ChemBioChem

Review
doi.org/10.1002/cbic.202200745

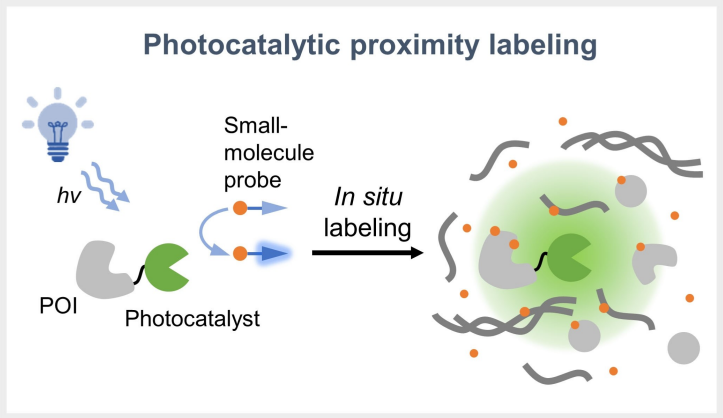
Chemistry Europe
European Chemical Societies Publishing

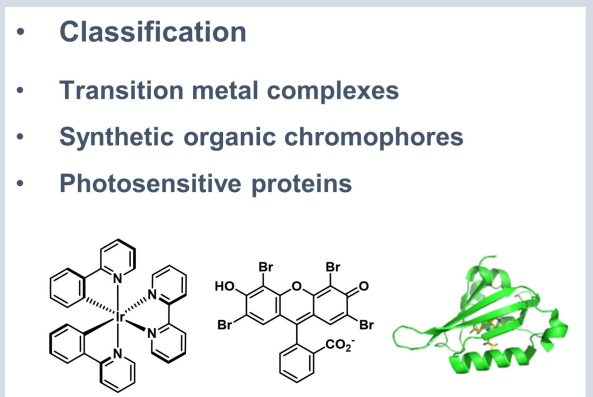
www.chembiochem.org

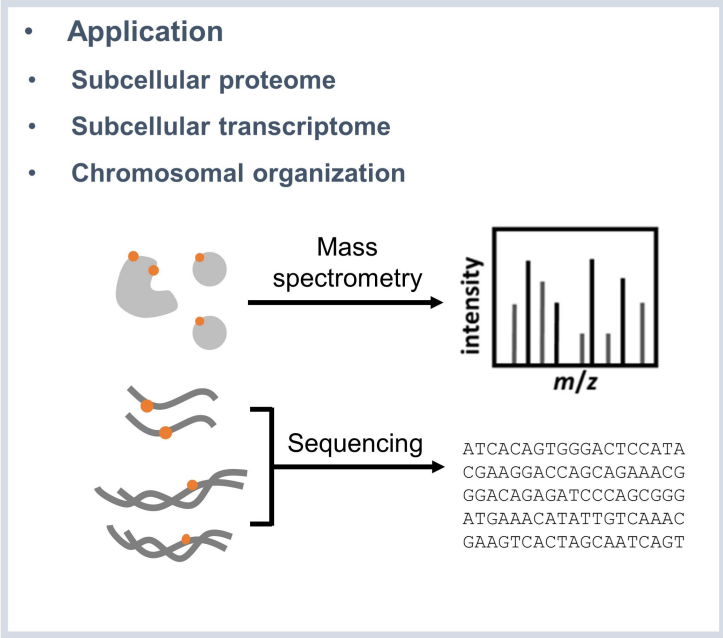
VIP *Very Important Paper*

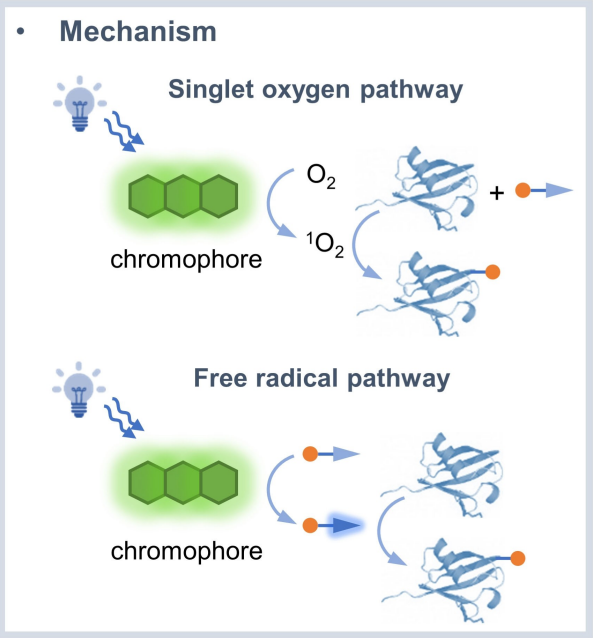
Photocatalytic Proximity Labeling for Profiling the Subcellular Organization of Biomolecules

Yuxin Fang[a] and Peng Zou*[a, b, c]









Investigating the subcellular organization of biomolecules is important for understanding their biological functions. Over the past decade, proximity-dependent labeling methods have emerged as powerful tools for mapping biomolecules in their native context. These methods often capitalize on the in-situ generation of highly reactive intermediates for covalently tagging biomolecules located within nanometers to sub-micrometers of the source of labeling. Among these, photocatalytic proximity labeling methods achieve precise spatial and temporal control of labeling with visible light illumination. In this review, we summarize the mechanisms and applications of existing photocatalytic proximity labeling methods and discuss future opportunities for improving the method.

1. Introduction

Eukaryotic cells are highly compartmentalized. Biomolecules and biochemical reactions are elaborately organized within different subcellular compartments. Therefore, the subcellular localization of biomolecules is crucial for their biological functions. Traditionally, researchers use physical isolation methods (e.g., subcellular fractionation) to study the spatial organization of biomolecules. However, such methods are prone to contamination and loss of material due to the often laborious procedure of organelle purification. Beside, physical isolation is only applicable to a handful of subcellular compartments that can easily be purified, such as the nucleus,[1] mitochondrion,[2] lipid droplet,[3] synaptosome,[4] but are less efficient when dealing with compartments that are not fully enclosed by lipid membrane, such as mitochondrial-endoplasmic reticulum contact sites and membraneless condensate.

