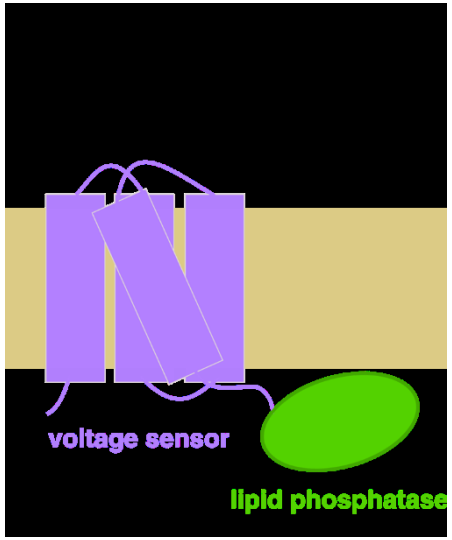
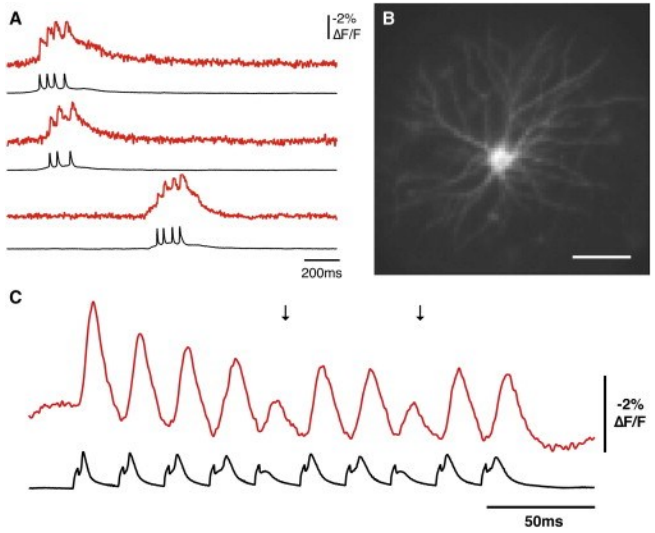
# More biosensors, actuators

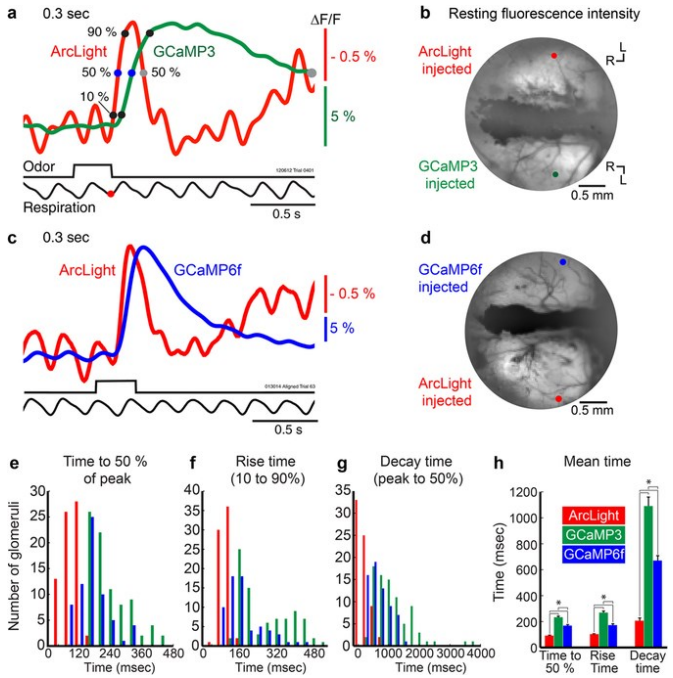
- Last class
  - Biosensing schemes
  - Dye sensors
  - FP sensors
- This class
  - More FP sensors
  - Actuators

