide bonds can be utilized for controlled drug release[35], a concept that has been translated to prepare disulfide-based PS[36] (Fig. 1c). Examples of this involve the use of PS (for example, tetraphenylethylene derivatives

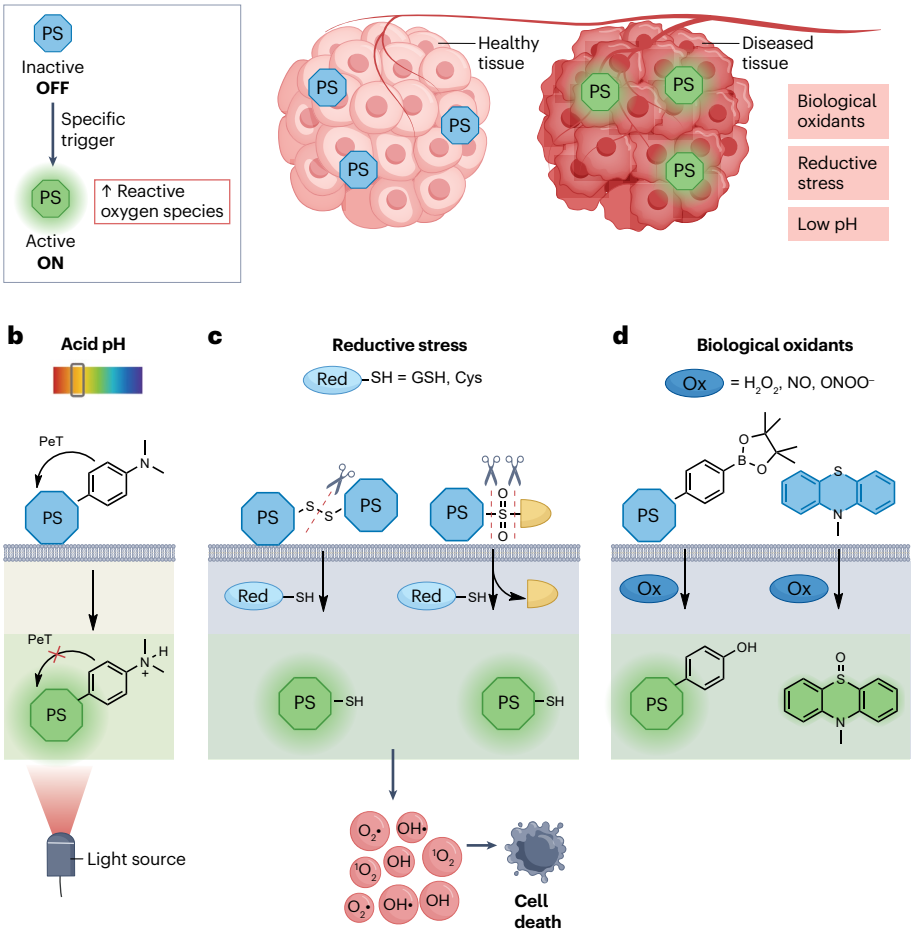


Fig. 1 | Biomarkers within the tumour microenvironment can be harnessed for activatable photodynamic therapy. a, Activatable photosensitizers (PS) initially exist in an inactive (OFF) state, wherein the generation of reactive oxygen species (ROS) is minimal under illumination. When exposed to specific triggers, the PS can be activated (ON) and the generation of ROS is enhanced (as indicated by the upward arrow). The tumour microenvironment has several characteristics, including low pH and reductive and oxidative stress, that can be exploited as activation triggers. **b**, Acidic pH can disrupt photoinduced electron transfer (PeT) quenching (as indicated by the 'X' mark) through protonation of amino or phenol functional groups. **c**, Reducing agents such as glutathione (GSH) and cysteine (Cys) can cleave disulfide bonds or act as nucleophiles towards sulfoxides to release active PS. **d**, Biological oxidants such as hydrogen peroxide can liberate hydroxyl groups or oxidize thiols to sulfoxides, enhancing ROS generation. Ox, oxidizing agent; Red, reducing agent.

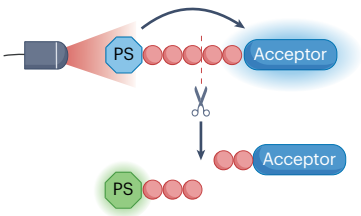
and phthalocyanines) linked through ferrocene (a PeT quencher) via disulfide bonds for efficient GSH-mediated activation[37,38]. Furthermore, to improve subcellular targeting, positively charged groups can favour mitochondrial localization[39,40], and quenching groups (for example, 2,4-dinitrobenzenesulfonate (DNBS)) that release sulfur dioxide following GSH-mediated cleavage show synergistic effects both in vitro and in vivo[39]. Other strategies have explored the reactivity of GSH (for example, in nucleophilic aromatic substitutions) to fine-tune anticancer PDT efficacy[41].

More recently, Kolemen and colleagues have investigated Cys as a trigger for the development of activatable anticancer PS[42,43]. Cys is involved in several physiological processes and is present at high concentrations in some disease states, including cancer and neurodegenerative disorders[44–46]. It was reported, for example, that Cys acts as a nucleophile in hetero-Michael addition reactions with unsaturated carbonyl groups in chlorinated hemicyanine PS. Upon reaction with Cys, the free hydroxyl group restored the photosensitivity of the PS, thereby amplifying ROS generation under light irradiation[43]. In a similar manner, hydrogen sulfide (H_2S) has been reported in reduction-activating strategies involving chromophores with azide, azo, nitro or nitroso groups[45–47]. For example, an H_2S-activatable resorufin PS was reported wherein the reduction of azide moieties to amines, followed by 1,6-elimination, released an active PS with increased ICT and photodynamic activity[48]. The high reactivity of H_2S has also been exploited to enable nucleophilic aromatic substitutions in activatable BODIPY PS containing sulfones and sulfoxides as H_2S-responsive quenchers[49,50].

Biological oxidants such as hydrogen peroxide, hypochlorous acid, nitric oxide or peroxynitrite can promote tumour progression[51] and represent attractive targets for anticancer PDT. For instance, molecular designs incorporating arylboronate groups into the structures of PS (for example, BODIPY) display PeT-mediated quenching with selective restoration of its photosensitive activity upon oxidative deboronylation by hydrogen peroxide[52,53]. Subsequent designs by Xing and co-workers have exploited the release of quinone methide as a GSH-depleting adjuvant to boost ROS production[54]. Another interesting approach reported by Feng, Wang and co-workers has exploited the reactivity of endogenous ROS to generate PS in situ from non-photosensitive precursors[55]. Briefly, this strategy relied on the accumulation of an iodinated 2,3,3-trimethyl-3*H*-indole in the mitochondria and its reactivity with hydrogen peroxide and hydroxy radicals to generate a cyanine PS inside cells. This strategy represents an interesting approach to leverage mitochondrial activity to accumulate PS in specific subcellular organelles.

