

Chapter 1

Optogenetics: Basic Concepts and Their Development

Yong Ku Cho and Dan Li

Abstract

The discovery of light-gated ion channels and their application to controlling neural activities have had a transformative impact on the field of neuroscience. In recent years, the concept of using light-activated proteins to control biological processes has greatly diversified into other fields, driven by the natural diversity of photoreceptors and decades of knowledge obtained from their biophysical characterization. In this chapter, we will briefly discuss the origin and development of optogenetics and highlight the basic concepts that make it such a powerful technology. We will review how these enabling concepts have developed over the past decade, and discuss future perspectives.

Key words Optogenetics, Light-activated protein, Photoreceptor, Channelrhodopsin, Protein engineering

1 Introduction

The word ‘optogenetics’ was initially used within the context of neuroscience [1], to describe the approach of using light to image and control neuronal activity in the intact, living brain. The idea of controlling biological function using light has been around for a long time, but it is in the field of neuroscience that its need as a truly enabling tool has been envisioned early on [2, 3], and remarkably to the realization of such potential [4–6] with wide acceptance over the past decade. The success of optogenetics in neuroscience has sparked interest of many scientists and engineers in other fields, and now the definition of optogenetics has broadened to encompass the general field of biotechnology that combines genetic engineering and optics to enable gain or loss of well-defined function, often in the intact animal [7, 8]. In this chapter, we will briefly go over the historical background of the origin of optogenetics and highlight the basic concepts of optogenetics that make it such a powerful technology. We will review how these enabling concepts have developed over the past decade, and discuss future perspectives.

2 Original Concepts: A Brief Historical Perspective

The seed for the birth of optogenetics in neuroscience may have been planted a long time ago by the pioneering work of Ramón y Cajal, who provided foundational evidence that neurons are the signaling units of the nervous system, and that they exist in many distinctive morphologies [9]. Since then, studies followed to show that indeed many different types of neurons exist, classified based on their physiological characteristics, anatomical location, morphology, and gene expression profile [10–12]. It is still unclear how many cell types exist in the human brain, but it is speculated that there are about 1000 neuronal cell types just within the mammalian cortex [13, 14]. In a general sense, it is agreed that a specific cell type carries out the same function within a neural circuit [15]. Therefore, identification of all cell types and mapping out their connectivity is essential in order to understand how the nervous system works. Identification of different cell types must be accompanied with functional characterization within their normal context, the nervous system. For this reason, neuroscientists have been looking for a way to perturb individual cell types within the intact brain. This was clearly expressed by Francis Crick in his insightful discussion published in 1979, in which he proposed the need for a ‘method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered’ [2].

Optogenetics is arguably the first technological breakthrough that enables such experiments. It makes the crucial connection between cell-type information and the ability to perform gain or loss of function experiments. Prior to the development of optogenetics, experimental approaches existed in neuroscience to use light as a way to control neural activity. For example, ‘caged’ compounds such as secondary messenger molecules, ions, and neurotransmitters have been developed that are initially inert, but become active upon light illumination [16, 17]. Even though these photochemical approaches did not provide ways to control specific cell types, they laid the groundwork for the use of millisecond timescale illumination in intact cells and tissue [17]. Cell-type specific activation of neurons was first achieved in a series of pioneering work by the Miesenböck group, by heterologously expressing an invertebrate rhodopsin with other interacting proteins [18], and ligand-gated ion channels that can be activated by synthetic photo-caged precursors [19, 20]. Around this time, microbial rhodopsins that function as single component light-gated ion channels were discovered [21–23]. Remarkably, these microbial photoreceptors could be heterologously expressed in mammalian neurons to optically trigger action potentials with millisecond timescale precision [4]. These findings, along with the fact that these rhodopsins contain a covalently bound all-*trans* retinal chromophore naturally

produced in nearly all cell types including mammalian cells and tissues [24, 25], catalyzed the wide adoption of these molecules in neuroscience.

3 Genetic Targeting: Cell-Type Specificity and Beyond

As discussed above, cell-type specificity is a defining feature of optogenetic experiments. In some ways, gain or loss of function experiments on distinct cell types are analogous to genetic knockin or knockout experiments in molecular biology. However, while the definition of gene is clear, the definition of cell type can be ambiguous and variable. This is particularly true in highly complex systems such as the mammalian neocortex [15, 26]. To account for such high complexity, many parameters have been used to describe each cell type, such as developmental lineage, anatomical location, dendritic and axonal morphology, electrophysiology, and gene expression. Nonetheless, recent advances in single cell analysis methods have provided evidence that gene expression patterns can capture many of these diverse cellular phenotypes [10, 27]. Therefore, the use of genetically encoded tools is well justified for probing the function of each cell type, and is likely to expand as we gather more gene expression data.

In principle, optogenetic tools can be driven by cell-type specific promoters or enhancers [28, 29] to achieve cell-type specific expression. However, this approach is not suitable in many cases for several reasons. First, it is rare to find a single genetic regulatory element exclusive for a given cell type. Cell identity in most cases seems to be defined by a combination of multiple gene expression patterns [8, 10, 15, 30]. Even when cell-type specific genetic regulatory elements are accessible, they may not drive high enough expression of optogenetic tools for efficient control [31]. Moreover, reproducing endogenous expression pattern of genes requires the entire transcription unit and all associated regulatory factors [11], which are undefined in many cases, and can be hard to transport. A widely used strategy to achieve cell-type specific expression that circumvents these problems is using viral vectors in transgenic animals expressing recombinase in genetically defined population of cells. In these approaches, viral vectors such as lentivirus or adeno-associated virus (AAV) carry an optogenetic tool under relatively strong promoters such as EF-1 α promoter (Fig. 1a). The optogenetic tool encoding gene is initially present in reverse frame to prevent expression, and requires recombinase activity for inversion and subsequent expression (Fig. 1a). The recombinase gene expression is driven in genetically defined cell types, for which the expression level is relatively unimportant. Cre-recombinase has been widely used for this purpose, due to the availability of increasing number of cell-type specific Cre-transgenic

