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Photo-Modulation of Gene-Editing Enzymes CRISPR/Cas9 with Bifunctional Small-Molecule Ligands†

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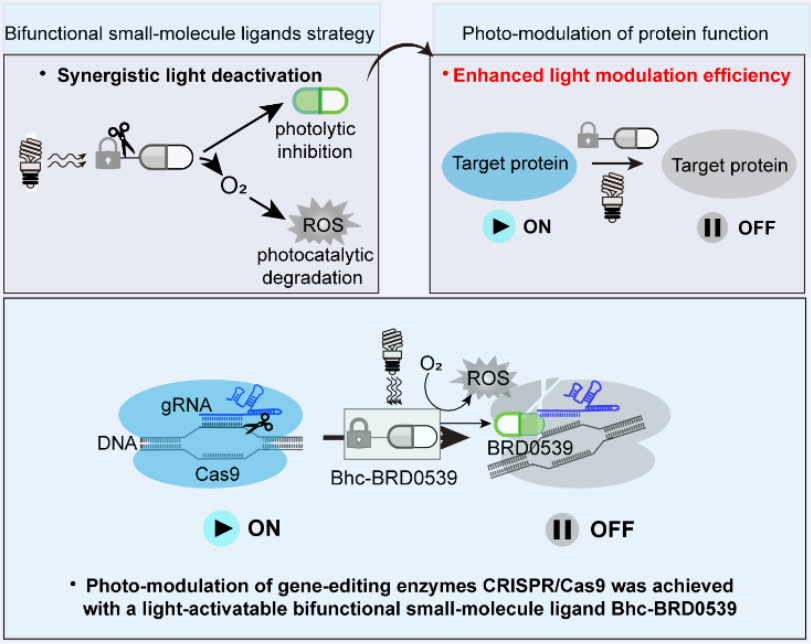
Keywords

Gene-editing | Small-molecule ligands | Enzyme modulation | DNA cleavage | Photoswitch | Inhibitors

Comprehensive Summary



The control of protein functions with light is valuable for spatiotemporal probing of biological systems. Current small-molecule photo-modulation methods include the light-induced uncaging of inhibitors and chromophore-assisted light inactivation with reactive oxygen species (ROS). However, the constant target protein expression results in inadequate photo-modulation efficiency, particularly for less potent inhibitors and chromophores. Herein, we report a novel bifunctional small-molecule ligands strategy to photo-modulate gene-editing enzymes CRISPR/Cas9. A coumarin-derived small-molecule ligand Bhc-BRD0539 is developed to uncage the active inhibitor upon light irradiation and to generate ROS in the Cas9 proximity for the dual inhibition of Cas9 activity. Our results highlight the synergistic photo-modulation with bifunctional small-molecule ligands, which offers a valuable addition to current CRISPR/Cas9 photo-modulation technologies and may extend to other protein classes.

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[‡] These authors contributed equally to this work.
[†] Dedicated to the Memory of Professor Xiyan Lu.

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Background and Originality Content

Precise modulation of target proteins in complex environments helps clarify their functions and aids in developing therapeutic strategies.[1-4] Small molecules are valuable resources for addressing these challenges due to their easy delivery and fast disruption of endogenous proteins.[5-7] The light-triggered release of small molecules is promising for external modulation of protein functions in high spatiotemporal resolution.[8-11] Typically, small-molecule inhibitors are caged with photoremovable protecting groups to restrict their inhibitory potency. The activity of these caged small-molecule inhibitors is restored upon light irradiation through photolysis (Figure 1a).[12] While effective, the efficiency of photolytic inhibition relies on the photolytic release rate. The constant expression of target proteins may result in poor deactivation and potential off-target effects, especially for small-molecule inhibitors with limited inhibitory potency.

The chromophore-assisted light inactivation (CALI) strategy offers a promising alternative for modulating protein functions through light-driven degradation.[13-14] Irreversible protein inactivation can be achieved via localized reactive oxygen species (ROS) generation upon light irradiation (Figure 1a).[15-16] Various ligand-directed photocatalysts have been employed for the target-selective CALI. However, the generated ROS may also bleach the photocatalysts, resulting in low photo-deactivation efficiency. It remains an intimidating challenge to discover photocatalysts for CALI-mediated deactivation.

