Published in final edited form as:

Cell Calcium. 2017 June ; 64: 36–46. doi:10.1016/j.ceca.2017.01.004.

Optogenetic toolkit for precise control of calcium signaling

Guolin Ma¹, Shufan Wen¹, Lian He¹, Yun Huang^{2,3}, Youjun Wang^{4,*}, and Yubin Zhou^{1,5,*}

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

²Center for Epigenetics and Disease Prevention, Institute of Biosciences and Technology, Texas A&M University, Houston, TX 77030, USA

³Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M University, Bryan, TX 77807, USA

⁴Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing 100875, China

⁵Department of Medical Physiology, College of Medicine, Texas A&M University, Temple, TX 76504, USA

Abstract

Calcium acts as a second messenger to regulate a myriad of cell functions, ranging from short-term muscle contraction and cell motility to long-term changes in gene expression and metabolism. To study the impact of Ca²⁺-modulated ‘ON’ and ‘OFF’ reactions in mammalian cells, pharmacological tools and ‘caged’ compounds are commonly used under various experimental conditions. The use of these reagents for precise control of Ca²⁺ signals, nonetheless, is impeded by lack of reversibility and specificity. The recently developed optogenetic tools, particularly those built upon engineered Ca²⁺ release-activated Ca²⁺ (CRAC) channels, provide exciting opportunities to remotely and non-invasively modulate Ca²⁺ signaling due to their superior spatiotemporal resolution and rapid reversibility. In this review, we briefly summarize the latest advances in the development of optogenetic tools (collectively termed as ‘genetically encoded Ca²⁺ actuators’, or GECAs) that are tailored for the interrogation of Ca²⁺ signaling, as well as their applications in remote neuromodulation and optogenetic immunomodulation. Our goal is to provide a general guide to choosing appropriate GECAs for optical control of Ca²⁺ signaling *in cellulo*, and in parallel, to stimulate further thoughts on evolving non-opsin-based optogenetics into a fully fledged technology for the study of Ca²⁺-dependent activities *in vivo*.

*MANUSCRIPT CORRESPONDENCE TO: Y Wang, Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing 100875, China; Tel: +86-10-5880-6258; wyounjun@bnu.edu.cn. Y Zhou, Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University Health Science Center, 2121 W Holcombe Blvd, Houston, TX 77030, USA; Tel: +1-713-677-7483; yzhou@ibt.tamhsc.edu.

Conflict of interest

The authors state no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Graphical abstract

1. Shedding light on Ca²⁺ signaling

Ca²⁺ acts as a versatile second messenger to regulate a myriad of cellular activities, ranging from short-term reactions occurring within seconds (*e.g.*, muscle contraction and neurotransmitter release) to long-term processes that last for hours or even days (*e.g.*, gene transcription) [1, 2]. The location, amplitude and frequency of Ca²⁺ signals in mammalian cells undergo constant changes to maintain Ca²⁺ homeostasis while meeting the diverse requirements of different Ca²⁺-modulated events. This challenging task is made possible through the coordinated actions of a repertoire of Ca²⁺ signaling components situated on the plasma membrane, across membranous organelles, or in the cytoplasm [2–4]. Over the past decade, the technical leap in Ca²⁺ imaging, propelled by the invention of a growing palette of genetically encoded Ca²⁺ indicators (GECIs), makes it a routine practice in numerous laboratories to monitor Ca²⁺ dynamics and report Ca²⁺-triggered activity in both model cellular systems and living organisms [5–11]. In contrast to the rapid progress in developing optical reporters for Ca²⁺, the quest for genetically encoded optical actuators to deliver Ca²⁺ signals with user-defined spatial and temporal properties remains relatively stagnant.

Earlier attempts to optically control Ca²⁺ signals in mammalian cells can be traced back to the invention of “caged” compounds in the 1980s. The caged substrates could be Ca²⁺ itself [12–17], or other signaling molecules, such as ATP [18], GTP [19], and IP₃ [20–23], that are intimately involved in the mobilization of intracellular Ca²⁺ in mammalian cells (Figure 1A). When shielded from light, the encapsulated biomolecule is trapped in an inert or less active state via chelation or formation of covalent bonds with photolabile “cages” [24–27]. For instance, Ca²⁺ cages can be synthesized by introducing photolabile groups, such as *o*-nitrophenyl (Figure 1A), into commonly used Ca²⁺ chelators (*e.g.*, BAPTA, EDTA or

EGTA) [12–16, 26]. In the dark, Ca^{2+} tightly binds to these modified metal chelators with a high affinity in the range of 5–150 nM [26]. Upon UV illumination, the photolytic products exhibit a dramatic decrease in affinity for Ca^{2+} by >10,000 fold, thereby unleashing the bound Ca^{2+} to produce Ca^{2+} spikes inside cells [12–16, 26]. These photolabile Ca^{2+} -releasing compounds, commercially branded as DM-Nitrophen, NP-EGTA or nitr-5, are often used to trigger or drive Ca^{2+} -dependent ‘ON’ reactions in living cells [28, 29]. Conversely, photoactivatable Ca^{2+} scavengers (*e.g.*, diazo-2) have been devised to chelate free intracellular Ca^{2+} to suppress or terminate Ca^{2+} -dependent activities [30, 31].

Photo-inducible control of Ca^{2+} signaling offers two major advantages over conventional pharmacological approaches. First, the high temporal resolution enables the dissection of kinetic requirements of Ca^{2+} signals during mechanistic studies of Ca^{2+} -dependent ‘ON’ and ‘OFF’ reactions *in cellulo* [20, 30, 31]. For example, the fast release of Ca^{2+} makes it possible to photo-activate the contraction of skeletal muscle fibers within tens of milliseconds, a speed that is five times faster than the most rapid solution change method [32]. Another desirable feature of photo-induced Ca^{2+} and IP_3 uncaging is that the amplitude of chemical signals can be conveniently tuned by varying the intensities of incident light. Second, photorelease technology makes it feasible to conveniently program the spatial profiles of Ca^{2+} signals. Both global and local Ca^{2+} signals can be generated to modulate Ca^{2+} -dependent activities at subcellular precision by applying a focused beam of light on the whole cell or at user-defined areas [25, 33–35]. However, the spatial resolution might be compromised owing to the rapid diffusion of caged compounds in the cytoplasm. Hurdles hampering the application of caged compounds *in vivo* include irreversibility, low delivery efficiency, limited depth of tissue penetration and strong phototoxicity associated with UV irradiation [24–26, 36].

Optogenetics, which combines the use of light and genetics to control cellular activities at high spatiotemporal precision [37], offers an ideal solution to overcome the aforementioned hurdles whilst still preserving the advantages of photorelease technology. Originally designed and most widely adopted to manipulate neuronal activities, optogenetic tools are now gaining wide popularity in biomedical research beyond neuroscience [36, 38–43]. At the heart of this revolutionary technology is the integration of genetically encoded photosensitive modules into cells of living tissues to achieve gain or loss of function of defined cellular events. Several photoactivatable domains and photosensory receptors derived from microbes or plants, including the most well-known channelrhodopsin 2 (ChR2) and its variants, light-oxygen-voltage-sensing domains (LOV), cryptochrome 2 (CRY2), phytochrome B (PhyB), UV-resistance locus 8 (UVR8) and Dronpa, have been successfully optimized and exploited to control a growing number of biological processes in mammals [36, 38–42]. Very recently, photosensitivity has been engineered into the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (*e.g.*, OptoSTIM1 and Opto-CRAC) [36, 44–46], G-protein coupled receptors (*e.g.*, melanopsin and Opto-XRs) [47, 48] and receptor tyrosine kinases (*e.g.*, Opto-RTKs) [49–51]. These exciting technical breakthroughs introduce a repertoire of highly Ca^{2+} -selective optogenetic tools to the Ca^{2+} signaling field (Figure 1B). We name these tools collectively as ‘genetically encoded Ca^{2+} actuators’ or GECAs, which complement the existing toolbox of GECIs to allow simultaneous perturbation and recording of Ca^{2+} signals. In this review, we will present the current state of the art of the optogenetic

toolkit tailored for Ca^{2+} signaling, outline engineering strategies and basic design principles for GECAs, and briefly discuss the strengths and weaknesses of the existing tools. Our goal is to provide a general guide to choosing appropriate GECAs based on the experimental requirements and the biological questions to be tackled.

2. Genetically-encoded photoactivatable Ca^{2+} releaser (PACR)

Inspired by photo-induced uncaging of Ca^{2+} with synthetic ‘caged’ compounds [13, 15–17], Fukuda *et al* devised a genetically encoded Ca^{2+} -releasing (PACR) molecular tool [52] by inserting a photosensitive domain LOV2 into a calmodulin (CaM)-M13 fusion protein (Figure 1B), the latter of which contains four Ca^{2+} -binding sites with each adopting a pentagonal bipyramidal geometry to coordinate Ca^{2+} [3, 4, 53]. In the dark, owing to the formation of a complex composed of CaM and its target peptide M13, PACR binds Ca^{2+} with a dissociation constant (K_d) of ~16 nM [54], which falls into the physiological range of resting Ca^{2+} and renders PACR to act as a Ca^{2+} chelator in mammalian cells. When exposed to blue light, photoexcited LOV2 disrupted the CaM-M13 interaction, and therefore, restored CaM to its target-free state with subsequent reduction in the affinity for Ca^{2+} ($K_d = 3.75 \mu\text{M}$) by >200 fold. Consequently, the rate constant (k_{off}) of Ca^{2+} release increased from 0.77 s^{-1} in the dark to 181 s^{-1} following photostimulation [52]. Unfolded PACR recovered to its dark state in a reversible manner with a half time of 41.7 s to allow repeated Ca^{2+} release. The expression of PACR in HeLa cells was shown to moderately elevate cytosolic $[\text{Ca}^{2+}]$ by 10–90 nM. The potential application of PACR *in vivo* was demonstrated in *C. elegans* by photo-triggering the firing of touch neurons to elicit a turning behavior [52].

