Microbial Rhodopsins as Multi-functional Photoreactive Membrane Proteins for Optogenetics

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In life science research, methods to control biological activities with stimuli such as light, heat, pressure and chemicals have been widely utilized to understand their molecular mechanisms. The knowledge obtained by those methods has built a basis for the development of medicinal products. Among those various stimuli, light has the advantage of a high spatiotemporal resolution that allows for the precise control of biological activities. Photoactive membrane protein rhodopsins from microorganisms (called microbial rhodopsins) absorb visible light and that light absorption triggers the trans–cis photoisomerization of the chromophore retinal, leading to various functions such as ion pumps, ion channels, transcriptional regulators and enzymes. In addition to their biological significance, microbial rhodopsins are widely utilized as fundamental molecular tools for optogenetics, a method to control biological activities by light. In this review, we briefly introduce the molecular basis of representative rhodopsin molecules and their applications for optogenetics. Based on those examples, we discuss the high potential of rhodopsin-based optogenetics tools for basic and clinical research in pharmaceutical sciences.

Key words rhodopsin; optogenetics; retinal; signal transduction

1. INTRODUCTION

Medicinal products continuously support the health of humans through the control of inherent biological activities in cells. In a broad sense, medicinal products can be simplified to stimuli that regulate biological activities. In addition to chemical stimuli (i.e., organic and inorganic compounds) as representative medicinal products, physical stimuli such as heat, pressure and light are also utilized. Thus, we assume that these stimuli are categorized as medicinal products. Among various stimuli, light has the advantage of a high spatiotemporal resolution that allows for the precise control of biological activities. In fact, light (electromagnetic wave) is widely used as a stimulus for various treatments, such as photodynamic therapy (PDT), photothermal therapy (PTT) and photoinmunotherapy (PIT). PDT, PTT and PIT utilize the conversion from light to produce molecular oxygen, to generate heat and to enhance immunostimulating responses, respectively. As a result, those methods can be applied to several diseases such as cancer. For detailed information about those methods, please see other extensive reviews.1,2 One of the most important characteristics of light is that it can control biological activities with a high spatiotemporal resolution.

Rhodopsins are seven-transmembrane proteins that are widely distributed in all domains of life (i.e., archaea, bacteria and eukaryotes).3,4 Rhodopsins commonly consist of an apoprotein opsin and a derivative of vitamin-A retinal as a chromophore (Fig. 1A) and are phylogenetically divided into two classes, animal rhodopsins and microbial rhodopsins.4 Microbial rhodopsins possess all-trans retinal, which is the most thermally stable isomer, as a chromophore and they have been widely discovered in microorganisms such as bacteria, algae and fungi.4 All-trans retinal covalently binds to a conserved lysine (Lys) residue on the seventh (also termed the G) helix of the opsin via a protonated retinal Schiff base (PRSB) linkage, where its positive charge is stabilized by a negatively charged carboxylate called the counterion (i.e., aspartic acid (Asp) or glutamic acid (Glu)).4 Visible light absorption at around 400–700 nm triggers the isomerization from all-trans to 13-cis retinal within an ultrafast time domain (i.e., several hundred femtoseconds).4 The photoisomerization induces a sequence of structural changes of the opsins, resulting in a variety of photobiological functions such as ion transporters, phototactic sensors, transcriptional regulators and enzymes (Fig. 1B). After that, the photoactivated rhodopsin returns to the initial state and the cyclic photoreaction sequence (called the photocycle) is completed5 (Fig. 1A). Ion transport rhodopsins are divided into two types, ion channel rhodopsins and ion pump rhodopsins. Ion channel rhodopsins transport ions across the membrane according to the ion concentration gradient while ion pump rhodopsins actively transport ions across the membrane against ion concentration gradient.

Historically, studies of microbial rhodopsins began with the discovery of the first microbial rhodopsin, bacteriorhodopsin (BR), in 1971.5 BR is abundantly expressed in the halophilic archaean Halobacterium salinarum (formerly halobium), which lives in a highly halophilic environment, and works as a light-driven outward proton pump to form a proton concentration gradient across the cellular membrane (Fig. 1B). The proton gradient generated by BR is utilized as an energy source to produce the molecular currency adenosine triphosphate (ATP) in cells. After the discovery of BR, halorhodopsin (HR) was identified in H. salinarum in 1977.6 HR works as a light-driven inward chloride pump and is thought to control osmotic pressure in cells through chloride ion transport across the membrane (Fig. 1B). Although the direction of ion trans-

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port is opposite between BR and HR, a point mutation around the retinal \( \text{i.e.,} \) D85T in BR converts it into an HR-like inward chloride pump, suggesting that they have a common mechanism of ion transport.

