

BACKGROUND: Rhodopsin, a member of the G protein-coupled receptor (GPCR) family found on rods in the retina, is necessary in detecting contrast in low-light conditions. This photoreceptor gets its function through its ability to bind G proteins (Heterotrimeric GTP binding proteins). G proteins are important molecules in signaling: acting as on/off switches for numerous cascades within the cell. A common way in which G proteins are effectively utilized is through transmembrane activation, where an external signal from the cytoplasm binds or activates some transmembrane receptor, typically causing a conformational change that allows for the subsequent aggregation of G protein within the extracellular fluid.

Rhodopsin acts as this receptor. It is located, and is the only photoreceptor, in the membrane of the double-layered disks within rod cells. The rod cell is a highly specialized neuron within the retina. At the head of this cell is the rod outer segment (ROS): a stack of 1-2 thousand disks enclosed by a plasma membrane. A large portion (~50%) of this disk membrane is occupied by rhodopsin.

Rhodopsin is activated through the absorption of photons, which induce a conformational change in its structure. The change allows for the binding of the membrane-bound G protein transducin to rhodopsin, initiating the visual cascade.

THE VISUAL CASCADE: When rhodopsin is activated by photons, it changes its conformation to create an active site capable of interaction with the binding site of transducin. This conformational change is driven by the isomerization of 11-*cis*-retinal, a chromophore within the transmembrane hydrophobic pocket of rhodopsin, to all-*trans*-retinal via photon energy. The following interaction of transducin at this newly formed active site initiates a release of GDP from its binding site, creating an open active site that can then bind readily to rhodopsin.

Transducin is activated in the presence of GTP. Upon activation, transducin picks up a GTP thus triggering the release of the heterodimer G protein portion of this complex. The resulting structure is the activated form of rhodopsin, termed metarhodopsin II (Meta II). Meta II decays rapidly to opsin (the transmembrane domain of rhodopsin), via the release of the all-*trans*-retinal. The chromophore releases because the Schiff base covalently binding the all-*trans*-retinal to rhodopsin is hydrolyzed after photoisomerization, causing the photobleached product to be unrestricted. It is proposed that this process is unidirectional, where 11-*cis*-retinal enters at an initiation site on the intermembrane intradiscal region, is bound to a hydrophobic site within the binding pocket, and is released upon photoactivation through a different intradiscal site.

Furthermore, the dissociation of the heterodimer of transducin from rhodopsin causes it to split into its α and $\beta\gamma$ domains, which then can activate portions of the visual cascade downstream.

The α subunit of transducin, importantly, binds to a cGMP phosphodiesterase enzyme (PDE), causing its activation. The activation of these PDEs trigger the hydrolysis of cGMP to GMP, thus lowering cGMP concentrations within the cytosol. Since cGMP plays a role in holding Na^+ channels open, low levels cause these plasma membrane cation channels to close, thus blocking Na^+ influx.

Inactivated rod cells are constantly depolarized, due to the positive charge associated with inflowing Na^+ . As a result, the rod cell is constantly releasing its neurotransmitter glutamate into its synaptic cleft. The inhibition of ion flow by reduced cGMP concentrations, therefore, results in increasingly negative voltage, triggering hyperpolarization across the membrane. This electrical signal then travels along the rod cell to the synaptic terminal to terminate glutamate release.

Bipolar cells, which synapse at the rod cell synaptic terminal, respond to this drop in glutamate by depolarizing and sending an electric signal through neurons across the optic nerve and into the brain. This creates the conceptualization of vision in a low-light environment.

Once more 11-*cis*-retinal is produced, opsin can be converted back into inactive rhodopsin. This process occurs very rapidly, and thereby terminates the signal activity rapidly. This allows for a low activation threshold, which explains the highly sensitive ability of rhodopsin to produce visual signal from as little as one photon of light.

