

REVIEW

Engineering synthetic optogenetic networks for biomedical applications

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Background: Recently, optogenetics based on genetically encoded photosensitive proteins has emerged as an innovative technology platform to revolutionize manipulation of cellular behavior through light stimulation. It has enabled user defined control of various cellular behaviors with spatiotemporal precision and minimal invasiveness, creating unprecedented opportunities for biomedical applications.

Results: This article reviews current advances in optogenetic networks designed for the treatment of human diseases. We highlight the advantages of these optogenetic networks, as well as emerging questions and future perspectives.

Conclusions: Various optogenetic systems have been engineered to control biological processes at all levels using light and applied for numerous diseases, such as metabolic disorders, cancer, and immune diseases. Continued development of optogenetic modules will be necessary to precisely control of gene expression magnitude towards clinical medical practice in the context of real-world problems.

Keywords: synthetic biology; mammalian designer cells; optogenetics; synthetic gene circuits; gene- and cell-based therapy

INTRODUCTION

Over the past decade, synthetic biology has emerged as an interdisciplinary field that addresses crucial challenges in human health and disease with an engineering approach [1,2]. Synthetic biology provides the platform and tools to design artificial regulators to rationally and precisely control the biological activities of cells [3]. To date, many complex synthetic devices have been designed and constructed such as toggle switches [4], sensor-effector device [5], oscillators [6,7], pattern detectors [8], and intercellular communication systems [8,9]. Most of the devices were responsive to small molecule inducers and had inherent limitations in regard to precise spatiotemporal control of gene expression. Recently, optogenetics has emerged as an innovative technology platform in the rapidly progressing field of synthetic biology to achieve highly precise spatiotemporal control and manipulation of cellular behavior through light stimulation, both *in vitro* and *in vivo* [10]. In addition, the target gene expressions

can also be quantitatively regulated by controlling the different light irradiances [11,12]. Some fine-tuned circuits based on bidirectional promoter were developed to quantitatively regulate multiple gene expression by modulating light irradiance [12].

The broad definition of optogenetics refers to combining optical and genetic principles together to control various cellular functions such as stimulation/inhibition of metabolic pathways [13,14], gene activation [15], intracellular signaling [16], and cellular migration [17] upon stimulation with a specific wavelength of light. Genetically encoded light-responsive systems exhibit various distinctive features, which make them stand out from other synthetic gene networks, such as fast activation and inactivation kinetics, low technical requirements for equipment and reagents besides illumination, and lack of side effects typically associated with drug administration. This technology platform has recently attracted much attention due to the unmatched properties of light as a signaling entity, which enables highly precise

spatio-temporal and quantitative control of cellular activities and gene expressions.

Since the debut of optogenetics about a decade ago, the field has developed rapidly. The history of optogenetic networks [18–22] is summarized in Figure 1. The first described microbial opsin, bacteriorhodopsin, dates back to the 1970s [23,24]. Over the subsequent years, a diverse variety of other light-sensitive proteins have been identified, such as halorhodopsin and channelrhodopsin. It was not until 2005 that the concept of fast optogenetic stimulation was realized with reliable control of millisecond-precision action potentials in mammalian neurons through light stimulation [21,25]. In 2010, the journal *Nature* declared optogenetics to be their “method of the year” [26], while the journal *Science* classified it as one of the major breakthroughs of the last decade [27].

Recently, optogenetics has turned early ideas into a powerful paradigm for cell biology [28,29]. In the most recent studies, several light-responsive gene expression systems designed with the engineering-driven approaches of modularization, rationalization and modeling have been created to monitor and control mammalian cellular activities with the unmatched precision of light stimulation [30–34]. These provide a trajectory for future development in the field. This review therefore aims to

highlight the latest state-of-the-art optogenetics-associated light-responsive synthetic networks for controlling gene expression in some important biomedical applications.

OPTOGENETIC NETWORKS FOR THE TREATMENT OF DIABETES MELLITUS

Type 2 diabetes is a chronic metabolic disease characterized by hyperglycemia and results from the combination of resistance to insulin, inadequate insulin secretion, and inappropriate glucagon secretion [35,36]. Diabetes has afflicted increasing numbers of people worldwide. Most people with type 2 diabetes will eventually need to inject insulin and take other medications, as well as strictly control their diet [37].

Recently, a number of promising optogenetic networks have been developed for the treatment of diabetes (Figure 2). In a pioneering study by Ye *et al.* [38], a blue-light responsive gene switch was created in human cells to activate transgene expression in an animal model (Figure 2A). In this design, a novel synthetic light-controlled transcription device could be used to treat type II diabetes. Under stimulation with blue light (~480 nm), melanopsin activates the nuclear factor of activated T cells (NFAT) through elevated calcium levels, which induces glucagon-

