JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

Conditionally activatable visible-light photocages

Márton Bojtár, Krisztina Németh, Farkas Domahidy, Gergely Knorr, András Verkman, Mihaly Kallay, and Peter Kele

J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.0c07508 • Publication Date (Web): 04 Aug 2020

Conditionally activatable visible-light photocages

Márton Bojtár,[a,*] Krisztina Németh,[a] Farkas Domahidy,[a] Gergely Knorr,[a,b] András Verkman,[a] Mihály Kállay[c] and Péter Kele[a,*]

[a] "Lendület" Chemical Biology Research Group, Institute of Organic Chemistry, Research Centre for Natural Sciences. Magyar tudósok krt. 2. H-1117, Budapest, Hungary. e-mail: bojtar.marton@ttk.hu; kele.peter@ttk.hu

[b] Faculty of Chemistry and Earth Sciences, Friedrich-Schiller-Universität Jena, Lessingstraße 8, D-07743 Jena, Germany

[c] Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, P.O. Box 91, H-1521 Budapest, Hungary

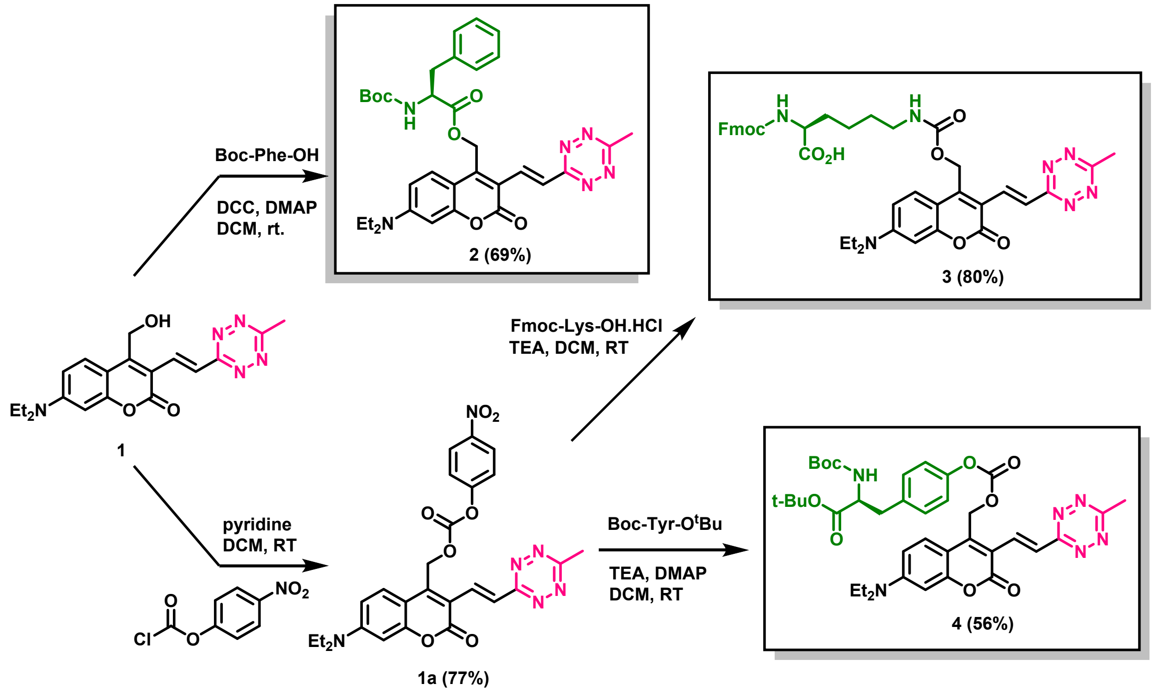
ABSTRACT: The proof-of-concept for conditionally activatable photocages is demonstrated on a new vinyltetrazine-derivatized coumarin. The tetrazine form is disabled in terms of light-induced cargo release, however, bioorthogonal transformation of the modulating tetrazine moiety results in fully restored photoresponsivity. Irradiation of such a "click-armed" photocage with blue light leads to fast and efficient release of a set of caged model species, conjugated via various linkages. Live-cell applicability of the concept was also demonstrated by the conditional release of a fluorogenic probe using mitochondrial pretargeting.

INTRODUCTION

The past decade has brought remarkable advances in light-related techniques allowing them to grow from simple means of observation to a precision tool in biology and medical sciences.[1–3] Its non-invasive nature, remote action together with easy control, fast and cost efficient operation make these techniques very appealing. These processes became possible by the development of photoresponsive materials that efficiently convert light to chemical energy. Among photoresponsive materials, photolabile protecting groups (PPGs) or photocages (PCs) play increasing role both in chemical biology studies and in therapeutic applications.[4–8] These photosensitive groups may be used to mask the biological function of small-molecular effectors,[8–15] proteins,[3,16] nucleotides[17,18] or drugs,[19–22] rendering them inactive. Light induced removal of these photolabile moieties by irradiation with a suitable wavelength, the activity of the caged substrate is restored. Manipulation of biological systems via photocaging has already revolutionized chemical biology. Nevertheless, the full potential of photocaging is yet to be exploited. To extend the use of these photoresponsive elements especially in the context of chemical biology, several limitations should be addressed, such as UV light activation,[23–26] poor water solubility,[19,27,28] and the lack of potential for targeting.[20,29–31] In addition to the impact on chemical biology, photocaging-based drug delivery systems, especially photoactivated chemoterapy (PACT) could also benefit from the development of such improved photocages possessing specific targeting elements.[23,32]

In recent years, a few notable examples were presented as 'clickable' photocages targeting various intracellular compartments.[31,33,34] However, in these instances click-chemistry (i.e., copper-catalyzed azide alkyne cycloaddition) was only used to facilitate the assembly of the organelle-targeting photocage, rather than to serve as the key element of the targeting process.[35-37] To the best of our knowledge, such clickable photocages where the clickable moiety is also the targeting element are not reported yet. Redefining the role of the clickable function, however, is rather an incremental step towards improved photocages. Exploiting the modulation power that certain biocompatible click handles (i.e., bioorthogonal functions) exert on chromophores, gives an extra twist to the story. Based on our extensive work on the development of bioorthogonal fluorogenic (turn on) probes,[38–40] we hypothesized that a similar concept can be applied to modulate the photoresponsivity of photocages. According to our foreseen concept termed "conditional photocaging", such switchable constructs become photocages solely by 'arming' by chemical transformation of the quencher moiety in a specific chemical reaction (i.e., a bioorthogonal reaction). Following this highly specific bioorthogonal ligation step to the target, the caged, biologically active molecule can be released upon light irradiation ('activation'). Non-specifically bound or free (disabled) constructs on the other hand, remain inactive even on exposure to light.

