

## Natural Resources for Optogenetic Tools

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### Abstract

Photoreceptors are found in all kingdoms of life and mediate crucial responses to environmental challenges. Nature has evolved various types of photoresponsive protein structures with different chromophores and signaling concepts for their given purpose. The abundance of these signaling proteins as found nowadays by (meta-)genomic screens enriched the palette of optogenetic tools significantly. In addition, molecular insights into signal transduction mechanisms and design principles from biophysical studies and from structural and mechanistic comparison of homologous proteins opened seemingly unlimited possibilities for customizing the naturally occurring proteins for a given optogenetic task. Here, a brief overview on the photoreceptor concepts already established as optogenetic tools in natural or engineered form, their photochemistry and their signaling/design principles is given. Finally, so far not regarded photosensitive modules and protein architectures with potential for optogenetic application are described.

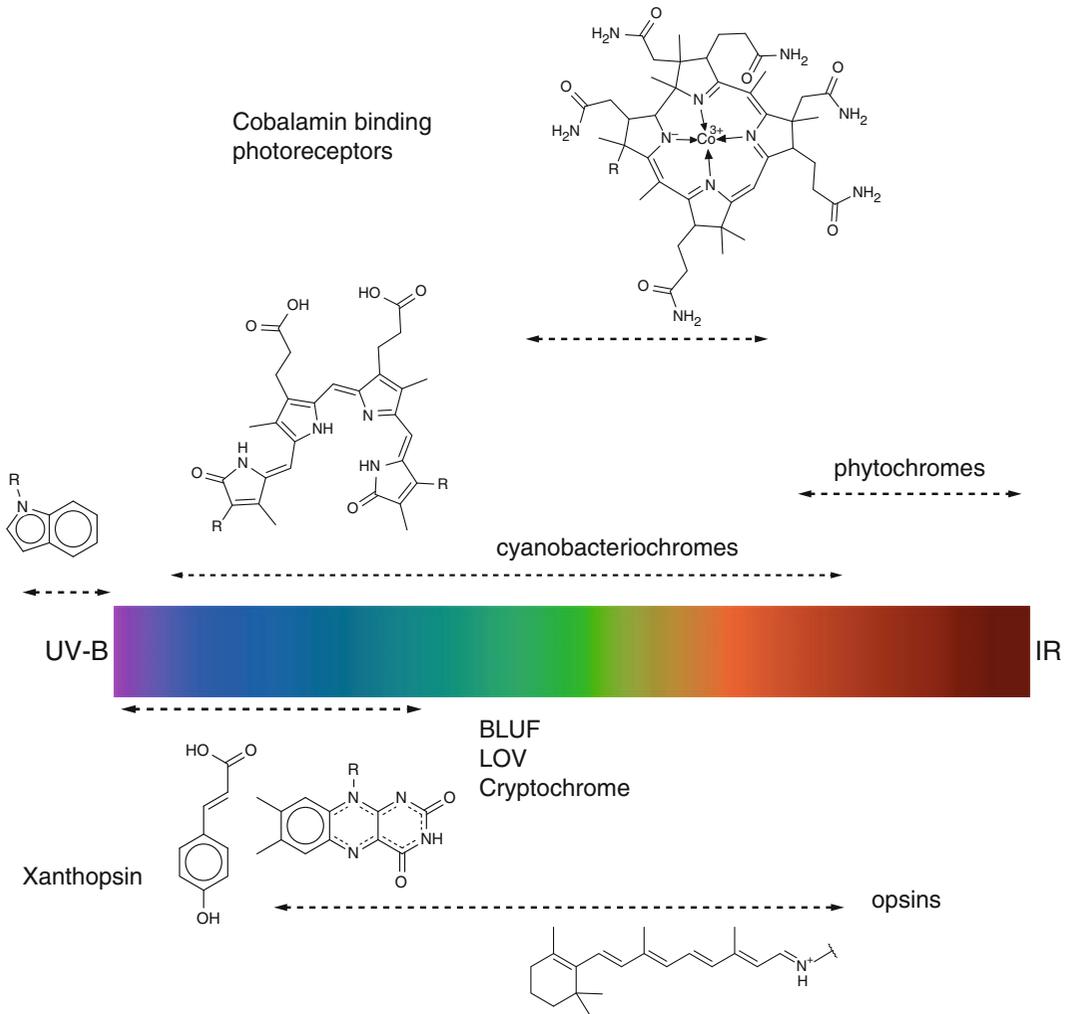
**Key words** Photoreceptor, Signal transduction, Optogenetics, Synthetic biology

