

Fluorescent Proteins

Nathan Shaner

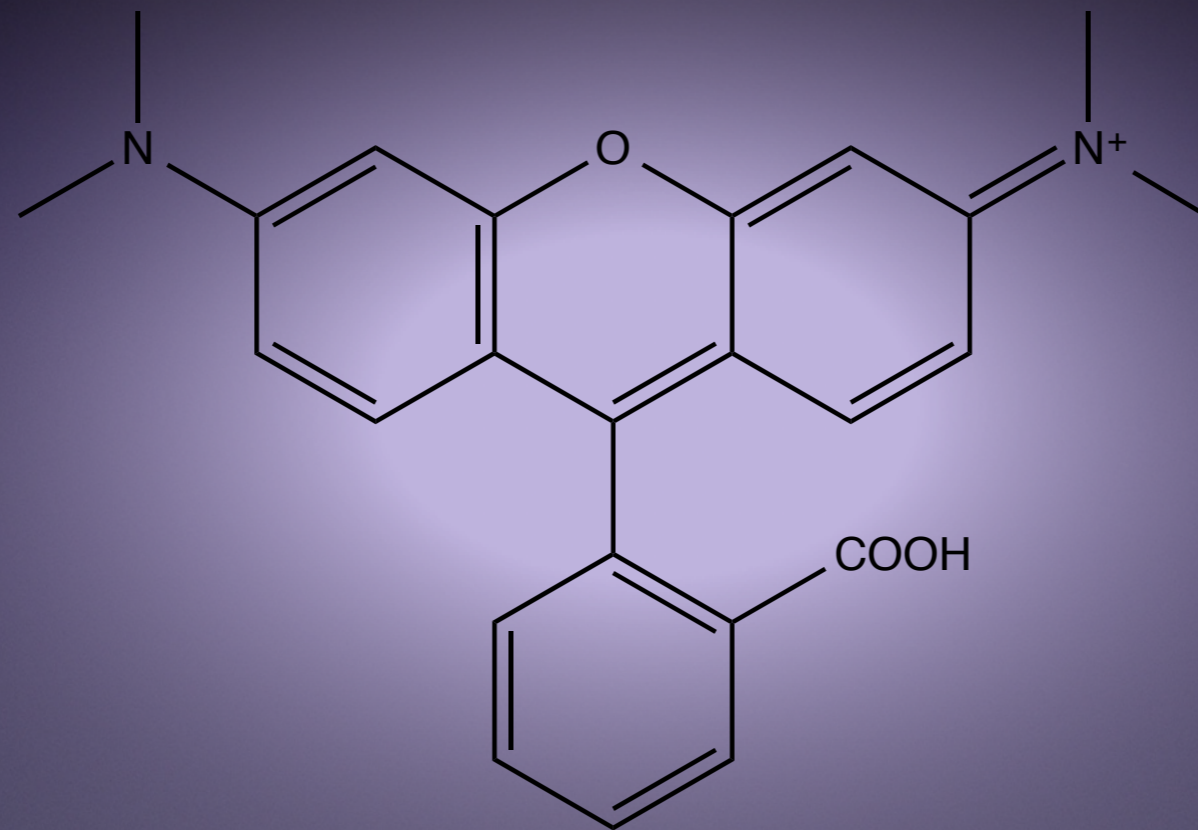


SCINTILLON INSTITUTE

FOR BIOMEDICAL AND BIOENERGY RESEARCH

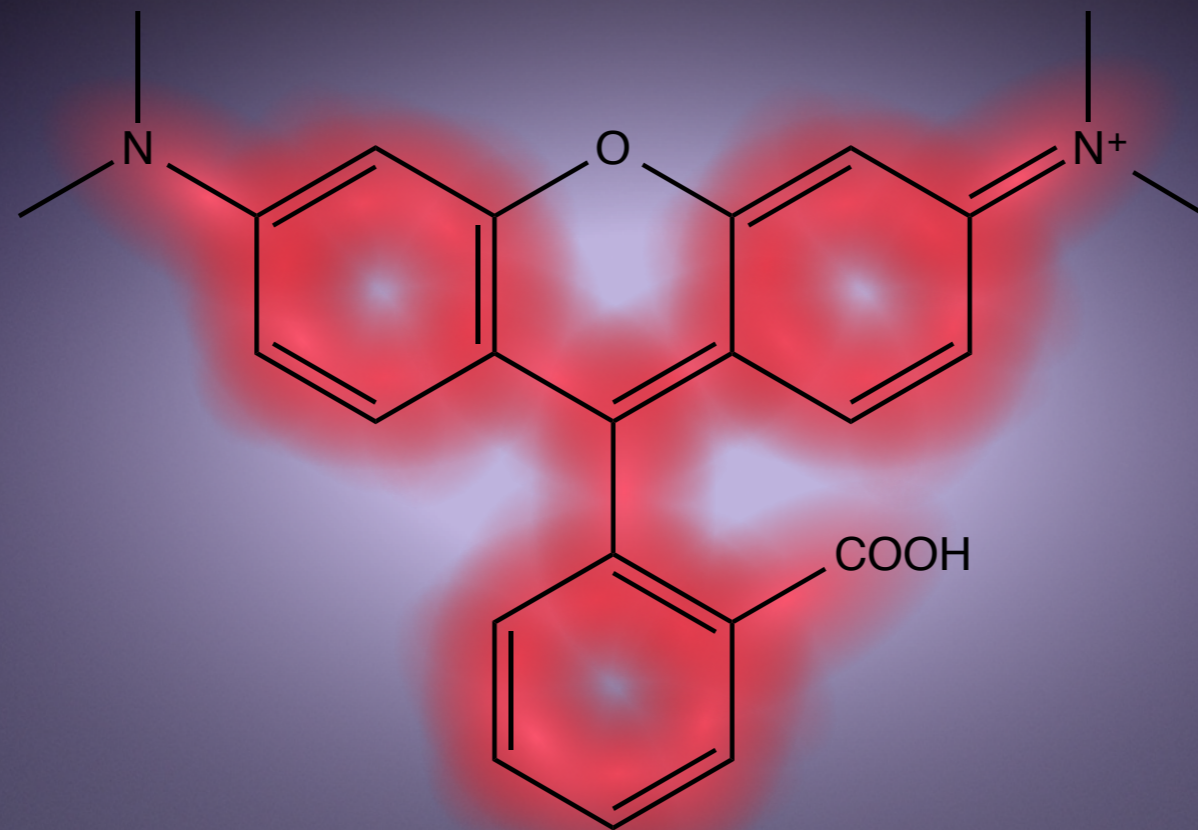
What is fluorescence?

What is fluorescence?



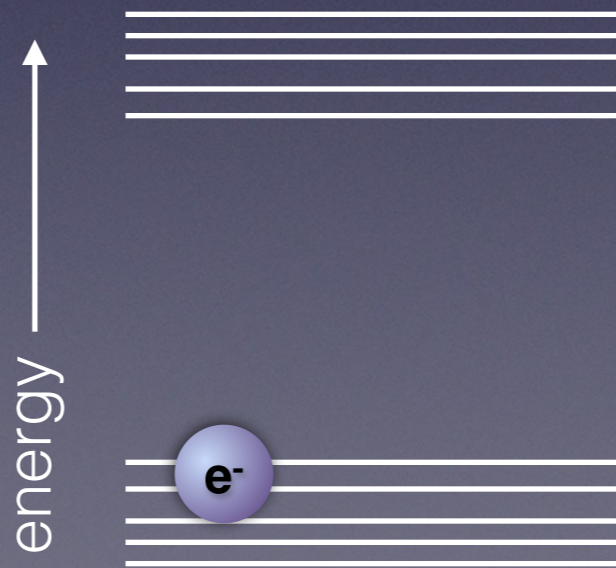
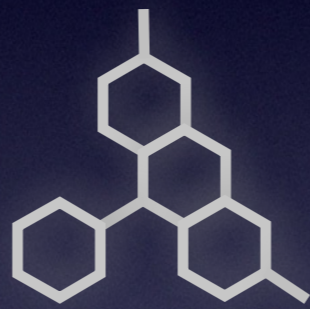
Planar molecules

What is fluorescence?



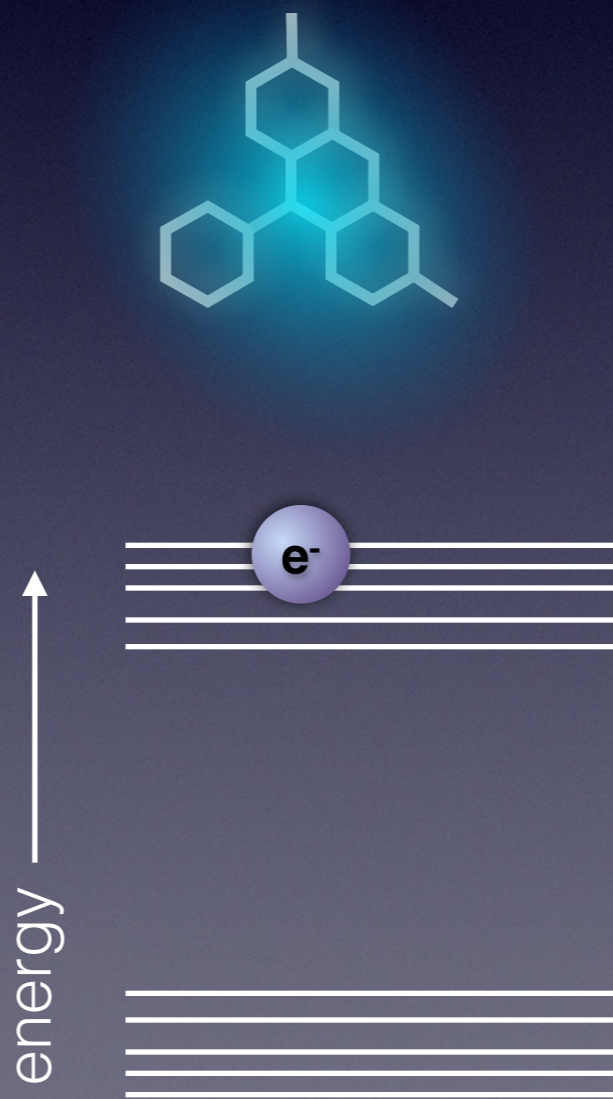
Planar molecules
Extended π -orbital system
("conjugated")

What is fluorescence?



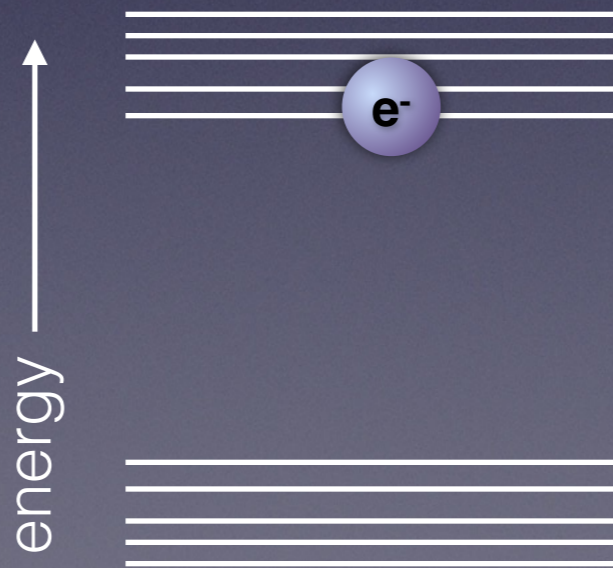
What is fluorescence?

Excitation



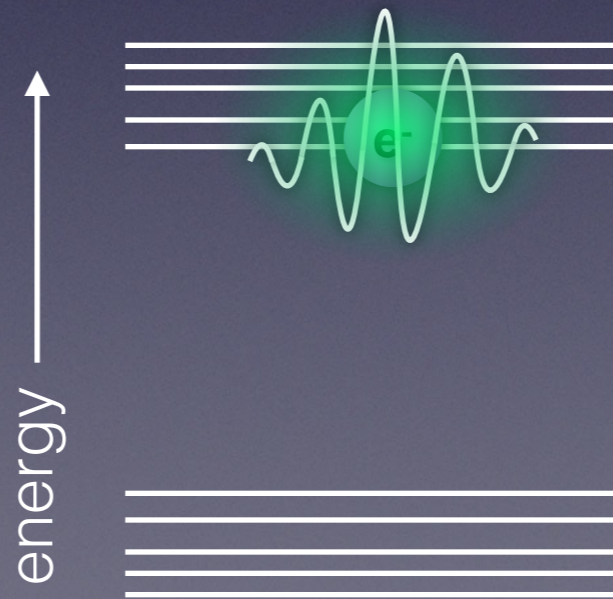
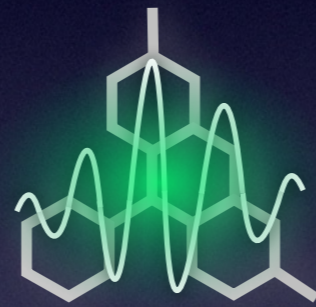
What is fluorescence?

Relaxation



What is fluorescence?

Emission



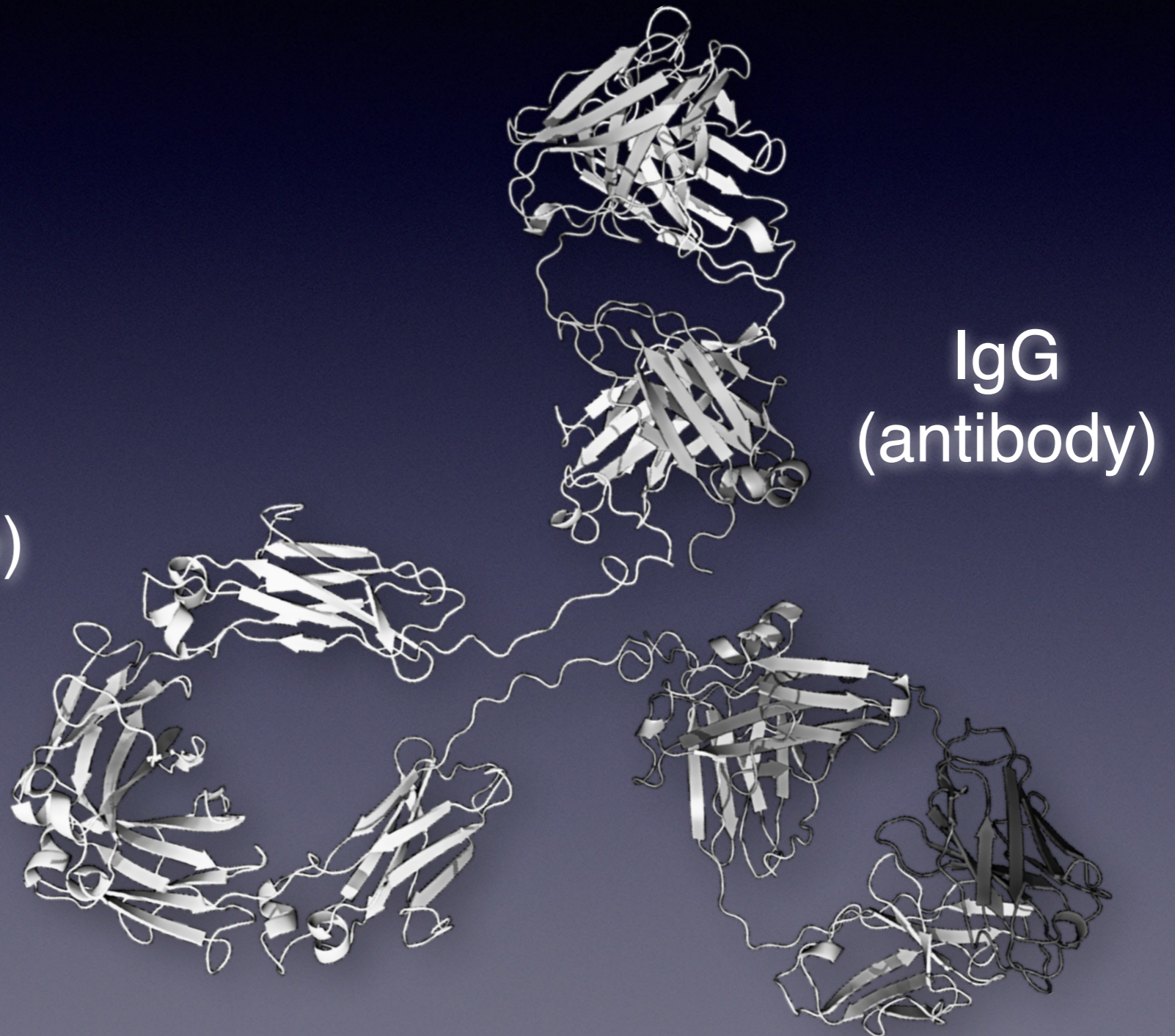
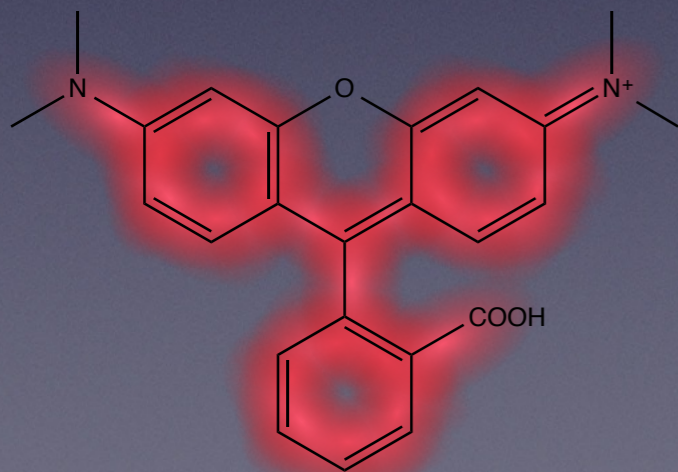
Dyes vs. genetically encoded labels

