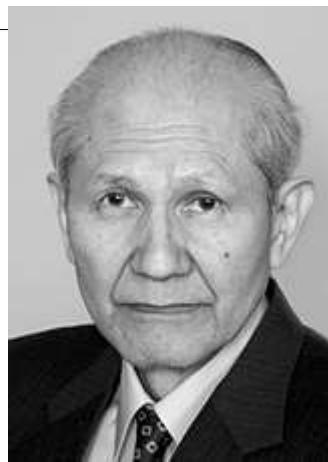


# Fluorescent proteins



Bo Huang  
2014.07.31



Osamu Shimomura



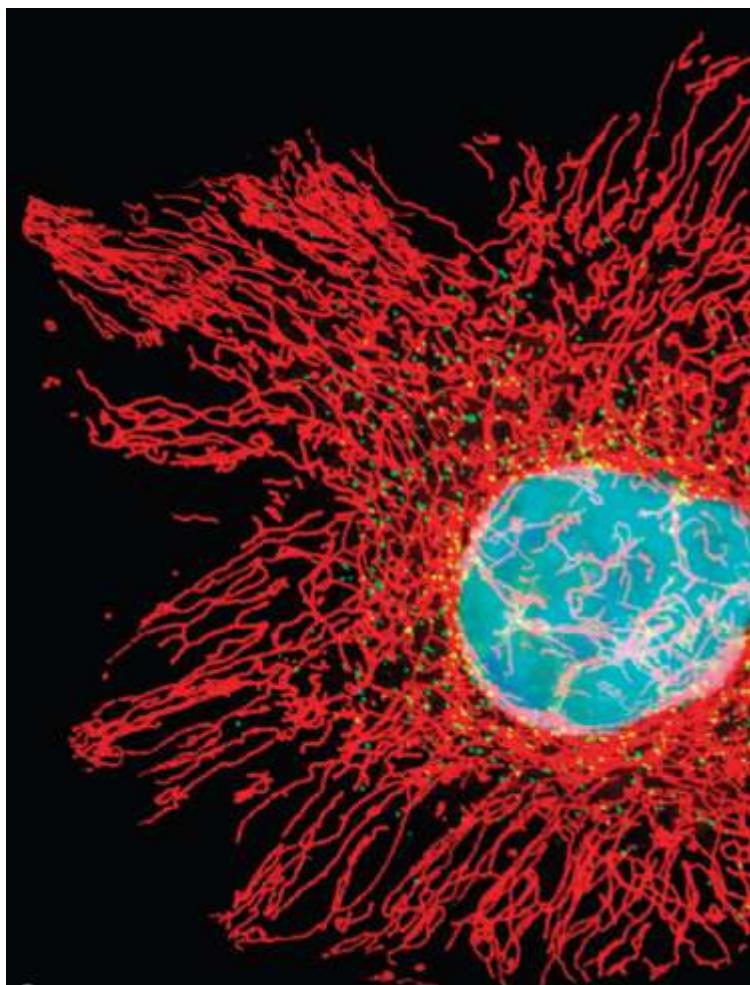
Martin Chalfie



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

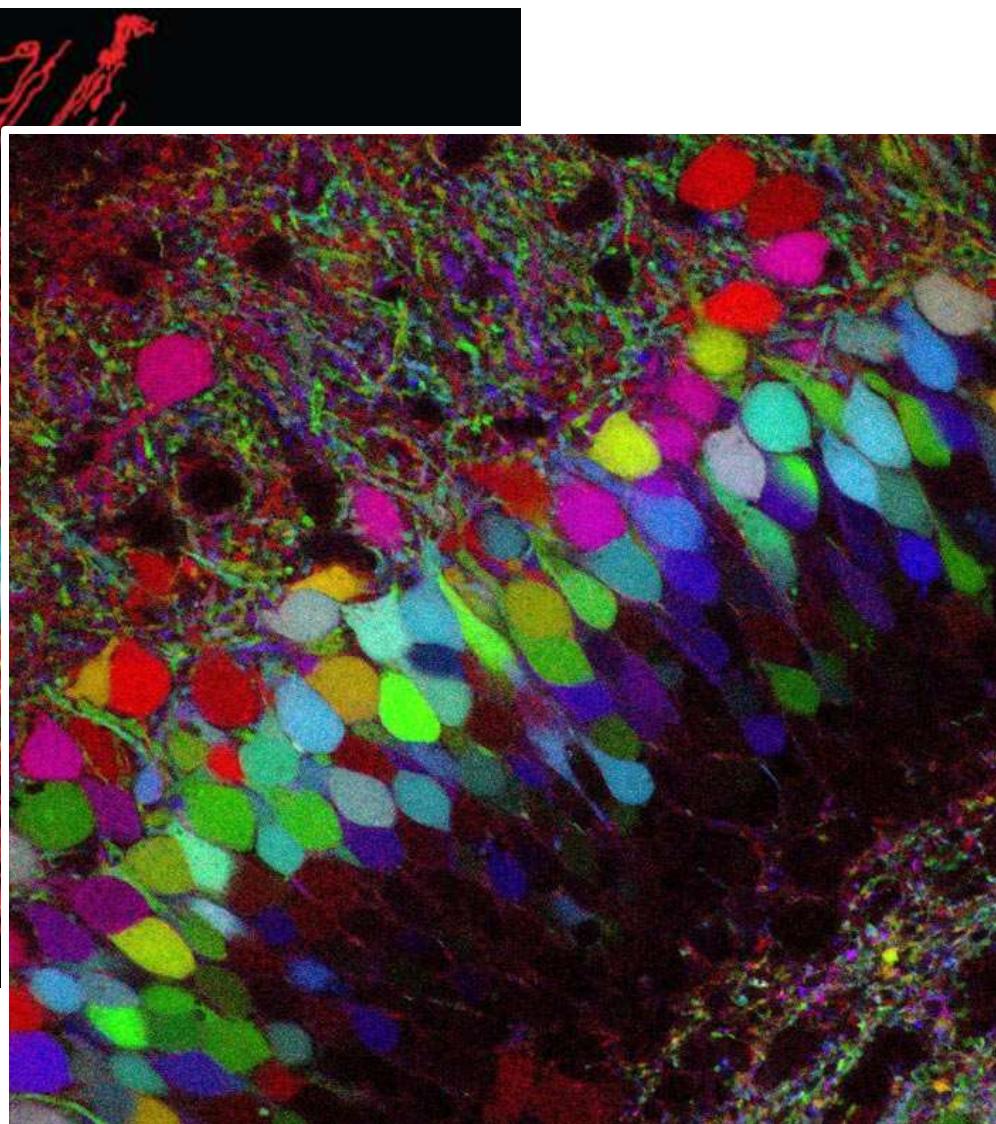
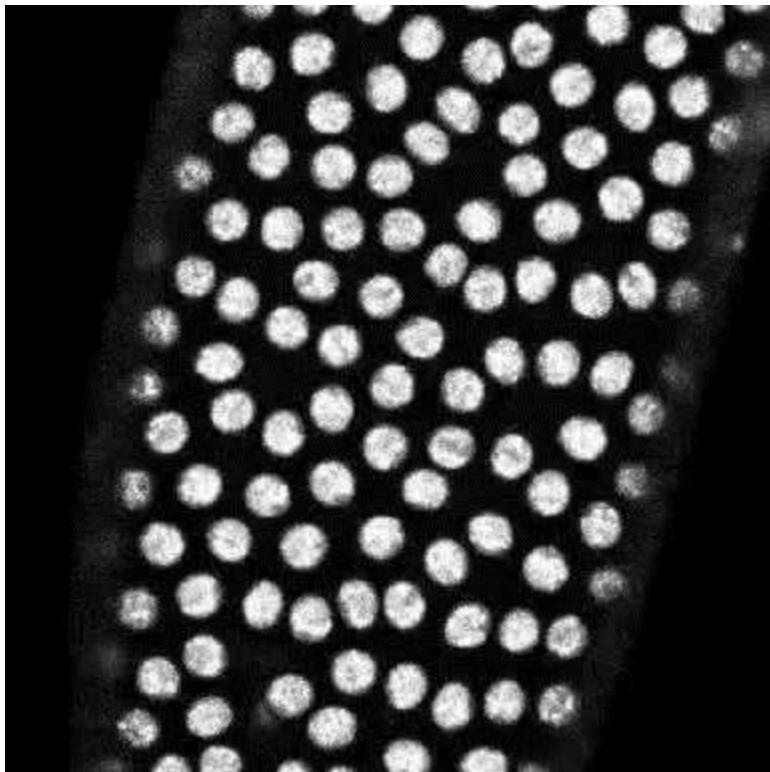


Image: Lichtman Lab

# GFP-based live microscopy

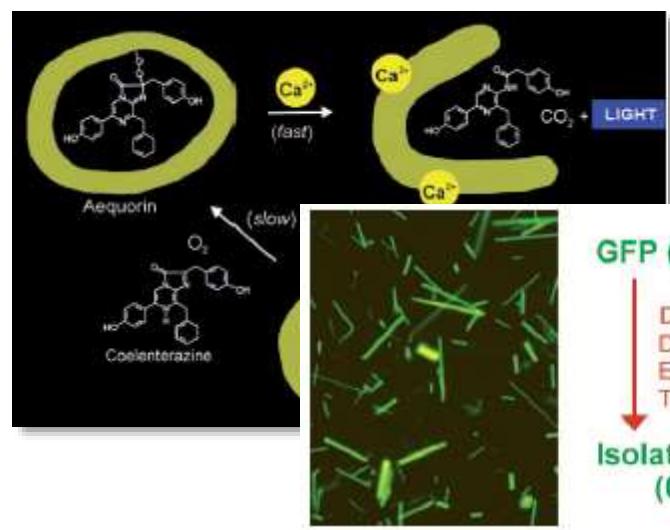


Ding et al., *Develop. Biol.* 2009(334):253  
*Chromator is required for proper microtubule spindle formation and mitosis in Drosophila*

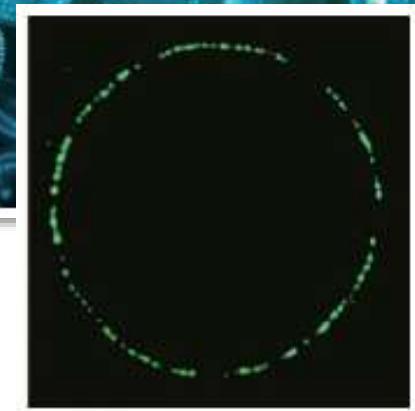


Kaech, Brinkhaus, and Matus, *Neurobiol.* 1999(96):10433  
*Volatile anesthetics block actin-based motility in dendritic spines*