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## 1 Natural Photoreceptors

### 1.1 Chromophores

Naturally occurring photoreceptors cover the spectral range from the UV-B to the near-IR (Fig. 1). For this purpose, different chromophores derived from metabolic compounds and enzyme cofactors are utilized and their electronic properties are fine-tuned by the protein environment. Until lately the retinal cofactor of visual and nonvisual opsins has been considered the most versatile chromophore spanning from the near UV regions (~360 nm) in short wavelength sensitive rhodopsins (SWS) [1] to ~700 nm in one of the mantis shrimp rhodopsins [2]. With the discovery algal phytochromes [3] and especially of a novel type of linear tetrapyrrole binding photoreceptor, cyanobacteriochrome, which expand the range of tetrapyrrole chromophores to cover all of the visible spectrum and the near UV, tetrapyrroles outperform retinal in spectral coverage [3, 4]. In the blue to near-UV region, flavin binding photoreceptors—LOV (light–oxygen–voltage), BLUF (blue light sensors using FAD), and cryptochrome photoreceptors—are



**Fig. 1** Spectral coverage (not to scale) of naturally occurring photoreceptor chromophores

prominent and cover a large spectral region with their first two electronic transitions in the oxidized form but are rather limited in color tuning [5]. However, in different reduced states, semiquinone or fully reduced form, flavins have been described to respond to green/yellow or UV-A, respectively [6–8]. In the UV-B region, a recently described photoreceptor concept, UVR8, uses no cofactor at all and employs the UV absorbing nature of tryptophan side chains as a proteinogenic chromophore [9]. In addition, a few special cases exist like the Xanthopsins that employ *p*-coumaric acid as a blue light absorbing chromophore [10] or the just recently discovered cobalamin binding photoreceptors absorbing in the green to red wavelength region [11].

The photochemical properties of most of these chromophores in isolated and protein-bound form are well known. The key biophysical determinants for photoactivation are the quantum

efficiency of signaling state generation and its deactivation reaction in the dark (Table 1). These properties directly affect the net biological signal/effect. The observed photoinduced reactions that lead to a signaling active conformation are isomerizations (retinal, tetrapyrrole, *p*-coumaric acid), proton coupled electron transfer (tryptophan, flavin), and chemical/redox reactions (flavin). The general photochemistry of the chromophores can be readily probed with state-of-the-art time-resolved spectroscopic techniques [12, 13]; however, the interplay with the protein and the relevant structural changes in signal transduction are in most cases more difficult to address. The latter are essential for the feasibility of a potential optogenetic tool, since the light-dependent modulation of the biological signal is not only determined by the quantum yield of the phototransformation of the chromophore itself but rather by the stability of the resulting signaling state and its impact on the protein structure/the extent of modulation of effector activity. In addition to the photoactivation properties, thermal/dark noise of the given photosensor as discussed in GPCR signaling is a relevant aspect [14].

Secondary aspects are of course phototoxicity by photoinduced generation of reactive oxygen species (ROS) byproducts in the photoactivation process itself or by absorption in the cell/tissue that have to be considered for application and naturally depend on the energy required for sufficient optical stimulation. The latter is especially crucial in tissue or animals, where penetration depth is limiting. In such cases, long-wavelength excitation is desirable to minimize diffusion/scattering of the stimulating light. Moreover, energy dissipation from the receptor protein and the tissue itself are crucial parameters for design and evaluation of optogenetic experiments [15].

