In the search for photocages cleavable with visible light: an overview of recent advances and chemical strategies

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Abstract: Photopharmacological tools enable the precise spatiotemporal control of small molecule drugs. Amongst them, caged compounds incorporate a photolabile moiety which is released under illumination, thus liberating the active molecule. Caging groups have long been known and many chemical scaffolds have already been used in different applications. However, most of the initial examples are cleaved with UV light, which suffers from low tissue permeability and cell damage. Recently, caging groups that are released under visible light have been reported, which expand their utility. In this review, we outline the chemical strategies that have been used to increase the absorption wavelengths; we compare their photophysical properties, discuss their synthetic accessibility, and exemplify some of their biological applications.

1. Introduction

The ability to control the precise spatiotemporal localisation and the concentration levels of small molecule drugs is still a big challenge, which often limits their therapeutic potential. To overcome this limitation, photopharmacology employs external light irradiation to activate small molecules in a specific region of interest at a desired time and intensity. This can be achieved reversibly (photoswitching) or irreversibly (photocaging). Photoswitches incorporate a functional group, usually an azo aromatic, which allows light to interconvert the molecule between two different isomeric configurations having distinct biological activity. In this manner, the bioactive molecule can be turned “on” and “off”. Photoswitching molecules, which have found numerous applications both *in vitro* and *in vivo*, have been extensively reviewed elsewhere.^[1–5] In the caging strategy, a bioactive molecule is covalently attached to a photoprotecting group (PPG), which renders it inactive. Upon illumination with a particular wavelength, the molecule is released in its active form in an irreversible manner and is free to exert its biological function locally. Even though this approach does not allow for deactivation, the chemical design strategy is often simpler. When irradiation is done at the area of interest, the active molecule reaches the surrounding tissues at lower concentrations, in a diffusion controlled process that can be regulated by adjusting illumination parameters. The drug effects at distant organs or tissues are often negligible due to dilution of the active molecule thus improving the therapeutic window. An interesting advantage of the caging strategy is that an approved drug or a fully validated active ligand can be chemically modified with different PPGs to obtain specific properties which increase biological and photochemical efficiencies. These can include, among others, specific tissue, cellular and subcellular targeting, the use of combined light strategies for a fine control of pharmacology or the localised simultaneous photorelease of different compounds. PPGs also permit tuning the physicochemical and pharmacokinetic properties more easily without affecting the biological activity of the active molecule.

o-Nitrobenzyl groups are amongst the most common caging groups used in biological studies – they are easily prepared and efficiently decay under UV light. However, a big limitation of this PPG is the low absorption wavelength required for uncaging; ultraviolet light suffers from limited penetration into tissues, and it can also lead to cell damage or death.^[6] Exposure to UV light has been shown to lead to the formation of reactive oxygen and nitrogen species that can damage DNA and membrane lipids, leading to cytotoxicity, mutations and alterations in cell signaling pathways.^[6–8] In terms of light penetration, the near-infrared (NIR) region of 650–900 nm has been suggested as optimal.^[9] At lower and higher wavelengths, hemoglobin and water are the major absorbers significantly diminishing the tissue depth reached by the irradiated photons. The first reported examples of visible-light-absorbing PPGs were based on organometallic ruthenium complexes,^[10,11] and in the last decade fully organic examples have also been introduced.

In this review, recent advances on photocaging groups are exposed, with a focus on the strategies that have been employed to obtain visible light excitable scaffolds. We do not offer an exhaustive analysis of all the PPGs that have been recently developed and applied to biological applications, but we instead explore the chemical modifications that have been utilised to shift the uncaging wavelengths towards higher values into the visible range. We also analyse the synthetic methods towards these groups, and the chemical modifications that can be introduced to fine tune their photochemical and physicochemical properties. We highlight some of their biological applications *in vitro* and *in vivo*. Even though we focus on organic PPGs, some inorganic PPGs such as ruthenium polypyridyl complexes can also photorelease under visible light.^[12] Recent reviews by Deiters and Moskalensky also discuss some of the strategies and biological applications of recently developed PPGs.^[13–15] We focus on examples using one-photon excitation (1PE); the alternative two-photon excitation (2PE) strategy has also been reviewed.^[16–18] An older review by Klán contains a thorough description of the big spectrum of available PPGs.^[19] During the revision of this manuscript, two other reviews on PPGs have been published – one by Weinstein, Klán and coworkers provides a comprehensive review of the PPGs that absorb visible and NIR light, including both organic and metal-containing groups,^[20] and another by Szymanski and coworkers discusses the optimal parameters of PPGs depending on their medical application.^[21]

We expect this analysis to be of use for researchers interested in adding a photocleavable group to a small molecule drug, as well as to guide researchers intending to increase the cleavable wavelength of existing caged groups. The study by P. Klán and coworkers showing that independent structural modifications can be additive, yielding PPGs with significantly improved properties,^[22] implies that it might be possible to combine learnings from the different reported structural changes to deliver PPGs with optimal properties for each application's needs.

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Besides maximum absorption wavelength, other parameters that are often used to evaluate the properties of PPGs are the quantum yield and the absorption coefficient. Quantum yield of uncaging (ϕ_u), also referred to as the efficiency of release, corresponds to the amount of released cargo over the amount of photons at a particular irradiation wavelength. Similarly to fluorescence quantum yield (ϕ_f), ϕ_u is higher in less polar solvents.^[23] The molar absorption coefficient (ϵ) is the amount of light absorbed by the substrate over the concentration and optical path, and is hence a measure of how easily a particular molecule absorbs light of a certain wavelength. The photoefficiency of a PPG is often quantified as the product of ϕ_u and ϵ , which is proportional to the amount of released substrate. For instance, the quantum yields of PPGs excitable under visible light are consistently lower than those of UV-excitable ones, but this is often compensated by high molar absorption coefficients, making them suitable for biological applications. These parameters have been used in this review to evaluate and compare the exposed PPGs.

There are a series of criteria that PPGs should fulfill for their applicability in biological systems.^[19] Apart from high absorption wavelength and photoefficiency, another parameter that is often optimised is the rate of release, quantified as the uncaging half-life. Ideally, the active drug should be released within a short time frame and with high chemical yields. The photochemical byproducts should be known, biocompatible and inert, and should ideally not absorb at the irradiation wavelength. The caged drug should also be soluble and stable in the targeted media in the dark, possess no or low biological activity, and have appropriate physicochemical and pharmacokinetic properties to reach the active site.

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2. BODIPY

Boron dipyrromethene (BODIPY) is a commonly used fluorophore; since its first example reported in 1968,^[24] it has found numerous applications as biological label, chemical sensor and in luminescent devices amongst others.^[25] Its popularity can be ascribed to excellent photochemical and thermal stability, high fluorescence quantum yield, low triplet state formation, sharp and intense absorption, high solubility and chemical robustness and versatility.

2.1. Cargo at the 4-position

While most of the PPGs based on BODIPY attach the drug at the 8-position, in the first reported example in 2014, Urano and coworkers prepared derivatives substituted at the boron (Figure 1).^[26] During their studies on the fluorescence properties of 4-aryloxy BODIPY derivatives, they serendipitously found that visible light led to photocleavage of the B-O bond. In this manner, they were able to cage phenol groups that can be photoreleased with blue-green light. Their PPG was also applicable to amines via introduction of a benzyloxycarbonyl linker. To further increase the uncaging wavelength, they extended the conjugated π system by preparing derivative **2**, which was able to release the aryloxy groups with orange-red light (600 nm). Although the limitation of this PPG is a significant decrease of ϕ_u , it was an unprecedented example of one-photon uncaging at this long wavelength and offered a chemical strategy to optimise PPGs. Similar conformationally locked cages have been prepared later with higher quantum yields having the cargo at the *meso* position (**8**, vide infra).^[27]

In terms of the fluorescence properties of these derivatives, they found an inverse correlation between ϕ_u and ϕ_f . Photoinduced electron transfer (PeT) from the alkoxy groups is responsible for both the uncaging properties and the quench of fluorescence, and therefore optimisation of one is at the expense of the other.

