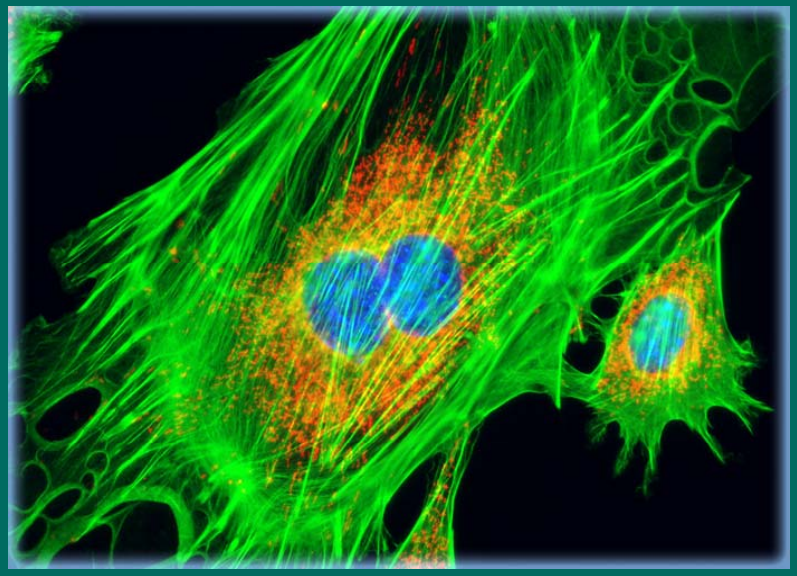
Protéines fluorescentes pour l'exploration cellulaire



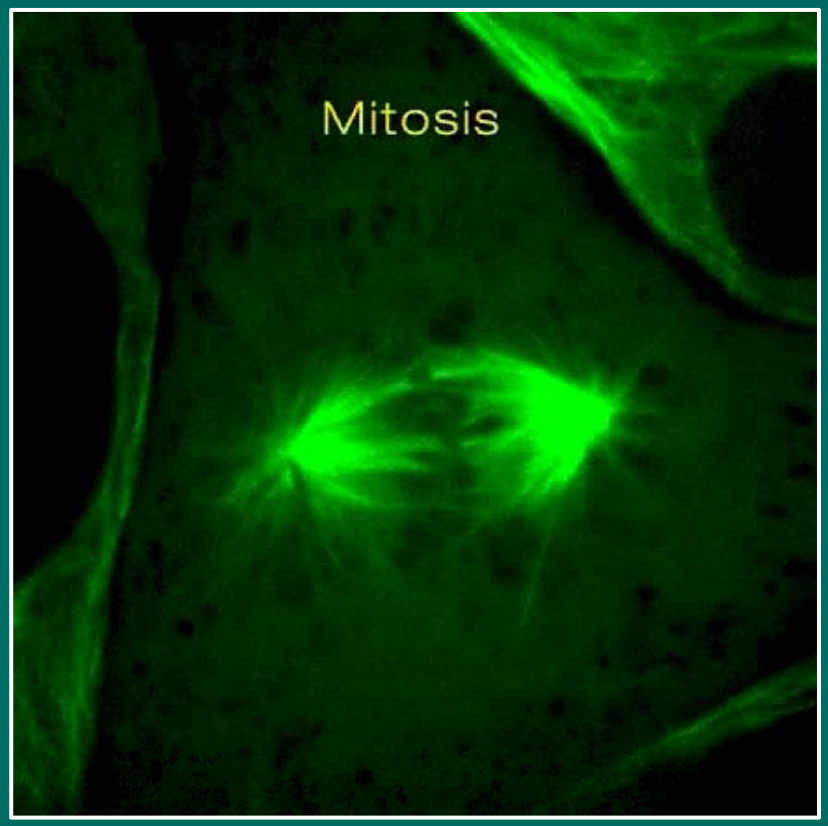
Catherine Leclerc & Marc Moreau
Centre de Biologie du développement
UMR 5547 Toulouse
&
GRDE 731

GDRE 731
Ca^{2+} and gene expression

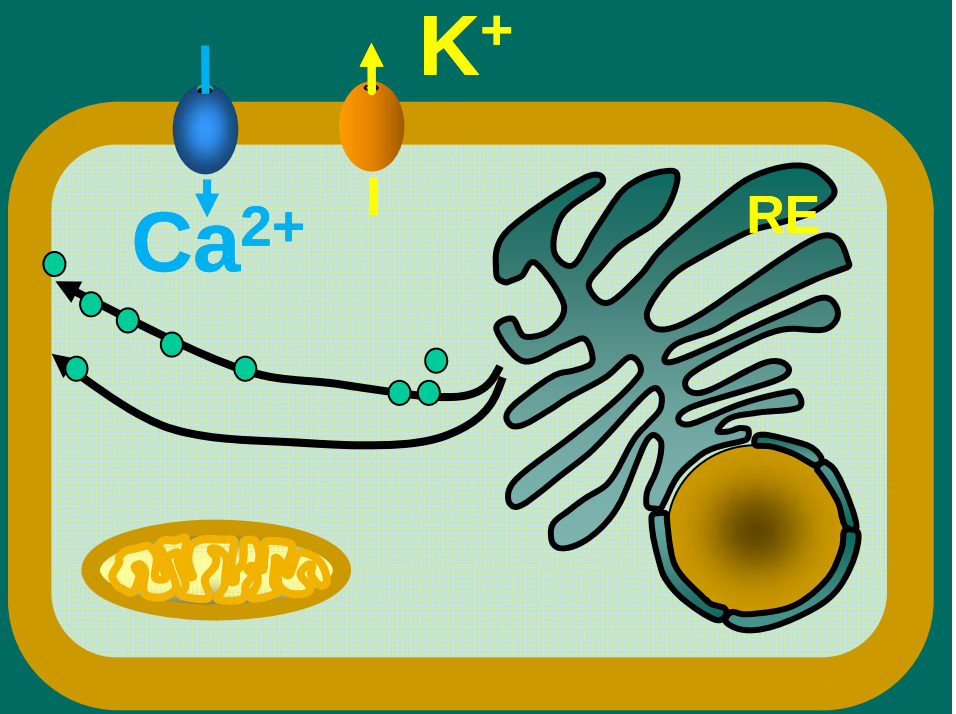
Intérêt de la fluorescence

- Mise en œuvre relativement facile
- Applicable au vivant

Études dynamiques
Fonctions

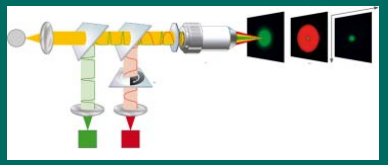


UltraView, PerkinElmer

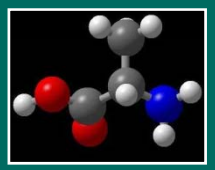


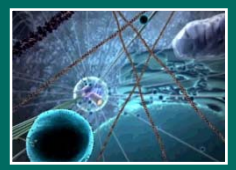
Fluorescence

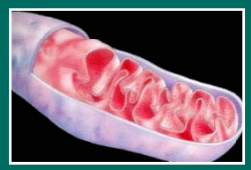
Super resolution

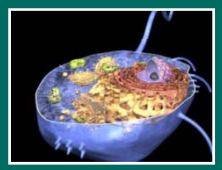


Micro.
électronique





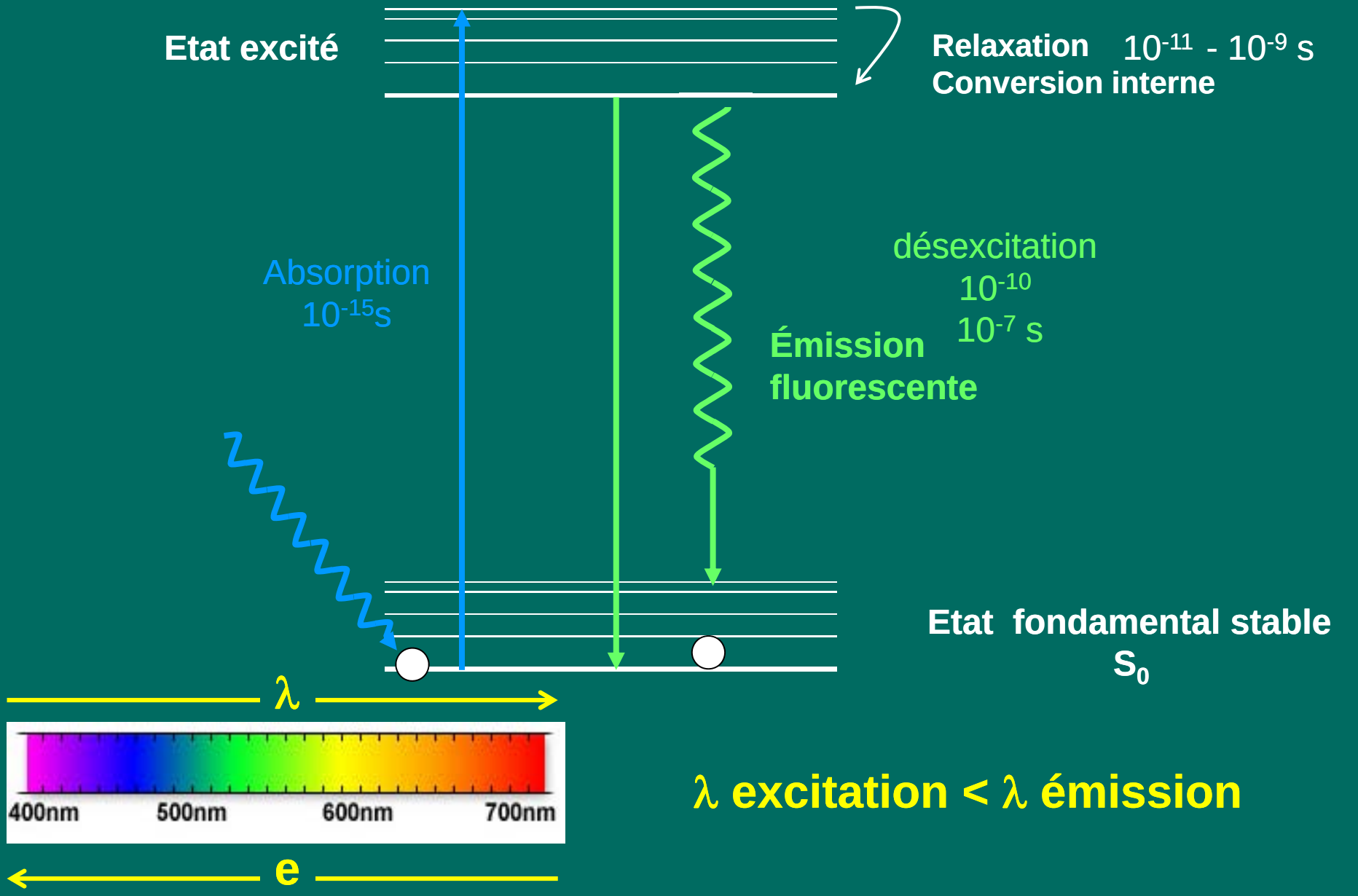




0.1 nm (1Å) 1 nm 10 nm 100 nm 1 µm 10 µm 0.1 mm

atomique moleculaire subcellulaire cellulaire

Fluorescence



λ excitation < λ émission

Rendement quantique & coefficient d'extinction

Rendement quantique
(efficacité de fluorescence)

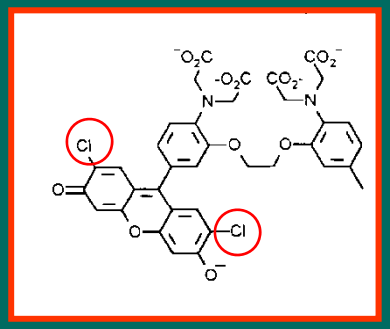
$$\Phi = \frac{I_f}{I_a}$$

0.05 - 1

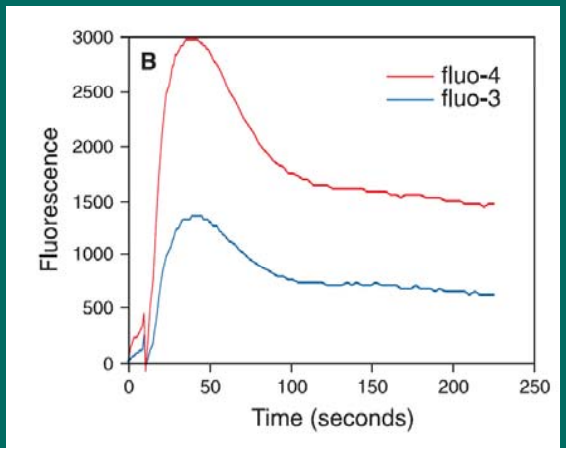
Coefficient d'extinction
(absorbance spécifique)

ε, reflète la probabilité d'absorption
($M^{-1}.cm^{-1}$ 5000 – 250 000)

Brillance = Φ x ε



	Φ	ε	B
Fluo-3	0.15	43 000	6450
Fluo-4	0.14	77 000	10 780

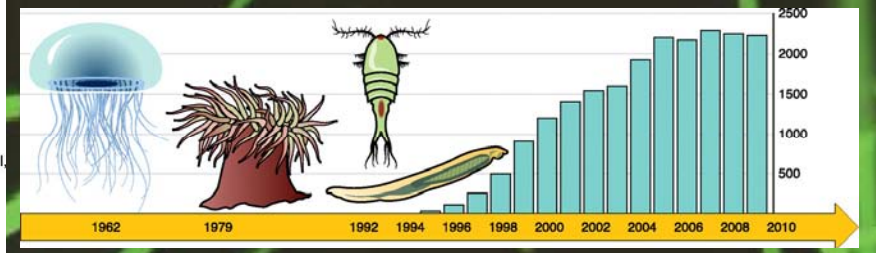


Protéines fluorescentes

M. Chalfie

R. Tsien

O. Shimomura



77 A.D.
Pliny the Elder writes of glowing jellyfish

1985
Douglas Prasher clones Aequorin

1992
Douglas Prasher clones AvGFP

1994
Martin Chalfie expresses AvGFP in bacteria and worms

1996
Mats Ormö, Jim Remington, Roger Tsien & co-workers solve GFP Crystal structure

1997
Jörg Wiedenmann proposes the existence of GFP-like proteins in sea anemones

1999
Mikhail Matz, Sergey Lukyanov & co-workers clone first RFP (DsRED)

2002
PA-GFP Developed by Jennifer Lippincott-Schwartz, & George Patterson

2002
Venus, Kaede developed by Takeharu Nagai, Ryoko Ando, Atsushi Miyawaki & co-workers

2004
mKO and Dronpa developed by Atsushi Miyawaki & co-workers

2004
PS-CFP2 developed by Konstantin Lukyanov, Dmitriy Chudakov, Vladislav Verkusha, Sergey Lukyanov & co-workers

2006
mTFP1 developed by Robert E. Campbell & co-workers

2006
Dendra developed by Nadya Gurskaya, Sergey & Konstantin Lukyanov, & co-workers

2007
TagRFP and mKate developed by Dmitriy Chudakov, Sergey Lukyanov, Konstantin Lukyanov & co-workers

2008
Fucci and mKO2 developed by Atsushi Miyawaki & co-workers

900 million years ago
Cnidari and Bilateria families diverge

1962
Osamu Shimomura observes autofluorescent protein & isolates Aequorin

AvGFP

1994
Roger Tsien, Roger Heim & Doug Prasher begin mutagenesis of AvGFP

GFP S65T **BFP** Y66H **CFP** Y66W

1996
YFP (T203Y) developed & characterized by Jim Remington, Roger Tsien, Rebekka Wachter & co-workers

2001
Citrine developed by Oliver Griesbeck, Robert Campbell, Roger Tsien & co-workers

2002
DsRED.T1 developed by Benjamin Glick & co-workers

2002
mRFP1 developed by David Zacharias, Amy Palmer, Robert Campbell, Paul Steinbach, Roger Tsien & co-workers

2004
Cerulean developed by David Piston & co-workers

2004
Development of "mFruit" series by Nathan Shaner, Roger Tsien, Robert Campbell & co-workers

mOrange tdTomato mStrawberry mCherry mPlum

2004 - 2006
EosFP developed by Anya Salih, Ulrich Nienhaus, Jörg Wiedenmann, Franz Oswald & co-workers

2006
Superfolder GFP developed by Geoffrey S. Waldo & co-workers

2008
TagBFP Developed by Vladislav Verkhusha, Dimitriy Chudakov & co-workers

2008
mOrange2, TagRFP-T, mApple, EBFP2 developed by Nathan Shaner, Robert E. Campbell, Michael Lin, Roger Tsien & co-workers

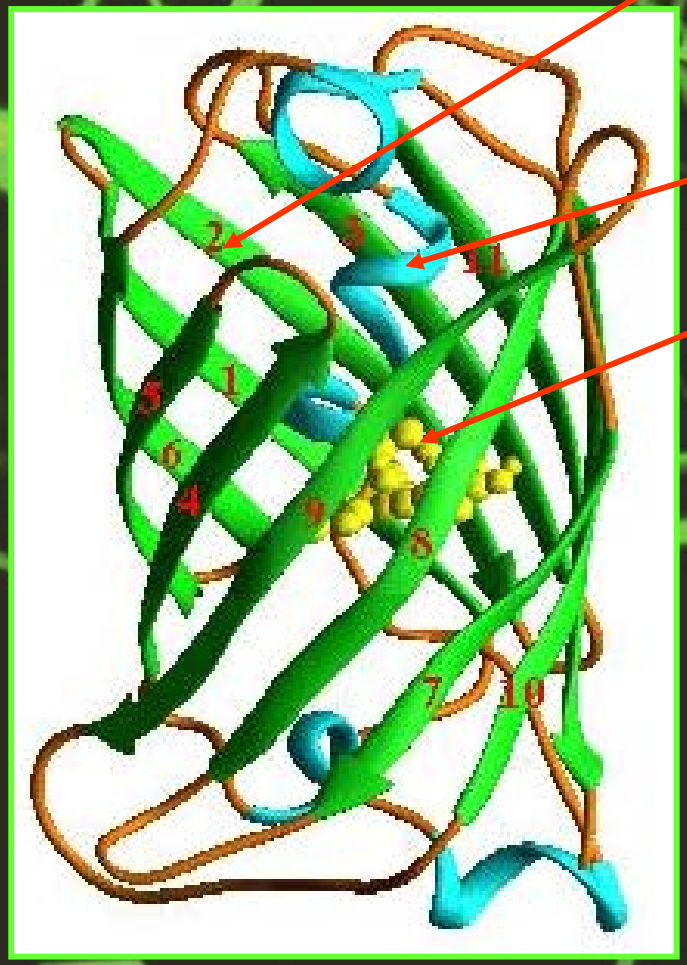
Day & Davidson, Chem. Soc. Rev. 2009

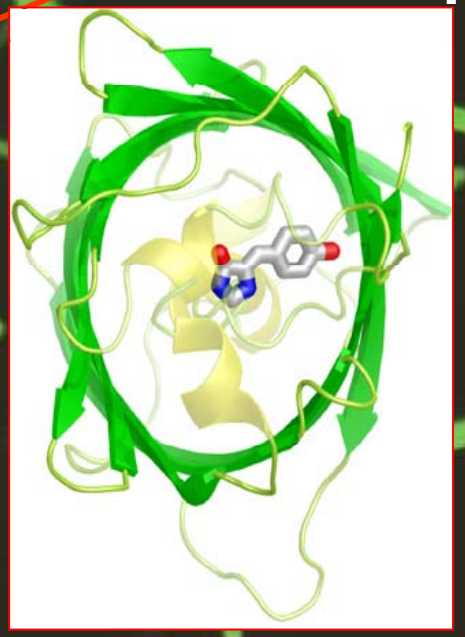
2008

Structure cristalline = tonneau β

GFP : 238 aa, 27 Kda,

- **11 feuillets β antiparallèles**
- **1 hélice contenant le chromophore**



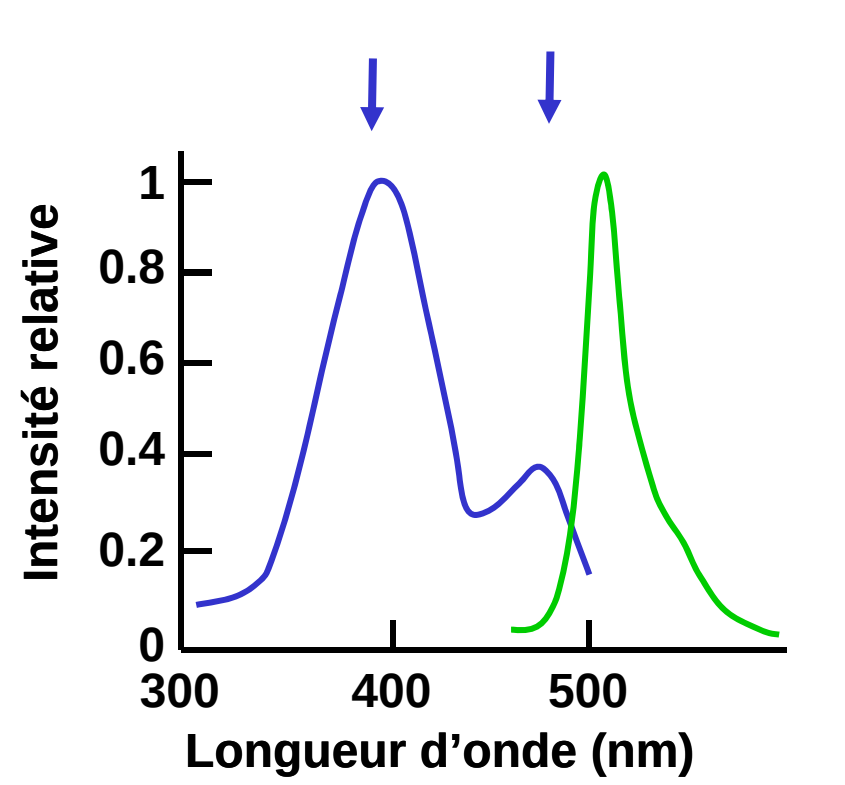


- Acides aminés 2 à 229 sont impliqués dans la réaction de fluorescence
- Chromophore composé de 3 résidus Ser65-Tyr66-Gly67 (SYG)

