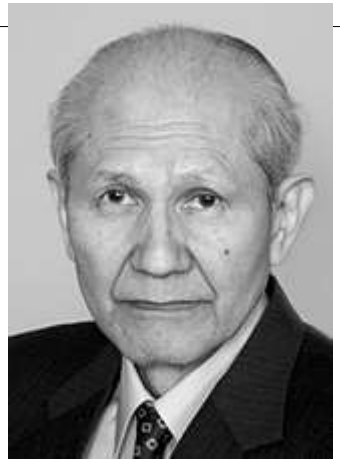
Fluorescent proteins

Bo Huang

2014.07.31



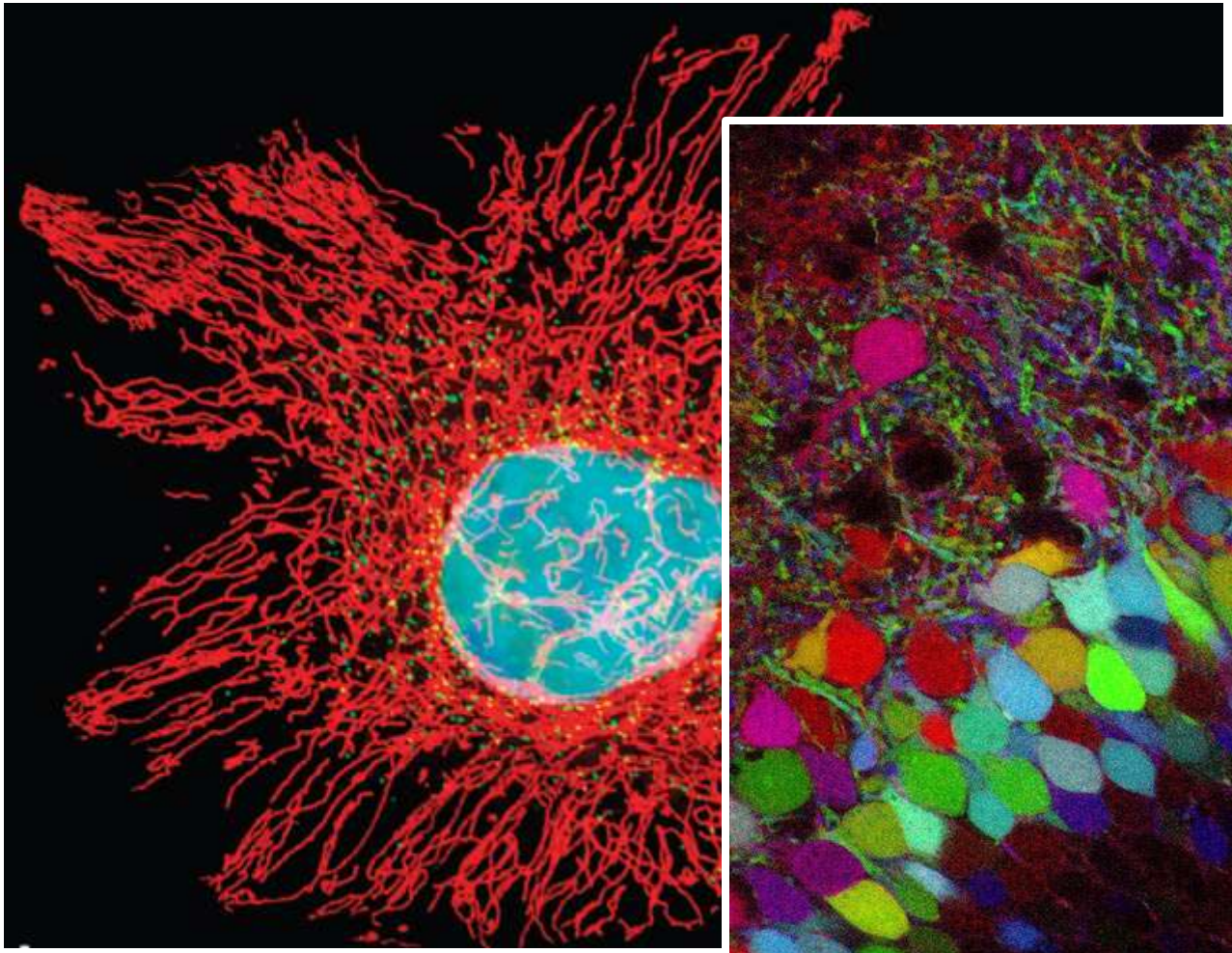
Osamu Shimomura

Martin Chalfie

Roger Y. Tsien

"for the discovery and development of the green fluorescent protein, GFP"

Fluorescent marker for cellular structures



Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

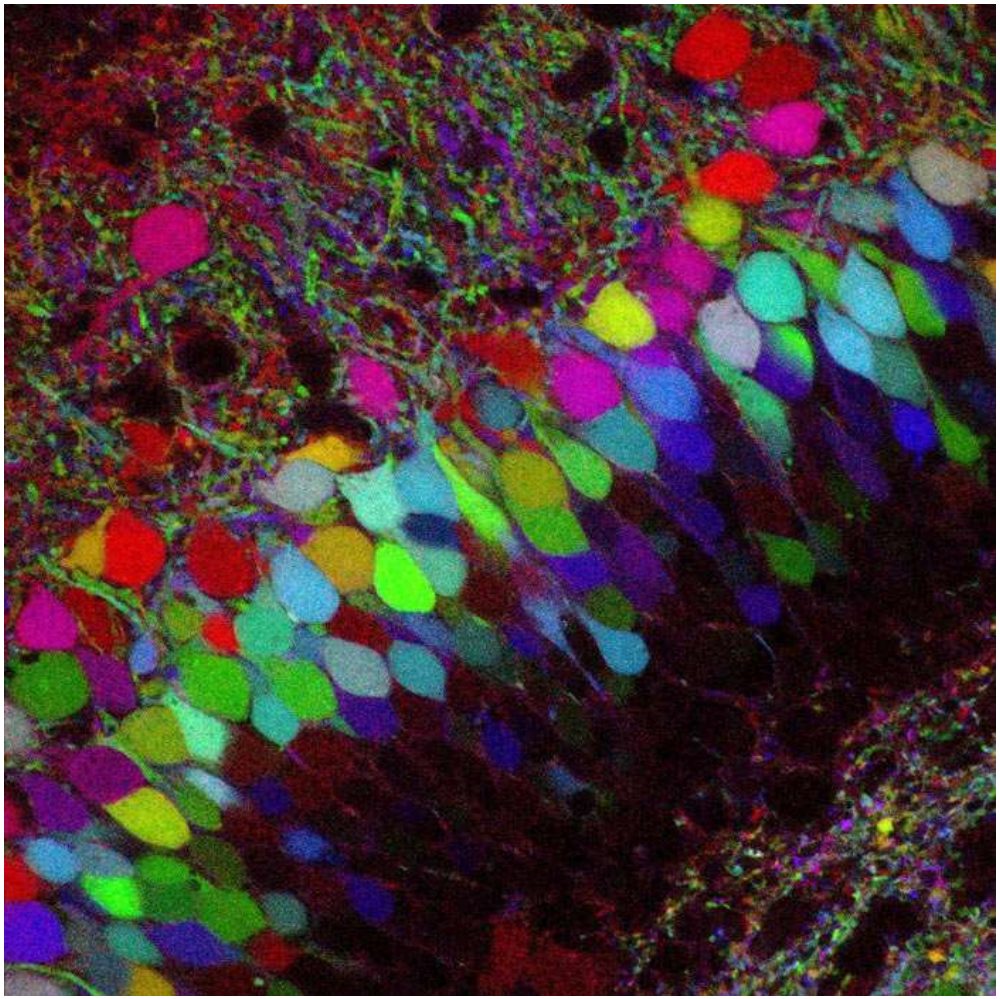
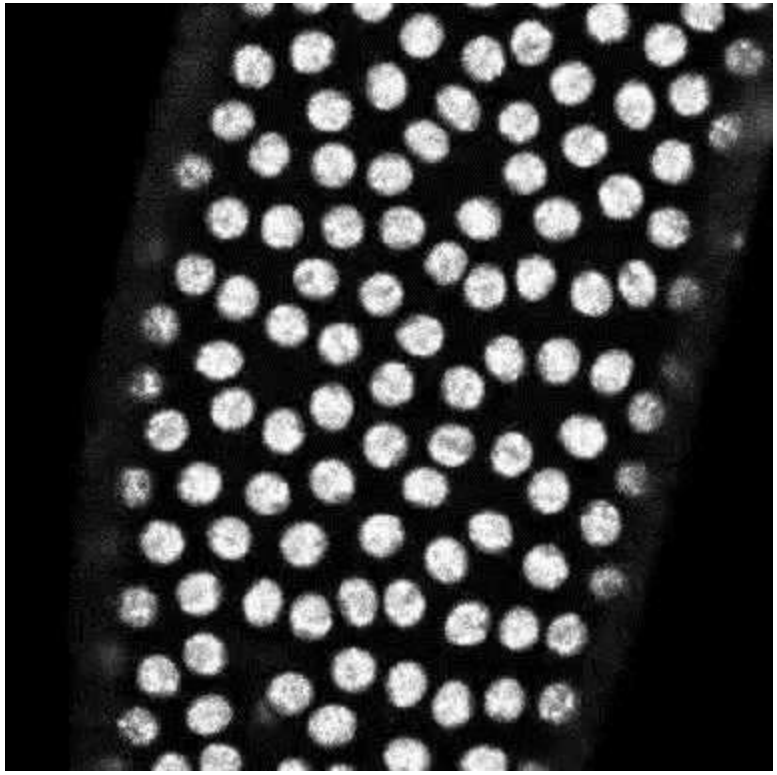
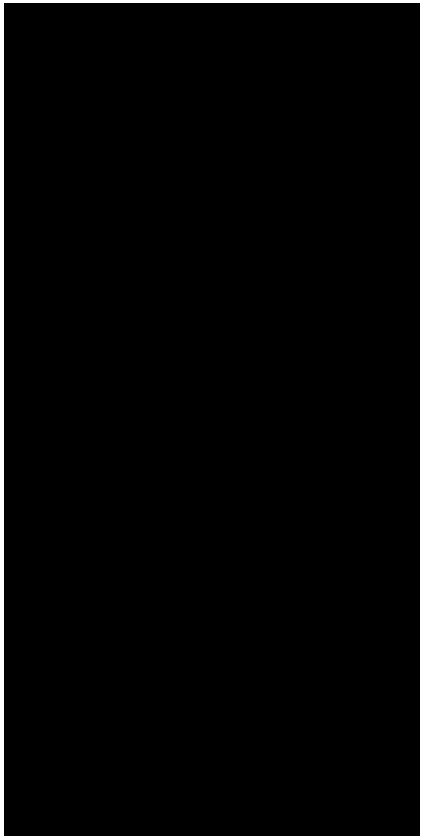


Image: Lichtman Lab

GFP-based live microscopy

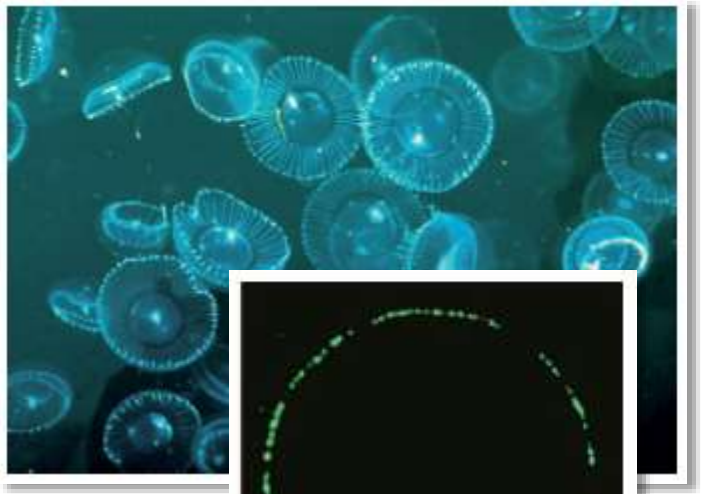


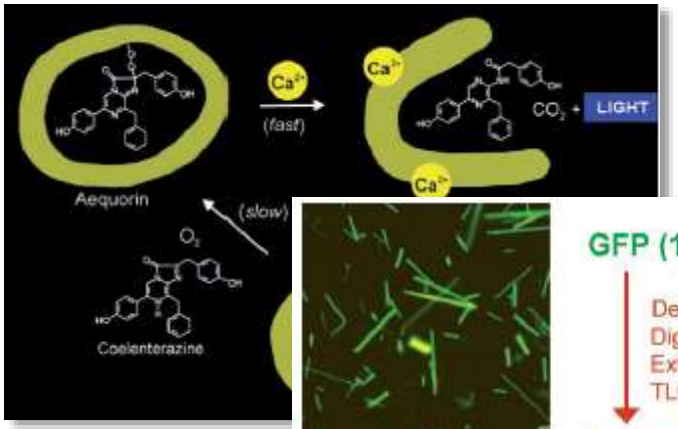
Ding et al., *Develop. Biol.* 2009(334):253
Chromator is required for proper microtubule spindle formation and mitosis in Drosophila