The application of PACR in cellular context, however, will likely be impeded because of its limited Ca^{2+} -releasing capacity and perturbation to the host physiology. The majority of cellular responses require the fluctuation of cytosolic $[\text{Ca}^{2+}]$ in the range of a few hundred nanomolar or micromolar, but PACR brings about no more than 90 nM increase in the cytosolic $[\text{Ca}^{2+}]$. Given that the amounts of sequestered Ca^{2+} is proportional to the intracellular concentrations of PACR, this concern might be partially alleviated through overexpression of PACR or PACR concatemers to push the Ca^{2+} -releasing capacity toward the upper limit. However, the presence of excessive amounts of PACR as a Ca^{2+} binding protein might run the risk of imposing buffering effects on intracellular Ca^{2+} and perturbing the host cell functions, particularly a multitude of biological processes that are dependent on CaM [3, 55]. Further tuning of kinetics of Ca^{2+} binding, release and recapture might be beneficial to improve this genetically encoded Ca^{2+} ‘cage’ and make it widely applicable.

3. Photoactivatable intracellular Ca^{2+} mobilization through the phospholipase C (PLC) pathway

Activation of cell-surface receptors, such as G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), results in mobilization of Ca^{2+} release from internal Ca^{2+} stores [56–59]. Upon ligand binding to these receptors, PLC is activated to hydrolyze the PM-bound lipid, phosphatidylinositol 4,5-bisphosphate (PIP2), generating two second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [56]. DAG is an activator of protein kinase C (PKC) and may directly activate certain types of transient

release potential (TRP) channels [60], resulting in Ca^{2+} influx from the extracellular space. Very recently, photoswitchable DAG and its analogs based on the azobenzene photoswitch (Figure 1A) have been developed to modulate PKC dependent pathways [61]. The second messenger IP_3 can diffuse to the ER where it binds and activates ER-resident IP_3 receptors to trigger Ca^{2+} release from ER Ca^{2+} stores [57], which is often followed by store-depletion induced Ca^{2+} entry from the extracellular space (discussed in Section 4). Receptor-induced Ca^{2+} signaling via the PLC pathway works in concert with other receptor activated pathways to modulate important cellular processes, such as synaptic transmission, fertilization, cell migration, and secretion [56, 57]. Here we highlight two notable examples of introducing light sensitivities into GPCRs or RTKs to enable photo-inducible intracellular Ca^{2+} mobilization (Figure 1B).

3.1 Photoactivatable GPCRs

As the largest family of transmembrane proteins in the human genome [62, 63], GPCRs, particularly those coupled to G_q protein ($G_{\alpha q}$ or $G_{q/11}$), mediate Ca^{2+} signaling via activation of PLC with subsequent hydrolysis of PIP_2 into DAG and IP_3 [2, 57]. IP_3 -dependent Ca^{2+} release from ER Ca^{2+} stores directly controls many physiological processes in both non-excitable and excitable cells [64]. Naturally-existing photosensitive melanopsin, a member of the opsin subgroup of GPCRs first characterized in retinal ganglion cells [65], can be expressed in other types of mammalian cells to drive Ca^{2+} dependent reactions when illuminated by blue or green light. Vertebrate melanopsin (or opsin 4, OPN4) and its variants have been used as intrinsic bistable optogenetic switches to generate both sustained and transient Ca^{2+} responses to control Ca^{2+} -dependent transgene expression and GPCR pathways [48, 66, 67]. By taking advantage of a light-gated human melanopsin (hOPN4) to generate Ca^{2+} oscillations with programmable frequency, peak amplitude, and duty cycle, Hannanta-anan *et al* resolved the decoding principle for NFAT by quantitatively assessing the impact of these parameters on NFAT-dependent transcriptional outputs [66]. Results from both optogenetic studies and mathematical modeling converge to support the conclusion that NFAT acts as a signal integrator of Ca^{2+} load rather than a frequency-selective decoder. In another application with the expression of melanopsin in beating embryoid bodies (EBs) derived from mouse ESCs, photoactivation of melanopsin and G_q signaling can generate local pacemaker activity in cardiomyocytes within EBs upon blue light illumination [68].

Given the common structure-function relationships between melanopsin and metabotropic glutamate receptor (mGluR), a chimeric receptor, Opto-mGluR6, has been recently generated by combining the light-sensing domain of melanopsin and the intracellular domain of mGluR6 [69]. Once expressed in retina, Opto-mGluR6 recovered vision in blind mice with retinal degeneration. With a similar engineering strategy, photosensitive GPCR chimeras were developed by capitalizing on the structural similarity between class A GPCRs and light-gated ChR2, as most prominently known as 'optoXRs' [47]. optoXRs were devised by replacing the intracellular region of G_i -coupled bovine rhodopsin to that of a human adrenergic receptors [47]. Using a chimeric photoactivatable GPCR named opto- $\alpha_1\text{AR}$, Airan *et al* showed that 1 min of light stimulation at ~504 nm at a power density of 7 mW mm^{-2} could generate prominent increase in cytosolic Ca^{2+} in HEK cells. These tools

can be further used to photo-tune the firing rate of neurons in mouse nucleus accumbens to control reward-related behaviors in living animals [47].

Since activation of GPCRs mobilizes Ca^{2+} through a series of signaling cascades, the resulting Ca^{2+} signal is greatly amplified [57]. This feature makes the Opto-GPCR tools very sensitive to light stimulus. However, the functional crosstalk between GPCRs in a heterodimeric assembly [70–72] within the host cell may activate multiple signaling pathways and/or ion channels, and thus, make it difficult to discriminate between Ca^{2+} -dependent and Ca^{2+} -independent effects. Furthermore, photo-activation of GPCRs leads to the generation of DAG, a second messenger that is implicated in a plethora of non- Ca^{2+} -dependent cellular signaling events through the activation of protein kinase C (PKC) and phorbol ester receptors [73–75]. This will likely confound the interpretation of the observed phenotypes following light stimulation. Some precautions, therefore, are needed when applying photoactivatable GPCRs to dissect the causal impact of Ca^{2+} -dependent signaling. In particular, control experiments to rule out the contribution of endogenous GPCRs and DAG-dependent signaling are required to reach an unequivocal conclusion.

3.2. Photoactivatable RTKs

RTKs belong to an important family of cell-surface receptors that share a similar molecular architecture, typically consisting of an extracellular ligand-binding domain (ED), a single-pass transmembrane domain (TM), and an intracellular tyrosine kinase domain (TKD; Figure 1B) [76]. RTKs play instrumental roles in the regulation of cell proliferation, differentiation, cell-cycle progression, survival, migration, and metabolism [77]. Mutations or aberrant expression of RTKs have been tightly linked to cancer and developmental syndromes [77, 78]. The signal transduction is often triggered upon the binding of ligands (*e.g.*, growth factors or hormones) to RTKs to induce the dimerization of the extracellular regions and ultimately activate the intracellular TKDs to mediate phosphorylation of downstream targets such as $\text{PLC}\gamma$ [59, 76, 79]. Activated $\text{PLC}\gamma$, as described above, cleaves its substrate PIP_2 into DAG and the Ca^{2+} -mobilizing secondary messenger IP_3 [58, 59]. Photoactivatable RTKs were generally designed by tagging the C-terminus of TKD with optical dimerizers derived from CRY2 (*e.g.*, the PHR domain) [51, 80], LOV domains from aureochrome photoreceptors (*e.g.*, VfAU1, OdPA1, or NgPA1) [49], or the cyanobacterial phytochrome 1 (Cph1) [81]. The extracellular domain was either retained or replaced by PM-targeting motifs (Figure 1B).

This optogenetic engineering strategy has been applied to a number of RTKs, including fibroblast growth factor receptor 1 (FGFR1) [49, 51, 81], epidermal growth factor receptor (EGFR) [49], rearranged during transfection (RET) [49] and the tropomyosin-related kinase (Trk) family [49, 80–82]. For example, Kim *et al* constructed a light-controlled optoFGFR1 by fusing the cytoplasmic TKD of human FGFR1 (aa 398–822) with CRY2-PHR, with the N-terminal extracellular region and the transmembrane segment of FGFR replaced by a myristoylation signal peptide in order to anchor the chimeric protein to plasma membrane [51]. In the absence of the extracellular ligand binding domain, the activation of optoFGFR1 is solely dependent on light-induced homodimerization of intracellular TKDs. When optoFGFR1 was transiently expressed in HeLa cells, reversible Ca^{2+} signals at high

spatiotemporal resolution could be generated in individual cells by toggling a blue light switch. This effect was dependent on TKD and the PLC pathway since either the addition of an FGFR-specific kinase inhibitor or the expression of a PLC-binding mutant (Y766F) of optoFGFR1 abolished photoswitchable changes in cytosolic Ca^{2+} [51].

