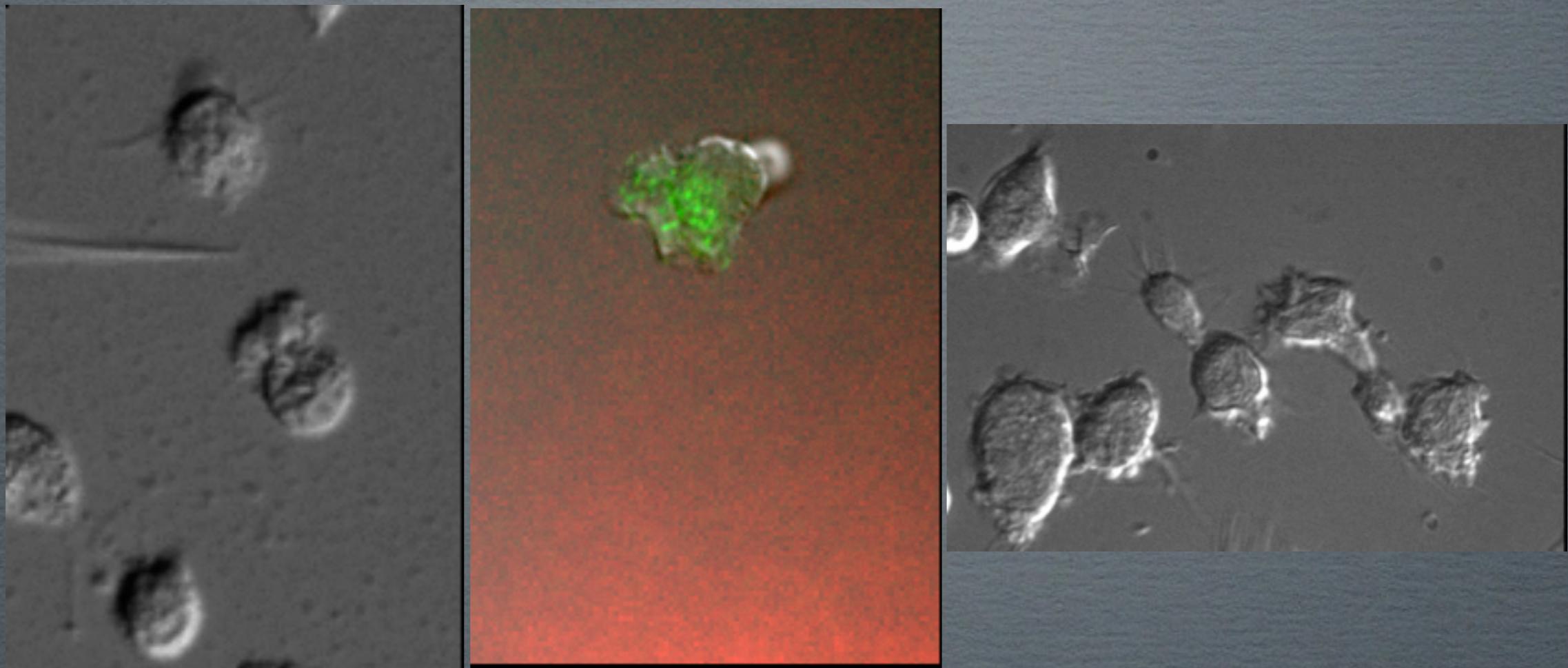
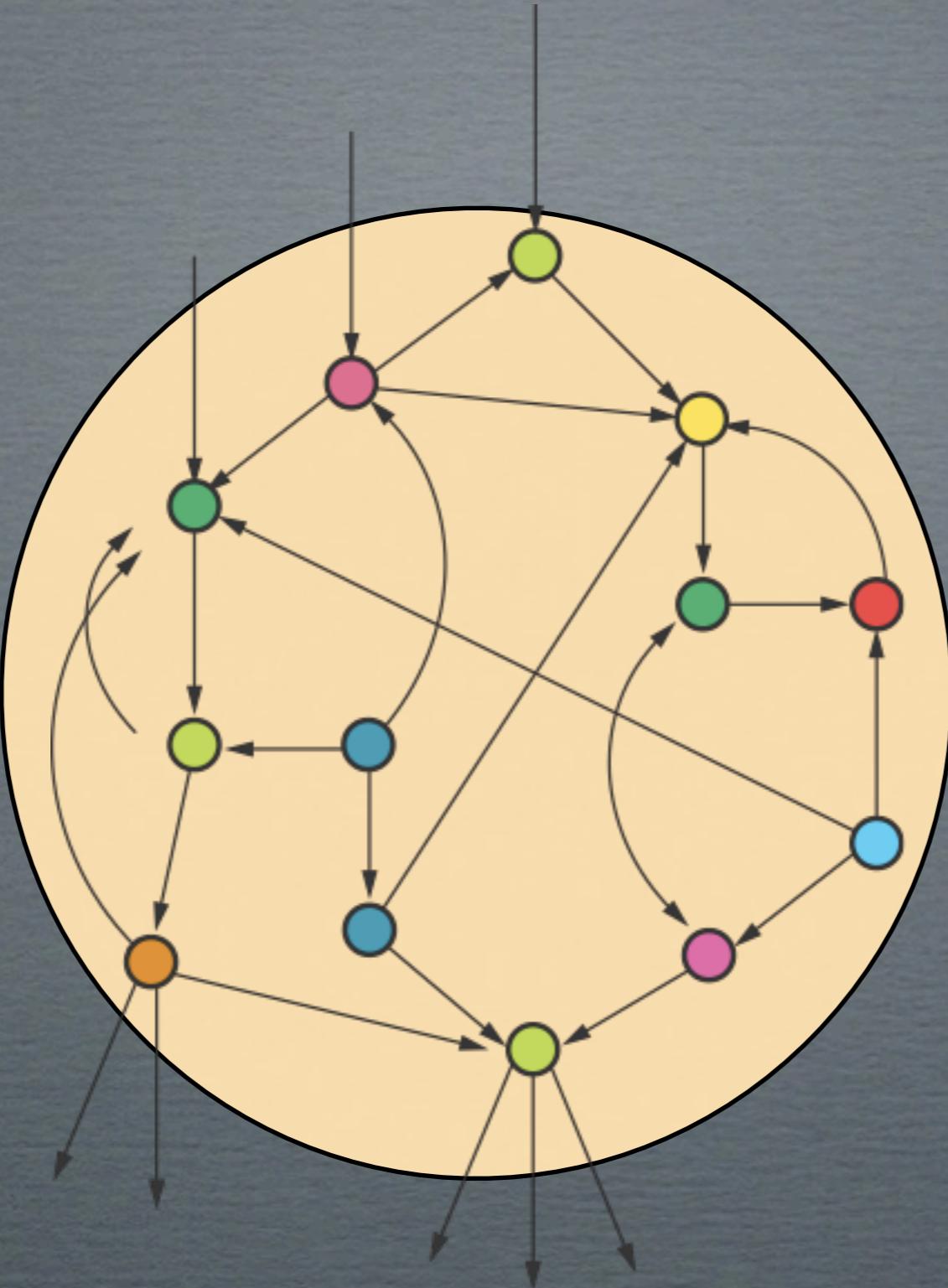


Why do we want/need spatial control of signaling?