Kaech, Brinkhaus, and Matus, *Neurobiol.* 1999(96):10433
Volatile anesthetics block actin-based motility in dendritic spines

The discovery of GFP





GFP (100 mg)

Denature at 90 °C
Digest with papain
Extraction with butanol at pH 1
TLC purification

Isolated chromophore
(0.1 mg)

Images from Osamu Shimomura

Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

77 A.D.
Pliny the Elder writes of glowing jellyfish

900 million years ago
Cnidari and Bilateria families diverge

1962
Osamu Shimomura [illegible] autofluorescent pr[illegible]

1985
[illegible]sher clones Aequorin

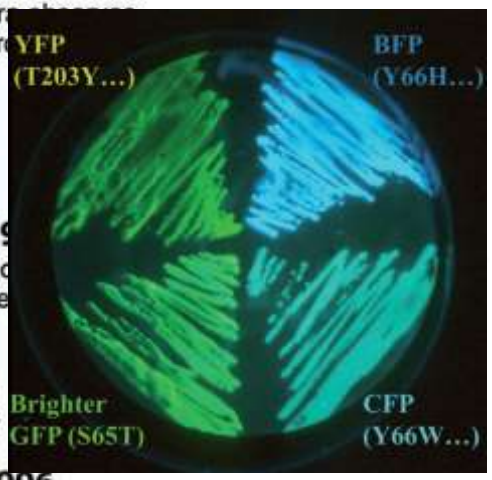
1992
[illegible]rasher clones AvGFP

AvGFP

1994
[illegible]FP
[illegible]ms

1996
[illegible]mington,
[illegible]-workers
[illegible]structure

GFP S65T | **BFP** Y66H | **CFP** Y66W



1996
YFP (T203Y) developed & characterized by Jim Remington, Roger Tsien, Rebekka Wachter & co-workers

1997
Jörg Wiedenmann proposes the existence of GFP-like proteins in sea anemones

2001
Citrine developed by Oliver Griesbeck, Robert Campbell, Roger Tsien & co-workers

1999
Mikhail Matz, Sergey Lukyanov & co-workers clone first RFP (DsRED)

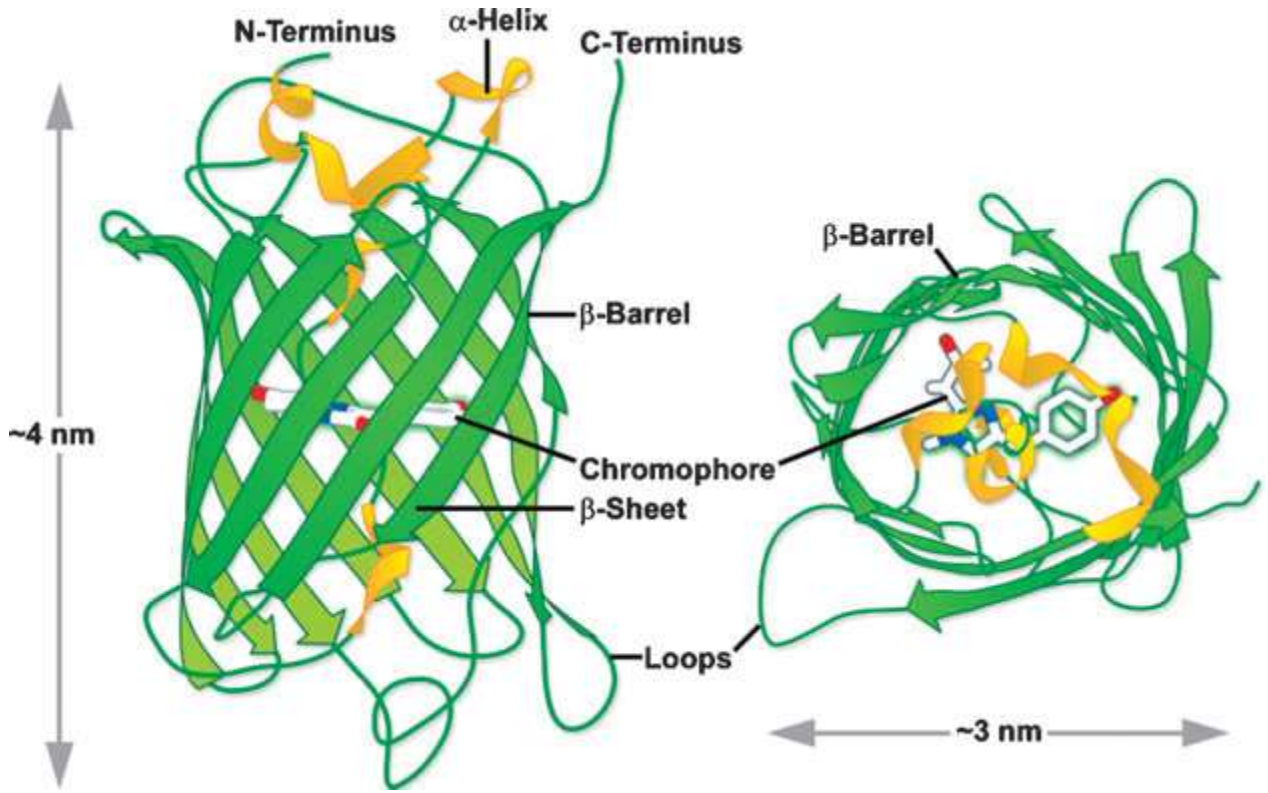
2002
DsRED.T1 developed by Benjamin Glick & co-workers

2002
PA-GFP Developed by Jennifer Lippincott-Schwartz, & George Patterson

2002
mRFP1 developed by David Zacharias, Amy Palmer, Robert Campbell, Paul Steinbach, Roger Tsien & co-workers

2002
Venus, Kaede developed by Takeharu Nagai, Ryoko Ando, Atsushi Miyawaki & co-workers

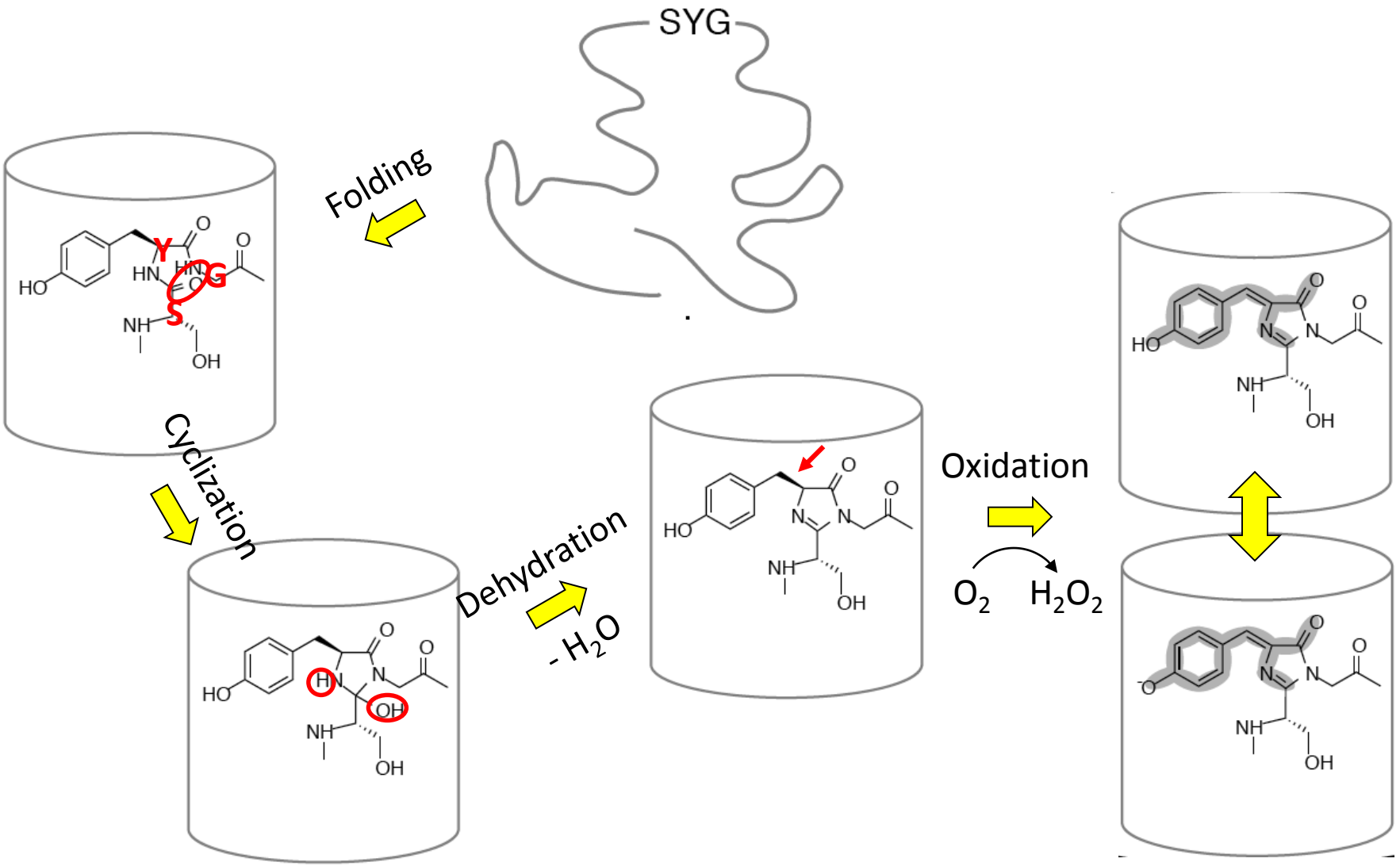
The green fluorescent protein



Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

~240 Amino acids, 27 kD

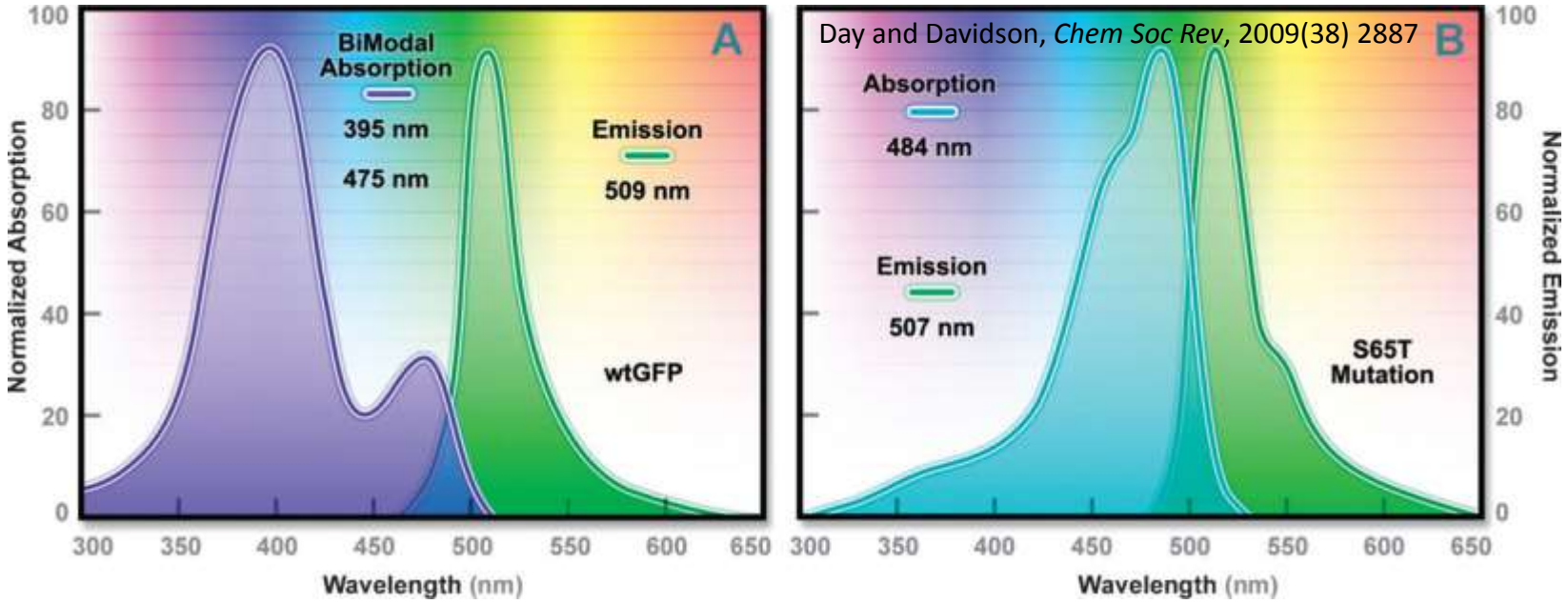
GFP chromophore formation



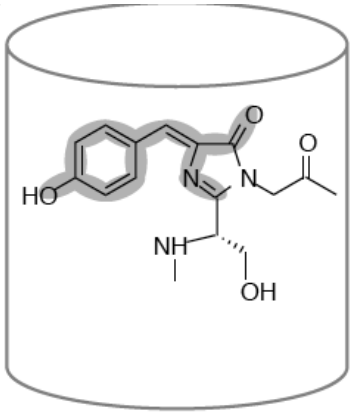
Improving the wild type GFP

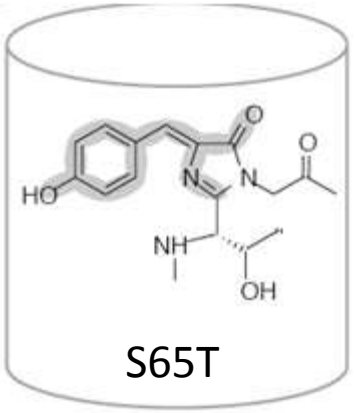
AvGFP

“GFP”