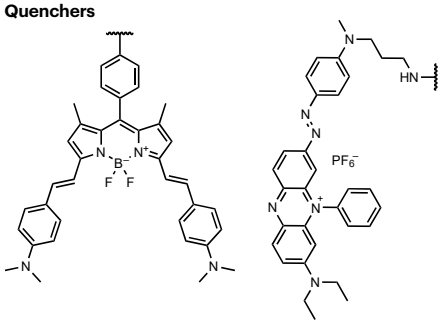
a

Enzyme-activatable PS (proteases)

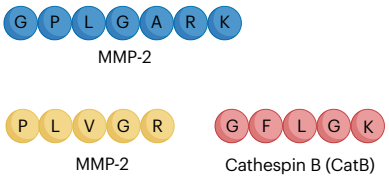


Energy transfer to acceptor (quencher)

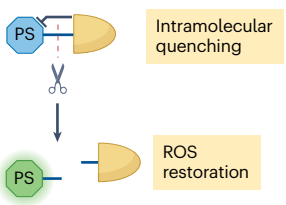
Quenchers



Peptide substrates



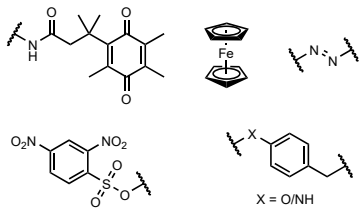
Enzyme-activatable PS (other enzymes)



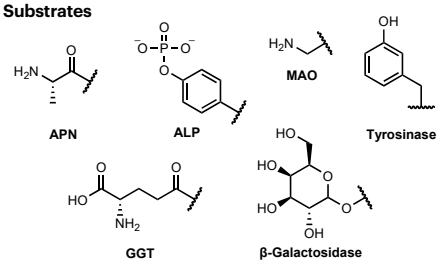
Intramolecular quenching

ROS restoration

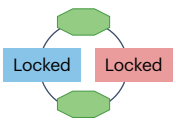
Quenchers



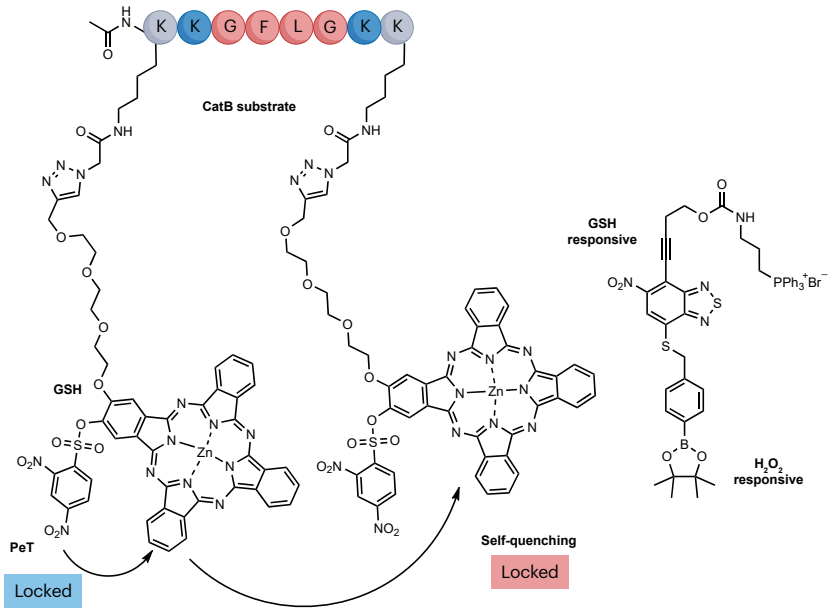
Substrates



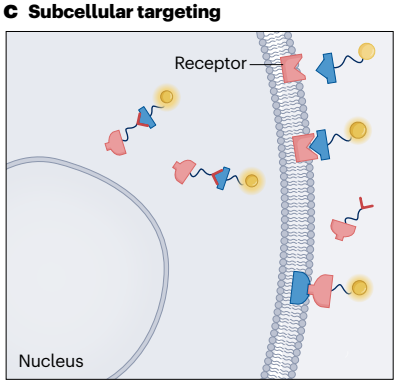
b Dual-locked PS



- Using two cancer-specific triggers
- Activation only after successive unlocking
- Minimizes the premature release of PS



c Subcellular targeting



Exploiting surface receptors for PS accumulation and activation

Biotin

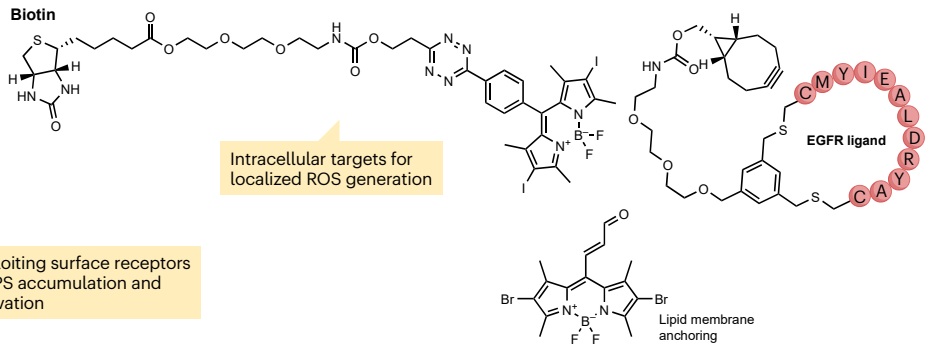


Fig. 2 | Activation mechanisms behind the differential behaviour of organic PS in their inactive and active states. a, Enzyme-activatable probes include acceptors with excitation wavelengths matching the emission wavelengths of the photosensitizers (PS). Upon spatial separation of the two groups, the quenching effect is minimized. Effective acceptors include boron dipyrromethene chromophores and black hole quenchers (for example, BHQ-3 shown). The PS and quencher can be linked via short peptides, facilitating activation via reaction with proteases. Other non-protease-activatable PS rely on intramolecular photoinduced electron transfer (PeT) and intramolecular charge transfer quenching mechanisms. In these cases, the quenchers are directly attached to free amines or alcohols via cleavable linkers. Enzyme substrates can be small molecules such as phosphates, L-glutamate or β-galactose. **b**, Dual-lock PS are responsive to two sequential biomarkers for enhanced discrimination between targeted cells and surrounding tissues. **c**, Receptor targeting (for example, biotin receptors or epidermal growth factor receptor (EGFR)) can accumulate the PS in cancer cells for subsequent activation via bioorthogonal reactions. Additionally, anchoring PS in lipid membranes (for example, thiol–acrolein adduct) or in subcellular organelles results in localized reactive oxygen species (ROS) production and effective phototoxicity. ALP, alkaline phosphatase; APN, aminopeptidase N; GGT, γ-glutamyl transpeptidase; MAO, monoamine oxidase; MMP, matrix metalloproteinase.

