Aequorin : a bioluminescent probe to monitor calcium changes in plant cells

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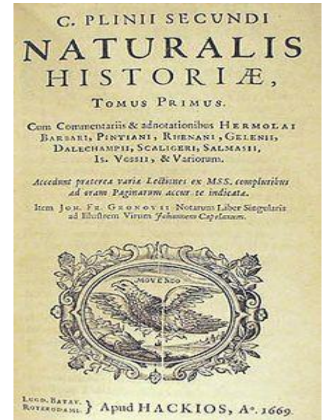
Bioluminescence : an old story!!

Pline l'Ancien (23-79) ***Naturalis Historiae***

.Livre IX [6] (XXXIII.) **«** *On range dans la même classe les peignes de mer, qui se cachent, eux aussi, pendant les grands froids et pendant les grandes chaleurs, et les ongles [pholades] qui brillent la nuit comme du feu, dans la bouche même de ceux qui les mangent. »*



Pholade

Pholas dactylus Linnaeus, 1758

Bioluminescence

« Dans la nuit les méduses flottent et changent de position …quand elles réalisent qu'une main s'approche elles changent de couleur et se contractent » *Plinius, G. S. Naturalis Historia Liber IX, §146 (77)*

Bioluminescence : widespread in oceans with multiple roles

Bioluminescence in the Ocean: Origins of Biological, Chemical, and Ecological Diversity
E. A. Widder
Science **328**, 704 (2010);
DOI: 10.1126/science.1174269

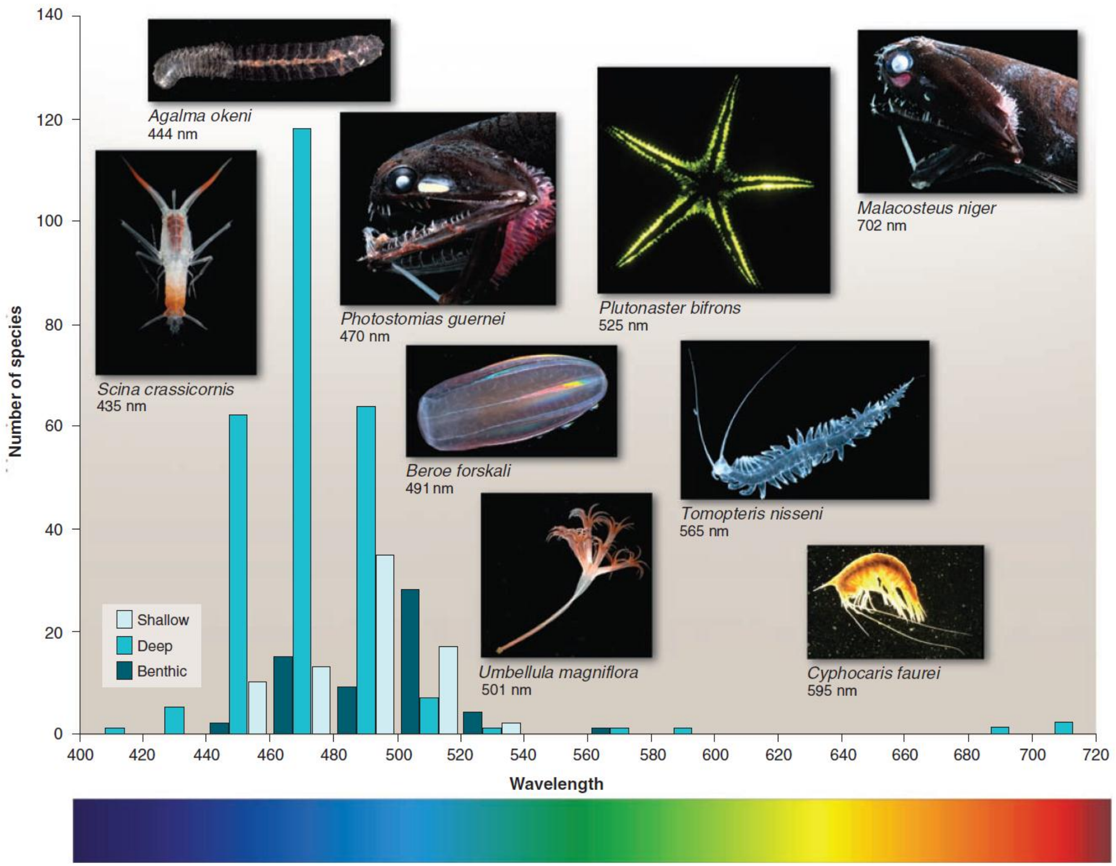


Fig. 1. The distribution of bioluminescence emission maxima varies by marine environment and organism type. Bioluminescent emissions extend over the full visible range and beyond. [Photo credits: J. Cohen for the photograph of *S. crassicornus*; P. Herring, *P. bifrons*; and P. Batson (DeepSeaPhotography.com), *C. faurei*]

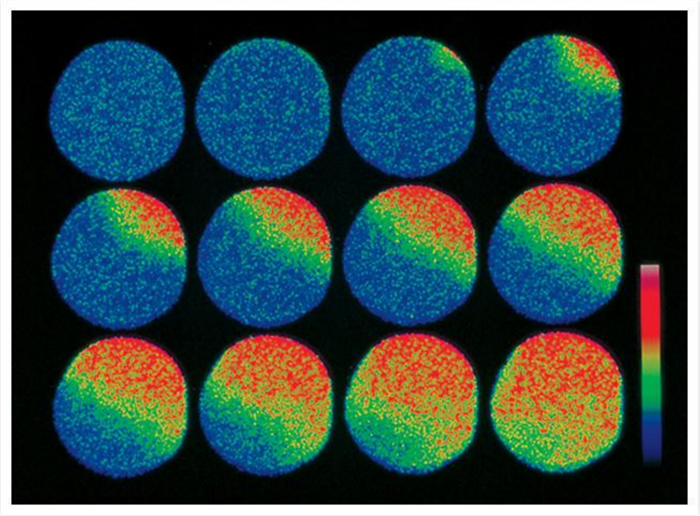
The vast majority of bioluminescent organisms reside in the ocean; of the more than 700 genera known to contain luminous species, some 80% are marine

Calcium : an old story too !!

Le Calcium, C'est la Vie: Calcium Makes Waves

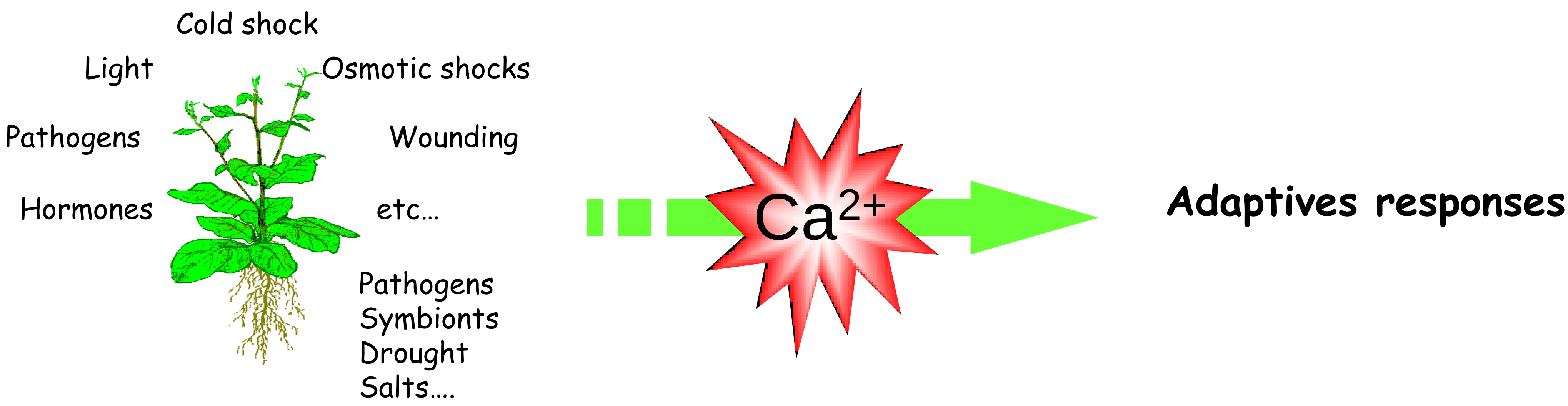
by Anthony Trewavas

Plantphysiol
Volume 120(1):1-6
May 1, 1999

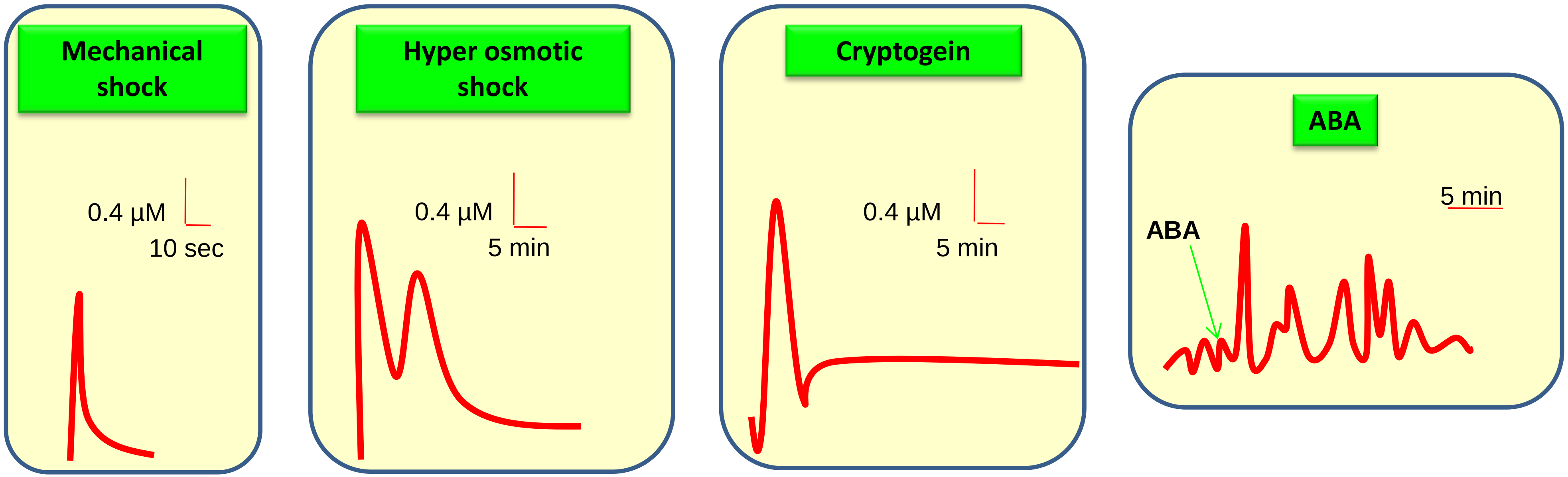


Fertilization-induced calcium wave in a starfish oocyte.
Confocal images taken at five-second intervals of a fertilization-induced calcium wave in a *Pisaster ochraceus* starfish oocyte that was microinjected with 10,000 MW Calcium Green™-1 dextran (Cat. No. C3713). The image was contributed by Stephen A. Stricker, University of New Mexico.

Calcium: a second messenger in plant signalling

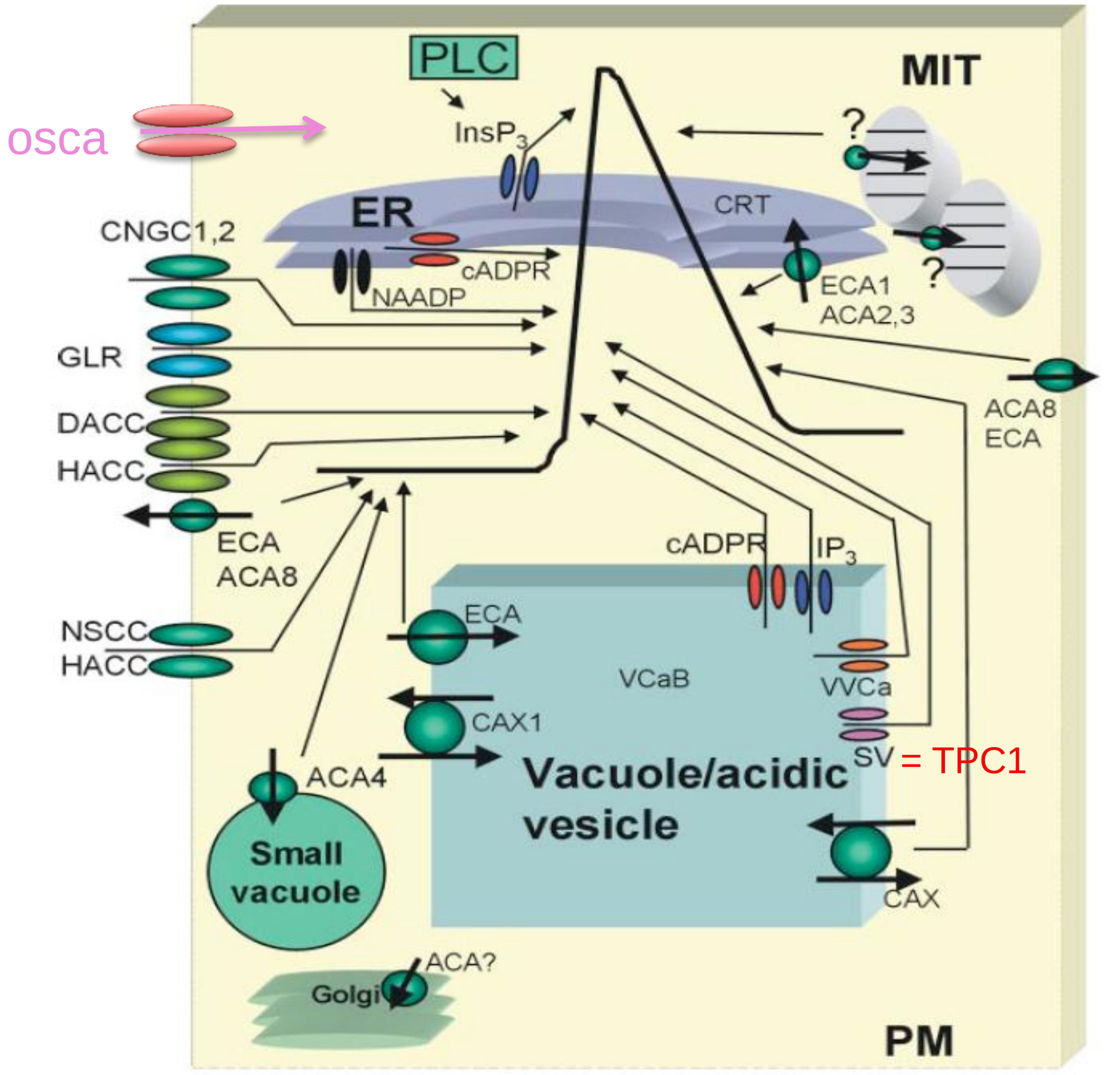


Concept of calcium fingerprint



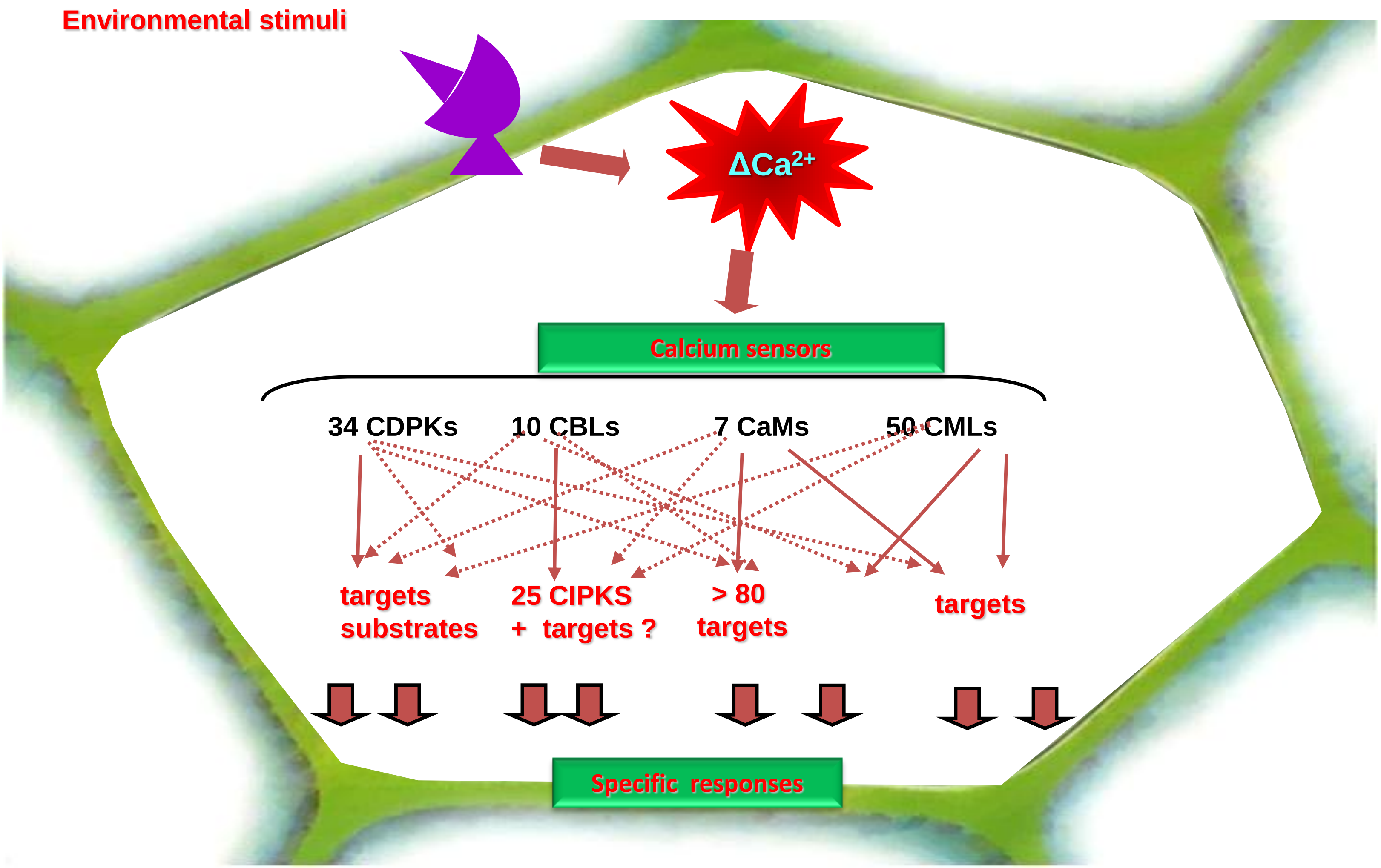
Parameters: **Shape**
Duration
Amplitude
Frequency
Compartmentation

How calcium concentration is regulated in cells?