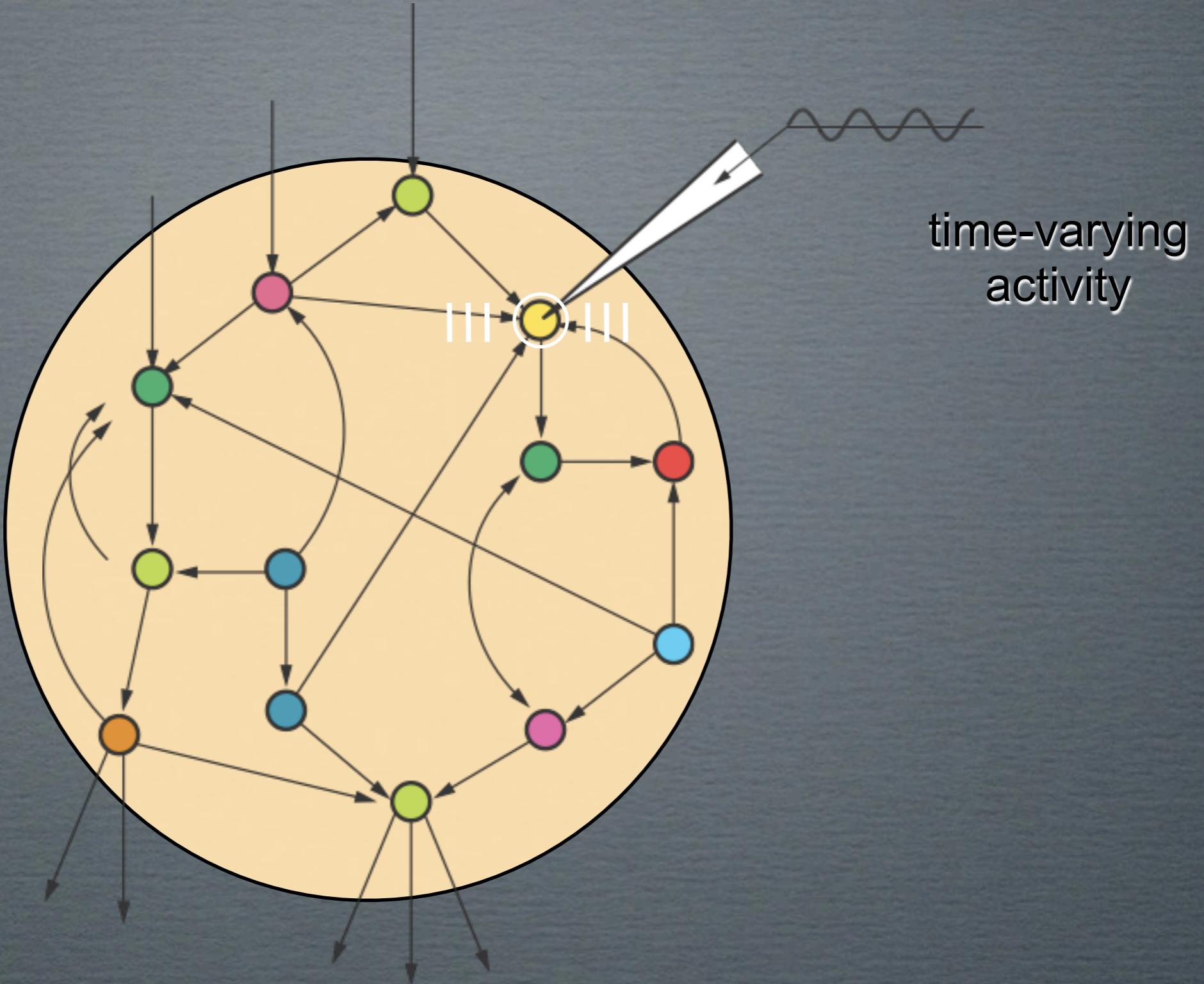


Orion Weiner

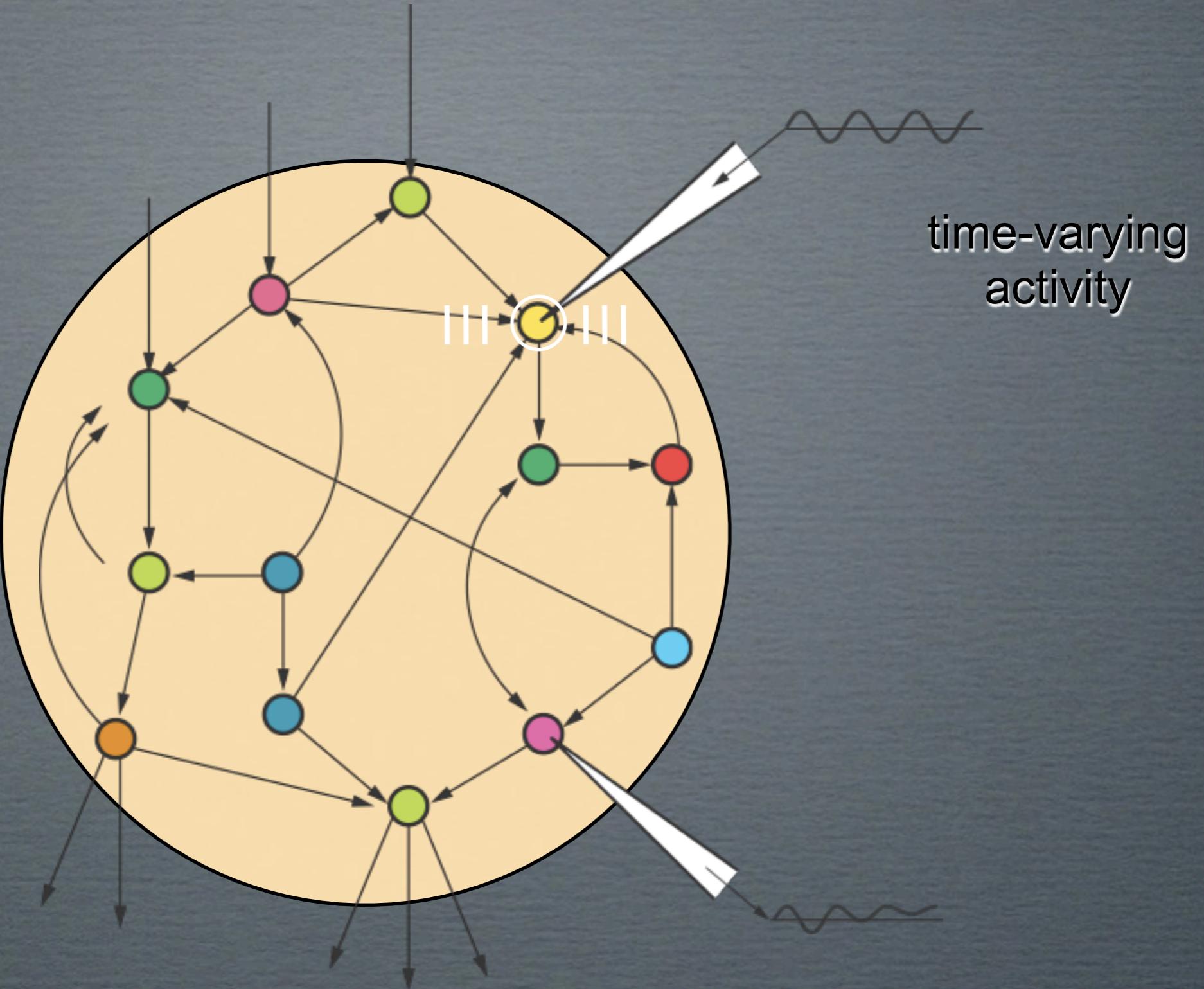
Dissecting cellular circuits



Dissecting cellular circuits: Perturbation

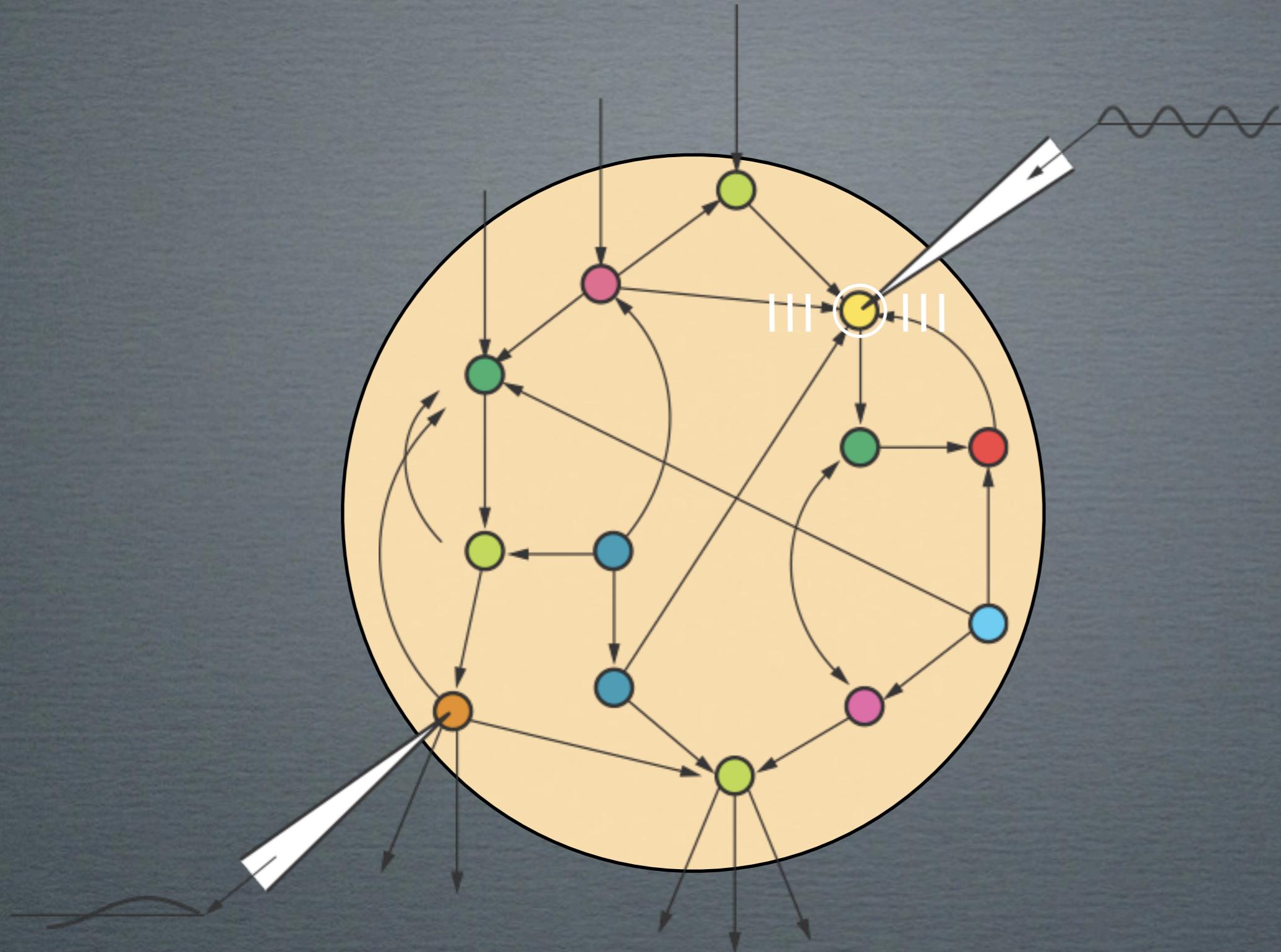


Dissecting cellular circuits: Measurement

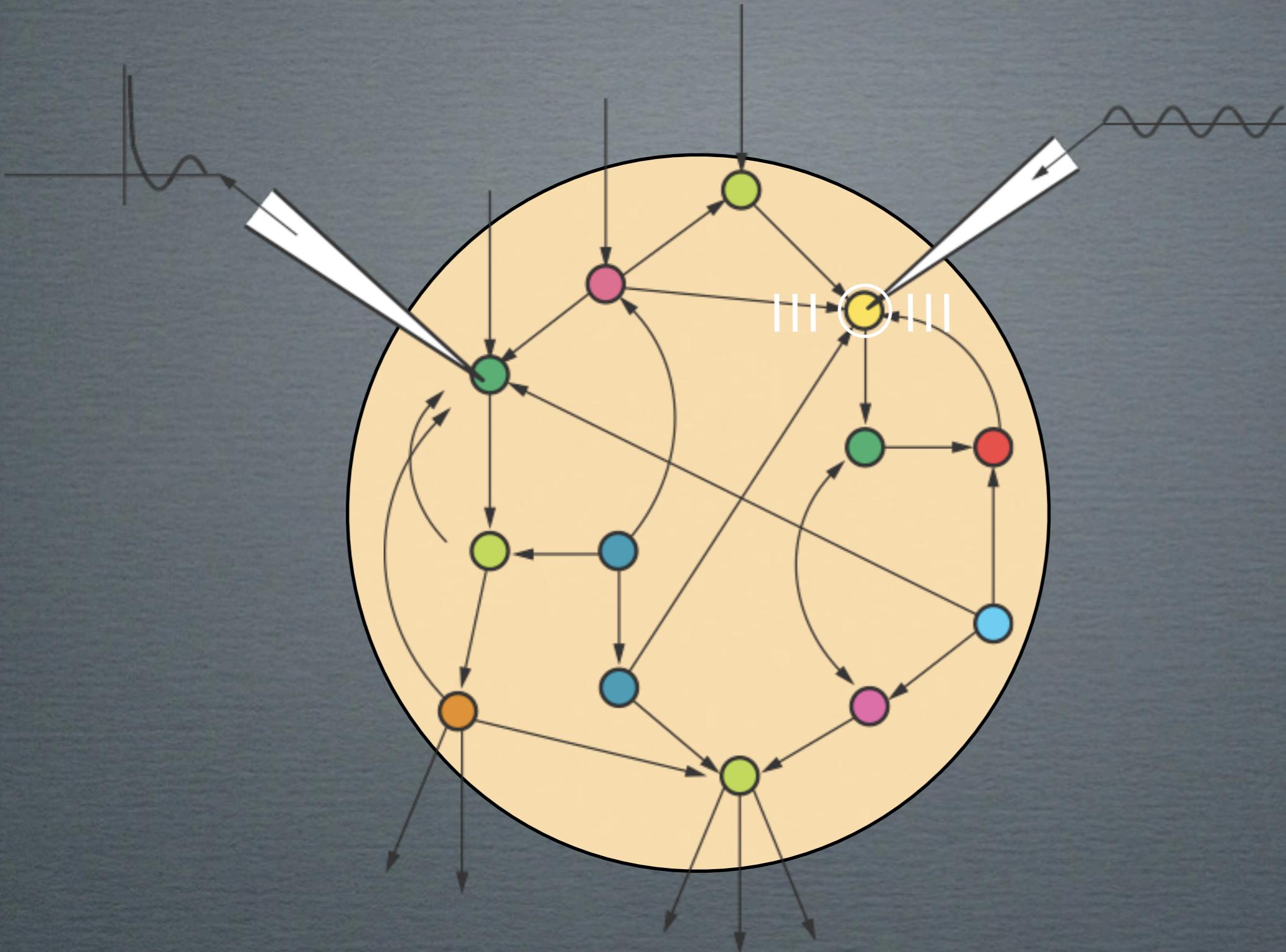


time-varying
activity

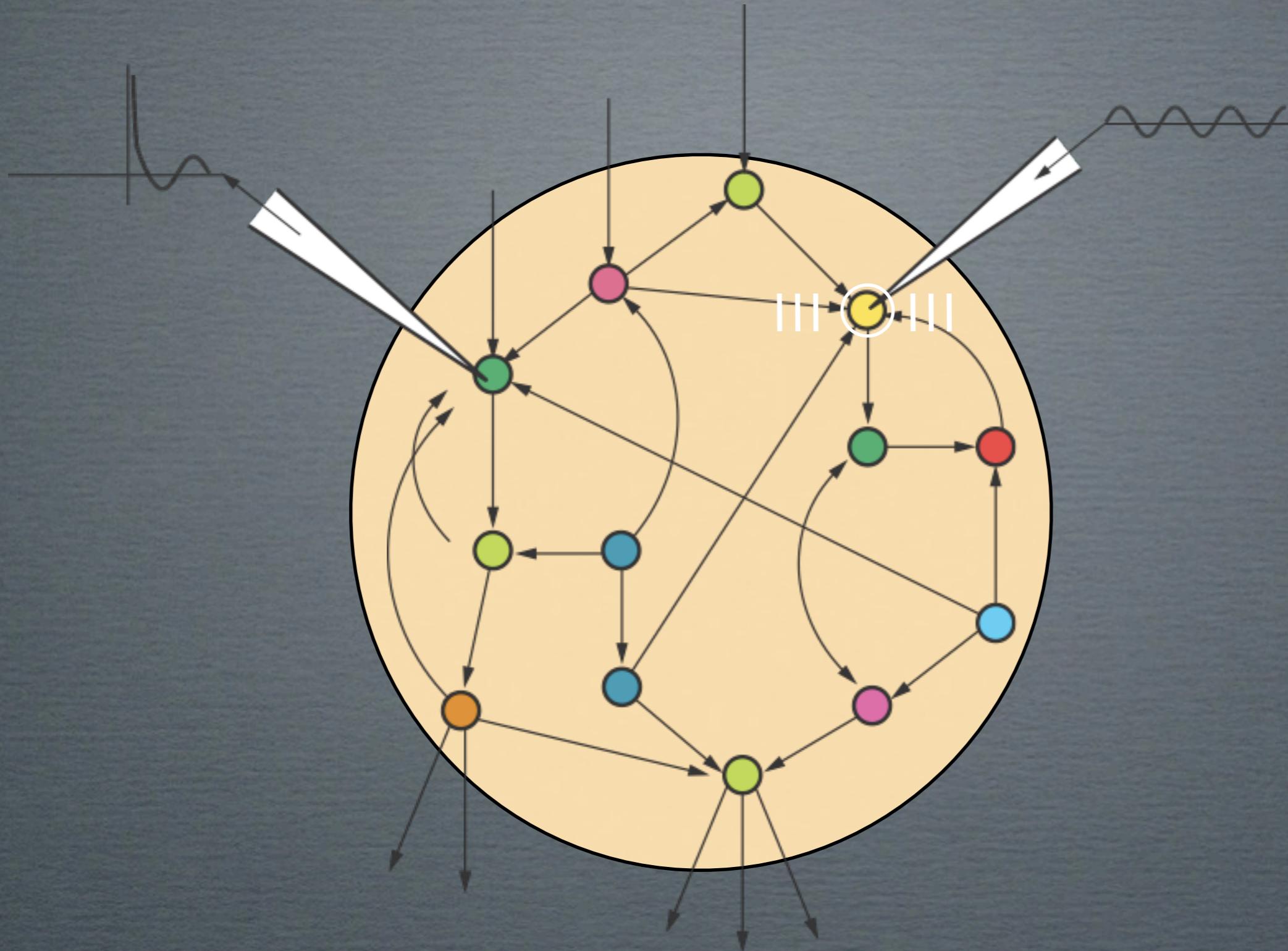
Dissecting cellular circuits: Measurement



Dissecting cellular circuits: Measurement

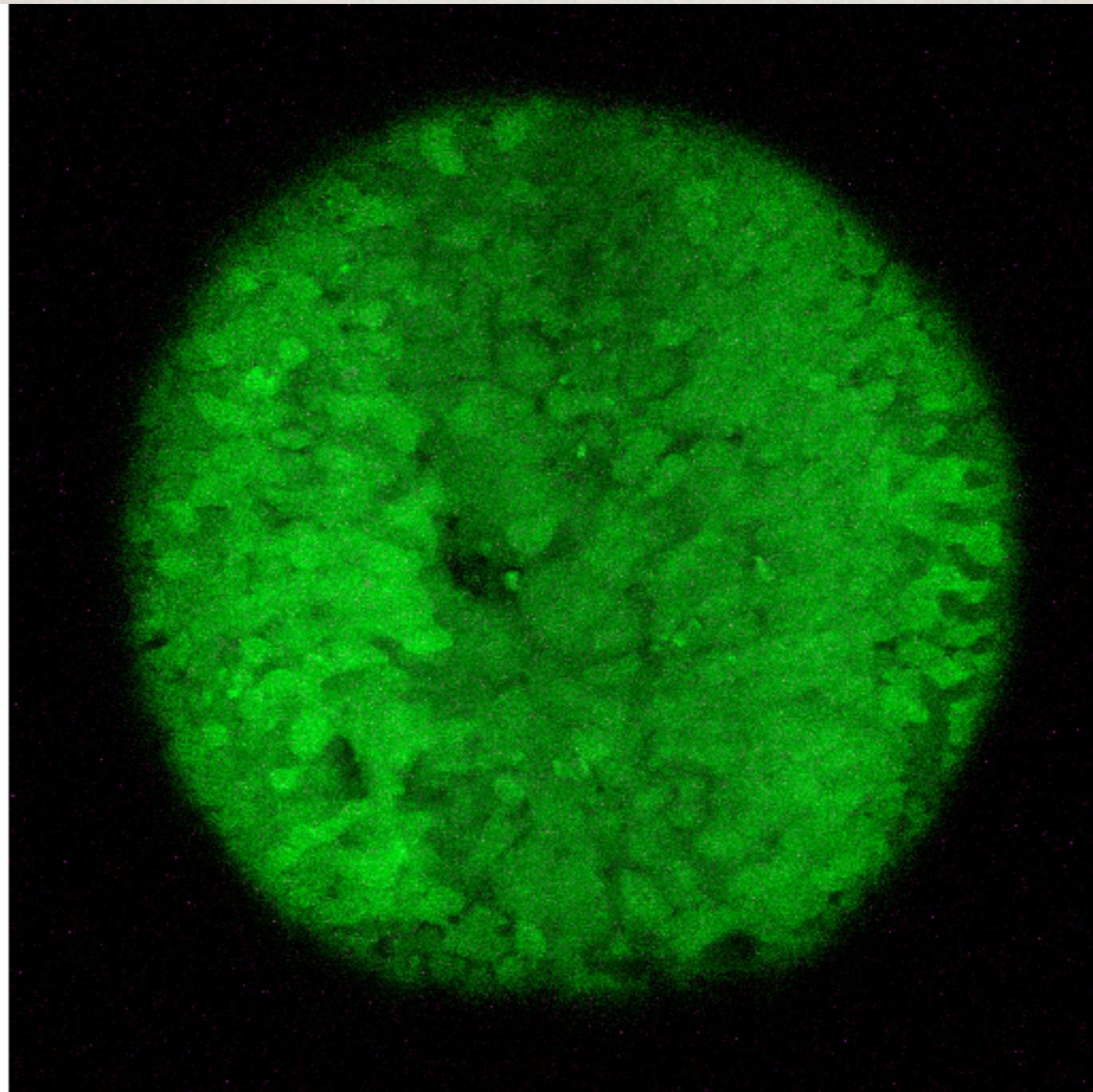


Dissecting cellular circuits: Measurement



Would like to generate inputs with spatial and temporal control.

LIGHT-CONTROL OF CELL SIGNALING



Want to couple light to signaling, not just fluorescence

1a. Uncaging small molecules with light

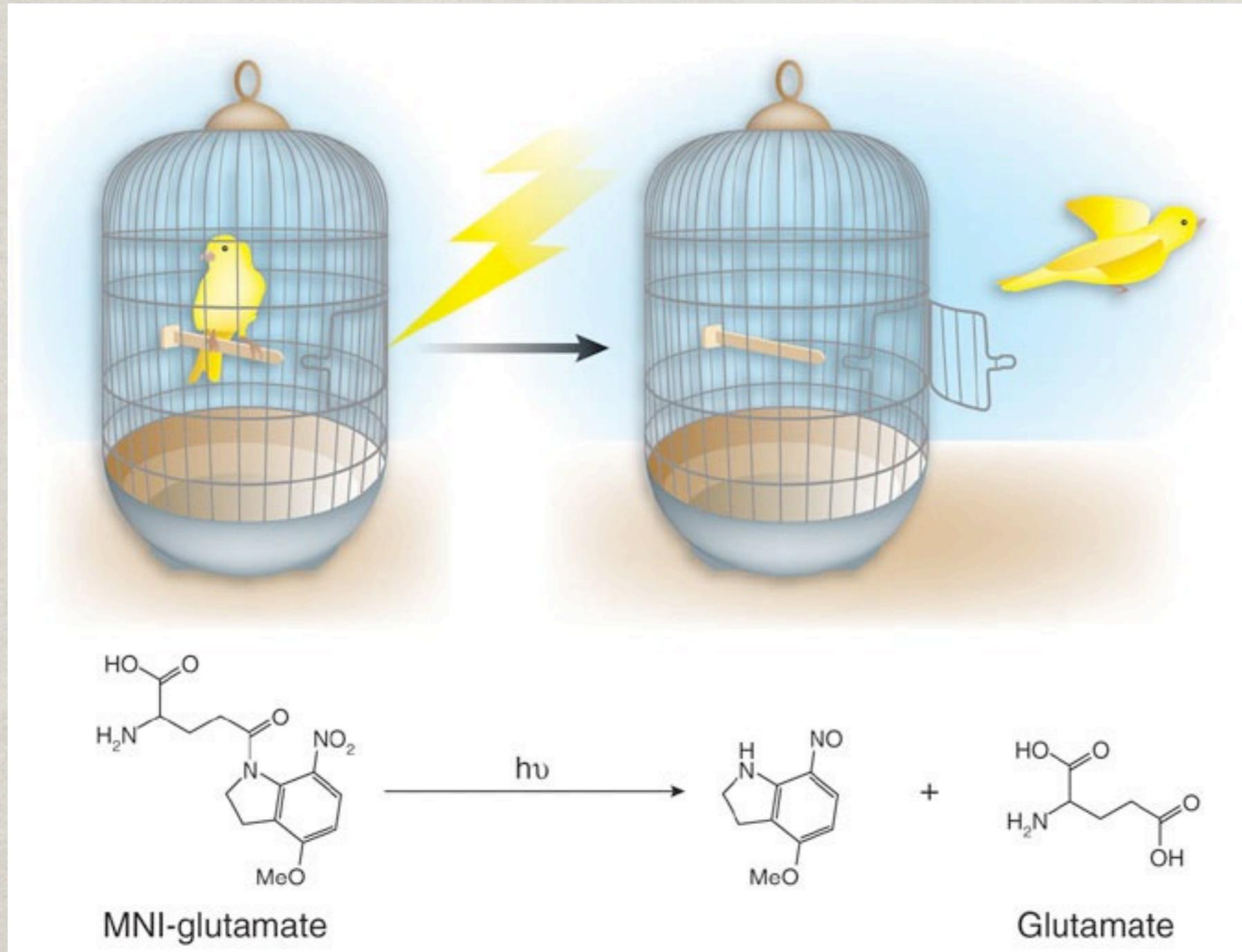


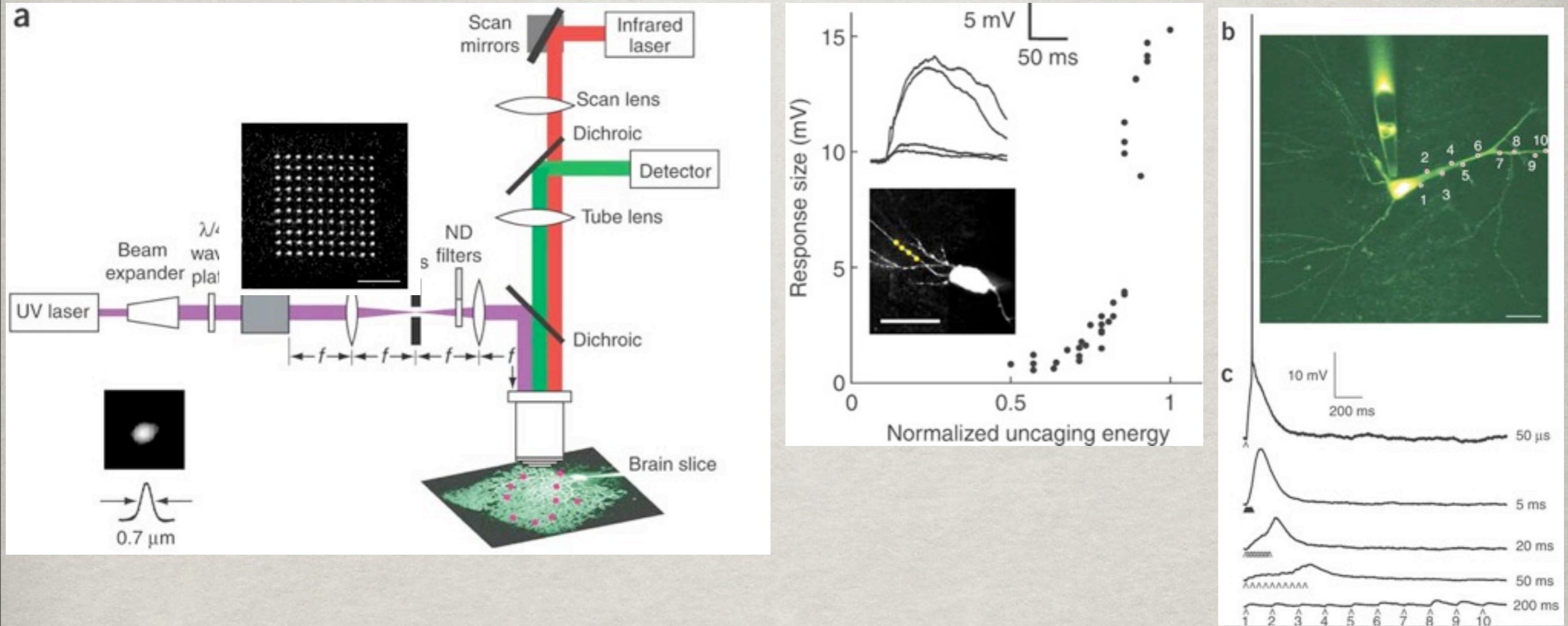
Figure 1. In photolysis of caged compounds, illustrated here by a metaphor and an example, the absorption of a photon leads to the opening of a 'cage', which liberates the trapped bioactive species.

In the neurosciences, photolysis is used to rapidly deliver neurotransmitters such as glutamate, shown here being liberated from 4-methoxy-7-nitroindolinyl (MNI)-glutamate by the absorption of a UV photon.

PMID: 16278648

engineering challenge, not reversible, not genetically encoded

Rapid multipoint control of neuronal signaling



This system uses TeO₂ acousto-optical deflectors to steer an ultraviolet beam rapidly and can uncage at over 20,000 locations per second. The uncaging beam is projected into the focal plane of a two-photon microscope, allowing combined patterned uncaging with imaging and electrophysiology.

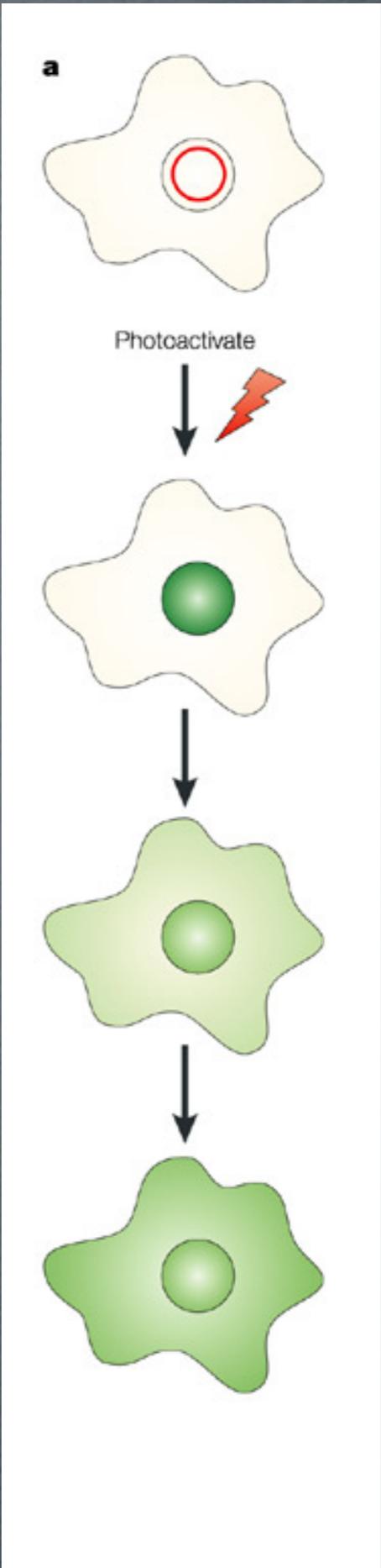
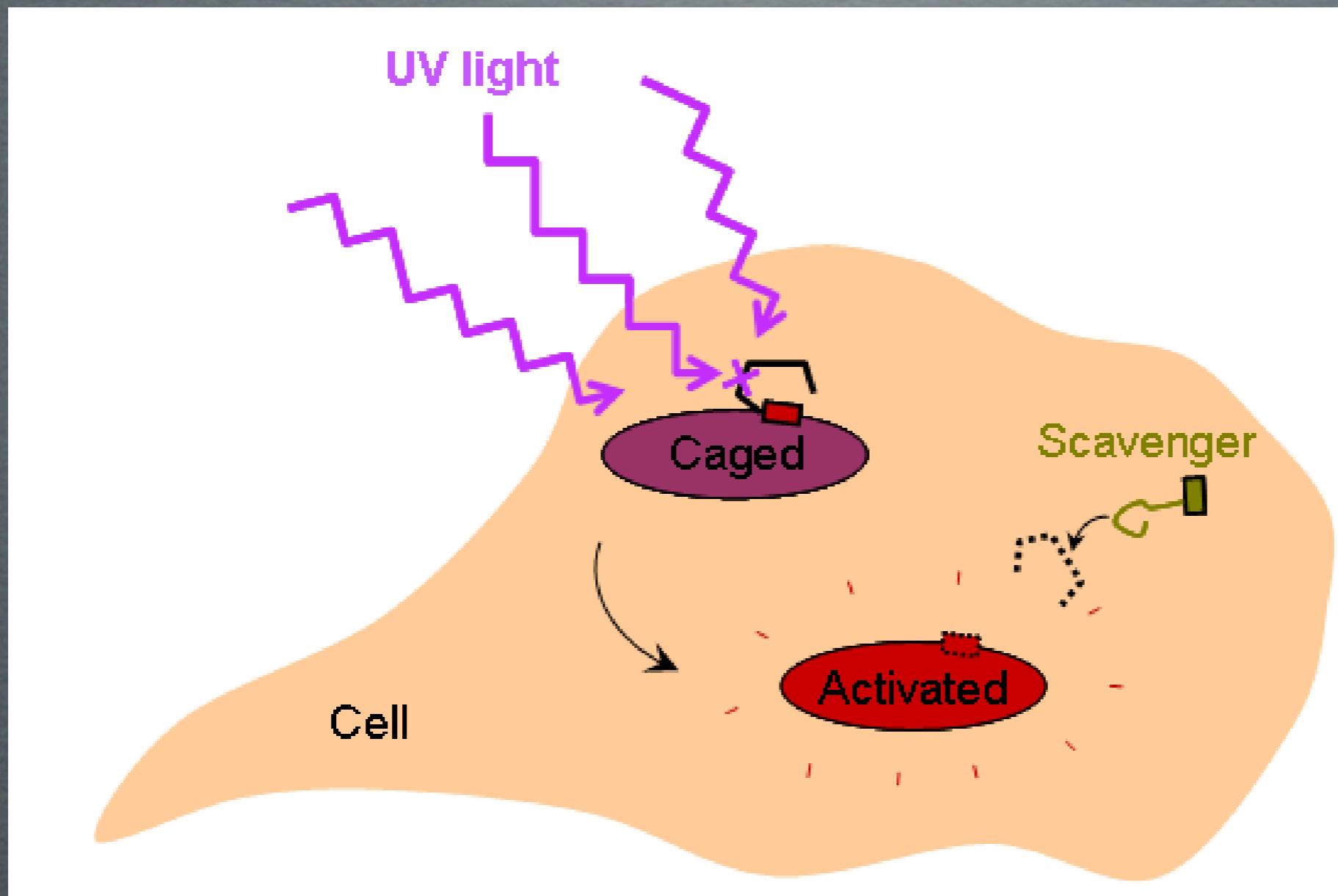
Nat Methods. 2005 Nov;2(11):837-43.