Photo S. Inouye, in Shimomura 2005, J of microscopy

Caractéristiques de la GFP native

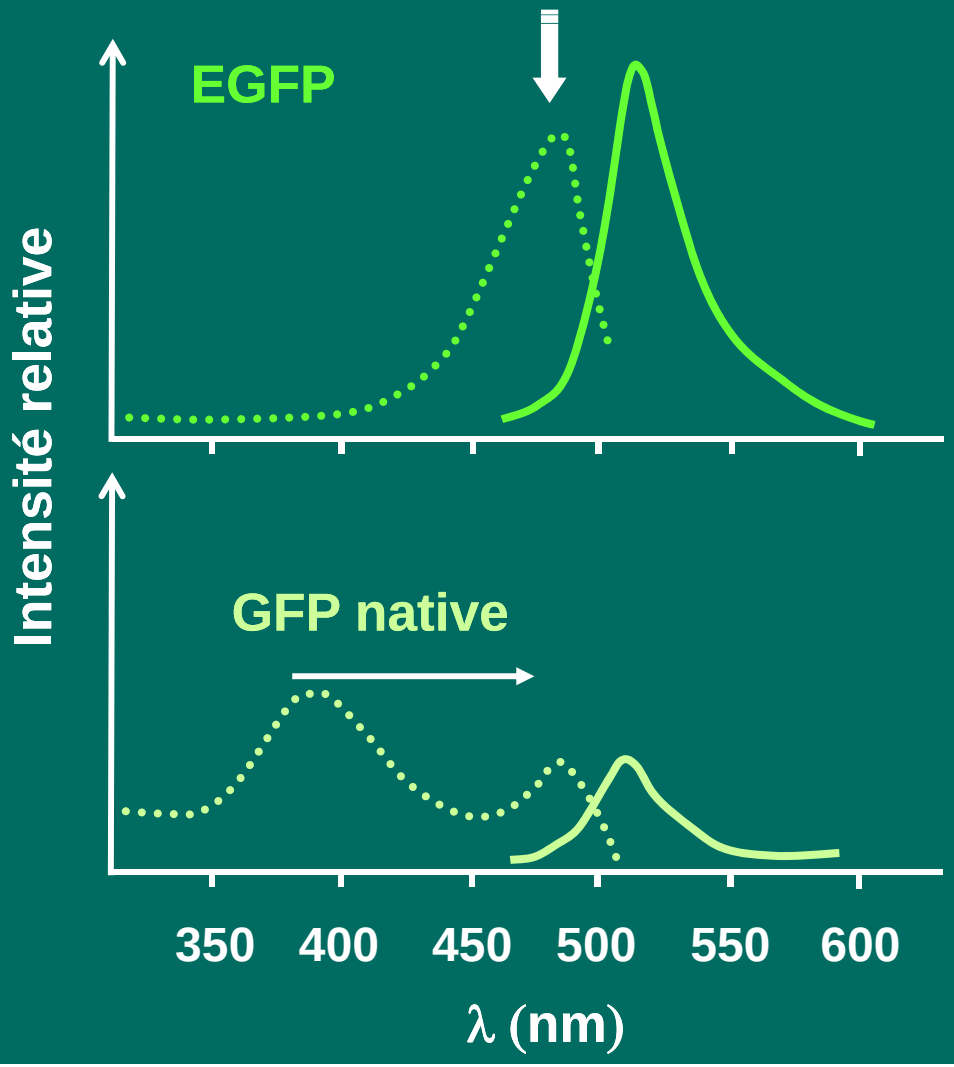
- Repliement efficace à T° ambiante ou <
- Mature GFP est stable et fluorescente pour T° > 65°C, pH 5.5 à 12
- stable en 8M urée, 6M guanidium hydrochloride, 1% de SDS
- GFP Fluorescente après fixation formaldehyde ou glutaraldehyde.
- Fluorescence ne nécessite ni substrat ni co-facteur



- 2 pics d'excitation
  395 & 477 nm
- 1 pic d'émission
  508 nm

La EGFP

Substitution de Ser 65 par Thr (mutant S65T)



excitation

émission

- Excitable à 488 nm
- Augmentation de la photostabilité
- Rendement quantique (intensité X6)

Maturation de la GFP

1- Un réarrangement par torsion

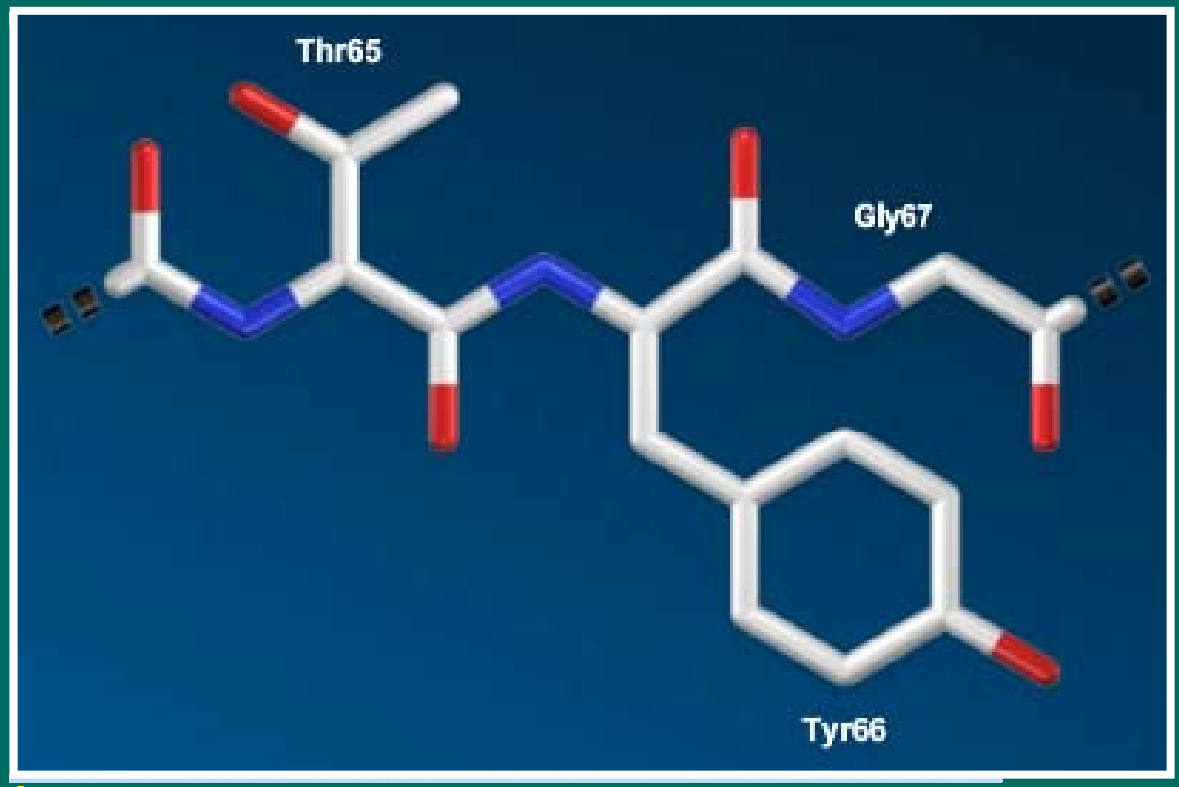
2- Une cyclisation

3- Une déshydratation

4- Une oxydation

Délocalisation du résidu carboxyl de la Thr65 à proximité du groupement amino de la Gly67.

Attaque de l'atome de carbone par le groupement amine de l'azote de la Gly 67

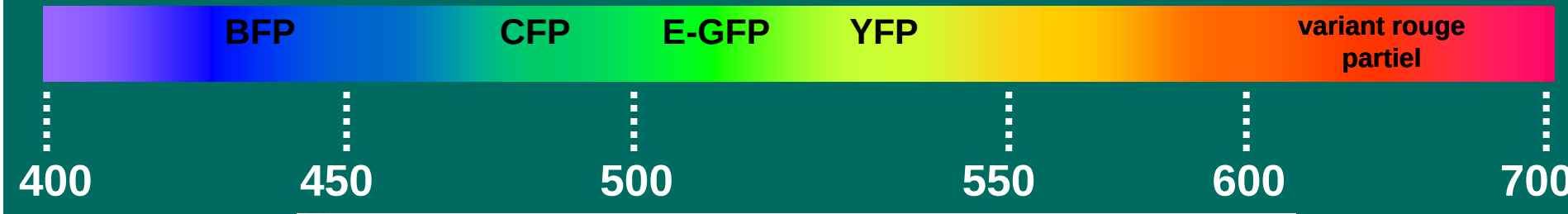


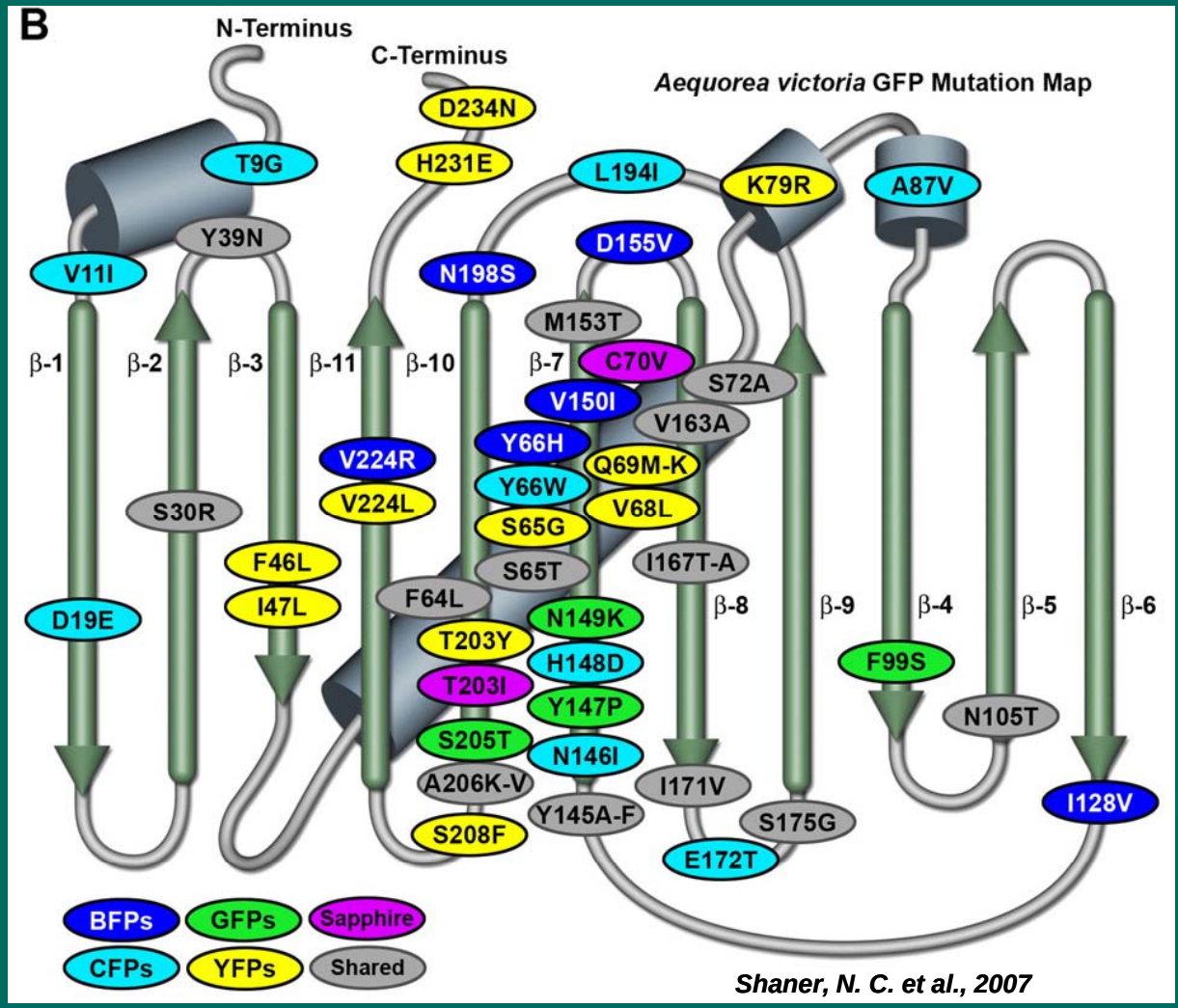
Oxydation par l'oxygène moléculaire du résidu de l'atome de carbone de la Tyr 66

Déshydratation et formation d'un anneau d'imidazole

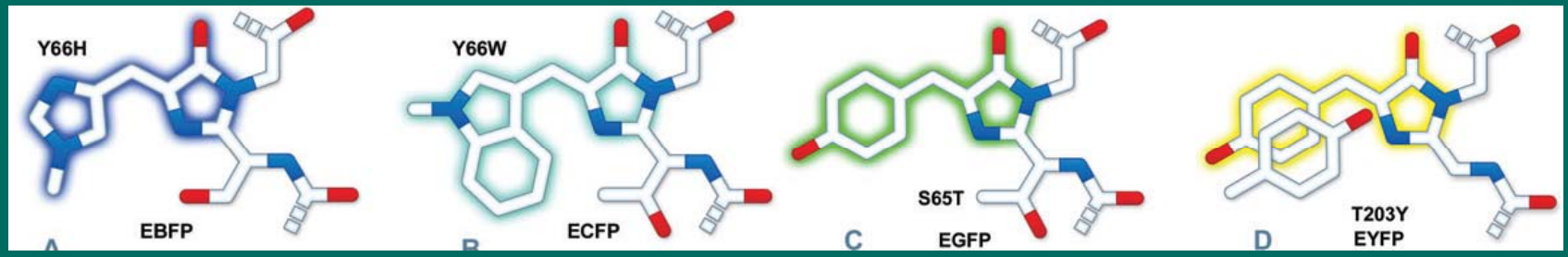
ZEISS Online Campus & Brice Ronsin

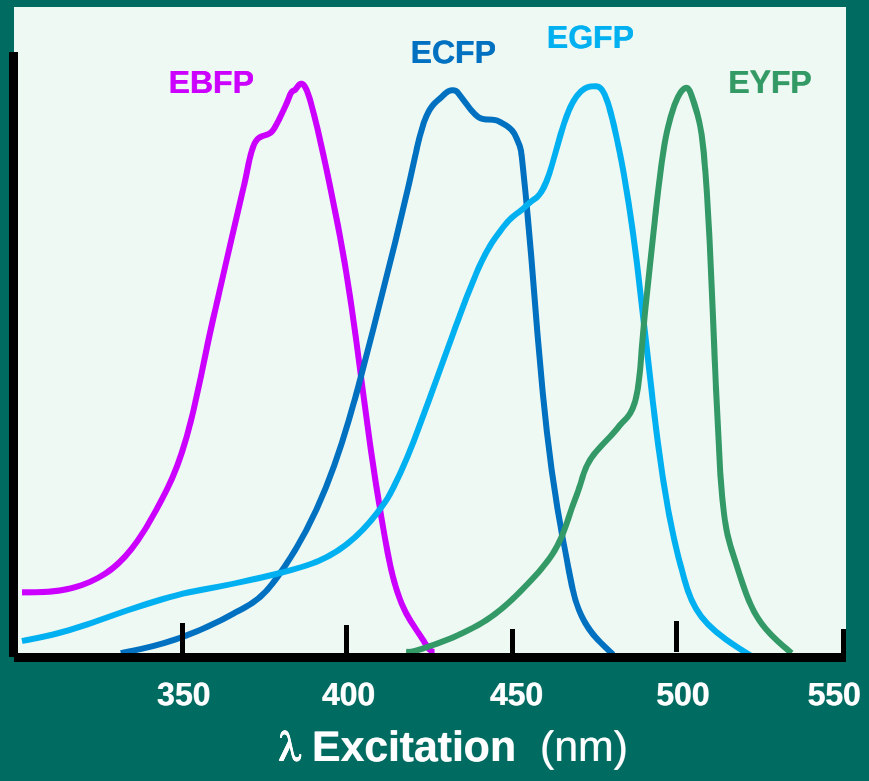
GFP variants





GFP variants





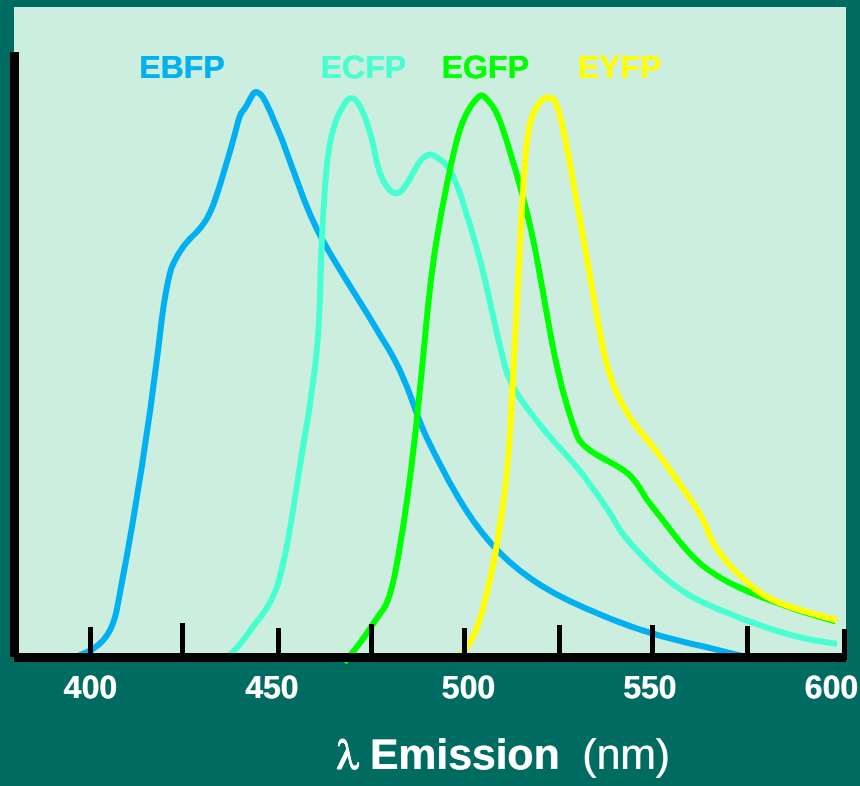


Table 1 Properties of selected *Aequorea*-GFP derivatives. The peak excitation (Ex) and emission (Em) wavelengths, molar extinction coefficient (EC), quantum yield (QY), relative brightness, and physiological quaternary structure are listed (* signifies a weak dimer, see Section 2.6). The computed brightness values were derived from the product of the molar extinction coefficient and quantum yield, divided by the value for EGFP

Protein (acronym)	Ex/nm	Em/nm	EC/10^{-3} M^{-1} cm^{-1}	QY	Quaternary structure	Relative brightness (% of EGFP)	Ref.
Blue fluorescent proteins							
Sirius	355	424	15.0	0.24	Monomer*	11	54
Azurite	384	448	26.2	0.55	Monomer*	43	52
EBFP	383	445	29.0	0.31	Monomer*	27	35
EBFP2	383	448	32.0	0.56	Monomer*	53	50
Cyan fluorescent proteins							
ECFP	439	476	32.5	0.40	Monomer*	39	22
Cerulean	433	475	43.0	0.62	Monomer*	79	56
CyPet	435	477	35.0	0.51	Monomer*	53	68
SCFP	433	474	30.0	0.50	Monomer*	45	60
Green fluorescent proteins							
EGFP	488	507	56.0	0.60	Monomer*	100	23
Emerald	487	509	57.5	0.68	Monomer*	116	
Superfolder avGFP	485	510	83.3	0.65	Monomer*	160	32
T-Sapphire	399	511	44.0	0.60	Monomer*	79	27
Yellow fluorescent proteins							
EYFP	514	527	83.4	0.61	Monomer*	151	62
Topaz	514	527	94.5	0.60	Monomer*	169	18
Venus	515	528	92.2	0.57	Monomer*	156	25
Citrine	516	529	77.0	0.76	Monomer*	174	67
YPet	517	530	104	0.77	Monomer*	238	68
SYFP	515	527	101	0.68	Monomer*	204	60
mAmetrine	406	526	45.0	0.58	Monomer	78	222