In recent years, proximity-dependent labeling methods have emerged as promising tools for investigating subcellular proteome and transcriptome in the context of live cells (Figure 1a). Two types of engineered enzymes have been employed for proximity labeling: peroxidases and biotin ligases. Peroxidases, as exemplified by horseradish peroxidase (HRP)[5] and engineered ascorbate peroxidase (APEX2),[6–7] catalyze the H_2O_2-mediated oxidation of biotin-phenol to locally generate highly reactive phenoxy free radicals, which then react with electron-rich amino acid residues such as tyrosine (Figure 1b). Biotin ligases, including BioID[8] and TurboID,[9] catalyze the conversion of biotin and adenosine triphosphate (ATP) into biotinyl-5′-AMP, a reactive adenylate intermediate that covalently labels lysine residues on proximal proteins (Figure 1c). While these two classes of proximity labeling methods have been widely used in the studies of subcellular proteomes,[10] protein complexes[11] and protein–protein interactions (PPIs) in cell signaling,[12,13] they each have limitations with respect to cellular toxicity of labeling and temporal control. For example, peroxidase-based proximity labeling requires millimolar concentrations of hydrogen peroxide, which is not suitable for investigating redox-sensitive pathways. Biotin ligases avoid cytotoxic reagents but could be activated by endogenous levels of biotin and ATP, leading to poor temporal resolution when studying dynamic processes. Recently, optical control of TurboID activity has been achieved through the development of a photocaged TurboID (photoTurbo) by incorporating a photolabile lysine derivative at the active site of the biotin ligase, which is readily converted to the native catalytic lysine residue upon UV illumination to reactivate the enzyme.[14] Labeling with photoTurbo offers temporal resolution of 10 min in the mitochondria and ER.[14]

Photocatalytic proximity labeling, which uses photosensitive compounds or proteins to catalyze the bioconjugation reactions between small-molecule probes and biological macromolecules, enables proximity labeling with nanometer to sub-micrometer spatial resolution (Figure 1d). In addition, the use of visible light is more flexible than chemical triggers, allowing precise temporal control over the initiation and termination of proximity labeling on the timescale of minutes to seconds.[15–17] Notably, photocatalytic proximity labeling described in this review should not be confused with photoaffinity labeling, which relies on the direct photolysis of photoactivatable probes (e.g., diazirine,[18] benzophenone[19]), resulting in one-to-one conjugations between the small molecule and its interacting protein targets.[20] In contrast, photocatalysis allows multiple rounds of proximity labeling from the protein of interest, leading to a more efficient one-to-many labeling mechanism of identifying protein–protein interactions.

Depending on the nature of catalysts, photocatalytic proximity labeling technologies can be divided into two categories: synthetic small-molecule photocatalysts and protein-based photocatalysts. In this review, we summarize existing photocatalytic proximity labeling methods and highlight their mechanisms of action, characteristics of performance, and biological applications in the profiling of subcellular organizations.

[a] *Dr. Y. Fang, Prof. Dr. P. Zou*
College of Chemistry and Molecular Engineering
Synthetic and Functional Biomolecules Center
Beijing National Laboratory for Molecular Sciences
Key Laboratory of Bioorganic Chemistry and
Molecular Engineering of Ministry of Education
Peking University
Beijing 100871 (P. R. China)
E-mail: zoupeng@pku.edu.cn
[b] *Prof. Dr. P. Zou*
Academy for Advanced Interdisciplinary Studies
PKU-Tsinghua Center for Life Science
PKU-IDG/McGovern Institute for Brain Research
Peking University
Beijing 100871 (P. R. China)
[c] *Prof. Dr. P. Zou*
Chinese Institute for Brain Research (CIBR)
Beijing 102206 (P. R. China)

This article is part of the Special Collection ChemBioTalents2022. Please see our homepage for more articles in the collection.

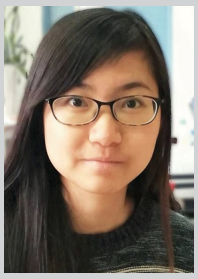
2. Small-Molecule Photocatalysts for Proximity Labeling

2.1. Transition metal complexes

Transition metal complexes have been used in photoredox catalysis for more than a decade. Notably, the visible-light-driven photochemistry is well suited for biological samples due to the long wavelength of peak absorbance, which avoid the UV bands that are damaging to nucleic acids and amino acids. Recently, McMillan and co-workers have developed a photocatalytic labeling strategy called μMAP, where an iridium complex is excited by blue light (450 nm) and subsequently converts diazirines into reactive carbenes by using the Dexter energy transfer mechanism[16] (Figure 2a). Carbenes can readily crosslink with nearby biomolecules and are rapidly quenched by water, resulting in a diffusion radius as small as 4 nm (a half-life of 2 ns in aqueous solution[21]), which is suitable for mapping the microenvironment surrounding the photocatalyst. In μMAP, the Ir complex was covalently conjugated with secondary antibodies. Using primary antibodies to recognize marker proteins on the cell membrane, the photocatalyst was successfully targeted for profiling the cell surface proteome. Similarly, photocatalytic labeling strategies using red light (660 nm) to generate aniline radicals or triplet nitrenes from aryl azides by electron transfer mechanisms have also been developed for microenvironment mapping on cell membranes[22,23] (Figure 2a). To better match with the azide reduction potential, the photocatalyst of μMAP was replaced with tin or osmium complexes in these derivative methods[22,23] (Figure 2a). CAT-Prox, a proximity labeling strategy developed by Chen and co-workers, undergoes a unique photo-decaging mechanism.[24] Upon the irradiation of blue light (450 nm), the Ir complex catalyzes the transformation of aryl azide to aniline, which is followed by the rapid decaging of para-azidobenzyl group to unveil a reactive quinone methide intermediate (Figure 2a). As a Michael acceptor, quinone methide primarily targets amino acid residues with nucleophilic side-chains, including lysine, serine, etc.[25] Owing to the selective accumulation of Ir complexes in the mitochondria, CAT-Prox allows profiling mitochondrial proteome dynamics in macrophages upon inflammatory stimulation.[24]