Rapid neurotransmitter uncaging in spatially defined patterns.

Shoham S, O'Connor DH, Sarkisov DV, Wang SS.

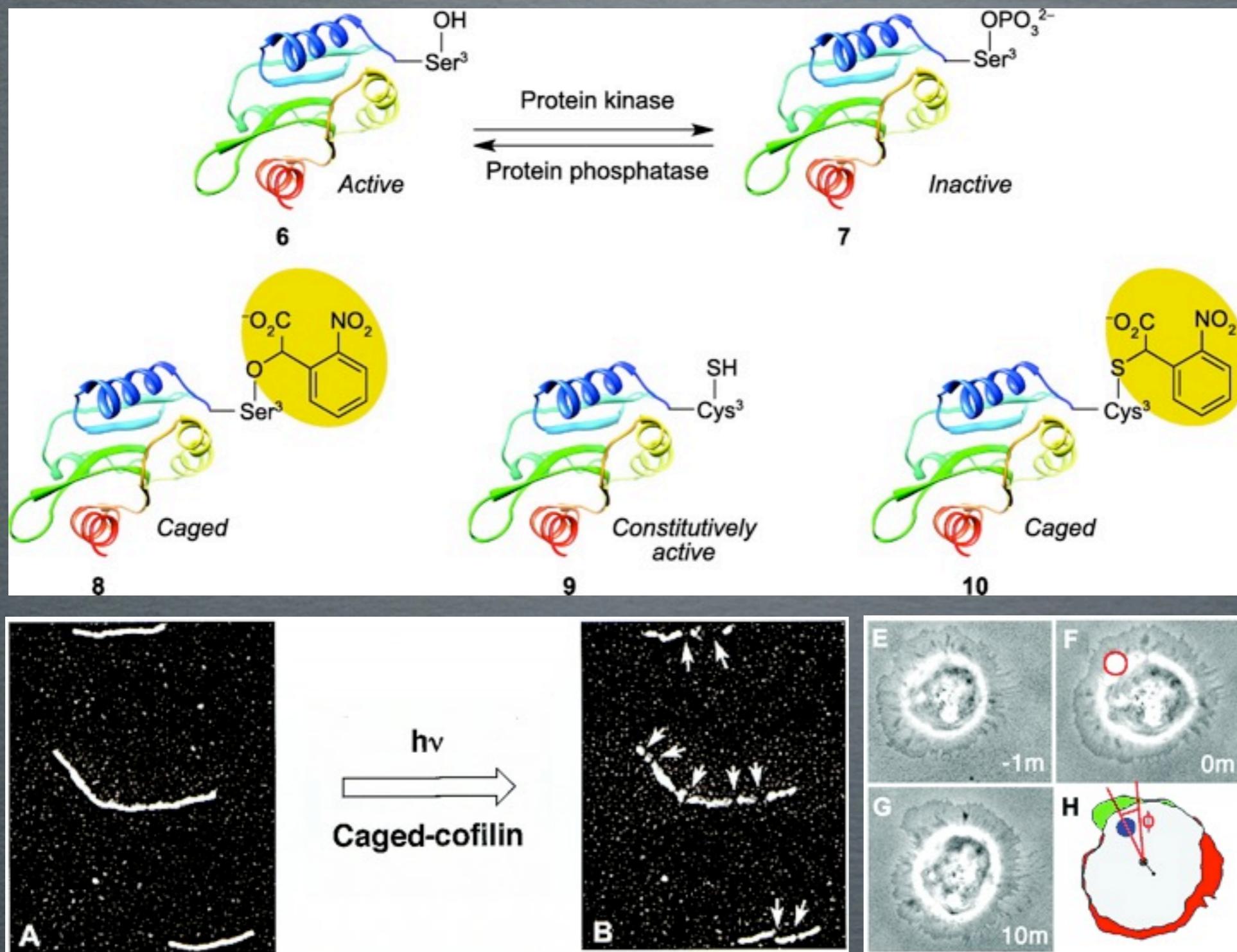
Current summation
in a pyramidal neuron

1b. Uncaging proteins with light



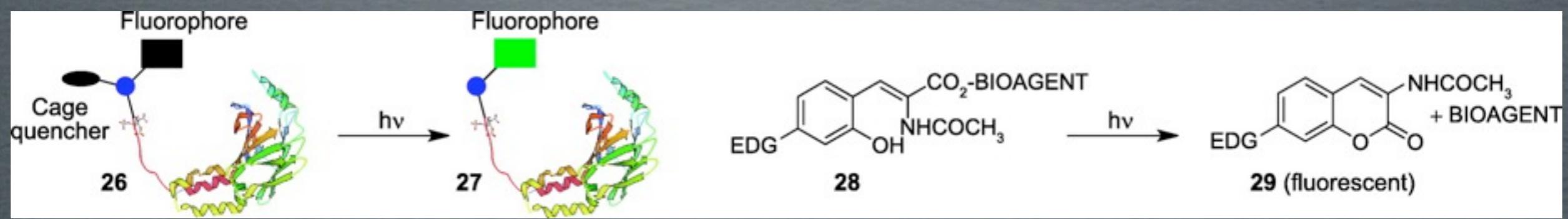
Without control of the off state, diffusion of the activated species limits spatial and temporal control

Caging a phosphoresponsive protein

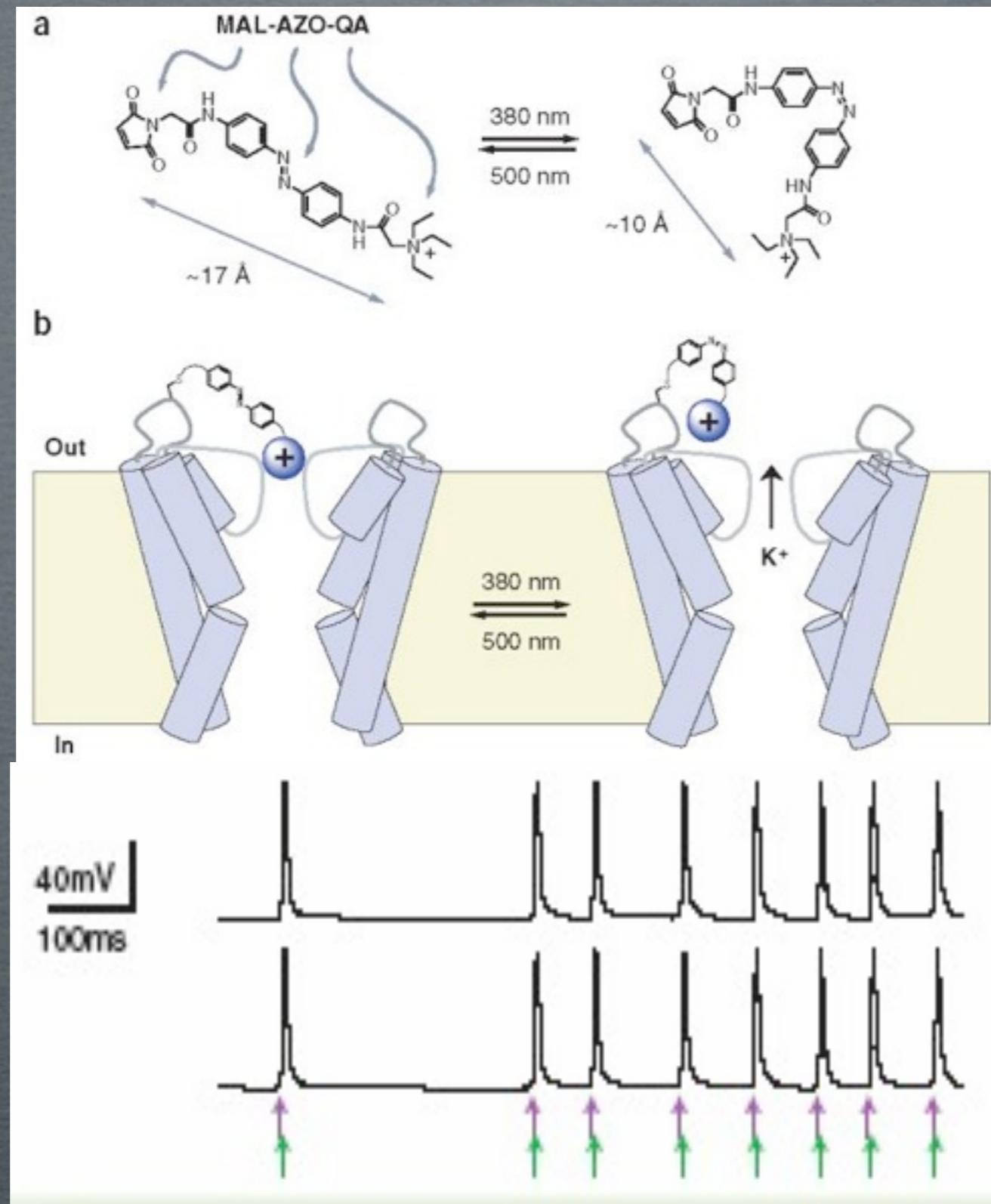


Cofilin promotes actin polymerization and defines the direction of cell motility.
Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS, Condeelis JS.
Science. 2004 Apr 30;304(5671):743-6.

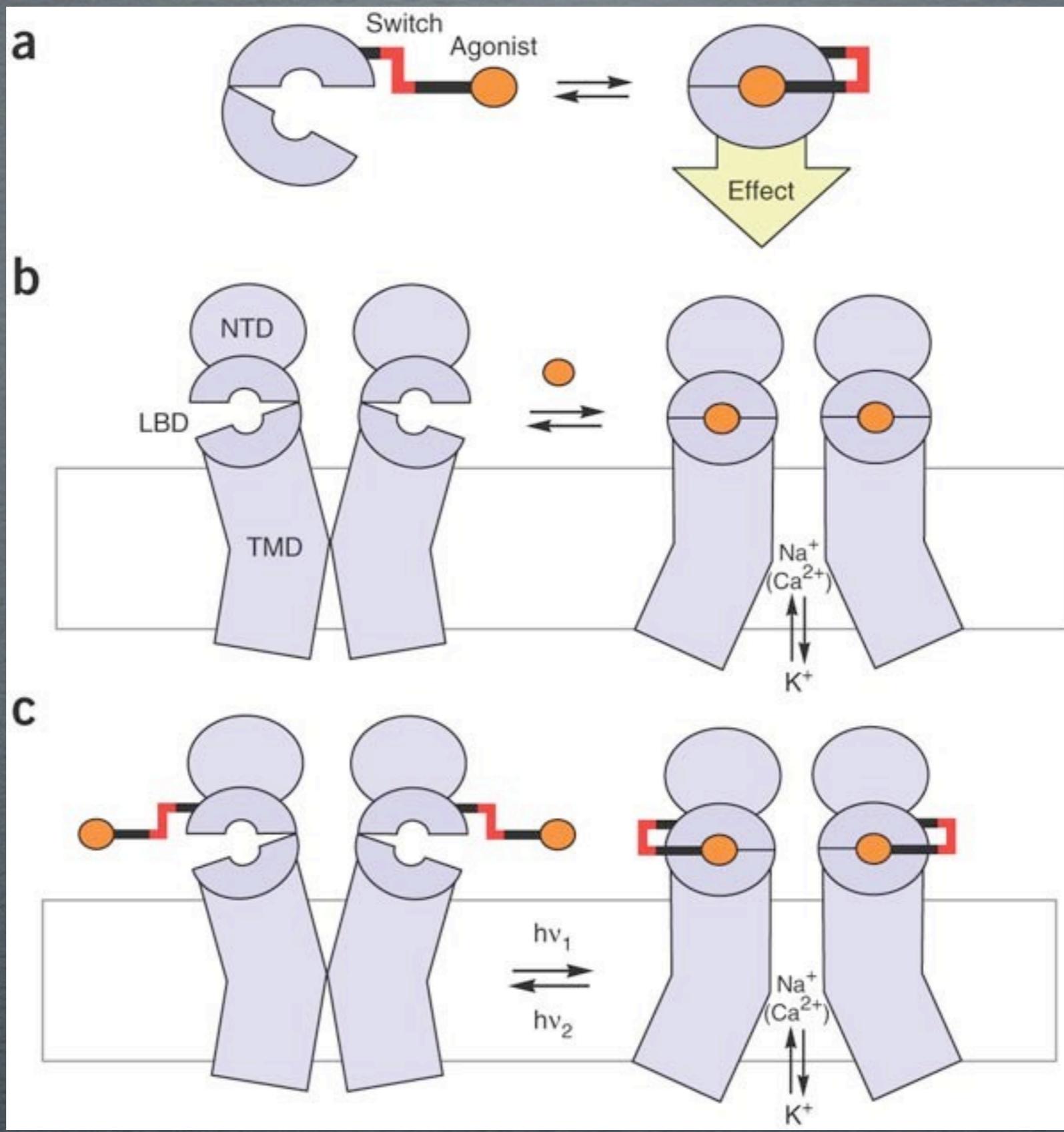
How to visualize the spatial/temporal dynamics of uncaging?



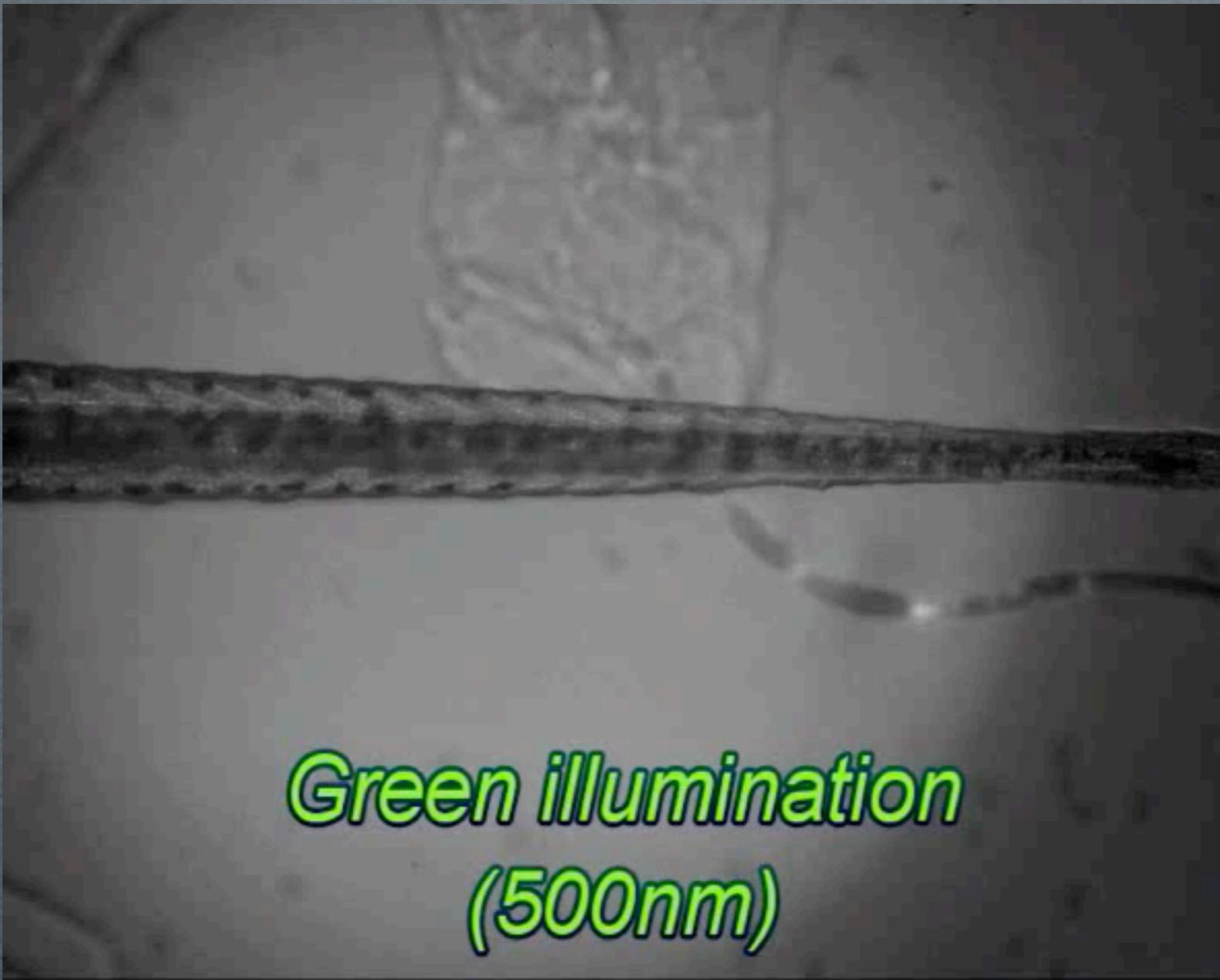
2. Reversible light control via photoisomerization



Reversible, engineering challenge, not genetically encoded, not cell permeable

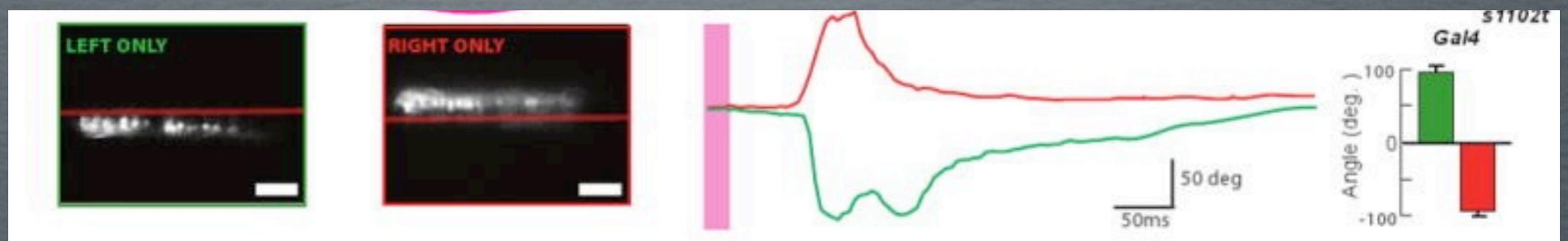


Cell-specific expression of azobenzene-regulated glutamate receptor can be used to dissect neuronal circuits