The dysregulated metabolism in cancer tissues also leads to higher levels of other oxidants, such as hypochlorite[56]. Phenothiazines are photosensitive structures that can be oxidized by hypochlorite to the corresponding sulfoxides, which exhibit enhanced fluorescence intensity[57]. This chemical transformation also shifts ROS generation from type II (that is, singlet oxygen mediated) to type I (that is, radical mediated), enabling synergistic imaging and anticancer PDT in preclinical models in vivo[57,58]. Biologically relevant radicals can also form peroxynitrite[59], another oxidative trigger that can be exploited for the design of activatable probes[60]. Li and colleagues have synthesized PS based on selenium-containing dicyanomethylene-4*H*-chromene as an electron donor and iodo-substituted phenolate as an electron acceptor[61], wherein blocking of the phenolate group can supress the photosensitivity by preventing effective ICT (Fig. 1d). Notably, the incorporation of selenium results in a red shift of the excitation wavelength and enhances the photosensitivity through the heavy atom effect. In this case, the phenolate was modified with a phenylboronate group to facilitate peroxynitrite-mediated activation of the PS. This represents a further example that a diverse pool of cancer biomarkers can be harnessed for the rational design of activatable PS with enhanced therapeutic outcomes.

Fine-tuning singlet oxygen localization

Molecular beacons were first introduced in 1998 as rationally designed molecular switches[62]. Existing initially in an OFF state, their interaction with their target leads to a change in chemical structure to produce an ON state with enhanced optical properties. Photodynamic molecular beacons were developed as an extension of fluorescent beacons in order to enhance the efficacy of PDT agents[63], and they combine a donor and an acceptor (or quencher) in close proximity to facilitate the inhibition of the optical signal via resonance energy transfer (RET). RET-based PS have garnered substantial attention because of enhanced light harvesting for increased ROS generation and reduction of off-target toxicity owing to specific activation[64]. The following section includes examples of activatable PS wherein the generation of singlet oxygen can be controlled by enzymatic activity, dual-locked activation strategies or specific subcellular localization.

Enzyme-activatable PS

The unique enzymatic signatures of the tumour microenvironment can provide good discrimination between healthy and malignant cells. Human quinone oxidoreductase 1 (hNQO1) is an enzyme that catalyses the reduction of quinone-related compounds, and it is found in high levels in cancer tissues[65]. Activatable PS reacting with hNQO1 have been synthesized by attaching trimethyl lock quinone quenchers to different PS, such as phenalenones and BODIPYs[66,67]. In these cases, the reaction between the hNQO1-responsive PS and the active enzyme leads to the reduction of quinone to hydroquinone followed by rapid lactonization and release of the active PS. Similar chemical strategies have been adapted to target cancer-associated proteases such as cathepsins[68,69] and matrix metalloproteinases (MMP)[70] by utilizing peptide sequences (that is, GFLGK and GPLGLARK, respectively) as spacers between the PS and their quenchers (Fig. 2a). Other cancer-targeting enzymes utilized as triggers in activatable PS include monoamine oxidase[25,71], alkaline phosphatase[72,73], tyrosinase[74,75], γ-glutamyltranspeptidase[76–79] and aminopeptidase[80–82] (Fig. 2a). In these cases, the caging of the PS occurs through ICT or PeT either directly (for example, via phenols or amines caged with small-molecule ligands) or indirectly through the presence of a self-immolative spacer (for example, *p*-aminobenzylalcohol) that is released upon reaction with the enzyme. For instance, Shen and Tung have prepared a derivative of the clinically approved PS methylene blue including an L-glutamic acid via a self-immolative linker[77]. By converting one amine group of methylene blue into a carbamate, the aromaticity and ROS-generating capacity of the PS were disrupted. The incorporation of the L-glutamate moiety enabled selective cleavage by γ-glutamyltranspeptidase, which triggered elimination and restored the conjugation within the PS core. This example highlights how linkers can be used to introduce stimulus-responsive caging units towards many different enzymes.

Azo-based linkers have also proven effective as enzymatic targets in cancer-specific activation. Azo functionalities can be directly incorporated into PS carrying free amine groups (for example, selenium-containing rosamines, hemicyanines and triphenylamines)[83–85] or through chemical linkers attached to the photosensitive scaffold (for example, rhodols and pyropheophorbide-α)[86,87]. In all cases, the PS is activated in hypoxic microenvironments with enhanced expression of reducing enzymes (for example, azoreductases). An interesting example was reported by Ping, Liu and co-workers, wherein a triphenylamine PS was functionalized with a trifluoromethyl-substituted arylazo group[85]. Under hypoxic conditions, the azo bond was partially reduced to the corresponding hydrazine, which triggered the ON state and yet maintained the spatial connectivity with the PS to enable re-oxidation to the OFF azo form as the PS migrated to healthy tissues over longer periods of time. This reversible mechanism enhanced the spatial selectivity and reduced any potential off-target effects of the PS.

Dual-locked PS

In order to minimize the premature release of active PS, significant efforts have been made towards dual-lock activation strategies (Fig. 2b). In these situations, the PS remains inactive until it recognizes or reacts with both of two target biomarkers, which can be either enzymes or small biomolecules. For example, Lo, Ng and colleagues have designed

a Zn phthalocyanine PS quenched by (1) a GSH-responsive DNBS moiety and (2) a cathepsin B-responsive peptide GFLG[88]. As a result, the photodynamic activity of the PS was suppressed through PeT by DNBS and the self-quenching effect of the two phthalocyanine units, whereas the sequential exposure to GSH and cathepsin B led to the restoration of ROS generation. This design has recently been expanded with the incorporation of cyclic peptide-targeting cancer-specific receptors (for example, epidermal growth factor receptors (EGFR)) to favour accumulation in malignant cells[89]. More recently, a loop system was developed wherein a distyryl BODIPY PS was linked to the commercial quencher Black Hole Quencher 3 through a peptide containing two cleavage sequences (PLGVR and GFLR) responding to MMP-2 and cathepsin B, respectively[90]. As a result of the loop structure, the PS and quencher remained in close proximity for effective RET quenching when only one of the sequences was cleaved while activation of the BODIPY PS took place when both enzymes were present. Another example of dual-locked PS was reported by Feng, Wang and colleagues with a mitochondria-targeting PS co-activated by reductive and oxidative stress[91]. In this design, a benzothiadiazole moiety consecutively reacted with two intracellular triggers GSH and H_2O_2 and underwent domino reactions to release an active PS under two-photon illumination.

Subcellular targeting for localized activation

One important aspect in the rational design of effective PS is the chemical tuning of their subcellular location, for instance, to facilitate binding to membrane receptors and transporters or to enable the reaction with intracellular enzymes. Examples of such chemical strategies include (1) exploiting the altered glycolytic metabolism in cancer cells through glucose–PS derivatives[92], (2) favouring the reaction with β-galactosidase with enzyme-activatable PS[93–95], (3) leveraging the high affinity of small-molecule inhibitors (for example, 1-bromo-2-naphthyl-*N*,*N*′-dimethylcarbamate for the breast cancer biomarker KAA1363)[96] or (4) using PS–peptide conjugate binding to EGFR or integrins[97] on the surface of cancer cells[98,99] (Fig. 2c).