Owing to the lack of tools with enough spatiotemporal resolution, the role of FGFR-mediated local Ca^{2+} signaling in the migration of HUVECs remains ill-defined [83]. To address this challenge, Kim *et al.* used optoFGFR1 to generate Ca^{2+} signals with superior spatial and temporal precision in HUVECs [50]. Local photostimulation of optoFGFR1-expressing HUVECs produced Ca^{2+} puffs around the illuminated area (defined as the “front” of the cell), but surprisingly induced Ca^{2+} sparklets in un-illuminated (or the “rear”) area of the cell, thereby creating a front-to-rear increasing Ca^{2+} gradient to maintain morphological polarity during directional migration [50]. The origin of Ca^{2+} sparklets was further traced to Ca^{2+} influx from the extracellular space through L-type Ca_v channels, as the frequency of Ca^{2+} sparklets was greatly diminished by treating HUVECs with a Ca^{2+} chelator EGTA or a Ca_v channel blocker nimodipine [50]. Nimodipine treatment disrupted the Ca^{2+} gradient and greatly inhibited light-directed migration of HUVECs, thus establishing a functional link between optoFGFR1-generated Ca^{2+} gradient and directed cell migration. This application represents an excellent example of combining the use of optogenetic tools with pharmacological agents to address biological questions that would otherwise be inaccessible with conventional approaches. Nonetheless, one potential caveat users should bear in mind is that photostimulation of OptoRTKs, similar to ligand-induced activation of canonical RTKs, simultaneously activates signaling pathways mediated by PLC, mitogen activated protein kinase (MAPK) and phosphoinositide 3 kinase (PI3K) [77]. Therefore, light will not only induce fluctuations in the cytosolic Ca^{2+} , but also trigger the MAPK/PI3K-associated signaling axis to program cell functions. The latter undesired effects call for caution during the use of optoRTKs to interrogate Ca^{2+} -dependence cellular processes.

4. GECAs engineered from CRAC channels

The CRAC channel is a prototypical example of store operated Ca^{2+} entry (SOCE) [84–90] and constitutes a major route for Ca^{2+} entry in many cell types. Store-operated CRAC channel comprises two major protein families (Figure 1B): ORAI as the pore-forming subunit on the plasma membrane (PM) [91, 92] and the ER-resident stromal interaction molecule (STIM) as the Ca^{2+} sensor for $[\text{Ca}^{2+}]_{\text{ER}}$ [93–95]. CRAC channels are among the most selective Ca^{2+} channels known thus far with a $\text{Ca}^{2+}/\text{Na}^{+}$ permeability ratio of >1000 under physiological conditions [96]. Over the past decade, the essential steps required to activate SOCE, particularly the dynamic coupling between STIM1 and ORAI1, have been worked out in great detail through the collective efforts from multiple groups (reviewed in [88–90, 97–99]). In brief, the depletion of ER Ca^{2+} store, triggered by ligand binding to cell-surface receptors or through pharmacological manipulation (*e.g.*, ionomycin or thapsigargin), causes the dissociation of Ca^{2+} from the canonical EF-hand Ca^{2+} binding motif, and subsequently induces the oligomerization of the STIM1 EF-SAM domain in the ER lumen [100]. These structural changes further prompt the reorganization of the single-pass transmembrane (TM) domain of STIM1 to transduce luminal signals toward its own

cytoplasmic domain (STIM1ct) [101, 102]. Next, STIM1ct undergoes a conformational switch to adopt an extended configuration by overcoming the intramolecular autoinhibition mediated by its juxtamembrane coiled coil region 1 (CC1) and the minimal ORAI-activating domain (SOAR, aa 344-442; or CAD, aa 342-448; Figure 2A) [101–104], thereby allowing further oligomerization and translocation toward the plasma membrane to directly engage and gate ORAI1 Ca^{2+} channels [105–109]. The efficient targeting of STIM1 from the ER network toward PM, as well as the stabilization of the CRAC channel complex, is facilitated by other protein regulators (*e.g.*, CRAC2A, septin and STIMATE) [110–114] and PM-resident lipids [102, 115–118].

Taking advantage of photosensitive domains derived from plants, light sensitivities can be installed into STIM1 to mimic two critical steps during STIM1 activation: oligomerization and conformational switch. Specifically, two engineering approaches have been employed: i) replacing the STIM1 luminal domain and the transmembrane domain with a light-inducible oligomerization domain (OptoSTIM1; Figures 1B and 2A) [44]; and ii) fusion of a photosensory module with SOAR/CAD to recapitulate reversible CC1-SOAR/CAD intramolecular trapping (LOVS1K, BACCS or Opto-CRAC; Figures 1B and 2B) [36, 45, 46, 119]. These tools enable light-dependent ORAI-STIM coupling to elicit Ca^{2+} influx through highly Ca^{2+} -selective CRAC channels in both excitable and non-excitable cells.

4.1 OptoSTIM1

The Ca^{2+} dissociation and subsequent oligomerization of the STIM1 luminal domain initiate STIM1 activation [100]. This scenario can be recapitulated by replacing the luminal domain, or the luminal domain plus TM domain of STIM1 [120], with a FKBP/FRB-based chemical inducible dimerization system. When the FKBP-STIM1 and FRB-STIM1 fusion chimeras were co-expressed in mammalian cells, rapamycin-induced heterodimerization of FKBP and FRB, much like Ca^{2+} -depletion induced oligomerization of the luminal EF-SAM domain, triggered STIM-ORAI coupling to evoke Ca^{2+} influx [120, 121]. Moreover, covalently crosslinking the N-terminus of STIM1ct at residue 233 has been shown to induce a conformational switch in STIM1ct [102]. Clearly, bringing the N-terminus of STIM1ct into close proximity, either through forced multimerization or via crosslinking, is sufficient to overcome the intramolecular autoinhibition to elicit the ensuing STIM1-ORAI1 functional coupling. Based on these pioneering studies, Kyung *et al*/hypothesized that an optical dimerizer could likewise oligomerize STIM1 to prompt STIM1-ORAI1 interaction and ultimately trigger Ca^{2+} influx via photostimulation [44]. To test this, the EF-SAM and TM domains of STIM1 were replaced by the N-terminal photolyase homology (PHR; aa 1-498) domain of CRY2 from *Arabidopsis thaliana*, the latter of which binds to the flavin adenine dinucleotide (FAD) cofactor (Figure 2A) and undergoes light-inducible monomer-to-oligomer transition in a reversible manner [122, 123]. After screening a handful of CRY2_{PHR}-STIM1ct chimeric constructs, Kyung *et al*/identified at least three variants (238-685, 238-463, and 342-685) that translocated to the plasma membrane and induced Ca^{2+} influx upon blue light illumination. The most effective construct comprising CRY2_{PHR} and the STIM1ct fragment 238-685 was termed as 'OptoSTIM1' (Figure 2A). OptoSTIM1 has an activation kinetics (the time to reach half-maximal Ca^{2+} -responsive R-GEQO1 intensity, $t_{1/2}$) of ~1 min and a deactivation kinetics of ~4–5 min upon the withdrawal of

blue light (Table 1). A similar construct generated in our laboratory by fusing CRY2_{PHR} with the STIM1ct fragment 233-685 displayed a faster activation kinetics ($t_{1/2, \text{on}} = 38$ sec) when assayed with R-GECO1.2 in HeLa cells (Figure 2C). In the near future, the activation and deactivation kinetics of OptoSTIM1 can be further tuned by introducing key mutations (e.g., L348F, W349R and E490G) into CRY2_{PHR} to expedite or slow down its photocycle [122–127].

The use of OptoSTIM1 to photostimulate Ca^{2+} influx has been demonstrated in various cell types including HeLa, NIH3T3, Cos-7, HEK293, human umbilical vein endothelial cells (HUVECs), human embryonic stem cells (hESCs) and astrocyte, as well as in zebrafish embryos and the CA1 hippocampus of mice [44]. When transiently expressed in cultured hippocampal neurons, OptoSTIM1 could trigger spatially-defined Ca^{2+} signals at selected regions of dendrites or soma that were exposed to blue light. OptoSTIM1-induced hippocampal Ca^{2+} influx was further shown to affect contextual fear memory formation and subsequent freezing behavior in mice [44]. This elegant proof-of-concept experiment points to the possibility of applying GECAs *in vivo* to modulate the emotion circuits and enhance the learning capacity of mammals [44, 128].

4.2 Opto-CRAC and BACCS

In another optogenetic engineering strategy, STIM1ct fragments bearing the minimal ORAI activating domain were fused to the LOV2 domain (aa 404-546) from *A. sativa* phototropin 1 (designated as 'Opto-CRAC'; Figure 2B and Table 1) [36, 45]. The AsLOV2 module contains a PAS (Period-ARNT-Single-minded) core that binds flavin mononucleotide (FMN) [129–132]. The chromophore FMN is an endogenous cofactor abundantly available in mammalian cells and renders LOV2 sensitive to blue light. In the dark, the J α helix tightly docks to the PAS core, and LOV2 has a high chance to mask the active site of fused effector domains due to steric hindrance (Figure 2B). Upon blue light illumination, LOV2 rapidly forms a covalent linkage between the C4a position of the flavin cofactor and the thiol moiety on a conserved cysteine residue (C450). This photo-induced reaction triggers the unfolding of the C-terminal J α helix to relieve the steric restrictions on the effector domain SOAR/CAD, and subsequently elicits Ca^{2+} flux through ORAI channels [45]. The LOV2-mediated photocycle is reversible owing to the hydrolysis of the cysteine-flavin bond in the dark at a timescale of dozens of seconds [38, 40, 45, 133]. After screening over 150 constructs through optimization of LOV2, STIM1ct fragments and the linkers, He *et al* identified one construct comprising LOV2₄₀₄₋₅₄₆ and STIM1₃₃₆₋₄₈₆ that underwent rapid translocation between the cytosol and plasma membrane in response to blue light illumination ($t_{1/2, \text{on}} = 6.8 \pm 2.3$ s; $t_{1/2, \text{off}} = 28.7 \pm 6.5$ s). Opto-CRAC and its variants generally have faster 'ON' and 'OFF' kinetics ($t_{1/2, \text{on}} = 10 \sim 36$ s; $t_{1/2, \text{off}} = 25 \sim 35$ s) compared with OptoSTIM1. The amplitude and speed of Ca^{2+} responses can be further fine tuned by varying the expression ratio of ORAI1 over Opto-CRAC, designing concatemers and ORAI1 + Opto-CRAC fusions, or tethering Opto-CRAC to PM (Table 1). Opto-CRAC has been successfully used to generate different temporal patterns of Ca^{2+} signals by varying the light pulse frequencies (Figure 2D), to deliver both global and localized Ca^{2+} signals at user-defined spatial resolutions, and most importantly, to phenocopy the hallmark Ca^{2+} /NFAT-dependent physiological responses in a number of cell types [45].

