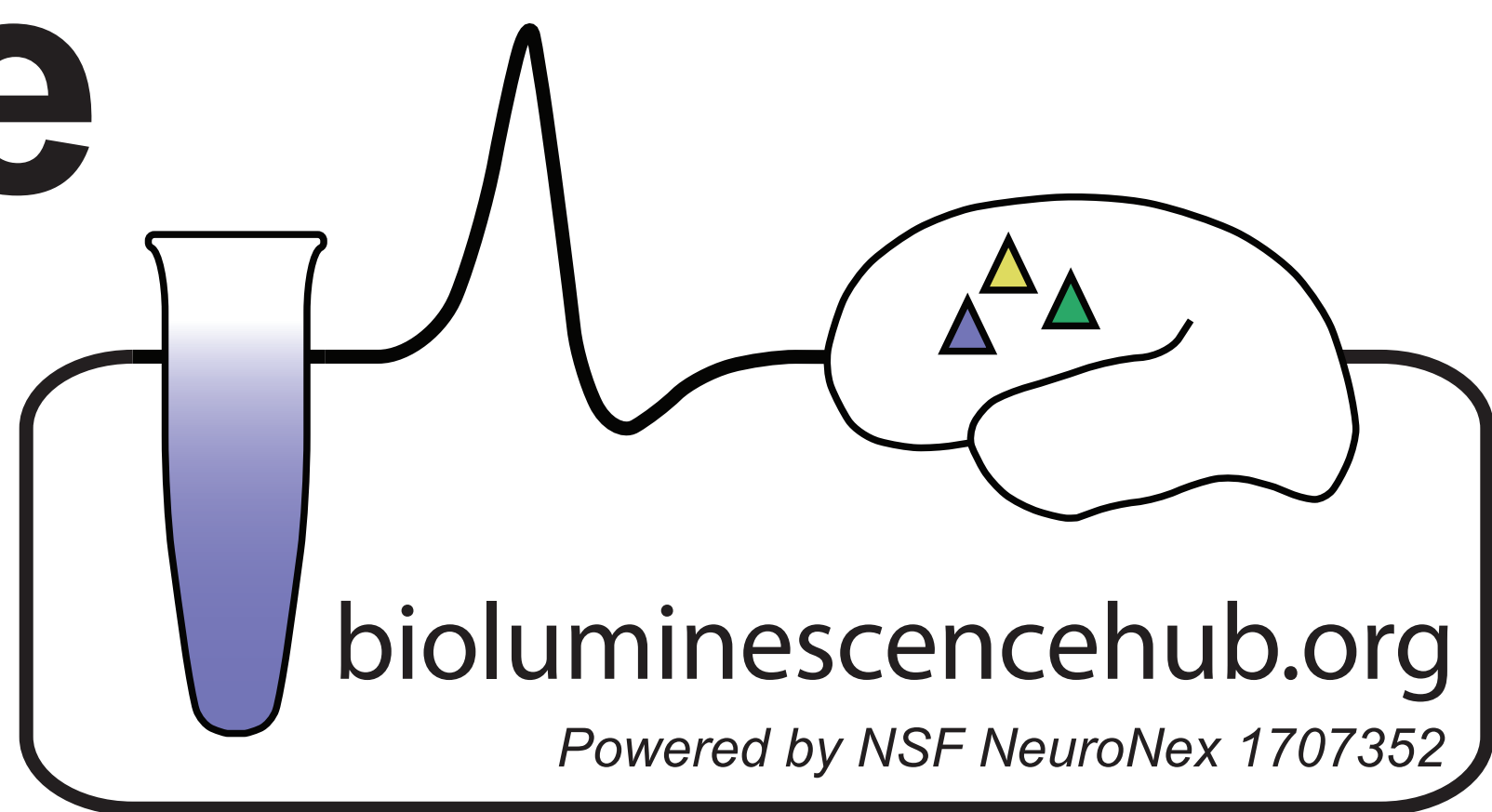


# Distinct Advantages of BioLuminescence for Neuroscience

Christopher I. Moore<sup>1</sup>, Diane Lipscombe<sup>1</sup>, Ute Hochgeschwender<sup>2</sup>, Nathan C. Shaner<sup>3</sup>, Justine J. Allen (Program Manager)<sup>1</sup>

1. Brown University, Providence, RI; 2. Central Michigan University, Mount Pleasant, MI; 3. University of California San Diego, San Diego, CA



