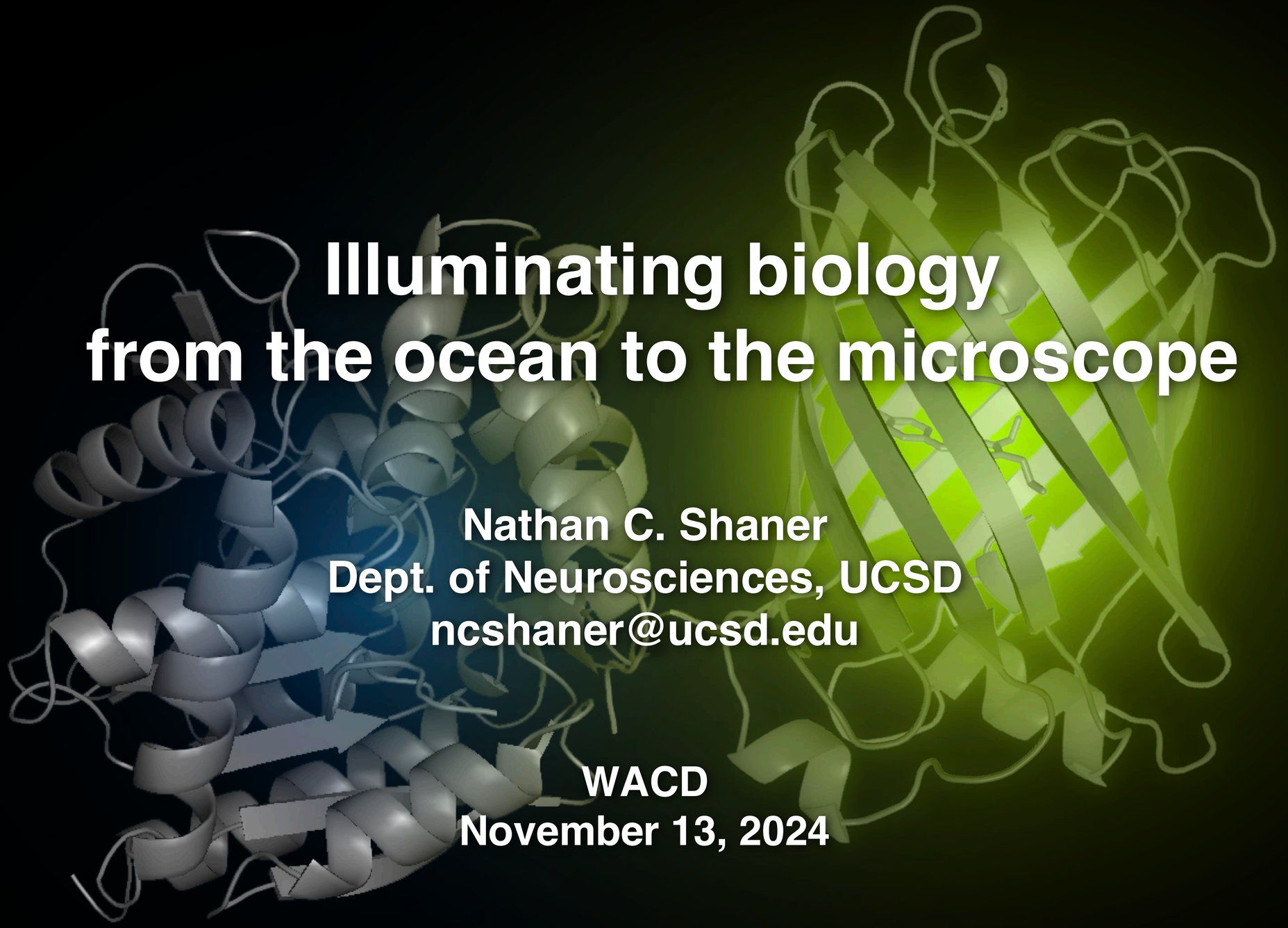
Organisms across the tree of life have evolved a diverse array of mechanisms to sense, modulate, and generate light. In this seminar, I will discuss how we discover and study these systems, and how we transform them into genetically encoded probes for imaging and controlling biological processes. Our research integrates three types of optical activity—fluorescence, bioluminescence, and optogenetics—to create versatile probes with a wide range of applications. These tools are designed not only for live-cell imaging but also for manipulating cellular activity with light, providing unprecedented control over complex biological systems. I will highlight several recent projects from my lab, where we've engineered these optical tools to tackle specific challenges in biomedical research. By combining insights from marine biology, protein engineering, and microscopy, we aim to push the boundaries of what is possible with light-based technologies. This talk will explore the exciting possibilities that emerge when fluorescence, bioluminescence, and optogenetics converge, and how these next-generation tools are opening new avenues for studying dynamic processes in complex biological systems.



Illuminating biology from the ocean to the microscope

Nathan C. Shaner
Dept. of Neurosciences, UCSD
ncshaner@ucsd.edu

WACD
November 13, 2024

Biology is not static!

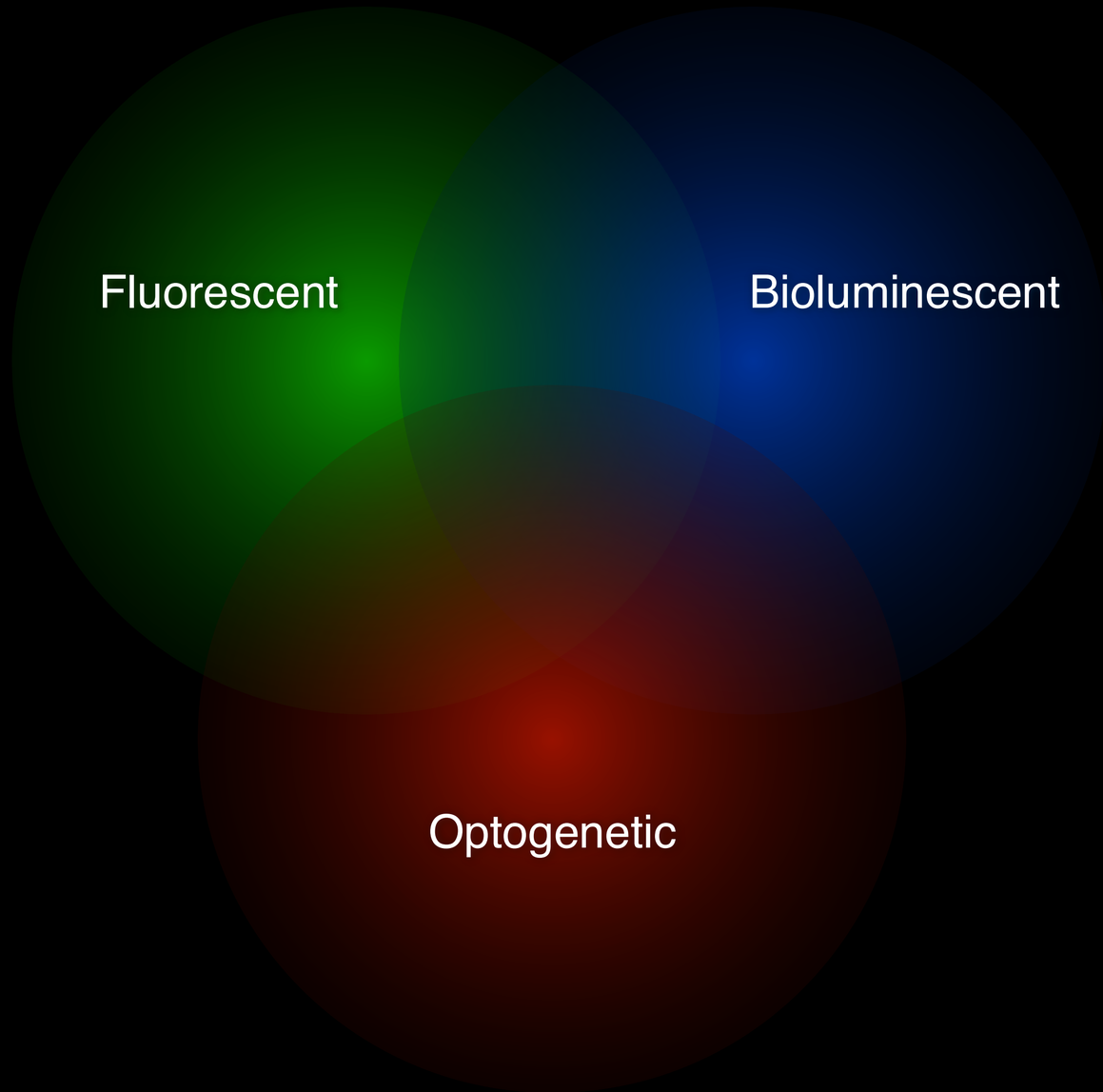
G. Lambert

We make new and better probes for living cells

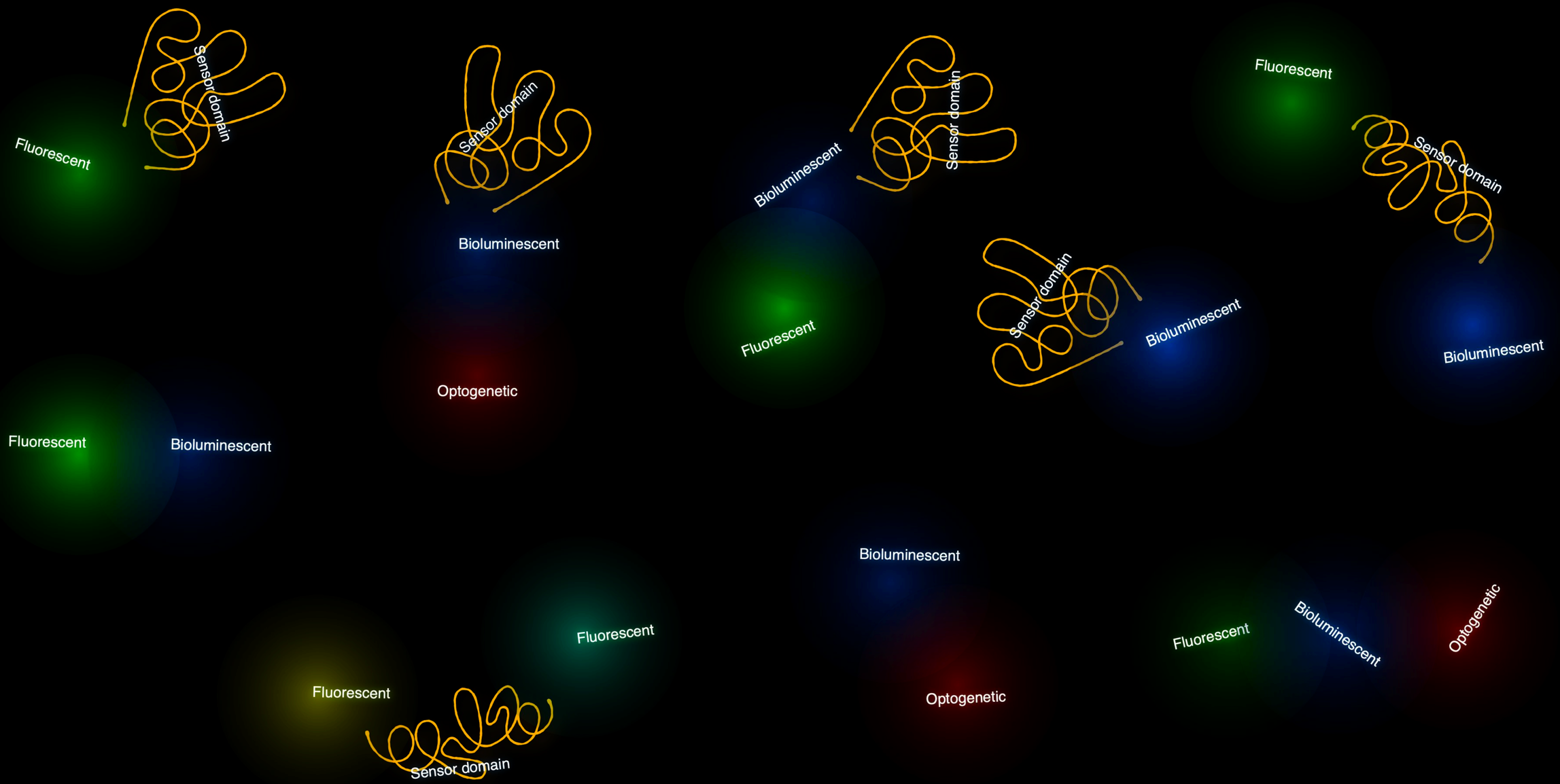
(among other things)

We make new and better probes for living cells

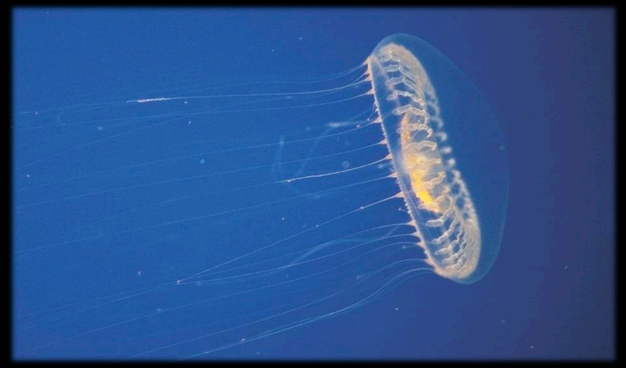
(among other things)



Genetically encodable tools: the LEGO of biology



Many animals produce genetically-encoded fluorescent pigments!