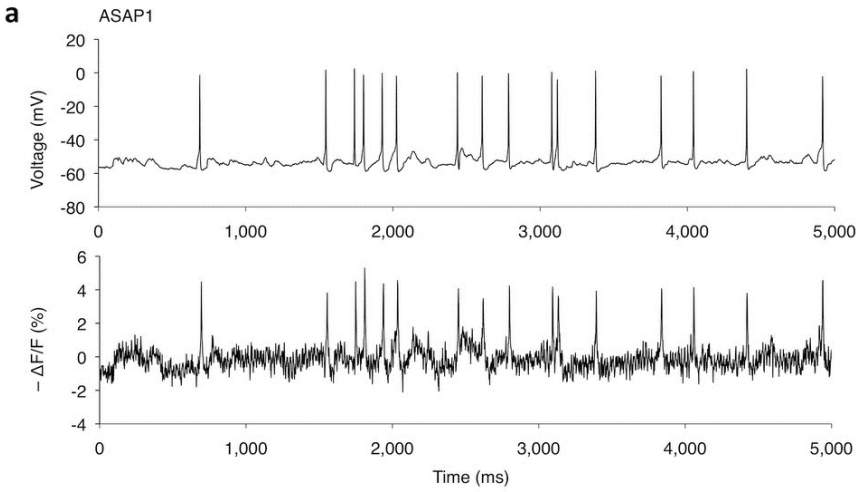
# Voltage FPs

- 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

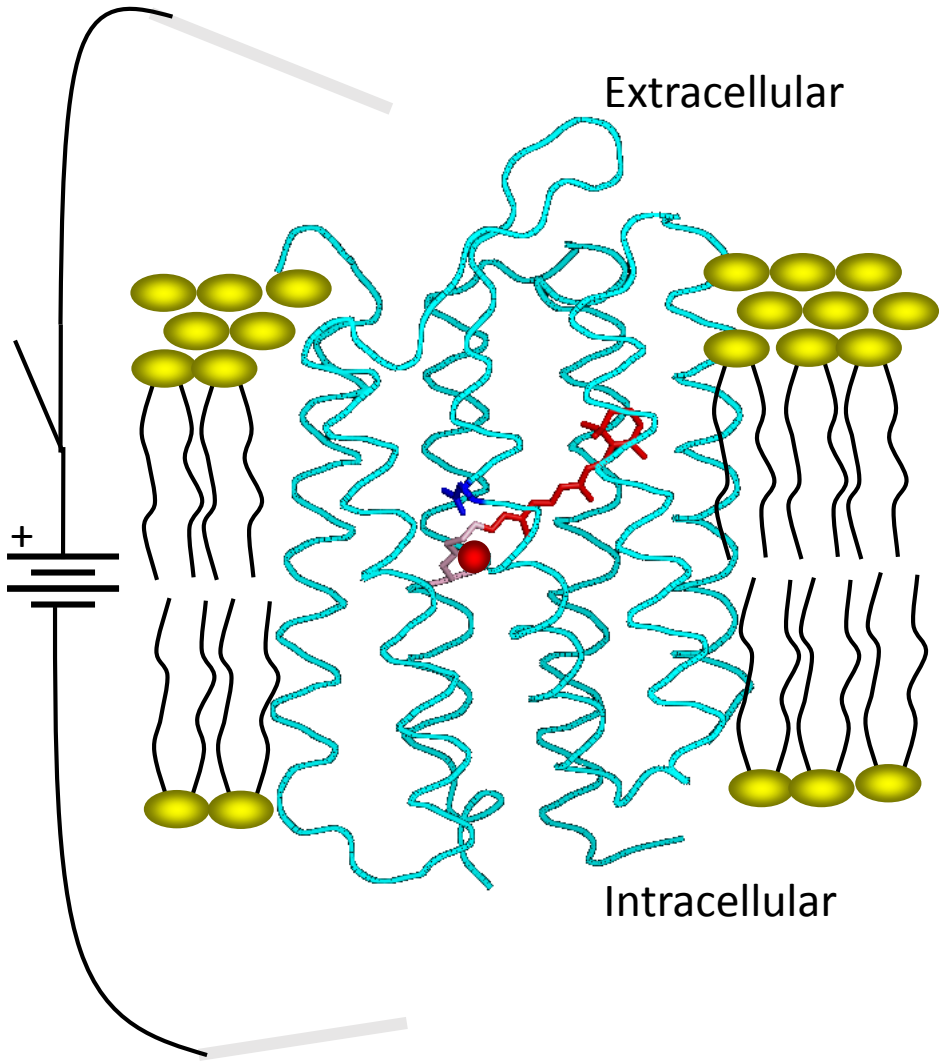


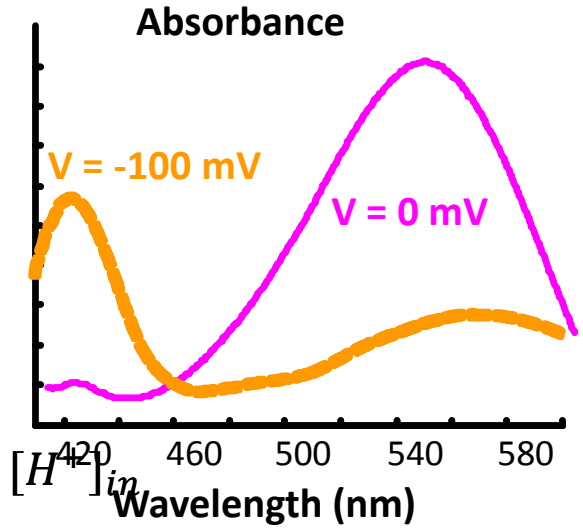



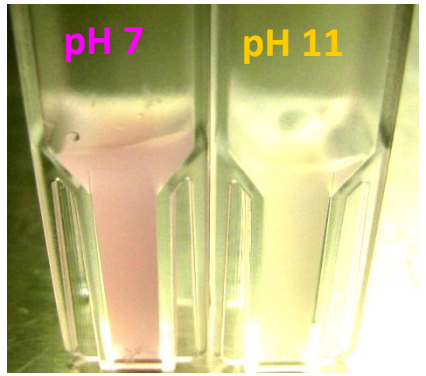



# Engineering voltage-induced color changes into microbial rhodopsins

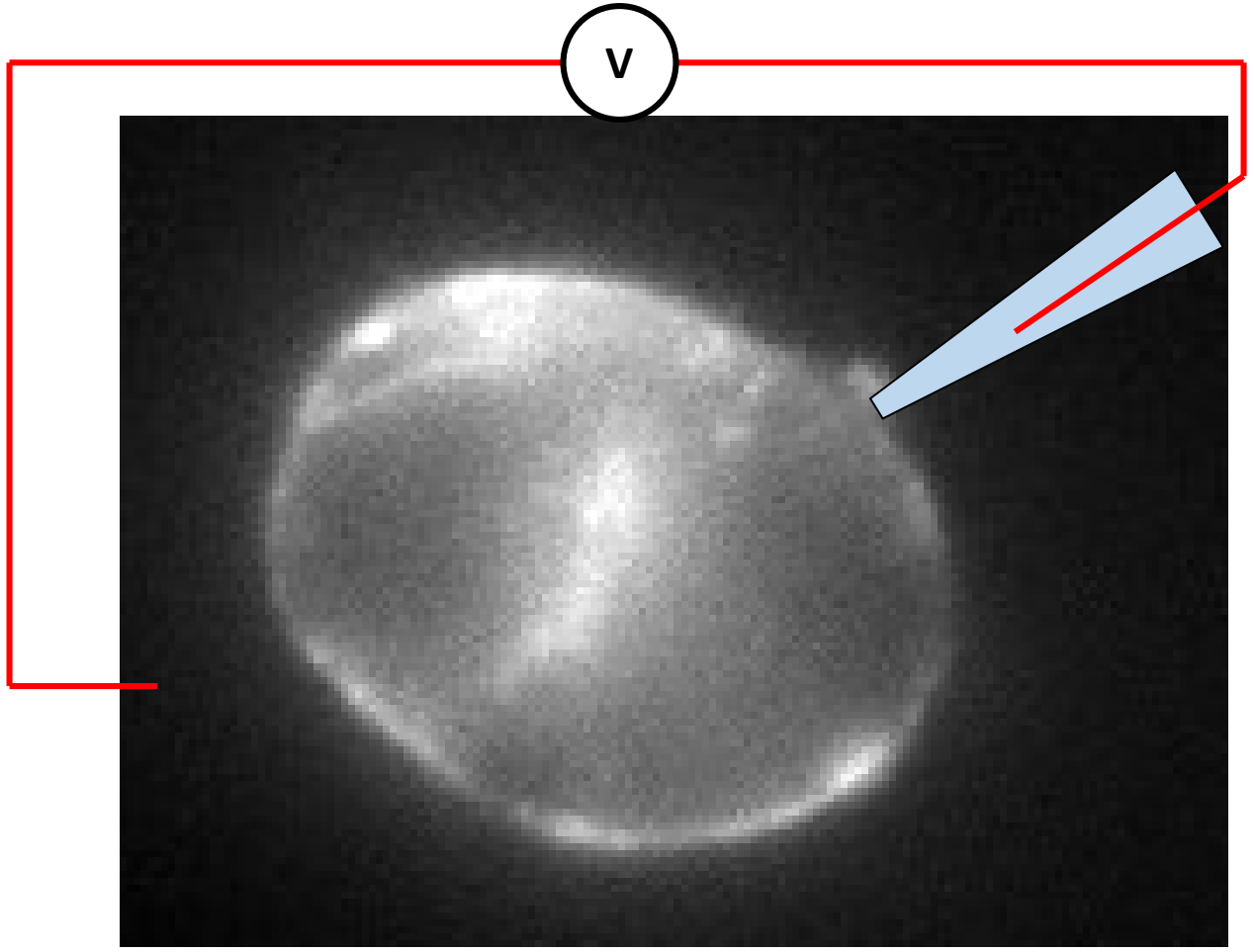


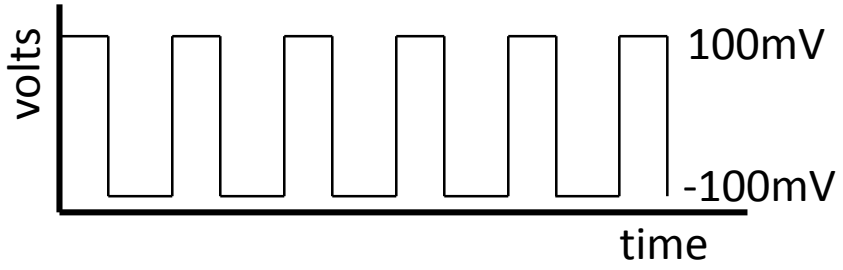




$$\Delta V = -59\ mV\ * \frac{[H^+]_{in}}{[H^+]_{out}}$$

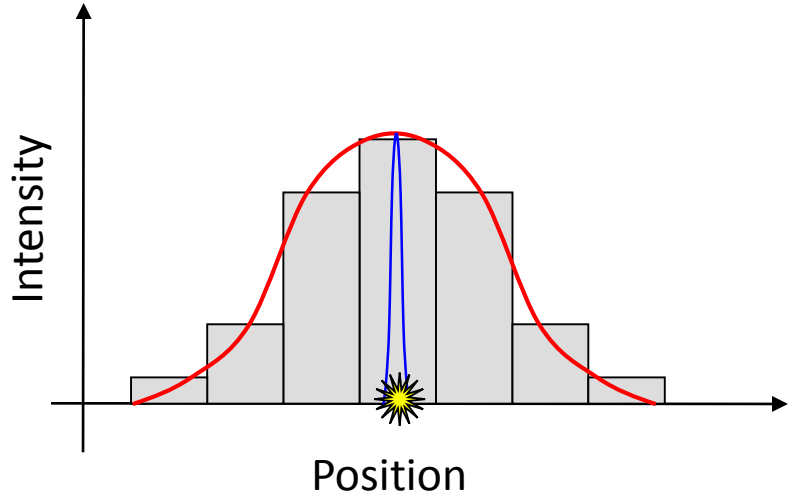
# Archaerhodopsin 3 is a fluorescent voltage indicator



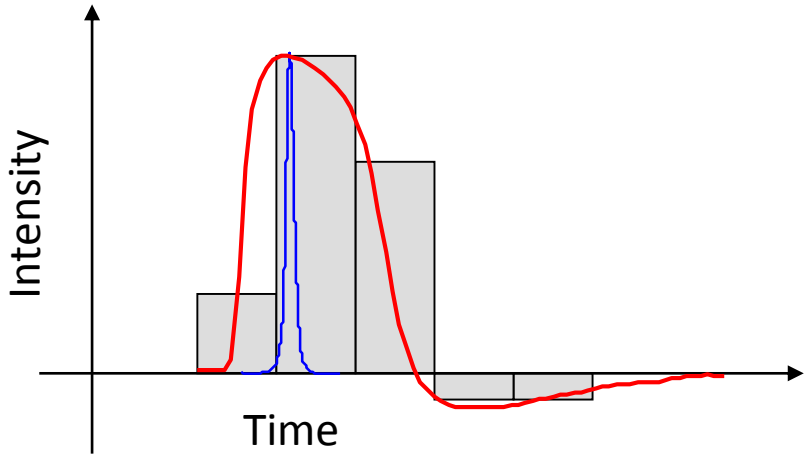
# Voltage imaging with Sub-Nyquist Action Potential Timing (SNAPT)

