

# Optogenetics: A Cellular Photoactivation Method and Its Applications in Biomedical Sciences

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## Abstract

In contrast to the classical activation by microelectrodes, Optogenetics uses light to control ion movement in a more robust manner across the membrane of an engineered cell, alternating the cells function in a desired manner. This review identifies optogenetics, the cascade of events that leads to alternating ion movement, and factors that influence microbial opsins protein function. It discusses factors needed to be considered for an application of optogenetics as a tool and the wavelength of light required to induce retinal isomerization on each type of opsin proteins that leads to photoactivation. Understanding the architecture of opsin proteins is critical in understanding the mechanism by which it transports individual ions. Therefore, in this article, it was necessary to focus on simplifying and explaining the crystal structure of the most common types of opsins (channelrhodopsin, halorhodopsin, and opsin-GPCR), and to explain the changes in protein structure and functional behavior when photo-excitation and inhibition takes place.

## Keywords

Optogenetics, Channelrhodopsin, Opsin, GPCR, Cell Signaling, Photo-Activation, Ion Movement

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## 1. Introduction

The term optogenetics refers to the use of both optical and molecular techniques to monitor cellular and tissue events with genetically engineered photosensitive tools, and involves three core features: I) microbial opsins, II) methods for targeting opsin gene expression and III) methods for targeting cells of living tissues through precise and fast optical control [1]. It has also been developed to manipulate protein transcription, protein-protein interactions and other cellular biochemical events [2]. The targeted control tool of optogenetics responds to light and delivers effector functions such as neural firing. Optogenetics arose from knowledge of an unmet need, regarding the study of biological systems. Addressing this need required a process that I) delivers light

to the tissues that are being investigated, II) targets the groups to the cells that are of interest and III) evokes activity that is then measured, from electrical recordings or targeted imaging. These steps facilitated the control of specific events in relative cell types at certain times in live, intact biological systems. So a defined cell in a system can be controlled, while leaving others unaltered unlike other delivery methods that may cause the recruitment or inhibition of arbitrary receptor channels [3].

The microbial molecules that allow the illumination of individual cells containing exogenous light-activated proteins is known as opsin proteins (seven-transmembrane helices that are found in various organisms such as archaea and plants [3]). As a result of the opsins, the cells of archaea and plants respond to light by the translocation of specific ions

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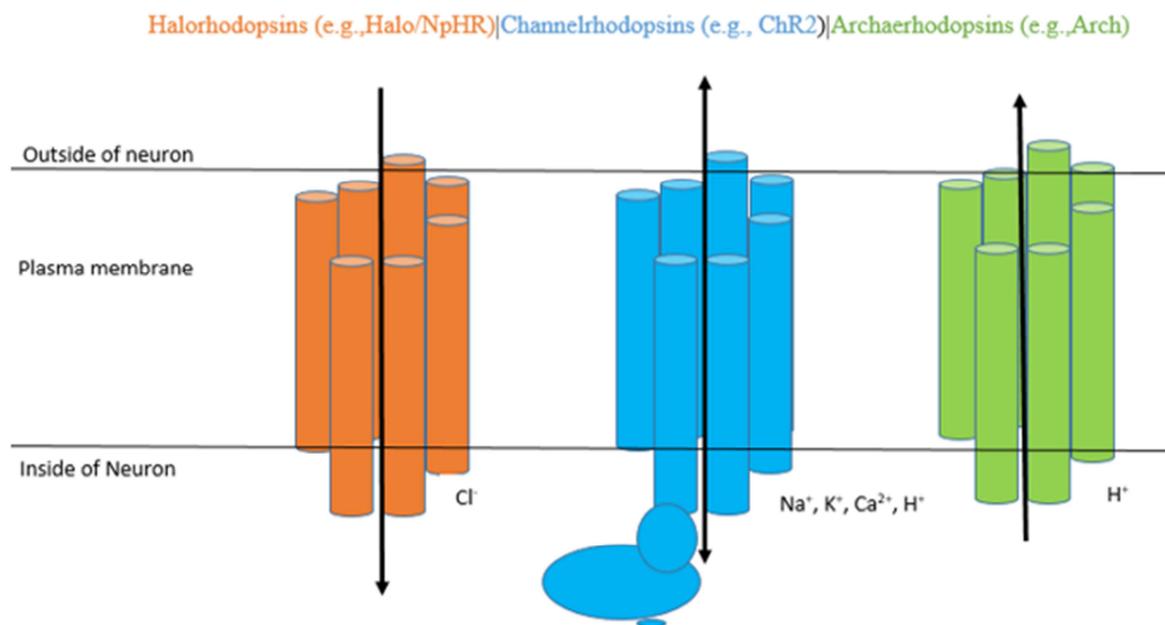
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from one side of the membrane to the other. The response of opsin proteins to light brings about ion transfer which is translated into cellular activity or inhibition depending on the type of opsins and ions involved in the transfer. Opsin genes can be divided into two types. Type 1 opsin genes found in prokaryotes, algae, and fungi which include channelrhodopsin and halorhodopsin photosensitive proteins. Type 2 opsin genes encode for G-protein coupled receptors (GPCR) chimeric molecule and a light-activated transgene system [4, 5].

