Visible Light Absorbing Photoremovable Protecting Groups

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ABSTRACT

Photoremovable protecting groups (or "photocages" or "phototriggers") are light-sensitive chemical moieties that provide control over the release of any number of reagents in a variety of environments. Photocages can provide orthogonal external control over when, where, and how much of a biological substrate is activated in cells using light irradiation. All commonly used commercially available photocages respond to UV-light. Researchers at Iowa State University have developed photocages that respond to light in the visible spectrum, in a so-called "biological-window"

Keywords: Photocage, biological sensor, fluorescent dye

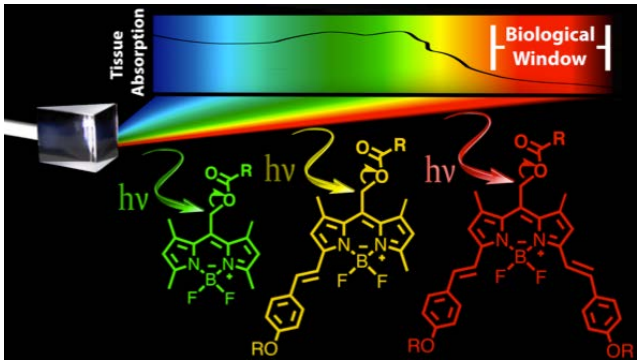
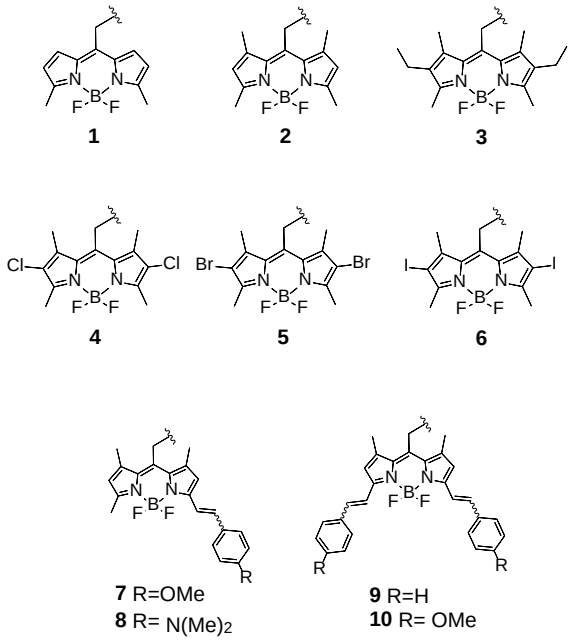


Figure 1: BODIPY derived photcages cleaved with different wavelength light

Specific control over biological studies can be difficult to obtain due to the complex spatial and temporal dimensions involved. Non-invasive techniques to trigger responses with spatial and temporal precision is therefore highly desirable. Light is an attractive trigger to achieve this control, as it gives the researcher the ability to conduct their study literally at a flick of a switch. Photocages are photo-sensitive moieties that control the release of covalently-linked substrates that are capable of taking advantage of the precision that light offers [1]. While photocages have important applications in areas such as organic synthesis [2], photolithography [3], and light-responsive organic materials [4], these structures are particularly prized for their ability to trigger in biological systems [5]. The most popular photocages in biological studies are based on the *o*-nitrobenzyl and its derivatives [6]. Alternatives to this photocage are phenacyl [7], acridinyl [8], benzoinyl [9], coumarinyl [10], and hydroxynapthyl [11] based structures. A major limitation of all these photocages is that they absorb light mostly in the ultraviolent range (UV-light). A photocage designed to use visible light provides several distinct advantages over the currently available UV-light absorbing photocages, including penetration of Pyrex glassware, activation with cheap lamps, greater depth of penetration in biological tissues, limited damage to biological tissue, increased researcher safety, and potential for use of multiple photocage-reagent combinations responsive to different light wavelengths in experimental design. Particularly for biological studies, there exists a window in which there is very little absorption from tissue and water, typically at wavelengths greater than 600 nm and less than 950 nm (red to near ir) (Figure 1) [12].

Several methods for exploiting visible light for photorelease have been studied using indirect schemes. The most common method is multiphoton absorption [13], wherein a UV-absorbing photocage is excited with two or more visible light photons. Other methods include photorelease initiated via photoinduced electron transfer [14], via metal-ligand photocleavage [15] or by using photosensitizers that generate reactive singlet oxygen that initiates a reaction cascade leading to substrate release [16]. However all of these methods rely on complex substrates or complicated experimental designs that are difficult to adapt to a laboratory setting.