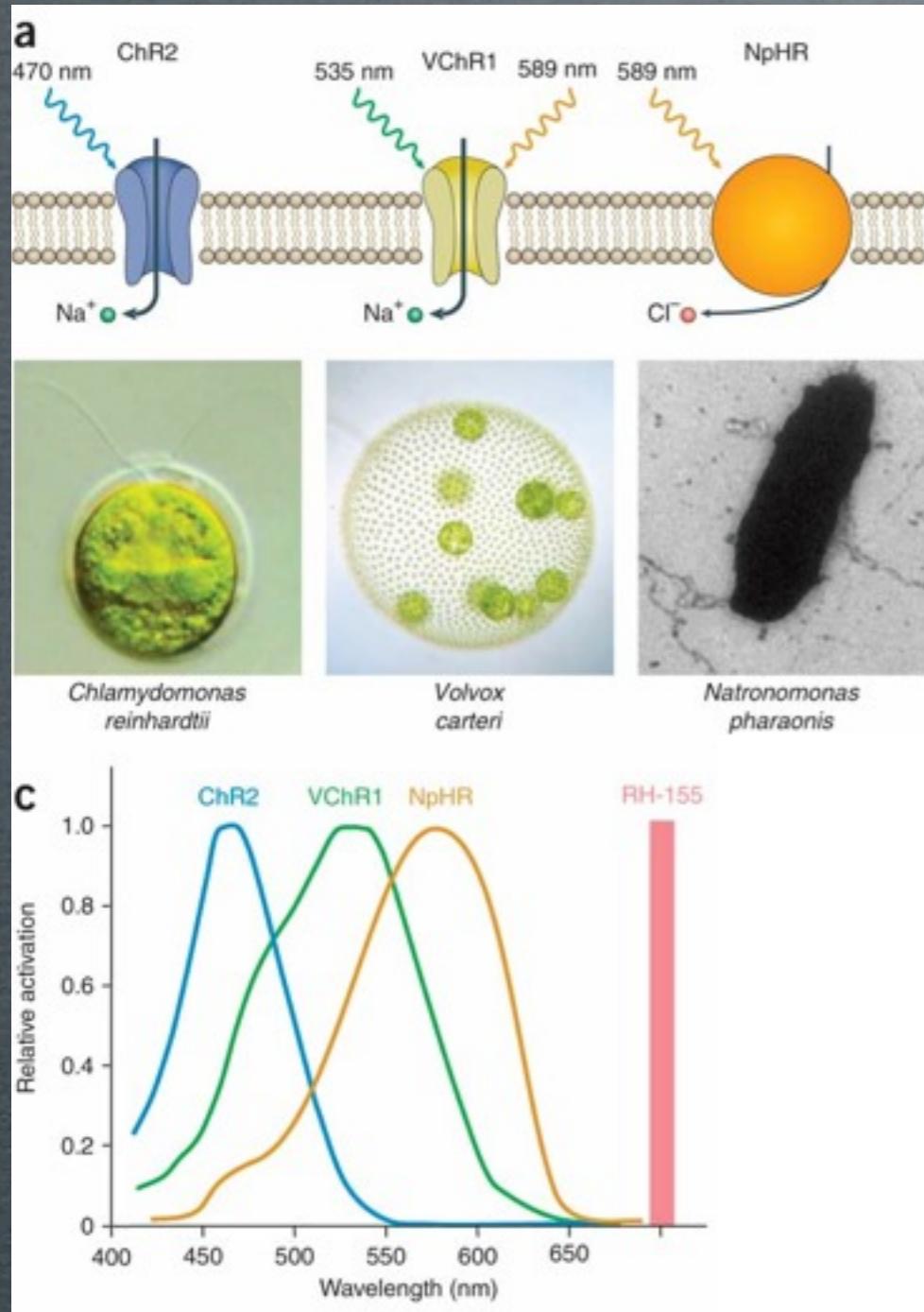


Isacoff Lab, PMID: 19759620

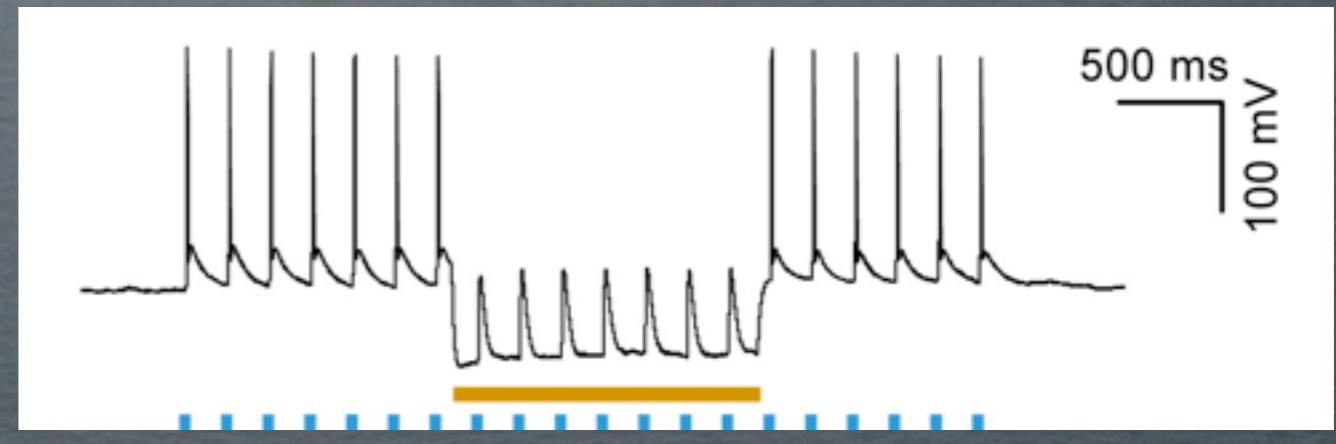
Can use light for spatial and temporal control within subset of cells expressing light-gated protein



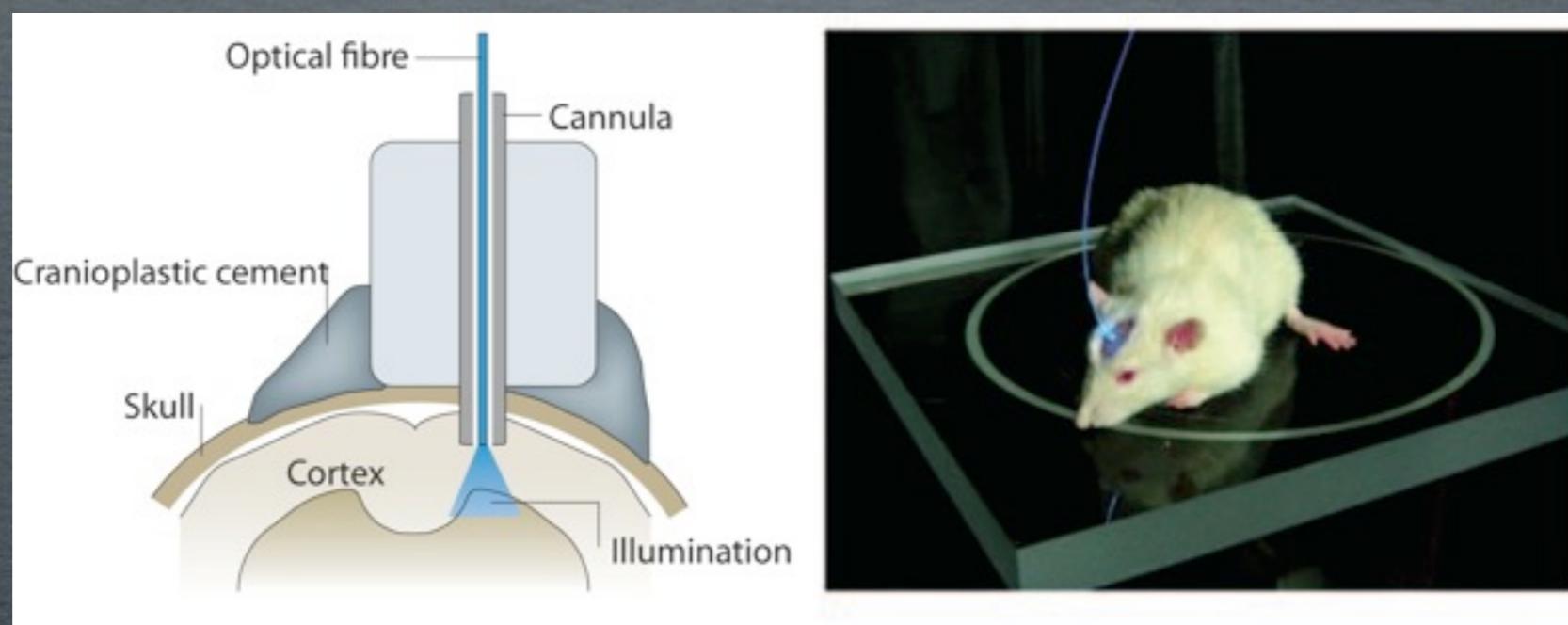
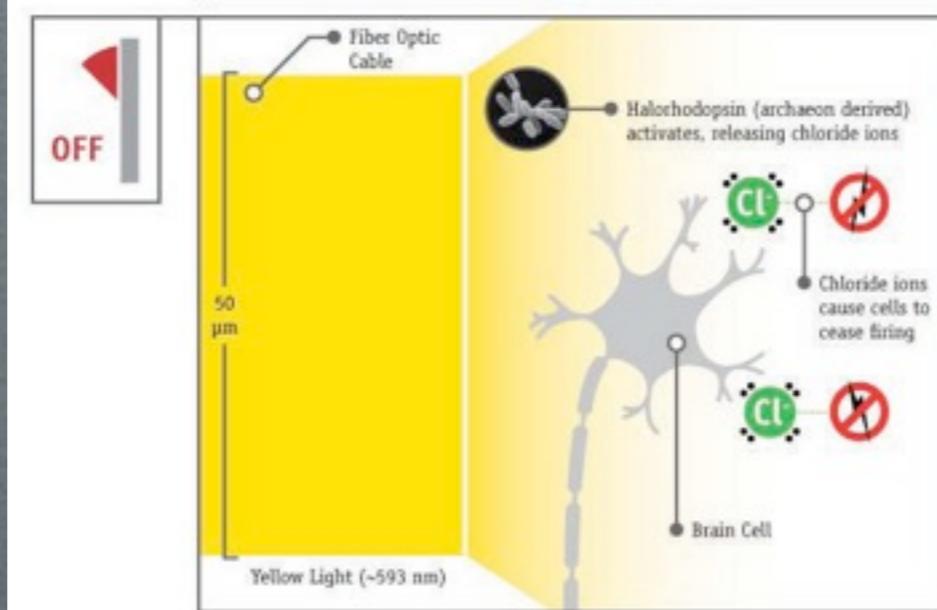
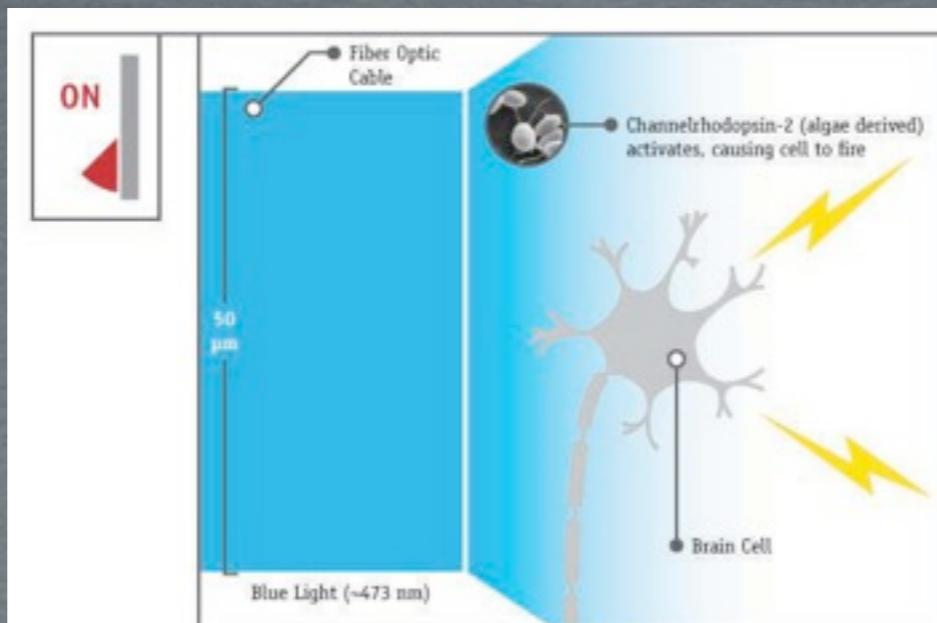
3. Borrowing naturally light responsive signaling molecules using their normal signaling function (primarily ion fluxes)



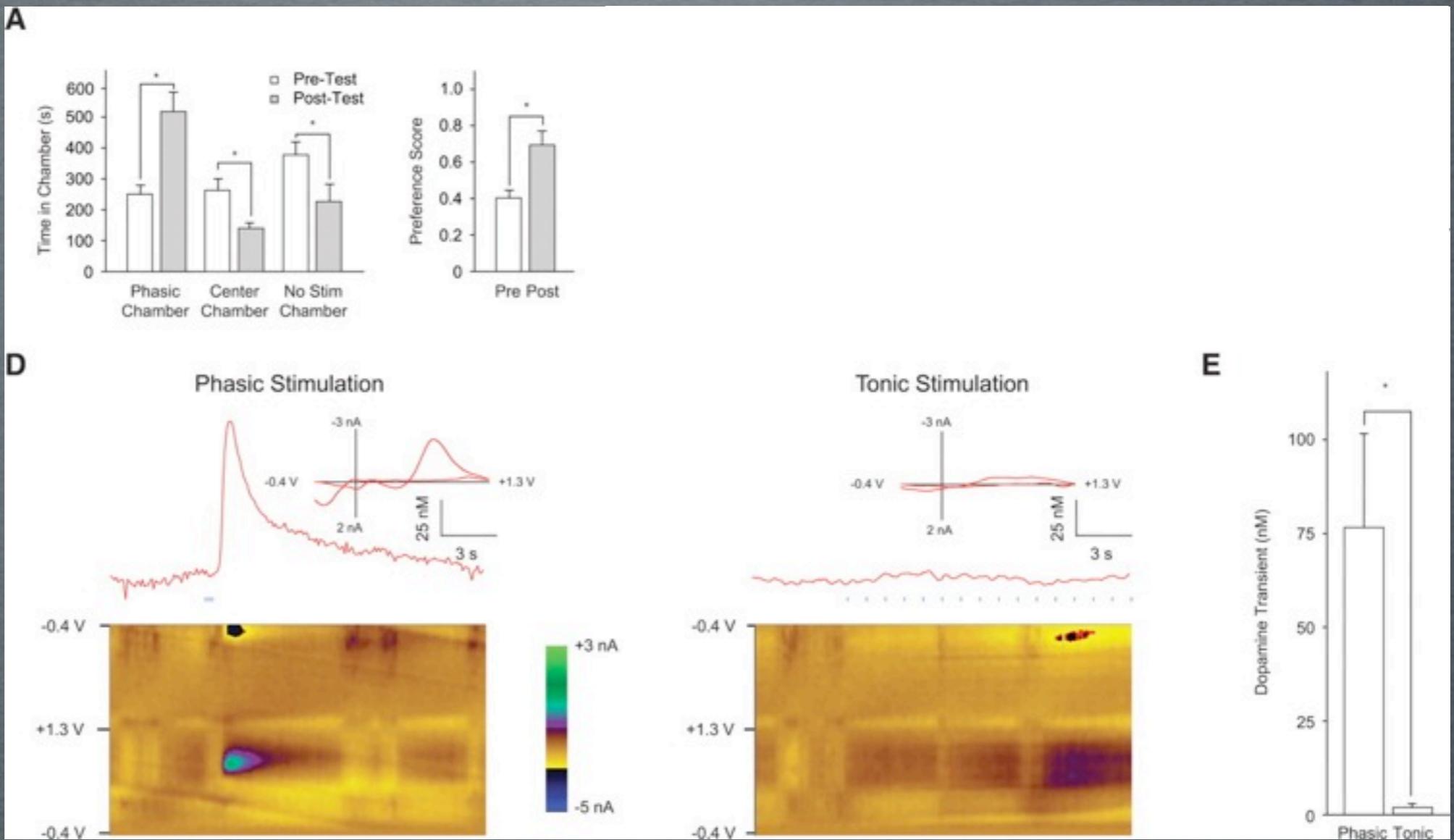
Zhang, et al: <http://www.nature.com/nature/journal/v446/n7136/abs/nature05744.html>



Molecular and cellular approaches for diversifying and extending optogenetics.
Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K.
Cell. 2010 Apr 2;141(1):154-65. Epub 2010 Mar 18.



Controlling animal behavior with optogenetics

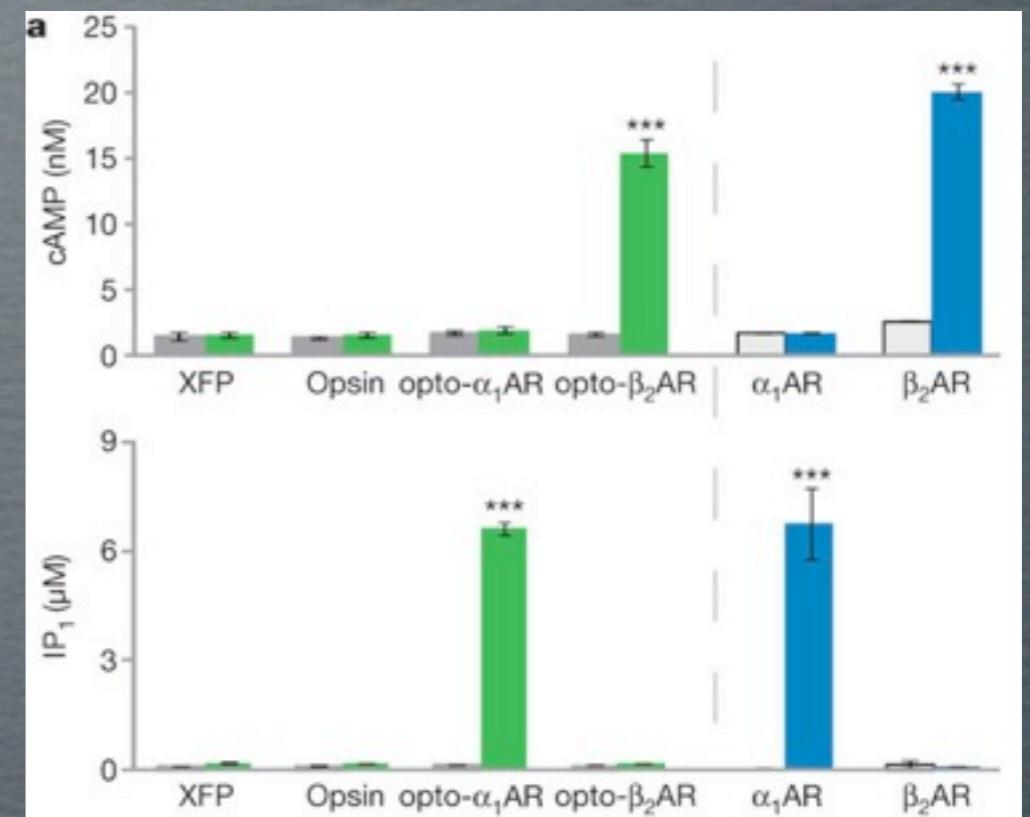
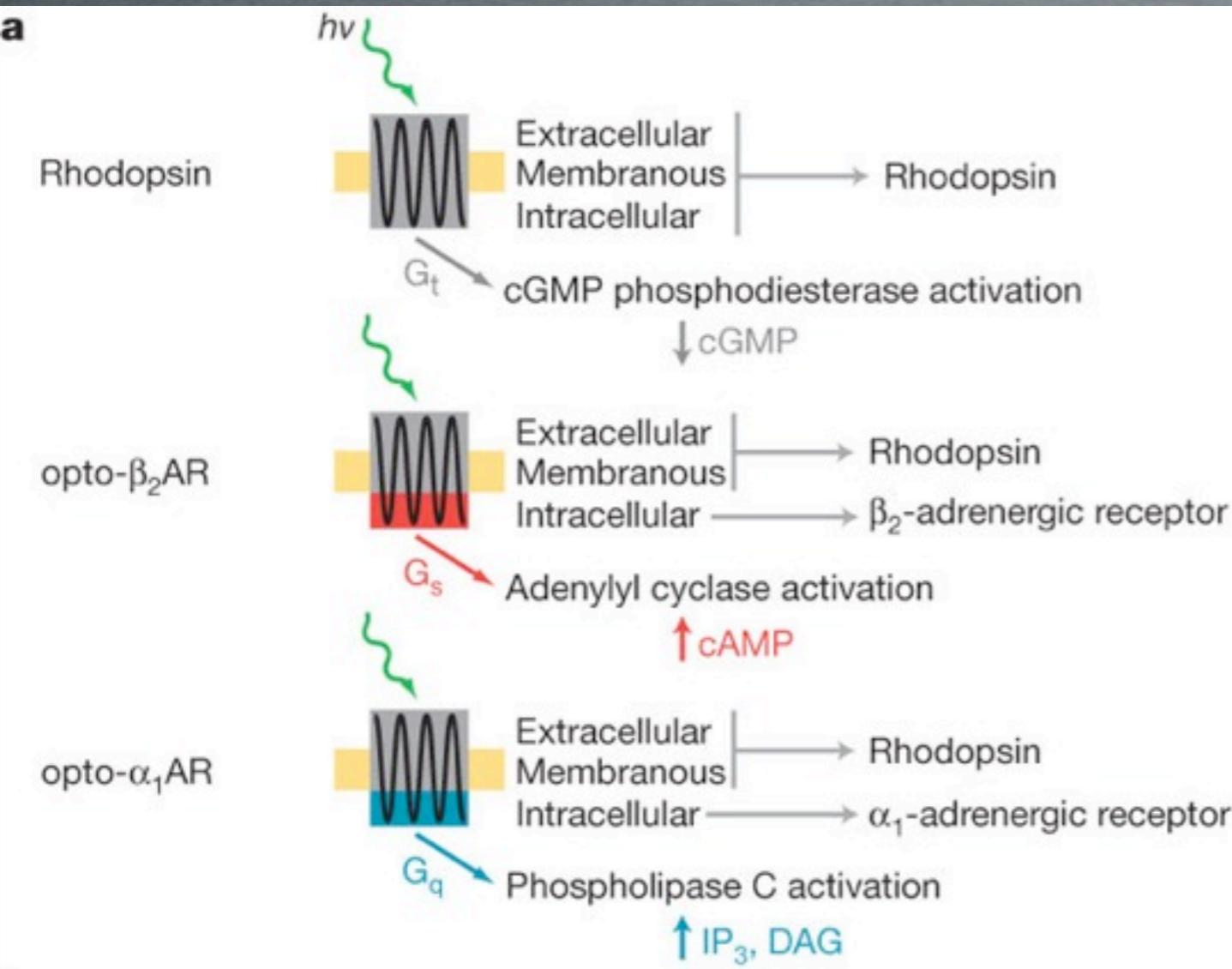


Science. 2009 May 22;324(5930):1080-4. Epub 2009 Apr 23.

Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning.

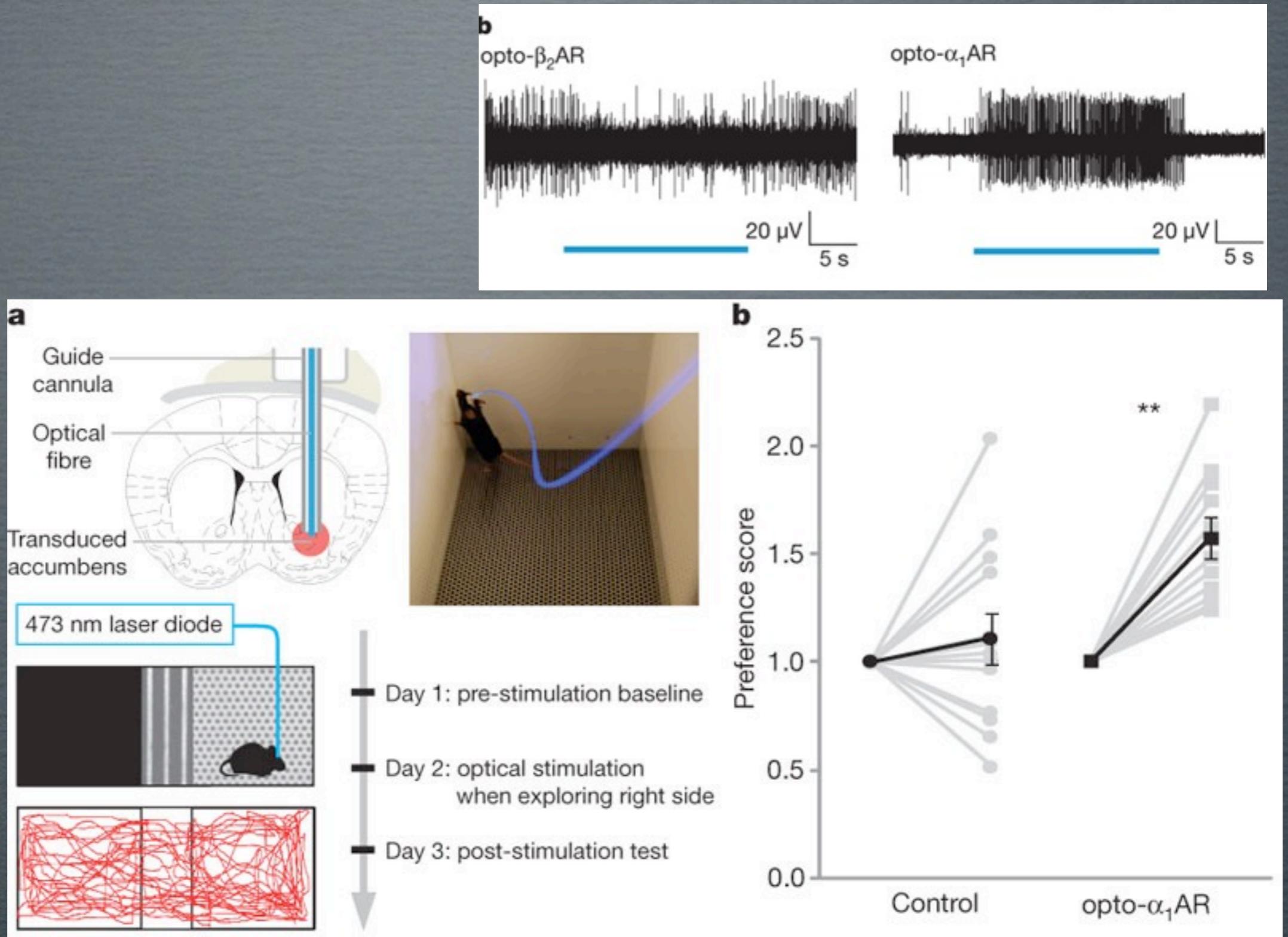
Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K.

4a. Adapting light-responsive proteins to new functions-- GPCRs

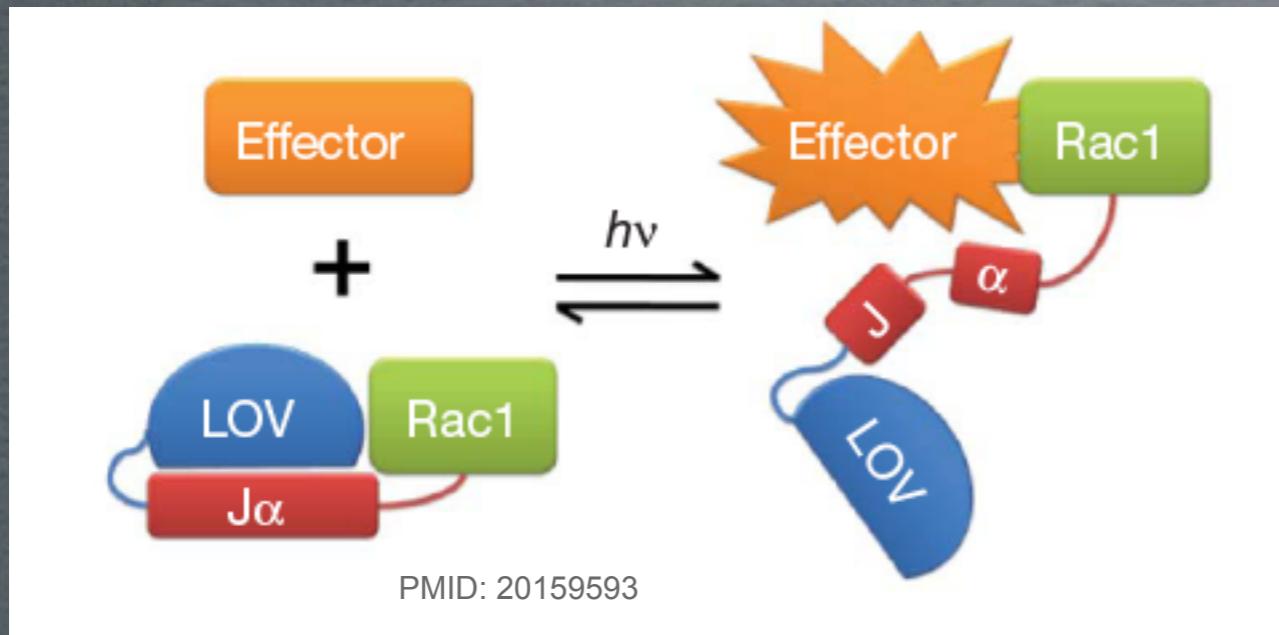


Deisseroth Lab-- OptoXRs

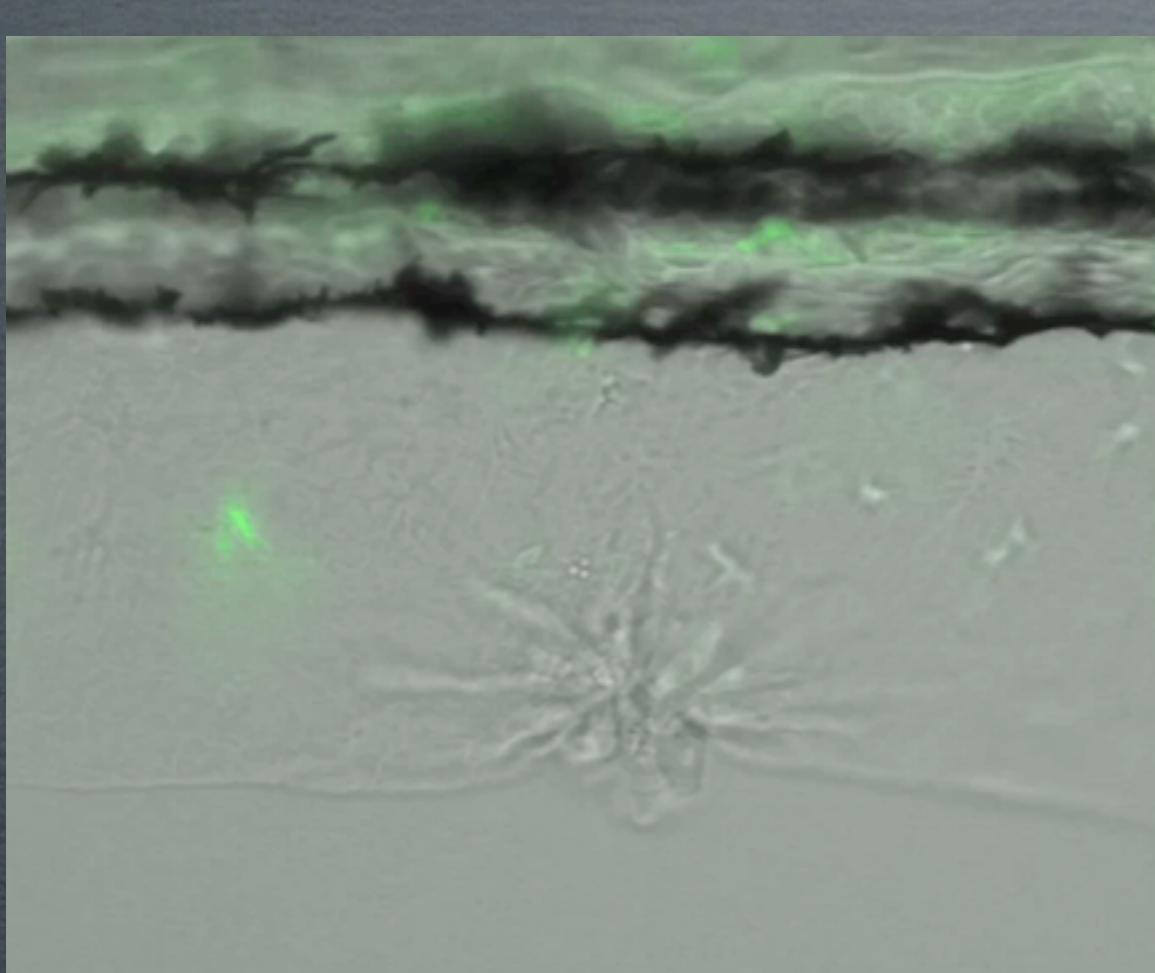
Can drive positional learning with these optogenetic tools



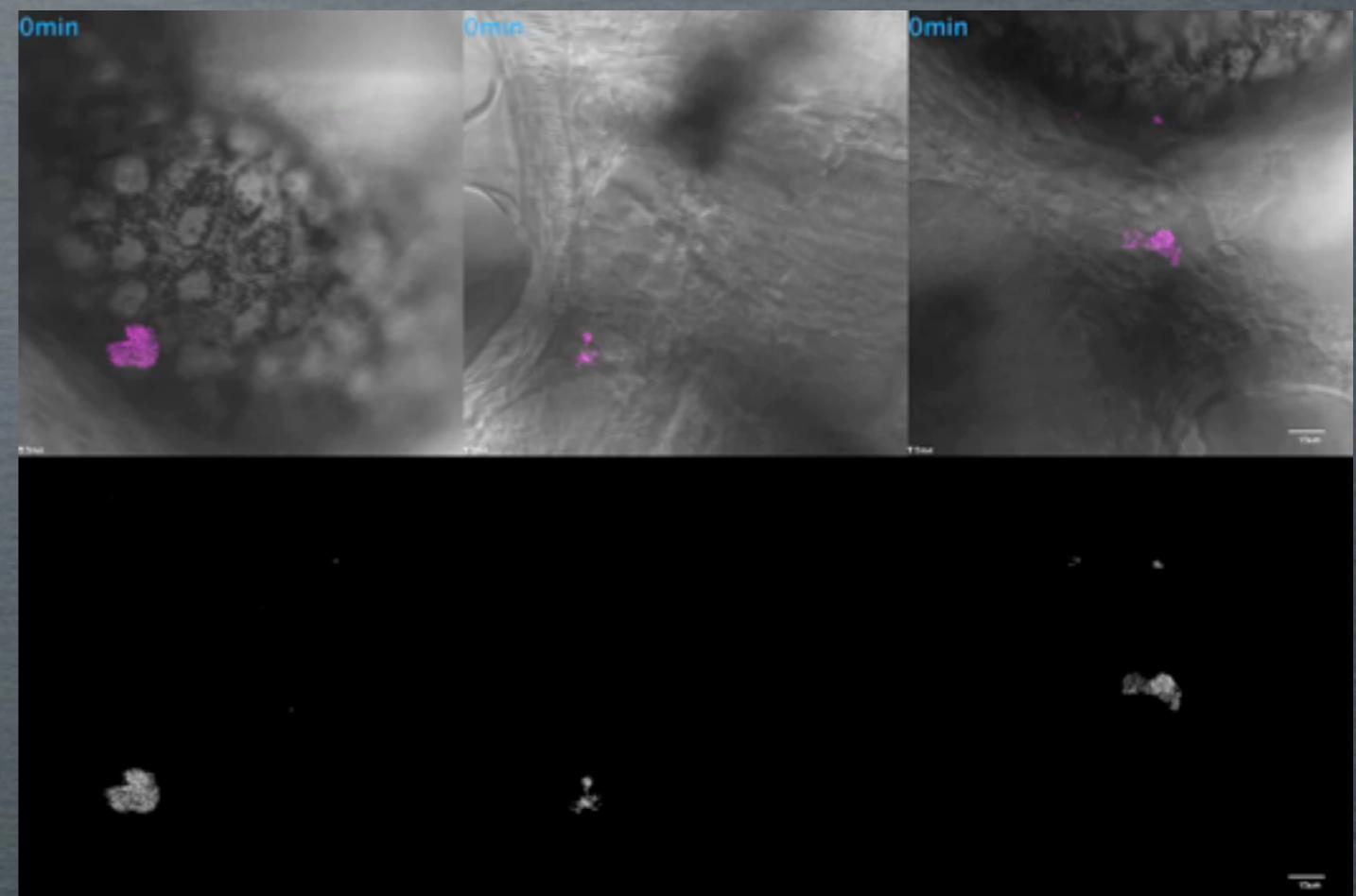
4b. Adapting light responsive proteins for allosteric control- LOV



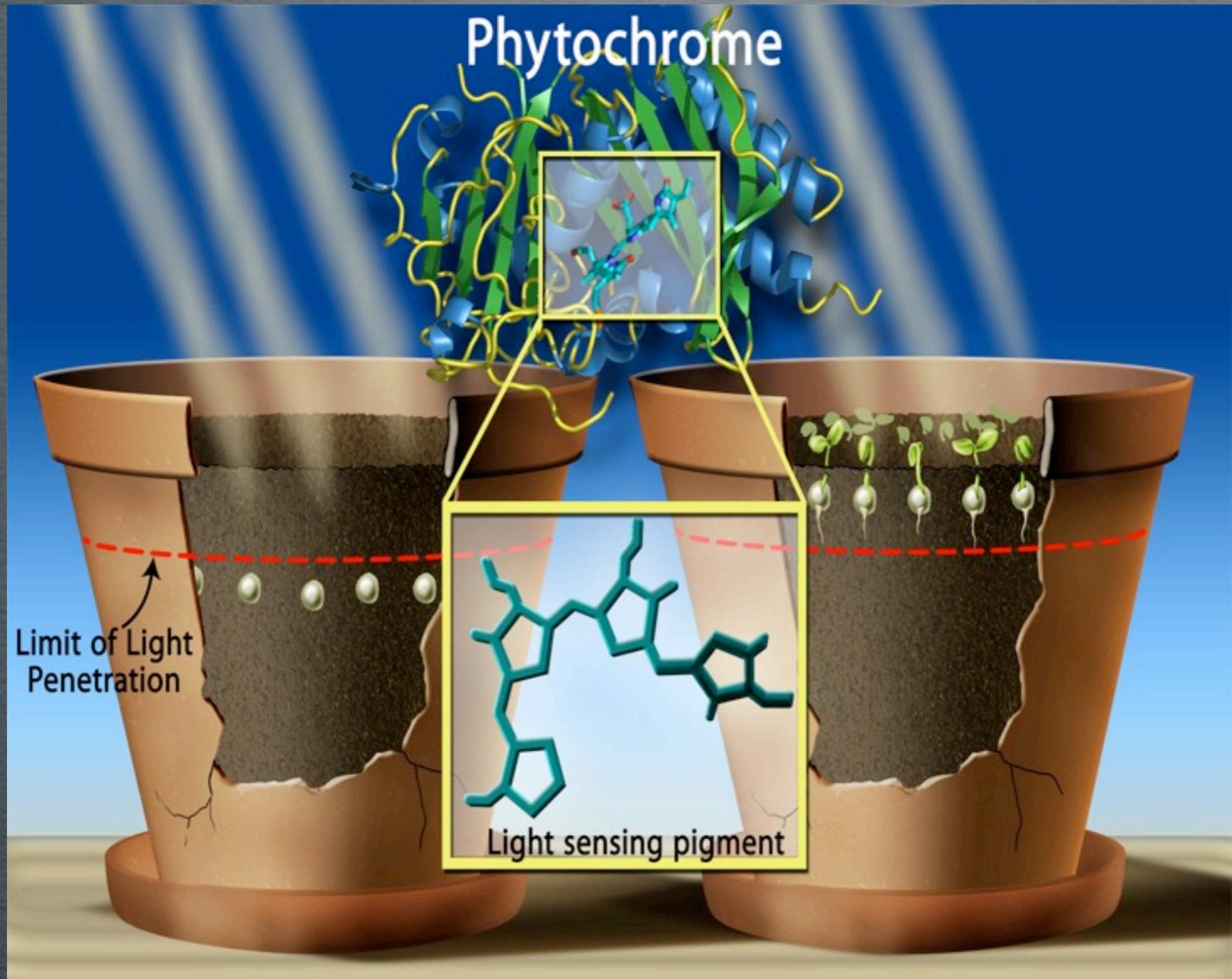
Allosteric control of normally non-light responsive protein
+ fully genetically encoded
*engineering challenge
*light only drives ON state
*can't directly visualize switch