Lately, several accounts reported on the development of so-called click-and-release systems that rely on the spontaneous elimination of caged compounds upon a bioorthogonal reaction (i.e., inverse electron demand Diels-Alder, IEDDA, reaction of tetrazines and strained alkenes).[37,41] Though it seems similar at first sight, our approach is conceptually different. Our click-and-uncage constructs are based on the quenched activity of the photocage, which is reinstated after the reaction of the quencher moiety.



Scheme 1 Synthesis and Structure of the Model Photocages

Moreover, the further necessity of light irradiation enables an extra level of temporal and spatial control over the release of the caged active species. During the course of this work, Vázquez et al. reported on the bioorthogonal modulation of the $^1O_2$ sensitizing potential of BODIPY derivatives allowing conditional photodynamic applications (i.e., PDT).[42,43] The above hypothesized biorthogonal modulation of photocages would enable the oxygen independent, complementary concept of conditional photoactivated chemotherapy (PACT).

Herein, we demonstrate the proof of concept of conditional photoactivation by disclosing the development and study of a bioorthogonal moiety- (tetrazine-) modulated, visible-light sensitive click-and-uncage platform with various caged compounds. Besides *in vitro* experiments, live-cell applicability of the concept is also demonstrated through the pretargeting-dependent conditional photorelease of a fluorogenic probe.

RESULTS AND DISCUSSION

Prompted by the above considerations, we turned our attention to coumarin-based photocages[13,23,24,28,45] and tetrazine quenched fluorogenic probes.[38,40,46,47] We assumed that both the fluorescence and light-induced bond dissociation originate from the same excited state, thus, we hypothesized that similarly to fluorescence, the photoresponsivity of photocages can also be modulated by the bioorthogonal and quencher tetrazine moiety. We have recently observed[48] that vinylene-linked methyl-tetrazine completely quenches the fluorescence of the 7-diethylaminocoumarin chromophore, which is then fully restored upon transforming the tetrazine in a bioorthogonal reaction. It was also observed that the vinylene linkage shifts the absorption wavelength of the related coumarin with ca. 60 nm towards the red range resulting in visible light absorption. Therefore, we designed compound **1**, which combined elements of coumarinyl photocages and bioorthogonally activatable vinylene linked coumarinyl-tetrazine fluorogenic probes.

Cage **1** was accessed through a synthetic route starting from 3-bromo-7-diethylamino-4-hydroxymethylcoumarin using the previously established procedure for the synthesis of vinyltetrazinylated frames[49] and further conjugated with three different amino acids as model caged molecules (Scheme 1). Boc-phenylalanine, Fmoc-lysine and Boc-tyrosine-tBu-ester were readily converted to their corresponding caged derivatives resulting in ester (**2**), carbamate (**3**) and carbonate (**4**) linked species, respectively. In accordance with our previous observations, absorption spectra of all derivatives were red-shifted compared to plain coumarin-caged congeners, with absorption maxima around 475 nm (tetrazine form) and medium molar absorption coefficients (35-40 000 $M^{-1}cm^{-1}$) in acetonitrile-HEPES 2:1 (pH 7.0). As expected, fluorescence of the tetrazine derivatives was found to be practically zero. Reaction with a strained alkyne, BCN ((1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol) resulted in blue-shifted absorption maxima (around 445 nm) and, very importantly, a ca. 1000-fold increase in bright green emission intensity at around 535 nm.

Next, we have compared the photo-uncaging features of the tetrazines and their respective BCN-conjugated congeners. Based on the near quantitative fluorescence quenching, we anticipated that the photo-dissociation is also suppressed. Gratifyingly, when the samples were irradiated with blue LED (463 nm, for details, please refer to the SI), neither the release of the caged amino acids nor photo-destruction could be observed in case of the unarmed (tetrazine) constructs. Irradiation, 'activation' of the BCN-conjugated, 'clicked and armed' forms under the same conditions,

Table 1. Spectroscopic Properties and Photochemical Quantum Yields of the Compounds in MeCN-HEPES 2:1 (pH 7.0).

	λ_{max} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	Φ_{flu}[a]	Φ_{u}[b]	Φ_{deg}[c]	$\varepsilon_{463} \times \Phi_{u}$ ($M^{-1}cm^{-1}$)
1 / 1-BCN	463 / 436 470 / 442[d]	527 / 531 536 / 538[d]	45 800 / 44 700 44 100 / 42 800	61%	-	-	-
2 / 2-BCN	470 / 442	535 / 534	34 500 / 30 100	69%	0.44%[e]	0.74%	107
3 / 3-BCN	468 / 440	534 / 535	42 600 / 40 800	62%	0.10%	0.42%	31
4 / 4-BCN	470 / 442	- / 553	37 600 / 30 800	38%	3.50%	4.40%	875

[a]fluorescence quantum yield [b] uncaging (release) quantum yield [c] degradation quantum yield [d] measured in HEPES buffer. Quantum Yields were Determined Only for the Clicked Derivatives. [e] see also Table S1

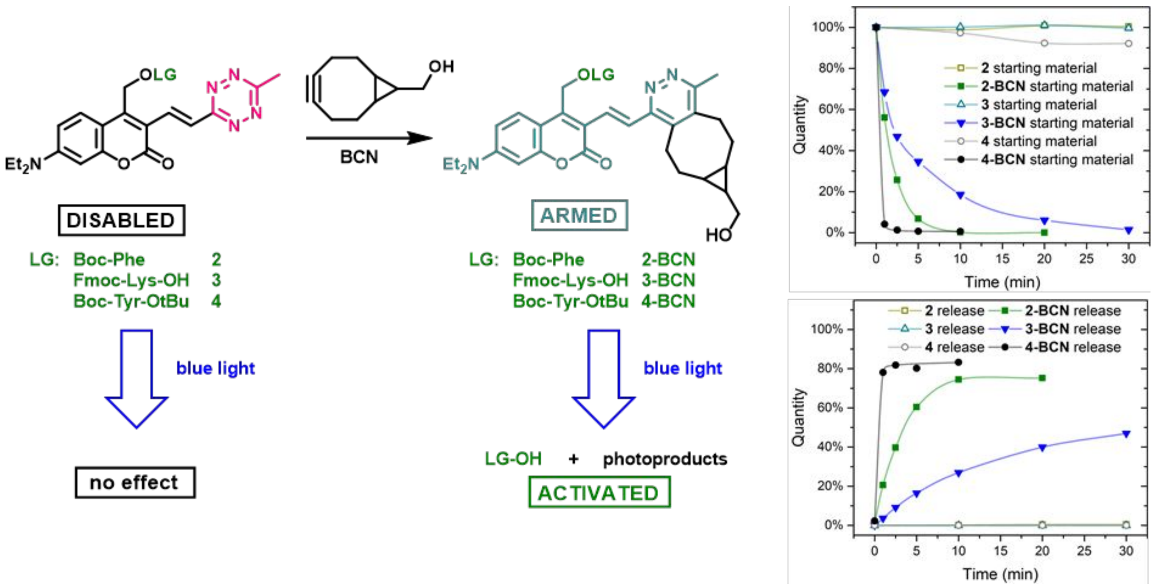


Figure 1. Scheme of the Conditional Uncaging and Degradation and Release Profiles of the Photocages Determined by HPLC

