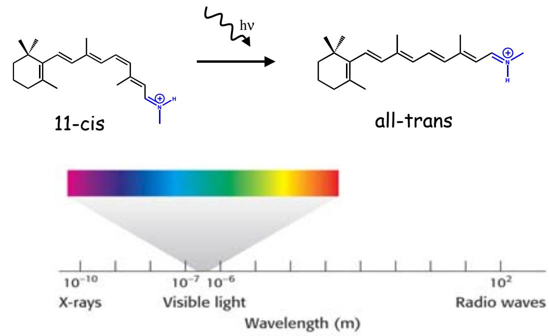
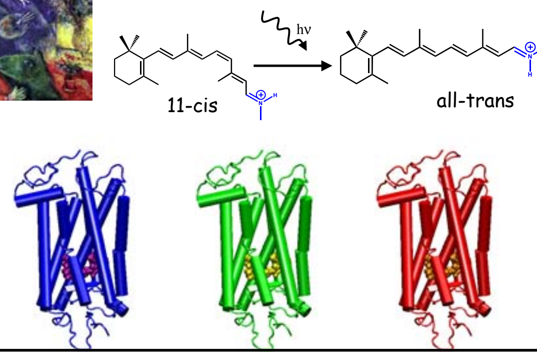
Spectral Tuning in Retinal Proteins



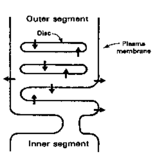
Color Vision



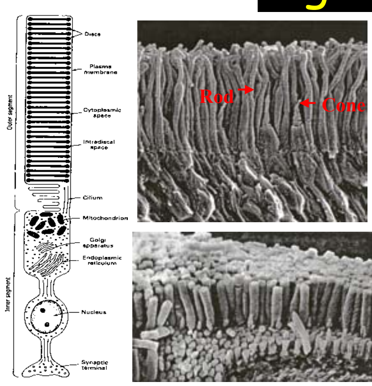
Visual Receptors

Light

G-protein signaling pathway

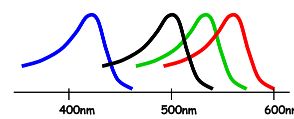


Rhodopsin

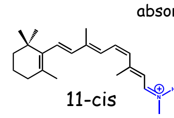


Spectral tuning in color visual receptors

Color is sensed by red, green and blue rhodopsin visual receptors.



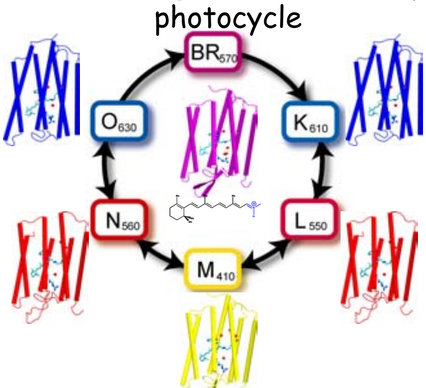
absorption spectrum



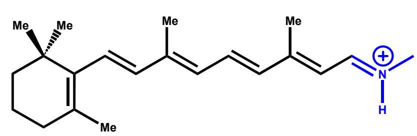
Their chromophores are exactly the same!

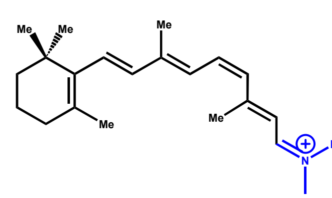
How does the protein tune its absorption spectrum?

Spectral Tuning in bacteriorhodpsin's photocycle

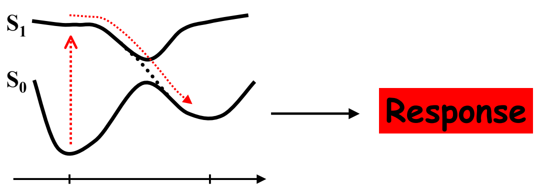


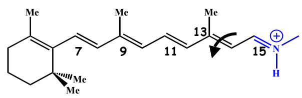
How can we change the maximal absorption of retinal chromophore?



