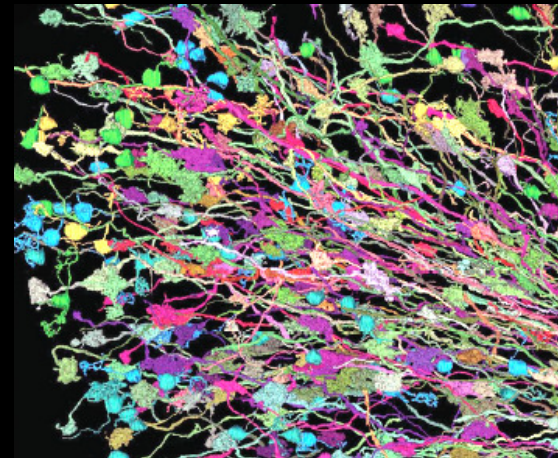


# De cómo las medusas nos enseñaron a colorear las células

Javier García-Sancho,  
Instituto de Biología y Genética Molecular  
(IBGM), Universidad de Valladolid y CSIC

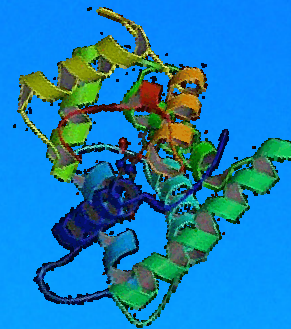
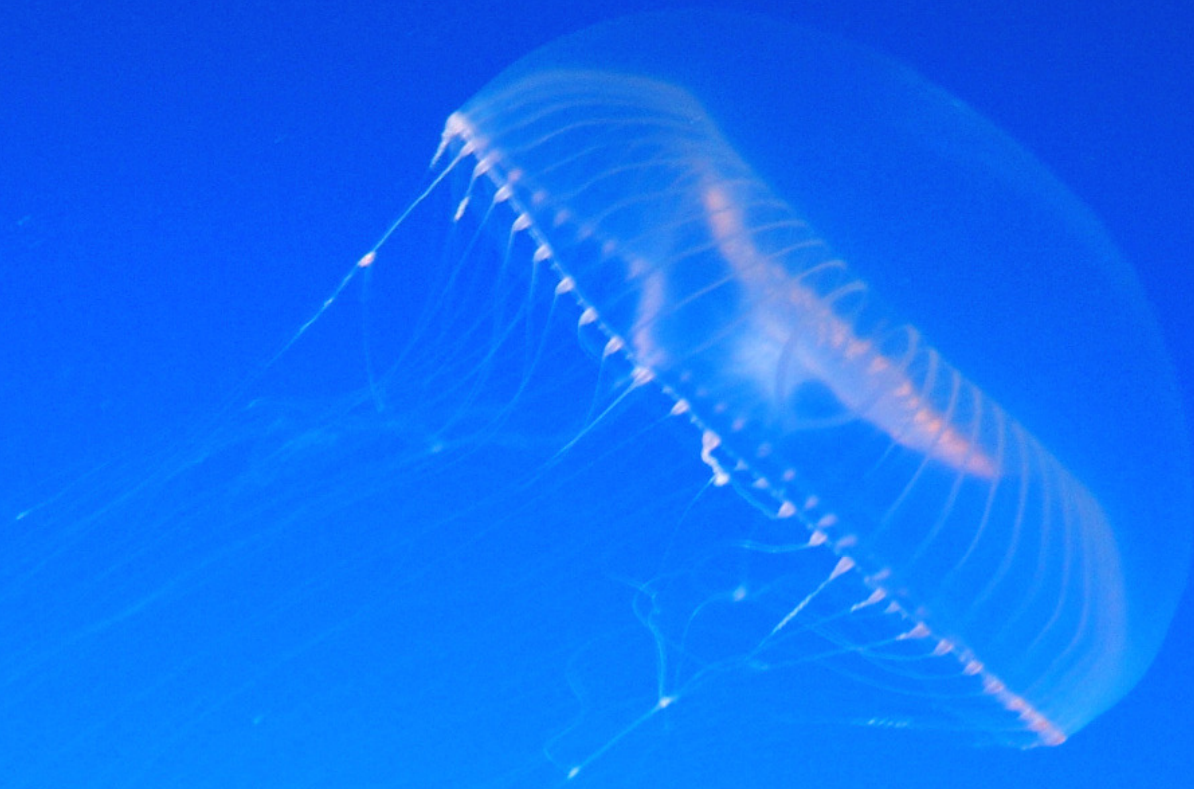


Universidad de Granada, 5 de Abril 2011

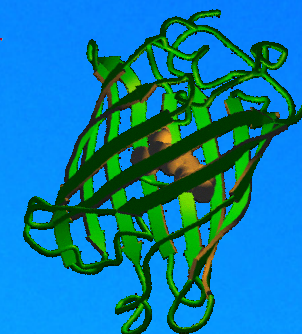


Osamu Shimomura,  
Martin Chalfie, y  
Roger Y. Tsien  
Premios Nobel de Química 2008

*Aequorea Victoria*



*Aequorin*

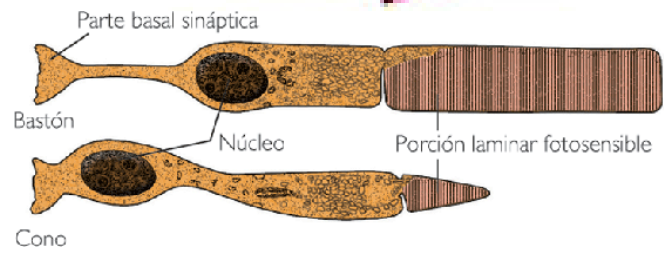
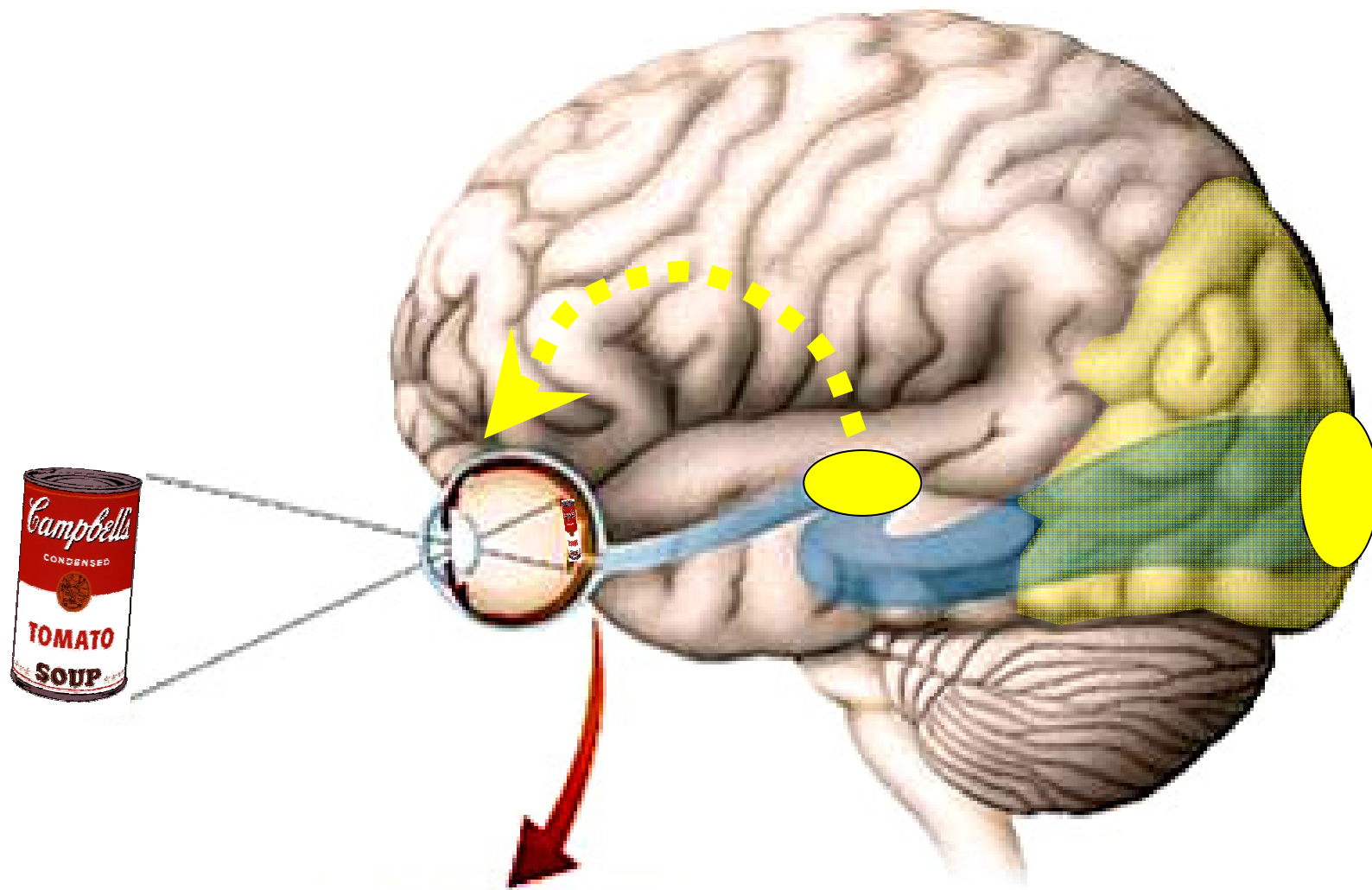


*GFP*

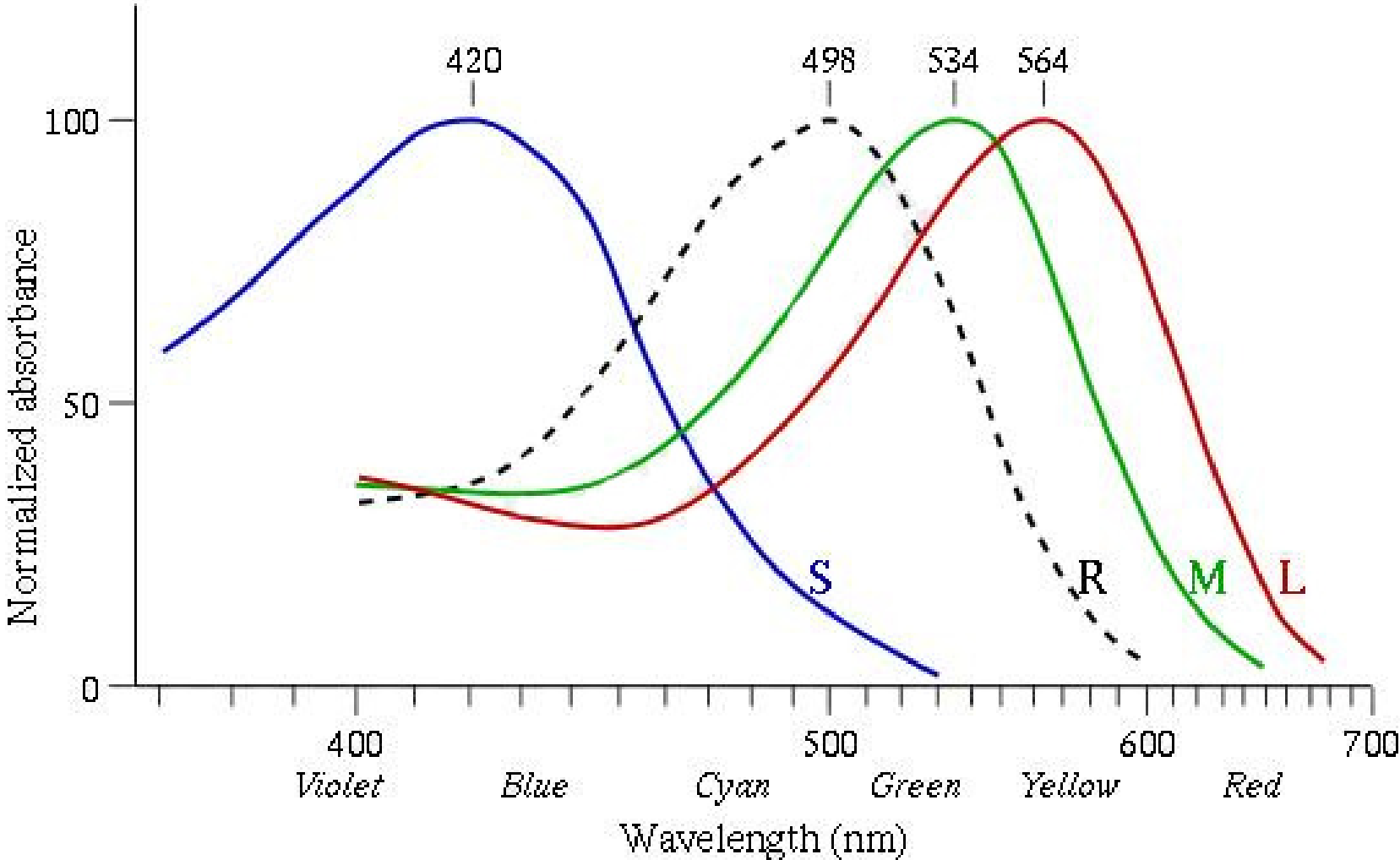




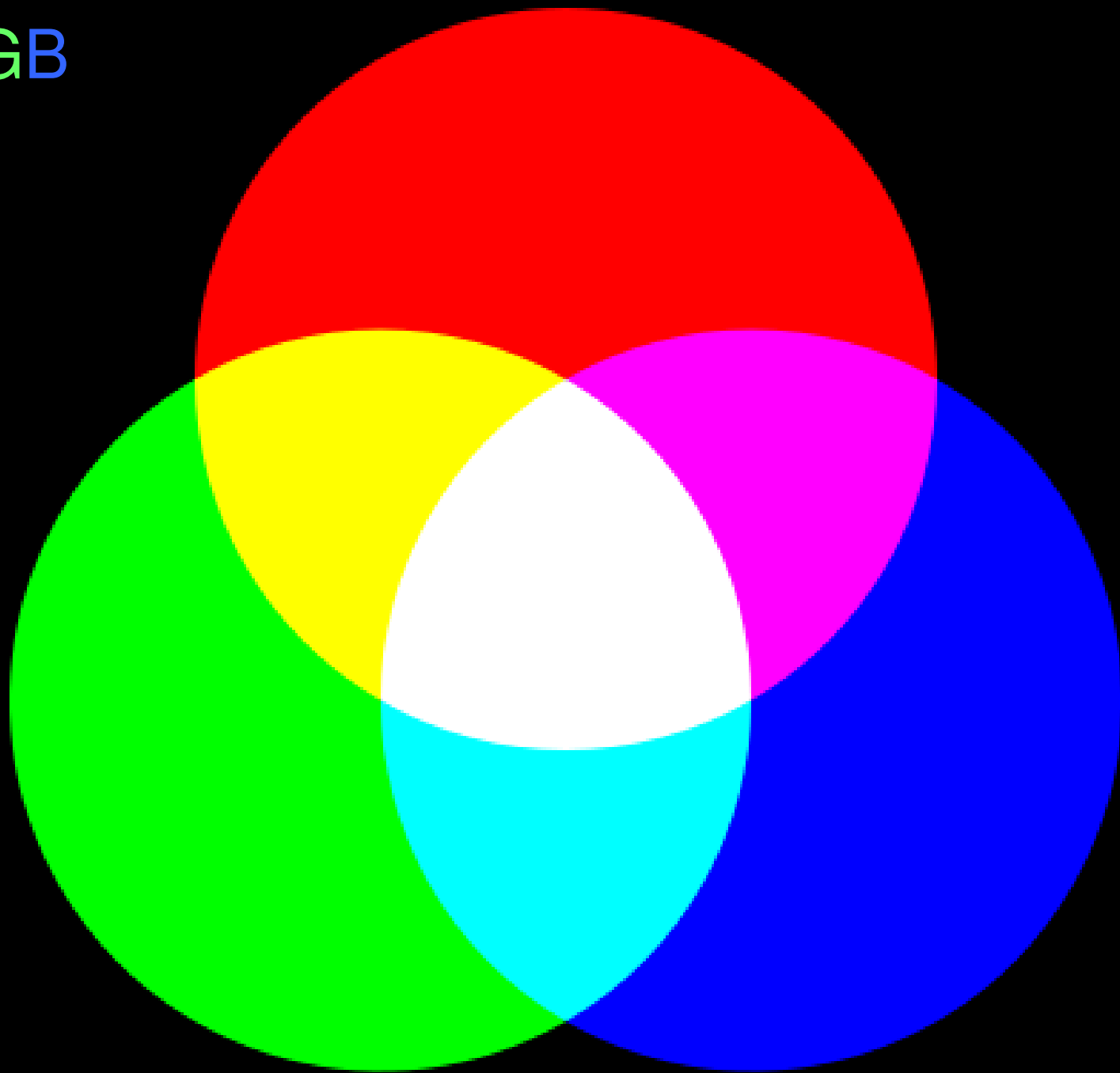
Mira como huele esta flor  
¿Ves que bien suena esta melodía?



