

# FRAIB : FEDERATION DE RECHERCHE AGROBIOSCIENCES INTERACTIONS ET BIODIVERSITE



Cécile POUZET\*, Christian BRIÈRE\*\* et Alain JAUNEAU\*  
[jauneau@lrsv.ups-tlse.fr](mailto:jauneau@lrsv.ups-tlse.fr), 05.34.32.38.37 \*FR-AIB 3450 CNRS, Plateforme de  
microscopie FRAIB \*\* Laboratoire de Recherche en Sciences Végétales,  
LRV – UMR 5546 UPS-CNRS, Campus INRA 24, chemin de Borde-Rouge BP  
42617 Auzeville, 31326 Castanet-Tolosan.

**5<sup>èmes</sup> Journées Scientifiques et Techniques du R<sub>pl</sub>  
Dijon – 11-14 Novembre 2014**

# FLUORESCENCE LIFETIME: DURÉE DE VIE D'UN FLUOROPHORE à L'ÉTAT EXCITÉ

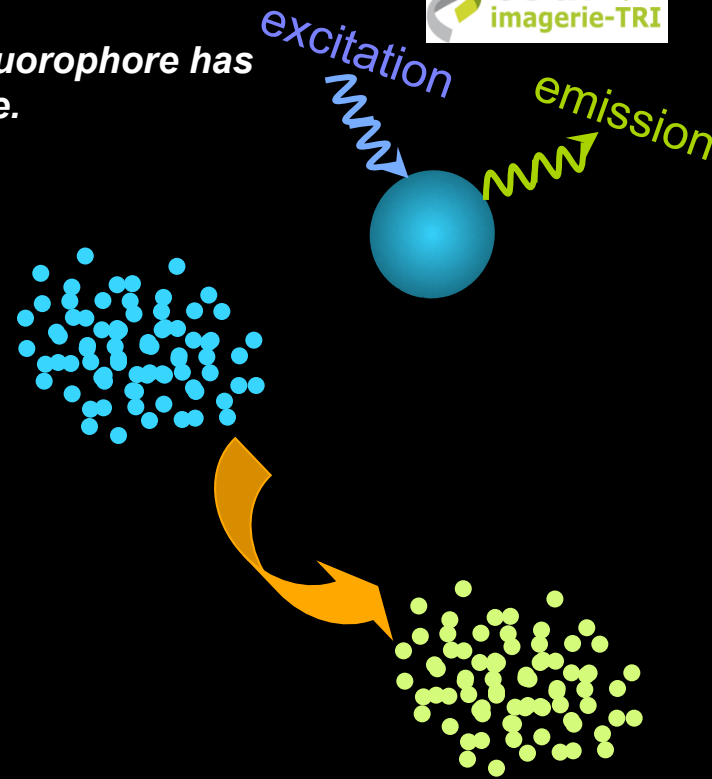
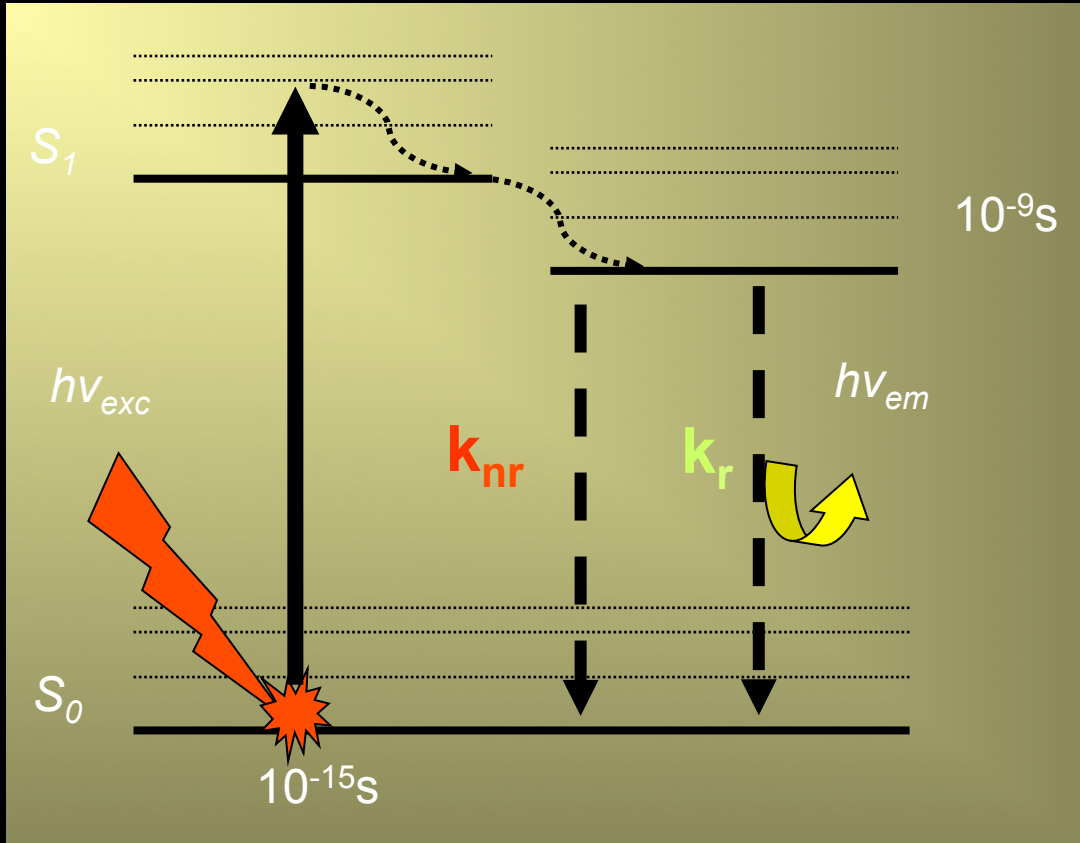
**Le FLIM:** Fluorescence lifetime imaging microscopy