One interesting example was reported by Urano and co-workers, who synthesized an activatable PS exploiting the intramolecular hydrolysis of a spirocyclic selenium–rhodamine linked to a β-galactosidase-responsive moiety[95]. Intracellular activation involves hydrolysis to the corresponding quinone methide, which rapidly reacts with nucleophiles and traps the PS inside the target cells[95]. Dual receptor targeting can also be used to improve cellular localization. For instance, Ng and co-workers have reported quenched tetrazine-BODIPY PS that were (1) conjugated to ligands selectively targeting biotin receptors on cancer cells and (2) activated in situ by reaction with dienophiles (for example, bicyclononyne and tricyclooctene) linked to an EGFR-binding cyclic peptide so that the release of active PS was limited to cells expressing both receptors[100] (Fig. 2c). Similar approaches have been further adapted to other biological targets (for example, enzymes)[101] and bioorthogonal reactions (for example, tetrazines)[102].

In addition to the accumulation of active PS in the target cells, the subcellular localization of the PS is important for their efficient performance as PDT agents given the short lifetime of singlet oxygen[103] (Fig. 2c). Activation strategies include both intrinsic properties of the PS such as their hydrophobicity[104,105] and extrinsic factors such as reactivity towards pH, redox mediators, enzymes and bioorthogonal pairs. In terms of localization, some reports have described the accumulation of PS in the plasma membrane[106], mitochondria[107–109], lysosomes[108,110] and the endoplasmic reticulum[111]. For instance, lipid-derived electrophiles inspired the use of acrolein, which is capable of forming adducts with free cysteines, both as a quencher and as a reactive centre for BODIPY PS[106]. Upon adduct formation with membrane-associated thiolates, the PS was activated and ROS damage to the membranes induced cell death. Alternatively, covalent binding to DNA can aid nuclear-activated PS through click chemistry, with ROS causing DNA damage, a phenomenon rarely observed with conventional PS owing to their inability to cross the nuclear membrane[112]. Additionally, multiple subcellular compartments can be targeted with one single molecular entity. This approach was demonstrated by Li et al. wherein two PS were attached through a linker cleaved by γ-glutamyltranspeptidase[108]. Upon cleavage and subsequent self-immolation, two distinct PS (that is, pyropheophorbide-α and phenothiazine) were released in mitochondria and lysosomes, respectively, and generated ROS to cause effective cell ablation.

Photosensitive warheads for antimicrobial PDT

Although most activatable PS have been described in the field of anticancer therapies, the use of PDT in the treatment of infectious diseases has emerged in recent years, particularly to kill multidrug-resistant pathogens. Antimicrobial PDT can offer an alternative to combat antimicrobial resistance that has arisen as a result of overuse of antibiotics. To date, most efforts in antimicrobial PDT have been focused on the synthesis and characterization of bacterial-targeted PS with good selectivity over host cells, and some of these molecular designs hold promise for the generation of future activatable PS for microbial pathogens.

From a chemical perspective, one of the most straightforward means for targeting bacterial pathogens is to incorporate cationic moieties that can interact electrostatically with the negatively charged bacterial cell envelope[113] (Fig. 3a). For instance, Tang and co-workers have reported PS featuring aggregation-induced emission to ablate bacterial cells[114]. Among these, NIR anion–π^+ PS stood out for their ability to eradicate multidrug-resistant bacteria[115]. These combined triphenylamine and thiophene fragments (donors) linked to cyano and pyridinium moieties (acceptors) through π-bridges. In addition to the inclusion of charged moieties, the chemical nature of the ROS generated by the PS can be also used to discern between bacterial strains, as shown in phenothiazinium PS containing sulfur (for type I PS) or selenium (for type II PS)[116]. Type I PS formed $O_2^{\bullet-}$ radicals that required intracellular conversion to the toxic hydroxy radicals, and their effectiveness was limited to Gram-negative bacteria. Type II PS, meanwhile, caused immediate cell death in both Gram-positive and Gram-negative bacteria by singlet oxygen generation, which was effective regardless of the composition of the outer membranes. This example illustrates how heteroatom selection can be used to fine-tune bacterial selectivity in antimicrobial PDT. The bacterial targeting capabilities of PS are not limited to extracellular domains. For instance, Beharry and co-workers introduced bromine atoms to the nucleic acid stain 4′,6-diamidino-2-phenyl-indole (DAPI) to generate intracellular PS able to oxidize nucleic acids and cause bacterial cell death[117]. Notably, this PS not only showed good water solubility and cell permeability but also demonstrated the ability to kill Gram-positive and Gram-negative bacteria with selectivity over mammalian cells.

The exploitation of bacterial biomarkers constitutes another strategy to generate targeted PS with suitable host–pathogen discrimination (Fig. 3b). The cell envelopes of bacteria exhibit a mixture of lipopolysaccharides, peptidoglycans, enzymes and receptors, with compositions differing between strains. Lectin–PS conjugates (for example, concanavalin A–Rose Bengal)[118], lectin–ligand mimetics (for example, composed of polygalactose groups that facilitate PS