Day and Davidson, *Chem Soc Rev*, 2009(38) 2887





Jellyfish (*Aequorea*) fluorescent protein family

AvGFP

↓

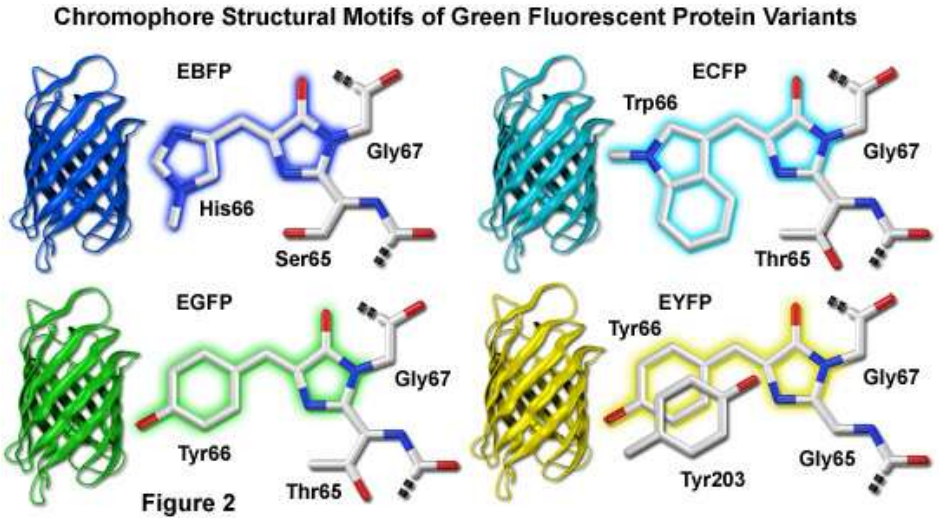
GFP (S65T), EGFP (S65T, F64L)

↓

BFP, CFP, YFP

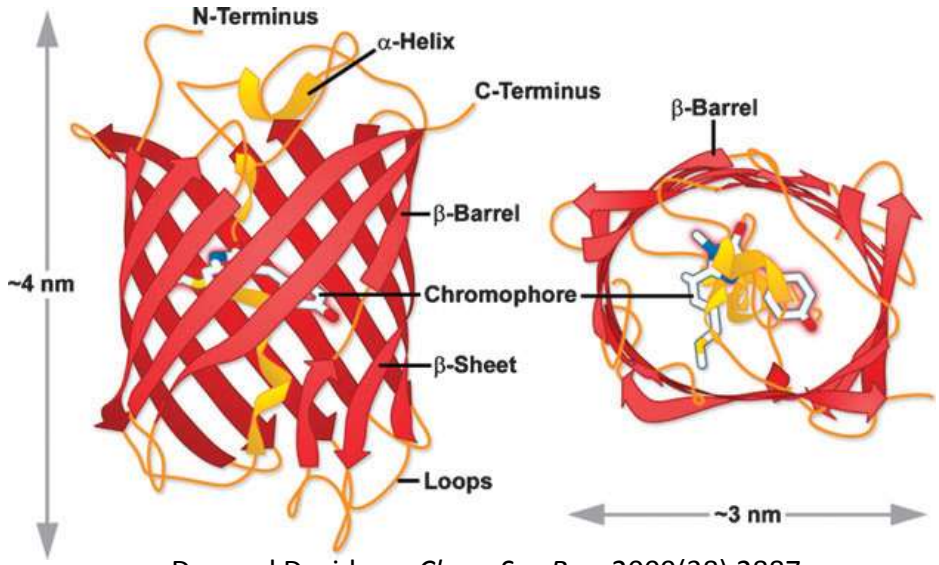
↓

Cerulean , CyPet, Sapphire, Venus, Citrine , Ypet…



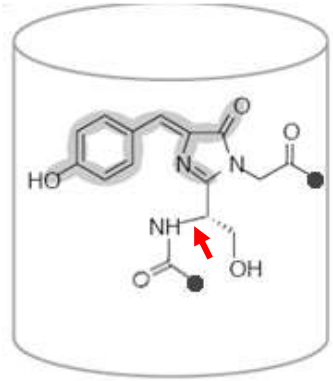

Seeking for red colors...

Red FPs from Anthozoa

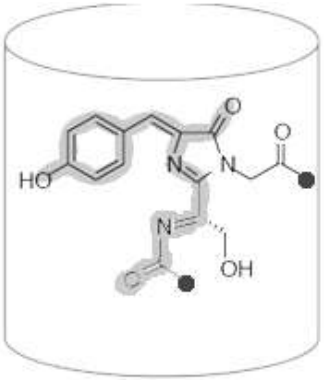


Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

dsRed
↓
mRFP
↓
mOrange, mCherry…



Second oxidation



The fluorescent protein pallet

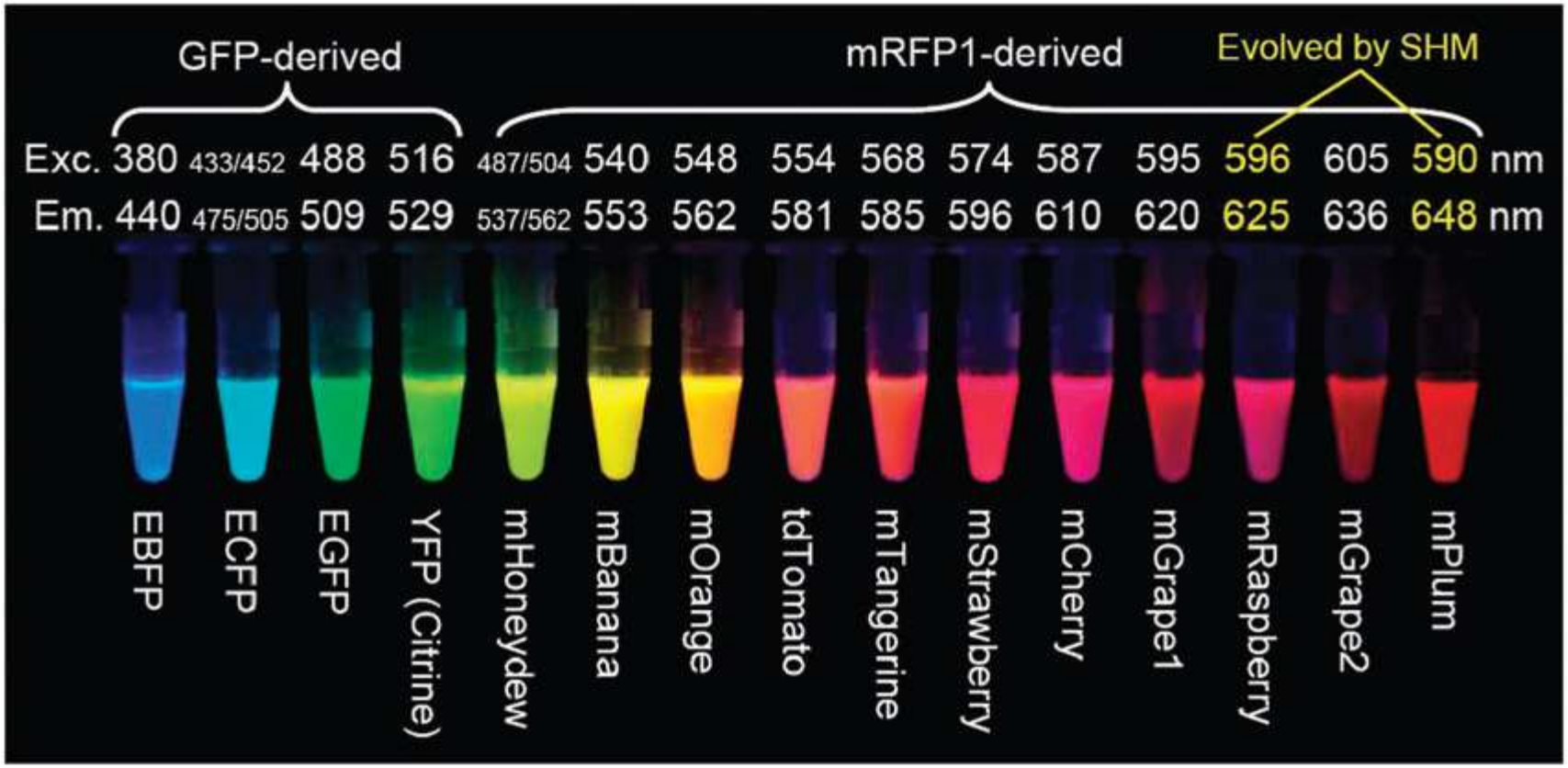


Image from Tsien lab

The fluorescent protein pallet

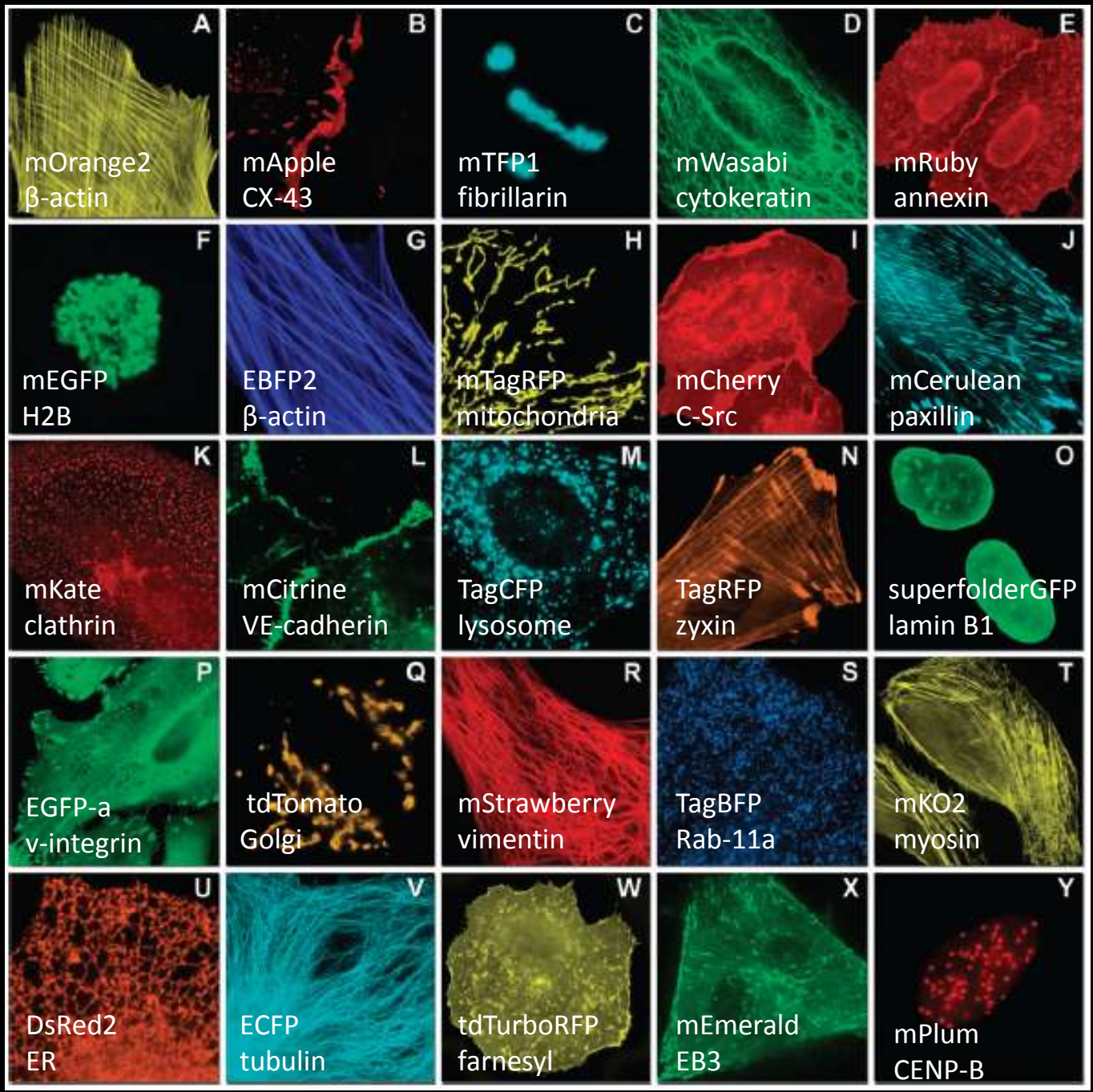
*N.C. Shaner, P.A. Steinbach, & R.Y. Tsien, Nature Methods **2**:905 (2005)*

