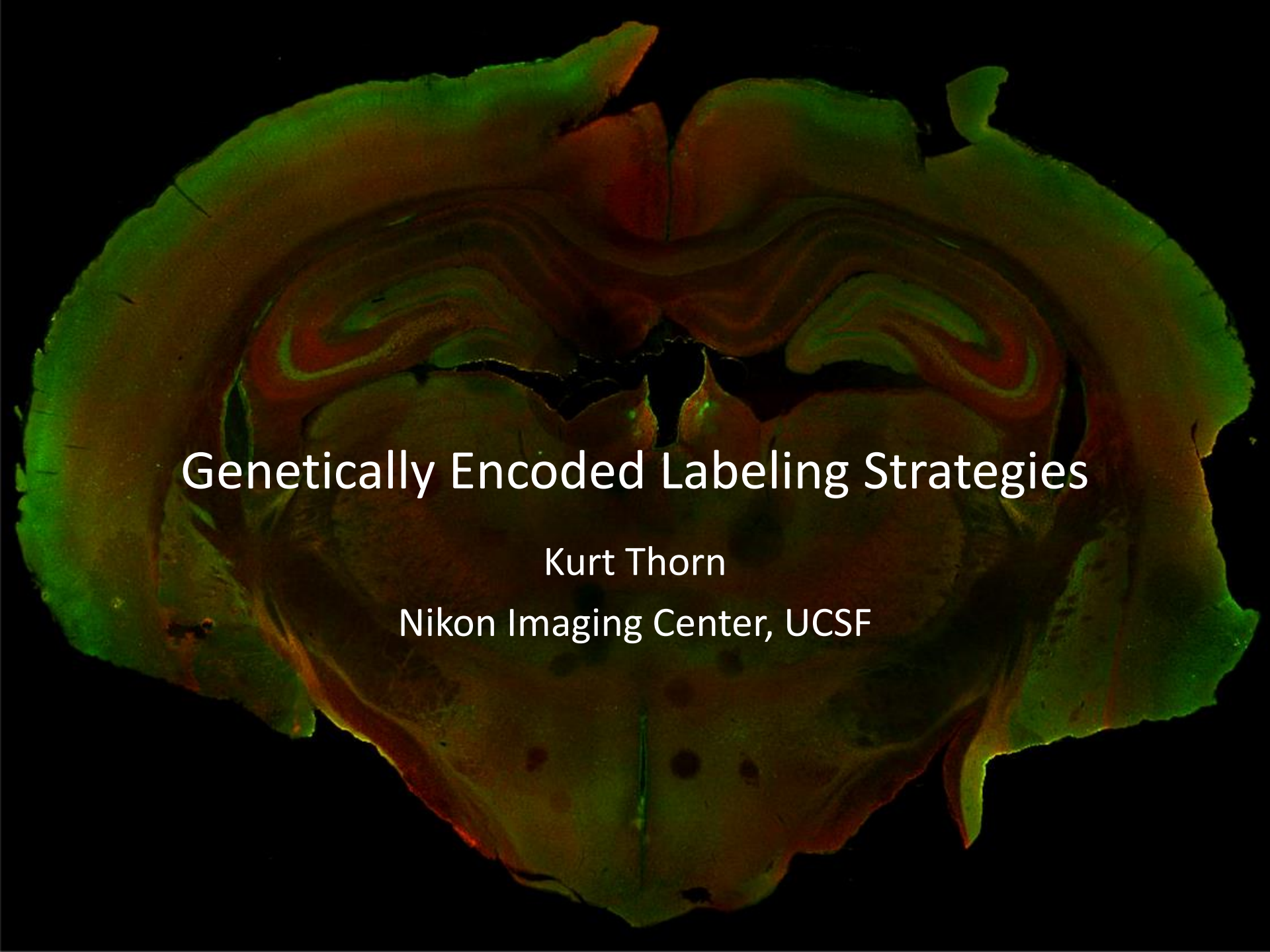
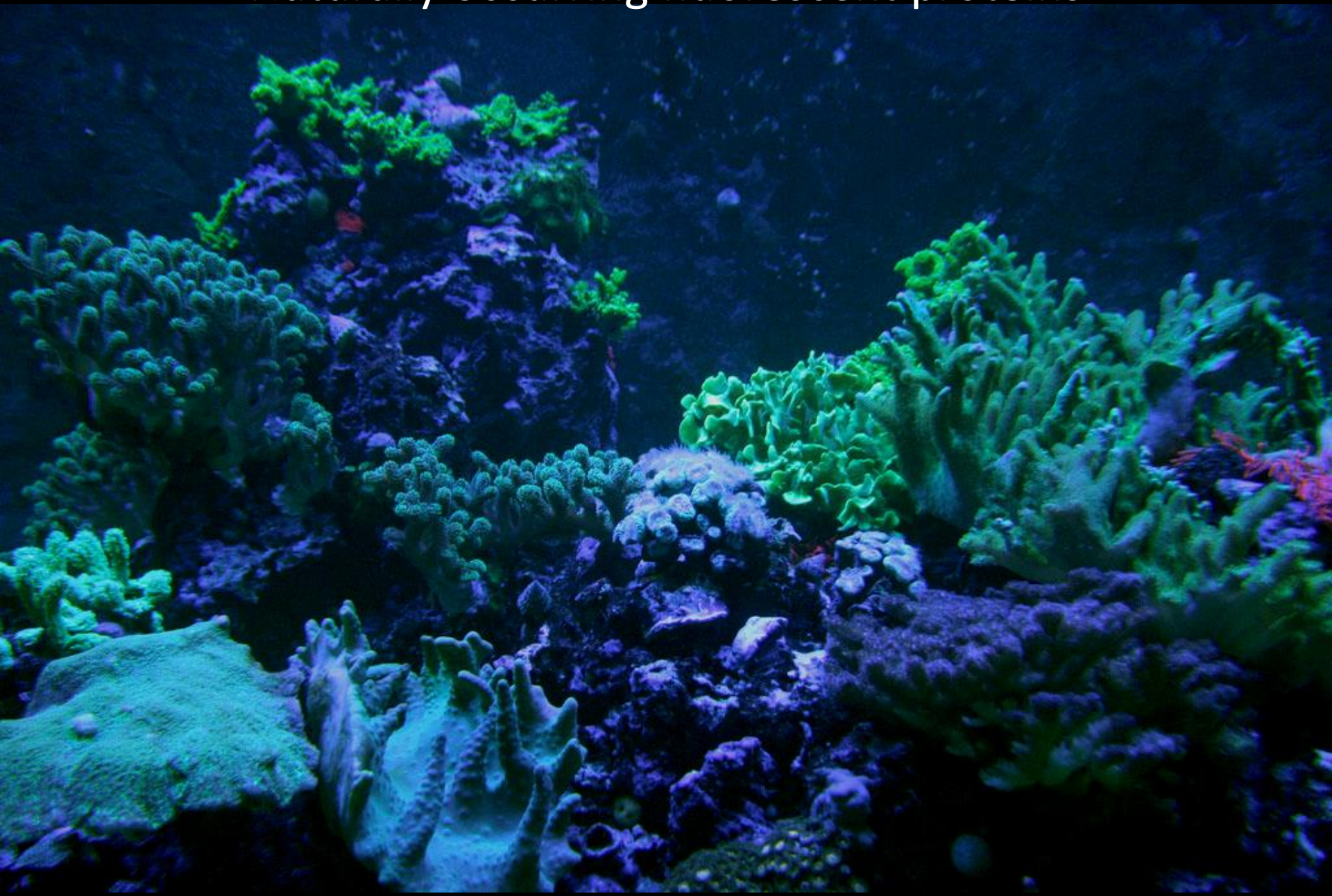