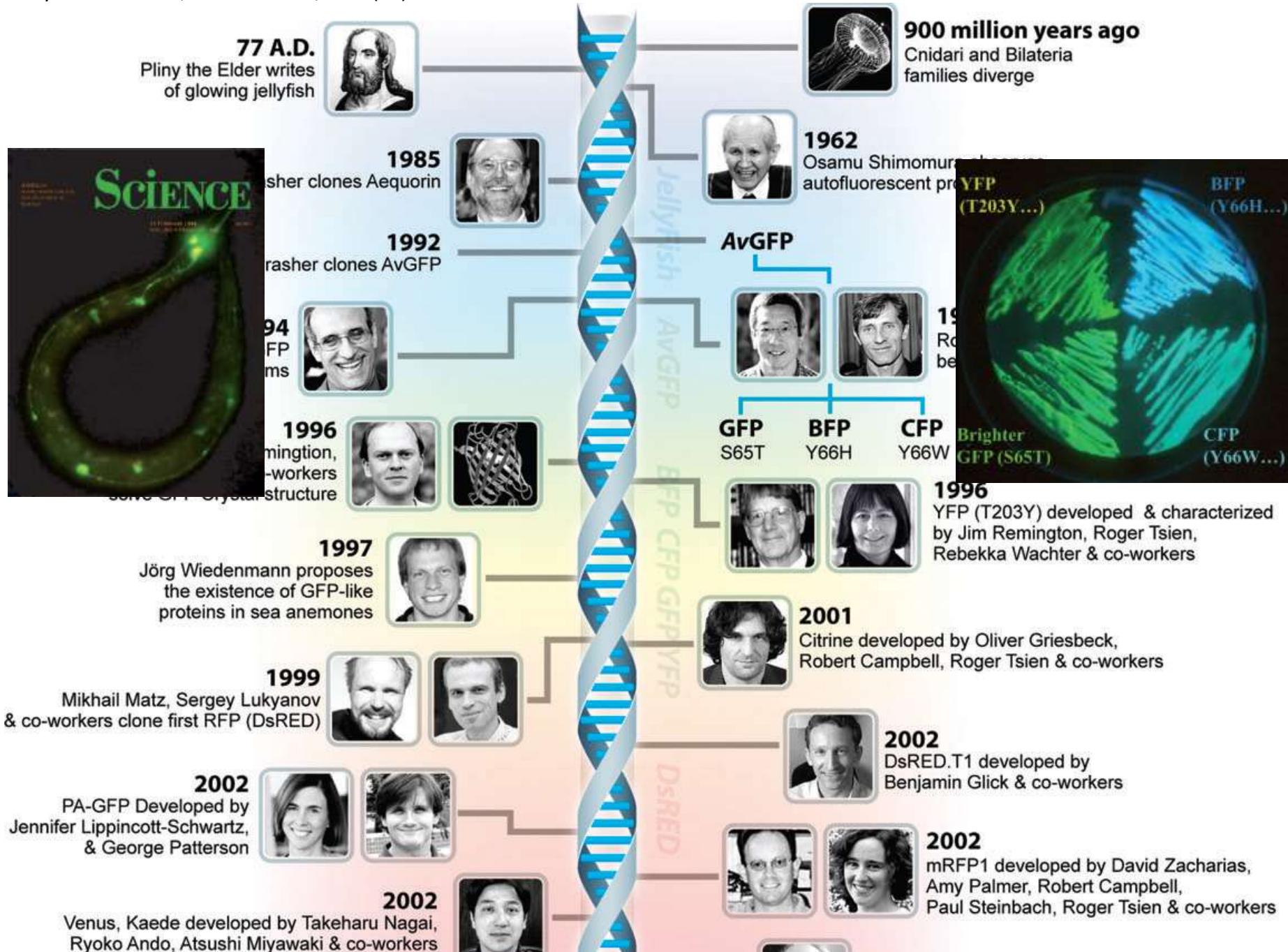
# The discovery of GFP



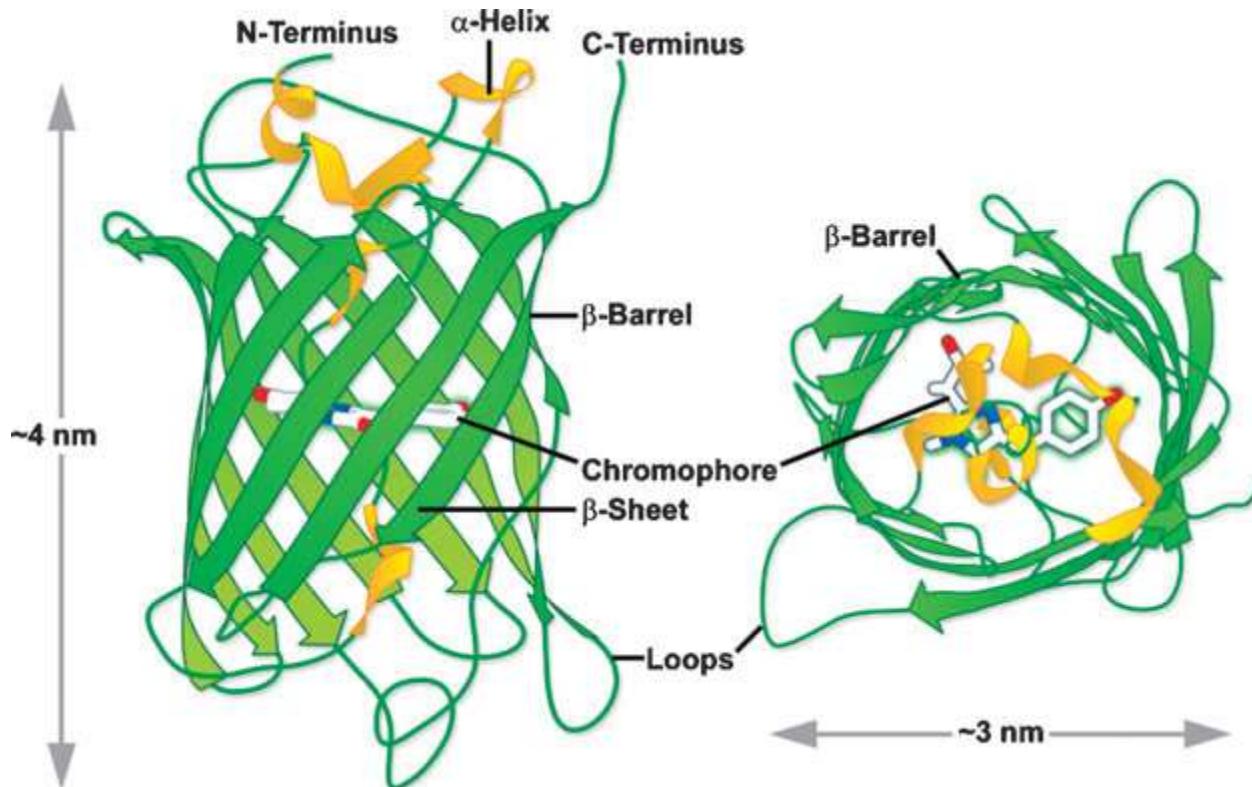
GFP (100 mg)  
↓  
Denature at 90 °C  
Digest with papain  
Extraction with butanol at pH 1  
TLC purification  
↓  
Isolated chromophore (0.1 mg)



