# Probes and Sensors

**BRAIN Initiative Investigators Pre-meeting:**
**Large Scale Recording and Modulation**

| Presentation Order | PI Name(s) All | Title | Project Number |
|---|---|---|---|
| 1 | ELLINGTON, ANDREW D (contact); ZEMELMAN, BORIS V | A robust ionotropic activator for brain-wide manipulation of neuronal function | 1 R21 EY026442-01 |
| 2 | HOCHGESCHWENDER, UTE H (contact); LIPSCOMBE, DIANE ; MOORE, CHRISTOPHER I | Employing subcellular calcium to control membrane voltage | 1 R21 EY026427-01 |
| 3 | KENNEDY, MATTHEW J (contact); TUCKER, CHANDRA L | Optical tools for extended neural silencing | 1 R21 EY026363-01 |
| 4 | KOBERTZ, WILLIAM R | Fluorescent Sensors for Imaging External Potassium in the Brain | 1 R21 EY026362-01 |
| 5 | SACK, JON THOMAS | Neuronal voltage tracers for photoacoustic imaging in the deep brain | 1 R21 EY026449-01 |
| 6 | TANTAMA, MATHEW | Optical Tools to Study Neuropeptide Signaling | 1 R21 EY026425-01 |
| 7 | WANG, SAMUEL SHENG-HUNG (contact); DIGREGORIO, DAVID A | Use of Calcium Indicator Proteins in Spike Counting Mode | 1 R21 EY026434-01 |
| 8 | ZEMELMAN, BORIS V (contact); DREW, MICHAEL R; MARTIN, STEPHEN | A viral system for light-dependent trapping of activated neurons | 1 R21 EY026446-01 |
| 9 | JASANOFF, ALAN | Calcium sensors for molecular fMRI | 5 U01 NS090451-02 |
| 10 | KRAMER, RICHARD H (contact); ISACOFF, EHUD | Optical control of synaptic transmission for in vivo analysis of brain circuits and behavior | 5 U01 NS090527-02 |
| 11 | LAM, KIT S (contact); TRIMMER, JAMES S | Genetically encoded reporters of integrated neural activity for functional mapping of neural circuitry | 5 U01 NS090581-02 |
| 12 | LIN, JOHN YU-LUEN (contact); KLEINFELD, DAVID | Optogenetic mapping of synaptic activity and control of intracellular signaling | 5 U01 NS090590-02 |
| 13 | PIERIBONE, VINCENT A | Development of Protein-based Voltage Probes | 5 U01 NS090565-02 |
| 14 | REBANE, ALEKSANDER (contact); CAMPBELL, ROBERT E.; DROBIJEV, MIKHAIL ; HUGHES, THOMAS E | Northern Lights collaboration for better 2-photon probes | 1 U01 NS094246-01 |
| 15 | ZHONG, HAINING (contact); MAO, TIANYI | A novel approach to examine slow synaptic transmission in vivo | 1 U01 NS094247-01 |
| 16 | PRASAD, PARAS N. (contact); XIA, JUN | Potentiometric photoacoustic imaging of brain activity enabled by near infrared to visible light converting nanoparticles | 1 R21 EY026411-01 |
| *10 min presentation* | *HUANG, CHENG; SCHNITZER, MARK J (contact); LIN, MICHAEL Z.* | *Protein voltage sensors: kilohertz imaging of neural dynamics in behaving animals* | *5 U01 NS090600-02* |
| *10 min presentation* | *TIAN, LIN* | *Genetically encoded sensors for the biogenic amines: watching neuromodulation in action* | *5 U01 NS090604-02* |

# A Robust Ionotropic Activator for Brain-wide Manipulation of Neuronal Function
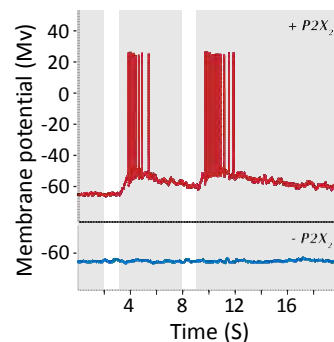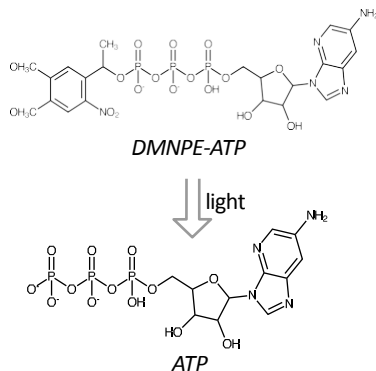
Andrew Ellington and Boris Zemelman
The University of Texas, Austin
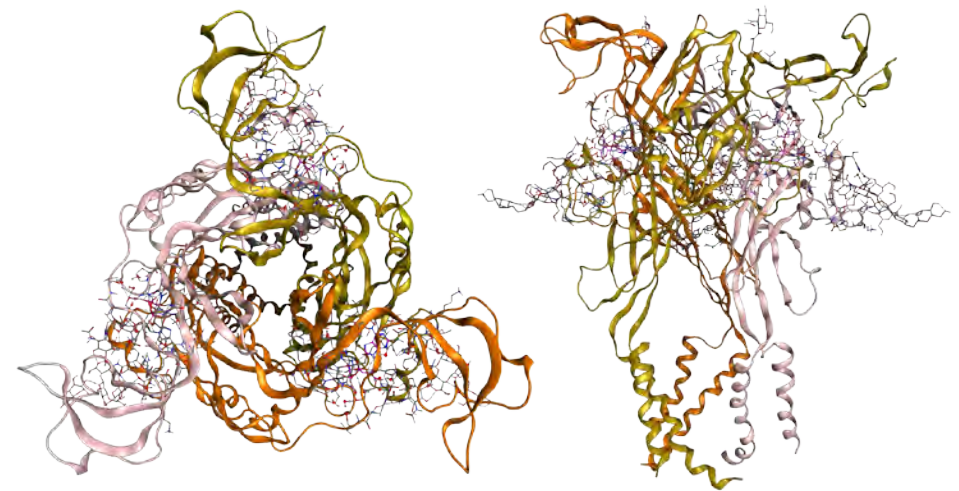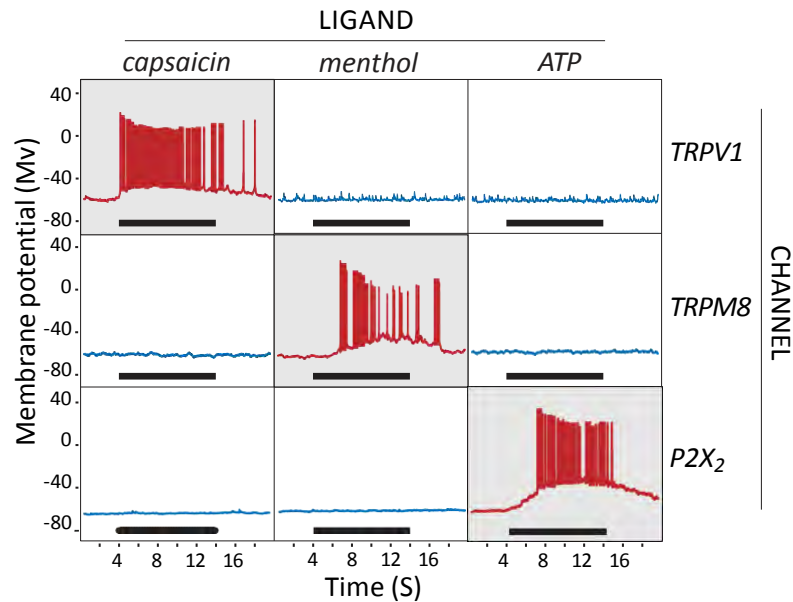
Trimeric P2X channel



DMNPE-ATP

light

ATP



## Activation of neurons with engineered P2X

Pros:
- (A) currents 100X> channelrhodopsin
- (B) does not inactivate
- (C) tunable gating
- (D) light or orthogonal ligand
- (E) systemic ligand administration

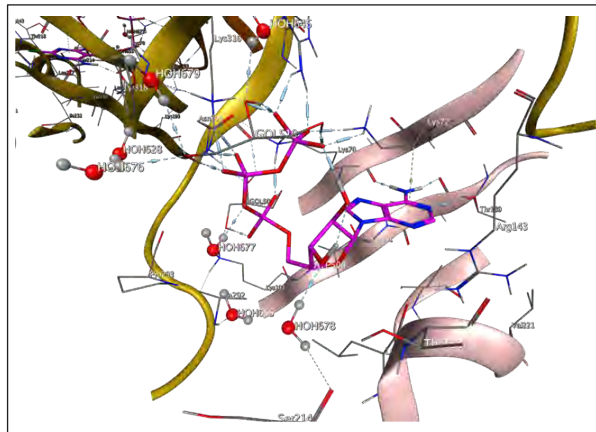**Photochemical Gating of Heterologous Ion Channels: Remote Control Over Genetically Designated Populations of Neurons**

*Zemelman, Nesnas, Lee, Miesenböck, PNAS 2003*

TEXAS

The University of Texas at Austin

# A Robust Ionotropic Activator for Brain-wide Manipulation of Neuronal Function
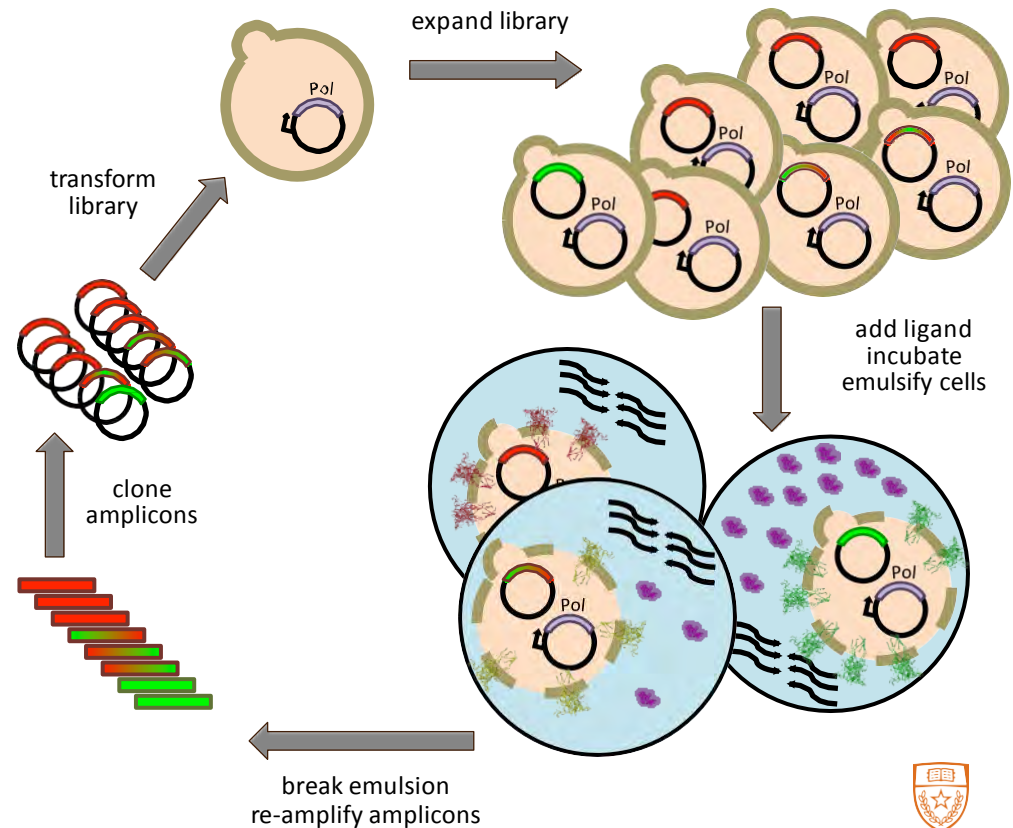


P2X ATP binding site
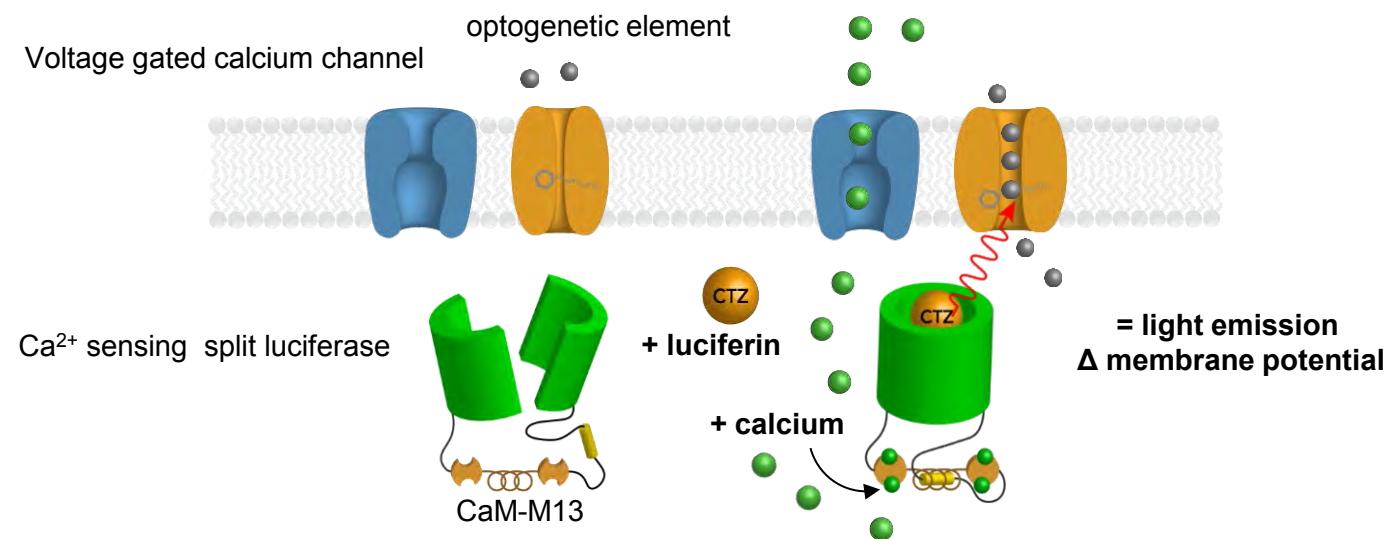
## Engineering novel P2X receptor/ligand pairs

Computational design ⟷ Directed evolution

## P2X receptor compartmentalized partnered replication (CPR) in yeast enables succesive enrichment



expand library

transform library

clone amplicons

add ligand incubate emulsify cells

break emulsion re-amplify amplicons



Agonist  Ca²⁺  P2X Receptors

Yeast Cell

Calmodium
Calcineurin
Crz1

Green

4xCDRE Promoter     KOD DNA Polymerase



-Ca²⁺  +Ca²⁺

yeast cell emulsion          promoter activation

TEXAS
The University of Texas at Austin

# Employing subcellular calcium to control membrane voltage

Ute Hochgeschwender, Diane Lipscombe, Christopher I Moore

**Ca$^{2+}$ sensitive light emitting molecules drive light sensing elements to control membrane voltage.**



optogenetic element

Voltage gated calcium channel

Ca$^{2+}$ sensing split luciferase

CTZ

**+ luciferin**

**+ calcium**

= **light emission**
**Δ membrane potential**

CaM-M13

**Device-free, molecular-based strategy for feedback control of neural activity.**

**Regulate neurons when they express maladaptive patterns, and specifically regulate those neurons exhibiting aberrant patterns (runaway activity or failure to amplify).**

**Light production can be made specific to large events such as bursts, or sensitive to individual spikes or single channel activity by adjusting calcium sensitivity and molecule location.**

