Multiwavelength Control of Mixtures Using Visible Light Absorbing Photocages

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Abstract
Selective deprotection of functional groups using different wavelengths of light is attractive for materials synthesis as well as for achieving independent photocontrol over substrates in biological systems. However, wavelength-selective activation is difficult to achieve with common UV-absorbing photoremovable protecting groups (PRPGs) because it is difficult to separate the chromophore absorption profiles. Moreover, deep UV irradiation of photocages can result in cellular phototoxicity. Here, we investigated the ability of recently-developed visible light absorbing BODIPY-derived PRPGs and a coumarin-derived PRPG to undergo wavelength selective activation in order to identify well-behaved pairs of PRPGs that allow independent optical control over a mixture of photocaged substrates using more biologically benign long-wavelength light. The three pairs of PRPGs tested have complete selectivities for cleaving the longerwavelength absorbing photocage first, and fair to excellent selectivities for releasing the lower-wavelength absorbing PRPG first when mixtures were irradiated in solution. When the PRPGs are attached to the same substrate, irradiating the shorter-wavelength absorbing PRPG results in energy transfer, but the PRPGs can be cleaved in a sequential manner starting by deprotecting the longest wavelength absorbing photocage first and then removing the lower-wavelength absorbing PRPG. A mixture of the three photocages could be sequentially reacted using common red, green, and far-UV (365 nm) LED irradiation.

Keywords
photochemistry, selective photorelease, photocages, BODIPY, Coumarin

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Supporting Information Placeholder

ABSTRACT: Selective deprotection of functional groups using different wavelengths of light is attractive for materials synthesis as well as for achieving independent photocontrol over substrates in biological systems. However, wavelength-selective activation is difficult to achieve with common UV-absorbing photoremovable protecting groups (PRPGs) because it is difficult to separate the chromophore absorption profiles. Moreover, deep UV irradiation of photocages can result in cellular phototoxicity. Here, we investigated the ability of recently-developed visible light absorbing BODIPY-derived PRPGs and a coumarin-derived PRPG to undergo wavelength selective activation in order to identify well-behaved pairs of PRPGs that allow independent optical control over a mixture of photocaged substrates using more biologically benign long-wavelength light. The three pairs of PRPGs tested have complete selectivities for cleaving the longer-wavelength absorbing photocage first, and fair to excellent selectivities for releasing the lower-wavelength absorbing PRPG first when mixtures were irradiated in solution. When the PRPGs are attached to the same substrate, irradiating the shorter-wavelength absorbing PRPG results in energy transfer, but the PRPGs can be cleaved in a sequential manner starting by deprotecting the longest wavelength absorbing photocage first and then removing the lower-wavelength absorbing PRPG. A mixture of the three photocages could be sequentially reacted using common red, green, and far-UV (365 nm) LED irradiation.

Biological systems consist of a multitude of systems acting in conjunction with each other. Orthogonal control of separate biological processes within the cellular environment is highly attractive for investigating such complex biological phenomena. Light is an ideal stimulus for achieving intracellular controlled activation of biological targets. It allows spatial and temporal control of activation, and it is a noninvasive external control so long as high wavelengths of light are used that are not absorbed by cellular biomolecules. The use of photocaged substrates is a popular way to achieve optical control over bioactive substrates. Applications for wavelength-controlled release include control of separate processes in biological systems (such as different inhibitors of signaling pathways or neurotransmitters), materials and surface patterning, drug delivery, peptide synthesis, and selective release of different protecting groups on a substrate with multiple reactive sites.

In reality, however, chromophores have overlapping absorbance bands, which makes selective deprotection difficult. Selectivity may be obtained from two chromophores with overlapping absorbances as long as one of them has an absorbance band that extends to a longer wavelength than the other (Figure 1a). In these cases, multiple levels of sequential release is possible if the irradiation source is carefully chosen.

Assuming that the chromophores do not interact during the lifetime of the excited state (e.g. no electron transfer, energy transfer, or exciplex formation occurs), the selectivity is governed by the relative quantum yields of photorelease if both chromophores absorb equal amounts of light. Under conditions of photon scarcity, which is more often the case in dilute biological experiments under moderate photon fluxes, the selectivity is related instead to the relative quantum efficiencies (Φ) of the photocages at the wavelength of irradiation. The quantum efficiency is defined as the product of the quantum yield of photorelease (Φ) and the extinction coefficient at the excitation wavelength, and takes into account both how strongly the photocage chromophore absorbs light and the percentage of release events that occur once light is absorbed.