Spatial Superresolution



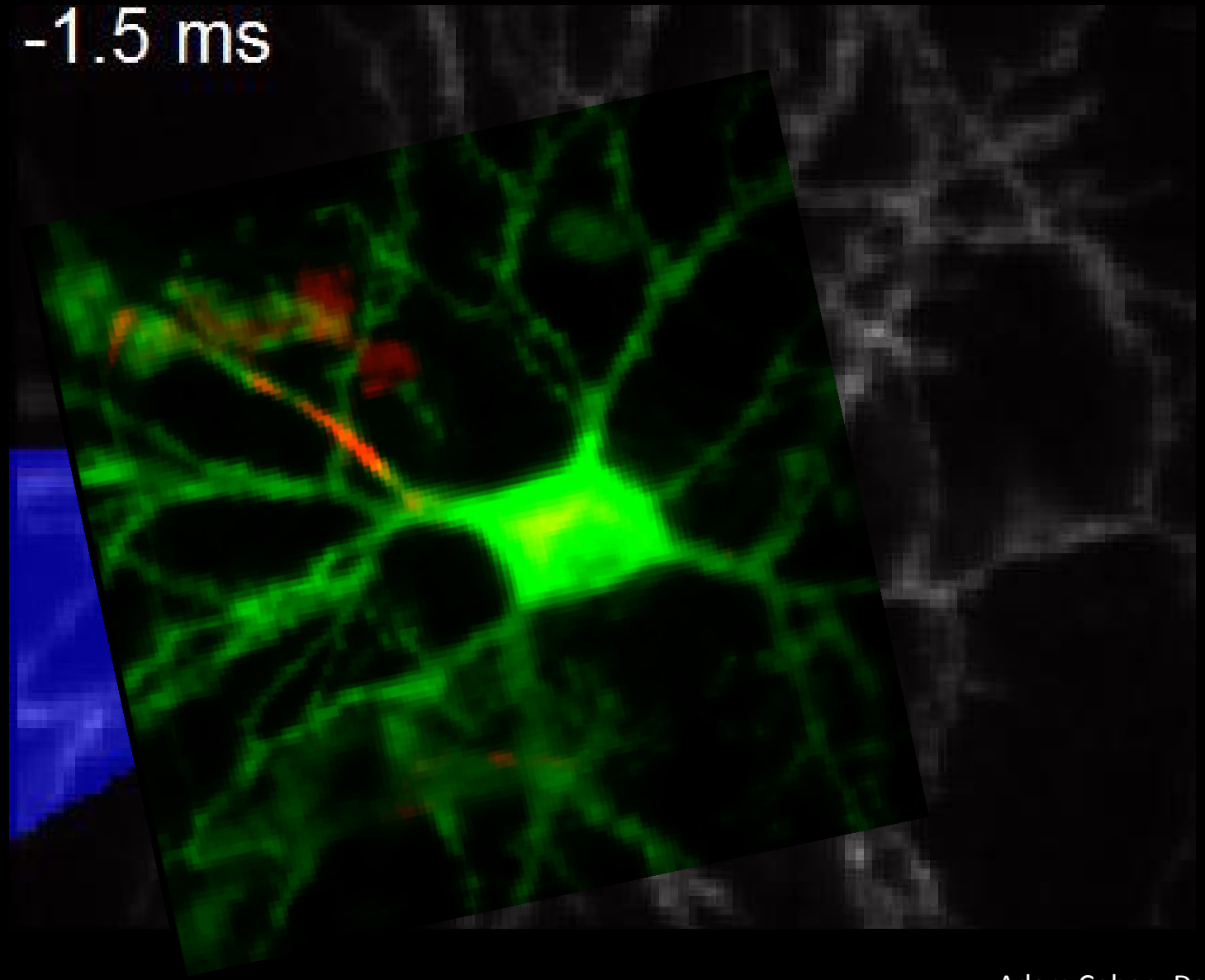

Bins = pixels

Temporal SNAPT




Bins = image frames

Dougal Maclaurin

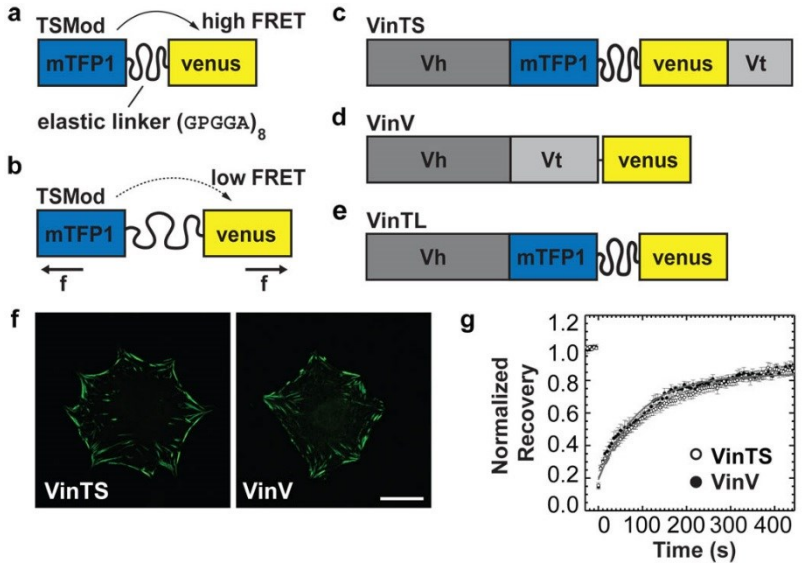


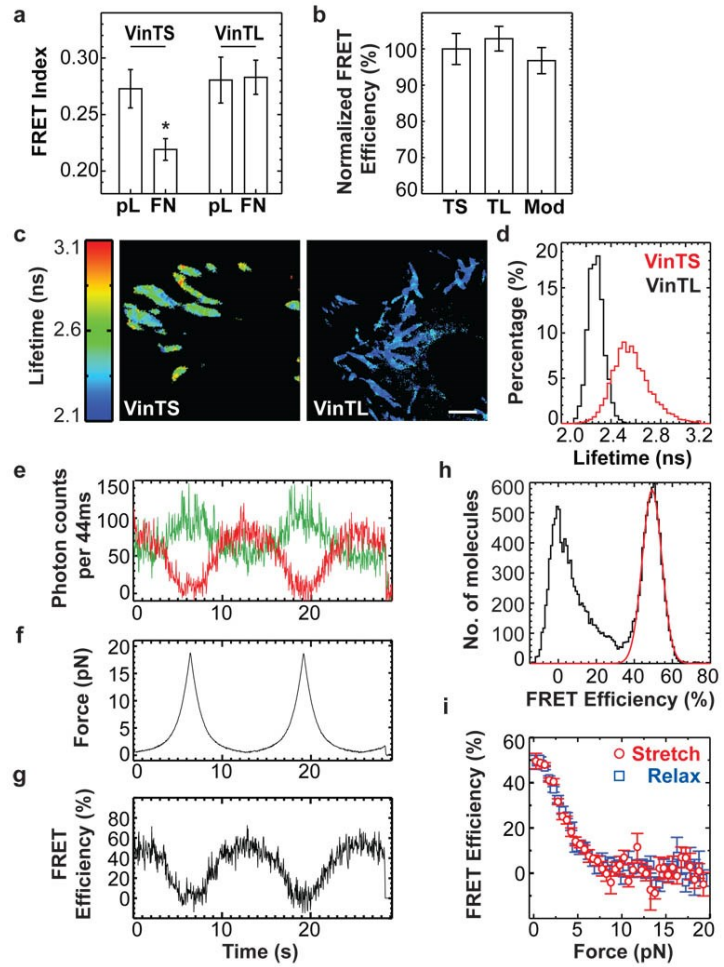

AnkG eGFP

Adam Cohen, Daniel Hochbaum, Sami Farhi, Dougal Maclaurin

# Mechanical strain

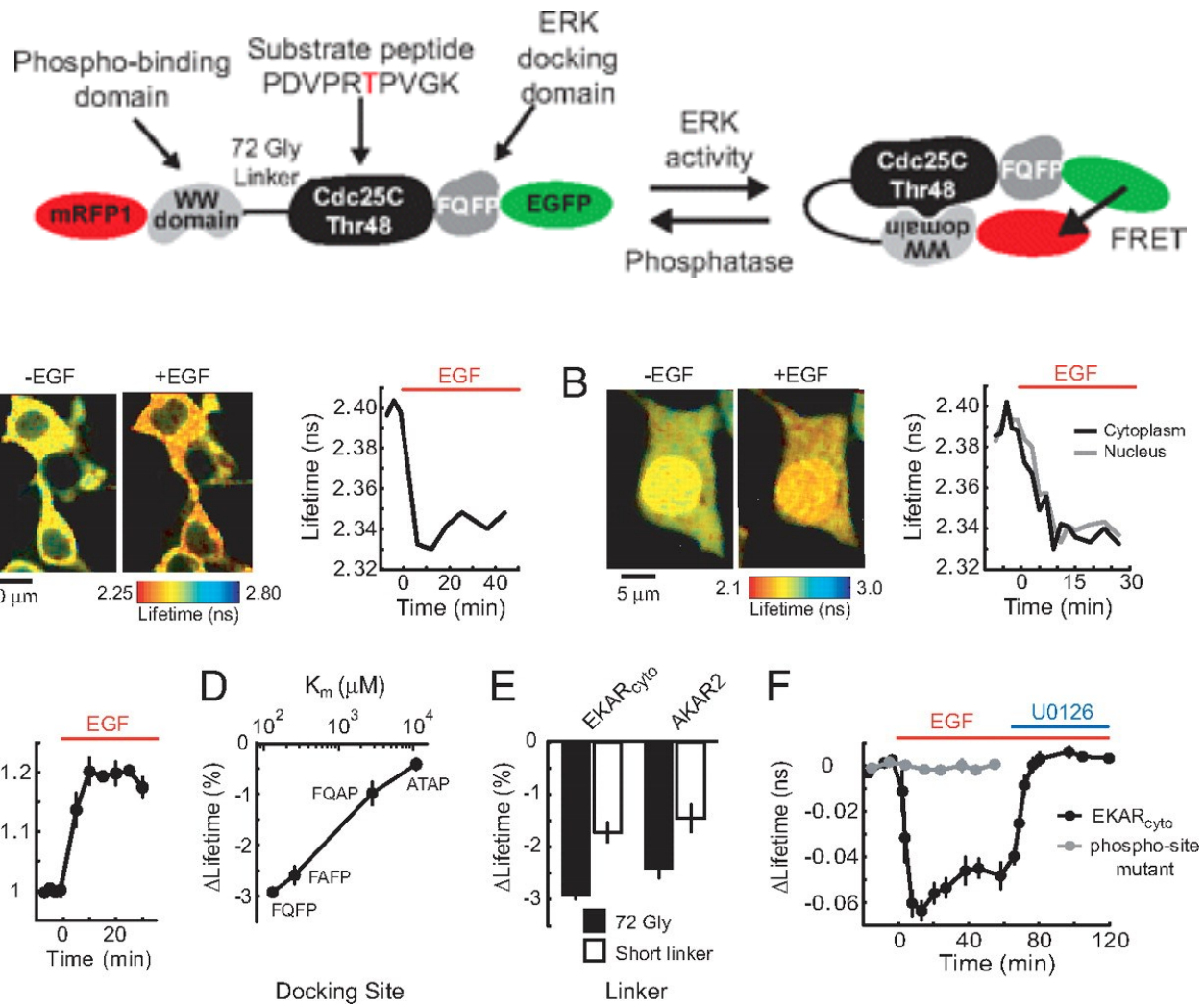
- Express sensors in membrane
- As membrane tension increases, FRET ratio changes
- None work particularly well
- Very important quantity – touches everything in the membrane



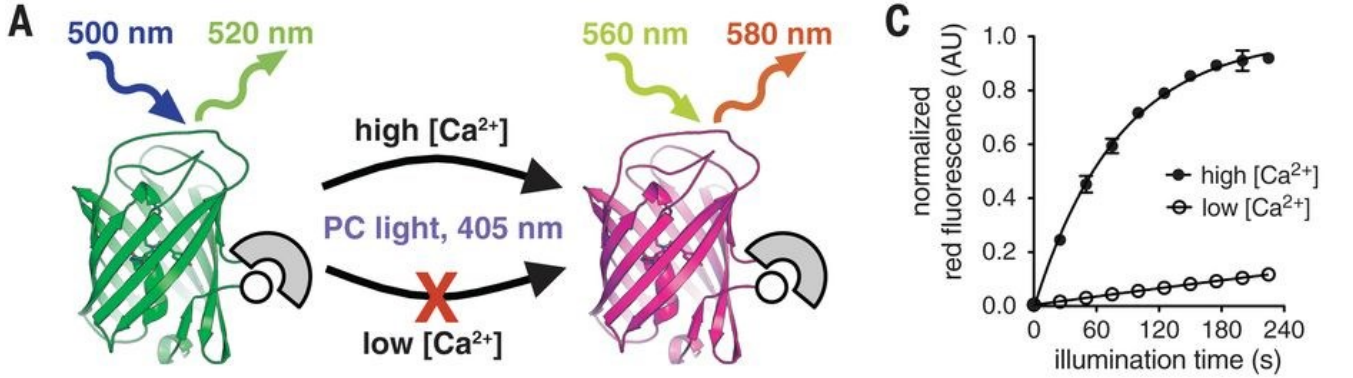
# Kinase activity

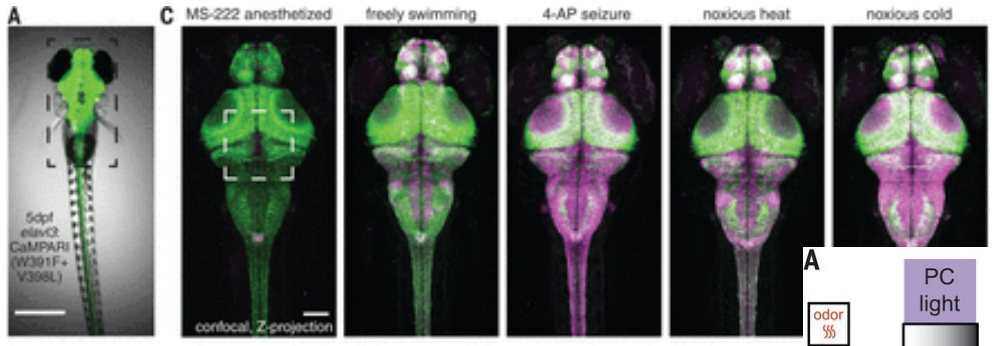
- Typically used in a FRET system
- Choose a substrate known to undergo remodeling upon phosphorylation
- Movements will change FRET ratio
- EKAR measures ERK activity
- Typically have a slow response, on the order of phosphorylation time

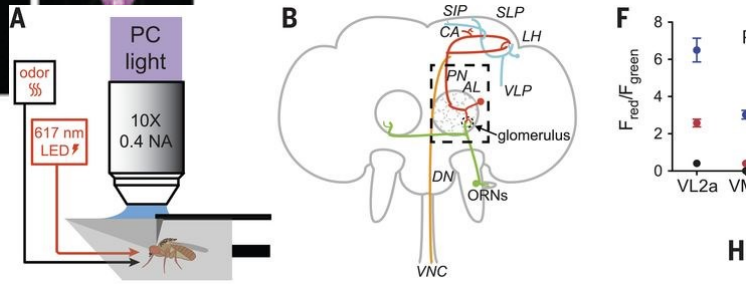


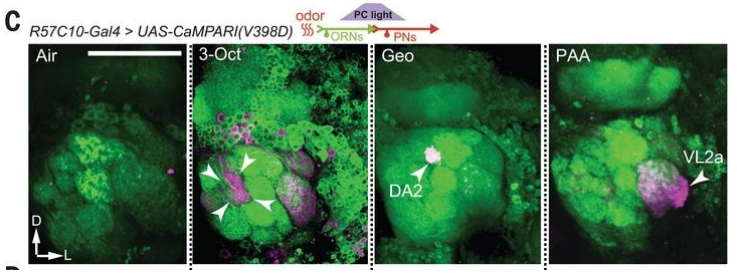
# Optical integrators

- 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



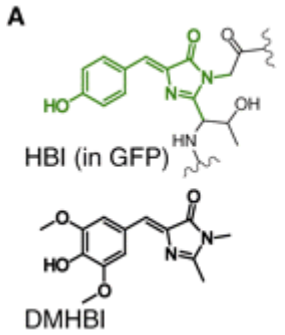



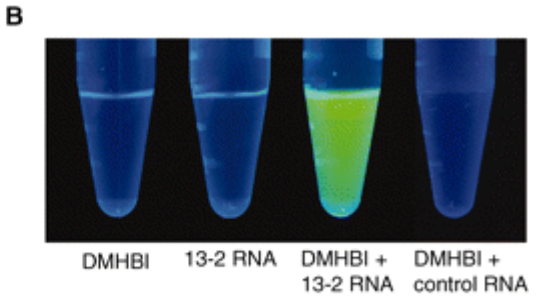



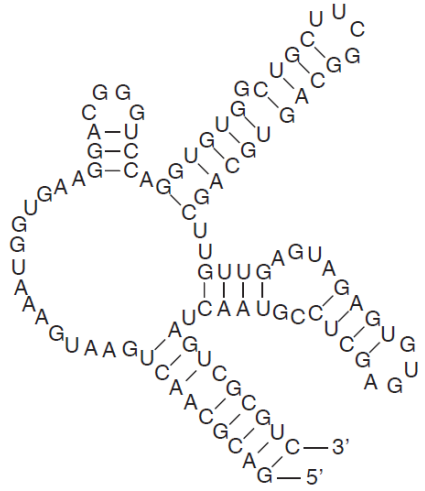



# RNA biosensors

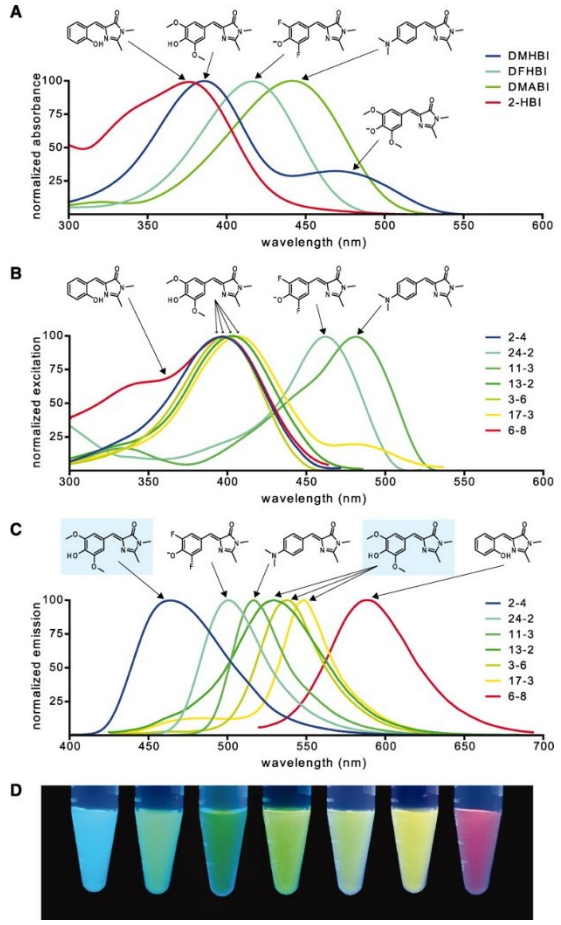
- v1 was Spinach
- GFP chromophore (4-hydroxybenzlidene imidazolinone, HBI) is non-fluorescent without GFP matrix
- Took GFP chromophore structure, and found RNA aptamer that bound this molecule and caused it to fluoresce
- Express aptamer, add DMHBI, and it new fluorescent RNA
- Different aptamers yielded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