Excitation proche UV

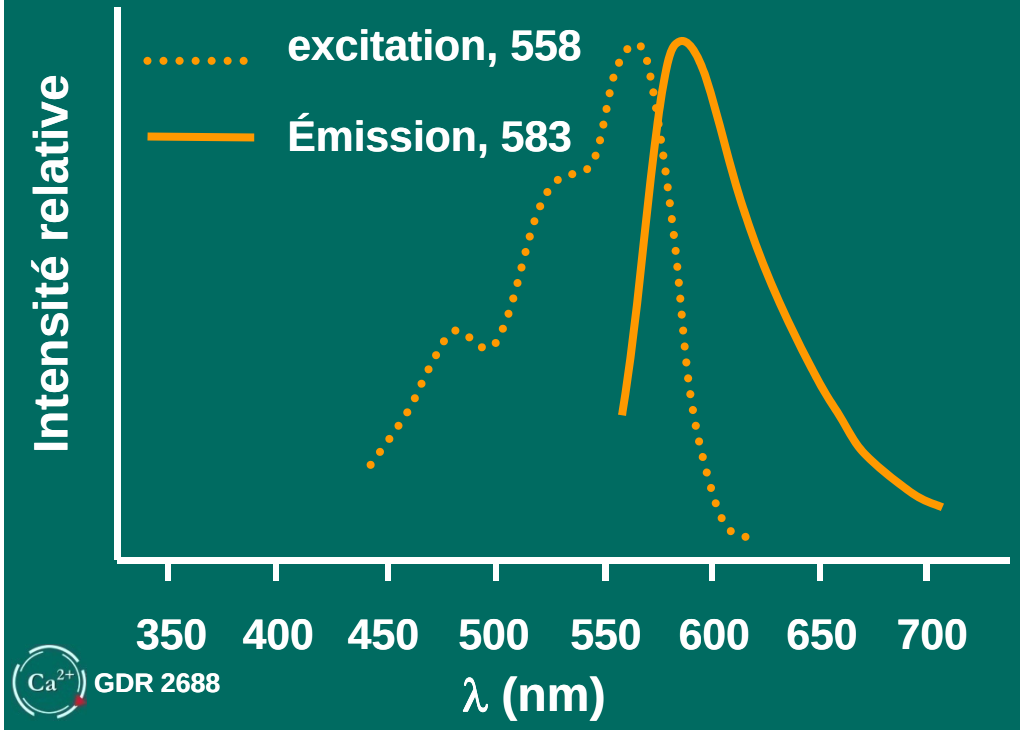
Moins photostable que EGFP

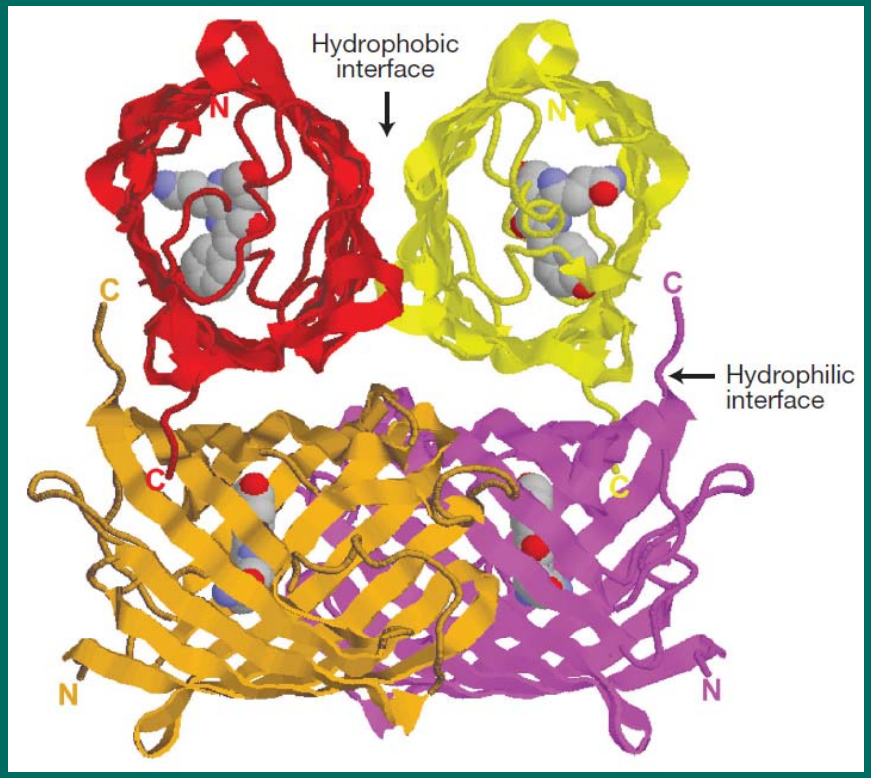
Red Fluorescent Protein (DsRed)

Discosoma s.

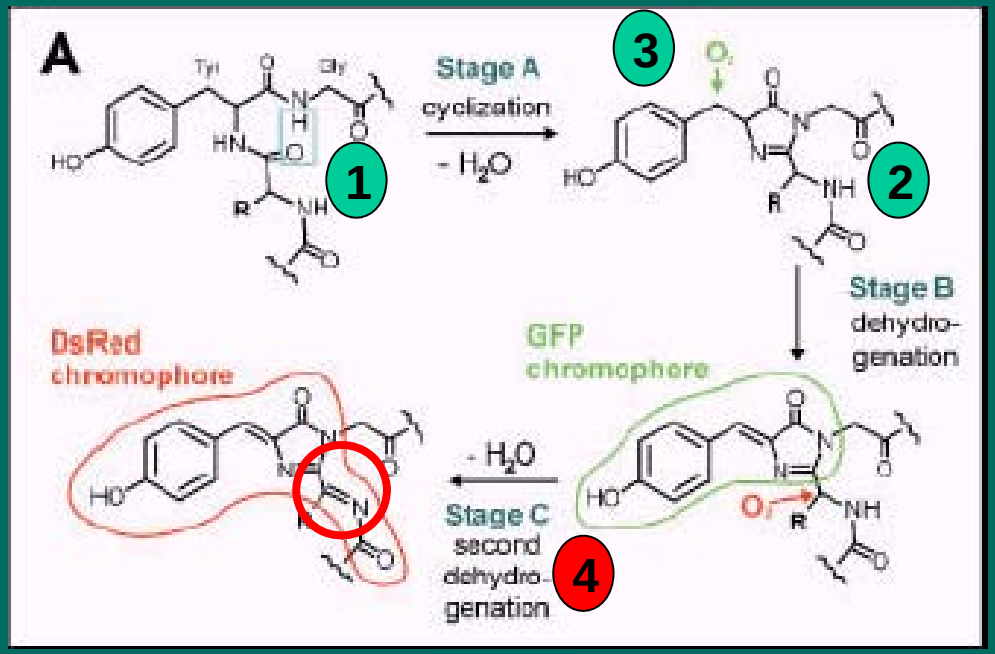
(Matz et al, Nat. Biotechnol. 1999, 17; 969-973)

Tétramère (120 kDa, 4 x 225 aa). chaque monomère est un tonneau-β comparable à la GFP (22%). Chromophore est formé des acides aminés 66-68 (Gln-Tyr-Gly)



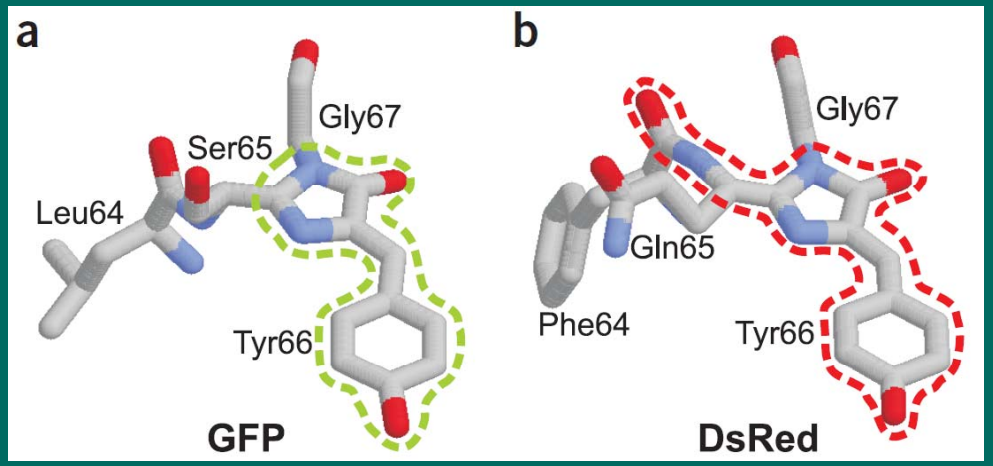


Chromophore de la DsRed

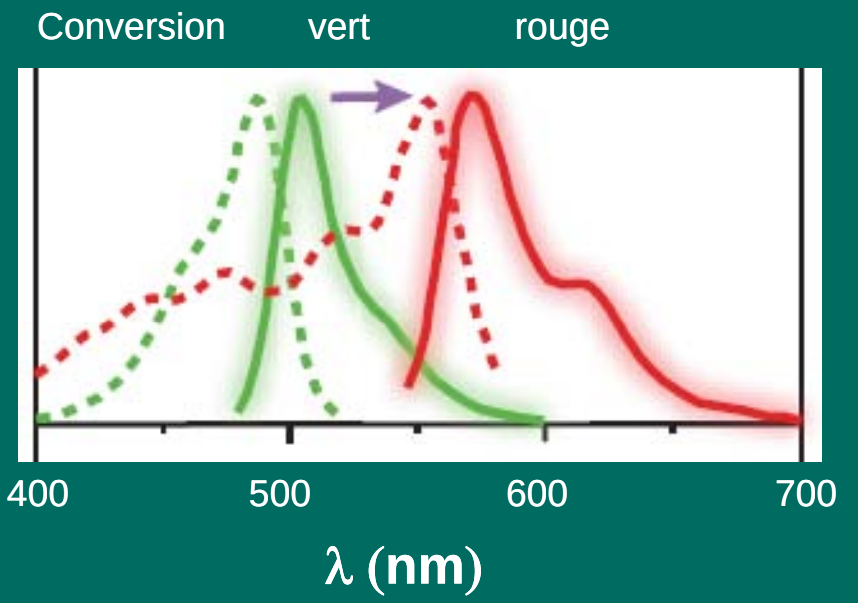


1- cyclisation entre Ser / Gln & Gly
2- dehydrogenation (formation noyau imidazole)
3- oxydation Tyr

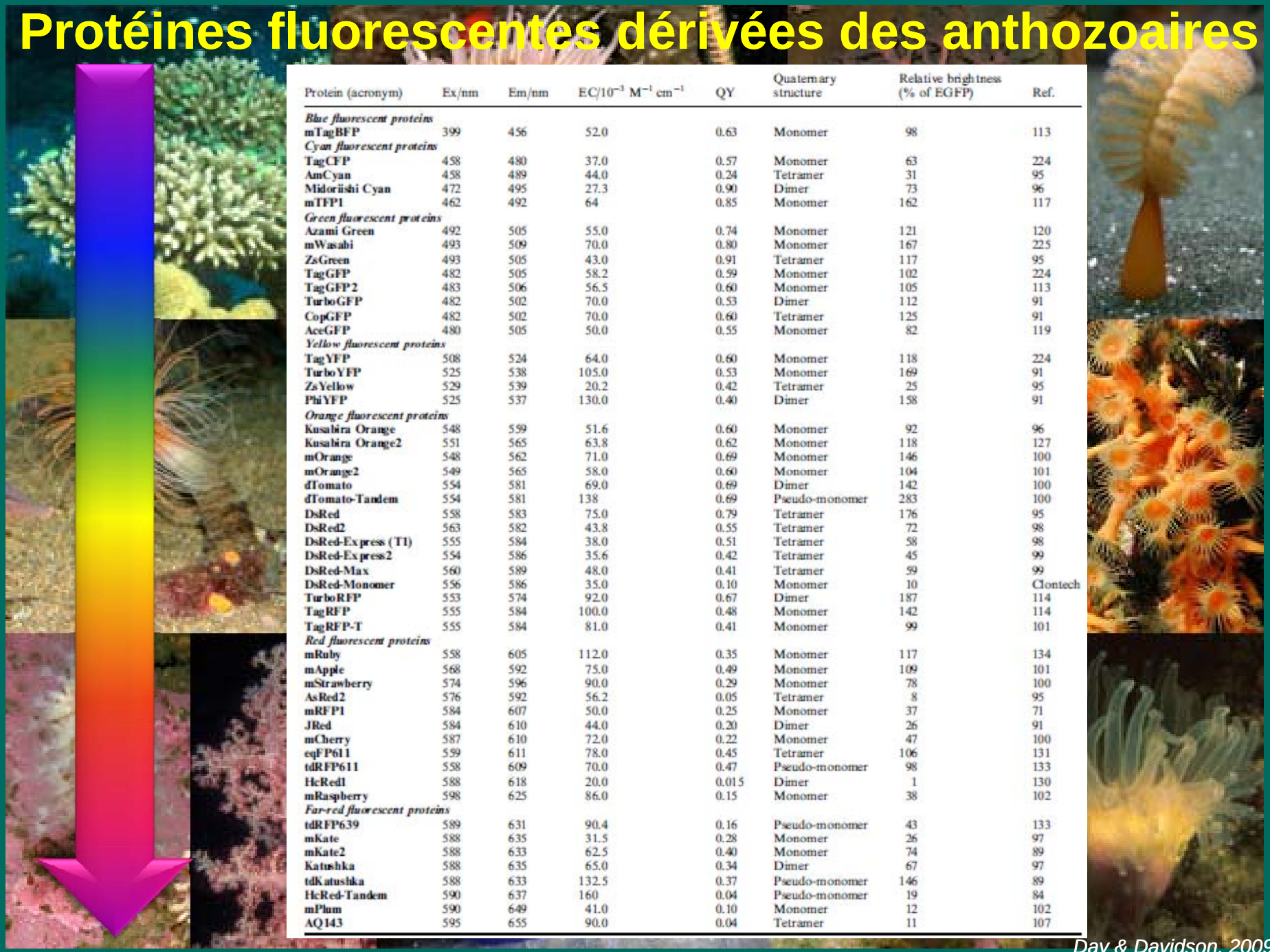
4- dehydrogenation au niveau de Gln



Verkhusha & Lukyanov, Nature Biotech 2004



Protéines fluorescentes dérivées des anthozoaires

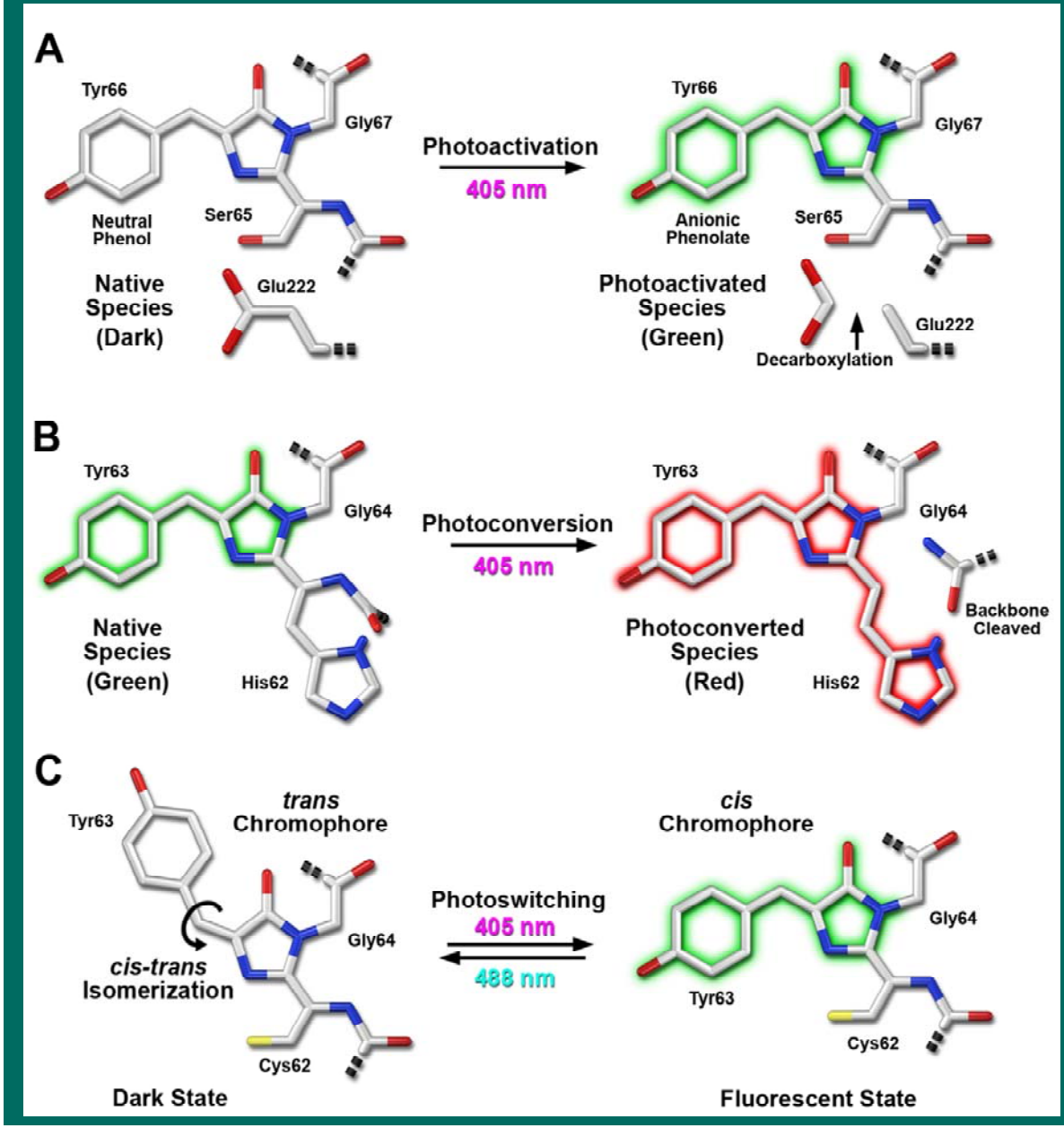


Protein (acronym)	Ex/nm	Em/nm	EC/10^{-3} M^{-1} cm^{-1}	QY	Quaternary structure	Relative brightness (% of EGFP)	Ref.
Blue fluorescent proteins							
mTagBFP	399	456	52.0	0.63	Monomer	98	113
Cyan fluorescent proteins							
TagCFP	458	480	37.0	0.57	Monomer	63	224
AmCyan	458	489	44.0	0.24	Tetramer	31	95
Midoriishi Cyan	472	495	27.3	0.90	Dimer	73	96
mTFP1	462	492	64	0.85	Monomer	162	117
Green fluorescent proteins							
Azami Green	492	505	55.0	0.74	Monomer	121	120
mWasabi	493	509	70.0	0.80	Monomer	167	225
ZsGreen	493	505	43.0	0.91	Tetramer	117	95
TagGFP	482	505	58.2	0.59	Monomer	102	224
TagGFP2	483	506	56.5	0.60	Monomer	105	113
TurboGFP	482	502	70.0	0.53	Dimer	112	91
CopGFP	482	502	70.0	0.60	Tetramer	125	91
AceGFP	480	505	50.0	0.55	Monomer	82	119
Yellow fluorescent proteins							
TagYFP	508	524	64.0	0.60	Monomer	118	224
TurboYFP	525	538	105.0	0.53	Monomer	169	91
ZsYellow	529	539	20.2	0.42	Tetramer	25	95
PhiYFP	525	537	130.0	0.40	Dimer	158	91
Orange fluorescent proteins							
Kusabira Orange	548	559	51.6	0.60	Monomer	92	96
Kusabira Orange2	551	565	63.8	0.62	Monomer	118	127
mOrange	548	562	71.0	0.69	Monomer	146	100
mOrange2	549	565	58.0	0.60	Monomer	104	101
dTomato	554	581	69.0	0.69	Dimer	142	100
dTomato-Tandem	554	581	138	0.69	Pseudo-monomer	283	100
DsRed	558	583	75.0	0.79	Tetramer	176	95
DsRed2	563	582	43.8	0.55	Tetramer	72	98
DsRed-Express (T1)	555	584	38.0	0.51	Tetramer	58	98
DsRed-Express2	554	586	35.6	0.42	Tetramer	45	99
DsRed-Max	560	589	48.0	0.41	Tetramer	59	99
DsRed-Monomer	556	586	35.0	0.10	Monomer	10	Clontech
TurboRFP	553	574	92.0	0.67	Dimer	187	114
TagRFP	555	584	100.0	0.48	Monomer	142	114
TagRFP-T	555	584	81.0	0.41	Monomer	99	101
Red fluorescent proteins							
mRuby	558	605	112.0	0.35	Monomer	117	134
mApple	568	592	75.0	0.49	Monomer	109	101
mStrawberry	574	596	90.0	0.29	Monomer	78	100
AsRed2	576	592	56.2	0.05	Tetramer	8	95
mRFP1	584	607	50.0	0.25	Monomer	37	71
JRed	584	610	44.0	0.20	Dimer	26	91
mCherry	587	610	72.0	0.22	Monomer	47	100
eqFP611	559	611	78.0	0.45	Tetramer	106	131
tdRFP611	558	609	70.0	0.47	Pseudo-monomer	98	133
HcRed1	588	618	20.0	0.015	Dimer	1	130
mRaspberry	598	625	86.0	0.15	Monomer	38	102
Far-red fluorescent proteins							
tdRFP639	589	631	90.4	0.16	Pseudo-monomer	43	133
mKate	588	635	31.5	0.28	Monomer	26	97
mKate2	588	633	62.5	0.40	Monomer	74	89
Katushka	588	635	65.0	0.34	Dimer	67	97
tdKatushka	588	633	132.5	0.37	Pseudo-monomer	146	89
HcRed-Tandem	590	637	160	0.04	Pseudo-monomer	19	84
mPlum	590	649	41.0	0.10	Monomer	12	102
AQ143	595	655	90.0	0.04	Tetramer	11	107

Day & Davidson, 2009

		λex. (nm)	λem. (nm)	Q. Yield	Brillance (% EGFP)	pH sensible	Maturation	Photo-stabilité (s)
Variants GFP-Aequorine								
GFP wt	m*	395/475	509	0.77	48			stable
EGFP	m*	488	509	0.60	100	5.9 +/-	< 15 min	174
Emerald	m*	487	509	0.68	116			Très faible
ECFP	m*	439	476	0.40	39	stable		
Cerulean	m*	433	475	0.62	79	stable	ND	36
EYFP	m*	514	527	0.61	151	sensible	ND	60
mcitrine	m	516	529	0.76	174	5.7	ND	faible
Variants Protéines Fluorescentes								
mOrange	m	548	562	0.69	146	sensible	2.5 h	9
tdTomato	m	554	581	0.69	283	4.7	1 h	98
DsREd	t	558	583	0.79	178	4.7	~ 10 h	16
mRFP1	m	584	607	0.25	37	stable	< 1 h	8.7
mStraw.	m	574	596	0.29	78	stable	50 min	15
mCherry	m	587	610	0.22	47	stable	15 min	96
mPlum	m	590	649	0.10	12	< 4.5	53	53
mKate2	m	588	6335	0.40	74	5.4	< 20 min	93

Protéines Fluorescentes Photoactivables, Photoconvertibles, photocomutables



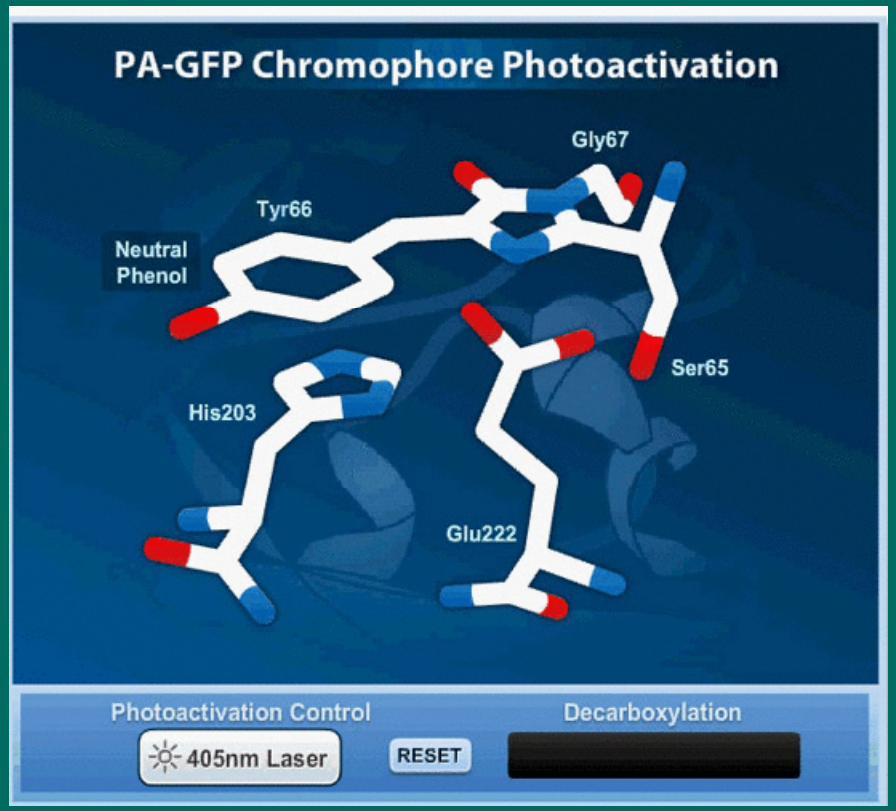
→ PA-GFP (Thr203His)

→ EosFP

→ Dronpa, Iris-FP

Mécanisme d'activation de PA-GFP

La PA-GFP (*Photoactivable GFP*) permet d'étudier la diffusion et la localisation de protéines dans les cellules en temps réel.