2.2. Synthetic organic chromophores

Besides transition metal complexes, many synthetic organic chromophores have served as photocatalysts, where they undergo singlet oxygen pathway and/or free radical pathway (Figure 2b). In both pathways, chromophores are excited by visible light into an excited singlet state (S_1), which subsequently transitions into a triplet state (T_1) through intersystem crossing. If the excited chromophore in T_1 state is quenched by ground state molecular oxygen through energy transfer, singlet oxygen could be generated, which causes oxidation of local biomolecules. The resulting photo-oxidation intermediates could be covalently captured by small-molecule probes harboring nucleophilic groups (e.g., hydrazide, aniline, primary amine; Figure 2b). In 2016, Tsien and co-workers achieved photocatalytic proximity labeling of NP41-binding proteins by conjugating methylene blue with the nerve-binding peptide NP41 and using a hydrazide probe for capturing the photo-oxidized targets.[26] Figure 2c lists several classic small-molecule singlet oxygen generators, including dibromofluorescein (DBF),[27] Eosin Y,[27] and Methylene blue.[28] In general, halogen substitutions or the replacement of oxygen with sulfur can increase singlet oxygen quantum yield (Φ_Δ) due to the "heavy atom effect".[27] For example, halogenation of fluorescein ($\Phi_\Delta = 0.03$) generates classic photosensitizers DBF ($\Phi_\Delta = 0.42$), eosin Y ($\Phi_\Delta = 0.57$) and Rose Bengal ($\Phi_\Delta = 0.75$).[27] When paired with suitable nucleophiles including hydrazide, aniline, primary amine and 1-methyl-4-arylurazole (MAUra; Figure 2d), these singlet oxygen generators have been successfully applied to profiling the nuclear[29,30] and cell-surface proteomes.[31] In the free radical pathway, chromophores in the T_1 state interacts with a small-molecule probe through either energy transfer or electron transfer, thus transforming the probe into a reactive free radical that can directly conjugate with nearby biomolecules (Figure 2b). Examples of proximity labeling through the free radical pathway are relatively new. In 2021, Chen and co-workers employed the photocatalytic energy transfer between organic dyes and aryl azides to generate triplet nitrenes for labeling proteins (Figure 2e).[32] In 2022, Fadeyi and co-workers reported a cell tagging approach where phenoxy free radicals were generated by single



Yuxin Fang received her Ph.D. in chemical biology from Peking University in 2022. She is currently a postdoctoral fellow at the College of Chemistry and Molecular Engineering, Peking University. Her research interest lies in protein engineering and nucleic acid modifications. Her current research project focuses on developing novel photocatalytic proximity labeling tools through directed evolution.

Peng Zou completed his Ph.D. at Massachusetts Institute of Technology in 2012. Following postdoctoral training at Harvard University, he joined Peking University in 2015 as an assistant professor in the College of Chemistry and Molecular Engineering, and was promoted to Associate Professor with tenure in 2021. His research focuses on inventing chemical tools for the high-resolution mapping of biomolecules and biophysical signaling that underlie neuronal functions. He was the recipient of the ACS C&EN's Talented 12 Award in 2019.

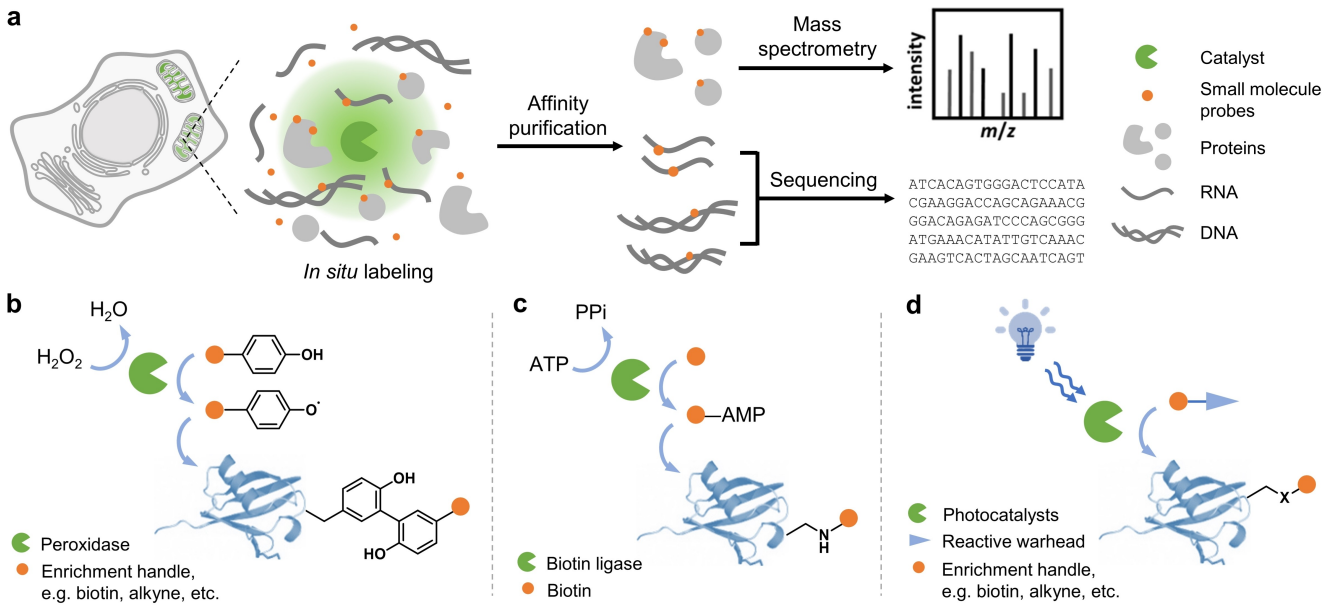


Figure 1. Mechanisms and categories of proximity labeling methods. a) Proximity labeling scheme for investigating subcellular proteomes, transcriptomes and chromosomal organization. Proximity labeling methods based on b) peroxidases, c) biotin ligases, and d) photocatalysts.

electron transfer (SET) from the biotin-phenol probe to the excited state flavin molecule (Figure 2e).[33]

2.3. Outlook for small-molecule photocatalysts

A common problem with small-molecule photocatalysts is the difficulty of achieving efficient subcellular targeting. Positively charged and hydrophobic small-molecule photocatalysts, such as rhodamine-123, have a high propensity of accumulating in the mitochondria owing to the electrostatic effect. However, targeting to other subcellular locations has remained challenging and requires conjugating the small-molecule photocatalysts with targeting moieties. For example, DNA intercalators or ligands to specific cell surface receptors have been used for targeting proximity labeling to the nucleus[29] or the cell membrane.[31] However, these approaches rely heavily on the availability of highly specific targeting molecules, thus constraining the applicability to many subcellular locations. Antibody-conjugated photocatalysts offer an alternative solution, but still suffer from poor cell membrane permeability of antibodies, limiting their applications to the cell membrane[16,22–23,33] and isolated nuclei.[34] Finally, self-labeling protein tags, such as Halo-tag[35] and SNAP-tag[36] have served as targeting moieties. For example, a DBF-conjugated Halo ligand could be covalently tethered to the genetically encoded Halo-tag, allowing proximity labeling in the nucleoplasm, nucleolus and cytoplasm.[37] It should be noted that applying small-molecule photocatalysts often causes high background labeling due to non-specific adsorption, which may introduce false-positives in the dataset. This situation is further complicated by the strong mitochondrial retention of positively charged molecules, thus disqualifying many photocatalyst candidates as generally applicable tools. Such inflexibility in targeting small-molecule photocatalysts has severely prohibited the inclusion of spatial controls for MS analysis, as is typically performed when using APEX/TurboID.[38]

Future optimization of small-molecule photocatalysts could rely on introducing novel chromophores with low nonspecific adsorption. The biophysical properties of small-molecule compounds can be modulated by rationally designing and screening various chemical derivatives. For example, to solve the problem of background adsorption in bioimaging, Chang and co-workers have developed a series of fluorescent dyes with minimal background through a combination of high-throughput screening and cheminformatics.[39,40] Recent work by Lavis and co-workers has revealed general principles for the rational design of photosensitizers from synthetic fluorescent dyes.[41] It is expected that photocatalysts with other optimized characteristics, such as boosted catalytic efficiency, better substrate selectivity, or longer wavelength of peak absorption could be similarly developed through rational design and screening.