# CONOS Y BASTONES



RGB

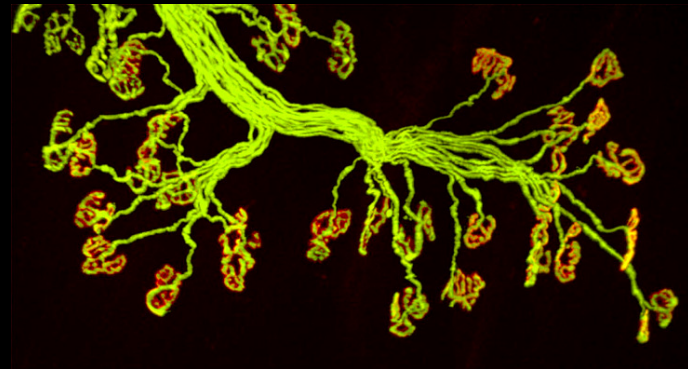
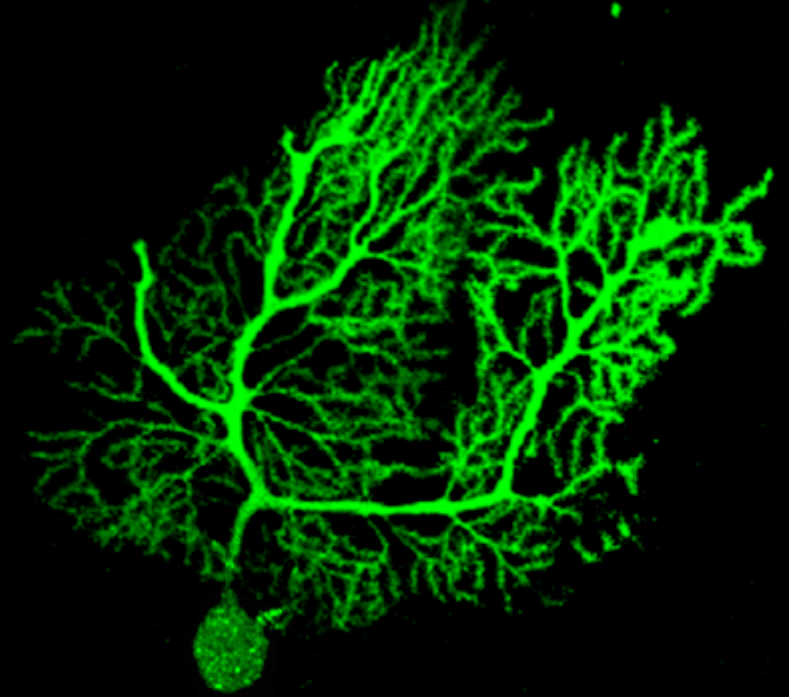
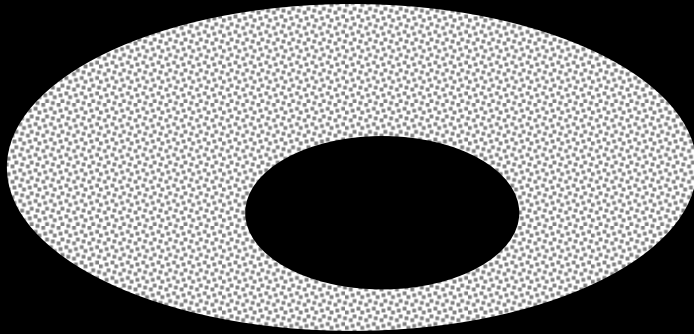
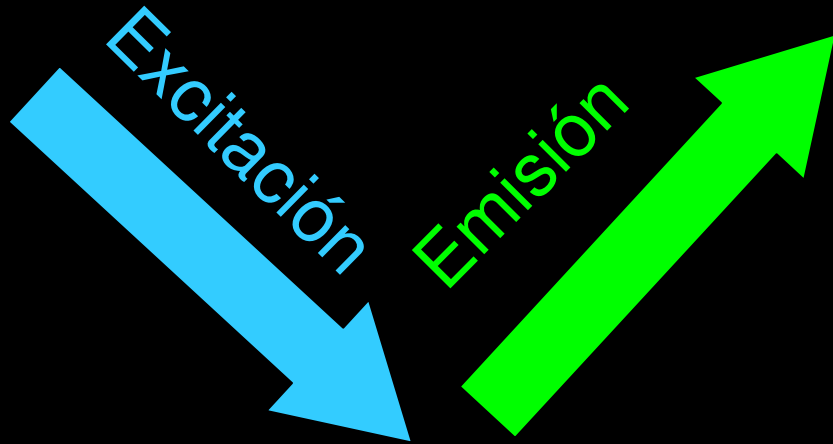


# COLORantes

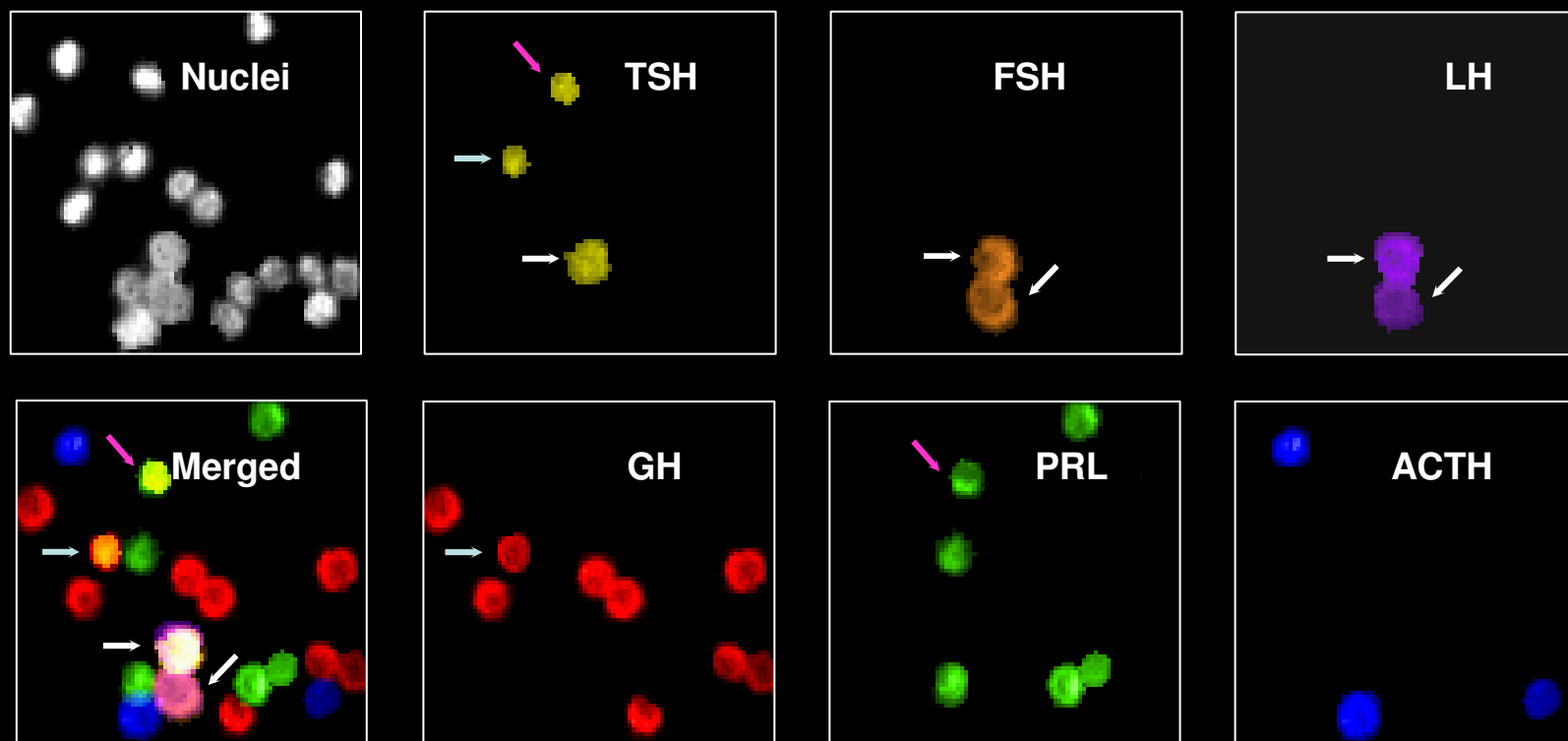




# Fluorescencia



# Inmunofluorescencia múltiple de 20 células adenohipofisarias



Nuñez *et al.* (2003) *J. Physiol.* **549**: 835–843

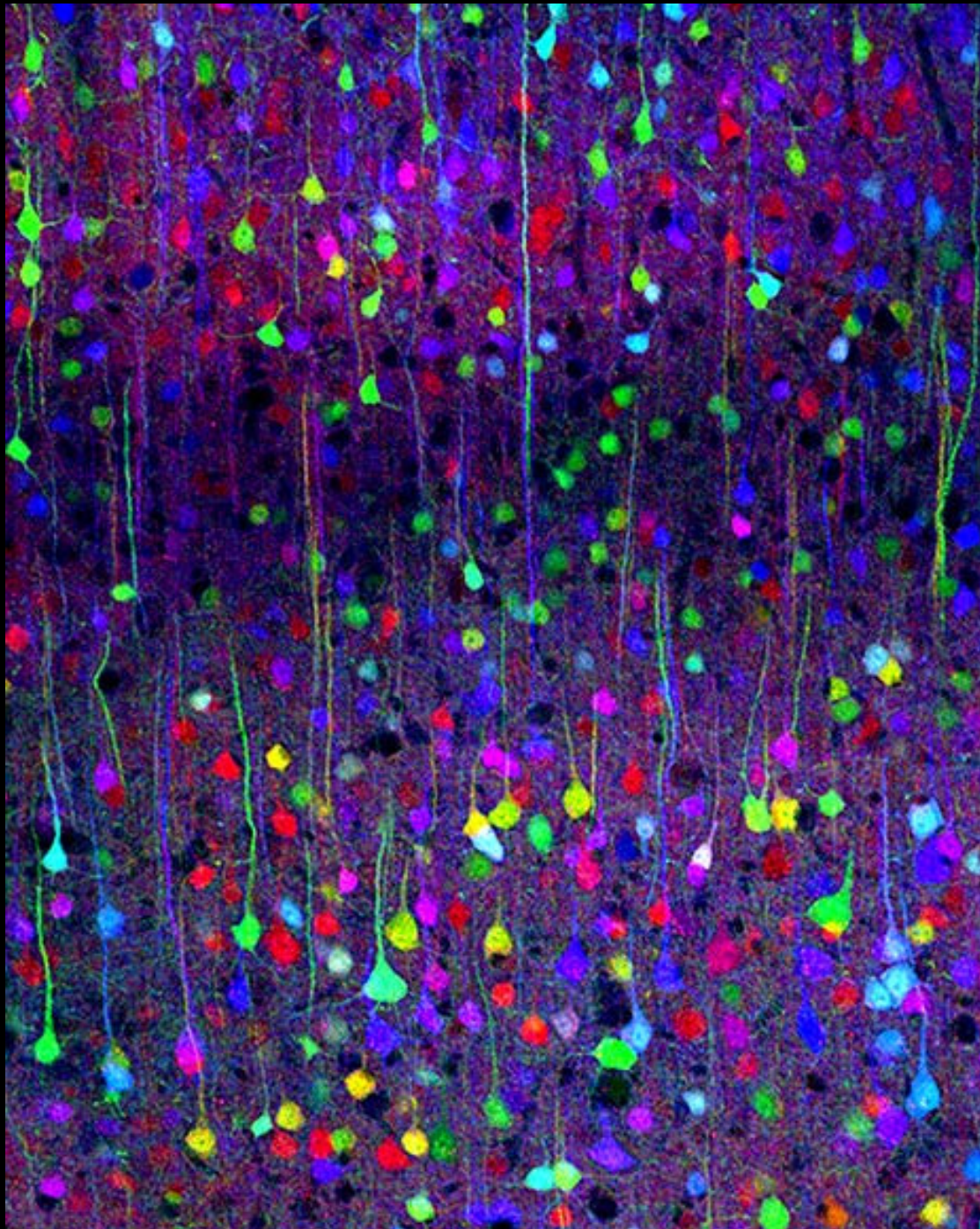
**a** XFP combinations

Outcome for  
each copy    Resulting  
                  colour

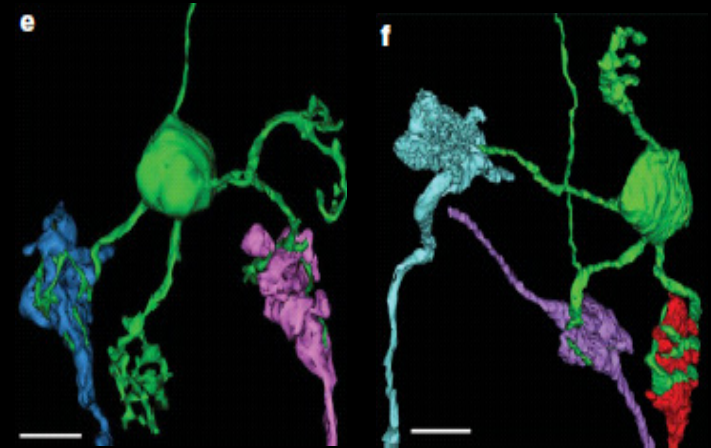
1	2	3	
C	C	C	Blue
C	C	Y	Light blue
C	Y	Y	Blue-green
Y	Y	Y	Green
Y	Y	R	Light green
Y	R	R	Orange
R	R	R	Red
R	R	C	Magenta
R	C	C	Purple
R	C	Y	Grey

**b**



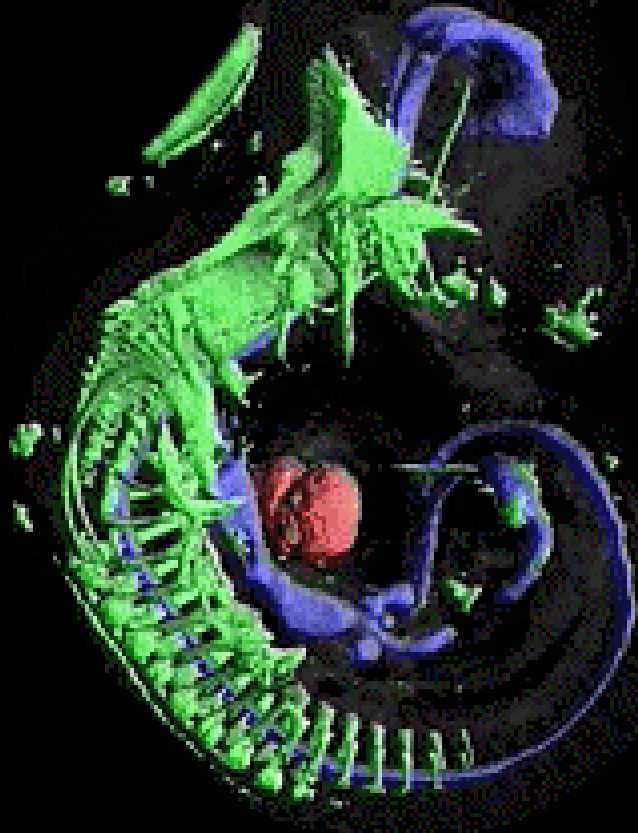


Expresión combinatoria de  
proteínas fluorescentes en  
el SNC



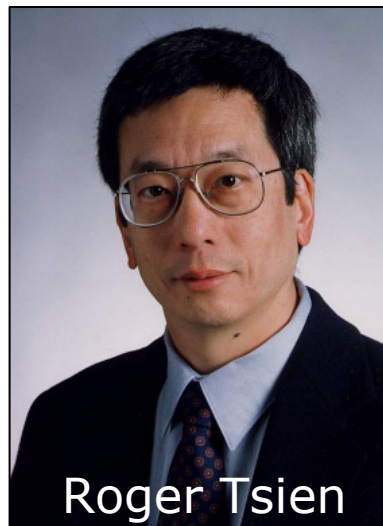
“Brainbow”  
Livet *et al.* (2007)  
*Nature* 450:56-62

# Reconstrucción tridimensional de un Embrión

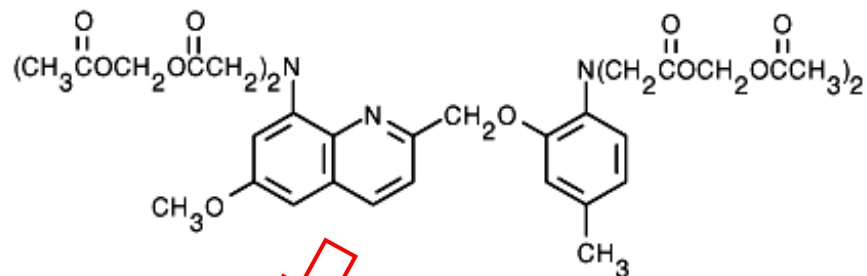


Cortesía de:  
Dr .James Sharpe ,  
MRC Human Genetics Unit,  
Edinburgh , U.K.

