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Light-Switchable Ion Channels and Receptors for Optogenetic Interrogation of Neuronal Signaling

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Abstract

Optogenetics is an emerging technique that enables precise and specific control of biological activities in defined space and time. This technique employs naturally occurring or engineered light-responsive proteins to manipulate the physiological processes of the target cells. To better elucidate the molecular bases of neural functions, substantial efforts have been made to confer light sensitivity onto ion channels and neurotransmitter receptors that mediate signaling events within and between neurons. The chemical strategies for engineering light-switchable channels/receptors and the neuronal implementation of these tools are discussed.

Graphical Abstract

1. INTRODUCTION

Nature employs a variety of photoreceptor proteins, such as opsins and phytochromes, to trigger signal transduction in response to light stimuli. These proteins are equipped with a chromophore (e.g., retinal, flavins, tetrapyrroles, etc.) which can reversibly isomerize upon exposure to light. For example, channelrhodopsin-2 from green algae is a light-gated cation channel, and halorhodopsin from archaea is a light-gated chloride pump.¹ Their light sensitivity is endowed by retinal which is covalently attached to a lysine residue through the formation of a Schiff base. Activation of these proteins is driven by the conversion of all-*trans*-retinal to its 13-*cis*-isomer, which is triggered upon exposure to ~470 nm light for channelrhodopsin-2 and ~570 nm light for halorhodopsin. Once the irradiation is terminated,

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13-*cis*-retinal spontaneously relaxes back to the *all-trans* configuration, and the proteins subsequently return to their inactive state (Figure 1a).

In recent years, several microbial opsins and their engineered variants have been widely used as tools for optogenetics, a revolutionary technique that employs light to precisely control biological functions in defined space and time.^{1–4} Using opsins as light-gated actuators, optogenetics was initially invented to elicit or silent action potential firing of neurons by triggering membrane depolarization or hyperpolarization. Benefitted by the high spatial and temporal resolutions of optical technologies, the manipulation of neuronal firing can be programmed with user-defined patterns.³ Moreover, photocontrol can be further confined by selectively expressing these tools in a defined cell type, enabling functional investigation of a specific neuron population or mapping of neuron–neuron connectivity within a complex circuit.³ These features together have made optogenetics exceptionally powerful for various neurobiological applications.

In addition to neuronal firing, optogenetics is also employed to manipulate other biological functions such as signal transmission, gene expression, and cellular physiology for the purpose of elucidating their impacts or mechanisms.^{4–6} In these applications, light targets the proteins that are endogenously involved in the signaling pathway or the physiological process, and the functions of the manipulated proteins will be manifested in the phenotypical changes upon illumination. In this Topical Review, we summarize the chemical strategies used for conferring light sensitivity onto ion channels and neurotransmitter receptors, two important classes of signaling mediators in the nervous system. Analogous to the assembly of opsins, light-switchable ion channels and neurotransmitter receptors (which are naturally “blind”) can be engineered by tethering a synthetic photoswitch to a genetically encoded conjugation site (Figure 1b–e). Through this built-in photoswitch, the function of the channel/receptor can be remotely and reversibly controlled in a light-dependent manner. These *chemical optogenetic* tools (alternatively named *optogenetic pharmacology* tools by neuroscientists)⁵ provide a powerful means for uncovering the unique roles of different signaling mediators in neural functions at the molecular, cellular, and organismic levels.

2. STRATEGIES FOR ENGINEERING LIGHT-SWITCHABLE ION CHANNELS AND RECEPTORS

The general principle of chemical optogenetics is that a synthetic photoswitch is attached to a genetically encoded bioconjugation site (e.g., cysteine or tag) in the protein of interest (Figure 1). For ion channels and neurotransmitter receptors, the installed photoswitches typically carry a ligand (pharmacophore) such as an agonist, antagonist, blocker, or allosteric modulator. The chromophore used in chemical optogenetics has mainly been azobenzene, a simple binary unit that undergoes reversible isomerization between *cis* and *trans* states in response to UV and visible light, respectively. *Trans*–*cis* isomerization significantly alters the geometry (shape and length) and the polarity of azobenzene. These changes in turn alter the accessibility or efficacy of the ligand linked/fused to azobenzene, thereby modulating channel/receptor function in a light-dependent manner. In some cases, the photoswitch does

not exert pharmacological effect but instead causes structural changes in the target protein upon light switching.

