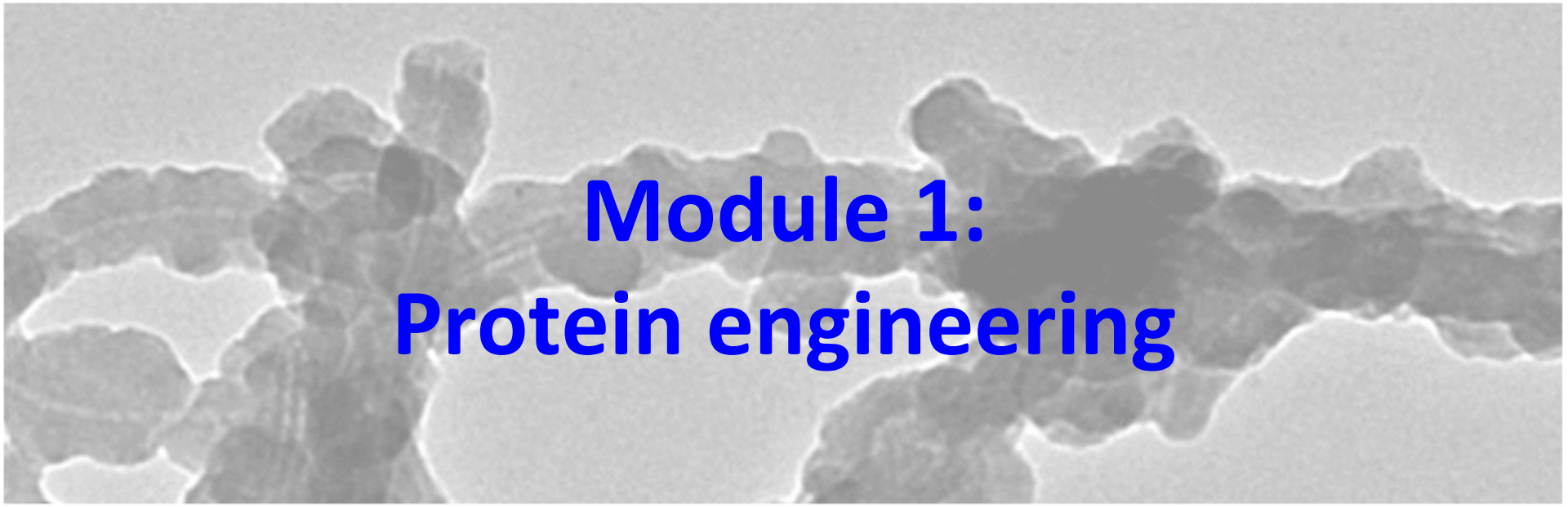


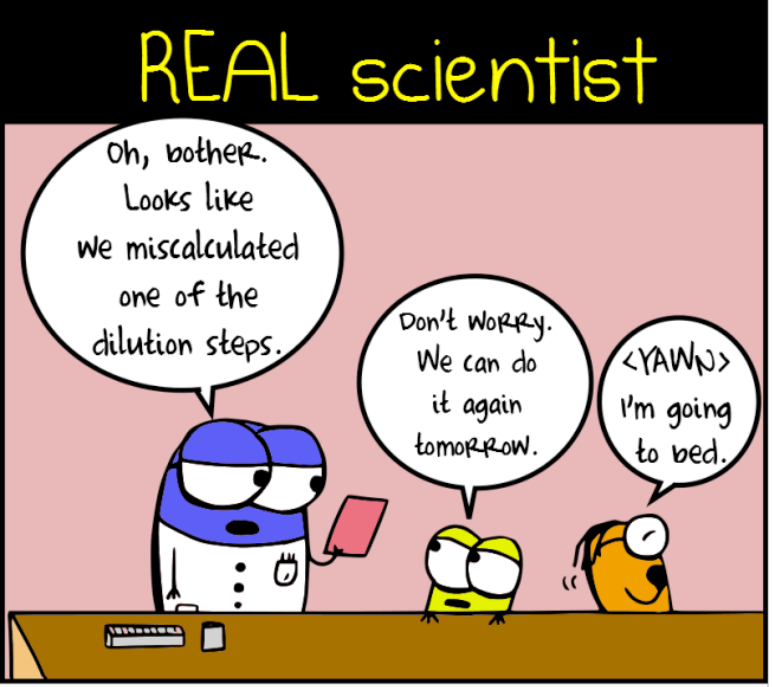
Module 1:
Protein engineering

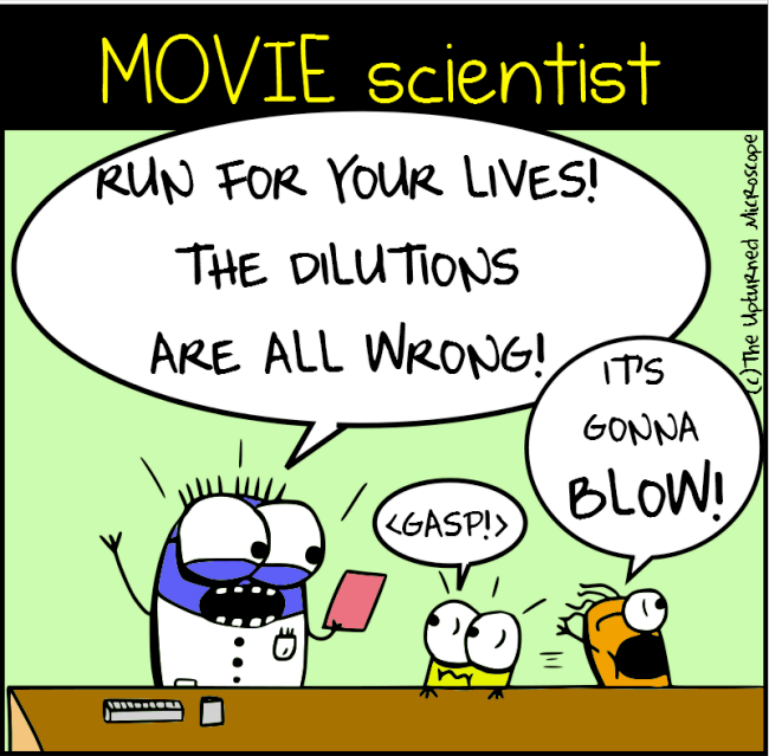
I Real science

II Protein engineering

III EGFP

2/11/16





We do *real* science

- No guarantee that your mutations will be incorporated
- No guarantee that your mutations will lead to an actual protein
- No guarantee that your mutations will alter protein function