A few small-molecule dyes can be used directly for labeling, e.g. DAPI

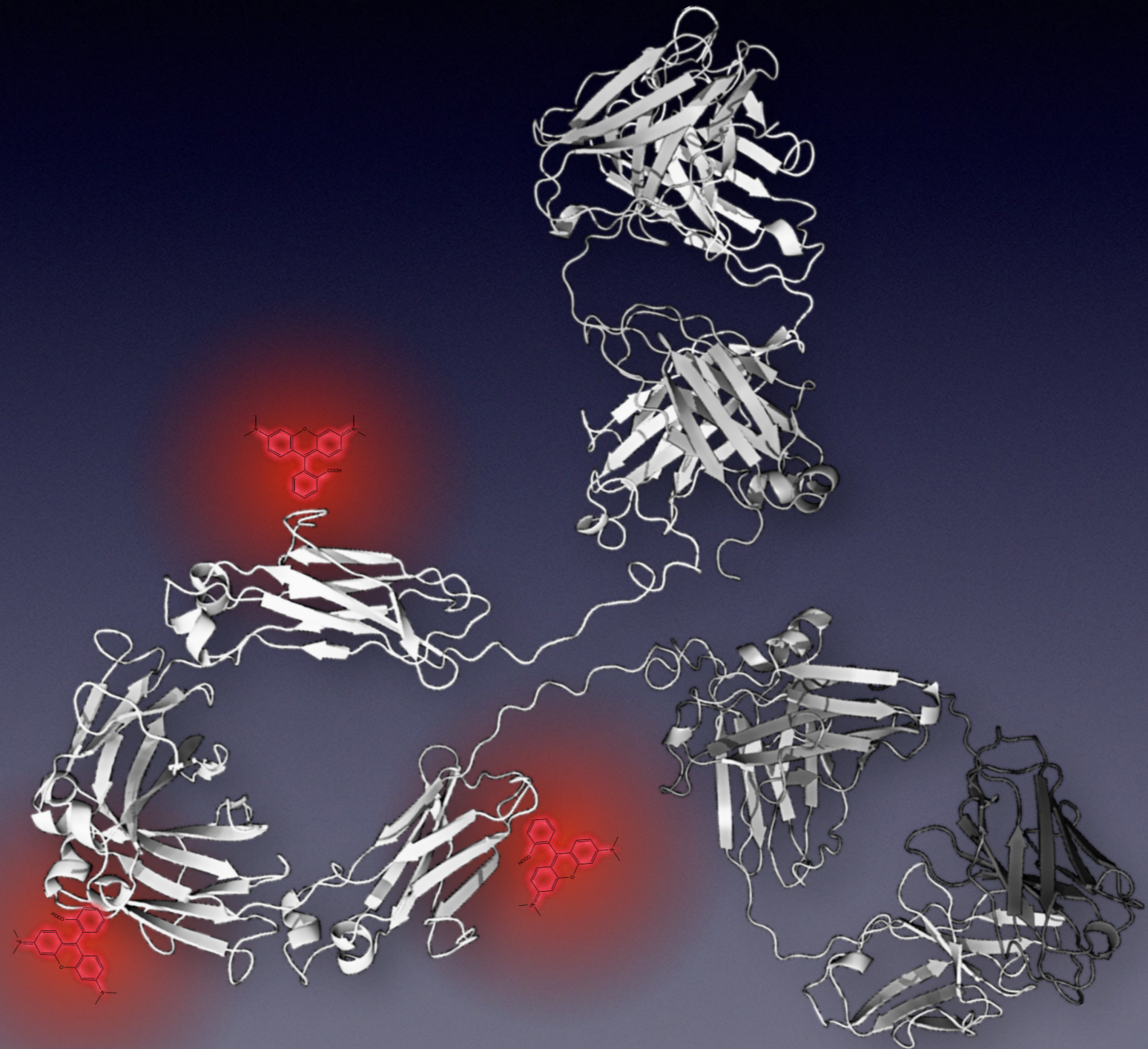
Most of the time, you need an antibody against your protein of interest!

Dyes vs. genetically encoded labels

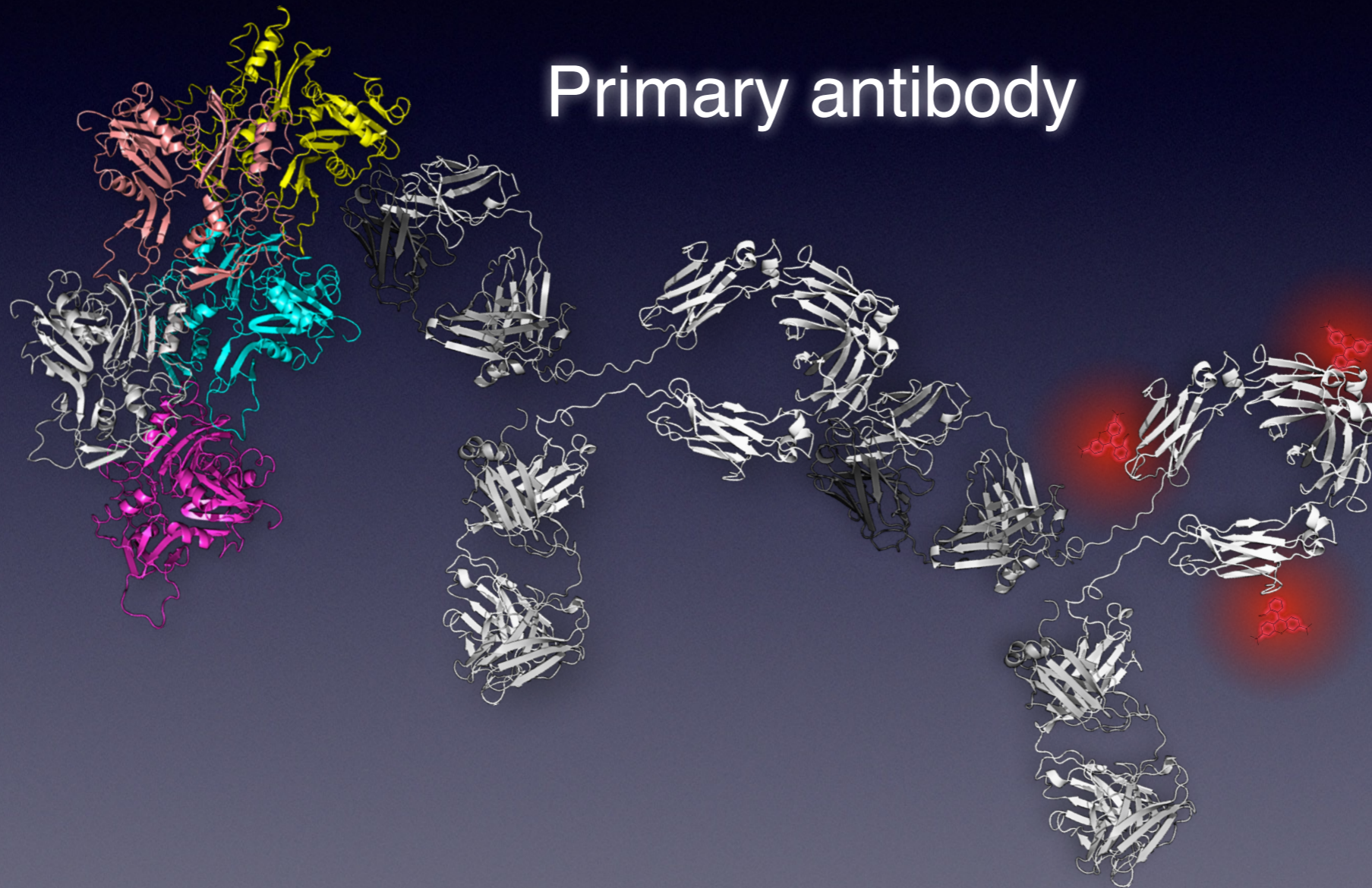
dye (e.g. tetramethylrhodamine)



Dyes vs. genetically encoded labels



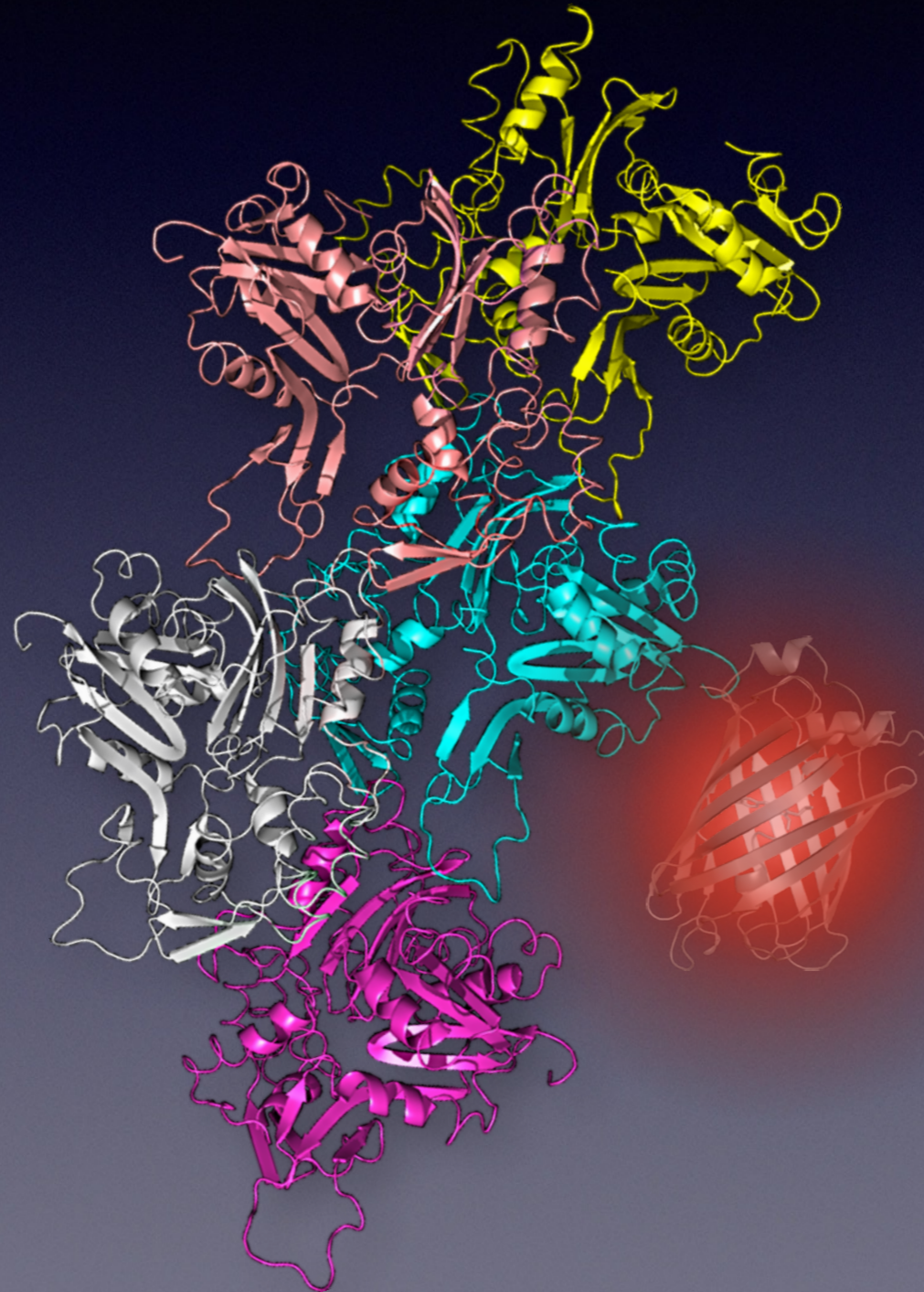
Dyes vs. genetically encoded labels



Mostly for fixed cells!

Secondary antibody

Dyes vs. genetically encoded labels



Live cells

Fluorescent Proteins

Incredibly diverse genetically encodable dyes

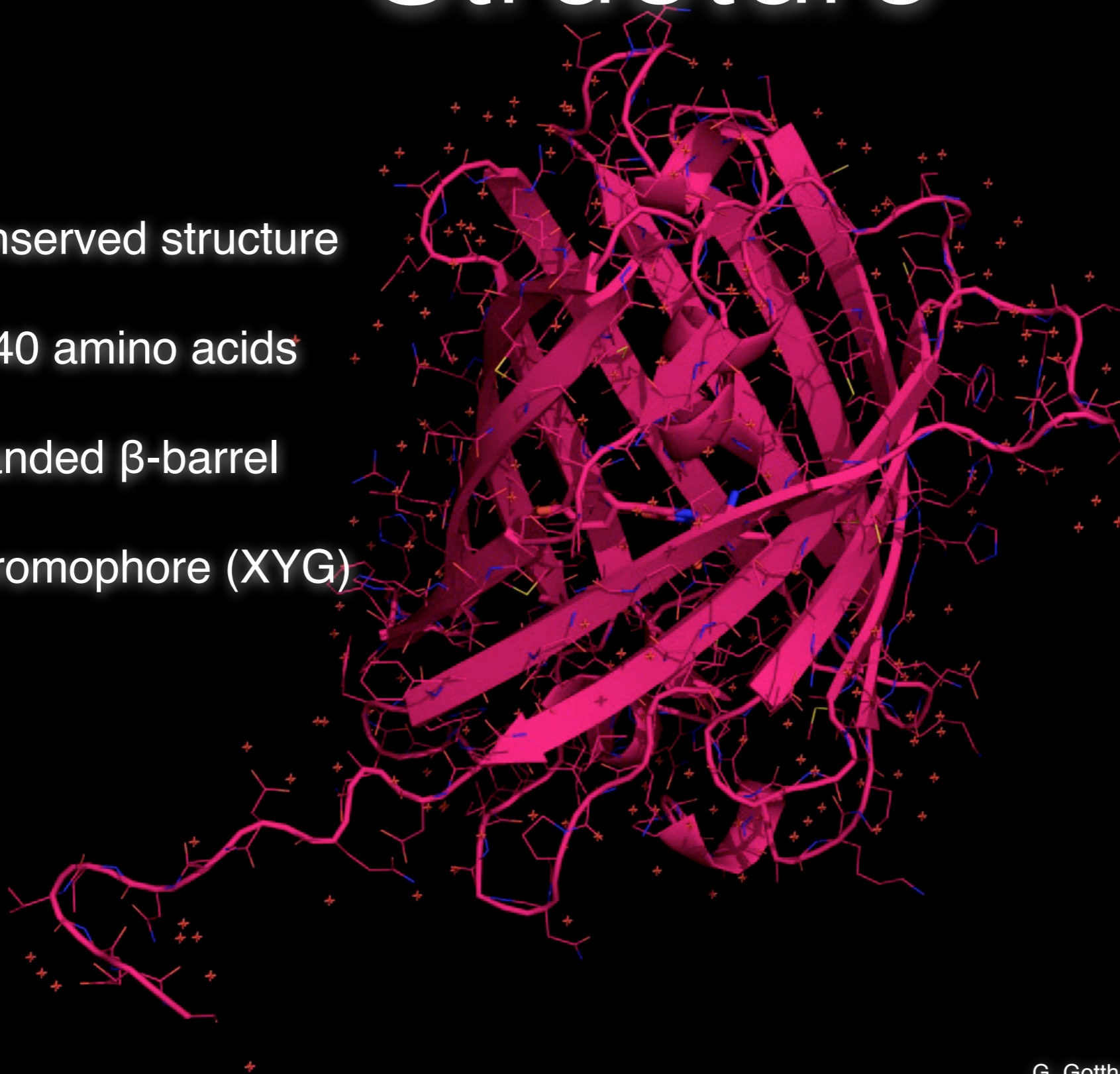
Structure

highly conserved structure

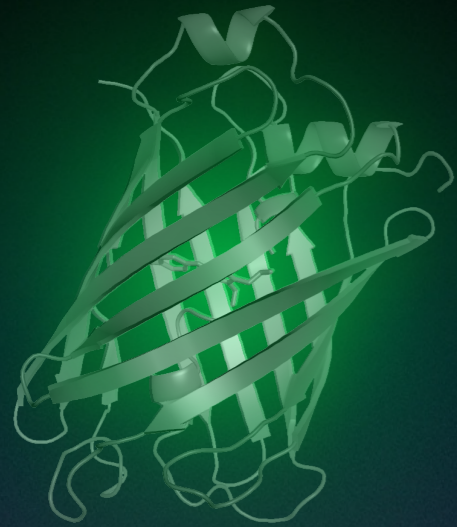
~220-240 amino acids

11-stranded β -barrel

central chromophore (XYG)



A little history

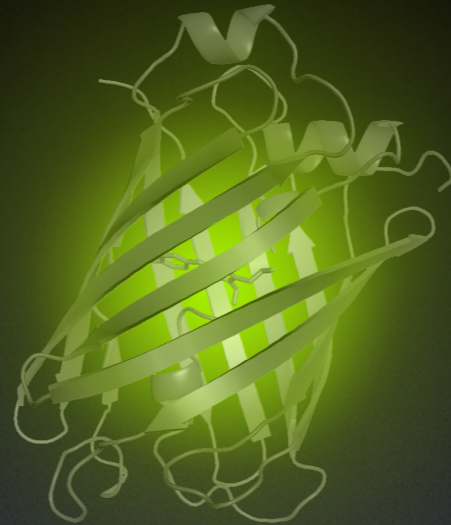


1994

Prasher et al.