PMID: 19693014



4c. Light-gated protein dimerization module from plants-- Phy/PIF



PMID: 19749742



Anselm Levskaya

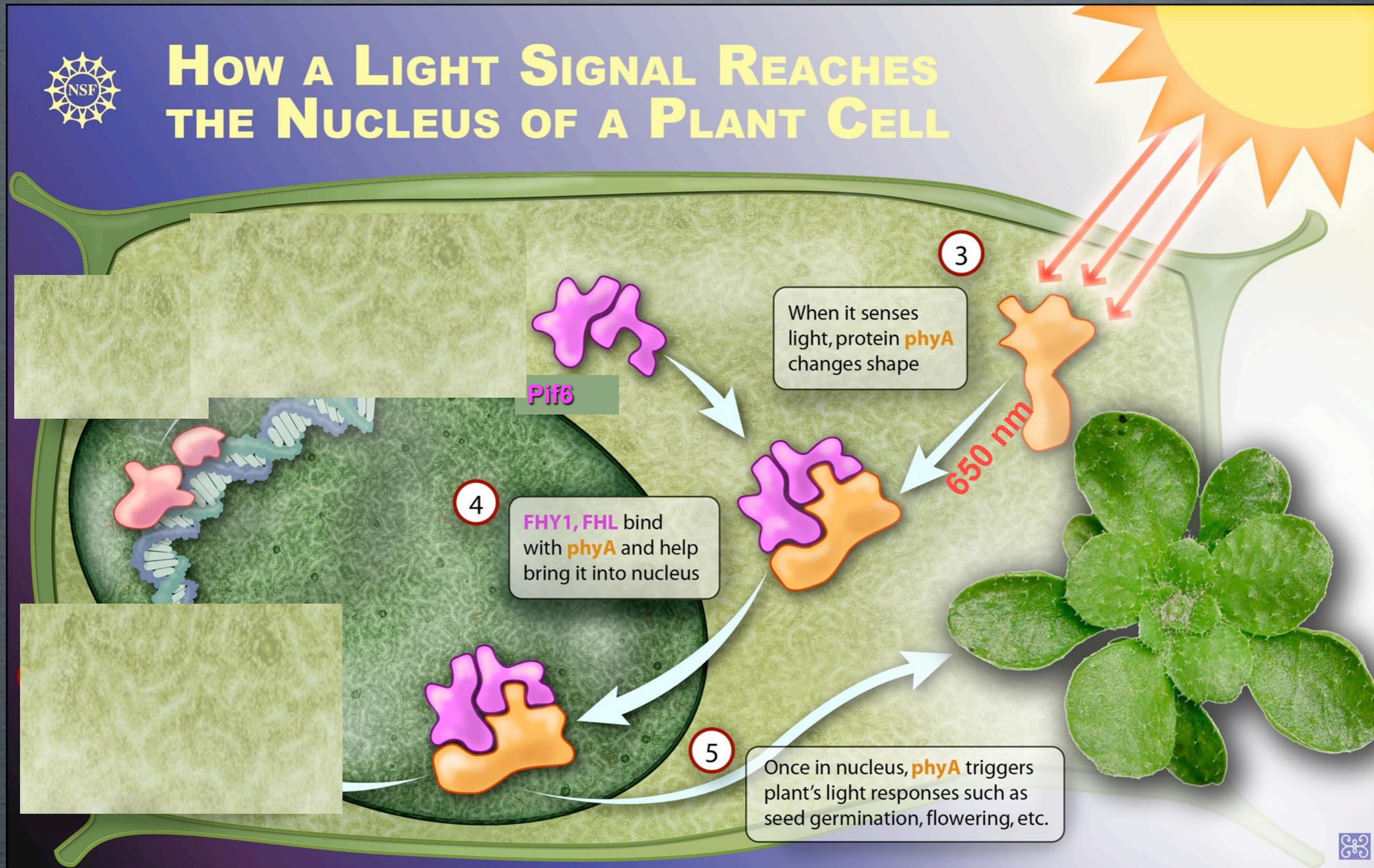


Chris Voigt



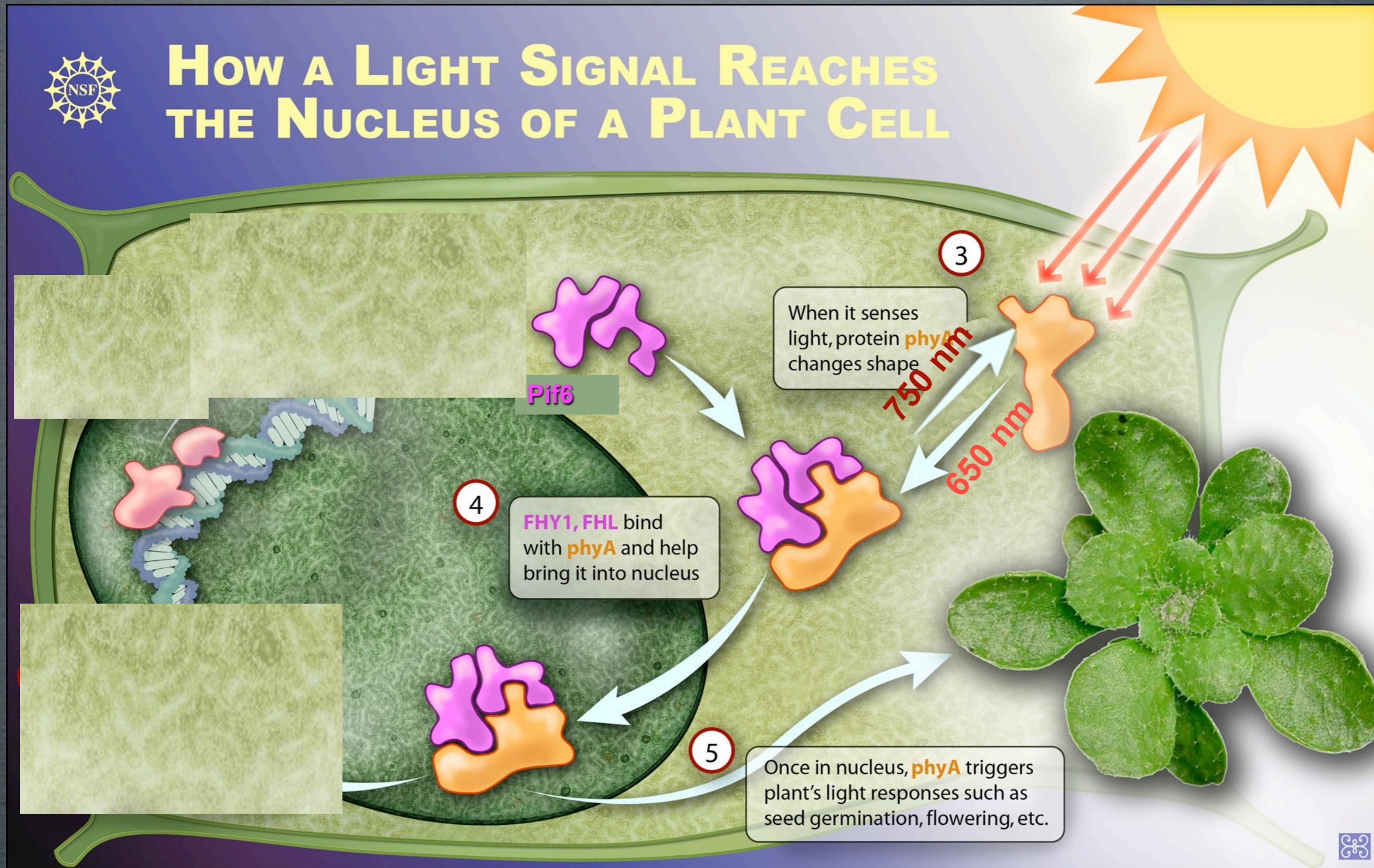
Wendell Lim

Borrowing a light-regulated protein interaction module from plants



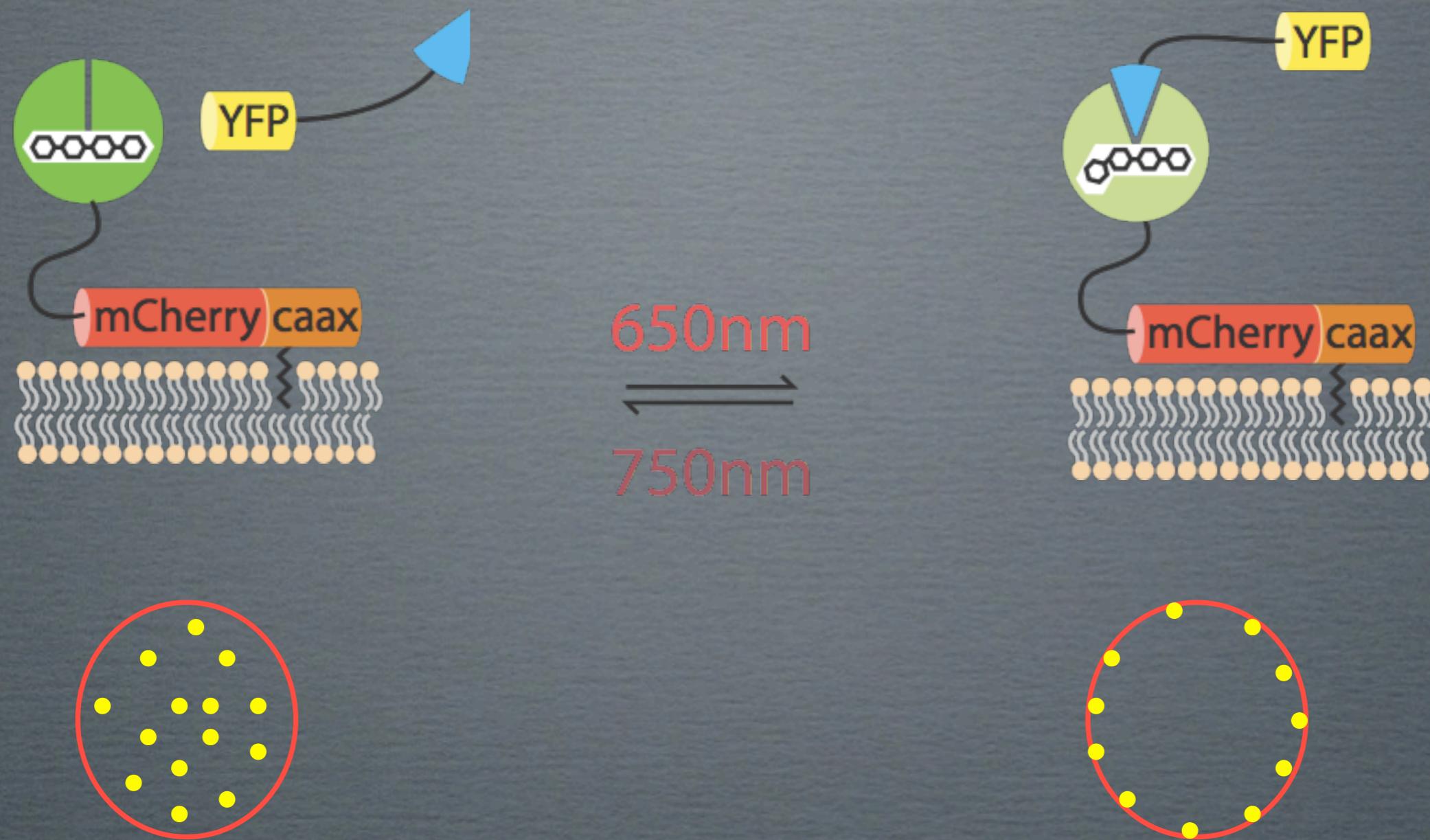
Peter Quail Lab, Lagarias, others

Borrowing a light-regulated protein interaction module from plants



Peter Quail Lab, Lagarias, others

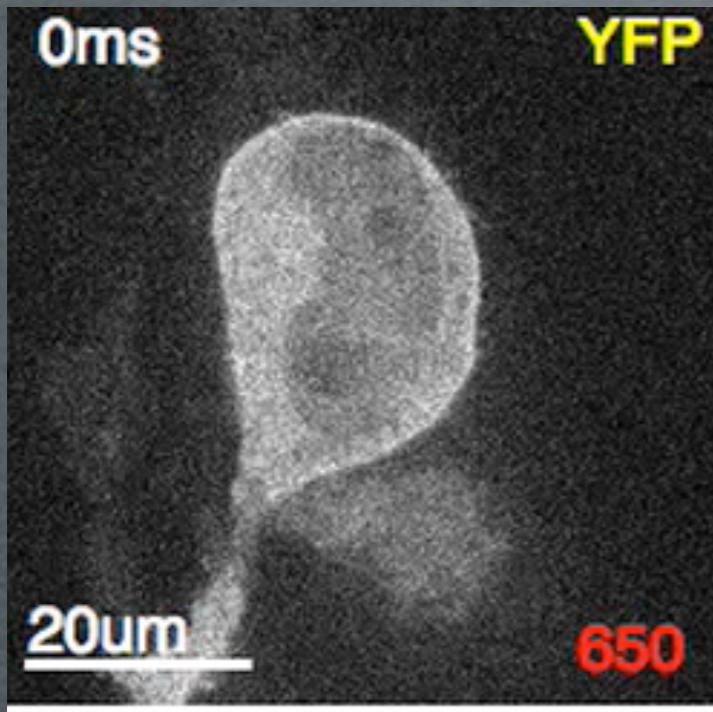
Building a light-gated membrane translocation system



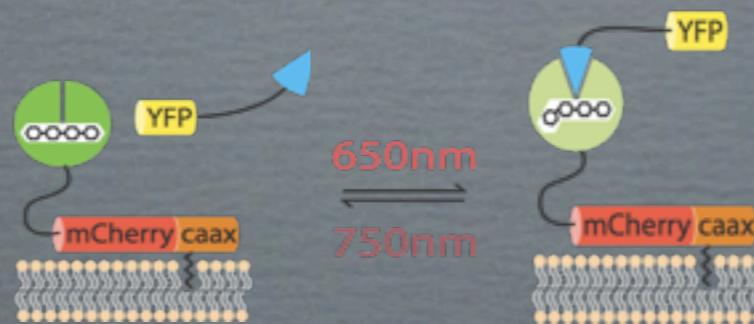
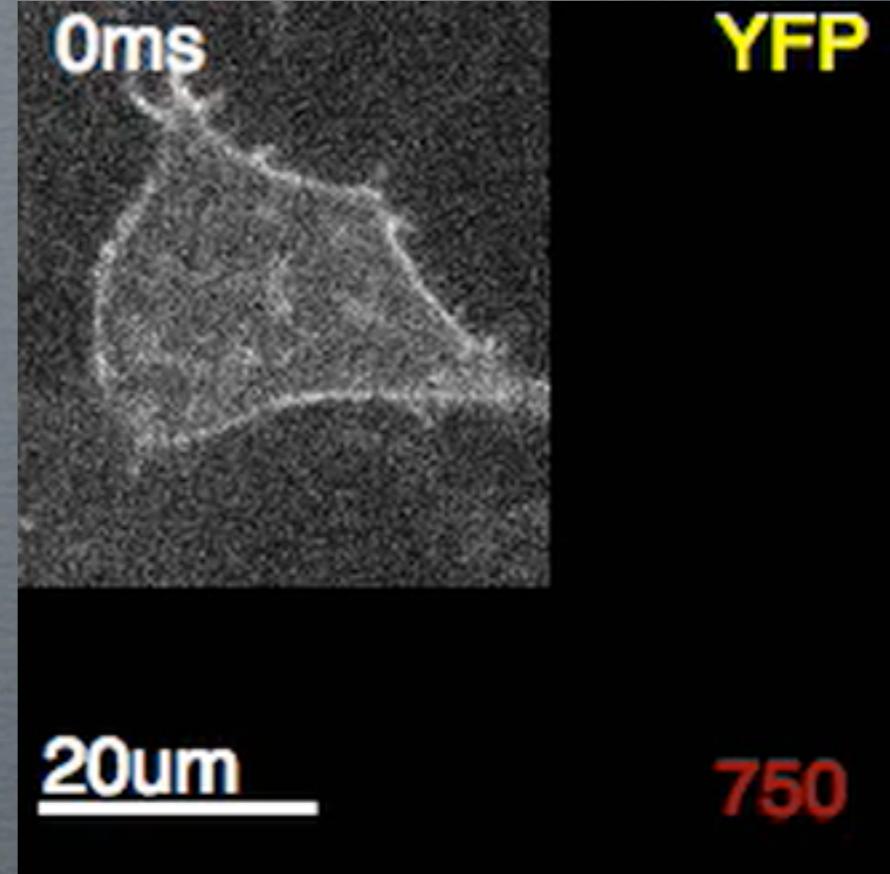
- + Genetically encoded
- + Can drive on and off with light
- + Highly modular, generalizable
- Requires exogenous chromophore

Light can direct proteins on and off the membrane in secs.

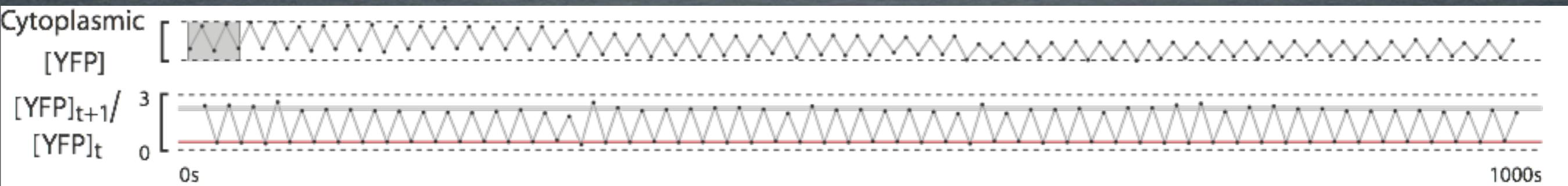
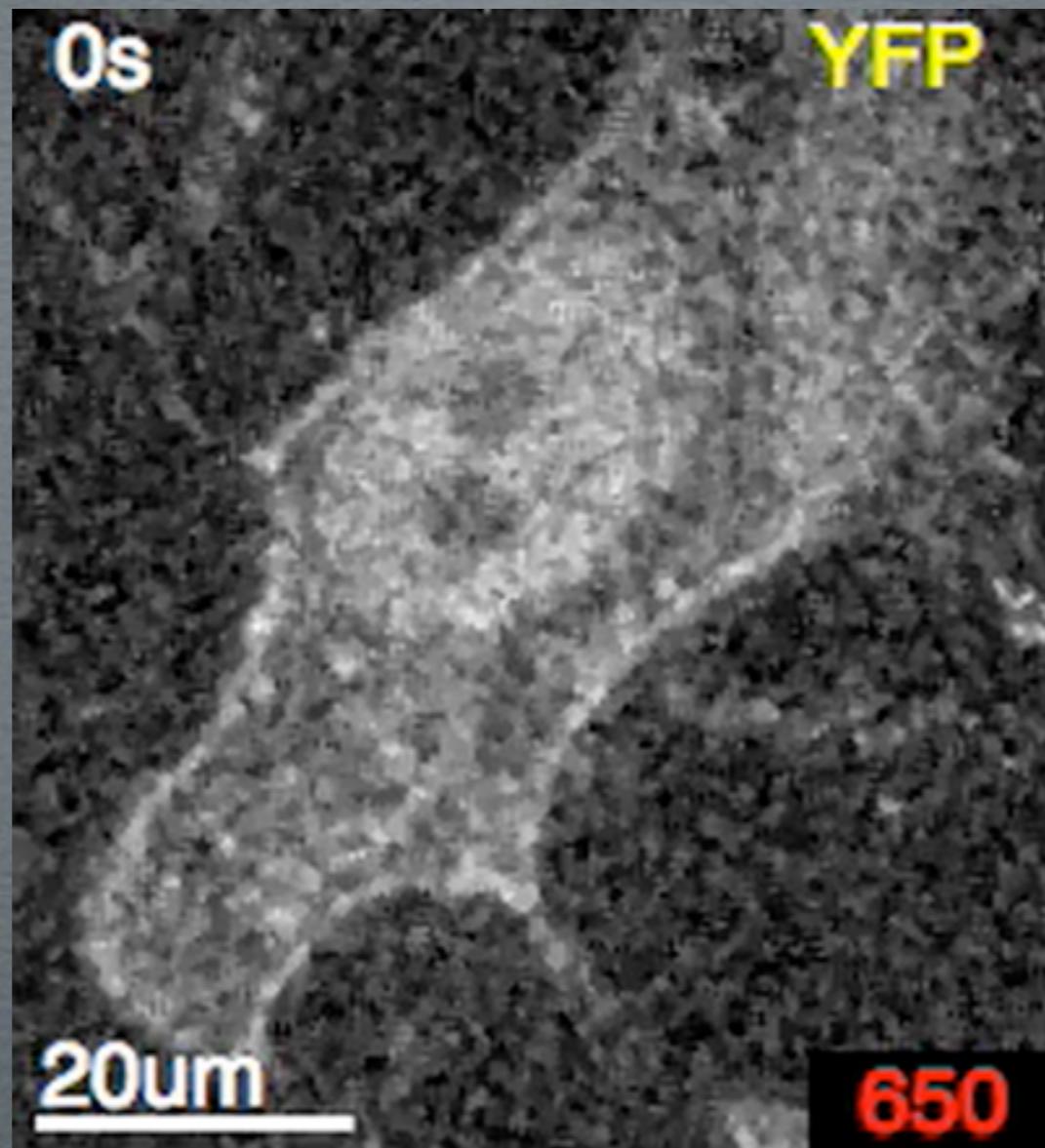
OFF to ON