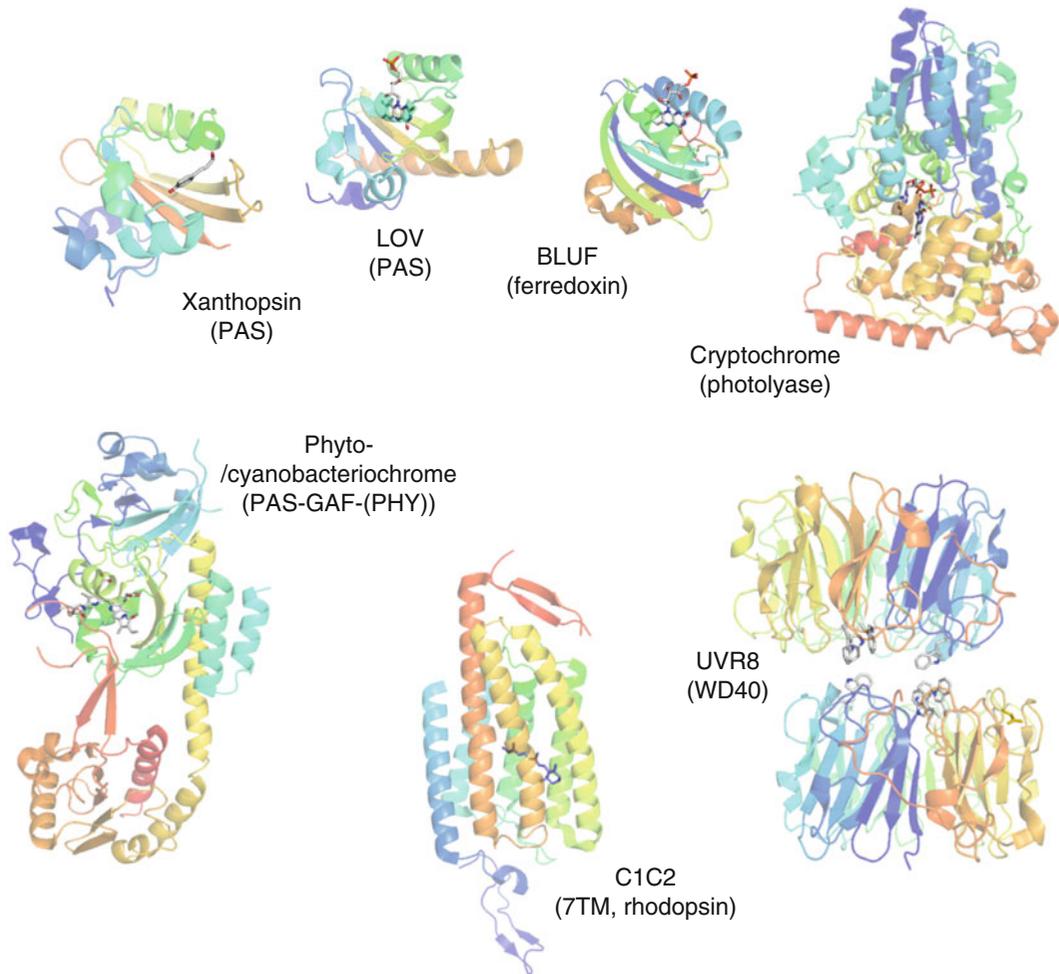
The ultimate requirement, however, is the bioavailability of the cofactor in the investigated cell type. While proteinogenic cofactors and flavins are ubiquitous in nature, the availability of retinal and tetrapyrrole based cofactors may be limiting for the given application. Moreover, spectral orthogonality may have to be considered for applications where multiple selective stimuli are desired.

## **1.2 Photosensitive Modules/Chromophore Binding Domains**

Representative crystal structural models of the binding domains for the above-described chromophores are shown in Fig. 2. While different architectures are found to bind flavins (PAS: Per-Arnt-Sim, BLUF: Blue Light sensors Using FAD, Cryptochrome/Photolyase), also some domain classes (e.g., PAS) are versatile in binding different chromophores (flavins, *p*-coumaric acid, tetrapyrroles). As mentioned above, the potential for optogenetics of these classes naturally depends on the given biophysical properties (Table 1). The small modular photoreceptors are usually more convenient to deliver into the given cell or organism, but most of these small molecule binding receptors feature a low molar