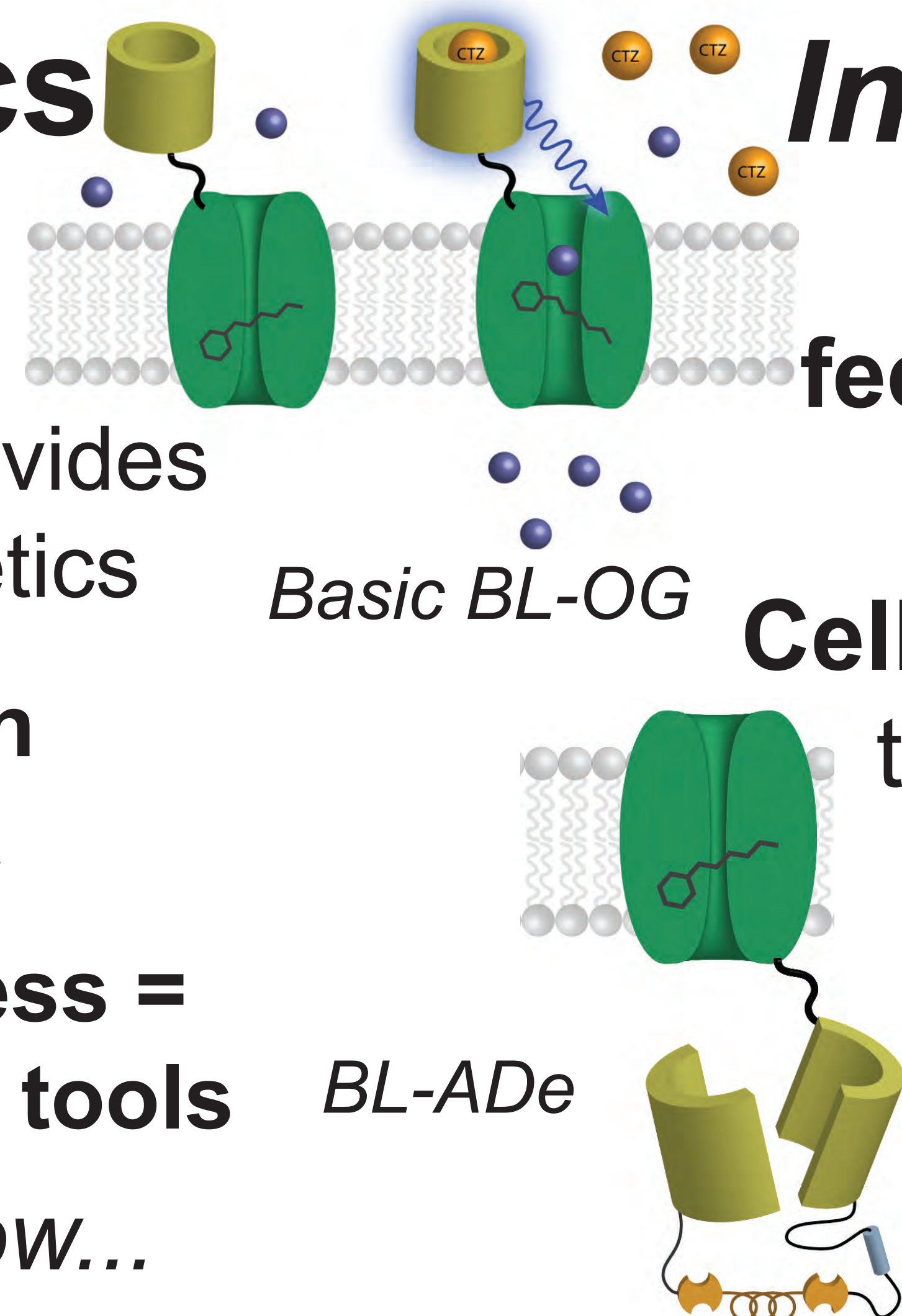
## BioLuminescent-OptoGenetics (BL-OG)

Single molecule provides Chemo- & OptoGenetics

Optical confirmation when drug hits target

OptoGenetic progress = new ChemoGenetic tools

Examples See below...



## Activity-Dependent BL-OG

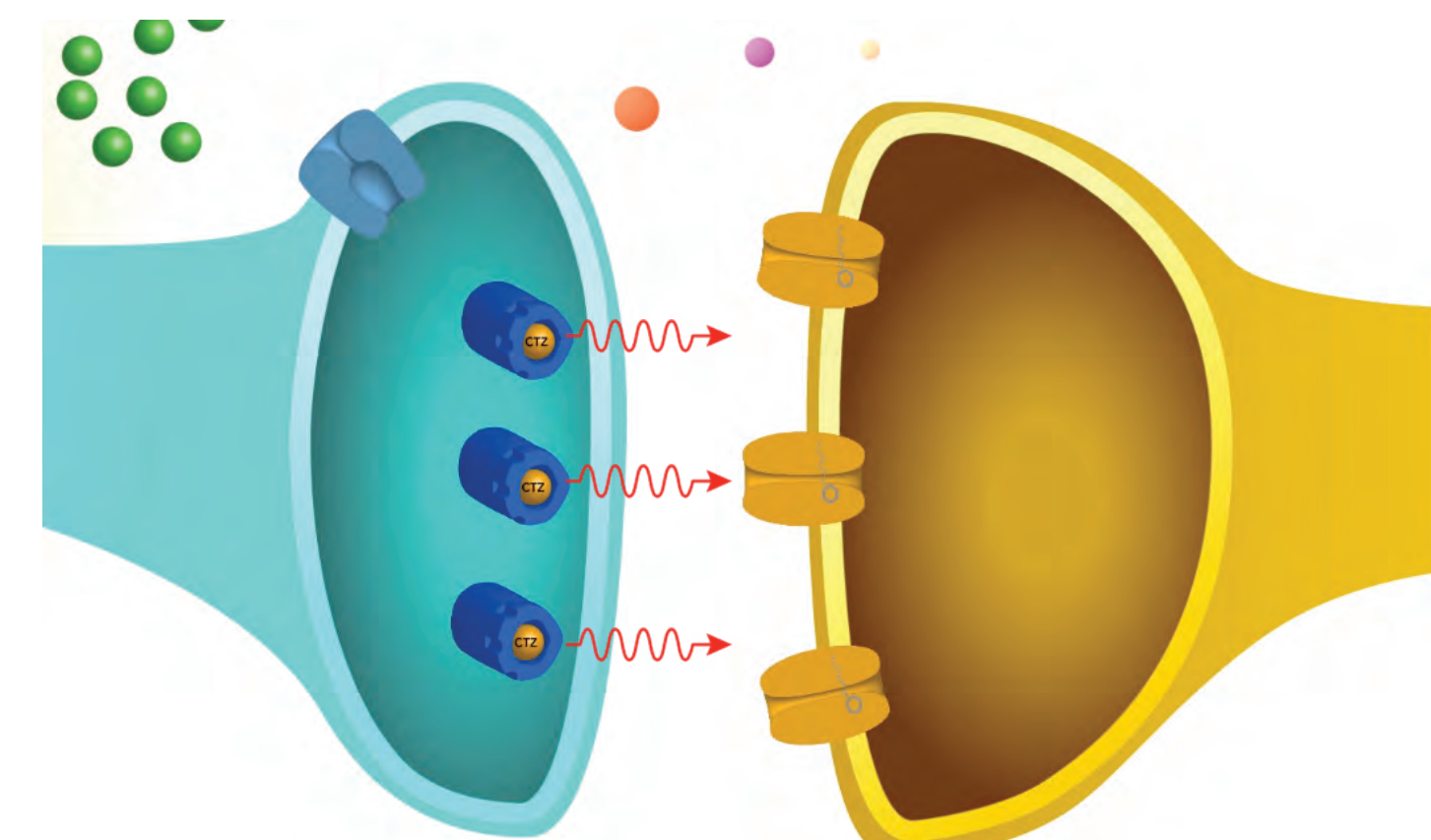
### Intraluminescence

Real-time, all-molecular feedback driven by a local increase in calcium

Cell-specific self-regulation to cease or perpetuate the cell's activity patterns

