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Engineered photoreceptors as novel optogenetic tools†‡

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Cellular processes and indeed the survival of entire organisms crucially depend on precise spatiotemporal coordination of a multitude of molecular events. A new tool in cell biology is denoted "optogenetics" which describes the use of genetically encoded, light-gated proteins, *i.e.* photoreceptors, which perturb and control cellular and organismal behavior in a spatiotemporally exact manner. Photoreceptors resemble fluorescent reporter proteins such as GFP in being genetically encoded, non-invasive, and applicable to intact cells and organisms. They are explicitly intended to modulate activity; in contrast, fluorescent proteins generally do not disturb the processes under study. Fluorescent proteins have revolutionized cell biology because they allow the monitoring of such processes by imaging techniques that offer superb spatiotemporal resolution and sensitivity. Optogenetics extends these advantages to offer control. The scope of optogenetics has recently been expanded beyond the use of naturally occurring photoreceptors by the biologically-inspired design of engineered (or synthetic) photoreceptors. These photoreceptors are derived by fusion of one or more light-absorbing sensor domains with an output or effector domain displaying the activity to be controlled. Here, we focus on the design and application of such engineered photoreceptors. We treat basic signaling principles and discuss the two photosensor classes which are currently most widely used in fusion-based design: LOV domains and phytochromes. Based on these principles, we develop general strategies for the engineering of photoreceptors. Finally, we review recently successful examples of the design and application of engineered photoreceptors. Our perspective provides guidelines for researchers interested in developing and applying novel optogenetic tools.

Introduction – Proteins that interact with light

Throughout evolution many organisms have developed the ability to interact with light, because light serves as a near-ubiquitous source of energy and information. Light can be utilized either for its energy content, *e.g.* in photosynthesis, or for its information content, *e.g.* in phototactic responses. Organisms are not only able to absorb light but also in certain circumstances to emit light signals *via* fluorescence, phosphorescence or bioluminescence. Interactions with light are mediated by proteins and require chromophore cofactors since neither the (unmodified) polypeptide backbone nor protein side chains absorb light of visible wavelengths. Chromophores can either be covalently or non-covalently bound by the protein moiety; they can be small organic molecules or derive from protein side chains as in green fluorescent protein (GFP). In this perspective article, we focus on photoreceptor proteins which utilize the information contained in light to elicit a physiological response; they are signaling photoreceptors. Signaling photoreceptors respond to light absorption with a change in biological activity: their activity is a function of light absorption. Since light readily traverses biological membranes, most signaling photoreceptors are intracellular and water-soluble; some such as channelrhodopsin and sensory rhodopsin, in which the activity to be controlled is located in a membrane, are themselves integral membrane proteins.

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Upon obtaining his diploma degree in biochemistry from the University of Regensburg, Germany, in 2001, Dr Möglich moved to the Biozentrum Basel, Switzerland, to pursue graduate research in protein folding under the supervision of Dr Thomas Kiefhaber. In 2005, Dr Möglich obtained his Ph.D. degree in biophysics. As a postdoc in Dr Keith Moffat's lab at the University of Chicago, USA, from 2006 until 2010, Dr Möglich studied the structure and design of photoreceptors. He has just taken up a junior professor position in the faculty of biology at Humboldt-University Berlin, Germany. His main research interests are the structural, functional and mechanistic characterization of photoreceptors, and the engineering and optogenetic application of photoreceptors.

Due to their relatively high abundance in certain organisms and the presence of distinctive chromophores, proteins that are either fluorescent or involved in harvesting light for energy purposes can readily be isolated and characterized. Facilitated by advances in molecular biology, many of these proteins have now been identified at the molecular level and their function is reasonably well understood. Detailed knowledge of the properties of these proteins subsequently allowed their targeted use in basic and applied science. In particular, applications of fluorescent proteins from jellyfish and other organisms,[1] of which GFP is the paradigm, have revolutionized biology. Fluorescent proteins can be genetically encoded and fused to target proteins, and thus they specifically label these cellular constituents with exquisite sensitivity. As light can readily penetrate cells and tissue to a certain depth, living cells can be observed non-invasively: lysis, fixation and staining of samples is not required. Light affords excellent spatiotemporal resolution, thus enabling the facile study of spatial localization and dynamic processes. Fluorescent proteins can be used in conjunction with recently developed super-resolution microscopy techniques[2] to achieve spatial resolution beyond the classic diffraction limit; however, further improvements are needed to study molecular interactions with atomic resolution.

In contrast to fluorescent or "energy-harvesting" proteins, photoreceptor proteins are generally much less abundant, which has hampered their identification and detailed study. Many photoreceptors have only been identified over the past two decades and their molecular characterization is still in progress. Since the biological activity of photoreceptors is light-dependent, light can be used to control their function and indeed the behavior of entire cells and organisms in which these photoreceptors are expressed. Fluorescent proteins have greatly improved our ability to *monitor* cellular processes but in marked contrast, photoreceptors now allow us to *control* cellular and even organismal behavior by light. The photoreceptors with the widest application at present are channelrhodopsins,[3] which function as light-gated ion channels and were first isolated from flagellate algae. When expressed in neurons, channelrhodopsins elicit action potentials upon light absorption which has led to their widespread use in neuroscience (see *e.g.* Gradinaru *et al.*[4]). Deisseroth and colleagues coined the term "optogenetics"[5] to describe the use of such natural, genetically encoded proteins to monitor and control the activity of "targets" within living neural circuits. While the term was originally confined to applications of natural photoreceptors in neuroscience, we deliberately extend the use of the term optogenetics to refer to all applications of a genetically encoded, light-gated molecule, natural or engineered, to modulate the activity or behavior of living cells and organisms of any type.

The arsenal of optogenetics has been greatly expanded recently by the design of engineered photoreceptors. Informed by the properties and architecture of natural photoreceptors, engineered photoreceptors with light-regulated function were successfully designed, generated by domain fusion and used to control molecular activity and cellular behavior. The use of natural photoreceptors in optogenetics has recently been reviewed.[6] We focus here on photoreceptors engineered by domain fusion and their application as novel optogenetic tools. We discuss different photoreceptors and their signaling properties, and provide examples of recently designed, novel photoreceptors. From these successful applications, we develop guidelines for the design and application of engineered optogenetic tools.

Chemical and biological approaches to control by light

Although the function of most proteins and biomolecules is not inherently light-dependent, in many cases it can be made so, *e.g.* through modification with a photosensitive chemical moiety.[7,8,9,10,11] Ideally, such moieties are held inactive (or "caged") until activated by light absorption. By analogy to these chemically caged compounds, engineered photoreceptors could be referred to as genetically caged compounds, in which the "cage" is provided by a photosensor domain. Since chemical and genetic caging differ in a number of important aspects, engineered photoreceptors represent valuable alternatives to the longer-established and versatile chemical caging approaches (Table 1). An in-depth treatment of chemical caging strategies has been provided elsewhere.[7,8,9,10,11] Here, we briefly outline some key aspects and contrast chemical and biological approaches.

Most chemically caged compounds are irreversibly activated by light, usually through photolysis of one or several covalent bonds. Although the primary photochemical event is ultrafast, subsequent structural rearrangements necessary to liberate the substrate and generate the desired, activated molecule can be much slower and require microseconds to milliseconds. Photolysis is frequently accompanied by the generation of reactive intermediates such as radical species, which can give rise to interfering side reactions. A different, reversible and ultrafast chemistry is exhibited by azobenzene compounds which undergo light-induced *cis-trans* isomerization that does not involve reactive intermediates.[9,12] Chemical approaches are versatile and allow the caging of low-molecular-weight substrates, *e.g.* Khan *et al.*,[13] and larger biomolecules including proteins, nucleic acids, and lipids. The size of the photosensitive cage is usually small. *In vivo* use of chemical approaches is frequently complicated by the necessity of smoothly and specifically introducing them into the cell or tissue of interest, and by the limited lifetime of chemically caged compounds under physiological conditions.

Keith Moffat

A biophysicist, Keith Moffat obtained his Ph.D. in protein crystallography with Max Perutz in the MRC Laboratory of Molecular Biology in Cambridge, UK, in 1970. After a brief postdoc in rapid reaction kinetics with Quentin Gibson at Cornell University, Prof. Moffat held faculty positions in biochemistry and molecular biology at Cornell University (1970–1990) and the University of Chicago (1990–present), where he is Louis Block Professor and, until 2010, Deputy Provost for Research. He is interested in time-resolved macromolecular crystallography, particularly of photoreceptors and other light-sensitive systems, and the design and optogenetic applications of photoreceptors.

Table 1 Properties of chemical and genetic caging

	Chemical caging	Genetic caging
Photochemistry		
Timescale	Initial photoreaction fast, subsequent steps μs to ms ps for azobenzene compounds	ps to μs for different photoreceptors
Reversibility	Usually irreversible Reversible for azobenzene compounds	Fully reversible
Side reactions	Frequent (reactive intermediates, radicals) None or minor for azobenzene compounds	None or minor
***In vivo* use**		
Size of cage	Small (photolabile chemical ligand)	Large (photosensor domain)
Target	Protein, nucleic acids, low-molecular-weight compounds	Protein
Caging specificity	Usually low to moderate High if caged compound can be introduced site-specifically, *e.g.* through use of modified tRNAs	High
Stability	Often limited lifetime *in vivo*	Stable *in vivo*
In vivo delivery	Usually exogenous addition Endogenous production possible in some cases, *e.g.* through use of modified tRNAs	Endogenous production through genetic encoding, if DNA can be delivered to the desired location
Cytotoxicity	Certain chemically caged compounds can be harmful to target cells and organisms	Usually low or no cytotoxicity

In contrast, in the case of natural and engineered photoreceptors, sensitivity to light is provided by one or more photosensor domains (as we discuss below), and hence is largely restricted to protein targets. Engineered photoreceptors offer perfect molecular targeting since the protein and its photosensor domain are genetically encoded and usually form parts of a single polypeptide. In contrast, the specificity of targeting by chemical caging of macromolecules is lowered by cross-reactivity, *i.e.* the chemical cage may react both with the desired macromolecule and with other chemically similar macromolecules. Targeting by chemical caging of low-molecular-weight substrates is further affected by their rapid diffusion, and by the fact that they could also be substrates of other biomolecules which would thus inadvertently be activated. The key advantage of engineered photoreceptors is their ability to be genetically encoded; they can be introduced to the target organism, tissue or cell as DNA templates and produced endogenously *in situ*. If the chromophore is a widely-available cellular constituent such as flavin mononucleotide (FMN) or biliverdin (BV, obtained as a degradation product of heme), then endogenous expression of the photosensor protein is usually followed by spontaneous, uncatalyzed incorporation of the chromophore to generate the light-sensitive photosensor species. In contrast, the use of chemically caged compounds normally involves exogenous addition although in some cases the caged compound can be produced endogenously, *e.g. via* use of modified tRNAs to introduce a non-natural, photosensitive amino acid.[14]