Figure 5 (Right)- Schematic Representation of Inactive Rhodopsin. Cytoplasmic loops (C-I, C-II, C-III), extracellular loops (E-I, E-II, E-III), transmembrane helices (H-I-H-VII) and a peripheral helix (H-VIII) are indicated. Two important stabilizing and binding regions are shown in grey (ERY salt bridge, and NPVIY G protein binding region, respectively). Two cysteine residues (lime green) also stabilize the inactive structure through palmitoylation to the membrane.

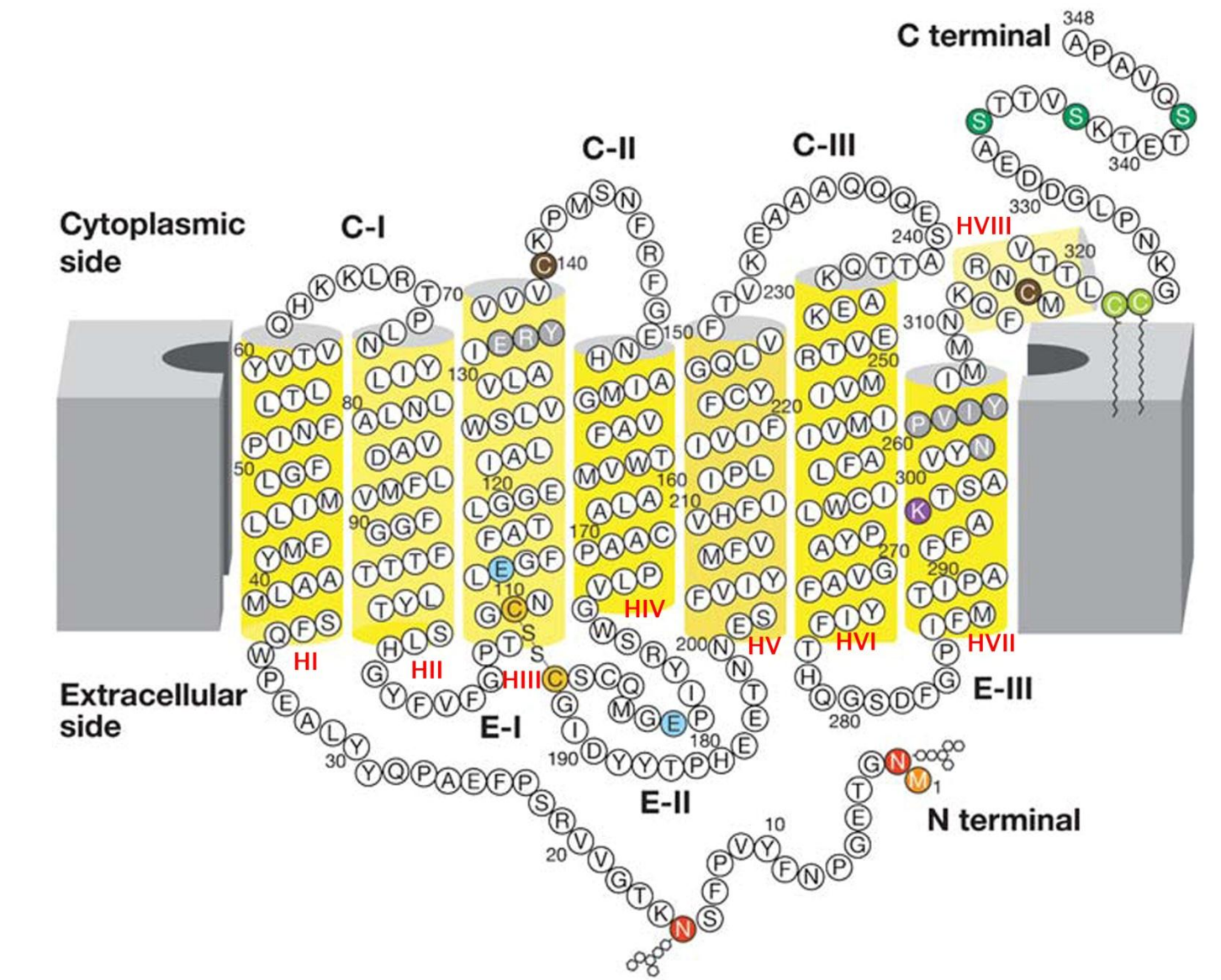
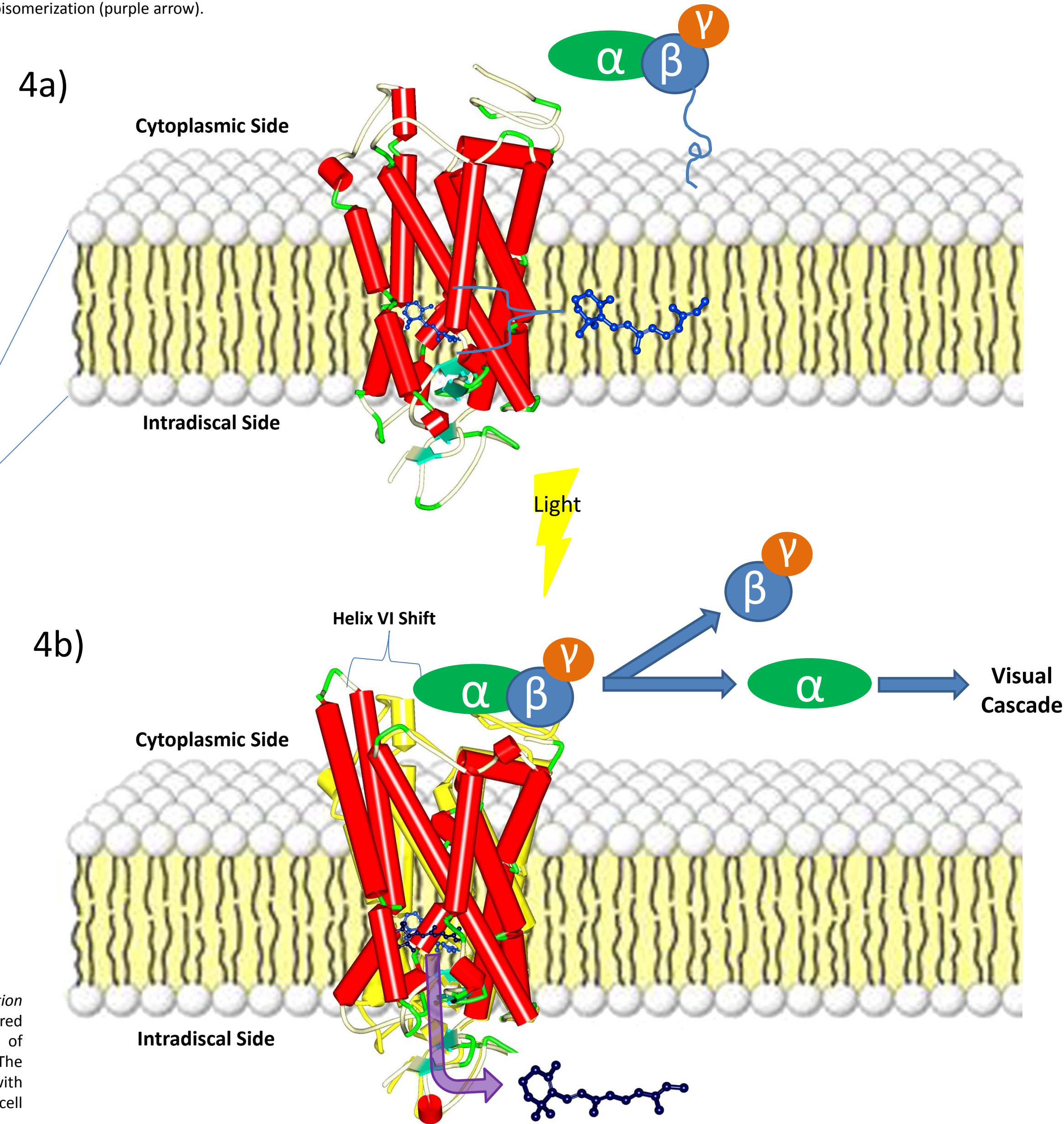