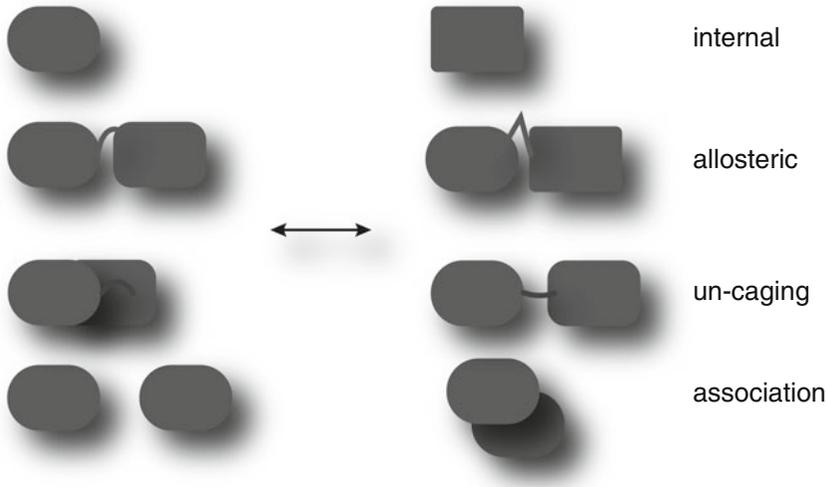
**Table 1**  
**Biophysical parameters of different photosensory modules**

Module	Size (kDa)	Absorption cross section $\epsilon$ ( $M^{-1} \text{ cm}^{-1}$ )	Quantum yield of signaling state formation (%)	Lifetime of signaling state (s)	Apparent modulation of biological activity in native or engineered form (as reported)
BLUF	~14	~11,000–12,000 (FAD) (~445 nm) [85]	~20–90 [86–88]	1–300	Up to 300-fold (bPAC) [27]
LOV	~14	~11,000–12,000 (FMN) (~445 nm) [85]	~30–80 [89, 90]	1–10,000	Up to 1000-fold (YFI) [49]
Cryptochrome	~60	~11,000–12,000 (FAD) (~445 nm, oxidized) ~4000 (~600 nm, semiquinone) [85]	2–14 (oxidized to neutral radical) [91, 92]	180–1000	Up to 200-fold [45]
PYP	~14	~45,500 (~445 nm) [93]	14–18 [94]	100–200	Up to 12-fold [95]
Phytochrome (cyanobacteriochrome)	~60 [40]	~40,000 (Pfr) to 90,000 (Pr) [96]	~10–17 phytochrome [97] (~8–30 cyanobacteriochrome [98])	~Bistable	Up to sevenfold (LaPD) [50]
Rhodopsin	~30	~40,000 [99]	~50 [100]	1–100	Up to 180-fold: Rh-GC [66, 67]
UVR8	~47	~154,000 calc.: $28 \cdot \epsilon_{380\text{nm}}$ (Trp) [101]	~20 [17]	> <i>d</i>	Up to 800-fold [102, 103]



**Fig. 2** Photoresponsive modules found in nature: Xanthopsins, LOV domains and phyto-/cyanobacteriochromes employ the PAS fold to assemble the light-sensitive module. Cyanobacteriochromes lack the PHY domain in contrast to phytochromes. BLUF domains use a ferredoxin-like fold to bind a flavin chromophore. Cryptochromes are structurally related to photolyases and bind FAD and an antenna cofactor (not shown). UVR8 belongs to the family of WD40 repeat proteins with a characteristic  $\beta$ -propeller structure. Pumping and channelrhodopsins belong to the greater family of seven transmembrane proteins and more specifically to the microbial rhodopsins. Structural data (PDB ID) used for illustration: Xanthopsin (1NWZ), AsLOV2 (2V1A), SyPixD (2HFO chain A), AtCry (4GU5), UVR8 (4D9S), C1C2 (3UG9), bacteriophytochrome (400P)

absorption cross section, which makes them generally less attractive for efficient photostimulation. Another important parameter is the quantum yield of phototransformation that illustrates the efficiency of the formation of the metastable signaling state. This is especially crucial in the case of the rhodopsin pumps where the phototransformation is stoichiometrically related to the number of translocated ions. However, as described above the apparent modulation of the biological activity in most other photoreceptors depends strongly on how well regulated the cognate effector



**Fig. 3** Signal transduction principles in naturally occurring photoreceptors

domain is and how strongly its activity becomes enhanced in the activated state. Therefore, even a photoreceptor with apparent low photochemical transformation efficiency (e.g., phytochrome) can be a potent optogenetic tool. Finally, the interaction with the given natural metabolism has to be considered as it might strongly lower the apparent activation compared to *in vitro* studies.