**Highly specific sensing of Ca$^{2+}$ at its source for temporal and spatial precision in feedback control.**

# Employing subcellular calcium to control membrane voltage

Ute Hochgeschwender, Diane Lipscombe, Christopher I Moore

## 1. Generate bright, fast, bioluminescent Ca²⁺sensors.
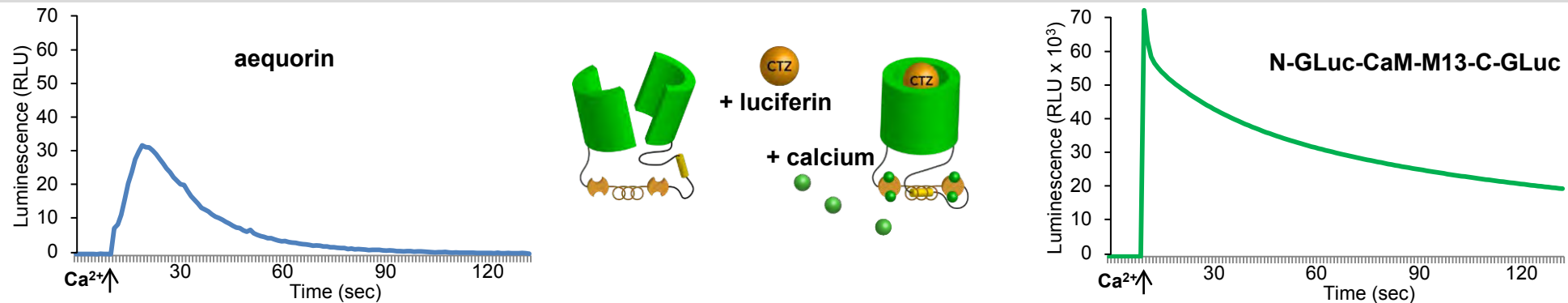


## 2. Localize bioluminescent Ca²⁺ sensors to specific calcium channels and to subcellular membrane regions: Highly specific biophysical event detection mechanism.
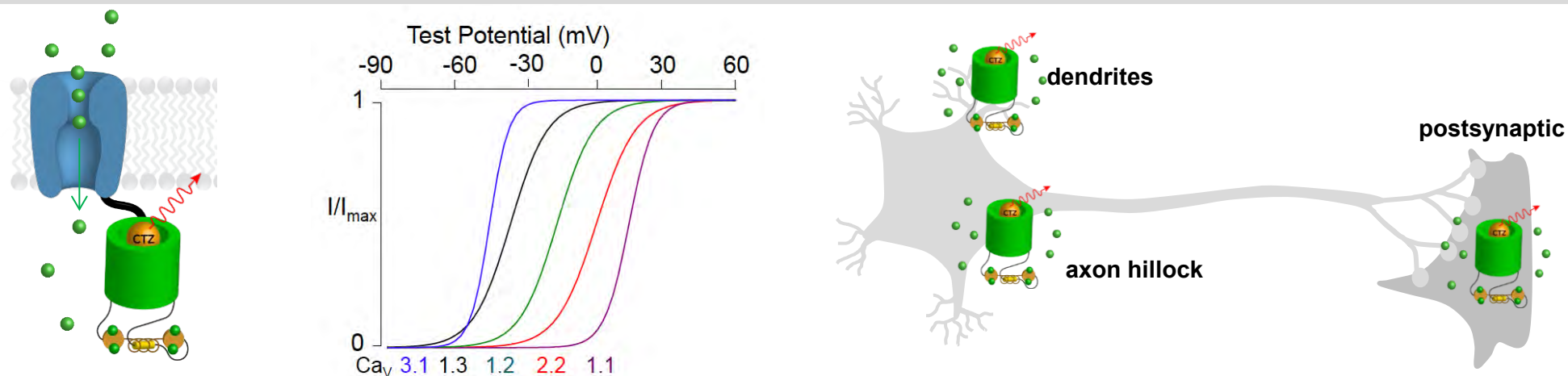


## 3. Couple bioluminescent Ca²⁺ sensors to optogenetic elements for rapid feedback control.
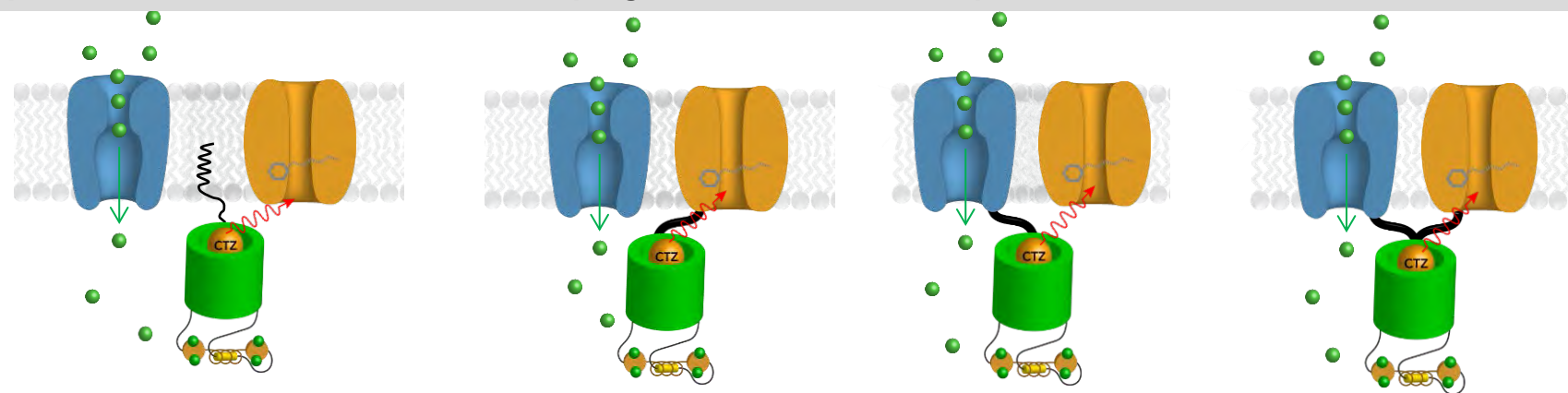
# Optical tools for conditional, local and long-term synapse silencing

Matt Kennedy, Chandra Tucker
University of Colorado School of Medicine

Currently available light-activated silencers (NpHR, Arch)

Problems with extended (min-hours) silencing:

-intracellular chloride build-up/pH alterations

-require continuous light exposure/phototoxicity

-Illuminating axon projections may not *locally* block activity

Traditional tools: *Clostridium* neurotoxins

-Silence neurotransmission by cleaving protein machinery required for neurotransmitter release

-Catalytic light chains can be genetically expressed but synaptic silencing could take tens of minutes to hours for the toxin to be transcribed/translated and to reach terminals
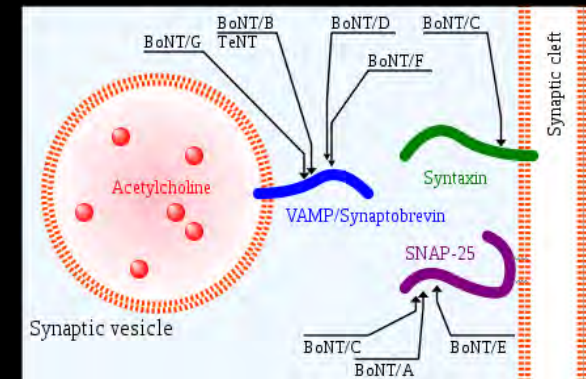


New technology for conditionally and locally blocking neurotransmission for extended times with a brief light exposure using either single or multiphoton excitation

-Engineered photoactivatable *Clostridium* toxins

# Engineering light-activated *Clostridium* neurotoxins

Light-triggered protein dimerization can be used to control protein function:
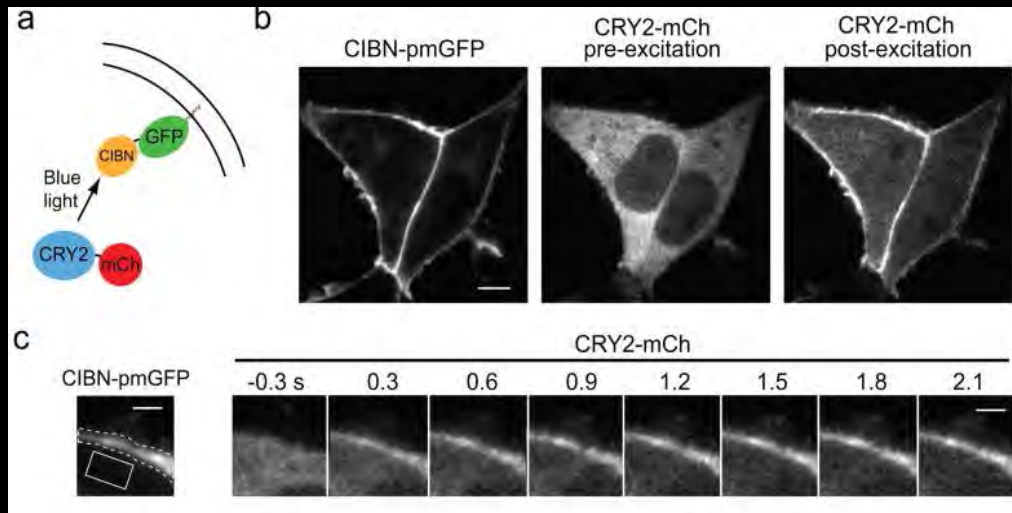


Kennedy et al., 2010 Nature Methods

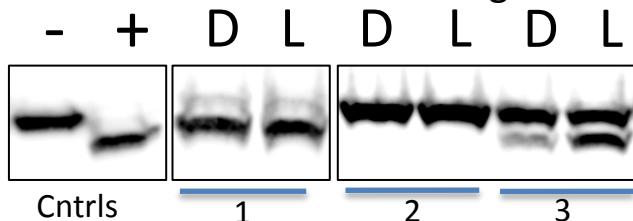Split protein complementation
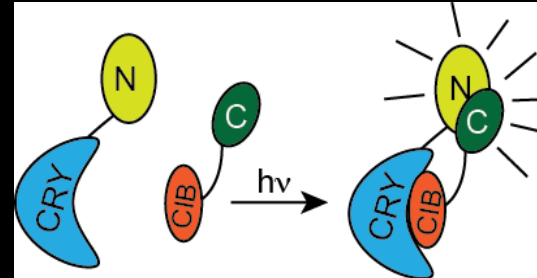


transcription
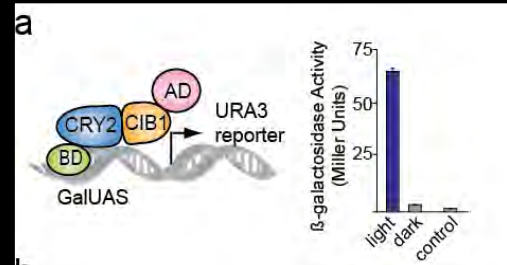


cre recombinase
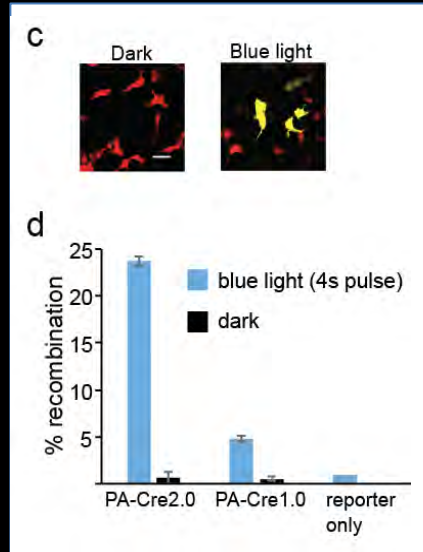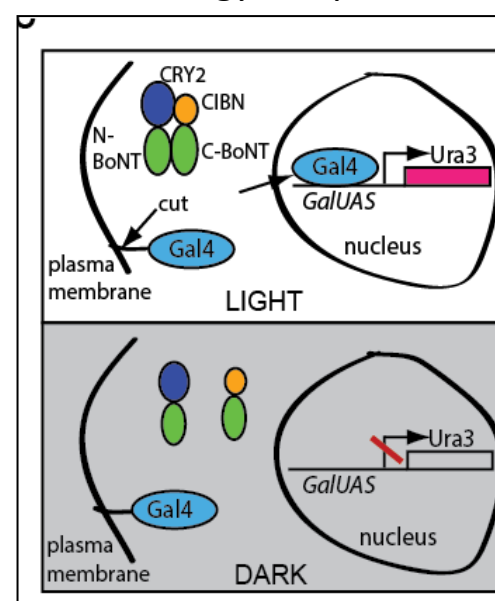


*Clostridium* neurotoxins?
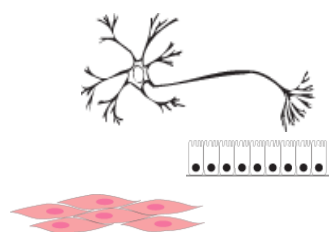
catalytic light chain BoNTB
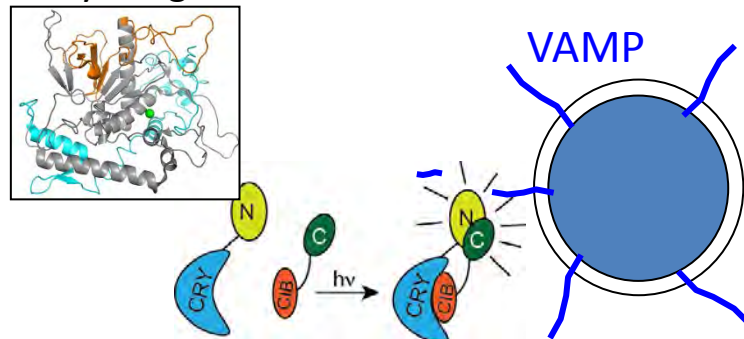


VAMP

Immunoblot for VAMP cleavage:

-  +  D  L  D  L  D  L



Cntrls    1    2    3

Genetic strategy to optimize/screen
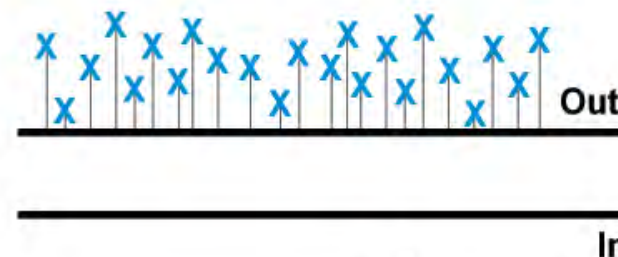
# Fluorescent Visualization of K⁺ Efflux



**Isolated Cells**

**Animals**

**X** = azide, -N₃

Out

In

K⁺

Out

In

K⁺

MeO

Y
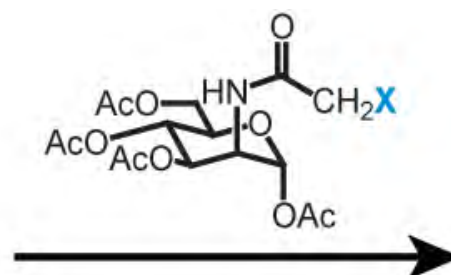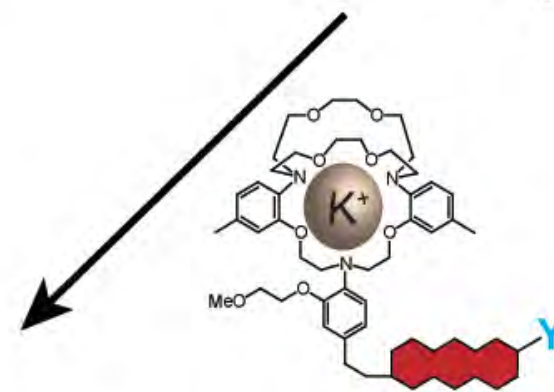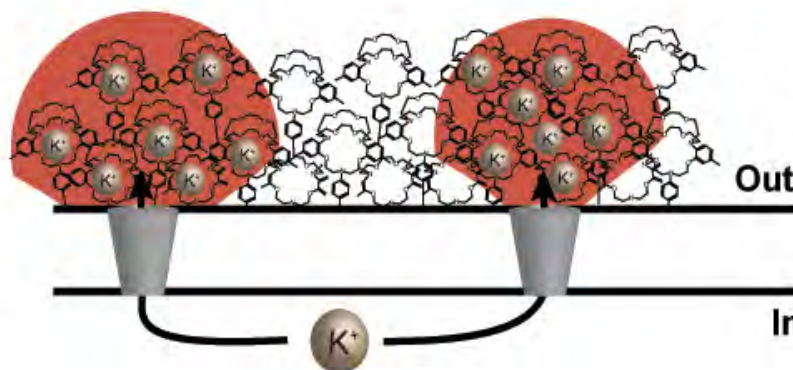
Copper free 'click' chemistry

R' —N₃  +  R''  →  R'  R''
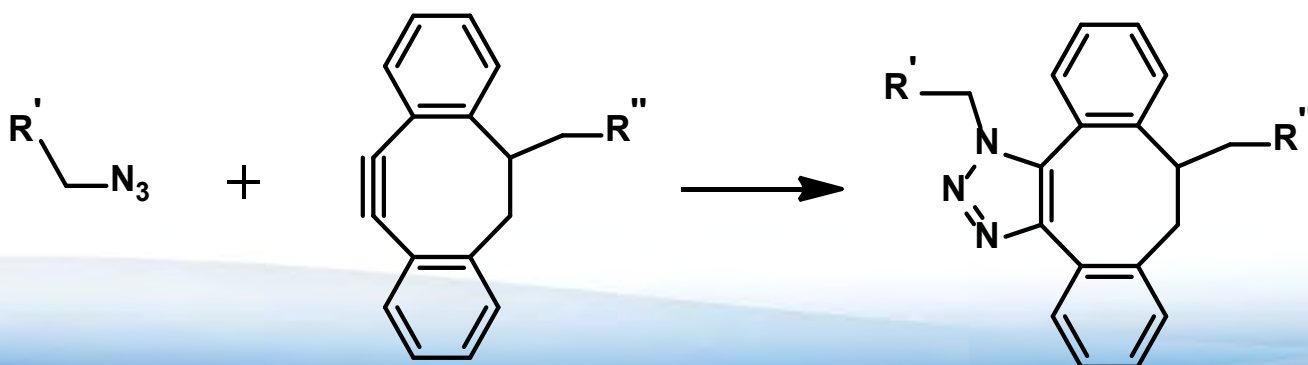
Dibenzocyclooctyne (DIBO)