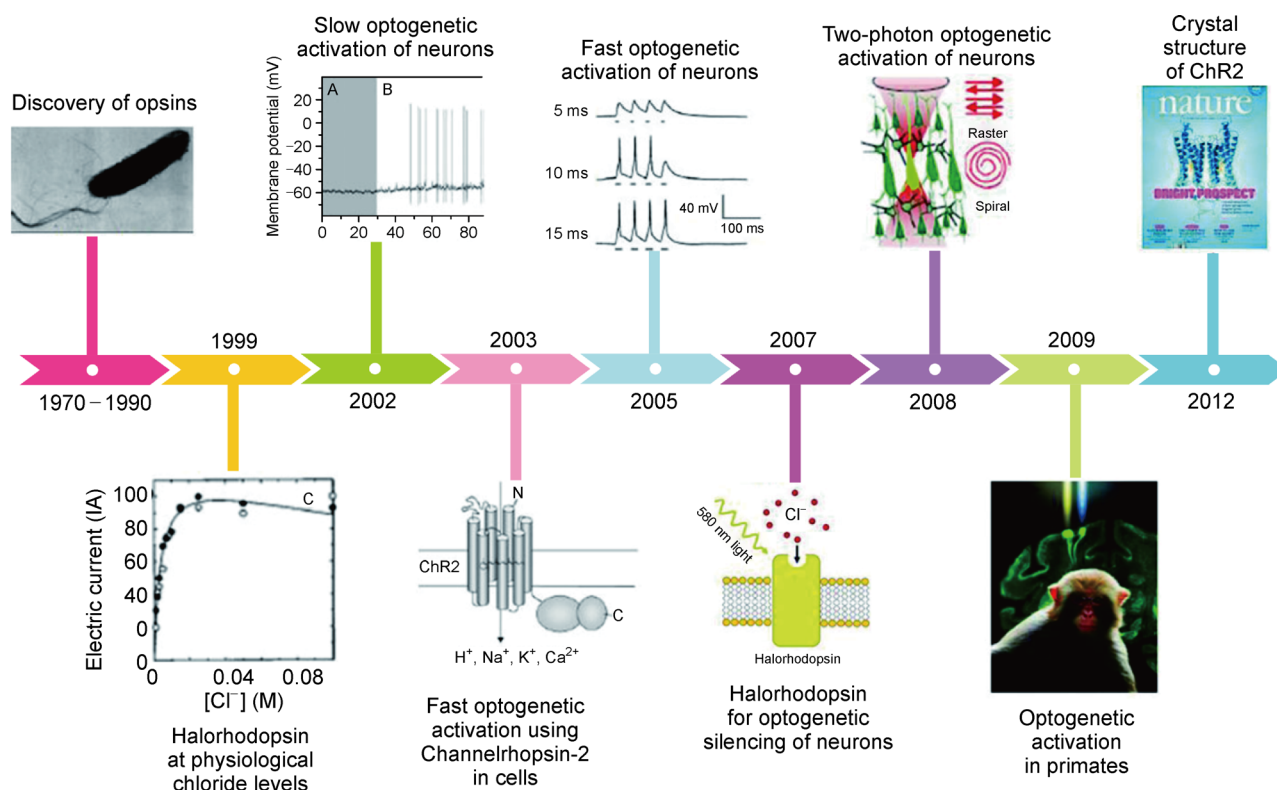


Figure 1. A brief summary of the history of optogenetics. 1999 – Reprinted with permission from Ref. [8]; 2002 – Reprinted with permission from Ref. [9]; 2003 – Reprinted with permission from Ref. [10]; 2005 – Reprinted with permission from Ref. [11]; 2012 – Reprinted with permission from Ref. [12].

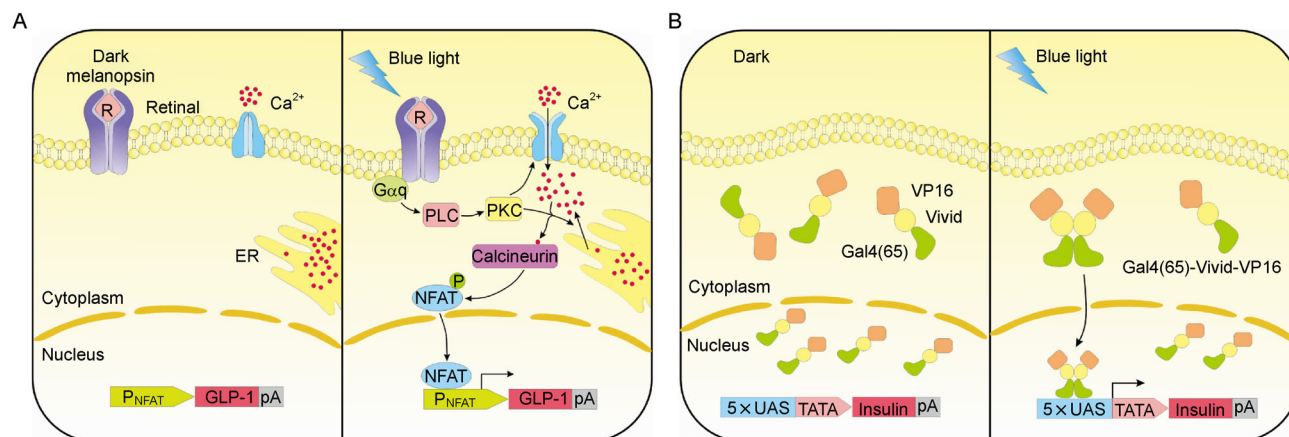


Figure 2. Synthetic optogenetic networks for diabetes therapy. (A) Synthetic optogenetic network for modulating glucose homeostasis in diabetic mice. In the dark, melanopsin do not trigger the phosphodiesterase-dependent cascade. Under blue-light illumination, the 11-*cis* retinal (R) chromophore is isomerized and changes the conformation of melanopsin, thus sequentially activating the Gαq-type G protein (Gαq), phospholipase C (PLC), and phosphokinase C (PKC), which in turn triggers calcium influx by transient receptor potential channels (TRPCs). The increased intracellular calcium levels activate the calcium sensor protein calmodulin (CaM) and calcineurin (CaN) and induce glucagon-like peptide-1 (GLP-1) expression via an NFAT-responsive promoter (P_{NFAT}). The transgenic HEK-293 cells containing this synthetic gene circuit were microencapsulated and subcutaneously implanted into diabetic mice, which lowered the blood-glucose levels after blue-light exposure. (B) A light-controlled transgene system based on light-oxygen-voltage (LOV) domain for the treatment of diabetes. In the dark, GAVP do not interact with UAS_G elements (5xUAS_G) and do not induce gene expression. After blue light activation, Vivid (VVD), one of the light-oxygen-voltage (LOV) domain, changes its structure to form a dimer and induces dimerization of the Gal4(65)-VVD fusion protein, which enhances binding to the UAS_G sequence and activates transcription of insulin. The system was transferred into type I diabetic mice by a hydrodynamic procedure, and the blood glucose levels were significantly decreased under blue-light illumination.

like peptide 1 (GLP-1) expression through a NFAT driven promoter. This can attenuate glycemic excursions in type II diabetic patients. When engineered cells containing this synthetic light-responsive network were subcutaneously implanted into type II diabetic mice, control of blood-glucose homeostasis was achieved by blue light stimulation.