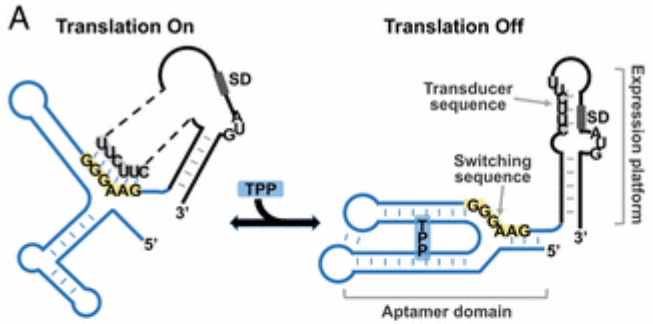



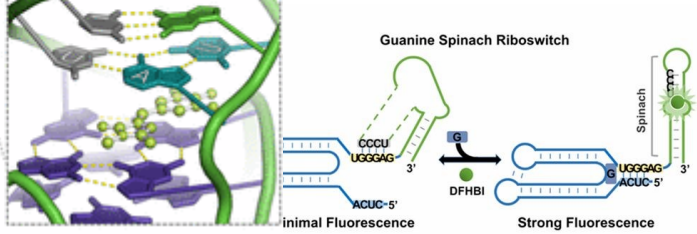
24-2 (Spinach)

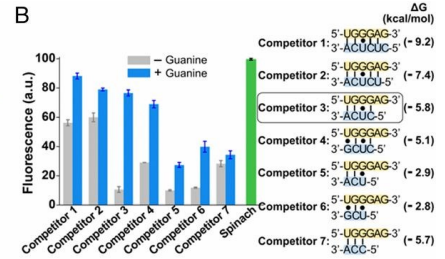


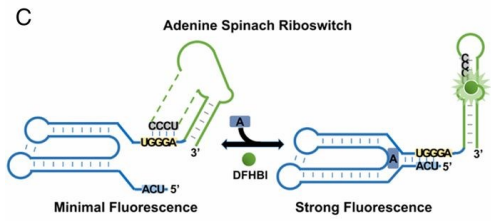
# RNA staples – easy to make modular biosensors

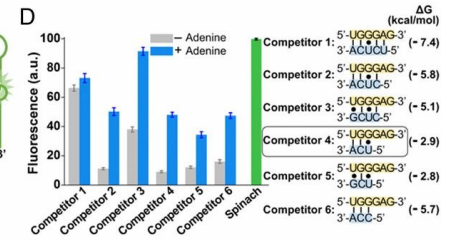
- Aptamers that bind other small molecules can be attached to Spinach domain
- Unbound form will prevent proper folding of the HBI domain, no fluorescence
- Binding of small molecule will twist the aptamer into the right shape and emit fluorescence

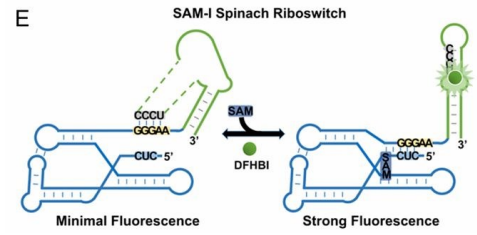


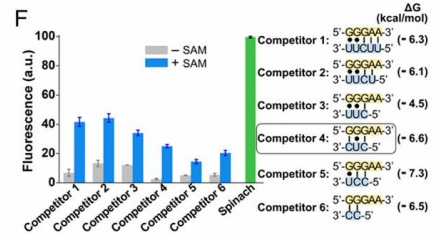



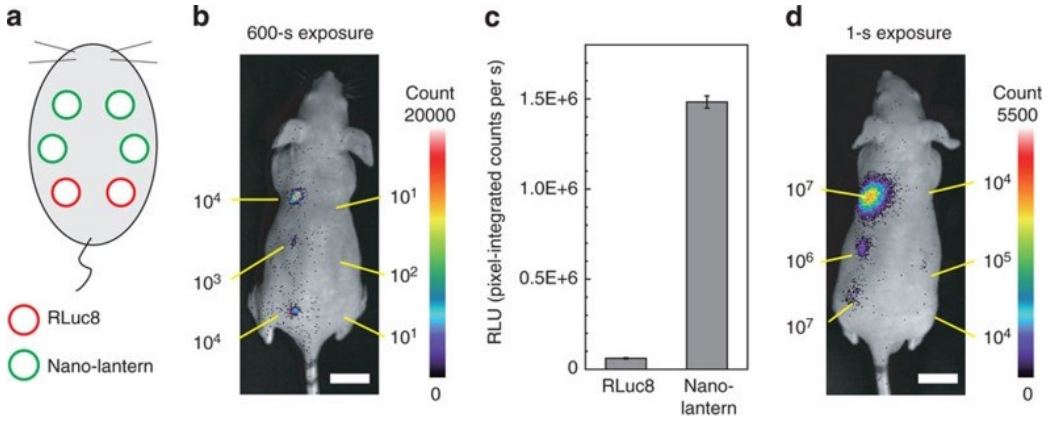



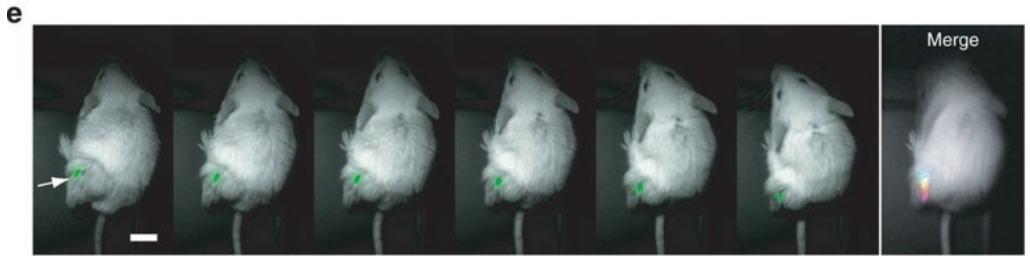



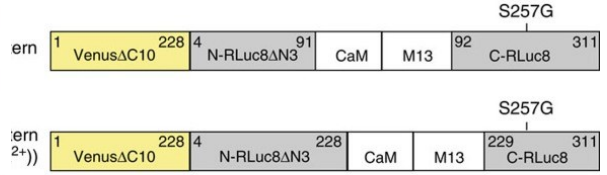



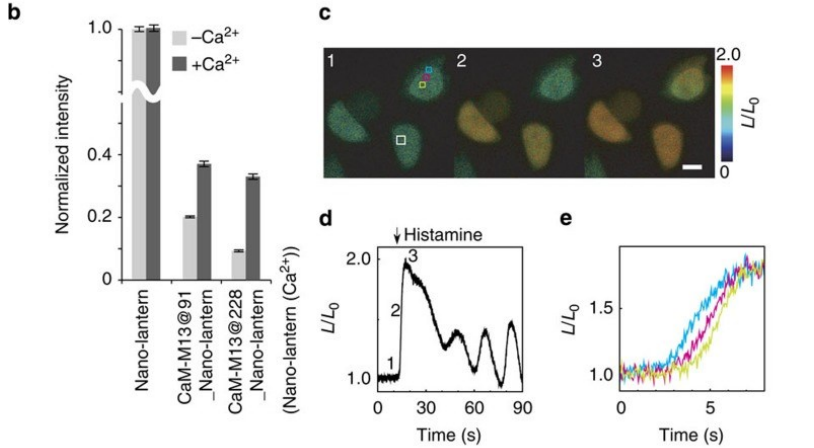
# Nano lanterns

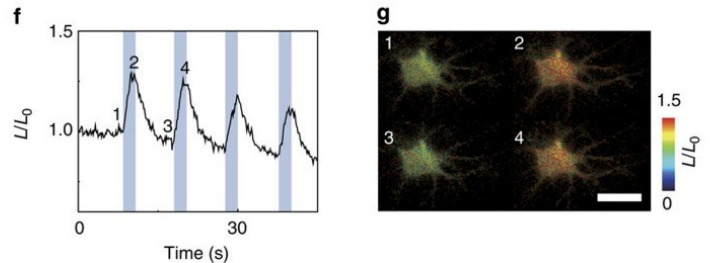
- Luminscence is a chemical reaction gives off light, no need for excitation
- Zero background, very high contrast technique
- Typically too dim to resolve individual cells
- Fusing luciferase to a YFP allows bioluminescent resonant energy transfer (BRET)
- BRET emission can be much higher than from 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

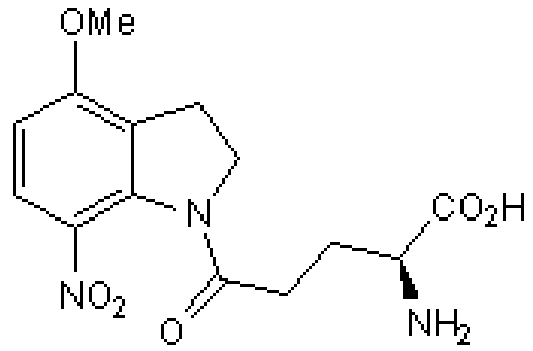
- Optically we have control of time (within milliseconds) and space, (microns)
- Much better resolution than with chemicals

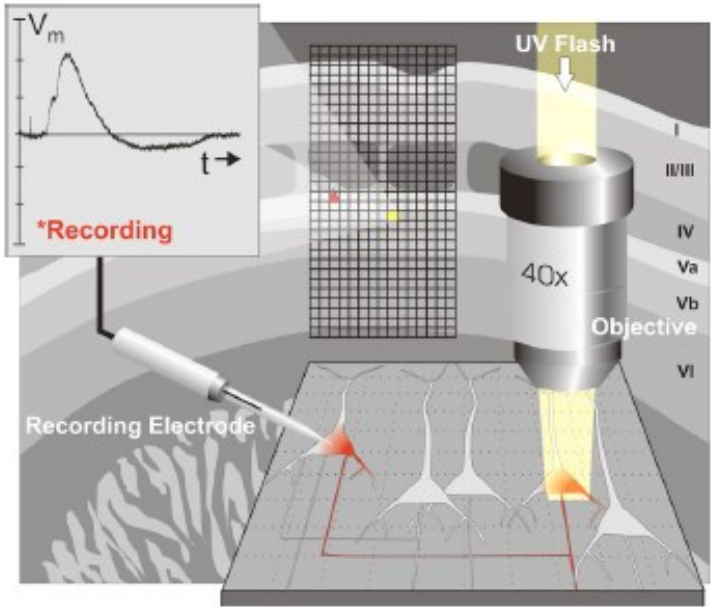
# Actuator parameters

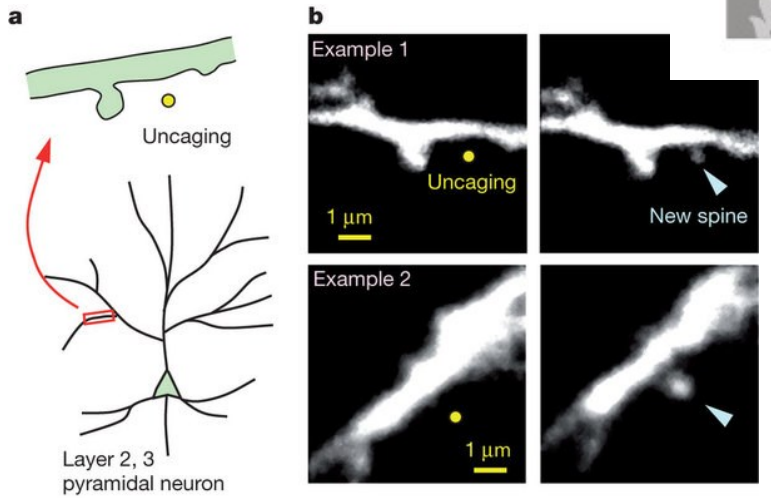
- Expression efficiency – how likely are you to get it into the cells you want, and how much is expressed?
- Activation wavelength – where in the spectrum, and what else will that do?
- Activation kinetics – how long after light does it actuate, and for how long?
- Dark activation – how much is happening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