The 8-phenyl BODIPY can be easily prepared in three steps from 2,4-dimethylpyrrole,^[28] which can also be done in a one-pot procedure (Scheme 1).^[29] Subsequent substitution of the fluorine atom with the required alcohol in the presence of a Lewis acid gives the desired 4-aryloxy derivative.^[30]

2.2. Cargo at the 2,6-positions

Another dose of serendipity led Urano and coworkers to find 2,6-sulfonamide-substituted BODIPYs **4** as PPGs.^[31] During their studies on the introduction of EWGs onto the BODIPY core, they identified that derivatives bearing sulfonamide groups on the 2 and 6 positions decomposed. After a series of mechanistic studies, they found that these derivatives can be used to release amines under visible light (490 nm). Although they were able to prepare this analogue in 4 steps using alanine, caging more complex amines might be more synthetically challenging.

Another example where the cargo has been introduced at the 2 position was reported in 2019 by Zhang and coworkers.^[32] They took advantage of the weak N-O bond of oxime esters^[33,34] to design a BODIPY photocage **7** that released aliphatic and aromatic carboxylic acids upon green light irradiation (500–520 nm). The photolysis mechanism proceeds at least partially via a triplet excited state, releasing the acid along with the 2-

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cyano-BODIPY photoproduct. With regards to the synthesis of these derivatives, incorporation of the necessary 2-substituents can be done in three steps after construction of the BODIPY core. The oxime is generated through Vilsmeier-Haack formylation of the BODIPY core followed by reaction with hydroxylamine, which is then reacted with the acid chloride deriving from the desired small molecule cargo.

Figure 1. Reported examples of longer wavelength BODIPY PPGs.

2.3. Cargo at the meso position

The most common substitution pattern of the most recently developed BODIPY PPGs is with installation of the substrate at the 8-position (also known as the *meso* position), which was first described by R. Weinstein and coworkers.^[35] They were inspired by other known protecting groups such as coumarins (*vide infra*) and xanthenes, where a fluorophore is equipped with a cargo through a methylhydroxy moiety, and they synthesised *meso*-methylhydroxy BODIPY analogues **3**. These released the substrate under green light illumination, and with properties in the range of other visible light PPGs (relatively low ϕ_{f} , high ϵ). They showed that their caging group can be used to release primary amines, anilines, phenols and carboxylic acids, and it should also be applicable to alcohols.^[36] The caged derivatives can easily be prepared in three steps starting from 3-ethyl-2,4-dimethyl-1*H*-pyrrole (Scheme 1), which is first condensed to the BODIPY core

10. Subsequent acetal hydrolysis gives alcohol **11**, a common intermediate for the introduction of the different functional groups.

Scheme 1. Synthetic approaches towards modification of the BODIPY core.

A thorough structure-reactivity relationships study performed by a joint effort between the groups of Weinstein, Winter and Klán on BODIPY substitution provided some insights into the structural modifications that can enhance the photophysical and photochemical properties of BODIPY photocages.^[22] The authors took advantage of the proposed photorelease mechanism of *meso*-cargo-BODIPYs,^[37] which upon irradiation undergo heterolysis to the *meso*-carbocation **17**, followed by solvolysis (Scheme 2). To increase quantum yields of known BODIPY-based photocages, structural motifs that could favor stability of the intermediate carbocation and release of the leaving group were investigated.

Replacement of the fluorine atoms by alkyl groups successfully increased the photoreaction efficiency (Table 1), consistent with an increase in the electron density leading to stabilisation of the intermediate carbocation. Methyl groups are optimal as increasing steric bulk (ethyl or phenyl) led to a decrease of ϕ_{f} , possibly due to distortion of planarity.^[38] In fact, the methyl groups present at positions 1 and 7 of most BODIPY derivatives probably also contribute towards an increase in electron density. *B*-alkylation also increases the rate of photorelease.^[39,40]

As expected for an $\text{S}_{\text{N}}1$ -type mechanism, the quality of the leaving group also played an important role in the efficiency of the photorelease; leaving groups with lower pK_{a} values had higher ϕ_{f} . 2,6-Diodination also significantly increased photoreaction quantum yields. A previous computational study by Smith, Winter and coworkers to identify PPGs with efficient visible light photocleavage had also identified that iodinated derivatives had a high quantum efficiency.^[37] Both publications attribute this result to intersystem crossing (ISC) to a carbocation triplet state, thus lowering the barrier of photorelease and increasing quantum yields. Indeed, substitution of a fluorophore with heavy atoms, and particularly with halogens, promotes ISC (the “heavy atom effect”), and in fact iodinated BODIPY analogues have been used as highly efficient photosensitisers applied to photodynamic therapy (PDT).^[41,42] Therefore a potential limitation of such compounds as photoremovable protecting groups are potential cellular phototoxicity via generation of singlet oxygen.^[43]

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Combination of two substituent effects that can independently increase photocage efficiency (boron alkylation and 2,6-diiodination) into analogue **5** resulted in unprecedentedly high $\varepsilon\phi_u$ ($9500 \text{ M}^{-1}\text{cm}^{-1}$) for a photocage absorbing visible light.

Both *B*-alkylated and 2,6-halogenated derivatives are easily accessed from the standard BODIPY core (Scheme 1). Fluorine replacement is achieved via reaction with the corresponding Grignard reagent, and halogenation via reaction with the corresponding *N*-halogensuccinimide.

Scheme 2. Uncaging mechanism of BODIPY PPGs with the cargo at the meso position.

Given the need for a good leaving group in the *meso*-derivatives, direct attachment of amines and alcohols does not lead to an efficient photorelease. W. Szymanski and coworkers proposed that primary and secondary amines can be photoprotected via a carbamate linker that facilitates the deprotection reaction and enables fast photocleavage (half-lives 1 – 2 min).^[44] Liberation of the amine takes place via a decarboxylation mechanism that releases CO_2 after initial photocleavage of the C–O bond. For alcohols, the corresponding carbonates can be used in a similar manner.^[45] Alternatively, Smith, Winter and coworkers have found that direct attachment of alcohols as ethers can be achieved with decent photorelease efficiencies ($\varepsilon\phi_u = 69 \text{ M}^{-1}\text{cm}^{-1}$) with the *B*-alkyl derivatives.^[36] While this approach avoids some of the disadvantages of the carbonate linker, such as potential non-photochemical ester cleavage by cellular esterases, lower hydrolytic stability of carbonates relative to ethers and reduced temporal control due to the two-step release mechanism after photolysis, the ethers undergo photocleavage at a slower rate (half-lives of hours).

Recent work by Truong and coworkers showed that thiols can also be photocaged with 8-substituted BODIPYs,^[46] with optical properties similar to other *meso*-cargo derivatives. The photoreleased thiolate product can be further reacted with an alkyne partner to give an alkene thioether, providing conditions for additive-free chemical ligation induced by light. The authors showed the utility of this strategy in biomaterial science; for example, they were able to prepare hydrogels with specific microchannel architectures using a photomask, and to encapsulate three-dimensional cell cultures. Synthetically, direct introduction of the thioether from the *meso*-Br-BODIPY was unsuccessful, and a longer route with at least 5 steps is necessary for these compounds. Selection of an appropriate ligation partner is critical as for instance acrylate esters and maleimides were not successful; the former are not electrophilic enough, and the latter possibly act as electron acceptors from the excited BODIPY and degrade.

