

A fluorescence micrograph of a coronal brain section. The image shows various brain structures with green and red fluorescent labeling. The green signal is widespread, while the red signal is more localized, appearing in specific regions of the brain. The overall image has a dark background with bright green and red areas.

Genetically Encoded Labeling Strategies

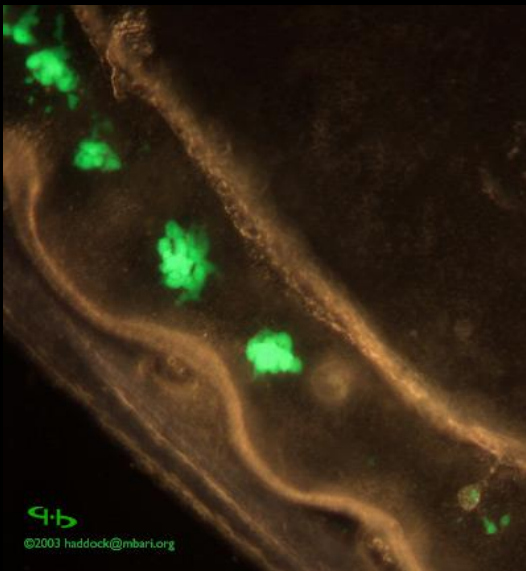
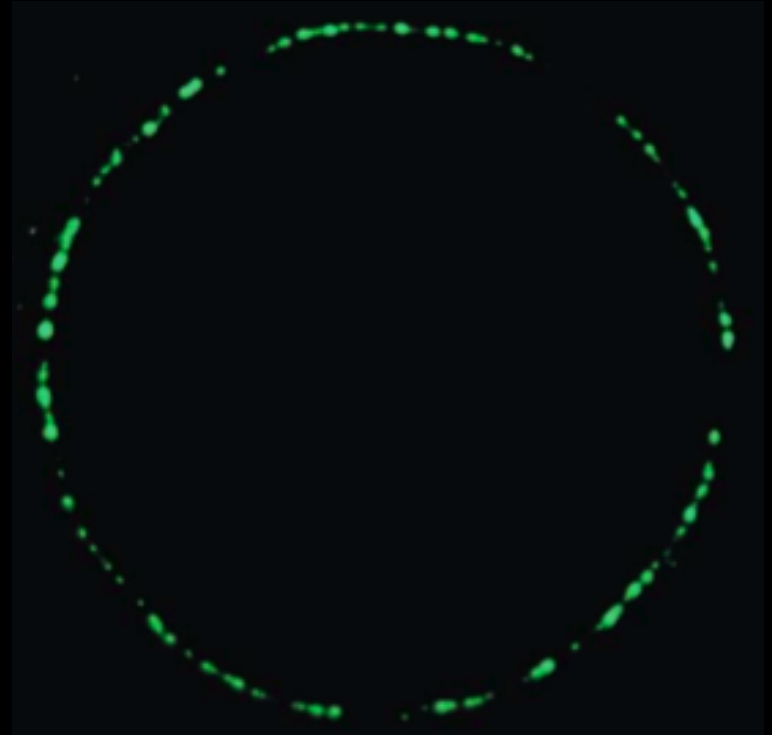
Kurt Thorn

Nikon Imaging Center, UCSF

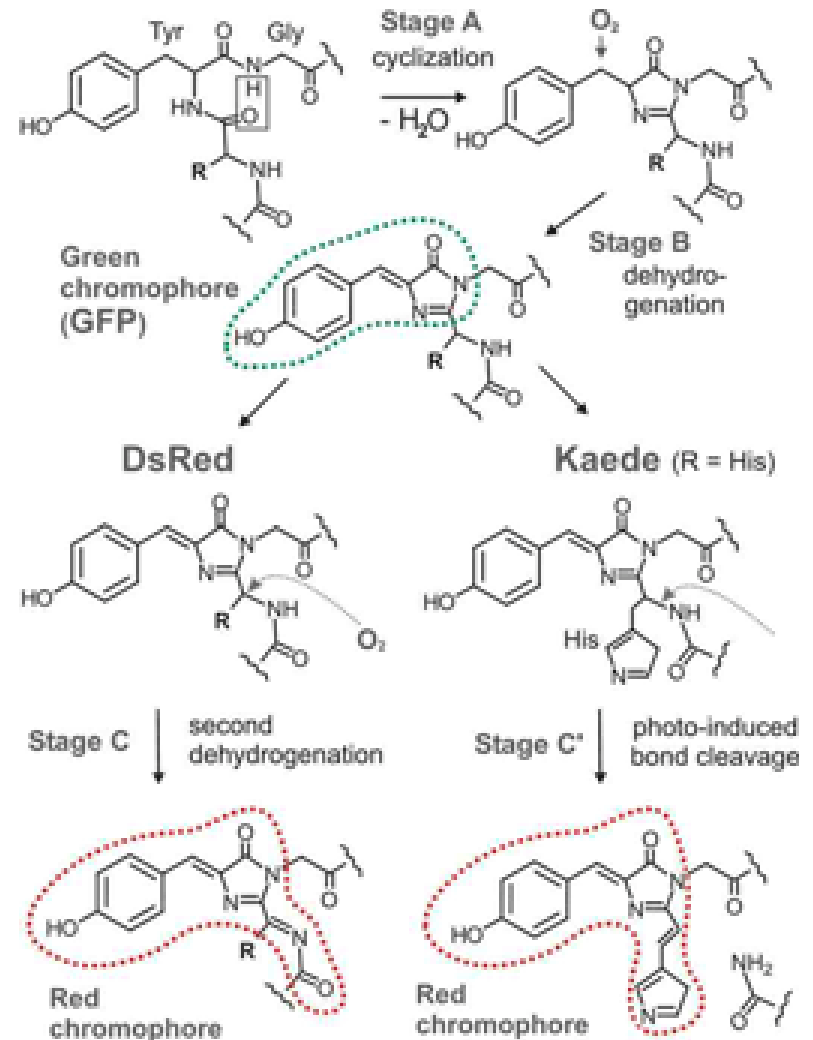
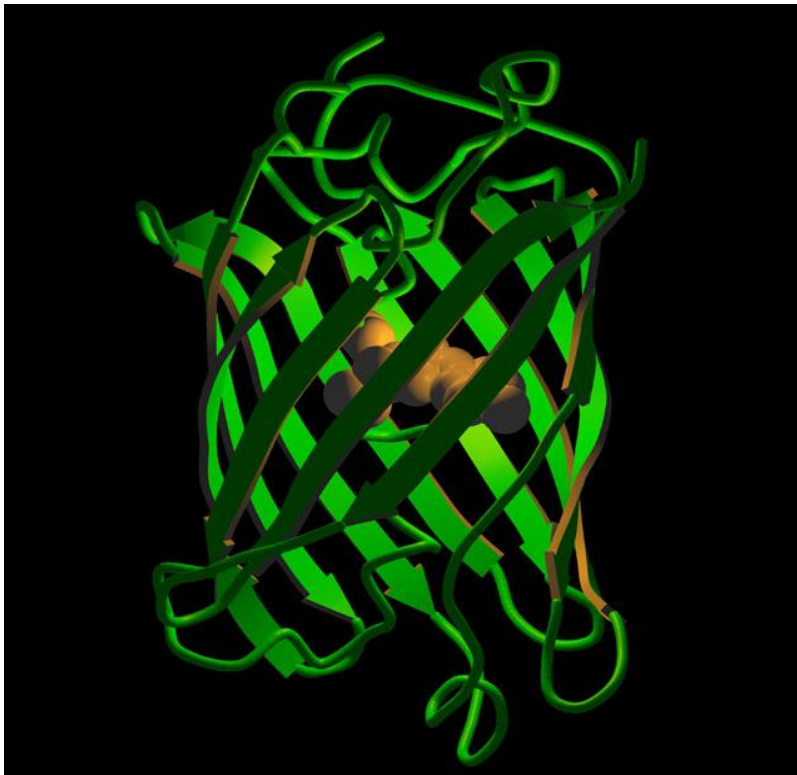
Naturally occurring fluorescent proteins



