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Optogenetic approaches to control Ca^{2+}-modulated physiological processes

Nhung T. Nguyen[1], **Guolin Ma**[1], **Yubin Zhou**[1,2,*], **Ji Jing**[1,*]

[1]Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, Houston, TX 77030, USA

[2]Department of Translational Medical Sciences, College of Medicine, Texas A&M University, Houston, TX 77030, USA

Abstract

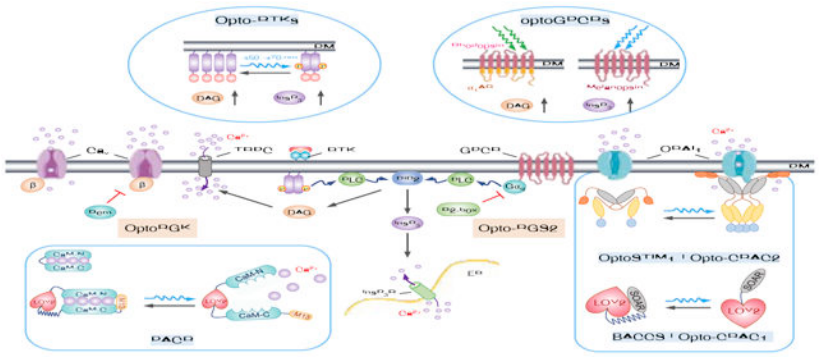
As a versatile intracellular second messenger, calcium ion (Ca^{2+}) regulates a plethora of physiological processes. To achieve precise control over Ca^{2+} signals in living cells and organisms, a set of optogenetic tools have recently been crafted by engineering photosensitive domains into intracellular signaling proteins, G-protein coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), and Ca^{2+} channels. We highlight herein the optogenetic engineering strategies, kinetic properties, advantages and limitations of these genetically-encoded Ca^{2+} channel actuators (GECAs) and modulators. In parallel, we present exemplary applications in both excitable and non-excitable cells and tissues. Furthermore, we briefly discuss potential solutions for wireless optogenetics to accelerate the *in vivo* applications of GECAs under physiological conditions, with an emphasis on integrating near-infrared (NIR) light-excitable upconversion nanoparticles (UCNPs) and bioluminescence with optogenetics.

Graphical Abstract

*MANUSCRIPT CORRESPONDENCE: J Jing TEL +1-713-677-7411; jing@tamu.edu; Y Zhou TEL +1-713-677-7483; yubinzhou@tamu.edu, Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, 2121 W Holcombe Blvd, Houston, TX 77030, USA.

Conflict of interest statement

The authors have no competing interests or conflict of interest to declare.



Keywords

optogenetics; Calcium signaling; protein design and engineering; LOV2; CRY2; Near-infrared light; upconversion nanoparticles; genetically-encoded calcium channel actuators; immune response; neuromodulation; CRISPRa; NFAT; synthetic biology; Ion channel

1. Introduction

Ca^{2+}, as a signal for life and death and secondary messenger, controls a myriad of cellular events, including muscle contraction, neurotransmitter release, lymphocyte activation, gene expression and metabolism[1–3]. In mammalian cells, the intracellular Ca^{2+} concentration is tightly regulated through coordinated actions of a repertoire of Ca^{2+}-signaling components, including G-protein coupled receptors (GPCRs), the receptor tyrosine kinases (RTKs), various Ca^{2+} channels and transporters/exchangers, and intracellular Ca^{2+} sensing and buffering proteins[1–3]. Upon engagement of membrane receptors by ligands or antigens, phospholipase C gamma (PLCγ) is phosphorylated and activated to catalyze the hydrolysis of phosphatidylinositol 4,5-biphosphate ($PI(4,5)P_2$), yielding two important secondary messengers, diacylglycerol (DAG) and inositol 1,4,5-triphosphate ($InsP_3$). $InsP_3$ further binds to ER-resident $InsP_3$ receptors to induce Ca^{2+} release from the ER Ca^{2+} store, followed by the activation of store-operated Ca^{2+} entry (SOCE)[2]. SOCE is best exemplified by the Ca^{2+} release-activated Ca^{2+} (CRAC) channel composed of ORAI1 and the stromal interaction molecule 1 (STIM1), and constitutes the primary route of Ca^{2+} entry in non-excitable tissues[4–6]. By taking advantage of naturally-existing photoreceptors derived from microbes, plants or mammals, these signalling events can be recapitulated through optogenetic engineering to achieve tailored function. Compared to pharmacological, chemogenetic and genetic approaches, these genetically-encoded Ca^{2+} channel actuators (GECAs) have the advantages of rapid reversibility, superior spatiotemporal resolution, and non-invasiveness[4]. In this review, we present the latest progress in this endeavor, with a focus on the design principles, kinetic features and caveats to be taken into consideration during the application of these tools. When used in combination with genetically-encoded Ca^{2+} indicators, GECAs permit the simultaneous monitoring and interrogation of Ca^{2+}-modulated physiological processes both *in vitro* and *in vivo*.

2. Engineered photoresponsive GPCRs

As the founding member of the optogenetic field, channelrhodopsin-2 (ChR2) derived from the green alga *Chlamydomonas reinhardtii* has transformed the neuroscience field by enabling precise control of neural circuits with light both *in vitro* and *in vivo*[7–9]. As a cation permeant channel, ChR2 permits the non-selective entry of Na^+, H^+, and Ca^{2+} into the cytosol upon blue-green light stimulation (450–510 nm), thereby depolarizing the plasma membrane (PM) to trigger neuronal firing[10] (Fig. 1a). However, given the over 75-fold higher concentrations of extracellular Na^+ over Ca^{2+} (136–151 mM *versus* 1.4–1.8 mM), the Ca^{2+} permeability of ChR2 under physiological conditions is very low (P_{Ca}/P_{Na}: 0.15)[11], thus greatly hampering its application in non-excitable cells and tissues. One ChR2 variant, designated CatCh for Calcium translocating Channelrhodopsin, harbors a point mutation L132C with the Ca^{2+} permeability enhanced by 1.6-fold (P_{Ca}/P_{NA}: 0.24)[11, 12], but still falls far behind voltage- or store-operated Ca^{2+} channels (P_{Ca}/P_{Na}: > 1000)[6]. More Ca^{2+}-specific ChR2 variants are yet to be developed.