Wavelength Class	Protein	Source Lab	Organism	Ex (nm)	Em (nm)	Extinction coefficient per chain, $M^{-1}cm^{-1}$	Fluorescence quantum yield	Brightness (EC*QY) (mM*cm)^-1	Brightness of fully mature protein (% of fluorescein)	$t_{0.5}$ for bleach, sec	photostability (fold improvement over fluorescein)	pKa	$t_{0.5}$ for maturation at 37°C	Oligomerization	References
Far-red	mPlum	Tsien	*Discosoma sp.*	590	649	41,000	0.10	4.1	5.9	53	7.3	<4.5	100 min	monomer	5
Red	mCherry	Tsien	*Discosoma sp.*	587	610	72,000	0.22	16	23	96	13.1	<4.5	15 min	monomer	4
	tdTomato	Tsien	*Discosoma sp.*	554	581	138,000	0.69	95	138	98	13.5	4.7	1 hr	tandem dimer	4
	mStrawberry	Tsien	*Discosoma sp.*	574	596	90,000	0.29	26	38	15	2.1	<4.5	50 min	monomer	4
	J-Red	Evrogen	Unidentified Anthomedusa	584	610	44,000	0.20	8.8	13	13	1.8	5	ND	*dimer*	*x*
	DsRed-Monomer	Clontech	*Discosoma sp.*	556	586	35,000	0.10	3.5	5.1	16	2.2	4.5	ND	*monomer*	*y*
Orange	mOrange	Tsien	*Discosoma sp.*	548	562	71,000	0.69	49	71	9.0	1.2	6.5	4.5 hr	monomer	4
	mKO	MBL Intl.	*Fungia concinna*	548	559	51,600	0.60	31	45	122	16.7	5	2.5 hr	monomer	10
Yellow	mCitrine	Tsien	*Aequorea victoria*	516	529	77,000	0.76	59	85	49	6.7	5.7	ND	monomer	16, 23
	Venus	Miyawaki	*Aequorea victoria*	515	528	92,200	0.57	53	76	15	2.0	6	ND	weak dimer	1
	YPet	Daugherty	*Aequorea victoria*	517	530	104,000	0.77	80	116	49	6.7	5.6	ND	weak dimer	2
	EYFP	Invitrogen	*Aequorea victoria*	514	527	83,400	0.61	51	74	60	8.3	6.9	ND	weak dimer	18
Green	Emerald	Invitrogen	*Aequorea victoria*	487	509	57,500	0.68	39	57	0.69	0.1	6	ND	weak dimer	18
	EGFP	Clontech*	*Aequorea victoria*	488	507	56,000	0.60	34	49	174	23.9	6	ND	weak dimer	*y*
Cyan	CyPet	Daugherty	*Aequorea victoria*	435	477	35,000	0.51	18	26	59	8.1	5	ND	weak dimer	2
	mCFP	Tsien	*Aequorea victoria*	433	475	32,500	0.40	13	19	64	8.8	4.7	ND	monomer	23
	Cerulean	Piston	*Aequorea victoria*	433	475	43,000	0.62	27	39	36	5.0	4.7	ND	weak dimer	3
UV-excitable green	T-Sapphire	Griesbeck	*Aequorea victoria*	399	511	44,000	0.60	26	38	25	3.5	4.9	ND	weak dimer	6
Reference	fluorescein pH 8.4			495	519	75,000	0.92	69	100	7.3	1.0	6.4			

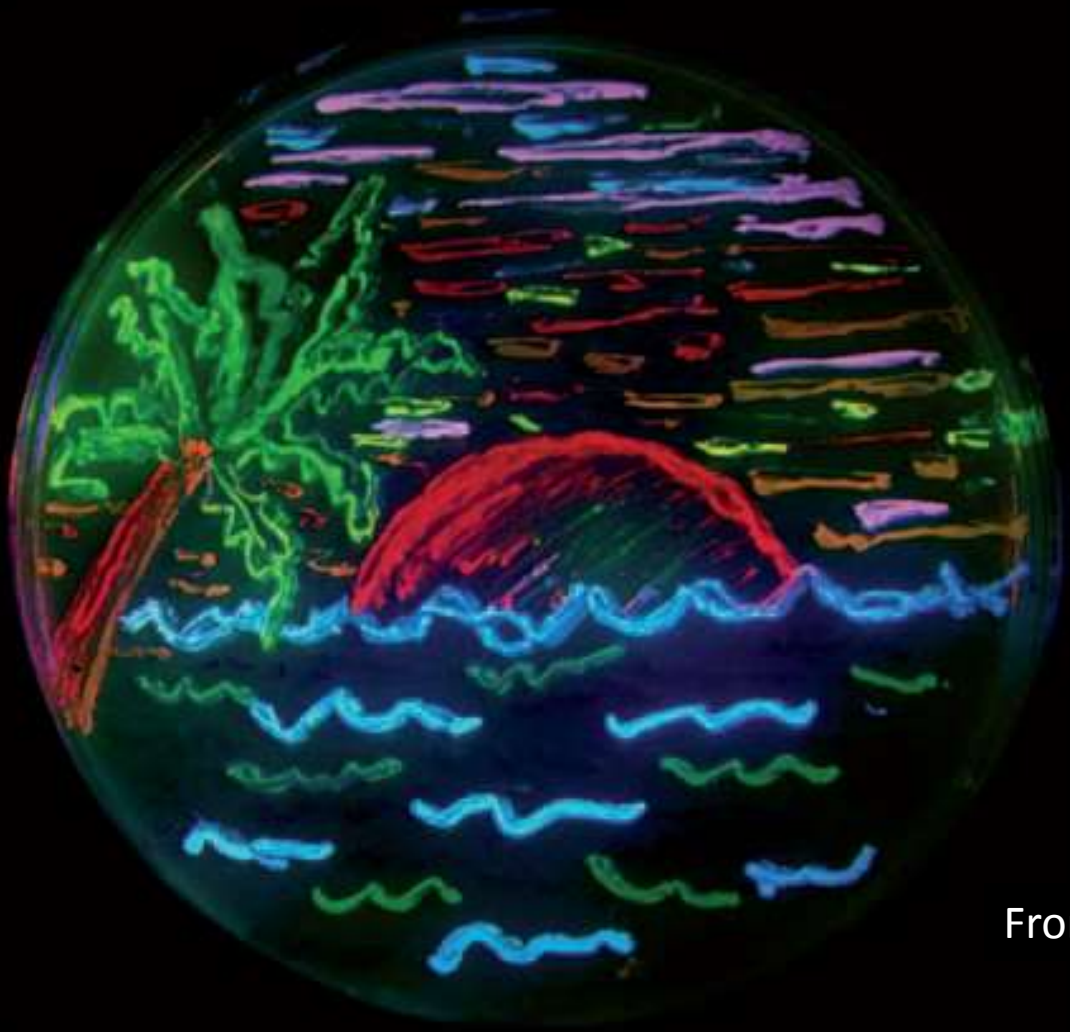
* No longer commercially available
x www.evrogen.com
y www.clontech.com
ND = not determined

*Other, **less** recommended FP:s*

Protein	Source	Comments
AceGFP	Evrogen	no clear advantage over well-validated Aequorea GFPs
AcGFP1	Clontech	no clear advantage over well-validated Aequorea GFPs
AmCyan1	Clontech	tetrameric
AQ143	Lukyanov	tetrameric
AsRed2	Clontech	tetrameric
Azami-Green/mAG	MBL Intl.	no clear advantage over well-validated Aequorea GFPs
cOFP	Stratagene	tetrameric
CopGFP	Evrogen	no clear advantage over well-validated Aequorea GFPs
dimer2, tdimer2(12)	Tsien	slower maturation than dTomato/tdTomato
DsRed/DsRed2/DsRed-Express	Clontech	tetrameric
EBFP	Clontech	Fast bleaching, dim, no longer commercially available
eqFP611	Weidenmann	poor folding at 37C, tetrameric
HcRed1	Clontech	dimeric, dim
HcRed-tandem	Evrogen	fast bleaching, dim
Kaede	MBL Intl.	dimmer and less efficient at photoconversion than KikGR
mBanana	Tsien	dim, fast photobleaching
mHoneydew	Tsien	dim, fast photobleaching
MiCy	MBL Intl.	dimeric, less spectral separation from YFPs than Aequorea GFP-derived CFPs
mRaspberry	Tsien	faster bleaching than mPlum
mRFP1	Tsien	dimmer and less photostable than mCherry
mTangerine	Tsien	fast bleaching, dimmer than mStrawberry
mYFP	Tsien	Chloride sensitivity
PhiYFP	Evrogen	suspected aggregation, faster bleaching than other YFPs, potential problems with fusion constructs
Renilla GFPs	various	dimeric, no clear advantages over well-validated Aequorea GFPs
TurboGFP	Evrogen	no clear advantage over well-validated Aequorea GFPs
ZsYellow1	Clontech	tetrameric

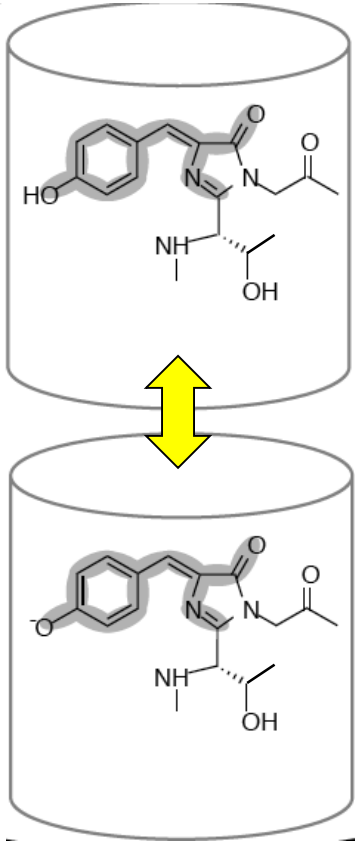

A
mOrange2
β-actin
B
mApple
CX-43
C
mTFP1
fibrillarin
D
mWasabi
cytokeratin
E
mRuby
annexin
F
mEGFP
H2B
G
EBFP2
β-actin
H
mTagRFP
mitochondria
I
mCherry
C-Src
J
mCerulean
paxillin
K
mKate
clathrin
L
mCitrine
VE-cadherin
M
TagCFP
lysosome
N
TagRFP
zyxin
O
superfolderGFP
lamin B1
P
EGFP-a
v-integrin
Q
tdTomato
Golgi
R
mStrawberry
vimentin
S
TagBFP
Rab-11a
T
mKO2
myosin
U
DsRed2
ER
V
ECFP
tubulin
W
tdTurboRFP
farnesyl
X
mEmerald
EB3
Y
mPlum
CENP-B

Even more fun with GFP?