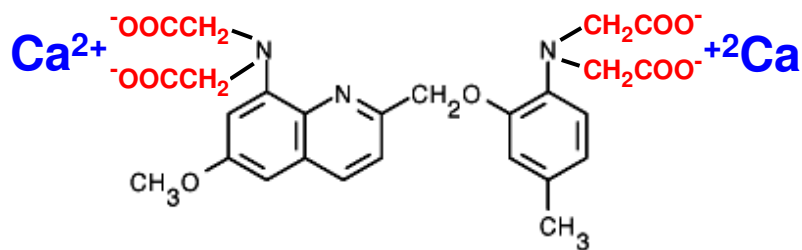
# SONDAS FLUORESCENTES



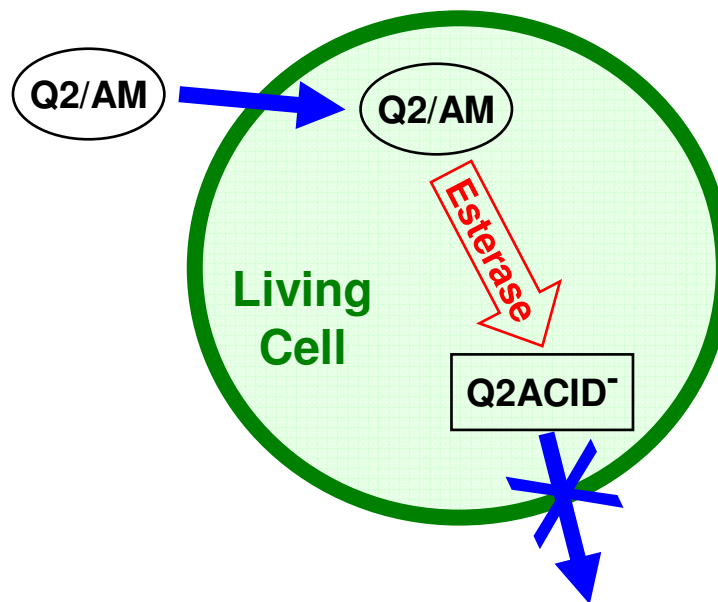
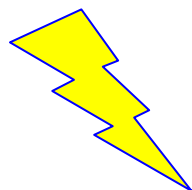
QUIN2/AM

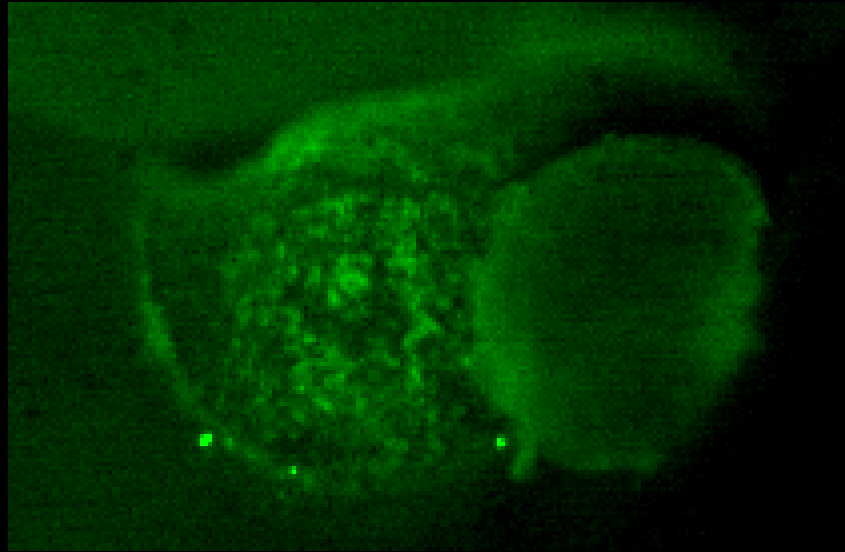


Loading into cells



QUIN2 ACID





Latidos espontáneos en el corazón embrionario  
de pollo cargado con la sonda Fluo-3.

(JGS & JM Pérez-Pomares 2008)

# LATIDOS ESPONTÁNEOS EN CARDIOMIOCITOS DE RATA CARGADOS CON FLUO-4

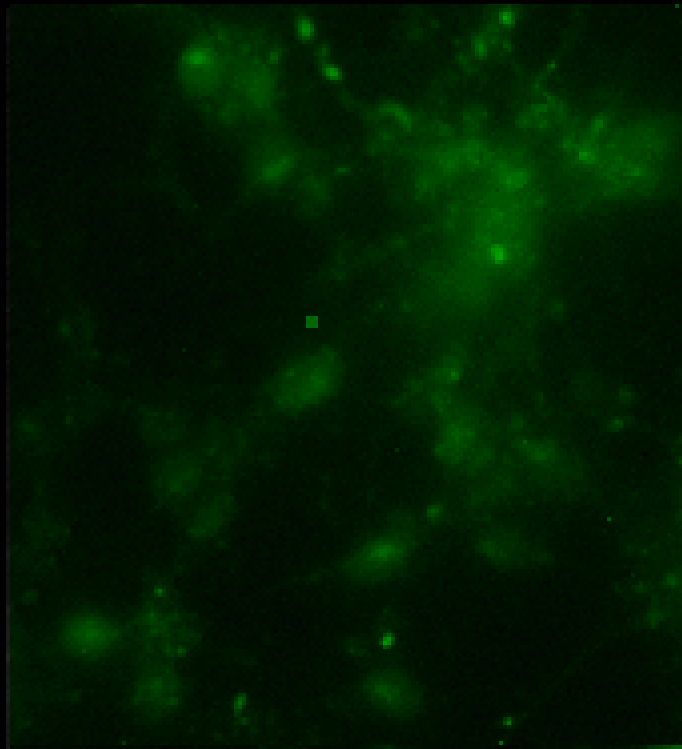
Experimento realizado por  
Christine Whalquist,  
IBGM, Universidad de Valladolid