3. Genetically Encoded Photocatalysts for Proximity Labeling

3.1. Mechanisms and applications of genetically encoded photocatalysts

MiniSOG is an engineered flavin-binding protein originally designed as a photosensitizer for generating electron microscopy contrast (Figure 3a). Upon blue light illumination, the flavin mononucleotide (FMN) cofactor of miniSOG is activated

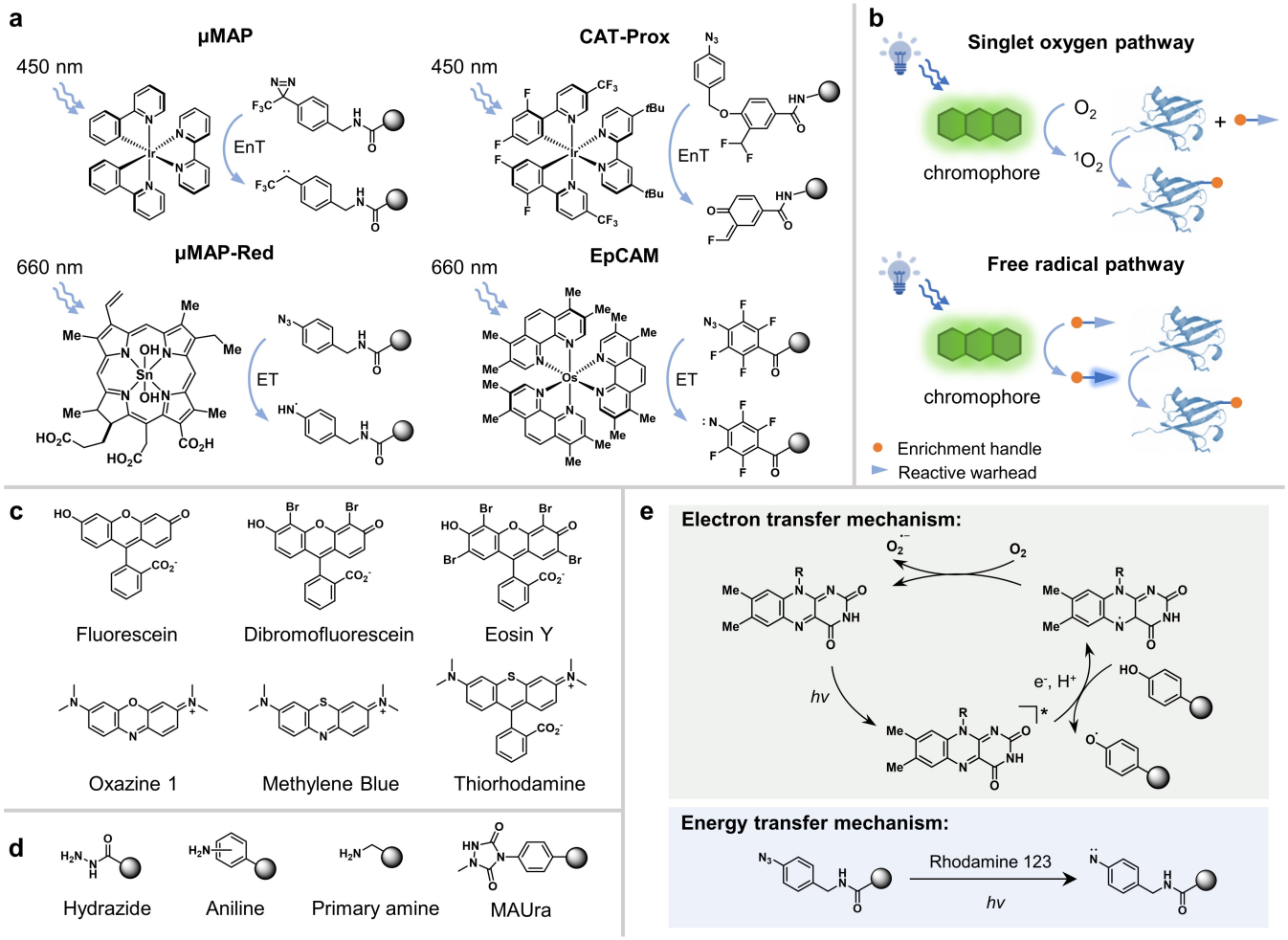


Figure 2. Small-molecule photocatalysts for proximity labeling. a) Photocatalytic reactions of transition metal complexes for the profiling of subcellular proteomes. EnT: energy transfer, ET: electron transfer. b) Two different mechanisms of photocatalytic proximity labeling by synthetic organic chromophores. c) Chromophores and d) chemical probes commonly used in photocatalytic proximity labeling by the singlet-oxygen-generating pathway. e) Mechanisms of radical generation in photocatalytic proximity labeling by synthetic organic chromophores.

and catalyzes the generation of singlet oxygen by the energy transfer pathway. In a putative mechanism, diaminobenzidine (DAB) is oxidized by singlet oxygen and forms the localized osmiophilic polymers which are resolvable by electron microscopy.[42]

In recent years, miniSOG has been employed as a genetically encoded photocatalyst for proximity-dependent RNA labeling.[15] The singlet oxygen generated in situ is capable of oxidizing guanosine base in nearby RNA molecules, resulting in the formation of guanine oxidative damages, including oxazolone, imidazolone and spiroiminodihydantoin (Sp).[15] As a major terminal product of 8-oxo-7,8-dihydroguanine (8-oxoG) oxidation, Sp is formed by the nucleophilic addition of water to the quinonoid intermediate 8-oxoguanine oxidized (8-oxoGox).[43] In the presence of an amine probe, the photo-oxidized RNA could be intercepted by the nucleophilic addition of amine groups, which enables the conjugation of bioorthogonal handles to RNA molecules (Figure 3b). The chemically tagged RNAs were enriched by affinity purification and identified by high-throughput sequencing. This labeling method was named as chromophore-assisted proximity labeling and sequencing (CAP-seq). miniSOG was genetically targeted to the mitochondrial matrix, outer mitochondrial membrane and endoplasmic reticulum membrane to capture local transcriptome, revealing that transcripts encoding ribosomal proteins and oxidative phosphorylation pathway proteins are highly enriched at the outer mitochondrial membrane.[15]