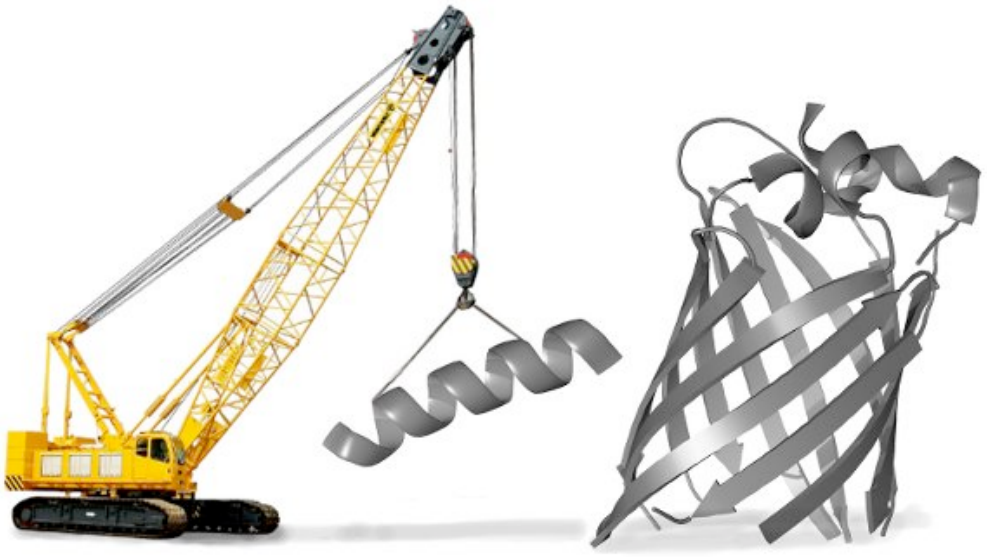
How will you test your progress?

- Were your mutations incorporated?
- Was your mutant protein produced?
- Did your mutant protein alter calcium binding?

Mechanisms for engineering proteins

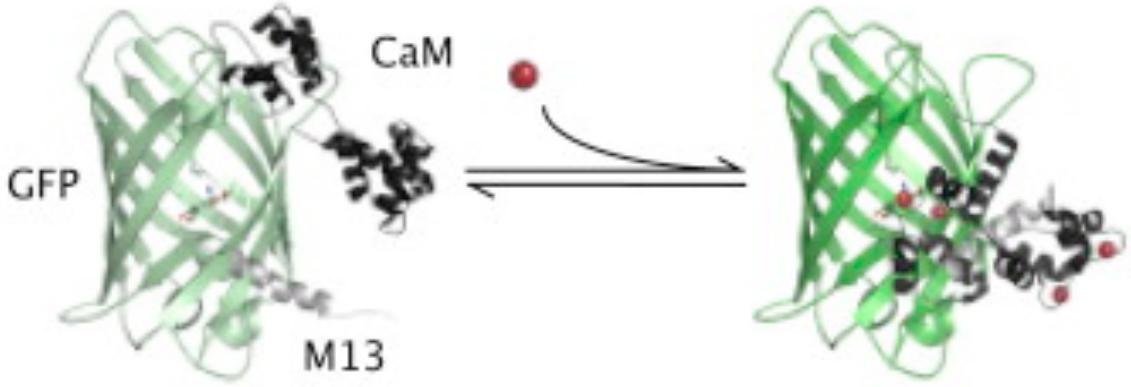
- Rational design

- 'Irrational design'
  - Random library screens
  - Directed evolution

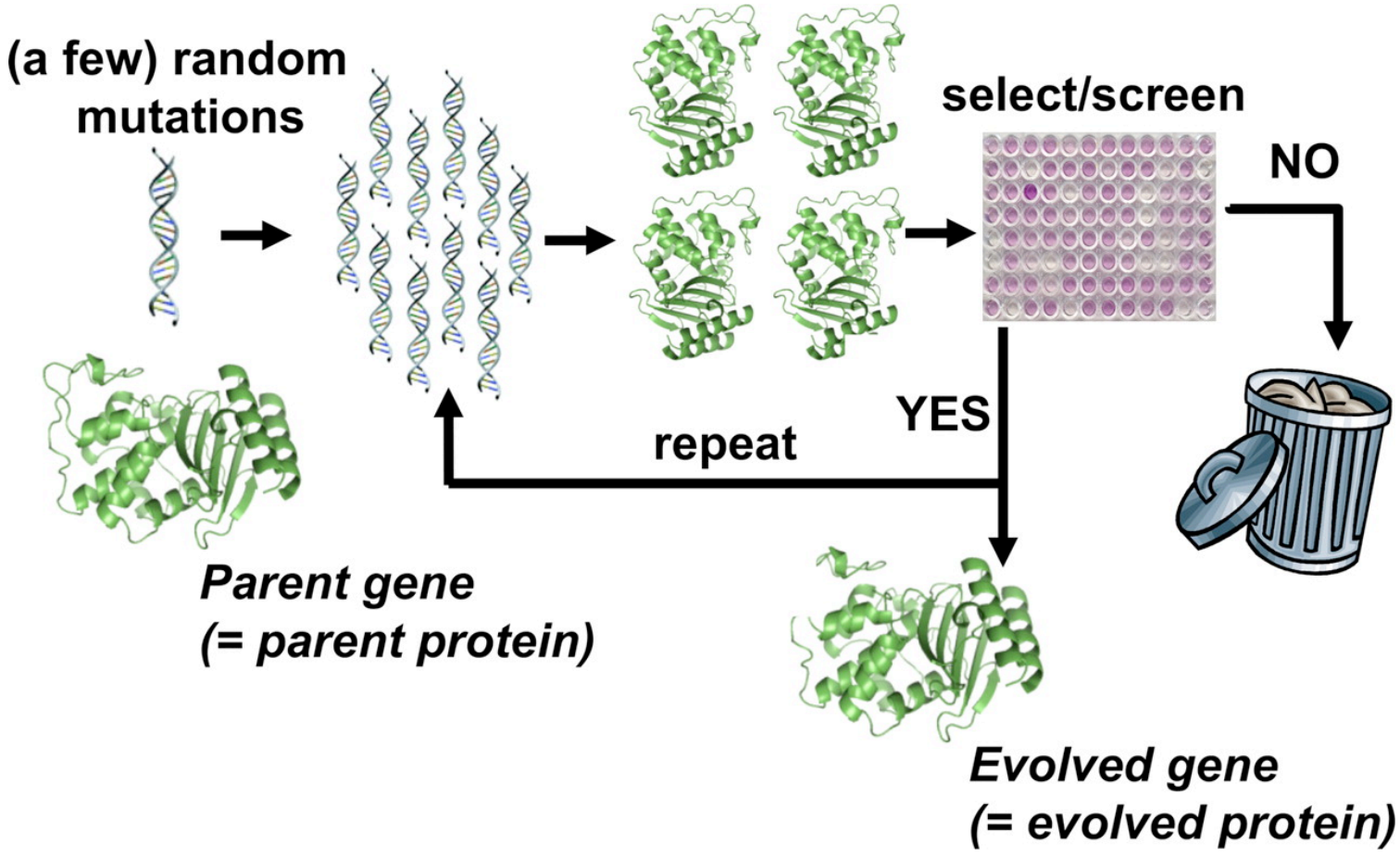


Rational design

- Use knowledge of protein sequence and structure to alter function
- What if you don't know the sequence or structure?