- Le chromophore non activé possédant un groupement phénol à l'état neutre est entouré par l'histidine 203 et un acide glutamique 222.

- L'activation à 405nm induit une décarboxylation de Glu222 et un changement du spectre d'absorption.

Exemples d'applications

Pourquoi faire? *Comment?*

Etudes structurales → Adressage

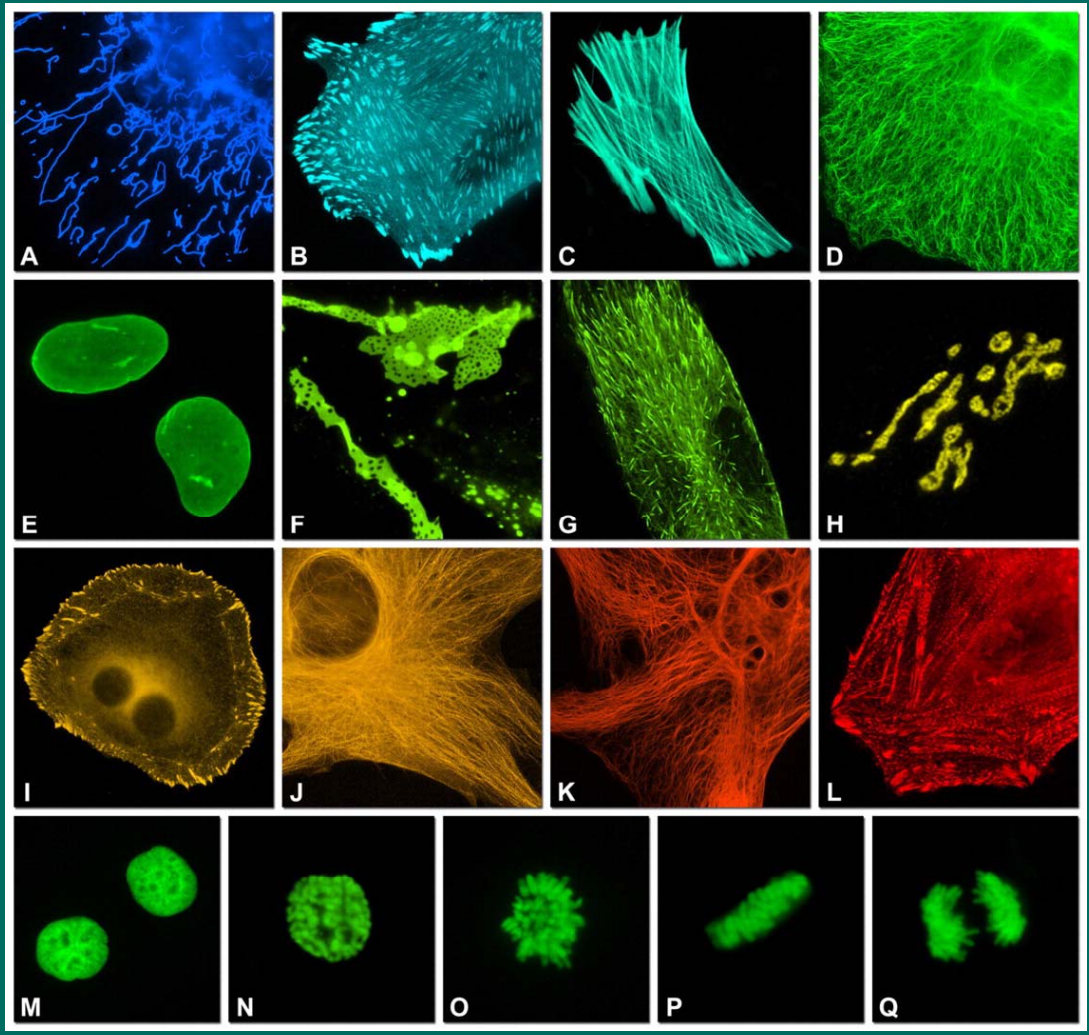
Etudes fonctionnelles → maturation

Etudes fonctionnelles → déstabilisation

Etudes fonctionnelles → Couple de FPs FRET

Possibilités d'adressage

(E) GFP-lamin
Enveloppe nucléaire



(B) Cerelean-paxilin
Focal adhesion
(D) Emerald-Keratin

(F) venus-connexin
(G) mKO-golgi

(J) RFP-tubulin
(K) mCherry-vimentin

M-Q
EGFP-histone H2B

Shaner N C et al. J Cell Sci 2007;120:4247-4260

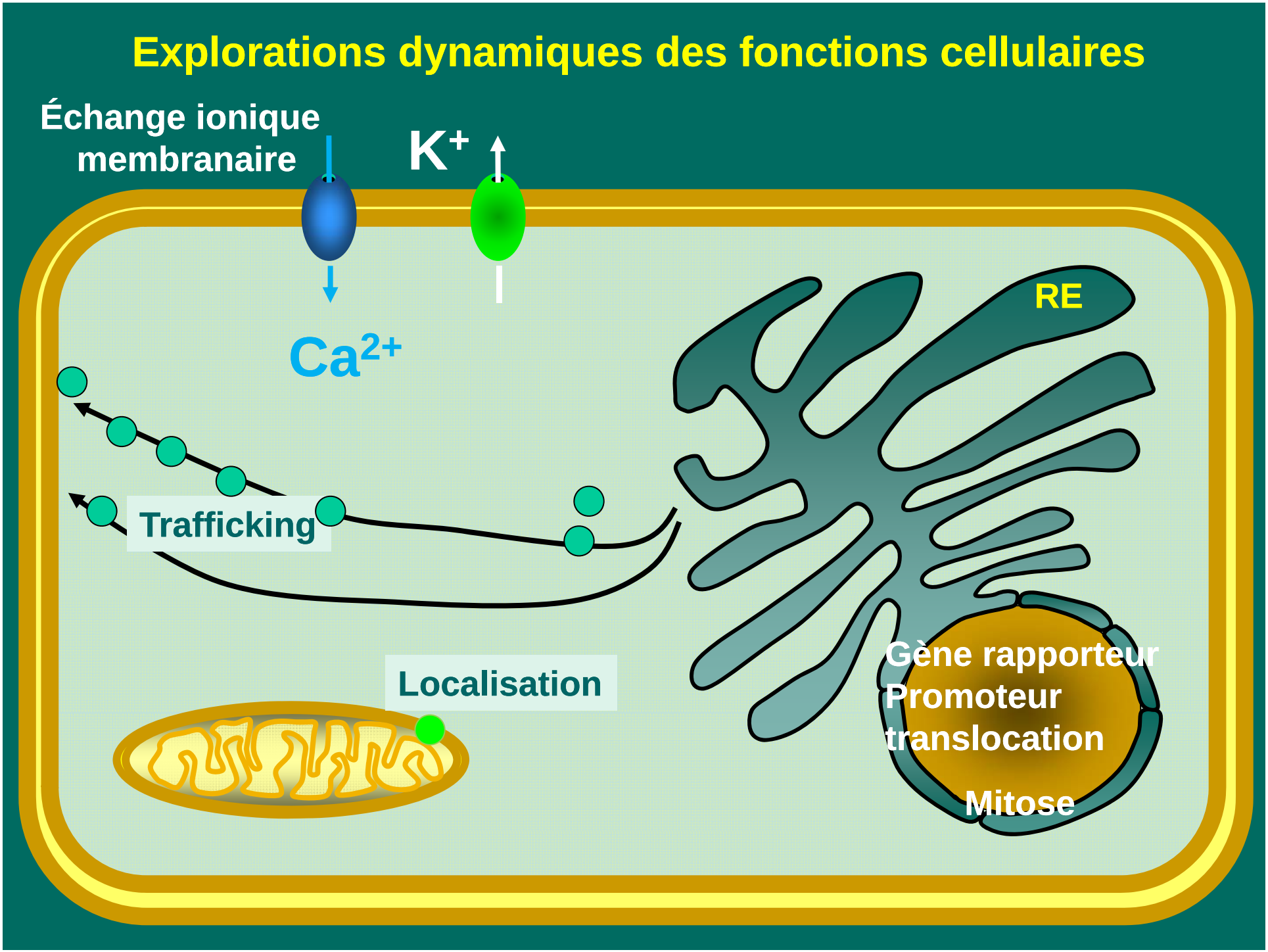
Journal of Cell Science
jcs.biologists.org

Motifs de localisation subcellulaire

Localisation	motifs	Source
Noyau	Cterm: PKKKRKVEDA Cterm: DPKKKRKV Cterm: (DPKKKRKV) 3-GSTGSR Séquence KR-rich en Cterm	NLS
Cytoplasme	Cterm: LALKLAGLDI	NES
Lumen RE	Nterm: MLLSVPLLLGLLGLAAAD Cterm: KDEL	calreticulin
Matrice mitochondriale	Nterm: MSVLTPLLLRGLTGSARRLPVPRAKIHSLGDP En tadem	Cytochrome c oxidase
Membrane mitochondriale	Nterm: MVGRNSAIAAGVCGALFIGYCIYFDRKRRSDPN MAIQLRSLFPLALPGMLALLGWWWFFSRKK	Tom20 DAKAP1a
Lumen Golgi	Nterm: 81 aa de la hβ1,4 galactosyltransferase	
Membrane Golgi	Nterm: GNLKSVAQEPGPPCGLGLGLGLGLCGKQGPA	eNOS
Membrane plasmique	Nterm:MGCIKSKRKDNLNDDGVDMKT Myristoylation et palmitoylation Cterm: KKKKKSKTKCVIM Farnesylation Cterm: KLNPPDESGPGCMSCKCVLS Nterm: MLCCMRRTKQVEKNDEDQKI	Lyn kinase K-ras4B V-Ha-Ras GAP-43
Matrice peroxisome	Cterm: SKL ou XKL	

Adapté de Chudakov, 2010

Explorations dynamiques des fonctions cellulaires



Études dynamiques

- Visualisation in vivo
- Méthode non destructrice
- Études de plusieurs promoteurs à la fois (plusieurs couleurs)

- Moins sensible que les méthodes enzymatiques

- Critères de choix de la FP : rapidité de **Maturation** et faible **Stabilité**
(Fast maturation / fast dégradation)

	½ repliement (s)	***½ maturation (s)***	***Constante de temps de maturation Kox 10^{-4} s^{-1}***
EGFP	90.6	3915 (~ 65 min)	1.77
Venus	46.2	4076	1.70
TurboGFP	11.2	1468 (~ 25 min)	4.72

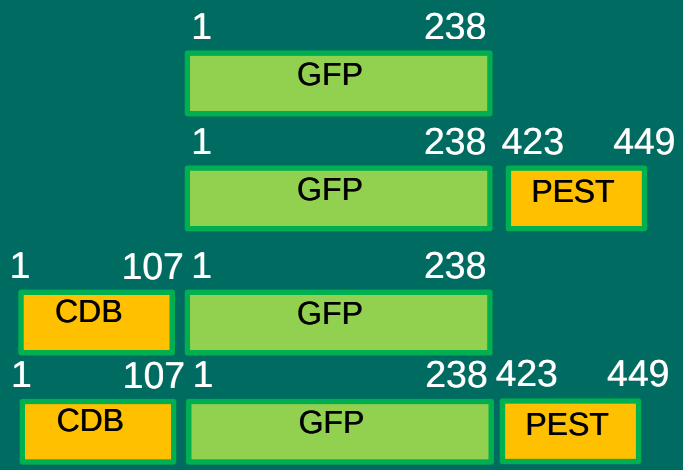
Données Evrogen

Déstabilisation

Protéine de fusion avec un signal de protéolyse:

Séquence PEST de mODC et/ou domaine CDB (cyclin destruction box) de la cyclin D1

Corish & Tyler-Smith, 1999



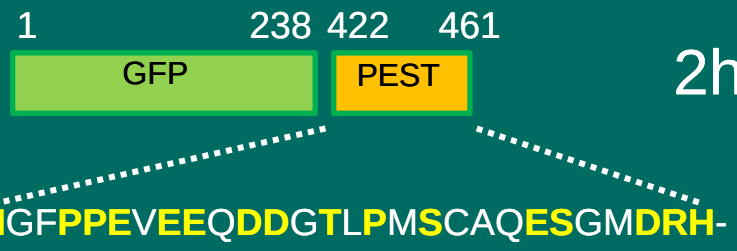
½ vie

26 h

9.8 h

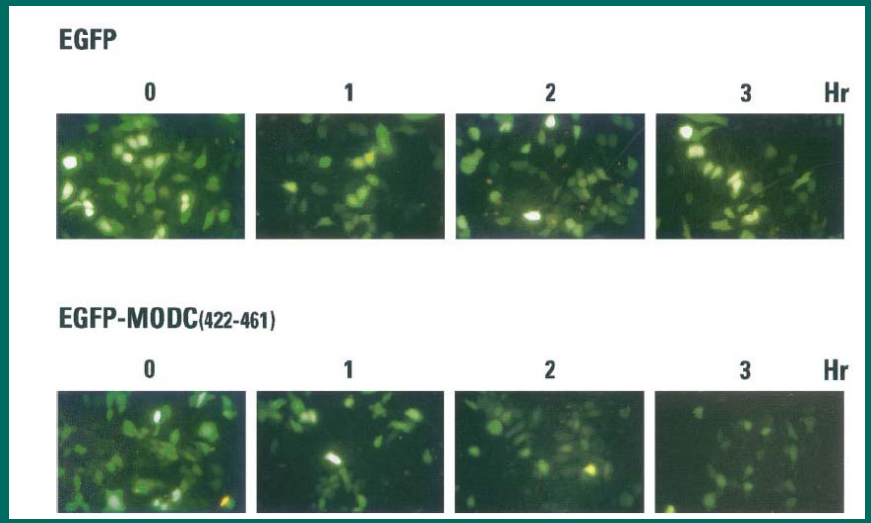
5.8 h

5.5 h



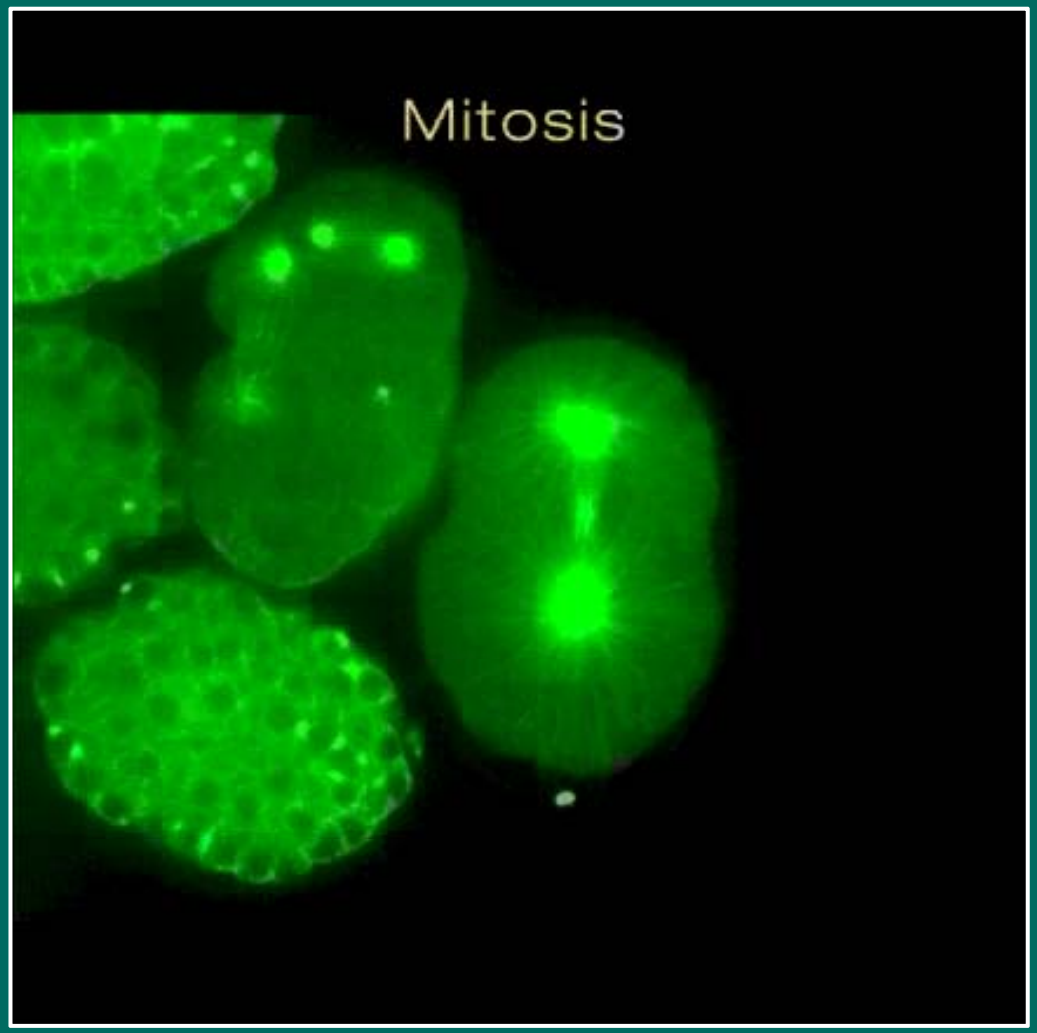
2h

Li et al., 1998



embryon de *C. elegans*
EGFP-Tubulin

Mitose

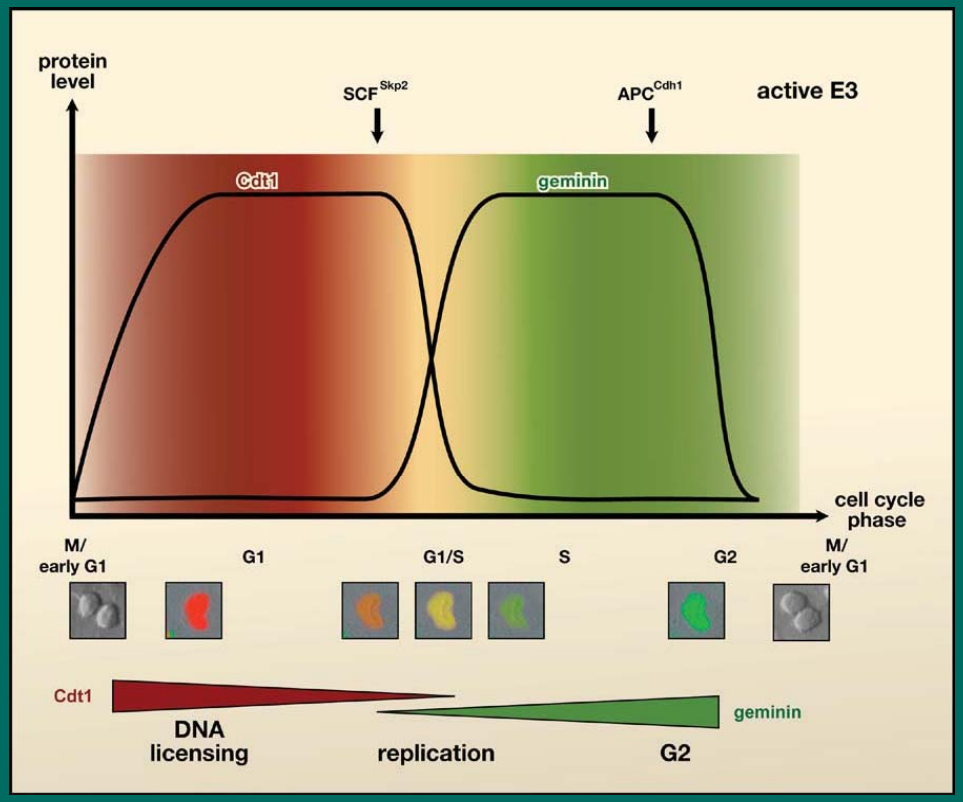


E. Hyman, EMBL,
UltraView, PerkinElmer

Le cycle cellulaire en couleur avec FUCCI

Fucci = Fluorescent Ubiquitination-based Cell-Cycle Indicator

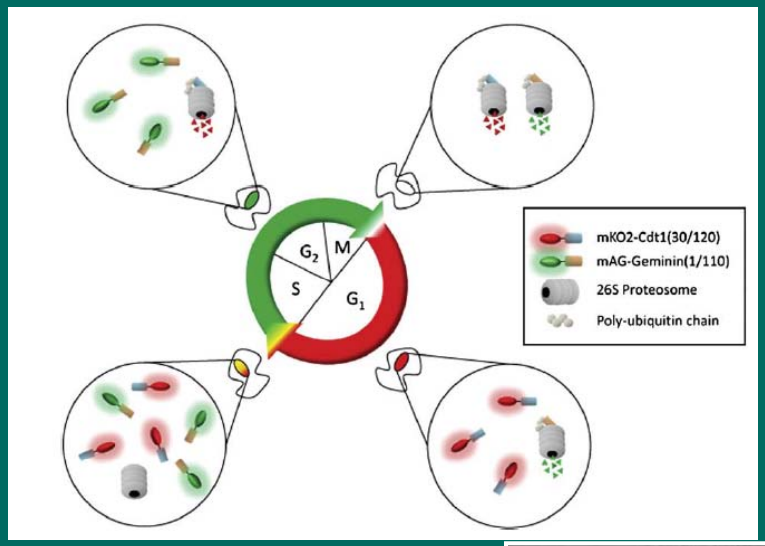
A. Miyawaki, 2008

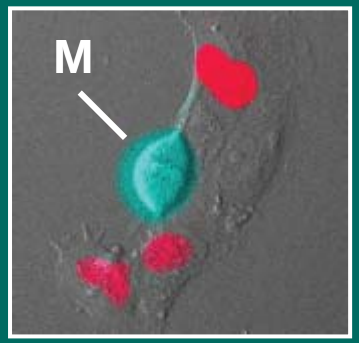


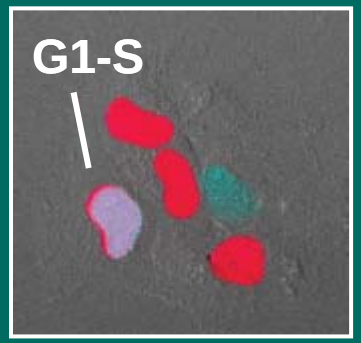
Méchali & Lutzmann, 2008

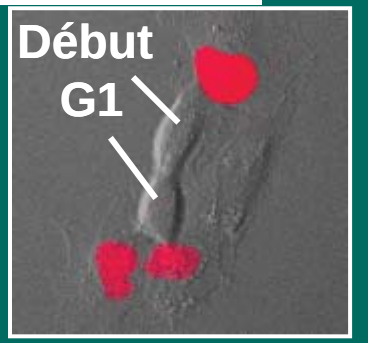
mKO2-cdt1(30/120)

mAG-geminin(10/1120)









Images Clontech

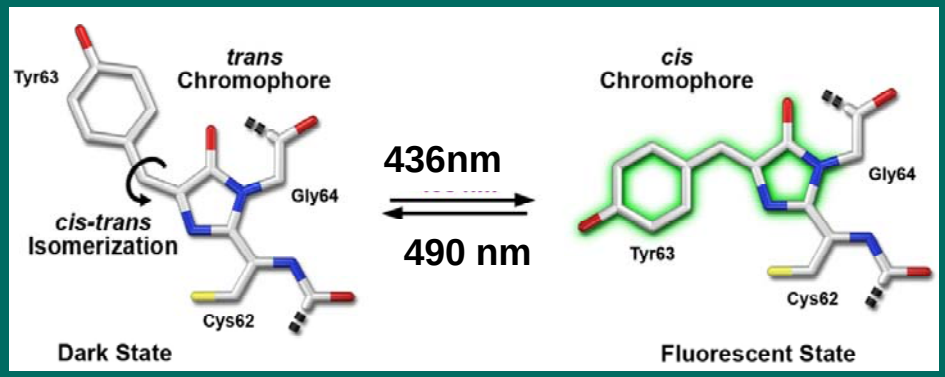
Gène rapporteur

γ-Crystallin GFP Transgenic Frog

Cardiac actin GFP transgenic frog

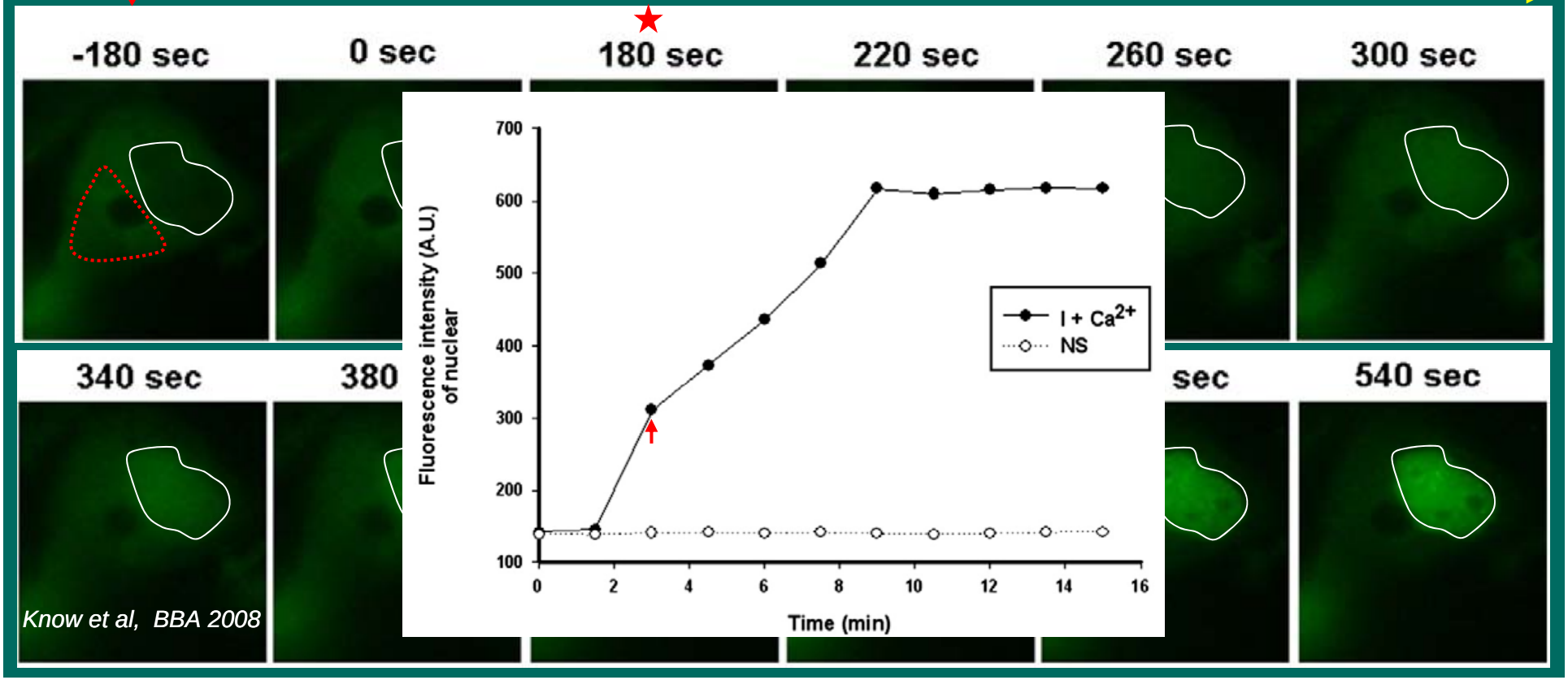
Enrique Amaya Lab

Transport nucleo-cytoplasmique de NF-AT avec Dronpa



Photoactivation
436 nm

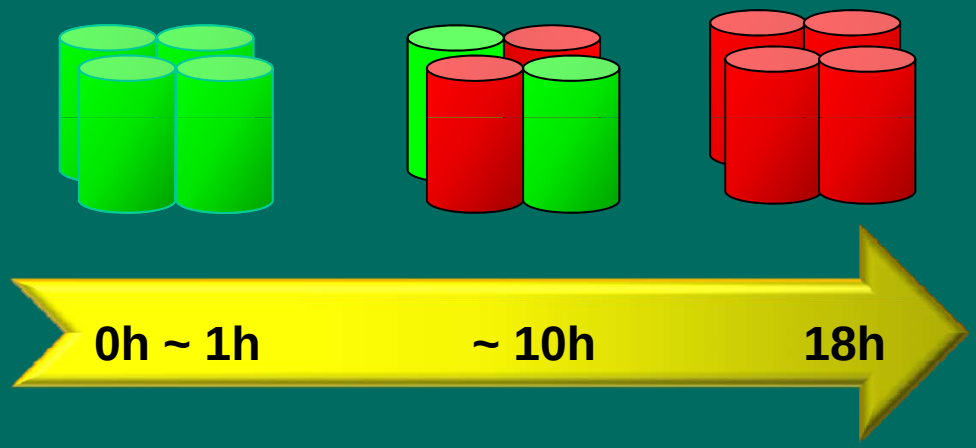
Stimulation Ionomycine + Ca^{2+}

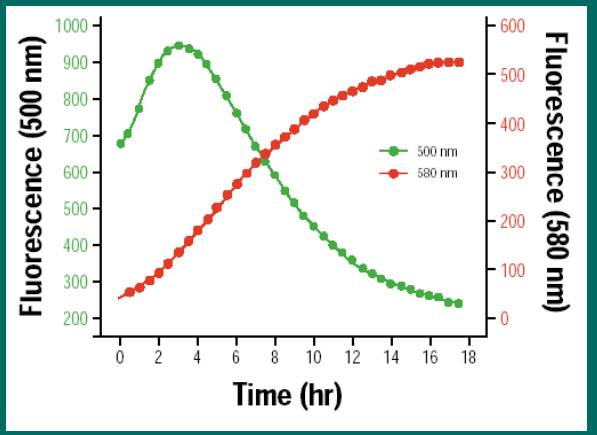


DsRed-E5 mutant = Fluorescent timer

(Terskikh et al, Science 2000, 290; 1585-1588)

- Mutant de la DsRed (V105A, S197T), tetramère stable
- Changement des propriétés spectrales

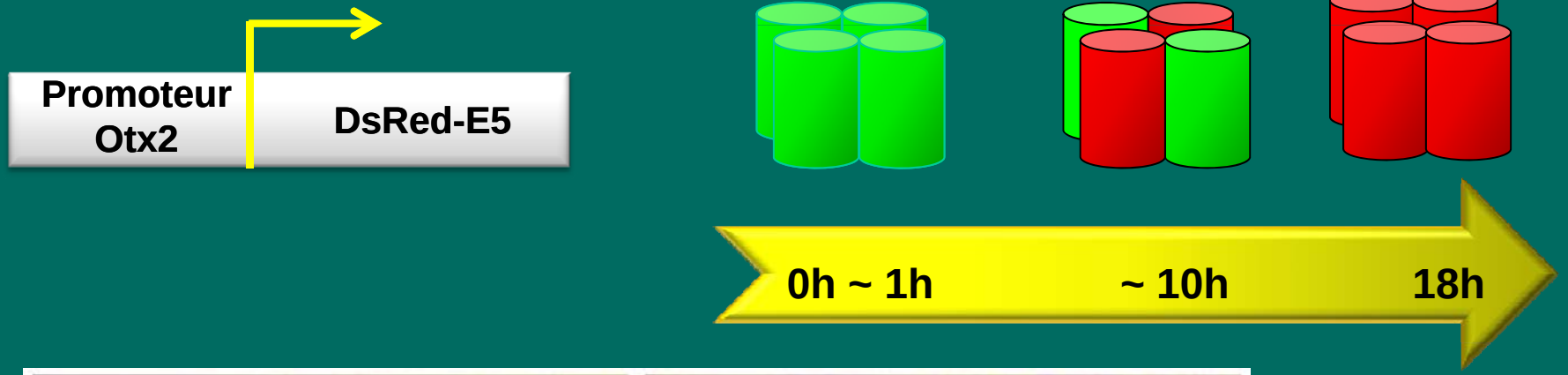


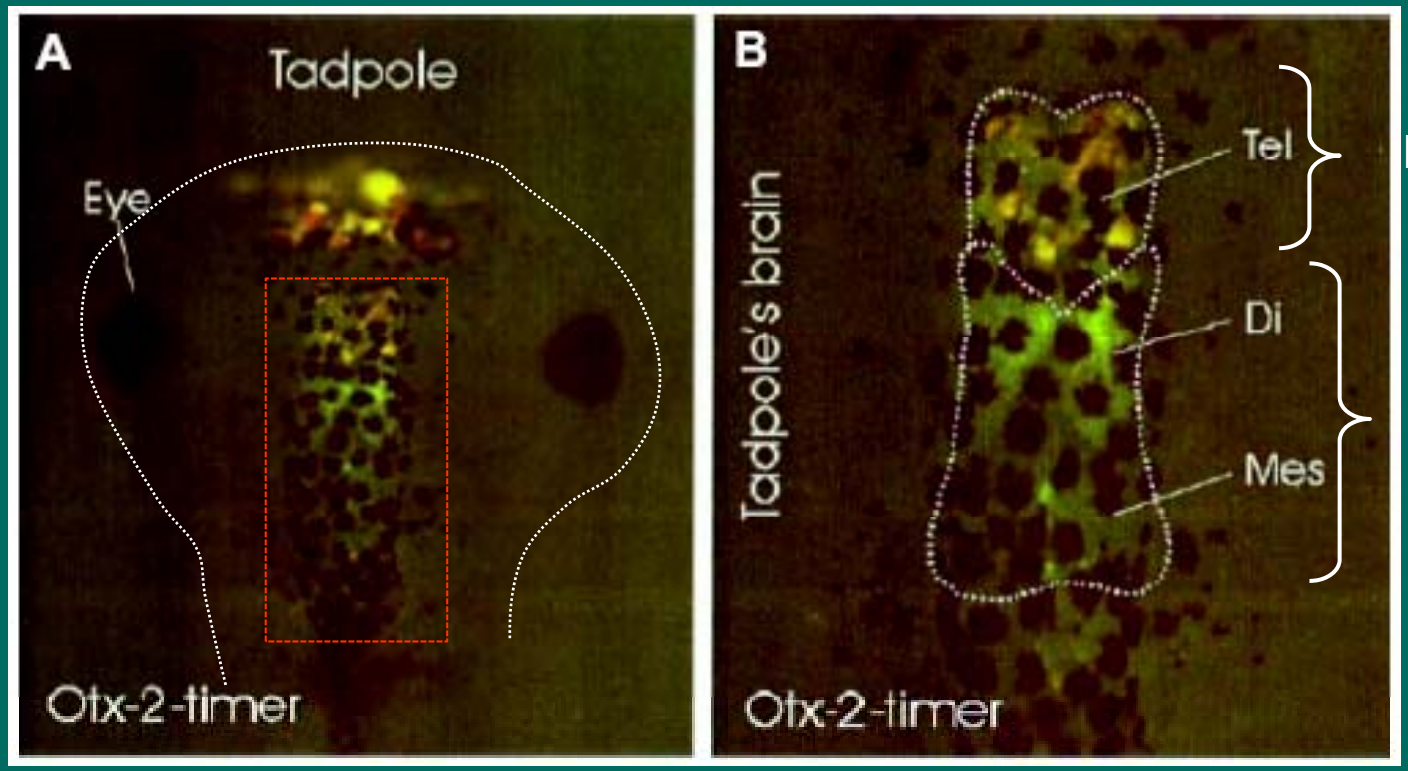


Applications

Suivi d'un évènement dans le temps (activité d'un promoteur) et dans l'espace (trafic intracellulaire)

DsRED-E5 pour un suivi dynamique de l'activité d'un gène





Expression précoce

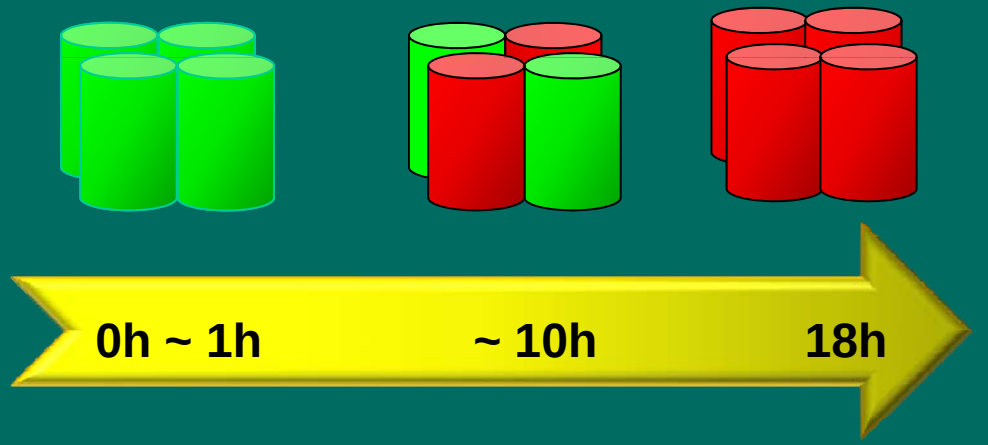
Expression tardive

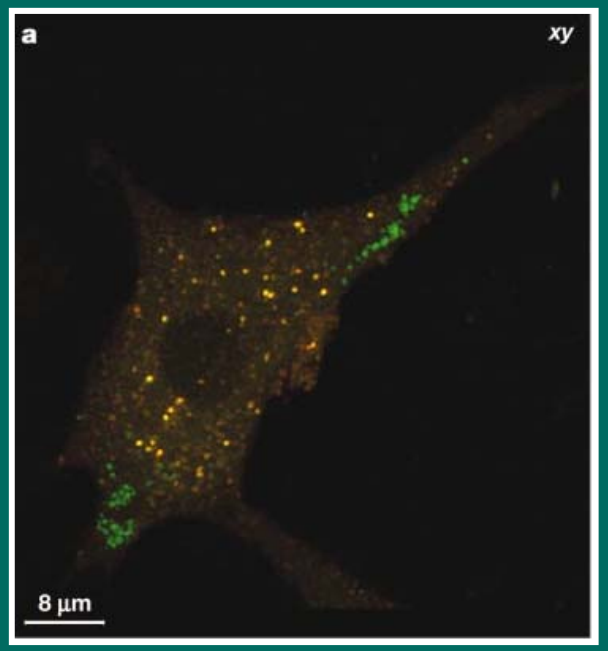
Terskikh et al, 2000

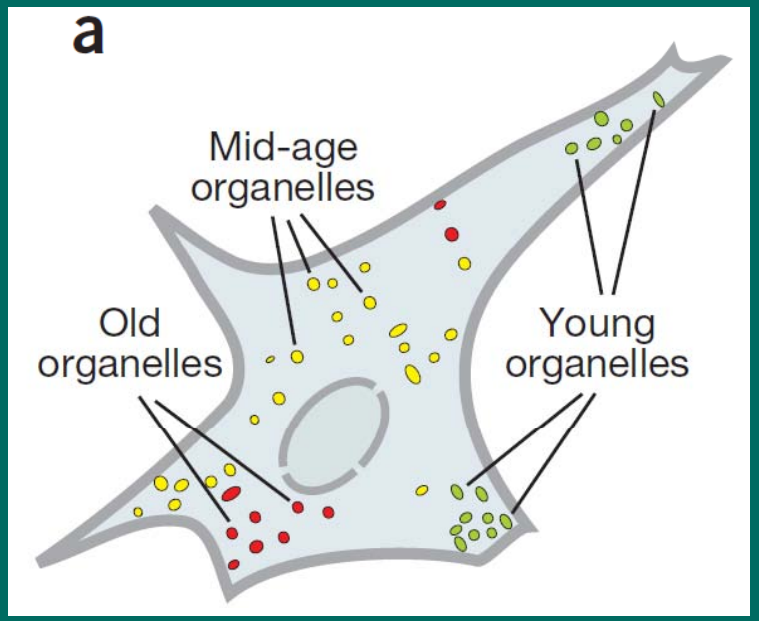
Functional and spatial segregation of secretory vesicle pools according to vesicle age

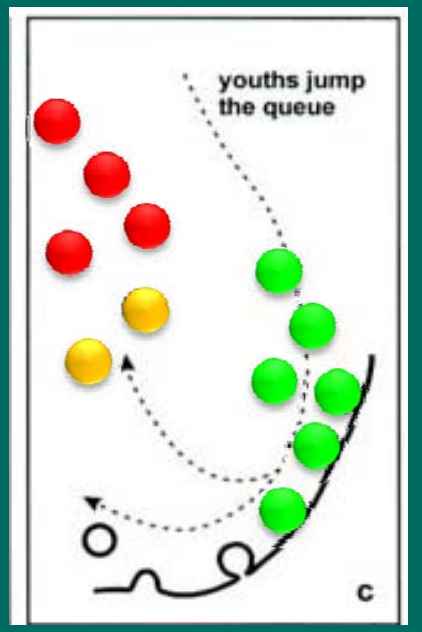
Rory R. Duncan*, Jennifer Greaves*, Ulrich K. Wiegand*, Ioulia Matskevich*, Georg Bodammer*†, David K. Apps*, Michael J. Shipston* & Robert H. Chow‡