Principles of signal transduction

We briefly review the thermodynamics of signaling processes as they provide the basis for function of both natural and engineered photoreceptors. Signal transduction by any receptor protein – be it a chemoreceptor or a photoreceptor – requires that the receptor protein can adopt at least two states which differ in their biological activity, and that the presence of the signal leads to a change of the relative populations of these states. Previously, we introduced a simple allosteric model to discuss receptor function (Fig. 1a).[15,16] Briefly, a receptor protein can be in equilibrium between a biologically less active state T ("tense") and a more active state R ("relaxed"). In the absence of signal, the ratio between the T and R states is determined by the free energy difference, ΔG_0, between them:

$$L_0 = [T]_0/[R]_0,\ \Delta G_0 = -RT\ln L_0 \quad (1)$$

where the fraction of molecules f_{R0} in the R state is given by:

$$f_{R0} = 1/(1 + L_0) \quad (2)$$

Introduction of a signal S – light in the case of photoreceptors – alters the stabilities of the T and R states by $\Delta G_{sig}{}^T$ and $\Delta G_{sig}{}^R$, respectively, thus giving rise to the new equilibrium L_S:

$$L_S = [T]_S/[R]_S,\ \Delta G_S = -RT\ln L_S \quad (3)$$

and

$$f_{RS} = 1/(1 + L_S),\ \Delta f_R = f_{RS} - f_{R0} \quad (4)$$

The total free energy derived from the signal, $\Delta\Delta G_{sig}$, is thus given by:

$$\Delta\Delta G_{sig} = \Delta G_{sig}{}^T - \Delta G_{sig}{}^R = \Delta G_S - \Delta G_0 = -RT\ln L_S/L_0 \quad (5)$$

Depending upon the sign and magnitude of $\Delta G_{sig}{}^T$ and $\Delta G_{sig}{}^R$, and hence of $\Delta\Delta G_{sig}$, introduction of a signal can thus lead to either an increase ($\Delta f_R > 0$) or decrease ($\Delta f_R < 0$) of biological activity. Our general model implies a pre-existing equilibrium between the T and R states even in the absence of signal,[17,18] which has been documented for at least some photoreceptors.[19] An alternative induced-fit model[20] represents a specialized case of the general scenario in which the receptor is completely in one state, *e.g.* T, in the absence of signal and completely in another state, *e.g.* R, in the presence of signal. Either model can be expanded to accommodate more than two states, *e.g.* for photoreceptors with complex photocycles or for oligomeric photoreceptors. Efficient regulation of biological activity necessitates that a significant fraction, α, of the total available free energy contained in the

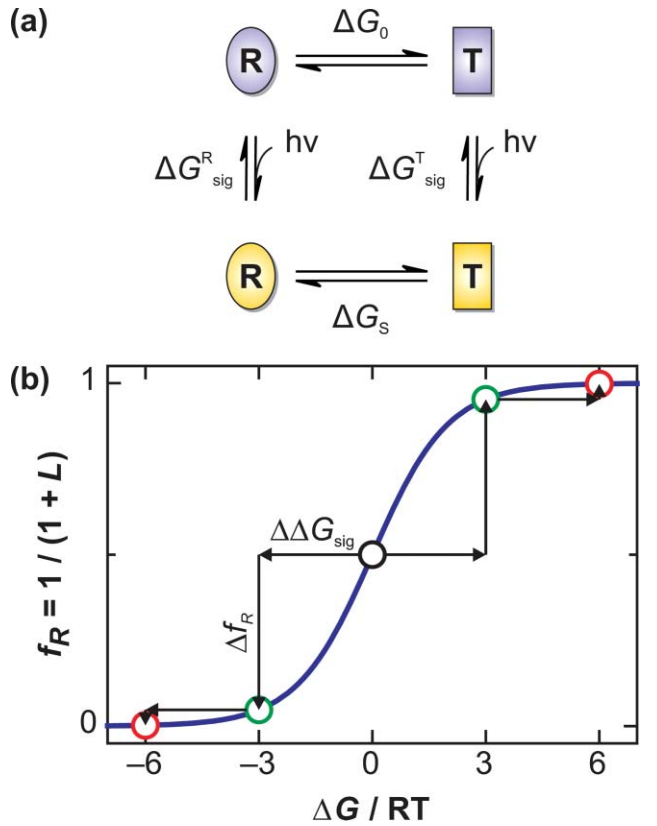

Fig. 1 Principles of signal transduction. (a) A signal receptor may exist in equilibrium between a biologically less active T (tense) and a more active R (relaxed) state. Introduction of a signal – light in the case of photoreceptors – alters the stability of T and R by $\Delta G_{sig}{}^{T}$ and $\Delta G_{sig}{}^{R}$, respectively. Thus, the equilibrium constant L between T and R is shifted and protein activity is modulated in a signal-dependent manner. (b) Sensitivity towards signal depends upon the intrinsic equilibrium between the T and R states in the absence of signal, determined by ΔG_0. A given signal with energy $\Delta\Delta G_{sig}$ induces a maximum change in the fraction of R, f_R, if ΔG_0 is close to zero (*e.g.* going from the black open circle to the green open circle). If $|\Delta G_0| \gg 0$, introduction of signal only slightly changes f_R (*e.g.* going from the green open circle to the red open circle).

signal, ΔG_{total}, is translated to a shift in equilibrium between the T and R states.

$$\Delta\Delta G_{sig} = \alpha \times \Delta G_{total},\ 0 \le \alpha \le 1 \qquad (6)$$

A key challenge for designers of synthetic photoreceptors is to maximize the coupling efficiency α (see below). For photoreceptors ΔG_{total} equals the energy of the incident photon itself.

As illustrated in Fig. 1b, the sensitivity of a receptor to signal not only depends upon the magnitude of $\Delta\Delta G_{sig}$ but also on the intrinsic equilibrium between T and R in the absence of signal. A receptor is maximally responsive to signal, *i.e.* $|\Delta f_R|$ is large, if ΔG_0 is close to zero. If, however, the intrinsic equilibrium lies far on the side of states T or R, even large $\Delta\Delta G_{sig}$ terms will only lead to small changes in the fractional populations of the T and R states, f_T and f_R.

These fundamental considerations already illustrate a key property of photoreceptors and signal receptors in general, namely that they are dynamic entities. For maximum sensitivity towards signal, photoreceptors must be delicately poised. As we discuss below, this represents a challenge to designers of engineered photoreceptors.

We recognize that dynamic aspects of receptor behavior, *i.e.* non-equilibrium thermodynamics, may also be important. Indeed, the rate at which the signaling state is attained, the nature of the short-lived intermediates en route to that state, and the rate at which signal disappears as that state reverts to the ground, dark state are important at both the biophysical and the biological level (see below and, for example, Moffat[21]).

LOV and phytochrome photoreceptors

Photoreceptors detect light through absorption of photons by their chromophore cofactor. Upon light absorption, a so-called photocycle is initiated which comprises a series of events that leads to changes of the conformation, dynamics and function of the chromophore and the surrounding protein moiety. At a minimum the photocycle comprises two states, the dark or ground state and the lit or signaling state, which differ in their biological activity. Often, additional states or intermediates form part of the photocycle. Usually, the photocycle is fully reversible and after light absorption the photoreceptor thermally reverts to its ground state. Progression through the photocycle is accompanied by distinct electronic changes and hence by spectroscopic changes of the chromophore in the UV/visible range. For example, photon absorption may lead to Z/E isomerization of the chromophore or formation of a covalent bond between the chromophore and the photoreceptor protein. Six classes of photoreceptors are currently distinguished by their chromophores and photochemistry: light-oxygen-voltage (LOV) sensors, xanthopsins, phytochromes, blue-light sensors using flavin adenine dinucleotide (BLUF), cryptochromes, and rhodopsins.[22,23] The first five of these are water-soluble; the last are integral membrane proteins. Cyanobacteriochromes[24] share aspects of their photochemistry with phytochromes yet could be considered to constitute their own seventh class. For the purpose of this perspective, we focus on the two classes which have been most widely used in the construction of engineered photosensors, LOV domains and phytochromes, and concentrate on the best studied examples. However, no fundamental reasons preclude other natural photosensor domains, including integral membrane proteins, from being used for the design of engineered photoreceptors. Once the properties and signaling mechanisms of all natural photoreceptors are better understood, we expect their photosensor domains to become part of the optogenetics toolkit.

In common with many chemoreceptors and certain other photoreceptors, LOV and phytochrome photoreceptors are composed of modules, that is, compactly folded protein domains arising from a contiguous amino acid sequence, that differ in their function.[25] Photosensor modules mediate light detection and serve as input domains; effector modules display biological activity, *e.g.* enzymatic activity, DNA or protein binding, transport or channel activity, and serve as output domains. Signal-dependent interactions between sensor and effector modules lead to a change in biological activity in response to signal. That is, the presence of signal (here, absorption of a photon) causes information to be transferred from the sensor to the effector domain. This information can be purely structural, *i.e.* signal causes a shift in mean atomic positions, or it can be purely dynamic, *i.e.* signal alters the extent of fluctuations in position of atoms without

affecting their mean positions; or of course, a combination of structural and dynamic effects. Whatever their exact bases, signals are essentially thermodynamic in nature and reflect a change in affinity between parts of one protein or between different proteins. Sensor and effector domains may be covalently linked, *i.e.* the complete photoreceptor comprises several domains, or non-covalently associated. Natural LOV and phytochrome photosensor domains are usually covalently linked to the N-terminus of their effector domain and occasionally to the C-terminus, but are never found inserted into, for example, a surface loop of the effector domain.[23] In diverse classes of chemo- and photosensors these links between individual modules are frequently formed by short amphipathic α-helical segments.[16,26]

The modularity of natural photoreceptors in which sensor and effector functions are located in separate domains provides the foundation of current design approaches, which are based on (careful) domain fusion. In the following we briefly cover structural and mechanistic aspects of LOV and phytochrome sensors as they apply to the design of engineered photoreceptors. A number of reviews offer an in-depth discussion of natural LOV and phytochrome photoreceptors.[16,27,28]

LOV domains

LOV sensors[29] derive from plant, fungal and bacterial proteins and form a subset of the versatile Per-ARNT-Sim (PAS)[16,30] family. They adopt the canonical PAS domain fold and non-covalently bind a flavin nucleotide cofactor (FMN or flavin adenine dinucleotide (FAD)) between the inner surface of a five-stranded antiparallel β sheet and several α helices (Fig. 2a). Upon blue-light absorption, a covalent thioether bond is formed on the microsecond time scale between the isoalloxazine ring of the flavin nucleotide and a conserved cysteine residue within the LOV domain to form the signaling state (Fig. 2b).[31] Removal of the active-site cysteine through site-directed mutagenesis abolishes the normal photochemistry and renders the LOV domain fluorescent.[32,33] Studies on different LOV domains reveal that the structural changes induced by light are small and largely confined to the vicinity of the flavin chromophore. Although the experimental results are largely consistent between different LOV domains, we caution that the precise nature and magnitude of observable light-induced changes may depend on the experimental technique used to measure them, *e.g.* X-ray crystallography.[16] Light-induced changes propagate to and through the β sheet to its outer surface, which serves as an interaction hub as in other PAS domains.[16] Light signals thus modulate the dissociation equilibrium between the outer surface of the β sheet and its interaction partner which, depending on the LOV domain, has different downstream consequences. In the *Neurospora crassa* Vivid protein an N-terminal α-helical cap structure forms the interaction partner and is packed on the outer surface of the β sheet in the dark state where it undergoes conformational changes upon light absorption.[34] The LOV domain of *Bacillus subtilis* YtvA dimerizes through the outer surface of its β sheet.[35] Small light-induced quaternary structural changes could propagate to the effector domain as torque within a coiled-coil linker which is formed by the so-called Jα helices immediately C-terminal to the core of the LOV domain.[36,37] In the best-studied LOV domain, LOV2 from *Avena sativa* phototropin 1, light absorption promotes unfolding of the C-terminal Jα helix which in the dark state is packed on the β sheet.[38] The signaling state is metastable: it thermally decays to the dark, ground state within tens to thousands of seconds depending upon the nature of the LOV domain. Alternatively, illumination of the signaling state with near-UV light can be used to photolyze the thioether bond and thus actively revert the signaling state to the ground state.[39]