University of
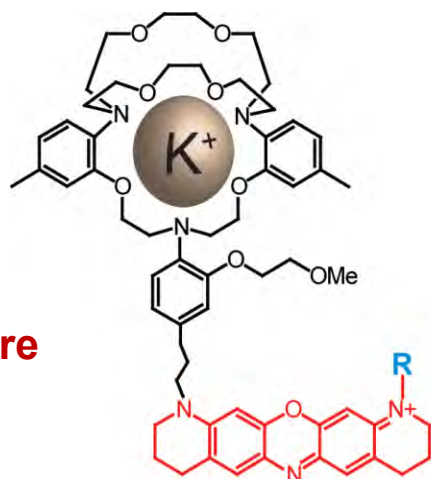Massachusetts
UMASS Medical School

# Progress Report

## A. Near-IR Fluorescent K+ Sensor

**Receptor:**
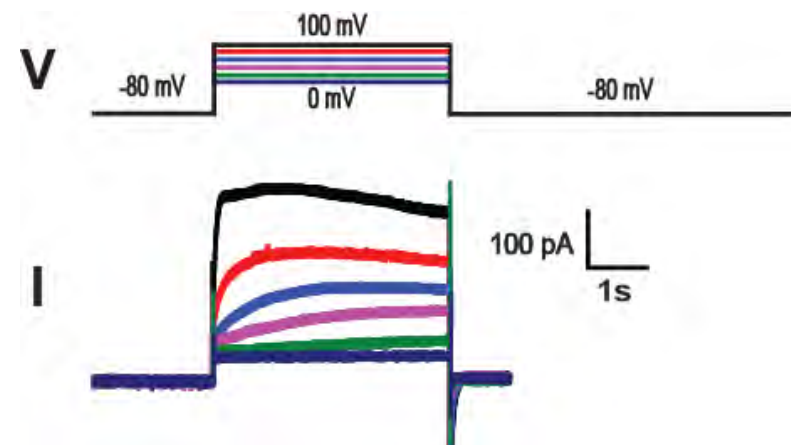**Triazacryptand (TAC)**
$K^+$ selective binding
domain

**Oxazine fluorophore**
$\lambda_{ex}/\lambda_{em}$ near IR
(630 – 800 nm)

### K+ Fluorescence
(with 1 mM $Mg^{2+}$ and 1.5 mM $Ca^{2+}$ and 150 mM $Na^+$)

Fluorescent Signal (au)

- 0 mM
- 1 mM
- 3 mM
- 5 mM
- 10 mM
- 30 mM
- 50 mM
- 100 mM
- 150 mM
- 200 mM

wavelength (nm)
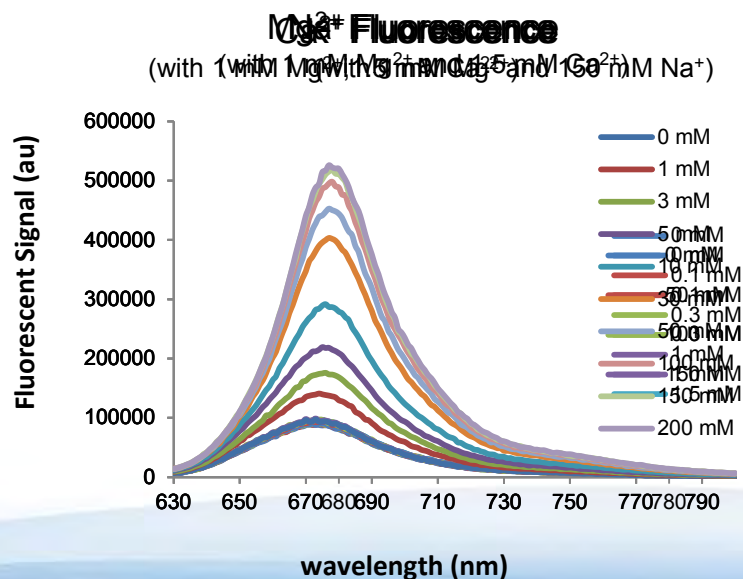
## B. Visualizing Cation Efflux with H+
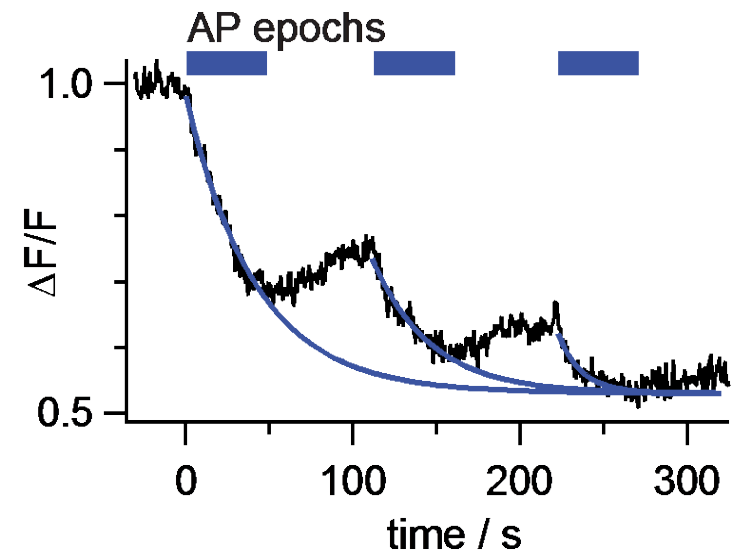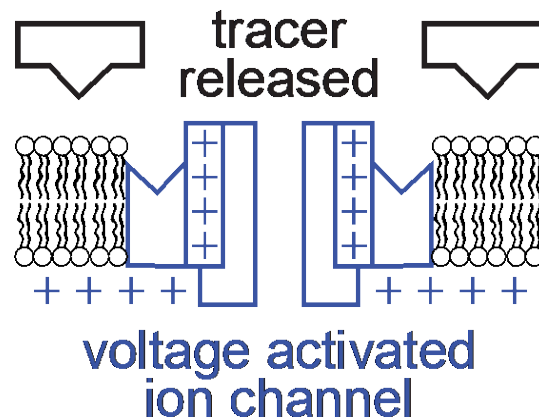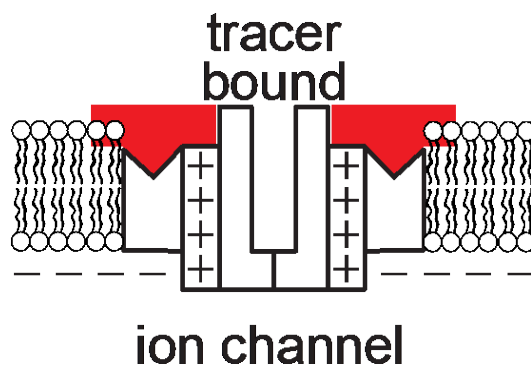
ΔF

-80 mV

pH 7.5  6.0
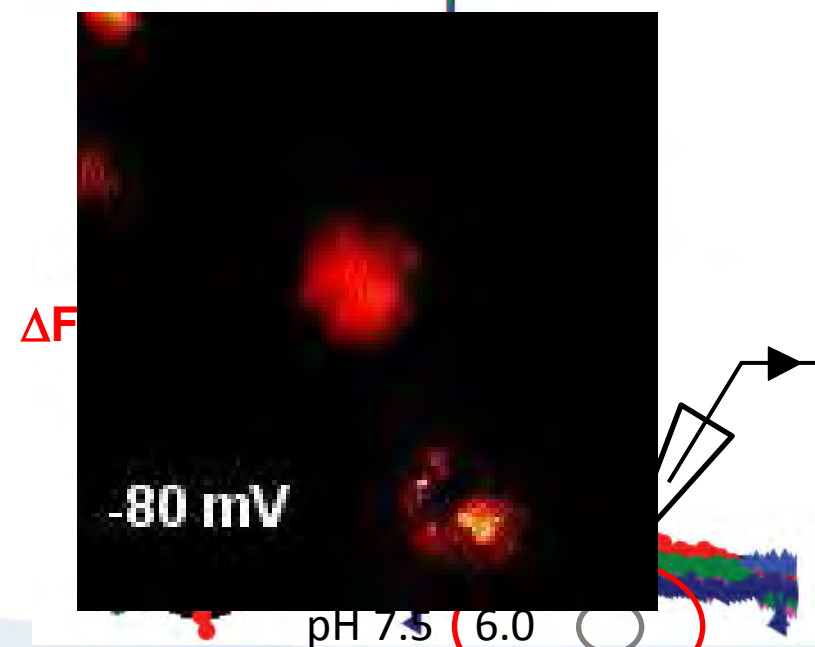
0.1 mM HEPES

# Neuronal voltage tracers for photoacoustic imaging in the deep brain

R21 EY026449  Jon Sack, Lin Tian, Lihong Wang, Laura Marcu

Motivation: photoacoustic imaging can exceed 1 mm depth in tissue

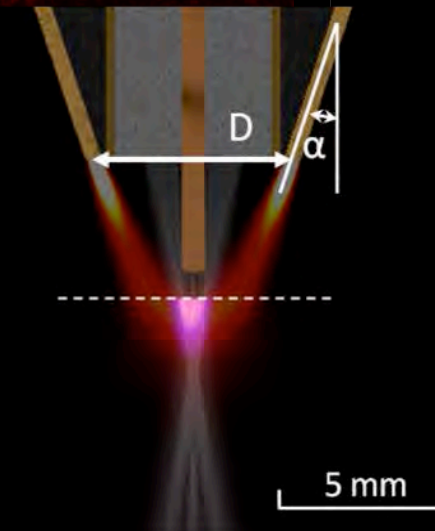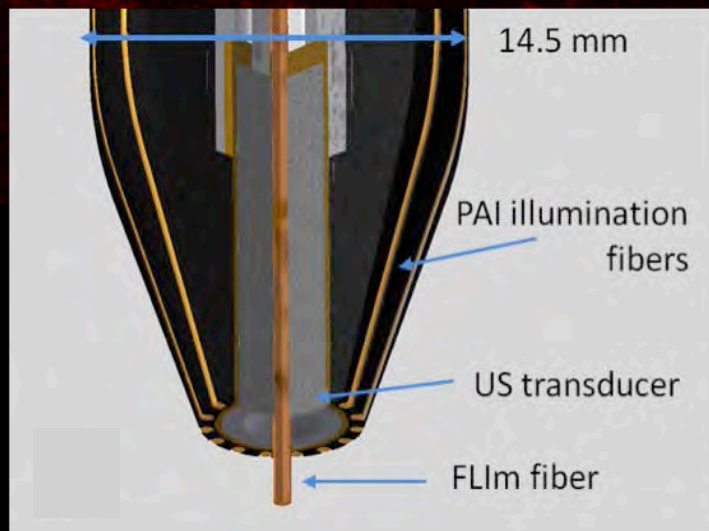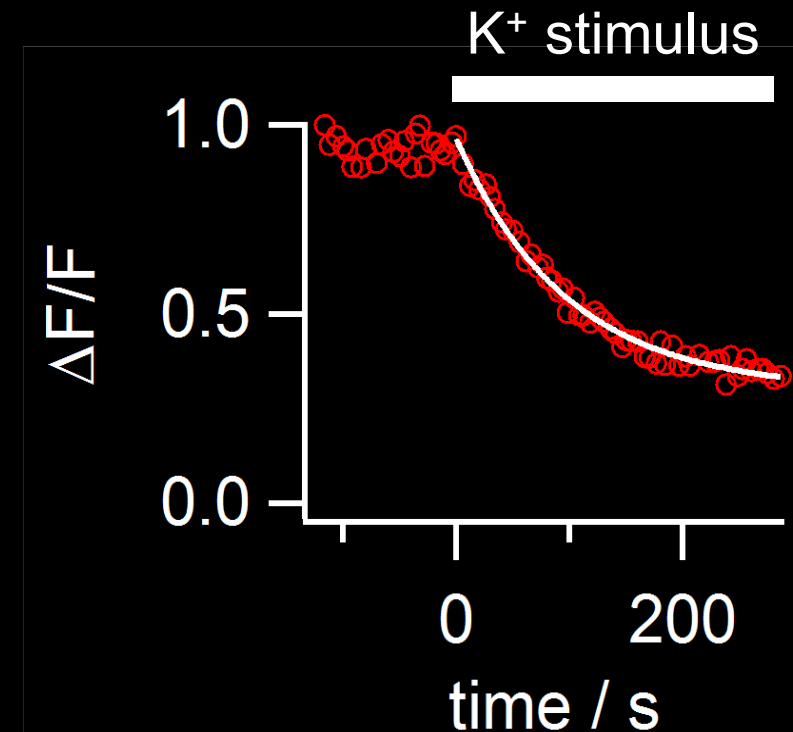Goal: photoacoustic contrast agents to resolve neuronal voltage changes
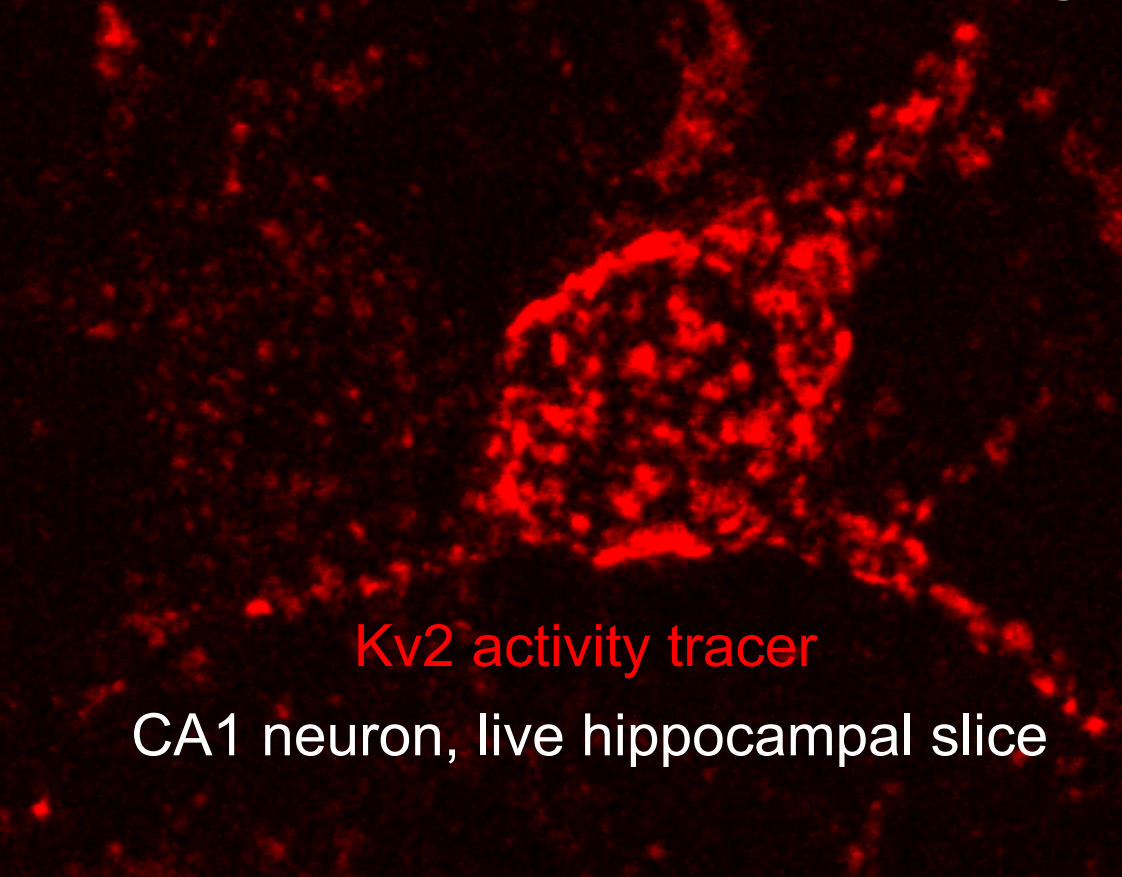
Strategy: image voltage activation of endogenous brain ion channels



PNAS 2014 111: E4789

**Aim 1: Screen and optimize voltage tracers for photoacoustic imaging**
**Aim 2: Validate photoacoustic voltage tracers for deep brain imaging**