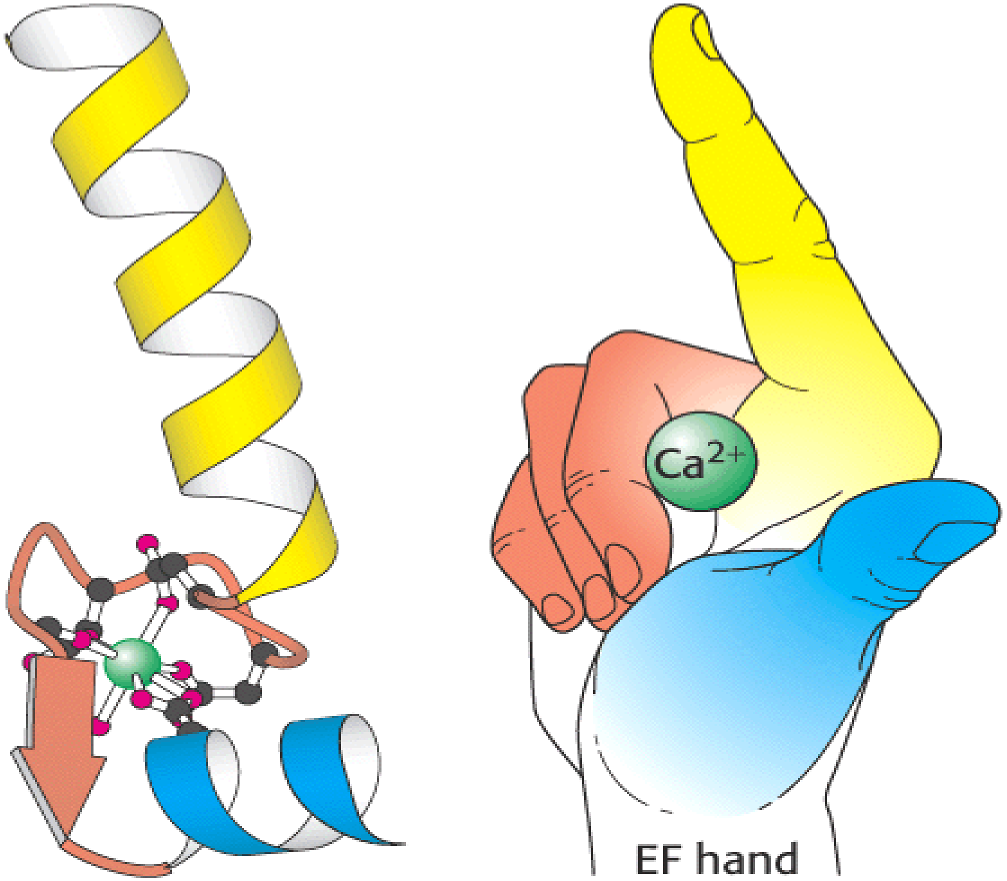


Hetherington A.M. and C. Brownlee
Ann. Rev. Plant. Biol. (2004)
55: 401-427

Decoding the calcium message



The E-F hand : a crucial motif required in calciproteins to decode changes in calcium concentration



EF Hand. Digital image. Biochemistry 492a. Web. 9 Mar. 2011. <http://www.biochem.arizona.edu/classes/bioc462/462a/NOTES/ENZYMES/enzyme_regulation.html>.

How to measure/monitor calcium changes in cells

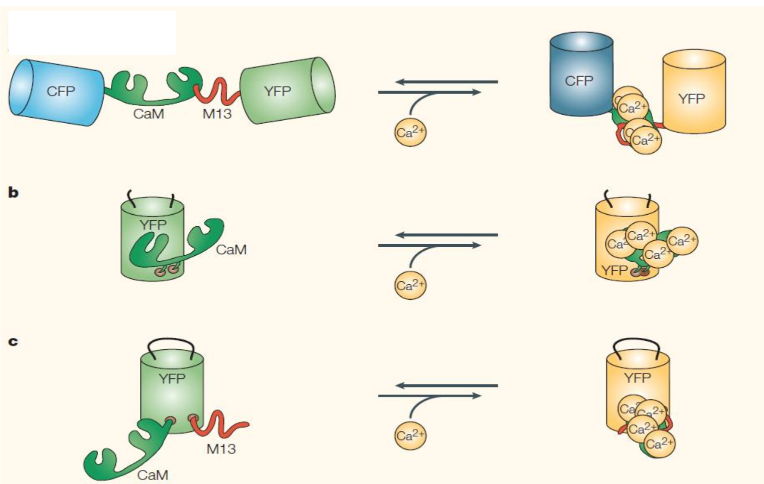
1 - Exploiting fluorescence

➢ Chemical fluorescent indicators

→ **Non ratiometric:** - ➢Fluo 4

→ **Ratiometric:** - Fura 2 (Excitation)
- Indo 1 (Emission)

➢ Genetically Engineered Calcium Indicators (GECIs): GFP-based proteins



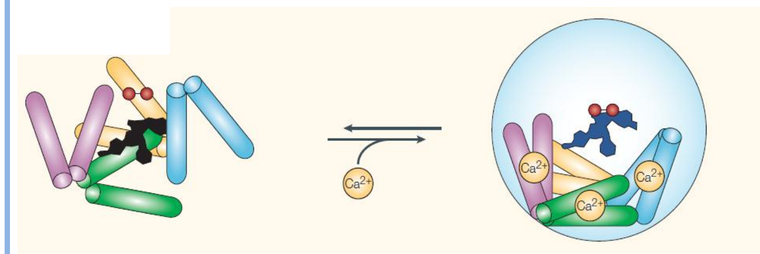
→ **Cameleon**

→ **Camgaroo**

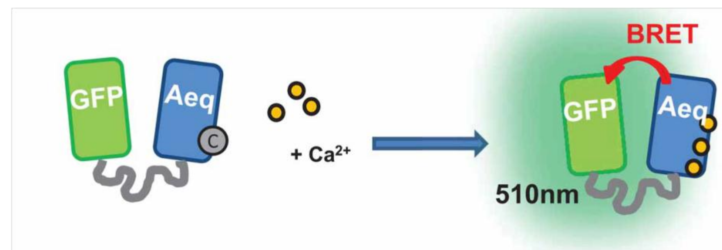
→ **cpYFP-Pericam**

2 - Exploiting luminescence

Photoproteins : aequorin



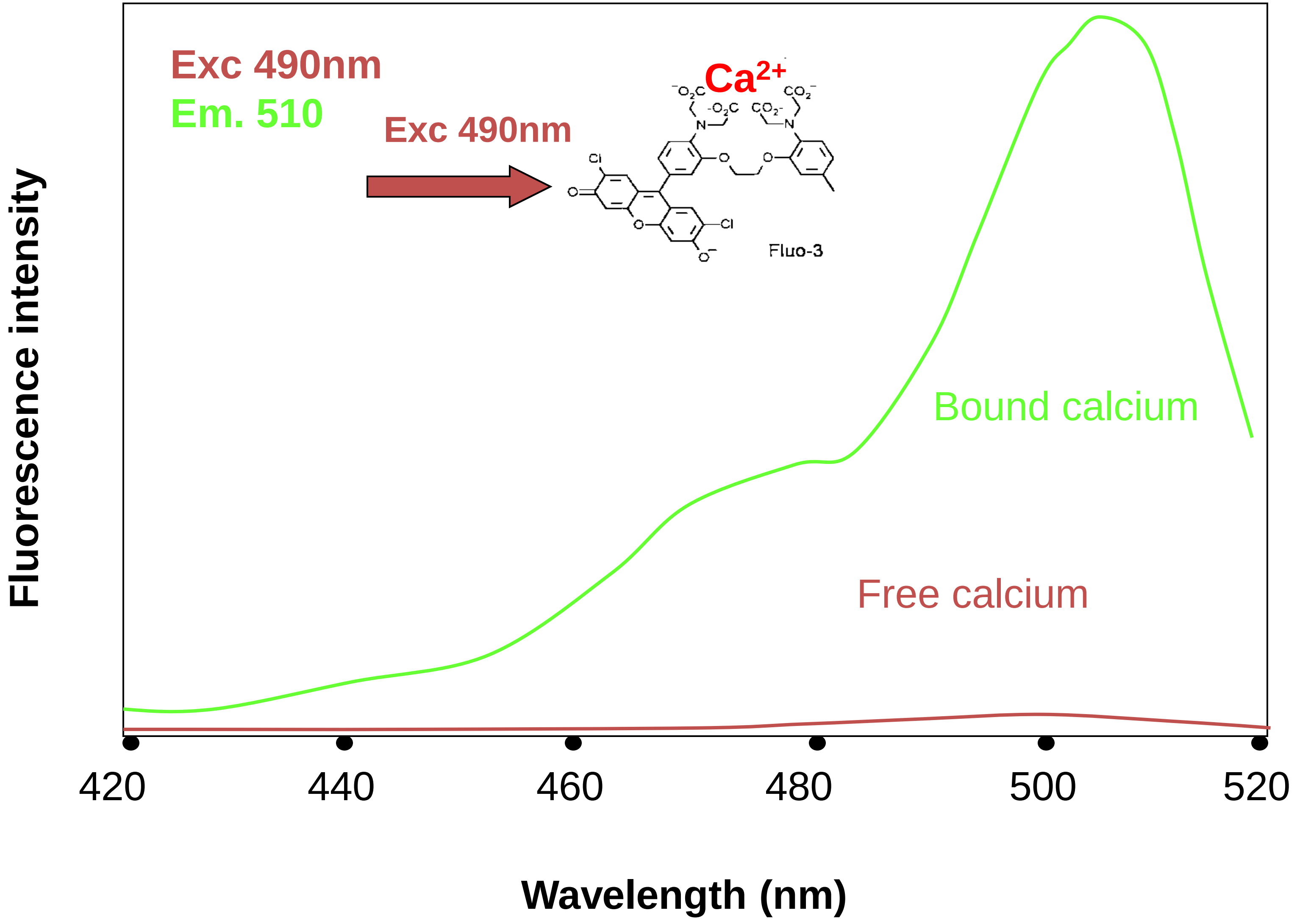
Photoproteins :G5A



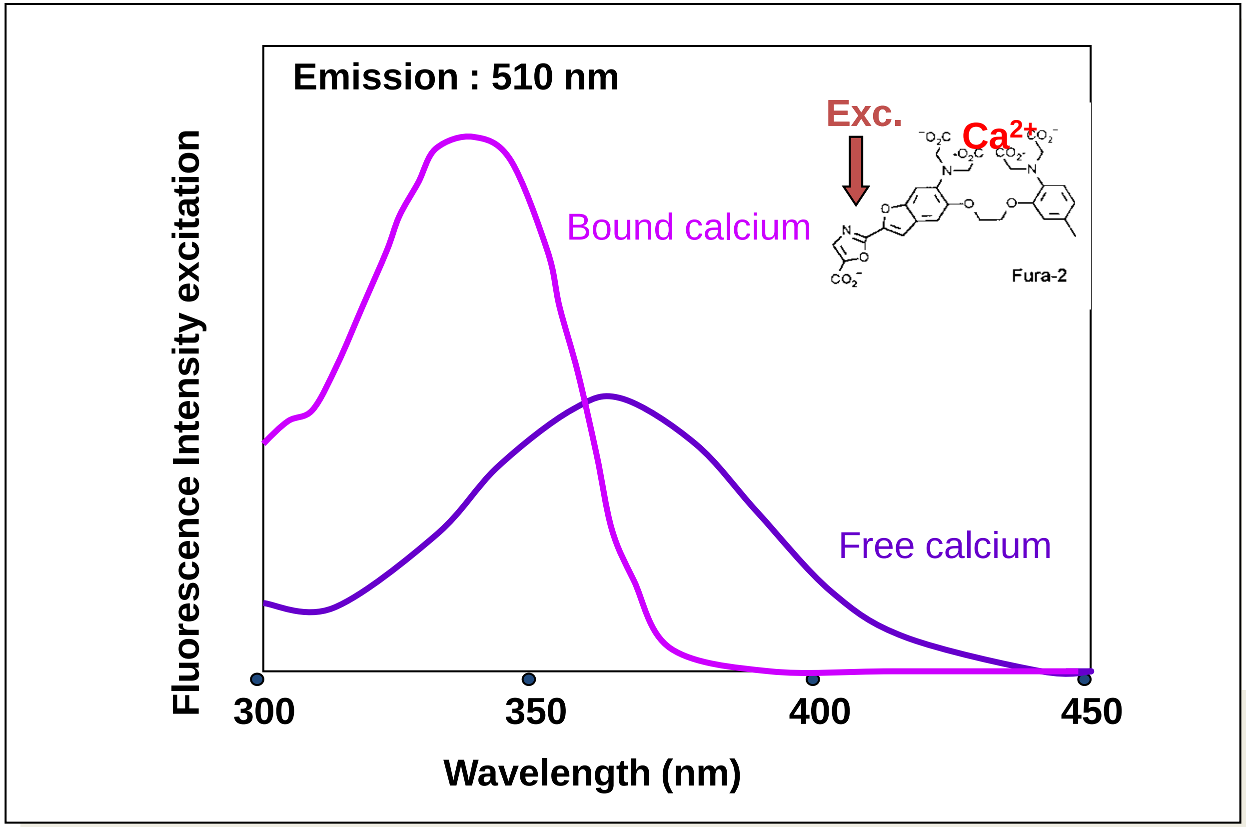
Adapted from : Rudolf R et al. (2003) Nature Cell Biol 4: 579

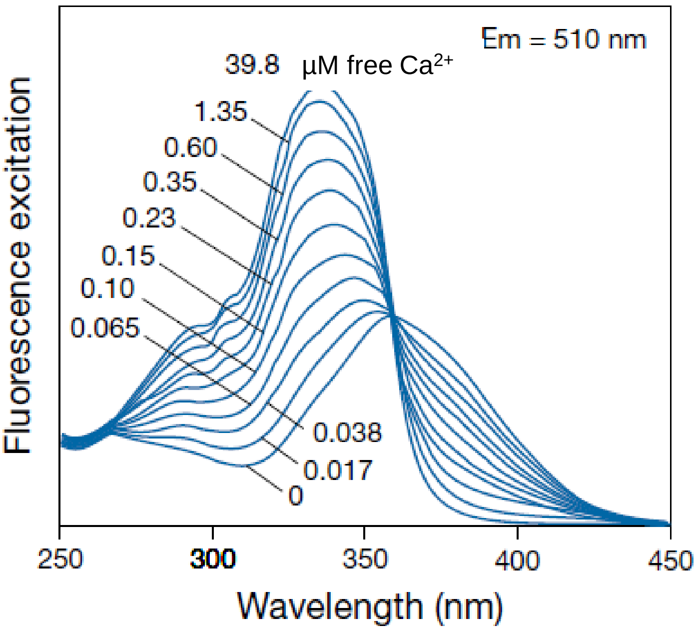
→ Tou Cheu Xiong
Mireille Chabaud
Fernanda De Carvalho

Non ratiometric chemical probes: Fluo-3



Ratiometric chemical probes : Fura-2





Changes in Fura-2 excitation spectra
in calcium solutions from 0 to 39,8µM

Ratio: 340/380

First generation of fluorescent calcium probes

Sondes Ca^{2+} - Mg^{2+}

Sonde	λ_1 exc	λ_2 exc	λ_1 em	λ_2 em	K_d (nM)
Fura-2	340	380	510		145
Bis-Fura-2	340	380	510		370
Fura-red	420	480	660		140
Quin-2	350		495		60
Mag-Fura-2	340	380	490		25 000
BTC	400	480	540		7 000
Indo-1	350		405	485	230
Mag Indo-1	350		405	485	35 000
Texas-red/Calcium green	488 568		535	615	370
Fluo-3	488		525		390
Rhod-2	540		570		570
Calcium green-1	488		530		190
Calcium orange	530		575		185
Calcium green-5N	488		530		14 000
Oregon green 488	488		520		170
Mag-Fura-2	340	380	490		25 000
Mag-Fura-5	330		340	385	28 000
Mag-green	475			530	1 mM
Mag-Fura red	480		660		2 mM