Images from Osamu Shimomura



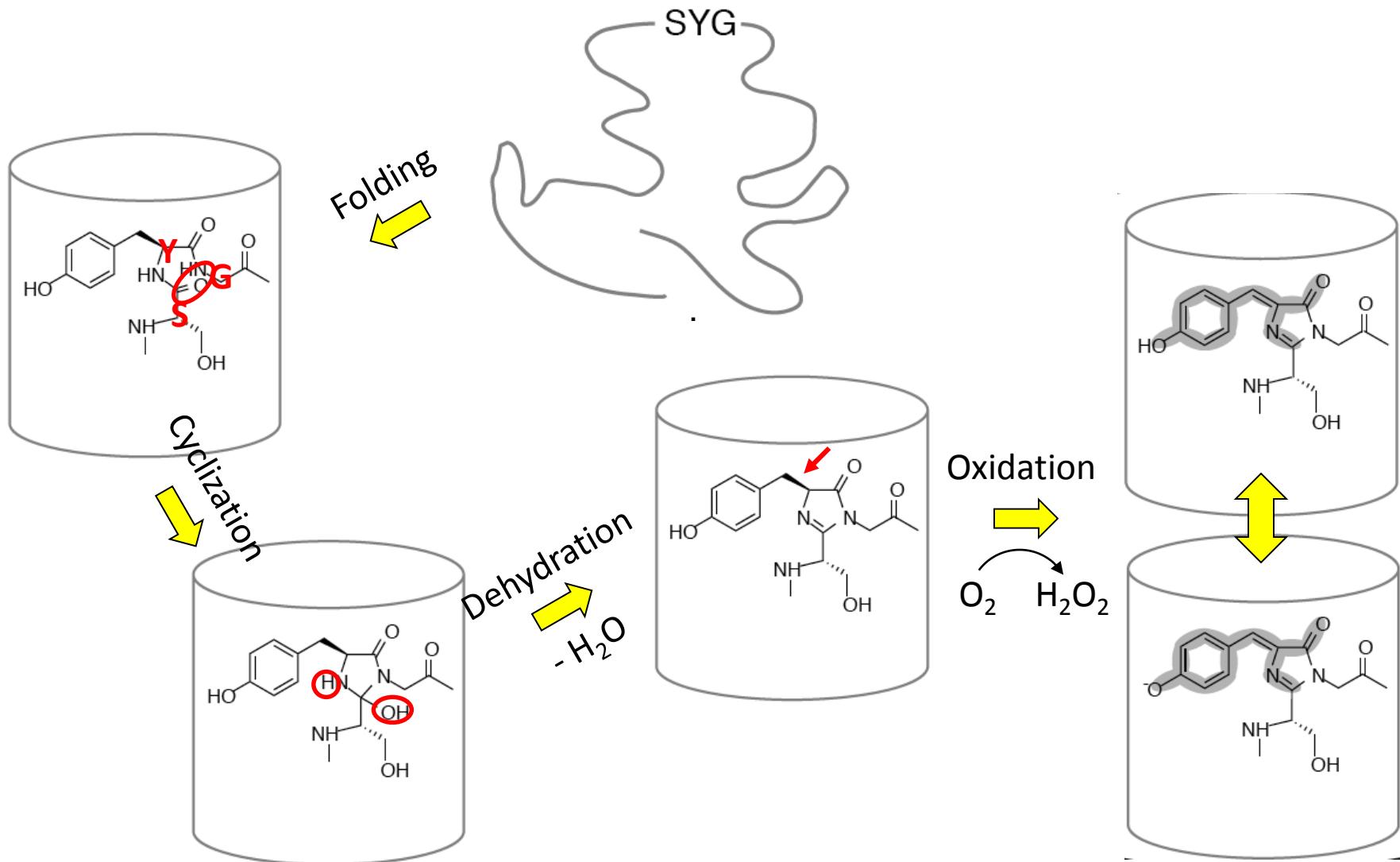
# The green fluorescent protein



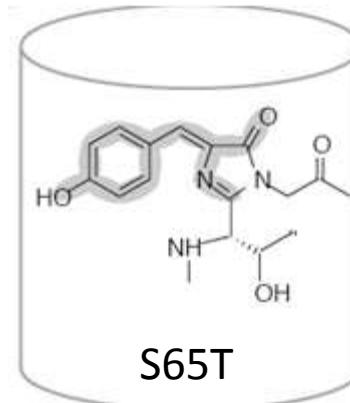
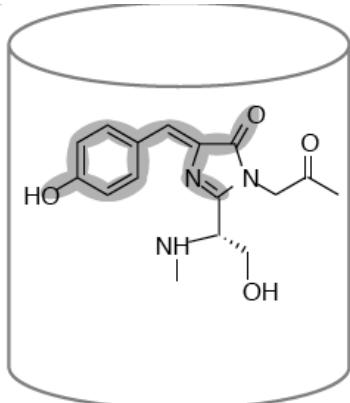
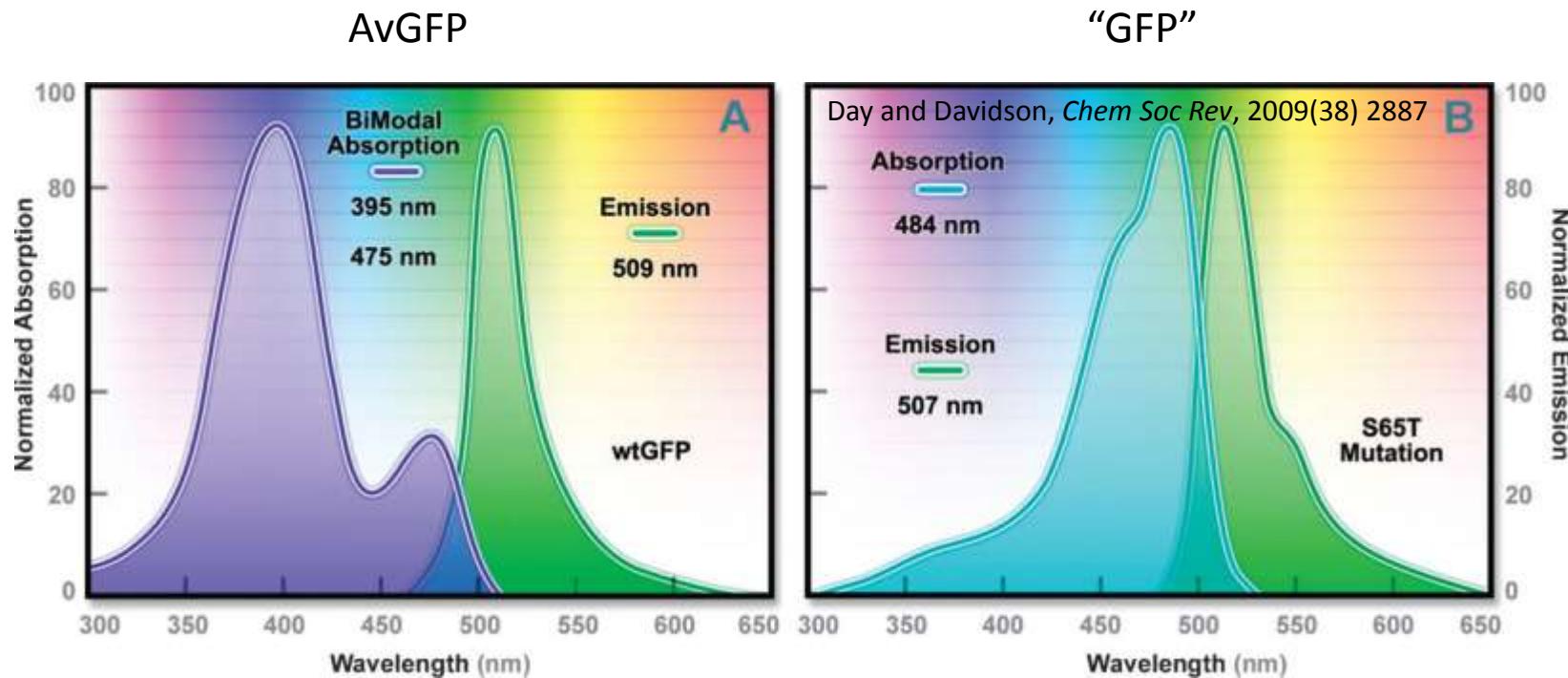
Day and Davidson, *Chem Soc Rev*, 2009(38) 2887

~240 Amino acids, 27 kD

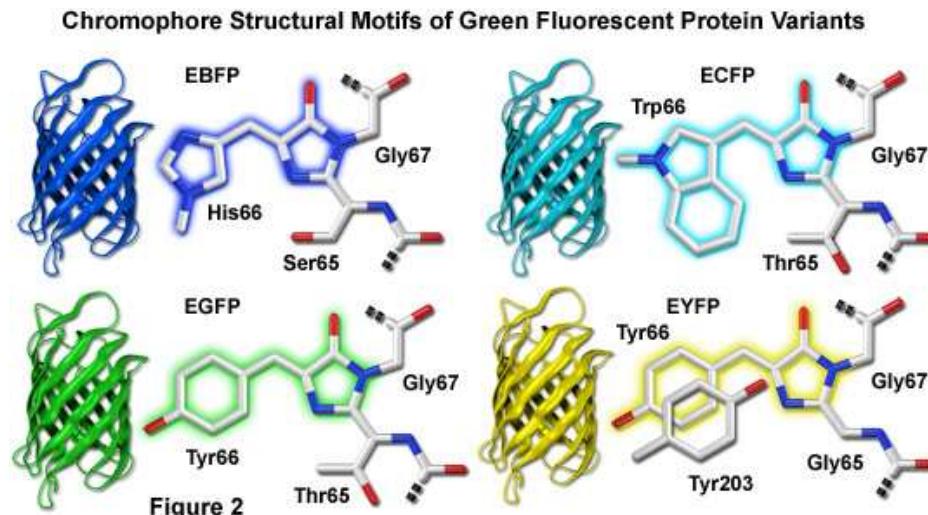
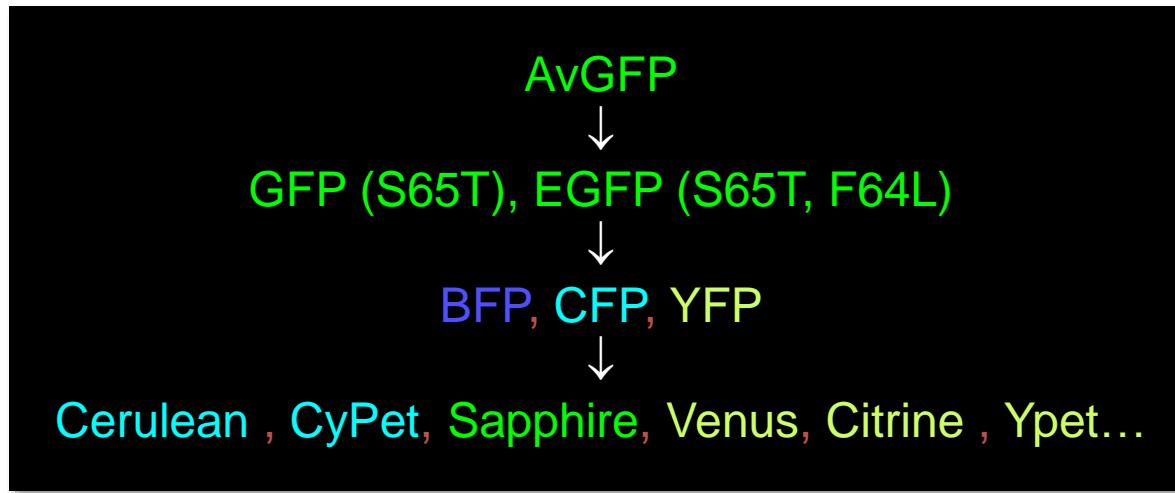
# GFP chromophore formation



# Improving the wild type GFP



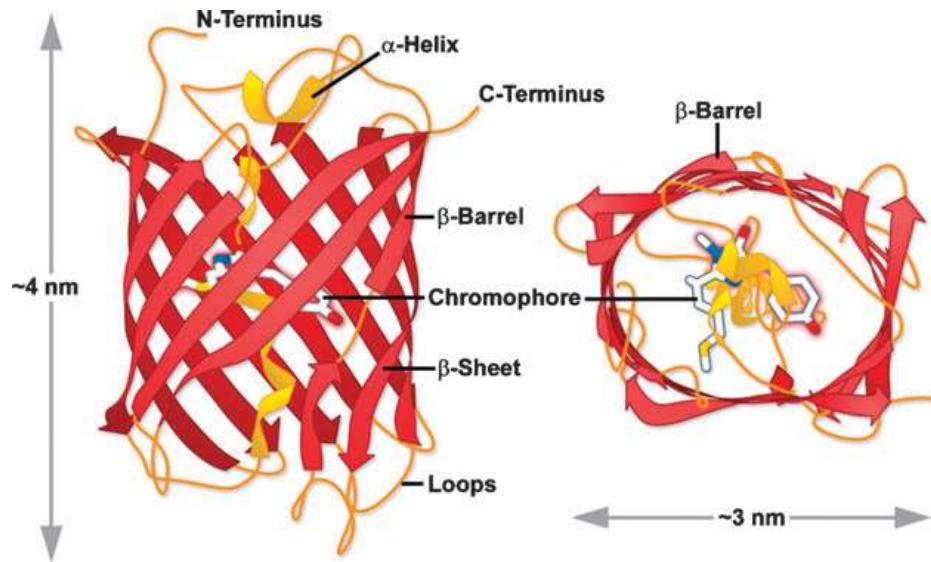
# Jellyfish (*Aequorea*) fluorescent protein family



A close-up photograph of a vibrant red coral polyp, likely a member of the genus *Acropora*. The polyp is densely covered in numerous small, white, finger-like tentacles (filamentous anemones) that are extended and slightly curved. The background is dark, making the bright red color of the coral stand out. The overall texture is soft and somewhat fuzzy due to the close proximity of the shot.

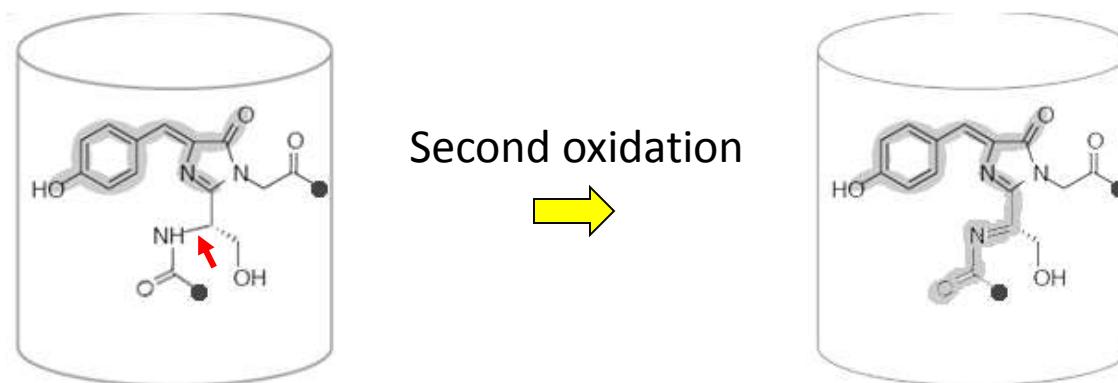
Seeking for red colors...

# Red FPs from Anthozoa



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

dsRed  
↓  
mRFP  
↓  
mOrange, mCherry...