### 1.3 *Signal Transduction Concepts*

The above described photoreceptor classes use various concepts to transform the local structural changes of the chromophore and its protein surrounding into a biological signal and can be roughly categorized in four categories (Fig. 3). In case of the channelrhodopsins and pumping rhodopsins, these processes are intricately entangled as their activity, channeling, or ion pumping, directly involves the chromophore. Similarly, the UV-B induced monomerization of UVR8 is accomplished by direct interaction of the excited chromophore with the cross dimer salt-bridges [16, 17]. In the remaining photoreceptor classes and the sensory rhodopsins, the chromophore is in most cases located distal from the biological effector part, which is especially obvious for modular photoreceptor designs. The signaling concepts of such receptors can be classified into directly allosteric or allosterically induced uncaging and association/dissociation mechanisms [18].

The so-to-speak integral and modular photoreceptors provide different opportunities and limitations for optogenetic application and customization, accordingly. Integral photoreceptors are limited for optogenetic application solely due to their characteristic dark/thermal noise which results in more or less pronounced background signaling activity [14]. In this sense, most modular photoreceptor designs are more prone to background activity since

the photosensory domain only modulates the activity of the effector domain and the dark activity thus depends strongly on the tightness of regulation by the photosensory domain [18]. The possibility to alter the principal functionality of an integral receptor is, however, very limited while modular photoreceptors are found in different sensor/effector combinations covering a wide range of biological activities already in their natural form. In addition, many photosensory modules elicit rather large scale structural changes like unfolding or (de-)oligomerization, which can be easily exploited in order to recruit or release other biomolecules.

#### **1.4 Natural Resources of Light-Responsive Modules and Signaling Architectures**

In contrast to the early times of genome analysis, the technological development in sequence analysis in combination with computational resources for automatic annotation available nowadays provides access to a vast and everyday increasing amount of information on the primary structure and, thus, on the function of proteins with known homologies. Especially the rise of metagenomics that does not rely on monoclonal samples provides access to diverse ensembles of organisms and their genomes and further increased the abundance of sequence information [19–21].

Currently, there are more than 3000 sequences available for the bacteriorhodopsin-like protein family PF01036 (<http://pfam.xfam.org/family/PF01036>) that feature ion pumps, ion channels, and sensory proteins. The greater family of seven transmembrane receptors (<http://pfam.xfam.org/family/PF00001>) with more than 58,000 sequences, covers about 8000 protein sequences of visual and nonvisual rhodopsins (<http://www.ebi.ac.uk/interpro/entry/IPR001760>). Even more remarkable is the greater family of PAS domains with more than 26,000 sequences (<http://pfam.xfam.org/family/PF13426>) that includes the LOV photosensory domains, Xanthopsins and phytochrome architectures. Less than 5 % of these sequences are found as single PAS domain containing proteins while the majority is arranged in a modular fashion in various architectures and with different effector domains. Given the high homology of PAS domains this abundance of structures provides a large number of naturally optimized architectures that can possibly be rendered light sensitive by replacing the given PAS domain with a suitable photosensitive domain, e.g., LOV. Similarly impressive are the phytochrome family (<http://pfam.xfam.org/family/PF00360>) that currently encompasses about 6300 sequences containing PAS-GAF(cGMP phosphodiesterase, adenylate cyclase, FhlA)-PHY(phytochrome-specific GAF related) modules and the BLUF family with about 3900 sequences (<http://pfam.xfam.org/family/PF04940>). Both classes feature a similar diversity in attached effector domains. The family of the cryptochromes (<http://pfam.xfam.org/family/PF12546>) currently features only 157 sequences, probably owing to their occurrence mainly in higher eukaryotic organisms for which less sequence information is available. The structurally closely

related photolyase family (<http://pfam.xfam.org/family/PF00875>), however, features about 15,000 sequences.

The current knowledge on photosensitive modules and receptor architecture thus allows us to extract homologous proteins from genomic or metagenomic data, predict to some extent their properties, screen for desired traits and learn about modular architecture that can be exploited for the rational design of artificial photoreceptors. However, proteins with novel photo-activation concepts cannot be easily discovered in this way and still require thorough photo- and cell biological research. A few recent examples are given below.

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## 2 Engineering of Photoreceptors and Artificial Signal Transduction Pathways

### 2.1 Photoreceptor Engineering

Besides directly harnessing the possibilities provided by nature itself, many photoreceptor functionalities have been extensively engineered. We only focus on a few selected examples, as concise review articles have been published recently (e.g., [18] and chapters 27 in this book). One of the most prominent examples is channelrhodopsin, which has been altered in its activation and deactivation properties, spectral sensitivity, and even ion selectivity [22–25]. Except for PACs, which have also been applied successfully in their natural form [26–29], most modular photoreceptors used in optogenetics have been cut down to the photosensitive module and fused with different interaction partners [18, 30]. The reason for this may be due to the related dark activity or unsuitable signaling activity of the given natural system.

