

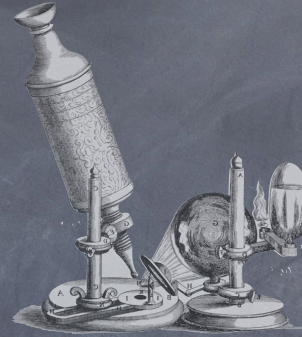
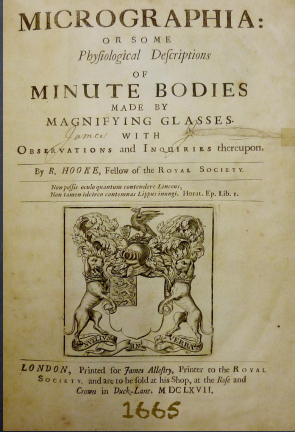
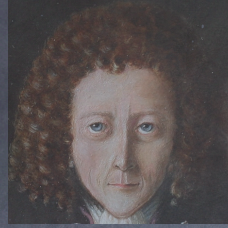


# Marcaje de células para estudios in vivo

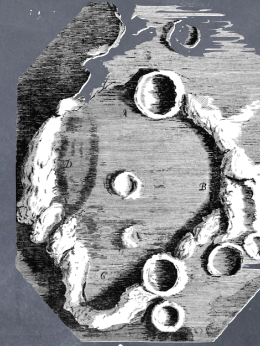
Germán Reig

# Introducción: Breve reseña histórica

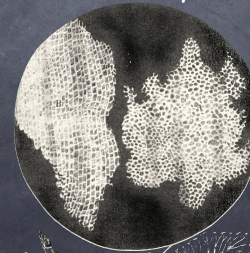
Robert Hooke,  
1635-1703



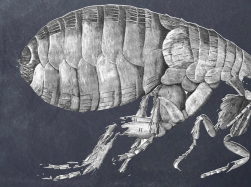
Microscopio



Luna

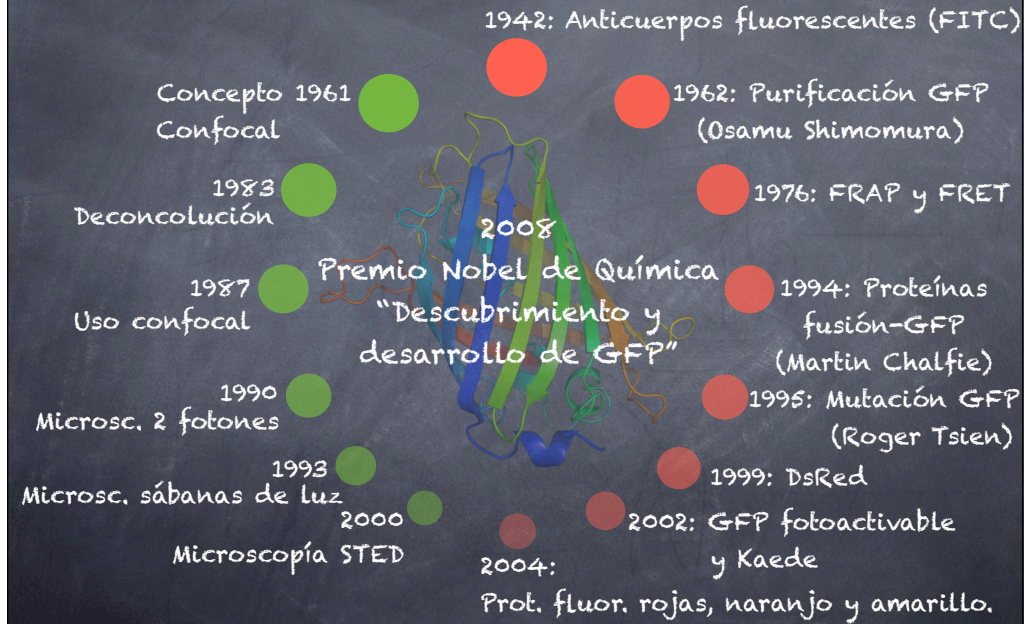


Corcho



Pulga

# Introducción: Breve reseña histórica



## Temas: Métodos basados en fluorescencia

Estructura

**Destinación a distintos organelos celulares**

Dinámica

**Movimiento de moléculas:**

- FRAP

**Recambio**

- **Cronómetros fluorescentes**

**Interacción proteína-proteína:**

- FRET

- **Complementación bimolecular**

Funcionalidad

**Control espacio-temporal**

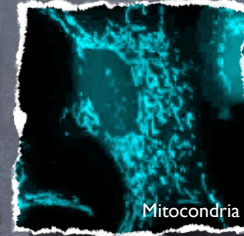
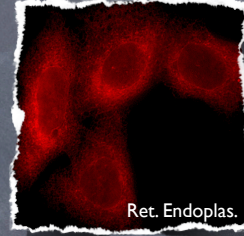