# The fluorescent protein pallet

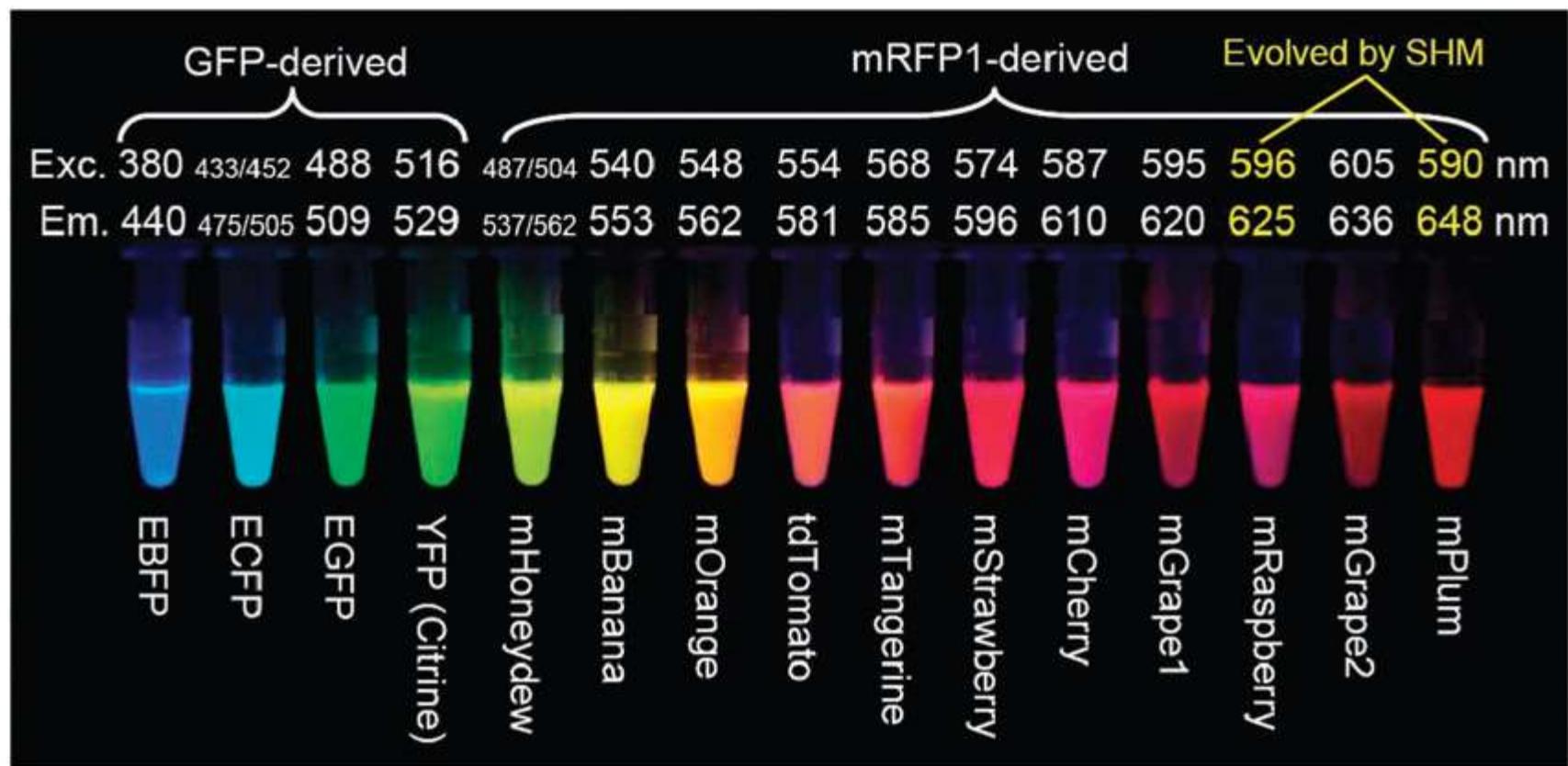


Image from Tsien lab

# 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 <sup>-1</sup> cm <sup>-1</sup>	Fluorescence quantum yield	Brightness (EC*QY) <sup>n-1</sup> (mM <sup>-1</sup> cm <sup>-1</sup> ) <sup>n-1</sup>	Brightness of fully mature protein (% of fluorescein)	t <sub>1/2</sub> for bleach, sec	photostability (fold improvement over fluorescein)	pKa	t <sub>1/2</sub> for maturation at 37°C	Oligomerization	References
Far-red	mPlum	Tsien	<i>Discosoma sp.</i>	590	649	41,000	0.10	4.1	5.9	53	7.3	<4.5	100 min	monomer	5
Red	mCherry	Tsien	<i>Discosoma sp.</i>	587	610	72,000	0.22	16	23	96	13.1	<4.5	15 min	monomer	4
	tdTomato	Tsien	<i>Discosoma sp.</i>	554	581	138,000	0.69	95	138	98	13.5	4.7	1 hr	tandem dimer	4
	mStrawberry	Tsien	<i>Discosoma sp.</i>	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	<i>Discosoma sp.</i>	556	586	35,000	0.10	3.5	5.1	16	2.2	4.5	ND	monomer	y
Orange	mOrange	Tsien	<i>Discosoma sp.</i>	548	562	71,000	0.69	49	71	9.0	1.2	6.5	4.5 hr	monomer	4
	mKO	MBL Intl.	<i>Fungia concinna</i>	548	559	51,600	0.60	31	45	122	16.7	5	2.5 hr	monomer	10
Yellow	mCitrine	Tsien	<i>Aequorea victoria</i>	516	529	77,000	0.76	59	85	49	6.7	5.7	ND	monomer	16, 23
	Venus	Miyawaki	<i>Aequorea victoria</i>	515	528	92,200	0.57	53	76	15	2.0	6	ND	weak dimer	1
	YPet	Daugherty	<i>Aequorea victoria</i>	517	530	104,000	0.77	80	116	49	6.7	5.6	ND	weak dimer	2
	EYFP	Invitrogen	<i>Aequorea victoria</i>	514	527	83,400	0.61	51	74	60	8.3	6.9	ND	weak dimer	18
Green	Emerald	Invitrogen	<i>Aequorea victoria</i>	487	509	57,500	0.68	39	57	0.69	0.1	6	ND	weak dimer	18
	EGFP	Clontech*	<i>Aequorea victoria</i>	488	507	56,000	0.60	34	49	174	23.9	6	ND	weak dimer	y
Cyan	CyPet	Daugherty	<i>Aequorea victoria</i>	435	477	35,000	0.51	18	26	59	8.1	5	ND	weak dimer	2
	mCFP	Tsien	<i>Aequorea victoria</i>	433	475	32,500	0.40	13	19	64	8.8	4.7	ND	monomer	23
	Cerulean	Piston	<i>Aequorea victoria</i>	433	475	43,000	0.62	27	39	36	5.0	4.7	ND	weak dimer	3
UV-excitable green	T-Sapphire	Griesbeck	<i>Aequorea victoria</i>	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

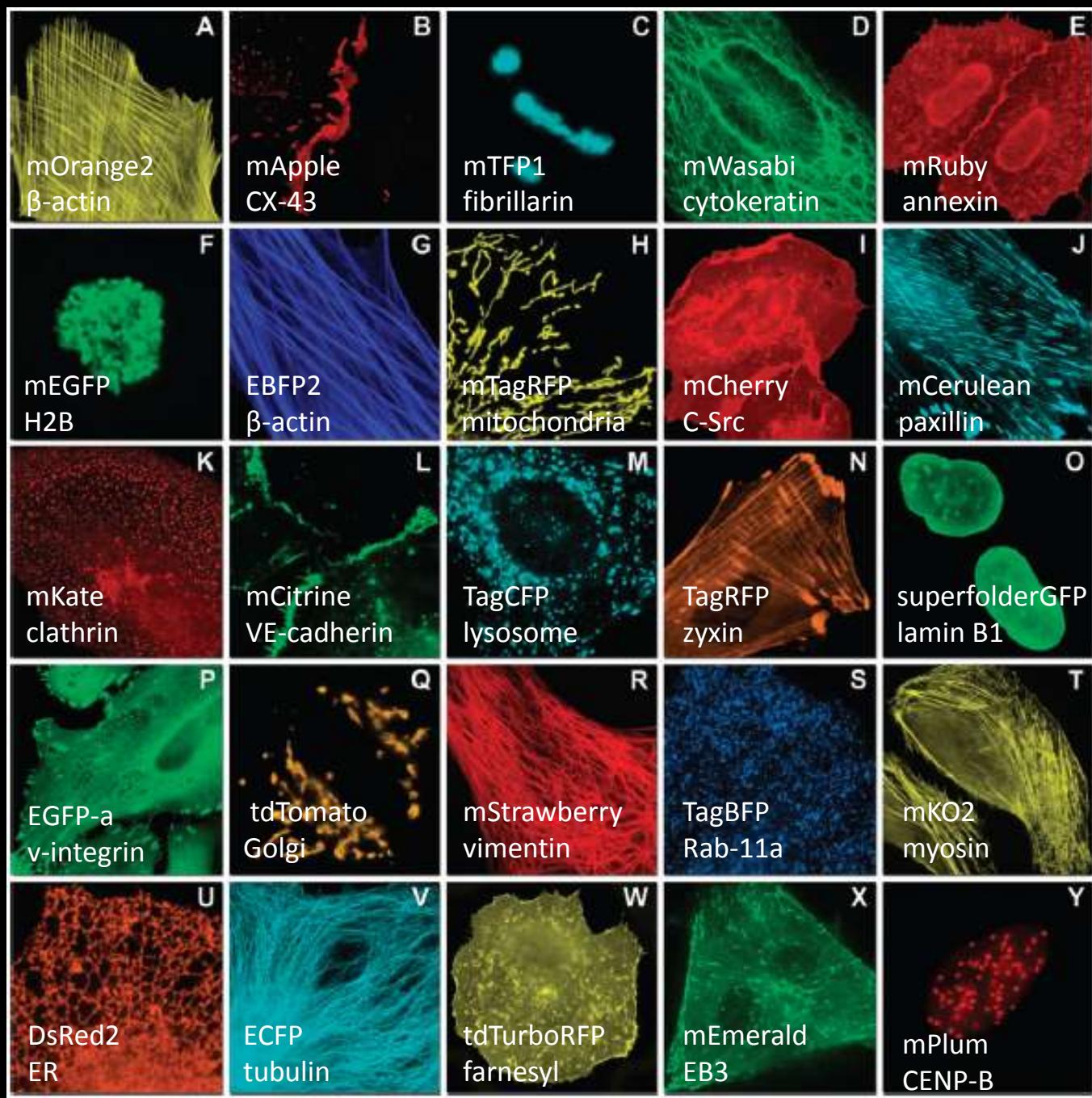
x [www.evrogen.com](http://www.evrogen.com)

y [www.clontech.com](http://www.clontech.com)