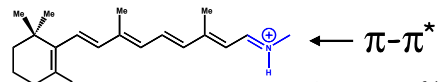


Excitation energy determines the maximal absorption

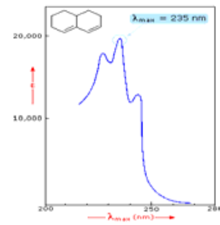


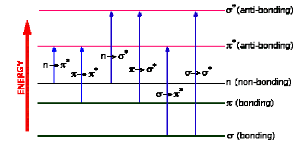


Electronic Absorption

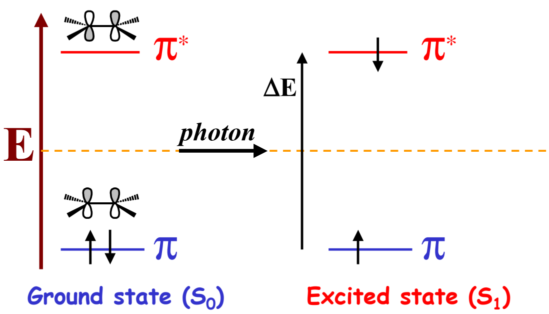


Absorption of light in the UV-VIS region of the spectrum is due to excitation of electrons to higher energy levels.



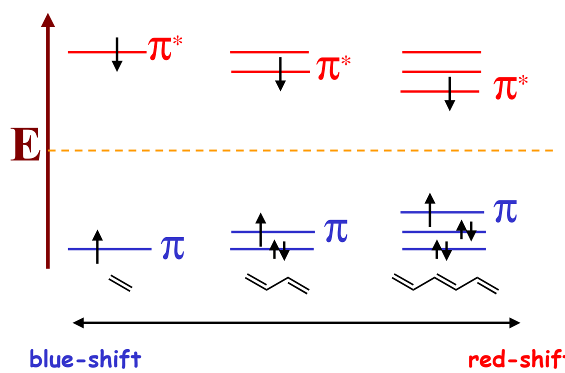


π-π* excitation in polyenes

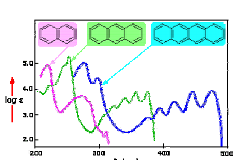


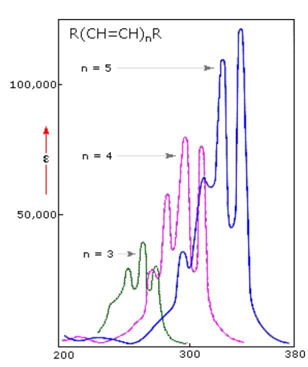
ΔE (excitation energy, band gap) = $h\nu = hc/\lambda$

π-π* excitation in polyenes

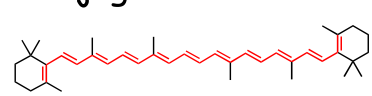


π-π* excitation in polyenes

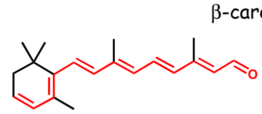




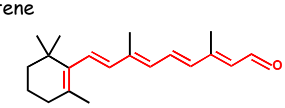
Tuning the length of the conjugated backbone



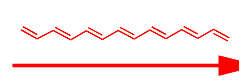
β-carotene



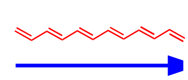
Vitamin A2 (retinal II)



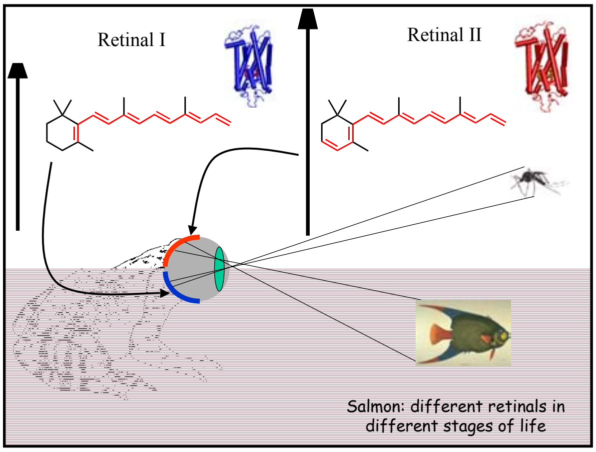
Vitamin A1 (retinal I)



Longer wavelength



Short wavelength



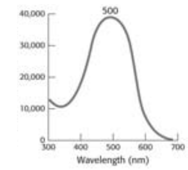
OPSIN SHIFT: how protein tunes the absorption maximum of its chromophore.

