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Optobiology: optical control of biological processes via protein engineering

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Abstract
Enabling optical control over biological processes is a defining goal of the new field of optogenetics. Control of membrane voltage by natural rhodopsin family ion channels has found widespread acceptance in neuroscience, due to the fact that these natural proteins control membrane voltage without further engineering. In contrast, optical control of intracellular biological processes has been a fragmented effort, with various laboratories engineering light-responsive properties into proteins in different manners. In the present article, we review the various systems that have been developed for controlling protein functions with light based on vertebrate rhodopsins, plant photoregulatory proteins and, most recently, the photoswitchable fluorescent protein Dronpa. By allowing biology to be controlled with spatiotemporal specificity and tunable dynamics, light-controllable proteins will find applications in the understanding of cellular and organismal biology and in synthetic biology.

Introduction
Light-controllable proteins have been of immense interest owing to their beneficial properties of studying cellular processes and biological events with precise spatial and temporal specificity [1,2]. Previously, small-molecule agonists or antagonists of signalling proteins have been used, but low spatial and temporal resolution, unexpected endogenous reactions, and off-target effects remain as inherent disadvantages when studying real-time events in living cells or organisms [3]. Thus developing tools that evade these shortcomings could greatly advance our understanding of biology.

Recently, many genetically encoded systems have been engineered to modulate protein activities in response to light. In general, in these systems, a light-induced conformational change in a chromophore-containing protein domain changes its interactions with other protein domains or influences the functional output of a fused protein domain. We present a short summary of recent developments of engineered light controllable proteins with a goal of pointing out the unique aspects of each system and providing an overview of current research in the field.

Vertebrate rhodopsins: light-induced G-protein activation
Rhodopsins are seven-pass transmembrane receptors that bind retinaldehyde as a cofactor. In bacteria and protists, many function as light-dependent ion channels or pumps. These have been enormously useful in mediating optical control of firing of vertebrate neurons with no or minimal modification, and the advantageous combination of controlling neuronal activity by light in genetically defined subpopulations of neurons gave rise to the term optogenetics [4]. In animals, rhodopsins are G-protein-coupled receptors that initiate light-induced intracellular signalling in photoreceptor cells by activating the specialized heterotrimeric G-protein transducin expressed only in photoreceptor cells.

Airan et al. [5] modified vertebrate rhodopsins to activate the ubiquitous heterotrimeric G-proteins G_q and G_s to allow control of G-protein-mediated processes in non-photoreceptor cells. G_q and G_s activate adenylate cyclase and phospholipase C respectively, inducing the second messengers cAMP and InsP_3. Airan et al. [5] replaced the intracellular loops of green-absorbing rhodopsin with those from the G_q-coupled α_1-AR (α_{1a}-adrenergic receptor) or the G_s-coupled β_2-AR (β_2-adrenergic receptor) (Figure 1). On green light stimulation, these chimaeras, Opto-α_1-AR and Opto-β_2-AR, significantly up-regulated levels of cAMP and InsP_3 respectively, as expected. Thus engineered rhodopsins are able to mediate optical activation of G-protein signalling in non-neuronal cells.

LOV (light/oxygen/voltage) domains: light-induced unfolding and heterodimerization
Plants and bacteria contain a variety of photoreceptors that activate intracellular signalling in response to light [6–8]. Among these, the most extensively studied are LOV domains, cryptochromes and phytochromes, which utilize the small