however, led to rapid release of all three amino acids, as seen by HPLC-MS (see Fig. 1 for the traces, Fig. S5-S7 for the HPLC chromatograms). Moreover, the tetrazine forms were found quite photostable, and no release of the amino acids could be detected after 30 minutes of irradiation. Comparison of the different linkages between the photocage and the amino acids suggests that carbonate **4-BCN** was the most photolabile with an uncaging quantum yield of 3.5%, followed by ester **2-BCN** and carbamate **3-BCN**. The uncaging quantum yields and efficiencies are summarized in Table 1. Solvent-dependency of the uncaging of **2-BCN** was also elaborated (Table S1). These results showed that higher water content results in increased photochemical quantum yields, which is advantageous for *in vivo* applications. It should be noted, however, that the release was not quantitative and slower photolysis resulted in lower efficiency, such as in the case of **3-BCN**. This can be rationalized by unwanted, rapid recombination of the photocage and the leaving group following homolytic bond cleavage as reported recently by Choi and coworkers.[50] This hypothesis was corroborated by the appearance of small peaks in the HPLC-MS chromatograms of the irradiated reaction mixtures of **3-BCN** with similar m/z values as the starting material. Comparison of the photochemical quantum yields of uncaging (release) with the degradation quantum yields (Table 1) suggest the occurrence of multiple photoreactions, which is more profound in the case of smaller efficiencies such as in the case of **3-BCN**. Increasing the distance between the cargo and the photocage by incorporating a self-immolative linker can be effective in enhancing the quantum yield by suppressing recombination (see below).[50]

We also wished to provide theoretical evidence for the experimental results. To this end, the low-lying excited states of a vinylene linked tetrazine-coumarin model system and its cyclooctyne conjugate were studied. We used the acetic acid ester of **1** for the calculations. The $-NEt_2$ group was replaced with $-NMe_2$ in order to decrease the number of conformers to be considered. The results showed that the vinylene linkage participates in the π-system of the chromophore, which explains the red-shifted absorbance. Furthermore, it was revealed that the S_1 state of the vinylene-linked tetrazine-coumarin corresponds to the dark $n \rightarrow \pi^*$ excitation of tetrazine (HOMO-1 → LUMO transition of the model compound, see Fig. 2), while the S_2 state is dominantly formed by promoting an electron from the highest π orbital of the vinylcoumarin to the lowest-lying π^* orbital of the tetrazine-vinylcoumarin system

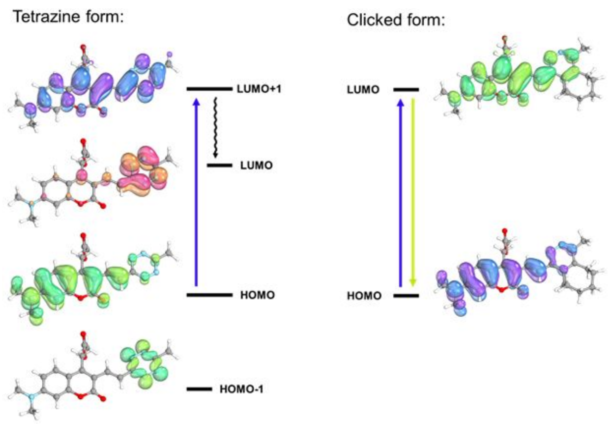


Figure 2. Low-Lying Excited States of the Model Tetrazine and its BCN-Clicked Product

(HOMO → LUMO+1 transition of the model). The probabilities of both the $S_0 \rightarrow S_2$ and the $S_0 \leftarrow S_2$ transitions are high, which suggests that the molecule gets into its S_2 state upon irradiation with blue light, followed by a rapid internal conversion to the dark S_1 and then to the ground state. The photoreaction presumably also take place on the S_2 surface, thus the presence of the tetrazine ring precludes both the reaction and the radiative decay of the excited state. After conjugation with cyclooctyne, the $n \rightarrow \pi^*$ type state does not exist anymore, and the $\pi \rightarrow \pi^*$ state of the vinylcoumarin (HOMO → LUMO transition of the cyclooctyne-conjugated model compound) becomes the lowest singlet excited state enabling both the fluorescence and the bond-dissociation.

As discussed above, not only do the constructs become photoresponsive after the click reaction, but their fluorescence is also restored (1000 × increase, see FigS1 in the SI). Such an inherently fluorogenic system is itself suitable to indicate the localization of the conjugated constructs, however, it does not provide any evidence of the uncaging process. In order to investigate the applicability of our concept in living systems, we wished to visualize both the pretargeting and uncaging processes through the liberation of a fluorogenic substrate that does not interfere with the activation/excitation of the coumarin cage. The use of rhodols as quenched fluorogenic markers is quite rare despite the fact that they are bright, easily accessible, and very importantly, require only one acyl/carbamoyl functionalization of the phenolic OH to render it fully quenched.[51,52] Taking spatial separation of the coumarin and the rhodol moieties into consideration in order to suppress recombination, we have designed compound **5** (Fig. 3). The well-established dimethylethylenediamine-carbamoyl self-immolative linker provides enough spatial separation and fast release kinetics (SI section 4).[53] Moreover, the carbamoyl-derived rhodol is practically non-emissive. LED irradiation of construct **5** and its 'click-armed' **5-BCN** congener was monitored by fluorescence spectroscopy and HPLC-MS. Both experiments revealed that unarmed construct **5** is not photoresponsive, while its click-armed BCN conjugate allows liberation of the rhodol upon LED activation.