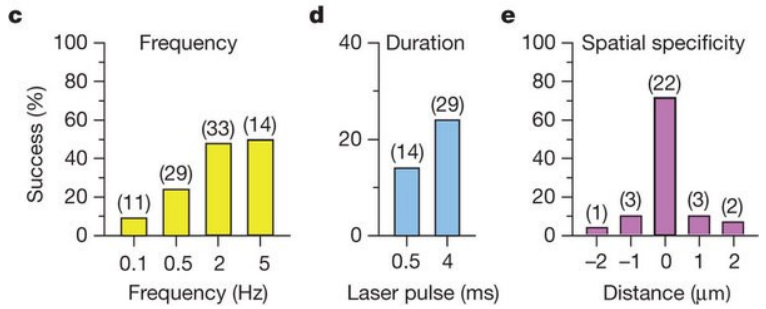
# Caged actuators

- 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



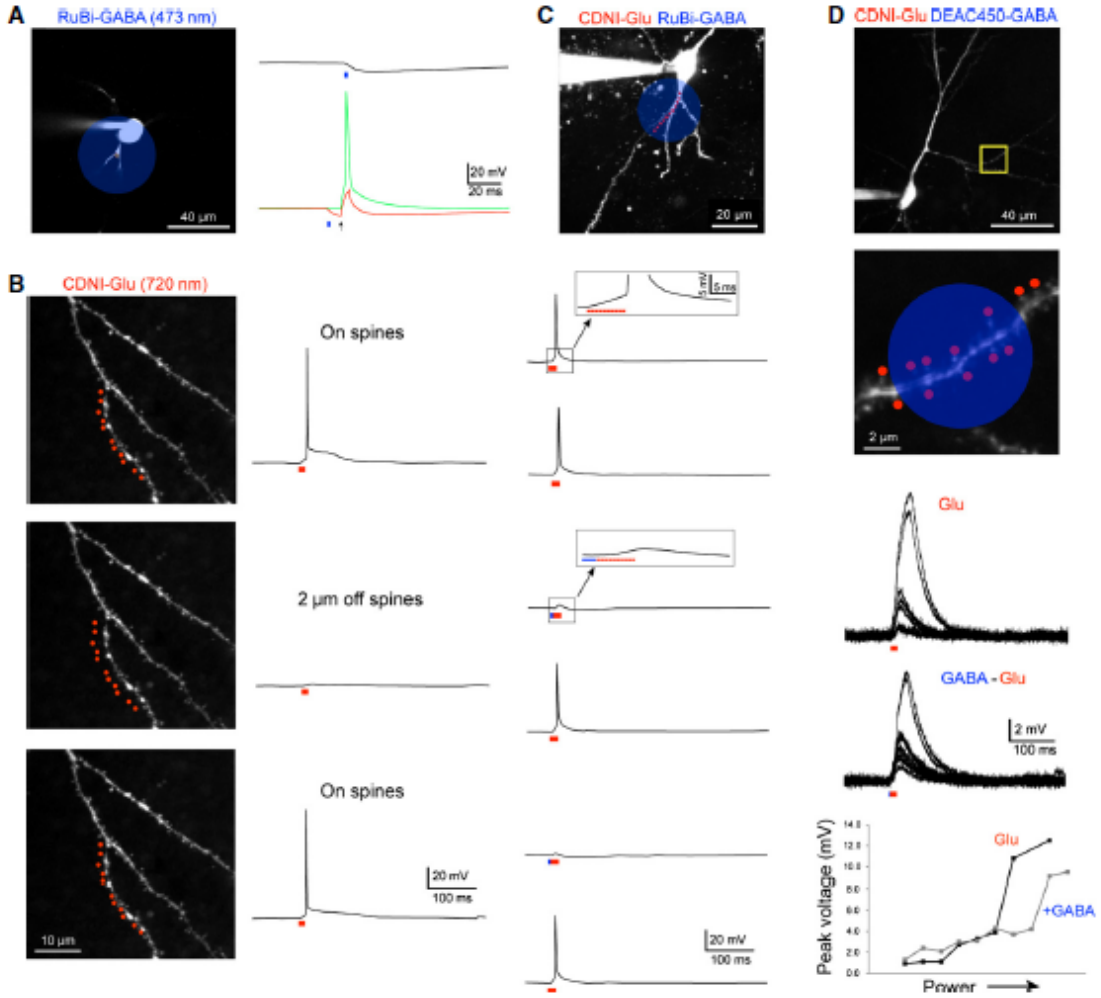



# Other caged actuators

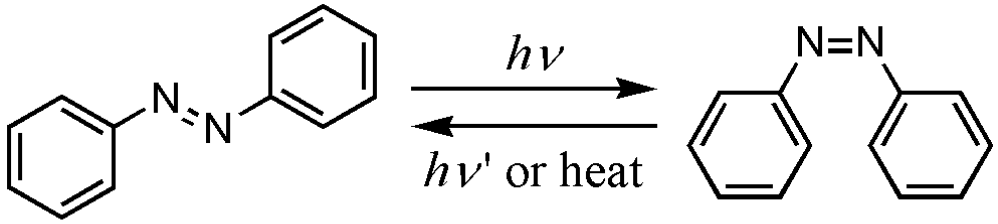
- Typically use 10ns – 1ms pulse to excite
- Quantum yields and absorption are given by vendor
- Typically want the release to be much faster than the rates of what you're trying to measure
- Using different cages, possible to have multi-color uncaging

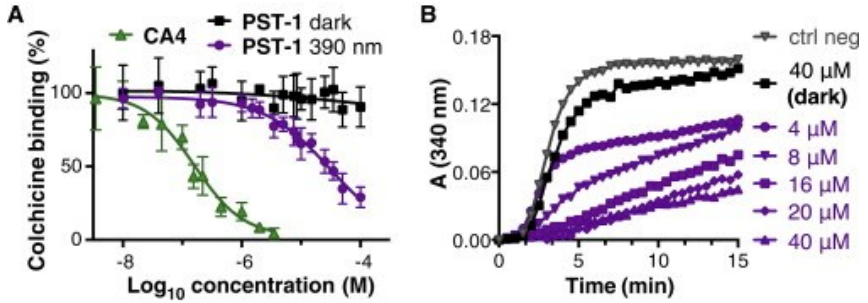


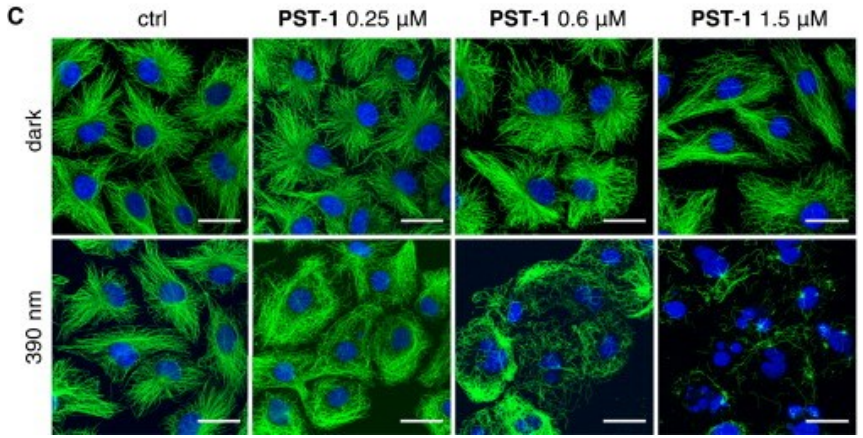

Some caged molecules:
-Glutamate
-GABA
-Kainate
-Caffeine
-AMPA
-Aspartate
-Proton

# Photoswitchable inhibitors

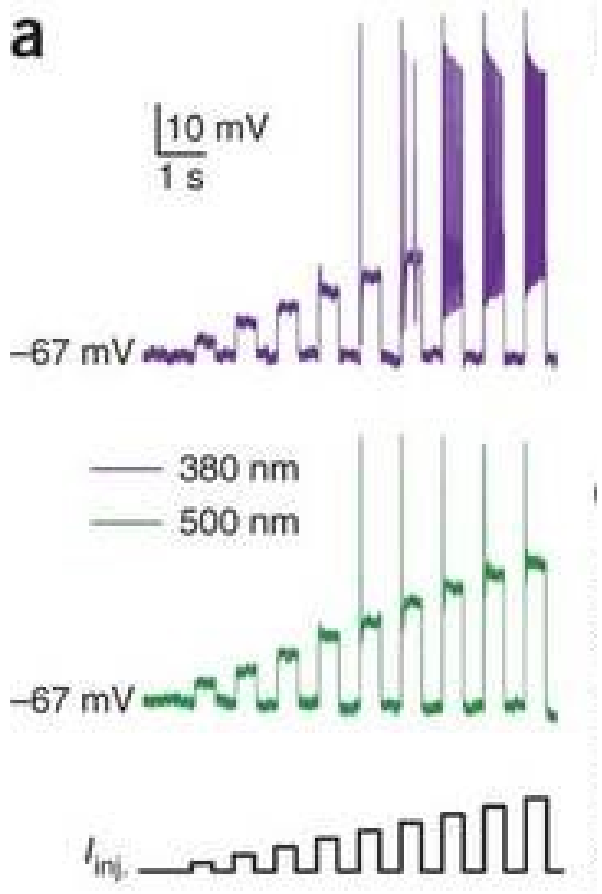
- Chemical toxins that can be activated upon exposure to light
- Often use an azobenzene group which switches between conformations with 400 or 500 nm light
- Goal is to optimize contrast ratio (on activity vs off activity)
- Filament polymerization, kinase activity, ion channel activity,