Aequorea victoria



Protein and chromophore structure



The 2004 palette of nonoligomerizing fluorescent proteins

	GFP-derived				mRFP1-derived								Evolved by SHM			
Exc.	380	433/452	488	516	487/504	540	548	554	568	574	587	595	596	605	590	nm
Em.	440	475/505	509	529	537/562	553	562	581	585	596	610	620	625	636	648	nm



EBFP

ECFP

EGFP

YFP (Citrine)

mHoneydew

mBanana

mOrange

tdTomato

mTangerine

mStrawberry

mCherry

mGrape1

mRaspberry

mGrape2

mPlum

High QY (~0.7), good FRET acceptor; acid-quenched, usable as exocytosis indicator

Highest overall brightness ($\epsilon \times QY$), but twice the MW

Closest successor to mRFP1; higher ϵ , faster maturing, several-fold more photostable

Easily and reversibly photoisomerizable by 470 nm illumination

Longest emission wavelength, largest Stokes' shift, quite photostable

Nathan Shaner, Lei Wang, Paul Steinbach

General fluorescent protein info

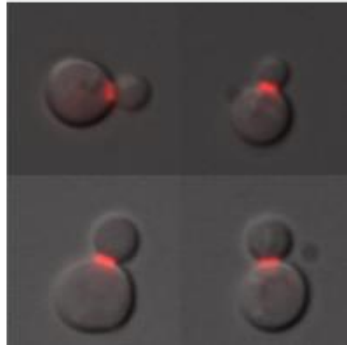
- Monomer size: ~ 240 a.a.; 27 kDa
- Many have been engineered from dimeric or tetrameric proteins and may have residual oligomerization
 - For GFP variants, A206K ensures monomer
- Require oxygen for maturation
- Maturation takes ~15 min to hours

How to evaluate a fluorescent protein?

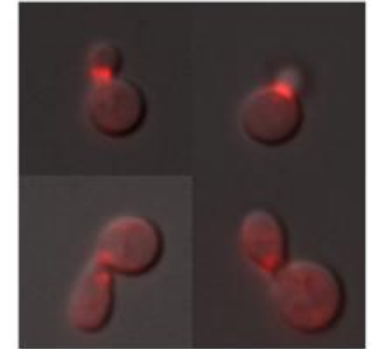
- Excitation and emission wavelengths
 - Compatible with filters / lasers?
 - Separable from other FPs / dyes?
- Brightness
- Maturation rate
 - May depend on organism and temperature
- pH / environment sensitivity
- Oligomerization state
- Does it perturb fusion protein function?

Perturbation of fusion proteins

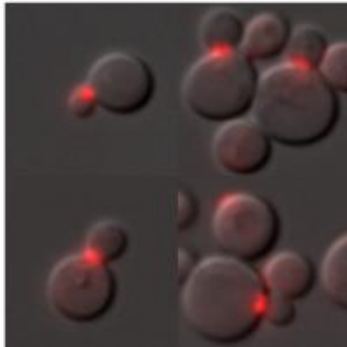
yEGFP



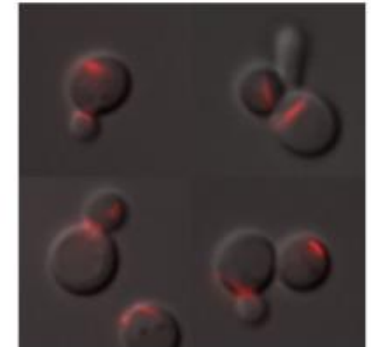
tdimer2



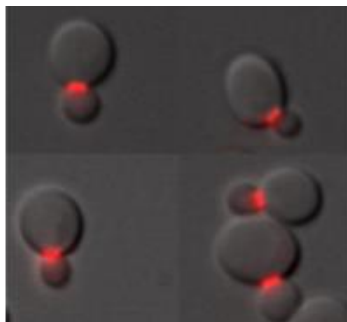
mCherry



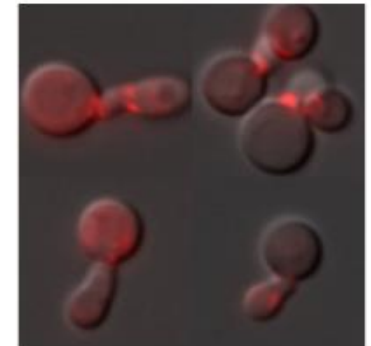
mKOrange



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

- mTagBFP: 499 ex / 454 em Brightness 32
 - Well-matched to 405nm laser
- EBFP2: 383 ex / 448 em Brightness 18
 - more photostable than mTagBFP

Recommended Cyan FPs

ECFP derived:

- mTurquoise2: 433 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:

- EGFP: 488 ex / 507 em Brightness 34
- mEmerald: 487 ex / 509 em Brightness 37
- Clover: 505 ex / 515 em Brightness 84

Others:

- mWasabi: 493 ex / 509 em Brightness 56
 - mTFP1 derivative
- mNeonGreen: 506 ex / 517 em Brightness 93

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
- TagRFP-T : 555 ex / 584 em Brightness 33
- mRuby2 : 559 ex / 600 em Brightness 43
- mKate2 : 588 ex / 633 em Brightness 25

Recommended Far-Red / near-IR FPs

(Capable of 640nm excitation)

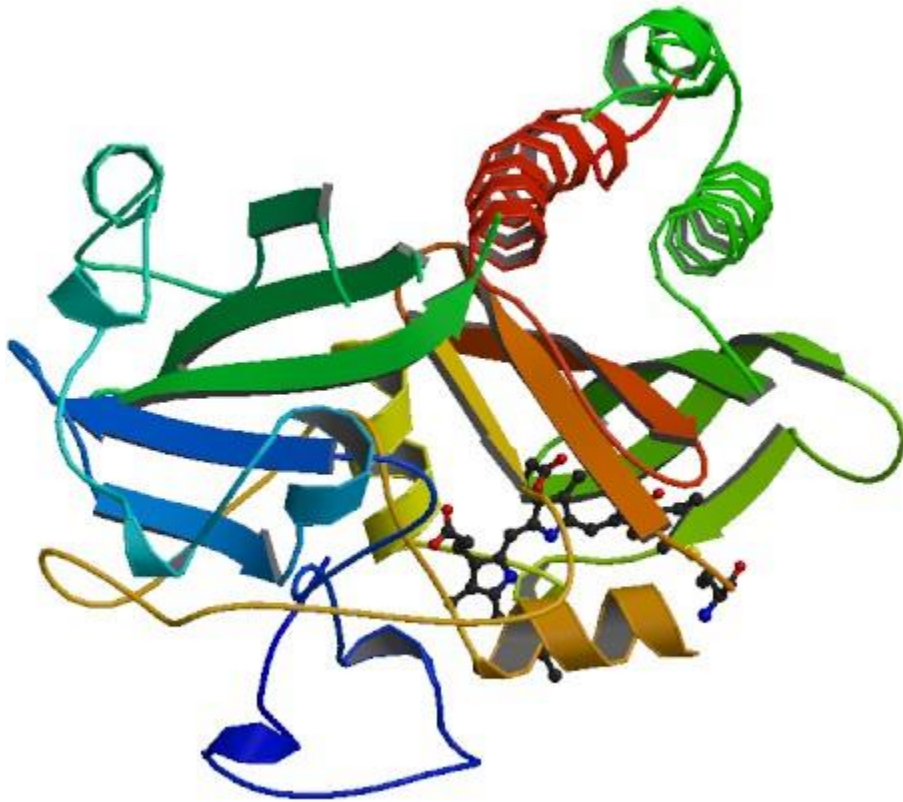
Intrinsically fluorescent:

- TagRFP657: 611 ex / 657 em Brightness 3

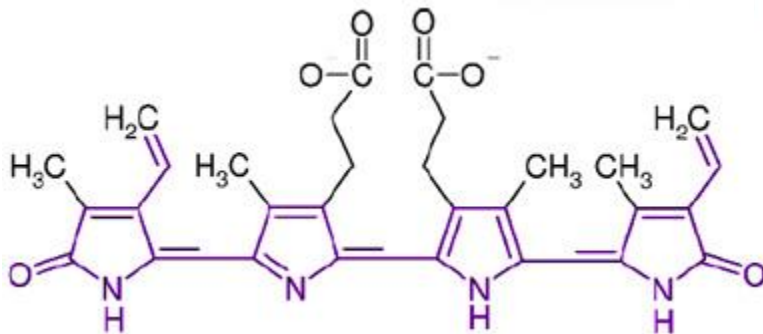
Biliverdin co-factor:

- IFP1.4: 684 ex / 708 em Brightness 8
- iRFP: 690 ex / 713 em Brightness 6
 - dimeric

IFP1.4

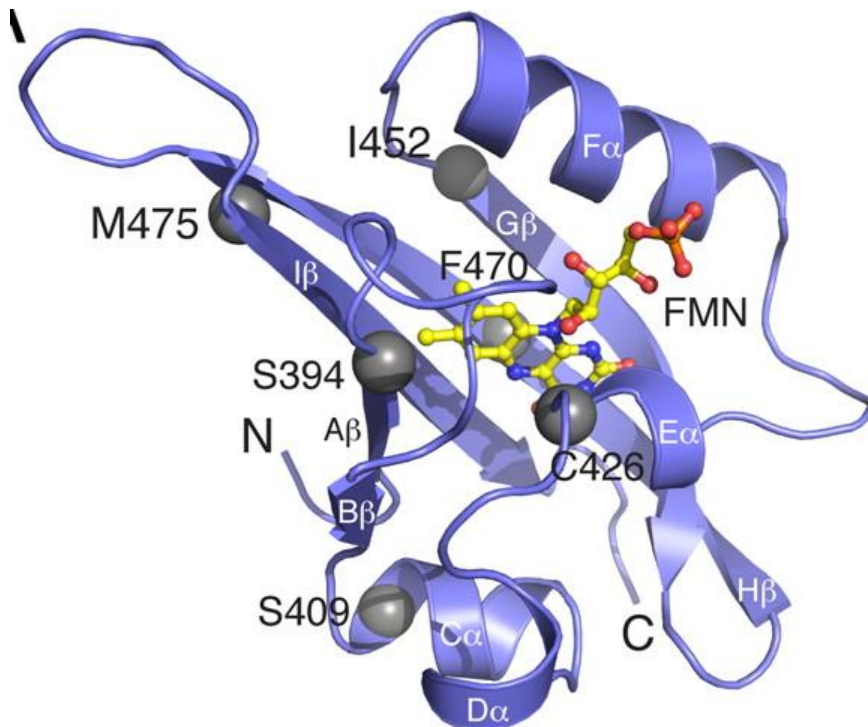


- Chromophore: biliverdin IXa
- Product of heme breakdown
- Membrane permeable
- 321 aa; 36.5kDa
- Typically fluorescent in mammalian cells but adding exogenous biliverdin increases fluorescence ~5-fold



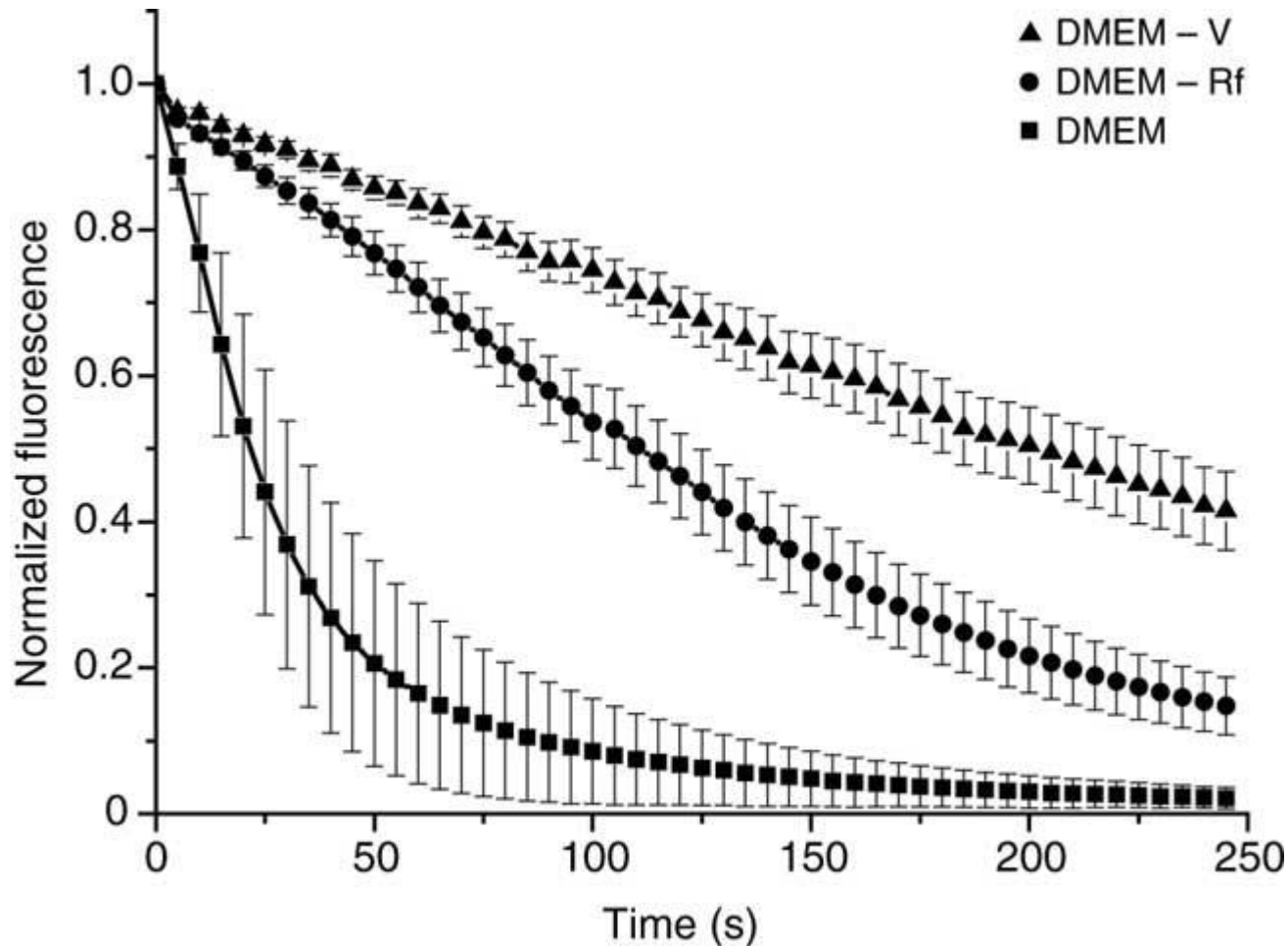
iLOV / phiLOV2.1

- Flavin-binding plant protein
- GFP-like fluorescence
- 10kD - about half the size of GFP



