Nobel Prize in Chemistry 2008

The green fluorescent protein: discovery, expression and development

O. Shimomura, M. Chalfie, R. Tsien

Overview

- Nobel Prize in Chemistry
  - GFP as a revolutionary tool in biology
- History of GFP: key contributors
- GFP chemical properties
  - Maturation of the fluorophore
  - Spectra, pH and pKa
  - XFPs chemical properties
- Applications
  - Optical highlighters (protein dynamics)
  - pH sensors (endocytosis)
  - Apoptosis
  - Protein interactions (FRET and PCA)
  - Pathways (Ca^{++} sensors, phosphorylation, etc.)

Nobel Prize in Chemistry

GFP as a revolutionary tool in biology

During the last century…

Development of biochemistry Enzyme structure and function	⟷	*Crystallography* *NMR*
Genetics revolution	⟷	*Bioinformatics*
Development of tools to study dynamic behaviour of living systems: GFP	⟷	*Light microscopy* *Computational power* *Molecular modelling*

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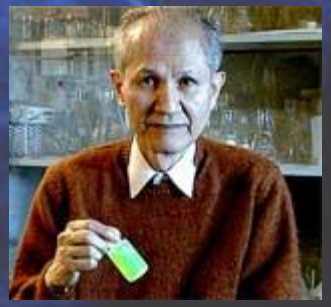
History of GFP: key contributors

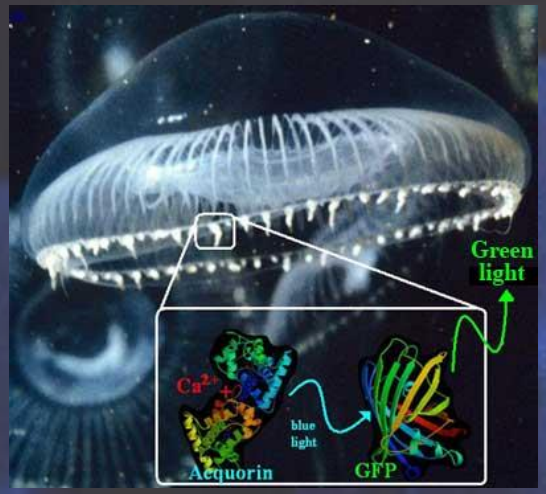


Osamu Shimomura: discovery, purification, characterization of GFP.

1960s isolated GFP, identified the fluorophore.

Interest: Aequorea bioluminescence,

its chemistry and biochemistry.



Aequorea Victoria

http://www.conncoll.edu/ccacad/zimmer/GFP-ww/shimomura.html

History of GFP: key contributors

Douglas Prasher 1980s-1990s
The first to realize the potential of GFP as a tracer. Isolated GFP gene but could not express fluorescent GFP in bacteria.

Martin Chalfie 1990s
Expressed fluorescent GFP in E.coli, then also in C.elegans, and demonstrated its use to monitor gene expression.

Fig. 1. Expression of GFP in *E. coli*. The bacteria on the right side of the figure have the GFP expression plasmid. Cells were photographed during irradiation with a hand-held long-wave UV source.

