A COMPREHENSIVE REVIEW

Bacteriorhodopsin

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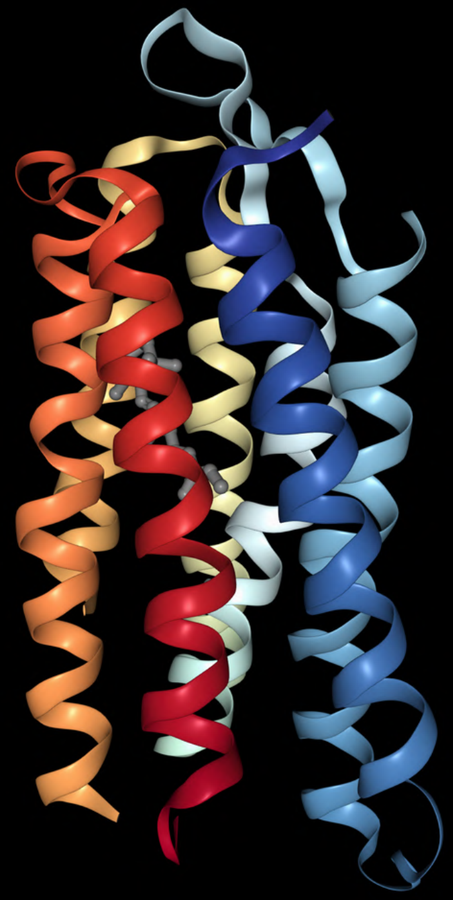
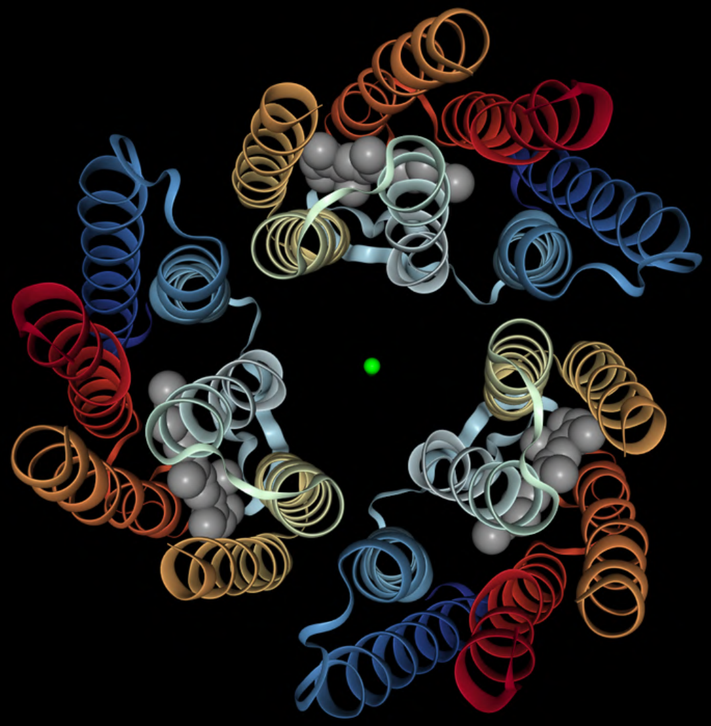
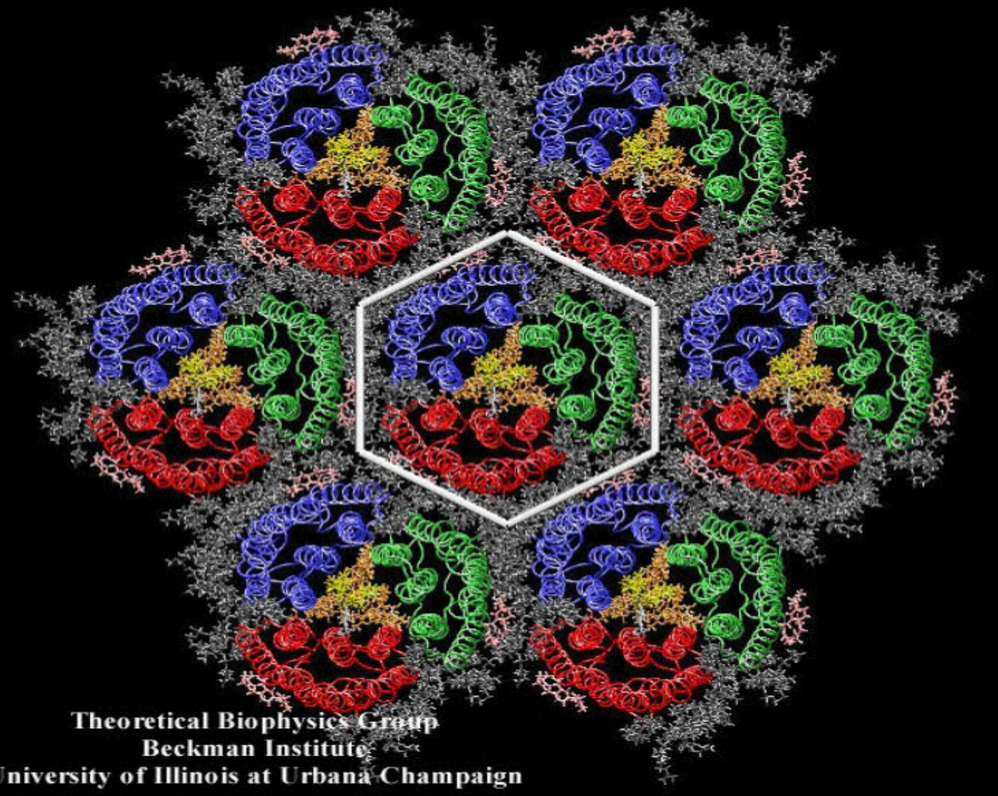
BACKGROUND