Example Detect calcium bursts to drive or terminate spindles

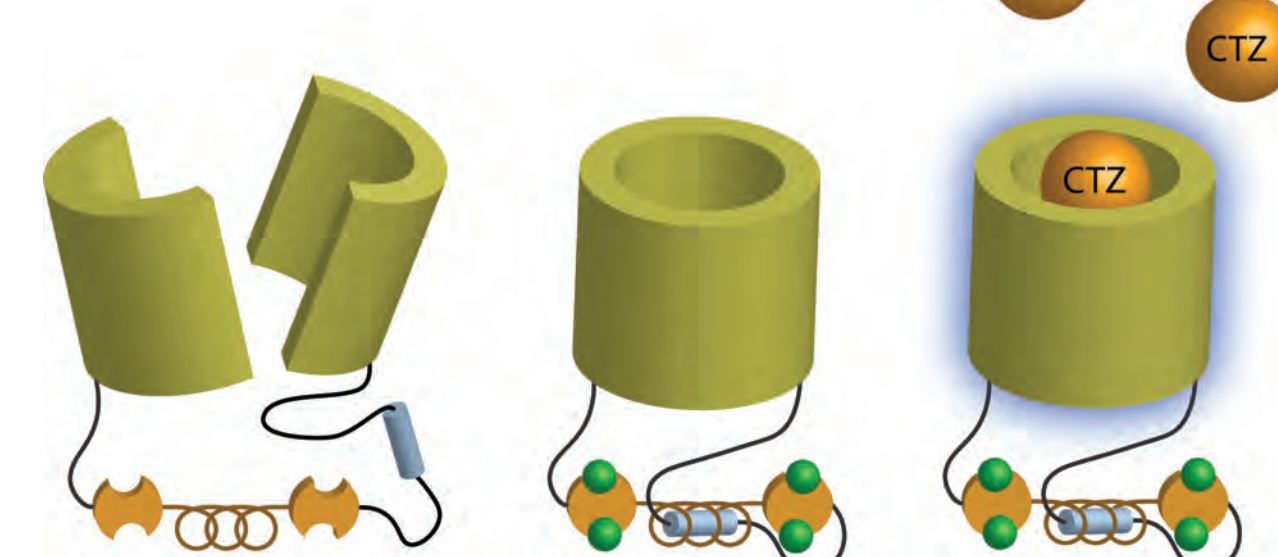
### Interluminescence



All-optical synapses Selectively modulate communication only with endogenous firing

Examples See below...

## Calcium Sensing



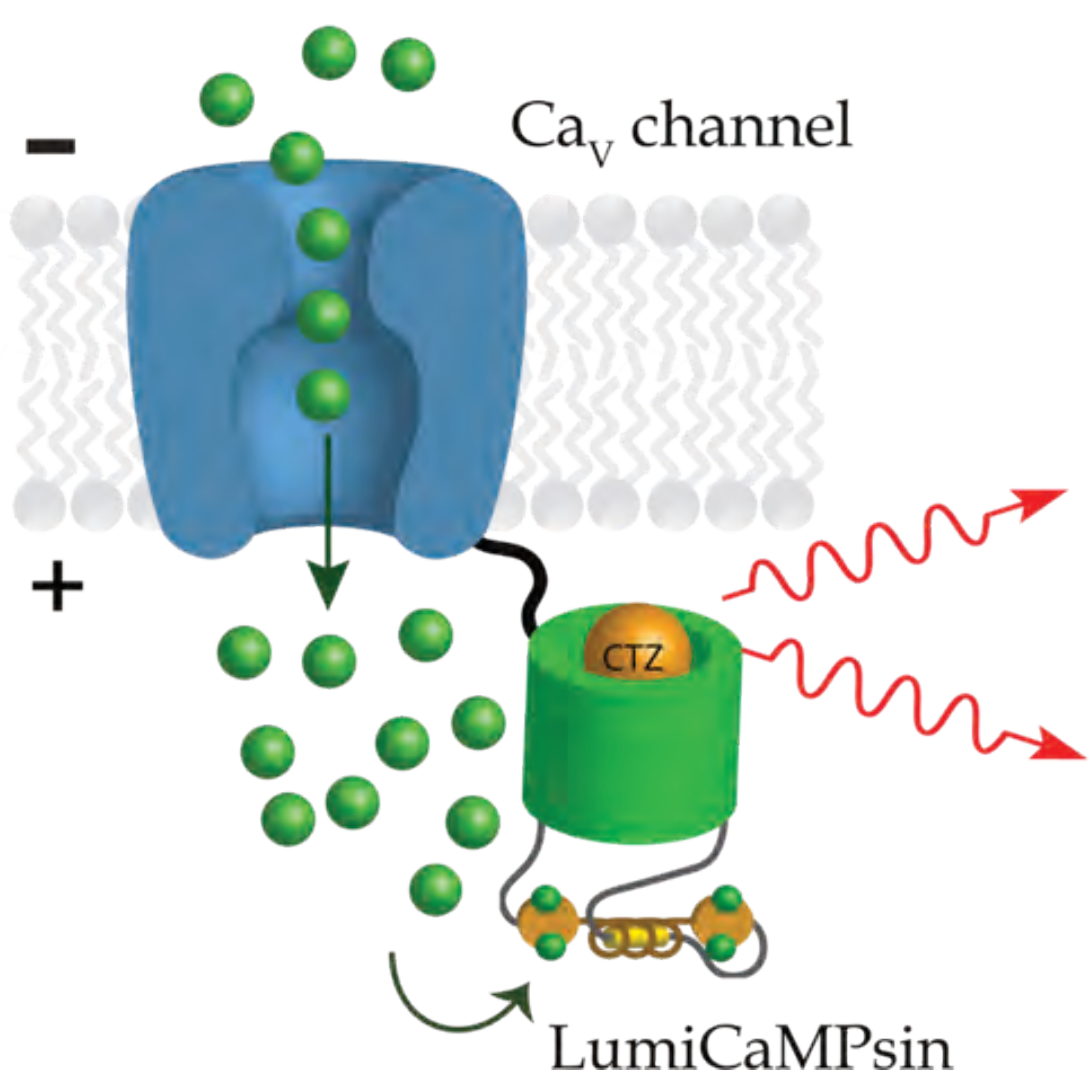
Generating novel BL calcium indicators with several advantages over 1P Fluorescent imaging

