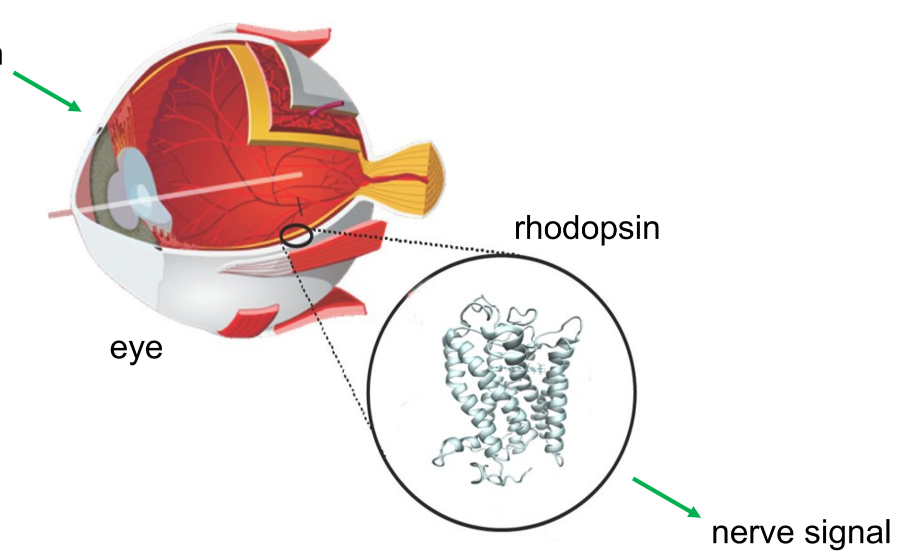
Study of the visual adaptation mechanism in marine species with the change of habitation depth. Demid Osipov, Daniil Moshnikov. Physical-Technical High School.

Introduction

Perception of light



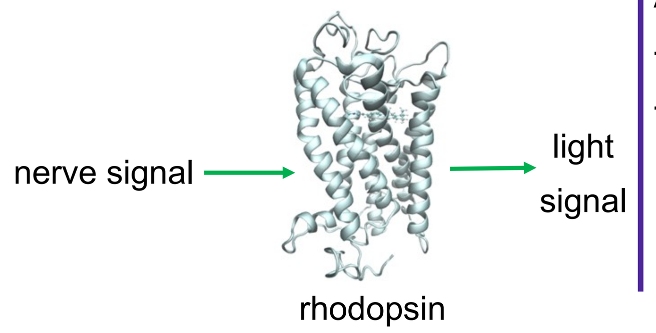
Rhodopsins, which are light-sensitive proteins located in our eye, transform incoming light (photon) into nerve signal to brain.

Molecular imaging of nerve systems

Special microbial rhodopsins can work in reverse direction—for imaging of nerve pulses. Applied voltage alters the intensity of their fluorescence.

Method of imaging nerves

- Potential-dependent rhodopsin is inserted into neuron
- Neuronal potential alters fluorescence

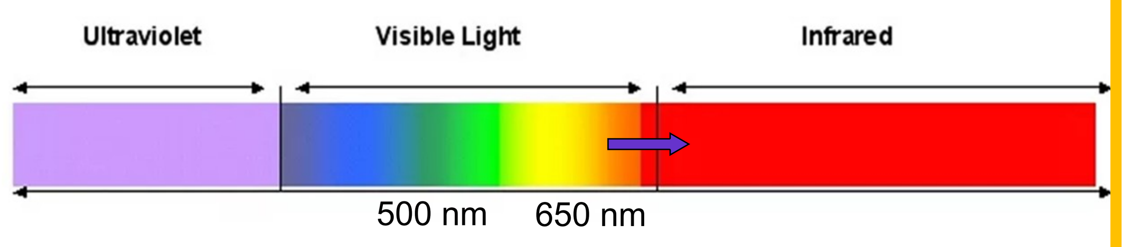


Applications of imaging:
- investigating brain diseases
- studying nervous system

- **Problem:** microbial rhodopsins absorb and radiate light, which can not go through tissue. Surgery is required to register the fluorescence.
- **Solution:** radiation in the IR range goes freely through biological tissues. We need to shift the absorption and fluorescence spectrum of rhodopsins towards IR.

