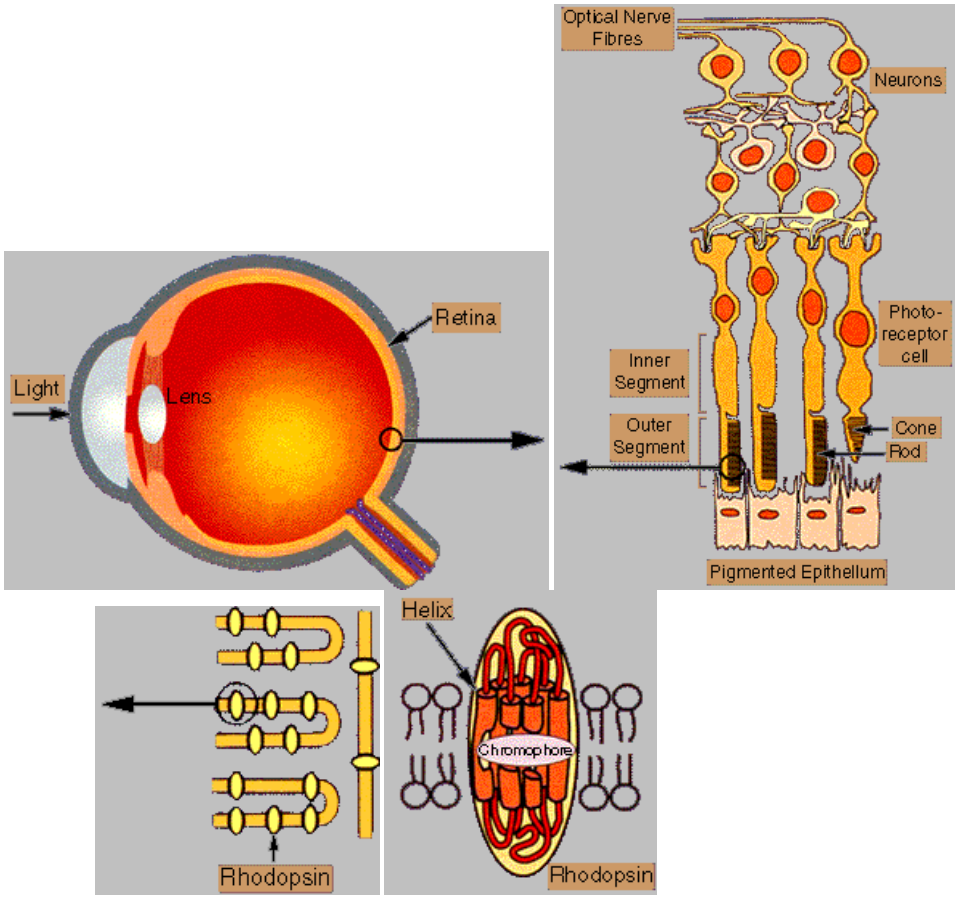
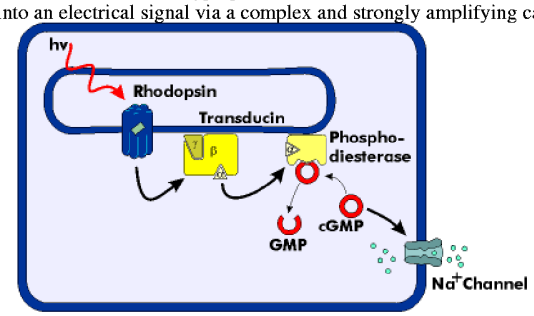
The process of Vision



Signal transduction in animal eyes

How does rhodopsin pass the light signal on to the cell? In the eye, the protein conformation change leads to a very sophisticated signal chain. The rod cells of the retina consist of stacks of disks. Each disk contains many rhodopsin molecules. Absorption of a photon, isomerization and conformation change lead to the activation of transducin. Transducin is a G protein, which activates cGMP phosphodiesterase. This enzyme cleaves cGMP (a small messenger molecule). The cGMP level drops. cGMP keeps Na^+ channels in the cell membrane open, thus depolarizing the cell. A light stimulus decreases the cGMP level, the Na^+ channels close and the cell hyperpolarizes relative to the dark level. In this way, the light signal is converted into an electrical signal via a complex and strongly amplifying cascade.