Genetically Encoded Labeling Strategies

Kurt Thorn

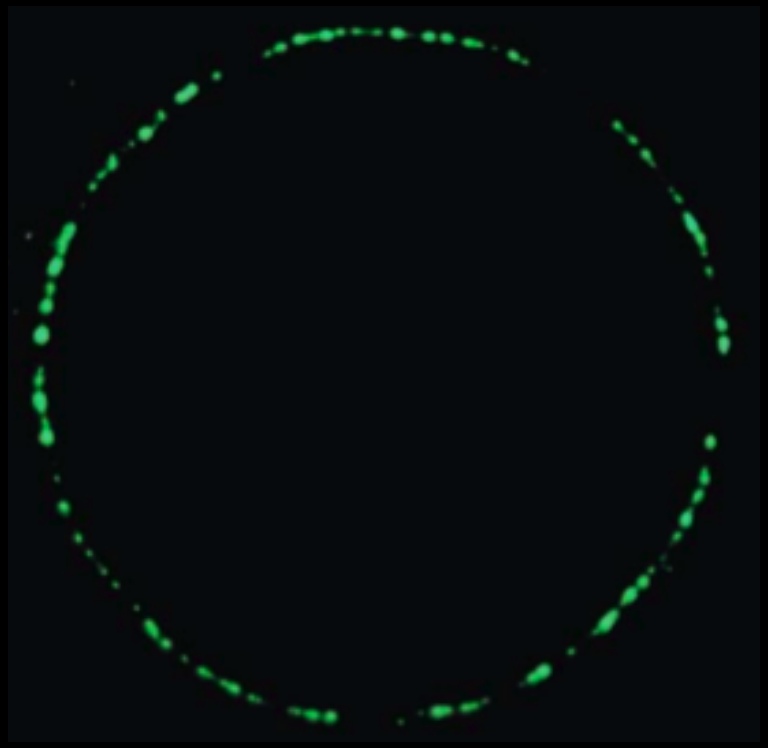
Nikon Imaging Center, UCSF

Naturally occurring fluorescent proteins

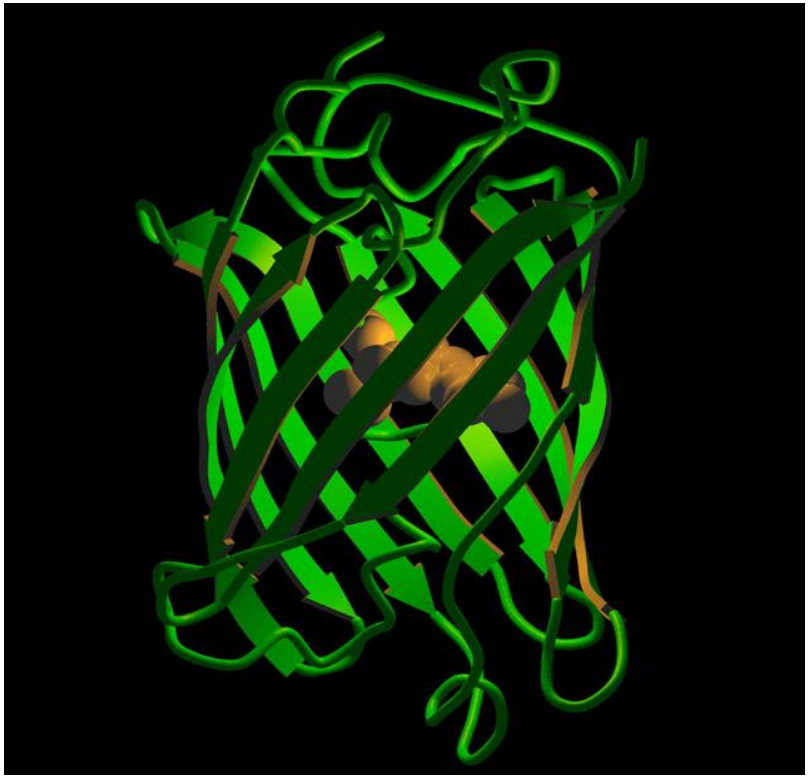


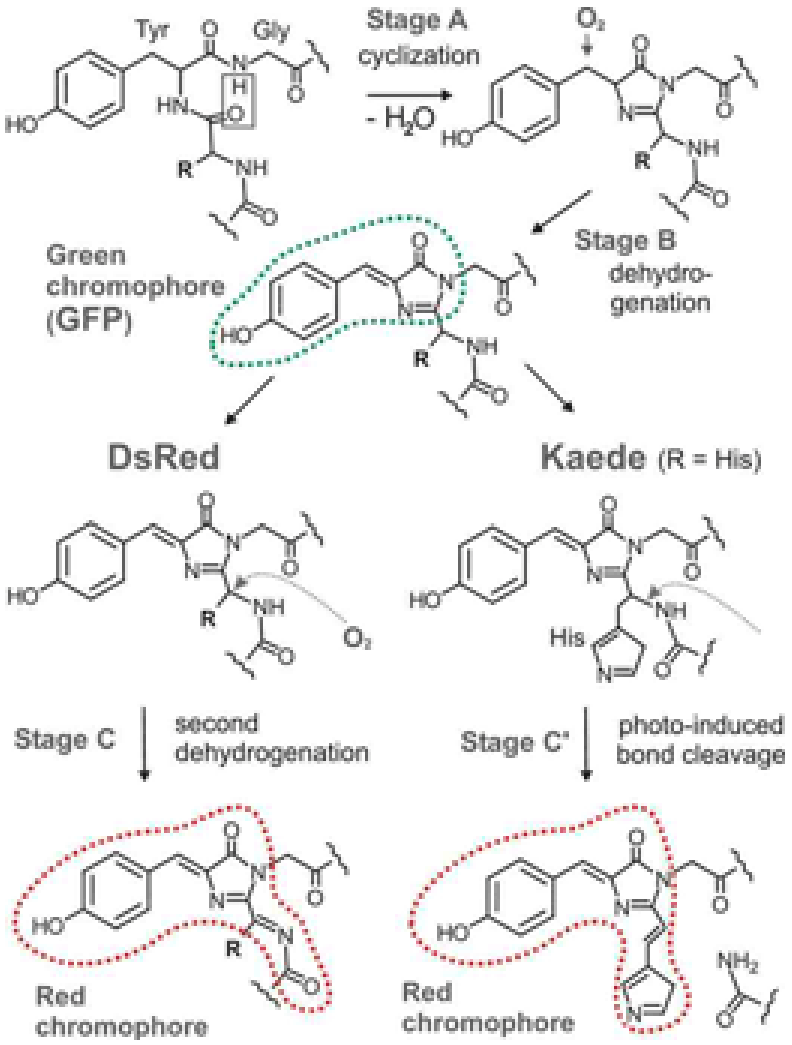
Fabio Gismondi / Flickr

Aequorea victoria

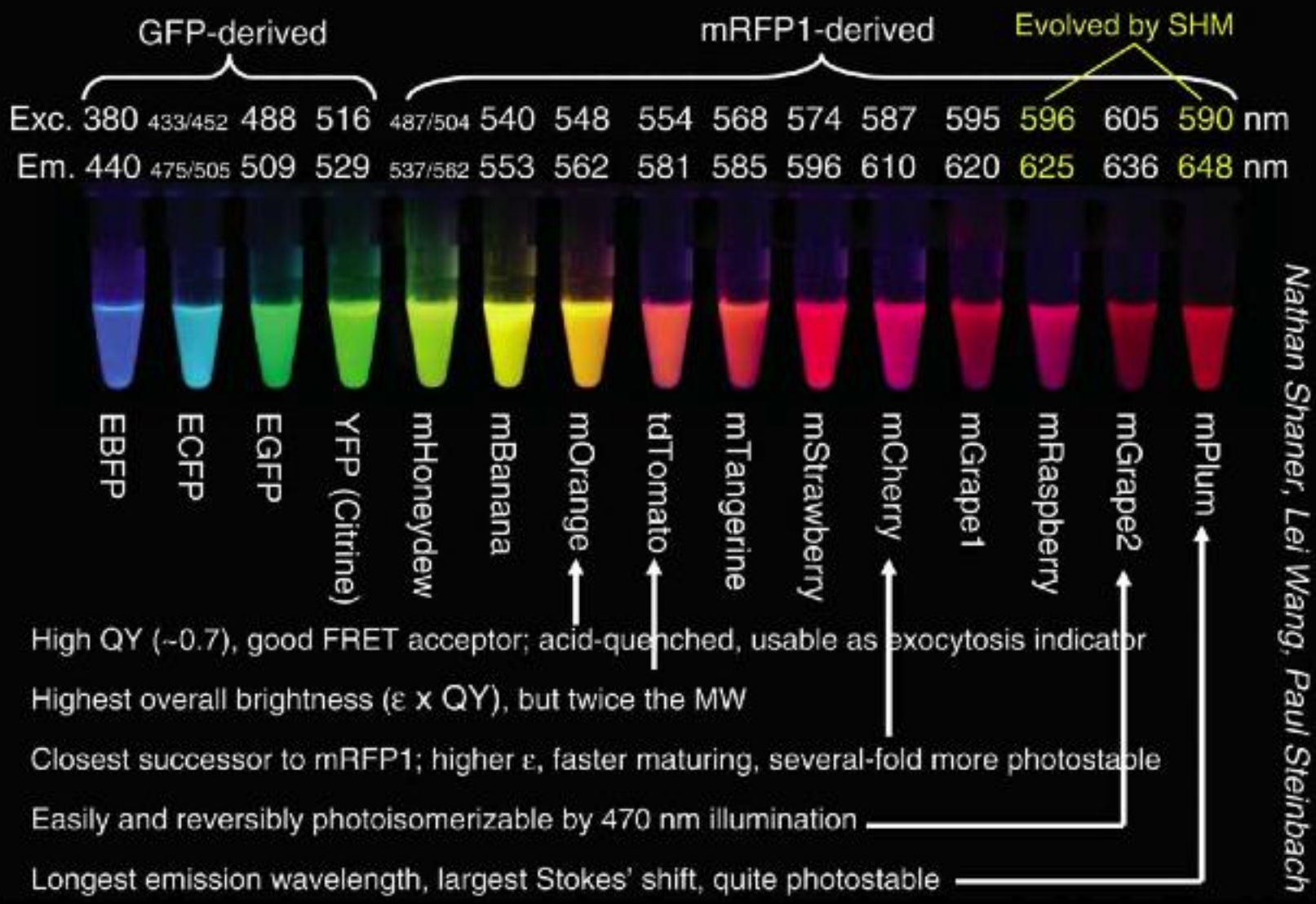


Protein and chromophore structure





The 2004 palette of nonoligomerizing fluorescent proteins



General fluorescent protein info

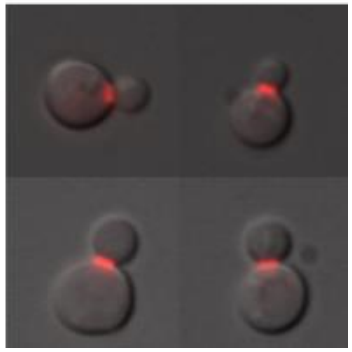
- Monomer size: ~ 240 a.a.; 27 kDa
- Many have been engineered from dimeric or tetrameric proteins and may have residual oligomerization
  - For GFP variants, A206K ensures monomer
- Require oxygen for maturation
- Maturation takes ~15 min to hours

How to evaluate a fluorescent protein?

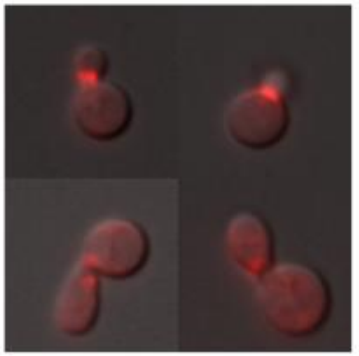
- Excitation and emission wavelengths
  - Compatible with filters / lasers?
  - Separable from other FPs / dyes?
- Brightness
- Maturation rate
  - May depend on organism and temperature
- pH / environment sensitivity
- Oligomerization state
- Does it perturb fusion protein function?

Perturbation of fusion proteins

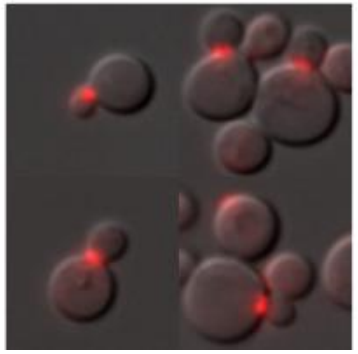
yEGFP



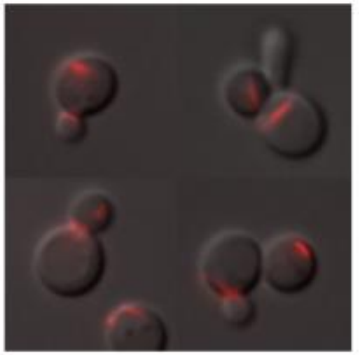
tdimer2



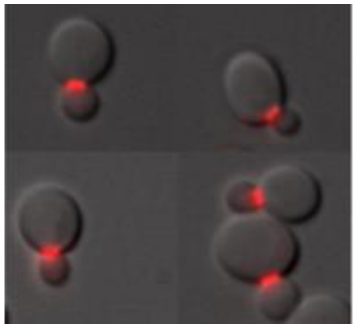
mCherry



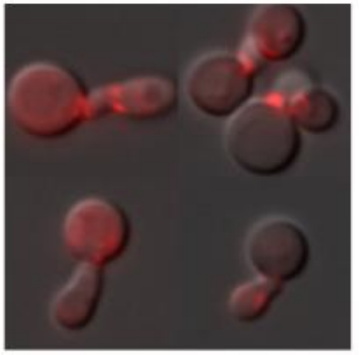