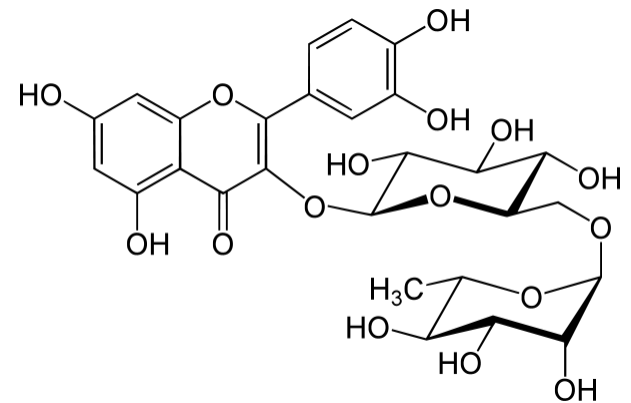
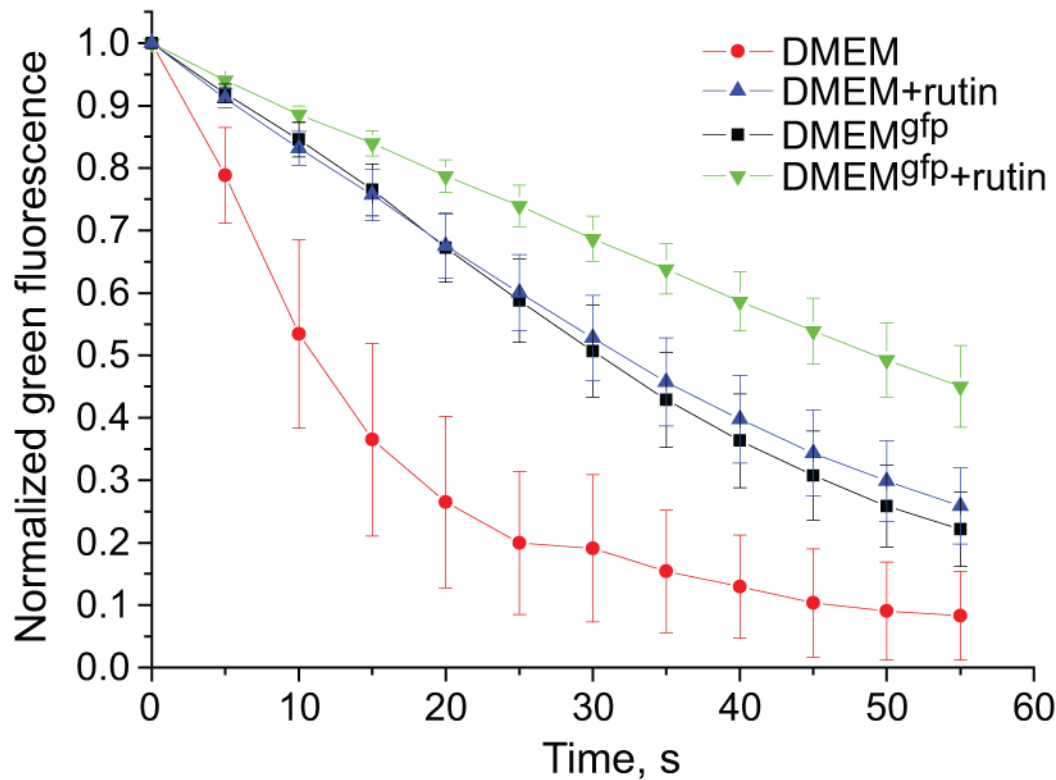
GFP Bleaching in Live Cells

Riboflavin and pyridoxal are bad, but do not affect RFPs



GFP Bleaching in Live Cells

Incubation of cells with rutin 30 minutes prior to imaging increases photostability



Optical Highlighter Proteins

Photoactivatable (Off \rightarrow On)

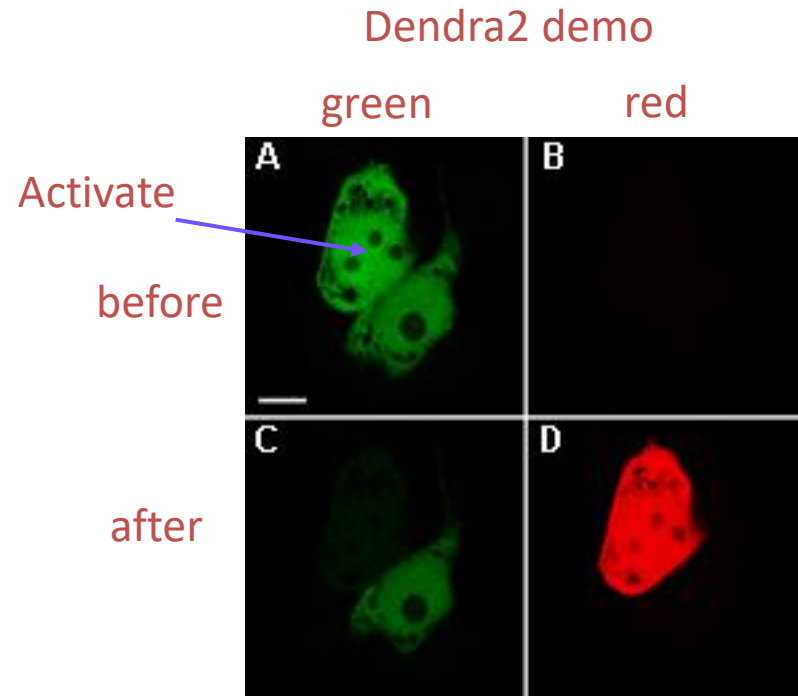
- PA-GFP, PAmCherry
- PTagRFP

Photoswitchable (Color change)