Scheme 1: Substrates available for licensing based on the BODIPY moiety.

Visible light absorbing organic structures that rely on simple photorelease schemes and structures, offer the ability to be more easily applicable to biological studies and simpler experimental design. Based on previous computational studies, Iowa State University researchers identified a class of molecules that undergoes photoheterolysis upon excitation with visible light, boron-dipyrromethene dyes or BODIPY [17].

The BODIPY scaffold affords the advantage of simple syntheses, a compact structure with known biological compatibility, and remarkably high extinction coefficients. Several BODIPY derivatives were synthesized and are available for licensing (scheme 1). These compounds are designed to have a range of absorbing wavelengths that span ~150 nm. While the simplest BODIPY (**1**) is green absorbing, the BODIPY core was modified and the conjugation was extended to encompass redder wavelengths (**7, 8, 9, 10**) (figure 2).

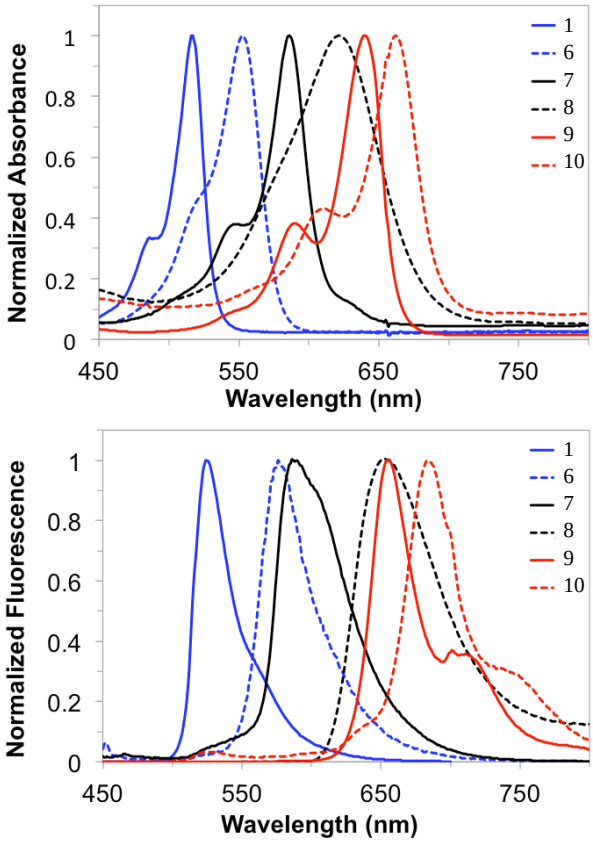


Figure 2: Normalized absorption and fluorescence of 1 and 6-10

To utilize the dyes, leaving groups are chemically attached to the dyes via simple coupling reactions. The alcohol derivative of the BODIPY compounds can be coupled to acids through DCC-coupling or any other of number of coupling reactions common for creating esters. The BODIPY alcohol is robust enough to survive most typical chemical environments used in ester coupling reactions.

Acetic acid was used as the test leaving group and quantum yields (ϕ) were determined by ferrioxalate actinometry and reported as the average value of three trials. With acetic acid as the leaving group, the dyes were found to be thermal stable, showing no change (monitored by NMR) when refluxed in the dark for 1 hour. The extinction coefficients (ε) at λ_{max} was determined with UV-vis spectroscopy. These values, as well as total quantum efficiency (εϕ) are summarized in table 1. The photocages feature high extinction coefficients typical for BODIPY dyes. The quantum yields are lower than *o*-nitrobenzyl photocaged structures or coumarinyl systems, but the high exctinction coefficient compensates so that the total quantum efficiency is competitive.