In addition to the ion transport rhodopsins, two types of sensory rhodopsins, sensory rhodopsin I (SRI) and sensory rhodopsin II (SRII), were discovered from the same organism \( H. \text{salinarum} \) in 1982 and 1985 as negative/positive and negative phototactic sensors, respectively. In the cell membrane, SRI and SRII form complexes with their cognate transducer proteins, named Halobacterial transducer protein for SRI (HtrI) and for SRII (HtrII), respectively (Fig. 1B). Light signals are transmitted from the SRI-HtrI and SRII-HtrII complexes to a cytoplasmic two-component signal transduction cascade composed of signaling molecules such as CheA and CheY. SRI and SRII regulate the rotational direction of the flagellar motor through activation of the cascade, which results in positive or negative phototaxis. Thus, in \( H. \text{salinarum} \), the four rhodopsins inherently control several biological activities through their different photo-induced molecular functions. Since then, numerous rhodopsin molecules have been identified from nature with a variety of distinct molecular functions, such as cation channels, anion channels, transcriptional regulators, enzymes, inward proton pumps, outward sodium pumps and a \( \text{SO}_4^{2-} \) transporter (Fig. 1B). For detailed biochemical and biophysical aspects of the above rhodopsins, please see other extensive reviews.

Of note, in 2005, an innovative method using visible light to control biological activities was developed and named optogenetics, in which microbial rhodopsins are heterologously expressed in target cells using genetic methods. In this review, we introduce representative rhodopsin molecules used for optogenetics.

## 2. MICROBIAL RHODOPSINS FOR OPTOGENETICS

### 2.1. Cation Channelrhodopsins (CCRs) for Neural Activation

In 2002 and 2003, channelrhodopsin-1 (CrChR1) and channelrhodopsin-2 (CrChR2) were identified in the single-cell algae \( Chlamydomonas \text{reinhardtii} \). CrChR1 and CrChR2 absorb blue light (around 460 nm) and work as light-gated cation (\( \text{H}^+ \) and \( \text{Na}^+ \)) channels, which induce membrane depolarization through their inward cation (mainly \( \text{Na}^+ \)) transport in cells (Fig. 2A and Table 1). In 2005, Boyden et al. focused on the characteristics of channelrhodopsins and produced rat hippocampal neurons expressing CrChR2 and successfully induced light-dependent neural activation via membrane depolarization as the first demonstration of optogenetics. After that, CrChR1 and CrChR2 (mainly CrChR2) have been introduced into various kinds of neurons (e.g., hippocampal neurons, retinal neurons, dopamine neurons and cortical neurons) by infection with a viral vector for the transient expression and generation of mouse transgenic lines (Fig. 2B). The neural activities of neurons were then regulated by light to elucidate their functions and neural circuits not only in brain slices, but also in living animals (Fig. 2B). In addition to CrChR1 and CrChR2, various related molecules (called cation channelrhodopsins, CCRs) have been identified from nature. In addition to the natural CCRs, the production of genetically modified variants...
For instance, cation channelrhodopsins from *C. noctigama* and *Tetraselmis striata* (named Chrimson and TsChR, respectively) absorb red and blue light (around 590 and 440 nm, respectively), and are employed as red and blue-sensitive neural activators, respectively. A cation channelrhodopsin from *Stigeoclonium helveticum* (named Chronos) shows fast channel closing kinetics, and is employed for high-frequency neural activation. Moreover, C128T mutant of CrChR2 shows slow channel closing kinetics, and is employed for long-term neural activation. These natural and non-natural molecules allow neuroscientists to precisely control neural activities with multi-color light (400–700 nm) and a wide time range (5 ms–100 s).