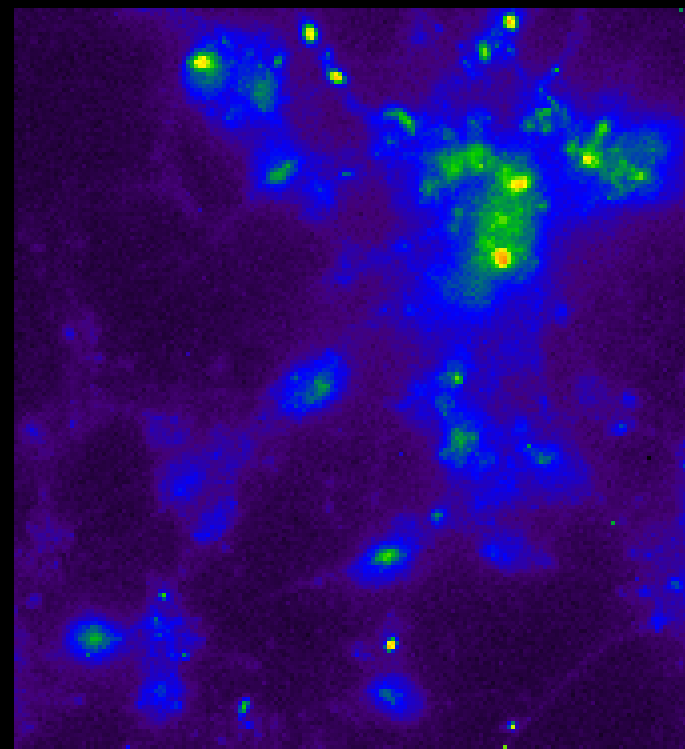


# $[Ca^{2+}]_c$ oscillations in cerebellar granule neurons networks

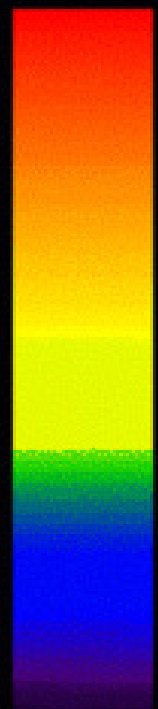
$[Ca^{2+}]_i$  ↑



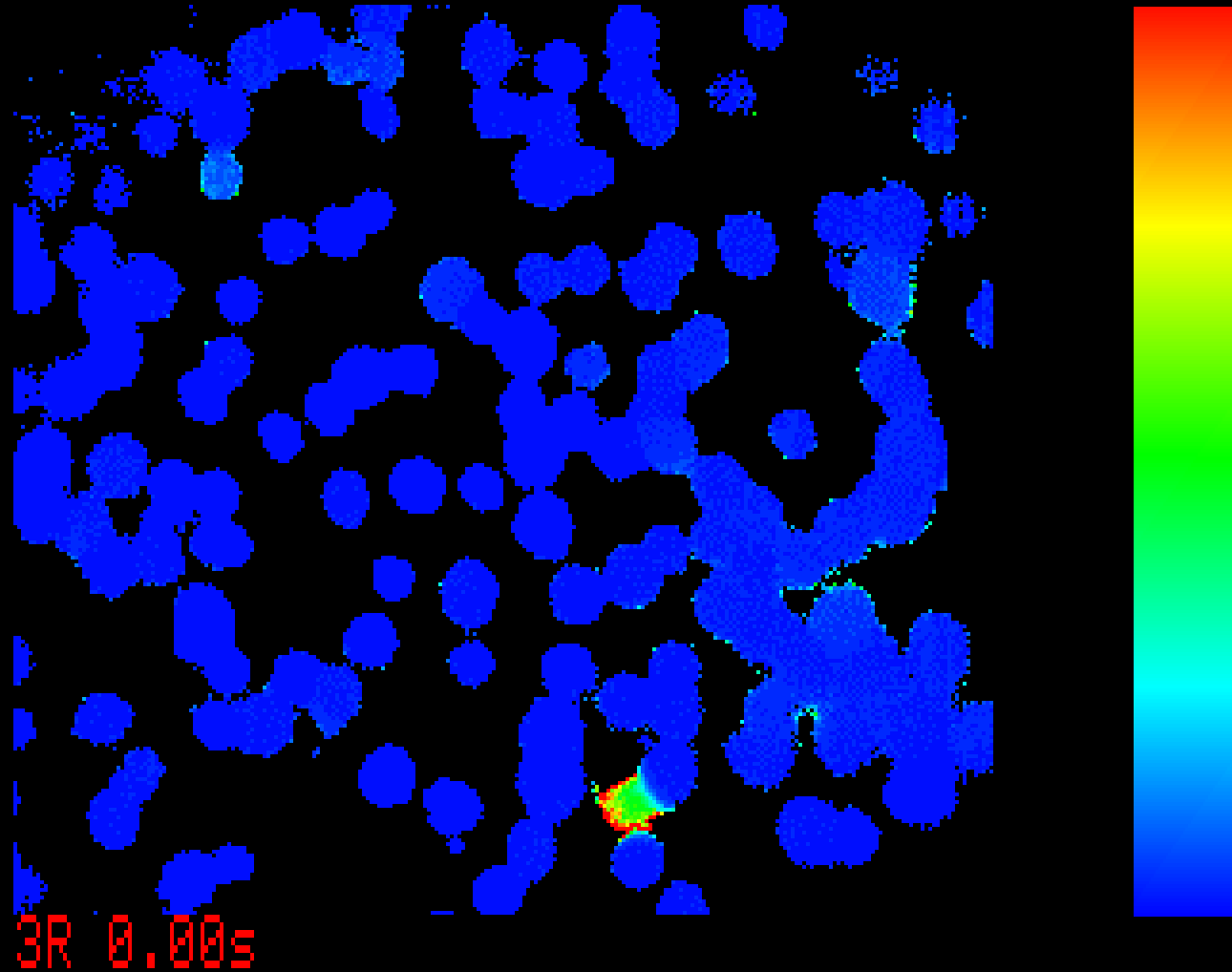
GREEN



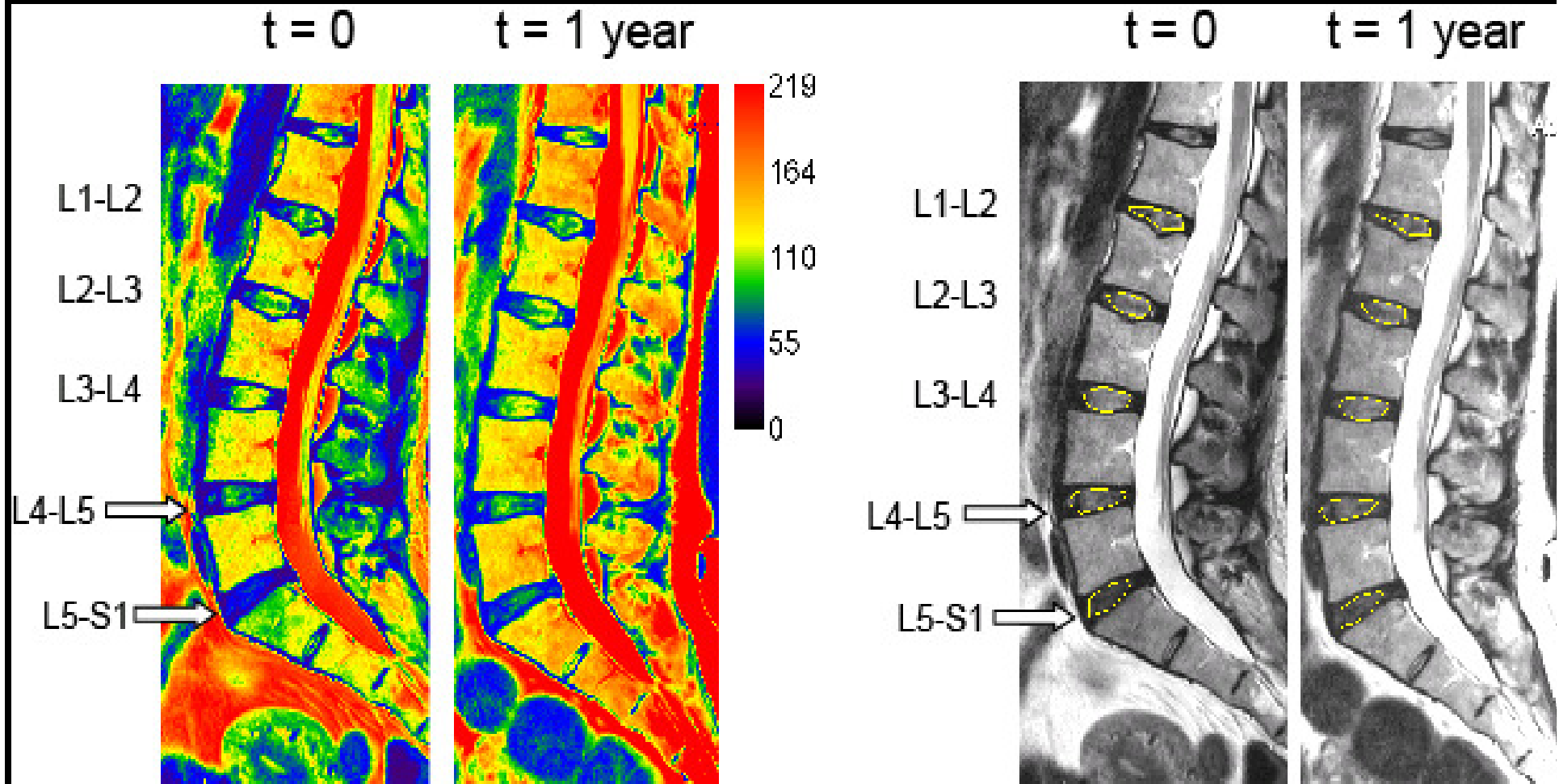
PSEUDOCOLOR



# Estimulación de linfocitos T por capsaicina y carbacol



# Quantification of the water content of intervertebral discs in T2-weighted MRI images



# Luminiscencia

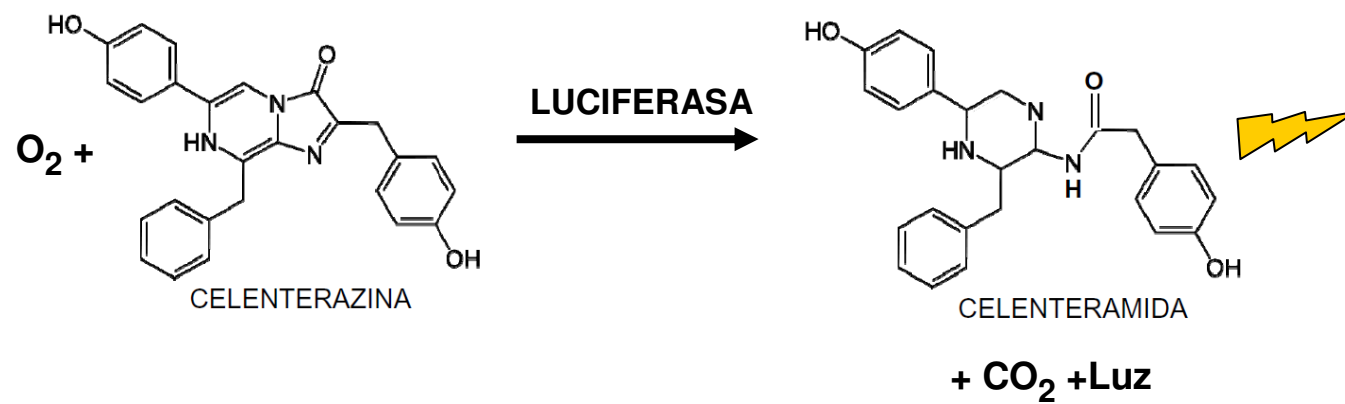
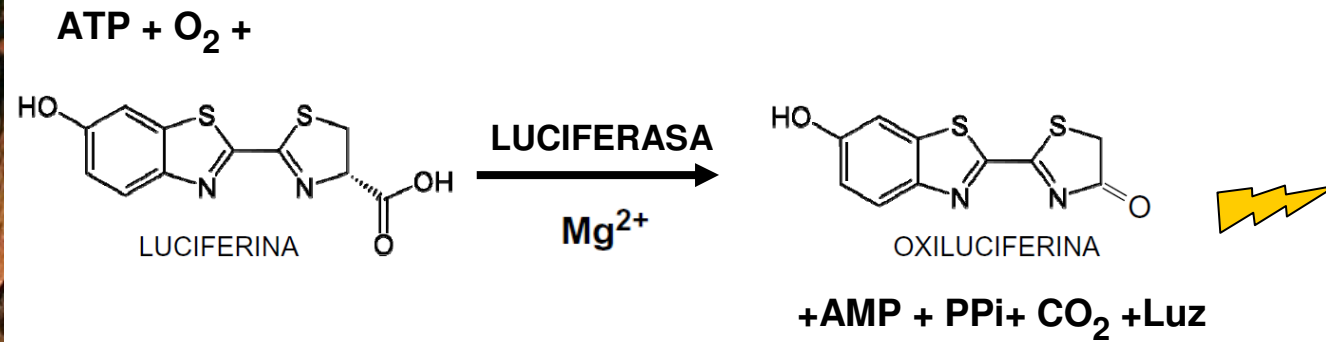
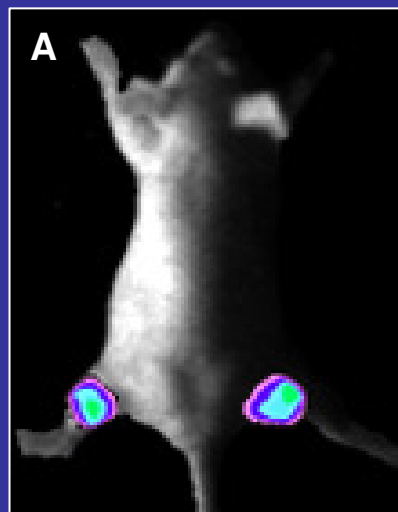
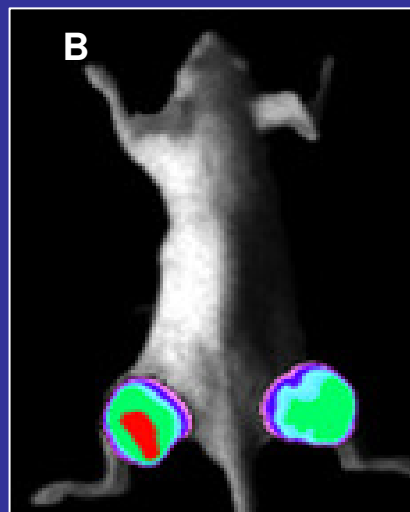


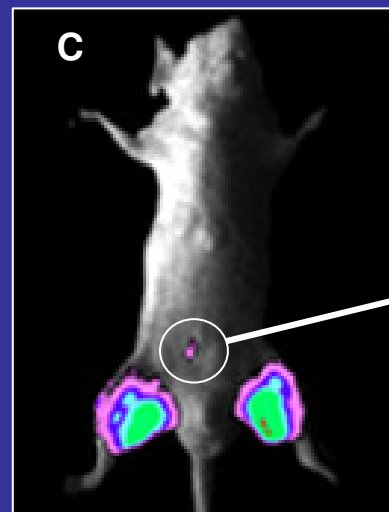
IMAGEN NO INVASIVA. Tumor sólido PC3-S-luc, inoculado en región inguinal de ratones immuno-deprimidos, seguido por fotoluminiscencia tras inyección de luciferina



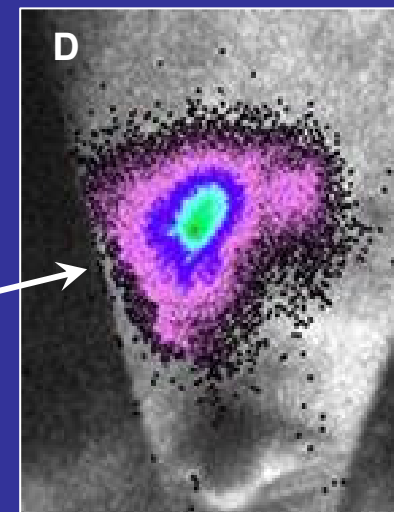
4 Semanas.  
Dorsal



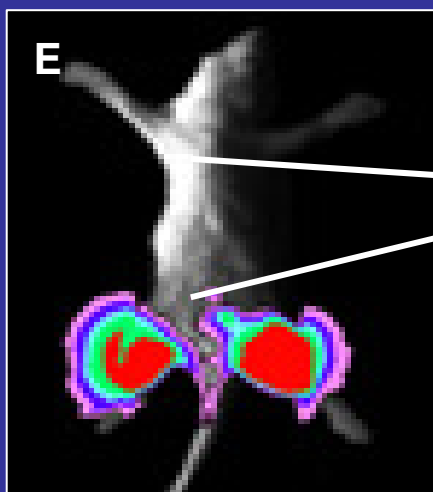
6 Semanas.  
Dorsal



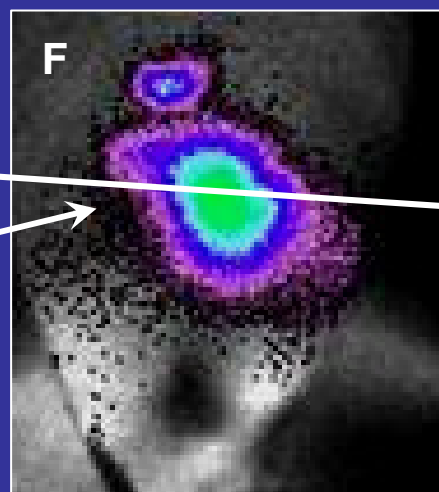
6 Semanas.  
Ventral.



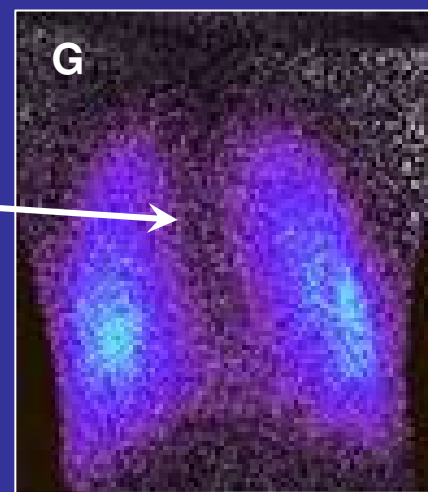
Metástasis en  
Nódulo linfático



10 Semanas. Ventral



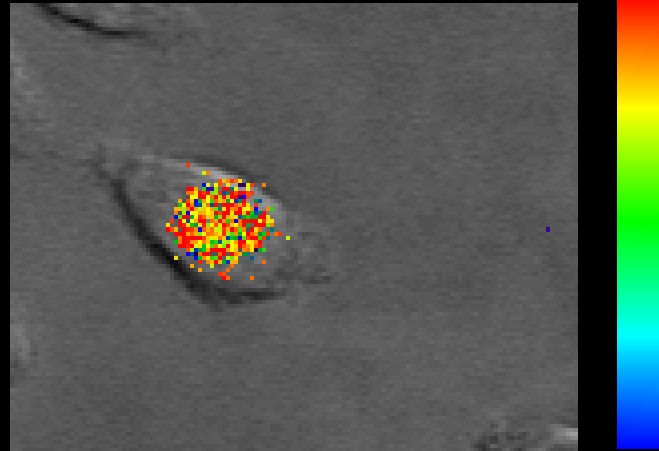
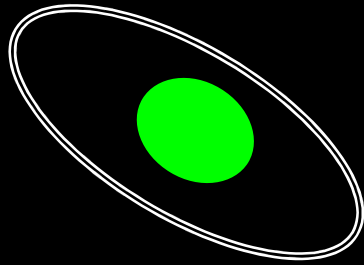
Metástasis en  
Nódulo linfático.



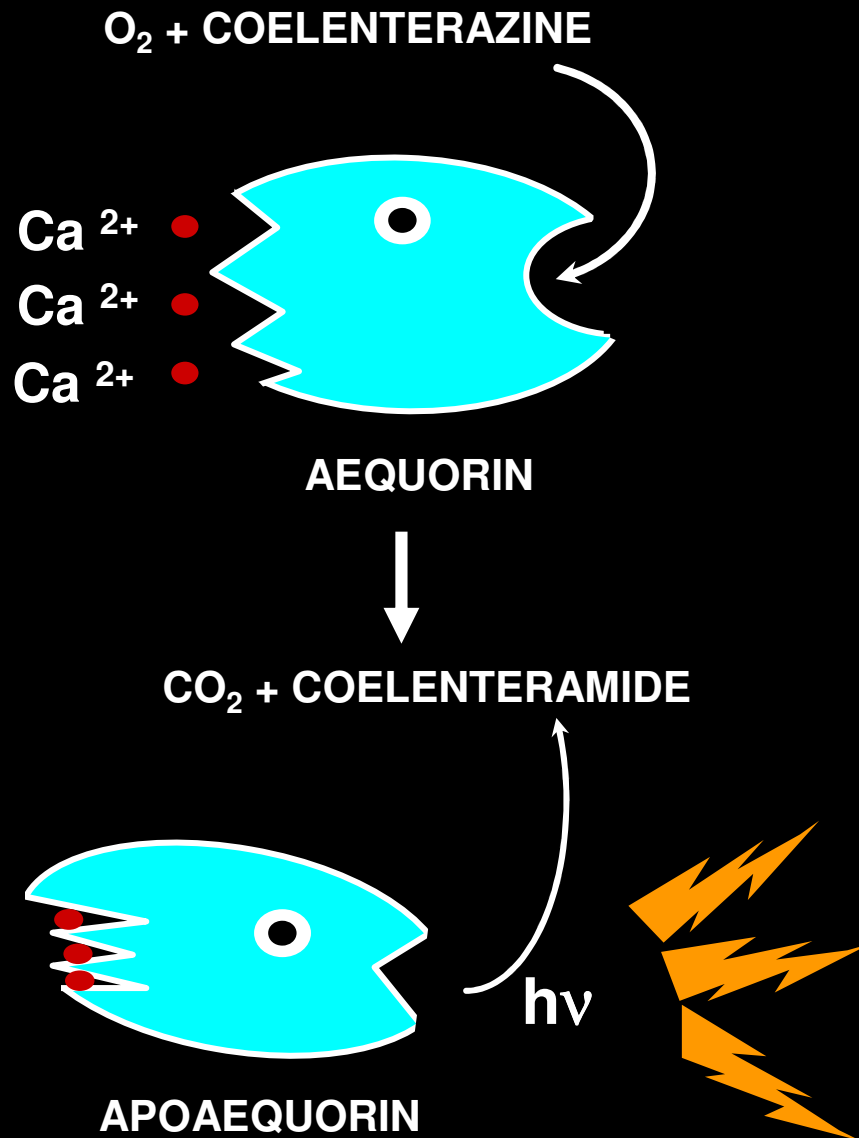
Pulmones



# SONDAS DIRIGIDAS



**Nuc-R-Pericam**



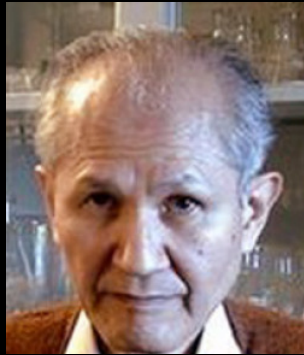
Viral expression +  
 Photon-counting camera  
 = IMAGING



**[Ca<sup>2+</sup>]<sub>mit</sub>**

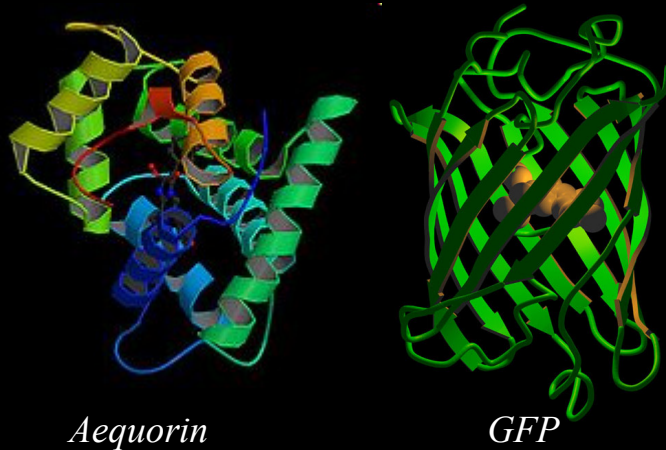
from Watanabe et al. (1989), FEBS Lett. 246:73-77

T. Pozzan & R. Rizzuto. Padova  
 A.K. Campbell. Cardiff



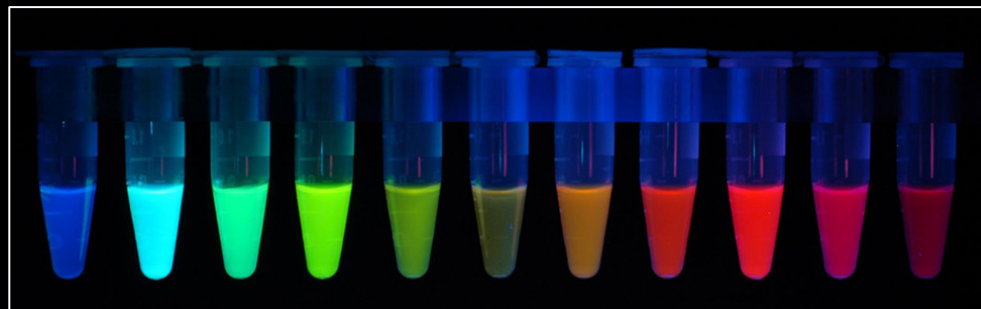
Osamu Shimomura,  
Martin Chalfie, y  
Roger Y. Tsien