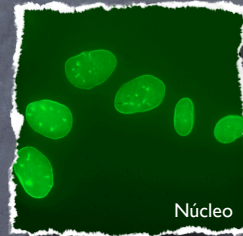
- **Optogenética**

# Estructura: Destinación de proteínas

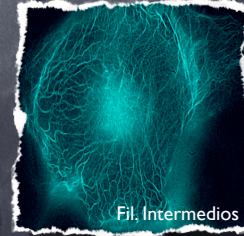
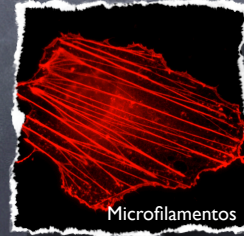
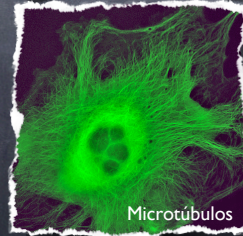
## 1. Proteína de fusión típica

Prot. fluorescente ⚡ Gen interés ⚡ Gen interés ⚡ Prot. fluorescente

Organelos



Citoesqueleto



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

## Estructura: Destinación de proteínas

### 2. Secuencias de destino y dominios de fusión

SD

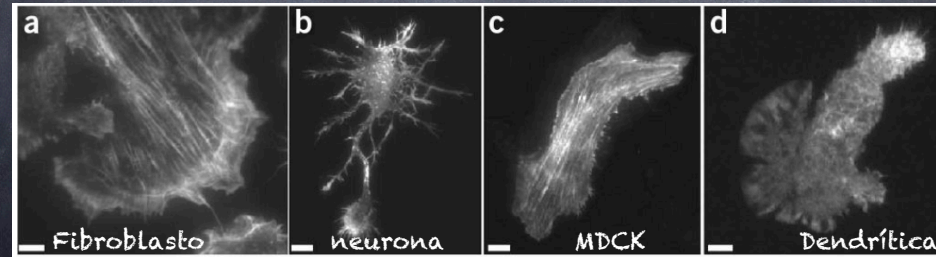
Proteínas

Núcleo -Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-  
Ret. Endop.-Lys-Asp-Glu-Leu-COOH  
Peroxisoma.-Ser-Lys-Leu-COOH

Lifeact: a versatile marker to visualize F-actin.

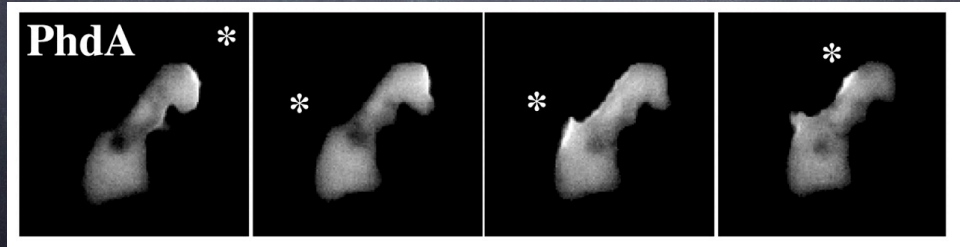
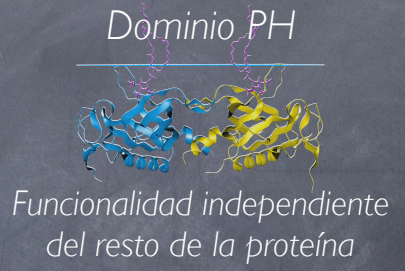
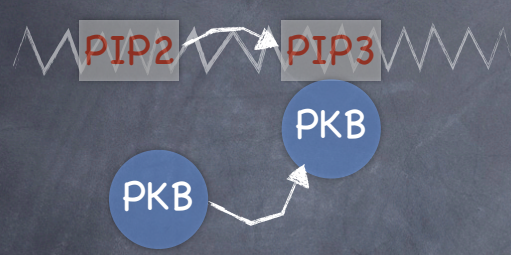
Nature Methods, 2008