### 2.2. Halorhodopsin (HR) for Neural Silencing

Halorhodopsin (HR) from *H. salinarum* absorbs green light (around 580 nm) and works as a light-driven inward chloride pump. Proteins homologous to HR have been identified from other archaea and bacteria. In 2007, HR from the archaeon *Na-tronomonas pharaonis* (NpHR) was shown to induce the neural silencing of mouse hippocampal neurons via hyperpolarization of the membrane potential through its inward chloride transport. Thus, NpHR has been utilized as a neural silencer to regulate various kinds of neurons (e.g., hippocampal neurons, cholinergic neurons, dopaminergic neurons and primary somatosensory cortex) in brain slices and in living animals.

Since AR3 is well expressed and localized in cellular membranes and induces a larger photocurrent compared to NpHR and other proton pump rhodopsins, it has been utilized as a typical neural silencer to regulate various kinds of neurons like NpHR. It should be noted that AR3 is also employed for voltage imaging of neurons (Fig. 3B and Table 1). Red light excitation of AR3 (around 560–620 nm) induces near-IR fluo-
rescence (around 660–760 nm). That fluorescence is thought to be derived from a highly fluorescent photointermediate (Q-intermediate) during the photocycle and it is noteworthy that its intensity is highly membrane voltage-sensitive. Therefore, AR3 can detect absolute membrane voltages ranging from -150 to 150 mV, while typical voltage indicators, Ca²⁺-sensors, cannot detect membrane voltage changes below the threshold in neurons. In addition, AR3-based voltage imaging has several advantages as follows: (i) it can be expressed in targeted neurons using genetics, and (ii) it is possible to visualize the membrane voltage with a high temporal resolution (500μs–40ms) resolution. While the wild-type AR3 has been utilized for voltage imaging, it shows light-induced proton pump activity and induce hyperpolarization. Thus, signals for voltage imaging with the wild-type AR3 contains unexpected effects from the membrane hyperpolarization. Recently, several AR3 mutants (e.g., QuasAr2, QuasAr3, Archon-1 and Archon-2) have been developed by random mutagenesis and directed evolution approaches. The light-induced proton pump activities of the mutants are eliminated by introducing mutation at a counterion residue Asp85 in the engineered proteins, allowing to trace the real voltage changes. Those engineered proteins show higher fluorescence, signal-to-noise levels and with improved membrane localization. Thus, the engineered AR3-based voltage indicators have been employed for real-time imaging of neural activities in various kinds of neurons even in living animals.

2.4. Anion Channelrhodopsins (ACRs) for Neural Silencing In 2015, anion channelrhodopsin-1 and -2 (GtACR1 and GtACR2, respectively) were found in the cryptophyte alga *Guillardia theta*. GtACR1 and GtACR2 absorb green and blue light (around 510 and 470 nm, respectively) and work as light-gated anion channels. Before the discovery of GtACR1 and GtACR2, ion pump rhodopsins (i.e., AR3 and NpHR) have been utilized as popular neural silencers. Of note, the ion pump rhodopsins transport only one ion during each photocycle, while ion channel rhodopsins (e.g., GtACR1 and GtACR2) transport thousands of ions during each photocycle, leading to high photocurrent. Thus, GtACR1 and GtACR2 have been recruited as powerful neural silencers that can hyperpolarize the membrane through their inward anion (mainly Cl⁻) transport, since they induce approximately a 1000-fold larger photocurrent compared to AR3 in mammalian cells (Fig. 3A, Table 1). Various related molecules (called anion channelrhodopsins, ACRs) have been identified in nature. In addition to the natural ACRs, the production of genetically modified variants has been reported that show characteristic molecular properties, such as red and blue light-absorption, fast-channel closing and slow-channel closing. These natural and non-natural molecules allow neuroscientists to optically suppress neural activities with multi-color light (400–700 nm) and a wide time range (3 ms–30 s). In fact, ACRs have been utilized as efficient neural silencers to regulate various kinds of neurons even in living animals. Nowadays, ACRs are widely employed as next generation neural silencers instead of NpHR and AR3.