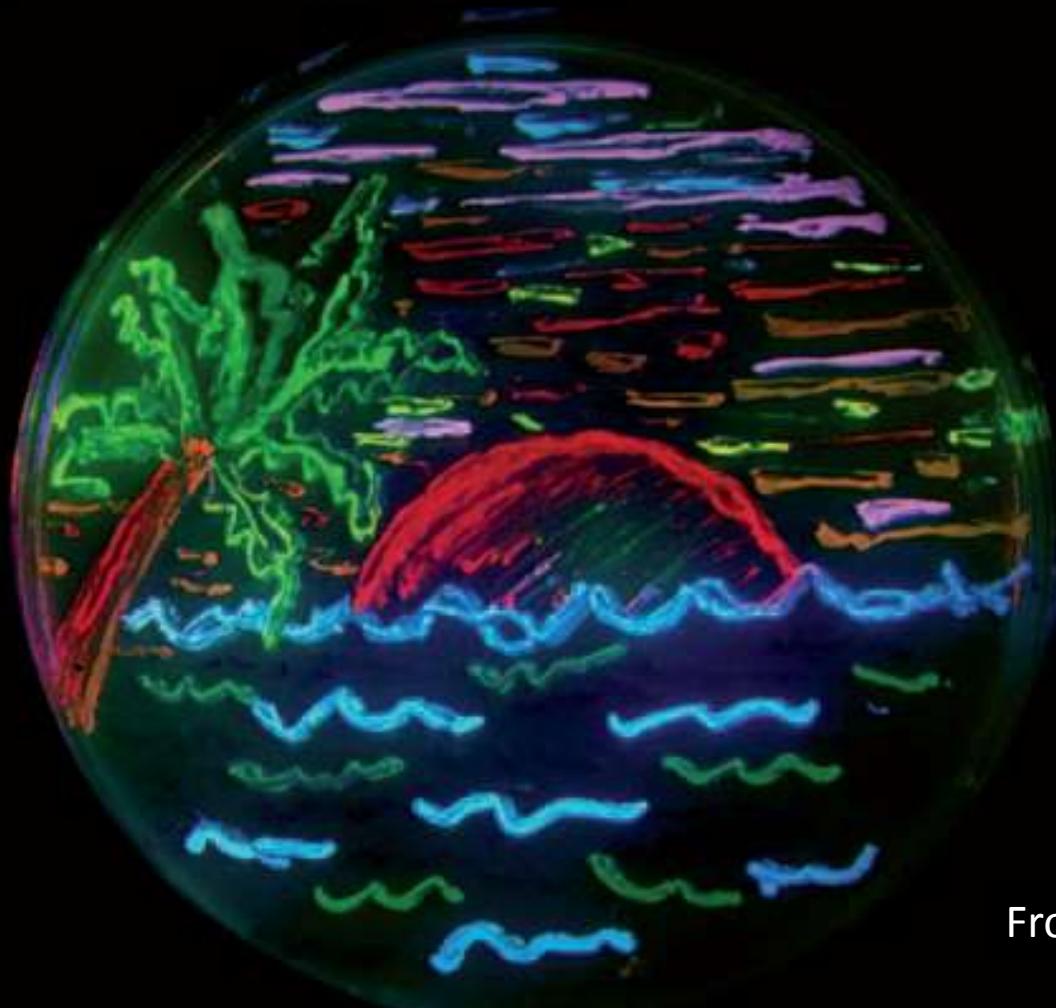
ND = not determined

## 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 37°C, 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
McY	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

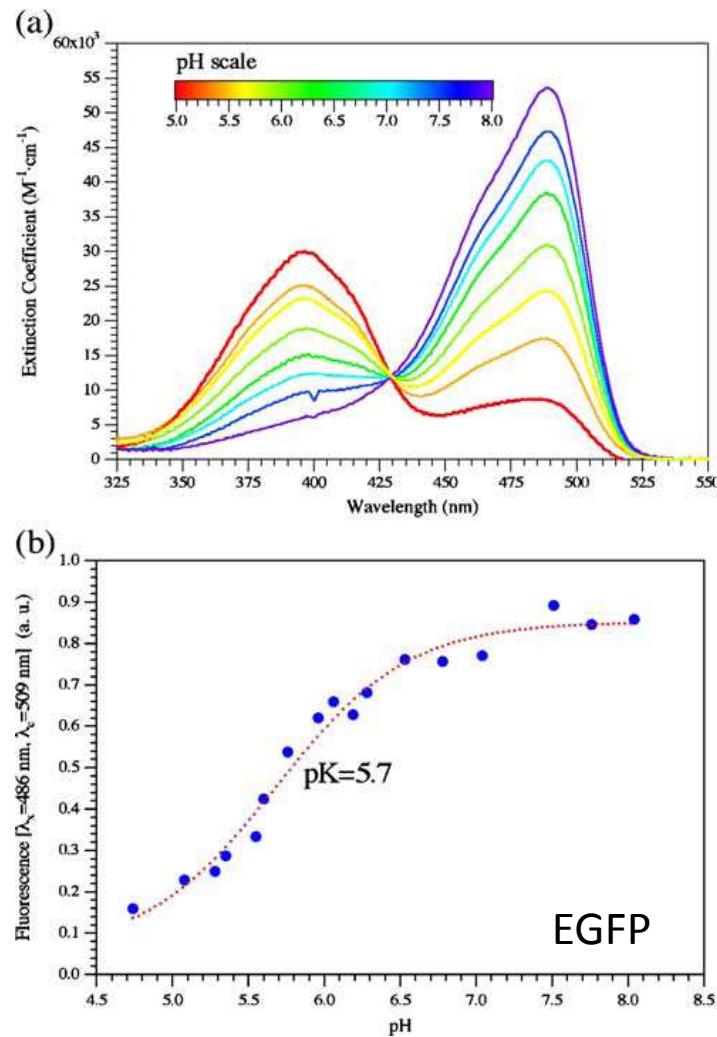
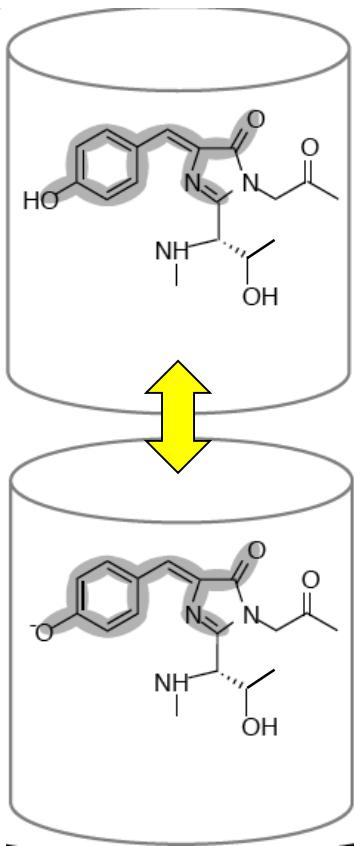


# Even more fun with GFP?

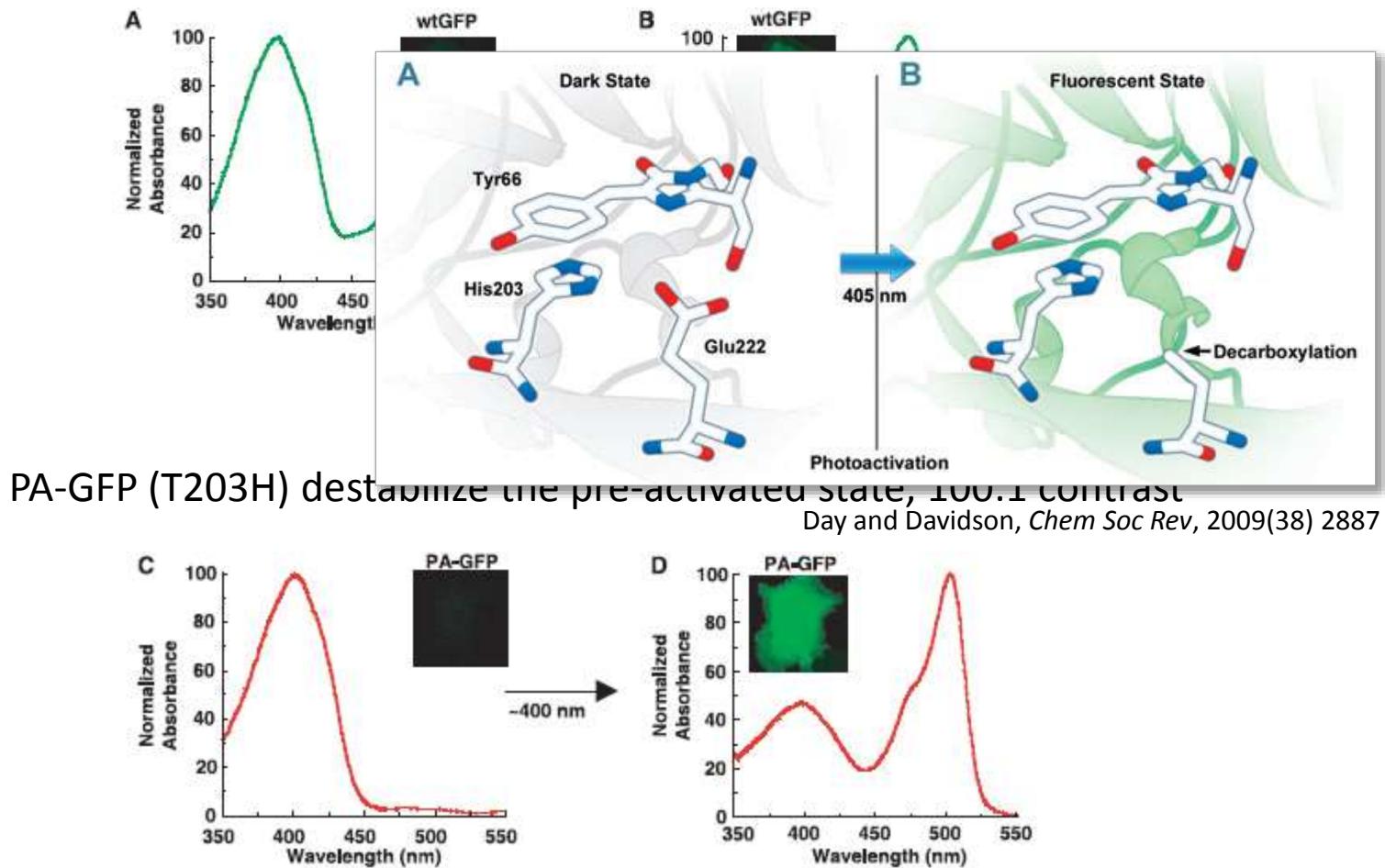


From Tsien lab

# pH sensitivity of GFP



# Photoactivatable fluorescent proteins

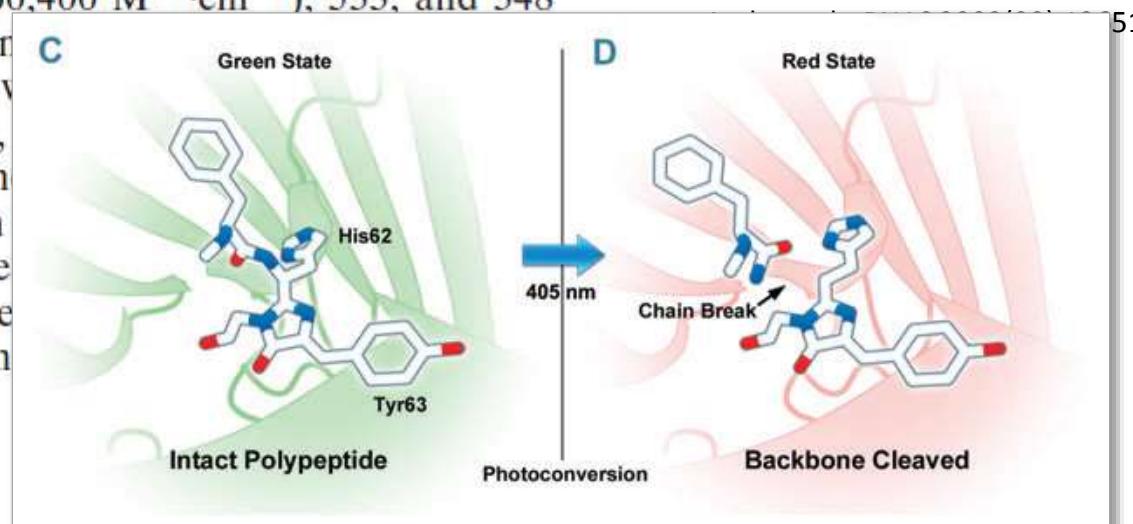
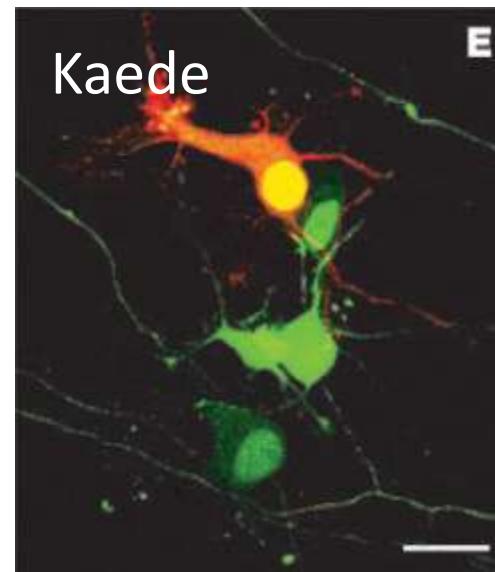