**Le FRET en FLIM:** Mise en évidence des interactions entre protéines, entre protéine et ADN.

# RAPPELS

## FLIM: Physical meaning of $\tau$ .

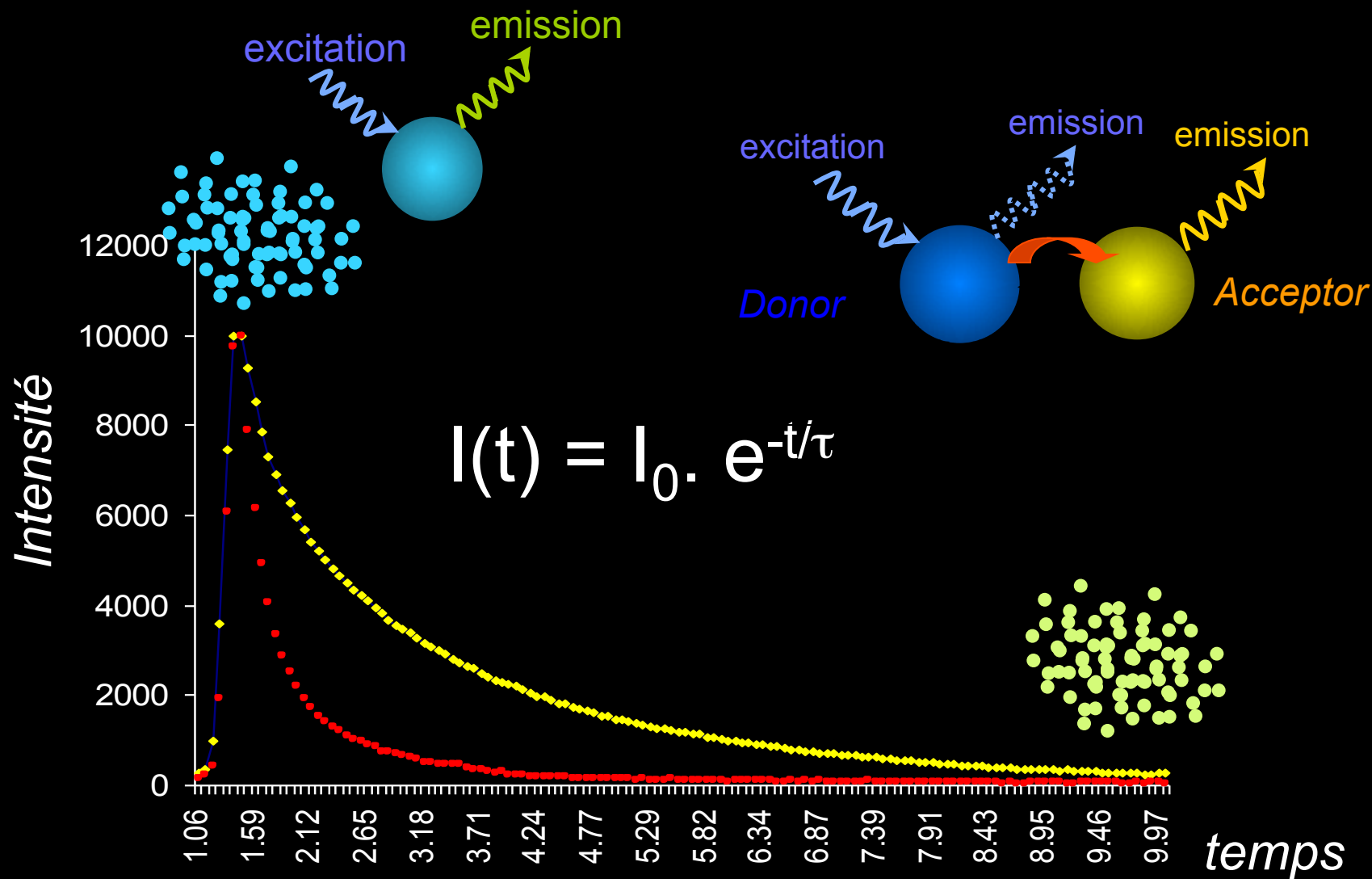
Emission of fluorescence is a statistical process. Each excited fluorophore has an equal probability of emitting a photon in a given period of time.



