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## TERM PAPER

### Enzymology

# Bacteriorhodopsin: A comprehensive review of its structure, enzymatic activity and its applications in Opto-Electronics

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Bacteriorhodopsin (bR) is a trans-membrane enzyme with light energy driven proton pump activity. bR has seven trans-membrane helices. It belongs to a class of proton pumps and is found in the purple membrane of *Halobacterium Salinarum*. Certain wavelengths of light cause photoisomerization of **Retinal** (present with bR) ligand from **trans** to **cis** which starts a cyclic reaction in which a proton is translocated across the membrane. This transfer of proton causes a pH difference across the membrane with more proton concentration outside the cell. Bacteriorhodopsin and its mutants have a lot of potential applications in the field of bioelectronics (particularly Opto-electronics), energy production, and bio-medical applications [1, 2].

**KEY WORDS**

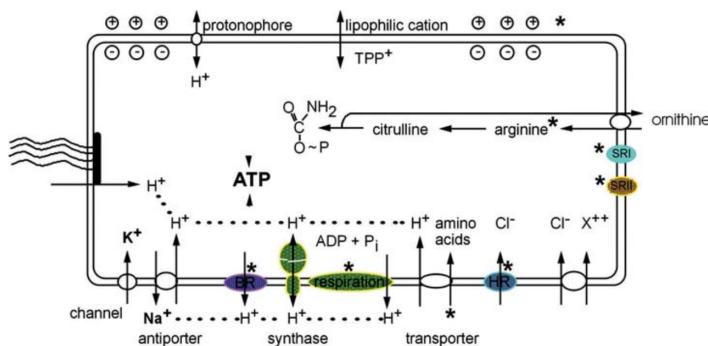
Bacteriorhodopsin, bR, Proton pump , Retinal, *Halobacterium Salinarum*, Opto-Electronics

## 1 | INTRODUCTION

Archaeal proteins have been of great interest due to their robustness and unique properties. The archaea live and reproduce in extreme environments, hence they have evolved to thrive in them with the help of their evolved proteins and enzymes. For instance, heat-stable enzymes are used as catalysts for the Polymerase Chain Reaction, which is performed at high temperatures. These enzymes are often obtained from archaea. There are six genera of archaea characterized as of now and all of them have retinal proteins in their membranes. Halophilic archaea require organic nutrients and oxygen to grow. With a sufficient supply of nutrients and oxygen, they respire normally [3].

Since, the solubility of oxygen in halophilic conditions is found to be five times lower than normal water, anaerobic condition is created. The halophilic archaea have evolved to produce energy through alternative methods. The most powerful method among them is the use of light energy with the help of retinal containing proteins (retinylidene moiety) where the retinal (chromophore) absorbs light energy. These proteins exist only in genus Halobacteria which belong to the order archaea [3].

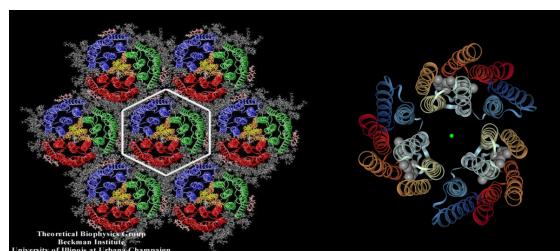
One such protein is Bacteriorhodopsin (bR). It is a trans-membrane, photo-active enzyme found in the outer membrane of *Halobacterium Salinarum*, a Halobacteria. There are three more structures of retinal proteins presently identified: halorhodopsin, sensory rhodopsin II, and visual pigment rhodopsin. The archaea makes use of all of them in combination during the energy production process.



**FIGURE 1** Bioenergetics of *Halobacterium Salinarum*. Source: [3].

When light falls on bR, energy is absorbed by the retinal which then isomerizes. This initiates a cycle of changes in the structure of bR (intermediates) in which one proton is pumped out into the extracellular space per cycle. This process is aided by halorhodopsin, a chloride pump which uses light energy to create a chloride gradient. This chloride gradient is used to produce electrochemical energy used to maintain the osmotic pressure. The proton gradient drives the membrane bound ATPase that is responsible for ATP synthesis [3].

Bacteriorhodopsin is overproduced under anaerobic conditions and presence of light. It covers up to 80 percent of the surface of the cell, hence imparting the characteristic purple color. The arrangement in the membrane follows a hexagonal lattice with three identical chains of bR (rotated by 120 degrees relative to each other) in each trimer subunit (Fig 2) [4].



**FIGURE 2** Hexagonal lattice arrangement consisting of trimer subunits (left) Cartoon representation of the bio-assembly of Bacteriorhodopsin. Ligand, retinal is represented as space fill models. (Right) Source: [2, 4].

## IMPORTANT QUALITIES OF bR

Bacteriorhodopsin has been extensively studied over the past two decades as an alternative material for technical applications. One of the biggest problems associated with bio-materials is its stability. Compared to organic and inorganic materials and semiconductor structures, bio-materials in general cannot match them in terms of stability. One solution is to implement repair mechanisms like in cells, but this is far into the future. Although most bio-materials need extensive stabilizing agents, bacteriorhodopsin is quite stable [3, 5]. This is one of its biggest advantages. Some important advantages of bR include -

- High quantum efficiency of conversion of light energy into a state charge.
- Absorption of light at 570nm coincides with the peak solar radiation.
- High thermal and photochemical stability.
- Resistant to harsh environmental conditions.
- Economically friendly.
- Mutants of bacteriorhodopsin exist with enhanced spectral properties for specific technical applications.