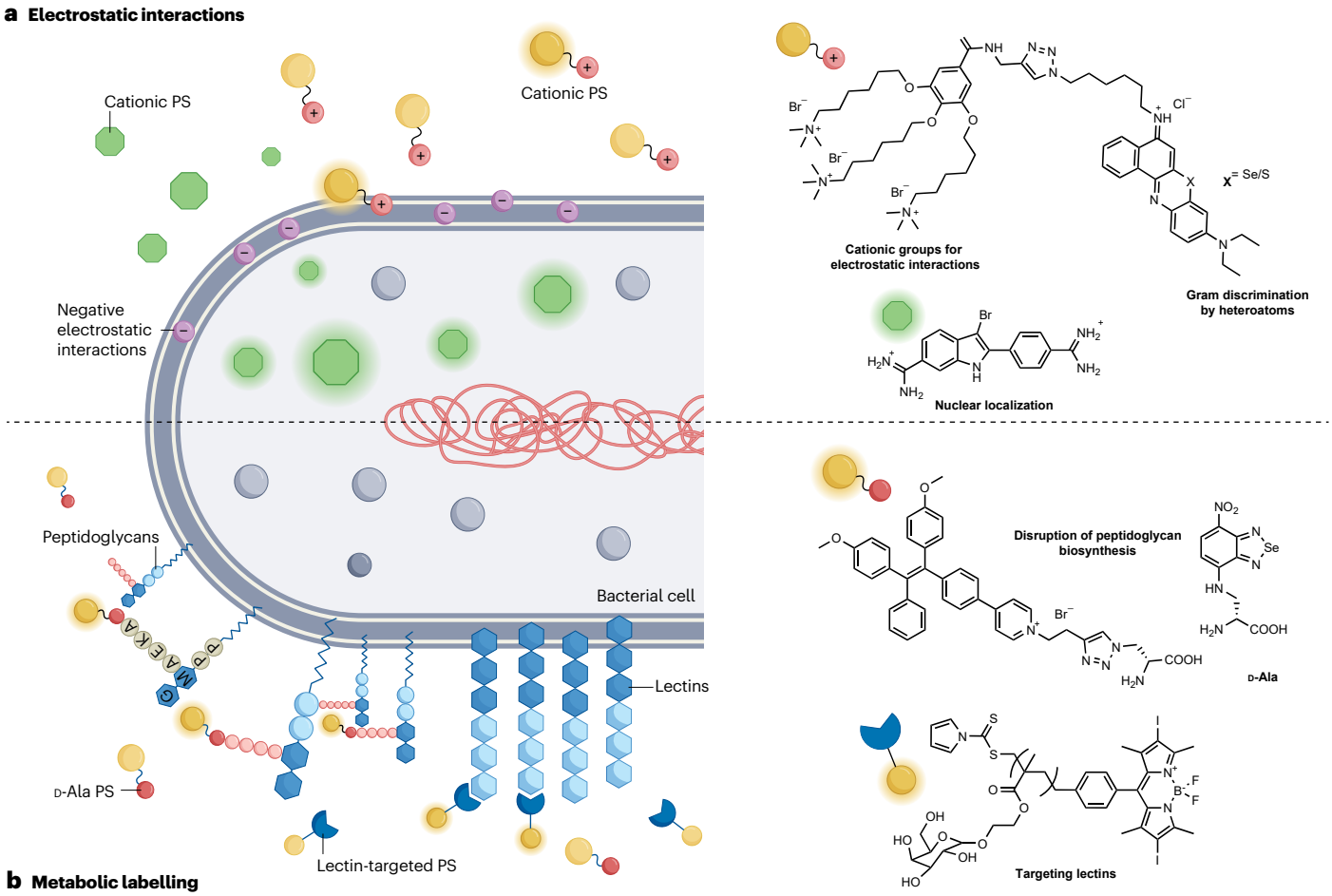


Fig. 3 | Electrostatic and metabolic labelling as targeting strategies for bacterial ablation in antimicrobial photodynamic therapy. a, Cationic groups facilitate electrostatic interactions with bacterial cells, and defined heteroatoms (that is, sulfur vs selenium atoms) can alter the selectivity for Gram-positive or Gram-negative bacteria. **b**, Metabolic targeting enables the incorporation of photosensitizers (PS) in the bacterial cell wall machinery, either covalently with the incorporation of D-Ala PS onto the peptidoglycan chains or with the introduction of glycomimetic groups for binding to bacterial lectins.

binding to lectin LecA)[119] and surface-expressed enzymes[120,121] have allowed covalent attachment of PS to bacteria and have all been used for antimicrobial PDT. Among these, the most exploited macromolecules are bacterial peptidoglycans. A number of D-alanine (D-Ala) PS have been reported that take advantage of the ability of bacterial machinery to recognize D-amino acids. Liu and co-workers have reported the TPEPy-D-Ala PS, wherein a pyridinium-substituted tetraphenylethylene (TPEPy) was incorporated into the peptidoglycan chains of methicillin-resistant *Staphylococcus aureus* and enabled the ablation of the pathogens in infected macrophages[122]. Subsequent designs incorporated the saccharide 3-deoxy-D-manno-octulosonic acid to improve its metabolic incorporation into the peptidoglycan of Gram-negative bacteria[123]. Alternatively, our group has focused on favouring metabolic incorporation by reducing the size of the PS and has reported nitrobenzoselenadiazoles as some of the smallest PS to date[92]. Importantly, the minimal size and neutral character of nitrobenzoselenadiazoles help to retain the molecular recognition properties and uptake of low molecular weight biometabolites, such as amino acids and saccharides. For instance, D-Ala analogues containing a nitrobenzoselenadiazole PS were effectively incorporated into peptidoglycan chains and were effective in the ablation of both Gram-positive and Gram-negative bacteria[92].

Finally, antibiotics can also be used as vectors to accumulate PS in bacterial pathogens. Bradley and co-workers have reported photosensitive analogues of polymyxin (a heptapeptide-based antibiotic that binds to lipopolysaccharides and disrupts bacterial membranes in Gram-negative bacteria)[124] and vancomycin (an antibiotic that interacts with peptidoglycan chains in Gram-negative bacteria)[125]. Our group has also described a nitrobenzoselenadiazole–vancomycin conjugate that combines the targeting abilities of the antibiotic and the environmental sensitivity of benzoselenadiazoles to enhance ROS production in hydrophobic environments and to magnify its photodynamic effect to kill antibiotic-resistant strains and biofilms[104].

Enhancing clinical translation

Near-infrared organic PS

One of the main shortcomings in the clinical translation of light-triggered photodynamic therapeutics is the limited tissue penetration

depth of visible light, which has prompted the engineering of activatable PS with excitation wavelengths in the NIR window[126,127]. Furthermore, given that most endogenous PS (for example, flavins) are excited at shorter wavelengths, NIR-absorbing PS can display more favourable selectivity profiles and signal-to-noise ratios.

Several chemical strategies have been reported to evolve NIR-activatable PS from UV–visible chromophores. One conventional approach involves the extension of π-conjugated systems, which can lead to large bathochromic shifts in the absorbance profile. This strategy was used to synthesize a series of NIR-absorbing halogenated BODIPY PS that could be activated by (1) thiol-mediated cleavage with GSH[128], (2) protonation of aniline groups[129] or (3) enzymatic activation after chemical ligation to quinone substrates for hNQO1 (ref. 67). In addition to extending π-conjugation, an effective means to produce NIR PS involves the replacement of oxygen atoms with heavier heteroatoms (for example, sulfur or selenium), which results in red-shifted absorbance and favours intersystem crossing to enhance photodynamic activity (Fig. 4a). Some examples of this strategy include sulfur-substituted derivatives of the fluorophore Nile blue with substrates of carboxylesterases for preferential cleavage inside liver cancer cells[130] and NIR-absorbing rhodamine PS including selenium atoms and azide masking groups that were bioorthogonally uncaged via Staudinger ligation[131].

