More biosensors, actuators
• Last class
  • Biosensing schemes
  • Dye sensors
  • FP sensors

• This class
  • More FP sensors
  • Actuators
Voltage FPs

- Based on voltage sensitive domains, or endogenous chromophores
- To report fast dynamics, HAVE to be embedded in membrane
- Jamming lots of extra proteins into membrane can have serious consequences
Engineering voltage-induced color changes into microbial rhodopsins

![Diagram showing extracellular and intracellular environments with a voltage source and absorbance graph.]

\[ \Delta V = -59 \text{ mV} \times \frac{[H^+]_{in}}{[H^+]_{out}} \]
Archaerhodopsin 3 is a fluorescent voltage indicator
Voltage imaging with Sub-Nyquist Action Potential Timing (SNAPT)

Spatial Superresolution

Temporal SNAPT

Bins = pixels

Bins = image frames

Dougal Maclaurin
Mechanical strain

- Express sensors in membrane
- As membrane tension increases, FRET ratio changes
- None work particularly well
- Very important quantity – touches everything in the membrane
Kinase activity

- Typically used in a FRET system
- Choose a substrate known to undergo remodeling upon phosphorylation
- Movements will change FRET ratio
- EKAR measures ERK activity
- Typically have a slow response, on the order of phosphorylation time
Optical integrators

- Combine photoactivatable FPs with sensing domain
- Photoconversion is now an AND gate – with 405 nm light AND signal, it will photoconvert
- Switches dynamic signal at the time of 405 into irreversible change that can be read out over hours
- CaMPARI is calcium integrator
RNA biosensors

• v1 was Spinach
• GFP chromophore (4-hydroxybenzidene imidazolinone, HBI) is non-fluorescent without GFP matrix
• Took GFP chromophore structure, and found RNA aptamer that bound this molecule and caused it to fluoresce
• Express aptamer, add DMHBI, and it new fluorescent RNA
• Different aptamers yielded plethora of different colors
RNA staples – easy to make modular biosensors

- Aptamers that bind other small molecules can be attached to Spinach domain
- Unbound form will prevent proper folding of the HBI domain, no fluorescence
- Binding of small molecule will twist the aptamer into the right shape and emit fluorescence
Nano lanterns

- Luminescence is a chemical reaction gives off light, no need for excitation
- Zero background, very high contrast technique
- Typically too dim to resolve individual cells
- Fusing luciferase to a YFP allows bioluminescent resonant energy transfer (BRET)
- BRET emission can be much higher than from luciferase
- Needs coelenterazine to be added to cells
- Can be made into biosensor by circularly permuting the luciferase
Actuators

• Opposite of sensing
• Use light to induce physiological change
• Nicely paired with optical sensing
Many timescales in biology

- Milliseconds – Voltage, calcium, diffusion
- Seconds – Receptor signaling, phosphorylation
- Minutes – Cell movement, cytoskeletal rearrangement, early gene transcription, post translational modifications
- Hours – Gene transcription, metabolic profile
- Days – Cell death, differentiation

- Optically we have control of time (within milliseconds) and space, (microns)
- Much better resolution than with chemicals
Actuator parameters

- Expression efficiency – how likely are you to get it into the cells you want, and how much is expressed?
- Activation wavelength – where in the spectrum, and what else will that do?
- Activation kinetics – how long after light does it actuate, and for how long?
- Dark activation – how much is happening when you don’t add light
- Activation intensity – how sensitive is the activity to light?
Caged actuators

- Compounds are chemically inert upon addition to the sample
- Photolysis releases “cage”, chemicals allowed to bind
- Uncaging is often in the UV
- Can use 1 or 2 photon to uncage
Other caged actuators

• Typically use 10ns – 1ms pulse to excite

• Quantum yields and absorption are given by vendor

• Typically want the release to be much faster than the rates of what you’re trying to measure

• Using different cages, possible to have multi-color uncaging

Some caged molecules:
- Glutamate
- GABA
- Kainate
- Caffeine
- AMPA
- Aspartate
- Proton
Photoswitchable inhibitors

- Chemical toxins that can be activated upon exposure to light
- Often use an azobenzene group which switches between conformations with 400 or 500 nm light
- Goal is to optimize contrast ratio (on activity vs off activity)
- Filament polymerization, kinase activity, ion channel activity,
Optogenetics

• Co-opting light activated proteins for freaky experiments
Channel rhodopsin

- Originally discovered in fresh water algae
- Used for light sensing in the organism (primitive eye)
- Absorption of photon causes channel opening, allows ions to flow through, changes the voltage
- Exact same process starts firing in neurons
Halorhodopsin – voltage inhibition

- Halorhodopsin is a light driven chloride pump
- Light hyperpolarizes cells, drives voltage more negative
- Prevents cells from firing
- It is a pump, so the activity can only occur with protein turnover (millisecond rate).
- One photon = 1 chloride
Optical protein localization

- Look for domains that respond to light
- Often found in plants
- Steal the genes, and use them to control cellular physiology
Phytochrome – Phy/PIF

- Phytochromes were originally found in arabadopsis – control red light behaviors

- Use a phycocyanobilin (PCB) chromophore that reversibly changes conformation

- As PCB changes, it will recruit or let go of the PIF domain

- Can be used to pull 2 proteins together or apart
Directing cellular movement

Local Recruitment of Tiam DPH by a 650 nm laser spot (red circle) moving in a line can cause production of a cell process that follows the light.
Actuators

Fiberoptic Control of Locomotion in ChR2 Mouse
Cryptochrome2 – CIB1 binding

- Cryptochrome2 is also found in Arabidopsis
- Blue light sensor
- Blue light activation induces binding to Cib1 domain
- Can be used to drive protein-protein interactions in cells
- Also has been used to drive gene transcription
- Requires no external cofactors
Other systems

- Light induced tetramerization of FPs - Dronpa
- LOV (light oxygen voltage domains) has slow kinetics
- VIVID fungal blue light receptor
- Phototropin
Patterning illumination

• Digital micromirror device – DMD
• Group of mirrors can reflect light in 1 of 2 ways
• Have to reform an image of the DMD on the objective
• Resolutions of 608x634 to full HD projectors (2M individual pixels)
Holographic illumination

- If you need all of your photons, can’t use DMD
- Possible to create arbitrary patterns using only phase mask in Fourier space
- It’s hard to calculate what phase should be to produce arbitrary pattern of light
- Spatial Light Modulator (SLM) is used to arbitrarily pattern phase, but allow 100% transmission
- Can be used in 2 photon, in 3 dimensions
- Can also use temporal focusing to illuminate in 3 dimensions
On to Matlab...