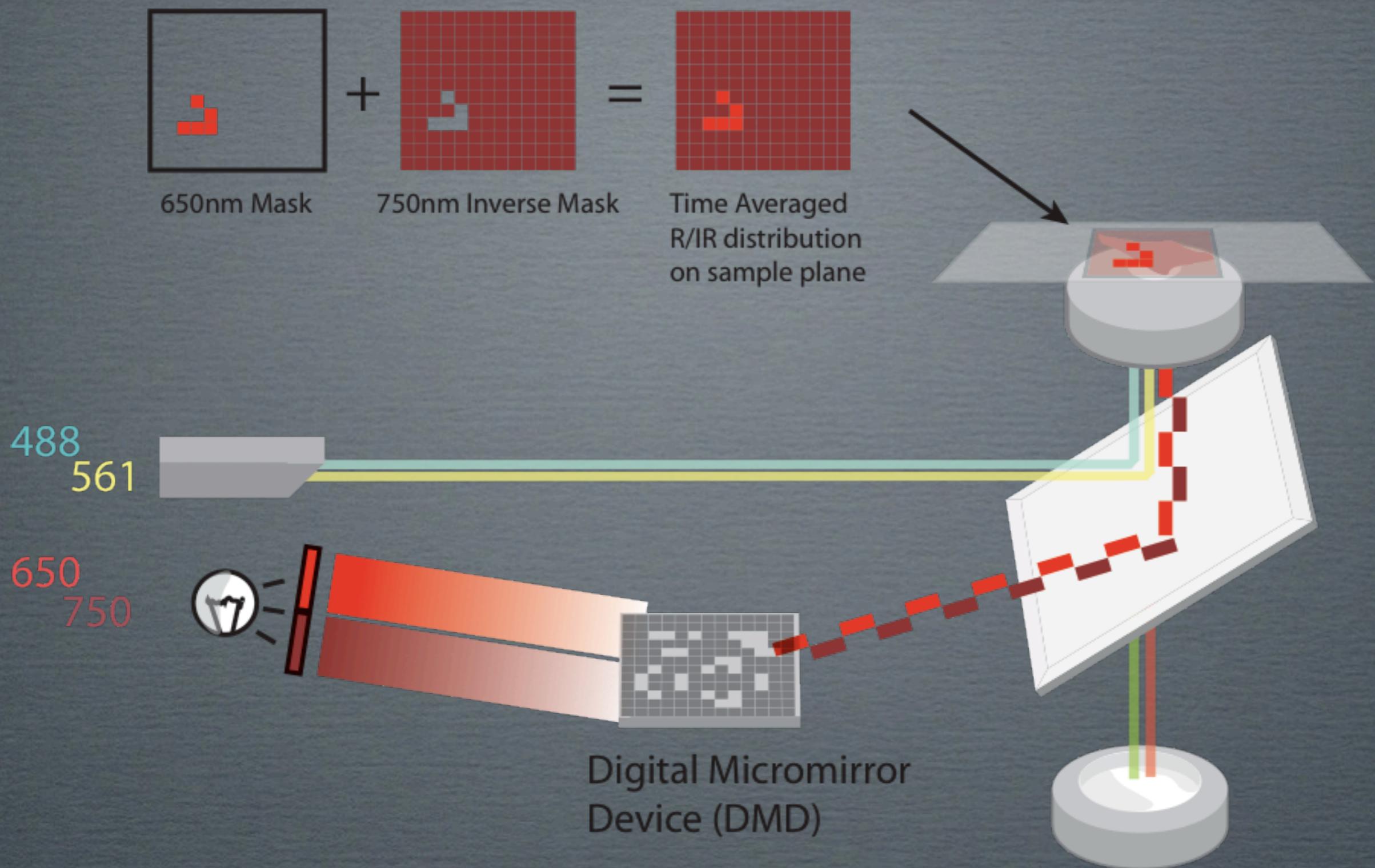
ON to OFF



The interaction is robust

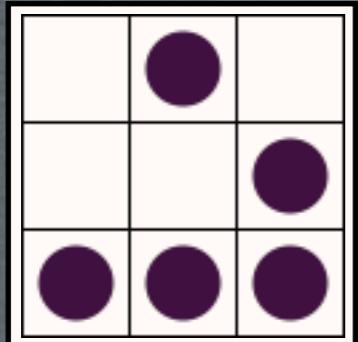


Mosaic DMD for more complex light patterning



Uses the same mirror-array chips as digital projectors to project an SVGA image onto the sample plane

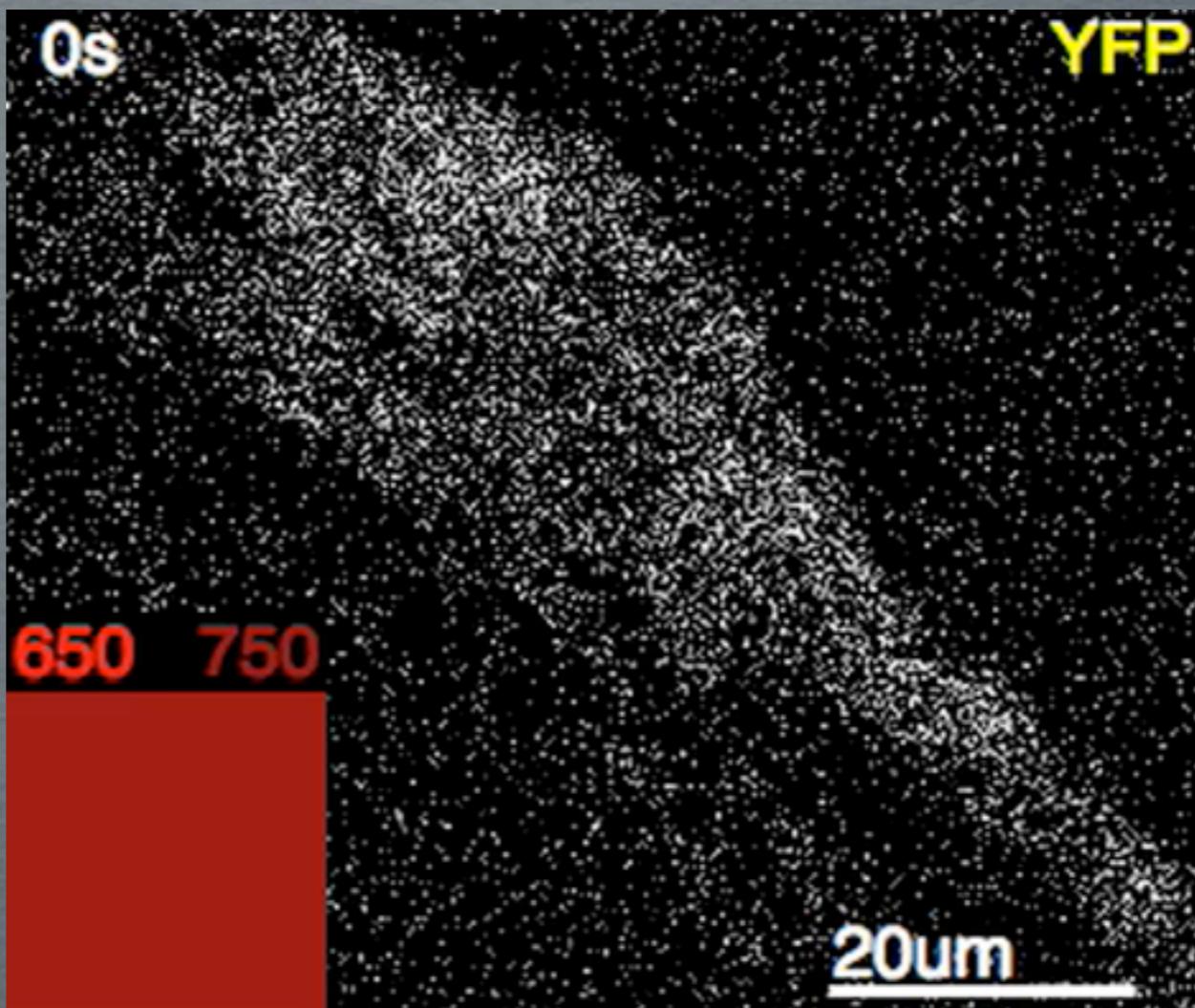
Light gives micron-level control of protein localization



Glider pattern from
Conway's
“Game of Life”

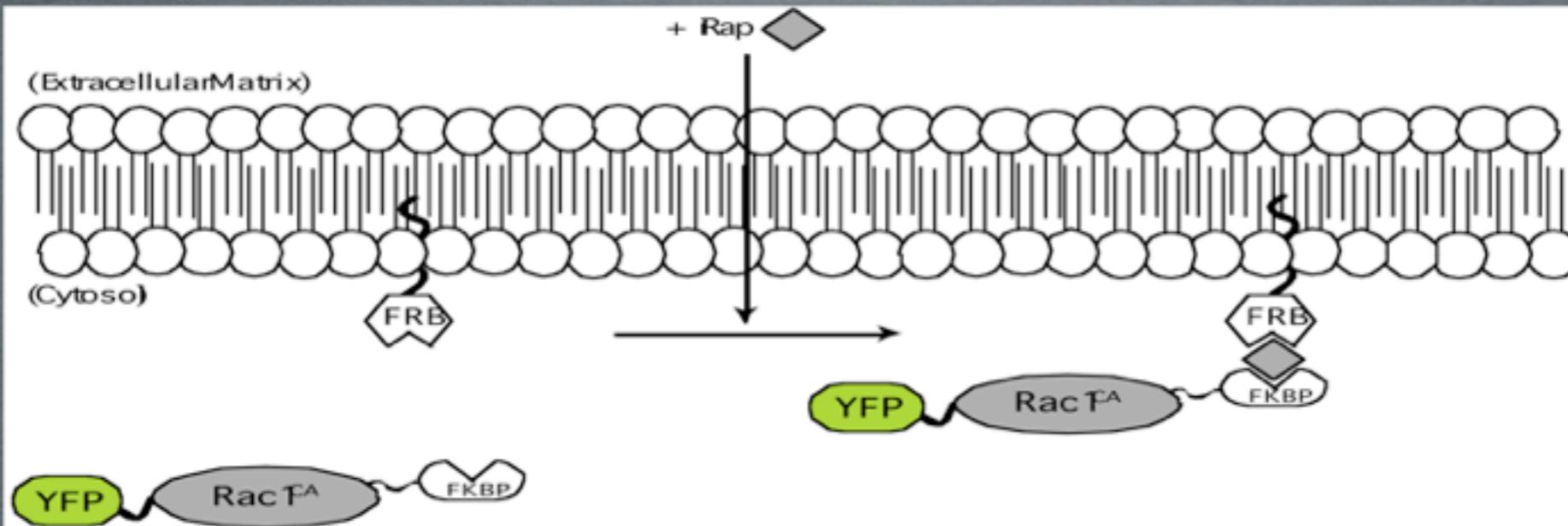
GFP APB_{piF6}

PhyB₉₀₈ mCherry K

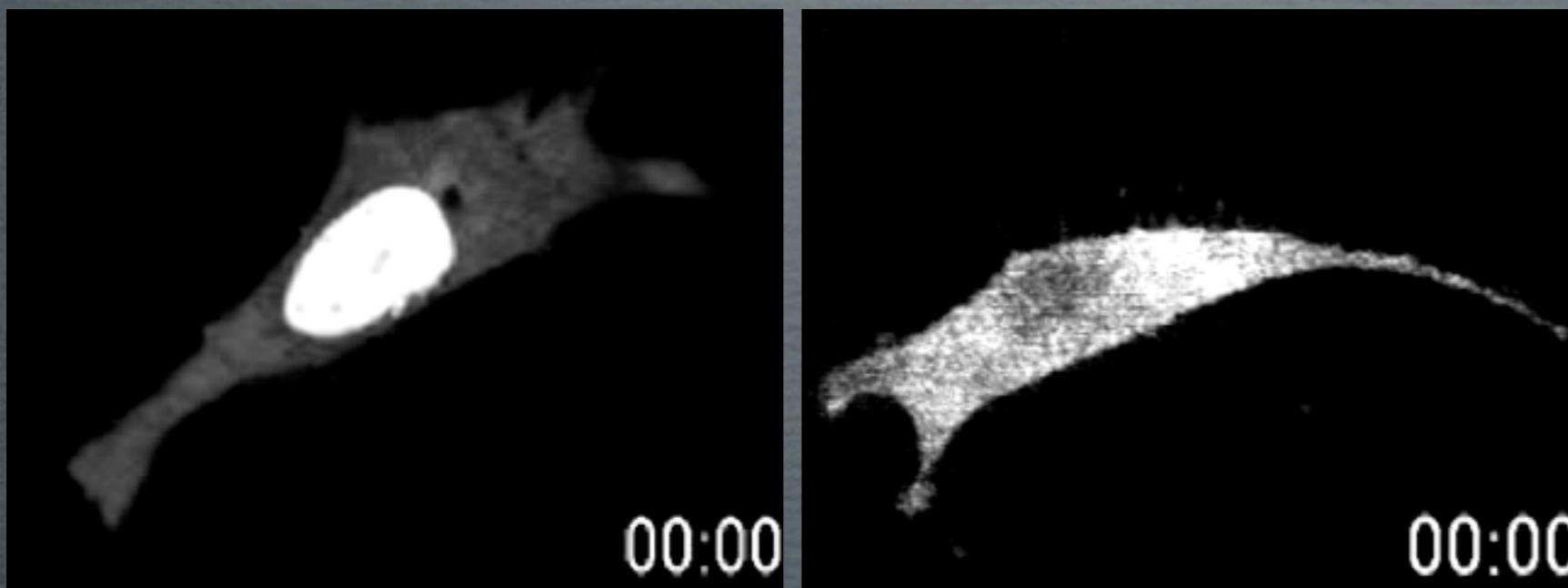


Signaling & Morphology Control

Can control signaling by controlling localization



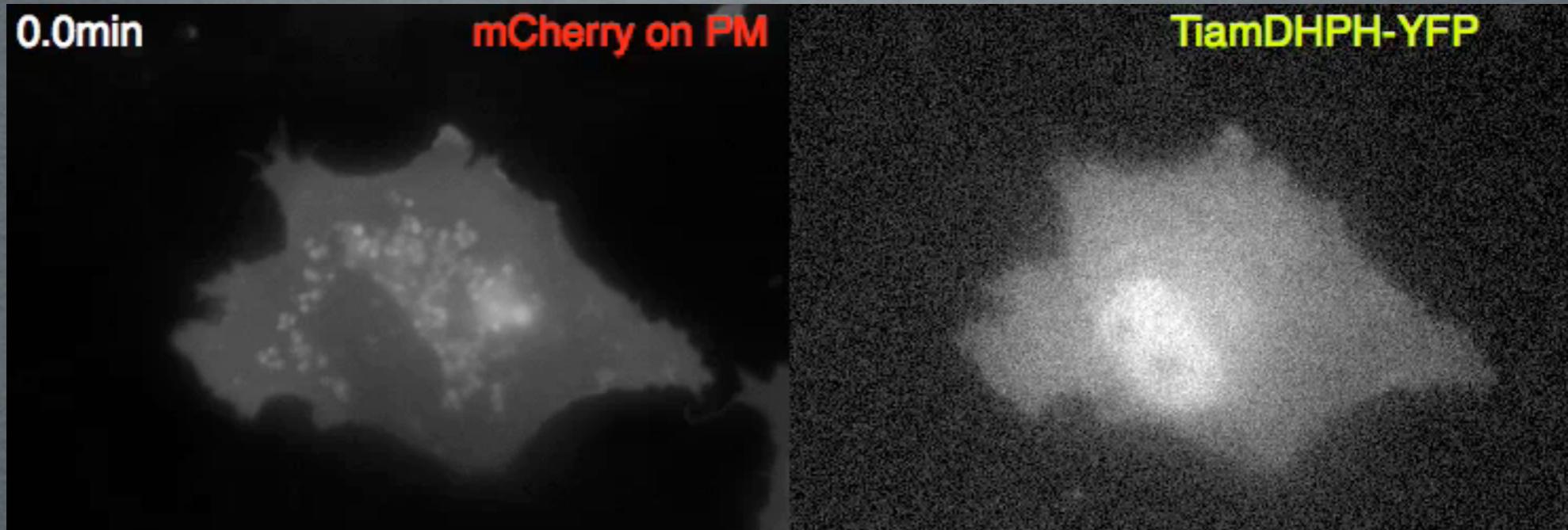
Chemical dimerizer
(rapamycin)



Inoue and Meyer

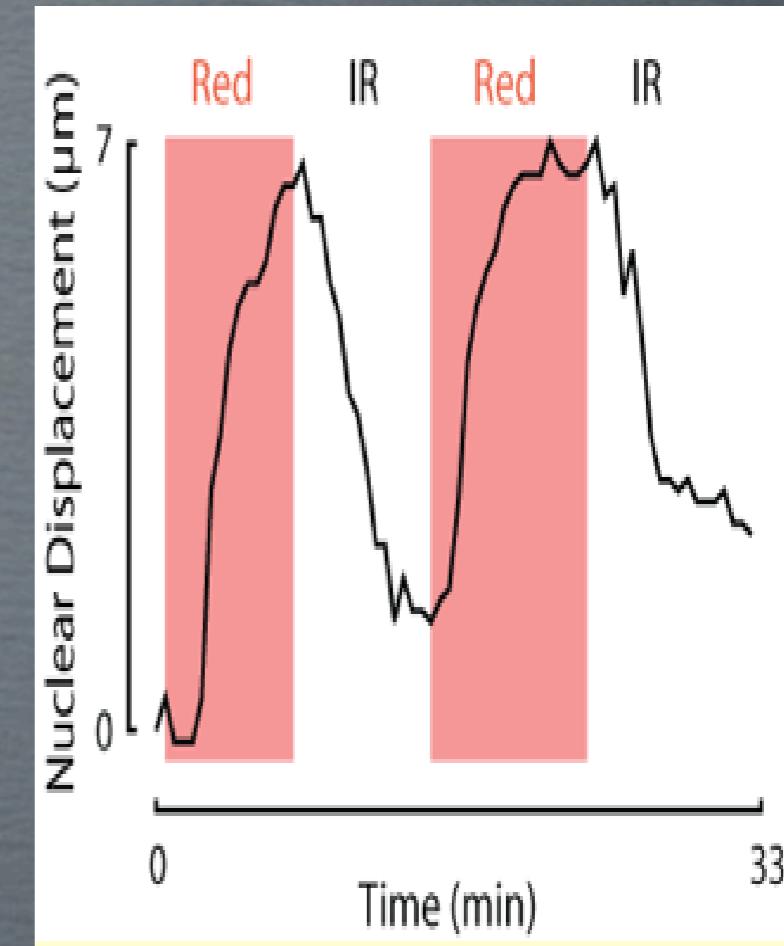
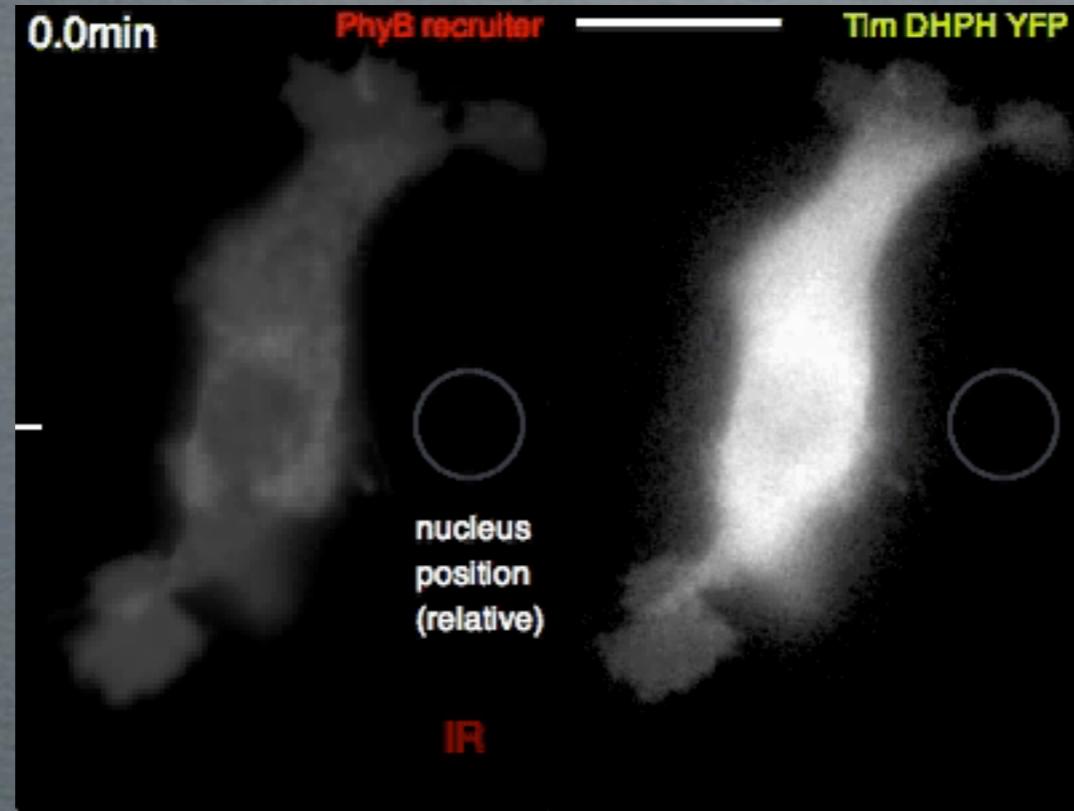
We reasoned that using an analogous approach with light system would give us greater spatial and temporal control of signaling pathways.