NIR chromophores can also be suitable starting points for the design of activatable PS, provided that their structures can be chemically modified with moieties that modulate their photodynamic activity. Some examples include (1) diketopyrrolopyrroles responding to aggregation-induced emission[132], (2) tricarbocyanine dyes reacting with the cancer-associated enzyme nitroreductase[133] and (3) GSH-activated methylene blue prodrugs for dual release of the PS and the anticancer drug camptothecin[134]. Among all NIR chromophores, the good optical properties and chemical modularity of the hemicyanine core have prompted its derivatization to generate activatable NIR PS for a wide range of biological targets. Our group recently described a generic platform to synthesize enzyme-activatable Se–hemicyanine PS. The selenium confers increased singlet oxygen generation upon NIR illumination and enzyme substrates were used as caging groups for controlled release of the PS only after reaction

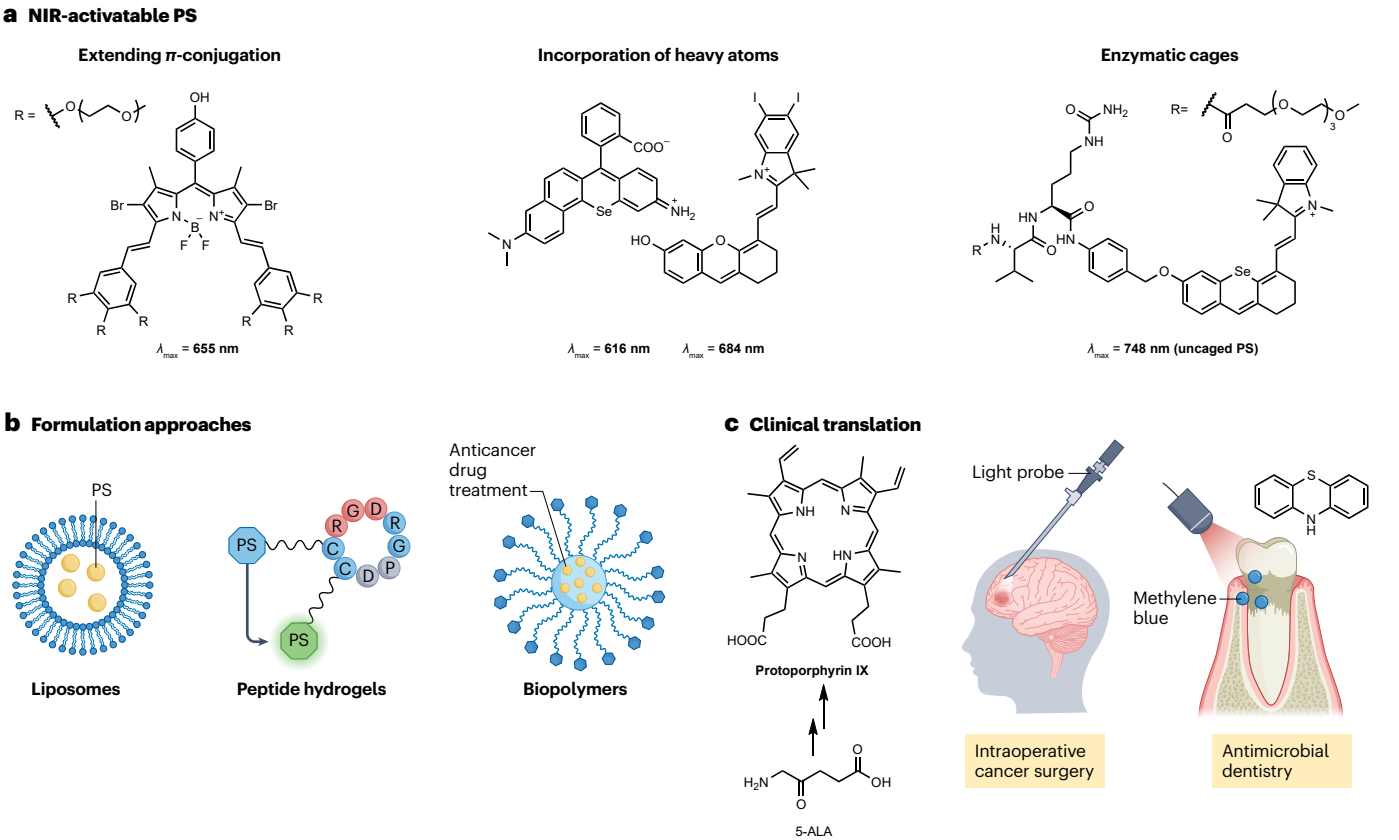


Fig. 4 | Clinical translation with NIR excitation and intelligent formulation designs. a, Representative examples of how existing photosensitizers (PS) can be improved to show excitation in the near-infrared (NIR) region of the electromagnetic spectrum. Some chemical strategies include extending electronic conjugation in distyryl boron dipyrromethenes, introducing heavy atoms in the rhodamine (for example, Se–rhodamine) or hemicyanine (for example, iodinated hemicyanine) scaffolds or the incorporation of enzymatic cages in NIR-activatable PS. **b**, Enhanced biocompatibility and uptake in target tissues can be achieved by optimal formulation, including liposomes, peptide hydrogels and biopolymers. These infer favourable properties to the PS (for example, stability and lipophilicity) and can include targeting moieties for effective uptake (for example, cyclic RGD for $\alpha_v\beta_3$ integrins or folic acid for cancer cell uptake). **c**, 5-Aminolevulinic acid (5-ALA), which is metabolized to the active PS protoporphyrin IX, has been tested for intraoperative treatment of glioblastoma, and phenothiazine derivatives have shown to be effective for the treatment of periodontitis (for example, oral bacterial infection). Part **c** created in BioRender.

Glossary

Activatable fluorophores
Fluorescent molecules whose emission varies in response to biological or chemical stimuli.

Bioorthogonal reactions
Chemical reactions that occur inside living systems without interfering with native biochemical processes, often used for labelling and tracking biomolecules.

Boron dipyrromethenes
(BODIPY). A class of fluorescent dyes with their high photostability and brightness, and used in various imaging applications.

Cyclic RGD peptides
Short peptides containing the amino acid sequence arginine–glycine–aspartic acid, which target integrins on cell membranes.

Dual-lock activation strategies
Methods that require two distinct triggers to activate a photosensitizer or drug, enhancing specificity and reducing off-target effects.

Enzyme-activatable PS
Photosensitizers that are activated by specific enzymes, allowing for targeted photodynamic therapy in areas wherein those enzymes are present.

Glutathione
(GSH). A tripeptide that acts as an antioxidant in cells, often used as a trigger for activating photosensitizers in anticancer therapy owing to its high levels in cancer cells.

Intramolecular charge transfer
(ICT). A process used in designing fluorescent probes and photosensitizers wherein a charge is transferred from an electron-rich donor moiety to an electron-poor acceptor moiety.

Near-infrared
(NIR). A region of the electromagnetic spectrum with wavelengths just beyond visible light and enhanced penetration in living tissues.

Photoinduced electron transfer
(PeT). A mechanism through which an electron is transferred between two chemical groups of photosensitizers and fluorophores, and used to quench or activate singlet oxygen generation or fluorescence emission, respectively.

Photosensitizers
(PS). Molecules that produce reactive oxygen species when exposed to light and used in photodynamic therapy to kill targeted cells.

Reactive oxygen species
(ROS). Chemically reactive molecules containing oxygen, which can cause cell damage and are used in photodynamic therapy to kill cancer cells or pathogens.