Chemical light production avoids: Photodamage Photobleaching Autofluorescence

allows decreased implanted microscope size

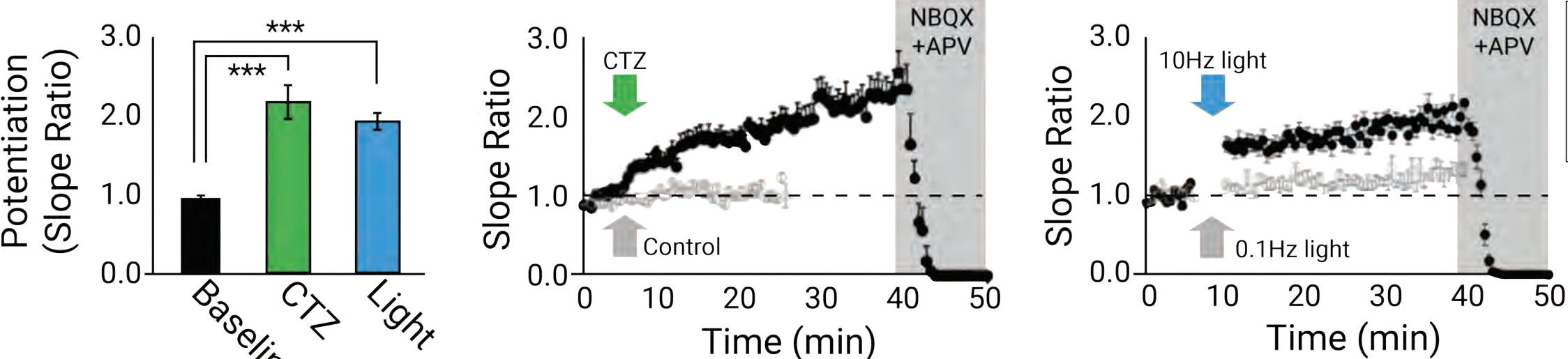
## LumiPoreIns

BL calcium indicators tethered to Ca<sub>v</sub> channels can provide highly selective, nano-domain sensing