Kv2 activity tracer

CA1 neuron, live hippocampal slice

K⁺ stimulus

$\Delta F/F$

time / s

14.5 mm

PAI illumination fibers

US transducer

FLIm fiber

D

α

Multimodal photoacoustic fluorescence imaging

5 mm

# Fluorescent Biosensors of Neuropeptides

## Personnel

R21EY026425

Mathew Tantama
Assistant Professor
Department of Chemistry
Purdue University

Stevie Norcross
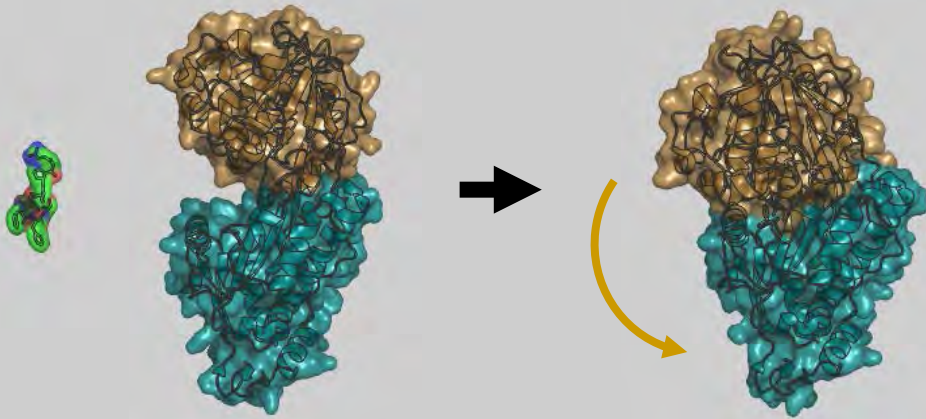Project Leader

Sara Doan

Diversity

Inclusion

you are welcome here

# Fluorescent Biosensors of Neuropeptides
## Proposed Work
R21EY026425

## Sensor Domain: Peptide Binding Protein



## Reporter Domain: Fluorescent Protein(s)



## Design Strategies
- Circular Permutation (cpFP)
- FRET
- Dimerization dependence (ddRFP)

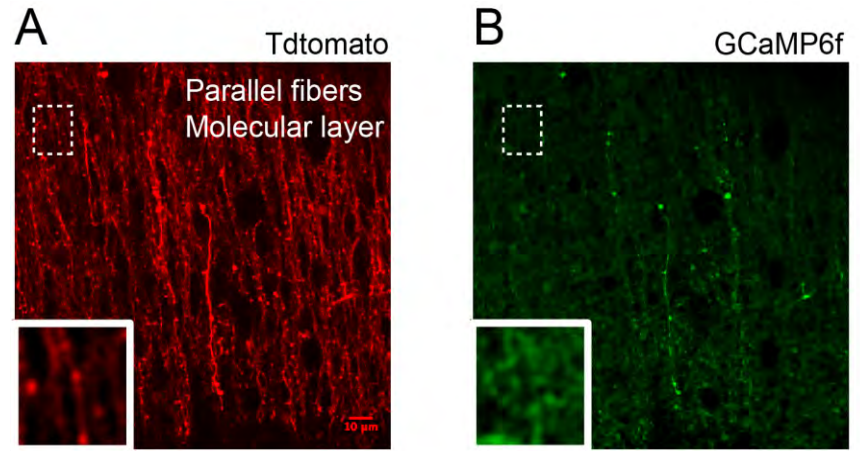## Imaging Modalities
- Intensiometric
- Ratiometric
- Lifetime

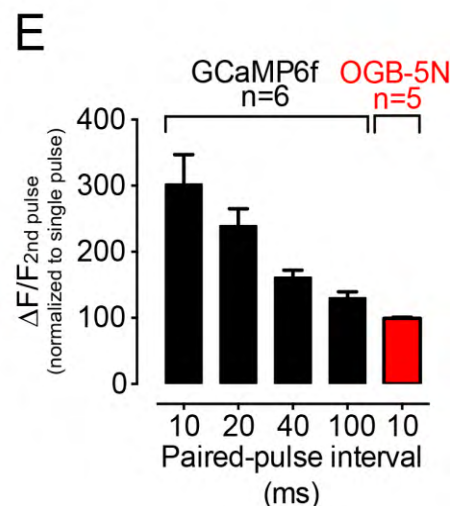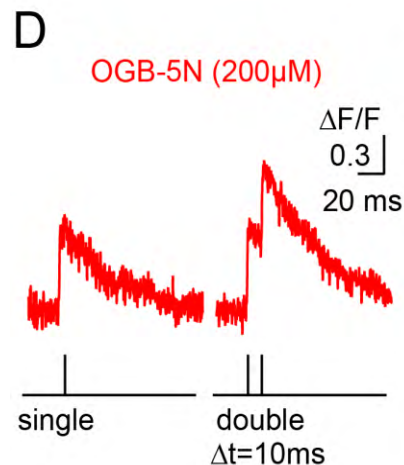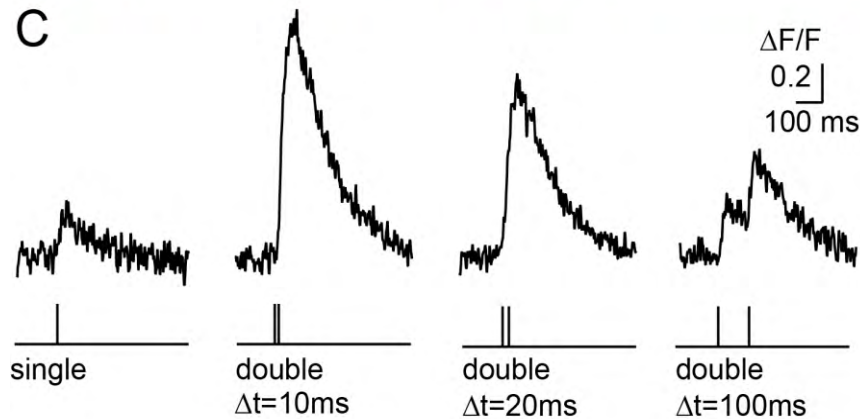## Challenges
- Affinity
- Specificity

## Methods
- "Semi"-Rational Mutagenesis
- Phage Display?
- Computational?

# Use of calcium indicator proteins in spike counting mode



A  Tdtomato
Parallel fibers
Molecular layer
10 μm

B  GCaMP6f

C

$\Delta F/F$
0.2
100 ms

single

double
$\Delta t = 10ms$

double
$\Delta t = 20ms$

double
$\Delta t = 100ms$

D

OGB-5N (200μM)

$\Delta F/F$
0.3
20 ms

single

double
$\Delta t = 10ms$

E

GCaMP6f   OGB-5N
n=6        n=5

$\Delta F/F_{2nd\ pulse}$ (normalized to single pulse)

400
300
200
100
0

10  20  40  100  10
Paired-pulse interval
(ms)

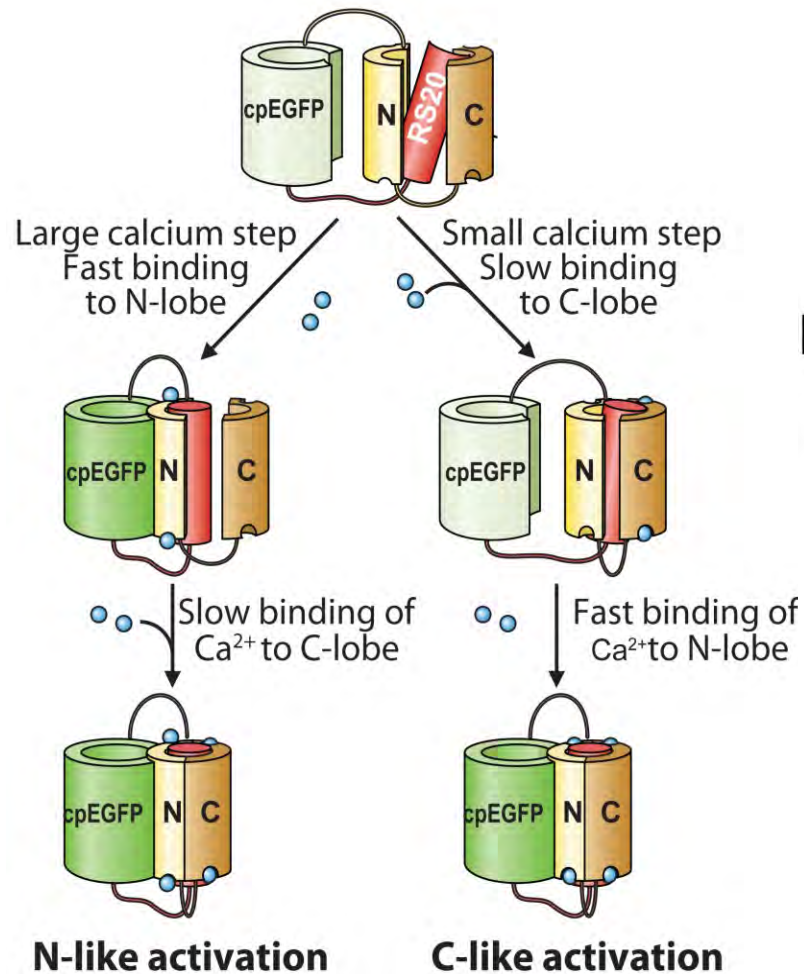In axon terminal boutons calcium signals are fast, but GCaMP6f cannot keep up.

We seek to use Fast-GCaMPs, whose off-responses are up to 30-fold faster, to achieve "spike-counting mode."
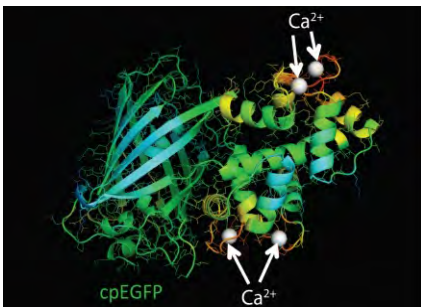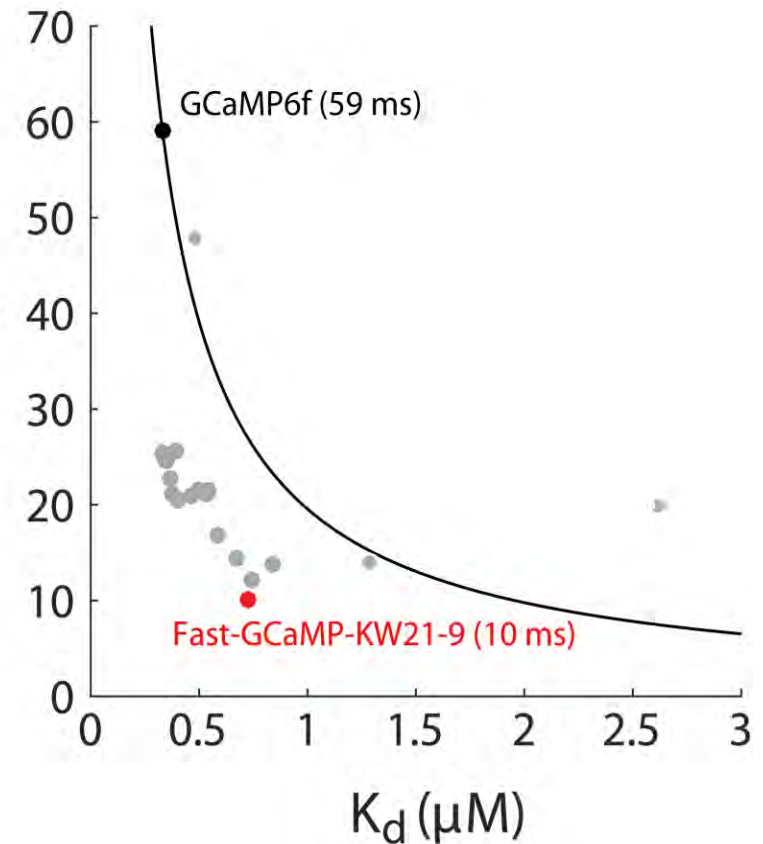
R21 EY026434
PIs: Sam Wang and David DiGregorio

# Use of calcium indicator proteins in spike counting mode
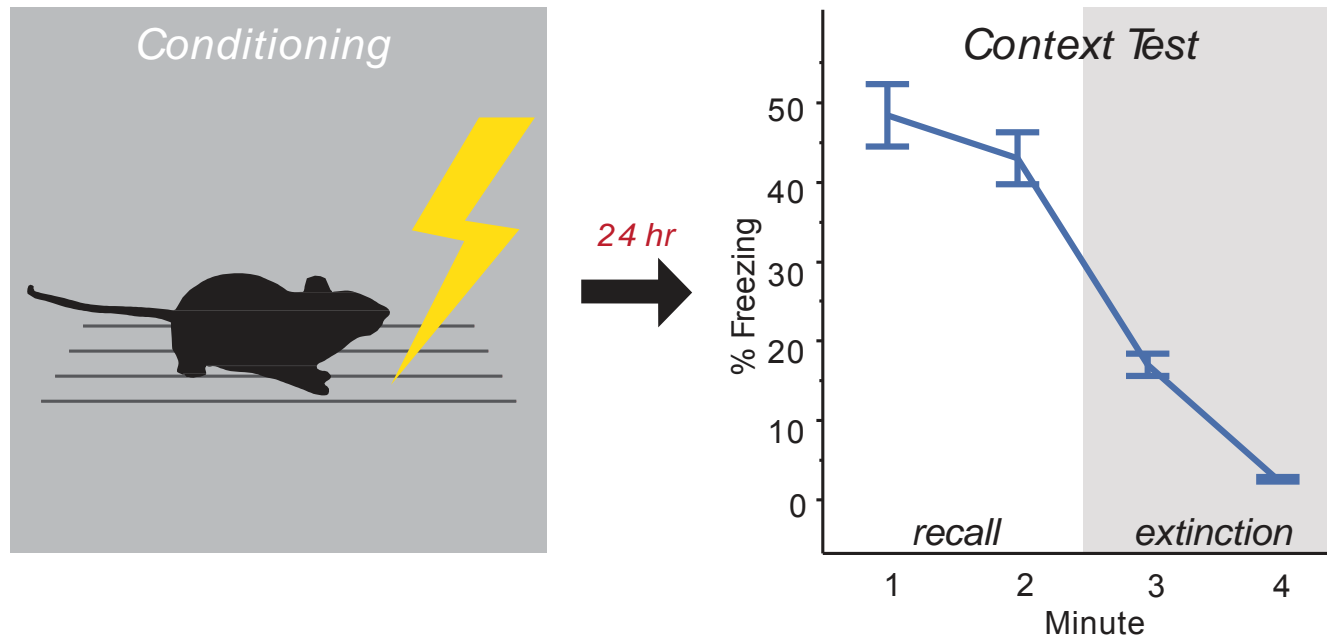


**Fast-GCaMPs have accelerated off-responses**

# A High Precision Method for Activity-dependent Neural Circuit Mapping

Michael Drew, Stephen Martin, Boris Zemelman
Center for Learning and Memory, The University of Texas, Austin

## Goal: to identify behaviorally relevant neurons



**Requirements:**

(A)     precise temporal control
(B)     universal cell detection
(C)     multiple timepoint selection
(D)     robust reporter expression
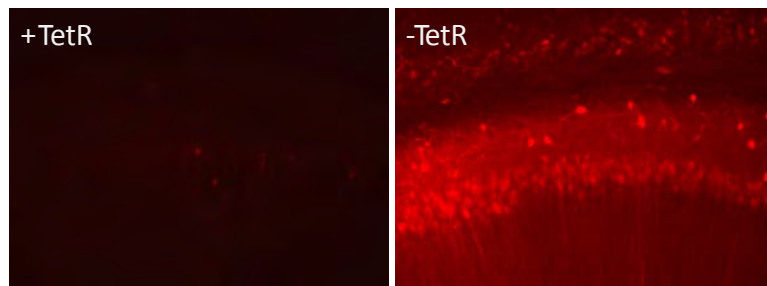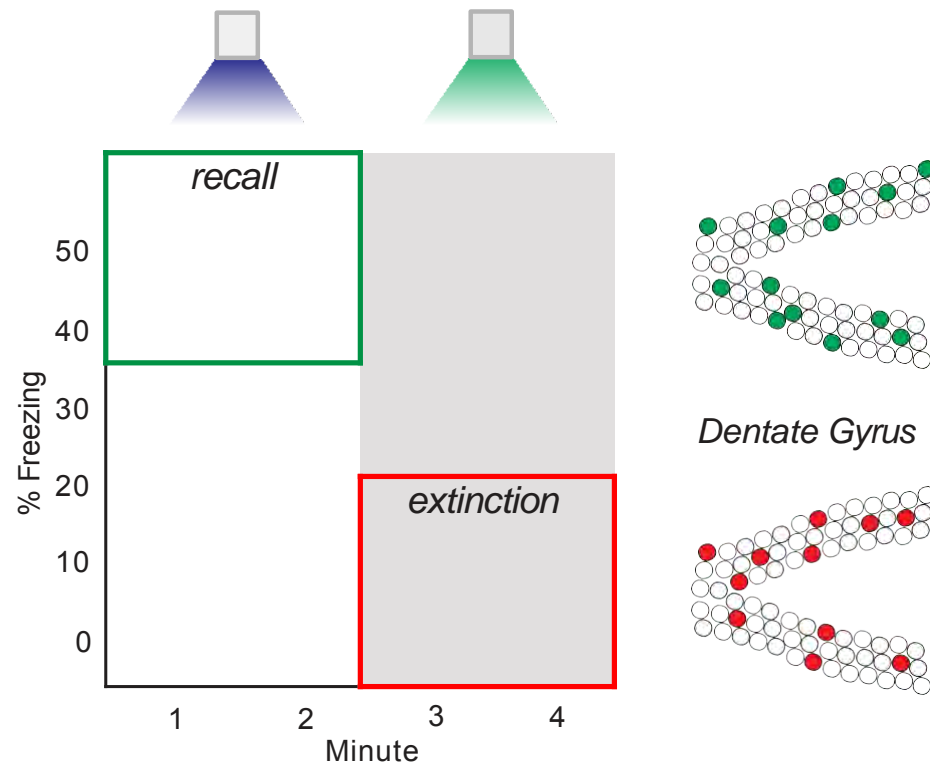
# A High Precision Method for Activity-dependent Neural Circuit Mapping