With a similar strategy, Ishii *et al* reported the development of a genetically engineered blue light-activated Ca^{2+} channel switch (BACCS), which is composed of a slightly truncated AsLOV2 (aa 404-538) and a STIM1ct fragment (aa 347-448) [46]. Three variants of BACCS were further designed: a dimeric form of BACCS (hBACCS2), a fusion protein of human ORAI1 and hBACCS2, and a *Drosophila* version of BACCS2 (dmBACCS2) that could only activate *Drosophila* Orai (dmOrai) but not human ORAI1. Among all the variants, dmBACCS2, when co-expressed with dmOrai, exhibited the highest Ca^{2+} amplitude and fastest kinetics. The ORAI1-hBACCS2 fusion protein, surprisingly, induced much less Ca^{2+} influx and took >1 min to reach the half-maximal Ca^{2+} response after blue light stimulation. BACCS is superior over an earlier version of LOV2-based GECA called LOVS1K (LOV2₄₀₄₋₅₄₆-STIM1₂₃₃₋₄₅₀) [119]. LOVS1K exhibited a lower dynamic range of Ca^{2+} response and failed to respond to blue light in primary cultured hippocampal neurons, HUVEC and NIH3T3 cells [44]. LOVS1K also showed a high basal activity when expressed in HEK293T cells, as reflected by the constitutive NFAT nuclear translocation in the dark [46]. The inclusion of CC1 in LOVS1K likely accounts for its low Ca^{2+} response and insufficient caging capability, because CC1 might compete with LOV2 for binding to SOAR/CAD and thus rendering the fusion protein less potent in activating ORAI1 channels.

LOV2-based GECAs have been applied to photo-induce Ca^{2+} influx and control Ca^{2+} -dependent biological processes both *in cellulo* and *in vivo*. They respond to blue light when expressed in cells of the immune system, neurons and cancer cell lines derived from various tissues [45, 46]. The relatively small sizes of LOV2-based GECAs (1~3 kb) make them compatible with almost all existing viral packaging systems. Adenoviruses encoding BACCS have been successfully used to infect mouse olfactory epithelium to photo-manipulate the activities of olfactory sensory neurons [46]. Lentiviruses expressing Opto-CRAC or OptoSTIM1 could be used to transduce cells of the immune system [45] and the hippocampus of mice [44]. Furthermore, Opto-CRAC can be retrovirally expressed in murine bone marrow-derived dendritic cells (DCs) to induce Ca^{2+} influx, and act as a genetically encoded 'photoactivatable adjuvant' to promote DC maturation and boost antigen-specific immune response both *in vitro* and *in vivo* [45]. This exciting progress attests to the feasibility of combining optogenetics with immunoengineering to develop light-controllable, smarter immunomodulatory therapeutics [36].

4.3 Practical considerations in the choice of GECAs

The choice of genetically encoded Ca^{2+} actuators (GECAs) may vary depending on the cell type, fluorescence imaging system and Ca^{2+} -dependent processes to be studied. A brief summary of biophysical characteristics of GECAs engineered from CRAC channels is listed in Table 1. In general, the following factors need to be taken into account during experimental design.

1. *Compatibility with existing Ca^{2+} -sensitive dyes and GECIs.* Since both LOV2- and CRY2-based GECAs have activation spectra centered around 400–500 nm, Ca^{2+} sensitive dyes excited in this range, such as Fluo-4, can only be used to monitor the 'ON' phase of photo-induced Ca^{2+} responses. Similarly, GECIs derived from BFP, CFP, GFP and YFP are incompatible with the recording of the 'OFF' phase of Ca^{2+} signals. The ratiometric Ca^{2+} indicator, Fura-2 AM, is

suitable for recording both the onset and decay of Ca^{2+} signals by switching on and off the light source. However, the use of Fura-2 tends to cause a drift in the baseline because the excitation at 380 nm overlaps with the photoactivation window of optoSTIM1 or Opto-CRAC [45], albeit to a much lesser extent when compared with blue or green light. Red-color GECIs, particularly R-GECO1, RCaMP, R-CEPIA and their variants [6, 10, 134, 135], are most compatible with optogenetic applications. R-GECO1 has a higher affinity for Ca^{2+} (K_d : 449 nM vs 1–4 μM) and exhibits a larger dynamic range than RCaMP ($F_{\text{max}}/F_{\text{base}}$: 2.5 vs 1.3–2.0 in HEK cells), but it shows significant photoactivation and may cause false-positive artefacts when excited by blue or green light [6, 134]. R-GECO1.2 is an improved version of R-GECO1 with higher fluorescence signal but reduced artefacts [134]. Using relatively low-intensity blue light (1–10 $\mu\text{J}/\mu\text{m}^2$) that was powerful enough to activate Opto-CRAC, we did not observe obvious artefacts associated with R-GECO1.2 during Ca^{2+} imaging [45]. Taken together, to avoid potential conflicts with the activation wavelengths of GECAs, we strongly recommend the use of red Ca^{2+} -responsive dyes, R-GECO1.2, RCaMP or further red-shifted GECIs for measurements of Ca^{2+} dynamics.

2. *Kinetic and spatial profiles of the delivered Ca^{2+} signals.* CRY2-based OptoSTIM1 has a slower activation and substantially prolonged deactivation speed than LOV2-based Opto-CRAC or BACCS2 (Table 1). The onset of hBACCS2-elicited Ca^{2+} influx often delays by 5–10 sec upon light stimulation. The deactivation kinetics largely follows the order of OptoSTIM1 $\ll$ hBACCS2 $<$ dmBACCS2 $<$ Opto-CRAC. For experiments requiring sustained and strong Ca^{2+} signals, OptoSTIM1 is a good choice since it can produce robust Ca^{2+} signals that may last for ~10 min with a single pulse of blue light (Figure 2C). Under similar experimental conditions, we observed that Opto-CRAC could generate six times more Ca^{2+} transients than OptoSTIM1 did in HeLa cells (Figure 2C), thus placing Opto-CRAC as an ideal GECA to generate repetitive Ca^{2+} signals to mimic physiologically-relevant Ca^{2+} oscillations in mammalian cells (Figure 2D). All the existing GECAs take advantage of cytosolic fragments of STIM1 that are able to constitutively activate ORAI1 channels without forming puncta at ER-PM junctions. GECAs that are capable of generating Ca^{2+} microdomains at membrane contact sites [136, 137] are yet to be developed. The recent development of a light-inducible ER-PM tethering tool (termed ‘LIMETER’) [110] might provide a potential solution to fulfill this unmet need.
3. *Endogenous levels of ORAI channels and heterogeneity of Ca^{2+} dynamics.* Since both OptoSTIM1 and Opto-CRAC rely on the endogenous ORAI channels to conduct photo-inducible Ca^{2+} influx, the expression levels of ORAI in the assayed cells will affect the amplitude and frequency of Ca^{2+} signals. In addition, following transient transfection, the variable expression of GECAs among cells may further contribute to the heterogeneity of photo-induced Ca^{2+} responses. This complication can be circumvented by generating stable cell lines or transgenic mouse strains. In cell types with very low or no detectable expression of ORAI, two strategies can be used: i) expressing the ORAI1::hBACCS2, or

ORAI1::Opto-CRAC fusion constructs; ii) co-expressing ORAI1 with Opto-CRAC, or dmBACCS2 with dmOrai, by using bicistronic expression systems.

4. *Dark activation and potential perturbations to host physiology.* Given that expressing STIM1ct fragments alone may cause varying degrees of constitutive Ca^{2+} influx [105–109], high expression of GECAs might cause a basal $[\text{Ca}^{2+}]_{\text{cytosol}}$ increase even in the dark, and thus, perturb Ca^{2+} -dependent reactions in the host cells. This ‘dark activation’ problem is most evident when co-expressing ORAI1 with OptoSTIM1 or expressing LOVS1K alone [44–46]. In addition, because OptoSTIM1 contains an SxIP EB1-binding motif, it shows light-dependent dynamic movement with the microtubule plus ends [44]. It remains unknown how this might affect the dynamics of cytoskeleton and cell migration of the host cells. This would be less of an issue with Opto-CRAC or hBACCS2 since the EB1-binding motif is not included in the constructs. Finally, because STIM1 has been reported to inhibit L-type voltage-gated Ca^{2+} channels through direct interactions [138, 139], we sound a note of caution to use STIM1-derived GECAs in excitable tissues.

5. Conclusions and future directions

The optogenetic applications discussed above have impressively demonstrated the general applicability of GECAs in mammalian cells. GECAs, particularly those engineered from *bona fide* Ca^{2+} channels, offer untapped potentials for remote and non-invasive control of Ca^{2+} signaling with subcellular precision at timescales of seconds to minutes. The combined use of GECAs and GECIs makes it possible to simultaneously perturb and observe intracellular Ca^{2+} signals, and offers novel approaches to aid the screening of chemical modulators for Ca^{2+} channels in the near future. These tremendous advances will greatly facilitate the study of Ca^{2+} signaling in a more quantitative and predictable manner, thereby enabling the dissection of causal relationships between Ca^{2+} signals and cellular or animal behaviors.