All opsin proteins require retinal (Vitamin -A derivative) for their function. When retinal binds to the functional pocket of an opsin, the protein complex is called rhodopsin. The substrate is attached to a lysine residue at helix 7 of the opsin protein. This binding of the substrate to the lysine residue specifies the spectral and kinetic properties of the particular

opsin. Illumination of the rhodopsin induces retinal isomerization (retinal has several isomers depending on the wavelength of light it is exposed to); this causes conformational changes within the protein itself and affects the subsequent functions of the protein. Since retinal is present in all vertebrate tissues, no chemical additives are required for this technique [6, 7].

The genetically engineered cells usually are stimulated by light, then the light-activation effects are observed on cellular behavior. The influence of light illumination and photosensitive protein activation can then be monitored by a group of cells or a whole organ. Therefore, optogenetics is a suitable method for monitoring the electrophysiological, and functional behavioral changes resulting from photo-stimulation [7].



**Figure 1.** A schematic diagram indicating direction of ion flow through ion channels as a result of the activation of three types of opsins used in optogenetic technology [adapted from Deisseroth, 2015].

## 2. Types of Photosensitive Proteins: Channelrhodopsin, Halorhodopsin and Archaerhodopsin

Channelrhodopsin (ChR) is a *Chlamydomonas* (genus of green algae) protein that selectively allows cations ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ ) to enter the cell in response to 470 nm wavelength light. When ChR is expressed in neurons, it supports light-driven neural depolarization as it is illuminated by blue light [3]. ChR2 is two times more potent than ChR1 in cation conductance and induces depolarization regardless of the extracellular pH. When ChR2 was engineered and expressed

in cells, it was demonstrated that it could be used to depolarize cells of various sizes by illuminating it with a suitable wavelength of light [8]. When ChR2 was expressed in nerve cells, it created action potentials when the engineered cells were illuminated by blue light. This illumination causes precise control and as a result allows altering of neural activity which can be performed at high precision [9]. ChR2 was mainly used to alter the membrane potential in neurons. Recently however, it has been applied to other cell types, such as cardiomyocytes and skeletal muscle cells. This approach has proven to be useful in biological micro-devices which regulate concentrations of light in tissues by targeted illumination [10]. Two other types of rhodopsins have been identified in *Volvox carteri* (VChR1-

another algal species). VChR2 is similar to ChR2 activated by the same wavelength of light. However, VChR1 is different from other channelrhodopsins as it is activated by 540 nm light, providing an advantage for optogenetic applications. Also, alteration of the amino acid sequence at the active position of the rhodopsin proteins leads to changes in photosensitivity of the resulting protein and ion conductance potency [11].

Halorhodopsins are involved in light driven inward chloride pumps, supporting hyperpolarization when activated by yellow light [3]. Two types of Halorhodopsins were isolated (*Halobacterium salinarium* and *Natronobacterium pharaonis*) which achieve antagonistic control through different wavelength sensitivities at various ion permeabilities as shown later in the description of the crystal structure of halorhodopsins [12].

Archaerhodopsin are involved in light driven outward proton pumps, when these opsins are expressed in neurons, they support light-driven hyperpolarization [3]. The wavelength of light needed for these molecules to be activated at typical levels for scientific experiments, falls in the range of 0.1-10mW/mm [3] This range allows for safe scientific experimentation and it is not high enough to cause background light that affects the molecules [3].