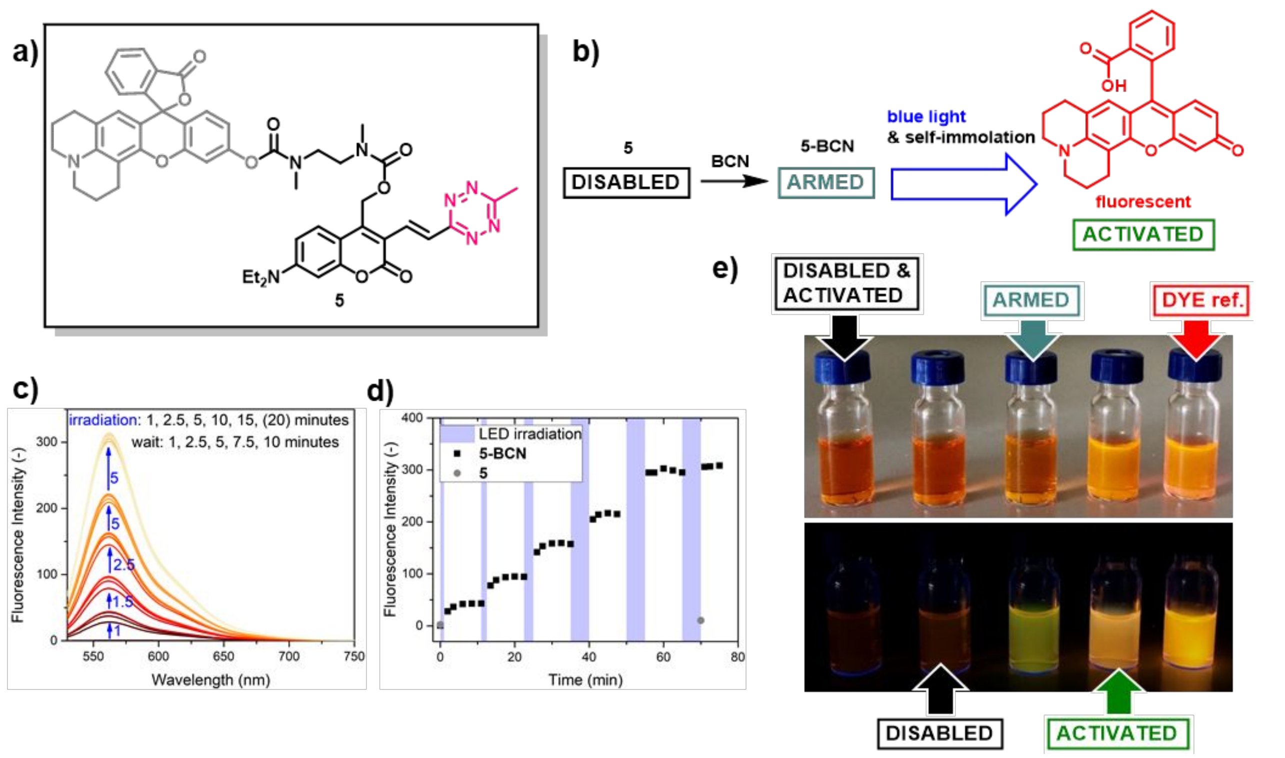


Figure 3. (a) Structure of **5** (b) Scheme for the Conditional Uncaging of **5** (c) Emission Spectra of the Uncaging of **5-BCN** upon Various Irradiation and Wait Time (1 μM in PBS, λ_{ex} = 515 nm); the Arrows Indicate Subsequent Irradiation of the Sample (d) Fluorescence Intensity of **5-BCN** at 566 nm, the Blue Lines Represent the Irradiation Time, and (e) Photographs of the Samples under Ambient and UV Light.

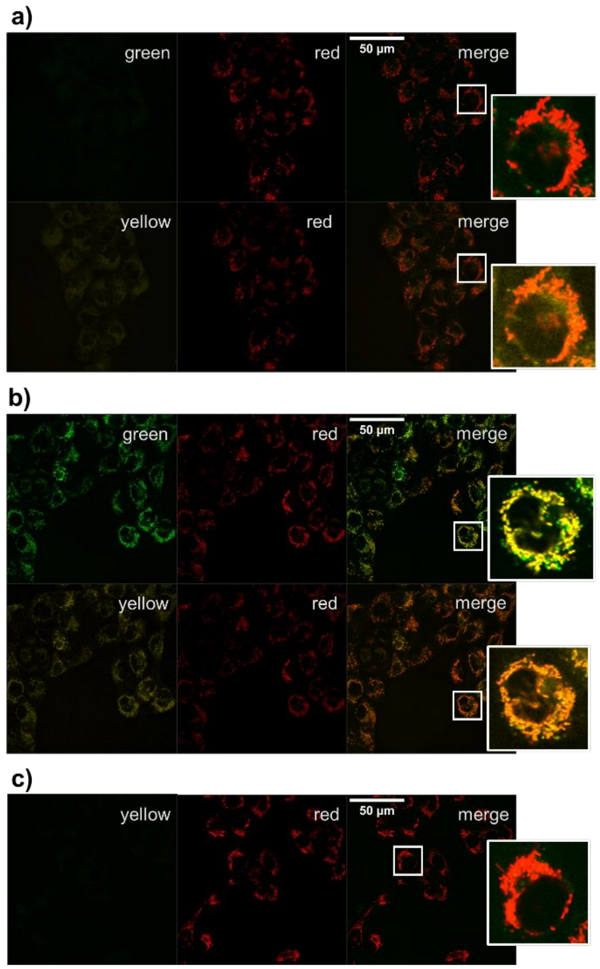


Figure 4. Confocal Images of the Colocalization of a) Cells Treated Only with Tetrazine **5** for 1h (200 nM); b) Cells Pretargeted with **TPP-BCN** (10 µM) for 1h, then with **5** (200 nM) c) Cells Treated with **TPP-5** (200 nM). The Colors Refer to the Corresponding Emission Channels (Green: Coumarin with 488 nm Excitation, Red: MitoTracker Deep Red (10 nM) with 638 or 552 nm Excitation, Yellow: Rhodol with 552 nm Excitation). The Brightness of the Insets is Enhanced for Better Visibility

Gratifyingly, uncaging of the rhodol resulted in an overall 1000 × increase of fluorescence intensity at the rhodol channel (λ_{exc} = 515 nm) after 15 minutes of irradiation. Fluorescence spectroscopy monitoring of the uncaging process revealed further information regarding the kinetics of the self-immolative destruction of the linker, i.e., following photolysis of the linkage between the coumarin and the linker. The self-immolation process requires a few more extra minutes to go to completion (Fig. 3c and d, Fig. S3 for further details on the kinetics).