GECAs can be applied to achieve remote neuromodulation or optogenetic immunomodulation when integrated into neurons or immune cells. However, the *in vivo* organismal application of existing GECAs is hampered by the inability of blue light to penetrate deep into the biological tissues. For neuromodulation, the use of invasive indwelling optical fibers or implantable miniature light-emitting diode may partially solve the issue. In addition, transcranial photoactivation of opsin-derived photoreceptors now becomes feasible by red-shifting their spectral sensitivity toward the red or far red range [140]. However, these approaches remain largely impractical for tissues deeply buried within the body or cells that are constantly migrating in the circulation system (*e.g.*, DCs or T lymphocytes). To remove this roadblock, we propose herein two potential strategies to shift the photosensitive window of GECAs toward the near infrared (NIR) range. NIR light is able to penetrate much deeper into biological tissues (up to 1–2 cm) compared to visible lights [36, 141–143]. The first approach involves the use of cell-targeting upconversion nanoparticles (UCNPs), which act as nanotransducers to convert (NIR) light to visible lights emitting in the range of 400–500 nm [143]. We have successfully demonstrated the use of

Opto-CRAC, in conjugation with UCNPs, to achieve *in vivo* NIR photoactivatable Ca^{2+} influx without causing significant heat generation [45]. The second approach requires the substitution of CRY2 or LOV2 in GECAs by NIR light-controllable optogenetic modules, such as the light-inducible heteromerization system made of BphP1 and PpsR2 [144]. Finally, to supplement the existing GECAs, optogenetic actuators tailored for the manipulation of organellar Ca^{2+} signaling in compartments, such as ER, lysosome, Golgi and mitochondria, are yet to be developed.

Acknowledgments

This work was supported by grants from the Leukemia & Lymphoma Society (LLS3013-12 to Y.Z.), the National Natural Science foundation of China (NSFC-31471279 to Y.W.), the Recruitment Program for Young Professionals of China (to Y.W.), the Program for New Century Excellent Talents in University (NCET-13-0061 to Y.W.), the Welch Foundation (BE-1913 to Y.Z.), the American Cancer Society (RSG-16-215-01-TBE to Y.Z.) and the National Institutes of Health (R01GM112003 to Y.Z.).

References

- Berridge MJ, Bootman MD, Lipp P. Calcium--a life and death signal. *Nature*. 1998; 395:645–648. [PubMed: 9790183]
- Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nature reviews. Molecular cell biology*. 2000; 1:11–21. [PubMed: 11413485]
- Zhou Y, Xue S, Yang JJ. Calciomics: integrative studies of Ca^{2+} -binding proteins and their interactomes in biological systems. *Metallomics*. 2013; 5:29–42. [PubMed: 23235533]
- Zhou Y, Frey TK, Yang JJ. Viral calciomics: interplays between Ca^{2+} and virus. *Cell Calcium*. 2009; 46:1–17. [PubMed: 19535138]
- Berlin S, Carroll EC, Newman ZL, Okada HO, Quinn CM, Kallman B, Rockwell NC, Martin SS, Lagarias JC, Isacoff EY. Photoactivatable genetically encoded calcium indicators for targeted neuronal imaging. *Nature methods*. 2015; 12:852–858. [PubMed: 26167640]
- Akerboom J, Carreras Calderon N, Tian L, Wabnig S, Prigge M, Tolo J, Gordus A, Orger MB, Severi KE, Macklin JJ, Patel R, Pulver SR, Wardill TJ, Fischer E, Schuler C, Chen TW, Sarkisyan KS, Marvin JS, Bargmann CI, Kim DS, Kugler S, Lagnado L, Hegemann P, Gottschalk A, Schreiter ER, Looger LL. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Front Mol Neurosci*. 2013; 6:2. [PubMed: 23459413]
- Seidemann E, Chen Y, Bai Y, Chen SC, Mehta P, Kajs BL, Geisler WS, Zemelman BV. Calcium imaging with genetically encoded indicators in behaving primates. *eLife*. 2016; 5
- Mank M, Griesbeck O. Genetically encoded calcium indicators. *Chem Rev*. 2008; 108:1550–1564. [PubMed: 18447377]
- Inoue M, Takeuchi A, Horigane S, Ohkura M, Gengyo-Ando K, Fujii H, Kamijo S, Takemoto-Kimura S, Kano M, Nakai J, Kitamura K, Bito H. Rational design of a high-affinity, fast, red calcium indicator R-CaMP2. *Nature methods*. 2015; 12:64–70. [PubMed: 25419959]
- Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, Abdelfattah AS, Fujiwara M, Ishihara T, Nagai T, Campbell RE. An expanded palette of genetically encoded Ca^{2+} indicators. *Science*. 2011; 333:1888–1891. [PubMed: 21903779]
- Lock JT, Parker I, Smith IF. A comparison of fluorescent Ca^{2+} indicators for imaging local Ca^{2+} signals in cultured cells. *Cell Calcium*. 2015; 58:638–648. [PubMed: 26572560]
- Morad M, Davies NW, Kaplan JH, Lux HD. Inactivation and block of calcium channels by photo-released Ca^{2+} in dorsal root ganglion neurons. *Science*. 1988; 241:842–844. [PubMed: 2457253]
- Adams SR, Lev-Ram V, Tsien RY. A new caged Ca^{2+} , azid-1, is far more photosensitive than nitrobenzyl-based chelators. *Chemistry & biology*. 1997; 4:867–878. [PubMed: 9384535]
- Gurney AM, Tsien RY, Lester HA. Activation of a potassium current by rapid photochemically generated step increases of intracellular calcium in rat sympathetic neurons. *Proceedings of the*

- National Academy of Sciences of the United States of America. 1987; 84:3496–3500. [PubMed: 2437582]
15. Tsien RY, Zucker RS. Control of cytoplasmic calcium with photolabile tetracarboxylate 2-nitrobenzhydrol chelators. *Biophys J*. 1986; 50:843–853. [PubMed: 3098316]
 16. Ellis-Davies GC, Kaplan JH. Nitrophenyl-EGTA, a photolabile chelator that selectively binds Ca^{2+} with high affinity and releases it rapidly upon photolysis. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:187–191. [PubMed: 8278362]
 17. Ellis-Davies GC, Barsotti RJ. Tuning caged calcium: photolabile analogues of EGTA with improved optical and chelation properties. *Cell Calcium*. 2006; 39:75–83. [PubMed: 16303177]
 18. Pierce DH, Scarpa A, Topp MR, Blasie JK. Kinetics of calcium uptake by isolated sarcoplasmic reticulum vesicles using flash photolysis of caged adenosine 5'-triphosphate. *Biochemistry*. 1983; 22:5254–5261. [PubMed: 6418200]
 19. Dolphin AC, Wootton JF, Scott RH, Trentham DR. Photoactivation of intracellular guanosine triphosphate analogues reduces the amplitude and slows the kinetics of voltage-activated calcium channel currents in sensory neurones. *Pflugers Arch*. 1988; 411:628–636. [PubMed: 2457868]
 20. Walker JW, Somlyo AV, Goldman YE, Somlyo AP, Trentham DR. Kinetics of smooth and skeletal muscle activation by laser pulse photolysis of caged inositol 1,4,5-trisphosphate. *Nature*. 1987; 327:249–252. [PubMed: 3494954]
 21. Dakin K, Li WH. Cell membrane permeable esters of D-myo-inositol 1,4,5-trisphosphate. *Cell Calcium*. 2007; 42:291–301. [PubMed: 17307252]
 22. Miyamoto A, Mikoshiba K. Probes for manipulating and monitoring IP3. *Cell Calcium*. 2016
 23. Wang Y, Deshpande M, Payne R. 2-Aminoethoxydiphenyl borate inhibits phototransduction and blocks voltage-gated potassium channels in *Limulus* ventral photoreceptors. *Cell Calcium*. 2002; 32:209–216. [PubMed: 12379181]
 24. Gurney AM, Lester HA. Light-flash physiology with synthetic photosensitive compounds. *Physiological reviews*. 1987; 67:583–617. [PubMed: 3031713]
 25. Adams SR, Tsien RY. Controlling cell chemistry with caged compounds. *Annu Rev Physiol*. 1993; 55:755–784. [PubMed: 8466191]
 26. Ellis-Davies GC. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nature methods*. 2007; 4:619–628. [PubMed: 17664946]
 27. Kaplan JH, Kennedy BG, Somlyo AP. Calcium-stimulated sodium efflux from rabbit vascular smooth muscle. *J Physiol*. 1987; 388:245–260. [PubMed: 3656192]
 28. Ellis-Davies GC. Development and application of caged calcium. *Methods Enzymol*. 2003; 360:226–238. [PubMed: 12622152]
 29. Rettig J, Neher E. Emerging roles of presynaptic proteins in Ca^{++} -triggered exocytosis. *Science*. 2002; 298:781–785. [PubMed: 12399579]
 30. Mulligan IP, Ashley CC. Rapid relaxation of single frog skeletal muscle fibres following laser flash photolysis of the caged calcium chelator, diazo-2. *FEBS Lett*. 1989; 255:196–200. [PubMed: 2507351]
 31. Adams SR, Kao JPY, Tsien RY. Biologically useful chelators that take up calcium(2^{+}) upon illumination. *J Am Chem Soc*. 1989; 111:7957–7968.
 32. Kaplan JH, Ellis-Davies GC. Photolabile chelators for the rapid photorelease of divalent cations. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:6571–6575. [PubMed: 3137570]
 33. Kaplan JH, Somlyo AP. Flash photolysis of caged compounds: new tools for cellular physiology. *Trends Neurosci*. 1989; 12:54–59. [PubMed: 2469211]
 34. Schneggenburger R, Neher E. Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature*. 2000; 406:889–893. [PubMed: 10972290]
 35. Finch EA, Augustine GJ. Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature*. 1998; 396:753–756. [PubMed: 9874372]
 36. Tan P, He L, Han G, Zhou Y. Optogenetic Immunomodulation: Shedding Light on Antitumor Immunity. *Trends in biotechnology*. 2016

37. Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. *Annual review of neuroscience*. 2011; 34:389–412.
38. Zoltowski BD, Vaccaro B, Crane BR. Mechanism-based tuning of a LOV domain photoreceptor. *Nature chemical biology*. 2009; 5:827–834. [PubMed: 19718042]
39. Deisseroth K. Optogenetics. *Nature methods*. 2011; 8:26–29. [PubMed: 21191368]
40. Tischer D, Weiner OD. Illuminating cell signalling with optogenetic tools. *Nature reviews. Molecular cell biology*. 2014; 15:551–558. [PubMed: 25027655]
41. Kianianmomeni A. UVB-based optogenetic tools. *Trends in biotechnology*. 2015; 33:59–61. [PubMed: 24985334]
42. Zhang K, Cui B. Optogenetic control of intracellular signaling pathways. *Trends in biotechnology*. 2015; 33:92–100. [PubMed: 25529484]
43. Guglielmi G, Falk HJ, De Renzis S. Optogenetic Control of Protein Function: From Intracellular Processes to Tissue Morphogenesis. *Trends Cell Biol*. 2016; 26:864–874. [PubMed: 27727011]
44. Kyung T, Lee S, Kim JE, Cho T, Park H, Jeong YM, Kim D, Shin A, Kim S, Baek J, Kim J, Kim NY, Woo D, Chae S, Kim CH, Shin HS, Han YM, Kim D, Heo WD. Optogenetic control of endogenous Ca(2+) channels in vivo. *Nature biotechnology*. 2015; 33:1092–1096.
45. He L, Zhang Y, Ma G, Tan P, Li Z, Zang S, Wu X, Jing J, Fang S, Zhou L, Wang Y, Huang Y, Hogan PG, Han G, Zhou Y. Near-infrared photoactivatable control of Ca(2+) signaling and optogenetic immunomodulation. *eLife*. 2015; 4
46. Ishii T, Sato K, Kakumoto T, Miura S, Touhara K, Takeuchi S, Nakata T. Light generation of intracellular Ca(2+) signals by a genetically encoded protein BACCS. *Nature communications*. 2015; 6:8021.
47. Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. Temporally precise in vivo control of intracellular signalling. *Nature*. 2009; 458:1025–1029. [PubMed: 19295515]
48. Ye H, Daoud-El Baba M, Peng RW, Fussenegger M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science*. 2011; 332:1565–1568. [PubMed: 21700876]
49. Grusch M, Schelch K, Riedler R, Reichhart E, Differ C, Berger W, Ingles-Prieto A, Janovjak H. Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. *EMBO J*. 2014; 33:1713–1726. [PubMed: 24986882]
50. Kim JM, Lee M, Kim N, Heo WD. Optogenetic toolkit reveals the role of Ca2+ sparklets in coordinated cell migration. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113:5952–5957. [PubMed: 27190091]
51. Kim N, Kim JM, Lee M, Kim CY, Chang KY, Heo WD. Spatiotemporal control of fibroblast growth factor receptor signals by blue light. *Chemistry & biology*. 2014; 21:903–912. [PubMed: 24981772]
52. 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 chemical biology*. 2014; 9:1197–1203. [PubMed: 24625002]
53. Zhou Y, Yang W, Kirberger M, Lee HW, Ayalasomayajula G, Yang JJ. Prediction of EF-hand calcium-binding proteins and analysis of bacterial EF-hand proteins. *Proteins*. 2006; 65:643–655. [PubMed: 16981205]
54. Martin SR, Bayley PM, Brown SE, Porumb T, Zhang M, Ikura M. Spectroscopic characterization of a high-affinity calmodulin-target peptide hybrid molecule. *Biochemistry*. 1996; 35:3508–3517. [PubMed: 8639501]
55. Yap KL, Kim J, Truong K, Sherman M, Yuan T, Ikura M. Calmodulin target database. *J Struct Funct Genomics*. 2000; 1:8–14. [PubMed: 12836676]
56. Rebecchi MJ, Pentylala SN. Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiological reviews*. 2000; 80:1291–1335. [PubMed: 11015615]
57. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nature reviews. Molecular cell biology*. 2003; 4:517–529. [PubMed: 12838335]
58. Carpenter G, Ji Q. Phospholipase C-gamma as a signal-transducing element. *Exp Cell Res*. 1999; 253:15–24. [PubMed: 10579907]

59. Patterson RL, van Rossum DB, Nikolaidis N, Gill DL, Snyder SH. Phospholipase C-gamma: diverse roles in receptor-mediated calcium signaling. *Trends Biochem Sci.* 2005; 30:688–697. [PubMed: 16260143]
60. Earley S, Brayden JE. Transient receptor potential channels in the vasculature. *Physiological reviews.* 2015; 95:645–690. [PubMed: 25834234]
61. Frank JA, Yushchenko DA, Hodson DJ, Lipstein N, Nagpal J, Rutter GA, Rhee JS, Gottschalk A, Brose N, Schultz C, Trauner D. Photoswitchable diacylglycerols enable optical control of protein kinase C. *Nature chemical biology.* 2016; 12:755–762. [PubMed: 27454932]
62. Gether U. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev.* 2000; 21:90–113. [PubMed: 10696571]
63. Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature.* 2009; 459:356–363. [PubMed: 19458711]
64. Berridge MJ. The Inositol Trisphosphate/Calcium Signaling Pathway in Health and Disease. *Physiological reviews.* 2016; 96:1261–1296. [PubMed: 27512009]
65. Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, Yau KW. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature.* 2009; 457:281–287. [PubMed: 19118382]
66. Hannanta-Anan P, Chow BY. Optogenetic Control of Calcium Oscillation Waveform Defines NFAT as an Integrator of Calcium Load. *Cell Syst.* 2016; 2:283–288. [PubMed: 27135540]
67. Spoida K, Eickelbeck D, Karapinar R, Eckhardt T, Mark MD, Jancke D, Ehinger BV, König P, Dalkara D, Herlitze S, Massek OA. Melanopsin Variants as Intrinsic Optogenetic On and Off Switches for Transient versus Sustained Activation of G Protein Pathways. *Curr Biol.* 2016; 26:1206–1212. [PubMed: 27068418]
68. Beiert T, Bruegmann T, Sasse P. Optogenetic activation of Gq signalling modulates pacemaker activity of cardiomyocytes. *Cardiovasc Res.* 2014; 102:507–516. [PubMed: 24576953]
69. 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.* 2015; 13:e1002143. [PubMed: 25950461]
70. Selbie LA, Hill SJ. G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. *Trends Pharmacol Sci.* 1998; 19:87–93. [PubMed: 9584624]
71. Hur EM, Kim KT. G protein-coupled receptor signalling and cross-talk: achieving rapidity and specificity. *Cell Signal.* 2002; 14:397–405. [PubMed: 11882384]
72. Prezeau L, Rives ML, Comps-Agrar L, Maurel D, Kniazeff J, Pin JP. Functional crosstalk between GPCRs: with or without oligomerization. *Curr Opin Pharmacol.* 2010; 10:6–13. [PubMed: 19962942]
73. Quest AF, Ghosh S, Xie WQ, Bell RM. DAG second messengers: molecular switches and growth control. *Adv Exp Med Biol.* 1997; 400A:297–303. [PubMed: 9547571]
74. Newton AC. Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem Rev.* 2001; 101:2353–2364. [PubMed: 11749377]
75. Yang C, Kazanietz MG. Divergence and complexities in DAG signaling: looking beyond PKC. *Trends Pharmacol Sci.* 2003; 24:602–608. [PubMed: 14607084]
76. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* 2010; 141:1117–1134. [PubMed: 20602996]
77. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature.* 2001; 411:355–365. [PubMed: 11357143]
78. McDonnell LM, Kernohan KD, Boycott KM, Sawyer SL. Receptor tyrosine kinase mutations in developmental syndromes and cancer: two sides of the same coin. *Hum Mol Genet.* 2015; 24:R60–66. [PubMed: 26152202]
79. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 1990; 61:203–212. [PubMed: 2158859]
80. Chang KY, Woo D, Jung H, Lee S, Kim S, Won J, Kyung T, Park H, Kim N, Yang HW, Park JY, Hwang EM, Kim D, Heo WD. Light-inducible receptor tyrosine kinases that regulate neurotrophin signalling. *Nature communications.* 2014; 5:4057.