MGVADLIKKFESISKEE



# Estructura: Destinación de proteínas

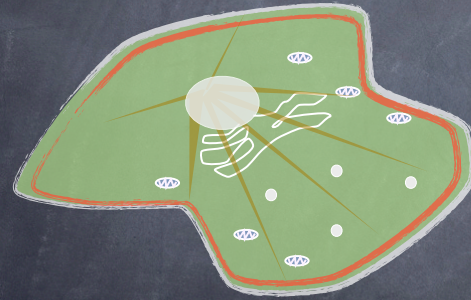
## 2. Secuencias de destino y dominios de fusión



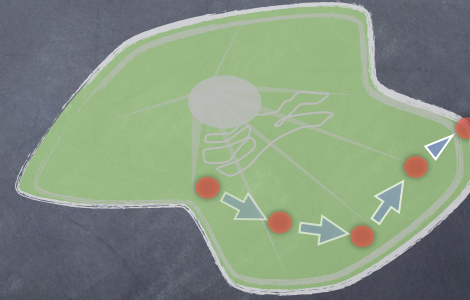
*Journal of Cell Science* 116, 2003

# Dinámica: Movimiento-recambio proteínas

Localización



Movimiento

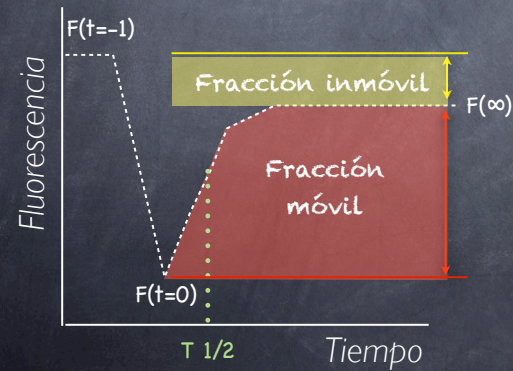
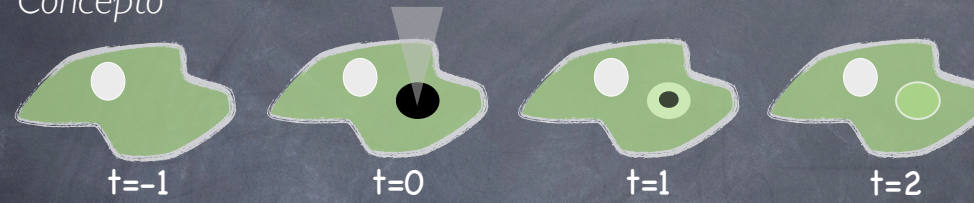