MiniSOG-mediated nucleic acid photo-oxidation has been extended to label DNA molecules in live cells.[44] To overcome the relatively low reactivity of double-stranded DNA, a mutant of miniSOG with improved singlet oxygen quantum yield, named SOPP2,[45] was used for labeling DNA in the mitochondrial matrix, nucleoplasm, nuclear lamina and nucleolus. Upon the blue light irradiation, DNA molecules were covalently tagged with propargylamine, clicked with biotin handles, enriched by affinity purification and identified by sequencing (Figure 3b). This labeling method allows the investigation of subnuclear chromosome organization in

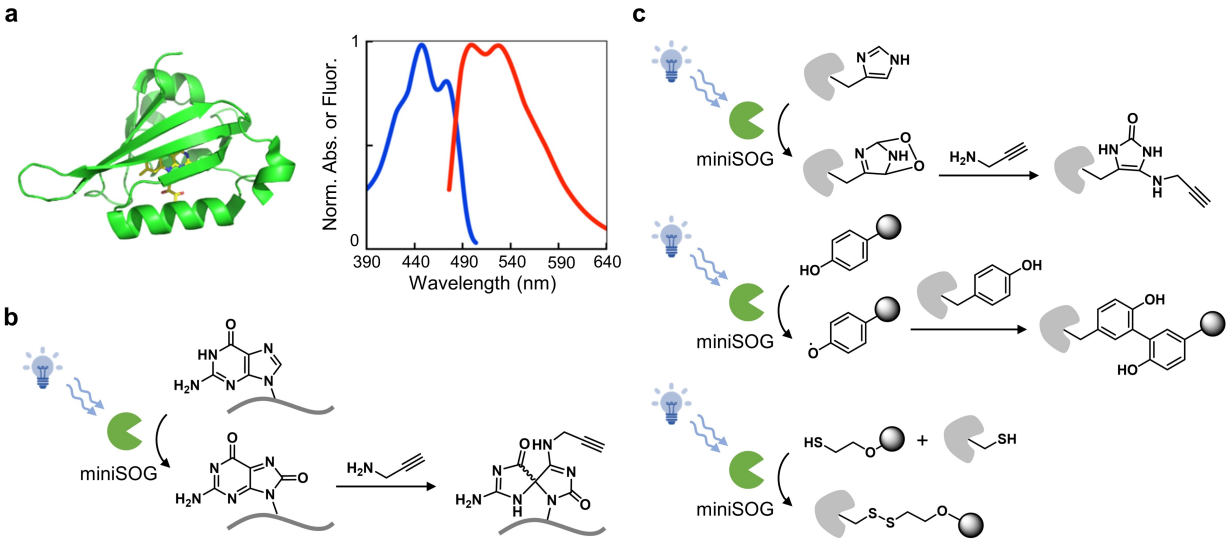


Figure 3. MiniSOG-mediated photocatalytic proximity labeling for the profiling of subcellular multi-omics. a) Crystal structure of miniSOG (PDB ID: 6GPU, left) and the normalized absorption and emission spectra of miniSOG (right, blue: absorption spectrum, red: emission spectrum). Reproduced with permission from ref. [42]. Copyright: 2011, PLOS. b) Mechanism of miniSOG-mediated photocatalytic labeling of nucleic acids with a primary amine probe. c) Mechanisms of miniSOG-mediated proximity labeling of amino acid residues with a propargylamine probe (upper), a phenol probe (middle), and a thiol probe (lower).

living cells. When applied to the nuclear lamina, CAP-seq successfully identify lamina-interacting DNA that are characterized with repressive heterochromatins, such as enrichment of histone 3 lysine 9 dimethylation (H3 K9me2) and depletion of CpG islands.[46]

More recently, miniSOG has been extended to label proteins in living cells. The singlet oxygen generated in situ could oxidize not only nucleobases, but also amino acid residues of nearby proteins. Histidine is one of the major targets of photo-oxidation. Diels–Alder addition of singlet oxygen to the imidazole ring of histidine yields an endoperoxide intermediate, which can crosslink with primary amines through a nucleophilic substitution at the C4 position (Figure 3c). Similar to the procedure of transcriptome profiling, the tagged proteins were enriched by affinity purification and identified by liquid chromatography-tandem mass spectrometry (LC–MS/MS). This labeling method was named as RinID (reactive oxygen species induced protein labeling and identification).[47] Besides primary amine, other nucleophiles like aniline and hydrazide have also been shown as efficient probes for miniSOG-mediated protein labeling.[48] Notably, miniSOG-based proximity labeling can be achieved through other mechanisms with altered selectivity towards amino acid residues. For example, Muir and co-workers took advantage of the SET reaction of FMN and performed the photocatalytic proximity labeling with a biotin-phenol probe, which primarily targeted tyrosine residues[17] (Figure 3c). Earlier in 2016, Shu and co-workers reported a labeling strategy using the biotin-conjugated thiol probe, which selectively formed disulfide bonds with cysteine residues[49] (Figure 3c).

3.2. Outlook for genetically encoded photocatalysts

Genetically encoded photocatalysts offer both convenience in subcellular targeting and high spatial specificity due to the avoidance of small-molecule adsorption. However, applications of genetically encoded photocatalysts have been hindered by limited choices of photosensitive proteins. While there are plenty of chromophore-containing proteins in nature, most of them exhibit little to no capability of generating reactive oxygen species (ROS). For example, green fluorescent protein (GFP) and many of its homologs have been shown to be inefficient photosensitizers.[50,51] Flavin-binding fluorescent proteins (FbFPs), which are derived from the same light, oxygen, voltage (LOV) photoreceptor domain as miniSOG, are regarded as potential photosensitizers but would require further engineering to improve labeling efficiency.[52] This is understandable as there was no evolutionary driving force for improving the ROS quantum yield in nature. Directed evolution is an effective strategy for improving or altering the activity of biomolecules. Therefore, development of genetically encoded photocatalysts could rely on the optimization of photosensitive proteins by directed evolution.