Based on rhodopsin-based neural activation and silencing tools, the optogenetic manipulation of neural activities has been extensively applied to study fundamental neural and brain functions (e.g., memory, learning, motion, sleep, awakening and sexual behavior) and their related diseases. 2.5. *Anabaena* Sensory Rhodopsin (ASR) for Transcriptional Regulation and Control of Endocytosis We now introduce a method to optically control non-neural biological activity. In 2003, the first bacterial sensory rhodopsin was identified from the freshwater cyanobacterium *Anabaena* PCC7120, and was named *Anabaena* sensory rhodopsin (ASR). ASR is encoded in an operon, along with a gene for a small soluble cytoplasmic protein, named the ASR transducer (ASRT). In 2012, we found that ASR represses the transcription of the chromatic adaptive gene cpcB through its C-terminal region in a light-dependent manner, when it is heterologously expressed in *Escherichia coli* cells. When the target gene is replaced by an arbitrary gene, ASR can be utilized as an optical method to regulate the expression of that arbitrary protein in bacterial cells (Table 1).
In 2009, the D217E mutant of ASR was shown to work as a light-driven inward proton pump.\(^{39}\) That engineered molecule is the first inward proton pump rhodopsin. One research group ectopically expressed the ASR mutant in endosomes of Purkinje cell synapses\(^{40}\) (Fig. 4A, Table 1). Since acidification of the endosomal lumen is essential for endocytosis in synapses, the inward proton transport activity of the ASR mutant can de-acidify the endosomal lumen when exposed to light, which induces the inhibition of endocytosis.\(^{40}\) Thus, the ASR mutant has been utilized for the control of synaptic endocytosis, which led to understanding the role of AMPA receptor endocytosis in motor learning in Purkinje cells.\(^{40}\) Recently, two groups of natural inward proton pump rhodopsins, named xenorhodopsins (XeRs) and schizorhodopsins (SzRs), were identified from several archaeal and bacterial species\(^{41,42}\) (Fig. 1B). The ASR mutant and other natural inward proton pump rhodopsins will be utilized as optical de-acidification tools in the endosomal lumen and as optical acidification tools in the intracellular region.

### 3. FUTURE DIRECTIONS

Since the 21st century, advances in genomic analysis have revealed that more than 100000 microbial rhodopsin genes exist in nature.\(^{3,4}\) As expansions of molecular diversity, many characteristic molecules showing novel functions have been identified. For instance, rhodopsins possessing catalytic domains of guanylyl/adenylyl cyclase and phosphodiesterase (called enzyme rhodopsins or Rh-GC, Rh-AC and Rh-PDE) were shown to function as a light-dependent enzymatic activity, which controls the intracellular concentrations of the cyclic nucleotides, cGMP and cAMP\(^{43,44}\) (Fig. 1B). As ion transport rhodopsins, outward sodium pump rhodopsins (NaRs) and a SO\(_4^{2-}\) transport rhodopsin have been discovered in nature\(^{45,46}\) (Fig. 1B). By introducing amino acid mutations in natural NaRs, outward potassium (K\(^+\)) and cesium pump (Cs\(^+\)) molecules have been engineered.\(^{47}\) Moreover, CrChR2 and its variants are permeable to Ca\(^{2+}\), although their permeability was still lower than those of other cations such as Na\(^+\) and H\(^+\).\(^{14,48}\) The production of improved mutants showing higher Ca\(^{2+}\) permeability would be useful to optically control Ca\(^{2+}\) concentrations in living cells and in animals.

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Fig. 4. Schematic Illustration of Optogenetic Control of Various Biological Activities

(A) ASR mutant induces inward proton transport across the endosomal membrane in a light-dependent manner. This proton transport causes the de-acidification of the endosomal lumen, which results in the inhibition of endocytosis in the synapse. (B) Outward and inward proton pump rhodopsins actively transport protons across the membrane, which induce intracellular alkalization and acidification, respectively, in a light-dependent manner. Since the intracellular pH value is related to cell homeostasis and cell death, the proton pump rhodopsins will enable us to optically control cell survival and death through intracellular pH changes. (C) Enzyme rhodopsins regulate intracellular concentrations of cGMP and cAMP in a light-dependent manner. Such types of rhodopsins will enable us to optically control the activation of kinases, increase intracellular Ca\(^{2+}\) concentrations and regulate transcription through the intracellular cyclic nucleotide concentration changes. (Color figure can be accessed in the online version.)
functional diversity of rhodopsins is progressively expanding, which allows us to optically control the intracellular concentrations of a variety of physiologically important ions and cyclic nucleotides (e.g., $H^+$, $Cl^-$, $Na^+$, $K^+$, cGMP and cAMP).