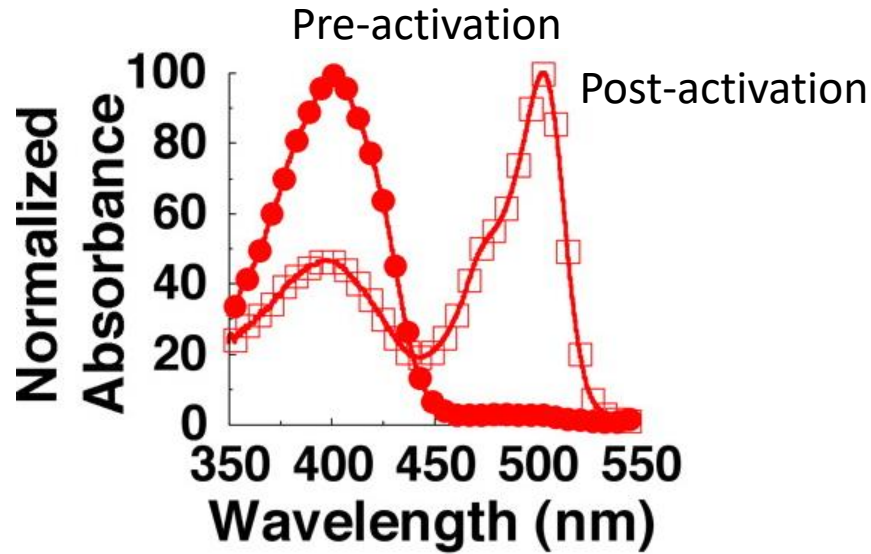
- Kaede, KikGR, Eos,
- Dendra (activatable by blue)
- PS-CFP

Photoswitchable (Off \leftrightarrow On)

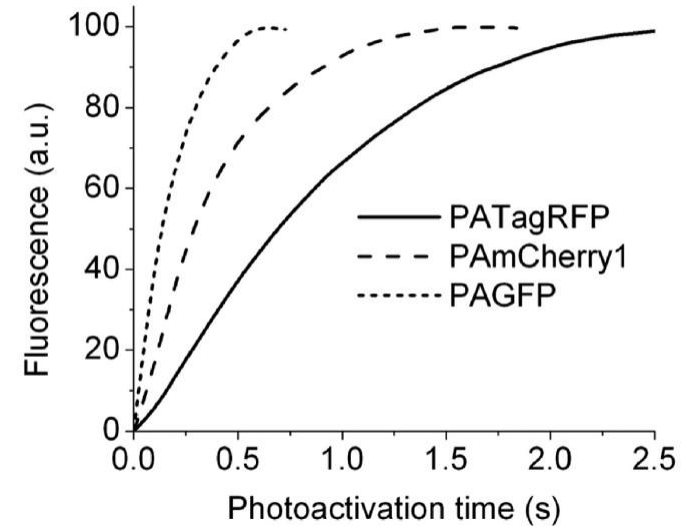
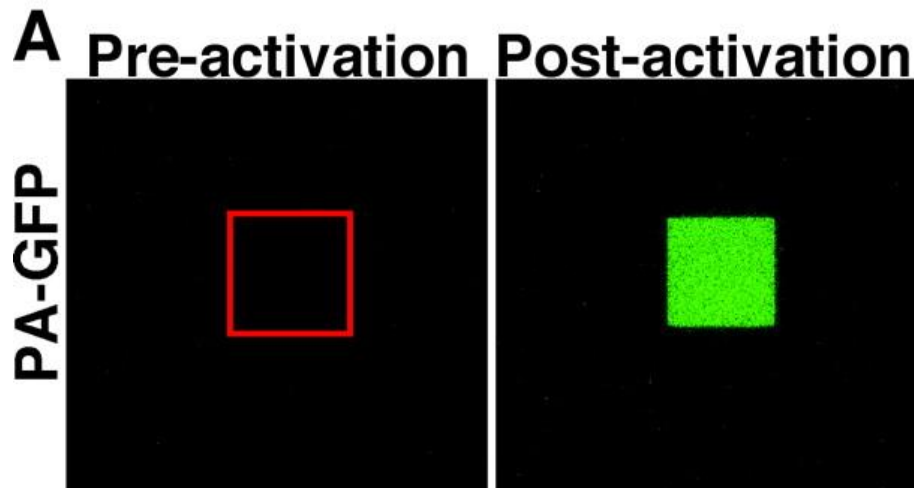
- asCP, KFP (tetrameric)
- Dronpa