It follows, then, that one way to allow for better independent selectivity when the absorbances of the PRPGs overlap is if the quantum efficiency of the shorter wavelength absorbing photocage is much higher than the photocage which has a longer wavelength absorbance (Figure 1b). In this case there is still complete selectivity for cleaving the longer-wavelength-absorbing PRPG as well as improved selectivity for cleaving the shorter wavelength-absorbing PRPG, assuming the chromophores do not interact during the lifetime of the excited state. The extent of selectivity with the shorter wavelength PRPG can be tuned based on the quantum efficiencies of the two.
PRPGs. In addition, sequential release may be obtained with a single wavelength depending on the rates of release of the chromophores.\textsuperscript{20, 21} In a classic example, Bochet and coworkers exploited the kinetic isotope effect on \(o\)-nitrobenzyl derivatives to successfully tune their reactivity and were able to achieve independent control of two \(o\)-nitrobenzyl PRPGs on a linker with two functional sites (Figure 1G).\textsuperscript{15} These cases, however, also require judicious selection of the irradiation wavelengths as well as the PRPGs. The photocage that absorbs longer wavelength light often cannot be irradiated at its \(\lambda_{\text{max}}\) and therefore requires long periods of irradiation (e.g. > 12 h) for full release to occur.\textsuperscript{13-16} True orthogonality between the two photocages could potentially occur if there is negligible overlap of the absorbances at the two wavelengths of irradiation (Figure 1E).

Ellis-Davies and coworkers successfully identified a coumarin derivative with a decreased absorbance in the UV region that gives excellent selectivity for photodeprotection with a UV absorbing photocage (Figure 1D), allowing independent control of cAMP and GABA. Another strategy to achieve selectivity is to exploit differences in the two-photon absorbing cross-sections of the photocages. For example, Heckel and coworkers exploited differential selectivity for a two-photon active PRPG that is surprisingly inactive to one-photon reactivity, which allows selective release by a combination of differential selectivity for photorelease of one- and two-photon absorbing cages.\textsuperscript{22} However, selective photorelease for a mixture of photocaged substrates using single photons of visible light would be highly desirable for many biological experiments.

**Figure 1.** A) Two separate chromophores with overlapping absorbances that can be activated sequentially, B) Chromophores that have selectivity based on differences in quantum efficiencies, C) Chromophores with separated absorbances that can be released orthogonally, D) A study done by Ellis-Davies et. al. of two PRPGs that were shown to give orthogonal release of GABA and cAMP,\textsuperscript{1} E) Schematic of a substrate with two reactive functional sites that can be caged and orthogonally released using different chromophores, F) Schematic of a substrate with two functional sites that can be released sequentially, G) An example of orthogonal uncaging of a substrate with two functional sites done by Bochet et. al.\textsuperscript{15}
Figure 2. Photocages investigated in this study based on coumarin, a green absorbing BODIPY and a red-absorbing BODIPY (top). These three chromophores have well separated absorbanes that match well with available red, green and UV LEDs (solid colored insets).

Recently, our group as well as Weinstain and coworkers reported that meso-substituted BODIPY derivatives could act as visible light PRPGs. More recently, we have shown that structural variants of these PRPGs can release substrates with red to near-infrared (NIR) light. We envisioned that the narrow absorption bands of BODIPY chromophores at various wavelengths may have the potential to achieve near orthogonality, or multi-layered sequential release with very high selectivities using more biologically benign visible light. Here, we show that indeed these PRPGs allow for long-wavelength photocontrol over a mixture of photocaged substrates. For our investigation we compared three PRPGs—one, using coumarin PRPG 1, which has a \( \lambda_{\text{max}} \) at \(~380 \text{ nm} \), and an absorption tail that extends above 400 nm into the blue range of the visible (for notational clarity, we color this one blue); a BODIPY-derived PRPG 2, which has a strong absorbance \(~520 \text{ nm} \) (colored green), and a redshifted BODIPY PRPG 3, which has a strong absorbance in the red to near IR region \(~690 \text{ nm} \) (colored red). These three PRPGs feature separated \( \lambda_{\text{max}} \) values and have \( \lambda_{\text{max}} \) values that fall near the emission of common LED wavelengths (See Figure 2). The BODIPY PRPGs 2 and 3 also have the advantage that their quantum efficiencies can be adjusted based on the substituents attached to the boron (BMe\(_2\) is much more efficient than BF\(_2\)), providing another handle with which to achieve selectivity. The plots of the absorbance and quantum efficiencies for each of the three sets of pairs are shown in Figure 3. As can be seen, all of them have reasonably separated absorbance bands. More importantly, plots of the quantum efficiencies for each pair suggests the possibility for very high selectivities (Figure 3, Figure S3).