Irrational design

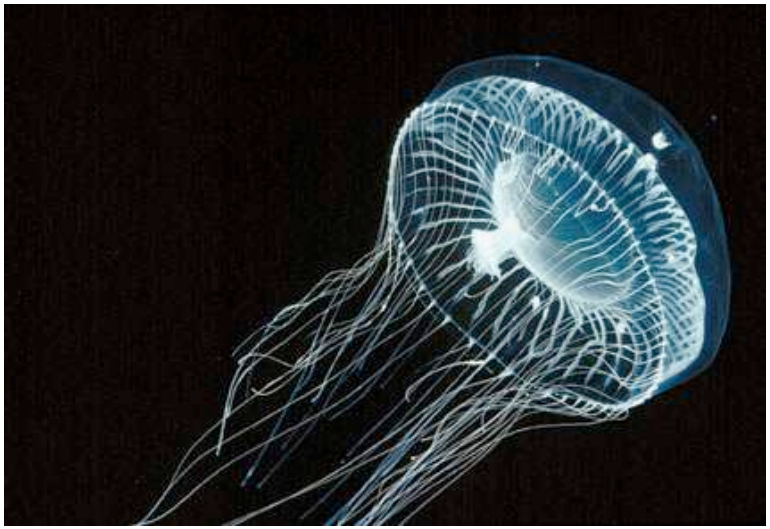


Bloom and Arnold. (2009) *PNAS* 106:9995-10000.

Which method is best?

GFP originally isolated from jellyfish

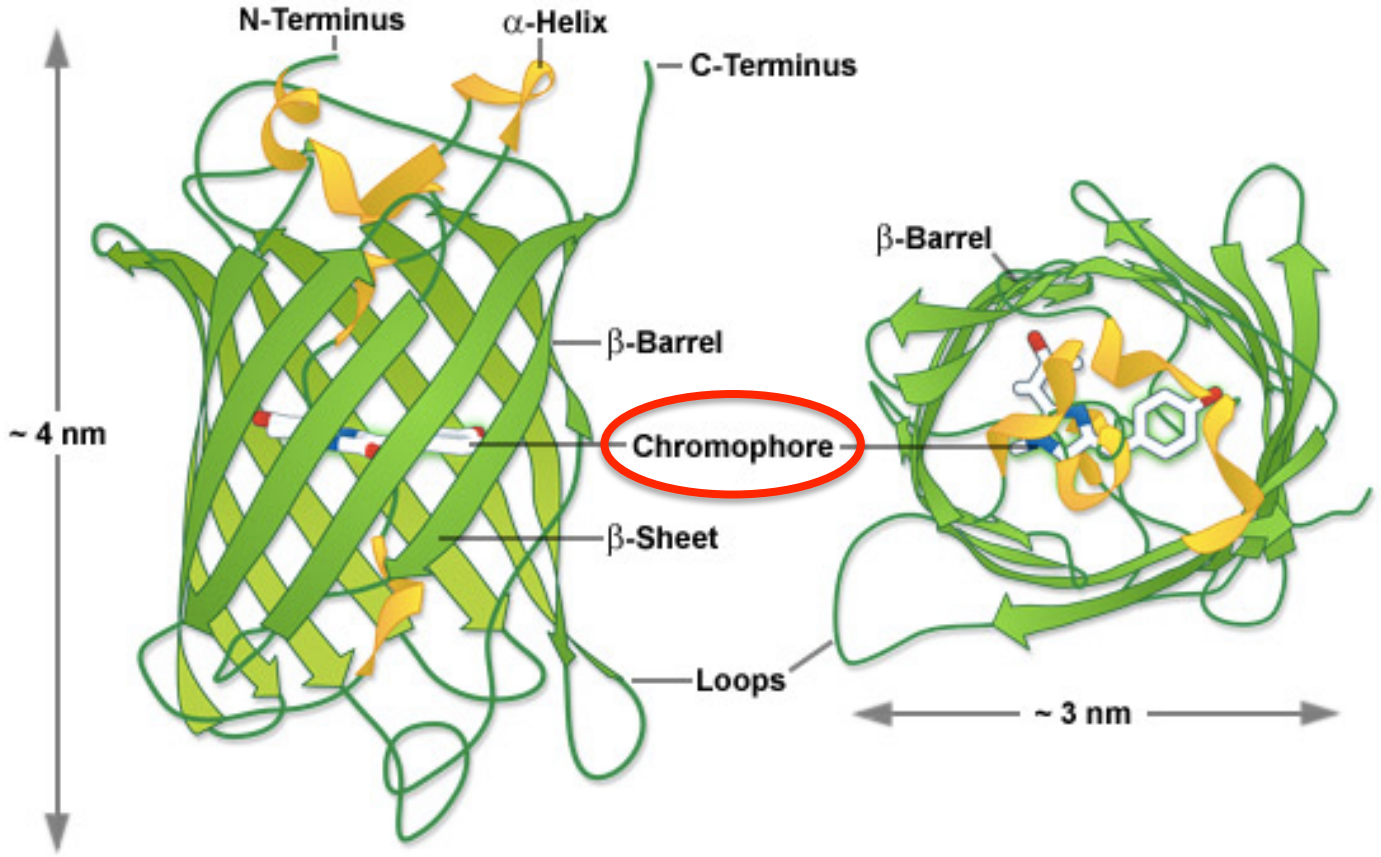
- Produces flashes of blue light via the photoprotein aequorin
  - Regulated by releases of calcium



Aequorea victoria (crystal jellyfish)