### 3. Other Types of Photosensitive Proteins: Opsin/ G – Protein Coupled Receptor (GPCR) Chimeras and Other Opsins

Opsin – GPCR chimeras are also known as OptoXRs, and activate corresponding GPCR signaling cascades (cAMP and IP3 pathways) in response to light. Different types of G-proteins can be used in OptoXR chimeras including Gi, Gs, and Gq [12, 13]. Therefore, OptoXR techniques have been used to precisely manipulate, monitor, or control intracellular signaling pathways in live and intact organisms. OptoXRs were also applied to investigate the roles of G-proteins in neurons, cardiac tissue, and immune cells [14].

### 4. Gene Delivery of Optogenetic Experiments

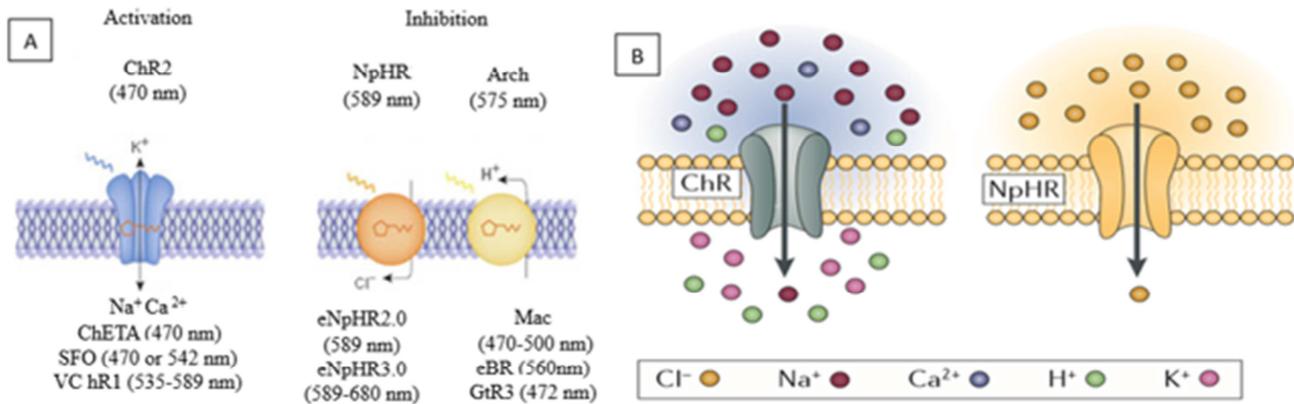
In optogenetic experiments, cells are genetically engineered to achieve expression of the opsin protein. Opsin genes can be transferred to target cells via viral vectors (transfection). The most common vectors used are retroviruses and adeno-

associated viruses (AAV) [6]. For optogenetic tissue targeting, viral vectors can be injected into the target tissue (example; a particular region of the brain) by a simple procedure [15, 16]. Although the use of viral vectors is so common, target genes can also be delivered by an expression plasmid into a single cell or tissue with the aid of an electric field in a process called electroporation. These methods allow rapid testing of the new genetically engineered cells with high levels of expression. Viral vectors also confer tissue or cell type specificity which could be achieved by several methods including; serotype – specific tropism or Cre recombinase expression, through which the opsin gene is linked to a fluorescent reporter and activated by the Cre expression. Although this approach seems to fulfill the requirements of opsin gene delivery, it has some limitations including infection efficiency depending on the viral quality and titer which may vary from one batch to another. In this case, high expressions rates may lead to accumulation of proteins to toxic levels which limits experimentation time and potential immunogenicity of viral particles [15, 17].

The limitations of viral vector gene delivery and electroporation can be overcome by using germline transgenesis. Germline transgenesis (such as testis electroporation or lentivirus – mediated transgenesis) confers protein expressions in animals without the need for further opsin gene delivery to specific tissue. However, this method achieves expression levels lower than that of viral vector gene delivery [15].

### 5. Light Targeting of Optogenetic Experiments

Another key element in an optogenetic research is the light used to illuminate target cells. Delivering the right wavelength of light at the correct intensity and scattering to the exact place is a key factor in the success of an optogenetic experiment. Different patterns of illumination have been reported. After choosing the appropriate wavelength of light, in vitro experiments use microscopic illumination with the desired wavelength of light (including fluorescence microscopy) [18]. One advantage is the use of the condenser or tube lens, in vitro, to focus the light and prevent scattering. In vivo experiments on the other hand are more complicated due to the difficulty of delivering the light to target cells in a tissue. In vivo, photostimulation can be achieved by one-photon or two-photon illumination and imaging by digital micromirror devices (DMD). This approach has utilized fiber optics to deliver targeted photostimulation [16].