To date, most of the light-switchable channels/receptors are engineered by conjugating a Photoswitchable Tethered Ligand (PTL) to a strategically located cysteine. A different approach has recently been reported, wherein a Photoswitchable Orthogonal Remotely Tethered Ligand (PORTL) is attached to a self-labeling tag (e.g., SNAP or CLIP) fused to one terminus of the target protein. The advantages and limitations of each approach are discussed in detail below.

Tethering PTL to a Cysteine.

As illustrated in Figure 1c, a PTL has three essential components linked in sequence: (1) a sulfhydryl-reactive group (e.g., maleimide) for conjugation to the cysteine; (2) an azobenzene for exerting photoswitching; and (3) a ligand for modulating the activity of the channel/receptor. The PTL conjugation site is located nearby the entrance of the channel pore (Figure 1b) or the binding pocket of agonist/allosteric modulator (Figure 1c). Because photoisomerization changes the length and dipole moment of azobenzene, the installed PTL can reversibly activate/inhibit/modulate the protein upon light switching by delivering or removing a ligand to/from its binding site. For example, MAQ (Maleimide-Azobenzene-Quaternary ammonium) is used as a photoswitchable tethered blocker for several voltage-gated potassium channels (Figure 1b).^{7,8,45} When conjugated nearby the channel entrance, the *trans* form of MAQ is long enough to occlude the pore via its quaternary ammonium group. Illumination of 380 nm light twists and shortens MAQ's azobenzene core, thereby relieving channel blockade. This process can be reversed rapidly by illuminating 500 nm light to drive *cis-to-trans* isomerization. In the case of neurotransmitter receptors, as exemplified by a light-gated kainate receptor (Figure 1c), L-MAG1 is installed next to the opening of the receptor's ligand-binding domain.⁹ Here the ligand (glutamate) is an agonist for the kainate receptor, a cation-conducting channel whose opening is induced allosterically upon glutamate binding. The conjugation site favors ligand entry from the *cis*-, but not the *trans* form, of L-MAG1. Consequently, the receptor can be activated by 380 nm light to trigger cation influx and be deactivated by 500 nm light.

The magnitude of photocontrol is highly dependent on the location of the PTL attachment site. Thus, a screening of cysteine mutants is needed when engineering a channel/receptor with a specific PTL. Moreover, the mode of photocontrol may be altered by changing the PTL and/or the conjugation site. For example, light-activated NMDA receptor is engineered by conjugating L-MAG1 onto GluN2A-(V713C), while light-antagonized NMDA receptor is obtained by conjugating L-MAG0 (a shorter congener) onto GluN2A-(G712C).¹⁰ For some GABA_A receptors (neurotransmittergated chloride channels), photoantagonism can be induced by green or UV light, respectively, by conjugating a shorter or a longer PTL onto the same site.¹¹

The main advantage of this approach is that it only requires the mutation of a single amino acid in the target protein. Such a small change often allows the function, expression, and trafficking of the mutant to resemble those of the wild-type. In addition, the effective concentration of the ligand, when PTL is in its active form, is very high (on the order of

mM)¹² due to the close proximity of its attachment site to the binding pocket. This feature offers more opportunities for a successful PTL design because a high-affinity ligand is not mandatory for enabling a strong effect.

However, this approach also has pitfalls that would potentially limit its use in a complex biological environment. (1) Cysteine is present not only in the mutant channel/receptor but also in many other endogenous proteins, and hence off-target conjugation of the PTL cannot be avoided. Users will need to perform careful control experiments to rule out artifacts in their own applications. (2) Sulfhydryl-reactive PTLs are not compatible with the high concentration of glutathione inside the cell, and hence PTL installation is limited to the extracellular side of the channel/receptor. (3) The maleimide group of PTL is prone to hydrolysis at physiological pH, which may constrain the conjugation efficiency in tissue and/or *in vivo* because PTL would be degraded during diffusion/partition of the reagent. (4) Cysteine is susceptible to oxidation,¹³ another factor that may reduce the yield of PTL conjugation. Although regenerating free cysteines on the cell surface can be achieved with reducing agents such as tris(2-carboxyethyl)phosphine (TCEP), this method should be used with caution to reduce toxicity or other adverse effects to the cells/tissue.