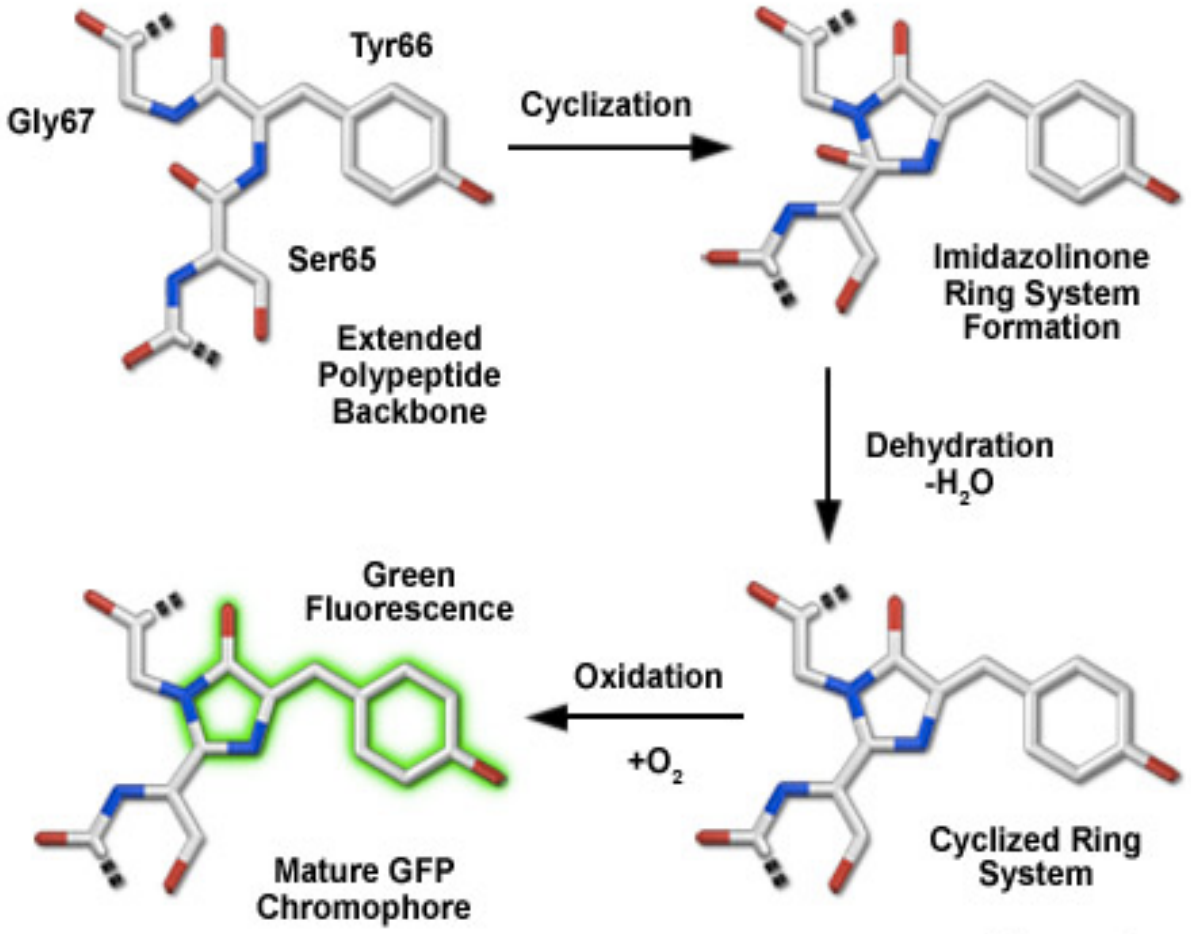
- Blue light is transduced to green by GFP

A case study in protein engineering: GFP



http://zeiss-campus.magnet.fsu.edu/articles/probes/jellyfishfps.html

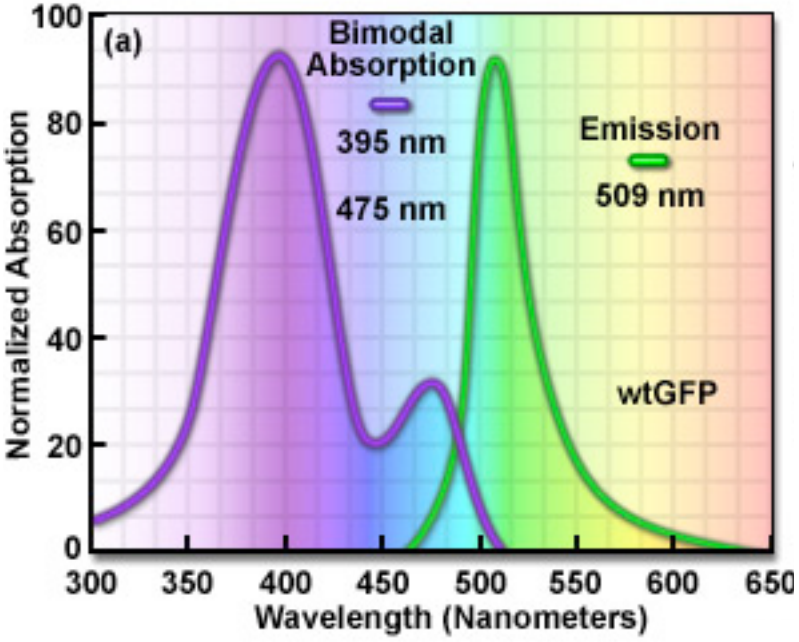
Chromophore dictates color of molecule



http://zeiss-campus.magnet.fsu.edu/articles/probes/jellyfishfps.html

WT GFP not ideal as research tool

- Complex absorption spectrum
  - Excited by both 378 and 475 nm
  - Exists in ground state and as ionized intermediate
- High extinction coefficient
  - Short lifetime
- Low quantum yields
  - Relatively dim



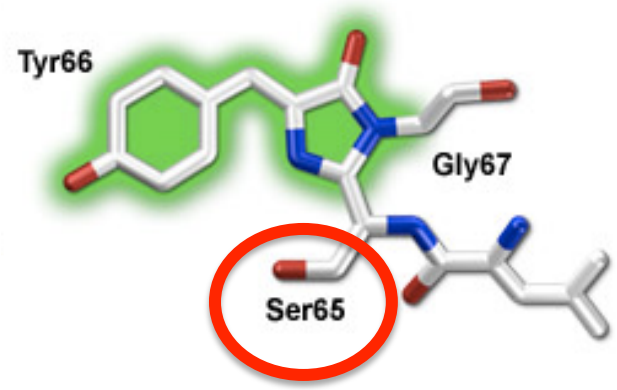
How can we engineer a better GFP?

(E)GFP is a revolutionary tool in science

Roger Y. Tsien

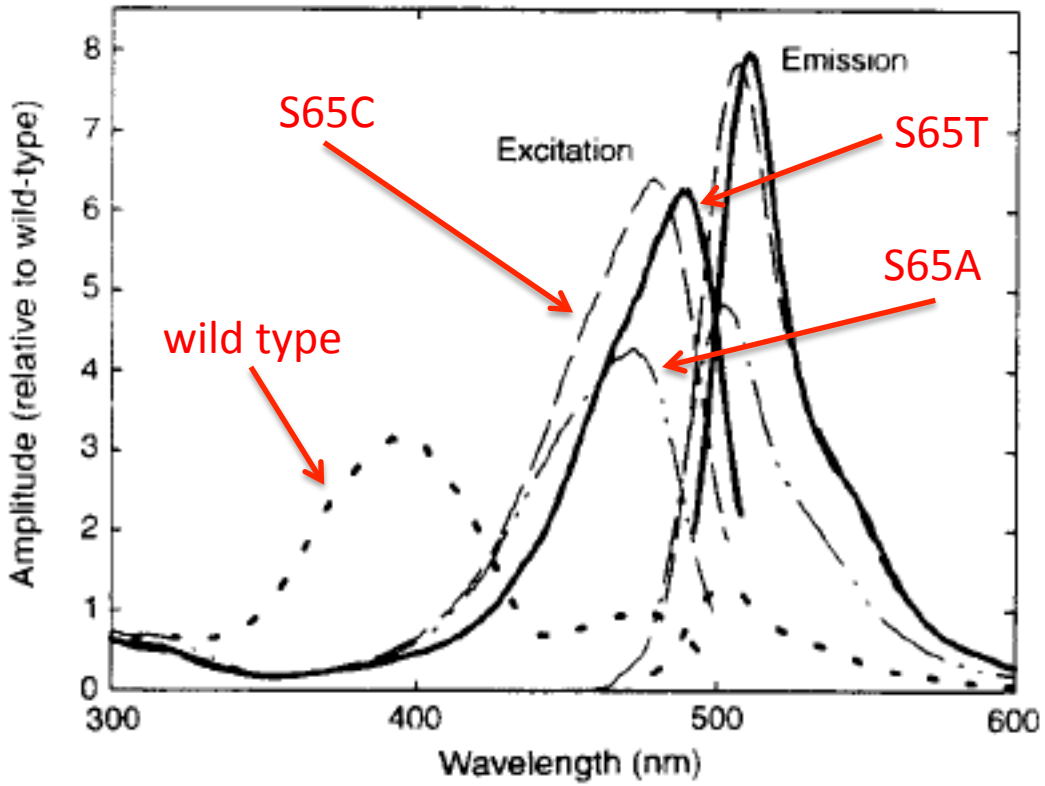
- Awarded Nobel Prize in Chemistry, with O. Shimomura and M. Chalfie
- Discovered and developed GFP