Premio Nobel de  
Química 2008



*Aequorin*

*GFP*







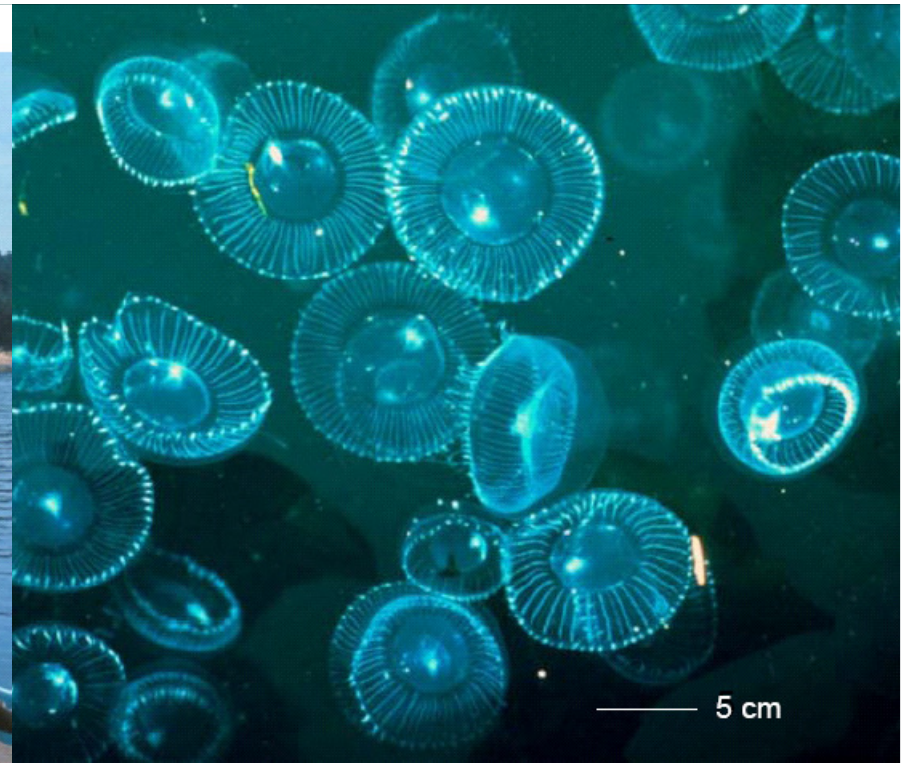
Dr. Osamu Shimomura

**Friday Harbor, San Juan Island, Washington State, 1961**



Ver notas

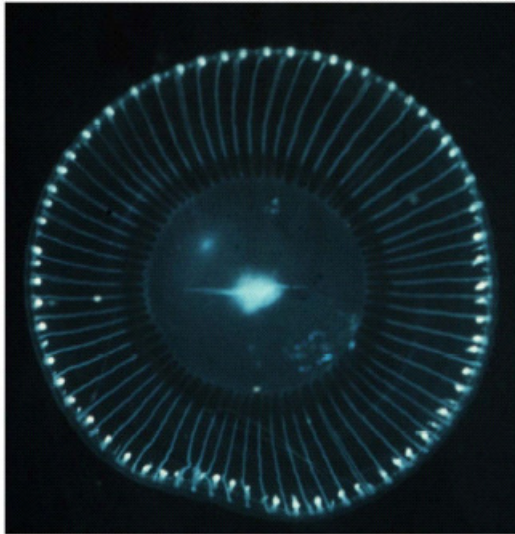
## Jellyfish Collectors (1974)



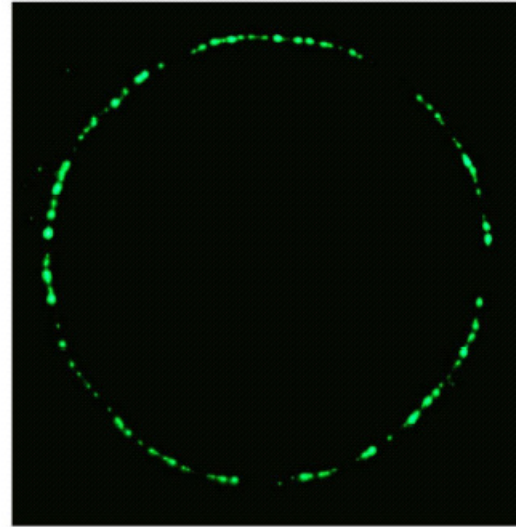
From left, my wife Akemi, Dr. Chang, myself, Mrs. Chang, Mrs. Johnson, Dr. Johnson, Debby (a helper). At lower right are my children, Tsutomu and Sachi. We collected 30–40 bucketfuls of jellyfish each day.

# *Aequorea aequorea*

In Daylight



Luminescence in Dark



Tissue of light organ (rings) **Weak light**

pH 4 buffer  
Filtration

Cell-free extract (pH 4) **No light**

NaHCO<sub>3</sub>

Cell-free extract (neutral pH) **Weak light**

Ca<sup>2+</sup> or sea water

**Bright light**

Rings of jellyfish (tissue of light organs)

Shake in saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
Squeeze through gauze  
Filtration

Granular light organs

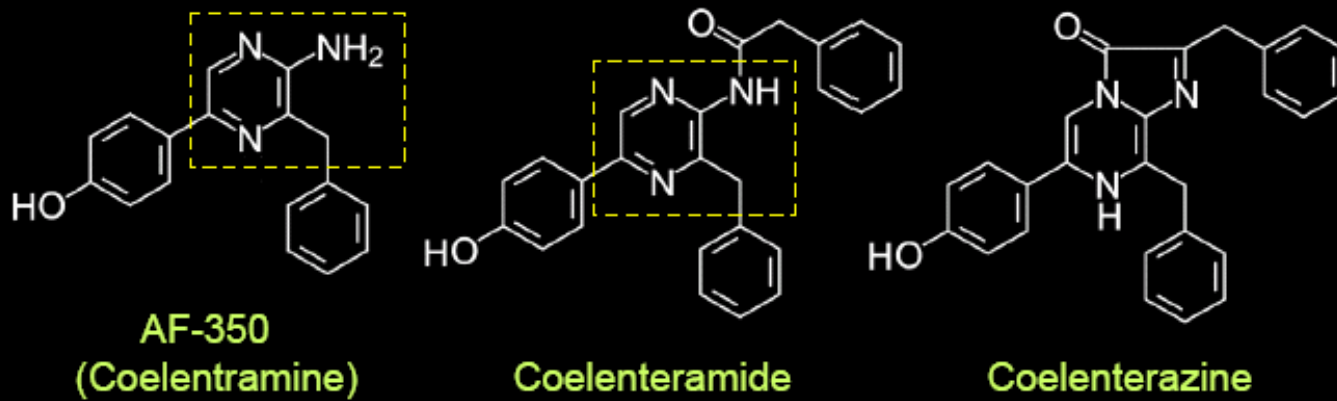
Shake in EDTA solution  
Filtration

Crude aequorin solution

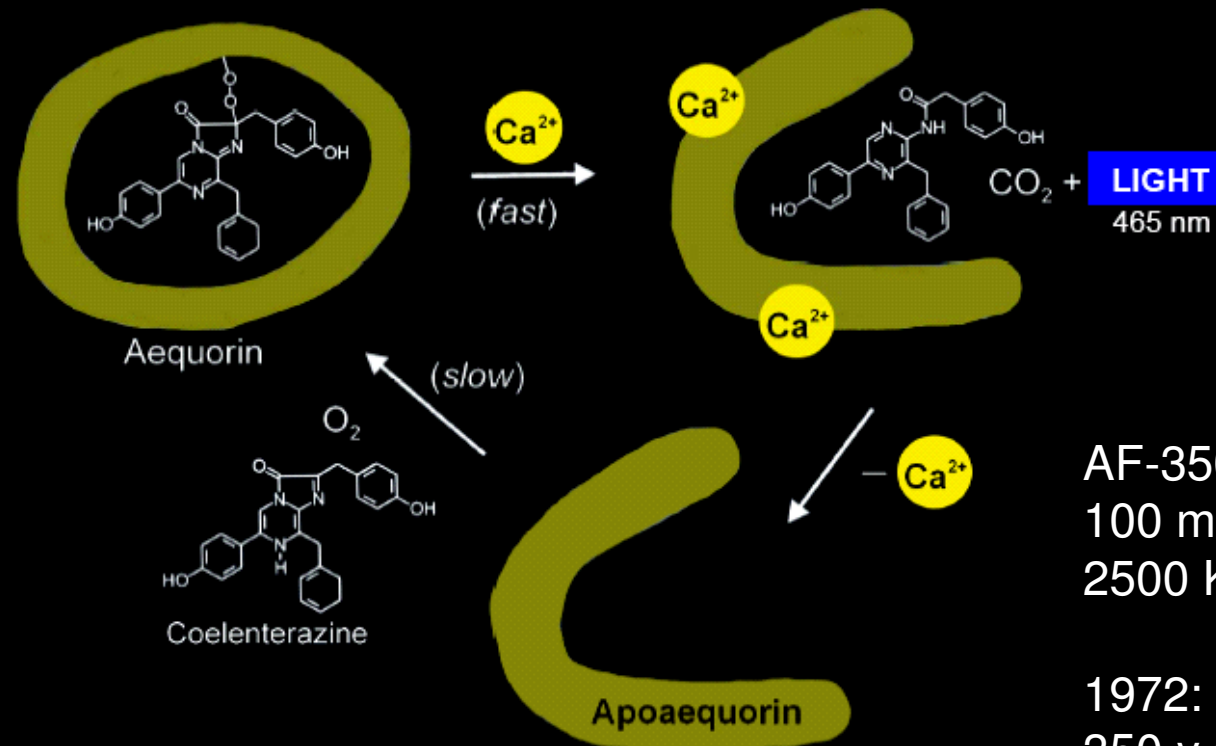
Purification

Aequorin and GFP

## Structure Elucidation of Coelenterazine

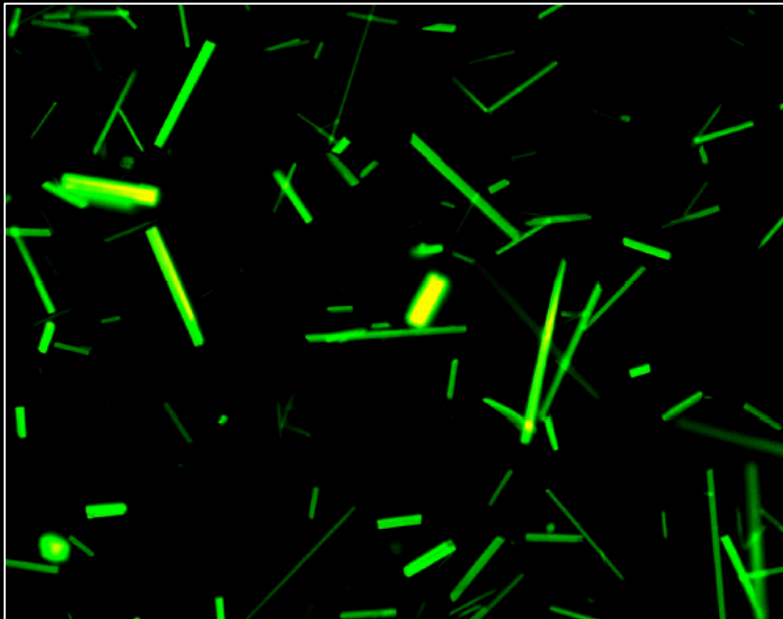


## Luminescence and Regeneration of Aequorin



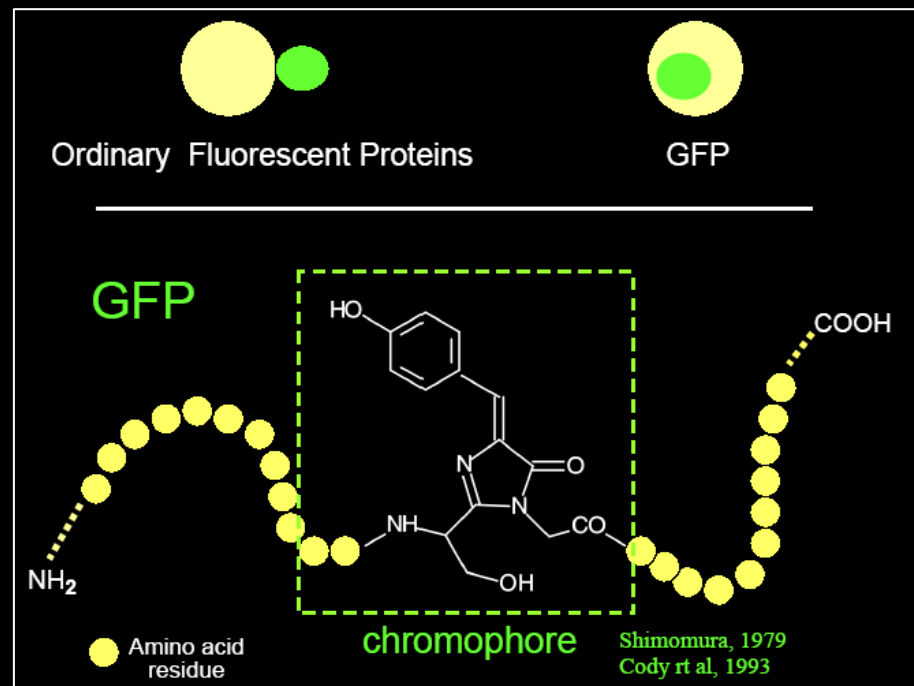
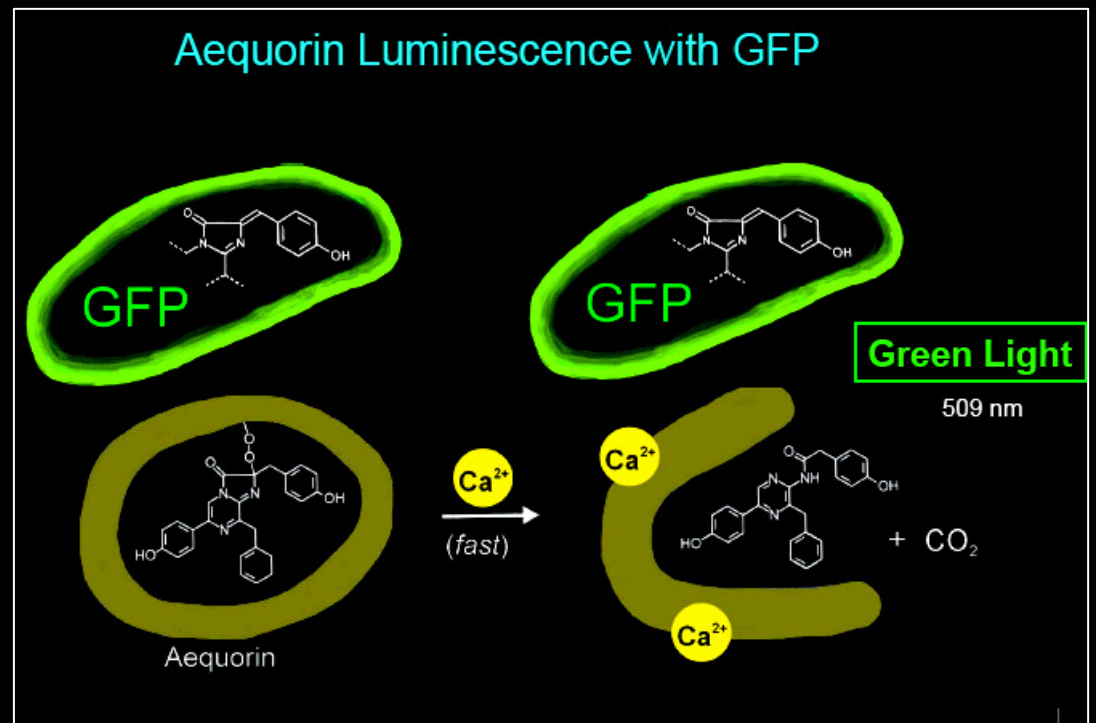
AF-350 (1969)  
 100 mg AEQ=  
 2500 Kg A.V.