In another study, Wang *et al.* [39] designed a light-controlled transgene system as a triggering mechanism to control insulin expression for type I diabetes treatment (Figure 2B). In this system, the light-oxygen-voltage domain Vivid (VVD) was used to control gene expression by blue light. The light-switchable transgene system contained VVD, a monomeric variant of the Gal4-DNA-binding domain and the activation domain p65. Upon stimulation with pulses of blue light, VVD can change its structure to form a dimer, thus dimerizing the Gal4-DNA binding domain to bind to its cognate promoter and activating insulin transcription. The inactive state was however maintained in the dark. When the system was transferred into type I diabetic mice by a hydrodynamic procedure, the blood glucose levels were significantly decreased under blue-light illumination. This light responsive system provides another example of controlling biological processes through light-switchable gene expression.

OPTOGENETIC NETWORKS FOR ONCOTHERAPY

Targeting the immune system to combat tumors is a promising therapeutic strategy [40]. T-cells are removed from a patient and modified, and then reintroduced back into the patient with the ability to recognize and kill targeted cancer cells [41–43]. Recently, optogenetic systems have been developed to enable immune cells to specifically attack tumor cells (Figure 3).

In the study of Zhou *et al.* [44], a near-infrared (NIR)-responsive optogenetic system (termed “Opto-CRAC”) was designed as a tool for immunomodulation and immunotherapy, by remotely controlling Ca²⁺-responsive gene expression in specialized immune cells, such as T lymphocytes, macrophages and dendritic cells (Figure 3A). In this study, the NIR-responsive optogenetic platform based on lanthanide-doped upconversion nanoparticles (UCNP) and the engineered calcium channel (Opto-CRAC channels) can remotely control engineered cells buried deep within tissues by UCNPs turning NIR light into visible light. The engineered CRAC channels consisted of ORAI1 and engineered stromal interaction molecule 1 (STIM1, ER-located Ca²⁺ sensor protein) fused with the light switch LOV2 (light, oxygen, voltage) domain. Upon illumination with blue light, C-terminal Jα