Instead of site-directed mutagenesis, Airan *et al.* explored an alternative approach by generating a series of rhodopsin-GPCR chimeras, designated “OptoXRs”[13], to photo-manipulate intracellular second messengers, including cAMP and Ca^{2+}. In a typical OptoXR, the intracellular loops of rhodopsin were replaced with the intracellular regions of G_q-coupled adrenergic receptors (Fig. 1b). Upon photostimulation at 504 nm, OptoXR evoked cytosolic Ca^{2+} elevation via the activation of the PLC pathway in HEK cells, and was further applied to photo-control behaviors in awake mice[13]. A limitation of ChR2 variants and OptoXRs is that the broad rhodopsin absorption peak (440–590 nm) may potentially conflict with biosensors used to report the functional consequences of optogenetic tools[14, 15]. In addition, owing to the rapid *trans*-to-*cis* isomerization of the retinal co-factor (tens of ms), sustained Ca^{2+} entry requires constant photostimulation and tends to cause undesired photo-toxicity. The latter concern is partially mitigated by the use of naturally-existing melanopsin (encoded by *OPN4*), a photopigment protein found in human retinal ganglion cells and the iris of mice and primates[16] (Fig. 1c). Melanopsin can generate both sustained and oscillatory Ca^{2+} signals within seconds ($t_{1/2, ON}$: < 10 s) to activate a downstream Ca^{2+}-responsive master transcription factor, the nuclear factor of activated-T cells (NFAT)[17]. Melanopsin has been introduced into mouse embryonic stem cell-derived embryoid bodies (EBs) to generate pacemaker-like activity in cardiomyocytes within EBs following photostimulation[18]. When expressed in non-excitable primary T cells, melanopsin could photo-boost tumor killing in a mouse model of hepatocarcinoma[19]. Similar to the design of OptoXRs, a chimeric receptor, Opto-mGluR6, composed of melanopsin and metabotropic glutamate receptor 6 (mGluR6), has been devised and expressed in retina to restore the vision of blind mice with retinal degeneration[20]. One potential caveat associated with these GPCR-based optogenetic actuators is the paralleled stimulation of the DAG/PKC pathway following PLC activation, which could cause non-Ca^{2+}-related functional crosstalk to complicate data interpretation.

3. Photoactivatable RTKs

Similar to GPCR, the engagement of RTKs by their cognate ligands often leads to receptor multimerization to activate the PLCγ pathway for Ca^{2+} mobilization. To faithfully recapitulate this process, optical multimerizers, including *Arabidopsis* cryptochrome 2 (CRY2), the light-oxygen-voltage domains (LOV) of aureochorme, and *Deinococcus* phytochrome BphP (DrBphP), have been fused to the catalytic intracellular kinase domain of RTKs to generate Opto-RTKs[21–24] (Fig. 1d). Most engineering efforts have been devoted to two RTKs, the fibroblast growth factor receptor 1 (FGFR1)[21, 22] and the tropomyosin-related kinase (Trk)[24]. Upon light stimulation, the photosensitive moieties undergo dimerization or oligomerization to drive the activation of the intracellular kinase domain of RTKs. Repetitive Ca^{2+} signals can be generated by switching on and off blue light for CRY2 or LOV-based tools[21–24], or toggling between near-infrared (780 nm) and far-red light (660 nm) for the case of DrBphP[25]. Opto-RTKs can thus be applied to control intracellular Ca^{2+} signals using both visible and non-visible light. The use of NIR light further enables multiplexed experiments using fluorescent probes and biosensors in the blue-green range without activation of the engineered receptors. Opto-RTKs, however, are fraught with the lack of Ca^{2+} signaling fidelity because of simultaneous activation of multiple downstream pathways. For example, aside from the Ca^{2+}-associated PLC pathway, photostimulation of Opto-FGFR1 could co-activate proliferation-and survival-related RAS-MAPK, PI3K-AKT and STAT-dependent signaling cascades[21–24]. Extra cautions, therefore, have to be taken when using Opto-RTKs to interrogate Ca^{2+}-regulated signaling events.

4. Photoswitchable Ca^{2+} releaser

Optogenetic engineering has also been extended to intracellular Ca^{2+} sensor proteins, such as the four EF-hand-containing calmodulin. A photoactivatable Ca^{2+} releaser (PACR) has been generated via optimized insertion of LOV2 into an engineered calmodulin-M13 fusion protein (CaM-M13) (Fig. 1e). In the dark, PACR interacts with Ca^{2+} with a dissociation constant (K_d) of approximately 16 nM. Upon blue light stimulation, structural changes within LOV2 disrupt the CaM-M13 interaction, which in turn causes Ca^{2+} release from PACR because of a pronounced reduction in the affinity for Ca^{2+} (K_d = 3.75 μM)[26], thereby leading to an elevation of $[Ca^{2+}]_{cyto}$. Even though highly specific for Ca^{2+}, the application of PACR faces three drawbacks: (i) the limited Ca^{2+} releasing capability (<90 nM increase of $[Ca^{2+}]_{cyto}$) and (ii) cell-to-cell variations because of varying levels of PACR expression; and (iii) the potential perturbation to resting cytosolic Ca^{2+} level and complications from other CaM-regulated targets.

5. Optogenetic actuators engineered from the CRAC channel

During SOCE activation, STIM1 dynamically couples to ORAI1 via two major molecular steps (Fig. 1f–g): (i) the initiation of STIM1 activation via Ca^{2+}-depletion induced oligomerization of the luminal domain containing the EF-SAM domain; (ii) inside-out signal propagation toward the STIM1 cytoplasmic domain (STIM1ct) to overcome an intramolecular inhibition primarily mediated by the interaction between the coiled-coil 1 region (CC1) and the STIM-Orai activating domain (SOAR)[2, 5, 6, 27]. Two major optogenetic

engineering approaches, using either CRY2 or LOV2 photosensory domains derived from plants, have been applied to reconstruct these key molecular steps to enable optical control of Ca^{2+} influx through CRAC channels (Fig. 1g).

5.1 CRY2-based OptoSTIM1 and variants

To recapitulate EF-SAM oligomerization during initiation of SOCE, the luminal and transmembrane domains of STIM1 have been replaced by the photolyase-homology region (PHR) of CRY2 to yield OptoSTIM1[28], which has an activation kinetics of <1 min in response to blue light stimulation and a deactivation kinetics of around 4–6 min upon the withdrawal of light (Fig 1g and Table 1). Very recently, two further improved versions, monster-OptoSTIM1 (monSTIM1) and enhanced OptoSTIM1 (eOS1), have been generated to enhance the light sensitivity and dynamic range of Ca^{2+} response by 55-fold and 5~10-fold, respectively. The deactivation half-life of the OptoSTIM1 toolset generally ranges from 4 to 7 min[28, 29]. For eOS1, it takes about 10 min to return to the basal level[30]. In addition to CRY2-mediated homo-oligomerization, the fusion of optical dimerizers, made of iLID/SspB or CRY2/CIBN, with STIM1ct can likewise photo-trigger Ca^{2+} influx in mammalian cells, with the activation and deactivation half-lives falling in the range of ~30 sec and 40–190 sec, respectively[31]. Furthermore, ER-tethered versions were developed to manipulate membrane contact site (MCS) assembly at ER-PM junctions and induce localized Ca^{2+} influx ($t_{1/2,\ on}$ = 37.8 ± 5.3 s and $t_{1/2,\ off}$ = 97.5 ± 16.5 s) in response to blue light stimulation[31]. These tools set the stage for more physiologically-relevant mimicry of native STIM1 puncta formation and Ca^{2+} microdomain generation.