# Dinámica: Movimiento de proteínas-FRAP

FRAP: Fluorescence Recovery After Photobleaching

Concepto



Coef. difusión

$$D = \frac{r_0^2 \gamma}{4\tau_{1/2}}$$

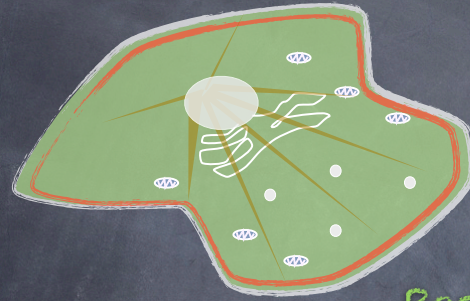
Restringido al plano

$$D = kT/6\pi\eta R_h$$

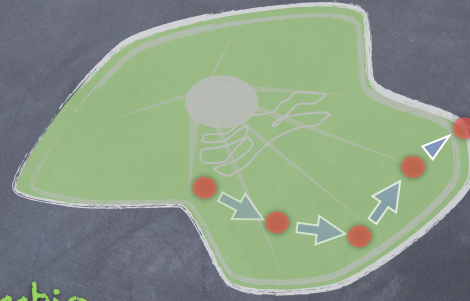
No restringido

# Dinámica: Movimiento-recambio proteínas

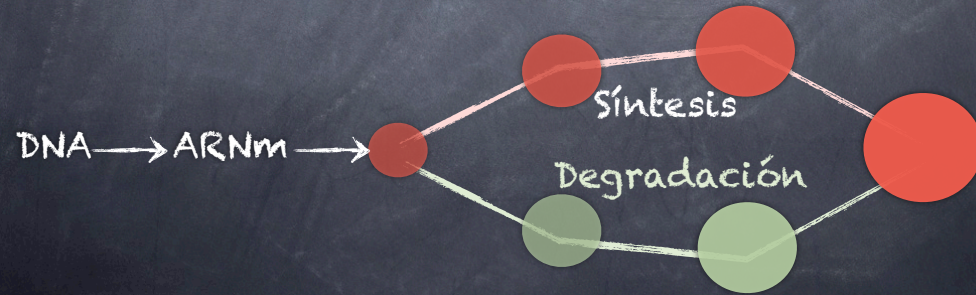
Localización



Movimiento

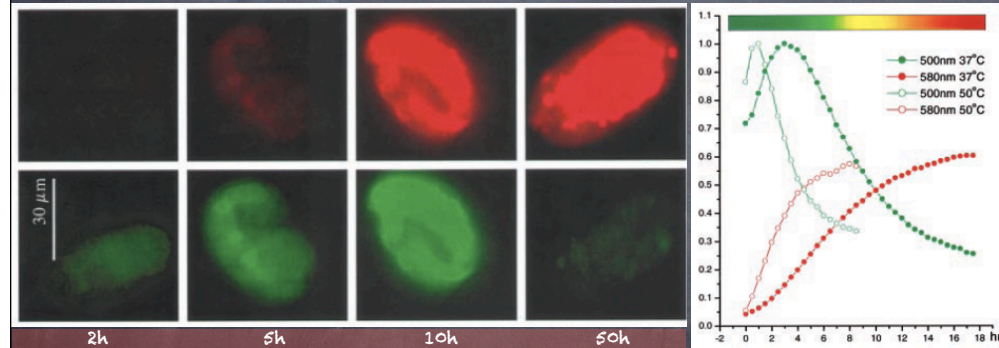


Recambio



## Dinámica: Recambio: Cronómetros fluorescentes

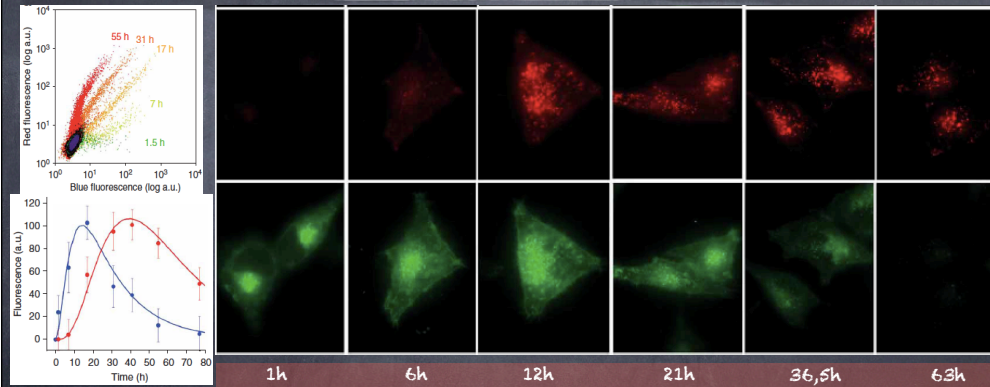
"Fluorescent timer": protein that changes color  
with time. Science 290, 2000.



Se basa en utilización de DsRed, cuya estructura tetramérica  
no permite su utilización en proteínas de fusión clásicas, aunque si  
como reportero de actividad transcripcional.