D. Prasher *et al. Gene* **1992**, 111, 229.
M. Chalfie *et al. Science* **1994**, 263, 802..

History of GFP: key contributors

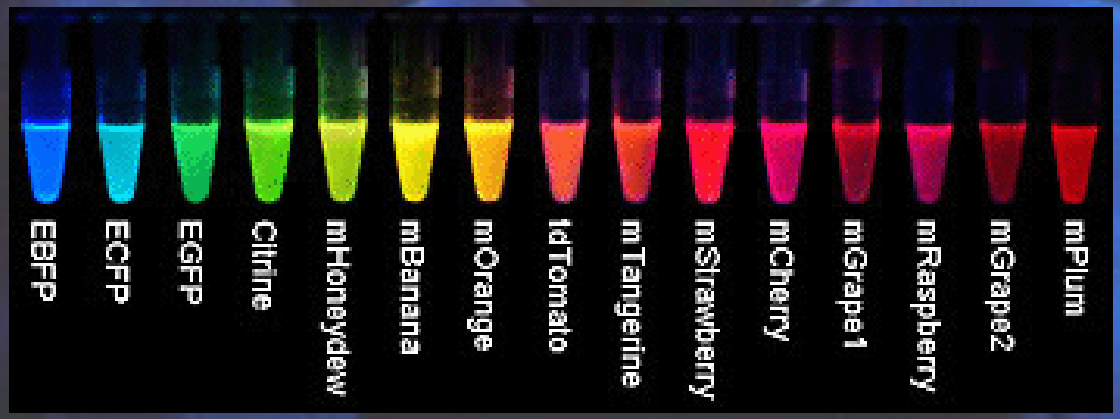
Sergey A. Lukyanov 2000s

Found GFP-like proteins in corals (Anthozoa species):

Found dsRed.

Roger Tsien 1990s-2000s http://www.tsienlab.ucsd.edu