5.2 Opto-CRAC series and other LOV2-based GECAs

To mimic the intramolecular autoinhibition of STIM1 mediated by the juxtamembrane CC1 and SOAR, CC1 was replaced by LOV2 and various STIM1ct fragments were optimized to generate LOV2-STIM1ct chimeras (Opto-CRAC[31, 32], BACCS[33], and LOVS1K[34]). These LOV2-based GECAs enable more rapid and readily reversible control of Ca^{2+} signals, with the activation kinetics ranging from 10 sec to 30 sec and the deactivation half-lives in the range of 30–50 sec (Table 1). In these chimeric constructs, LOV2 cages the fused STIM1ct fragment in a largely inactive conformation presumably through steric hindrance. Upon photostimulation, the photochemical reaction within LOV2 leads to the unfolding of the C-terminal Jα helix to unleash the caged STIM1ct fragments, thereby exposing the SOAR/CAD region to engage and gate ORAI Ca^{2+} channels in the PM (Fig. 1g). Opto-CRAC[32] or BACCS variants[33], either anchored to PM or using STIM1ct fragments derived from species other than human and rodent (fruit fly[33], chicken[35], and zebrafish[36]) further diversify the kinetic properties of the LOV2-based GECAs. In addition, these LOV2-based GECAs can be directly fused to ORAI1 channels, in the form of a single or two copies[32, 35], to enable optogenetic control of Ca^{2+} signaling in cells or tissues with no or little expression of endogenous ORAI proteins.

Collectively, compared to Opto-GPCRs and Opto-RTKs, STIM1-based GECAs directly gate one of the most Ca^{2+}-selective channels, the ORAI protein family, and therefore, retain high Ca^{2+} selectivity over other cations[32]. Nonetheless, one has to bear in mind the caveat that STIM1, and presumably STIM1-derived GECAs too, might cross-talk with other types of

Ca^{2+} channels (e.g., certain types of transient receptor potential (TRP) channels and voltage gated Ca^{2+} channels) in selected cell types.

6. Genetically-encoded photoswitchable inhibitors of Ca^{2+} signaling

Complementary to the GECA toolkit, two optogenetic tools capable of suppressing Ca^{2+} entry and/or ER Ca^{2+} release have been generated. To develop a light-inducible OFF-switch for voltage-gated Ca^{2+} (Ca_V) channel, Ma *et al.* developed an optogenetic construct, termed OptoRGK[37] (Fig. 2a), by using a light-sensitive heterodimerization system (iLID/SspB) to control the subcellular localization of a small G protein, $Rem_{1–266}$. Rem belongs to the RGK family of G proteins and functions as negative regulator of Ca^{2+} entry mediated by Ca_V channels in excitable cells. OptoRGK has been successfully used to perturb Ca^{2+} oscillations in HL-1 cardiomyocytes, and shows promise to rescue cardiac arrhythmia[37]. In a second case, Hannanta-anan *et al.* utilized a similar strategy using the CRY2/CIBN optical dimerizer to recruit regulators of G-protein signaling (RGS) toward PM, thereby reducing the lifespan of GTP-bound α-subunits to terminate agonist-evoked calcium oscillations via inhibition of $G\alpha_q$-mediated downstream signaling (including Ca^{2+} mobilization from internal stores) in response to blue light stimulation[38] (Fig. 2b). Complementary to the genetic approach, photopharmacological tools building upon azopyrazoles have also been invented to selectively suppress CRAC channel activity in a light-dependent manner[39].

7. Applications of GECAs and wireless optogenetics

Given the strict Ca^{2+} selectivity and high signalling fidelity, STIM1-derived GECAs have been used *ex vivo* to control Ca^{2+}/NFAT-dependent gene expression (Fig. 3a) and cytokine production in cells of the immune system (Fig. 3b). When coupled with the CRISPR activation (CRISPRa) technique[40], Opto-CRAC can further be repurposed to control the expression of endogenous genes. The calcium-responsive transcriptional reprogramming tool (CaRROT)[36], made of NFAT1–460-dCas9-VP64, has been devised to serve this purpose, which allows precise control of transcriptional outputs at user-defined genomic loci, thus expanding the Ca^{2+}-responsive effectors to almost any endogenous genes (Fig. 3c). Moving beyond *in vitro* applications, recent studies have reported encouraging progress in the use of non-opsin-based optogenetics in living animals.

7.1 Neuromodulation in awake mice

A bottleneck with the existing optogenetic approach is a situation where the depth of tissue penetration of visible light is shallow (<2–3 mm)[41], requiring optogenetic protocols to use invasive fiber optic probes or microLED to deliver visible light (in the range of 400–500 nm) into tissues (Fig. 3d). These invasive procedures are technically demanding, tend to elicit inflammatory responses, and remain incompatible with chronic experiments. To overcome these issues, monSTIM1 was generated by using a hyper-sensitive CRY2 variant with one mutation and nine additional amino acids in the C-terminus of $CRY2_{PHR}$ (CRY_{2E281A}-A9) to boost its clustering capability. The new construct displayed 55-fold increase in light-sensitivity compared to the parental OptoSTIM1[29] (Fig 1g). Hence, monSTIM1 was utilized to manipulate Ca^{2+} signaling and activate Ca^{2+}-dependent gene expression in deep-brain regions (hippocampal and thalamic regions), and to boost the social learning capability of

awake mice without invasive surgical procedures[29] (Fig. 3d). However, upon non-invasive light stimulation, how deep the tissues can be reached to effectively photoactivate monSTIM1 remains elusive. Furthermore, blue light emitting at 450–470 nm may generate local heat and oxidative stress while STIM1 is also sensitive to both heat and ROS[27]. It remains to be rigorously validated if the observed behavior changes are indeed caused by Ca^{2+} signals per se rather than undesired side effects of photostimulation. Another attractive strategy to overcome this bottleneck is to exploit optogenetic modules that can be switched on and off by NIR that has a greater depth of tissue penetration (up to centimeters)[42].

7.2 Immunomodulation in living animals

STIM1-based GECAs have been successfully applied *in vivo* to control the motility and function of cells of the immune system. eOS1 has been employed to manipulate Ca^{2+} signals in single T cells and photo-tune T cell migration, adhesion and chemokine release in vivo via two-photon-based photoactivation[30]. Although two-photon optogenetics provide a better spatial specificity and deep penetration in scattering tissue[43], potential heat generation during photostimulation and special equipment requirements make it non-ideal for daily use in the laboratory setting, let alone further clinical applications. A wireless nano-optogenetic approach based on NIR-excitable upconversion nanoparticles (UCNPs; Fig. 3e) has been developed to accelerate in vivo application using immune cells. The most significant advantage of UCNPs is their unusual inverse excitation and emission profiles: UCNPs are excited using low power (mW) and deep tissue-penetrating (1–2 cm) NIR light. This low energy input is efficiently converted to a higher energy output emission at diverse shorter wavelengths, including blue light emission at 470 nm that can activate most optogenetic constructs (Fig. 3e). Since UCNPs can be illuminated from NIR sources located outside the body, they can act as “nano-illuminator relays” to activate optogenetic constructs in a wireless manner. The nano-optogenetic concept has been validated in a dendritic cell-based immunomodulatory therapy against tumor in a mouse mode of melanoma[32] (Fig. 3e).