## Cellular substrate identification:

*recall*

*extinction*

% Freezing — 50, 40, 30, 20, 10, 0

Minute — 1, 2, 3, 4

*Dentate Gyrus*

+TetR
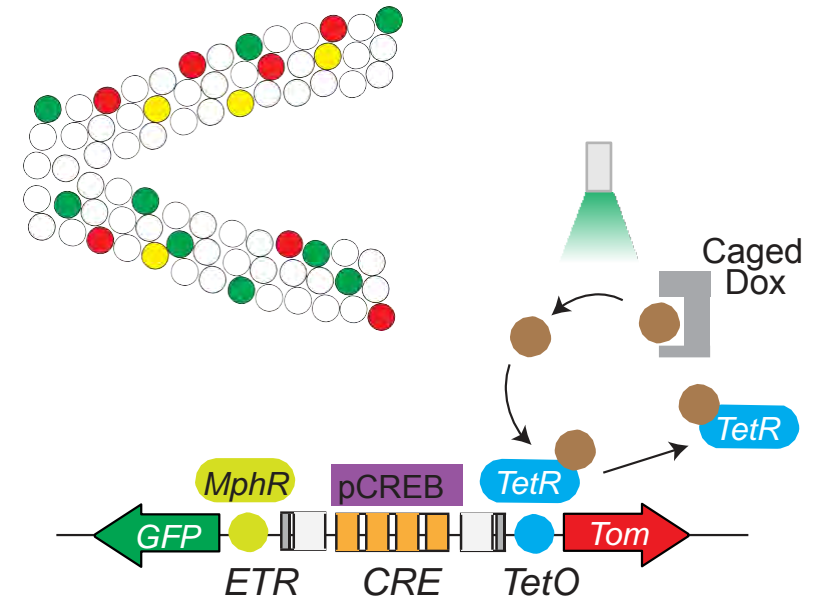
-TetR

modulated reporter expression in an enriched environment

## Proposal:

(A) rAAV cross-species reporter
(B) light-based temporal control
(C) rapid, robust and universal
    expression of reporter or actuator
(D) bidirectional/bitemporal vector
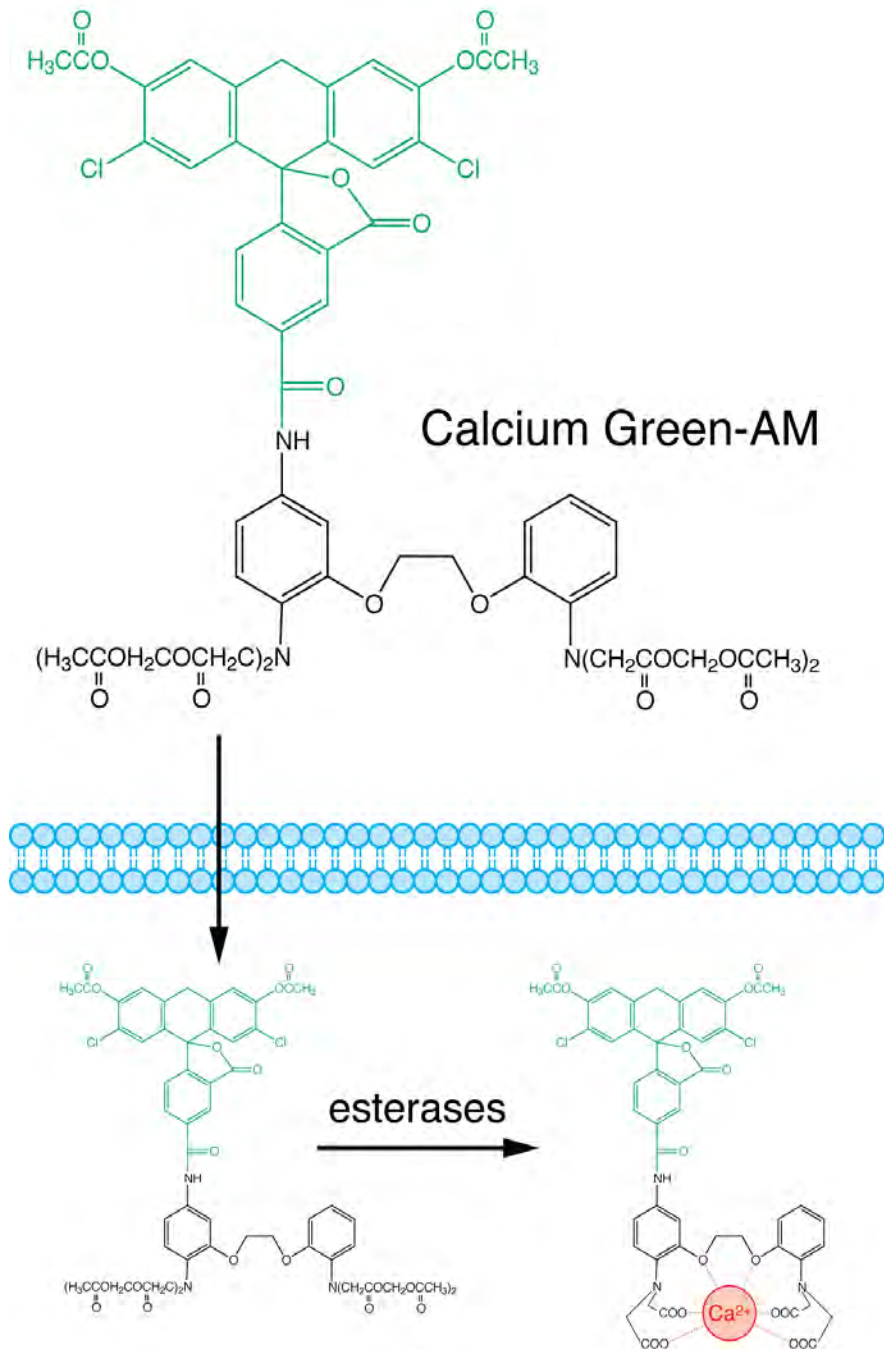    for reproducible distillation of cell activity
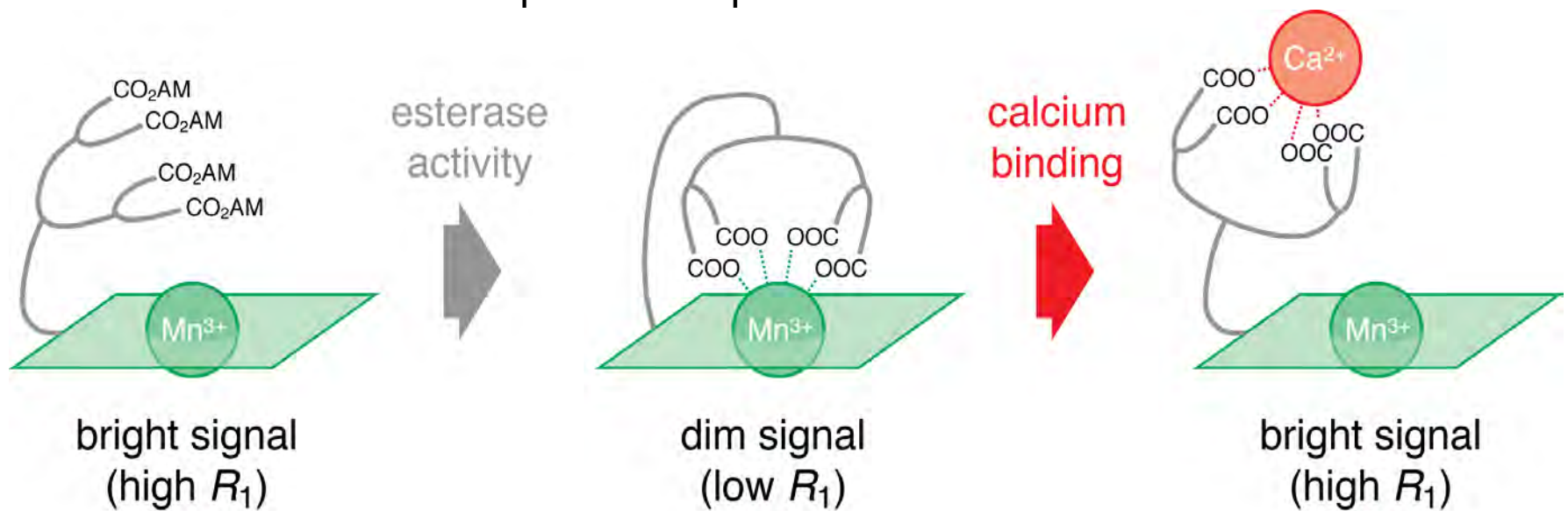
Caged Dox

TetR

TetR

MphR

pCREB

GFP

Tom

ETR

CRE

TetO

# Calcium sensors for molecular fMRI (U01-NS090451)
*A. Barandov, B. B. Bartelle, B. A. Gonzalez, S. J. Lippard, & A. Jasanoff*



Calcium Green-AM

esterases

retention of intracellular agents shown

# calcium sensors based on cell-permeable platform

CO$_2$AM
CO$_2$AM
CO$_2$AM
CO$_2$AM

Mn$^{3+}$

**bright signal**
**(high $R_1$)**

esterase
activity

COO OOC
COO OOC

Mn$^{3+}$

**dim signal**
**(low $R_1$)**

calcium
binding

Ca$^{2+}$
COO
COO
OOC
OOC

Mn$^{3+}$

**bright signal**
**(high $R_1$)**

promising candidates; further work in progress...

# Optogenetic Pharmacology:   Controlling neurotransmitter receptors with light

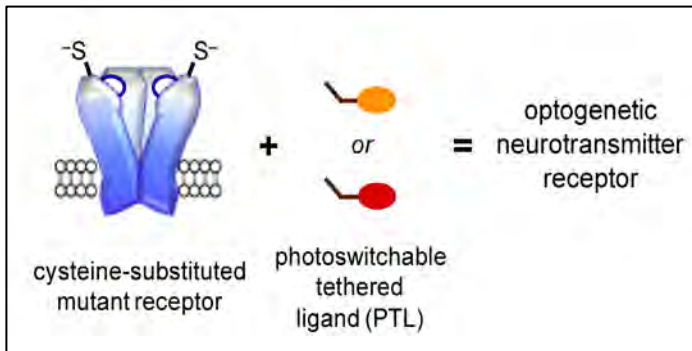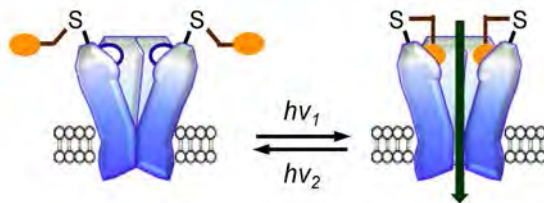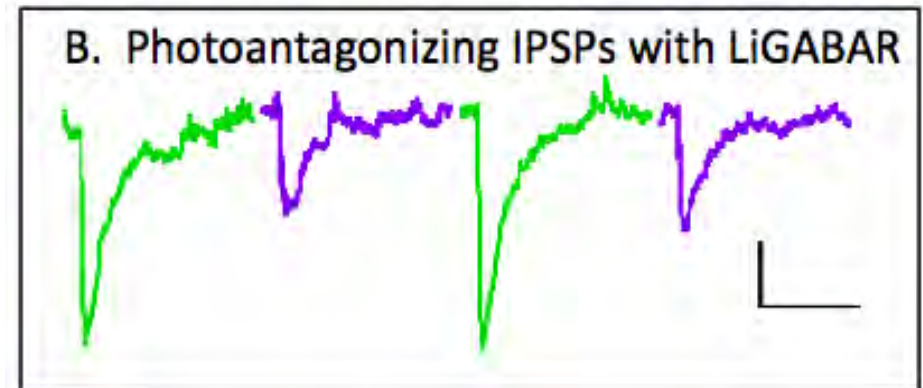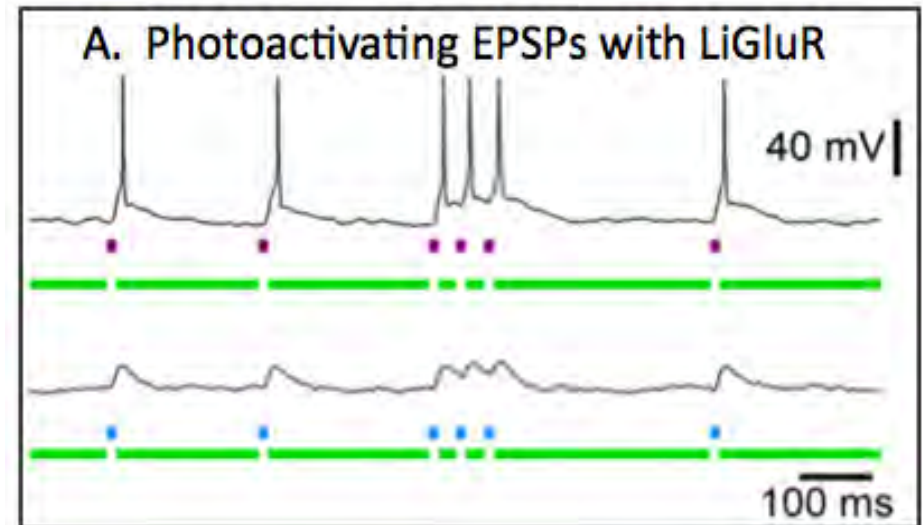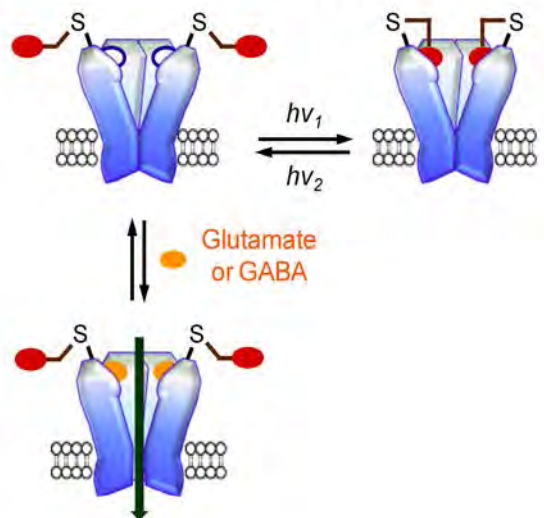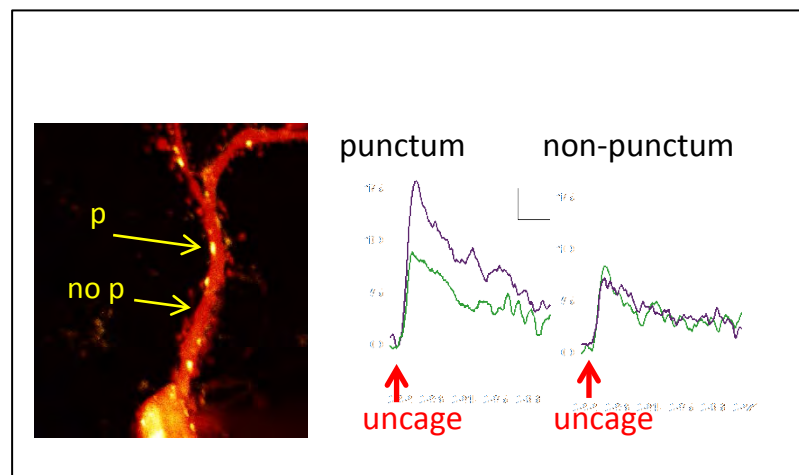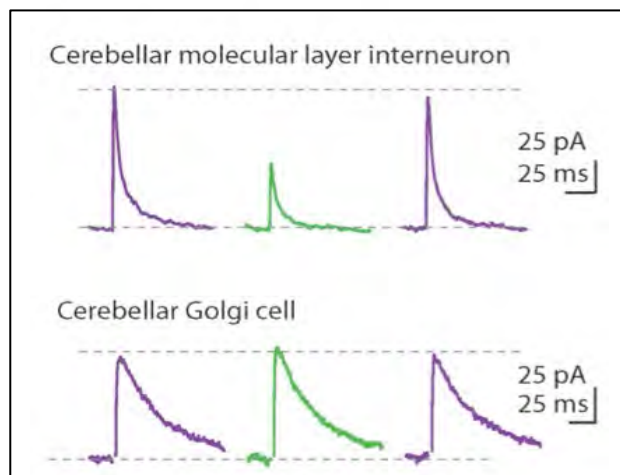Richard H. Kramer and Ehud Isacoff, UC Berkeley