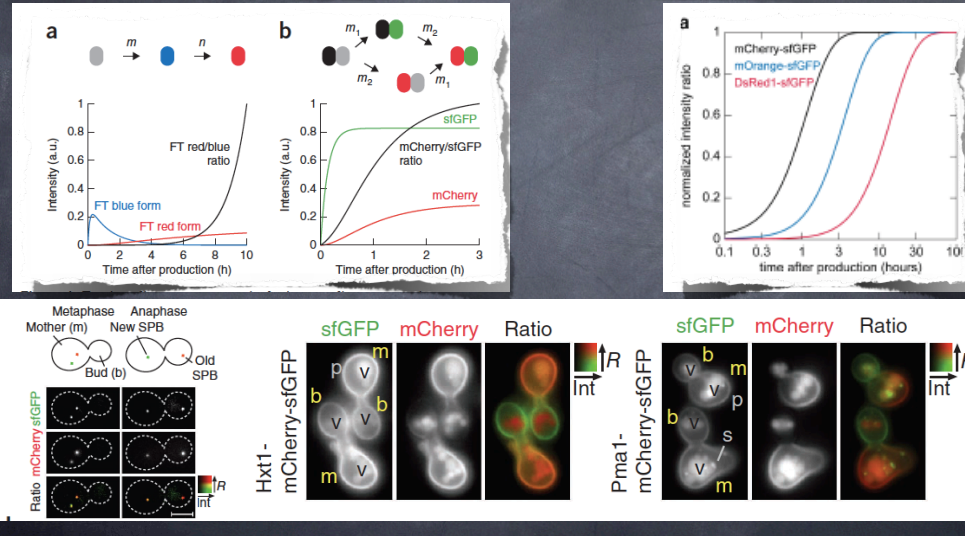
# Dinámica: Recambio: Cronómetros fluorescentes

Monomeric fluorescent timers that change color from blue to red report on cellular trafficking. Nat. Chem. Biol. 2009.



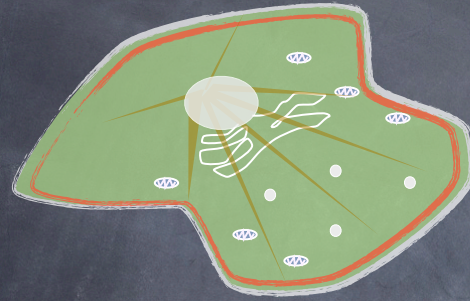
# Dinámica: Recambio: Cronómetros fluorescentes

Tándem fluorescent protein timers for in vivo analysis of protein dynamics Nat. Biotech 2012.