- Bacteriorhodopsin (bR) is a trans-membrane enzyme with light energy driven proton pump activity. It is a retinal protein.
- Archael proteins - Evolution in extreme environments (Robust, heat resistant, etc.
- All six genera of Archae have retinal proteins.
- Uptake on nutrients and oxygen is difficult in halophilic conditions, alternative method of energy production is through sunlight
- 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. The proton gradient created is used for energy production through membrane bound ATPase.



- 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.
- Hexagonal lattice of trimers (120 degrees with each other)



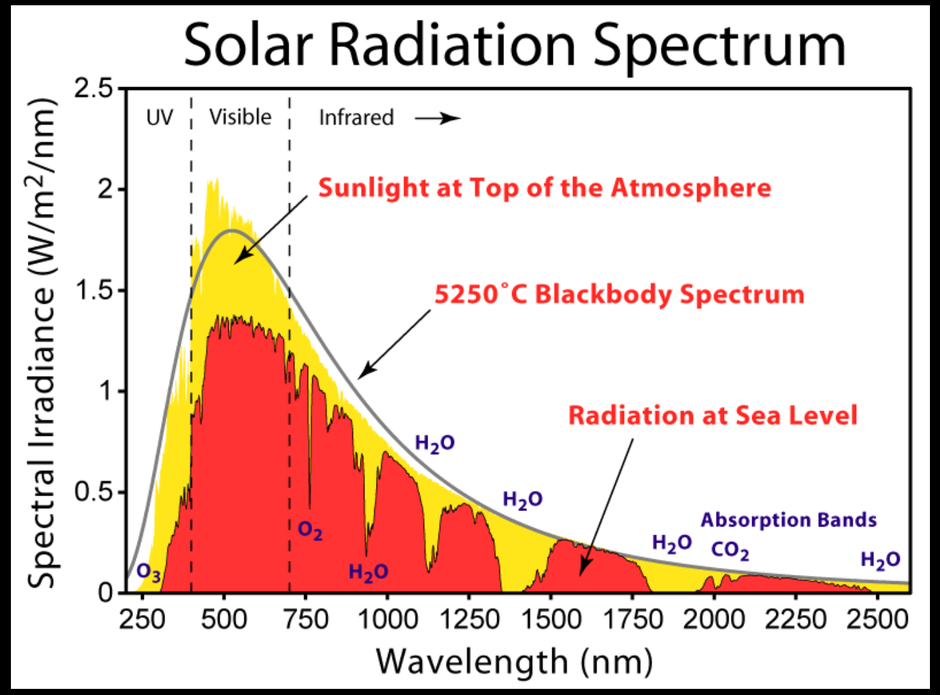


WHY BACTERIORHODOPSIN

- 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.

ABSORPTION SPECTRUM

- 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.