Due to the robustness and unique properties of bR, scientists are constantly experimenting with it. Various tools for its genetic modification and rapid production have also been developed.

## GENETIC MODIFICATION

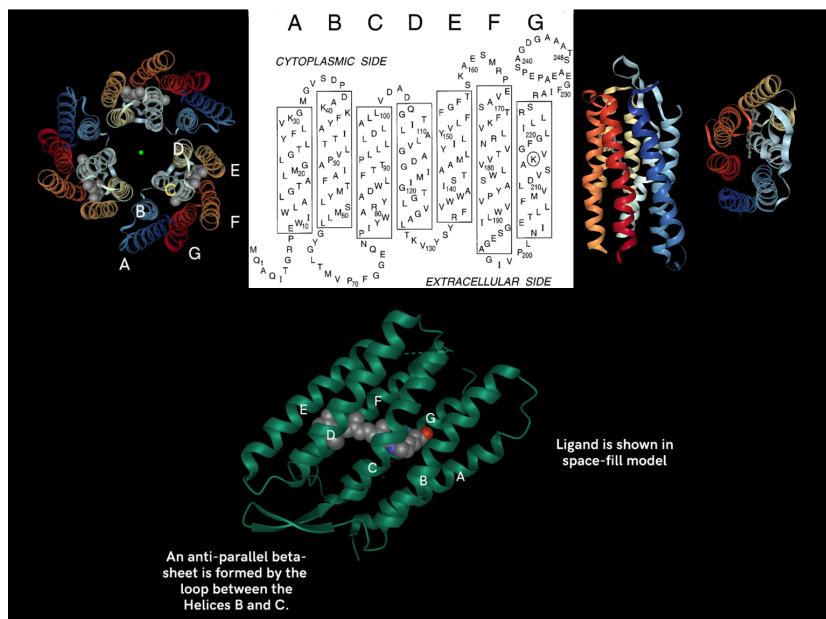
In order to find the function of a particular residue in the enzyme, a technique called site-specific mutagenesis along with spectroscopic studies is used. Once a residue is mutated, the rest of the residues are untouched and the enzyme's functions and kinetics are studied. If there is a change or disruption in the activity of the enzyme, then the residue is crucial for that particular activity of the enzyme. Site-specific mutagenesis was made possible by the development of transformation techniques for halophilic archaea. In earlier experiments, before the development of transformation techniques, mutants of bR were isolated by random mutagenesis *in vivo*. The basic process of transformation begins with the incubation of Halobacterium cells with EDTA to remove magnesium ions. The protoplasts obtained are incubated with a mixture of vector DNA with an antibiotic resistance gene and polyethyleneglycol (Induces transformation in bacteria without the need for cell wall removal). Next, the transformed protoplasts are kept in complex medium for curing. The cells are then plated with antibiotics to kill the non-transformed cells. Site-specific mutagenesis is also used to create useful mutant bR which have different useful properties [3].

## STUDY OF RESPONSE TO LIGHT

As mentioned earlier, to understand the mechanism of bacteriorhodopsin, site-specific mutagenesis, and spectroscopic studies are performed. To understand specifically the response of bR to light, spectroscopic studies are the way to go. To activate bR, a nanosecond pulse of laser light is used and the resulting structural changes are observed spectroscopically over a time period of nanoseconds to milliseconds. UV-Visible spectroscopy is used to identify changes in the chromophore and its surroundings. Resonance Raman Vibrational Spectroscopy is used to observe the retinal structure and Schiff base protonation state at a better resolution. Fourier Transform Infrared (FTIR) spectroscopy is used to obtain information about the residues and the chromophore. Finally, pH-sensitive dyes have been used to analyze the proton release and uptake in the two phases (Release phase and Uptake phase) of the photocycle of bR. These methods along with site-specific mutagenesis are used to discuss the mechanism of the photocycle upon illumination [6].

## 2 | STRUCTURE, MECHANISM AND KINETICS

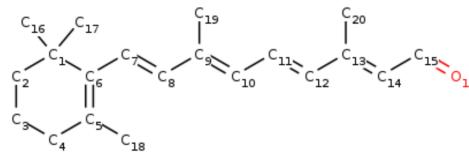
Bacteriorhodopsin is a trans-membrane protein with a molecular weight of 26 kDa and a residue count of 248. Electronic diffraction readings (at 2.3 Angstroms resolution) of the purple membrane of certain *Halobacteria* were used to model the tertiary structure of bR. The tertiary structure of bR contains 7 alpha-helices with bulky hydrophobic residues. Helices B, C, D form the inner arc and helices A, E, F, G form the outer arc. Lys216 which is circled in the Helix G is where the ligand RET (Retinal) is attached as a positively charged Schiff base. An anti-parallel beta-sheet is formed by the loop between the Helices B and C [3, 6].