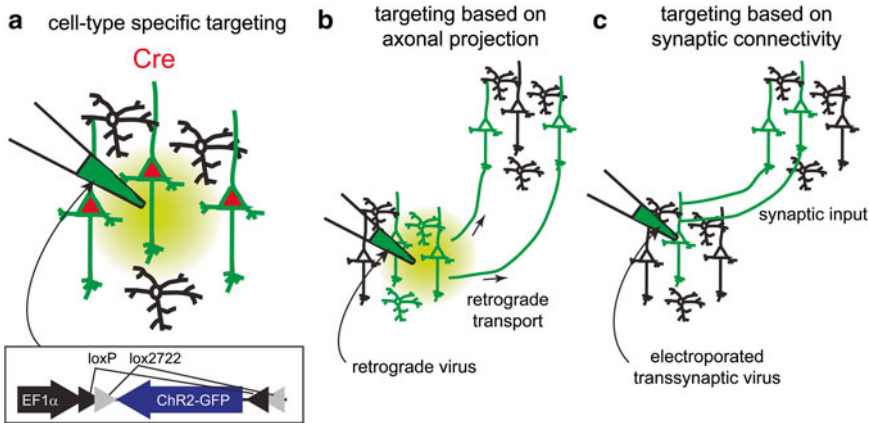


Fig. 1 Genetic targeting strategies used in optogenetics. **(a)** Cell-type specific targeting using a viral vector carrying an optogenetic construct (e.g., ChR2-GFP, indicated in *green*) in reverse frame. The optogenetic construct is expressed only in cells that express Cre-recombinase (indicated in *red*). **(b)** Targeting neurons that project in defined brain regions. Modified rabies virus can retrogradely transduce neurons. **(c)** Targeting neurons based on synaptic connectivity. Modified rabies virus can be electroporated into a single neuron, and spread transsynaptically to label cells making synaptic connections

lines [32]. Recently, this approach has been further developed to enable targeting of neural cell types defined by multiple genetic markers. Transgenic animals that express multiple recombinases driven by independent promoters are infected with viral vectors carrying a combination of these recombinase sites, resulting in intersectional targeting [33, 34].

Using viral vectors also enable other useful modes of targeting. One approach is to target neurons based on their axonal projections or synaptic connectivity (Fig. 1b, c). For example, modified rabies virus can retrogradely transduce neurons [35, 36], while herpes simplex virus and vesicular stomatitis virus can anterogradely transduce across axon terminals [37, 38]. These viruses can be used to deliver Cre-recombinase transsynaptically [37, 39], allowing the targeting of neurons projecting to a genetically defined populations. Recently, it was also shown that certain serotypes of AAV also can mediate efficient retrograde transduction [40]. These tools enable targeting optogenetic tools based on their axonal projection [41–43] and potentially synaptic connectivity.

Another targeting strategy is to express genes using promoters and enhancers induced by neuronal activity. These genetic elements were identified from immediate early genes that are known to rapidly induce expression upon neuronal activity [44, 45]. In many cases, these elements are triggered by calcium influx that activates calcium-dependent kinases [46]. These promoters have been used to specifically express optogenetic tools in neurons that gain activity during specific behavioral tasks in rodents, such as fear conditioning [47]. In a set of impressive demonstrations, Liu and others

showed that these neurons could be specifically reactivated using the light-gated ion channel channelrhodopsin-2 (ChR2) driven by the activity-dependent promoter of c-Fos [47, 48]. This reactivation recreated the original behavior with only light activation, indicating optically controlled memory recall [47]. Ramirez and others later used the same approach to trigger fear response by optically activating a set of neurons activated during fear conditioning, demonstrating the creation of false memory [49]. Conceptually, these demonstrations show that optogenetics can be used to reactivate cell populations specifically activated during a behavioral task, enabling activity-based targeting. Considering the diverse functionality of transcription factors that can sense a wide range of input signals [50–52], such responsive transcription may be a generalizable approach to express optogenetic tools to control cell populations that drive a specific functional output.

4 Customizing Photoreceptors for Designed Control: Modes of Action

The use of microbial rhodopsins in mammalian neural cells is an inspiring demonstration of customizing naturally existing photoreceptors for controlling physiological properties in a completely new context. Owing to the diverse modes of action found in photoreceptors (Fig. 2), the concept of repurposing naturally existing parts to control new biological functions has been implemented in many applications, and is expected to further develop as new photoreceptors continue to be discovered.

Microbial rhodopsins (type I rhodopsins) mediate either transmembrane ion transport (Fig. 2a, b) or light sensing through signal transduction (Fig. 2c). Microbial rhodopsins that mediate light-driven ion transport have been widely used to control membrane potential in mammalian neurons [5]. They can be categorized into two mechanistically distinct forms: ion pumps (Fig. 2a) that can transport ions against their gradient and channels (Fig. 2b) that passively conduct ions along the gradient established by other active transporters. Interestingly, all known light-driven ion pumps result in hyperpolarization of membrane potential through outward transport of proton or inward transport of chloride ion [53]. When heterologously expressed in mammalian neurons, they generate enough current to enable optical silencing [5, 54–56]. Recently, a light-driven chloride pump with high sensitivity for far-red light named Jaws has been described that enabled noninvasive neural silencing through the intact mouse skull [57]. A light-driven outward sodium pump (KR2) has also been found recently [58], that may be used for optical silencing of neurons. Unlike ion pumps, microbial rhodopsin ion channels cannot transport ions against their gradient, but enable control of membrane potential through selective conduction of specific ions. First described