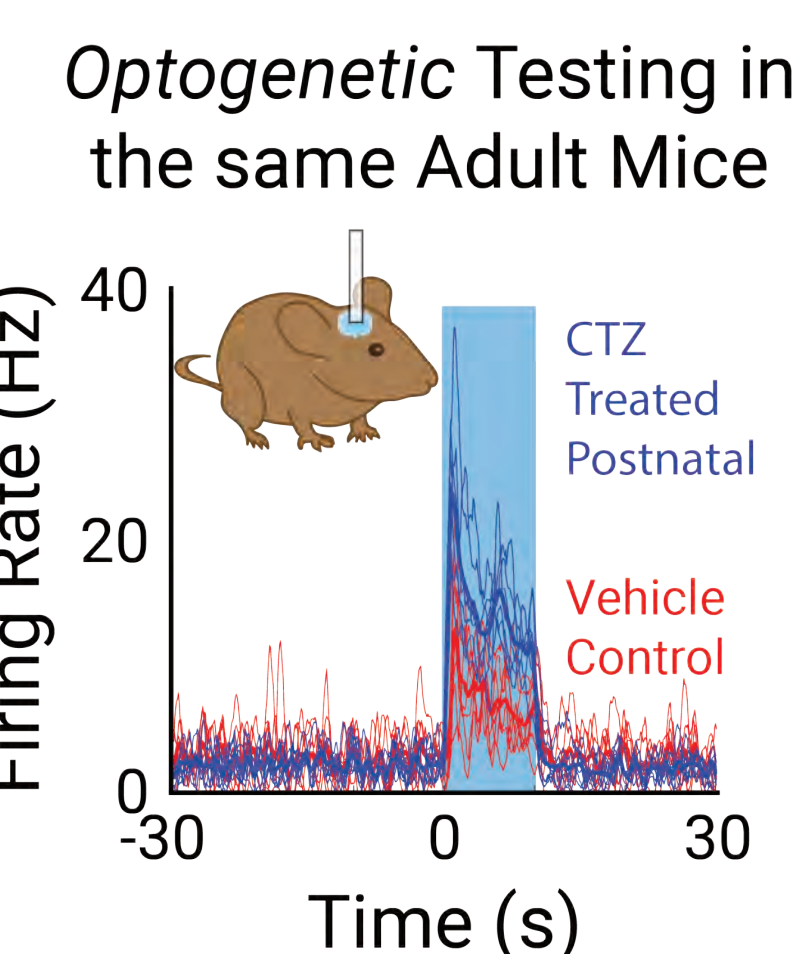
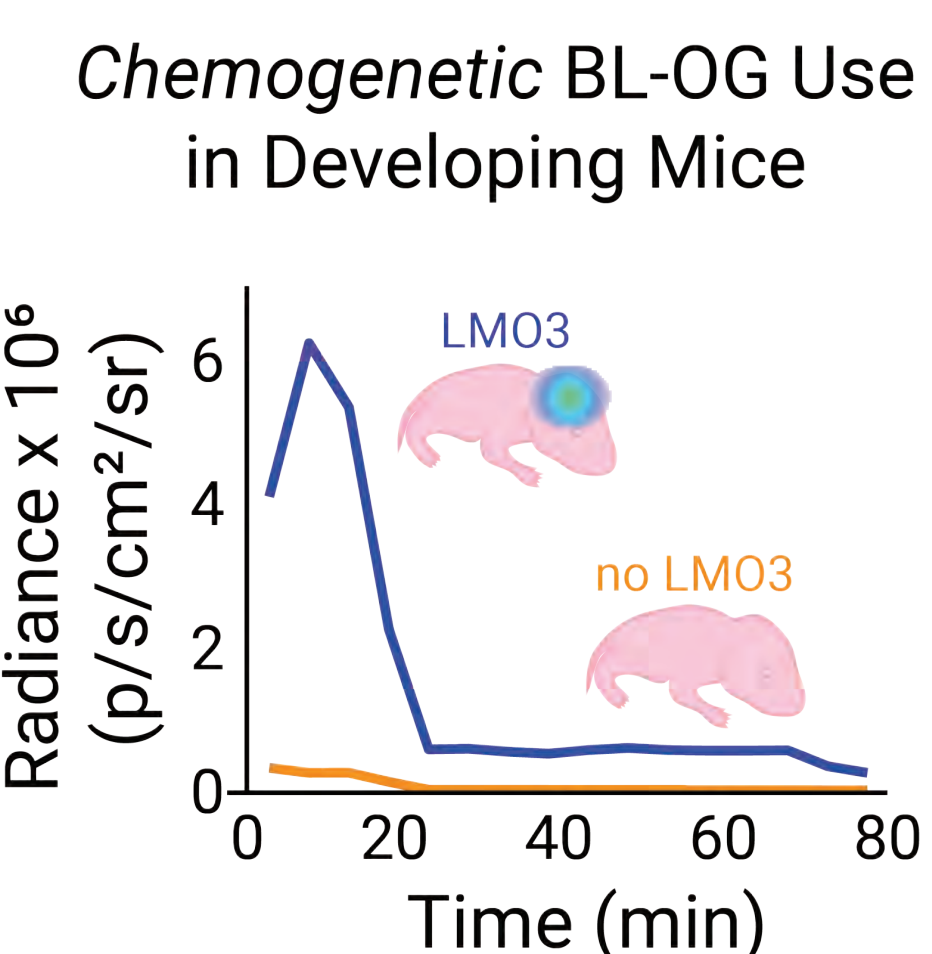
## Chronic *in vivo* BL-OG Enables New Kinds of Hypothesis Testing

IPSC control by LMO3 Post-Stroke: *Chemogenetic* or *Optogenetic* activation drives *Behavioral Recovery* and *Synaptic Enhancement*



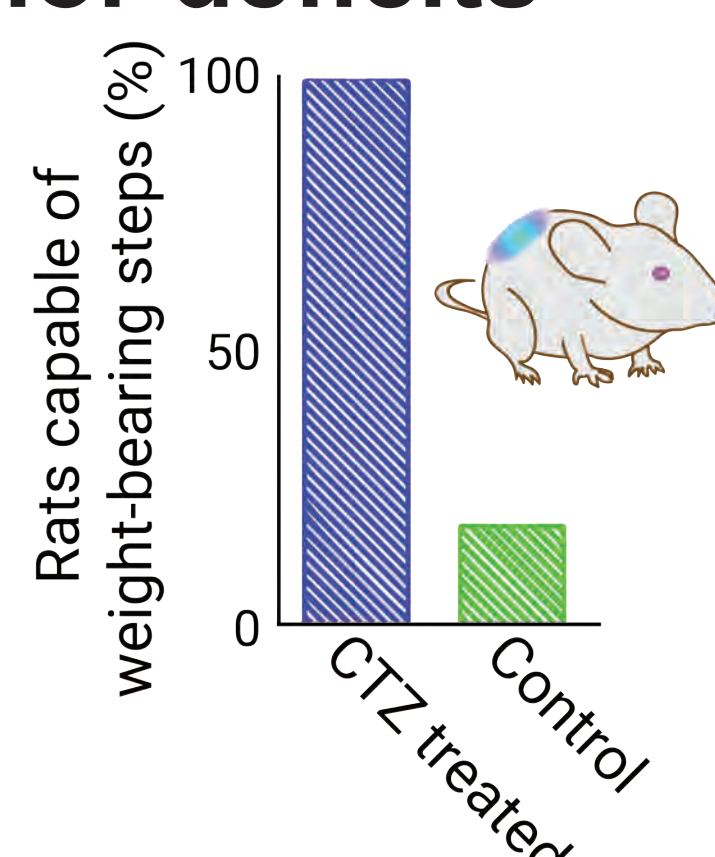
Medendorp...Moore, Hochgeschwender, *iScience* 2021