Inhibiting tubulin polymerization



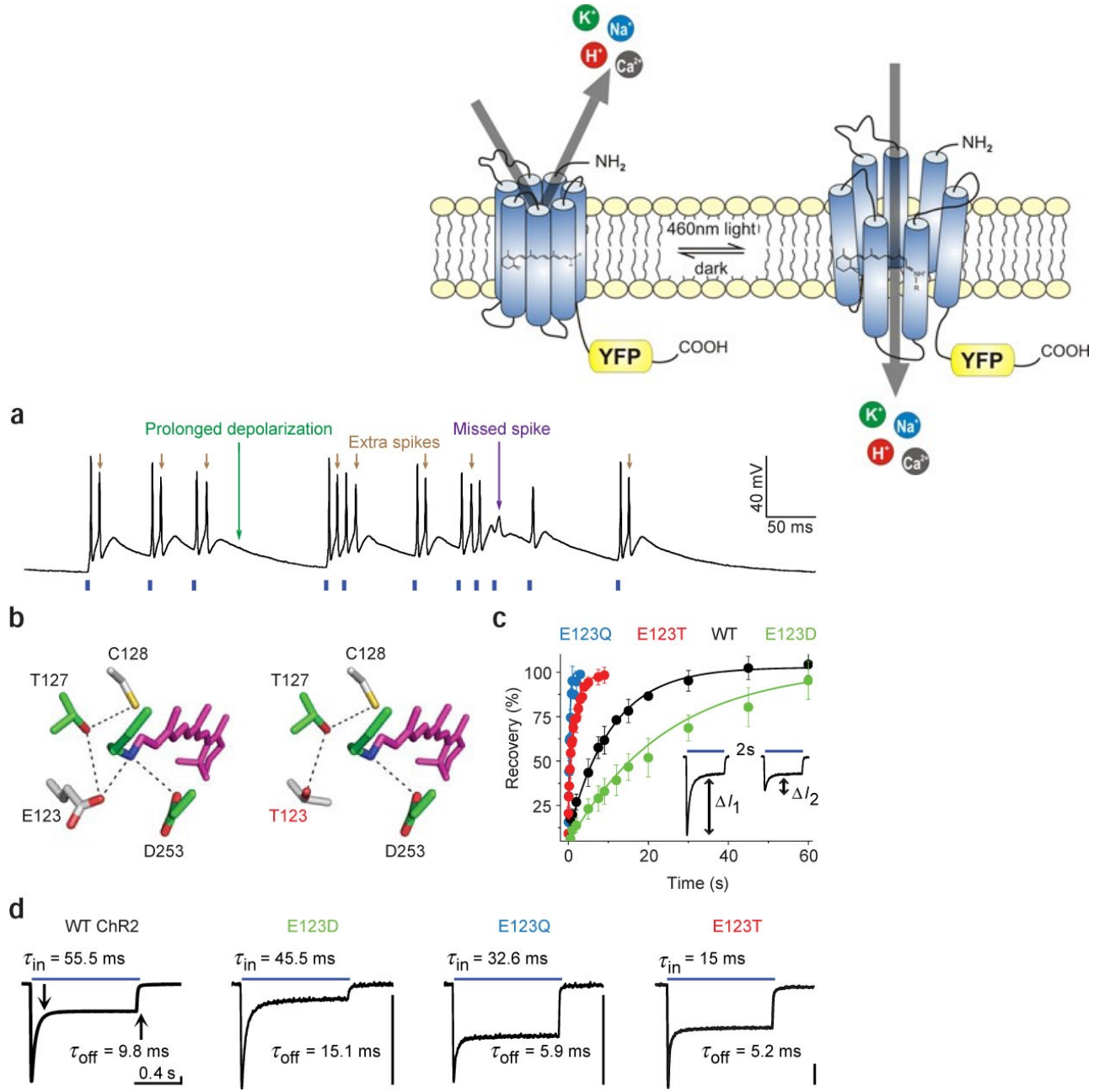

Photoswitchable lidocaine

# Optogenetics

- Co-opting light activated proteins for freaky experiments

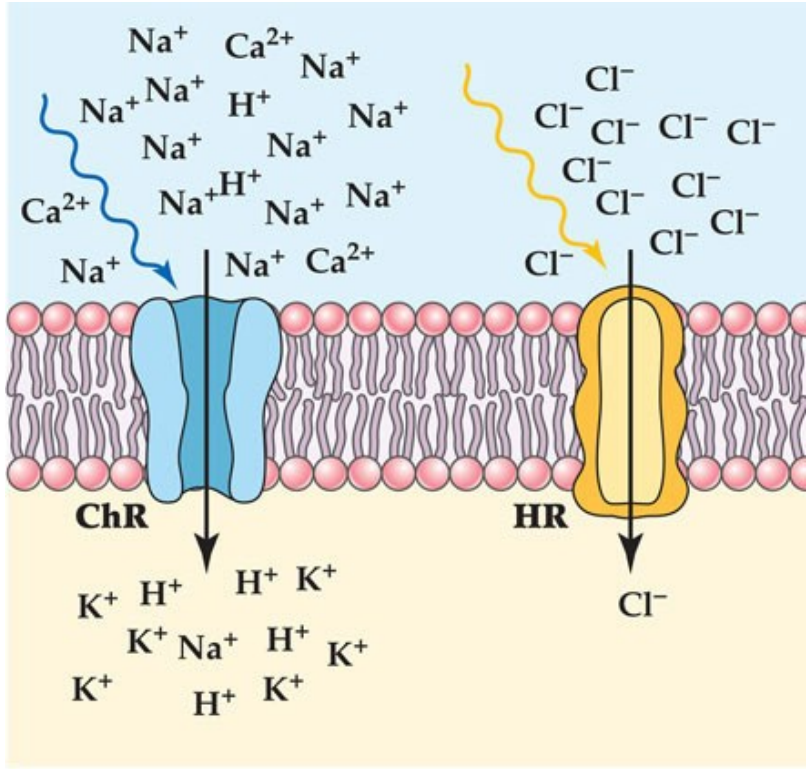
# Channel rhodopsin

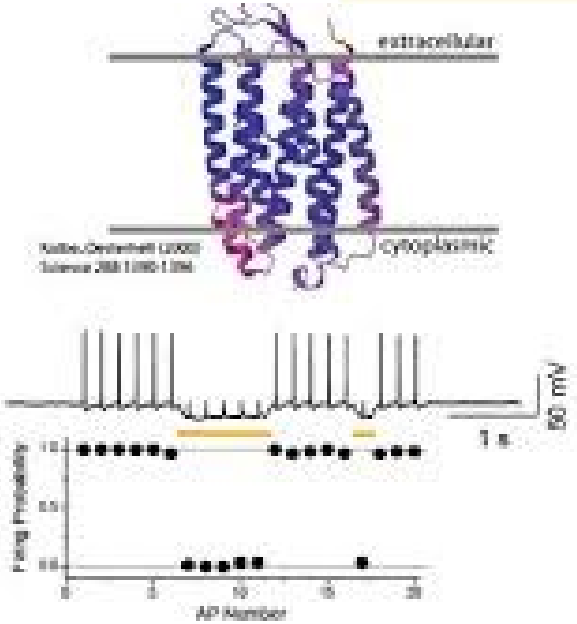
- Originally discovered in fresh water algae
- Used for light sensing in the organism (primitive eye)
- Absorption of photon causes channel opening, allows ions to flow through, changes the voltage
- Exact same process starts firing in neurons

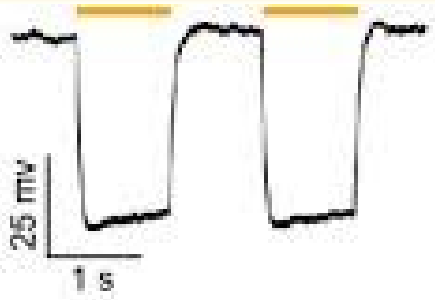


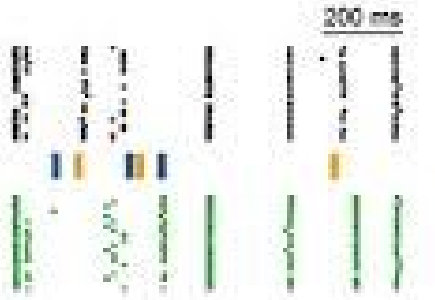
# Halorhodopsin – voltage inhibition

- 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



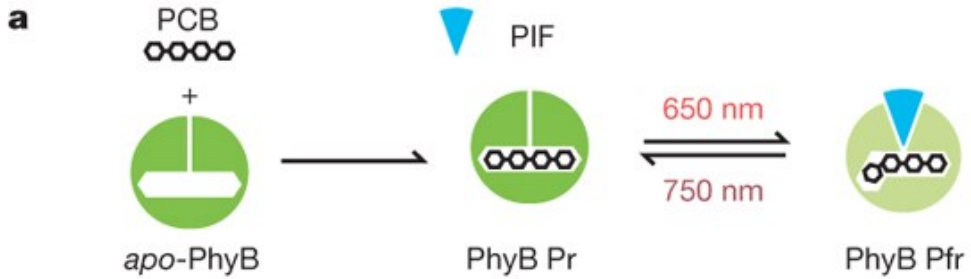



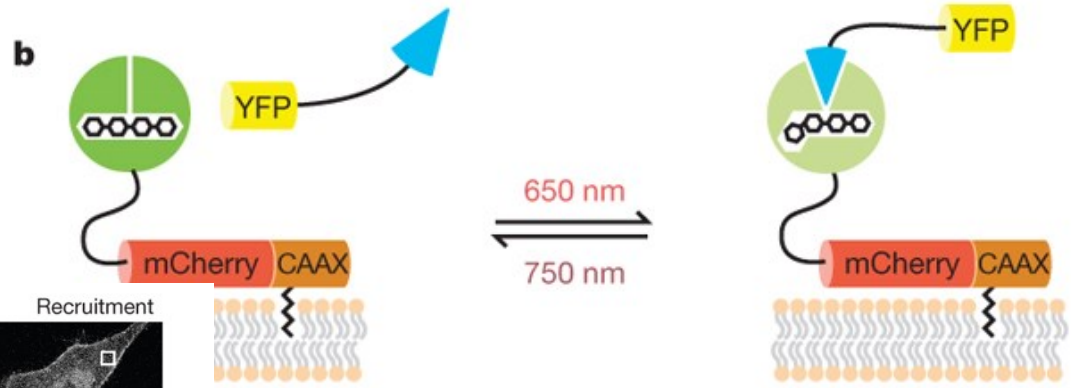
# Optical protein localization

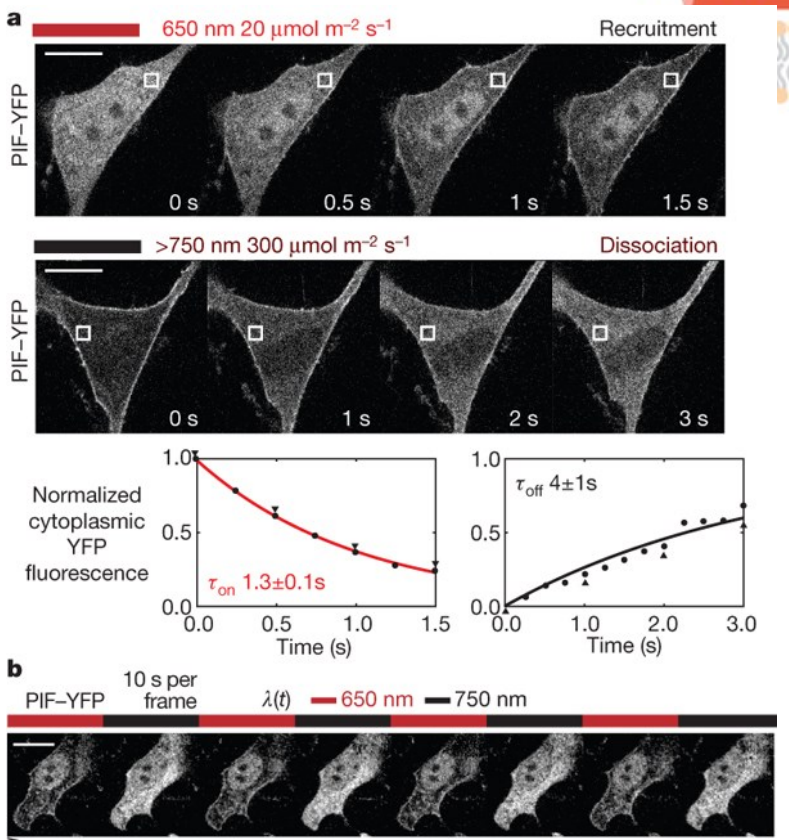
- Look for domains that respond to light
- Often found in plants
- Steal the genes, and use them to control cellular physiology