Identified the chemistry of maturation of the GFP fluorophore. Developed enhanced mutants of GFP.



Y. Yanushevich *et al. FEBS lett.* **2002**, 511, 11.

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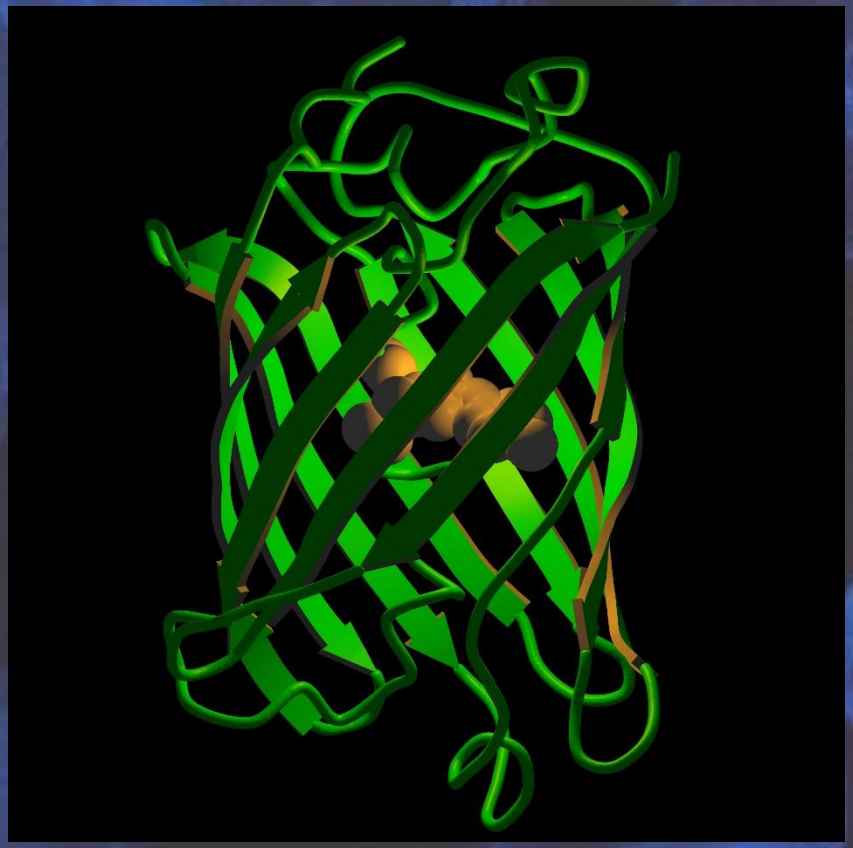
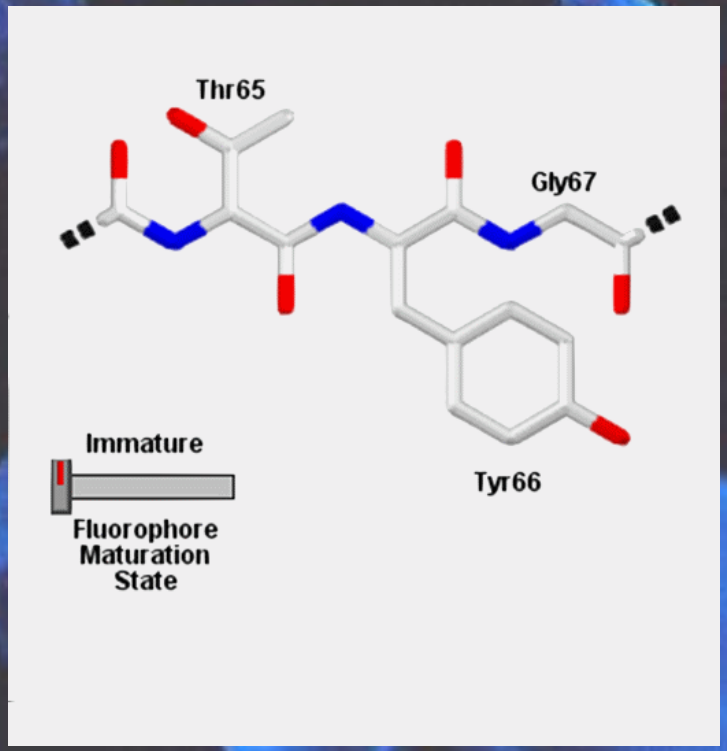
GFP chemical properties