**FIGURE 3** Figure 3B: Fragmented structure of bR. The length and correct alignment of the A-G segments are derived from X-Ray and electron diffraction. Lys216 which is circled in the segment G is the site of Ligand (Retinal) attachment. The N-terminal sequence of wild type bR is Pyro-Glu-Ala-Gln. But, in the figure the N-Terminal sequence is shown to be Met-Gln-Ala-Gln encoded by the synthetic BO gene. Source: [7] Figure 3A & C: Cartoon representation of bR. The seven trans-membrane helices are shown and the ligand is shown in gray (Ball and stick). 3D: Cartoon representation with the helix labelling Source: [2].

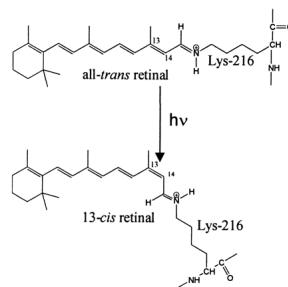
### LIGAND

The light absorbing ability of the bacteriorhodopsin is credited to Retinal (Fig 4). Retinal (Vitamin-A aldehyde) is the result of oxidative cleavage in the center of beta-carotene. The aldehyde (with five conjugated double bonds) formed in free state is chemically unstable and oxygen sensitive. When light falls on the retinal, it isomerizes from all-trans to 13-cis form (Fig 5). Retinal is prone to destruction in its free state due to photooxidation (Oxydation due to the action of light). All retinal proteins including bacteriorhodopsin protect the molecule against photooxidation [6, 8]. The retinal spontaneously returns to the all-trans form after the proton transfer is completed and the photo-cycle is repeated. Retinal, along with bR and its side chains is called a chromophore. The color of the retinylidene moiety



**FIGURE 4** Structure of Ligand RET (Retinal). A Schiff base has the form R2C=NR. The 15th Carbon of retinal is covalently bound to Lys216 in the Helix G of bR. The retinal and Lys216 together is of the form R2C=NR. Hence, RET is attached as a Schiff base. All rhodopsins consist of two building blocks - retinaldehyde (retinal) and a protein moiety covalently bound with the retinal, hence the chromophore is called retinylidene moiety. Source: [2].

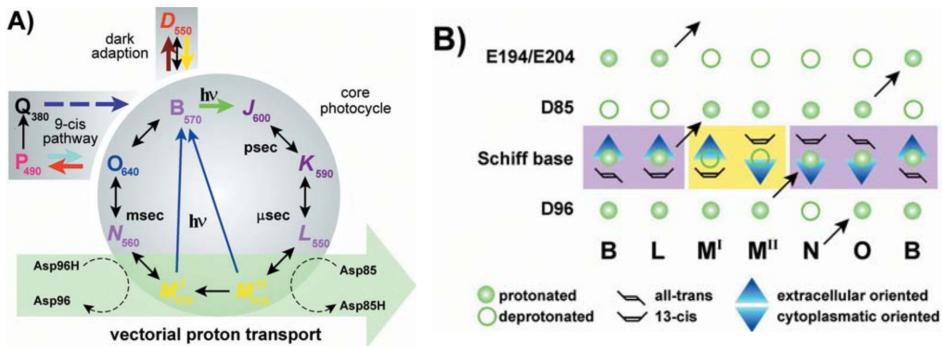
is the result of electrostatic interactions between the retinal and a complex counterion comprising of amino acids in proximity. In order to produce energy with a high efficiency, the absorbance spectrum of a chromophore must coincide with the solar radiation spectrum which peaks at around 500 nm and retinal fits the bill since the retinal linked through a protonated Schiff base has a peak absorbance at around 570 nm [6, 8].



**FIGURE 5** Retinal, covalently bound to Lys216 as protonated Schiff base. Absorption of photon isomerizes retinal from all-trans to 13-cis form. Source: [9].

## 2.1 | MECHANISM

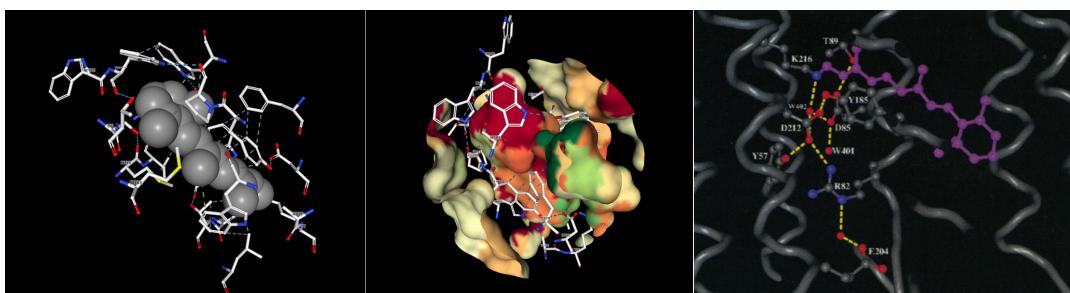
The photoisomerization of retinal causes a change in configuration resulting in the protonated Schiff base sliding into a new protein environment. Now, the bR is at a meta-stable state (Higher energy). From this state, the moiety relaxes into the original configuration (B570) following a pathway consisting of at least five intermediates (J600, K590, L550, M, N560, O640). Extensive spectroscopic and mutational studies have been used to determine the mechanism of the pathway at the residue level. This pathway (photocycle) happens in two steps- (1) Release phase (Release of proton from the extracellular side of bR into the extracellular space); (2) Uptake phase (Uptake of proton from the cytoplasmic side of bR to reprotonate the Schiff base) (Fig 6) [6, 8].