Phytochromes

Phytochromes occur in plants, fungi and bacteria (where they are denoted bacteriophytochromes) and covalently bind linear tetrapyrrole (bilin) chromophores that absorb red/far-red light. The four pyrrole rings of the bilin cofactor are denoted A–D. Bacterial and fungal phytochromes bind biliverdin, plant phytochromes bind phycocyanobilin (PCB) or phytochromobilin (PΦB), and cyanobacterial phytochromes bind PCB, PΦB and other linear tetapyrroles.[24,28] In plant and many bacterial phytochromes the photosensor comprises three individual domains denoted PAS, GAF and PHY, where PHY is closely similar to the GAF domain (Fig. 2c),[40,41,42] both of which in turn topologically resemble the PAS domain family.[16,43] While the bilin chromophore largely makes contact with residues in the GAF domain, residues in the other two domains also contribute, and the presence of the PAS and PHY domains is required for full photoactivity.[28] However, several cyanobacterial phytochromes entirely lack either the PAS domain, the PHY domain or both.[24] The photocycle of conventional phytochromes involves transitions between red-light-absorbing (denoted Pr) and far-red-light-absorbing spectral states (Pfr). In some proteins Pr is the thermally stable, dark state and in others Pfr. The states can be reversibly interconverted by illumination with red or far-red light, respectively. Thus, the full photocycle involves absorption of two photons, one to drive the forward transition, *e.g.* Pr→Pfr, and the other, the backward transition, *e.g.* Pfr→Pr. Alternatively, the signaling state can thermally revert back to the ground state. At the molecular level, the primary photochemical event in the Pr↔Pfr transition involves isomerization of the bilin chromophore. In the traditional view, isomerization occurs around the C15═C16 double bond between the C and D rings, thus leading to a flip of ring D (Fig. 2d). However, recent evidence suggests that in certain phytochromes isomerization may mainly involve the linkage between the A and B rings.[44] Yet other types of photochemistry occur in the diverse group of cyanobacteriochromes which to date have been largely untapped for design purposes.[24] Similar to LOV domains, impairment of the normal photochemistry through mutagenesis can convert phytochromes to fluorescent proteins.[45,46,47] Signal transduction mechanisms in phytochromes are only beginning to emerge. As evidenced by crystal structures, several bacterial phytochromes form parallel dimers in which individual domains are linearly arranged around a central spine formed by an extensive α-helical bundle (Fig. 2c). Propagation of light signals to a histidine kinase effector domain could involve generation of torques about the C-terminal helices of the sensor domains and their propagation to the histidine kinase (or kinase-like) effector domain where they modulate its activity, in a manner similar to that discussed for the YtvA LOV domain (*cf.* above).[36]

Although key aspects of function and signal transduction remain unclear, the recent successful design and implementation of

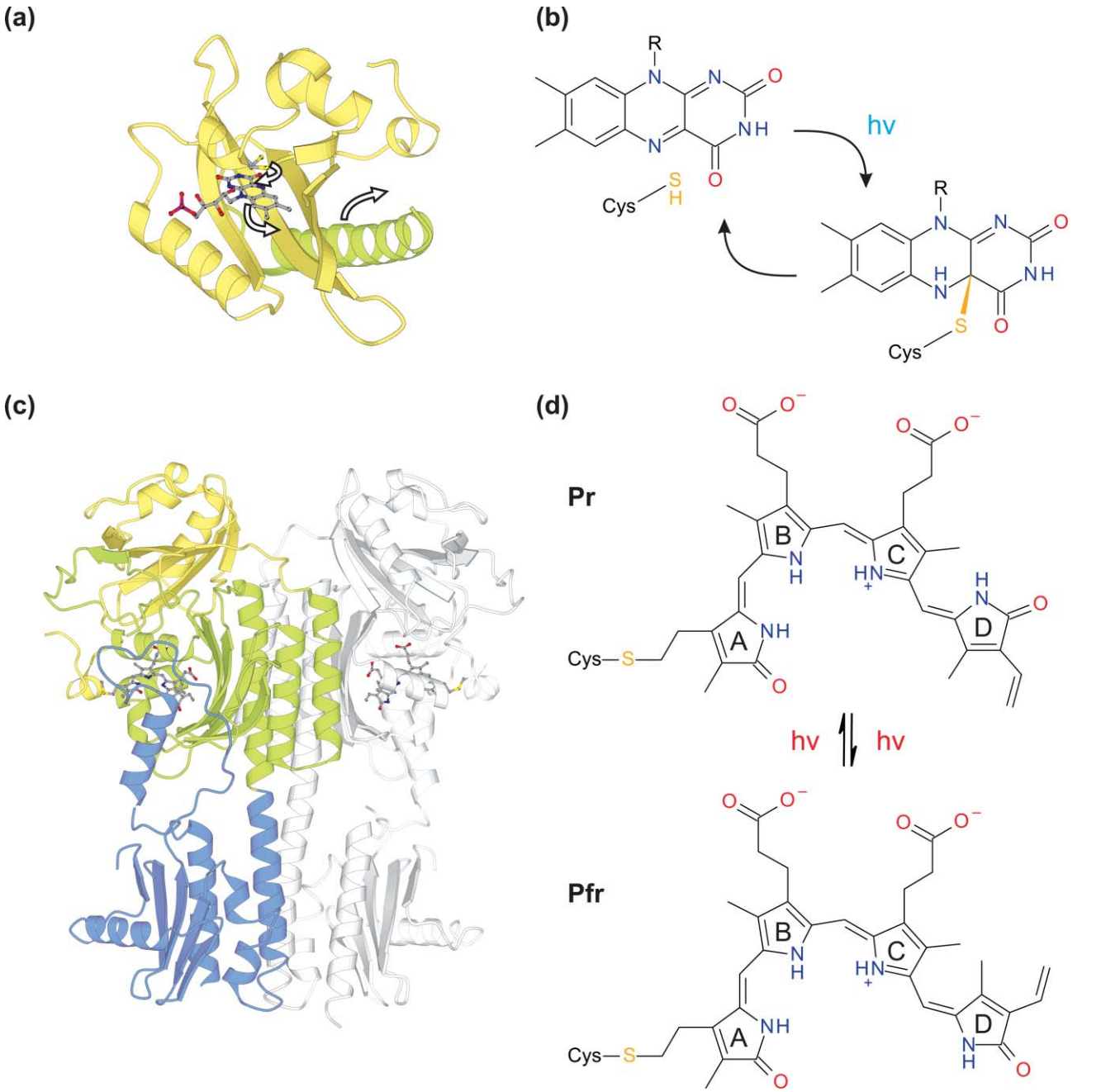

Fig. 2 Selected photosensors used for the generation of engineered photoreceptors. (a) LOV domains such as LOV2 from *A. sativa* phototropin 1 (PDB 2V0U) adopt the canonical PAS fold and bind flavin nucleotides.[79] For clarity, the loop between strands Aβ and Bβ is not shown. Blue-light absorption promotes formation of a covalent bond between the flavin ring and a conserved cysteine residue (first open arrow). Light signals are predominantly propagated to and through the β sheet (second open arrow). Here, they promote unfolding of the C-terminal Jα helix (green; third open arrow).[38] (b) The LOV photocycle involves blue-light-induced formation of a thioether bond between a conserved cysteine residue and atom C(4a) of the flavin cofactor. The lit state reverts thermally to the ground state. (c) As illustrated for the bacteriophytochrome from *P. aeruginosa* (3C2W),[42] phytochrome red-light sensors comprise a bilin-binding GAF domain (green), often followed by another GAF domain denoted PHY (blue) and preceded by a PAS domain (yellow). (d) Absorption of red and far-red light promotes transitions between the Pr and Pfr spectroscopic states. At the molecular level, the Pr↔Pfr transition involves *Z*/*E* isomerization of the bilin chromophore around the C15═C16 double bond, thus leading to a flip of ring D.

engineered photoreceptors clearly demonstrates that our current knowledge – even though partial – suffices to generate novel optogenetic tools. On the other hand, it is certain that ongoing research into natural photoreceptors will provide an even sounder basis for further design approaches.

General design strategies

From the structure and function of LOV, phytochrome and other natural photoreceptors, several general principles emerge which form the biologically-inspired bases for the design and engineering

Table 2 Design principles of natural photoreceptors

Sensor (input) and effector (output) functions are located in different structural modules (proteins or protein domains) (exception: rhodopsins)
Covalent linkage of sensor and effector domains where the sensor is usually N-terminal to the effector, occasionally C-terminal, but never inserted into the effector domain
Light-dependent structural and dynamic changes often involve N- or C-terminal helices in the photosensor domain
Linkers between domains play important roles in signal transduction
A single type of sensor domain is found linked to many types of effector domains; conversely, a single effector type is found linked to many types of sensor domains
Full-length photoreceptors may contain more than one sensor domain allowing integration of multiple signals
Full-length photoreceptors may contain domains other than sensors and effector, *e.g.* "interaction" domains
Photoreceptors may interact non-covalently with other proteins to transmit the signal downstream, *e.g.* response regulators

of novel photoreceptors. These principles are summarized in Table 2. The modular nature of photoreceptors (and chemoreceptors, to which many of these principles also apply) has almost certainly been of importance in the evolution of these molecules,[25,48] and is central to design through domain fusion. The fact that a wide variety of sensor–effector pairs occur naturally argues against tertiary-structure-specific interactions, and argues for the likelihood that domain fusion may be productive, and for the importance of linkers. Were tertiary-structure-specific interactions to exist, the specific mechanism of signal transduction would be unique to each photosensor and the prospects for successful engineering of photosensors by domain fusion would be greatly reduced, contrary to results. Successful design will offer a number of desirable outcomes. A better understanding of these general principles will in turn enhance subsequent design; provide useful new tools for cell biology, systems biology, biophysics and perhaps even the clinical sciences; extend the optogenetic approach; and generate new targets for time-resolved experiments that rely on light for fast, readily-controlled reaction initiation, such as time-resolved, biological X-ray crystallography.[21]