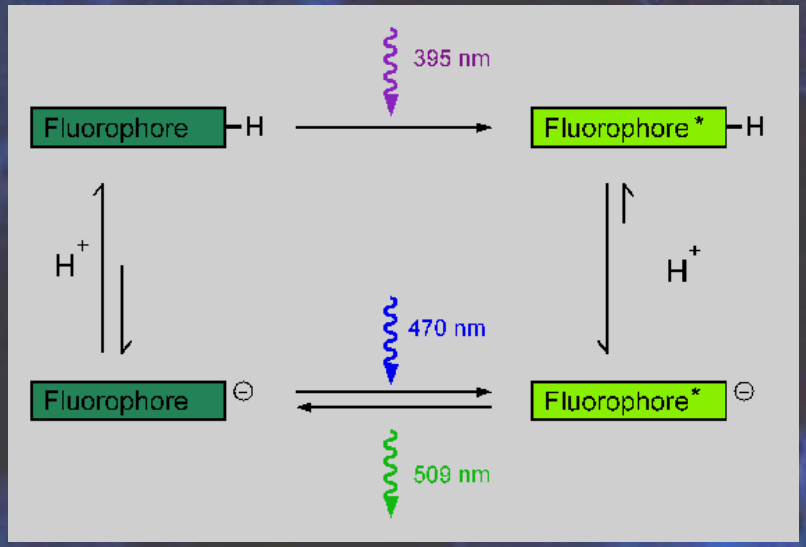


Maturation of EGFP fluorophore

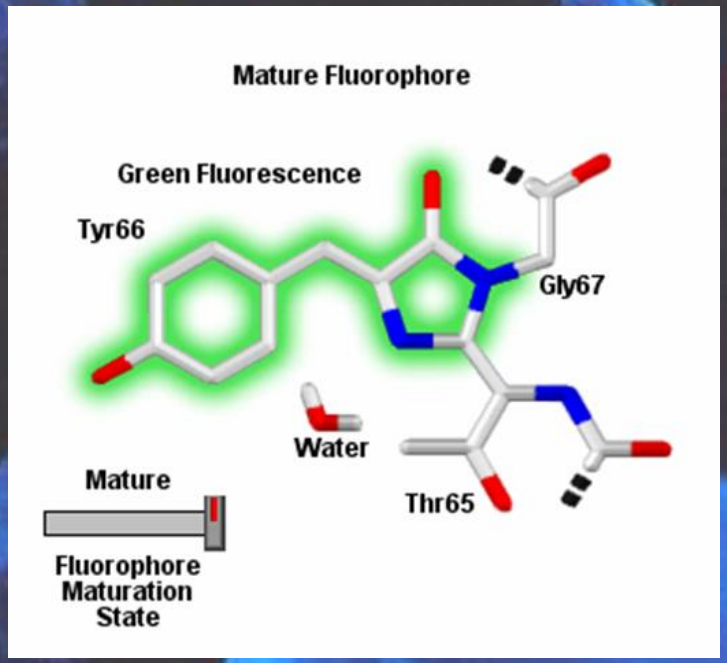


http://www.olympusconfocal.com/java/fpfluorophores/gfpfluorophore/index.html

GFP chemical properties



Maturation of EGFP fluorophore



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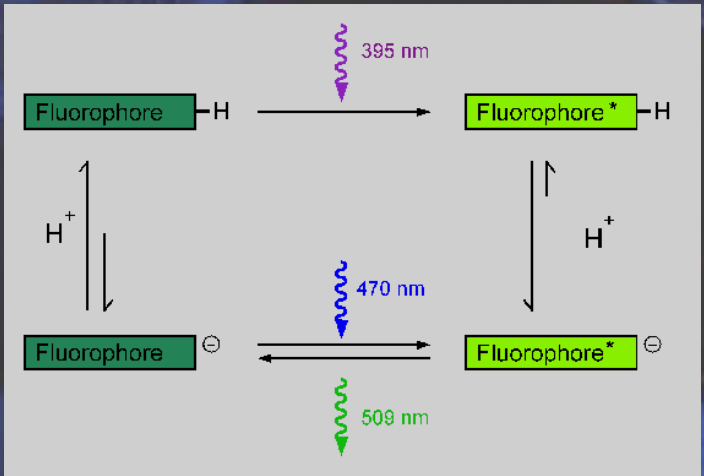
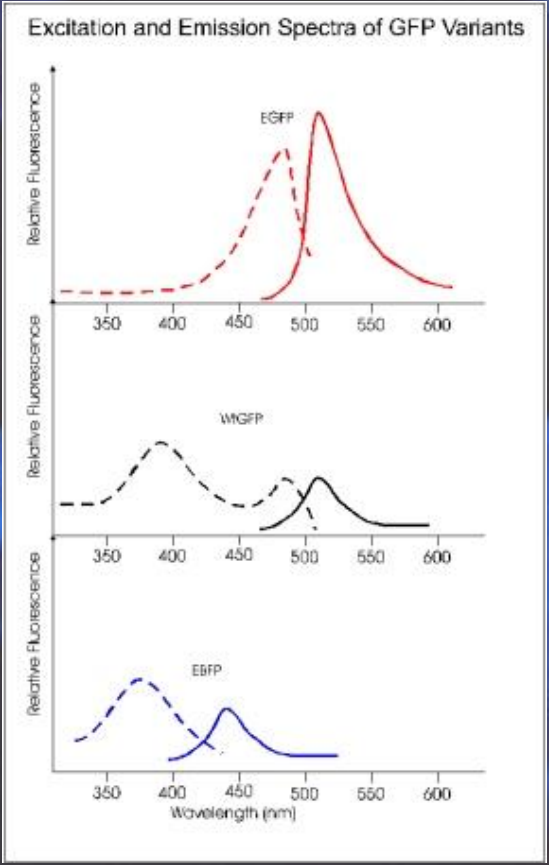
GFP chemical properties