Recently, coumarins have emerged as potent singlet oxygen photocatalysts to induce cell apoptosis and autophagy upon light exposure.[17-18] Coumarin derivatives have also found widespread applications as photocleavable protecting groups.[19] We hypothesize that a judicious combination of small-molecule ligands and dual functional coumarins may enhance the photo-deactivation efficiency with synergistic photo-released inhibitors and localized ROS generation.

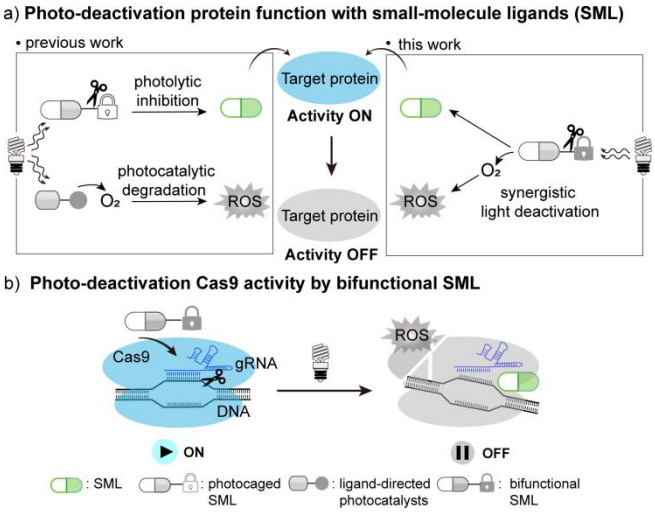

Figure 1 Methods for light-modulating protein functions with small molecules and their applications on CRISPR/Cas9 modulation. a) Photolysis of caged small-molecule ligands (SML) and chromophore-assisted light inactivation (previous work). Bifunctional small-molecule ligands enable light-triggered release of small-molecule inhibitors and localized reactive oxygen species (ROS) generation (this work). b) Photo-deactivation of Cas9 activity with light-activatable bifunctional small-molecule ligands.

The CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated protein 9) system is a highly efficient and powerful gene-editing tool, which has revolutionized the genome engineering in biotechnology and biomedical research.[20-22] However, the reliability and safety of the CRISPR/Cas9 system are hindered by the overactivity of CRISPR complexes after on-target editing.[23] Therefore, the development of on/off-switches to modulate CRISPR/Cas9 activity is highly desirable. Currently, various light control methods have been explored to activate CRISPR/Cas9 as on-switches, including split Cas9,[24] photocaged Cas9,[25] and light-responsive RNA.[26-28] However, light-induced off-switches to modulate CRISPR/Cas9 have been less reported, which require rational modifications of the gRNA backbone with multiple photocleavable groups.[29-30] In contrast to the intricate design of gRNA-based switches, the chemical synthesis of photocaged small-molecule ligands may provide unique advantages as off-switches. Herein, we present a bifunctional small-molecule ligands strategy to photo-modulate gene-editing enzymes CRISPR/Cas9 by enhancing the inhibitory potency of existing small-molecule inhibitors (Figure 1b).[31]

Results and Discussion

We started studying the small-molecule ligands of Cas9, among which BRD0539 was one of the few reported inhibitors to disrupt the interaction of Cas9 with the target DNA, thus preventing DNA cleavage.[31] The hydroxyl group of BRD0539 is supposedly crucial for the Cas9 inhibition. However, the biotinylated form of the hydroxyl group has been used for Cas9 protein pull-down from cell lysate,[31] suggesting that the hydroxyl group modification on BRD0539 decreases its inhibitory activity but does not interfere with its Cas9 binding.

To test this hypothesis, we covalently connected 6-bromo-7-hydroxy-4-hydroxymethylcoumarin to the hydroxyl group of BRD0539 via a carbonate ester linker to synthesize Bhc-BRD0539 as a light-activatable bifunctional small-molecule ligand (Figure 2a). The light-induced conversion of Bhc-BRD0539 is expected to reveal BRD0539 owing to the well-documented photocleavable property of coumarin moiety.[17] At the same time, it should