Photoactivation



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

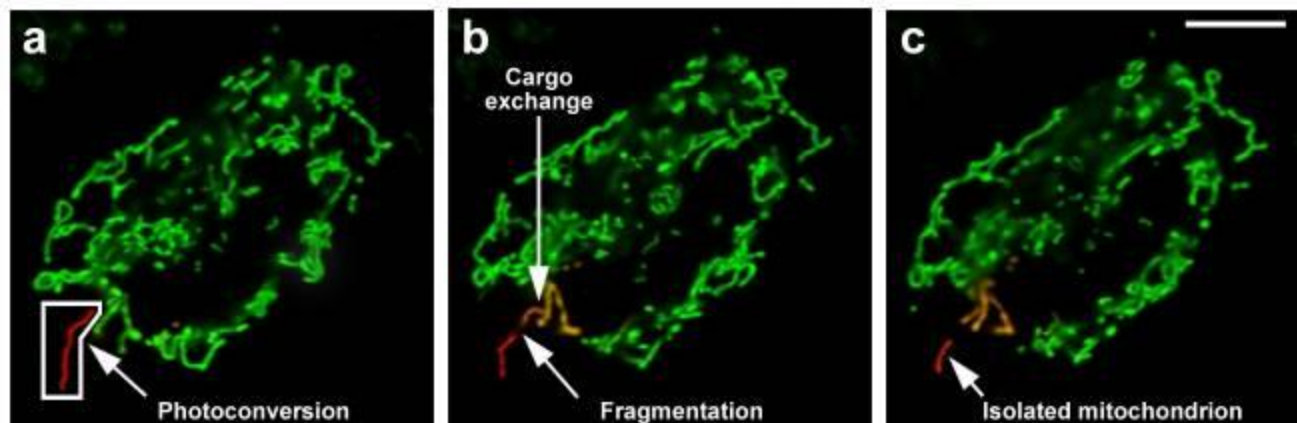


Recommended Photoactivatable Proteins

- 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

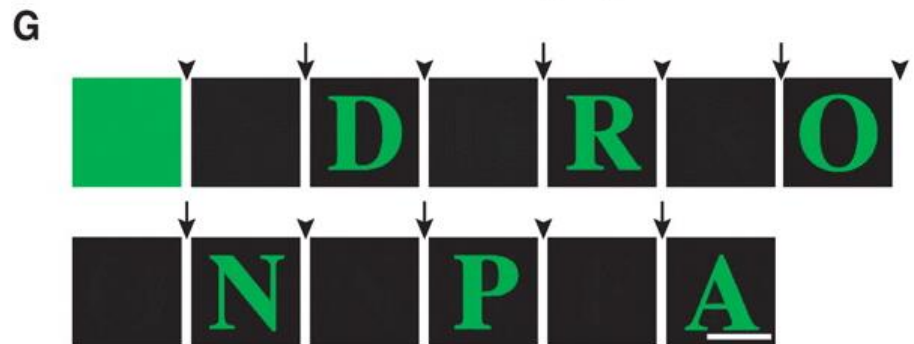
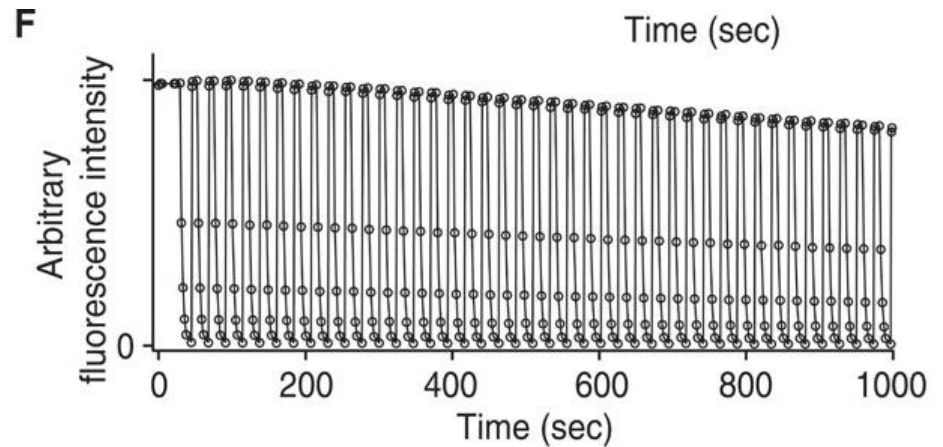
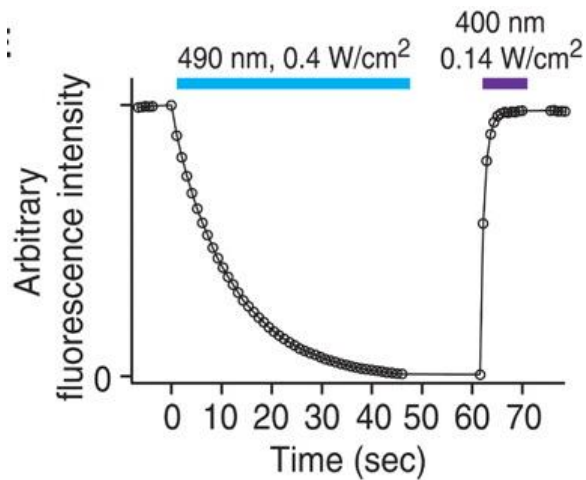
Photoconversion

- Generally: Green $\xrightarrow{405\text{ nm}}$ Red
- Best monomer is probably mEos2; multiple tetrameric proteins available
- mEos2 (green): 506 ex / 519 em Brightness 47
- mEos2 (red): 573 ex / 584 em Brightness 30 Contrast 2000?

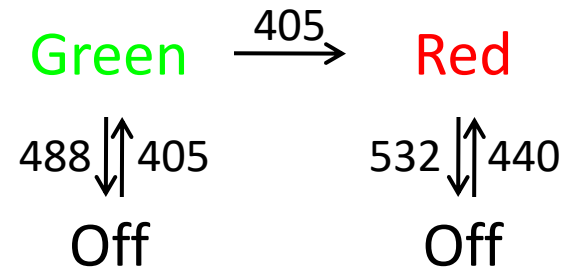


Photoswitchable – Dronpa

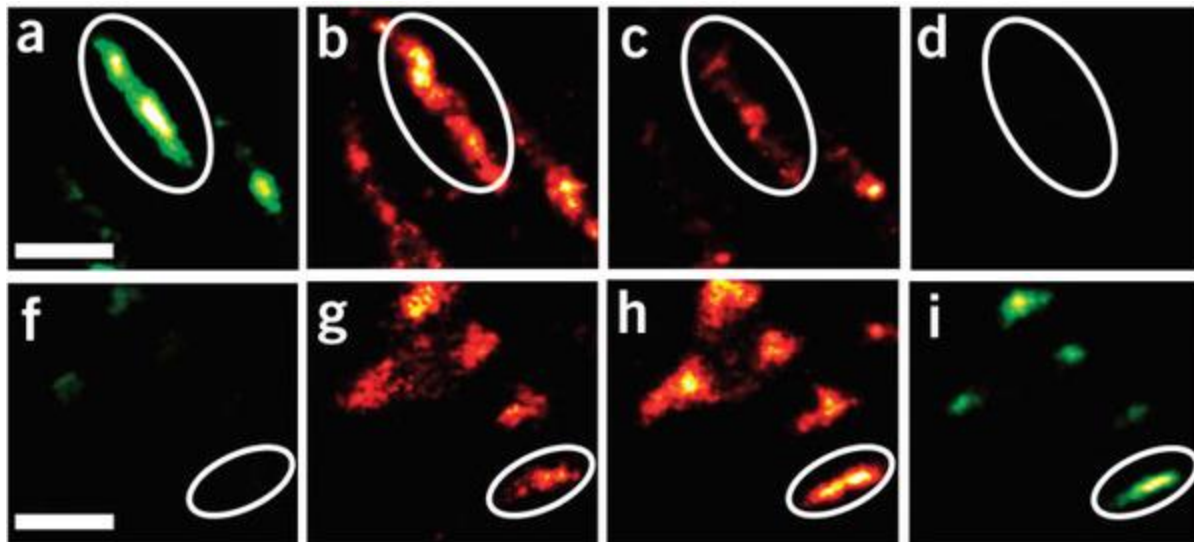
Generally: Off $\xrightleftharpoons[\lambda_{\text{ex}}]{405 \text{ nm}}$ On



mIrisFP – Photoswitchable and Photoconvertible



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

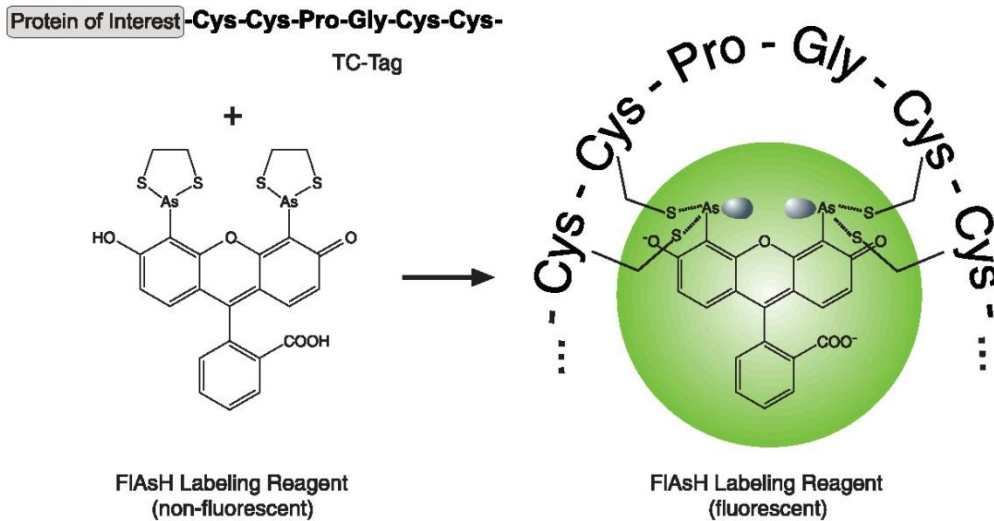


Fluorescent proteins – pros / cons

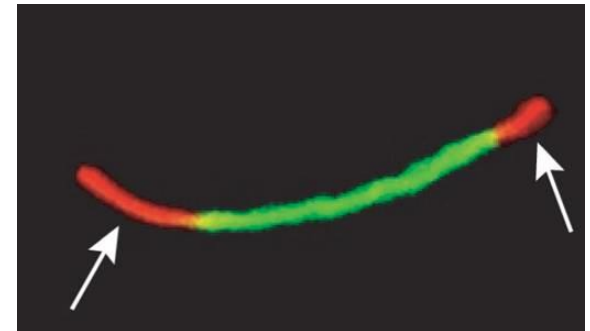
- Can be easily introduced into live cells
 - Minimally perturbative
 - Photoactivatable/photoconvertible versions exist
 - Avoids fixing / staining
-
- Require genetically tractable system
 - Folding and maturation can be slow
 - Some are pH and Cl^- sensitive
 - Some have very complicated photophysics (strange photoactivation / photobleaching behavior)