$$\frac{dN(t)}{dt} = -(k_r + k_{nr}) \cdot N(t)$$

$$\tau = 1 / (k_r + k_{nr})$$

$$\frac{dI(t)}{dt} = -(k_r + k_{nr} + k_{FRET}) \cdot I(t)$$



# FLUORESCENCE LIFETIME MICROSCOPY STREAK FLIM SYSTEM

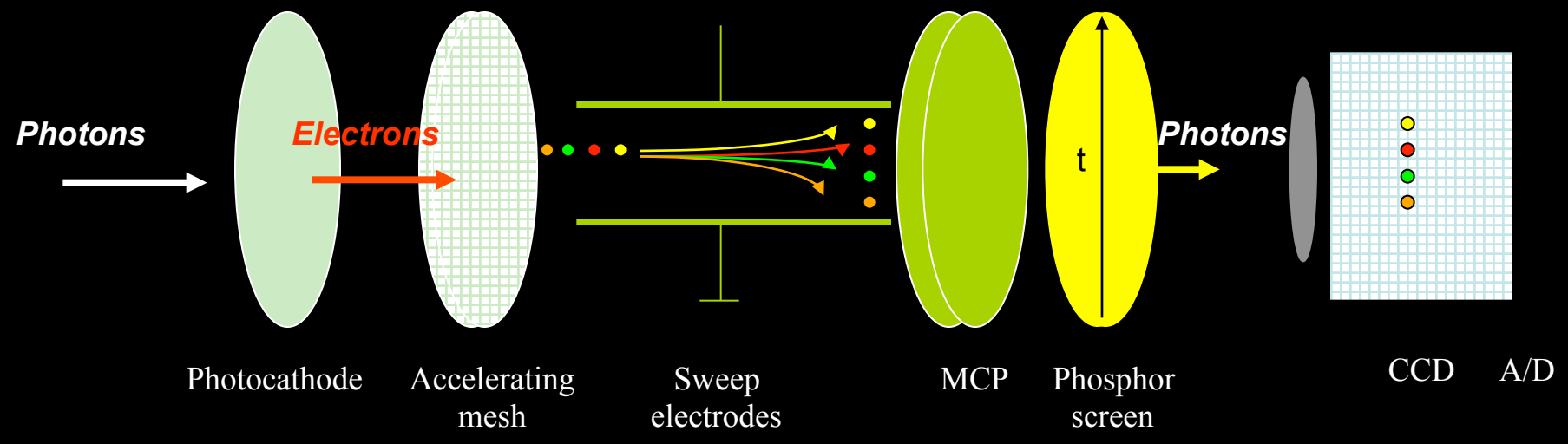
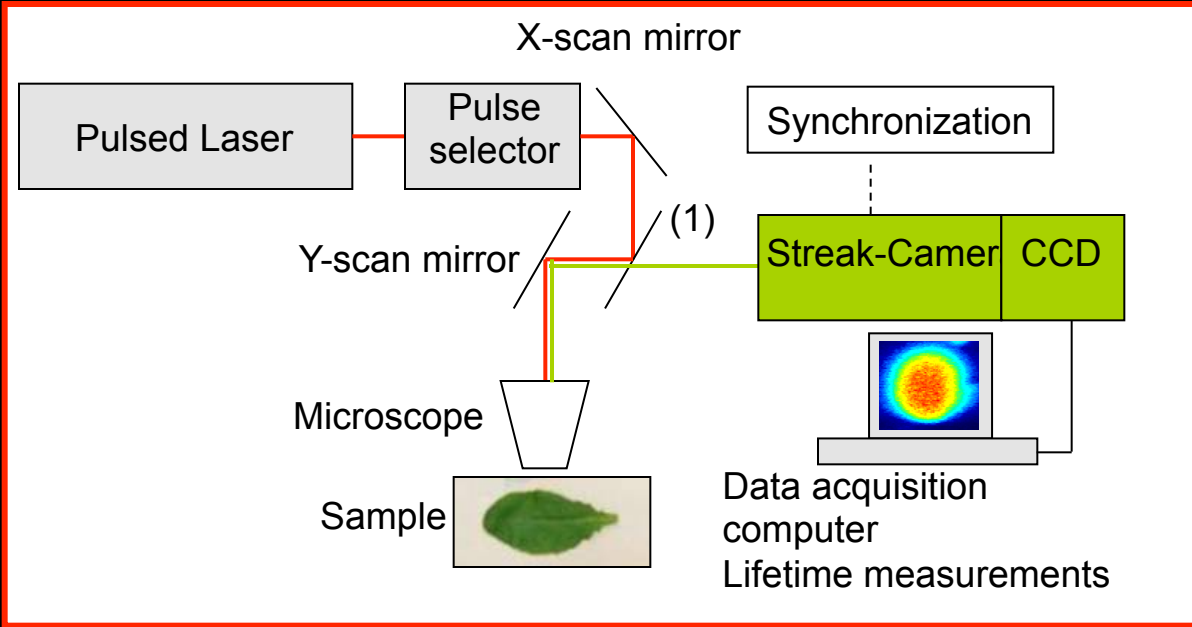