UV	Visible

Important parameters:

1) Affinity (Kds)
2) Excitation (UV-visible)
3) Emission (UV-visible)
4) Dynamics
5) Specificity (Ca^{2+} – Mg^{2+})

An extended choice of fluorescent calcium probes

Ca^{2+} Indicator	Water-Soluble Salt *	Cell-Permeant Ester †	Dextran ‡	Mode §	K_d (nM) **	Notes
Bis-fura-2t	B6810			Ex 340/380	370	1
BTC	B6790	B6791		Ex 400/480	7000	2
Calcium Green™-1	C3010MP	C3011MP, C3012	C6765, C3713, C3714	Em 530	190	3, 4
Calcium Green™-2	C3730	C3732		Em 535	550	3, 5
Calcium Green™-5N	C3737	C3739		Em 530	14,000	3
Calcium Orange™	C3013	C3015		Em 575	185	2
Calcium Crimson™		C3018		Em 615	185	2
Fluo-3	F1240, F3715	F1241, F1242, F14218, F14242, F23915		Em 525	390	3, 4
Fluo-4	F14200	F14201, F14202, F14217, F23917	F14240 ††, F36250 ‡‡	Em 520	345	3, 6
Fluo-5F	F14221	F14222		Em 520	2300	3
Fluo-4FF	F23980	F23981		Em 520	9700	3
Fluo-5N	F14203	F14204		Em 520	90,000	3
Fura-2	F1200, F6799	F1201, F1221, F1225, F14185	F3029	Ex 340/380	145	2
Fura-4F	F14174	F14175		Ex 340/380	770	2
Fura-6F	F14178			Ex 340/380	5300	2
Fura-FF	F14180	F14181		Ex 340/380	5500	2
Fura Red™	F14219	F3020, F3021		Ex 420/480	140	2, 7
Indo-1	I1202	I1203, I1223, I1226		Em 405/485	230	2
Mag-fluo-4	M14205	M14206		Em 520	22,000	3
Mag-fura-2	M1290	M1291, M1292		Ex 340/380	25,000	2
Mag-indo-1		M1295		Em 405/485	35,000	2, 8
Magnesium Green™	M3733	M3735		Em 530	6000	3
Oregon Green® 488 BAPTA-1	O6806	O6807	O6798	Em 520	170	3
Oregon Green® 488 BAPTA-2	O6808	O6809		Em 520	580	3, 9
Oregon Green® 488 BAPTA-6F	O23990			Em 520	3000	3
Oregon Green® 488 BAPTA-5N	O6812			Em 520	20,000	3
Quin-2	Q23918			Em 495	60	2, 10
Rhod-2	R14220	R1244, R1245MP	R34676 ‡‡	Em 580	570	3, 11
Rhod-3		R10145		Em 580	570	3
Rhod-FF		R23983		Em 580	19,000	3
Rhod-5N	R14207			Em 580	320,000	3
X-rhod-1		X14210		Em 600	700	3
X-rhod-5F	X23984	X23985		Em 600	1600	3

* Catalog number(s) for the cell-impermeant salt. † Catalog number(s) for the cell-permeant AM ester. ‡ Catalog number(s) for the dextran conjugates. § Measurement wavelengths, in nm, where Ex = fluorescence excitation and Em = fluorescence emission. Indicators for which a pair of wavelengths are listed have dual-wavelength, ratio-measurement capability. ** Ca^{2+} dissociation constant, measured *in vitro* at 22°C in 100 mM KCl, 10 mM MOPS, pH 7.2, unless otherwise noted. K_d values depend on temperature, ionic strength, pH and other factors, and are usually higher *in situ*. Because indicator dextrans are intrinsically polydisperse and have variable degrees of substitution, these values may vary; lot-specific K_d values are printed on the vial in most cases. †† Low-affinity dextran conjugate. ‡‡ High-affinity dextran conjugate.

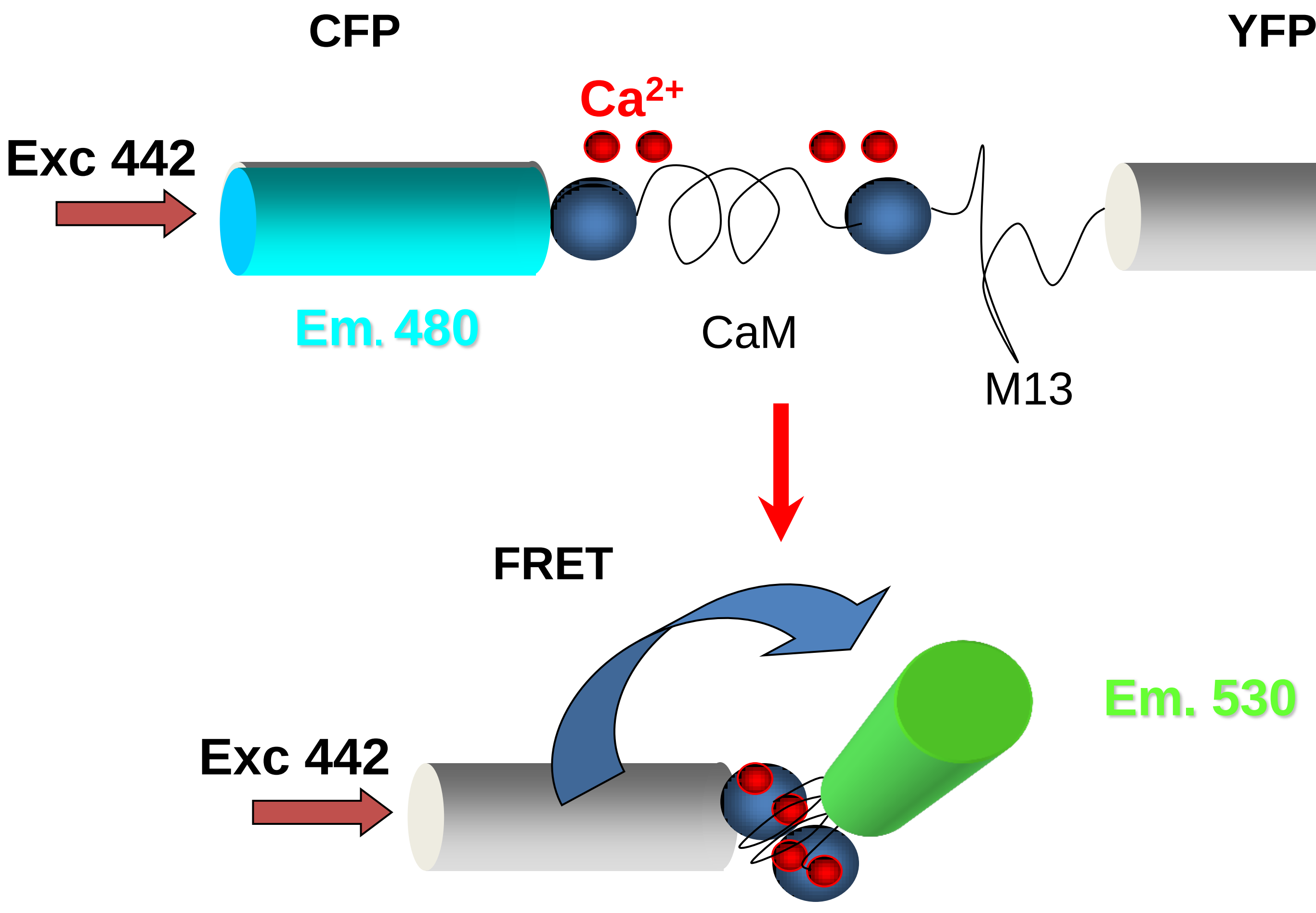
Notes: **1**. Ca^{2+}-dependent fluorescence response similar to fura-2 but ~75% greater molar absorptivity. **2**. The AM ester form is fluorescent (a major potential source of error in Ca^{2+} measurements). **3**. The AM ester form is nonfluorescent. **4**. Calcium Green™-1 is more fluorescent than fluo-3 in both Ca^{2+}-bound and Ca^{2+}-free forms. The magnitude of the Ca^{2+}-dependent fluorescence increase is greater for fluo-3; see Section 19.3. **5**. Larger Ca^{2+}-dependent fluorescence increase than Calcium Green™-1. **6**. The K_d value for the low-affinity fluo-4 dextran (F14240) is ~3 µM, which is much higher than that of the free dye. The K_d value for the high-affinity fluo-4 dextran (F36250) is ~600 nM. **7**. Can also be used in combination with fluo-3 for dual-wavelength ratio measurements, Ex = 488 nm, Em = 530/670 nm (Cell Calcium (1995) 18:377; Cytometry (1994) 17:135; Cell Calcium (1993) 14:359). **8**. K_d determined in 100 mM KCl, 40 mM HEPES, pH 7.0 at 22°C (Biochem Biophys Res Commun (1991) 177:184). **9**. Larger Ca^{2+}-dependent fluorescence increase than Oregon Green® 488 BAPTA-1. **10**. K_d determined in 120 mM KCl, 20 mM NaCl, pH 7.05 at 37°C (Methods Enzymol (1989) 172:230). **11**. The K_d value for the high-affinity rhod dextran (R34676) is ~780 nM.

Non-ratiometric indicators extending the range of Ca^{2+} concentrations measurement (Fluo-3, Fluo-4, Fluo-5F, Fluo-4FF, Fluo-5N)

Ratiometric indicators extending the range of Ca^{2+} concentrations measurement (Fura-2, Fura-4F, Fura-6F, Fura-FF)

From: The Molecular Probes® Handbook
A GUIDE TO FLUORESCENT PROBES AND LABELING TECHNOLOGIES
11th Edition (2010)

Genetically encoded calcium probe: cameleon



Aequorin: a bioluminescent calcium probe

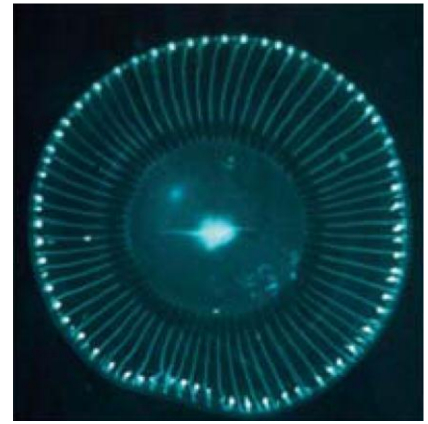
Aequorea victoria

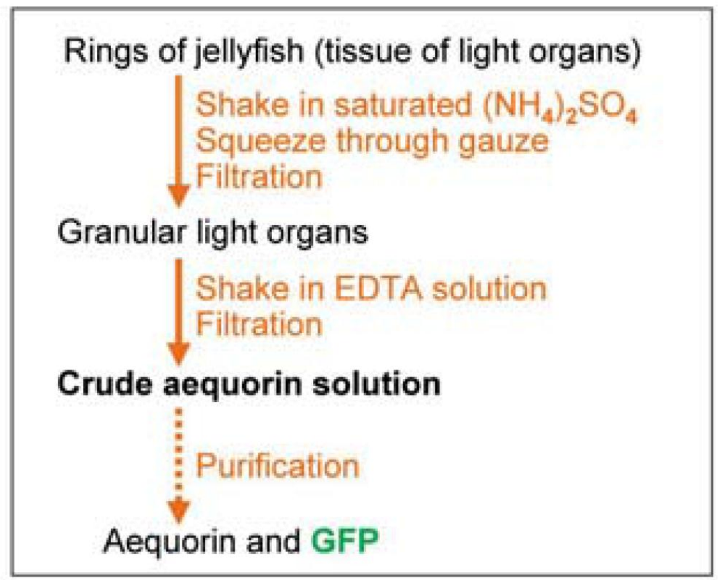
Luminescence described for the first time in 1775 by Forskal