In another publication by Smith, Winter and coworkers, the authors aimed to further increase the photorelease wavelength of the BODIPY PPGs by extending the π -conjugation,^[45] a strategy that had been previously used to photorelease carbon monoxide.^[47] They incorporated 3,5-styryl groups easily

accessible from 3,5-dimethyl BODIPY via Knoevenagel condensation with the corresponding aldehyde (Scheme 1). The conjugated derivatives were indeed found to absorb at red-shifted wavelengths up to 660 nm, albeit at the expense of quantum yields (0.01%). Taking advantage of the previous work showing a substantial increase in ϕ_u with the *B*-alkylated derivatives,^[22] further optimisation of the styryl analogues was achieved upon *B*-methylation. The resulting compounds **6** had exceptional overall properties, with $\varepsilon\phi_u$ similar to commonly used photocages, but at much higher absorption wavelengths (Figure 1). Throughout their photorelease studies, they noticed that other than the cargo, irradiation led to small amounts of benzaldehyde deriving from oxidative cleavage of the alkene. Given that the resulting BODIPY lacking one of the styryls has a different fluorescence profile, this can in fact be a means to effectively track the extend of photorelease *in vitro*. However, it might be necessary to investigate the potential toxic effects of the released aldehyde before further progression of these derivatives.

Table 1. Effect of different substitution strategies on the photochemical properties of BODIPY PPGs. ε , ϕ_u and $\varepsilon\phi_u$ are measured at $\approx\lambda_{\text{max}}$ unless specified.

	R	λ_{max} [nm]	ε [$\times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$]	ϕ_u [%]	$\varepsilon\phi_u$ [$\text{M}^{-1} \text{ cm}^{-1}$]
Leaving group ^[36]	Aniline	545	3.5	0.06	21
	Amine	544	4.5	0.015	6.7
	Phenol	547	4.8	0.016	77
	Alkyl (acid release)	545	3.9	0.018	7
2,6-Halogens ^[22]	H	517	7.1	0.14 (365)	
	Cl	544	4.8	0.29 (365)	
	Br	545	5.6	0.70	392
	I	553	4.9	0.99	485
B-Alkyl ^[22]	F	514	10.4	0.15	156
	Me	512	6.8	5.5	3740
3,5-Styryls ^[45]	Me	515	7.1	0.099	70
	3-Me, PMP	586	6.1	0.0098	6
	PMP	661	6.5	0.0041	2.7

Recently, Smith, Winter and coworkers have been able to increase the uncaging quantum yields of their conjugated analogues.^[27] Rather than modifying the structure to favour a more efficient photorelease, their strategy consisted in inhibiting access to competitive excited state decay channels. Conformational restraint of fluorophores has been shown to improve ϕ_f by blocking the major deactivation pathways,^[48–50] including *cis-trans* double bond isomerisation. Thus, to block unproductive energy losses from the alkene bonds of **6**, they prepared more conformationally restrained analogues such as **8**,

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which was indeed found to have a quantum yield 30-fold higher than **6** (3.8 vs 0.1%).

2.4. Modification of physicochemical properties and cellular localisation

Other than photochemical properties, favorable physicochemical properties are also essential for the biological application of PPGs. Many of the reported BODIPY photocages are highly hydrophobic, which can limit their aqueous solubility. Recent publications on BODIPY PPGs also report possible chemical modifications that can be utilised to optimise these properties. An increase in hydrophilicity can be achieved by introducing carboxylate groups onto the 2 and 6 positions,^[26] such as in **20** (Figure 2), incorporating short PEG chains to the analogues with 3,5-extension^[45] (**19**) and adding remote sulfonation to the 3 and 5 positions (**21**).^[51] The *B*-methyl derivatives are also more soluble than the corresponding *B*-F.

Besides decreasing solubility, hydrophobicity can also increase cell permeability. This can be desirable for the photorelease of small molecules with intramolecular targets, but it can negatively affect the biological response when the target is extracellular or in the plasma membrane. For instance, when Urano and coworkers used their 4-substituted BODIPY to cage histamine, illumination failed to induce the expected cellular response in HeLa cells.^[26] They ascribed this lack of effect to the hydrophobic nature of the caged precursor leading to accumulation in intracellular membranous structures, while histamine H1 receptors exist on the cell surface. The necessary hydrophilicity to achieve localisation in the extracellular space was obtained with the 2,6-dicarboxy **23** (Figure 3), which was indeed able to increase intracellular Ca^{2+} levels consistent with histamine receptor binding.

Figure 2. Modifications of the BODIPY core that modify organelle localisation and increase hydrophilicity.

In a more recent example by Miller, Weinstein and coworkers, the cellular permeability of photocages, and consequently their biological activity, was shown to be tunable via the number of incorporated remote sulfonate groups.^[51] Using small molecule drugs with a range of polarities, their non-sulfonated derivatives had a range of permeabilities but mostly accumulated intracellularly, while the di-sulfonated ones were completely cell impermeable. For a more precise control on the localisation of the photoreleased small molecules, the BODIPY PPGs can also be modified to incorporate functional groups that permit photorelease at a specific organelle, thus achieving greater spatial control. Weinstein and coworkers pioneered this approach in BODIPY

photocages **22**,^[52] which had been previously applied to 2-nitrobenzyl derivatives.^[53,54] Introduction of triphenylphosphonium, phenyl sulfonamide and morpholine allowed accumulation of the photocages in mitochondria, endoplasmic reticulum (ER) and lysosomes respectively without significantly changing their photochemical properties. The cellular application was shown by directing the protonophore 2,4-dinitrophenol to mitochondria, and the protein synthesis inhibitor puromycin to the ER of HeLa cells. These targeting groups can be easily installed via a late stage functionalisation strategy that allows the introduction of a large variety of functional groups.

2.5. Cellular assays

A common strategy to test the biological applicability of PPGs in cellular assays is to firstly demonstrate efficient photorelease in cells, and secondly show that the release of a bioactive molecule enables the induction of a particular cellular effect only upon illumination.

For the former, one common approach is to photocage a group that quenches the fluorescence of BODIPY, such as nitrobenzoic acid.^[37,45] Upon uncaging, the increase in fluorescence from the BODIPY photoproduct can be detected to monitor release of the cargo. An alternative approach is to cage a cargo molecule that gains fluorescence after uncaging; for instance, fluorescence of 7-hydroxycoumarin is dependent on its substitution pattern. When loaded through the 7-position onto BODIPY, fluorescence is only recovered after photorelease.^[36] In both of the mentioned examples, the authors detect some decrease of fluorescence in their controls (BODIPY not attached to quencher or fluorophore), which is due to photobleaching caused by the intense focused light.

Figure 3. BODIPY PPGs used in cellular assays.

So far, the biological applicability of the BODIPY class of photocages has been evaluated only in cell lines (Figure 3). In the

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first example by Urano, uncaging histamine in HeLa cells with 500 nm light irradiation was able to increase intracellular Ca^{2+} levels consistent with histamine receptor binding.^[26] Caged GABA has been used to stimulate neuronal cells in mouse brain slices under 470 nm illumination.^[31] Photocages of the histone deacetylase inhibitor (HDACi) valproic acid (VPA)^[32] and cysteine protease cathepsin B (CTSB) inhibitor CA-074^[39] showed more efficient cytotoxicity than the non-caged inhibitors in HeLa and MDA-MB-231 cells respectively. In a recent example using the styryl derivatives, photocaged dopamine was able to increase beating frequency of spontaneously beating hESC derived cardiomyocytes under red light illumination.^[40]

2.6. Summary

Since its first example in 2014, significant progress has been made in the use of BODIPY as a PPG and red-absorbing derivatives have already been identified.