## Developmental Chemogenetic drive & Adult Optogenetic circuit interrogation



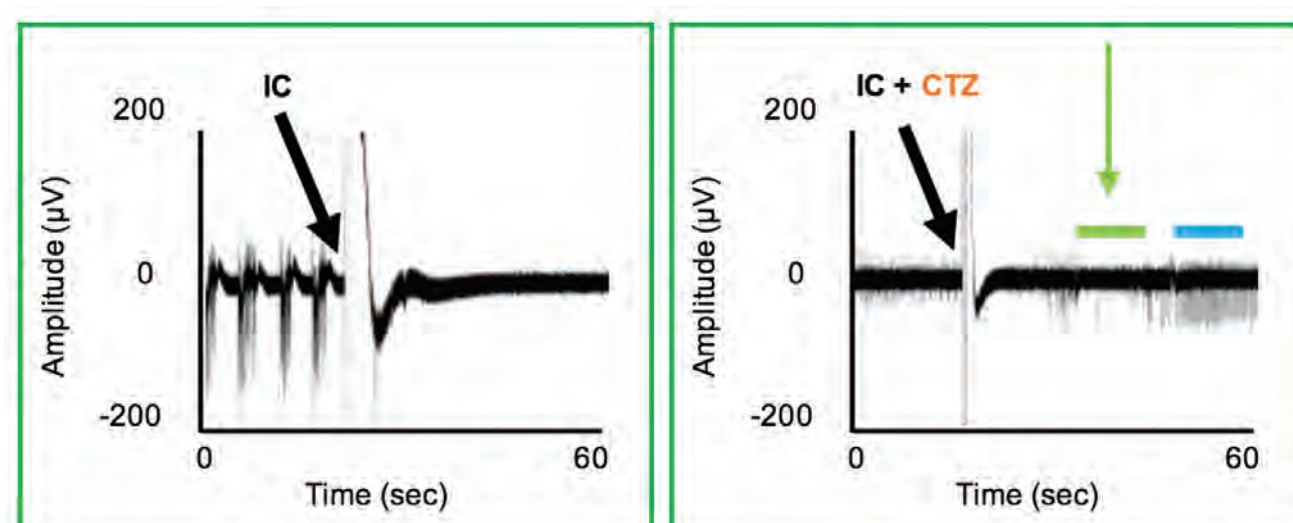
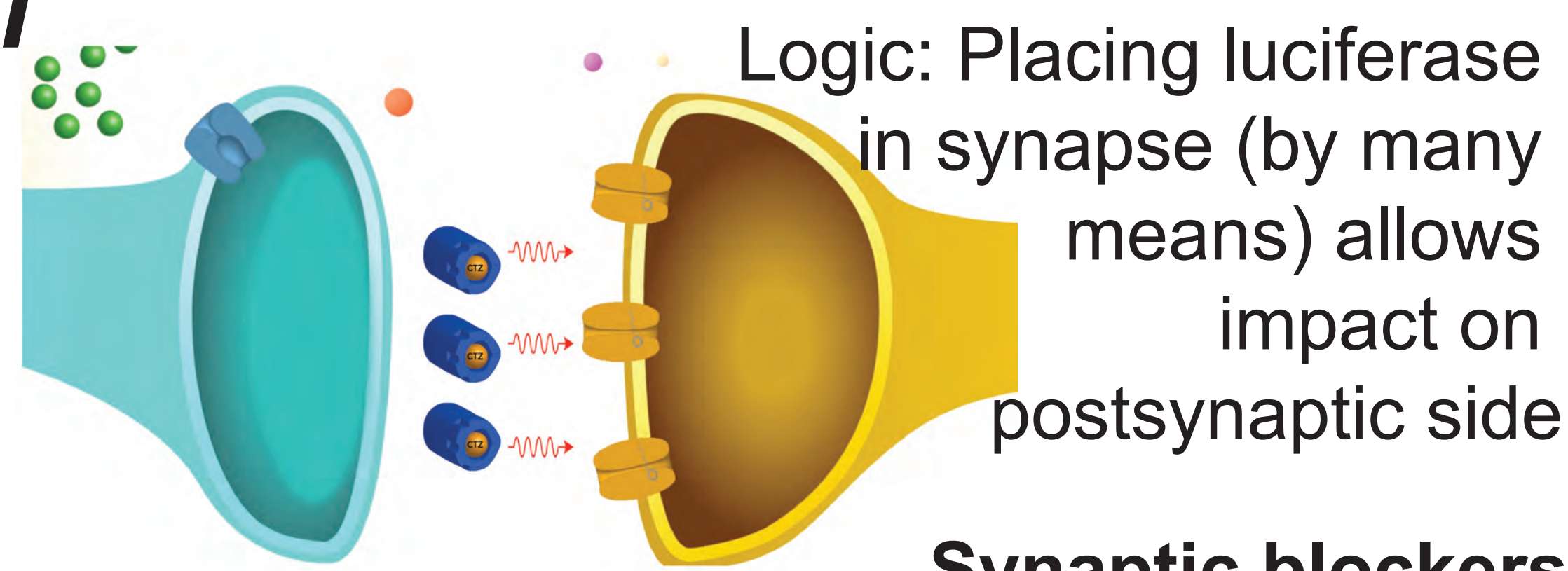
Petersen...Moore, Hochgeschwender, *in review*