Tethering PORTL to a Protein Tag.

The PORTL approach was recently developed to address some practical challenges encountered by the use of PTL, namely, the off-target installation of the photoswitch and the instability of the chemical reagent under physiological conditions. Analogous to the PTL design, a PORTL comprises a functional group for bioconjugation, an azobenzene for photoswitching, and a ligand for the target protein (Figure 1d). However, PORTL and PTL are substantially different in their conjugation strategy and the mechanism of photocontrol. Instead of using a genetically engineered cysteine, this approach employs a self-labeling protein tag¹⁴ (e.g., SNAP and CLIP) for PORTL installation. These tags are “single-turnover” enzymes that form stable adducts with their substrates (e.g., O⁶-benzylguanine derivatives for SNAP tag and O²-benzylcytosine derivatives for CLIP tag). They are comparable to GFP in size and are used as N- or C-terminal fusion of many membrane proteins. Owing to the nature of enzymatic catalysis, the conjugation reaction is mild, efficient, and specific. Unlike the maleimide group of PTL, which is prone to hydrolysis and nucleophilic attack by glutathione, the substrates of self-labeling tags are stable in the physiological environment. Because the tag is fused at the protein terminus, a long and flexible linker is needed for PORTL to span the distance between its installation and ligand-binding sites.

In the PTL approach, photoisomerization of azobenzene changes the end-to-end distance of the molecule by a few angstroms. Because PTL is anchored right next to the ligandbinding site, this difference in molecular length is sufficient to cause a profound impact on ligand accessibility. However, this principle is not applicable to PORTL because the sites for conjugation and ligand binding are separated by a few nanometers. Rather, photocontrol is achieved by altering the efficacy/binding affinity of the ligand (which is fused or closely coupled to azobenzene). Note that the effective ligand concentration provided by PORTL is

lower (<mM) due to the longer linker length. Hence, the ligand needs to be sufficiently potent when PORTL is in the active form.

The PORTL approach has been applied to engineer light-activated metabotropic glutamate receptors (mGluRs; Figure 1d).^{15,16} For example, N-terminally SNAP-tagged mGluR2 is conferred light sensitivity by reacting with O⁶-benzylguanine containing, glutamate-based PORTLs (BGAG_n).¹⁵ In this case, the optimal linker size (*n*) for PORTL is 0–12 ethylene glycol units. A CLIP-tagged variant is also created using an O²-benzylcytosine analogue of BGAG₁₂.¹⁶ In practice, the PORTL approach may be advantageous for its efficiency and target specificity of conjugation, as well as the stability of the reagent it uses. It may also simplify the engineering procedure because the search for an optimal conjugation site is not needed. However, this approach also has potential limitations and challenges. First, PORTL is not suitable for proteins that are not tolerant to the fusion of a bulky tag, or for channels/receptors whose taggable terminus is geographically hindered from the ligand-binding site. Second, the design of PORTL might be technically demanding in some cases because strong photoswitching of its pharmacological effect needs to occur within the range of effective ligand concentration. A critical evaluation of these factors is important when engineering channels/receptors with this method.

Which approach (PTL or PORTL) should be used for the channel/receptor of interest depends on not only the feasibility but also other factors. The PTL approach, which requires only a point mutation in the target protein, may be more favorable for channels/receptors that are within a compact environment (e.g., synaptic cleft) or a multicomponent complex, where addition of a bulky tag might disrupt the arrangement or interactions of the signaling machineries. The PORTL approach would be more advantageous if a user wishes to ensure target specificity of the photoswitch compound (and thus the photocontrol) in the biological applications. Owing to the catalytic nature of the conjugation reactions and the stability of the reagents, the PORTL approach would in principle be more suitable for an efficient generation of light-switchable channels/receptors in the brain. However, the *in vivo* applicability of PORTL remains to be examined further.

Other Approaches.