	λ_{max} (nm)	λ_{em} (nm)	ε (X10^4 M^{-1} cm^{-1})	Φ(X10^{-5})	εΦ (M^{-1} cm^{-1})
1	519	527	5.7	64	37
2	515	526	7.1	99	70
3	544	560	6.2	95	59
4	544	570	4.8	40.	19
5	545	575			
6	553	576	4.9	238	117
7	586	607	6.1	9.8	6.0
8	633	650	6.0	6.9	4.1
9	640	656	6.5	4.5	2.9
10	661	684	6.5	4.5	2.9

Table 1: Optical properties and quantum efficiencies of 1-10 with acetic acid leaving groups. Quantum yield (ϕ) determined by ferrioxalate actinometry in methanol with a 532 ND:YAG laser source and release followed using quantitative LC-UV (average of 3 runs)

Proof of concepts studies were performed in Drosphilia S2 cells (**1**) and HeLa cells (**10**) summarized in figures 3 which demonstrates the biological efficacy for these dyes. The leaving group used in these studies was a known photoquencher for BODIPY dyes, 2,4-dinitrobenzoic acid and was compared to acetic acid leaving group. The dyes loaded with the photoquencher started fluorescent silent, as expected, and upon irradiation with a mercury lamp, there is an increase in fluorescence as the quencher releases. Release of the quencher was verified by NMR. Studies were also performed with bovine GM07373 cells to similar results.

Cytotoxicity studies showed no decrease in cell viability (trypan blue exclusion assay) after 1 hour when compared to standard cells with no BODIPY dye. Cells had 90±3% viability for HeLa cells with no treatment of dye, 92±5% viability with treatment with **10**, 95±2% viability for S2 cells without treatment, 93±2% for S2 cells with **10**, 90±1% for GM07373 without treatment and 92±5% for GM07373 with **10**.

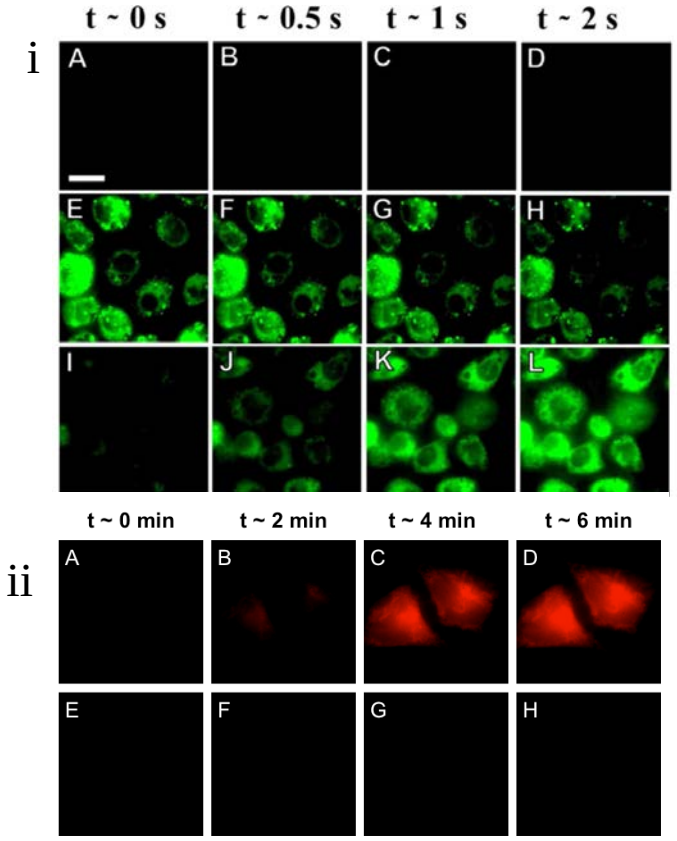


Figure 2: **(i)** Fluorescence images of S2 cells with no BODIPY compound (A-D), cells incubated with compound **1** and an acetate leaving group (E-H), and cells incubated with compound **1** with a photoquencher leaving group (I-L) as a function of irradiation time. Scale bar in panel A is 20 µm. **(ii)**Fluorescence images of HeLa cells incubated with **10** irradiated with 635 nm light (A-D) and cells incubated with **10** with no irradiation.

In conclusion Iowa State University researchers synthesized a series of photocages derived from BODIPY that are triggered with visible light. These photocages have been demonstrated to release carboxylic acids efficiently and are an attractive replacement for the current commercially used *o*-nitrobenzyl photocage systems in that they are easy to synthesize, utilize biocompatible chromophores and have superior optical properties. Of particular advantage is the tunable visible light absorption and activation. The wide range of wavelengths of absorbance allows photorelease to be achieved using essentially any color of visible light. These dyes have exciting applications in targeted and controlled release in a number of applications, particularly in pharmaceuticals or other biomolecular application.

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