Increasing conjugation and iodinating the core has increased the excitation wavelength up to the red region (Table 1). Alkylation on the boron, iodination and conformational restraint increase uncaging quantum yields. However, these modifications do not come without challenges: conjugation decreases ϕ_u , and *B*-alkylation can also cause a decrease in the rate of release over time due to photolability of the alkyl groups on the boron.^[36,55] With respect to 2,6-iodination, although significant increase in λ_{abs} and ϕ_u has been observed, there are some reported exceptions where appending iodines to the BODIPY core decreased ϕ_u .^[36] These analogues have also been shown to be less soluble^[44] but photochemically faster.^[46] Photobleaching of the BODIPY core is also often detected.^[35,37]

With biological applications involving solely cellular assays, further applicability still remains to be seen. We expect to see reports on the application of BODIPY in more complex *in vitro* and *in vivo* systems, which will cast light on the translational potential of this PPG, and on the optimal core pattern.

3. Coumarin

The first example of coumarin being used as a photoprotecting group for biomolecules dates back to 1984 where it was established as an efficient phosphate-releasing group (Figure 4).^[56] Since then, many examples have been reported which release a wide range of functional groups loaded at the 4/*meso* position including other phosphates,^[57] carboxylates,^[58] sulfates and sulfonates,^[59] diols^[60] and carbonyls^[61] with short UV irradiation wavelengths. Early efforts were made to improve some of the optical properties of this PPG, in particular by optimising the substituents at the 6 and 7 positions. Important improvements were obtained with 6-bromo-7-hydroxy analogues such as **29**.^[62] A plausible explanation is that the bromine atom promotes ISC to a triplet state, and it also contributes to lower the $\text{p}K_a$ of the 7-hydroxy group, thereby causing complete deprotonation at physiological pH, which both further increases the absorption maximum and enhances water solubility. Later analogues bearing 7-dialkylamino substituents (**30**) provided higher absorption wavelengths of 400 nm and a substantial increase in quantum yields.^[63]

Coumarins have been used for both one-photon and two-photon uncaging. Herein the one-photon absorption properties of recently developed coumarin analogues will be discussed.

Figure 4. First reported examples of coumarin PPGs.^[56,62,63]

Similar to other arylalkyl-type PPGs, the photolysis mechanism occurs through cleavage of the C-O bond. Upon excitation, photoheterolytic bond cleavage releases the cargo as the leaving group and the positively charged coumarin, which is trapped by the solvent to produce as a byproduct a coumarinylmethyl alcohol in the case of aqueous media.^[58,64] Consistent with the involvement of a carbocation intermediate, an additional methyl group at the 4 position has been shown to increase the uncaging rate.^[65] The nature of the leaving group also plays an important role, where more acidic cargos better stabilise the ion pair generated upon bond cleavage and are released faster.^[65]

Scheme 3. Synthetic approaches towards modification of the coumarin core.

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The common coumarin-OH core **34** is usually prepared from commercially available 7-diethylamino-4-methylcoumarin **31** through oxidation with selenium dioxide and subsequent aldehyde reduction in one pot (Scheme 3).^[66] Recently, an alternative 3-step route has been proposed that avoids the use of selenium and is stated to give higher and more robust yields.^[67] This route involves preparing the aldehyde through initial condensation to enamine **33**, which undergoes oxidative cleavage with sodium periodate to aldehyde **32**.

Most of the core modifications require previous alcohol protection, which is usually done either as the acetate ester or as a silyl ether. After final deprotection, the cargo molecules can be introduced.

3.1. Extension of conjugation at the 3 and 7 positions

In the last years, significant progress has been made with the coumarin PPG to shift the photorelease wavelength onto the visible range. In one of the first examples by Zhu and coworkers, the authors incorporated styryl groups at the 7 position of the coumarin core and used it to photorelease alcohols via a carbonate linker.^[68] They expected that increasing π -conjugation and electron donation would both red-shift the absorbance and increase absorption coefficient. Indeed, analogue **42** bearing a strong electron donating group led to the biggest increase in λ_{max} to 407 nm, however with a concomitant decrease in quantum efficiency as compared to the 7-diethylamine analogue (Table 2). The styryl groups can be introduced via Heck coupling between the required alkene and 7-iodocoumarin with previous protection of the 4-methylhydroxy.

Extension of π -conjugation has also been described at the 3-position. In the first example, Ellis-Davis and coworkers prepared the acrylamide derivatised coumarin DEAC450, absorbing at 450 nm with remarkably high uncaging quantum yields^[69] (39% for caged glutamate **44** and GABA,^[70] 78% for caged cAMP^[71]). On top of high one-photon quantum efficiency of uncaging with visible light, a real strength of this conjugated coumarin is under two-photon excitation, which occurs at wavelengths as high as 900 nm. The design of DEAC450 is based on an EDG- π -EWG system, with the 7-diethylamine acting as the electron donor, and the acrylamide as electron acceptor. Such electronic push-pull character has in fact been used in the design and optimisation of other fluorophores.^[72–74]

Klausen, Blanchard-Desce and coworkers later intensified the dipolar nature of this design by introducing 3-styryl heterocycles, which were used to cage Fmoc-protected glycine attached as an ester.^[75] Most of their derivatives successfully increased the absorption wavelength and absorption coefficient, and in particular benzothiazole **48** with a λ_{max} of 472 nm, albeit with lower uncaging yields as compared to DEAC450. The authors note that EWGs are detrimental to photorelease efficiency, and despite causing the highest red-shift, their strongest electron withdrawing benzothiadiazolyl analogue did not proceed to uncaging upon irradiation. These results are attributed to large photoinduced intramolecular charge-transfer (ICT) leading to a decrease of uncaging efficiency. On the other hand, the added π extension in **49** leads to similar uncaging efficacy to DEAC450 and a slightly higher λ_{max} of 458 nm. These derivatives have unprecedentedly high λ_{max} values of two-photon absorption at 940–970 nm.

Figure 5. Reported examples of longer wavelength coumarin PPGs.

Further work on π -conjugation at the 3 position has established an alternative EDG- π -EDG system,^[76,77] which had previously been successful in two-photon excitation of stilbenes.^[78] In the work from Zhu, introducing electron donating substituents at the *para*-position of the styryl group increased the λ_{max} by 76 nm as compared to the 3-unsubstituted coumarin (Table 2), with the best substituent being the conformationally locked julolidinestyryl with λ_{max} of 456 nm. The electron-rich styryls probably contribute to stabilise the intermediate carbocation. The bathochromic shift was further enhanced by combining it with a thiocoumarin core (*vide infra*) and by conformationally locking the electron donating aniline at the 7 position with a julolidine **47**, achieving photorelease of *para*-methoxybenzoic acid at 515 nm. Similar to the previous 3-styryl analogues from Ellis-Davis, these derivatives had high quantum yields, as well as a fast photorelease, with half-lives of less than 2 min.

Recently, 3-vinyltetrazine coumarins have been designed by Bójtár and Kele.^[79] These release the cargo under blue light illumination only after biorthogonal reaction of the tetrazine moiety, thus providing an extra level of spatiotemporal control to target specific cells or cellular structures.

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The styryl group can be introduced through regioselective bromination/iodination of the coumarin alcohol DEAC-OH (which requires previous alcohol protection in some cases) followed by Heck coupling with the corresponding alkene (Scheme 3).