mKOrange



yECitrine



tdTomato



Good FP combinations

- Blue / Green / Red / near IR
  - For 405 / 488 / 561 / 640 lasers
  - (like DAPI / Fluorescein / Rhodamine / Cy5)

- Cyan/ Yellow / Red / near IR
  - Good for CFP/YFP FRET

Recommended Blue FPs

- mTagBFP2: 399 ex / 454 em Brightness 32
  - Well-matched to 405nm laser

- EBFP2: 383 ex / 448 em Brightness 18

Recommended Cyan FPs

ECFP derived:

- mTurquoise2: 434 ex / 474 em Brightness 28

- SCFP3A: 433 ex / 474 em Brightness 17
  - Folds better than Cerulean and ECFP

Others:

- mTFP1: 462 ex / 492 em Brightness 54

- TagCFP: 458 ex / 480 em Brightness 21

Recommended Green FPs

EGFP derived:

- mEmerald: 487 ex / 509 em Brightness 37

- Superfolder GFP: 485 ex / 510 em Brightness 54

- Clover: 505 ex / 515 em Brightness 84

Others:

- mWasabi: 493 ex / 509 em Brightness 56
  - mTFP1 derivative

Recommended Yellow FPs

EYFP derived:

- mCitrine: 516 ex / 529 em Brightness 58

- SYFP2: 515 ex / 527 em Brightness 69
  - Improved Venus

Recommended Red FPs

DsRed derived:

- mCherry: 587 ex / 610 em Brightness 16

- mApple: 568 ex / 592 em Brightness 37

Others:

- TagRFP-T : 555 ex / 584 em Brightness 33

- mRuby(2) : 558 ex / 605 em Brightness 39

Recommended Far-Red / near-IR FPs

(Capable of 640nm excitation)

Intrinsically fluorescent:

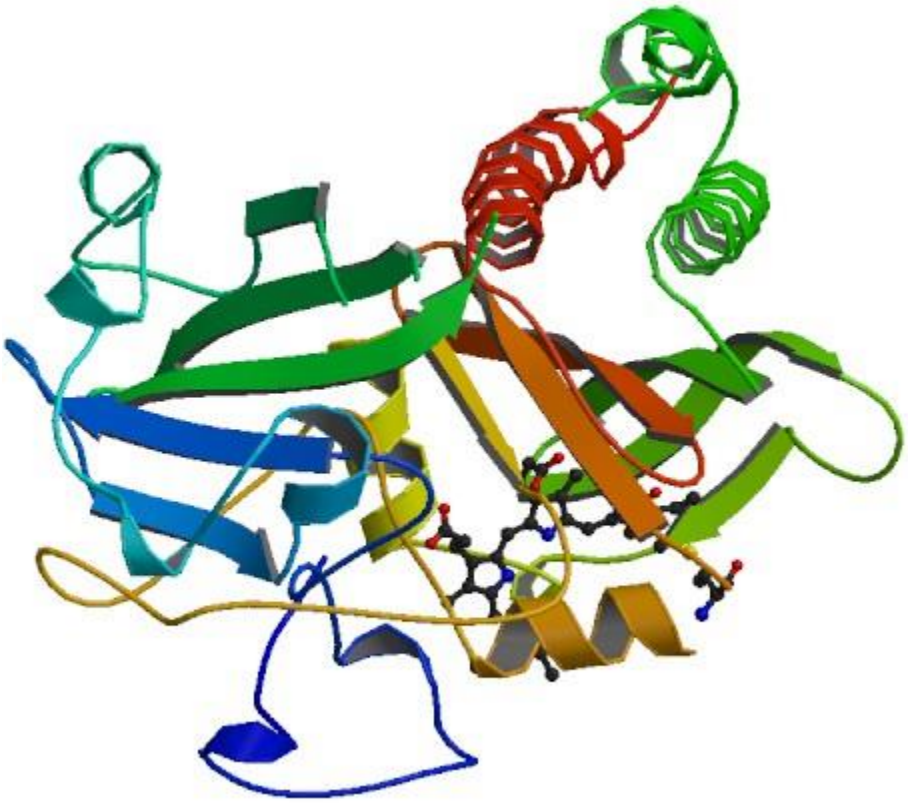
- TagRFP657: 611 ex / 657 em Brightness 3
  - Probably not bright enough for routine use

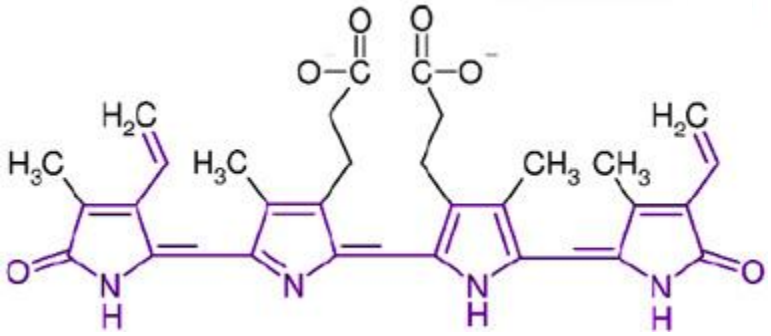
Biliverdin co-factor:

- IFP1.4: 684 ex / 708 em Brightness 6

- iRFP: 690 ex / 713 em Brightness 6
  - Dimeric

IFP1.4





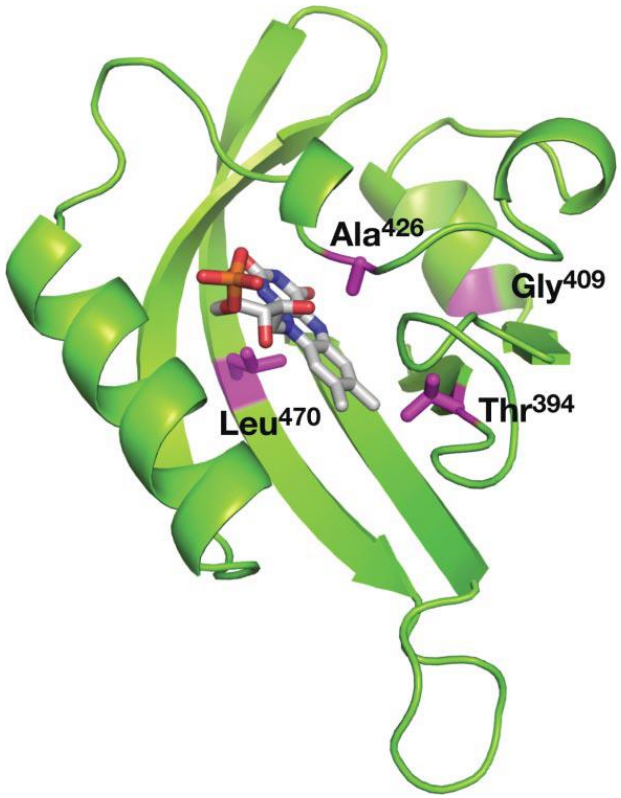
- Chromophore: biliverdin Ixa
- Product of heme breakdown
- Membrane permeable

- 321 aa; 36.5kDa

- Typically fluorescent in mammalian cells but adding exogenous biliverdin increases fluorescence ~5-fold

iLOV and relatives

- Plant Light, Oxygen, or Voltage sensing (LOV) domains
- Bind flavin mononucleotide (FMN)
- ~100 amino acids
- Does not require oxygen



Optical Highlighter Proteins

Photoactivatable (Off → On)

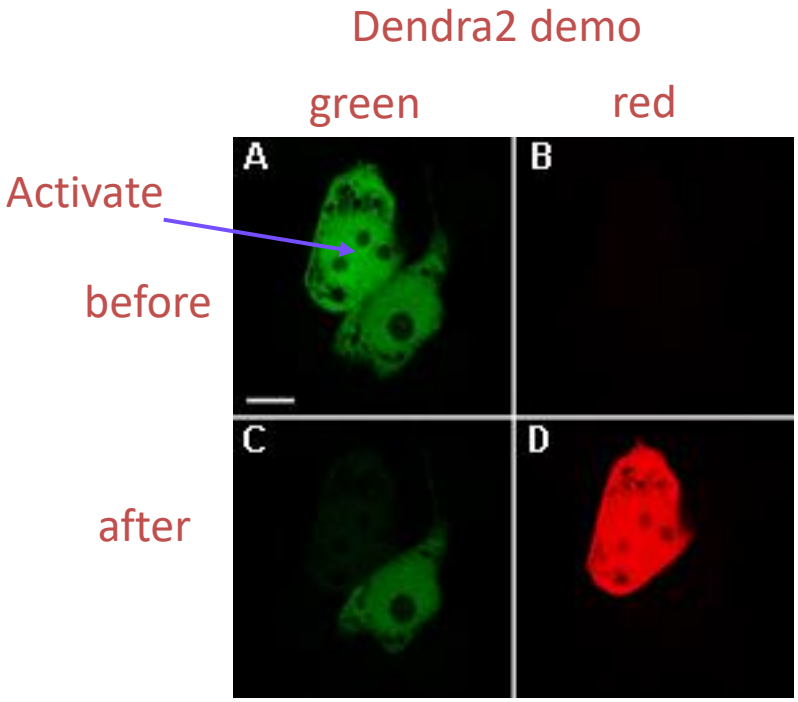
- PA-GFP, PAmCherry
- PATagRFP

Photoswitchable (Color change)