Our goal

Investigate the mechanism that can be used for spectral tuning of rhodopsins in order to shift radiation towards IR.



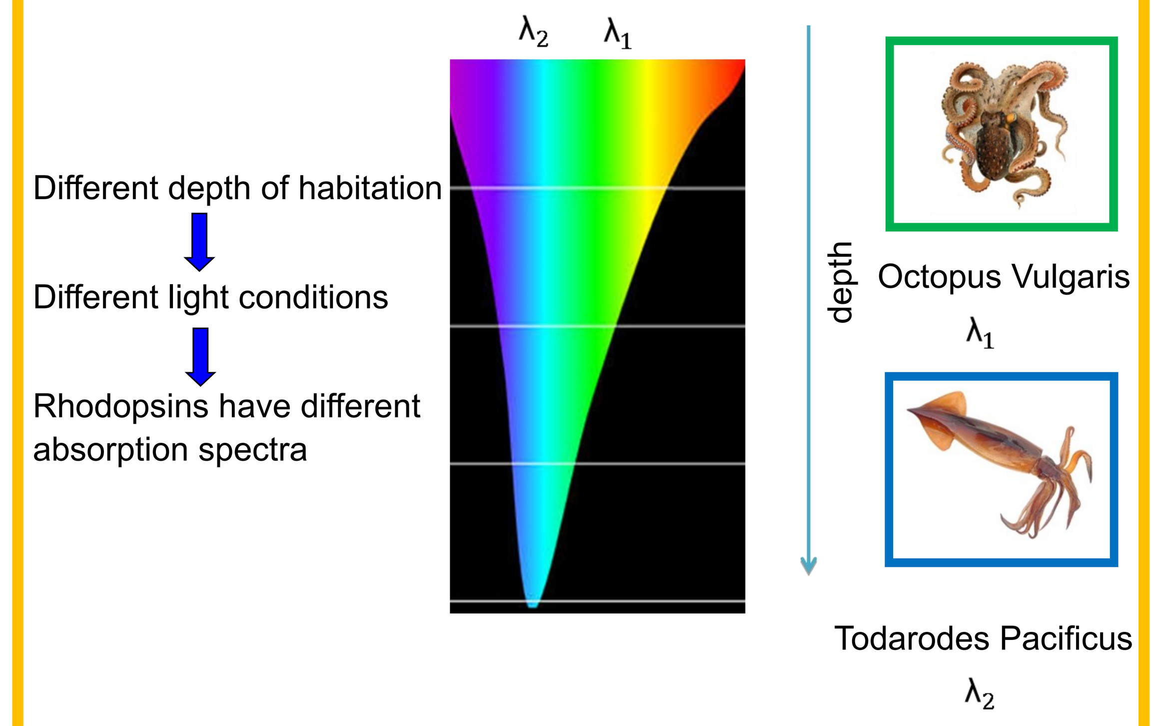
Approach to the task

- Wavelength of radiated photon ≈ wavelegth of absorbed photon.
- Absorption spectra of rhodopsins in an eye defines visual range of an animal.

To investigate the differences between the rhodopsins that have different absorption spectra we decided to study the differences in rhodopsins of species which have different visual range.

Todarodes Pacificus — Octopus Vulgaris

Differences in color perception

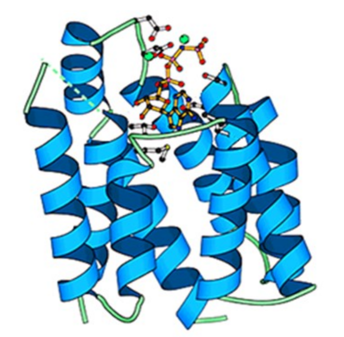


Rhodopsin

Rhodopsin is a protein. It consists of amino acids. Sequence of amino acids uniquely determines

Properties of rhodopsin
- Chemical
- Physical (including spectrum)
- Biological functions