81. Reichhart E, Ingles-Prieto A, Tichy AM, McKenzie C, Janovjak H. A Phytochrome Sensory Domain Permits Receptor Activation by Red Light. *Angew Chem Int Ed Engl.* 2016; 55:6339–6342. [PubMed: 27101018]
82. Ingles-Prieto A, Reichhart E, Schelch K, Janovjak H, Grusch M. The optogenetic promise for oncology: Episode I. *Mol Cell Oncol.* 2014; 1:e964045. [PubMed: 27308360]
83. Stockwell BR. Chemical genetics: ligand-based discovery of gene function. *Nat Rev Genet.* 2000; 1:116–125. [PubMed: 11253651]
84. Putney JW Jr. A model for receptor-regulated calcium entry. *Cell Calcium.* 1986; 7:1–12. [PubMed: 2420465]
85. Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiological reviews.* 2005; 85:757–810. [PubMed: 15788710]
86. Vig M, Kinet JP. The long and arduous road to CRAC. *Cell Calcium.* 2007; 42:157–162. [PubMed: 17517435]
87. Cahalan MD, Zhang SL, Yeromin AV, Ohlsen K, Roos J, Stauderman KA. Molecular basis of the CRAC channel. *Cell Calcium.* 2007; 42:133–144. [PubMed: 17482674]
88. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annual review of immunology.* 2010; 28:491–533.
89. Soboloff J, Rothberg BS, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. *Nature reviews. Molecular cell biology.* 2012; 13:549–565. [PubMed: 22914293]
90. Prakriya M, Lewis RS. Store-Operated Calcium Channels. *Physiological reviews.* 2015; 95:1383–1436. [PubMed: 26400989]
91. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature.* 2006; 441:179–185. [PubMed: 16582901]
92. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP. CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science.* 2006; 312:1220–1223. [PubMed: 16645049]
93. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. *The Journal of cell biology.* 2005; 169:435–445. [PubMed: 15866891]
94. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Current biology: CB.* 2005; 15:1235–1241. [PubMed: 16005298]
95. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD. STIM1 is a Ca²⁺ sensor that activates CRAC channels and migrates from the Ca²⁺ store to the plasma membrane. *Nature.* 2005; 437:902–905. [PubMed: 16208375]
96. Hoth M, Penner R. Calcium release-activated calcium current in rat mast cells. *J Physiol.* 1993; 465:359–386. [PubMed: 8229840]
97. Deng X, Wang Y, Zhou Y, Soboloff J, Gill DL. STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *J Biol Chem.* 2009; 284:22501–22505. [PubMed: 19473984]
98. Gudlur A, Zhou Y, Hogan PG. STIM-ORAI interactions that control the CRAC channel. *Curr Top Membr.* 2013; 71:33–58. [PubMed: 23890110]
99. Frischauf I, Fahrner M, Jardin I, Romanin C. The STIM1: Orai Interaction. *Adv Exp Med Biol.* 2016; 898:25–46. [PubMed: 27161223]
100. Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell.* 2008; 135:110–122. [PubMed: 18854159]
101. Ma G, Wei M, He L, Liu C, Wu B, Zhang SL, Jing J, Liang X, Senes A, Tan P, Li S, Sun A, Bi Y, Zhong L, Si H, Shen Y, Li M, Lee MS, Zhou W, Wang J, Wang Y, Zhou Y. Inside-out Ca(2+) signalling prompted by STIM1 conformational switch. *Nature communications.* 2015; 6:7826.
102. Zhou Y, Srinivasan P, Razavi S, Seymour S, Meraner P, Gudlur A, Stathopulos PB, Ikura M, Rao A, Hogan PG. Initial activation of STIM1, the regulator of store-operated calcium entry. *Nature structural & molecular biology.* 2013; 20:973–981.

103. Muik M, Fahrner M, Schindl R, Stathopoulos P, Frischauf I, Derler I, Plen P, Lackner B, Groschner K, Ikura M, Romanin C. STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. *EMBO J.* 2011; 30:1678–1689. [PubMed: 21427704]
104. Fahrner M, Muik M, Schindl R, Butorac C, Stathopoulos P, Zheng L, Jardin I, Ikura M, Romanin C. A coiled-coil clamp controls both conformation and clustering of stromal interaction molecule 1 (STIM1). *J Biol Chem.* 2014; 289:33231–33244. [PubMed: 25342749]
105. Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nature cell biology.* 2009; 11:337–343. [PubMed: 19182790]
106. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell.* 2009; 136:876–890. [PubMed: 19249086]
107. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, Romanin C. A Cytosolic Homomerization and a Modulatory Domain within STIM1 C Terminus Determine Coupling to ORAI1 Channels. *J Biol Chem.* 2009; 284:8421–8426. [PubMed: 19189966]
108. Kawasaki T, Lange I, Feske S. A minimal regulatory domain in the C terminus of STIM1 binds to and activates ORAI1 CRAC channels. *Biochemical and biophysical research communications.* 2009; 385:49–54. [PubMed: 19433061]
109. Zhou Y, Meraner P, Kwon HT, Machnes D, Oh-hora M, Zimmer J, Huang Y, Stura A, Rao A, Hogan PG. STIM1 gates the store-operated calcium channel ORAI1 in vitro. *Nature structural & molecular biology.* 2010; 17:112–116.
110. Jing J, He L, Sun A, Quintana A, Ding Y, Ma G, Tan P, Liang X, Zheng X, Chen L, Shi X, Zhang SL, Zhong L, Huang Y, Dong MQ, Walker CL, Hogan PG, Wang Y, Zhou Y. Proteomic mapping of ER-PM junctions identifies STIMATE as a regulator of Ca(2+)(+) influx. *Nat Cell Biol.* 2015; 17:1339–1347. [PubMed: 26322679]
111. Hooper R, Soboloff J. STIMATE reveals a STIM1 transitional state. *Nat Cell Biol.* 2015; 17:1232–1234. [PubMed: 26419802]
112. Sharma S, Quintana A, Findlay GM, Mettlen M, Baust B, Jain M, Nilsson R, Rao A, Hogan PG. An siRNA screen for NFAT activation identifies septins as coordinators of store-operated Ca2+ entry. *Nature.* 2013; 499:238–242. [PubMed: 23792561]
113. Srikanth S, Jung HJ, Kim KD, Souda P, Whitelegge J, Gwack Y. A novel EF-hand protein, CRACR2A, is a cytosolic Ca2+ sensor that stabilizes CRAC channels in T cells. *Nat Cell Biol.* 2010; 12:436–446. [PubMed: 20418871]
114. Quintana A, Rajanikanth V, Farber-Katz S, Gudlur A, Zhang C, Jing J, Zhou Y, Rao A, Hogan PG. TMEM110 regulates the maintenance and remodeling of mammalian ER-plasma membrane junctions competent for STIM-ORAI signaling. *Proceedings of the National Academy of Sciences of the United States of America.* 2015; 112:E7083–7092. [PubMed: 26644574]
115. Maleth J, Choi S, Muallem S, Ahuja M. Translocation between PI(4,5)P2-poor and PI(4,5)P2-rich microdomains during store depletion determines STIM1 conformation and Orai1 gating. *Nature communications.* 2014; 5:5843.
116. Cao X, Choi S, Maleth JJ, Park S, Ahuja M, Muallem S. The ER/PM microdomain, PI(4,5)P(2) and the regulation of STIM1-Orai1 channel function. *Cell Calcium.* 2015; 58:342–348. [PubMed: 25843208]
117. Chang CL, Liou J. Homeostatic regulation of the PI(4,5)P2-Ca(2+) signaling system at ER-PM junctions. *Biochim Biophys Acta.* 2016; 1861:862–873. [PubMed: 26924250]
118. Pacheco J, Dominguez L, Bohorquez-Hernandez A, Asanov A, Vaca L. A cholesterol-binding domain in STIM1 modulates STIM1-Orai1 physical and functional interactions. *Sci Rep.* 2016; 6:29634. [PubMed: 27459950]
119. Pham E, Mills E, Truong K. A synthetic photoactivated protein to generate local or global Ca(2+) signals. *Chemistry & biology.* 2011; 18:880–890. [PubMed: 21802009]
120. Korzeniowski MK, Manjarres IM, Varnai P, Balla T. Activation of STIM1-Orai1 involves an intramolecular switching mechanism. *Science signaling.* 2010; 3:ra82. [PubMed: 21081754]

121. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature*. 2008; 454:538–542. [PubMed: 18596693]
122. Bugaj LJ, Choksi AT, Mesuda CK, Kane RS, Schaffer DV. Optogenetic protein clustering and signaling activation in mammalian cells. *Nature methods*. 2013; 10:249–252. [PubMed: 23377377]
123. Taslimi A, Vrana JD, Chen D, Borinskaya S, Mayer BJ, Kennedy MJ, Tucker CL. An optimized optogenetic clustering tool for probing protein interaction and function. *Nature communications*. 2014; 5:4925.
124. Lee S, Park H, Kyung T, Kim NY, Kim S, Kim J, Heo WD. Reversible protein inactivation by optogenetic trapping in cells. *Nature methods*. 2014; 11:633–636. [PubMed: 24793453]
125. Bugaj LJ, Spelke DP, Mesuda CK, Varedi M, Kane RS, Schaffer DV. Regulation of endogenous transmembrane receptors through optogenetic Cry2 clustering. *Nature communications*. 2015; 6:6898.
126. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL. Rapid blue-light-mediated induction of protein interactions in living cells. *Nature methods*. 2010; 7:973–975. [PubMed: 21037589]
127. Taslimi A, Zoltowski B, Miranda JG, Pathak GP, Hughes RM, Tucker CL. Optimized second-generation CRY2-CIB dimerizers and photoactivatable Cre recombinase. *Nature chemical biology*. 2016; 12:425–430. [PubMed: 27065233]
128. LeDoux JE. Emotion circuits in the brain. *Annual review of neuroscience*. 2000; 23:155–184.
129. Harper SM, Neil LC, Gardner KH. Structural basis of a phototropin light switch. *Science*. 2003; 301:1541–1544. [PubMed: 12970567]
130. Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature*. 2009; 461:104–108. [PubMed: 19693014]
131. Mills E, Chen X, Pham E, Wong S, Truong K. Engineering a photoactivated caspase-7 for rapid induction of apoptosis. *ACS synthetic biology*. 2012; 1:75–82. [PubMed: 23651071]
132. Renicke C, Schuster D, Usherenko S, Essen LO, Taxis C. A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chemistry & biology*. 2013; 20:619–626. [PubMed: 23601651]
133. Pudasaini A, El-Arab KK, Zoltowski BD. LOV-based optogenetic devices: light-driven modules to impart photoregulated control of cellular signaling. *Frontiers in molecular biosciences*. 2015; 2:18. [PubMed: 25988185]
134. Wu J, Liu L, Matsuda T, Zhao Y, Rebane A, Drobizhev M, Chang YF, Araki S, Arai Y, March K, Hughes TE, Sagou K, Miyata T, Nagai T, Li WH, Campbell RE. Improved orange and red Ca(2)+/- indicators and photophysical considerations for optogenetic applications. *ACS Chem Neurosci*. 2013; 4:963–972. [PubMed: 23452507]
135. Suzuki J, Kanemaru K, Ishii K, Ohkura M, Okubo Y, Iino M. Imaging intraorganellar Ca2+ at subcellular resolution using CEPIA. *Nature communications*. 2014; 5:4153.
136. Hogan PG. The STIM1-ORAI1 microdomain. *Cell Calcium*. 2015; 58:357–367. [PubMed: 26215475]
137. Berridge MJ. Calcium microdomains: organization and function. *Cell Calcium*. 2006; 40:405–412. [PubMed: 17030366]
138. Wang Y, Deng X, Mancarella S, Hendron E, Eguchi S, Soboloff J, Tang XD, Gill DL. The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels. *Science*. 2010; 330:105–109. [PubMed: 20929813]
139. Park CY, Shcheglovitov A, Dolmetsch R. The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science*. 2010; 330:101–105. [PubMed: 20929812]
140. Lin JY, Knutsen PM, Muller A, Kleinfeld D, Tsien RY. ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nature neuroscience*. 2013; 16:1499–1508. [PubMed: 23995068]