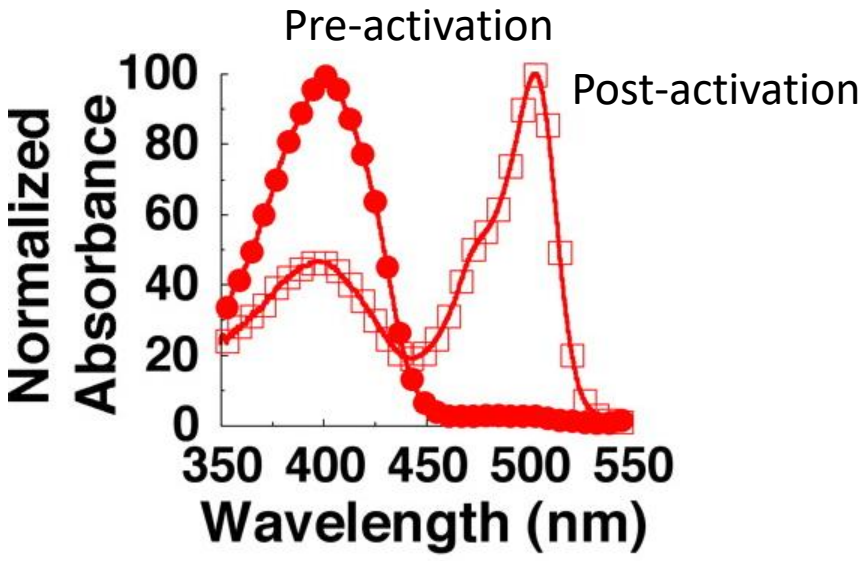
- mEos2/3
- PS-CFP

Photoswitchable (Off ↔ On)

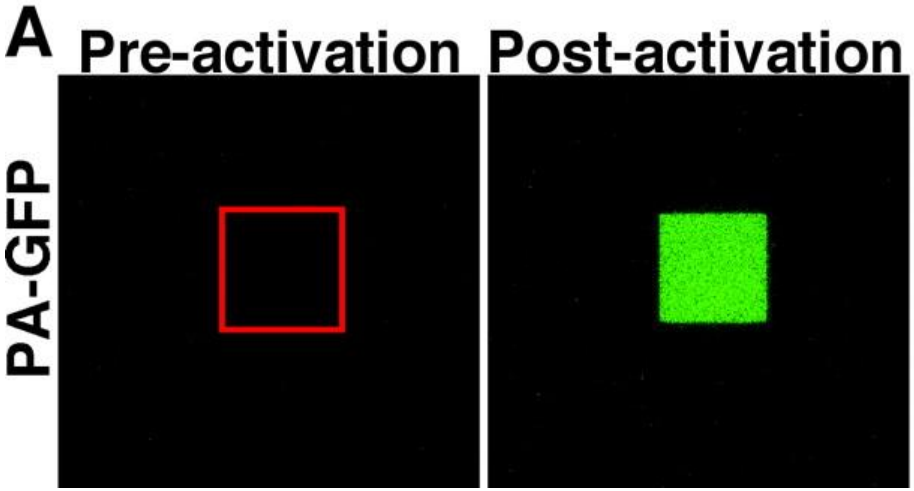
- asCP, KFP (tetrameric)
- Dronpa

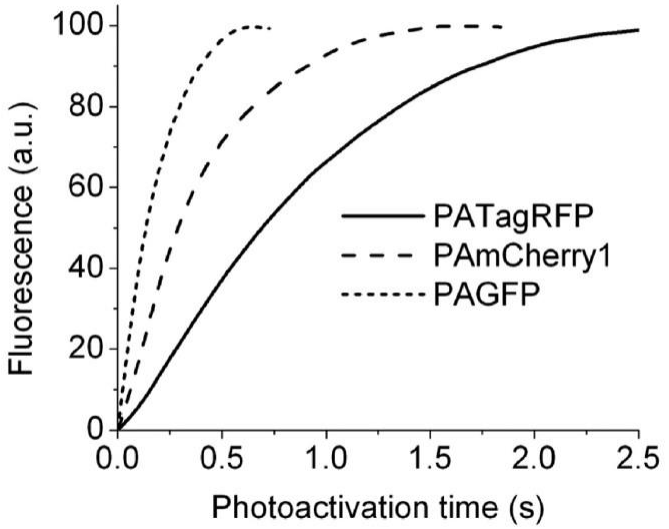


Photoactivation



Generally: Off $\xrightarrow{405\text{ nm}}$ On





Science 2002 297: 1873-1877

Recommended Photoactivatable Proteins

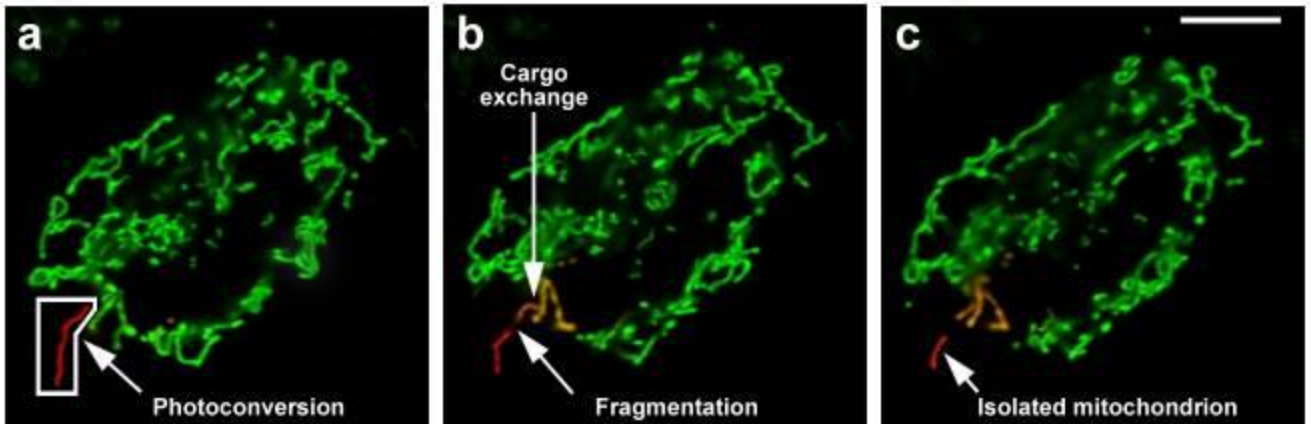
- PAGFP: 504 ex / 517 em Brightness 14 Contrast 100
- PAmCherry: 564 ex / 595 em Brightness 8 Contrast 4000
- PAtagRFP: 562 ex / 595 em Brightness 25 Contrast 500

Photoconversion

- Generally: Green $\xrightarrow{405\ nm}$ Red

- Best monomer is probably mEos2 / mEos3.2; multiple tetrameric proteins available

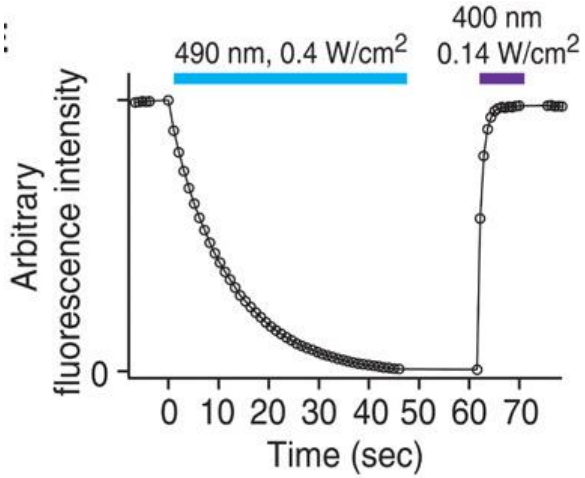
- mEos2 (green): 506 ex / 519 em Brightness 47
- mEos2 (red): 573 ex / 584 em Brightness 30 Contrast 2000?