1992

Heim et al.

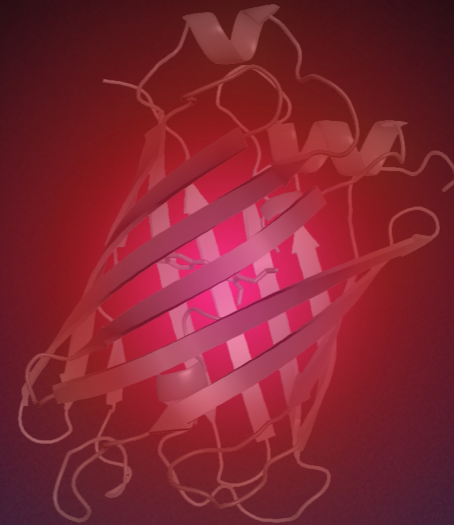


Cubitt et al.

1995

1999

Matz et al.

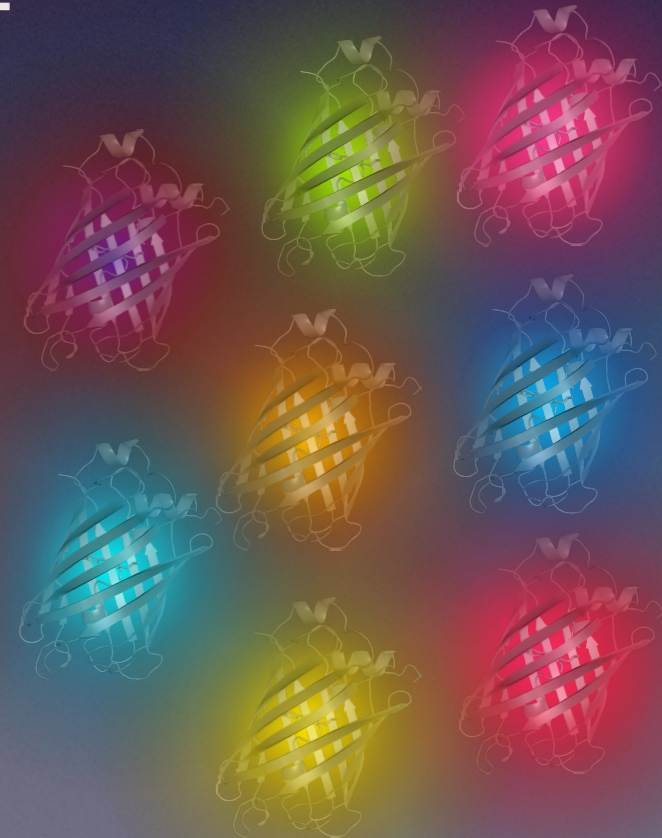
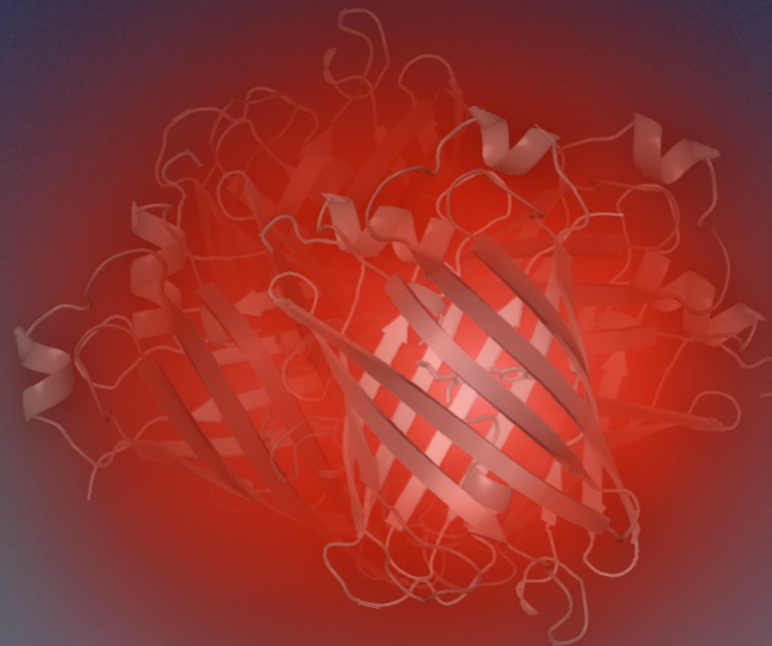
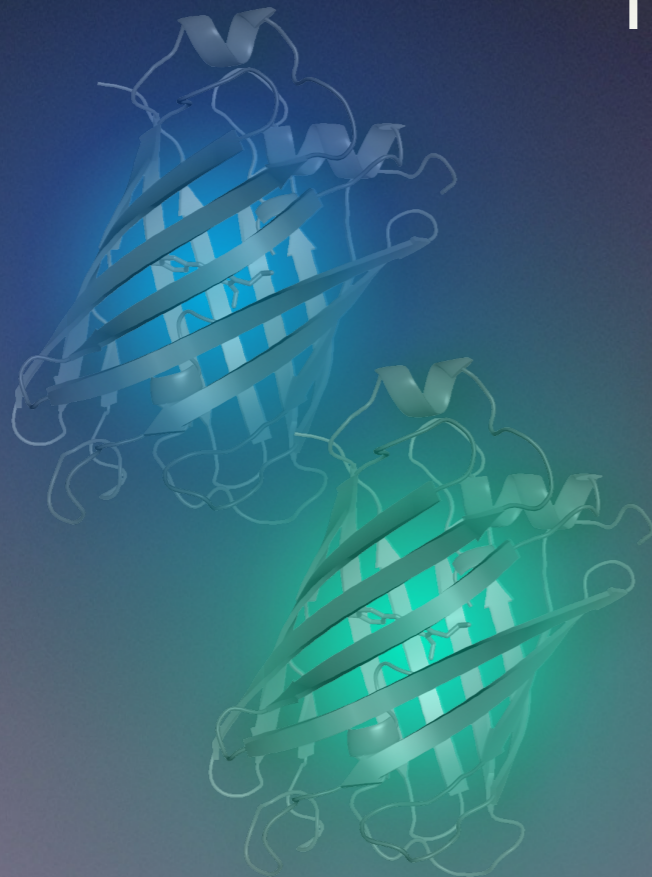


Campbell et al.

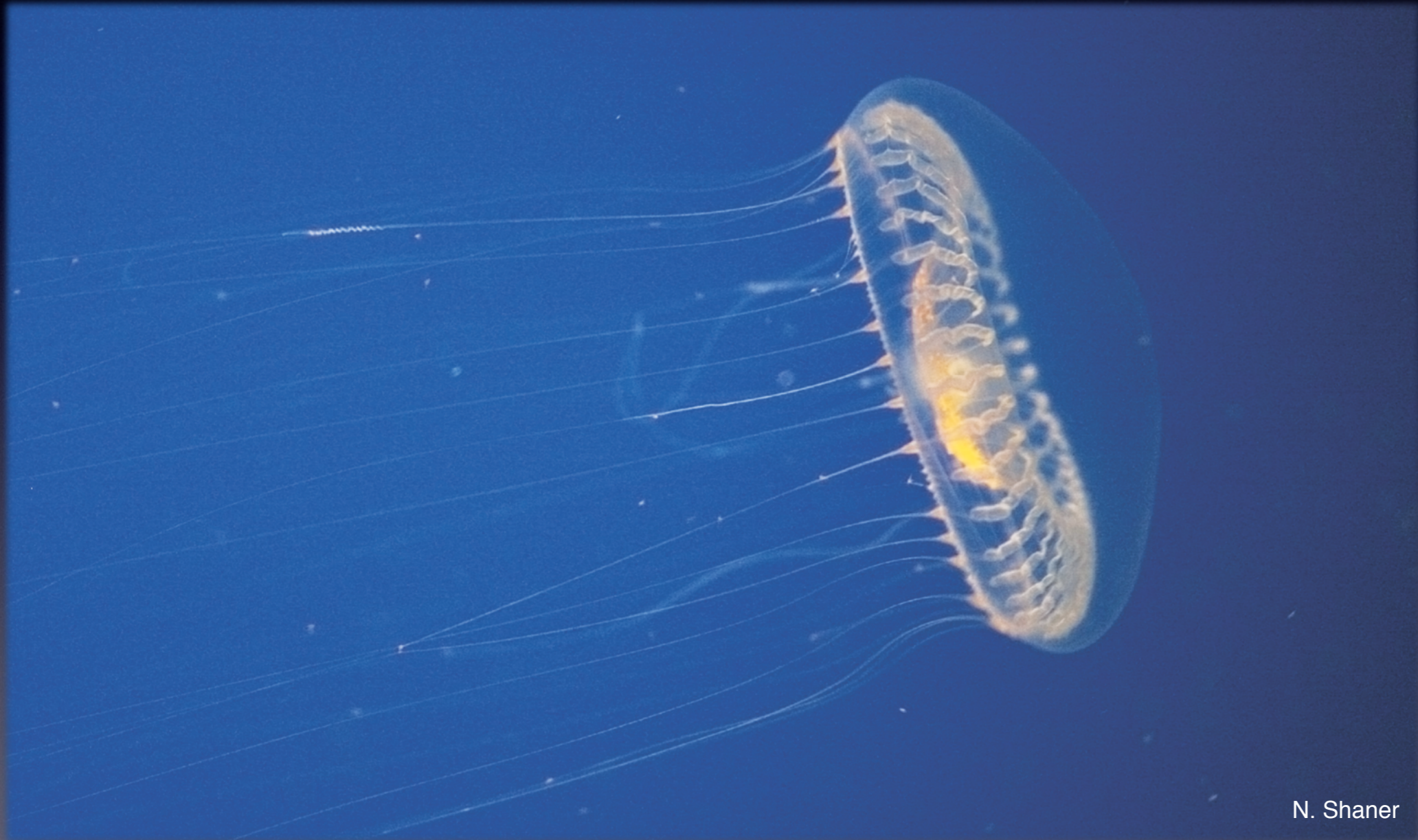
2002

2004+

Shaner et al., Wang et al.,
many, many, many others....



A little history

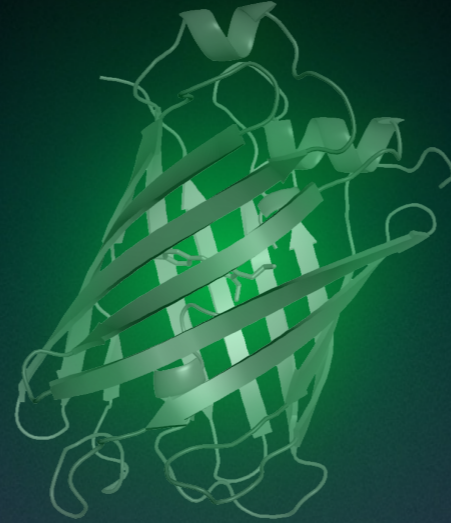


N. Shaner

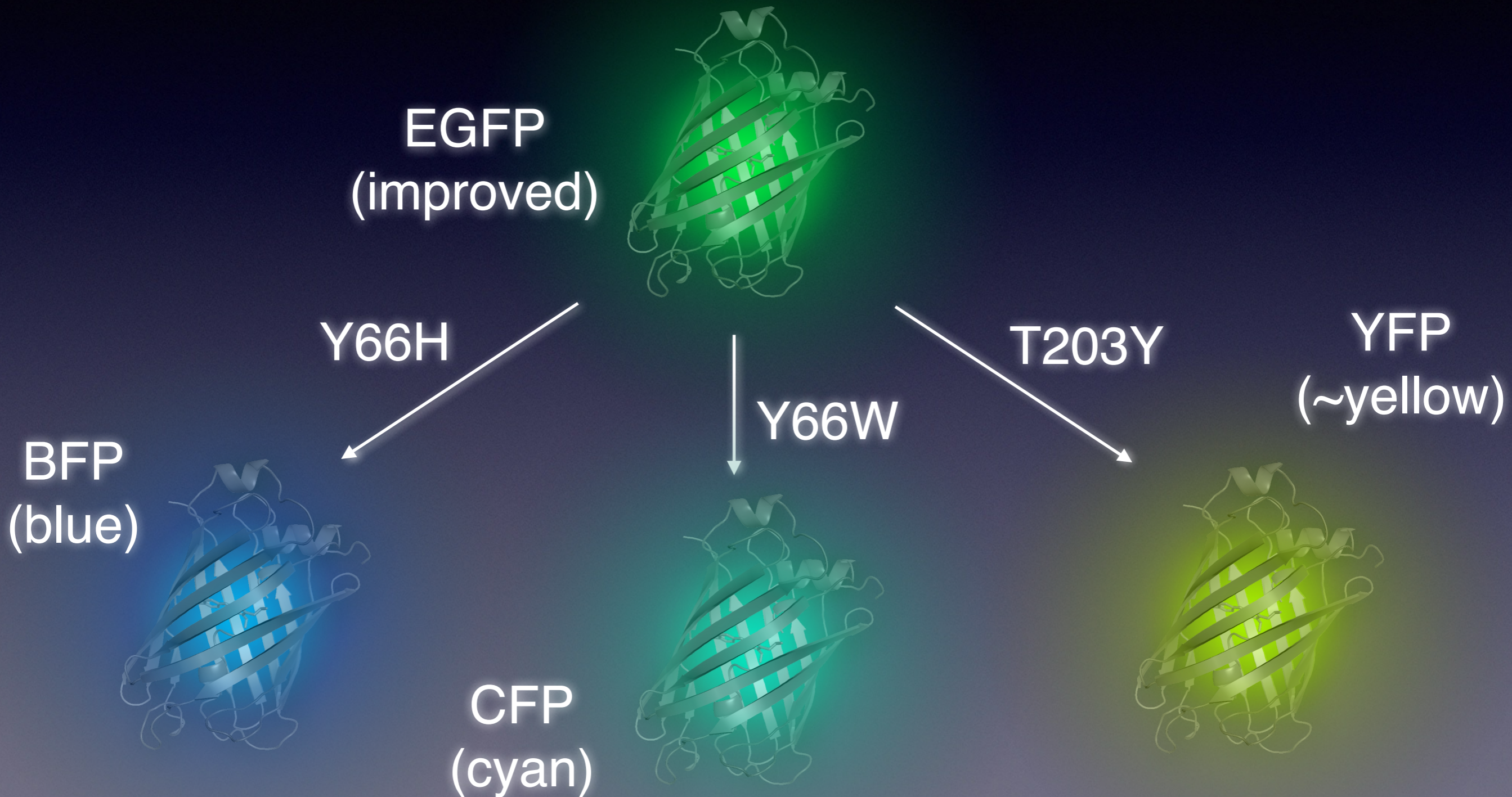
Aequorea victoria

Aequorea GFP

GFP
(green)