Sequence and structure revealed target



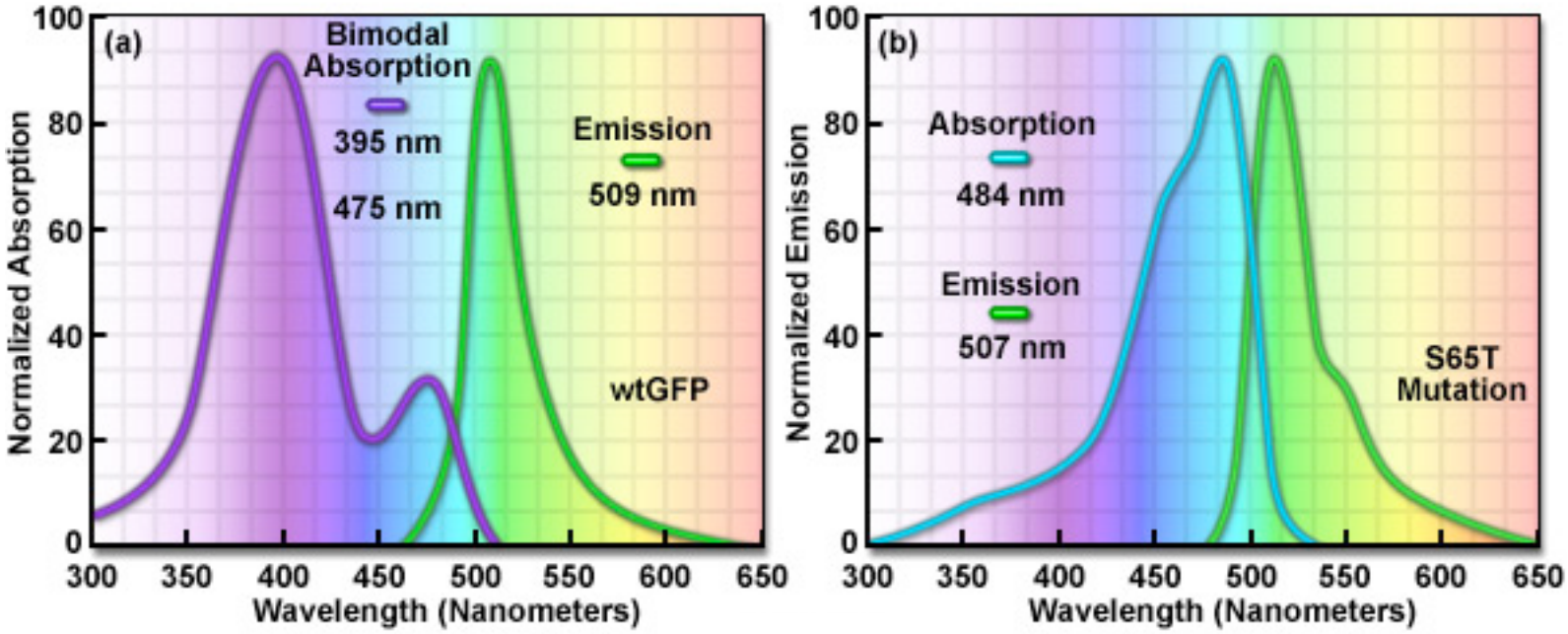
- Point mutations (PMs) introduced:
  - Ser 65 Ala
  - Ser 65 Cys
  - Ser 65 Thr
  - Ser 65 Arg
  - Ser 65 Asn
  - Ser 65 Asp
  - Ser 65 Phe
  - Ser 65 Trp

PMs altered GFP absorption and emission



Heim *et al*. (1995) *Nature* 373:663-664.

S65T resulted in longest emission, excitation wavelengths

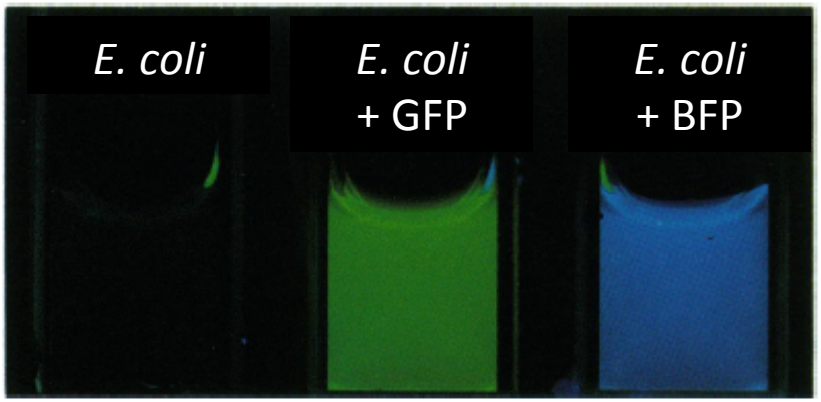


http://zeiss-campus.magnet.fsu.edu/articles/probes/fpintroduction.html

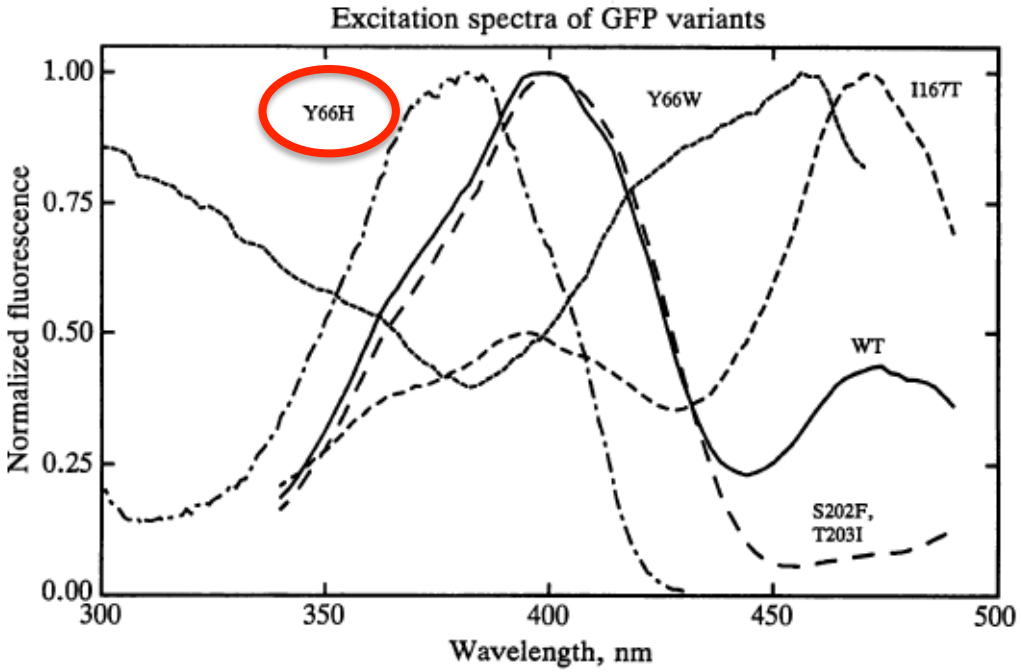
How might you engineer different colors?