Hydrozoan jellies

Anemones

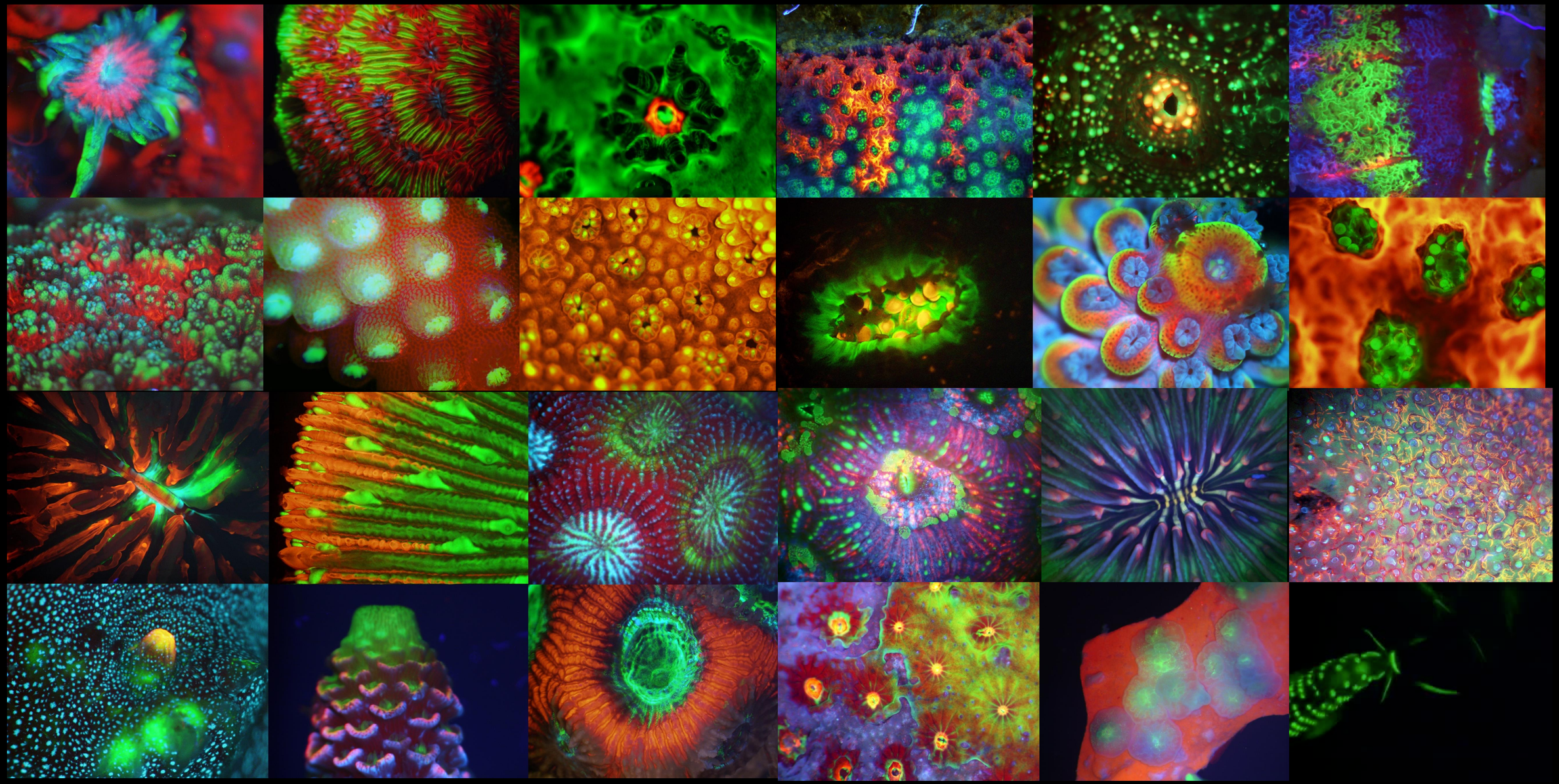
Corals

Lancelets

Copepods

The Reef at Night

A. Salih, N. Shaner

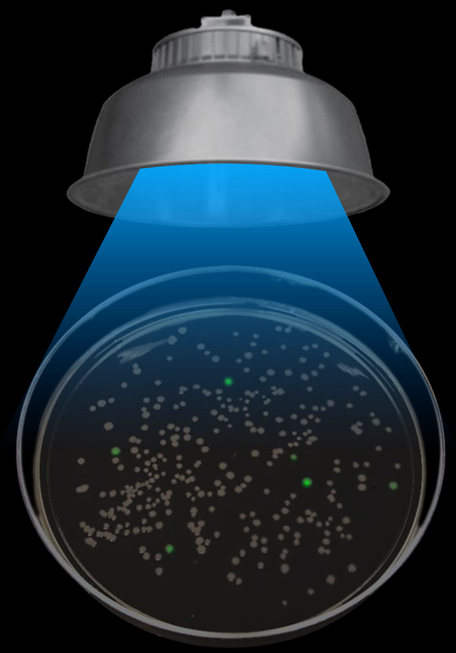


A. Salih, N. Shaner

Directed evolution

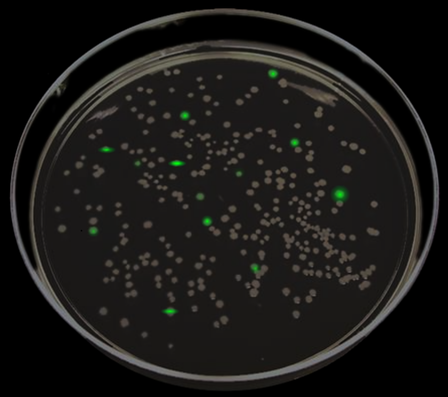
wild-type FP

random mutagenesis



sequence improved mutants

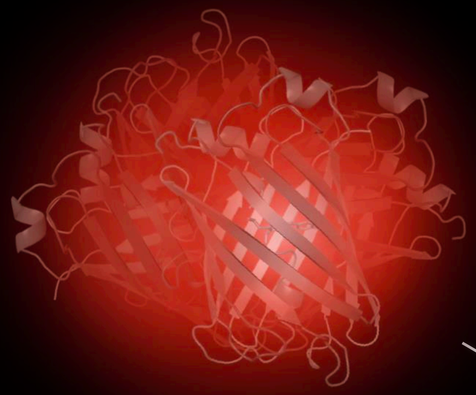
site-directed library + shuffling



select best clones

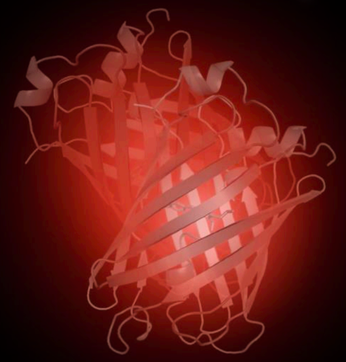
repeat...

Discosoma sp. RFP



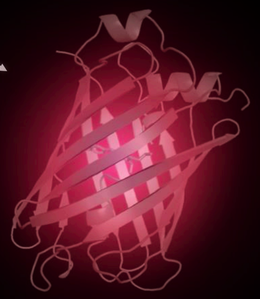
DsRed.T1
tetramer

dimer2
(17 mutations)



Break A/B interface
Rescue fluorescence

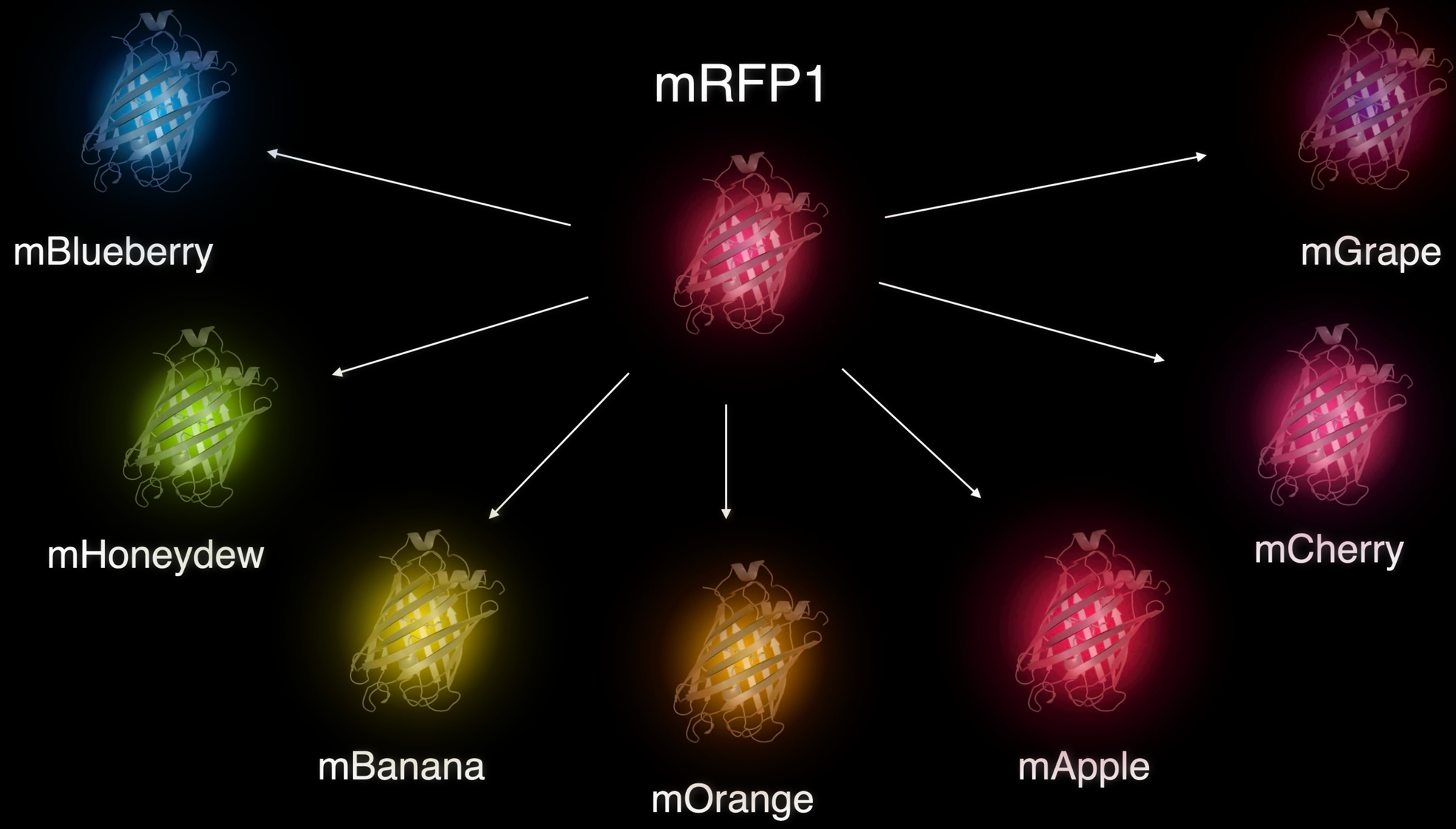
Break A/C interface
Rescue fluorescence



mRFP1
(33 mutations)

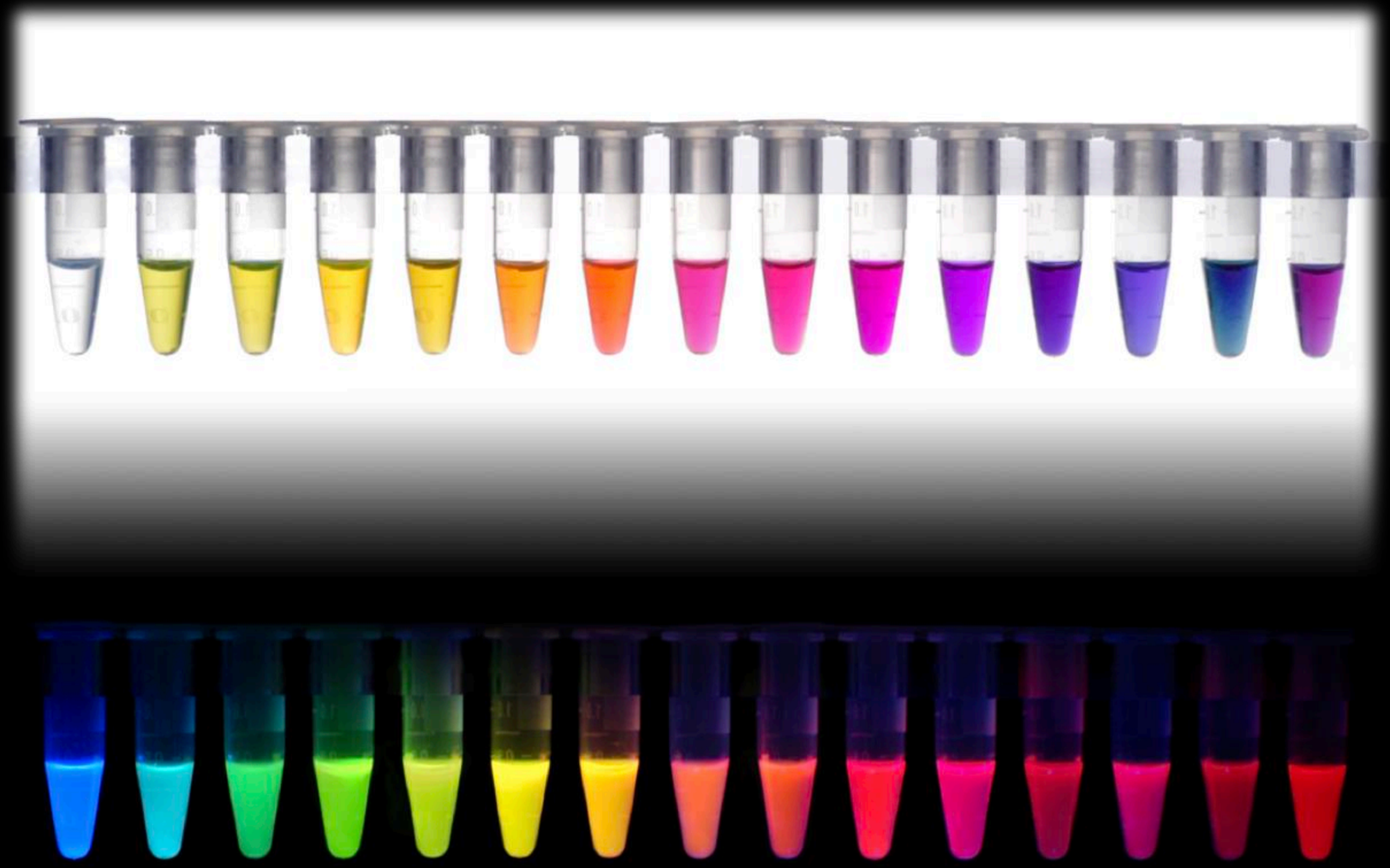
Matz et al (1999); Bevis & Glick (2002); Campbell et al (2002)

The "mFruits"



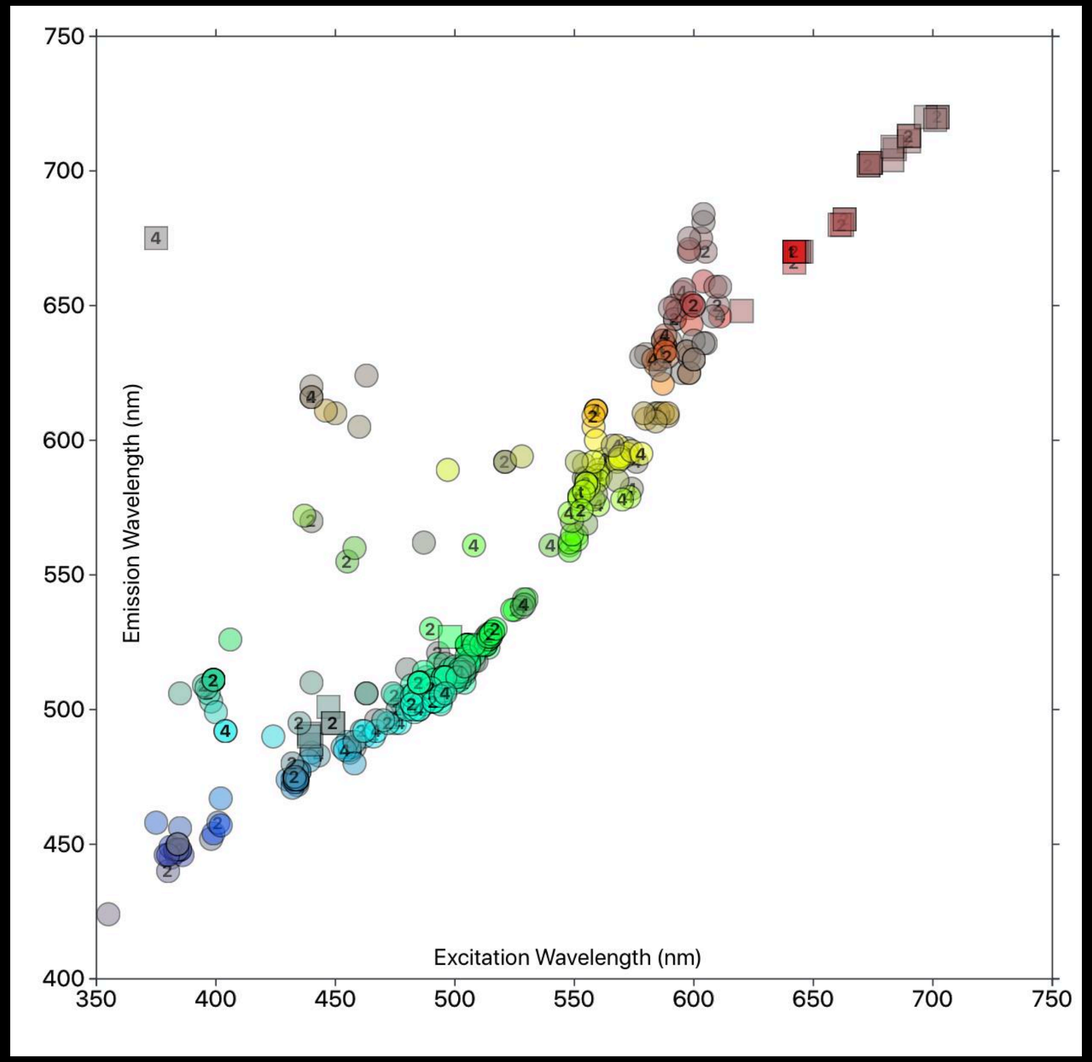
Campbell et al (2002); Shaner et al (2004); Wang et al (2004); Ai et al (2007); Shaner et al (2008); Lin et al (2009)