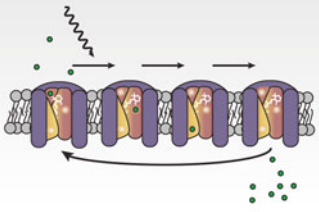
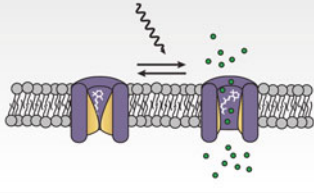
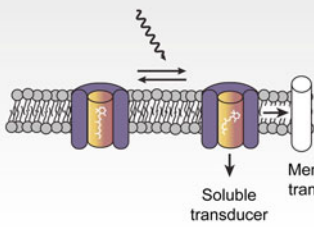
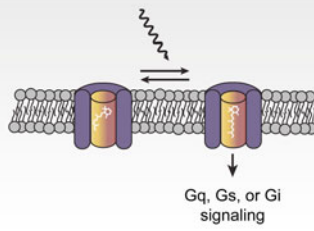
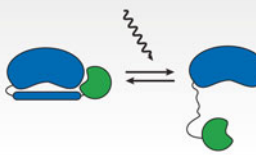
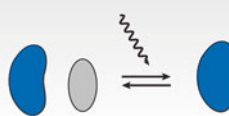
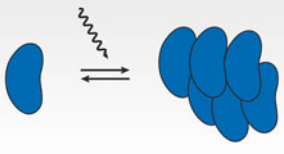
Mode of action		Representative examples	Refs
a			
Ion pump		Halorhodopsin (NpHR) Archaeorhodopsin-3 (Arch) Cruxhalorhodopsin (Jaws) KR2	[54] [56] [57] [58]
b			
Ion channel		Channelrhodopsin-2 (ChR2) Chronos Chrimson Anion Channel Rhodopsins	[4] [61] [61] [62]
c			
Light sensors		Sensory rhodopsins	[53]
d			
G-protein signaling		Light-activated β 2-AR OptoXR	[67] [68]
e			
Conformational change		LOV domain Dronpa	[77] [82]
f			
Homo/hetero dimerization		Cry2 PhyB LOV domain Dronpa UVR8	[91] [89] [83] [82] [85]
g			
Multimerization		Cry2 Cry2olig	[93] [95]

Fig. 2 Modes of action found in photoreceptors. **(a)** Light-activated ion pumps. **(b)** Light-gated ion channels. **(c)** Microbial sensory rhodopsins for light-activated signal transduction. **(d)** Animal rhodopsins that function as light-activated G-protein coupled receptors. **(e)** Light-triggered conformational change, leading to regulation of protein domain function through allosteric coupling or uncaging. **(f)** Homo/heterodimerization controlled by light. Dronpa and UVR8 monomerize in the lit-state. **(g)** Multimerization upon light activation. Figure panels modified from [63] with permission

examples are channelrhodopsins found in the green alga *Chlamydomonas reinhardtii* [21, 22], which conduct cations including proton, sodium, potassium, and calcium ions [22]. Even though the conductance of these channels are relatively low (about 100-fold lower than high-conductance ion channels) [59, 60], their current depolarize mammalian neurons above their threshold to initiate action potentials [4]. Recently, more than 60 channelrhodopsin homologues were identified by conducting a systematic search of transcriptome from 127 species of alga [61]. This study resulted in a high sensitivity channelrhodopsin with extremely fast channel kinetics named Chronos, and a red-sensitive channelrhodopsin named Chrimson. As a pair, these channelrhodopsins enabled independent multicolor activation of two distinct neural populations [61]. Another recent study identified anion-conducting light-gated channels in the cryptophyte *Guillardia theta* that enabled rapid and reversible optical silencing of rat neurons [62]. So far, microbial sensory rhodopsins have not been applied in optogenetic experiments, perhaps due to the requirement of transmembrane or soluble transducers that are not readily compatible with other cell types. In depth discussion of biophysical properties of microbial rhodopsins and their impact as optogenetic tools have been discussed elsewhere [63].

Animal rhodopsins (type II rhodopsins) share structural homology to microbial rhodopsins, but act as G-protein coupled receptors (GPCRs) that upon light illumination, catalyze GDP to GTP exchange in heterotrimeric G proteins (Fig. 2d). In fact, rhodopsins constitute the largest GPCR family—over 700 rhodopsin-like GPCRs have been found in humans [64]. Based on the extensive structure–function relationship studies of GPCRs [65, 66], Khorana and colleagues demonstrated that the cytoplasmic domains of a bovine rhodopsin can be replaced by analogous sequences from a non-light sensitive GPCR, such as β_2 -adrenergic receptor (β_2 -AR) to create a chimeric light-sensitive β_2 -AR [67]. This strategy has been extended to create light-driven rhodopsins coupled to Gq and Gs signaling pathways [68], Gi/o coupled pathways [69, 70]. It was also found that heterologous expression of unmodified forms of rhodopsins enables light-driven activation of endogenous G proteins of the host cell [71]. Unlike microbial rhodopsins that keep the retinal chromophore throughout the photocycle, certain animal rhodopsins such as bovine rhodopsin lose the chromophore after light activation, requiring consistent supply of retinal cofactors [72]. Fortunately, in mammalian cells and brain tissue, sufficient concentration of retinal cofactors are present to generate enough functional rhodopsins. However, even in cell types where the retinal chromophore is less abundant, the retinal cofactor supplementation may be avoided by using ‘non-bleaching’ G-protein coupled opsins that remain associated with the chromophore [73].