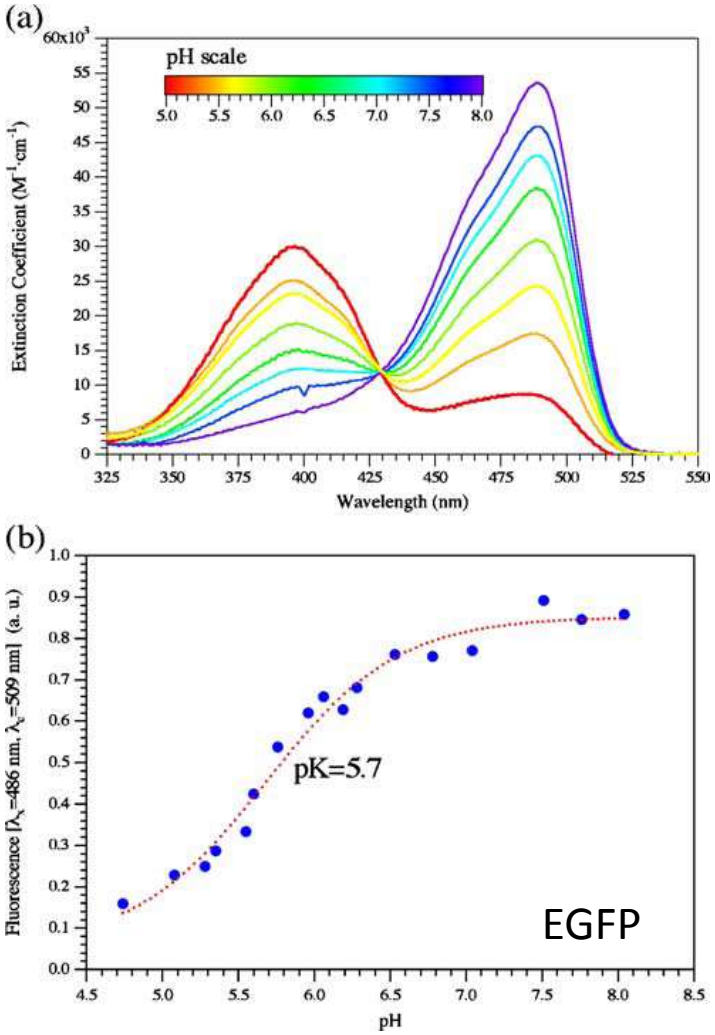
From Tsien lab

pH sensitivity of GFP



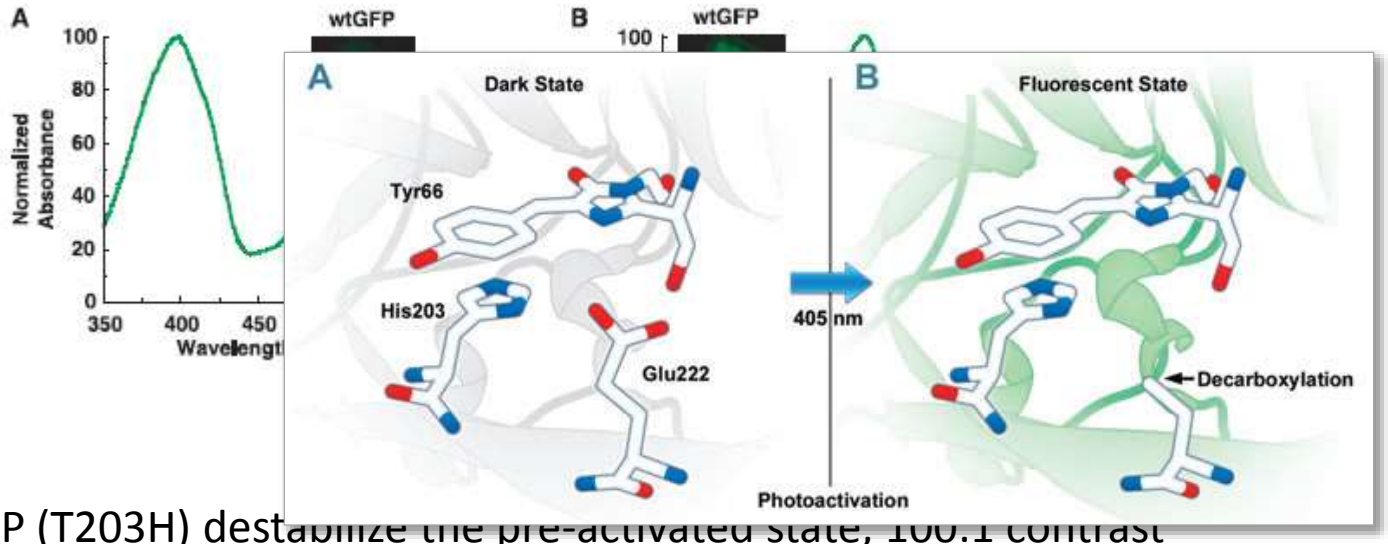
Low pH

High pH



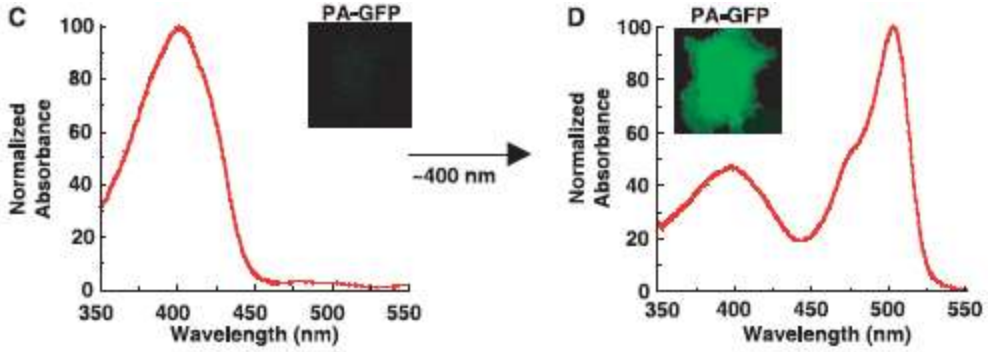
Bizzarri, et al., *Anal Bioanal Chem* (2009) 393:1107

Photoactivatible fluorescent proteins



PA-GFP (T203H) destabilize the pre-activated state, 100:1 contrast

Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

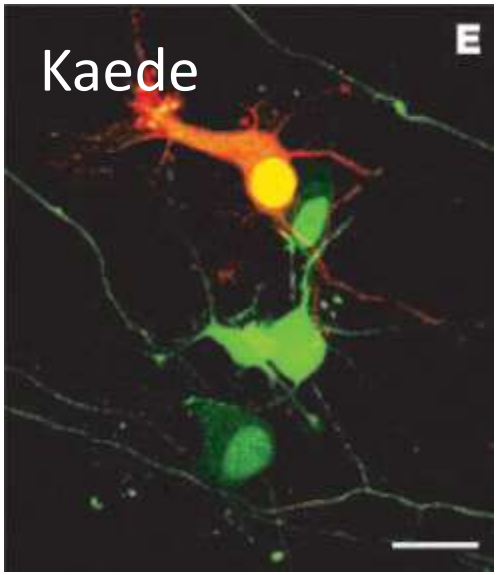


How discovery can be made…

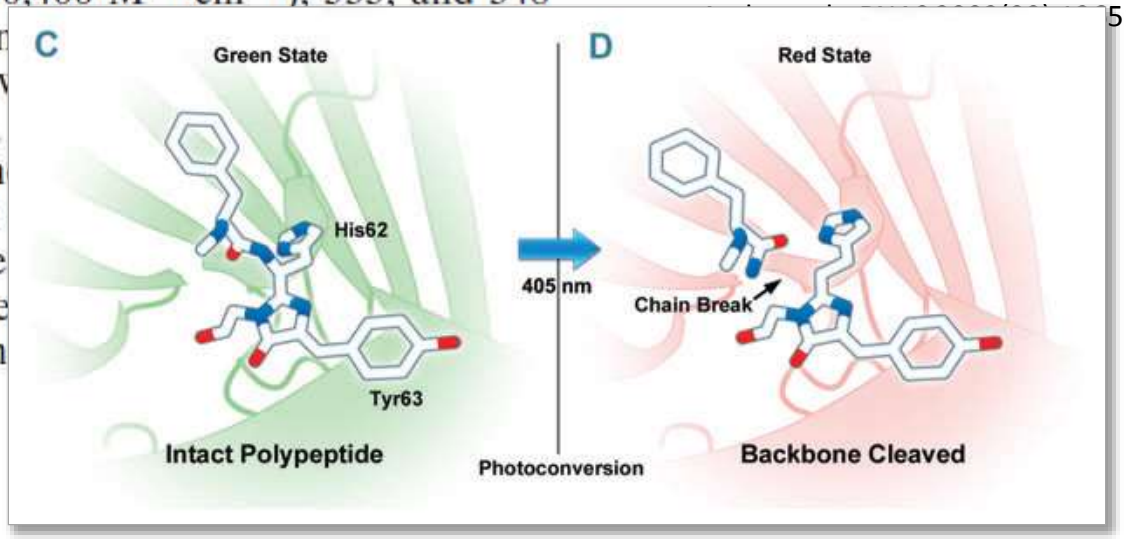
An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein

Ryoko Ando*†, Hiroshi Hama*, Miki Yamamoto-Hino*, Hideaki Mizuno*, and Atsushi Miyawaki*‡

We happened to leave one of the protein aliquots on the laboratory bench overnight. The next day, we found that the protein sample on the bench had turned red, whereas the others that were kept in a paper box remained green. Although the sky had been partly cloudy, the red sample had been exposed to sunlight through the south-facing windows. The absorption spectrum of the red protein was measured at pH 7.4 (Fig. 2*A*, red line). The major absorption peak at 508 nm and minor peak at 380 nm were reduced in size, and new peaks appeared at 572 (ε = 60,400 $M^{-1}\cdot cm^{-1}$), 533, and 348 nm. These peaks reflect the ion
they were augmented equally
of 5.7. When excited at 480 nm,
maximum at 582 nm with a sh
line) in addition to the 518-nm
627 nm were also pH-sensitive
shown), indicating that the re
(Φ = 0.33) is also pH-resistan



51



Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

“Good” PA-FPs

Catagory	Name	Abs	Em
Dark → Green	PA-GFP	400 → 504	517
	PS-CFP2	400 → 490	470 → 511
Dark ⇔ Green	Dronpa	503	518
	rsEGFP, rsEGFP2	491	510
Green → Red	Dendra2	490 → 553	507 → 573
	mEos2, mEos3.2	506 → 573	519 → 584
	mMaple3	489 → 566	505 → 583
Dark → Red	PAmCherry	564	595
Other	Dreiklang	515	529

Nearly 50% of the FPs show reversible photobleaching (reactivation)

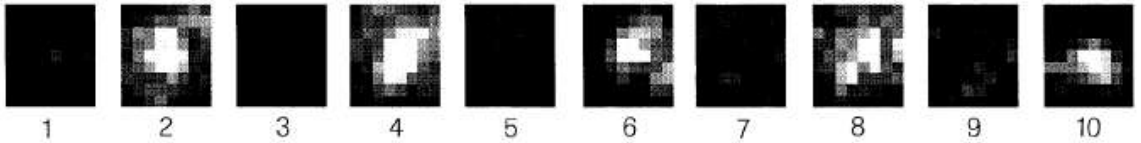


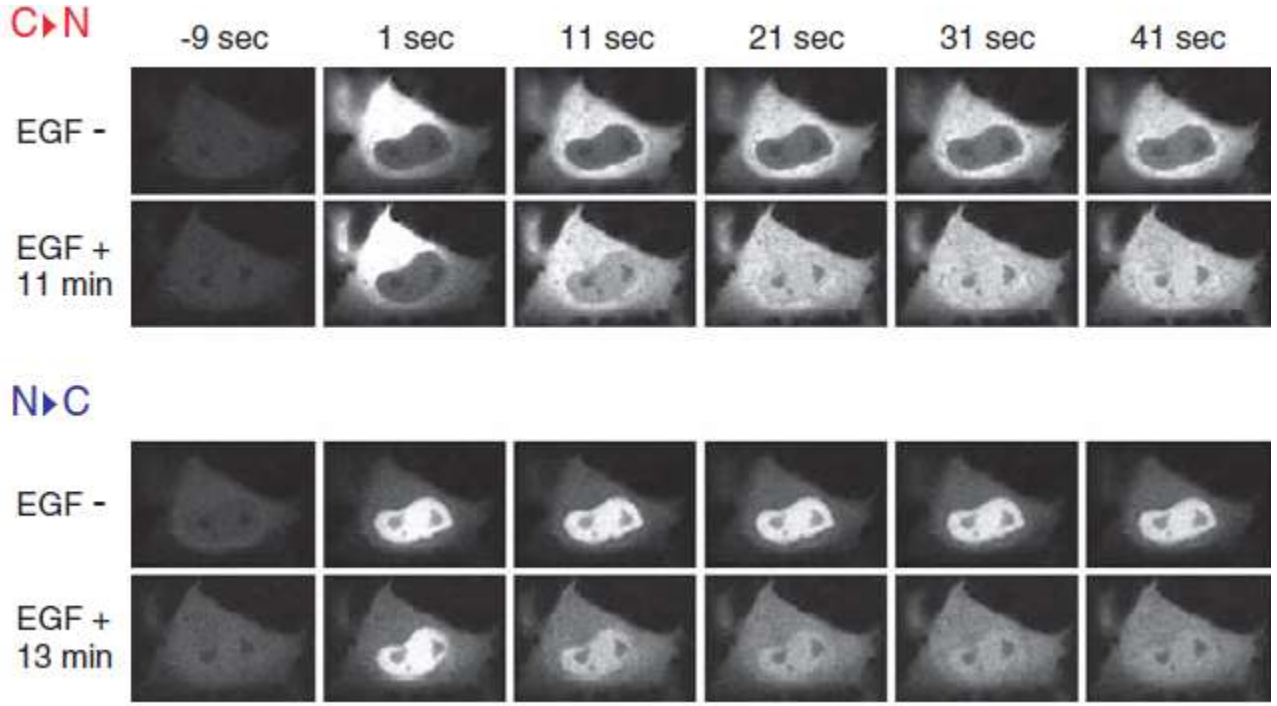
Figure 2 The switching behaviour of a single T203F molecule, illustrated by a series of ten consecutive experiments on the same molecule. Each 100-ms frame