**FIGURE 6** Photocycle of bR. In absence of light bR relaxes thermodynamically to the D State which has 13-cis configuration. The formation of 9-cis retinal (Q state) is possible from the O state which does not return to B state spontaneously. Source: [3].

### 2.1.1 | RELEASE PHASE

Exposure to light causes the initial state of bR (B state) to convert to a short-living J state and then quickly proceeds to K state. The intermediates K, L, and M are part of the release phase of the proton. Resonance Raman spectroscopy showed that in all the above intermediates, the chromophore is in 13-cis form with only slight differences in rotation around the bonds present in the polyene chain. FTIR studies showed that the Aspartic acid residues act as likely proton acceptors from Schiff base during the L->M transition. Site-specific substitution of Asp85 confirmed that it acts as the proton acceptor since neutral substitutions of Asp85 stopped proton translocation (Fig 6) [6, 8].



**FIGURE 7** Figure 7A and 7B: The ligand binding pocket [2] Figure 7C: Schematic of the active site and part of the extracellular proton translocation pathway. The distances of relevant groups (in angstroms) are as follows: Schiff base (NZ) to W402, 2.7; W402 to Asp85 (OD1), 2.8 W402 to Asp212 (OD1), 3.4; W402 to Asp212 (OD2), 3.6; Asp85 (OD1) to W401, 2.6; Asp85 (OD2) to Thr89 (OH), 2.7; Arg82 (NH2) to Asp212 (OD1), 3.3; Asp212 (OD1) to Tyr57 (OH), 2.8; Asp212 (OD2) Tyr185, 2.6; Arg82 (NE) to W403, 3.2; W403 to Glu204 (OE2), 3.5; Arg82 (NH2) to W401, 4.2; Asp212 (OD1) to W401, 4.9; and Tyr57 (OH) to W401, 5.1 [2, 8].

### EXPLANATION

Structure models show that the Asp85 is hydrogen bonded to a water molecule (W401) and another water molecule (W402) which in turn accepts a hydrogen bond from the Schiff base. W402 and Thr89 (Hydrogen bonded to OD2

of Asp85) together stabilize the otherwise unstable Schiff base-Asp85 pair in the unphotolyzed moiety.[Figure 5A] The breaking of these hydrogen bonds post photoisomerization of retinal causes the pKa of Asp85 to rise and results in its protonation by the Schiff base. The release of the proton from the extracellular side of bR is induced by the protonation of Asp85 (Fig 7C, 13) [6, 8].

### **ROLE OF ARG82, GLU204 and GLU194 RESIDUES**

pH sensitive dye analysis suggests that Asp85 remains protonated in M state. One possible source of released proton is Arg82. Arg82 and Asp85 may form a salt bridge and may lose a proton in the L->M transition. There is also some structural evidence which suggests that the source of proton is a water molecule (W403) and Arg82 is hydrogen bonded to W403 through NE. W403 is at a hydrogen bonding distance of 3.5 angstroms (moderately long) from OE2 of Glu204 which is within 4.0 angstroms of OE2 of Glu194, hence providing a possible pathway for the proton headed for the extracellular space. Extensive spectroscopic and mutational studies have suggested that Arg82, Glu204 and Glu194 are key in the pathway of proton release (Fig 7C, 13) [6, 8].

### **2.1.2 | UPTAKE PHASE**

In this phase, the structural changes that occurred in the release phase is reversed and the bR is returned to the original state. N and O are the intermediates of this phase. During the M->N transition, Asp96 and Thr46 are found to play the important role of reprotonation of the deprotonated Schiff base in the cytoplasmic region. This is confirmed by mutagenic studies. The mutants slowed the proton uptake and the M->N transition by 10-fold at neutral pH. But, when the proton concentration was increased artificially, the effects were reversed. This suggests that Asp96 acts as a proton donor in the cytoplasmic side. FTIR studies also suggest that Asp96 is deprotonated (Fig 6) [6, 8].

### **EXPLANATION**

Asp96 is surrounded by Phe42 and Phe219 with a hydrophobic environment in the region which is consistent with the high Pka value of 11 observed for Asp96 in the unphotolyzed bR. The Thr46 residue is at a distance of 3.5 angstroms to Asp96 which is consistent with hydrogen bonding distance. But, Thr46 is hydrogen-bonded to Phe42 (2.7 angstroms). When it is replaced by Val residue by site specific mutation, it makes the protonation of Schiff base more rapid and reprotonation of Asp96 much slower which suggests that Thr46 and Asp96 may form hydrogen bonds during the photocycle even if the hydrogen bonding did not exist initially. Hence, Asp96 and Thr46 together aid in reprotonation of Schiff base (Fig 7C, 13) [6, 8].

### **ROLE OF TRP182 AND LEU93**

The 9-methyl and 13-methyl of retinal are 3.6 and 3.7 angstroms from the closest heavy atoms - Trp182 and Leu93 respectively (Fig 13C). These residues are found to aid in thermal reisomerization to all-trans form after the formation of 13-cis form due to photoisomerization. This process happens during the N->O transition, hence completing the photocycle (Fig 7C, 13) [6, 8].