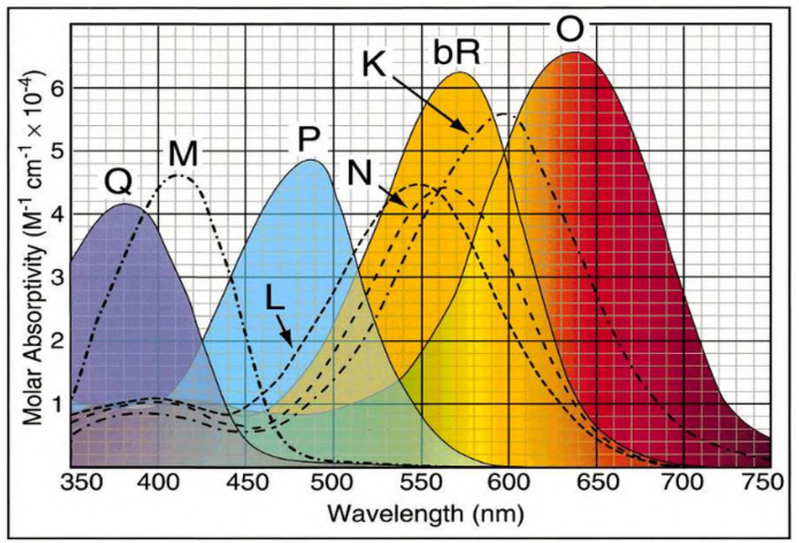
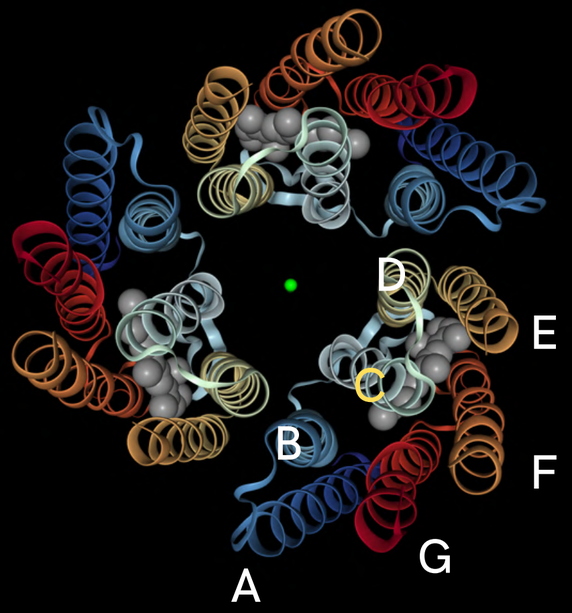
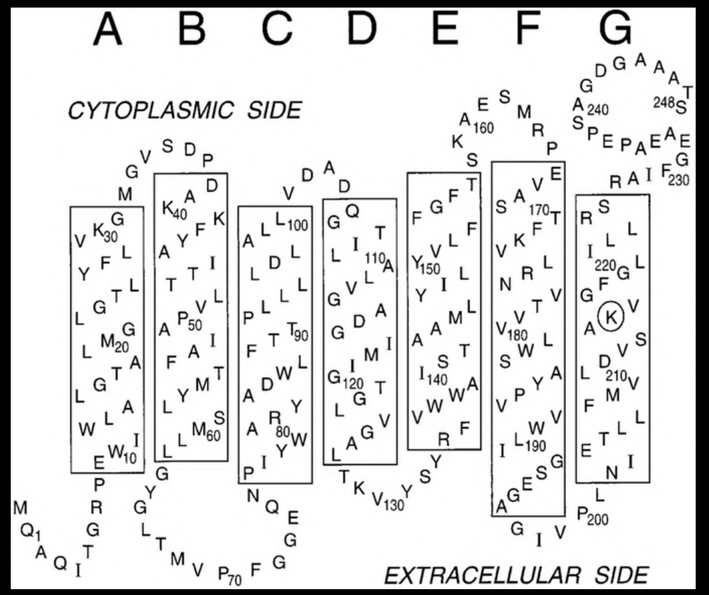


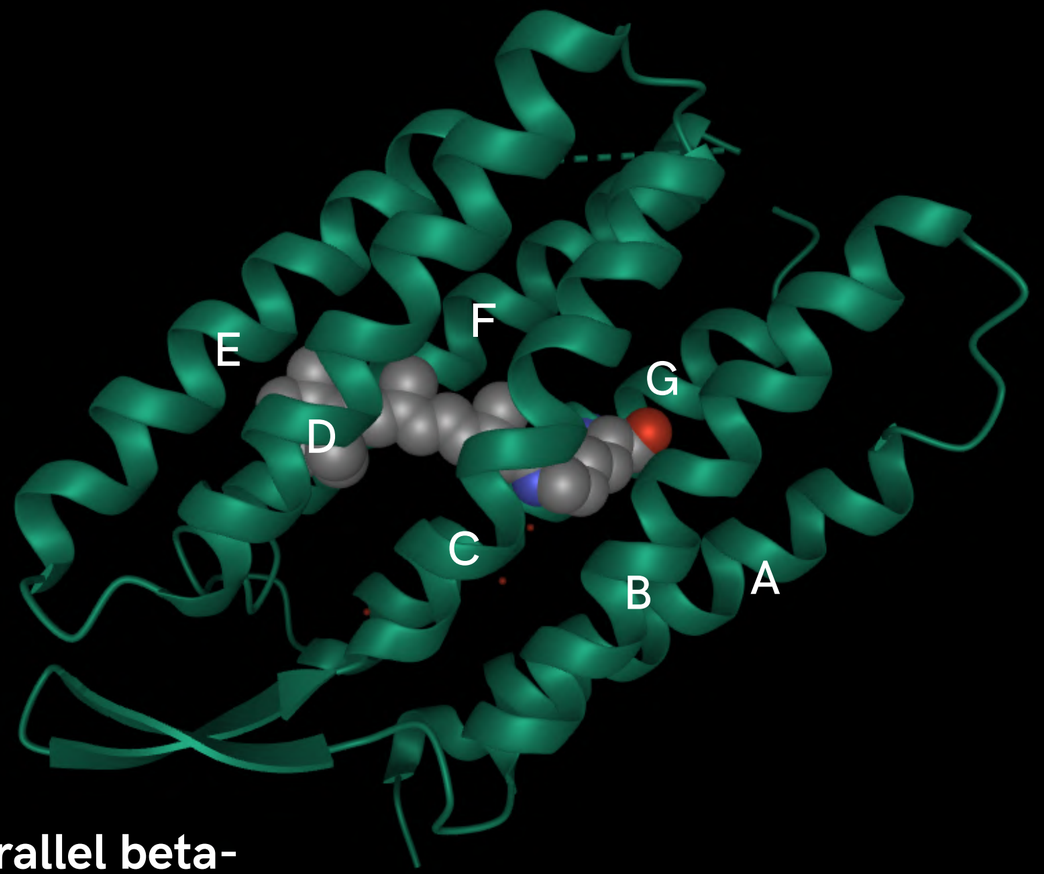
Fig. 5. Schematic illustrating typical absorption spectra of the five key states, bR, M, O, P, and Q, in the photocycle of BR.