Photochemical requirements and optimization

The choice of photosensor domain for design approaches will be governed in part by the spectral region to be used for photoreceptor activation in optogenetic experiments. Red-light sensors have the advantage of deeper tissue penetration of light of longer wavelengths.[46] However, certain spectral regions may be reserved for observation channels, *e.g. via* fluorescent markers, or for excitation of another photoreceptor. For LOV blue-light sensors there is only limited potential for color tuning (that is, for adjusting their absorption characteristics) since their flavin chromophore is rather small and of rigid structure. On the other hand, for phytochrome sensors it should be possible to considerably alter their absorption spectra by varying substituents on the four pyrrole rings, as occurs naturally in plant phytochromes and within the diverse group of cyanobacteriochromes, and by constraining the extent to which conformational changes can occur between the four pyrrole rings. However, this may require the exogenous provision of the chromophore with suitably modified substituents or interlocked rings, rather than relying on endogenous incorporation of a natural chromophore (see below). To reduce the light dose required for photoreceptor activation (*i.e.* illumination time and intensity), photoreceptors should have high extinction coefficients, ε, and high quantum yields for undergoing productive photochemistry, Φ_P, at the excitation wavelength. While this is by and large true for natural photoreceptors, care must be taken to retain these favorable properties in the design of engineered photoreceptors. The photocycle should be fully reversible to allow temporary and repetitive activation of the photoreceptor. Formation of the signaling state occurs within pico- to microseconds in different photoreceptors, considerably shorter than the timescales of most biological processes. Of more practical relevance is the rate of reversion to the ground state, since it determines the time for which the signaling state persists. The lifetime of the signaling state, τ_S, thus affects the overall light sensitivity of the photoreceptor. If τ_S is long, low light levels will lead to substantial photoreceptor activation and are less likely to be damaging to other molecular, cellular or tissue-level processes. For different LOV domains τ_S is between hundreds and ten thousands of seconds. By introducing point mutations in the vicinity of the flavin cofactor, the LOV photocycle can be greatly accelerated or decelerated.[49] For example, the crystal structures of the isolated YtvA LOV domain under dark and light conditions[35] reveal that residue Phe46 undergoes a light-induced ring flip of its side chain (Fig. 3a). This conformational change indicates flexibility in this protein region and implies that amino acid substitutions might be tolerated at this site. Since addition of imidazole greatly accelerates the dark recovery of LOV domains,[50] we prepared the Phe46His mutant which contains an imidazole moiety in the side chain of residue 46. Indeed, the photocycle of this mutant is accelerated by 25-fold. In combination with a previously discovered mutation (Ile39Val),[51] the photocycle is accelerated by 75-fold from (3900 ± 20) s at 22 °C in wild type to (52 ± 2) s in the double mutant Phe46His/Ile39Val (Fig. 3b). Similarly, in a bacteriophytochrome from *Pseudomonas aeruginosa* the lifetime of the signaling state is ~300 s; mutations have been identified that slow down or accelerate the photocycle by up to 80-fold.[42] Finally, photoreceptors should be photostable, able to undergo many cycles of excitation without degradation. This is usually the case for natural photoreceptors but must be tested and optimized, if needed, for engineered photoreceptors.

Requirements for genetic encoding

For use in optogenetics, photoreceptors must be readily expressible in their target organism or tissue. To achieve appropriate expression levels may require optimization, *e.g.* of the encoding DNA sequence through improving codon usage. Promoters of different strength can be used to adjust expression levels and to confine expression to specific cell types. Correct folding of photoreceptor proteins in the target location under the target conditions must be achieved. Suitable truncation of photoreceptor constructs or use of homologous proteins may facilitate expression and correct folding. Almost all natural photoreceptors incorporate their chromophore cofactors autonomously without depending on

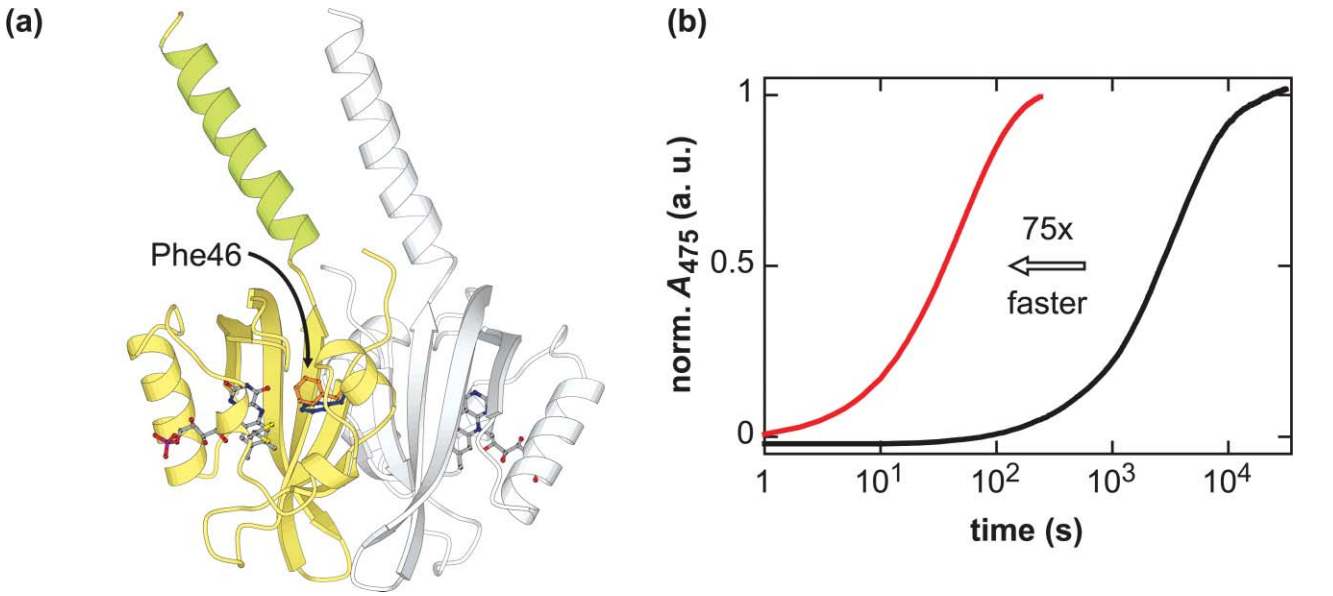

Fig. 3 Accelerating the LOV photocycle in *B. subtilis* YtvA. (a) Residue Phe46 in the vicinity of the flavin mononucleotide cofactor undergoes a light-induced ring flip indicating conformational flexibility in this protein region.[35] The orientations of Phe46 in the dark and in the light are indicated in blue and orange, respectively. (b) Mutating residues Phe46 to His and Ile39 to Val accelerates the dark recovery by 75-fold (red curve) in comparison to the wild-type protein (black).

any accessory proteins. (The xanthopsins may form an exception: although the 4-hydroxy cinnamic acid chromophore can be covalently incorporated chemically *in vitro*, the *in vivo* reaction may be more complex.) Cofactor availability can become limiting, especially for chromophores that are not common metabolites in the target location. In such cases, chromophores could be supplied either exogenously or endogenously through introduction of corresponding chromophore-synthesis genes. An example is the co-expression of bacteriophytochrome constructs with heme oxygenase, to provide the essential biliverdin chromophore through degradation of heme.[52] Some photoreceptors can be functionally reconstituted with non-natural chromophores which could be exploited; *e.g.* plant phytochromes naturally incorporate PΦB but are also functional with PCB as a chromophore which can readily be isolated from cyanobacteria. The correct subcellular location of photoreceptors can be ensured through translocation signals, such as nuclear export or import tags or membrane anchors. Expression of certain photoreceptors may prove cytotoxic which could be resolved by adjusting expression levels or using another photoreceptor. Furthermore, the effector domain has to be sufficiently active in the target location to elicit the desired physiological response. Site-directed mutations can attenuate or increase activity as required; effector domains from different origins may be more or less suited for application in certain target organisms.

Linking photosensor and effector

It is important to estimate how large the effect of light on photoreceptor activity need be to trigger a meaningful reaction in optogenetic experiments. Recent studies on both natural and engineered photoreceptors indicate that even small activity changes on the order of 10-fold or less can mediate relevant light-induced behavioral changes *in vivo*.[53,54] Thus, it may suffice if only a small fraction α of the total energy provided by photon absorption is translated into a change of photoreceptor activity (*cf.* eqn (6)). Engineered photoreceptors should be maximally sensitive to light signals; their equilibrium between the *T* and *R* states should be relatively close to unity in the dark (Fig. 1b). Gardner, Sosnick and co-workers recently showed that the intrinsic equilibrium between the *T* and *R* states of engineered LOV photoreceptors can be readily adjusted by site-directed mutagenesis of the photosensor domain (Yao *et al.*;[19] Strickland *et al.*[55]).

Successful design of photoreceptors puts the activity of a given effector under the control of a photosensor domain. While not strictly required, it is favorable to covalently link the photosensor and effector domains to allow regulation *in cis* and to achieve enhanced specificity. Despite sharing common photochemistry, photosensors of one class differ in important aspects such as their tertiary and quaternary structure which makes them more or less suited for coupling to specific effector domains. For example, while the LOV domain from *B. subtilis* YtvA forms a dimer in solution, the widely used LOV2 domain from *A. sativa* phototropin 1 appears to be monomeric.[38,56] How shall the sensor domain be covalently linked to the effector domain? In contrast to fluorescent protein tags which should be inert, in photoreceptors the photosensor and effector domains need to be carefully linked so as to enable efficient interaction and communication of signals between these domains, *i.e.* to maximize α in eqn (6). Studies on natural systems and photoreceptors engineered by domain fusion have shown that their properties are crucially determined by these linkers (Table 2). Thus in general, a greater amount of specific knowledge about the mechanism, structure and function of sensor and effector is required for the engineering of photoreceptors than for labeling of cellular constituents with fluorescent proteins.

Although no guarantee for success, we advise reproducing the natural architecture of photosensors and not deliberately departing from it without a compelling reason (Table 2). This suggests that having identified a target effector domain in a given organism,

one should first explore whether that effector is linked to a photosensor domain, *e.g.* a LOV domain, in any other organism. If so, that natural photosensor–linkage–effector construct provides a powerful starting point for design, in which the strategy is to replace the effector domain that occurs naturally in this construct by the target effector domain. If no such construct exists, one can explore less restrictively whether the target effector domain is linked to any relative of a photosensor domain, *e.g.* to a PAS domain of otherwise unknown function, in any other organism. Given the fact that LOV domains form a subclass of PAS domains, the sequence homology between them may be sufficiently high that sequence-based (or, better yet, structure-based) replacement of the PAS domain by the LOV domain will provide a starting point. One should explore sequence databases widely. For example, LOV domains are found both N-terminal and C-terminal to effector domains. This suggests that depending on the detailed environment, LOV domains may exert a signal directed towards either their C-terminus or their N-terminus, or even towards both. In many natural signaling proteins, the linkers between sensor and effector modules predominantly form α-helices,[16,36,57] of which the so-called "signaling helix" is a particularly widespread representative.[26] Thus, sequence analyses should be conducted of candidate linkers, to identify such properties as propensity to form an α-helix or a coiled-coil, amphipathic nature, and length distribution.