Other than rhodopsin based photoreceptors, light-sensing proteins found in plants and microbes have been applied in controlling cell signaling in diverse cell types, including mammalian cells, yeast, and bacteria. These proteins function by inducing light-triggered conformational change coupled to other protein domains (Fig. 2e), homo/heterodimerization (Fig. 2f), or multimerization (Fig. 2g). A prominent example that undergoes conformational change upon light activation is the Light-Oxygen-Voltage (LOV) domains found in photoreceptor systems of plants, fungi, and bacteria [74, 75]. In these systems, LOV domains may control the activity of an effector domain directly fused to it through allosteric coupling or steric inhibition. For example, the bacterial chemosensor FixL was made light-activatable by replacing its Per-ARNT-Sims (PAS) motif (which is structurally homologous to LOV domains) with the LOV domain Ytva [76]. In this example, an α -helical coiled coil linker of the LOV domain undergoes a rotational movement upon light activation, which activates a histidine kinase domain fused to it. In other examples, the conformational change in the LOV domain leads to unfolding of an α -helix in the lit-state, resulting in ‘uncaging’ of the effector domain fused to it (Fig. 2e). Such light-activated uncaging approach has been successfully applied in several synthetic constructs including a photoactivatable GTPase Rac1 and Cdc42 [77], peptide binding motifs [78], and tethered toxins [79]. Even though such photo-uncaging approach seems to be a generally applicable design, in many cases it requires several levels of optimization customized to each molecule for effective control. For instance, in the photoactivatable Rac1, Ca^{2+} -mediated interaction between residues at the interface of the LOV domain and Rac1 turned out to be crucial for effective light control [80]. Since such conformation-dependent interactions are hard to be predicted, de novo design of a photoactivatable construct still remains a challenge. Such difficulties may be overcome by using Dronpa, which is a photoactivated fluorescent protein that seems to allow modular design of optical control. Upon light activation, Dronpa not only changes fluorescence, but also monomerizes through the unfolding of a β -sheet [81]. This feature has been used to design photoactivatable GTPases and proteases [82].

Another mode of action found in photoreceptor domains is light-induced interaction. Certain LOV domains, such as the fungal LOV domain Vivid (VVD) [83] and bacterial LOV domain EL222 [84], homodimerize upon light activation (Fig. 2f). Plant photoreceptors such as *Arabidopsis thaliana* UVR8 exist as dimers in the dark but monomerize upon UV illumination [85]. Other photoreceptor domains such as *Arabidopsis thaliana* Cryptochrome-2 (CRY2) and Phytochrome B (PhyB) heterodimerize with a specific partner (Fig. 2f), cryptochrome-interacting basic helix–loop–helix 1 (CIB1) and phytochrome interacting factor (PIF), respectively [74]. Such light-induced interactions have

been used primarily to control intracellular signaling, by recruiting signaling proteins to or away from a specific intracellular site of action, resulting in signal activation or inhibition through sequestration. Activities of various kinases, including Ras/ERK [86], phosphoinositide-3 kinase (PI3K) [87], and receptor tyrosine kinase (RTK) [88] have been controlled using this approach. Several studies also demonstrated optical control of DNA transcription using light-induced binders that mediate the recruitment of transcription activation domains [89, 90] or activation of Cre-recombinase [91, 92]. Interestingly, CRY2 has been shown to undergo multimerization upon illumination (Fig. 2g), which has been used to mediate light-dependent activation of Rho GTPase [93], RTK fibroblast growth factor receptor [94], and actin polymerization [95]. The use of photoreceptors for optogenetic control of cellular signaling pathways has been extensively reviewed recently [63, 96, 97].

5 Engineering Photoreceptors: A Multidimensional Problem

Even though it seems that natural photoreceptor systems rely on modularity of light-activated protein domains for controlling diverse signaling pathways [74, 98], development of a new optogenetic tool in general requires significant engineering efforts for effective control. One major challenge is achieving adequate expression of optogenetic tools. As seen in the case of microbial opsins, the expression level of an optogenetic construct may be one of the limiting factors in achieving effective control. Heterologous expression of photoreceptor domains depends on the host cell type, and may yield low levels of functionally active form of the optogenetic tool. For example, a study that systematically compared channelrhodopsin homologues found that less than half of them showed detectable ion conduction when expressed in a mammalian cell line, and even less were functionally active in neurons [61]. One strategy to improve functional expression is to enhance the protein trafficking to the desired compartment using targeting sequences [56, 99]. However, this approach is not generalizable [100], and achieving adequate expression of optogenetic tools in the desired cell type remains a challenge.

Another major challenge is the fact that optogenetic tools require multiple properties to be optimized. For example, critical properties of channelrhodopsins that impact their effectiveness in controlling neural activity include ion conductance, light sensitivity, spectral sensitivity, channel kinetics, and ion selectivity. Structure-guided mutagenesis studies have resulted in improved channelrhodopsins with fast kinetics [101], enhanced photocurrent [102, 103], red-shifted spectral sensitivity [104], and altered ion selectivity [105, 106]. These studies demonstrated that key

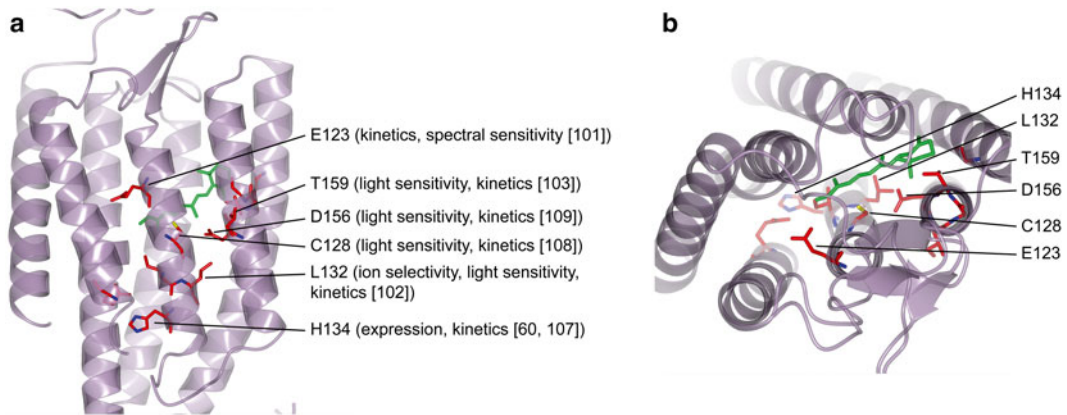


Fig. 3 ChR2 residues that affect multiple properties when mutated. Residue numbers based on ChR2 sequence are labeled on the X-ray structure obtained from a chimera between ChR1 and ChR2 (PDB code 3UG9). (a) Side-view. (b) Top-view