# How discovery can be made...

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

Ryoko Ando<sup>\*†</sup>, Hiroshi Hama\*, Miki Yamamoto-Hino\*, Hideaki Mizuno\*, and Atsushi Miyawaki<sup>\*‡</sup>

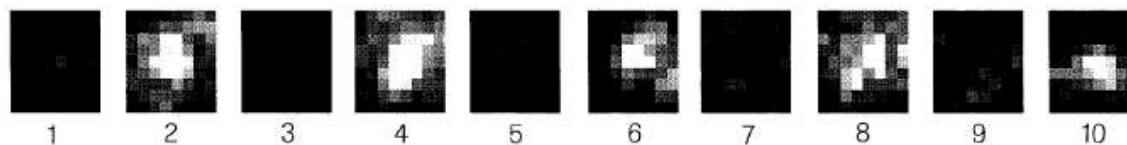
We happened to leave one of the protein aliquots on the laboratory bench overnight. The next day, we found that the protein sample on the bench had turned red, whereas the others that were kept in a paper box remained green. Although the sky had been partly cloudy, the red sample had been exposed to sunlight through the south-facing windows. The absorption spectrum of the red protein was measured at pH 7.4 (Fig. 2A, 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 ( $\epsilon = 60,400 \text{ M}^{-1}\cdot\text{cm}^{-1}$ ), 533, and 348 nm. These peaks reflect the ionization of the chromophore, which they were augmented equally well at pH 5.7. When excited at 480 nm, the emission maximum at 582 nm with a shoulder at 518 nm (red line) in addition to the 518-nm peak (green line) at 627 nm were also pH-sensitive (Fig. 2B, red line), indicating that the red fluorescence quantum yield ( $\Phi = 0.33$ ) is also pH-resistant.



# “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)

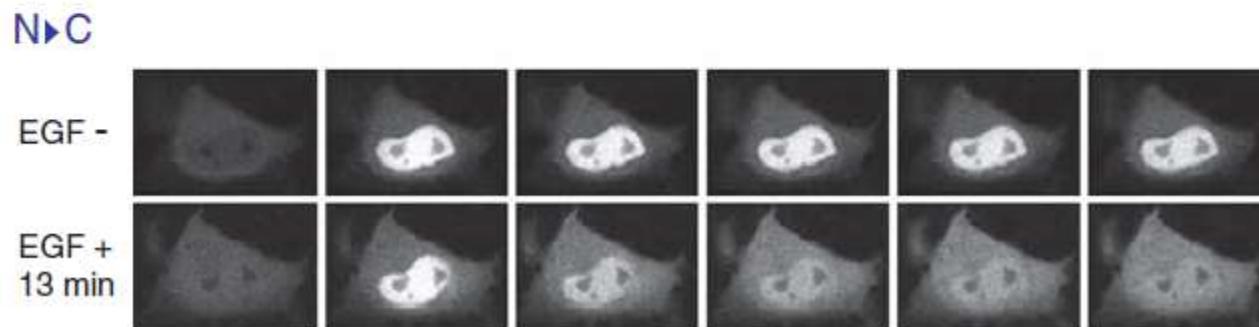
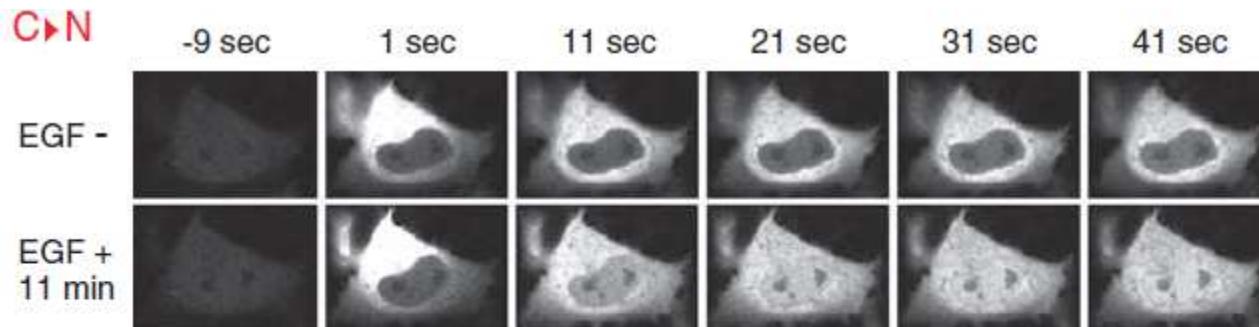


**Figure 2** The switching behaviour of a single T203F molecule, illustrated by a series of ten consecutive experiments on the same molecule. Each 100-ms frame

# Applications of PA-FPs

## Visualization of protein trafficking

ERK-Dronpa

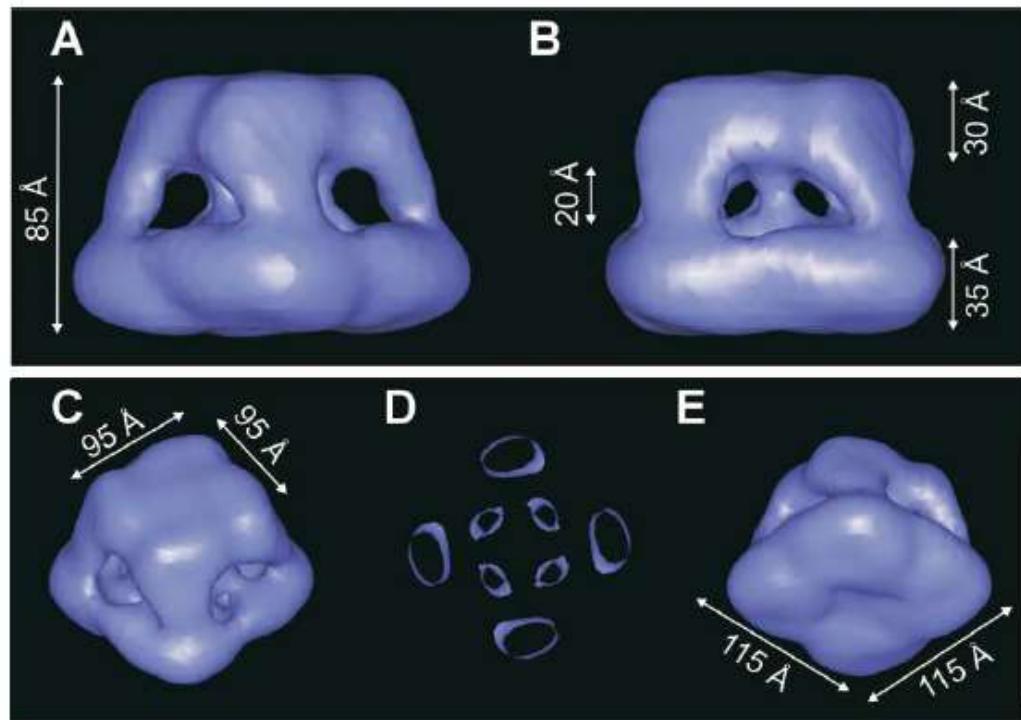


Ando, Mitzuro and Miyawaki, Science 2004(306): 1370

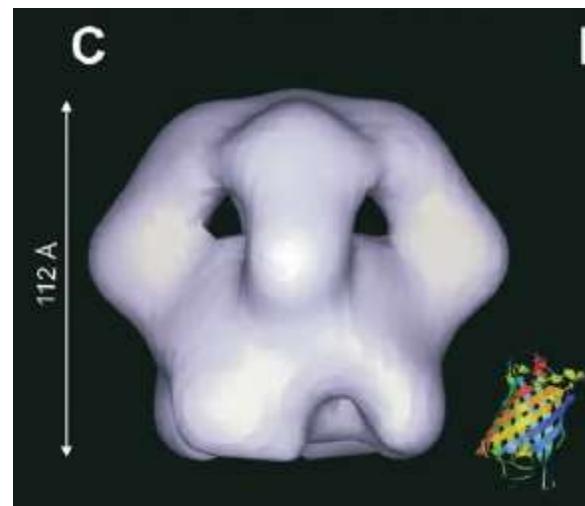
Super-resolution microscopy

# Nonfluorescent applications of GFP

Kv4.2 + KChIP2

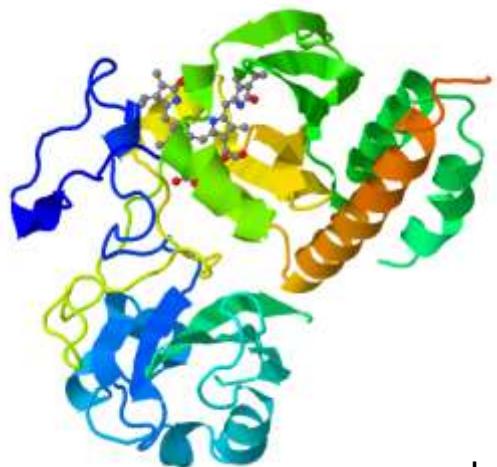


Kv4.2 + GFP-KChIP2

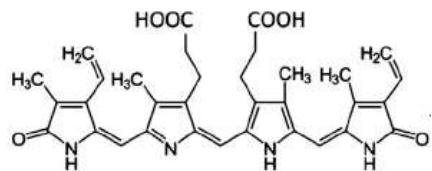


# Beyond GFP

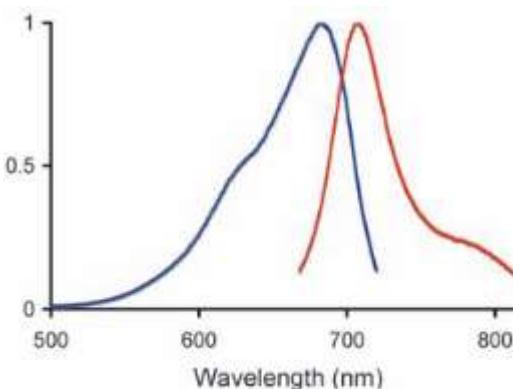
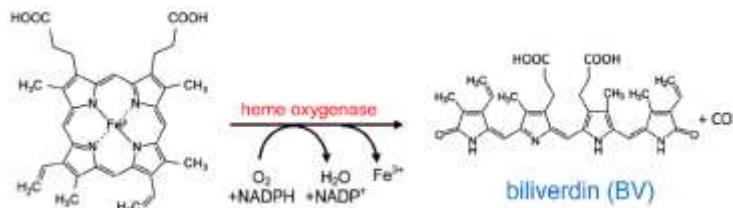
Bacterial phytochrome