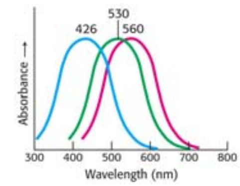
Maximal absorption of protonated retinal Schiff base in:

Water/methanol solution: 440 nm

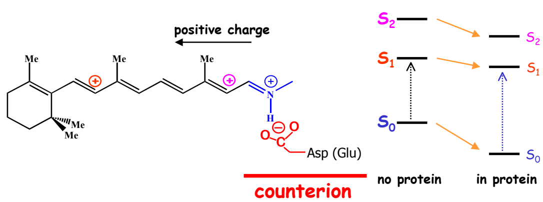
bR: 568 nm

rod Rh: 500 nm
red receptor: 560 nm
green receptor: 530 nm
blue receptor: 426 nm



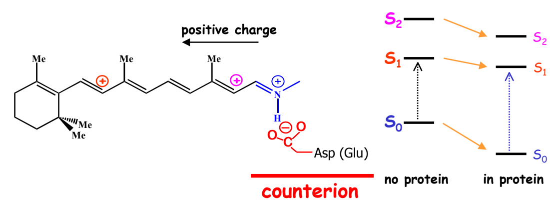


Electrostatics and opsin shift



- The counterion stabilizes the positive charge of the chromophore.
- The position of the counterion determines how and how much the band gap energy changes.

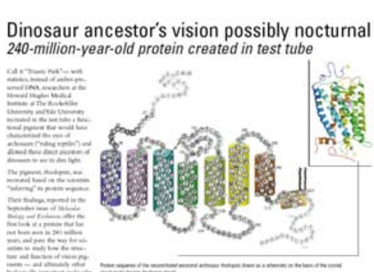
Electrostatics and opsin shift



Maximal absopriton of protonated retinal Schiff base can be changed by D85N (Purple to blue shift)

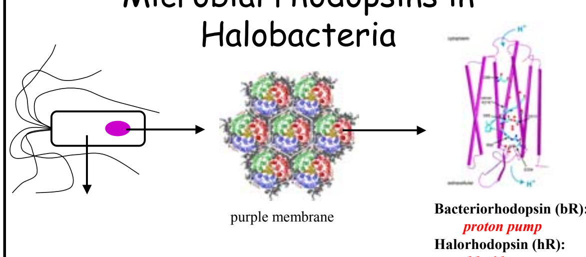
Red shift from 568 to 605 nm at pH = 3

Dinosaurs had red-shifted visual receptors!



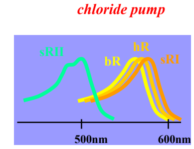
Howard Hughes Medical Institute
at The Rockefeller University and
Yale University

Microbial rhodopsins in Halobacteria



Sensory rhodopsin I (sRI):
attractant (repellent) to orange (near UV) light
Sensory rhodopsin II (sRII):
repellent to blue-green light

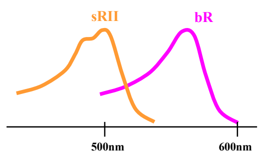
phototaxis
(color vision of halobacteria)



Spectral Tuning in Bacterial Rhodopsins

Sensory Rhodopsin II (sRII) Phototaxis	Bacteriorhodopsin (bR) Proton pump

• Large blue shift of absorption maximum in sRII (70 nm)



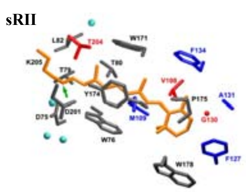
Structures of bR and sRII

X-ray crystallography shows that structures are very similar. Both include protonated *all-trans* retinal Schiff base