- Also, metabotropic Glu and GABA receptors
- Nicotinic acetylcholine receptors

# Photo-control of endogenous GABA$_A$ receptors across all spatial scales



Photo-control phenocopies the normal expression profile

α1-GABA$_A$ is only at synapses

Cellular scale

Systems scale

Photoswitch-ready α1 subunit (T125C) knock-in mouse

Photo-control of the stimulus-elicited gamma oscillation *in vivo*

Lin et al., Neuron 2015 (in press)

# Genetically Encoded Reporters of Integrated Neural Activity for Functional Mapping of Neural Circuitry

Overarching goal: develop *in vivo* reporters of the recent history of neuronal activity based on the Kv2.1 channel



Kv2.1 is highly expressed in brain neurons

Kv2.1 phosphorylation can be extremely cell-specific

A dephosphospecific Kv2.1 indicator should report recent neuronal activity
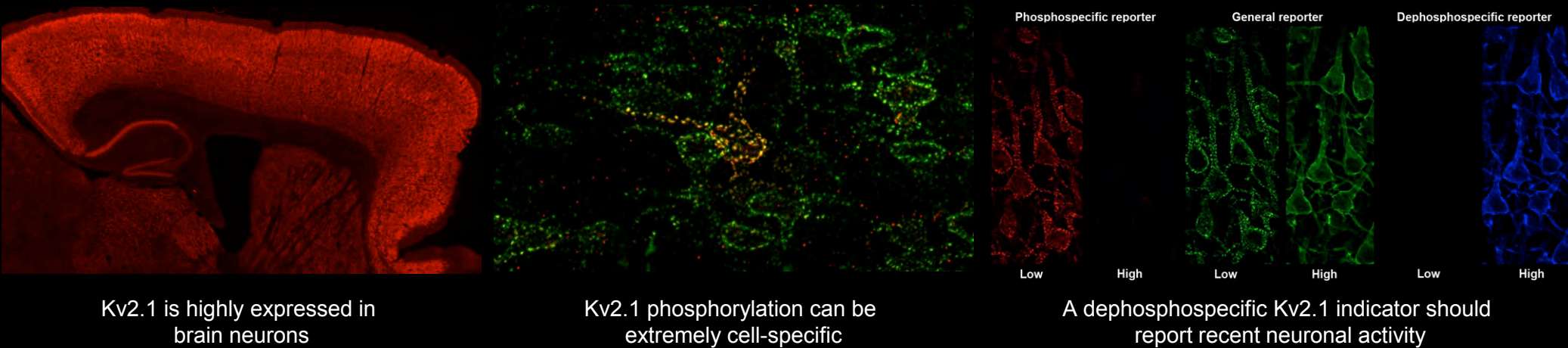
Develop reporters of Kv2.1 conformational changes during voltage activation, and activity-dependent Kv2.1 phosphorylation.

Genetically Encoded Small Illuminants (GESIs), peptides that increase dye fluorescence by state-dependent binding.

Screen combinatorial peptide libraries using one-bead, one compound (OBOC) technology modified for GESI peptides.

Insert GESI sequences into Kv2.1 as state-dependent reporters of conformational changes during voltage activation, and activity-dependent Kv2.1 dephosphorylation.
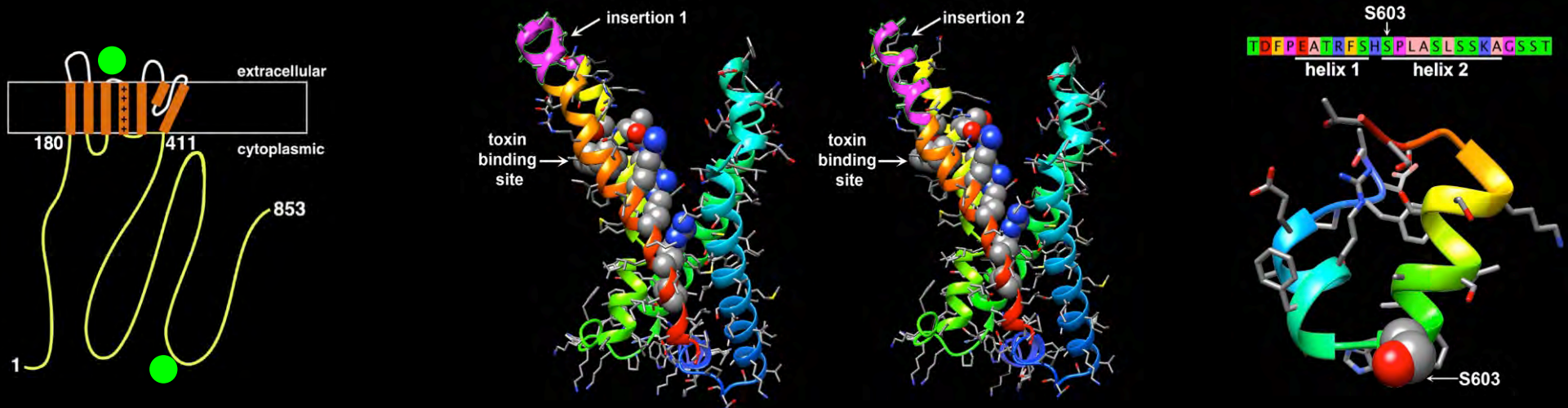
# Novel sensors of integrated neural activity-ion channel variants that capture and turn-on fluorescent dyes when activated

## Identification of Voltage Sensor Reporter by OBOC

≈2.5M beads have been screened to identify OBOC voltage sensor reporters from both 6 and 9 amino acid insertion sequences.
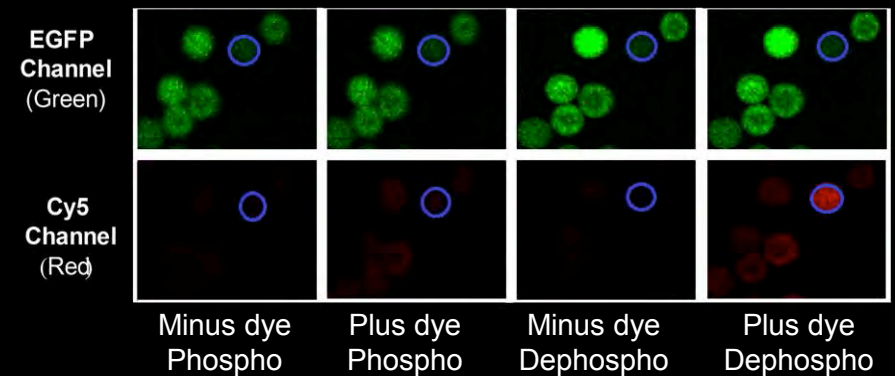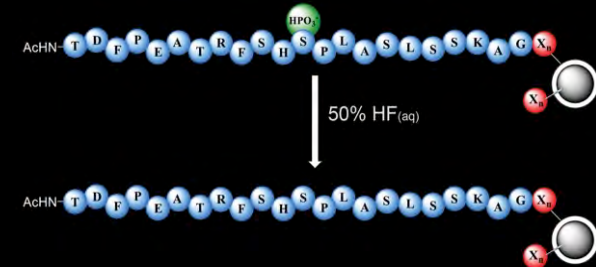
6 amino acid insertion

9 amino acid insertion
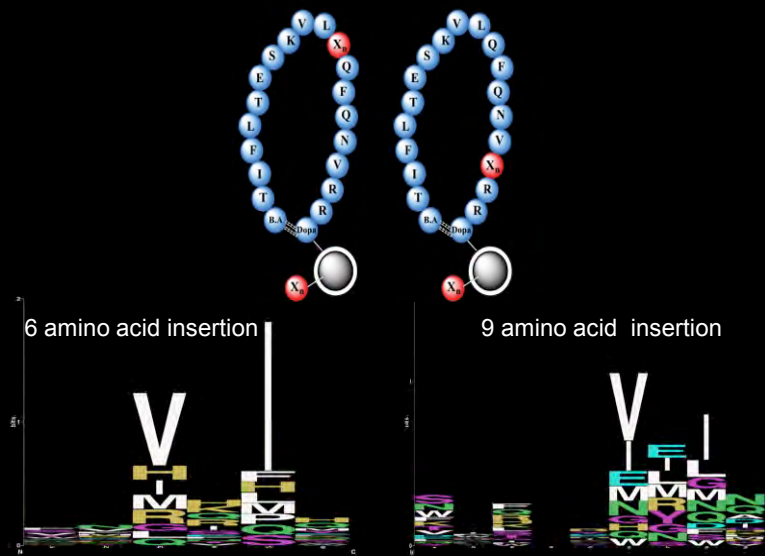
Voltage sensor GESI hit peptides show sequence convergence

Before BCP

After 1 uM BCP

After 100 mM KCl

EGFP

BCP

A  B  C  D
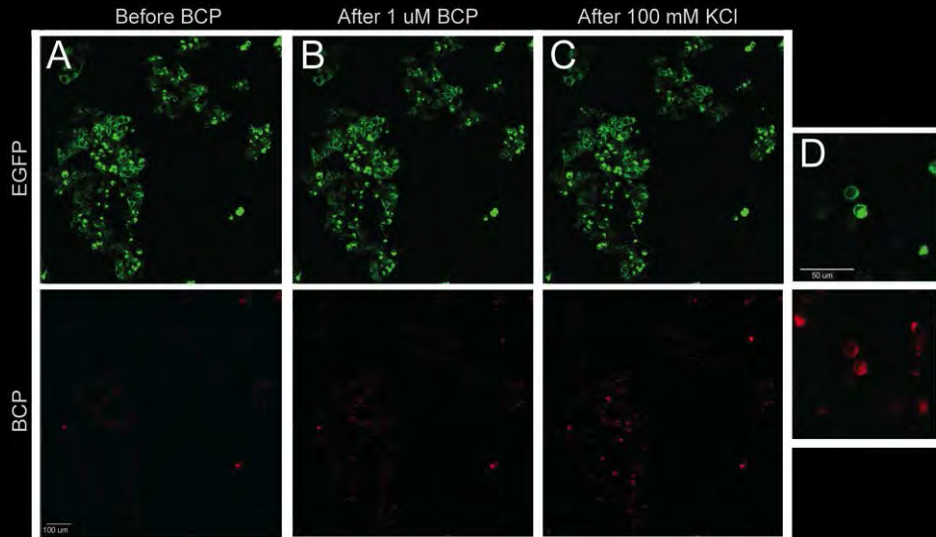
Dye binding to Kv2.1 with NFRHIE insertion expressed in HEK293-Kir cells.

## Identification of S603 Dephosphorylation Reporter by OBOC

≈700K beads have been screened to identify OBOC dephosphorylation reporters from both 6 and 9 amino acid insertion sequences.
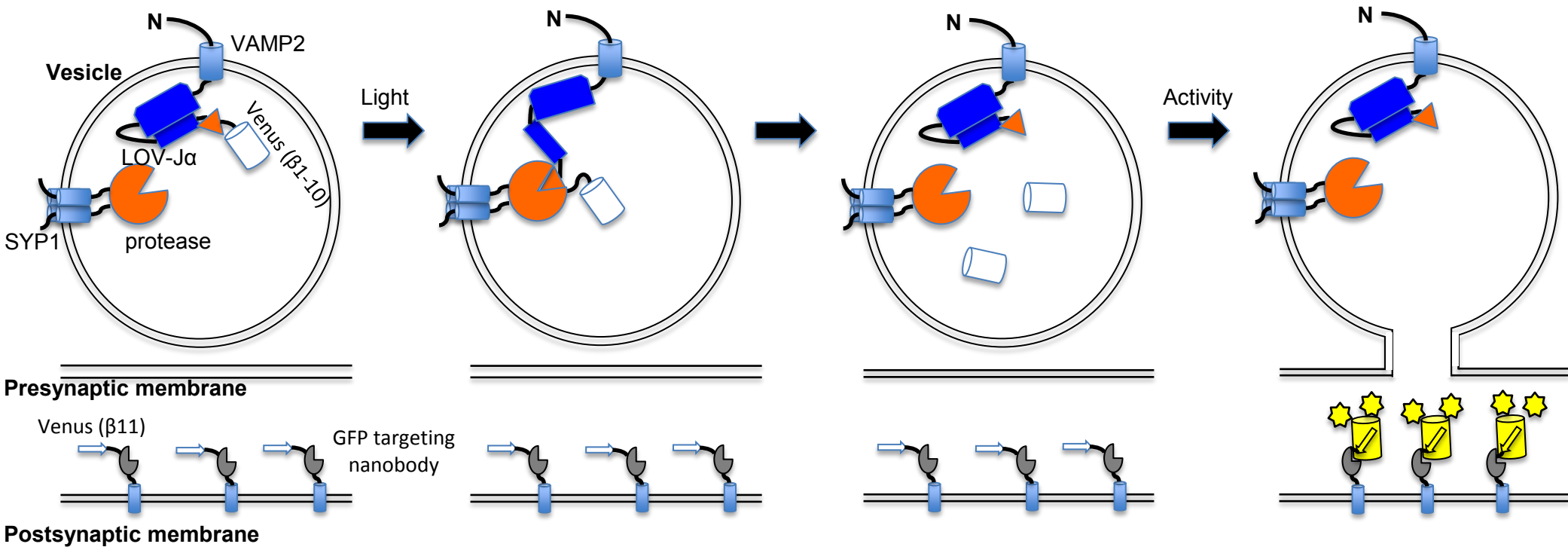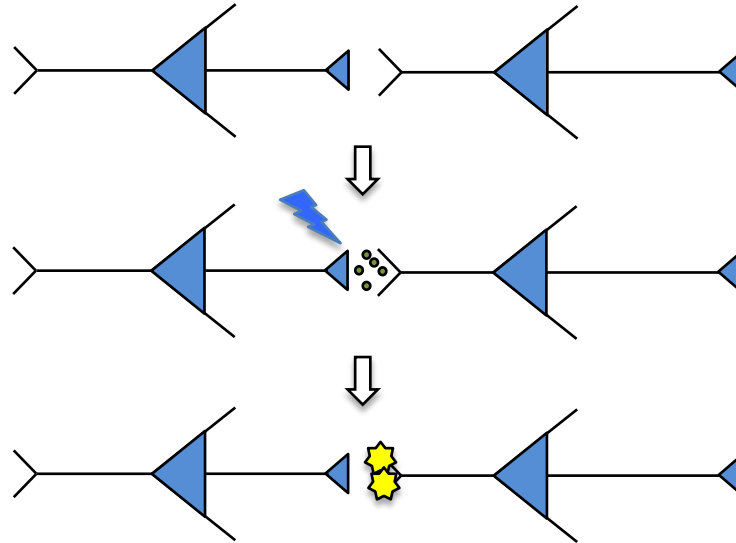
AcHN–T D F P E A T R F S H S P L A S L S S K A G $X_n$

HPO$_3$

50% HF$_{(aq)}$

AcHN–T D F P E A T R F S H S P L A S L S S K A G $X_n$

EGFP Channel (Green)

Cy5 Channel (Red)

Minus dye Phospho | Plus dye Phospho | Minus dye Dephospho | Plus dye Dephospho

| Bead | Delta F | Tau | $X_6$ | $X_5$ | $X_4$ | $X_3$ | $X_2$ | $X_1$ |
|---|---|---|---|---|---|---|---|---|
| 5521 | 0.627949 | 9331.453 | M | L | N | F | I | Q |
| 32719 | 0.537649 | 12240.21 | K | L | S | A | K | L |
| 97949 | 0.520705 | 10374.31 | H | D | L | D | F | I |
| 74057 | 0.518227 | 6915.307 | N | D | I | A | F | F |
| 11486 | 0.494928 | 10723.37 | M | L | N | D | F | I |
| 21769 | 0.490665 | 10419.91 | V | Q | E | N | I | L |