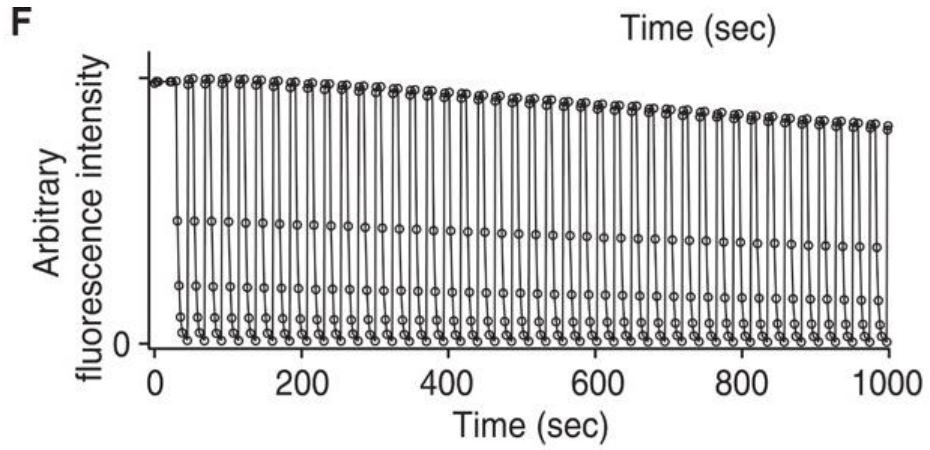


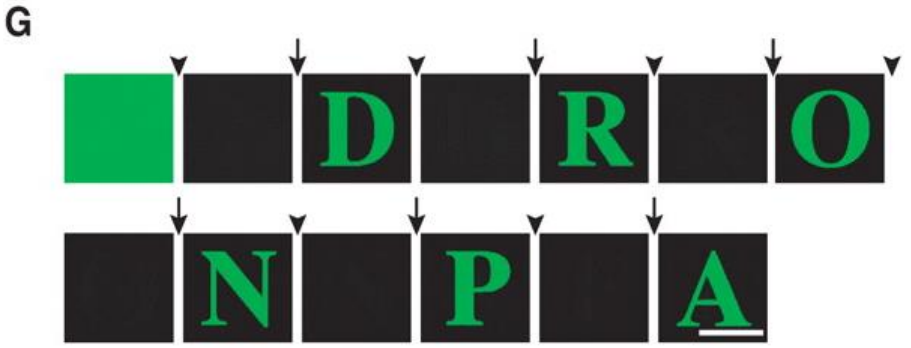
McKinney et al. Nature Methods 6, 131–133 (2009)

Photoswitchable – Dronpa

Generally: Off $\underset{\lambda_{ex}}{\overset{405\ nm}{\rightleftharpoons}}$ On

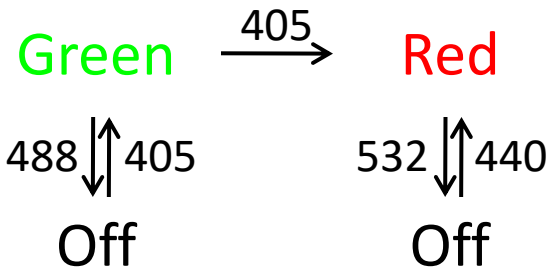




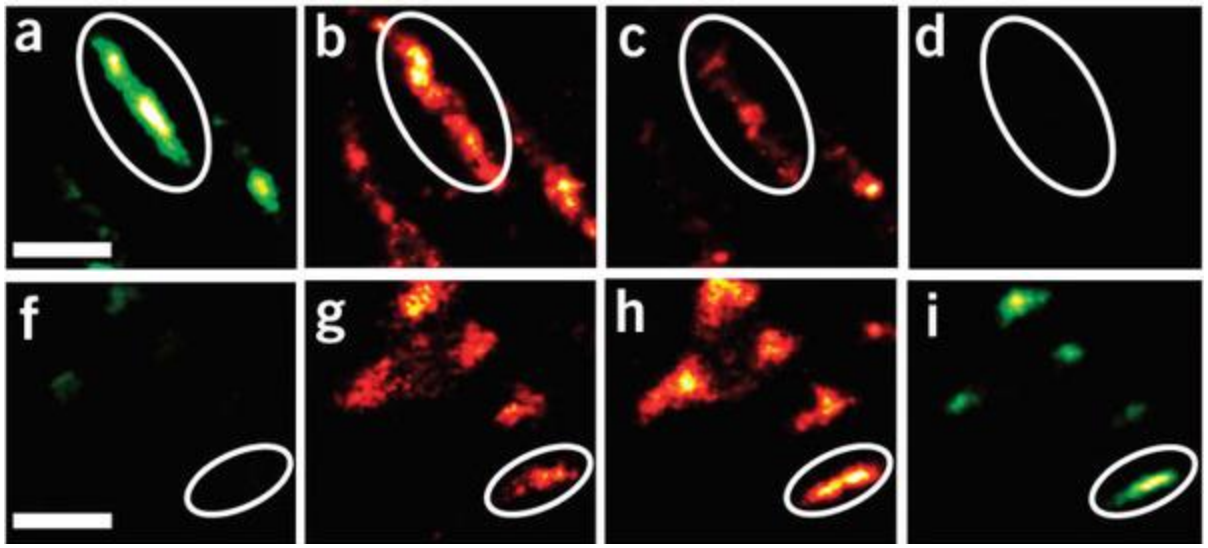


Ando et al. 2004, Science 306: 1370-1373

mIrisFP – Photoswitchable and Photoconvertible



- Eos derivative
- Both green and red states are decently bright



Fuchs et al. Nature Methods 7, 627–630 (2010)

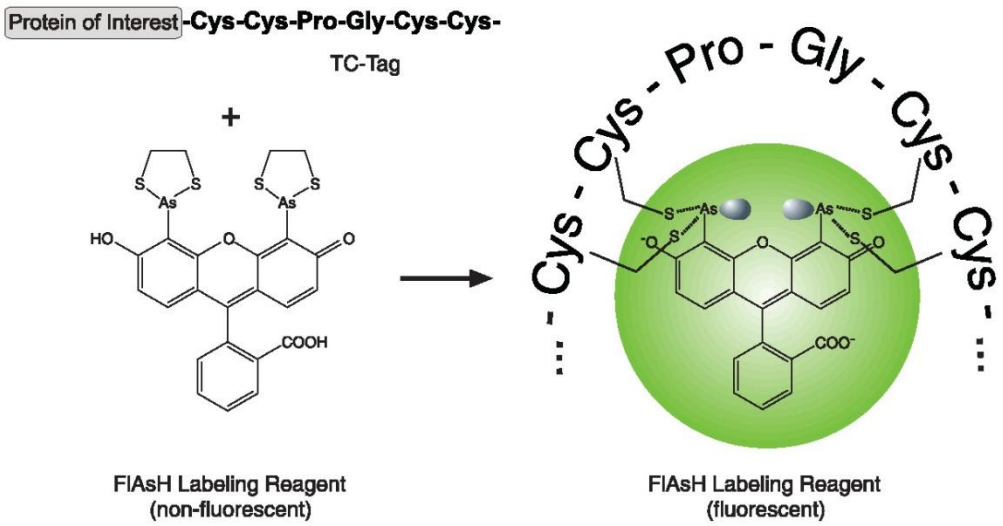
Fluorescent proteins – pros / cons

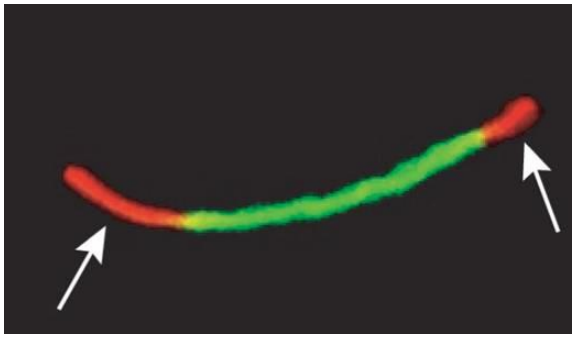
- Can be easily introduced into live cells
- Minimally perturbative
- Photoactivatible/photoconvertible versions exist
- Avoids fixing / staining