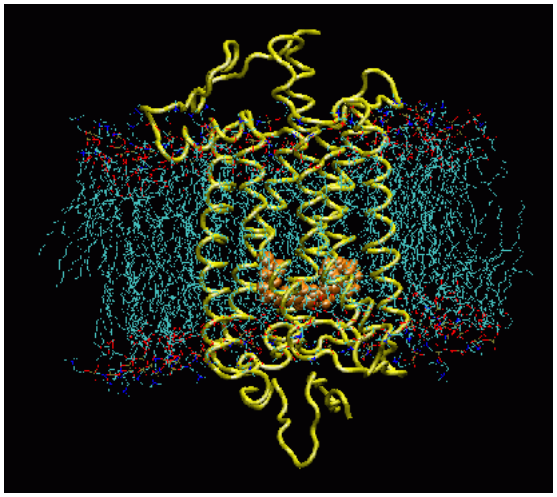


While Na+ carries most of the current into the cell, a small amount of Ca^{++} also enters the cell through the same channels. During light stimulation, Ca^{++} influx is also blocked. The Ca^{++} concentration in the cell drops. This accelerates some reactions, e.g. the synthesis of cGMP. Thus, Ca^{++} plays a minor part in depolarizing the cell, but is very important in adaptation and return to the resting state.

After isomerization, the retinal is cleaved from the protein, leaving behind an opsin without chromophore. It takes some time to replenish the protein with retinal in the correct 11-cis conformation. This explains the long-lasting bleaching effects of bright light to our eyes.

Opsins

- Rhodopsins (Rh) are a class of proteins whose common feature is the presence of a *retinyl cromophore* (retinal, or similar, attached somehow to the protein) - the proteins without retinal are called *opsins*.



Rhodopsin embedded in a patch of POPC membrane. Retinal is shown in orange

- Rhodopsin is a membrane protein, i.e. it sits inside a lipid bilayer. It has seven trans-membrane helices which are approximately parallel. Thus, rhodopsin resembles a cylinder with its axis at a right angle against the membrane surface. The protein itself does not absorb much light.
- In most cases, the cromophore is 11-*cis*-retinal. It is attached to *opsin* in a Schiff base linkage through an amino group of lysine.
- Different kinds:
  - Animal rhodopsin. Bovine rhodopsin is one of the most studied systems, in the late 1960s it was established that irradiation of Rh isomerizes the 11-*cis*-retinal cromophore to *all-trans* retinal (Wald, Science **162**, 230 (1968)). Afterwards, the mechanism is a signal transduction.
  - Rhodopsin in algae. Mechanisms are different, and quite unknown.
  - Bacteriorhodopsin. The bacterium Halobacter halobium produces a special version of rhodopsin, the so-called bacteriorhodopsin. This protein is not a signal processor, but a light-driven proton pump. As such, it converts light energy into an electrochemical gradient across the bacterial plasma membrane. The proton gradient can be used to synthesize ATP. Isomerization of retinal is different to that in animal rhodopsin: *all-trans* retinal to 13-*cis*-retinal.

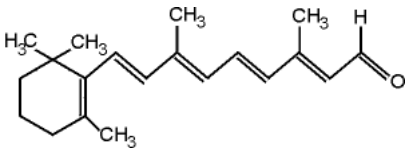
    The structure of bacteriorhodopsin has been elucidated. This was possible, because the protein - in contrast to other rhodopsins - occurs as an ordered two-dimensional crystal in the membrane, allowing electron crystallography
  - ...

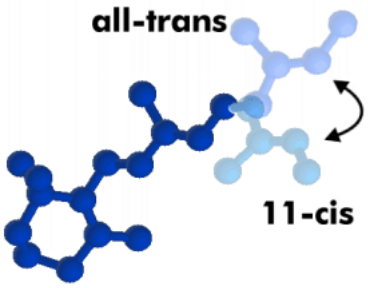
Retinal

Retinal is a small molecule derived from retinoic acid, vitamin A. It cannot be synthesized de novo in the human body, hence our dependency on vitamin A for unhindered vision. It has two interesting properties:

- First, it is an aromatic system, i.e. the double bonds form large orbitals that extend over the whole molecule. The electrons occupying these orbitals can be excited by light very easily. Whereas ”normal” electrons cannot interact with visible light, the electrons in retinal can absorb photons in the visible range of wavelengths (400 - 800nm). solution of retinal has an intensive yellow colour.

- Second, the double bonds can isomerize upon absorption of photons. Isomerization means that for a short moment the atoms can freely rotate along a double bond, thereby changing the geometry of the whole molecule. When retinal is bound to opsin (rhodopsin without retinal is just called ”opsin”), only one isomerization is possible: 11-cis retinal is transformed into all-trans retinal. This leads to a conformation change of the whole protein, thereby activating a signal pathway.





- The absorption spectra of isolated retinals are characterized by three bands: a *principal* band, broad, at about 3.3 eV, and two additional weaker ones at about 4.4 and 5 eV. Oscillator strenghts of these transitions are isomer-dependent. There seem to be 16 known isomers of retinal.

- Isomerization process in vision: irradiation of Rh → 11-*cis*-retinal isomerizes to all-*trans*-retinal : in appr. 200 fs, the photo-Rh is produced, a bathocromically shifted photo-product, which has a highly distorted *trans* conformation. This thermally relaxes to batho-Rh. Thermal relaxation keeps going → all-*trans*-retinal + opsin. This is colourless (in contrast to former oranged rhodopsin). The all-*trans*-retinal is reduced to retinol, esterified, isomerized to 11-*cis*-retinol and oxidized to 11-*cis*-retinal → recombination with opsin, and back to the beginning.