Based on the excellent ability of **5** for monitoring the uncaging process, we selected mitochondria as an intracellular target due to its well established targetability with triphenylphosphonium (TPP) moiety.[54] In order to achieve specific organelle localization, we synthesized **TPP-BCN** (Scheme S3) for delivering a bioorthogonal platform into the mitochondria. Conditional uncaging was investigated using confocal fluorescence microscopy imaging of A-431 (skin cancer) cells either with or without pretreatment with **TPP-BCN**. We also investigated the effects of extracellularly pre-assembled **TPP-5**. In each case, the cells were treated with the photocaged-constructs for 1 hour (200 nM) then imaged directly without removal of unreacted tetrazines (no-wash condition). As can be seen in Figure 4, only cells pretargeted with **TPP-BCN** show clear colocalization with MitoTracker Deep Red (present in all experiments), confirming successful biorthogonal-targeting of the photocage inside the mitochondria. It can also be seen that the green emission of the coumarin upon excitation with the blue laser (488 nm) is only visible in the case of pretargeting demonstrating the fluorogenicity of the coumarin photocage upon bioorthogonal conjugation. In contrast, pre-assembled derivative **TPP-5** was not taken up by the cells indicating the often overlooked importance of the 2-step assembly of active species inside cells. Possibly due to its large size and increased molecular weight, the pre-clicked triphenylphosphonium-containing conjugate is unable to cross the cell membrane.

Live-cell photo uncaging of the fluorogenic rhodol was investigated using the built-in blue metal halide lamp of the microscope (λ_{max}~488 nm). Each field-of-view was irradiated for 5s, then the images were taken at least 1 minute after irradiation. To clearly see the highly localized effect of uncaging, we obtained 3 x 3 tile scans before and after irradiation of the central area (Fig. 5). The cells treated only with tetrazine **5** showed a small fluorescence enhancement in the yellow (rhodol) channel that is dispersed evenly throughout the cells. Contrary to this, cells pretargeted with **TPP-BCN** displayed bright fluorescence after irradiation that is mostly located inside the mitochondria. Similar results were obtained by visualizing the uncaging process in real time, using the built-in laser (488 nm with continuous imaging at both the red and the yellow channels, see the Supporting Videos and Figure S22). Importantly, the confined irradiation area combined with the subcellular pre-targeting can serve as dual control for highly localized manipulation as demonstrated by our fluorogenic click and uncage platform.

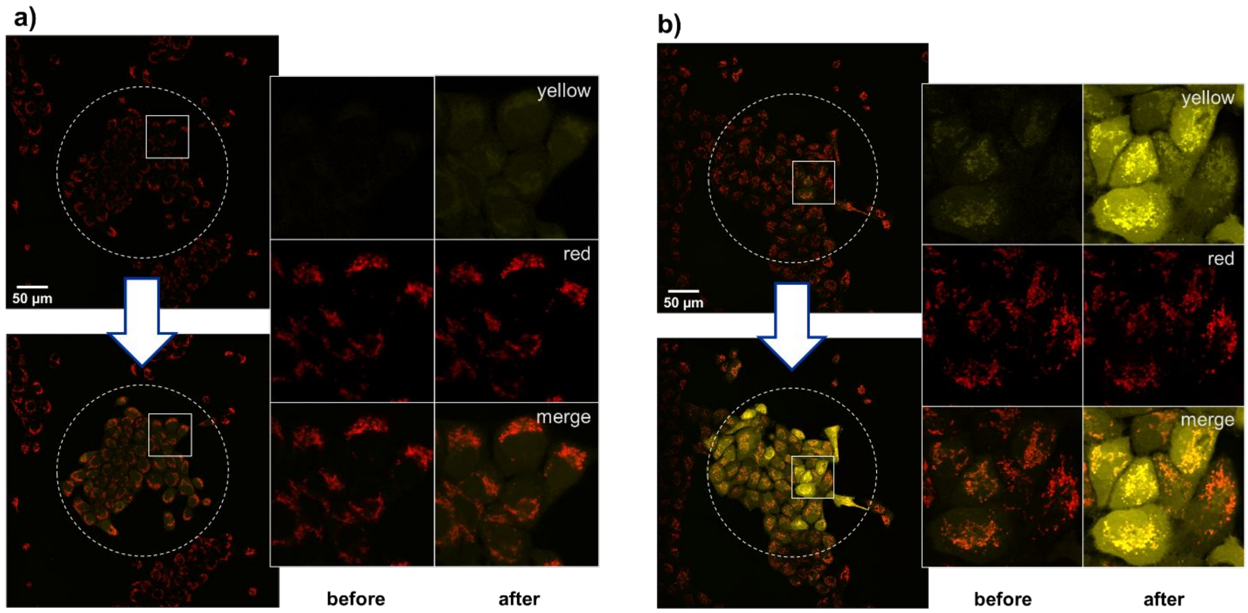


Figure 5. Tile Scan Experiments Before (Upper Image) and After (Lower Image) Irradiation of the Central Area (Marked with the Dotted Circle) with the Built-in Blue Lamp (488 nm, 5 s) of the Microscope. a) Cells Treated Only with Tetrazine **5** for 1h (200 nM); b) Cells Pretargeted with **TPP-BCN** (10 µM) for 1h, then with **5** (200 nM). The Colors Refer to the Corresponding Emission Channels (Yellow: Rhodol, Red: MitoTracker Deep Red). The White Squares Indicate the Magnified Area of the Images. Further Images are Shown in SI Section 7

CONCLUSION

In conclusion, we have demonstrated the proof of concept study of a bioorthogonal click reaction activatable photocage system. Experimental evidence and theoretical calculations suggested that the presence of the bioorthogonal tetrazine motif efficiently quenches the excited state of the coumarin necessary for photolysis resulting in disabled photoresponsivity (both in terms of photocaging and fluorescence). Transformation of the tetrazine moiety in a bioorthogonal click-reaction fully restores its sensitivity for light. Since bioorthogonal reactions enable highly specific targeting of cells or cellular structures, such conditionally activatable photocages provide an extra level of spatial and temporal control. This was demonstrated in live cells using a fluorogenic, conditionally activatable construct that solely became light sensitive when the cells were pretargeted with a mitochondria directed, complementary bioorthogonal function. These results confirm the applicability of our concept in biological systems and also clearly demonstrate the advantage of pretargeting and bioorthogonal chemistry. The applicability of this system in photoactivated chemotherapy involving the conditional release of drugs is currently under investigation in our laboratory and results will be reported in due course.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website. Experimental details, synthetic procedures, spectroscopic characterization, physical data determination, details on the imaging experiments, further images and viability assessment.