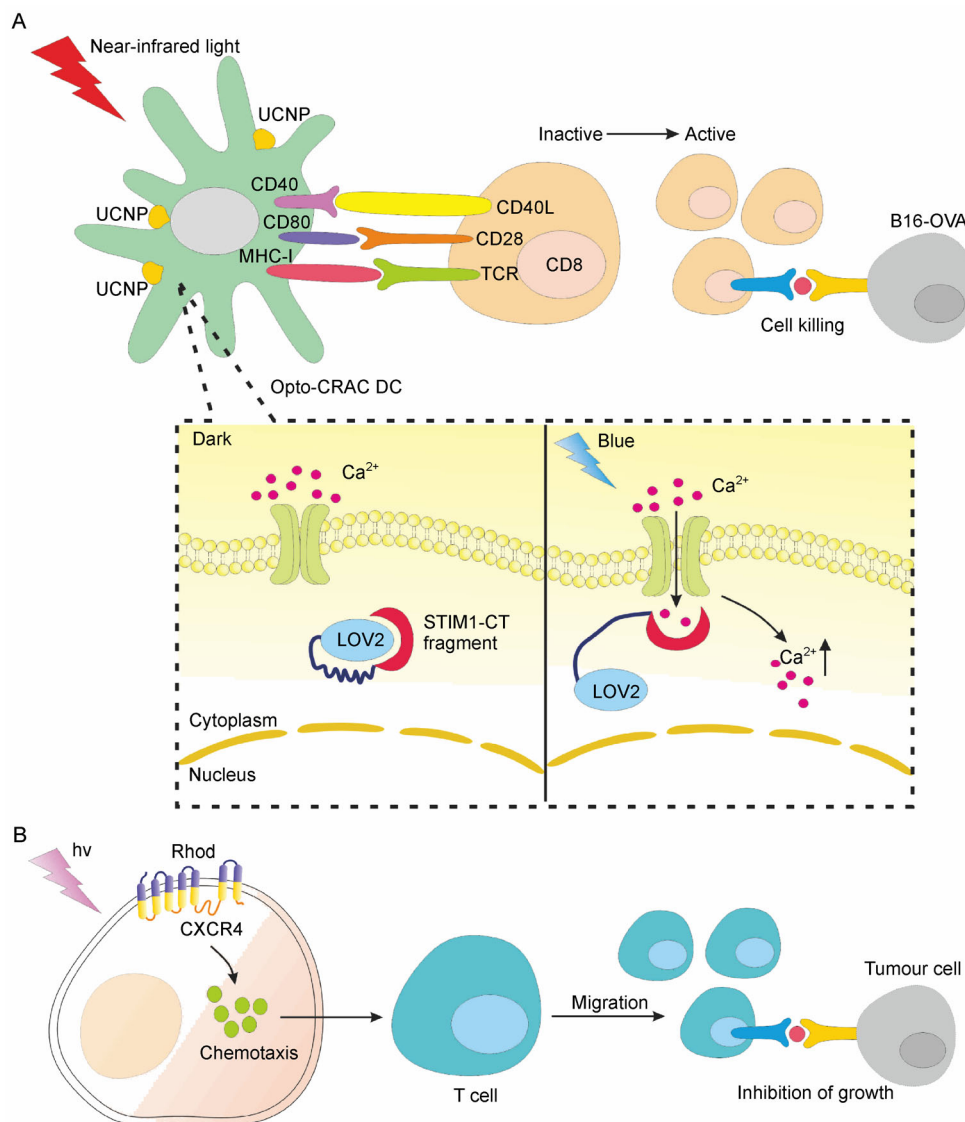


Figure 3. Synthetic gene circuits for cancer therapy. (A) A synthetic near infrared (NIR)-stimulable optogenetic platform (termed Opto-CRAC) designed for treatment of melanoma in a B16-OVA murine model. Photosensitive STIM1 can be engineered by fusing STIM1-CT fragments with the LOV2 (light, oxygen, voltage) domain (residues 404–546) of *Avena sativa* phototropin 1. In the dark, STIM1-CT fragments are kept quiescent by docking toward the LOV2 domain. Upon blue light illumination, the undocking and unwinding of the LOV2 C-terminal α helix exposes the STIM1-CT fragments, enabling their interaction with ORAI1 Ca^{2+} channels to induce calcium ion influx across the plasma membrane. When coupled to lanthanide-doped upconversion nanoparticles (UCNP), the optogenetic excitation window is converted to visible light from tissue-penetrable NIR light to enable wireless photo activation of Ca^{2+} -dependent signaling and modulation of immune cells activities. NIR-stimulated calcium ion influx in Opto-CRAC DCs facilitate immature dendritic cell maturation and promote antigen presentation, thereby sensitizing T lymphocytes towards tumor antigens to improve anti-tumor immune response to selectively kill tumor cells in the B16-OVA melanoma model. (B) Optical control of chemokine receptor used for cancer immunotherapy in B16/OVA tumor bearing mice. Under 505 nm light irradiation, a photoactivatable chemokine receptor CXCR4 (PA-CXCR4) based on rhodopsin and chemokine receptor is activated to recruit transferred T cells and make them migrate into the core of B16 tumors, promoting local effector functions and suppressing tumor growth.

helix of LOV2 was unwinded to enable mobility of STIM1-CT fragments, resulting in activation of ORAI1 Ca^{2+} channels and influx of calcium ions into the cells,

which in turn activate calcium dependent gene expression to elicit a series of immune responses. Additionally, upon injection of the Opto-CRAC system into the mouse model

of melanoma by utilizing ovalbumin as an antigen, the system can elicit anti-tumor immune responses to specifically kill tumor cells. This approach will pave the way for broader applications of optogenetic tools in synthetic biology that enables the remote and wireless regulation of gene networks.

Another study [45] also reported an optical chemokine receptor system based on photoactivatable-chemokine C-X-C motif receptor 4 (PA-CXCR4) that can induce highly specific chemokine signals and guide cell migration through light stimulation (Figure 3B). The genetic circuit was carefully designed to couple an extracellular optical signal to intracellular chemokine effector functions, which in turn can control chemotactic signals and modulate directional cell migration towards the source of light stimulation. This synthetic chemotaxis system was validated in a mouse model. PA-CXCR4 was shown to recruit transfused tumor-specific CD8⁺ T cells to a tumor site *in vivo*, resulting in significant reduction of tumor growth in mice after light stimulation. These findings thus suggest that photoactivatable chemokine receptors provide a unique platform to remotely control cell trafficking to enable accurate lymphocyte chemotaxis and thus gain access to a specific tissue site in response to localized stimulation with a specific light wavelength. The utilization of antitumor T-cell migration to localize chemokine signals for treatment of tumors was also confirmed by another study [46,47].

OPTOGENETIC NETWORKS FOR THE TREATMENT OF RETROGRADE AMNESIA

Retrograde amnesia is a form of memory loss in patients with closed head injury, Korakoff's syndrome, and other memory disorders, in which remote memories are more easily accessible than events occurring just prior to the trauma. Retrograde amnesia usually follows damage to areas of the brain other than the hippocampus that deal largely with memory consolidation [48–50], because existing long-term memories are stored in the neurons and synapses of various different brain regions. Memory consolidation is the phenomenon characterized by newly formed memory transitions from a fragile state to a stable and persistent state.

Recently, several groups demonstrated that specific activation of hippocampal cells for encoding memory are both sufficient and necessary to drive future recall of a contextual fear memory and thus represent a component of a distributed memory engram [51–56]. Tonegawa *et al.* [57] designed an optogenetic stimulation system based on channelrhodopsin-2 (ChR2). In this study, dentate gyrus (DG) and CA3 engram cells were labeled with TRE-ChR2-EYFP and AAV9-TRE-mCherry in amnesic mice

induced by a protein synthesis inhibitor. Under light illumination, DG engram cells were induced to restore retrograde amnesia caused by disruption of reconsolidation of a contextual fear memory [58], and the physiological connectivity between DG and CA3 or basolateral amygdala (BLA) engram cells was activated. Together, these findings suggest that engram cells induced by contextual fear conditioning (CFC) training are crucial for retrieving memory recall.