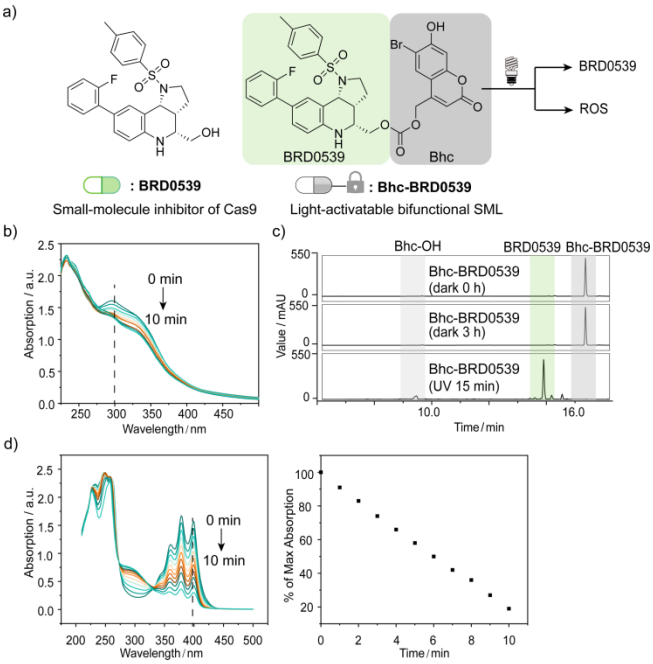

Figure 2 Design, synthesis, and evaluation of the photochemical properties of Bhc-BRD0539. a) Structures of BRD0539 and Bhc-BRD0539. b) UV-vis absorption spectra analysis of Bhc-BRD0539 after UV lamp irradiation (365 nm, 12 mW·cm^{-2}) at various times. c) HPLC characterization of light-induced generation of BRD0539 and Bhc-OH. d) Absorption spectra analysis of the singlet oxygen generation during the irradiation of Bhc-BRD0539 (left panel). Time-dependent ADPA absorption at 400 nm after UV lamp irradiation (365 nm, 12 mW·cm^{-2}) (right panel).

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release ROS due to its singlet oxygen-generating capacity. To assess the photochemical properties of the synthesized Bhc-BRD0539, we monitored its UV-visible absorption spectra in 50 mmol/L Tris-HCl buffer at pH 7.4. Bhc-BRD0539 exhibited absorption wavelength ranging from 200 to 450 nm, with a characteristic peak at 300 nm. After continuous irradiation with a handheld UV LED lamp (365 nm, 12 mW·cm^{-2}) for 10 min, the absorption peak at 300 nm gradually decreased and ultimately disappeared, indicating the conversion of Bhc-BRD0539 (Figure 2b). LC-MS analysis further confirmed the generation of BRD0539 upon light irradiation. After a 15-minute light treatment, complete photolysis of Bhc-BRD0539 was observed, leading to the formation of BRD0539 and Bhc-OH (Figure 2c). In contrast, Bhc-BRD0539 remained stable after incubation in Tris-buffer for 3 h without exposure to light, with over 95% retention. Additionally, BRD0539 exhibited stability under UV light irradiation (Figure S1).

We further investigated if ROS was generated during light irradiation. To this end, 9, 10-anthracenedipropionic acid (ADPA, 100 μmol/L)[32] was exposed to Bhc-BRD0539 (20 μmol/L) under UV LED lamp irradiation. UV-visible absorption spectra revealed ADPA underwent photochemical oxygenation, which indicated that Bhc-BRD0539 generated singlet oxygen upon light irradiation (Figure 2d). These results confirmed the synergistic photochemical reactivity of Bhc-BRD0539 to dually release the Cas9 inhibitor and the singlet oxygen.

We next evaluated the inhibitory activity of Bhc-BRD0539 on Cas9-guided DNA cleavage. Bhc-BRD0539 (0.45 mmol/L) was incubated with Cas9 protein-gRNA complex (0.15 μmol/L) in NEB 3.1 buffer at 37 °C for 1 h.[33] Afterward, the target-DNA fragment (11 nmol/L) was subjected to an extra hour of incubation following the general procedures outlined for Cas9-guided DNA cleavage.[27] The Cas9 cleavage activity was assessed by the fluorescence analysis of the DNA fragments with gel electrophoresis (Figure S2). The incubation of Cas9-gRNA complex with Bhc-BRD0539 resulted in the cleavage of the full-length target DNA fragments (720 bp) to 551 bp and 169 bp fragments, respectively (Figure 3a and Figure S3). Similarly, the cleavage products were observed in the Cas9-gRNA complex sample treated without Bhc-BRD0539 (Figure S4). In contrast, when an equivalent of BRD0539 was employed to treat Cas9-gRNA complex, the minimal Cas9-guided DNA cleavage was observed. Further analysis of dose-dependent inhibition revealed that the Cas9 inhibition required more than twice the concentration (1.05 mmol/L) of Bhc-BRD0539 as that of BRD0539 (Figure 3b).