FIAsH/ReAsH

Labeling protein with
tetra-cysteine motifs:



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

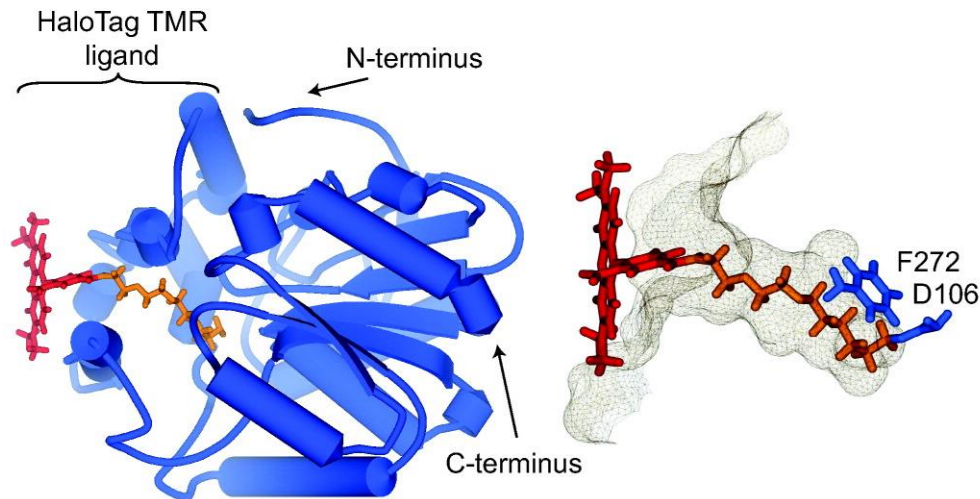


Newly synthesised connexins
(ReAsH:Red) are added to the outer
edges of existing gap junctions
(FIAsH:Green).

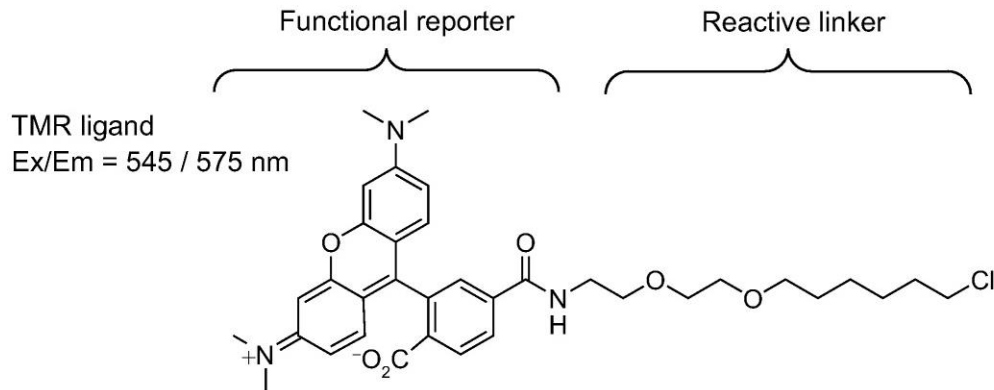
Gaietta et al 2002

Covalent attachment of dyes to genetic tags

HaloTag (Promega)

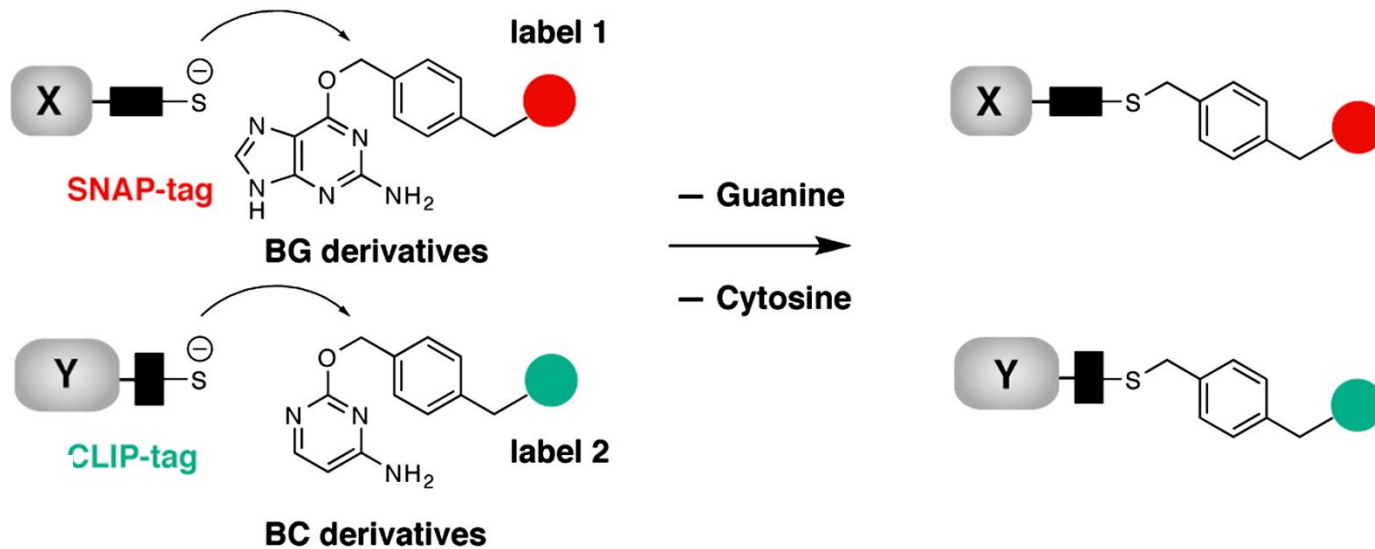


Modified haloalkane
dehalogenase
~31 kDa



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:

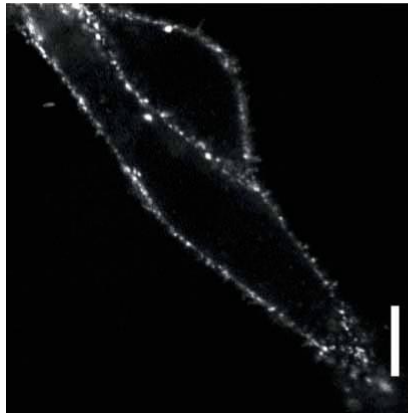
- Same protein can be labeled with almost anything you want
 - Easy to couple ligands to any NHS or maleimide compound
 - Many dye/biotin ligands commercially available
- Label same protein in different colours (avoid recloning)
- Label different compartments
 - Cell-permeable and impermeable probes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