Can pattern dorsal ruffling by local Rac GEF recruitment



Tiam → RacI → WAVE → Arp2/3 → F-Actin

System is general-- also works for controlling contraction



Tim \longrightarrow RhoA \longrightarrow Rho Kinase $\longrightarrow \longrightarrow$ Myosin-P

Tools for *in vivo* biochemistry-- Greyscale control



Jared
Toettcher



Delquin
Gong



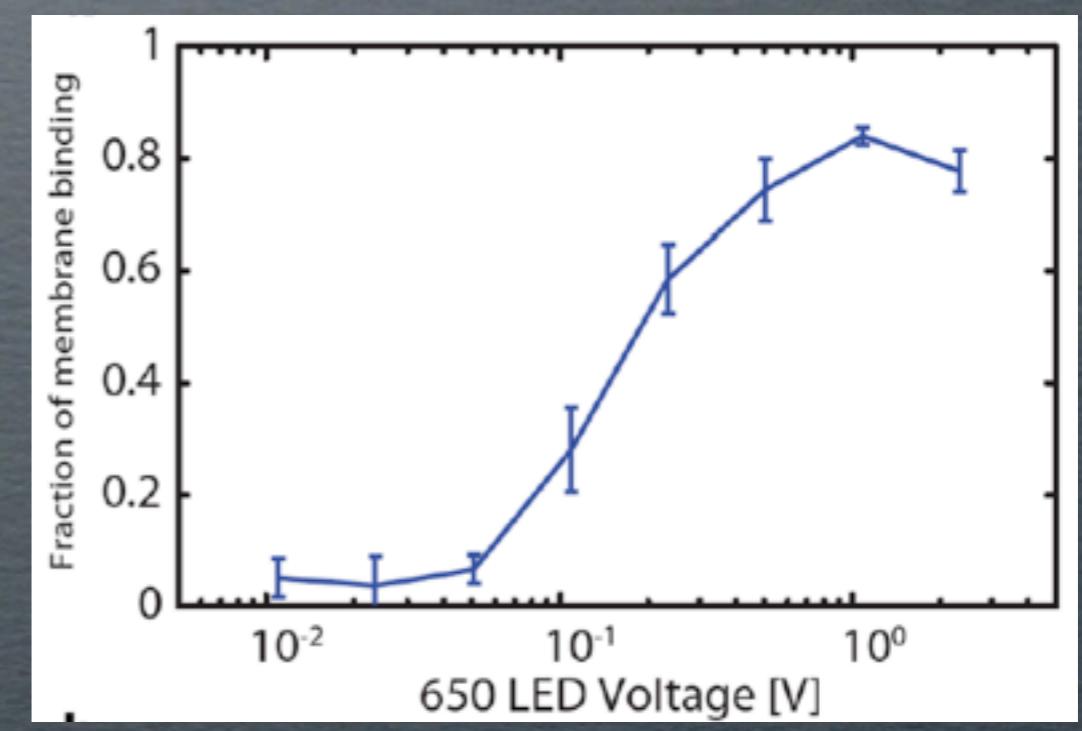
Ron Vale



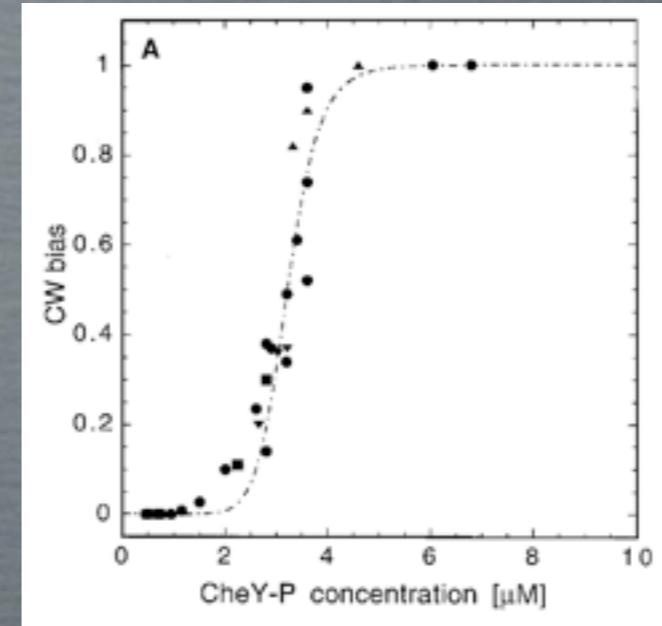
Nico
Stuurman



Arthur
Edelstein



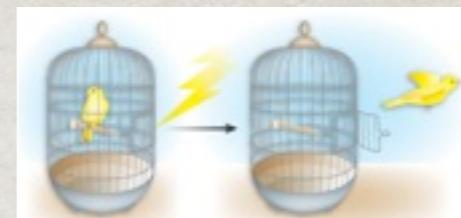
Tools for *in vivo* biochemistry



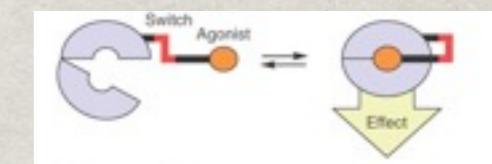
Leibler

SUMMARY

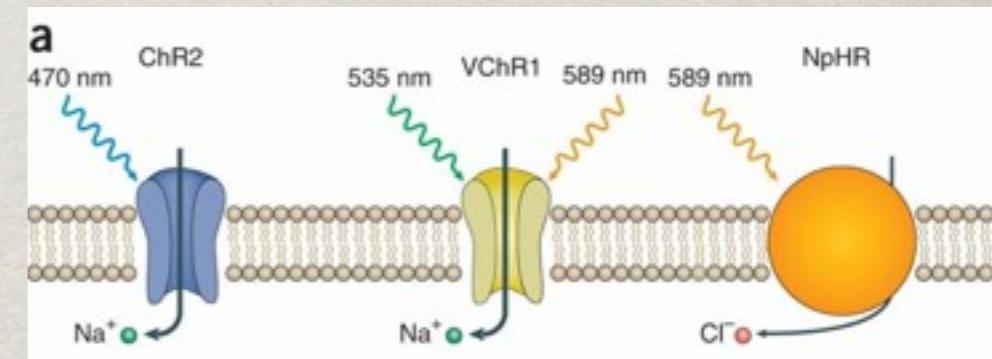
1. chemical/protein uncaging
engineering challenge, not reversible, not genetically encoded



2. azobenzene-- engineering challenge, not genetically encoded but is light reversible,
usually requires agonist or antagonist against signaling molecule of interest

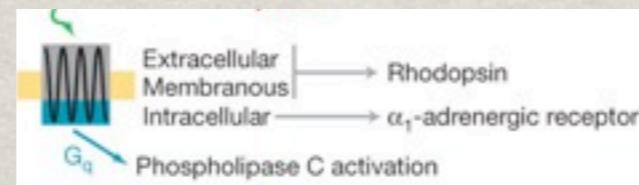


3. Genetically encoded, naturally light responsive proteins
channel/ halorhodopsin --
borrow existing signaling function-- specific currency



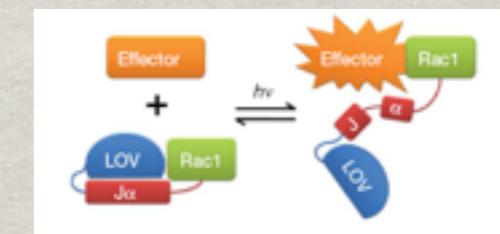
4. Adapting light responsive proteins to new functions

- a. OptoXR-- specific currency

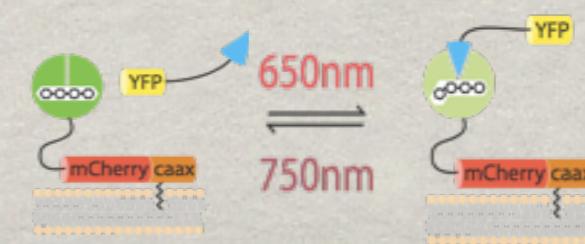


- b. LOV proteins (not reversible)--

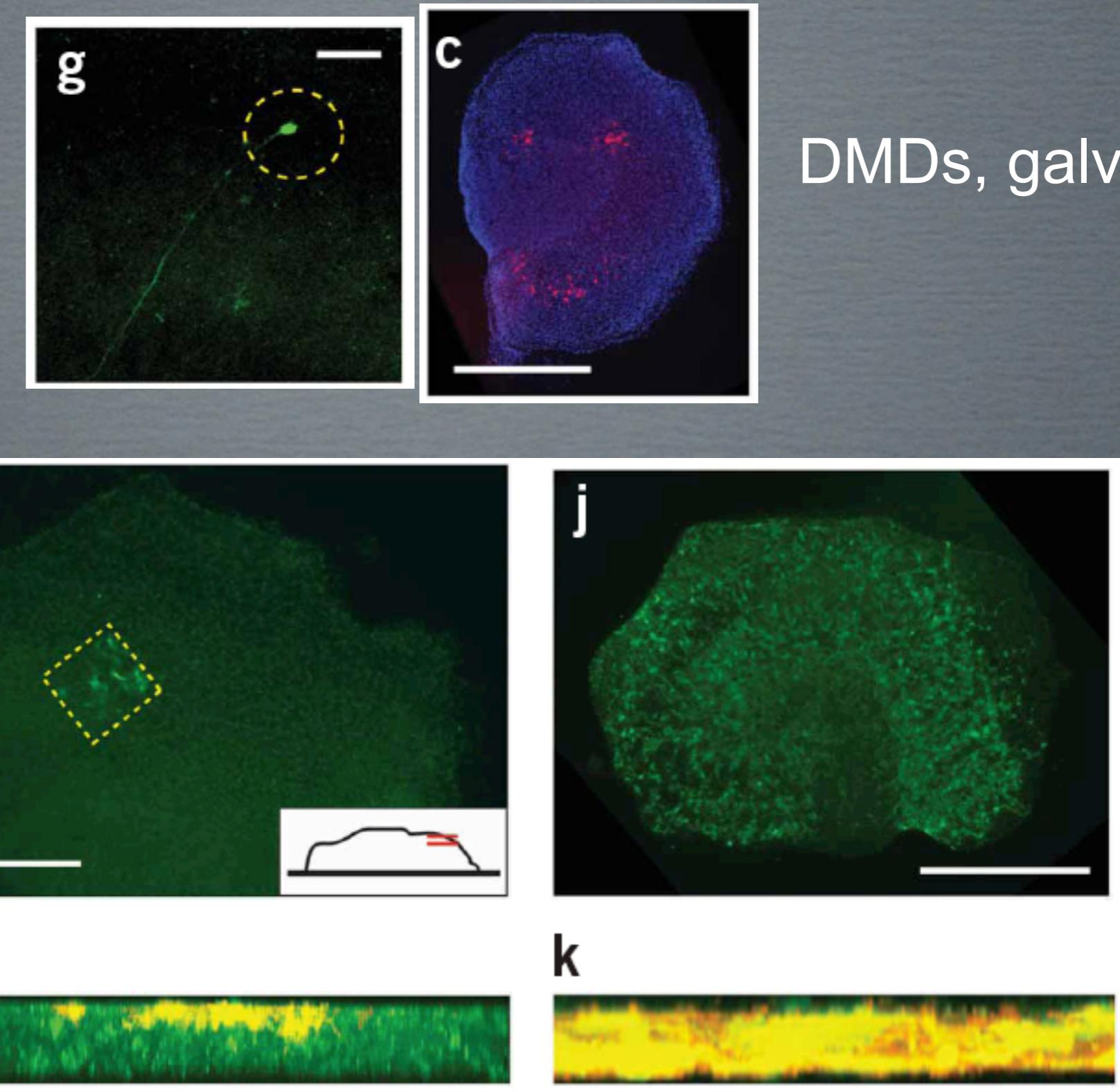
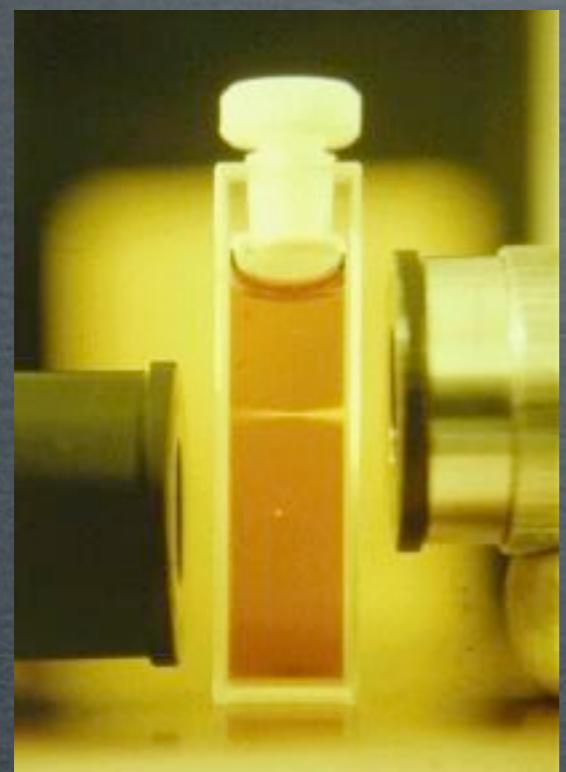
allosteric control, completely genetically encoded, engineering challenge



- c. plant phytochromes-- reversible, genetically encoded (but requires exogenous chromophore).
can drive in both directions with light, modular/general.



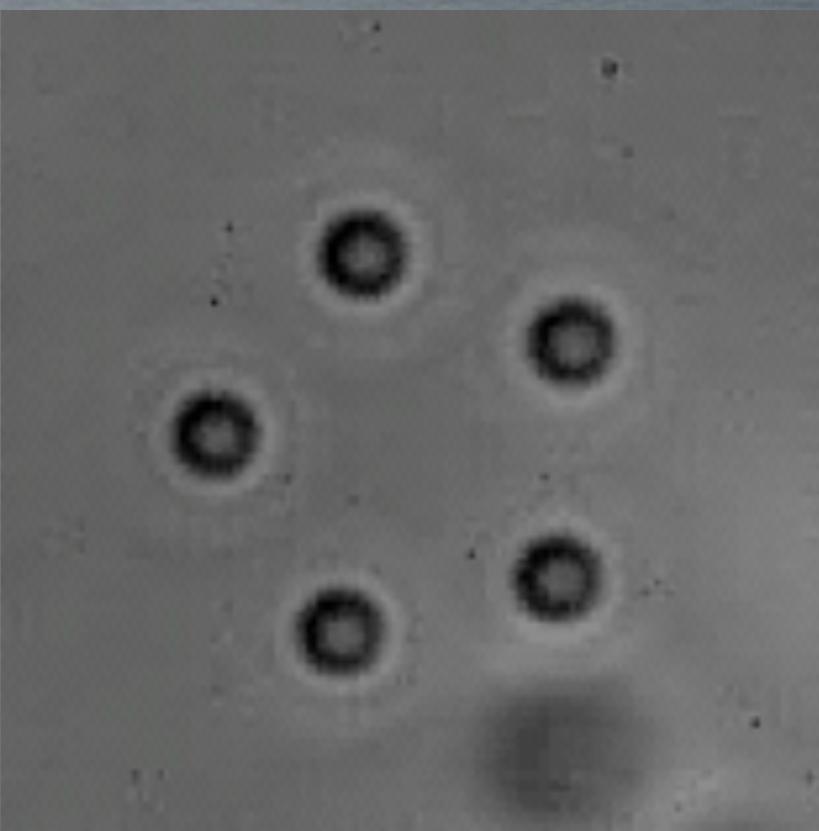
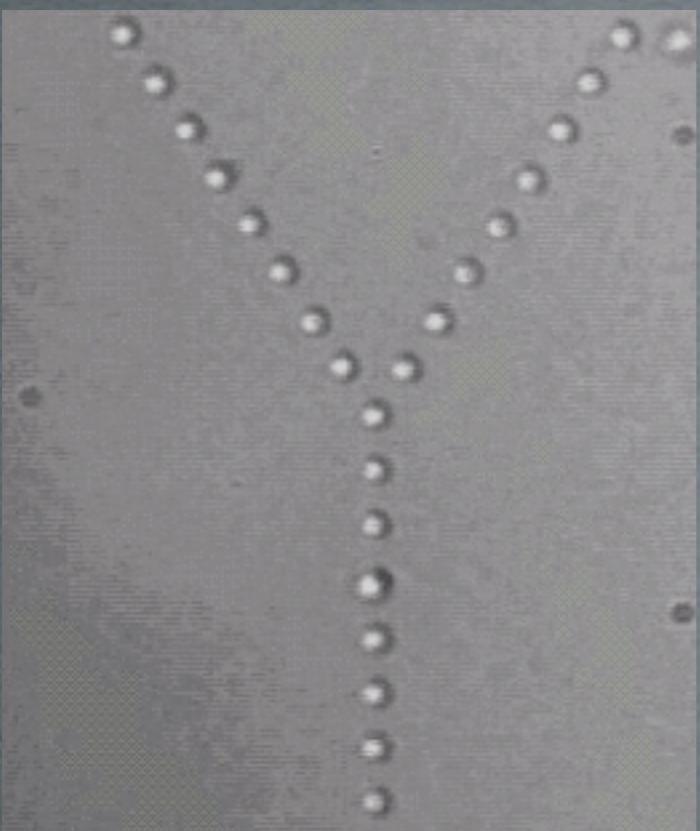
How to deliver the light?



2-photon

40

Spatial Light Modulator in back focal plane for holographic control of light



Holographic optical traps



Eric Dufresne Lab (Yale)



Holger Kress

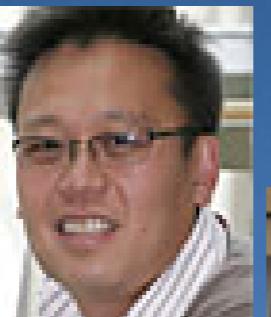
THANKS!



Anselm
Levskaya



Chris
Voigt



Wendell
Lim



Ron Vale



Nico
Stuurman



Arthur
Edelstein



Jared
Toettcher



Anna
Reade



Anna
Payne-Tobin

Steve Altschuler
Lani Wu (UTSW)

Andrew
Houk

Ben Rhau

Orion
Weiner

Delquin
Gong

Arthur
Millius

Scott
Hansen

Oliver
Hoeller

Sheel
Dandekar



Grace
Peng



SEARLE SCHOLARS

American Heart
Association



National Institute of
General Medical Sciences

the
Cell Propulsion
lab

THANKS!



Nico Stuurman



Kurt Thorn



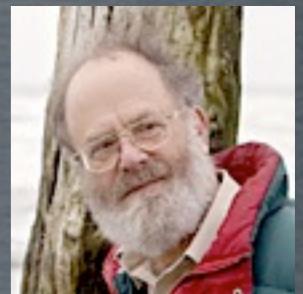
Orion Weiner



Susanne
Rafelski



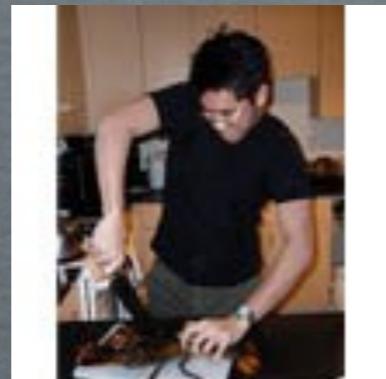
Alice Thwin



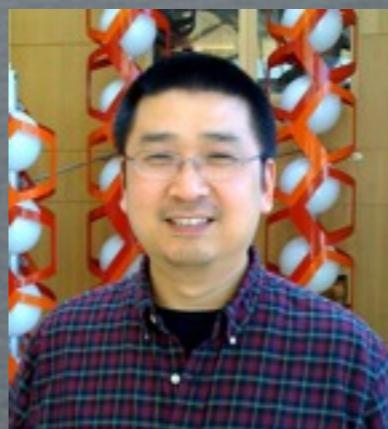
John Sedat



Dave Agard



Bryant Chhun



Yifan Cheng



Bo Huang



Max Krummel



Steve Ross



Silvia Foppiano

Reese Allen

NIKON
IMAGING CENTER

@ UCSF

University of California
San Francisco

MICROSCOPES AND IMAGING SOLUTIONS
 Technical Instruments