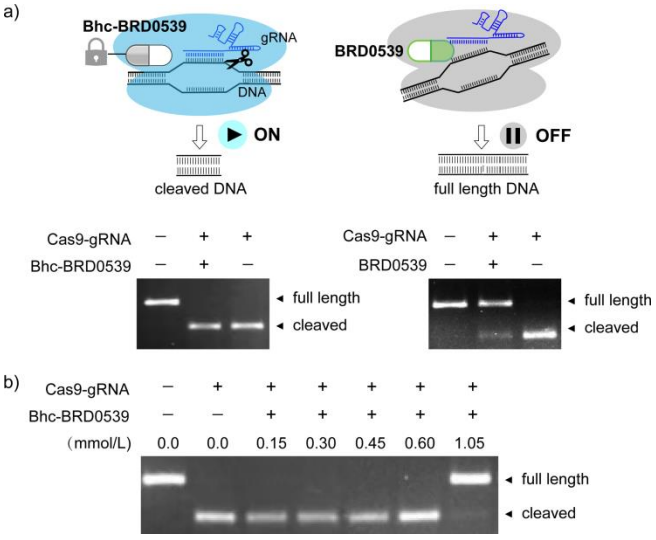

Figure 3 Evaluation of Bhc-BRD0539 on Cas9 cleavage activity disruption. a) Schematic illustration and in-gel DNA-fragment fluorescence analysis of Cas9 cleavage activity after treatment with Bhc-BRD0539 (0.45 mmol/L, left panel) or BRD0539 (0.45 mmol/L, right panel). The gel analysis revealed the presence of the full-length DNA fragments of 720 bp and one of the cleaved fragments of 551 bp. b) In-gel DNA-fragment fluorescence analysis of Cas9 cleavage activity disruption after treatment with different concentrations of Bhc-BRD0539 for 1 h.

We then investigated the photo-modulation of Cas9-guided DNA cleavage by Bhc-BRD0539 (Figure 4a). With Bhc-BRD0539 (0.45 mmol/L), the Cas9-guided DNA cleavage proceeded smoothly after a 1-hour incubation (Figure 4b). However, when the Bhc-BRD0539-treated CRISPR system was exposed to a handheld UV LED lamp irradiation for 15 min (365 nm, 12 mW·cm^{-2}), the DNA cleavage was effectively inhibited, resulting in no DNA cleavage products (Figure 4b, upper panel). Higher-intensity irradiation with a UV dot light (365 nm, 650 mW·cm^{-2}) accelerated the light-induced inhibition process, leading to the complete abolishment of Cas9 cleavage activity within 2 min (Figure 4b, lower panel).[34] In the negative control experiments, the identical light irradiation without Bhc-BRD0539 showed minimal effect on Cas9-guided DNA cleavage (Figure S6).

We further explored the dual functions of Bhc-BRD0539 in enhancing light-induced deactivation. The Cas9 cleavage activity was reduced by Bhc-BRD0539 irradiated for 5 min before the CRISPR incubation (Figure S7). However, the extended light pre-treatment of Bhc-BRD0539 did not completely abolish DNA cleavage. In addition, the pre-treated Bhc-BRD0539 by light