**Figure 2.** Illustrates the most common types of photosensitive proteins; Channelrhodopsin (ChR2) and Halorhodopsin (NpHR). (A) depicts the excitation of membrane-bound proteins with the appropriate wavelength of light [19, 20]. (B) represents ion movement across the protein channels upon illumination [21].

## 6. Further Applications

There are several other applications in optogenetics that are intriguing such as light induced apoptosis. This is regulated by a cellular suicidal mechanism. Regulation of apoptosis is as a result of caspases which are a family of cysteine proteases that can be activated. The initial caspases cleave and activate the effector caspases that are downstream; these downstream effector caspases then degrade other cellular proteins and cause apoptosis. It was realized through the process of optogenetics that caspases and their subsequent effect on apoptosis, can be controlled by light. This can be done as a result of fusing a Light- Oxygen-voltage-sensing (LOV) domain to the apoptosis-executing domain of caspase-7. Under dark conditions, the LOV domain would be expected to block the caspase activity; alternatively, blue light would cause conformational changes in the LOV domain, releasing inhibition of the caspase activity and causing cell apoptosis. Using optogenetics in this manner can potentially lead to induced cell apoptosis of transfected cancer cells which can specifically be targeted for destruction [22, 23].

Optogenetic approaches also led to new ways to specifically study signal transduction in live biological systems. Multiple signaling pathways are examples of this as they are activated by growth factors/inputs that regulate outputs of proliferation, migration, differentiation and apoptosis. Conventional methods used to facilitate this input and output requirements are intrinsic or chemical means (such as making use of agonists and antagonists) and genetic perturbations (gain- or loss-of-function). These approaches reveal the components that are essential for signaling pathways, but they lack the spatial and temporal control that is needed to decode the dynamic information of intracellular signal transduction. These limitations are eliminated in optogenetics [23].

Another intriguing application of optogenetics is the light controlled production, inactivation and degradation of proteins. In this system of optogenetics, both light mediated heterodimerization of CRY2 and CIB1 and homodimerization of CRY2 is used. CIB1 is fused to a multimeric protein Ca<sup>2+</sup> calmodulin-dependent protein kinase IIa (CIB1-MP). The target protein was shown to be fused to CRY2 and photo-activated CRY2 proteins were oligomerized and bound to CIB1-MP, inducing the formation of higher order clusters by promoting the interconnection among MPs. The clusters serve as traps that inactivate target proteins (notwithstanding these results, it should be noted that the light that is used for excitation, may also induce photo-toxicity based on the wavelength of light, exposure time, and intensity) [22].

Optogenetics have also been applied in understanding neural cell functions, neural firing and neural cell inhibition. It is also applied in determining certain nerve loci or clusters that control the function of a specific tissue. For example, optogenetics stimulation in the prefrontal cortex was found to initiate rapid anti depression like responses in mice [28, 34]. Optogenetics has also been implemented to investigate understanding of social behavior [29] and memory formation [40]. Optogenetics as a tool can be implemented in many other applications, treating seizures, Parkinson's disease and early-stage dementia.

Lack of a proper beta-carotenoid cleavage enzyme (beta-carotene 15,15'-monooxygenase) in plant kingdom that is required to produce retinal limits the use optogenetics in plant biotechnology application. However, it is known that many physiological processes (photosynthesis, respiration, guard cell movement etc) in the plant kingdom are regulated by the ion movements across organelle/cell membranes. To apply the technology in plant kingdom, application of retinal to plants expressing proper opsins in the targeted

tissues can be utilized. In addition, the beta-carotene cleavage enzyme from animals can be over-expressed in the targeted plant tissues expressing the opsins to allow the channels regulate the physiological process.

## 7. Crystal Structure of Opsin Proteins

After the discovery of opsin proteins and promising applications in optogenetics, studying the crystal structure of these proteins has become a necessity. The need for studying structures lies in their critical applications, especially in neuroscience and the need to find other light-gated channels that respond to different wavelengths, or new channels that allow the passage of various cations. The following sections will discuss some of the light-driven ion channels and highlight opsin crystal structures.