Two additional strategies have been reported for engineering light-activated P2X receptors (ATP-gated cation channels). Although these methods also involve the conjugation of photoswitches to genetically engineered cysteines, receptor photocontrol is independent of ligand binding in both cases. The first method, named optogating, is achieved by installing positively charged azobenzene derivatives to the transmembrane domain of the P2X2 receptor. Photoisomerization of azobenzene changes the local charge distribution, thereby inducing channel opening or closing.¹⁷ In the second method, 4,4'-bis(maleimido)azobenzene was used to cross-link the transmembrane channel-forming helices from two adjacent subunits (Figure 1e).¹⁸ Photoisomerization of azobenzene provides mechanical force to move the gating elements and subsequently triggers channel opening or closing. This approach has been applied to optically gate homomeric P2X2 receptor, heteromeric P2X2/3 receptor, and acid-sensing ion channels.

3. THE PHOTOSWITCH CHROMOPHORES

So far, most of the light-switchable channels/receptors use regular azobenzene as the photoswitch chromophore, with the bioconjugation and the ligand modules linked at the two *para*-positions via amide coupling. Azobenzenes have several features that make them popular for biological applications.¹⁹ They are photostable and synthetically tractable, and their photochemical properties can be tuned by structural modifications. Photoisomerization is fast, reversible, and reliable, allowing multiple cycles of photoswitching with high fidelity. When completely adapted in darkness (i.e., thermally relaxed), azobenzenes are in the extended *trans* configuration. For the regular azobenzene used in PTL and PORTL, *trans-to-cis* isomerization is triggered by UV light (typically 360–380 nm), and the metastable *cis* isomer (which has a bent structure) can be reverted to the *trans* state rapidly with blue-green light or through thermal relaxation in darkness on a longer time scale.

One common concern about employing azobenzene photoswitches in biological systems, especially in tissues or in organisms, is the use of UV light. Prolonged exposure to UV light (to maintain azobenzene in the *cis* state) may lead to cell damage or toxicity. Moreover, UV light does not penetrate deeply into tissue, and thus the spatial range of *in vivo* photocontrol is constrained. The issue of cell damage/toxicity is technically manageable because most light-switchable channels/receptors are bistable to some degree. Namely, the installed photoswitches can be locked in the *trans* or *cis* state in darkness after a brief pulse of visible or UV light, respectively. The extent of bistability is determined by the half-life of the metastable *cis*-azobenzene, which is often >10 min for light-switchable channels/receptors.^{11,12,20,21} At an intensity commonly used for neuronal photocotrol (1–10 mW/mm²), less than 1 s of UV illumination is sufficient to cause a complete switching.^{11,31} Hence intermittent pulses of UV light over the dark period would maintain the state of the channel/receptor for at least hours. This protocol was used to optically silent NMDA receptors in the brain of zebrafish over days of development.¹⁰

A more straightforward way to address the above issues is to red-shift the spectral profile of azobenzene. Several strategies of azobenzene derivatization have been reported to enable photoisomerization without UV light,²² and two of them have been applied to engineer light-switchable glutamate receptors. The first approach relies on the “push-pull” electronic effect to lower the energy barrier of photoisomerization. For example, by replacing one of the *para*-amido groups in L-MAG0 with an electron-donating tertiary amine, the absorbance spectrum of the resulting PTL (L-MAG0₄₆₀) in the *trans* state is red-shifted by ~100 nm (Figure 2).²³ This chemical modification lowers the energy barrier for both directions of isomerization, and thus the thermal stability of the *cis* isomer is also reduced. Consequently, *trans-to-cis* isomerization of L-MAG0₄₆₀ can be driven by blue-green light, and *cis*-L-MAG0₄₆₀ reverts to the *trans* state spontaneously in the dark within a second.²³ L-MAG0₄₆₀ therefore enables reversible manipulation of light-gated kainate receptor with single-color irradiation. The same strategy has also been applied to red-shift light-switchable mGluRs.^{15,16,24} Notably, *trans-to-cis* isomerization can also be driven by two-photon excitation for this type of PTL,^{24,25} offering higher spatial resolution of photocontrol in a biological context.