3.2. Modification of the 2 and 3 positions

Jullien and coworkers have taken advantage of earlier reports showing that thiocarbonyls absorb at red-shifted wavelengths with respect to carbonyls.^[80,81] They prepared a 2-thiocoumarin caging group for an amine cargo via a carbamate linker, which absorbed blue light (469 nm) with reasonable $\epsilon\phi_u$.^[82] The empty 3d atomic orbitals of the sulfur atom probably enhance ICT, producing a bathochromic shift of the uncaging absorbance. Thiocoumarins can be accessed directly from the corresponding coumarins using the Lawesson's reagent.

In a later report, the same group looked at the ability of different substituents at the 2 and 3 positions to further red-shift the maximum absorption wavelength.^[83] The strategy was based on increasing the photoinduced ICT, by maintaining the electron donating diethylamine at the 7 position while introducing 2- and 3-electron-withdrawing groups. A range of analogues with different substituents were prepared and photocharacterised. Indeed, those analogues having stronger electron withdrawing groups gave the biggest shifts to higher absorption values and molar absorption coefficients. The best performing substituents were cyano at C3 ($\lambda_{\max}$ 443 nm) and dicyanomethylene at C2 ($\lambda_{\max}$ 487 nm) (Table 2). However, these PPGs had weak fluorescence properties and relatively low $\epsilon\phi_u$, which is consistent with the previous reports showing a decrease in quantum yields when ICT is favoured.

Both chemical modifications can be done at late-stage after construction of the coumarin core and attachment of the cargo. Cyanation involves 3-bromination with *N*-bromosuccinimide followed by substitution with copper cyanide. Dicyanomethylene is accessible through reaction of the thiocoumarin with malonitrile in the presence of 4-dimethylaminopyridine and lead oxide (Scheme 3).

The group of Marchán proposed that replacing one of the cyano groups in the 2-dicyanomethylene derivatives with aromatic groups bearing electron withdrawing substituents at *ortho* or *para* could further increase the bathochromic shift.^[84] They found that addition of 1-3 nitro groups gradually increased the absorption wavelength, with the trinitro derivative absorbing in the red region (596 nm). However, limitations of these compounds are low quantum yields, loss of fluorescence and slow uncaging rates. With the mono-nitro analogue loaded with benzoic acid as the cargo, only 4% was photoreleased after 10 h of irradiation, which could only be increased to 18% by incorporation of an extra methyl group at position 4 (*vide supra*) to **48** (Figure 5). These compounds are accessible from thiocoumarin with the appropriately substituted phenylacetonitrile, which in most examples requires previous synthesis in 1-2 steps.

In some of the analogues functionalised at the 2 position, such as thiocoumarin and dicyanomethylene groups, the released photoproduct upon excitation consequently degrades to the corresponding 2-oxocoumarin.^[83] This observation is of particular

relevance when the released coumarin-OH is used to monitor the release of the cargo molecule.

Table 2. Effect of different substitution strategies on the photochemical properties of coumarin PPGs. ϵ , ϕ_u and $\epsilon\phi_u$ are measured at $\lambda_{\max}$ unless specified.

	R	$\lambda_{\max}$ [nm]	ϵ [$\times 10^4$ M ⁻¹ cm ⁻¹]	ϕ_{f} [%]	$\epsilon\phi_{\text{f}}$ [M ⁻¹ cm ⁻¹]
6-Styryl ^[88]	NEt ₂	377	1.2	4.5 (420)
	407	2.9	0.08
2- and 3-EWGs ^[83]	R ₁ =O	385	2.4	0.21	50	
	R ₁ =S	472	3.1	0.50	155	
	R ₁ =	487	3.3	0.024	7.9	
	R ₁ =O, R ₂ =CN	443	2.6	0.010	2.6
meso-Methyl ^[65]	H	487	3.2	0.15	48	
	Me	489	3.2	0.33	106
3-Styryl ^[77]	H	380	2.0	7	1400
	430	3.0	45	13500
	456	4.4	19	8360	

3.3. Physicochemical properties

The common coumarin scaffold has been generally found to have favourable physicochemical properties, with suitable aqueous solubility and cell permeability. However, in some examples where either the nature of the cargo molecule or the core modifications contribute to increase hydrophobicity, certain modifications have been described that can improve solubility.

The 7-*N*-diethylamine used in most of the latest examples can be substituted by a 7-bis(carboxymethyl)amine, which has much higher aqueous solubility by virtue of its anionic nature, without significantly altering the optical properties.^[85]

For 3-acrylamidecoumarins, the common DEAC450 scaffold has an appended dicarboxylate moiety aimed at increasing solubility.^[71] In the case where the caged derivatives are still not soluble enough, a polyethyleneglycol (PEG) chain can be used instead.^[70]

Aqueous solubility of 3-heterocyclic styryls can be enhanced through formation of the corresponding methylated nitrogen salts such as **50**^[86] (Figure 5). This modification also causes a bathochromic shift but a decrease of quantum yield of uncaging and fluorescence.

In an example by Fournier and coworkers, the *in vivo* experiments were performed in the presence of (2-hydroxypropyl)- β -cyclodextrin,^[82] which produces a 1:1 complex with the caged drug and improves its disponibility.

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3.4. Biological assays

There are numerous examples where coumarins have been used to photochemically control biological functions in cellular assays. Only some representative examples will be exposed. The initial design cleavable under UV light was used to photocontrol protein kinase C (PKC) activation in a particular region of interest within CHO-K1 cells with a caged 1,2-diacylglycerol (DAG) lactone,^[87] to control cell migration of A375M cells with a caged lysophosphatidic acid (LPA), where cells were found to migrate towards the source of photorelease,^[88] and to control antibacterial activity.^[89] The dicyanomethylene derivatives have been used to cage a cyclic RGD peptide conjugated to the metallodrug ruthenocene, which has the potential to be used as a targeted anticancer strategy activable under green light (496 nm).^[90]

Figure 6. Biological applications of coumarin PPGs. A) Whole-cell current clamp recordings from acute mouse brain slices were used to monitor spontaneous action potential firing in striatal cholinergic interneurons. In the presence of both DEAC450-cAMP and CDNI-GABA, sequential illumination with 355 nm and 473 nm light produces bidirectional changes in action potential firing.^[71] Adapted with permission from ^[71]. Copyright (2013) American Chemical Society. B) En2 positive phenotype (absent or reduced eye) observed in En2-ERT² mRNA-injected embryos treated with thiocoumarin-caged cyclofen only after blue illumination.^[82] Adapted with permission from ^[82]. Copyright (2013) American Chemical Society. C) Combinatorial regulation of *spt* and *flh* activity using wavelength-selective cMOs. (a) Classification of myod1 expression patterns associated with wild-type (adaxial/paraxial), *flh* null (axial/paraxial), and *spt* null (partial adaxial) phenotypes in 12 hpf embryos (dorsal view, anterior top); (b) corresponding phenotypic distributions for embryos subjected to the indicated cMOs and irradiation conditions.^[91] Reprinted with permission from ^[91]. Copyright (2014) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

The recent optimisation of this class of PPG allowing visible light activation has opened the door to multiwavelength activation of small molecules and the first examples of *in vivo* applications in zebrafish.

A pronounced absorption minimum of some coumarin PPGs in the UV region, where some of the older PPGs (eg *o*-nitroaryls) are photolysed, allows for orthogonal uncaging of different bioactive molecules depending on the irradiated wavelength. This has been used for instance to modulate neuronal firing rates,^[71] which can be either inhibited by GABA or upregulated by cAMP. Ellis-Davis and coworkers prepared a previously reported violet-cleavable CDNI-GABA^[92] and a blue-cleavable cAMP based on a 3-styrylcoumarin, DEAC450-cAMP. The membrane potential of neurons in acutely isolated brain slices treated with both compounds could be bidirectionally modulated depending on the applied light colour (Figure 6A).