Purification of aequorin from jellyfish

O. Shimomura
Nobel prize in Chemistry 2008

Purification from 10000 Jellyfish in 1961

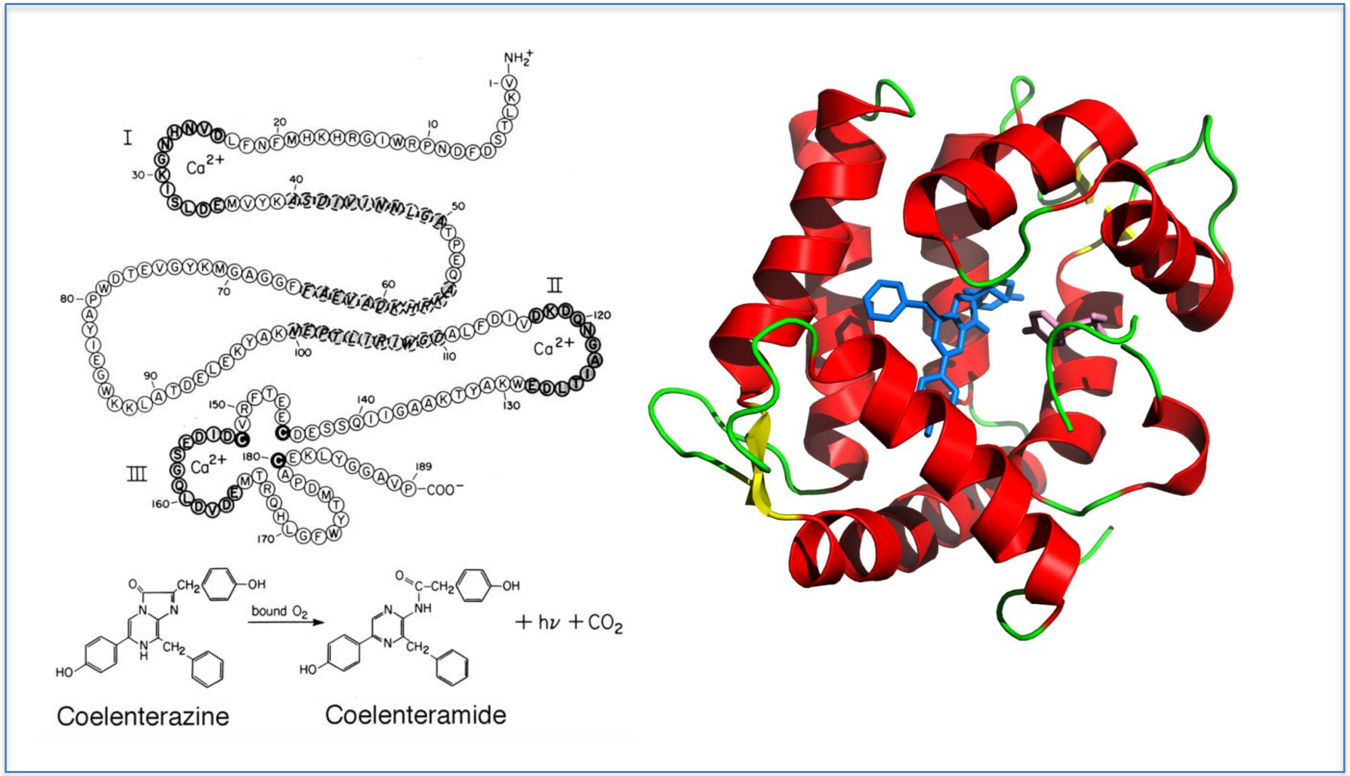




Structure of apo-aequorin

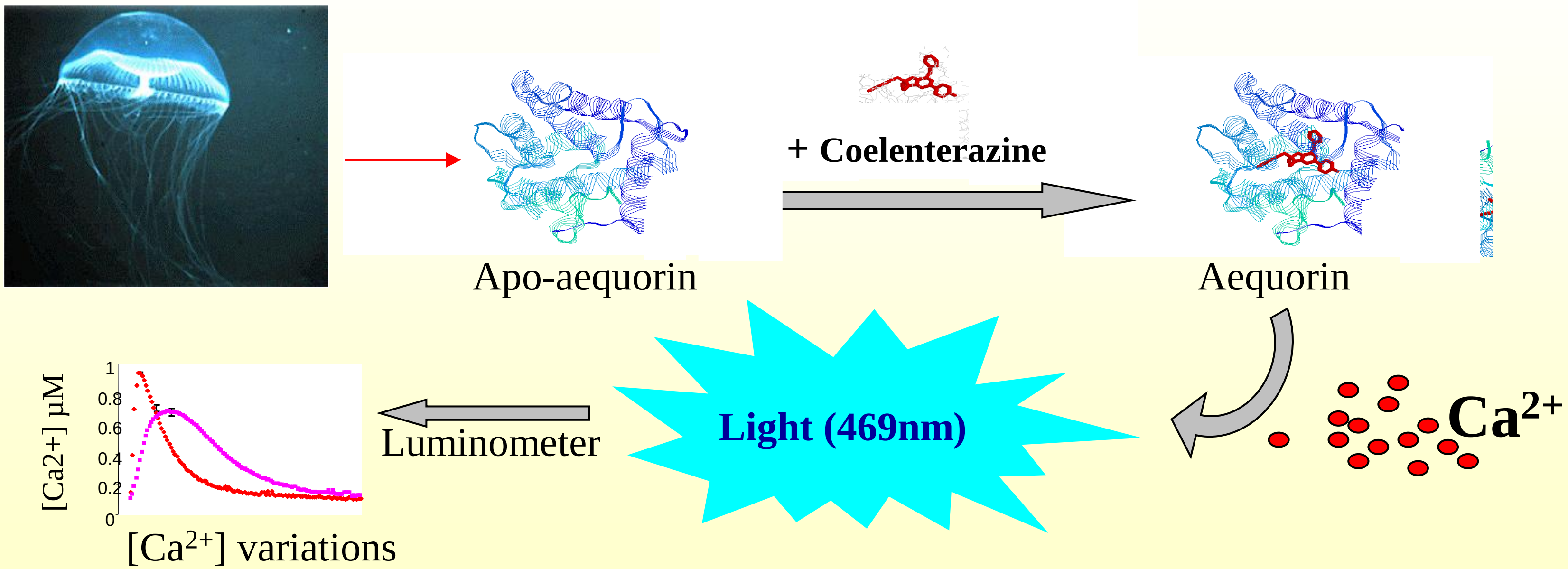
Aequorin: 189 AA, 22 kDa

Coelentarazine: 423 Da

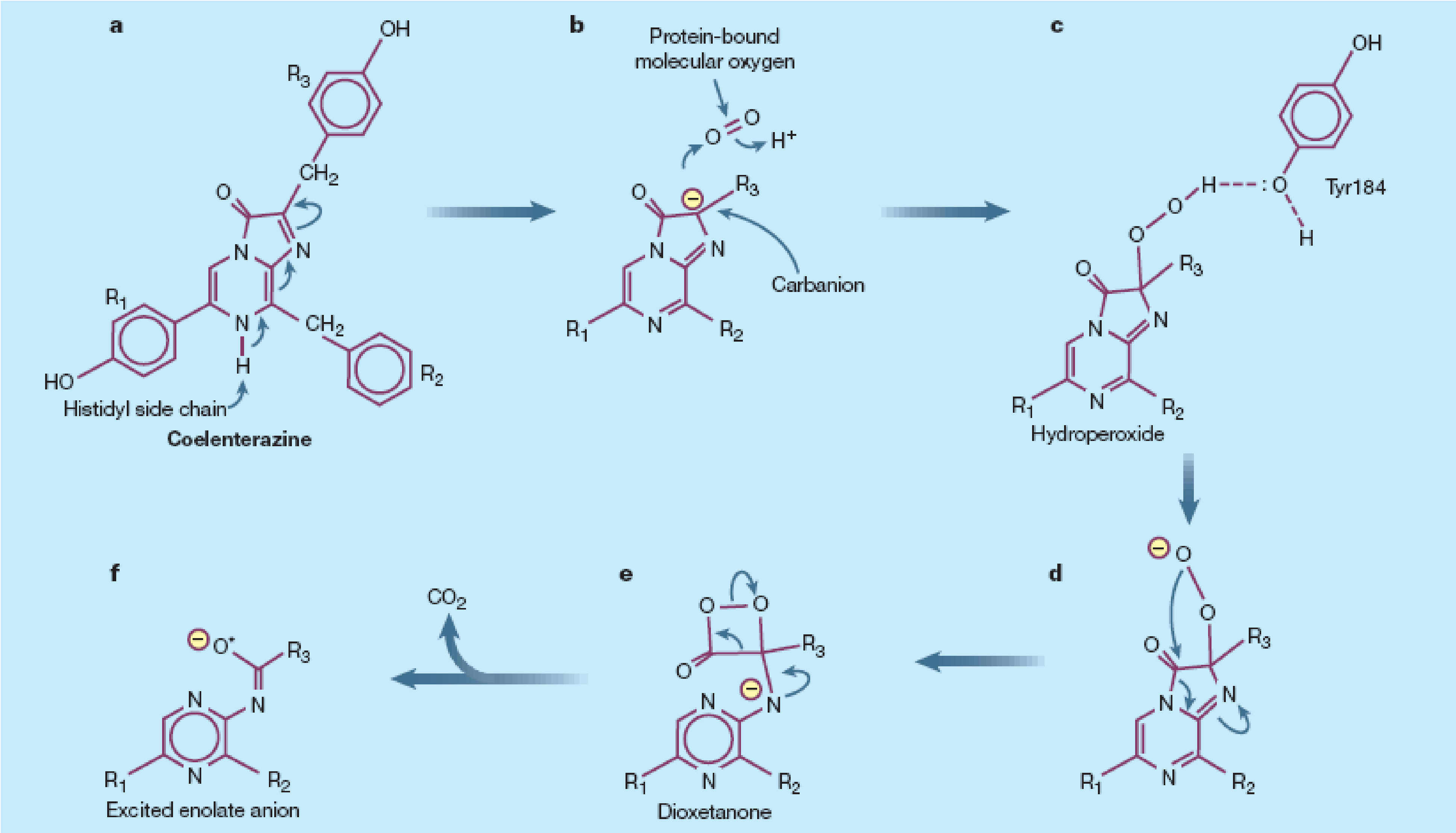


Kendall J.M. and Badminton M. 1998.
TIBTECH 16: 216-224

Calcium monitoring with aequorin



Chemical reactions underlying bioluminescence



Prendergast F.G. 2000. Nature 405: 291-293

Set of available coelenterazines to modulate aequorin sensitivity

Table 1. Coelenterazines and their properties.

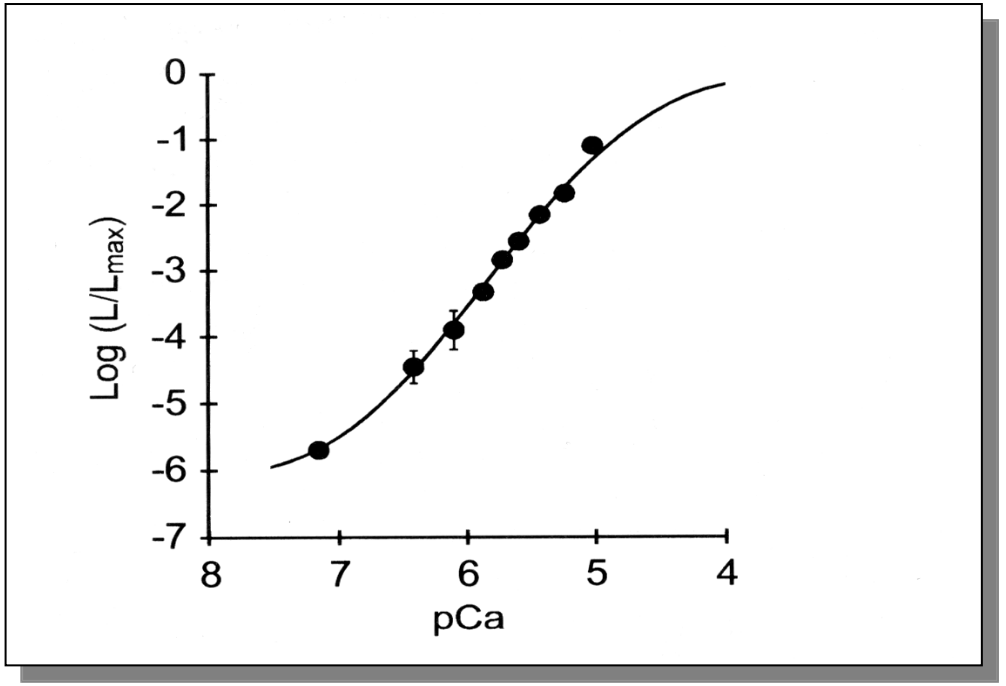
Cat #	Coelen-terazine Analog	Em (nm)	RLC *	Relative Intensity †	Half-Rise Time ‡ (ms)
C-2944	native	466	1.00	1	6–30
C-14260	*cp*	442	0.63	28	2–5
C-6779	*f*	472	0.80	20	6–30
C-6780	*h*	466	0.75	16	6–30
C-14261	*hcp*	445	0.65	500	2–5
C-6776	*n*	468	0.25	0.15	6–30

* RLC = relative luminescence capacity: Total time-integrated emission of aequorin in saturating Ca^{2+} relative to native aequorin = 1.0. † Ratio of the luminescence of aequorin reconstituted with coelenterazine analog relative to native aequorin at 100 nM Ca^{2+}. ‡ Half-Rise Time: The half-rise time is the time for the luminescence signal to reach 50% of the maximum after addition of 1 mM Ca^{2+} to a standard of aequorin reconstituted with the coelenterazine analog of interest. All data are from O. Shimomura in Cell Calcium 14, 373 (1993).

http://probes.invitrogen.com/media/pis/mp02944.pdf

Calibration

1. Prepare :
   - Ca^{2+} buffers with EGTA or HEDTA
   - Lysates of cell expressing aequorin
2. Reconstitute aequorin with coelentarazine
3. Dilute (1:10) cell lysates in Ca^{2+} buffers
4. Measure emitted light with a luminometer



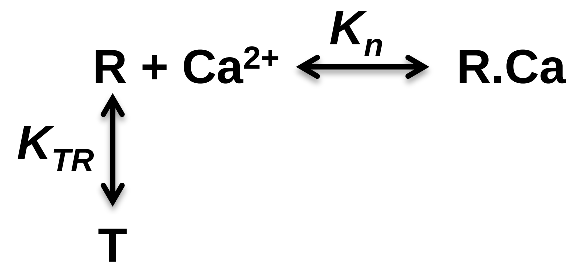
From: Brini et al.1995. Transfected aequorin in the measurement of cytosolic ca concentration
J. Biol. Chem 270: 17 9896-9903

See also: Allen et al. 1977 : Aequorin luminescence: Relation of light emission to calcium concentration - A calcium-independent component. Science 195:996-998

From light to calcium concentration

Allen method (Allen et al. 1977. Science, 195: 996-998)

This method postulates that Ca^{2+} binding has two possible states and light is emitted only when all the sites are in R state :



$$[Ca^{2+}] = \{(L_0/L_{max})^{1/3} + [K_{TR}(L_0/L_{max})^{1/3}] - 1\}/\{K_R - [K_R(L_0/L_{max})^{1/3}]\},$$

K_R = The calcium association constant = $7.10^6\ M^{-1}$

$K_{TR} = [T]/[R] = 118$

L_0 = $count.s^{-1}$ L_{max}=Total counts

Knight empirical method (Knight et al. 1977. The Plant Cell, 8: 489-503) :

$$pCa = 0.332588(-\log k) + 5.5593$$

K= $count.s^{-1}$ at each time / total counts of the whole experiment

Advantages and disadvantages of aequorin

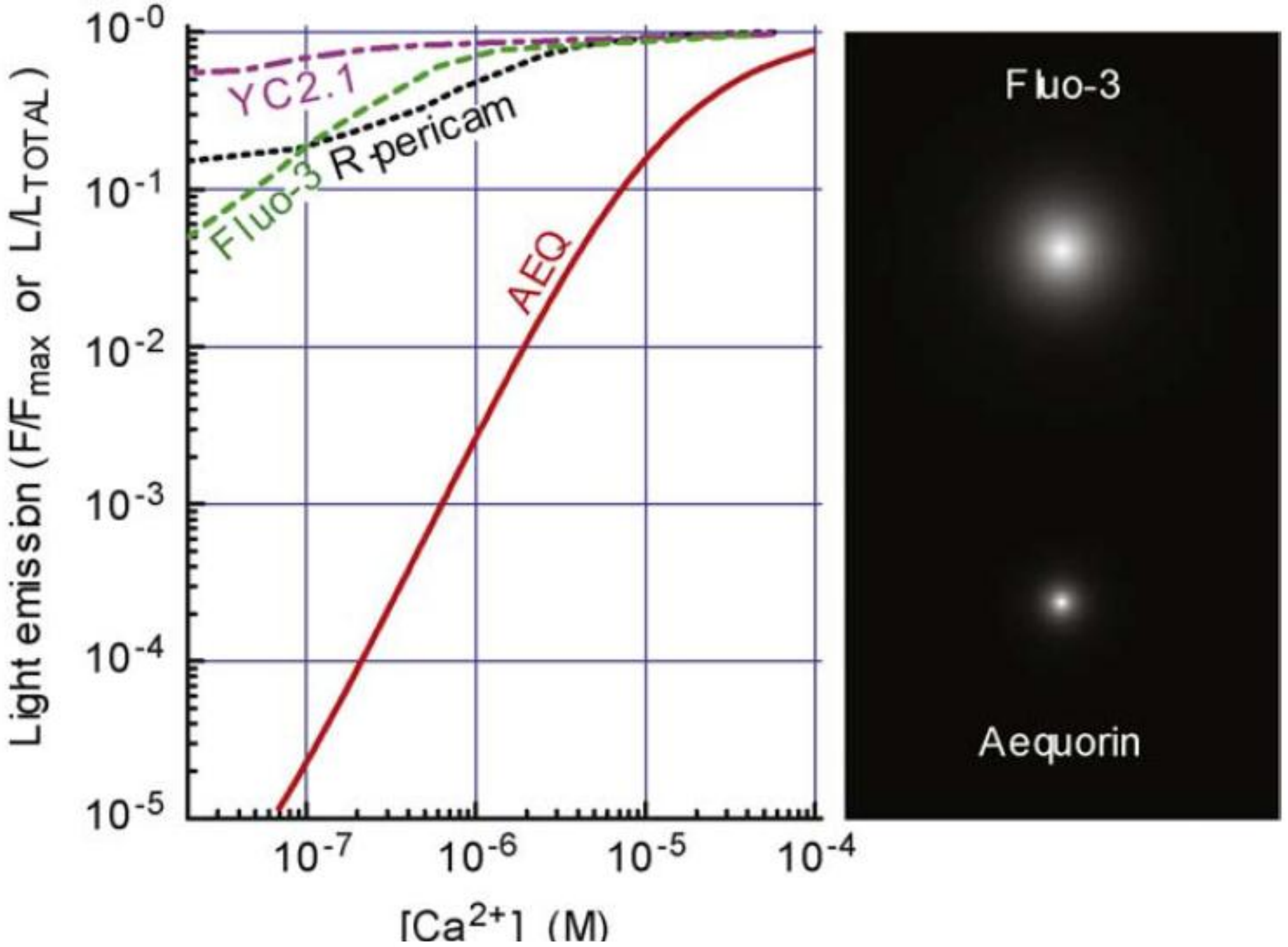
•PROS:

- Excellent dynamics (0.1 - 100 µM → 10 000 x)
- Excellent signal-to-noise ratio
- Absence of chemiluminescent proteins in living cells
- Very low [Ca^{2+}] buffering capacity
- Possibility of organelle targeting
- Stability increases upon reconstitution
- Expression of 10^4-10^5 molecules/cell
- Possibility to monitor calcium changes over long periods
- No cell toxicity

•CONS:

- Low light emission : 1 photon/mol.
  (fluorescent probes: 10^4 photons/mol.)
- Requires transformation or injection
- Requires a specific equipment (luminometer - High sensitivity camera)

Dynamic range of calcium probes



M.T. Alonso, J. García-Sancho / Cell Calcium 49 (2011) 280–289

Pros and cons of calcium probes

Pros		Cons
	Chemical fluorescent probes	
-Good dynamics (40 for Fluo3) -Detection at the level of a single cell -Availability of permeant probes (AM conjugates) - Strong fluorescent signal		- Pb of permeability (injection) -Pb of compartmentation, leakage, stability (bleaching) -Buffering effects and toxicity with AM conjugates (release of formaldehyde and acetic acid) -Quantification only with ratiometric probes
	Fluorescent proteins	
- Possibility of organelle targeting - Measurement at the level of a single cell		-Low dynamics (2-7) - Requirement of specific equipment (FRET) - Calcium buffering and /or CaM competition - pH sensitivity - Possibility of bleaching
	Luminescent probes (aequorin)	
- No need of excitation wavelength - Very high dynamics (up to 10,000) - Possibility of organelle targeting - Measurement at the whole tissu or organ level - No toxicity, no buffering, no pH sensitivity - Calcium monitoring over long periods		-Single cell measurement still difficult - Light detection equipment required (luminometer) - Low energy photons→imaging requires high sensitivity cameras

Applications

Aequorin probe, a widely used tool in plant field

Stimulus	Organism	Reference
Mechanical stress (touch,wind)	N. plumbaginifolia (cyt)	22,30
Cold	N. plumbaginifolia (cyt)	22
	A. thaliana (cyt)	31
Heat	N. plumbaginifolia (cyt)	32
Blue light	A. thaliana (cyt)	33
	N. plumbaginifolia (cyt)	34
Darkness	N. plumbaginifolia (chl, cyt)	35
Drought	A. thaliana (cyt)	36
Osmotic shocks	N. tabacum (cyt)	16
	A. thaliana (cyt)	36
	N. plumbaginifolia (cyt)	37
	N. tabacum (nuc)	25
Circadian rhythms	N. plumbaginifolia (chl, cyt)	38
Anoria	A. thaliana	39
Oxidative stress	N. plumbaginifolia (cyt)	40
	A. thaliana (cyt)	41
Gravity	A. thaliana (cyt)	42
Acidification of external medium	A. thaliana (cyt)	43,44
Alkalinisation of external medium	A. thaliana (cyt)	45
Elicitors	N. plumbaginifolia (cyt)	22
	N. plumbaginifolia (cyt)	46
	L. esculentum (cyt)	20
	G. max (cyt)	5,47
	P. crispum (cyt)	6
Nod factors	G. max (cyt)	48
Phytohormones (ABA)	N. plumbaginifolia (cyt)	49
(auxin)	A. thaliana (cyt)	44
Sucrose	A. thaliana (cyt)	50
Glutamate	A. thaliana (cyt)	51
cAMP, cGMP	N. plumbaginifolia (cyt)	52
Lanthanum	A. thaliana (cyt)	44
Mastoparan	N. tabacum (cyt)	53
	N. plumbaginifolia (cyt, nuc)	14

Intracellular localization of aequorin expression: cytosol (cyt); chloroplast (chl); nucleus (nuc).