### **ROLE OF ASP212**

Another residue Asp212 is known to be of importance probably for the stability of bR. The function of Asp212 has not been clearly defined. But, neutral point substitution severely affected the activity of the enzyme. The mutants also fail to form the chromophore altogether, suggesting that the Asp212 is aiding in stability of the Schiff base linkage during the photocycle. Moreover, the Asp212 is invariant in many known halobacterial retinylidene moiety which

makes it an even stronger case for its importance (Fig 7C, 13) [6, 8].

## 2.2 | KINETICS

The intermediates formed during the photocycle of bR and their absorbance changes have been measured over wide range of wavelengths, temperatures, pH and over a broad time period. These values have been globally fitted to the kinetic model with the help of experimentally determined rate constants and activation energies for forward and reverse reaction intermediates [Table 1] [10, 11]:



### METHOD

In this study [11], the photocycle of bR was studied at three pH values (5, 7, 9) in a total salt concentration of 0.01 M at seven different temperatures ranging from 5 to 30 degrees Celsius. Data were documented from 1 microsecond until the end of the photocycle, followed by excitation with a 10 ns 490 nm laser flash. Measurements were taken at four different wavelengths 420, 540, 600 and 660 nm and at 5, 15, 25 and 35 degrees Celsius (The transient absorbance changes are significant and they represent the intermediate states). Additional readings were taken at 11 wavelengths between 380 to 700 nm, at temperatures of 10, 20 and 30 degrees Celsius. The data set at each pH has 61 kinetic traces with each describing the absorbance changes at 47 time points equally spaced on a logarithmic scale (from 1 microsecond to 300 milliseconds) which brings it to a total of 183 vectors and 8601 data points. The data were fitted simultaneously to different kinetic models by using a program FACSMILE.

### RESULTS

Table 1. Rate constants and activation energies obtained from fits of the model  $K \rightleftharpoons L \rightleftharpoons X \rightleftharpoons M \rightleftharpoons N \rightleftharpoons O \rightarrow bR$  (Eq. 3)

Reaction	pH 5		pH 7		pH 9	
	$k$ (20°C)	$E_A$	$k$ (20°C)	$E_A$	$k$ (20°C)	$E_A$
<b>Forward</b>						
$K \rightarrow L$	$5.42 \pm 0.10 \times 10^5$	$9.0 \pm 0.3$	$5.38 \pm 0.09 \times 10^5$	$8.9 \pm 2.7$	$5.97 \pm 0.11 \times 10^5$	$8.9 \pm 0.3$
$L \rightarrow X$	$2.46 \pm 0.06 \times 10^4$	$17.7 \pm 0.4$	$5.03 \pm 0.78 \times 10^4$	$12.6 \pm 1.9$	$9.60 \pm 0.55 \times 10^4$	$17.6 \pm 1.0$
$X \rightarrow M$	$1.14 \pm 0.14 \times 10^4$	$15.3 \pm 1.7$	$6.42 \pm 1.2 \times 10^4$	$10.3 \pm 1.9$	$1.93 \pm 0.10 \times 10^4$	$12.1 \pm 0.8$
$M \rightarrow N$	$1.69 \pm 0.18 \times 10^2$	$16.4 \pm 1.2$	$1.79 \pm 0.47 \times 10^3$	$6.1 \pm 1.9$	$2.54 \pm 0.04 \times 10^2$	$19.7 \pm 0.2$
$N \rightarrow O$	$3.68 \pm 1.60 \times 10^3$	$13.5 \pm 5.7$	$1.69 \pm 0.12 \times 10^3$	$29.5 \pm 1.0$	$71.5 \pm 45$	$18.0 \pm 9.1$
$O \rightarrow bR$	$1.33 \pm 0.03 \times 10^2$	$5.9 \pm 0.4$	$2.57 \pm 0.06 \times 10^2$	$7.5 \pm 0.5$	$5.06 \pm 7.6 \times 10^2$	$0.8 \pm 3.2$
<b>Reverse</b>						
$K \rightarrow L$	$4.14 \pm 0.35 \times 10^4$	$21.8 \pm 1.3$	$3.68 \pm 0.34 \times 10^3$	$22.6 \pm 1.4$	$5.70 \pm 0.44 \times 10^3$	$21.5 \pm 1.1$
$L \rightarrow X$	$1.26 \pm 0.25 \times 10^3$	$15.7 \pm 2.9$	$2.16 \pm 0.79 \times 10^4$	$-7.4 \pm 3.0$	$1.48 \pm 0.16 \times 10^4$	$10.2 \pm 1.8$
$X \rightarrow M$	$3.00 \pm 0.14 \times 10^3$	$8.3 \pm 0.8$	*	*	$1.58 \pm 0.07 \times 10^3$	$12.3 \pm 0.7$
$M \rightarrow N$	$5.43 \pm 2.8 \times 10^2$	$29.1 \pm 3.9$	$1.61 \pm 0.51 \times 10^4$	$16.0 \pm 2.0$	$20.5 \pm 3.5$	$30.2 \pm 2.4$
$N \rightarrow O$	$1.62 \pm 1.2 \times 10^2$	$(-13.8) \pm 5.1$	$27.4 \pm 7.1$	$6.8 \pm 5.1$	$5.26 \pm 6.8 \times 10^2$	$0.7 \pm 17.6$

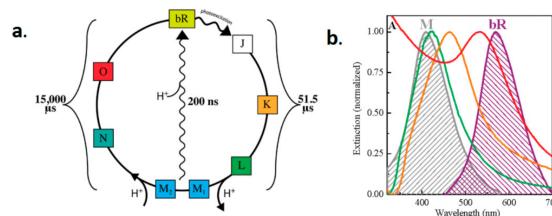
Errors are reported as the 5%-95% confidence limits from the final iteration of the fit. Trial calculations using subsets of the data suggest that these values are, in most cases, reasonable estimates of the actual errors.