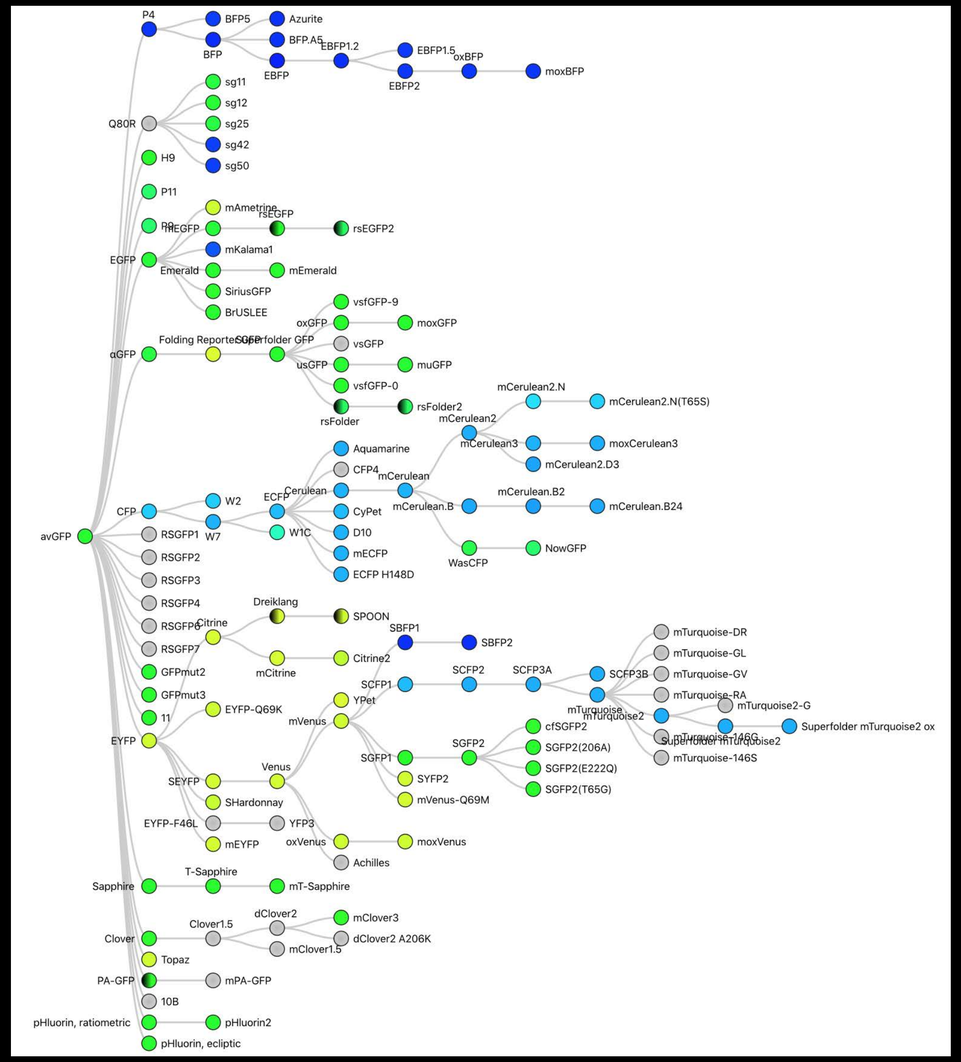
~20 years ago



N. Shaner, P. Steinbach, R. Y. Tsien

Today

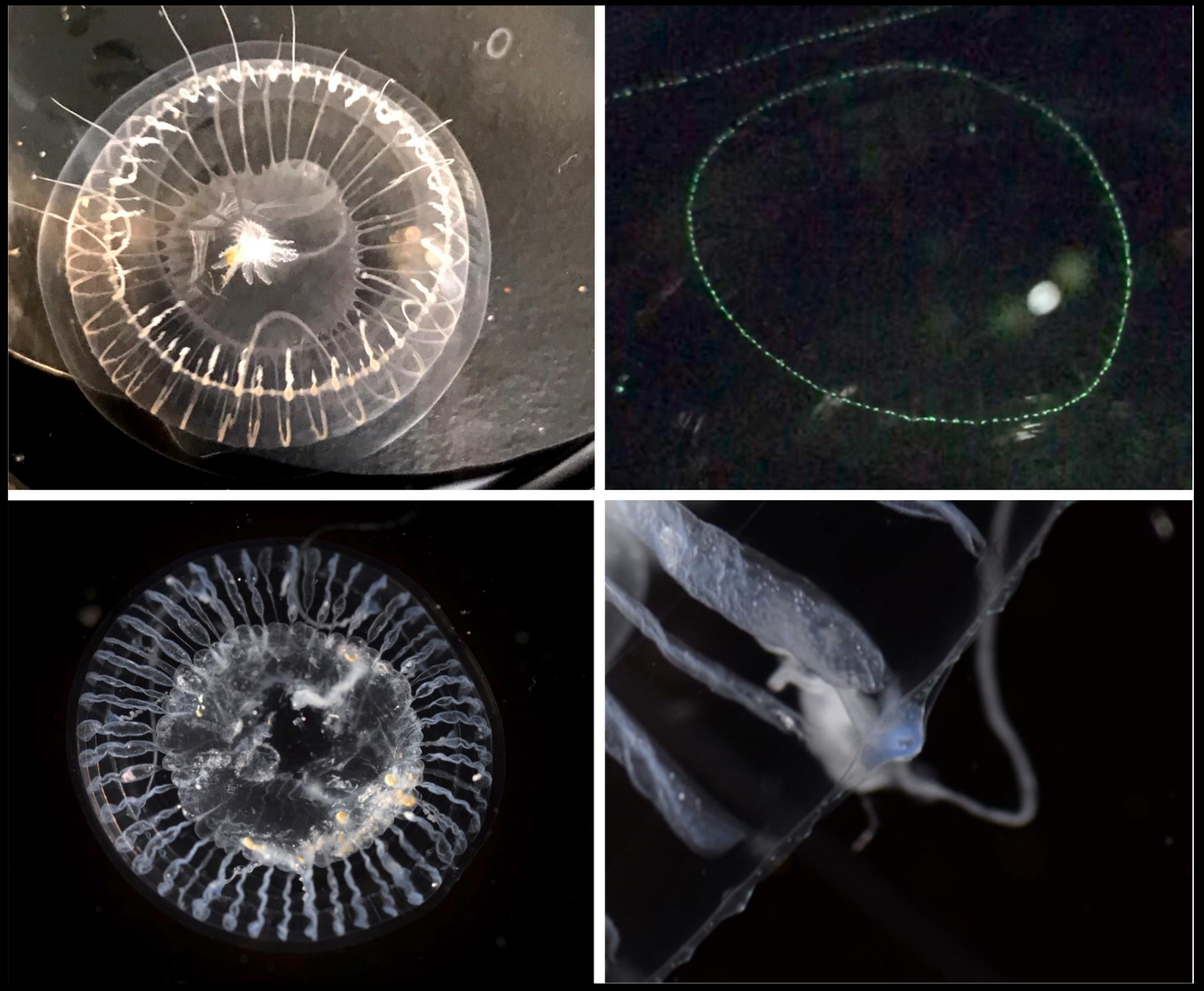




FPbase.org

Recent developments in FPs

Diverse new FPs from *Aequorea* species

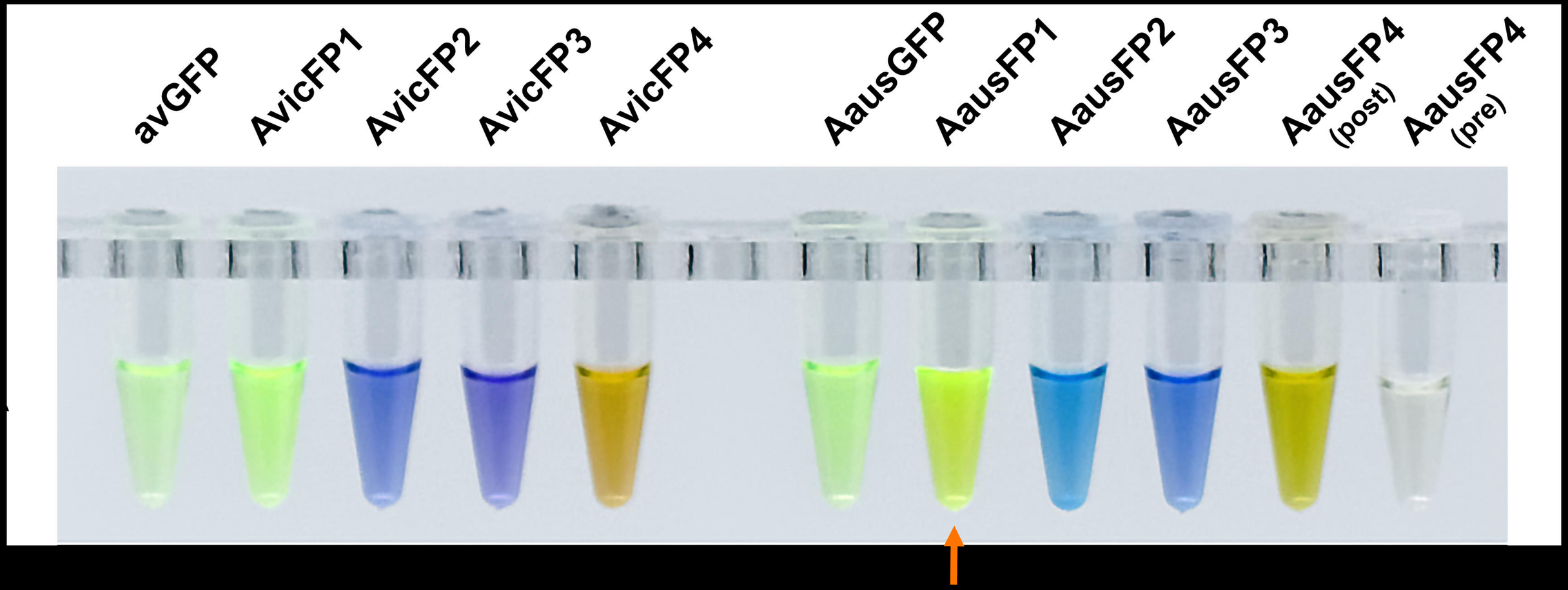


Aequorea victoria

Aequorea (cf.) *australis*

Lambert GG et al., *PLoS Biology* (2020). https://doi.org/10.1371/journal.pbio.3000936

Diverse new FPs from *Aequorea* species



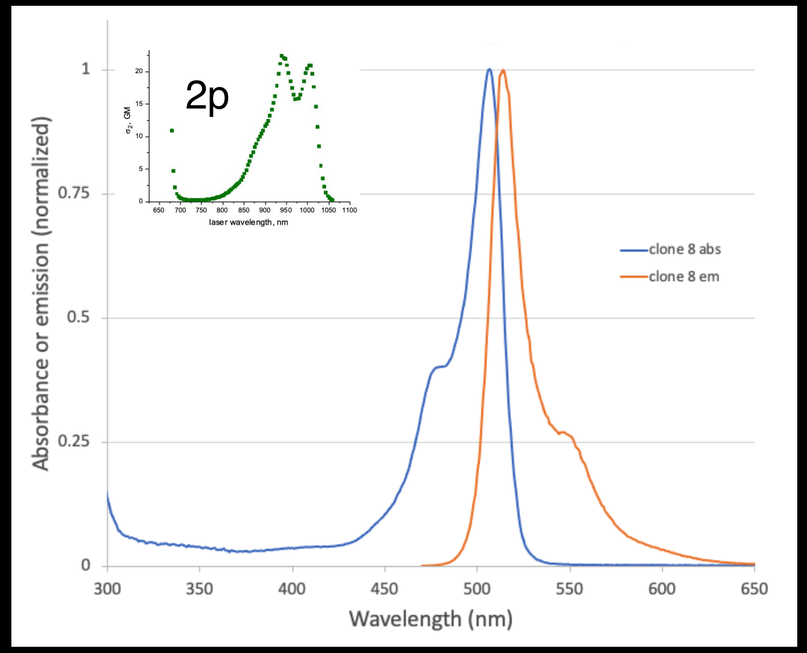
Ultra-bright with very narrow spectra

Lambert GG et al., *PLoS Biology* (2020). https://doi.org/10.1371/journal.pbio.3000936

New monomeric variants of AausFP1 from *Aequorea* cf. *australis*

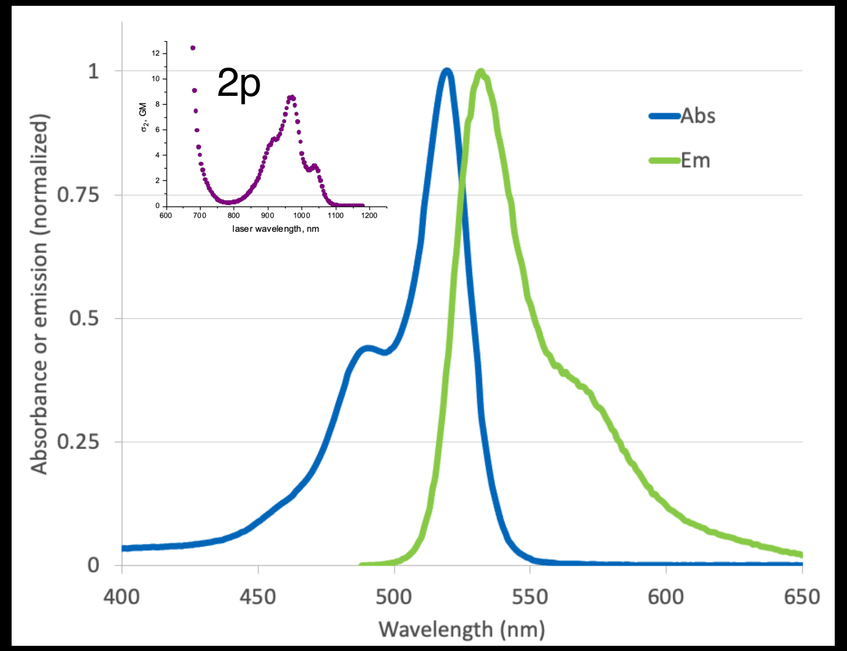
00:00:00

“mAbbsinthe”