Key words: cryptochrome, fluorescent protein, light/oxygen/voltage domain (LOV domain), optogenetics, phytochrome, ULTRAVIOLET RESPONSE 8 (UVR8).
Abbreviations used: α_1-AR, α_1-adrenergic receptor; β_2-AR, β_2-adrenergic receptor; CIB1, calcium and integrin-binding protein 1; COP1, CONSTITUTIVELY MORPHOGENIC 1; CRY, cryptochrome; FKF1, flavin-binding/Kelch repeat/F-box 1; GI, GIGANTEA; HCV, hepatitis C virus; LOV, light/oxygen/voltage; Pfr, phytochrome in far-red-absorbing state; PIF, phytochrome-interacting protein; Pr, phytochrome in red-absorbing state; UVR8, ULTRAVIOLET RESPONSE 8.
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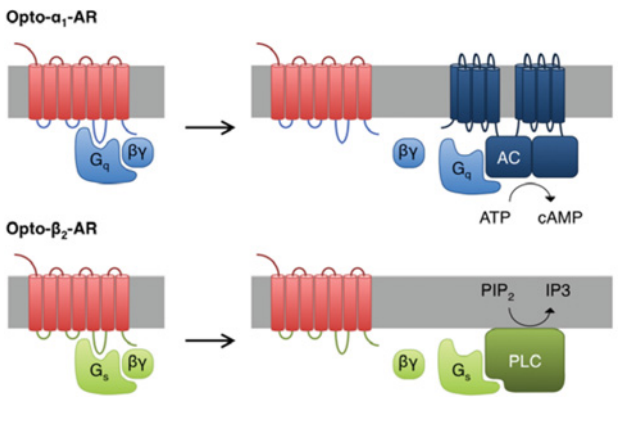
Figure 1 | Optical control of G-protein signalling by engineered vertebrate rhodopsins

Opto-α_1-AR (chimaera of green-absorbing rhodopsin and α_1-AR) activates adenylate cyclase via G_q, whereas Opto-β_2-AR (chimaera of green-absorbing rhodopsin and β_2-AR) activates phospholipase C via G_s. The rhodopsin chromophore is the cofactor retinaldehyde. AC, adenylate cyclase; PLC, phospholipase C.



chemical compounds FMN, FAD and phytochromobilin respectively as cofactors.

On blue light (peak 450 nm) absorption, the FMN cofactor in the LOV domain forms a covalent bond with a cysteine residue [9]. This process reverts in the dark over the course of seconds to minutes via hydrolysis of the FMN–cysteine bond. In plant FKF1 (flavin-binding/Kelch-repeat/F-box 1), the FMN–cysteine bond formation leads to interaction of the FKF1 N-terminus with GI (GIGANTEA) [10]. Yazawa et al. [10] took advantage of this feature to relocalize protein domains of interest to the specific locations within mammalian cells in response to light. They used light to recruit a FKF1 fusion with Rac1 to a GI fusion with a membrane anchor, enabling Rac1 activation by endogenous membrane-bound activators (Figure 2A). They similarly recruited an FKF1 fusion to a transcriptional activation domain to a GI fusion to a DNA-binding domain to activate transcription (Figure 2A). This transcriptional regulation scheme using FKF1 and GI has been recently reproduced and extended by another group [11].

In the LOV2 domain of plant phototropin, the FMN–cysteine bond formation leads to partial unfolding of the C-terminal α-helix (Jα) from the rest of the LOV2 domain. This was initially used to confer light responsiveness in an allosteric manner, specifically on the enzyme DHFR (dihydrofolate reductase) by linkage to a surface site and to a transcription factor by mutually exclusive folding of Jα and the N-terminal α-helix of the transcription factor [12,13]. Subsequently, phototropin LOV2 has been fused to the N-terminus of target domains or peptides to create steric blocks that can be relieved by light-induced Jα unfolding (Figure 2A). In the first example of this approach, Wu et al. [14] tested different linkages of the LOV2 domain to the N-terminus of the GTPase Rac1 and selected for those that showed Rac1 activation by blue light. The resulting construct, PA-Rac1 (photoactivable Rac1), enabled optical control of membrane ruffling in mammalian cells [14] and optical guidance of cell migration in zebrafish embryos [15]. Crystallography revealed unexpected surface contacts between the LOV2 and Rac1 domains and enabled the rational development of a photoactivatable Cdc42 through targeted mutation of residues on LOV2 [14]. Recently, a light-responsive caspase 7 has been engineered by fusion to the C-terminus of the LOV2 domain [16]. Strickland et al. [17] created LOV2 fusions capable of light-mediated heterodimerization by fusing the LOV2 domain to peptides that bind to PDZ domains. Light induced peptide exposure and PDZ binding in yeast and mammalian cells, as demonstrated by recruitment of PDZ fusion proteins to LOV2–peptide fusions located at the plasma membrane.