For example, the light-modulated transcription factor Aureochrome found in yellow algae and diatoms at first glance seems like a perfect minimal system to regulate transcription in heterologous systems [31]. However, the short recognition sequence of the bZIP (Basic Leucine Zipper) domain and the ratio of light/dark activity prevent an efficient transfer of these systems into heterologous environments [32]. In its natural context probably other transcription effectors or regulatory elements are engaged to facilitate a physiologically sufficient light-induced change in activity. Therefore, a more efficient approach was to employ a hybrid approach using a (heterologous) LOV domain that undergoes light-induced dimerization, fused to a homologous DNA binding and a transactivator domain of the target cell type. Accordingly, light-induced binding of the homologous fusion protein to a specific sequence and a subsequent expression induction is facilitated [33]. Similarly, the naturally occurring LOV–HTH (helix–turn–helix) protein EL222 that naturally uses light-induced dimerization was fused with a transactivator domain, most likely to enhance optogenetic activation in the heterologous system [34]. Both systems have been applied

successfully but differ in their dynamic range, mainly due to the lifetime of the signaling state of the given LOV domain.

In other artificial fusion constructs several modes of activation, uncaging, (de-)oligomerization, and even allostery directly have been efficiently employed. The most prominent uncaging scenario originates from the plant phototropins. Photoactivation of the LOV2 domain induces dissociation and unfolding of a short C-terminal helix [35] attached to the LOV-domain core and can be efficiently used, e.g., for uncaging of a RAC GTPase [36], a peptide toxin [37] or a cation channel [38]. Partial light-induced unfolding of a PAS domain is also witnessed in the photoactive yellow protein (PYP) and has been exploited for controlling protein/protein interactions [39].

Some naturally occurring LOV domains show light-induced dimerization that was successfully employed by fusion to DNA-binding motifs as described above [40, 41]. PYP in contrast can be engineered to feature such a photoinduced transformation [42]. Especially powerful also proved to be light-dependent heterodimerization of the Arabidopsis photoreceptors cryptochrome 2 (Arabidopsis) and CIB1 (cryptochrome-interacting basic-helix-loop-helix) [43] or phytochrome B and PIF (phytochrome-interacting factors) proteins [44], that was efficiently used to recruit and release proteins. Cryptochrome 2 (CRY2) also features homodimerization/clustering that was optimized and applied for spatial accumulation of proteins [45–47]. Similarly powerful proved to be the UV-B induced dissociation reaction of UVR8, that was used to drive expression or nuclear localization using the natural interaction partner COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) [48]. Despite its large size and slow dark reversion, the specific excitation with UV-B light, which is unbiased by the observation light source in light microscopic experiments, and the missing need for an exogenous cofactor makes UVR8 especially attractive as a fully genetically encoded photoswitch.

The above-described engineering approaches employ secondary effects of light-induced allosteric changes. Engineering allostery directly, however, is significantly more difficult due to the lack of knowledge on the molecular prerequisites in most cases. In the successful cases, careful homologies between different sensor/effector constructs have been investigated and applied in the construction of the artificial protein [49–53].

## **2.2 Orthogonal Signal Engineering**

Besides changing the photosensory properties of a given photoreceptor or de- and reconstructing of sensor/effector combinations, nature also provides us with elegant ways of introducing complete artificial signaling cascades into a heterologous system. These approaches are especially interesting since highly orthogonal systems can be designed, that ideally prevent interference with the

natural metabolism in a given cell. In this regard, especially modular photoreceptor architectures are appealing as an effector domain with a suitable activity can be chosen or engineered.

The most versatile approach in this regard is the optogenetic regulation of expression. An elegant example is the hybrid light-activated histidine kinase YF1, that has been engineered by exchanging a redox sensitive PAS domain from the oxygen activated histidine kinase FixL from *Bradyrhizobium japonicum* for a LOV domain from *Bacillus subtilis* [49, 54]. Together with its downstream phosphorylation target FixK and the well-described activation of its cognate FixK<sub>2</sub> promoter an orthogonal light-activated expression systems for *E. coli* was established. Due to the high specificity of such bacterial, two component systems, a cross talk with endogenous signaling cascades is unlikely.