Key steps of directed evolution include gene diversification, protein expression and screening or selection for mutants with desired phenotypes. Genes of the winner mutants were replicated and serve as starting points for subsequent rounds of evolution (Figure 4). During the directed evolution of photosensitizers, since the enrichment handles of the small-molecule probes are often biotin or other clickable groups that can be conjugated with biotin, labeling activities can be determined by the level of biotinylation, which can be further converted into fluorescence or binding affinities as the readout of screening

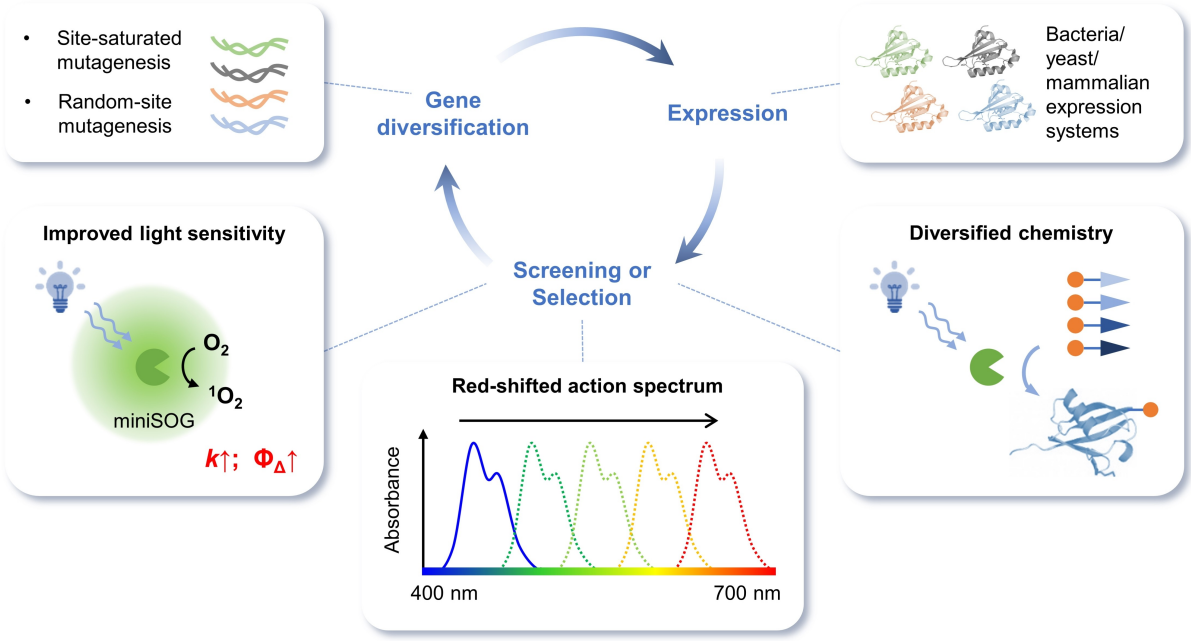


Figure 4. Extending the toolbox of photocatalytic proximity labeling by directed evolution of photosensitive proteins.

or selection. Taking the yeast-display platform as an example, a mutant library can be displayed on the surface of yeast cells and promiscuously biotinylate the yeast surface proteins through photocatalytic proximity labeling. After staining with fluorescently labelled streptavidin, active mutants with stronger fluorescence can be selected by fluorescence-activated cell sorting (FACS). It is worth noting that the directed evolution of proximity labeling enzymes, such as the engineering of APEX2[7] and TurboID,[9] typically focuses on improving the activity of labeling protein targets. However, labeling of RNA and DNA is equally appealing in the applications of photocatalysts. Therefore, novel selection platforms are required for optimizing the activities of labeling nucleic acid targets.

Improvements to genetically encoded photocatalysts could be made on the following fronts (Figure 4). First, published tools generally require 15–20 min illumination for the activation of miniSOG.[15,44,48] It is reported that the time window of labeling can be shorten down to 5 min when an engineered miniSOG mutant, SOPP3 was used.[47] Evolving more efficient photosensitizers with improved light sensitivity could decrease the required dosage of light (i.e., reduce the light intensity and/or shorten the illumination time), which not only reduces the cytotoxicity caused by blue light, but also allows the investigation of cellular processes with fast dynamics. Second, none of the current photocatalytic proximity labeling tools have been demonstrated in living animals, which can be attributed to the poor tissue penetration of blue light. To overcome this problem, engineering of photosensitizers with red-shifted action spectra is highly sought after. Third, considering the diverse photochemistry of chromophores, it is expected that the substrates of photocatalytic proximity labeling can be further expanded through using a variety of small-molecule probes with altered chemical properties (e.g., redox potentials, nucleophilic reactivity, electrical properties, etc.). Directed evolution of photosensitizers can be performed to acquire different engineered versions that best match with each probe.

4. Summary

Overall, small-molecule photocatalysts and genetically encoded photocatalysts have complementary strengths and limitations. One of the major advantages of small-molecule photocatalysts is the avoidance of genetic manipulation, which is valuable when dealing with experimental materials that are difficult to be transfected, such as clinical tissue samples. However, applications of small-molecule photocatalysts are often limited to cell-surface proteomes due to their poor targeting abilities. In contrast, genetically encoded photosensitizers are more suitable and flexible for intracellular applications, especially when reliable antibodies are lacking.

A critical factor in the selection of photocatalytic proximity labeling methods is the labeling radius, which depends heavily on the nature of reactive intermediates generated by photocatalytic reactions. Generally, reactive intermediates with shorter half-lives are quenched more rapidly in solvents, thus resulting in a smaller labeling radius. For example, the highly reactive carbene radicals (with a half-life of ~2 ns) have the smallest labeling radius of the probes discussed in this review, and yield fewer identified proteins with higher enrichment levels than other methods.[53] In comparison, triplet nitrenes,

aniline radicals and phenol radicals are relatively long-lived, leading to larger labeling scopes.[53] It is also reported that the labeling radius can be fine-tuned by altering the length of the poly(ethylene glycol) (PEG) linker within the small-molecule probes.[53] However, increasing linker lengths might cause a reduction in cell membrane permeability,[54] which should be considered carefully if the photocatalytic proximity labeling is carried out within cells.

Acknowledgements

This work was supported by the Ministry of Science and Technology (2022YFA1304700, 2018YFA0507600, 2017YFA0503600), the National Natural Science Foundation of China (32088101, 21727806). P.Z. is sponsored by Bayer Investigator Award.

Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: photocatalysts · proteomics · proximity labeling · subcellular organization · transcriptome profiling

[1] M. L. Qi, K. Tagawa, Y. Enokido, N. Yoshimura, Y. Wada, K. Watase, S. Ishiura, I. Kanazawa, J. Botas, M. Saitoe, E. E. Wanker, H. Okazawa, *Nat. Cell Biol.* **2007**, *9*, 402–414.
[2] J. Palmfeldt, P. Bross, *Mitochondrion* **2017**, *33*, 2–14.
[3] Y. Ding, S. Zhang, L. Yang, H. Na, P. Zhang, H. Zhang, Y. Wang, Y. Chen, J. Yu, C. Huo, S. Xu, M. Garaiova, Y. Cong, P. Liu, *Nat. Protoc.* **2013**, *8*, 43–51.
[4] C. Biesemann, M. Gronborg, E. Luquet, S. P. Wichert, V. Bernard, S. R. Bungers, B. Cooper, F. Varoqueaux, L. Li, J. A. Byrne, H. Urlaub, O. Jahn, N. Brose, E. Herzog, *EMBO J.* **2014**, *33*, 157–170.
[5] K. H. Loh, P. S. Stawski, A. S. Draycott, N. D. Udeshi, E. K. Lehrman, D. K. Wilton, T. Svinkina, T. J. Deerinck, M. H. Ellisman, B. Stevens, S. A. Carr, A. Y. Ting, *Cell* **2016**, *166*, 1295–1307, e1221.
[6] J. D. Martell, T. J. Deerinck, Y. Sancak, T. L. Poulos, V. K. Mootha, G. E. Sosinsky, M. H. Ellisman, A. Y. Ting, *Nat. Biotechnol.* **2012**, *30*, 1143–1148.
[7] S. S. Lam, J. D. Martell, K. J. Kamer, T. J. Deerinck, M. H. Ellisman, V. K. Mootha, A. Y. Ting, *Nat. Methods* **2015**, *12*, 51–54.
[8] K. J. Roux, D. I. Kim, M. Raida, B. Burke, *J. Cell Biol.* **2012**, *196*, 801–810.
[9] T. C. Branon, J. A. Bosch, A. D. Sanchez, N. D. Udeshi, T. Svinkina, S. A. Carr, J. L. Feldman, N. Perrimon, A. Y. Ting, *Nat. Biotechnol.* **2018**, *36*, 880–887.
[10] H. W. Rhee, P. Zou, N. D. Udeshi, J. D. Martell, V. K. Mootha, S. A. Carr, A. Y. Ting, *Science* **2013**, *339*, 1328–1331.
[11] D. I. Kim, K. C. Birendra, W. Zhu, K. Motamedchaboki, V. Doye, K. J. Roux, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E2453–2461.
[12] B. T. Lobingier, R. Huttenhain, K. Eichel, K. B. Miller, A. Y. Ting, M. von Zastrow, N. J. Krogan, *Cell* **2017**, *169*, 350–360 e312.
[13] J. Paek, M. Kalocsay, D. P. Staus, L. Wingler, R. Pascolutti, J. A. Paulo, S. P. Gygi, A. C. Kruse, *Cell* **2017**, *169*, 338–349 e311.
[14] Y. Liu, R. Zeng, R. Wang, Y. Weng, R. Wang, P. Zou, P. R. Chen, *Proc. Natl. Acad. Sci. USA* **2021**, *118*.
[15] P. Wang, W. Tang, Z. Li, Z. Zou, Y. Zhou, R. Li, T. Xiong, J. Wang, P. Zou, *Nat. Chem. Biol.* **2019**, *15*, 1110–1119.
[16] J. B. Geri, J. V. Oakley, T. Reyes-Robles, T. Wang, S. J. McCarver, C. H. White, F. P. Rodriguez-Rivera, D. L. Parker Jr., E. C. Hett, O. O. Fadeyi, R. C. Oslund, D. W. C. MacMillan, *Science* **2020**, *367*, 1091–1097.
[17] N. Hananya, X. Ye, S. Koren, T. W. Muir, *bioRxiv* **2022**, preprint, DOI: 10.1101/2022.07.30.502153.
[18] S. S. Ge, B. Chen, Y. Y. Wu, Q. S. Long, Y. L. Zhao, P. Y. Wang, S. Yang, *RSC Adv.* **2018**, *8*, 29428–29454.
[19] G. Dorman, H. Nakamura, A. Pulsipher, G. D. Prestwich, *Chem. Rev.* **2016**, *116*, 15284–15398.
[20] N. R. Burton, P. Kim, K. M. Backus, *Org. Biomol. Chem.* **2021**, *19*, 7792–7809.
[21] A. Admasu, A. D. Gudmundsdóttir, M. S. Platz, D. S. Watt, S. Kwiatkowski, P. J. Crocker, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1093–1100.
[22] B. F. Buksh, S. D. Knutson, J. V. Oakley, N. B. Bissonnette, D. G. Oblinsky, M. P. Schwoerer, C. P. Seath, J. B. Geri, F. P. Rodriguez-Rivera, D. L. Parker, G. D. Scholes, A. Ploss, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2022**, *144*, 6154–6162.
[23] N. E. S. Tay, K. A. Ryu, J. L. Weber, A. K. Olow, D. C. Cabanero, D. R. Reichman, R. C. Oslund, O. O. Fadeyi, T. Rovis, *Nat. Chem.* **2022**, *15*, 101–109.
[24] Z. Huang, Z. Liu, X. Xie, R. Zeng, Z. Chen, L. Kong, X. Fan, P. R. Chen, *J. Am. Chem. Soc.* **2021**, *143*, 18714–18720.
[25] J. Liu, L. Cai, W. Sun, R. Cheng, N. Wang, L. Jin, S. Rozovsky, I. B. Seiple, L. Wang, *Angew. Chem. Int. Ed.* **2019**, *58*, 18839–18843; *Angew. Chem.* **2019**, *131*, 19015–19019.
[26] H. L. Glasgow, M. A. Whitney, L. A. Gross, B. Friedman, S. R. Adams, J. L. Crisp, T. Hussain, A. P. Frei, K. Novy, B. Wollscheid, Q. T. Nguyen, R. Y. Tsien, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 12774–12779.
[27] E. Gandin, Y. Lion, A. Vandevorst, *Photochem. Photobiol.* **1983**, *37*, 271–278.
[28] C. M. L. Francisco, J. M. L. A. Gonçalves, B. S. Brum, T. P. C. Santos, A. Lino-dos-Santos-Franco, D. F. T. Silva, C. Pavani, *New J. Chem.* **2017**, *41*, 14438–14443.
[29] T. Tamura, M. Takato, K. Shiono, I. Hamachi, *Chem. Lett.* **2020**, *49*, 145–148.
[30] M. Tsushima, S. Sato, K. Miura, T. Niwa, H. Taguchi, H. Nakamura, *Chem. Commun. (Camb.)* **2022**, *58*, 1926–1929.
[31] M. Muller, F. Grabnitz, N. Barandun, Y. Shen, F. Wendt, S. N. Steiner, Y. Severin, S. U. Vetterli, M. Mondal, J. R. Prudent, R. Hofmann, M. van Oostrum, R. C. Sarott, A. I. Nesvizhskii, E. M. Carreira, J. W. Bode, B. Snijder, J. A. Robinson, M. J. Loessner, A. Oxenius, B. Wollscheid, *Nat. Commun.* **2021**, *12*, 7036.
[32] H. Wang, Y. Zhang, K. Zeng, J. Qiang, Y. Cao, Y. Li, Y. Fang, Y. Zhang, Y. Chen, *JACS Au* **2021**, *1*, 1066–1075.
[33] R. C. Oslund, T. Reyes-Robles, C. H. White, J. H. Tomlinson, K. A. Crotty, E. P. Bowman, D. Chang, V. M. Peterson, L. Li, S. Frutos, M. Vila-Perello, D. Vlerick, K. Cromie, D. H. Perlman, S. Ingale, S. D. O. Hara, L. R. Roberts, G. Piizzi, E. C. Hett, D. J. Hazuda, O. O. Fadeyi, *Nat. Chem. Biol.* **2022**, *18*, 850–858.
[34] C. P. Seath, A. J. Burton, D. W. C. MacMillan, T. W. Muir, *bioRixv* **2021**, preprint, DOI: 10.1101/2021.09.28.462236.
[35] G. V. Los, L. P. Encell, M. G. McDougall, D. D. Hartzell, N. Karassina, C. Zimprich, M. G. Wood, R. Learish, R. F. Ohana, M. Urh, D. Simpson, J. Mendez, K. Zimmerman, P. Otto, G. Vidugiris, J. Zhu, A. Darzins, D. H. Klaubert, R. F. Bulleit, K. V. Wood, *ACS Chem. Biol.* **2008**, *3*, 373–382.
[36] A. Keppler, S. Gendreizig, T. Gronemeyer, H. Pick, H. Vogel, K. Johnsson, *Nat. Biotechnol.* **2003**, *21*, 86–89.
[37] K. L. Engel, H. G. Lo, R. Goering, Y. Li, R. C. Spitale, J. M. Taliaferro, *Nucleic Acids Res.* **2022**, *50*, e24.
[38] V. Hung, N. D. Udeshi, S. S. Lam, K. H. Loh, K. J. Cox, K. Pedram, S. A. Carr, A. Y. Ting, *Nat. Protoc.* **2016**, *11*, 456–475.
[39] S. H. Alamudi, R. Satapathy, J. Kim, D. Su, H. Ren, R. Das, L. Hu, E. Alvarado-Martinez, J. Y. Lee, C. Hoppmann, E. Pena-Cabrera, H. H. Ha, H. S. Park, L. Wang, Y. T. Chang, *Nat. Commun.* **2016**, *7*, 11964.
[40] S. H. Alamudi, D. Su, K. J. Lee, J. Y. Lee, J. L. Belmonte-Vazquez, H. S. Park, E. Pena-Cabrera, Y. T. Chang, *Chem. Sci.* **2018**, *9*, 2376–2383.
[41] T. C. Binns, A. X. Ayala, J. B. Grimm, A. N. Tkachuk, G. A. Castillon, S. Phan, L. Zhang, T. A. Brown, Z. Liu, S. R. Adams, M. H. Ellisman, M. Koyama, L. D. Lavis, *Cell Chem. Biol.* **2020**, *27*, 1063–1072 e1067.
[42] X. Shu, V. Lev-Ram, T. J. Deerinck, Y. Qi, E. B. Ramko, M. W. Davidson, Y. Jin, M. H. Ellisman, R. Y. Tsien, *PLoS Biol.* **2011**, *9*, e1001041.
[43] W. Luo, J. G. Muller, E. M. Rachlin, C. J. Burrows, *Org. Lett.* **2000**, *2*, 613–616.