Wavelength-selective experiments with 3-styryl coumarin analogues were also used in 2014 for orthogonal uncaging of GABA and glutamate.^[70] In this case, the two neurotransmitters were rendered light-responsive through caging with a blue-cleavable coumarin (DEAC450-GABA) and a nitroaromatic group cleavable under two-photon excitation at 720 nm (CDNI-Glu). Both compounds were applied to a brain slice, and currents evoked to pyramidal neurons were monitored via whole-cell patch clamp. Under blue light illumination, inhibitory postsynaptic potentials were detected, while red light evoked membrane depolarisation.

The *in vivo* applicability of the coumarin PPG has been demonstrated in zebrafish embryos, a commonly used model in photopharmacology due to their transparent body.^[93] Jullien and coworkers used their thiocoumarins to photocage cyclofen (tcInd), an inducer of the transcription factor En2.^[82] Overexpression of En2 results in reduction of the size of the diencephalon causing a reduction of the size of the eyes. They observed that in En2-ERT²-injected embryos incubated with tcInd, those illuminated by blue light had a considerable increase in the En2 phenotype (Figure 6B) as compared to those kept in the dark.

A combination of orthogonal nitrobenzyl and coumarin based probes has been used by Deiters and Chen for sequential gene silencing in zebrafish (Figure 6C).^[91] The authors prepared caged morpholino oligonucleotides (cMOs) to study mesoderm patterning in zebrafish embryos. Antisense MO are designed to target specific RNA sequences, thereby silencing gene expression. Cyclisation with a photocleavable linker inactivates them, while illumination induces relineralisation and restores their function. In this particular example, the cMOs were directed to the genes *spadetail* (*spt/Tbx16*) and *floating head* (*flh*), which regulate mesoderm development. Irradiation at 365 nm led to a drastic loss of myod1 expression, consistent with *spt* loss-of-function, while at 405 or 470 nm resulted in aberrant myod1 expression, consistent with *flh* silencing. Sequential irradiation at 365 nm and 405/470 nm at defined timepoints of embryonic development and analysis of the obtained phenotypes allowed for a detailed understanding of gene function and gene interactions with precise temporal control.

3.5. Summary

In the last decade, numerous SAR studies around the coumarin core have been performed, which provide insights into the effect of different structural modifications. Similar to what has been observed for BODIPY PPGs, extended conjugation is an effective

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strategy to increase the λ_{abs} . The collateral effect on quantum yield depends on the position and the styryl substitution. The examples at the 7 position containing EDGs, and styryls at the 3 position bearing electron withdrawing heterocycles decreased Φ_{u} . Contrarily, the acrylamide in DEAC450 and 3-styryls with EDGs increase Φ_{u} . Some of these modifications also decreased or practically abolished fluorescence properties, while some reported groups still retain reasonably high Φ_{r} , such as some heterocyclic 3-styryls. These studies highlight the challenge of increasing uncaging wavelength of PPGs without compromising other key photochemical parameters.

Examples absorbing red light under one-photon excitation, with reasonable uncaging efficacies, have not been reported yet. Nonetheless, many examples of biological applicability both *in vitro* and in zebrafish models have been described. The visible light absorbing coumarin PPGs with narrow absorption bands have also opened the door to wavelength-selective experiments controlling the release of different biomolecules depending on the applied light colour.

4. Cyanine

Cyanines are valuable fluorophores with well-established biocompatibility.^[94–96] They have a characteristic polyene linker that confers them with unique optical properties, including absorption and emission maxima in the near-IR window. They also have high aqueous solubility and brightness. Cyanines are routinely used for numerous biomedical applications, including *in vivo* imaging,^[97] and indocyanines Cy3, Cy5, Cy7 and Alexa 647 are often used in fluorescence-based experiments. Also, by virtue of their preferential accumulation in cancer cells vs normal cells,^[98] cyanines have been conjugated to chemotherapy drugs and used as theranostic tools.^[98,99]

4.1. Modification of the cyanine core

Cyanine fluorophores are prone to light-induced decomposition via photooxidative cleavage of the polyene,^[100,101] which Schnermann and coworkers have leveraged to transform this fluorophore into a PPG.^[102] The mechanism of their PPG involves initial formation of dioxetane at two possible double bonds (C1–C1' and C2'–C3'), which thermally decompose to aldehydes **55** and **56** (Scheme 4). This oxidation is possibly mediated by singlet oxygen generated through energy transfer from the cyanine triplet excited state. Subsequent enamine hydrolysis and intramolecular cyclisation liberate the cargo molecule. The authors proposed a clever design where the enamine gains electrophilic character after disruption of the polyene system, and thus hydrolysis is favoured after irradiation-driven polyene cleavage.

Cyanine **58** (Figure 7) successfully released phenol-containing small molecules under red light (λ_{max} 668–705 nm depending on the cargo and the indoline *N*-substituent).^[102] Derivatives were prepared bearing either *n*-propyl or 4-butesulfonate at the indoline nitrogen. Although the former had a bathochromic shift (30 nm), the latter have been shown to be more water soluble and less prone to aggregation.^[103]

The high absorption wavelength of this first example of cyanine PPG was promising, and the molar absorption coefficient is in the same range as previously mentioned PPGs. It will be of interest

to see examples of reported quantum yields. Other than phenols, a later report has shown the release of a tertiary alcohol.^[104]

One of the limitations that were found with this cyanine PPG is low difference between light and dark activity, probably deriving from background C–N hydrolysis and subsequent release of the cargo. In a follow-up paper, these limitations were addressed by taking advantage of previous reports showing that sterics around the carbamate can affect the rate of cleavage,^[105] and that the enamine substituents can impact on absorption wavelength.^[106] Thus, changing the ethylenediamine linker from *N,N'*-dimethyl to *N,N'*-diethyl decreased the background hydrolysis rates, and it also increased λ_{max} by 40 nm.^[107] Earlier work had also shown that additional fused benzenesulfonate rings on the indole groups can shift the absorption to longer wavelengths.^[108] Indeed, sulfonated benz[e]indazole derivatives provided another bathochromic shift of 18 nm, taking the λ_{max} of **59** up to 732 nm (Figure 7).

Scheme 4. Uncaging mechanism of C4'-dialkylamine-substituted heptamethine cyanines.

The cellular uptake and localisation of these cyanine PPGs can be enhanced by replacing the sulfonate groups by *n*-butyl esters, without altering λ_{max} .^[109] The utility of ester prodrugs to enhance drug biodistribution had been previously demonstrated.^[110] These lipophilic esters improve cell permeability, while subsequent intracellular ester cleavage promotes retention.

Other than phenols, the photorelease of aromatic amines has also been investigated by Schnermann.^[111] Direct attachment of the aniline-containing small molecule onto the heptamethine resulted in slow release. Instead, the authors proposed an alternative approach with a merocyanine **60** which undergoes a photooxidation/ β -elimination sequence, a release strategy previously used in other contexts.^[112] This modified scaffold maintains red light absorption and high molar absorption coefficient with modest release yields. This work provides a proof of concept for the release of amines from cyanines, and opens the door to structural optimisation for an improvement of release efficiency and extension to other functional groups. Cyanines

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have also recently been used to release carbon monoxide by fusing a CO-releasing flavonol moiety to the coumarin core.^[113]

Figure 7. Reported examples of longer wavelength cyanine PPGs.