Figure 4 (below)- Photon Activation of Rhodopsin. (a) The transmembrane receptor rhodopsin is shown prior to photon activation. The membrane-bound transducin protein is represented by its three subunits: α , β and γ . 11-*cis*-retinal is shown bound within the transmembrane helices, and then magnified for clarification (baby blue and baby blue with bracket, respectively). (b) Rhodopsin is shown after activation with photon energy. The conformation prior to activation is shown in yellow (with retinal in baby blue). After activation the conformation is shown in red (with retinal in dark blue). Also apparent is the significant shift in helix VI. This shift allows for the binding of transducin. After activation transducin is broken into its respective components, and the α -subunit may progress through the visual cascade (blue arrows). Also, the all-*trans*-retinal (dark blue) exits the hydrophobic membrane core after photoisomerization (purple arrow).



STRUCTURE: Rhodopsin, in its inactive state, is composed of a seven α -helical transmembrane receptor with an 11-*cis*-retinal chromophore covalently bound at a Lys296 residue via a Schiff base within the hydrophobic center of the transmembrane helices (Figure 5). The transmembrane helices of rhodopsin are highly irregular, showing significant bending around Gly-Pro residues. The most pronounced distortion is around a proline at residue 267 on helix VI. A salt bridge formed between the protonated Schiff base and Glu133 is an important constraint holding rhodopsin in its inactive state. However, when this salt bridge is disrupted by the isomerization of retinal the flexible helix VI moves into its active position.

The N-terminal tail of rhodopsin (and opsin) is situated intradiscally (extracellularly). Also on the extracellular side of rhodopsin are three interhelical loops (E-I, E-II, and E-III). These regions act as a chromophore-binding site that is important for the reincorporation of 11-*cis*-retinal into opsin after Meta II has degraded at the conclusion of a cycle of the visual cascade.

The cytoplasmic portion of rhodopsin also contains a C-terminal tail and three interhelical loops (C-I, C-II, and C-III). Additionally, there also exists a peripheral helix VIII. This helix is not bound within the membrane, but is fastened to the membrane by the palmitoylation of two contiguous cysteine residues (Cys³²² and Cys³²³). These structures play an important role in the binding of transducin. More specifically, an NPXXY region is homologous in the rhodopsin of all organisms, and is thought to be the key binding site of transducin. The conformational shift upon activation, primarily occurring in helix VI, projects this region toward the active site, thus allowing the binding of transducin (Figure 3b).

REFERENCES: (1) Palczewski, K., 2006. "G Protein-Coupled Receptor Rhodopsin." *Annu. Rev. Biochem.* 75:743-67. (Figure 5).
 (2) Jastrzebska, B., et al, 2010. "Complexes between photoactivated rhodopsin and transducin: progress and questions." *Biochem. J.*, 428(1) pp. 1-10.
 (3) Palczewski, K., et al., 2000. "Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor." *Science*, 28(5480), pp. 739-745.
 (4) Janz, J.M., et al., 2004. "Rhodopsin Activation Exposes a Key Hydrophobic Binding Site for the Transducin α -subunit C Terminus." *J. of Bio. Chem.*, 279(28), pp. 29767-73.
 (5) Molecular structures obtained from Protein Data Bank (1U19 and 3PXO), www.rcsb.org
 (6) Stock membrane photo from Anderson Labs, www.andersonlab.qb3.berkeley.edu.

Figure 1: The human eye. A portion of the retina, located on the dorsal posterior region of the eye, is selected (blue rectangle) and magnified in Figure 2.

