**Vibrational Spectroscopy of Optogenetic Rhodopsins**

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**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.

**Why study microbial rhodopsins?**

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

**Microbial rhodopsin**

(Pictured left: bacteriorhodopsin)

- Light-sensitive proteins
- Chromophore: retinal (red)
- 7-transmembrane α-helices
- Various types
  - Ion pumps
  - Bacteriorhodopsin (H+): Archaebacterium (H+)
  - Halorhodopsin (Cl-)
- Signaling
  - Sensory rhodopsin I & II
- Light-gated Channels
  - CaChR1, CaChR2 (cations)
  - GACR1, GACR2 (anions)

**Optogenetics**

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

**Examples of optogenetic proteins**

- Channelrhodopsin 1/2 (Chlamydomonas reinhardtii channelrhodopsin-2)
  - Cation channel widely used for cell activation
  - One of first two channelrhodopsins discovered
- ACR1 (Chlamydomonasaugustae channelrhodopsin)
  - Cation channel
  - Studied by our group in 2014:105
- HR (Halorhodopsin)
  - Anion pump, used as an inefficient cell silencer
- QuasAR1/QuasAR2 (Quality superior to AR3 – 1/2)
  - Membane potential dependent fluorescent protein
- GACR1 & GACR2 (Guillardia theta anion-channelsphotopsin)
  - Anion channel discovered in 2015, >1000 times more efficient than HR as cell silencer

**How do optogenetics work**

A light-sensitive protein from algae

Take the gene for this protein...

... and insert the DNA into specific neurons in the brain.

Neurons communicate by **“flashing blue light!”**

With the right combination of neurons, you can rewire an entire brain circuit to control specific behaviors (like movement)

http://neurobyn.blogspot.se/2011/01/controlling.html

**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

**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

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**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**