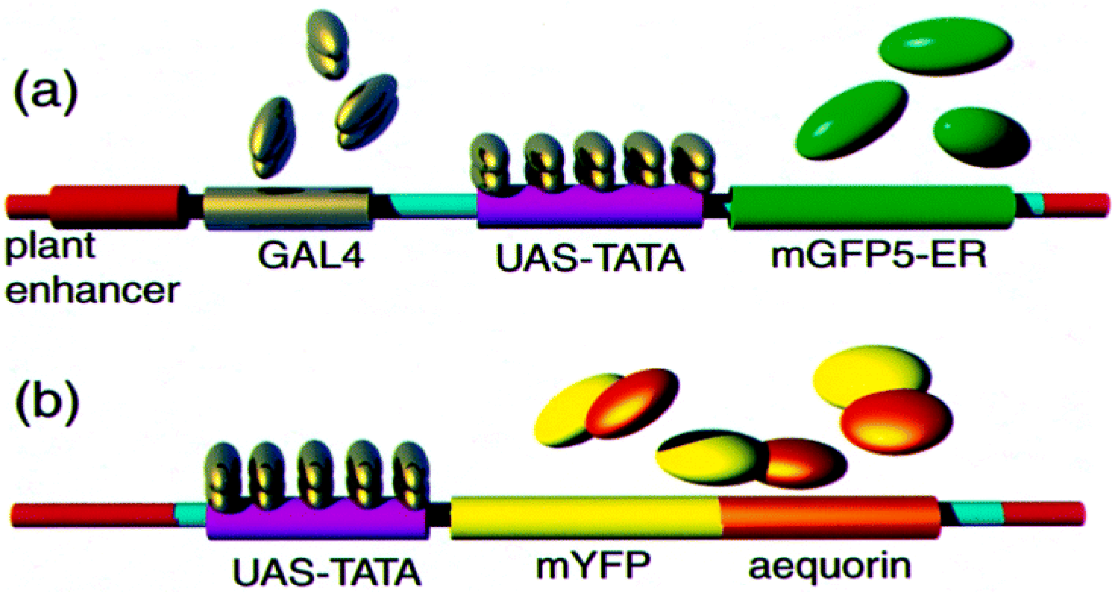
Biological Procedures Online (2002) 4: 1

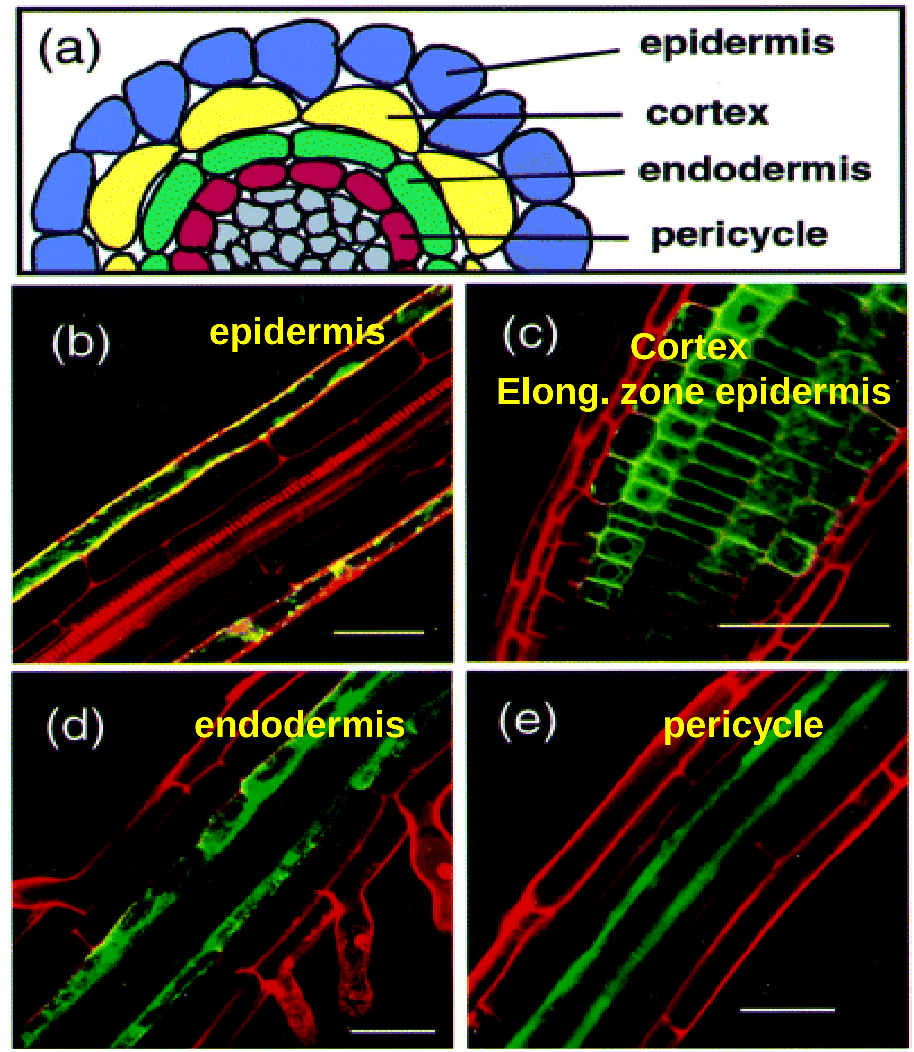
Specific tissue and compartment targeting of aequorin in plants

Localisation	Targeting strategy
Tissue targeting	
Root epidermis	« Enhancer trapped GAL4 »
Root endoderm	« Enhancer trapped GAL4 »
Root pericycle	« Enhancer trapped GAL4 »
Stomata guard cells	KST1 promoter
Pollen	G10 promoter
Compartment targeting	
Cytosol*	CaMV 35S promoter
Chloroplast*	Small subunit of RuBisCo
ER	chitinase containing HDEL motif
Nucleus (nucleoplasm)	nucleoplasmin fusion
Tonoplast	H^+-Ppase fusion
Cell Wall (apoplaste)	targeting signal of chitinase
Mitochondria	N-term 90AA Beta –ATPase fusion

*Plasmids from Molecular Probes [**Web site (http://www.probes.com**)].

Tissue targeting of aequorin using GAL4 transactivation strategy

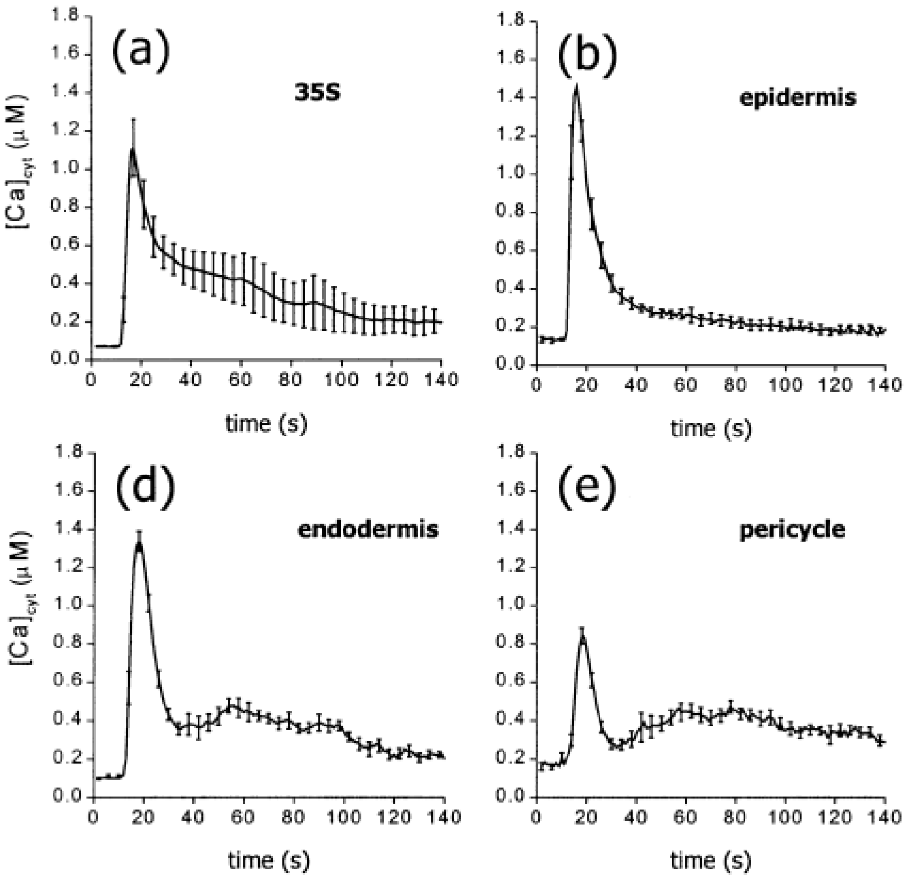


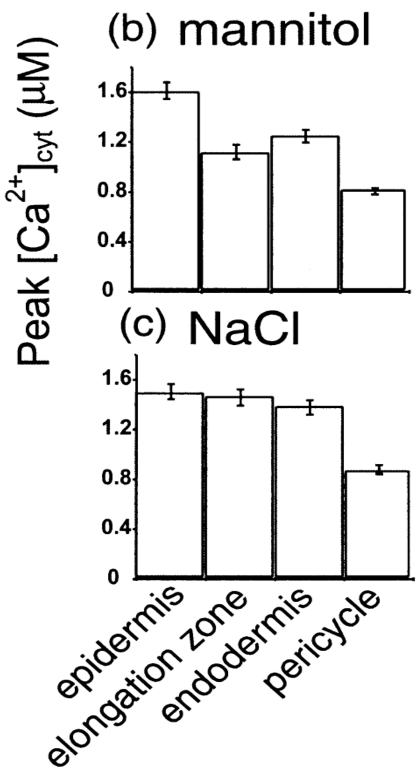


1. Selection of « enhancer trap » lines (GFP)
2. Selection of strong phenotypes (mYFP)
3. Measuring luminescence in specific lines

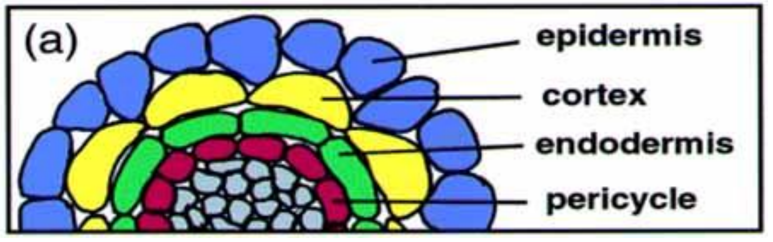
Kiegle et al. (2000) Plant J., 23, 267-278

Ca^{2+} responses to osmotic shocks in various tissues



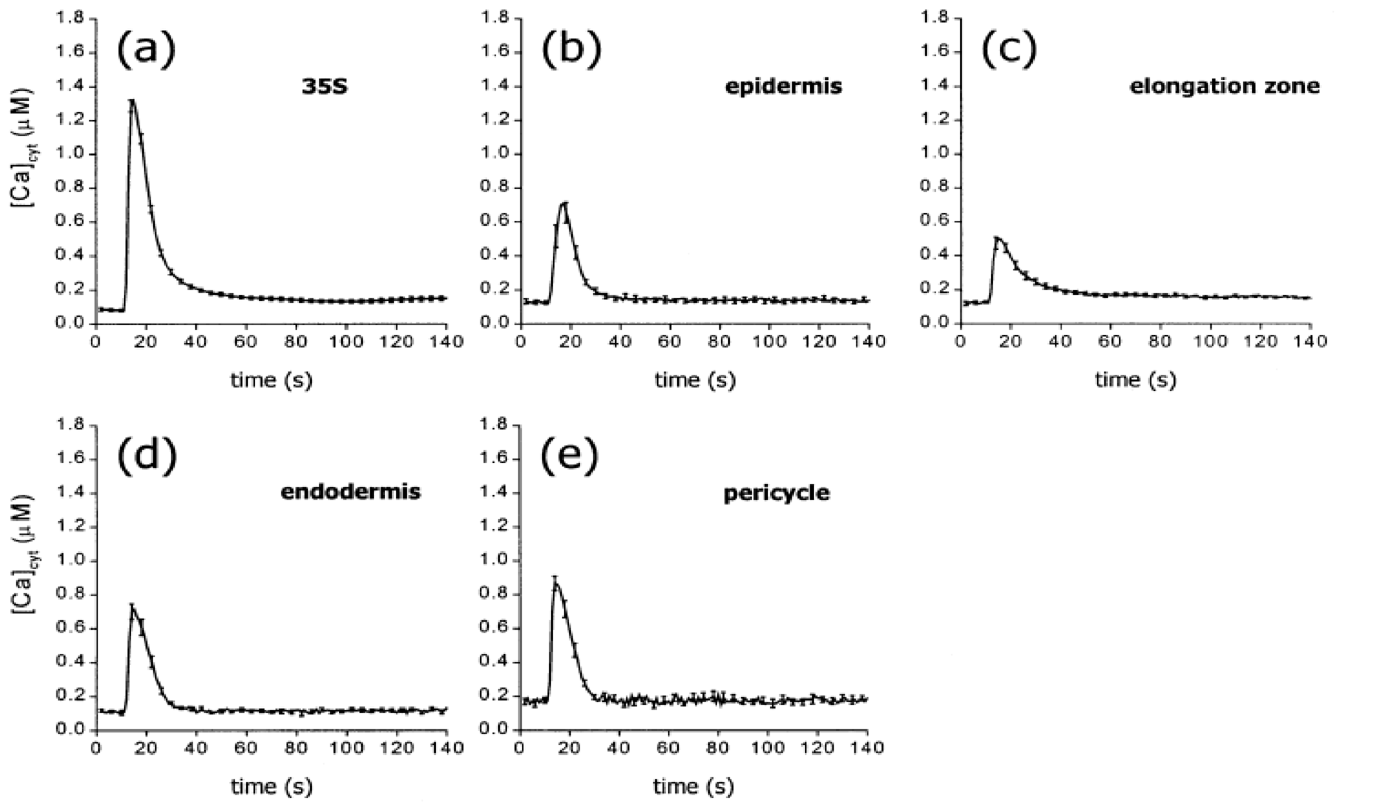


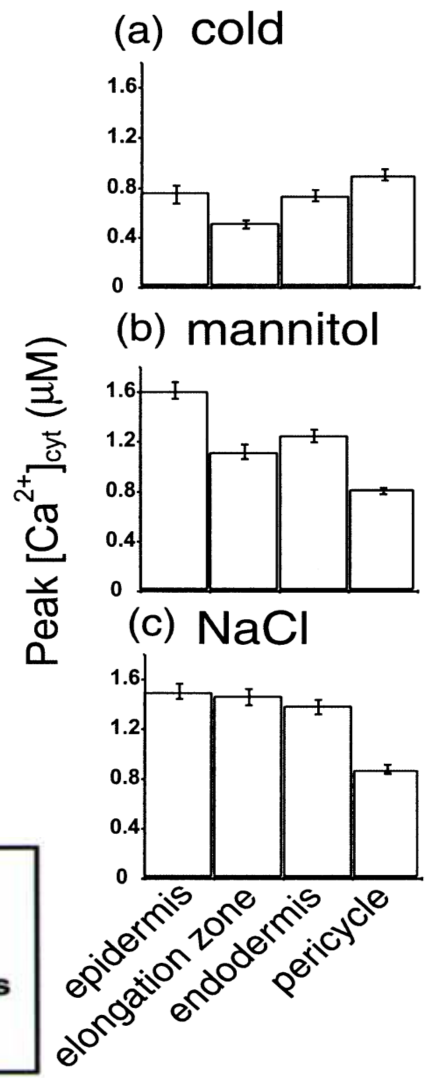
NaCl



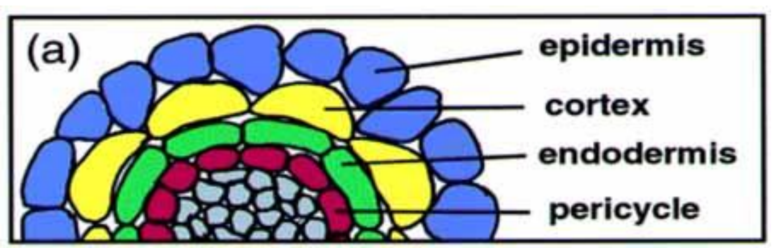
Kiegle et al. (2000) Plant J., ***23****, 267-278*