Cryptochrome domains: light-induced heterodimerization and aggregation

Cryptochromes are FAD-binding proteins that participate in growth processes in plants and regulate circadian clocks in animals [18]. In plant cryptochromes, blue light induces reduction of FAD and a conformational change in the protein, with the process reversing spontaneously in seconds in the dark. In *Arabidopsis* CRY2 (cryptochrome 2), the light-induced conformational change allows binding of CIB1 (calcium and integrin-binding protein 1) [19]. Similarly to Yazawa et al. [10], Kennedy et al. [20] used the light-inducible interaction of CRY2 and CIB1 to relocalize a protein to the cell membrane and a transcriptional activation domain to a DNA-binding domain (Figure 2B). They also used CRY2 and CIB1 to induce reconstitution of the enzyme Cre recombinase from two fragments in response to light, enabling optical control of recombination at loxP sites (Figure 2B). CRY2–CIB1 heterodimerization is robust and rapid, as demonstrated by optical control of phosphoinositide distributions via light-induced recruitment of a inositol phosphatase to the membrane [21] and by its applicable to control gene expression in zebrafish [22].

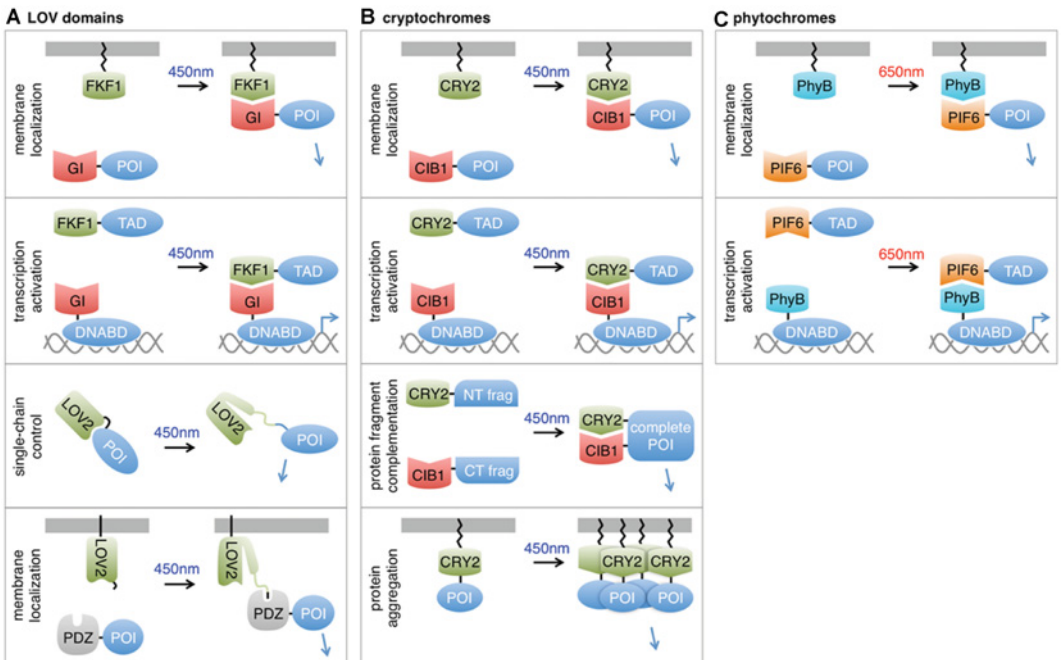
Bugaj et al. [23] discovered that CRY2 undergoes aggregation in response to light (Figure 2B). They found that light-induced aggregation of CRY2 fusions to the Wnt receptor LRP6 (low-density lipoprotein receptor 6) or to the GTPase RhoA was sufficient to activate downstream effectors. This method may enable optical control of certain cellular pathways with the expression of a single fusion protein whose construction may be fairly simple.

Phytochrome domains: light-induced heterodimerization

Phytochromes are light-responsive signalling proteins in plants and cyanobacteria that contain a covalently linked tetrapyrrole as the chromophore. Plant phytochromes

Figure 2 | Systems for optical control of proteins based on plant photosensory proteins using chemical cofactors