The second approach red-shifts azobenzene by substituting the four *ortho*-positions with methoxy groups or halides.^{26–30} Distinct from the “push-pull” azobenzenes, tetra-*ortho*-substituted azobenzenes are highly bistable (half-life of the *cis* state is at least hours) and are driven by two colors of visible light. A tetra-*ortho*-chloro version of L-MAG1 (*t*Cl-MAG1) has been used for red-shifting light-gated kainate receptor (Figure 2).²⁶ At a light intensity of ~1 mW/mm², the engineered receptor can be rapidly activated by green-yellow light and deactivated by blue light. Due to the low absorbance by *t*Cl-MAG1, red light only elicits a very small current. However, when the receptor is exposed to a high intensity of 625 nm light (12.5 mW/mm²) for a longer duration, a large current can be produced. It is thus possible to develop light-switchable channels/receptors driven by long wavelengths of light; however, more structure–function studies of azobenzene will be needed to discover the types and combinations of substituents that offer rapid and robust photoswitching in the desired wavelength ranges.

Currently, most light-switchable channels/receptors use azobenzene-based PTLs or PORTLs. While strategies have been discovered, as discussed above, to diversify the photochemical properties of azobenzenes (and thus the engineered proteins), the repertoire of chemical-optogenetic tools may be further expanded by using other types of chromophores. There are some promising candidates suggested by the recent development of photochromic drugs. For instance, arylazopyrazole-based amindohydrolase inhibitors were found to exhibit better performance (e.g., higher photoconversion yield and bistability) than the azobenzene-based analogues.⁴² In another report, a photochromic inhibitor of ATP-sensitive potassium (K_{ATP}) channel, which is based on a “push-pull” heterocyclic azobenzene, enabled insulin secretion from rodent islets upon illumination of 560 nm light.⁴⁶ Chromophores used in these examples, and perhaps other recently discovered photoswitches,^{38–41} should also be suitable for PTLs and/or PORTLs.

4. OPTOGENETIC MANIPULATION OF ION CHANNELS AND RECEPTORS IN NEURONS

Ion channels and neurotransmitter receptors play pivotal roles in various aspects of neurophysiology. They may generate an ionic conductance that modulates neuronal excitability, or trigger/prevent a biochemical event underlying a neuronal function. Due to their enormous diversity, elucidating the roles of these signaling mediators in the nervous system is a highly challenging task. Chemical optogenetics provides an unprecedented means to address this challenge. The use of light enables a precise control (achievable on the micrometer and millisecond scales) that is specific for the channel/receptor of interest within a complex biological environment.

Since the initial development of the SPARK (Synthetic Photoisomerizable Azobenzene-Regulated K⁺) channel,⁷ the PTL strategy has been applied to confer light sensitivity onto a variety of ion channels and neurotransmitter receptors. Together with those engineered by PORTL and other approaches, a versatile collection of tools (Table 1 and Table 2) are now available for optogenetic interrogation of neural function and signaling. Some achievements of implementing these tools in neurons are summarized below.

Glutamate Receptors.

Glutamate is the major excitatory neurotransmitter which has 18 ionotropic receptors (ligand-gated cation channels) and 8 metabotropic receptors (G-protein coupled receptors). So far, the PTL approach has been applied to confer light sensitivity onto two classes of ionotropic glutamate receptors: the kainate type (LiGluR; Table 1)^{9,23,25,26,31} and the NMDA type (LiGluN; Table 1).¹⁰ Photoactivation of LiGluR in neurons, which causes membrane depolarization, can trigger action potential firing both in culture and in the mouse brain.^{31,32} LiGluR can also be introduced into degenerating mouse retina to restore light sensitivity,^{33,34} or into zebrafish larvae to photocontrol escape responses to touch.³² NMDA receptors are implicated to play an important role in learning and memory as they regulate the formation of excitatory synapse and the strength of synaptic transmission. Consistent with this notion, photocontrol of LiGluNs in mouse hippocampal slice can regulate the growth of dendritic spines as well as the induction of long-term synaptic plasticity.¹⁰ LiGluN can also be introduced into developing zebrafish to control the refinement of retinal ganglion cell axonal arbors.¹⁰

Several members of metabotropic glutamate receptors (mGluRs) have been engineered by either the PTL^{24,35} or the PORTL^{15,16} approach (Table 1 and Table 2), but only the tools for mGluR2 have been tested in neurons. Activation of mGluR2 is coupled to several downstream signaling events, such as the opening of G-protein-coupled inwardly rectifying potassium channels (GIRKs) or the suppression of voltage-gated calcium channels. The former leads to reduced firing of action potential and the latter results in suppression of neurotransmitter release, both can be optically manipulated in neurons expressing light-switchable mGluR2s.^{15,16,35} One of the light-switchable mGluR2s has also been introduced into degenerated mouse retina to restore light sensitivity *in vitro* and *in vivo*.³⁶

GABA_A Receptors.