Similarly, it is conceivable that non-native second messenger modules may be used for a given optogenetic task, e.g., to regulate the activity of a protein. At first glance, promising candidates seemed to be represented by dicyclic nucleotides, which regulate various processes in bacteria and corresponding (light-activated) cyclases and phosphodiesterases have been already described and functionally investigated [55–57]. However, only recently receptor proteins for c-di-GMP, c-di-AMP, and cGAMP have been discovered and functionally described in eukaryotes and even in mammals [58]. Accordingly, dicyclic nucleotides cannot be considered orthogonal second messengers in eukaryotic systems.

A more promising approach for orthogonality may therefore be the integration with chemical biology. Using so-called optochemical genetics it is possible to render an endogenous compound selectively light responsive with only minimal perturbation of the natural system [59, 60]. Moreover, it is possible to investigate the effect of light on the given cell independently of optical stimulation as in most cases the chromophore/photoactive ligand can be added independently of the expressed modified receptor. Similarly, it should be possible to generate non-natural enzyme activities that produce, in a light-responsive manner, artificial second messengers that are recognized by a cognate, non-natural designed receptor.

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### 3 Potential Future Optogenetic Tools Found in Nature

The recent advances in optogenetics and optochemical genetics demonstrate that the combination of natural resources with knowledge driven molecular engineering provides seemingly endless opportunities for synthetic biology and biotechnology. Still, completely new photoreceptor principles are likely to be discovered and will add to the palette of the optogenetic construction kit in the future.

Apart from the so far described and successfully applied opsin based tools several new microbial and also some longer known opsins represent very promising candidates for optogenetics. The histidine kinase rhodopsins discovered in green algae, for example, show a unique multidomain architecture [61]. Directly fused to the transmembrane rhodopsin part are several soluble domains that constitute a complete phosphotransfer cascade on a single polypeptide. The terminal effector domain is a GTP cyclase and thus a promising natural light-activated cGMP synthase. Its function has yet to be demonstrated but the signaling network given by the protein architecture suggests an intricately regulated cyclase activity that offers many levers for optimization/adaptation in an optogenetic context. Especially intriguing is furthermore the bimodal nature of the rhodopsin photosensory domain of HKR1 that can be switched permanently between two states by UV and blue light, respectively. In that sense, also the long known non-visual rhodopsins like melanopsins are highly appealing as they also represent bimodal switches [62] and can be used to drive various G-protein signaling cascades. Melanopsins have recently been applied in modified forms in optogenetic assays for vision restoration [63] and controlling cardiomyocyte activity [64].

Another interesting modular photoreceptor candidate is a recently discovered directly rhodopsin coupled guanylyl cyclase identified in the fungus *Blastocladiella emersonii* [65]. The protein may be considered as a short-cut  $G\alpha_s$ GPCR that usually requires G-proteins to stimulate cyclase activity. Its potential for optogenetics has been recently demonstrated [66, 67]. In contrast to the BLUF regulated photoactivated cyclases, the protein is intrinsically membrane localized and thus enables subcellularly localized stimulation. It is very likely that the cGMP synthesis activity can be adapted to cAMP synthesis as shown vice versa for the BLUF activated PACs [68] and would add a novel membrane localized PAC to the optogenetic toolbox.

A noteworthy subclass of microbial rhodopsins, the xanthorhodopsins [69], employ carotenoid antennas (salinixanthin) to extend the spectral sensitivity to facilitate light-induced proton pumping. This concept is highly interesting for color and sensitivity tuning of potentially any kind of rhodopsin, but has yet to be explored in optogenetics. Another carotenoid employing photoreceptor is the orange carotenoid protein (OCP), which has been found to regulate non-photochemical quenching in cyanobacteria. The carotenoid chromophore (3'-hydroxyechinenone) enables photochromic switching between an orange and a red form [70]. A model involving a large scale translocation of the carotenoid and accompanying structural changes at the C-terminus has been recently proposed [71], which would enable the protein to interact in a regulatory manner with the phycobilisome [72]. OCP belongs to a smaller protein family of about 216 sequences containing a

cyanobacteria-specific N-terminal domain (<http://pfam.xfam.org/family/PF09150>). The remaining protein belongs to the class of Nuclear transport factor 2 (NTF2) domain (~6200 sequences; <http://pfam.xfam.org/family/PF02136>). So far this photoactivation concept has not been applied in an optogenetic context, but clearly provides interesting novel starting points for the design of optogenetic tools regarding new chromophores and specific light-induced structural changes.