8. Conclusions

In summary, a variety of optogenetic tools, based on both opsin or non-opsin photoreceptors, have been crafted to enable remote control of intracellular Ca^{2+} signaling both *in cellulo* and in living organisms (summarized in Table 1). Most tools are engineered from membrane receptors (GPCRS and RTKs) and signaling proteins (CaM, G proteins and STIM1) that are directly or indirectly involved in Ca^{2+} signaling. Among these tools, GECAs inspired by STIM1 are regarded as the most Ca^{2+} selective and have been most widely applied to modulate the central nervous system and the immune system. The *in vivo* application of these tools will likely be accelerated by developing NIR-responsive optogenetic modules and combining existing tools with nanotechnologies or bioluminescence to enable in vivo optogenetics. The feasibility of bioluminescence-aided optogenetic stimulation (Fig. 3f), at the cost of partially sacrificing the spatial resolution, has been validated in several recent studies[44–46]. These innovative platforms might represent alternative solutions for future wireless optogenetics.

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References

1. Clapham DE Calcium signaling. Cell 131, 1047–1058 (2007). [PubMed: 18083096]
2. Hogan PG, Lewis RS & Rao A Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. Annu Rev Immunol 28, 491–533 (2010). [PubMed: 20307213]
3. Berridge MJ, Bootman MD & Roderick HL Calcium signalling: dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 4, 517–529 (2003). [PubMed: 12838335]
4. Ma G et al. Optogenetic toolkit for precise control of calcium signaling. Cell Calcium 64, 36–46 (2017). [PubMed: 28104276]
5. Nguyen NT et al. CRAC channel-based optogenetics. Cell Calcium 75, 79–88 (2018). [PubMed: 30199756]
6. Prakriya M & Lewis RS Store-Operated Calcium Channels. Physiol Rev 95, 1383–1436 (2015). [PubMed: 26400989]
7. Nagel G et al. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A 100, 13940–13945 (2003). [PubMed: 14615590]
8. Boyden ES, Zhang F, Bamberg E, Nagel G & Deisseroth K Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8, 1263–1268 (2005). [PubMed: 16116447] *A pioneering study describing ChR2 as the founding member of the optogenetic field, which revolutionizes the way neuroscientists to interrogate neuronal activities and dissect neural circuits.
9. Zhang F et al. Multimodal fast optical interrogation of neural circuitry. Nature 446, 633–639 (2007). [PubMed: 17410168]
10. Fenno L, Yizhar O & Deisseroth K The development and application of optogenetics. Annu Rev Neurosci 34, 389–412 (2011). [PubMed: 21692661]
11. Beck S et al. Synthetic Light-Activated Ion Channels for Optogenetic Activation and Inhibition. Front Neurosci 12, 643 (2018). [PubMed: 30333716]
12. Kleinlogel S et al. Ultra light-sensitive and fast neuronal activation with the Ca(2)+-permeable channelrhodopsin CatCh. Nat Neurosci 14, 513–518 (2011). [PubMed: 21399632]
13. Airan RD, Thompson KR, Fenno LE, Bernstein H & Deisseroth K Temporally precise in vivo control of intracellular signalling. Nature 458, 1025–1029 (2009). [PubMed: 19295515] *The first study describes the design of rhodopsin-GPCR chimeras, termed optoXRs, to remotely control GPCR signaling with high spatiotemporal precision.
14. Deisseroth K & Hegemann P The form and function of channelrhodopsin. Science 357 (2017).
15. Shichi H Spectrum and purity of bovine rhodopsin. Biochemistry 9, 1973–1977 (1970). [PubMed: 5442166]
16. Hankins MW, Peirson SN & Foster RG Melanopsin: an exciting photopigment. Trends Neurosci 31, 27–36 (2008). [PubMed: 18054803]
17. Hannanta-Anan P & Chow BY Optogenetic Control of Calcium Oscillation Waveform Defines NFAT as an Integrator of Calcium Load. Cell Syst 2, 283–288 (2016). [PubMed: 27135540]
18. Beiert T, Bruegmann T & Sasse P Optogenetic activation of Gq signalling modulates pacemaker activity of cardiomyocytes. Cardiovasc Res 102, 507–516 (2014). [PubMed: 24576953]
19. Zhao B et al. An Optogenetic Controllable T Cell System for Hepatocellular Carcinoma Immunotherapy. Theranostics 9, 1837–1850 (2019). [PubMed: 31037142]
20. van Wyk M, Pielecka-Fortuna J, Lowel S & Kleinlogel S Restoring the ON Switch in Blind Retinas: Opto-mGluR6, a Next-Generation, Cell-Tailored Optogenetic Tool. PLoS Biol 13, e1002143 (2015). [PubMed: 25950461]