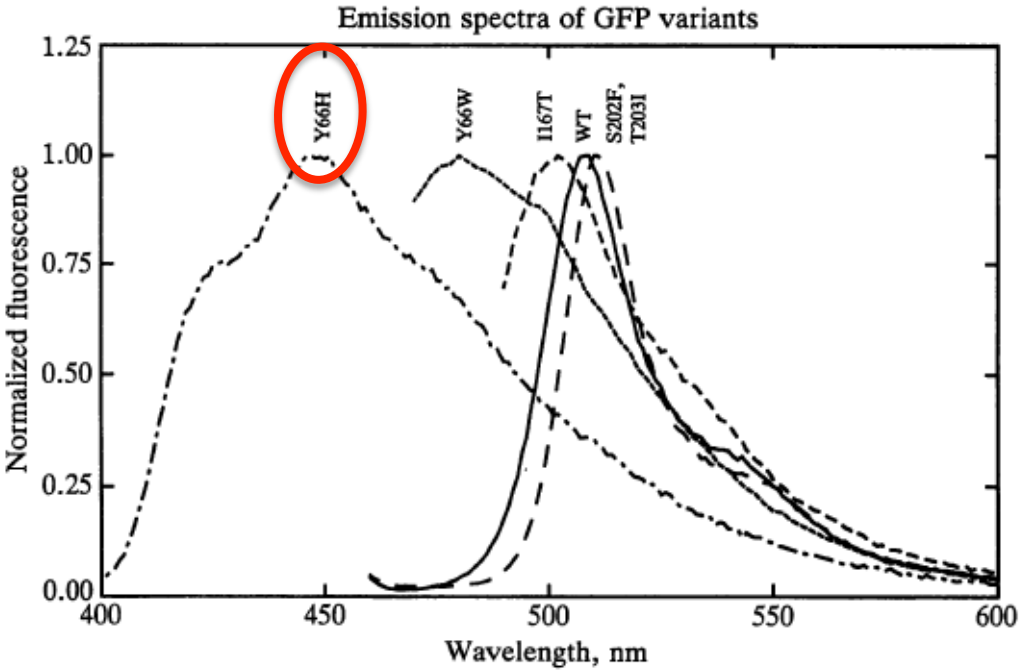
GFP sequence randomly mutagenized

- Employed hydroxylamine treatment and error-prone PCR amplification
- Products ligated into vector and transformed into *E. coli* strain
- Colonies visually screened following excitation

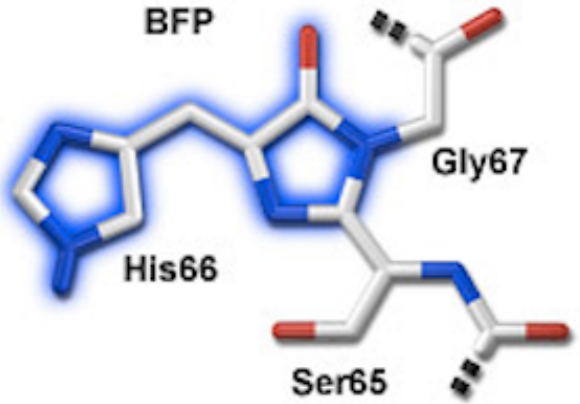


Heim *et al*. (1994) *PNAS* 91:12501-12504.