OPTOGENETIC NETWORKS FOR THE TREATMENT OF PENILE ERECTION

Precise spatiotemporal control of physiological processes by optogenetic networks may provide a strategy for gene- and cell based therapies. Optogenetics has become a key technology to modulate neuronal activities managing memory [52], learning [59], and olfactory processing [60]. Penile erection is the result of a complex neurovascular process involving various central and peripheral neural and/or humoral endocrine mechanisms [61,62]. It is widely accepted that nitric oxide plays an important role in penile erection [63–65]. Nitric oxide stimulates soluble guanylyl cyclase (sGC), subsequently inducing the second messenger 3', 5'-cyclic guanosine monophosphate (cGMP) to reduce intracellular calcium ion concentration by closure of voltage-gated calcium channels, which leads to relaxation of the smooth muscle of the corpus cavernosum and triggering penile erection [66–68].

Erectile dysfunction is a sexual dysfunction affecting more than 100 million people worldwide. Erectile dysfunction can occur due to both physiological and psychological reasons including diabetes, kidney disease, chronic alcoholism, multiple sclerosis, atherosclerosis, vascular disease, and neurologic disease [69–72]. cGMP specific phosphodiesterase inhibitors such as Viagra® have been used to treat erectile dysfunction by suppressing cGMP and extending erection time. However, these drugs often exert adverse side effects in some patients suffering from hypotension or severe hepatic dysfunction and heart disease, which thus limited their clinical applications. With the advent of synthetic biology, genetic devices can provide an alternative method to precisely control the physiological process of this particular disease, thus providing novel therapeutic strategies for erectile dysfunction.

Recently, Kim *et al.* [73] designed and validated a blue-light-responsive synthetic erectile optogenetic circuit in human cells (Figure 4). This erectile optogenetic stimulator (EROS) biosynthesizes guanylate cyclase acid upon blue-light illumination, leading to high levels of cGMP in mammalian cells and reduced intracellular calcium levels. When introducing EROS to the corpus

cavernosum of male rats, the treated animals exhibited obvious erectile response and ejaculation upon exposure to blue light. Compared with other methods [74–78], the EROS device provides trigger-inducible erection on demand with less side effects, but needs to be evaluated in clinical trials for the treatment of erectile dysfunction.

OPTOGENETIC NETWORKS FOR ORGANELLE TRANSPORT

Peroxisomal disorders represent genetically heterogeneous metabolic diseases characterized by the absence of normal peroxisomes in the cells of the body. Peroxisomes are cellular organelles involved in important metabolic pathways, such as beta-oxidation of very-long-chain fatty acids (VLCFA) and detoxification of hydrogen peroxide [79]. Impaired peroxisome transport causes compromised peroxisome function resulting in oxidative stress in many neurodegenerative diseases [80]. Recently, optogenetic networks have been developed to directly regulate protein trafficking, including nuclear-cytoplasmic trafficking using light (Figure 5) [81–84].

Spiltoir *et al.* [85] used blue-light-responsive LOV2 domain of *Avena sativa* phototropin1 (AsLOV2) to directly control peroxisomal import with light. They developed caged GFP-LOV-PTS1 constructs containing a GFP reporter, AsLOV2- α motif and peroxisomal targeting signal (PTS1) protein bound to the Pex5 peroxisomal import receptor to localize peroxisome with light (Figure 5A). When proteins or peptides are attached to the C-terminus of the α -helix, they can be blocked and could not interact with effectors in the dark, but the block can be uncaged upon illumination and α -helix unfolding. This system provides a tool to elicit peroxisomal protein

trafficking at specific time points or within defined locations in specific cells through optical activation and deactivation.

Another example is optical control of organelle transport and positioning (Figure 5B) [86]. The function of various organelles depends on their proper spatial arrangement. Despite the importance of local positioning, the precise correlation between position and function is not yet fully understood. In order to solve this problem, many studies have developed different strategies to manipulate organelle distributions. Recent technical advances, such as optogenetics, are beginning to provide newer and more accurate ways to manipulate organelle positioning. One strategy is to control the motility of kinesin 5 and kinesin 1 *in vitro* by photocontrol ATP hydrolysis through incorporating photochromic molecules into the ATPase domain of motor proteins [87,88]. In addition, another study reported that both the speed and directionality of myosin VI and kinesin 14 motors could be modulated by post exposure to blue light [89]. However, these methods have not been applied to reposition organelles. Recently, Bergeijk *et al.* [86] have established a light-responsive intracellular transport system by using light-sensitive heterodimerization to control the connection between selected organelles and specific motor proteins such as kinesin, dynein or myosin. Under light illumination, the synthetic systems containing blue light sensitive LOV2 (light- oxygen-voltage-sensing) domain with an engineered PDZ domain and cryptochrome 2 together with CIB1 (cryptochrome-interacting basic-helix-loop-helix 1) can induce the motility of peroxisomes, mitochondria, and recycling endosomes. Selected areas from peroxisomes or mitochondria could be depleted by localized laser illumination, whereas

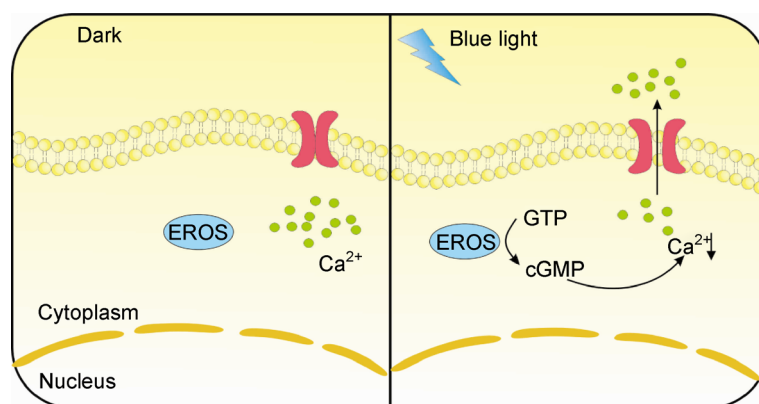


Figure 4. Synthetic erectile optogenetic stimulator for the treatment of male erectile dysfunction. A synthetic erectile optogenetic stimulator (EROS) containing a blue-light sensor domain BLUF (blue-light using FAD) activates an adjacent catalytic nucleotide cyclase domain. In the dark, EROS is inactivated. Under blue light illumination, the synthetic erectile optogenetic stimulator (EROS) enables conversion of GTP to cGMP, which activates protein kinase C to trigger Ca^{2+} -outflux decrease by closure of voltage-gated calcium channels. This process ultimately results in the relaxation of the corpus cavernosum smooth muscle and subsequent penile erection.