properties can be tuned using mutagenesis, but also revealed that a single mutation often affects multiple properties (Fig. 3). For example, mutations at E123 in ChR2 (corresponding to D85, which is the Schiff base counter-ion in bacteriorhodopsin), affect channel kinetics and red-shifts the action spectra [101]. Mutation H134R in ChR2 enhances photocurrent perhaps due to improved membrane expression, but slows channel kinetics [60, 107]. Mutations C128 and D156 slow down the channel kinetics drastically, which helps to keep open channels for longer periods of time, effectively enhancing the light sensitivity [108, 109]. The trend of multiple property modulation by a single mutation is also found in LOV domains. For example, in the *Avena sativa* phototropin 1 LOV2, mutations in the highly conserved residue Q513 reduce the structural changes between the dark and the lit-state and slow down the dark state return rate [110]. In addition, in the LOV domain VVD, mutations in M135 and M165 slow recovery kinetics and enhance the affinity of the lit-state VVD dimer [111]. Therefore, optimization of an optogenetic tool requires multidimensional characterizations to measure all essential parameters [109], and in certain cases individual properties may not be independently optimized. In other words, the fitness landscape of photoreceptors is a multidimensional space composed of potentially dependent parameters. Since typical screens used for protein engineering rely on rapid measurement of one or two parameters, strategies such as directed evolution to optimize one parameter may result in de-optimization of others that are critical for tool performance. Currently, the general strategy is to combine random mutagenesis-based methods with structure-guided approaches and rely on additive beneficial effects of multiple mutations to yield an optimal construct [105, 106, 112, 113]. In the future, screens that can characterize multiple parameters may be developed to perform multidimensional optimizations.

6 Analogies to Optical Imaging: Multicolor Imaging Versus Optogenetics

Another major direction in optogenetics has been to use multiple colors of light to control two independent cell types or processes using photoreceptor domains that have distinctive spectral sensitivities. This approach has been extensively explored using microbial opsins. For instance, ChR2, which has maximal sensitivity for blue light has been combined with a halorhodopsin which can be activated with yellow light to enable bidirectional control of neural activity [54, 55]. Proton pumps with separation in their spectral sensitivity have been used to achieve two-color neural silencing [56]. Even though these studies demonstrated that triggering or inhibiting action potentials in neurons can be controlled by leveraging the difference in spectral sensitivity of microbial rhodopsins, a recent study showed that achieving multicolor control relying on spectral separation alone may result in cross talk, due to the inherent blue-light sensitivity found in rhodopsins [61]. Even the red-sensitive channelrhodopsin Chrimson was shown to trigger action potentials under strong blue light in neurons with high expression levels [61, 63]. However, detailed biophysical characterization of Chrimson revealed that its channel opening rate under blue light is substantially slower than that under red light, and is also dependent on light intensity [61]. Therefore, cross-activation of neurons under blue light was minimized by pairing Chrimson with the fast and light-sensitive channelrhodopsin Chronos and limiting the intensity and duration of blue light [61, 63]. The blue-light sensitivity of microbial rhodopsins may not be completely abolished unless the chromophore itself is altered. Many photoreceptors, including LOV domains and cryptochromes are also maximally sensitive to blue light, which may be hard to eliminate as in the case for microbial rhodopsins. Therefore, strategies other than relying on spectral separation alone, such as leveraging differences in light sensitivity and kinetics under blue light may be necessary to reduce cross talk for multicolor control using other photoreceptors.

Although the concept of multicolor control in optogenetics may seem analogous to multicolor fluorescence imaging, practical differences exist that require caution in designing optogenetic experiments with multiple light sources. In multicolor fluorescence imaging, cross-activation of fluorophores occurs quite often, but the emission from multiple fluorophores can be filtered to obtain cross talk free images. In experiments using light-activated proteins, any cross-activation causes change that cannot be filtered or eliminated. Depending on the biological question asked, small changes induced by cross-activation may become important, such as in experiments that focus on subthreshold changes in membrane potential. Therefore, when designing experiments that require multiple sources of light, such as using light-gated ion channels with calcium reporters [114], the wavelength and intensity of light

used for imaging should be tested for cross-activation of optical actuators. It is notable that blue-sensitive channelrhodopsins that seem to have negligible red-sensitivity can depolarize membrane potential under intense red light [113]. As described above, strategies that capitalize on all aspects of biophysical properties of photoreceptors may be used to prevent cross-activation, but may be difficult to completely eliminate small cross talks induced.

7 Conclusion and Future Perspectives

Over the past decade, optogenetics has made a major impact on neuroscience research, by enabling the control of specific cell types in the intact brain. A major advancement enabled using these tools was the ability to control specific cell types in intact systems with high spatial and temporal resolution. One potentially transformative future direction would be the integration of optical control methods with various detection methods to enable closed-loop control of biological systems, as achieved by several studies that demonstrated closed-loop control of neural circuit [115–117] and feedback-regulated control of cell signaling [118] and gene expression levels [119]. These studies suggest that optogenetic approaches will enable us to reveal the underlying mechanisms behind complex intracellular signaling systems or multicellular dynamics and precisely tune it to achieve a desired outcome. They will also allow us to develop and test models of intact, complex biological systems, opening an era of real-time ‘process engineering’ in biological systems. Optogenetics may enable the application of advanced process engineering approaches widely used in chemical and electrical systems to biological systems.

As discussed in this chapter, successful development of new optogenetic tools and their implementation is an interdisciplinary effort that requires tools tailored to the specific biological process studied. Targeting and achieving optimal expression in the desired cell type need to be tested, and the biophysical properties of photoreceptors used need to be optimized to the spatial and temporal properties to be controlled. Therefore, users of optogenetic tools need to pay close attention to their biophysical characteristics, and tool developers need to identify ways to tune them.

Acknowledgements

This work was funded by the University of Connecticut and the Brain and Behavior Research Foundation (NARSAD Young Investigator grant).