Among them, intracellular concentrations of $H^+$ are strictly regulated to maintain the physiological pH (around 7.4) in mammalian cells. The alteration of intracellular pH would lead to the disruption of homeostasis and cell death.49) We expect that outward and inward proton pump rhodopsins will enable us to optically control cell death and survival through intracellular pH changes (Fig. 4B). As another example, cGMP and cAMP play roles as second messengers to induce various signal transduction cascades, such as the activation of kinases, increase of intracellular $Ca^{2+}$ concentrations and transcriptional regulation50) (Fig. 4C). These cascades are universally involved in regulating basic cellular activities, such as cell development, differentiation, growth and death. It is expected that enzyme rhodopsins will be utilized as optical tools to control broad cellular activities through changes of intracellular cyclic nucleotide concentrations.

The disruption of fundamental cellular activities leads to severe diseases such as cancers and immune disorders. The rhodopsin-based optogenetics tools allow us not only to understand the molecular mechanisms of various cellular activities, but also to develop medicinal products for diseases.3,36) In combination with gene therapy, optogenetics has a potential to partially recover in blind patients by introducing the cation pump.40) In green algae, the long-lifetime photointermediate of s-rhodopsin is a receptor for photosensitivity in genetically engineered neurons expressing green algae light-gated channels.59)

In summary, optogenetics can broadly contribute to basic and clinical research in pharmaceutical sciences. We hope that the expanding optogenetic applications of microbial rhodopsins will shed light on new approaches for drug discovery and therapeutics.

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**Conflict of Interest** The authors declare no conflict of interest.

**REFERENCES**

1) Alsaab HO, Alghamdi MS, Alobaii AS, Alzhrani R, Alwathayanni F, Althobaiti YS, Almalki AH, Saeed A, Iyer AK. Progress in clinical trials of photodynamic therapy for solid tumors and the role of nanomedicine. *Cancers*, 12, 2793 (2020).

2) Wang M, Yao J, Wang M, Li X, Liu K, Naylor MF, Nordquist RE, Chen WR, Zhou F. Cancer photo-immunotherapy: from bench to bedside. *Theranostics*, 11, 2218–2231 (2021).

3) Kojima K, Shibukawa A, Sudo Y. The unlimited potential of microbial rhodopsins as optical tools. *Biochemistry*, 59, 218–229 (2020).

4) Ernst OP, Łodowski DT, Elstner M, Hegemann P, Brown LS, Kandori H. Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. *Chem. Rev.*, 114, 126–163 (2014).

5) Oesterhelt D, Stoeckenius W. Rhodopsin-like protein from the purple membrane of Halobacterium halobium. *Nat. New Biol.*, 233, 149–152 (1971).

6) Matsuno-Yagi A, Mukohata Y. Two possible roles of bacteriorhodopsin; a comparative study of strains of Halobacterium halobium differing in pigmentation. *Biochem. Biophys. Res. Commun.*, 78, 237–243 (1977).

7) Sasaki J, Brown LS, Chon YS, Kandori H, Maeda A, Needleman R, Lanyi JK. Conversion of bacteriorhodopsin into a chloride ion pump. *Science*, 269, 73–75 (1995).

8) Bogomolni RA, Spudich JL. Identification of a third rhodopsin-like pigment in phototactic Halobacterium halobium. *Proc. Natl. Acad. Sci. U.S.A.*, 79, 6250–6254 (1982).

9) Takahashi T, Michizuki Y, Kamo N, Kobatake Y. Evidence that the long-lifetime photointermediate of s-rhodopsin is a receptor for negative phototaxis in Halobacterium halobium. *Biochem. Biophys. Res. Commun.*, 127, 99–105 (1985).

10) Inoue K, Tsukamoto T, Sudo Y. Molecular and evolutionary aspects of microbial sensory rhodopsins. *Biochim. Biophys. Acta*, 1837, 562–577 (2014).

11) Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.*, 8, 1263–1268 (2005).