\*Parameters not optimized in the final fit because they were found in the initial fitting stages to be ill-determined by the data. The value selected prior to discarding this parameter was effectively zero.

Subsets of the data (Table 1) was used to conduct preliminary fitting on various unbranched reversible models. The model:  $A \leftrightarrow B \leftrightarrow C \leftrightarrow D \rightarrow bR$  yielded residuals with <1 percent of the maximum data amplitudes. Further analysis after combining the subsets of data showed that the model with six intermediates:  $K \leftrightarrow L \leftrightarrow X \leftrightarrow M \leftrightarrow N \leftrightarrow O \rightarrow bR$  fits best and matches the globally accepted model.

## EFFECT OF SILVER NANOPARTICLES

Silver nanoparticles absorb and scatter light with incredible efficiency due to the phenomenon of surface plasmon resonance (SPR). This phenomenon of silver nanoparticles can affect the bR photocycle and significantly speed up the photocycle process. The long lived intermediate M is generated quickly (around 70 microseconds) from the bR (or B) state [12]. The rest of the cycle (M->N->O->bR) takes 15 ms which is much slower compared to the bR->M conversion (bR->J->K->L->M). The SPR speeds up the process of proton release and bypasses the slow part of the photocycle by allowing the M state to decay in 200 ns to the original state (bR) directly (Fig 8).



**FIGURE 8** (a) Photocycle of bR; and (b) Normalized Surface Plasmon Resonance spectra of Ag NPs(425 nm in green), Ag-Au NPs (465 nm in orange) and Au NPs (530 nm in red). Gray and violet shadows represented the absorption of M412 state and bR570 state, with maxima corresponding 412 and 570 nm respectively Source: [12].

## 3 | TECHNICAL APPLICATIONS OF BACTERIORHODOPSIN

Opto-electronics is the inter-conversion of light energy and electrical energy. It spans the areas of energy storage and production, optical sensors, optical information storage devices, etc. In this section, the various applications of bacteriorhodopsin in opto-electronics has been discussed in a semi detailed manner. There are three important molecular functions occurring in the photocycle which are possible to exploit [3, 13]. They comprise of -

- Photoelectric events- caused by the change in structure of the Schiff base due to photoisomerization and transfer of proton, a photovoltage up to 250 mV per single purple membrane layer is generated.
- Photochromatic events- The change in color, it can be used to store and process information
- Proton transport events- The change in pH across the membrane as a result of transfer of proton

### 3.1 | PHOTOELECTRIC APPLICATIONS

When light energy in the form of photons is absorbed by the bacteriorhodopsin molecule, it undergoes rapid changes in molecular structure. It also leads to changes in the orientation of molecular dipole moments triggered by the photoisomerization of retinal in a time scale of femtosecond. This results in the generation of photovoltage of up to 250 mV for every single layer of bacteriorhodopsin. The proton released in the first phase of the photocycle may either end up in the extracellular space or conducted along the surface of the purple membrane (PM). In photoelectric applications, often the water content is reduced in order to increase the surface conductivity of protons. A single layer of PM contains thousands of unidirectionally oriented bR molecules. Hence, the voltage generated over the PM layer remains constant irrespective of the intensity of light falling on it. The number of bR molecules excited to

accomplish the proton translocation is directly proportional to intensity of light falling on the PM layer. Hence, the photon current is directly dependent on the intensity of light. The voltage generated can be measured by inserting the PM layer between two fairly transparent electrodes. The photovoltage induces a balancing polarization voltage in the transparent electrodes. This voltage can be detected as low resistance voltage signal [3, 13].

### **APPLICATION EXAMPLES**

Ultrafast Light sensor was one of the earliest technical applications of bacteriorhodopsin. Light falling on the PM layer can be detected as electric current and in turn the intensity of light can be determined. Devices that make use of photoelectric properties of bacteriorhodopsin are called artificial retinas. The reason is not necessarily because of the presence of retinal, it is due to some pre-processing features from the retinal like edge detection and novelty filtering (Motion detection). Other applications of artificial retina include 3D memory devices and Electro-optically controlled spatial light modulators [3, 13].