Maud Bernoux *et al.* **Plant Cell.** 2008  
Solène Froidure *et al.*, **PNAS**, 2010.  
Céline Tasset, *et al.*, **PlosPathogens.** 2010.  
Alexandre Perochon, *et al.*, **BBRC** 2010.  
Joanne Canonne *et al.*, **Plant Cell**, 2011.  
Katharina Heidrich *et al.*, **Science** 2011.  
Rivas *et al.* **Nature Comm** 2013  
Cesari *et al.*, **Plant Cell** 2013

Rose Boutros *et al.* **Biol Cell.** 2011  
Yvan Canitrot *et al.*, **JCB.** 2012

Novaleads (2010-2011)  
LED – LAAS (2013)

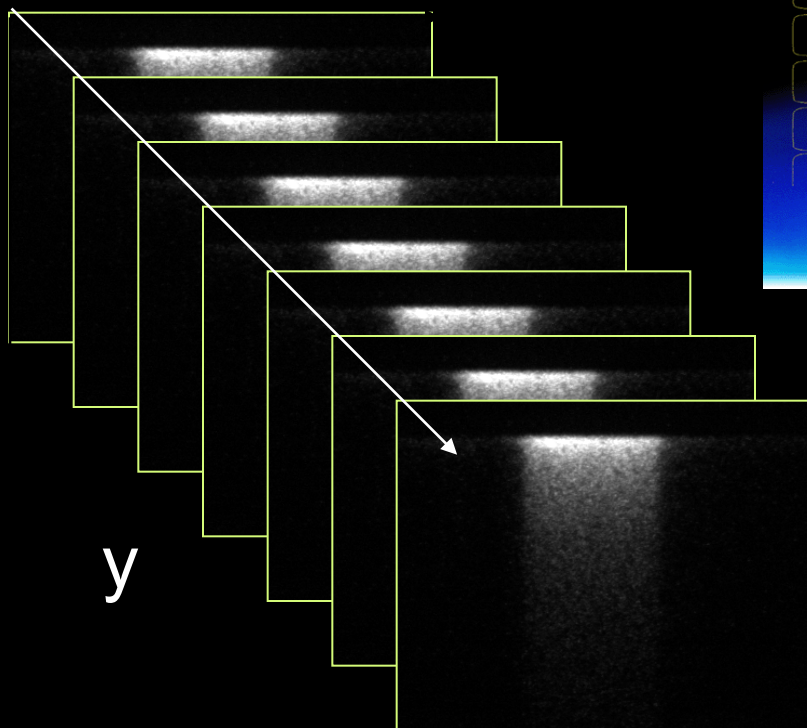
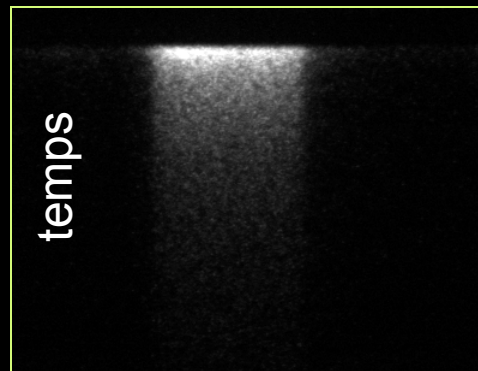
# STREAK-FLIM SYSTEM



# STREAK-FLIM SYSTEM

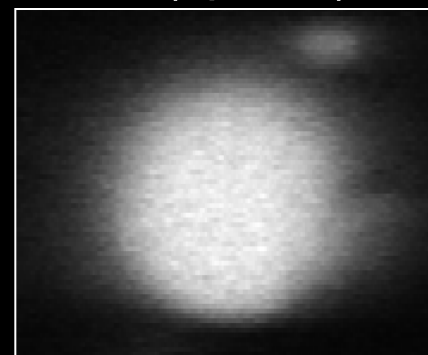
X (spatial)

temps



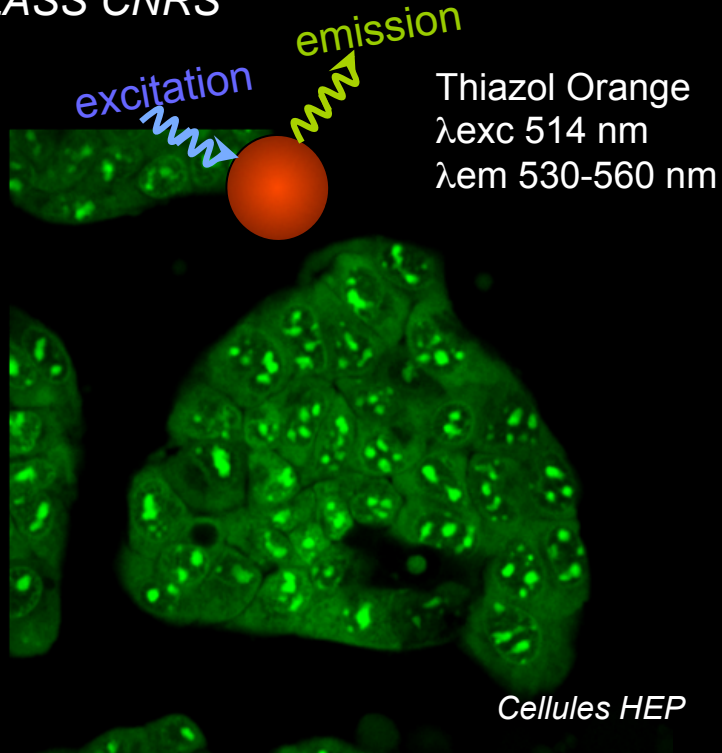
X (spatial)

y (spatial)