Dickson, Cubitt, Tsien, Moerner, *Nature*, 1997(388):355

Applications of PA-FPs

Visualization of protein trafficking

ERK-Dronpa

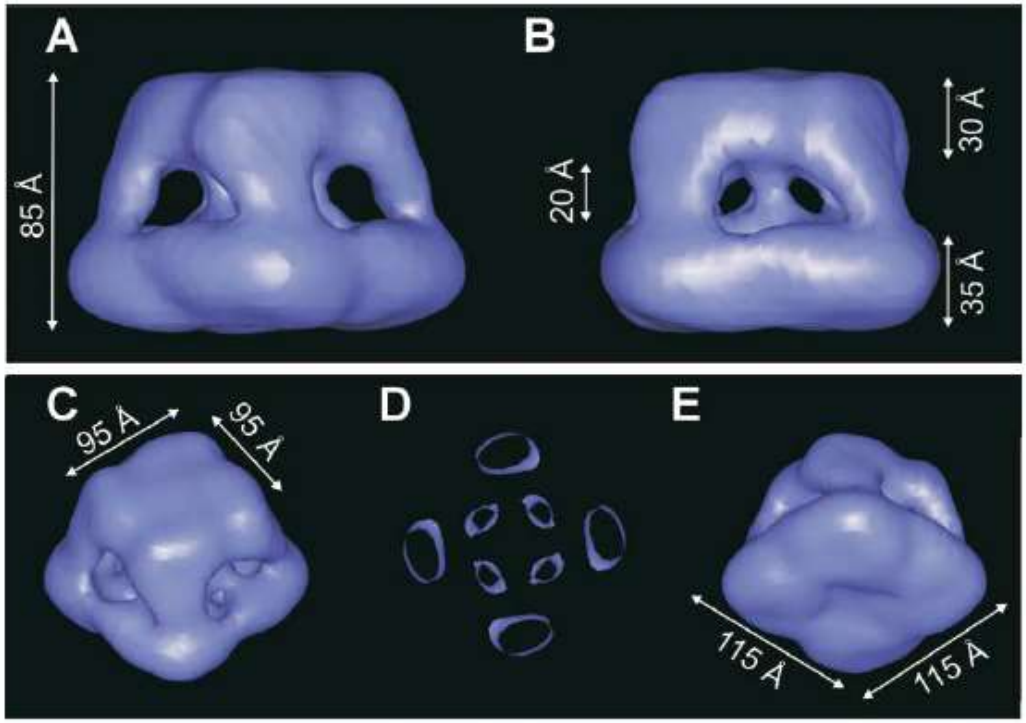


Ando, Mitzuro and Miyawaki, Science 2004(306): 1370

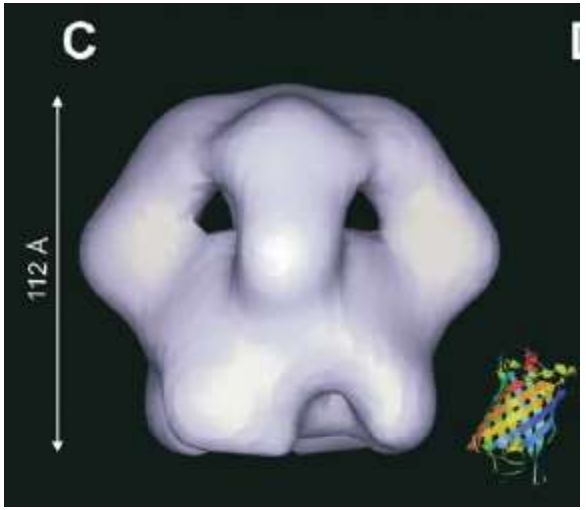
Super-resolution microscopy

Nonfluorescent applications of GFP

Kv4.2 + KChIP2



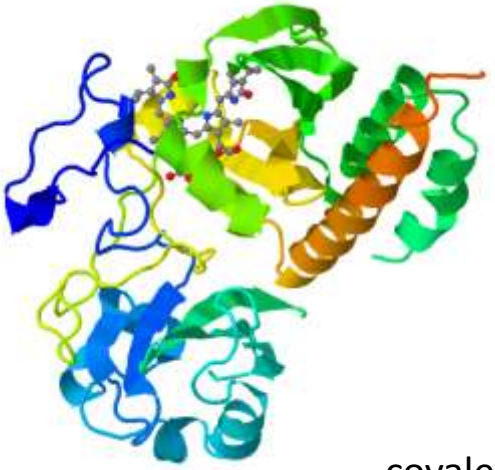
Kv4.2 + GFP-KChIP2



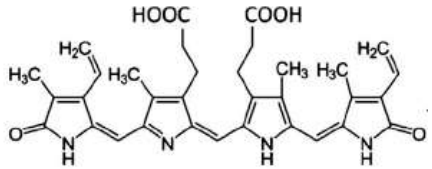
Kim et al., *Neuron*, 2004(41): 513

Beyond GFP

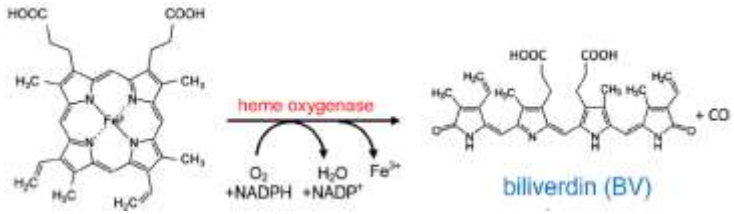
Bacterial phytochrome

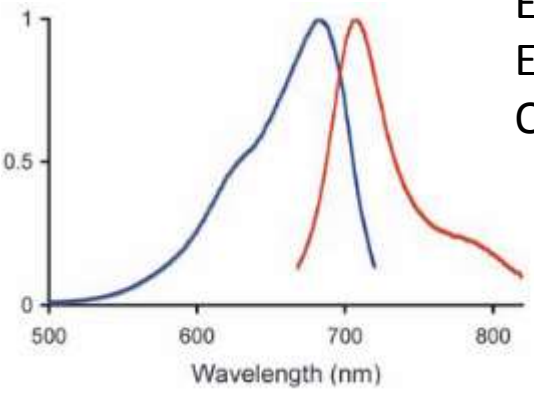


covalent binding



biliverdin (BV)

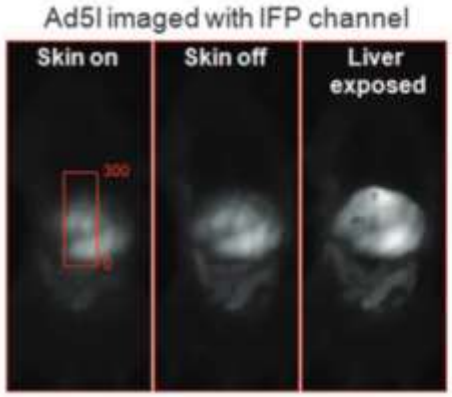


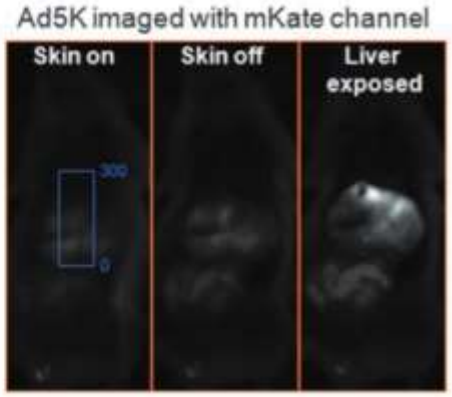


Shu et al., Science 2010

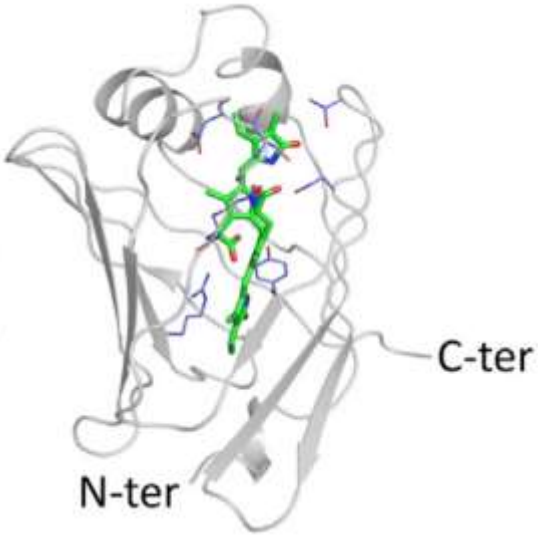
	IFP1.4	Cy5.5
Ab:	684 nm	675 nm
EC:	92,000	230,000
Em:	708 nm	695 nm
QE:	0.07	0.28

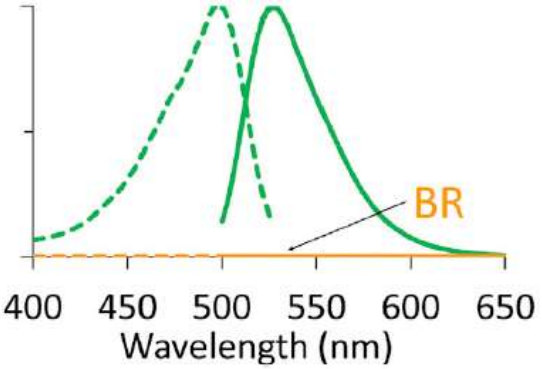
iRFP, IFP2.0, mIFP…

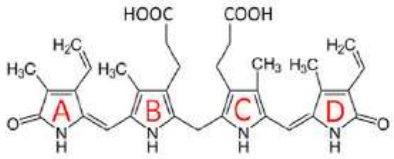




UnaG from Japanese eel (unagi)

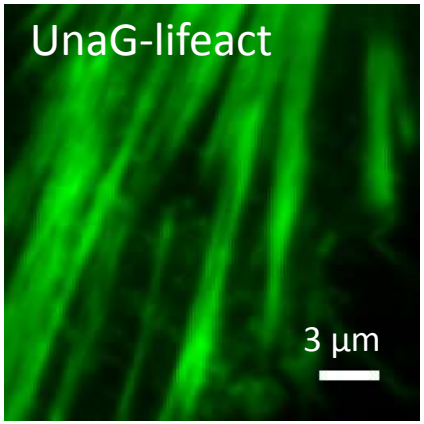






bilirubin (BR)