## **3.2 | PHOTOCROMATIC APPLICATIONS**

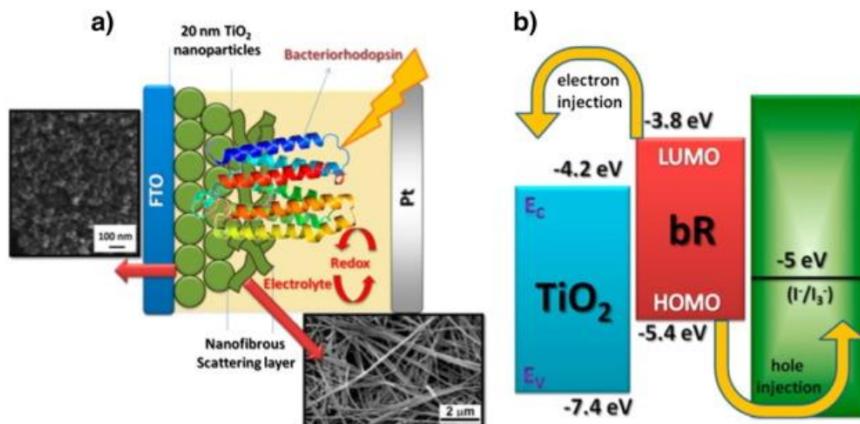
Currently, most applications experimented with are utilizing the photochromatic properties of bR. When bR undergoes light induced photocycle, bR cycles through intermediates which have different absorption maximum that is different from that of the initial state. However, most applications are based on photochromism (particularly purple to yellow absorption change) of bR which happens during the M state formation. When placed in an acid medium, the membrane turns blue with a 9-cis retinal containing state which is significantly different from the normal photocycle. There are three types of photochromic changes described which have different applications. The first one is the photochromic shift between B and M states which is used for optical processing tasks. The next one is photoerasable data storage using 9-cis containing states of bR with the blue membrane. Unfortunately, they have a very low quantum efficiency of less than 1 percent which is a major limitation. And the third one, permanent data storage through permanent photochromic changes obtained through two-photon absorption in bR [3, 4, 13].

### **APPLICATION EXAMPLES**

Since the bR film can be completely sealed for photochromatic applications, it can be easily interfaced with other optical systems and hence widening the potential applications. Photochromic color classifier (color sensor) have been developed by using different bR variants with different absorption maxima coupled with a neural network for color recognition. Photochromic inks, electrochromic inks, photochromic photographic film, long term photo-rewritable storage, 3D storage are some more examples of promising applications in opto-electronics [3, 4, 13].

## **3.3 | BIOMOLECULE-SENSITIZED SOLAR CELLS**

Biomolecule-sensitized solar cells (BSSCs) are promising eco-friendly and low cost photovoltaics. In bacteriorhodopsin based BSSCs, the photoanode is made of porous semiconductor (often Titanium Dioxide) nanoparticles sensitized with bR. The problem with Titanium Dioxide is its absorption spectrum lies in the high energy (UV) region. Since UV rays make up a small portion of the sun's radiation hitting the Earth's surface, most of the radiation is left unutilized. This is where bR comes into the picture. bR has its absorption range in the visible light coinciding with the solar radiation. Hence, bR can harvest a much higher amount of radiation. Upon absorption, bR gets into an excited state, and injects photo-excited electrons into the conduction band (CB) of the semiconductor (Titanium Dioxide). (Fig 10) The lowest occupied molecular orbital (LUMO) of bR is located at a higher energy than the CB of Titanium Dioxide and hence



**FIGURE 9** Schematic illustration of biomolecule-sensitized solar cell (BSSC) Source: [5].

the injection of the photoelectrons into the CB is energetically favorable. The electrons pass through the circuit and ends up in the counter electrode. The bR accepts electron from the electrolyte to return to ground state from the oxidized state. A study has demonstrated that by mutating Glu9, Glu194 and Glu204 to Gln residues, the bR and Titanium Dioxide binding improved due to changes in the surface potential map of bR. This led to improved efficiency. There have been many studies demonstrating various combinations and techniques to improve the efficiency of BSSCs [3, 4, 5, 13].

#### 4 | CONCLUSION AND OUTLOOK

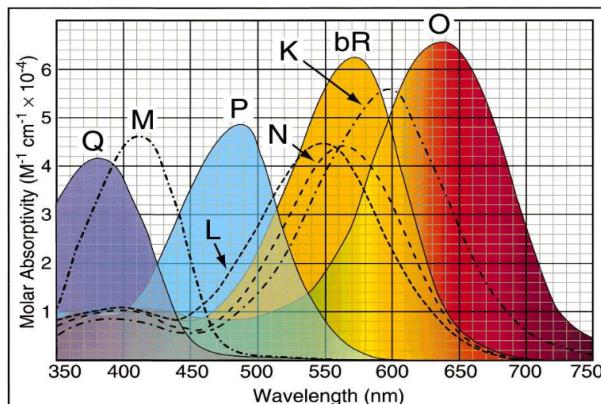
Bacteriorhodopsin is the result of archaea evolving over millions of years in extreme conditions. It is efficient, robust and possesses unique properties leading to applications in fields like opto-electronics which has been dominated by semiconductors for decades. With research output high, we could see a lot more interesting applications of bacteriorhodopsin in bioelectronics and advancements in bio-materials in general. Bio-materials are promising future materials that are environmental friendly and have more advanced structures that can be tweaked or customized to perform a particular function. Bacteriorhodopsin has come a long way from helping halobacteria in producing energy to living in solar cells to help produce energy for the world. This is just the beginning of the journey of bacteriorhodopsin and bioelectronics.