STRUCTURE

- Bacteriorhodopsin is a trans-membrane protein.
- Molecular weight of 26 kDa
- A residue count of 248
- Electronic diffraction readings (at 2.3 Angstroms resolution) of the purple membrane 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.





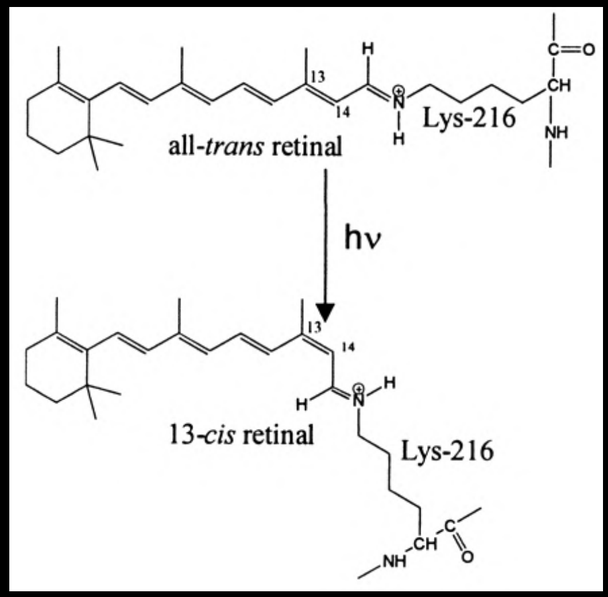


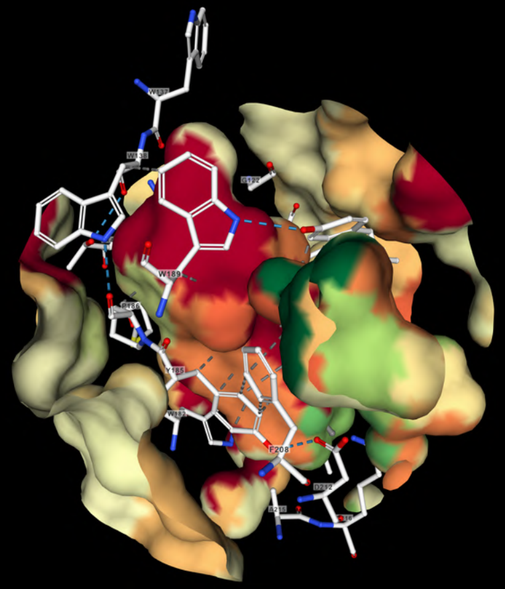
Ligand is shown in space-fill model

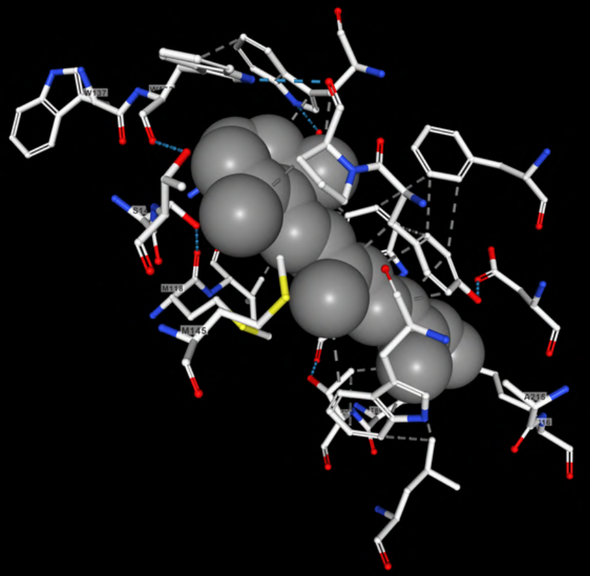
An anti-parallel beta-sheet is formed by the loop between the Helices B and C.