- Require genetically tractable system
- Folding and maturation can be slow
- Some are pH and Cl^- sensitive
- Some have very complicated photophysics (strange photoactivation / photobleaching behavior)

FlAsH/ReAsH

Labeling protein with tetra-cysteine motifs:





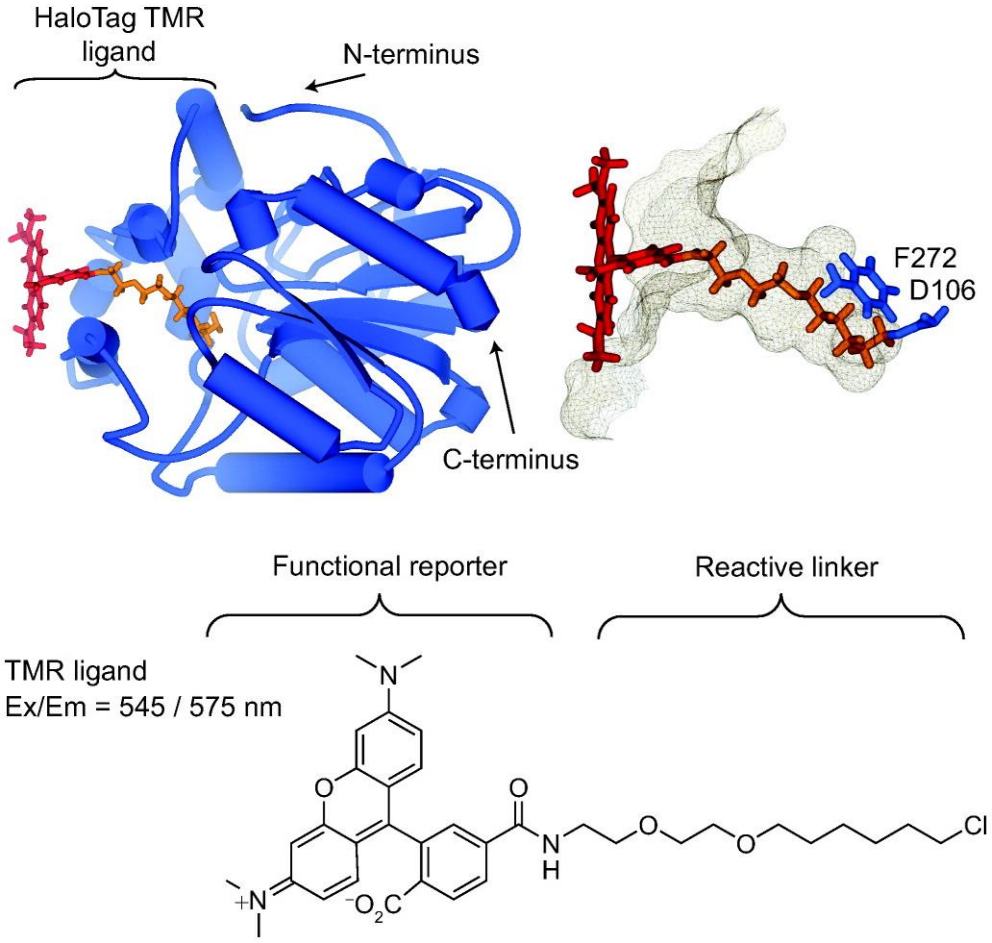
Newly synthesised connexins (ReAsH:Red) are added to the outer edges of existing gap junctions (FlAsH:Green).

Gaietta et al 2002

Commercially available from Invitrogen as Lumio or TC-FlAsH II / TC-ReAsH II

Covalent attachment of dyes to genetic tags

HaloTag (Promega)

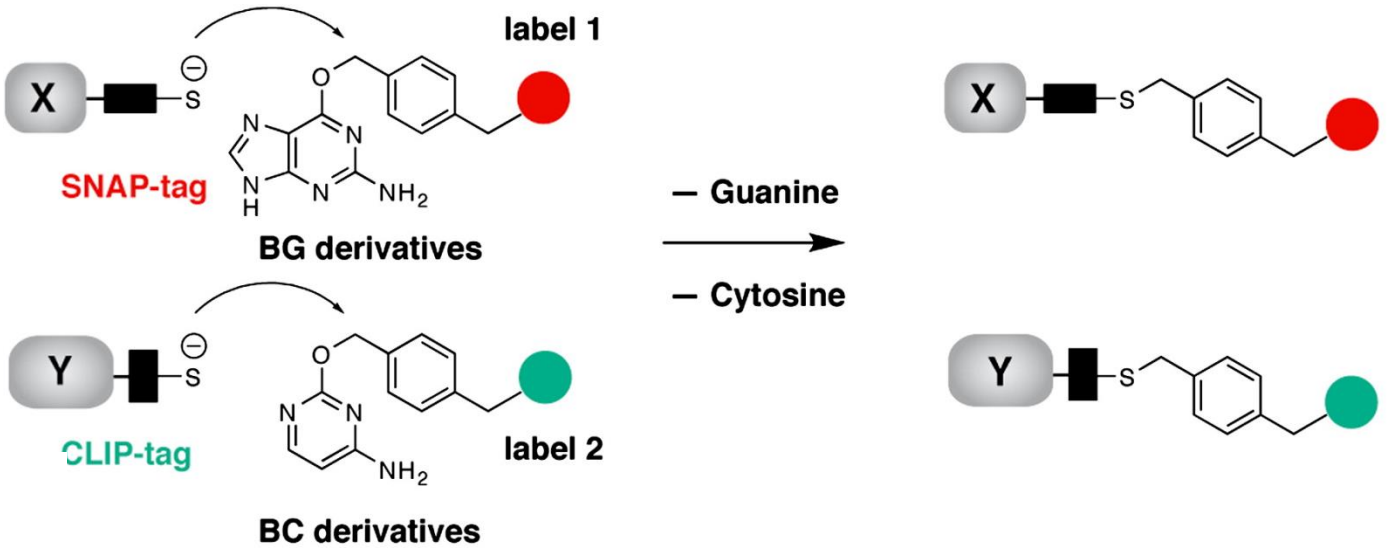


Modified haloalkane dehalogenase

~31 kDa

Covalent attachment of dyes to genetic tags

SNAP- and CLIP-tags (NEB)



Modified *O*^{6}-alkylguanine-DNA alkyltransferase; ~20 kDa

Advantages of HaloTag / SNAP-Tag fusions over GFP:

- Same protein can be labeled with almost anything you want
  - Easy to couple ligands to any NHS or malemide compound
  - Many dye/biotin ligands commercially available
- Label same protein in different colours (avoid recloning)
- Label different compartments
  - Cell-permeable and impermeable probes available
- Pulse chase experiments
  - Add two different dyes at different time points

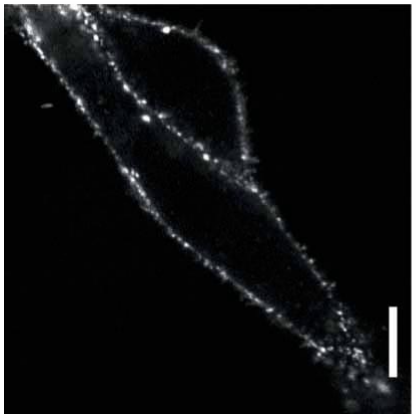
Dye-binding antibodies

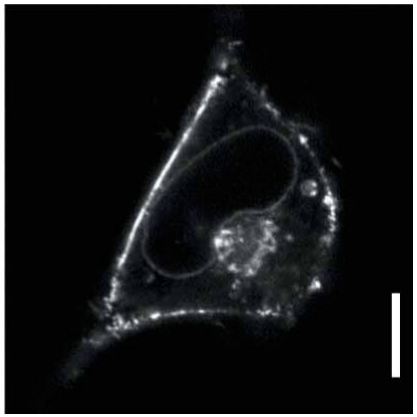
- Single chain antibodies (scFv) that bind dye molecules
- Dyes are only fluorescent in their bound state
  - 10,000-fold activation possible
- Different scFvs bind different dye families
  - Can do multicolor labeling
- But multiple color dyes can bind same scFv
- 14 – 26 kDa
- Not yet commercially available