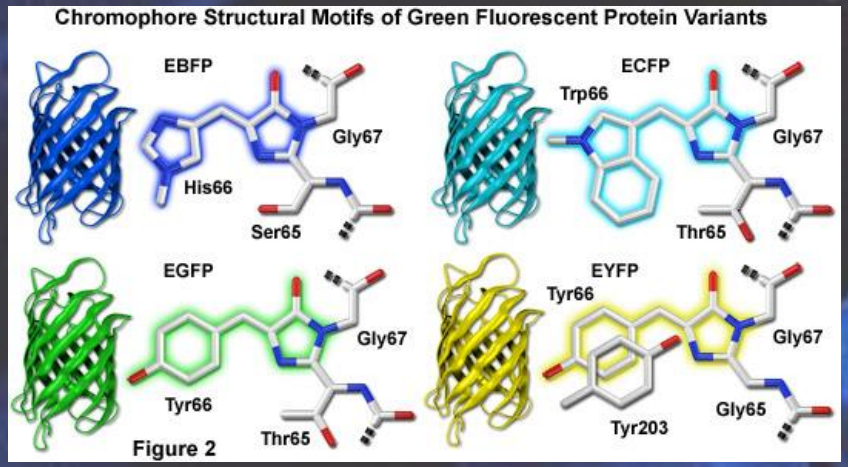


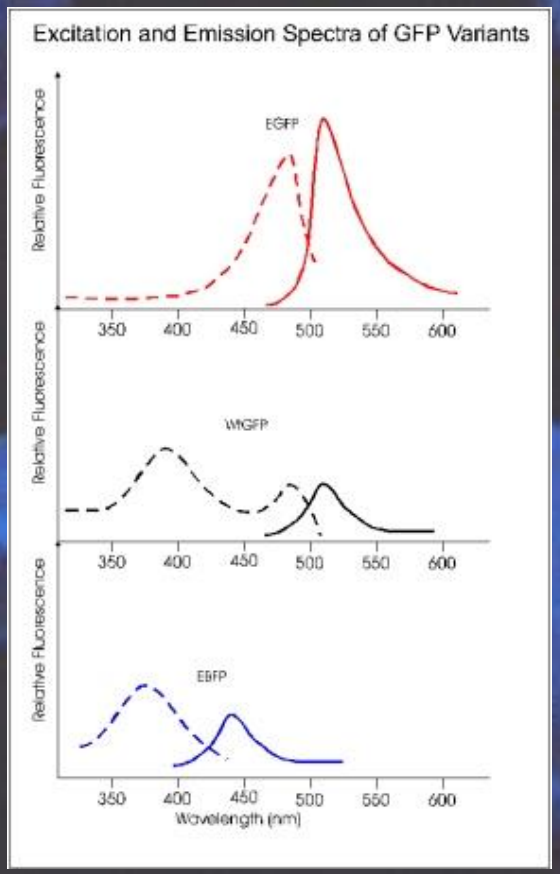
Ser 65 Thr

Tyr 66 His

http://www.microscopyu.com/articles/livecellimaging/fpintro.html

GFP chemical properties





Ser 65 Thr

Tyr 66 His

http://www.olympusconfocal.com/applications/fpcolorpalette.html

GFP chemical properties

Protein (Acronym)	Excitation Maximum (nm)	Emission Maximum (nm)	Molar Extinction Coefficient	Quantum Yield	*in vivo* Structure	Relative Brightness (% of EGFP)
GFP (wt)	395/475	509	21,000	0.77	Monomer*	48
Green Fluorescent Proteins						
EGFP	484	507	56,000	0.60	Monomer*	100
Emerald	487	509	57,500	0.68	Monomer*	116
Blue Fluorescent Proteins						
EBFP	383	445	29,000	0.31	Monomer*	27
Sapphire	399	511	29,000	0.64	Monomer*	55
Cyan Fluorescent Proteins						
mCFP	433	475	32,500	0.40	Monomer	39
Cerulean	433	475	43,000	0.62	Monomer*	79
Yellow Fluorescent Proteins						
EYFP	514	527	83,400	0.61	Monomer*	151
mBanana	540	553	6,000	0.7	Monomer	13
Venus	515	528	92,200	0.57	Monomer*	156
Orange and Red Fluorescent Proteins						
mOrange	548	562	71,000	0.69	Monomer	146
dTomato	554	581	69,000	0.69	Dimer	142
mCherry	587	610	72,000	0.22	Monomer	47
DsRed	558	583	75,000	0.79	Tetramer	176