Aequorea GFP



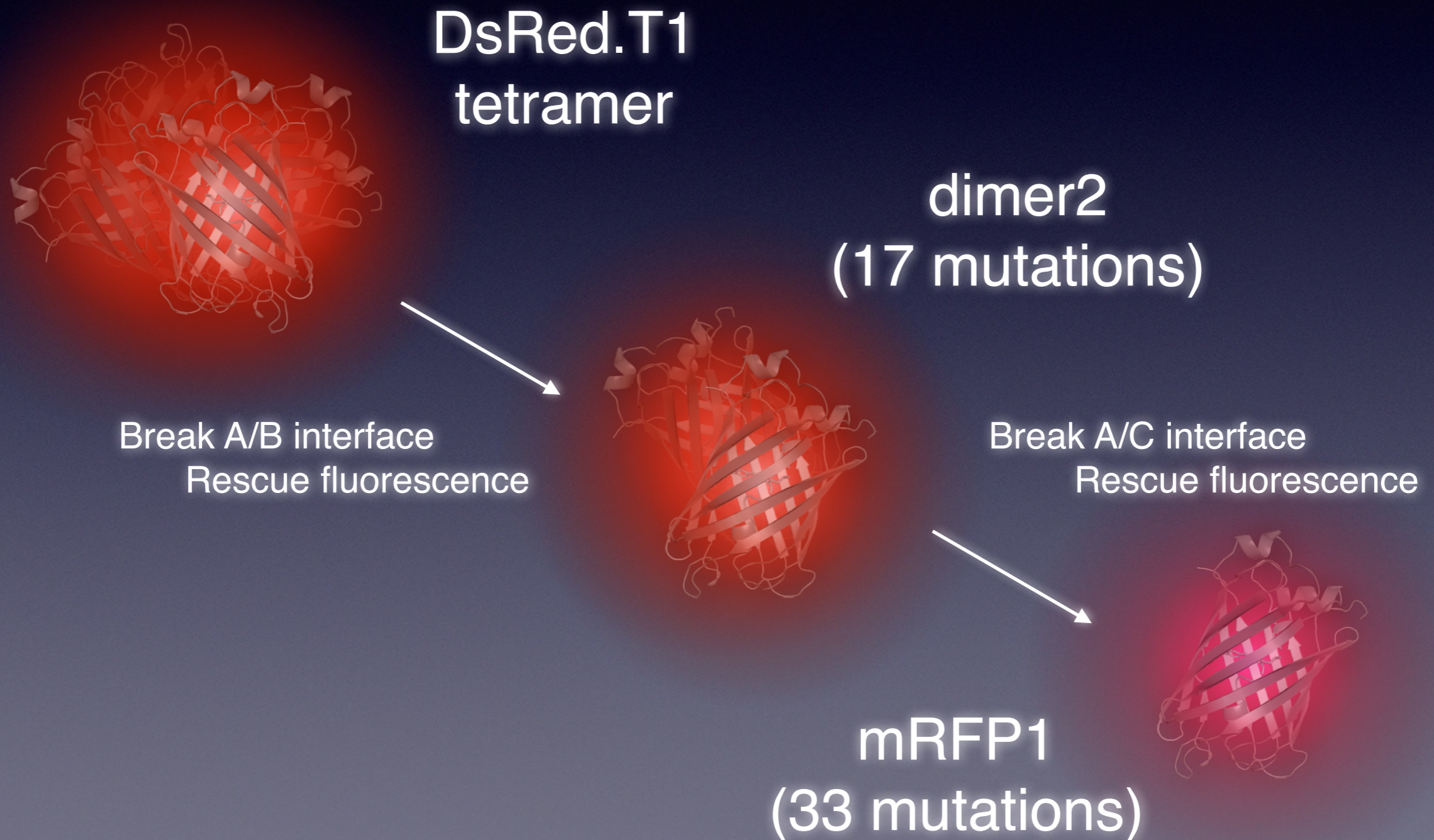
Discosoma RFP



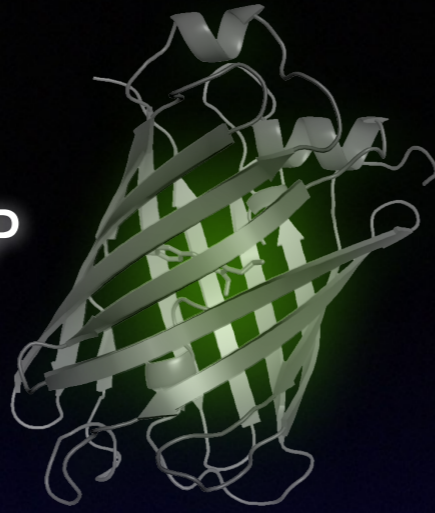
Flickr: Fauna NYC

Discosoma sp.

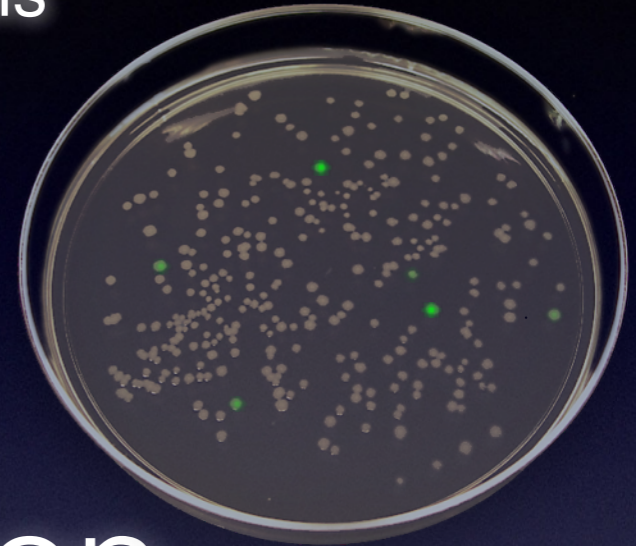
Discosoma RFP



wild-type FP

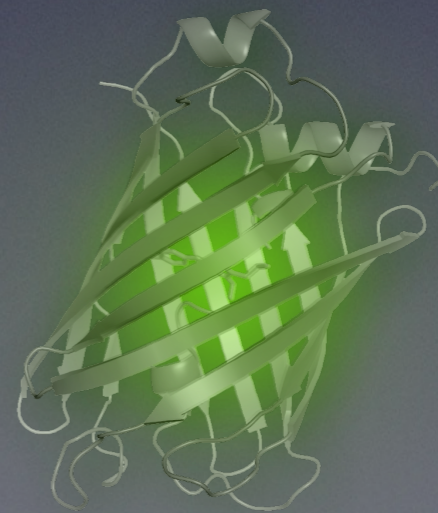


random
mutagenesis

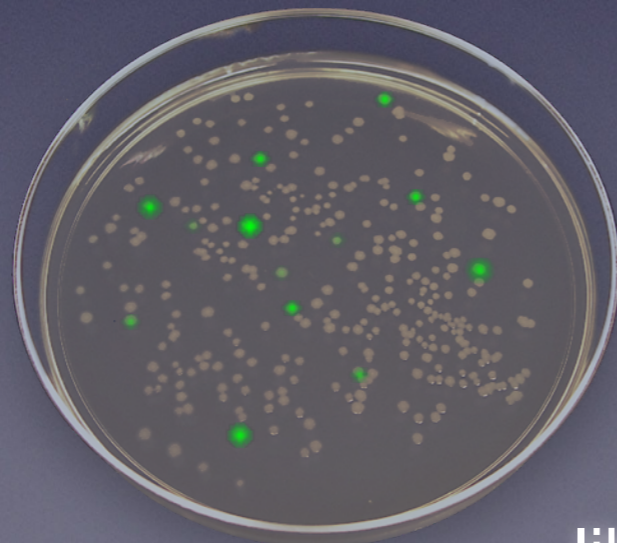


Directed evolution

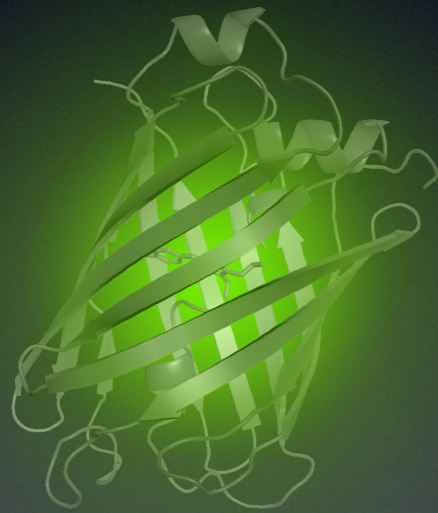
sequence
improved
mutants

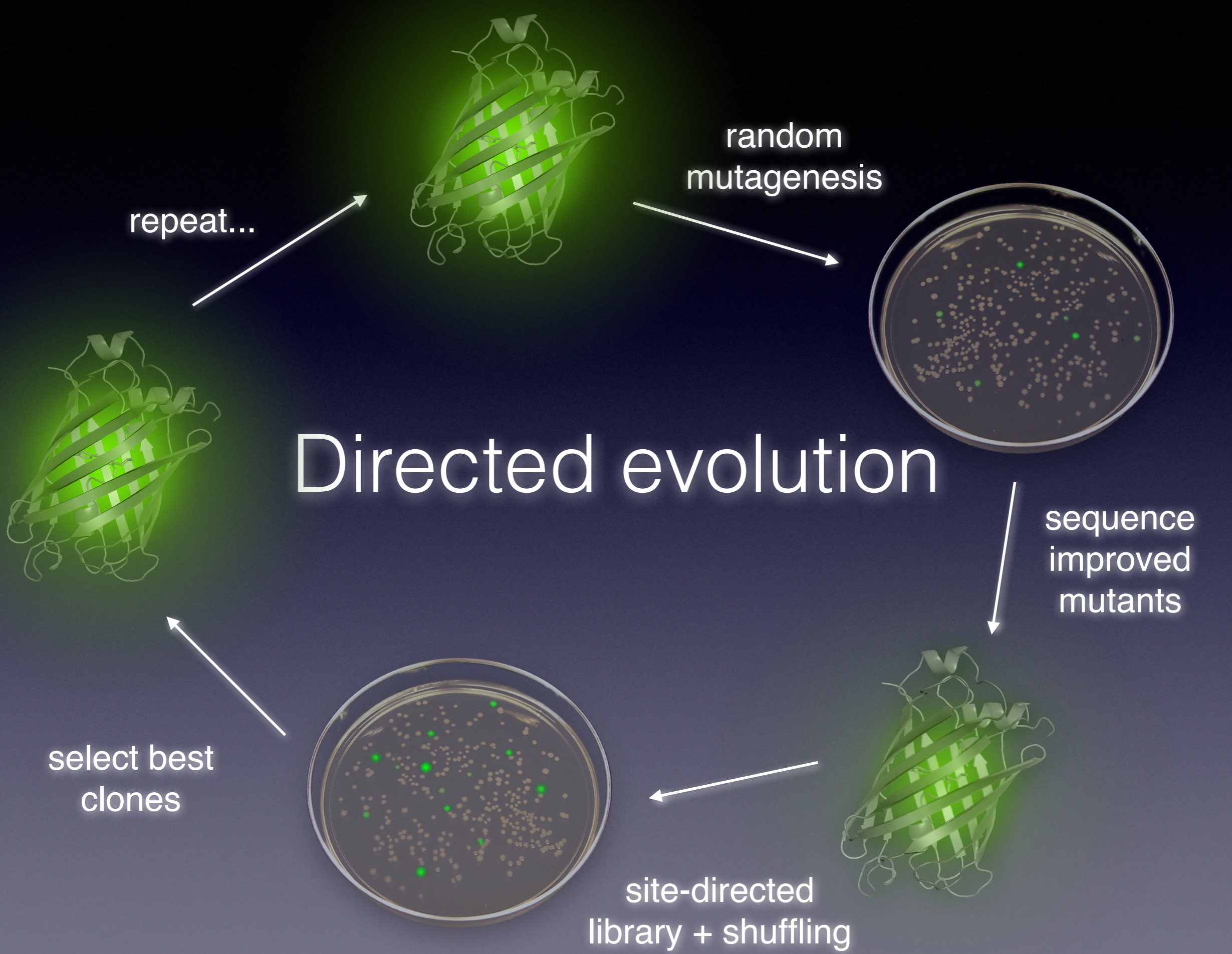


site-directed
library + shuffling



select best
clones





Directed evolution

repeat...