"This material is available free of charge via the Internet at http://pubs.acs.org."

AUTHOR INFORMATION

Corresponding Author

* Márton Bojtár, e-mail: bojtar.marton@ttk.hu; Péter Kele, e-mail: kele.peter@ttk.hu

Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

Present work was supported by the "Lendület" Program of the Hungarian Academy of Sciences (LP2013-55/2013) and the National Research, Development and Innovation Office (NKFIH-K-123917). MK is grateful for the financial support from the National Research, Development, and Innovation Office (NKFIH, Grant No. KKP126451). This work was also supported by the BME-Biotechnology FIKP grant of EMMI (BME FIKP-BIO).

REFERENCES

(1) Paoletti, P.; Ellis-Davies, G. C. R.; Mourot, A. Optical Control of Neuronal Ion Channels and Receptors. *Nat. Rev. Neurosci.* **2019**, *20* (9), 514–532. https://doi.org/10.1038/s41583-019-0197-2.

(2) Hüll, K.; Morstein, J.; Trauner, D. In Vivo Photopharmacology. *Chem. Rev.* **2018**, *118* (21), 10710–10747. https://doi.org/10.1021/acs.chemrev.8b00037.

(3) Gautier, A.; Gauron, C.; Volovitch, M.; Bensimon, D.; Jullien, L.; Vriz, S. How to Control Proteins with Light in Living