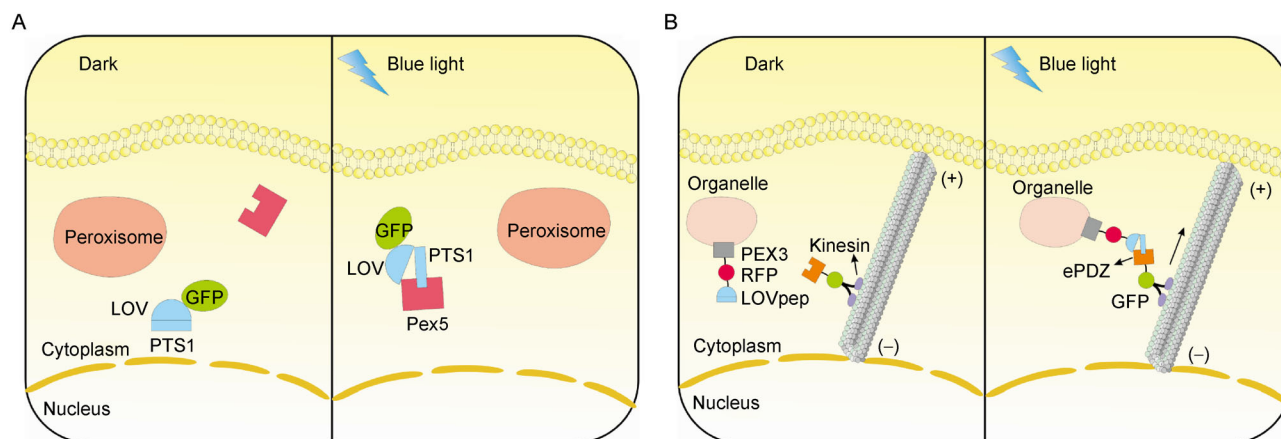


Figure 5. Optical control of organelle transport and positioning. (A) Optical control of peroxisomal trafficking based on LOV2 (light, oxygen, voltage) domain of *Avena sativa* phototropin1 (AsLOV2). LOV-PTS1 construct contains C-terminus of the α -helix that can be released from the LOV core in the presence of light and uncages the PTS1 sequence. When Pex5 binds to PTS1, the protein is translocated to the peroxisome. (B) Local and reversible activation of organelle transport with light. PEX-LOV fusion protein consists of a peroxisomal targeting signal of PEX3 and a photosensitive LOV domain. In the dark, the photosensitive protein is in the free state and do not control organelle positioning. Upon exposure to blue light, PEX-LOV cages a small peptide that binds the engineered PDZ domain ePDZb1 fused to the plus-end-directed kinesin-3 KIF1A, leading to relocation of peroxisomes from the cell centre to the periphery.

recycling endosomes may be induced to enter into specific dendritic spines, targeted away or moved towards axonal growth cones. A subsequent study extended this optogenetic tool to lysosomes [90]. Different optogenetic systems can be used to manipulate organelle positioning with precise spatiotemporal control.

OPTOGENETIC GENE TARGETING

Synthetic biology provides a powerful platform to assemble biological components into well-controlled systems to explore the intricate mechanisms underlying cellular behavior. Recently, a variety of microbial and plant-derived light-sensitive proteins have been engineered as optogenetic actuators to spatially and temporally control gene expression in various cells (Figure 6). Using different types of opsins and viral delivery vectors for devising new optogenetic tools, investigators can engineer cells to detect and respond to a variety of extracellular and intracellular signals to manipulate the cellular machinery towards novel purposes with precise spatiotemporal control [91–94]. For example, designable transcription factors such as zinc fingers (ZF) and transcription-activator like effectors (TALE) can be engineered to bind any DNA segment of choice. By combining the TALE technology with optogenetic control elements, very fast regulation of mammalian endogenous transcription could be achieved (Figure 6A) [95].

Another example, the light-inducible transcriptional effectors (LITEs) system consists of TALE DNA-binding domain from *Xanthomonas* sp. [96–98], and the light-

sensitive cryptochrome 2 (CRY2) protein from *Arabidopsis thaliana* (TALE-CRY2) [99] and its interactive partner CIB1-effector [99,100]. Upon blue light illumination, CRY2 changes its conformation and subsequently recruit CIB1-VP64 to the target promoter to regulate downstream gene expression. Furthermore, the LITE system has been applied to *in vitro* and *in vivo* tests. A Grm2-targeted LITE was applied into primary cortical neurons by adeno-associated viruses (AAVs) [95,101], which upregulated Grm2 mRNA and mGluR2 protein levels after light stimulation. Additionally, AAV vectors carrying TALE(Grm2)-CIB1 and CRY2PHR-VP64 were introduced into the prefrontal cortex (PFC), triggering light-dependent increase of Grm2 mRNA after illumination with light. The epigenetic mark-modified TALE-histone effector constructs (epiTALEs) have induced endogenous Grm2 for interplay between epigenetic and transcriptional dynamics. This LITE system provides a powerful tool for engineering and modifying mammalian genomes.