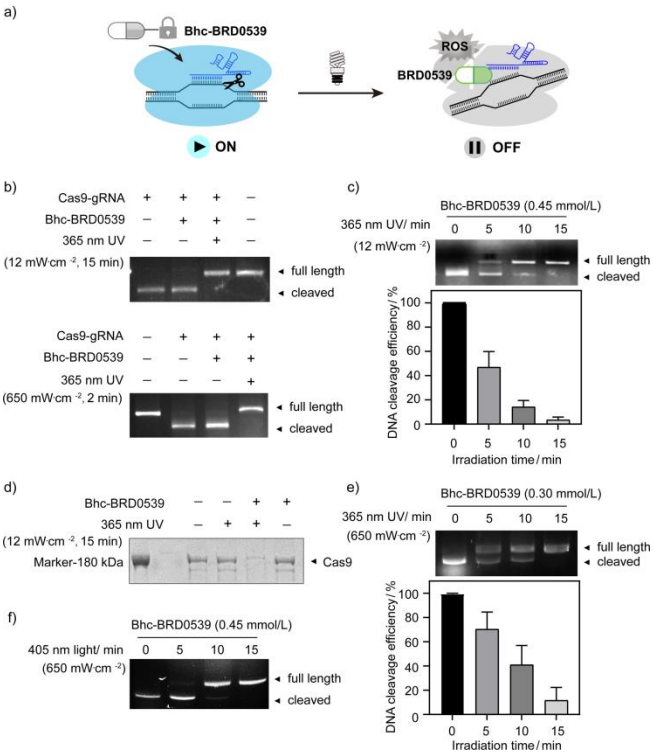

Figure 4 Photo-modulation of Cas9-guided DNA cleavage with Bhc-BRD0539. a) Schematic illustration for Bhc-BRD0539 used as light-inducible off-switches on Cas9 cleavage activity. b) In-gel fluorescence analysis of light-controlled CRISPR inhibition after 365 nm UV LED light irradiation (12 mW·cm^{-2}, 15 min, upper panel) or 365 nm UV LED dot light irradiation (650 mW·cm^{-2}, 2 min, lower panel). c) Dose-dependency of light irradiation (365 nm, 12 mW·cm^{-2}) on light-controlled CRISPR inhibition. Error bars represent the standard error of the mean (±SEM) from technical replicates (n = 3). d) In-gel Coomassie Blue Staining analysis of Cas9 degradation after light irradiation (365 nm, 12 mW·cm^{-2}, 15 min). e) In-gel DNA-fragment fluorescence analysis of light-controlled CRISPR inhibition with lower dose Bhc-BRD0539 (0.30 mmol/L) under UV LED dot light irradiation (365 nm, 650 mW·cm^{-2}). Error bars represent the standard error of the mean (±SEM) from technical replicates (n = 3). f) In-gel fluorescence analysis of light-controlled CRISPR inhibition by violet LED dot light irradiation (405 nm, 650 mW·cm^{-2}).

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irradiation did not lead to light-driven Cas9 degradation (Figure S8b, lane 4). These results demonstrated the necessity of photochemically generated ROS in enhancing the photo deactivation. In contrast, the light-driven Cas9 degradation was observed when the CRISPR system was incubated with Bhc-BRD0539, as shown in Figure 4d using in-gel Coomassie Blue Staining analysis. We also added BSA and other bystander proteins to the CRISPR system, and found no detectable degradation of these proteins other than the Cas9 protein (Figure S8). These results together suggest that BRD0539-conjugated coumarin acts as photocatalysts to induce ROS production only in proximity to Cas9 for selective Cas9 degradation.

As it remains challenging to identify new small-molecule inhibitors for Cas9,[35] we investigated if the inhibitory activity of existing ones could be enhanced through the synergistic photochemical strategy. With the lower-dose Bhc-BRD0539 or BRD0539 (0.30 mmol/L), 1 h incubation did not disrupt Cas9-guided DNA cleavage (Figure 4e and Figure S9). However, nearly complete CRISPR inhibition was achieved after a 15-minute UV light irradiation (365 nm, 650 mW·cm^{-2}) with Bhc-BRD0539. The synergistic effect of CALI-mediated deactivation and photo-released BRD0539 was attributed to the complete photo-inhibited Cas9-guided DNA cleavage.

The longer-wavelength light irradiation may enhance the biocompatibility and cellular penetration.[36-37] We found that the violet LED dot light irradiation (405 nm, 650 mW·cm^{-2}) for 10 min resulted in the similar inhibition of Cas9 cleavage activity (Figure 4f). The violet LED dot light condition demonstrated excellent biocompatibility with a mammalian cell survival rate exceeding 95% after 10-minute irradiation (Figure S10). These findings suggest the potential of Bhc-BRD0539 for further photo-modulation of Cas9 gene editing with longer wavelength. While the synthesis of Bhc-BRD0539 only requires a one-step modification of BRD0539, the synthesis of BRD0539 itself is complex involving eight steps from commercially available materials in low yields.[35] The challenges associated with the large-scale production of Bhc-BRD0539 may hinder their further applications *in vivo*. Nevertheless, the synergistic light deactivation process offered by Bhc-BRD0539 presents a valuable small-molecule strategy for photo-modulation of CRISPR/Cas9.