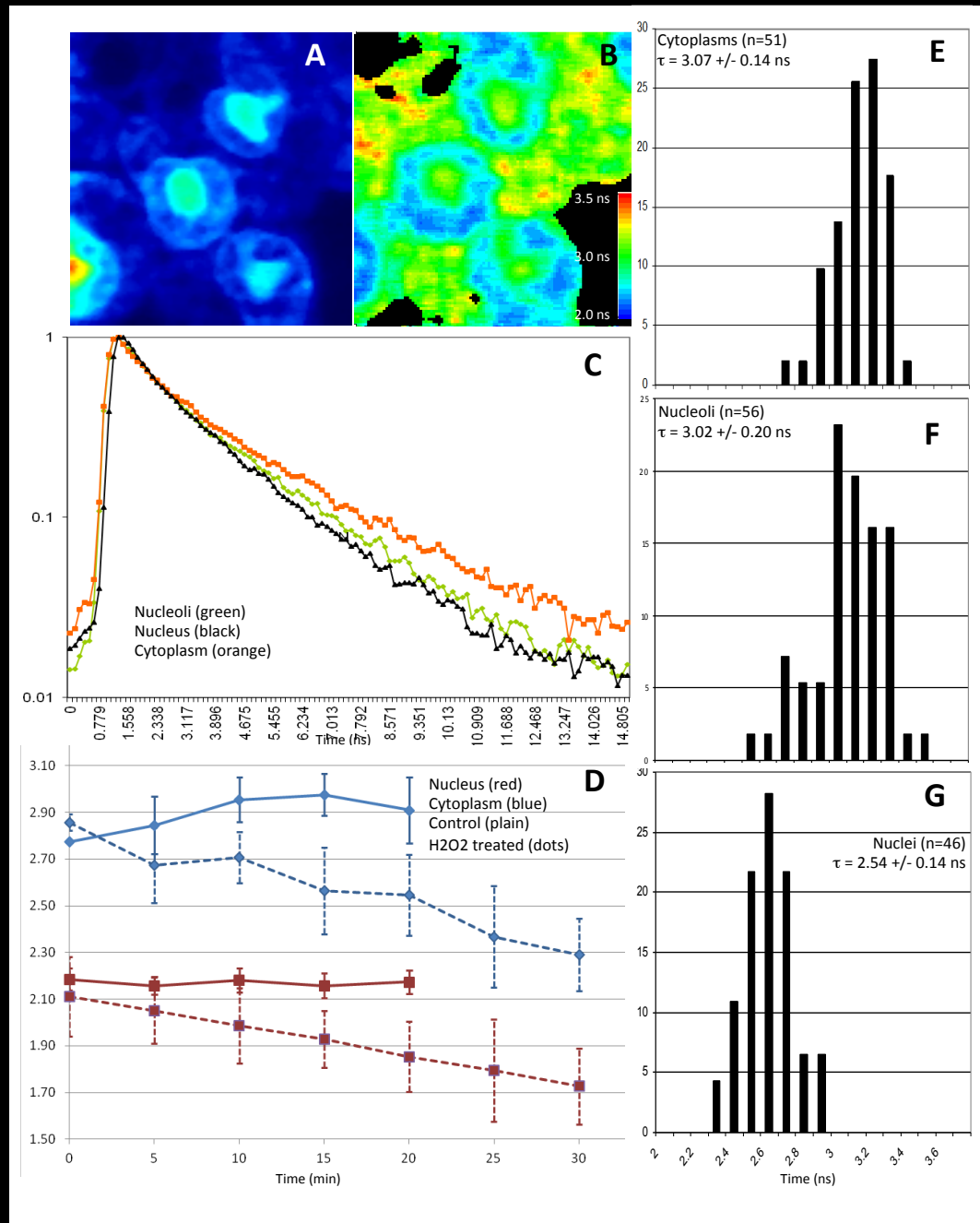
# ASSAY FOR TOXICITY EVALUATION IN LIVE CELLS

Coll. Christophe FURGER LED  
LASS CNRS



La durée de vie,  $\tau$ , du thiazol orange est différente selon le compartiment cellulaire.

La durée de vie,  $\tau$ , du thiazol orange diminue lorsque les cellules sont en présence d'un composé toxique.