covalent binding



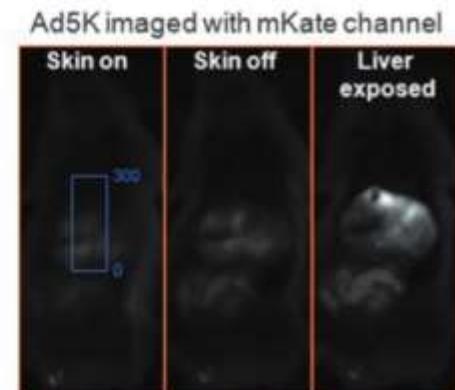
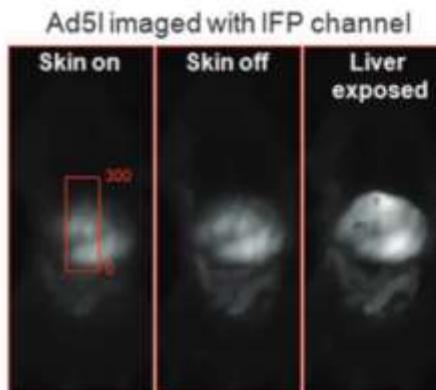
biliverdin (BV)



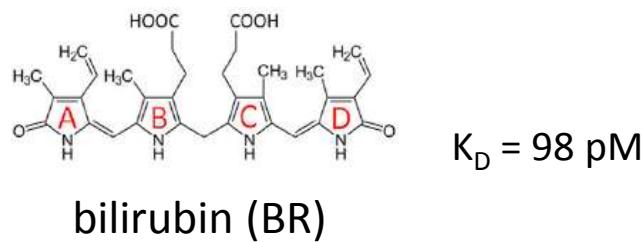
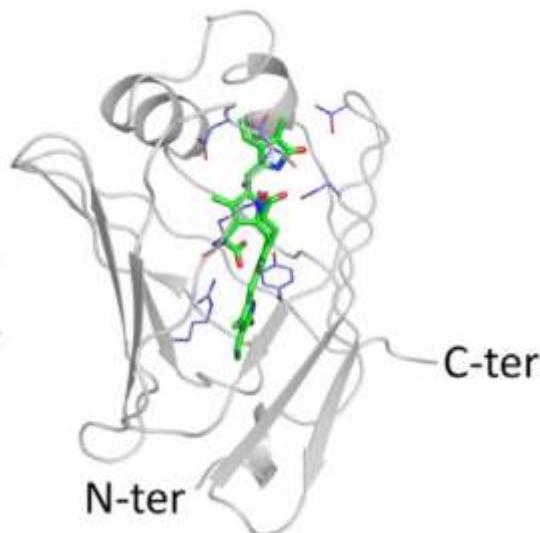
Shu et al., Science 2010

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...

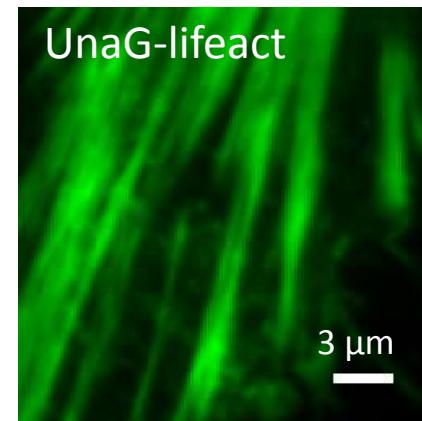
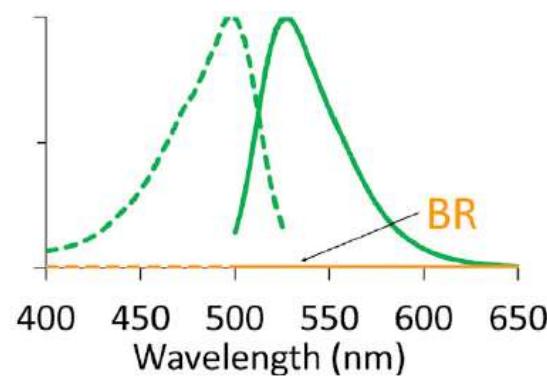


# UnaG from Japanese eel (unagi)



$K_D = 98 \text{ pM}$

bilirubin (BR)



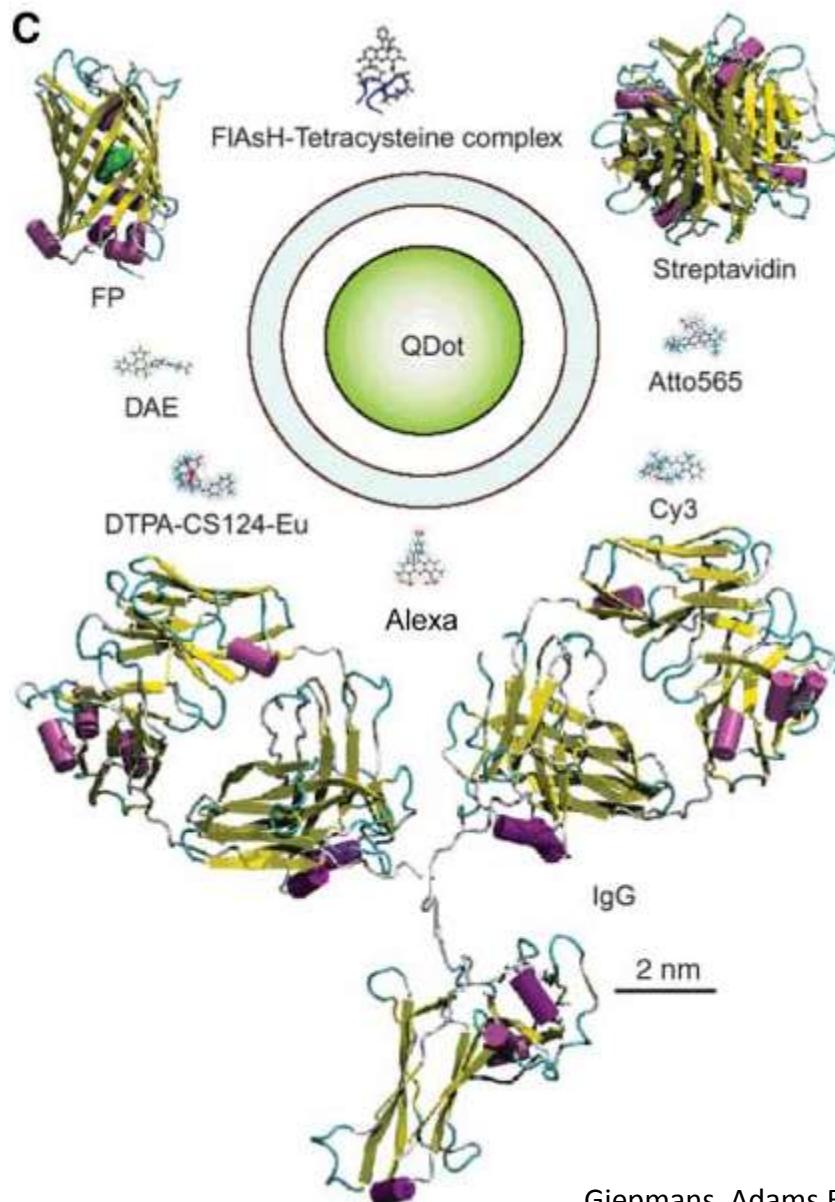
	ex/em maxima (nm)	Molar extinction coefficient	Fluorescence quantum yield	pKa	O <sub>2</sub> requirement	Number of amino acids	Mr
UnaG	498/527	77,300 (498 nm)	0.51	< 4.0	no	139	16.5 K
EGFP	490/509	49,550 (490 nm)	0.60	5.8	yes	238	27 K



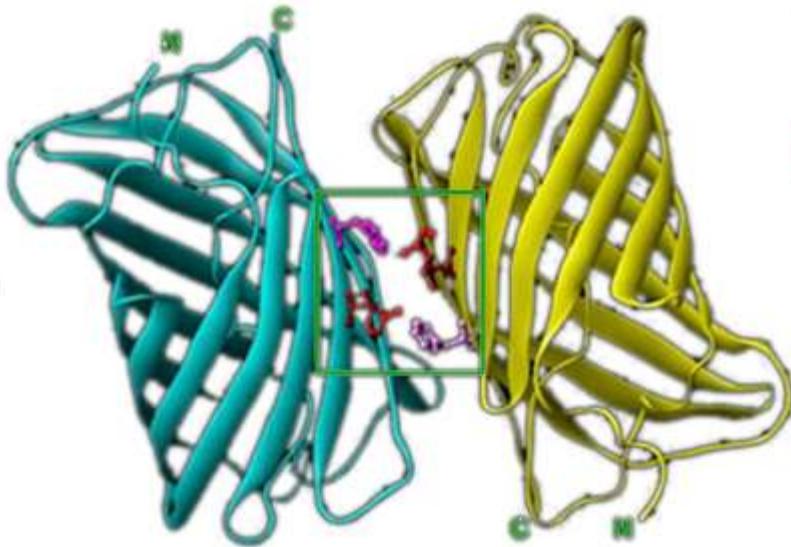
**CAUTION**

**GFP  
ARTIFACTS**

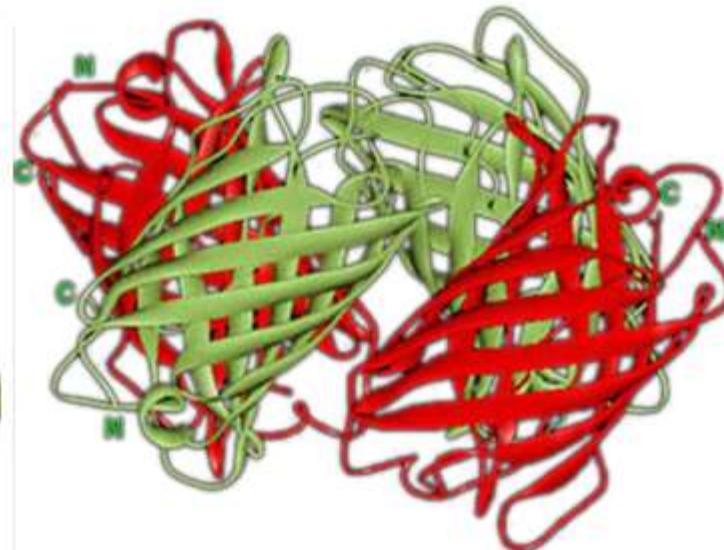
# Labeling without disturbing the system