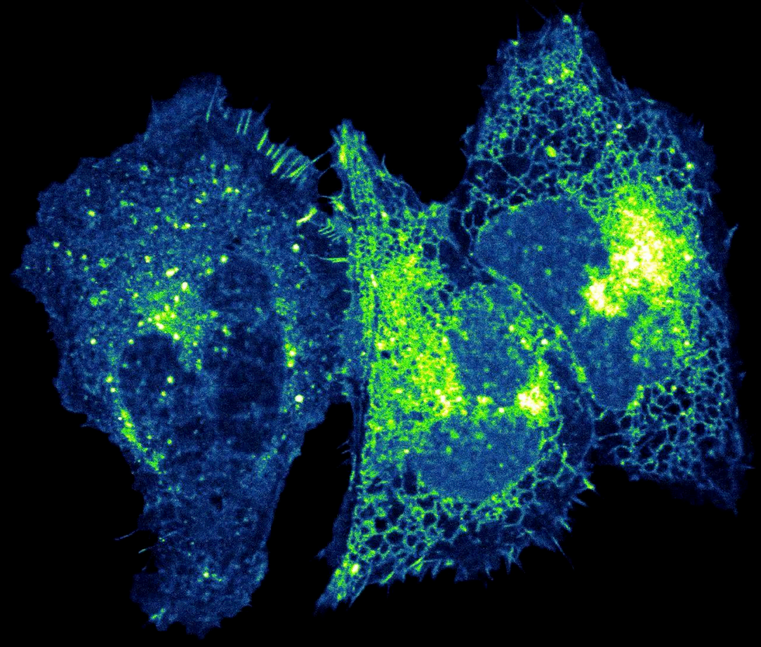


Ex 505nm, Em 514nm, QY 0.85
As photostable as mEGFP

Yellow name TBD



Ex 520nm, Em 532nm, QY 0.70
Super photostable



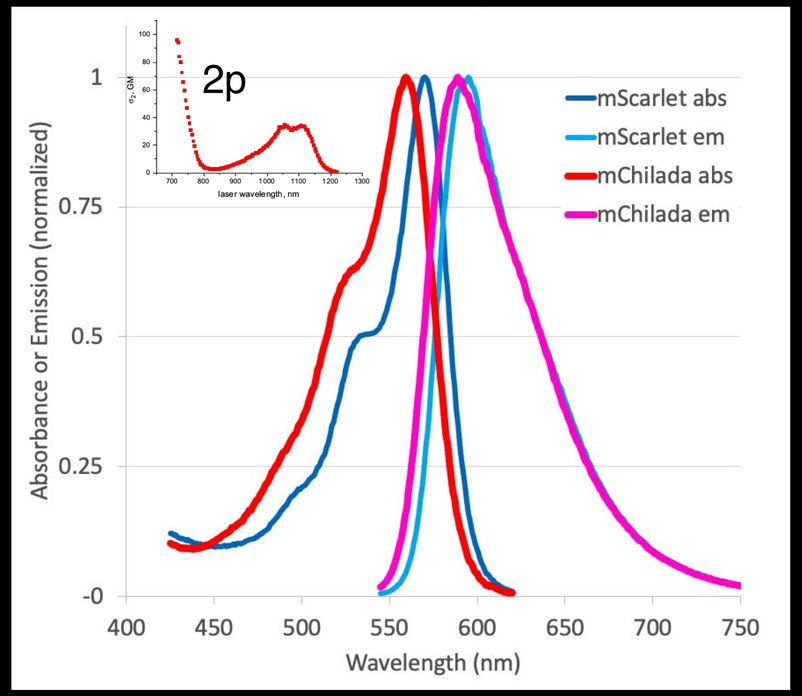
mAbbsinthe-CD63

10 µm

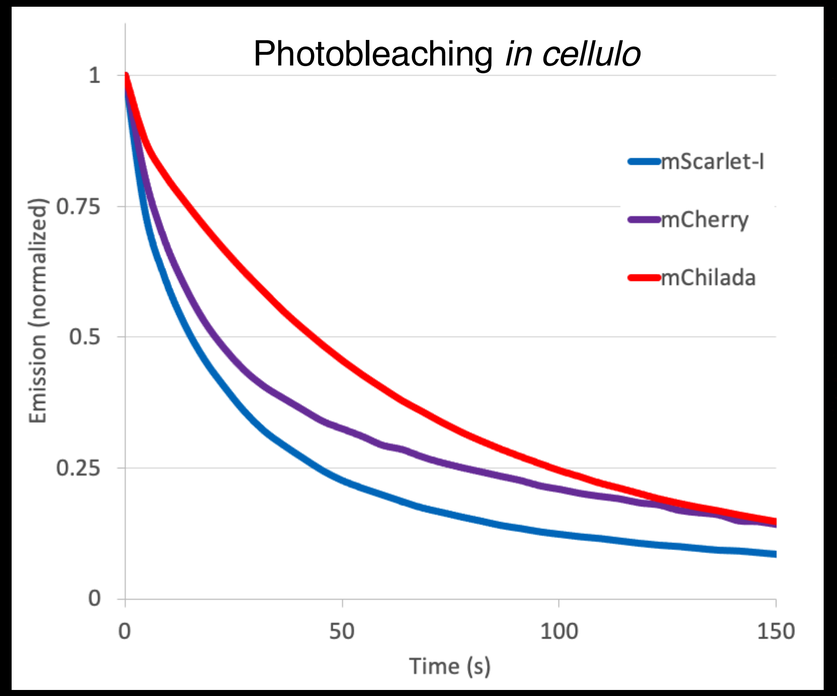
(Unpublished)
data from Gerard Lambert (UCSD)

Ultra-evolved mCherry derivative

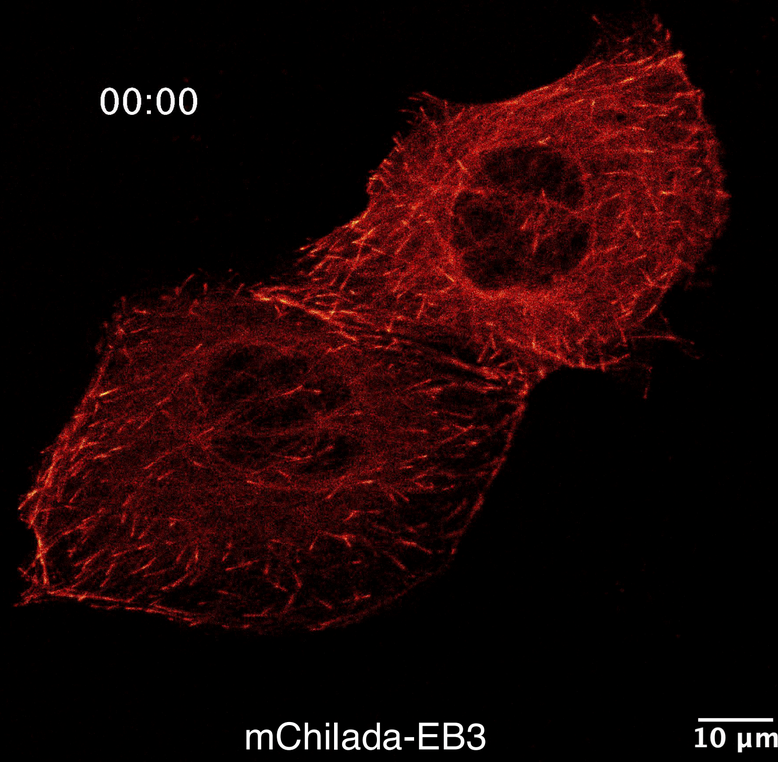
“mChilada”



Ex 562nm, Em 590nm, QY ≥ 0.75

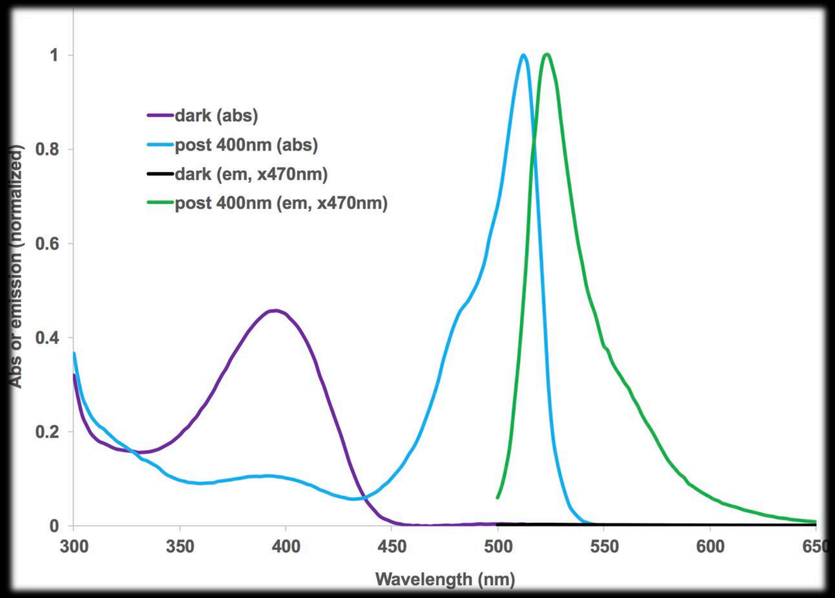


> 3-fold more photostable than mSc-I/I3 with similar *in cellulo* brightness



(Unpublished) data from Gerard Lambert (UCSD)

High-contrast photoswitching

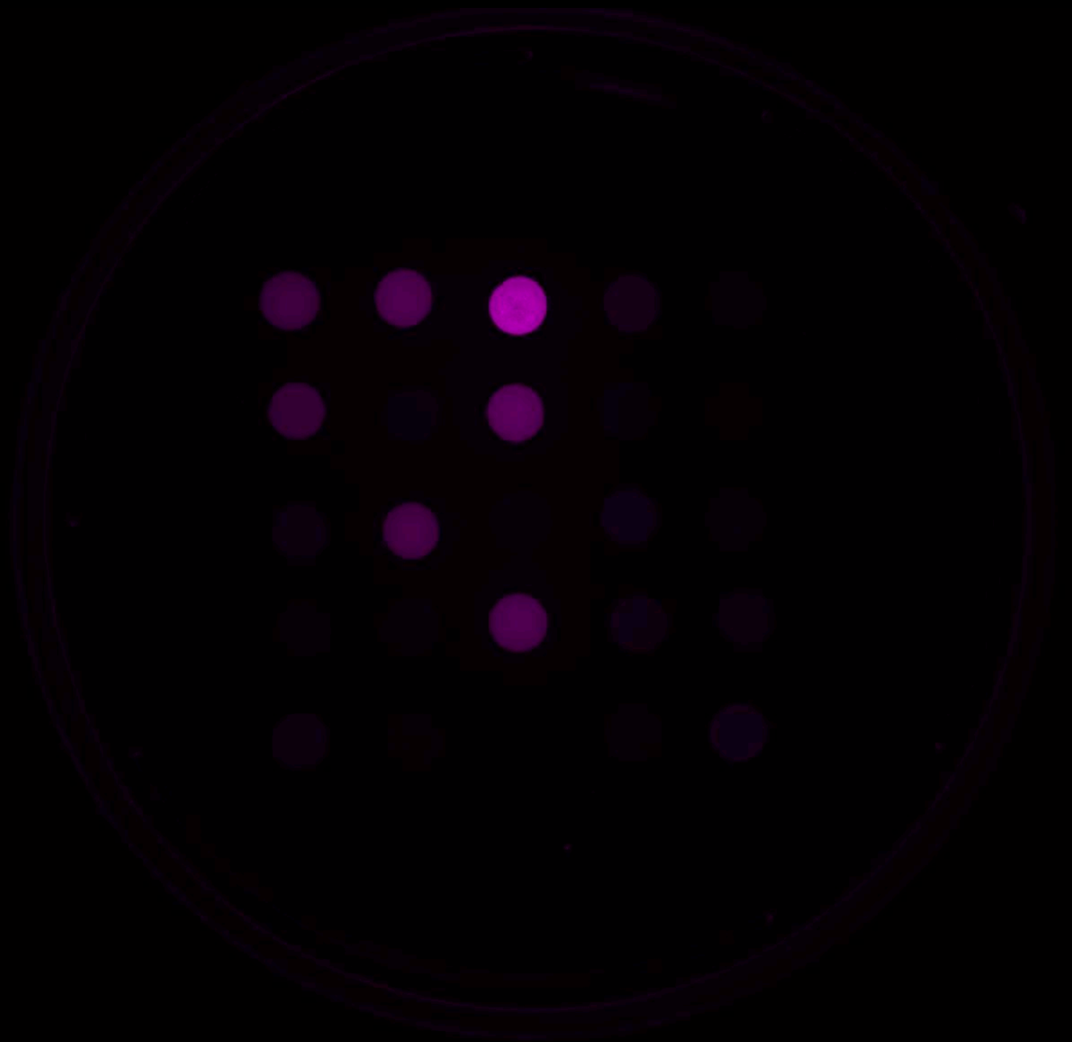


"PSmOfo"
Engineered from a novel green FP
from *Olindias formosus*

- Reversibly photoswitchable (405nm/488nm)
- contrast ratio > 500:1
- "Off" thermal equilibrium
- Monomeric, QY ~0.80
- Multiple variants with a variety of switching kinetics

(Unpublished)

High-contrast photoswitching



“PAmFRank”
Engineered from a hybrid of red FPs
mChilada and mRuby3

- Photoactivatable (dark to bright)
  405nm or 440nm

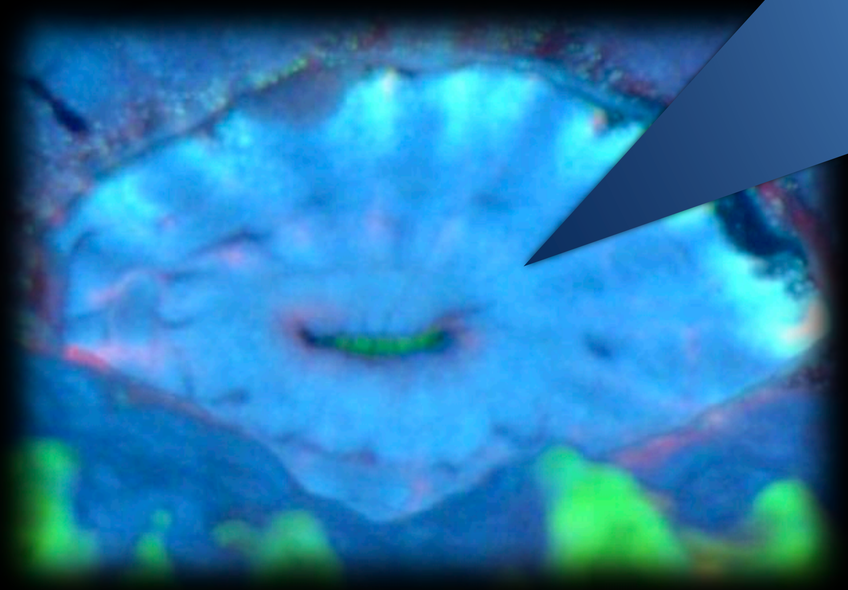
- Still under development - sensitivity, contrast, and oligomeric state need optimization

(Unpublished)

All Shaner Lab tools are freely available to academic users

(including unpublished and preliminary constructs)

Requests - ncshaner@ucsd.edu



Why care about bioluminescence?