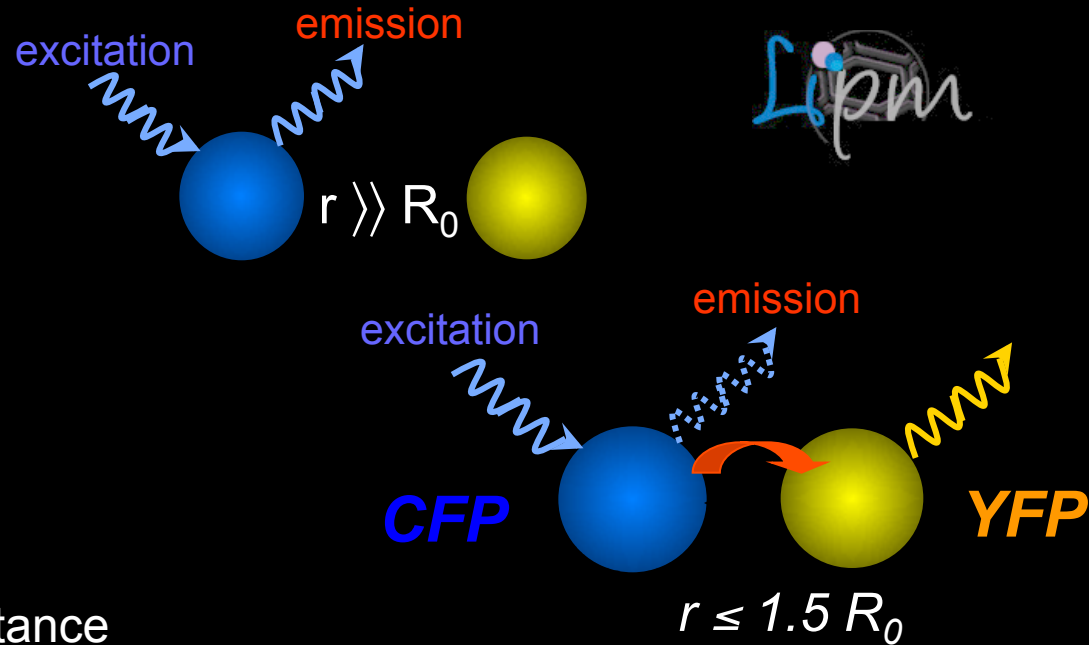
# INTERACTIONS ENTRE PROTEINES IN PLANT CELLS

GMI1000 Inoculation



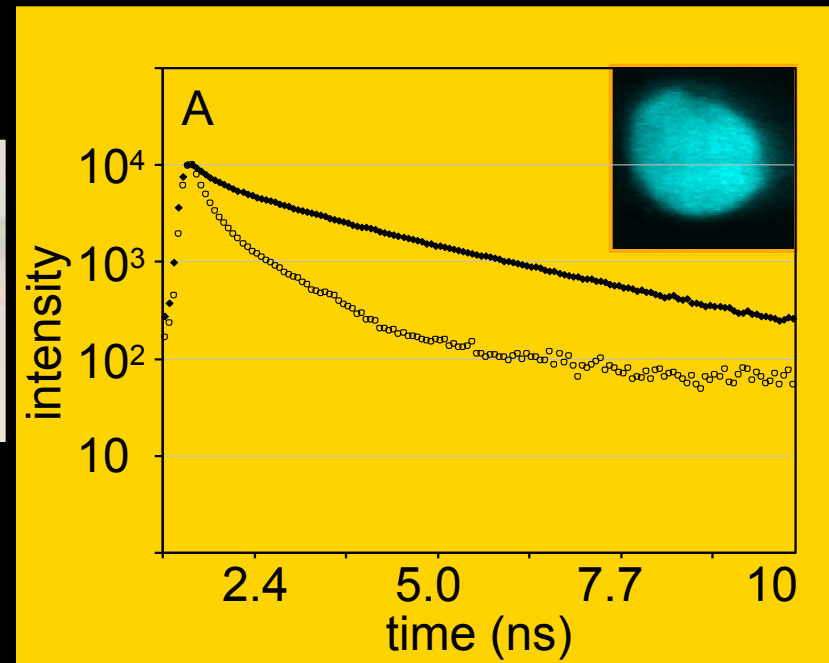
**Nd-1  
Resistant**

**Col-5  
Susceptible**



Identification de protéines de résistance  
RRS1-R (*Résistance à Ralstonia solanacearum*)

- Maud Bernoux *et al.* *Plant Cell*. 2008
- Solène Froidure *et al.*, *PNAS*, 2010.
- Céline Tasset, *et al.*, *PlosPathogens*. 2010.
- Alexandre Perochon, *et al.*, *BBRC* 2010.
- Joanne Canonne *et al.*, *Plant Cell*, 2011.
- Katharina Heidrich *et al.*, *Science* 2011.
- Rivas *et al.* *Nature Comm* 2013.
- Cesari *et al.*, *Plant Cell* 2013.
- Vilarrasa-Blasi *et al.*, *Developmental Cell* 2014.