Figure 3. Structures of the photocages dissolved in a 1:1 ratio in 50:50 DMSO:MeOH for irradiation (top), Normalized absorbance, quantum efficiencies of photocages based on similar structures (middle), and reactivity over time of the mixtures irradiated with red, green, or UV LEDs. (A) Irradiation of a 0.02 mM mixture of 1-Succ and 2-Succ, (B) irradiation of 2-Me-OAc and 3-OAc with green and red LEDs.
Indeed, when a mixture of 2-Succ and 1-Succ were irradiated with green light, 2-Succ reacted with no evidence of reaction of 1-Succ. When the mixture was irradiated with an LED centered around 365 nm, 1-Succ reacted quickly with only a small amount of 2-Succ reacted. Selectivity is nearly perfect at short irradiation times, but diminishes as irradiation times increase because the ratio of the absorbances of the unreacted photocages changes in favor of the longer-wavelength absorbing photocage as more of the shorter-wavelength absorbing photocage is consumed. This pair has excellent selectivity with both long and short wavelengths (Figure 3a).

For 2-Me-Succ with 3-Me-Succ (Figure 3b), the methylation of the boron on the BODIPY chromophores has been shown to increase quantum yields of release.\textsuperscript{25,26} We envisioned that selectivity could be obtained based on the much higher quantum efficiency of 2-Me-Succ compared to that of 3-Me-Succ. This mixture had a good selectivity from red to green irradiation; however, 2:1 selectivity for 2-Me-Succ over 3-Me-Succ was achieved at full completion of 2-Me-Succ when irradiated with an LED centered around 520 nm. We then tested a mixture of 2-Me-OAc with 3-OAc (Figure 3c). Since the fluorinated derivative has an even lower quantum efficiency compared to the methylated derivative, the mixture should give better selectivity. Indeed, this pair does give better selectivity (~4:1 selectivity after completion of 2-Me-OAc). However, in both of these cases, the selectivity is lower than that predicted based on the quantum efficiencies at the wavelengths of irradiation, suggesting that there may be interaction of the chromophores during the lifetime of the excited state (e.g., energy transfer between the chromophores of 2 and 3).

We then chose the two pairs that had the best selectivity in order to demonstrate alternating activation upon irradiation of different wavelengths of light. Figure 4a shows the relative reactivity of the 1-Succ with 2-Succ by alternating between irradiating with the 360 nm LED and the 520 nm LED. Similarly, Figure 4b shows the reactivity of a mixture of 2-Me-OAc with 3-OAc (Figure 4a-b) by alternating irradiation with the 520 nm LED and the 620 nm LED. In both cases, selective reactivity is observed, and demonstrates that the two photocages can be controlled independently.

While mixtures of these photocages in solution do not demonstrate complete orthogonality, they all show perfect selectivity when irradiating the longer wavelength absorbing photocage first. In order to demonstrate their viability to be sequentially cleaved, compounds 1-Succ, 2-Me-Succ, and 3-Me-Succ were irradiated together in a cuvette with red, green, and UV LEDs to show that they show sequential selectivity (Figure 4c). As expected, 3-Me-Succ selectively reacted with red light, green light selectively cleaved 2-Me-Succ, and UV light cleaved the remaining 1-Succ.