* *Membrane Biology Group, University of Edinburgh, George Square, Edinburgh EH8 9XD, UK*
‡ *Department of Physiology and Biophysics, Keck USC School of Medicine, Los Angeles, California 90089-9142, USA*





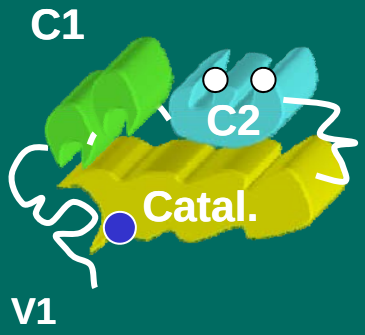


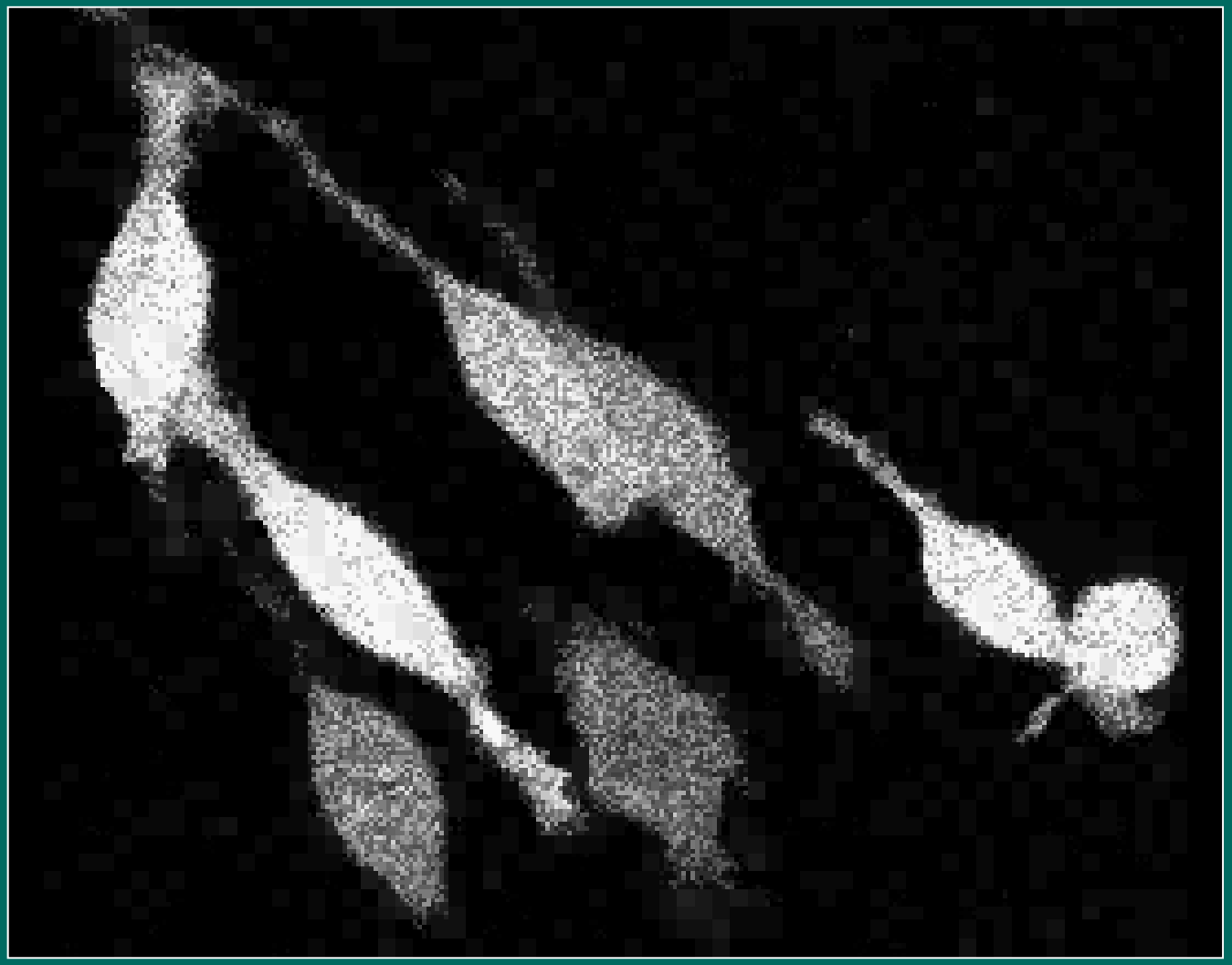


Duncan et al, 2003, Nature

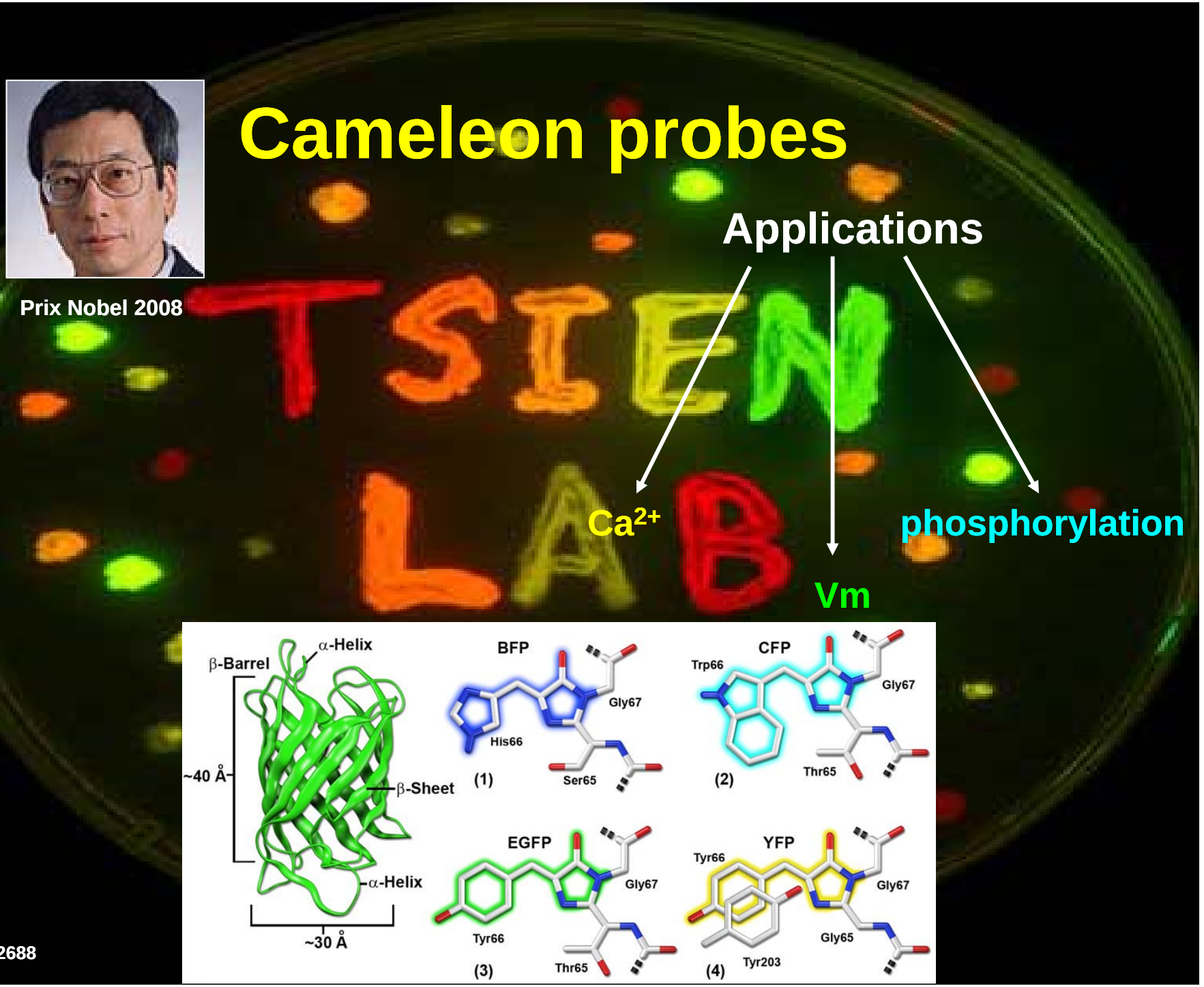
Translocation transitoire de la PKCγ à la membrane

Domaine C1A fusionné avec GFP





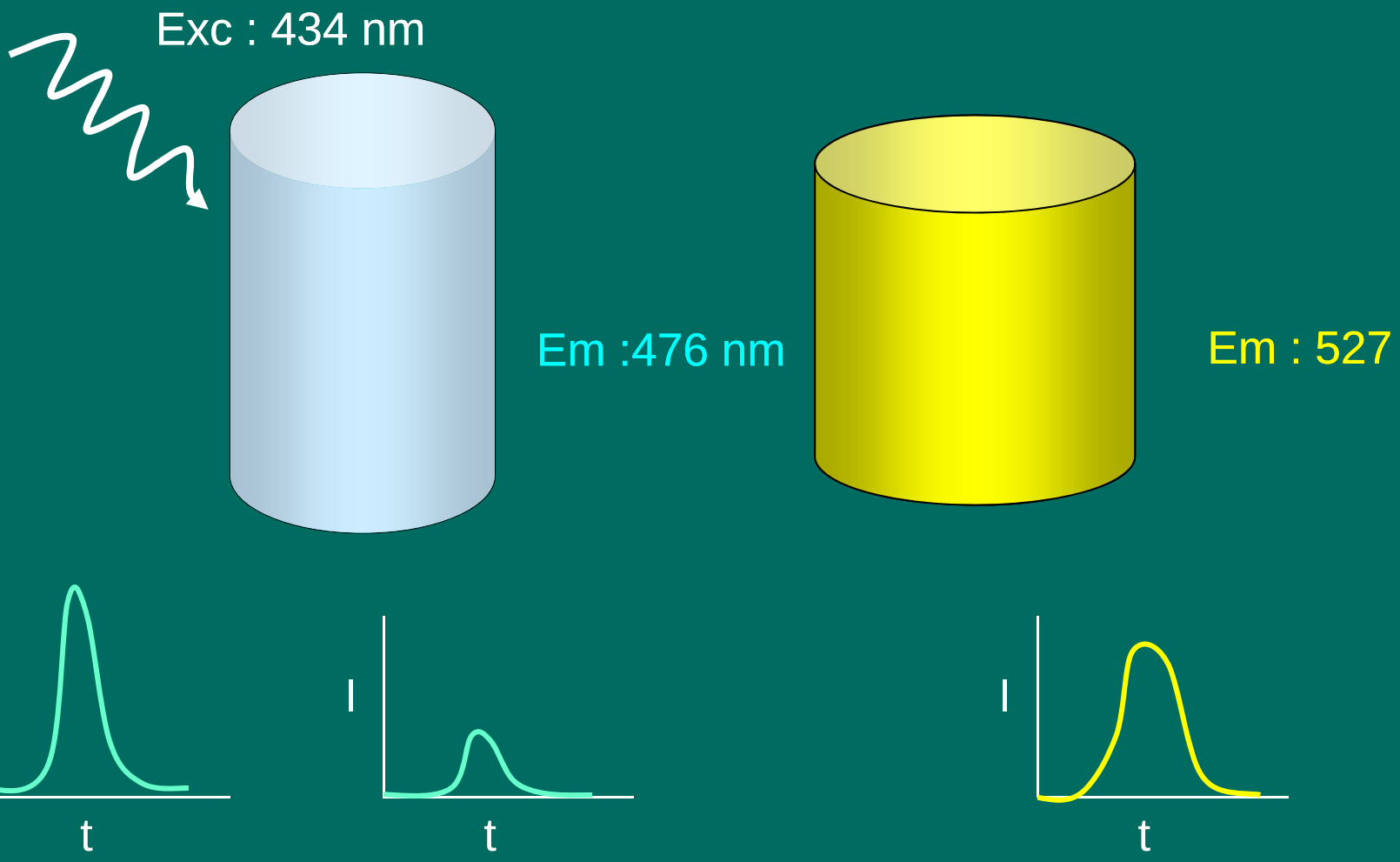
Oancea & Meyer Cell *95*, 307-318


Cameleon probes
Prix Nobel 2008
TSIEN
LAB
Applications
Ca2+
Vm
phosphorylation
β-Barrel
α-Helix
~40 Å
β-Sheet
α-Helix
~30 Å
BFP
Gly67
His66
Ser65
(1)
CFP
Trp66
Gly67
Thr65
(2)
EGFP
Gly67
Tyr66
Thr65
(3)
YFP
Tyr66
Gly67
Gly65
Tyr203
(4)

GDR 2688

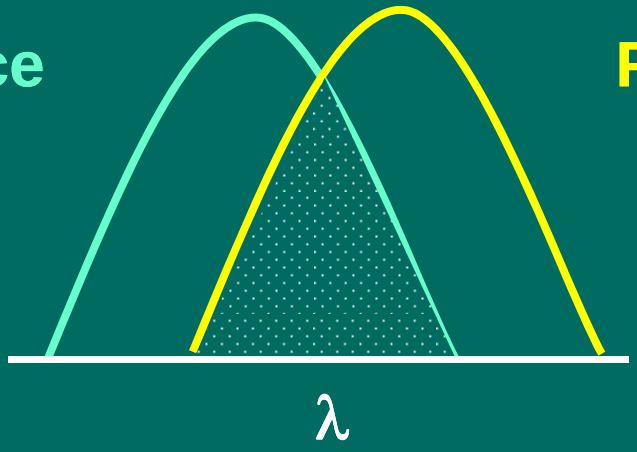
FRET

Le FRET est un processus dans lequel l'énergie est transférée, de façon non radiative, d'un fluorochrome dans un état excité (le donneur) à un second fluorochrome (l'accepteur).



Conditions pour le FRET

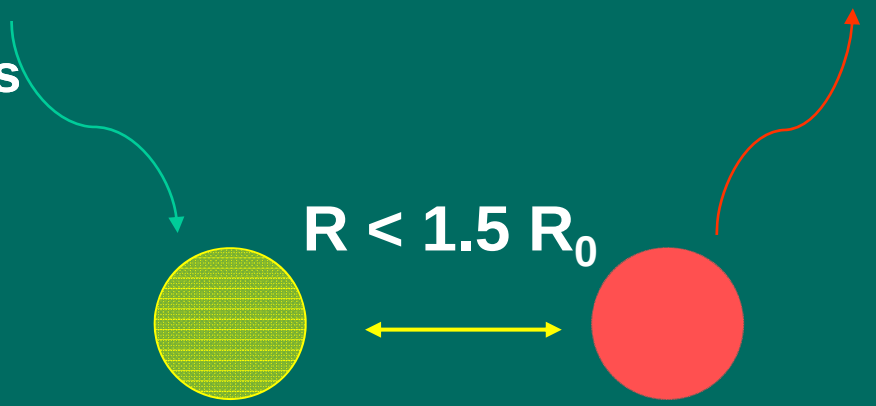
Fluorescence donneur



Fluorescence accepteur (absorption)

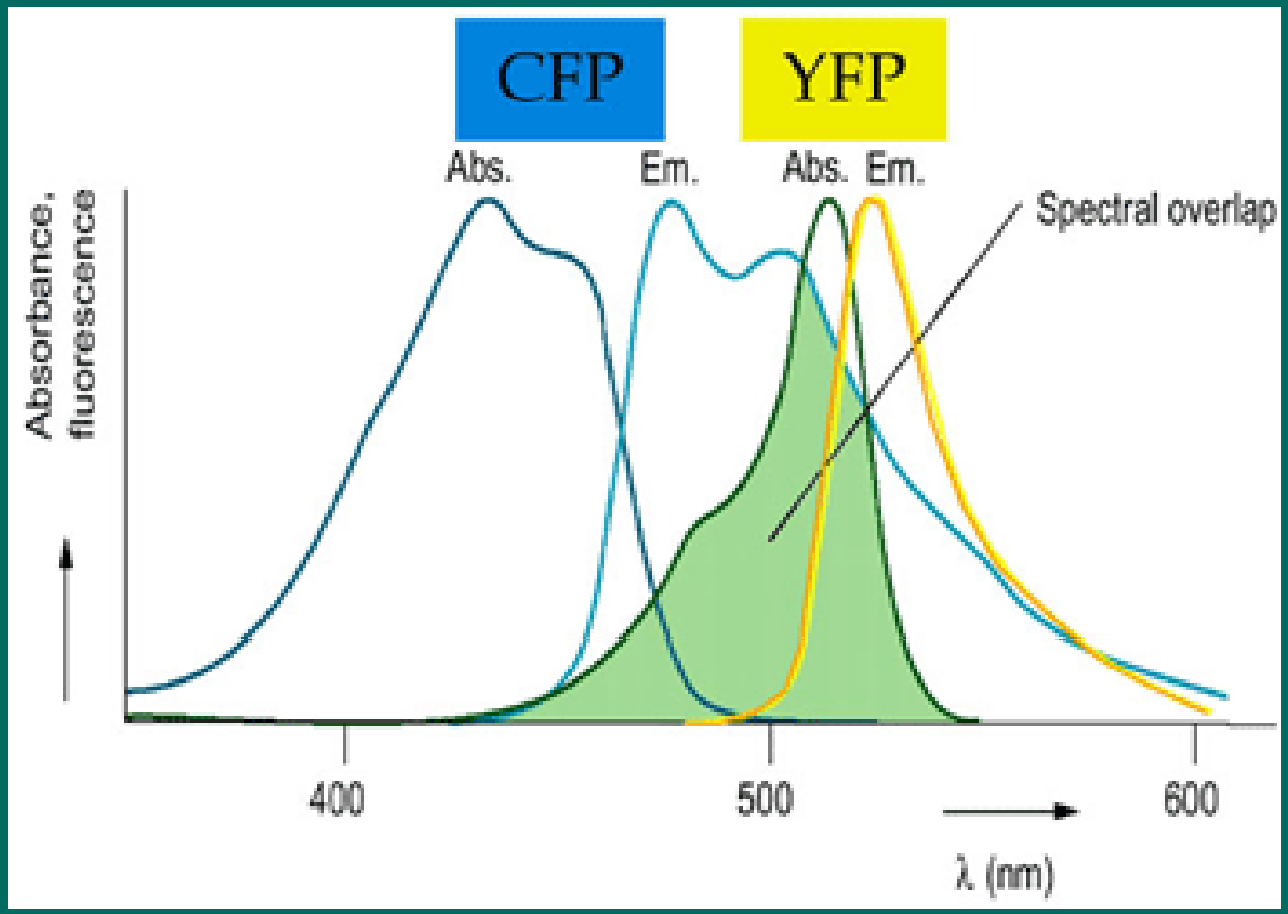
- Proximité des molécules (rayon de Förster)
- Recouvrement des spectres
- Dipôles donneur / accepteur parallèles

$$E = \frac{1}{1 + (R/R_0)^6}$$



R_0 est généralement compris entre 30 et 60 Å

GFP variants



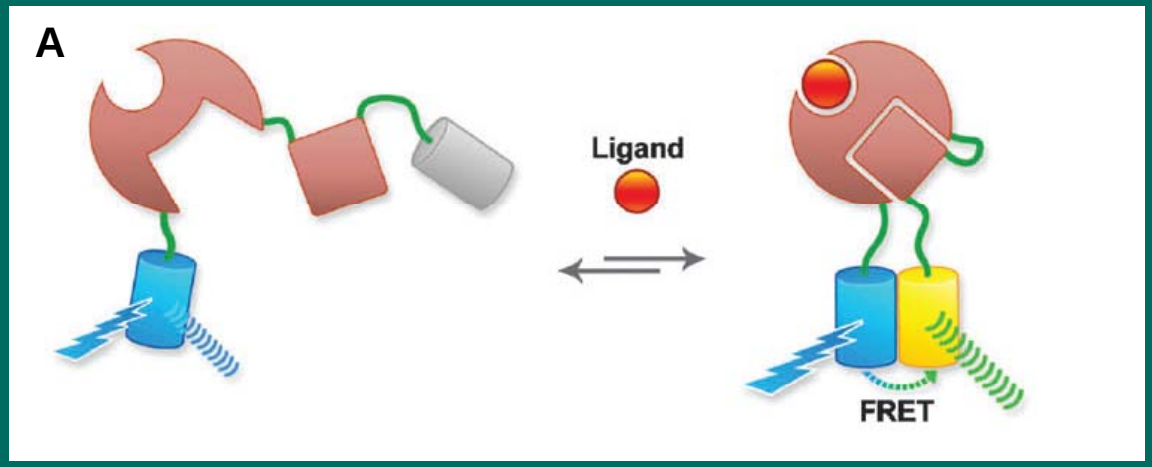
Couples de Protéines fluorescentes pour le FRET

FRET pair	Donor excitation peak, nm	Donor emission peak, nm	Acceptor excitation peak, nm	Acceptor emission peak, nm	Donor QY	Acceptor EC, M^{-1} cm^{-1}	Forster radius (Ro), nm	comments
EBFP2-mEGFP	383	448	489	509	0.56	57,500	4.8	
TagBFP-TagGFP2	402	457	483	506	0.63	56,500	5.3	
PS-CFP2-phiYFP	400	468	525	537	0.20	130,000	6.1	Very good spectral separation. phiYFP is a weak dimer.
GFP2-YFP	~400	~510	~515	~530	0.55	84,000	-	Good spectral separation
T-Sapphire-DsRed	399	511	563	582	0.60	43,800	-	Very good spectral separation. DsRed is a tetramer.
T-Sapphire-TdTomato	399	511	554	581	0.60	138,000	-	Very good spectral separation. tdTomato is a tandem
E0GFP-mCherry	400	508	587	610	0.60	72,000	5.1	Very good spectral separation.
mAmertine-TagRFP	406	526	555	584	0.58	100,000	-	Very good spectral separation.
mAmertine-tdTomato	406	526	554	581	0.58	138,000	-	Very good spectral separation. tdTomato is a tandem
ECFP-EYFP	434	477	515	527	0.40	83,400	4.9	
CyPet-Ypet	435	477	517	530	0.51	104,000	5.1	enhanced dimerization
mTFP1-mCitrine	462	492	516	529	0.85	64,000	-	
mECFP-cp157Venus	434	477	515	528	0.40	92,200	5.1	
mCerulean-cp157Venus	433	475	515	528	0.62	92,200	5.3	
mTFP1-cp157Venus	462	492	515	528	0.85	92,200	5.7	
mTFP1-mOrange	462	492	548	562	0.85	71,000	5.7	Good spectral separation
MiCy-mKO	472	495	548	559	0.90	51,600	5.3	Good spectral separation. MiCy is a dimer
mUKG-mKO	483	499	548	559	0.72	51,600	-	Good spectral separation
TagGFP-TagRFP	482	505	555	584	0.59	100,000	5.7	Very good spectral separation
EGFP-mCherry	489	509	587	610	0.60	72,000	5.1	Very good spectral separation.
Venus-tdTomato	515	528	554	581	0.57	138,000	5.9	tdTomato is a tandem
Venus-mCherry	515	528	587	610	0.57	72,000	5.7	Very good spectral separation.
mCitrine-mKate2	516	529	588	633	0.57	62,500	-	Very good spectral separation.
Venus-mPlum	515	528	590	649	0.57	41,000	5.2	Very good spectral separation. Plum is dim.
TagRFP-mPlum	555	584	590	649	0.48	41,000	-	Plum is dim.