Currently generating/characterizing full length Kv2.1 with insertions in voltage sensor and near S603 phosphorylation site for changes in dye binding as induced by membrane depolarization to trigger Kv2.1 voltage-induced activation, and stimuli that trigger Kv2.1 dephosphorylation, respectively.
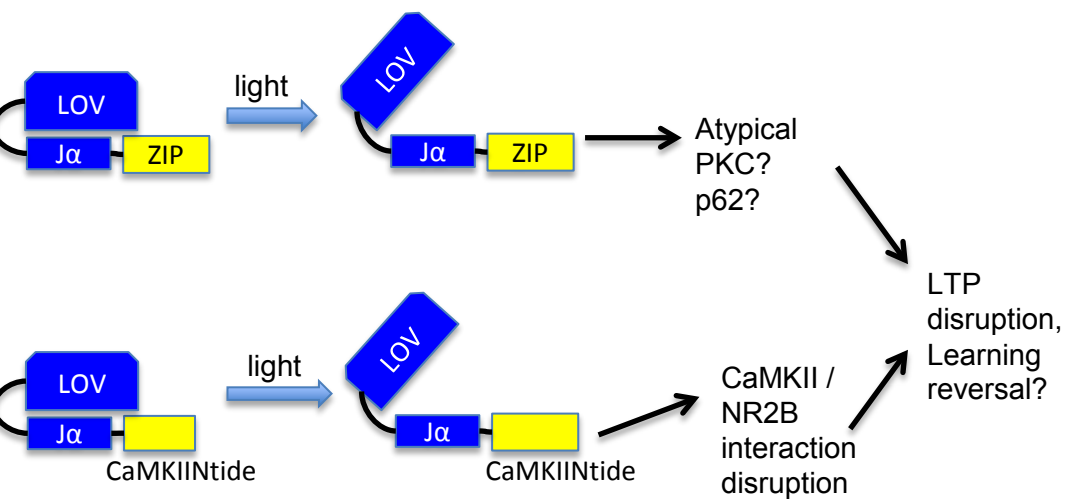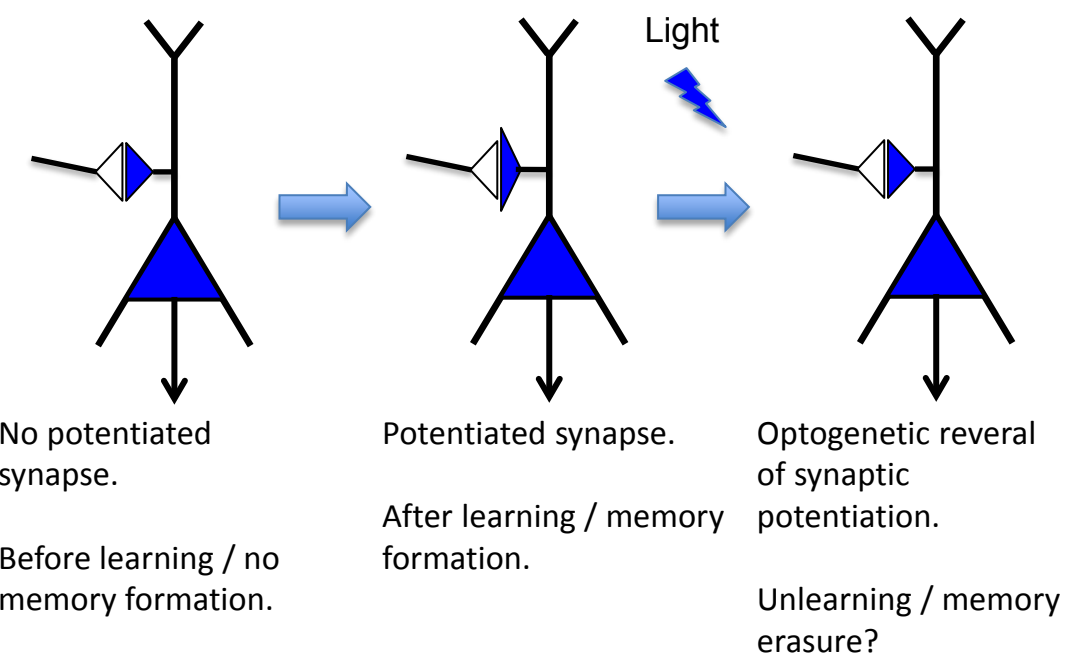
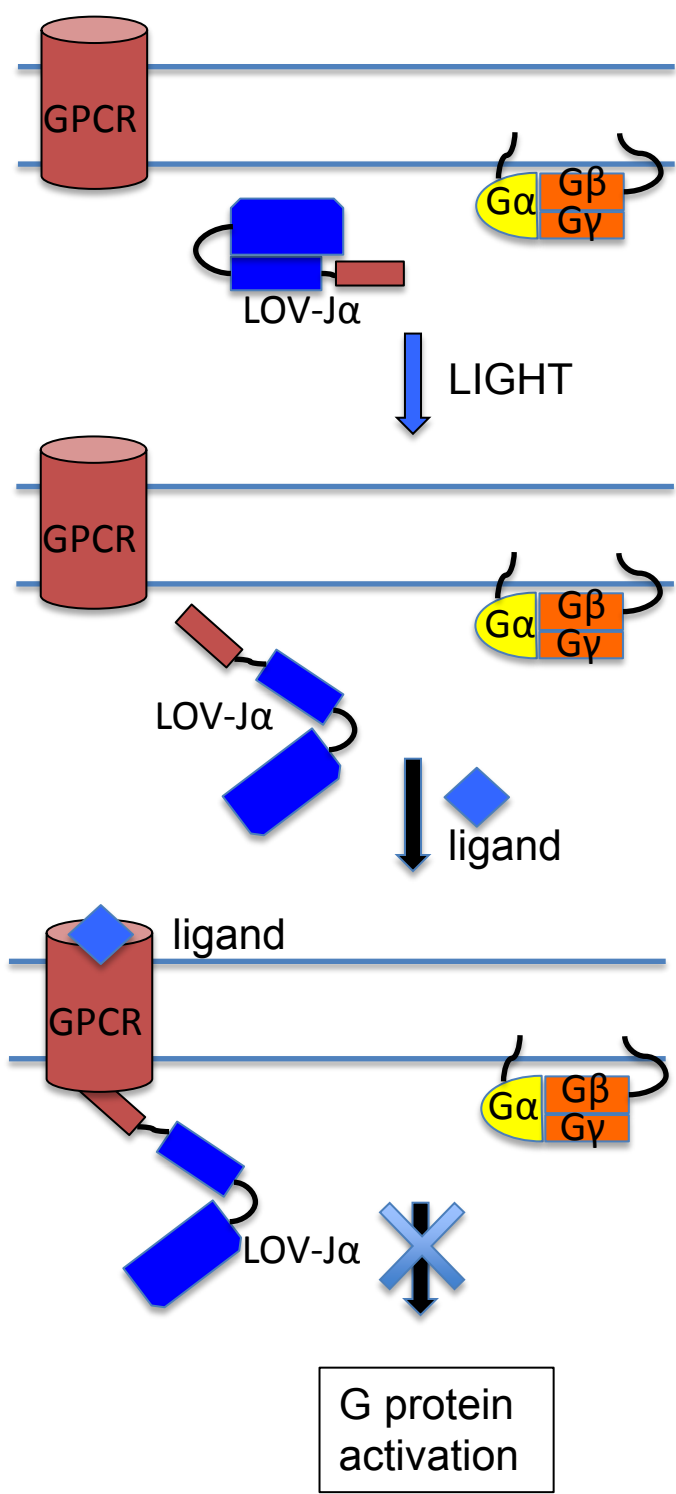# Optogenetic mapping of synaptic activity and control of intracellular signaling

**1. Mapping of synaptic activity**

## 2. Optogenetic reversal of Long-term potentiation



Light

No potentiated synapse.

Before learning / no memory formation.

Potentiated synapse.

After learning / memory formation.

Optogenetic reveral of synaptic potentiation.

Unlearning / memory erasure?

LOV

Jα  ZIP

light

LOV

Jα  ZIP

Atypical PKC? p62?

LOV

Jα

CaMKIINtide

light

LOV

Jα

CaMKIINtide

CaMKII / NR2B interaction disruption

LTP disruption, Learning reversal?

## 3. Optogenetic disruption of GPCR pathways



GPCR

Gα  Gβ Gγ

LOV-Jα

LIGHT

GPCR

Gα  Gβ Gγ

LOV-Jα

ligand

ligand

GPCR

Gα  Gβ Gγ

LOV-Jα

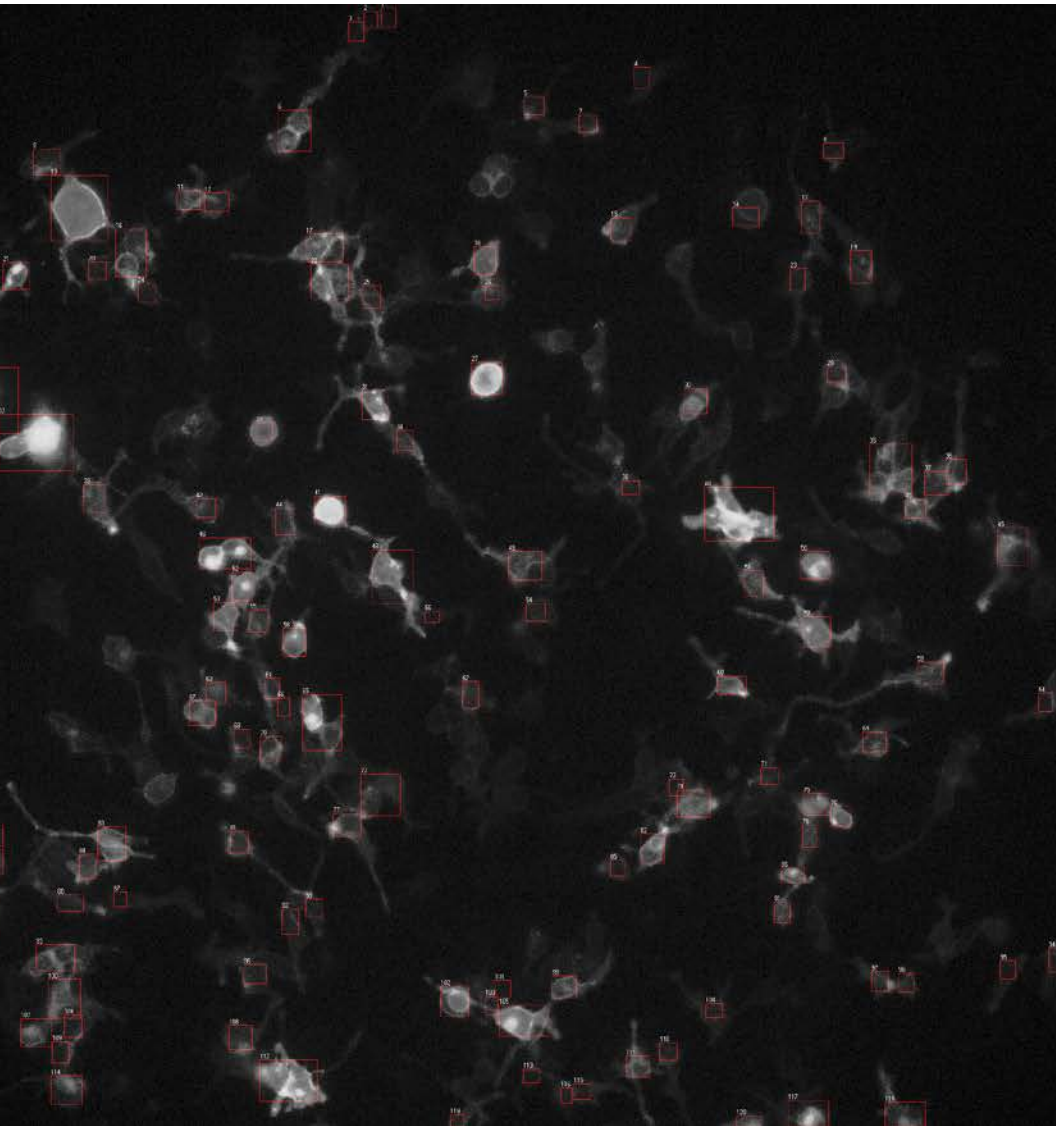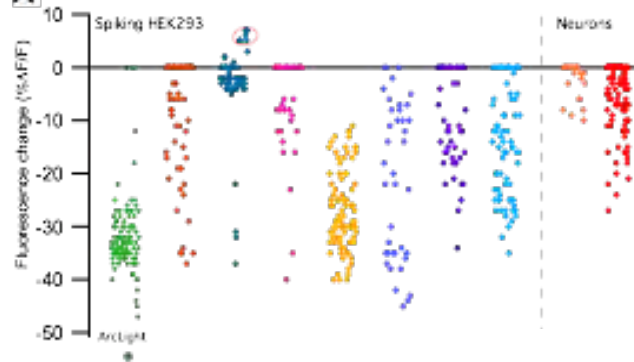G protein activation

# Development of Protein Based Voltage Probes (U01NS090565+S1)

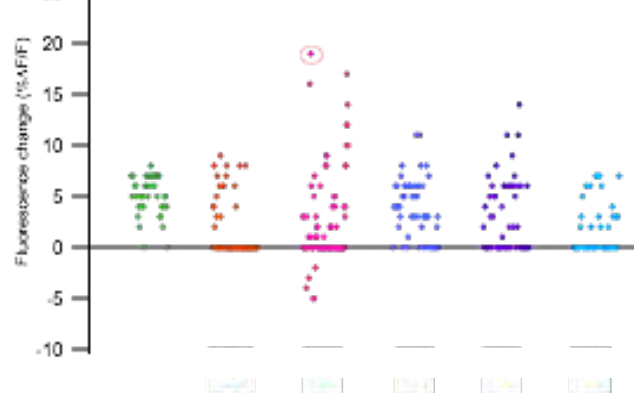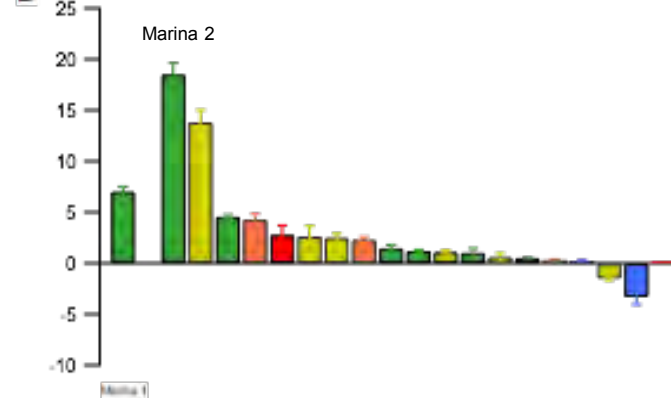Vincent A. Pieribone[1,2], Michael Nitabach[2], and Robert Campbell[3]

**A** Marina 1

Spiking HEK293       Neurons

Fluorescence change (%ΔF/F)

ArcLight

**B**

Marina 1

- Nonpolar, aliphatic side group
- Polar, uncharged side group
- Aromatic side group
- Negatively charged side group
- Positively charged side group

ArcLight

**C** Marina 2

Fluorescence change (%ΔF/F)

**D**

Marina 2

Marina 1

**E** Marina 3

Fluorescence change (%ΔF/F)