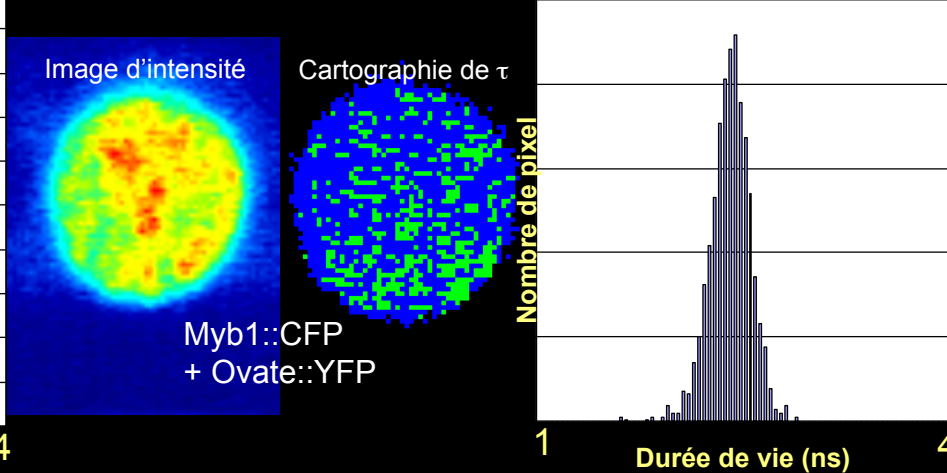
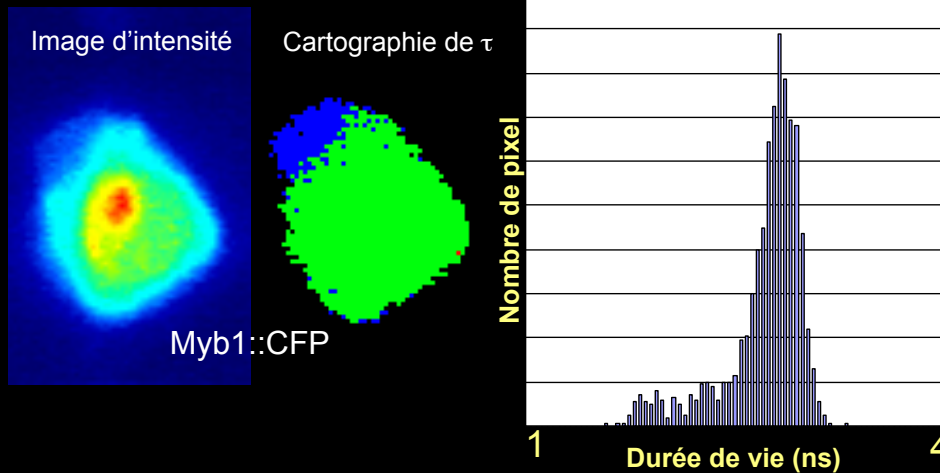
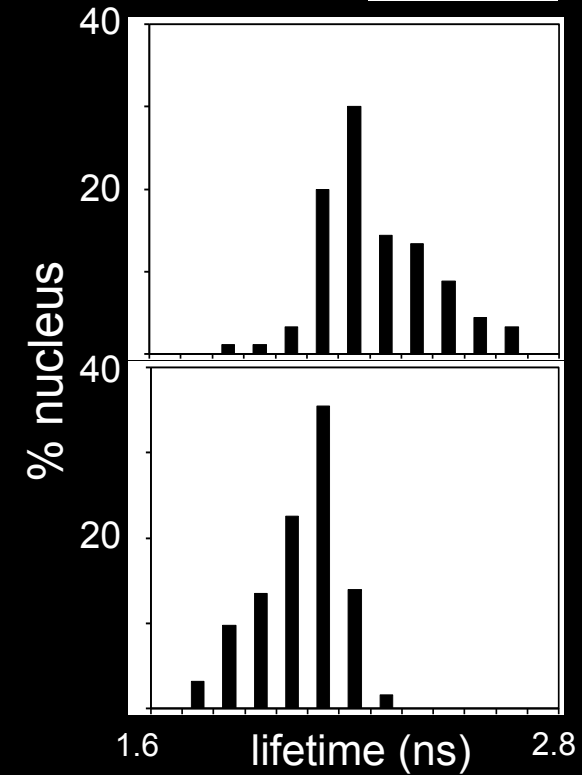