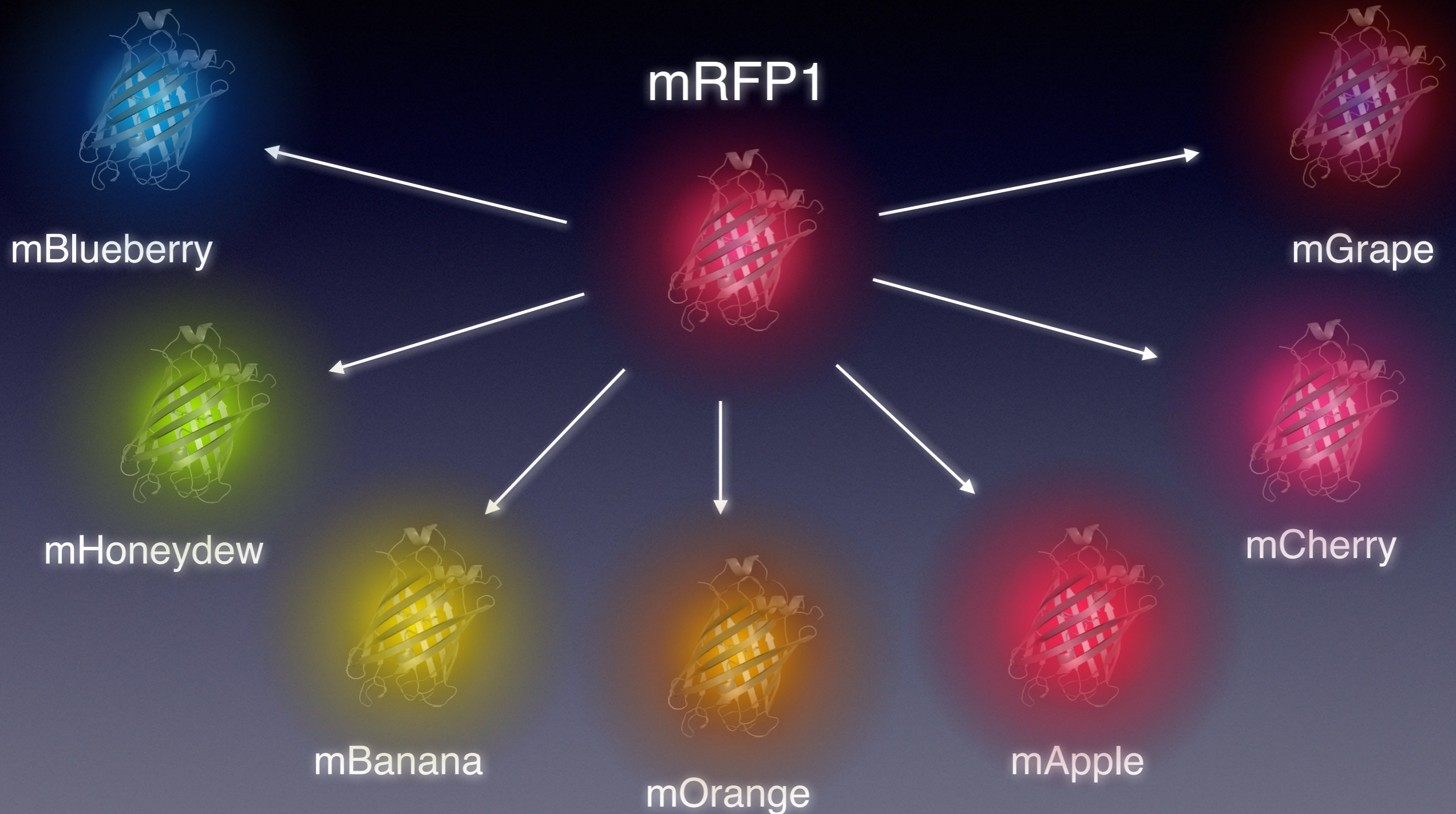
random
mutagenesis

sequence
improved
mutants

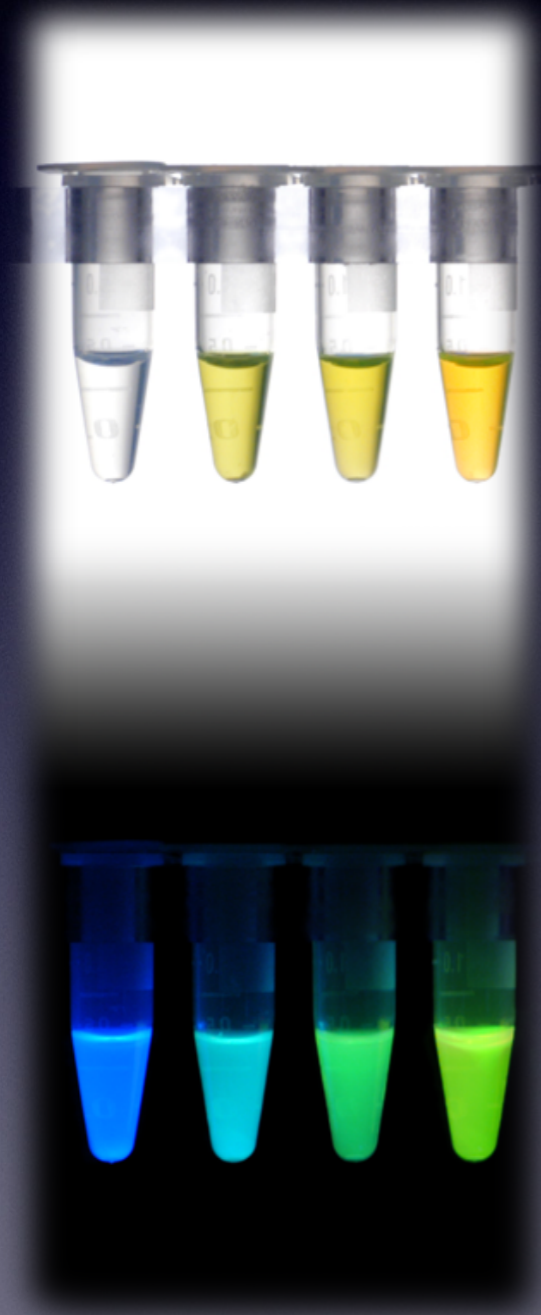
select best
clones

site-directed
library + shuffling

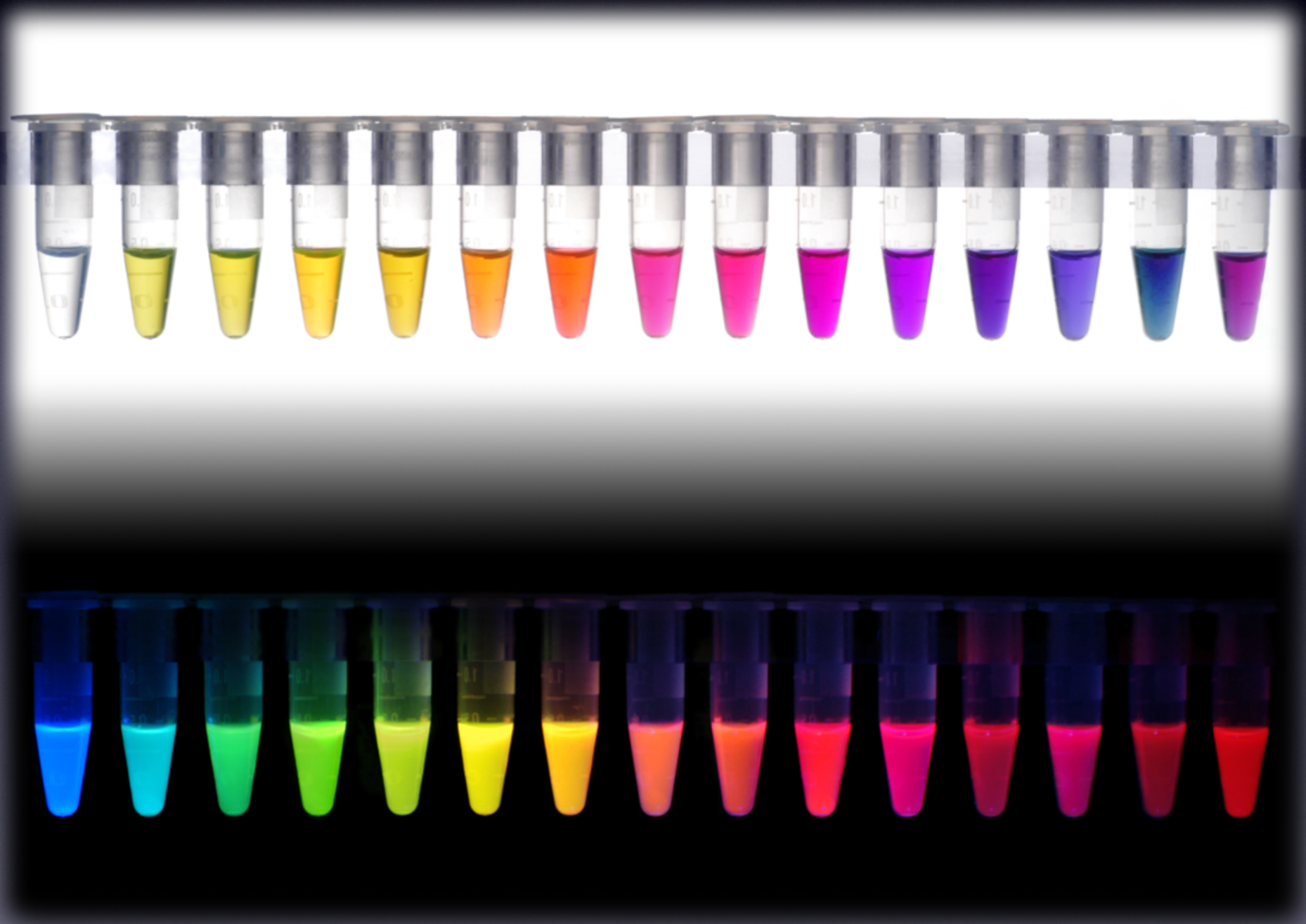
The "mFruits"



20 years ago



10 years ago



Today

mNeonGreen

mKeima

mCitrine/
mVenus



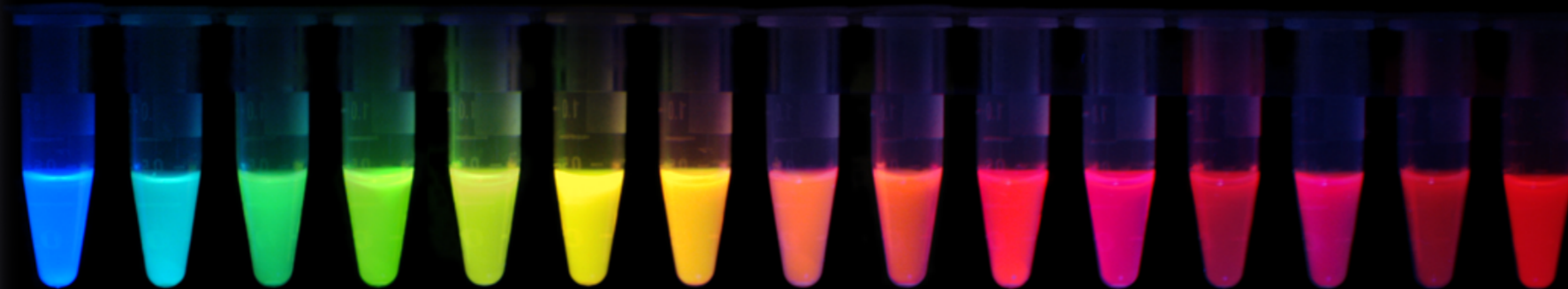
mRuby2

mNeptune

“Regular” FPs

mKate2

EBFP2



many, many,
many other
good FPs

mTurquoise2

mEmerald +
sfGFP, etc.

mOrange2

mKO variants

LSSmOrange

Today



Photoactivatable



Today

A 2x14 grid of test tubes. The top row shows a color gradient from light yellow to dark purple. The bottom row shows a color gradient from light blue to dark red. The text 'Photoswitchable' is centered between the two rows.

Photoswitchable

Today

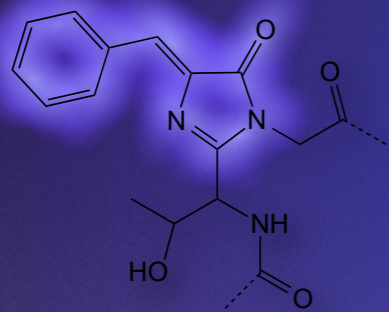
A 2x15 grid of test tubes. The top row shows a color gradient from light yellow to dark purple. The bottom row shows a color gradient from light blue to dark red. The text 'Photoconvertible' is centered between the two rows.

Photoconvertible

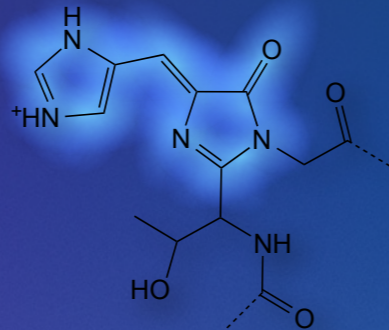
Don't forget to upgrade!



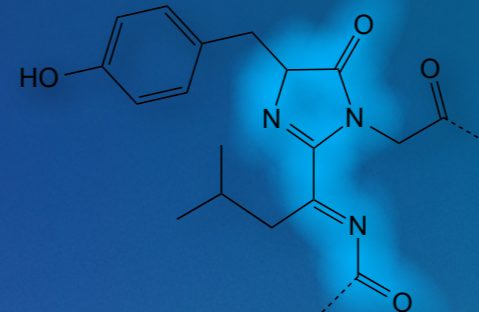
Chromophore diversity



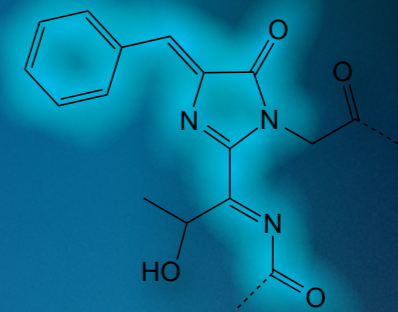
UMFP (Sirius)
x355/m424



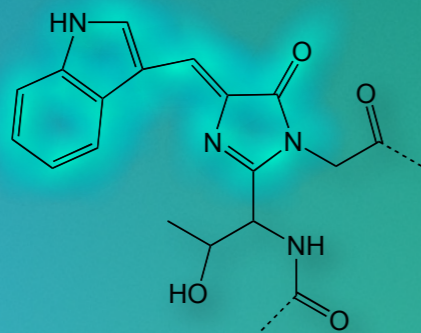
BFP
x380/m440



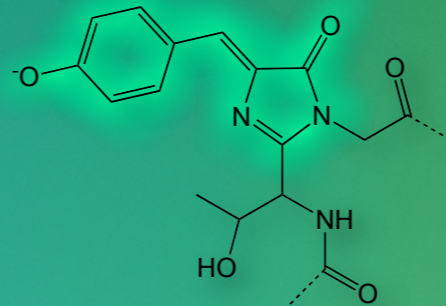
TagBFP
x402/m457



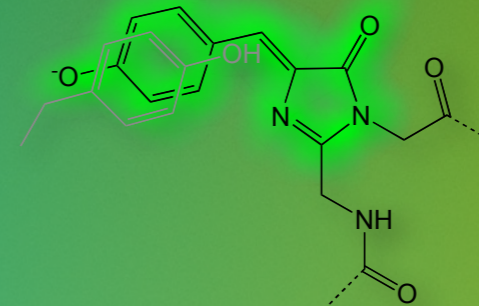
mBlueberry
x402/m467



CFP
x434-452/m476-505

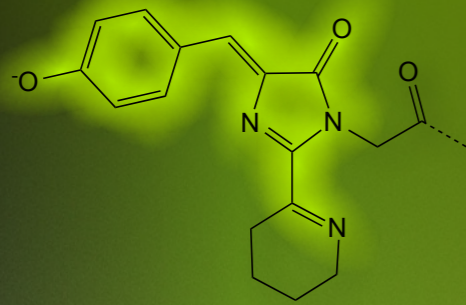


(E)GFP
x488/m510

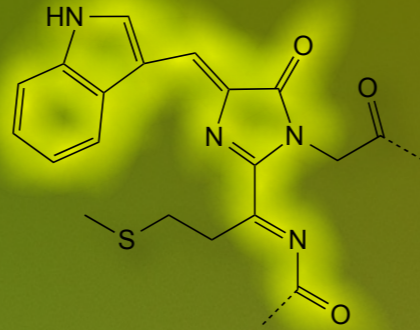


YFP
x514/m529

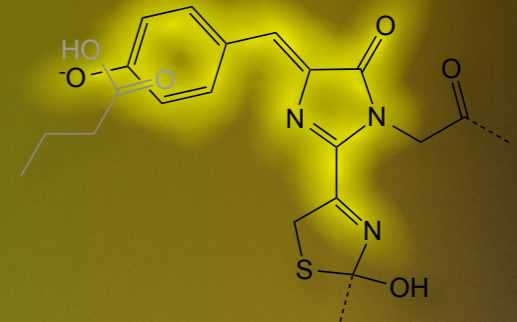
Chromophore diversity



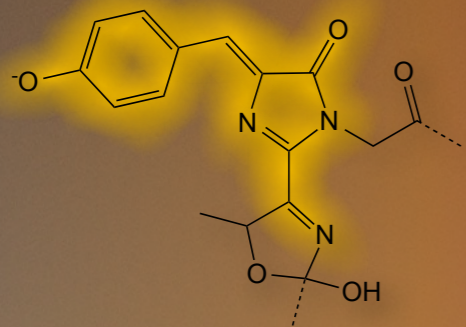
zFP538
x525/m538



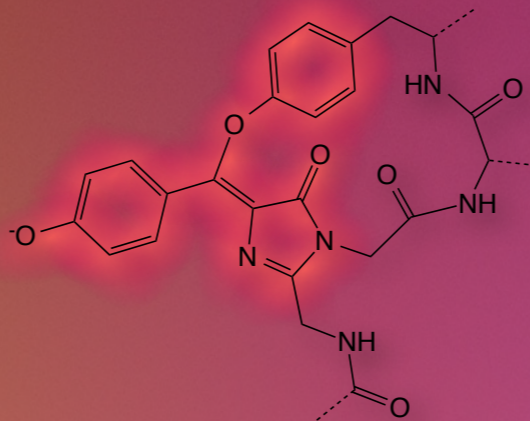
mHoneydew
x480-504/m537-562



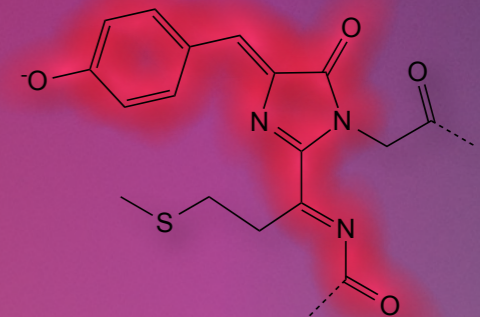
mBanana
x540/m553



mOrange
x548/m562

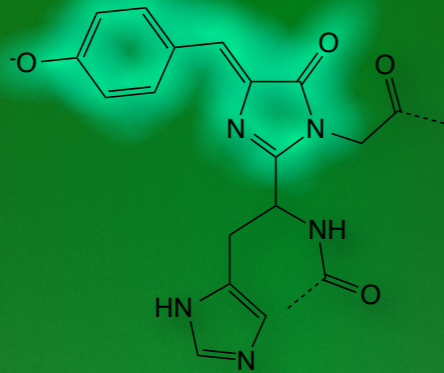


LanRFP
x520/m600

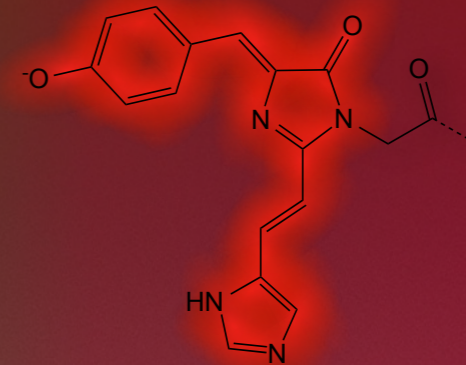


mCherry
x587/m610

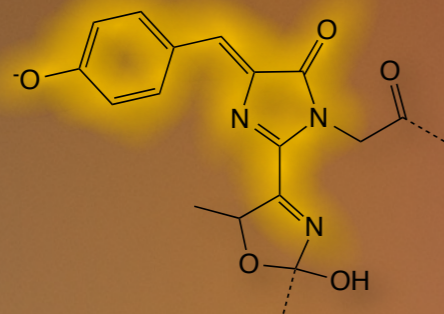
Chromophore diversity



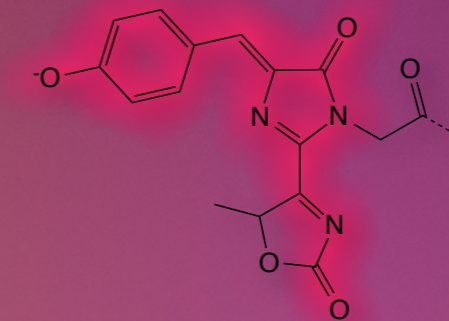
Kaede (pre-conversion)
x508/m518



Kaede (post-conversion)
x572/m582



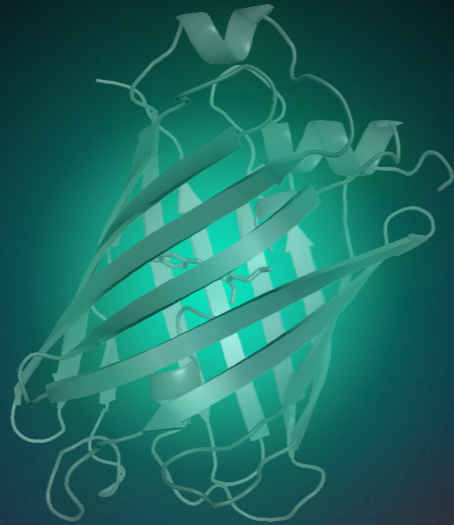
PSmOrange (pre-conversion)
x548/m565



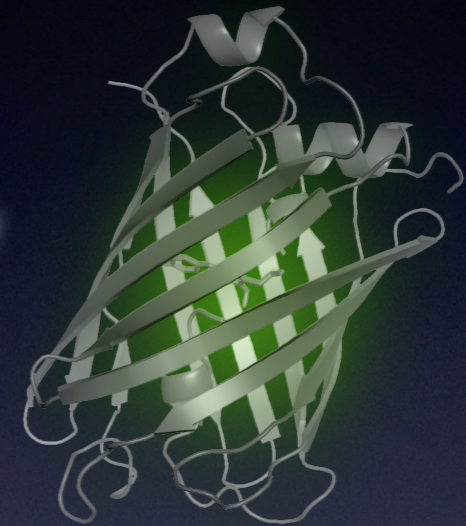
PSmOrange (post-conversion)
x634/m662

What's important?