Systems. *Nat. Chem. Biol.* **2014**, *10* (7), 533–541. https://doi.org/10.1038/nchembio.1534.
(4) Ellis-Davies, G. C. R. Caged Compounds: Photorelease Technology for Control of Cellular Chemistry and Physiology. *Nat. Methods 2007*, *4* (8), 619–628. https://doi.org/10.1038/nmeth1072.
(5) Yu, H.; Li, J.; Wu, D.; Qiu, Z.; Zhang, Y. Chemistry and Biological Applications of Photo-Labile Organic Molecules. *Chem. Soc. Rev.* **2010**, *39* (2), 464–473. https://doi.org/10.1039/b901255a.
(6) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable Protecting Groups in Chemistry and Biology: Reaction Mechanisms and Efficacy. *Chem. Rev.* **2013**, *113* (1), 119–191. https://doi.org/10.1021/cr300177k.
(7) Slanina, T.; Shrestha, P.; Palao, E.; Kand, D.; Peterson, J. A.; Dutton, A. S.; Rubinstein, N.; Weinstain, R.; Winter, A. H.; Klán P. In Search of the Perfect Photocage: Structure–Reactivity Relationships in meso-Methyl BODIPY Photoremovable Protecting Groups. *J. Am. Chem. Soc.* **2017** *139* (42), 15168-15175. https://doi.org/10.1021/jacs.7b08532.
(8) Bort, G.; Gallavardin, T.; Ogden, D.; Dalko, P. I. From One-Photon to Two-Photon Probes: “Caged” Compounds, Actuators, and Photoswitches. *Angew. Chem. Int. Ed.* **2013**, *52* (17), 4526–4537. https://doi.org/10.1002/anie.201204203.
(9) Becker, Y.; Unger, E.; Fichte, M. A. H.; Gacek, D. A.; Dreuw, A.; Wachtveitl, J.; Walla, P. J.; Heckel, A. A red-shifted two-photon-only caging group for three-dimensional photorelease. *Chem. Sci.*, **2018**, *9*, 2797–2802. https://doi.org/10.1039/C7SC05182D.
(10) Hansen, M. J.; Velema, W. A.; Lerch, M. M.; Szymanski, W.; Feringa, B. L. Wavelength-Selective Cleavage of Photoprotecting Groups: Strategies and Applications in Dynamic Systems. *Chem. Soc. Rev.* **2015**, *44* (11), 3358–3377. https://doi.org/10.1039/C5CS00118H.
(11) Štacko, P.; Muchová, L.; Vítek, L.; Klán, P. Visible to NIR Light Photoactivation of Hydrogen Sulfide for Biological Targeting. *Org. Lett.* **2018**, *20* (16), 4907-491. https://doi.org/10.1021/acs.orglett.8b02043.
(12) Olson, J. P.; Banghart, M. R.; Sabatini, B. L.; Ellis-Davies, G. C. R. Spectral Evolution of a Photochemical Protecting Group for Orthogonal Two-Color Uncaging with Visible Light. *J. Am. Chem. Soc.* **2013**, *135* (42), 15948–15954. https://doi.org/10.1021/ja408225k.
(13) Olson, J. P.; Kwon, H. B.; Takasaki, K. T.; Chiu, C. Q.; Higley, M. J.; Sabatini, B. L.; Ellis-Davies, G. C. R. Optically Selective Two-Photon Uncaging of Glutamate at 900 Nm. *J. Am. Chem. Soc.* **2013**, *135* (16), 5954–5957. https://doi.org/10.1021/ja4019379.
(14) Adatia, K. K.; Halbritter, T.; Reinfelds, M.; Michele, A.; Tran, M; Laschat, S.; Heckel, A.; Tovar, G. E. M.; Southan, A. Coumarin-4-ylmethyl- and p-Hydroxyphenacyl-Based Photoacid Generators with High Solubility in Aqueous Media: Synthesis, Stability and Photolysis. *ChemPhotoChem* **2020** , *4*, 207–217. https://doi.org/10.1002/cptc.201900258.
(15) Hammer, C. A.; Falahati, K.; Jakob, A.; Klimek, R.; Burghardt, I.; Heckel A.; Wachtveitl, J. Sensitized Two-Photon Activation of Coumarin Photocages. *J. Phys. Chem. Lett.* **2018**, *9* (6), 1448–1453 https://doi.org/10.1021/acs.jpclett.7b03364.
(16) Ankenbruck, N.; Courtney, T.; Naro, Y.; Deiters, A. Optochemical Control of Biological Processes in Cells and Animals. *Angew. Chem. Int. Ed.* **2018**, *57* (11), 2768–2798. https://doi.org/10.1002/anie.201700171.
(17) Agarwal, H. K.; Zhai, S.; Surmeier, D. J.; Ellis-Davies, G. C. R. Intracellular Uncaging of CGMP with Blue Light. *ACS Chem. Neurosci.* **2017**, *8* (10), 2139–2144. https://doi.org/10.1021/acschemneuro.7b00237.
(18) Park, C.-H.; Givens, R. S. New Photoactivated Protecting Groups. 6. p-Hydroxyphenacyl: A Phototrigger for Chemical and Biochemical Probes 1 , 2. *J. Am. Chem. Soc.* **1997**, *119* (10), 2453–2463. https://doi.org/10.1021/ja9635589.
(19) Noguchi, M.; Skwarczynski, M.; Prakash, H.; Hirota, S.; Kimura, T.; Hayashi, Y.; Kiso, Y. Development of Novel Water-Soluble Photocleavable Protective Group and Its Application for Design of Photoresponsive Paclitaxel Prodrugs. *Bioorg. Med. Chem.* **2008**, *16* (10), 5389–5397. https://doi.org/10.1016/j.bmc.2008.04.022.
(20) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Yamamoto, T.; Ivanic, J.; Kobayashi, H.; Schnermann, M. J. In Vivo Activation of Duocarmycin–Antibody Conjugates by Near-Infrared Light. *ACS Cent. Sci.* **2017**, *3* (4), 329–337. https://doi.org/10.1021/acscentsci.7b00026.
(21) van Rixel, V. H. S.; Ramu, V.; Auyeung, A. B.; Beztsinna, N.; Leger, D. Y.; Lameijer, L. N.; Hilt, S. T.; Le Dévédec, S. E.; Yildiz, T.; Betancourt, T.; et al. Photo-Uncaging of a Microtubule-Targeted Rigidin Analogue in Hypoxic Cancer Cells and in a Xenograft Mouse Model. *J. Am. Chem. Soc.* **2019**, *141* (46), 18444–18454. https://doi.org/10.1021/jacs.9b07225.
(22) Silva, J. M.; Silva, E.; Reis, R. L. Light-Triggered Release of Photocaged Therapeutics - Where Are We Now? *J. Control. Release* **2019**, *298*, 154–176. https://doi.org/10.1016/j.jconrel.2019.02.006.
(23) Klausen, M.; Dubois, V.; Clermont, G.; Tonnelé, C.; Castet, F.; Blanchard-Desce, M. Dual-Wavelength Efficient Two-Photon Photorelease of Glycine by π-Extended Dipolar Coumarins. *Chem. Sci.* **2019**, *10* (15), 4209–4219. https://doi.org/10.1039/C9SC00148D.
(24) Lin, Q.; Yang, L.; Wang, Z.; Hua, Y.; Zhang, D.; Bao, B.; Bao, C.; Gong, X.; Zhu, L. Coumarin Photocaging Groups Modified with an Electron-Rich Styryl Moiety at the 3-Position: Long-Wavelength Excitation, Rapid Photolysis, and Photobleaching. *Angew. Chem. Int. Ed.* **2018**, *57* (14), 3722–3726. https://doi.org/10.1002/anie.201800713.
(25) Peterson, J. A.; Wijesooriya, C.; Gehrmann, E. J.; Mahoney, K. M.; Goswami, P. P.; Albright, T. R.; Syed, A.; Dutton, A. S.; Smith, E. A.; Winter, A. H. Family of BODIPY Photocages Cleaved by Single Photons of Visible/Near-Infrared Light. *J. Am. Chem. Soc.* **2018**, *140* (23), 7343–7346. https://doi.org/10.1021/jacs.8b04040.
(26) Gorka, A. P.; Nani, R. R.; Zhu, J.; Mackem, S.; Schnermann, M. J. A Near-IR Uncaging Strategy Based on Cyanine Photochemistry. *J. Am. Chem. Soc.* **2014**, *136* (40), 14153–14159. https://doi.org/10.1021/ja5065203.
(27) Kand, D.; Liu, P.; Navarro, M. X.; Fischer, L. J.; Rousso-Noori, L.; Friedmann-Morvinski, D.; Winter, A. H.; Miller, E. W.; Weinstain, R. Water-Soluble BODIPY Photocages with Tunable Cellular Localization. *J. Am. Chem. Soc.* **2020**, jacs.9b13219. https://doi.org/10.1021/jacs.9b13219.
(28) Bojtár, M.; Kormos, A.; Kis-Petik, K.; Kellermayer, M.; Kele, P. Green-Light Activatable, Water-Soluble Red-Shifted Coumarin Photocages. *Org. Lett.* **2019**, *21* (23), 9410–9414. https://doi.org/10.1021/acs.orglett.9b03624.
(29) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Kobayashi, H.; Schnermann, M. J. Near-IR Light-Mediated Cleavage of Antibody-Drug Conjugates Using Cyanine Photocages. *Angew. Chem. Int. Ed.* **2015**, *54* (46), 13635–13638. https://doi.org/10.1002/anie.201507391.
(30) Feng, S.; Harayama, T.; Chang, D.; Hannich, J. T.; Winssinger, N.; Riezman, H. Lysosome-Targeted Photoactivation Reveals Local Sphingosine Metabolism Signatures. *Chem. Sci.* **2019**, *10* (8), 2253–2258. https://doi.org/10.1039/C8SC03614D.
(31) Kand, D.; Pizarro, L.; Angel, I.; Avni, A.; Friedmann-Morvinski, D.; Weinstain, R. Organelle-Targeted BODIPY Photocages: Visible-Light-Mediated Subcellular Photorelease. *Angew. Chem. Int. Ed.* **2019**, *58* (14), 4659–4663. https://doi.org/10.1002/anie.201900850.
(32) Bonnet, S. Why Develop Photoactivated Chemotherapy? *Dalt. Trans.* **2018**, *47* (31), 10330–10343. https://doi.org/10.1039/C8DT01585F.
(33) Wagner, N.; Stephan, M.; Höglinger, D.; Nadler, A. A Click Cage: Organelle-Specific Uncaging of Lipid Messengers. *Angew.*

Chem. Int. Ed. **2018**, *57* (40), 13339–13343. https://doi.org/10.1002/anie.201807497.

(34) Suzuki, A. Z.; Sekine, R.; Takeda, S.; Aikawa, R.; Shiraishi, Y.; Hamaguchi, T.; Okuno, H.; Tamamura, H.; Furuta, T. A Clickable Caging Group as a New Platform for Modular Caged Compounds with Improved Photochemical Properties. *Chem. Commun*. **2019**, *55* (4), 451–454. https://doi.org/10.1039/C8CC07981A.

(35) Xu, L.; Li, Y.; Li, Y. Application of "Click" Chemistry to the Construction of Supramolecular Functional Systems. *Asian J. Org. Chem*. **2014**, *3*, (5), 582–602. https://doi.org/10.1002/ajoc.201300245.

(36) Trindade, A. F.; Frade, R. F. M.; Maçôas, E. M. S.; Graça, C.; Rodrigues, C. A. B.; Martinho, J. M. G.; Afonso, C. A. M. "Click and Go": Simple and Fast Folic Acid Conjugation. *Org. Biomol. Chem*. **2014**, *12* (20), 3181–3190. https://doi.org/10.1039/C4OB00150H.