### 7.1. Channelrhodopsins

Channelrhodopsins (ChRs) were the first identified opsin proteins found in *Chlamydomonas reinhardtii*, other green algae, fungi archaea, and eubacteria. Many types of channelrhodopsins have been identified, and their molecular machinery studied, leading to the construction of ChR chimeras with optimal light sensitivity and cation selectivity. ChRs consist of seven transmembrane folds (7-TM); designated the letters A through G, with a retinal C<sub>13</sub>=C<sub>14</sub> chromophore linked to a lysine to form a protonated Schiff base (SB) [7, 24]. Light-driven retinal isomerization leads to channel opening due to both conformational and electrostatic changes of the ion channel, called a photocycle.

The transmembrane domain of ChRs confined between amino acids 1 – 315 consist the ion channel of the protein. Also, the protein contains a cytoplasmic domain of  $\approx$  400 amino acids. The function of the cytoplasmic domain has still not been identified. Of the most commonly studied type of ChRs is ChR2, which encouraged researchers to form channelrhodopsin chimeras to have a different cation conductivity and light sensitivity. C1C2 chimera is a type of channelrhodopsin that consists of two helices (F and G) of ChR2 and five helices (A to E) of ChR1 with some alterations in the C-terminus. C1C2 chimeric structure shares helix similarity and properties with ChR2. Therefore, the following explains the crystal structure based on research findings in ChR2 and C1C2 chimeric structure and imposes it with other types of ChRs [17, 25].

In ChR2, helices A and B have little sequence homology with other types of ChRs and contain a large number of charged amino acid residues. These two helices lean outward by 3 – 4 Å compared to the bacteriorhodopsin structure. The protein is

surrounded by helices A, B, C, and G with a cavity towards the extracellular medium. These helices create an electronegative pore rich in charges and polar amino acid residues, mostly contributed by helix B. Helix B also contain five glutamate residues (E82, E83, E90, E97, and E101). At neutral pH, E90, E83, and E97 are present in the protonated form, while E82 and E101 exist in an ionic form. Depending on the protonation state of E90, it has been found that E90 serves another two purposes: it plays a role in cation selectivity along with the other helices, and it works as a pH sensor to adapt ChR2 to pH of the medium on both sides of the cell membrane. The cavity of the protein contains several restriction sites, including the bulk side chain of helix A (Y70 position), the hydrogen bonds between E90 (of helix B) and N258 (of helix G), and the E83 (of helix B) and H134 (of helix C), which also behaves as a Schiff base proton donor. The cavity opens to the extracellular side, creating a pathway for cation crossing, but the intracellular side is blocked [21, 26].

Helices C and D make a dimer called the helix C-helix D dimer gate (DC gate), which is essential for ChR function. Experiments show that mutations in the DC gate cause  $10^2 - 10^5$  fold increase in conduction lifetime, particularly in C128 and D156, which are conserved in all types of ChRs. In D156 and C128, the aspartic acid and the cysteine residue side chains, respectively, are linked by hydrogen bonds. The carbonyl group of aspartic acid can be a proton acceptor, and the thiol group of cysteine can be the proton donor (type I). On the other hand, the sulfur atom of the thiol group in cysteine can be a hydrogen acceptor and the hydroxyl group of aspartic acid can be a hydrogen donor (Type II). Energy calculations, statistical analyses, and X-ray structures show that type II interaction is more favorable over type I. The complete nature of the DC gate is still a research controversy. Studies suggest that the hydrogen bond is between the C128 and D156 on one side and the  $\pi$  bonds of the retinal from the other side. Other studies suggested a water molecule links the C128 and D156 residues like a bridge. Therefore, a complete resolution of the controversy is yet to be determined, however, all tests agree that the DC gate is highly essential to the mechanism of ChRs [25, 26].

Passage of cations through the channel is governed by alterations in helices A-B-G and the DC gate. Retinal isomerization affects the DC gate primarily at C128, which has Van der Waals attractions with retinal. The cis-trans structure of retinal undergoes light-induced isomerization of its C-C and C=C bonds. On ChR2, retinal exists in its all-trans conformation specifically during the dark state. Upon illumination, most of the all-trans retinal will be converted to 13-cis retinal (a small fraction will be 11-cis and 9-cis retinal). Since retinal forms a protonated Schiff base (SB) with the lysine residue at C128, the change in retinal

conformation is mediated by the deprotonation/ reprotonation of the SB. Under illumination, “on-gating” occurs: the SB deprotonates and retinal turns to its 13-cis conformation causing structural changes of the protein. Helices B and F play a significant role in protein conformational changes along with the DC gate protonation state. The intracellular fragment of helix B moves outward, helix F undergoes few helix rearrangements, and deprotonation of D156 at the DC gate occurs. These changes allow the influx of water and cations through the channel. On the other hand, “off-gating” causes retinal to revert back to the all-trans structure due to reprotonation of the SB, protonation of D156 and its hydrogen bonding with C128 at the DC gate, and deprotonation of E90 at helix B which, hypothetically, could lead to stronger interaction with both; N258 at helix G and S63 at helix A. Therefore, it restrains the flow of water and cations [26, 27].