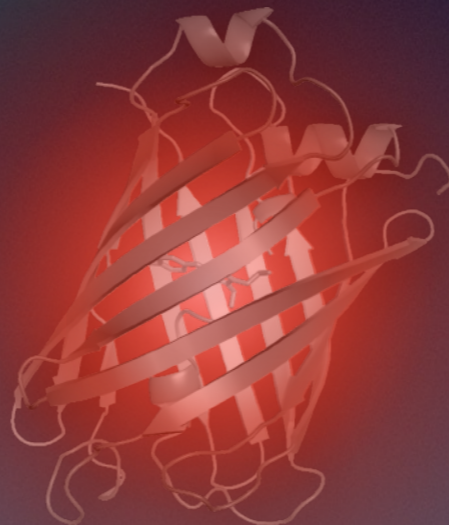
Brightness



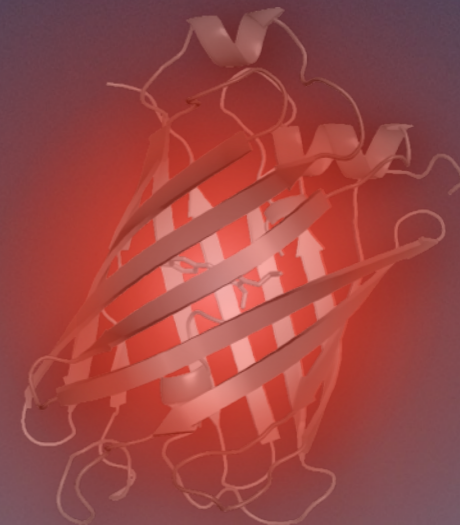
Photostability



Wavelength

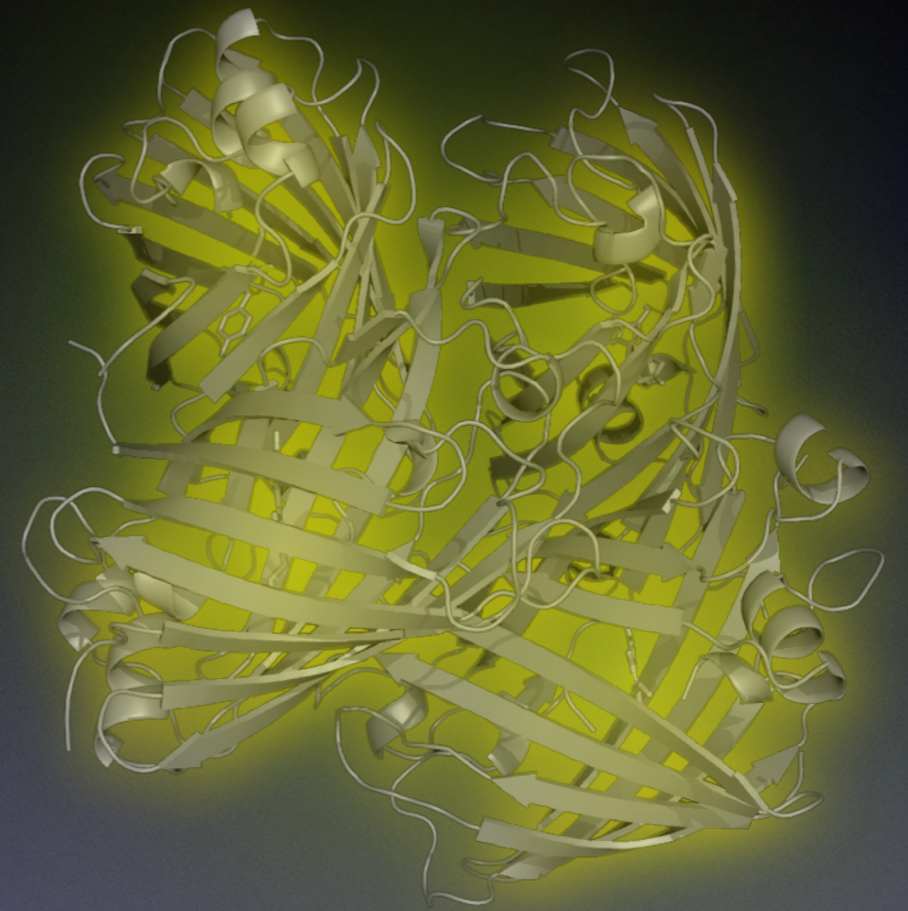
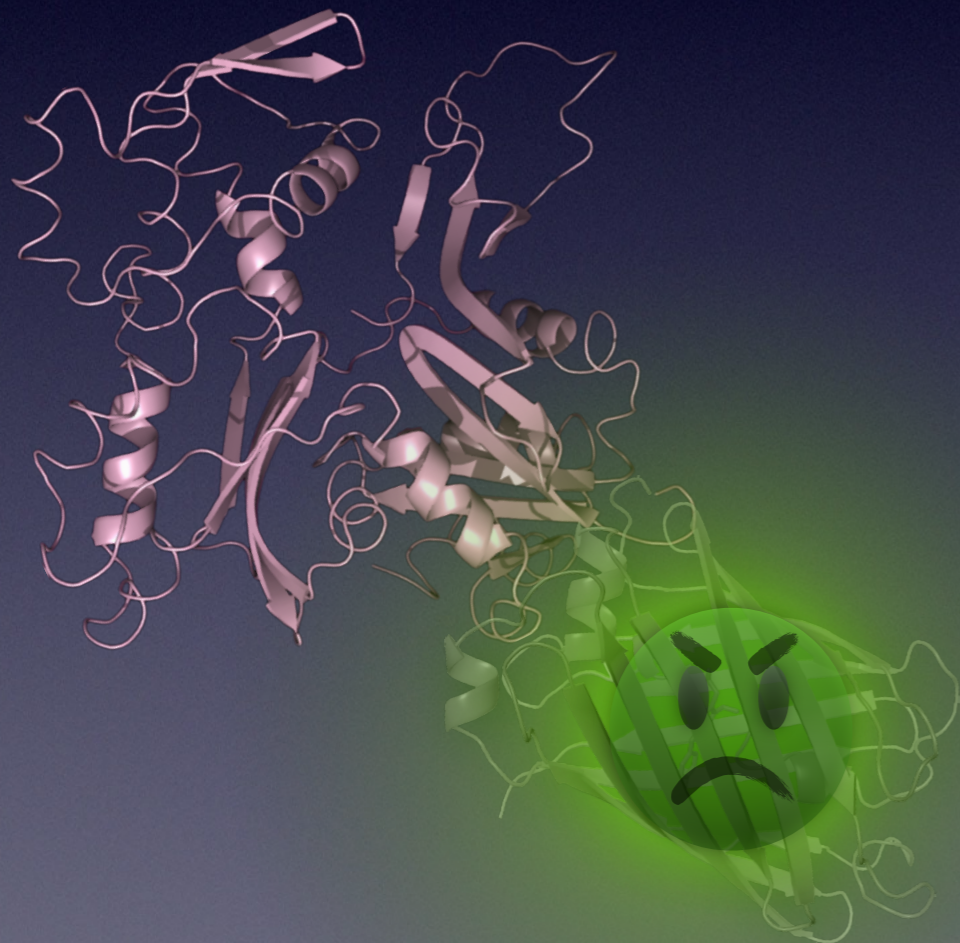


Switching/conversion kinetics



Fluorescent Proteins

Is it really a monomer?



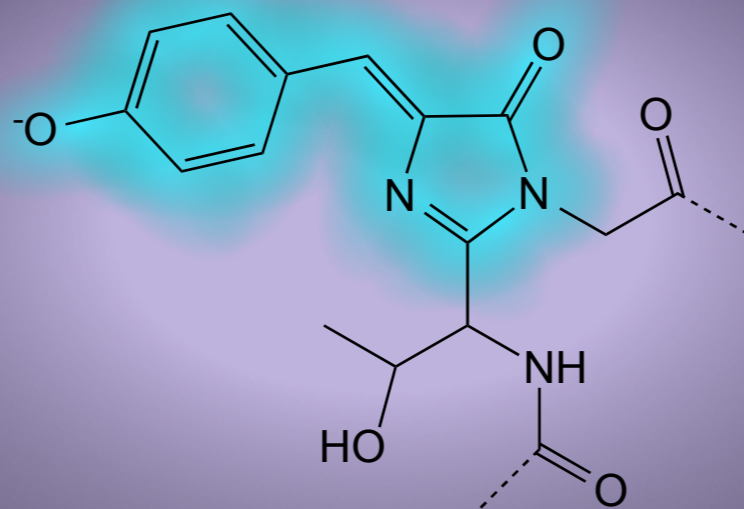
LanYFP crystal structure *

Is it well-behaved?

What is "brightness"?

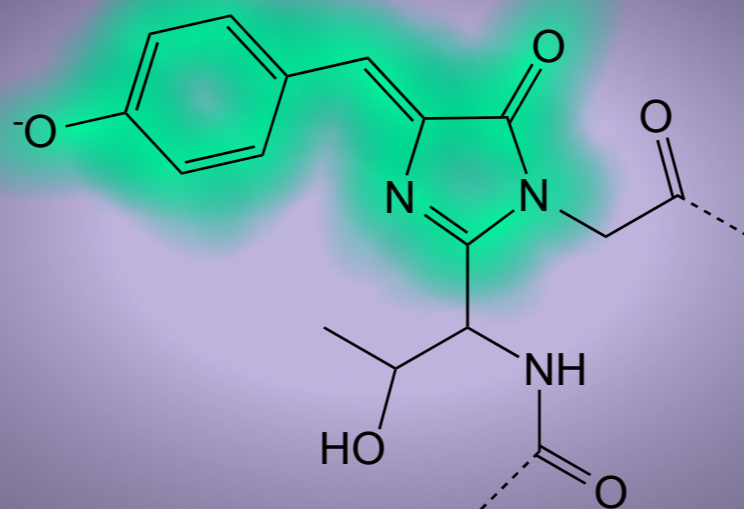
Brightness \sim Extinction Coefficient \times Quantum Yield

What is "brightness"?



Extinction coefficient – how well does it absorb?

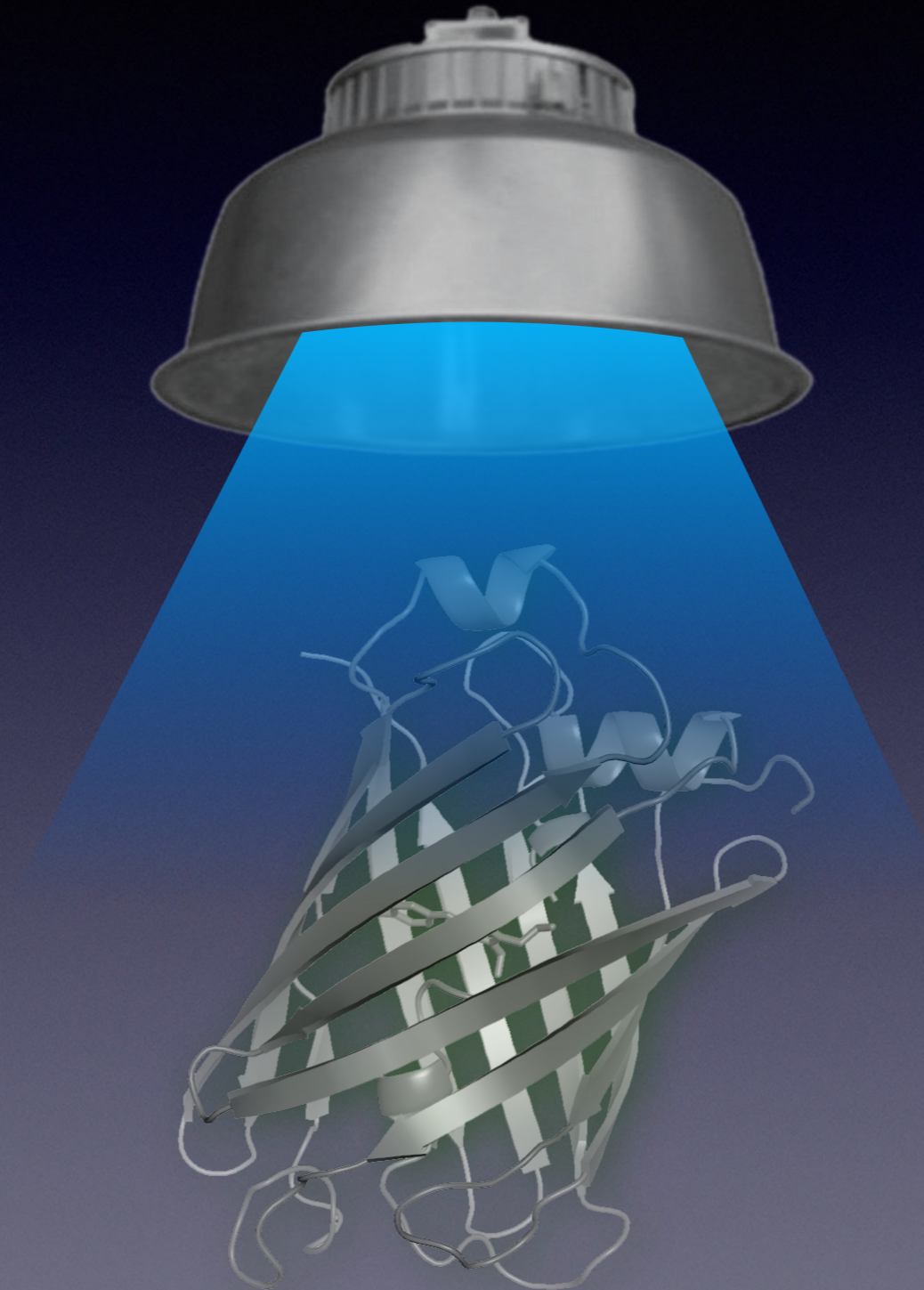
What is "brightness"?



Quantum yield – what fraction of excitations leads to emission?

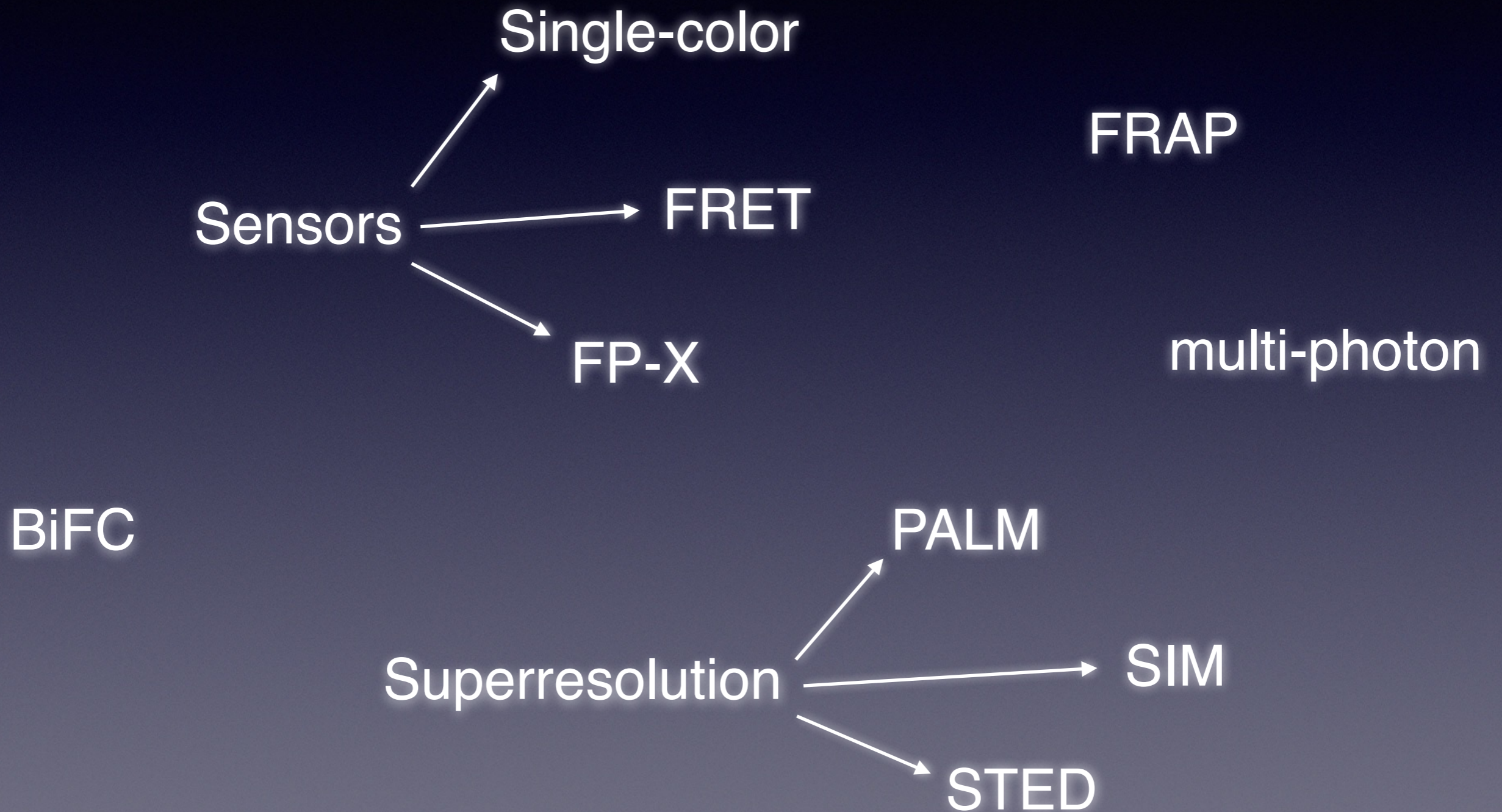
Photostability

Beware when
comparing
photostability
data!