LIGAND

- The light absorbing ability of the bacteriorhodopsin is credited to Retinal. It is covalently bound to Lys216 as protonated Schiff base.
- Retinal (Vitamin-A aldehyde) is the result of oxidative cleavage in the center of beta-carotene.
- Unstable in free state. All retinal proteins including bacteriorhodopsin protect the molecule against photo-oxidation.
- When light falls on the retinal, it isomerizes from all-trans to 13-cis form and returns to all-trans form spontaneously.

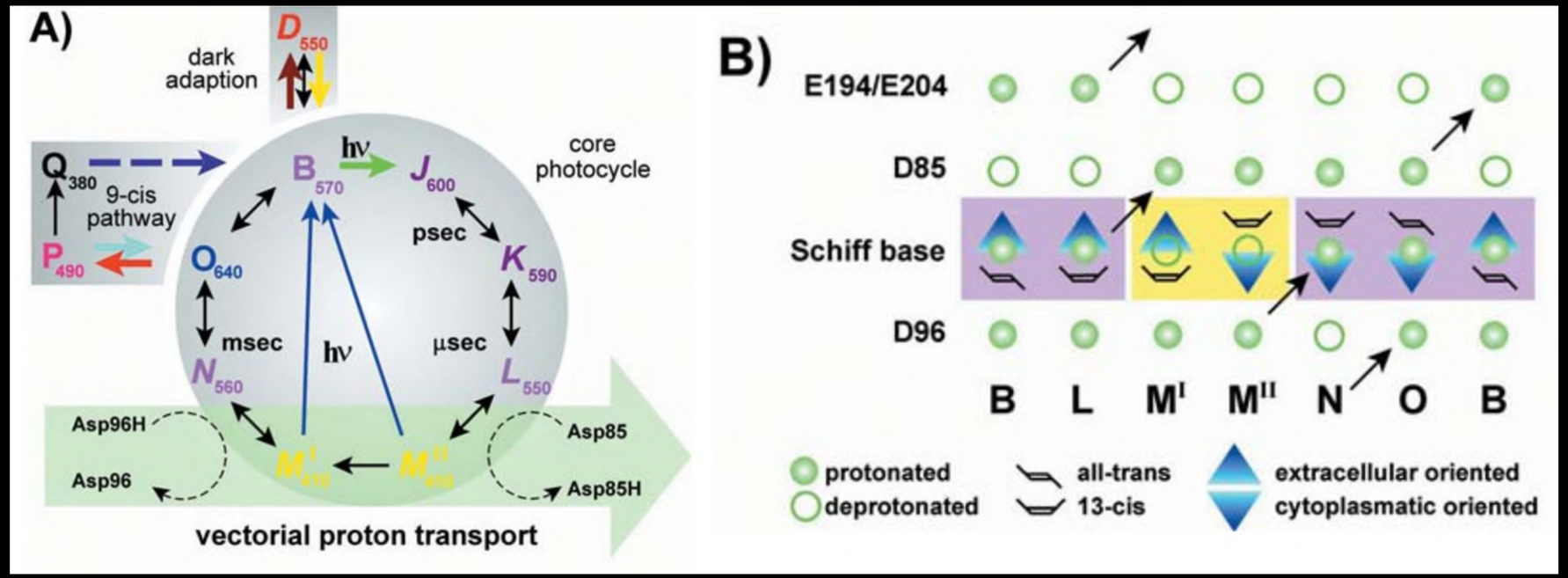






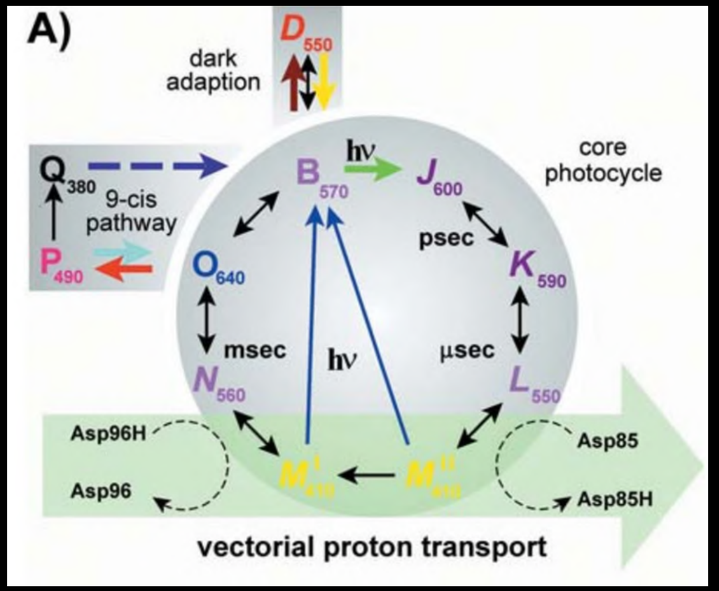
THE PHOTOCYCLE

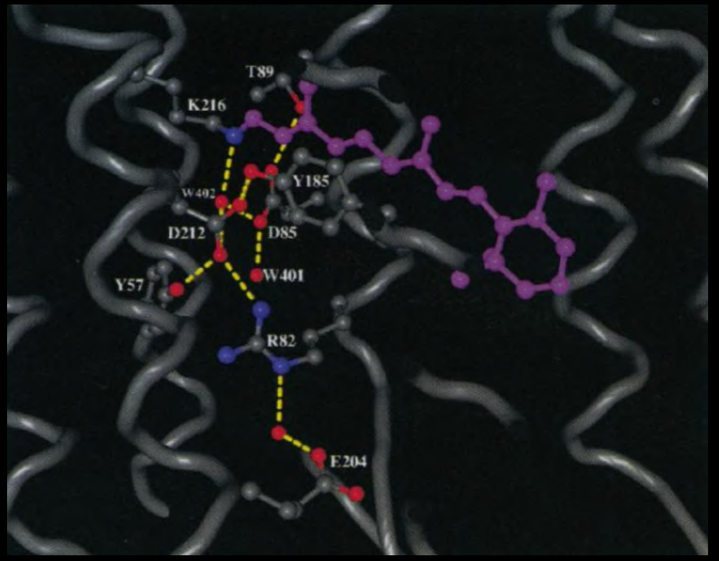
- The photoisomerization of retinal causes a change in configuration resulting in the protonated Schiff base sliding into a new protein environment. Now, the bR is at a meta-stable state (Higher energy).