In general, the question of how sensor and effector domains are to be connected is intricately linked to the principal design strategy one pursues. Target photosensor and effector domains can be linked in different ways that achieve the desired control of activity *via* quite diverse structural routes. For example, photosensor domains have been used to modulate the folding of effector domains[58] or access to their active site,[54] or through light-controlled association.[59]

Libraries of engineered photoreceptors

Engineering of photoreceptors usually involves generating a family or library of variants and screening them for members with favorable properties. Libraries of photoreceptor variants may be generated systematically or randomly, for example by random mutagenesis. Library members differ from each other in their primary structure and may thus differ in their activity and response to light. For example, individual members may covalently connect photosensor and effector domains by linkers of different length and sequence. Once variants with some – even if far from optimal – light-regulated activity have been identified, *i.e.* variants with $\alpha > 0$ in eqn (6), they provide the basis for more focused libraries aimed at further optimization.

It might be thought that production of an engineered photoreceptor suitable for optogenetic applications would require large library sizes. Strikingly, in all cases reported so far the successful design of photoreceptors engineered by domain fusion required only very small library sizes containing on the order of 10–100 members. Thus, either the design principles were surprisingly powerful or these cases represent low-hanging fruit. This library size is sufficiently small that active, engineered photoreceptors could be identified by manual screening. Careful initial design ensured that all members retained protein regions or individual residues associated with essential properties of both the photosensor domain, *e.g.* its ability to readily incorporate the natural chromophore and to undergo productive photochemistry, and the effector domain, *e.g.* its enzymatic or DNA-binding activity. Small variations in sequence largely restricted to residues in the linker then identified library members with the critical additional property: light-dependent modulation of the activity of the effector domain.

We expect that for "more difficult" targets and for photoreceptors that require highly optimized properties, substantially larger library sizes will be needed. Such libraries would quickly exceed all manual screening capabilities and require improved screens that allow higher throughput. Ideal screens are highly parallel and give rise to a readily assayable signal. For example, Fischer and Lagarias used fluorescence-activated cell sorting to successfully screen more than 10^4 bacteriophytochrome variants and identify a highly fluorescent species.[45] Similarly, multiple variants of jellyfish fluorescent proteins have been produced.[1] Related approaches may also be applied to screen for libraries of engineered photoreceptors. The success of a given design approach will be determined by two factors: how many members the library must comprise to include sufficiently active representatives (difficulty of the problem); and how efficiently one can screen such libraries (difficulty of achieving high throughput).

Specific design examples

Design with LOV domains

***Avena sativa* phototropin 1 LOV2.** Sparked by the seminal report of Gardner and coworkers[38] that light absorption induces unfolding of its C-terminal Jα helix, the LOV2 domain of *A. sativa* phototropin 1 has found particularly wide use in the design of engineered photoreceptors. Strickland *et al.* employed this LOV domain to regulate the DNA-binding activity of the *Escherichia coli* Trp repressor protein, TrpR, by light.[58] Neither TrpR itself nor homologous proteins in other organisms are known to be regulated by light, which at first glance makes this a high-hanging fruit. Twelve protein variants were generated in which the LOV domain was fused *via* its Jα helix to the N-terminus of TrpR such that the two protein domains shared one common, linking helix. One of these twelve variants, denoted LOV-TAP, displayed light-regulated activity: LOV-TAP bound to DNA weakly in its dark state and with ~5-fold higher affinity in its illuminated state. The authors propose that the shared linker helix predominantly docks onto the LOV core domain in the dark and the TrpR domain is distorted; upon light absorption, the linker helix dissociates from the LOV core domain and restores structure and function of TrpR (Fig. 4a). While this work pioneered the use of LOV domains in the design and engineering of photoreceptors and thus provided an important proof of concept, the degree of light activation is modest and the overall DNA-binding affinity of LOV-TAP is about two orders of magnitude lower than that of the wild-type TrpR.[60] This limits its practical application and light-regulated activity *in vivo* was not demonstrated. Recently, the regulatory effect of light on DNA binding by LOV-TAP could be improved to ~64-fold.[55] Using site-directed mutagenesis, the DNA affinity of LOV-TAP in the dark was lowered by a factor of 10 whereas the affinity of the light state was left nearly unchanged.

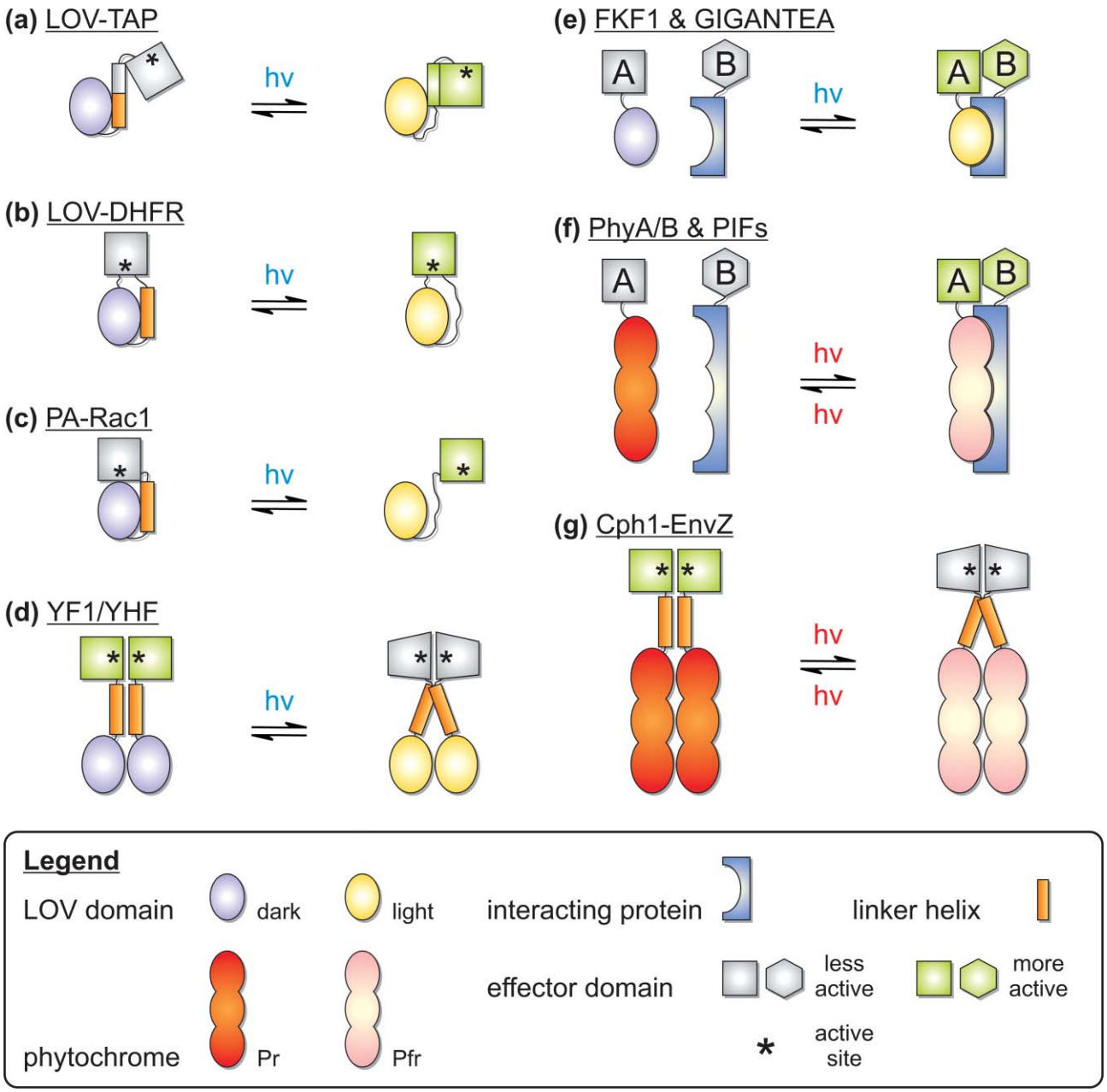

Fig. 4 Examples of engineered photoreceptors based on LOV domains (a–e) and phytochromes (f, g). Domain symbols are indicated in the legend. Note that the signal transduction mechanisms are depicted as proposed in the original publications; further mechanistic and structural studies are required to confirm or falsify these models. See main text for detailed discussion. (a) LOV-TAP;[58] (b) LOV-DHFR;[61] (c) PA-Rac1;[54] (d) YF1/YHF;[36,57] (e) FKF1 and GIGANTEA;[71] (f) PhyA/B and PIFs;[72,73,74,59] (g) Cph1-EnvZ.[75]

Using the same LOV domain from *A. sativa* phototropin 1, Benkovic, Ranganathan and colleagues generated variants of the enzyme dihydrofolate reductase (DHFR) that showed only low levels of light regulation.[61] This is not altogether surprising. In a clear departure from the architecture of natural photoreceptors, the LOV2 domain was inserted into a loop of DHFR known to be critically involved in enzymatic function (Fig. 4b). Consequently, the resulting chimeric proteins were severely impaired in their catalytic activity, in which both enzymatic turnover and substrate affinity were decreased by two orders of magnitude. On average, only a 1.5- to 2-fold increase of catalytic activity was observed *in vitro* upon light absorption, and no light-regulated activity *in vivo* was demonstrated. The authors based their design strategy on statistical coupling analysis (SCA) which seeks to identify networks of co-evolving residues within proteins.[62] They proposed that bringing such coupled networks of residues within a photosensor and an effector domain into spatial proximity will be sufficient to achieve light-regulated function. While this proposal remains to be more widely tested, we are doubtful that it will be of general utility. Co-evolution between residues, which under positive circumstances can be detected by SCA, may arise from multiple reasons some of which are entirely unrelated to

signal transduction. For example, certain residues might co-evolve because they both form part of a conserved structural scaffold.