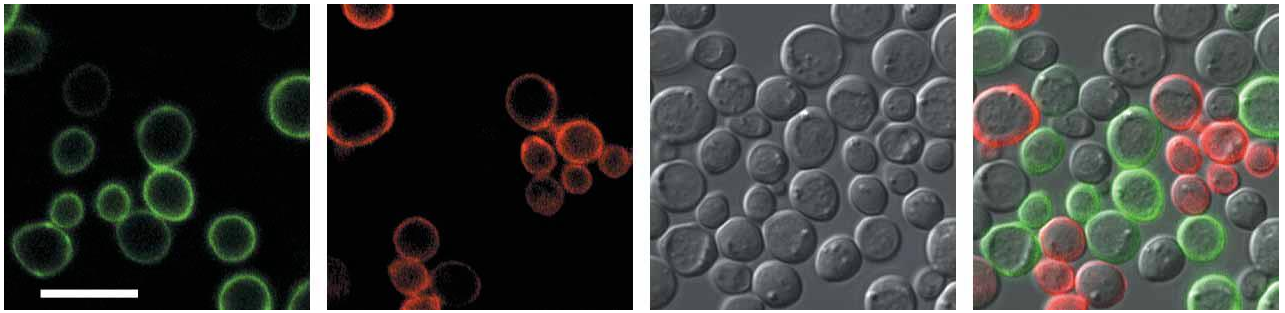
Dye binding antibodies

Membrane impermeant dye

Membrane permeable dye

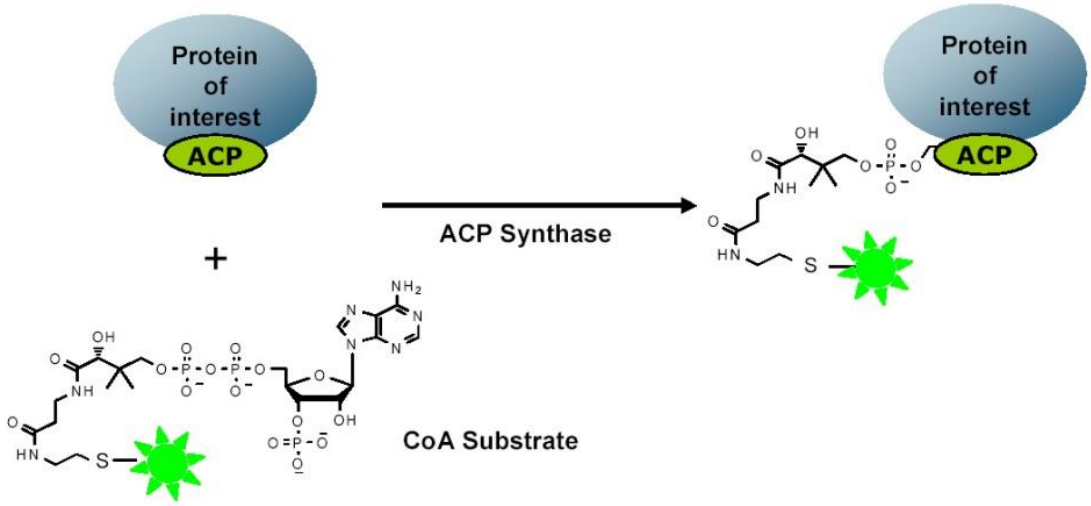






Enzyme catalyzed labeling

ACP/SFP synthase (NEB)

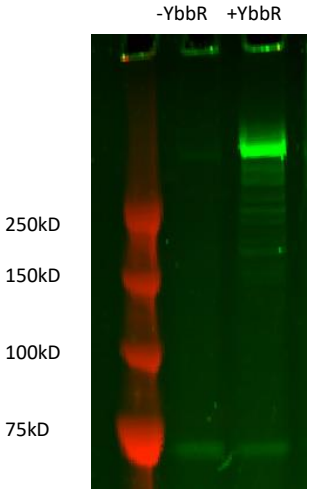


Genetic Tags:

- ACP / MCP Tag (77aa)
- YbbR peptide (11aa) (Christopher Walsh)

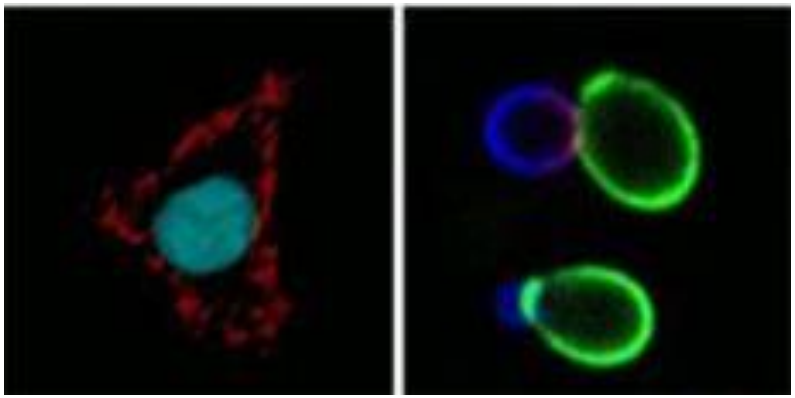
Uses of enzyme catalyzed labeling

1) Site specific labeling of proteins with short peptides



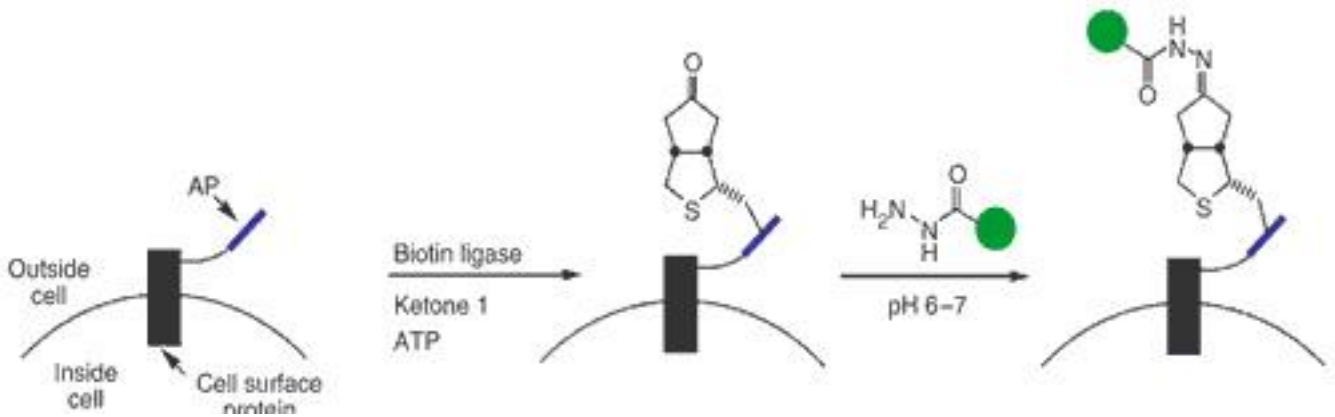
Artificial dimerised dynein with and without YbbR peptide labelled with SFP synthase and 547-CoA (Vale lab)

2) Labelling of cell surface proteins



Enzyme catalyzed labeling

Biotin and Lipoic Acid Ligase (Alice Ting)



- Biotin or keto-biotin labeling
- Ketone group can be specifically labeled
- Not yet commercially available

Further Reading

- http://nic.ucsf.edu/dokuwiki/doku.php?id=fluorescent_proteins

Acknowledgements

- Nico Stuurman
- Mike Davidson