## 7.2. Halorhodopsins

Halorhodopsin (hR) are retinal bound transmembrane archaeal proteins, identified in *Halobacterium salinarum* (shR) and *Natronomonas pharaonis* (phR) and other archaeal species. They are composed of seven transmembrane helices with a central lysine residue that binds to retinal by a SB at the core of the protein. These helices are designated letters A through G and are connected by loops: the helix B-helix C (BC) loop between helix B and helix C, and interchain interactions including hydrogen bonds and hydrophobic interactions [27]. The protein also contains a short N-terminal helix and a C-terminal region. In phR, hydrophobic residues of the N-terminal helix form a hydrophobic cap along with nonpolar residues of the BC loop which, in turn, interacts with nonpolar residues from helices A, B, D, F and G. This cap covers the extracellular surface of the protein and prevents a rapid exchange of charged ions (chloride) between the active center and the extracellular space. shR lacks the hydrophobic cap. Instead, it contains a secondary binding site of chloride that is surrounded by Arg 24, Arg 103, and Glu 105 [27, 28]. Upon illumination, retinal isomerization causes conformational changes in the protein resulting in inward transport of chloride ion. Retinal lies in a pocket surrounded by the protonated SB and several aromatic residues. It is not directly bound to the SB. Instead, it is attached to the  $\epsilon$ -amino group of Lys 256 residue. In phR, another chromophore, bacterioruberin, is bound to the protein. Therefore, phR exhibits variable colors depending on the direction of the polarization plane when investigating its crystal form. Bacterioruberin is located between adjacent protomers in the trimeric structure of phR. On one side, it is located between helices A and B. The other end of bacterioruberin is located between helices D and E. The terminal hydrophilic group of bacterioruberin interacts with a chloride ion located between

Asn 147, Lys 148, and Lys 65 residues. The other terminal of bacterioruberin interacts weakly with polar residues of the protein and lipids on the extracellular surface of the protein [7, 27, 29]. Although it does not participate in chloride ion transfer, bacterioruberin protects the protein against the harmful intensities of sunlight in archaeal species. It also acts as a sensor for membrane potential. On the other hand, in shR, palmitate is suggested to do bacterioruberin's function. However, the lipid component of the protein is lost during protein purification or crystallization [28].

Halorhodopsin contains ionizable residues that facilitate chloride transport across the cell membrane. These residues are the Schiff base, T111 (in shR) or T126 in phR), D 212, E 194, T230 and V122. Chloride binding sites differ between phR and shR. The primary chloride binding site is conserved between all types of halorhodopsin. However, it is the only binding site in phR, while in shR, there are three chloride binding sites; T111 (site B) on the extracellular side of the protein, T230 (section C), near which the other chloride ion is bound, and R108 (site A). Halorhodopsins can be converted to light – driven proton pumps when the chloride ion in the primary binding site is replaced by an inorganic base (azide) [28, 29, 30].

Another four ionizable residues (Arg 123, Arg 176, Glu 234, and His 100) are conserved in all homologs of halorhodopsins. These residues align the core of the protein between the chloride binding site and the extracellular space. Therefore, it has been suggested that it plays a central role in chloride ion uptake from the extracellular space. Several water molecules are hydrogen bonded to the protein core and extend to the extracellular surface. Since His 100 lies at the chloride inlet of the protein, it was noticed that replacement of His 100 with alanine results in a significant decrease in chloride transport activity. Water distribution also differs between phR and shR due to the difference in helix C (T126 in phR and T111 in shR). This difference explains why the affinity of chloride to the primary binding site is much higher in phR than in shR. Moreover, phR contains a proline residue (P132) causing a kink in the middle of helix C which plays a role in chloride ion transport [29, 30].