21. Kim JM, Lee M, Kim N & Heo WD Optogenetic toolkit reveals the role of Ca2+ sparklets in coordinated cell migration. Proc Natl Acad Sci U S A 113, 5952–5957 (2016). [PubMed: 27190091]
22. Kim N et al. Spatiotemporal control of fibroblast growth factor receptor signals by blue light. Chem Biol 21, 903–912 (2014). [PubMed: 24981772] * One of the first studies to describe the design of optoFGFR1 to control intracellular Ca2+ signals and other downstream effectors associated with RTK signaling.
23. Grusch M et al. Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. EMBO J 33, 1713–1726 (2014). [PubMed: 24986882] * One of the first studies to describe the use of LOV domains from aureochrome to photo-manipulate RTK signaling.
24. Chang KY et al. Light-inducible receptor tyrosine kinases that regulate neurotrophin signalling. Nat Commun 5, 4057 (2014). [PubMed: 24894073]
25. Leopold AV, Chernov KG, Shemetov AA & Verkhusha VV Neurotrophin receptor tyrosine kinases regulated with near-infrared light. Nat Commun 10, 1129 (2019). [PubMed: 30850602] * The authors describe the use of NIR light-controllable optogenetic modules derived from a bacterial phytochrome to modulate RTK signaling.
26. Fukuda N, Matsuda T & Nagai T Optical control of the Ca2+ concentration in a live specimen with a genetically encoded Ca2+-releasing molecular tool. ACS Chem Biol 9, 1197–1203 (2014). [PubMed: 24625002]
27. Soboloff J, Rothberg BS, Madesh M & Gill DL STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol 13, 549–565 (2012). [PubMed: 22914293]
28. Kyung T et al. Optogenetic control of endogenous Ca(2+) channels in vivo. Nat Biotechnol 33, 1092–1096 (2015). [PubMed: 26368050] * This study describes the first development of CRY2-STIM1 chimeras to enable optical control of Ca2+ influx by taking advantage of endogenous ORAI channels into mammalian cells. The authors further apply the tool to remotely modulate neuronal activities both in vitro and in vivo.
29. Kim S et al. Non-invasive optical control of endogenous Ca(2+) channels in awake mice. Nat Commun 11, 210 (2020). [PubMed: 31924789]
30. Bohineust A, Garcia Z, Corre B, Lemaitre F & Bousso P Optogenetic manipulation of calcium signals in single T cells in vivo. Nat Commun 11, 1143 (2020). [PubMed: 32123168] * This study describes an improved version of optoSTIM1 termed eOS1 to control the migration, adhesion and chemokine production in single T cells by utilizing two-photon photoactivation.
31. Ma G et al. Optogenetic engineering to probe the molecular choreography of STIM1-mediated cell signaling. Nat Commun 11, 1039 (2020). [PubMed: 32098964] * A comprehensive study illustrating the use of optogenetic engineering approaches to recapitulate key signaling events during store-operated calcium entry, which validates oligomerization and conformational switch as two essential steps to drive CRAC channel activation.
32. He L et al. Near-infrared photoactivatable control of Ca(2+) signaling and optogenetic immunomodulation. Elife 4 (2015).*One of the first studies demonstrating a LOV2-fusion strategy to mimic STIM1 intramolecular trapping. The first proof-of-concept study demonstrates the feasibility of wireless nano-optogenetics to modulate the immune system in living animals.
33. Ishii T et al. Light generation of intracellular Ca(2+) signals by a genetically encoded protein BACCS. Nat Commun 6, 8021 (2015). [PubMed: 26282514] *One of the first studies illustrating the design of LOV2-STIM1 chimeras to reversibly control Ca2+ signaling.
34. Pham E, Mills E & Truong K A synthetic photoactivated protein to generate local or global Ca(2+) signals. Chem Biol 18, 880–890 (2011). [PubMed: 21802009]
35. Li A et al. Calcium oscillations coordinate feather mesenchymal cell movement by SHH dependent modulation of gap junction networks. Nat Commun 9, 5377 (2018). [PubMed: 30560870]
36. Nguyen NT, He L, Martinez-Moczygemba M, Huang Y & Zhou Y Rewiring Calcium Signaling for Precise Transcriptional Reprogramming. ACS Synth Biol 7, 814–821 (2018). [PubMed: 29489336] *This study integrates genetically-encoded calcium channel actuators with the CRISPR-based transcriptional activation technique to generate CaRROT, which extends Ca2+-controllable gene transcription toward any genomic loci in a light-dependent manner.

37. Ma G et al. Optogenetic Control of Voltage-Gated Calcium Channels. Angew Chem Int Ed Engl 57, 7019–7022 (2018). [PubMed: 29569306] *One of the few studies describing an OFF-switch to photo-suppress calcium influx mediated by voltage-gated calcium channel in excitable cells.

38. Hannanta-Anan P & Chow BY Optogenetic Inhibition of Galphaq Protein Signaling Reduces Calcium Oscillation Stochasticity. ACS Synth Biol 7, 1488–1495 (2018). [PubMed: 29792810]

39. Yang X et al. Optical Control of CRAC Channels Using Photoswitchable Azopyrazoles. J Am Chem Soc 142, 9460–9470 (2020). [PubMed: 32330031]

40. Qi LS et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152, 1173–1183 (2013). [PubMed: 23452860]

41. BASHKATOV AN, GENINA EA & TUCHIN VV OPTICAL PROPERTIES OF SKIN, SUBCUTANEOUS, AND MUSCLE TISSUES: A REVIEW. Journal of Innovative Optical Health Sciences 04, 9–38 (2011).

42. Redchuk TA, Omelina ES, Chernov KG & Verkhusha VV Near-infrared optogenetic pair for protein regulation and spectral multiplexing. Nat Chem Biol 13, 633–639 (2017). [PubMed: 28346403]

43. Oron D, Papagiakoumou E, Anselmi F & Emiliani V Two-photon optogenetics. Prog Brain Res 196, 119–143 (2012). [PubMed: 22341324]

44. Tan P, He L, Han G & Zhou Y Optogenetic Immunomodulation: Shedding Light on Antitumor Immunity. Trends Biotechnol 35, 215–226 (2017). [PubMed: 27692897]

45. England CG, Ehlerding EB & Cai W NanoLuc: A Small Luciferase Is Brightening Up the Field of Bioluminescence. Bioconjug Chem 27, 1175–1187 (2016). [PubMed: 27045664]

46. Kim CK, Cho KF, Kim MW & Ting AY Luciferase-LOV BRET enables versatile and specific transcriptional readout of cellular protein-protein interactions. Elife 8 (2019).*This study demonstrates the feasibility of combining NanoLuc-catalyzed bioluminescence with LOV2-based optogenetic devices to photo-stimulate cell signaling.

47. Ye H, Daoud-El Baba M, Peng RW & Fussenegger M A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. Science 332, 1565–1568 (2011). [PubMed: 21700876] *A pioneering study describing the use of melanopsin as an optogenetic tool to control Ca2+ signaling and blood glucose metabolism in awake mice.

48. Kumbalasiri T, Rollag MD, Isoldi MC, Castrucci AM & Provencio I Melanopsin triggers the release of internal calcium stores in response to light. Photochem Photobiol 83, 273–279 (2007). [PubMed: 16961436]

Highlights

- Overview of optogenetic tools to control Ca^{2+}-modulated physiological processes
- Engineering strategies for genetically-encoded Ca^{2+} channel actuators (GECAs)
- Guidelines for selecting appropriate GECAs to achieve tailored function
- Insights into potential solutions for wireless optogenetics in vivo

