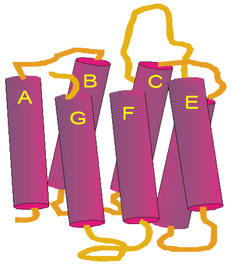


BU MBL

Vibrational Spectroscopy of Optogenetic Rhodopsins

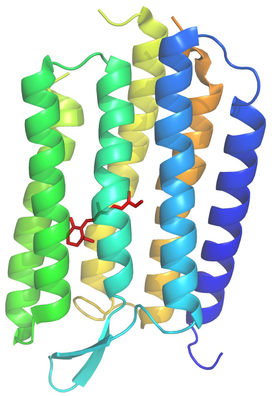
Adrian Yi, Natalia Mamaeva, Kenneth J. Rothschild

Department of Physics, Photonics Center and Molecular Biophysics Laboratory, Boston University, Boston, MA 02215, USA

BOSTON UNIVERSITY

What are rhodopsins?

1) Animal rhodopsins
   - Rods & cones in the retina responsible for vision
2) Microbial rhodopsins
   - Membrane proteins in microbes responsible for ion transport, energy transduction, signaling, etc.



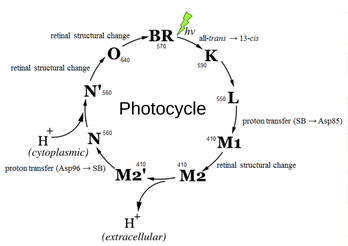
Microbial rhodopsin

(Pictured left: bacteriorhodopsin)

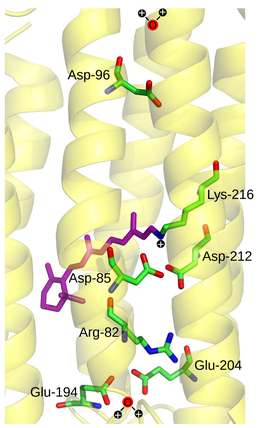
- Light-sensitive proteins
  - Chromophore – retinal (red)
- 7-transmembrane α-helices
- Various types
  - Ion pumps
    - Bacteriorhodopsin (H^+)
    - Archaerhodopsin (H^+)
    - Halorhopsin (Cl^-)
  - Signaling
    - Sensory rhodopsin I & II
- Light-gated Channels
  - *Cr*ChR1, *Cr*ChR2 (cations)
  - *Ca*ChR1, *Ca*ChR2 (cations)
  - *Gt*ACR1, *Gt*ACR2 (anions)

*ChR: channelrhodopsin, ACR: anion-channelrhodopsin

Bacteriorhodopsin Photocycle



Bacteriorhodopsin absorbs photons near 570nm, which drives it into its photocycle resulting in a displacement of a proton out of the cell.