(**A**) Light-induced heterodimerization by the LOV domain of FKF1 and light-induced conformational changes in the LOV domain of phototropin has been used to control protein localization, transcription and the activity of a fused protein domain. The chromophore of LOV domains is FMN. (**B**) Light-induced heterodimerization or aggregation by cryptochromes has been used to control protein localization, transcription, protein fragment complementation and signalling protein activity. The chromophore of cryptochromes is FAD. (**C**) Light-induced heterodimerization by phytochromes has been used to control protein localization and transcription. The chromophore of phytochromes is phytochromobilin. POI, protein of interest; DNABD, DNA-binding domain; TAD, transcriptional activation domain; NT frag, N-terminal protein fragment; CT frag, C-terminal protein fragment. The right-angled blue arrow indicates transcription, whereas the diagonal blue arrow indicates downstream protein activities.



utilize phytochromobilin, whereas cyanobacteria use the structurally similar phycocyanobilin [6]. Red light induces isomerization of the tetrapyrrole cofactor, inducing a conformational change in the protein from a red-absorbing [Pr (phytochrome in red-absorbing state)] to a far-red-absorbing [Pfr (phytochrome in far-red-absorbing state)] state. The Pfr state then relaxes slowly back to the Pr state, or the conversion can be induced quickly by far-red light. In plant phytochromes, the Pfr state selectively binds to PIFs (phytochrome-interacting factors).

Shimizu-Sato et al. [24] used the light-dependent interaction of PhyA with PIF3 to control transcription in yeast cells fed with phytochromobilin. Light-induced recruitment of a transcriptional activation domain fused with PIF3 to a DNA-binding domain fused with PhyA-induced gene transcription (Figure 2C). Levskaya et al. [25] used the PhyB–PIF6 interaction to control recruitment of the Rac activator Tiam to the membrane in mammalian cells fed with phytochromobilin (Figure 2C). Local illumination resulted in local lamellipodia formation, as expected for Rac activation.

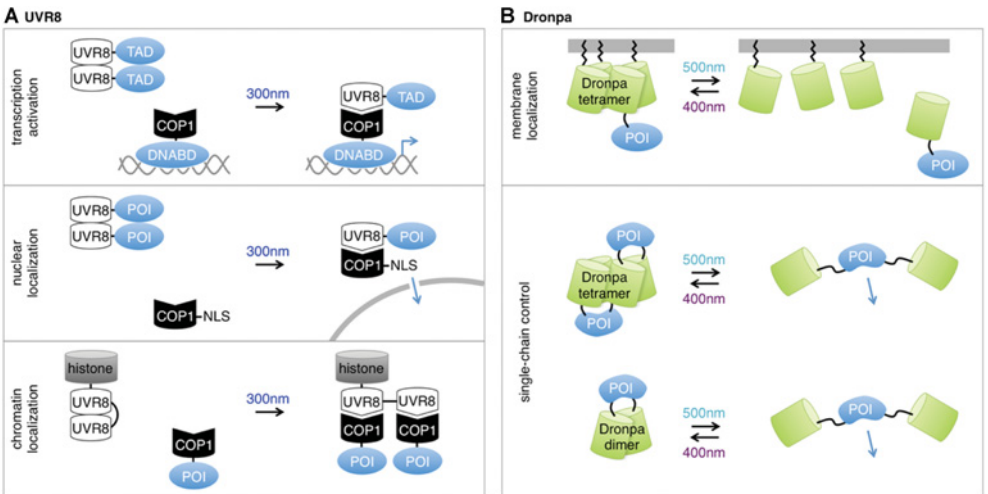
The absorption of red light by phytochromes contrasts with the blue light absorption by flavin-binding proteins and would be desirable for the lower phototoxicity and enhanced tissue penetration of red light. However, this system has seen slower adoption by cell biologists than the LOV domain or cryptochrome-based systems. The only other report of phytochrome–PIF interactions being regulatable in animal cells comes from a recent study [26] reproducing the approach of Shimizu-Sato et al. [25] in mammalian cells. One reason for the slow adoption of phytochrome–PIF could be sensitivity of phytochrome domains to fusion and a requirement for high phytochrome expression levels [27].