## BL-OG stimulation Recovers Locomotor Ability following Spinal Cord Injury

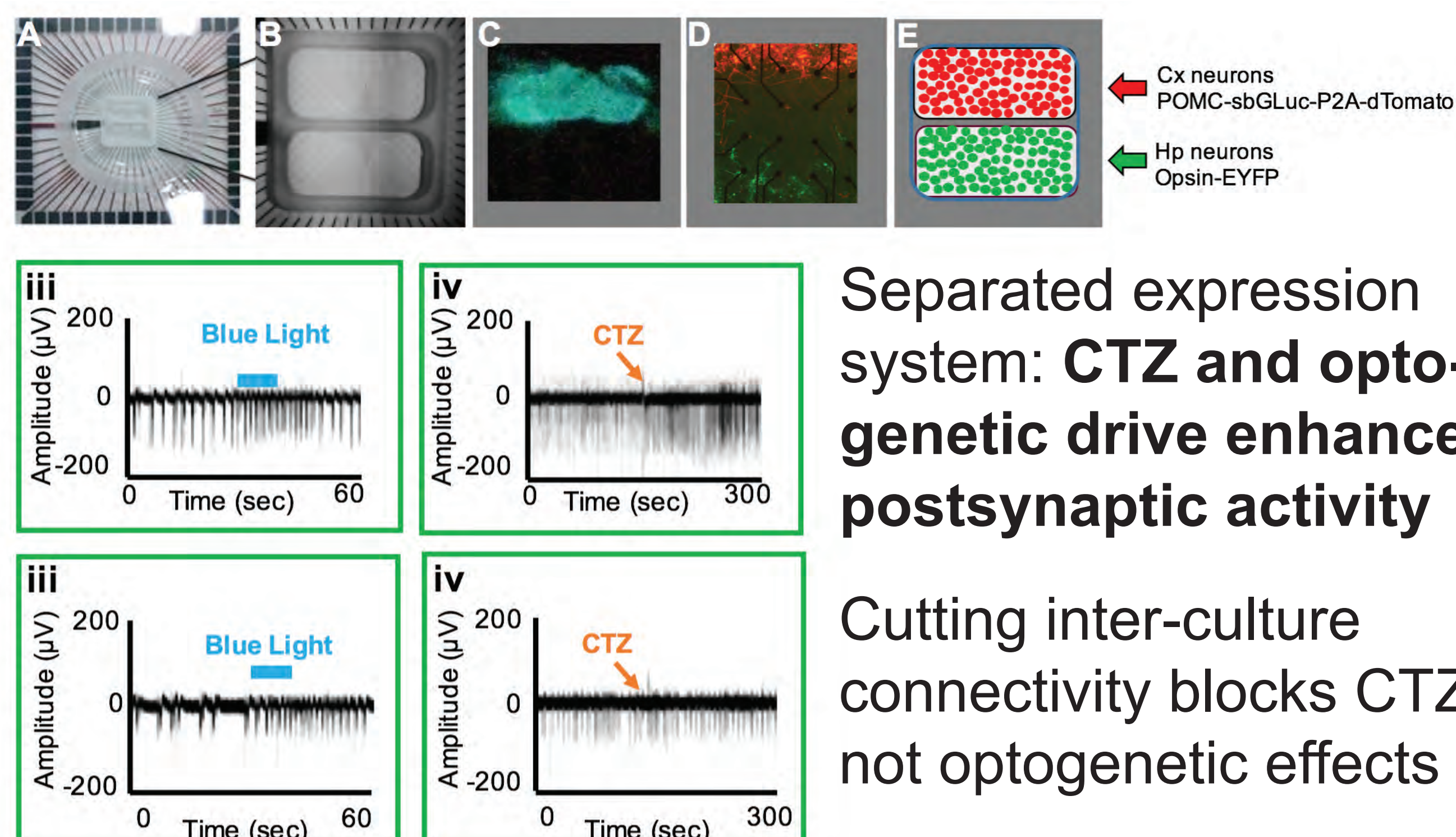


## BL TransEmitters (B-LiTE): Synaptic Luciferase Drives Postsynaptic Optogenetic Targets

Prakash, St. Laurent...Moore, Hochgeschwender, *in revision*



Synaptic blockers do not interfere with CTZ: Green inactivation of step f(n) opsins does block effect



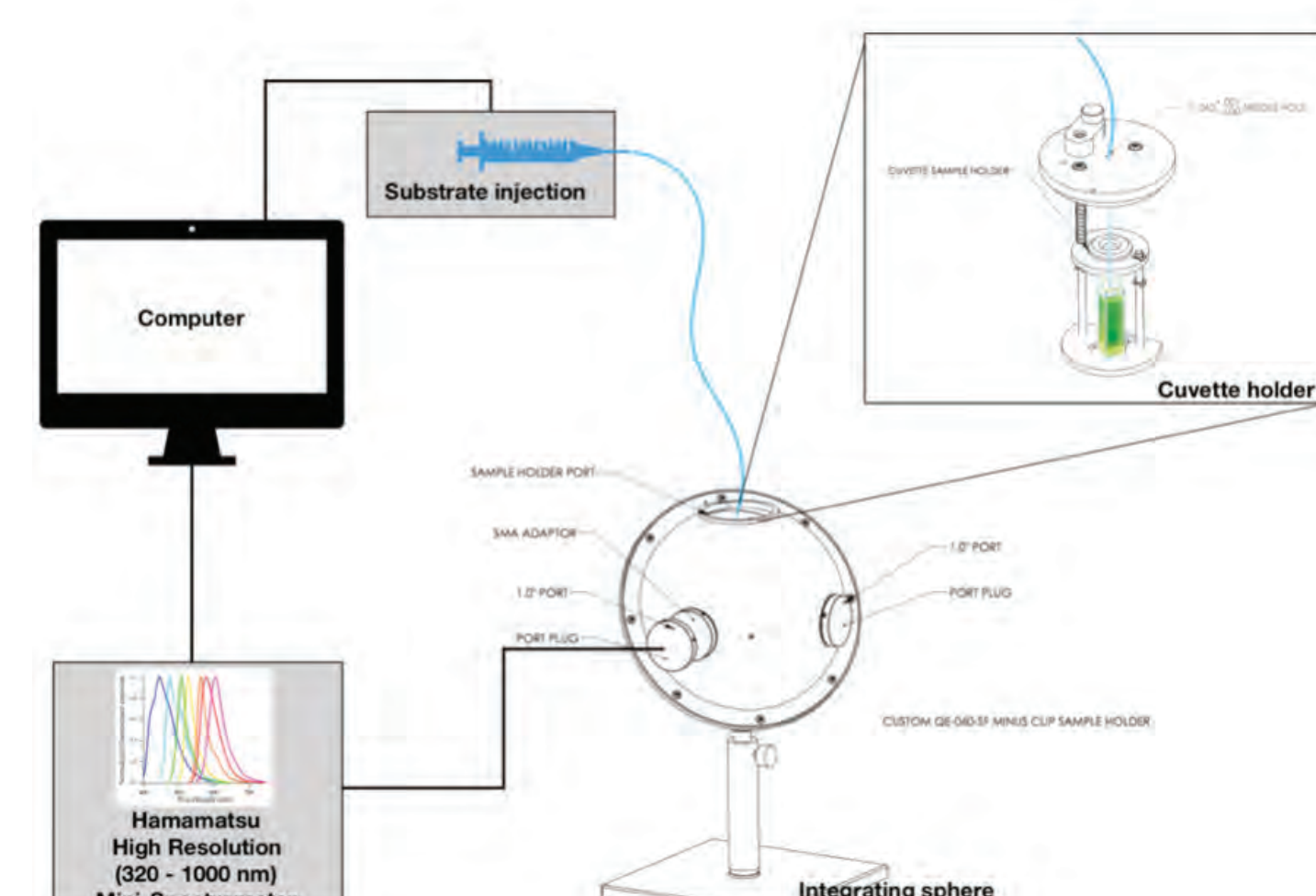
Separated expression system: CTZ and opto-genetic drive enhance postsynaptic activity