[44] T. Ding, L. Zhu, Y. Fang, Y. Liu, W. Tang, P. Zou, *Angew. Chem. Int. Ed.* **2020**, *59*, 22933–22937; *Angew. Chem.* **2020**, *132*, 23133–23137.
[45] M. Westberg, M. Bregnhoj, M. Etzerodt, P. R. Ogilby, *J. Phys. Chem. B* **2017**, *121*, 9366–9371.
[46] A. J. Bannister, T. Kouzarides, *Cell Res.* **2011**, *21*, 381–395.
[47] F. Zheng, C. Yu, X. Zhou, P. Zou, *bioRxiv* preprint, **2022**, DOI: 10.1101/2022.08.01.502286v1.
[48] Y. Zhai, X. Huang, K. Zhang, Y. Huang, Y. Jiang, J. Cui, Z. Zhang, C. K. C. Chiu, W. Zhong, G. Li, *Nat. Commun.* **2022**, *13*, 4906.
[49] T. L. To, K. F. Medzihradszky, A. L. Burlingame, W. F. DeGrado, H. Jo, X. Shu, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3359–3363.
[50] A. Jimenez-Banzo, S. Nonell, J. Hofkens, C. Flors, *Biophys. J.* **2008**, *94*, 168–172.
[51] X. Ragas, L. P. Cooper, J. H. White, S. Nonell, C. Flors, *ChemPhysChem* **2011**, *12*, 161–165.
[52] S. Endres, M. Wingen, J. Torra, R. Ruiz-Gonzalez, T. Polen, G. Bosio, N. L. Bitzenhofer, F. Hilgers, T. Gensch, S. Nonell, K. E. Jaeger, T. Drepper, *Sci. Rep.* **2018**, *8*, 15021.
[53] J. V. Oakley, B. F. Buksh, D. F. Fernandez, D. G. Oblinsky, C. P. Seath, J. B. Geri, G. D. Scholes, D. W. C. MacMillan, *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2203027119.
[54] V. G. Klein, C. E. Townsend, A. Testa, M. Zengerle, C. Maniaci, S. J. Hughes, K. H. Chan, A. Ciulli, R. S. Lokey, *ACS Med. Chem. Lett.* **2020**, *11*, 1732–1738.

Manuscript received: December 14, 2022
Revised manuscript received: February 3, 2023
Accepted manuscript online: February 10, 2023
Version of record online: March 24, 2023