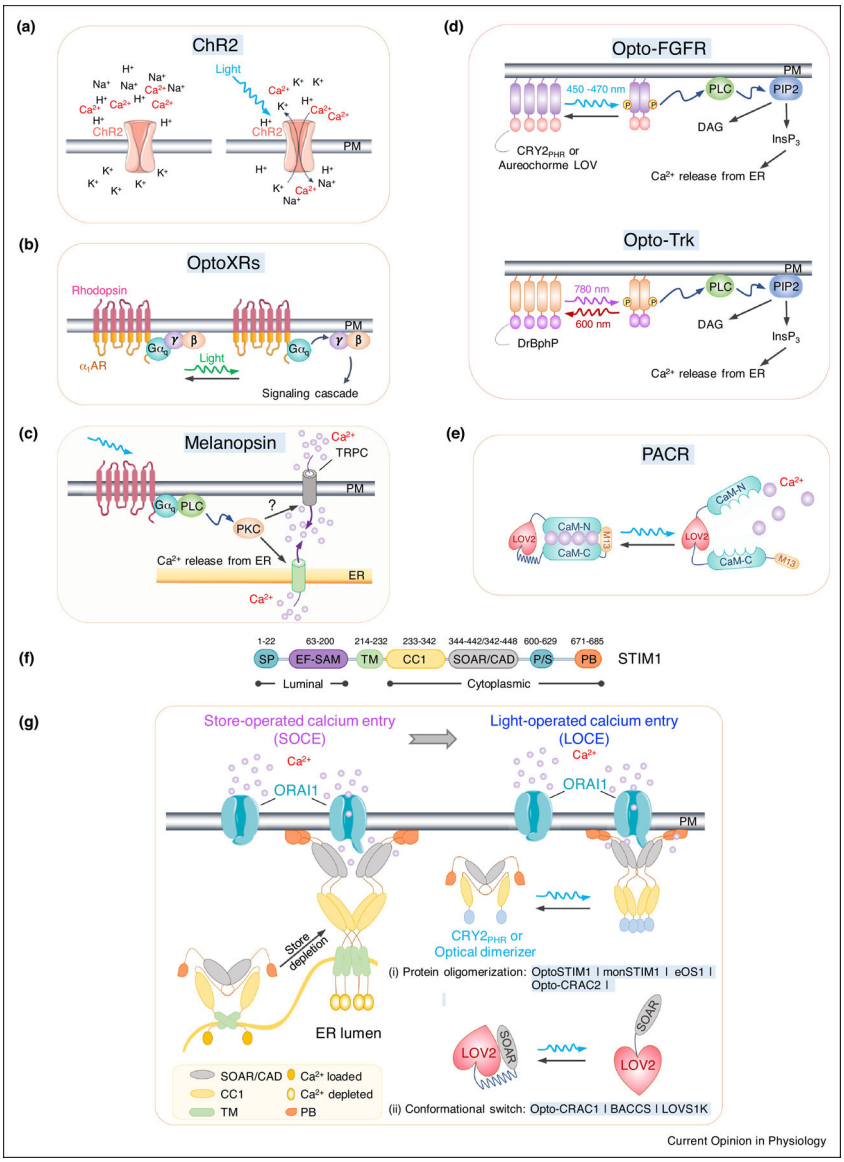

Figure 1. Optogenetic tools for remote activation of intracellular Ca^{2+} signaling.

a. Channelrhodopsin-2 (ChR2) as a cation-permeant channel is non-selective toward H^+, K^+, Na^+, and Ca^{2+}.

b. OptoXRs as light-sensing rhodopsin-GPCR chimeras. The extracellular and TM regions are derived from a light-sensitive rhodopsin, while intracellular regions from different adrenergic receptors (β_2AR or α_1AR).

c. Blue light-mediated isomerization of the light-absorbing retinal cofactor triggers the conformation change of melanopsin (OPN4) and subsequent activation of $G\alpha_q$-phospholipase C (PLC) to induce Ca^{2+} influx via TRPC channel.

d. Opto-RTKs as photoactivatable membrane-anchored receptor tyrosine kinases. The intracellular domain of RTKs (FGFR or TrK), is fused to CRY2 or other optical dimerizers, thereby enabling light-inducible clustering to activate downstream signaling, including the PLC pathway and subsequent Ca^{2+} mobilization.

e. LOV2-CaM-M13 hybrid protein as a photoactivatable Ca^{2+} releaser (PACR). Photostimulation results in the release of bound Ca^{2+} into the surrounding environment.
f. Domain architecture of STIM1. SP, signal peptide; EF, EF-hand Ca^{2+}-binding motif; SAM, sterile alpha motif; TM, transmembrane domain; CC1, coiled-coil domain 1; SOAR, STIM-Orai activating region; PS, proline-serine rich domain; PB, polybasic domain.
g. Genetically-encoded Ca^{2+} channel actuators (GECAs) derived from STIM1. (*Left*) schematic illustrating functional STIM1-ORAI1 coupling in response to store depletion. Ca^{2+} depletion within the ER lumen induces oligomerization of the luminal EF-SAM domain to initiate STIM1 activation and triggers conformational switch to expose SOAR/CAD and the C terminal PB domain. Next, STIM1 undergoes further oligomerization and migration toward the PM, a process facilitated by the association between SOAR/CAD and ORAI1, as well as the interaction between positively-charged PB and negatively-charged phosphoinositides embedded in PM. SOAR/CAD is responsible for direct engagement and activation of ORAI channels to mediate Ca^{2+} influx. (*right*) Optogenetic engineering to convert store-operated Ca^{2+} entry (SOCE) into light-operated Ca^{2+} entry (LOCE). (i) A protein oligomerization-based engineering strategy using various optical dimerizers (Opto-CRAC2) and CRY2-STIM1 fusion proteins (OptoSTIM1, monSTIM1, and eOS1). Upon light illumination, CRY2 or optical dimerizers undergoes oligomerization, mimicking Ca^{2+} -depletion induced EF-SAM multimerization in ER lumen, to trigger STIM1 activation and the subsequent opening of ORAI Ca^{2+} channels in the PM. (ii) The design of GECAs based on LOV2 conformational switch (Opto-CRAC1, LOVS1K or BACCS), in which the CC1-SOAR interaction-mediated intramolecular autoinhibition is mimicked by LOV2-SOAR fusion in the dark. Upon photostimulation, LOV2 undergoes conformational changes to expose SOAR/CAD, which engages PM-resident ORAI Ca^{2+} channel to induce Ca^{2+} influx.