Scale half-time to
initial light output!

Advanced applications

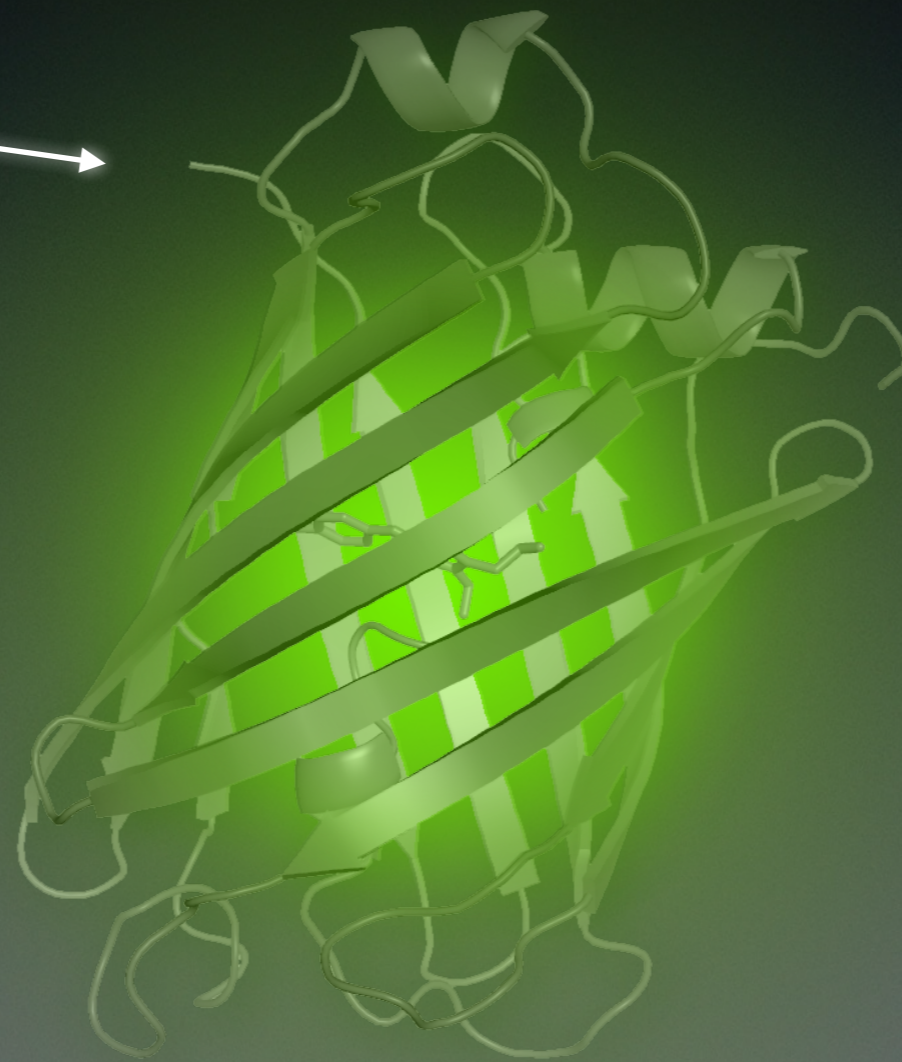


Genetically-encoded sensors

Making a new sensor is not easy!

Circularly permuted FPs

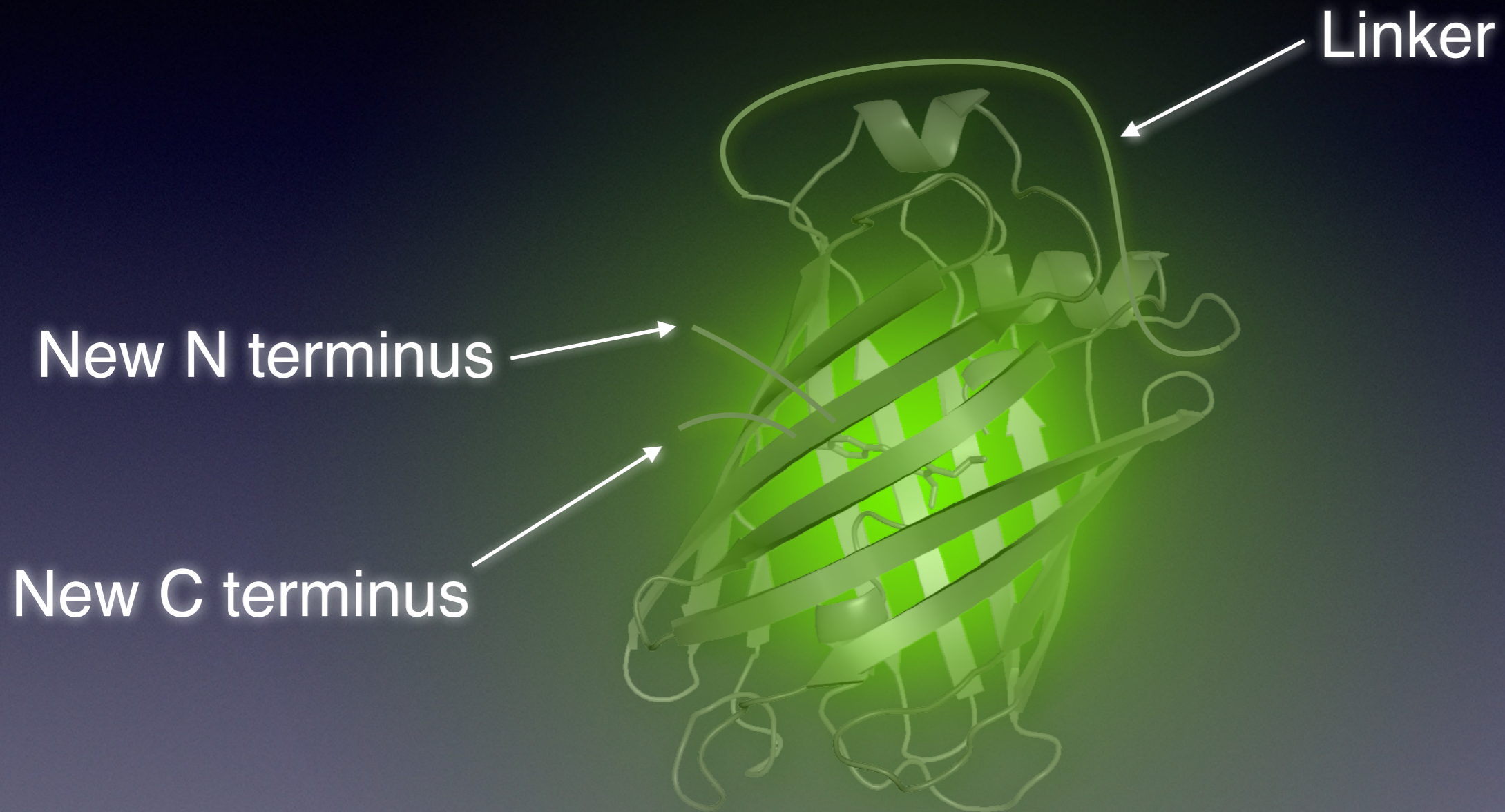
Original C
terminus



Original N
terminus

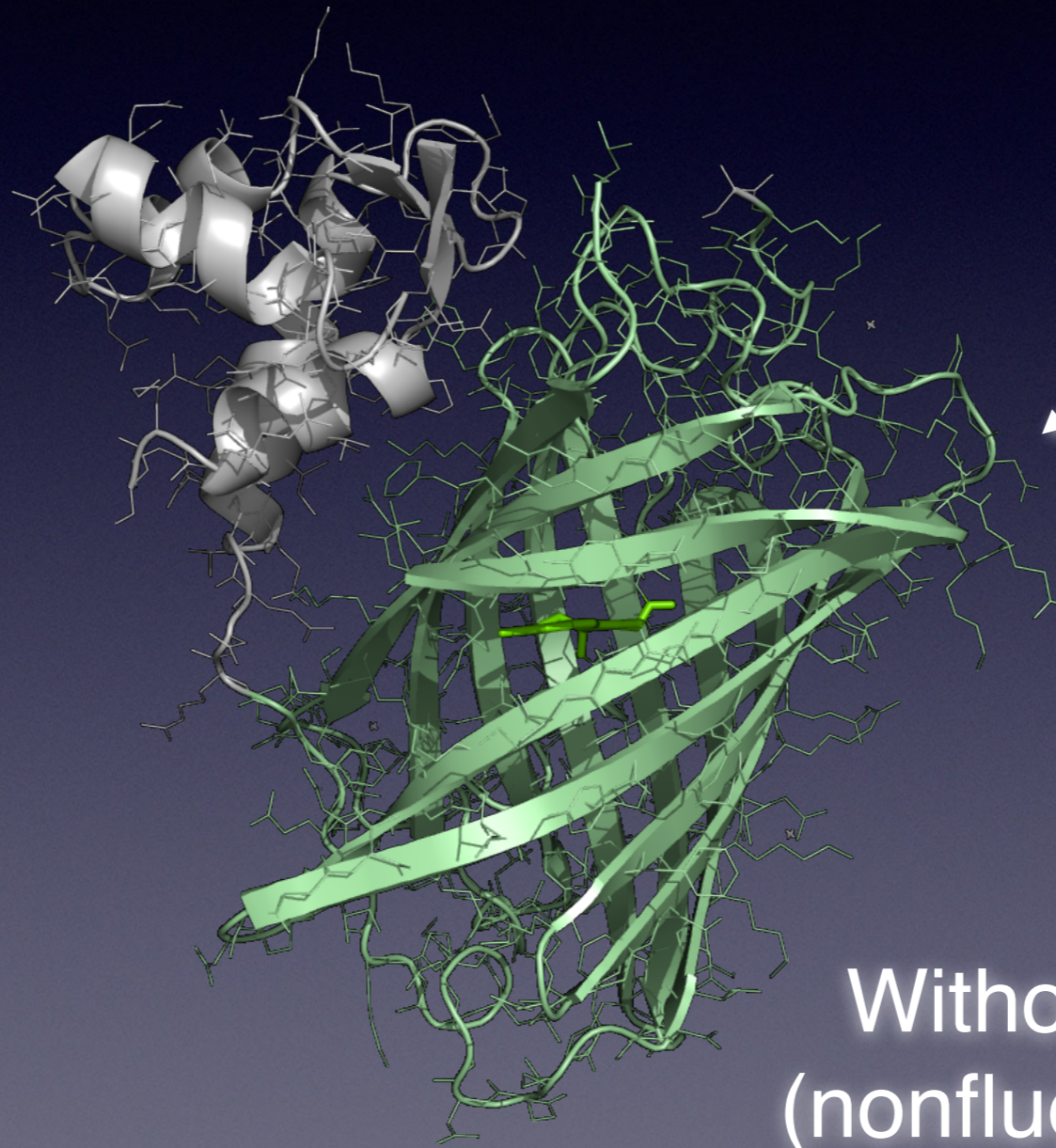


Circularly permuted FPs

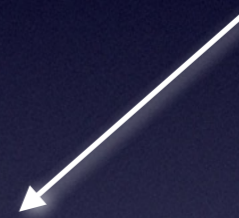


Single-color FP sensors

Calmodulin +
CaM-binding
peptide



Circularly permuted
GFP

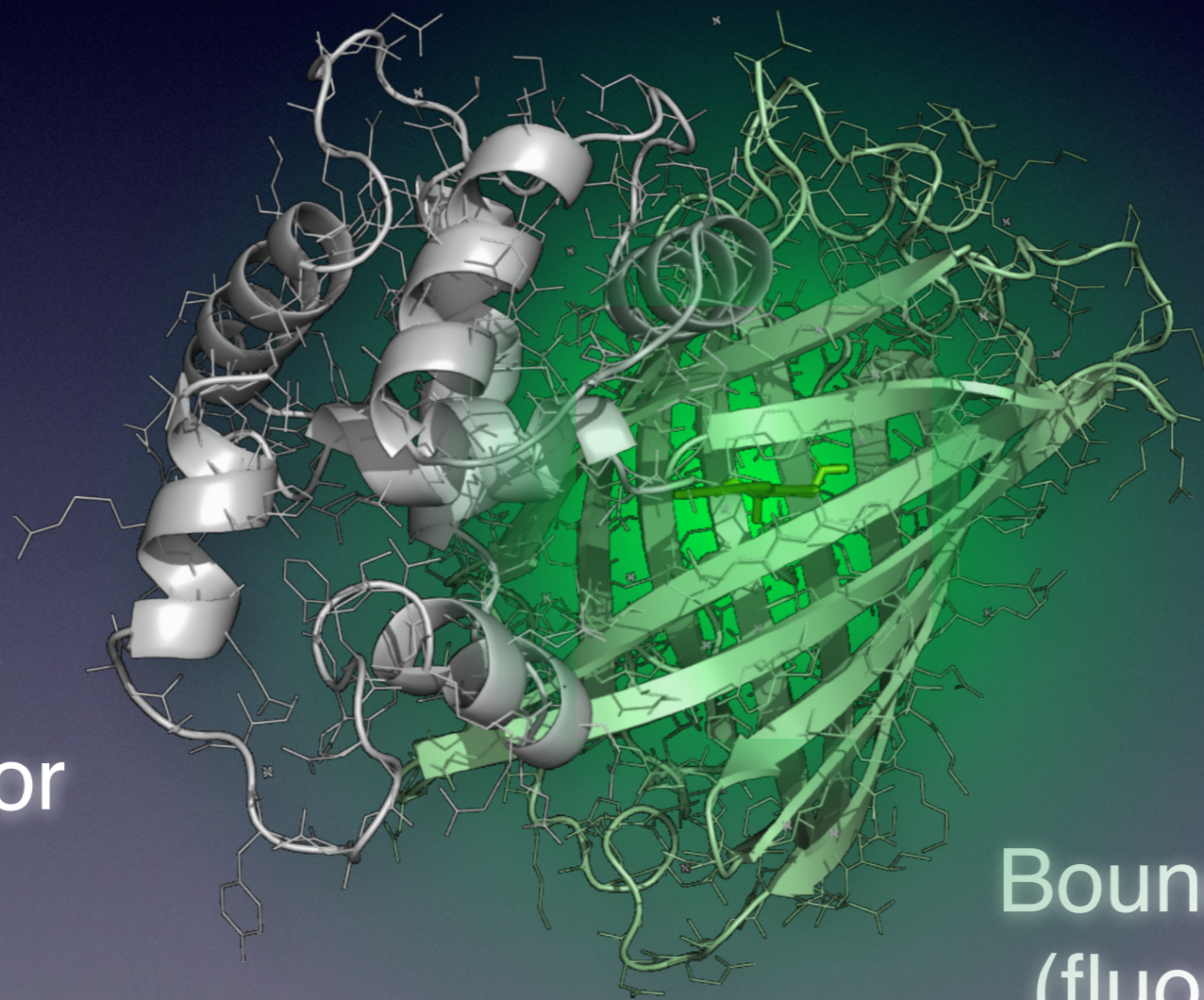


GCaMP
Ca²⁺ sensor

Without Ca²⁺
(nonfluorescent)