Tertiary structure of rhodopsin

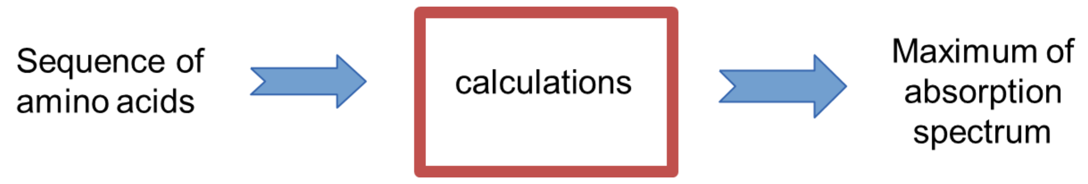


In order to change the spectrum of rhodopsin one has to change its amino acid sequence.

Methodology

For this problem we used computational modeling of rhodopsin structure and spectrum starting from its amino acid sequence.

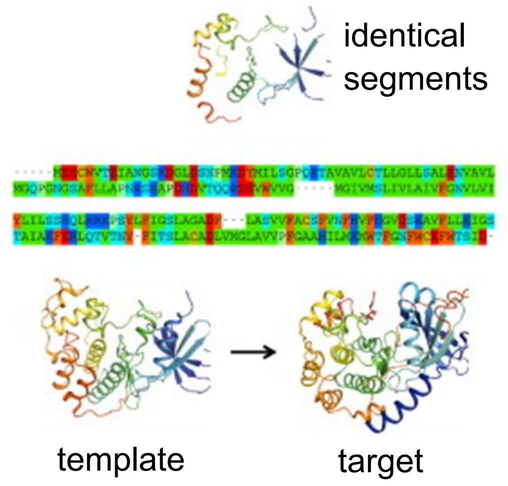
1. Firstly on the basis of amino acid sequence (taken from database) we created the structures (spatial position of atoms) of rhodopsin and optimized them.
2. Secondly we calculated absorption spectrum of rhodopsin on the basis of these structures.



How is the structure of rhodopsin predicted using computer modeling?

- Search for template with known experimental structure
- Align sequences of target and template
- Build model
- Refine model (inserting water and hydrogen network)
- Optimize the structure by minimizing energy function

$E(x_1, y_1, z_1, \ldots, x_n, y_n, z_n)$, where x_i, y_i, z_i - are the coordinates of atom i

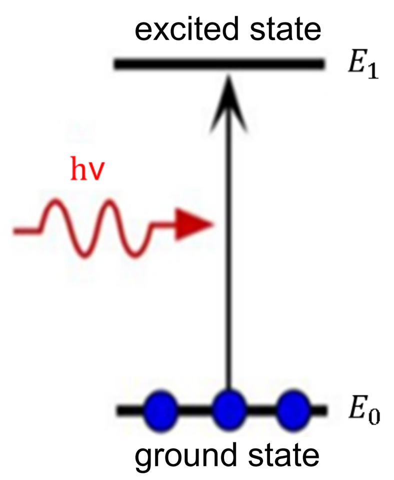


How is the rhodopsin spectrum calculated?

We find the energies of rhodopsin in its ground and excited states by solving the Schrödinger equation with a program package ORCA.

Absorption maxima

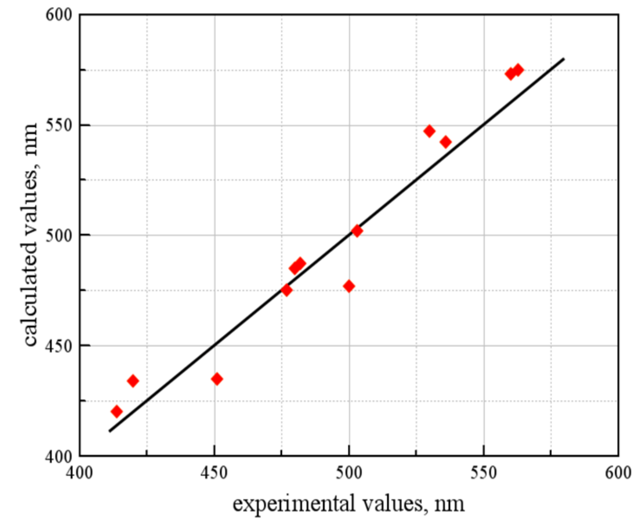
$$\lambda = \frac{ch}{(E_1 - E_0)}$$



Testing the methodology

The method was used to calculate the absorption spectra of some rhodopsins with known spectra. Calculated values correlate well with the experimental data. In our test pool we took rhodopsins from rods and/or cones from the following species:

- *Brachydanio Rerio*
- *Carassius Auratus*
- *Careproctus Rhodomelas*
- *Bufo Bufo*
- *Bos Taurus*
- *Homo Sapiens*

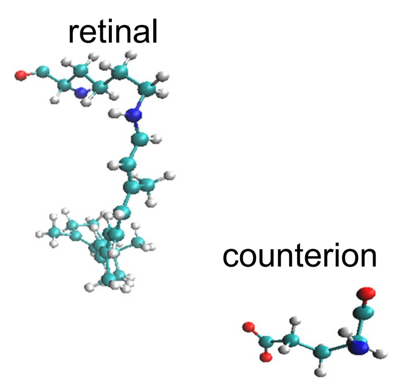


Mechanism of spectral shift

In rhodopsin there is a **retinal** that absorbs photons.

Near retinal there is a **counterion** – negative amino acid which accepts proton from retinal right after photon absorption.

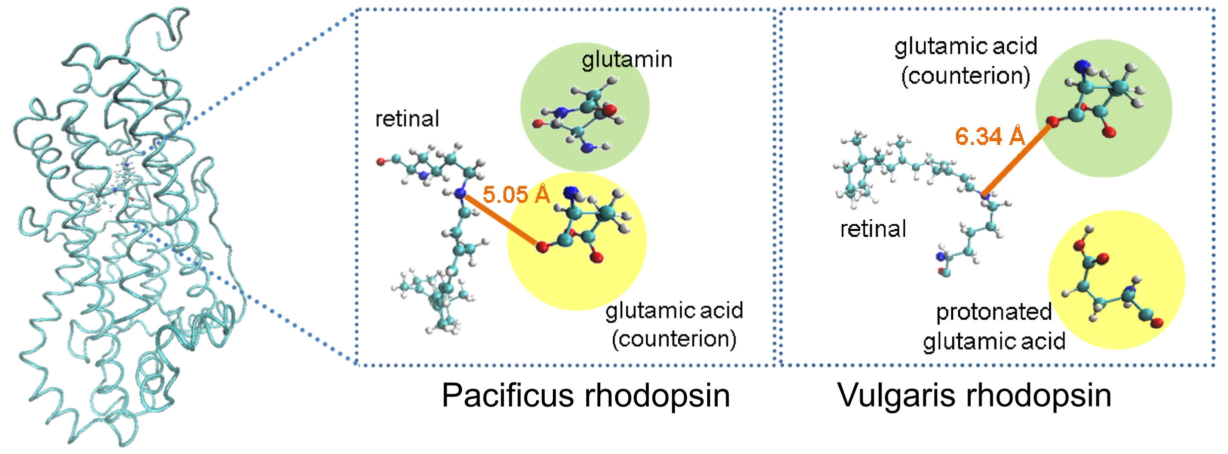
- The wavelength of absorbed photon is defined by energy gap in ground and excited states of retinal.
- This difference is affected by electrical field from negative counterion.
- According to the Coulomb law, the shorter the distance between counterion and retinal, the bigger the field from counterion.



Hypothesis

Difference in distance between counterion and retinal is responsible for difference in absorption spectra.