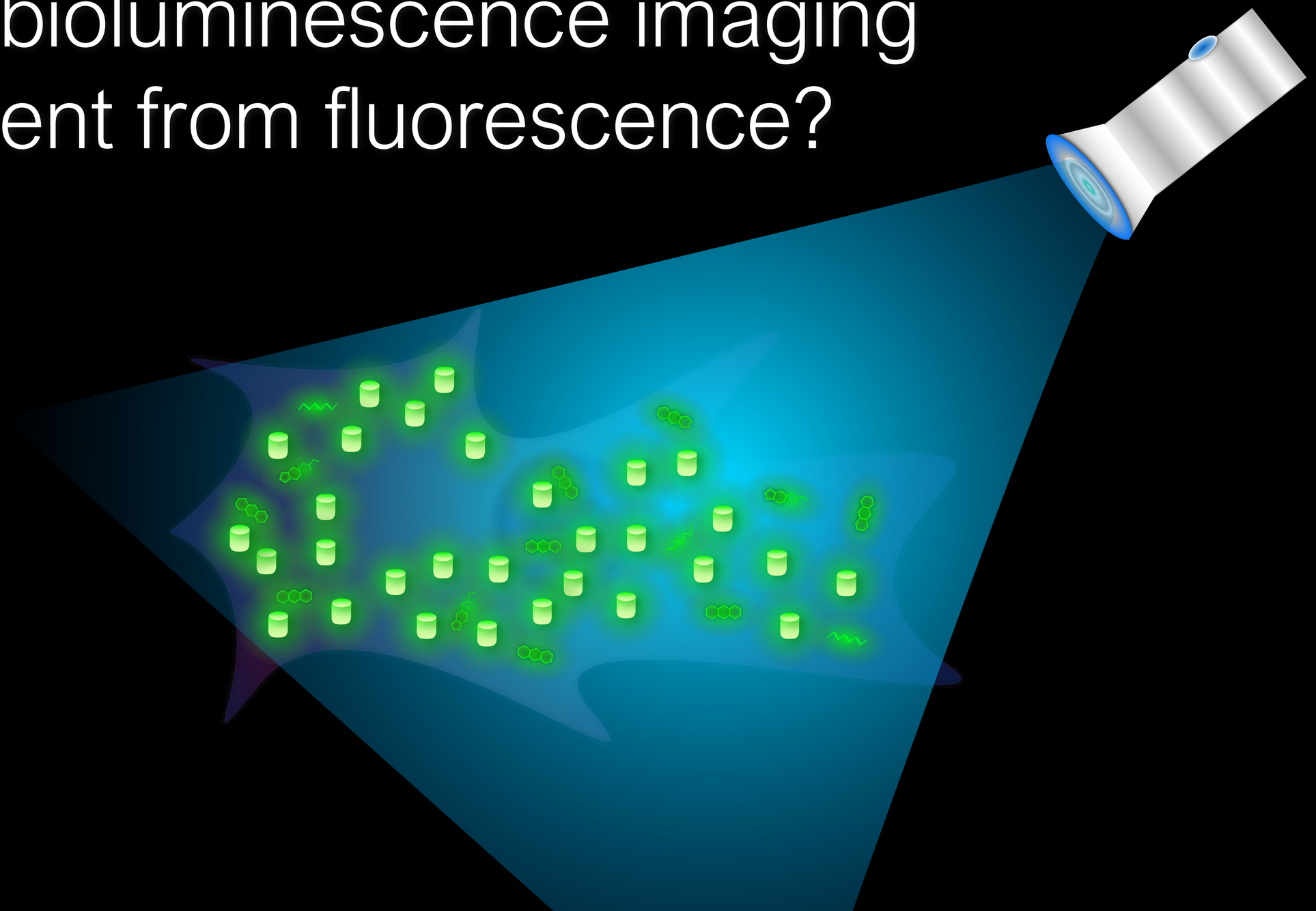
Bioluminescent organisms from the reef

D. Schultz

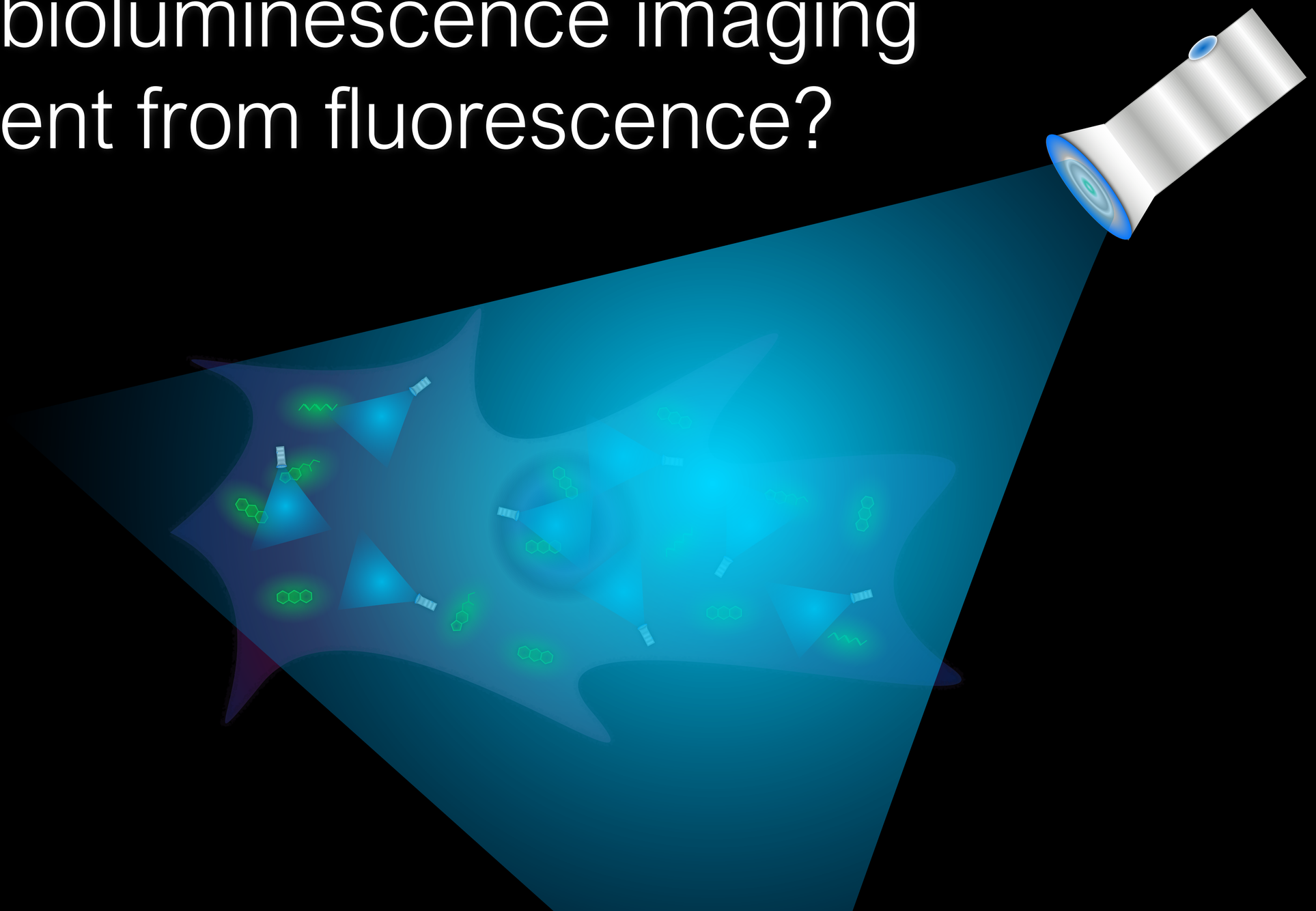
Bioluminescence: local light generation

$$\text{quantum yield (QY)} = \frac{\text{photons emitted}}{LH_2 \text{ molecules oxidized}}$$

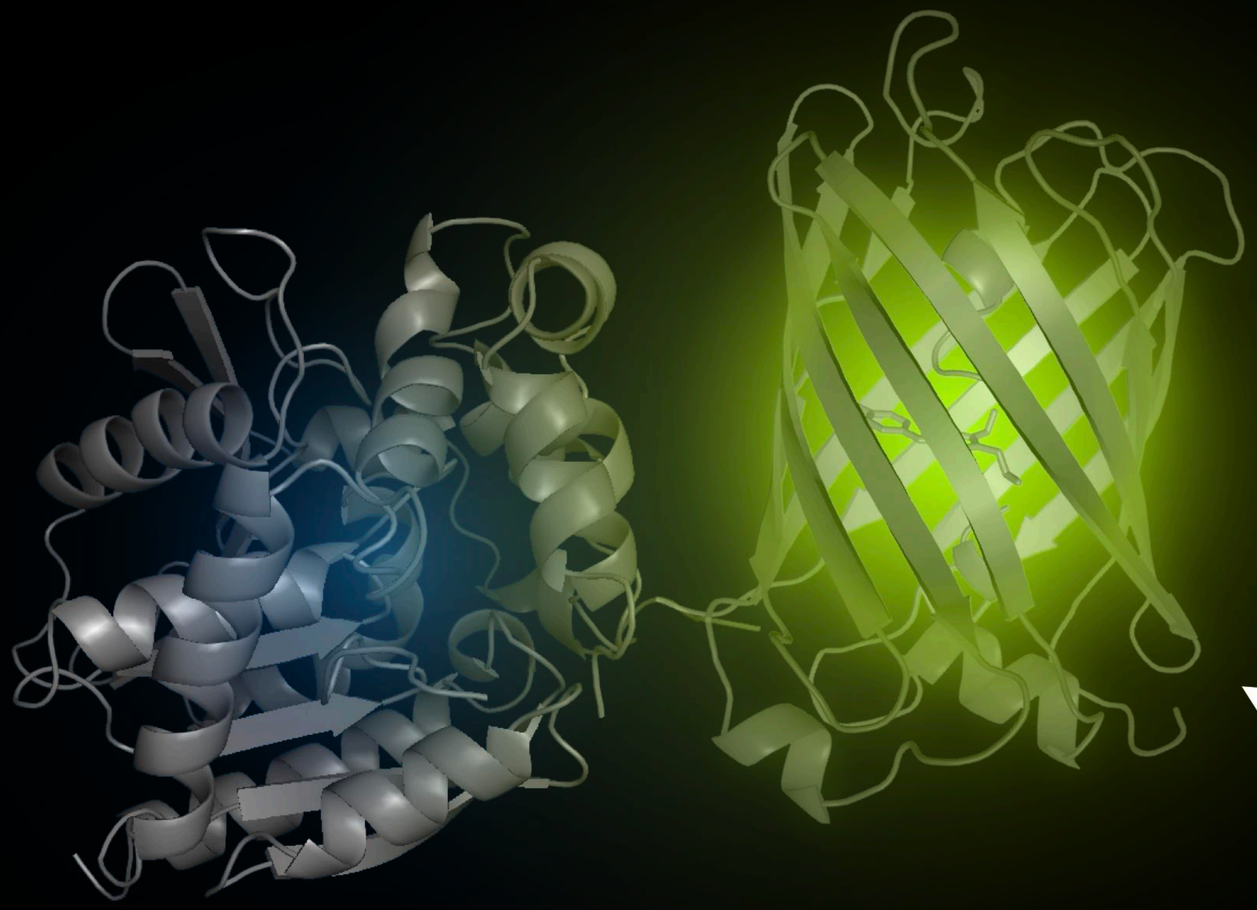
How is bioluminescence imaging different from fluorescence?



How is bioluminescence imaging different from fluorescence?