References

1. Deisseroth K, Feng G, Majewska AK et al (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. *J Neurosci* 26(41):10380–10386
2. Crick FH (1979) Thinking about the brain. *Sci Am* 241(3):219–232
3. Crick F (1999) The impact of molecular biology on neuroscience. *Philos Trans R Soc Lond B Biol Sci* 354(1392):2021–2025
4. Boyden ES, Zhang F, Bamberg E et al (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 8(9):1263–1268
5. Boyden ES (2011) A history of optogenetics: the development of tools for controlling brain circuits with light. *F1000 Biol Rep* 3:11
6. Deisseroth K (2010) Controlling the brain with light. *Sci Am* 303(5):48–55
7. Deisseroth K (2011) Optogenetics. *Nat Methods* 8(1):26–29
8. Miesenbock G (2009) The optogenetic catechism. *Science* 326(5951):395–399
9. Ramón y Cajal S (1911) *Histology of the nervous system of man and vertebrates*. Oxford University Press, 1995 translation
10. Fishell G, Heintz N (2013) The neuron identity problem: form meets function. *Neuron* 80(3):602–612
11. Gong S, Zheng C, Doughty ML et al (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425(6961):917–925
12. Hawrylycz MJ, Lein ES, Guillozet-Bongaerts AL et al (2012) An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 489(7416):391–399
13. Koch C (2004) *The quest for consciousness: a neurobiological approach*. Roberts and Co., Denver, CO
14. Stevens CF (1998) Neuronal diversity: too many cell types for comfort? *Curr Biol* 8(20):R708–R710
15. Luo L, Callaway EM, Svoboda K (2008) Genetic dissection of neural circuits. *Neuron* 57(5):634–660
16. Ellis-Davies GC (2007) Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat Methods* 4(8):619–628
17. Kaplan JH, Somlyo AP (1989) Flash photolysis of caged compounds: new tools for cellular physiology. *Trends Neurosci* 12(2):54–59
18. Zemelman BV, Lee GA, Ng M et al (2002) Selective photostimulation of genetically chARGed neurons. *Neuron* 33(1):15–22
19. Zemelman BV, Nesnas N, Lee GA et al (2003) Photochemical gating of heterologous ion channels: remote control over genetically designated populations of neurons. *Proc Natl Acad Sci U S A* 100(3):1352–1357
20. Lima SQ, Miesenbock G (2005) Remote control of behavior through genetically targeted photostimulation of neurons. *Cell* 121(1):141–152
21. Nagel G, Ollig D, Fuhrmann M et al (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 296(5577):2395–2398
22. Nagel G, Szellas T, Huhn W et al (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A* 100(24):13940–13945
23. Sineshchekov OA, Jung KH, Spudich JL (2002) Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 99(13):8689–8694
24. Blomhoff R, Blomhoff HK (2006) Overview of retinoid metabolism and function. *J Neurobiol* 66(7):606–630
25. Zhang F, Wang LP, Boyden ES et al (2006) Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods* 3(10):785–792
26. Markram H, Toledo-Rodriguez M, Wang Y et al (2004) Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* 5(10):793–807
27. Sugino K, Hempel CM, Miller MN et al (2006) Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nat Neurosci* 9(1):99–107
28. Spergel DJ, Kruth U, Hanley DF et al (1999) GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neurosci* 19(6):2037–2050
29. Oliva AA Jr, Jiang M, Lam T et al (2000) Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. *J Neurosci* 20(9):3354–3368
30. McGarry LM, Packer AM, Fino E et al (2010) Quantitative classification of somatostatin-positive neocortical interneurons identifies three interneuron subtypes. *Front Neural Circuits* 4:12

31. Zeng H, Madisen L (2012) Mouse transgenic approaches in optogenetics. *Prog Brain Res* 196:193–213
32. Gong S, Doughty M, Harbaugh CR et al (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 27(37):9817–9823
33. Fenno LE, Mattis J, Ramakrishnan C et al (2014) Targeting cells with single vectors using multiple-feature Boolean logic. *Nat Methods* 11(7):763–772
34. Madisen L, Garner AR, Shimaoka D et al (2015) Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron* 85(5):942–958
35. Wickersham IR, Finke S, Conzelmann KK et al (2007) Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat Methods* 4(1):47–49
36. Wickersham IR, Lyon DC, Barnard RJ et al (2007) Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* 53(5):639–647
37. Lo L, Anderson DJ (2011) A Cre-dependent, anterograde transsynaptic viral tracer for mapping output pathways of genetically marked neurons. *Neuron* 72(6):938–950
38. Beier KT, Saunders A, Oldenburg IA et al (2011) Anterograde or retrograde transsynaptic labeling of CNS neurons with vesicular stomatitis virus vectors. *Proc Natl Acad Sci U S A* 108(37):15414–15419
39. Wall NR, Wickersham IR, Cetin A et al (2010) Monosynaptic circuit tracing in vivo through Cre-dependent targeting and complementation of modified rabies virus. *Proc Natl Acad Sci U S A* 107(50):21848–21853
40. Rothermel M, Brunert D, Zabawa C et al (2013) Transgene expression in target-defined neuron populations mediated by retrograde infection with adeno-associated viral vectors. *J Neurosci* 33(38):15195–15206
41. Osakada F, Mori T, Cetin AH et al (2011) New rabies virus variants for monitoring and manipulating activity and gene expression in defined neural circuits. *Neuron* 71(4):617–631
42. Apicella AJ, Wickersham IR, Seung HS et al (2012) Laminarly orthogonal excitation of fast-spiking and low-threshold-spiking interneurons in mouse motor cortex. *J Neurosci* 32(20):7021–7033
43. Kress GJ, Yamawaki N, Wokosin DL et al (2013) Convergent cortical innervation of striatal projection neurons. *Nat Neurosci* 16(6):665–667
44. Smeyne RJ, Schilling K, Robertson L et al (1992) *fos-lacZ* transgenic mice: mapping sites of gene induction in the central nervous system. *Neuron* 8(1):13–23
45. Kawashima T, Okuno H, Nonaka M et al (2009) Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons. *Proc Natl Acad Sci U S A* 106(1):316–321
46. Bito H, Deisseroth K, Tsien RW (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* 87(7):1203–1214
47. Liu X, Ramirez S, Pang PT et al (2012) Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484(7394):381–385
48. Kubik S, Miyashita T, Guzowski JF (2007) Using immediate-early genes to map hippocampal subregional functions. *Learn Mem* 14(11):758–770
49. Ramirez S, Liu X, Lin PA et al (2013) Creating a false memory in the hippocampus. *Science* 341(6144):387–391
50. Sellick CA, Reece RJ (2005) Eukaryotic transcription factors as direct nutrient sensors. *Trends Biochem Sci* 30(7):405–412
51. Chandel NS, Maltepe E, Goldwasser E et al (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A* 95(20):11715–11720
52. Tamura T, Yanai H, Savitsky D et al (2008) The IRF family transcription factors in immunity and oncogenesis. *Annu Rev Immunol* 26:535–584
53. Ernst OP, Lodowski DT, Elstner M et al (2014) Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. *Chem Rev* 114(1):126–163
54. Han X, Boyden ES (2007) Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS One* 2(3):e299
55. Zhang F, Wang LP, Brauner M et al (2007) Multimodal fast optical interrogation of neural circuitry. *Nature* 446(7136):633–639
56. Chow BY, Han X, Dobry AS et al (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* 463(7277):98–102
57. Chuong AS, Miri ML, Busskamp V et al (2014) Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat Neurosci* 17(8):1123–1129
58. Inoue K, Ono H, Abe-Yoshizumi R et al (2013) A light-driven sodium ion pump in marine bacteria. *Nat Commun* 4:1678