Ca^{2+} responses to a cold shock in various tissues





Kiegle et al. (2000) Plant J., ***23***, *267-278*



Aequorin can be specifically targeted in various plant compartments

Tic40 – inner envelope of chloroplasts : *130 AA of Tic 40*

OEP7 – outer envelope of chloroplasts : *FL OE protein 7*

NTRC – chloroplast stroma : *85 AA NADPH-TRX reductase C*

AKDE1- mitochondrial matrix : 66 AA of oxoglutarate deshydrogenase

CPK17 – plasma membrane : *58 AA of CPK17*

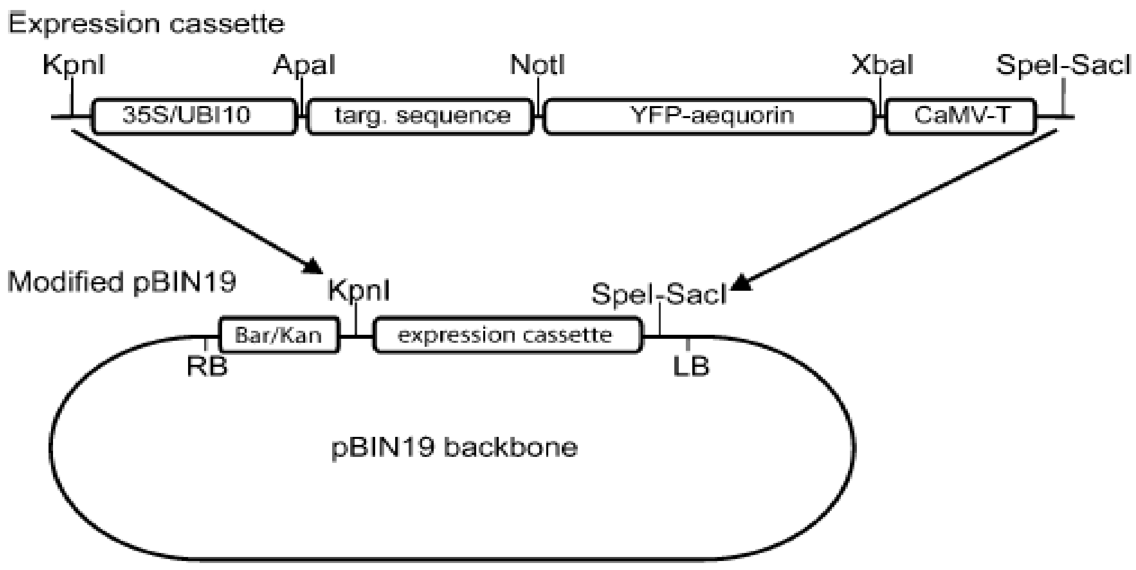
CPK17G2A – cytosol

NLS – nucleus : *NLS of SV40*

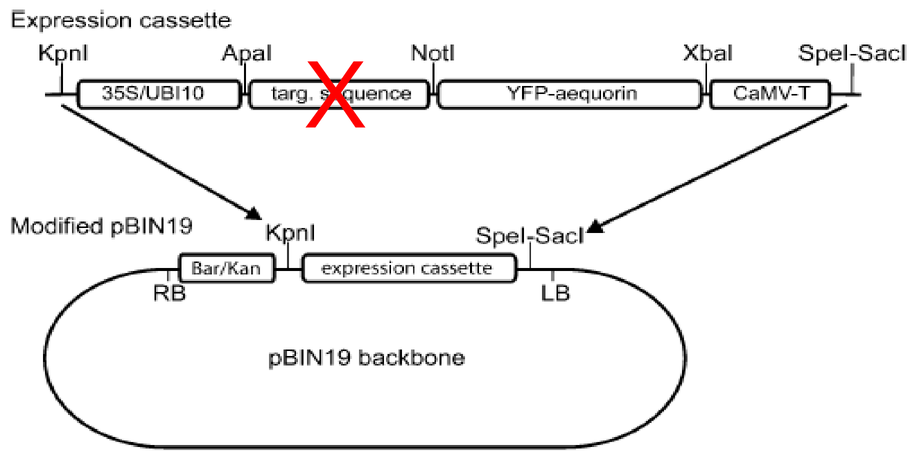
NES – cytosol : NES from Heat Stable Kinase inhibitor

Mehlmer et al. J. Exp.Bot. 2012: 63 (4): 1751–1761

Construct structure of YFP-Aequorin (YA)

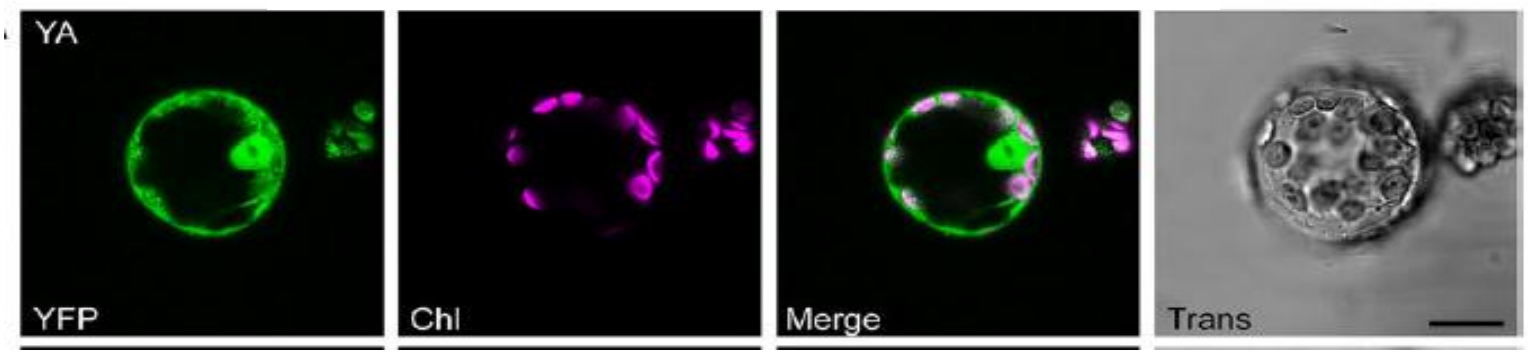


Cytosol and nucleus targeting of aequorin in *Brassica rapa* protoplasts using YA construct



YA fusion alone

Cytosolic and nuclear calcium monitoring



Bar = 20µm

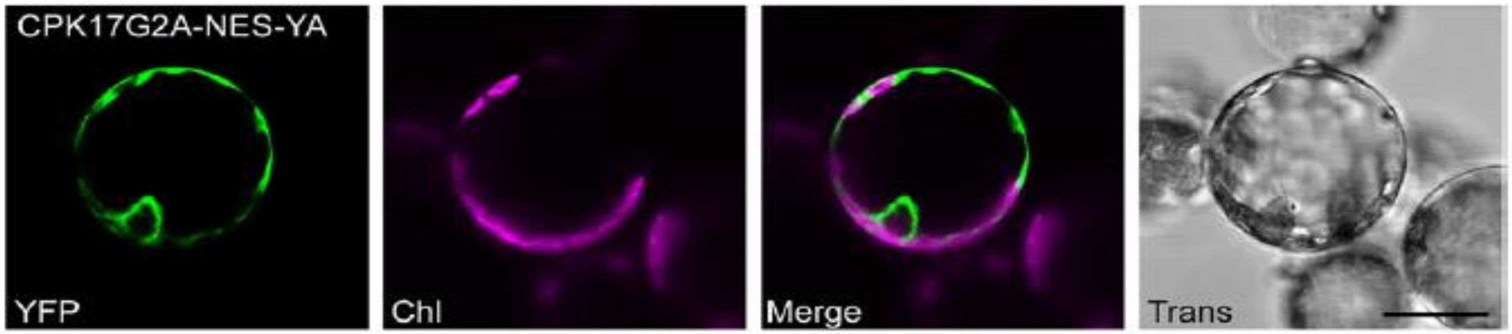
YFP : λ_{ex} = 514nm, λ_{em} = 525-546 nm

Chl : λ_{ex} = 514nm, λ_{em} = 657-726 nm

Mehlmer et al. J. Exp.Bot. 2012: 63 (4): 1751–1761

Cytosol targeting of aequorin in *Brassica rapa* protoplasts using the CPK17G2A-NES-YA construct

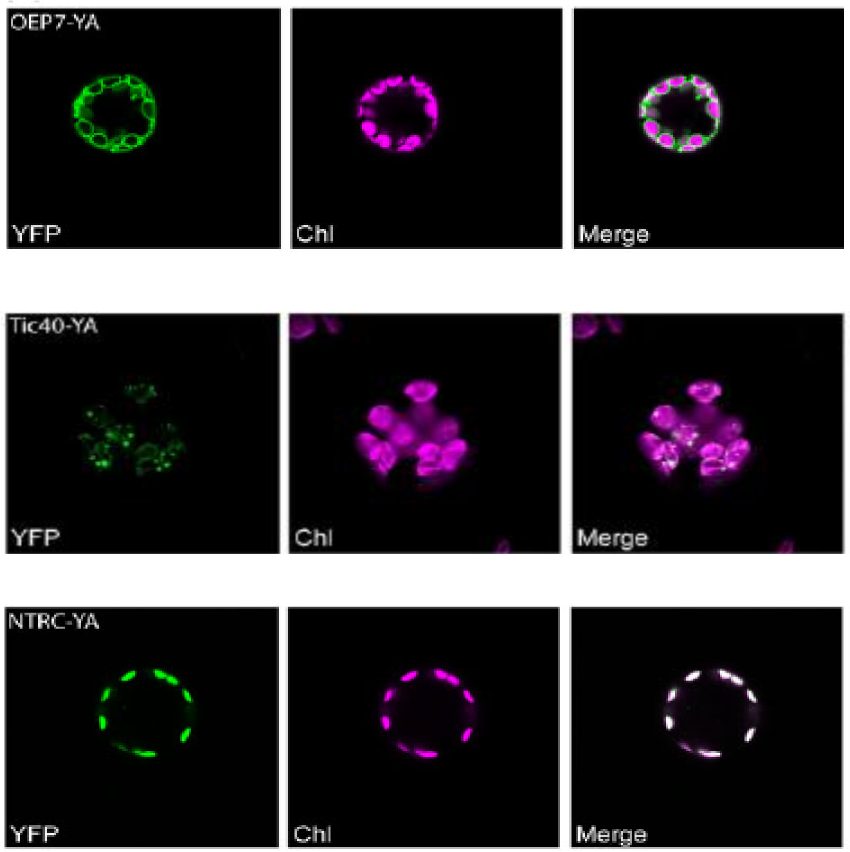
CPK17G2A – cytosol (Benetka *et al., 2008)*



Bar = 20µm
YFP : λ_{ex} = 514nm, λ_{em} = 525-546 nm
Chl : λ_{ex} = 514nm, λ_{em} = 657-726 nm

Mehlmer et al. J. Exp.Bot. 2012: 63 (4): 1751–1761

Localization of YA fusion proteins in various chloroplastic compartments in *Brassica rapa* protoplasts



OEP7 – outer envelope of chloroplasts (Schleiff *et al., 2001) FL OE protein 7*

Tic40 – inner envelope of chloroplasts (Chou *et al., 2003) 130 AA of Tic 40*

NTRC – chloroplast stroma (Perez-Ruiz *et al., 2009) 85 AA NADPH-TRX reductase C*

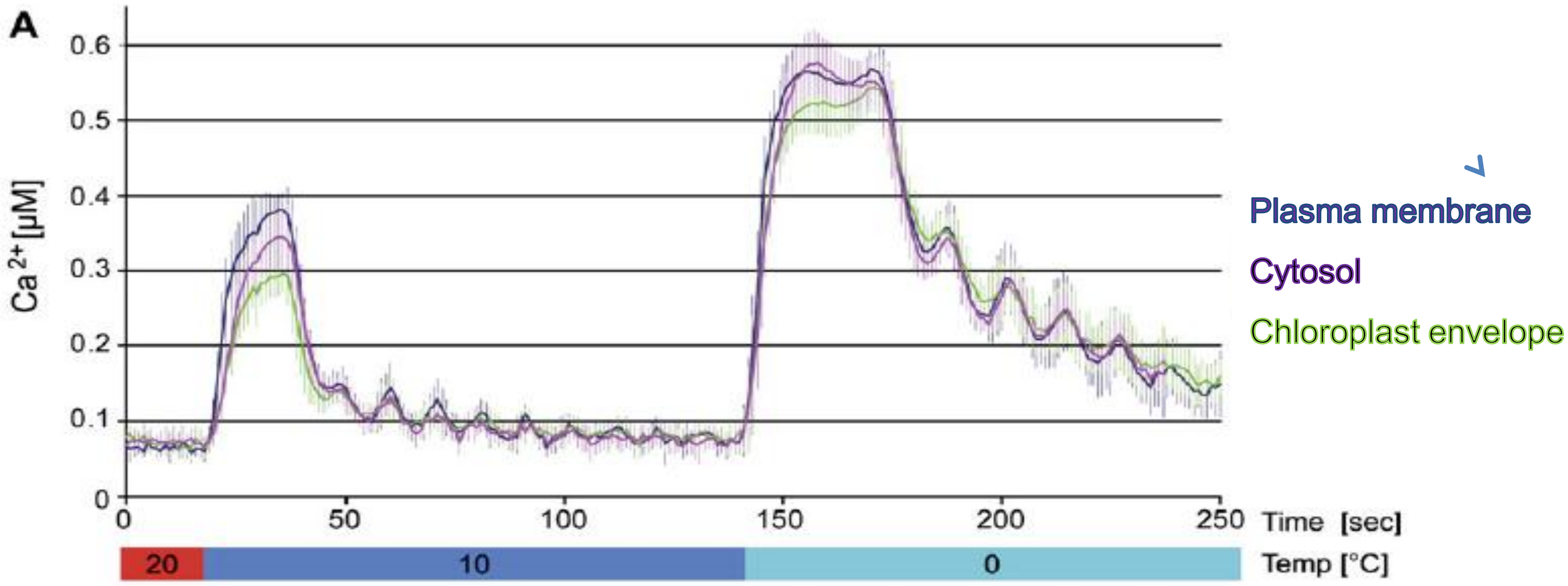
Bar =20µm

YFP : λex = 514nm λem = 525-546 nm

Chl: λex = 514nm λem = 657-726 nm

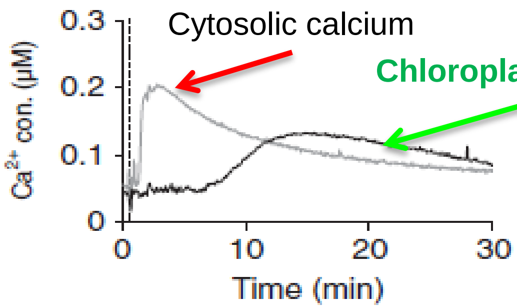
Mehlmer et al. J. Exp.Bot. 2012: 63 (4): 1751–1761

Calcium responses to cold shock in various cell compartments



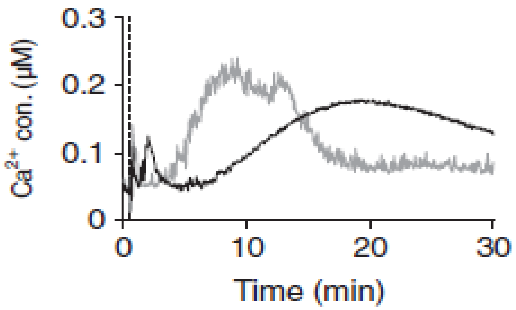
Mehlmer et al. J. Exp.Bot. 2012: 63 (4): 1751–1761

Comparison of cytosolic and chloroplastic calcium responses

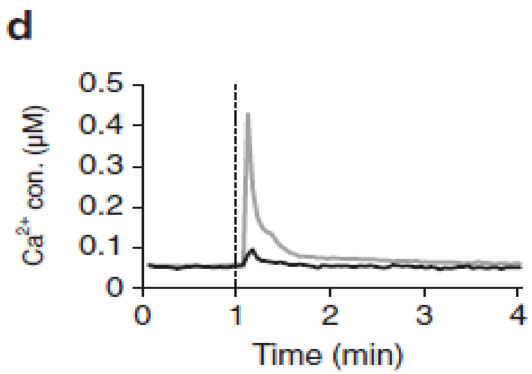


1µM flg22

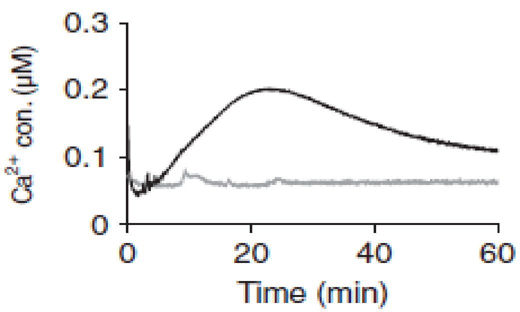
Targeted aequorin to stroma with signal peptide from RBCS (ribulose-1-5-biphosphate carboxylase)



500mM sorbitol



Cold-shock



Light to dark transition

Chloroplast-mediated activation of plant immune signalling in *Arabidopsis*

Hironari Nomura[1,2], Teiko Komori[1], Shuhei Uemura[1], Yui Kanda[1], Koji Shimotani[1], Kana Nakai[1], Takuya Furuichi[3], Kohsuke Takebayashi[4], Takanori Sugimoto[4], Satoshi Sano[1], I Nengah Suwastika[1], Eiichiro Fukusaki[4], Hirofumi Yoshioka[2,5], Yoichi Nakahira[1] & Takashi Shiina[1]

NATURE COMMUNICATIONS | 3:926 | DOI: 10.1038/ncomms1926 |

Cytosolic and nuclear calcium signalling in plants

Objectives of the group:

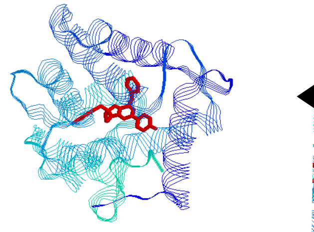
To understand how calcium signatures and compartmentation can control response specificity to biotic and abiotic stimuli in plants