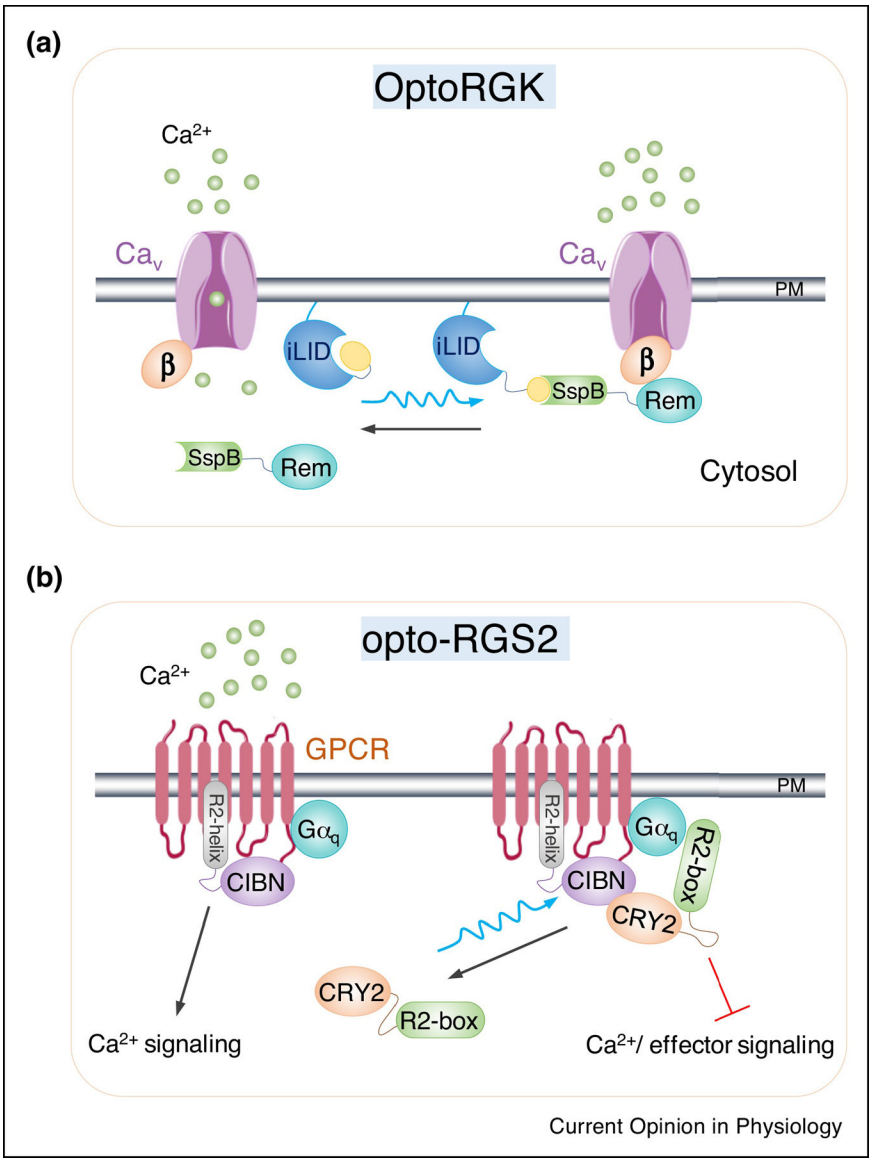

Figure 2. Genetically-encoded photoswitchable inhibitors of intracellular Ca^{2+} signaling.

a. Cartoon illustration of OptoRGK to photo-control Ca_V channel activity in cardiomyocytes. An optical dimerizer pair made of LOV2-SsrA (iLID) and SspB is utilized to induce the translocation of Rem1 core domain (a member of the RGK family of GTP-binding proteins) from the cytosol to PM, where it engages the auxiliary beta subunits of Ca_V channel to suppress its activity upon photostimulation.

b. Design of optogenetic regulators of G-protein signaling (Opto-RGS2). In this design, RGS2 is split into two components, including the catalytic box (R2-box) which is critical for inhibition of $G\alpha_q$ signaling and the N-terminal amphipathic helix (R2-helix). The two split parts are fused with either $CRY2_{PHR}$ or its binding partner CIBN. In the dark, R2-box is distributed in the cytoplasm. Upon blue light illumination, the forced dimerization between CIBN-R2-helix and CRY2-R2-box triggers reconstitution of the active protein and

subsequently inhibit $G\alpha_q$ signaling and terminates Ca^{2+} release originated from GPCR signaling.

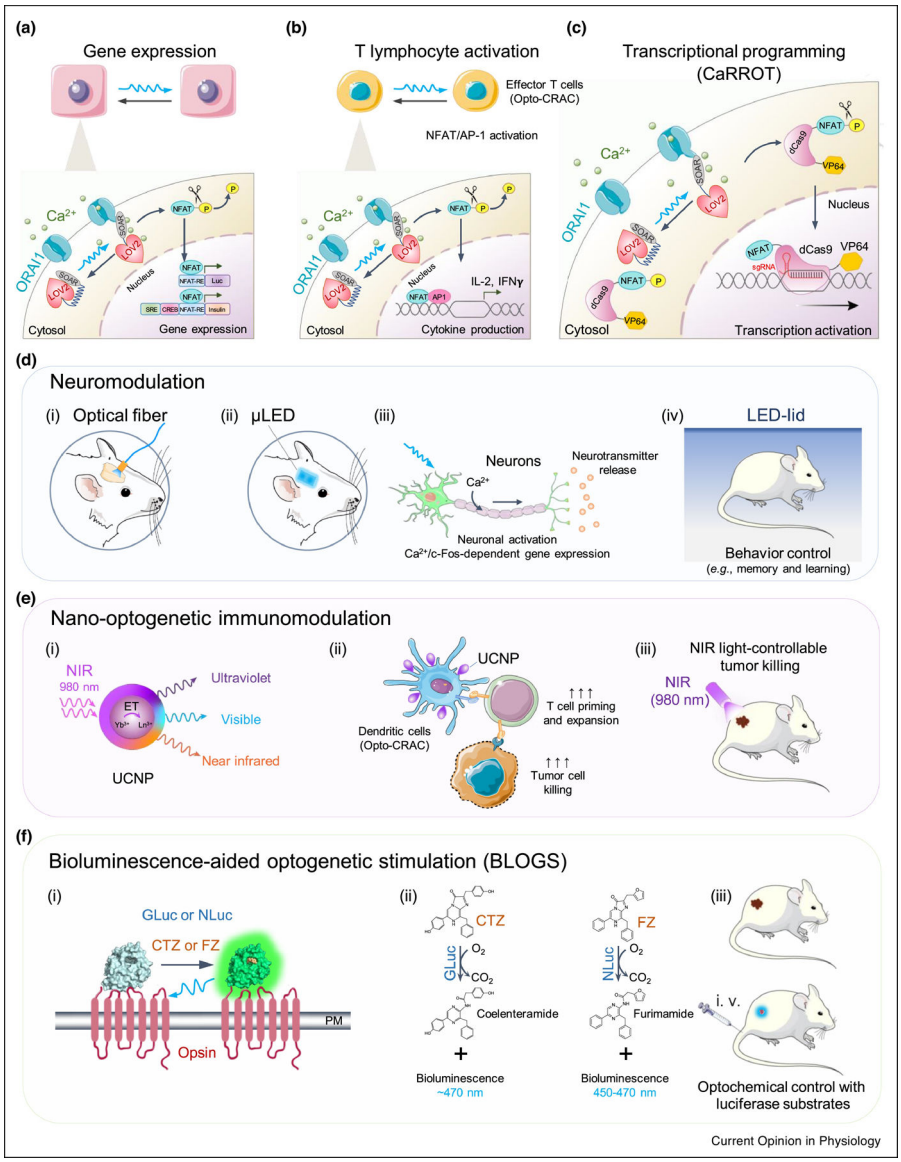

Figure 3. Applications of GECAs and potential solutions for wireless optogenetics.

a. GECAs (e.g., Opto-CRAC) utilized to control Ca^{2+}-dependent gene expression using synthetic response elements (RE) derived from various Ca^{2+}-responsive transcription factors. NFAT, nuclear factor of activated-T cells; CREB: cAMP response element-binding domain; SRE, serum response element.

b. GECAs used to control Ca^{2+}/NFAT-driven cytokine production in T cells in the presence of co-stimulatory signals to activate activator protein 1 (AP-1).

c. Coupling GECAs with CRISPRa to enable light-tunable transcriptional programming. Upon blue light illumination, Opto-CRAC1 induces cytosolic Ca^{2+} influx, which in turn activates CaM/calcineurin to dephosphorylate the N-terminal non-DNA binding fragment of NFAT ($NFAT_{1–460}$) and eventually drive the nuclear entry of the fusion protein ($NFAT_{1–460}$-dCas9-VP64) – a calcium-responsive transcriptional reprogramming tool (designated as CaRROT). In the presence of small guide RNAs (sgRNAs), CaRROT can be precisely

targeted to the promoter regions of any desired genomic loci to photo-tune the expression of endogenous genes.