141. Shen J, Zhao L, Han G. Lanthanide-doped upconverting luminescent nanoparticle platforms for optical imaging-guided drug delivery and therapy. *Adv Drug Deliv Rev.* 2013; 65:744–755. [PubMed: 22626980]
142. Chen G, Qiu H, Prasad PN, Chen X. Upconversion nanoparticles: design, nanochemistry, and applications in theranostics. *Chem Rev.* 2014; 114:5161–5214. [PubMed: 24605868]
143. Zhang Y, Huang L, Li Z, Ma G, Zhou Y, Han G. Illuminating Cell Signaling with Near-Infrared Light-Responsive Nanomaterials. *ACS nano.* 2016; 10:3881–3885. [PubMed: 27077481]
144. Kaberniuk AA, Shemetov AA, Verkhusha VV. A bacterial phytochrome-based optogenetic system controllable with near-infrared light. *Nature methods.* 2016; 13:591–597. [PubMed: 27159085]

Highlights

- Overview of genetically encoded Ca^{2+} actuators (GECAs) for control of calcium signaling
- Comparing engineering strategies and biophysical characteristics of GECAs
- Guidelines for selecting appropriate GECAs for context-dependent applications

Figure 1. Tools for photoactivatable control of Ca²⁺ signaling

(A). Examples of caged Ca²⁺ chelator, caged IP₃ and a photoswitchable DAG (PhoDAG).

(B). Genetically encoded optical actuators engineered from membrane receptors (opto-RTKs and opto-GPCRs), Ca²⁺ sensory proteins STIM1 (OptoSTIM1 and Opto-CRAC) and CaM (PACR).

Figure 2. Optogenetic tools engineered from the CRAC channel activator STIM1

(A). Schematic of the design of OptoSTIM1. *Left*, the 3D structural model of the PHR domain of atCRY2 generated from the crystal structure of its counterpart in atCRY1 (PDB entry: 1U3D). The cofactor FAD is highlighted in orange. *Right*, the light-inducible multimerization domain CRY2-PHR is fused with STIM1ct. In the dark, the intramolecular interaction between CC1 (cyan) and SOAR (red) keeps CRY2-STIM1ct inactive. Upon blue light illumination, CRY2 undergoes oligomerization to trigger the conformation switch of STIM1ct, thus exposing SOAR toward the PM to activate Ca²⁺ influx through ORAI channels.

(B). Schematic of the design of Opto-CRAC. *Left*, 3D structure of AsLOV2 (PDB entry: 2V0W), with its cofactor FMN highlighted in yellow and the cysteine residue at position 450 in blue. *Right*, LOV2 masks the active site in SOAR to prevent its interaction with ORAI in the dark. Following blue light stimulation, the Jα helix unfolds to release the effector domain, leading to the functional restoration of SOAR, a potent activator of ORAI Ca²⁺ channels on the plasma membrane.

(C). Representative examples of light-induced Ca^{2+} transients generated by OptoSTIM1 (upper panel; light stimulation at 470 nm programmed at 2 min ON + 14 min OFF) or Opto-CRAC (lower panel; light stimulation at 470 nm: 0.5 min ON and 2 min OFF) in HeLa cells when exposed to repeated light-dark cycles. The red GECI, R-GECO1.2, enabled the recording of the whole course of intracellular Ca^{2+} fluctuations. Blue bar indicates light stimulation at 470 nm with a power density of $40 \mu\text{W mm}^{-2}$. Error bars denote s.e.m.

(D). Examples of photo-induced Ca^{2+} oscillations in HeLa cells expressing Opto-CRAC. HeLa cells were exposed to repeated blue light pulses, with the ON time fixed at 30 sec while varying the interpulse intervals (top to bottom: 0.5, 1, 2, 3 to 4 min).

Table 1

Summary of STIM1-based GECAs tailored for optical control Ca^{2+} signaling in a reversible manner.

Name	Photosensitive module	Linker	STIM fragment	ON ($t_{1/2}$, sec)	OFF ($t_{1/2}$, sec)	Ca^{2+} reporter	Fold change (F/F_0)	Cell types	Ref
CRY2-based GECAs *									
OptoSTIM1	CRY2 ₁₋₄₉₈	24 aa	238-685	64.5 ± 4.8	274 ± 23.7	R-GECO1	~ 10	HeLa	[44]
	CRY2 ₁₋₄₉₈	24 aa	238-463	43.2 ± 3.8	383 ± 52.3	R-GECO 1	~ 8	HeLa	[44]
	CRY2 ₁₋₄₉₈	24 aa	342-685	48.2 ± 5.4	344 ± 34.6	R-GECO 1	~ 1	Hela	[44]
	CRY2 ₁₋₄₉₈	24 aa	233-685	38.4 ± 2.1	320 ± 37.2	R-GECO1.2	8.2	HeLa	Unpubl***
LOV2-based GECAs **									
Opto-CRAC	LOV2 ₄₀₄₋₅₄₆	KL	336-486	23.4 ± 4.2	24.9 ± 4.8	R-GECO 1.2	3.0	HeLa	[45]
				36.4 ± 2.4	30.0 ± 1.1	Fura-2 AM	1.2	HeLa	[45]
				31.1 ± 4.9	34.7 ± 4.9	R-CaMP2	0.16	HeLa	[45]
	Lyn-LOV2 ₄₀₄₋₅₄₆	KL	336-486	10.2 ± 3.0		GCaMP6s	4.05	HeLa	[45]
	2 * LOV2 ₄₀₄₋₅₄₆	KL	336-486	2.9 ± 1.1		GCaMP6s	2.52	HeLa	[45]
				46.2 ± 8.5		GCaMP6s	2.75	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆		336-486			GCaMP6s	1.35	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KLAAA	336-486			GCaMP6s	0.95	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	336-450			GCaMP6s	1.85	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	336-460			GCaMP6s	2.01	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	336-473			GCaMP6s	0.97	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	342-486			GCaMP6s	1.78	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	344-486			GCaMP6s	3.51	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆	KL	344-442			GCaMP6s	0	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆ , C450A	KL	336-486			GCaMP6s	0.11	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆ , G528A	KL	336-486			GCaMP6s	2.35	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆ , I532E	KL	336-486			GCaMP6s	0.46	HeLa	[45]
	LOV2 ₄₀₄₋₅₄₆ , N538E	KL	336-486			GCaMP6s	0.32	HeLa	[45]

Name	Photosensitive module	Linker	STIM fragment	ON (t _{1/2} , sec)	OFF (t _{1/2} , sec)	Ca ²⁺ reporter	Fold change (F/F ₀)	Cell types	Ref
	LOV2 ₄₀₄₋₅₄₆ I539E	KL	336-486			GCaMP6s	0	HeLa	[45]
LOVSIK	LOV2 ₄₀₄₋₅₄₆	KL	233-450			GCaMP6s	1.79	HeLa	[45, 119]
hBACCS1	LOV2 ₄₀₄₋₅₃₈		347-448			Fluo-8 AM	~ 1.8	HEK293T	[46]
hBACCS2	2 * LOV2 ₄₀₄₋₅₃₈		347-448			Fluo-8 AM	~ 3.0	HEK293T	[46]
dmBACCS2	2 * LOV2 ₄₀₄₋₅₃₈		413-514			Fluo-8 AM	0	HEK293T	[46]
dmBACCS2	2 * LOV2 ₄₀₄₋₅₃₈		413-514	<30		Fluo-4 AM	~ 1.6	S2	[46]
dmBACCS2+dmOrai	2 * LOV2 ₄₀₄₋₅₃₈		413-514	<30		Fluo-8 AM	~ 5.4	HEK293T	[46]
dmBACCS2+dmOrai	2 * LOV ₄₀₄₋₅₃₈		413-514	<30	~ 50-60	Rhod-3 AM		HEK293T	[46]
dmBACCS2NS+dmOrai	2 * LOV2 ₄₀₄₋₅₃₈ (N425S)		413-514	<30	< 30	Rhod-3 AM		HEK293T	[46]
dmBACCS2VL+dmOrai	2 * LOV2 ₄₀₄₋₅₃₈ (V416L)		413-514	<30	> 480	Rhod-3 AM		HEK293T	[46]

GECA, genetically encoded Ca²⁺ actuator; h, *Homo sapiens*; dm, *Drosophila melanogaster*.

* Tested in HeLa, NIH3T3, COS-7, HEK293, HUVEC, hESCs, astrocyte, zebrafish embryo and mouse hippocampus.

** Tested in HEK293, COS-7, MEF, HIT-T15, S2 cells, hippocampal neurons, macrophage, dendritic cells, primary and leukemic T cells, and cancer cell lines derived from ovary, prostate, breast, skin, and brain, as well as in olfactory epithelium and lymphoid organs.

*** unpublished data by Ma G and Zhou Y.