Singlet oxygen
A highly reactive form of oxygen generated by photosensitizers during photodynamic therapy, responsible for inducing cell death.

with the targeted enzyme[135] (Fig. 4a). Other hemicyanine PS include halogen atoms (for example, chlorine, bromine or iodine) to enhance their otherwise limited photosensitivity[136]. Peng and co-workers have reported one of the first activatable hemicyanine PS for anticancer PDT under hypoxic conditions[137]. Briefly, the protection of the phenol group with a nitrophenyl moiety was used to enhance the release of an active PS in the presence of nitroreductase. Similar chemical designs have been reported by Miao and co-workers, who used phosphate cages reacting with alkaline phosphatase[138]. Zhang, Qu and co-workers have reported a brominated hemicyanine structure caged with a hydrogen peroxide-responsive group for the dual release of the PS and 5′-deoxy-5-fluorouridine as a bioactive drug[139] (Fig. 5). More recent reports include chlorinated hemicyanines for anticancer photodynamic and photothermal therapy upon activation with cysteine[43] and quinone-quenched hemicyanines for targeted activation with hNQO1 (ref. 140) (Fig. 5). Finally, Xie and co-workers have described an enzyme-activatable hemicyanine PS wherein the reaction the pro-PS and alkaline phosphatase triggered a large increase in hydrophobicity that (1) enhanced cell permeability and (2) enabled covalent binding with proximal proteins to maximize accumulation in target cells[73] (Fig. 5). This strategy proved effective for in vivo ablation of tumours using NIR illumination, and this chemical design could be, in principle, adapted to the preparation of other activatable PS targeting cancer biomarkers.

The roadmap towards clinical applications

The translational roadmap for testing new optical agents in clinical studies is lengthy[141]; as a result, most PDT clinical studies to date have used already clinically approved PS, including 5-aminolevulinic acid (5-ALA), porphyrins or methylene blue. The minimally invasive nature of PDT makes it a useful resource for cell ablation during surgical procedures close to vital structures (for example, nerves and the brain). For instance, 5-ALA, which is metabolized by cancer cells to generate the PS protoporphyrin IX[142], has been tested for intraoperative procedures in glioblastoma patients[143] (Fig. 4c), and talaporfin (that is, a chlorin-based PS) has been tested in patients with disseminated lumbar medulloblastoma[144].

Some other clinical PDT applications have investigated the treatment of neovascularization or the killing of pathogenic cells (for example, bacteria) in tissues wherein light can be readily applied. Notable examples include the use of pyropheophorbide-α for the treatment of age-related macular degeneration in ophthalmology[145], the application of phenothiazine PS for antimicrobial PDT on chronic periodontitis in dentistry[146] (Fig. 4c), or different indications in gastrointestinal disorders[147]. Finally, in addition to enhancing the accumulation of PS in target cells to minimize off-target toxicity[148], the successful translation of organic PS for clinical PDT requires the optimization of suitable formulations (Fig. 4b). Successful examples have described the encapsulation of BODIPY PS in biodegradable polymers to generate water-soluble and tumour-targetable agents for low-power NIR PDT in vivo[149] and in liposomal formulations[150]. More recent reports have begun to integrate bioengineering approaches into this field, including peptide-based implants for controlled release of PS[151] and poly(ethylene glycol) diacrylate nanocomposites for wireless brain PDT[152].

Conclusions

Activatable organic PS promise a reduction in potential off-target toxicity of ROS-producing chromophores and, thus, improved safety and efficiency of PDT agents for clinical applications. In the same fashion

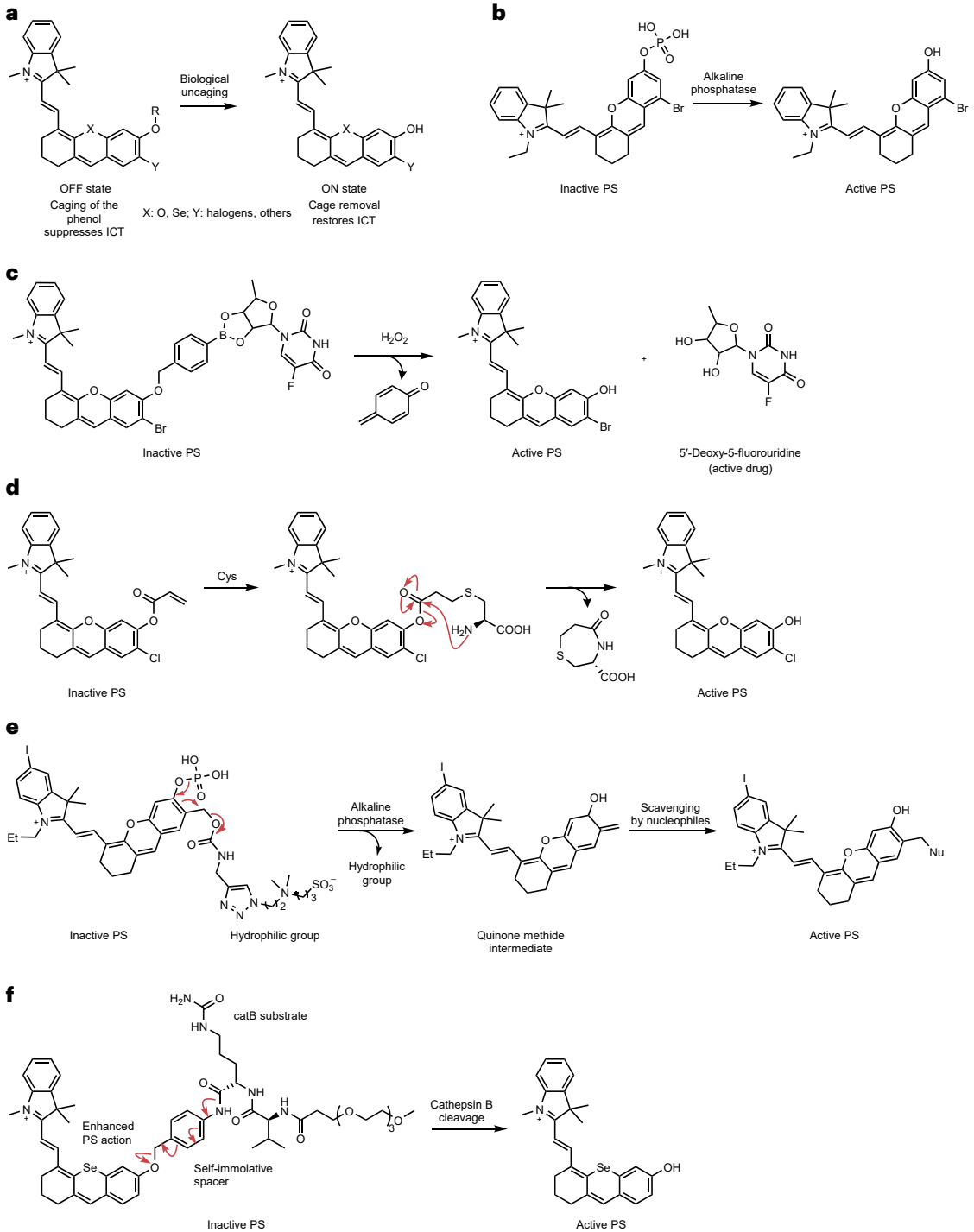


Fig. 5 | Chemical strategies to modulate ICT and PS activity in NIR hemicyanines. The caging of phenol groups in hemicyanine photosensitizers (PS) and subsequent uncaging by biological triggers. Representative examples of caged O-bridged (parts **a**–**e**) and Se-bridged (part **f**) hemicyanine PS and their activation in the presence of different biomolecules to release active near-infrared (NIR) PS. Red arrows (part **f**) show the enzyme-mediated cleavage of a self-immolative spacer. ICT, intramolecular charge transfer.