# Phytochrome – Phy/PIF

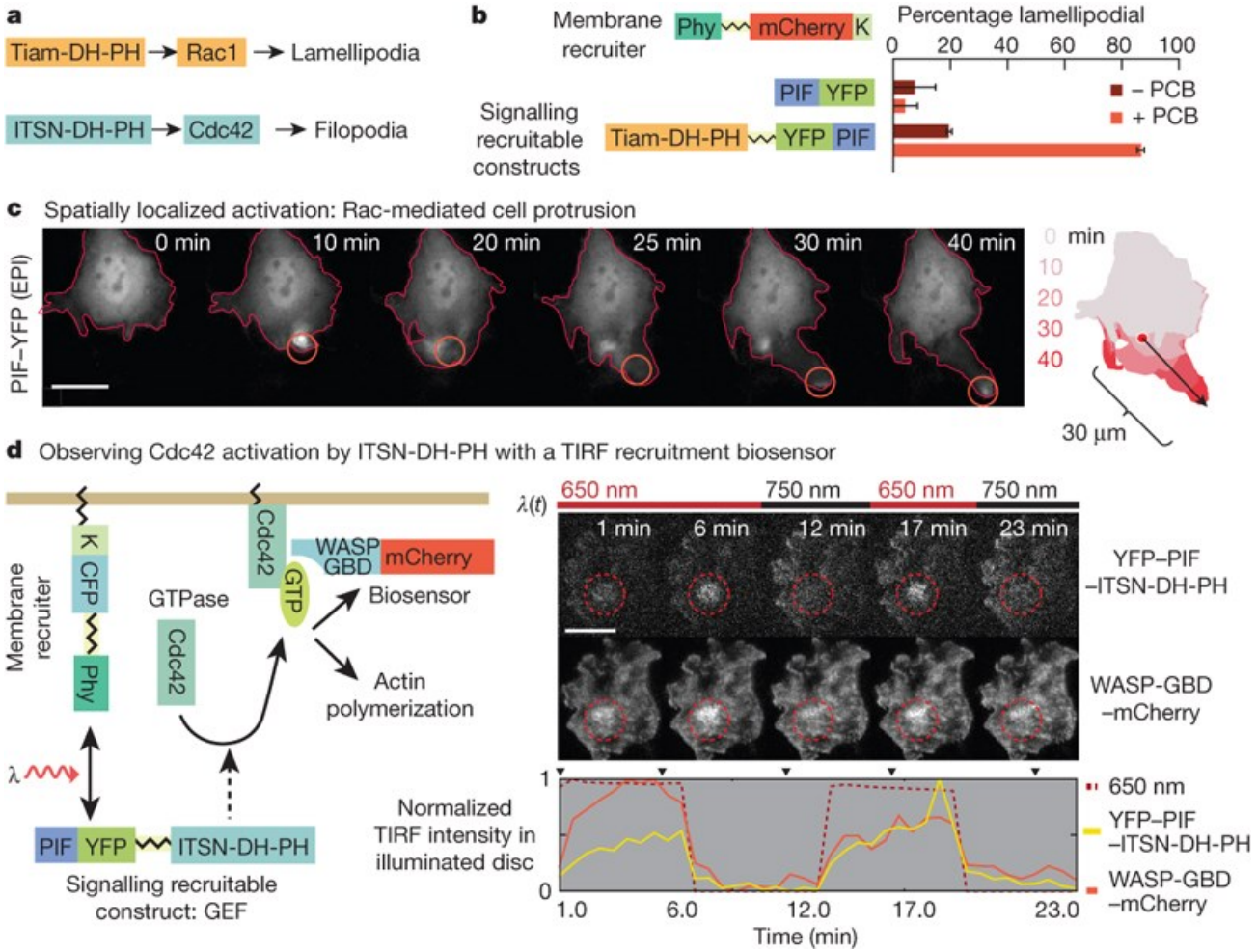
- Phytochromes were originally found in arabadopsis – control red light behaviors
- Use a phycocyanobilin (PCB) chromophore that reversibly changes conformation
- As PCB changes, it will recruit or let go of the PIF domain
- Can be used to pull 2 proteins together or apart



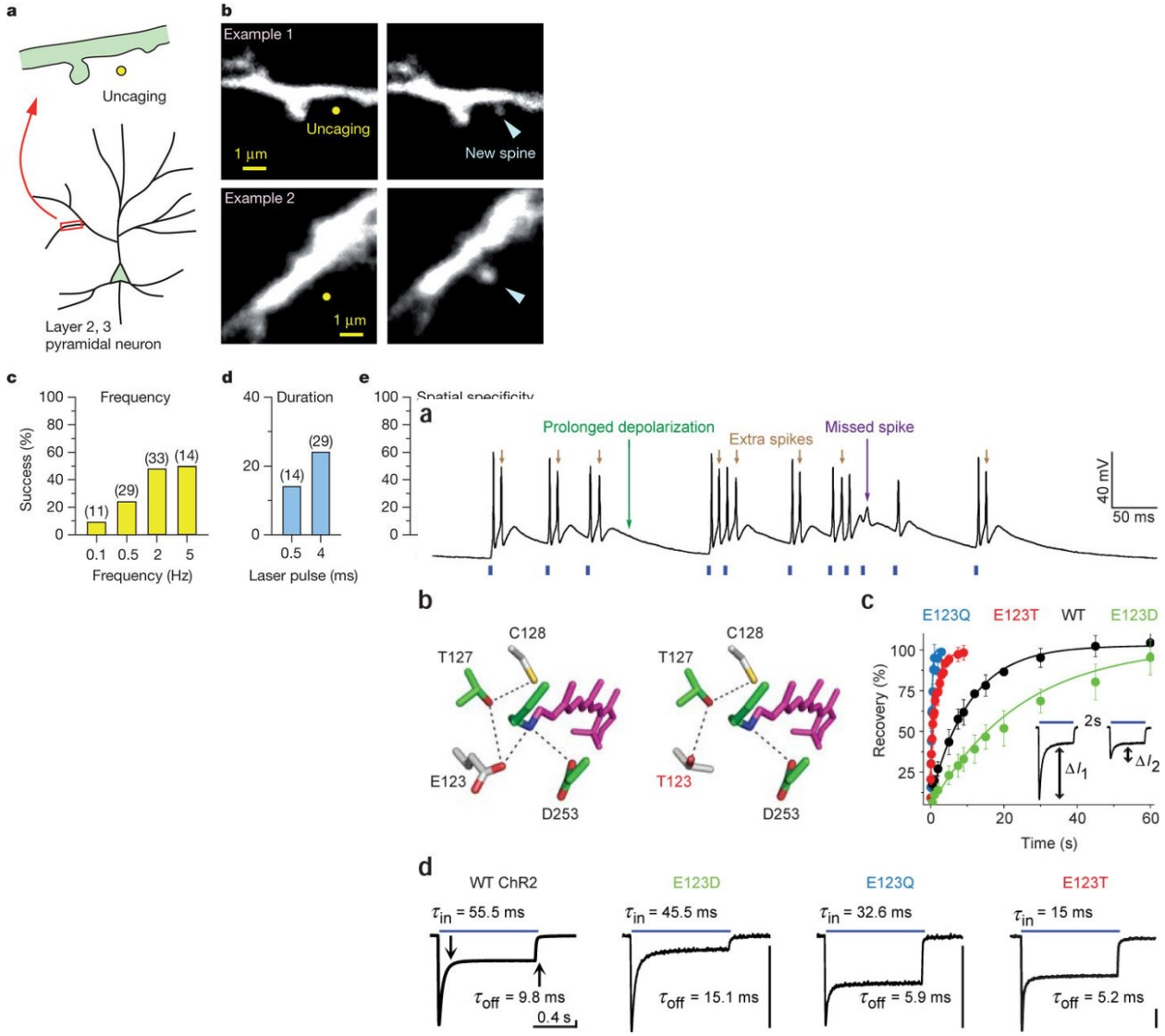
# Directing cellular movement




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

Epi Mode

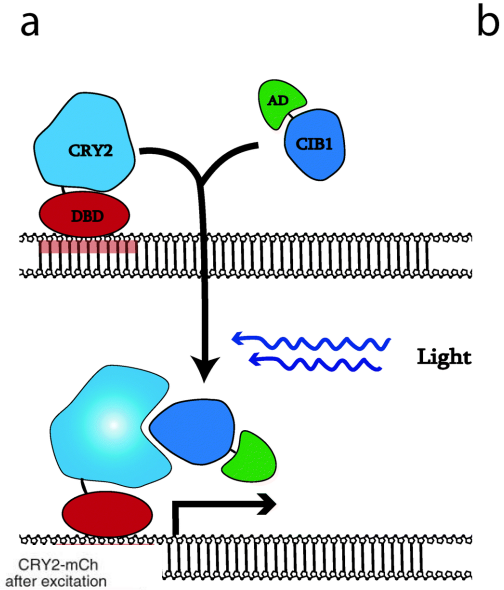
# Actuators

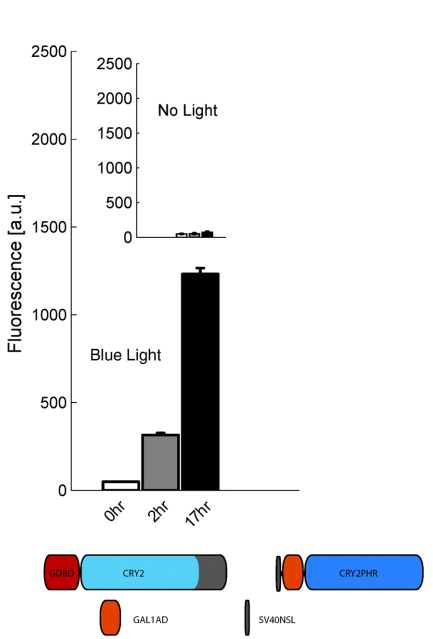


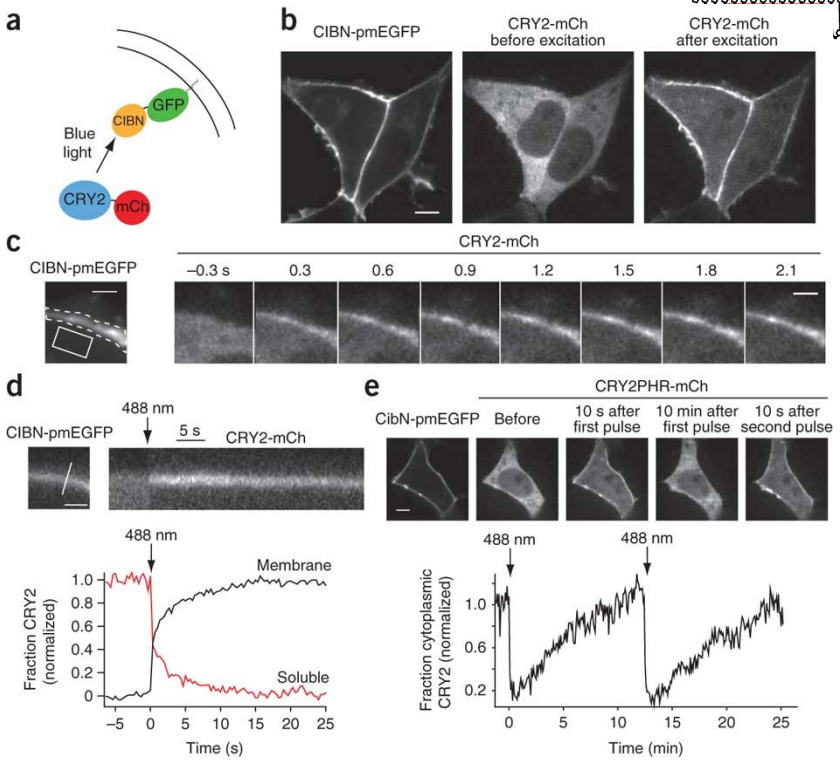

Fiberoptic Control of Locomotion in ChR2 Mouse

# Cryptochrome2 –CIB1 binding

- Crytptochrome2 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 co-factors