The photocycle in halorhodopsin is divided into intermediate states. During the dark state, chloride is located between the protonated Schiff base and Arg 108 guanidino group. Upon illumination, isomerization of retinal to 13-cis configuration induces conformational changes in the protein, changing the Schiff base location and disrupting the anion-binding pocket interaction (K-state). This leaves the anion to bind to other residues reversibly (L state). At this state, photoactivation decreases the anion affinity of Site B (T111). Thus, chloride moves from site B to site C (T230), where it will be released to the cytoplasm. Then, site B will be replenished with

chloride from site A, and site A will acquire a chloride ion from the extracellular space [27, 30].

### 7.3. Bacteriorhodopsin

Like halorhodopsin, bacteriorhodopsin is also composed of seven transmembrane helices with an ionizable core (D85, D96, E194, and E204) necessary for proton pumping. Retinal exists in bacteriorhodopsin in the all-trans form. Upon illumination, retinal isomerization takes place, changing its configuration to 13-cis [28]. Retinal isomerization induces conformational changes in bacteriorhodopsin and the beginning of the photocycle. Similar to halorhodopsin, the photocycle in bacteriorhodopsin is divided into states that involve series of ionizable residues that mediate the transfer of a proton extracellularly, according to changes in charge and pH of these residues. At the beginning of the photocycle, a proton is transferred from the Schiff base to D85. This is referred to as the early M state, and this proton is replenished by deprotonation of D96 at the N state. Protonated D85 loses its proton to E194/E204 at the late M state. This shared proton between E194 and E204 could be in the form of hydronium ion. The shared proton is expelled to the extracellular space. During the O state, re-protonation of D96 occurs through acquisition of a proton from the cytoplasm. The function of bacteriorhodopsin can be altered to transport chloride into the cell by substituting Asp 85 (D85) with Serine, Threonine, or Asparagine [28, 31, 32].

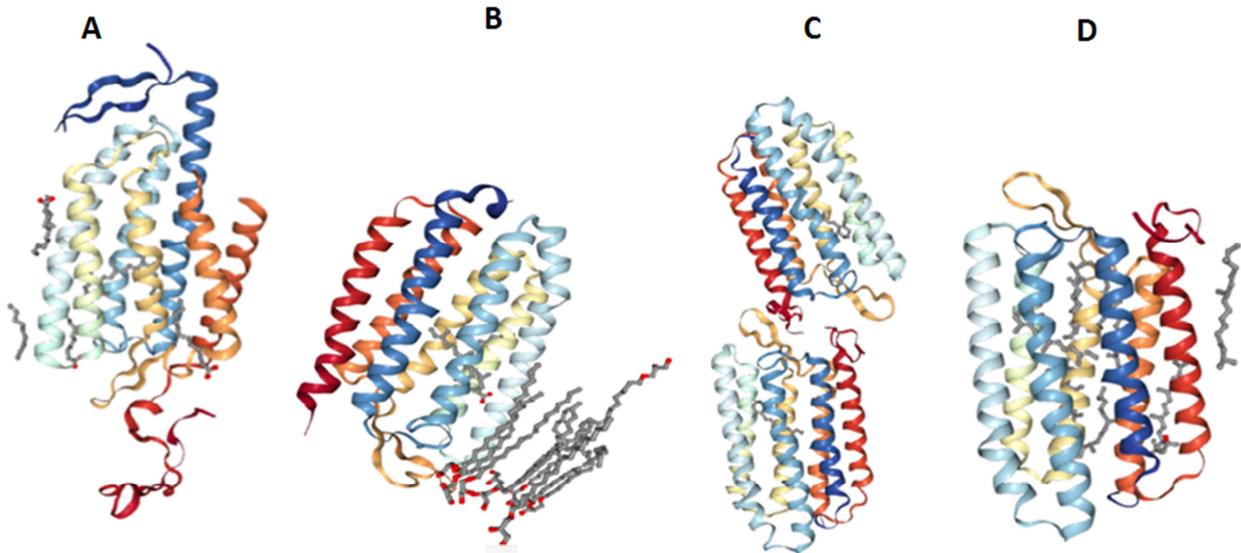
### 7.4. Opsin – GPCR (optoXR)

G-protein coupled receptors are a large family of transmembrane proteins associated with  $G\alpha$ ,  $G\beta$ , and  $G\gamma$  protein subunits intracellularly. The most common type of these proteins is rhodopsin. It is also composed of seven – transmembrane helices designated I through VII, which span the membrane bilayer, like other opsin proteins. Many rhodopsin helices contain kinks introduced by proline residues on these helices. These helices are IV, V, VI, and VII. In some helices, more than one proline exists; one example is helix VII which contain two kinks and helix IV with two adjacent proline residues (Pro170, Pro 171). On the other hand, helix II contains two adjacent glycine residues (Gly89, Gly90) introducing a bend in the polypeptide chain [16, 33].