UVR8 (ULTRAVIOLET RESPONSE 8) domains: light-induced dissociation and heterodimerization

Recently, a plant protein that demonstrates light-regulated binding and does not utilize any cofactors has been characterized. UVR8 forms homodimers that dissociate

Figure 3 | Systems for optical control of proteins that do not require chemical cofactors

(**A**) Light-induced heterodimerization by UVR8 has been used to control transcription and protein localization. (**B**) Bidirectional light-controlled switching between tetrameric and monomeric states in Dronpa mutants, or between dimeric and monomeric states, has been used to control protein localization and the activity of single-chain fusion proteins. NLS, nuclear localization sequence.



upon UV illumination, after which the monomers are able to bind COP1 (CONSTITUTIVELY MORPHOGENIC 1) [28,29]. The responsible chromophores in UVR8 are actually a pair of tryptophan residues that are engaged in cation–π interactions with arginine residues at the dimeric interface. It is believed that photon absorption induces breakage of the cation–π interactions, leading to subtle conformational changes that in turn break hydrogen-bond interactions at the dimeric interface [28,29]. The conformational change is remarkably long-lived, lasting many hours.

Two groups used UVR8 and COP1 to control protein heterodimerization in mammalian cells with UV light. Müller et al. [30] fused UVR8 to a DNA-binding domain and COP1 to a transcriptional activation domain, whereas Crefcoeur et al. [31] reversed the positions of UVR8 and COP1 (Figure 3A). Going beyond transcriptional activation, Crefcoeur et al. [31] also tagged COP1 with a nuclear localization signal to enable UV-induced localization of UVR8 fusions, and created a UVR8 tandem dimer fused to histone to enable UV-induced chromatin localization of COP1 fusions (Figure 3A). However, the UVR8–COP1 system has the disadvantage that prolonged exposure to UV light is mutagenic and phototoxic, although acute phototoxicity can be minimized by applying UV light intermittently [30,31].

Fluorescent protein domains: light-induced dissociation

Photoswitchable fluorescent proteins are fluorescent proteins that alter their optical properties in a reversible manner upon illumination [32]. Like other fluorescent proteins, the chromophore in photoswitchable fluorescent proteins is created autocatalytically from three adjacent amino acids. In Dronpa, an extensively studied photoswitchable green fluorescent protein, fluorescence is switched off upon illumination with cyan light and switched on again upon illumination with violet light. The off-switching is associated with a *cis*-to-*trans* isomerization of the chromophore and an unfolding of the portion of the β-barrel wall of the protein adjacent to the chromophore [33,34]. Thus Dronpa has the essential characteristic of natural photoregulatory proteins: light-induced conformational change.

In dimeric and tetrameric coral fluorescent proteins such as 22G, the natural precursor to Dronpa, the region of the β-barrel adjacent to the chromophore forms a dimerization interface [34]. We hypothesized that mutants of Dronpa that formed weak tetramers at baseline would undergo dissociation upon blue light illumination and reassociation upon violet light illumination. We indeed found this to be the case with purified protein *in vitro* [35]. When we expressed in mammalian cells a fusion of the weakly tetramerizing Dronpa mutant with a plasma membrane anchor, and another fusion of Dronpa to a fluorescent protein, we found that the fluorescent protein was membrane-localized at baseline. Cyan light induced immediate release from the membrane and violet light induced re-recruitment to the membrane, demonstrating that Dronpa interactions could be regulated by light in cells (Figure 3C). Interestingly, as the photoswitching behaviour of Dronpa was evolved in the laboratory rather than in Nature, the light-regulated tetramerization of Dronpa may be the first example of an artificial light-regulated protein–protein interaction.

We used this property of light-induced dissociation as the basis for creating single-chain light-regulatable proteins. Specifically, we fused one copy of weakly tetramerizing Dronpa via flexible linkers to each termini of domains whose activity we desired to regulate. We hypothesized that, if the N- and C-termini of a domain flanked a functional interface, then assembly of the Dronpa tetramer in the dark would occur in front of the interface and inhibit protein function via steric blockade. The light-induced disassembly of the Dronpa tetramer could then remove the steric block and allow protein activity (Figure 3C). We tested this idea on the catalytic domain of the Cdc42 activator intersectin and on the HCV (hepatitis C virus) protease domain, as these domains have N- and C-termini flanking their active sites. Using a cellular morphology assay for intersectin and a substrate membrane release assay for HCV protease, we found that both proteins were inactivated by the fusion of weakly tetramerizing Dronpa to both termini, and reactivated by illumination with cyan light [35]. In each case, only a few constructs with linkers of different lengths needed to be constructed and tested, and several variants in each case showed light-dependent activity. The creation of single-chain light-controllable proteins by fusion at each end to Dronpa thus appears to be a generalizable strategy.