K_D = 98 pM

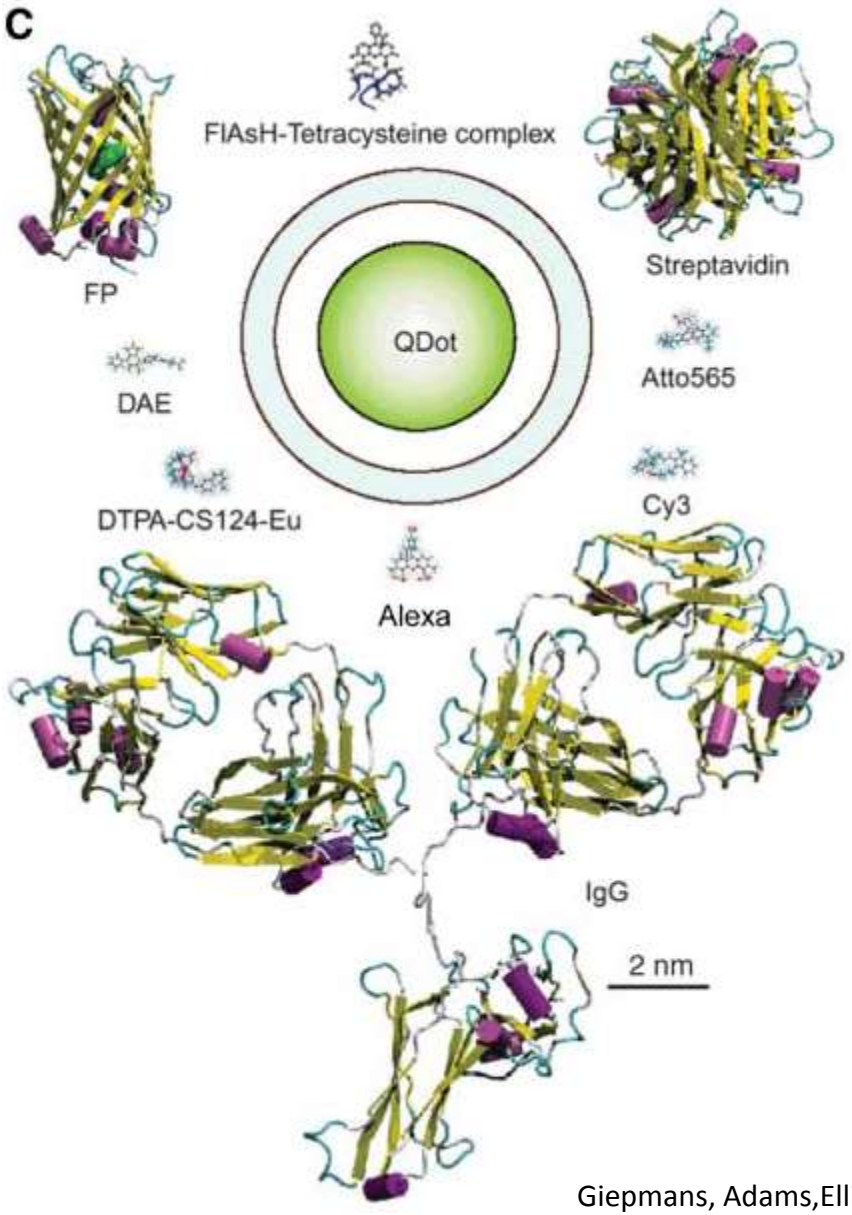


	ex/em maxima (nm)	Molar extinction coefficient	Fluorescnece quantum yield	*pKa*	O_2 requirement	Number of amino acids	*Mr*
UnaG	498/527	77,300 (498 nm)	0.51	< 4.0	no	139	16.5 K
EGFP	490/509	49,550 (490 nm)	0.60	5.8	yes	238	27 K

Kumagai et al., Cell 2013

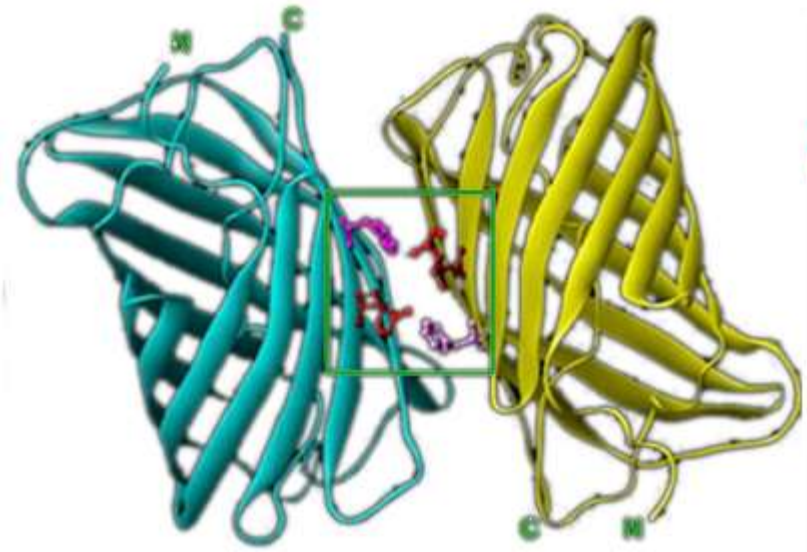

CAUTION
GFP
ARTIFACTS

Labeling without disturbing the system

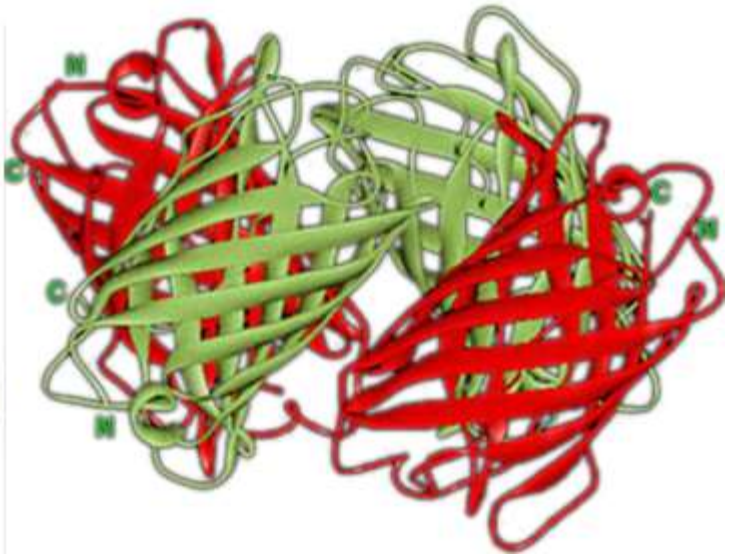


Giepmans, Adams,Ellisman,Tsien, Science 2006(312): 217

Oligomerization



GFP: weak dimer

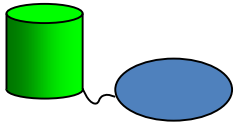


dsRed: Tetramer

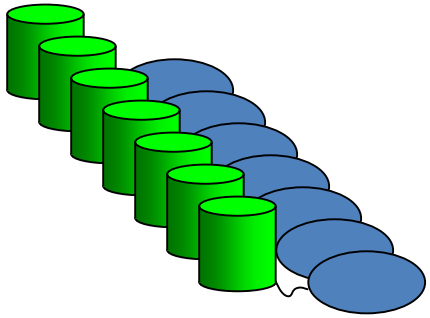
tdTomato, Kaede, tdEosFP, etc.

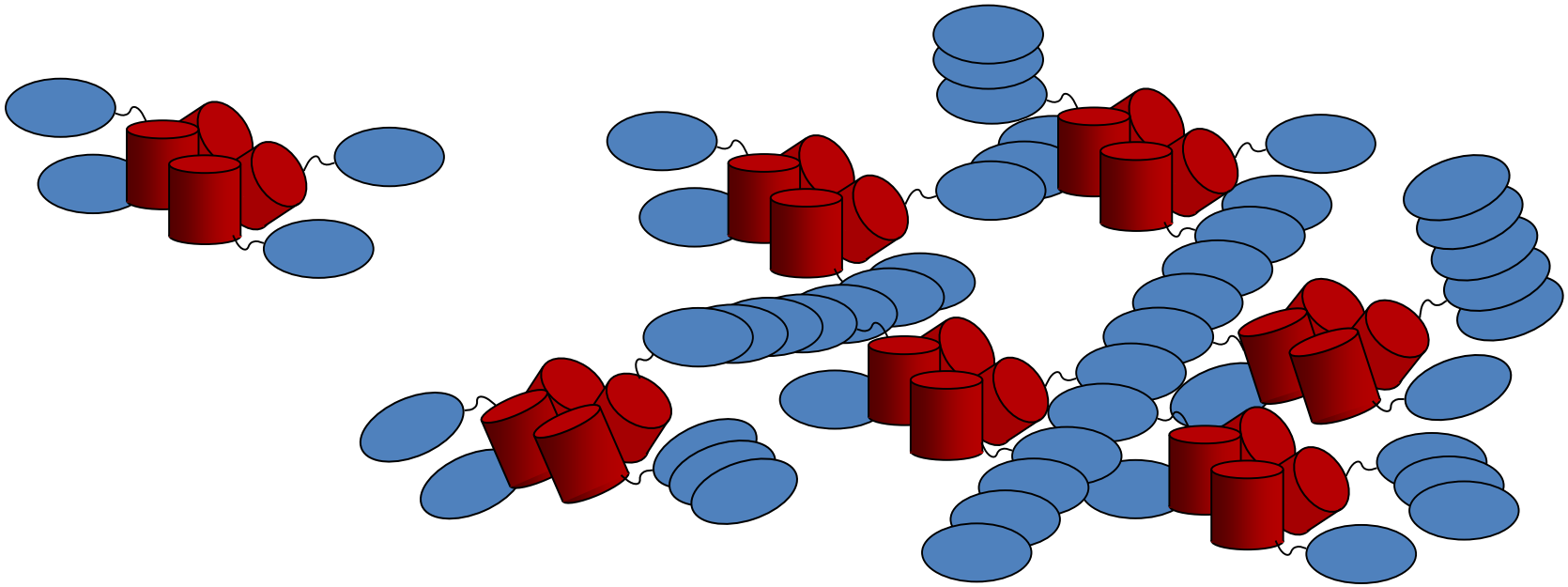
Why is oligomerization a problem?