# Oligomerization



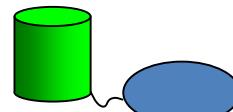
GFP: weak dimer



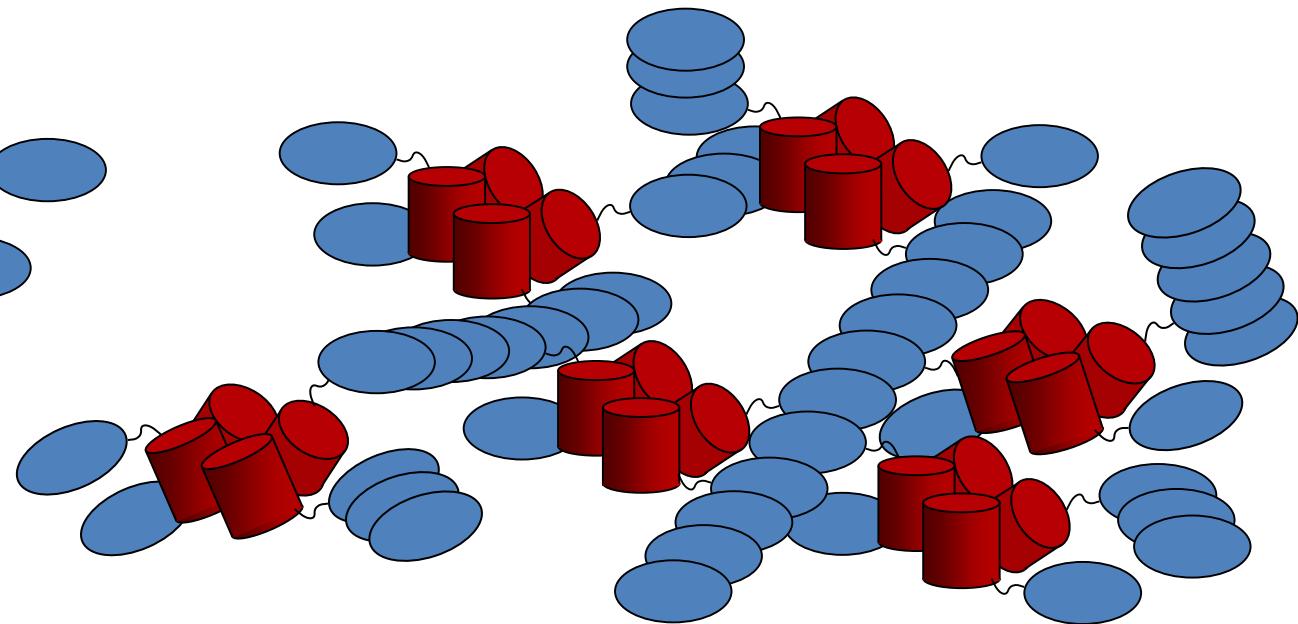
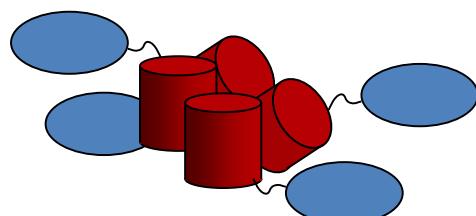
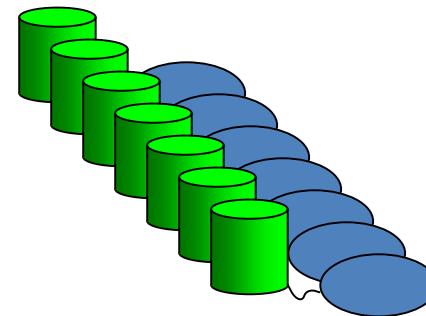
dsRed: Tetramer  
tdTomato, Kaede, tdEosFP, etc.

# Why is oligomerization a problem?

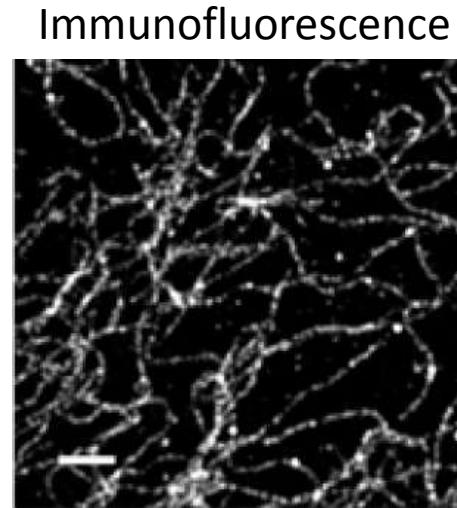
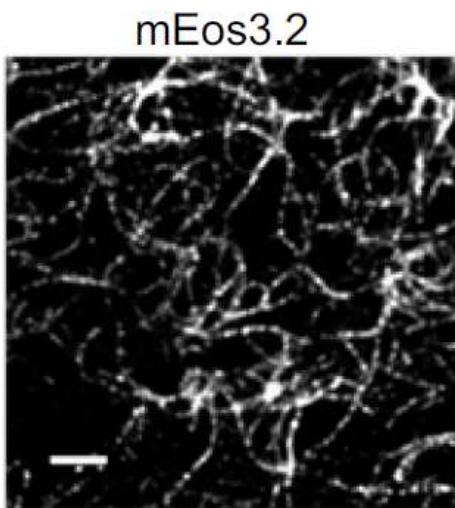
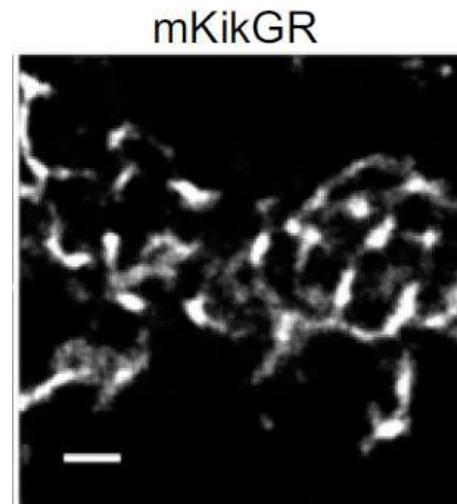
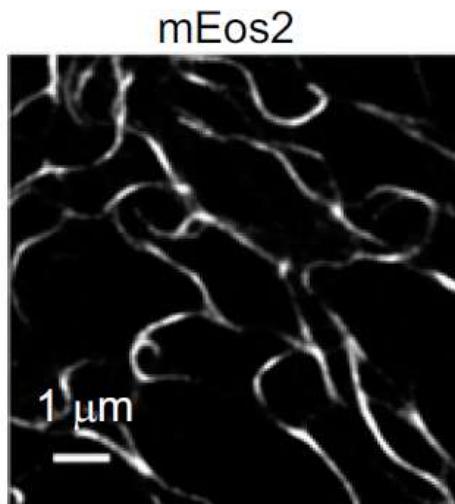
Fusion to inert protein  
or targeting sequence



Fused to interacting protein



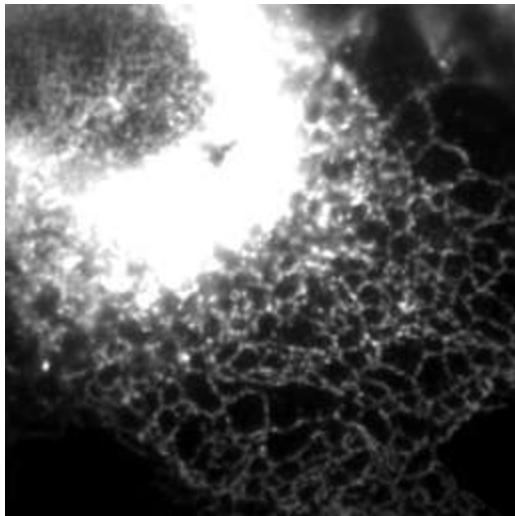
# Oligomerization artifacts



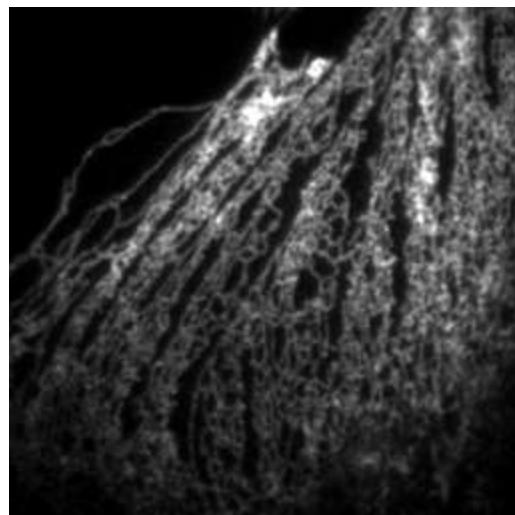
Super-resolution image of vimentin with various FP fusion and immunostaining

# Over expression of fused protein

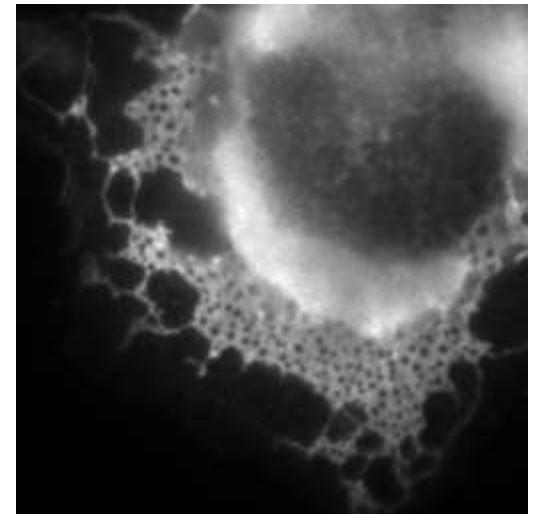
WT, immunofluorescence



GFP-rtn4a, stable cell line



FLAG-climp, transient expression



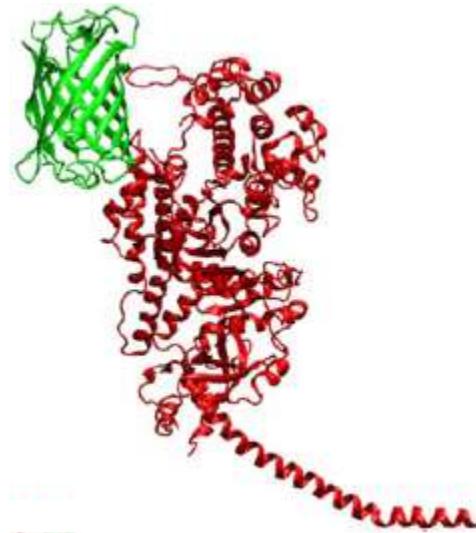
# Over expression of free FP

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

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

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 interpretation of data.



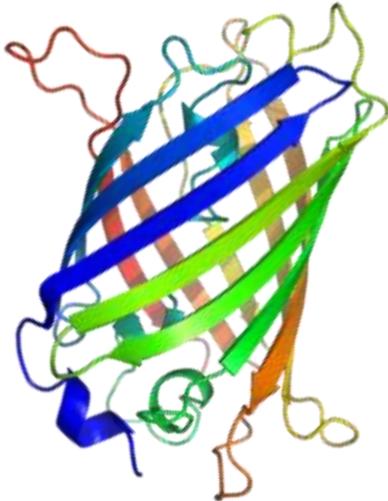
Agbulut et al., *J. Biol. Chem.* 2007(282) 10465

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

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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!