Dye-binding antibodies

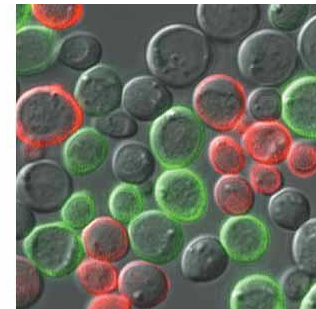
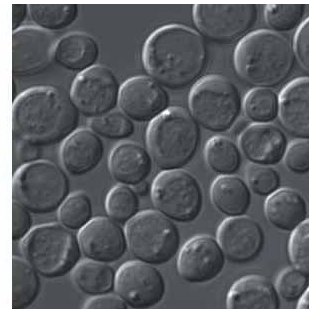
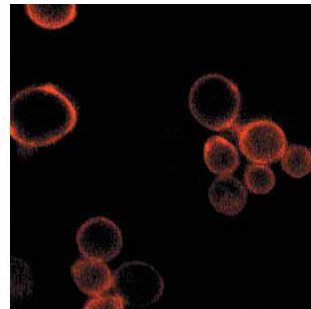
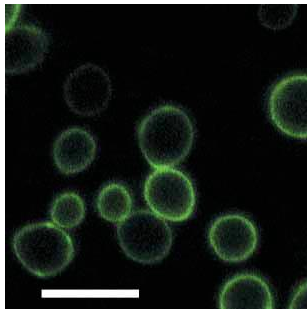
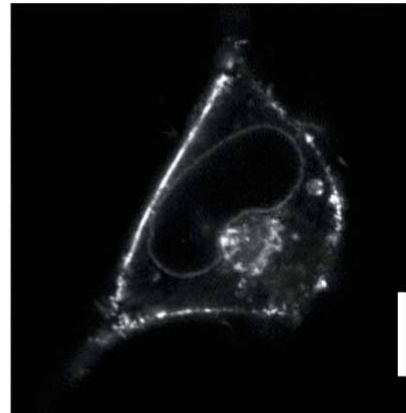
- Single chain antibodies (scFv) that bind dye molecules
- Dyes are only fluorescent in their bound state
 - 10,000-fold activation possible
- Different scFvs bind different dye families
 - Can do multicolor labeling
- But multiple color dyes can bind same scFv
- 14 – 26 kDa
- Commercially available from Spectragenetics.com

Dye binding antibodies

Membrane
impermeant dye

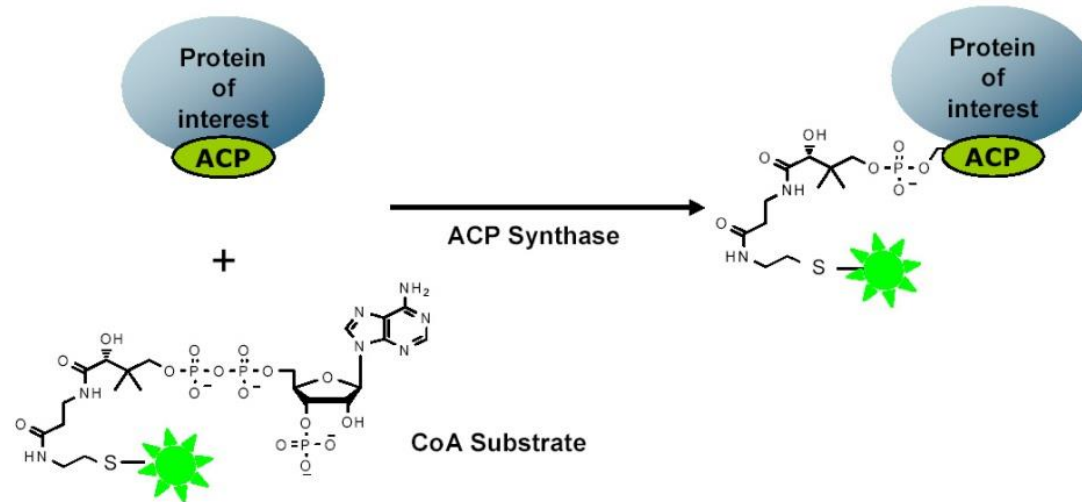


Membrane
permeable dye



Enzyme catalyzed labeling

ACP/SFP synthase (NEB)

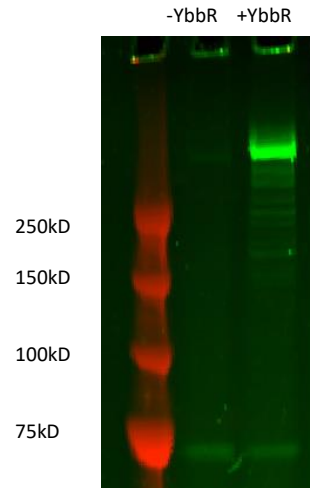


Genetic Tags:

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

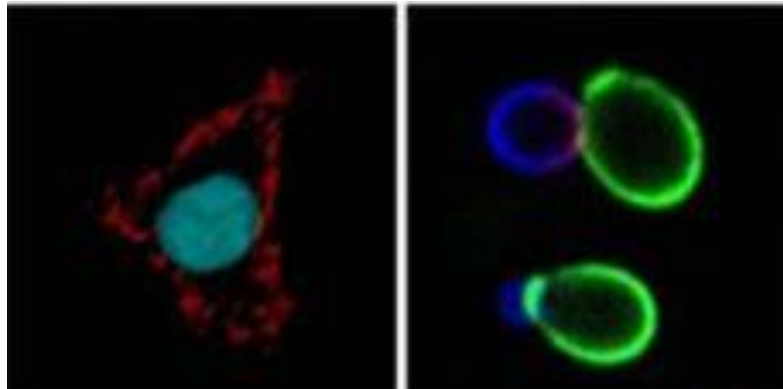
Uses of enzyme catalyzed labeling

1) Site specific labeling of proteins with short peptides



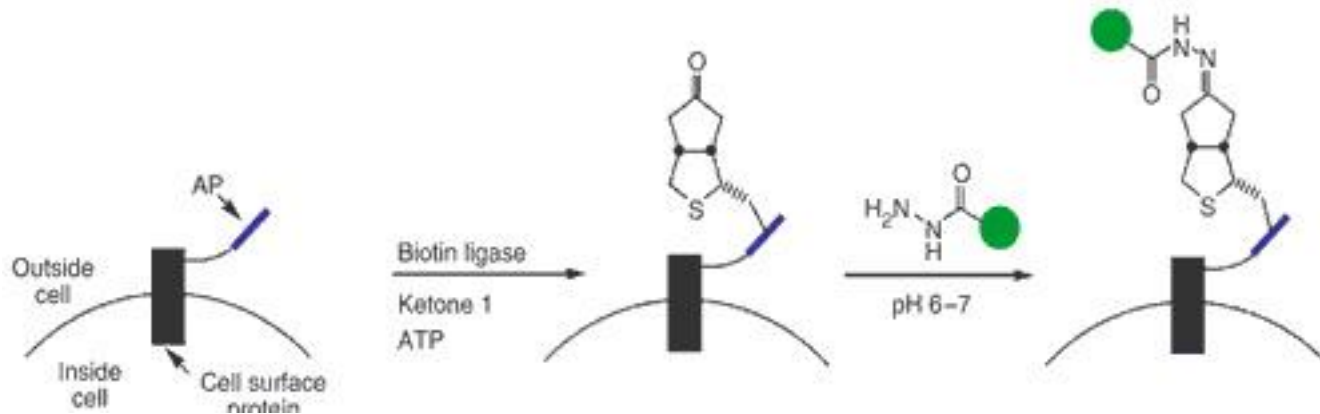
Artificial dimerised dynein with and without YbbR peptide labelled with SFP synthase and 547-CoA (Vale lab)

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