The polyene chromophore “retinal” exists in its 11-cis configuration during the dark state. The binding pocket of

retinal is mainly composed of hydrophobic residues. Two phenylalanine side chains (Phe212 and Phe261) and Glu212 located near the  $\beta$ -ionone ring of retinal. Also, the Trp265 side chain lies near the center of the polyene chain and retinal bends around it during the dark state (11-cis). The chromophore is bound to Lys296 residue of the protonated SB in the middle of helix VII. Since the protonated SB is positively charged, Glu113 (the counterion) lies near the protonated SB and is highly conserved between rhodopsin homologs. It forms a salt bridge with the SB and plays a vital role in protein activation and resting. Upon photoactivation, a disruption to the salt bridge and movement of helix VI takes place. The counterion inhibits spontaneous hydrolysis of the protonated SB. Also, several water molecules are found in the transmembrane portion of the protein. These water molecules play a role in stabilizing interhelical interactions, the response to a particular wavelength of light, and some play a role in transferring the counterion from Glu113 to Glu181 [33, 34].

It is proposed that rhodopsin exists in a dimer form. Rhodopsin dimers contact each other via transmembrane helices IV and V. Activation of the dimer occurs by conformational changes within helix VI and the AsnProXaaXaaTyr (NPXXY) sequence at the end of helix VII in only one rhodopsin molecule within the dimer. Rhodopsins interact with the G protein subunits via the N and C-terminal regions of the G protein’s  $\alpha$ -subunit. Only hydrophobic regions of  $\alpha$  and  $\gamma$  – subunits are anchored to the membrane. The  $\alpha$ -subunit C-terminal tail is bound to the inner face of helix VI in an activation-dependent manner. Upon illumination, isomerization of 11-cis retinal takes place changing the configuration of the chromophore to all-trans retinylidene. This alters the position of the  $\beta$ -ionone ring which causes deprotonation of the SB and disruption of the salt bridge between the SB and Glu113. These changes cause transient protein activation before the release of all-trans retinal from the protein. These changes include alteration in the position of helix III and a subsequent change in helix VI (Meta I state). The conformational changes cause Trp126 and Trp 265 to be weakly hydrogen bonded (Meta II state). Finally, all-trans retinal is released from the binding pocket to be replaced with a new 11-cis retinal chromophore. The rapid regeneration and recombination of 11-cis with opsin restores the dark state of the protein (inactivation), which allows for subsequent photoactivation [34].



**Figure 3.** Illustrates the crystal structure of channelrhodopsin (closed state- A), halorhodopsin (B) and archaerhodopsin-1, 2 (C, D) proteins [35, 36].

## 8. Conclusion

In 2006, the term optogenetics was first used by Karl Deisseroth, who utilized genetic engineering and illumination to investigate neural dynamics. In 2007, the emergence of fiber-optic neural interfaces allowed for the use of bacterial opsins to influence behavior in intact, freely behaving mammals. Since then, the field has been expanding with many applications, especially in neuroscience. In optogenetics, ion transport across the cell membrane is a critical process that stimulates or inhibits other cellular processes including: cell excitation, release of neurotransmitters or proteins, and gene expression. Optogenetics opens the chance to control the flow of certain ions to subsequently control cellular processes in response to changing that ion concentration in the cell. Of the most challenging factors are gene delivery and illumination of modified cells *in vivo*. The selection of ions mainly depends on the type of opsin proteins employed in that cell and the wavelength and intensity of light used to illuminate the protein. Optogenetics is a promising technique in controlling cellular function to treat various conditions. Many researchers have used this method in cell and disease control, especially in neural cells. Meanwhile, other studies revealed new types of opsin proteins with different characteristics including different ion selectivity, response to different color or intensity of light, and different cellular membrane localization. Several studies have altered the crystal structure of existing opsin proteins by substitution, deletion, or addition of amino acids, altered the retinal chromophore at the core of the protein, and have created opsin protein chimeras. These studies have innovated a broad spectrum of opsins with variable affinities for their corresponding ions,

changed the ion transport direction, or altered responses to light. Since optogenetics provides many methodological advantages, it is believed that many more applications will be created in this rapidly advancing field.

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