Chudakov D M et al. Physiol Rev 2010;90:1103-1163

Principe d'un biocapteur de type FRET

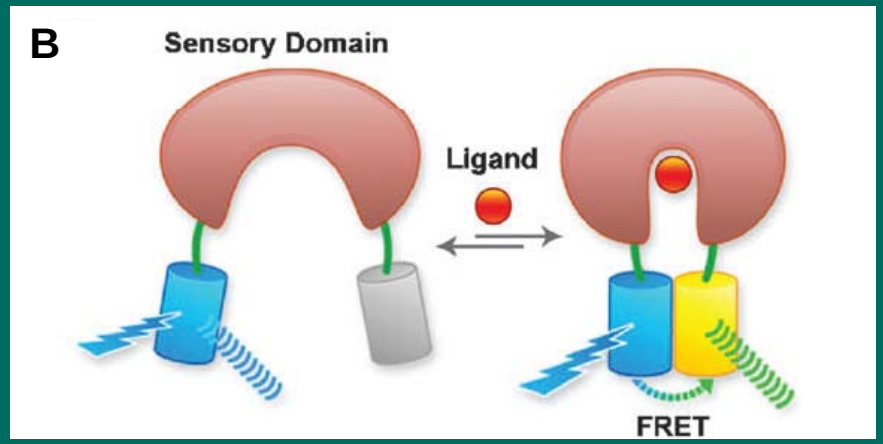
Interaction protéine-protéine dépendant d'un ligand



Exemples d'applications

Cameleon
(Calcium/CaM)

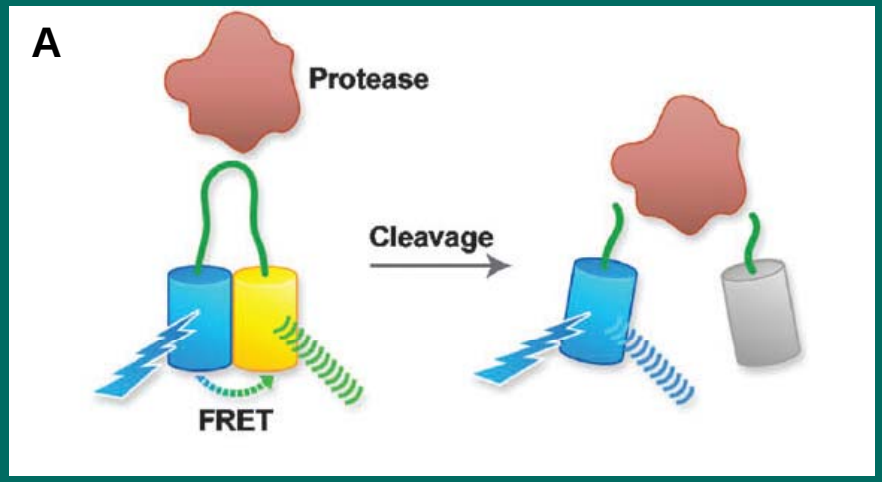
Changement de conformation dépendant ou non d'un ligand



GTPase (GKI)
ATP (ε SU)
Potentiel membranaire
cAMP

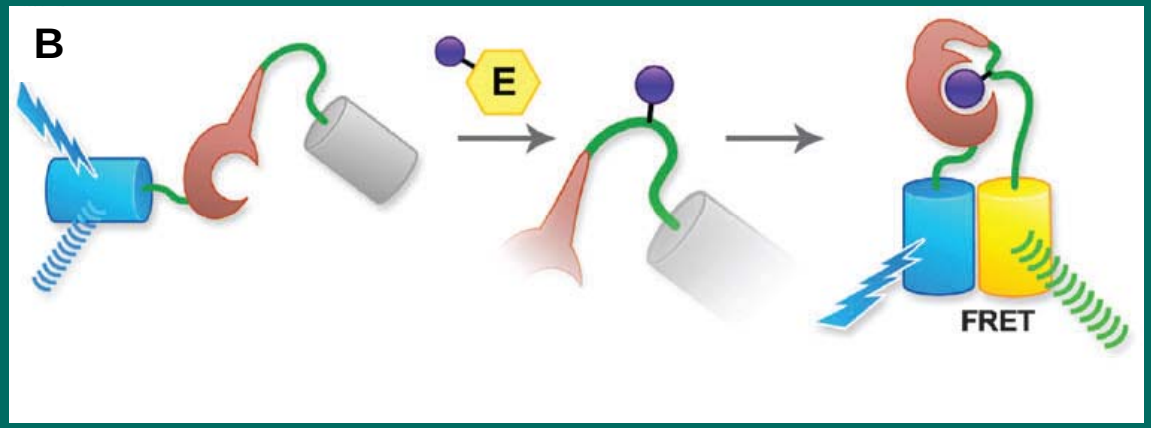
Frommer et al, 2009

Exemples d’applications



Metalloproteinase
Caspase

Modification post-traductionnelle

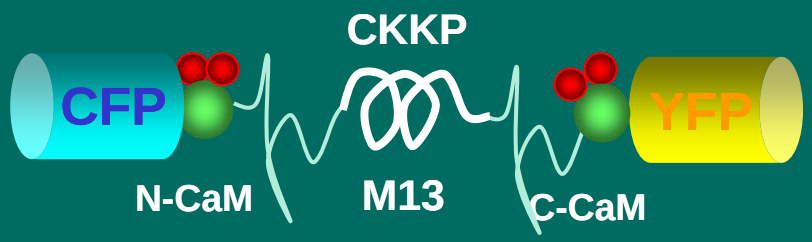


Activité Kinase
Acetylation

Bio-capteurs Ca^{2+} -dépendants

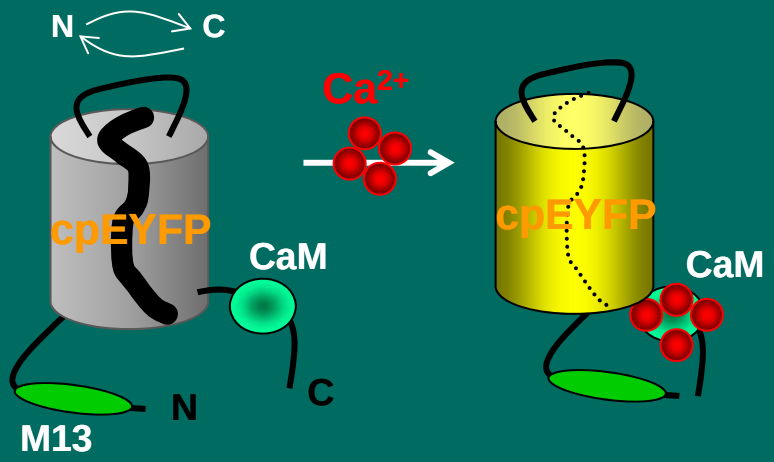
Capteurs doubles type FRET

- Cameleons, troponeons, D1

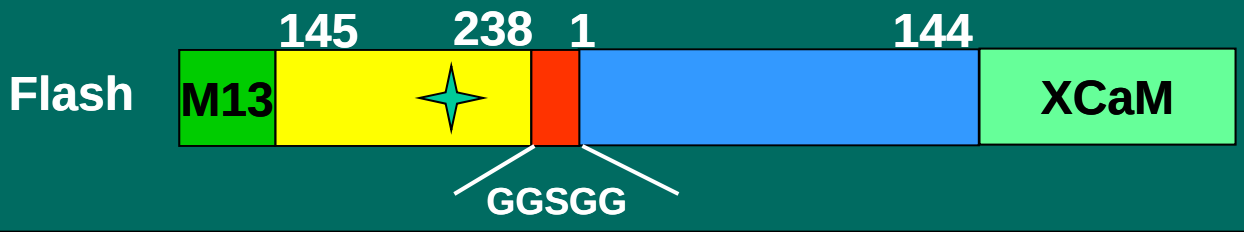


Capteurs simples

- Pericams, camgaroos, G-CAMPs



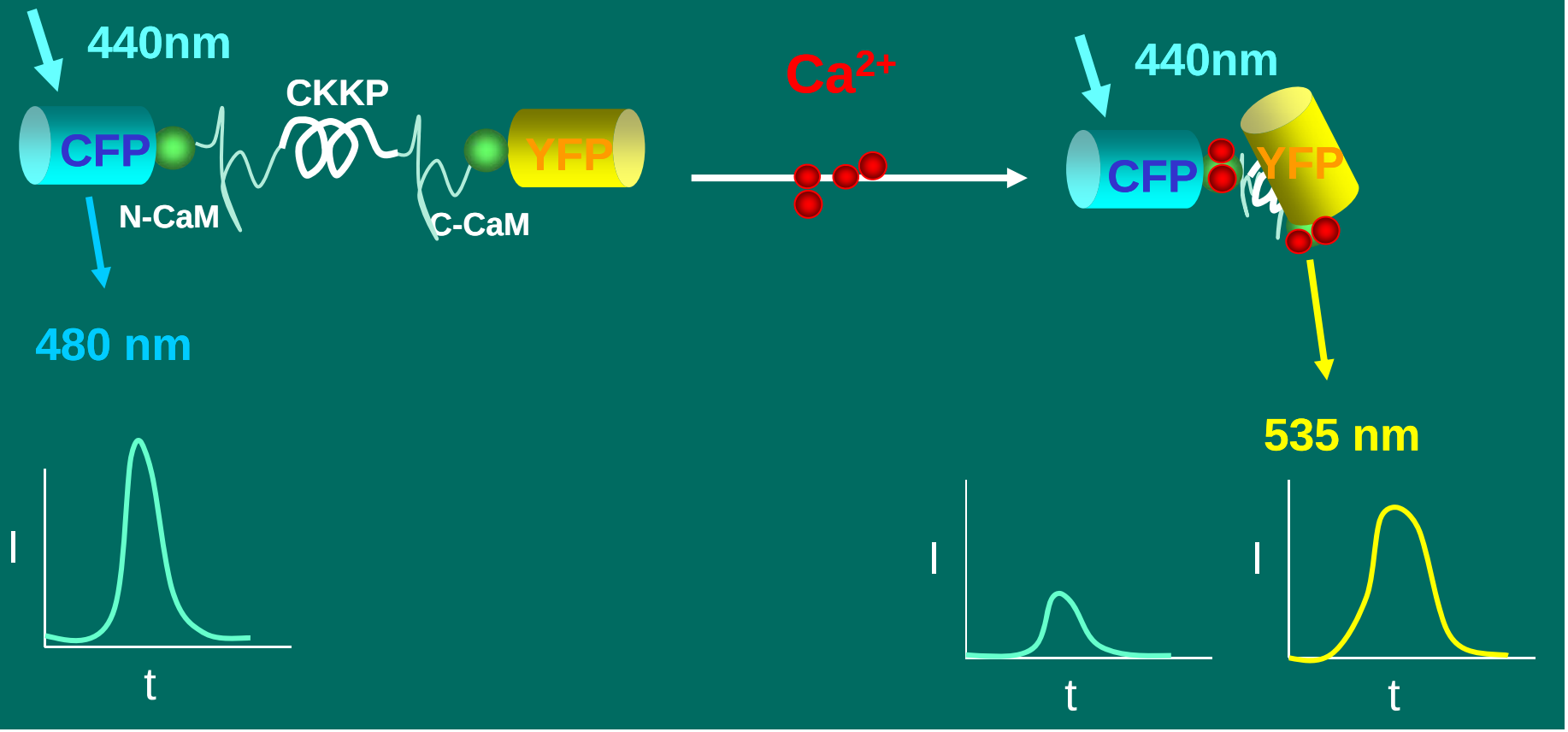
Capteurs		Dynamique
Double type FRET	YC2	1.6
	YC3.3 (citrine)	1.7
	YC6.1	2.1
Simple	YC 3.6 (cp venus)	5.6
Sonde organique	Fluo3, Fluo4	> 100



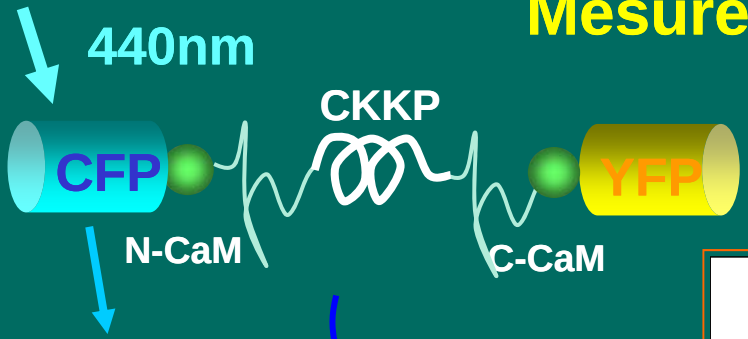
Sondes Ca^{2+} fluorescentes adressables
Dérivées de GFP

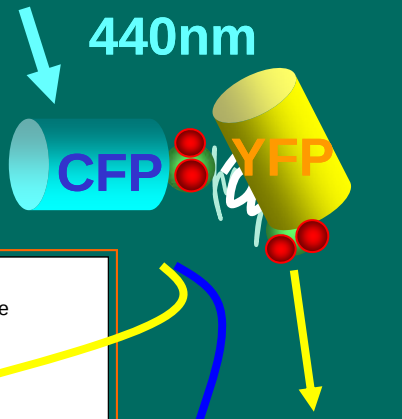
Double tonneaux (FRET)