d. Optogenetic neuromodulation with GECAs (e.g., OptoSTIM1 or monSTIM1). Blue light can be delivered into a specific brain region via implantation of optical fiber (i) or μLED (ii). Photoactivatable Ca^{2+} influx can lead to induction of Ca^{2+}-responsive gene expression (e.g., c-Fos) in both neurons and astrocytes in deep-brain regions such as hippocampal and thalamic regions (iii), thereby modulating the behavior (e.g., social fear learning) in awake mice (iv).

e. A nano-optogenetic platform for wireless optogenetic immunomodulation in a mouse model of melanoma. (i) schematic illustration of the core/shell structure of lanthanide doped upconversion nanoparticles (UCNPs), which act as nanoilluminator to emit visible light upon near-infrared irradiation (NIR); (ii-iii) NIR light-controllable activation of Ca^{2+} influx in dendritic cells (DCs) expressing Opto-CRAC1 could boost antigen presentation and DC maturation to more efficiently prime T cells in the draining lymph nodes, thereby facilitating dendritic cell-based immunomodulatory therapy against melanoma.

f. Bioluminescence-aided optogenetic stimulation (BLOGS). (i) Cartoon illustrating the photoactivation of a chimeric luminopsin (LMO) receptor comprising a microbial opsin and Gaussia luciferase (GLuc) or NanoLuc luciferase (NLuc) in the presence of their cognate substrates coelenterazine (CTZ) or furimazine (FZ). (ii) Photochemical reactions catalyzed by GLuc or NLuc. (iii) Schematic depiction of wireless optogenetics using BLOGS in mice administered with luciferase substrates.

Table 1.

Summary of optogenetic tools for noninvasive and precise control Ca^{2+} signals in mammals.

Names	Engineering templates	Photosensitive components	Stimulation	Activation ($t_{1/2}$, sec)	Deactivation ($t_{1/2}$, sec)	Cell / Tissue Types	Refs
Photoswitchable GPCRs and G proteins							
ChR2	Channelrhodopsin-2	opsin	Blue light	~0.002	~0.01	Multiple mammalian cells, model organism, rodents and primates	7
OptoGPCR / OptoXRs	G_q-coupled α1AR	opsin	Blue/green light	N/A	N/A	HEK293 and mice	13
	Melanopsin	opsin	Blue light	< 10	N/A	Multiple mammalian cells and mice	47, 48
Photoactivatable RTKs							
OptoRTKs	FGFR1$^{398–822}$	CRY2PHR	Blue light	<30	N/A	HeLa/HUVECs	21, 22
	TrkA/B/C					HeLa	24
	TrkA	DrBphP-PCM	NIR	~7	~10	HeLa	25
STIM1-based GECAs							
BACCS variants	STIM1$^{347–448}$	LOV2	Blue light	< 30	~30–60	HEK293T	33
	STIM1$^{347–448}$	2*LOV2					
	ORAI1 + STIM1$^{347–448}$	2*LOV2					
	dOrai + dmStim$^{413–514}$	2*LOV2				S2	
LOVS1K	STIM1$^{233–450}$	LOV2	Blue light	N/A	N/A	HeLa	34
Opto-CRAC1 variants	Mouse STIM1$^{336–486}$	LOV2	Blue light	23.4 ± 4.2	24.9 ± 4.8	HeLa and multiple cell lines	32
			NIR	<30	<30	BMDCs and mice	
	Human STIM1$^{336–685}$	LOV2	Blue light	36.3 ± 6.4	N/A	HeLa	
	chicken STIM1$^{262–412}$	LOV2	Blue light	~10	<30	Skin mesenchymal cells	35
	zebrafish STIM1$^{341–442}$	LOV2	Blue light	18.7	24.5	HeLa	36
Opto-CRAC2 variants	STIM1$^{233–685}$	iLID/SspB	Blue light	28.5 ± 3.2	48.6 ± 5.4	HeLa	31
	STIM1$^{233–685}$	$CRY2_{PHR}$/CIBN		23.4 ± 2.6	153.0 ± 26.2		
	ER-tethered-STIM1$^{233–685}$	$CRY2_{PHR}$		37.8 ± 5.3	97.5 ± 16.5		
OptoSTIM1 variants	STIM1$^{238–685}$	$CRY2_{PHR}$	Blue light	64.5 ± 4.8	274 ± 23.7	HeLa and multiple cell lines	4, 28
	STIM1$^{238–463}$			43.2 ± 3.8	383 ± 52.3		

Names	Engineering templates	Photosensitive components	Stimulation	Activation ($t_{1/2}$, sec)	Deactivation ($t_{1/2}$, sec)	Cell / Tissue Types	Refs
	$STIM1^{342–685}$			48.2 ± 5.4	344 ± 34.6		
monSTIM1	$STIM1^{238–685}$	$CRY2_{PHR-E281A}$-9A	Blue light	24.8 ± 1.0	513.5 ± 25.8	HEK and neurons and mice	29
eOS1	$STIM1^{238–685}$	$CRY2_{PHR-E490G}$	Blue light	<60	<600	B3Z T cell	30
			Two-photon			*In vivo* $CD8^+$ T dells	
Light-switchable inhibition of Ca^{2+} signaling							
OptoRGK	$Rem^{1–266}$	iLID/SspB	Blue light	3.2 ± 1.0 *	23.0 ± 2.4 *	HEK293; HL-1	37

* cytosol-plasma membrane translocation

Abbreviations: BACCS, blue light-activated Ca^{2+} channel switch; BMDCs, bone marrow-derived DCs; CaM-M13, calmodulin fused with the M13 peptide; ChR2, Channelrhodopsin-2; CIBN, the N-terminal domain of CIB1; CRY2, cryptochrome2; eOS1, enhanced OptoSTIM1; FGFR1, fibroblast growth factor receptor 1; GECA, genetically encoded Ca^{2+} actuator; HUVECs, human umbilical vein endothelial cells; iLID, improved light-induced dimer (LOV2-SsrA); LOV2, light-oxygen-voltage sensing) domain; LOVS1K, LOV2 fused STIM1 fragments (233–450); monSTIM1, monster-OptoSTIM1; PACR, photoactivatable Ca^{2+} releaser; Opto-α_1AR, Optogenetic G_q-coupled α1-adrenergic receptor; Opto-CRAC, Optogenetic Ca^{2+} release-activated Ca^{2+} channel; OptoRGK, Optogenetic Ras-like GTPase Rad/Rem/Gem/Kir; Opto-RGS2, Optogenetic regulators of G-protein signaling; Opto-RTKs, Optogenetic receptor tyrosine kinases; OptoSTIM1, Optogenetic stromal interaction molecule 1; OptoXRs, optogenetic control of intracellular signal transduction; S1, somatosensory cortex; S2, *Drosophila* S2 cells; SP, Signal peptide of STIM1; NIR, near-infrared; TM, transmembrane domain; Trk, tropomyosin-related kinase;