Besides its relatively easy generalizability, two additional advantages of this approach may arise from the use of a fluorescent protein as the light-responsive domain. First, the chromophore absorbs visible rather than UV light, yet is completely autocatalytically generated without the need for any endogenous metabolites such as flavins or exogenous chemicals such as phytochromobilin or phycocyanobilin. The lack of involvement of flavins not only makes the maturation of the light-controllable protein independent of cellular metabolism, but also assures that wavelengths used for optical control do not excite flavins in endogenous flavoproteins, which is the major cause of phototoxicity in mammalian cells [36,37]. The second potential advantage is that it may be possible to evolve photoswitchable fluorescent proteins that absorb yellow light to also undergo light-induced dissociation, providing the possibility for multiple channels for optical control and further reducing phototoxicity versus cyan light.

Conclusion

The goal of the present review was to provide an overview of the various approaches for implementing optical control over intracellular signals in animal cells, with an emphasis on recent research. Efforts in optical control have been focused on adapting natural light-responsive proteins from plants and micro-organisms, but recent discoveries suggest that light-dependent interactions can be evolved in fluorescent proteins artificially. For a more comprehensive description of natural light-responsive systems, recent detailed reviews are available [38,39].

Light-controllable proteins offer the potential for novel insights into biology, due to the ability to control protein activities with high spatiotemporal resolution and tunable dynamics. For optical control of proteins to become a widespread approach, however, it will be critical for to move beyond light-regulated transcription and light-regulated subcellular localization to achieve light-regulated control of a larger gamut of protein functions. The ability to easily create light-responsive versions of proteins of interest is important. Protein relocalization by CRY2–CIB1 or FKF1–GI and protein aggregation by CRY2 appear to be generally reliable approaches, when the pathway being investigated can be activated by relocalization or aggregation. Light-induced protein fragment complementation, as demonstrated with CRY2–CIB1, is, in principle, also possible for any light-dependent heterodimerization system, and may be another general approach to create light-activatable proteins, although with protein fragment complementation, activation is typically permanent. When regulation of protein activity needs to be transient and cannot be achieved through relocalization or aggregation, creating light-controllable single-chain proteins through fusion of the phototropin LOV2 domain or photoswitchable fluorescent protein domains may be necessary. How generalizable these two approaches are remains to be determined.

As optical approaches to biology are becoming more common, it may be appropriate at this time to introduce a new term. Although the term optogenetics is used by some scientists to include a wide variety of efforts to confer light-responsiveness on proteins [39–41], in most usages, optogenetics refers to its original meaning of functional dissection of neuronal circuits using expression of microbial rhodopsins in genetically defined populations of neurons [4,42,43]. To avoid confusion, we propose using the term ‘optobiology’ to refer to efforts to investigate and control biology using light-responsive proteins. The term optobiology has the further benefit of improved accuracy. It reflects the idea that the field is defined by the interaction of light with biological processes, similar to how the term optoacoustics refers to the interaction of light with sound and optoelectronics to the interaction of light with electrons, something that optogenetics, as mentioned by others, does not [44].

In summary, recent years have witnessed the creation of a wide variety of tools for manipulating protein activity with light. Similarly to how fluorescent proteins transformed the study of biology by making optical sensing of protein localization and activity routine, the tools described in the present review are poised to enable another light-based revolution where optical control of protein localization and activity enables the investigation and control of biology at very fine time and length scales. Another analogy can be made to molecular biology. Just as technical breakthroughs in DNA engineering enabled molecular biology to become a routine method, bringing control of protein expression and structure to biological experimentation, likewise advances in protein engineering may enable optobiology to become a standard method, bringing control of protein activity in space and time to the understanding of biological processes.