- This pathway (photocycle) happens in two steps-
  - Release phase (Release of proton from the extracellular side of bR into the extracellular space).
  - Uptake phase (Uptake of proton from the cytoplasmic side of bR to reprotonate the Schiff base).



THE PHOTOCYCLE: THE RELEASE PHASE

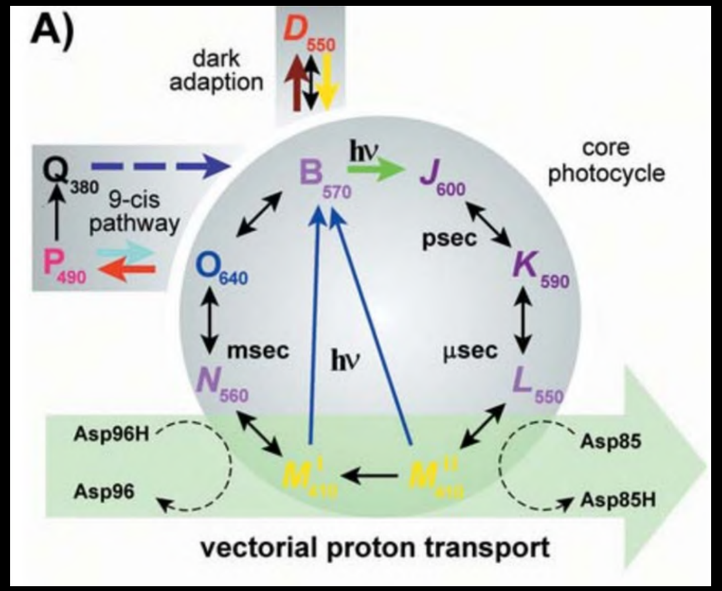
- The exposure to light causes the initial state of bR (B state) to convert to a short-living J state and then quickly proceeds to K state.
- The intermediates K,L and M are part of the release phase of the proton.
- Resonance Raman spectroscopy showed that in all the above intermediates, the chromophore 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.