1972: Estructura AF-  
 350 y Celenterazina



GFP crystals. Photo by Dr. Shinya Inoue

- 100 mg GFP en 1979
- 1990 desaparición de A.V. en F.H.
- 1992 Clonación de GFP (Prasher)
- 1994 Expresión en cel. vivas (Chalfie)





**Martin Chalfie**

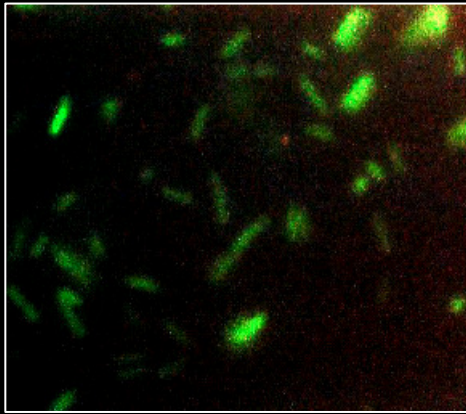


**Caenorhabditis elegans**

Chalfie se graduó en Harvard y es profesor de Biología en la Universidad de Columbia. El laboratorio de Chalfie utiliza al nematodo *Caenorhabditis elegans* (*C. elegans*) para investigar el desarrollo y funcionamiento de las neuronas.

Mientras trabajaba en el MBL de Cambridge en 1984 publicó un influyente artículo «The Neural Circuit for Touch Sensitivity in *C. Elegans*», junto a John Sulston y Sydney Brenner.

Chalfie fue el primero que logró introducir la GFP en otros organismos, en los cuales la proteína también emitió la fluorescencia.



Expresión de GFP  
en bacterias  
(1992-1993)

Green Fluorescent Protein as a Marker  
for Gene Expression

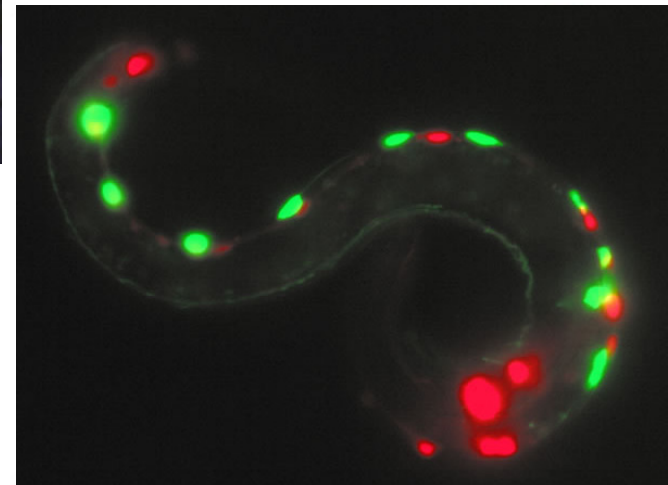
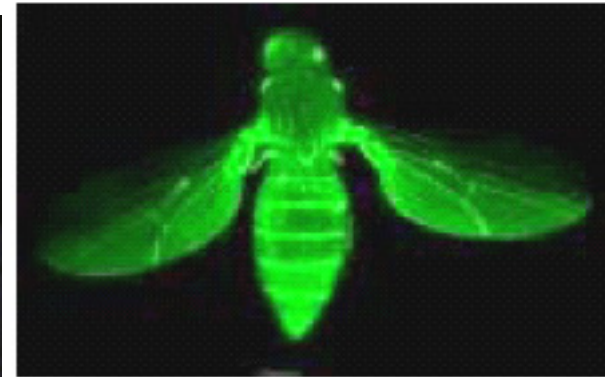
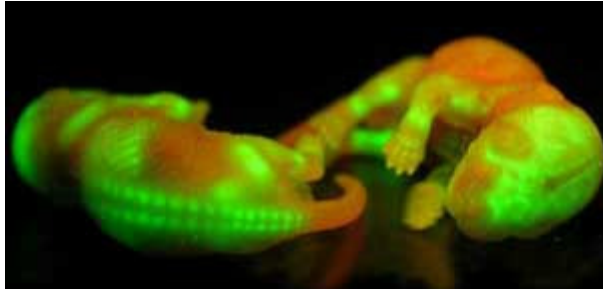
Martin Chalfie, Yuan Tu, Ghia Euskirchen,  
William W. Ward, Douglas C. Prasher

*Science* **263**: 802-805, 1994





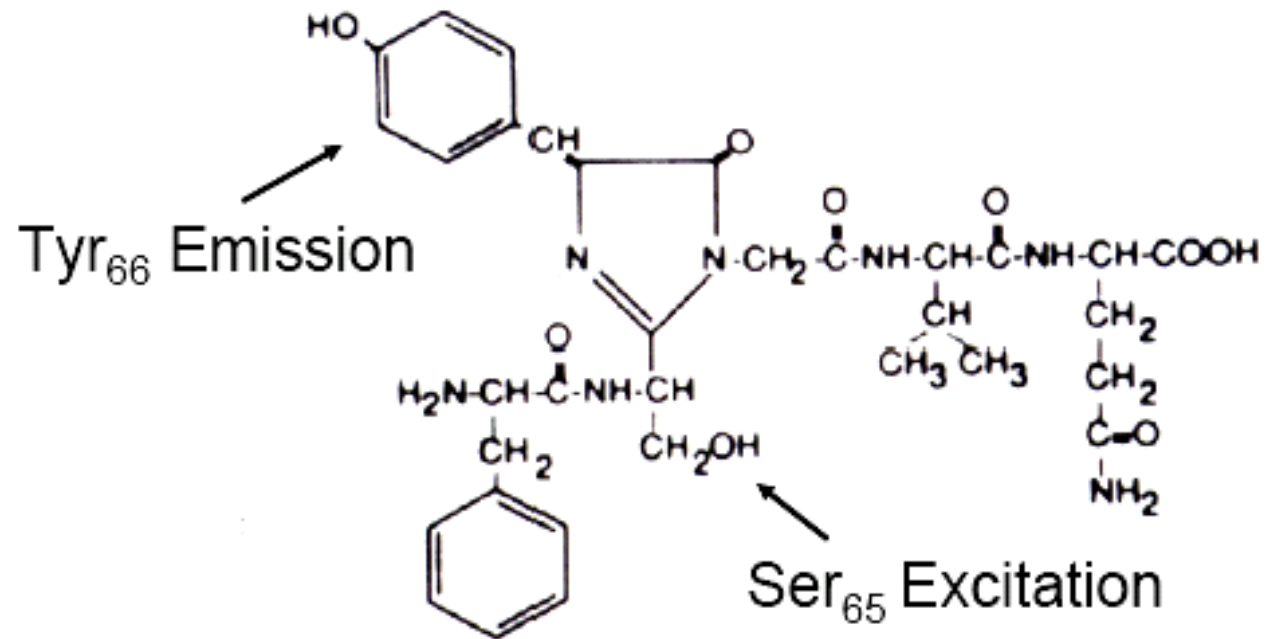
# TRANSGÉNICOS





Roger Tsien

## Improving GFP



Tsien también tuvo la idea de expresar la GFP en la células como marcador, pero se le adelantó Chalfie (1992). Hizo mutaciones en el cromógeno y encontró variantes mas brillantes y estables (EGFP; YFP; CFP; BFP).

4 colors of GFP mutants expressed in *E. coli*

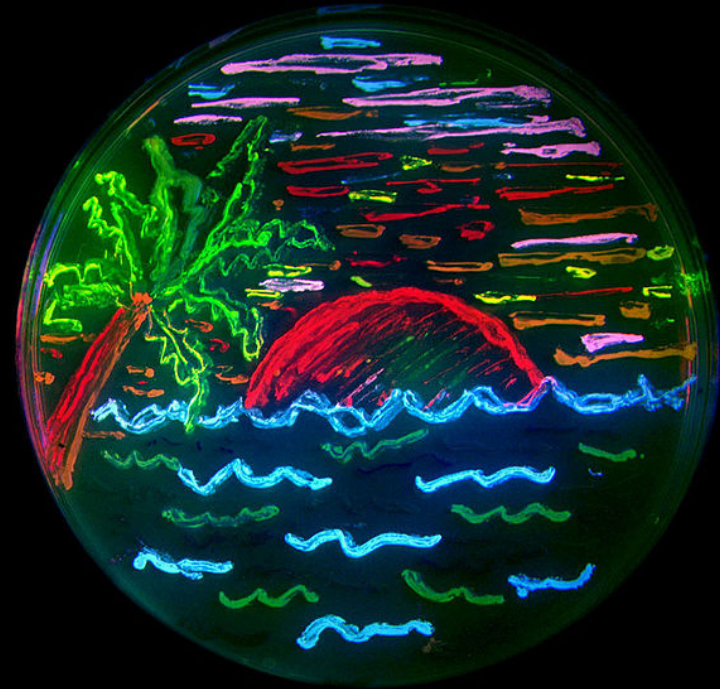
YFP  
(T203Y...)

BFP  
(Y66H...)

Brighter GFP (S65T)

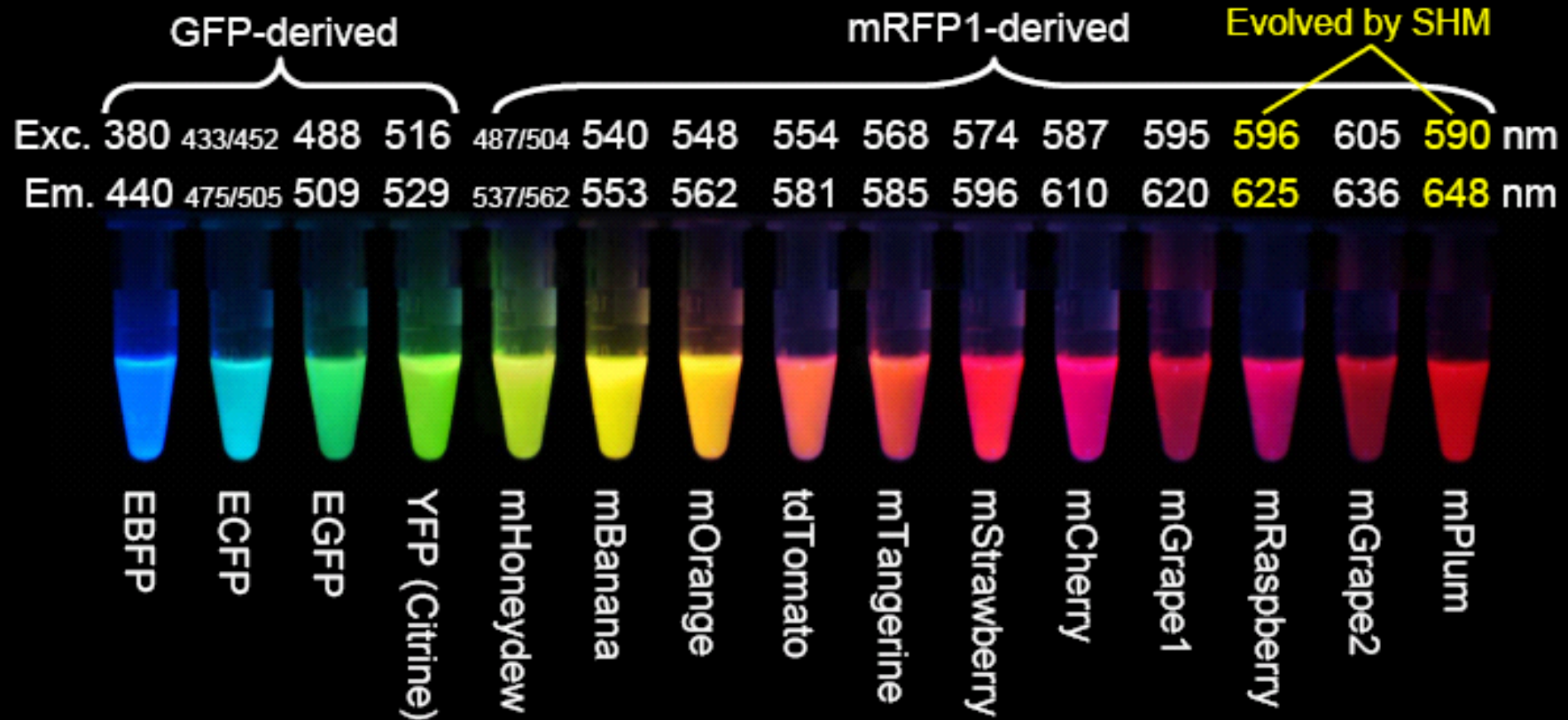
CFP  
(Y66W...)

*R. Heim, A. Cubitt*



Contribuciones a la estructura de estas proteínas

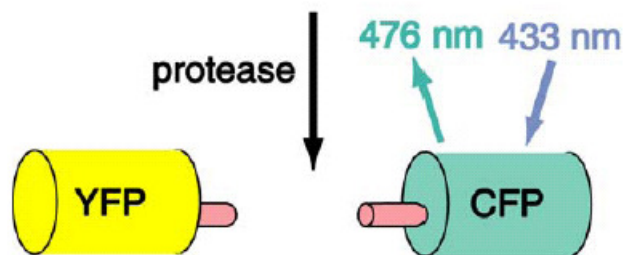
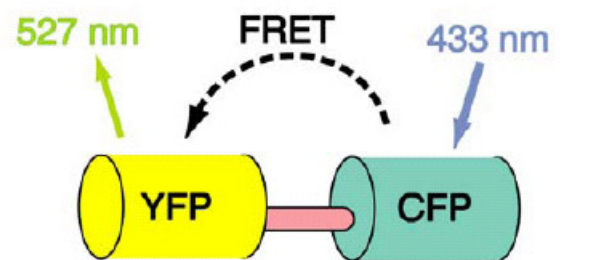
# The 2004 palette of nonoligomerizing fluorescent proteins



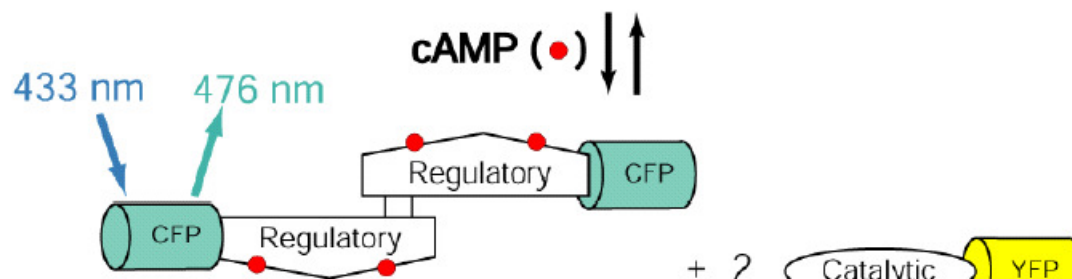
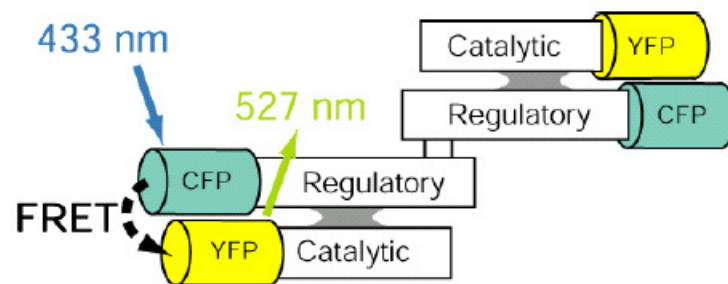
*Nathan Shaner et al (2004) Nature Biotech. 22: 1567-1572*

*Lei Wang et al (2004) Proc. Natl. Acad. Sci. USA 101: 16745-16749*

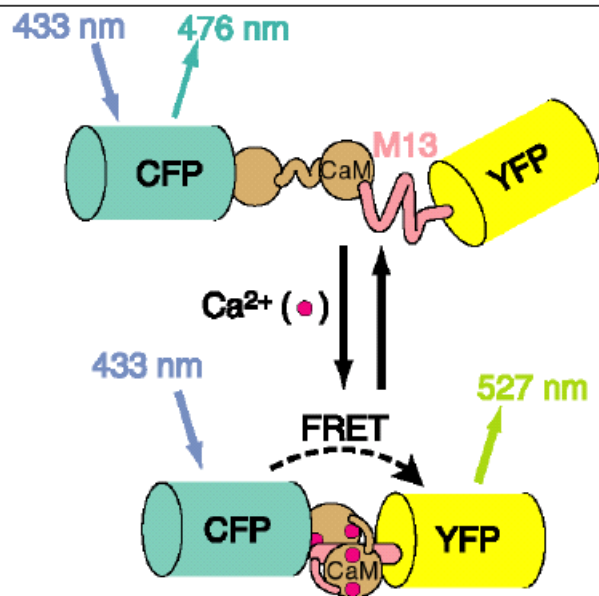
# Examples of genetically encoded FRET sensors