Conclusions

In conclusion, we have developed a bifunctional small-molecule ligands strategy to enhance the efficiency of protein photo-modulation. A case study of gene-editing enzymes CRISPR/Cas9 photo-modulation was achieved with Bhc-BRD0539, which released active inhibitor BRD0539 upon light irradiation and generated ROS near the Cas9 enzyme. This bifunctional small-molecule ligands strategy holds potential for enhancing the photo-modulation sensitivity of existing small-molecule inhibitors, which may also extend to other protein classes.

Experimental

General procedures

The detailed procedures for Bhc-BRD0539 synthesis, Cas9 expression and purification, gRNA transcription and purification, and full-length target DNA fragments amplification and purification were presented in the Supporting Information.

Photochemical properties evaluation of Bhc-BRD0539

Bhc-BRD0539 (100 μmol/L) was dissolved in a mixture of DMSO and water (V : V = 5 : 95) and then subjected to UV irradiation using a handheld UV lamp (365 nm, 12 mW·cm^{-2}). Samples irradiated for varied duration were collected and analyzed by HPLC and UV-vis absorption spectroscopy to monitor the release of BRD0539.

To detect the ROS generation during the irradiation of Bhc-BRD0539, the singlet oxygen indicator probe ADPA (100 μmol/L) was subjected to 50 mmol/L Tris-HCl buffer with Bhc-BRD0539 (20 μmol/L). The mixture was exposed to various durations of a hand-held UV light (365 nm, 12 mW·cm^{-2}) under room temperature at air atmosphere. The absorption spectra of the samples were recorded using a Nanodrop 2000c UV-vis spectrophotometer, with the peak absorption at 400 nm chosen for visualization.

Cas9-guided DNA cleavage assay

Cas9 protein (500 ng, 0.15 μmol/L) was preincubated with gRNA (120 ng, 0.15 μmol/L) at 37 °C for 10 min. Subsequently, the full-length target DNA fragments (100 ng, 11 nmol/L) were introduced into NEB 3.1 buffer (50 mmol/L Tris-HCl, 100 mmol/L NaCl, 10 mmol/L $MgCl_2$, 100 μg/mL BSA), followed by incubation at 37 °C for 1 h. Finally, the reaction mixtures were heated at 65 °C for 10 min and analyzed using 2% agarose gel electrophoresis.

Light-modulating Cas9-guided DNA cleavage with Bhc-BRD0539

Cas9 protein (500 ng, 0.15 μmol/L) was preincubated with gRNA (120 ng, 0.15 μmol/L) in NEB 3.1 buffer for 10 min. Subsequently, Bhc-BRD0539 was added to the mixture and incubated at 37 °C for 30 min. The reaction mixtures were then subjected to UV irradiation for varying durations, followed by an additional incubation at 37 °C for 30 min. The full-length target DNA fragments (100 ng, 11 nmol/L) were then added and incubated at 37 °C for 1 h. Finally, the reaction mixtures were heated at 65 °C for 10 min and analyzed using 2% agarose gel electrophoresis.

Supporting Information

The supporting information for this article is available on the WWW under https://doi.org/10.1002/cjoc.202300452.

Acknowledgement

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References

[1] Fehrentz, T.; Schönberger, M.; Trauner, D. Optochemical Genetics. *Angew. Chem. Int. Ed.* **2011**, *50*, 12156–12182.
[2] Ankenbruck, N.; Courtney, T.; Naro, Y.; Deiters, A. Optochemical Control of Biological Processes in Cells and Animals. *Angew. Chem. Int. Ed.* **2018**, *57*, 2768–2798.
[3] Wang, H.; Li, W. G.; Zeng, K.; Wu, Y. J.; Zhang, Y.; Xu, T. L.; Chen, Y. Photocatalysis Enables Visible-Light Uncaging of Bioactive Molecules in Live Cells. *Angew. Chem. Int. Ed.* **2019**, *58*, 561–565.
[4] Yang, L.; Ozawa, T.; Dong, H.; Zhang, X. Optogenetic Control of Phosphatidylinositol (3,4,5)-Triphosphate Production by Light-Sensitive Cryptochrome Proteins on the Plasma Membrane. *Chin. J. Chem.* **2021**, *39*, 1240–1246.
[5] Stockwell, B. R. Exploring biology with small organic molecules. *Nature* **2004**, *432*, 846–854.
[6] Rakhit, R.; Navarro, R.; Wandless, T. J. Chemical biology strategies for posttranslational control of protein function. *Chem. Biol.* **2014**, *21*, 1238–1252.
[7] Zeng, K.; Han, L.; Chen, Y. Endogenous Proteins Modulation in Live Cells with Small Molecules and Light. *ChemBioChem* **2022**, *23*, e202200244.