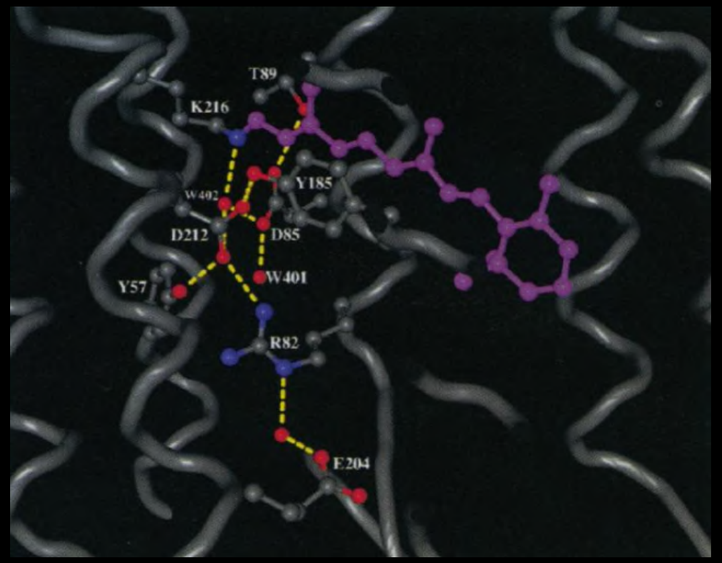




THE PHOTOCYCLE: THE RELEASE PHASE

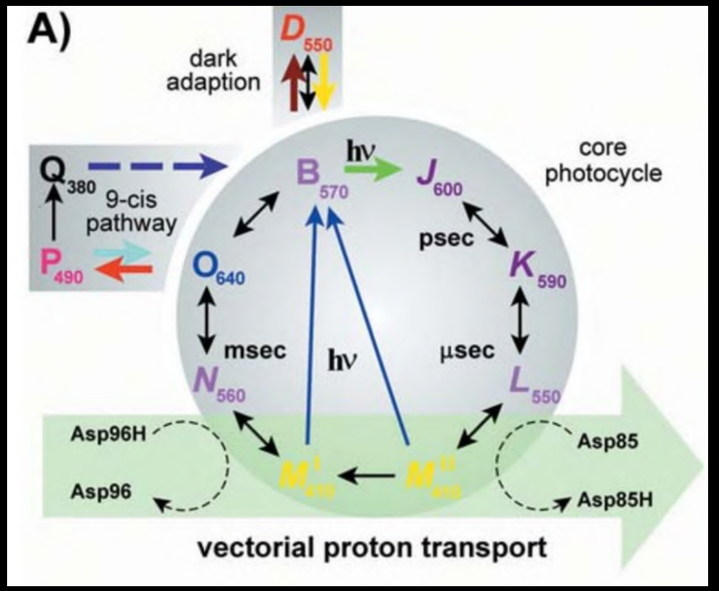
- Asp85 is hydrogen bonded to a water molecule (W401) and another water molecule (W402).
- W402 and Thr89 (Hydrogen bonded to Asp85) together stabilize the Schiff base-Asp85 pair in the unphotolyzed moiety.The breaking of these hydrogen bonds post photoisomerization of retinal causes the pKa of Asp85 to rise and results in its protonation by the Schiff base.
- pH sensitive dye analysis suggests that Asp85 (Hence, not the source of proton) remains protonated in M state.
- Arg82 and Asp85 may form a salt bridge and 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.
- Extensive spectroscopic and mutational studies have suggested that Arg82, Glu204 and Glu194 are key in the pathway of proton release.

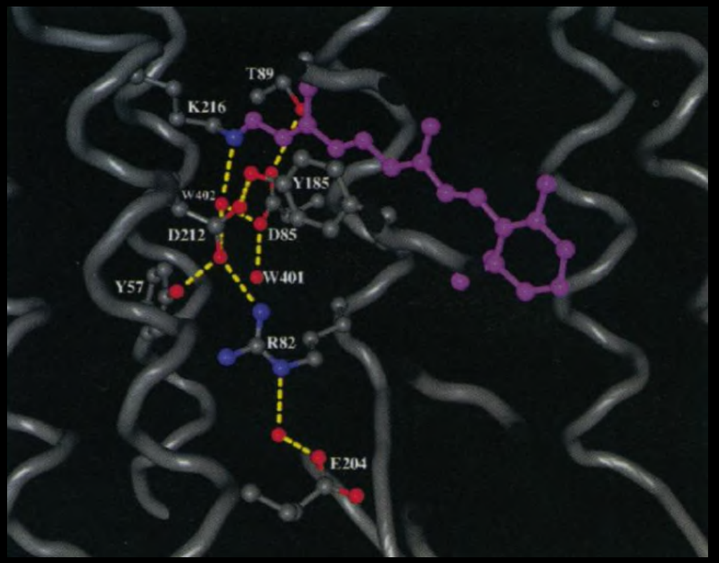




THE PHOTOCYCLE: THE UPTAKE PHASE

- The structural changes that occured 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 propagate reprotonation of the 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.
- The 9-methyl and 13-methyl of retinal are 3.6 and 3.7 angstroms from the closest heavy atoms - Trp182 and Leu93 respectively. These residues help in thermal reisomerization to all-trans form. This process happens during the N->O transition, hence completing the photocycle.





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: K <-> L <-> X <-> M <-> N <-> O -> bR

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)