In a ground-breaking study, Wu *et al.* put the activity of the small GTPase Rac1 under the control of blue light through fusion of this effector domain with the *A. sativa* phototropin 1 LOV2 sensor domain.[54] The principal design strategy was to restrict access to the active site of the effector domain in a light-dependent manner. To this end, a small library of fusion variants with several tens of members (Yi Wu, personal communication) was prepared and screened manually. In one member denoted PA-Rac1, LOV2 was linked *via* its C-terminal Jα helix to the N terminus of Rac1 such that it occludes the active site in the dark, as confirmed by a crystal structure of PA-Rac1[54] (Fig. 4c). Upon blue-light absorption, the Jα helix unfolds, which presumably releases the Rac1 domain and allows access to its active site. *In vitro*, the lit-state PA-Rac1 bound its downstream effector protein PAK with approximately the same affinity as wild-type Rac1; in the dark the affinity was 10-fold lower. Strikingly, this seemingly small effect was sufficient to control by blue light the motility of fibroblasts which expressed PA-Rac1. To spectacular effect, fibroblast movement could be remote-controlled by light. More recently, PA-Rac1 was also used to control neutrophil movement in developing zebra fish embryos[63] and to induce polarization in *Drosophila* ovary cells.[64]

***Bacillus subtilis* YtvA.** We have used the sole LOV sensor domain of the *B. subtilis* YtvA protein to engineer blue-light-regulated variants of the histidine kinase FixL from *Bradyrhizobium japonicum*.[36] FixL forms part of the FixL/FixJ two-component system which regulates nitrogen metabolism in response to oxygen levels by adjusting the level of phospho-FixJ, which acts as a transcription factor. By replacing both the PAS A and PAS B domains of FixL by this LOV domain, we reprogrammed the signal specificity of FixL from oxygen to blue light while retaining its catalytic efficiency (Fig. 4d, 5a). The most thoroughly studied fusion protein YF1 was active as a histidine kinase in the dark. Light absorption led to a more than 1000-fold reduction in net kinase activity as measured by phosphotransfer to the response regulator FixJ, and converted YF1 to a net phosphatase. Out of 16 variants differing in the length and sequence of the L4 linker between the N-terminal LOV sensor and the C-terminal histidine kinase effector domains, several displayed robust light-regulated activity. Kinase activity and light regulation in these variants showed a remarkable dependence on linker length displaying the seven-residue, heptad periodicity highly suggestive of a coiled coil (see Fig. 4 of Möglich *et al.*[36]). We propose that in the dimeric fusion kinases, the linkers form amphipathic α helices which assemble into coiled coils. This model is supported by heptad periodicities of linker length and hydropathy in a large group of natural PAS histidine kinases.[36] We suggest that light absorption leads to conformational changes within the LOV domains which are propagated to the histidine kinase domain through the coiled-coil linker as a 40–60° rotation (Fig. 5b). A similar rotation model was also proposed for the four-helix bundle of HAMP domains[65] that are often found in association with membrane-bound histidine kinases. By changing the length of the linker L4 (Fig. 5a), we could alter the signal response of YF1.[36] YF1 is not only active *in vitro* but also *in vivo*. An engineered YF1/FixJ system regulated gene expression in *E. coli* by light, with a more than 70-fold reduction of gene expression upon light absorption.[36]

More generally, any variation of a general parameter X such as a structural property or any intensive thermodynamic variable could be suited to modulate the signal-response function of photoreceptors (Fig. 5c). If the activity in the dark and signaling states are affected differently by variations of X, photoreceptor variants might be obtained in which presence of signal leads to an increase of biological activity (solid arrow in Fig. 5c) or to a decrease (dashed arrow).

Recently, we demonstrated that multiple PAS sensor domains can be combined in one protein to enable several signals to be detected and integrated.[57] We generated the protein YHF by replacing the PAS A domain of FixL with the blue-light-sensitive LOV domain of YtvA while retaining the oxygen-sensitive PAS B domain of FixL (Fig. 5a). YHF responded to blue light and oxygen in a positive heterotropic, cooperative manner. As separate signals, blue light and oxygen moderately suppressed YHF kinase activity. When both signals were present they largely abolished kinase activity, an effect that greatly exceeded the product of the separate signal effects. Structural and sequence analysis of tandem PAS domains in natural proteins such as FixL suggests that PAS domains are connected by short amphipathic α helices and oriented in a head-to-tail manner. Based on these findings, we propose a structural model for YHF and illustrate how integration of signals could be achieved (Fig. 5d). We suggest that the LOV, PAS B, and histidine kinase domains are linked by short helices and are linearly arranged around the dyad axis of dimeric YHF. Signals detected in either sensor domain would give rise to torque which could propagate through the helical linkers to the effector domain (Fig. 5b, 5d). The linear arrangement of individual domains would allow integration of torque signals arising from detection of multiple signals. In the absence of high-resolution structures of full-length YF1, YHF or related kinases, the structural and functional consequences of signal detection within the effector domain remain unclear. However, recent crystal structures of several histidine kinases[66,67,68,69] indicate that the DHp domain is plastic, *i.e.* its four-helix bundle can adopt different conformations under different conditions. In particular, two conformations which differ in the relative orientation of the four DHp helices were observed in the crystal structures of the isolated *Thermotoga maritima* HK853 kinase domain[66] and in its complex with the response regulator RR468.[67] We propose that in YHF, YF1 and FixL, signal-induced torques could give rise to similar conformational transitions which would modulate kinase activity. Structures of full-length histidine kinases comprising sensor and effector domains in both the presence and absence of signal(s) are necessary to test this model and to provide molecular details.

Another photoreceptor design based on the YtvA LOV domain was reported by Krauss *et al.*[70] Here, the YtvA LOV domain was fused *via* its C-terminal Jα helix to the N terminus of *B. subtilis* lipase A. Preliminary data suggest that lipase activity can thus be controlled by blue light.[70]

***Arabidopsis thaliana* FKF1.** Yazawa *et al.*[71] exploited the blue-light-dependent interaction between the LOV protein FKF1 and GIGANTEA (GI), both from *Arabidopsis thaliana*. In a readily generalizable approach, the interaction between two target

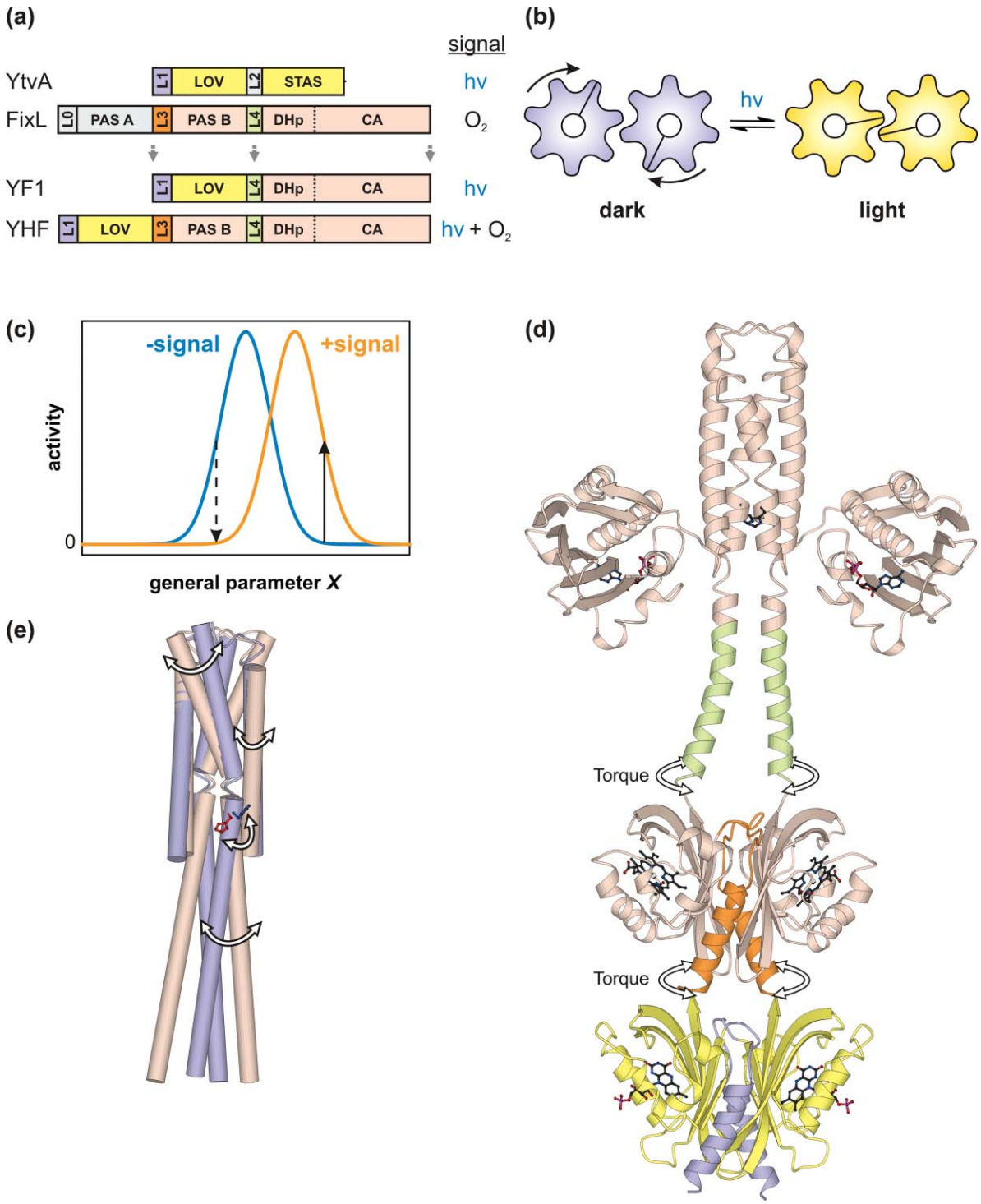

Fig. 5 Design and signaling mechanism of blue-light-regulated histidine kinases. (a) The engineered photoreceptors YF1 and YHF were designed by modular recombination of domains from the parent proteins *B. subtilis* YtvA and *B. japonicum* FixL which detect blue light and oxygen, respectively. YF1 responds to blue light, YHF synergistically responds to blue light and oxygen. (b) Within the dimeric histidine kinases FixL, YF1, and YHF the linker L4 between the sensor and effector domains adopts α-helical coiled-coil conformation. The helices L4 are here depicted as gear-wheels. Signals, such as light, could induce a 40–60° torque within this linker (gear-wheel model). (c) Variation of a general parameter *X*, *e.g.* a structural property or an intensive thermodynamic variable, could be used to adjust the signal response of engineered photoreceptors. Depending upon the value of *X*, the presence of signal could lead to an increase of biological activity (solid arrow) or to a decrease (dashed arrow). (d) In the structural model of YHF, the sensor and effector domains are connected by amphipathic α helices and are linearly arranged around the dyad symmetry axis. Signal detection in the LOV and PAS B domains could generate torques around the dyad axis which can be integrated and propagated to the histidine kinase domains. Individual domains and linkers are colored as in panel a. (e) Signal-induced torque could cause conformational changes in the DHp domain of the histidine kinase resembling those observed in the structures of *T. maritima* HK853 (red)[66] and of its complex with the response regulator RR468 (blue).[67] Open arrows highlight differences between the two structural models.

proteins A and B could be rendered light-dependent by fusing them with the interaction domains from FKF1 and GI, respectively (Fig. 4e). In one demonstration, this strategy was used to localize the Rac1 GTPase to the plasma membrane of fibroblasts upon blue-light absorption. When recruited to the membrane, Rac1 induced actin polymerization and thus cell movement. As in the work by Wu *et al.*[54] noted above, fibroblast movement could thus effectively be controlled by blue light. In a second demonstration, FKF1 and GI domains were fused to the VP16 transactivation domain and the GAL4 DNA-binding domain, respectively, to achieve light-regulated transcription. Activity levels of a reporter gene were increased by up to 4-fold upon blue-light absorption.

Design with phytochromes

Plant phytochromes. Quail and coworkers[72] utilized the red-light-regulated interaction between the *A. thaliana* phytochromes PhyA and PhyB with the phytochrome-interacting factor denoted PIF3 to generate a light-inducible promoter system (Fig. 4f). Conceptually similar to the later work by Yazawa *et al.*[71] discussed above, the N-terminal domains of PhyB and PIF3 were fused to the DNA-binding and transactivation domains of GAL4, respectively. Red-light absorption induced colocalization of the two hybrid proteins and thus transcriptional activation. In yeast, the activity of a reporter gene could be increased by more than 1000-fold following red-light absorption. As phytochromes are reversible photochromic systems, illumination with far-red light could be used to disrupt the interaction between PhyB and PIF3 and to thus switch off transcription. Since the plant phytochromes PhyA and PhyB employ the modified tetrapyrroles PCB or PΦB which are not available in most tissues and cell types, these chromophores must be supplied either exogenously or endogenously (*cf.* above).