OPTOGENETIC NETWORKS FOR GENE EDITING

A follow-up study by other groups demonstrated how to use light to control the powerful gene-editing tool-the clustered regularly interspaced short palindromic repeats (CRISPR) system [15,102–104]. In contrast to classical designer nucleases based on chimeric fusions between a ZF or TALE-domain to a non-specific endonuclease, the Cas9 protein is by nature a site-specific endonuclease.

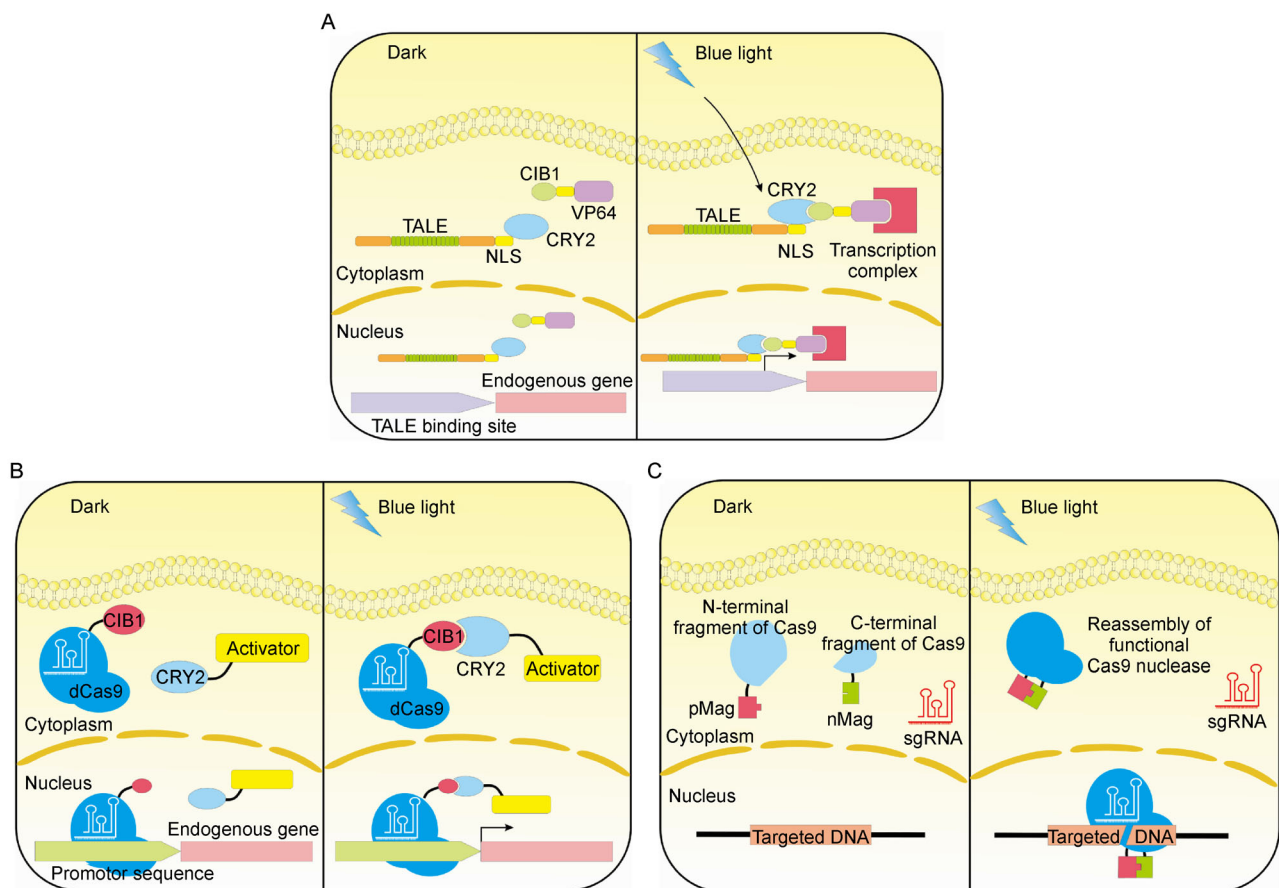


Figure 6. Optogenetic tools for Genome editing. (A) Optical control of mammalian endogenous transcription based on the LITE system. Light-inducible transcriptional effectors (LITEs) consisting of transcription activator-like effectors (TALEs), DNA-binding domain with the light-sensitive cryptochrome 2 (CRY2) protein and its interactive partner CIB1 from *Arabidopsis thaliana*. In the dark, TALE-CRY2 binds to the target gene promoter while the CIB1-effector remains inactive. When illuminated with blue light, CRY2 conformation is changed and recruits CIB1-effector (VP64) to the target region to induce downstream gene expression. (B) Photoactivatable transcription system based on CRISPR-Cas9. This CRISPR-Cas9-based photoactivatable transcription system includes two fusion proteins and sgRNAs. One fusion protein contains dCas9 and CIB1, and binds to the targeted genome sequence by sgRNAs, while the other fusion protein consists of the photolyase homology region of CRY2 (CRY2PHR) and the transcriptional activator domain. In the dark, the dCas9-CIB1 complex binds to the targeted gene promoter by sgRNAs while the activator probe is free in the nucleus and cannot activate the target genes. Upon blue light illumination, the downstream gene expression is activated by heterodimerized CRY2PHR and CIB1 and then the transcriptional activator domain is recruited to the target gene region. (C) Engineered photoactivatable Cas9 (paCas9) genome editing in human cells. Optically controlled Cas9 is based on the split Cas9 fragments fused with light-responsive dimerization domains (pMag and nMag). In the dark, Cas9 is split into two fragments without gene editing. Under blue light illumination, pMag and nMag are heterodimerized to make the split Cas9 fragments active, leading to resumption of RNA-guided nuclease activity to activate genome editing.