Single-color FP sensors

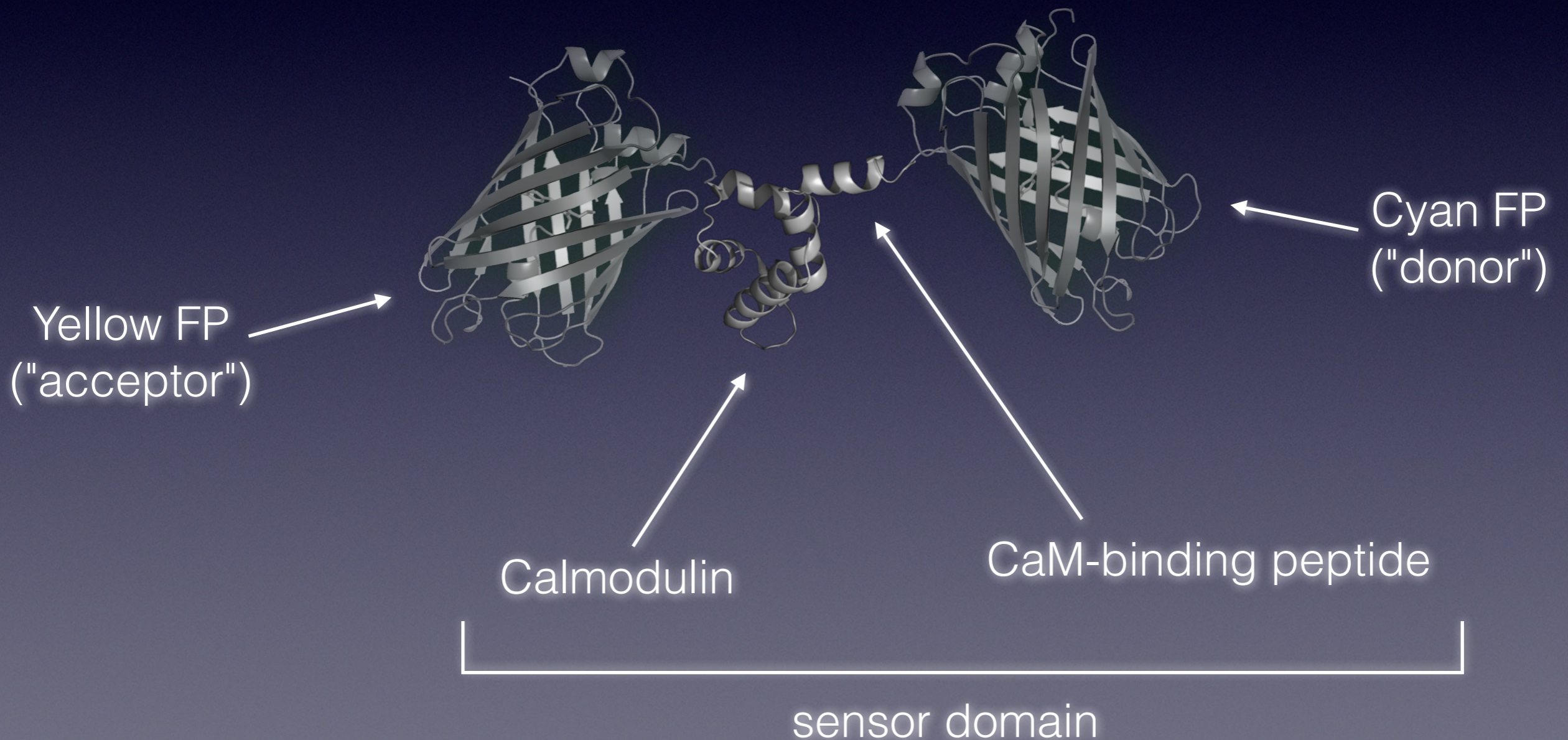
GCaMP
Ca²⁺ sensor

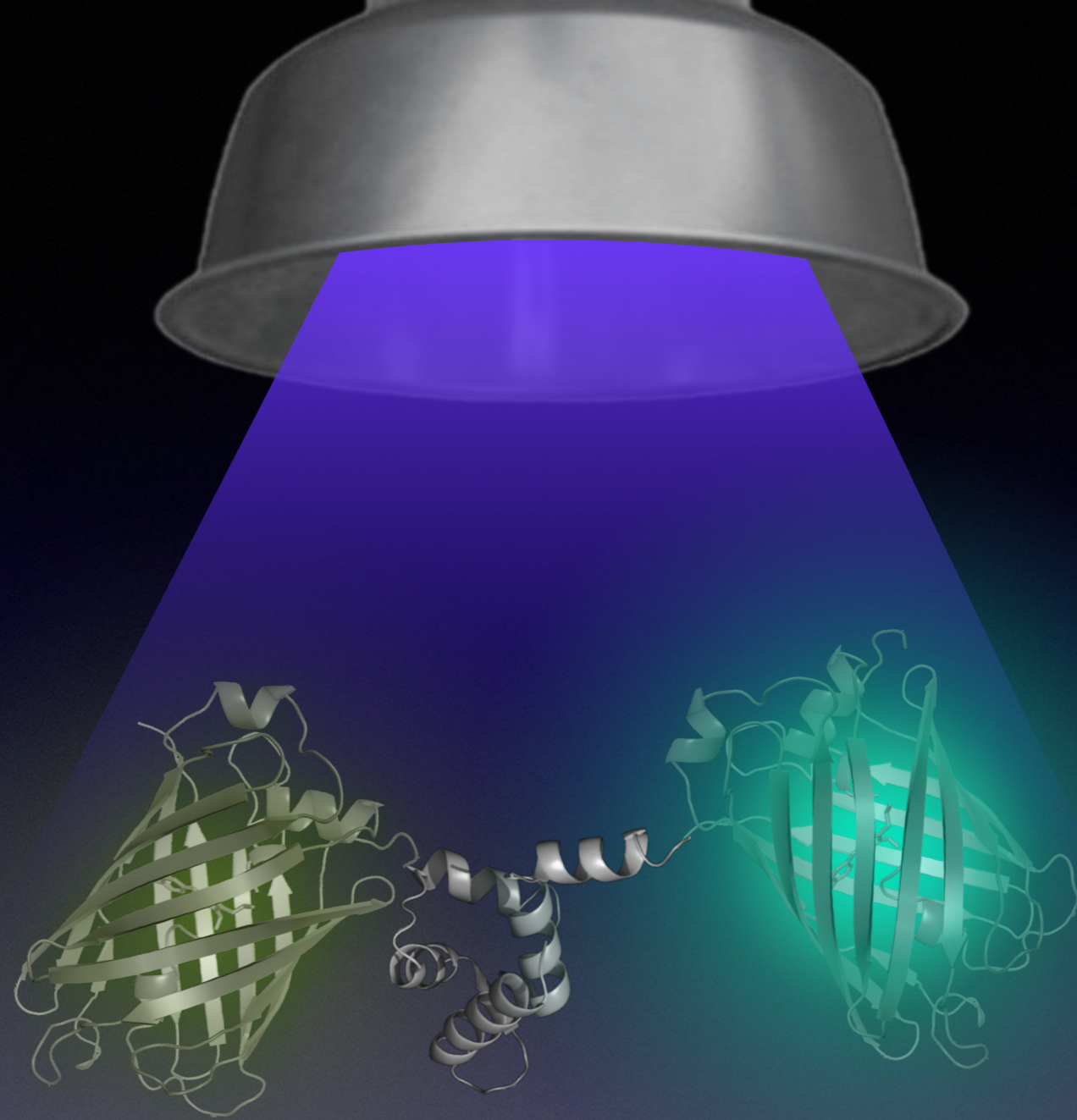


Bound to Ca²⁺
(fluorescent)

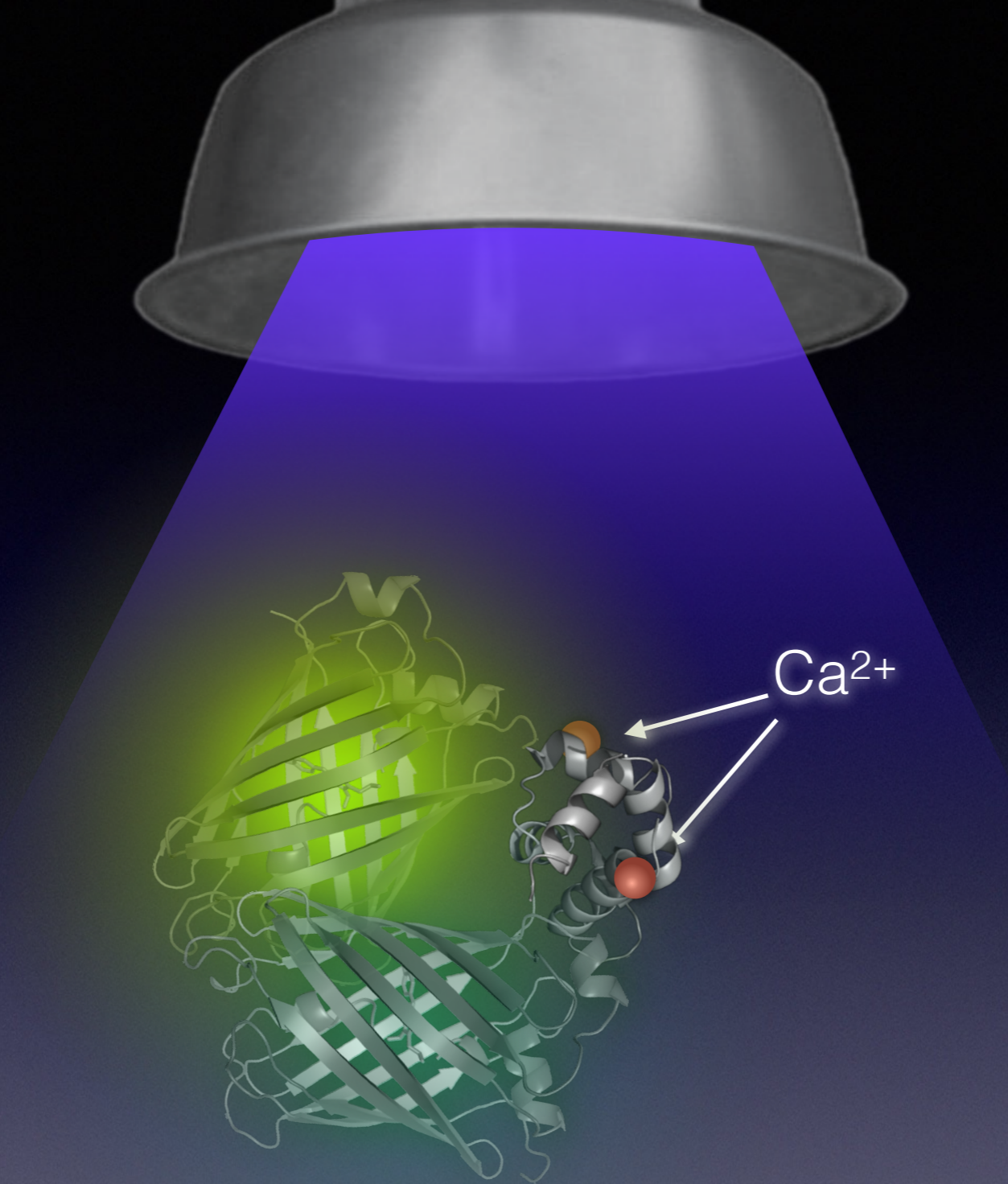
FRET

(Fluorescence/Förster Resonance Energy Transfer)





Mostly cyan (donor) emission

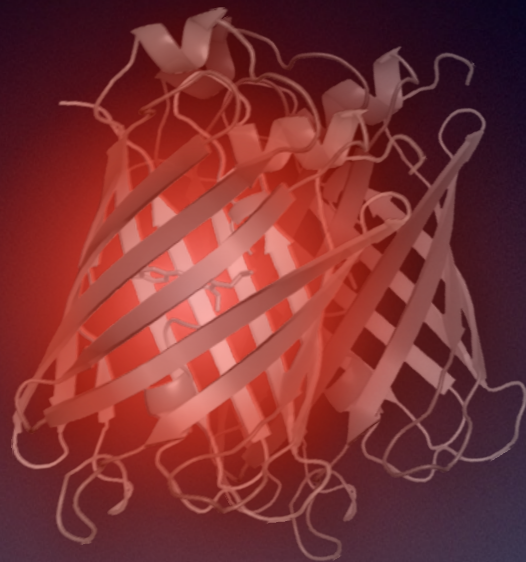


Mostly yellow (acceptor) emission

FP-X

(Fluorescent protein exchange)

RA (red 'A')



GA (green 'A')



B (non-fluorescent partner)

$K_d \sim 40 \mu\text{M}$

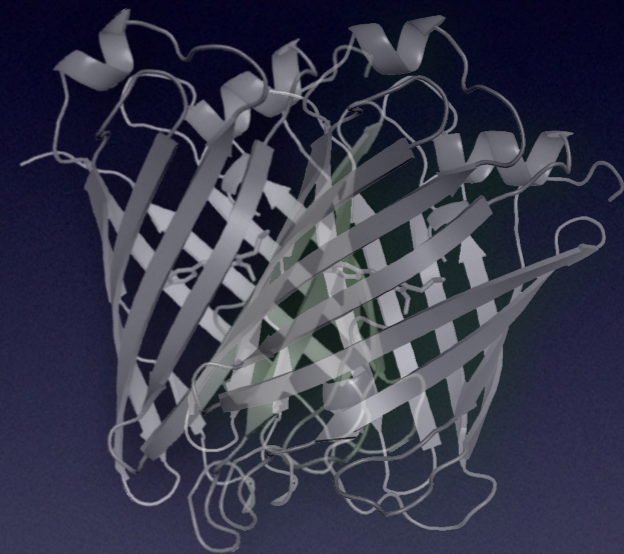
Heterodimerization-dependent fluorescence

FP-X

RA (red 'A')



GA (green 'A')

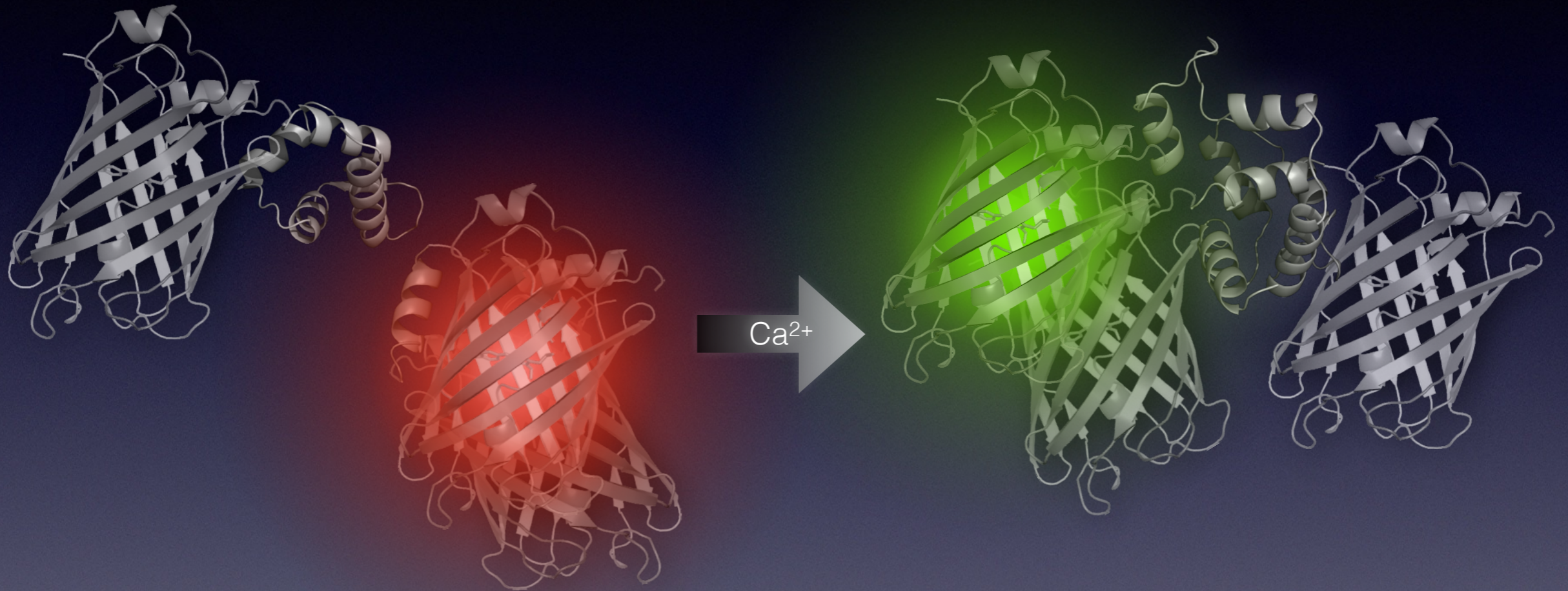


B (non-fluorescent partner)

$K_d \sim 40 \mu\text{M}$

Heterodimerization-dependent fluorescence

FP-X



Heterodimerization-dependent fluorescence