A particular advantage of this design approach is its versatility: target proteins are activated through light-controlled colocalization. Three later studies also utilized the interaction between PhyB and PIFs to achieve light regulation of target proteins. Leung *et al.* put the association of the GTPase Cdc42 with its effector protein WASP under red-light control.[73] When in complex with PhyB-Cdc42, PIF3-WASP promoted actin polymerization *in vitro*; use *in vivo* was not demonstrated. Based on the interaction between PhyB and PIF3, Muir and coworkers established a protein-splicing system that was moderately regulated by red light *in vitro*.[74] Lastly, Voigt and colleagues employed the light-dependent interaction between PhyB and PIF6 to activate target proteins *in vivo*.[59] The nucleotide exchange factors Tiam and intersectin were recruited to the plasma membrane in a red-light-controlled manner where they activated their GTPase effectors Rac1 and Cdc42, respectively. In their activated form, the GTPases promoted formation of cell protrusions, and thus the motility of fibroblasts could be controlled by red light.

Bacteriophytochromes. In earlier work, Voigt and coworkers used a bacterial phytochrome to furnish a red-light-dependent gene expression system[75] (Fig. 4g). The phytochrome sensor of the histdine kinase Cph1 from *Synechocystis sp.* was fused to the histdine kinase portion of *E. coli* EnvZ *via* a common coiled-coil linker. Out of approximately 20 variants made one was studied in detail. In this variant, the originally osmo-sensitive EnvZ histidine kinase was put under control of red light and could regulate expression of a reporter gene in *E. coli*. While this engineered photoreceptor has not been studied in any mechanistic detail, signal transduction could involve a mechanism similar to that of the engineered blue-light receptor YF1 (see above).[36]

Design with xanthopsin

A recent study[76] reports the design of a light-activated, DNA-binding protein through fusion with photoactive yellow protein (PYP) which belongs to the xanthopsin photoreceptor class. In contrast to most LOV sensor domains which display light-dependent structural changes at their C-terminal helix, PYP exhibits light-dependent structural changes in its N-terminal region. The leucine zipper GCN4 was therefore fused *via* its C-terminal helix to the N-terminus of PYP. In the dark, the fusion protein bound its target DNA about 10-fold more weakly than wild-type GCN4; upon light absorption, DNA binding affinity was enhanced by a factor of 2.

Conclusions

Into a bright future

The fast-growing number of successful design examples indicates that light signals originating in photosensor domains can readily be coupled to effector domains by domain fusion to achieve light-induced changes in biological activity. Further, these fusion proteins can be used in optogenetic applications *i.e.* to control the behavior of living systems by light. Interestingly, functional coupling can be obtained *via* a variety of routes, some of which may involve signal transduction mechanisms not realized in nature. An improved mechanistic understanding of different, natural photoreceptor classes and further examples of engineered photoreceptors – both successful and unsuccessful – will provide an improved basis for the design and application of yet other engineered photoreceptors with light-regulated functionality.

How general are such approaches? Can any effector functionality be subjected to light control? While definitive answers obviously await further study, the multiple and apparently ready success of current design approaches indicates that diverse biological activities can be placed under the control of light, thus making engineered photoreceptors versatile and flexible tools.

Design approaches are not confined to the currently most widely used LOV domains and phytochromes but can be extended to other photoreceptors and possibly beyond. New photoreceptor proteins and classes could be identified by selection of mutant organisms that are defective in their photoreception. In general, any protein (or possibly any other biomolecule) that interacts with light might be suitable for generating a novel light switch. The essential feature is that light absorption by the chromophore generates a structural and/or dynamic signal which can be thermodynamically coupled to a change in effector domain activity. For example, certain variants of fluorescent proteins can be switched on and off or their fluorescence emission spectrum can be altered, by light.[77] The underlying structural and dynamic changes accompanying such switches might be harnessed to regulate the activity of a suitable effector domain. However, naturally occurring proteins that interact with light for purposes other than sensing may require significant modification and optimization to enable them to serve as an efficient photosensor. In principle, one

could also envision employing photosensor domains that bind altogether different chromophores with desirable properties such as metal cofactors or chromophores with an even more extended, conjugated π-electron system designed to absorb in the infrared spectral region and hence to be applicable for use in tissue.

Take a photon, not a pill!

Recent years have seen spectacular *in vivo* applications in optogenetics based on the use of natural photoreceptors, primarily the light-sensitive cation channel channelrhodopsin, *e.g.* Gradinaru *et al.*[4] and Lagali *et al.*[78] Key to this success was that channelrhodopsin can be genetically encoded and expressed in the desired location, where it affords non-invasive and reversible control over neural processes with superb spatiotemporal resolution. The recent generation and application of engineered synthetic photoreceptors now extends the repertoire of light-regulated tools and thus the utility of optogenetics in general. If any arbitrary biological functionality may be rendered light-dependent in the desired cell type and tissue *in vivo* through appropriate photoreceptor engineering – admittedly a big "if" – then optogenetics may extend beyond its present applications as a clever tool in cell biology and the neurosciences to a new clinical modality: take a photon, not a pill. Exploring that "if" will happily occupy many scientists for some time.

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Notes and references

1 R. Y. Tsien, Constructing and exploiting the fluorescent protein paintbox (Nobel Lecture), *Angew. Chem., Int. Ed.*, 2009, **48**, 5612–5626.
2 T. A. Klar and S. W. Hell, Subdiffraction resolution in far-field fluorescence microscopy, *Opt. Lett.*, 1999, **24**, 954–956.
3 G. Nagel, D. Ollig, M. Fuhrmann, S. Kateriya, A. M. Musti, E. Bamberg and P. Hegemann, Channelrhodopsin-1: a light-gated proton channel in green algae, *Science*, 2002, **296**, 2395–2398.
4 V. Gradinaru, M. Mogri, K. R. Thompson, J. M. Henderson and K. Deisseroth, Optical deconstruction of parkinsonian neural circuitry, *Science*, 2009, **324**, 354–359.
5 K. Deisseroth, G. Feng, A. K. Majewska, G. Miesenböck, A. Ting and M. J. Schnitzer, Next-generation optical technologies for illuminating genetically targeted brain circuits, *J. Neurosci.*, 2006, **26**, 10380–10386.
6 V. Gradinaru, F. Zhang, C. Ramakrishnan, J. Mattis, R. Prakash, I. Diester, I. Goshen, K. R. Thompson and K. Deisseroth, Molecular and cellular approaches for diversifying and extending optogenetics, *Cell*, 2010, **141**, 154–165.
7 M. Goeldner and R. Givens, *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*, Wiley-VCH, Weinheim, Germany, 2005.
8 G. C. R. Ellis-Davies, Caged compounds: photorelease technology for control of cellular chemistry and physiology, *Nat. Methods*, 2007, **4**, 619–628.
9 P. Gorostiza and E. Y. Isacoff, Optical Switches for Remote and Noninvasive Control of Cell Signaling, *Science*, 2008, **322**, 395–399.
10 A. Deiters, Principles and applications of the photochemical control of cellular processes, *ChemBioChem*, 2009, **11**, 47–53.
11 C. W. Riggsbee and A. Deiters, Recent advances in the photochemical control of protein function, *Trends Biotechnol.*, 2010, **28**, 468–475.
12 D. Balasubramanian, S. Subramani and C. Kumar, Modification of a model membrane structure by embedded photochrome, *Nature*, 1975, **254**, 252–254.
13 S. Khan, K. Amoyaw, J. L. Spudich, G. P. Reid and D. R. Trentham, Bacterial chemoreceptor signaling probed by flash photorelease of a caged serine, *Biophys. J.*, 1992, **62**, 67–68.
14 N. Wu, A. Deiters, T. A. Cropp, D. King and P. G. Schultz, A genetically encoded photocaged amino acid, *J. Am. Chem. Soc.*, 2004, **126**, 14306–14307.
15 J. Monod, J. Wyman and J. P. Changeux, On the Nature of Allosteric Transitions: A Plausible Model, *J. Mol. Biol.*, 1965, **12**, 88–118.
16 A. Möglich, R. A. Ayers and K. Moffat, Structure and Signaling Mechanism of Per-ARNT-Sim Domains, *Structure*, 2009, **17**, 1282–1294.
17 V. A. Feher, J. W. Zapf, J. A. Hoch, J. M. Whiteley, L. P. McIntosh, M. Rance, N. J. Skelton, F. W. Dahlquist and J. Cavanagh, High-resolution NMR structure and backbone dynamics of the Bacillus subtilis response regulator, Spo0F: implications for phosphorylation and molecular recognition, *Biochemistry*, 1997, **36**, 10015–10025.
18 B. F. Volkman, D. Lipson, D. E. Wemmer and D. Kern, Two-state allosteric behavior in a single-domain signaling protein, *Science*, 2001, **291**, 2429–2433.
19 X. Yao, M. K. Rosen and K. H. Gardner, Estimation of the available free energy in a LOV2-J alpha photoswitch, *Nat. Chem. Biol.*, 2008, **4**, 491–497.
20 D. E. Koshland, G. Nemethy and D. Filmer, Comparison of experimental binding data and theoretical models in proteins containing subunits, *Biochemistry*, 1966, **5**, 365–385.
21 K. Moffat, Time-resolved biochemical crystallography: a mechanistic perspective, *Chem. Rev.*, 2001, **101**, 1569–1581.
22 M. A. van der Horst and K. J. Hellingwerf, 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.*, 2004, **37**, 13–20.
23 A. Möglich, X. Yang, R. A. Ayers and K. Moffat, Structure and Function of Plant Photoreceptors, *Annu. Rev. Plant Biol.*, 2010, **61**, 21–47.
24 N. C. Rockwell and J. C. Lagarias, A brief history of phytochromes, *ChemPhysChem*, 2010, **11**, 1172–1180.
25 T. Pawson and P. Nash, Assembly of cell regulatory systems through protein interaction domains, *Science*, 2003, **300**, 445–452.
26 V. Anantharaman, S. Balaji and L. Aravind, The signaling helix: a common functional theme in diverse signaling proteins, *Biol. Direct*, 2006, **1**, 25.
27 S. Crosson, S. Rajagopal and K. Moffat, The LOV domain family: photoresponsive signaling modules coupled to diverse output domains, *Biochemistry*, 2003, **42**, 2–10.
28 N. C. Rockwell, Y. S. Su and J. C. Lagarias, Phytochrome structure and signaling mechanisms, *Annu. Rev. Plant Biol.*, 2006, **57**, 837–858.
29 J. M. Christie, P. Reymond, G. K. Powell, P. Bernasconi, A. A. Raibekas, E. Liscum and W. R. Briggs, Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism, *Science*, 1998, **282**, 1698–1701.
30 B. L. Taylor and I. B. Zhulin, PAS domains: internal sensors of oxygen, redox potential, and light, *Microbiol. Mol. Biol. Rev.*, 1999, **63**, 479–506.
31 M. Salomon, W. Eisenreich, H. Dürr, E. Schleicher, E. Knieb, V. Massey, W. Rüdiger, F. Müller, A. Bacher and G. Richter, An optomechanical transducer in the blue light receptor phototropin from Avena sativa, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 12357–12361.
32 T. Drepper, T. Eggert, F. Circolone, A. Heck, U. Krauss, J. K. Guterl, M. Wendorff, A. Losi, W. Gärtner and K. E. Jaeger, Reporter proteins for *in vivo* fluorescence without oxygen, *Nat. Biotechnol.*, 2007, **25**, 443–445.
33 S. Chapman, C. Faulkner, E. Kaiserli, C. Garcia-Mata, E. I. Savenkov, A. G. Roberts, K. J. Oparka and J. M. Christie, The photoreversible fluorescent protein iLOV outperforms GFP as a reporter of plant virus infection, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 20038–20043.
34 B. D. Zoltowski, C. Schwerdtfeger, J. Widom, J. J. Loros, A. M. Bilwes, J. C. Dunlap and B. R. Crane, Conformational switching in the fungal light sensor Vivid, *Science*, 2007, **316**, 1054–1057.
35 A. Möglich and K. Moffat, Structural Basis for Light-dependent Signaling in the Dimeric LOV Domain of the Photosensor YtvA, *J. Mol. Biol.*, 2007, **373**, 112–126.
36 A. Möglich, R. A. Ayers and K. Moffat, Design and Signaling Mechanism of Light-regulated Histidine Kinases, *J. Mol. Biol.*, 2009, **385**, 1433–1444.
37 M. Avila Pérez, J. Vreede, Y. Tang, O. Bende, A. Losi, W. Gärtner and K. J. Hellingwerf, In vivo mutational analysis of YtvA from bacillus