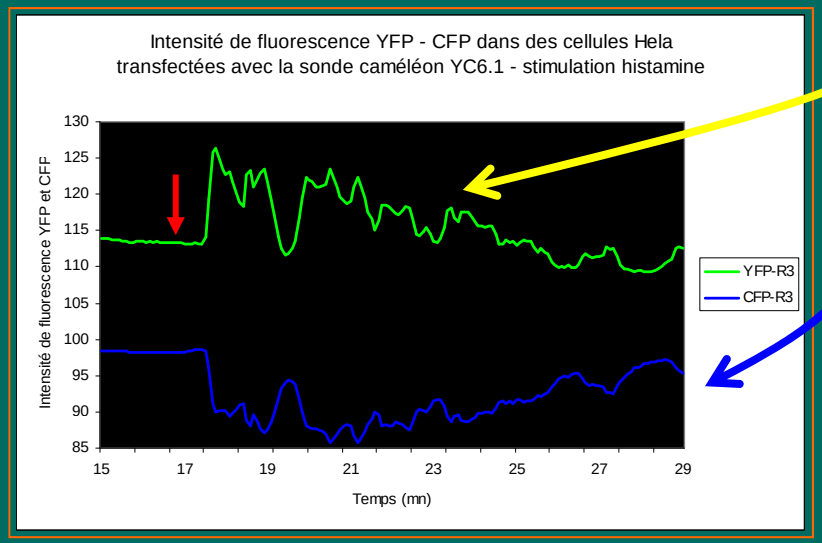
Cameleons, troponeons, D1

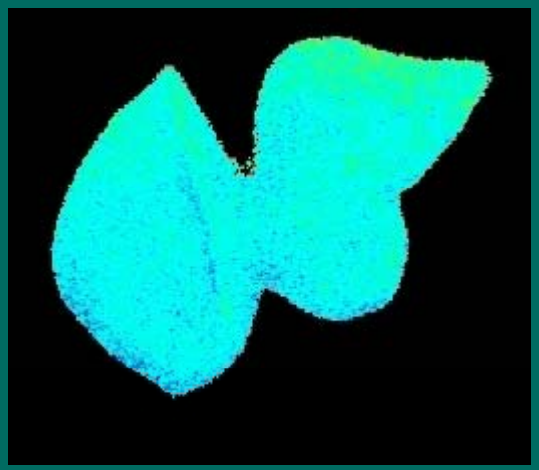


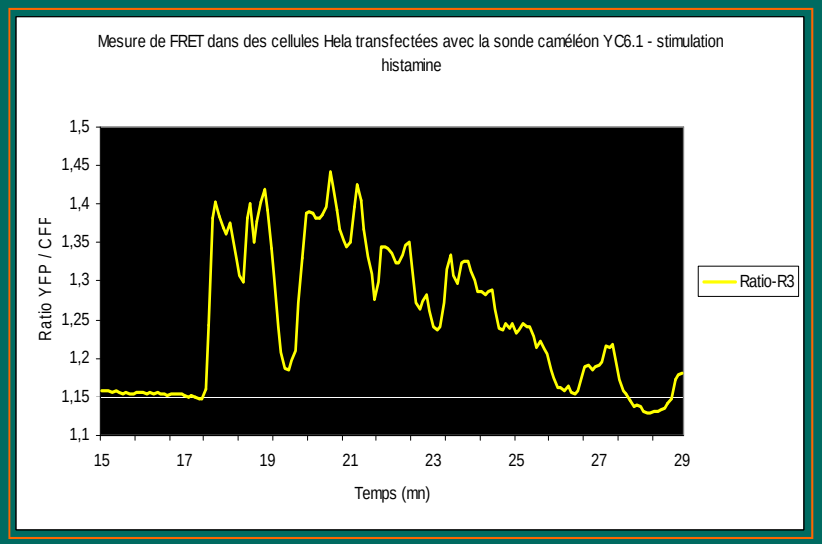
Mesure de Ca^{2+} avec YC6.1





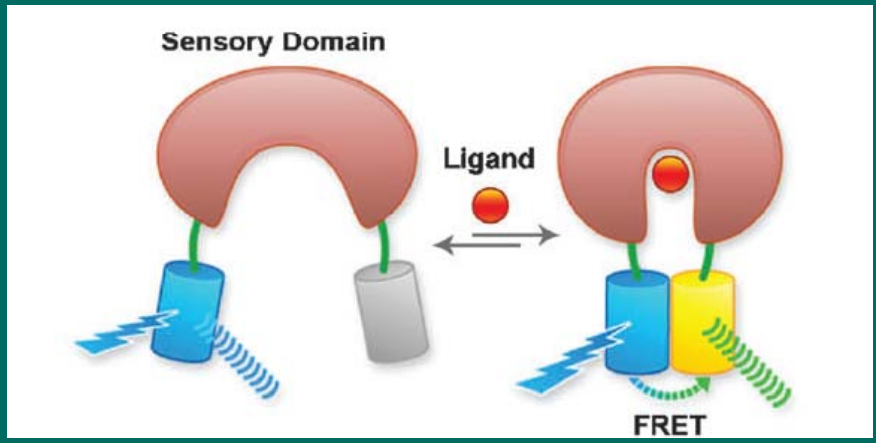




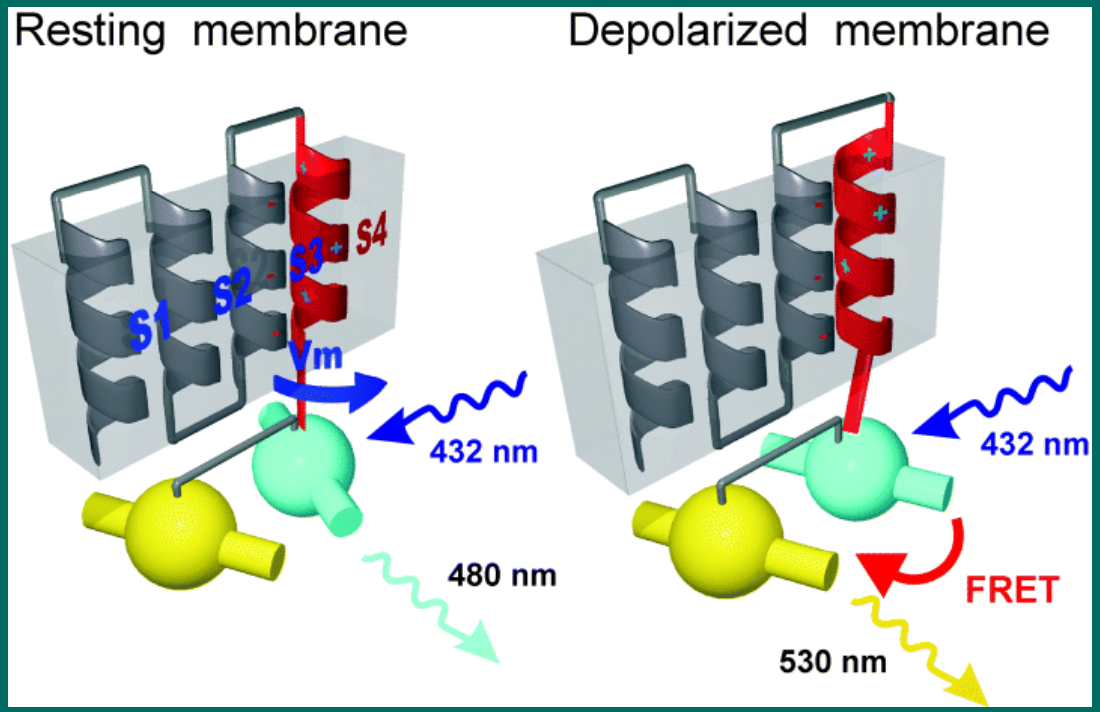


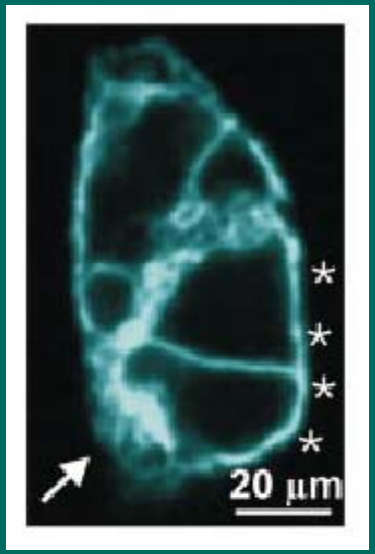
Système Hamamatsu Ashura

Membrane potential measurement with fluorescent protein based on FRET

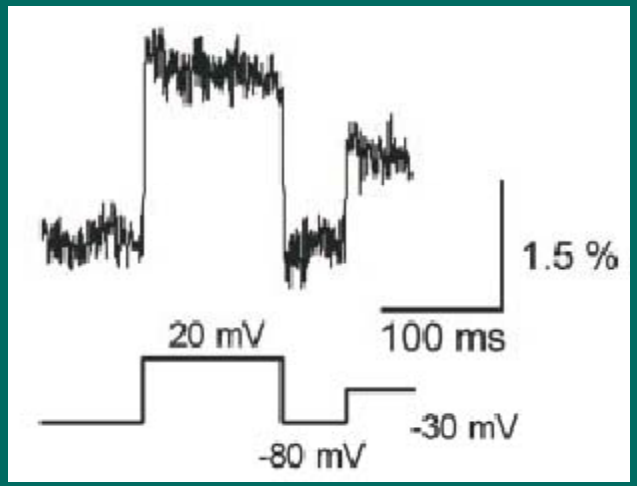


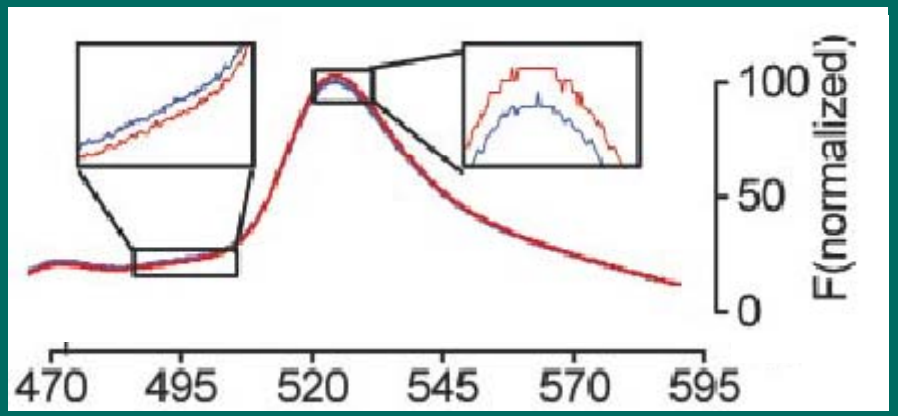
Voltage-sensitive fluorescent protein based on FRET





HEK 293 cell

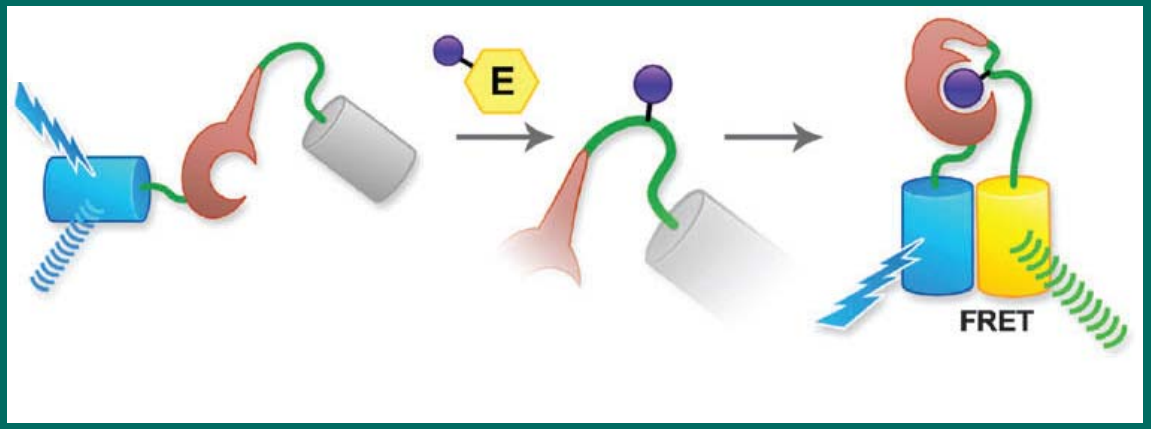




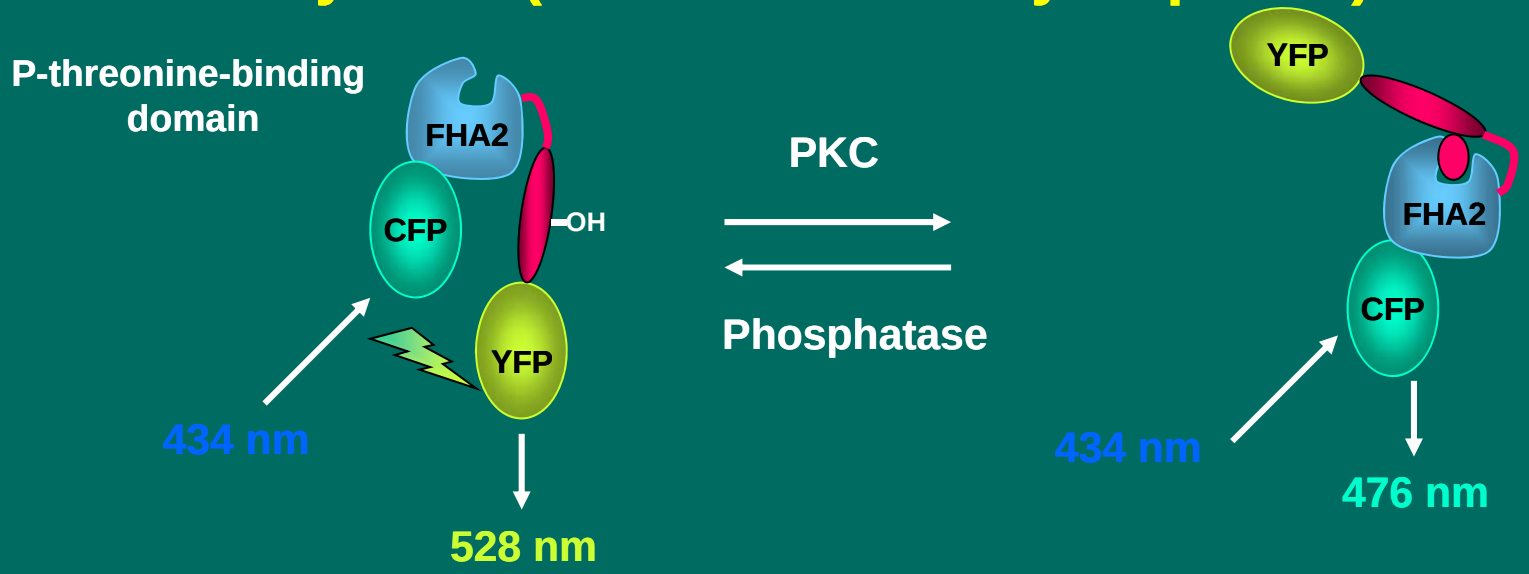
Sakai et al, Eur. J. Neuroscience 2001, 13; 2314-2318

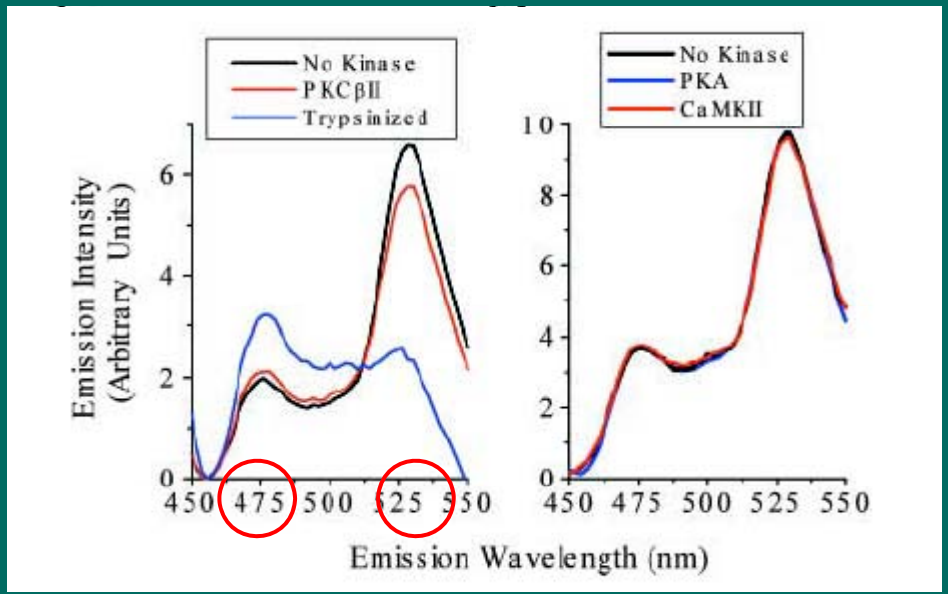
Fluorescent reporter to follow oscillatory phosphorylation by PKC (CKAR)

Modification post-traductionnelle



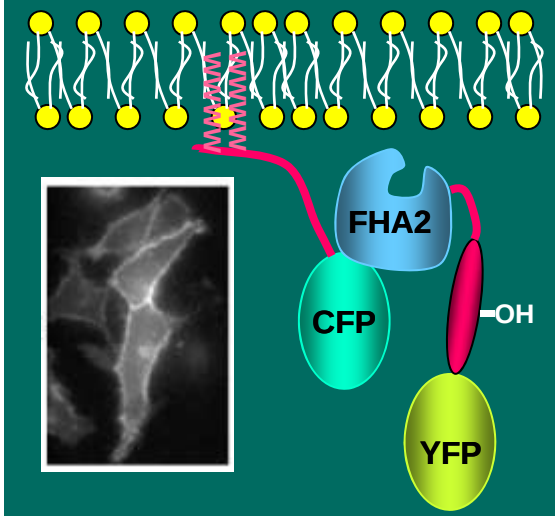
Fluorescent reporter to follow oscillatory phosphorylation by PKC (C-Kinase Activity Reporter)

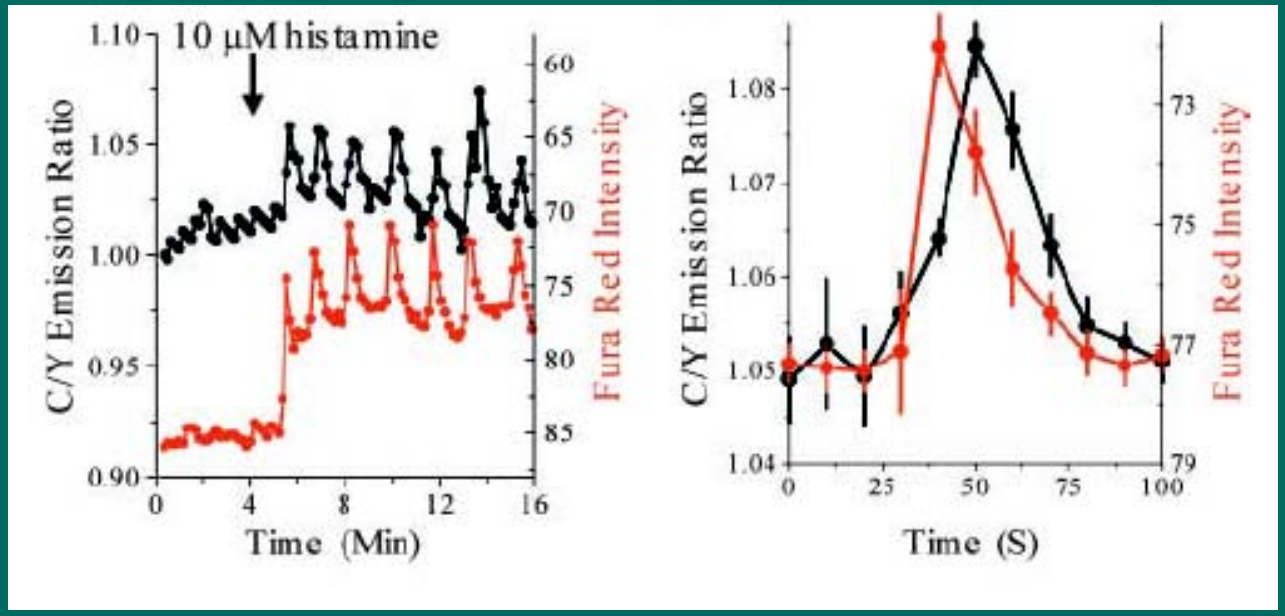


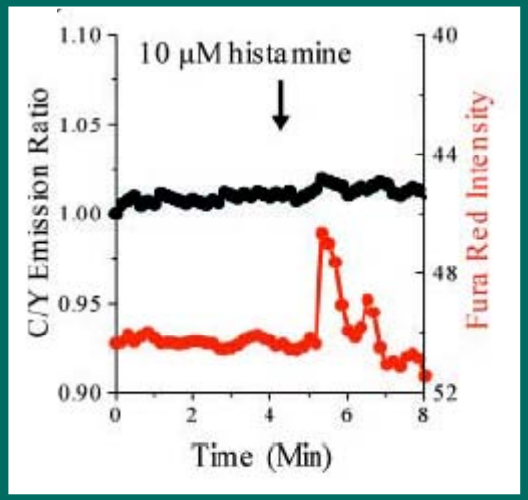


Violin et al, JCB 2003, 161; 899-909

Fluorescent reporter to follow oscillatory phosphorylation by PKC (C-Kinase Activity Reporter)

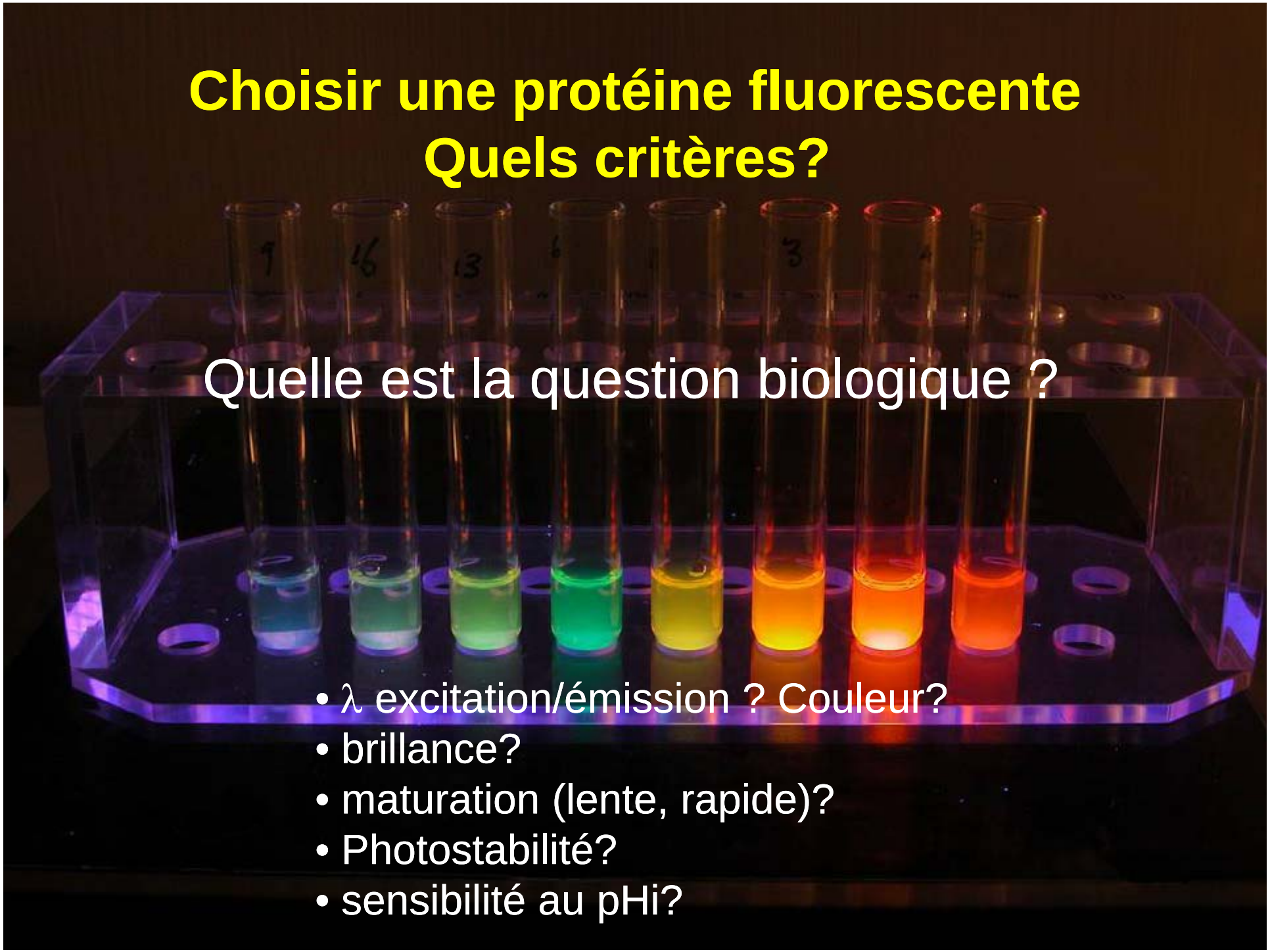






Mutation in substrat peptide

Choisir une protéine fluorescente
Quels critères?

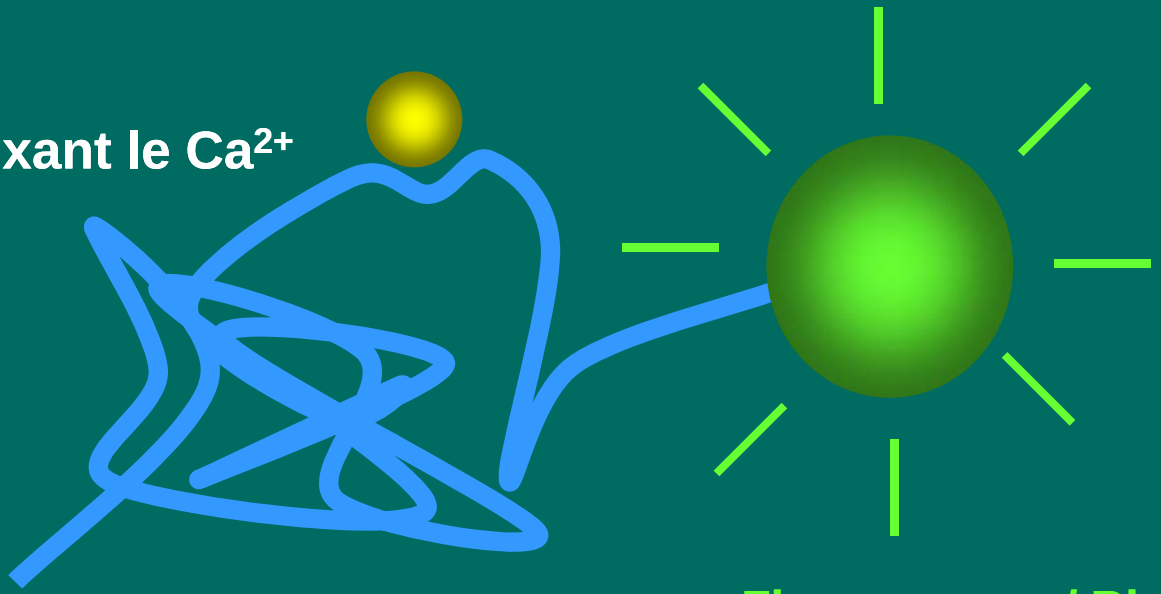


Quelle est la question biologique ?

- λ excitation/émission ? Couleur?
- brillance?
- maturation (lente, rapide)?
- Photostabilité?
- sensibilité au pHi?

Principe d'une sonde pour mesurer Des variations de calcium intracellulaire

Molécule fixant le Ca^{2+}



chromophore

Fluorescence / Bioluminescence

- Dérivé du BAPTA
- Protéine de liaison au Ca^{2+}
- Aequorine, Obéline

- Dérivés de fluorescéine, Rhodamine …
- Dérivés de GFP, YFP…
- Coelenterazine