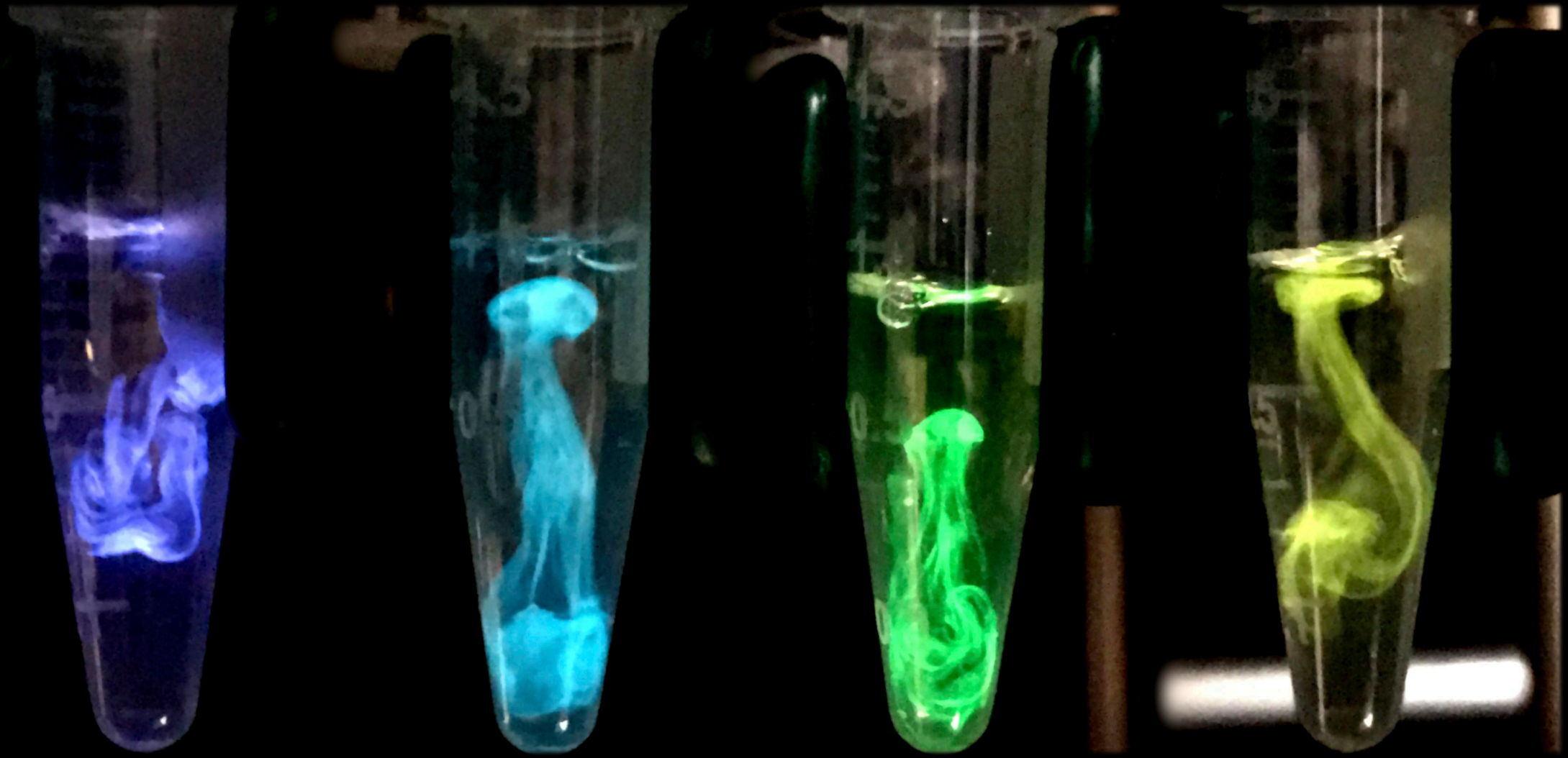
Enhancing bioluminescence QY via FRET



low QY, high k_{cat}
luciferase

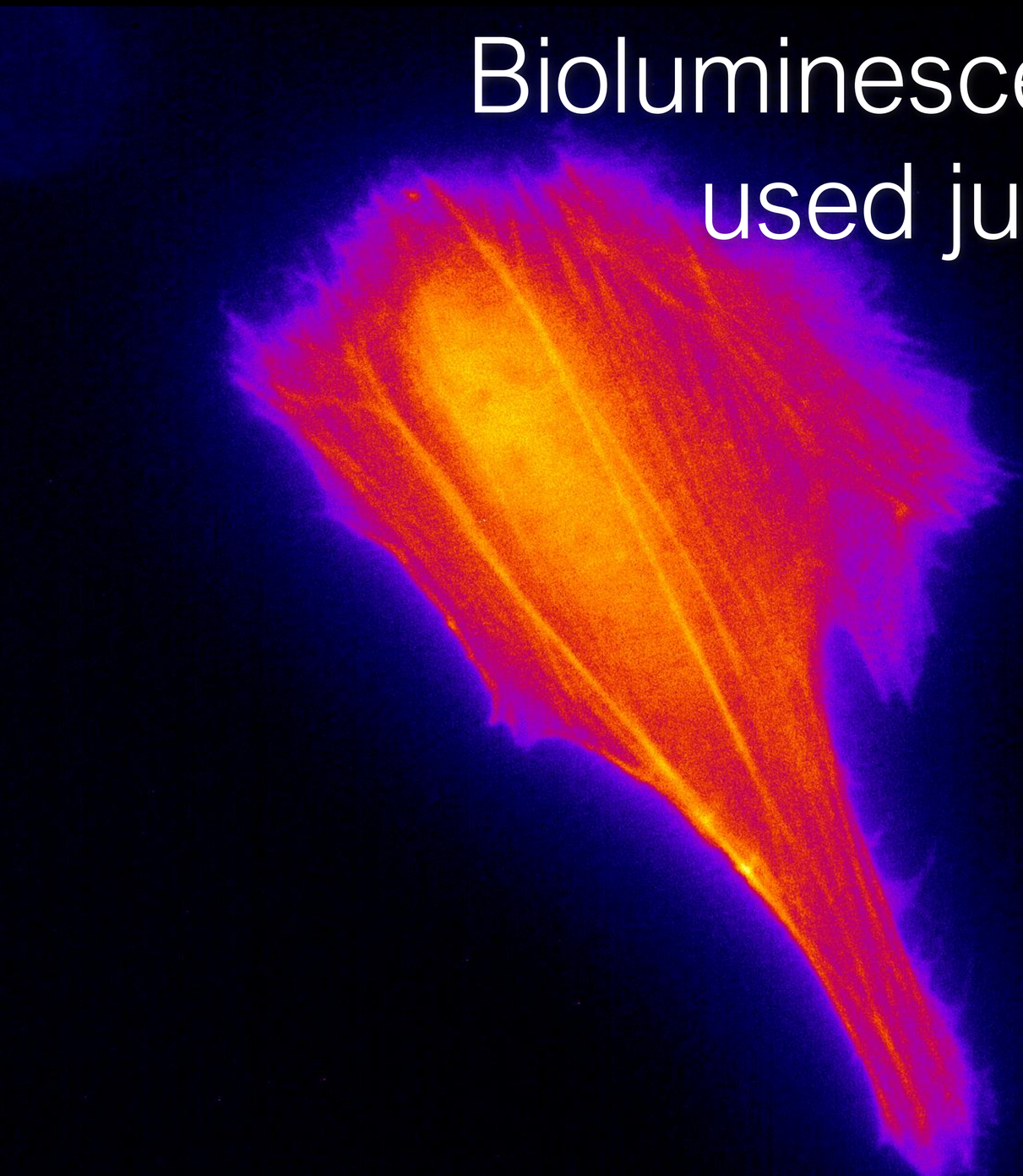
high QY
fluorescent protein

Brighter, more colorful bioluminescent probes



G. Lambert

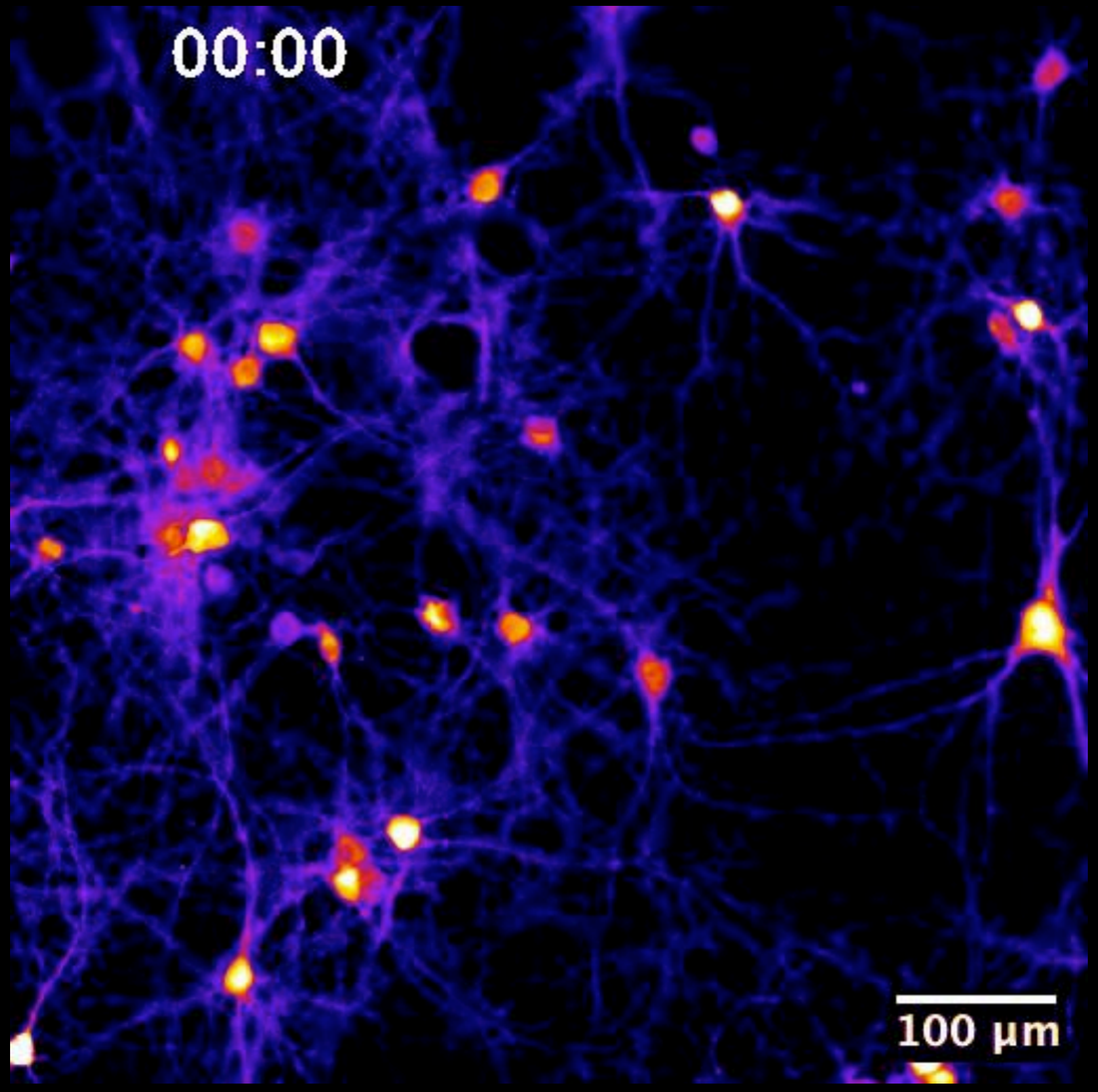
Bioluminescent tags can be used just like FPs



LifeAct-mNGΔ10C-Δ5NeKL9h
In U2OS cells

NCS

Genetically encoded calcium sensors (GECIs)



GCaMP8s in primary rat hippocampal neurons

Aequorin – first-in-class, bioluminescent

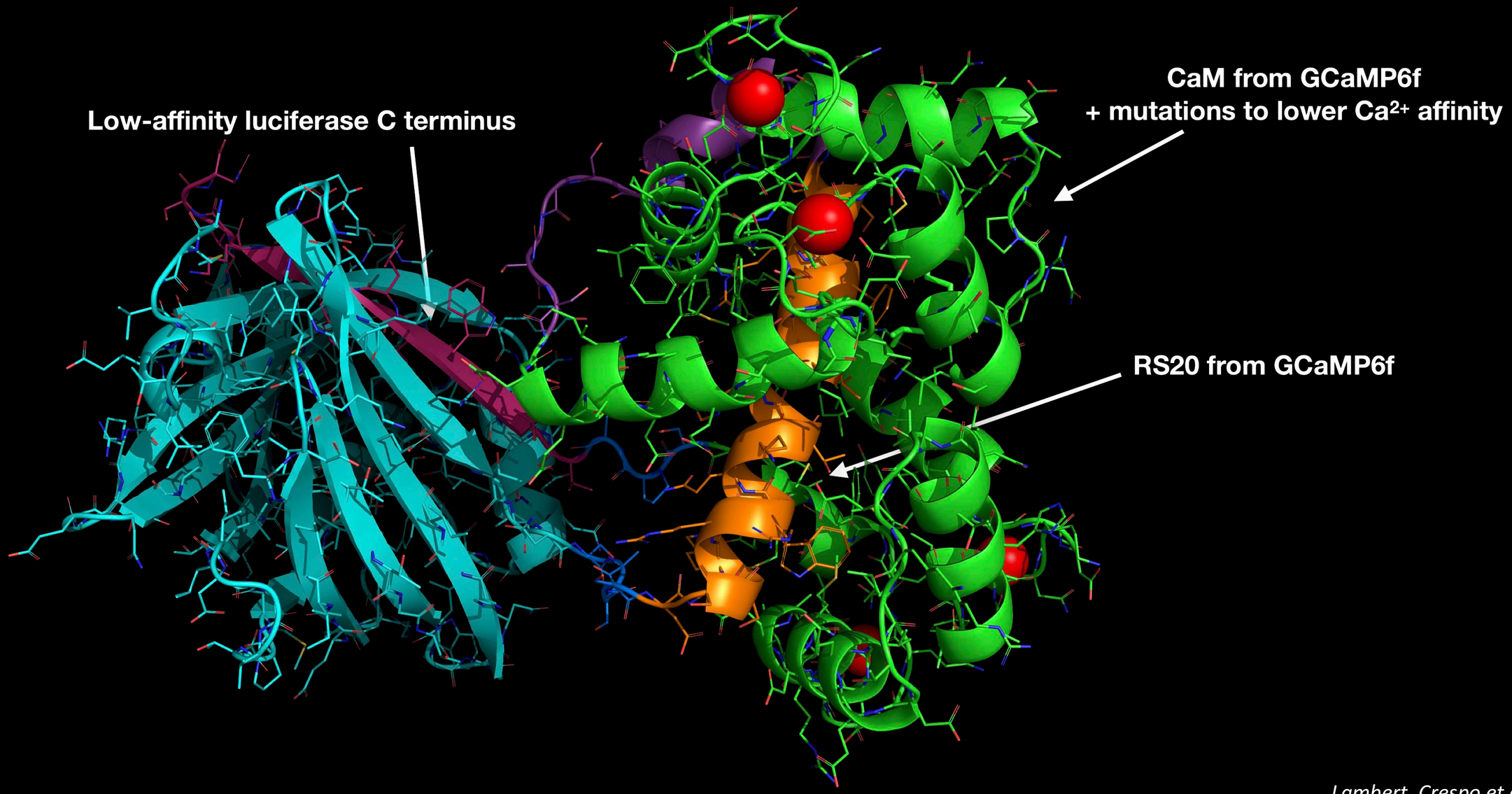
GCaMP series – standard fluorescent GECIs
(among many other fluorescent GECIs)

Newer bioluminescent GECIs include:

- GeNL-Ca2+ series
- CaMBI series
- BRIC
- GLICO (BL/fluo)
- CalFluxVTN (BL FRET)
- LUCI-GECO1 (BL FRET)
- ReLICO (BL FRET)

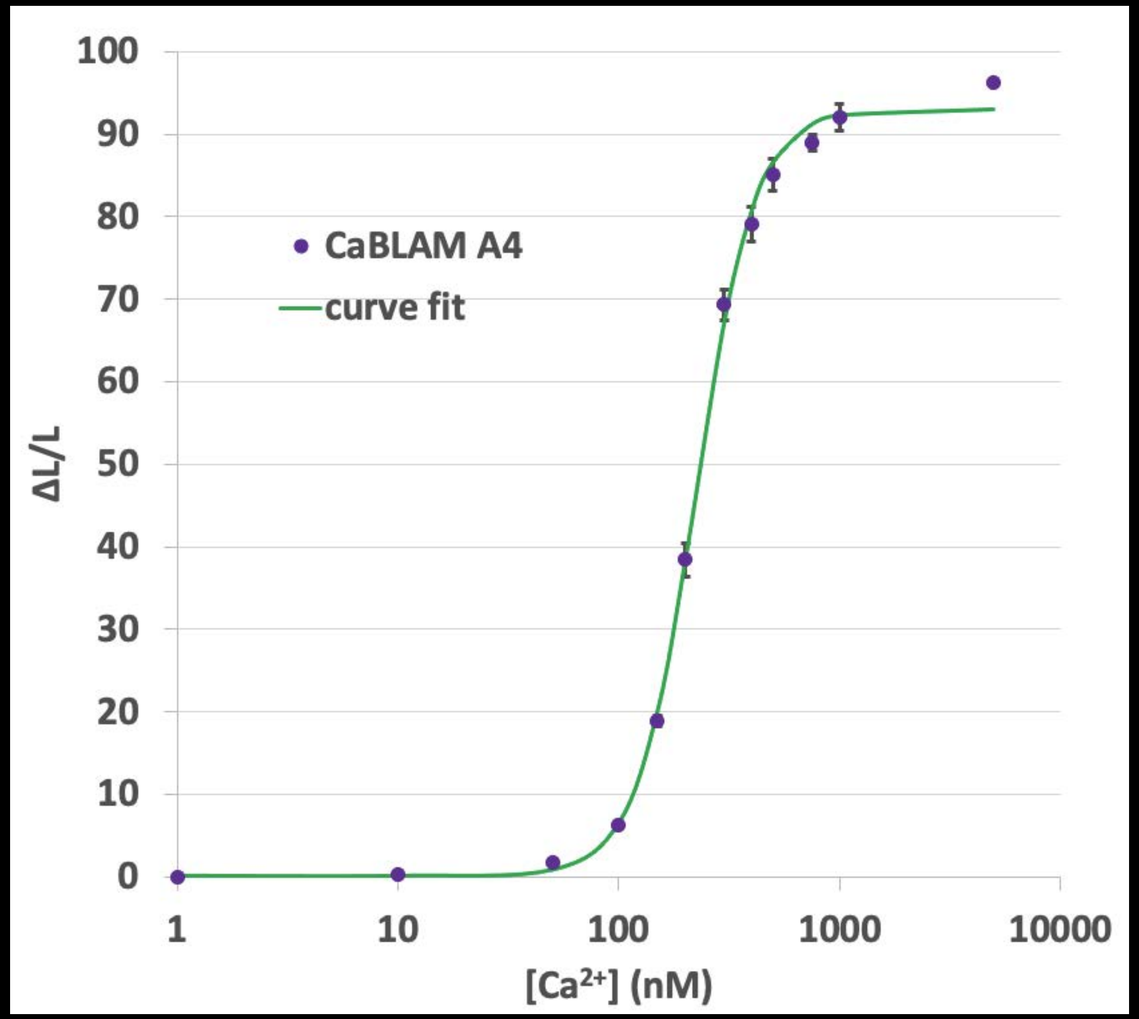
data from Emmanuel L. Crespo (Central Michigan University)