GABA (γ -aminobutyric acid) is the major inhibitory neurotransmitter which targets ionotropic GABA_A receptors (ligand-gated chloride channels) and metabotropic GABA_B receptors (G-protein coupled receptors). The GABA_A receptors are heteropentameric proteins (assembled from 19 possible subunits) with a typical stoichiometry of two α , two β , and one tertiary subunit (usually γ or δ). The α subunit, which has six isoforms, largely determines the function, expression, and pharmacological profile of a GABA_A receptor. Due to the lack of subtype-specific drugs and the technical limitations of gene knockout (e.g., compensation and irreversibility), it has been challenging to elucidate the roles of different GABA_A members in neural functions and disorders. To address this challenge, the PTL approach has been applied to engineer light-regulated GABA_A receptors (LiGABARs; Table 1) for all six α isoforms,¹¹ providing a comprehensive optogenetic toolkit for subtype-specific manipulation of GABA_A-receptor signaling. Implementation of LiGABAR in the brain has been demonstrated both *ex vivo* and *in vivo* with the $\alpha 1$ isoform.¹¹ Light switching can remotely and reversibly modulate inhibitory synaptic responses in brain slices, as well as neuronal firing and rhythmic activities in the brain of behaving mice.

Introducing a light-switchable channel/receptor into the nervous system is a two-step process. The mutated or tagged protein is first expressed and later treated with the PTL or

PORTL to endow light sensitivity. Expression of the mutant/tagged protein has mainly been achieved exogenously via transfection or viral transduction of neurons. While practically convenient, this approach may not allow the mutant/tagged protein to fully resemble its wild-type counterpart in terms of expression level and subcellular distribution. Moreover, this method could alter the expression profile of other endogenous proteins, especially those in the same family as the light-switchable channel/receptor, in the host neurons. To eliminate these concerns, it would be ideal to make the mutant/tagged protein endogenous. The first knock-in mouse, in which the gene of GABA_A α 1 subunit is replaced by its “PTL-ready” mutant, has been created for optogenetic interrogation of GABAergic signaling.¹¹ By specifically and precisely manipulating a GABA_A receptor in its natural context, its physiological roles and distribution profiles can be unambiguously revealed with light.

5. FUTURE PERSPECTIVES

The physiological significance of ion channels and neurotransmitter receptors has long been recognized and appreciated. However, it remains challenging to elucidate whether and how exactly each member plays a role in a biological phenomenon. Chemical optogenetics, which allows precise and specific control over these proteins, opens the door to advancing our understanding of neural function and signaling. At the molecular level, it allows the identification of functional interactions between a specific channel/receptor and other signaling components in neurons.⁴⁵ At the cellular level, it enables the probing of a specific channel/receptor in different types of neurons, or at various subcellular locations of a neuron.¹¹ Notably, the role of a specific channel/receptor in neurophysiology can be precisely interrogated by light: the timing, duration, and spatial coverage of optical control can be programmed to gain deeper kinetic and mechanistic insights.³ At the tissue or organism level, chemical optogenetics can be used to reveal the causal relationship between the activity of a specific channel/receptor and a neural function such as longterm synaptic plasticity,¹⁰ sensory-evoked network activity,¹¹ or behavior. The functional impact of a channel/receptor in a neural circuit may be further elucidated by targeting photocontrol to a defined population of neurons through genetic manipulation.³