as fluorescent probes, organic PS can be intramolecularly quenched through PeT or ICT mechanisms and subsequently released using biomarkers (for example, pH, redox mediators and non-proteolytic enzymes) as triggers[153] (Table 1). Activatable constructs can harness energy transfer mechanisms between organic PS and energy-matching quenchers to render PDT probes modulated by enzymatic activity, thus

generating ROS preferentially in target cells and reducing off-target cytotoxicity. More recent molecular designs (for example, dual-locked PS) consider the sequential activation by two different biomarkers. These molecules can remarkably improve cellular selectivity, but their complex structures involve long synthetic procedures and can potentially hamper water solubility or biodistribution.

Although most activatable PS have been described for light-controlled ablation of cancer cells, recent reports have extended their

Table 1 | Chemical structures of activatable organic photosensitizers reviewed in this work

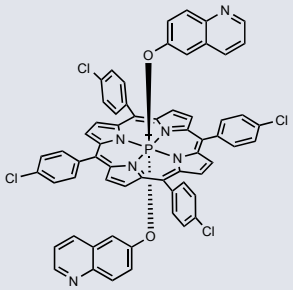
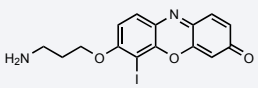
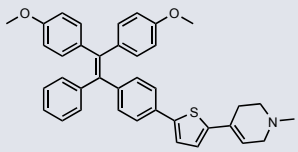
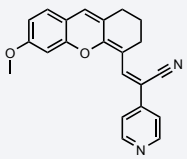
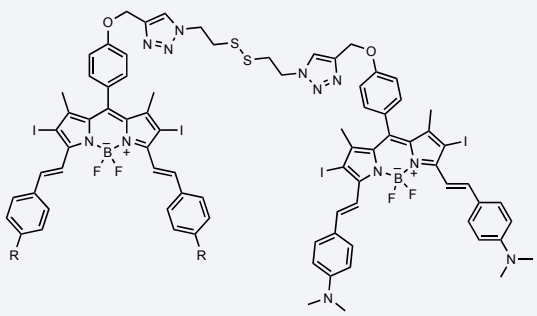
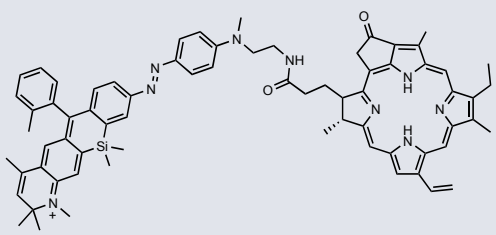
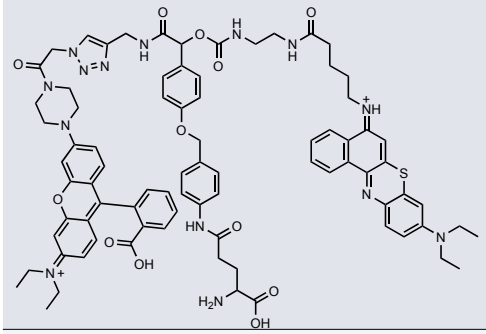
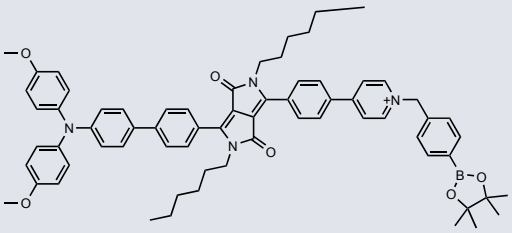
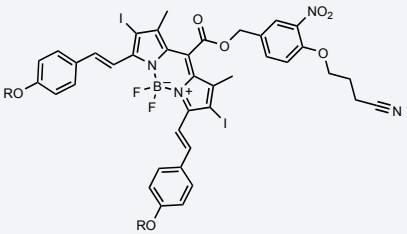
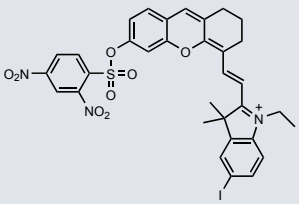
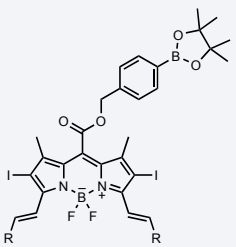
Photodynamic therapy agent	Excitation wavelength (nm)	Cellular target	Ref.
Intramolecular charge transfer suppression			
	585	Human serum albumin	28
	595	SH-SY5Y and NIH-3T3	71
	430	SH-SY5Y, HepG2 and NIH-3T3	25
	580	HepG2	29
Resonance energy transfer			
	670	A549 and HELF	36
	670	BEL-7402	87

Table 1 (continued) | Chemical structures of activatable organic photosensitizers reviewed in this work

Photodynamic therapy agent	Excitation wavelength (nm)	Cellular target	Ref.
Resonance energy transfer (continued)			
	640	HepG2 and U87	78
Photoinduced electron transfer			
	530	MCF-7, HeLa and ZJU0430	132
	680	ASGPR+ HepG2	102
	488	MCF-7	40
	670	HeLa and HELF	52

application to antimicrobial therapy, wherein PDT may prove advantageous in combating multidrug resistance. Such an approach might be particularly suited to the treatment of superficial infections (for example, skin and eye infections)[154], wherein PS can be applied topically and the efficacy of the treatment is not hindered by the limited tissue penetration of visible light. Current designs discriminate pathogens from host cells by conjugation of PS to biomolecules that can bind cell-surface biomolecules (for example, lectins) or form covalent linkages with the cell wall (for example, peptidoglycans). Future molecular designs might adopt strategies similar to those reported in anticancer

PDT and previously validated in fluorescent probe development (for example, enzymatic cleavage by bacterial proteases)[155].

Finally, current efforts to translate these designs into the clinic have prompted the optimization of both their formulation — for enhanced biodistribution — and their optical properties (for example, by shifting their excitation wavelengths to the more favourable NIR range). Organic fluorophores with excitation in the shortwave infrared window (1,000–2,000 nm)[156,157] may be adapted to build activatable PS for enhanced penetration to cure lesions in deeper tissues. In parallel, the emergence of sonodynamic therapy[158] will create opportunities to generate chemical sensitizers that are activated by exposure to low-intensity ultrasound rather than light and, therefore, enable the non-invasive treatment of less accessible tumours or infections. Several examples of conventional organic PS are already in use clinically and the next generations of activatable organic PS will improve safety and efficacy and will accelerate the consolidation of light-controlled therapeutics in biomedicine.

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