The phenol-releasing cyanines **63** (Scheme 5) can be easily prepared in 2-3 steps from the chloro precursor IR-783, which is commercially available. Initial conjugation with *N,N*-dimethylethylenediamine is followed by reaction with either the chloroformate or the mixed nitrophenyl carbonate of the cargo molecule. Preparation of the aniline-releasing merocyanines involves a longer 6-step synthesis.

Scheme 5. Preparation of cyanine PPGs.

4.2. Biological assays

The biological applicability of the cyanine PPG has been proven by Schnermann both *in vitro* and *in vivo*. In one of the first examples in a cellular context, cyclofen-OH **64**, which has been shown to have cytotoxic activity in some breast cancer cell lines, was photocaged through the phenol moiety (Figure 8A).^[102] MCF-7 cells treated with **64** showed a decrease in cell viability comparable to the free drug only when cells were irradiated for 30 min at 690 nm. The inherent fluorescence of cyanine can be used to track the location of the photocage.

The same authors have also used these cyanine cages to pioneer the near-IR light-mediated cleavage of antibody-drug conjugates (ADCs).^[107,114] They attached both a small molecule drug and a monoclonal antibody (mAb) to the cyanine core to achieve targeted release of the drug through dual selectivity. The mAb directs the drug to particular cells via antigen recognition, and the cyanine photocage provides external spatiotemporal control. In one of their examples, panitumumab was used as a mAb for the epidermal growth factor receptor (EGFR), which is overexpressed in some cancers, and duocarmycin, a potent DNA-alkylating agent, as the anticancer drug.^[107] Both antibody labeling and cytotoxicity were confirmed in MDA-MB-468 breast cancer cells *in vitro*. The *in vivo* activity was evaluated in an MDA-MB-468-*luc* mouse xenograft model. The ADC was administered by tail vein injection, and 690 nm light was irradiated onto the tumour region with a commercial 800 mW/cm² PDT laser system. Fluorescence imaging at 800 nm revealed that the conjugate accumulated at the tumour, the liver and the bladder. A single 100 µg dose led to a significant decrease of tumour volume as compared to the vehicle and the non-illuminated groups (Figure 8B), as well as an increase in survival.

Figure 8. Biological applications of cyanine PPGs. (A) Light-dependent cytotoxicity of cyanine-caged cyclofen: dose-dependent viability of MCF-7 cells assessed with the MTT assay and confocal microscopy images of live MEF cells

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treated with **64** (1 μ M) and Hoechst 33342.^[102] Adapted with permission from ^[102]. Copyright (2014) American Chemical Society. (B) *In vivo* efficacy of a caged ADC in MDA-MB-468-luc tumor-bearing mice: mice dosed on day -4 and irradiated once on day 0 for 1.7 min showed decreased tumour volume and increased survival (not shown) compared to the non-irradiated and vehicle groups.^[107] Reprinted with permission from ^[107]. Copyright (2017) American Chemical Society. (C) Imaging of HeLa tumour-bearing mice after intratumour injection of Cy-CPT-Biotin under light irradiation; "Rainbow" denotes fluorescent signal at $\lambda_{\text{ex}} = 720$ nm, $\lambda_{\text{em}} = 820$ nm from **67**, and "Yellow-red" at $\lambda_{\text{ex}} = 510$ nm, $\lambda_{\text{em}} = 535$ nm from the photoproduct after uncaging.^[104] Reprinted with permission from ^[104]. Copyright (2018) Springer Nature.

Another example of *in vivo* application has been presented by Zhu, who prepared a cyanine-based theranostic prodrug.^[104] The cyanine core was conjugated to the topoisomerase inhibitor camptothecin (CPT) via carbamate as the cargo, and to biotin, a tumour-targeting agent. Upon light irradiation and subsequent photooxidation, cyclisation and release of CPT, the fluorescent signal shifted from $\lambda_{\text{em}} = 810$ nm to $\lambda_{\text{em}} = 535$ nm. This shift enabled monitoring the release of the active molecule through dual-channel fluorescence. In a subcutaneous HeLa xenograft model, their conjugate allowed site-specific real-time tracking of conjugate accumulation and drug release with NIR light (Figure 8C).

4.3. Summary

Despite the relatively low number of publications on cyanine PPGs, the known examples successfully photorelease the cargo molecules under red light and have already proven to be applicable to *in vivo* mouse models. Such quick progression into *in vivo* studies is somewhat striking, and has probably been expedited by the prior extensive use of cyanine dyes in biomedical applications, which circumvents some of the concerns about biocompatibility and toxicity.

Full direct comparison with other red absorbing PPGs cannot be made as the uncaging quantum yields of these derivatives are unknown. In addition, cyanine PPGs might be incompatible with the caging of singlet oxygen sensitive cargo groups or when this oxidative damage needs to be minimised. So far, the examples have been limited to alcohols and aniline cargos, but given the promising *in vivo* results we foresee an expansion of the functional group scope. Further progression would also benefit from short synthetic routes from cheap starting materials.

5. Other PPGs

Xanthene derivatives substituted with aromatic rings at the 9-position, such as fluorescein, are used as fluorophores that absorb visible light. Klán and Wirz showed that derivatives bearing a 9-leaving group, similar to other arylalkyl derivatives described herein, could be used as PPGs. Xanthenes such as **68** (Figure 9), which are isolated as 1:1 complexes with DDQ, release carboxylates and phosphates under green light.^[115] A 9-COOH-xanthene analogue has been used to release carbon monoxide upon irradiation at 500 nm.^[116]

1,4-Benzoquinones substituted with sulfides^[117] and dialkylamines^[118] can be reduced with visible light.^[119] Steinmetz has used this property to design benzoquinone-based PPGs for carboxylate and phenolate cargos, which are incorporated via a

5-hydroxymethyl group.^[120,121] Initial photocyclisation with visible light activates the system towards elimination of the cargo (Scheme 6A). Although this approach had some drawbacks, such as low efficiency in polar solvents and formation of an *o*-quinone methide intermediate, it introduced amino-1,4-benzoquinones (AQ) as visible light PPGs.

Figure 9. Reported examples of recent PPGs absorbing in the visible range.

A 10-fold increase in quantum yield of these compounds can be achieved through benzannulation of the cyclic amines to **70**.^[122]

Almutairi later showed that the water incompatibility of AQs can be addressed by formulating the caged molecules into water-dispersible nanoparticles.^[123] Irradiation with red light through mammalian tissue filters allowed photorelease of paclitaxel, with uncaging yields dependent on the tissue properties.

The group of Dougherty has incorporated a trimethyl lock (TML) into 1,4-benzoquinones.^[124,125] TML is a trimethyl-substituted *o*-hydroxydihydrocinnamic acid derivative that undergoes lactonisation under certain conditions to give a dihydrocoumarin (Scheme 6B). Release of unfavourable steric interactions from the pendant methyl groups upon lactonisation ensures fast rates, hence the name. The caging group is based on blocking the phenolic oxygen with a moiety that can be removed under an enzymatic, chemical or photolytic trigger, and attaching the cargo to the carboxyl group. Initial examples of photolytic release with TML were based on protecting the phenol with an *o*-nitrobenzyl group, cleavable under UV light. The first example cleavable under visible light was based on the aforementioned photochemical reduction of benzoquinones. Dougherty and coworkers prepared sulfide-1,4-benzoquinone derivatives **69** (Figure 9), which upon irradiation undergo reduction, liberating the phenol group for a thermal TML reaction (Scheme 6B).^[125,126] Even though the λ_{max} of the reported compounds is in the UV region, they have an additional broad absorption band in the visible range, which can be used to trigger reduction of the quinone core under a range of wavelengths. Such broad absorption bands lead to low ϵ values. However, the ϕ_{f} values are much higher than the first AQs, giving $\epsilon\phi_{\text{f}}$ in the same range as other PPGs. The authors show the applicability of their PPG for the photorelease of alcohols, phenols and primary amines, including the neurotransmitter GABA, but it could also be applicable to other functional groups that have been previously caged with TML.^[127,128] Later amine-substituted analogues by the

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same authors having a less sterically demanding cyclisation allowed the photorelease of alcohols at longer wavelengths, up to 600 nm.^[126]

Scheme 6. Uncaging mechanism of (A) amino-1,4-benzoquinones and (B) 1,4-benzoquinones trimethyl lock (TML).