Tools available in the group

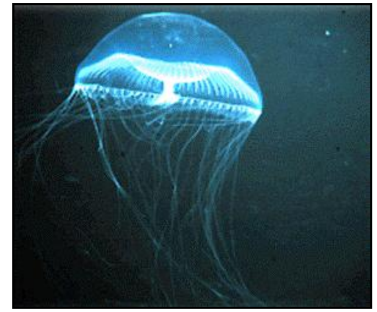
Aequorin tobacco BY-2 cell lines

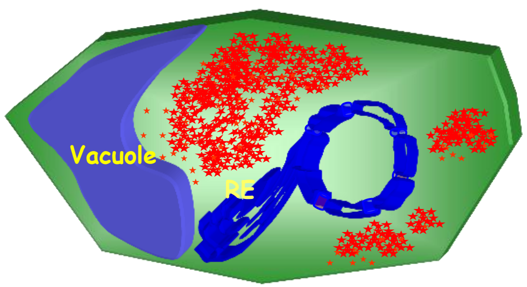
Tobacco cells (BY-2)

aequorin

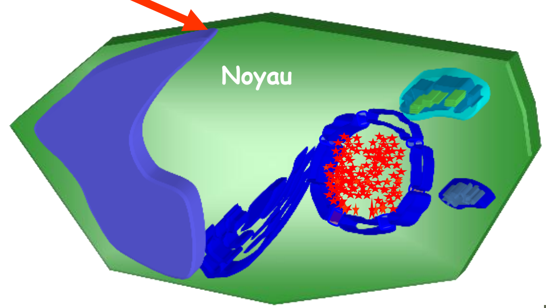


Aequorea victoriae



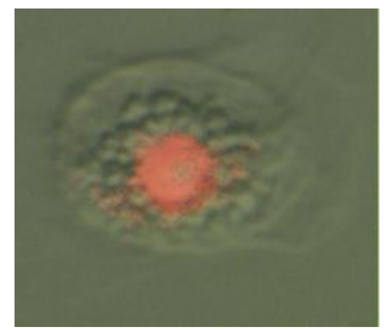


Ca^{2+}_{cyt}



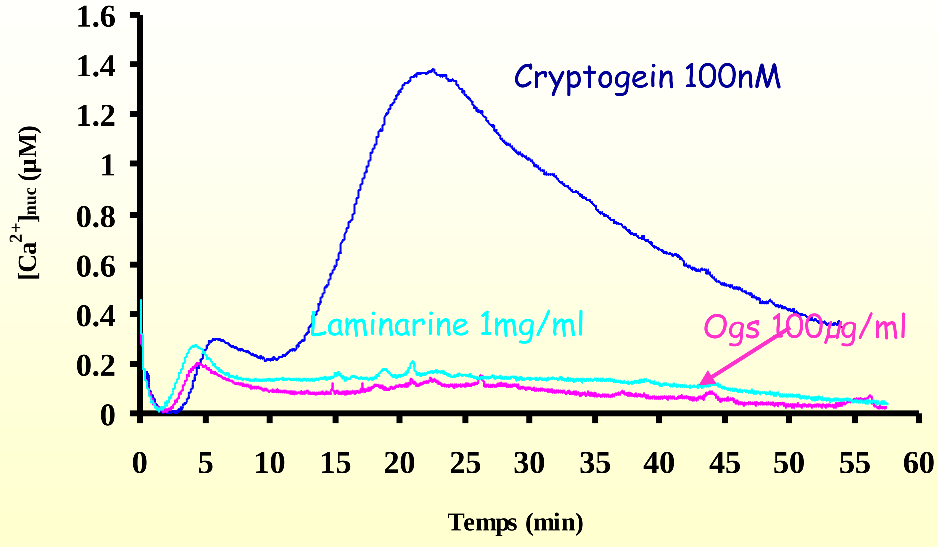
Ca^{2+}_{nuc}

Immunodetection of nuclear aequorin (Secondary Ab Texas red labelled)

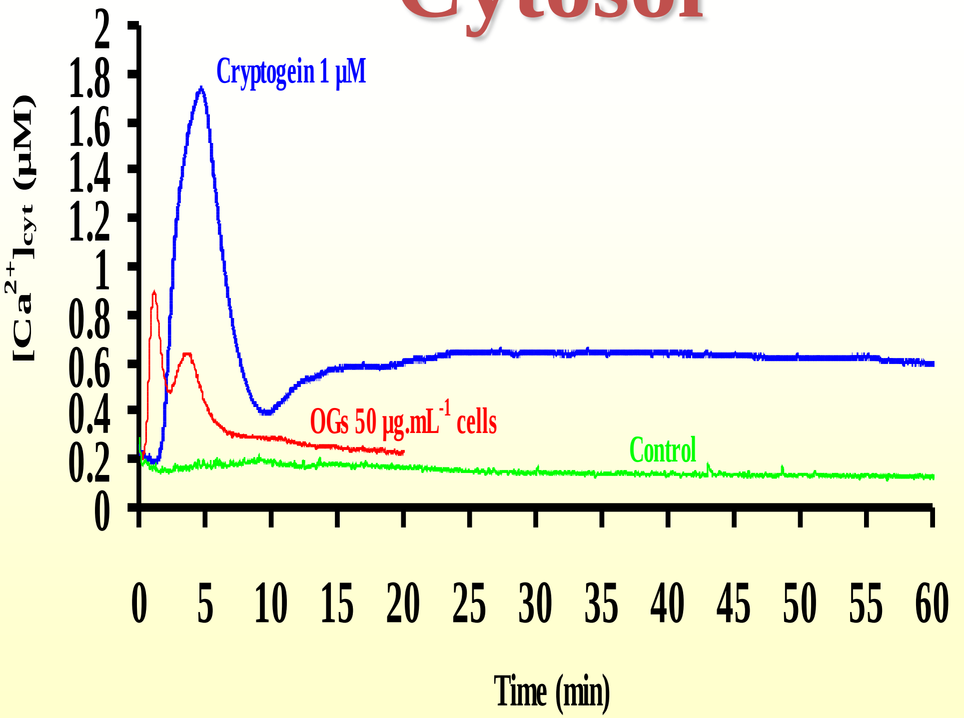


Elicitors-induced cytosolic and nuclear calcium transients in BY-2 tobacco cells

Nucleus

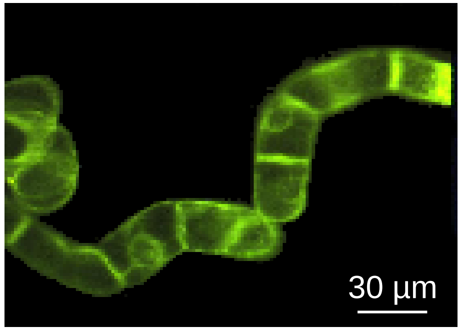


Cytosol

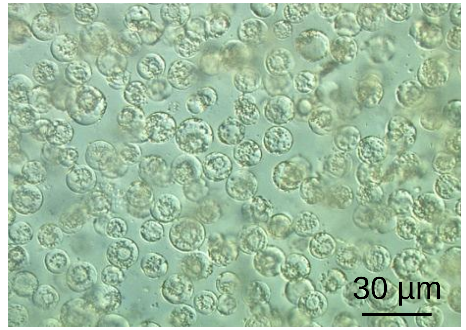


Nuclei preparation from tobacco BY-2 cells

BY-2 tobacco cells

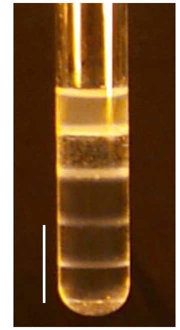


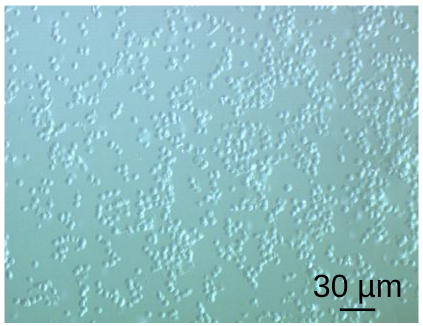
Protoplasts



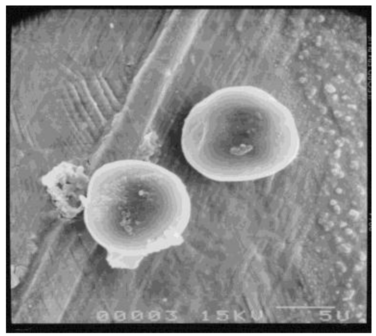
Iodixanol gradient

0%
10%
25%
30%
36%

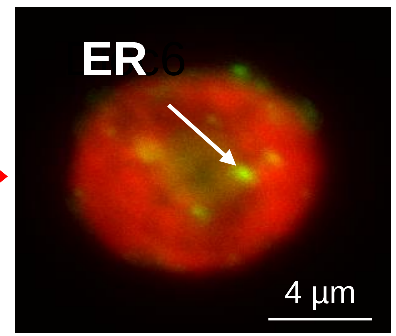




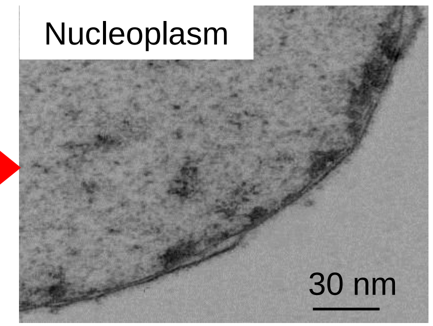
purified nuclei



Scanning microscopy



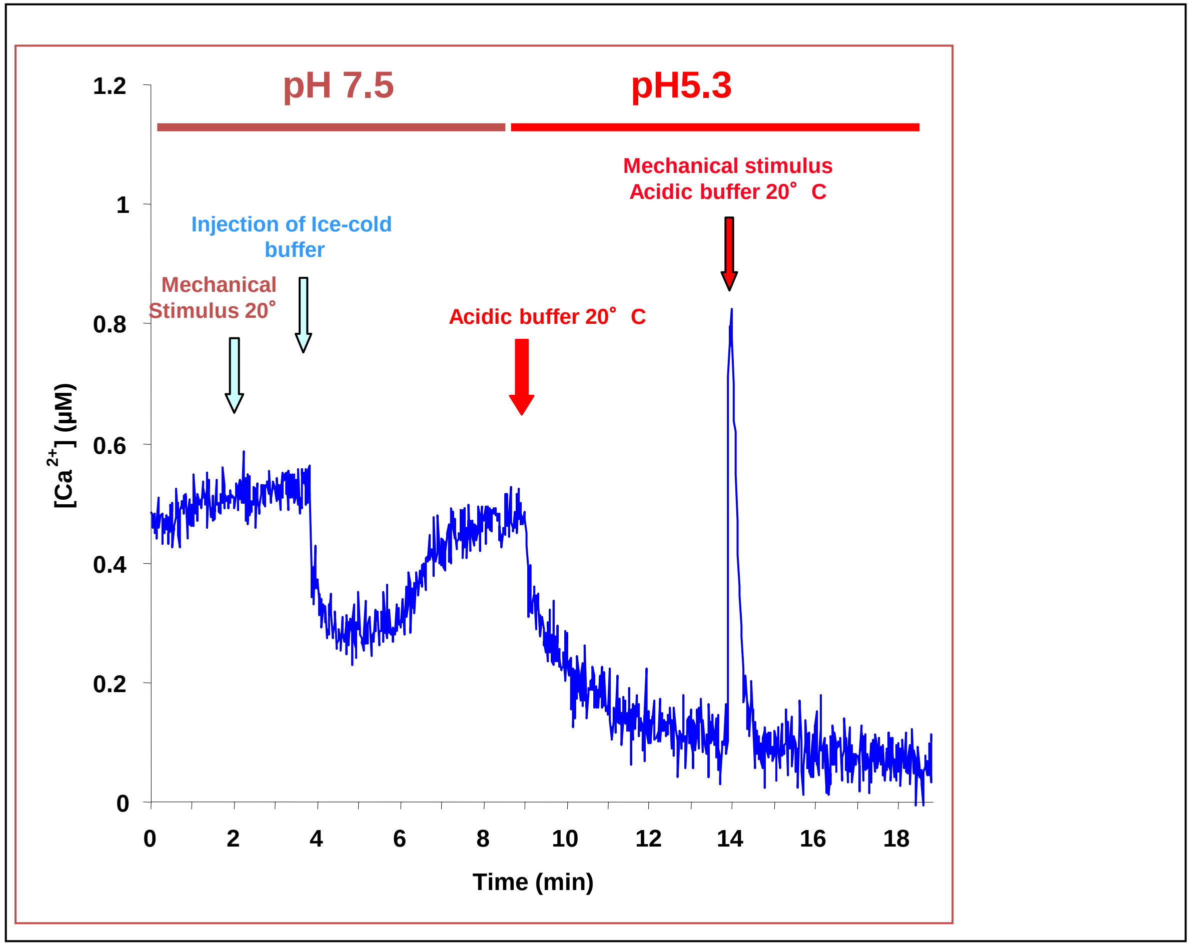
Confocal microscopy
DiOC6 labelling of ER



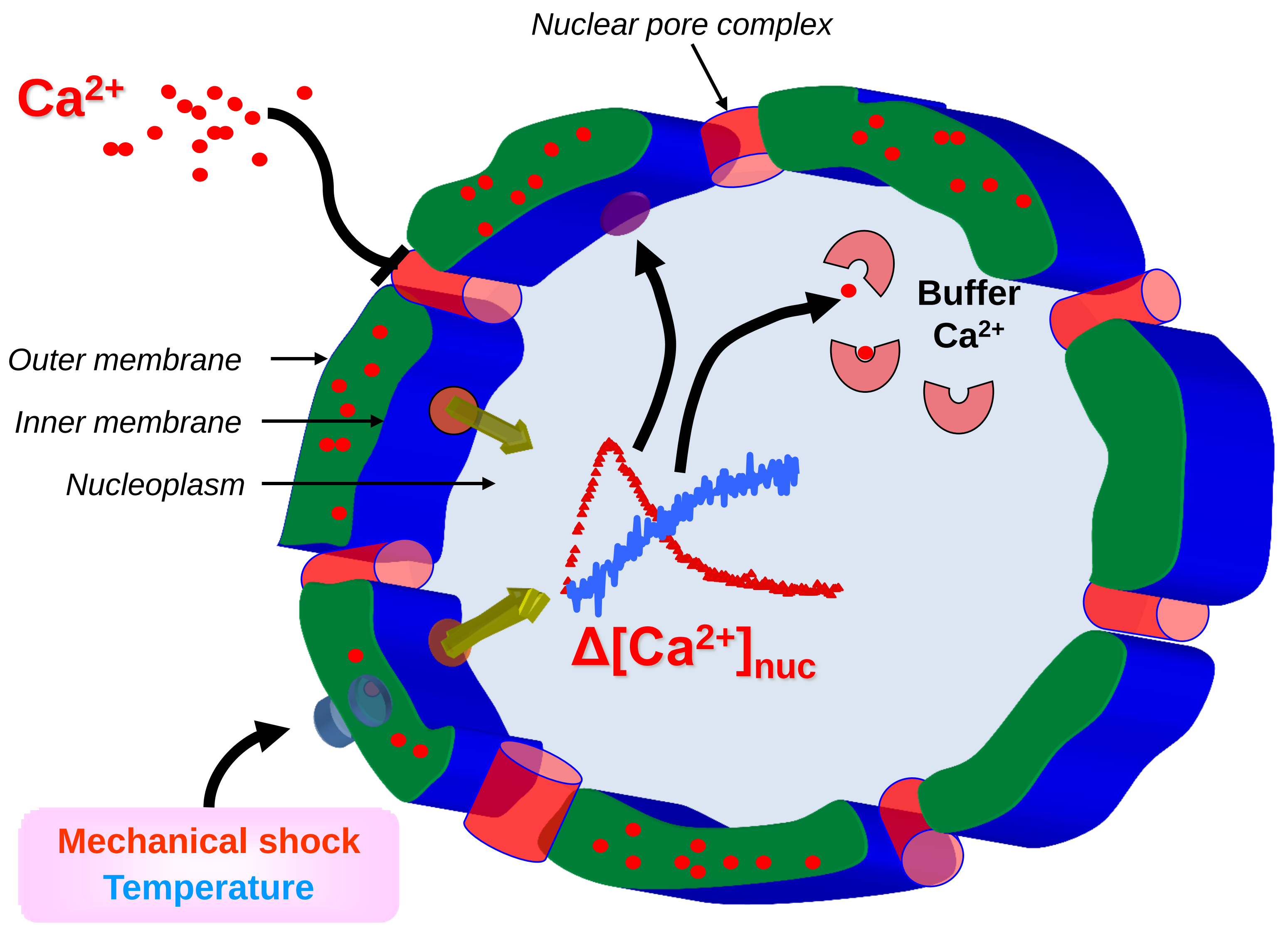
TEM

Xiong TC et al. (2004) Plant J. 40 : 12-21

pH of external medium controls nuclear calcium responses to T° and mechanical shocks