Following the milestones summarized in this review, there are a few directions for the future development of light-switchable channels/receptors. The PTL and PORTL approaches together shall enable the engineering of many other ion channels and receptors that have modifiable pharmacophores. Alternatively, methods such as optogating may be suitable for channels that have well-characterized structural and biophysical properties. Other chemical strategies will also be promising for conferring light sensitivity onto channels/receptors. For example, the issue of off-target conjugation for the current PTL approach may be solved by replacing the engineered cysteine with an unnatural amino acid that enables bio-orthogonal reactions (such as click chemistry).³⁷ The palette of photoswitch chromophores can also be expanded further. In addition to variants of modified azobenzenes, some recently discovered photoswitches may serve the same role and offer opportunities to manipulate biomolecules with longer wavelengths of light^{38–40} or nearquantitative photoisomerization yields.^{41,42} With multiple photosensitization methods and photoswitch chromophores in hand, it will be possible to (1) engineer light-switchable channels/receptors with user-selected features; and (2) orthogonally manipulate more than one protein target with different colors of light in the

same cell or neural circuit. Collectively, the maturation of chemical optogenetics will enable more logical and systematic interrogations of neuronal signaling in the future.

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Figure 1.

Optical manipulation of an ion channel or a neurotransmitter receptor through a covalently installed photoswitch. (a) Photoactivation of channelrhodopsin-2 (a microbial opsin; through retinal). (b) Photoblocking/unblocking of a potassium channel (through a PTL, i.e., photoswitchable tethered ligand). The azobenzene core of *trans*- and *cis*-MAQ is highlighted in green and violet, respectively. (c) Photoactivation/deactivation of a kainate receptor (an ionotropic glutamate receptor; through a PTL). (d) Photoactivation/deactivation of metabotropic glutamate receptor 2 (through a PORTL, i.e., photoswitchable orthogonal remotely tethered ligand). (e) Optical gating of a P2X receptor (through a photoswitchable cross-linker).

Figure 2.

Strategies for red-shifting the spectral profile of a PTL. Replacing a *para*-amido group in the parent PTL (L-MAG0; top) with an electron-donating tertiary amine results in a fast-relaxing variant (L-MAG0₄₆₀; middle) that enables single-color optical control.²³ Introducing chlorides to the four *ortho*-positions of azobenzene yields a bistable analogue of MAG1 (toCl-MAG1; bottom) that can be switched by a brief pulse of green or blue light and remained “locked” in darkness after irradiation.²⁶

Table 1.

Chemical Optogenetic Tools Engineered through Cysteine Conjugation

protein	name of the tool(s)	type of control	light for switching	fast-relaxing or bistable	remarks	ref.
voltage-gated potassium channels	SPARK and designer K ⁺ channels	channel block	~380 nm (to <i>cis</i>) ~500 nm (to <i>trans</i>)		Light-switchable Shaker (SPARK), Kv1.3, Kv3.1, Kv3.4, Kv7.2, SK2, and TREK1 channels. PTL = MAQ. Photocontrol of action potential firing in hippocampal neurons (SPARK and light-switchable Kv3.1).	7,8,45
kainate receptor (GluK2; formerly iGluR6)	LiGluR	activation	~380 nm (to <i>cis</i>) ~500 nm (to <i>trans</i>)	bistable	PTL = L-MAG0 or L-MAG1. <i>In vivo</i> photocontrol in zebrafish and mice. Additional variants carrying mutations to reduce glutamate sensitivity or calcium permeability available.	9,12,31–34
			violet-green light (to <i>cis</i>) dark (to <i>trans</i>)	fast-relaxing	PTL = L-MAG0 ₄₆₀ , MAG _{2p} , or MAGA _{2p} . Activatable by two-photon excitation. <i>In vivo</i> photocontrol in blind mouse retina.	23–25,34
			520–580 nm (to <i>cis</i>) ~440 nm (to <i>trans</i>)	bistable	PTL = α Cl-MAG1	26
NMDA receptors	LiGluN	activation	360–405 nm (to <i>cis</i>) 460–560 nm (to <i>trans</i>)	bistable	PTL = L-MAG1. Mutant = GluN2A(V713C), GluN2B(V714C). Photoregulation of structural plasticity in hippocampal neurons.	10
		antagonism	360–405 nm (to <i>cis</i>) 460–560 nm (to <i>trans</i>)	bistable	PTL = L-MAG0. Mutant = GluN2A(G712C), GluN1a(E406C). Photoregulation of synaptic plasticity in hippocampal slice and refinement of retinal ganglion cell axonal arbors in larval zebrafish <i>in vivo</i> .	
GABA _A receptors	LiGABAR	antagonism	380–395 nm (to <i>cis</i>) 470–540 nm (to <i>trans</i>)	bistable	Cysteine mutants and complementary PTLs available for all six α -isoforms. Transgenic mice enabling photocontrol of endogenous inhibitory synaptic transmission available. <i>In vivo</i> photocontrol of vision-evoked cortical activities in mice.	11
nicotinic acetylcholine receptors (β 2 and β 4)	LinAChR	activation or antagonism	~380 nm (to <i>cis</i>) ~500 nm (to <i>trans</i>)	bistable	PTL = MAACH (for activation) or MAHoCh (for antagonism)	21
P2X receptors and ASIC channel		activation (agonist-independent)	365 nm (to <i>cis</i>) 525 nm (to <i>trans</i>)	bistable	P2X2 receptors with tethered positively charged azobenzene photoswitches in the transmembrane pore region. Photocontrol of action potential firing in hippocampal neurons.	17
			360 nm (to <i>cis</i>) 440 nm (to <i>trans</i>)		P2X2/3 receptors and ASIC1 channel in which transmembrane domains were	18