(37) Kim, E.; Koo, H. Biomedical Applications of Copper-Free Click Chemistry: In Vitro , in Vivo , and Ex Vivo. *Chem. Sci.* **2019**, *10* (34), 7835–7851. https://doi.org/10.1039/C9SC03368H.

(38) Kozma, E.; Girona, G. E.; Paci, G.; Lemke, E. A.; Kele, P. Bioorthogonal Double-Fluorogenic Siliconrhodamine Probes for Intracellular Superresolution Microscopy. *Chem. Commun*. **2017**, *53* (50), 6696–6699. https://doi.org/10.1039/C7CC02212C.

(39) Kormos, A.; Kern, D.; Egyed, A.; Söveges, B.; Németh, K.; Kele, P. Microscope Laser Assisted Photooxidative Activation of Bioorthogonal ClickOx Probes. *Chem. Commun*. **2020**. https://doi.org/10.1039/D0CC01512A.

(40) Knorr, G.; Kozma, E.; Schaart, J. M.; Németh, K.; Török, G.; Kele, P. Bioorthogonally Applicable Fluorogenic Cyanine-Tetrazines for No-Wash Super-Resolution Imaging. *Bioconjug. Chem*. **2018**, *29* (4), 1312–1318. https://doi.org/10.1021/acs.bioconjchem.8b00061.

(41) Versteegen, R. M.; ten Hoeve, W.; Rossin, R.; de Geus, M. A. R.; Janssen, H. M.; Robillard, M. S. Click-to-Release from trans-Cyclooctenes: Mechanistic Insights and Expansion of Scope from Established Carbamate to Remarkable Ether Cleavage. *Angew. Chem. Int. Ed*. **2018**, *57*, 10494–10499. https://doi.org/10.1002/anie.201800402.

(42) Zhou, Y.; Wong, R. C. H.; Dai, G.; Ng, D. K. P. A Bioorthogonally Activatable Photosensitiser for Site-Specific Photodynamic Therapy. Chem. Commun. **2020**, *56* (7), 1078–1081. https://doi.org/10.1039/C9CC07938F.

(43) Linden, G.; Zhang, L.; Pieck, F.; Linne, U.; Kosenkov, D.; Tonner, R.; Vázquez, O. Conditional Singlet Oxygen Generation through a Bioorthogonal DNA-Targeted Tetrazine Reaction. *Angew. Chem. Int. Ed*. **2019**, *58* (37), 12868–12873. https://doi.org/10.1002/anie.201907093.

(44) Yu, B.; Wang, B.; Pan, Z.; De La Cruz, L. K.; Ke, B.; Zheng, Y.; Ji, X. Click and Release: Bioorthogonal Approaches to "on-Demand" Activation of Prodrugs. *Chem. Soc. Rev*. **2019**, *48* (4). https://doi.org/10.1039/c8cs00395e.

(45) Fournier, L.; Aujard, I.; Le Saux, T.; Maurin, S.; Beaupierre, S.; Baudin, J. B.; Jullien, L. Coumarinylmethyl Caging Groups with Redshifted Absorption. *Chem. Eur. J*. **2013**, *19* (51), 17494–17507. https://doi.org/10.1002/chem.201302630.

(46) Meimetis, L. G.; Carlson, J. C. T.; Giedt, R. J.; Kohler, R. H.; Weissleder, R. Ultrafluorogenic Coumarin-Tetrazine Probes for Real-Time Biological Imaging. *Angew. Chem. Int. Ed*. **2014**, *53* (29), 7531–7534. https://doi.org/10.1002/anie.201403890.

(47) Wieczorek, A.; Werther, P.; Euchner, J.; Wombacher, R. Green- to Far-Red-Emitting Fluorogenic Tetrazine Probes-Synthetic Access and No-Wash Protein Imaging inside Living Cells. *Chem. Sci*. **2017**, *8* (2), 1506–1510. https://doi.org/10.1039/C6SC03879D.

(48) Németh, E.; Knorr, G.; Németh, K.; Kele, P. A Bioorthogonally Applicable, Fluorogenic, Large Stokes-Shift Probe for Intracellular Super-Resolution Imaging of Proteins. *Biomolecules 2020*, *10*, 397. https://doi.org/10.3390/biom10030397.

(49) Wu, H.; Yang, J.; Šečkutė, J.; Devaraj, N. K. In Situ Synthesis of Alkenyl Tetrazines for Highly Fluorogenic Bioorthogonal Live-Cell Imaging Probes. *Angew. Chem. Int. Ed*. **2014**, *53* (23), 5805–5809. https://doi.org/10.1002/anie.201400135.

(50) Tang, S.; Cannon, J.; Yang, K.; Krummel, M. F.; Baker, J. R.; Choi, S. K. Spacer-Mediated Control of Coumarin Uncaging for Photocaged Thymidine. *J. Org. Chem*. **2020**, *85* (5), 2945–2955. https://doi.org/10.1021/acs.joc.9b02617.

(51) Kathayat, R. S.; Cao, Y.; Elvira, P. D.; Sandoz, P. A.; Zaballa, M.-E.; Springer, M. Z.; Drake, L. E.; Macleod, K. F.; van der Goot, F. G.; Dickinson, B. C. Active and Dynamic Mitochondrial S-Depalmitoylation Revealed by Targeted Fluorescent Probes. *Nat. Commun*. **2018**, *9* (1), 334. https://doi.org/10.1038/s41467-017-02655-1.

(52) Poronik, Y. M.; Vygranenko, K. V; Gryko, D.; Gryko, D. T. Rhodols – Synthesis, Photophysical Properties and Applications as Fluorescent Probes. *Chem. Soc. Rev.* **2019**, *48* (20), 5242–5265. https://doi.org/10.1039/C9CS00166B.

(53) Dal Corso, A.; Pignataro, L.; Belvisi, L.; Gennari, C. Innovative Linker Strategies for Tumor-Targeted Drug Conjugates. *Chem. Eur. J.* **2019**, *25* (65), 14740–14757. https://doi.org/10.1002/chem.201903127.

(54) Werther, P.; Yserentant, K.; Braun, F.; Kaltwasser, N.; Popp, C.; Baalmann, M.; Herten, D.-P.; Wombacher, R. Live-Cell Localization Microscopy with a Fluorogenic and Self-Blinking Tetrazine Probe *Angew. Chem. Int. Ed*. **2020**, *59*, 804–810. https://doi.org/10.1002/anie.201906806.

Insert Table of Contents artwork here