Nuclear calcium homeostasis

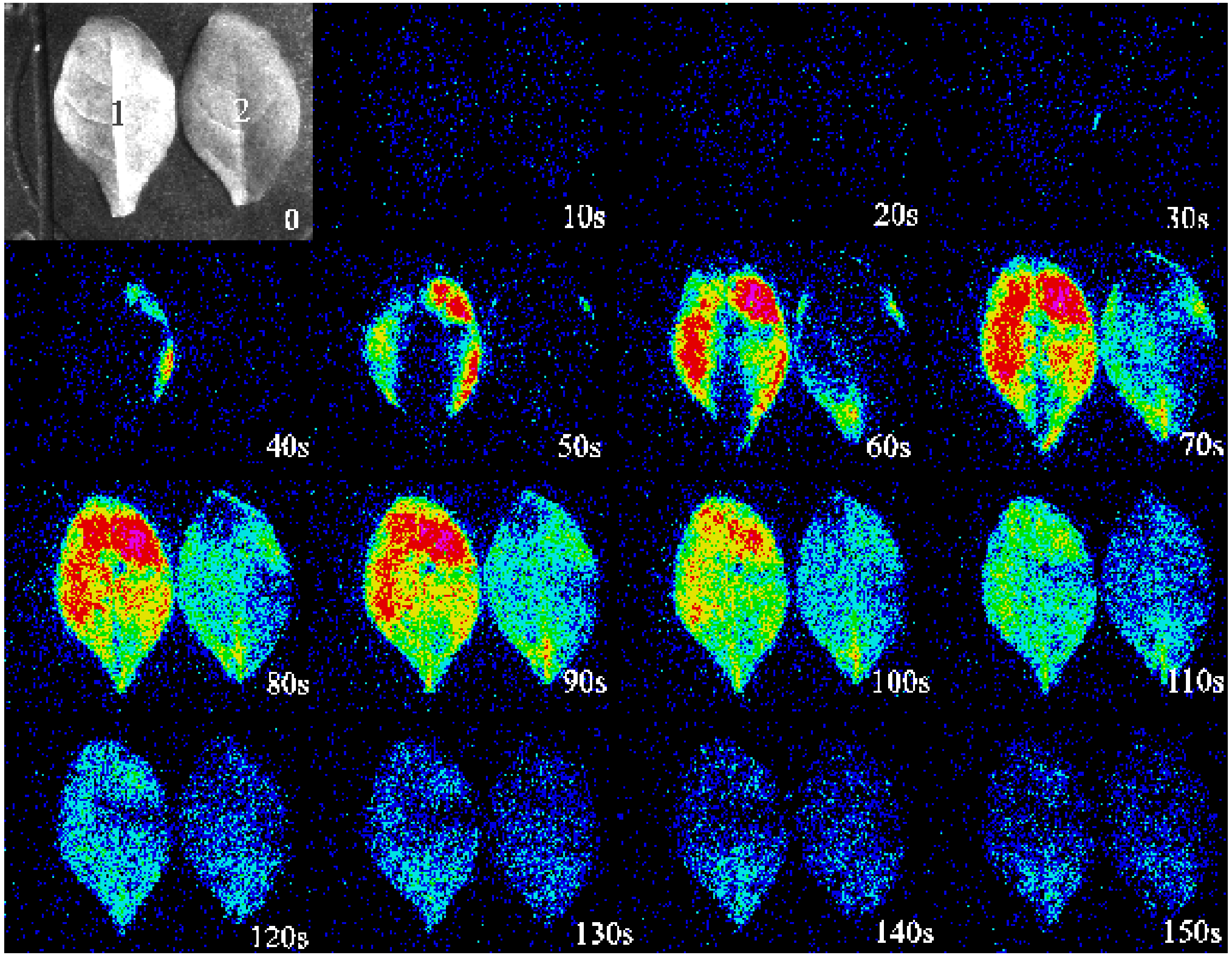


Xiong et al. (2004) Plant J. 40: 12-21
Briere et al. (2006) Cell Calcium 39: 293-303

Aequorin imaging
at the organ or whole plant level

Cold shock (0°C) induced calcium transients on tobacco leaves

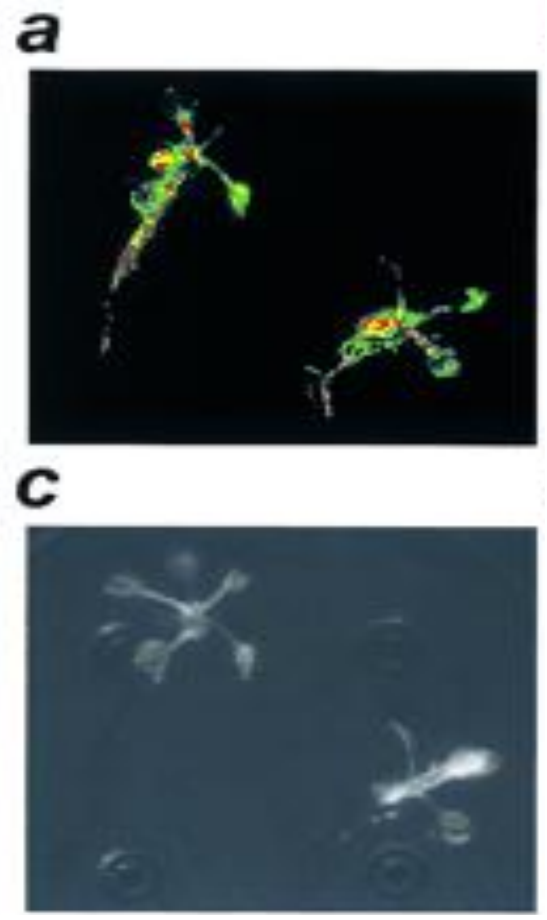
Plants: ***N. plumbaginifolia***
Calcium probe : **Aequorin**
Stimulus : **Cold shock**
Monitoring: **Intensified CCD**
(Photek 216)
Integration time: **10 s**



Campbel et al. 1996 Cell calcium 19(3),211-216

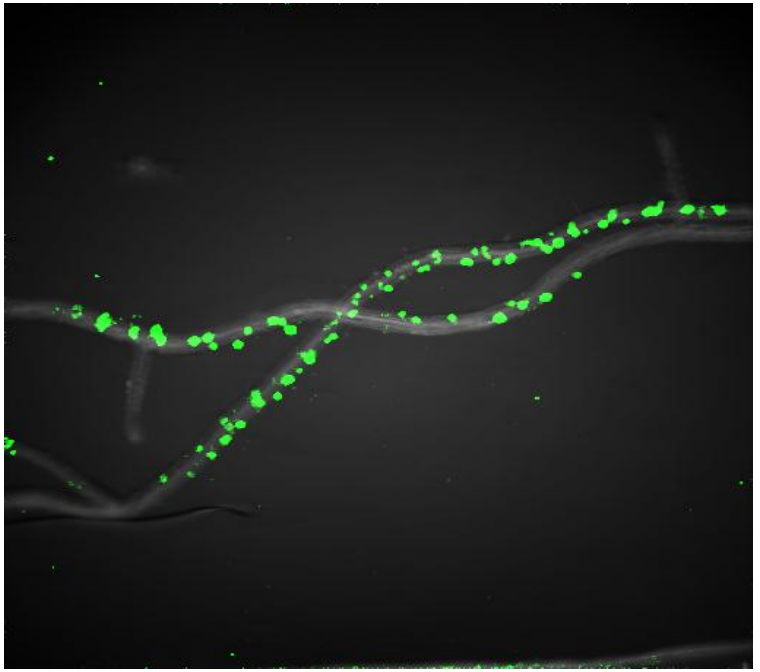
Cold shock- induced calcium variation on whole seedlings of A. thaliana

Plants: seedlings of ***Arabidopsis thaliana***
Calcium probe : **Aequorin**
Stimulus : **Cold shock 17° → 6°**
Monitoring: **Intensified CCD camera** (Photek)
Integration time: **60 s**



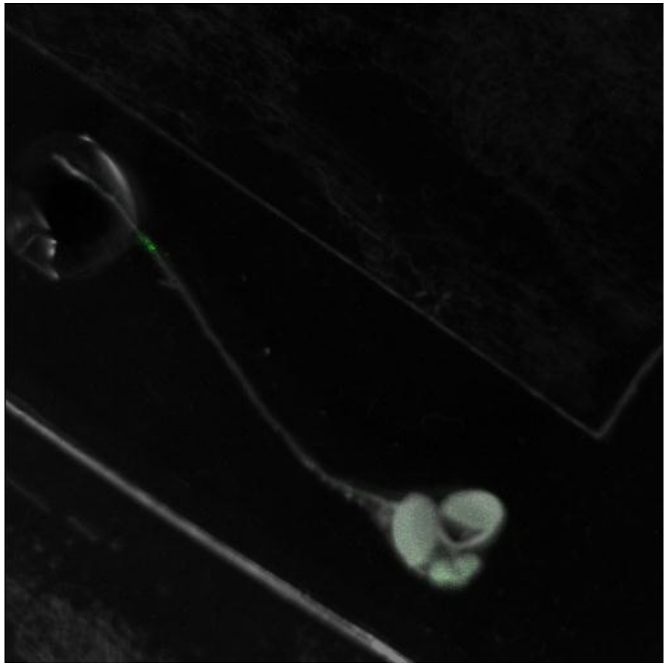
Knight H. et Knight M. (2000) J. Exp. Bot., ***51****, 1679-1685*

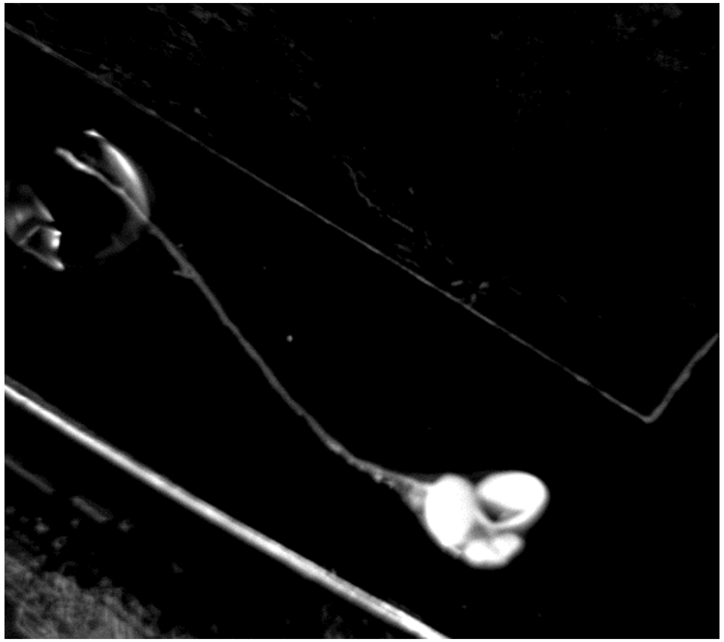
Chitosan-induced nuclear calcium responses in *A. thaliana* roots expressing Nuc-Aequorin



Monitoring: Olympus system LV200 with EmCCD Andor camera
Lens:10X
Stimulus:Chitosan: 0.5mg.ml^{-1}
Integration: 5 s

Oligogalacturonides-induced cytosolic calcium responses in *A. thaliana* seedlings expressing Cyt-Aequorin



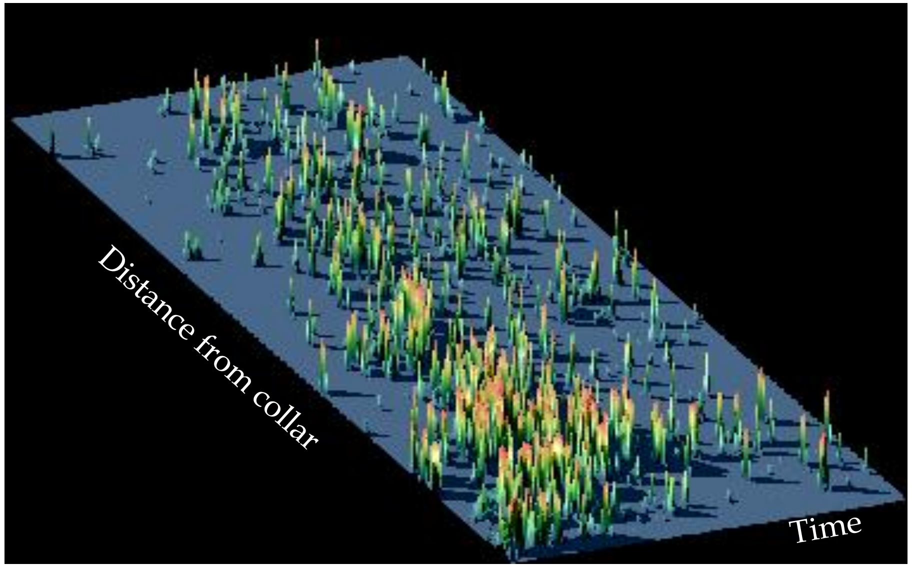


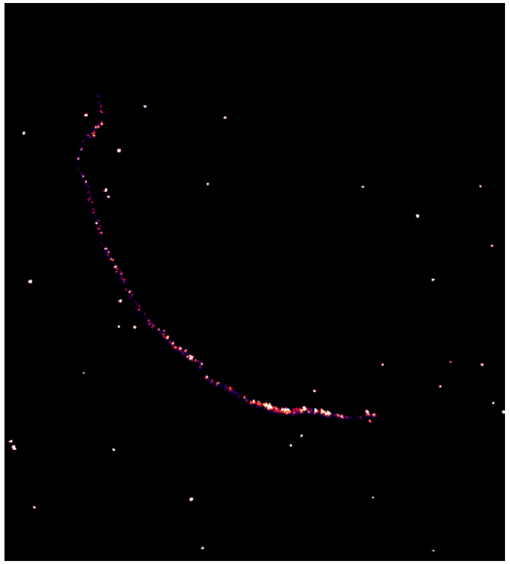
Monitoring: EmCCD Hamamatsu camera
Lens: macro
Stimulus: OGs:1mg.ml^{-1}
Integration: 5 s

Phytosphingosine-induced nuclear calcium responses in *A. thaliana* seedlings

Monitoring: EmCCD Hamamatsu camera
Lens: macro
Stimulus: PHS: 25µM
Integration: 5 s

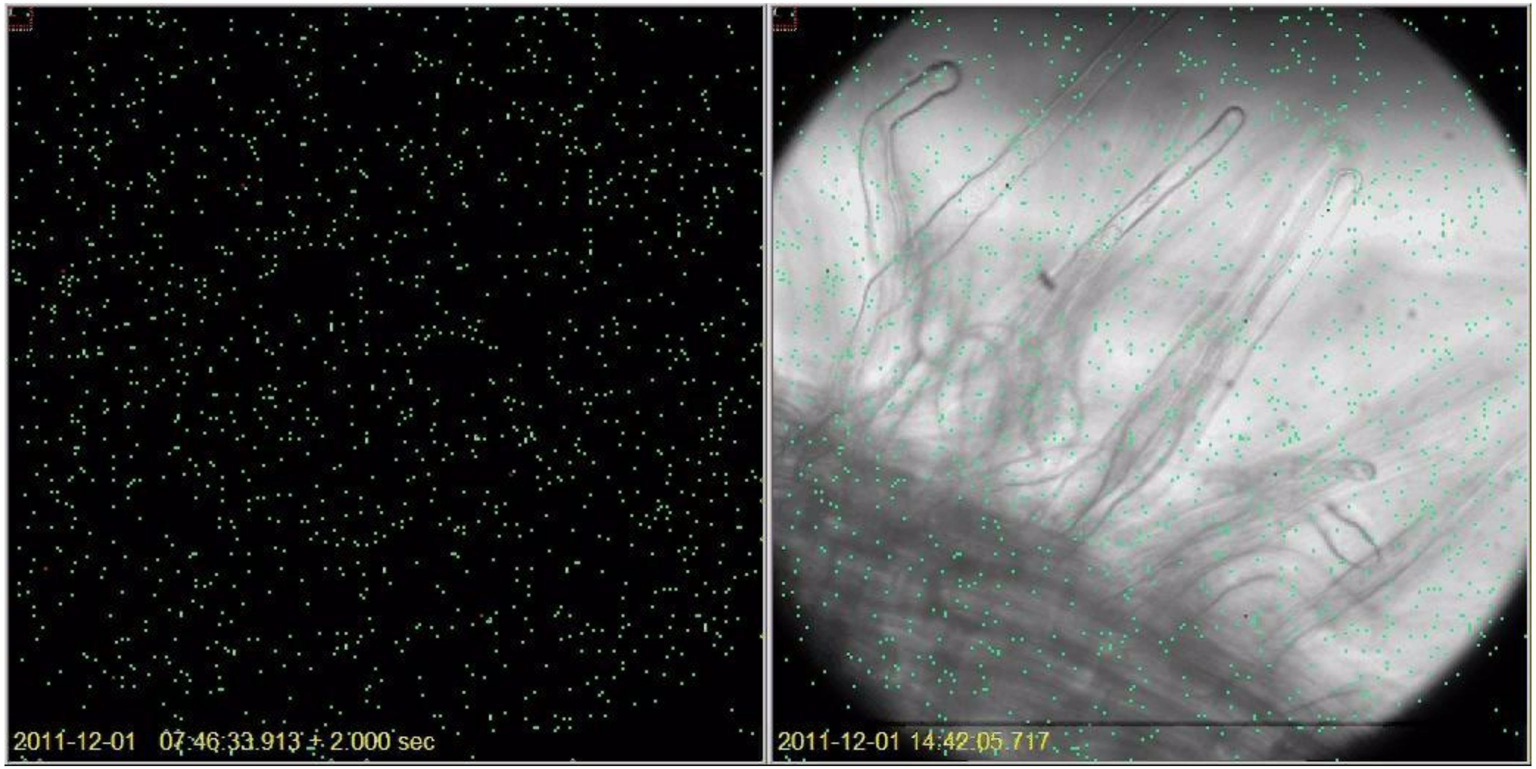
Kymograph





Time integration

Chitosan-induced nuclear calcium responses in *A. thaliana* hair roots



Monitoring: ScienceWares system with EmCCD Andor camera in photon-counting mode
Lens: 40x
Stimulus: Chitosan:0.5 $mg.ml^{-1}$

Acknowledgements

Calcium group

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Université de Toulouse

RSV Laboratoire de Recherche en Sciences Végétales

Thank you for your attention