protein	name of the tool(s)	type of control	light for switching	fast-relaxing or bistable	remarks	ref.
metabotropic glutamate receptors	LimGluR	activation	380 nm (to <i>cis</i>) 500 nm (to <i>trans</i>)	bistable	cross-linked by bis (maleimido)azobenzene. PTL = D-MAG-0 for mGluR2(L300C) and mGluR3(Q306C). Photoregulation of action potential firing and neurotransmitter release in hippocampal neurons. Photocontrol of zebrafish behavior.	35
dopamine receptors	LimGluR-block	antagonism	~470 nm (to <i>cis</i>) dark (to <i>trans</i>) 380 nm (to <i>cis</i>) 500 nm (to <i>trans</i>)	fast-relaxing bistable	PTL = D-MAG-0 ₄₆₀ for mGluR3(Q306C). Activatable by two-photon excitation. PTL = D-MAG-1 for mGluR2(S302C) and D-MAG-0 for mGluR6(K306C). Photoregulation of action potential firing in hippocampal neurons.	24 35
chimera of iGluR6 and sGluR0	Lid1R and Lid2R Hylighter	inhibition activation	360 nm (to <i>cis</i>) 460 nm (to <i>trans</i>) 380 nm (to <i>cis</i>) 500 nm (to <i>trans</i>)	bistable	PTL = MAP for DIR(I183C) (antagonism), DIR(G88C) (inverse agonism), and D2R(I184C) (inverse agonism). PTL = L-MAG0. Operating like LiGluR but providing potassium conductance for silencing action potential firing in neurons.	44 43

Table 2.
Chemical Optogenetic Tools Engineered through Conjugation of Self-Labeling Tags

protein	name of the tool(s)	type of control	light for switching	fast-relaxing or bistable	remarks	ref.
metabotropic glutamate receptors	SNAG-mGluR	activation	380 nm (to <i>cis</i>) 500 nm (to <i>trans</i>)		PORTL = BGAG _n ($n = 0-12$) for SNAP-tagged mGluR2, and BGAG ₁₂ for SNAP-tagged mGluR6 and SNAP-tagged mGluR7(N74K). Photoregulation of action potential firing and neurotransmitter release in hippocampal neurons (SNAG-mGluR2).	15,16
			~460 nm (to <i>cis</i>) dark (to <i>trans</i>)	fast-relaxing	PORTL = BGAG _{12,460} for SNAP-tagged mGluR2. Triggering light responses in blind mouse retina both <i>ex vivo</i> and <i>in vivo</i> .	15,36
metabotropic glutamate receptors		activation	380 nm (to <i>cis</i>) 500 nm (to <i>trans</i>)		PORTL = BGAG ₁₂ for CLIP-tagged mGluR2. Photoregulation of action potential firing in hippocampal neuron.	16
			~460 nm (to <i>cis</i>) dark (to <i>trans</i>)	fast-relaxing	PORTL = BGAG _{12,460} for CLIP-tagged mGluR2.	16