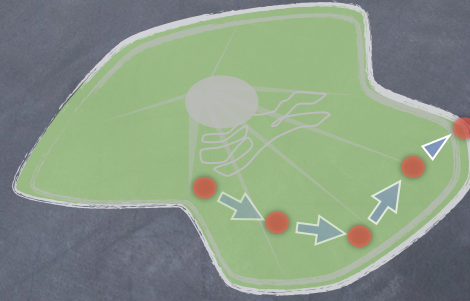


# Dinámica: Movimiento-recambio-prot-prot

Localización



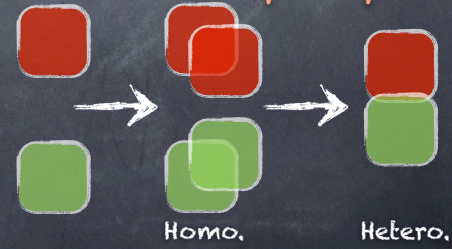
Movimiento



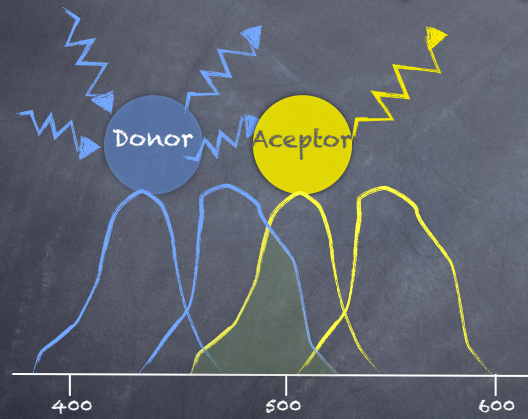
Recambio



Interacción prot-prot



# Dinámica: Movimiento-recambio-prot-prot Fluorescence Resonance Energy Transfer (FRET)



## Tipos

Inter-molecular

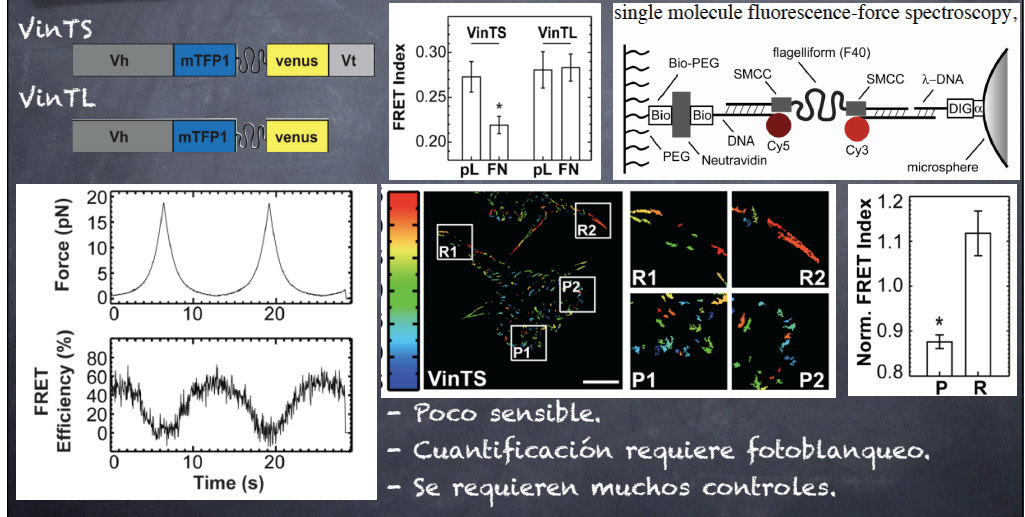
Intra-molecular



- Emisión donor se debe sobrelapar con excitación aceptor.
- Distancia entre moléculas menor a 10 nm.
- Orientación moléculas

# Dinámica: Movimiento-recambio-prot-prot

Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics.  
Nature, 2010.



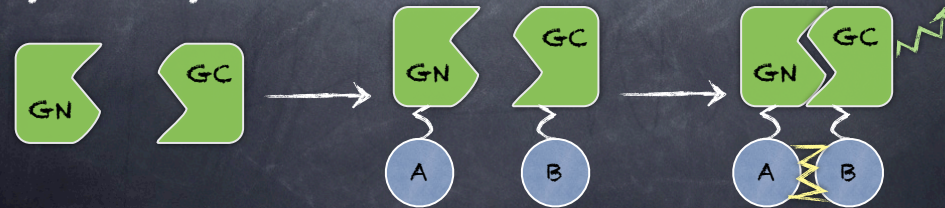


# Dinámica: Movimiento-recambio-prot-prot

## Complementación fluorescente bimolecular (BiFC)

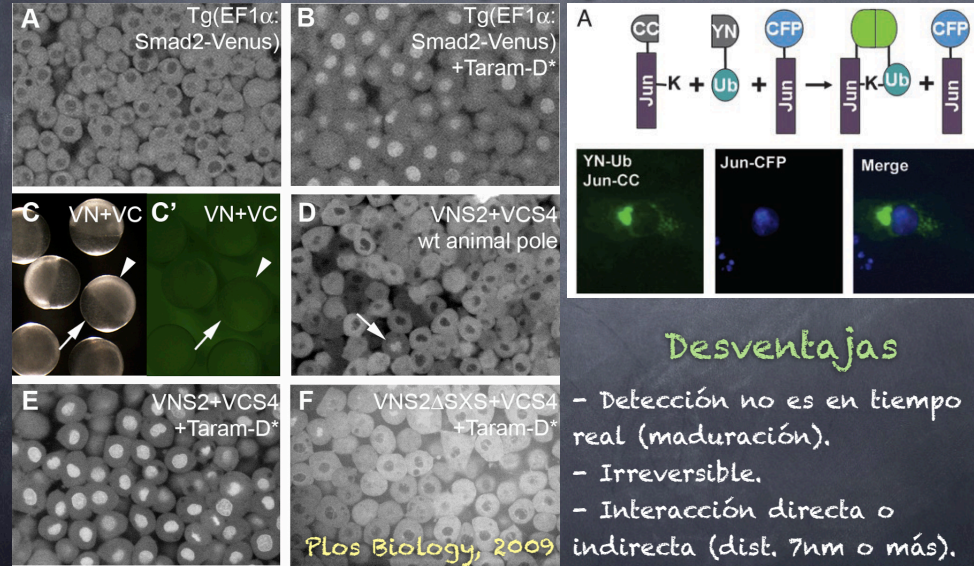
On the enzymatic activity of subtilisin-modified ribonuclease. PNAS 1957.