12) Zhang F, Vierock J, Yizhar O, Ferno LE, Tsunoda S, Kianianmo Meni A, Prigge M, Berndt A, Cushman J, Polie J, Magnuson J, Hegemann P, Deisseroth K. The microbial opsin family of optogenetic tools. *Cell*, 147, 1446–1457 (2011).

13) Nagel G, Ollig D, Feuermann M, Kateri S, Musti AM, Bamberg E, Hegemann P. Channelrhodopsin-1: a light-gated proton channel in green algae. *Science*, 296, 2395–2398 (2002).

14) Nagel G, Szellas T, Huhn W, Kateri S, Adeeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 13940–13945 (2003).

15) Ishizuka T, Kakuda M, Araki R, Yawo H. Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels. *Neurosci. Res.*, 54, 85–94 (2006).

16) Madisen L, Mao T, Koch H, et al. A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nat. Neurosci.*, 15, 793–802 (2012).

17) Berndt A, Yizhar O, Gunaydin LA, Hegemann P, Deisseroth K. Bi-stable neural state switches. *Nat. Neurosci.*, 12, 229–254 (2009).

18) Klapoetke NC, Murata Y, Kim SS, et al. Independent optical excitation of distinct neural populations. *Nat. Methods*, 11, 338–346 (2014).

19) Lanyi JK, Oesterhelt D. Identification of the retinal-binding protein in halobacterium. *J. Biol. Chem.*, 257, 2674–2677 (1982).

20) Zhang F, Wang LP, Brauner N, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K. Multimodal fast optical interrogation of neural circuitry. *Nature*, 446, 633–639 (2007).

21) Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell*, 141, 154–165 (2010).

22) Ibara K, Umemura T, Katagiri I, Kitajima-Ibara T, Sugiyma Y, Kimura Y, Mukohata Y. Evolution of the archaeal rhodopsins: evolution rate changes by gene duplication and functional differentiation. *J. Mol. Biol.*, 285, 163–174 (1999).

23) Sudo Y, Okazaki A, Ono H, Yagasaki J, Sugo S, Kamiya M, Reissig L, Inoue K, Ibara K, Kandori H, Takagi S, Hayashi S. A blue-shifted light-driven proton pump for neural silencing. *J. Biol. Chem.*, 288, 20624–20632 (2013).

24) Chow BY, Han X, Dobry AS, Qian XF, Chuong AS, Li MJ, Henninger MA, Belfort GM, Lin YX, Monahan PE, Boyden ES. High-
32) Govorunova EG, Sineshchekov OA, Hemmati R, Janz R, Morelle O,
Hashimoto N, Shimono K, Yamashita K, Yamamoto M, Miyasuchi S, Takagi S, Hayashi S, Murata T, Sudo Y. X-ray crystallographic structure of thermophilic rhodopsin: implications for high thermal stability and optogenetics function. J. Biol. Chem., 291, 12223–12232 (2016).

26) Kralj JM, Douglass AD, Hochbaum DR, Maclaurin D, Cohen AE. Optical recording of action potentials in mammalian neurons using a microbial rhodopsin. Nat. Methods, 9, 90–95 (2011).

28) Piatkevich KD, Jung EE, Straub C, Kralj JM, Douglass AD, Hochbaum DR, Maclaurin D, Cohen AE. Structure of thermophilic rhodopsin: implications for high thermal stability and optogenetics function. J. Biol. Chem., 291, 12223–12232 (2016).

29) Kojima K, Kurihara R, Sakamoto M, Takanashi T, Kuramochi H, Zhang XM, Bito H, Tahara T, Sudo Y. Comparative studies of the fluorescence properties of microbial rhodopsins: spontaneous emission versus photointermediate fluorescence. J. Phys. Chem. B, 124, 7361–7367 (2020).

25) Tsukamoto T, Mizutani K, Hasegawa T, Takahashi M, Honda N, Kralj JM, Douglass AD, Hochbaum DR, Maclaurin D, Cohen AE. Optical recording of action potentials in mammalian neurons using a microbial rhodopsin. Nat. Methods, 9, 90–95 (2011).

27) Kojima K, Kurihara R, Sakamoto M, Takanashi T, Kuramochi H, Zhang XM, Bito H, Tahara T, Sudo Y. Comparative studies of the fluorescence properties of microbial rhodopsins: spontaneous emission versus photointermediate fluorescence. J. Phys. Chem. B, 124, 7361–7367 (2020).