# INTERACTIONS ENTRE PROTEINES IN PLANT CELLS

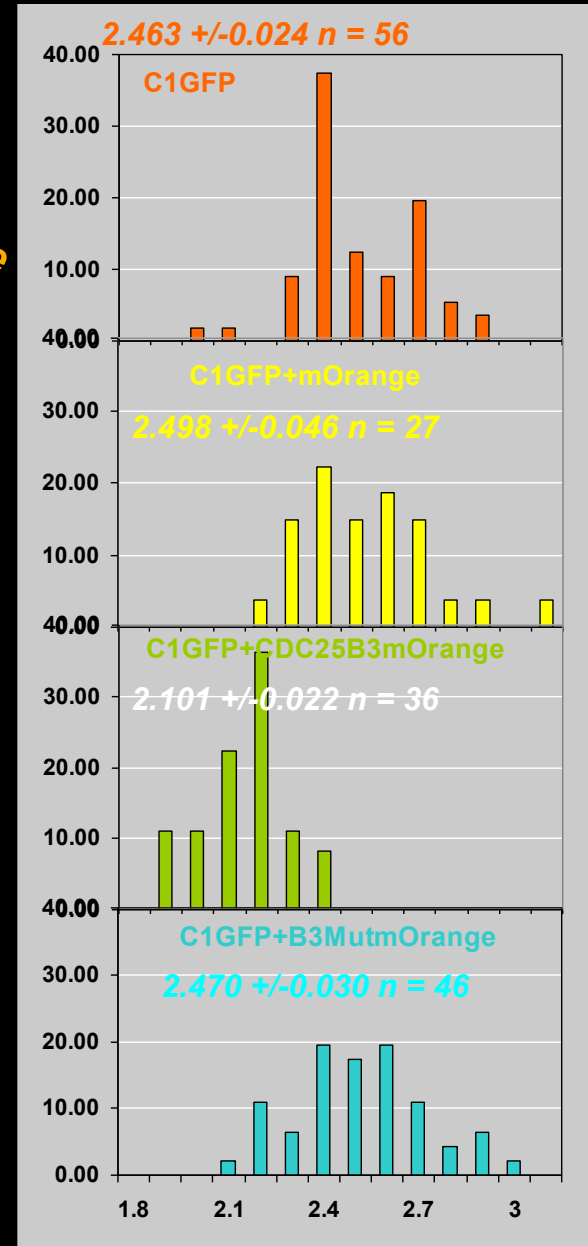
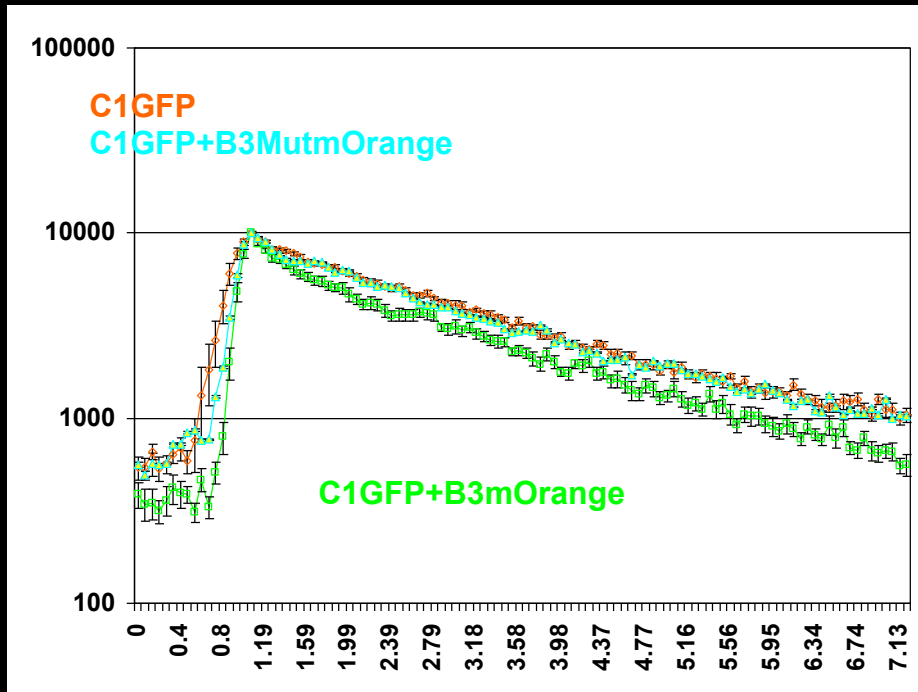
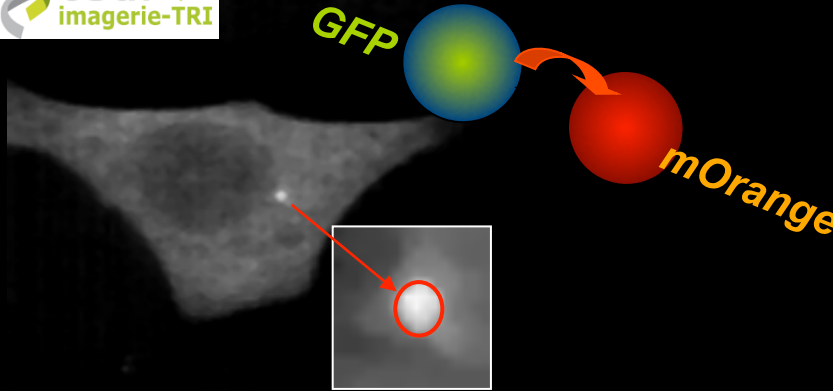


Les contrôles **positifs**, les contrôles **négatifs**

Donor	Acceptor	$\tau_m$ moyen ns	sem	NB	FRET %	p-value
MybX CFP	-	2.580	0.026	107	-	-
<b>MybX CFP</b>	<b>O- YFP</b>	2.052	0.029	70	20	$3.910^{-27}$
<b>MybX CFP</b>	<b>Histone YFP</b>	2.161	0.051	58	16	$2.8 \cdot 10^{-13}$
<b>MybX CFP</b>	<b>35S YFP</b>	2.566	0.029	56	-	0.75
<b>Myb96 CFP</b>	-	2.647	0.053	54	-	-
<b>Myb96 CFP</b>	<b>O- YFP</b>	2.568	0.064	28	-	0.36
<b>Myb96 CFP</b>	<b>Histone YFP</b>	2.693	0.046	38	-	0.56



# INTERACTIONS ENTRE PROTEINES IN HUMAN CELLS



Rose Boutros *et al.* Biol Cell. 2011  
 Yvan Canitrot *et al.*, JCB. 2012