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References

1 Krauss, U., Drepper, T. and Jaeger, K.E. (2011) Enlightened enzymes: strategies to create novel photoresponsive proteins. Chemistry **17**, 2552–2560
2 Riggsbee, C.W. and Deiters, A. (2010) Recent advances in the photochemical control of protein function. Trends Biotechnol. **28**, 468–475
3 Keiser, M.J., Irwin, J.J. and Shoichet, B.K. (2010) The chemical basis of pharmacology. Biochemistry **49**, 10267–10276
4 Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A. and Schnitzer, M.J. (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. J. Neurosci. **26**, 10380–10386
5 Airan, R.D., Thompson, K.R., Fenno, L.E., Bernstein, H. and Deisseroth, K. (2009) Temporally precise *in vivo* control of intracellular signalling. Nature **458**, 1025–1029
6 Moglich, A., Yang, X., Ayers, R.A. and Moffat, K. (2010) Structure and function of plant photoreceptors. Annu. Rev. Plant Biol. **61**, 21–47
7 Purcell, E.B. and Crosson, S. (2008) Photoregulation in prokaryotes. Curr. Opin. Microbiol. **11**, 168–178
8 van der Horst, M.A. and Hellingwerf, K.J. (2004) Photoreceptor proteins, 'star actors of modern times': a review of the functional dynamics in the structure of representative members of six different photoreceptor families. Acc. Chem. Res. **37**, 13–20
9 Christie, J.M., Gawthorne, J., Young, G., Fraser, N.J. and Roe, A.J. (2012) LOV to BLUF: flavoprotein contributions to the optogenetic toolkit. Mol. Plant **5**, 533–544
10 Yazawa, M., Sadaghiani, A.M., Hsueh, B. and Dolmetsch, R.E. (2009) Induction of protein–protein interactions in live cells using light. Nat. Biotechnol. **27**, 941–945
11 Polstein, L.R. and Gersbach, C.A. (2012) Light-inducible spatiotemporal control of gene activation by customizable zinc finger transcription factors. J. Am. Chem. Soc. **134**, 16480–16483
12 Lee, J., Natarajan, M., Nashine, V.C., Socolich, M., Vo, T., Russ, W.P., Benkovic, S.J. and Ranganathan, R. (2008) Surface sites for engineering allosteric control in proteins. Science **322**, 438–442
13 Strickland, D., Moffat, K. and Sosnick, T.R. (2008) Light-activated DNA binding in a designed allosteric protein. Proc. Natl. Acad. Sci. U.S.A. **105**, 10709–10714
14 Wu, Y.I., Frey, D., Lungu, O.I., Jaehrig, A., Schlichting, I., Kuhlman, B. and Hahn, K.M. (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. Nature **461**, 104–108
15 Yoo, S.K., Deng, Q., Cavnar, P.J., Wu, Y.I., Hahn, K.M. and Huttenlocher, A. (2010) Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish. Dev. Cell **18**, 226–236
16 Mills, E., Chen, X., Pham, E., Wong, S. and Truong, K. (2012) Engineering a photoactivated caspase-7 for rapid induction of apoptosis. ACS Synth. Biol. **1**, 75–82
17 Strickland, D., Lin, Y., Wagner, E., Hope, C.M., Zayner, J., Antoniou, C., Sosnick, T.R., Weiss, E.L. and Glotzer, M. (2012) TULIPs: tunable, light-controlled interacting protein tags for cell biology. Nat. Methods **9**, 379–384
18 Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.O., van der Horst, G.T., Batschauer, A. and Ahmad, M. (2011) The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. **62**, 335–364
19 Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D. and Lin, C. (2008) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. Science **322**, 1535–1539
20 Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D. and Tucker, C.L. (2010) Rapid blue-light-mediated induction of protein interactions in living cells. Nat. Methods **7**, 973–975
21 Idevall-Hagren, O., Dickson, E.J., Hille, B., Toomre, D.K. and De Camilli, P. (2012) Optogenetic control of phosphoinositide metabolism. Proc. Natl. Acad. Sci. U.S.A. **109**, E2316–E2323
22 Liu, H., Gomez, G., Lin, S., Lin, S. and Lin, C. (2012) Optogenetic control of transcription in zebrafish. PLoS ONE **7**, e50738