Cutting inter-culture connectivity blocks CTZ not optogenetic effects

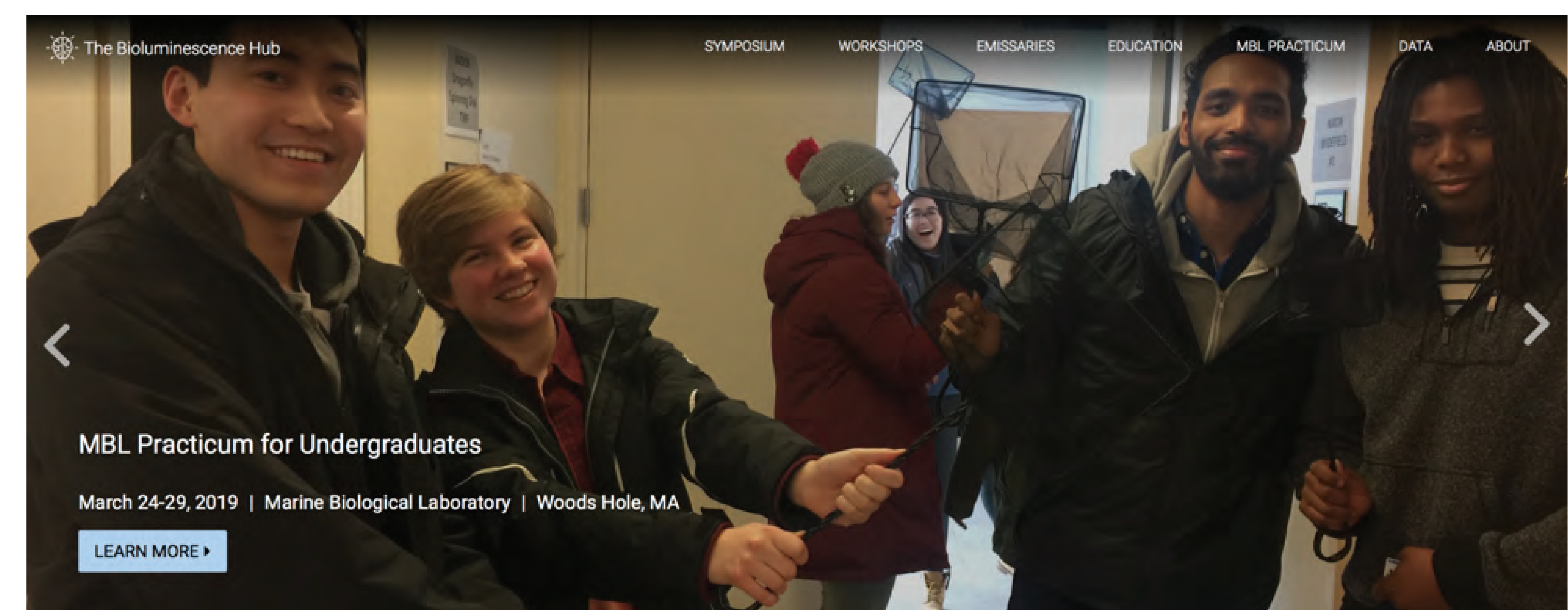
## New Brighter, & Ca<sup>2+</sup>-Sensitive Luciferases by Natural Selection and FRET

Discovery of several new luciferases and fluorescent proteins, and unique combinations to use Resonant Energy for amplification and color choice

Development of new testing sphere for quantitative, systematic comparison of photon output from new molecules



## Education, Dissemination



We have hosted 42 students from 37 colleges over 3 iterations of our intensive, week-long Practicum. Our lectures and labs explore bioluminescence at the organismal, chemical, physical, and molecular level.

Symposium Next Generation Technologies for Neuroscience June 11-12, 2018, Brown University

Workshop for Scientists on Bioluminescence Methods August 6, 2018 Brown University

We disseminate BL tools and know-how by Workshops, Symposia, and via our Website: [www.bioluminescencehub.org](http://www.bioluminescencehub.org)

## BL Constructs Database

Filter	Refine Search	Results:
Type	BL-OG	(LMO3) pAAV-hSyn-sbGlu-eYFP
Function	Actuator	Type: BL-OG
Actuator	Ca <sup>2+</sup> reporter	Parent: LMO3
Ca <sup>2+</sup> reporter		Constitutive: LMO3
		Features: Blue light responsive
		Activity: Excitatory
		Vector: pAAV
		Promoter: hSyn
		Luciferase: sfGlu
		Opsin: VCN1
		Reporter: eYFP
		Requires more information
Constitution		(LMO3) pAAV-CAG-sbGlu-eYFP
LMO3		Type: Bioluminescent Ca <sup>2+</sup> sensor
LMC4f		Parent: CAG reporter
		Parent: LMC4f
		Constitutive: LMC4f
		Features: Blue light responsive
		Activity: Reporting calcium
		Vector: pAAV
		Promoter: CAG
		Luciferase: sfGlu
		Opsin: No opsins
		Reporter: sfGlu