"..... By careful treatment with trichloroacetic acid it has been possible to separate this N-terminal peptide (RNase-S-Pep) from the rest of the molecule (RNase-S-Prot). These fractions have only traces of residual enzymic activity. On mixing a solution of the trichloroacetic acid precipitate with an equivalent amount of the supernatant fluid, the full enzymic activity of the unfractionated material is regenerated".



# Dinámica: Movimiento-recambio-prot-prot

## Complementación fluorescente bimolecular (BiFC)



# Dinámica: Movimiento-recambio-prot-prot

Localización

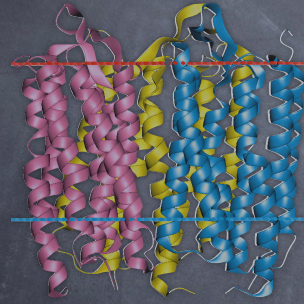
Movimiento



# Funcionalidad: Control espacio-temporal

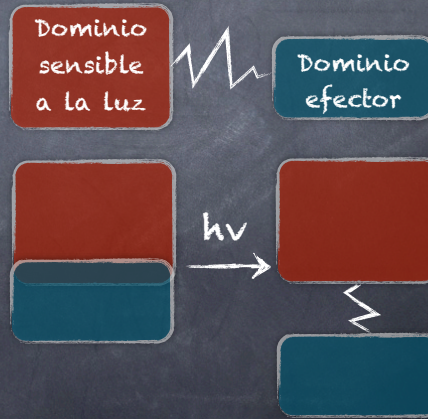
## Optogenética

Opto: Opsinas



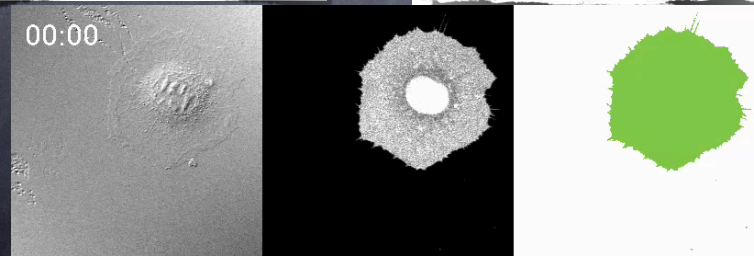
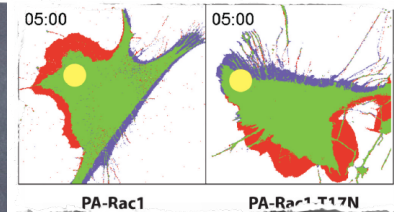
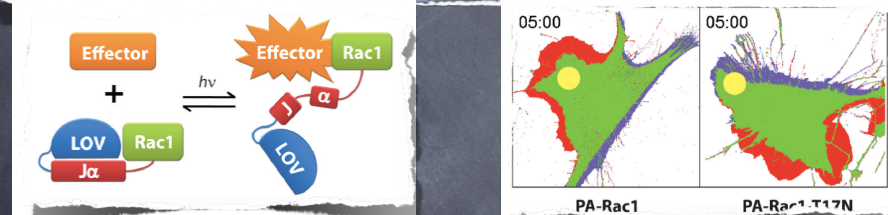
Genética

Proteínas fotoreceptoras artificiales



# Funcionalidad: Control espacio-temporal Optogenética

A genetically-encoded photoactivatable Rac controls the motility of living cells. Nature, 2009



# Dinámica: Movimiento-recambio-prot-prot

Localización

Movimiento