* Weak Dimer

http://www.microscopyu.com/articles/livecellimaging/fpintro.html

XFPs chemical properties

- ***pH sensitivity***
  - ***pKa ~ 7 are more sensitive to pH changes in the cell***
- ***Anions sensitivity (chloride)***
  - ***Chloride binding pocket near chromophore***
  - ***Interconnected with pKa***
- ***Folding at 37 C (fluorophore maturation)***
  - ***Important especially for PCA techniques***
- ***Photobleaching stability and reversible photobleaching***
- ***SDS-PAGE artefacts***
  - ***e.g. DsRed, if boiled, hydrolyses into 2 fragments***

O. Griesbeck *et al. JBC* **2001**, 276, 29188.

XFPs chemical properties

Wavelength Class	Protein	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
Far-red	mPlum	5.9	53	7.3	<4.5	100 min
Red	mCherry	23	96	13.1	<4.5	15 min
	tdTomato	138	98	13.5	4.7	1 hr
	mStrawberry	38	15	2.1	<4.5	50 min
	J-Red	13	13	1.8	5	ND
	DsRed-Monomer	5.1	16	2.2	4.5	ND
Orange	mOrange	71	9.0	1.2	6.5	2.5 hr
	mKO	45	122	16.7	5	4.5 hr
Yellow	mCitrine	85	49	6.7	5.7	ND
	Venus	76	15	2.0	6	ND
	YPet	116	49	6.7	5.6	ND
	EYFP	74	60	8.3	6.9	ND
Green	Emerald	57	0.69	0.1	6	ND
	EGFP	49	174	23.9	6	ND
Cyan	CyPet	26	59	8.1	5	ND
	mCFP	19	64	8.8	4.7	ND
	Cerulean	39	36	5.0	4.7	ND
UV-excitable green	T-Sapphire	38	25	3.5	4.9	ND
Reference	fluorescein pH 8.4	100	7.3	1.0	6.4	

N. C. Shaner *et al. Nature Methods* **2005**, 12, 905.

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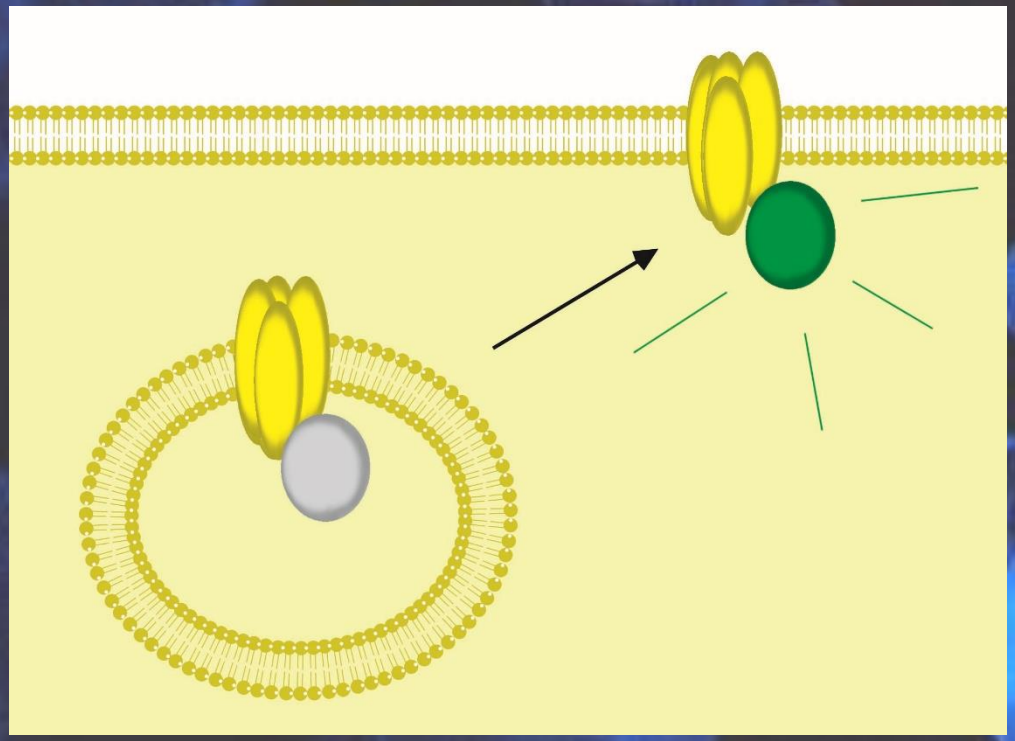
Applications