subtilis: Mechanism of light-activation of the general stress response, *J. Biol. Chem.*, 2009, **284**, 24958–24964.
38 S. M. Harper, L. C. Neil and K. H. Gardner, Structural basis of a phototropin light switch, *Science*, 2003, **301**, 1541–1544.
39 J. T. Kennis, I. H. van Stokkum, S. Crosson, M. Gauden, K. Moffat and R. van Grondelle, The LOV2 domain of phototropin: a reversible photochromic switch, *J. Am. Chem. Soc.*, 2004, **126**, 4512–4513.
40 J. R. Wagner, J. S. Brunzelle, K. T. Forest and R. D. Vierstra, A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome, *Nature*, 2005, **438**, 325–331.
41 L. O. Essen, J. Mailliet and J. Hughes, The structure of a complete phytochrome sensory module in the Pr ground state, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 14709–14714.
42 X. Yang, J. Kuk and K. Moffat, Crystal structure of Pseudomonas aeruginosa bacteriophytochrome: Photoconversion and signal transduction, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 14715–14720.
43 Y. Ho, L. M. Burden and J. H. Hurley, Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor, *EMBO J.*, 2000, **19**, 5288–5299.
44 A. T. Ulijasz, G. Cornilescu, C. C. Cornilescu, J. Zhang, M. Rivera, J. L. Markley and R. D. Vierstra, Structural basis for the photoconversion of a phytochrome to the activated Pfr form, *Nature*, 2010, **463**, 250–254.
45 A. J. Fischer and J. C. Lagarias, Harnessing phytochrome's glowing potential, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17334–17339.
46 X. Shu, A. Royant, M. Z. Lin, T. A. Aguilera, V. Lev-Ram, P. A. Steinbach and R. Y. Tsien, Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome, *Science*, 2009, **324**, 804–807.
47 K. C. Toh, E. A. Stojkovic, I. H. M. van Stokkum, K. Moffat and J. T. M. Kennis, Proton-transfer and hydrogen-bond interactions determine fluorescence quantum yield and photochemical efficiency of bacteriophytochrome, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 9170–9175.
48 L. Aravind, L. M. Iyer, and V. Anantharaman, in *Sensory Mechanisms in Bacteria: Molecular Aspects of Signal Recognition*, ed. S. Spiro and R. Dixon, Horizon Scientific Press, Norwich, UK, 2010.
49 B. D. Zoltowski, B. Vaccaro and B. R. Crane, Mechanism-based tuning of a LOV domain photoreceptor, *Nat. Chem. Biol.*, 2009, **5**, 827–834.
50 M. T. Alexandre, J. C. Arents, R. van Grondelle, K. J. Hellingwerf and J. T. Kennis, A base-catalyzed mechanism for dark state recovery in the Avena sativa phototropin-1 LOV2 domain, *Biochemistry*, 2007, **46**, 3129–3137.
51 J. M. Christie, S. B. Corchnoy, T. E. Swartz, M. Hokenson, I. S. Han, W. R. Briggs and R. A. Bogomolni, Steric interactions stabilize the signaling state of the LOV2 domain of phototropin 1, *Biochemistry*, 2007, **46**, 9310–9319.
52 Y. Chung, S. Masuda and C. E. Bauer, Purification and reconstitution of PYP-phytochrome with biliverdin and 4-hydroxycinnamic acid, *Methods Enzymol.*, 2007, **422**, 184–189.
53 T. R. Barends, E. Hartmann, J. J. Griese, T. Beitlich, N. V. Kirienko, D. A. Ryjenkov, J. Reinstein, R. L. Shoeman, M. Gomelsky and I. Schlichting, Structure and mechanism of a bacterial light-regulated cyclic nucleotide phosphodiesterase, *Nature*, 2009, **459**, 1015–1018.
54 Y. I. Wu, D. Frey, O. I. Lungu, A. Jaehrig, I. Schlichting, B. Kuhlman and K. M. Hahn, A genetically encoded photoactivatable Rac controls the motility of living cells, *Nature*, 2009, **461**, 104–108.
55 D. Strickland, X. Yao, G. Gawlak, M. K. Rosen, K. H. Gardner and T. R. Sosnick, Rationally improving LOV domain-based photoswitches, *Nat. Methods*, 2010, **7**, 623–626.
56 V. Buttani, A. Losi, T. Eggert, U. Krauss, K. E. Jaeger, Z. Cao and W. Gärtner, Conformational analysis of the blue-light sensing protein YtvA reveals a competitive interface for LOV-LOV dimerization and interdomain interactions, *Photochem. Photobiol. Sci.*, 2007, **6**, 41–49.
57 A. Möglich, R. A. Ayers and K. Moffat, Addition at the Molecular Level: Signal Integration in Designed Per-ARNT-Sim Receptor Proteins, *J. Mol. Biol.*, 2010, **400**, 477–486.
58 D. Strickland, K. Moffat and T. R. Sosnick, Light-activated DNA binding in a designed allosteric protein, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 10709–10714.
59 A. Levskaya, O. D. Weiner, W. A. Lim and C. A. Voigt, Spatiotemporal control of cell signalling using a light-switchable protein interaction, *Nature*, 2009, **461**, 997–1001.
60 A. Joachimiak, R. L. Kelley, R. P. Gunsalus, C. Yanofsky and P. B. Sigler, Purification and characterization of trp aporepressor, *Proc. Natl. Acad. Sci. U. S. A.*, 1983, **80**, 668–672.
61 J. Lee, M. Natarajan, V. C. Nashine, M. Socolich, T. Vo, W. P. Russ, S. J. Benkovic and R. Ranganathan, Surface sites for engineering allosteric control in proteins, *Science*, 2008, **322**, 438–442.
62 S. W. Lockless and R. Ranganathan, Evolutionarily conserved pathways of energetic connectivity in protein families, *Science*, 1999, **286**, 295–299.
63 S. K. Yoo, Q. Deng, P. J. Cavnar, Y. I. Wu, K. M. Hahn and A. Huttenlocher, Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish, *Dev. Cell*, 2010, **18**, 226–236.
64 X. Wang, L. He, Y. I. Wu, K. M. Hahn and D. J. Montell, Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo, *Nat. Cell Biol.*, 2010, **12**, 591–597.
65 M. Hulko, F. Berndt, M. Gruber, J. U. Linder, V. Truffault, A. Schultz, J. Martin, J. E. Schultz, A. N. Lupas and M. Coles, The HAMP domain structure implies helix rotation in transmembrane signaling, *Cell*, 2006, **126**, 929–940.
66 A. Marina, C. D. Waldburger and W. A. Hendrickson, Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein, *EMBO J.*, 2005, **24**, 4247–4259.
67 P. Casino, V. Rubio and A. Marina, Structural insight into partner specificity and phosphoryl transfer in two-component signal transduction, *Cell*, 2009, **139**, 325–336.
68 D. Albanesi, M. Martín, F. Trajtenberg, M. C. Mansilla, A. Haouz, P. M. Alzari, D. de Mendoza and A. Buschiazzo, Structural plasticity and catalysis regulation of a thermosensor histidine kinase, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 16185–16190.
69 S. Yamada, H. Sugimoto, M. Kobayashi, A. Ohno, H. Nakamura and Y. Shiro, Structure of PAS-linked histidine kinase and the response regulator complex, *Structure*, 2009, **17**, 1333–1344.
70 U. Krauss, J. Lee, S. J. Benkovic and K. Jaeger, LOVely enzymes - towards engineering light-controllable biocatalysts, *Microb. Biotechnol.*, 2010, **3**, 15–23.
71 M. Yazawa, A. M. Sadaghiani, B. Hsueh and R. E. Dolmetsch, Induction of protein-protein interactions in live cells using light, *Nat. Biotechnol.*, 2009, **27**, 941–945.
72 S. Shimizu-Sato, E. Huq, J. M. Tepperman and P. H. Quail, A light-switchable gene promoter system, *Nat. Biotechnol.*, 2002, **20**, 1041–1044.
73 D. W. Leung, C. Otomo, J. Chory and M. K. Rosen, Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 12797–12802.
74 A. B. Tyszkiewicz and T. W. Muir, Activation of protein splicing with light in yeast, *Nat. Methods*, 2008, **5**, 303–305.
75 A. Levskaya, A. A. Chevalier, J. Tabor, Z. B. Simpson, L. A. Lavery, M. Levy, E. A. Davidson, A. Scouras, A. D. Ellington, E. M. Marcotte and C. A. Voigt, Synthetic biology: engineering Escherichia coli to see light, *Nature*, 2005, **438**, 441–442.
76 S. Morgan, S. Al-Abdul-Wahid and G. A. Woolley, Structure-Based Design of a Photocontrolled DNA Binding Protein, *J. Mol. Biol.*, 2010, **399**, 94–112.
77 D. M. Chudakov, V. V. Verkhusha, D. B. Staroverov, E. A. Souslova, S. Lukyanov and K. A. Lukyanov, Photoswitchable cyan fluorescent protein for protein tracking, *Nat. Biotechnol.*, 2004, **22**, 1435–1439.
78 P. S. Lagali, D. Balya, G. B. Awatramani, T. A. Munch, D. S. Kim, V. Busskamp, C. L. Cepko and B. Roska, Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration, *Nat. Neurosci.*, 2008, **11**, 667–675.
79 A. S. Halavaty and K. Moffat, N- and C-terminal flanking regions modulate light-induced signal transduction in the LOV2 domain of the blue light sensor phototropin 1 from Avena sativa, *Biochemistry*, 2007, **46**, 14001–14009.