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[8] 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.

[9] Wang, H.; Zhang, Y.; Zeng, K.; Qiang, J.; Cao, Y.; Li, Y.; Fang, Y.; Zhang, Y.; Chen, Y. Selective Mitochondrial Protein Labeling Enabled by Biocompatible Photocatalytic Reactions inside Live Cells. *JACS Au* **2021**, *1*, 1066–1075.

[10] Zhang, Y.; Han, L.; Tian, X.; Peng, C.; Chen, Y. Ligand-Directed Caging Enables the Control of Endogenous DNA Alkyltransferase Activity with Light inside Live Cells. *Angew. Chem. Int. Ed.* **2022**, *61*, e202115472.

[11] Cheng, B.; Wan, Y.; Tang, Q.; Du, Y.; Xu, F.; Huang, Z.; Qin, W.; Chen, X. A Photocaged Azidosugar for Light-Controlled Metabolic Labeling of Cell-Surface Sialoglycans. *Chin. J. Chem.* **2022**, *40*, 806–812.

[12] Ellis-Davies, G. C. R. Reverse Engineering Caged Compounds: Design Principles for their Application in Biology. *Angew. Chem. Int. Ed.* **2023**, *62*, e202206083.

[13] Jacobson, K.; Rajfur, Z.; Vitriol, E.; Hahn, K. Chromophore-assisted laser inactivation in cell biology. *Trends Cell Biol.* **2008**, *18*, 443–450.

[14] Jay, D. G. Selective destruction of protein function by chromophore-assisted laser inactivation. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 5454–5458.

[15] Davies, M. J. Singlet oxygen-mediated damage to proteins and its consequences. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 761–770.

[16] Lee, J.; Udugamasooriya, D. G.; Lim, H.-S.; Kodadek, T. Potent and selective photo-inactivation of proteins with peptoid-ruthenium conjugates. *Nat. Chem. Biol.* **2010**, *6*, 258–260.

[17] Guerrero, T.; Vázquez-Ortega, F.; Lagunes, I.; Ortiz-Blanco, E.; Sosa-Ortiz, G.; Tovar-Miranda, R.; Medina, M. E.; Trigos, Á. Antagonistic activity of hydroxycoumarin-based antioxidants as possible singlet oxygen precursor photosensitizers. *Dyes Pigm.* **2021**, *192*, 109447.

[18] Vellakkaran, M.; Hong, S. Visible-light-induced Reactions Driven by Photochemical Activity of Quinolinone and Coumarin Scaffolds. *Asian J. Org. Chem.* **2021**, *10*, 1012–1023.

[19] Klan, P.; Solomek, 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*, 119–191.

[20] Wang, J. Y.; Doudna, J. A. CRISPR technology: A decade of genome editing is only the beginning. *Science* **2023**, *379*, eadd8643.

[21] Hsu, P. D.; Lander, E. S.; Zhang, F. Development and Applications of CRISPR-Cas9 for Genome Engineering. *Cell* **2014**, *157*, 1262–1278.

[22] Komor, A. C.; Badran, A. H.; Liu, D. R. CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. *Cell* **2017**, *168*, 20–36.

[23] Gangopadhyay, S. A.; Cox, K. J.; Manna, D.; Lim, D.; Maji, B.; Zhou, Q.; Choudhary, A. Precision Control of CRISPR-Cas9 Using Small Molecules and Light. *Biochemistry* **2019**, *58*, 234–244.

[24] Truong, D. J.; Kuhner, K.; Kuhn, R.; Werfel, S.; Engelhardt, S.; Wurst, W.; Ortiz, O. Development of an intein-mediated split-Cas9 system for gene therapy. *Nucleic Acids Res.* **2015**, *43*, 6450–6458.

[25] Hemphill, J.; Borchardt, E. K.; Brown, K.; Asokan, A.; Deiters, A. Optical Control of CRISPR/Cas9 Gene Editing. *J. Am. Chem. Soc.* **2015**, *137*, 5642–5645.