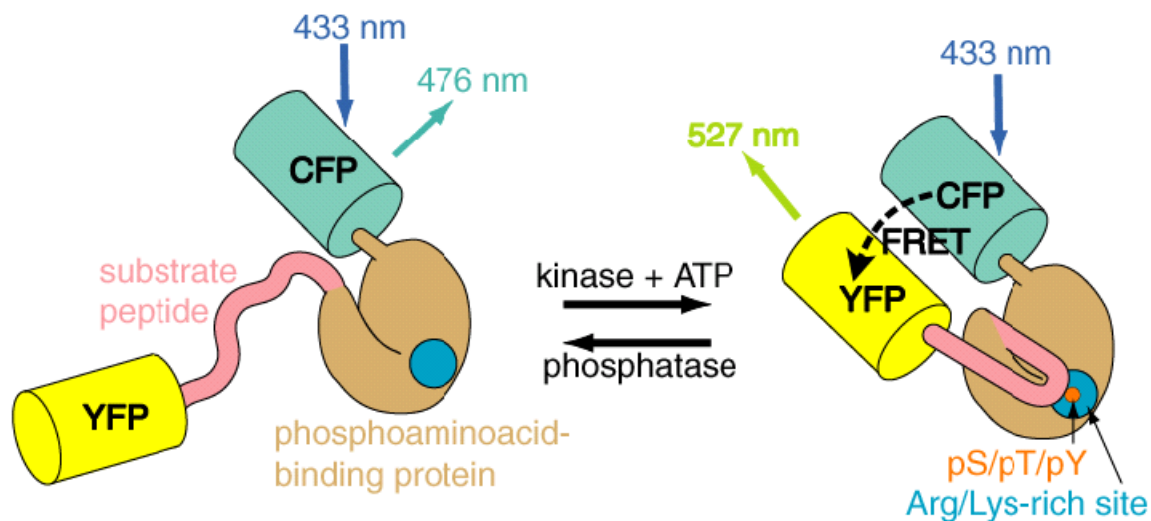
Protease disrupts FRET (R. Heim)



cAMP disrupts FRET (M. Zaccolo, T. Pozzan (Padova))

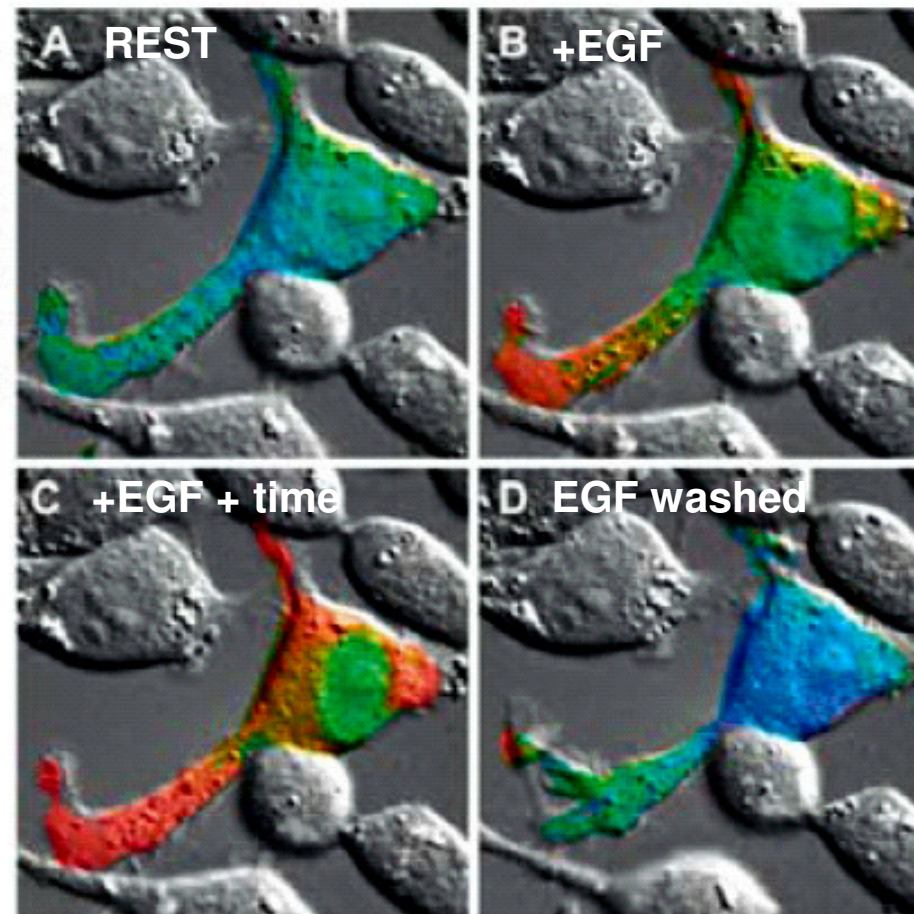


Ca<sup>2+</sup> increases FRET (A. Miyawaki)



Phosphorylation increases or decreases FRET (J. Zhang, A. Ting)

Reporter for EGF receptor kinase activity shows signaling from plasma membrane to nucleus

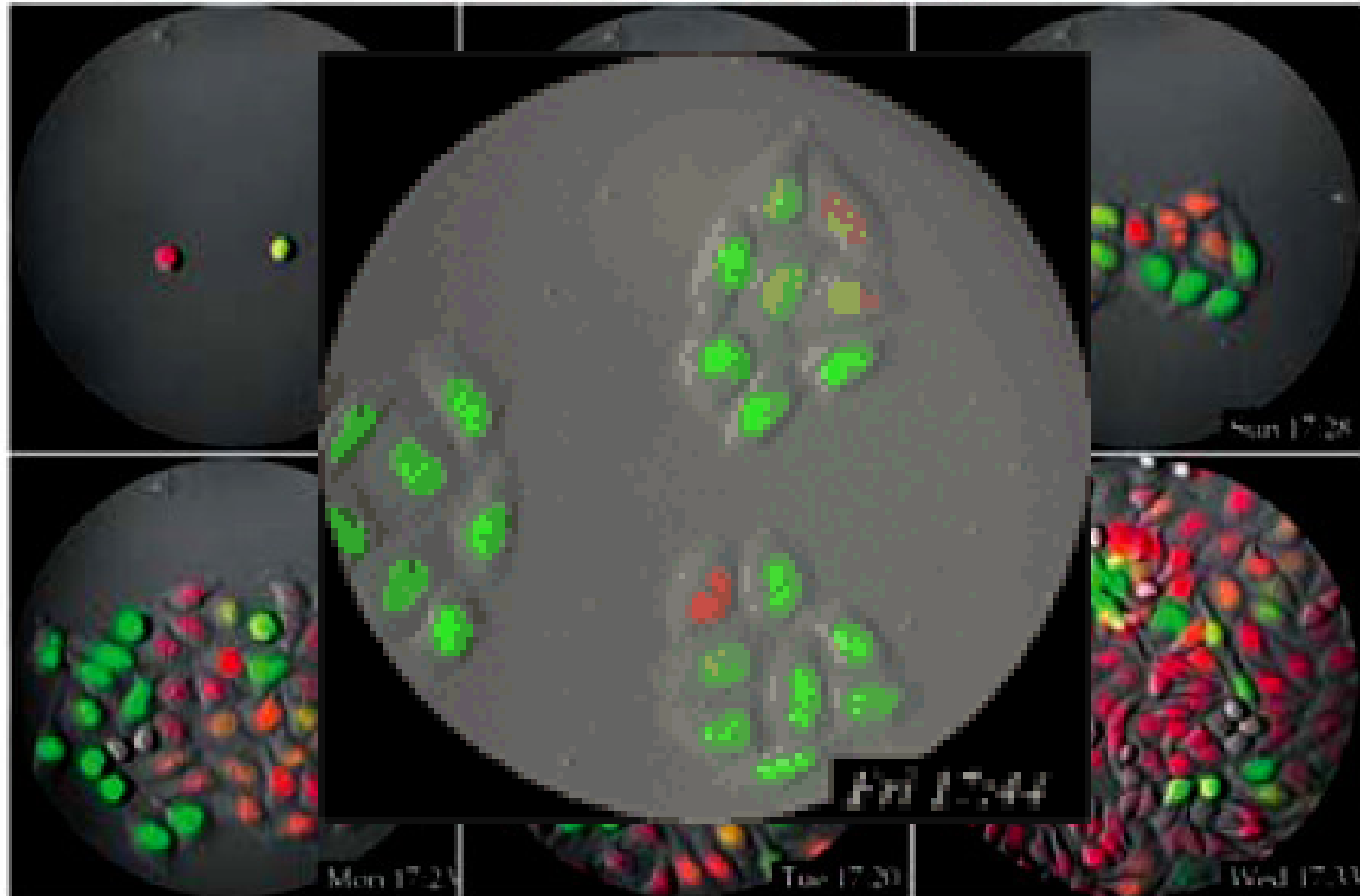


Alice Ting

Figure 10. A fibroblast transfected with a reporter<sup>47</sup> for the tyrosine kinase activity of the **receptor for epidermal growth factor (EGF)**. The extent of FRET, indicating the extent of phosphorylation of the reporter, is shown in pseudocolors ranging from blue (negligible phosphorylation) to red (maximal phosphorylation). These colors are superimposed

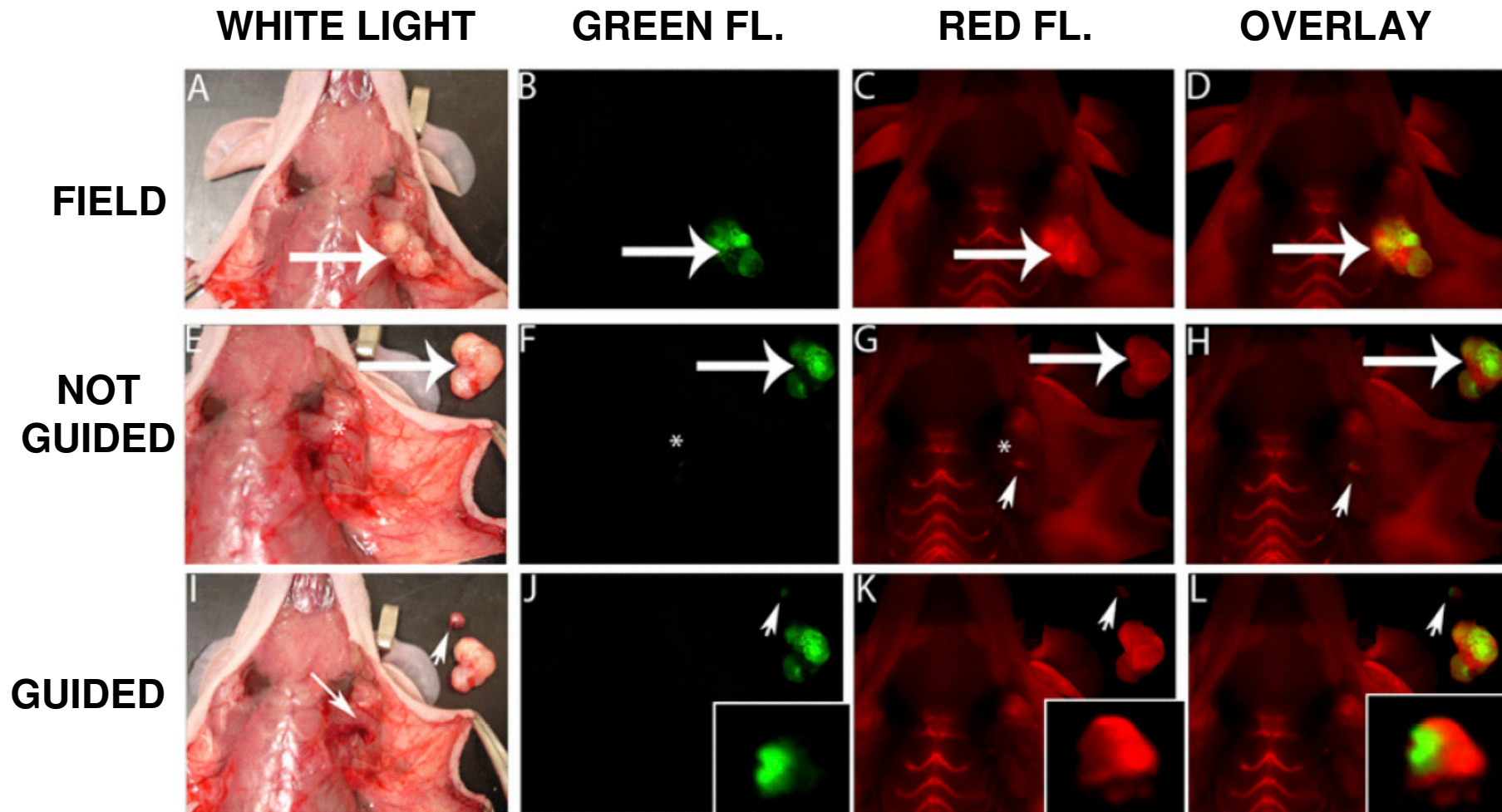
# Cell cycle indicator using YFP and mCherry

Green = in mitosis; Red = interphase



Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides (ACPP). Tumour cells labeled with GFP.

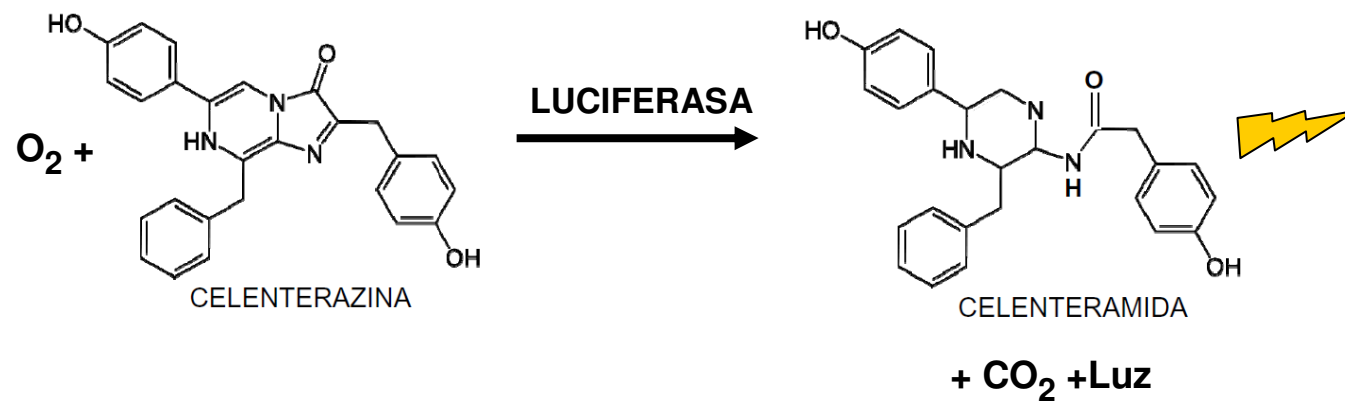
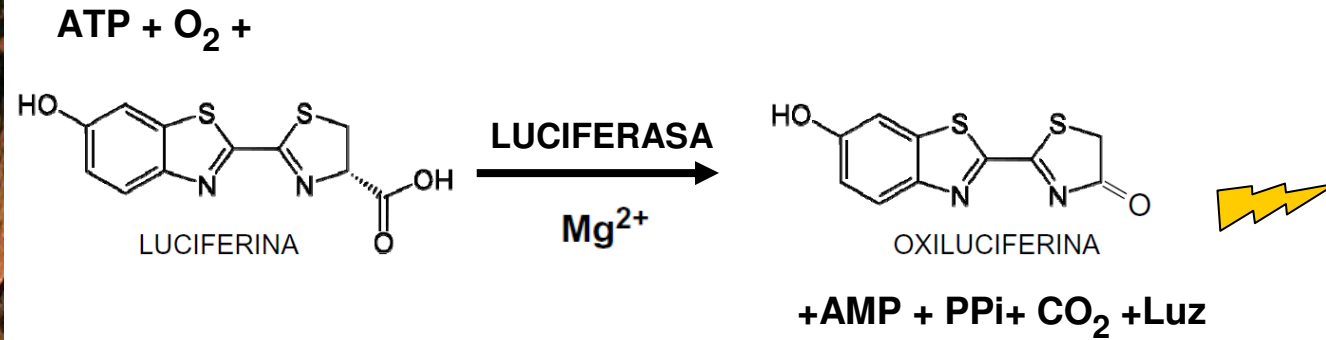
Cy5- free ACPP injected 5 hours before guided surgery