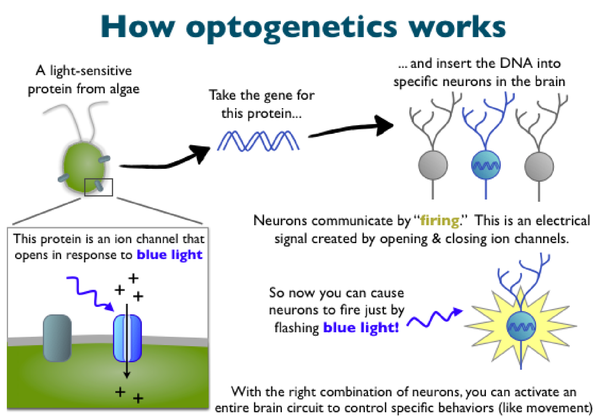


Recent papers

- Ogren, J., Mamaev, S., Russano, D., Li, H., Spudich, J. L., Rothschild, K. J., Retinal Chromophore Structure and Schiff Base Interactions in Red-Shifted Channelrhodopsin-1 from Chlamydomonas augustae. *Biochemistry*, **53** (24), 3961-3970 (2015).
- Ogren, J., Yi, A., Mamaev, S., Lugtenburg, J., DeGrip, W. J., Spudich, J. L., Rothschild, K. J., Comparison of the Structural Changes Occuring during the Primary Phototransition of Two Different Channelrhodopsins from Chlamydomonas Algae. *Biochemistry*, **54** (2), 377-388 (2015).
- Ogren, J., Yi, A., Mamaev, S., Li, H., Spudich, J. L., Rothschild, K. J., Proton Transfers in a Channelrhodopsin-1 Studied by Fourier Transform Infrared (FTIR) Difference Spectroscopy and Site-directed Mutagenesis. *The Journal of Biological Chemistry*, **290**,12719-12730 (2015).
- Yi, A., Mamaeva, N., Li, H., Spudich, J. L., Rothschild, K. J., Resonance Raman Study of an Anion Channelrhodopsin: Effects of Mutations near the Retinylidene Schiff Base. *Biochemistry*, **55** (16), 2371-2380 (2016).

Why study microbial rhodopsins?

1) Model for key cellular processes
   - Ion transport, energy transduction, signaling, etc.
2) Optogenetics
   - New interdisciplinary field revolutionizing neuroscience



*http://neurobyn.blogspot.se/2011/01/controlling-brain-with-lasers.html

Optogenetics

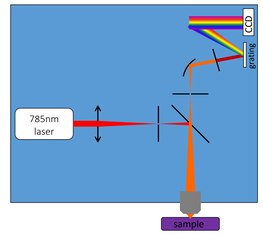
- Developed in 2005
- Microbial rhodopsins are expressed in specific cells of interest by DNA insertion
- Cells are excited by shining light, which activates photocycles of expressed microbial rhodopsins
- Provides spatial and temporal specificity advantages over electrical stimulations

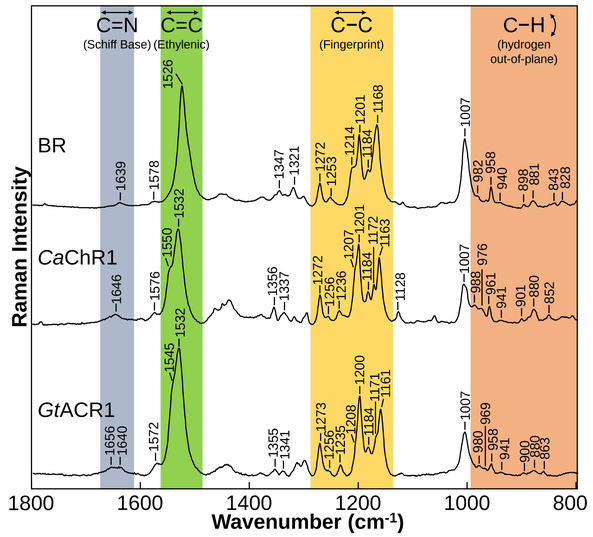
Examples of optogenetic proteins

- *Cr*ChR2 (*Chalmydomonas reinhardtii* channelrhodopsin-2)
  - Cation channel widely used for cell activation
  - One of first two channelrhodopsins discovered
- *Ca*ChR1 (*Chalmydomonas augustae* channelrhodopsin-1)
  - Cation channel, studied by our group in 2014-2015
- HR (Halorhodopsin)
  - Anion pump, used as an inefficient cell silencer
- QuasAR1/QuasAR2 (Quality superior to AR3 – 1/2)
  - Membrane potential dependent fluorescent protein
- *Gt*ACR1 & *Gt*ACR2 (*Guillardia theta* anion-channelrhodopsin)
  - Anion channel discovered in 2015, >1000 times more efficient than HR as cell silencer

Resonance Raman Spectroscopy

- Inelastic scattering technique
- 785nm laser excitation
- Chromophore bands are resonantly enhanced, enabling us to probe near the chromophore without the absorption from rest of the protein
- Used to probe ground state structure and protonation states of the protein