[26] Zhou, W.; Brown, W.; Bardhan, A.; Delaney, M.; Ilk, A. S.; Rauen, R. R.; Kahn, S. I.; Tsang, M.; Deiters, A. Spatiotemporal Control of CRISPR/Cas9 Function in Cells and Zebrafish using Light-Activated Guide RNA. *Angew. Chem. Int. Ed.* **2020**, *59*, 8998–9003.

[27] Jain, P. K.; Ramanan, V.; Schepers, A. G.; Dalvie, N. S.; Panda, A.; Fleming, H. E.; Bhatia, S. N. Development of Light-Activated CRISPR Using Guide RNAs with Photocleavable Protectors. *Angew. Chem. Int. Ed.* **2016**, *55*, 12440–12444.

[28] Wang, S. R.; Wei, L.; Wang, J. Q.; Ji, H. M.; Xiong, W.; Liu, J.; Yin, P.; Tian, T.; Zhou, X. Light-Driven Activation of RNA-Guided Nucleic Acid Cleavage. *ACS Chem. Biol.* **2020**, *15*, 1455–1463.

[29] Zou, R. S.; Liu, Y.; Wu, B.; Ha, T. Cas9 deactivation with photocleavable guide RNAs. *Mol. Cell* **2021**, *81*, 1553–1565.

[30] Carlson-Stevermer, J.; Kelso, R.; Kadina, A.; Joshi, S.; Rossi, N.; Walker, J.; Stoner, R.; Maures, T. CRISPRoff enables spatio-temporal control of CRISPR editing. *Nat. Commun.* **2020**, *11*, 5041.

[31] Maji, B.; Gangopadhyay, S. A.; Lee, M.; Shi, M.; Wu, P.; Heler, R.; Mok, B.; Lim, D.; Siriwardena, S. U.; Paul, B.; Dancik, V.; Vetere, A.; Mesleh, M. F.; Marraffini, L. A.; Liu, D. R.; Clemons, P. A.; Wagner, B. K.; Choudhary, A. A High-Throughput Platform to Identify Small-Molecule Inhibitors of CRISPR-Cas9. *Cell* **2019**, *177*, 1067–1079.

[32] Lindig, B. A.; Rodgers, M. A. J.; Schaap, A. P. Determination of the lifetime of singlet oxygen in water-d2 using 9,10-anthracenedipropionic acid, a water-soluble probe. *J. Am. Chem. Soc.* **1980**, *102*, 5590–5593.

[33] BRD0539 displays dose-dependent Cas9 inhibition (apparent IC_{50} = 22 μmol/L) and completely inhibits Cas9 protein-gRNA complex (5 nmol/L) at 30 μmol/L, see ref [31]. Our initial tests with BRD0539 at varying concentrations revealed the significant inhibition of Cas9 (0.15 μmol/L) by 0.45 mmol/L of BRD0539, however, no inhibition was observed at 0.30 mmol/L (Figure 3a and Figure S9). Therefore, we assessed Bhc-BRD0539's inhibitory potential at 0.45 mmol/L and explored lower-dose Bhc-BRD0539 modulation at 0.30 mmol/L.

[34] Photolysis of Bhc-BRD0539 was observed upon exposure to higher-intensity UV dot light irradiation (365 nm, 650 $mW \cdot cm^{-2}$) (Figure S5) .

[35] Lim, D.; Zhou, Q.; Cox, K. J.; Law, B. K.; Lee, M.; Kokkonda, P.; Sreekanth, V.; Pergu, R.; Chaudhary, S. K.; Gangopadhyay, S. A.; Maji, B.; Lai, S.; Amako, Y.; Thompson, D. B.; Subramanian, H. K. K.; Mesleh, M. F.; Dančík, V.; Clemons, P. A.; Wagner, B. K.; Woo, C. M.; Church, G. M.; Choudhary, A. A general approach to identify cell-permeable and synthetic anti-CRISPR small molecules. *Nat. Cell Biol.* **2022**, *24,* 1766–1775.

[36] Sinha, R. P.; Richter, P.; Faddoul, J.; Braun, M.; Häder, D.-P. Effects of UV and visible light on cyanobacteria at the cellular level. *Photochem. Photobiol. Sci.* **2002**, *1*, 553–559.

[37] Lemke, E. A.; Summerer, D.; Geierstanger, B. H.; Brittain, S. M.; Schultz, P. G. Control of protein phosphorylation with a genetically encoded photocaged amino acid. *Nat. Chem. Biol.* **2007**, *3*, 769–772.

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