59. Feldbauer K, Zimmermann D, Pintschovius V et al (2009) Channelrhodopsin-2 is a leaky proton pump. *Proc Natl Acad Sci U S A* 106(30):12317–12322
60. Lin JY, Lin MZ, Steinbach P et al (2009) Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys J* 96(5):1803–1814
61. Klapoetke NC, Murata Y, Kim SS et al (2014) Independent optical excitation of distinct neural populations. *Nat Methods* 11(3):338–346
62. Govorunova EG, Sineshchekov OA, Janz R et al (2015) Natural light-gated anion channels: a family of microbial rhodopsins for advanced optogenetics. *Science* 349(6248):647–650
63. Schmidt D, Cho YK (2015) Natural photoreceptors and their application to synthetic biology. *Trends Biotechnol* 33(2):80–91
64. Fredriksson R, Lagerstrom MC, Lundin LG et al (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 63(6):1256–1272
65. Farrens DL, Altenbach C, Yang K et al (1996) Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science* 274(5288):768–770
66. Rosenbaum DM, Rasmussen SG, Kobilka BK (2009) The structure and function of G-protein-coupled receptors. *Nature* 459(7245):356–363
67. Kim JM, Hwa J, Garriga P et al (2005) Light-driven activation of beta 2-adrenergic receptor signaling by a chimeric rhodopsin containing the beta 2-adrenergic receptor cytoplasmic loops. *Biochemistry* 44(7):2284–2292
68. Airan RD, Thompson KR, Fenno LE et al (2009) Temporally precise in vivo control of intracellular signalling. *Nature* 458(7241):1025–1029
69. Oh E, Maejima T, Liu C et al (2010) Substitution of 5-HT1A receptor signaling by a light-activated G protein-coupled receptor. *J Biol Chem* 285(40):30825–30836
70. Siuda ER, Copits BA, Schmidt MJ et al (2015) Spatiotemporal control of opioid signaling and behavior. *Neuron* 86(4):923–935
71. Cao P, Sun W, Kramp K et al (2012) Light-sensitive coupling of rhodopsin and melanopsin to G(i/o) and G(q) signal transduction in *Caenorhabditis elegans*. *FASEB J* 26(2):480–491
72. Terakita A, Koyanagi M, Tsukamoto H et al (2004) Counterion displacement in the molecular evolution of the rhodopsin family. *Nat Struct Mol Biol* 11(3):284–289
73. Koyanagi M, Terakita A (2014) Diversity of animal opsin-based pigments and their optogenetic potential. *Biochim Biophys Acta* 1837(5):710–716
74. Moglich A, Yang X, Ayers RA et al (2010) Structure and function of plant photoreceptors. *Annu Rev Plant Biol* 61:21–47
75. Herrou J, Crosson S (2011) Function, structure and mechanism of bacterial photosensory LOV proteins. *Nat Rev Microbiol* 9(10):713–723
76. Moglich A, Ayers RA, Moffat K (2009) Design and signaling mechanism of light-regulated histidine kinases. *J Mol Biol* 385(5):1433–1444
77. Wu YI, Frey D, Lungu OI et al (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 461(7260):104–108
78. Strickland D, Lin Y, Wagner E et al (2012) TULIPs: tunable, light-controlled interacting protein tags for cell biology. *Nat Methods* 9(4):379–384
79. Schmidt D, Tillberg PW, Chen F et al (2014) A fully genetically encoded protein architecture for optical control of peptide ligand concentration. *Nat Commun* 5:3019
80. Winkler A, Barends TR, Udvarhelyi A et al (2015) Structural details of light activation of the LOV2-based photoswitch PA-Rac1. *ACS Chem Biol* 10(2):502–509
81. Mizuno H, Mal TK, Walchli M et al (2008) Light-dependent regulation of structural flexibility in a photochromic fluorescent protein. *Proc Natl Acad Sci U S A* 105(27):9227–9232
82. Zhou XX, Chung HK, Lam AJ et al (2012) Optical control of protein activity by fluorescent protein domains. *Science* 338(6108):810–814
83. Zoltowski BD, Schwerdtfeger C, Widom J et al (2007) Conformational switching in the fungal light sensor Vivid. *Science* 316(5827):1054–1057
84. Nash AI, McNulty R, Shillito ME et al (2011) Structural basis of photosensitivity in a bacterial light-oxygen-voltage/helix-turn-helix (LOV-HTH) DNA-binding protein. *Proc Natl Acad Sci U S A* 108(23):9449–9454
85. Muller K, Engesser R, Schulz S et al (2013) Multi-chromatic control of mammalian gene expression and signaling. *Nucleic Acids Res* 41(12):e124
86. Toettcher JE, Weiner OD, Lim WA (2013) Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. *Cell* 155(6):1422–1434