Benzothiadiazoles **71** take advantage of the “*meta-ortho* effect”^[129] to cleave a bond at the benzyl position with release of a leaving group. They have been used to photorelease alcohols and carboxylic acids under blue light (430 nm) with uncaging quantum yields of 4–6%.^[130]

Uncaging of alcohols and carboxylic acids has also been achieved with push-pull stilbenes **72**.^[131] Upon irradiation, isomerisation to the *cis* isomer occurs followed by cyclisation to a dihydrophenanthrene, which aromatises releasing the cargo. The generated phenanthrene is fluorescent and can be used to monitor drug release. Biological applicability has been demonstrated in MCF-7 breast cancer cells treated with caged chlorambucil, an alkylating agent. Exposure to visible light led to a decrease of cell viability and an increase of fluorescence.

7-Hydroxyquinolines (HQ) have been used to cage carboxylates, phosphates and diols, releasable either by 1PE with UV light or 2PE.^[132] Narumi and coworkers have recently been able to increase the 1PE wavelength of this PPG into the blue region.^[133] The authors took advantage of previous reports on 7-hydroxyquinolines as fluorophores, which had shown that *N*-methylation reduces the pK_a of the 7-OH, promoting formation of zwitterions and increasing both photosensitivity and absorption wavelength.^[134] Their best derivative **73** was able to release amines, attached via a carbamate linker, with blue light and modest $\epsilon\phi_{\text{U}}$ values.

6. Nitroaryl

ortho-Nitrobenzyl and *ortho*-nitrophenethyl groups have been used as PPGs since the 1960s^[135] and have been extensively utilised in biological applications. An important limitation of this PPG is its excitation in the low UV range – the parent groups absorb at ≈ 260 nm^[136,137] – and efforts have also been made to increase their absorption wavelengths. Considerable bathochromic shifts have been achieved into the high UV region, and particularly the dimethoxy derivatives – absorbing at ≈ 350 nm^[138,139] – are commonly used, but there is a limited number of examples with λ_{max} of 1PE in the visible range. Some

have also been used for 2PE. These efforts have been recently reported in detail^[20] and are not discussed here. Nonetheless, it is of interest to indicate that some of these examples (Figure 10) employ structural modifications similar to the strategies already outlined for other PPGs, such as extension of the π -electron system.^[140–143]

Figure 10. Reported examples of longer wavelength nitroaryl PPGs.

7. Summary and outlook

For a translational clinical application of photocaged drugs, lack of tissue damage caused by light is critical, as well as sufficient light penetration to reach the tissue of interest. For this, the ability to photocage with visible light is necessary. Photocages have been used for several decades, but the most popular groups suffer from low excitation wavelengths in the UV region, limiting their applicability. In the last decade, massive progress has been made in the design and optimisation of PPGs that release the cargo molecule at wavelengths in the visible range. Even though the optimal optical window has been suggested to be 650–900 nm,^[9] arguably some of the exposed examples absorbing at lower values in the visible spectrum (400–650 nm) could still find clinical use. Firstly, at adequate intensities, these do not suffer from the same acute and chronic effects of shorter wavelength UV light. Secondly, despite their low tissue penetration they could still be applicable for skin and superficial tissues, as well as areas reachable by endoscopic techniques or implantable optic fibers. In fact, it is known that some tissues, such as human skin, eye, or bladder, are less colored than muscles or blood, and diseased tissues of this type may be suitable for blue- or green-light PPGs.^[144]

Throughout this review, we have outlined the chemical strategies that have permitted the discovery and optimisation of PPGs absorbing in the visible range. Two major approaches can be identified. One strategy has been to chemically modify known UV-absorbing photocages with functionalities that increase the absorption wavelength, such as extending conjugation of the chromophore. This has been sometimes limited by a concomitant decrease in uncaging efficacy or other photochemical properties. Another strategy is to transform known visible-absorbing fluorophores into PPGs, by attaching them to a light-removable

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leaving group. In some cases, such as in cyanines and 1,4-benzoquinones, this transformation has been based on taking advantage of the photochemical instability of the known fluorophores.

Three PPG scaffolds that have received much attention are BODIPY, coumarin and cyanine. Other less explored PPGs (Figure 9) are also emerging, and further studies into their biological use will provide insights into their biocompatibility and where they stand with respect to the more widely used scaffolds. One of the concerns of some of the presented PPG classes is that certain derivatives have been used for PDT. Cyanines,^[145,146] halogenated BODIPY molecules^[42] and some coumarin derivatives^[147] are efficient PDT photosensitisers. While in the case of photoreleasing anticancer drugs this might even be an opportunity to provide an additional level of potency, in most applications this potential inherent phototoxicity is undesirable. Further *in vivo* studies are probably necessary to understand which of the visible light PPGs presented can be activated at safe regimens that do not cause cytotoxic side effects, especially in chronic long term uses.

We could argue that it is still not fully understood what the optimal properties for *in vivo* application of photocages are. Publications reporting new PPGs absorbing in the visible range with lower uncaging efficacies of previous examples often justify that this decrease is well compensated by the bathochromic shift in absorption. It has even been argued that a high photorelease efficacy under visible light could also make manipulation in the laboratory (and in the clinic) challenging.^[22] In terms of the absorption spectra, broad bands enable photolysis at wavelengths above λ_{max} , but narrow bands are necessary for instance in dual systems. The optimal values for translatable use are open to discussion. Further examples where these PPGs are used in advanced biological applications will certainly shed light into the optimal properties that should be pursued. These will most likely depend upon each particular application, such as the targeted tissue, localisation of the specific target, short vs long-term treatments and whether monitoring through fluorescence is necessary.

Further progression will also require the development and clinical approval of appropriate medical devices that allow for controlled irradiation into the area of interest. This field is also continuously expanding and huge advancements have already been made.^[148,149] Optical fibers,^[150] endoscopic systems^[151] and implantable optoelectronic technologies with LED systems^[152,153] are being developed. This has been largely driven by optogenetics in neuroscience, but is also quickly expanding to other disease areas and phototherapeutic applications.^[154] Technologies that are already in clinical use for PDT can also be adapted for releasing therapeutic agents from PPGs with light.

Overall, the high spatiotemporal precision by which drugs can be delivered using photoprotecting groups is remarkable, and huge progress has been made in the last years towards improved biocompatible and clinical applicability. Even though many questions still remain to be answered and further development of optimal caging groups and medical devices remains to be done, we envisage that the field will continue to progress in the near

future. This will surely support the huge potential of photocages to solve some of the limitations of conventional drugs.

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Photoprotecting groups (PPGs) permit the activation of small molecules at a precise space and time using external light. Most of the first PPGs are cleaved with UV light, but in the last years massive progress has been made towards PPGs cleavable with visible light. This review describes some of these examples, with a focus on the chemical strategies and modifications that have enabled this shift towards higher wavelengths.

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