**F**

Marina 3

Marina 2

Y   L   V   A   M   C

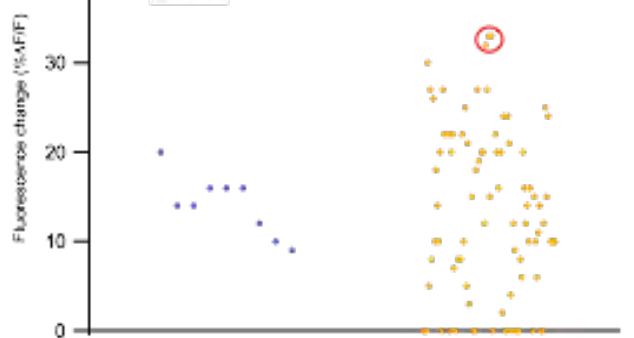Marina3
Marina2
Marina1
ArcLight

Marina3      Hippocampal neurons

1 %

Electrical
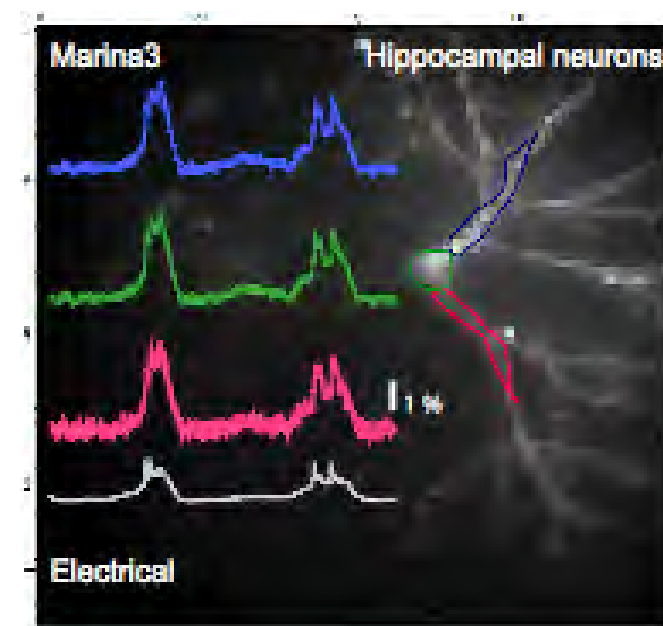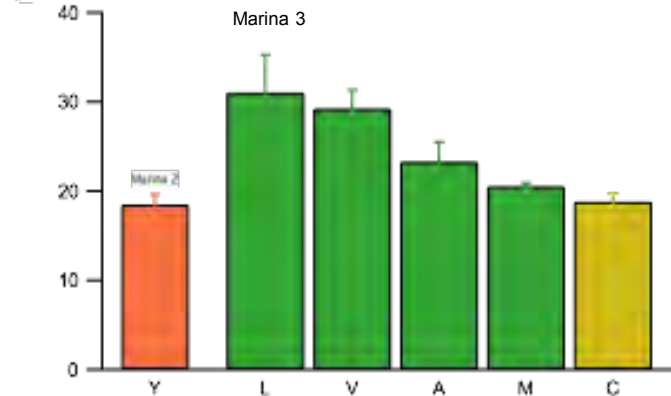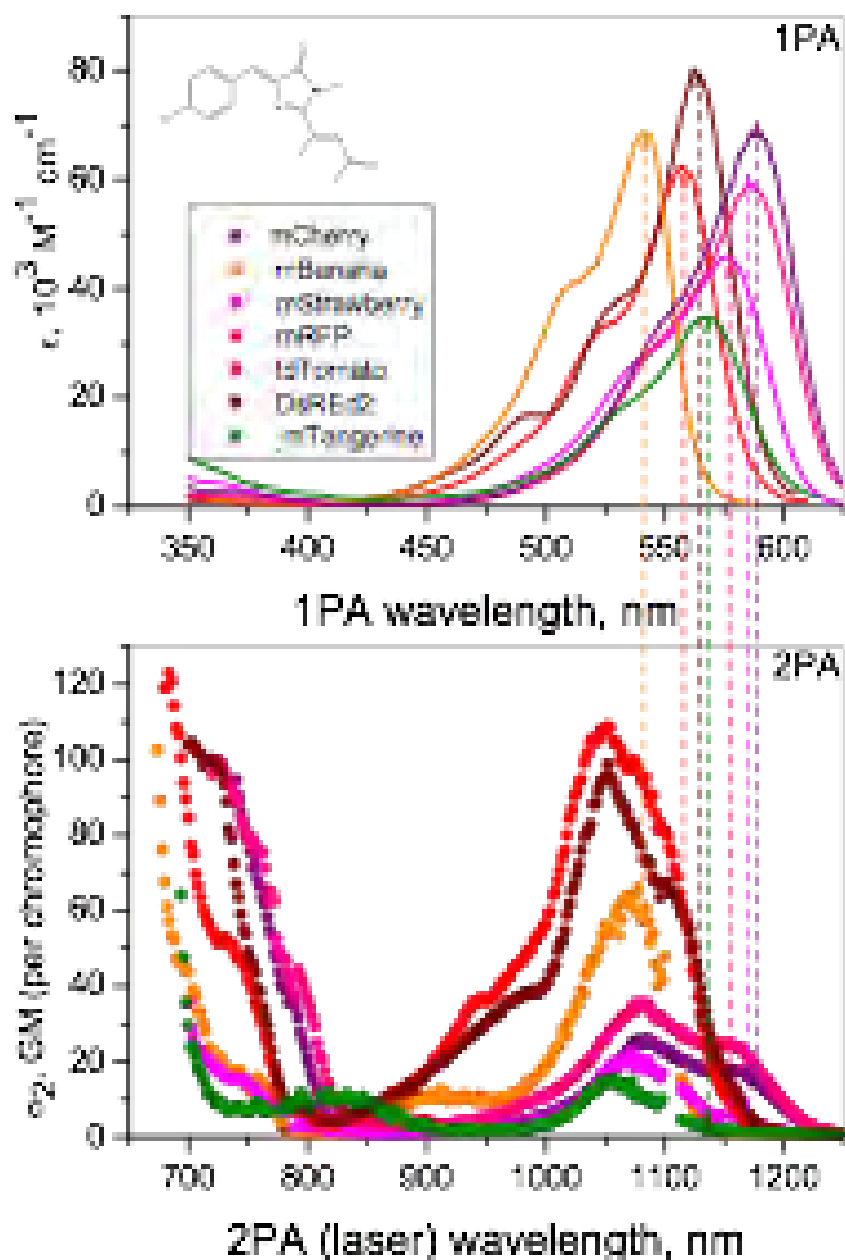
# One and two photon absorption involve different molecular parameters. Our goal is to optimize fluorescent proteins and sensors for multi-photon microscopy.
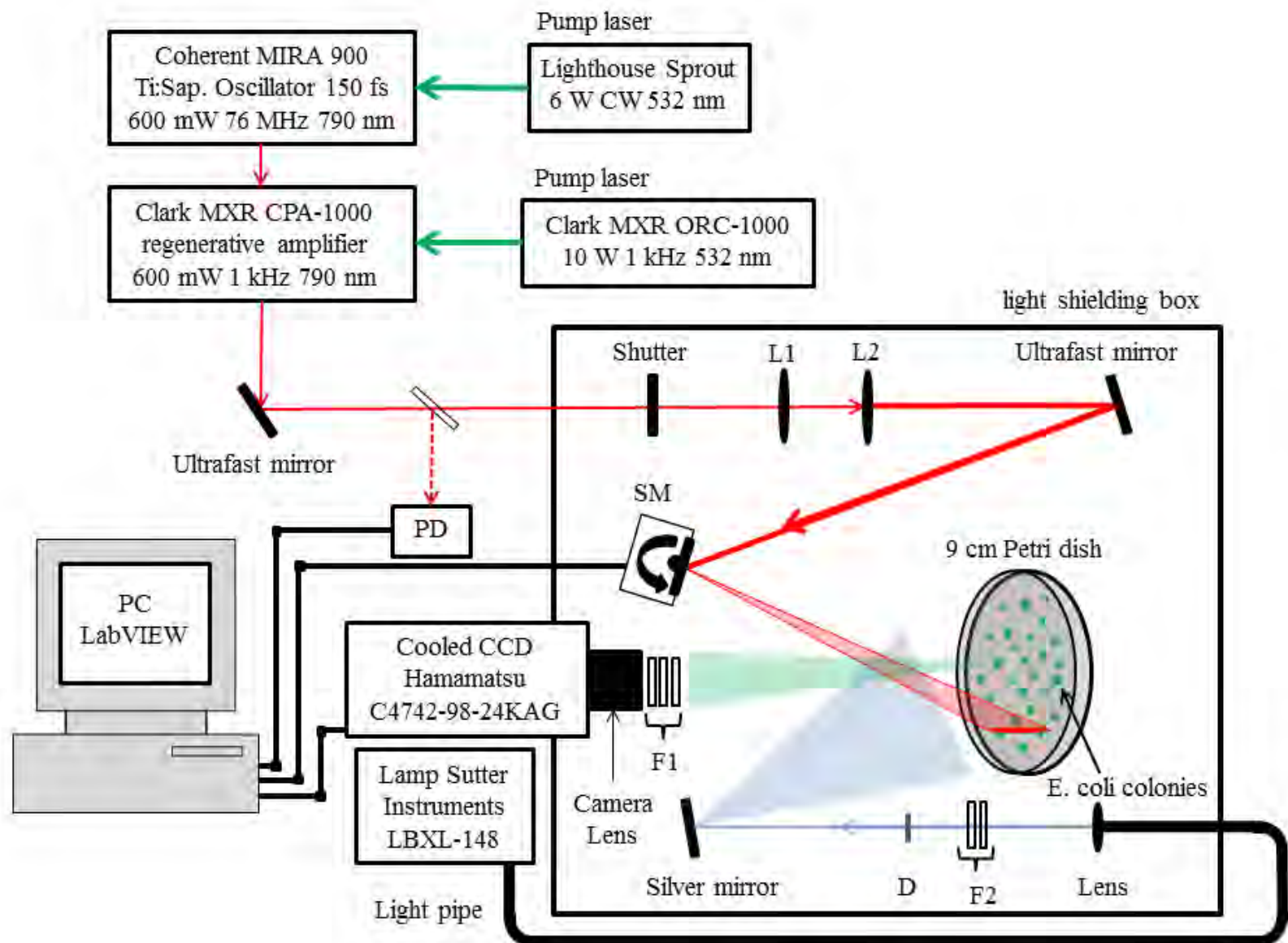
Investigators: Aleksander Rebane, Robert Campbell, Mikhail Drobijev, Thomas Hughes
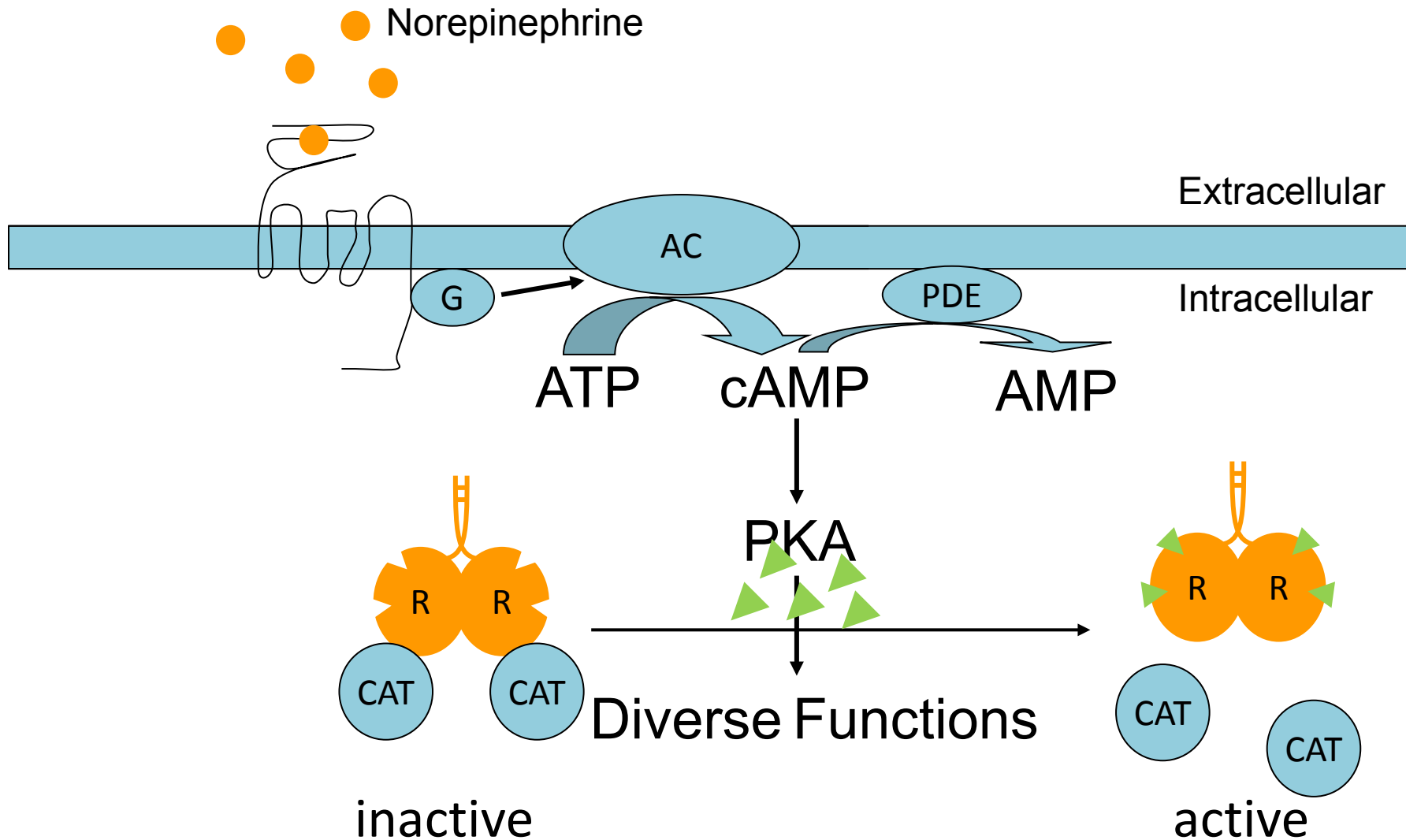
$$\varepsilon = B\mu^2$$

$$\sigma_2 = A|\Delta\mu|^2|\mu|^2$$

# The Bazooka: a new way to evolve FPs for 2P brightness.
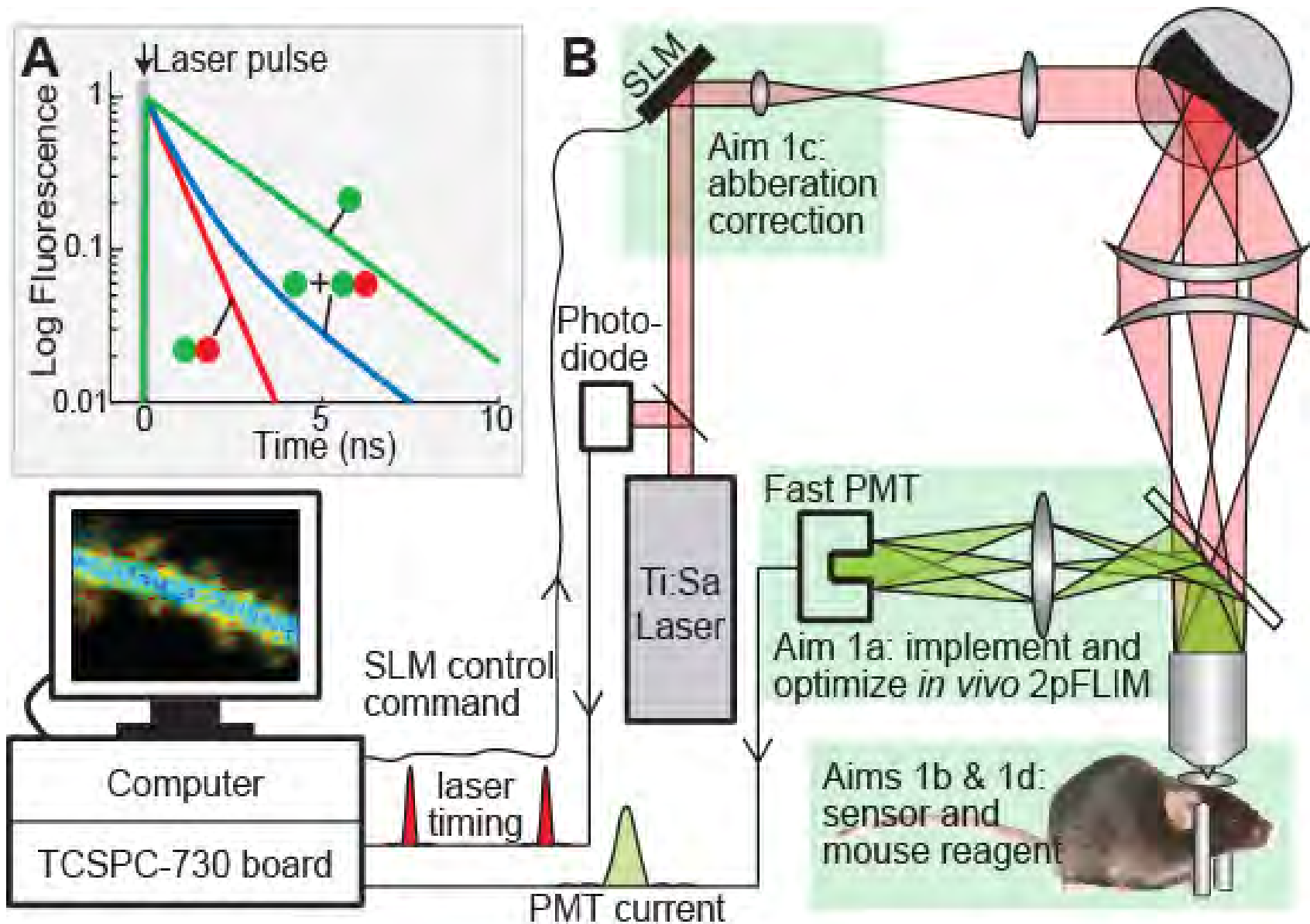
# *In vivo* monitoring of neuromodulation by imaging subcellular signaling pathways



*Investigators: Haining Zhong and Tianyi Mao*

# Approach: *in vivo* FRET imaging of novel PKA sensors

# POTENTIOMETRIC PHOTOACOUSTIC IMAGING OF BRAIN ACTIVITY ENABLED BY NEAR INFRARED TO VISIBLE LIGHT CONVERTING NANOPARTICLES (1R21EY026411, 09/01/2015 – 06/30/2017)

PIs: Paras Prasad[1] and Jun Xia[2]

[1]Department of Chemistry

[2]Department of Biomedical Engineering

University at Buffalo, The State University of New York

# The problem

- Voltage-sensitive dye (VSD) imaging allows for real-time probing of the neuronal activity via non-invasive optical methods.

- However, VSDs have limited use in deep brain imaging, because they require excitation in the visible range.

- Development of NIR-sensitive VSDs is hampered as the larger π-electron system in NIR-active dyes implies smaller sensitivity towards changes of the cell membrane potential.

- VSD imaging with tissue-penetrating near-infrared light will vastly advance neuroimaging.

# The solution

- We propose to address this problem through the convergence of biocompatible upconversion (UC) nanoparticles (NP) and photoacoustic imaging (PAI).

- UCNPs will serve as nanotransformers that convert skull penetrating NIR light to VIS light, which will be absorbed by the VSDs.

- Photoacoustic effect transforms absorption into acoustic waves, which have low scattering in tissue, allowing for high resolution imaging of VSD absorption.

- Objective of this project is to validate voltage-sensitive upconverting photoacoustic imaging (VSUPAI) *in vitro* and *in vivo*