87. Idevall-Hagren O, Dickson EJ, Hille B et al (2012) Optogenetic control of phosphoinositide metabolism. *Proc Natl Acad Sci U S A* 109(35):E2316–E2323
88. Grusch M, Schelch K, Riedler R et al (2014) Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. *EMBO J* 33(15):1713–1726
89. Shimizu-Sato S, Huq E, Tepperman JM et al (2002) A light-switchable gene promoter system. *Nat Biotechnol* 20(10):1041–1044
90. Liu H, Gomez G, Lin S et al (2012) Optogenetic control of transcription in zebrafish. *PLoS One* 7(11):e50738
91. Kennedy MJ, Hughes RM, Peteya LA et al (2010) Rapid blue-light-mediated induction of protein interactions in living cells. *Nat Methods* 7(12):973–975
92. Boulina M, Samarajeewa H, Baker JD et al (2013) Live imaging of multicolor-labeled cells in *Drosophila*. *Development* 140(7):1605–1613
93. Bugaj LJ, Choksi AT, Mesuda CK et al (2013) Optogenetic protein clustering and signaling activation in mammalian cells. *Nat Methods* 10(3):249–252
94. Kim N, Kim JM, Lee M et al (2014) Spatiotemporal control of fibroblast growth factor receptor signals by blue light. *Chem Biol* 21(7):903–912
95. Taslimi A, Vrana JD, Chen D et al (2014) An optimized optogenetic clustering tool for probing protein interaction and function. *Nat Commun* 5:4925
96. Tischer D, Weiner OD (2014) Illuminating cell signalling with optogenetic tools. *Nat Rev Mol Cell Biol* 15(8):551–558
97. Zhang K, Cui B (2015) Optogenetic control of intracellular signaling pathways. *Trends Biotechnol* 33(2):92–100
98. Losi A, Gartner W (2012) The evolution of flavin-binding photoreceptors: an ancient chromophore serving trendy blue-light sensors. *Annu Rev Plant Biol* 63:49–72
99. Gradinaru V, Zhang F, Ramakrishnan C et al (2010) Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* 141(1):154–165
100. Kralj JM, Douglass AD, Hochbaum DR et al (2012) Optical recording of action potentials in mammalian neurons using a microbial rhodopsin. *Nat Methods* 9(1):90–95
101. Gunaydin LA, Yizhar O, Berndt A et al (2010) Ultrafast optogenetic control. *Nat Neurosci* 13(3):387–392
102. Kleinlogel S, Feldbauer K, Dempski RE et al (2011) Ultra light-sensitive and fast neuronal activation with the Ca²⁺-permeable channelrhodopsin CatCh. *Nat Neurosci* 14(4):513–518
103. Berndt A, Schoenenberger P, Mattis J et al (2011) High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels. *Proc Natl Acad Sci U S A* 108(18):7595–7600
104. Prigge M, Schneider F, Tsunoda SP et al (2012) Color-tuned channelrhodopsins for multiwavelength optogenetics. *J Biol Chem* 287(38):31804–31812
105. Berndt A, Lee SY, Ramakrishnan C et al (2014) Structure-guided transformation of channelrhodopsin into a light-activated chloride channel. *Science* 344(6182):420–424
106. Wietek J, Wiegert JS, Adeishvili N et al (2014) Conversion of channelrhodopsin into a light-gated chloride channel. *Science* 344(6182):409–412
107. Nagel G, Brauner M, Liewald JF et al (2005) Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol* 15(24):2279–2284
108. Berndt A, Yizhar O, Gunaydin LA et al (2009) Bi-stable neural state switches. *Nat Neurosci* 12(2):229–234
109. Mattis J, Tye KM, Ferenczi EA et al (2012) Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat Methods* 9(2):159–172
110. Nash AI, Ko WH, Harper SM et al (2008) A conserved glutamine plays a central role in LOV domain signal transmission and its duration. *Biochemistry* 47(52):13842–13849
111. Zoltowski BD, Vaccaro B, Crane BR (2009) Mechanism-based tuning of a LOV domain photoreceptor. *Nat Chem Biol* 5(11):827–834
112. Gleichmann T, Diensthuber RP, Moglich A (2013) Charting the signal trajectory in a light-oxygen-voltage photoreceptor by random mutagenesis and covariance analysis. *J Biol Chem* 288(41):29345–29355
113. Hochbaum DR, Zhao Y, Farhi SL et al (2014) All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. *Nat Methods* 11(8):825–833
114. Akerboom J, Carreras Calderon N, Tian L et al (2013) Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Front Mol Neurosci* 6:2
115. Krook-Magnuson E, Szabo GG, Armstrong C et al (2014) Cerebellar directed optoge-

- netic intervention inhibits spontaneous hippocampal seizures in a mouse model of temporal lobe epilepsy. *eNeuro* 1(1):pii: e.2014
116. Paz JT, Davidson TJ, Frechette ES et al (2013) Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nat Neurosci* 16(1):64–70
117. Sohal VS, Zhang F, Yizhar O et al (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459(7247):698–702
118. Toettcher JE, Gong D, Lim WA et al (2011) Light-based feedback for controlling intracellular signaling dynamics. *Nat Methods* 8(10):837–839
119. Miliadis-Argeitis A, Summers S, Stewart-Ornstein J et al (2011) In silico feedback for in vivo regulation of a gene expression circuit. *Nat Biotechnol* 29(12):1114–1116