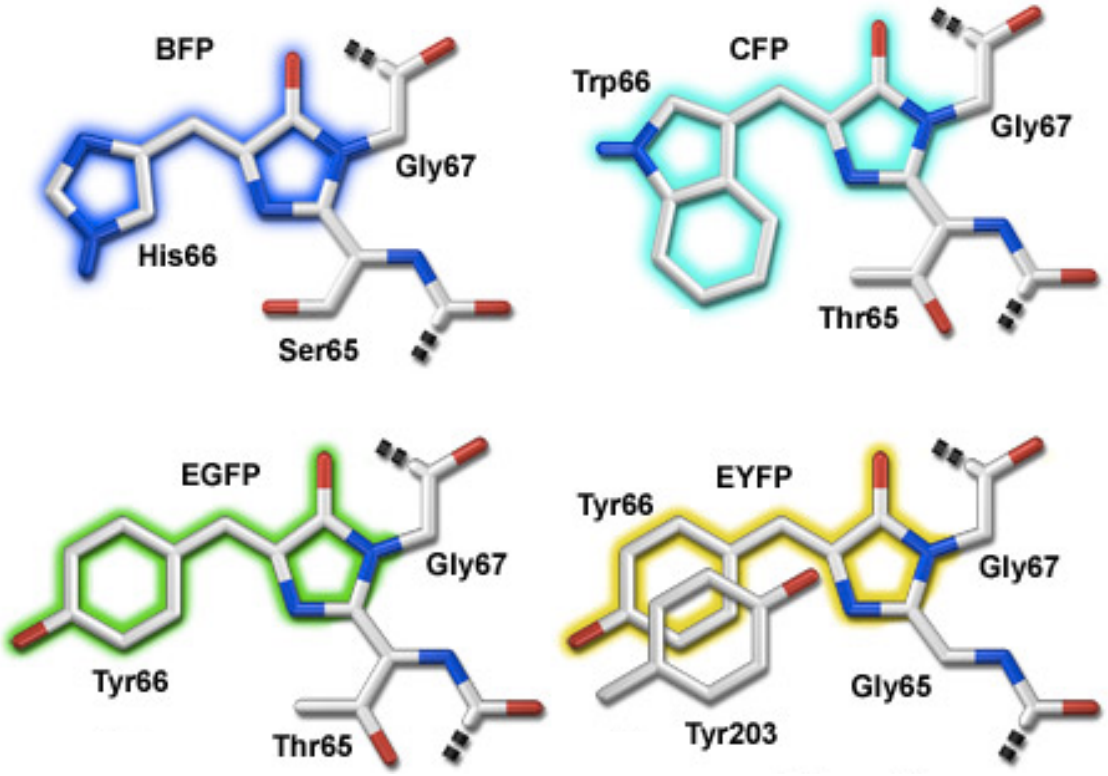


Y66H resulted in BFP



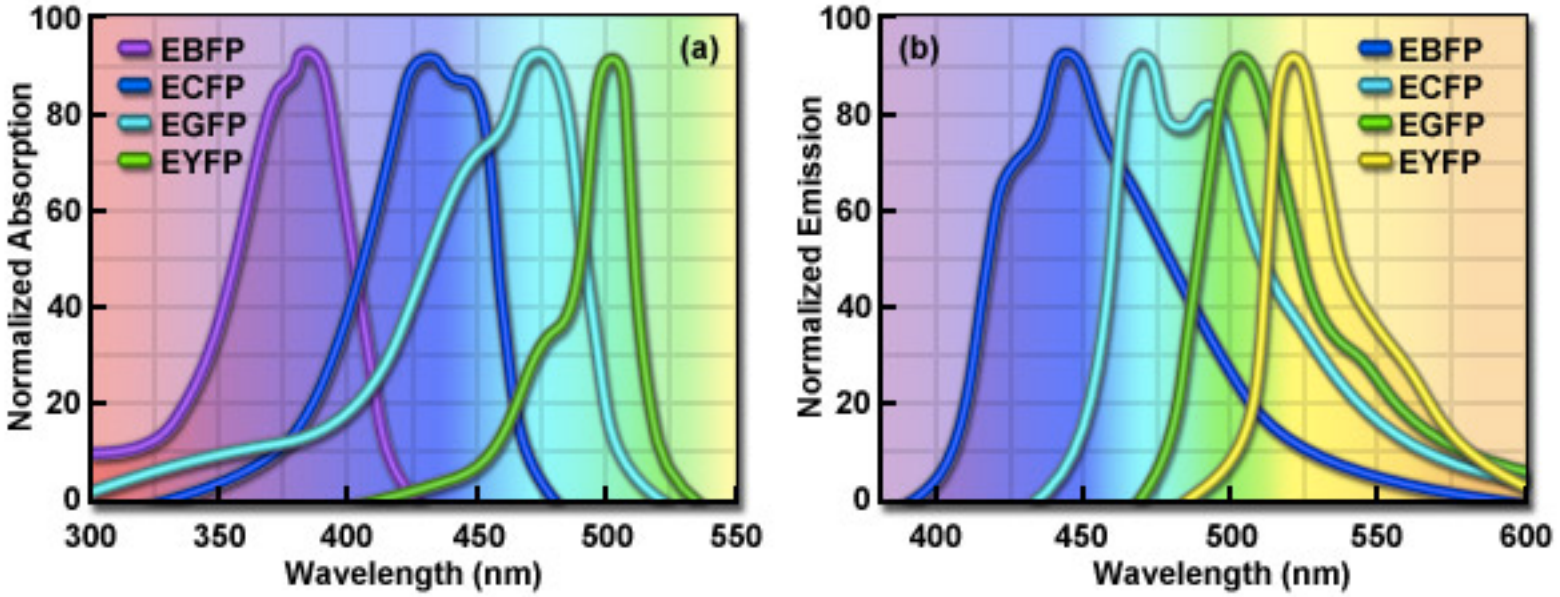
Heim *et al*. (1994) *PNAS* 91:12501-12504.