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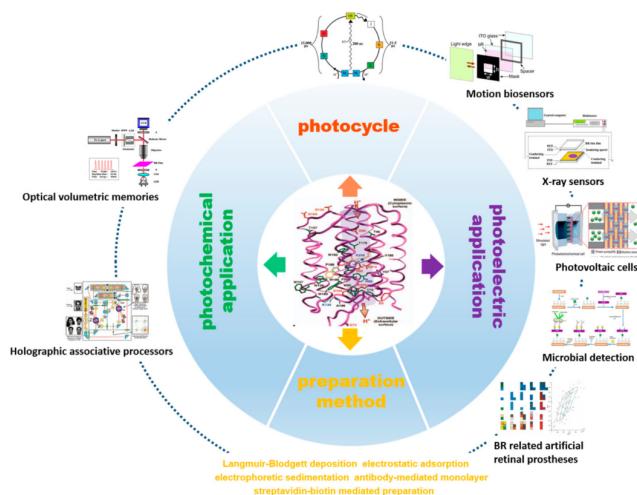
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## 5 | APPENDIX



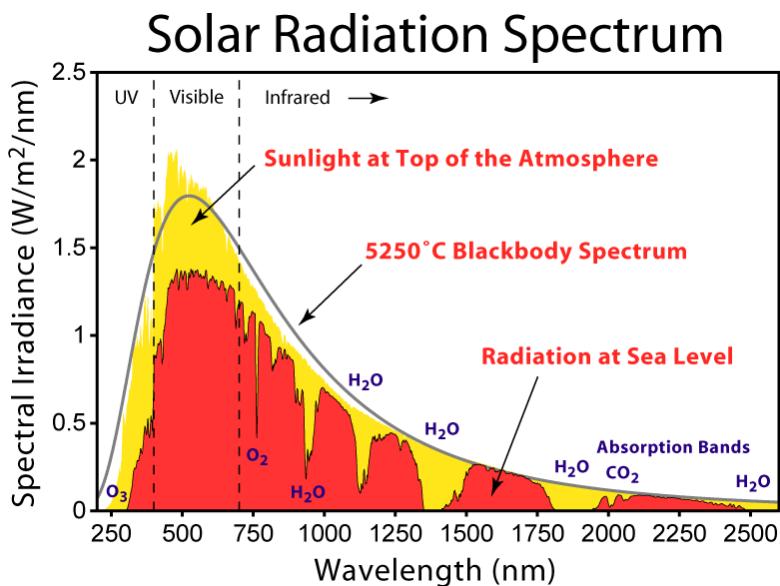
**Fig. 5.** Schematic illustrating typical absorption spectra of the five key states, bR, M, O, P, and Q, in the photocycle of BR.

**FIGURE 10** Source: [4].



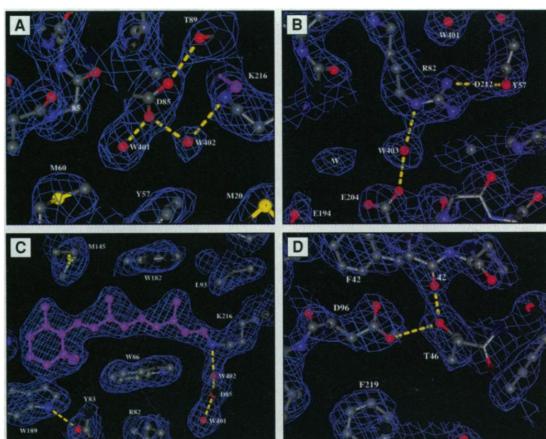
**Figure 2.** An overview of bR-based bioelectronic devices showing the photocycle, preparation method, and photochemical and photoelectric applications.

**FIGURE 11** Summary of Technical Applications of bR. Source: [12].



**FIGURE 12** Source: Wikimedia

Electron density maps ( $2|F_o| - |F_c|$ , contoured at  $1\sigma$ ) and corresponding molecular models of three regions of interest. Dashed yellow lines indicate hydrogen bonds. (A) The region of the Schiff base and the initial proton acceptor, Asp<sup>85</sup>. OD1 of Asp<sup>85</sup> is within hydrogen-bonding distance of a water molecule (W401,  $B = 3.1 \text{ \AA}^2$ ) (7). Another water (W402,  $B = 24.7 \text{ \AA}^2$ ) molecule was identified between the Schiff base and OD1 of Asp<sup>85</sup>. (B) Region between Arg<sup>82</sup> and Glu<sup>204</sup>. Water W403 ( $B = 23.8 \text{ \AA}^2$ ) hydrogen-bonds to both NE of Arg<sup>82</sup> and OE1 of Glu<sup>204</sup> and is the potential source of the proton released to the surface when Asp<sup>85</sup> becomes protonated. The feature labeled as W is one of three disconnected densities in this immediate region. Although they are likely to be water, we did not model them because their ligand arrangement is uncertain at the present resolution. (C) Region of the retinal (magenta), showing residues Trp<sup>162</sup>, Leu<sup>193</sup>, Met<sup>145</sup>, and Trp<sup>98</sup>, which flank it. The OH of Tyr<sup>93</sup> forms a hydrogen bond with the indole N of Trp<sup>162</sup>. (D) Region near Asp<sup>98</sup> in the cytoplasmic portion of the proton translocation pathway. The hydroxyl of Thr<sup>146</sup> forms a hydrogen bond with the peptide C=O of Phe<sup>92</sup>. The distance between Asp<sup>98</sup> and the Thr<sup>146</sup> hydroxyl (drawn in) is 3.5 Å, which may be sufficient for a hydrogen bond. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; E, Glu; F, Phe; L, Leu; M, Met; R, Arg; T, Thr; W, Trp; and Y, Tyr.



**FIGURE 13** Source: [2].