Using Cas9 and a set of user-defined guide RNA molecules (gRNA), specific DNA sequences can be targeted, cleaved and modified with unprecedented precision [105–107]. Now, methods for selectively deploying CRISPR have been developed with the flick of a light switch.

Hemphill *et al.* pioneered the development of genetically encoded light-activated Cas9 to investigate biological processes such as signal transduction and gene

expression [103]. In this study, the light-sensitive lysine residue was inserted near a gRNA binding site on Cas9 proteins in human embryonic kidney cells. The modified lysine blocked gRNA binding, thereby preventing Cas9 from activating gene editing. Upon UV light exposure, however, the lysine changes shape to allow gRNA to bind to Cas9, thereby enabling gene editing. This light-activated system can also be used to silence gene expression.

Nihongaki *et al.* [102] have also developed a CRISPR-Cas9-based photoactivatable circuit based on an catalytically inactive Cas9 variant (dCas9), the light-sensitive cryptochrome 2 (CRY2) and its binding partner CIB1 from *Arabidopsis thaliana* [99], enabling blue light-triggered activation of user-defined endogenous genes (Figure 6B). This optogenetic system can rapidly and reversibly activate the target gene, as well as various endogenous genes in mammalian cells, thus making optogenetic regulation of mammalian genome editing possible for various synthetic biology applications.

An engineered photoactivatable Cas9 (paCas9) system was also reported in human embryonic kidney cells by Nihongaki *et al.* [15]. In this study, paCas9 was generated from two split Cas9 fragments and light-responsive dimerization domains named Magnets, which could modify the targeted genome sequence through joining of both non-homologous ends and activation of homology-directed repair pathways under blue light irradiation (Figure 6C). Moreover, paCas9 can temporally and reversibly control gene editing in response to light. Several reports have shown that programming dCas9 to a transcription site can block RNA polymerase and transcription (termed CRISPR interference or CRISPRi) [108]. A photoactivatable CRISPRi (paCRISPRi) was designed to optically control RNA-guided transcription for targeted gene silencing.

CONCLUSION AND PERSPECTIVES

Synthetic biology provides a powerful platform to understand and harness biology, with influences from many scientific and engineering disciplines, and can exert a profound impact on various aspects of daily life and society in the future. Recently, two design principles are mainly applied for various light-inducible systems to control gene expression. One is based on light-dependent recruitment of transcriptional activation domain to DNA-bound proteins, the other is based on light-modulated dimerization of a photoreceptor for reconstitution a DNA-binding domain to activate the target gene transcription. Nowadays, synthetic biologists have designed complex artificial gene circuits assembled into biosensing devices to monitor cellular behavior, which have promising therapeutic applications [3,109,110]. Compared to chemical or microwave systems, optogenetic tools triggered by specific wavelengths of light offer unprecedented spatiotemporal precision without the addition of chemical inducers that might perturb the system under investigation [95,111]. Consequently, the field of optogenetics has experienced a big upsurge over the last few years, and have begun to revolutionize biomedical research. Various optogenetic approaches have already been applied for numerous diseases, such as metabolic disorders, cancer,

and immune diseases. A dual-input genetic circuit was reported to simultaneously modulated by utilizing the benefits of a chemical and light inducer. Moreover, the target gene of the reported circuits can be quantitatively expressed by regulating the chemical concentration and light irradiance both in the cells and mice [11].

Yet, can the optogenetic networks be validated outside the laboratory? To achieve the goals, standardized genetic circuits could be designed to ensure more robust and safe for patients. Moreover, most of the current genetic circuits were engineered into human embryonic kidney cells, the clinically proven mesenchymal stem cells from the patients could be used in clinic in the future. In addition, therapeutic proteins could be modulated upon light illumination in the patients.

To date, various optogenetic networks have been engineered to control biological processes using light. Two types of devices can perform these functions: light-driven actuators control electrochemical signals, while light-emitting sensors report them. These range from light-inducible ion channels and receptors to the targeted interaction of proteins with light-induced proteolysis and light-induced second messenger products for recruitment of the related proteins. Most mammalian light-responsive gene circuits are controlled by blue light-responsive gene switches [38,39,112]. However, these are limited by lower efficiency in deep tissue. Red light systems could prove to be highly influential because of its selective targeting capacity and higher efficiency and viability [29,113]. However, some red light-responsive gene networks need phytochromobilin, which is not only difficult to acquire but also unlikely to be clinically licensed because of the side effects caused by this plant-derived co-factor. Therefore, a more concerted effort towards advancing optogenetic devices in mammalian cells will be crucial for the next-generation of therapeutic switches, such as the choice of clinically safe light sources and/or using optogenetic systems that do not interfere with other metabolic processes. Multichromatic control circuits will further broaden the scope of light-controlled circuits used in biomedical applications.

Further development of optogenetic modules will be necessary to combine light-responsive gene expression systems with multi-spectral imaging without the need for exogenous compounds. Despite these challenges, future work using these novel tools and techniques will undoubtedly boost the development of next-generation synthetic biology in biotechnological applications. Lastly, optogenetic tools are maturing towards clinical medical practice, where they could induce therapeutic protein synthesis in patients subjected to light stimulation.

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COMPLIANCE WITH ETHICS GUIDELINES

The authors Meiyan Wang, Yuanhuan Yu, Jiawei Shao, Boon Chin Heng and Haifeng Ye declare they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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