After observing selectivity with irradiation of a mixture in solution, we asked whether we could protect two different functional groups on the same substrate to achieve wavelength-selective release (Figure 1e). Orthogonal protecting groups have a wide variety of applications for synthesis on single molecules\textsuperscript{27} including small molecule, carbohydrate,\textsuperscript{28-30} and peptide synthesis.\textsuperscript{31,32} Photoremovable protecting groups are
formed. Indeed, succinic acid served because it was able to react much faster than it was time, for efficient energy transfer of the reactivity of the coumarin derivative in 4 to be diminished (plots of the absorption of 2-Succ and the emission of 1-Succ show good overlap, indicating the possibility for efficient energy transfer (Figure S2). At the same time, 1-Succ may have been formed, but was not observed because it was able to react much faster than it was formed. Indeed, succinic acid increased steadily over time with UV irradiation (Figure S1). When irradiated with green light, however, there was good selectivity. The green light-absorbing BODIPY chromophore was able to react and release 1-Succ nearly quantitatively. Furthermore, although 4 is not able to be selectively cleaved first with 365 nm light, it can be activated sequentially, first with the activation of BODIPY photocage with green light, and then with the activation of coumarin with UV light to release succinic acid.

We also synthesized 5, which has 2-Me green-absorbing BODIPY photocage linked with the read-absorbing 3-Me photocage (Figure 6). We chose 3-Me over the fluorinated 3 because it has a much higher quantum yield, leading to shorter irradiation times. However, when irradiating 5 with green light no selectivity was observed. In fact, we observed growth of 2-Me-Succ without a measurable amount of 3-Me-Succ, suggesting that energy transfer followed by cleavage of the longer-wavelength protecting group was occurring. However, selectivity was obtained when first using red light to cleave 3-Me. As was the case for 4, we were able to sequentially cleave 5 by first irradiating with red light followed by green light to release succinic acid. In both cases of 4 and 5 there was selectivity when the chromophores were separated, but not when linked together, presumably due to the higher efficiency of intramolecular energy transfer that leads to loss of selectivity.

In conclusion, we have demonstrated that BODIPY PRPGs along with a coumarin photocage can be used for selective release with longer wavelength LEDs. While particularly attractive in solid phase synthesis as they can give excellent spatial and temporal control in order to create microarrays. Some examples have been published with sequential deprotection of substrates including in liquid and solid phase peptide synthesis. Bochet and coworkers identified pairs that undergo orthogonal deprotection on a linked compound, including an example that takes advantage of isotope rate changes to achieve selectivity with two different o-nitrobenzyl derivatives (Figure 1g). We envisioned that the systems in this study may be candidates for selective release of two functional groups on a single substrate but using longer-wavelength light.

We chose to investigate the pairs of 1 and 2 (compound 4) as well as 2-Me and 3-Me (compound 5), using succinic acid as the linking substrate, as both pairs had fair to excellent selectivity and reasonable rates of photorelease. When irradiating 4 with green light, the BODIPY photocage was selectively cleaved to release 1-Succ (Figure 5). Interestingly, when irradiating 4 with UV light, much slower photoreactivity was observed. 2-Succ was observed and 1-Succ was never observed; however, the percent of conversion was less than 10% over a much longer time period than 1-Succ had reacted in a mixture. Likely, energy transfer from coumarin to BODIPY caused the reactivity of the coumarin derivative in 4 to be diminished (plots of the absorption of 2-Succ and the emission of 1-Succ show good overlap, indicating the possibility for efficient energy transfer (Figure S2). At the same time, 1-Succ may have been formed, but was not observed because it was able to react much faster than it was formed. Indeed, succinic acid increased steadily over time with UV irradiation (Figure S1). When irradiated with green light, however, there was good selectivity. The green light-absorbing BODIPY chromophore was able to react and release 1-Succ nearly quantitatively. Furthermore, although 4 is not able to be selectively cleaved first with 365 nm light, it can be activated sequentially, first with the activation of BODIPY photocage with green light, and then with the activation of coumarin with UV light to release succinic acid.

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In conclusion, we have demonstrated that BODIPY PRPGs along with a coumarin photocage can be used for selective release with longer wavelength LEDs.
excellent selectivity was obtained in mixed solutions, the linked compounds did not exhibit selectivity for irradiating the lower-wavelength absorbing PRPG first due to energy transfer. However, the PRPGs could be removed sequentially starting with removal of the longer-wavelength absorbing PRPG. A mixture of red and green BODIPY PRPGs with a coumarin PRPG exhibited excellent sequential selectivity. Overall, well-behaved pairs of photocages absorbing longer-wavelength light were identified that provide independent photocontrol over a mixture of photocaged substrates.

ASSOCIATED CONTENT
Supporting Information. Synthetic procedures and compound characterization data. Details for monitoring substrate release.
The Supporting Information is available free of charge on the ACS Publications website.

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