23 Bugaj, L.J., Choksi, A.T., Mesuda, C.K., Kane, R.S. and Schaffer, D.V. (2013) Optogenetic protein clustering and signaling activation in mammalian cells. Nat. Methods **10**, 249–252
24 Shimizu-Sato, S., Huq, E., Tepperman, J.M. and Quail, P.H. (2002) A light-switchable gene promoter system. Nat. Biotechnol. **20**, 1041–1044
25 Levskaya, A., Weiner, O.D., Lim, W.A. and Voigt, C.A. (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature **461**, 997–1001
26 Müller, K., Engesser, R., Metzger, S., Schulz, S., Kampf, M.M., Busacker, M., Steinberg, T., Tomakidi, P., Ehrbar, M., Nagy, F. et al. (2013) A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells. Nucleic Acids Res. **41**, e77
27 Toettcher, J.E., Gong, D., Lim, W.A. and Weiner, O.D. (2011) Light control of plasma membrane recruitment using the Phy–PIF system. Methods Enzymol. **497**, 409–423
28 Christie, J.M., Arvai, A.S., Baxter, K.J., Heilmann, M., Pratt, A.J., O'Hara, A., Kelly, S.M., Hothorn, M., Smith, B.O., Hitomi, K. et al. (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. Science **335**, 1492–1496
29 Wu, D., Hu, Q., Yan, Z., Chen, W., Yan, C., Huang, X., Zhang, J., Yang, P., Deng, H., Wang, J. et al. (2012) Structural basis of ultraviolet-B perception by UVR8. Nature **484**, 214–219
30 Müller, K., Engesser, R., Schulz, S., Steinberg, T., Tomakidi, P., Weber, C.C., Ulm, R., Timmer, J., Zurbriggen, M.D. and Weber, W. (2013) Multi-chromatic control of mammalian gene expression and signaling. Nucleic Acids Res. **41**, e124
31 Crefcoeur, R.P., Yin, R., Ulm, R. and Halazonetis, T.D. (2013) Ultraviolet-B-mediated induction of protein–protein interactions in mammalian cells. Nat. Commun. **4**, 1779
32 Bourgeois, D., Regis-Faro, A. and Adam, V. (2012) Photoactivated structural dynamics of fluorescent proteins. Biochem. Soc. Trans. **40**, 531–538
33 Andresen, M., Stiel, A.C., Trowitzsch, S., Weber, G., Eggeling, C., Wahl, M.C., Hell, S.W. and Jakobs, S. (2007) Structural basis for reversible photoswitching in Dronpa. Proc. Natl. Acad. Sci. U.S.A. **104**, 13005–13009
34 Mizuno, H., Mal, T.K., Walchli, M., Kikuchi, A., Fukano, T., Ando, R., Jeyakanthan, J., Taka, J., Shiro, Y., Ikura, M. and Miyawaki, A. (2008) Light-dependent regulation of structural flexibility in a photochromic fluorescent protein. Proc. Natl. Acad. Sci. U.S.A. **105**, 9227–9232
35 Zhou, X.X., Chung, H.K., Lam, A.J. and Lin, M.Z. (2012) Optical control of protein activity by fluorescent protein domains. Science **338**, 810–814
36 Godley, B.F., Shamsi, F.A., Liang, F.Q., Jarrett, S.G., Davies, S. and Boulton, M. (2005) Blue light induces mitochondrial DNA damage and free radical production in epithelial cells. J. Biol. Chem. **280**, 21061–21066
37 Hockberger, P.E., Skimina, T.A., Centonze, V.E., Lavin, C., Chu, S., Dadras, S., Reddy, J.K. and White, J.G. (1999) Activation of flavin-containing oxidases underlies light-induced production of H_2O_2 in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **96**, 6255–6260
38 Müller, K. and Weber, W. (2013) Optogenetic tools for mammalian systems. Mol. Biosyst. **9**, 596–608
39 Pathak, G.P., Vrana, J.D. and Tucker, C.L. (2013) Optogenetic control of cell function using engineered photoreceptors. Biol. Cell. **105**, 59–72
40 Dugue, G.P., Akemann, W. and Knopfel, T. (2012) A comprehensive concept of optogenetics. Prog. Brain Res. **196**, 1–28
41 Yin, T. and Wu, Y.I. (2013) Guiding lights: recent developments in optogenetic control of biochemical signals. Pflügers Arch. **465**, 397–408
42 Packer, A.M., Roska, B. and Hausser, M. (2013) Targeting neurons and photons for optogenetics. Nat. Neurosci. **16**, 805–815
43 Yawo, H., Asano, T., Sakai, S. and Ishizuka, T. (2013) Optogenetic manipulation of neural and non-neural functions. Dev. Growth Differ. **55**, 474–490
44 Miesenbock, G. (2009) The optogenetic catechism. Science **326**, 395–399

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