pH sensors: imaging of endocytosis



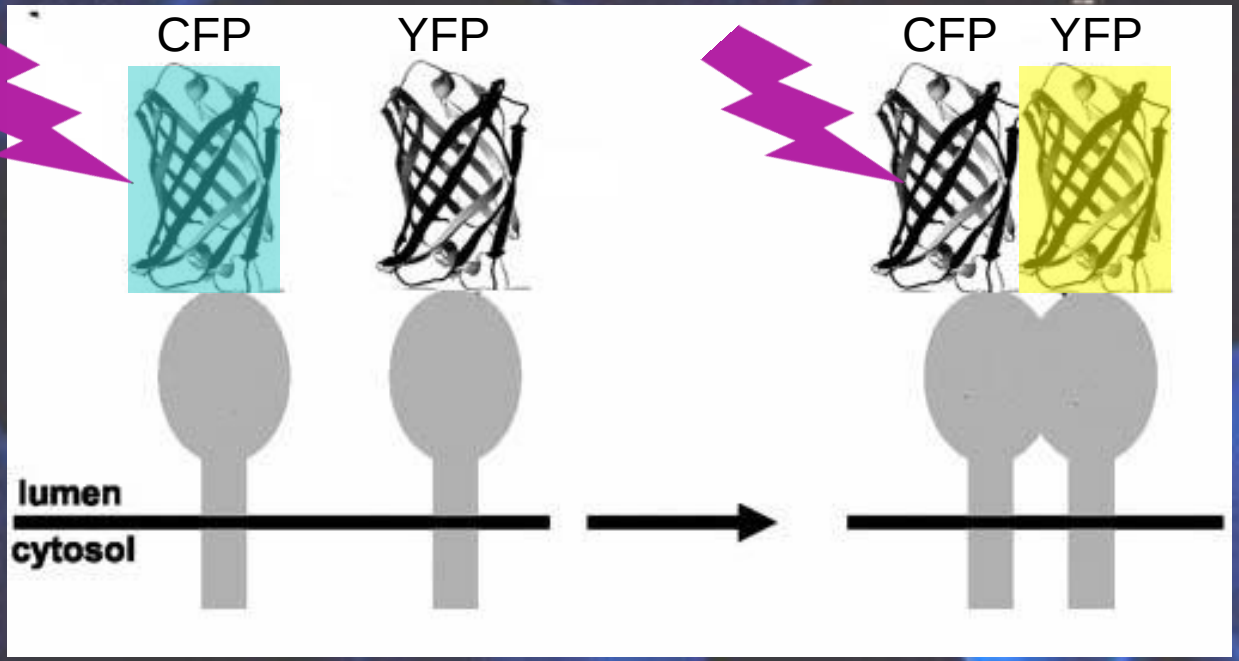
Intracellular vesicles have a pH of 5.0 - 5.5 (pHluorin is **not** visible).
When the receptor is placed on the cell surface, the pHlourin is exposed to the extracellular pH (~7.4),
it becomes
visible under blue illumination.

G. Miesenbock *et al. Nature* **1998**, 394, 192. www.tsienlab.ucsd.edu

Applications

Protein interactions: FRET and PCA

Fluorescence Resonance Energy Transfer



B. S. Nyfeler *et al., PNAS* **2005**, 102, 6350.

Useful References

- Tsien's lab ***www.tsienlab.ucsd.edu***
- Phogemon ://www.path1.med.kyoto-u.ac.jp/mm/e-phogemon
- Olympus www.olympusconfocal.com
- Nikon www.microscopyu.com