Protein engineering generated toolkit of fluorescent proteins



http://zeiss-campus.magnet.fsu.edu/articles/probes/jellyfishfps.html

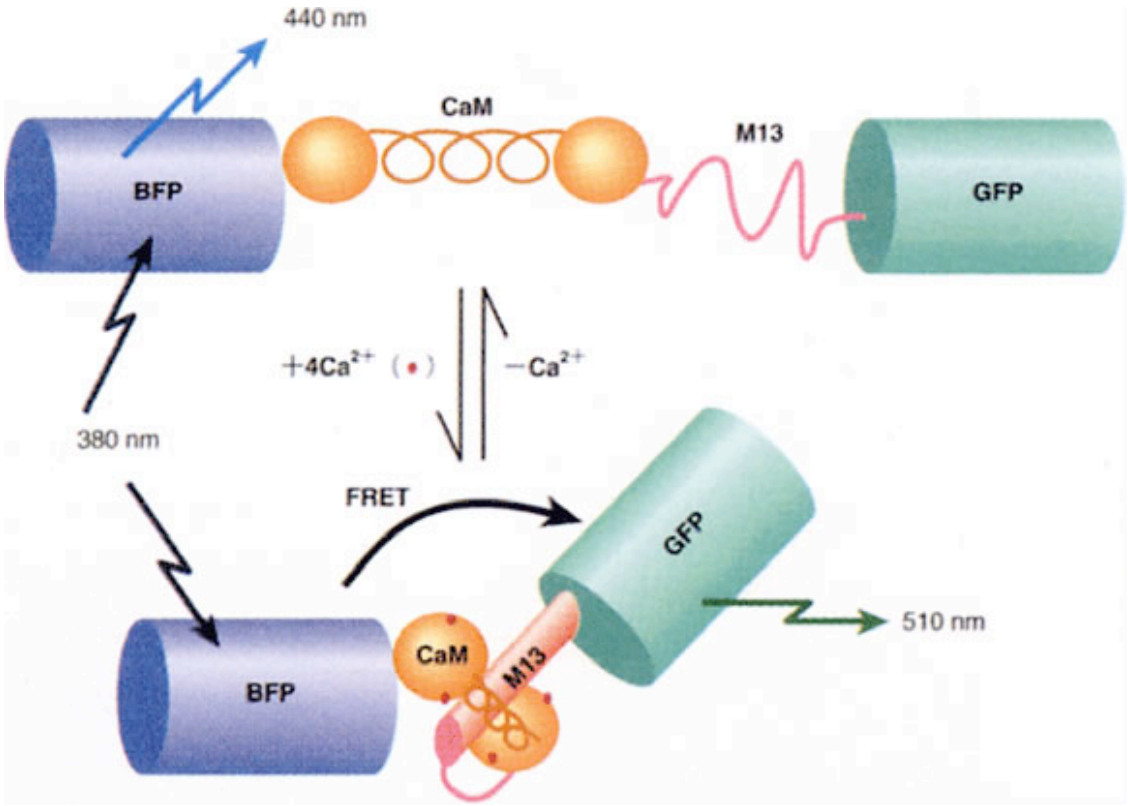
Utility of fluorescent protein toolkit



- Fluorescence resonance energy transfer (FRET)
- Multicolor labeling

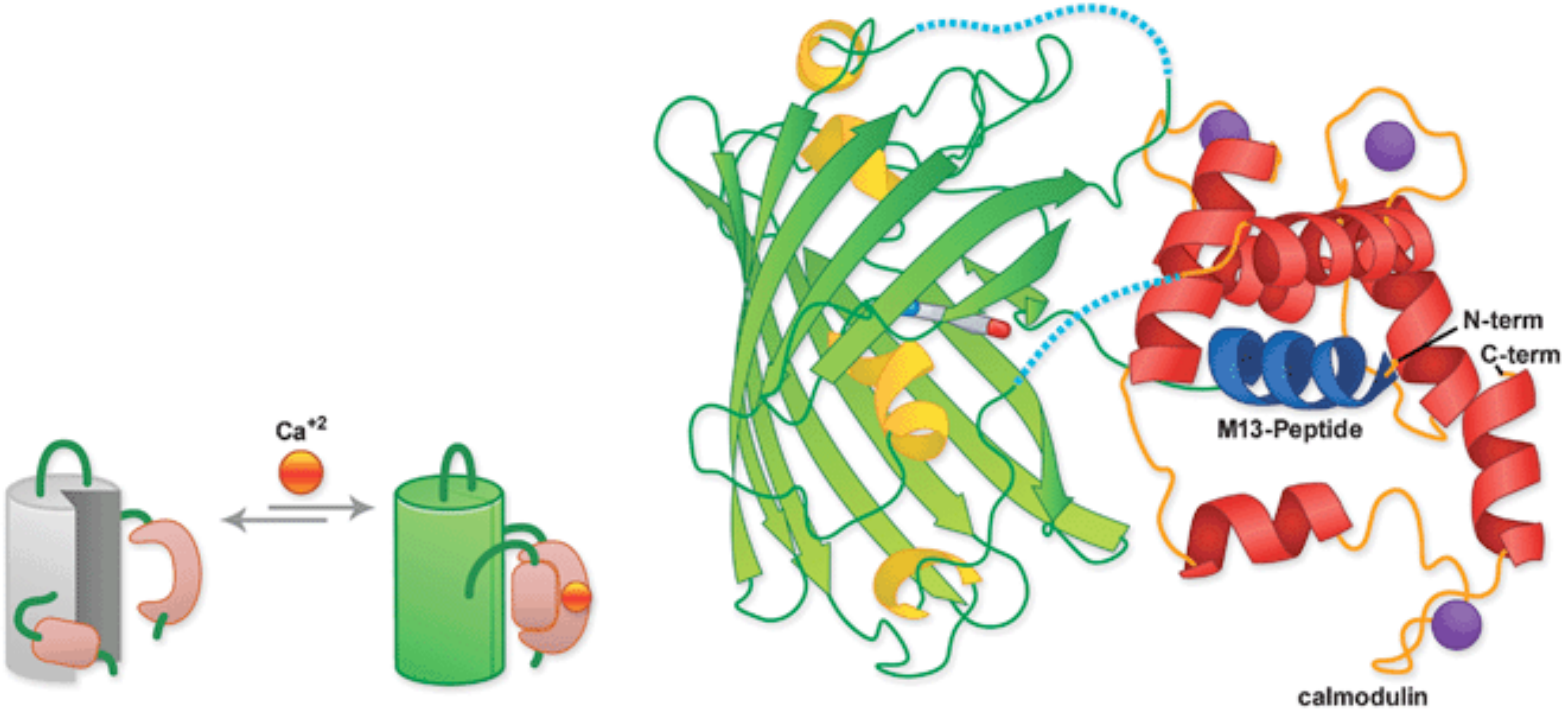
http://zeiss-campus.magnet.fsu.edu/articles/probes/jellyfishfps.html

FRET quantification of [Ca^{2+}]

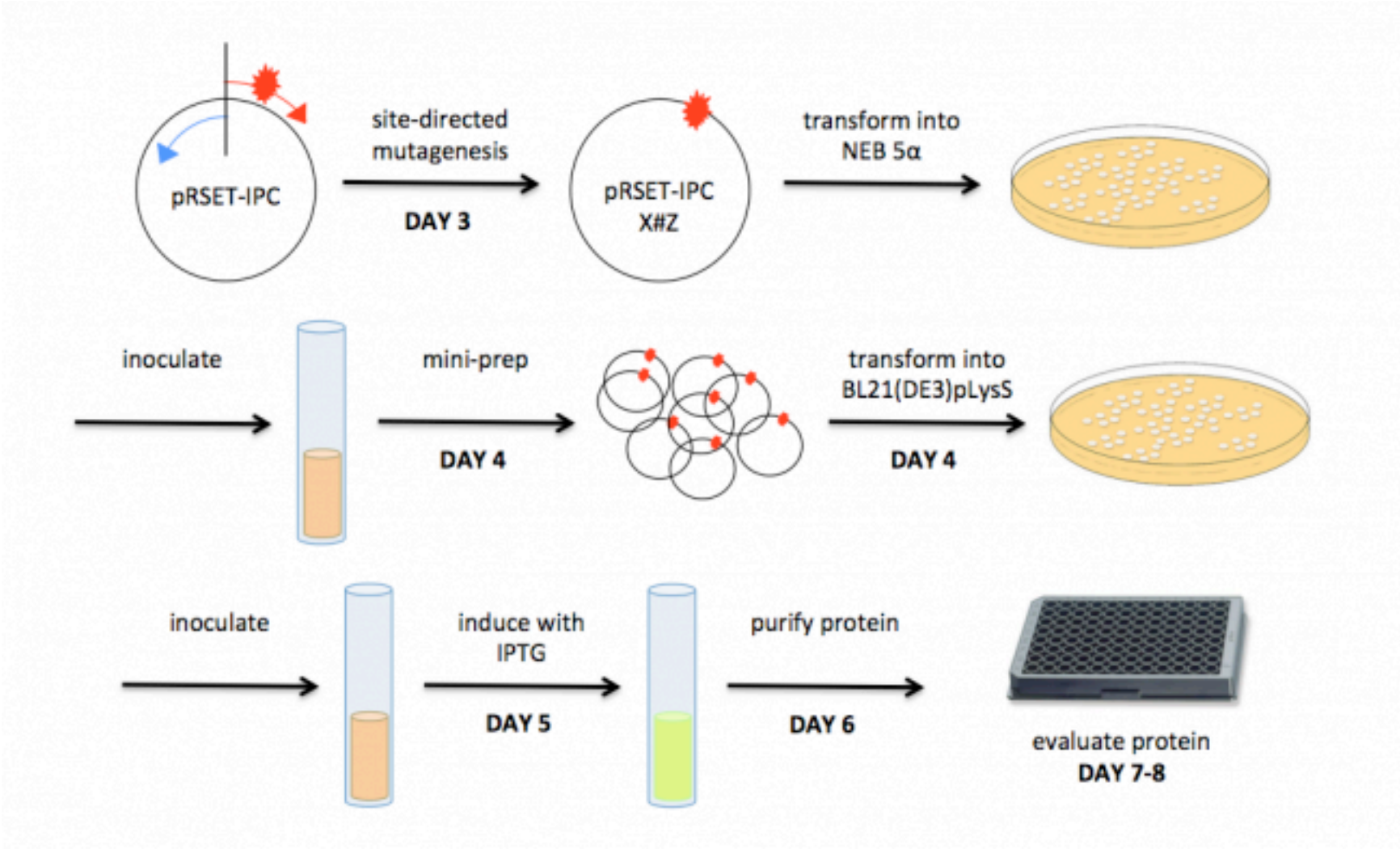


How does this differ from our sensor?

Which brings us to Mod 2…



Mod 2 overview



In the laboratory…

- Prepare the primers you designed on M1D2
- Setup site-directed mutagenesis reaction
- Discuss article by Nagai et al.