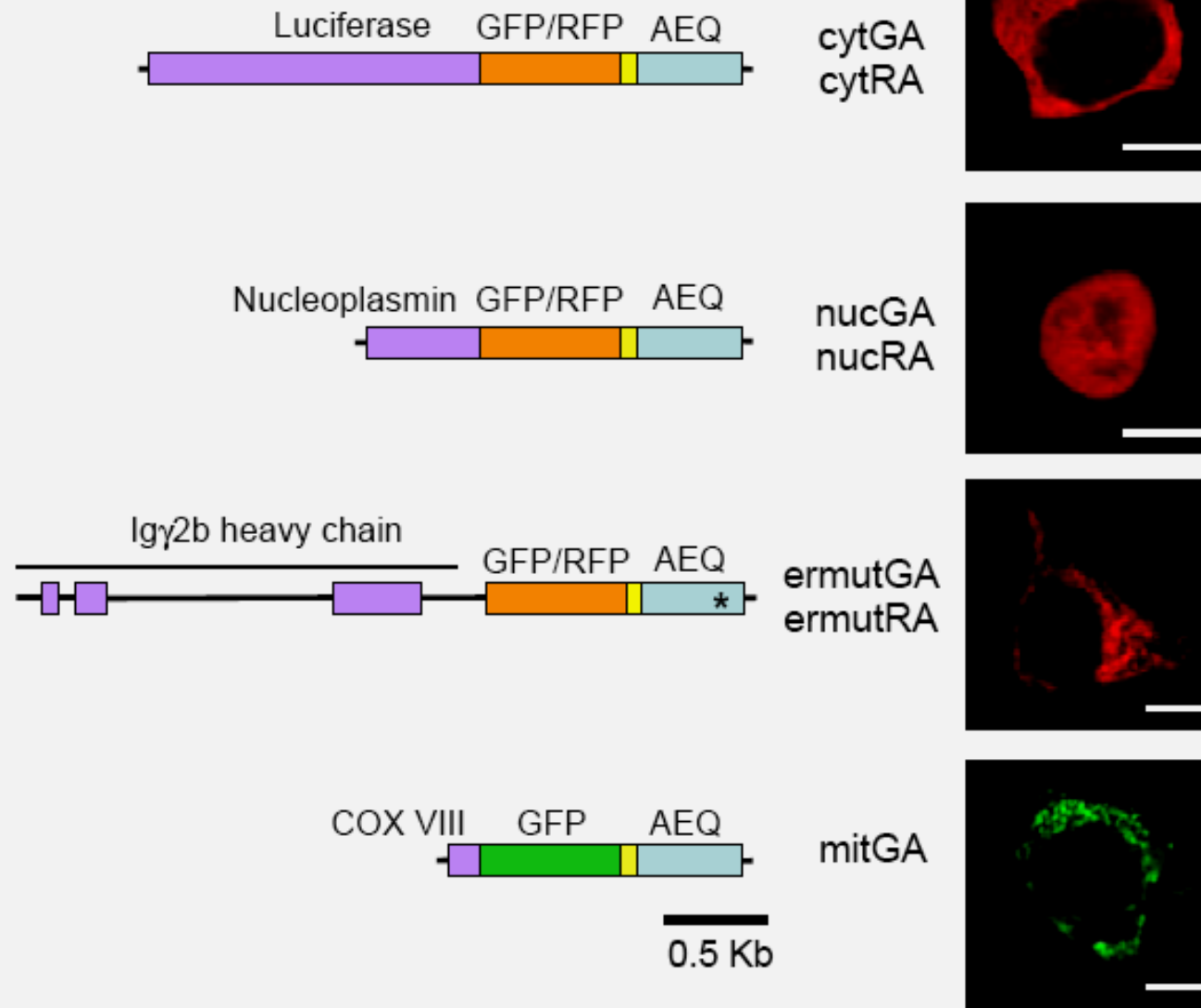
Nguyena *et al.*, (2010) *PNAS* **107**: 4317–4322



# Luminiscencia



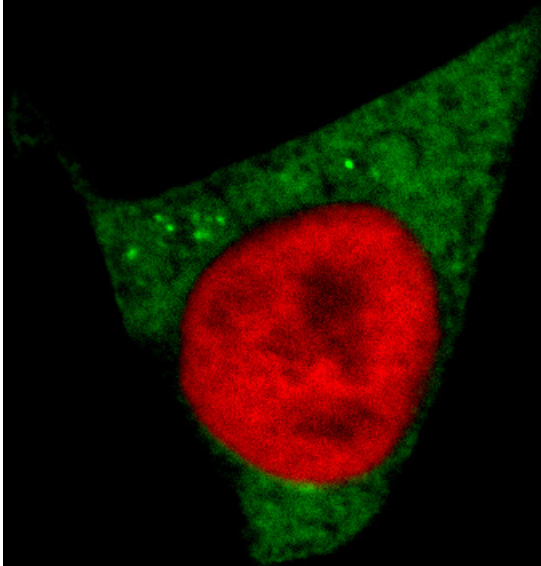
## Chimeric aequorin-GFP/RFP proteins



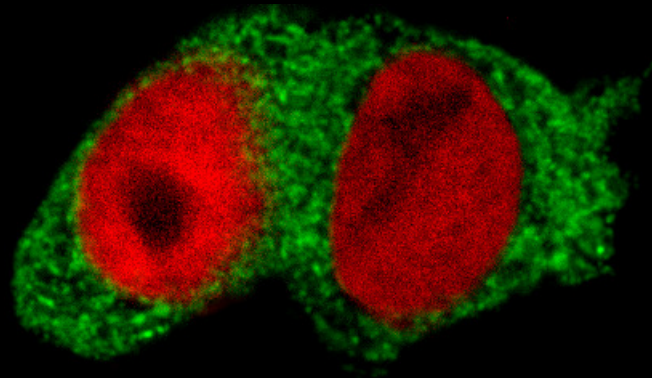
Manjarres et al. (2008) Red and green aequorins for simultaneous monitoring of  $\text{Ca}^{2+}$  signals from two different organelles. *Pflugers Arch.* **455**: 961-970

# Red and Green aequorins

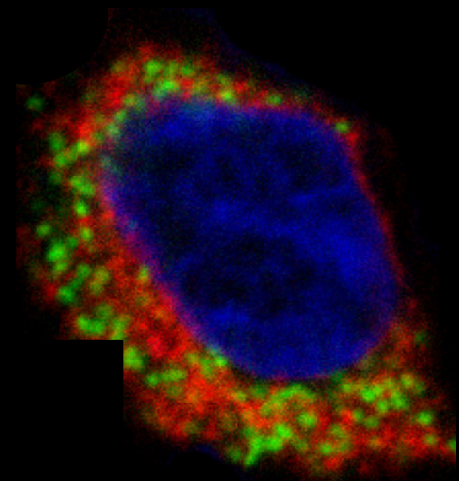
cytAEQ-GFP  
nucAEQ-RFP




erAEQ-GFP  
nucAEQ-RFP



mitAEQ-GFP  
erAEQ-RFP  
DAPI

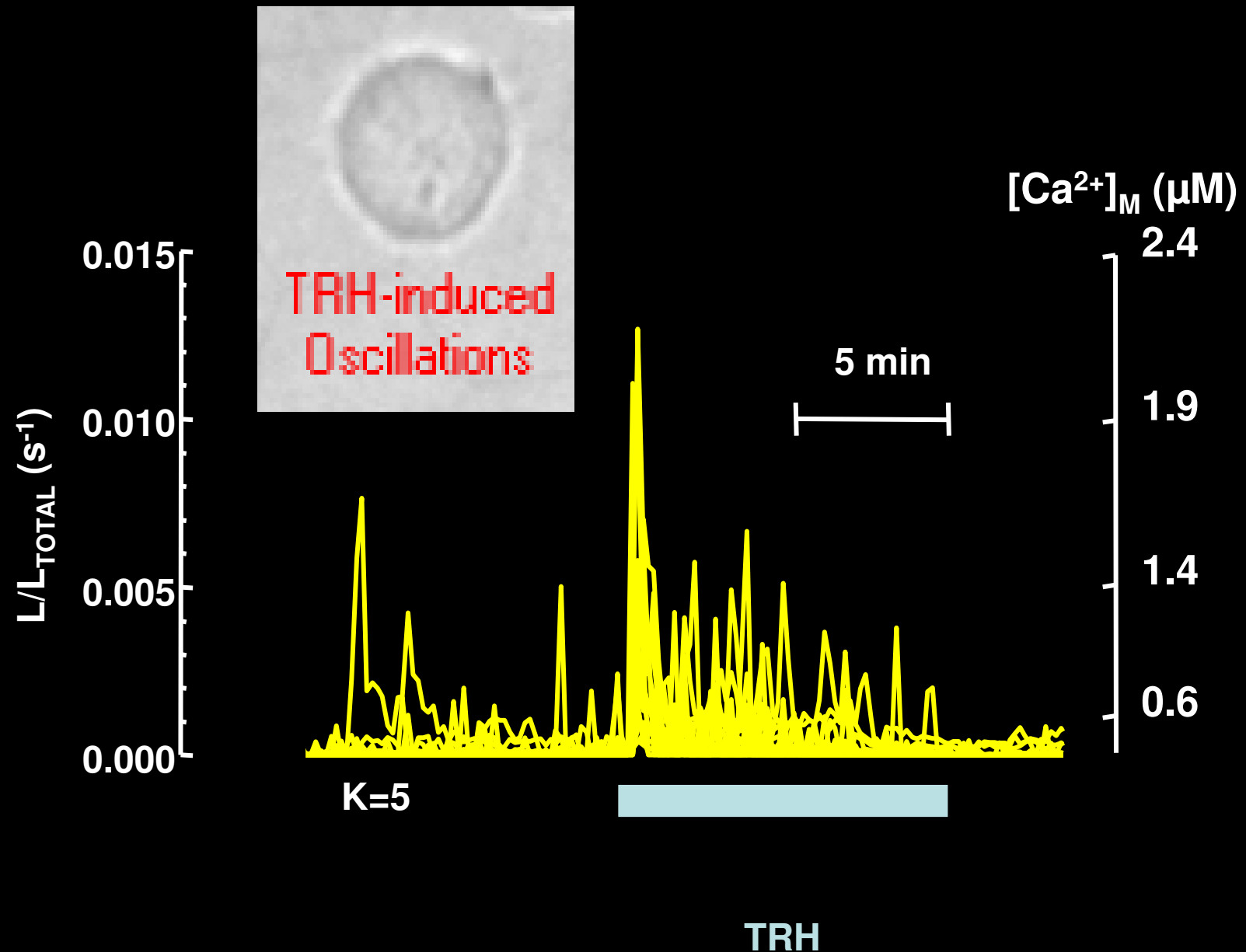


A fluorescence microscopy image showing a cell with green and red signals. The green signal is distributed throughout the cell, while the red signal is concentrated in a specific region, likely the mitochondria. The text "Mitochondrial calcium oscillations" is overlaid on the image in a red, pixelated font.

Mitochondrial  
calcium  
oscillations

VILLALOBOS et al. *J. Biol. Chem.* **276**, 40293-40297; 2001

# Changes of electrical activity modify $[Ca^{2+}]_M$ oscillations







Pablo Chamero

Isabel M. Manjarrés

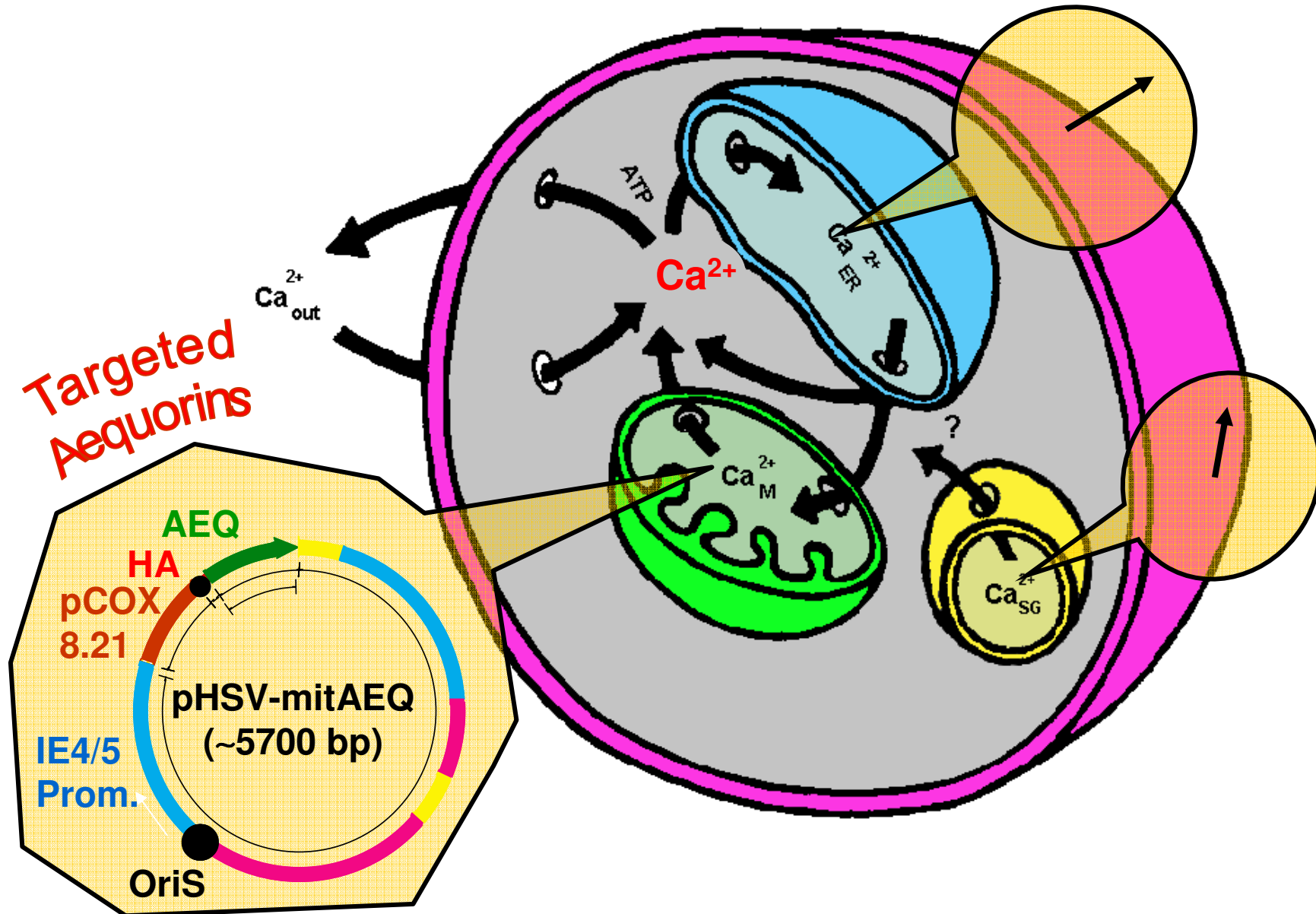
María Teresa Alonso

Javier García-Sancho



IBGM is a joint venture of the University of Valladolid  
and the Spanish National Research Council

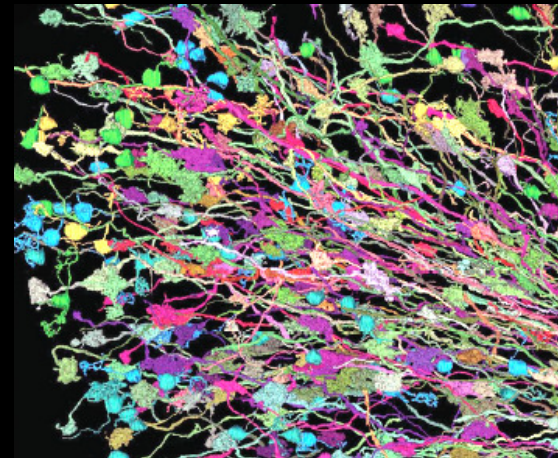
# Contribution of organelle to $Ca^{2+}$ signalling





# De cómo las medusas nos enseñaron a colorear las células

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Universidad de Granada, 5 de Abril 2011