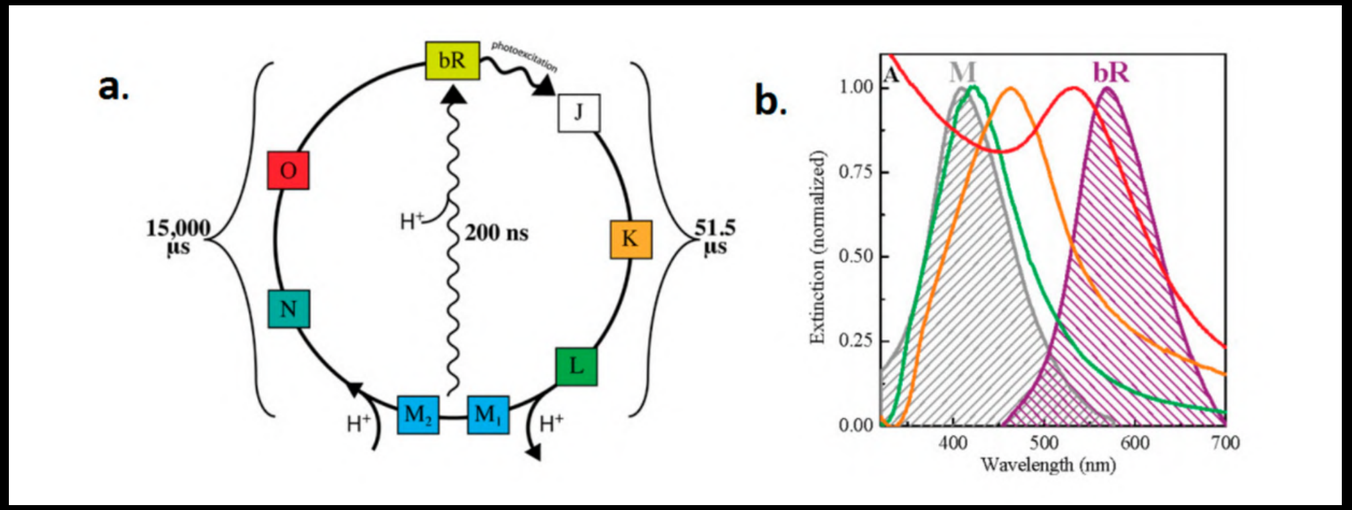
	pH 5		pH 7		pH 9	
Reaction	k (20°C)	E_A	k (20°C)	E_A	k (20°C)	E_A
Forward						
K → L	$5.42 \pm 0.10 \times 10^5$	9.0 ± 0.3	$5.38 \pm 0.09 \times 10^5$	8.9 ± 2.7	$5.97 \pm 0.11 \times 10^5$	8.9 ± 0.3
L → X	$2.46 \pm 0.06 \times 10^4$	17.7 ± 0.4	$5.03 \pm 0.78 \times 10^4$	12.6 ± 1.9	$9.60 \pm 0.55 \times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

SILVER NANO-PARTICLES

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. 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.



APPLICATIONS IN OPTO-ELECTRONICS

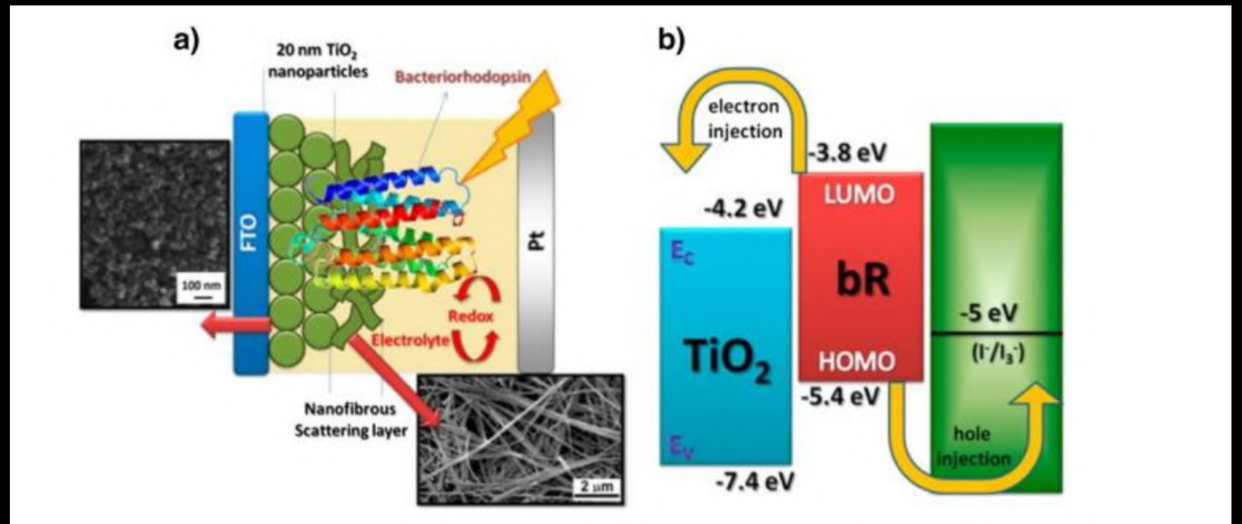
- Opto-electronics is the inter-conversion of light energy and electrical energy. It spans the areas of energy storage and production, optical sensors, optical information storage devices, etc.
- There are three important molecular functions occurring in the photocycle which are possible to exploit. 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

APPLICATIONS IN OPTO-ELECTRONICS

- 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.
- 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.

BIOMOLECULE-SENSITIZED SOLAR CELLS

- Low cost and eco-friendly.
- In bacteriorhodopsin based BSSCs, the photoanode is made of porous semiconductor (often Titanium Dioxide) nanoparticles sensitized with bR.
- Upon absorption, bR gets into an excited state, and injects photo-excited electrons into the conduction band (CB) of the semiconductor (Titanium Dioxide).
- The lowest occupied molecular orbital (LUMO) of bR is located at a higher energy than the CB of Titanium Dioxide and hence the injection of the photoelectrons into the CB is energetically favorable.
- 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.



CONCLUSION

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.