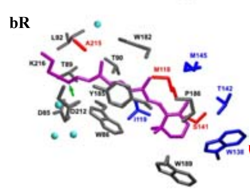
orange: sRII
purple: bR

Binding Sites of bR and sRII

sRII



bR



Similar structure
• Aromatic residues.
• Hydrogen-bond network. (counter-ion asparatates, internal water molecules)

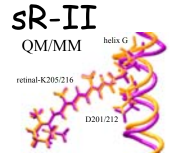
Mutagenic substitutions

T204A/V108M/G130S of sRII produces only 20 nm (30%) spectral shift.

What is the main determinant(s) of spectral tuning?

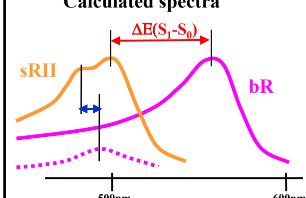
QM/MM Calculation of spectral shift in bR and sR-II

• Refinement of X-ray structures by HF (retinal, 2Asp, $3H_2O$)
• Excitation energy calculations for retinal



bR: purple, sRII: orange

Calculated spectra



Spectral shift

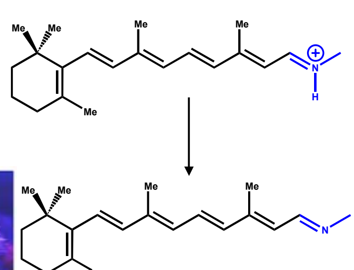
$\Delta E(S_1\text{-}S_0)$: 6.1 (exp. 7.2) kcal/mol

$\Delta E(S_2\text{-}S_0)$: 1.7 (exp. 4.0) kcal/mol

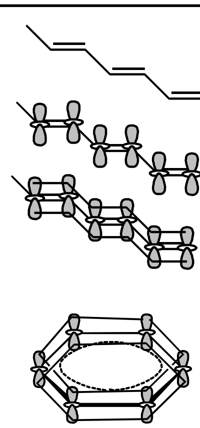
A sub-band in sRII is due to the second excited state (S_2).

Deprotonation of the Schiff base

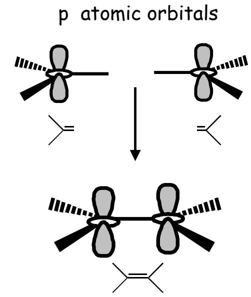
UV vision
birds, honeybee



Strong blue shift

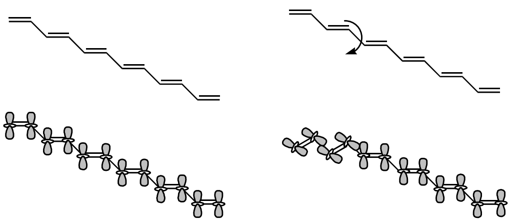


p atomic orbitals



Planarity is essential for maximal overlap of p orbitals in a double bond (π molecular orbital)

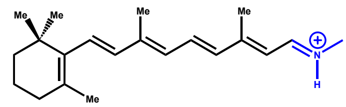
Steric interactions and spectral shift?

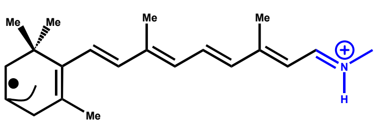


A highly twisted structure can decrease the overlap of p orbitals and effectively decrease the length of the conjugation, i.e., blue shift.

Summary of Mechanisms of Spectral Tuning

- Using a different chromophore with a longer or a shorter conjugated chain
- Modifying the amino acid composition of the binding pocket (electrostatics)
- Manipulating the distance and/or conformation of charged/polar groups in the vicinity of retinal
- Steric interaction with the chromophore so that some of the double bonds go out of plane (a similar effect to using a shorter chromophore)
- Protonation state of retinal Schiff base (Strong blue shift upon deprotonation)





The chromophore retinal adopts different colors in different environments. Doesn't it remind you of something?