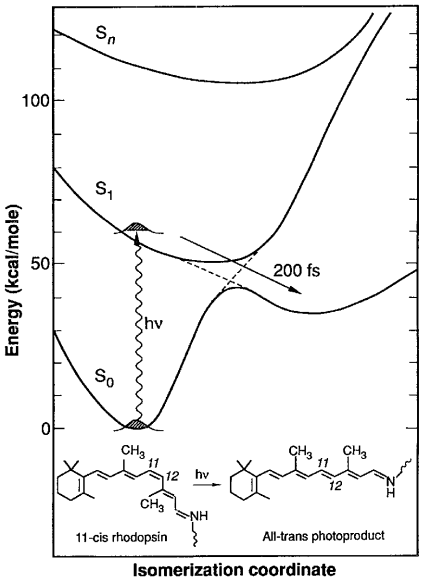


Fig. 1. Schematic ground-state and excited-state potential energy surfaces for the 11-*cis* → 11-*trans* isomerization in rhodopsin, adapted from (*14*). The reaction path of the photoisomerization is indicated by the nonadiabatic potential surfaces (broken lines).

Schoenlein *et al*, Science **254**, 412 (1991)

Some things that have been done

- Study of the kinetics of the femtosecond isomerization of retinal in rhodopsin, resolving it with the use of femtosecond opcical measurement techniques.

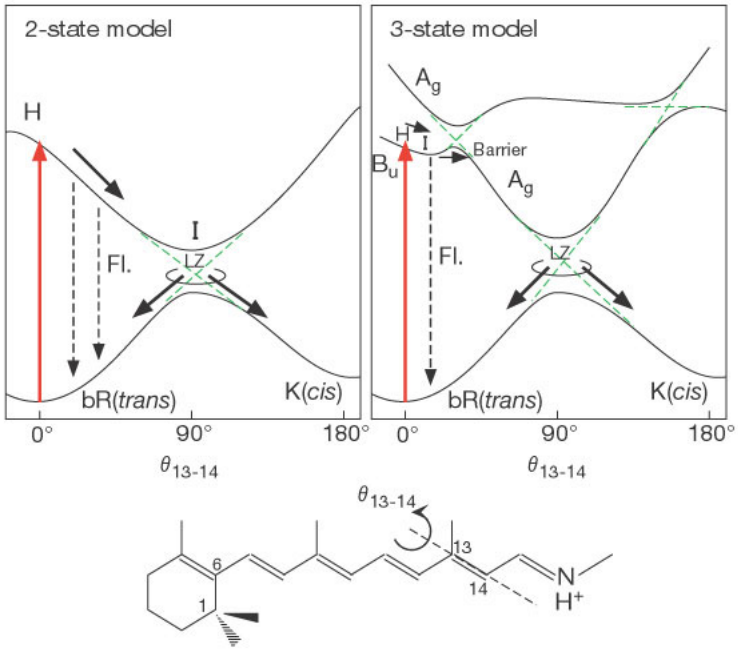
  Schoenlen *et al*, Science **254**, 412 (1991).

  Excitation of the rhodopsin with a 35 fs pump at 500 nm, and observe within 200 fs an increased absorption, which signals the formation of photoproduct. So the first step in vision (11-*cis* → 11-*trans*) is complete in 200 fs.

- Real time spectroscopy of transition states in bacteriorhodopsin during retinal isomerization.

  Kobayashi *et al*, Nature **414**, 531 (2001).

  Use of visible-light pulses of less than 5 fs in duration to monitor changes in the vibrational spectra of the molecule. Observations lead to favour the theory of a three-state model for the isomerization.



- *Ab initio* study on the lowest excited states of retinal.

  Merchán *et al*, J. Chem. Phys. **106**, 1112 (1997).

  Determination of ground states geometries, and vertical excitation energies using multiconfigurational second-order perturbations theory through the CASPT2 formalism.

- Quantum dynamics of the femtosecond photoisomerization of retinal in bacteriorhodopsin.

  Ben-Num *et al*, Faraday Discuss. **110**, 447 (1998).

  Quantum chemical calculations suggest that three coupled electronic states are involved in the motion. Use of "multiple spawning method" for simulating these non-adiabatic dynamics. Conclusion: most of the population transfer occurs within 300 fs.

- *Ab initio* photo-isomerization dynamics of a simple retinal chromophore model.

  Vreven *et al*, J. Am. Chem. Soc. **119**, 12687 (1997).

- Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor

  Palczewski *et al*, Science **289**, 739 (2000).

  Determination of the structure of rhodopsin from diffraction data extending to 2.8 angstroms resolution. Interactions of the chromophore with a cluster of key residues determine the wavelength of the maximum absorption.