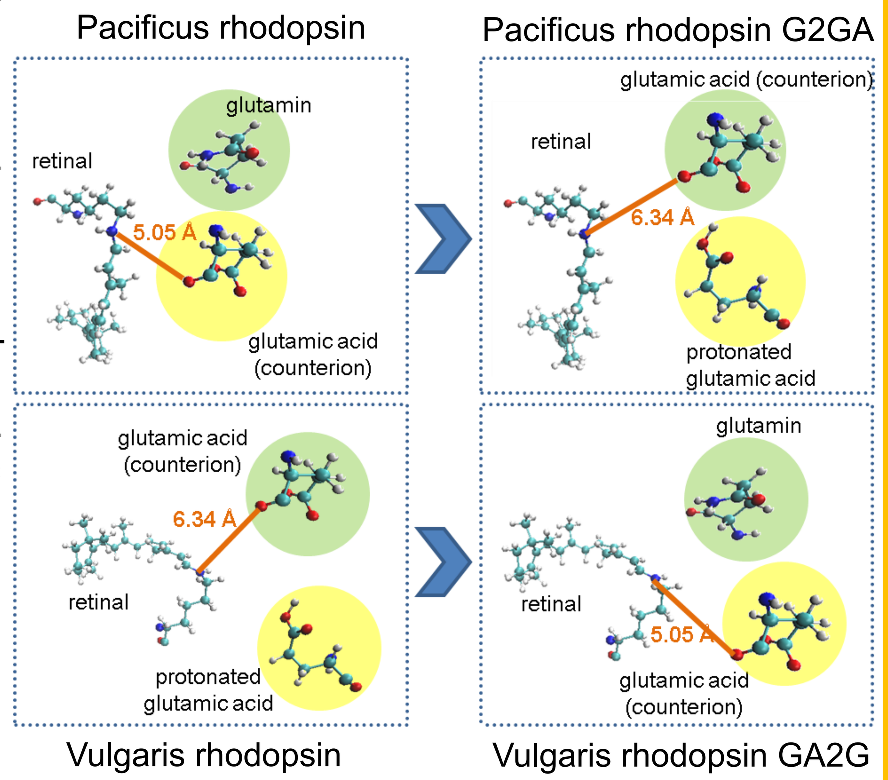
We created the structures of Vulgaris rhodopsin and Pacificus rhodopsin. Then we compared them and found that they have counterions in different places. The distances between retinal and counterion differ.



It's important that substitution of glutamin to glutamic acid causes protonation of counterion. In comparison to Pacificus rhodopsin there is a lower electric potential at site 2. Therefore glutamic acid at site 2 is protonated and no longer a counterion.

Confirmation of the hypothesis

To prove the hypothesis we created two structures of mutants. The first mutant was derived from Pacificus rhodopsin by substituting of glutamin for glutamic acid and protonating counterion. Its calculated spectrum turned out to be almost the same as the spectrum of Vulgaris rhodopsin. The second one was derived from Vulgaris rhodopsin by substituting counterion for glutamin and deprotonating protonated glutamic acid. Its calculated spectrum turned out to be almost as the spectrum of Pacificus rhodopsin. Next we created dozens of other mutants in which other replacements were carried out. Other substitutions barely shifted the spectra. This means that only the substitution of glutamic acid shifts the spectrum. So our hypothesis is correct.



Protein	Absorption spectrum, nm
Pacificus rhodopsin	480 ± 10
Vulgaris rhodopsin	550 ± 10
Pacificus rhodopsin G2GA	540 ± 10
Vulgaris rhodopsin GA2G	480 ± 10
Other mutants from P. rhodopsin	480 ± 10
Other mutants from V. rhodopsin	550 ± 10

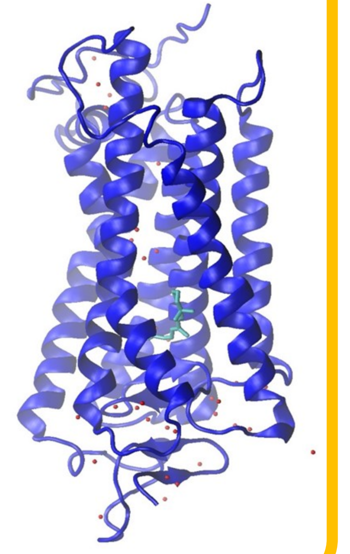
Conclusions

Results

- The mechanism of visual adaptation in marine animals was discovered.
- The hypothesis that the absorption spectrum of rhodopsin depends on the distance between the retinal and the counterion was confirmed.
- The same amino acid changes made in microbial rhodopsins could be used for shifting radiation spectrum to IR range.

Futher work

In the future, we are going to carry out the similar amino acid substitutions in microbial arherorodopsin-3 to shift its spectrum of radiation towards the IR range.



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