CaBLAM – a GECI from an improved luciferase



Lambert, Crespo et al., (2023)

CaBLAM – highest contrast BL indicator, so far



Calcium titration *in vitro*

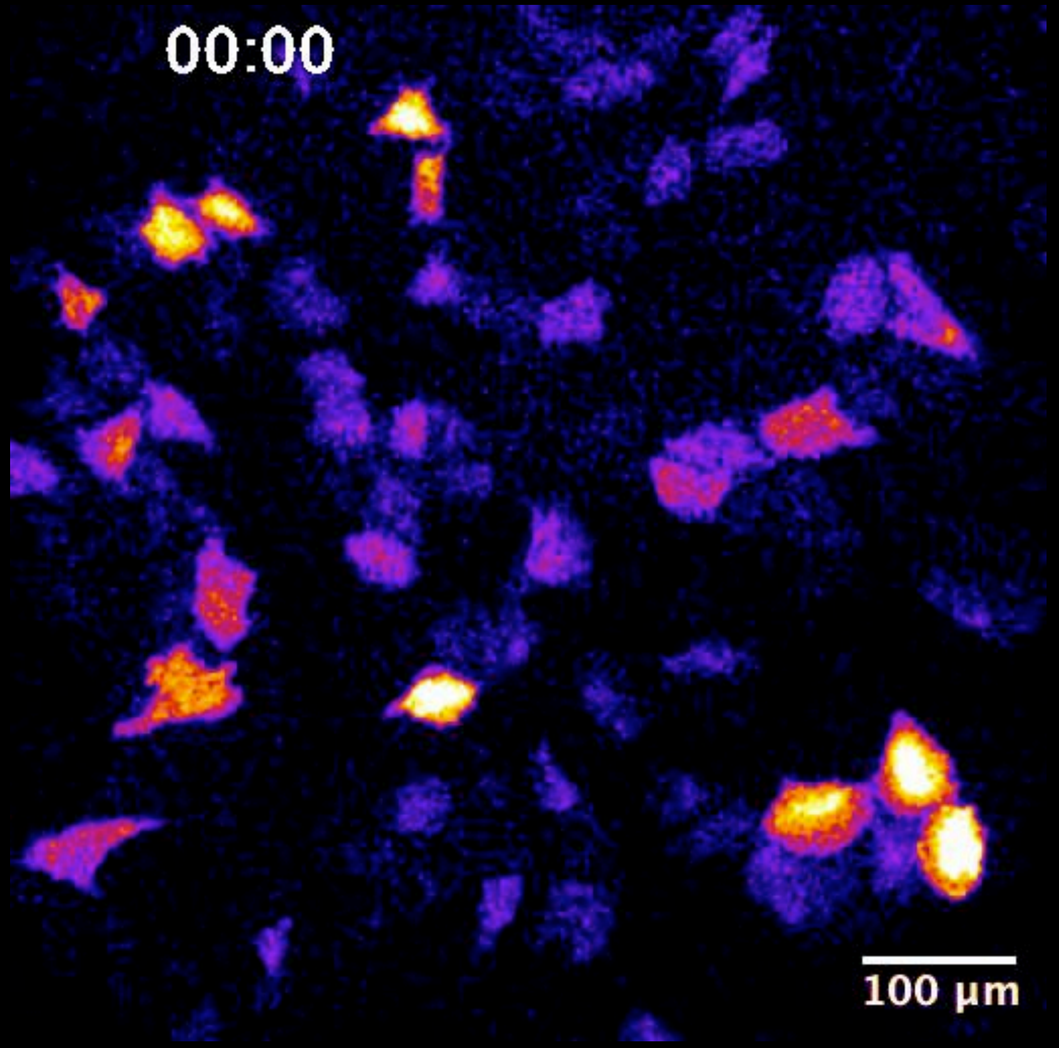
Reported contrast *in vitro*:

GeNL-Ca^{2+}(480)	~5
CaMBI	~8
GLICO	~23
CaBLAM	~95

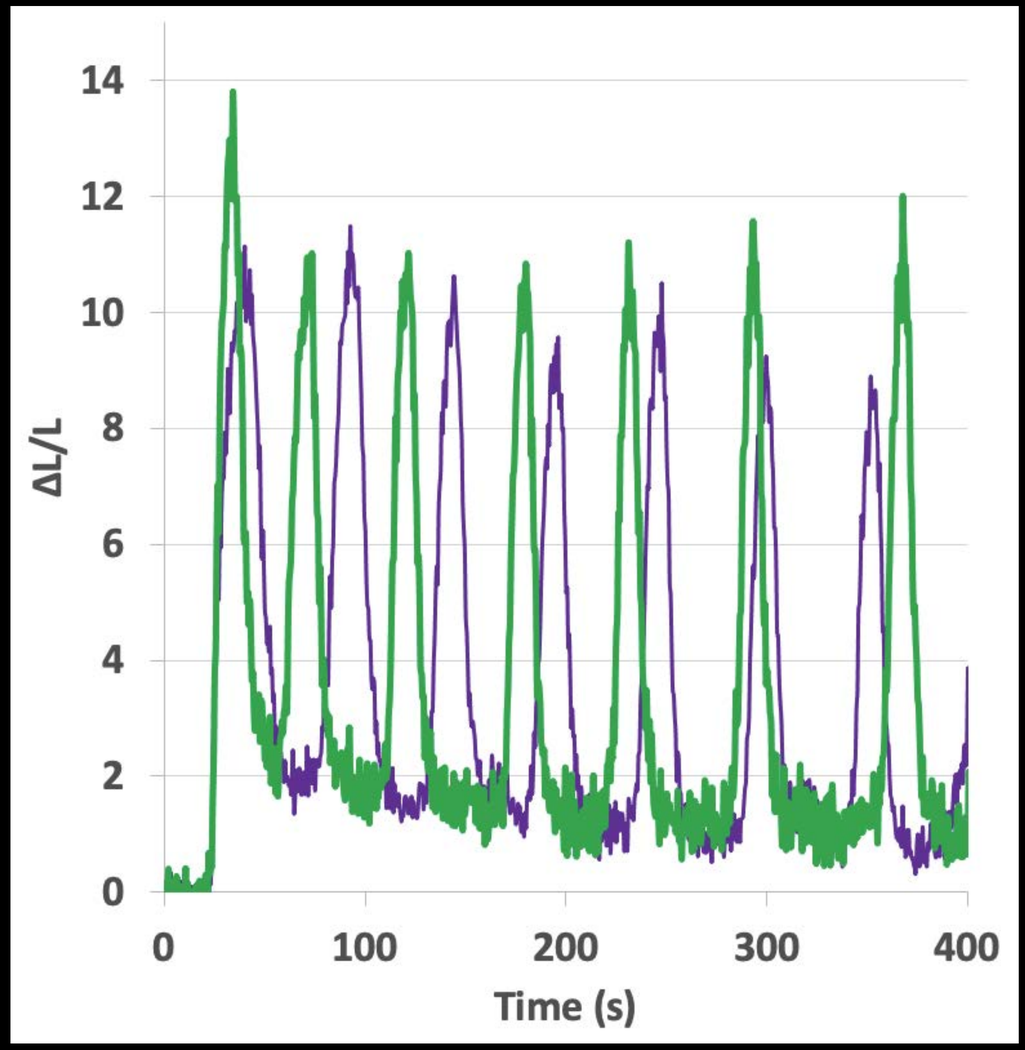
High contrast is critical for downstream optogenetic/signal integration applications!!

Lambert, Crespo et al., (2023)

CaBLAM reports single-cell Ca^{2+} dynamics

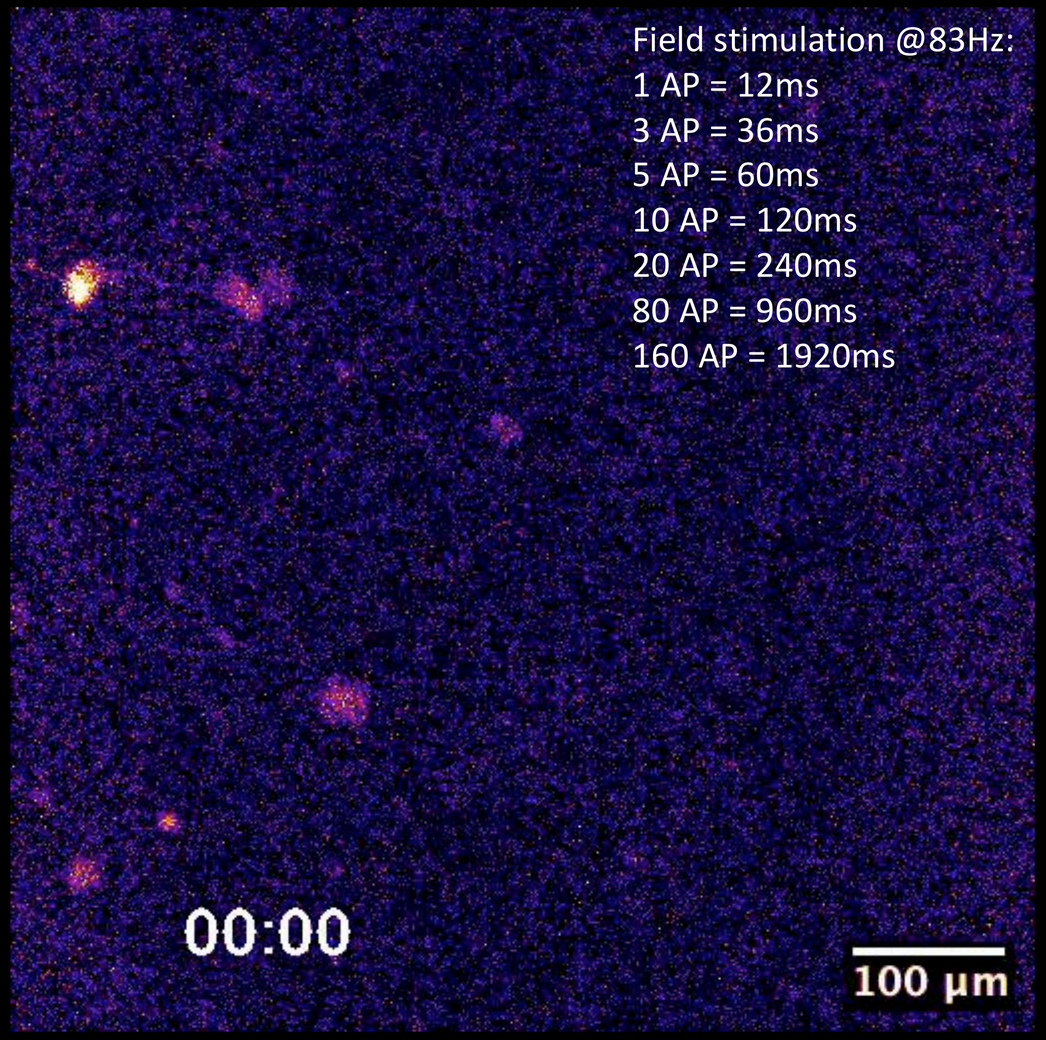


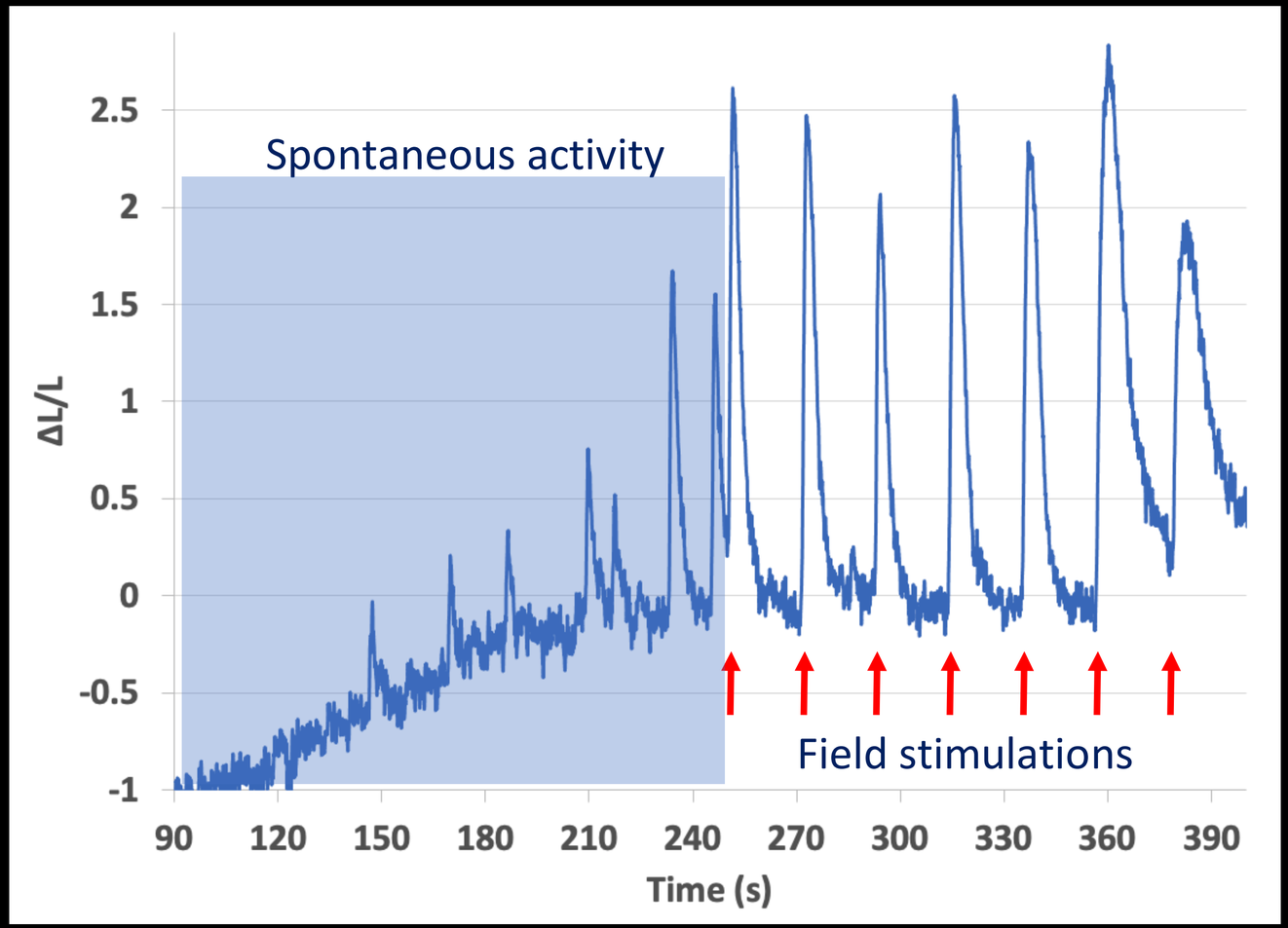
HeLa cells treated with L-histamine, $\Delta L/L_0$