Fingerprint region

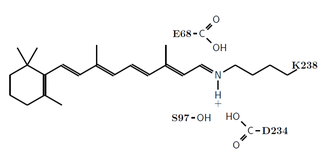
Fingerprint region is very sensitive to retinal configuration. BR, *Ca*ChR1, and *Gt*ACR1 all have similar fingerprint region, especially the high intensity peaks near 1168 and 1201 cm^{-1} and other smaller peaks. BR is known to have all-*trans* retinal configuration from many studies including x-ray crystallography, high-performance liquid chromatography, and molecular dynamics simulations. This shows that *Ca*ChR1 & *Gt*ACR1 also have all-*trans* retinal configuration at ground state.

Schiff Base

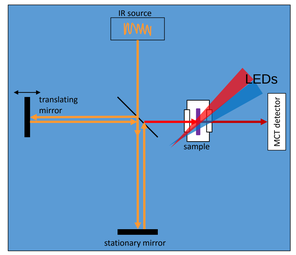
Schiff base (SB) is the C=N bond that connects the retinal with the lysine-216 (numbering for BR). The nitrogen can attract a proton, which would give it an extra positive charge. To test if the SB is protonated, hydrogen can be replaced with deuterium through H_2O–D_2O exchange, which causes the C=N peak to downshift. In BR, 1639 cm^{-1} band downshifts to 1622 cm^{-1}; in *Ca*ChR1, 1646 cm^{-1} band downshifts to 1620 cm^{-1}; in *Gt*ACR1, 1640 cm^{-1} band downshifts to 1622 cm^{-1}.

Example (*Gt*ACR1, pictured right)

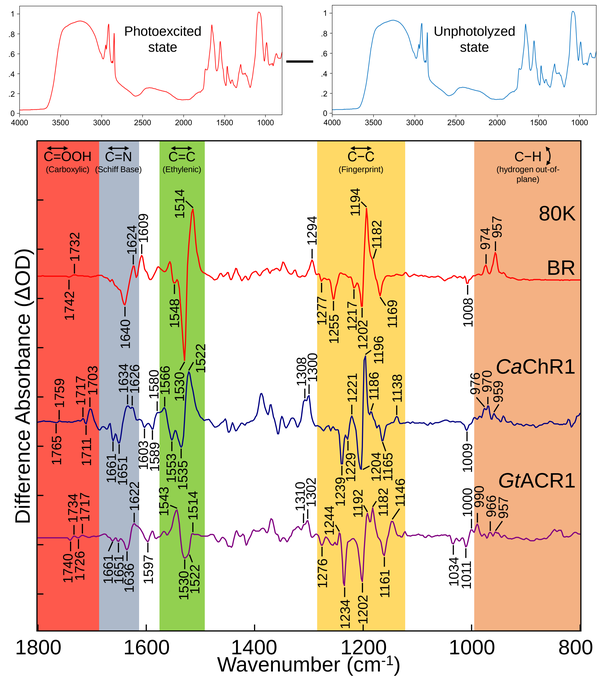
- All-*trans* retinal, protonated SB, protonated E68 and D234.



Low-temperature Fourier-transform Infrared (FTIR) Difference Spectroscopy



- Direct absorption technique using film samples
- Liquid nitrogen cooled cryostat sample holder allows temperature to be controlled from 80 to 300 K.
- LED lights are used to switch between photo-intermediate states
- Used to probe changes between different photo-intermediate states (both protein and chromophore)



Carboxylic acid region

This region shows changes in C=O vibrations in carboxylic acids, such as aspartic acid or glutamic acids nearby the SB. These changes occur from protonation changes or hydrogen bonding strength changes.

Example (*Ca*ChR1, pictured below)

- *Ca*ChR1 → P_1 : all-*trans* to 13-*cis* retinal structure, proton transfer from E169 to D299
- P_1 → P_2 : proton transfer from SB to E169 (SB, E169, D299 all neutral in P_2)