Fusion to inert protein
or targeting sequence

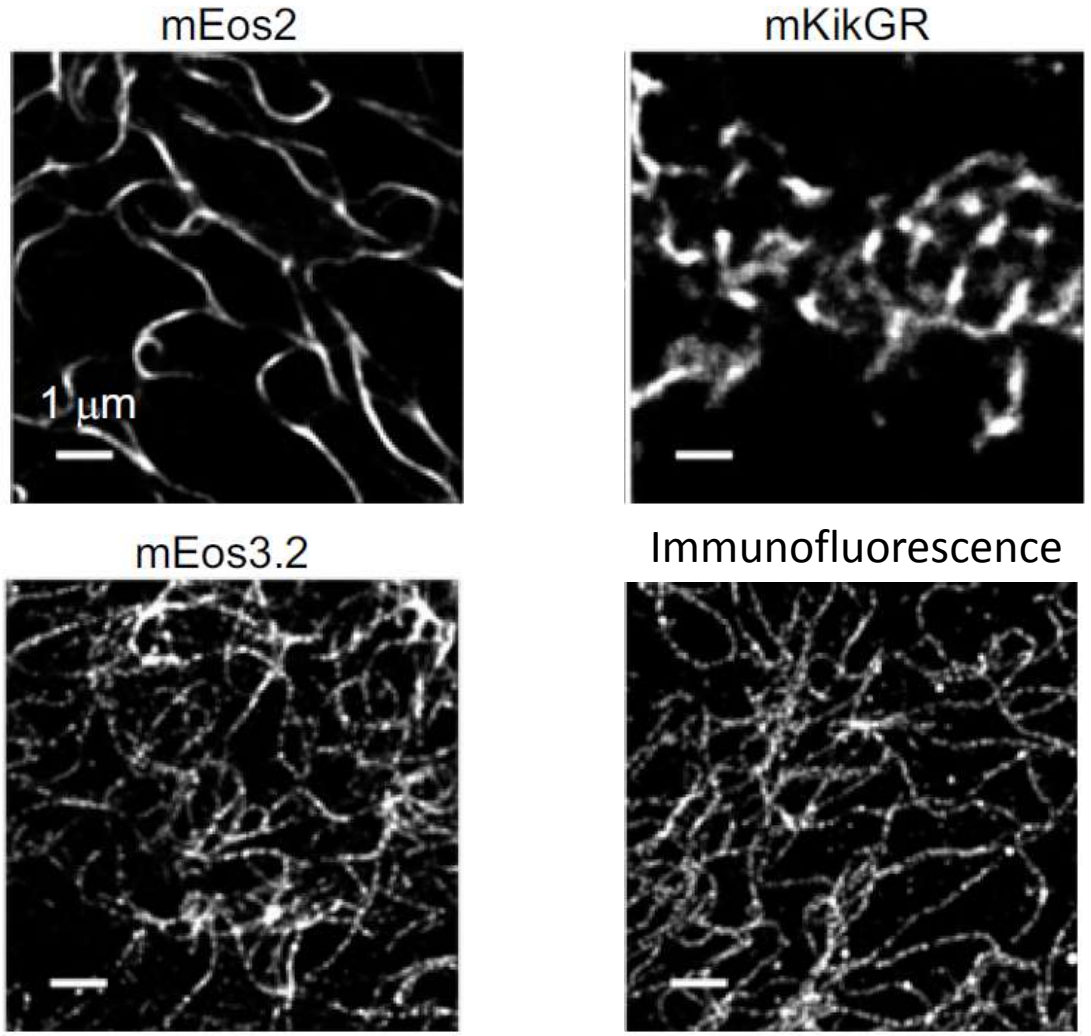


Fused to interacting protein





Oligomerization artifacts

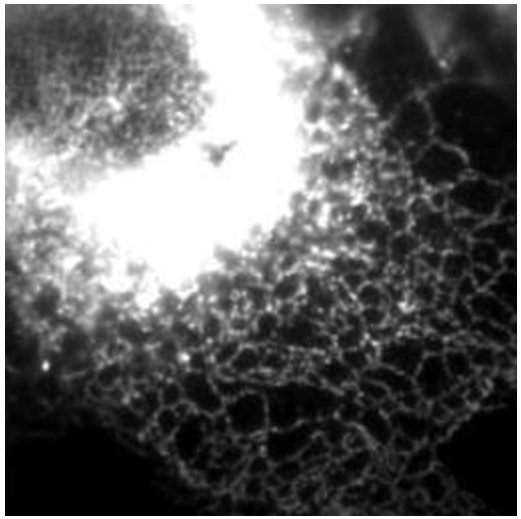


Super-resolution image of vimentin with various FP fusion and immunostaining

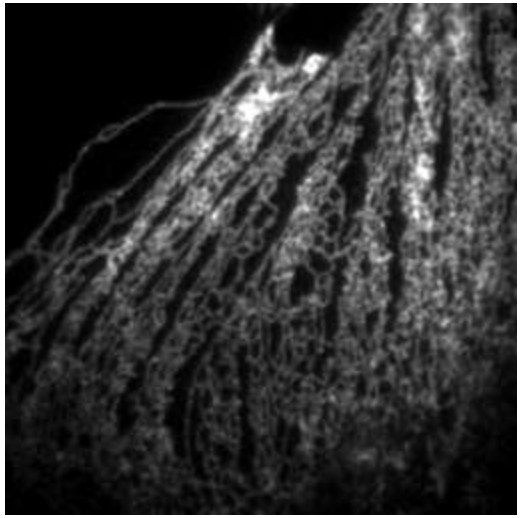
Wang et al., PNAS 2014

Over expression of fused protein

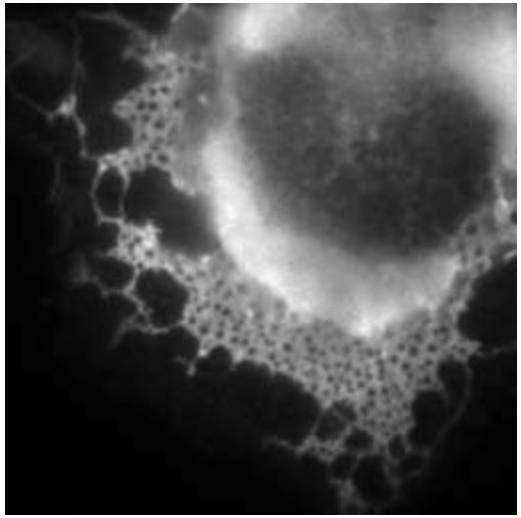
WT, immunoflurescence



GFP-rtn4a, stable cell line



FLAG-climp, transient expression



Collaboration with T. Rappaport

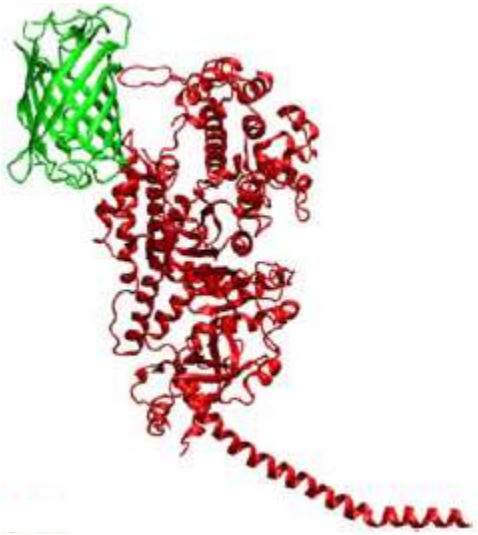
Over expression of free FP

GFP expression in muscle cells impairs actin-myosin interactions: implications for cell therapy

Onnik Agbulut[1,7], Catherine Coirault[2,7], Nicolas Niederländer[3], Alexis Huet[1], Patrick Vicart[1], Albert Hagège[4,5], Michel Puceat[3] & Philippe Menasché[5,6]

NATURE METHODS | VOL.3 NO.5 | MAY 2006 | 331

To conclude, our results show that eGFP expression impairs actin-myosin interactions, thereby causing excitation-contraction uncoupling and impaired contractile function of muscle cells. This adverse effect of eGFP should be kept in mind when using this marker to track cells after transplantation because the induced changes in cellular function may confound interpretati
tional data.



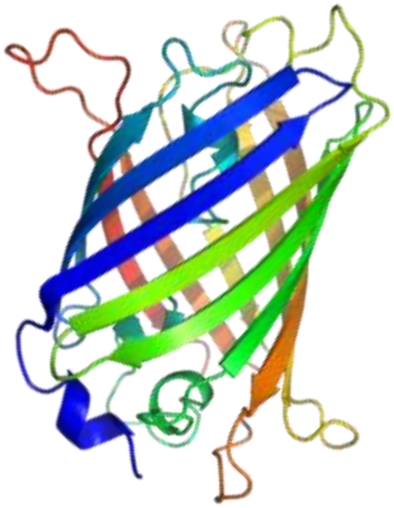
Agbulut et al., *J. Biol. Chem.* 2007(282) 10465

GFP fails to inhibit actin-myosin interactions *in vitro*

Daniel I Resnicow[1], Anneka M Hooft[2], Brooke C Harrison[1], Josh E Baker[2] & Leslie A Leinwand[1]

212 | VOL.5 NO.3 | MARCH 2008 | NATURE METHODS

Fluorescent protein



Good:

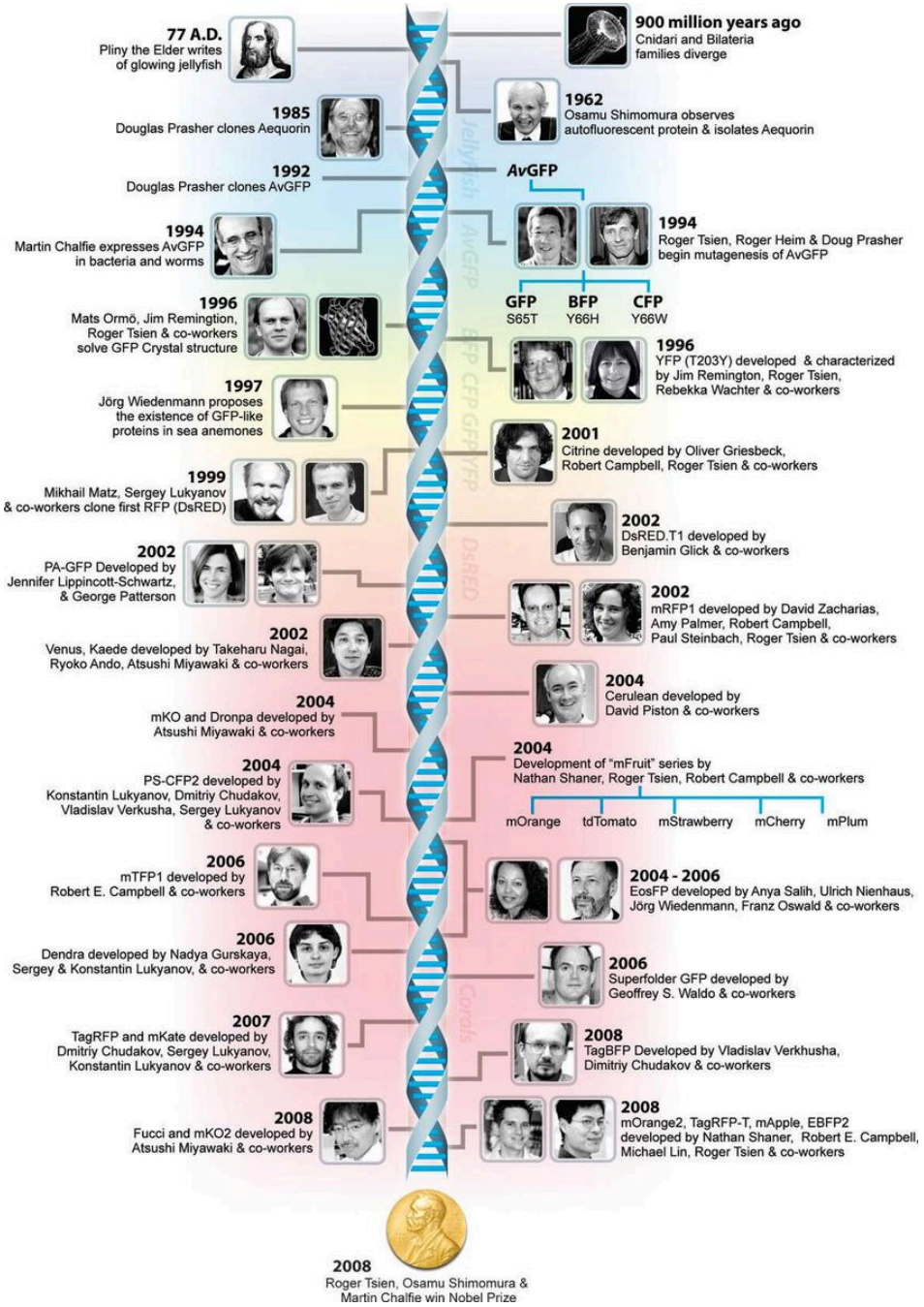
- Genetically encoded
- Live cell/animal labeling
- Simple and versatile

Not perfect yet:

- Sometimes still perturbative
- Not as bright and photostable
- Maturation can be slow
- Not applicable to…

  human samples

Thanks!


77 A.D.
Pliny the Elder writes of glowing jellyfish
900 million years ago
Cnidari and Bilateria families diverge
1962
Osamu Shimomura observes autofluorescent protein & isolates Aequorin
1985
Douglas Prasher clones Aequorin
1992
Douglas Prasher clones AvGFP
AvGFP
1994
Martin Chalfie expresses AvGFP in bacteria and worms
1994
Roger Tsien, Roger Heim & Doug Prasher begin mutagenesis of AvGFP
GFP S65T
BFP Y66H
CFP Y66W
1996
Mats Ormö, Jim Remington, Roger Tsien & co-workers solve GFP Crystal structure
1996
YFP (T203Y) developed & characterized by Jim Remington, Roger Tsien, Rebekka Wachter & co-workers
1997
Jörg Wiedenmann proposes the existence of GFP-like proteins in sea anemones
2001
Citrine developed by Oliver Griesbeck, Robert Campbell, Roger Tsien & co-workers
1999
Mikhail Matz, Sergey Lukyanov & co-workers clone first RFP (DsRED)
2002
DsRED.T1 developed by Benjamin Glick & co-workers
2002
PA-GFP Developed by Jennifer Lippincott-Schwartz, & George Patterson
2002
mRFP1 developed by David Zacharias, Amy Palmer, Robert Campbell, Paul Steinbach, Roger Tsien & co-workers
2002
Venus, Kaede developed by Takeharu Nagai, Ryoko Ando, Atsushi Miyawaki & co-workers
2004
Cerulean developed by David Piston & co-workers
2004
mKO and Dronpa developed by Atsushi Miyawaki & co-workers
2004
Development of "mFruit" series by Nathan Shaner, Roger Tsien, Robert Campbell & co-workers
mOrange
tdTomato
mStrawberry
mCherry
mPlum
2004
PS-CFP2 developed by Konstantin Lukyanov, Dmitriy Chudakov, Vladislav Verkusha, Sergey Lukyanov & co-workers
2006
mTFP1 developed by Robert E. Campbell & co-workers
2004 - 2006
EosFP developed by Anya Salih, Ulrich Nienhaus, Jörg Wiedenmann, Franz Oswald & co-workers
2006
Dendra developed by Nadya Gurskaya, Sergey & Konstantin Lukyanov, & co-workers
2006
Superfolder GFP developed by Geoffrey S. Waldo & co-workers
2007
TagRFP and mKate developed by Dmitriy Chudakov, Sergey Lukyanov, Konstantin Lukyanov & co-workers
2008
TagBFP Developed by Vladislav Verkhusha, Dimitriy Chudakov & co-workers
2008
Fucci and mKO2 developed by Atsushi Miyawaki & co-workers
2008
mOrange2, TagRFP-T, mApple, EBFP2 developed by Nathan Shaner, Robert E. Campbell, Michael Lin, Roger Tsien & co-workers
2008
Roger Tsien, Osamu Shimomura & Martin Chalfie win Nobel Prize