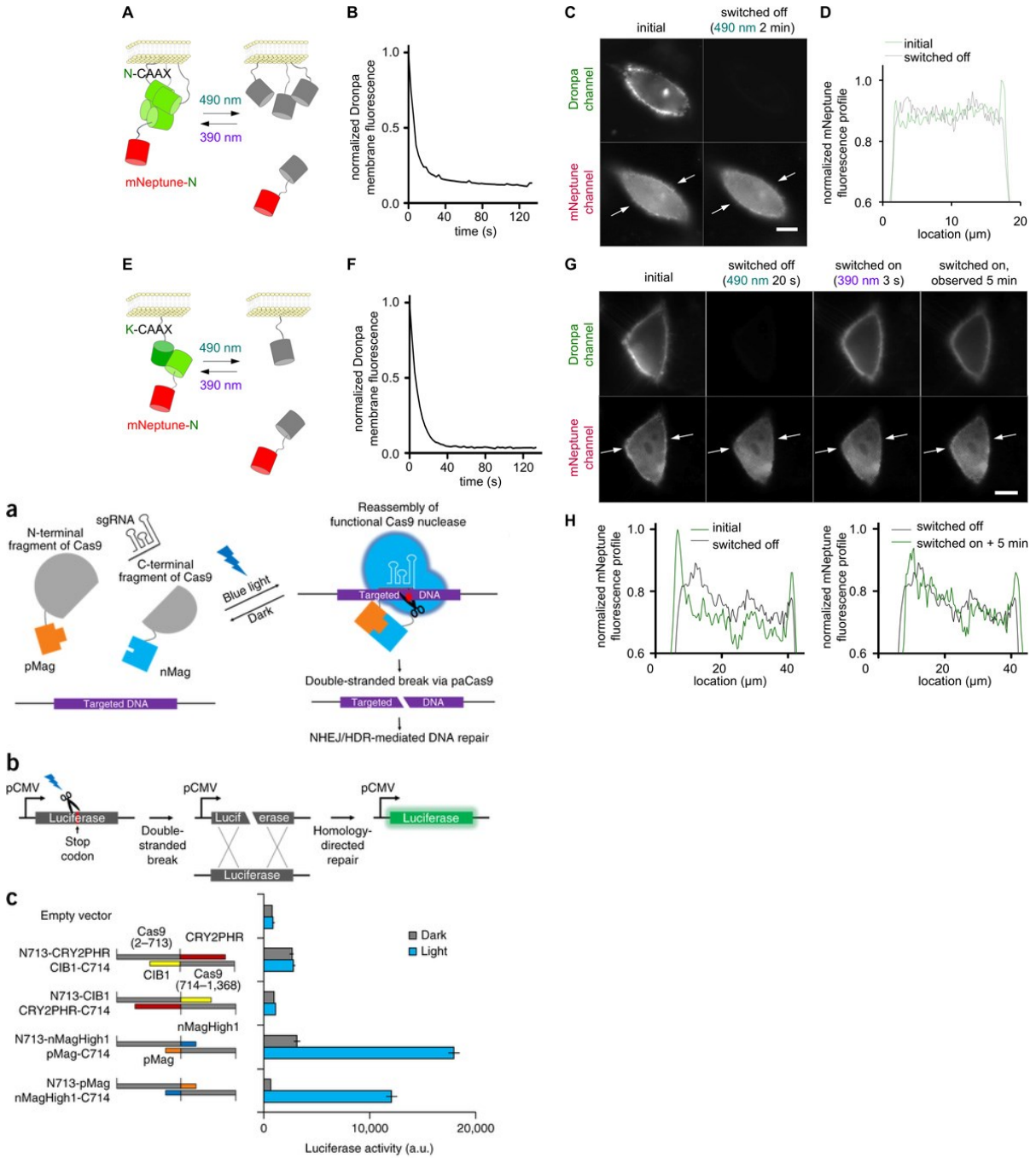


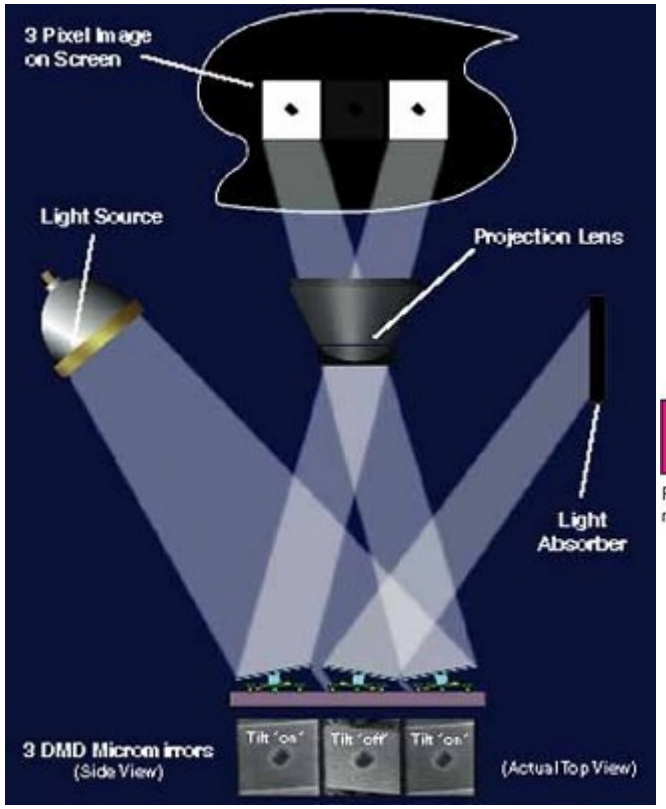
# Other systems

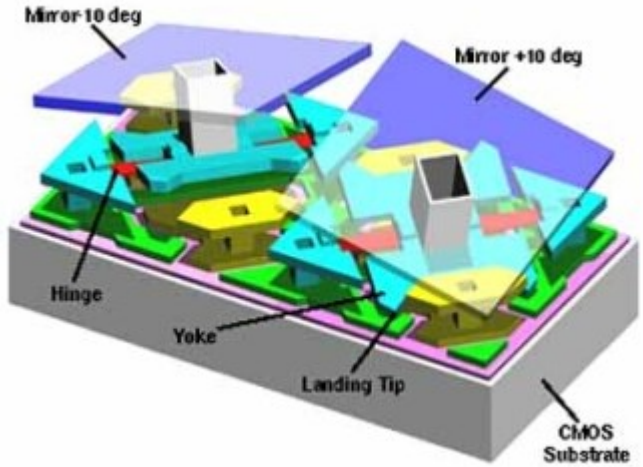
- Light induced tetramerization of FPs - Dronpa
- LOV (light oxygen voltage domains) has slow kinetics
- VIVID fungal blue light receptor
- Phototropin

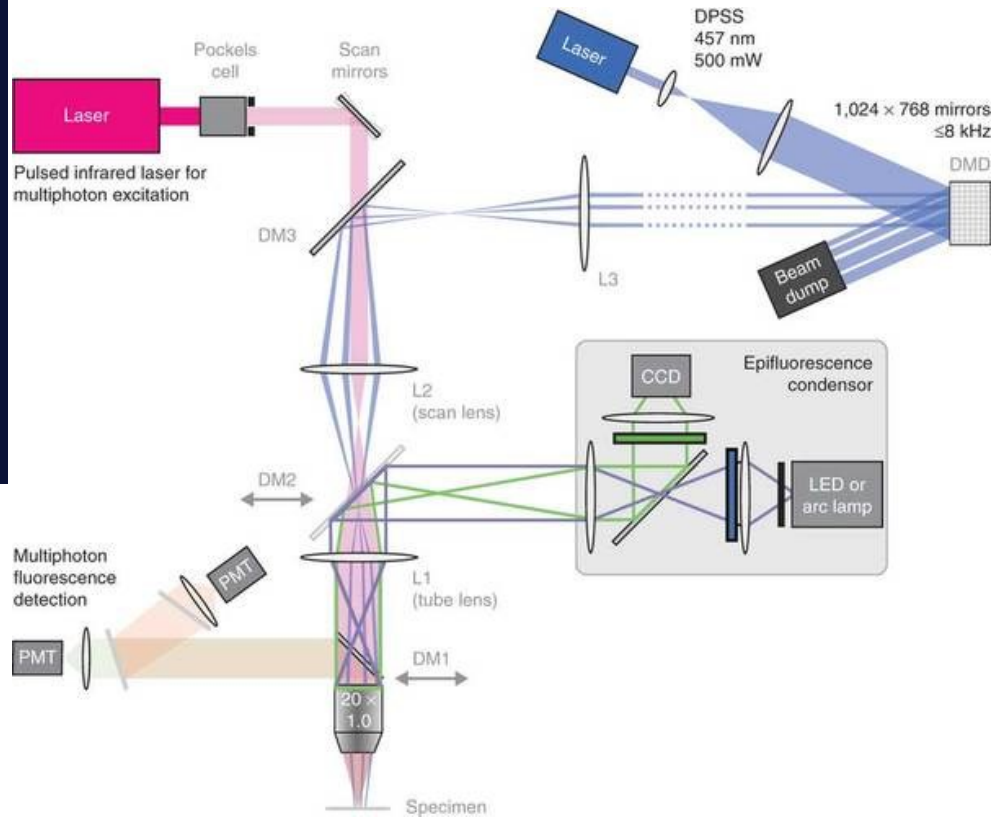


# Patterning illumination

- 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)

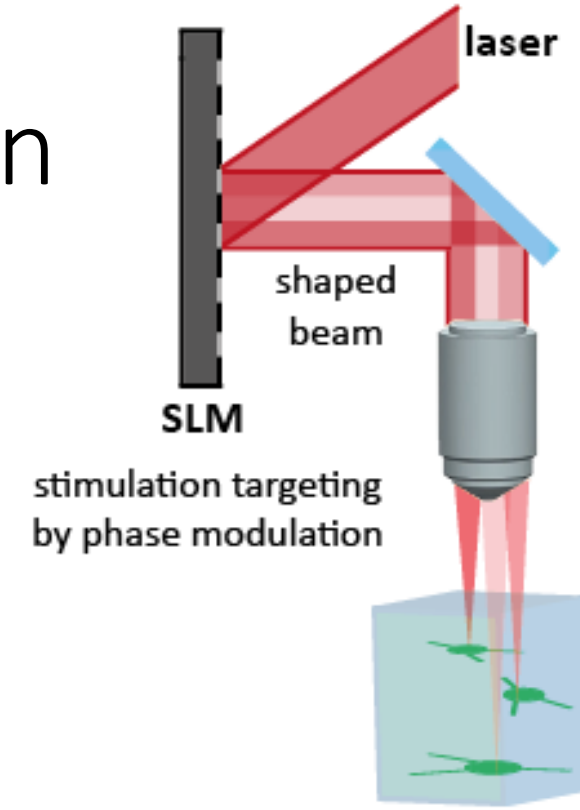


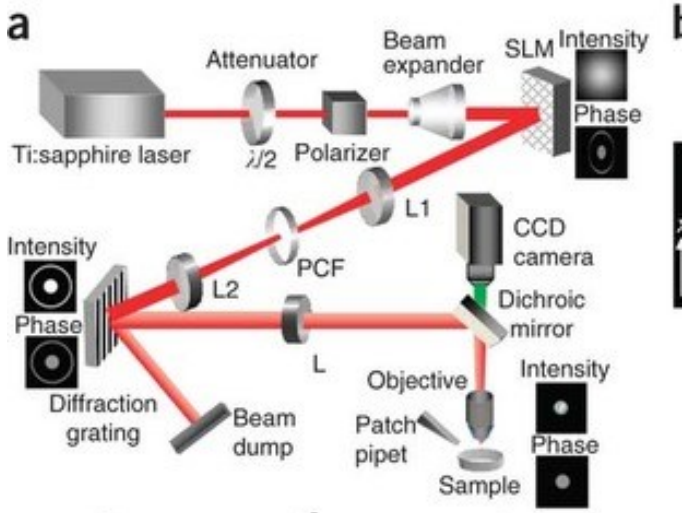



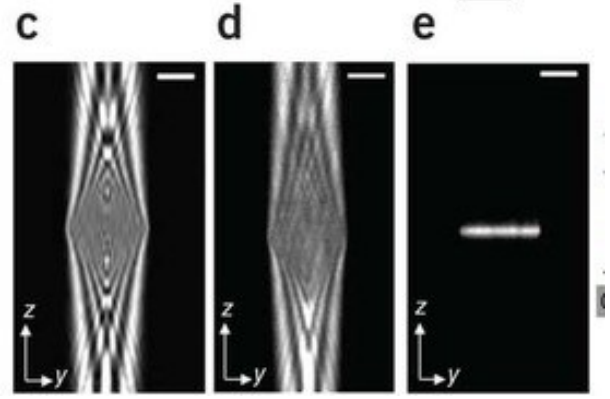



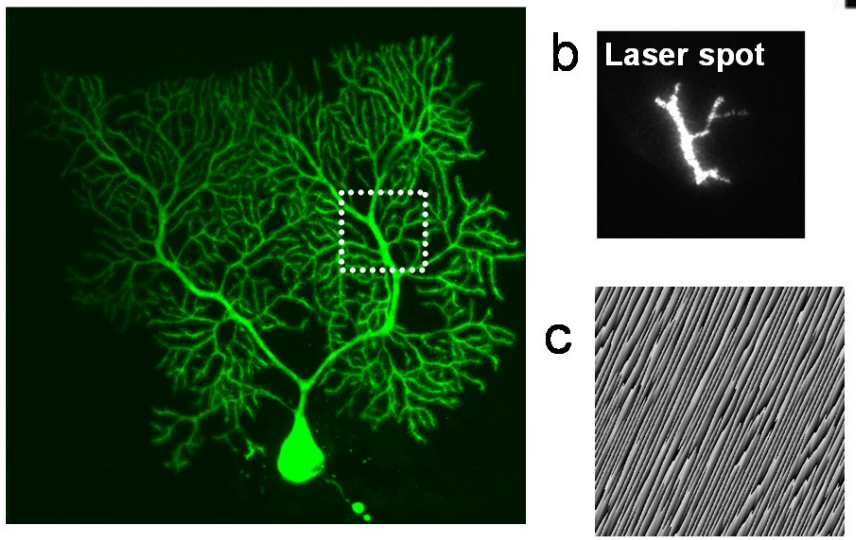
# Holographic illumination

- 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 focusir to illuminate in 3 dimensions



# On to Matlab…