33) Kato HE, Kim YS, Paggi JM, Fenno LE, Yamashita K, Hilger I, Inoue K, Ono H, Abe-Yoshizumi R, Yoshizawa S, Ito H, Kogure K, Kandori H. Light-driven sodium ion pump in marine bacteria. Nat. Commun., 4, 1678 (2013).

38) Irieda H, Morita T, Maki K, Homma M, Aiba H, Sudo Y. Photo-induced regulation of the chromatic adaptive gene expression by Anabaena sensory rhodopsin. J. Biol. Chem., 287, 32485–32493 (2012).

39) Kawanabe A, Furutani Y, Jung KH, Kandori H. Engineering an inward proton transport from a bacterial sensor rhodopsin. J. Am. Chem. Soc., 131, 16439–16444 (2009).

40) Kakegawa W, Katoh A, Narumi S, Miura E, Motohashi J, Takahashi A, Kohda K, Fukazawa Y, Yuzaki M, Matsuda S. Optogenetic control of synaptic AMPA receptor endocytosis reveals roles of LTD in motor learning. Neuron, 99, 985–999.e6 (2018).

41) Inoue S, Yoshizawa S, Nakajima Y, Kojima K, Tsukamoto T, Kikukawa T, Sudo Y. Spectroscopic characteristics of Rubricoccus marinus xenorhodopsin (RmXeR) and a putative model for its inward transport mechanism. Phys. Chem. Chem. Phys., 20, 3172–3183 (2018).

42) Kojima K, Yoshizawa S, Hasegawa M, Nakama K, Kurihara M, Kikukawa T, Sudo Y. Lokiarchaeota archaeon schizorhodopsin-2 (LaSzR2) is an inward proton pump displaying a characteristic feature of acid-induced spectral blue-shift. Sci. Rep., 10, 20857 (2020).

43) Avelar GM, Schluncken RJ, Zaini PA, Leonard G, Richards TA, Goes SL. A rhodopsin-guanyl cyclase gene fusion functions in visual perception in a fungus. Curr. Biol., 24, 1234–1240 (2014).

44) Yoshida K, Tamoda SP, Brown LS, Kandori H. A unique chloro- flagellate enzyme rhodopsin exhibits light-dependent cyclic nucleotide phosphodiesterase activity. J. Biol. Chem., 292, 7531–7541 (2017).

45) Inoue K, Ono H, Abe-Yoshizumi R, Yoshizawa S, Ito H, Kogure K, Kandori H. A light-driven sodium ion pump in marine bacteria. Nat. Commun., 4, 1678 (2013).

46) Niho A, Yoshizawa S, Tsukamoto T, Kuroki H, Tahara T, Sudo Y, Kojima K, Mizuno M, Kuramochi H, Tahara T, Mizutani Y, Sudo Y. Demonstration of a light-driven SO + transporter and its spectroscopic characteristics. J. Am. Chem. Soc., 139, 4376–4389 (2017).

47) Konno M, Kato Y, Kandori H. Mutant of a light-driven sodium ion pump can transport cesium ions. J. Phys. Chem. Lett., 7, 51–55 (2016).

48) Kleinlogel S, Feldbauer K, Dempski RE, Fotis H, Wood PG, Bamann C, Bamberg E. Ultra light-sensitive and fast neuronal activation with the Ca 2 + -permeable channelrhodopsin CatCh. Nat. Neurosci., 14, 513–518 (2011).

49) Lagadic-Gossmann D, Huc E, Lecureur V. Alterations of intracellular pH homeostasis in apoptotic: origins and roles. Cell Death Differ., 11, 953–961 (2004).

50) Francis SH, Corbin JD. Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. Curr. Rev. Clin. Lab. Sci., 36, 275–328 (1999).

51) Sabel JA, Boulanger-Schemama E, Pagot C, Arleo A, Galluppi F, Martel JM, Esposti S, Delaux A, de Saint Aubert JB, de Montaleau C, Gutman E, Audo J, Duebel J, Pecaud S, Dalkara D, Blouin L, Tazel M, Roska B. Partial recovery of visual function in a blind patient after optogenetic therapy. Nat. Med., 27, 1223–1229 (2021).