Lambert, Crespo et al., (2023)

CaBLAM detects spontaneous and stimulated activity in hippocampal neurons



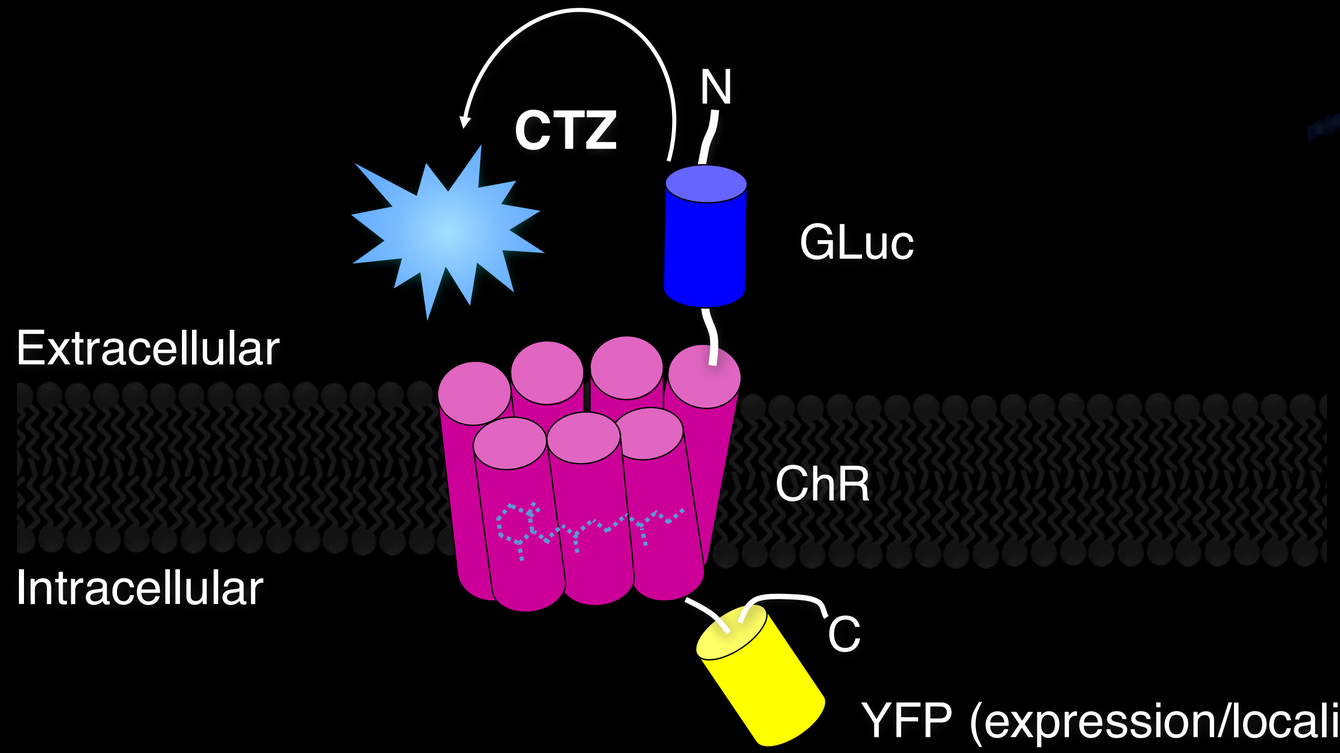


Single neuron trace, 10Hz

Lambert, Crespo et al., (2023)

Combining bioluminescence with optogenetics

Using BioLuminescence to Drive OptoGenetics: BL-OG

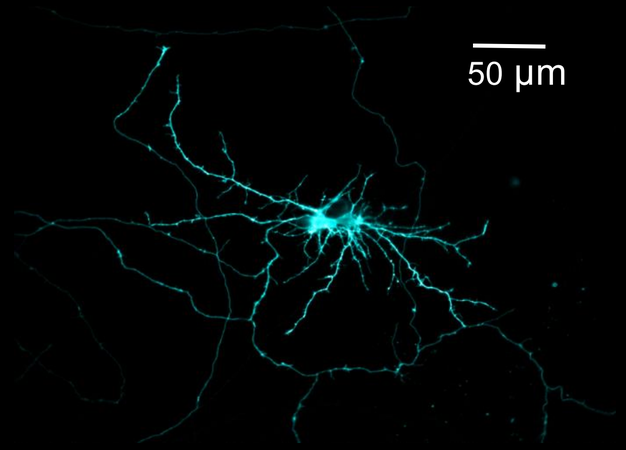


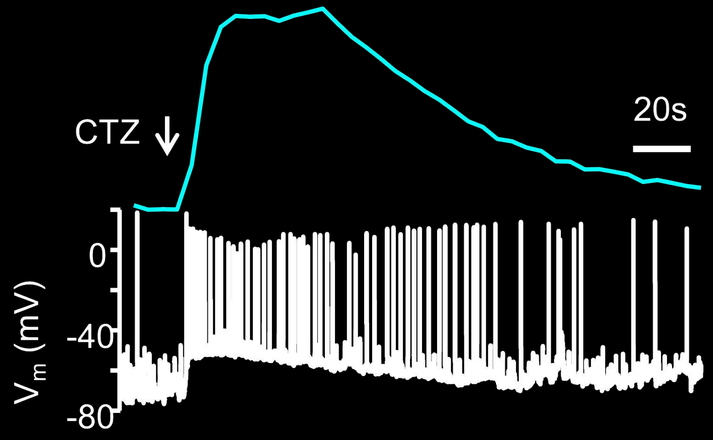
luciferase + channelrhodopsin = luminescent channelrhodopsin = Luminopsin (LMO)

U. Hochgeschwender

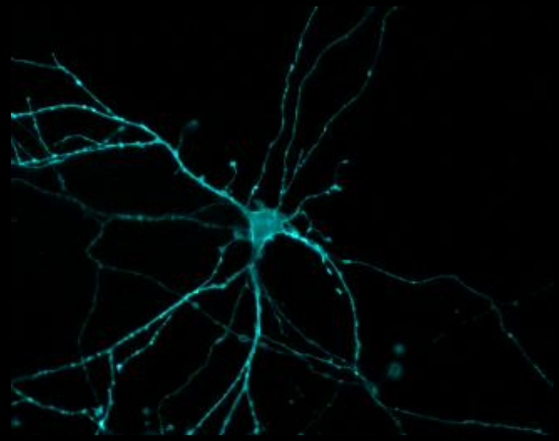
BL-OG in primary neurons

LMO3: sbGLuc-VChR1



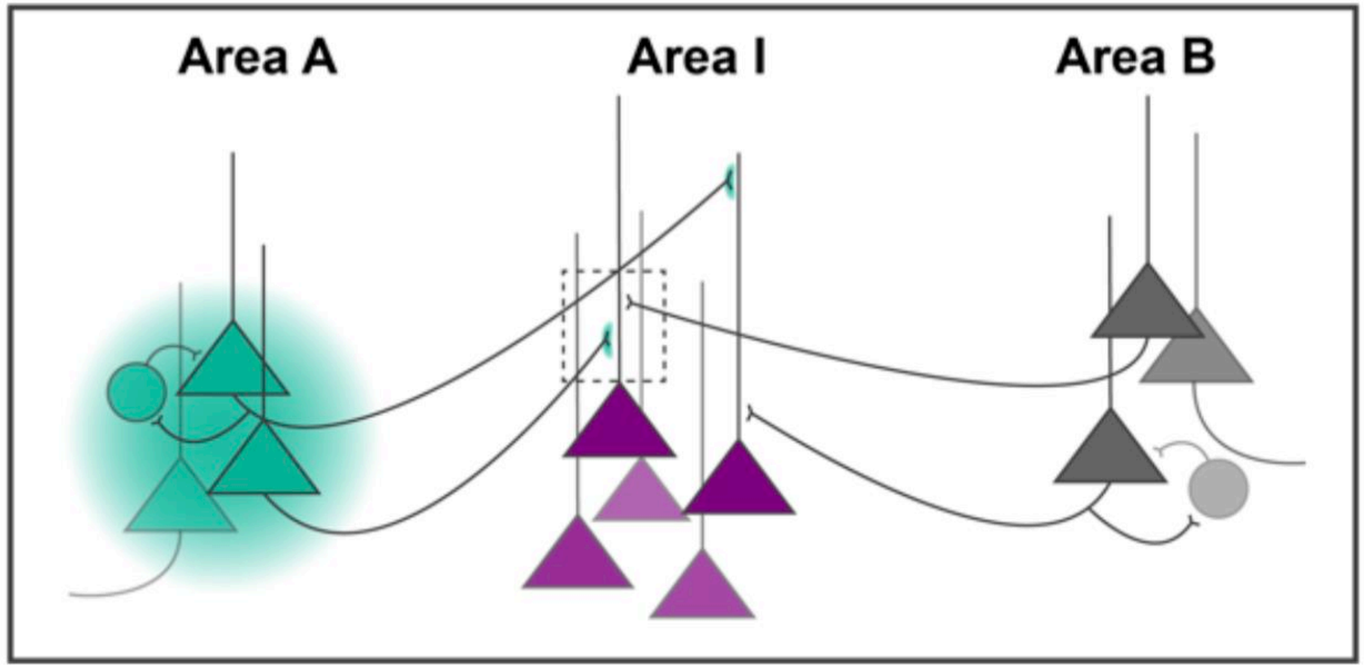


iLMO: slGLuc-Mac

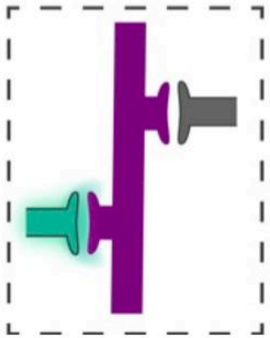


Berglund K, Clissold K, Li HE, / … / Augustine GJ, Yin HH, Hochgeschwender U. Proc Natl Acad Sci USA 113: E358-67, 2016

Interluminescence: Optical synapses



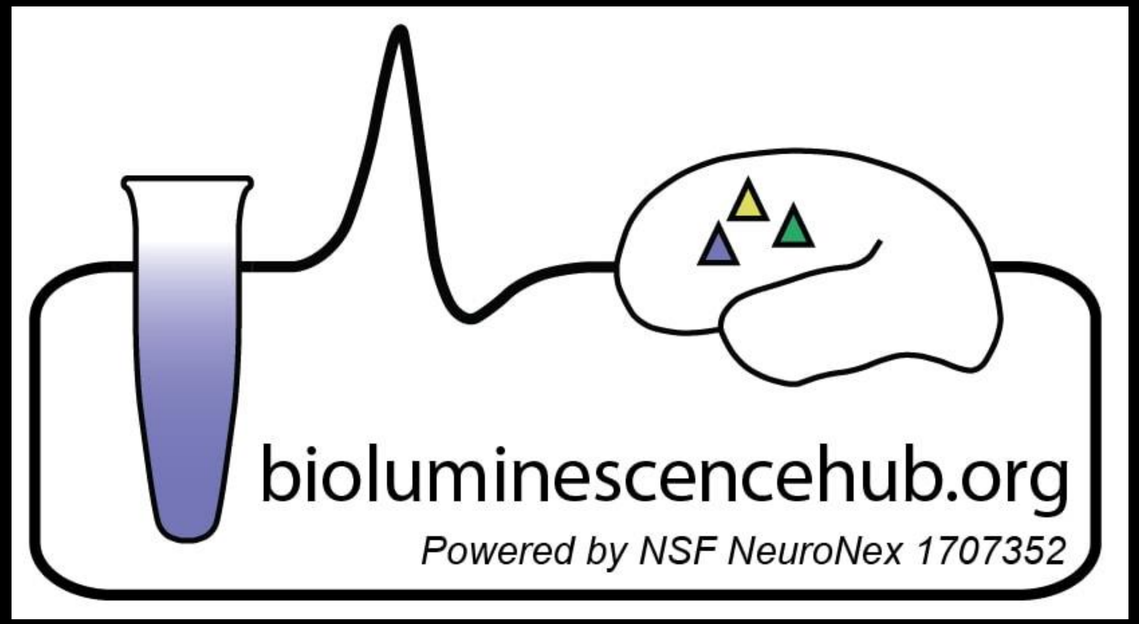
Presynaptic neurons express activity-responsive BL



Postsynaptic neurons express Optogenetic actuator

Light from presynaptic neurons activates OG elements in postsynaptic neurons

https://doi.org/10.1038/s42003-021-02981-7



BL-OG tools are freely available!

Thank you

Shaner Lab members (current): **Gerard Lambert, Brittany Sprecher, Daniel Nguyen, Sylvia To, Jaein Kwon,** and **Juan Jacobo Mejia**

Shaner Lab (alumni): Alexus Grey, Ernesto Alcala, Jerick Kim, Skylar Laube, Camille Phares, Frida Lucía Padilla Rodríguez, Alexia Jazmín López Pillado, Selena Luong, Matthew Tate, Frederic-Khoi Nguyen, Aidan Bell, Lauren Barnett, Daphne Bindels, Andreas Björefeldt, Albertina Torreblanca Zanca, Meihua Chu, Daniel Heydari, Richard Orcutt, Tariq Brown, Ryann Felton, Hira Choudhury, Dylan Gregory, Patrick Friend, Sabrina Tran, Alicia Liu, and Ali Hakimi

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Todd Martz (Scripps), Carly Kenkel (USC), Phillip Cleves (Carnegie Science), Tracy Larson (UVa), and Trevor Hamilton (MacEwan U)

Steven Haddock, Wyatt Patry, Darrin Schultz (MBARI, UCSC, Monterey Bay Aquarium)

Chris Moore, Diane Lipscombe, Ian Moore, Jeremy Murphy, and Justine Allen (Brown U)

Ute Hochgeschwender, Emmanuel Crespo, Stephanie Venn, Akash Pal, Eric Petersen, and Mansi Prakash (Central Michigan U)

Antoine Royant, Damien Clavel, Céline Lafaye, Sylvain Aumonier, Hadrien Depernet, and Guillaume Gotthard (IBS/ESRF)

Anya Salih (Western Sydney University) and Robert Brennan (independent adventurer)

Robert Campbell, Yusuke Nasu, Liang-da Chu, and Katsumasa Fujita (U Tokyo)

Jennifer Waters and Talley Lambert (Harvard Med School)

Thom Hughes, Mikhail Drobizhev, and Rosana Molina (Montana State U)

Jennifer Prescher (UC Irvine), Peter Yingxiao Wang (USC, UCSD)

Vincent Levesque (Birch Aquarium at SIO)

François St-Pierre (Baylor College of Medicine)

and many, many, many others