Similarly to the photochemical diversity of retinal binding photoreceptors, also novel photochemical traits of the tetrapyrrole binding receptors have been discovered recently. Although phytochromes already represent near-IR/far-IR bimodal switches, the cyanobacteriochromes can be tuned over a broad range in the visible spectrum and feature well separated spectra of both states. Moreover, these proteins do not require the PHY domain that is needed for efficient photoswitching in phytochromes. These properties are clearly attractive for optogenetics and a widespread use of cyanobacteriochrome in optogenetic application is expected in the future. In addition, novel insights into the monomerization/dimerization prerequisites for phytochromes have been found recently. Inspired by the search for monomeric phytochrome based infrared fluorescent proteins [73], a photochromic monomer/dimer switchable phytochrome module has been described and clearly has a high potential for building tightly regulated bimodal optogenetic mono-/dimerizers [74].

In the first optogenetic experiments, complete visual rhodopsin signaling cascades [75] and modified rhodopsins [76–78] have been employed to stimulate G-protein induced responses. Because of the abundance of GPCRs in higher animals and their relevance for disease and metabolic disorders, GPCRs are naturally attractive targets for optogenetic studies or even therapeutics. A noteworthy completely new type of photoactivatable GPCRs has been found to be responsible for the photoavoidance of *Caenorhabditis elegans* and larvae of *Drosophila melanogaster* [79–82]. Apparently, these gustatory GPCR paralogs (family of 7TM chemosensory receptors; <http://pfam.xfam.org/family/PF08395>; currently 1900 sequences) facilitate the UV-induced photophobic behavior via different G-protein cascades. LITE-1 found in *C. elegans* mediates photoavoidance via  $G_{i/o}$  proteins that stimulate the activity of membrane associated guanylyl cyclases which in turn affect CNG channels [80]. Although action spectra of the LITE-1 mediated photoresponse have been described, the nature of its chromophore is completely unclear. From the crude action spectra recorded by behavioral experiments showing a maximum in the UV-A region at around 350 nm but also a significant response at up to 450 nm, many potential chromophores can be discussed (*see* Fig. 1). Besides

using classical prosthetic chromophores LITE-1 may in principle also transiently bind small molecules that have been chemically altered by short wavelength/high-energy light. The discussion whether LITE-1 and the related Gur-3 may also be activated by ROS as a photoinduced by-product is discussed controversially [83]. In *D. melanogaster*, the close homolog Grb28 (isoform B) also employs G-protein mediated signaling, but the classification of the interacting G-protein remains unknown. In contrast to LITE-1, the observed  $\text{Ca}^{2+}$  photocurrents are not mediated by CNG channels. Instead, the transient receptor potential (TRP) channel TrpA1 was identified to be responsible. Clearly, this class of proteins features promising candidates to stimulate various G-protein related activities in optogenetic approaches.

Another completely new class of soluble photoreceptor that was found in *Myxococcus xanthus* uses a closed ring tetrapyrrole cofactor, Cobalamin (coenzyme B12), and is the first appearance of this chromophore in the photoreceptor world [11]. CarH requires coenzyme B12 for light-dependent gene repressor activity by oligomerization and subsequent DNA binding. Upon illumination the cofactor is photolysed and the oligomer dismantles and dissociates from its cognate operator. The underlying photodynamics and crystal structures in dark and light-activated states have been recently described [84]. Therefore, a solid basis for application of CarH as a novel optogenetic tool has been already established.

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## 4 Concluding Remarks

Up to now nature has provided us with a vast toolbox for optogenetic application and most likely has further so far unknown photoactivation concepts in its repertoire. Moreover, even for well-known photoreceptor concepts novel photochemical traits or signal transduction scenarios are discovered constantly. Most of these proteins provide new opportunities for optogenetic application, either to be harnessed from nature directly or in the sense that we learn about different molecular solutions for photoreception that can be applied in the rational design of new optogenetic tools. Furthermore, combination of synthetic biology with chemical biology is expected to add significantly to the optogenetic toolbox.

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