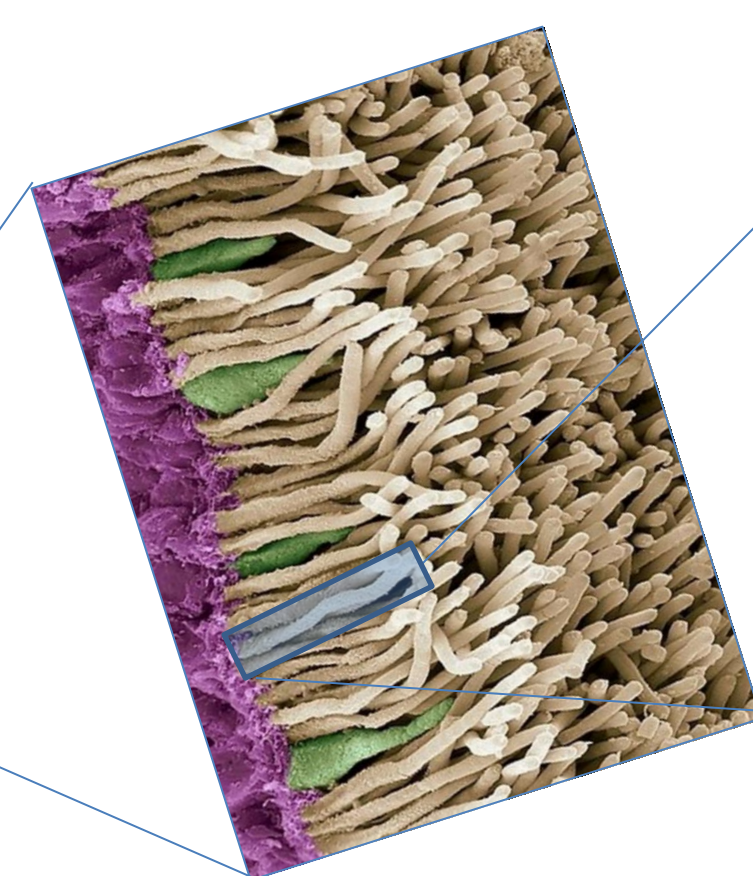
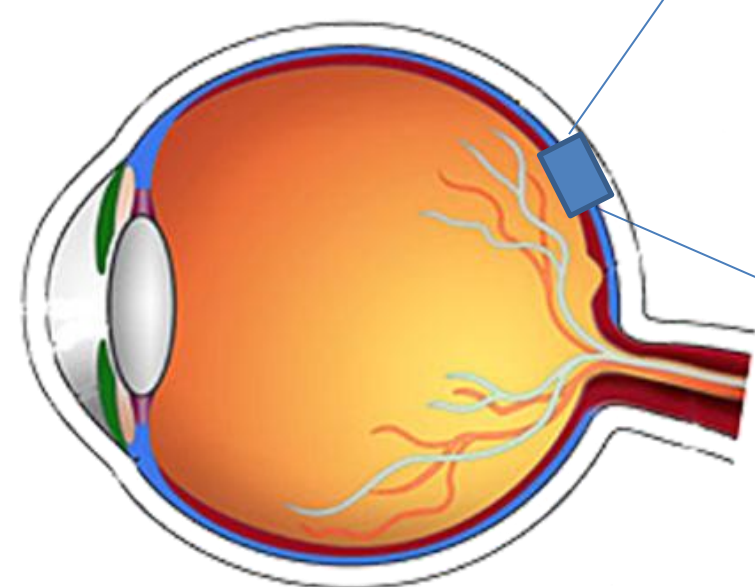


Figure 2: Magnification of the retina of the human eye. The white finger-like projections are numerous rod cells, which are densely packed within the retina. A rod cell is selected (blue rectangle) and magnified in Figure 3.

Figure 3- Diagram representation of the rod cell. The ROS region (red rectangle) contains thousands of membrane enclosed disks. The synaptic terminal is indicated with a red asterisk and the neuronal cell body with a blue asterisk.